आज़ादी का अमृत महोत्सव

वार्षिक प्रतिवेदन 2022-2023
ANNUAL REPORT 2022-2023

सी डी एफ डी
... नवीन शोध प्रक्रियाएं जनहित में

C D F D
... Innovating to benefit society

डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र
(जैव प्रद्योगिकी विभाग, विज्ञानं एवं प्रद्योगिकी भारत सरकार का स्वायत्त संस्थान)

Centre for DNA Fingerprinting and Diagnostics
(An autonomous institute of the Dept. of Biotechnology, Ministry of Science and Technology, Govt. of India)

www.cdfd.org.in

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CDFD

वार्षिक प्रतिवेदन
अप्रैल 2022 से मार्च 2023

ANNUAL REPORT
April 2022 to March 2023

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CDFD

डी एन ए फिंगरप्रिंटिंग एवं निदान केन्द्र
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Uppal, Hyderabad - 500 039

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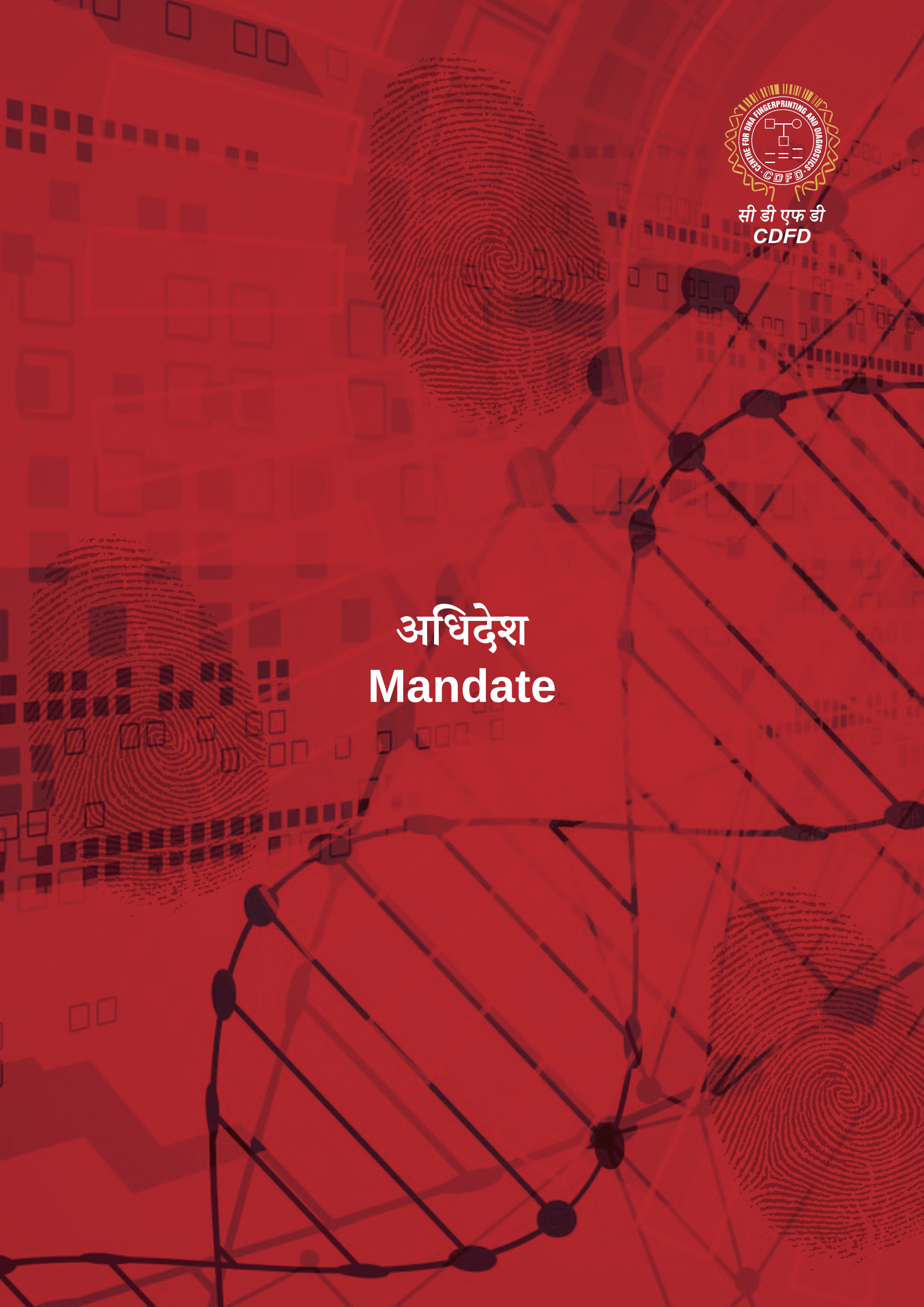


अधिदेश
Mandate

अधिदेश

जिन उद्देश्यों के लिए डीएनए फिंगरप्रिंटिंग एवं निदान केंद्र (सीडीएफडी) की स्थापना की गई थी, जैसा कि बहिर्नियमावली और सीडीएफडी सोसाइटी के नियमों और विनियमों में बताया गया है, वे इस प्रकार हैं:

I. निजी पक्षों सहित विभिन्न एजेंसियों के लिए, उचित भुगतान पर, पितृत्व विवाद, आप्रवासन और अस्पतालों में नवजात शिशुओं के आदान-प्रदान जैसे नागरिक मामलों में डीएनए प्रोफाइलिंग और संबंधित विश्लेषण से संबंधित वैज्ञानिक अनुसंधान करना;

II. अपराध जांच एजेंसियों को डीएनए फिंगरप्रिंटिंग और संबंधित विश्लेषण और सुविधाएं प्रदान करना;

III. अपराध जांच और पारिवारिक मामलों में डीएनए प्रोफाइल विश्लेषण और संबंधित तकनीकों के साक्ष्य मूल्य को समझने में पुलिस कर्मियों, फोरेंसिक वैज्ञानिकों, वकीलों और न्यायपालिका की सहायता करना;

IV. आनुवंशिक विकारों का पता लगाने के लिए डीएनए निदान पद्धतियां स्थापित करना और ऐसी पहचान के लिए जांच विकसित करना;

V. पादप और पशु कोशिका सामग्री, कोशिका रेखाओं के प्रमाणीकरण के लिए डीएनए फिंगरप्रिंटिंग तकनीकों का उपयोग करना और ऐसे उद्देश्यों के लिए जहां आवश्यक हो नई जांच विकसित करना;

VI. डीएनए फिंगरप्रिंटिंग तकनीकों में प्रशिक्षण प्रदान करना;

VII. बुनियादी, व्यावहारिक और विकासात्मक अनुसंधान एवं विकास कार्य करना;

VIII. देश में चिकित्सा संस्थानों, सार्वजनिक स्वास्थ्य एजेंसियों और उद्योग को परामर्श सेवाएं प्रदान करना;

IX. केंद्र के उद्देश्यों से संबंधित क्षेत्रों में विदेशी अनुसंधान संस्थानों और प्रयोगशालाओं और अन्य अंतरराष्ट्रीय संगठनों के साथ सहयोग करना;

X. अनुसंधान विद्वानों को स्नातकोत्तर डिग्री के लिए पंजीकरण करने में सक्षम बनाने के उद्देश्य से मान्यता प्राप्त विश्वविद्यालयों और उच्च शिक्षा संस्थानों के साथ संबद्धता स्थापित करना;

XI. भारत सरकार, राज्य सरकारों, धर्मार्थ संस्थानों/न्यासों, व्यक्तियों और देश के भीतर उद्योग से नकद या अन्य रूपों में अनुदान, दान और योगदान प्राप्त करना;

XII. केंद्र सरकार की पूर्व अनुमति से, प्रशिक्षण कार्यक्रमों, वैज्ञानिक अनुसंधान और अन्य गतिविधियों के लिए अंतरराष्ट्रीय संगठनों सहित विदेशी स्रोतों से मौद्रिक सहायता प्राप्त करना;

XII. किसी भी चल या अचल संपत्ति को उपहार, खरीद, विनिमय, पट्टे, किराए पर या किसी भी तरह से प्राप्त करना या केंद्र की गतिविधियों को चलाने के लिए आवश्यक या सुविधाजनक इमारतों और संरचनाओं का निर्माण, सुधार, परिवर्तन, विध्वंस या मरम्मत करना;

XIII. केंद्र के प्रयोजन के लिए, भारत सरकार और अन्य वचन पत्र, विनिमय बिल, चेक या अन्य परक्राम्य लिखतों को आकर्षित करना और स्वीकार करना, बनाना और समर्थन करना, छूट देना और बातचीत करना;

XIV. केंद्र को सौंपे गए निधि या धन का निवेश करने के लिए, ऐसी प्रतिभूतियों को खोलने के लिए या ऐसे तरीके से जो समय-समय पर शासी परिषद द्वारा निर्धारित किया जा सकता है और ऐसे निवेश को बेचने या स्थानांतरित करने के लिए;

XV. ऐसे सभी अन्य वैध कार्य करना जो उपरोक्त सभी या किसी भी उद्देश्य की प्राप्ति के लिए आवश्यक, आकस्मिक या अनुकूल हों;

XVI. केंद्र के उद्देश्यों को साकार करने के लिए प्रोफेसरशिप, अन्य संकाय पदों, विजिटिंग फेलोशिप सहित फेलोशिप, अनुसंधान और कैडर पदों, छात्रवृत्ति आदि की स्थापना करना;

XVII. केंद्र के वैज्ञानिक और तकनीकी कार्यों के लिए प्रयोगशालाओं, कार्यशालाओं, दुकानों, पुस्तकालय, कार्यालय और अन्य सुविधाओं की स्थापना, रखरखाव और प्रबंधन करना;

XVIII. उद्यमियों और उद्योगों से तकनीकी जानकारी प्राप्त करना या हस्तांतरित करना; और

XIX. केंद्र द्वारा विकसित किए जा सकने वाले पेटेंट, डिज़ाइन और तकनीकी जानकारी को पंजीकृत करना और ऐसे पेटेंट/डिज़ाइन/तकनीकी जानकारी के किसी भी हिस्से को केंद्र के हित में स्थानांतरित करना।

Mandate

The objectives for which the Centre for DNA Fingerprinting and Diagnostics (CDFD) was established, as enumerated in Memorandum of Association and Rules and Regulations of CDFD Society, are as follows:

I. To carry out scientific research pertaining to DNA profiling and related analysis in civil cases like paternity disputes, immigration, and exchange of newborns in hospitals, for various agencies including private parties, on appropriate payment;

II. To provide DNA fingerprinting and related analysis and facilities to crime investigation agencies;

III. To assist police personnel, forensic scientists, lawyers and the judiciary in understanding the evidential value of the DNA profile analysis and related techniques in crime investigation and family matters;

IV. To establish DNA diagnostic methods for detecting genetic disorders and to develop probes for such detection;

V. To use DNA fingerprinting techniques for the authentication of plant and animal cell material, cell lines and to develop new probes where necessary for such purposes;

VI. To provide training in DNA fingerprinting techniques;

VII. To undertake basic, applied and developmental R & D work;

VIII. To provide consultancy services to medical institutions, public health agencies and industry in the country;

IX. To collaborate with foreign research institutions and laboratories and other international organizations in fields relevant to the objectives of the Centre;

X. To establish affiliation with recognized universities and institutions of higher learning for the purpose of enabling research scholars to register for post-graduate degrees;

XI. To receive grants, donations and contributions in cash or in other forms from the Government of India, State Governments, Charitable Institutions/Trusts, individuals and industry within the country;

XII To receive, with the prior approval of the Central Government, monetary assistance from foreign sources including international organizations for training programmes, scientific research and other activities;

XIII. To acquire by gift, purchase, exchange, lease, hire or otherwise howsoever, any property movable or immovable or to construct, improve, alter, demolish or repair buildings and structures as may be necessary or convenient for carrying on the activities of the Centre;

XIV. For the purpose of the Centre, to draw and accept, make and endorse, discount and negotiate Government of India and other Promissory Notes, Bills of Exchange, Cheques or other Negotiable Instruments;

XV. For investing the funds of or money entrusted to the Centre, to open such securities or in such manner as may from time to time be determined by the Governing Council and to sell or transpose such investment;

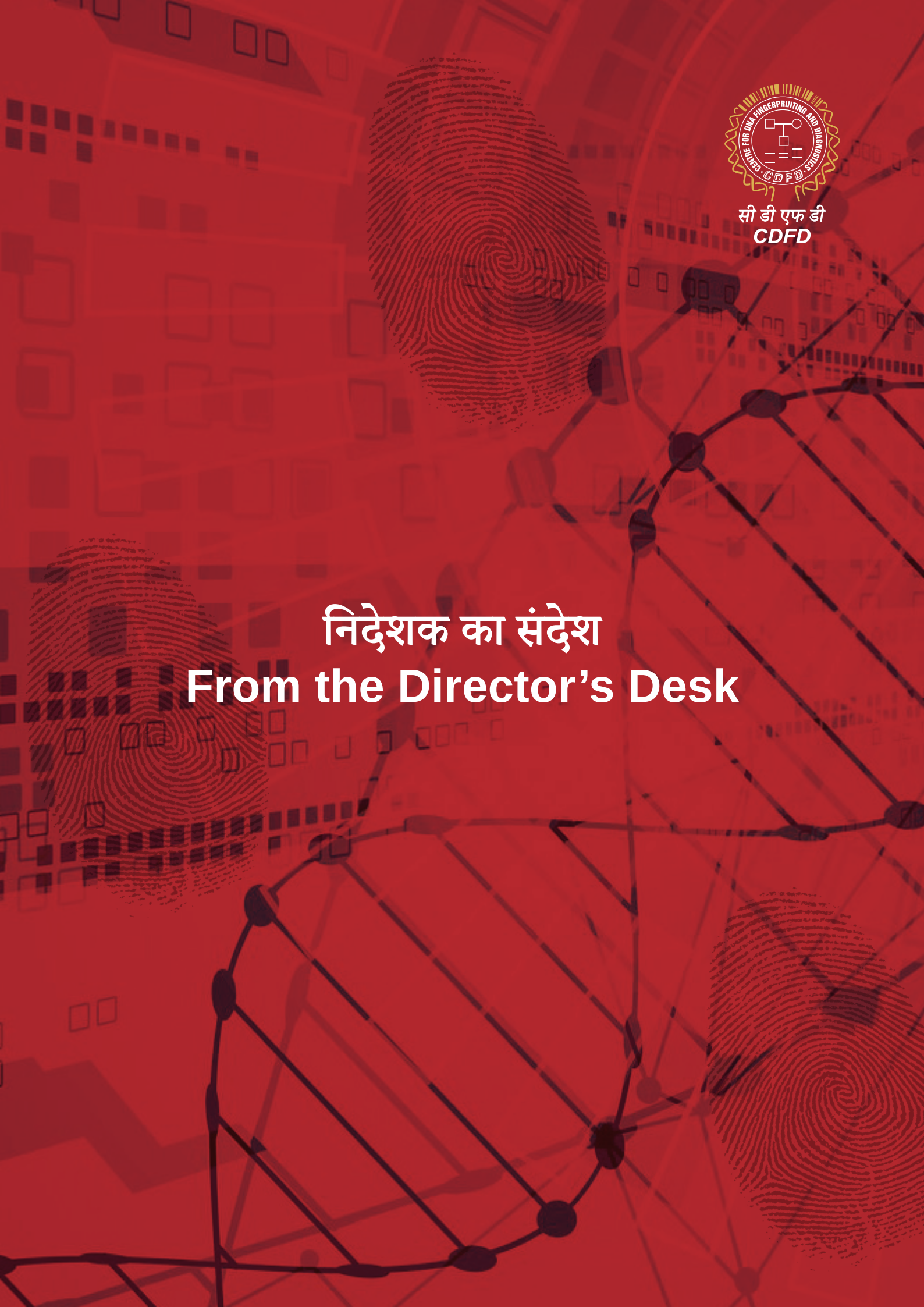
XVI. To do all such other lawful acts as may be necessary, incidental or conducive to the attainment of all or any of the above objectives;

XVII. To institute Professorships, other faculty positions, fellowships including visiting fellowships, research and cadre positions, scholarships, etc. for realizing the objectives of the Centre;

XVIII. To establish, maintain and manage laboratories, workshops, stores, library, office and other facilities for scientific and technological work of the Centre;

XIX. To acquire or transfer technical know-how from/to entrepreneurs and industries; and

XX. To register patents, designs & technical know-how that may be developed by the Centre and transfer any portion of such patents/designs/ technical know-how in the interest of the Centre.



निदेशक का संदेश
From the Director's Desk

निदेशक का संदेश

सी डी एफ डी
CDFD

मुझे अपने सहकर्मियों की ओर से और व्यक्तिगत तौर पर वर्ष 2022-2023 के लिए डीएनए फिंगरप्रिंटिंग एवं निदान केंद्र (सीडीएफडी) की वार्षिक रिपोर्ट प्रस्तुत करते हुए बहुत प्रसन्नता हो रही है। इस केंद्र में विशिष्ट रूप से दो प्रकार की गतिविधियों को पूरा किया जाता है, पहला कानून-प्रवर्तन एजेंसियों के लिए डीएनए प्रोफाइलिंग और आनुवंशिक विकारों के लिए नैदानिक परीक्षणों के क्षेत्रों में सेवा प्रावधान और दूसरा आण्विक जीव विज्ञान के विभिन्न विषयों में किए जाने वाले अग्रणी स्तर के अनुसंधान और ये सब कार्य इस तरह किए जाते हैं कि प्रत्येक आपस में पूरक होते हैं और बदले में दूसरे से समृद्धि हासिल करते हैं। मुझे विश्वास है कि रिपोर्ट में बताए गए अधिकांश कार्यों में यह सहजीवन पाठक के लिए स्पष्ट साक्ष्य होगा।

केंद्र में अंतरराष्ट्रीय सहकर्मी-समीक्षित पत्रिकाओं में प्रकाशनों का एक प्रभावशाली रिकॉर्ड हासिल किया गया है और इसे अनेक पुरस्कार और सम्मान प्राप्त हुए हैं। केंद्र द्वारा किए गए शोध अध्ययनों के विस्तृत विवरण जो रिपोर्ट में अन्यत्र दिए गए हैं, मैं आगे कुछ महत्वपूर्ण शोध की झलकों की जानकारी दे रही हूं।

डीएनए फ़िंगरप्रिंटिंग सेवा प्रयोगशाला में केंद्र और विभिन्न राज्य सरकारों की न्यायपालिका और कानून प्रवर्तन एजेंसियों द्वारा अग्रेषित कुल 75 मामलों का विश्लेषण किया गया है। कुछ उल्लेखनीय मामले हैं, बाहरी दिल्ली के इलाके में विभिन्न स्थानों पर जमा किए गए शारीरिक अवशेषों का उपयोग कर क्रूरतापूर्वक हत्या किए गए पीड़ित की पहचान, राजस्थान के बाड़मेर जिले में मिग -21 लड़ाकू विमान दुर्घटना के बाद अवशेषों से वायु सेना के पायलटों की पहचान और अरुणाचल प्रदेश में हेलीकॉप्टर के दुर्घटनाग्रस्त होने के बाद दो मृत सेना कर्मियों की पहचान। इस अवधि में छात्रों, डॉक्टरों और राज्य/केंद्रीय फोरेंसिक प्रयोगशालाओं के अधिकारियों के लिए डीएनए फ़िंगरप्रिंटिंग पर दो कार्यशालाएँ आयोजित की गईं।

पादप डीएनए फ़िंगरप्रिंटिंग के सेवा क्षेत्र में, कुल 684 बासमती नमूनों का विश्लेषण किया गया और प्लांट डीएनए फ़िंगरप्रिंटिंग सेवाएं प्रदान करने के लिए चावल की 24 किस्मों, खजूर के 4 क्लोनों की डीएनए फ़िंगरप्रिंटिंग की गई।

डायग्नोस्टिक्स सेवाओं के जरिए विभिन्न आनुवंशिक रोगों के लिए 4084 रोगियों को आनुवंशिक मूल्यांकन प्रदान किया। कुल 1326 साइटोजेनेटिक, 2431 आण्विक आनुवंशिकी और 327 जैव रासायनिक आनुवंशिक परीक्षण कराए गए। बहुत गौरव के साथ यह घोषणा की जा रही है कि सीडीएफडी के नैदानिकी प्रभाग में गुणवत्ता और रोगी सुरक्षा में सुधार के लिए इस प्रभाग में अपनाई जाने वाली मानक प्रक्रियाओं की प्रणाली को दोहराते हुए नेशनल एक्रिडिटेशन बोर्ड फॉर टेस्टिंग एंड कैलिब्रेशन लेबोरेटरीज (एनएबीएल) में सफलतापूर्वक आवेदन किया और मान्यता प्राप्त की। निज़ाम इंस्टीट्यूट ऑफ मेडिकल साइंसेज, हैदराबाद में स्थापित मेडिकल जेनेटिक्स विभाग आनुवंशिक सेवाएं प्रदान करने के लिए सफलतापूर्वक कार्य कर रहा है और 8 छात्रों के प्रशिक्षण के साथ मेडिकल जेनेटिक्स में एक डीएनबी प्रशिक्षण कार्यक्रम सफलतापूर्वक चलाया जा रहा है। मेडिकल जेनेटिक्स विभाग, एनआईएमएस, हैदराबाद में जेनेटिक काउंसलिंग में 2 साल के एमएससी प्रशिक्षण कार्यक्रम में चार छात्रों को प्रशिक्षित किया गया है। डीबीटी प्रायोजित "वंशानुगत विकारों के प्रबंधन और उपचार के विशिष्ट तरीके" (यूएमएमआईडी) परियोजना में 'चिकित्सकों के प्रशिक्षण' कार्यक्रम के तहत जेनेटिक डायग्नोस्टिक्स में छह महीने की अध्येतावृत्ति सहित 8 संकाय सदस्यों को प्रशिक्षित किया है। इसके अलावा सीडीएफडी ने आकांक्षी जिलों में रोग की जांच गतिविधियों के लिए यादगीर जिला अस्पताल और रायचूर इंस्टीट्यूट ऑफ मेडिकल साइंसेज में एक डीबीटी निदान केंद्र और रायचूर में एक विज्ञान संग्रहालय की स्थापना की है।

इंसाकॉग के हिस्से के रूप में सीडीएफडी कोविड लैब ने अब तक 60,758 कोविड-19 आरटीपीसीआर परीक्षण सफलतापूर्वक किए हैं और अब तक 17,000 से अधिक कोविड जीनोम को संसाधित और अनुक्रमित किया है।

बैक्टीरियल जेनेटिक्स प्रयोगशाला में संशोधित न्यूक्लियोटाइड्स (पी) पीपीजीपीपी और इसके प्रोटीन सह-कारक डीकेएसए द्वारा विनियमित प्रक्रियाओं की जांच की जा रही है, जिसे लोकप्रिय रूप से जीवाणु एस्चेरिचिया कोलाइ का उपयोग करके कठोर प्रतिक्रिया कारकों के रूप में जाना जाता है। इसमें विशेष रूप से प्रयोगशाला में कठोर कारकों की भूमिका का अध्ययन किया जा रहा है। इसमें कोशिका आकार और विभाजन के साथ फैटी एसिड चयापचय का समन्वय और फैटी एसिड चयापचय में शामिल एक नवीन जीन फैबवाय का विनियमन शामिल है। प्रयोगशाला के एक अन्य समूह ने इस आधार का अध्ययन किया है कि PtsP-PtsO-PtsN फॉस्फो रिले के टर्मिनल फॉस्फो एसेप्टर प्रोटीन, PtsN की अनुपस्थिति ई. कोलाइ में ल्यूसीन संवेदनशीलता की ओर क्यों ले जाती है। इन अध्ययनों से संकेत मिलता है कि ΔptsN उत्परिवर्ती में कम से कम दो विक्षोभ के संयुक्त और सहक्रियात्मक प्रभाव आइसोल्यूसिन जैव संश्लेषण में ख़राबी पैदा करते हैं। अन्य अध्ययनों में, K+ ट्रांसपोर्टरों की फोल्डिंग की मध्यस्थता में SecD/SecF प्रोटीन की भूमिका उनके द्वारा प्रस्तावित की गई है।

कोशिका चक्र विनियमन प्रयोगशाला में शोध अध्ययनों से पता चला है कि एमएलएल और सेटडी1ए सेंट्रोमियर पर आर-लूप को हल करने में अलग-अलग व्यवहार करते हैं जिससे पता लगता है कि एमएलएल के निषेध को ट्यूमर में संभावित चिकित्सीय के रूप में इस्तेमाल किया जा सकता है।

सभी मानव फॉस्फेटेस के लिए इंटरैक्टोम डेटा का उपयोग करके कोशिका मृत्यु और कोशिका उत्तरजीविता प्रयोगशाला में विभिन्न फॉस्फेटेस के लिए नए कार्य सौंपे गए हैं, और महत्वपूर्ण बात एक आण्विक सेतु के रूप में फॉस्फेटस ईवाईए कॉम्प्लेक्स की पहचान है जो रेट्रोग्रेड कोशिकाओं में वेस्कुलर ट्रेफिकिंग के दौरान एंडोसोम को गोल्गी नेटवर्क से जोड़ता है।

सेल सिग्नलिंग प्रयोगशाला में प्रदर्शित किया है कि IP6K1 द्वारा संश्लेषित इनोसिटॉल पाइरोफॉस्फेट 5-IP7 स्तनधारी कोशिकाओं में समजात पुनर्संयोजन मध्यस्थता डीएनए मरम्मत के पूरा होने को बढ़ावा देने के लिए RAD51 और BRCA2 के बीच अंत-क्रिया को नियंत्रित करता है। प्रयोगशाला में SERPINA11 का प्रारंभिक कार्यात्मक लक्षण वर्णन भी किया, जिसकी पहचान एक नवीन सर्पिनोपैथी से संबद्ध है।

सिर्टुइन्स और एफपीसी घटकों को कैंसर में नियंत्रणमुक्त कर दिया गया है तथा क्रोमेटिन जीवविज्ञान और एपिजेनेटिक्स प्रयोगशाला द्वारा अपने शोध अध्ययनों के आधार पर सुझाव दिया गया है कि ये कैंसर-रोधी चिकित्सा विज्ञान के लिए संभावित लक्ष्य हो सकते हैं। इसके विनियामक तंत्र को समझने से नए कैंसर उपचारों को डिजाइन करने में मदद मिलने की उम्मीद है। कई कैंसर में कुछ सिर्टुइन की अभिव्यक्ति बढ़ जाती है, इसलिए, सिर्टुइन अवरोधक संभावित कैंसर विरोधी एजेंट हैं। उन्होंने मानव सिर्टुइन्स के एक पेप्टाइड अवरोधक की खोज की है। इससे अवरोधक कैंसर कोशिकाओं को कुशलता से समाप्त किया जा रहा है। वे आगे कैंसर कोशिकाओं को मारने की क्रियाविधि का अध्ययन कर रहे हैं।

कम्प्यूटेशनल और कार्यात्मक जीनोमिक्स प्रयोगशाला में ऑटोफैगी द्वारा पॉलीनेडिलेटेड एग्रीगेटेड मिसफोल्डेड प्रोटीन के क्षरण को समन्वित करने में हंटिंग्टिन अंत-क्रियात्मक प्रोटीन K (HYPK) की एक नई भूमिका दिखाई गई है। इसके अलावा, उन्होंने HYPK के अंत-क्रियात्मक पार्टनर N-α एसिटाइल ट्रांसफेरेज़ 10 (NAA10) प्रोटीन के संरचनात्मक खंडों की गतिशील प्रकृति की भी जांच की है। संक्रामक रोगों (तपेदिक) के क्षेत्र में उन्होंने एम. ट्यूबरकुलोसिस से प्रतिलेखन नियामक जैसे आईसीएलआर के तीन परलोकों की कार्यात्मक भूमिका को समझने के लिए अपने प्रयासों को आगे बढ़ाया। परजीवी रोगों (मलेरिया) के क्षेत्र में वे एसीबीपी फ़ंक्शन के संभावित रासायनिक अवरोधकों की पहचान और लक्षण वर्णन करने का प्रयास कर रहे हैं। उन्होंने संभावित मेजबान हेपेटोसाइट्स रीमॉडलिंग में मूनलाइटिंग फंक्शन पीएफसीएसपी की भी जांच की।

ड्रोसोफिला न्यूरल डेवलपमेंट की प्रयोगशाला में दर्शाया गया कि बेसिक-हेलिक्स-लूप-हेलिक्स टीएफ ग्रेनीहेड (जीआरएच) एक सामान्य हॉक्स कोफ़ेक्टर के रूप में कार्य कर सकता है और विकास के दौरान उनकी इन-विवो (जीवे) भूमिकाओं को निष्पादित करने में मदद मिल सकती है।

कवक रोगजनन प्रयोगशाला में पहली बार दर्शाया गया कि कैंडिडा ग्लेबराटा एर्गोस्टेरॉल बायोसिंथेसिस मार्ग को डाउनरेगुलेट करके सेल वॉल-टार्गेटिंग इचिनोकैंडिन दवाओं पर प्रतिक्रिया करता है। उनके निष्कर्ष आपस में जुड़े ट्रांसक्रिप्शनल नेटवर्क को रेखांकित करते हैं जो दो अलग-अलग तनावों, कोशिका दीवार की हानि और एर्गोस्टेरॉल संश्लेषण अवरोध के प्रति कोशिकीय प्रतिक्रिया को नियंत्रित करते हैं, साथ ही एजोल एंटी फंगल एर्गोस्टेरॉल जैव संश्लेषण को बाधित करते हैं।

जीनोम संगठन में महत्वपूर्ण भूमिका निभाने वाले कोहेसिन पर जीनोम वास्तुकला प्रयोगशाला में किए गए शोध अध्ययन में कोइसिन के एक नए विनियमन की खोज की गई, जहां डीएनए सुपरकोलिंग क्रोमैटिन से कोइसिन की लोडिंग और निर्मुक्ति दोनों का मार्गदर्शन करता है।

जीनोम सूचना विज्ञान प्रयोगशाला में विभिन्न स्रोतों से जीनोमिक्स डेटा प्राप्त करने के लिए बड़े डेटा विज्ञान, कृत्रिम बुद्धिमत्ता और गहन शिक्षण की शक्ति का उपयोग किया जाता है। इस प्रयोगशाला का उद्देश्य विभिन्न फेनोटाइपिक लक्षणों, विशेष रूप से मनुष्यों, पौधों में बीमारियों का कारण बनने वाले जीनों और रोगजनक की खोज करके नई जानकारी हासिल करना है।

मानव और चिकित्सा आनुवंशिकी प्रयोगशाला में अनुसंधान गुणसूत्र और एकल जीन विकारों के लिए नवीन उत्परिवर्तन/जीन पहचान पर केंद्रित है। उन्होंने संपूर्ण एक्सोम/जीनोम अनुक्रमण विश्लेषण के विश्लेषण के लिए घरेलू डेटा विश्लेषण पाइपलाइनों का विकास और उपयोग किया है। उन्होंने बाल चिकित्सा दुर्लभ आनुवंशिक विकारों पर मिशन कार्यक्रम के तहत दुर्लभ अस्पष्टीकृत आनुवंशिक इटियोलॉजी वाले 196 परिवारों का चयन किया है और 8 मामलों में बीमारी के कारण की सफलतापूर्वक पहचान की है। कार्यक्रम की जरूरतों को पूरा करने और जागरूकता लाने के लिए पीआरएजीईडी वेबसाइट को सीडीएफडी द्वारा विकसित और होस्ट किया गया है। उन्होंने माइटोकॉन्ड्रियल बीमारी के लिए माइटोकॉन्ड्रियल एनजीएस पैनल के विकास पर भी काम किया है।

मानव आण्विक आनुवंशिकी प्रयोगशाला का कार्य माइटोकॉन्ड्रियल विकारों से जुड़े नए जीनों का पता लगाने के एक विशिष्ट उद्देश्य के साथ मानव स्वास्थ्य और बीमारी में माइटोकॉन्ड्रियल शिथिलता को समझने पर केंद्रित है। एनजीएस विश्लेषण में नवीन माइटोकॉन्ड्रियल और नाभिकीय जीन वेरिएंट की पहचान की गई है।

प्रतिरक्षा विज्ञान प्रयोगशाला द्वारा ऐसे साक्ष्य प्रदान किए गए हैं जो सुझाव देते हैं कि एजीई का ऊंचा स्तर कई तरीकों से न्यूरोडीजेनेरेशन, मोटापा, एपोप्टोसिस आदि को बढ़ाता है और एजीई -मध्यस्थता सिग्नलिंग के विनियमन से इन बीमारियों में सुधार होना चाहिए, जिन्हें विवो में और अधिक मान्य करने की आवश्यकता है।

संक्रामक रोग प्रयोगशाला में मानव रोगजनक ई. हिस्टोलिटिका में सेलुलर निबलिंग की विकासवादी संरक्षित प्रक्रिया की जांच की जा रही है। उन्होंने ई. हिस्टोलिटिका वैक्यूलर (वी) एटीपीस सब यूनिट्स की एक विशिष्ट भूमिका की पहचान की है जो सीधे मेजबान सेल निबलिंग के प्रारंभिक चरण में चुने जाते हैं। उनके प्रारंभिक परिणाम बताते हैं कि एह वी-एटीपीस सबयूनिट्स बाह्य कोशिकीय सूक्ष्म वातावरण और मेजबान कोशिका कठोरता को महसूस करने पर अपने स्थानीयकरण को ठीक करते हैं।

आण्विक कोशिका जीव विज्ञान प्रयोगशाला द्वारा किए गए अध्ययनों से संकेत मिलता है कि माइकोबैक्टीरियम ट्यूबरकुलोसिस के पुनः संयोजी रूप से शुद्ध पीपीई 2 (आरपीपीई 2) प्रोटीन और पीपीई 2 से प्राप्त सिंथेटिक पेप्टाइड चोट के स्थान पर मास्ट कोशिका की आबादी को कम करके फॉर्मेलिन प्रेरित पॉ की सूजन को रोकता है। आरपीपीई 2 गैर विषैला होता है और लीवर और किडनी के कार्यों को प्रभावित नहीं करता है। आरपीपीई2/पेप्टाइड फ़ाइब्रोब्लास्ट के केंद्रक में स्थानीयकृत होता है और स्टेम सेल कारक के प्रवर्तक से प्रतिलेखन को रोकता है, जो मास्ट कोशिका रखरखाव और प्रवासन के लिए महत्वपूर्ण है। इस प्रकार, पीपीई2 प्रोटीन/पेप्टाइड का उपयोग सूजन और ऊतक की चोट के उपचार के लिए एक शक्तिशाली गैर-स्टेरायडल विरोधी इनफ्लेमेटरी चिकित्सीय कारक के रूप में किया जा सकता है।

आण्विक ऑन्कोलॉजी प्रयोगशाला में कोलोरेक्टल कैंसर में जीन फ्यूजन और क्रोमैटिन आर्किटेक्चर के बीच एक महत्वपूर्ण संबंध की पहचान करने का कार्य शुरू किया गया है, और कई प्रकार के कैंसर में इसकी पुष्टि की है। क्रोमैटिन रीमॉडलर ARID1B के टर्नओवर और डीएनए मरम्मत के बीच एक नया लिंक सामने आया है।

पादप सूक्ष्म जीव अंत-क्रिया प्रयोगशाला ने पहली बार रिपोर्ट दी कि एक्स. ओरिजे पी.वी. **Oryzae** एक नवीन आसंजन को कूटबद्ध करता है जिसे XadM के नाम से जाना जाता है जो रोग के प्रारंभिक चरण में बायोफिल्म निर्माण और संक्रमण में शामिल होता है। किसी भी रोगजनक बैक्टीरिया में XadM प्रकार के चिपकने की यह पहली रिपोर्ट है।

अनुलेखन प्रयोगशाला में माइकोबैक्टीरियोफेज प्रोटीन, जीपी49 के कार्यों को स्थापित किया, आरएचओ और आरएनएएसईएच के बीच आनुवंशिक संपर्क स्थापित किया और ट्रांसक्रिप्शन रिप्रेसर्स के रूप में पीएसयू-व्युत्पन्न पेप्टाइड के कार्य को स्थापित किया।

सीडीएफडी ने अपने बारह अनुसंधान अध्येताओं को मणिपाल एकेडमी ऑफ हायर लर्निंग (एमएएचई) और हैदराबाद विश्वविद्यालय (यूओएच) से पीएचडी की डिग्री प्रदान करने के

लिए सफलतापूर्वक मार्गदर्शन और नेतृत्व किया है। सीडीएफडी में अनेक पोस्ट डॉक्टरल अध्येताओं, परियोजना सहयोगियों और ग्रीष्मकालीन प्रशिक्षुओं ने काम किया और केंद्र के विकास में महत्वपूर्ण योगदान दिया।

इस रिपोर्ट में वर्णन किए गए सभी कार्यों में, मुझे वैज्ञानिक, तकनीकी और प्रशासनिक संवर्ग के अपने सहयोगियों के साथ-साथ केंद्र में विभिन्न परियोजनाओं में काम करने वाले छात्रों और कर्मचारियों के योगदान और सहयोग को निष्ठापूर्वक स्वीकार करना है। वर्ष के दौरान केंद्र को जैव प्रौद्योगिकी विभाग के अधिकारियों और शासी परिषद् और अनुसंधान क्षेत्र पैनल-सीडीएफडी की वैज्ञानिक सलाहकार समिति के सदस्यों की सलाह, समर्थन और प्रोत्साहन से भी काफी लाभ हुआ है।

सीडीएफ के सभी कार्मिकों का लक्ष्य पूरी ईमानदारी के साथ आने वाले वर्षों में अनुसंधान और सेवा गतिविधियों दोनों में अधिक ऊंचाइयों तक पहुंचने का प्रयास जारी रखना है।

संगीता मुखोपाध्याय

निदेशक-अतिरिक्त कार्यभार

From the Director's Desk

On behalf of my colleagues and on my personal behalf, I have great pleasure in presenting the Annual Report of the Centre for DNA Fingerprinting and Diagnostics (CDFD) for the year 2022-2023. This Centre uniquely combines two kinds of activities, the first being that of service provision in the areas of DNA profiling for law-enforcement agencies and of diagnostic tests for genetic disorders, and the second of frontier-level research in various disciplines of molecular biology, in such a way that each complements and in turn is enriched by the other. I am sure that this symbiosis will be in clear evidence to the reader in much of the work that is described in the Report.

The Centre has achieved an impressive record of publications in international peer-reviewed journals and several awards and honours have come its way. From the exhaustive details of the research studies undertaken by the Centre that are given elsewhere in the Report, I give below a few significant research highlights.

The Laboratory of DNA Fingerprinting Services has analysed a total of 75 cases forwarded by the judiciary and law enforcing agencies of the central and different state governments. The notable cases are, identification of a brutally murdered victim using bodily remains collected at different places in the outskirts of Delhi, the identification of Air Force pilots from remains after the MIG-21 fighter crash in Barmer district, Rajasthan and two deceased army personnel after a helicopter crash in Arunachal Pradesh. Two workshops on DNA Fingerprinting were conducted in this period for Students, Doctors and Officers from State/Central forensic laboratories.

In the service area of Plant DNA Fingerprinting, a total of 684 Basmati samples were analysed and DNA fingerprinting of 24 rice varieties, 4 date palm clones was undertaken for Plant DNA Fingerprinting Services.

Diagnostics services provided genetic evaluation to 4084 patients for various genetic diseases. A total of 1326 cytogenetic, 2431 molecular genetics and 327 biochemical genetic tests were conducted. With great pride, it is to announce that the Diagnostics division of CDFD successfully applied and obtained the accreditation from National Accreditation Board for Testing and Calibration Laboratories (NABL) reiterating the system of standard procedures implemented in the division to improve the quality and patient safety. The Medical Genetics department established at Nizam's Institute of Medical Sciences, Hyderabad is functioning successfully to provide genetic services and a DNB training program in Medical Genetics is running successfully with training of 8 students. A 2 year M.Sc training programme in Genetic Counseling at Department of Medical Genetics, NIMS, Hyderabad has trained four students. A six-month Fellowship in Genetic Diagnostics under the 'Training of Clinicians' programme in DBT sponsored "Unique methods of management and treatment of inherited disorders" (UMMID) project has trained 8 faculties. In addition CDFD has established a DBT Nidan Kendra at Yadgir District hospital and Raichur Institute of Medical Sciences, for disease screening activities in aspirational districts and a Science Museum at Raichur.

CDFD COVID lab, as part of the INSACOG, has successfully conducted 60,758 COVID-19 RTPCR tests to date and have processed and sequenced more than 17,000 COVID genomes till date.

The Laboratory of Bacterial Genetics is investigating processes regulated by the modified nucleotides (p)ppGpp and its protein co-factor DksA, popularly referred as the stringent response factors using the bacterium *Escherichia coli*, Specifically, the laboratory is studying role of stringent factors in the co-ordination of fatty acid metabolism with cell size and division and the regulation of *fabY* a novel gene involved in fatty acid metabolism. Another group of the laboratory has studied the basis behind why absence of PtsN, the terminal phosphoacceptor protein of the PtsP-PtsO-PtsN phosphorelay leads to leucine sensitivity in *E. coli*. These studies indicate that joint and synergistic effects of at least two perturbations in the Δ*ptsN* mutant impair isolecucine biosynthesis. In other studies, a role for the SecD/SecF proteins in mediating folding of K^+ transporters has been proposed by them.

Research studies in the Laboratory of Cell Cycle Regulation revealed that MLL and SetD1A behave differently in resolving R-loops at the centromere which suggests that inhibition of MLL can be used as a potential therapeutic in tumours.

New functions for various phosphatases have been assigned in the Laboratory of Cell Death and Cell Survival by utilizing interactome data for all the human phosphatases, and of significant note is the identification of a phosphatase EYA complex as a molecular bridge that connects endosomes with Golgi network during retrograde vesicular trafficking in cells.

Laboratory of Cell Signalling has demonstrated that the inositol pyrophosphate 5-IP7 synthesized by IP6K1 modulates the interaction between RAD51 and BRCA2 to promote completion of homologous recombination mediated DNA repair in mammalian cells. The lab also conducted preliminary functional characterization of SERPINA11, identified to be associated with a novel serpinopathy.

Sirtuins and FPC components are deregulated in cancer which, the Laboratory of Chromatin Biology and Epigenetics based on their research studies, suggests that these could be potential targets for anti-cancer therapeutics. Understanding the regulatory mechanisms is expected to help design new cancer therapeutics. The expression of certain sirtuin increase in several cancers, therefore, sirtuin inhibitors are potential anti-cancer agents. They have discovered a peptide inhibitor of human sirtuins This inhibitor is killing cancer cells efficiently. They are further studying the mechanism of killing cancer cells.

Laboratory of Computational and Functional Genomics has shown a novel role of Huntingtin interacting protein K (HYPK) in coordinating the degradation of polyneddylated aggregated misfolded proteins by autophagy. In addition, they have also examined the dynamic nature of structural segments of N-α acetyltransferase 10 (NAA10) protein an interacting partner of HYPK. In the area of infectious diseases (tuberculosis), they advanced their efforts to understand the functional role of three paralogues of *iclR* like transcription regulator from *M. tuberculosis.* In the area of parasitic diseases (malaria), they are making efforts to identify and characterise potential chemical inhibitors of ACBP function. They also investigated a moonlighting function PfCSP in a possible host hepatocytes remodelling.

Laboratory of Drosophila Neural Development showed that basic-helix-loop-helix TF Grainyhead (Grh) can function as a generic Hox cofactor and help them perform their in-vivo roles during development.

Laboratory of Fungal Pathogenesis showed for the first time that *Candida glabrata* responds to cell wall-targeting echinocandin drugs by downregulating the ergosterol biosynthesis pathway. Their findings underscore the intertwined transcriptional networks that regulate cellular response to two seemingly distinct stresses, cell wall impairment and ergosterol synthesis inhibition, with azole antifungals impeding ergosterol biosynthesis.

Research studies conducted in the Laboratory of Genome Architecture on cohesin which plays a crucial role in genome organization, yielded discovery of a new regulation of cohesin, where DNA supercoiling guides both the loading and release of cohesin from chromatin.

Laboratory of Genome Informatics harnesses the power of big-data science, artificial intelligence, and deep learning to mine genomics data from diverse sources The lab aims to extract novel information by exploring genes associated with various phenotypic traits, particularly those causing diseases in humans, plants, and pathogens.

The research in Laboratory of Human and Medical Genetics focusses on novel mutation/gene identification for chromosomal and single gene disorders. They have developed and used in house data analysis pipelines for analysis of whole exome/ genome sequencing analysis. They have recruited 196 families with rare unexplained genetic etiology under the Mission program on Paediatric Rare Genetic Disorders and have successfully identified cause of disease in 8 cases. PRaGed website has been developed and hosted by CDFD to cater to the needs of the program and to create awareness. They have also worked on the development of mitochondrial NGS panel for mitochondrial disease.

Laboratory of Human Molecular Genetics focuses on understanding the mitochondrial dysfunction in human health and disease with a specific aim to explore the new genes associated with mitochondrial disorders. The NGS analysis has identified novel mitochondrial and nuclear gene variants.

Laboratory of Immunology provided the evidences that suggest the elevated level of AGE increases neurodegeneration, obesity, apoptosis, etc. in

multiple ways and regulation of AGE-mediated signalling should ameliorate these ailments which needs to be further validated in vivo.

Laboratory of Infectious Diseases is investigating the evolutionary conserved process of cellular nibbling in human pathogen E. *histolytica*. They have identified a typical role of *E. histolytica* vacuolar (V) ATPase subunits that directly recruited at the early stage of host cell nibbling. Their preliminary results suggest that the Eh V-ATPase subunits fine-tune their localization upon sensing the extracellular microenvironment and host cell stiffness.

Studies conducted by Laboratory of Molecular Cell Biology indicates that recombinantly purified PPE2 (rPPE2) protein of *Mycobacterium tuberculosis* and a synthetic peptide derived from PPE2 inhibits formalin induced paw-inflammation by reducing mast cell population to the site of injury. The rPPE2 is non-toxic and does not affect liver and kidney functions. The rPPE2/peptide localizes to the nucleus of fibroblasts and inhibits transcription from the promoter of stem cell factor, important for mast cell maintenance and migration. Thus, PPE2 protein/peptide can be used as a potent non-steroidal anti-inflammatory therapeutic agent for the treatment of inflammation and tissue injury.

The laboratory of Molecular Oncology has pioneered the identification of a significant association between gene fusions and chromatin architecture in colorectal cancer, and confirmed the same across several cancer types. A novel link between turnover of the chromatin remodeler ARID1B and DNA repair has been revealed.

Laboratory of Plant Microbe Interaction reported for the first time that in *X*. *oryzae* pv. *oryzae encodes a novel adhesion known as* XadM is involved in biofilm formation and infection in the early stages of disease. This is the first report of XadM type of adhesin in any pathogenic bacteria.

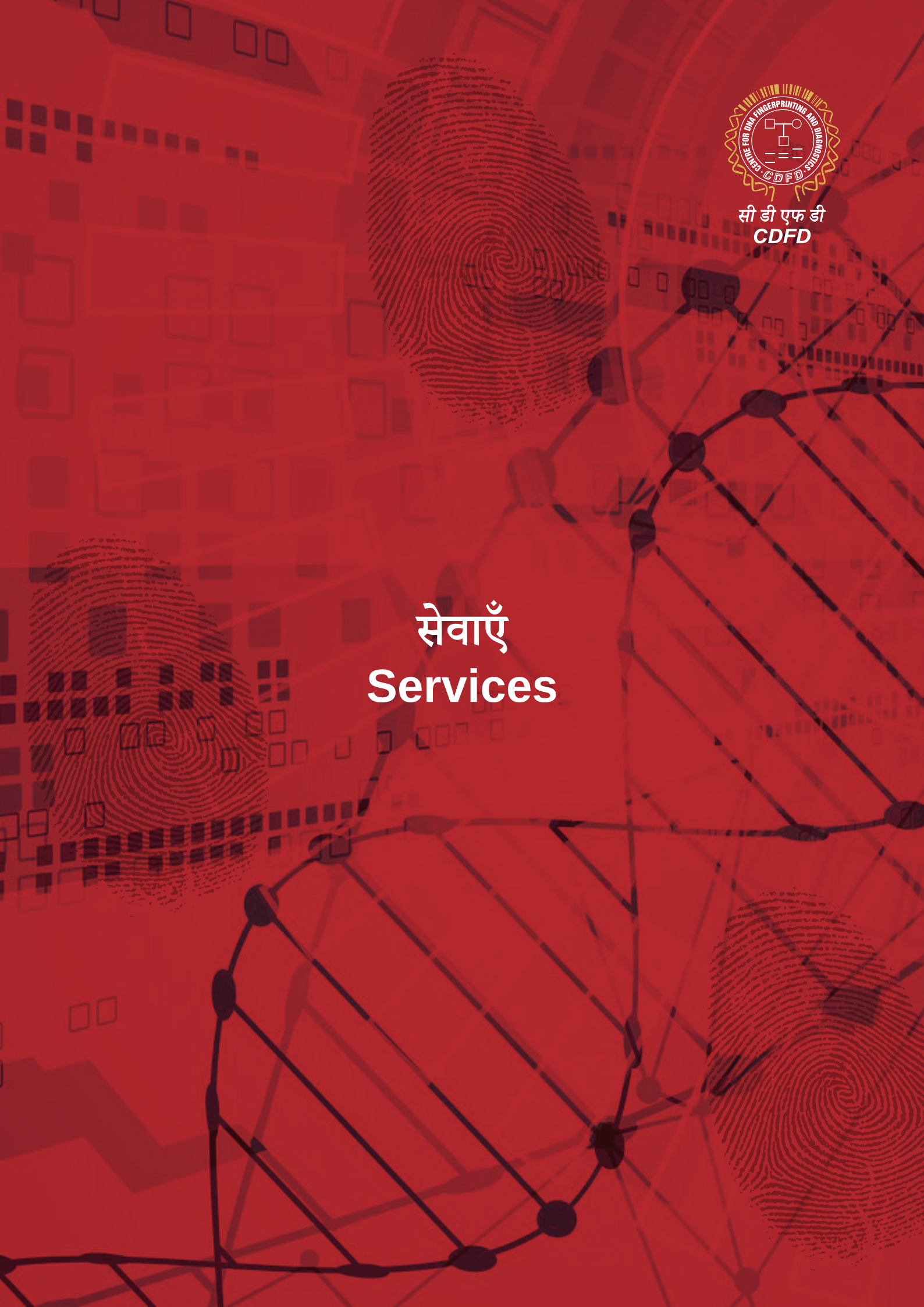
Laboratory of Transcription established the functions of mycobacteriophage protein, Gp49, established genetic interactions between Rho and RNAseH and established the function of Psu-derived peptide as transcription repressors.

CDFD has successfully guided and mentored twelve of its research scholars to be conferred PhD degrees from the Manipal Academy of Higher Learning (MAHE) and University of Hyderabad (UoH). Many postdoctoral fellows, project associates and summer trainees worked at CDFD and contributed significantly in the Centre's development.

In all of the work described in this Report, I must sincerely acknowledge the contributions of and co-operation from my colleagues in the scientific, technical, and administrative cadres as well as from students and staff working in various projects at the Centre. The Centre has also benefitted immensely during the year from the advice, support, and encouragement from the officers of the Department of Biotechnology, and members of the Governing Council and the Research Area Panels – Scientific Advisory Committee of CDFD.

With all sincere earnestness, CDFD aims to continue to strive to greater heights in both research and service activities in the years ahead.

