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

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MINIREVIEW

Repeated Sequence Elements in Zebrafish and Their Use in Molecular Genetic Studies

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Although zebrafish (*Danio rerio*) has many advantages over other organisms for studies in developmental genetics (1, 2), certain well-developed methodologies for genetic analysis are lacking. Repetitive sequences in general, and transposable elements in particular, have been found in all metazoan genomes in which they have been sought (3). Transposons have many potential applications in genetic research and are routinely used for insertional mutagenesis, gene mapping, gene tagging and gene transfer in other well-established model systems. These methods, once developed for zebrafish, would greatly facilitate the identification and mapping of genes involved in vertebrate embryogenesis and to investigate the evolutionary processes that have been shaping eukaryotic genomes (4, 5). Accordingly, we decided to characterize repetitive elements from zebrafish.

The first repetitive elements characterized in the zebrafish genome were the **AluI repeats** (6, 7). These **satellite-like**, high copy-number elements form long, tandem arrays. They come in two types. Type I is A+T-rich and composes 5-8% of the zebrafish genome whereas Type II is G+C-rich and composes about 1% of the *Danio rerio* genome. Both types are highly polymorphic, are suitable for identifying individuals within a species, and can be used for mapping genes and other DNA sequences. We attempted to use these sequences to enhance integration of transgenic DNA constructs through homologous recombination, but were unsuccessful (6). Therefore, we investigated two other types of repetitive elements that are mobile in genomes, elements that move directly through DNA copies and elements that transpose through an RNA intermediate, retroposons. Figure 1 shows the various families of repeated sequences that have been found in zebrafish; microsatellite (simple) sequences such as poly(A) and TG/CA sequences are not shown

Cont'd on Page 2

REGULATIVE INTERACTIONS IN ZEBRAFISH NEURAL CREST

(in press in Development*)*

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Zebrafish trunk neural crest cells that migrate at different times have different fates: early-migrating crest cells produce dorsal root ganglion neurons as well as glia and pigment cells, while late-migrating crest cells produce only non-neuronal derivatives. When presumptive early-migrating crest cells were individually transplanted into hosts such that they migrated late, they retained the ability to generate neurons. In contrast, late-migrating crest cells transplanted under the same conditions never generated neurons. These results suggest that, prior to migration, neural crest cells have intrinsic biases in the types of derivatives they will produce. Transplantation of presumptive early-migrating crest cells does not result in production of dorsal root ganglion neurons under all conditions, suggesting that these cells require appropriate environmental factors to express these intrinsic biases. When early-migrating crest cells are ablated, late-migrating crest cells gain the ability to produce neurons, even when they migrate on their normal schedule. Interactions among neural crest cells may thus regulate the types of derivatives neural crest cells produce, by establishing or maintaining intrinsic differences between individual cells.

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

or discussed. We use the terms "transposon" and "retroposon" if the respective element has structural and/or sequence characteristics of known and active transposable elements, regardless of whether the particular sequence has been shown to transpose in the laboratory.

We first examined **Tc1-like transposable elements** (**TCE**s) which had been identified and characterized in salmonid fish species and zebrafish (8). TCEs transpose directly via a DNA intermediate, similar to P elements and *hobo* from *Drosophila*. In nematodes, Tc1 is widely used for insertional and deletional mutagenesis, transposon-tagging and enhancer trapping. In an attempt to employ such an element in zebrafish for the same purposes, we characterized the zebrafish **Tdr1** element (9). Tdr1 was successfully amplified about 1000-fold in the zebrafish, constituting a little less than 0.1% of the haploid genome. However, all of the isolated copies isolated so far (and the search has been intensive) are mutated and do not encode an active transposase. Phylogenetic analysis suggests that the zebrafish genome contains multiple TCE families, *e.g.*, **Tdr2** (10). Analysis of several copies of Tdr1 and TCEs isolated from other fish species (10) helped us to reconstruct a consensus sequence of a fish TCE transposase gene which, when expressed as a protein, has many of the known enzymatic activities of the transposases (11). The synthetic Tc1-like element should prove useful as an efficient vector for delivery of transgenes as well as for insertional mutagenesis and enhancer/promoter trapping and gene mapping.

