MBL Zebrafish Course August 2005

Mapping a mutation

We will map the *you*^{ty97} mutation, which disrupts cell fate specification in the somites and the ventral spinal cord. DNA from wild-type and mutant diploid sibling embryos has already been prepared. Our first task is to score 48 markers on linkage groups 4-7 to identify those that may be linked to the mutation. Polymorphic markers linked to mutation will amplify different fragments from pools of genomic DNA from wild-type and mutant embryos. Thus we can score 48 markers on 2 pools in one set of 96 PCR assays.

After determining which markers could be linked to the mutation, the next step is to check candidate markers for linkage by scoring them in individual diploid wild-type and mutant individuals. We will show representative examples of assays scoring 16 diploids (8 wild-type and 8 mutant) for some potentially linked markers identified in the initial analysis.

PCR with pooled genomic DNA and arrayed primers.

1. Prepare two "premixes," one with wild-type DNA, the other with mutant DNA.

For 48 reactions:

PCR mix	960 microliters
water	80 microliters
genomic DNA (1:5 dilution)	25 microliters
Taq polymerase	5 microliters

2. Using the repeating pipettor, distribute 20 microliters of premix into each well of a 96-well microtiter plate. Alternate rows with wild-type and mutant samples (Row A with wild-type, Row B with mutant, etc.). Keep the plate on ice until you are ready to start the thermocycler.

3. Using the 8-channel pipettor, add 5 microliters of 1 micromolar primer mix from the arrayed plate to each well in your 96-well plate. Forward and reverse primers are already mixed, and the primers are arrayed in pairs in columns (each column contains 8 wells: 4 primer pairs in pairs of adjacent wells). With this format, each primer will be tested on one sample from a wild-type row and one sample from a mutant row.

4. Remove the unlabeled, pink-colored backing from the sealing film and apply the sealing film to the plate, place the plate in a thermocycler with a heated lid, and amplify for 45 cycles with a 55˚C annealing temperature and a 30 second extension reaction at 72˚C.

5. Electrophorese the amplified fragments on a 2.5% agarose gel in TAE buffer. USE CAUTION: Ethidium bromide has been added to the gels. Add 50 microliters of 10 mg/ml ethidium bromide to the running buffer.

6. Photograph gels and identify potentially linked markers by looking for those that amplify different fragments in the wild-type and mutant pools.

FYI: PCR mix recipe

171.12 mL sterile H20
0.393 mL 1M MgCl2
2.618 mL 1 M Tris-HCl (pH 8.4)
13.092 mL 1M KCl
0.262 mL 1% Gelatin
Total Volume: 187.490 mL

*Autoclave 20 minutes to sterilize. Do not allow solution to boil over (slow exhaust).
*Cool to room temperature and check volume, maintain sterile conditions.

Chill the solution on ice and add:
3.468 mL 100mg/mL BSA
0.262 mL dATP (100 mM)
0.262 mL dCTP (100 mM)
0.262 mL dGTP (100 mM)
0.262 mL dTTP (100 mM)
FINAL VOLUME: 192 mL

*Aliquot 1000uL per 1.5 mL sterile eppendorf tube
*Store at –20˚C

References and sources for more detailed protocols

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