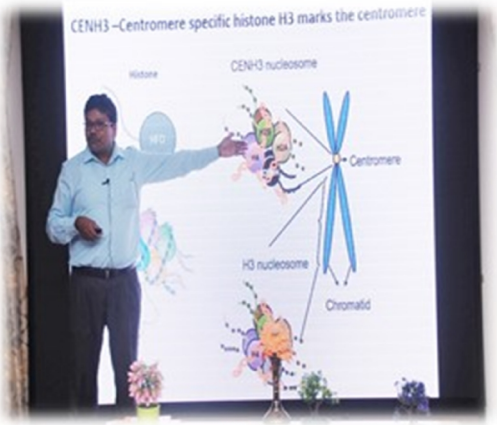
DBT SPONSORED WORKSHOP ON

CROP GENOME EDITING USING CRISPR/Cas9




DBT Sponsored workshop on
Crop Genome Editing using CRISPR/Cas9
September 05 - 09, 2017
Organized by
TAL SAMY UNIT FOR PLANT TISSUE CULTURE AND MOLECULAR BIOLOGY
DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY
LOYOLA COLLEGE (AUTONOMOUS), CHENNAI - 600034

Department of Biotechnology sponsored workshop for young scientists, academicians and research scholars working in the area of Plant Biotechnology and Molecular Genomics, at TAL Samy Unit of Plant Tissue Culture and Molecular Biology, Department of Plant Biology and Biotechnology, Loyola College (Autonomous), Chennai, Tamil Nadu, India

CROP GENOME EDITING USING CRISPR/Cas9

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MEETING REPORT

DBT sponsored workshop on **Crop Genome Editing using CRISPR/Cas9** was organized between September 05 - 09, 2017 (5 days) by TAL Samy Unit of Plant Tissue Culture and Molecular Biology, Department of Plant Biology and Biotechnology, Loyola College, Chennai to provide hands-on training to professors, young scientists and researchers in the emerging area of New Plant Breeding Techniques. The main objective of the training was to provide adequate skills to plant researchers and in-depth guidance for sgRNA design, construction of CRISPR vectors, PEG-mediated transfection in protoplasts and mutation analysis of genome-edited crops.

The workshop started with an inaugural function on 5th September, 2017 at the Department of Plant Biology and Biotechnology, Loyola College, Chennai. Dr. R. Ravindhran, Convener & Head of the Department welcomed the gathering. Rev. Dr. M. Arockiasamy Xavier, S.J., Principal and Prof. T. A. Lourdusamy, Former Vice-Principal and Dr. S. Vincent, Dean of Research, Loyola College, felicitated the gathering. Dr. Ravi Maruthachalam Asst. Professor, School of Biology, CET Transit

Campus IISER-TVM, Trivandram, Kerala delivered the inaugural address for the training titled '***Editing centromeres to produce haploid plants'***. A training manual authored and edited by Dr. R. Ravindhran, titled **'Crop Genome Editing using CRISPR/Cas9'** with standardized protocols and supporting theoretical notes was released during the inauguration and the same was distributed to the participants. Dr. R. Ravindhran, Convener of the training course highlighted the dynamics of the training course.

The training course mainly consisted of the following types of activities: Thematic lectures, Special Lectures and hands-on training. Totally Sixteen participants (Professors, Research scholars and mid-career scientists) from various Institutions and Universities across the state (Tamil Nadu) actively participated in the training. Thematic lectures and workshops covered a substantial range of topics, examining numerous aspects used in genome editing, tools for designing the sgRNAs and advanced cloning techniques.

In the special lecture (1) **Dr. Ravi Maruthachalam**, Asst. Professor, School of Biology, CET Transit campus IISER-TVM delivered lecture on "**Engineering centromeres to induce uniparental genome elimination to produce haploids in plants**". In his lecture he explained about the modulation of Centromere – kinetochore complex that lead to the production of Haploid seeds. (2) **Dr. M. Harikrishnan**, Senior Research Scientist, Pondicherry Biotech Pvt Ltd briefed about "**Tools and Resources for Genome Engineering in Plants**". His lecture was focused on Several resources and Tools used in genome engineering and also stated the Mechanism of gene editing using CRISPR/Cas9. (3) **Dr. X. Baskaran**, Post Doctoral Fellow Sun Yat Sen University, P. R. China, discussed on "**Apomictic plants and**

their mechanism". He highlighted the advantages of producing Apomictic plants in ferns. (4) **Dr. S. Kirankumar**, Assistant Professor, Department of Genetic Engineering, SRM University, Kattankulathur, emphasized on "**CRISPR/Cas9 mediated mutagenesis in Zebrafish**". He discussed about generating Transgenic lines using CRISPR/Cas9 mediated Knock-in strategy and also the testing efficiency of *fh* CRISPR by PCR-RFLP analysis. (5) **Dr. R. Sathishkumar**, Associate Professor, Plant Genetic Engineering Laboratory, Department of Biotechnology, Bharathiar University, Coimbatore spoke on "**Gene Cloning and Plant Genetic Transformation**". He discussed about the advancements in Gene cloning (i.e) Gateway Cloning system and its key benefits. (6) **Dr. A. Vinoth**, Assistant Professor, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai presented on "**Recent Advancements in CRISPR/Cas9 Genome Editing**". He emphasized on Modular Cloning System for Multiplex gene editing and Direct delivery of purified CRISPR/Cas9 ribonucleoproteins (RNPs) to protoplast generating DNA free genome editing in Plants.

The thematic lectures were mainly focused on current and advanced techniques in genome engineering, production of transgene free genome editing crops through Protoplast culture, Tools and resources for genome engineering in Plants as well as Animals, Efficient gene cloning techniques and recent advancements in genome Editing. All the lectures were followed by hands-on-training with the assistance of four laboratory supporting staff. Most of the sessions were interactive where participants were given the liberty to share their ideas and suggestions. They were also encouraged to handle the processing on their own under the guidance of supporting staff.

In the hands-on training session, participants were given a general introduction on CRISPR/Cas9 and were trained to design a Single Guide RNA using CRISPR-P and CRISPR-P V2.0 followed by Cloning of CRISPR vector (Oligo duplex), Restriction Digestion using BsaI Enzyme, Ligation using Ligation Mighty mix, Transformation of Ligated Product to *E.coli* (TOP10), PCR analysis of transformed colonies, Isolation of Plasmid, Quantification of Plasmid using Qubit Flurometer. Isolation of Plant Protoplast using Macrozyme R-10 and Cellulase R-10, Protoplast cell count in hemocytometer, PEG-mediated Protoplast transfection, DNA isolation from Protoplast using CTAB buffer, PCR analysis of Protoplast DNA and Mutant Analysis.

The training course was concluded on 9th September 2017, with a valedictory function presided over by Dr. S. Vincent, Dean of Research, Loyola College, Chennai. Participants gave a written feedback about the training and certificates were distributed to the participants.

Dr. R. Ravindhran
Convener