We have identified a third family of repeated elements in the zebrafish genome, the DANA elements (12). DANA is a **retroposon** (retroelement) that lacks long terminal repeats and apparently transposes by reverse transcription of an RNA intermediate. DANA is specific for the genus *Danio*, and was the first retroposon isolated from zebrafish. DANA exhibits all the hallmarks of a tRNA-derived SINE (short interspersed element). In

Classification of repetitive elements in zebrafish

	Name	Length (bp)	Copy# /haploid genome	% of genome	Distribution in the genome	Reference
Satellite-like						
	BglI-Type I/a	~180	$5-8\times10^5$	5-8	frequently form tandem repeats	4,7
	Type I/b	165	2.2×10^4	0.2		7
	BglI-Type II	188	9×10^4	1		6
Tc1-like transposon						
	Tdr1	~1250	1×10^3	0.07	interspersed	8,9
	Tdr2	~1500	?	?		10
Retroposon						
	DANA	~350	$4-5\times10^5$	10	interspersed	12
Unclassified						
(a) composite element	"no tail-transposon"	~1550	~80-100	<0.01	interspersed	12,15,16
(b) "fold-back"-like	Oops	~350	?	?	interspersed	17

▷-short internal repeat ▶-short terminal inverted repeat 8 8-target site duplication ▶◀ terminal inverted repeat with directly repeated motifs at the ends C U-conserved/variable regions

contrast to generic SINE elements, DANA appears to have been formed by insertions of short sequences (Cv blocks in Fig. 1) into a progenitor, tRNA-derived element, and amplified further as a transposable unit. DANA can be particularly suitable as a genetic marker because it has high copy-number (comprising about 10% of the zebrafish genome), has an apparently random distribution in the genome, and has a Mendelian segregation pattern suggesting that the elements are stable in the genome. With DANA-specific PCR-primers (12), the method of SINE-PCR (13) can be used for genome mapping, fingerprinting and YAC cloning. Association of DANA with microsatellite-like repeats permits the combination of SINE-PCR and microsatellite marker mapping (13). "DANA-PCR" can be employed in combination with RAPD markers (14) for genetic and phylogenetic analyses.

Transposable elements are capable of inserting into each other to form **composite elements** that can be amplified. Evidence for one such event has been found in zebrafish; a DANA-related element apparently inserted into another sequence that subsequently was amplified and inserted into the zebrafish *ntl* gene (15). The particular composite element that integrated into the *ntl* gene is amplified about 80- to 100-fold in the genome (16), although the mechanism by which the element spread is not known. Other, unidentified, composite elements surely exist in the zebrafish genome; their structures and modes of amplification remain to be determined. Their importance is demonstrated by the observation that the DANA-composite element is responsible for one of only three spontaneous phenotypic mutants that have been characterized at the sequence level.

A fourth type of repeated element, named **Oops** (17), has been found in the zebrafish genome. Oops is a short repetitive sequence with the potential to form a cruciform structure. These **fold-back-like elements**, similar to the Tourist/Stowaway families, have heretofore been found only in fungi and plant genomes (18). Oops was found to accompany other repetitive elements such as DANA, thereby forming complex structures. Oops is found in and around expressed genes such as growth hormone, eIF-4E, and *ntl*. Like other repeated elements (19), Oops may be able to influence expression of nearby genes.

In conclusion, about 15-20% of the zebrafish genome has been characterized in terms of several types of repeated elements. Understanding the dynamics and evolution of these sequences will allow us to harness them, or derivatives thereof, for further genetic manipulations and analyses in zebrafish.

Acknowledgements

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Cont'd on Page 4

MiniReveiw... *(cont'd from Page 3)*

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HOW YOU CAN HELP BUILD THE ZEBRAFISH DATABASE

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

A group of us were appointed at the 1994 Cold Spring Harbor meeting on zebrafish genetics and development, to establish an on-line database of information for zebrafish researchers. We have obtained funding from the National Science foundation and The Zebrafish Database Project is underway. We are currently in the process of implementing the database which will run over the Internet. We need two kinds of help:

1. We ask that you help us with this project by making suggestions for the kinds of data that will be included in the database.

