

# Genotyping protocol for Adamts13-KO mice

-- Recipe from the original developer of Adamts13 KO mice --

Three primers are used for genotyping Adamts13-KO alleles:

Adamts 5'F                    5' -ACC CTA TCT CTG GCC TGT ATT CCT-' 3  
Adamts 5'R1                  5' -TAC TGA CTT GTG ACC ACA AGC CCT-' 3  
Adamts 5'R2                  5' -ATC GAG TCT AGC TTG GCT GGA CGT-' 3

Adamts 5'F and Adamts 5'R1 produce 616-bp band, derived from wild alleles.

Adamts 5'F and Adamts 5'R2 produce 350-bp band, derived from knockout alleles.

Reaction mixture:

Primer Adamts 5'F (10 $\mu$ M)	1 $\mu$ L
Primer Adamts 5'R1 (5 $\mu$ M)	1 $\mu$ L
Primer Adamts 5'R2 (5 $\mu$ M)	1 $\mu$ L
HotStarTaq Master Mix (Qiagen)	10 $\mu$ L
DNA sample	1 $\mu$ L
Water	6 $\mu$ L
Total	20 $\mu$ L

PCR conditions: Veriti Thermal cycler (ABI).

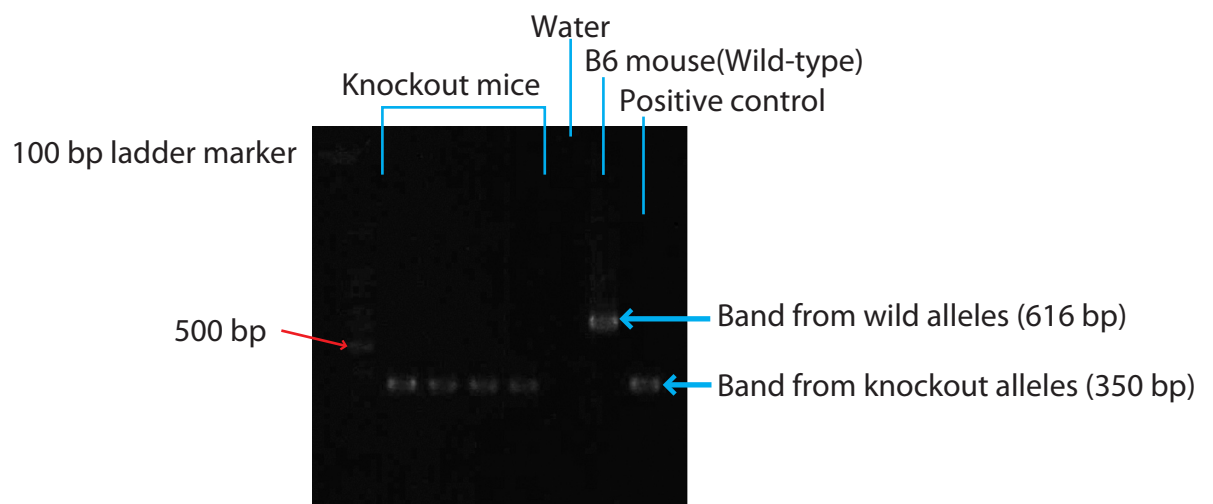
(95°C: 15 min.) x 1 cycle

(94°C: 20 sec., 57°C: 20 sec., 72°C: 40 sec.) x 35 cycles

(72°C: 5 min.) x 1 cycle

Then, keep at 4°C

## Examples of PCR results



Separated by electrophoresis on a 2% agarose gel with TAE buffer

Note: both 616-bp and 350-bp bands were produced with genomic DNA from heterozygous mice (not shown in this figure).