

### **Ligase 4 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>	KMO62	CTC TGT AGG GCT TAG TGA CAT CTC
	KMO63	GCG CTC ACC ATC AAG CGC AGT TTC GAT GTA G
<b>mut</b>	KMO64	ACC GCT ATC AGG ACA TAG CGT TGG
	KMO65	GAC TCT TTT TAC CCT GCA ATG AGA CTC ATT C

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

12.5 uL Promega GoTaq Mastermix  
1.0 uL Primer Mix (=10pmol each primer)  
10.5 uL 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° :45 sec		
55° :45 sec	}	35 cycles for mutant reaction
72° 1:30		
72° 10 min		
10° forever		

### **Expected band sizes:**

WT: approx. 150bp  
Mut: approx. 700bp