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

THE ZEBRAFISH SCIENCE MONITOR

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CSH MEETING ON ZEBRAFISH DEVELOPMENT AND GENETICS: SYNOPSIS OF COMMUNITY ORGANIZATIONAL SESSION

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The first open invitation meeting on *Zebrafish Development and Genetics* was held April 27-May 1 at Cold Spring Harbor Laboratory. The attendance of the meeting was phenomenal (337 registrants) and there were 186 poster and slide presentations covering topics including cell fate, genetics, methodology, gastrulation, patterning, morphogenesis, growth control, nervous system development and function, and organogenesis.

One session of the meeting was devoted to *Community Organization*. At this session, we discussed a number of issues of importance to the zebrafish research community. The main goal of the session was to establish working groups to propose solutions to these issues.

A brief synopsis of each discussion is reported below, along with the names of the members of the working group. If you have specific concerns about a particular area, you should contact a member of that working group directly. Addresses and phone numbers can be obtained from Pat Edwards: FAX (503) 346-4548; e-mail EDWARDS@UONEURO.UOREGON.EDU. The working groups will report their progress in upcoming issues of the *Monitor*.

1. Information Transfer:

A. *Zebrafish Science Monitor*: 1 . **Monte Westerfield**, the editor, re-

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TEMPERATURE AND THE RATE OF EMBRYONIC DEVELOPMENT

By C.B. Kimmel and B. Ullmann, Institute of Neuroscience, 1254 University of Oregon, Eugene, OR 97403-1254, USA.

We have used the staging series (*The Zebrafish Book*, Edition 2.1, p. 3.27, 1994) to learn how zebrafish development varies as a function of incubation temperature. This information is useful because different studies do not all use the same temperature, and moreover, one might use temperature as a tool to change developmental rate, e.g. to bring embryos from one batch to two different stages at once for heterochronic transplantation.

We found that between 25˚C and 33˚C the relationship between development and temperature is approximately linear. In the accompanying figure, we show selected developmental stages along the ordinate and incubation time along the abscissa. Note that the time scale is expanded in A relative to B, but that the slopes of the lines are the same in both panels. (Data points represent averages from groups of embryos, as we plan to describe in more detail elsewhere.) Each figure is scaled so that the diagonal line (i.e. a line with a slope of 45˚) corresponds to the rate of development at 28.5˚C, the standard temperature described in the staging series. We reconfirmed the developmental rate at this temperature for a set of control embryos developing at 28.5˚C (not shown).

Because of the apparent linearity, a simple calculation allows one to determine approximately when embryos developing at any temperature between 25˚C and 33˚C will reach a desired state of interest:

$$H_T = h/(0.055T - 0.57)$$

where H_T = hours of development at temperature T, and h = hours of development to reach the stage at 28.5˚C, as set out below, and in more detail in Table 2 from the staging series.

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Electronic Zebrafish

By M. Westerfield, Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254 USA

In response to discussions at the Cold Spring Harbor meeting about putting various zebrafish databases onto the Internet (see article by J. Eisen, this issue), two servers have been established. Both are available on the World Wide Web and accessible with programs like *Mosaic*.

One server is accessible at: 'http://zfish.uoregon.edu/'. It contains information about:

The Zebrafish Book - a guide for the laboratory use of zebrafish.

The Zebrafish Genetic Map - information on the map and on mapping genes and mutations.

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THE ZEBRAFISH SCIENCE MONITOR

MONTE WESTERFIELD, *Editor*
PAT EDWARDS, *Publications*
DAVID BRUMBLEY, *Bulletin Board*

