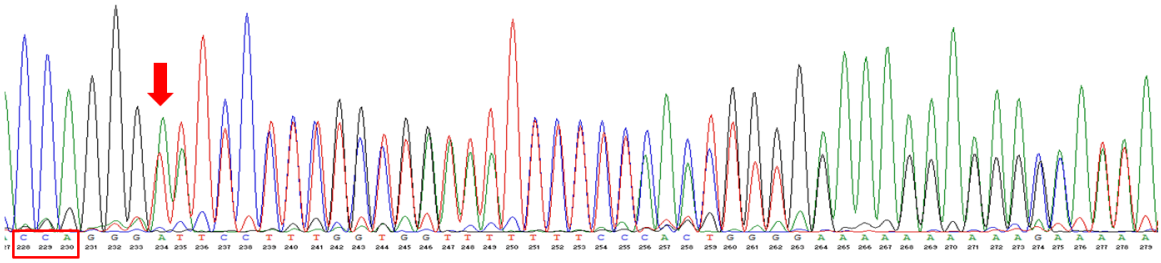
C6-19

Step 5-1: Single cell clones genotyping--Sequencing of sgRNA target regions

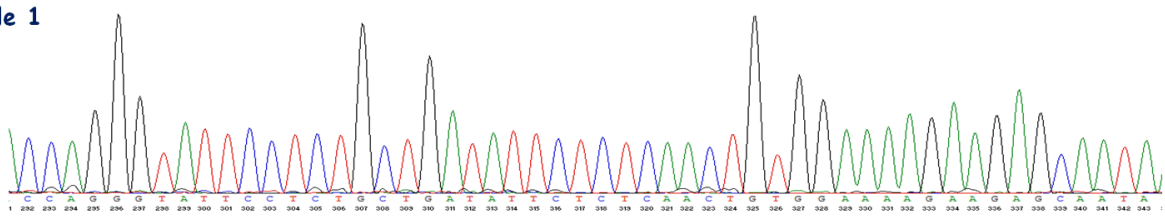
Each of the individual clonal lines were further cultured and analyzed by genomic DNA isolation for sequencing and by western blot for protein detection (No service provided). The genomic DNA was extracted using a QIAamp DNA mini kit (QIAGEN) according to the manufacturer's instructions. The genomic region flanking the CRISPR target site for the gene of interest was amplified and sequenced individually.

Step 5-2: Single cell clones genotyping--TA cloning

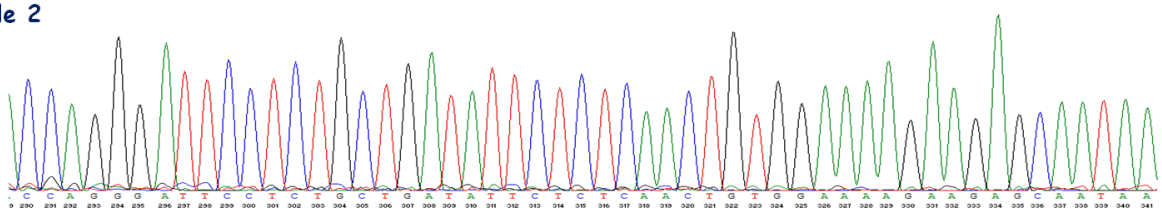
TA cloning was used to identify the heterozygous of the knockout alleles. The PCR amplicon flanking the CRISPR/Cas9-targeted sites was generated and the PCR products was cloned into the TA cloning vector pJeT1.2/blunt (Life Technologies) and multiple (about >8) cloned alleles were sequenced.



Allele 1



Allele 2



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WT        ACTGCCATTTTTTATTTTCTTCTTCTTTTTCGCTCCTGTAGGAACACCAGGGCTATTCCTCTGCTGATATTCTCTCAACTGTGGAAAAGAAGAGCAATA

Allele 1  ACTGCCATTTTTTATTTTCTTCTTCTTTTTCGCTCCTGTAGGAACACCAGGG-TATTCCTCTGCTGATATTCTCTCAACTGTGGAAAAGAAGAGCAATA
Allele 2  ACTGCCATTTTTTATTTTCTTCTTCTTTTTCGCTCCTGTAGGAACACCAGGG--ATTCCTCTGCTGATATTCTCTCAACTGTGGAAAAGAAGAGCAATA
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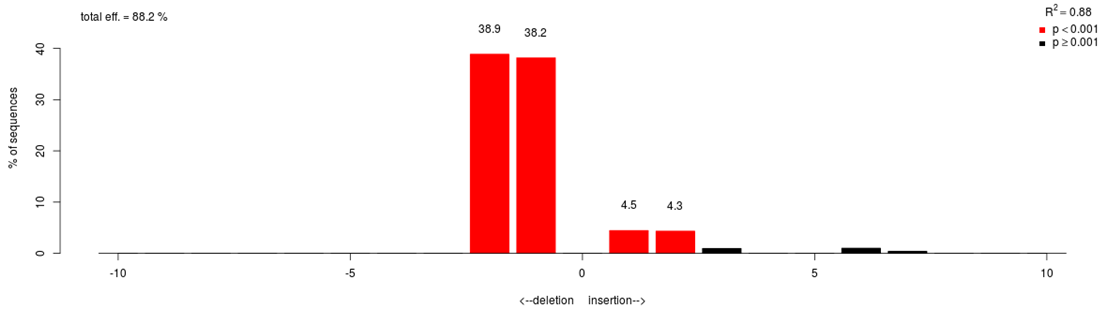

Flowchart of CRISPR/Cas9-mediated genome editing