Sangita Mukhopadhyay
Director-Additional Charge



सेवाएँ
Services

Diagnostics Division

Faculty

Ashwin Dalal
Staff Scientist

Adjunct Faculty

Prajnya Ranganath	Additional Professor, NIMS
Shagun Aggarwal	Additional Professor, NIMS

Other Members

P. Rajitha	Technical Officer
Angalena R	Senior Technical Officer
Usha Rani Dutta	Technical Officer
M Muthulakshmi	Technical Officer
Jamal Md Nurul Jain	Technical Officer
Vasantha Rani	Technical Officer
C. Krishna Prasad	Technician I

Objectives

1. To conduct genetic evaluation for patients/families with genetic disorders
2. To develop new methods and assays for genetic analysis and engage in research on chromosomal and single gene disorders
3. To act as national referral center for analysis and quality control of genetic tests for few genetic diseases
4. To impart training in genetic evaluation of patients with genetic disorders

Services provided and Training programs during the year 2022-2023

Clinical Genetics

A total of 4084 patient samples were analyzed for genetic testing, during the year 2022-23 (1/4/2022 to 31/3/2023). These consisted of patients with chromosomal disorders, monogenic disorders, mental retardation, congenital malformations, inborn errors of metabolism, and other familial disorders. The Department of Medical Genetics established at Nizam's Institute of Medical Sciences, Hyderabad is functioning successfully. A total of 9905 patients, of which 3930 were new registrations, were examined and counseled in the department during April 2022- March 2023. In addition, 519 antenatal ultrasonograms, 436 antenatal invasive procedures (chorionic villus sampling and amniocentesis) and 117 fetal autopsies were done. A 3 year training program for Diplomate of National Board (DNB) in Medical Genetics initiated with affiliation to National Board of Examinations, New Delhi is running successfully and 8 students have completed the course and are placed at different institutions across the country. The Diagnostics division provided genetic evaluation to 4084 patients for various genetic diseases. A total of 1326 cytogenetic, 2431 molecular genetics and 327 biochemical genetic tests were conducted.

Achievements

- Diagnostics division of CDFD obtained accreditation from National Accreditation Board for Testing and Calibration Laboratories (NABL).
- Diagnostics division organized a hands on workshop in Molecular cytogenetics and another on Oxford Nanopore technology which was attended by delegates from across the country
- CDFD has been designated as one of the Centres of Excellence under the Rare Disease Policy 2021 of Government of India. More than 155 patient details have been uploaded on the MOHFW portal and more than 20 patients have benefitted from therapy for rare genetic diseases.

MSc training programme in Genetic counseling

A MSc Genetic Counseling program has been initiated at Medical Genetics department established at NIMS, Hyderabad. It is a two year masters program and the course objective is to provide academic and vocational training to become professional genetic counselors. The students trained under this program will be able to cater to comprehensive clinical genetics clinics in tertiary level hospitals. Four students have completed the training.

Fellowship in Genetic Diagnostics

A six month Fellowship in Genetic Diagnostics has been started under the 'Training of Clinicians' programme in DBT sponsored "Unique methods of management and treatment of inherited disorders" (UMMID) project. Clinicians from government

medical colleges/hospitals are being trained in cytogenetics and molecular genetics. Eight faculty from Government medical colleges have completed training by March 2023. New batch of two faculty is expected to join in July-August 2023.

Outreach programme for Aspirational Districts

CDFD has established a DBT Nidan Kendra at Yadgir District hospital and Raichur Institute of Medical Sciences, Raichur, Karnataka under a DBT funded proposal called UMMID (Unique methods of management and treatment of inherited disorders). The plan of the DBT-UMMID initiative is to link the well-established centres of Medical Genetics in India to upcoming centres and to establish clinical genetics facilities in district hospitals. The activities being conducted under the programme include screening of 10,000 antenatal mothers annually attending the district hospital of the aspirational district for thalassemia followed by prenatal diagnosis for prevention of Thalassemia, screening of 5000 newborns annually for 5 common and treatable genetic diseases i.e. G6PD, Congenital hypothyroidism, Galactosemia, Biotinidase deficiency and Congenital adrenal hyperplasia and start early therapy, detection of high risk pregnancies for birth defects and genetic diseases using a questionnaire and referral for free prenatal diagnosis to CDFD and sensitization of school and college students by way of lectures/ presentations in the identified schools /colleges regarding genetic diseases and new advancements. CDFD has established a Science Museum at RIMS, Raichur.

Group of Diagnostics


Laboratory of DNA Fingerprinting

Dr. R Harinarayanan	Scientist In-charge
Dr. D P Kasbekar	Co-ordinator

Other Members

S P R Prasad	Senior Technical Officer
D S Negi	Technical Officer
V A Girnar	Technical Officer
Shruti Dasgupta	Technical Assistant

Objectives

1. To provide DNA fingerprinting services in cases forwarded by law-enforcing agencies/ judiciary of State and Federal Governments, relating to murder, sexual assault, paternity, maternity, child swapping, deceased identification, organ transplantation, etc.;
2. To develop human resources skilled in DNA fingerprinting, to cater to the needs of State and Federal Government agencies;
3. To impart periodical training to manpower involved in DNA fingerprinting sponsored by State and Federal Government agencies;
4. To provide advisory services to State and Federal Government agencies in establishing DNA Fingerprinting facility;
5. To create DNA marker databases of different populations of India.

Details of services provided in the current reporting year (1st April 2022 to 31st March 2023):

A total of 75 cases were received for DNA fingerprinting examination in the current reporting period. Of these, 13 cases were related to maternity/paternity, 30 cases were related to identity of deceased, 31 cases were related to biological relationship and one case is of sexual assault. 10 States and 3 Union Territories of India have availed DNA fingerprinting services from CDFD in this reporting period. Telangana State has forwarded the highest number of cases (32) followed by Uttar Pradesh (13), Andhra Pradesh (9), Maharashtra (4), Chhattisgarh and Goa 3 of each, Delhi, Karnataka, Madhya Pradesh and Leh-Ladak 2 of each and Manipur, Puducherry and Tamil Nadu one of each respectively as shown in Figure 2. The state-wise break-up of cases received are shown in Table 2.

Breakup of the types of cases received during this reporting period is given in Table – 1 and percentage (of the total) of each type of case is given in the pie chart (Figure – 1).

Table – 1

Biological Relationship	31
Identity of Deceased	30
Paternity/Maternity	13
Sexual Assault (Rape)	01
Total number of Cases	**75**

Figure – 1

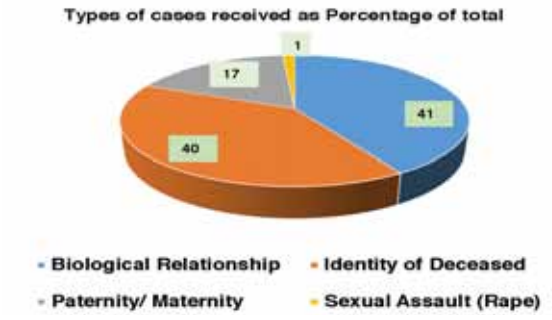
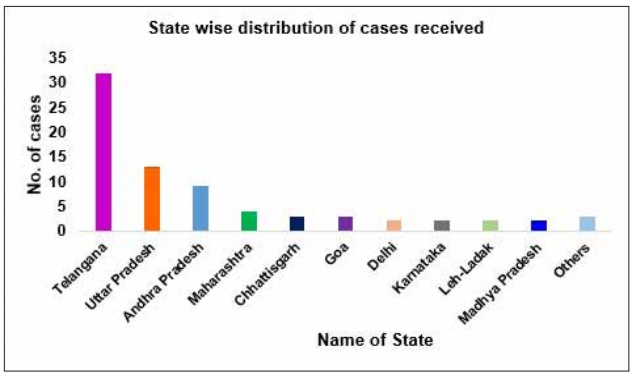


Figure – 2



Prominent cases

1. Establishing identity of a victim, who was brutally murdered and her body parts were thrown at different places in the outskirts of Delhi.
2. Establishing identity of a victim, who was brutally killed and cut into pieces by a man and kept in an abandoned house in Visakhapatnam, AP.
3. Two deceased pilots were identified in MIG-21 fighter crash in Barmer district, Rajasthan on 28-07-2022.

Table – 2: Summary of the State-wise breakup of DNA Fingerprinting cases

Name of the State	Biological Relationship	Identity of Deceased	Paternity/ Maternity	Sexual Assault (Rape)	No. of Cases
Andhra Pradesh	1	7	1	-	09
Chhattisgarh	-	-	3	-	03
Delhi	-	2	-	-	02
Goa	-	-	2	-	03
Karnataka	-	2	-	-	02
Leh-Ladak	-	-	2	-	02
Madhya Pradesh	-	1	1	1	02
Maharashtra	-	4	-	-	04
Manipur	-	-	1	-	01
Puducherry	-	-	1	-	01
Tamil Nadu	1	-	-	-	01
Telangana	29	1	2	-	32
Uttar Pradesh	-	13	-	-	13
Total No. of Cases	**31**	**30**	**13**	**01**	**75**

4. Triple murder case forwarded by CBI, New Delhi
5. Rape & murder case forwarded by CBI, Bhopal.
6. Two deceased pilots were identified in Army Aviation Helicopter crash on 16th March 2023 at Arunachal Pradesh.

Deposition of evidence in Courts of Law

During this reporting year, the DNA experts defended their reports in 9 cases in various Honorable Courts of Law throughout the country.

Training/Lectures/Workshops: 2022 – 2023

1. "Workshop on Forensic DNA Fingerprinting: from Crime Scene to Courtroom" at CDFD during 23-27 May 2022.
2. "Hands-on Workshop on Human Forensic DNA Fingerprinting Training at CDFD during 31st October to 4th November 2022.
3. Demonstration and training on Forensic DNA Fingerprinting to Medical Doctors from Indian Air Force and Institute of Aerospace Medicine, Benguluru during 09-10 June 2022.
4. Lectures on DNA evidence in sexual assault cases given at Central Detective Training Institute, Hyderabad on 22.12.2022 and 19.01.2023.
5. Visit of Air Force officials from Air Force Intelligence School, Pune to CDFD on 03.02.2023.
6. Two-day workshop on "Introduction of DNA Expert System - TrueAllele® Technology for DNA Mixture Interpretation and DNA Database conducted by experts from M/s. Cybergenetics, USA during 06-07 Feb, 2023

Revenue generated

During this reporting period, an amount of **Rs. 24,35,800/-** (Rupees Twenty-four lakhs thirty-five thousand and eight hundred only) has been received towards DNA fingerprinting analysis charges, which is inclusive of GST (18% at present) as levied by the Govt. of India.

Group of Laboratory of DNA Fingerprinting Services

Plant DNA Fingerprinting Services

Chairperson : **Subhadeep Chatterjee**

Scientist in-charge: **K. Anupama**

Other Members : R. Lakshmi Vaishna

M. Sri Lalitha

P. Chandrashekar

Objectives

1. Testing the purity of Basmati samples received from Export Inspection Council (EIC), Ministry of Commerce, Government of India, Basmati rice exporters from India, and other countries;
2. DNA fingerprinting of varieties and hybrids of rice and other crops.
3. To generate new panels of markers for varietal identification and accurate detection of adulteration in Basmati rice.

Details of progress made in the current reporting year (April 1, 2022 - March 31, 2023)

Objective 1: Testing the purity of Basmati samples received from Export Inspection Council (EIC), Ministry of Commerce, Govt. of India, Basmati rice exporters from India and other countries.

During the current reporting year, a total of 684 samples were analyzed of which 68% of the samples were pure and 32% of the samples were adulterated with non-Basmati rice (Figure 1).

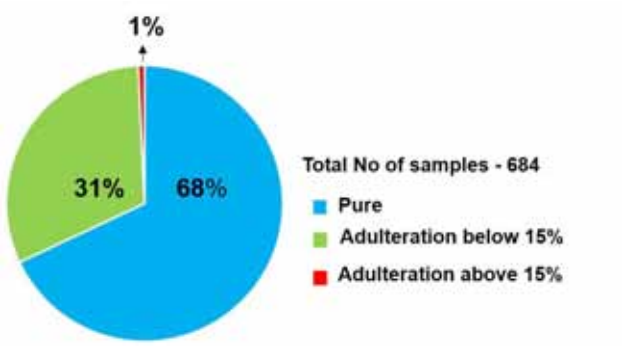


Figure 1. Basmati samples analyzed in the current reporting year.

Objective 2: DNA fingerprinting of varieties and hybrids of rice and other crops.

1. Fingerprinting of 19 rice varieties from Seed Association of Bengal, West Bengal was carried out with 10 SSR markers
2. Fingerprinting of 4 rice varieties from Pan Seeds, Kolkata was carried out with 25 SSR markers
3. It was tested whether markers can be identified for dwarf phenotype or non-flowering phenotype arising during micro-propagation of Date palm using two dwarf non-flowering clones, one normal non-flowering clone and a normal flowering clone as a control with two RAPD and two ISSR markers.

Revenue generated:

An amount of **₹ 96,16,320/-** which includes GST (18%) is received towards purity testing of Basmati samples and **₹ 2,40,889/-** (including 18% GST) is received towards fingerprinting of varieties and hybrids of rice and other crops.

Total revenue generated from April 1, 2022 - March 31, 2023 is **₹ 98,57,209/-** which includes 18% GST as levied by the Govt. of India.

Objective 3: To Generate new panels of markers for varietal identification and accurate detection of adulteration in Basmati rice

Genotyping with SNP markers *alk*4330, *wx*-1, *wx*-6, *wx*-10, *Badh2*, Os03g0717600-SNP-T/C, *Badh1* SNP-6, SNP-10, Os03g0717700 SNP-T/A, *Gw*5 SNP-C/T, and *Wtg* SNP-G differentiated Basmati varieties from potential non-Basmati varieties. Additionally, LOC_Os04g08390SNP-G/C and *PG1* SNP-G/A helped in giving different profiles for Vallabh Basmati 22, Vallabh Basmati 23 and Basmati564 Basmati varieties. Since most of the Basmati varieties now have different profiles it was tested whether HRM method could be used for genotyping and multiplexing of different markers. For each SNP marker both homozygous alleles and heterozygous alleles have to be kept as control while studying the test samples, which is actually increasing the number of reactions and further multiplexing was not giving expected results. Therefore, fluorescent tagged tails

for allele specific primers were tested. If this works, there is no need to tag fluorophore to each allele specific primer, instead the tagged tail can be used for all the markers. Of all the five tested tails, M13F and T7 tails have worked with few markers which will now be tested on all the other SNP markers and multiplexing will be standardised.

Other research

Molecular characterization of two elite water chestnut genotypes (*Trapa spp*.) using RAPD and ISSR markers

Water chestnut (2n=48) is an aquatic plant of Trapaceae family, the fruits of which are nutritious and have medicinal properties. Dr. B.R. Jana from Centre for Makhana, Darbhanga, Bihar has developed two varieties, Improved Red Spineless (IRS) and Improved Green Spineless (IGS) by selecting from Green Spineless and Red Spineless Biotypes for high TSS and yield. These two improved varieties along with the well-known local varieties were analysed in PDFS division using 10 RAPDS and 10 ISSR markers. A total of 78 polymorphic fragments with an average PIC of 0.25 and an average MI of 1.3 were produced by the RAPD and ISSR markers. The dendrogram analysis based on individual and combined RAPD and ISSR markers has demonstrated that the new improved varieties are molecularly distinct from the locally popular varieties.

Publication:

Submitted article:

B. Jana, K.Anupama, RL Vaishna A Das K, Development of two elite water chestnut genotypes (*Trapa spp.)* and their molecular characterization using RAPD and ISSR markers. Submitted to Genetic resources and Crop Evolution.

Group of Plant DNA Fingerprinting Services


CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
CDFD
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CDFD

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Research

Laboratory of Bacterial Genetics

Studies on integral membrane proteins of *Escherichia coli* involved in adaptive solute transport

Principal Investigator **Abhijit A Sardesai**

Staff Scientist

Members

Neeraj Kumar	Senior Research Fellow
Yogesh Patidar	Senior Research Fellow
Suchitra Upreti	Senior Research Fellow
Sayani Ghosh	Junior Research Fellow
Amit Kumar	Junior Research Fellow
Bayya Shirisha	Technical Assistant

Objectives

Research in the laboratory is concerned with the study of integral membrane proteins of *E. coli* involved in adaptive solute transport. In this regard we are studying the interplay between a three protein phosphorelay comprising the proteins PtsP, PtsO and PtsN and potassium (K^+) transport systems of *E. coli*. Extensions in these studies have led to research in the areas of K^+ transporter biogenesis and the interplay between cellular K^+ pools and L-isoleucine biosynthesis. Another component of research deals with the study of basic amino acid export in *E. coli*.

Escherichia coli (*E. coli*) and members of *Enterobacteriaceae* utilize active K^+ uptake systems for uptake of the essential cytoplasmic cation K^+. K^+ is believed to exert regulatory effects on multiple physiological processes. We have been studying the PtsP-PtsO-PtsN phosphorelay and its impact on cellular K^+ ion homeostasis. These studies have led us into the study of the relationship between cellular K^+ and L-isoleucine biosynthesis. The Δ*ptsN* mutant has been reported to display a leucine-sensitive growth phenotype (Leu^S) in a minimal medium of low K^+ content (10 to 20 mM). The basis behind the Leu^S of the Δ*ptsN* mutant is not clear. Earlier we had reported on mutant TrkH proteins bearing the T201, L80Q and P151R amino acid substitutions whose expression suppressed of the Leu^S of the Δ*ptsN* mutant. TrkH is the K^+ channel component of the TrkH K^+ uptake transporter.

In this year we noted that aforementioned TrkH mutant proteins hyperactivated K^+ uptake via the Trk transporter. Moreover, K^+ uptake via these TrkH mutant proteins occurred in the absence of SapD, the ATP binding component of the Trk transporter. This observation also points to the possibility that PtsN may also positively regulate the Trk K^+ uptake transporter, besides the Kdp transporter. Furthermore, we found that the Leu^S was also alleviated by mutations leading to hyperactivation of Kdp K^+ uptake transporter. The Leu^S of the Δ*ptsN* mutant in K_1 (K^+ content 1 mM) medium, was alleviated by either L-isoleucine (Ile) or by α-ketobutyrate, the product of threonine deaminase (IlvA) catalysing the first step of Ile biosynthesis, in the medium. This indicated that the perturbation caused by external Leu in the Δ*ptsN* mutant leads to Ile auxotrophy and at the least impairs IlvA function. Expression either of the IlvA G360V variant or overexpression of IlvA alleviated the Leu^S. Over expression of other biosynthetic enzymes of the Ile pathway namely IlvD or IlvE did not alleviate the Leu^S. Activity of IlvA was found to be K^+ dependent, activated by L-valine (Val) inhibited by Ile, as expected and not inhibited by Leu. On the other hand, activity of the IlvA G360V variant showed a lesser dependence on K^+, was not inhibited by Leu, not activated by Val but was hyperactivated by Ile. These observations implicate IlvA function as one casualty of the attenuated K^+ uptake in the Δ*ptsN* mutant

In *E. coli* K-12 the activities of the two acetohydroxyacid synthases isozymes (AHASs) encoded by the *ilvBN* (AHASI) and *ilvIH* (AHASIII) operons, catalyse the first step common to the biosynthesis of Leu and Val and the second step in the biosynthesis of Ile respectively. We found that overexpression of either AHAS I or AHAS III alleviated the Leu^S.

Our studies support a scenario wherein joint and synergistic effects of at least two perturbations in the Δ*ptsN* mutant impair Ile biosynthesis. Reduced K^+ uptake via the Kdp and Trk K^+ transporters in

the Δ*ptsN* mutant attenuates IlvA function, whereas exogenous Leu supplementation likely perturbs either the expression and/or activity of AHAS I and AHAS III, jointly leading to Ile starvation. The latter presumption is currently under study.

Previously we have described linkages between the auxiliary components of protein secretion namely the SecD and the SecF proteins, and K^+ metabolism. These were based on studies with a SecD/SecF function perturbing lesion *yajC**. *yajC** represents a transposon insertion in *yajC*, the first gene of the *yajC secD secF* operon, that severely attenuates expression of *secD* and *secF.* The outcome of this lesion is that it led to a K^+ requiring phenotype (K^{Req}). We have shown that the K^{Req} is associated with reduced levels of the K^+ channel components of the Trk systems TrkG/TrkH and the Kup K^+ transporter and attenuates K^+ uptake through Trk and Kup. The K^{Req} could be alleviated by the absence of the HslUV protease or by overexpression of the membrane protein insertase/chaperone YidC.

In this year we studied the membrane localization a TrkH::mNeonGreen hybrid under conditions of depletion of components of membrane protein biogenesis, by fluorescence microscopy. We noted that envelop localization of the TrkH::mNeonGreen was not perturbed by depletion of either SecD/SecF or YidC but was perturbed when Ffh and SecE were limiting *in vivo*. These studies indicate that SecD/SecF may not play a role in the membrane integration TrkH, rather they may play a role in folding of TrkH upon its entry into the membrane via the Ffh, SecE pathway. Overall our studies support a scenario wherein attenuated SecD/F activity leads to misfolded K^+ transporters, rendered labile to HslVU degradation and shielded from degradation by overexpression of YidC.

Group of Laboratory of Bacterial Genetics

Laboratory of Bacterial Genetics

Studies on the physiological functions modulated by the stringent response factors (p)ppGpp/DksA in *Escherichia coli*.

Principal Investigator **R Harinarayanan**
Staff Scientist

Members

Vani Singh	SRF
Karthika Shiraz	Project Associate
Shaffiqu	Technical Officer

Objectives

Escherichia coli is a model bacterium amenable to experimental manipulation. We are using it for addressing fundamental questions in bacterial physiology. We are studying processes regulated by the modified nucleotides (p)ppGpp and its protein co-factor DksA, popularly referred as the stringent response factors. We are also investigating the metabolic significance of having the transketolase mediated link between the pentose phosphate pathway and glycolysis. Accordingly, the objectives of the studies in the present reporting period are,

1. To investigate role of (p)ppGpp and DksA in the transcriptional regulation of novel gene *fabY* involved in fatty acid metabolism and to identify amino acid residues required for catalytic activity of the protein.
2. To understand the role of (p)ppGpp in co-ordinating fatty acid metabolism with cell size and division in *Escherichia coli*.

In *E. coli*, metabolism of the modified nucleotides (p)ppGpp is primarily governed by three enzymes, namely, RelA, SpoT and GppA. RelA and SpoT are (p)ppGpp synthases and SpoT is also a (p)ppGpp hydrolase that converts ppGpp and pppGpp to GDP and GTP respectively. GppA is a hydrolase that converts pppGpp to ppGpp. Multiple studies have presented evidence that transcriptional regulation by (p)ppGpp was facilitated by the RNA polymerase binding protein DksA. In previous work, we had identified two phenotypes using mutant strains of E. coli, (i) loss of the *fabH* function conferred synthetic growth defect in strains compromised for (p)ppGpp synthesis (ii) loss of the *fabH* function conferred synthetic growth defect in strains lacking *yiiD*, which was renamed as *fabY* (iii) loss of the *fadR* function conferred synthetic growth defect in strains compromised for (p)ppGpp synthesis. Studies undertaken in this reporting period to characterize the molecular basis of these phenotypes are described below.

Studies to understand the transcriptional regulation of *fabY* gene

In a previous study we had identified an ORF of unknown function, annotated as *yiiD* and renamed it as *fabY* following genetic evidence for its role in fatty acid biosynthesis in the (p)ppGpp deficient strain lacking the *fabH*. To explain the ppGpp^0 *fabH* synthetic lethality, it was proposed, in the absence of FabH activity, initiation of fatty acid biosynthesis was primarily mediated thorough FabY and that the expression of FabY was positively regulated by (p)ppGpp and/or DksA. *fabY* is the last gene in an operon comprising the *yihW*, *yihX*, *yihY*, and *dtd* genes. To map the location of promoter(s) within the *yihW-yihX-yihY-dtd* operon, the pKD3-Cm cassette that confers transcriptional polarity was inserted immediately after the stop codon of each gene in the operon. These insertions introduced into the *E. coli* chromosome by homologous recombination in a *yiiD*$^+$ and *yiiD-lac* strains were designed such that ORFs before and after insertion were not disrupted and then subsequently moved into the the Δ*fabH* / pRC*fabH* strain by phage P1 transduction for performing the blue-white colonies segregation assay to monitor the strains ability to survive loss of the unstable pRC*fabH* plasmid. It was observed that the *dtd*$^+$::Cm insertion did not give white colonies indicating that the insertion was synthetically lethal with *fabH* mutation and probably due to the reduced *fabY* expression. This was corroborated by the low β-galactosidase activity observed using the *fabY-lac* fusion and the *dtd*$^+$::Cm insertion (see below). However, insertions between the other upstream ORF's did not affect viability of the strain.

To study transcriptional regulation of *fabY*, a *fabY-lac-kan* fusion was generated at the chromosomal *fabY*::FRT allele and FLP-mediated recombination using plasmid pKG137. The effect of pKD3-Cm

insertion that confers transcriptional polarity beyond its point of insertion on *fabY*-lac expression was studied by β-galactosidase assay. The *fabY-lac* expression was unaltered when pKD3-cm insertion was placed immediately downstream of *yihW*. There was a 7-fold decrease in the *fabY*-lac expression when the polar pKD3-cm insertion was placed immediately after the *dtd* ORF as compared to that placed immediately downstream of *yihW* ORF. These results suggest, promoter elements controlling *fabY* expression are located after the *yihW* ORF.

In silico based prediction of FabY (YiiD) function

To understand the basis for Δ*fabH* Δ*fabY* synthetic lethality, we looked at the in silico functional annotation of the FabY protein. No similarity was found between FabY protein sequence and three β-Ketoacyl-ACP synthase genes reported in *E. coli*, namely, FabH, FabB or FabF. Thus, YiiD may belong to a new class of proteins involved in fatty acid synthesis. The *yiiD* gene was annotated as a putative acetyltransferase. When a Pfam analysis was performed to look for domain(s) in the protein sequence, two hits were obtained – an N-terminal acetyltransferase domain and a C-terminal thioesterase domain. Additionally, a Coenzyme A binding pocket was annotated at the N terminal half of the protein in the acetyltransferase domain. It is, therefore, possible to make the argument that the protein could catalyze the synthesis of acetoacetyl-ACP by cleaving the acetyl moiety out of acetyl-CoA using the thioesterase activity and transferring it to malonyl-ACP using the acetyltransferase activity. In this manner, YiiD (FabY) may be able to compensate for loss of FabH function.

Characterization of amino acid residues required for the catalytic activity of FabY

The FabY protein sequence was analyzed to identify the conserved residues. For this, the *E. coli yiiD* nucleotide sequence was obtained from NCBI (NC_000913.3: 4077449-4078438). The translated sequence was used to obtain orthologs by sequence similarity from the EGGNOG v4.5.1 database (Huerta-cepas *et al.*, 2016). In total 172 protein sequences across 169 species were obtained. Based on the multiple sequence alignment using ClustalW (MEGA7.0.26; Kumar, Stecher and Tamura, 2016) 4 residues that were conserved across all the sequences analyzed were identified. These were Proline at 186, Asparagine at 212 and 214, and Glycine at 222 position. It would be reasonable to assume these residues would be important for the activity of the protein. Through oligonucleotide mediated site-directed mutagenesis followed by overlapping PCR, the mutations were individually introduced into the *yiiD* DNA sequence. The corresponding change expected in the protein sequence is the following: Proline at 186 was replaced with Glycine (P186G); Asparagine at 212 and 214 replaced with Alanine (N212A and N214A, respectively); and Glycine at 222 replaced with Alanine (G222A). The PCR products carrying the mutations were digested with *SfiI* and cloned into the pCAyiiD plasmid (ASKA collection) to replace the wild type *yiiD* gene in the plasmid. Cloning in the pCA24N vector allows IPTG dependent regulation of gene expression. Following transformation, the plasmid clones were verified by sequencing to ensure that only the desired mutation was introduced and that the clones had no other mutation(s). While it was possible to introduce mutations that would cause the following changes P186G, N212A, and N214A in the FabY protein, clones carrying the mutation expected to produce the G222A change could not be recovered. Interestingly, sequencing the PCR product used for cloning showed the presence of the mutation. Remarkably, the clones encoding for the G222A substitution could be recovered only together with another mutation that encodes for the N214A substitution, although the PCR product did not have the latter change.

These clones having the mutations were transformed into the strain Δ*fabY* Δ*fabH*/pRCfabH in order to test their ability to suppress the Δ*fabY* Δ*fabH* synthetic lethality using the blue white plasmid segregation assay. As earlier described, pCAyiiD was able to suppress the Δ*yiiD* Δ*fabH* synthetic lethality in the absence of IPTG (Fig. 1). However, growth was inhibited in the presence of IPTG, suggesting that an increase in YiiD expression conferred growth inhibition. This can arise because of two possible reasons, first, increased in the YiiD activity could be detrimental to growth, second, the increased expression could inhibit growth non-specifically, for reasons such as protein misfolding and aggregation, etc. The two possibilities are not mutually exclusive.

As compared to pCAyiiD, suppression of Δ*yiiD* Δ*fabH* synthetic lethality as determined using the blue white plasmid segregation assay by pCAyiiD-P186G expressing the mutant gene, was very weak. In the absence of IPTG, growth of white colonies were much slower than that of the blue colonies. Growth of the white colonies improved on plates containing 0.1 mM IPTG, and furthermore, unlike in the case of pCAyiiD, the growth of the blue colonies was unaffected. In plates containing 0.5 mM IPTG, the growth of the colonies were greatly inhibited and therefore blue and white colonies could not be distinguished and with 1 mM IPTG, no colonies could be visualized after 24 hours of incubation. Given the conserved nature of the substituted residue, these results are most consistent with the idea that the YiiD function is compromised by P186G substitution, although, other possibilities such as a shorter half-life

of the mutant protein cannot be ruled out. Expression of the mutant gene from pCAyiiD-N212A was unable to suppress the ΔyiiD ΔfabH synthetic lethality in the absence of IPTG, however, suppression was evident in plates containing 0.1 mM IPTG but not higher IPTG concentration. Similarly, expression of pCAyiiD-N214A suppressed the ΔyiiD ΔfabH synthetic lethality only in the presence of 0.1 mM IPTG but the growth of the white and blue colonies was inhibited. While the ΔyiiD ΔfabH/ pCAyiiD N212A colonies (blue as well as white) from the 0.1 mM IPTG plate could be purified further on LB Cm 0.1 mM IPTG plates the ΔyiiD ΔfabH/ pCAyiiD N214A colonies did not grow under similar conditions indicating that greater toxicity was associated with the expression of the N214A variant. Expression of double mutant from the pCAyiiD N214A G222A plasmid did not suppress the ΔyiiD ΔfabH synthetic lethality in the absence of IPTG and as well as 0.1 mM IPTG. However, growth was progressively inhibition at higher IPTG concentrations. Overall, a correlation can be observed between the efficiency of the mutant alleles to suppress the ΔyiiD ΔfabH synthetic lethality and toxicity associated with their overexpression. That is, as compared to the wild type protein, in the case of mutants, increased expression was required to observe suppression of synthetic lethality, and correspondingly, the toxicity associated with the increased expression was alleviated. This observation can be best explained by assuming, (i) the function carried out by YiiD (FabY) is inherently growth inhibitory at higher expression and (ii) the mutant alleles have lower catalytic efficiency than the wild type gene and therefore growth was unaffected despite an increase in the protein expression. Our data also suggests to the possibility that the G222A variant may be catalytically more active (therefore more toxic) than the wild type protein. This idea is supported by the finding that clones carrying the G222A mutation could be recovered only together with the N214A mutation that reduced the activity of YiiD (out of ten plasmid clones screened, six clones did not have any mutation while four clones have both N214A and G222A mutations). Further studies are needed to understand the contribution of these residues to the biochemical activity of YiiD protein and through which it could compensate for the loss of FabH function.

Growth defect of (p)ppGpp deficient *fadR* mutant was rescued and accentuated by the increase and decrease respectively of growth medium osmolarity

FadR is a protein involved in fatty acid metabolism. While the acronym FadR reflects the proteins role as a repressor of genes involved in fatty acid degradation, later studies have highlighted its role in the positive transcriptional regulation of overall fatty acid biosynthesis and especially the two genes involved in biosynthesis of unsaturated fatty acids, namely, fabA and fabB. The FadR protein activates transcription of all genes involved in fatty acid biosynthesis and represses genes involved in fatty acid degradation (β-oxidation). Recent reports have suggested a link between the fatty acid biosynthetic capacity of cells and its size - a decrease in fatty acid biosynthesis being associated with decreased cell size. Cell size control is also an intrinsic feature of the cell cycle.

We had earlier observed, loss of the *fadR* function conferred synthetic growth defect in strains compromised for (p)ppGpp synthesis. We examined if growth phenotype of (p)ppGpp deficient *fadR* strains in LB medium at the different temperatures was influenced by osmolarity of growth medium. The osmolarity of LB was lowered by omitting NaCl (this medium is referred as LBON), and increased by adding NaCl or $(NH_4)_2SO_4$. The ΔrelA Δ*fadR* strain showed growth defect in LBON medium at 25°C and 30°C but not 37°C, while the ΔrelA ΔspoT Δ*fadR* / pRC*spoT* strain, in the absence of IPTG, showed growth defect in LBON medium at 25°C, 30°C and 37°C. Strains deficient either for (p)ppGpp synthesis, namely, ΔrelA mutant and ΔrelA ΔspoT mutant or in fatty acid metabolism, namely Δ*fadR* mutant, did not exhibit growth defect in LBON medium at 25°C, 30°C and 37°C indicating growth defect was observed only when (p)ppGpp synthesis and fatty acid metabolism was perturbed together. The NaCl concentration in the routine LB medium used is 0.17 M. To increase osmolarity of the growth medium, NaCl concentration was raised to 0.5 M, or $(NH_4)_2SO_4$ was added in the LB medium to a final concentration of 0.3M. The growth defect of ΔrelA Δ*fadR* strain in LB medium at 25°C and that of ΔrelA ΔspoT Δ*fadR* /pRC*spoT* strain in LB medium at 30°C was rescued by an increase in the osmolarity of the medium. To ask if growth rescue was due to the osmotic effect of solutes, 0.8M glycerol, a solute freely permeable across *E. coli* membrane was added to the LB medium. Unlike NaCl or $(NH_4)_2SO_4$, glycerol did not rescue the growth defect of the strains, indicating growth rescue could arise from osmotic effect of the solutes.

During osmotic downshifts, the increase in turgor pressure activates MscL, the mechanosensitive channel of large conductance, leading to solute exit from cells. Gain-of-function mutants that constitutively leaks solutes from the cells have been identified. Since growth defect of ΔrelA Δ*fadR* and ΔrelA ΔspoT Δ*fadR* /pRC*spoT* strains was relieved by increase in medium osmolarity, we speculated increase in turgor pressure may contribute to the growth defect. To test this, wild type and gain-of-function *mscL* mutants, *K31E, G26S* and *V23A* were expressed from plasmid. The gain-of-function mutants, but not the wild type

rescued the growth defect of *relA fadR* mutant in LB at 25°C. It is possible, turgor pressure under the growth conditions used was insufficient to activate the wild type MscL protein while the gain-of-function proteins which remain constitutively active supported solute export and reduction of turgor pressure. The results support the idea, solute extrusion and reduction in turgor pressure was required for growth rescue.

Publications:

Evidence for role of transketolase function in the maintenance of pyridine nucleotide levels in *Escherichia coli.* bioRxiv preprint doi: https://doi.org/10.1101/2023.03.15.532724.

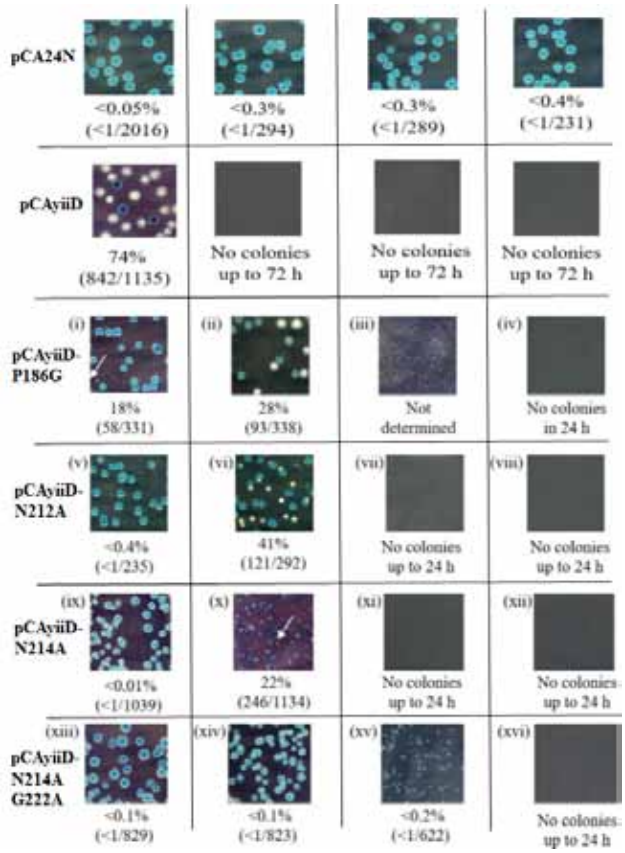

Fig. 1. Testing suppression of Δ*yiiD* Δ*fabH* synthetic lethality by wild type (pCAyiiD) and mutant clones pCAyiiD P186G, pCAyiiD N212A, pCAyiiD N214A, and pCAyiiD N214A G222A at various expression levels. The assay was performed as described in the methods. All strains have the genotype of ΔyiiD ΔfabH/pRCfabH. The relevant genotype of the strain, the percentage of white colonies, and the total number of colonies (blue+white) used to calculate the ratios are indicated. The cultures were plated on LB Cm X-gal plates and incubated at 37°C for 24 hours. The white arrows indicate small, white colony. When colonies were slow-growing, the plates were incubated longer in order to calculate the percentage of blue and white colonies. See text for details.

Group of Laboratory of Bacterial Genetics

Laboratory of Cell Cycle Regulation

Elucidating the role of chromatin modifying proteins in cell cycle regulation

Principal Investigator	**Shweta Tyagi** Staff Scientist & DBT-Wellcome Trust IA Senior Fellow

Ph. D. Students

Kausika Kumar Malik	Senior Research Fellow
Akash Nitin Chinchole	Senior Research Fellow
Kaiser Ahmed Lone	Senior Research Fellow
Aditi Arora	Senior Research Fellow
Avishek Katariya	Junior Research Fellow
Bijaya Ta	Junior Research Fellow
Shreyta Gupta	Junior Research Fellow
Pradeep Nile	Junior Research Fellow (since Feb 2023)

Other Members

V N Sailaja	Technical Officer
Geethanjali Ravindran	Research Associate
Ramesh Gogulothu	Research Associate
Deepshika Pulimamidi	Project-JRF
Neeraja H	Project-JRF

Collaborators

Debabrata Biswas	Indian Institute of Chemical Biology, Kolkatta
Sanjeev Galande	Indian Institute of Science Education and Research, Pune
Himanshu Goel	Hunter Genetics, New South Wales, Australia
Ajay Mahato	CDFD, Hyderabad

Objectives

1. Study of non-canonical roles of MLL in cell cycle.
2. Role of MLL in regulation of repetitive non-coding regions.

Project 1: Study of non-canonical roles of MLL in cell cycle.

Leukemia or blood cancer can be caused due to multiple reasons. One such reason is when a gene called Mixed Lineage Leukemia (MLL) located on chromosome 11, breaks from between and both halves of this gene fuse with random regions of other chromosomes. This process is called translocation and it gives rise to 'unnatural' fusion proteins. These fusion proteins are believed to cause leukemia. Sadly, this type of leukemia is mostly found in infants and children. Often these children have poor prognosis and do not respond well to standard therapies of leukemia.

It has been puzzling the researchers how these random translocations with more than 100 different regions (in MLL-based leukemia) produce the same disease? The function assigned to MLL in 'normal' cell is transcription. It is believed that MLL-fusion protein also participates in transcription and deregulate it. The cure for this kind of leukemia will only be effective once we fully understand about the MLL protein and then apply that knowledge to appreciate which processes the MLL-fusions proteins are disturbing.

Details of the progress made in the current reporting year (April 1, 2022 –March 31, 2023)

MLL is present in most cells of the body. Hence to study its function, we artificially create cells where MLL is destroyed by siRNA technology. After siRNA treatment, the levels of MLL are very low (20-30%) and observing these cells can help us understand which processes are disturbed. By correlation, MLL is required in those processes.

In this ongoing project, we are investigating the role of H3K4 HMTs in determining cell shape and cell migration by affecting the homeostasis of Rho GTPases. We showed that MLL directly regulates Rho GTPases chaperon protein RhoGDI1. Upregulated expression of RhoGDI1 is linked with many different cancers, associated with enhanced

invasion, metastasis, and chemoresistance. We noted that MDA-MB-231 cells exhibit high expression of RhoGDI1. The MDA-MB-231 cell line is commonly used to model, late-stage triple- negative breast cancer (TNBC). To assess if our findings here can have clinical relevance, we used a small molecule inhibitor, OICR-9429, which binds specifically to WDR5 and inhibits its interaction with MLL, thereby the methyltransferase activity of MLL complex for the treatment of these TNBC cells. Treating MDA- MB-231 cells with OICR-9429 for 72 hours significantly reduced the expression of RhoGDI1 transcript (Figure 1A). To investigate the efficacy of OICR-9429/ depletion of MLL on TNBC in vivo, we performed xenograft assays in nude mice. MDA-MB-231 cells were treated with either MLL or RhoGDI1 shRNA and engrafted in breasts of female nude mice by subcutaneous injection. MLL or RhoGDI1 shRNA treated cells showed substantially small tumor size compared to control set (Figure 1B). Similarly, in mice engrafted with MDA- MB-231 cells, intravenous injections with 4 mg/Kg OICR-9429, showed a significant reduction in size of tumors as compared to those injected with vehicle (Figure 1C). Taken together, our results identify MLL as a potential new target to treat TNBC (or any other cancers) with upregulated expression of RhoGDI (Figure 1D).

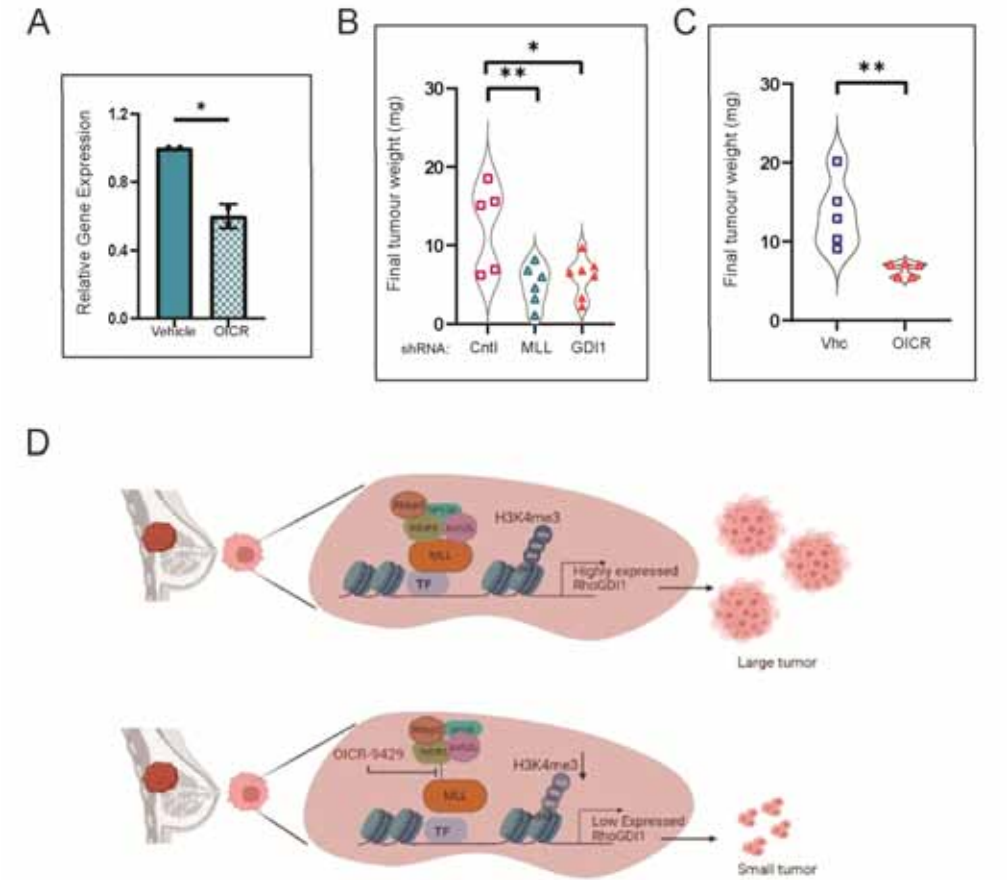

Figure 1. Inhibition of MLL can reduce tumors in xenografts.

(A) Shown is RT-qPCR analysis of gene expression of RhoGDI1 in MDA-MB-231 cells upon treatment with 25μM OICR-9429. Data represents mean ± SD, *P = .0323 (Student's unpaired t-test; m=2 experiments). **(B)** Tumours obtained from xenografts of MDA-MB-231 cells, treated with control, MLL shRNA or RhoGDI1 shRNA were harvested, weighed and plotted. **P =.007, *P = .014 one-way ANOVA test was performed. (n = 5, 6 and 7 animals for control, MLL and RhoGDI1 shRNA treatment groups respectively) **(C)** Tumours obtained after treatment with vehicle (DMSO) or 4 mg/kg OICR-9429 were harvested, weighed and plotted. **P =.008, Student's unpaired t-test was performed. (n = 5 animals each). Cntl, control; vhc, vehicle; mg, milligrams; OICR, OICR- 9429. **(D)** MDA-MB-231 cells are TNBCs with high RhoGDI1 expression, which promotes tumour formation. MLL with its core complex proteins promotes the transcription of RhoGDI1 by methylating the RhoGDI1 promoter. Treatment with OICR-9429, a non-peptide inhibitor of MLL-WDR5 interaction, reduces the activity of MLL at RHOGDI1 promoter, thus reducing the expression of RhoGDI1 gene. This results in tumour regression. Thus, inhibition of MLL can be used as a potential therapeutic in tumours showing high expression of RhoGDI1.

Publication:

Malik K K, Sridhara S C, Lone K A, Katariya P D, Pulimamidi D and Tyagi S. (2022) KMT2 family members regulate H3K4 methylation to ensure kinetochore activity at human centromeres. ***BioRxiv*.** https://doi.org/10.1101/2022.06.20.496844

Chinchole A, Lone KA and Tyagi S. (2022) MLL regulates the actin cytoskeleton and cell migration by stabilising Rho GTPases via the expression of RhoGDI1. ***J Cell Sci***. 135 (20) https://doi.org/10.1242/jcs.260042

Group of Laboratory of Cell Cycle Regulation

Laboratory of Cell Death & Cell Survival

Functional protein networks controlling cellular pathways and their role in human diseases

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Objectives

1. To identify new cellular functions for phosphatases and assess their role in human diseases

2. To map the functions of ubiquitin system in cells and evaluate its aberrations in human diseases

Research Summary

Theme 1: Functional phosphatase network in cells

Proteins in general are synthesized as inactive molecules in the cells. Once synthesized, they need to be modified to mediate their functions. Phosphorylation (attachment of a chemical group of phosphate) is one such protein modification required for them to function in the cell. Kinases are the enzymes, which add phosphate group to the proteins, while phosphatases are enzymes that oppose this process. Phosphatases play a crucial role in biological functions and controls nearly every cellular process, including metabolism, gene transcription, translation, cell-cycle progression, protein stability, signal transduction, and apoptosis. Phosphatases are so far studied in isolation to assess their function in the cell, but in reality, they work in a network of protein complexes. In this theme, we aim to map the functional phosphatase network with the identification of interacting partners of every phosphatase in human cell. By using a biochemical and proteomic approach we identified the associated protein complexes of more than 140 phosphatases. During earlier years, we assigned several novel cellular functions to different phosphatases based on their interacting partners. During this year, we expanded the role of phosphatases during vesicular trafficking. In this work, we discovered EYA phosphatase complex as a molecular bridge that interacts with the retromer complex and promotes the retrograde vesicular trafficking of Wntless cargo by directing it specifically to TGN. Although EYA phosphatases were known to be essential for cell-fate determination processes and organ development, their role in vesicular trafficking is not documented so far. By using various cellular and biochemical approaches, we demonstrated that EYA proteins (EYA 1-4) form a hetero-tetrameric complex that interacts with the retromer complex on early endosomes. We show that the retromer bound EYA complex loads SCAMP3 to endosomes which is essential for docking and fusion of Wntless loaded endosomes to TGN. The retrograde trafficking of Wntless through EYA-SCAMP3 axis facilitates Wnt ligand secretion and promotes Wnt signalling. In conclusion, our study discovered a new multiprotein phosphatase complex that assists retromer in determining the destination of cargos during retrograde vesicular trafficking (Figure 1).

Theme 2: Network of ubiquitin system

Ubiquitin is a small protein that attaches to other proteins via a covalent addition. Similar to phosphorylation, ubiquitin attachment to substrate proteins acts as a regulatory protein modification. Ubiquitin attaches to target proteins through the activity of three different sets of enzymes: ubiquitin activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). Ubiquitin E3 ligases are the most critical enzymes in this pathway where they facilitate the activation and transfer of ubiquitin either directly to the target protein or to other ubiquitin proteins that already have been attached to the target protein. Ubiquitin linked to the substrates serves as a molecular tag that marks proteins for either degradation by proteasome (a multi-subunit complex that degrades proteins in cells) dependent pathway or to function in wide variety of processes in a proteasome independent manner. When a chain of more than one ubiquitin molecule attaches to the same target protein, that protein is said to be poly-ubiquitinated. Poly-ubiquitin chains appear to serve multiple purposes, of which the best understood is marking target proteins for degradation through the proteasome. However, seven different kinds of ubiquitin-ubiquitin attachments are possible in the cell that can provide wide variety of topologies, each of which signal a different outcome. In this theme, we are interested in identifiying new functions for ubiquitin system by mapping the interaction network of different E3 ligases as well as various ubiquitin chain types in cells. We have reported several new complexes in this pathway during previous years. In the current reporting year, we established a new function for ubiquitin linkage in cells. We identified an essential role of a non-canonical K63 ubiquitin linkage in liquid-liquid phase separation of Dvl2. Our study denoted WWP2 as an E3 ligase that mediates Dvl2 ubiquitination via K63 ubiquitin chain linkage, which is required for Dvl2 Phase separation. In conclusion, our study revealed an ubiquitination-dependent liquid-liquid phase separation as a new functional identity for Dvl2 in cells, which is critically necessary for activation of Wnt pathway.

Publications

Tathe P, Chowdary KVSR, Murmu KC, Prasad P, Maddika S (2022). SHP-1 dephosphorylated histone H2B to facilitate its ubiquitination during transcription. ***EMBO J.*** 41(19): e109720.

Vamadevan V, Chaudhary N, Maddika S (2022). Ubiquitin-assited phase separation of dishevelled-2 promotes Wnt signaling. ***J Cell Sci.*** 135(24): jcs260284.