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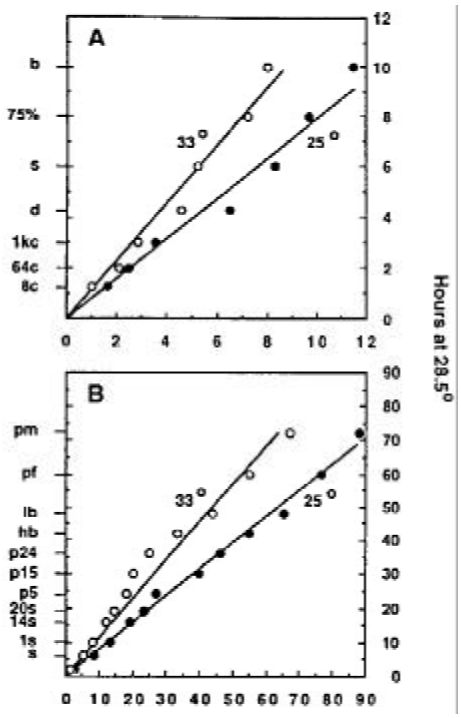
TEMPERATURE...*(cont'd from Page 1)*

We have not systematically investigated developmental rate outside of this temperature range. We selected these extremes because unpublished experiments from the Streisinger lab suggested they were near the limits at which development can proceed normally. In fact, 33˚C appears to be at the upper limit (not shown), but development may be normal somewhat below 25˚C (see Shiorone and Gross, 1968, *J. Exp. Zool.* **169**, 43-52).

We did not find any differences in when or how various features of the embryo develop, relative to one another, at different temperatures. However, one should perhaps be cautious about making direct comparisons between embryos reared at different temperatures: there is no assurance that rates of development of more cryptic features, such as when a marker gene is expressed or when a cell becomes committed to this or that fate, are all coordinately regulated.

Stage abbreviations in the figure (including time to reach the stage at 28.5˚C): **8c**: 8-cell (1.25h), **64c:** 64 cell (2 h), **1kc:** 1,000-cell (3 h), **d:** dome



(4.3 h), **s:** shield (6 h), **75%:** 75%-epiboly (8 h), **b:** bud (10 h), **1s:** 1 somite (10.3 h), **14s:** 14-somite (16 h), **20s:** 20 somite (19 h), **p5:** prim-5 (24 h), **p15:** prim-15 (30 h), **p24:** prim-24 (36 h), **hb:** high bud (42 h), **lb:** long bud (48 h), **pf:** pec fin (60 h), **pm:** protruding mouth (72 h). See also, *The Zebrafish Book*.

ELECTRONIC...*(cont'd from Page 1)*

Zebrafish Genetic Strains - a listing of zebrafish wild-type and mutant strains.
Zebrafish Molecular Probes - information on DNA libraries, cloned genes, and antibodies.
The Zebrafish Science Monitor - back issues and the upcoming issue.
Zebrafish References - a listing of zebrafish research publications.
Zebrafish Research Community - an address book of zebrafish researchers.

The other server can be accessed at: 'http://zebra.scarolina.edu/'. It provides zebrafish news, updates and postings and pointers to a wide range of other information databases about fish care, etc.

If you don't know how to access a database, don't worry. You can either ask a local internet guru or wait for a detailed description of 'how to', which will be forthcoming by email distribution and in the next *Monitor* issue.

LARGE-SCALE MUTAGENESIS IN THE ZEBRAFISH: IN SEARCH OF GENES CONTROLLING DEVELOPMENT IN A VERTEBRATE

By M.C. Mullins, M. Hammerschmidt, P. Haffter, and C. Nüsslein-Volhard; Max-Planck-Institut für Entwicklungs-biologie, Spemannstrasse 35/III, 72076 Tübingen, GERMANY

(Current Biology 1994, **4**:189-202)

Background: In *Drosophila melanogaster* and *Caenorhabditis elegans*, the elucidation of developmental mechanisms has relied primarily on the systematic induction and isolation of mutations in genes with specific functions in development. Such an approach has not yet been possible in a vertebrate species, owing to the difficulty of analyzing and keeping a sufficiently high number of mutagenized lines of animals.

