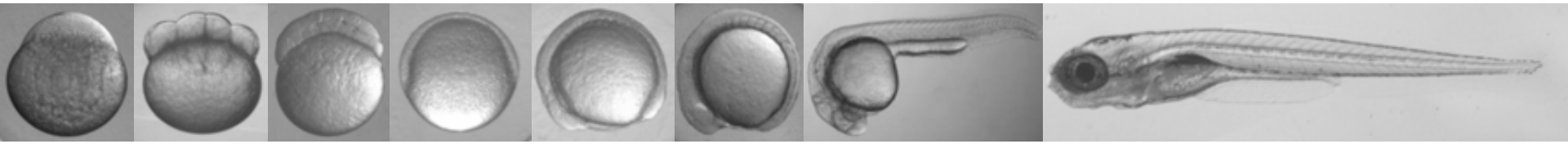
Zebrafish Procedures and Techniques

Carrie Barton

Sinnhuber Aquatic Research Laboratory

Oregon State University



5th Annual International Zebrafish Husbandry Course
Buguggiate, Italy 2016

Outline

- Anesthesia with tricaine
- Squeezing females
- Squeezing males
- IVF –Fresh Samples
- IVF – Frozen Samples
- Cryopreservation of Sperm
- Fin Clipping
- Microinjection
- Embryo Bleaching
- Histology dissection and fixation
- Histology dissection video
- Dechorionation
- Shipping Fish

Anesthesia w/Tricaine

Materials Needed:

- **Tricaine bath**
- Slotted spoon
- Paper towels
- Metal Spatula
- Dish of clean fish water
- Recovery tank
- Fish

Tricaine-S (MS222)

Concentration:
~4mls 3X buffered tricaine per 100mls fish water

Anesthesia w/Tricaine

Materials Needed:

- Tricaine bath
- **Slotted spoon**
- Paper towels
- Metal Spatula
- Dish of clean fish water
- Recovery tank
- Fish

Anesthesia w/Tricaine

Materials Needed:

- Tricaine bath
- Slotted spoon
- **Paper towels**
- **Metal Spatula**
- Dish of clean fish water
- Recovery tank
- Fish

Anesthesia w/Tricaine

Materials Needed:

- Tricaine bath
- Slotted spoon
- Paper towels
- Metal Spatula
- **Dish of clean fish water**
- **Recovery tank**
- Fish

Shallow container w/ fish water
-spawning baskets work well

Anesthesia w/Tricaine

Materials Needed:

- Tricaine bath
- Slotted spoon
- Paper towels
- Metal Spatula
- Dish of clean fish water
- Recovery tank
- **Fish**

http://zdmsociety.org/zebrafish-and-human-disease/

Euthanasia w/Tricaine

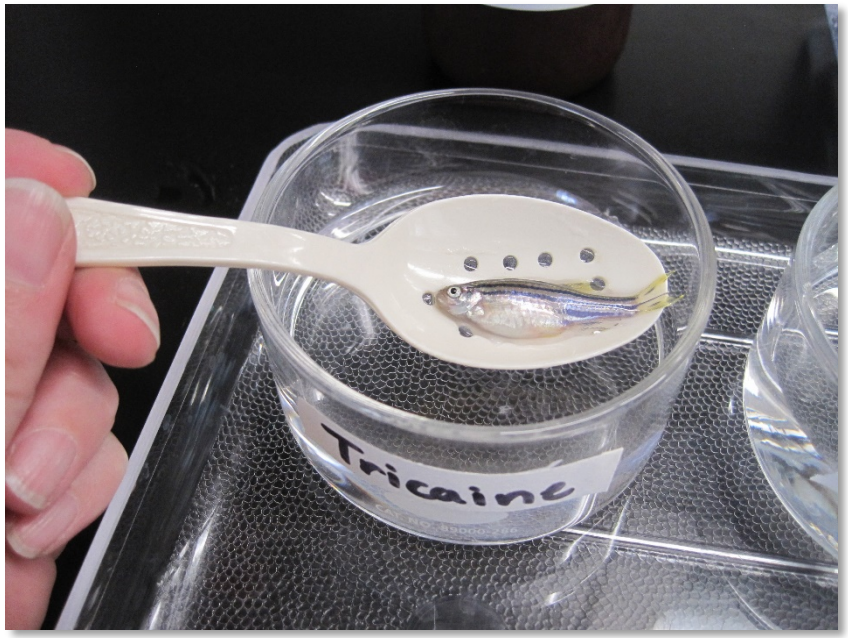
Euthanasia can be achieved with a lethal dose of Tricaine S (MS 222). Increase the dose up to ~13ml 3x buffered tricaine per 100 mls fish water. A secondary method of euthanasia is always recommended.

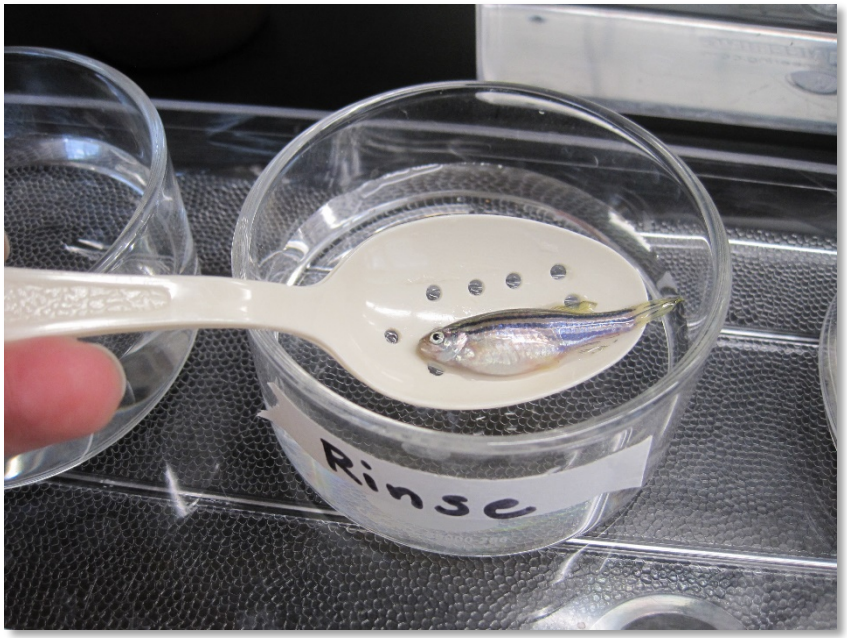
Anesthesia w/Tricaine

1. Place fish into the tricaine bath

2. Wait for gill movement to slow down and remove fish with the plastic spoon.

3. Dip the fish in a dish of clean fish water to remove tricaine from exterior of fish





Anesthesia w/Tricaine

4. Transfer fish from spoon to towel, to remove excess water to ease with handling.

5. Using metal spatula, transfer fish to whatever surface protocol requires.

6. When done with procedure, using metal spatula, return fish to a shallow recovery tank and observe until fully revived.

Anesthesia w/Tricaine

Considerations:

Use only freshly made Tricaine

Small aliquots frozen until just before procedure are best

Observe fish for signs of stress:

http://zdmsociety.org/zebrafish-and-human-disease/

Extracting Embryos and Sperm: *"Squeezing"*

Proficiency in squeezing male and female zebrafish is essential for successful cryopreservation and in vitro fertilization

Squeezing Females - Supplies

Materials Needed:

- **Anesthesia supplies & protocol**
- 35 mm petri dishes
- Metal spatula
- Humidity chamber
- Female Fish

Tricaine-S (MS222)

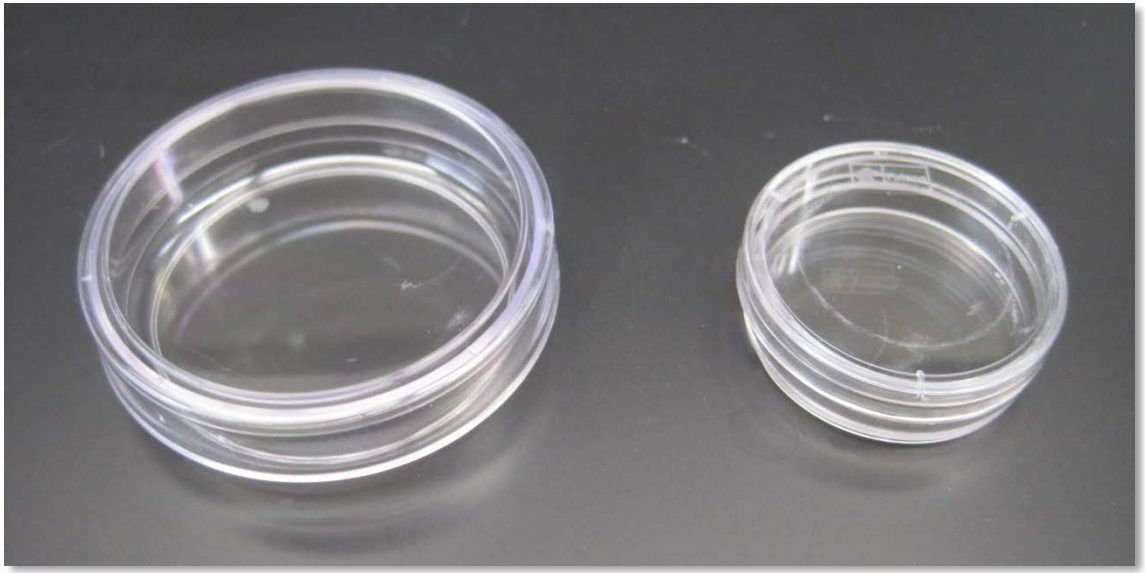
~4mls 3X buffered tricaine per 100mls fish water

Shallow container w/ fish water
-spawning baskets work well

Squeezing Females - Supplies

Materials Needed:

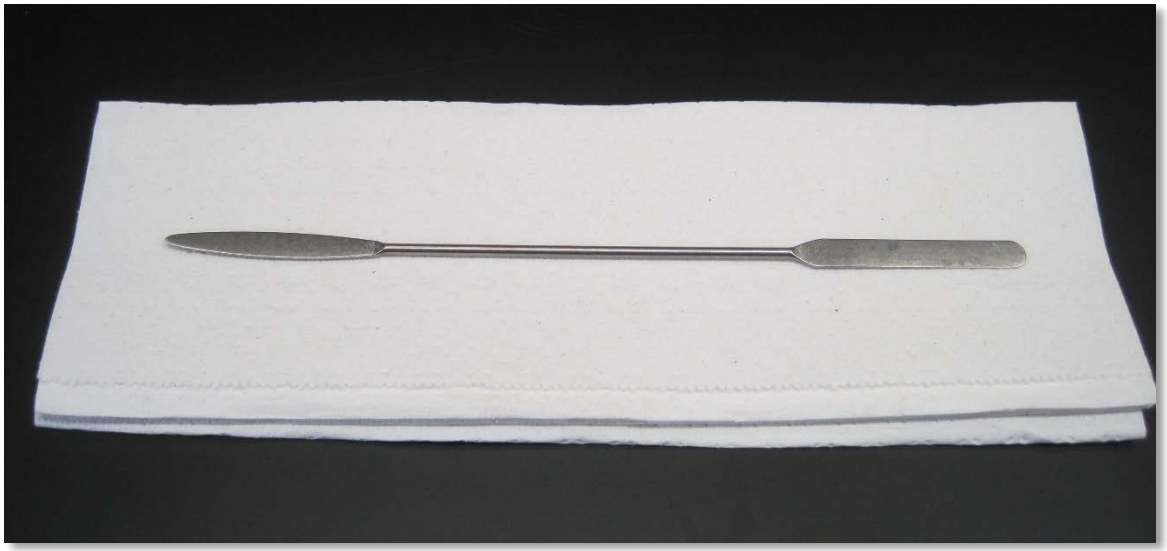
- Anesthesia supplies & protocol
- **35 mm petri dishes**
- Metal spatula
- Humidity chamber
- Female Fish



