DR. D. Y. PATIL BIOTECHNOLOGY & BIOINFORMATICS INSTITUTE
DR. D. Y. PATIL VIDYAPEETH, PUNE
(Deemed to be University)
(Re-accredited by NAAC with a CGPA of 3.62 on a four- point scale at 'A' Grade)
(Category I University approved by UGC)
(An ISO 9001: 2015 & 14001:2015 Certified University),
Institute supported by DST-FIST & Approved by AICTE, Govt. of India

Date: 08/04/2021

A REPORT ON

ADVANCED WORKSHOP ON GENE CLONING EXPRESSION AND CHARACTERIZATION

(GCEC 2021)

Organized by: Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Pune

Day & Date: Tuesday & Wednesday, April 06 & 07, 2021

Time: 09:30 to 16:30

Platform: Online on Zoom

Organizing Chairman and Convener: Dr. J. K. Pal, Director, DYPBBI, Pune

Coordinator: Dr. Tanushree Banerjee, Associate Professor, DYPBBI, Pune

Co-Coordinator: Dr. Neelu Nawani, Professor, DYPBBI, Pune

Honorable Guests:

Chief Patron: Dr. P.D. Patil, Chancellor, Dr. D. Y. Patil Vidyapeeth, Pune
Guest-of-Honor: Dr. (Mrs.) Smita Jadhav, Hon. Trustee and Director, Dr. D. Y. Patil Vidyapeeth, Pune
Chief Guest: Dr. N. J. Pawar, Hon. Vice-Chancellor, Dr. D. Y. Patil Vidyapeeth, Pune
Distinguished Guest: Dr. R. R. Bhonde, Director Research, Dr. D. Y. Patil Vidyapeeth, Pune
Chief Guest (Valedictory): Dr. Srikanth Tripathy, Director, Medical Research, Dr D Y Patil Medical College Hospital and Research Centre

Participants: 163 nos., from all over India

Objectives:

1. Demonstration of Central Instrumentation Facility funded by DST-FIST
2. Demonstration of various applications of Equipment at CIF with complete guidelines on their usability, software functions, and real time problem solving

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WORKSHOP OVERVIEW

DAY 1

INAUGURAL FUNCTION

The function began with offering prayers through Saraswati Vandana, followed by felicitation of the dignitaries, a welcome address, inaugural address and some wise words by the guests and concluded by a vote of thanks.

Insight:

GCEC 2021 was a national level workshop designed for students, researchers and faculty, initially conceptualized for providing a hands-on experience on advanced equipment in the facility developed at the institute, funded and supported by DST FIST. However, due to the pandemic situations, the workshop was eventually conducted online, but in an effective way so that the participants could get the real feel of handling the instruments and the equipment.

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Four sessions were conducted, one for each equipment/instrument. The application specialists from the makers of the instruments explained the instrument design, principles, working and uses. This was followed by the study design and demonstration of an experiment using the same instrument, by the instrument in charge and their respective teams.

The sessions also had cloning methodologies and *in silico* characterization explained.

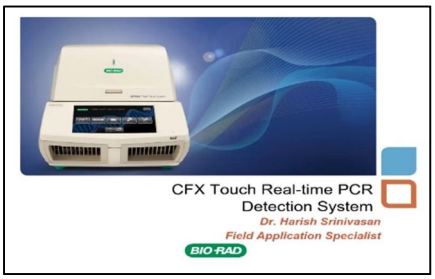
SCIENTIFIC SESSIONS

SESSION 1

CLONING METHODOLOGIES:

- Conducted by Dr. Amit Ranjan
- Concepts of central dogma of life, cloning, types of cloning, gene cloning and steps involved in gene cloning were explained. Applications of gene cloning were mentioned.
- Demonstration included RNA isolation using TRIzol method, estimation of total RNA with multimode plate reader, performing denaturing agarose gel electrophoresis for separation of RNA, cDNA synthesis using RT PCR, amplification of the target gene CD151 using its specific probes, double restriction digestion along with vector using Hind III and KPN I, ligation of gene into the expression vector, transformation into the competent cells (*E.coli* K12), their culturing in media containing Kanamycin and screening of the cloned colonies through colony PCR.

REAL-TIME PCR DETECTION SYSTEM: Model: CFX96 TouchTm, Company: Bio – Rad



Equipment design

- Application specialist: Dr. Harish Srinavasan
- Brief introduction about the difference between PCR and RT PCR, stating the advantage of the latter being able to quantify the PCR products as the reaction is in process.

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- Various mechanisms of detection of the amplified gene like detection chemistry using dyes, Taqman based chemistry (probe- primer) were explained.
- A sample data of probe-based detection with an example of covid sample process was shown.
- The instrumentation part covered its components- optical block, thermal cycler, block design, thermal gradient, optical technology, filter sets, detection, modes of scanning etc.
- Setting up the program using inbuild software and analysis of data were shown through the actual interface.

