

"All the Zebrafish News
That's Fit to Print"

THE ZEBRAFISH SCIENCE MONITOR

Volume 1 Issue 2

Monday, August 30, 1991

Eugene, Oregon, USA

HIGH FREQUENCY GERMLINE TRANSMISSION OF PLASMID DNA SEQUENCES INJECTED INTO FERTILIZED ZEBRAFISH EGGS

By Patricia Culp, Christiane Nusslein-Volhard, and Nancy Hopkins

With the goal of developing techniques for DNA insertional mutagenesis in zebrafish, we established procedures for rapidly obtaining and injecting large numbers of fertilized eggs. Using either of two plasmid constructs, we injected uncut DNA into fertilized eggs at the one- or two-cell stage. Injected eggs were raised to sexual maturity and frequency of transgenic founder fish determined by pair mating the fish and testing DNA extracted from pools of their 16-hour-old offspring using PCR and then Southern analysis. Eggs injected with one of two different plasmids yielded no transgenic fish, but 7-23% (18/114 overall) of the eggs injected with the other transmitted the injected sequences to their offspring (F1). Of seven lines studied further, all were able to pass the foreign DNA sequences to the next F2 generation. Inheritance in the F2 was Mendelian in the four lines tested. PCR and Southern analysis indicated that the plasmid sequences were present in multiple copies, probably tandemly arranged. Two founder fish carried more than one independent integration of the plasmid sequences. The line studied in more detail was a mosaic carrying two independently segregating copies of the transgene in one germ cell, and another integration in another germline precursor cell. The ability to obtain and inject large numbers of zebrafish eggs combined with a high frequency of germline integration may be steps towards the goal of being able to perform insertional mutagenesis with this organism. In press, PNAS.

ELECTRONIC BULLETIN BOARD TURNED ON IN EUGENE

The Zebrafish Bulletin Board is up and running in Eugene. The Bulletin Board provides on-line access to a variety of services including back issues of the Monitor, updated chapters from *The Zebrafish Book: A Laboratory Manual*, contributed recipes, results, lists of mutants, genes, antibodies, address lists, etc. It also provides a Message Section which allows users to leave and get messages or to make specific or general requests for information.

Presently, you can access the Bulletin Board with a computer modem by calling (503)-346-4951. Hopefully, a toll-free 800 number will be available in the future.

The Bulletin Board answers the phone and responds with a greeting which provides information for logging on

to the system. Once logged on, the user is provided with the main menu which offers a choice of either the Message Section or File Section.

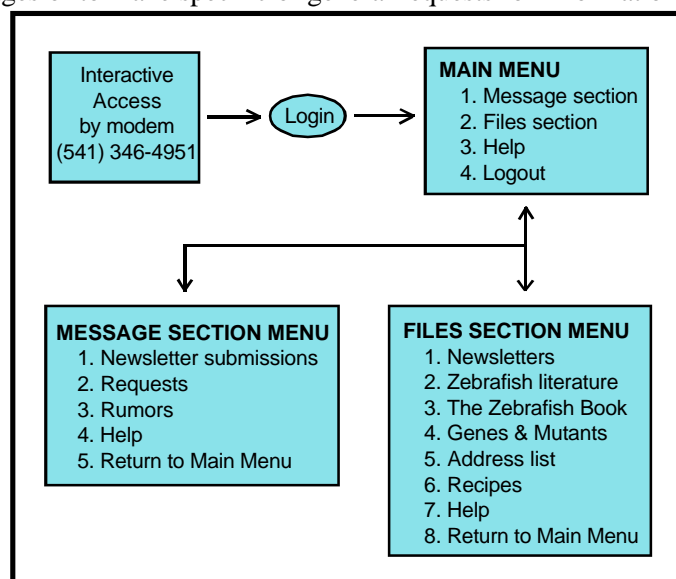
The purpose of the Message Section is to stimulate a dialogue between users. This section offers a menu of various types of conferences. Once a conference is selected, the user can read messages, add a message, reply to a specific message, or scan all the messages for a specific text string.

The Files Section is designed to provide a means for users to submit articles for the *Monitor* and to share data. This section offers a menu of possible file areas. Once an area is selected, the user can look at the directory of files in that area, search for a specific file, or send in or get a specific file.

The system is simple to use and we are eager to make it even simpler. Suggestions and comments are welcome!

As before, the email system is still available for off-line requests.

Write to: ZEBRAFISH@UONEURO.UOREGON.EDU.



THE ZEBRAFISH SCIENCE MONITOR

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...an informal vehicle dedicated to communicating zebrafish news. References to information appearing in the Monitor should be made as personal communications and only if explicit permission of the authors is obtained.

Published at the Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403 USA

WESTERN BLOTS OF ZEBRAFISH EMBRYOS

From Sherry O'Shea & Monte Westerfield

I. Dechorionating & de yolking

1. Dechorionate embryos in batches of ~100 by placing in 1 mg/ml of protease and swirling occasionally (5-10 minutes for 24 h embryos, 10-20 minutes for 3 day embryos). Finish dechorination by gentle trituration using a Pasteur pipette. The chorions float and can be decanted. Rinse 3x in cold Ringer's solution.

2. Transfer the embryos to cold Ringer's with EDTA and PMSF. Remove yolks by trituration with a glass pipette that has been drawn out to have a tip diameter approximately the size of the yolk.