Squeezing Females

Materials Needed:

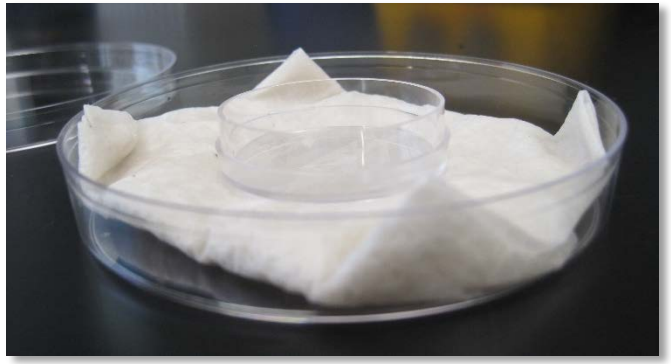
- Anesthesia supplies & protocol
- 35 mm petri dishes
- **Metal spatula**
- Humidity chamber
- Female Fish



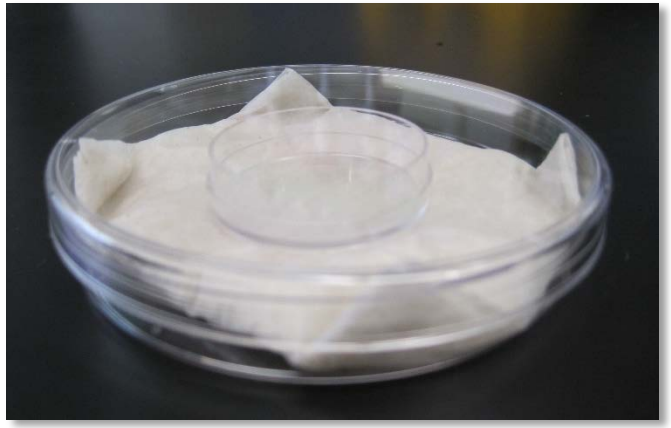
Squeezing Females - Supplies

Materials Needed:

- Anesthesia supplies & protocol
- 35 mm petri dishes
- Metal spatula
- **Humidity chamber**
- Female Fish



- 100mm petri dish
- Paper towel nested in bottom
- Saturated in fish water
- 35mm petri dish (with eggs) nests inside
- Lid goes on while collecting embryo clutches



Squeezing Females - Supplies

Materials Needed:

- Anesthesia supplies & protocol
- 35 mm petri dishes
- Metal spatula
- Humidity chamber
- **Female Fish**

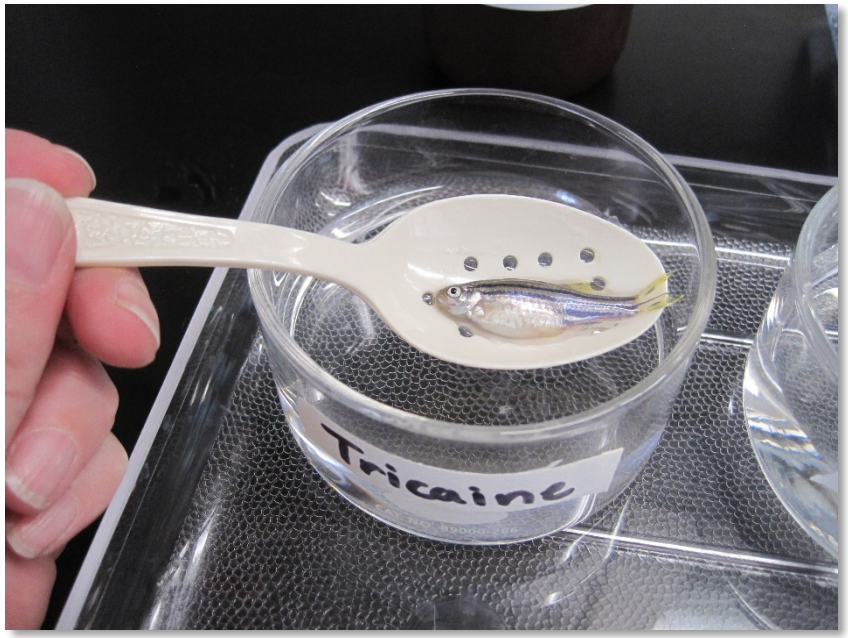
Separated from male fish prior to procedure

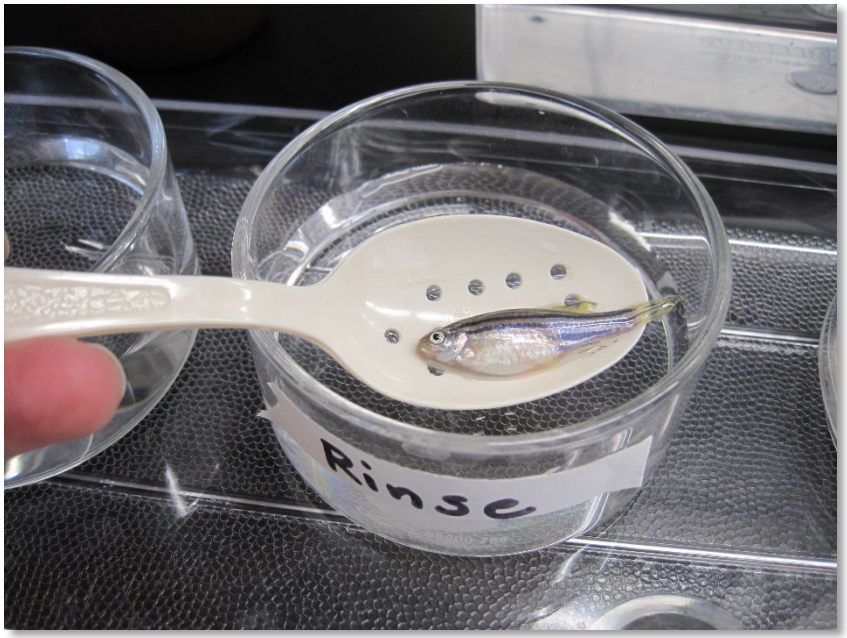
Squeezing Females - Procedure

1. Place female fish into the tricaine bath

2. Wait for gill movement to slow down and remove fish with the plastic spoon.

3. Dip the fish in a dish of clean fish water to remove tricaine from exterior of fish





Squeezing Females - Procedure

4. Gently transfer fish from spoon to towel, roll once to dry surface.

5. Transfer fish from paper towel to 35 or 60 mm petri dish using the spatula

6. Slightly dampen your fingers with fish water

Squeezing Females - Procedure

7. Place index finger of non-dominant hand on the dorsal side of the fish.

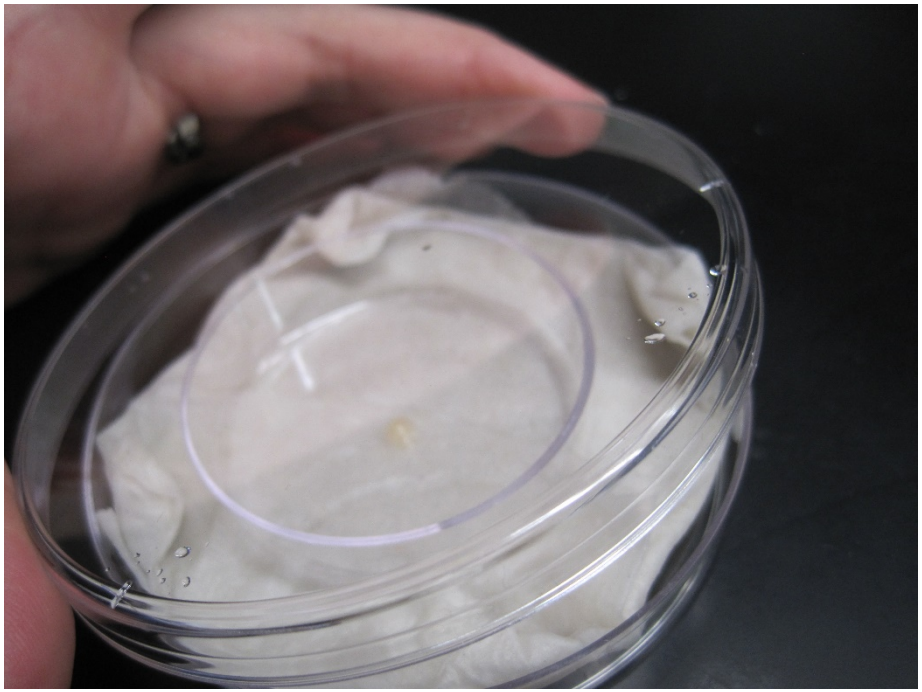
8. Using index finger of the dominant hand, press gently from the belly (~mid trunk) towards the vent. If gentle pressure yields no embryos, do not continue to squeeze.

9. If eggs are procured, use the metal spatula to gently move the mass away from the fish's body. Then slide the fish out of the dish.

Squeezing Females - Procedure

10. Place the fish into a recovery tank. Wait until all fish are fully revived before putting them back on the system. (follow up observation later that day is suggested)

11. If pooling multiple clutches, place embryos in humidity chamber or keep in small dish w/lid.



Squeezing Males - Supplies

Materials Needed:

- **Anesthesia supplies and protocol**
- 35 mm petri dishes w/foam fish holder
- Kimwipes
- Millipore smooth forceps
- Glass capillary tubes
- Mouth pipette (or pipetman)
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- Male fish

Tricaine-S (MS222)

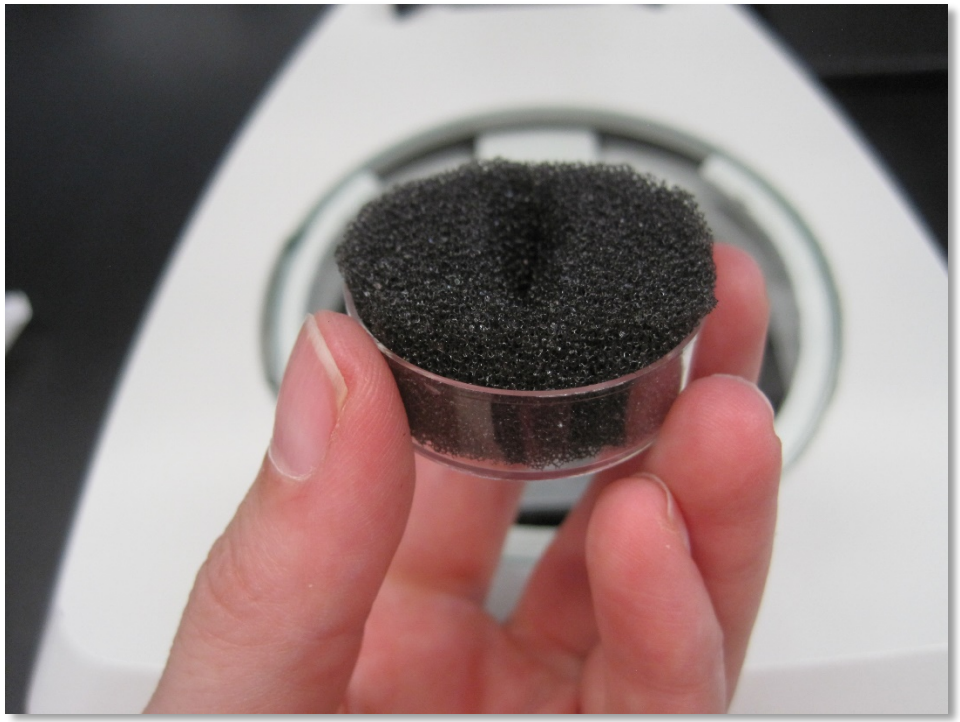
~4mls 3X buffered tricaine per 100mls fish water

