Induction of mutations and introduction of reporter genes in the genome of murine embryonic stem cells with the CRISPR/Cas9 editing system

The perturbation of genetic loci in order to dissect their underlying function and determine their contribution to a phenotype has long been an efficient strategy in the field of “forward genetics”. Extensive attempts for the development of effective gene editing systems have been made in the past (cre/loxP recombination, ZFN and TALEN proteins), however their wide application was hindered due to low accuracy, specificity as well as difficulties in their synthesis. The discovery of the bacterial CRISPR adaptive immune system against viruses and its adaptation for conventional laboratory use has revolutionized the field of genetics and enabled scientists to manipulate virtually any DNA sequence in the genome. Deletions or duplications of large genomic areas (Structural variations SVs) and SNPs that have been found to contribute to the development of pathogenic conditions can be selectively induced in embryonic stem cells. In addition, widely used reporter genes (GFP, mcherry) can be inserted downstream of promoters of a gene of interest in order to dissect its spatial and temporal expression properties as well as localize and determine the migration of the encoded protein. By following a protocol which includes conventional techniques such as cloning, qRT PCR, and embryonic cell culture, transgenic cell lines and organisms (mouse model) can be generated in a time period of 10 weeks. The effectiveness and convenience of this procedure can be utilized to study the effect of numerous SNPs that are annotated as risk factors for diseases as well as those that affect the metabolism of pharmaceutical subastances, thus contributing to the advance of the field of pharmacogenomics and personalized medicine. (*Doudna and Charpentier, 2014*, *Kraft et al., 2015, He et al., 2016*)