2. We hope you can tell us how you use the current Fish Net WWW server (http://zfish.uoregon.edu) and what sort of things you would like to be able to do with an on-line database.

Please send suggestions to either Monte Westerfield (monte@uoneuro.uoregon.edu) or Wolfgang Driever (driever@helix.mgh.harvard.edu).

Thanks for your help.

PROPOSED ZEBRAFISH DATA TYPES

People: name, address, lab, phone, fax, email, bio, publications, research interests

Labs: name, location, members, publications, contact person, WWW URL, research interests

Publications: authors, date, title, source, keywords, abstract

Genes: gene name, gene abbreviation, cDNA sequence, cDNA sites, start codon, intron locations, stop location, protein sequence, genomic sequence, GenBank accession #, human homologue, map location, comments, publications

Map markers: marker name, type (gene, SSCP, RAPD, SST), primer sequence, location, map cross, polymorphic stocks and allele sizes, sequence, lab of origin, comments, publications

Stocks: name, lineage, origin, comments, publications

Mutants: name, allele, locus, segregation, map location, chromosome change (Dp, Df, T, In), breakpoints, phenotypes and expressivity, mutagen, genetic background, image, lab of origin, comments, publications

Antibodies: name, type, structures labeled, immunogen, source (person), comments, publications

RNA probes: name, structures labeled, source (person), vectors, sense/antisense sites, enzyme, stages analyzed so far, comments, publications

Developmental Atlas: stage, image, strain, description

Staging series: stage, image, section/orientation, plane, strain, description

Adult Atlas: region/structures, image, strain, description

Images: stage, specimen (section, whole-mount), type (still, movie, optical series, 3-D), orientation, labeling, comments, publications

Anatomical parts: name, abbrevation(s), description, image

Physiological records: kind (current, voltage, optical), record, description, publications

Methods:

WHOLE-MOUNT *IN SITU* HYBRIDIZATION OF THICK TISSUE SECTIONS

By Qiling Xu and David Wilkinson; Randall Institute, King's College London, 26-29 Drury Lane, London WC2B 5RL, UNITED KINGDOM

This is a method involving RNA *in situ* hybridization of unmounted tissue sections by a whole-mount procedure. It is suitable for large embryos, but may be difficult to carry out with small pieces of tissue that can easily be damaged or lost during the washes. It provides more sensitivity for detection of RNAs than obtained using thin tissue sections mounted on slides, and avoids the penetration problems with whole-mounts of large embryos.

1. Fix embryos in 4% paraformaldehyde in PBS overnight at 4°C.

2. Rinse in PBS, then process for sectioning as described below. We have used embryos embedded in paraffin wax, but it is likely that cryostat sections work at least equally well. Cut ~50 m sections.

3. If wax sections have been cut, dewax with Histoclear 3 times for 5 min, then wash in 100% methanol 3 times for 5 min, and go to step 5.

4. If cryostat sections have been cut, dehydrate in 5 min washes of 25%, 50%, 75% methanol in PBS, then twice in 100% methanol.

5. Rehydrate in 5 min washes of 75%, 50%, 25% methanol in PBS and 3 times in PBT.

6. Treat with 10 g/ml proteinase K in PBT for 10-15 min at room temperature.

7. Rinse briefly with PBT and then refix with 4% paraformaldehyde in PBS for 20 min.

8. Wash 4 times for 5 min each with PBT.

9. Prehybridize in hyb mix at 65°C for 2-3 h.

10. Replace with hyb mix containing DIG-labelled probe and hybridize overnight at 65°C.

11. Wash for 10 min each at 65°C in:

solution 1
- 3:1 solution 1: 2 x SSC
- 1:1 solution 1: 2 x SSC
- 1:3 solution 1: 2 x SSC
- 2 x SSC

12. Wash twice for 30 min each at 65°C in 0.2 x SSC

13. Wash for 10 min each at room temp in:
- 3:1 0.2 x SSC: PBT
- 1:1 0.2 x SSC: PBT
- 1:3 0.2 x SSC: PBT
- PBT

14. Block with blocking solution for 60 min.

15. Replace with 1/5000 diluted AP-coupled anti-DIG antibody in blocking solution and incubate at 4°C overnight.

16. Wash in TBT 6 times for 20 min each.

17. Equilibrate with NTMT, 3 times for 5 mins.

18. Stain with NBT/BCIP in NTMT.

19. After appropriate amount of staining has been obtained, wash three times with PBT.

20. Equilibrate in 70% glycerol in PBT and mount under a coverslip. The sections may appear wrinkly, but by placing them on a slide in a minimum amount of liquid and gently unfolding any creases, they can be nicely flattened under a coverslip (use silicon grease to support the edges of the coverslip).