Shallow container w/ fish water
-spawning baskets work well

Squeezing Males - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- **35 mm petri dishes w/foam fish holder**
- Kimwipes
- Millipore smooth forceps
- Glass capillary tubes
- Mouth pipette (or pipetman)
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- Male fish



Squeezing Males - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- 35 mm petri dishes w/foam fish holder
- **Kimwipes**
- Millipore smooth forceps
- Glass capillary tubes
- Mouth pipette (or pipetman)
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- Male fish

Squeezing Males - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- 35 mm petri dishes w/foam fish holder
- Kimwipes
- **Millipore smooth forceps**
- Glass capillary tubes
- Mouth pipette (or pipetman)
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- Male fish

Squeezing Males - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- 35 mm petri dishes w/foam fish holder
- Kimwipes
- Millipore smooth forceps
- **Glass capillary tubes**
- **Mouth pipette (or pipetman)**
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- Male fish

Squeezing Males - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- 35 mm petri dishes w/foam fish holder
- Kimwipes
- Millipore smooth forceps
- Glass capillary tubes
- Mouth pipette (or pipetman)
- **Hank's sterile saline**
- **Micro tubes or cryovials**
- **Ice bucket**
- Male fish

https://wiki.zfin.org/display/prot/Recipes

0.05 ml of Hank's per clutch

Squeezing Males - Supplies

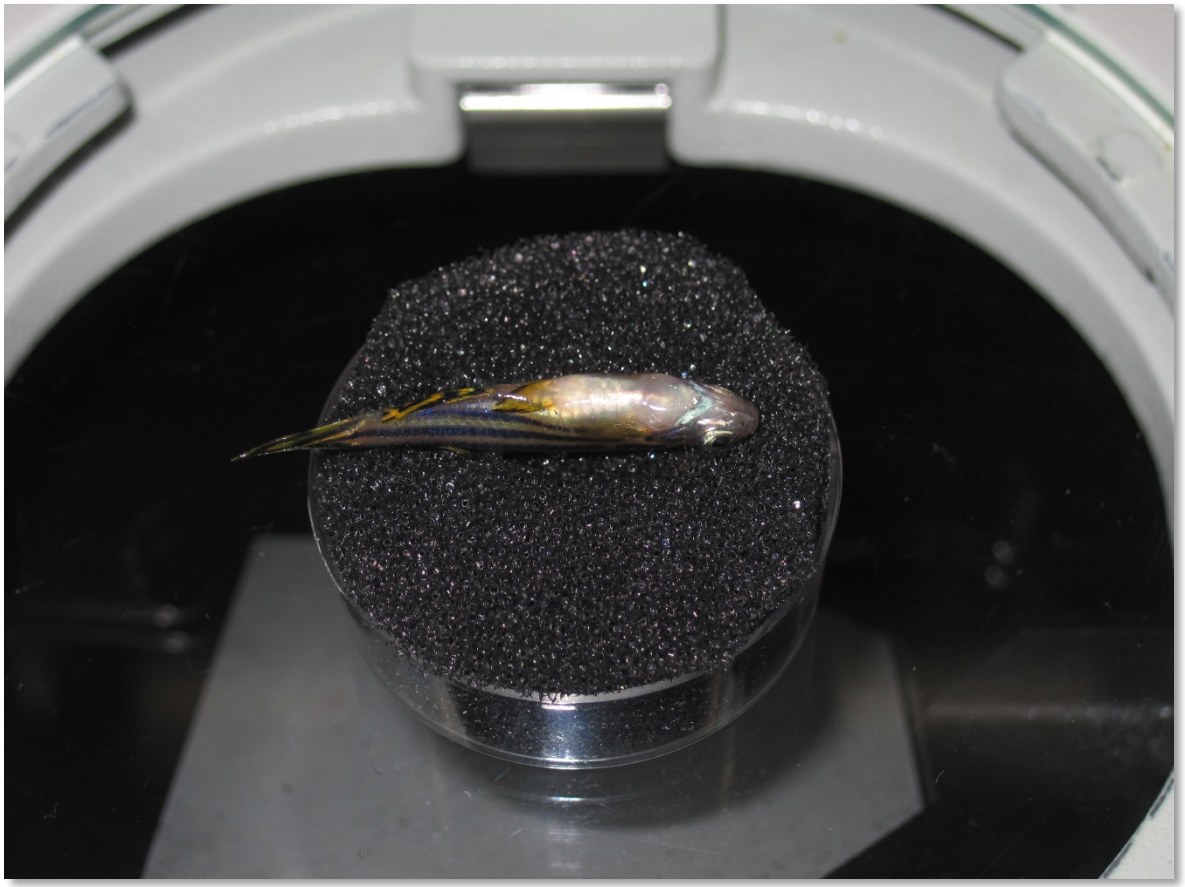
Materials Needed:

- Anesthesia supplies and protocol
- 35 mm petri dishes w/foam fish holder
- Kimwipes
- Millipore smooth forceps
- Glass capillary tubes
- Mouth pipette (or pipetman)
- Hank's sterile saline
- Micro tubes or cryovials
- Ice bucket
- **Male fish**

Separated from female fish prior to procedure

Squeezing Males - Procedure

1. Anesthetize fish per institutional protocol.
2. Place the fish, belly up into slit of foam holder.
3. Place the dish w/fish on the microscope stage and use top light for illumination.



Squeezing Males - Procedure

4. Gently wipe the ventral side of fish with the corner of the Kimwipe.
   -completely dry underside of fish as water activates sperm

5. Using the microcapillary, move the anal fins aside to expose the urogenital opening

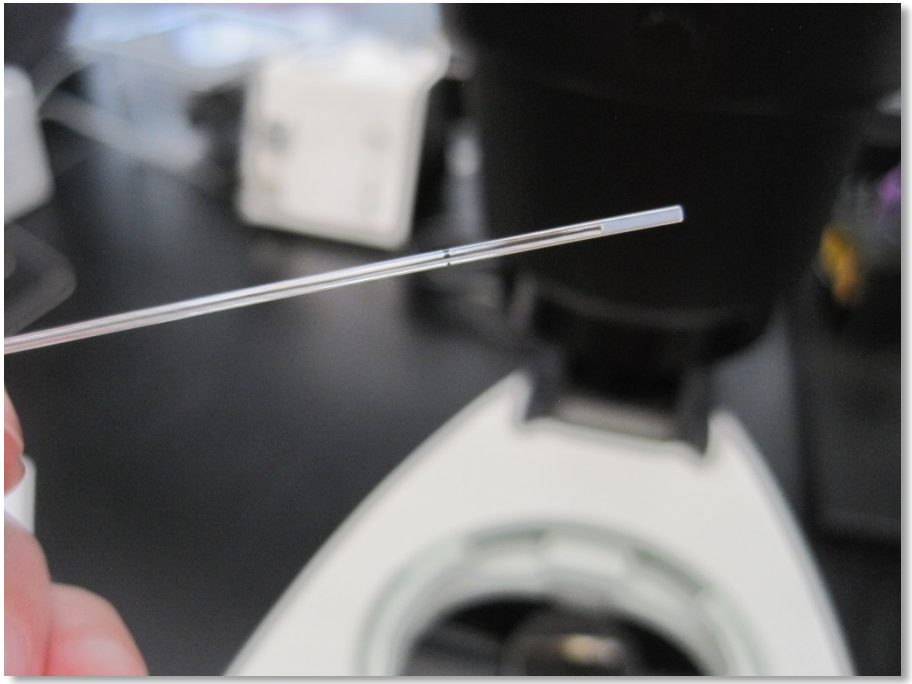
6. Use the smooth forceps to gently squeeze side of fish
   -collect sperm using capillary action of the tube.

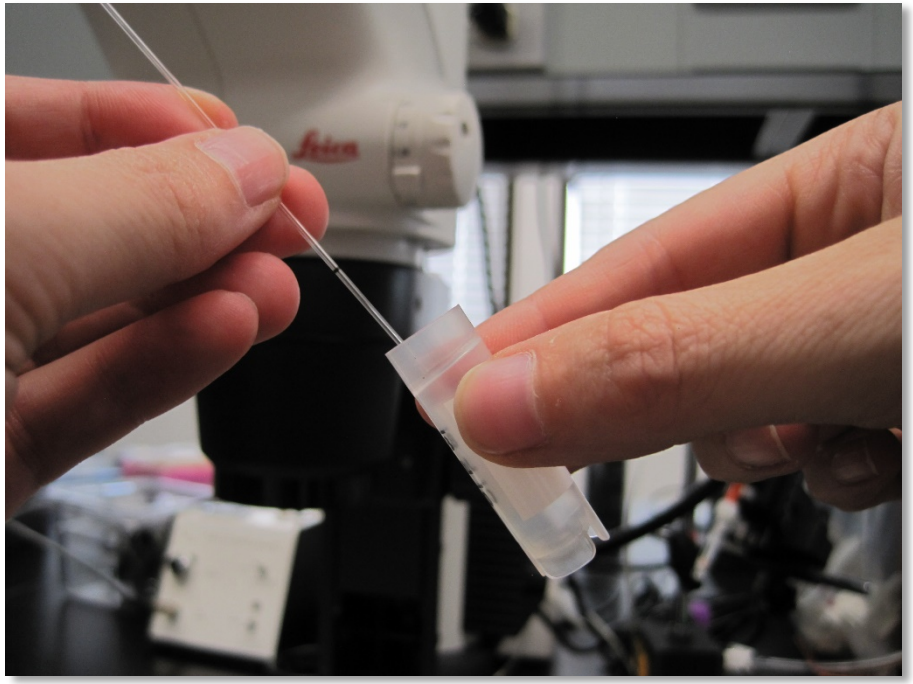
Squeezing Males - Procedure

7. Return fish to recovery tank.

8. Expel the sample into the tube of Hank's solution and keep on ice

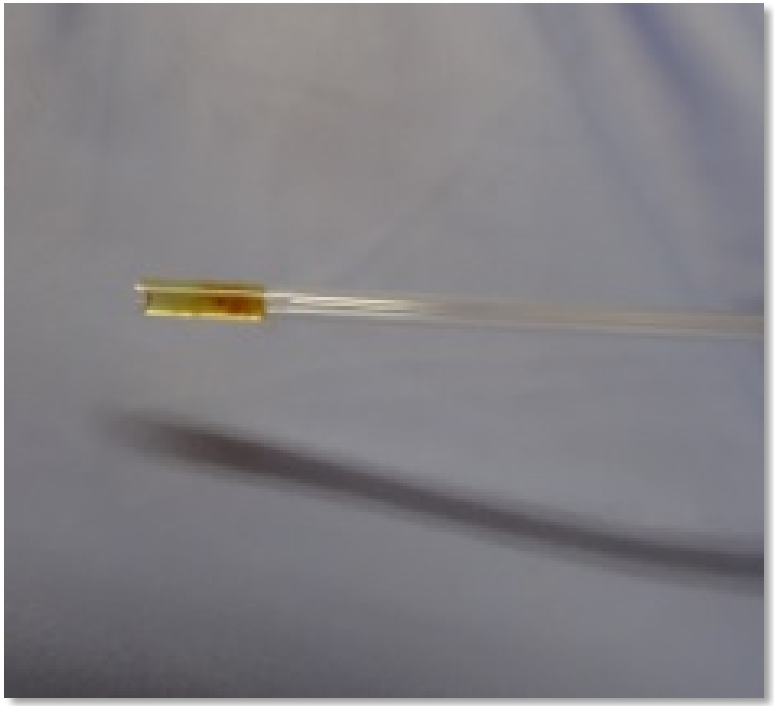
-samples on ice can be used for ~90 minutes post collection
-each vial should contain 0.05 ml of Hank's for each clutch of embryos



