

**University of Washington**  
**Warren C. Ladiges**  
**MMRRC #10323**

### **P58<sup>IPK</sup> Null PCR Protocols**

There are two protocols needed to adequately genotype for P58<sup>IPK</sup> Null:

1) This protocol checks for the targeted disruption of P58 by amplifying the region between the B-Gal gene on the construct and the genomic DNA outside of the targeted site. A band of approximately 2000 bp should be visible if the sample contains at least one disrupted allele.

#### Primers:

**P58ES**            5'- CCT ATG TGT TGC AGC TAT GGC AGG CAA G -3'

**B-GAL#2-5'R** 5'- GCG CCA TTC GCC ATT CAG GCT GCG -3'

#### Reaction Mix (For one reaction):

H2O	9.1ul
Lu's 5X Buffer*	3.33ul
25mM MgCl <sub>2</sub>	1.0ul
25uM P58ES primer	0.3ul
25uM B-GAL primer	0.3ul
Betaine	5.0ul

Hot start:

Mix Well. Add 10ul mix to each reaction tube

Add 1ul DNA to each tube

Begin PCR reaction

At thermocycler pause, add and mix 0.2 ul/rxn of 25mM dNTP and 0.2ul/rxn of Taq Polymerase to remaining reaction mix in master mix tube. Add 10ul to each reaction tube. Continue thermocycler.

#### Thermocycler Settings:

100C for 3 min

72C Pause

Cycle 35 times

94C for 30 sec

65C for 30 sec

72C for 1.5 min

72C for 5 min  
4C forever

Electrophoresis: Run on 1% Gel

2) This protocol checks for zygosity of P58 and shows that the targeting construct has integrated. A 330 bp band represents the wildtype P58 allele and a 280 bp band represents the P58KO construct (indicating a disrupted allele). There was a 50 bp deletion in the targeting arm of the construct for the 330 bp region represented in the wildtype DNA. One 330 bp band indicates wildtype, one 330 bp band and one 280 bp band indicates heterozygosity, and one 280 bp band indicates homozygosity for the knocked out allele.

Primers:

**P58-Sma-del-5'** 5'- AGC CCG GCC TCC CCA GCC TCT TC -3'

**P58-Sma-del-3'** 5'- CCC GTC CAC TCG CTC GCT CGC TC -3'

Reaction Mix (For one reaction):

H2O	4.8ul
Lu's 5X Buffer*	4.0ul
25mM MgCl <sub>2</sub>	1.2ul
25uM P58 -5' primer	1.0ul
25uM P58 - 3' primer	1.0ul
Betaine	4.0ul
5X R.L. Buffer**	4.0ul

Hot start:

Mix Well

Add 10ul mix to each reaction tube

Add 1ul DNA to each tube

Begin PCR reaction

At thermocycler pause, add and mix 0.2 ul/rxn of 25mM dNTP and 0.2ul/rxn of Taq Polymerase to remaining reaction mix in master mix tube. Add 10ul to each reaction tube. Continue thermocycler.