Applied StemCell

Generation of mPklr-KO Mouse Model

Customer Information

Project Title	mPklr KO
Job Ticket	MC107
Client Name	Dr. Martina Klevstig
Client Institute	Wallenberg Laboratory for Cardiovascular and Metabolic Research Dep. of Molecular and Clinical Medicine Institute of medicine, The Sahlgrenska academy University of Gothenburg
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Summary

This project is to generate a knockout (KO) model in mouse Pklr gene. The model specifies a Guanine (G) deletion immediate downstream of the ATG start codon in mouse Pklr L-isozyme, not R-isozyme. To achieve this model, three major steps were carried out. First, a mixture containing active guide RNA molecules (gRNA), one single stranded oligodeoxylnucleotide (ssODN) donor and Cas9 protein (refer to report 1) was injected into the cytoplasm of C57BL/6 embryo of Jackson laboratory (B6J) (refer to microinjection memorandum). The second step was to screen new mice born from the microinjection by PCR and sequencing, and to confirm potential founders by additional sequencing. Third, founder(s) were mated with wild type (WT) mouse to test germline transmission in F1 mice.

On 01/16/2017, 163 B6 embryos were injected through cytoplasmic route with a CRISPR targeting cocktail containing g2 and its matching donor DNA (refer to report 1). One hundred and sixteen of the injected embryos well developed *in vitro* and were then transferred into five surrogate mice. From this round of microinjection, twenty-five mice were born on 02/05/2017. Genotyping results from PCR-sequencing suggested that twelve of them carry the ATGG>ATG mutation.

The female founders were mated with WT mice.

Method - Identification of mPklr KO Mouse model

Tail tissues from F0 mice were collected and DNA extraction was performed individually. One set of PCR primers, mPklr 1F/2R amplifying a 382bp fragment, was used to identify mPklr KO (Table 1 and Figure 1). PCR products were submitted for sequencing to screen and confirm 1bp deletion (ATGG>ATG).

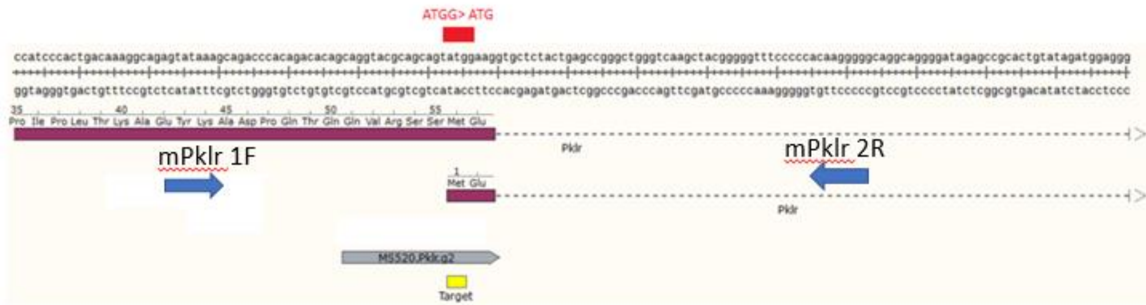


Figure 1. PCR-sequencing scheme to identify mutants with mPklr KO genotype

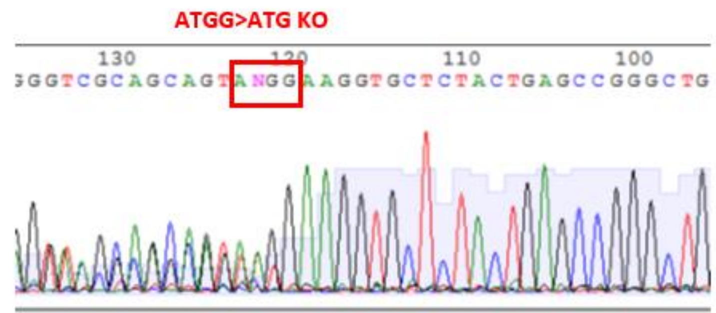
Table 1. Primers used in identification of mPklr KO mutants

	Primer sequences
mPklr 1F	5'- CCGTGGTTCCTGGACTCTGG -3'
mPklr 2R	5'- CTCAAGTCTCAAGGCATCTCATTCTCTAGG -3'

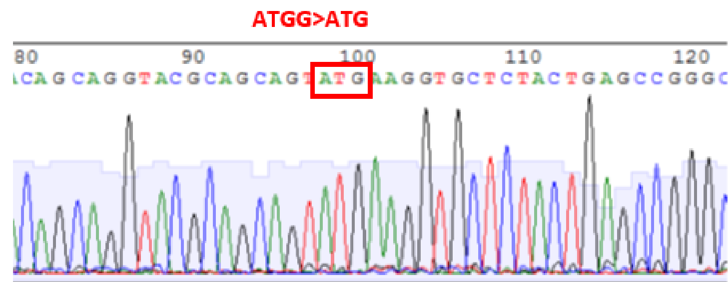
Results - Identification of mPklr KO mice

Screening and confirmation of mPklr KO founders

Twenty five mice were born from embryos microinjected with mPklr KO CRISPR microinjection cocktail. Genomic DNA was extracted from individual mouse and subjected to PCR amplification using mPklr 1F/2R. The PCR products were subjected to Sanger sequencing. After TOPO cloning, sequence results from bacterial clones showed that twelve mice, 2302#4, 2302#7, 2302#8, 2302#9, 2302#10, 2302#12, 2302#14, 2302#15, 2303#3, 2303#5, 2303#6, and 2303#7, carry mPklr KO. Sequencing data from raw PCR products indicated that they are heterozygous founders (Figure 2a and 2b).



(a)



(b)

Figure 2. Representative chromatograms for heterozygous mPklr KO mice. (a) sequence reading of raw PCR products; (b) sequence reading of a PCR fragment cloned into TOPO vector

Animal transfer information

The identifications of mPklr KO founders are entailed in Table 2. We will indicate F1 animals to be transferred when positive F1 mice will be identified.

Table 2. Information of mPklr KO mice

Mouse ID	DOB	Gender
2302#4*	02/05/2017	Male
2302#7*	02/05/2017	Male
2302#8*	02/05/2017	Male
2302#9*	02/05/2017	Male
2302#10*	02/05/2017	Male
2302#12**	02/05/2017	Male
2302#14**	02/05/2017	Male
2302#15**	02/05/2017	Male
2303#3***	02/05/2017	Female
2303#5***	02/05/2017	Female
2303#6	02/05/2017	Female
2303#7	02/05/2017	Female

* keep as backups
** will be sacrificed due to large litter
*** will be sacrificed to donate oocytes to produce F1 mice through embryo re-derivation.