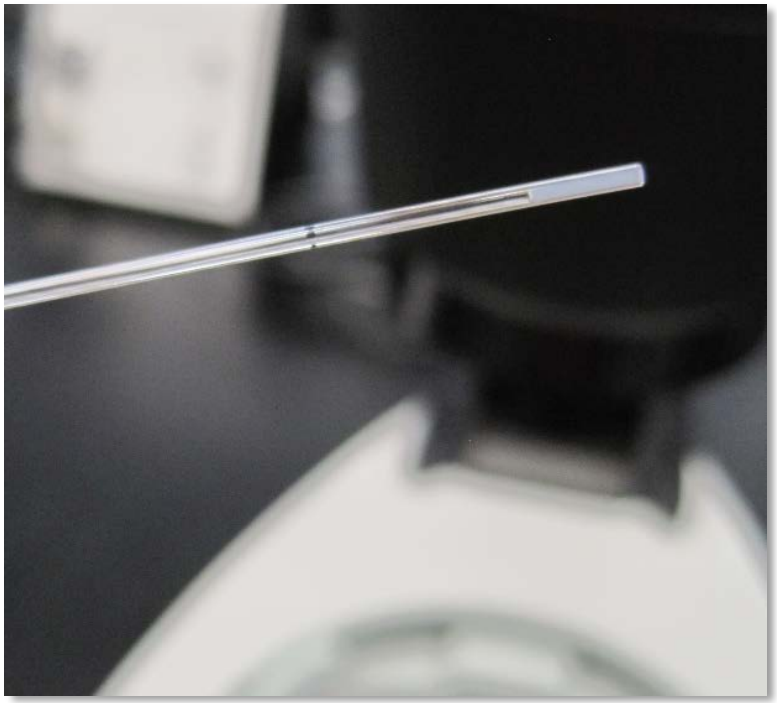


Squeezing Males – Good vs. Bad Sperm

BAD! DO NOT USE!



GOOD!



IVF and Cryopreservation

With a few additional supplies, the techniques of squeezing males and females can be used to execute:

- IVF with fresh samples
- Cryopreservation
- IVF with frozen samples

in *Vitro* Fertilization w/fresh samples

Order of operations:

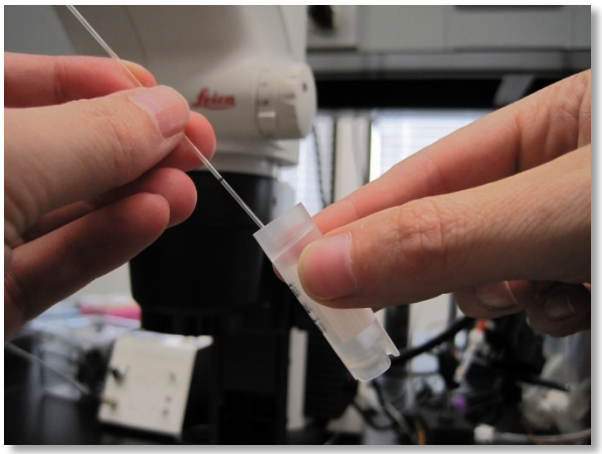
1. Separate male and female fish and house on system separately prior to IVF event

2. 30 minutes prior to “dawn” set up stations for squeezing females and males

3. Squeeze male fish (using technique previously discussed)

4. Pool the sperm from several males in ice-cold, full-strength Hank's solution.

-Sperm from 5-10 males is adequate for fertilization of several hundred eggs.
-Concentration of sperm can be estimated and should look like a cloudy suspension.



in *Vitro* Fertilization w/fresh samples

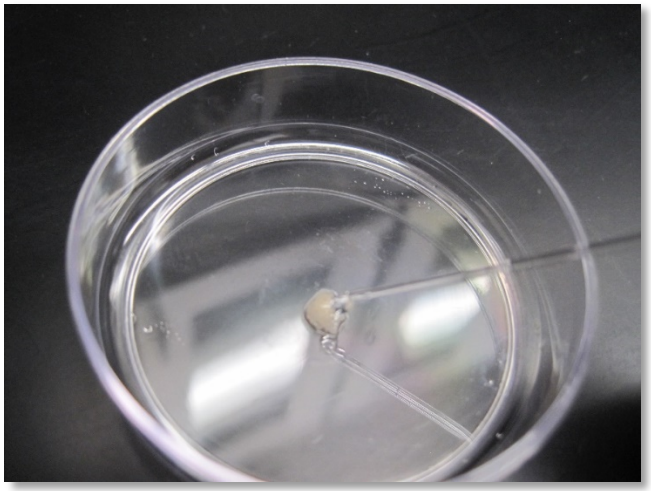
5. Begin squeezing female fish (pooled or individual depending on need)

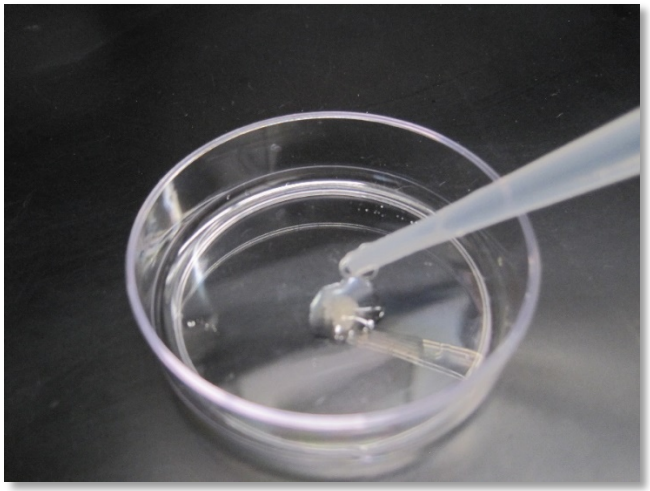
-eggs not immediately being worked with should remain in humidity chamber

6. Add 30-50µl of the sperm in Hank's directly over the clutch in a 35mm petri dish.

7. Mix gently with the capillary or pipette tip, and then add ~0.5ml of fish water.

8. Wait 1-2 minutes and add an additional 2ml fish water.





Cryopreservation - Supplies

Additional Materials Needed:

- **Watch glasses**
- **Crushed dry ice**
- **15ml conical tubes**
- Freeze media w/and w/o methanol
  - *http://www.jove.com/video/1395*
- Cryofreezer or storage doer

Cryopreservation - Supplies

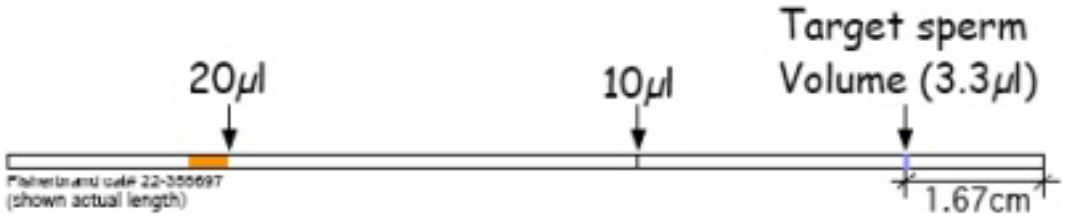
Additional Materials Needed:

- Watch glasses
- Crushed dry ice
- 15ml conical tubes
- **Freeze media w/and w/o methanol**
  - ***http://www.jove.com/video/1395***
- **Cryofreezer or storage doer**

Moens & Draper Method
http://www.jove.com/video/1395

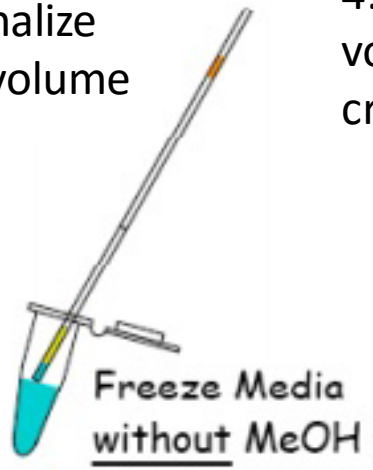
Cryopreservation - Procedure

1. Mark capillary tubes with target volumes

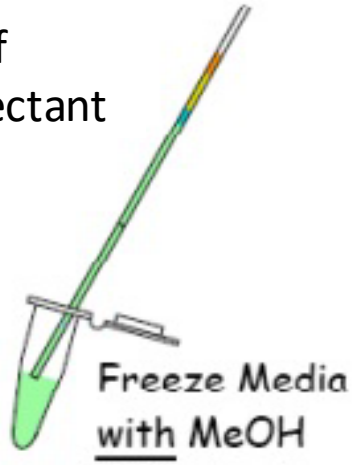


2. Squeeze sperm from male

3. Normalize sperm volume



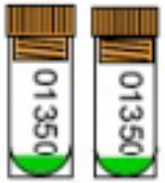
4. Add 5X volume of cryoprotectant



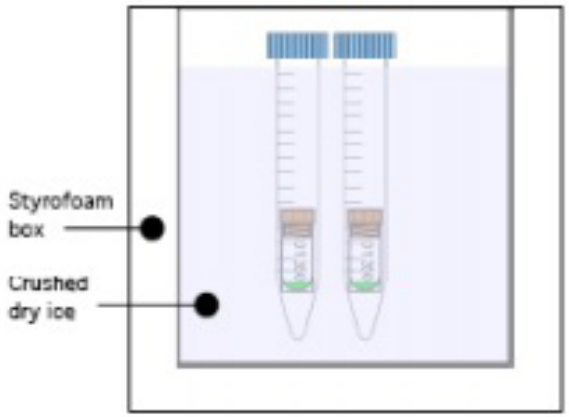
Moens & Draper Method, http://www.jove.com/video/1395

Cryopreservation - Procedure

5. Mix on clean watch glass and aliquot into 2 cryovials



6. Place cryovials in 15ml conical tubes, cap and ram into crushed dry ice.



7. After 20 min on dry ice, transfer cryovials to liquid nitrogen

Moens & Draper Method, http://www.jove.com/video/1395

in *Vitro* Fertilization w/frozen samples

Protocol for squeezing females
and
IVF fertilization

Additional Materials Needed:

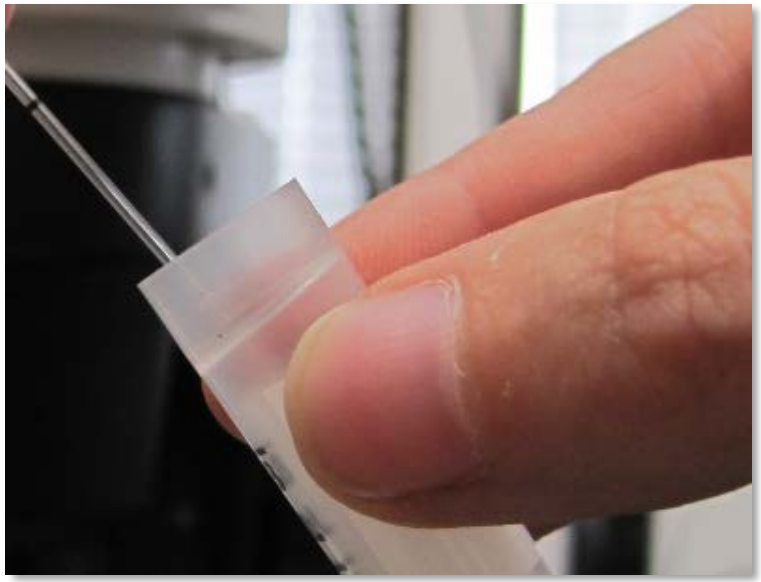
- Liquid nitrogen
- Cryo trays
- Water bath @ 33 °C

in *Vitro* Fertilization w/frozen samples

1. Use females that have been housed separately from males for several days
2. 30 minutes prior to “dawn” set up stations for squeezing females
3. Remove frozen sample from freezer and place in insulated tray of liquid nitrogen
4. Start by squeezing several clutches of eggs and pooling.

in *Vitro* Fertilization w/frozen samples

5. Remove frozen vial from liquid nitrogen and remove cap.
6. Immerse vial half way into 33 °C water bath for 8-10 seconds.
7. Quickly add 70µL Hank's solution to vial. Mix by pipetting up and down.