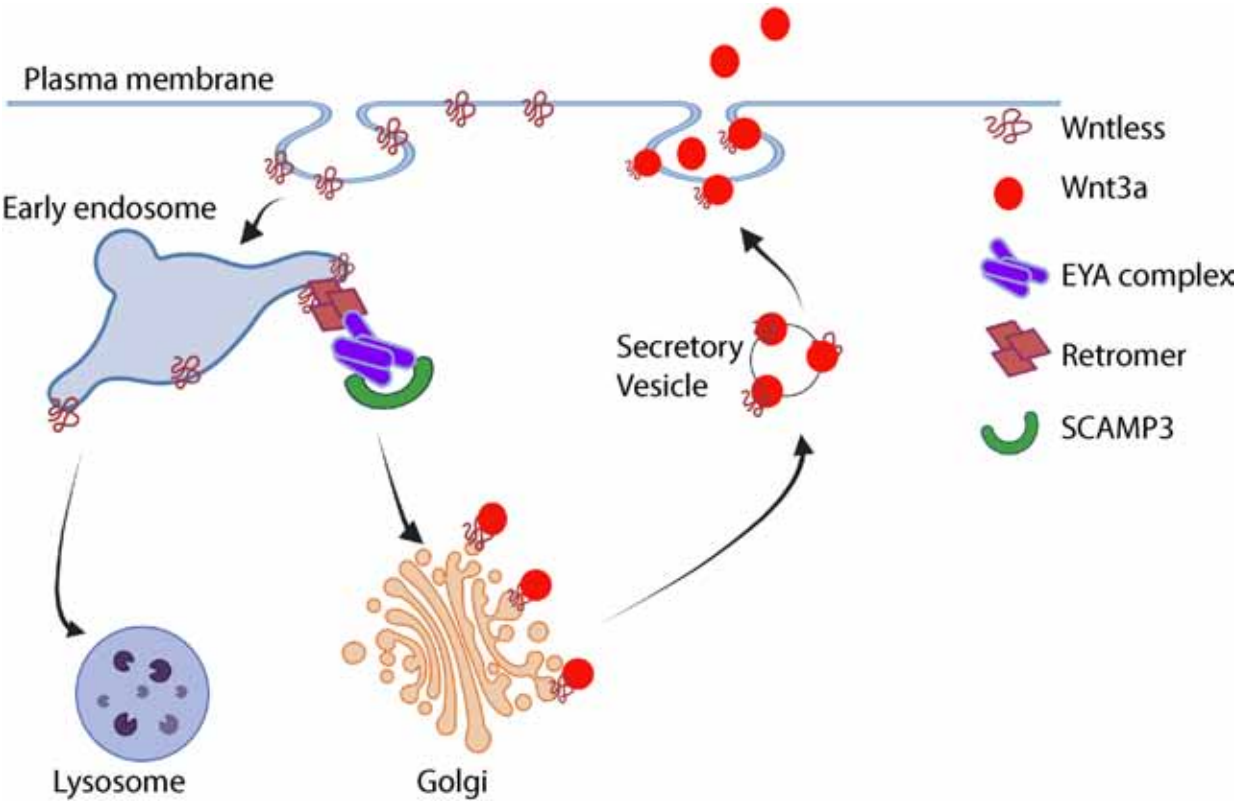

Figure-1: A working model to depict the role of retromer-EYA complex-SCAMP3 in directing Wntless from endosomes to TGN.

Group of Laboratory of Cell Death & Cell Survival

Laboratory of Cell Signalling

Investigating the functions of phosphate-rich biomolecules in eukaryotic cells

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Our laboratory studies the functions of two phosphate-rich biomolecules: (i) the inositol pyrophosphate, 5-IP_7 (5PP-IP_5), and (ii) inorganic polyphosphate (polyP). Our broad objectives are (a) to understand the cellular processes by which the levels of these small molecules are regulated, and (b) investigate the cellular and physiological processes that these phosphate-rich molecules influence. We are also involved in functional characterization of novel mutations underlying rare genetic disorders in the Indian population.

Cellular functions of inositol pyrophosphates

5-IP_7 is synthesised from IP_6 and ATP by a family of enzymes known as inositol hexakisphosphate (IP6) kinases, of which there are three isoforms in mammals – IP6K1, 2, and 3. 5-IP7 can modulate protein function by serine pyrophosphorylation, a post-translational modification in which the β-phosphate moiety is transferred from 5-IP7 to a pre-phosphorylated serine residue to generate pyrophosphoserine. We use mammalian cell lines and knockout mouse strains as model systems to investigate the signalling and metabolic pathways that are altered when 5-IP_7 levels are perturbed.

We have previously reported that IP6K1 supports homologous recombination-mediated DNA repair in mouse embryonic fibroblasts, and that this effect is dependent on 5-IP7 synthesis by IP6K1 (Jadav *et al*., J. Biol. Chem. 2013). We used U-2 OS cells depleted for IP6K1 as a model system to investigate the molecular mechanism by which 5-IP7 regulates homologous recombination (HR) mediated DNA repair. We found that synthesis of 5-IP7 by IP6K1 is necessary for cells to recover from DNA damage induced by the inter-strand crosslinker mitomycin C. It is known that a reduction in the interaction between the C-terminal domain (CTD) of BRCA2 and the HR marker protein RAD51 helps dislodge RAD51 from the sites of DNA damage post-repair. We demonstrated that 5-IP7 can pyrophosphorylate two sites in the N-terminal disordered region of RAD51. We conducted in vitro reconstitution assays to demonstrate that RAD51 that is pyrophosphorylated by 5-IP7 exhibits reduced interaction with BRCA2 CTD, whereas 5-IP7 has no effect on the interaction of BRCA2 CTD with mutant versions of RAD51 that do not undergo pyrophosphorylation. Overall, our data suggest that 5-IP7 synthesized by IP6K1 pyrophosphorylates RAD51 in its N-terminus to reduce its interaction with BRCA2 CTD, promoting the removal of RAD51 from the DNA damage foci after repair (see Figure 1).

Functional characterization of SERPINA11 underlying a novel serpinopathy

In collaboration with the Diagnostics division at CDFD, we are working to conduct functional characterization of novel genes and mutations identified as being causative of monoallelic disorders. The Diagnostics division identified a perinatal lethal phenotype associated with biallelic loss of function variants in SERPINA11, and characterised by gross and histopathological features of extracellular matrix disruption. SERPINA11 is a poorly characterized protein with 41% amino acid sequence homology with SERPINA1 (Alpha1 antitrypsin) and a predicted molecular weight of ~47kDa. The mutant SERPINA11 gene identified in the fetus encoded a truncated protein Y224X, which would lack the predicted RCL region that is essential for the anti-proteinase function in serpin family proteins. We conducted protein expression studies in HEK293T cells to confirm that the Y224X mutant version of SERPINA11 is indeed truncated. Western blot analysis of mouse tissues showed expression of SERPINA11 in liver, lung, kidney, heart, brain, testis, and ovary of adult C57BL/6 mice. The expression pattern of SERPINA11 in mice mirrors the tissues in which gross pathologies were observed in the affected fetus. Although Serpina11 transcript has been reported only in the liver of adult C57BL/6 mice, our detection of the protein in different mouse tissues could arise from SERPINA11 transport to these tissues via circulation, or low level of Serpina11 transcription in these tissues. We are presently conducting immunofluorescence analyses to identify the cell types in which Serpina11 is expressed in mouse tissues.

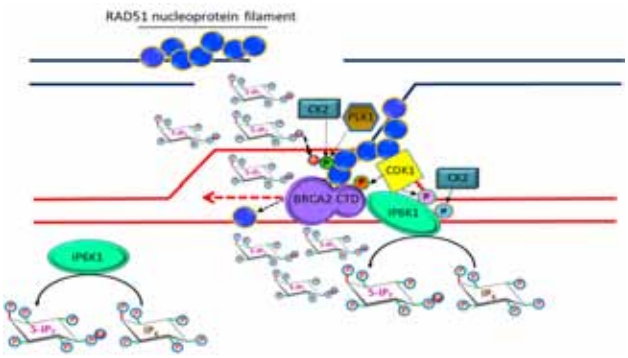

Figure 1. RAD51 undergoes phosphorylation by the acidophilic Ser/Thr kinases CK2 and PLK1. IP6K1 interacts with proteins involved in the disassembly of RAD51 nucleoprotein filaments, like BRCA2 CTD and CDK1. IP6K1 may synthesise 5-IP7 in the vicinity of DNA damage sites, leading to a local increase in the 5-IP7 concentration and hence bringing about pyrophosphorylation of pre-phosphorylated RAD51. This additional modification on RAD51 mediates the disruption of BRCA2 CTD - RAD51 interaction, resulting in RAD51 eviction from DNA damage sites.

Publications

Morgan J.A.M.*, Singh A.*, Kurz L., Nadler-Holly M., Penkert M., Krause E., Liu F., Bhandari R.†, and Fiedler D.† Pyrophosphoproteomics: extensive protein pyrophosphorylation revealed in human cell lines (2022) bioRxiv 2022.11. 11.516170

Sarkar S., Sharma H., Ladke J.S., Raran-Kurussi S., Bhandari R.†, and Jaiswal M.† Development of Drosophila as a metazoan model to study inorganic polyphosphate biology (2023) bioRxiv, 2023.03. 26.534266

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Group of Laboratory of Cell Signalling

Laboratory of Chromatin Biology and Epigenetics

Understanding the functions and regulation of sirtuins in maintenance of genomic integrity

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Research in the laboratory is broadly aimed at understanding the molecular functions and mechanisms of regulation of Sirtuins during normal growth, proliferation of cells as well as under stress such as DNA damage. We use fission yeast, *Schizoschharomyces pombe* and human cell lines as model systems. Reversible acetylation/deacetylation of proteins regulates numerous important cellular processes. The Sirtuin family NAD+ dependent protein/histone deacetylases (HDAC) are conserved from yeast to human cells carry out a broad range of crucial cellular functions ranging from transcriptional silencing to DNA damage response, cell cycle regulation, metabolism and aging etc. During DNA metabolic processes such as DNA replication and DNA repair, the expression level of specific Sirtuins are known to alter, indicating conditional regulation of these proteins. However, the molecular functions and mechanisms of regulation of sirtuins under these conditions remain elusive. There is a need to study these regulatory mechanisms as sirtuins are often deregulated in various diseases including cancer. Deciphering these mechanisms will help in designing novel cancer therapeutics.

We are currently focused on the following objectives:

1) Understanding the molecular functions and regulation of human sirtuins in DNA Double Strand Break Repair pathways
2) Investigation of novel molecular mechanisms by which sirtuins family protein deacetylases regulate DNA metabolic processes such as DNA replication and repair. We are also studying regulation of sirtuins during DNA replication stress response in fission yeast.
3) Discovery of new epigenetic therapeutics targeted to sirtuin family histone deacetylases.

Understanding the molecular functions and regulation of Human Sirtuins in DNA Double Strand Break Repair Pathways

DNA double strand breaks are deleterious in nature, if not repaired can result in diseases such as Cancer. Histone modifications, especially, acetylation of various histones has been linked to DNA Double Strand break (DSB) Repair pathways. Nuclear sirtuins, SIRT1, SIRT3, SIRT6 and SIRT7 are known to function in DNA repair. How DNA repair pathway is selected for repair of specific types of DNA damage in chromatin context still remains elusive. End resection is an important step of DSB repair pathway which is crucial for choice of DSB repair pathway. This is an important step which directs DNA repair to the pathway called homologous recombination (HR) repair and requires opening of chromatin. In response to DNA damage, H3K56Ac is rapidly deacetylated by SIRT6, thereby reducing the level of this modification and facilitating the recruitment of other DNA repair enzymes to the damaged foci. We have observed that, absence of H3K56Ac hinders the recruitment of early DNA damage sensors. Our results indicate that in absence of ASF1, a chaperone needed for H3K56Ac, this process is severely affected. Cells lacking H3K56ac shows reduced number of foci of HR pathway proteins and poor ssDNA formation. This study deciphers a novel mechanism that affects HR which can be a target for various cancer treatments.

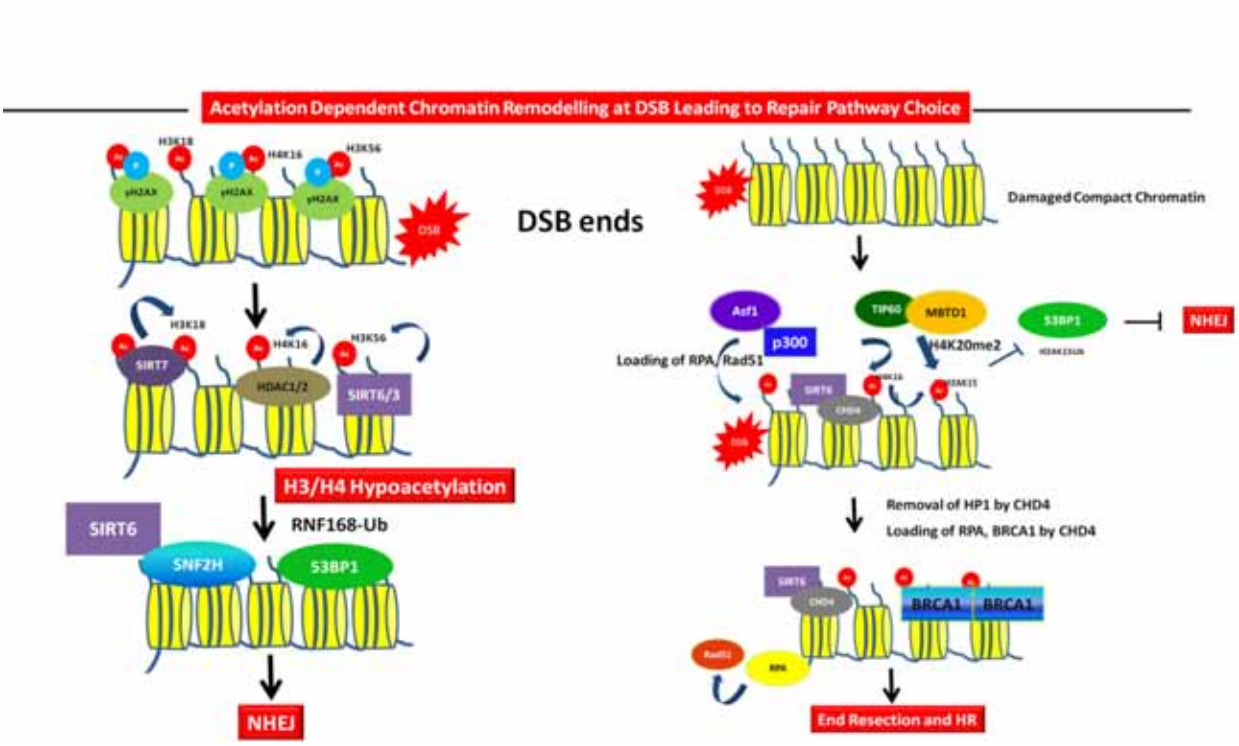

Figure. Histone acetylation/deacetylation in DNA repair pathway choice. Recruitment of HDACs like sirtuins, SIRT6, SIRT3 leads to deacetylation of histones, leading to chromatin compaction and recruitment of NHEJ factors 53BP1 and Ku70/80. The repair pathway choice for HR through acetylation is mediated via acetylation switch at H2AK15, through H3K20me3 inhibiting binding of 53BP1 and thus inhibiting NHEJ. Repair of damage in G2 or at compact chromatin regions require removal of heterochromatin protein like HP1 by CHD4. CHD4 is recruited by SIRT6 and this leads to removal of HP1 leading to chromatin decompaction, recruitment of RPA and BRCA1 to facilitate HR. Chaperon Asf1 and Histone acetyltransferase, p300 also facilitates the recruitment of Rad51 and RPA at DSBs.

To understand the molecular functions and mechanisms of regulation of fission yeast sirtuin Hst4 in replication stress response.

DNA replication stress is one of the hallmarks of cancer. The DNA replication machinery encounters variety of obstacles during the unperturbed DNA replication including damaged template DNA and various difficult to replicate chromosome regions due to the presence of DNA secondary structures. These conditions stall the replication fork, generating replication stress. Recent studies have indicated that chromatin regulators may play active part in replication stress response. In fission yeast, *Schizosaccharomyces pombe,* a sirtuin family histone deacetylase (HDAC), Hst4, functions in the maintenance of genome stability by promoting cell survival upon replication stress. We have earlier reported that sirtuin *hst4* deficient cells are sensitive to replication stress generated on methyl methanesulfonate (MMS) treatment and Hst4 is downregulated during replication stress. However, the molecular mechanism and significance of this regulation is not known. The aim of this study is to decipher the molecular mechanism of regulation of Hst4 upon replication stress and significance of this degradation. We have discovered that DDK kinase phosphorylates and targets Hst4 for degradation by SCF complex upon replication stress. This degradation increase histone H3K56ac (target of Hst4) which is required for stabilization and recovery of stalled replication forks through recruitment and stable association of fork protection complex (FPC) components Swi1 (Timeless, human homolog) and Mcl1 (hAND1) to the chromatin. In this work, we have discovered a novel mechanism for maintenance of genomic integrity during replication stress via induction of degradation of histone deacetylase Hst4 to stabilize the fork protection complex (FPC) for protecting stalled replication forks and promoting recovery of stalled forks following stress. Our results indicate that this mechanism is conserved in human cells. It is known that sirtuins and FPC components (Timeless and Claspin) are deregulated in cancer, therefore, these could be potential targets for anti-cancer therapeutics. Interestingly, *hst4* deletion mutants are sensitive to replication stress caused by methyl methanesulfonate (MMS) and Camptothecin (CPT). Hst4 is degraded during replication stress in response MMS but not in response to CPT. However, the molecular mechanism and significance

of this regulation is not known. The aim of the current study is to decipher the molecular mechanism of differential regulation of Hst4 in response to replication stress causing agents. We are working towards understanding the signalling and molecular mechanism, why cells degrade this protein upon replication stress MMS (causes replication fork stalling) but does not degrade it upon CPT treatment (causes fork collapse) when Hst4 is required for cell survival during replication stress caused by both, role of checkpoint in this differential damage signalling is also under investigation.

Discovery of new epigenetic therapeutics targeted to sirtuin family histone deacetylases.

Discovery of new epigenetic anti-cancer therapeutics targeted to sirtuin family histone deacetylases. Epigenetic therapeutics of cancer such as inhibitors of DNA methyltransferases and histone deacetylases (class I and classII) are already being used in combination with the standard cytotoxics with encouraging results. The Sirtuins (class III NAD+ dependent deacetylases) are being considered as important targets for cancer therapeutics as they are up-regulated in many cancers. Inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. However, very few sirtuin inhibitors have entered into the clinic yet as an anticancer agent. In this project, we are working towards identifying novel small molecule inhibitors of Sirtuins and characterize their potential as anti-cancer agents using budding yeast as model system for compound screening. Our have discovered 4bb, a new class of human SIRT1 inhibitor and results suggest that inhibition of SIRT1 by 4bb induces apoptosis of colon cancer cells at least in part via activating p53 by preventing p53 deacetylation, increasing Bax expression and inducing caspases. Therefore, this molecule provides an opportunity for lead optimization and may help in development of novel, non-toxic epigenetic therapeutics for colon cancer. We have also identified very potent hit peptide inhibitors for sirtuins using yeast cell based reporter silencing assay. Our data indicates these peptides can inibit human SIRT1 and SIRT2. We are currently investigating mechanism of inhibition and testing the effect these peptides on different types of cancer cells and also working towards understanding their mechanism of action.

Publications

Shalini Aricthota, Paresh Priyadarshan Rana, **Devyani Haldar (2022)** Histone acetylation dynamics in repair of DNA double-strand breaks. **Front in Genetics** 13:926577.

Group of Laboratory of Chromatin Biology and Epigenetics

Computational and Functional Genomics Laboratory

Computational and functional genomics approach to understand disease biology

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Research in our laboratory use computational biology and functional genomics approach to uncover novel molecular mechanisms underlying disease biology

Computational and functional characterization of molecular players associated with activated fatty acids storage, transport as well as host remodeling in malaria biology

We have earlier characterised ACBP as an important molecular players associated with activated fatty acid storage and its intracellular transport. Towards this, we have initiated the testing of the screened novel compound for its potential to serve as an inhibitor of *Pf*ACBP function. Further, we have studied the stability and the dynamics of ACBP at a molecular level, upon the binding of select novel chemical compounds using molecular dynamics simulation studies. Further, we have characterised various pharmacoinformatics properties of some these chemical compounds.

Additionally, we are investigating a novel function of the Circumsporozoite Protein (CSP) of *Plasmodium falciparum* in host cell remodelling. An interesting idea is that the parasite remodels their hepatocyte host environment upon infection. In order to test this idea, we are investigating the role of malaria parasite exported proteins such as CSP and others in host remodelling. These studies are expected to reveal critical molecular mechanism that are important for parasite infection and that could be blocked at the initial stage of parasite infection using novel chemotherapeutic strategies. One of these proteins is a well-known immunodominant antigen and is an essential constituent of the sporozoite surface coat, circumsporozoite protein (CSP) that gets released inside the cytoplasm of the host cell and their release in the host hepatocyte enhances the growth of liver stage parasite.

We have earlier shown that the CSP of *P.falciparum* has at least two nuclear localization signals (NLS). One is of the monopartite type and the other is bipartite. We experimentally demonstrated this by fusing the predicted NLS sequences with *Pf* Aldolase that normally localised within cytoplasm and expressed them in human hepatoma cell line HepG2 cells. We demonstrated that individually both the monopartite and the bipartite NLS are functional NLS but show weak nuclear localization, whereas when used together they can drive synergistically enhanced nuclear accumulation of reporter protein. The two NLS identified from *P. falciparum* CSP is different from the monopartite type NLS of *P. yoelii* CSP. We have now generated a structural model of how the C terminal domain of *P. falciparum* CSP may interact with human importin proteins (Figure 1).

Computational and functional characterization of molecular players associated IclR regulon in the biology of mycobacteria

M. tuberculosis genome consists of at least three IclR like proteins- Rv1719, Rv1773c, and Rv2989. Among these, Rv2989 was earlier characterized and its upregulation was reported to induce dormancy-like features. Further, we carried out a phylogenetic study using sequence-based clustering of all published IclR

like proteins of various species, including Rv2989, Rv1719 and Rv1773c. Based on this sequence-based clustering we showed that Rv1719 and Rv1773c cluster with other IclR like proteins involved in antibiotic resistance where as Rv2989 was clustered with a protein involved in biosynthesis that agreed with our earlier result that Rv2989 regulates *leu*CD operon. Further using a β-galactosidase reporter assay, we have investigated the activity of Rv1719 and Rv1773c promoters in response to antimicrobial drugs Rifampicin and Isoniazid. Some of the functionally-characterized IclR family proteins are reported to be auto-regulated. Therefore, we looked into the activity of Rv1719 and Rv1773c promoters, with and without ectopic expression of Rv1719 and Rv1773c respectively. The β-galactosidase reporter assay showed that Rv1773c is autoregulatory, whereas Rv1719 is not autoregulatory. Using a β-galactosidase reporter assay and electrophoretic mobility shift assay (EMSA) we demonstrate the autoregulation of Rv1773c through a direct interaction of Rv1773c gene product with DNA element upstream of the gene. Further, we are assessing the possibility of developing a tightly regulated engineered cells modeling some of the physiology of IclR regulon.

Computational and functional characterization of molecular players associated with the biology of human neurodegenerative/ neurodevelopmental diseases

We have worked on reported pathogenic mutations of the human N-α-acetyltransferase 10 (NAA10), the catalytic subunit of the ribosome-associated NatA complex. NatA catalyses irreversible Nα-acetylation on about one-half of the human proteome co-translationally. Many missense mutations in the NAA10 gene have been reported to be associated with X-linked rare genetic disorders constituting a broad spectrum of phenotypes. According to the biochemical studies, F128I and F128L mutations show a loss of function and poor cellular stability of the NAA10 protein. Even so, the mechanism of this mutant-associated loss of function and stability is poorly understood. Therefore, we conducted molecular dynamics simulation and in-silico analyses on wild-type and mutant NAA10 proteins to delineate the possible mechanism of loss of function and instability.

Our data suggest that although both mutations occur at the same residue of the protein and are similar, they have different folding patterns. In addition, they impact the different regions of the protein. According to our analyses, F128I reduces flexibility in the substrate peptide binding region and impairs the substrate peptide binding. However, the other mutation, F128L, reduces the flexibility of the region that contains acetyl-CoA binding sites. Consequently, these two mutations occurring at the same position take up two different mechanisms to cause decreased enzymatic activity of NAA10 (Figure 2).

Previously, we reported a mechanism by which HYPK helps in the clearance of toxic aggregated proteins. HYPK induces aggrephagy by acting as scaffolds for the Nedd8 and LC3 proteins to initiate the formation of autophagosomes around the polyneddylated Huntingtin aggregates. In addition, HYPK is stable interactor of subunits of N-terminal acetyltransferase A complex HYPK forms a triheteromeric NatA complex along with NAA10 and NAA15. In this complex, NAA10 is the catalytic subunit, and NAA15 is an auxiliary subunit. NatA-mediated N-terminal acetylation of nascent proteins is one of the most ubiquitous covalent modifications in humans, occurring on ~80% of human proteins. This modification affects many protein functions, including protein half-life, folding, complex formation, and localisation. Multiple studies showed that HYPK acts as an intrinsic inhibitor of human NAA10.

Further, through a bioinformatics approach, we identified an ortholog of HYPK in Drosophila (Figure 3). Currently, we are examining whether the orthologous protein CG9922 is expected to share a similar role in sensing, regulating, and clearing protein aggregation in Drosophila.

Figure 1. A molecular interaction complex model for Importin alpha3 (gray) and its interaction partner the bipartite NLS sequence from *P. falciparum.* Circumsporozoite protein (CSP) localized in the C terminal domain (CTD) with (red).

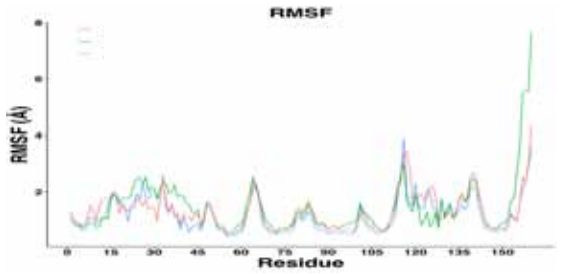

Figure 2. Comparisons of the root mean square fluctuation (RMSF) show that the mutations affect the local structure in several regions of the mutant proteins: NAA10F128I mutant shows low flexibility at the substrate binding region and NAA10F128L mutant shows loss of flexibility at the neighbouring residues of the mutation.

Figure 3. Sequence alignment of HYPK and its Drosophila homolog-CG9922

Publications:

Ghosh DK, Ranjan A (2022) HYPK coordinates degradation of polyneddylated proteins by autophagy. ***Autophagy,*** 18(8):1763-1784.

Ghosh DK, Pande S, Kumar J, Yesodharan D, Nampoothiri S, Radhakrishnan P, Reddy CG, Ranjan A, Girisha KM (2022) The E262K mutation in Lamin A links nuclear proteostasis imbalance to laminopathy-associated premature aging. ***Aging Cell.*** 21(11):e13688.

Ghosh DK, Udupa P, Shrikondawar AN, Bhavani GS, Shah H, Ranjan A, Girisha KM. (2023) Mutant MESD links cellular stress to type I collagen aggregation in osteogenesis imperfecta type XX. ***Matrix Biol.*** 115:81-106.

Group of Computational and Functional Genomics

Laboratory of Fungal Pathogenesis

Understanding the pathobiology of the human opportunistic fungal pathogen *Candida glabrata*

Principal Investigator: **Rupinder Kaur**
Staff Scientist

Ph.D Students:

Fizza Askari	Senior Research Fellow (Till 10 January 2023
Mahima Sagar Sahu	Senior Research Fellow
Sandip Patra	Senior Research Fellow
Aditi Pareek	Senior Research Fellow
Mayur Raney	Senior Research Fellow
Asmita Sarowgi	Junior Research Fellow
Manisha Ghosh	Junior Research Fellow (Since 14 July 2022)
Sayan Naskar	Junior Research Fellow (Since 14 July 2022)

Other Members:

S Surya Vamshi	Technical Officer
Kundan Kumar	Research Associate
Anamika Battu	Research Associate (Till 14 October 2022)
Adarsh Goel	Project-JRF
Anjali Prajapati	Project Associate – I (Since 18 April 2022)

Collaborators: Rajendra Prasad
Amity University Haryana, Gurgaon

Candida species are the most prevalent cause of bloodstream fungal infections, with *Candida glabrata* being the second to fourth most frequently isolated *Candida* species depending upon the geographical location. Evolutionarily, *C. glabrata* is closer to the non-pathogenic yeast *Saccharomyces cerevisiae* than to the most common *Candida* species, *C. albicans*. Research in our laboratory is aimed at a better understanding of pathogenesis and antifungal drug resistance mechanisms in *C. glabrata*.

Objectives

1. Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in *Candida glabrata*: role in pathogenicity
2. Elucidating the role of histone H3 lysine methylation in antifungal drug resistance

Research summary

Details of the progress made in the current reporting year (1st April 2022 - 31st March 2023)

Project 1: Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in *Candida glabrata*: role in pathogenicity

CgYPS gene family is comprised of eleven genes, *CgYPS1-11*, that code for putative glycosylphosphatidylinositol-linked, cell surface-associated aspartyl proteases (CgYapsins). CgYapsins are essential for survival of *C. glabrata* in macrophages as these aid in suppression of pro-inflammatory cytokine IL-1β production. Recently, we found that the ATPase subunit of the SWI/SNF chromatin remodelling complex, CgSnf2, is also indispensable for intracellular survival and virulence. To elucidate the underlying basis, we profiled the transcriptomes of human THP-1 macrophage-internalized *wild-type* (*wt*) and *Cgsnf2*Δ cells, via RNA-Sequencing approach. We found that *C. glabrata* upregulates and downregulates the seven mannosyltransferase-cluster (*CgMT-C*) and the cell surface adhesin *EPA1* genes, respectively, in response to the macrophage internal milieu. Further, *CgSNF2* deletion led to a differential transcriptional response, and evoked increased IL-1β secretion in macrophages, that led to intracellular killing of *C. glabrata*.

Further, transcriptome analysis revealed that of known 81 adhesins, 21 adhesin genes, including 9 subtelomeric adhesin genes, were differentially expressed in *Cgsnf2*Δ (Fig. 1A). To examine the role of adhesins in immunosuppression, we selected *EPA1* gene for further analysis, as its gene product is the major adhesin for the adherence of *C. glabrata* to host cells. We first verified RNA-Seq results by qRT-PCR. *EPA1* transcription was activated and repressed upon *CgSNF2* deletion and macrophage-internalization of *wt* cells, respectively (Fig. 1B and C), thereby raising two possibilities: (1) *EPA1*

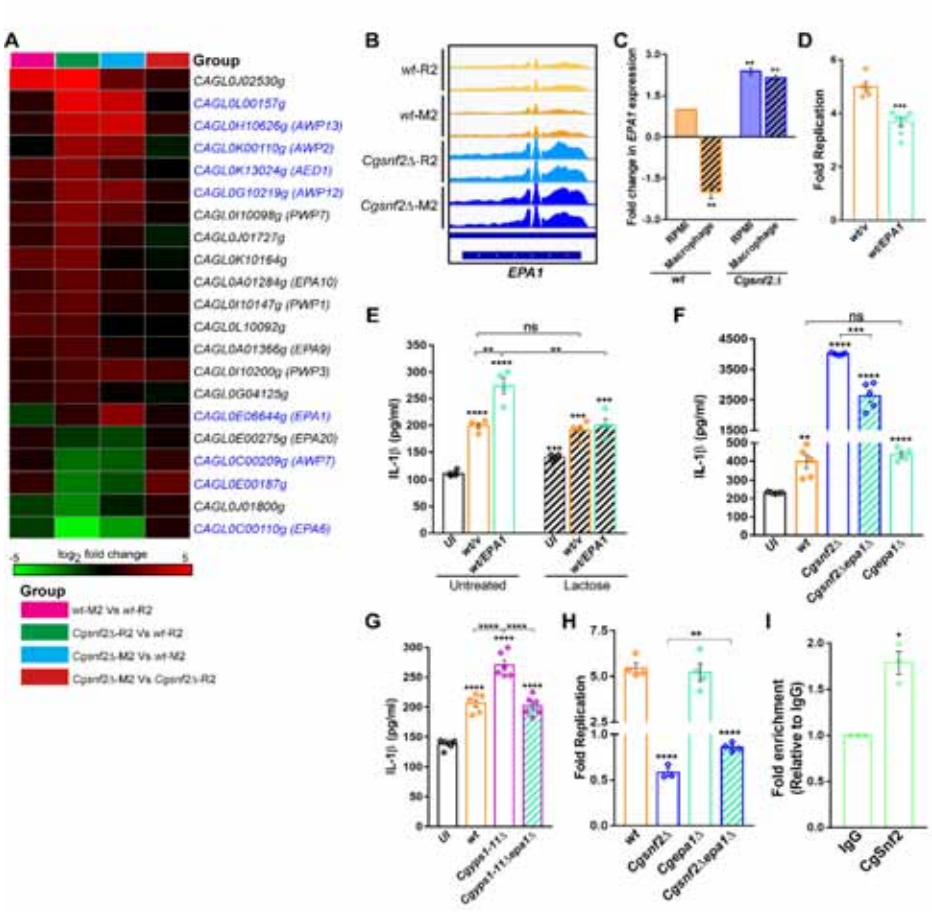

Figure 1: *EPA1* expression is deleterious for *C. glabrata* survival in macrophages. A, Heatmap showing differential expression of 21 adhesin genes. Adhesin genes encoded at subtelomeric regions (25 kb from the chromosome ends) are marked in blue colour. R2 and M2 refer to *Cg* grown in RPMI medium and infected to THP-1 macrophages for 2 h, respectively. **B,** Integrative genome viewer (IGV) snapshot of RNA-seq signal at *EPA1* locus (ChrE: 682420 to 685524 bp). All IGV tracks have the same scaling factor [0-750] for the Y-axis. **C,** qRT-PCR-based analysis of *EPA1* expression after 2 h growth in RPMI medium or macrophage internalization. Data mean ± SEM (n = 3) were normalized with *ACT1* mRNA control, and plotted as fold change in gene expression, compared to RPMI-grown *wt* (considered as 1.0). Paired two-tailed Student's t-test. **D,** Colony-forming unit (CFU)-based measurement of *C. glabrata* replication in THP-1 macrophages. Fold replication represent ratio of 24 h CFUs to 2 h CFUs. Data represent mean ± SEM (n = 5-7). Unpaired two-tailed Student's t-test. **E,** IL-1β secretion in uninfected (UI) and *C. glabrata*-infected THP-1 macrophages. *C. glabrata* strains were either untreated- or treated with 10 mM lactose, 1 h prior to THP-1 infection, and the infection was continued for 24 h in the presence of lactose. Data represent mean ± SEM (n = 4). Unpaired two-tailed Student's t-test. **F and G,** IL-1β secretion in uninfected (UI) and *C. glabrata*-infected THP-1 macrophages. Data represent mean ± SEM (n = 4-6). Unpaired two-tailed Student's t-test. **H,** *C. glabrata* survival in THP-1 macrophages. Data represent mean ± SEM (n = 3-4). Unpaired two-tailed Student's t-test. **I,** ChIP-qPCR quantification of the level of bound, ectopically expressed SFB-tagged CgSnf2 to *EPA1* promoter. Y-axis label is fold enrichment, with immunoglobulin G (IgG)-control and anti-FLAG (CgSnf2) antibodies. Data represent mean ± SEM (n = 3). Paired two-tailed Student's t-test.

downregulation aids in suppressing the macrophage pro-inflammatory response, and (2) Increased *EPA1* expression is deleterious for survival of *C. glabrata.*

To address these, we performed five experiments. First, we overexpressed *EPA1* from the strong *PDC1* promoter in *wt* and profiled growth in THP-1 cells. We found *wt/EPA1* to display reduced proliferation (Fig. 1D), and 3-fold increased IL-1β secretion in macrophages (Fig. 1E). Since Epa1 is a calcium-dependent lectin, lactose treatment inhibits its binding to host asialo-lactosyl-containing carbohydrates. Consistently, THP-1 infection with lactose-treated *wt/EPA1* cells reduced IL-1β secretion significantly (Fig. 1E), reinforcing the role of Epa1 in modulating IL-1β production. Second, we deleted *EPA1* gene in the *Cgsnf2*Δ background, and found that *Cgsnf2*Δ*epa1*Δ

infection invoked 1.5-fold less IL-1β production in THP-1 macrophages, compared to infection with the single *Cgsnf2*Δ mutant (Fig 1F). IL-1β secretion was similar in response to *wt* and *epa1*Δ infection, probably due to functional redundancy among Epa adhesins (Fig. 1F).

Third, we performed the same analysis with *Cgyps1-11*Δ after deleting *EPA1*, and found that *Cgyps1-11*Δ*epa1*Δ-infected macrophages secreted 1.3-fold less IL-1β than *Cgyps1-11*Δ-infected cells (Fig. 1G). Notably, CgYapsins are required for Epa1 processing from the cell wall, and *Cgyps1-11*Δ contained increased Epa1 levels in its cell wall. Fourth, we checked the intracellular survival of *epa1*Δ in THP1-macrophages, and found *wt*-like intracellular proliferation, while *Cgsnf2*Δ*epa1*Δ showed 27% better survival than *Cgsnf2*Δ (Fig. 1H), highlighting Epa1's adverse contribution to *Cgsnf2*Δ survival in macrophages. Finally, to demonstrate that CgSnf2 directly regulates *EPA1* expression, we performed chromatin immunoprecipitation analysis, and found 2-fold enrichment of CgSnf2 on *EPA1* promoter (Fig. 1I). Altogether, these data suggest that Epa1 is immunostimulatory, and acts as a fungal activator of IL-1β induction, and that *EPA1* levels are probably regulated transcriptionally by CgSnf2 via nucleosome repositioning, and post-translationally by CgYapsins through its processing off the cell wall. Currently, we are trying to delineate Epa1-responsive signalling pathways in macrophages.

Project 2: Elucidating the role of histone H3 lysine methylation in antifungal drug resistance

The main goal of this project is to delineate the epigenetic regulation of resistance mechanisms towards two mainstream antifungals, ergosterol biosynthesis-inhibitory azole and cell wall-targeting echinocandin drugs. During the current reporting period, we have elucidated a pivotal role for a SET domain-containing protein CgSet4 in azole and echinocandin resistance. We demonstrated that of six SET-domain proteins in *C. glabrata*, CgSet4 uniquely acts as a repressor of CgPdr1-dependent multidrug resistance, and ergosterol biosynthesis pathways, as *CgSET4* deletion resulted in decreased susceptibility to fluconazole (azole drug) and caspofungin (echinocandin drug) antifungals, elevated ergosterol levels and reduced virulence. We further showed, through genetic and transcriptional analyses, that CgSet4-dependent negative regulation of *CgPDR1* and *CgERG* genes is mediated via CgSet4 binding to promoter of the transcriptional activator of ergosterol biosynthesis, CgUpc2a, thereby establishing CgUpc2a as a key target of CgSet4. Studies are ongoing to delineate the role of CgSet4 in echinocandin resistance.

Publications

Bhakt, P., Raney, M. and **Kaur, R.** (2022) The SET-domain protein CgSet4 negatively regulates antifungal drug resistance via the ergosterol biosynthesis transcriptional regulator CgUpc2a. *Journal of Biological Chemistry* **298:**102485.

Patra, S., Raney, M., Pareek, A. and **Kaur, R.** (2022) Epigenetic regulation of antifungal drug resistance. *Journal of Fungi* **8:** 875.

Askari, F.¶, Rasheed, M.¶ and **Kaur, R.** (2022) The yapsin family of aspartyl proteases regulate glucose homeostasis in *Candida glabrata*. *Journal of Biological Chemistry* **298:** 101593. ¶Equal contribution.

Group of Laboratory of Fungal Pathogenesis

Laboratory of Genome Architecture

Impact of DNA topology in genome organization and functional regulation

Principal Investigator: **Yathish J Achar**
Staff Scientist

PhD students:

Md. Altamash	Junior Research Fellow
Nilay Bhowal	Junior Research Fellow
Kanchan Sahu	Junior Research Fellow

Other Members:

Pooja Tripathi	Technical Officer-I

Genome organization encompasses a diverse interplay of genetic and structural components, orchestrating an intricate network of chromatin contacts. This intricate folding of chromatin in three-dimensional space has emerged as a pivotal factor in governing genome function, thereby influencing developmental processes and cellular identities. In instances where cellular functionality is compromised, such as in cancer cells, the higher-order organization of the genome undergoes disruptions, resulting in the suppression of tumor suppressor genes or the activation of oncogenes. However, comprehending the underlying mechanisms and functionalities associated with the three-dimensional genome remains a formidable task, as the internal structures and elements contributing to chromatin organization within the nucleus are yet to be fully elucidated. By employing integrated genomics approaches, our objective is to unravel the intricate mechanisms governing chromatin folding within three-dimensional space. We seek to decipher how these distinctive features actively contribute to essential processes like cellular differentiation and the establishment of cellular identity. Furthermore, we aim to investigate how these mechanisms become disrupted under pathological conditions, particularly in diseases such as cancer. Through our research, we aspire to gain insights into the deregulation of chromatin folding and its implications for disease progression.

Research Summary

Cohesin plays a crucial role in genome organization by facilitating the structural integrity and proper functioning of the genome. Cohesin is a multi-protein complex composed of four core subunits: SMC1A, SMC3, RAD21, and STAG1/2. It is primarily known for its role in mediating sister chromatid cohesion during DNA replication and cell division. However, cohesin also contributes significantly to the organization and regulation of chromatin in the interphase nucleus. One of the key functions of cohesin is establishing and maintaining long-range chromatin interactions. Cohesin binds to specific genomic regions called cohesin anchor sites or CTCF (CCCTC-binding factor) binding sites, which are typically present at enhancers and promoters. By forming loops, cohesin brings distant DNA sequences into close spatial proximity, facilitating regulatory interactions between these regions. These chromatin loops are important for gene regulation, as they enable enhancers to interact with their target genes and modulate their expression.

In budding yeast, cohesin is predominantly loaded onto centromere and promoters of RNAPII transcribing regions during interphase. Cohesin translocates from the promoter to the transcription termination site, suggesting for loop extrusion model at the single gene level. Cohesin-mediated loop extrusion is halted upon encountering a barrier or when released by Wapl. CTCF boundaries are thought to be the predominant barrier for cohesin-mediated extrusion, however recent finding advocate for alternate structural barriers including MCM and RNAPII complexes. We hypothesized cohesin complexes are loaded and released from chromatin in a DNA supercoil-dependent manner. By controlling cohesin association DNA supercoil events might dictate gene loop formation as well as genome organization.

DNA supercoil controls cohesin association with chromatin

We analyzed the binding profile of cohesin (Scc1-10X Flag) in budding yeast using wild-type and topoisomerase double mutant (*top1Δtop2-1*), in the presence or absence of E. coli TopA. TopA specifically acts on negative supercoils, converting them into positive supercoils. Expression of TopA in *top1Δtop2-1* alleviates negative supercoiling at gene boundaries, leading to increased accumulation of

positive supercoils and nucleosome repositioning. In wild-type cells, Scc1 peaks are predominantly observed at centromeres and pericentromeric regions, where cohesin complexes are trapped by convergent genes. Similarly, a higher proportion of Scc1 peaks accumulate at converging genes across the chromosome arms. Converging genes consist of smaller intergenic spaces and accumulate positive supercoils at the expense of negative supercoils. We found that converging genes with shorter intergenic spaces (<250 bp) accumulate higher levels of Scc1 compared to those with medium-sized intergenic spaces (251-500 bp) and larger intergenic spaces (>500 bp).

We performed a meta-analysis of Pol II-transcribed genes (6706 genes) to analyze the distribution of Scc1. Scc1 accumulation gradually increases within the ORF, while negative supercoil-enriched transcription starts sites (TSSs) and transcription termination sites (TTSs) show reduced accumulation. This suggests that cohesin complexes have a preference for positive supercoiling, as it allows for more efficient compaction of the DNA. Previously, we found a bias towards negative supercoiling in highly expressed genes, but we failed to observe a direct dependency on transcription activity and DNA supercoil build-up. Similarly, we found no correlation between gene expression and Scc1 peaks, as all three groups of

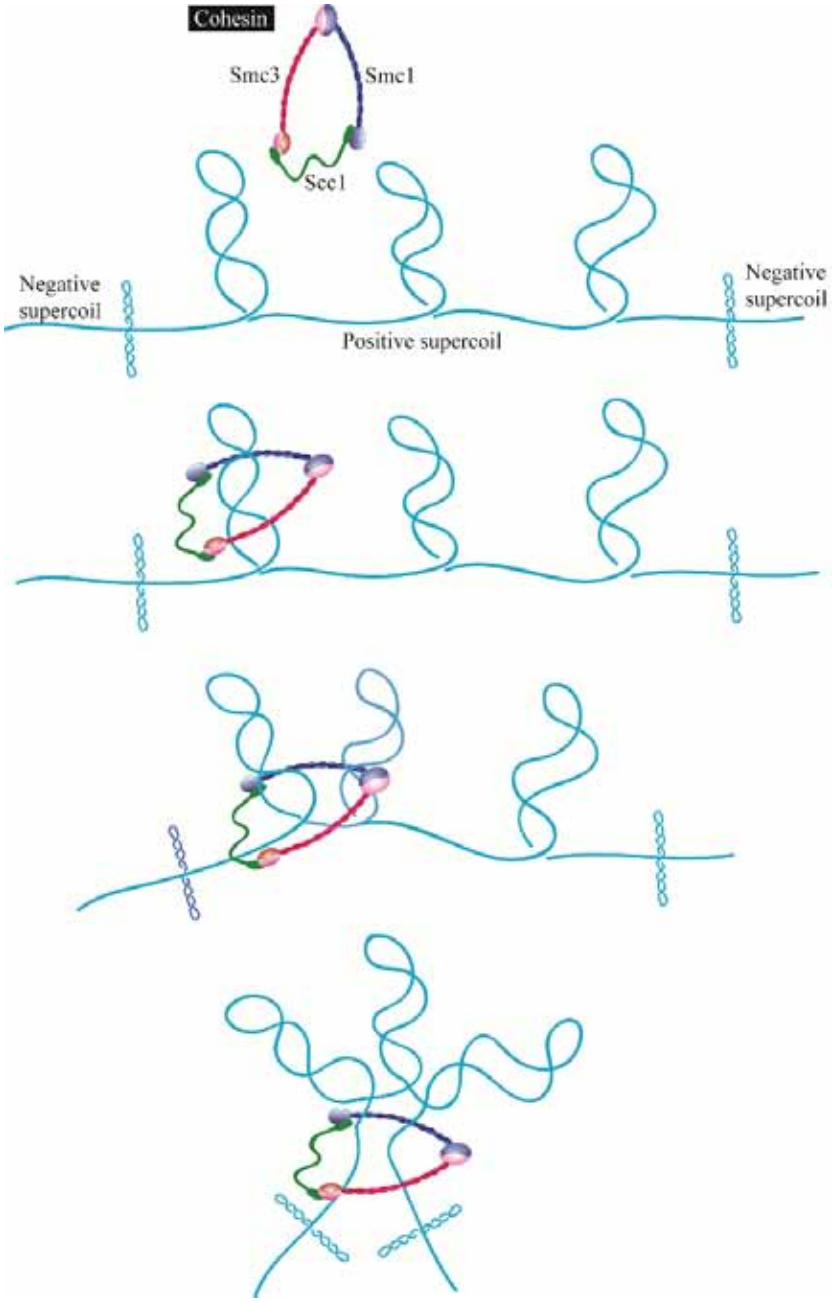

Figure 1: Schematic representation of DNA supercoil-dependent cohesin loading and sliding across a gene body leading to gene loop model.

genes (high, medium, and low expression) showed similar binding patterns. This implies that cohesin association with chromatin, like DNA supercoiling, is not directly dependent on transcription activity per se.

Expression of TopA in wild-type cells had a nominal effect, as Scc1 levels showed a reduction, but the majority of peaks remained intact across the chromosome arms and at centromeres. This reduction could be attributed to the diffusion of supercoil waves causing a decrease in positive supercoiling within the ORF. The functional loss of Top1 and Top2 only partially reduces negative supercoiling at gene boundaries. We observed a major reduction in Scc1 accumulation at the centromere but not at the Pol II coding genes. Top2 protein accumulates in higher proportions at the centromere and pericentromeric regions, and it is crucial for resolving cohesin-dependent topological stress at centromeres. Expression of TopA in *top1Δtop2-1* drastically reduced cohesin peaks at the centromere and chromosome arms. A decrease in Scc1 levels was observed across the chromosome, particularly at its major accumulation sites, including centromeres. Conversely, we observed a higher proportion of Scc1 at telomeres, as cohesin sliding from neighboring regions would be trapped by telomere secondary structures. Scc1 peaks are scattered across the chromosome in lower amounts but are not randomly distributed. This suggests that DNA supercoiling guides both the loading and release of cohesin from chromatin; however, we did not observe any new peaks. Overall, these data suggest that negative supercoiling at gene boundaries acts as a barrier for trapping positive supercoiling, which in turn assists in loading and sliding cohesin molecules.

Group of Laboratory of Genome Architecture

Laboratory of Genome Informatics

Application of big data, artificial intelligence, and deep learning in medical and agricultural genomics

Principal Investigator: **Ajay Kumar Mahato**
Staff Scientist

Ph.D Students:

E. Ramesh	Junior Research Fellow
Priyanka Kushwaha	Junior Research Fellow

Other members:

Satyam Shrivastava	Computer Programmer

Collaborators:

National

Dr. Sabhyta Bhatia	DBT-NIPGR, Delhi
Dr. Rakesh Singh	ICAR-NBPGR, Delhi
Dr. Mamta Sharma	ICRISAT, Hyderabad
Dr. Satya Pal Yadav	ICAR-DPR, Hyderabad
Prof. Devarshi Gajjar	The Maharaja Sayajirao University of Baroda, Vadodara.

International

Fei Zhao	Shanghai Institute of Plant Physiology and Ecology, Shanghai

Objectives:

Harnessing the Power of Big Data, Artificial Intelligence, and Genomics to develop new computational tools/pipelines, and genomic resources, focused on human disease diagnostics and plant disease resistance.

Our cutting-edge In-silico laboratory harnesses the power of Big-data science, artificial intelligence, and deep learning to mine genomics data from diverse sources - humans, plants, pathogens, and more. We aim to extract novel information by exploring genes associated with various phenotypic traits, particularly those causing diseases in humans, plants, and pathogens. In addition, we are committed to decoding new genomes of species vital for national food security, nutrition, and human health. The novel genomic resources we generate are valuable for both Indian and global scientific communities, enabling the development of better cultivars and breeds through methods such as QTL mapping, genome-wide SSR/SNP mining for marker development, linkage map creation, GWAS, and more. To accomplish this, we utilize state-of-the-art open-source software to process and analyze large-scale genomic Big-data.

Beyond processing and analysis, our lab is also dedicated to generating vast genomic datasets and sourcing data from public repositories. We use this wealth of information to develop innovative algorithms and methodologies for AI-based or deep learning-based models using an object-oriented programming language. Once created, these models undergo rigorous training, refinement, and benchmarking on our on-premise 5 Petaflop GPU server. The goal is to convert these models into user-friendly web applications and genomic resources made freely available to researchers worldwide. In doing so, we aim to push the boundaries of genomics and help expedite ground-breaking research globally.

Project 1: De Novo Transcriptome Profiling for Microsatellite Markers, Transcription Factors, and Database Development: A Study on *Andrographis paniculate*

Andrographis paniculata, a therapeutic plant from the Acanthaceae family, is recognized for its medicinal properties owing to its distinct chemical constituents. The plant's leaves contain andrographolide, a critical therapeutic component with antimicrobial and anti-inflammatory properties. Using advanced 454 GS-FLX pyrosequencing, we produced a complete transcriptome profile of A. paniculata leaves, yielding 22,402 high-quality transcripts. Functional annotation was successfully carried out for 86% of the total transcripts. Transcription factor analysis unveiled 6669 transcripts across 57 different transcription factor families, with NAC, MYB, and bHLH TF categories verified via RT PCR amplification. Our in-depth in silico analysis identified 102 transcripts associated with terpenoid biosynthesis, a group of chemicals with medicinal value. Furthermore, we identified 4254 EST-SSRs from 16.34% of the total transcripts, which facilitated the assessment of genetic diversity among 18 A. paniculata accessions. Finally, we

established a comprehensive database incorporating EST transcripts, EST-SSR markers, and transcription factors. This database, a combination of our study's data and publicly available transcriptomic resources, serves as a one-stop resource for researchers studying this medicinal plant.

