GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
- *Choose File, Save As*
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These instructions are very generic. The menu options for your graphics program may be different.

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Telephone **713-798-2717**	FAX **713-798-3946**	
Strain Name **DBH_p2a_FlpO**	MMRRC Stock Number **41575**	

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NAME OF PCR: FLPo genotyping **MMRRC:** 0-CTR

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	10.4
10x Buffer	1.25
$MgCl_2$ (stock concentration is mM)	0
Betaine (stock concentration is 5M) *Optional*	0
dNTPs (stock concentration is 10mM)	0.25
DMSO *Optional*	0
Primer 1. (stock concentration is 20µM)	0.25
Primer 2. (stock concentration is 20µM)	0.25
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	0.1
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**12.5 µL**

Comments on protocol:

- We use DNA prepped by ear punch followed by boiling lysis prep. Add 25 µl boiling lysis buffer (25mM NaOH, .2mM EDTA in water). Incubate at 95C for 1 hour. Add 25 µl neutralization buffer (40 mM Tris acid in water).

Strategy:

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

Primers:

Name	Nucleotide Sequence (5' - 3')
1. FLPo-F	CACGCCCAGGTACTTGTTCT
2. FLPo-R	CCACAGCAAGAAGATGCTGA
3.	
4.	
5.	

Electrophoresis Protocol:

Argarose: 3.5% V:150

Estimated Running:Time: 10 min.

Primer Combination	Band	Genotype
FLP0_F/FLPo_R	226 bp	+
	bp	
	bp	

Please size gel images to fit in this space

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Protocol / Gel Comments:

Gel pictures: