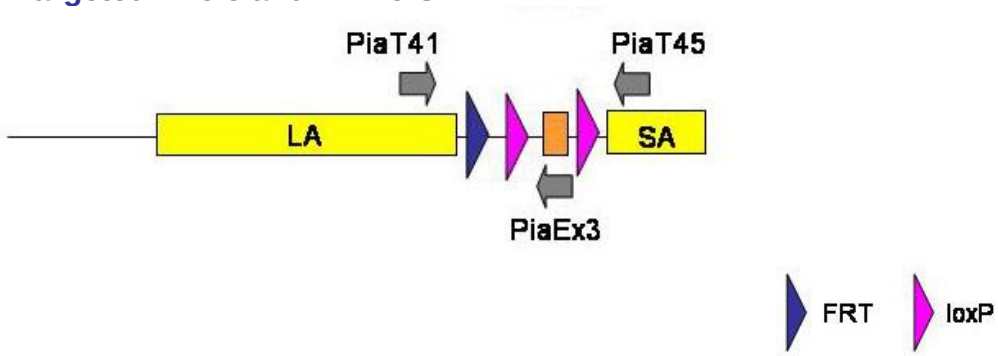
PCR Genotyping Protocol for *Rictor*^{Flox} (*Rictor*$^{tm1.1Mgn}$) Mice

The following conditions are used by the Mark Magnuson Lab to genotype mouse genomic DNA for *Rictor* gene targeting and recombination events.

Map of Targeted Allele and Primers



PCR reagents:

1. PCR primers:
   oligo primers at 20 uM amplify a 466 bp *Rictor*^{WT} &/or a 554 bp *Rictor*^{Flox} alleles
   PiaT41 5'-ACT GAA TAT GTT CAT GGT TGT G-3' (top)
   PiaEx3 5'-GAA GTT ATT CAG ATG GCC CAG C-3' (bottom)
2. Perkin Elmer PCR buffer with $MgCl_2$
3. dNTP premix (I make my own dNTP premix using 100 mM NEB dNTP's. The premix contains 250 ul of each dNTP - A, C, G, &T - and 19 ml sterile water and is stable at -20ºC. I freeze this in 1 ml aliquots and thaw and refreeze as needed.)
4. Perkin Elmer Amplitaq Gold
5. genomic DNA samples diluted to 50 ng/ul with sterile water

PCR reaction mixture:

15.8 ul sterile water
2.5 ul 10X PCR buffer
4 ul dNTP premix
0.75 ul primer PiaT41
0.75 ul primer PiaEx3
1 ul dil. DNA template
0.2 ul Amplitaq Gold
25 ul total volume

Cycling conditions:

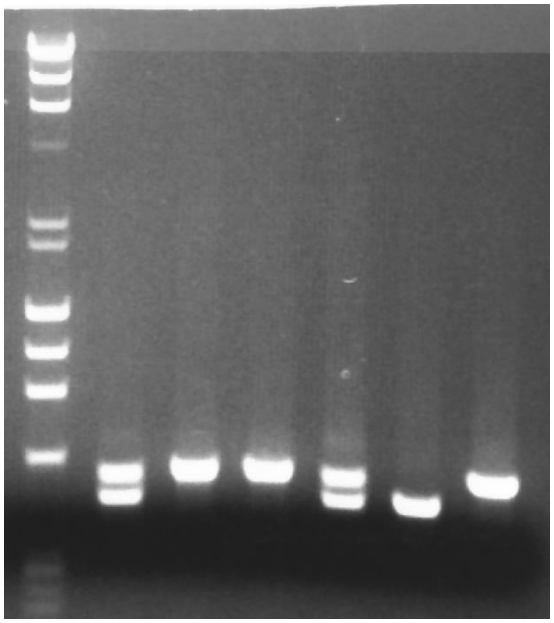
1 cycle - 94º x 6 min.
40 cycles - 94º x 1 min., 60º x 30 sec., 72º x 30 sec.
1 cycle - 72º x 7 min.
hold at 4ºC.

Analysis of PCR products:

Load 10 ul aliquots of reactions + 2 ul gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.

Standards = Hind III digested λDNA + Hae III digested PhiX 174 DNA

Primers PiaT41 + PiaEx3



← 554 bp *Rictor*^{Flox}
← 466 bp *Rictor*^{WT}