

## **Online Resources**

CRISPRtools: A flexible computational platform for performing CRISPR/Cas9 experiments in the mouse

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**ESM 1.**

Screen shot for CRISPRtools web-based submission form. Ensembl exon IDs or genomic coordinates can be used as input. Ensembl exon identifiers can be entered individually or comma separated when attempting to delete two adjacent exons. The user can then select several different parameters to restrict the search region and/or limit filtering to take into account linked off-target hits. In addition, the user can choose which scoring algorithm to use to prioritize guide selection.

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## Run CRISPR Knock Out Pipeline

Use this form to submit Ensembl Exon IDs (mouse) to target for deletion.

Ensembl Exon IDs:

ENSMUSE00000279350  
ENSMUSE00001252117,ENSMUSE00001230697

Max 500

Input format:

Ensembl Exon ID

Upstream Exon ID,Downstream Exon ID

chr[:start-end optional\_target\_name

If the "Internal Deletion" option is specified, only single exon IDs are valid input.

Options:

- Internal Deletion
- Extended Stem Loop
- Use Repeat-Masked Genome
- Allow Designs Without Backup Guides

Linked Off-Target  
Filtering

- None
- Filter Guides With Linked Off-Targets
- Filter Guides With Exonic Linked Off-Targets

Flanking Sequence  
Length:

100

integer from 50 to 300

Rank By:

- sgRNA designer score (in vitro; mouse/human)
- CRISPRscan score (in vivo; zebrafish)

Email:

Optional, notification will be sent with download link upon completion.

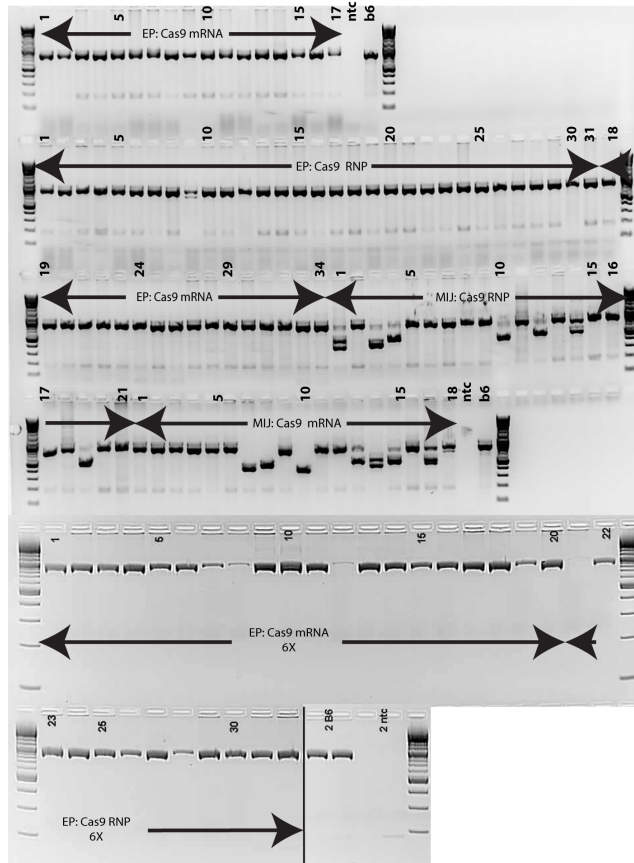
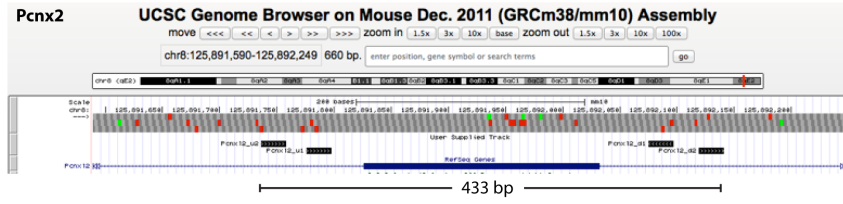
Submit

This may take a while.

## ESM 2

UCSC genome browser view indicating location of paired guides relative to the critical exon of *Pcnx2* with the maximal sized deletion indicated below. The complete genotyping results for each experiment are shown below each locus display with the number of live born animals analyzed for each condition indicated between the arrows. Vertical lines indicate junctions between different gels. The conditions are abbreviated as microinjection (MIJ), electroporation (EP), Cas9 ribonucleoprotein (RNP) and the different EP pulse numbers are indicated as either 1X or 6X iterations of the program.

