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PKR Transgenic Genotyping Protocol

Description:

PKR tg mice contain the human PKR cDNA with a CMV-T7 promoter. The 5' primer CMV-JM1 is located in the CMV promoter, and the 3' primer PKR-CMV spans parts of exons 11 and 12 of the human PKR gene. Amplified product should be approximately **1.4KB**

Primers:

CMV-JM1 5' - CGC TAT TAC CAT GGT GAT GCG G -3'

PKR-CMV 5' - TAC TTC ACG CTC CGC CTT CTC G -3'

Reaction Mix (For one 25ul reaction):

H2O	12.65ul
10X PCR Buffer	2.5ul
25mM MgCl ₂	1.35ul
25uM 5'Primer	1.0ul
25uM 3'Primer	1.0ul
25mM dNTP	0.2ul
5X Rapid-Load Buffer*	5.0ul
Taq Polymerase	0.3ul
DNA	1.0ul

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

Thermocycler Settings:

95C for 4 min

cycle 30 times

95C for 1 min

55C for 1 min

72C for 2 min

72C for 10 min

4C forever

Electrophoresis:

Run on 1% Gel