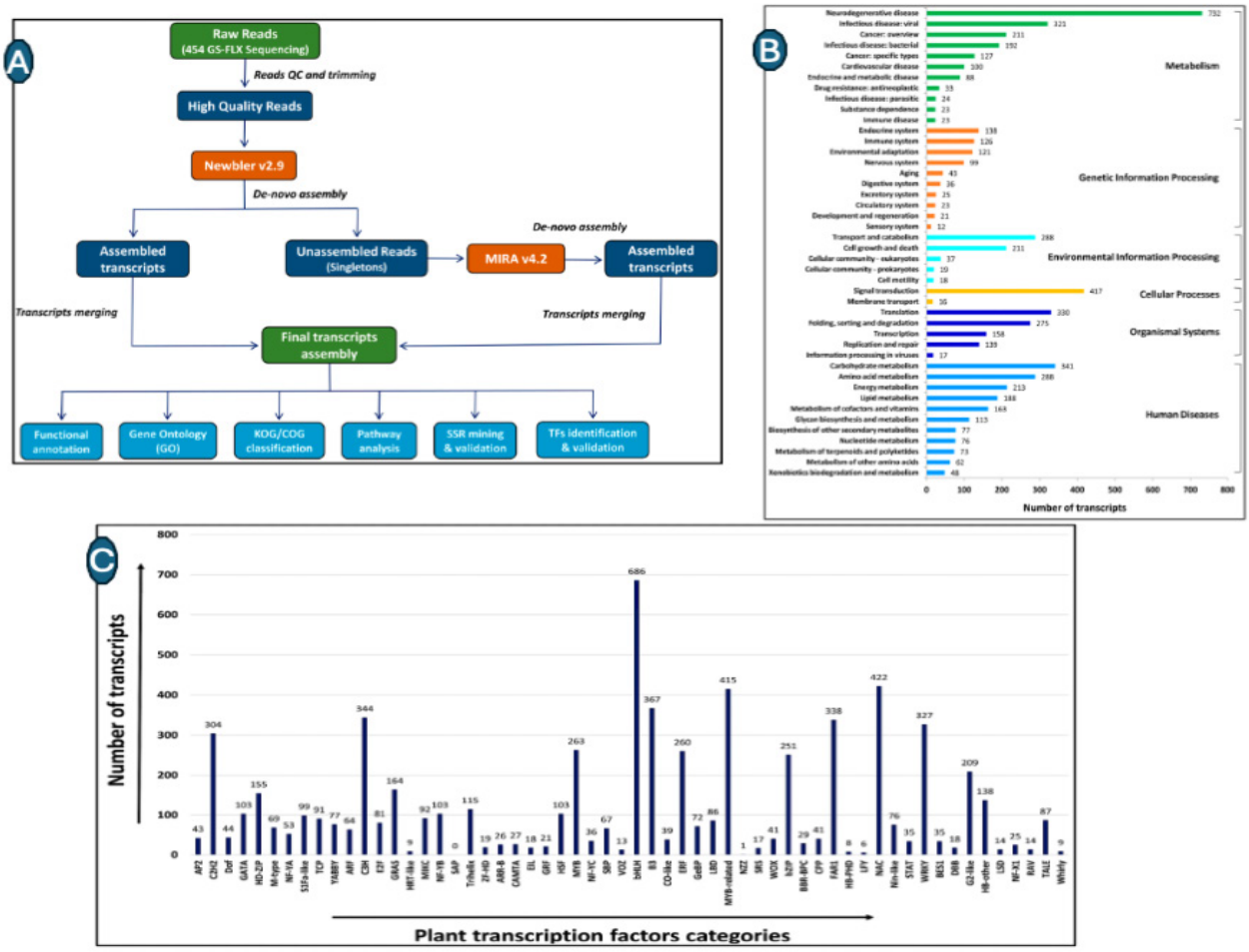

Figure 1: **(A)** Process flow diagram of the complete materials and methodology followed in this study; (**B)** KEGG metabolic pathway mapping of A. paniculata transcripts. The y-axis shows the name of the KEGG metabolic pathway, and the x-axis shows the number of transcripts; **(C)** Functional classification of transcripts in to 58 different transcription factor categories.

Project 2: De novo transcriptome assembly and identification of brassinosteroid biosynthetic pathway in safflower

Safflower (Carthamus tinctorius L.), recognized for its superior oil and drought tolerance, is of increasing importance in today's climate-challenged world. Brassinosteroids (BRs), plant steroid hormones instrumental in plant growth, development, and stress responses, can potentially increase crop yields by up to 40% and enhance stress tolerance. Yet, our understanding of BR biosynthesis and signaling pathways in safflower is currently limited. To bridge this knowledge gap, we conducted a pioneering de novo transcriptomic analysis on untreated and 24-epibrassinolide (EBR)-treated safflower leaves, using the Illumina sequencing platform. Our investigation generated about 5 GB of clean data from both sets of samples, assembling into a combined total of 50,630 transcripts and 43,637 coding sequences (CDS). Over 71% of the CDS received annotations, with the majority referencing Cynara cardunculus var. scolymus, a relative in the safflower family. A total of 74 KEGG pathways were identified in safflower. Significantly, six genes, including DWF4, pivotal in BR biosynthesis, were traced to the BR biosynthesis pathway using the KEGG mapper. Our study represents a crucial step in utilizing functional genomics to bolster safflower's productivity and resilience, thereby contributing to global food security.

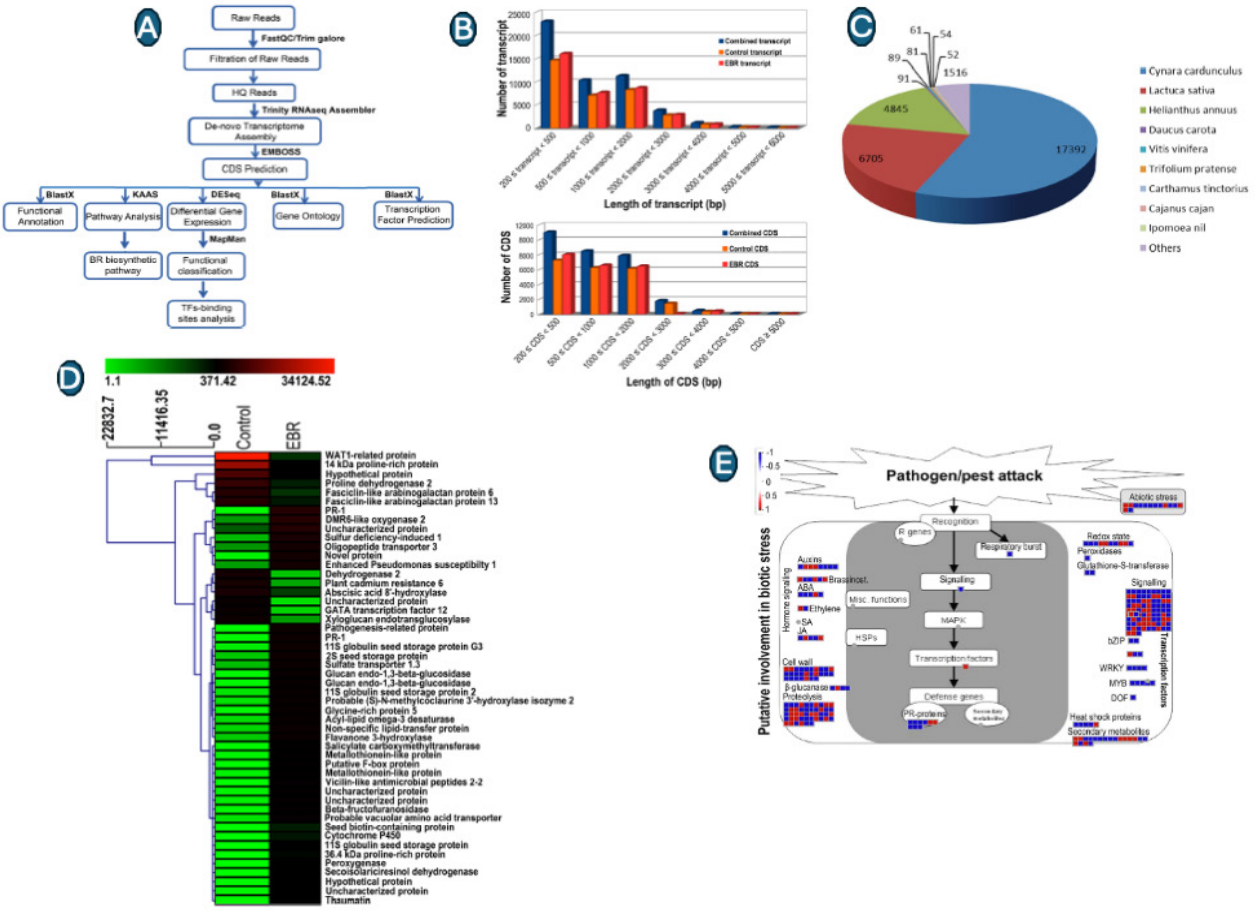

Figure 2: **(A)** The flow diagram of the complete methodology with software details followed in this study; (**B)** Length distribution of transcript and CDS length in control and EBR samples; **(C)** Top BLASTx hits of the combined transcriptome of untreated and EBR-treated safflower samples; **(D)** Heatmap showing the differentially expressed genes (DEGs) in Control and EBR treated safflower samples; **(E)** Functional annotation of genes related to stress pathways in MapMan analysis.

Project3: Decoding of the Indian black chicken genome "Kadaknath" and identification of genes related to its nutritional quality

The primary objective is to create a reference genome of the "Kadaknath", a unique Indian black chicken breed known for its health benefits. The Kadaknath has evolved naturally and has been maintained by the tribal communities, or Adivasis, of Madhya Pradesh, India. The breed is distinct in having entirely black internal and external organs. In order to construct the Kadaknath's whole genome sequence, we used a combination of the long-read but low-depth PacBio Sequel II platform and the high-depth Illumina short-read platform.

The sequencing data has been rigorously quality-checked, assembled via trio-binning genome assembly, and submitted to the NCBI SRA under a specified Bioproject number. Currently, we're conducting further secondary and tertiary analysis of the data. Our aim is to complete this process and have our findings published in a peer-reviewed journal within the current academic year. This study's intention is to contribute to our understanding and preservation strategies for the fascinating "Kadaknath" chicken breed by providing a robust genomic reference.

Publications: ***(April 2022 – March 2023)***

R Singh, A Singh, **AK Mahato**, R Paliwal, G Tiwari, A Kumar (2023). De Novo Transcriptome Profiling for the Generation and Validation of Microsatellite Markers, Transcription Factors, and Database Development for Andrographis paniculata.**s** 24,9212. https://doi.org/10.3390/ijms24119212

A Singh, **AK Mahato**, A Maurya, R Subramani, AK Singh, R Bhardwaj, SK Kaushik, S Kumar, V Gupta, K Singh, R Singh (2023). Amaranth Genomic Resource Database (AGRDB): an integrated database resource of Amaranth genes and genomics. **Frontiers in Plant Science**. Volume 14 - 2023 | doi: 10.3389/fpls.2023.1203855

A Kumar, PK Jayaswal, **AK Mahato**, A Arya, PK Mandal, NK Singh, SK Sinha (2022). Growth stage and nitrate limiting response of NRT2 and NAR2 gene families of bread wheat, and complementation and retrieval of nitrate uptake of atnrt2. 1 mutant by a wheat NRT2 gene. **Environmental and Experimental Botany**. https://doi.org/10.1016/j.envexpbot.2022.105205.

BD Prasad, S Sahni, P Krishna, D Kumari, **AK Mahato**, SJ Jambhulkar, P Kumar T Ranjan, AK Pal (2022). De Novo Transcriptome Assembly and Identification of Brassinosteroid Biosynthetic Pathway in Safflower. **Journal of Plant Growth Regulation**. https://doi.org/10.1007/s00344-021-10429-9.

Group of Laboratory of Genome Informatics

Laboratory of Human and Medical Genetics

Genomic studies in chromosomal and single gene discorders

Principal Investigator: **Ashwin Dalal**
Staff Scientist

Adjunct Faculty:

Prajnya Ranganath	Additional Professor, NIMS
Shagun Aggarwal	Additional Professor, NIMS

Ph.D Students:

A Sandeep	Senior Research Fellow
Shrutika Padwal	Junior Research Fellow
Aparna Roy	Junior Research Fellow (From 29/06/2022)

Other Members:

Anjana Kar	Research Associate
Sundarvadivel	Research Associate (From 21/11/2022)
Mugdha Singh	Research Associate (Until 22/08/2022)
Pragna Lakshmi	Research Associate (From 11/10/2022)
Upasana	Senior Research Fellow (Until 11/11/2022)
Mohini Annapurna	Project Assistant (Until 28/02/2023)
B. Siddhardha	Project Assistant (Until 28/02/2023)

Objectives

1. To conduct genetic evaluation for patients/families with genetic disorders
2. To develop new methods and assays for genetic analysis and engage in research on chromosomal and single gene disorders
3. To act as national referral center for analysis and quality control of genetic tests for few genetic diseases
4. To impart training in genetic evaluation of patients with genetic disorders

Mission Program on Pediatric Rare Genetic Disorders (PRaGeD)

Rare genetic diseases are rare by themselves but collectively they are significant cause of morbidity and mortality and serious public health problem in India. India is home to an estimated 72 million people affected by rare disease. But there is lack of awareness and understanding of these conditions. This has led to significant challenges in terms of diagnosis, treatment and access to care. Due to large diversity in India and practices of consanguineous marriages, India has a pool of indigenous genetic variants and many of which may be cause of unexplained genetic conditions found only in Indian population. Till date many genes, causing single gene disorders have been identified but still a large number remains to be characterized. Next generation sequencing has revolutionized the field of gene identification by exome sequencing and/or genome sequencing.

Mission program on Pediatric Rare Genetic Disorder (PRaGeD) is a PAN- India initiative to create awareness, achieve genetic diagnosis, discover and characterize new gene/variants, provide counselling and to develop new therapies for pediatric rare genetic diseases in India. CDFD in collaboration with 15 centres across India plans to recruit patients and their families with rare genetic disorders study. The outcome of this study will not only provide unique opportunity for identification of novel genes for various known as well as unexplained inherited phenotypes but also help the patient and family with management of disease and prenatal diagnosis. In addition, functional characterization of novel genes/variants using different metazoan model systems is likely to establish causality between the novel mutation and the phenotype. A database of sequence variants corresponding to phenotypes in Indian pediatric patients will serve as an invaluable resource for genetic diagnostics labs, clinicians and researchers within Indian and outside the country. In parallel, an effort will be made to develop technologies for novel therapeutics for rare disorders, and affordable methods for diagnostics and screening of genetic disorders. This wholistic approach, combining basic, applied, and translational research will ultimately enhance delivery of diagnostics services and genetic counselling to reduce the disease burden of pediatric rare genetic disorders.

A total of 196 families with unexplained genetic conditions were recruited by various centres. Using conventional molecular testing i.e. targeted sequencing, MLPA, Genotyping, Chromosomal analysis etc 8 cases were solved and 133 cases

are under investigation. A total of 45 cases with unexplained genetic etiology were planned for whole exome sequencing and 10 for whole genome sequencing. We have created and hosted website for PRaGeD Mission program: www.praged.cdfd.org.in Under this website all collaborative institutes were given user ID and passcode to access. In the same website we have created portal for tracking various activities under PRaGed Program such as recruitment of cases, EBV transformation, Exome sequencing, Exome Analysis etc. We have given access to every collaborator to document their cases for exome sequencing at PRaGeD website using login. We have created database of phenotypes present in cases recruited for Mission Program by documenting them in Phenotips database. The same database has been linked to our website and portal. Fully functional lab set up for Genome sequencing as well as function analysis is under progress at CDFD. This lab will cater need of the project including collaborating institutes for doing WES and WGS as well as functional validation of findings in cell lines, drosophila, zebrafish etc. Functional analysis projects have been initiated which include development of a mouse model for SERPINA11 gene, Drosophila model for AIMP2 gene and studies on Wiedemann-Steiner Syndrome (WSS) caused by mutation in MLL(KMT2A) gene by performing the centrosomal microtubule regrowth assay which indicates the nucleating capabilities of the centrosome. We have created infographics flyers in English, Hindi and 6 other regional languages for display in OPD, Institutes information board and for distribution under awareness program. Informative Videos are also prepared to be displayed in website, OPD and institutes information desk wherever display units are available

Undiagnosed diseases Program

The genetic diversity of India, coupled with inbreeding practices and founder effects, creates an environment conducive to the accumulation of deleterious genetic variations leading to rare diseases. Exome sequencing serves as a powerful tool in unraveling the genetic etiology of these rare genetic disorders and expanding the knowledge of rare disease biology, by focusing on the protein-coding regions of the genome. In our research, exome sequencing, in conjunction with our in-house pipeline, encompasses the identification of single nucleotide variants (SNVs), small insertions/deletions (indels), copy number variations (CNVs), and structural variations (SVs). With this, we are able to improve diagnostic rates and uncover novel variants in genes associated with unexplained inherited phenotypes. This newfound knowledge has the potential to better disease management, patient care and influence the personalized treatment approaches.

Patients with unknown genetic etiology inspite of basic genetic investigations were recruited for exome sequencing. A total of 110 individuals from 101 families, exhibiting clinical features suggestive of a genetic disease with unknown diagnosis, were included in this study. Through whole exome sequencing, a definitive diagnosis was obtained for 55 individuals from 50 families. Within this cohort, we identified a set of 32 novel and 18 previously reported rare deleterious variants (31 homozygous, 1 hemizygous, 2 compound heterozygous, and 16 heterozygous variants, distributed across 50 families). Among these rare variants, 21 were missense, 29 loss-of-function variants (including insertions, deletions, stop gain, exon deletion, and duplication). To understand the inheritance pattern of identified variant we conducted segregation analyses within the families and to gain insights into the effect of variant on protein structure, *in silico* structural investigation was performed.

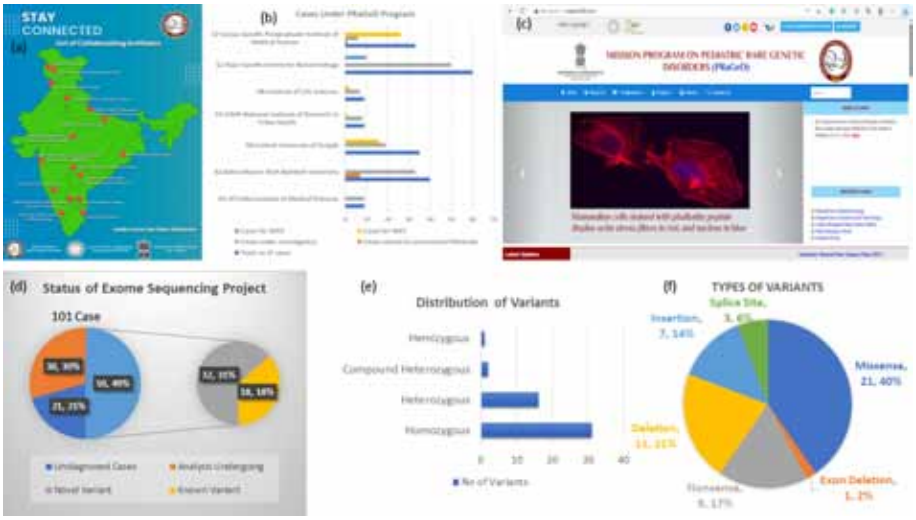

Figure 1: (a) PRaGeD Program Collaborating Centers (b) Cases under PRaGeD program 2022-2023 (c) PRaGeD website (d) Status of cases under exome sequencing project (e) Distribution of variants zygosity (f) Distribution chart of type of variants identified under the project.

Publications

Research papers published in 2022:

Kemp SA, Cheng MTK, Hamilton WL, Kamelian K; Indian SARS-CoV-2 Genomics Consortium (INSACOG), Singh S, Rakshit P, Agrawal A, Illingworth CJR, Gupta RK. (2022) Transmission of B.1.617.2 Delta variant between vaccinated healthcare workers. ***Scientific Reports*** 12(1):10492.

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Sarma AS, Siddardha B, T PL, Ranganath P, Dalal A. (2023) A novel homozygous synonymous splicing variant in SELENOI gene causes spastic paraplegia 81. ***Journal of Gene Medicine*** (In Press)

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Priestley JRC, Deshwar AR, Murthy H, D'Agostino MD, Dupuis L, Gangaram B, Gray C, Jobling R, Pannia E, Platzer K, Prescott K, Redman M, Rippert AL, Rosenfeld JA, Scott DA, Wang YW, Schmederer Z, Dalal A, Sarma AS, Skraban C, Dowling JJ, Mendoza-Londono R, Slavotinek A, Bhoj EJ. Monoallelic loss-of-function BMP2 variants result in BMP2-related skeletal dysplasia spectrum. ***Genetics in Medicine*** (In Press)

Sarma AS, Peter Mathew R, Dalal A, Bhat V, Patil SJ. Familial monoallelic CYP26B1 truncating variant causes a syndromic craniosynostosis due to haploinsufficiency? ***European Journal of Medical Genetics*** (In Press)

Singh A, Saini N, Behl G, Aggarwal S, Kolar G (2022) Recurrent Vein of Galen Aneurysmal Malformation as a Presentation of Hereditary Hemorrhagic Telangiectasia. ***Molecular Syndromology*** (In Press)

Group of Laboratory of Human and Medical Genetics

Laboratory of Human Molecular Genetics

Understanding the mitochondrial dysfunction in human health and disease

Principal Investigator: **P Govindaraj**
Staff Scientist

Ph.D Students:

Rohan Peter Mathew

B Disha

Other members:

A Vasanthakumar

Pothina Amarnadh

Mulla Khayum Khan

Collaborators:

Dr. Madhu Nagappa	NIMHANS, Bangalore
Dr. Sireesha Yareeda	NIMS, Hyderabad
Dr. Bhupesh Mehta	NIMHANS, Bangalore

Objectives:

Our laboratory focuses on understanding the mitochondrial dysfunction in human health and disease. In particular, with a specific aim to explore the new genes that are associated with mitochondrial disorders, understand the molecular mechanisms, and develop theragnostics (diagnosis and treatment). We use next-generation sequencing to investigate the interaction between mitochondrial DNA and nuclear DNA. Further, we use patient-derived cell lines (fibroblasts) for generating transmitochondrial cybrids for mtDNA mutations and other cellular models to delineate the molecular mechanism leading to neuronal loss and neurological defects. In addition, our group is also involved in identifying the novel genetic cause of other rare genetic disorders.

Project 1: The Identification and characterization of newer pathogenic variants associated with mitochondrial diseases of the nervous system

Last decade of biomedical research, there has been a remarkable convergence of interest in the powerhouse of cells, the mitochondria. Mitochondrial dysfunction is associated with a broad spectrum of human disorders, ranging from rare, inborn errors of metabolisms to common, age-related conditions, including cardiovascular and neurodegenerative diseases. However, the emerging field of mitochondrial medicine is hindered by the complexity of these organelles and the breadth of implication in disorders, leading to a lack of mechanistic insights, biomarker discovery, and therapeutic targets.

Mitochondrial diseases are multi-systemic, heterogeneous group of disorders affecting children and adults with 1 in 5000 individuals. Because of the clinical and genetic heterogeneity; and tissue-specificity, often rendering the diagnostic process protracted and challenging. Despite advances in understanding the pathophysiology, varied phenotype-genotype relationships have limited the development of effective therapies. During the current year (April 2022- March 2023), we have collected 33 patients suspected of mitochondrial disorders along with relatives from the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore and Nizam's Institute of Medical Sciences (NIMS), Hyderabad. DNA isolation, quantification and whole mitochondrial genome / Whole exome sequencing (WES) analysis was performed using next-generation sequencing (NGS). Data analysis of mtDNA and nuclear DNA revealed several known and novel variants. Further, patients with novel/ VUS variants; skin fibroblast and lymphocytes were collected for functional characterization.

Interestingly, WES of a patient who was born of a consanguineous marriage presented with clinical features such as failure to thrive, microcephaly, motor delay, bronchiolitis and metabolic acidosis (s/o Leigh disease) revealed a digenic variant, *NDUFA11* (c.586G>A, p.Gly196Arg) & *PET100* (c.115-3C>G, 3' splice site) **(Figure 1).** In addition, WES analysis also revealed eight variants (two *TARS2*, one *NARS2*, one *FARS2*, two *WARS2*, one *KARS1* and one *IARS2*) in different tRNA synthetase genes in seven unrelated patients with a wide range of clinical manifestations. Few of the novel missense variants observed were analyzed using various in silico prediction tools and found to be highly conserved and pathogenic. Among these variants, four are novel, and though the other four are already reported, they lack functional

validation. Further, functional characterization using patients-derived fibroblasts is underway to study the role of these variants in the pathogenesis of disease. As part of mitochondrial genetic diagnosis, we have provided 16 mtDNA analysis report to NIMS, Hyderabad.

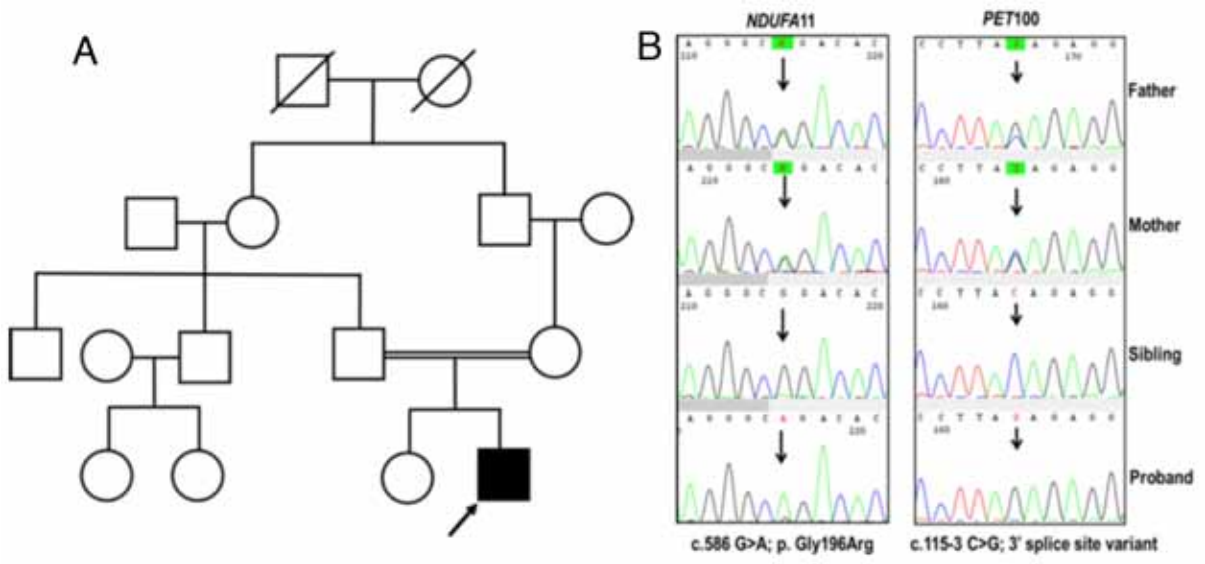

Figure 1: The pedigree (A) and the sequencing electropherogram (B) of a Leigh patient.

Project 2: Lead-toxicity-induced memory impairments

Lead (Pb2+), a ubiquitously present heavy metal toxin, has various detrimental effects on memory and cognition. However, the molecular processes affected by Pb2+ causing structural and functional anomalies are still unclear. To explore this, we employed behavioral and proteomic approaches using rat pups exposed to lead acetate through maternal lactation from postnatal day 0 (P0) until weaning. Behavioral results from three-month-old rats clearly emphasized the early life Pb2+ exposure induced impairments in spatial cognition. Further, proteomic analysis of synaptosomal fractions revealed differential alteration of 289 proteins, which shows functional significance in elucidating Pb2+ induced physiological changes. Focusing on the association of Small Ubiquitin-like MOdifier (SUMO), a post-translational modification,

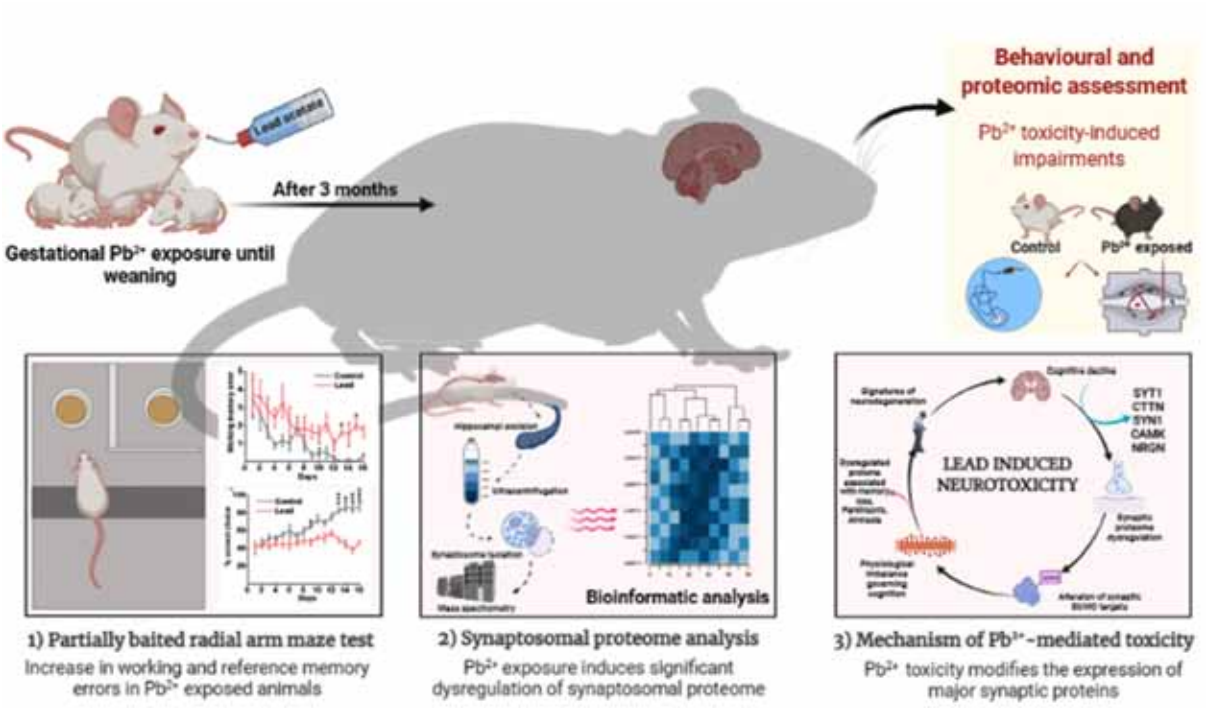

Figure 2: The graphical representation of the early life lead exposure alters hippocampal memory functioning and synaptic proteome.

with Pb2+ induced cognitive abnormalities, we identified 45 key SUMO target proteins. The significant downregulation of SUMO target proteins such as metabotropic glutamate receptor 3 (GRM3), glutamate receptor isoforms 2 and 3 (GRIA 2 and GRIA3) and flotilin-1 (FLOT1) indicates SUMOylation at the synapses could contribute to and drive Pb2+ induced physiological imbalance. These findings identify SUMOylation as a vital protein modifier with potential roles in hippocampal memory consolidation and regulation of cognition. In addition, human disease enrichment analysis showed various mitochondrial diseases such as Leigh syndrome due to mitochondrial complex I-V deficiencies, alteration in NADH dehydrogenase [ubiquinone] flavoprotein 3 expression (NDUFV3), providing more reasons to investigate Pb2+ toxicity on mitochondrial physiology **(Figure 2).**

Publications:

De S, Rai D, Tamang S, Sherpa RD, Subba S, Lepcha DR, **Govindaraj P,** Thangaraj K, Chaubey G, Tamang R (2023). Signatures of high altitude adaptation in Tibeto-Burman tribes of the Darjeeling Hill Region. *American Journal of Human Biology (Inpress).* https://doi.org/10.1002/ajhb.23858.

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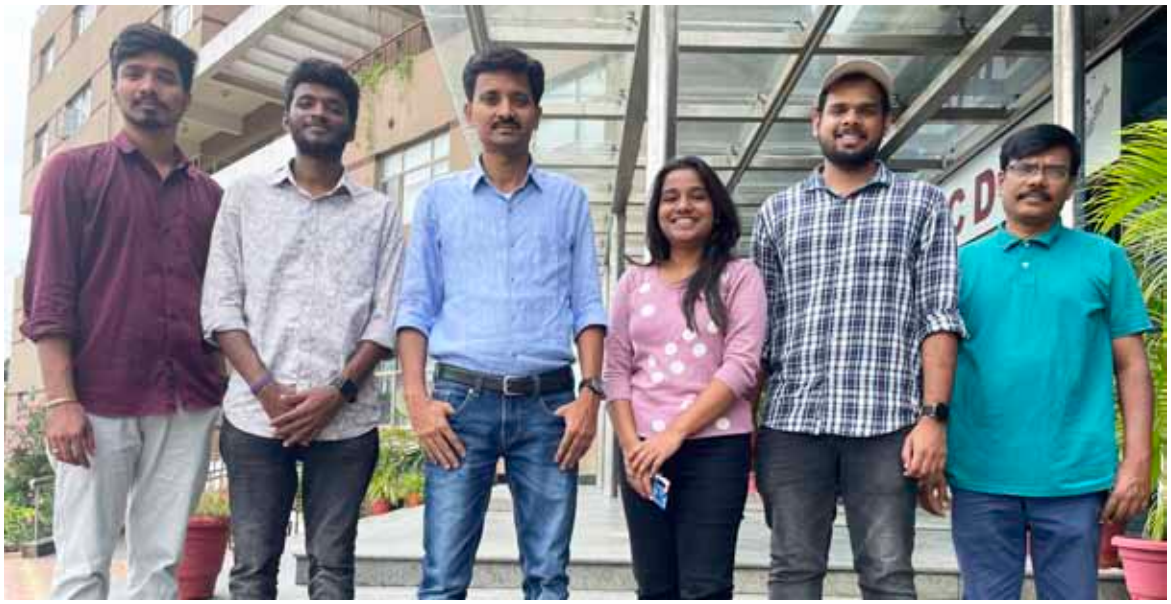


Group of Laboratory of Human Molecular Genetics

Laboratory of Immunology

Advanced Glycation End products mediated Lipogenesis and its Regulation

Principal Investigator: **Sunil K Manna**
Staff Scientist

Ph.D Students

Shashank Saurav	Senior Research Fellow (till August 2022)
Aher Abhishek Taterao	Senior Research Fellow
Saphy	Senior Research Fellow
V Chandana Praneetha	Senior Research Fellow
Bindi Goradia	Senior Research Fellow
Homagni Dey	Senior Research Fellow
Suraja Kumar Das	Junior Research Fellow
Neelamadhaba Pani	Junior Research Fellow

Other Members

T Navaneetha	Technical Officer

Collaborators

Tushar Basu Baul	NEHU, Shilong
Pulakesh Bera	Vidyasagar University, WB
Sudit Mukhopadhyay	NIT, Durgapur, WB

Objectives

1. Understanding and regulation of advanced glycation end products (AGE) - mediated deleterious effects.
2. Understanding the role of Profilin in regulation of tumorigenesis.
3. Understanding and regulation of inflammatory and tumorigenic responses.

Research Summary

Profilin, a 15 kDa globular protein, regulates actin polymerization and interacts with proline-rich ligands through its poly-L-proline binding domain to regulate organ development, wound healing and immune functions. Most of the cancers express a lower amount of profilin, which results in reduction of focal adhesion and increased malignancy. Although reduced expression of profilin increases cancer aggressiveness, complete ablation of this protein results in compromised growth and viability. Profilin expression was found to be very low in a triple-negative breast cancer (TNBC), MDA MB-231 cells and its overexpression results in inhibition of tumor initiation and growth. Autophagy is a well-organized, multi-step cellular recycling event, which is controlled by more than 18 autophagy regulating genes (ATGs). Autophagosome maturation is important step to renew energy especially in the rapid growing tumor cells. Our study shows that ATRA-mediated profilin expression increases anti-tumor potential by impairing autophagy through AMPK stabilization. Taken this as proof of concept from both cell-based and '*in vivo*' data, ATRA may be a potent and safe agent which can be utilized for future combination therapeutics. Therapeutic utilization of ATRA-induced cytotoxic autophagy to drive cancer cell death especially for the triple-negative cancer, could be an emerging paradigm for cancer therapy.

Details of progress in the current reporting year (April 1, 2022 - March 31, 2023)

Role of Advanced Glycation End products in inducing Lipogenesis.

Advanced glycation end (AGE) products are formed by covalently attaching reducing sugars or its reactive carbonyl metabolites such as methylglyoxal (MGO) and glycolaldehyde, to amino group of the basic amino acids present in the proteins. The mechanism for AGE formation involves the formation of Schiff base between amino terminal of the basic amino acids and the carbonyl group of sugar moiety. AGEs are known to interact with their specific receptors, Receptors for AGE (RAGE), members of super immunoglobulin family. The signal induced by AGE-RAGE binding is tissue and disease specific. Depending on the intensity and duration of AGE-RAGE ligation, various pathways get activate such as ERK1/2, P38MAPK, CDC42/RAC, SAPK/JNK and NF-κB. During natural aging, AGEs get accumulated inside the human body triggering various pathological consequences ranging from retinopathy, diabetes, kidney failure to Alzheimer.

AGE treatment disturbs lipid homeostasis in neuronal cells: The effect of AGE treatment in

dysregulating lipid homeostasis was investigated in human neuroblastoma cell line IMR-32 using nile red dye. Cells treated with different concentrations of AGE for 24 were stained with Nile Red Dye to detect the neutral lipid droplets. The number and size of the lipid droplets per cell was increasing with increasing the concentration of AGE treatment (**a** & **b**). The number of lipid droplets per cell was also increasing with increasing the time of AGE treatment (3 µM) (**c**). Cells treated with glucose was taken as positive control. Nile red staining data suggest that AGE promotes the formation of lipid droplets in dose and time dependent manner.

Analysis of Diabetic Peripheral Neuropathy (DPN) Patient Samples: Dysregulation of lipid metabolism in diabetic neuropathy is often reported phenomenon responsible for diabetes related complications. Transcriptome data of Diabetic Peripheral Neuropathy Patients was retrieved from NCBI-SRA database (Bioproject ID: PRJNA767371) to analyse the genes involved in lipid metabolism pathway. Upregulated genes were annotated to pathways using pathway browser available on web-based server reactome (https://reactome.org) and about 95 genes were found to be involved in metabolism of lipid pathway. Venn diagram was plotted between genes involved in metabolism of lipid pathway and T2DiACoD database containing gene curated for onset of diabetic neuropathy. AKR1B1, SREBP1 and SYNJ1 emerged as a common factor between both the analyses (**d**). AKR1B1 was taken up for further studies as SREBP1 is well-known player for lipogenesis and other SYNJ1 is mostly implicated in neurodegenerative disorders.

AGE up-regulates AKR1B1 expression: IMR-32 cells treated with different concentrations AGE has shown the significant increase in AKR1B1 protein expression level with increasing the AGE concentration. Quantification of western blots showed about 1.5-fold increase in AKR1B1 protein expression in 3 µM AGE treated cells compared control cells (**e**).

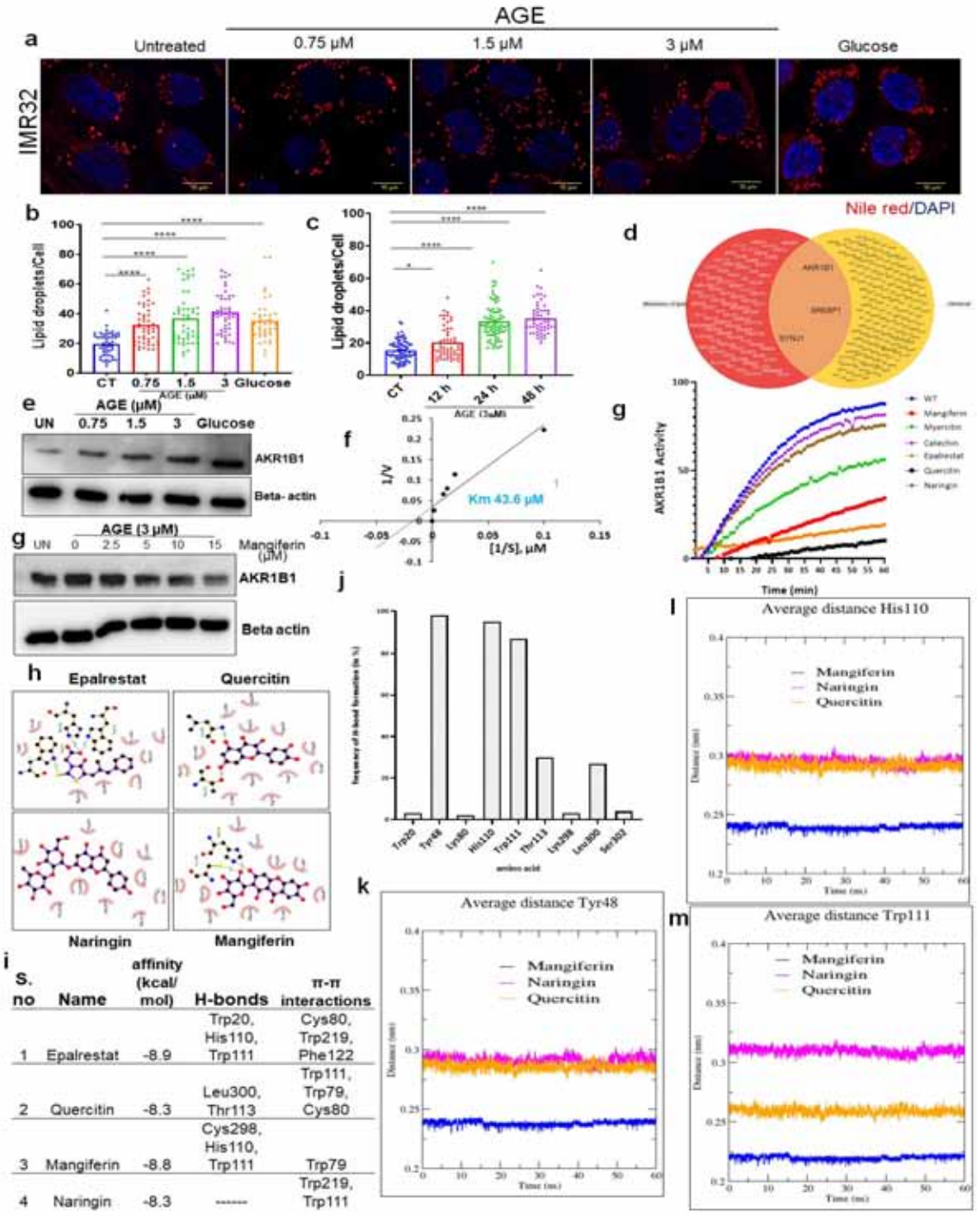
Standardization of enzymatic assay of recombinant AKR1B1: The purified recombinant wild type human AKR1B1gave band of expected size ~36 kDa on SDS-PAGE gel and the k_m value of purified protein was calculated using Lineweaver Burk plot. The calculated k_m value 43.6 µM was approximate to the k_m value of AKR1B1 previously reported (**f**).

Screening of AKR1B1 inhibitors: Epalrestat is only FDA approved AKR1B1 inhibitor used to control AKR1B1 induced pathogenesis and only used in India, Japan, and China. In present study, various herbal compounds were taken based on their anti-diabetic role known in literature and screened to inhibit the AKRIBI activity. Mangiferin was inhibiting the AKR1B1 activity close to its known inhibitors Epalrestat (synthetic) and Quercitin (herbal) (**g**). Then, Different concentration of Mangiferin were used to check the inhibition of AKR1B1 activity. Mangiferin inhibits AKR1B1 activity on dose dependent manner but attains saturation beyond 10 µM. Protein level of AKR1B1 was also assessed in IMR-32 cells treated with 3 µM AGE and different concentration of Mangiferin. AKR1B1 protein level was significantly reducing with increasing the Mangiferin concentration as shown by western blots probed with AKR1B1 antibody (**g**).

Molecular docking studies: The molecular docking between AKR1B1 and different ligands was performed to evaluate its interaction with various compounds taken for inhibitor screening (**h**). Data obtained in molecular docking was aligning with *in vitro* screening of AKR1B1 inhibitors. Mangiferin, Quercitin and Epalrestat showed AKR1B1 inhibition in *in vitro* assays makes either hydrogen bonding or pi-pi interactions with amino acids present in the active site of AKR1B1 (**i**). Naringin that fails to inhibit AKR1B1 enzymatic activity in *in vitro* assays did not make any significant hydrogen bond with amino acids present in AKR1B1 active site. The molecular docking studies suggest that Mangiferin and known inhibitors (Epalrestat and Quercitin) makes hydrogen bond with the active site residues to replace the AKR1B1 substrate whereas Naringin does not make any hydrogen bond with active site residues might be the reason for its failure in inhibiting the AKR1B1 activity. So, Naringin was taken as negative control and Quercitin as positive control for further *in silico* analysis.

Molecular dynamic (MD) simulation studies: Hydrogen bond formation between AKR1B1 and co-crystalized inhibitors/substrate was analysed in around 100 AKR1B1 structures submitted to RCBS-PDB using Ligplot+. Three amino acids (Trp48, His110, Trp111) emerged as most frequently involved in formation of hydrogen bond between AKR1B1 and co-crystalized molecule (**j**). It is previously reported that AKR1B1 binds with its substrate by making bonds through these three amino acids only, thus making them crucial for AKR1B1 activity and potential inhibitor should compete for these amino acids to competitively replace AKR1B1 substrate resulting in inhibition of AKR1B1 activity. MD simulation data was analysed to calculate the distance between these three amino acids of AKR1B1 and different herbal compounds. The average distance between amino acid and ligand residue should be less than 0.3 nm to make effective bond between them. Mangiferin residues were present less than 0.25 nm apart from these three crucial amino acids, whereas Quercitin residues were around 0.3 nm apart from Trp48, His110 and 0.26 nm from Trp111. Naringin residues

were around 0.3 nm apart from Trp48, His110 and more than 0.3 nm from Trp111 (**k-m**). Again, MD simulation studies showed that Mangiferin has high chances of making efficient bond with these three crucial amino acids.



S. no	Name	affinity (kcal/ mol)	H-bonds	π-π interactions
1	Epalrestat	-8.9	Trp20, His110, Trp111	Cys80, Trp219, Phe122
2	Quercitin	-8.3	Leu300, Thr113	Trp111, Trp79, Cys80
3	Mangiferin	-8.8	Cys298, His110, Trp111	Trp79
4	Naringin	-8.3	------	Trp219, Trp111

Figure 1: AGE treatment disturbs lipid homeostasis in neuronal cells: (a) Nile red staining of IMR32 treated with different concentrations of AGE; (b-c) dose and time dependent quantification of Nile red staining. **Screening of inhibitors of AKR1B1:** (d) AKR1B1 is overlapping gene between genes involved in metabolism of lipid in Diabetic Neuropathy patients and disease targets for Diabetes neuropathy; (e) AGE induces the protein level of AKR1B1; (f) Purification of recombinant AKR1B1 from *E. coli* BL21; (g) in-vitro Screening of AKR1B1 inhibitors; (h) Mangiferin also inhibit the protein expression of AKR1B1 in human neuronal cell line. **Screening of inhibitors of AKR1B1: (**h) ligplot+ analysis to study the hydrophobic interaction between AKR1B1 and its inhibitors; (i) amino acids of AKR1B1 involved in Hydrogen and pi-pi interaction with inhibitors; (j) analysis of PBD database to analyze the frequently involved AKR1B1 amino acids in hydrogen bond formation with ligand; (k-m) Molecular Dynamic simulation studies to calculate the distance between frequently involved AKR1B1 amino acid and inhibitors.

Publications:

Saurav S and Manna SK* (2022). Increased expression of Profilin potentiates chemotherapeutic agent-mediated tumour regression. ***British Journal of Cancer*** 126: 1410-1420. (DOI: 10.1038/s41416-021-01683-5).

Saurav S and Manna SK* (2022). Profilin upregulation induces autophagy through stabilization of AMP-activated protein kinase. ***FEBS Letters*** 596: 1765-1777. **(DOI:** 10.1002/1873-3468.14372).

Jana A, Aher A, Brandão P, Bera P, Sharda S, Phadikar U, Manna SK, Mahapatra AK and Bera P* (2022). Evaluation of anticancer activities varying ligand's substituents in Co(II/III)-picolyl phenolate derivatives: Synthesis, characterization, DFT, DNA cleavage and molecular docking studies. ***Dalton Transactions*** 51(6): 2346-2363. (DOI: 10.1039/D1DT02825A).

Bera P, Aher A, Brandao P, Debnath U, Dewaker V, Manna SK*, Jana A, Pramanik C, Mandal B and Bera P* (2022). Instigating the In Vitro Anticancer Activity of New Pyridine–Thiazole-Based Co(III), Mn(II), and Ni(II) Complexes: Synthesis, Structure, DFT, Docking, and MD Simulation Studies. ***Journal of Chemical Information and Modeling*** 62(6): 1437-1457. (DOI: 10.1021/acs.jcim.1c01280).

Jana A, Aher A, Brandao P, Sharda S, Bera P, Phadikar U, Manna SK, Mahapatra AP and Bera P* (2022). Dissociation of a tripodal pyridyl-pyrazole ligand and assortment of metal complex: Synthesis, structure, DFT, thermal stability, cytotoxicity, DNA cleavage, and molecular docking studies. ***Journal of Molecular Structure*** 1256 (2022): 132479. (DOI:10.1016/j.molstruc.2022.132479).

Tushar S. Basu Baul, Maheswara Rao Addepalli, Bietlaichhai Hlychho, Antonin Lyčka, Praseeda Vamadevan, Shashank Saurav, Sunil K. Manna, M. Fátima C. Guedes da Silva. (2022). O,N,S-tris-chelating ligand scaffolds flanked with cyclohexyl or adamantyl substituents anchored with diorganotin(IV) moieties: synthesis, structures and cytotoxicity. ***Inorganica Chimica Acta*** 537: 120935. (DOI: 10.1016/j.ica.2022.120935).

Group of Laboratory of Immunology

Laboratory of Infectious Diseases

Understanding the biology of human pathogens *Entamoeba histolytica* and *Naegleria fowleri*

Principal Investigator: **Kuldeep Verma**
Staff Scientist

Ph.D Students

Amisha Sharma	Junior Research Fellow (since 20.02 2023)
Meena Khatri	Junior Research Fellow (since 24.02.2023)

Project Students

Bhagyashree Chordiya	Junior Research Fellow
P Navyaka	Junior Research Fellow

The pathogenic amoeba, *Entamoeba histolytica* and *Naegleria fowleri*, are a class of human pathogens that cause life threatening infections, amoebiasis and primary amoebic meningoencephalitis, respectively. The objective of our lab is to understand how host cues modulate the invasive nature of pathogenic amoebas and how it contributes to tissue destruction in a complex host environment.

Research Summary

Project 1: Understanding the functional role of vacuolar ATPase in trogocytosis and tissue invasion mediated by *E. histolytica*

Trogocytosis is often called partial phagocytosis, an evolutionary conserved process from amoeba species to higher eukaryotic cells. Trogocytosis is a cellular process in which the target cell physically captures and engulfs a piece of cellular material from donor cells. *E. histolytica*, an enteric protozoan parasite that causes amoebic colitis and liver abscesses, uses trogocytosis to invade and hijack the immune system to spread infections in different organs. During this year, we have established that *Eh*V-ATPase subunits are directly involved in the early stages of trogocytosis and phagocytosis. Interestingly, *Eh*V-ATPase subunits distinctly localize to host cells upon the nibbling of hepatocyte cells, compared to phagocytosis. Our preliminary results suggest that the V-ATPase subunits fine-tune their localization upon sensing the extracellular microenvironment. Currently, we are trying to identify how the physical property of the host cell regulates amoebic V-ATPase mediated trogocytosis in *E. histolytica*.