Solutions

PBT: PBS, 0.1% Triton X-100

Hyb mix: 50% formamide, 5 x SSC, pH 6.0, 0.5 mg/ml yeast RNA, 0.1% Triton X-100, 50 g/ml heparin (This is the hyb mix we have used, but your favorite hyb mix for whole-mounts should also work)

Solution 1: 50% formamide, 5 x SSC

Blocking solution: 2 mg/ml BSA, 5% sheep serum, 1% DMSO in PBT

TBT: 50 mM TrisHCl, pH 7.5, 150 mM NaCl, 0.1% Triton X-100.

NTMT: 100mM NaCl, 100mM TrisHCl pH 9.5, 50mM MgCl2, 0.1% Triton X-100. Make from concentrated stocks on day of use (because the pH will decrease during storage due to the absorption of carbon dioxide).

NBT: 75 mg/ml in 70% dimethylformamide (store at -20°C); use 4.5 l per ml NTMT.

BCIP: 50 mg/ml in dimethylformamide (store at -20°C); use 3.5 l per ml NTMT.

Preparation of wax sections

1. After fixation and washing in PBS, dehydrate by washing for 30 min each in 25%, 50%, 75% methanol in PBS, 3 times in 100% methanol, then 2 times in Histoclear.

2. Incubate for 20 min at 60°C in 1:1 Histoclear: paraffin wax, then 3 times in paraffin wax.

3. Transfer to suitable mould, orientate as required and allow wax to set.

4. Cut 50 m sections on a microtome. Continue the hybridization protocol at step 3.

Preparation of cryostat sections

1. After fixation and washing in PBS, embed in 5% sucrose, 1.5% LMP agarose (Sigma) and then leave the blocks in 30% sucrose, 1% paraformaldehyde overnight at 4°C to equilibrate.

2. Cut 50 m thick sections on a cryostat, incubate in 4% paraformaldehyde in PBS for 20 min, then continue hybridization protocol at step 4.

CONSTRUCTION OF ZEBRAFISH SPAWNING CAGES

By P. Ham and K. Cheng; Department of Pathology, C7804, Hershey Medical Center, 500 University Drive, Hershey, PA 17033

We have received several requests for detailed instructions for construction of durable, autoclavable, economical spawning cages, which we summarize here. We use a design similar to one described by Solnica-Krezel *et al*. (*Genetics* 1994, **136**:1404). The end-product is an opaque cage with a wire-mesh bottom, placed inside of a transparent cage. As the fish spawn, eggs drop through the mesh to a space in the outer cage that the adults cannot reach.

Tools required include pliers, glue gun, medium steel shears, hotplate stirrer, large file, razor blade, and carbide-tipped blade on a table saw. Materials include stainless steel wire mesh (McMaster-Carr, 9226T79; 8 x 8 per inch), 9 3/8 x 5 7/16 x 5 1/8" polycarbonate (Nalgene 6602277) and polypropylene (Nalgene No. 6621177) animal cages. Melt the plastic to the mesh in a fume hood, in case of smoke.

The steps:

1) Slowly and precisely remove the bottoms of the polypropylene cages using a carbide-tipped blade on a table saw.

2) Remove large burrs with the razor.

3) Create a template and cut wire-mesh bottoms from the stainless steel mesh using sheet metal shears. The edges of the mesh should meet the outer edges of the cage walls for a proper fit.

4) With a hot glue gun, temporarily glue the wire-mesh to the polypropylene cage bottom in about six places. We suggest liberal glue application in six spots: in the middle of one of the shorter sides, and 3-4 cm. away from each corner along the long sides. Excess glue provides a handle for subsequent glue removal.

