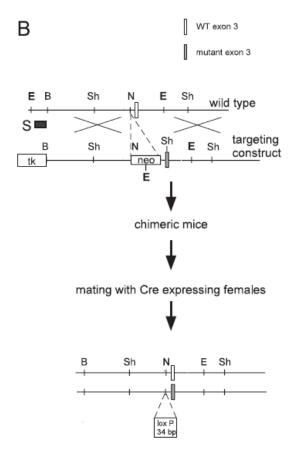
# Genotyping Protocol: MMRRC 34289

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

**DNA Extraction**: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

**Strain Description:** This strain carries a knocked-in mutation within Exon 3 of the of the estrogen receptor 1 (alpha) gene (*Esr1*) on Chromosome 10. The knock-in of the E207A/G208A mutation results in substitution of glutamate (207) and glycine (208) with alanine at both positions. Details can be found in Jakacka et al (2002) Mol Endocrinol 16:2188-2201.

Please note: this mutation is being maintained on a C57BL/6 genetic background. The mutation is also available on the 129 genetic background (MMRRC 34288).



Sequence Comparison (Codons 207 and 208 highlighted in yellow) WT Sequence:

## Mutant Sequence:

#### 1.19.11 EB

#### **Primer Information:**

1) Name: Esr1.F Sequence: 5'-GTG TTC TGA AAT CCC ACC CAG CTG C-3'
2) Name: Esr1.R Sequence: 5'-GAA ATG GAC TGC ATA CCC CAG AAC AG-3'

Primer location: Esr1.F and Esr1.R are located on either side of the 34bp loxp site, which is located upstream of exon 3.

Assay name: M34288 PCR

## **PCR Master Mix Components:**

component	manufacturer	concentration	μl/rxn
10X Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
Esr1.F	Sigma	25µM	0.3
Esr1.R	Sigma	25µM	0.3
FastStart Taq	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

# PCR Setup:

Final Reaction: 19µl master mix & 1µl 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
 20 seconds

 3)
 58°C
 25 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

#### **Product Analysis:**

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

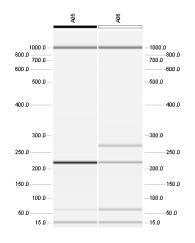
Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521) Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)

Method: AM320 Injection: 10s at 5KV Separation: 320s at 6KV

**Expected products:** 

WT: 200 bp Mut: 235 bp

Example gel:



Lane A05 displays a WT sample (200bp product). Lane A06 displays a heterozygous sample (200bp and 235bp products).

\*Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel method and do not represent expected products.\*