Project 2: Understanding the spatiotemporal dynamics and ultrastructure details of ECM degrading device “amoebic invadosomes” and their crosstalk with Rab GTPases and cell surface proteases trafficking machinery in *E. histolytica*

Invadopodia are F-actin-rich, concentrated foci important for tissue invasion through the secretion of proteases. *E. histolytica* trophozoites also displayed an invertasome-like structure (actin dot) upon contact with extracellular matrix (ECM) proteins. We have identified that EhRab35 localizes to an invadosome-like structure and leads to the biogenesis of actin dots in an ECM-independent manner. Our proteomics-based study identified that amoebic *Eh*Rab35 interacts with atypical *Eh*RasGEF and localizes to actin dots. Similarly, it has been observed that ectopic *Eh*RasGEF expression upregulates the biogenesis of actin dots in the absence of ECM cues. Currently, we are trying to investigate how the Ras and Rab mediated signalling cascade regulates the biogenesis of amoebic invadosomes and coordinates the trafficking of proteases in tissue invasion.

Group of Laboratory of Infectious Diseases

Molecular Cell Biology

Signal transduction pathways in macrophages and host-pathogen interaction in tuberculosis

Principal Investigator: **Sangita Mukhopadhyay**
Staff Scientist

Ph.D Students

Manoj Kumar	Senior Research Fellow
Priyanka Dahiya	Senior Research Fellow
S. Brahmaji Senior	Research Fellow
G. Akshay Senior	Research Fellow
Pooja Kushwaha	Senior Research Fellow
Shahid Aziz	Senior Research Fellow
Sajal Dey Junior	Research Fellow
Ruhi Gupta	Junior Research Fellow
Rituparna Chatterjee	Junior Research Fellow (since 14th July 2022)

Other Members

Niteen Pathak	Senior Technical Officer
Sivapriya Pavuluri	Research Associate
KM Rohini	Research Associate
Ravi Pal	Senior Research Fellow (upto 31st May 2022)
Rahila Qureshi	ICMR Research Associate
Katherin Steffy	DBT RA (since13th January 2023)

Collaborators

Prof. K N Balaji	IISc, Bangalore
Dr. Sudip Ghosh	NIN, Hyderabad
Dr. Vinay K. Nandicoori	CCMB, Hyderabad
Dr. Sunil K Manna	CDFD, Hyderabad
Dr. S. Aparna	BPHRC, Hyderabad
Dr. Santosh Kumar	CCMB, Hyderabad

Objectives:

i) Signal transduction pathways in macrophages regulating its innate-effector functions and how various candidate proteins of *Mycobacterium tuberculosis* (*M.tb*) interfere with macrophage signaling cascades to modulate host's protective responses against the bacilli.

ii) Identification of therapeutics against tuberculosis and inflammatory diseases.

Summary of the work done until the beginning of this reporting year

In our earlier studies, we very specifically have demonstgrated that one of the PE/PPE (proline glutamic acid/proline proline glutamic acid) family proteins of *Mycobacterium tuberculosis* (Mtb), PPE2 is a secretory protein having a Nuclear localization signal and DNA binding property (***Bhat et al., [2013] Annals of the New York Academy of Sciences 1283:97; Bhat et al., [2017] Scientific Reports, 7:39706***). We showed that during infection PPE2 is secreted by the bacterium and localizes to the macrophage nucleus by exploiting the classical importin-α/β-dependent import system. Once inside the nucleus, it binds to the promoter region of *inos* (inducible nitric oxide synthase) gene to inhibit transcription from the *inos* promoter by physically masking the GATA-1-binding sites critical for transcription (***Bhat et al., [2017] Scientific Reports, 7:39706***). iNOS is responsible for production of nitric oxide (NO) which is known to be cytotoxic against the microbes. Expectedly, PPE2-null mutants caused higher production of nitric oxide in infected macrophages indicating a direct role of PPE2 in inhibiting NO production. Thus, PPE2 very strongly inhibits NO production and favors survival of the bacilli (***Bhat et al., [2017] Scientific Reports, 7:39706***). In addition to the cytotoxic effect of NO against microbes, NO is also known to play a key role in the pathogenesis of inflammation. Large amount of NO is produced at sites of inflammation through the action of *inos* present in both infiltrating leucocytes and activated, resident tissue cells. Nitric oxide and its oxidation products are known to cause tissue injury. This work highlights the role of PPE2 not only to contribute to TB pathology by directly inhibiting nitric oxide, but also the possibility of PPE2 to use as a therapeutic to inhibit NO production and thus in the treatment of inflammation/tissue injury.

In addition to NO, during infection, activated macrophages also generate reactive oxygen species (ROS) which are shown to be cytotoxic against *M. tuberculosis* and *M. tuberculosis* employs strategies

to inhibit ROS production also in addition to inhibition of NO to safely persist and multiply inside macrophages. We observed a novel mechanism by which PPE2 can directly inhibit ROS production by destabilizing NADPH-oxidase complex in the phagosome. During infection, PPE2 is secreted into the cytoplasm and binds to the $p67^{phox}$ subunit of NADPH-oxidase complex via its SRC Homology 3 (SH3) domain. The PPE2-$p67^{phox}$ interaction results in inhibition of translocation of p67 molecule from cytosol to the membrane leading to reduced NADPH activity and ROS production (***Srivastava et al., [2019] Journal of Immunology, 203:1218***). This results in higher mycobacterial burden in macrophages. Thus, *M. tuberculosis* exploits PPE2 to its own advantage and this is an example of how cunning pathogens coevolve to adapt our physiology. Thus, PPE2 was shown to act as an important anti-inflammatory molecule inhibiting both NO and ROS that helps the bacilli to survive better inside the host. Since it ROS generation by polymorphonuclear neutrophils at the site of inflammation is known to cause endothelial dysfunction and tissue injury, PPE2 may be considered as an important therapeutic to be used to prevent inflammation acting as an inhibitor of both NO and ROS.

Details of progress made in the current reporting year (April 1, 2022 - March 31, 2023).

PPE2 protein of *Mycobacterium tuberculosis* inhibits Inflammation and Tissue injury

In a detail study published in *Immunobiology* (***Pal and Mukhopadhyay, [2021] Immunobiology, 226:152051***), we have shown that Mast cell population is lower in mice infected with *M. smegmatis* expressing PPE2 protein of *M. tuberculosis* (M. smeg-PPE2) as compared to mice infected with *M. smegmatis* harboring the vector control pVV16 (M. smeg-pVV16) (Figure 1). Since mast cell plays a crucial role in innate immunity and the role of mast cells is eminent in tissue inflammation, inhibition of mast cells by PPE2 is very crucial for the bacilli to persist better inside the host environment. Expectedly, a higher bacterial load of M. smeg-PPE2 was observed in lung, liver and spleen tissues compared to M. smeg-pVV16 (Figure 1). Thus, PPE2 acts as a crucial anti-inflammatory molecule inhibiting mast cells, NO and ROS which eventually helps in the better persistence of the bacterium in the host (***Pal et al. [2021] Journal of Immunology, 207:2393***). Though these properties of PPE2 (inhibition of NO, ROS and mast cells) are helpful for the *M. tuberculosis* to create a favorable niche for the bacilli to survive and multiply inside the host, the same properties of PPE2 can be exploited to use PPE2 protein or a synthetic peptide derived from PPE2 as a therapeutic to treat inflammatory disorders like acute and chronic inflammation and tissue injury.

Excessive inflammation can damage the surrounding healthy cells, tissues, and organs. The conventional anti-inflammatory drugs available on the market are often associated with adverse side effects when used for a long time. Therefore, there is a need to develop anti-inflammatory drugs/molecules with better efficacy and least side effects. Biological anti-inflammatory molecule is in demand as it is effective and with lesser side effects. Keeping this in mind, we, examined whether recombinantly purified PPE2 (rPPE2) and a PPE2-derived synthetic peptide can be used as a biologic therapeutic to treat chemical (formalin) induced tissue injury and inflammation.

We have demonstrated that rPPE2 indeed can be used as a potent anti-inflammatory drug to treat pathophysiological disorders associated with inflammation like acute and chronic inflammation/ tissue injury (*Pal et al [2022], EMBO Molecular Medicine, e1489*; *Filed Indian patent [2020], Patent No. 201941000876; Filed USA patent [2020], Patent no 16737012*). Interestingly, in this study, we demonstrated that mice injected intraperitoneally with a single dose of rPPE2 had significant reduction in formalin induced paw inflammation within 3 hours of formalin injection as compared to paw inflammation in mice treated with PBS alone (Figure 2). Administration of single dosage of rPPE2 also prevented inflammation and tissue damage for later time points (21 days). PPE2 showed its beneficial effect even when injected 48 hours post tissue injury. Levels of various mast-mediators and inflammatory molecules like TNF-α, IL-6, and MPO activity were found to be lower in the paw-tissue of mice treated with PPE2 when compared to the untreated mice with inflammation. PPE2 at 3 mg/kg showed a better and faster healing than the commercial anti-inflammatory drug, Diclophenac which showed its potent effect only at 10 mg/kg. Interestingly Diclophenac at 3 mg/kg was unable to heal tissue injury. Though Diclophenac at 10 mg/kg dose showed liver and kidney toxicity, PPE2 did not show any liver and kidney toxicity.

PPE2 exerts its anti-inflammatory activity by affecting Fibroblast-mast cell communication. It mainly induces its anti-inflammatory activity by suppressing the mast cell population in the injured tissue and inhibits mast cell degranulation (Figure 2). Interestingly, through PPE2 inhibited mast cells, the commercial anti-inflammatory drug Diclophenac did not show any effect on inhibition of mast cells. The levels of various mast cell mediators like β-hexosaminidase, MCP-3 and Mcpt4 were lower in injured tissue in PPE2 injected mice as compared to PBS control. Bone marrow-derived mast cells (BDMCs) transplantation

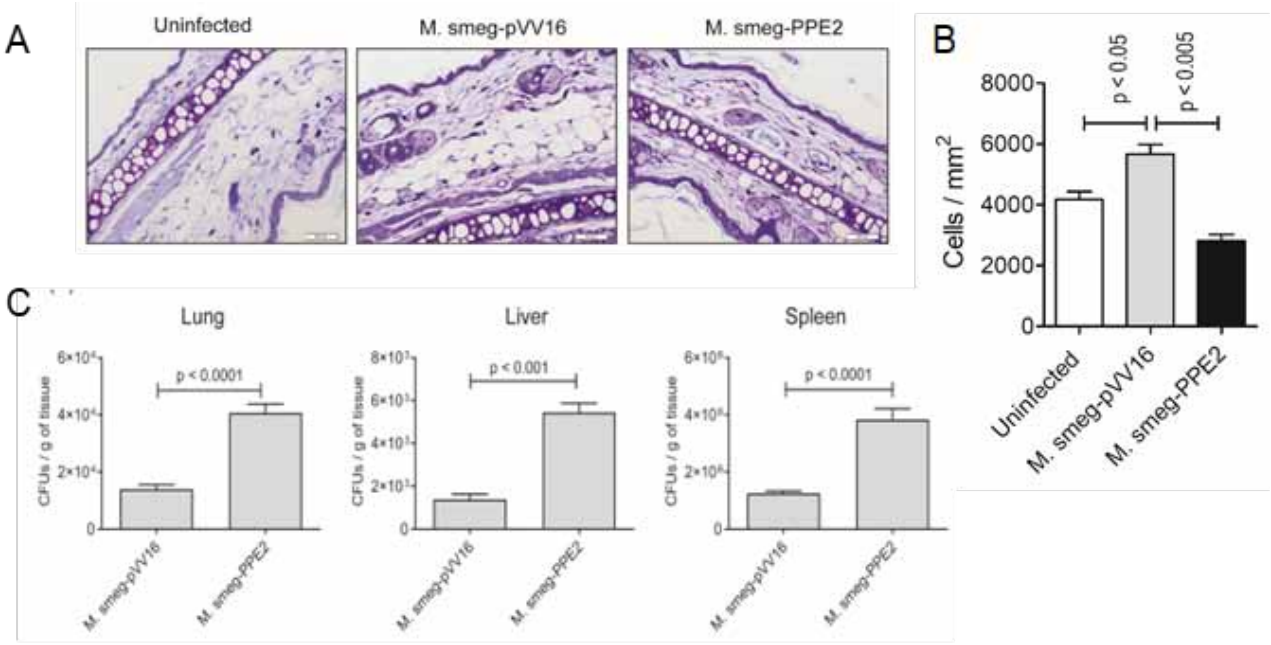


Figure 1. PPE2 protein of *Mycobacterium tuberculosis* downregulates mast cell population in mice and confers survival advantage to the bacteria in mice. About 8-10 weeks old Balb/c mice were infected with 10 × 10^7 CFUs of either M. smeg-pVV16 or M. smeg-PPE2 *via* intravenous route. Uninfected mice were kept as heathy controls. After 5 days of infection, mice were sacrificed and ear pinna was collected from each group. Sections were prepared and stained with toluidine blue. (**A**) Photographs of representative sections were visualized at 40X. (**B**) Counting of mast cells was performed in toluidine blue stained paw sections using ImageJ software and were normalized per unit area (mm^2). Data represent mean ± SEM of 5 mice per group. Unpaired *t*-test is used to calculate the p values. (**C**) After 5 days, from the sacrificed mice lung, liver and spleen tissues were harvested and homogenates were prepared for CFU determination. CFUs were counted as per gram of the tissue. Data represent mean ± SEM of 7 mice per group. Unpaired *t*-test was used to calculate the p values.

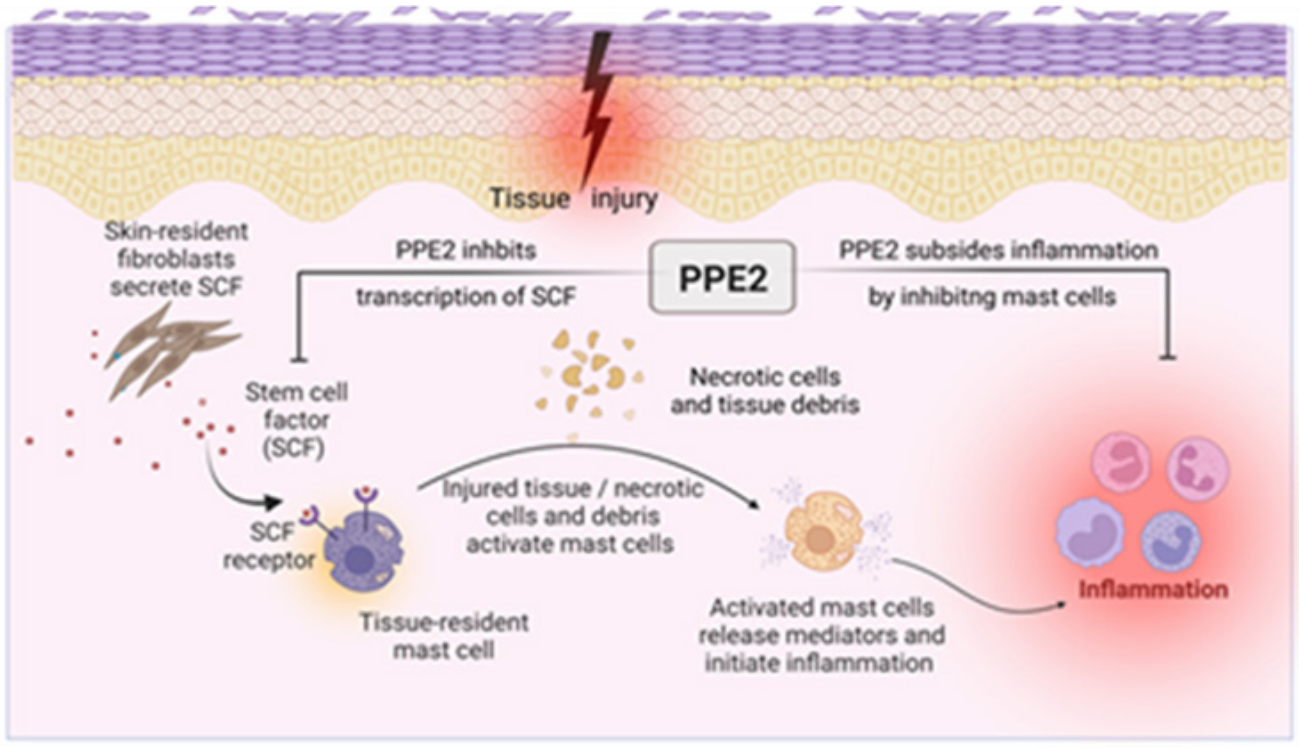


Figure 2. PPE2 reduces injury induced inflammation. PPE2 protein inhibits mast cell population in the site of injury through inhibiting SCF transcription factor from fibroblast, and thus inhibits production of mast cell-induced inflammatory mediators. This results in reduced inflammation in the site of tissue injury.

experiments clearly demonstrated that PPE2 specifically targets mast cells for its anti-inflammatory properties. The stem cell factor (SCF) is required for mast cell proliferation, maintenance, and migration at the site of injury. PPE2 was found to localize to the nucleus of fibroblasts, binds to the SCF promoter, and inhibits SCF transcription (Figure 2). Thus, PPE2 inhibits mast cells by directly inhibiting SCF transcription.

For easy synthesis, better stability and easy cellular delivery, we next designed a synthetic peptide derived from PPE2 which is a 36 amino acid long peptide. Based on the nuclear migration and DNA binding property of PPE2, the PPE2-derived peptide was designed. It was observed that the synthetic peptide derived from PPE2 showed similar anti-inflammatory property and was able to suppress formalin-induced paw inflammation, redness and swelling in mice. The peptide also suppressed SCF transcription and mast cells population in paw tissue. Mast cells play an important role in pathology caused by inflammation. Recently, FDA has approved large number of recombinant protein therapeutics to treat clinical problems including autoimmunity/inflammation, infection, cancer and genetic disorders. There are drugs available in the market that suppress mast cells activity by neutralizing one or more mast cell mediators (like anti-histamines) to lower inflammation but, at present there is no drugs available to limit mast cell population at the site of injury. Focusing on mast cell as a whole seems a better solution rather than focusing on its mediators, as the previous strategy will not only be more efficient in curbing inflammation but also be effective for a longer duration. Thus, PPE2 protein or the peptide may be an important non-steroidal biological molecule to be used successfully in the treatment of inflammation and tissue injury.

Future study: It would be interesting to further examine the therapeutic use of PPE2 peptide in the treatment of wound healing and inflammatory bowel disease that are associated with extreme inflammation.

Publications:

Research papers published in the calendar year 2020-2021

Shrivastava R, Pavuluri S, Ghosh S and Mukhopadhyay S (2023). Rab7l1 plays a role in regulating surface expression of Toll like receptors and downstream signaling in activated macrophages. ***Biochemical and Biophysical Research Communications*** 640:125-133

Shrivastava R, Pradhan G, Ghosh S and Mukhopadhyay S (2022). Rabaptin5 acts as a key regulator for Rab7l1-mediated phagosome maturation process. ***Immunology*** 165:328-340.

Pal R, Battu MB and Mukhopadhyay S (2022). Therapeutic application of PPE2 protein of Mycobacterium tuberculosis in inhibiting tissue inflammation. ***EMBO Molecular Medicine*** 14:e14891

Pal R, Bisht MK and Mukhopadhyay S (2022). Secretory proteins of Mycobacterium tuberculosis and their roles in modulation of host immune responses: Focus on therapeutic targets. ***The FEBS Journal*** 289:4146-4171.

Group of Laboratory of Molecular and Cell Biology

Laboratory of Molecular Oncology

Genomics and Molecular Genetics of Cancer

Principal Investigator: **Murali Dharan Bashyam**
Staff Scientist

Ph.D Students

Sara Anisa George	Senior Research Fellow
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Shaily Agrawal	Senior Research Fellow
Sanjana Sarkar	Senior Research Fellow
Mudodi Devaunshi Sadanand	Senior Research Fellow
Sumaiya Sabnam	Senior Research Fellow
Rinita Dutta	Junior Research Fellow
Rikita Karar	Junior Research Fellow
Bambhaniya Sandipkumar Mohanlal	Junior Research Fellow

Other Members

Ajay Kumar Chaudhary	Technical Officer-II
Asmita Gupta	Research Associate
Padmavathi Kavadipula	Project SRF (Till 31-08-2022)
Shivani Yadav	Project JRF (Till 20-06-2022)
Rupin Gangadhar Shelke	Project JRF (Till 15-01-2023)
Sumedha Avadhanula	Project JRF (Since November 2022)
Swathi Vadlamani	Project JRF (Since January 2023)
Collaborators	Swarnalata Gowrishankar Apollo Hospitals, Hyderabad
Shantveer G Uppin	NIMS Hyderabad

Objectives

Identification and characterization of important deregulated genes/pathways in cancers prevalent in India.

Research Summary:

Project title: Characterization of Gene Fusions (GFs) in early-onset sporadic rectal cancer (EOSRC).

Concise report: A network analysis of all genes participating in GF formation in EOSRC and CRC datasets revealed a major network centred around *EEF1A1*, *CEACAM6*, *WWOX*, *PDIA3*, *KRT8*, and *PIGR* (Figure 1A, B). Of the top 10 genes with highest network degree, *WWOX*, *ACTB* and *KRT8* were found to encompass or be located near known chromosomal fragile sites (CFSs). Subsequent analysis revealed several recurrent GFs whose breakpoints overlapped with known CFS loci (Figure 1C). Further, we extended the correlation analysis between genome architecture and GFs to three additional cancer types *viz.* Breast (BRCA), Prostate (PRAD), and Pancreatic (PAAD) adenocarcinomas. Hi-C data generated earlier for representative cell lines namely MCF-7 (BRCA), PC3 (PRAD), and Panc-1 (PAAD) were analyzed to estimate open ('A') and closed ('B') chromatin compartments. A majority of GFs arose from open chromatin regions in these three cancer types validating our previous results from EOSRC and TCGA-CRC. More importantly, a statistically significant elevation in the expression of 3'- partner genes participating in 'A-B' GFs (compared to when not participating in GFs) was observed in these three cancer types validating our previous observations in CRC (Figure 1D). Further, the expression levels of selected 3'- partner genes belonging to 'A-B' GFs in EOSRC were confirmed by quantitative PCR analysis (Figure 1E).

Future plans and directions

Functional studies on novel EOSRC gene fusions.

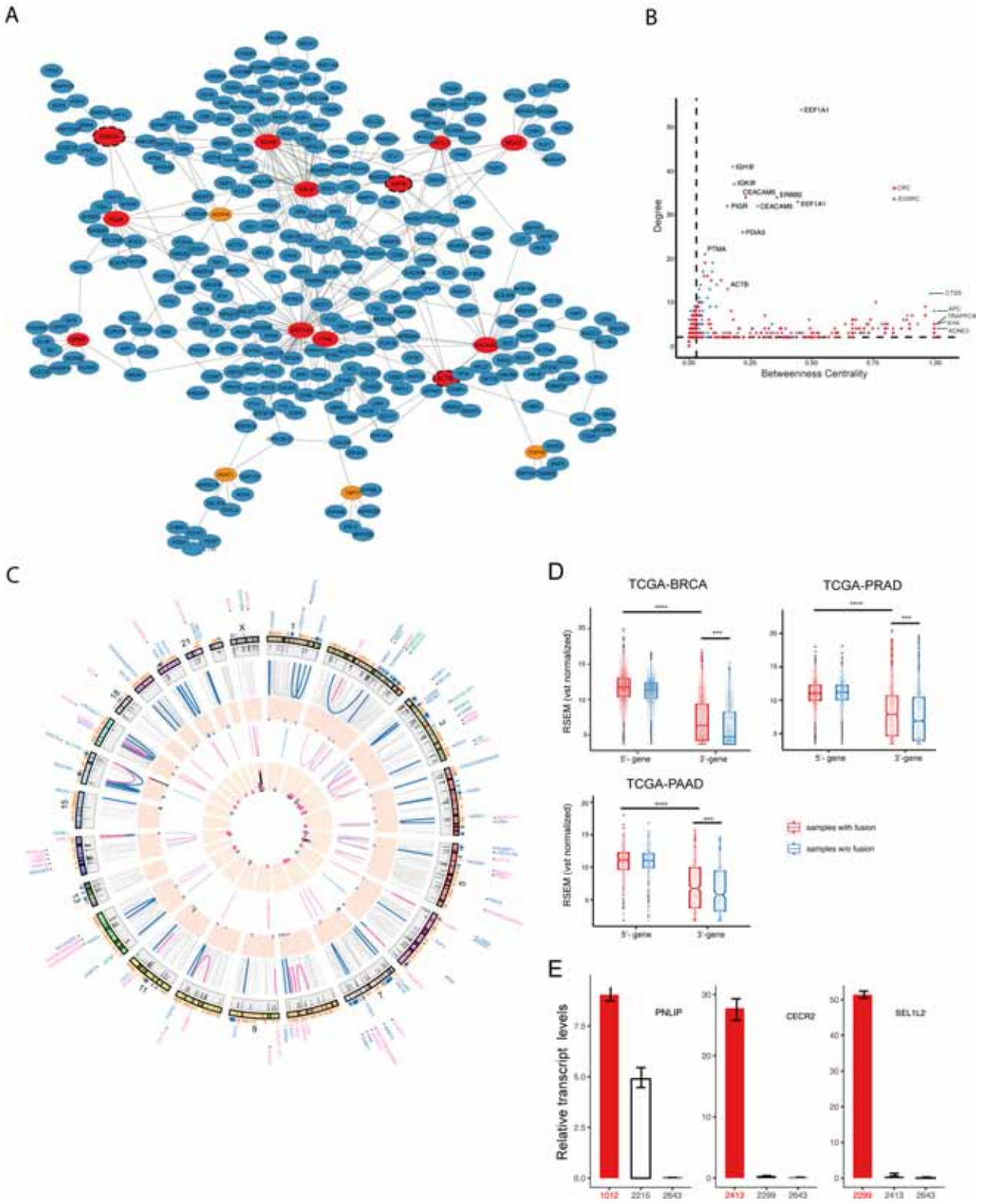


Figure 1. Panel A, EOSRC GF network comprising of major nodes highlighted in red (nodes with degree > 10) and orange (nodes with degree >=5). Panel B, EOSRC network features. Panel C, distribution of 5'-(blue links) and 3'- (pink links) fusion gene breakpoints overlapping with CFS markers. From outermost to innermost circle - ChIP-seq track showing FANCD2 binding sites, followed by chromosome ideograms, MiDas-Seq loci tracks, intra-chromosomal links showing overlap with FANCD2 binding, sample wide recurrence of the fusion involving the corresponding gene, intra-chromosomal links overlapping with MiDAS-Seq regions, and finally sample wide recurrence of the fusion involving the corresponding gene. Panel D, genes from 'B/closed' chromatin compartment exhibit elevated expression when participating as 3' partner in A-B fusions as determined from TCGA data for Breast (BRCA), Prostate (PRAD) and Pancreatic (PAAD) cancers. Panel E, RT-qPCR based validation of elevation of transcript level of 3' partner in A-B gene fusions compared to its level in samples where it is not participating in gene fusion formation; sample exhibiting fusion is shown in red colour.

Publications

Research papers published in 2022

SA Kemp, MTK Cheng, WL Hamilton, K Kamelian, INSACOG, S Singh, P Rakshit, A Agrawarl, CJR Illingworth, RK Gupta. Transmission of B.1.617.2 Delta variant between vaccinated healthcare workers. **Sci Rep**, 2022; 12:10492. doi: 10.1038/s41598-022-14411-7.

P Bala, P Kavadipula, S Sarkar, **M D Bashyam**. To β or not to β: Lack of correlation between APC mutation and β-catenin nuclear localization in colorectal cancer. **J Gastrointestinal Cancer**, 2022 Dec 31. doi: 10.1007/s12029-022-00886-0.

Research papers published in 2023

Asmita Gupta; Reelina Basu; **Murali Dharan Bashyam**. Assessing the evolution of SARS-CoV-2 lineages and the dynamic associations between nucleotide variations. **Access Microbiology**, 2023 Feb 23. https://doi.org/10.1099/acmi.0.000513.v2.

SA George, V Kotapalli, P Ramaswamy, R Kumar, S Gowrishankar, SG Uppin, **MD Bashyam.** Identification of novel oncogenic transcriptional targets of mutant p53 in Esophageal Squamous Cell Carcinoma. 2023 March 12. **BioRxiv** preprint doi: https://doi.org/10.1101/2023.03.12.532255.

Group of Laboratory of Molecular Oncology

Laboratory of Neuroscience and Cell Biology

Understanding the generation of cellular diversity in developing Central Nervous System of *Drosophila melanogaster*

Principal Investigator: **Rohit Joshi**
Staff Scientist-V

Ph D Students:

Yamini Rawal	Senior Research Fellow
Punam Bala	Senior Research Fellow
Jiban Barman	Senior Research Fellow
Savita	Senior Research Fellow
Vandana Chaurasia	Junior Research Fellow

Other Members:

Chandra Shekhar Singh	Technical Officer
Vishakha Kurlawala	Project Associate
Ravalika Silveri	Project Associate
Prakeerthi Abburi	Project Associate
Kabila Nagaraju	Project Associate

Collaborators:

Anuradha Ratnaparkhi	ARI Pune
Deepti Jain	RCB, Faridabad
Ashwin Dalal	CDFD Hyderabad

Bilaterian organisms (insects, vertebrates, and mammals-humans) require a complex Central Nervous System (CNS) to execute sophisticated functional behaviours necessary for their propagation and survival. The generation of region-specific cellular diversity during development is cardinal for assembling a functional CNS.

Objective

The key objective of our lab is to understand how region-specific cellular diversity is generated in developing CNS. The primary cells in CNS are Neural Stem Cells (NSCs), Intermediate progenitor cells (INPs), Neurons and Glia. The NSCs regenerate by asymmetric cell division to give rise to another NSC and a smaller intermediate progenitor cell. Latter, than symmetrically divide to give rise to a pair of differentiated neurons or glia. Precise coordination of the proliferation, differentiation, and apoptosis of NSCs is critical for normal neurogenesis and functional brain development. Misregulation of any of these processes results in developmental disorders and malignancies.

We use *Drosophila melanogaster* as our model organism, whose CNS resembles its vertebrate counterpart in its constitution and organisation. We specifically focus on *Drosophila* Neural Stem Cells (Neuroblast-**NBs**) to understand the molecular mechanisms underlying the generation of region-specific cellular diversity in developing CNS. To this end, the specific aims of our lab are as follows:

1. Understanding the molecular basis of Hox-dependent patterning in Drosophila CNS.
2. Investigating the role of Grainyhead in Neural Stem Cell proliferation in Drosophila.
3. Investigating the role of Drosophila AIMP2 in CNS development.

Understanding the molecular basis of Hox-dependent patterning in *Drosophila* CNS.

A highly conserved family of homeodomain-containing transcription factors (**TFs**) called Hox genes express segmentally along with the head-to-tail axis of CNS and play a critical role in generating region-specific cellular diversity during development. Hox genes do this by controlling the proliferation, differentiation, and apoptosis of NSCs. Hox factors are known to execute many of these functions with the help of their well-characterised TALE-HD containing cofactors Pbx/Exd and Meis/Hth. However, the absence of these factors from specific cells emphasises the need to identify and validate new Hox cofactors. In *Drosophila,* there are 8 Hox genes which sequentially express along the head-to-tail axis of the developing body plan (including CNS).

The primary goal of this part of the work has been to understand the molecular basis of the Hox-dependent NB apoptosis as a mode of regulation of cell numbers in developing CNS. Even though Hox-dependent NB apoptosis happens in 5 different regions of developing *Drosophila* CNS, its effect is most apparent in abdominal and terminal segments of the CNS (expressing Hox factors Abdominal-A and

Abdominal-B), where the majority of the NBs undergo apoptosis by mid-larval stages, thereby resulting in fewer neurons in these regions. While NBs in other regions are still generating neurons necessary for adult life.

Using this cellular context, we have shown that two non-TALE-HD TFs, Grainyhead (**Grh**-a bHLH TF) and Doublesex (**Dsx**-a DM-domain TF), can function as Hox cofactors in executing NB apoptosis. More specifically, we had shown that Grh could function as a cofactor for abdominal and terminal Hox genes Abdominal-A (Khandelwal et al., 2017) and Abdominal-B (Bakshi et al., 2020). We have also shown that in a specialised population of NBs in terminal segments, Abdominal-B uses DsxF (a female-specific isoform of Dsx) for executing NB apoptosis, specifically in females. At the same time, these NB in male CNS continues dividing, forming male-specific serotonergic neurons crucial for male mating behaviour (Ghosh et al. 2019).

Details of progress made in the current reporting year (1 April 2022-31 March 2023)

Our most recent work expanded on the idea that Grh could function as a Hox cofactor not just for Abdominal-A and Abdominal-B but for all other Hox factors. To this end, we first elucidated the mechanistic details of the physical interaction of Abdominal-A with Exd and Grh to execute NB apoptosis. We find that AbdA and Grh interact through their highly conserved DNA binding domains, and the DNA binding specificity of AbdA-HD is vital for its interaction with Grh and essential for executing NB apoptosis. Subsequently, we showed in vitro that Grh can physically interact with all the Hox proteins. This is supported by our in vivo results, showing that Hox-dependent NB apoptosis in the remaining three regions of CNS also requires Grh. These observations established that all the five regions of developing CNS rely on Grh for Hox-dependent apoptosis, establishing that Grh can function as a general Hox cofactor during development (Sipani and Joshi, Genetics (*2022*)).

Future Plans: We are working to understand the molecular basis of the continued proliferation of Dsx expressing NBs in male CNS and how these cells generate neurons responsible for male mating behavior.

Investigating the role of Grainyhead in Neural Stem Cell proliferation in *Drosophila*.

Grh is a helix-loop-helix pioneer transcription factor that has been researched in Drosophila and vertebrates owing to its wide-ranging role in epithelial cell differentiation, wound healing, barrier formation and tumorigenesis. Studies in humans indicate that Grh orthologs play a role in adult-onset of autosomal dominant deafness, cleft palate, cancer and congenital neural tube defect spina bifida. In *Drosophila* CNS, Grainyhead is expressed in larval NBs, intermediate progenitors but not in their neuronal progeny. While the contribution of Grh to NB apoptosis is well characterised, it is not clear how Grh molecularly contributes to cell proliferation and subsequent cellular diversity generation in CNS. Considering its importance across the species in both neural and epithelial tissues, it is crucial to understand Grh regulation and the mechanistic basis of its cellular functions. To this end, we are trying to understand the transcriptional regulation and mechanisms that keep *grh* “on” in the NBs and “off” in its neuronal progeny. The goal is to identify CNS-specific enhancers (or cis-regulatory elements CRE), establish their importance for in vivo gene expression, and study their transcriptional and epigenetic regulations. We are also working to identify Grh targets which help it to execute its other cellular functions like cell proliferation and generation of cellular diversity.

We have identified eight genomic regions of *grh* based on sequence conservation across multiple *Drosophila* species and made *reporter-lacZ* lines for these regions. We find that these enhancers express in larval NBs and thus may regulate *grh* expression. Three of these enhancers (*grhF1, grh*-2F3 and *grh-1up*) which show strong expression in larval NBs are being analysed further by generating deletions using CRISPR-Cas9. We find that two of these single deletions (*grhF1* and *grh*-2F3) are homozygous viable and had no impact on the expression of Grh protein in NBs, suggesting that *grh* expression in NBs relies on multiple enhancers during development.

Details of progress made in the current reporting year (1 April 2022-31 March 2023)

More recently we have generated a double deletion for *grh-2F3* and *grhF1*. We find that unlike the single deletions, the double deletion is homozygous lethal and dies in the embryonic stages. Detailed analysis of the double deletion is ongoing. Work is ongoing to generate the double deletion of *grhF1-grh-1up* enhancers. Thus far, our results indicate that multiple CREs regulate Grh during larval CNS development, and we have narrowed two CREs vital for *grh* expression and larval survival.

To understand the role of Grh in NB proliferation, we have used the Targeted DamID technique to identify tissue-specific direct targets of Grh in NBs. Targeted DamID relies on the tissue-specific expression of very low levels of a fusion protein of bacterial Dam enzyme with Grh. This fusion protein will bind to the cognate genomic binding sites of the TF and Dam fusion will methylate “A” in the nearest GATC sequence, which

is monitored by high throughput sequencing. Using this method, we find that multiple cycle regulators are direct targets of Grh in NB.

Future plan: Double (*grhF1-grh-1up and grh-2F3-grh-1up)* deletion combination for *grh* enhancers will be attempted to understand the tissue specificity of the enhancers.

Grh targets which regulate cell cycle are being analysed.

Investigating the role of AIMP2 in Drosophila CNS development.

Microcephaly is the condition wherein the newborn's head is much smaller than the expected average size at birth. It has a developmental origin, and depending on the severity of the condition, it may lead to seizures, developmental delay, intellectual disability, and problems with movement, balance, hearing, and vision. Various genes have been implicated in causing

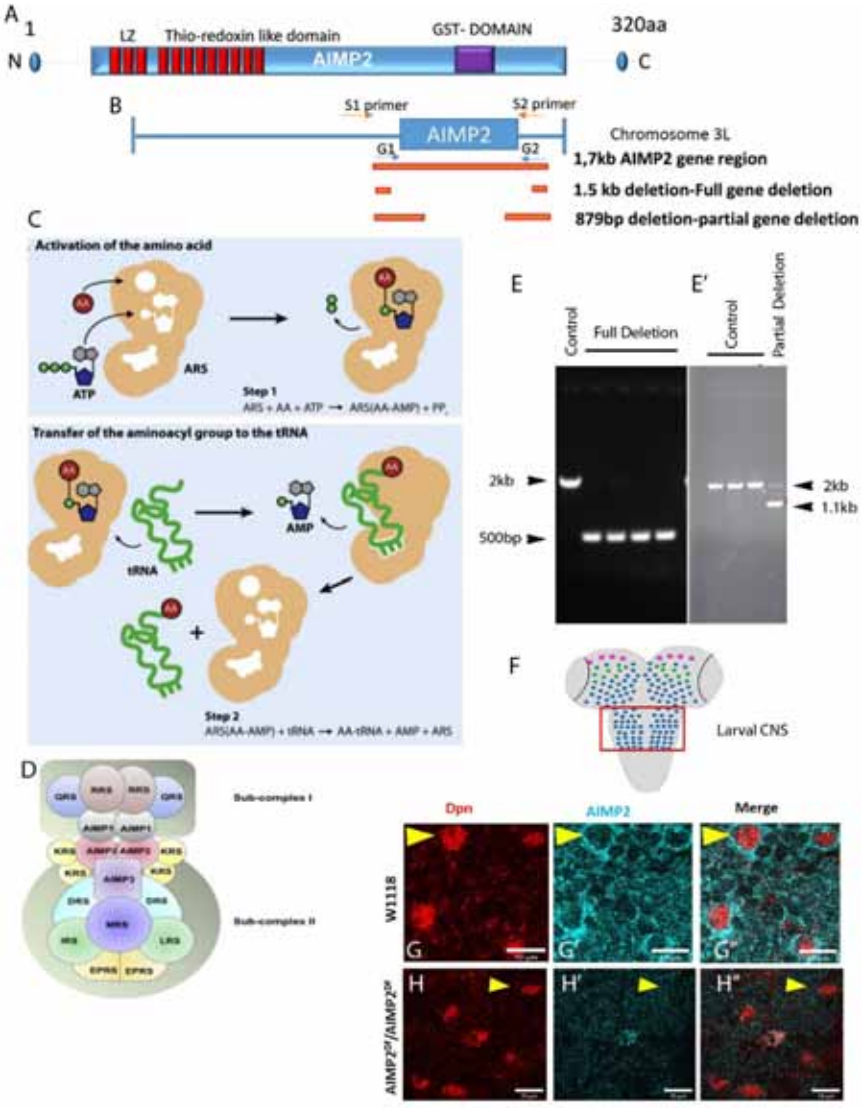


Fig-1: AIMP2 expresses in Neural Stem Cells of *Drosophila* larval CNS. (A) Structural organisation of Drosophila AIMP2. (B) CRISPR-Cas9 Strategy employed for deletion of *Drosophila* AIMP2 gene. G1 and G2 show the approximate position of two sets of gRNA used for generating deletion. S1 and S2 are the primers used for screening the deletion in Drosophila. (C) Mode of the function of AIMP2 gene, MSC ligate their cognate amino acids to tRNAs for protein synthesis. (D) Schematic representation of the organisation of multi-synthetase complexes (MSC), which is composed of 9 Amino acyl t-RNA synthetases (QRS, RRS, KRS, DRS, IRS, EPRS, MRS, EPRS, LRS) and 3 scaffolding proteins (AIMP1, AIMP2 and AIMP3). (E and E') PCR was done with primers S1 and S2 to confirm the AIMP2 deletion with genomic DNA isolated from the control and AIMP2 deletion. Complete deletion has an amplicon of 500bp, and partial deletion has a 1.1kb amplicon. (F) Schematic of larval CNS. (G and H) AIMP2 expresses in NSC cytoplasm. Expression of AIMP2 protein in the cytoplasm of thoracic Neural Stem Cells of Drosophila larvae in control (G) and homozygous deletion larvae for *AIMP2* gene (H) is shown. Yellow arrowhead indicates NSC marked by Deadpan staining (Dpn). Bar is 10 microns. Fig.1C+1D are taken from Rajendran et. al. 2018.

microcephaly. However, the molecular basis of these gene functions has not always been characterised in detail. AIMP2 is one such gene for which the first human disease-associated mutation was reported in 2017 by Dr. Ashwin Dalal's group. The homozygous null mutant of AIMP2 results in premature termination of the protein. The mutation resulted in atrophy in the cerebral cortex, spinal cord, and cerebellum, leading to severe neurodevelopmental disorder with microcephaly, seizures, and spasticity.

Aminoacyl-tRNA synthetases (ARSs) are traditionally known as housekeeping enzymes responsible for protein synthesis and cellular homeostasis. ARS and ARS interacting multi-functional proteins (AIMP) form a multi-tRNA synthetase complex (MSC). There are 3 AIMPs known as AIMP1/p43, P2/p38, and P3/p18 categorized based on molecular weights. AIMPs are known for their non-enzymatic scaffolding function.

AIMP2 and other members of MSC are conserved in *Drosophila,* and ARSs have been shown to be essential mediators of Myc regulated growth control in *Drosophila* wing development. However, the role of AIMP2 in neural development has not been characterised. This part of the work aims to understand the molecular mechanism behind AIMP2-mediated microcephaly and spasticity.

Details of progress made in the current reporting year (1 April 2022-31 March 2023)

We have generated partial and full deletion for *Drosophila* AIMP2 gene (CG12304) using CRISPR-Cas9 (Fig-1E and E'). An antibody generated against *Drosophila* AIMP2 show that protein is expressed in the cytoplasm of *Drosophila* NBs and neurons (Fig-1G). This specificity of the staining is underlined by its absence from NBs and neurons of the larvae homozygous for *AIMP2* deletion (Fig-1H). The larvae homozygous for *AIMP2* deletion show no apparent developmental delay or lethality and go through pupal stages to eclose as adult insects. This is not unexpected considering the microcephaly phenotype seen in adults in humans with AIMP2 mutation. The detailed phenotypic and molecular analysis of the homozygous deletion of *AIMP2* is ongoing.

Future Plan: We are working to check the size of larval brains in wildtype and larvae homozygous for *AIMP2* deletion. We also intend to check adults' homozygous for *AIMP2* deletion for behavioural phenotypes like fertility, locomotion and flight.

Publication

Rashmi Sipani and Rohit Joshi. "Hox genes collaborate with helix-loop-helix factor Grainyhead to promote neuroblast apoptosis along the anterior-posterior axis of the Drosophila larval central nervous system." Genetics 2022 Aug 30; 222(1): iyac101.

https://pubmed.ncbi.nlm.nih.gov/35792854/

Group of Laboratory of Neuroscience and Cell Biology

Laboratory of Plant Microbe Interaction

Understanding virulence mechanisms of *Xanthomonas* plant pathogens and interaction with host plants

Principal Investigator: **Dr. Subhadeep Chatterjee**
Staff Scientist

Ph.D Students:

Yasobanta Padhi	Senior Research Fellow
Chayan Bhattacharjee	Senior Research Fellow
Kanishk Saraf	Senior Research Fellow
Arkaprabha China	Junior Research Fellow
Sudiksha	Junior Research Fellow
Kurma Devakrishna	Junior Research Fellow
Ayesha Faraz	Junior Research Fellow

Project

Dr K B Durga Bhavani	Project Investigator - DST/WOS -A
Parimala Gundu	Junior Research Fellow
Dr Parnoshree Dey	Research Associate I

Other Members

Binod Bihari Pradhan	Technical officer
Krishnamurty	Tradesman
K V Rao	Gardener (on Contract)

Objectives

1. Identification and characterization of virulence factors of Xanthomonas
2. Role of cell-cell communication in Xanthomonas colonization and virulence
3. Function of protein secretion system in Xanthomonas and its role in virulence
4. Role of PAMP in pathogen recognition and plant defense response

Summary of work done until the beginning of this reporting year (up to March 31, 2022)

The diffusible signal factor synthase, RpfF, in *Xanthomonas oryzae* pv. *oryzae* is required for the maintenance of membrane integrity and virulence. The *Xanthomonas* group of phytopathogens communicate with a fatty acid-like cell–cell signalling molecule, *cis*-11-2-methyl-dodecenoic acid, also known as diffusible signal factor (DSF). In the pathogen of rice, *Xanthomonas oryzae* pv. *oryzae*, DSF is involved in the regulation of several virulence-associated functions, including production and secretion of several cell wall hydrolysing type II secretion effectors. To understand the role of DSF in the secretion of type II effectors, we characterized DSF synthase-deficient (*rpfF*) and DSF-deficient, type II secretion (*xpsE*) double mutants. Mutant analysis by expression analysis, secretion assay, fatty acid analysis, and physiological studies indicated that *rpfF* mutants exhibit hypersecretion of several type II effectors due to a perturbed membrane and DSF is required for maintaining membrane integrity. The *rpfF* mutants exhibited significantly higher uptake of 1-*N*- phenylnapthylamine and ethidium bromide, and up-regulation of *rpoE* (σE). Increasing the osmolarity of the medium could rescue the hypersecretion phenotype of the *rpfF* mutant. The *rpfF* mutant exhibited highly reduced virulence. We report for the first time that in *X*. *oryzae* pv. *oryzae* RpfF is involved in the maintenance of membrane integrity by playing a regulatory role in the fatty acid synthesis pathway. using QS-responsive whole-cell bioreporters of *Xcc,* we present a detailed chronology of QS-facilitated *Xcc* colonization in the mesophyll region of cabbage (*Brassica oleracea*) leaves. We report that QS-enabled localization of *Xcc* to parenchymal chloroplasts triggers leaf chlorosis and promotion of systemic infection. Our results indicated that the QS response in the *Xanthomonas* group of vascular phytopathogens maximizes their population fitness across host tissues to trigger stage-specific host chlorophagy and establish a systemic infection.

Details of progress made in the current reporting year (April 1, 2022 - March 31, 2023)

Project 1: Understanding the Mechanisms of Xanthomonas virulence

By screening a transposon induced mutant library of *Xanthomonas oryzae* pv. *oryzae*, the bacterial blight pathogen of rice, we have identified a novel 5.241 kb open reading frame (ORF) named *xadM* that is required for optimum virulence and colonization. This ORF encodes a protein, XadM, of 1746 amino-

acids that exhibits significant similarity to Rhs family proteins. The XadM protein contains several repeat domains similar to a Wall-Associated Surface Protein (WASP) of *Bacillus subtilis*, which has been proposed to be involved in carbohydrate binding. We have further characterized a novel virulence deficient mutant of Xoo, XadM (Xanthomonas adhesin M). XadM is involved in attachment to the EPS and adhesion to form biofilm. XadM mutants also exhibited hyper motility due to reduced stickiness. As XadM is required for virulence and for colonization, we studied the localization of XadM in Xoo using antibody against this protein. Immunofluorescence microscopy indicated that XadM is localized on the surface of the Xoo cells (Fig. 1A and B). To see the regulation of expression and localization, different fractions of extracellular, whole cell lysate and outer membrane was isolated from Xoo cells grown under different conditions (Fig. 1C). A 130 kDa band corresponding to the XadM protein is detected in the outer membrane and the whole cell lysate (Fig. 1C). No signal was detected in the extracellular fraction indicating that XadM is primarily localized in the outer membrane of Xoo. Relative expression analysis indicated that XadM is expressed 4 fold higher in the plant growth mimicking media as compare to the rich medium, indicating that the XadM expression is influenced by conditions inside the host plant. This will be the first report of *XadM* like gene as a virulence factor, which is involved in attachment and probably biofilm formation in any bacteria. Analysis of XadM adhesns indicated that it is primarily present in xylem vessel colonizing pathogens. In order to gain more insight into its role in xylem colonization, we used Xoc, a rice parenchyma tissue colonization pathogen, as a gain of function approach to study the significance of this adhesin in the biology of vascular vs. non-vascular pathogen. Interestingly, ectopic expression of XadM in a non-vascular pathogen of rice *Xanthomonas oryzae* pv. *oryzicola* (Xoc; a pathogen of rice leaf parenchyma), significantly increased migration indicating gain of function advantage towards a vascular pathogen lifestyle.