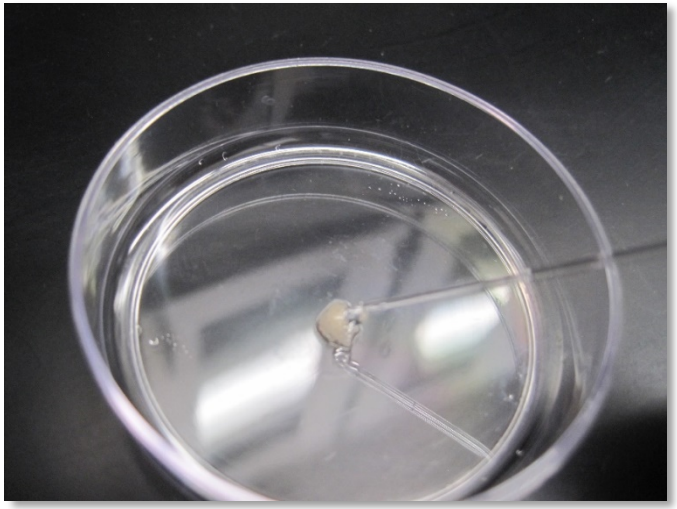


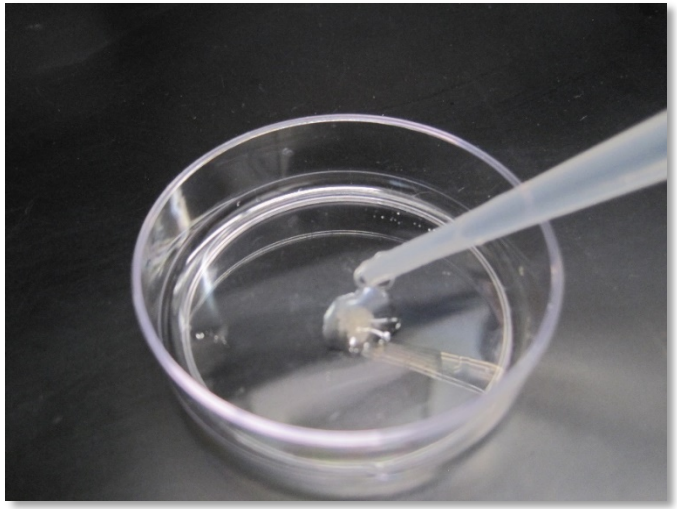
in *Vitro* Fertilization w/frozen samples

8. Immediately add to eggs and stir gently with pipette tip.

9. Activate sperm by adding 750µL fish water. Swirl water in dish to mix.

10. After incubating for 5 minutes at room temp, fill the dish with fish water and transfer to incubator (28°C)





Fin Clipping - Supplies

Materials Needed:

- **Anesthesia supplies and protocol**
- Gated spawning baskets/# 1-88
- 96 well PCR plate
- Plate template for notes/results
- Several square sheets of Parafilm
- Scalpel or razor blade
- Sharp forceps
- Beaker of water and kimwipes
- Ice bucket

Tricaine-S (MS222)

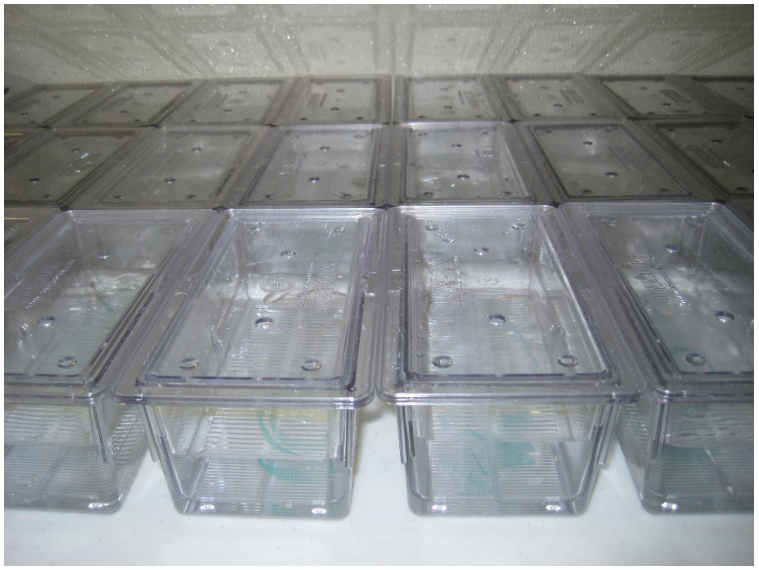
~4mls 3X buffered tricaine per 100mls fish water

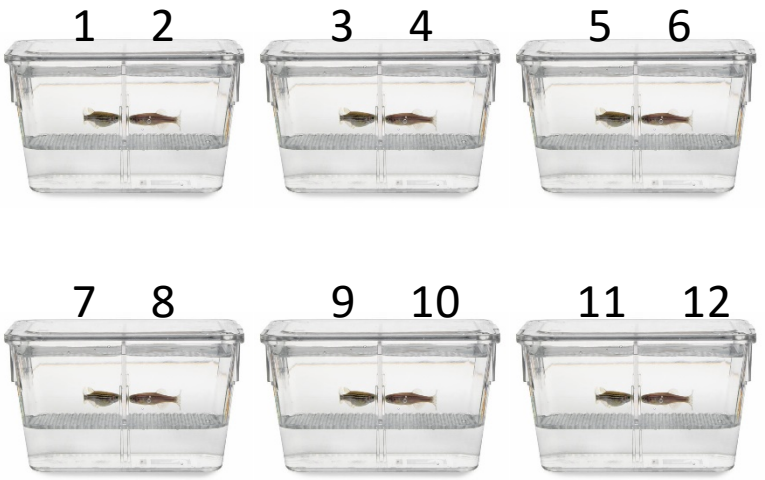
Shallow container w/ fish water
-spawning baskets work well

Fin Clipping - Supplies

Materials Needed:

- Anesthesia supplies and protocol
- **Gated spawning baskets/# 1-88**
- 96 well PCR plate
- Plate template for notes/results
- Several square sheets of Parafilm
- Scalpel or razor blade
- Sharp forceps
- Beaker of water and kimwipes
- Ice bucket

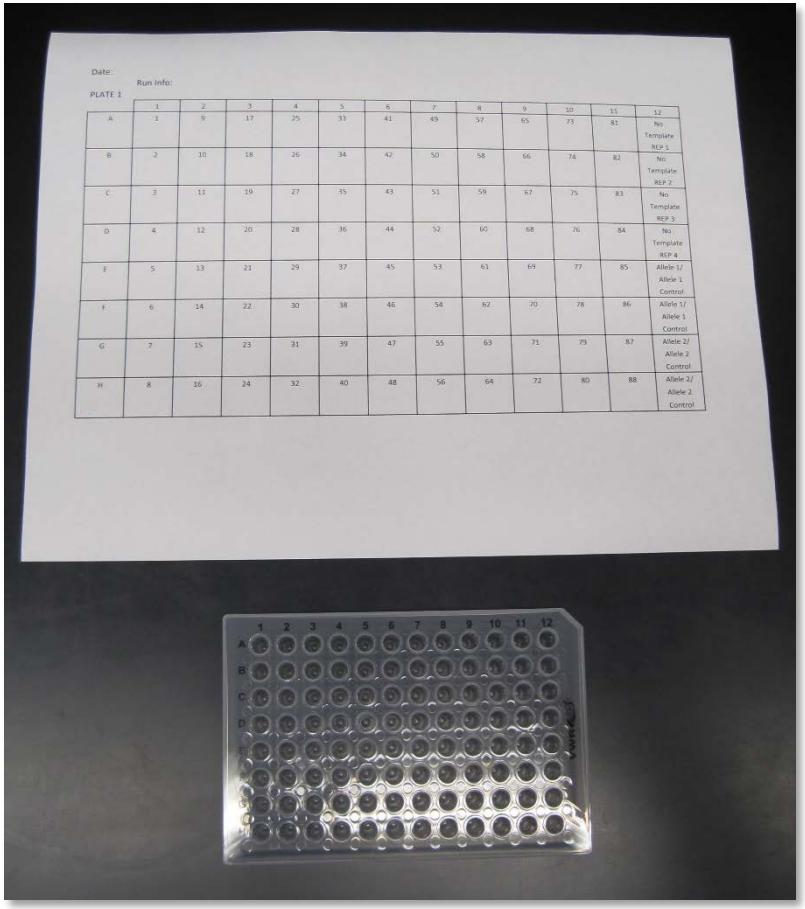




Fin Clipping - Supplies

Materials Needed:

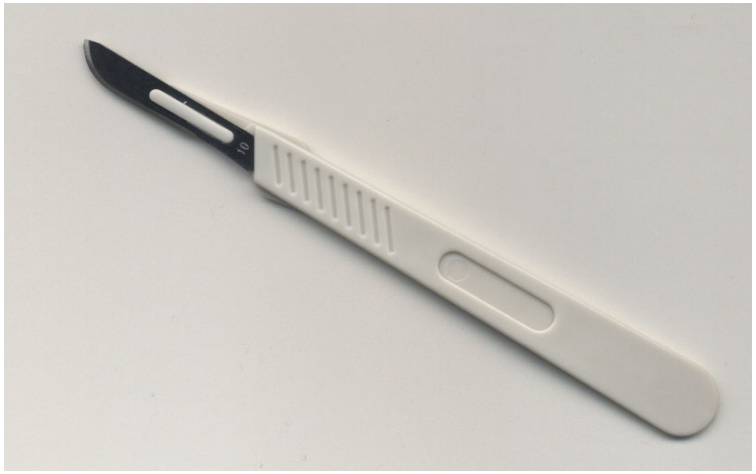
- Anesthesia supplies and protocol
- Gated spawning baskets/# 1-88
- **96 well PCR plate**
- **Plate template for notes/results**
- Several square sheets of Parafilm
- Scalpel or razor blade
- Sharp forceps
- Beaker of water and kimwipes
- Ice bucket



Fin Clipping - Supplies

Materials Needed:

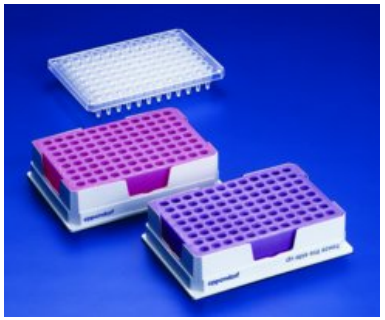
- Anesthesia supplies and protocol
- Gated spawning baskets/# 1-88
- 96 well PCR plate
- Plate template for notes/results
- **Several square sheets of Parafilm**
- **Scalpel or razor blade**
- **Sharp forceps**
- Beaker of water and kimwipes
- Ice bucket



