GENOTYPING PROTOCOL

UC Davis Mouse Biology Program

Protocol Name: **036512-UCD FLPO and Universal CSD Post FLP Protocol**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0.5
Primer 4. (stock concentration is 20µM)	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1x**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**30X**
7. Elongation		72	0:40	
8. Finish		4	∞	n/a

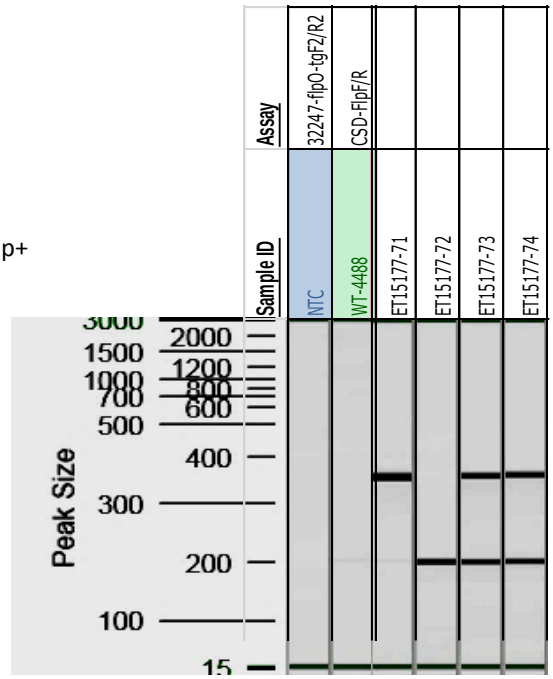
Primers:

Name	Nucleotide Sequence (5' - 3')
1. 32247-flpO-tgF2	GCCACCTTCATGAGCTACAACACC
2. 32247-flpO-tgR2	AACAGGAACTGGTACAGGGTCTTGG
3. CSD-FlpF	CGCATAACGATACCACGATATCAACAAG
4. CSD-FlpR	CCGCCTACTGCGACTATAGAGATATC

Electrophoresis Protocol:

Agarose:	**1.5%**	**9**
Estimated Running	**90**	min.
Primers	**Band** (bp)	**Genotype**
1 & 2	383	36512 FLPO positive
3 & 4	214	CSD Post Flp positive

#71=36512 FLPO+
#72=CSD Post Flp+
#73 & 74=Both 36512 Flpo as well as CSD Post Flp+



This protocol is designed for use with CSD and EUCOMM Knockout First (tm1a) alleles that are being crossed with MMRRC FLPo strain 036512-UCD.