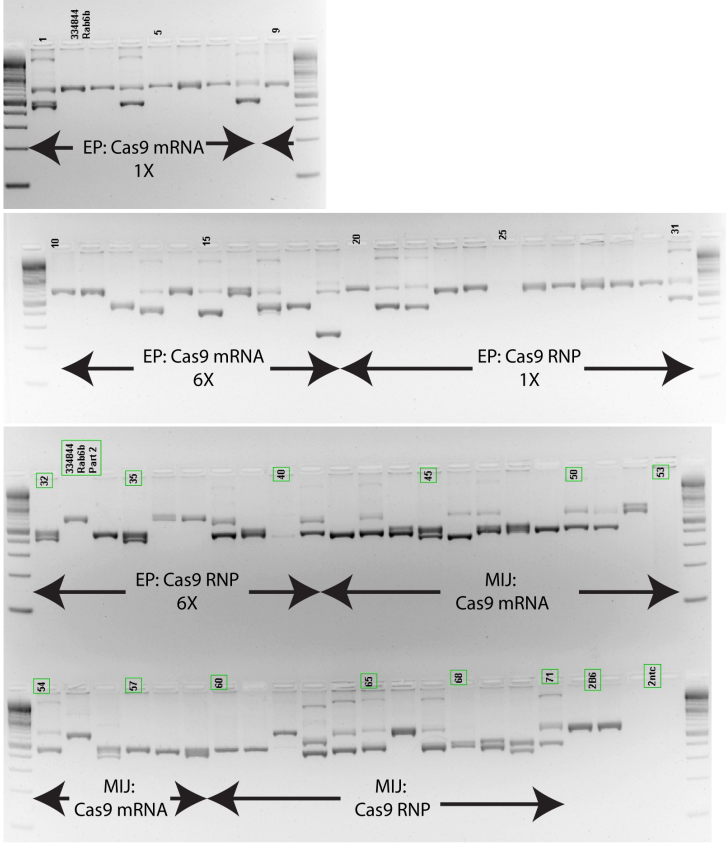
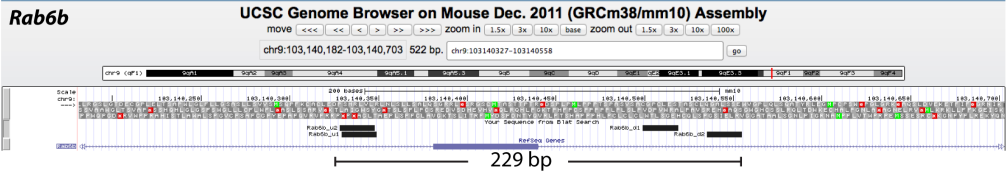
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### **ESM 3**

UCSC genome browser view indicating location of paired guides relative to the critical exon of *Rab6b* with the maximal sized deletion indicated below. The complete genotyping results for each experiment are shown below each locus display with the number of live born animals analyzed for each condition indicated between the arrows. The conditions are abbreviated as microinjection (MI), electroporation (EP), Cas9 ribonucleoprotein (RNP) and the different EP pulse numbers are indicated as either 1X or 6X iterations of the program.