Fin Clipping - Supplies

Materials Needed:

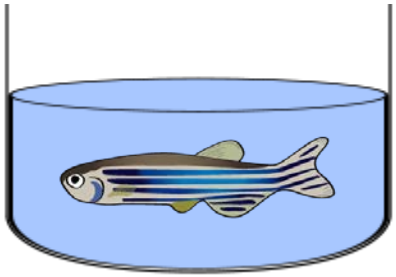
- Anesthesia supplies and protocol
- Gated spawning baskets/# 1-88
- 96 well PCR plate
- Plate template for notes/results
- Several square sheets of Parafilm
- Scalpel or razor blade
- Sharp forceps
- **Beaker of water and kimwipes**
- **Ice bucket**



Fin Clipping - Procedure

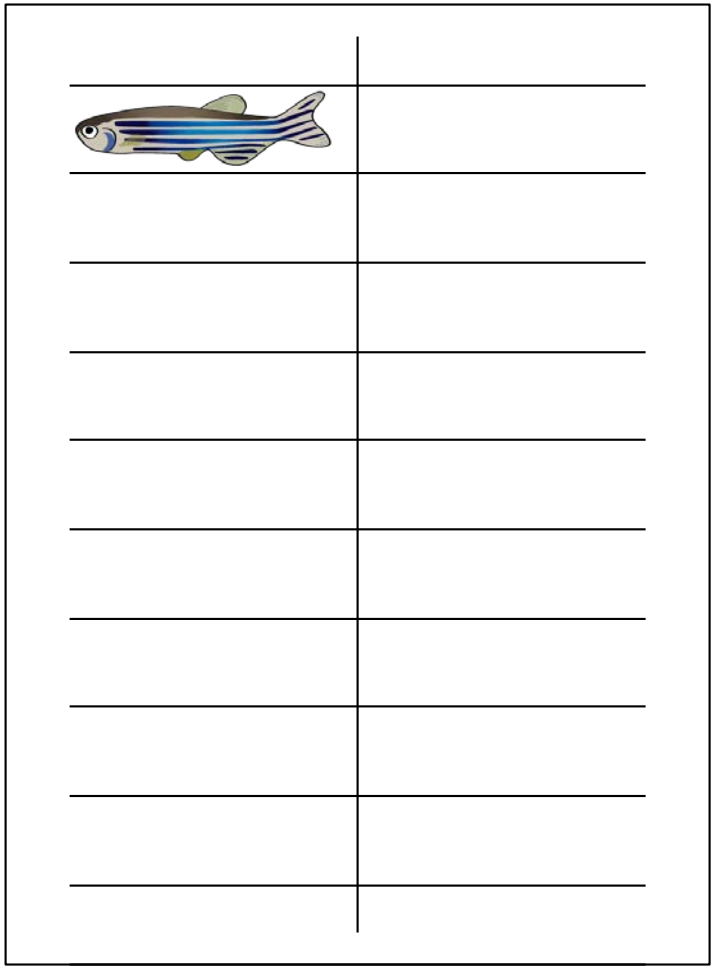
1. Set up clipping station, gated baskets and fish

2. Put fish in tricaine until gill movement slows



3. Remove from tricaine with spoon, remove excess water with paper towel

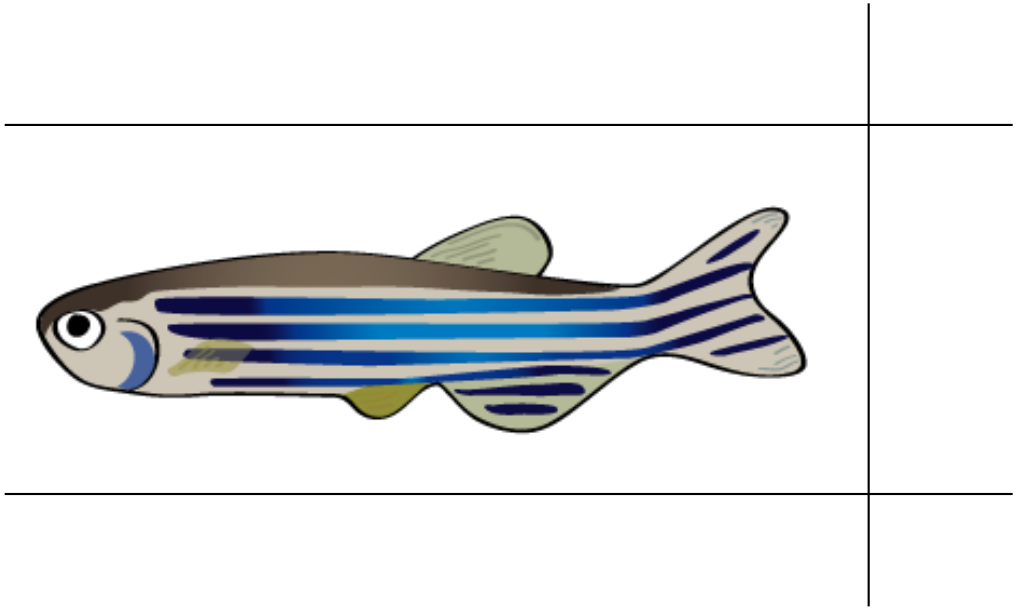
4. Transfer fish to top left spot on parafilm



Fin Clipping - Procedure

5. Firmly cut a portion of the tail off using the scalpel.

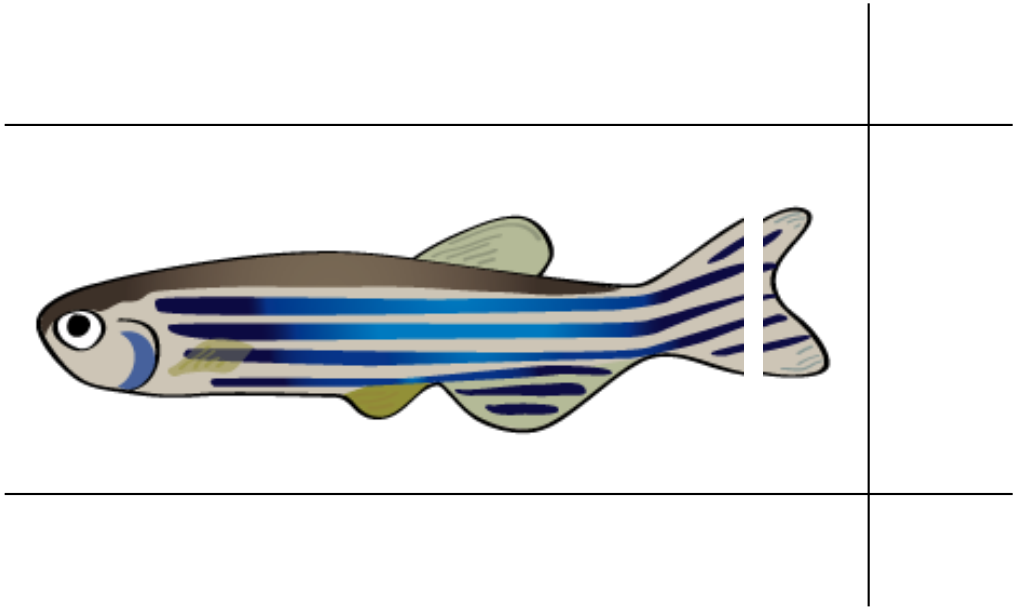
6. Use spoon to scoop fish up, and place in numbered spawning basket



Fin Clipping - Procedure

5. Firmly cut a portion of the tail off using the scalpel.

6. Use spoon to scoop fish up, and place in numbered spawning basket



Fin Clipping - Procedure

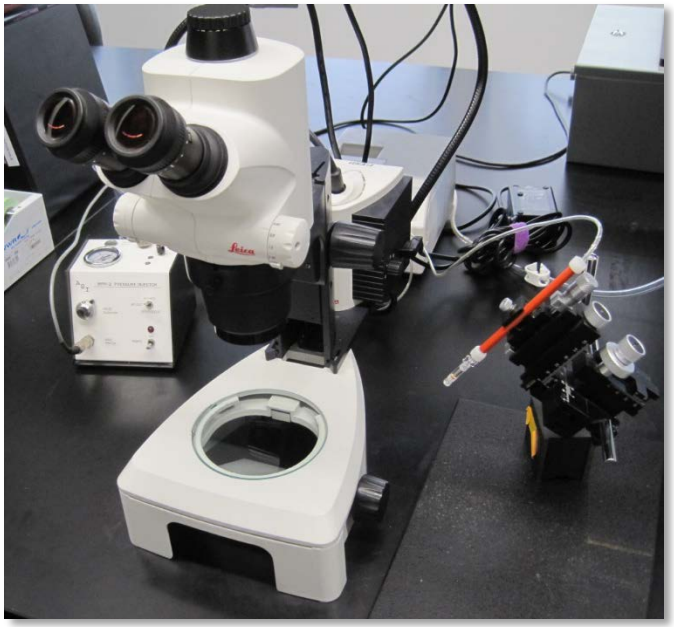
7. Using forceps, pick up fin clip and place in corresponding well on plate
8. Dip forceps and scalpel in water, dry on kimwipe
9. Repeat this process, each time moving down to a fresh space on parafilm.

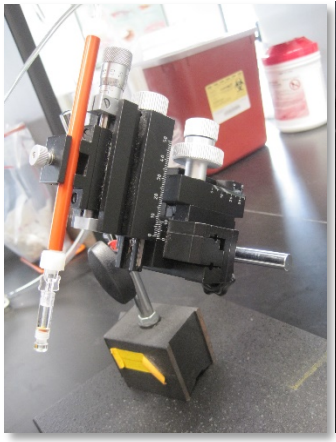
Consistent clip sizes will make the PCR process easier

Microinjection - Supplies

Materials Needed:

- **Microscope**
- **Injection apparatus**
- **Pressure injector/air tanks**
- 100mm petri dishes
- Glass microscope slide
- Agar injection molds
- Pipette
- Fish in gated crosses or iSpawn
- Injection needles
- Compound – Vector - Dye

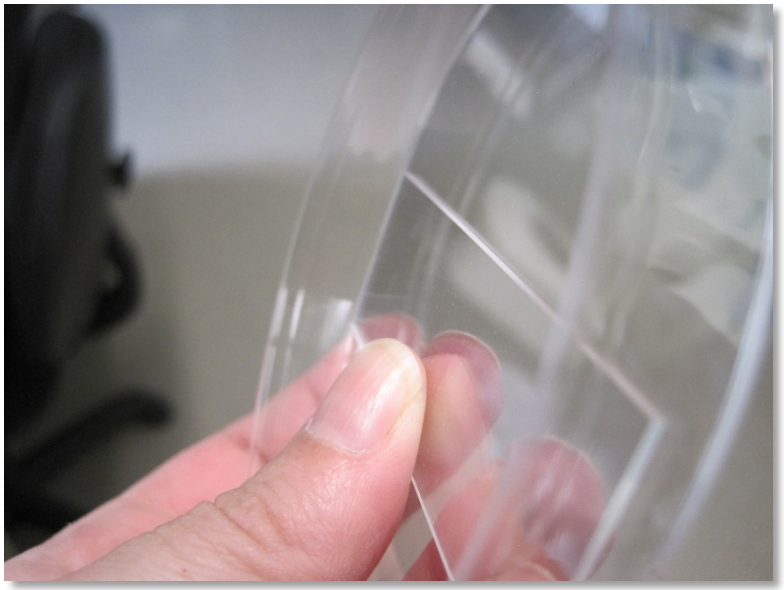




Microinjection - Supplies

Materials Needed:

