GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **Generic EGFP Genotyping Protocol for GENSAT BAC Transgenic Strains**

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	9.2
10x Buffer (Qiagen)	2.0
$MgCl_2$ (stock concentration is 15mM) (Qiagen)	0.8
dNTPs (stock concentration is 2.5mM) (Qiagen)	1.6
Primer 1 (stock concentration is 50µM) EGFP forward primer	0.2
Primer 2 (stock concentration is 50µM) EGFP reverse primer	0.2
Primer 3 (stock concentration is 50µM) Actin PCR control/forward	0.4
Primer 4 (stock concentration is 50µM) Actin PCR control/reverse	0.4
Taq Polymerase (Qiagen)	0.2
Q solution (Qiagen)	4.0
DNA sample extracted	1.0
TOTAL VOLUME OF REACTION:	**20µL**

***Comments on protocol**:*

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	3:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	60	0:45	**30x**
4. Elongation		72	0:45	
5. Amplification		72	10:00	**1**
6. Finish		4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: EGFP forward	CCT ACG GCG TGC AGT GCT TCA GC
2: EGFP reverse	CGG CGA GCT GCA CGC TGC GTC CTC
3: Actin PCR control/forward	GAT GAC GAT ATC GCT GCG CTG GTC G
4: Actin PCR control/reverse	GCC TGT GGT ACG ACC AGA GGC ATA CAG

Electrophoresis Protocol:

Agarose: 2.5% **V:** 200-250 **Estimated Running Time:** 30-45 min.

Primer Combination	Band	Genotype
1 and 2	~300 bp	EGFP
3 and 4	1000 bp	Actin (control)