5) Melt the wire-mesh into the bottom of the polypropylene cage on the unglued end using a hotplate. We use a Corning PC-320 hotplate stirrer at a setting of about 4, and hold the cage at both sides at an angle of approximately 30 . Softening of the plastic proceeds from the edges towards the middle; continue to press down until the two flows meet. Cooling may be accelerated on a cold surface. When a lip of melted plastic forms on the outside edge of the cage, one can push the plastic over the edges of the wire using a flat tool.

6) Melt the other sides one half length at a time, removing the temporary hot glue beforehand (remaining glue will smoke).

7) File protruding sharp edges towards the cage openings to prevent dislodging the mesh from the cages.

While using these cages for spawning zebrafish during the past three years, we have found that plastic “greens” appear to miminize trauma by providing refuge, and cloth covers help to minimize distraction of the fish by lab personnel.

REMEMBER!

1996
Zebrafish Meeting
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AN ECONOMICAL ZEBRAFISH GENETICS FACILITY

By E.G. Gestl and K.C. Cheng; Division of Experimental Pathology and Department of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA 17033.

General Description

Our goal was to set up a zebrafish facility at minimum cost that would support a mutant hunt, maintain healthy fish with minimum effort, and offer modular flexibility and the greatest efficiency with regard to feeding, water changes, and system maintenance. We have just finished construction of a facility with a capacity of 51 ten gallon tanks and 144 five gallon tanks at a cost of about $36,000. The water temperature is regulated by a mixer valve and by room temperature, and is usually set at 28°C. A light/dark cycle of 14h/10h is achieved using electronic timers. System water recirculates at a rate of up to 5 water changes per hour with standard use being 3-4 changes per hour. The system was designed to fit in an existing room in the Animal Facility at the Penn State College of Medicine, Hershey Medical Center, and was slowly brought to 50% capacity over about 6 months. Our fish appear to be very healthy.

Tanks and Stands

The tanks were made by All-Glass Aquarium to be 1" shorter than the standard 10- and 5-gallon tanks (11½” and 9¼”, respectively) to allow more working space above the tanks. The tank walls are approximately 1/8" thick with a 1¼” hole drilled in an upper corner (centered at 1" down from the plastic lip and 1½” in from the side) into which a ½” bulkhead (Aquatic Eco-Systems,

TFK1) is siliconed in place. The lids consist of 2 overlapping glass pieces sliding on a plastic rail purchased from Tropical Isle. Each glass lid has a corner cut off, the rear lid for a water inlet into the tank and the front lid for liquid feeding such as brine shrimp.

The stands are constructed of grade 304 #4 polished stainless steel (Staco). Other labs would use local metal companies to minimize shipping costs. The structural supports for the stands are made of 1¼" square tubing and the shelves from 18 gauge stainless steel sheet metal. The shelves have a ½" lip and a ¾" hole in one corner so that in the case of an overflowing tank the water is contained and exits through the hole into the drain. Each leg also has a 2½" square footplate to distribute the weight evenly. The stands for the five gallon tanks have 4 rows of tanks with 3, 4, or 5 tanks in a row, while the ten gallon tank stands have 3 rows of tanks with either 3 or 4 tanks per row.

Description of the Recirculation System

Water leaving the tanks exits through the bulkhead by gravity and enters an angled 1" common drainage pipe through open tees. These lead to vertical 1½" drainage pipes which in turn exit into 90 gallon fiberglass sump tanks (Aquanetics, 18" D x 16" W x 75"L). Before entering the sumps the water passes through DSL pad filters (Aquanetics) to eliminate large particles. Two of the sumps are interconnected via a 2" PVC pipe which is below the water line. To make efficient use of the available space, tanks had to be placed on either side of a walkway. The sumps on each side are connected by a siphon system (3" PVC pipe) which transports 25 gpm or 40% of the system's water back to the side of the room containing the pump. This inverted "U" also has a small peristaltic pump which pumps out the air that accumulates at the top of the "U." This is necessary for maintaining the siphon.

