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Mouse Conditional Knockout User Manual

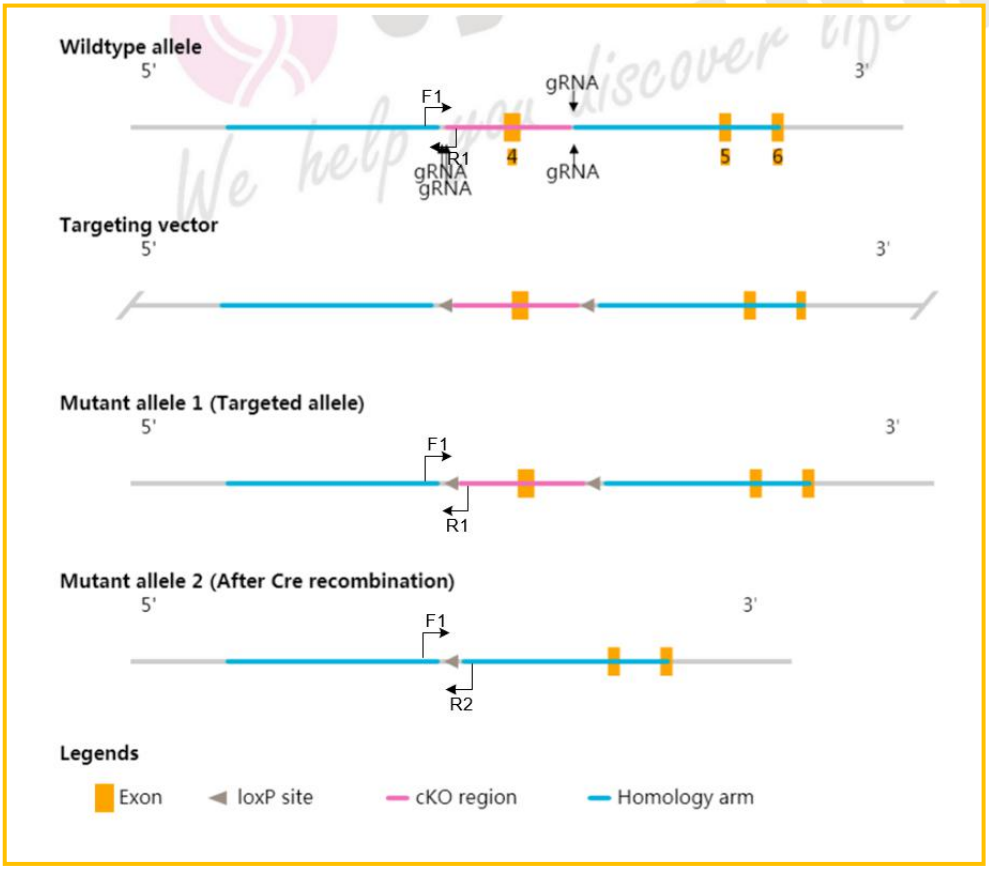
Quote: CKOAI190919YZ7-B

- Confidential -

1. Product Information

Name	C57BL/6N-Nr1h4^{em1cyagen}
Serial Number	CKOCMP-20186-Nr1h4-B6N-VA
Gene	Nr1h4
NCBI ID	20186
Strain	C57BL/6N
Type	conditional knockout

2. Targeting Strategy



3. Genotyping strategy

Primers1: (Annealing Temperature 60.0 ºC)

F1: 5'-GTATACAGGATACCCAAGGAGGC-3'
R1: 5'-TGGCATTTAAAAGGAGAAGGCCAG-3'

Homozygotes: one band with 228 bp
Heterozygotes: two bands with 228 bp and 197 bp
Wildtype allele: one band with 197 bp

Primers2: (Annealing Temperature 60.0 ºC)

PVillin-M-F : 5'-GTGTTTGGTTTGGTTTCCTCTGCATAAGA-3'
PVillin-M-R : 5'-GCAGGCAAATTTTGGTGTACGGTCA-3'

Cre amplicon: ~500 bp

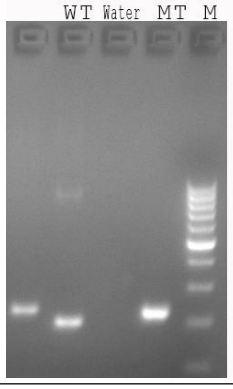
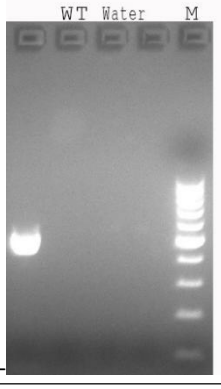
The tissue-specific gene deletion can be confirmed by adding one additional primer to the PCR assay:

Primers4: (Annealing Temperature 60.0 ºC)

F1: 5'-GTATACAGGATACCCAAGGAGGC-3'
R2: 5'-CTTTCCTTCTTAAGGGCAAGAGAG-3'

With Cre activity: one band with 230 bp

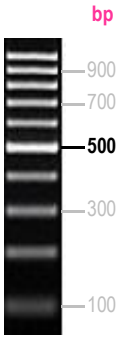
4. Expected Results

Genotyping	Primers1	Primers2
Flox/Flox, PVillin-Cre: Homozygotes, Cre		

Note:

1) PCR was carried out in 25 μL volume for 35 cycles under standard conditions, with all two primers listed above added to each reaction.

