Generation of Ciz1-S264G TARGATT Knockin Mouse Model

Customer information

Project	Ciz1-S264G
Job ticket	MT095
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1. Summary

The generation of Ciz1-S264G transgenic mouse model involved four steps. The first step is to generate a genetically modified mouse line, designated H11-3XattP (H11P3), by knocking in three tandem attP sequences, namely landing pad, into mouse Hipp11 (H11) locus of C57BL/6J (B6J) strain. The second step integrated the Ciz1-S264G transgene sequence into H11P3 sites. This step was accomplished by microinjecting integration cocktail into the pronuclei of heterozygous H11P3 embryos harvested from B6 mice. The integration cocktail consisted of a plasmid pBT-Ciz1-S264G donor vector DNA and *in vitro* transcribed ϕC31 integrase mRNA. Because the Ciz1-S264G donor construct contains two attB sites, and the mouse genome has three attP sites, ϕC31 integrase will catalyze site-specific DNA integration between attB and attP sequences, resulting in transgene integration into the H11P3 locus in the mouse genome. In the third step, the zygotes injected with the integration cocktail were implanted into CD1 foster mice to produce new mice. The fourth step was to identify Ciz1-S264G transgenic mice by PCR-based genotyping.

On 04/06/2017, 179 heterozygous C57BL/6 H11/H113P embryos were injected with the integration cocktail, containing pBT-Ciz1-S264G DNA and PhiC31 integrase mRNA, 112 of the injected embryos developed well *in vitro* and were transferred into four surrogate mice. From this round of microinjection, 25 mice were produced on 04/26/2017. Among them, one male mouse was identified as Ciz1-S264G founder by PCR genotyping.

F1 generation is in progress. The founder will be mated with two wild type B6 female mice.

2. Method - Identification of Ciz1-S264G model

Tail tissues from F0 mice were collected and DNA extraction was performed individually. A panel of PCR primer pairs (Table 1) was designed to identify and confirm 1) attP site-specific insertion by the amplification of novel junction sequences at the Ciz1-S264G-targeted H11P3 allele; and 2) the insertion of Ciz1-S264G. Following PCRs illustrated in Figure 1 were performed. Their expected fragment sizes to corresponding primer sets are listed (Table 2). Different PCR fragment sizes for the same reaction reflect different integration options, e.g. integration at one of the three attP sites, or cassette exchange using any two of the three attP sites (Figure 1).

Table 1. PCR primers used in genotyping

Primers	Sequence
PR425N	5′-GGTGATAGGTGGCAAGTGGTATTCCGTAAG-3′
PR436N	5′-CCACCTCGACCCGTTCATCATGATG-3′
CAG-R	5′- CATATATGGGCTATGAACTAATGACCCCGT-3′
Ciz1-F1	5′- GTGGCCTGGATTTGGGAGAATGTG-3′
Ciz1-R1	5′- GCTGGATCAAGTCTCCCACGTAG-3′
Ciz1-F2	5′-CAGGTGAAGCCGAGAGAAACATCC-3′
polyA-Ru	5′-ATGTGGTATGGCTGATTATGATCAGTTATCTAG-3′
polyA-Fu	5′-CTAGATAACTGATCATAATCAGCCATACCACAT-3′
PR522N	5′-GACGATGTAGGTCACGGTCTCGAAG-3′
Frt-R2	5′-CTTCAGATTCGAGCCCTAGCCAC-3′

All PCR amplifications were prepared in 25 µL using MyTaq™ Red Mix (Bioline, Cat#, BIO-25044) (Table 3), and the amplifications were carried out using the following program: 95°C, 2 min; 35 cycles of [95°C, 15 s; 60°C, 15 s; 72°C, elongation duration varies by amplicon size], 72°C, 5 min; 4°C, indefinite.

