MMRRC @ UCDavis
Mutant Mouse Resource & Research Centers supported by NIH

Mouse Biology Program (MBP)
University of California, Davis
2795 Second Street, Suite 400, Davis, CA 95618

MMRRC Stock #: 050307-UCD

C57BL/6N-Atm1Brd Uts2btm1.1(KOMP)Mbp/Mmucd

Mouse genotyping Protocol

Project ID: CSD69135

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM $MgCl_2$	1.7
5 M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: (20uM)	0.5
Primer 2: (20uM)	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25 µl**

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

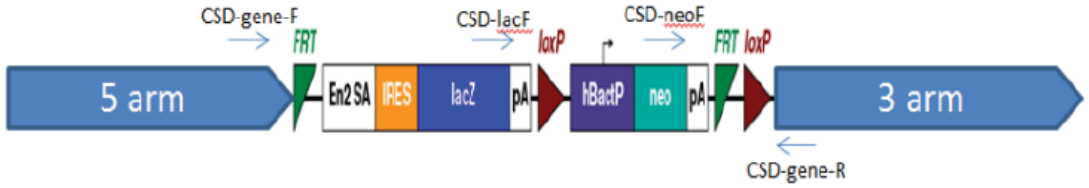
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	94	5:00	**1**
2. Denaturation	94	0:15	\
3. Annealing steps 2-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	> **x 40**
4. Extension	72	0:40	/
5. Final Extension	72	5:00	**1**
6. Finish	4	Hold	--

Primer Name	Nucleotide sequence (5'– 3')
1: CSD-lacF:	GCTACCATTACCAGTTGGTCTGGTGTC
2: CSD-neoF	GGGATCTCATGCTGGAGTTCTTCG
3: CSD-Uts2d-R	ACTATTGCTGTCATTAGAGGCTCAGG
4: CSD-Uts2d-F	CGAAAGTAAAATCCCCAGCAGTCCC
5: WT-1	CTGAGAGCATAAATGCATATCCTGACG
6: WT-2	CTTGGGTCACTAGTACCACATTTGC

Electrophoresis Protocol: **Run for 90 Min at 90 Volts on 1.5% Agarose.**

Primer Combinations	Band size (bp)	Genotype
CSD-neoF & CSD-Uts2d-R	621	PreCre
CSD-lacF & CSD-Uts2d-R	621	PostCre
WT-1 & WT-2	358	Wildtype
CSD-Uts2d-F & CSD-Uts2d-R	644	PostFlp

Primer Strategy



Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

We recommend running primers singleplex. For screening of pups prior to any Flp or Cre recombination, the PreCre primers may be used to identify the mutant mice. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line. The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).