- Microscope
- Injection apparatus
- Pressure injector/air tanks
- **100mm petri dishes**
- **Glass microscope slide**
- Agar injection molds
- Pipette
- Fish in gated crosses or iSpawn
- Injection needles
- Compound – Vector - Dye



Glass slide methods is ideal for injections into the yolk stream

- Morpholinos
- Emulsions
- Dyes

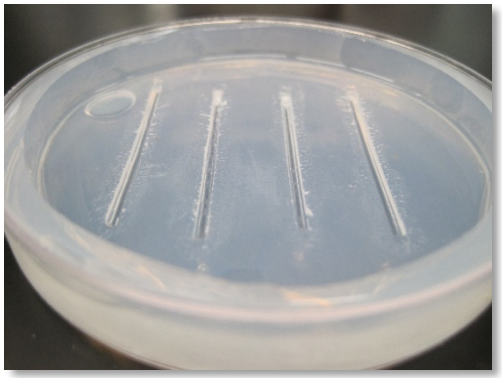
Microinjection - Supplies

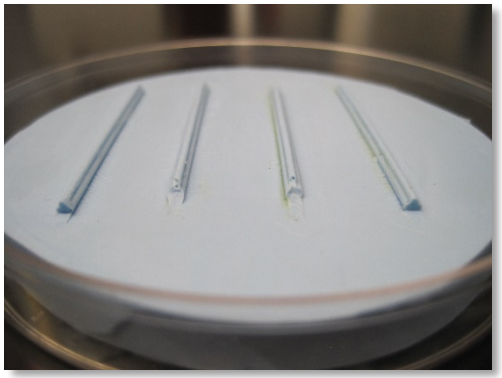
Materials Needed:

- Microscope
- Injection apparatus
- Pressure injector/air tanks
- 100mm petri dishes
- Glass microscope slide
- **Agar injection molds**
- Pipette
- Fish in gated crosses or iSpawn
- Injection needles
- Compound – Vector - Dye

Agar mold methods is ideal for injections into the single cell, which requires orienting the embryo more precisely

- **cDNA**
- **RNA**





Microinjection - Supplies

Materials Needed:

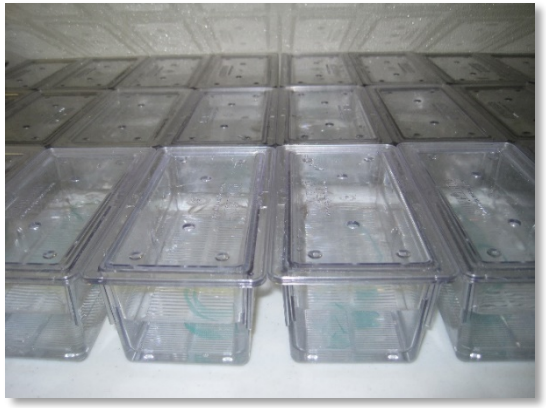
- Microscope
- Injection apparatus
- Pressure injector/air tanks
- 100mm petri dishes
- Glass microscope slide
- Agar injection molds
- **Pipette**
- Fish in gated crosses or iSpawn
- Injection needles
- Injection material - Dye

Brand: Kimble Chase
Product # is 63A53WT
Description: 5 ¾ borosilicate (Boro) glass Pasteur pipette

Microinjection - Supplies

Materials Needed:

- Microscope
- Injection apparatus
- Pressure injector/air tanks
- 100mm petri dishes
- Glass microscope slide
- Agar injection molds
- Pipette
- **Fish in gated crosses or iSpawn**
- Injection needles
- Injection material- Dye



Microinjection - Supplies

Materials Needed:

- Microscope
- Injection apparatus
- Pressure injector/air tanks
- 100mm petri dishes
- Glass microscope slide
- Agar injection molds
- Pipette
- Fish in gated crosses or iSpawn
- **Injection needles**
- Injection material- Dye

1.0mm OD glass capillary w/filament
Micropipette puller
Or
Pre-pulled needles

Microinjection - Supplies

Materials Needed:

- Microscope
- Injection apparatus
- Pressure injector/air tanks
- 100mm petri dishes
- Glass microscope slide
- Agar injection molds
- Pipette
- Fish in gated crosses or iSpawn
- Injection needles
- **Injection material - Dye**

CRISPR/Cas9

CDNA, RNA

Morpholinos

Microinjection – Media and Volumes

Compound

+

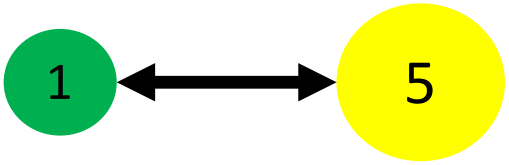
Sterile liquid

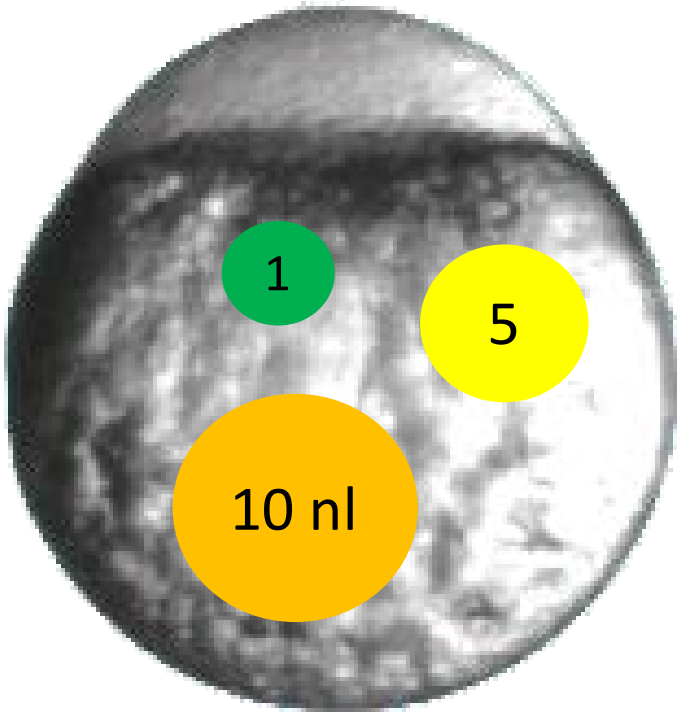
+

Tracer dye (phenol red)

=

Total injection volume





Microinjection – Media and Volumes

Compound

+

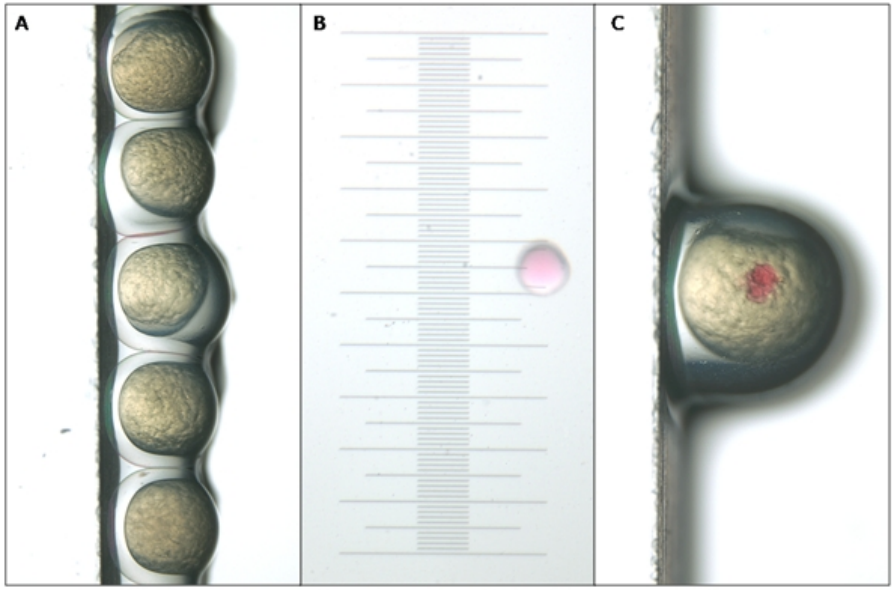
Sterile liquid

+

Tracer dye (phenol red)

=

Total injection volume



http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2762901/

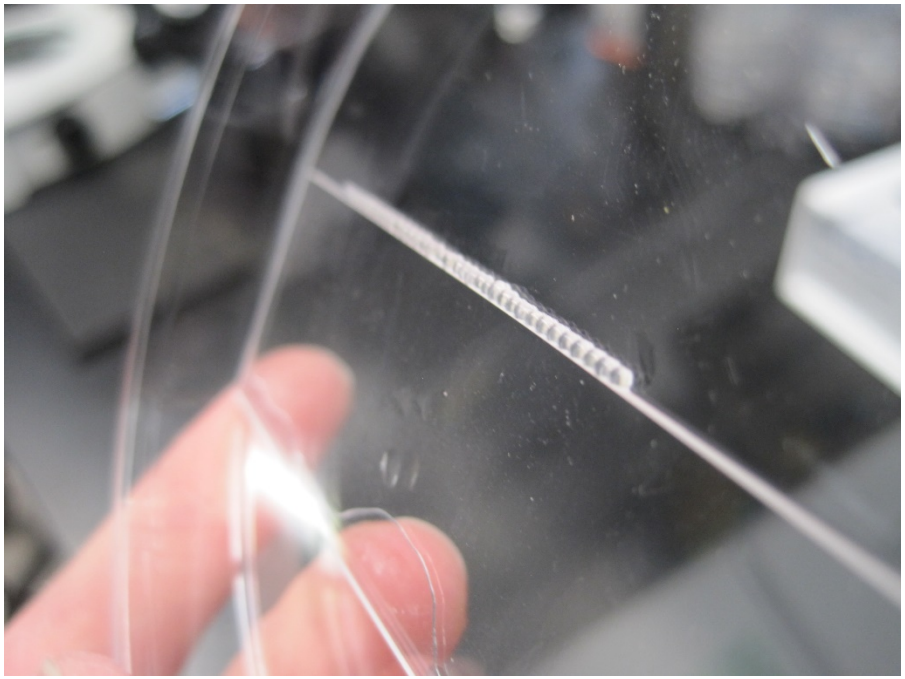
- Place a drop of mineral oil on a micrometer
- Break tip of needle with forceps
- Injection into the oil
- a bead with a diameter of 0.1 mm contains 1nL

Microinjection – Procedure

1. Pull gates from spawning baskets or activate iSpawn
2. Wait ~15-20 minutes for fish to spawn, set up injection station during this time
3. Load needles with compound, and brake tip with fine forceps
4. Once fish are visibly producing embryos, collect with strainer