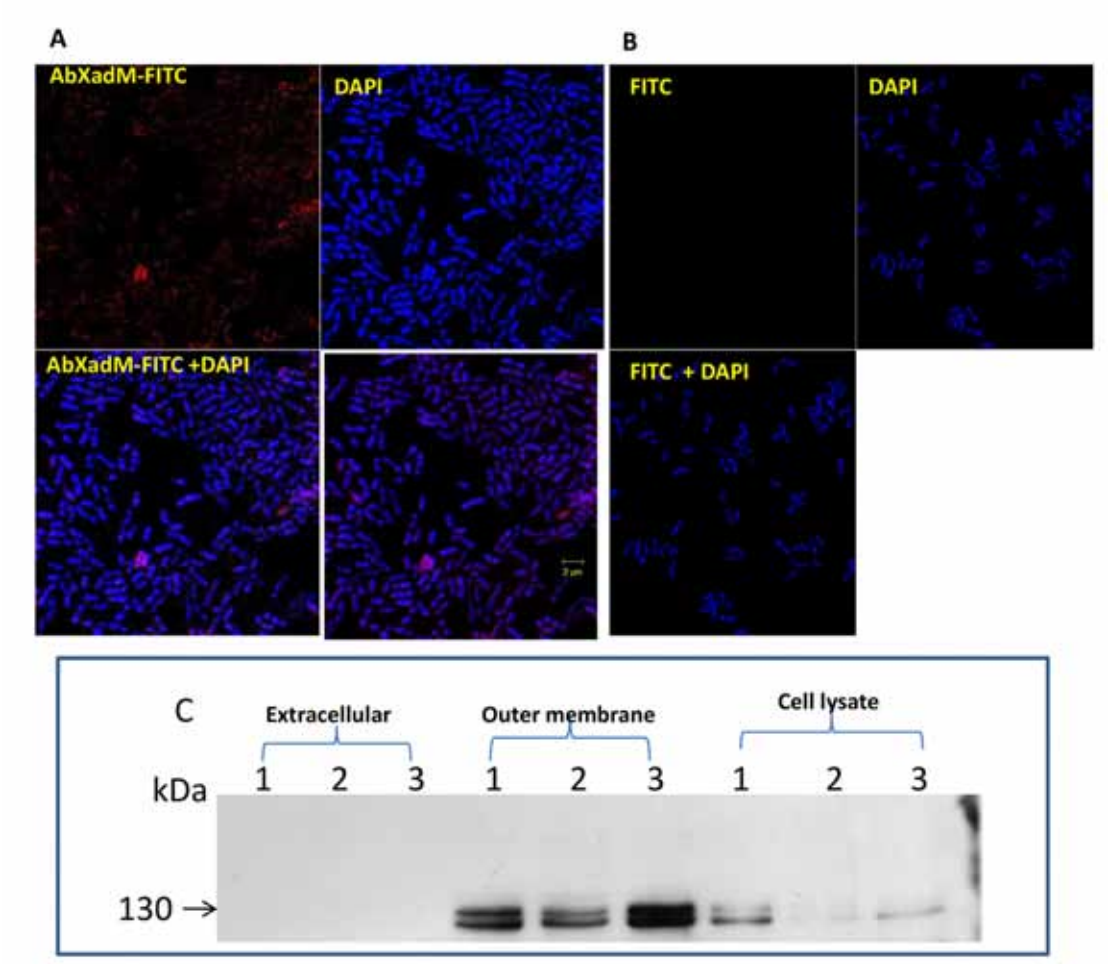


Figure 1. (A and B) Immunofluorescence localization of XadM in Xoo cells. Cells were stained with Antibody against Xad M and probed with anti-rabbit FITC conjugate secondary antibody. DAPI was used to stain nucleic acid in the cells which appears blue. For control, Xoo cells were stained with DAPI and were probed with secondary antibody. C. Western blot analysis for the localization and expression of XadM protein in Xoo cells grown in 1) Minimal Media; 2) PS (rich media); 3) XOM2 media (Plant growth mimicking media)

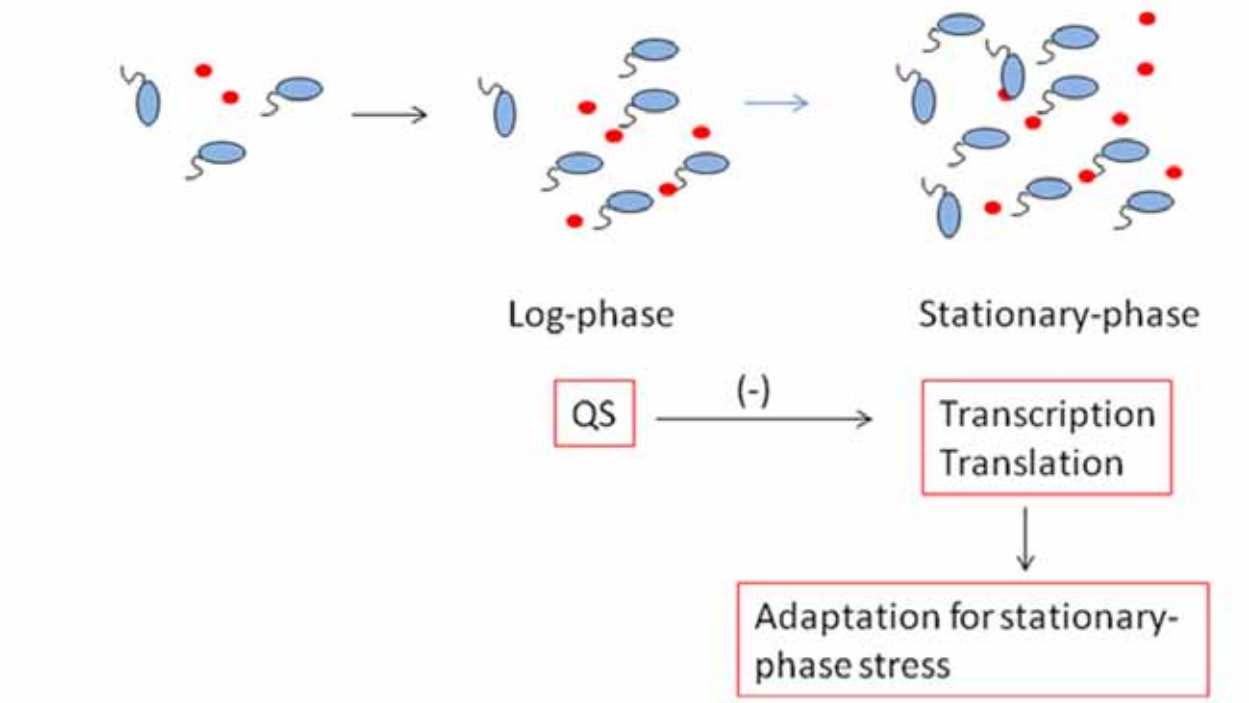


Figure 2. A proposed model for the role of QS as a signal for anticipation of stationary-phase. At high cell-density, the concentration of QS signal increases and mediate down regulation of transcription and translation, as a preparative step to counter long-term survival under stationary-phase stress. QS- mutants exhibit increased production of ribosomal proteins, protein synthesis, metabolic enzymes and poor survival under prolong stationary-phase stress.

In future, we are going to study detailed biofilm assays to see, at what stage, XadM is required for the biofilm formation.

Project: Role of quorum sensing and heterogeneity in environmental adaptation of bacteria.

Bacteria coordinate their social behavior in a density dependent manner by production of diffusible signal molecules by a process known as quorum sensing (QS). We have shown that bacteria exhibit reversible non gebnetic heterogeneity in QS. We have proposed a model based on our studies and evolutionary theory, which predicts that maintaining phenotypic heterogeneity in performing social tasks is advantageous as it can serve as a bet-hedging survival strategy. To gain more understanding of the role of QS in adaptation to different environmental conditions, we performed co-inoculation and competition experiments using mixed population of wild type and QS deficient mutants. Co inoculation studies indicate that under rich media condition, there is no significant difference in the growth rate of wild type and QS $^{-}$ mutants. However, in coculture, the QS- mutant exhibited significant growth advantage which indicates that cost of signal production may be disadvantageous for the wild type strain when nutrients are available in sufficient amount. In recent experiments we have observed that the wild type cells exhibit increased viability during late stationary-phase, which is generally associated with nutrient limitation, compared to the QS^{-} mutants. In general, it appears that QS^{-} mutants exhibit growth disadvantage at early log phase and compromised viability at late stationary phase. Our transcriptome analysis by microarray and translation assays indicate that QS promotes transition to stationary phase by slowing down the metabolism (transcription and translation), as an anticipation of stationary-phase stress (Figure 2).

In future, we are interested to study the role of QS in stationary–phase adaptation and contribution of QS heterogeneity in this process.

Publications:

Research papers published in the year 2022:

Singh, P., Verma, R.K., & Chatterjee, S. (2022) The diffusible signal factor synthase, RpfF, in *Xanthomonas oryzae* pv. *oryzae* is required for the maintenance of membrane integrity and virulence. **Molecular Plant Pathology**. 23: 118–132. 2. https://doi.org/10.1111/mpp.13148

Research papers in press as on 31st March 2023

Padhi Y, Chatterjee S. (2023) XdfA, a novel membrane associated DedA family protein of *Xanthomonas campestris* is required for optimum virulence, maintenance of magnesium and membrane homeostasis**. MBio**: DOI: 10.1128/mbio.01361-23, Manuscript ID: mBio01361-23

He YW, Chatterjee S, et al. (2023) DSF-family quorum sensing signal-mediated intraspecies, interspecies, and inter-kingdom communication. **Trends Microbiol**. 31:36-50. S0966-842X(22)00188-3. doi: 10.1016/j.tim.2022.07.006.

Group of Laboratory of Plant Microbe Interaction

Laboratory of Transcription

Bacterial transcription terminator Rho and mycobactericidal proteins from mycobacteriophages

Principal Investigator: **Ranjan Sen**
Staff Scientist

Ph.D Students:

Passong Immanual	Senior Research Fellow
Ajay Khatri	Senior Research Fellow
Saddam Ansari	Senior Research Fellow
Pankaj Sharma	Junior Research Fellow
Ankita Bhosale	Junior Research Fellow
Abhijeet Behera	Junior Research Fellow

Other Members

Shriyans Jain	Postdoctoral Fellow
Naveen Kumar	Postdoctoral Fellow
N. Yogesh Balakarthick	Technical Assistant-I

Collaborators

Prof. Markus Wahl	Freie Universität Berlin, Germany.
Prof. Udayaditya Sen	SINP, Kolkata, India
Prof. Ageneiszka	Szalewska-Palasz University of Gnask, Poland.

Objectives

Our laboratory is at present focused to understand the mechanism of action, physiology, and inhibition of the conserved bacterial transcription terminator, Rho. The following studies are underway in our laboratory. 1) Mechanism of action of transcription termination factor, Rho both *in vivo* and *in vitro*. 2) Molecular basis of Rho-NusG interaction. 3) Characterization of peptides from Psu for new properties. 4) Involvements of Rho in different physiological processes. In a translational project, we are exploring novel therapeutic protein molecules from mycobacteriophages.

Summary of the work done until the beginning of this reporting year (April 1, 2021 - March 31st, 2022).

In vivo regulation of bacterial Rho-dependent transcription termination by the nascent RNA.

Bacterial Rho is an RNA-dependent ATPase that functions in the termination of DNA transcription. However, the *in vivo* nature of the bacterial Rho-dependent terminators, as well as the mechanism of the Rho-dependent termination process, are not fully understood. Here, we measured the *in vivo* termination efficiencies of 72 Rho-dependent terminators in *E. coli* by systematically performing qRT-PCR analyses of cDNA prepared from mid-log phase bacterial cultures. We found that these terminators exhibited a wide range of efficiencies, and many behaved differently *in vivo* compared to the predicted or experimentally determined efficiencies *in vitro*. Rho-utilization sites (*rut* sites) present in the RNA terminator sequences are characterized by the presence of C-rich/G-poor sequences, or C>G bubbles. We found that weaker terminators exhibited a robust correlation with the properties (size, length, density, etc.) of these C>G bubbles of their respective *rut* sites, while stronger terminators lack this correlation, suggesting a limited role of *rut* sequences in controlling *in vivo* termination efficiencies. We also found that *in vivo* termination efficiencies are dependent on the rates of ATP hydrolysis as well as Rho-translocation on the nascent RNA. We demonstrate that weaker terminators, in addition to having *rut* sites with diminished C>G bubble sizes, are dependent on the Rho-auxiliary factor, NusG, *in vivo*. From these results, we concluded that *in vivo* Rho-dependent termination follows a nascent RNA-dependent pathway, where Rho-translocation along the RNA is essential and *rut* sequences may recruit Rho *in vivo*, but Rho-*rut* binding strengths do not regulate termination efficiencies.

Details of the progress in the current reporting year (April 1, 2022, to 31st March 2023).

A novel nucleic acid-binding protein, Gp49, from mycobacteriophage with mycobactericidal activity has the potential to be a therapeutic agent.

The mycobacteriophages encode unique proteins that are potent to be therapeutic agents. We screened several clones with mycobactericidal properties from a genomic library of mycobacteriophages. Here we report the properties of one such clone coding the gene product, Gp49, of the phage Che12. Gp49 is a 16 kD dimeric protein having an HTH motif at its C-terminal and is highly conserved among mycobacteriophages and likely to be part of phage DNA replication machinery. Alphafold predicts it to be an a-helical protein. However, its CD spectrum showed it to be predominantly b-sheeted. It is a high-affinity heparin-binding protein having similarities with the macrophage protein Azurocidin. Its b-sheeted apo-structure gets transformed into a -helix upon binding to heparin. It binds to linear dsDNA as well as ssDNA and RNA cooperatively in a sequence non-specific manner. This DNA binding property enables it to inhibit both *in vitro* and *in vivo* transcription. The c-terminal HTH motif is responsible for binding to both heparin and nucleic acids. Its *in vivo* localization on DNA could cause displacements of many DNA-binding proteins from the bacterial chromosome. We surmised that the bactericidal activity of Gp49 arises from its non-specific DNA binding leading to the inhibition of many host-DNA-dependent processes. Its heparin-binding ability could have therapeutic/ diagnostic usages in bacterial sepsis treatment (figure 1).

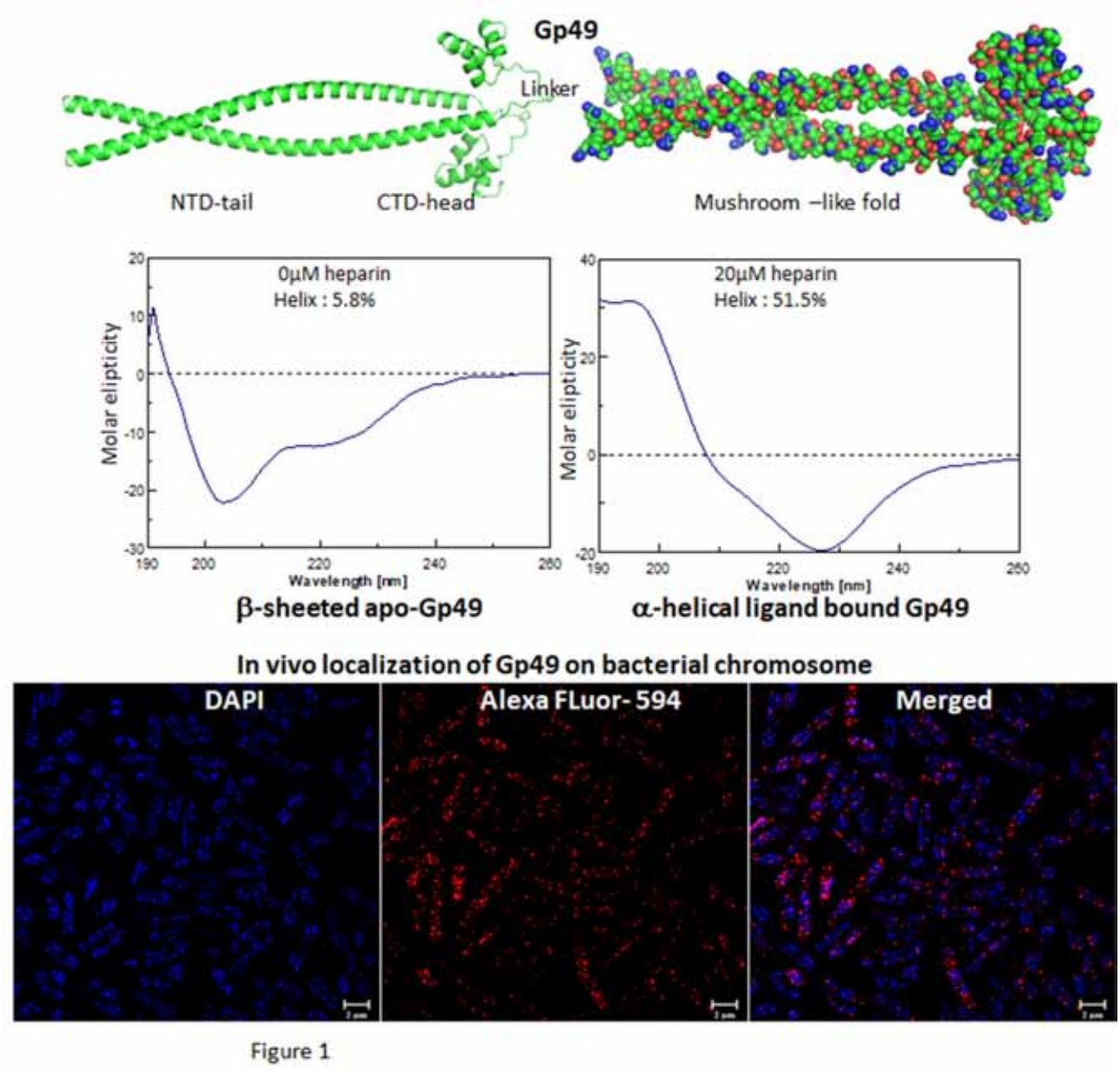


Figure 1

Peptides designed from a bacteriophage capsid protein function as synthetic transcription repressors.

The bacteriophage capsid protein, Psu, inhibits the bacterial transcription terminator, Rho. We designed peptides from the c-terminal of the Psu that function as the inhibitor of the Rho These peptides have positive surface-charge densities, and upon expression, they downregulate many genes in *E. coli*. We hypothesized that these peptides could bind to nucleic acids and repress gene expressions. One of these peptides, peptide 33, represses i*n vitro* transcription from the *T7A1* and P_{lac} promoters efficiently. This inhibition occurred by blocking the access of RNA polymerase to the promoter, a mode of transcription repression akin to many bacterial repressors. *In vivo,* expressions of the peptides reduce the total RNA level as well as transcription from P_{lac} and P_{osm} promoters significantly. However, they are less efficient in repressing transcription from the *rRNA* promoters that have a very high turnover of RNA polymerase. The peptide 33 binds to both single and double-stranded DNA as well as to RNA with dissociation constants ranging from 1 to 5 μM exhibiting preferences for the single-stranded DNA and RNAs. These interactions are sequence non-specific and salt-resistant. Interactions with dsDNA are entropy-driven, while it is enthalpy-driven for the ssDNA. This mode of interaction with nucleic acids is similar to many non-specific ssDNA-binding proteins. Expression of peptide 33 induces cell elongation and impaired cell division might be due to dislodging of the DNA-binding proteins. These effects might be one of the major reasons for the cytotoxic effects of these peptides. We surmised that these synthetic transcription repressors would function like bacterial nucleoid-associated proteins (NAP).

Future plans/directions:

The following projects, being pursued in my lab, are in different stages of completion. i) Characterizations of Rho-DNA interactions, ii) characterization of different myco-bacteriocidal factors from mycobacteriophages, iii) characterization of the Rho-RNAP-NusA-NusG interaction during the transcription termination process, and iv) involvement of Rho in resolving RNA: DNA hybrids and RNA metabolism pathways.

Publications 2022-23:

Husain MSA, N. Jain, S., Balakarthick, YN and **Sen, R**. (2023). A novel nucleic acid-binding protein, Gp49, from mycobacteriophage with mycobactericidal activity has the potential to be a therapeutic agent. ***International Journal of Biological Macromolecules***. 236, 124025. doi: 10.1016/j.ijbiomac.2023.124025

Chhakchhuak, P. I. R. and **Sen, R**. (2022). *In vivo* regulation of bacterial Rho-dependent transcription termination by the nascent RNA**.** ***Journal of Biological Chemistry,*** 298(6) 102001. doi: 10.1016/j.jbc.2022.102001.

Group of Laboratory of Transcription

अन्य वैज्ञानिक सेवाएँ / सुविधाएँ

Other Scientific Services / Facilities

Bioinformatics

In-charge

Dr. Ajay Kumar Mahato	Staff Scientist

Members

R Chandra Mohan	Technical Officer
Prashanthi Katta	Junior Assistant
Murali Mohan	Skilled Work Assistant
B Laxminarayana	HPC Administrator
Kamal	Computer Engineer

Objectives

This section provides critical IT services to all the users in CDFD. The primary job is to manage and maintain CDFD cyberspace, various servers, workstations, PCs, printers, and other peripherals devices. Apart from managing and maintaining the CDFD in-house official website, we also designed and maintained several online web applications to properly manage academics and research activities, such as project associates/project training/summer training and employee recruitment web applications.

The CDFD is also a collaborating institute in the Genome India Project (GIP), supported by the Department of Biotechnology, Government of India. For the GIP, we manage and maintain the server and other IT requirements for exchanging high-volume sequencing data across partner institutes and with the nodal canter via a dedicated private virtual LAN over the National Knowledge Network (NKN) infrastructure provided by Govt. of India. We also manage and maintain the Honey port sensor deployment infrastructure deployed by the National Cyber Coordination Centre (NCCC), Government of India, for monitoring, capturing, and enrichment of increased trends in the cyber ecosystem with the ultimate goal of supporting the secured India Cyberspace. We also manage the procurement of IT-related equipment via the GeM portal, followed by its installation, quality check, and activation of requisite software /license.

Details of progress made in the current reporting year (April 1, 2022 - March 31, 2023)

Our activities have encompassed the installation, administration, and maintenance of advanced servers responsible for delivering various services, including databases and computational tasks. We have also installed antivirus software on newly purchased PCs. We handle the in-house maintenance of internet, web, and other intranet services, ensuring they are continuously enhanced and made available to users. We have developed and managed the NGC website and the Paediatric Rare Genetic Disorder website. Furthermore, we provide regular updates to our self-maintained CDFD website. In alignment with the Government of India Guidelines, we undertook to redesign the CDFD website. We have started offering services related to the high-computational power requirement (CPU/GPU) for various projects the institute all research group and other institutions (CSIR-NIN, HCU etc.) To facilitate the sharing of Genomic sequencing data generated from our HPC infrastructure, we developed an in-house dedicated FTP server to upload/download high-throughput sequencing data. We have completed the AMC support renewal of domain services for existing high-end servers and the SSL certificate renewal. Our e-mail server migration to the NIC server, hosted and maintained by the National Informatics Centre Govt of India, was successfully carried out. We started the CDFD intranet services e-portal for online IT-related complaint registration.

Fig1. Newly developed PRaGeD website, CDFD-intranet information and service e-portal and summary of services delivered by Bioinformatics section.

Inauguration of CDFD-Advanced Supercomputing Facilities (ASF)

The Centre for DNA Fingerprinting and Diagnostics (CDFD) has launched the 'Mission on Paediatric Rare Genetic Disorders (PraGeD)' in collaboration with 16 other institutions. The mission aims to address the

challenges posed by rare genetic diseases prevalent in India. It involves a nationwide screening program to identify unknown genetic mutations causing such disorders. The mission intends to create awareness, achieve genetic diagnosis, discover novel genes, provide counseling, and develop new therapies for rare pediatric genetic diseases.

These disorders are especially common in regions with a history of endogamous marriages, and many affected children may not live beyond the age of five. Unfortunately, around 95% of these rare genetic diseases currently lack approved treatments. The initiative has already identified 5,600 families for screening over a five-year period. Once genetic mutations are detected in children, parents will receive counselling, and scientists will conduct further studies to understand the underlying mechanisms. To support the mission's objectives, a CDFD-Advanced Supercomputing Facilities (ASF) has been inaugurated at CDFD by Secretory DBT Dr. Rajesh Gokhale for high throughput genomic/proteomics data storage, and analysis of the raw data will be shared with the Indian Biotechnology Data Centre (IBDC) to aid researchers in better understanding genetic mutations in Indian populations.

Fig 2. Dr. Rajesh Gokhale (Secretory, DBT) inaugurated CDFD-Advanced Supercomputing Facility (ASF) on 1st November 2022.

Computation capacity of CDFD-Advanced Supercomputing Facilities (ASF)

Master – 2 No. (Lenova Server model 650)	2 X 2.5 GHz Intel Xeon Gold 6248 with Cores, RAM 12 * 32 GB DDR4 2933 MHz, Hard disk - 5 * 4TB, 7.2 K SAS 12 GB Hot swapable,
Compute – 20 No. (Lenova Server model 650)	2 X 2.5 GHz Intel Xeon Gold 6248 with Cores, RAM 12 * 32 GB DDR4 2933 MHz, Hard disk - 5 * 4TB, 7.2 K SAS 12 GB Hot swapable.
Cloud compute – 4 No (Lenova Server model 650)	2 x 2.5 GHz Xeon Gold (Cascadelake) 6248 with 20Cores,4 X 4 TB 2.5/3.5" 7.2 K RPM 6 Gbps SATA hot pluggable hard disks.
Storage 2 PiB (DDN Storage)	HPC Data 2PiB @40 GB/s,(DDN) Storage –PES Solution,ES7990X hardware RAID Storage Array
GPU Server (NVIDIA DGX A100)	8 * 40 GB GPU, application installed Parabricks pipeline and Ganana cluster suite Interconnect with Mellanox QM8700 infinite band Mellanox 40-port Non-blocking HDR 200 gb/s

Group of Bioinformatics

Covid 19 Testing Laboratory

Contributions of the Centre for DNA Fingerprinting and Diagnostics (CDFD) towards Diagnostics and Genomics research on COVID-19

Principal Investigators:

Kumarasamy Thangaraj	Director
Murali Dharan Bashyam	Staff Scientist
Ashwin Dalal	Staff Scientist

Present Members:

M Vidhyadhari	Senior Project Associate
Sivakumar Pandian	Project Associate II
B Himasri	Project Associate II
Sumedha Avadhanula	Project Associate II

Past Members:

Arunkumar Karunanidhi	Project Scientist
Prajakta Meshram	Project Associate II
Salava Hymavathy	Project Associate II
Rajeshwar Rao M	Data Analyst
Shankar Lavudia	Lab Assistant

- CDFD initiated RT-PCR based diagnostics of SARS-CoV-2 causing COVID-19 infection from 19th April 2020 by establishing a state-of-the-art laboratory with a maximum testing capacity of 450 samples per day. Identification of positive samples has helped the State and the Indian Government in contact tracing and containment measures.
- In addition to COVID-19 RT-PCR testing, we were also actively involved in the Indian SARS-CoV-2 genomics consortium (INSACOG) to identify the existing and upcoming variants for SARS-CoV-2 by sequencing method.

COVID-19 Genomics Research:

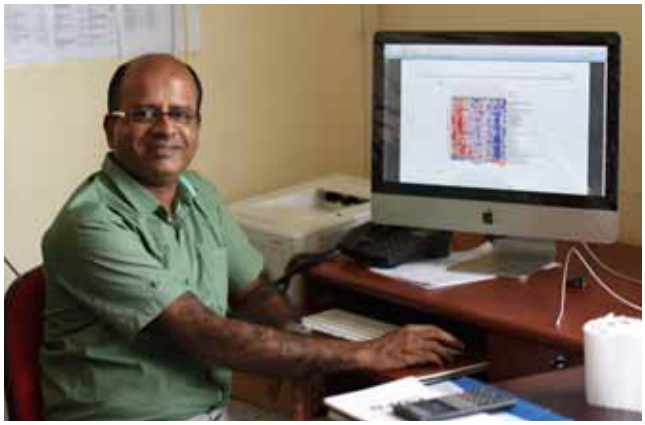
- This is the first comprehensive study from the state of Telangana on the dynamics of SARS-Cov-2 genomic evolution observed during the period of March, 2020 to March, 2023.
- Comprehensive profiling of SARS-CoV-2 genomes from COVID-19 infected patients using both Illumina and Nanopore sequencing platforms, revealed dominant viral lineages as well as important spike protein mutations.
- A total of 60,758 cases have been successfully analyzed at the COVID-19 diagnostics based on RT-PCR approach. As part of the INSACOG initiative, CDFD had sequenced 17,228 SARS-CoV-2 genomes, collected from the states of Telangana, Tamil Nadu, Rajasthan, Himachal Pradesh, Punjab, Andhra Pradesh, Goa, Uttar Pradesh, and Manipur with the overarching objective of identifying unique mutations, in addition to determining the dominant viral lineages circulating in the population. These sequences have been submitted to the national data hub maintained at NIBMG, Kalyani, West Bengal (earlier) and Indian Nucleotide Data Archive - Controlled Access (INDA-CA) maintained by the Indian Biological Data Centre of RCB, Haryana as well as to the GISAID international database. Our COVID team was also involved in updating IGSL results in Integrated Health Information Platform (IHIP) portal which is maintained by IDSP and NCDC, New Delhi.

- The sequencing strategy included sentinel surveillance of positive SARS-CoV-2 samples from hospitalized patients (THSTI-led hospital network study) as well as evaluation of samples from sudden clusters/surge events and international travellers tapped from airports. In addition, special efforts have been undertaken to meticulously monitor and collect samples which are suspected and/or confirmed to be vaccination breakthroughs and reinfection cases. The genomic analyses of such samples are expected to shed light into possible mechanisms of viral immune escape.
- During the period from April 2022 till March 2023, a total of 7954 samples from Telangana and Tamil Nadu along with airport surveillance were collected. Of these, 6707 samples were sequenced successfully and the results indicated that there is a consistent increase in the Omicron sub-lineages such as XBB.1, XBB1.9.1, XBB.1.5, XBB.2.3 with a major predominance of XBB.1.16 and XBB.1.16.1 lineages.

Publications:

SA Kemp, MTK Cheng, WL Hamilton, K Kamelian, INSACOG, S Singh, P Rakshit, A Agrawarl, CJR Illingworth, RK Gupta. Transmission of B.1.617.2 Delta variant between vaccinated healthcare workers. **Sci Rep**, 2022; 12:10492. doi: 10.1038/s41598-022-14411-7.

Asmita Gupta; Reelina Basu; **Murali Dharan Bashyam**. Assessing the evolution of SARS-CoV-2 lineages and the dynamic associations between nucleotide variations. **Access Microbiology**, 2023 Feb 23. https://doi.org/10.1099/acmi.0.000513.v2.



Group of Covid-19 Testing Laboratory

Experimental Animal Facility

Principal Investigator: **Dr. Pranjali Pore**
Other Members: Arikothan Sheeba
Kadingula Pavan
Faculty Co-coordinator: Dr. Rashna Bhandari,
Staff Scientist, CDFD

Objectives:

Our objective of the Experimental Animal Facility (EAF) is to(i)breed, maintain and supply laboratory animals to institutional scientists. Breeding and experimentation of all strains of mice is undertaken in individually ventilated caging systems;(ii)to support research programmes that promote the health and wellbeing of people and animals by facilitating high quality and scientifically sound research with animals; (iii)to comply with regulatory government body (CPCSEA) requirements for animal experimentation and breeding. Our goals include maintaining state-of-the-art facilities, implementing rigorous veterinary care, and adhering to stringent ethical guidelines.

Details of the progress made in the current reporting year (April 1, 2022 - March 31, 2023)

During this reporting year, the CDFD Experimental Animal Facility was working smoothly in compliance with regulatory government body CPCSEA for animal experimentation. All the mice were housed in IVC caging system. The CDFD Institutional Animal Ethics Committee (IAEC) was held on March 28, 2022, for an annual inspection of the facility and explaining the new rules of CPCSEA to conduct of an experiment. As per these new rules, CDFD Experimental Animal Facility went completely under CCTV surveillance and all the procedures for better experimentation and wellbeing of the animals. The meeting of CDFD Institutional Animal Ethics Committee (IAEC) was held on May 21, 2022 for review and approval of all ongoing and new studies conducted by CDFD and outside scientists.

Following approval from CPCSEA, the CDFD Experimental Animal Facility acquired rabbits of New Zealand White strain to generate polyclonal antibodies. As per Standard Operating Procedures, rabbits were quarantined for 14 days and then shifted to Experimental rooms for further procedures.

No health-related issues and no mortalities were noticed during the transfer and the quarantine period. Standard Operating Procedures (SOPs) were prepared, revised for the CDFD EAF as per new CPCSEA guidelines and all EAF staff were trained accordingly. The EAF was fumigated periodically. All the essential equipments of Experimental Animal facility were validated annually for better performance. Breeding colonies were established for all the five strains of mice (Table 1), all mice are breeding well.

Mice were bred to expand the colonies and 1,462 mice were supplied to users for IAEC approved experimentation. 12 Rabbits were brought from CPCSEA authorized vendor and housed for further experimentation.

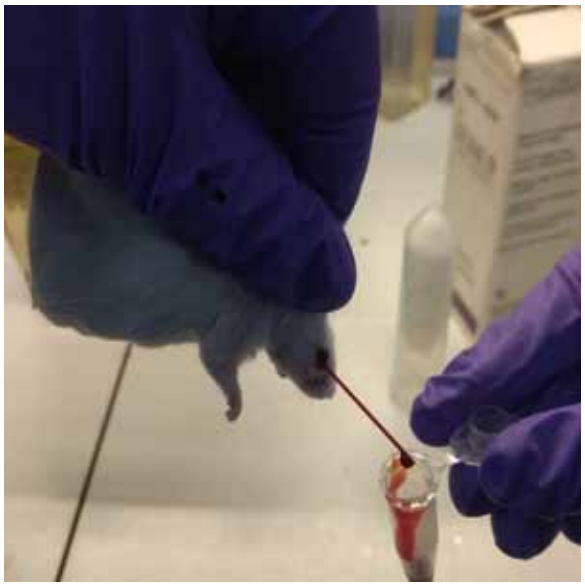
Table 1. Strain-wise break up of adult mice and rabbit housed at CDFD Experimental Animal Facility during 1st April 2022 to 31st March 2023, and supplied to users during 1st April 2022 to 31st March 2023.

Strain	Breeding (Male + Female)	Supplied
BALB/c	61+122	707
C57BL/6	36+72	552
Ip6k1	58 + 116	115
NnatΔNEO/Δl^{2}	07+14	Only Maintenance
Foxn1^{nu}	35 + 70	88
NZW Rabbits	Only Supply	12

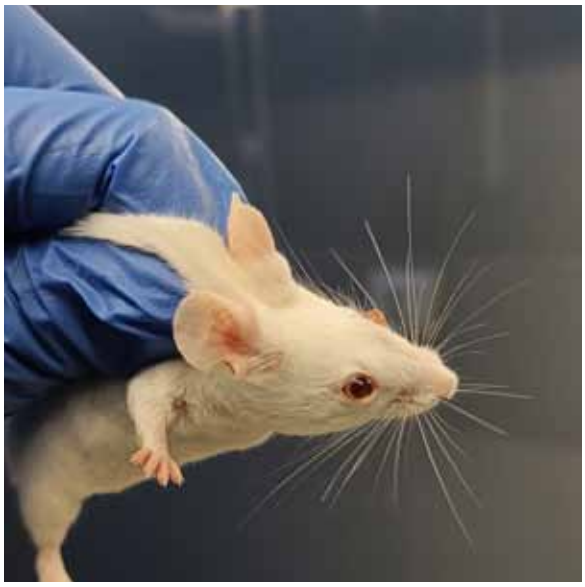
The experiments conducted during this period are listed below:

- 74 BALB/c mice were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.
- 115 *Ip6k1* mice were used for histo-pathological characterization of *Ip6k1* knockout mice.
- 156 C57BL/6 mice were used to study inorganic phosphate in eukaryotes synthesis detection metabolism and physiology

- 74 BALB/c mice were used to study *in vivo* immunomodulatory roles of some candidate PE/PPE proteins of *Mycobacterium tuberculosis*
- 44 BALB/c mice were used to study efficacy of PPE2 protein in the treatment of inflammation and tissue injury
- 71 BALB/c mice were used to study in vivo wound healing activity of recombinant purified PPE2 and PPE18 proteins of *Mycobacterium tuberculosis.*
- 237 C57BL/6 mice were injected orally with *Candida glabrata* for studies on comparative bio-burden of different *Candida* strains.
- 282 BALB/6 mice were injected orally with *Candida glabrata* for studies on comparative bio-burden of different *Candida* strains
- 88 *FoxN1*^{nu} athymic mice were injected with oncogenic cell lines to study tumor progression and metastasis.
- 55 C57BL/6 mice were used to study antimicrobial peptides in understanding immune responses and treatment of fungal ocular infections.
- 12 C57BL/6 mice were used to study molecular mechanisms involved in the anti-tumerogenic effects of PPE2 protein.
- 92 C57BL/6 and 162 BALB/c mice were injected with thioglycolate by intra-peritoneal route for the generation of macrophages.
- 12 NZW rabbits were injected subcutaneously with protein antigens and polyclonal antibodies were generated successfully.



Retro-Orbital blood collection in BALB/c mouse.



Ear punching in BALB/c mouse.

Metabolic cage for rodents

Markings of subcutaneous injections in rabbit for Polyclonal Antibody Generation.

Future direction

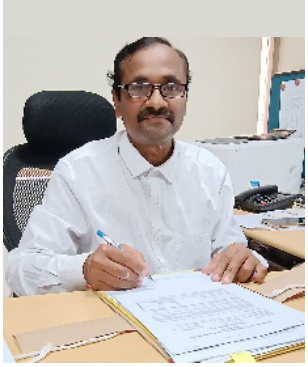
As the CDFD EAF achieves full functionality, our trajectory is defined by visionary expansion. The enrichment of our breeding colonies and incorporation of novel transgenic mouse strains form the bedrock, amplifying the tapestry of experimental animal research at CDFD.

Concurrently, our ambition extends beyond our walls, as we forge collaborative bonds with academic institutions. This synergy fosters a dynamic exchange of expertise, propelling innovative research and experimentation towards uncharted realms.

Central to our evolution is the development of cryopreservation, archiving, and retrieval systems for transgenic mouse strains within the EAF. This pioneering endeavour safeguards genetic resources, ensuring their enduring availability for future scientific exploration.

In these collective endeavours, we epitomize dedication to scientific advancement, ethical stewardship, and unwavering commitment to shaping a future where knowledge flourishes, collaborations thrive, and responsible animal welfare takes precedence.

Group of Experimental Animal Facility



Instrumentation

In-charge: **R N Mishra**

Member: S D Varalaxmi

M Laxman

R M K Satyanarayana

T Ramakrishna Reddy

Shailesh R Kamble

P Kranthi

G Prasad

Objectives

To upkeep all the Instruments in the laboratory by preventive maintenance, breakdown maintenance, repair and calibration. To provide technical specifications as per end user research requirements for the newly purchased Instruments. Technical comparative statement along with ordering information. To provide pre-installation requirements for the newly purchased instruments and to co-ordinate with the manufacturer/ local agents in installation and warranty service of the new instruments. Also to provide test/ installation reports for newly installed instruments.

Work undertaken during 2022-23

During the year 2022-23, we have installed 176 Nos new equipment including Single channel Variable Pipettes, Micro lit motorized controller, Gel Electrophoresis, Small refrigerator, Autoclaves, Tube Rotatory Units, I Shake 3d-Shaker, Magnetic Stirrer with Hot plate, Laboratory Refrigerator, Dual Chamber Water Bath, Fire Boy Plus, Vacuum Aspiration System, Mini Protein Tetra Cell, Thermal Cyclers, Thermo Mixers, Refrigerated Incubators, Weighing Balances, Refrigerated Table Top Centrifuge, Contamination Monitors, Digital Microscope Camera, Sorval Centrifuges, CO2 Incubators, pH Meters, Rotating Mixers, Biosafety Cabinets, Micro Centrifuges, Vacuum Pump with Trap Kit, Stationary Water Bath, Olympus Cell Counter, Olympus Cell Imaging System, Gel Rocker, Micro Balance, Conductivity Meter, LED Screen, Multi-Function Copiers, Microwave Oven, ULT Freezers, Hematology Analyzer, Ultrasonic Probe Sonicator, Laminar Flow Hood, Analytical Balances, Electrophoresis Power Packs, Stereo Zoom Microscope, UV Cross Linker, Ice Flaking Machine, Vacuum Concentrator, Electric Fumigator, Video Conferencing Facility, Laboratory Water Purification System, Bacterial Incubator, ID Card Printer, UV Trans Illuminator, N2 Storage Container, Gel Rocker etc.

Adding Instruments in CDFD Government e Marketing (GeM) Cart with technical specifications. We have completed more than 380 maintenance work orders, 308 Pipette calibrations, processed 176 Purchase Indents for purchase of new Instruments, maintaining the communication system etc. We have maintained most of the Instruments for maximum uptime in the Laboratory by replacing the local compatible electronics and electromechanical components. Most of the instruments are maintained by our Instrumentation Engineers, thereby saving on expensive AMCs and with very little downtime. In addition to above, we have involved in organizing the audio visual requirements for presentation in various seminar, lectures and workshops.

Group of Instrumentation

National Genomics Core

Principal Investigator: **Dr. K Thangaraj**

Co-Principal Investigator: Dr. Ashwin Dalal

Chief Executive Officer: Dr. Divya Vashisht
Until: 16.09.2022

Experimental Lab:

Manager: Dr. Priyanka K — Until: 31.12.2022

Associate: Mr. Vinay D — Until: 03.12.2022

Computational Lab:

Associate: Dr. B. Divya Bhanu

Associate: Mr. Avinash Dhar — Until: 04.07.2022

Project Coordinator:

Admin: Ms. Swetha G — Until: 31.01.2023

About NGC

National Genomics Core (NGC), is the establishment of Department of Biotechnology (DBT), India to act as a facilitator of genomics-driven discovery and application, and to accelerate the ushering in of a vibrant bio economy in our nation. South- Central regional core at CDFD, Hyderabad has been established along with central core-NIBMG (National Institute of Biomedical Genomics, Kolkata) and North-Central regional core (University of Allahabad, Prayagraj) to provide genomics services such as genome-scale DNA and RNA sequencing, genome-wide microarrays and gene-panel assays to institutes and the industry. The Core is intended to be a one-stop shop for all genomics services.

NGC Objectives

- Provide high-throughput platform facilities and expertise for generation of genome-scale data, using massively-parallel nucleic acid sequencing platforms
- Provide facilities and expertise for big data analysis, storage, management and access.
- Develop genomics skills using a pyramidal approach and taking advantage of India's recent membership of international molecular biology organizations (e.g., EMBO)

Summary of work for 2022-2023

- Accomplished MOUs with different university and private laboratories and various hospitals for genomics and sequencing services
- Organized Hands-on workshop on "Next Generation Sequencing" – A walk through From Sample QC to Data QC" in June'2022 as part of skill development programme on NGS to various researchers and doctors
- More than 150 different genomics services have been offered to various research groups from CDFD and other places like IFGTB, IISER, UAS etc.
- Around 4973 samples have been sequenced generating 3.5 Tb of data and business of ~1.13 Cr INR.
- Submission of RNA-seq transcriptome data of recurrent pregnancy loss from Institute of Genetics, Hyderabad to National Centre for Biotechnology Information database

Highlights of NGC-CDFD's work in COVID-19

NGC-CDFD has actively participated in Nation's initiative against pandemic of COVID-19 by performing whole genome sequencing of a total of 6707 SARS-COV-2 samples for the year 2022-23 under the following initiatives:

a) DBT-PAN-INDIA 1000 genome SARS-CoV-2 RNA consortium

b) Indian SARS-COV-2 Genome Consortium (INSACOG). The sequenced samples are submitted to openly available database GISAID (Global Initiative on Sharing Avian Influenza Data)

c) Strengthening COVID task force for various state institutes by training for COVID-genomics protocols

MoUs signed

Memorandum of Understanding, (MOUs) have been signed with different laboratories for sequencing and genomics services which include:

1. Bionivid Technology Pvt Ltd, Bengaluru, Karnataka

2. P.D. Hinduja Hospital and Research Centre, Mumbai
3. CSIR-Institute of Microbial Technology, Chandigarh
4. Asian Healthcare Foundation, Hyderabad
5. Eurofins India, Hyderabad

Workshops

A successful 5-day workshop was conducted from 20-24 June, 2022 on “Next Generation Sequencing” – A walk through From Sample QC to Data QC”. The session was completely hands on, training 30 participants from institutes like AIIMS, New Delhi & Jodhpur, National Institute of Immunology, New Delhi, Dr. D.Y.Patil Medical College and Hospital, Kolhapur, Kasturba Medical College, Manipal, Nizam Institute of Medical Sciences, Hyderabad, ICAR-CRIDA, Hyderabad and other renowned institutions and diagnostics around the country on next generation sequencing techniques.

Research

Transcriptome data of six samples from Institute of Genetics, Hyderabad has been submitted to NCBI with title “RNA-Sequencing of Placental Decidua of RPL” with NGC as data submitters and service providers under the following accession numbers

Bioproject ID: PRJNA973821

Biosample IDs: SAMN35151992-SAMN35151997

SRA Accession Number:
SRR24630373-SRR24630378

Group of National Genomics Core

Science Communication

Head: **Dr Varsha**
Staff Scientist VI

Other members: K Shirisha
Junior Assistant

Main activity of Science Communication at CDFD is:

- Science Communication & Outreach activities
- Media coverage of scientific papers/ events/ press releases
- Organizing scientific events
- Development of content for articles/Infographics
- Managing Social Media platforms
- Pre & Post Event reporting in all social media handles
- Support for press notes, and media reports
- Preparation of statuary reports

Science Communication & Outreach activities

Science communication and outreach is communicating scientific research and its outcome to general public. It is very essential to connect the common man with science. CDFD organises many institutional visits and outreach activities to create awareness and encourage curiosity about science among school and college students. In order to make the students aware about the career prospects in science and to give students and educators an experiential understanding of research, we conduct visits from more than 30 colleges in our campus. During the reporting period students from more than 30 schools and colleges across the nation visited us including Banaras Hindu University, Govt. Degree college, Khairatabad, Hyderabad public school, RBVRR Women's College, NAARM, Rajendranagar, Hyderabad, Pillai College of Science and Commerce, Mumabi, Dept. of Biotechnology, Karnataka Science College etc.

As part of our outreach program, we are engaged in following activities:

Open days:

During these days, school and college students, educators or anyone from general public can visit our labs and interact with our scientists / researchers to learn more about the world of research. In order to provide information regarding the job options in science and to give students and educators an experiential understanding of research, we conduct visits for them on our campus. During the reporting period students from various schools and colleges visited us including RBVRR Women's College, Karnatak Science College, Dharwad, Department of Biochemistry, Mangalore University, Hyderabad Public School, Pillai College of Science and Commerce, Mumbai, Banaras Hindu University and many more.

Science Setu: Our scientists will visit various schools/ colleges and educational institutions and deliver talk and interact with students. It gives them a chance to get exposed to the cutting edge research which is being carried out at the Centre and also inspires them to opt science as a career. Our scientists also visit the schools and colleges in the twin cities under 'Bridge'and 'Vigyan- Jyothi' programs and teach the students. The webinars have been arranged for DBT STAR Colleges and virtual Open Days have been organised for the benefit of the students who were far from Hyderabad and were not able to visit us.



Institutional visits:

CDFD encourages the visits of students from various schools and college across the nation to give them exposure about the cutting edge biological research being carried out at the Centre.

In the reporting period students from more than 30 schools and colleges across the nation have visited us.

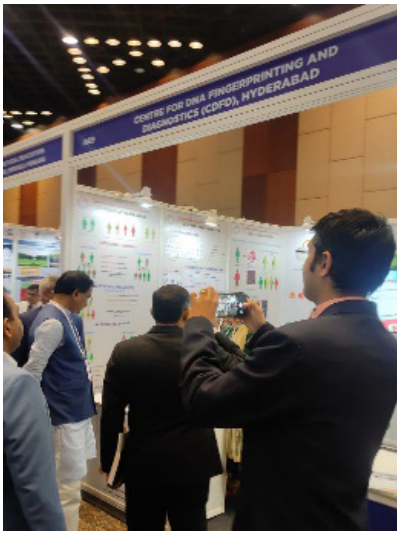


Popular science talks and lecture series:

Popular talks are being organised on different occasions like the Foundation Day, National Science Day, the India International Science Festival, Lalji's Memorial Lecture on his birth anniversary, visits of eminent scientists etc. This is always an opportunity to the staff and students to interact with such eminent personalities of scientific fraternity.

Other Outreach activities:

CDFD takes part in science exhibitions like India International Science Festival" (IISF), India Science Festival (ISF), Global Bio-India, Vigyan Manthan Yatra under Mission Excellence Program with Madhya Pradesh Council of Science and Technology, Science Congress organised by Kendriya Vidyalaya for school children and various other programs organised under the aegis of under Azadi ka Amrit Mahotsav.



Social Media Objectives:

With the objective of creating awareness, CDFD is having Facebook, Twitter, YouTube, Linked In and Instagram handles and we keep updating all these handles. The posts about any new publications, PhD defense, awards and honors, events, seminars / lectures / training / workshops / outreach activities including Open Days and institutional visits/MoU executed/Science Outreach etc. are regularly updated. We have initiated the following new series which are uploaded on every Friday:

1. **Meet the young scientist**
2. **Research Team of the month**
3. **Alumni Spotlight**
4. **Experts behind the scenes**
5. **Science Art**

We regularly update our social media handles with new findings, outcomes, achievements and various events. Also, we disseminate the scientific knowledge through other media, including science articles in magazines and newspapers, TV programmes with Rajya Sabha TV, Yadgiri TV Channel etc. We are also initiating popular science talks/ podcasts etc with our scientists and students on our social media handles.

Under the societal outreach activities, we are into an organ donation awareness programme under “Jeevandhan Scheme” with Gandhi Medical College, Secunderabad.

Media coverage /news clips:

GENETIC TESTS FOR RARE ILLNESS AT CDF

DC CORRESPONDENT
HYDERABAD, NOV. 1

A nationwide programme to reduce the prevalence of rare genetic disorders in children through genetic analysis was launched at the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad on Tuesday.

పిల్లల్లో అనువంశిక వ్యాధులకు అడ్డుకట్ట

INBRIEF

Bones, hair from forest match with Shraddha's family DNA

The Delhi police on Wednesday said that the results of a fresh DNA profiling test on a set of bones and hair strands that were recovered from forests in south Delhi's Mehrauli forest and Chhattarpur during investigation into the murder of Shraddha Walker, matched the samples provided by her family. Special Commissioner of Police (Land and Order Zone II) Sagar Preet Hooda said that the set of bones and hair strands, where the DNA couldn't be extracted, were sent to Centre for ... Fingerprinting and Diagnostics (CDFC) in H... mitrocondrial profiling ar... bones will now b... that will b... AIIMS," ...

CDFD organizes three-days confere... on mitochondria in health and disea...

(Capital Information)

Hyderabad, June 21 : Centre for DNA Fingerprinting and Diagnostics (CDFD), in collaboration with the Society for Mitochondrial Research and Medicine, India (SMRM) is organizing the 9th Annual Conference on the theme "Mitochondria in Biology and Medicine" during 21 - 23 June 2023. In this conference, the scientists, clinicians and young researchers discuss contemporary science in mitochondrial biology and medicine.

Mitochondria are popularly known as the powerhouse of an organism, essential for energy. Mitochondrial dysfunction leads to several diseases. Many people in India, particularly children, are affected by mitochondrial diseases. However, there needs to be more awareness about the disease among the public. Therefore, it is essential to spread knowledge about mitochondrial diseases and awareness among the public.

The 9th Annual Conference of the Society for Mitochondrial Research and Me... inaugurated at the Centre for DNA F... ing and Diagnostics (CDFD) on ... 2023. Dr. K. Thangaraj, Director, ... Founder of the SMRM, welcom... gates.

The Presidential address wa... Dr K. Satyamoorthy who emp... cent updates in this field. Dr. P... ganizer of the Conference ... SMRM, briefed about the sci... the Conference.

Hyderabad

'Indians have gene traces of ancient 'humans' like Neanderthals, Denisovans'

...ad gears up to host P... ...o from ...

Group of Science Communication

Sophisticated Equipment Facility (SEF)

Head **Vinod Kumar Mishra**
Staff Scientist

Other Members

Ch V Goud	Technical Officer
K Sreethi Reddy	Technical Officer
Bala Maddileti C	Technical Officer (Out sourcing)
Mohd. Mudassir	Technical Officer (Out sourcing)
Abhijeet Singh	Technical Officer (Out sourcing)
Viswa Kalyan	Technical Officer (Out sourcing)
Tripti Sharma	Technical Assistant (Out sourcing)

Objectives

- In order to maximize the utilization of all high end equipments and their better management, these equipments are brought under one umbrella “Sophisticated Equipment Facility” (SEF).
- To extend testing and analysis facility to research personnel, doctoral students and faculty members of CDFD
- To extend its facilities to other academic institutions, R & D laboratories and industries.
- To organize short term courses/workshops on the use and application of various instruments and analytical techniques.
- To train technicians for maintenance and operation of sophisticated instruments.
- The initiative minimizes duplication of expensive equipment and lead to better utilization of instruments.

Summary of work done till March 2023

- Activities related to installation, administration and maintenance of various sophisticated equipment’s in the facility.
- The list of services offered with the major equipments available under the scope of this Sophisticated Equipment Facility (SEF) are as follows:
- **Genomics Services: DNA** Sequencers and Real-Time PCR Machines.
- **Proteomics Services:** HPLC System, Circular Dichroism spectropolarimeter.
- **Cellomics Services:** Confocal Microscope with multiphoton laser, Live Cell Imaging and FACS ARIA Flow cytometer with Sorter.
- **Tissue processing unit:** Microtome
- We have carried out outreach programmes for educating children of various schools and colleges regarding the services offered by us and efficient use of such high end equipments
- Efficiently propagated the idea of using centralized facility for various R & D activities within CDFD as well as various academic institutes and private research organizations.
- Various companies had the opportunity to display their high end equipment in CDFD.