Results: We have developed the methods necessary to perform large-scale saturation screens for mutations affecting embryogenesis in the zebrafish, *Danio (Brachydanio) rerio*. Firstly, a new aquarium system was developed to raise and keep large numbers of strains of genetically different fish safely and with little maintenance care. Secondly, by placing adult male fish in water containing the chemical mutagen, ethylnitrosourea, we induced point mutations in premeiotic germ cells with a rate of one to three mutations per locus per 1,000 mutagenized haploid genomes. This rate, which is similar to the mutagenesis rates produced by ethylmethane-sulfonate in *Drosophila* was determined for alleles of four different pigmentation genes. Finally, in a pilot screen in which mutagenized fish are inbred for two generations and scored for embryonic mutants, we isolated 100 recessive mutations with phenotypes visible in the homozygous embryos.

Conclusion: The high rate of induction and recovery of point mutations, in addition to an efficient aquarium system to house large numbers of mutagenized lines, means that it is now possible to perform saturation mutagenesis screens in a vertebrate, similar to those done in invertebrates.

CSH MEETING...*(cont'd from Page 1)*

ported that the cost per issue is about $600 for postage and printing.

2. Meeting attendees felt that the *Monitor* is doing an excellent job of communicating information within the community, although **Monte** would like to see more unpublished communications; everyone is reminded that the *Monitor* cannot be cited, except as personal communication with the permission of the author.

3. Once an electronic network is established, the *Monitor* will be available electronically even before publication.

4. A question arose as to the appropriateness of researchers using other organisms (eg *Xenopus, medaka, pufferfish*) contributing to the *Monitor*; **Igor Dawid** was designated as the contact person.

5. Further information on the *Monitor* can be obtained from **Pat Edwards** at: EDWARDS@UONEURO.UOREGON.EDU.

B.Interactive electronic network:

Perry Hackett, **Paul Myers**, **Dick Vogt**, and **Monte Westerfield** will work together to establish an interactive electronic network, probably modeled on the one used by *C. elegans* researchers.

C. Access to the mailing list:

Once the network is up and running, the mailing list will be available to anyone who can access the network.

2. Stock center:

A. The need for a centralized stock center was unresolved. However, if we want to have one in place within 2-3 years, now is the time to start planning, decide what a stock center would carry and how to maintain quality control, identify funding sources, write proposals, etc.

B. **Janni Nüsslein-Volhard** stressed the importance of having the stock centers associated with labs. If the decision is to establish stock centers, she would probably be willing to have a stock center associated with her facility.

C. It may be necessary to have multiple centers to carry various types of stocks. In addition, it is difficult to get fish into the UK from the US, thus, it may be necessary to have duplicate stock centers in the US and Europe.

D. A committee composed of **A. Chandrasekhar, Wolfgang Driever, Marnie Halpern, Linda Ross, Charlene Walker, Jim Weston** and **Steve Wilson** will look into the possibilities for initiating and funding stock centers.

E. **Bob Karp** from the *National Institute of Alcoholism and Alcohol Abuse* volunteered to provide the names of contacts for federal funding sources.

3. Wild-type and lethal-free lines:

A.Several wild-type and lethal-free lines currently exist; more information and fish can be obtained by contacting the indicated persons:

1. Oregon AB and AB* (**Charline Walker**, University of Oregon)

2. AB subline (MGH)

3. Tübingen (**Mary Mullins**, Max-Planck-Institut)

4. Darjeeling (**Steve Johnson**, University of Oregon; still not entirely lethal-free)

5. Longfin (**Mary Mullins and Steve Johnson**)

B. AB x Tübingen is a very vigorous stock

4. Central Reagent Facility:

A. Antibodies should be made available through the Developmental Studies Hybridoma Bank.

B. Information about reagents should be part of the database that is being established.

C. **David Grunwald** (email GRUNWALD@GENE1.MED.UTAH.EDU) is collecting information on reagents, including genomic libraries, cDNA libraries, etc.

5. Nomenclature:

A. The name of the species appears to be *Danio rerio*.

B. Naming of genes and mutations was previously discussed and the decisions published in the *Monitor* **2**:4-5. This article will be reprinted on a regular basis.

