Genome editing using programmable nucleases: current developments from Sigma-Aldrich and the Czech Centre for Phenogenomics (CCP)

SIGMA-ALDRICH

Czech Centre for Phenogenomics

Milan Hašek lecture hall of the Institute of Molecular Genetics of the ASCR in Prague
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9:30 **Visualizing endogenous protein interactions and translocation in screening**
Dr. Rainer Ebel, Sigma-Aldrich

Insight into protein pathways is critical in the drug discovery process. Protein interactions and relocation have traditionally been studied using over-expressed cell models or cell lysates measurements. We will present the Duolink® PLA® method which allows the visualization of endogenous protein-protein interactions. Targeted gene editing methods such as Zinc Finger Nucleases can also be used to create fluorescent tagged endogenous cell lines where protein translocation can be monitored.

10:10 **Genome Editing with the CRISPR/Cas9 System**
Dr. Rainer Ebel, Sigma-Aldrich

The discovery of the type II prokaryotic CRISPR/Cas immune system has allowed for the development of an RNA guided genome editing tool that is simple, easy and quick to implement. Sigma Aldrich have developed a single vector system with a Cas9-GFP (or Cas9-RFP) and a U6-guideRNA expression cassette for CRISPR/Cas9 delivery and expression. CRISPR/Cas systems along with our CompoZR ZFN technology provide a quick and affordable solution to targeted genome editing in a wide variety of species and cell types.
We will present how Sigma CRISPRs (and ZFN technology) can be utilised to generate precisely targeted genome edits. Topics will include an overview of (i) the CRISPR system, (ii) examples of how the technology has been used for gene knockout and targeted integration, (iii) design, efficiency and specificity of CRISPR, (iv) and available CRISPR/Cas9 products for genome engineering.

10:50 **Gene & Genome manipulation in mice using programmable nucleases: a practical cookbook and examples of fast generation of mouse mutants**
Radislav Sedlacek, IMG/BIOCEV/CCP

Homologous recombination in embryonic stem cells is routinely used to mutate desired loci in the mouse genome. To establish a mutant mouse line from ES cells is a long-lasting, costly and often inefficient process. The recent development of targeting strategies based on programmable nucleases (zinc-fingers nucleases, transcription-activator-like effector nucleases (TALENs) and CRISPR/Cas9 RNA-guided nucleases) is opening new possibilities for the efficient generation of mutant mouse lines in just a fraction of time and costs.
Frame-shifts caused by insertions or deletions (Indels) are used for fast generation of knockout mice that can be generated typically within six months. Additional modifications can be introduced including insertion of ssOligonucleotides with single point mutations or dsDNA-constructs allowing the tagging of proteins or creation of fusion proteins.