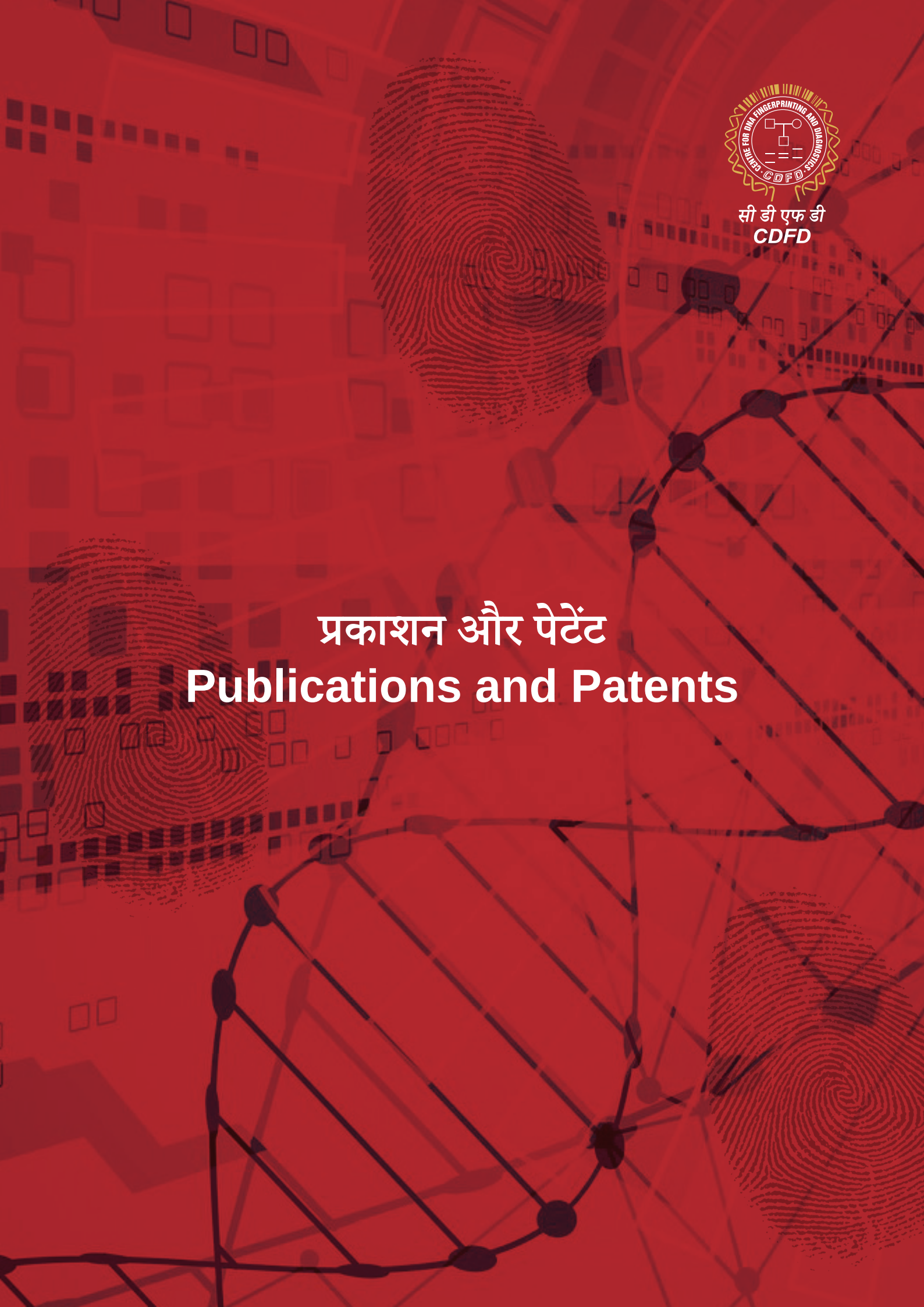
Details of Progress made in current reporting year (April 1, 2022 to March 31, 2023)

- A new addition to the SEF Facility is the new Phosphoimager Cytiva Amersham TYPHOON.
- An Outreach activity was done to promote the use of centralized facility by making the SEF Posters. The Posters were attached pertaining to each machine and visitors from various background had feasibility in knowing the equipments.
- Many schools and outside personnel visited the facility for acquiring knowledge of various equipment in the facility.
- Co-ordination with various users and the instrumentation department for AMC/ CMC requirements for smooth functioning of SEF facility.
- The facility was used by various inside as well as outside users and the list are as follows:

Sequencing and Genotyping	1756 users (18039 Samples)
Confocal LSM 700/Leica SP-8	1093 users
Super Resolution LSM 980	360 users
FACS	167 users
CD Spectropolarimeter	13 users
RT-PCR	439 users
Histopathology	19 users

- Revenue generated for the year April 2022 – March 2023 was **Rs. 3779928/-** (Rupees Thirty-seven lakhs seventy-nine thousand nine hundred twenty-eight).

Group of Sophisticated Equipment Facility



प्रकाशन और पेटेंट
Publications and Patents

CDFD Publications FY 2022-23

(1 April 2022 to 31 March 2023)

1. Achar YJ, Foiani M (JUNE-2022). Topology of RNA:DNA Hybrids and R-Loops in Yeast. ***Methods in Molecular Biology*** **(IF-1.167).** 2022; 2528:317-328. doi: 10.1007/978-1-0716-2477-7_21.

2. Ansari MSH, Kumar N, Jain S, Balakarthick NY, Sen R (MAR-2023). A novel nucleic acid-binding protein, Gp49, from mycobacteriophage with mycobactericidal activity has the potential to be a therapeutic agent. ***International Journal of Biological Macromolecules*** **(IF-6.953)**. **2023 Mar** 13;236:124025. doi: 10.1016/j. ijbiomac.2023. 124025.

3. Aricthota S, Rana PP, Haldar D (SEPT-2022). Histone acetylation dynamics in repair of DNA double-strand breaks. ***Frontiers in Genetics*** **(IF- 4.599)**. **2022 Sep 9**; 13:926577. doi: 10.3389/fgene.2022.926577.

4. Bala P, Kavadipula P, Sarkar S, Bashyam MD (DEC-2022). To ß or Not to ß: Lack of Correlation Between APC Mutation and ß-Catenin Nuclear Localization in Colorectal Cancer. ***Journal of Gastrointestinal Cancer*** **(IF-Not Available)**. **2022 Dec 31**. doi: 10.1007/s12029-022-00886-0.

5. Balakrishnan S, Aggarwal S, Muthulakshmi M, Meena AK, Borgohain R, Mridula KR, Yareeda S, Ranganath P, Dalal A (JUNE-2022). Clinical and Molecular Spectrum of Degenerative Cerebellar Ataxia: A Single Centre Study. ***Neurology India*** **(IF- 2.117). 2022 May-Jun**; 70(3): 934-942. doi: 10.4103/ 0028-3886.349660.

6. Basnet R, Rai N, Tamang R, Awasthi NP, Pradhan I, Parajuli P, Kashyap D, Reddy AG, Chaubey G, Das Manandhar K, Shrestha TR, Thangaraj K (OCT-2022). The matrilineal ancestry of Nepali populations. ***Human Genetics*** **(IF- 4.132)**. **2022 Oct 15**. doi: 10.1007/s00439-022-02488-z.

7. Bhakt P, Raney M, Kaur R (OCT-2022). The SET-domain protein CgSet4 negatively regulates antifungal drug resistance via the ergosterol biosynthesis transcriptional regulator CgUpc2a. ***Journal of Biological Chemistry*** **(IF- 5.157)**. **2022 Oct** : 298(10) : 102485. doi: 10.1016/j.jbc.2022.102485.

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मानव संसाधन विकास
Human Resource Development

PhD Program

The Institute offers a vibrant multidisciplinary research scholars Program. Keeping in view the interdisciplinary nature of scientific research, the Centre especially encourages persons from different scientific disciplines to take up challenges in various areas of modern biology. The Research scholars are encouraged to take admission in the PhD program of Manipal Academy of Higher Education, Regional Centre of Biotechnology, or University of Hyderabad.

The eligibility for the program is Masters degree in any branch of Science, Technology or Agriculture from a recognized University / Institute or MBBS. Candidates must have cleared National Eligibility Test (NET) with a valid fellowship. Eligible candidates are Interviewed for selection as research scholars.

As of March 31, 2023 the Centre has 104 Research Scholars working for their doctorates in different areas of research. In the reporting year, 12 Research Scholars have completed PhD and are pursuing careers elsewhere in India or abroad.

Postdoctoral Program

In addition to the JRF program, the Centre also carries out training at the post-doctoral level. The post-doctoral fellows are funded through extramural grants that CDFD receives. Some are also selected competitively by various schemes of Government of India such as the DST WoS-A program, the DST N-PDF program, the DBT post-doctoral fellowship program and others.

Summer Training Program

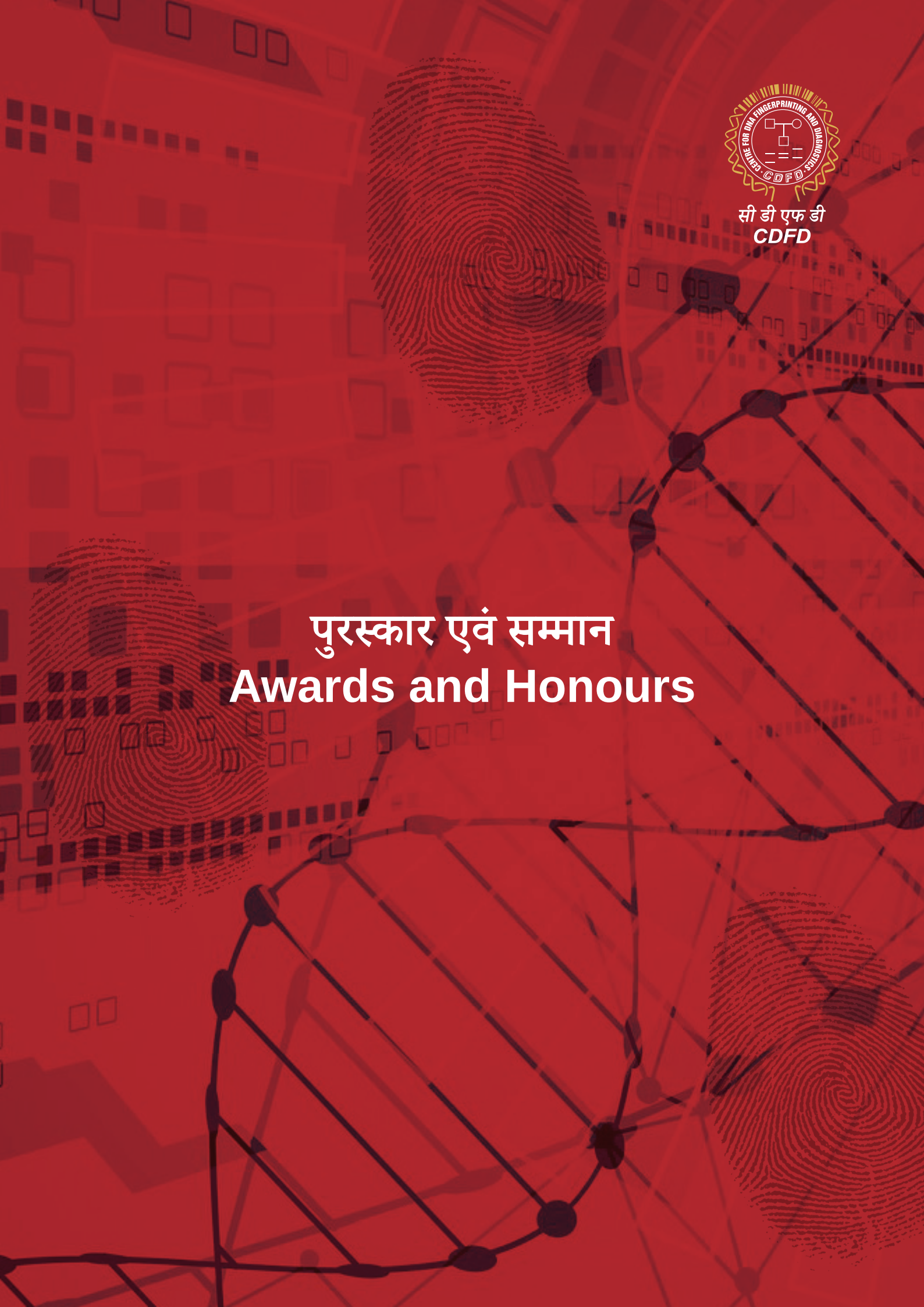
CDFD provides admissions to summer training program to those students who are supported either by the three Indian Science Academies or Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore or the Kishore Vigyanik Protsahan Yojna, New Delhi. In the reporting year 09 students received summer training at the Centre.

Dissertation based Research Training for students

Under this programme, the students spend 4 - 6 months at CDFD and work on active projects being carried out by CDFD faculty. This training helps the students in gaining hands-on experience in modern biology. In the reporting year, 20 students were given the opportunity to avail training under this programme.

SERB-SSR Training for students

Under this programme, the students spend 2 months at CDFD and work on active projects being carried out by CDFD faculty. This training helps the students in gaining hands-on experience in frontier areas of modern biology. In the reporting year, 02 students availed training under this programme.



पुरस्कार एवं सम्मान
Awards and Honours

AWARDS AND HONORS – 2022-23

S.No.	FACULTY & STAFF	
1.	Dr. K. Thangaraj	Sir. DR. U. N. Brahmachari Award – 2022 for remarkable contribution to biological sciences by NIPER, Kolkata
2.	Dr. Sangita Mukhopadhyay	Felicitated by the Governor of Telangana in the program 'Women Scientists Conclave: Self Reliance' organised by the 'National Academy of Sciences, India – Hyderabad chapter' jointly with the 'Academy of Science, Technology & Communication (ASTC) on 10.10.2022.
3.	Dr. Rupinder Kaur	Fellowship of Indian National Science Academy
4.	Dr. M Subba Reddy	Fellowship of National Academy of Sciences
5.	Dr. Subhadeep Chatterjjee	1) Fellowship of Indian Academy of Sciences 2) Dr Lalji Singh Memorial Award by Association for the Promotion of DNA Fingerprinting and other DNA Technologies (ADNAT)
6.	Dr. Usha Dutta	Fellowship of Telangana Academy of Sciences
	PhD STUDENTS & PROJECT PERSONNEL	
1.	Mr. Hilal A Reshi	Professor A.S Mukherjee Memorial award for the best oral presentation at 44th All India Cell Biology Conference held at University of Kashmir on 2-3 September 2022.
2.	Ms. Devanshi Gupta	Prof B.S. Sheshachar Memorial Award for the best poster presentation at 44th All India Cell Biology Conference held at University of Kashmir on 2-3 September 2022.
3.	Ms. Arpita Singh	Poster prize at IDPosters22, an international online meeting organized by the Intrinsically Disordered Proteins (IDP) Seminars group.
4.	Mr. Yashobanta Padhi	Outstanding Oral Presentation (Second Prize) at International Conference on Current Trends and Future Prospects of Plant Biology (CTFPPB-2023), organized by the Department of Plant Sciences, School of Life Sciences, University of Hyderabad from 23-25 February, 2023.
5.	Mr. Kanishk Saraf	Outstanding Poster Presentation (First Prize) at International Conference on Current Trends and Future Prospects of Plant Biology (CTFPPB-2023), organized by the Department of Plant Sciences, School of Life Sciences, University of Hyderabad 23-25 February, 2023.
6.	Mr. Kundan Kumar	Best Poster award at Fundamentals to applications of yeast and fungi-Yeast India 2023' conference organized by the Indian Institute of Science Education and Research (IISER) Mohali from 10-13 March, 2023.
7.	Ms. Fizza Askari	Best Poster award at Fundamentals to applications of yeast and fungi-Yeast India 2023' conference organized by the Indian Institute of Science Education and Research (IISER) Mohali from 10-13 March, 2023.

विभिन्न कार्यक्रम
Various Events

IMPORTANT EVENTS – 2022-2023

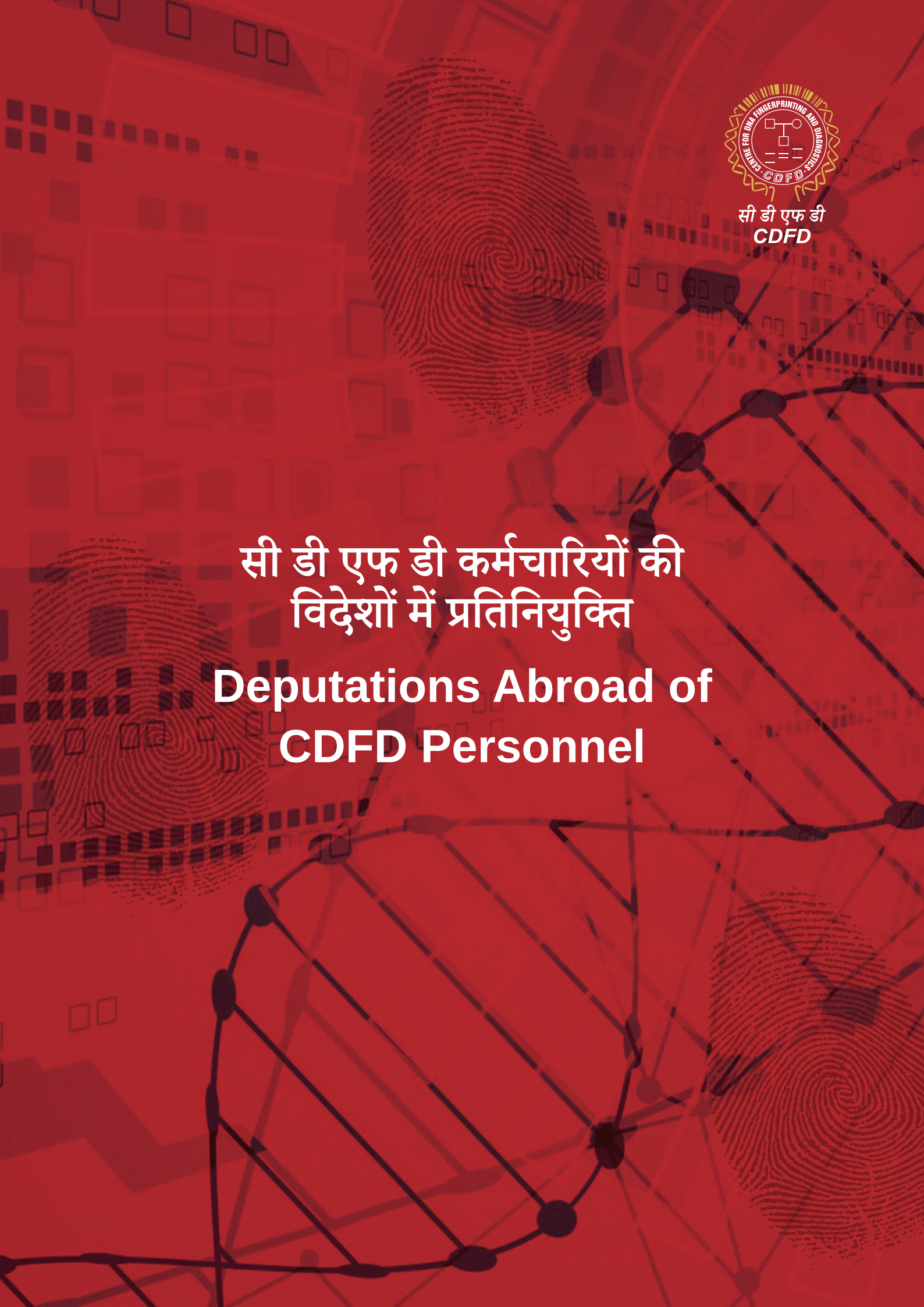
S.No.	Event	Date
1.	Finance Committee meeting	08.04.2022
2.	Governing Council meeting	12.04.2022
3.	MoU between CDFD and Institute of Bioresources and Sustainable Development (IBSD), Imphal	20.04.2022
4.	Talk by Shri Justice Gunda Chandraiah, Chairperson, Telangana State Human Rights Commission in connection with Dr. B.R. Ambedkar Jayanthi celebrations	22.04.2022
5.	Swachhta Pakhwada	01.05.2022 to 15.05.2022
6.	Hy-Sci 2022	14.05.2022
7.	Observance of Anti Terrorism day	20.05.2022
8.	Workshop on Human Forensic DNA Fingerprinting: From Crime Scene to Courtroom	23.05.2022 to 27.05.2022
9.	MoU between CDFD, Hyderabad and AIG Hospital, Hyderabad	01.06.2022
10.	Participation in the Biotech Startup Expo2022, New Delhi organized by BIRAC & DBT	09.06.2022 to 10.06.2022
11.	International Yoga Day celebrations	21.06.2022
12.	Hands on workshop on Next Generation Sequencing	20.06.2022 to 24.06.2022
13.	Dr Lalji Singh Memorial Lecture by Prof Subramaniam Ganesh, Department of Biological Sciences and Bioengineering, IIT Kanpur	05.07.2022
14.	Open Day celebrations	06.07.2022
15.	MoU with International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad	28.07.2022
16.	Har Ghar Thiranga campaign on the occasion of 75th Independence Day	15.08.2022
17.	Sadbhavana Diwas	18.08.2022
18.	Hands on workshop on Clinical Applications of Cytogenetics and Molecular Cytogenetics	22.08.2022 to 27.08.2022
19.	RAP -SAC meeting	01.09.2022 to 02.09.2022
20.	Hindi Day celebrations	14.09.2022
21.	Finance Committee meeting	28.09.2022
22.	Fit India Freedom Run 3K as part of Azadi ka Amrit Mahotsav	14.10.2022 & 21.10.2022
23.	Ayurveda Day Celebrations	25.10.2022

S.No.	Event	Date
24.	MoU with P.D. Hinduja National Hospital & Medical Research Centre, Mumbai	26.10.2022
25.	Rashtriya Ekta Diwas (National Unity Day)	31.10.2022
26.	Vigilance Awareness Week	31.10.2022 to 06.11.2022
27.	Hands-On Workshop on Human Forensic DNA Fingerprinting	31.10. 2022 to 04.11. 2022
28.	Launching of Mission Programme on Pediatric Rare Genetic Diseases by Dr. Rajesh S Gokhale, Secretary DBT in the presence of Media	01.11.2022
29.	Governing Council meeting	01.11.2022
30.	MoU with Osmania Medical College	25.11.2022
31.	“Constitution Day” Celebrations	26.11.2022
32.	Society meeting	01.12.2022
33.	MoU with ICMR – National Institute for Research & Child Health (ICMR-NIRRCH), Mumbai	12.12.2022
34.	MoU with Indo US Organisation for Rare Diseases (IndoUSrare), Neil Armstrong Ave Herndon VA 20171	10.01.2023
35.	MoU with Organisation for Rare Diseases (ORDI), Bangaluru	11.01.2023
36.	Participation of CDFD in India Science Festival, Hyderabad Public School, Begumpet, Hyderabad	20-22 January 2023
37.	Participation of CDFD in India International Science Festival, at MANIT Bhopal	21-23 January 2023
38.	Foundation Day lecture by Shri Sanjeev Sanyal, Member, Economic Advisory Council to the Prime Minister of India & Secretary, Government of India on 28.01.2023 in the august presence of Dr. Rajesh Gokhale, Secretary, DBT, Govt. of India.	28.01.2023
39.	MoU with CSIR-CCMB and CDFD	30.01.2023
40.	Observance of Martyr’s day	30.01.2023
41.	DBT Symposia on “Opportunities for frontier research collaborations” by the Human Frontier Science Program (HFSP)	11.02.2023
42.	Participation in BioAsia-2023 Exhibition at HICC, Hyderabad.	24-26 February 2023
43.	Hands-on Workshop on - Long Range Genome Sequencing by Nanopore Technology	27th February to 3rd March 2023
44.	Open Day	01.03.2023
45.	Women’s Day Celebrations	09.03.2023
46.	MoU with Govt. of Goa and CDFD	09.03.2023

OUTREACH ACTIVITIES-2022-23

S.No.	Activity	Date
1.	Visit of students from RBVRR Womens college, Hyderabad	22.04.2022
2.	Visit of students from TTWRDC, college, Devarakonda, Nalgonda dist	25.04.2022
3.	Visit of students from RBVRR Womens college, Hyderabad	27.04.2022
4.	Webinar entitled “Use and the importance of DNA Fingerprinting in investigation with case studies”, delivered to Police Training College Moradabad, UP	19.05.2022
5.	Hy-Sci 2022 by the students, for the students	14.05.2022
6.	DNA Fingerprinting training imparted to Air force medical officers	09.06.2022
7.	Participation in the Biotech Startup Expo2022, New Delhi organized by BIRAC & DBT	09.06.2022 to 10.06.2022
8.	Visit of students from Karnatak **Science College, Dharwad,** Karanataka.	22.06.2022
9.	Webinar on “Understanding the social language of bacteria: speak or not to speaker?” for Parul University of Applied Sciences, Dept. of Microbiology, Gujarat	01.07.2022
10.	Open Day Celebration	06.07.2022
11.	Lecture on “Mitochondrial diseases: an integrative approach for diagnosis and treatment” in the symposium “Advances in Mitochondrial Research: From Bench to Bedside” organized by Alva’s college, Moodubidire, and Society of Mitochondrial Research and Medicine (SMRM)	19.08.2022
12.	Visit of students from Department of Biochemistry, Mangalore University	25.08.2022
13.	Talk at the 44th All India Cell Biology Conference at the University of Kashmir	02.09.2022 to 03.09.2022
14.	Visit of students from RBVRR Women’s College, Narayanaguda, Hyderabad	16.09.2022
15.	Visit of students from Hyderabad Public School, Begumpet, Hyderabad	28.09.2022
16.	Talk on Tuberculosis and Immunological Therapy jointly organized by the National Academy of Sciences, India-Hyderabad and Academy for Science, Technology & Communication (ASTC)	10.10.2022
17.	Visit of students from Pillai College of Science and Commerce, Navi Mumbai	13.10.2022 & 14.10.2022
18.	Visit of students from Banaras Hindu University	14.10.2022
19.	Visit of students from Rockwoods International School, Ghatkesar, Hyderabad.	15.11.2022 & 16.11.2022
20.	Visit of NAARM, Rajendranagar, Hyderabad	17.11.2022
21.	Visit of students from Birla Open Mind International School, L. B. Nagar, Hyderabad	21.11.2022
22.	Visit of students from Govt. Degree College, Khairatabad, Hyderabad	28.11.2022
23.	Visit of MBBS Students from Apollo Institute of Medical Sciences and Research, Apollo Health City, Jubilee Hills, Hyderabad	08.12.2022

S.No.	Activity	Date
24.	Seminar on Science and Society organised by Indo-german nochkontakt Association (IGNA)	16.12.2022
25.	Trip of CDFD students and postdocs to Sai Life Sciences, Shamirpet, Hyderabad	17.12.2022
26.	Open Day at Experimental Animal Facility, CDFD	22.12.2022 to 23.12.2022
27.	Visit of Students from College of Horticulture & Forestry, Neri, Hamirpur, Himachal Pradesh	29.12.2022
28.	Visit of students from K.C. College, Mumbai	05.01.2023
29.	Inaugural lecture by Dr. K. Thangaraj, Director, CDFD on “Population Genomics and Public Health” at DST STUTI workshop, School of Life Sciences, University of Hyderabad	16.01.2023
30.	Visit of students from Nagarjuna School, Sai Nagar, Nagole, Hyderabad	19.01.2023 & 20.01.2023
31.	Visit of students from Dept. of Biotechnology, School of Sciences (CPGS), Bengaluru	31.01.2023
32.	Visit of students from Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Chennai	02.02.2023
33.	Visit of students from Department of Molecular Biology and Genetic Engineering, RTM Nagpur University	08.02.2023
34.	Visit of students from EMEA College of Arts And Science, Malapuram, Kerala	09.02.2023
35.	Visit of students from G.N. Khalsa College, Matunga, Mumbai	10.02.2023
36.	Talk on Coordination of social behaviour in bacteria: How social are bacteria? (Online mode) at 5-days Faculty Development Programme (FDP) under the theme “Innovation in Health Sciences: Challenges and Future Trends” organized by Faculty of Allied Health Sciences (FAHS) SGT University, Gurugram.	14.02.2023
37.	Visit of students from Department of Clinical Biochemistry, University of Kashmir	14.02.2023
38.	Visit of Students from Department of Biotechnology, P. C. Jabin Science College, Hubli, Karnataka	23.02.2023
39.	Talk at C-DAC conference on Accelerating Biology 2023.	01.03.2023
40.	Visit of Students from Agricultural College & Research Institute, Tamil Nadu Agricultural University, Coimbatore.	01.03.2023
41.	Visit of Students from Mandsaur University	03.03.2023
42.	Visit of students from B.N.S. Science College, Dist. Ahmednagar	21.03.2023

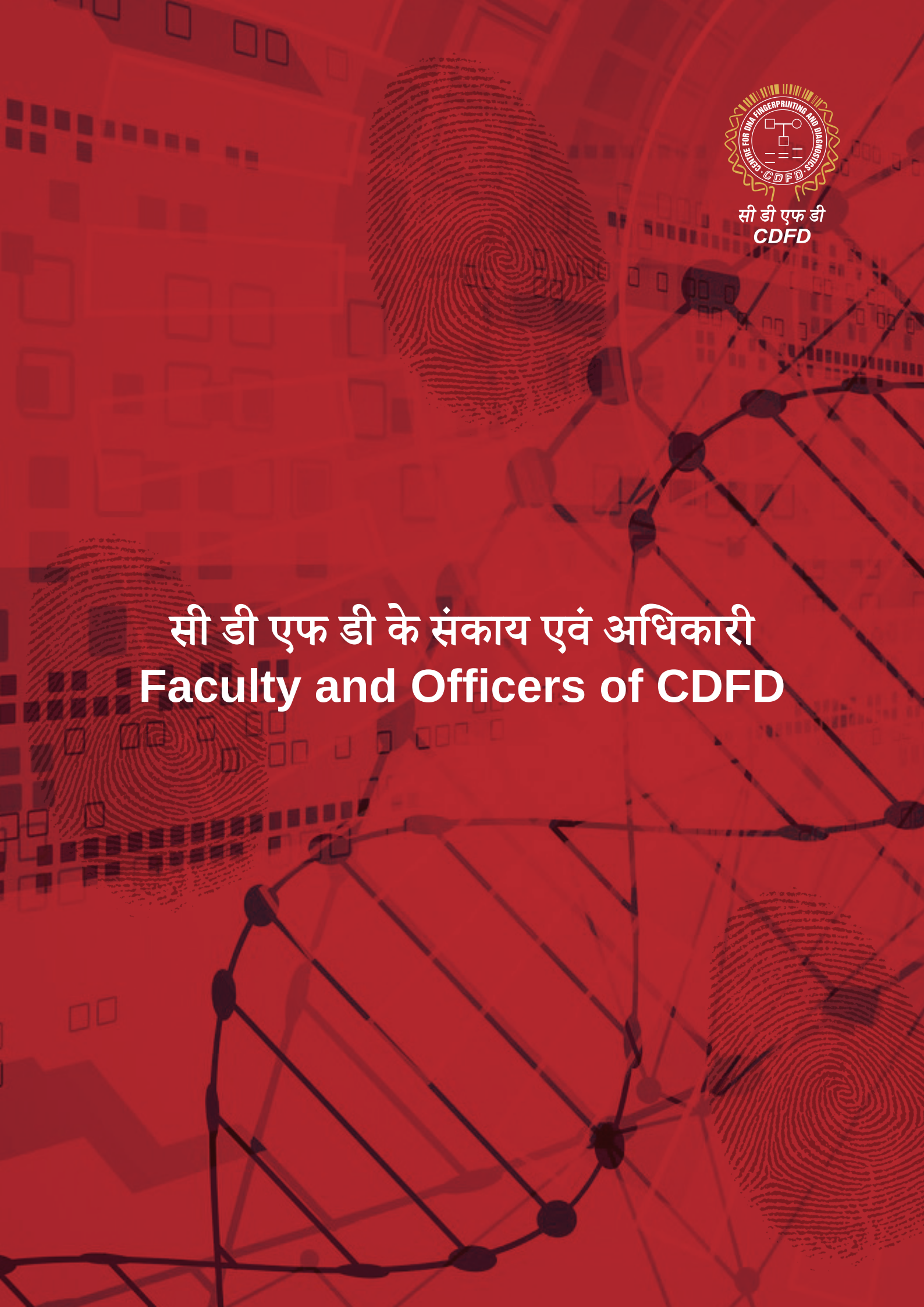


सी डी एफ डी कर्मचारियों की विदेशों में प्रतिनियुक्ति

Deputations Abroad of CDFD Personnel

List of Staff Members who had been abroad on deputation or attended the International conferences during the period from 01.04.2022 to 31.03.2023

S. No.	Name of the Employee & Designation	Duration of visit / conference		Conference attended
1.	Dr. Murali Dharan Bashyam, Staff Scientist	06.04.2022	19.04.2022	(i) To present his work during 08-12 April, 2022 at "American Association for Cancer Research (AACR) Annual Meeting 2022" held during 08-13 April, 2022 at New Orleans, Louisiana, USA. (ii) To visit Northwestern University, USA on 13.04.2022.
2.	Dr. Ashwin B Dalal, Staff Scientist	10.06.2022	20.06.2022	To attend European Society of Human Genetics Annual meeting (ESHG) held during 11-14 June, 2022 at Vienna, Austria.
		15.11.2022	17.11.2022	To attend one day scientific event focusing on population genomics scheduled to be held on 16.11.2022 at Genome Institute of Singapore (GIS), Singapore.
		27.03.2023	31.03.2023	To attend International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) meeting and Translational Research Conference held during 28-30 March, 2023 at University College London (UCL), London, UK.
3.	Dr. Dutta Usha Rani, Technical Officer	15.11.2022	17.11.2022	To attend one day scientific event focusing on population genomics scheduled to be held on 16.11.2022 at Genome Institute of Singapore (GIS), Singapore.
4.	Dr. Rashna Bhandari, Staff Scientist	20.01.2023	28.01.2023	To attend the HFSP Research Grant Review Committee Meeting held during 23-25 January, 2023.
5.	Dr. K Thangaraj, The then Director	27.03.2023	31.03.2023	To attend International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) meeting and Translational Research Conference held during 28-30 March, 2023 at University College London (UCL), London, UK.



सी डी एफ डी के संकाय एवं अधिकारी
Faculty and Officers of CDFD

Scientific Group Leaders (Faculty)

Dr. K Thangaraj
Dr. Ranjan Sen
Dr. Sangita Mukhopadhyay
Dr. Murali Dharan Bashyam
Dr. Sanjeev Khosla
Dr. Sunil Kumar Manna
Dr. Akash Ranjan
Dr. Rupinder Kaur
Dr. Ashwin B Dalal
Dr. Rashna Bhandari
Dr. Devyani Halder
Dr. N Madhusudan Reddy
Dr. Shweta Tyagi
Dr. M V Subba Reddy
Dr. Subhadeep Chatterjee
Dr. Rohit Joshi
Dr. Sardesai Abhijit Ajit
Dr. R Harinarayanan
Dr. Yathish Jagadheesh Achar
Dr. Yelagandula Ramesh
Dr. P Govindaraj
Dr. Kuldeep Verma
Dr. Ajay Kumar Mahato
Dr. Pore Pranjali Milind

Adjunct Faculty

Prof. Anuradha Lohia,	VC of Presidency University
Dr. Renu Wadhwa,	National Institute of Advanced Industrial Science & Technology
Dr. Prajnya Ranganath,	Nizam's Institute of Medical Sciences
Dr. Shagun Aggarwal,	Nizam's Institute of Medical Sciences

Other Service Group Leaders

Dr. Varsha
Mr. Vinod Kumar Mishra
Ms. M Kavita Rao
Dr. V Punnaiah
Mr. K Arun Kumar
Mr. Rabinarayan Mishra

Administrative Group Leaders

Mr. G Ravindar
Mr. E V Rao

Directors Office

Administration Section

DDO Section

Estate Section

Security Section

Finance and Account Section

Academics Section

EMPC Section

Stores and Purchase Section

Library Section

Electrical Engineering Section

Civil Engineering Section

Transport Section

Canteen Section

केन्द्र की समितियाँ
Committees of the Centre

1. Members of CDFD Society

1.	Dr. Jitendra Singh	The Hon'ble Union Minister of State (Independent Charge) of Science & Technology and Earth Sciences	President
2.	Sri Allola Indra Karan Reddy	The Hon'ble Forests & Environment and Science & Technology Minister, Telangana State	Member – Ex-officio
3.	Dr. Rajesh S Gokhale	Secretary, DBT	Member – Ex-officio
4.	Prof. Balram Bhargava	Secretary, DHR & DG, ICMR	Member – Ex-officio
5.	Dr. N Kalaiselvi	Secretary, DSIR & DG, CSIR	Member – Ex-officio
6.	Dr. Rajat Kumar	IAS, Special Chief Secretary Environment, Science & Technology Department, Telangana State	Member – Ex-officio
7.	Shri Chaitanya Murti	JS (Admin), DBT	Member – Ex-officio
8.	Shri Vishvajit Sahay	Additional Secretary & Financial Advisor, DBT	Member – Ex-officio
9.	Dr. K Thangaraj	Director, CDFD	Member – Secretary
10.	Dr. J M Vyas	Vice-Chancellor, National Forensic Sciences University	Nominated members
11.	Dr. Vineet Ahuja	Professor, Department of Gastroenterology, AIIMS, New Delhi	Nominated Member
12.	Dr. M R S Rao	Honorary Professor, Chromatin Biology Laboratory, Neuroscience Unit (NSU), Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru	Nominated Member
13.	Prof. V Nagaraja	Former President, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), and Hon. Professor, IISc, Bengaluru	Nominated Member
14.	Prof. P Appa Rao	Former Vice-Chancellor, University of Hyderabad, Hyderabad	Nominated Member
15.	Shri Dilip S Shanghvi	Managing Director, Sun Pharma, Goregaon, Mumbai	Nominated Member

2. Members of CDFD Governing Body

1.	Dr. Rajesh S Gokhale, Secretary, DBT	·	Chairperson
2.	Shri Chaitanya Murti, JS (Admin), DBT	·	Member – Ex-officio
3.	Shri Vishvajit Sahay, Additional Secretary & Financial Advisor, DBT	·	Member – Ex-officio
4.	Dr. K Thangaraj, Director, CDFD	·	Member – Ex-officio
5.	Dr. Ranjan Sen, Staff Scientist – VII, CDFD	·	Member – Ex-officio
6.	Dr. Sandhya Shenoy, Scientist –'F', DBT	·	Member – Ex-officio
7.	Dr. Onkar N. Tiwari, Scientist 'F', DBT	·	Member – Ex-officio
8.	Shri G. Ravindar, Head – Administration, CDFD	·	Member – Secretary
9.	Dr. Sanjeev Khosla, Director, CSIR-Institute of Microbial Technology (CSIR-IMTech), Chandigarh	·	Nominated Member
10.	Dr. Anurag Agrawal, Director, Ashoka University, New Delhi	·	Nominated Member
11.	Lieutenant General (Dr.) Madhuri Kanitkar, Vice Chancellor, Maharashtra University of Health Sciences, Nasik	·	Nominated Member
12.	Dr. Subeer S Majumdar, Distinguished Professor, National Institute of Animal Biotechnology (NIAB), Hyderabad	·	Nominated Member

3. CDFD Scientific Advisory Committee (SAC) – Oct. 2021

1.	Prof M R S Rao, JNCASR, Bangalore	-	Chairperson
2.	Dr. Suchita Ninawe DBT, New Delhi (DBT Representative)	-	Member
3.	Dr. Sanjeev Khosla CSIR-IMTech, Chandigarh	-	Member
4.	Dr. Anurag Agrawal CSIR-IGIB, New Delhi	-	Member
5.	Lieutenant General (Dr.) Madhuri Kanitkar Maharashtra University of Health Sciences, Nashik	-	Member
6.	Dr. Subeer S Majumdar NIAB, Hyderabad	-	Member
7.	Dr. Rajan Sankaranarayanan CSIR-CCMB, Hyderabad	-	Member
8.	Prof. Usha Vijayaraghavan IISc., Bengaluru	-	Member
9.	Prof. Suman Kumar Dhar JNU, New Delhi	-	Member
10.	Dr. Eric Green NHGRI, NIH, USA	-	Member
11.	Prof Dipshikha Chakravortty IISc., Bangalulu	-	Special Invitee
12.	Dr K Thangaraj Director, CDFD	-	Member Secretary

4. Members of CDFD Finance Committee

1.	Shri Vishvajit Sahay	Additional Secretary & Financial Advisor, DBT	Chairperson
2.	Ms. Kapavarapu Ganga	IA & AS (1981) (Retired), Former Deputy Comptroller and Auditor General, Government of India	Nominated Member
3.	Shri Atul Kumar Gupta	Former President, Institute of Chartered Accountants of India	Nominated Member
4.	Dr. G Taru Sharma	Director, NIAB, Hyderabad	Nominated Member
5.	Dr. K Thangaraj	Director, CDFD	Member –Ex-officio
6.	Dr. Onkar N Tiwari	Scientist 'F', DBT	Member –Ex-officio
7.	Shri G Ravindar	Head – Administration, CDFD	Member –Ex-officio
8.	Shri E V Rao	I/c – Finance & Accounts, CDFD	Member – Secretary

5. Institutional Biosafety Committee (IBSC)

1.	Dr. Sangita Mukhopadhyay, Staff Scientist – VII, CDFD	-	Chairperson
2.	Dr. Arvind Kumar, Principal Scientist, CCMB	-	DBT Nominee
3.	Dr. Ashwin B Dalal, Staff Scientist – VII, CDFD	-	Biosafety Officer
4.	Dr. Shweta Tyagi, Staff Scientist – VI, CDFD	-	Member Secretary
5.	Prof. Krishnaveni Mishra, Professor, UoH	-	Outside Expert
6.	Dr. Sardesai Abhijit Ajit, Staff Scientist – V, CDFD	-	Internal Expert
7.	Dr. P Govindaraj, Scientist – IV, CDFD	-	Internal Expert

6. Sexual Harassment Complaints Committee (SHCC)

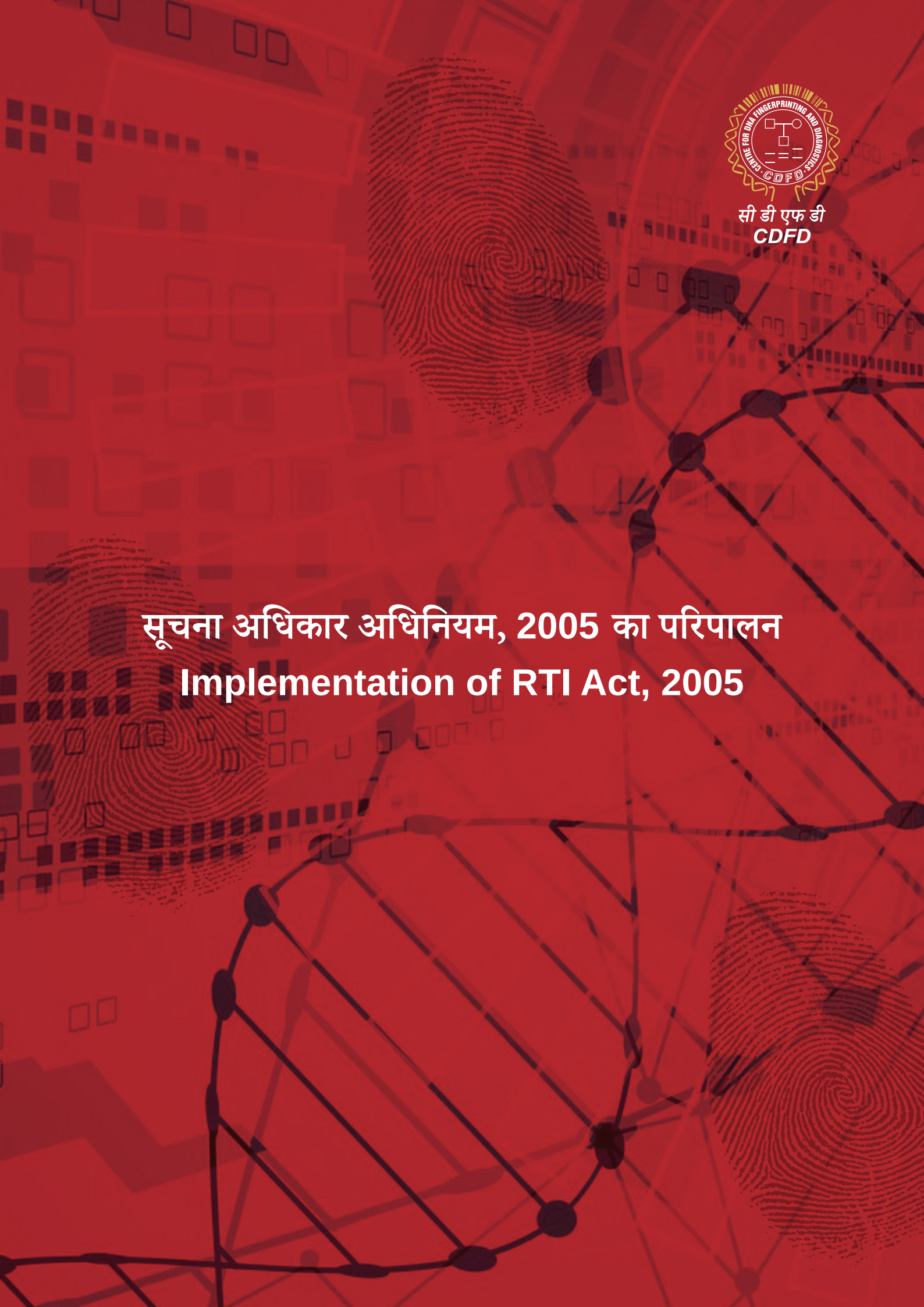
1.	Dr. Shweta Tyagi, Staff Scientist – VI	-	Chairperson
2.	Ms. Jyoti Das, Advocate, A.J.Legal	-	External Member
3.	Dr. Subhadeep Chatterjee, Staff Scientist – VI	-	Member
4.	Dr. Rohit Joshi, Staff Scientist – V	-	Member
5.	Ms. Angalena Ramachandran, Senior Technical Officer	-	Member
6.	Ms. A Kalyani, Management Assistant	-	Member
7.	Ms. T Navaneetha, Technical Officer – II	-	Member

7. Institutional Ethics Committee

1.	Prof. G B Reddy University College of Law, Osmania University, Hyderabad	-	Chairperson
2.	Prof. Sheela Prasad Associate Professor, Centre for Regional Studies, School of Social Sciences, University of Hyderabad	-	Member
3.	Dr. Mahtab S Bamji Emeritus Scientist, Dangoria Charitable Trust, Hyderabad	-	Member
4.	Mrs. Amita Kasbekar VP, Deloitte Consulting India Pvt. Ltd., RMZ, Hitech City, Hyderabad	-	Member
5.	Dr. M D Bashyam Staff Scientist – VII, CDFD	-	Member
6.	Dr. P Govindaraj, Staff Scientist – IV,CDFD	-	Member
7.	Dr. Ashwin B Dalal Staff Scientist – VII, CDFD	-	Member Secretary

8. Institutional Animal Ethics Committee (IAEC)

1.	Dr. Murali Dharan Bashyam Staff Scientist – VII, CDFD	-	Chairperson
2.	Dr. N Harishankar Ex-Scientist – G, NIN	-	Member (Main Nominee)
3.	Dr. A Gopala Reddy College of Veterinary Sciences, Ranjendra Nagar, Hyderabad	-	Member Dean (Link Nominee)
4.	Dr. Ashok Kumar Devarasetti Asst. Professor Department of Biochemistry, College of Veterinary Sciences, Rajendra Nagar, Hyderabad	-	Member
5.	Mr. A Madhava Rao Senior Advocate, High Court of Telangana	-	Member
6.	Dr. Rashna Bhandari Staff Scientist – VI, CDFD	-	Member
7.	Dr. R Harinarayanan Staff Scientist – V, CDFD	-	Member
8.	Dr. Pore Pranjali Milind Scientist – II (Veterinarian), CDFD	-	Member & Convenor



सूचना अधिकार अधिनियम, 2005 का परिपालन
Implementation of RTI Act, 2005

Implementation of RTI Act, 2005

We maintain transparency in the system and in order to achieve this we have provided following information in our website:

1) CDFD Society: Memorandum of association and rules and regulations
2) Particulars of organisation, functions and duties
3) Powers and duties of officers and employees
4) Norms for discharge of functions
5) Categories of documents held or under control
6) Formulation of policy or implementation thereof
7) Statement of the boards, councils, committees and other bodies
8) Directory of scientists, officers and employees
9) Monthly remuneration of scientists, officers and employees and system of compensation
10) Budget allocations (all plans, proposed expenditures and reports on disbursements made)
11) Execution of subsidy programmes (including amounts allocated, details and beneficiaries)
12) Names, designations and other particulars of the Public Information Officers
13) CDFD Recruitment Rules 2018-19 & Bye laws 2019.
14) Recipients of concessions, permits or authorisations granted
15) Particulars of facilities available to citizens for obtaining information (library/reading room)
16) Procedure followed in decision making process
17) Monthly RTI Returns
18) Immovable property returns statement
19) Details of CDFD purchase orders valuing more than Rs. 10 lakh
20) CDFD Policy on research misconduct
21) Procedure for handling of complaints under Public Interest Disclosure and Protection of Informers (PIDPI) Resolution to be followed by Chief Vigilance Officer (CVO)
22) Vigilance Manual

Below table gives a detailed description of the receipt of RTI cases at CDFD and their disposal.

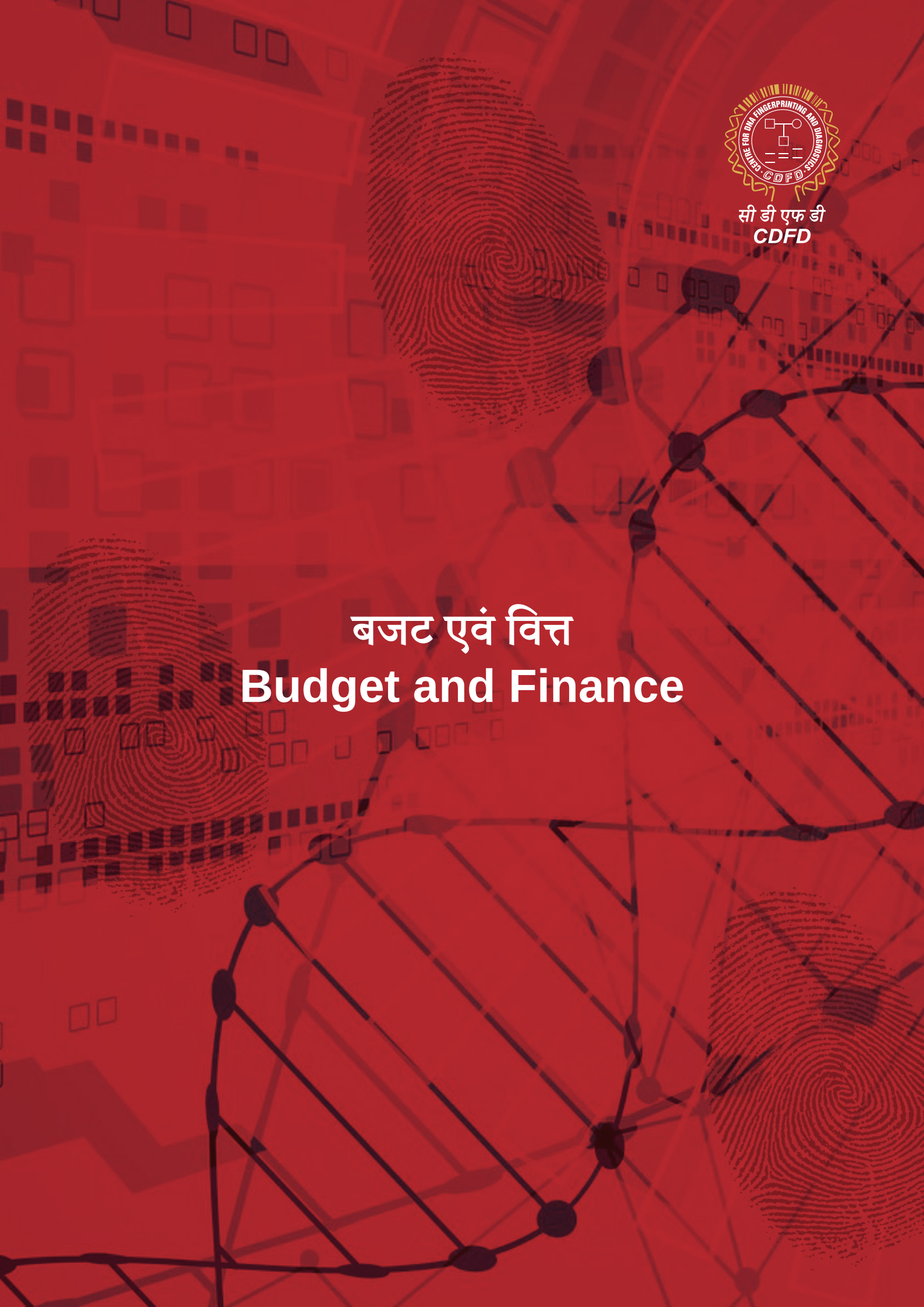
IMPLEMENTATION OF RIGHT TO INFORMATION (RTI) ACT, 2005

Dr. M.D. Bashyam
Appellate Authority

Dr. Varsha
Central Public Information Officer

Details about the RTI applications and appeals received in CDFD

As received under the RTI Act 2005	Opening Balance as on 01-04-2022	Received during the year 2022-23			Disposed off during the year 2022-23				Closing Balance as on 31-03-2023
		Received directly	Received as transfer from other Public Authorities [U/s 6(3) of Act]	Total	Decisions where applications accepted/ appeals upheld	Decisions where applications accepted/ appeals rejected	Transferred to other Public Authorities [U/s 6(3) of Act]	Total	
Applications	03	81	16	97	97	NIL	NIL	97	03
Appeals	01	11	01	12	12	NIL	NIL	12	01



बजट एवं वित्त
Budget and Finance

लेखा परिक्षक की रिपोर्ट
Auditor's Report

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

AUDITOR'S REPORT

To

The Director,
Centre for DNA Fingerprinting and Diagnostics,
Hyderabad.