Operating only one of two M1000-H pumps (Aquanetics), the water then goes through two sets of two filters (Aquanetics, 420) each set containing a 150 µm and 25 µm bag filter (Aquanetics, 400-150 and 400-5). The water is then sterilized using 2 ultraviolet (UV) sterilizing units placed in series (Aquanetics, Q480IL and Q240IL). The sterilizing power of the large unit at the maximum flow rate is 45,000 µwsec/cm^2, 3-fold the killing power needed for viruses and bacteria (15,000 µwsec/cm^2) so that many fungi, protozoa, and spores are also destroyed (*Aquacultural Engineering*, Fredrick Wheaton, 1985). The UV units are installed in series so that when bulbs burn out, water will still be sterilized. Therefore, the small unit has an output of at least 15,000 µwsec/cm^2 and is placed in series with the first to ensure that the water is sterilized at a minimal level. The water leaving the UV system enters a network of PVC piping whose pressure is equalized by circular loops throughout the system. The water flow into the individual tanks is controlled by no kink style globe valves (Aquatic Eco-systems, VK-2), and the cycle begins again.

The aeration system uses a 1/6 H.P. turbo-blower (Aquanetics, 104P) with silencer and filter. Air hose leads to every tank, and is plumbed in circular loops to yield equal pressure. The air exits the PVC pipe from plated brass valves (Aquatic Ecosystems, VN2), goes through 1/8" silicone tubing (McMaster- Carr, 51135K16), and enters the water through Jungle glass-beaded airstones (Jungle Laboratories, NJ297). The usual rate of aeration per tank is 0.7 L/min while 4L/min is its maximum rate.

Water Changes

To ensure that ammonia and other harmful toxins do not accumulate, the system is designed for continuous automatic water changes; we exchange about 10% of the system volume per day. Waste water exits the system through a valve which is located between the filtration and UV systems. The temperature of the replacement water is regulated using a mixing valve. This water then passes through a pressure reducing valve and two carbon filters (Aquanetics, model 220) in series before entering the sump. The flow rate is regulated by two parallel float valves (AREA, FLV550-050) in one sump.

In our second fish room, we wished to have automatic water changes in tanks in which babies are raised. Because babies are fed powdered food and paramecia, we did not want small food to plug the particulate filters. Therefore, we raise babies in the flow-through portion of our system. To keep things simple, we decided to use "conditioned" water from the recirculating system in the flow-through system. We also use flow-through tanks for quarantine purposes when new fish are brought into the system. The water leaving the tanks in the flow-through system goes to waste drains. In our basement system, 28 five-gallon tanks are used as flow through tanks. Another 44 five-gallon and 33 ten-gallon tanks will have this capability in the future. The flow through of these tanks represents from 0 to 100% of the water exchange in the system, with the remainder draining from the recirculating portion of the water flow. System water pressure is adjusted using two lines which bring water

directly back to the sump either after the filter bags or after the UV system.

In order to maintain the maximum number of fish per volume of water, the dissolved oxygen level of greater than 96% saturation is obtained using a bioreactor trickle filter (Aquanetics, B126) which draws about 20% of the system flow following the bag filters, and returns aerated water to the sump.

Individual Adult Tank System

In the recirculation section of our separate fish room, we recently added 160 one liter tanks, of polystyrene. These tanks (model T49F) were purchased from Alpack Inc. for $1.25/tank. The unhinged lids were drilled with 2 holes, one 3/8" in diameter in a back corner for a water inlet line and the other 1 3/8" in diameter in the front center for feeding (we would recommend using a 1' hole next time, since fish occasionally jump out of the 1 3/8" holes). A 1/8" wide, 1" deep slit was cut in the front center of each tank for water to exit. The shelf frame was constructed of materials previously mentioned and has a pitch of ½" over the length of 51" to aid in drainage. The shelves are constructed of ¼" thick acrylic with 1" high by ½" thick acrylic sides. Each shelf contains 2 rows of 10 tanks with access from either side of the rack.

UV-sterilized water is pumped through 1" PVC pipe to the individual adult tank system where it is regulated by a ball valve for each shelf. The water exits the common water inlet by a 1/4" threaded adapter with a 1/8" barbed end (Aquatic Eco-Systems, 62001). A pipette tip attached to 1/8" tubing transports the water to the tank. Water flows onto the acrylic shelf through the tank slits, where gravity takes the water to one end of the shelf. It then exits through a pair of 1/2" bulkheads, enters a common drainage pipe, and empties into a sump which is connected to the recirculation system.

