H11-CAG-mmu_circ_0000953-IRES-EGFP-PolyA
cas9-ki(H11) Strategy

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Reviewer: Jia Yu

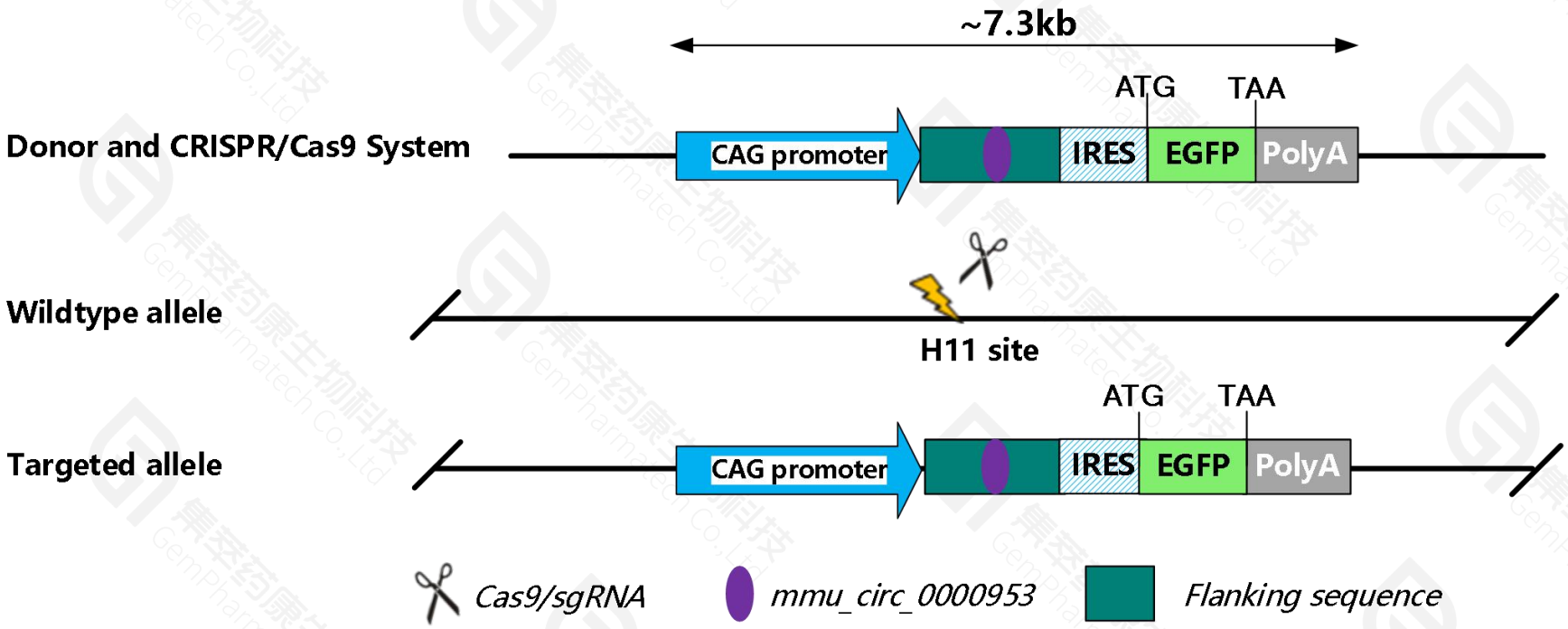
Design Date: 2021-6-2

Project Overview

Project Name	**H11-CAG-mmu_circ_0000953-IRES-EGFP-PolyA**
Project type	**cas9-ki(H11)**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit. The schematic diagram is as follows:



Technical routes

➢ The *CAG* promoter used in this strategy is a broad-spectrum promoter with a length of about 1.7kb[1].

➢ H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene[2].

➢ In this project we use CRISPR/Cas9 technology to modify H11 localization. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

- *mmu_circ_0000953* is transcripted systemically by the CAG promoter, and the phenotype of this model is unknown.
- The *mmu_circ_0000953* sequence is from UCSC, and used directly in the production of mouse models. The transcription pattern and phenotype of the mice were unknown.
- Please confirm the sequence of *mmu_circ_0000953* gene, and the sequence needs to be synthesized.
- There are repeats sequence in flanking sequence of *mmu_circ_0000953*, and mutation may occur in the process of mouse model making.
- The IRES-linked genes will be transcripted together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.
- The H11 localization is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

mmu_circ_0000953 sequence (3790bp)