Study design and demonstration

- Conducted by Dr. Priyanka Guru
- An experiment using the CFX96 touch RT PCR was demonstrated with an aim "To confirm overexpression of the target gene CD151" where it was checked if the cloned gene was expressed at the transcript level.
- mRNA of the target gene was isolated from the transfected cells- MFC7, which was used for cDNA synthesis and quantification using RT PCR through probe-based detection, specific to the target gene. A reference gene GAPDH was also studied.
- Sample was set up using transfected cells (MCF7) for target and reference gene, and a control of un transfected cells for same genes.
- Reaction was set up using master mixes, placed in the thermocycler, protocol was set up and the reaction was initiated. On completion, the results were analyzed for the Cq values, concluding that the gene was expressed in the transfected cells.

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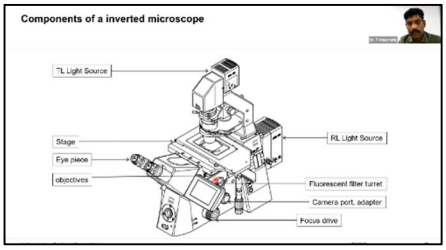
SESSION 2

INVERTED RESEARCH MICROSCOPE: Model: Axio Observer 5, Company: Carl Zeiss



Equipment design

- Application specialist: Dr. T. Amarnath
- Fundamentals of microscopy, nature of light and its properties for designing a microscope, interaction of light with optics and magnification and resolution of a microscope were explained, along with differences between upright and inverted microscope.
- Components of the inverted microscope- Stage, eye piece, objectives, light source, filter turret, camera port, focus drive, field diaphragm, condenser etc. were elaborated.
- Contrast techniques for viewing and capturing explained phase contrast, differential interference contrast and fluorescence microscopy.
- Various filter sets and cameras that can be used for the purpose were stated.

Study design and demonstration

- Conducted by Dr. Tanushree Banerjee
- All the parts of microscope were demonstrated. Operation of the microscope was shown for observing a fixed tissue sample using immunofluorescence, to check the expression of a protein.
- Demonstration was given for microscopy on live cells, which covered DMEM/F 12 medium preparation, filtration, processing human blastoma cell line, staining the cells with trypan blue solution, cell counting, slide preparation- sterilization, treating with poly L lysin for 24hrs, permeabilization, blocking, and staining with primary and secondary antibodies, mounted with coverslip with mounting agent- DAPI.

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- The slide was observed under lower magnification first, then under higher. Images were captured for bright field then with checking signals for DAPI and Alexa 488. All the images were merged and processed. This helped localization of beta actin inside the cells.
- Similarly, microscopy on live cells: Hek293t cells transfected with clone of CDK151 gene was carried out and studied.
- Also, a study design of fluorescence microscopy experiment was explained. Steps involved in processing live cells with fluorophore included fixation, permeabilization, blocking, immunostaining with fluorophore, multicolor staining, counter staining and mounting.

A talk on The Biotalk Magazine and Competition

Ms. Deepakshi Kasar, founder of The Biotalk Magazine, was invited to speak about the magazine and the competition to be held for the participants regarding the workshop. The aim, mission, topic areas and features of the magazine were explained. The competition wherein the participants could submit an article on any of the topics covered in the two days' workshop was mentioned and the best articles would be featured in the magazine. This would promote the students in article writing experience, research and learning and networking opportunities.

With this, the day one of the workshop ended.

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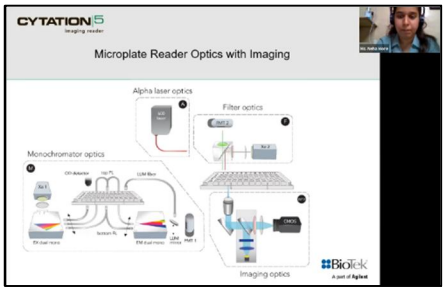
Day 2

SESSION 3

MULTIMODE MICROPLATE READER: Model: Cytation5, Company: BioTek Instruments Inc



Equipment design

- Application specialist: Ms. Neha More
- Equipment can be used for both quantitation as well as imaging.
- Range for quantifying proteins/ nucleic acids were explained- UV/Vis absorbance, fluorescence and luminescence along with components like Monochromator, filters, dichroic mirror, detection mode, variable bandwidth monochromators and alpha screen assay.
- Special feature of the equipment explained was the Take3 Multi volume plate, used for quantifying samples with volume as low as 2 microliters.
- Imaging optical path for various modes of imaging were stated. Imaging helps improve sensitivity as in signal to background ratio is increased
- Application diversity- biochemical assays, cell-based assays, kinetics, time-lapse imaging, 3D biology.
- Other features of the equipment included- advanced shaking movements, live cell assay optimization like maintaining CO_2/ O_2 levels, temperature & condensation controlling, automation, stacking etc.
- Software- Gen5 with actual interface was demonstrated for functionality, reading, analysis, imaging etc.

Study design and demonstration

- Conducted by Dr. Nilesh Sharma and Dr. Rajesh Gupta
- The equipment was explained for multimode plate reader with imaging, advanced microscopy, assay-ready, hit-picking, hybrid plate reader, micro volume analysis, sensitivity and specificity.

