Generation of gRNAs and Donor DNA: 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**
Report Date	**01/03/2017**

Summary

This project is to generate a 1bp deletion for (KO) model in mouse mPklr L-isozyme and keep R-isozyme gene intact. The model specifies a nucleotide remove of G just after the ATG start codon in exon 1. Two guide RNA candidates were cloned and evaluated for directing Cas-9 cleavage activity targeting mPklr gene. A single stranded oligo deoxylnucleotide (ssODN) donor was designed and generated based on the active gRNA sequences. Qualified gRNA transcripts, ssODN donor, and Cas-9 mRNA will be used for microinjection to generate mPklr L-isozyme KO model.

Generation of Guide RNAs

1. **Strategy of guide RNA (gRNA) selection**
   Two gRNA sequences were found to have decent activities for targeting the sequence area of mPklr L-isozyme start codon. The sequences of the candidate gRNAs, mMS520.Pklr.g1 and g2, and the Protospacer Adjacent Motif (PAM), in bold, are listed in Table 1. The genome sequence targeted by the gRNAs are depicted in Figure 1.

Table 1. Guide RNA candidates for mPklr gene modification

Name	Sequence
MS520.Pklr.g1	5′– ACAGCAGGTACGCAGCAGTA**TGG** –3′
MS520.Pklr.g2	5′– CAGGTACGCAGCAGTATGGA**AGG** –3′

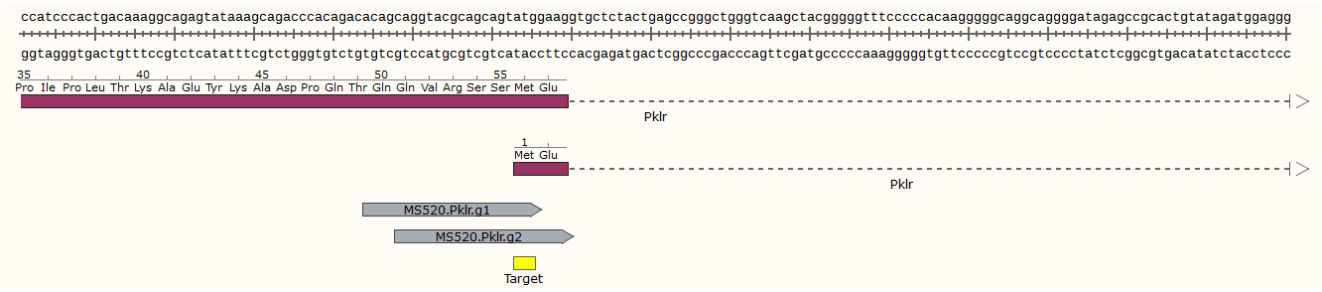


Figure 1. Illustration of gRNA positions and gene modification site

2. Construction of gRNA expressing vectors

The two selected, mMS520.Pklr.g1 and g2, were cloned into a gRNA/cas9 expression vector by inserting double-stranded oligo cassettes of their sequences into the *Bbs* I sites in the expression vector (Figure 2). Each oligo cassette contains 20bp guide RNA sequence with a G (guanosine) at the 5'-end for optimal expression, and adherent ends for cloning at *Bbs* I sites.

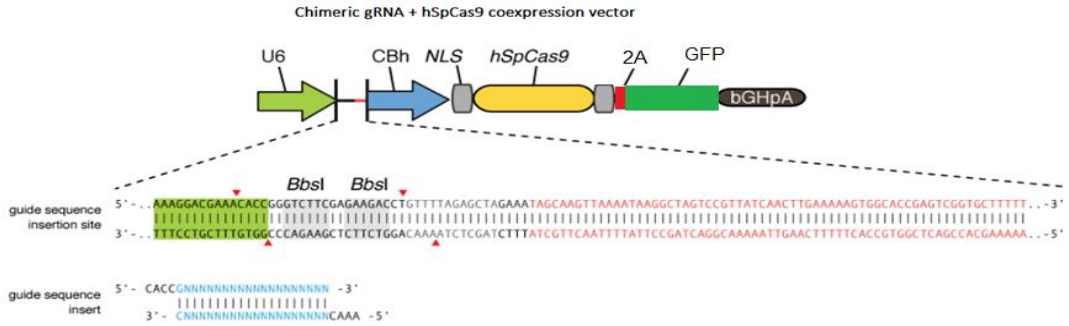


Figure 2. Illustration of targeting vector construction

3. Assessment of gRNA by Next generation sequence (NGS) analysis

After the double-stranded oligos of the two gRNAs were cloned into the gRNA expression vector, pBT-U6-Cas9-2A-GFP, each construct was transfected into mouse N2A cells, individually. DNA was extracted and PCR was performed to test g1 and g2 activity. Next generation sequence (NGS) analysis was employed. Mutation rate of each nucleotide of one PCR product was curated and normalized against GFP-transfected cells used as a wild type control. Occurrence of deletion, addition or mutation of one or two nucleotides at the expected cut site was evaluated and gRNA activity was determined. The results showed non-homologous end joining (NHEJ) frequency of 93% for MS520.Pklr.g1 and 90% for MS520.Pklr.g2, respectively (Figure 3). The results qualified both gRNA candidates. MS520.Pklr.g2 gRNA was taken into consideration to design and generate donor DNA and to produce gRNA transcripts for microinjection.

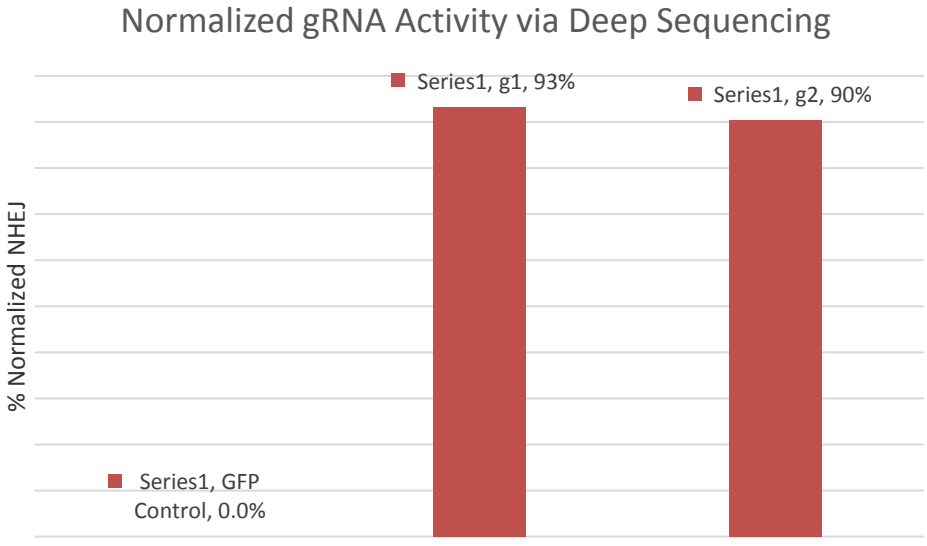


Figure 3. *In vitro* evaluation of gRNA activity for MS520.Pklr.g1 and g2 using NGS

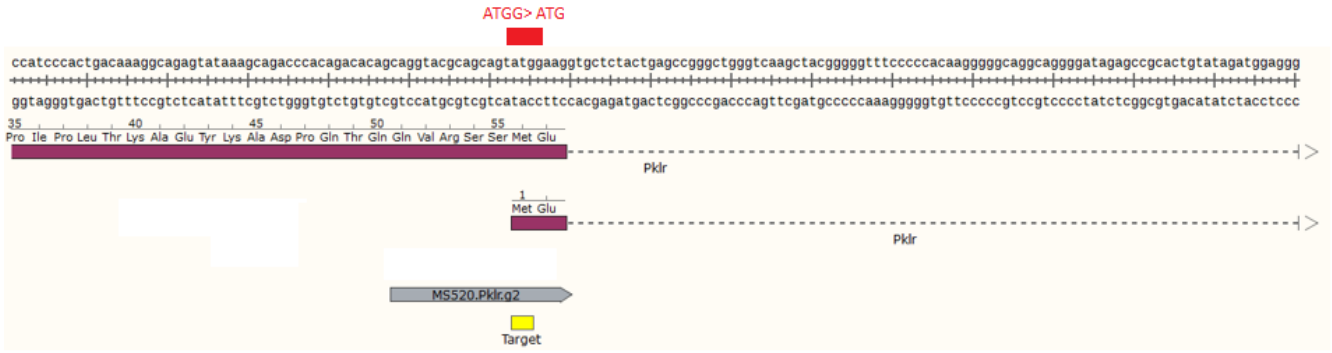
Genome Editing Scheme

Based on gRNA validation results indicating the location of an active gRNA, a single stranded oligo deoxylnucleotide (ssODN) was designed, which removes a G after the start codon in exon 1 (Figure 4a). It serves as a donor during Homology-Directed-Repair (HDR) process. The gene editing strategy is illustrated in Figure 4b.

Pklr.g2.KO.ssODN:

TCTCGTCTGTGCCAGGCCCCATCCCACTGACAAAGGCAGAGTATAAAGCAGACCCACAGACACAGCAGGTACGCA
GCAGTATGAAGGTGCTCTACTGAGCCGGGCTGGGTCAAGCTACGGGGGTTTCCCCCACAAGGGGGCAGGCAGGGG
ATAGAGCCGC

(a)



(b)

Figure 4. Homology Directed Repair (HDR) through embryonic injection. (a) donor oligo DNA; (b) editing scheme to create mPklr L-isozyme KO
Legend:ATGG>ATG (1bp deletion)