C. A committee composed of **Chuck Kimmel, Mary Mullins, Jose Campos-Ortega, John Postlethwait** and **Nigel Holder** will continue to consider issues involving nomenclature.

6. Embryological Atlases:

Several groups are considering or in the process of putting together anatomical or staging atlases. Each group should email information about their atlas to the *Monitor* (EDWARDS@UONEURO.UOREGON. EDU). At least some of the atlases will be made available on the electronic network.

7. Genomics:

A. Over the summer, **John Postlethwait** and **Howard Jacob** will get together and decide how to put the RAPD and SSR maps together.

B. RAPD primers for mapping are available from:

Operon (1 800-688-2248)
1000 Atlantic Ave
Alameda, CA 94501

For advice contact **John Postlethwait** (JPOSTLE@OREGON.UOREGON.EDU)

C. A committee composed of **Mark Ekker, Denice Smith, Trevor Jowett** and **Keith Cheng** will deal with mapping issues

8. Tanks:

A. The *Monitor* will devote an issue to tank design, under the direction of a committee composed of **Janni Nüsslein-Volhard, Stephan Schulte-Merker, Bill Trevarrow** and **Robert Riggleman**.

B. **Paul Linser** will find out about raising rotifers from his colleague at Whitney Marine Lab

9. Next meeting:

A. We decided to meet every 2 years

B. The next meeting will be at Cold Spring Harbor and will be organized by: **Janni Nüsslein-Volhard, Monte Westerfield, Phil Ingham** and **Nigel Holder**.

TWO COLOR WHOLE-MOUNT IN SITU HYBRIDIZATION

G. Hauptmann, Biozentrum der Universitat Basel, Abteilung Zellbiologie, Klingelbergstr. 70, 4056 Basel, SWITZERLAND

(*Trends in Genetics* **10**: in press)

I. Preparation of RNA Probe

Reagents:

NTPmix: ATP, CTP, GTP 10 mM each, UTP 6.5 mM (all Boehringer)
Digoxigenin-11-UTP 10 mM (Boehringer)
Fluorescein-12-UTP 10 mM (Boehringer)
RNAsin 20-40 u/ul (Promega)
T7 RNA-Polymerase 15 u/ul (Promega)
T3 RNA-Polymerase 15 u/ul (Promega) or 40 u/ul (Boehringer)
5x transcription buffer (Promega):
200 mM Tris-HCl pH 7.9
30 mM $MgCl_2$
10 mM spermidine
50 mM NaCl
DNAse 1 u/ul (Promega)
TNE: 20 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM EDTA
NucTrapTM push columns (Stratagene)
HB4-H2O: 5 ml formamide, 2.5 ml 2xSSC, 10 ul heparin (50 mg/ml), 10 ul Tween-20, 50 mg torula RNA (Sigma) final volume is 7.6 ml

· *Add in the following order:*
1.0 ug linearized, phenol extracted and precipitated template DNA in 9.5 ul H_20_{DEPC}
2 ul 100 mM DTT
1.3 ul NTPmix
0.7 ul Digoxigenin-11-UTP or Fluorescein-12-UTP
0.5 ul RNAsin
· Mix and spin down
· *Add:*
4 ul 5x transcription buffer
2 ul T7 RNA-Polymerase or T3 RNA-Polymerase
final volume is 20 ul
· Incubate 2-3 hr at 37°C.
· Add 4 ul DNAse and incubate 30 min at 37°C.
· Add 40 ul H_20_{DEPC}.
· *Prepare push column device:*
Push 70 ul TNE through column.
Push probe through column.
Push 70 ul TNE through column.
Volume of purified probe will be about 110 ul.
· Precipitate with 0.5 vol 7.8 M NH_4Ac and 3 vol 100% EtOH at -20°C for 30 min.
· Wash with 80% EtOH.
· Redisolve in 25 ul H_20_{DEPC}.
· Remove 1 ul and analyze on a 1% agarose gel. To check for incorporation you can blot and stain the gel or just do a dot blot.
· Add 76 ul HB4-H20 and store at -20°C.
· First try 1 ul probe in 100 ul HB4 for *in situ* hybridization.