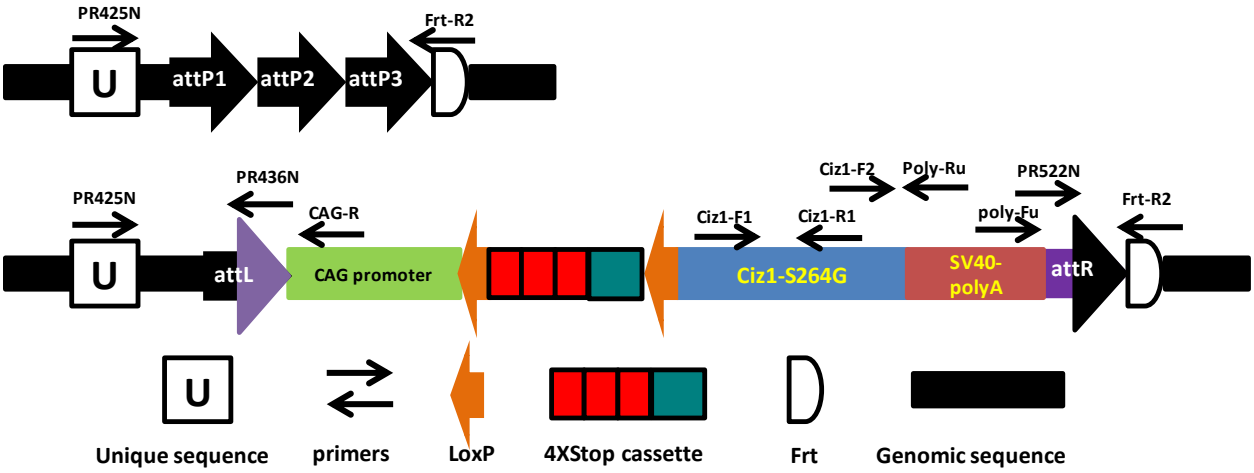

Figure 1. Transgene integration and genotyping scheme of Ciz1-S264G transgenic model. Upper panel: genomic sequence with H11P3 landing pad; Lower panel: founder genotype with Ciz1-S264G transgene integrated at the 1st and the 3rd attP sites

Table 2. Description of genotyping PCRs illustrated in Figure 1

PCR#	Description	Forward primer	Reverse primer	Fragment size(s), bp
1	5'-junction	PR425N	PR436N	136/206
2	5'-GoI*	PR425N	CAG-R	377/447
3	GoI-1	Ciz1-F1	Ciz1-R1	438
4	GoI-2	Ciz1-F2	polyA-Ru	452
5	3'-GoI-1	polyA-Fu	Frt-R2	507/437
6	3'-junction	PR522N	Frt-R2	225/155

***GoI: Gene-of-Interest**

Table 3. Preparation of PCRs

	25 µl reactions
MyTaq™ Red Mix 2X	12.5 µl
Nuclease-free water	8.5 µl
Forward Primer (20µM)	1 µl
Reverse Primer (20µM)	1 µl
Template DNA	2 µl
Total Volume	**25 µl**

3. Results and interpretation- Identification of Ciz1-S264G model

3.1. Identification of Ciz1-S264G founders

On 04/26/2017, 25 mice were produced from the round of microinjection conducted on 04/06/2017. They are separated by two cage IDs, 2530 for male and 2531 for females. Genotyping screening was done using PCRs 1 and 6 to detect junctions after transgene insertion at H11P3 site. PCRs 1 and 6 suggest H11 site-specific insertions (Figures 2a). PCRs 3 and 4 indicate transgene integration, randomly or at the H11 locus (Figures 2b). PCRs 2 and 5 confirm the site-specific integration of Ciz1-S264G transgene (Figure 2c). Collectively, the results suggested that mouse of cage 2450#1 is a founder, while others of the same cage and the entire female cage (data not shown) are negative.

