

### **Trp53 Genotyping**

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Make a Primer Mix containing both **wt** or both **mut** primers at 10 pmol/  $\mu$ l (each) using ultrapure H<sub>2</sub>O.

<b>wt</b>	KMO57	GTG TTT CAT TAG TTC CCC ACC TTG AC
	KMO58	ATG GGA GGC TGC CAG TCC TAA CCC
<b>mut</b>	KMO53	GTG GGA GGG ACA AAA GTT CGA GGC C
	KMO54	TTT ACG GAG CCC TGG CGC TCG ATG T

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

12.5  $\mu$ L Promega GoTaq Mastermix  
1.0  $\mu$ L Primer Mix (=10pmol each primer)  
10.5  $\mu$ L H<sub>2</sub>O



X No. of samples

4. Add 1 $\mu$ l template DNA to corresponding well in PCR plate.
5. Add 24 $\mu$ 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°.

### **PCR Program**

94° 3 min.	} 35 cycles for WT reaction
94° :30 sec	
67° :45 sec	} 35 cycles for mutant reaction
72° :30 sec	
72° 2 min	
10° forever	

### **Expected band sizes:**

WT: approx. 320bp  
Mut: approx. 150bp