PCR Protocol for Genotyping Cre: (Cre200 can be used to genotype all Cre lines.)

A. Primers:

Forward: ATT GCT GTC ACT TGG TCG TGG C
Reverse: GGA AAA TGC TTC TGT CCG TTT GC

B. Digestion of mouse tail: (Sigma-Aldrich Extract-N-Amp™ Tissue PCR Kits)

1. Add 50ul of Extraction solution
2. Add 12.5ul of Tissue Preparation Solution
3. Incubate at room temperature for 10min.
4. Incubate at 95°C for 3min.
5. Add 50ul of Neutralization Solution B
6. Vortex for 20 sec.
7. Store at -20°C (Digest is now ready for PCR)

C. PCR Reactants:

Biomix	10.0ul
ddH_2O	4.2ul
Cresol Red Dye	2.0ul
Primer 1	0.4ul
Primer 2	0.4ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

*DNA is added last

D. PCR Conditions:

Heated Lid:	105°C	
Initial Denaturation:	94°C	2 min
Cycles:	35x	
	94°C	20 sec
	59.5°C	30 sec
	72°C	30 sec
Final Extension:	72°C	10 min
Final Hold:	4°C	

E. Product:

Cre: ~200 base pair

F. Notes:

1. Tails can be store at -20°C until digested
2. When genotyping embryo tails, do not add dye in the PCR reactants and reduce the DNA amount to 1.0ul