Comments

If you get high background with this probe concentration after a short staining time, dilute your probe. If you get no staining at all, this usually means the probe is not very good. Make a new one.

T7 RNA-Polymerase from Promega usually works and will produce about 10 ug of RNA. In my hands T3 RNA-Polymerase from Promega for some strange reason often gives a very low amount of RNA. But with good templates like krx-20 you still get a very good probe. For difficult templates try T3 RNA-Polymerase from Boehringer which appears to produce more RNA. All in all it is better to use T7 RNA-Polymerase to produce antisense RNA.

I use Wizard Miniprep DNA to make the templates and dissolve it in H_2O_{DEPC}. It is best to use 1 ug of template. More than 2 ug does not increase the amount of RNA significantly and less than 0.5 ug is not very effective.

You can also precipitate with NaAc instead of NH_4Ac. If you omit the push column and just precipitate with NH_4Ac, to save time you will also get a good separation of nucleotides. However, precipitation with LiCl only is not very effective in removing free nucleotides. Hydrolysis of the probe is not advisable because this reduces the sensitivity of the probes. I used probes of a length from 0.5-2.5 kb without hydrolysis. Probes longer than 1 kb work best. If you want to combine different fragments of the same gene to get a stronger probe, mix them before the push column step.

II. Fixation and Storage of Zebrafish Embryos

· Transfer embryos of the desired stage to a small petri dish.
· Remove embryos from their chorions using watchmaker forceps.
· Fix embryos with 4% paraformaldehyde in PBS overnight at 4°C.
· Wash 4 x 5 min in PBSTw at RT.

· Transfer embryos to 100% methanol. Replace with fresh methanol after 5 min. Store embryos at -20°C in a 24 well tissue culture plate (Falcon 3047) sealed with parafilm. Embryos should be cooled to -20°C for at least 30 min for permeabilization even if you don't want to store them.

III. **Proteinase Digestion and Postfixation**

All steps are performed at RT on a shaker.
· Transfer embryos to a small petri dish.
· Immerse 5 min in 75% MeOH in PBSTw at RT.
· Immerse 5 min in 50% MeOH in PBSTw.
· Immerse 5 min in 25% MeOH in PBSTw.
· Rinse 2 x 5 min in PBSTw.
· Digest zebrafish embryos with Proteinase K (10 ug/ml in PBSTw) at RT for 1 to 15 min depending on the stage: 1 cell to high for 1 min; 30% epiboly to 10 somites for 2-3 min; 10-20 somites for 3-4 min 24-32 h for 5-6 min; 40-50 h for 10-15 min.
· Rinse 2 x shortly in 2 mg/ml glycine in PBSTw.
· Fix in 4% paraformaldehyde in PBSTw for 20 min.
· Wash 5 x 5 min in PBSTw.

IV. **Hybridization**

Following steps are performed at 55°C for zebrafish.
· Transfer embryos into 2.0 ml Eppendorf tubes.
· Prehybridize in 350 ul Hb4 for 1-8 h.
· Heat the probe in 100 ul Hb4 at 80°C for 5-10 min, quickly cool on ice/ethanol, spin down and keep on ice/ethanol. Replace prehybridization solution with probe solution. Incubate overnight.

V. **Washes**

· Wash embryos 2 x 30 min in 50% formamide in 2xSSCTw at 55°C or at 65°C.
· Wash 15 min in 2xSSCTw at 55°C or at 65°C.
· Wash 2 x 30 min in 0.2xSSCTw at 55°C or at 65°C.

