

GluR5 Genotyping PCR protocol

To distinguish between GluR5 wildtype, heterozygous, and knockout mice.

Date:

Tail #s:

Controls: +/
 +/-
 -/-
 H₂O

Products:

Wild Type allele gives ~694bp PCR product.

Knockout allele gives ~380bp PCR product.

Primers: (There are 15uM stocks.) (15uM is same as 15pmol/ul)

R5 sense 5' ccagaagagcaaaagacc 3' T_m=54° C

R5 antisense 5' ctctacttctgcctttgtc 3' T_m=56° C

Neo 5' gagtagaaggtggcgcg 3' T_m=56° C

Reaction (25ul reactions)

Reagents	For 1 reaction (1X)	Master Mix ___X
dH ₂ O	17.85 ul	
10X PCR Buffer	2.5 ul	
dNTPs (25 mM)	0.2 ul	
Primer R5 sense (15 uM stock)	1.25 ul	
Primer R5 antisense (15 uM stock)	1.25 ul	
Primer neo (15 uM)	1.25 ul	
Taq polymerase	0.2 ul	

Pipette 24.5 ul Master Mix into each tube.

Add 0.5ul of of tail DNA/per tube.

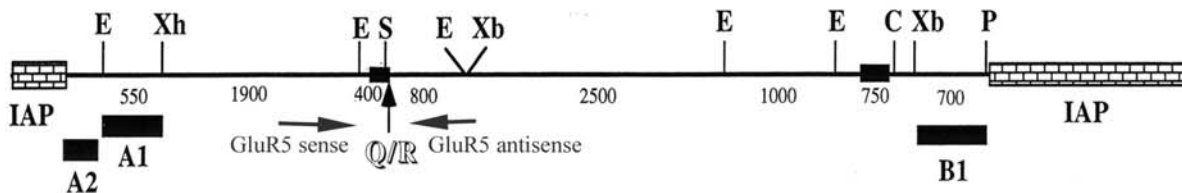
Cycles:

Step 1 92°C load
Step 2 95°C 2 minutes

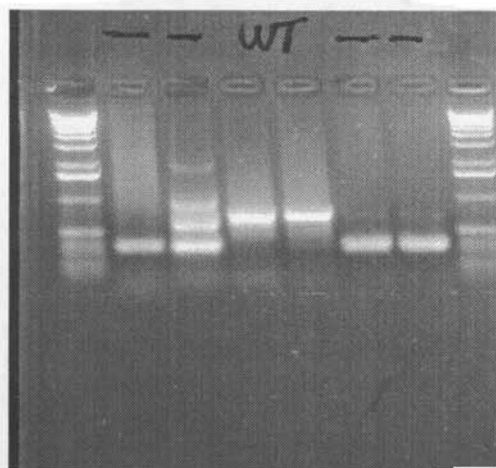
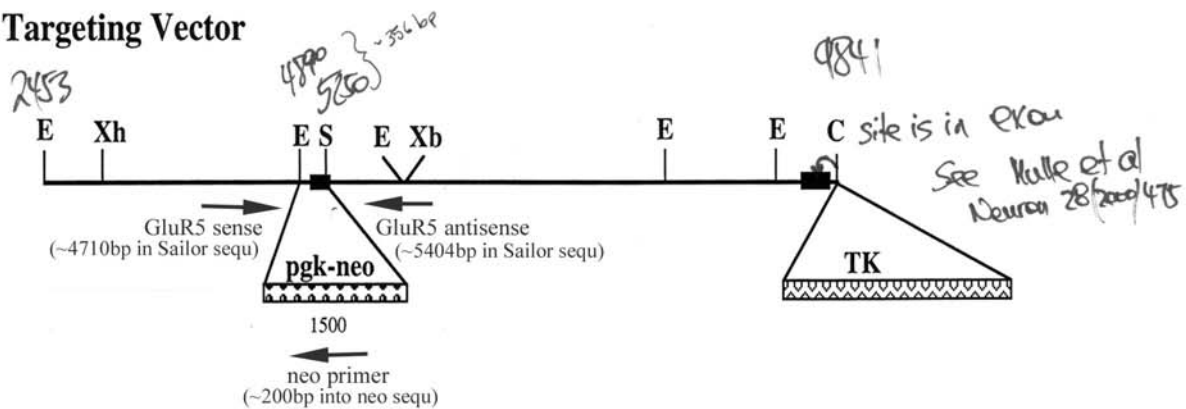
Step 3 95°C 25 seconds
Step 4 52°C 1 minute
Step 5 72°C 2 min
Step 6 Go To step 3, 34 times

Step 7 72°C 10 minutes
Step 8 7°C hold

GluR5 Genomic Map around TM2 Exon



Targeting Vector



← WT ~694 bp
 ← Mut ~380 bp