

sgRNA design & quality control

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Target - Rattus norvegicus **T-type Cav3.2 subunit alpha1 H**, *Cacna1h* gene (transcript ID NM153814.2, Gene ID: 114862)

sgRNA

Delivery of dual sgRNA cassettes and Nme2Cas9 in a single AAV vector, mouse targeting (addgene plasmid #159537).

Existing gRNA's spacers were designed by rat *Cacna1h* mRNA sequence.

Instead of a check of single spacer sequences I prefer to design a new set of spacers. I used a highly recommended online tool for sgRNA design CRISPick (<https://portals.broadinstitute.org/gppx/crispick/public>).

The top 10 results are presented in **sgRNA_top_10.xlsx** file with all supplementary information including potential cut efficacy and off-target cutting sites (columns legend you may find by link <https://portals.broadinstitute.org/gppx/crispick/public/how-to-use>).

Was selected sequence 1 (target exon **17**), 2 (target exon **6**) and 4 (target exon **8**). To finalize sgRNA design I added BspQI or BsmBI cohesive ends to double-strand sgRNA sequences.

- **gRNA I** (BspQI)
5'-AAC / CCA-5' << U6
- **gRNA II** (BsmBI)
U6 >> 5'-CACC / CAAC-5'

Deletion/inversion region size with different sgRNAs pairs:

sgRNA pair	Flanked region size	Size of CDS with deletion, bp
6+8	1058 bp	6023 bp
6+17	2984 bp	4096 bp
8+17	1926 bp	5155 bp

As a negative control, it is recommended to use the sgRNA for a gene absent in the model organism (GFP, luciferase, etc.). All sgRNA sequences with cohesive ends including negative control with GFP saved in **sgRNA.fasta**.