Other Features of the System

An important advantage of the system is its flexibility. First, back-up systems are important for flood prevention and to ensure continuous running. The room is divided into 4 zones with the ability to isolate and stop water or air flow to that zone when the need arises. The water pump, bag filtration, and UV systems all have bypass routes which can be activated without stopping water flow when damage occurs or routine maintenance is required. For example, when the siphon action in the inverted "U" siphon is broken, there is a back-up pump (Aquanetics, TE-6-MO-SC) activated by a seesaw switch (AREA, LP19), which returns water to the other side of the room. In addition, when the water level of the sumps becomes abnormally high for any reason, two parallel condensate pumps (McMaster-Carr, 9907K11), each capable of pumping 100 gph, are activated. The sterilization power of the UV system was designed to be higher than normally used, with two UV sterilizing power units in series. This decreases the possibility of cross-contamination when bulbs inevitably burn out.

Monitoring of the system is eased by plumbing T's and valves so that water can be collected and chemically analyzed at critical points. Valves between and after the carbon filters allow us to test their efficiency. At other locations, branches have been added to allow measurement of water exchange and bioreactor trickle flow rates. True unions and true union ball valves were used in many areas to create easy access to many parts of the system. The low cost of this system must be balanced against the time needed for design and construction. However, the knowledge gained from setup has already helped us to make changes and correct problems as required.

Businesses/Companies

All-Glass Aquarium Co., Inc.
9675 South 60th Street
Franklin, WI 53132
(414) 421-9670
(414) 421-9682 - FAX

Alpack, Inc.
7 Overhill Rd.
Natick, MA 01760
(508) 653-9131
(508) 650-3696 - FAX

Aquaculture Research/Environmental Associates, Inc. (AREA)
P.O. Box 1303
Homestead, FL 33090
(305) 248-4205
(305) 248-1756 - FAX

Aquanetics Systems
5252 Lovelock Street
San Diego, CA 92110
(619) 291-8444
(619) 291-8335 - FAX

Aquatic Eco-Systems, Inc.
2056 Apopka Blvd.
Apopka, FL 32703
(407) 886-3939
(800) 422-3939 - Order
(407) 886-6787 - FAX

Jungle Laboratories Corporation
Box 630
Cibolo, TX 78108-0630
(210) 658-3505
(800) 245-1446 - Order
(210) 658-8413 - FAX

McMaster-Carr Supply Co.
P.O. Box 440
New Brunswick, NJ 08903-0440
(908) 329-3200
(908) 329-3772 - FAX

Staco
P.O. Box 216
Route 501 North
Schaefferstown, PA 17088
(717) 949-2630
(717) 949-3103 - FAX

Tropical Isle
4 Pierce Street
Framingham, MA 01701
(508) 875-5303
(508) 872-1916 - FAX

1996 ZEBRAFISH DEVELOPMENT & GENETICS

Wednesday, April 24 - Sunday, April 28, 1996
Registration Deadline:
Abstract Deadline: February 7, 1995

ORGANIZED BY:

Nigel Holder, *King's College London*
Nancy Hopkins, *Massachusetts Institute of Technology*
Philip Ingham, *Imperial Cancer Research Fund*
Christiane Nusslein-Volhard, *Max-Planck-Institut Entwicklungsbiologie*
Monte Westerfield, *University of Oregon*

You are cordially invited to participate in the 2nd biennial meeting on Zebrafish Development and Genetics, which will be held at Cold Spring Harbor Laboratory. The meeting will begin with dinner and the first session on the evening of Wednesday, April 24, and will conclude with lunch on Sunday, April 28. The meeting is devoted to research on zebrafish and will focus on the cellular, molecular, and genetic regulation of embryonic development and growth, with sessions devoted to the following topics and chaired by:

Epiboly and gastrulation: Mary Mullins, *Max-Planck-Institut fur Entwicklungsbiologie*
Inductive interactions: Christine Thisse, *Institut de Genetique et de Biologie, CNRS*
Morphogenesis: Charles Kimmel, *University of Oregon*
Pattern regulation: Denis Duboule, *University of Geneva*
Neural crest and head development: Judith Eisen, *University of Oregon*
CNS development: Steve Wilson, *King's College London*, United Kingdom
Blood, heart, and intermediate mesoderm: Mark Fishman, *Massachusetts General Hospital*
Genetic mapping and cytogenetics: John Postlethwait, *University of Oregon*
Gene transfer, gene expression and new methodologies: Nancy Hopkins, *Massachusetts Institute of Technology*.

