

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: C57BL/6-Tg(CYP19A1-cre)1Jri/Mmucd MMRRC: 037059-UCD

Protocol: *PCR protocol provided by Donating Investigator*

Reagent/Constituent	Volume (μL)
Water	5.4
5x Buffer	3.0
MgCl ₂	1.5
dNTPs (0.6mM each)	1.5
Primer Mix (2μM each)	2.0
Taq Polymerase 5Units/μL	0.1
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	
	15.0 μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	60	0:25	35x
4. Elongation	72	0:30	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. Cyp19Cre-F	TACAGCACCTCTGAAGCAA	Estimated Running: Time: 90 min.		
2. Cyp19Cre-R	ACTTGGTCAAGTCAGTGCG	Primer Combination	Band (bp)	Genotype
		1 and 2	580	transgenic