TTTTGATCTTTATCAATCTCATCAGTTTATGTCAGCTGGACTTTTGTTTTTCGCACTCTTTTGCTTATTGTATGTATCTGGCTGTCATATAAAGTATGTTAAATTTGAAAGGCTGTGGGGATGAAAGTCCATACTGT
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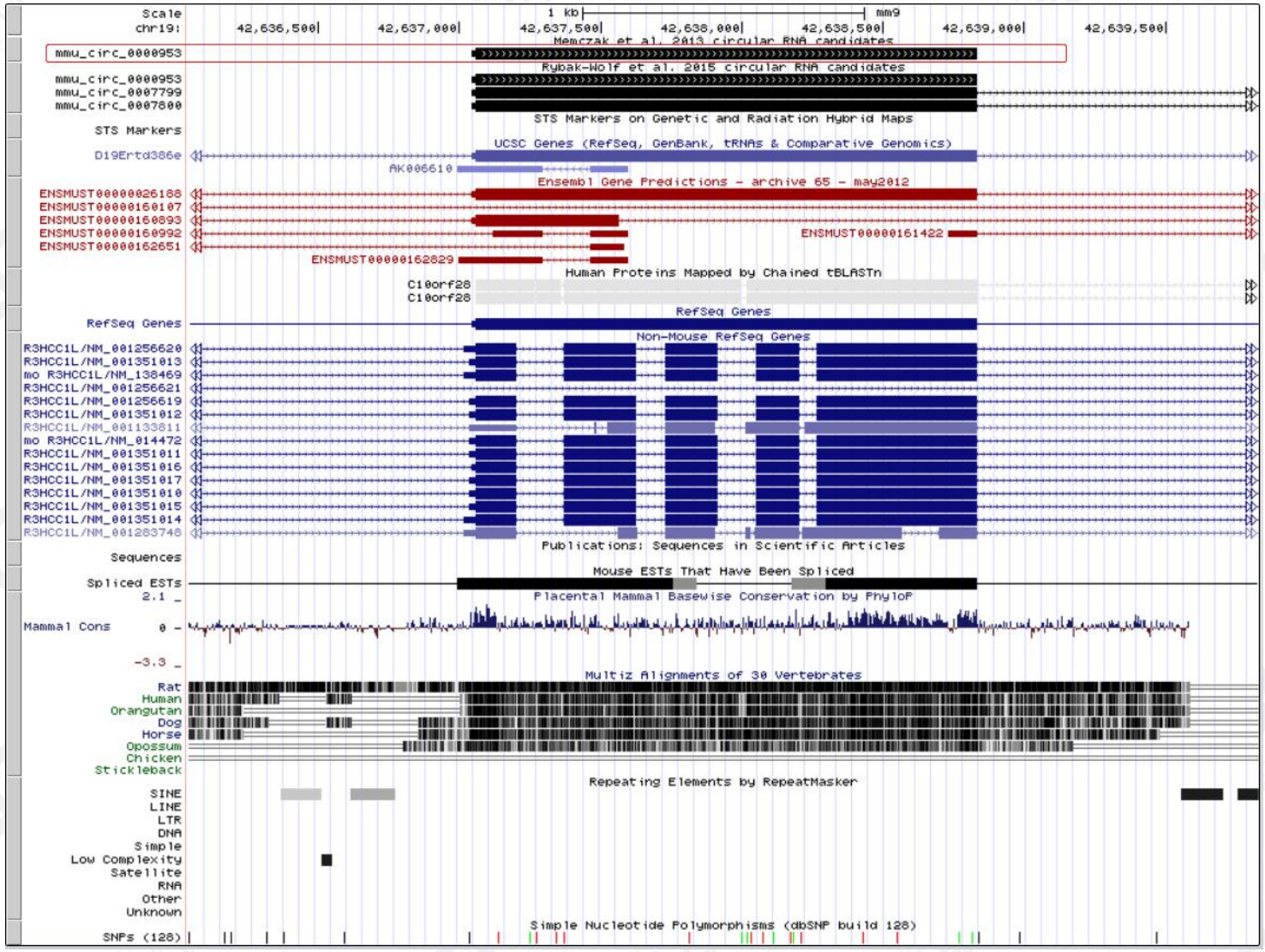
GACACTCCAGAAG

Red=Mouse *mmu_circ_0000953*

Black=Flanking sequence

Gene information(UCSC)



References

[1] Alexopoulou, A. N., J. R. Couchman, et al. (2008). "The CMV early enhancer/chicken beta actin (CAG) promoter can be used to drive transgene expression during the differentiation of murine embryonic stem cells into vascular progenitors." BMC Cell Biol 9: 2.

[2] Hippenmeyer, S., et al., Genetic mosaic dissection of Lis1 and Ndel1 in neuronal migration. Neuron, 2010. 68(4): 695-709.

Additional cycles and costs

Additional itmes	cycle（month)	cost（¥)
mmu_circ_0000953 sequence	1	5685

If you have any questions, you are welcome to inquire.

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