

**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.

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Strain Name		MMRRC Stock Number
B6D2F1-Bsph2<tm1b(EUCOMM)Osb>/12FOsb		037628

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NAME OF PCR: mBsph2 genotyping **MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	7
USB Taq PCR Master Mix (2X) (101375-538)	10
Primer 1. (stock concentration is 10µM)	0.5
Primer 2. (stock concentration is 10µM)	0.5
DNA extracted w/ NaOH-EDTA (http://jaxmice.jax.org/support/genotyping/dna-isolation-protocols.html)	2
<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: 20 µL

Comments on protocol:

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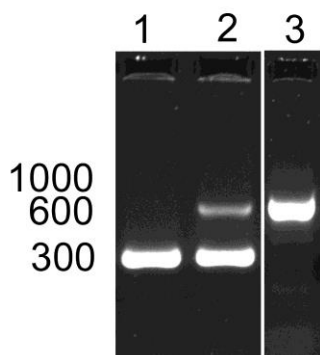
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	1:00	1
2. Denaturation	94	0:30	
3. Annealing steps 2-3-4 cycle in sequence	65	0:30	40x
4. Elongation	72	0:30	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V:100
1. Bsph2a-both	CCTACAGATATGCCACAGACCA	Estimated Running Time: 30 min.	
2. Bsph2a-WT	CCAATCATAGTCACTGTGGAGG	Primer Combination	Band
3. LAR3	CACAACGGGTTCTTCTGTTAGTCC	1&2	600 bp
4.		1&3	300 bp
5.			bp



mBSPH2A Genotyping

1. LAR3 + Bsph2a-both
2. LAR3 + Bsph2a-WT + Bsph2a-both
3. Bsph2a-WT + Bsph2a-both

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Protocol / Gel Comments:

Multiplex PCR can be performed.

Gel pictures:

