MASH1

Mutant Allele (468bp)

MASH1jm-1: CCA ACT GGT TCT GAG GAC
MASH1jm-3: CCC ATT TGA CGT AGT TGG

Neomycin resistance gene (270bp)

MASH1neo-1: GAT CTC CTG TCA TCT CAC CT
MASH1neo-2: ATG GGT CAC GAC GAG ATC CT

94  5m

94  1m
60  1m
72  2m
35 cycles

72  10m

Reaction mixture should contain both primer pairs, one of which identifies the intact Mash-1 gene and the other the presence of the neomycin resistance gene.
1. **From Yolk Sacs – use Yolk Sac Buffer (YSB)**

   A. Digest yolk sacs in 500μl of non-SDS ProK Buffer containing 100μg/ml Proteinase K (10 l of Pro K stock kept at -20°C) overnight at 55°C with shaking (on Speci mixer).

   For embryos at 12.5dpc or older, cut a small piece of the tail and process similarly.

   **YSB**
   - 50mM KCL
   - 10mM Tris HCl – pH8.3
   - 2mM MgCl2
   - 0.1mg/ml gelatin
   - 0.45% NP4O
   - 0.45% Tween-20

   **NOTE:** Store YSB in 50ml aliquots at -20°C. Keep a working tube at 4°C.

2. **From Tails – use Tail Buffer**

   Tails can be cut starting from 3 week – old mice.

   A. Add 500μl of Tail Buffer containing 100μg/ml Proteinase K (10 l stock Pro K kept at -20°C). Incubate at 55°C with shaking overnight.

   **Tail Buffer**
   - 100mM Tris HCl – pH8.5
   - 200mM NaCl
   - 5mM EDTA
   - 0.2% SDS

   B. Spin for 10 minutes.
   C. Pour off supernatant into a new tube.
   D. Add 500μl of Isopropanol.
   E. Spin for 5 minutes.
   F. Wash with 70% ETOH
   G. Resuspend the pellet in 500μl of TE.
   H. Take 2μl for PCR
   I. See PCR protocol for 1B.

3. **PCR reaction**
DNA template in ProK Buffer  2.5µl

**Master Mix**
- dNTP’s (10 mM)  3.75 µl
- LIM (10X)  2.5 µl
- DMSO  2.5 µl
- Primer 1 (150 ng/µl)  1.25 µl
- Primer 2 (150 ng/µl)  1.25 µl
- H₂O to Total Volume  25 µl
- Taq  0.25 µl

Add Half of the Master Mix (without Taq) to the DNA
Incubate at 95°C for 10 minutes (to denature ProK) in the PCR machine.
When machine is at 80°C - add 2nd half of the PCR mix (with Taq).
Start the PCR reaction (35-40 cycles) at the appropriate conditions for your primers.

**10X PCR Buffer (LIM)**
- 670mM Tris HCl – pH8.8
- 67mM MgCl2
- 1.7mg/ml BSA
- 166mM (NH₄)₂SO₄

Primers – 200ng/each
dNTP – 1.5mM final
DMSO – 10% final
Taq Polymerase (5u/µl) – 0.25µl/reaction

**To genotype Mash1 mutant mice:**

**Wild-type:**
- **upper** : CTC CGG GAG CAT GTC CCC AA
- **lower** : CCA GGA CTC AAT ACG CAG GG

35 cycles of:
- 94°C/1 min
- 64°C/1 min
- 72°C/1 min

**Mash1 Mutant:**
- **upper** : GCA GCG CAT CGC CTT CTA TC
- **lower** : CCA GGA CTC AAT ACG CAG GG

35 cycles of:
- 94°C/1 min
- 60°C/1 min
- 72°C/1 min