VI. **Detection**

All detection steps are performed at RT and on a shaker except the staining reactions.
· Block for 1-8 h with PBSTw plus 5% sheep serum.
· Incubate embryos in 100 ul preabsorbed sheep anti-Fluorescein-AP Fab fragments at a 1:2000 dilution in PBSTw. You can reuse antibody 2x.
· Shake for 2 hr at RT or overnight at 4°C. Save used antibody in a new tube designated 1x used or 2x used.
· Wash 6 x 20 min with PBSTw (you can perform one of the washes overnight).
· Wash 2 x 5 min in 0.1 M Tris-HCl pH 8.2, 0.1% Tween.
· Dissolve Fast Red tablets in 0.1 M Tris-HCl pH 8.2, 0.1% Tween (2 ml/tablet) and sterile filter.
· Stain in Fast Red solution for up to 48 h.
· Wash 3 x 5 min with PBSTw. (Storage at 4°C possible.)
· Incubate 10 min in 0.1 M glycine-HCl pH 2.2 plus 0.1% Tween at RT to remove first antibody.
· Wash 4 x 5 min in PBSTw.
· Incubate embryos in 100 ul preabsorbed sheep anti-Digoxigenin-AP Fab fragments at a 1:2000 dilution in PBSTw. You can reuse antibody two times.
· Shake for 2 hr at RT or overnight at 4°C.
· Wash 6 x 20 min with PBSTw (one of the washes overnight possible).
· Wash 2 x 5 min in SB.
· Stain in SS for up to 48 hr.
· Wash 3 x with PBSTw.
· Embryos may be fixed for at least 30 min and stored at 4°C in PBSTw (add N_aNO_3).
· Mount in glycerol.
Do not clear embryos in alcohol because Fast Red precipitate is supposed to be unstable in ethanol.

Comments

To detect two different RNA transcripts in whole embryos make one RNA probe using Fluorescein-UTP and the other using Digoxigenin-UTP. Both probes are hybridized at the same time and detected one after another in two rounds of detection. The crucial step is the inactivation and removal of the first applied Fab-AP conjugate using the low pH step after the first staining round. Inactivation of the first applied Fab-AP conjugate by heating is not recommended, since incubation at 65°C for 20 min or at 80°C for 10 min causes fading of the red precipitate resulting in a weaker and more diffuse signal. In contrast, the blue precipitate darkens resulting in an increased background in the embryo and a nearly black yolk.

Because Fast Red is less sensitive than BCIP/NBT (5-10x) the stronger probe is always visualized in red. Because the second round of detection is less sensitive than the first, I recommend staining the red first.

Nevertheless, if necessary, it is also possible to do the purple staining first.

Fluorescein labeled probes are slightly less sensitive than digoxigenin probes. Therefore the stronger probe is usually detected in red using a fluorescein probe.

To detect transcripts of a single gene use just one probe and one round of detection with BCIP/NBT as substrate.

Antibodies, Stock Solutions and Reagents

Preadsorption of antibody with zebrafish embryos
Zebrafish embryos need not to be dechorionated. The number of embryos you use for preabsorption depends on the amount of antibody you want to preabsorb. The stages used for preabsorption should include the same or older stages you will use in your *in situ* hybridizations. 2-3 d embryos work well.

· Fix embryos in 4% paraformaldehyde at 4°C overnight.
· Wash 4 x 5 min in PBSTw.
· Store embryos in 100% methanol at -20°C.
· Rehydrate 1 ml of embryos by rinsing 3 x 5 min in PBSTw.
· Transfer embryos to a 2.0 ml Eppendorf tube.
· Homogenize embryos with a pestle and adjust to about 1.0 ml with PBSTw.
· Add 10 ul antibody (final 1:100).
· Preabsorb antibody by shaking at least overnight or store for a few days at 4°C. Spin down embryonic debris and sterile filter (Millex-GV 0.22 um, Millipore) supernatant.
· Resuspend embryonic debris in PBSTw and sterile filter again.
· Fill up with PBSTw to a final 1:2000 dilution of antibody.
· Store preabsorbed antibody (20 ml) at 4°C.
· The antibody preabsorbed with zebrafish embryos works well also for staining of *Drosophila embryos.*

