

## **JH Genotyping**

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Make a Primer Mix containing both **wt** or both **mut** primers at 10 pmol/  $\mu\text{l}$  (each) using ultrapure  $\text{H}_2\text{O}$ .

<b>wt</b>	KM0318 JH3	CAC AGT AAC TCG TTC TTC TCT GC
	KM0320 JH1	CAG TGA ATG ACA GAT GGA CCT CC
<b>mut</b>	KM0319 JH2	GCA GAA GCC ACA ACC ATA CAT TC
	KM0320 JH1	CAG TGA ATG ACA GAT GGA CCT CC

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°.

## **PCR Program**

94° 4 min.	}	35 cycles for WT reaction
94° 1 min.		
60° 1 min.	}	35 cycles for mutant reaction
72° 1 min.		
72° 5 min		
10° forever		

### **Expected band sizes:**

WT: approx. 400bp  
Mut: approx. 650bp