2) DNAmarker:Thermo Scientific GeneRuler 100 bp DNA Ladder #SM0242



3) Controls used in PCR genotyping are:

- Water control: No DNA template added.
- Wildtype control: Mouse genomic DNA.
- Mutation control: Donor vector DNA.

5. PCR reaction

5.1 DNA Extraction

➢ Method One:

We recommend that using TaKaRaMiniBEST Universal Genomic DNA Extraction kit (Ver.5.0_Code No. 9765) to gain high purity of genomic DNA.

a. Add 180 μL of Buffer GL, 20 μL of Proteinase K and 10 μL of RNase A per tail piece (2-5 mm) in a microcentrifuge tube. Be careful not to cut too much tail.

b. Incubate the tube at 56℃ overnight.

c. Spin in microcentrifuge at 12,000 rpm for 2 minutes to remove impurities.

d. Add 200 μL Buffer GB and 200 μL absolute ethyl alcohol with sufficient mixing.

e. Place the spin Column in a collection tube. Apply the sample to the spin and centrifuge at 12,000 rpm for 2 min. Discard flow-through.

f. Add 500 μL Buffer WA to the spin column and centrifuge at 12,000 rpm for 1 min. Discard flow-through.

g. Add 700 μL Buffer WB to the spin column and centrifuge at 12,000 rpm for 1 min. Discard flow-through. (Note: Make sure the Buffer WB has been premixed with 100% ethanol. When adding Buffer WB, add to the tube wall to wash off the residual salt.)

h. Repeat step g.

i. Place the spin Column in a collection tube and centrifuge at 12,000 rpm for 2 min.

j. Place the spin Column in a new 1.5ml tube. Add 50~200 μL sterilized water or elution buffer to the center of the column membrane and let the column stand 5min. (Note: Heating sterilized water or elution buffer up to 65℃ can increase the yield of elution.)

k. To elute DNA, centrifuge the column at 12,000 rpm for 2 min. To increase the yield of DNA, add the flow-through and/or 50~200 μL sterilized water or elution buffer to the center of the spin column membrane and let the column stand 5 min. Centrifuge at 12,000 rpm for 2 min.

l. Quantify to genomic DNA. Eluted genomic DNA can be quantified by electrophoresis or electrophoresis.

➢ Method Two:

A low-cost and sample method to gain rough genomic DNA.

a. Add 100 μL of tail digestion buffer per tail piece (2-5 mm) in a microcentrifuge tube. Be careful not to cut too much tail.

b. Incubate the tube at 56℃ overnight.

c. Incubate the tube at 98℃ for 13 minutes to denature the Proteinase K.

d. Spin in microcentrifuge at top speed for 15 minutes. Use an aliquot of supernatant straight from the tube (1 μL in a 12.5 μL reaction) for PCR.

Final concentration of tail digestion buffer:

➢ 50 mMKCl
➢ 10 mM Tris-HCl (pH 9.0)

- 0.1 % Triton X-100
- 0.4 mg/mL Proteinase K

5.2 PCR Mixture (primer concentration: 10μM):

Component	x1	
ddH_2O	9.0	μl
Product primer F	1.0	μl
Product primer R	1.0	μl
Premix Taq	12.5	μl
DNA	1.5	μl
Total	25	μl

5.3 PCR Reaction Conditions:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30 s	35 x
Annealing	60 °C	35 s	
Extension	72 °C	35 s	
Additional extension	72 °C	5 min	

5.4 Relevant Reagents:

TrizmaHydrochloride Solution	Sigma, Cat. No. T2663
Proteinase K	Merck, Cat. No. MK539480
Triton X-100	Sigma, T8787-50 mL
2 × Taq Master Mix (Dye Plus)	Vazyme, P222
Agarose	BIOWEST AGAROSE, REGULAR
DNA Marker	Thermo Scientific GeneRuler 100 bp DNA Ladder #SM0242
0.5×TBE	Tris Bio Basic Inc, TBO194-500g EDTA Shanghai Sangon, 0105-500g Boric Acid, Shanghai Sangon, 0588-500g