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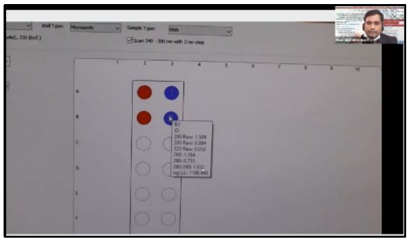

- With respect to gene cloning expression the limitations were mentioned like low volume of sample, low expression, degradation and purity can be efficiently overcome by this equipment.
- Demonstration included quantifying the nucleic acids and proteins: sample loading, reading n analysis using Take3 plate as well as 96 well microplates.

IN SILICO STRUCTURAL CHARACTERIZATION:

- Conducted by Dr. Shuchi Nagar
- After cloning and expression of a gene, a direction towards determining its function can be achieved through *in silico* characterization. This helps minimizing time and is also cost effective.
- When the gene sequence under the study becomes available, it could be used for determining the protein sequence, checking if the structure is available, designing the structure, and eventually predicting the function of the protein.
- Demonstration was given for using the tools. The gene sequence was analyzed using NCBI- BLAST to check if the sequence of the target gene was already present in the database, exact match was chosen considering the parameters like e Value, identity etc.
- The protein sequence obtained from above was used for finding similarity, predicting the structure and function. The best match was chosen and used for modelling.
- Since high similarity sequence was not available, threading was preferred over homology modelling, using HH pred. The sequence was pasted and a 3D structure was obtained.
- Lastly, the quality of the protein generated was checked using SAVES and compared with the similar proteins.

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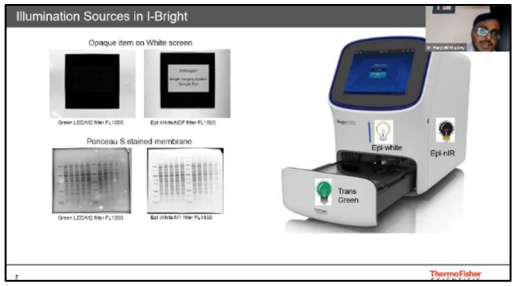
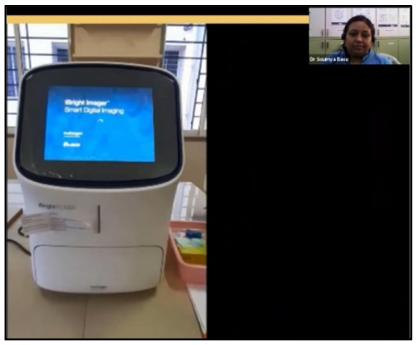
IMAGE ANALYZER: Model: IBright, Company: Thermo Fisher Scientific



Equipment design

- Application specialist: Dr. Punyatirtha Dey
- The equipment can be used for imaging gels, plates, blots, transgenic organisms etc.
- Features with respect to the technology explained were simple & intuitive use, multi-detection platform, automation, resolution, technology and quantitation.
- The hardware components covered illumination sources (trans and epi), filters (excitation and emission) and camera.
- Detailed features of binning were explained.
- The analysis that can be performed with the equipment include background correction, absolute and relative quantification, molecular weight estimation and normalization.

Study design and demonstration



- The study design included expression analysis of the protein using multimode imaging system and validation of cloned CD151 protein expression.
- Western blot analysis was used for identifying the expressed protein using labelled antibodies. Detailed experiment was explained and demonstrated which included cell lysis for extraction of proteins by physical or chemical disruption, determining the concentration of proteins with multimode plate reader, SDS PAGE gel preparation, loading of samples, running them on SDS-PAGE for separation of the proteins, transferring the proteins from gels to the PVDF membrane through western blotting, blocking for non-specific binding, treatment of the blots with primary and secondary antibody, detection of antibody- protein complex by chemiluminescence.
- Beta actin was used as a loading control. Detection by chemiluminescence was carried out usingIBright image analyzer, images were captured, files were exported and analyzed.

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With this, the scientific sessions ended. And the workshop proceeded to the valedictory function

VALEDICTORY FUNCTION

The valedictory function began with a workshop report summarizing the scientific sessions in brief, followed by welcome of the chief guest, valedictory address by the chief guest, feedback on the workshop by some participants, concluded by an address by the director, followed by a vote of thanks.

END

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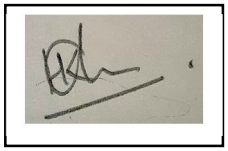
Report prepared by

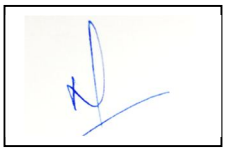


Ms. Harsha Chandwani
Scientific Administrative Officer, DYPBBI, Pune

Workshop Coordinator

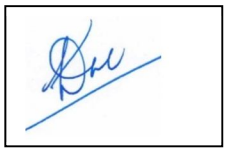
Dr. Tanushree Banerjee
Associate Professor, DYPBBI, Pune

Workshop Co-Coordinator



Dr. Neelu Nawani
Professor, DYPBBI, Pune

Workshop Organizing Chairman and Convener



Dr. J. K. Pal
Director, DYPBBI, Pune