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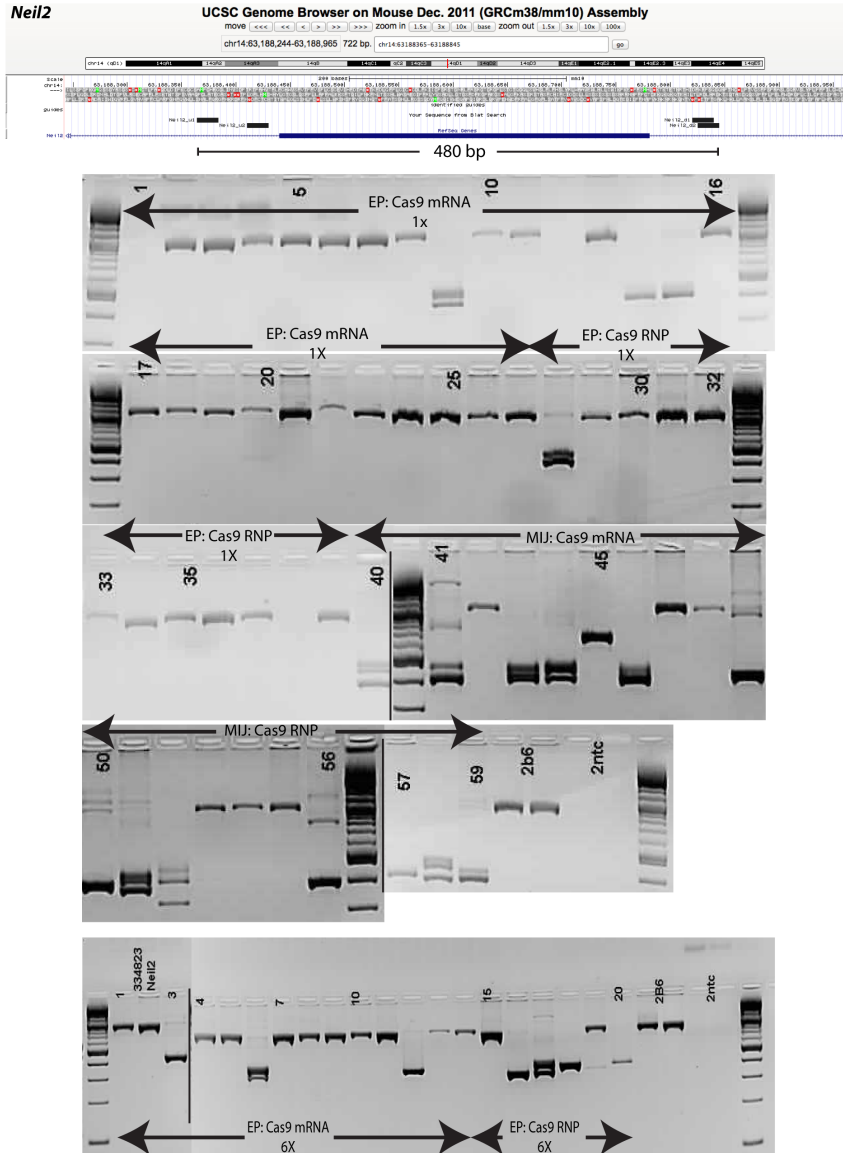


#### **ESM 4**

UCSC genome browser view indicating location of paired guides relative to the critical exon of *Neil2* with the maximal sized deletion indicated below. The complete genotyping results for each experiment are shown below each locus display with the number of live born animals analyzed for each condition indicated between the arrows. Vertical lines indicate junctions between different gels. Note, a subset of the results are shown in main Figure 3d. The conditions are abbreviated as microinjection (MIJ), electroporation (EP), Cas9 ribonucleoprotein (RNP) and the different EP pulse numbers are indicated as either 1X or 6X iterations of the program.



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## **ESM 5.**

Examples of deletion breakpoints obtained from founders injected with four guides targeting *Rab6b* exon ENSMUSE00000320561 and Cas9 mRNA. Individual guide sequences are shown in turquoise and PAM sequences are colored red. Alignments were obtained using BLAT. Founder animal numbers correspond to numbers in ESM 3. Note the variability in deletion sizes and evidence of small indels not resulting in deletion events for founder #55.

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```
u1/u2                                     d1                                     d2
B6      GTTTGAAGAGCCCTAGCCATAGGCTGGTCTTACGGCTGACTGACCTCTCTCT...GCCTCTGCTCTGCCTGTGGACCCTGCTGGAGAGCACTGCCAGCTGCTCTCCAGGGAGCATTGAGCTCAGAGCTGGGGTG
Rab6b #42 GTTTGAAGAGCCCTAGCCATAG-----189 bp del-----CTGCTCCAGGGAGCATTGAGCTCAGAGCTGGGGTG
Rab6b #46 GTTTGAAGAGCCCTAGCCATAG-----208 bp del-----AGTGGGGTG
Rab6b #49 GTTTGAAGAG-----178 bp del-----CCTGCTGGAGAGCACTGCCAGCTGCTCTCCAGGGAGCATTGAGCTCAGAGCTGGGGTG
Rab6b #50 GTTTGAAGAGCCCTAGCCATAG-----164 bp del-----GACCCTGCTCTGGAGAGCACTGCCAGCTGCTCTCCAGGGAGCATTGAGCTCAGAGCTGGGGTG
Rab6b #55 GTTTGAAGAGCCCTAG-----GCTGGCTTACGGCTGAACTCTCTCT...GCCTCTGCTCTG-----CCCTGTCTGGAGAGCACTGCCAGCTGCTCTCCAGGGAGCATTGAGCTCAGAGCTGGGGTG
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