

Arispe Lab  
 PCR Protocol for PR-CRE Recombinase Genotyping January 25,  
 2007

MIX

	Vol. / rxn	Total req'd
H2O	16.3 $\mu$ L	
10x buffer (Hotmaster)	3.0 $\mu$ l	
2.5 mM dNTP	2.5 $\mu$ l	
10 pmol/ $\mu$ L Fwd Primer	2.0 $\mu$ l	
10 pmol/ $\mu$ L Reverse1 Primer	2.0 $\mu$ l	
10 pmol/ $\mu$ L Reverse2 Primer	3.0 $\mu$ l	
Taq polymerase (Hotmaster)	0.2 $\mu$ l	

Add mix to 2  $\mu$ L tail DNA or 2  $\mu$ L  
 H2O. Total volume: 30  $\mu$ L

EXPECTED BANDS

WT (-/-)	500 bp band
PRCRE HET (+/-)	200 bp band, 500 bp band
PRCRE HOMOZY. (+/+)	200 bp band

PRIMERS

Forward primer	5-AGG GAA GGA GGC AGA AAT TCC AGA-3
Reverse primer	5-ATC ACT CGT TGC ATC GAC CGG TAA-3
Reverse2 primer	5-TTC CAG TGC TCG AGG TTT GCT CTT-3

PCR PROGRAM

1. 2 min at 94°C
2. 20 s at 94°C
3. 20 s at 59°C
4. 40 s at 68° C
5. Repeat 2-4 24x additional cycles
6. 5 min at 68°C
7. 4°C inf.