CRISPR/Cas Genome Editing

Before starting

Step 1:

→ Check that the model (whether organism or cell line) has not been produced yet.

For example for mouse lines, you may want to check here: http://www.findmice.org; http://www.mousephenotype.org; https://www.infrafrontier.eu; http://www.informatics.jax.org.

Step 2:

→ Get to know everything about your target. The more you know about the function, expression pattern, localization, splice variants and functional domains, the better will be your targeting strategy.

Useful sites: http://www.ncbi.nlm.nih.gov/gene; http://www.uniprot.org; http://www.genecards.org

Step 3:

→ Get the DNA sequence you're going to target. You also want to know everything that is located within the genomic locus you're aiming to modify. You need to know the structure of the target gene, putative splice variants and overlapping genes or regulatory sequences which might be within the target gene locus.

Useful sites: http://www.ensembl.org/index.html; http://vega.sanger.ac.uk/index.html; http://www.ncbi.nlm.nih.gov

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Step 4:

→ Once you've established the region you want to target, you have to find the perfect CRISPR for your project

The perfect CRISPR is the one closest to the target site with the highest specificity (lowest number of off- targets).

Useful sites: http://crispr.mit.edu; http://crispor.tefor.net; http://www.sanger.ac.uk/htgt/wge; https://chopchop.cbu.uib.no;

Step 5:

→ If your aim is to generate a KO by frameshift mutations you can use online tools which predict the outcome of NHEJ repair based on micro-homology arms in proximity to the cutting site: http://www.rgenome.net/mich-calculator

Step 6:

→ Repetitive sequence in the targeting locus might cause some difficulties especially for KI projects. To avoid or at least to be prepared for it you can analyse the target region for repetitive sequences using: http://www.repeatmasker.org

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Step 7:

→ Protection of the correction: when using donor repair templates to insert ssOligos or dsDNA constructs you may want to prevent cutting of the donor template by the CRISPR. This can be achieved by introducing silent mutations into the PAM or CRISPR sequence. The following link helps you to find suitable silent mutations in the CRISPR sequence and also indicates the restriction sites generated by the mutations. This restriction site can be of great use for genotyping.

http://watcut.uwaterloo.ca/template.php

Step 8:

→ Decide what approach to use: what to deliver (plasmid DNA, RNA, ribonucleoprotein complex) and how (microinjection, electroporation, transfection).

Step 9:

→ Order the reagents (crRNA, tracrRNA and Cas). For example https://eu.idtdna.com/pages/products/crispr-genome-editing

Done !!!

(modified from the CCP programmable nucleases (CRISPR/Cas9) transgenesis workshop TT2016)