

## Rag2 genotyping PCR protocol

### Primers

RagA: GGG AGG ACA CTC ACT TGC CAG TA

RagB: AGT CAG GAG TCT CCA TCT CAC TGA

RagNeo: CGG CCG GAG AAC CTG CGT GCA A

Reagent	Amount (multiply by number of samples)
ddH <sub>2</sub> O	11.5 $\mu$ L
25 mM MgCl <sub>2</sub>	1.5 $\mu$ L
Fermentas 10x Taq buffer (+ (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> )	2.5 $\mu$ L
2.5 mM dNTP	2.5 $\mu$ L
10 $\mu$ M primers	1.0 $\mu$ L
DMSO	2.5 $\mu$ L
Taq polymerase	0.5 $\mu$ L
<b>Total</b>	

Add 3  $\mu$ L of DNA to each tube to bring total volume to 25  $\mu$ L

PCR parameters			
	Time	Temperature	Number of cycles
<b>Denature</b>	5 min	95°C	1
<b>Denature</b>	30 sec	94°C	35
<b>Anneal</b>	45 sec	62°C	
<b>Extend</b>	1 min	72°C	
<b>Final extension</b>	10 min	72°C	1
<b>Cool</b>	Indefinitely	4°C	1

### Products

WT Band: ~ 300bp

KO Band: ~ 400bp