Stock solutions and other reagents

0.5 M PO_4 buffer pH 7.3: 80 ml 0.5 M Na_2HPO_4 + 20 ml 0.5 M Na_2HPO_4
10x PBS: 8% NaCl, 0.2% KCl, 0.2 M PO_4 buffer pH 7.3
1xPBSTw: 1x PBS + 0.1% Tween-20
100 mg/ml glycine in $_{dd}H_2O$ store at -20°C
20 mg/ml proteinase K in $_{dd}H_2O$ store at -20°C in aliquots
4% paraformaldehyde in 1x PBS is prepared under a fume hood: mix in a beaker 2 g paraformaldehyde, 5 ml 10x PBS and 45 ml ddH20, heat to 70°C and stir for about 2 hr until all paraformaldehyde is dissolved, cool to 4°C, add 4 ul 1N NaOH and mix, store at -20°C in 5 ml aliquots
HB4: 50% formamide (Merck), 5x SSC, 50 ug/ml heparin, 0.1% Tween-20, 5 mg/ml torula RNA (Sigma)
50 mg/ml **heparin**, store at -20°C
2x SSCTw: 2xSSC + 0.1% Tween-20
0.2x SSCTw: 0.2xSSC + 0.1% Tween-20
Sheep serum: heat inactivated at 56°C for 30 min, store at -20°C
Fast Red tablets (Boehringer): 0.5 mg napthol substrate, 2 mg Fast Red chromogen, 0.4 mg levamisole per tablet
SB: 100 mM NaCl, 50 mM $MgCl_2$, 100 mM Tris-HCl pH 9.5, 1mM levamisol, 0.1% Tween-20
NBT (Boehringer): 75 mg/ml in 70% DMF/H_2O
BCIP (Boehringer): 50 mg/ml in 100% DMF
SS: 4.5 ul NBT + 3.5 ul BCIP in 1 ml SB
Final: 337.5 ug/ml NBT and 175 ug/ml BCIP

Reference:

Hauptmann, G. and Gerster, T. (1994) 2 Color whole-mount *in situ* hybridizations on zebrafish and *Drosophila embryos. Trends Genet.* **10**: in press.

ZEBRAFISH CANCER RESEARCH

There is some interest in a dialogue among zebrafish cancer researchers to engender interaction and to foster research support. Bulletin Board and gopher interaction have been suggested as a means. Please send suggestions and comments to:

Keith C. Cheng
Department of Pathology
C7804
The M.S. Hershey Medical Ctr.
Pennsylvania State University
500 University Drive
Hershey, PA 17033
(717) 531-5635
FAX (717) 531-5301
KCHENG@PSUHMC.HMC.PSU.EDU

A GENETIC LINKAGE MAP FOR THE ZEBRAFISH

By J.H. Postlethwait, S. Johnson, C.N. Midson, W.S. Talbot, M. Gates, E.W. Ballinger, D. Africa, R. Andrews, T. Carl, J.S. Eisen, S. Horne, C.B. Kimmel, M. Hutchinson, M. Johnson, and A. Rodriguez; Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254

Science **264**:219-234 (1994)

To facilitate molecular genetic analysis of vertebrate development, haploid genetics was used to construct a recombination map for *Danio (Brachydanio) rerio*. The map consists of 401 random amplified polymorphic DNAs (RAPDs) and 13 simple sequence repeats spaced at an average interval of 5.8 cM. Novel strategies exploiting the advantages of haploid genetics and RAPD markers were developed which quickly map lethal and visible mutations and which place cloned genes on the map. The map is useful for position-based cloning of mutant genes, the characterization of chromosome rearrangements, and the investigation of evolution in vertebrate genomes.

POSTDOCTORAL POSITION

University of Auckland, New Zealand

A postdoctoral position for a person interested in studying muscle differentiation using the zebrafish as a model system is available within a small group in the School of Biological Sciences at the University of Auckland. Candidates must have practical expertise in molecular biological techniques. The position is available for two years at $NZ35,000 pa. Assistance with travel expenses will be provided.

For further information, interested persons should contact Clive Evans, Developmental Biology and Cancer Research Group, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.
e-mail C.EVANS@AUCKLAND.AC.NZ
tel +64 9 373 7599 ext 7245
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