

MMRRC Strain #16135
Parp1 PCR Genotyping Protocol

Description: Parp 1 wildtype band ~ 353 and gene trap band ~ 177. F-Parp 1 and R-Parp 1a detect endogenous wildtype sequence in the intron between exon 7 and exon 8. F-Parp1 and R-GEO Parp 1 detects presence of gene trap in the intron between exon 7 and exon 8.

Primers:

F- Parp 1 5' –GTT GCT CTT CAC CAG ACG TCA TCA – 3'
R- Parp 1 a 5' –CCC TAC AGA CTG CAC AAC GCC – 3'
R- GEO Parp 1 5' –ACT CCA ACC TCC GCA AAC TCC TAT – 3'

Reaction Mix (For one 25ul reaction):

H2O	12.85 ul
10X PCR Buffer	2.5 ul
25mM MgCl ₂	1.5
25 mM dNTP	0.2 ul
25 uM F Parp 1	0.75 ul
25 uM R Parp 1a	0.5ul
25 uM R GEO Parp 1	0.5 ul
5X Rapid-Load Buffer*	5.0ul
Taq DNA Polymerase	0.2 ul
DNA	1.0 ul

*= 5X R.L. Buffer – OriGene Technologies, Catalog # RL-105

Thermocycler Settings:

95C for 2 min

Cycle 35 times

95C for 20 sec

62C for 20 sec

72C for 30 sec

72C for 2 min

10C forever

Electrophoresis:

Run on 2% Gel