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 µg/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 4.5µl per ml NTMT.

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

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:

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 ìm and 25 ìm 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 ìwsec/cm², 3-fold the killing power needed for viruses and bacteria (15,000 ìwsec/cm²) 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 ìwsec/cm² 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/FAX (414) 421-9682

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

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

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

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

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

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

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

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