

Genome Editing Technologies

Genome editing techniques can be exploited to engineer genome by insertions, deletions, modifications or replacements of DNA segments in a site specific location in the genome of a living organism. Such techniques lead to development of genetically engineered organisms for biomedical and agricultural applications. In past few years, recognizing the potential of such technologies to study and manipulate the genome, DBT has been engaged in promoting research and innovation in the area of genome engineering and its applications in both animals and plants.

More than 40 new R&D projects have been initiated with different research institutes and laboratories. Research work in this direction has helped in development of haploid inducer line, and enhancement of seed meal quality in *Brassica juncea* through CRISPR/Cas mediated genome editing. The CRISPR-based editing has been applied to study the regulatory region and expanded triplet repeats as therapeutic approach in India's most common hereditary ataxias. Other genome editing tools are used for generating semen favoring production of cows, and establishment of an efficient platform for precise genome editing in rice and wheat. Such techniques have been used to study the both biotic and abiotic stress in different crops grown in India.



Genome editing using the latest genome editing tools such as CRISPR/Cas 9 system

Some of the outstanding accomplishment of genome editing tools achieved by different research institutes/laboratories funded by DBT is discussed.

At All India Institute of Medical Sciences (AIIMS), New Delhi, scientists have identified MicroRNAs (miRNAs) as biomarkers in chronic pancreatitis patients with risk for developing pancreatic cancer. The defective phospholipid signaling has been found to be involved in developmental defects, cancers, diabetes and neurological diseases.

At National Centre for Biological Sciences (NCBS), Bengaluru, scientists are using genome editing tools to understand the role of different molecular entities involved in phosphoinositide signaling using CRISPR in *Drosophila melanogaster*. They have designed and cloned gRNA sequences which would be tested for efficacy and used for generation of a germ line knockout of each selected gene.

At team of researchers at Centre for Cellular and Molecular Biology (CCMB) are working hard on the functional analysis of Casein locus in mouse using CRISPR technology, and its role in regulation of lactation. The ECR knockout mouse generated successfully mouse may provide understanding of the use of the metabolic space for expression of pharmaceutically important gene in place of casein.

Scientist at National Centre for Cell Science (NCCS), Pune have successfully dissected the individual roles of Clta and Cltb in early mammalian development in order to understand the clathrin dependent endocytosis with help of CRISPR-Cas9-based knock-out and knock-in models. Development of such knocks out and knock in models will help understand the process of endocytosis in multiple scenario and such models can be further modified for specific disease conditions or to explore the therapeutic potential of different molecules.

Genome editing tool have been highly explored to understand deferent physiological and molecular processes in plants. Using CRISPR-Cas9 mediated genome editing tools, scientists at scientists at National Institute of Plant Genome Research (NIPGR), New Delhi, have developed a protocol on biofortification of branched chain amino acid leucine in Brassica crops in order to enhance its leucine content for nutritional requirements. The CRISPR Cas9 has been employed in engineering of potato virus Y resistance at Assam Agricultural University, Guwahati. Similarly, researchers at Punjab Agricultural University, Ludhiana are engaged in RNAi mediated gene silencing for identification of novel and potential targets in whitefly. Researchers at Indian Institute of Science (IISc), Bengaluru have developed genome-wide RNAi screens to

help researchers in understanding proteostasis, cellular senescence and chemoresistance. Genome editing of tomato using CRISPR/CAS9 system have been jointly started at University of Hyderabad, Hyderabad, Indian Institute of Horticultural Research, Bangalore, National Institute of Technology, Durgapur and University of Delhi South Campus, New Delhi. Genes which can inhibit tomato ripening (ripening inhibitor gene) have been targeted in such study.

Researchers have also employed targeted genome editing tools to study the role of ganglioside GM2 in mediating AIG, anoikis resistance and metastasis. The microRNA modulated gene expression has been explored to study the myeloma genome. Microarray based comparative genomic hybridization analysis is used to study multiple myeloma (MM). Several studies have been carried out to characterize the novel regulators of cell survival signaling in cancer cells. The miRNA's have been fully exploited to study multiple cancers at molecular levels. Other genomics guided novel diagnostic and targeted therapeutic strategies have been developed to study gastric cancer and gene expression markers would also be investigated to understand their diagnostic potential at protein levels.

The list of studies using genome editing techniques is long and is impossible to mention all major breakthroughs at one place. However, the RNA interference (RNAi), Transcription Activator-Like Effector-Based Nucleases (TALEN), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) system are some of the most widely used genome editing approaches.

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