3. Transfer the dechorionated, de yolked embryos to fresh, cold Ringer's solution and rinse 2x.

4. Embryos can be frozen in liquid nitrogen and stored at -70 C at this point by transferring to a microcentrifuge tube and removing as much liquid as possible.

Solutions:

Protease: 5 mg/ml pronase diluted to 1 mg/ml in embryo medium

PMSF: Stock - 100 mM PMSF in isopropanol. Add 30 µl of stock/10 ml Ringer's (final conc. 0.3 mM PMSF) immediately before use.

EDTA: Stock - 10 mM EDTA, pH 7.0. Add 1 ml of stock/10 ml Ringer's (final conc. 1 mM EDTA)

Ringer's: (recipe in *The Zebrafish Book*)

II. Preparation of gel sample

1. Remove from freezer & thaw frozen, dechorionated, de yolked fish.

2. Microfuge for 1-2 minutes to pellet.

3. Remove excess liquid.
4. Add 150-200 µl sample buffer (for example, ~50-100 3 day embryos or 100-150 24 h embryos; this will yield enough for a 1.5 cm curtain or four 0.4 cm lanes of about 25-30 µl each).

5. Homogenize with microfuge pestle until uniform in consistency.

6. Repeat step 5 until sample is no longer stringy.

7. Boil 5 minutes in a water bath.

8. Microfuge, 1-2 minutes.

9. Transfer supernatant to a new microfuge tube. Discard pellet or add more sample buffer and homogenize again if significant pellet remains.

10. Freeze at -70 C or run immediately on gel.

Solutions:

Sample buffer: 0.63 ml 1M Tris-HCl pH 6.8

1.0 ml glycerol

0.5 ml -mercaptoethanol

1.75 ml 20% SDS

6.12 ml H₂O

(10 ml total)

Store at -20 C in aliquots.

For cytoskeleton/extracellular matrix preps:

Add an extraction step between steps 3 & 4 (above).

1. Homogenize dechorionated, de yolked embryos in extraction buffer.

2. Incubate overnight at 4 C.

3. Centrifuge 20 min at 5000 x g.

4. Remove supernatant.

Solutions:

Extraction buffer:

10 mM tris pH 7.4

2% Triton-X 100

1 mM PMSF

1 mM aprotinin

1 mM leupeptin

1 mM trypsin inhibitor

III. Load, run and transfer gel

IV. Immunoblotting (using PBDF blotting paper)

1. After the antigen is blotted, immerse the membrane at a 45° angle, into the blocking solution. Gently agitate for 60 minutes at RT or overnight at 4 C.

2. Decant the blocking solution and add TTBS to the membrane. Wash for 10 minutes with gentle agitation at RT.

3. Decant the TTBS and add the 1 antibody diluted in 1% dried milk in TTBS. Incubate 4 hours at RT (or over night at 4 C) with gentle agitation.

4. Remove the unbound 1 antibody by washing 2x 5 minutes in TTBS.

5. Add alkaline-phosphatase conjugated 2 antibody solution (diluted 1:3000 in 1% dried milk in TTBS). Incubate 1-2 hours at RT with gentle agitation.

6. Wash membrane 2x 5 minutes in TTBS and then, just prior to color development, 1x 5 minutes in TBS to remove the Tween-20.

7. Immerse membrane in color development solution. Proteins present at 100 ng or greater will immediately become visible as purple bands. Lower amounts will take longer, but should be visible within 30 minutes. Staining can continue up to 4 hr.

8. Rinse membrane 4x 5 minutes in dH₂O.

Solutions:

Blocking solution: 3% dried milk in TBS

TBS: 20 mM Tris, pH 7.5

500 mM NaCl

TTBS: 20 mM Tris, pH 7.5

500 mM NaCl, 0.05% Tween-20

Color development solution:

66 µl NBT stock

33 µl BCIP stock

10 ml color development buffer

Both NBT and BCIP stocks are 50 mg/ml in DMF

Color development buffer:

102 mg MgCl₂

4.2 g NaHCO₃

500 ml dH₂O

PREPARATION OF DNA FROM EMBRYOS FOR PCR AMPLIFICATION

From Uwe Strahle

1. Transfer embryo(s) in chorion into an Eppendorf tube and remove as much liquid as possible with a drawn-out pasteur pipette.

2. Add 50 μ l (for 1 embryo or 100 μ l for 25) of 1x PCR buffer supplemented with 0.3% Tween-20 and 0.3% NP-40. The PCR buffer does not seem to be critical, so it can be adapted to the needs of the polymerase you use. The Taq polymerase (Perkin Elmer Cetus) and the new thermostable polymerase (Replinas) from NEN work well with this concentration of detergent. Other polymerases have not been tested.

3. Incubate at 98 C for 5 minutes.

4. Add 5 μ l (for 1 embryo or 10 μ l for 25) 10 mg/ml Proteinase K.

5. Incubate at 55 C for at least 30 minutes to over night.

6. Heat-inactivate the Proteinase K for 5-10 minutes at 98 C.

7. Use 10 μ l for amplification in a 50 μ l reaction.

PCR-Buffer: 10mM Tris/Hcl, pH 8.4, 50 mM KCl, 3mM mgCl₂.

FISH R US

Manfred Scharl is moving from the Max-Planck-Institut in Munich to the Biochemistry Department at the University of Würzburg.

The zebrafish group at the Biocenter in Basel is growing with the addition of Thomas Gerster to the Cell Biology Department. Jochen Wittbrodt has joined Frederic Rosa's lab in the same department.

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