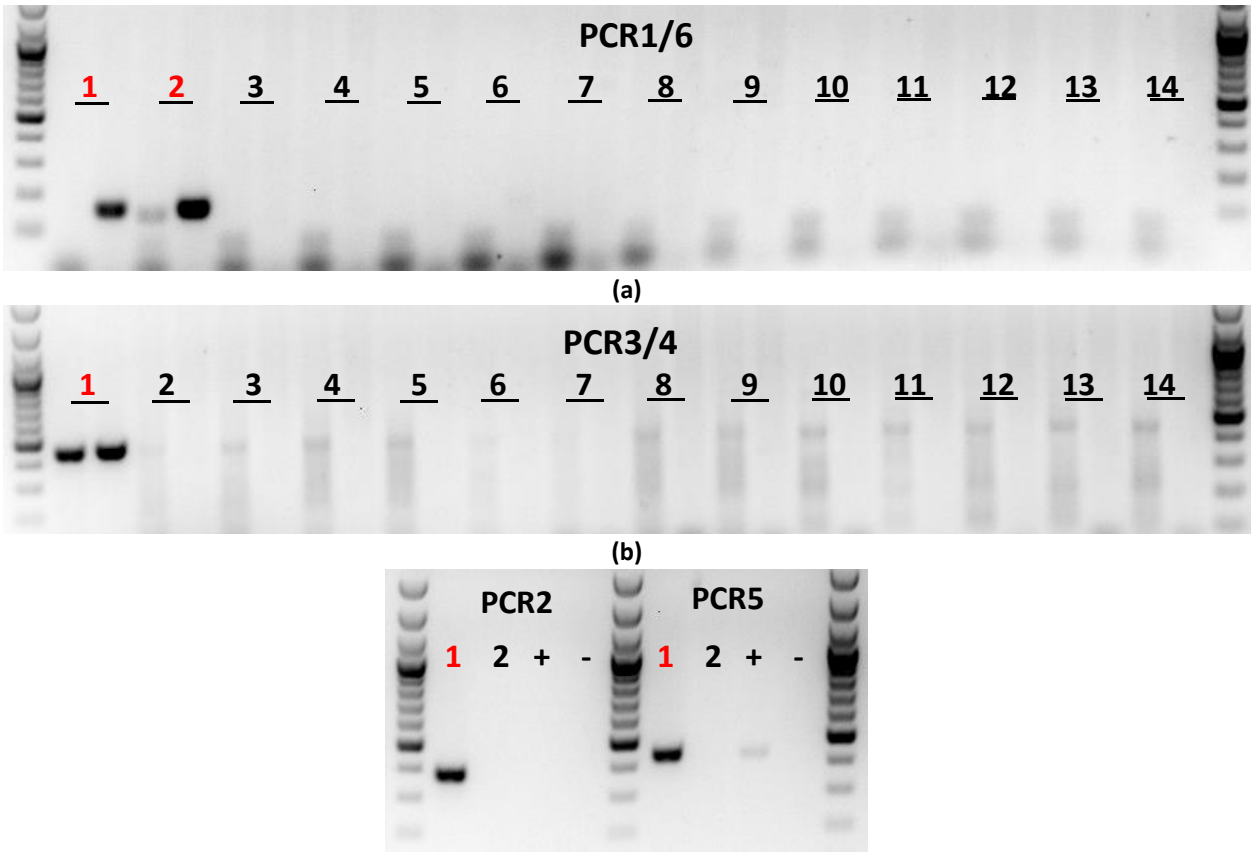

Figure 2. Identification of Ciz1-S264G founder #1 of cage 2530

3.2. Germline transmission and identification of Ciz1-S264G F1 mice

Germline testing and generating positive F1 mice was accomplished by breeding the male founder with two WT female mice.

4. Shipping information for Ciz1-S264G mice

The identifications of the Ciz1-S264G founders and F1 mice are entailed in Table 4.

Table 4. Ciz1-S264G founder and positive F1 mice

Mouse ID	DOB	Gender
2530#1	04/26/2017	Male

*recommended to be kept at ASC as backups