

**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
- Select No Compression in the save options.
- Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.
- Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI		
Russell Ray		
Email		
Russell.ray@bcm.edu		
Institution		
Baylor College of Medicine		
Address		
1 Baylor Plaza, T707		
City	State	Zip
Houston	TX	77030
Lab Contact		
Ronda Kram		
Email		
kram@bcm.edu		
Telephone	FAX	
713-798-2717	713-798-3946	
Strain Name		MMRRC Stock Number
DBH_p2a_FlpO		41575

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER**

sacoord@mmrrc.org

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

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) <i>Optional</i>	0
dNTPs (stock concentration is 10mM)	0.25
DMSO <i>Optional</i>	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
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	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 (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	60	0:15	30x
4. Elongation	72	0:30	
5. Amplification			1
6. Finish		∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 3.5%	V:150
1. FLPo-F	CACGCCCAGGTACTTGTCT	Estimated Running:Time: 10 min.	
2. FLPo-R	CCACAGCAAGAAGATGCTGA	Primer Combination	Band
3.		FLP0_F/FLPo_R	226 bp
4.			bp
5.			bp
			Genotype
			+

Please size gel images to fit in this space

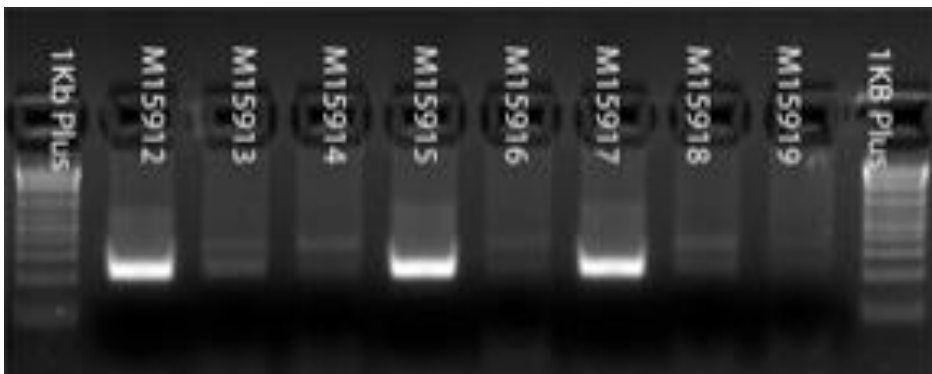
**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER**

sacoord@mmrrc.org

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

Protocol / Gel Comments:

Gel pictures:



PCR protocol provided by Donating Investigator