

Genotyping Protocol: MMRRC 30139

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 μ l of Extraction Solution, 12.5 μ l Tissue Preparation Solution and 50 μ l of Neutralization Solution B.

Mutation Information: This strain carries a knock-out allele of the CD72 antigen gene (*Cd72*). Exons 1 through 4 of this gene were replaced by the pGK-MC1-*neo* cassette. Details can be found in Pan et al (1999) *Immunity* 11:495-506.

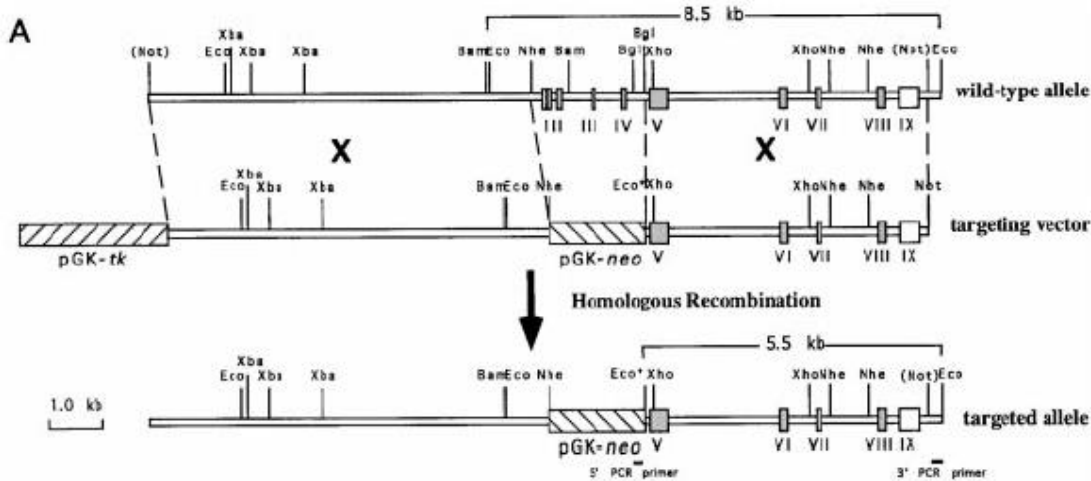


Image from Pan et al (1999) *Immunity* 11:495-506.

Primer Information:

- | | |
|---------------------|--|
| 1) Name: CD72 WT-UP | Sequence: 5'-ATA CAG GTG TGT GGT GCT AC-3' |
| 2) Name: CD72-LO | Sequence: 5'-GGT GGC TTC CCA AAT CCT GG-3' |
| 1) Name: NEO F | Sequence: 5'-CAT TCG ACC ACC AAG CGA AAC ATC -3' |
| 2) Name: NEO R | Sequence: 5'-ATA TCA CGG GTA GCC AAC GCT ATG -3' |

Primer Location: CD72 WT-UP is located in intronic region 4-5 of the *Cd72* gene. CD72-LO is located at the beginning of Exon 5 of *Cd72*. Neo F and R are both located in neomycin.

Assay Name: CD72 PCR

PCR Master Mix Components:

Wild-type PCR

component	manufacturer	concentration	μ l/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
CD72 WT-UP	Sigma or IDT	25 μ M	0.3
CD72-LO	Sigma or IDT	25 μ M	0.3
sterile water			5.4

Cycle Parameters:

Wild-type PCR

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 68°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Wild-type: 100 bp

Homozygous mutant: no product

Heterozygous: 100 bp

Neo PCR (mutant allele)

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
Neo F	Sigma or IDT	25µM	0.3
Neo R	Sigma or IDT	25µM	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

Neo PCR

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 59°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Wild-type: no product

Homozygous: 289 bp

Heterozygous: 289 bp

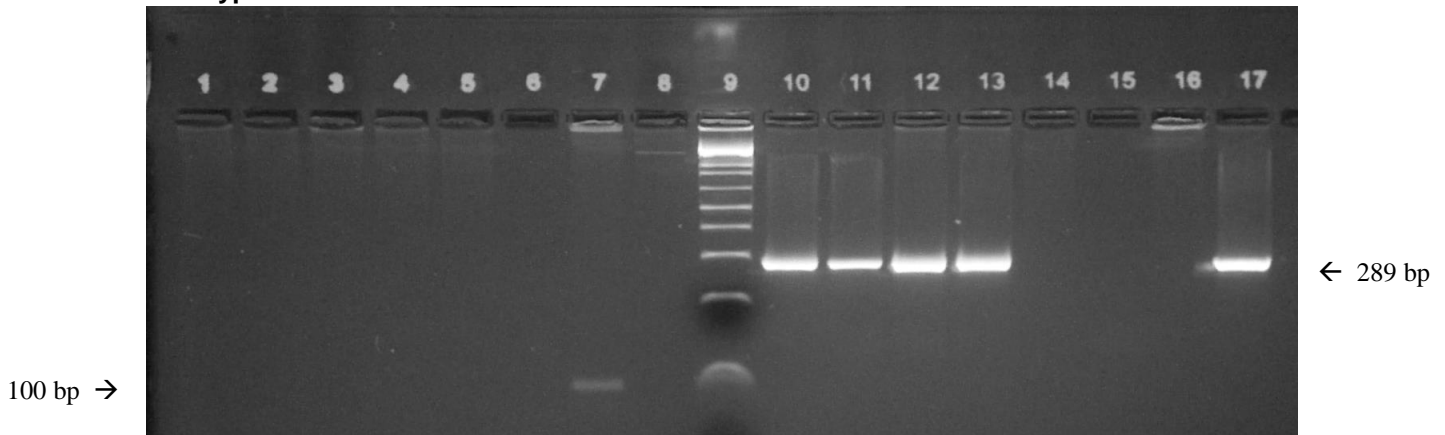
Result analysis:

	Wild-type PCR	Neo PCR
Wild-type	100bp product	no product
Heterozygous	100bp product	289bp product
Homozygous Mutant	no product	289bp product

Example of Gel:

Wild-type PCR

Neo PCR



Wells 1-4 are negative for the wild-type PCR. Well 7 is a wild-type control. Well 8 is a homozygous control. Well 9 is 1Kb Plus DNA ladder (Invitrogen Cat. # 10787-018). Wells 10-13 are positive for the Neo PCR. Well 16 is a wild-type control. Well 17 is a homozygous control.