

08.07.13 MLS
04.07.17 MLS

Genotyping Protocol: **MMRRC 36517**

Assay Type: PCR – can distinguish between heterozygous and homozygous samples.

DNA Extraction: DNA from tail snips was extracted using the following protocol:

1. Place a ~3mm piece of mouse or rat tail in 180ul of 50mM NaOH in a 0.2mL tube and vortex
2. Incubate at 95°C for 10 minutes, then at 40°C for 5 minutes to prevent popped tube caps.
3. Add 20ul of 1M Tris-HCl (pH 8.0) and vortex well. Use this lysate as your DNA template in KOD Xtreme PCR reactions.

Strain Description: This strain carries an ENU-induced 19 base pair deletion in exon 10 of the mouse solute carrier family 5, member 2 gene (*Slc5a2*). This results in the creation a premature stop codon in exon 10 of *Slc5a2*. Details can be found on the Mutagenetix database under 'jimbee.'

Wild type *Slc5a2* sequence – the 19 deleted base pairs are colored in red.

CAGCAGTAGC ACGCTCTTCA CC**ATGGATAT CTACACGCGC** CTGCGGCCCC GTGCAGGTGA

Primer Information:

- 1) Name: M36517 seq F Sequence: 5'-CAG CAG TAG CAC GCT CTT CA-3'
- 2) Name: M36517 seq R Sequence: 5'-CCC AGA GCC TGT AGT TGA GG-3'

Primer location: Primers are located on either side of the 19bp deletion in exon 10 of *Slc5a2*.

Assay Name: M36517 Jimbee PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	EMD Millipore	2X	10
KOD Xtreme dNTPs	EMD Millipore	2mM	4
M36517 seq F	Sigma or IDT	25µM	0.3
M36517 seq R	Sigma or IDT	25µM	0.3
KOD Xtreme <i>Taq</i>	EMD Millipore (Cat# 71975-3)	1.0 U/µl	0.4
sterile water			3

PCR Setup:

Final Reaction: 18µl master mix & 2µl DNA template (10-20ng/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:

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 63°C 30 seconds
- 4) 72°C 30 seconds
- 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

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Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320

Injection: 10s at 5KV

Separation: 320s at 6KV

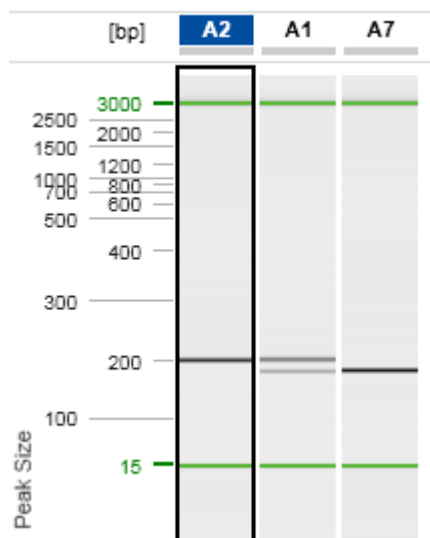
Expected products:

Wild-type: 205bp

Heterozygous: 205bp, 186bp

Homozygous mutant: 186bp

Example gel:



Lane A2 displays a wild-type sample (205bp product)
Lane A1 displays a heterozygous sample (205bp and 186bp products)
Lane A7 displays a homozygous mutant product (186bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.