

### **P53R172 Genotyping**

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Bring final volume of each oligo to 100 pmol/  $\mu\text{l}$  (each) using ultrapure  $\text{H}_2\text{O}$ .

pL5    KMO272 ACCTGTAGCTCCAGCACTGG  
pL6    KMO273 ACAAGCCGAGTAACGATCAGG

3. Set up a master mix (on ice) for each primer as follows:

12.5  $\mu\text{L}$  Promega GoTaq Mastermix  
1.0  $\mu\text{L}$  Primer Mix (=10pmol each primer)  
10.5  $\mu\text{L}$   $\text{H}_2\text{O}$



X No. of samples

4. Add 1 $\mu\text{l}$  template DNA to corresponding well in PCR plate.
5. Add 24 $\mu\text{l}$  master mix to each well in PCR plate
5. Seal using Microseal film.
6. Keep reactions on ice and load onto PCR block pre-heated to 94 $^\circ$ .

### **PCR Program**

95 $^\circ$  5 min.  
95 $^\circ$  1 min  
60 $^\circ$  1 min  
72 $^\circ$  3 min  
72 $^\circ$  8 min  
10 $^\circ$  forever

} Steps 2-4 for 35 cycles

### **Expected band sizes:**

WT: approx. 380bp  
Mut: approx. 450bp