The format of the meeting will include morning and evening sessions consisting of short talks, strictly limited to no more than 10 slides and 10 - 15 minutes each including discussion, principally on unpublished work. Oral presentations will be selected by the organizers in consultation with the session chairs from the submitted abstracts. The other submitted abstracts will be presented in poster sessions in the three afternoon sessions. As usual at Cold Spring Harbor meetings, all abstracts of both poster and platform sessions will be published in an abstract book given to all participants.

Abstract and registration materials are available from the address below or can be downloaded or printed from the Cold Springs Harbor WWW site at: <http://www.cshl.org/> (under Meetings and Courses). The submission of an abstract is not required for attendance. The abstract deadline is February 7, 1996. In order to make the abstract book more useful, we wish to provide an index. Please choose the three most appropriate keywords (see list at <http://www.cshl.org/meetings/96zebra.htm>) and type the corresponding numbers at the bottom of your abstract in a separate area outside the main body of your abstract. We are especially eager to have as many young people as possible attend since they are likely to benefit most from this meeting. To assist them, we have applied for funds from government and industry that we hope will allow us to provide at least partial support for the attendance of graduate students and postdocs.

We look forward to seeing you at Cold Spring Harbor.

For Additional Information, please contact:

Cold Spring Harbor Laboratory
The Meetings & Courses Office
1 Bungtown Road, PO Box 100
Cold Spring Harbor, NY 11724-2213
Meetings Phone: 516-367-8346
Fax Number: 516-367-8845
E-Mail: MEETINGS@CSHL.ORG

FINAL ANNOUNCEMENT

15th Annual Singer Symposium on

"Models of Regeneration: Lower Versus Higher Vertebrates"

to be held at

Institute of Child Health
University of London
London, UK

March 21-22, 1996

The 1996 Singer Symposium will be a satellite of two international conferences, "Limb Development and Regeneration" (organized by M. Maden, March 25-28, 1996) and "Regeneration, Growth and Pattern" (Spring Meeting of the British Society for Developmental Biology organized by V. French and J. Slack, March 27-30, 1996) both to be held at the University of York, which is about two and a half hours from London by rail.

The conference format will include 30 minute talks and short platform presentations selected from the submitted abstracts. Abstracts, which will be included in the Abstract Book, must be submitted by the 15th of January 1996 together with the registration form. Registration and poster mounting will be between 1 and 2 pm and the scientific session will start at 2 pm on Thursday 21 March.

Preliminary Programme: Relationship between development and regeneration / CNS regeneration through evolution / Limb and muscle regeneration in lower and higher vertebrates. Speakers will include: H. Wallace (University of Birmingham, UK); R. Nordlander (Ohio State University, USA); B.M. Carlson (University of Michigan, USA); J.P. Brockes (Ludwig Institute London, UK); S. Dunnett (CTR Brain Repair, MRC Cambridge, UK), J.D.W. Clarke (University College London, UK); R. Borgens (Purdue University, USA), J.S. Edwards (University of Washington, USA); A. Forge (Institute of Laringology and Otology, London, UK); K. Muneoka (Tulane University, USA); T.A. Partridge (Royal Postgraduate Medical School, London, UK); H-G Simon (Squibb-Myers, Princeton, USA).

Registration Fees :

A330.00 for members of the Singer Society
A335.00 for non-members
A325.00 for students (evidence of status is required)

Registration covers the two days of the meeting and includes the programme and abstracts, lunch, tea and coffee and reception. Registration and abstract forms can either be supplied by post or by e-mail as attached documents in Word. The number of participants will be limited to 120. I look forward to seeing you in London next spring.

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E-mail: FERRETTI@ION.BPMF.AC.UK