

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	2
<b>MMRRC Strain Name</b>	B6.129P2- <i>Esr2</i> <sup>tm1Unc</sup> /Mmnc
<b>Gene Name(s)</b>	Estrogen receptor 2 ( <i>Esr2</i> )
<b>Breeding Protocol(s)</b>	Intra-strain Mating
<b>Protocol Date</b>	8/26/13

### MMRRC #2 PCR Reaction

	<b>1X</b>
ddH <sub>2</sub> O	14.5
5X Buffer (1.5 mM MgCl <sub>2</sub> in the final 1X concentration)	5.0
25mM MgCl <sub>2</sub>	0.5
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

Note: Final concentration of MgCl<sub>2</sub>: 2 mM

**Thermal Cycler:**

Step 1: 98°C for 30 sec  
 Step 2: 98°C for 5 sec  
 Step 3: 69°C for 30 sec  
 Step 4: 72°C for 30 sec  
 Step 5: Step 2 to 4; Cycles: 35  
 Step 6: 72°C for 1 min

**Taq: Finnzyme Phire<sup>®</sup> Hot Start DNA Polymerase (Cat# F-122)**

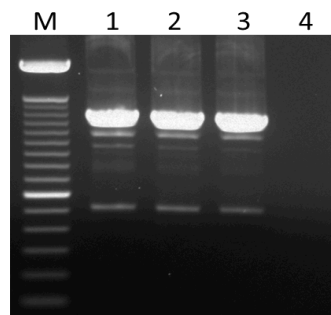
**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

Com-F (2): GTGATGAGCTGAGGTGGTGCTT  
 WT-Rev (2): CATCCTTCACAGGACCAGACAC  
 Mut-Rev (2): GCAGCCTCTGTTCCACATACAC

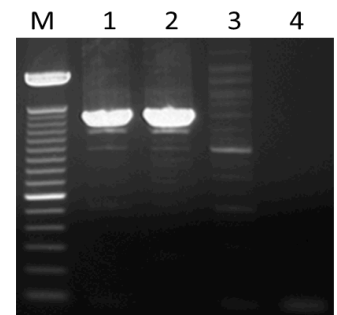
**Bands expected:**

Com-F (2) + WT-Rev (2), WT 1313 bp  
 Com-F (2) + Mut-Rev (2), Mut 1479 bp

Run on 0.8% agarose gel in TAE.



Primers: Com-F (2) + WT-Rev (2)



Primers: Com-F (2) + Mut-Rev (2)

Lane 1, 2: Het; Lane 3: WT; Lane 4: H<sub>2</sub>O; M: 100 bp DNA ladder Invitrogen