

Genotyping protocol

EM:11180

B6.129X1(Cg)-Esr1^{tm1.1Gust}/Kctt

Primers

ER α in1SP 5' **GGA ATG AGA CTT GTC TAT CTT CGT** 3'
ER α 3'ASP 5' **CCT GGC ATT ACC ACT TCT CCT** 3'

PCR profile

95 °C, 5 min

94 °C, 30 s 30 cycles
50 °C, 45s
72 °C, 90s

72 °C, 10 min
4 °C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	3.0 μ l
MgCl ₂ (25 mM)	1.8 μ l
dNTP mix (25 mM of each nucleotide)	0.24 μ l
ER α in1SP (100 μ M)	0.15 μ l
ER α 3'ASP (100 μ M)	0.15 μ l
AmpliTaq Gold (5 U/ μ l)	0.15 μ l
DNA template (~ 0.5 μ g tail DNA)	2.0 μ l
H ₂ O	<u>22.51 μl</u>
	30 μ l

Post-PCR analysis

2% agarose gel.
Expected pattern is **wt: 800bp**
KO: 300bp