We have audited the attached financial statements of **CENTRE FOR DNA FINGER PRINTING AND DIAGNOSTICS**, Hyderabad, which comprises of Balance Sheet as at 31st March 2023 and the Income & Expenditure Account and the Receipts and Payments Account for the year ended on that date annexed there to. These Financial Statements are the responsibility of the organization's management. Our responsibility is to express an opinion on these Financial Statements based on our audit.

ORGANIZATION'S RESPONSIBILITY FOR FINANCIAL STATEMENTS

The management of the organization is responsible for the preparation of these Financial Statements. This responsibility includes the design, implementation, and maintenance of Internal Control relevant to the preparation of the Financial Statements that are free from material misstatement.

AUDITOR'S RESPONISBILITY

Our responsibility is to express an opinion on these financial statements based on our Audit. We conducted our audit in accordance with the Standards on Auditing specified by ICAI. Those standards require that we comply with ethical requirements and plan and perform the Audit to obtain reasonable assurance about whether the financial statements are free from material misstatement.

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel,
Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

As part of an audit in accordance with SAs, we exercise professional judgment and maintain professional skepticism throughout the Audit. We also:

Identify and assess the risks of material misstatement of the Financial Statements, whether due to fraud or error, design and perform audit procedures responsive to those risks, and obtain audit evidence that is sufficient and appropriate to provide a basis for our opinion. The risk of not detecting a material misstatement resulting from fraud is higher than for one resulting from error, as fraud may involve collusion, forgery, intentional omissions, misrepresentations, or the override of internal control.

Evaluate the appropriateness of accounting policies used and the reasonableness of accounting estimates and related disclosures made by management.

Conclude on the appropriateness of management's use of the going concern basis of accounting and, based on the Audit evidence obtained, whether a material uncertainty exists related to events or conditions that may cast significant doubt on the Company's ability to continue as a going concern. If we conclude that a material uncertainty exists, we are required to draw attention in our auditor's report to the related disclosures in the Financial Statements or, if such disclosures are inadequate, to modify our opinion. Our conclusions are based on the Audit evidence obtained up to the date of our Auditor's report. However, future events or conditions may cause the Company to cease to continue as a going concern.

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

H.No. 3-6-84/12&13, Flat # 402, Legend Venkatesha, Beside Taj Mahal Hotel, Narayanguda, Hyderabad - 500 029. Telangana, India.
Phone : 040-40151768, E-mail: kprauditors@yahoo.com ; www.kprandco.com

We communicate with those charged with governance regarding, among other matters, the planned scope and timing of the audit and significant audit findings, including any significant deficiencies in internal control that we identify during our Audit.

Report on the Audit of the standalone Financial Statements

Qualified Opinion

We have audited the financial statements of "Centre for DNA Fingerprinting and Diagnostics", which comprises the Balane Sheet as at 31st March 2023, and the Income and Expenditure Account for the year then ended, and notes to the financial statements, including a summary of significant accounting policies.

In our opinion and to the best of our information and according to the explanations given to us, except for the effects of the matter described in the Basis for Qualified Opinion section of our report, the accompanying financial statements give a true and fair view of the financial position of the Institute as at 31st March 2023, and of its financial performance for the year ended in accordance with the Accounting Standards issued by the Institute of Chartered Accountants of India (ICAI).

Basis for Qualified Opinion is based on the following reservations:

1. We have observed that current year Objection Register (OB) has an outstanding amount of Rs. 2.99 Crores in respect of from advances for equipment, consumables and other advances which must be reconciled. As per the previous audit report objection register has advances to the tune of Rs.8.61 crores as on 31-03-2022 in respect of advances for equipment, consumables and other advances are pending for clearance. Management has initiated steps to clear such outstanding balances and to the tune of Rs. 7.47 Crores have been reconciled and identified.

BRANCH OFFICE : 47-9-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

K. PRAHLADA RAO & CO.

CHARTERED ACCOUNTANTS

1-2-288/41, FLAT N0.301, 302, SURYA RESIDENCY,
INDIRA PARK 'X' ROADS, DOMALGUDA, HYDERABAD - 500 029.
PHONE : 040-66661496, 66661497, FAX : 27662749, E-MAIL: kprauditors@yahoo.com

2. Bank reconciliations are not completed for the past years in SBI Current Account, and there are still unidentified long outstanding entries to be reconciled.

3. We are unable to comment on Fixed Assets as physical verification of assets is not done by the management and there are differences found in the Fixed Asset Register maintained by the Stores department with the Fixed Asset schedule in the books of accounts.

For K. Prahlada Rao & Co.,
Chartered Accountants
F R No-002717S

K. Prahlada Rao
Partner
M.No -018477
UDIN: 23018477BGPXCG5499
Place : HYDERABAD
Date : 26.06.2023

BRANCH OFFICE : 47-3-28/19, FLAT NO. 2, II FLOOR, BHARAT TOWERS,
5th LINE, DWARAKA NAGAR, VISAKHAPATNAM - 530 016.
PHONE NO'S. : 0891-2549314, 2546419

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

BALANCE SHEET AS ON 31st MARCH 2023

(Amount - ₹.)

	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
Corpus / Capital Fund	1	2,35,81,40,082	2,26,41,72,167
Reserves and Surplus	2	10,64,02,302	8,28,93,791
Earmarked / Endowment funds	3	27,89,44,983	12,99,85,300
Secured Loans & Borrowings	4	-	-
Unsecured Loans & Borrowings	5	-	-
Deffered Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	18,84,80,262	17,95,31,233
TOTAL		2,93,19,67,628	2,65,65,82,491
ASSETS			
Fixed Assets	8	1,70,21,59,844	1,70,68,03,510
Investments- From Earmarked / Endowment Funds	9	-	-
Investments - Others	10	23,51,78,007	12,07,78,393
Current Assets, Loans, Advances etc.	11	99,46,29,776	82,90,00,588
Other Payments		-	-
TOTAL		2,93,19,67,628	2,65,65,82,491
Significant Accounting Policies	24		
Contingent Liabilities and Notes on Accounts	25		

I/c - FINANCE & ACCOUNTS
CDFD

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी वित्त एवं लेखाधिकारी/In-charge Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार का स्वायत्त संस्थान)
(Dept. of Biotechnology Ministry of Science & Technology, Govt. of India)
इनर रिंग रोड, उप्पल, हैदराबाद-500 039, तेलंगाना
Inner Ring Road, Uppal, Hyderabad-500 039. Telangana State.

PLACE : HYDERABAD
DATE :

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
M.No - 018477
UDIN : 23018477BGXCG5499

K. PRAHLADA RAO & CO. Chartered Accountants FRN No: 002717S Hyderabad

DIRECTOR
CDFD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

INCOME & EXPENDITURE FOR THE YEAR ENDED 31st MARCH 2023

(Amount - Rs.)

	Schedule		Current Year		Previous Year
INCOME					
Income from Sales/Services	12		2,35,08,511		1,44,40,947
Grants/Subsides	13		40,67,66,764		42,41,00,000
Fees/Subscriptions	14		-		-
Income from Investments	15		-		83,63,715
Income from Canteen	16		37,49,630		-
Interest Earned	17		1,02,07,685		34,37,793
Other Income	18A		2,68,46,419		1,24,36,093
Increase/(decrease) in stock of Finished goods and works-in-progress	19		-		-
TOTAL (A)			47,10,79,009		46,27,78,548
EXPENDITURE					
Establishment Expenses	20 A		23,01,91,704		18,58,41,038
Administrative Expenses	21		18,85,12,245		22,62,57,347
Expenditure on Grants, Subsides etc.	22				-
Canteen Purchases	23		44,14,566		-
Depreciation (Net Total at the year-end -corresponding to Schedule 8)		7,51,15,558		7,31,31,720	
Less:Transferred to Grants-in-Aid		7,51,15,558	-	7,31,31,720	-
Provision for Expenses			1,19,18,029		-
Provision For Salaries			1,05,39,493		94,91,937
TOTAL (B)			44,55,76,037		42,15,90,322
Balance being excess of Income over Expenditure (A-B)			2,55,02,972		4,11,88,226
Transfer to Special Reserve (Specify each)					
Transfer to/from General Reserve			2,35,08,511		1,44,40,947
BALANCE BEING SURPLUS/(DEFLICT) CARRIED TO CORPUS/CAPITAL FUND			19,94,461		2,67,47,279
SIGNIFICANT ACOUNTING POLICIES	24				
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS	25				

I/c - FINANCE & ACCOUNTS
CDFD

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखा/IC-Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार का स्वायत्त संस्थान)
(An Autonomous Institute of Biotechnology Ministry of Science & Technology, Govt. of India)
इनर रिंग रोड, उप्पल, हैदराबाद-500 039, तेलंगाना
Inner Ring Road, Uppal, Hyderabad-500 039. Telangana State.

PLACE : HYDERABAD
DATE :

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
M.No - 018477
UDIN : 230184

K. PRAHLADA RAO & CO. Chartered Accountants FRN No: 002717S Hyderabad

DIRECTOR
CDFD

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2023

(Amount - Rs.)

RECEIPTS	Current Year	Previous Year	PAYMENTS	Current Year	Previous Year
1.Opening Balances			1. Expenses		
a) Cash in hand	-	-	a) Establishment Expenses (SCH 20)	19,21,19,252	18,58,41,098
b) Bank Balances			b) Administrative Expenses (SCH 21)	18,85,12,245	22,62,57,547
i) In current accounts	12,67,51,547	9,60,19,069			
ii) In deposit accounts	-	-			
iii) Savings accounts	36,48,70,047	32,81,44,981			
2. Grants Received			2. Payments made against funds for various projects		
a) From Government of India	40,67,68,764	44,06,00,000	(Name of the fund or project should be shown along with		
b) From State government			the particulars of payments made for each project)		
c) From other sources (details)	-		Projects	11,88,08,896	15,18,01,888
(Grants for capital & revenue			Projects - CIB Clearances	-	-
exp. To be shown seperately)			Research Fellow Associates Payments	-	-
Research Fellow Associates Receipts		5,09,000	Grant in Aid Capital(Refund)	38,984	1,83,00,000
Project Grants	19,81,01,790	18,91,53,169			
Project Grants - CIB Clearances	-	-			
Capital Grant	8,00,00,000				
			3. Investments and deposits made		
			a) Out of Earmarked/Endowement funds	5,00,00,000	8,43,99,614
			b) Out of Own Funds (Investments-Others)		
			c) Deposit made in CPF A/C	-	
3. Income on investments from			4. Expenditure on Fixed Assets & Capital Work-in-Progress		
a) Earmarked/Endow. Funds	-	88,63,715	a) Purchases of Fixed Assets:		
b) Own Funds (Oth. Investment)			Books & Journals	24,019	
Investments EnCashed	2,00,00,000	27,48,99,614	Equipment -Lab/Office/Furniture	6,99,65,107	7,38,86,114
			b) Expenditure on Capital Work-in-Progress:	-	-
4. Interest Received					
a) On Bank deposits	1,02,07,685	34,97,799			
b) Loans, Advances etc			5. Refund of surplus monies/Loans		
c) Interest on Computer Advance, Conveyance advance and HBA		29,612	a) To the Government of India	18,99,76,000	-
d) Interest on CPF			b) To the State Government		-
			c) To other providers of funds	1,27,38,058	-
5. Other Income(Specify)					
a) Analysis Charges	78,47,011	1,44,40,847	6. Finance Charges (Interest)		
b) Other Income (SCH 18)	2,63,76,793				
6. Any Other Receipts(Give Details)			7. Other Payments (Specify)		
i-Remittances (Annexure-A)	78,25,483	2,99,88,122	Advances (Annexure-D)	4,60,72,642	10,84,85,564
CPF-SUB,Arrears and adv.Refund	78,35,280	53,08,649	i-Remittances (Annexure-E)	7,10,20,118	3,43,89,680
Sundry Receipts	-	15,29,022	CPF A/c	1,27,02,552	3,40,40,902
Application Fee		22,251	New Pension Scheme		2,34,46,854
Sale Of Tender Forms		14,500			
Contingencies Students	69,613	66,150	8. Closing Balances		
NGC Charges		15,25,341	a) Cash in hand	-	-
i-Remittances (Annexure-B)	2,24,84,727				
NPS		-	b) Bank Balances		
Advance/Refunds/Recovery/Adj		6,21,19,078	i) In current accounts	40,04,89,563	12,67,51,547
Canteen sales	37,49,630	-	ii) In deposit accounts	-	-
		-	iii) Savings accounts	19,09,97,896	36,48,70,047
TOTAL	1,82,24,05,329	1,43,56,70,995	TOTAL	1,82,24,05,329	1,43,56,70,995

I/c - FINANCE & ACCOUNTS
CDFD

वि सुकन्या/M.V. SUKANYA
प्रभारी वित्त एवं लेखा अधिकारी
डी.एन.ए. फिंगरप्रिंटिंग एवं डिटेक्शन केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भा.स.प्र.सं. का स्वायत्त संस्थान)
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PLACE : HYDERABAD
DATE :

For K.PRAHLADA RAO & CO
CHARTERED ACCOUNTANTS
F R No - 002717S

K.PRAHLADA RAO
M.No - 018477
UDIN : 23018477BGPX[illegible]5499

K.PRAHLADA RAO & CO. CHARTERED ACCOUNTANTS FRN No. 002717S Hyderabad

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Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
BALANCE SHEET AS ON 31st MARCH 2023

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 1 - CORPUS/CAPITAL FUND :				
Balance as at the begining of the year		2,26,41,72,167		2,22,79,02,694
Add : Contribution towards Corpus/Capital Fund				
CDFD Core - Plan (Non-Recurring)	8,00,00,000		-	
Capitalised portion of Capital Expenditure of projects	8,71,27,506	16,71,27,506	8,26,53,914	8,26,53,914
Less : Depreciation For the Year		7,51,15,558		7,31,31,720
Less : Fund returned to DBT		38,494		-
Add : Excess of Income over Expenditure		19,94,461		2,67,47,279
BALANCE AS AT THE YEAR - END		**2,35,81,40,082**		**2,26,41,72,167**

एम. वि. सुकन्या/M.V. SUKANYA
वित्त एवं लेखा/Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
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K. PRAHLADA RAO & CO. Chartered Accountants Firm No: 002717S Hyderabad

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Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 2 -RESERVES AND SURPLUS :				
1.Capital Reserve :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
2.Revolution Reserve :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
3.Special Reserves :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
4.General Reserve - Lab Reserve :				
As per last Account	8,28,93,791		6,84,52,844	
Addition during the year	2,35,08,511		1,44,40,947	-
Less : Deductions during the year	-	10,64,02,302	-	8,28,93,791
Total		**10,64,02,302**		**8,28,93,791**

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखा/IC-Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 3 - EARMARKED/ENDOWMENT FUNDS :				
(Refer Annexures)				
(a) Opening balance of the Funds		**12,99,85,300**		**15,05,56,541**
(b) Additions to the Funds :				
i. Donations /grants (net)	36,13,48,942		16,91,53,163	
ii. Income from investments made on account of funds	-		-	
iii. Other additions (OB clearances)	55,91,147	36,69,40,089	-	16,91,53,163
TOTAL (a+b)		49,69,25,389		31,97,09,704
(c) Utilisation/Expenditure towards objective of funds				
(i) Capital Expenditure (Refer Annexures I & II)				
- Fixed Assets	8,71,27,506		8,26,53,914	
- Others	-	8,71,27,506	-	8,26,53,914
- Total				
(ii) Revenue Expenditure (Refer Annexures I & II)				
- Salaries, Wages and allowances etc.	3,09,22,342		3,94,33,914	
- Rent/ REFUNDS	-		-	
- Project Consumales & Other Expenses	8,71,92,491	11,81,14,833	6,26,98,364	10,21,32,278
Total				
(iii) Refund of Project grants		1,27,38,068		49,38,212
TOTAL (c)		21,79,80,407		18,97,24,404
NET BALANCE AS AT THE YEAR-END [(a + b)-c]		**27,89,44,983**		**12,99,85,300**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 7 - CURRENT LIABILITIES AND PROVISIONS :				
A. CURRENT LIABILITIES				
1. Acceptances				
2. Sundry Creditors	19,15,903		7,49,361	
3. Advances Received				
4. Interest accured but not due on:				
5. Statutory Liabilities:				
TDS on salaries	19,08,090		18,02,993	
TDS others	3,13,472		4,25,246	
Service Tax	24,325		24,325	
Works Tax	16,80,631		16,80,631	
PM Cares Fund Payable	5,95,935		5,95,935	
6. Other current Liabilities			-	
CDFD.CP Fund A/C	13,50,78,350		13,94,08,534	
Contract Staff security deposit	5,78,049		5,78,049	
ECCS	-		9,520	
EMD	24,95,235		21,73,734	
Festival Advance	450		450	
GSLI	120		2,596	
House Building Advance	1,29,831		1,29,831	
Lab Security Deposit & Hostel Security Deposit	16,75,741		15,29,741	
LIC	1,24,643		20,22,145	
Performance Guarantee Deposit	6,71,786		39,436	
Other Payables	2,85,070		-	
Other Out Standing Liabilities	18,68,560		64,37,523	
PT Payable	22,550		43,650	
Public Provident Fund	3,91,158		3,91,158	
Royalty & Consultancy	15,31,642		15,31,642	
Security Deposit	1,03,97,467		1,03,13,709	
STAFF BENEVOLENT FUND	1,34,073		1,15,333	
GST TDS	3,44,536			
Gst	32,18,343			
ECCS Loan Payable	3,45,380			
ECCS Subscription Payable	1,55,000			
Staff Welfare Fund Payable	1,28,400			
Contributory Provident Fund	8,000			
TA-DA-Hon within India [Advance]			33,754	17,00,39,296
TOTAL (A)		16,60,22,740		17,00,39,296
B.PROVISIONS				
1. For Taxation		-		-
2. Gratuity		-		-
3. Superannuation/Pension		-		-
4. Accumulated Leave Encashment		-		-
5. Trade Warranties/Claims		-		-
6. Others - Salary & Other Provisions		2,24,57,522		94,91,937
TOTAL (B)		2,24,57,522		94,91,937
TOTAL (A+B)		**18,84,80,262**		**17,95,31,233**

वि. सुकन्या/M.V. SUKANYA
प्रभारी वित्त एवं लेखा/IC-Finance & Accounts
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Biotechnology Ministry of Science & Technology, Govt. of India)
र रिंग रोड, उप्पल, हैदराबाद-500 039, तेलंगाना
g Road, Uppal, Hyderabad-500 039. Telangana State.

K. PRUHLADA RAO & CO. Chartered Accountants FRN No: 002217S Hyderabad

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - ₹.)

SCHEDULE 8 - FIXED ASSTES :	GROSS BLOCK					DEPRECIATION				NET BLOCK	
	Cost/valuation As at begining of the year	Addition during Before September	After September	Deductions during the year	Cost/valuation at the year end	As at the begining of the year	during the year	On Deducti during year	Total up to the year end	As at the Current year end	As at the Previous year end
INTANGIBLE ASSETS											
1. Firewall Cord	-	-			-	-	-	-	-	-	-
TANGIBLE ASSETS											
A. FIXED ASSETS:											
1. LAND:											
a) Freehold	39,00,000	-			39,00,000	-	-	-	-	39,00,000	39,00,000
b) Leasehold	-	-			-	-	-	-	-	-	-
2. BUILDINGS											
a) On Freehold Land	22,00,52,369	6,63,912	6,76,246		22,13,92,527	14,97,12,234	71,34,217	-	15,68,46,451	6,45,46,076	7,03,40,135
b) On Leasehold Land	-	-			-	-	-	-	-	-	-
c) Ownership Flats/Premises	-	-			-	-	-	-	-	-	-
d) Superstructures on Land not belongs to the entity	-	-	-		-	-	-	-	-	-	-
3. PLANT MACHINERY & EQUIPMENT	1,06,59,08,418	-	-		1,06,59,08,418	69,28,32,967	5,59,61,318	-	74,87,94,285	31,71,14,133	37,30,75,451
4. VEHICLES	56,23,446		-		56,23,446	40,82,298	2,31,172	-	43,13,470	13,09,976	15,41,148
5. FURNITURE, FIXTURES	1,91,80,055	10,81,445	2,64,300		2,05,25,800	1,39,56,005	6,43,765	-	1,45,99,769	59,26,031	52,24,050
6. OFFICE EQUIPMENT	1,37,53,462	3,37,99,902	3,84,53,241	1,66,55,615	6,93,50,990	1,15,65,157	57,83,882	-	1,73,49,039	5,20,01,951	21,88,305
7. COMPUTER/PERIPHERALS	77,53,174	50,52,809	6,42,618		1,34,48,601	30,51,134	40,30,463	-	70,81,597	63,67,004	47,02,039
8. SOFTWARE	22,03,138	7,28,286	7,82,336		37,13,760	16,68,575	6,61,606	-	23,30,181	13,83,579	5,34,562
9. ELECTRIC INSTALLATIONS	-	22,73,042	26,85,350		49,58,392	-	5,42,358	-	5,42,358	44,16,034	-
10. LIBRARY BOOKS	2,13,46,712	24,019	-		2,13,70,731	2,13,45,137	25,595	-	2,13,70,731	-	1,576
11. TUBEWELLS & WATER SUPPLY	-	-			-	-	-	-	-	-	-
12. OTHER FIXED ASSETS	92,78,548	-			92,78,548	86,03,988	1,01,184	-	87,05,172	5,73,376	6,74,560
Airconditioning works	-	-			-	-	-	-	-	-	-
Aluminium partition work	-	-			-	-	-	-	-	-	-
DG Set	-	-			-	-	-	-	-	-	-
Paintings	-	-			-	-	-	-	-	-	-
Typewriters	-	-			-	-	-	-	-	-	-
Miscellaneous non consumables	46,400	-			46,400	-	-	-	-	46,400	46,400
Other Assets	-	-			-	-	-	-	-	-	-
EMB Net	-	-			-	-	-	-	-	-	-
TOTAL	1,36,90,45,722	4,36,23,415	4,35,04,091	1,66,55,615	1,43,95,17,613	90,68,17,494	7,51,15,558	-	98,19,33,052	45,75,84,560	46,22,28,226
B. CAPITAL WORK-IN-PROGRESS	1,24,45,75,284			-	1,24,45,75,284	-	-	-	-	1,24,45,75,284	1,24,45,75,284
TOTAL	**2,61,36,21,006**	**4,36,23,415**	**4,35,04,091**	**1,66,55,615**	**2,68,40,92,897**	**90,68,17,494**	**7,51,15,558**	-	**98,19,33,052**	**1,70,21,59,844**	**1,70,68,03,510**

एम. वि. सुकन्या/M.V. SUKANYA
अपर वित्त एवं लेखा/AC-Finance & Accounts
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Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 10 - INVESTMENTS - OTHERS :		
(Annexure-J)		
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures and Bonds : UTI Bonds		
5. Subsidiaries and Joint Ventures	-	-
6. Others (to be specified) - STDRs,(CPF),CDFD CP FUND A/C	23,51,78,007	12,07,78,393
TOTAL	**23,51,78,007**	**12,07,78,393**

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखा/IC-Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार का स्वायत्त संस्थान)
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K. PRAHLADA RAO & CO. Chartered Accountants Regn No. 0027175 Hyderabad

डॉ. के. थंगराज
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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 11 - CURRENT ASSETS AND LOANS , ADVANCES & OTHER ASSETS :	Current Year		Previous Year	
A. CURRENT ASSETS				
1. Inventors				
a) Stores and Spares	-		-	
b) Loose Tools	-		-	
c) Stock-in-trade				
Finished Goods	-		-	
Work-in-progress	-		-	
Raw Materials	-	-	-	-
2. Sundry Debtors:				
a) Debts Outstanding for a period exceeding six months	43,70,688	-	-	
b) Others-Life Membership Fees	-	43,70,688	1,69,236	1,69,236
3. Cash balances in hand (including cheques/drafts and imprest)				
4. Bank Balances:				
a) With Scheduled Banks:				
-On Current Accounts	40,04,89,563		12,67,51,547	
-On Deposit Accounts (includes margin money)	-		8,43,99,614	
-On Savings Accounts	29,09,37,896	69,14,27,458	36,43,70,047	57,55,21,208
b) With non-Schedules Banks:				
-On Current Accounts	-	-	-	
-On Deposit Accounts	-	-	-	
-On Savings Accounts	-	-	-	-
5. Post Office-Savings Accounts				
TOTAL (A)		69,57,98,146		57,56,90,444
B.LOANS, ADVANCES AND OTHER ASSETS				
a) Staff (Annexure-L)	3,89,302		4,38,162	
b) Other Entities engaged in activities/objectives similar to that of the Entity	-	3,89,302	-	4,38,162
2. Advances and other amounts recoverable in cash or in kind or for value to be received				
a) On Capital Account (Annexure-H)	15,35,79,968		12,24,75,096	
b) Prepayments - Deposits (Annexure-I)	2,04,77,878		2,19,55,233	
c) TDS Receivable	12,30,223		9,43,109	
d) Others (Annexure-K)	9,37,53,451		9,17,52,127	
Misc. Expenditure (Schedule 21B)	2,94,00,808	29,84,42,328		23,71,25,565
a) Income Accrued				
a) On Investments from Earmarked/Endowments Funds	-		-	
b) On Investments - Others	-		-	
c) On Loans and Advances	-		-	
d) Others	-	-	-	-
4. NPS Contribution		-		1,57,46,417
TOTAL (B)		29,88,31,630		25,33,10,144
TOTAL (A+B)		**99,46,29,776**		82,90,00,588

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 12 - INCOME FROM SALES/SERVICES :	Current Year	Previous Year
1) Income from sales		
a) Sale of Finished Goods	-	-
b) Sale of Raw Material	-	-
c) Sale of Scraps	-	-
2) Income from Services		
a) Labour and Processing Charges	-	-
b) Professional/Consultancy Services (Analysis & Diagnostics Charges)	1,40,65,880	1,44,40,947
c) Agency Commission and Brokerage	-	-
d) Maintenance Services (Equpiment/Property)	-	-
e) Others (Specify)	94,42,631	-
TOTAL	**2,35,08,511**	**1,44,40,947**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 13 - GRANTS/SUBSIDES : (Irrevocable Grants & Subsides Received)		
1) Central Government (DBT Plan Grant-in-Aid)	40,67,66,764	42,41,00,000
2) State Government(s)	-	-
3) Government Agencies	-	-
4) Institutions/Welfare Bodies	-	-
5) International Organisations	-	-
6) Others (Specify)	-	-
TOTAL	**40,67,66,764**	**42,41,00,000**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 16 - INCOME FROM CANTEEN ETC. :		
1) Income from Canteen	37,49,630	-
2) Income from Publications	-	-
3) Others (Specify)	-	-
TOTAL	**37,49,630**	-

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31ST MARCH, 2023

(Amount - Rs.)

SCHEDULE 17 - INTEREST EARNED :	Current Year	Previous Year
1) On Term Deposits		
a) With Schedule Banks	1,02,07,685.00	1,18,01,508.00
b) With Non-Scheduled Banks	0.00	0.00
c) With Institutions	0.00	0.00
d) Others	0.00	0.00
2) On Saving Accounts		
a) With Schedule Banks	0.00	0.00
b) With Non-Scheduled Banks	0.00	0.00
c) post Office Savings Accounts	0.00	0.00
d) Others	0.00	0.00
3) On Loans		
a) Employees/Staff	0.00	0.00
b) Others	0.00	0.00
	0.00	
4) Interest on Debtors and Other Receivables	0.00	0.00
TOTAL	**1,02,07,685.00**	**1,18,01,508.00**
Note :- Tax deducted at source to be indicated		

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF RECEIPTS & PAYMENTS AS ON 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 18 - OTHER INCOME :	Current Year	Previous Year
1) Profit on Sale/disposal of Assets:	-	-
a) Owned assets	-	-
b) Assets acquired out of grants, or received free of cost	-	-
2) Export Incentives realized	-	-
3) Fees for Miscellaneous Services	-	-
4) Miscellaneous Receipts	15,146	-
5) Other Receipts	4,49,304	29,612
Sundry Receipts	1,16,58,335	15,27,832
Application Fee and collaboration with UCL	6,81,438	22,251
Sales Of Tender Forms	-	14,500
Tution fee& Ra Ship Stipend& road show& hfsp	4,70,253	-
Fellowship Income	1,31,02,275	-
Contingencies(Students)	-	66,150
NGC CHARGES	-	1,07,75,748
TOTAL	**2,63,76,751**	**1,24,36,093**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 18A - OTHER INCOME :	Current Year	Previous Year
1) Profit on Sale/disposal of Assets:	-	-
a) Owned assets	-	-
b) Assets acquired out of grants, or received free of cost	-	-
2) Export Incentives realized	-	-
3) Fees for Miscellaneous Services	-	-
4) Miscellaneous Receipts	620	-
5) Other Receipts	-	29,612
Sundry Receipts	1,25,91,833	15,27,832
Application Fee and collaboration with UCL	6,81,438	22,251
Sales Of Tender Forms	-	14,500
Tution fee& Ra Ship Stipend& road show& hfsp	4,70,253	-
Fellowship Income	1,31,02,275	-
Contingencies(Students)	-	66,150
NGC CHARGES	-	1,07,75,748
TOTAL	**2,68,46,419**	**1,24,36,093**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF RECEIPTS & PAYMENTS AS AT 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 20 - ESTABLISHMENT EXPENSES :	Current Year	Previous Year
a) Salaries and Wages	11,77,58,192	10,83,64,486
b) Allowances and Bonus	47,48,595	37,82,204
c) Contribution to Provident Fund	-	48,65,960
d) Contribution to Other Fund (NPS)	5,99,06,058	6,34,03,715
e) Staff Welfare Expenses - Medical charges	58,90,793	44,68,946
f) Expenses on Employees Retirement and Terminal Benefits	12,56,213	-
g) Others (specify) -	25,59,401	9,55,727
h) EPF Employer Contribution		-
TOTAL	**19,21,19,252**	**18,58,41,038**

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखा/IC-Finance & Accounts
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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

SCHEDULE 20A- ESTABLISHMENT EXPENSES :	Current Year	Previous Year
a) Salaries and Wages	15,27,14,112	10,83,64,486
b) Allowances and Bonus	43,21,564	37,82,204
c) Contribution to Provident Fund	-	48,65,960
d) Contribution to Other Fund (NPS)	6,34,49,621	6,34,03,715
e) Staff Welfare Expenses - Medical charges	58,90,793	44,68,946
f) Expenses on Employees Retirement and Terminal Benefits	12,56,213	-
g) Others (specify) -	25,59,401	9,55,727
h) EPF Employer Contribution		-
TOTAL	**23,01,91,704**	**18,58,41,038**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF RECEIPTS & PAYMENTS AS ON 31st MARCH 2023

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 21 - OTHER ADMINISTRATIVE EXPENSES :		
1) Purchases	4,89,47,085.58	5,93,10,036.00
2) Electricity and power	3,56,99,268.00	2,93,10,318.00
3) Water charges	33,92,034.00	46,01,208.00
4) Insurance	81,997.00	1,02,030.00
5) Repairs and maintenance	1,87,55,811.00	3,74,09,337.00
6) Rent, Rates and Taxes	52,71,503.00	2,42,50,837.00
7) Vehicles Running and Maintenance	24,80,279.00	19,89,713.00
8) Postage, Telephone and Communication Charges	21,25,227.00	37,25,061.00
9) Printing and Stationary	11,65,360.00	3,12,896.00
10) Travelling and Conveyance Expenses	1,35,042.00	4,194.00
11) Expenses on Seminar/Workshops	42,066.00	8,33,600.00
12) Testing Charges	8,82,000.00	0.00
13) Expenses on Fees & Renewals	4,43,029.00	3,94,623.00
14) Auditors Remuneration	1,01,000.00	96,000.00
15) Hospitality Expenses	5,42,934.00	4,75,973.00
16) Professional Charges	1,82,820.00	9,333.00
17) Advertisement and Publicity	16,32,474.00	14,30,354.00
18) Bank Charges	64,896.00	27,486.00
19) Security & Cleaning Contract Charges	74,00,491.00	2,63,32,162.00
20) CDFD Contract Staff Salaries	53,96,069.00	57,89,267.00
21) Other Contingencies	33,285.00	6,39,392.00
22) AMC	40,47,349.00	21,29,949.00
23) Other Research Expenses	57,47,751.00	80,73,940.00
24)Office Books	28,004.00	4,368.00
26)Contract Staff	0.00	16,42,699.00
27)Manpower Outsourcing(Staff)	3,07,38,511.00	81,39,376.00
28) Prior Period Expenses	0.00	92,23,195.00
29)Meeting	53,766.00	
30)Works and Servives	6,54,125.00	
31)Student contingency fund	8,27,975.00	
32)Consultancy Service	16,19,027.00	
33)Legal Expenses	1,13,150.00	
34)webnar /hosting	96,525.00	
35)Incentives	1,69,400.00	
36)TADA	11,01,336.00	
37)Clearing and Custom	8,69,115.00	
38)Administrative expenses	41,14,750.00	
39)Accredation fee	18,700.00	
40)Foundation day expenses	10,54,338.00	
41)Guest house expenses	19,700.00	
42)Diagnostic expenses	4,11,000.00	
43)Diem charges	31,671.00	
44)Exhibition	3,90,964.00	
45)Other Misc Expenses	2,31,853.00	
46)Hiring charges	1,77,738.00	
	12,20,826.00	
TOTAL	**18,85,12,244.58**	**22,62,57,347.00**

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निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD, Hyderabad.

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

SCHEDULES FORMING PART OF INCOME & EXPENDITURE AS ON 31st MARCH 2023

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 23 - CANTEEN PURCHASES ETC. :		
1) Purchase of Canteen Items	44,14,566	-
	-	-
	-	-
TOTAL	**44,14,566**	-

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखा/IC-Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार का स्वायत्त संस्थान)
(Dept. of Biotechnology Ministry of Science & Technology, Govt. of India)
इनर रिंग रोड, उप्पल, हैदराबाद-500 039, तेलंगाना
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K. PRAHLADA RAO & CO. Chartered Accountants FRN No: 002717S Hyderabad

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CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2023

Annexure: A Forming part of Receipts and Payment a/c

RECEIPTS

Previous Year	Particulars	Current Year
	I-Remittances	
44,32,029.00	TDS other than Salaries	13,33,761.00
20,66,779.00	TDS on Salaries	18,25,032.00
1,76,170.00	Works Tax	
81,000.00	LIC	
3,336.00	GSLI	
0.00	Gst Tds	29,11,972.00
2,01,250.00	Professional Tax	1,86,350.00
0.00	salary linked Allowance	10,09,516.00
2,15,15,752.00	Others (I-Remittances)	
0.00	Health Insurance	
0.00	ECCS	
15,11,806.00	Contract Staff security deposit	
0.00	STAFF BENEVOLENT FUND	
0.00	EPF	
0.00	GST	5,58,852.34
2,99,88,122.00		**78,25,483.34**

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CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2023

Annexure: B Forming part of Receipts and Payment a/c

RECEIPTS

Previous Year	Particulars	Current Year
	Advance refunds/recovery/Adjst.	
1,31,397.00	Advance for Expenses- purchases by Staff	1,39,424.00
0.00	Other Research Expenses	
0.00	Computer Advance [Research Fellows]	1,52,279.00
0.00	Other Advances	1,00,235.00
0.00	Cdfd Staff Welfare	13,200.00
90,48,414.00	Debtors	1,35,56,002.00
0.00	court attachment Amount	61,336.00
96,03,050.00	Margin Money	
0.00	EMD	3,25,000.00
1,62,77,008.00	Equipment [Advance]	
0.00	Festival Advance	
4,56,202.00	GDA [Others]	9,72,241.00
4,30,402.00	General Deposits And Advances	0.00
0.00	Human Resource Develpment - Training of Staff - Conferences [Advance]	
0.00	Inter Bank Transfer	
2,36,822.00	Lab Security Deposit & Hostel Security Deposit	2,91,000.00
1,96,136.00	LTC [Advance]	3,41,790.00
0.00	Miscellaneous Salary [Advance]	
1,04,14,549.00	Diagnostic Collab with NIMS	
0.00	Pay of Establishment [Advance]	
0.00	Revolving Advance	
0.00	Security Deposit	1,47,725.00
0.00	TA Abroad [Advance]	50,000.00
0.00	TA-DA-Hon within India [Advance]	
4,000.00	Trainee Security Deposit	3,000.00
1,53,04,098.00	Misc Advances	3,38,819.00
0.00	Workshop & Conference	
0.00	Leave Salary & Pension	
17,000.00	Performance Guarantee Deposit	6,43,204.00
	LTC Margin Money	53,49,472.00
6,21,19,078.00		**2,24,84,727.00**

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CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2023

Annexure: D Forming part of Receipts and Payment a/c

PAYMENTS

Amount in Rs.

Previous Year	Particulars	Current Year
	Advances	
13,28,021.00	Advance for Expenses- purchases by Staff	14,94,121.00
	Chemicals [Advance]	
50,000.00	Computer Advance [Research Fellows]	
	Performance Guarantee Deposit	10,854.00
1,57,79,739.00	Consumables, glassware and Spares [Advance]	
	EXPENSES Payable	61,200.00
45,000.00	EMD	3,500.00
8,32,17,078.00	Equipment [Advance]	13,56,816.00
91,704.00	GST	
2,11,684.00	GDA [Others]	20,000.00
	staff welafre and Benovalant fund	11,500.00
	Lab Security Deposit & Hostel Security Deposit	1,45,000.00
	Man Power Services	1,88,028.00
1,14,800.00	LTC [Advance]	8,28,367.00
46,85,525.00	Margin Money LC	2,65,39,257.00
5,55,035.00	Others [Advances]	34,28,692.00
	Others [Contingencies Advance]	3,62,267.00
	Debtors	17,930.00
	collabaration with NIMS	1,04,12,049.00
5,12,390.00	Diagnostic Services CCMB	
7,61,737.00	Security Deposit	63,967.00
	GPF	6,00,000.00
	TA Abroad [Advance]	1,02,448.00
26,025.00	TA-DA-Hon within India [Advance]	
1,83,293.00	TDS Receivable	2,71,514.00
4,000.00	Trainee Security Deposit	500.00
7,60,585.00	Student CF	
56,088.00	TA Arrears	
1,02,860.00	Workshop & Conference	
	GIS	1,32,500.00
	GSU	22,132.00
10,84,85,564.00		**4,60,72,642.00**

एम. वि. सुकन्या/M.V. SUKANYA
वित्त एवं लेखा/Finance & Accounts
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Director, CDFD, Hyderabad.

CENTER FOR DNA FINGERPRIINT AND DIAGNOSTIC SERVICES

For the Year Ended 31st MARCH 2023

Annexure: E Forming part of Receipts and Payment a/c

PAYMENTS

Amount in Rs.

Previous Year	Particulars	Current Year
	I-Remittances	
18,000.00	Contract Staff security deposit	
42,78,473.00	ECCS	43,78,050.00
0.00	CANTEEN PURCHASES	44,05,238.00
0.00	ECCS subscription	16,67,000.00
9,74,008.00	HRA DA Arrears	
1,45,000.00	Health Insurance	
2,04,70,891.00	TDS on Salaries	2,66,47,551.00
17,15,092.00	LIC	16,74,611.00
7,20,000.00	Others (I-Remittances)	5,557.00
4,43,700.00	Professional Tax	4,36,950.00
0.00	Contributory Provident Fund	1,08,690.00
23,09,012.00	GST	2,84,68,324.00
3,30,014.00	CPF advance recovery	1,33,332.00
29,85,490.00	TDS on Others	0.00
	Creditors	2,50,589.00
0.00	GST TDS	28,44,224.00
3,43,89,680.00		**7,10,20,116.00**

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प्रभारी वित्त एवं लेखा/IC-Finance & Accounts
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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2023

Annexure: H Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
4,310.00	Advance for Expenses- purchases by Staff	0.00
0.00	Advances [Previous Years]	0.00
0.00	Chemicals [Advance- Proj Consumables)	0.00
0.00	Computer Advance [Research Fellows]	0.00
0.00	Computer Advance [Staff]	0.00
1,55,33,582.00	Consumables, glassware and Spares [Advance]	1,08,06,559.00
0.00	Conveyance Advance	0.00
10,43,28,715.00	Equipment [Advance]	11,74,31,858.00
1,446.00	Festival Advance	1,446.00
0.00	Health Insurance	0.00
0.00	Liveries & Blankets [Advance]	0.00
26,07,043.00	LTC [Advance]	25,92,170.00
0.00	LC Margin Money	2,27,47,935.00
0.00	Miscellaneous Salary	0.00
0.00	NPS Subscription	0.00
0.00	Office Equipment [Advance]	0.00
0.00	Others [Advances]	0.00
0.00	Pay of Establishment	0.00
0.00	Rent [Advance]	0.00
0.00	Research Fellows-Associates	0.00
0.00	Revolving Advance	0.00
0.00	Scientific Workshops - Symposiums - Seminars [Advance]	0.00
0.00	Telephone [Advance]	0.00
0.00	Trainee Security Deposit	0.00
0.00	Transport maintenance [Advance]	0.00
0.00	Workshop & Conference	0.00
12,24,75,096.00		**15,35,79,968.00**

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखाधिकारी/Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
Centre for DNA Fingerprinting and Diagnostics
(जैव प्रौद्योगिकी विभाग, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भा...
(Dept. of Biotechnology Ministry of Science & Technology, ...
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For PRAHLADA RAO & CO
Chartered Accountants
Firm No: 007175
Hyderabad

डॉ. के. थंगराज
Dr. K. Thangaraj
निदेशक, सी डी एफ डी, हैदराबाद
Director, CDFD,Hyderabad

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2023

Annexure: I Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	DEPOSITS	
2,09,85,107.00	General Deposits And Advances	2,04,77,878.00
9,70,126.00	GDA[Others]	
2,19,55,233.00		**2,04,77,878.00**

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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2023

Annexure: K Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
4,310.00	Advances [Previous Years]	4,310.00
2,14,35,274.00	Chemicals [Advance]	1,14,35,274.00
1,14,49,940.00	Consumables, glassware and Spares [Advance]	1,14,49,940.00
96,50,585.00	Diagnostics Collabration With NIMS	2,00,62,634.00
1,92,678.00	ECCS	1,92,678.00
0.00	GST on Reverse Charge	
6,63,909.00	Health Insurance	6,63,909.00
1,58,200.00	Liveries & Blankets [Advance]	1,58,200.00
26,53,205.00	LTC [Advance]	26,53,205.00
854.00	Magzines [Advance]	854.00
1,54,333.00	Others (I-Remittances)	1,54,333.00
0.00	Others [Advances]	17,98,693.00
17,453.00	Others [Contingencies Advance]	4,99,833.00
1,63,800.00	Printing & Stationery [Advance]	1,63,800.00
3,04,569.00	Rent [Advance]	3,04,569.00
4,37,58,727.00	Research Fellows-Associates	4,36,06,448.00
1,00,482.00	Revolving Advance	
8,000.00	Scientific Workshops - Symposiums - Seminars [Advance]	8,000.00
3,75,400.00	Software [Advance]	3,75,400.00
84,913.00	TA Abroad [Advance]	1,37,361.00
50,000.00	Telephone [Advance]	50,000.00
25,000.00	Trainee Security Deposit	22,500.00
11,510.00	Transport maintenance [Advance]	11,510.00
4,88,985.00	Workshp & Conference	
9,17,52,127.00		**9,37,53,451.00**

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प्रशासी वित्त एवं लेखा/C-Finance & Accounts
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CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS

For the Year Ended 31st MARCH 2023

Annexure: L Forming part of Balance sheet

Amount in Rs.

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
2,23,011.00	Advance for Expenses- purchases by Staff	2,27,321.00
1,35,445.00	Computer Advance [Research Fellows]	1,35,445.00
46,528.00	Computer Advance [Staff]	26,536.00
33,178.00	Conveyance Advance	
4,38,162.00		**3,89,302.00**

एम. वि. सुकन्या/M.V. SUKANYA
प्रभारी-वित्त एवं लेखाधिकारी/In-charge-Finance & Accounts
डी.एन.ए. फिंगरप्रिंटिंग एवं निदान केन्द्र
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Director, CDFD, Hyderabad.

सी डी एफ डी
CDFD

फोटो गैलरी

Photo Gallery

MoU between CDFD and Institute of Bioresources and Sustainable Development (IBSD), Imphal on 20.04.2022

Talk by Shri Justice Gunda Chandraiah, Chairperson, Telangana State Human Rights Commission in connection with Dr. B.R. Ambedkar Jayanthi celebrations on 22.04.2022

Swachhta Pakhwada
from 01.05.2022 to 15.05.2022

Workshop on Human Forensic DNA Fingerprinting: From Crime Scene to Courtroom from 23.05.2022 to 27.05.2022

Hy-Sci 2022 by the students, for the students on14.05.2022

MoU between CDFD, Hyderabad and AIG Hospital, Hyderabad on 01.06.2022

Participation in the Biotech Startup Expo2022, New Delhi organized by BIRAC & DBT from 09.06.2022 to 10.06.2022

International Yoga Day celebrations on 21.06.2022

Hands on workshop on Next Generation Sequencing from 20.06.2022 to 24.06.2022

Dr Lalji Singh Memorial Lecture by Prof Subramaniam Ganesh, Department of Biological Sciences and Bioengineering, IIT Kanpur on 05.07.2022

Open Day celebrations on 06.07.2022

Har Ghar Thiranga Compaign and 75th Independence Day Celebrations on 15.08.2022

Hands on workshop on Clinical Applications of Cytogenetics and Molecular Cytogenetics from 22.08.2022 to 27.08.2022

Hindi Diwas Samaroh on 14.09.22

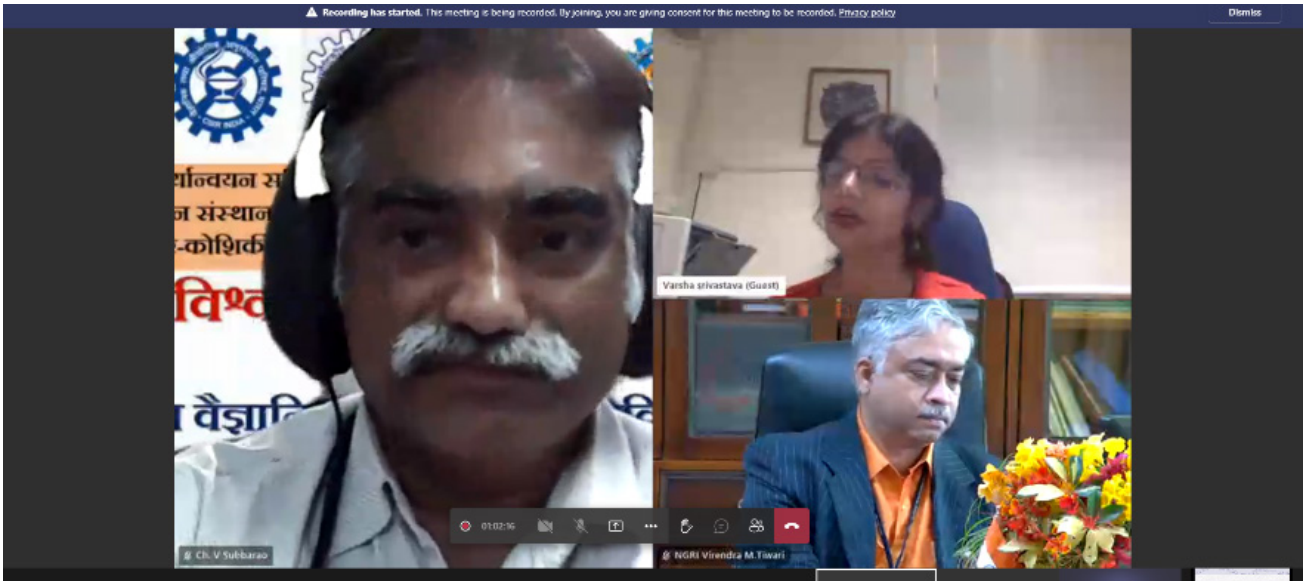
Fit India Freedom Run 3K as part of Azadi ka Amrit Mahotsav from 14.10.2022 & 21.10.2022

Ayurveda Day Celebrations on 25.10.2022

Launching of Mission Programme on Pediatric Rare Genetic Diseases by
Dr. Rajesh S Gokhale, Secretary DBT in the presence of Media on 01.11.2022

Hands-On Workshop on Human Forensic DNA Fingerprinting from 31. 10. 2022 to 04. 11. 2022

Governing Council meeting on 01.11.2022



Participation in Vishwa Hindi Diwas

MoU with Organisation for Rare Diseases (ORDI), Bangaluru on 11.01.2023

Participation in ISF, Hyderabad Public School, Begumpet, Hyderabad from 20.01.2023 to 22.01. 2023

Foundation Day (28 January 2023)

Foundation Day lecture by Shri Sanjeev Sanyal, Member, Economic Advisory Council to the Prime Minister of India & Secretary, Government of India in the August presence of Dr. Rajesh Gokhale, Secretary, DBT, Govt. of India.

Lecture entitled: “The Civilizational Importance of Intellectual Risk-Taking”

Participation in IISF, at MANIT Bhopal from 21.01.2023 to 23.01. 2023

DBT Symposia on opportunities for frontier research collaborations by the Human Frontier Science Program (HFSP) on 11.02.2023


Participation in BioAsia-2023 at HICC, Hyderabad, from 24.02.2023 to26.02.2023




Hands-on Workshop on - Long Range Genome Sequencing by Nanopore Technology from 27.02.2023 to 03.03.2023.

Women's Day Celebrations on 09.03.2023

Open Day on 01.03.2023

MoU with Govt. of Goa and CDFD on 09.03.2023

डीएनए फिंगरप्रिंटिंग एवं निदान केन्द्र

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