UCSF ES Cell Targeting Core

Custom CRISPR/TALEN Request Form

1. Project Summary (Please describe the goal of your project. For guidance, please see example project summary form found at http://ESCore.ucsf.edu/forms.)

2. Scientific Information

Project Title	
Name of Gene/locus to be Modified*	
Application: NHEJ or donor-derived HR	
Model genome (examples: Mouse, human, C.elegans, and zebrafish)	
DNA transfection or RNA injection	

3. Services you need

Services	Requested services
Custom TALENs	
CRISPR gRNA cloning	
Cell-based functional assay	
In vitro RNA synthesis	

4. Custom CRISPR/TALEN Request Form instruction

1. Consultation with Core personnel before submitting a service request is strongly recommended. There is no consultation fee if you submit the order afterwards.

2. Client chooses a target location and submits its sequence data along with Project Summary Form.

***Important! Please upload a sequence file of your target region (400~500nt) from cell line or species you are going to use to MyCORES. SNPs will interfere with binding and cutting of TALEN and Cas9/CRISPR. The file should be annotated with a preferred cutting area for knockouts or an insertion point for knockins and saved as .gb or .ape file.**

The Core recommends that you clone the PCR product into a vector such as TA cloning plasmid and then sequence about 10 colonies. This way you can detect even low-level SNPs in a heterogenous pool of cell lines.

3. TALEN: The Core designs and makes two Forward and two Reverse TALENs per target site. These can be used as 4 pairs in most cases. Typical TALEN target site will have 16-20 bp binding site/15-18 spacer/16-20 bp binding site. It takes 2 weeks from designing to sequence verification of TALEN plasmids.

4. CRISPR/Cas9: The CRISPR gRNA cloning takes about a week from design to sequence verification. We design 3~4 sgRNAs per target site.

5. To test the cutting efficiency, the Core can provide a cell-based functional test (Surveyor assay) as a service.

6. For some applications (embryos injection), the use of RNAs instead of DNA would be a lot more efficient. We can make RNAs for your injection and transfection as services.