Sg RNA design instruction

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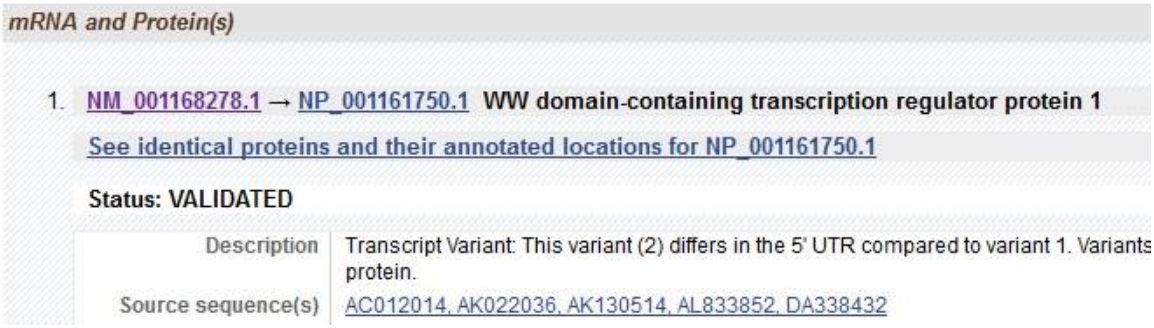
1. Input gene name in NCBI gene;
2. Go to the section of ***mRNA and Protein(s)*** ;
3. Click on the link for the gene, for example NM_001168278.1 for WWTR1 (see the image below)



4. Go to the section of **FEATURES** to get the information regarding exon and CDS;
5. Get the sequence of the first exon inside the CDS; (Note: the exon is not necessarily be the first exon, since the first exon might not be in the CDS);
6. Copy the above sequence to the following web page: http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design-v1

7. Download the data in .txt;
8. Open a blank excel, File> open the above downloaded file, and follow the below order: Delimited---next----Tab----General ---finish;
9. Sort the result with **Score** from largest to the smallest; (We only use the sequence and score information, just ignore the information in the other columns);
10. The first 3 sequences with the score being > 0.5 will be adopted.
11. Design the oligo sequence:
    Forward oligo: CACCG+ the sequence we get from the above procedure;
    (Note: If the first nucleotide is G in the sequence, just add CACC before the sequence)
    Reverse oligo: AAAC + reverse complement of the sequence we get + C
    (Note: If the first nucleotide is G in the sequence, do not add C after the sequence).

CRISPR cloning notes

Note：

1. In step 1, we don't use DTT;
2. In step 3, for annealing reaction, a new protocol named ANNEAL-C is saved in the MAIN folder on the new PCR machine.
3. In step 5, we normally have 3 sgRNA plus 1 negative control ligation, totally 4.
4. Mix & go competent cells (Zymo Research T3011) are used in place of Stbl3 bacteria. 25ul competent cells for each ligation.

Brief Instruction for Mix & go cells

Single Tube Aliquots
1. To a tube of Mix & Go cells thawed on ice, add 1-5 µl plasmid DNA1 , (add 1.25 ul plasmid into 25 ul mix &go cells) and then mix2 gently for a few seconds.

2. Spread 25 µl onto a pre-warmed culture plate (Ampicillin selection only, see note below). Incubate the plate at the appropriate temperature (e.g., 37°C) for the colonies to grow.

Notes:
1 Keep the added volume of DNA less than 5% of the total.

2 To mix cells after DNA addition gently tap the tube with your fingers and then shake the tube downwards in a single motion from the elbow to collect the mixture at the bottom of the tube. Avoid exposing the cells to room temperature for more than a few seconds at a time.

Product Information

Product name	Item No. in Cell Culture Core Facility
FastDigest *BsmB*I (Fermentas)	MBIZR141
FastAP(Fermentas)	MBIZR074
T4 PNK (NEB M0201S)	NEBZR160
Quick Ligase (NEB M2200S)	NEBZR187
BsmBI (for enzyme digestion)	NEBZR280

Sequencing Primer Information

Sequencing primer after inserting sgRNA into H138 vector:
GAGACG CGTCTC is the BsmBI sequence, located at 2858-2869 bp on H138 .
We design the forward primer at 2740-2757 bp, Crispr-F: CTTGGGTAGTTTGCAGTT ;
However, the forward primer does not work.

We design the reverse primer at 2973-2990 bp (Sequence ---GAAAGGAGTGGGAATTGG)
By reverse complement of the above sequence, we get the reverse primer---
Crispr-R: CCAATTCCCACTCCTTTC, which works well.