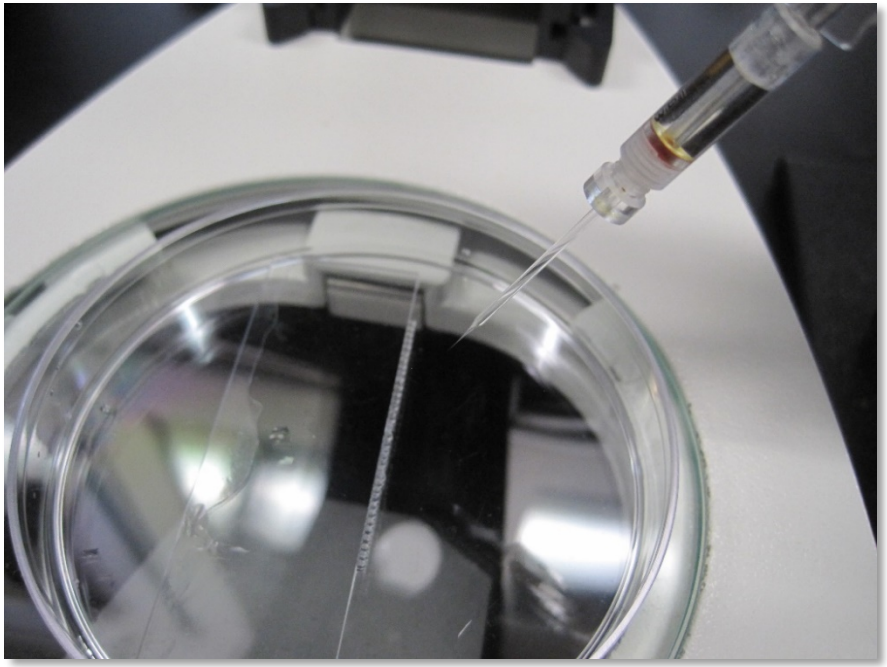
Microinjection – Procedure

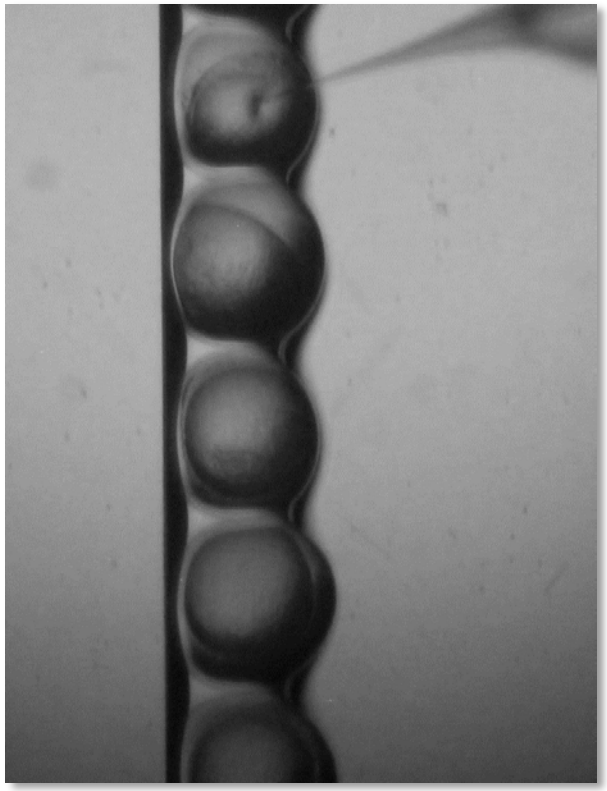
5. Select fertilized embryos with a glass pipette
6. Dispense embryos along the shelf created by the slide and petri dish
7. Place petri dish on microscope stage and begin injecting



Microinjection – Procedure

8. Lower the needle toward the top embryo
9. Pierce the surface of the chorion and enter the yolk in one motion
10. Depress injector pedal and smoothly retract the needle
11. Repeat down the column of embryos
12. Rinse embryos into petri dish w/embryo media





Embryo Bleaching - Supplies

Materials Needed:

- **5 small containers**
- **Embryo strainers**
- Clean fish water or EM
- RO water
- 5-6% scientific grade bleach
- Household bleach
- Sodium Thiosulfate

Embryo Bleaching - Supplies

Materials Needed:

- 5 small containers
- Embryo strainers
- **Clean fish water or EM**
- **RO water**
- 5-6% scientific grade bleach
- Household bleach
- Sodium Thiosulfate

RO Water

Embryo media
Or
Sterile fish water

Embryo Bleaching - Supplies

Materials Needed:

- 5 small containers
- Embryo strainers
- Clean fish water or EM
- RO water
- **5-6% scientific grade bleach**
- **Household bleach**
- **Sodium Thiosulfate**

Embryo Bleaching - Protocol

1. Sanitize all work surfaces and supplies with household bleach at a concentration of 500 ppm (500mg/liter)
   –neutralize with sodium thiosulfate and rinse well

2. Make all stock solutions (bleach water, rinse water, neutralizing bath)

3. Set up bleaching station in the following configuration:

   Bleach 1 - Rinse 1 - Bleach 2 - Thio Rinse - Rinse 2

4. Pre-clean embryos, removing any that are dead or damaged

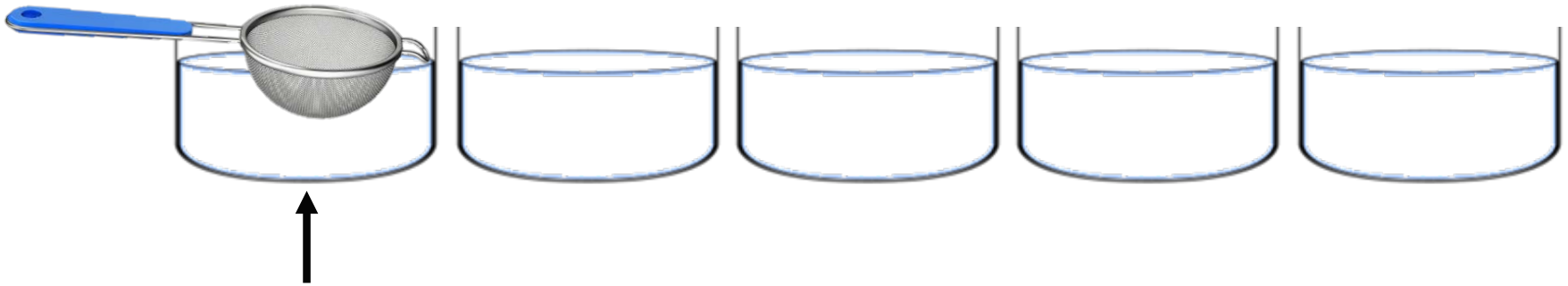
5. Rinse embryos into strainer

Embryo Bleaching - Protocol

Steps:

6. Place strainer in 1st bleach bath for 5 minutes
   -gentle swirl to expose all surfaces to bleach

- Add 1ml of 5-6% sodium hypochlorite to 1 liter RO water
- Target concentration is 50ppm



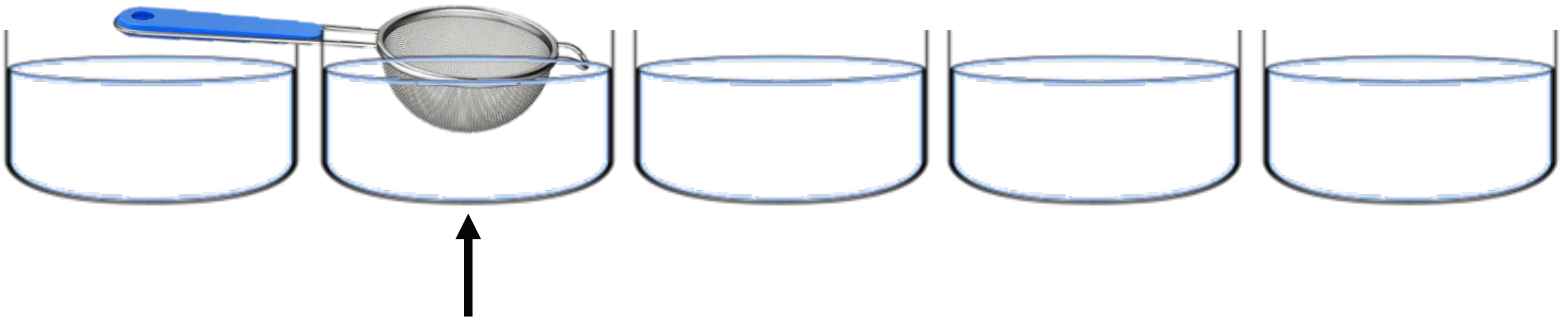
Bleach bath #1

Steps:

7. Transfer strainer to 1st rinse bath for 5 minutes

   -gently swirl to expose all surfaces to rinse water

- Rinse baths can be any source of clean fish water
- EM, autoclaved system water, clean fish water



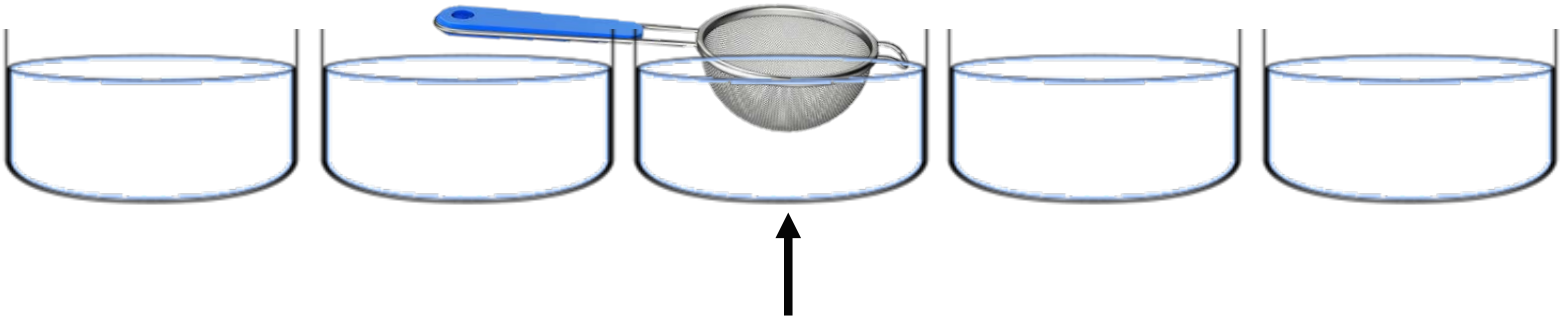
Rinse bath #1

Steps:

8. Transfer strainer to 2nd bleach bath for 5 minutes

-gently swirl to expose all surfaces to bleach water

- Add 1ml of 5-6% sodium hypochlorite to 1 liter RO water
- Target concentration is 50ppm



Bleach bath #2

Embryo Bleaching - Protocol

Steps:

9. Transfer strainer to Sodium Thiosulfate bath for 5 min.

   -gently swirl to expose all surfaces to neutralizer

- Add 500 mg of sodium thiosulfate to 1 Liter of clean system water

Sodium Thio Bath

Steps:

10. Transfer strainer to final rinse bath for 5 minutes

-gently swirl to expose all surfaces to rinse water

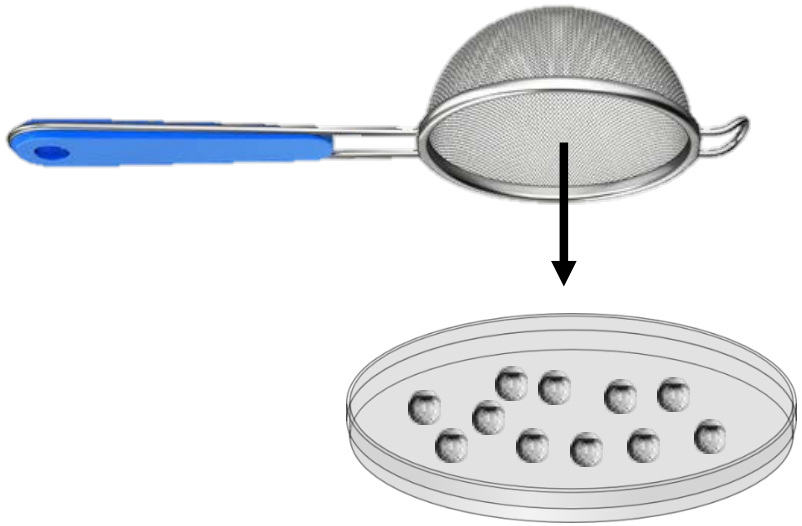
- Rinse baths can be any source of clean fish water
- EM, autoclaved system water, clean fish water

Final rinse bath

Steps:

12. Rinse embryos into petri dish w/embryo media

- Keep in densities no greater than 50 per 100mm dish



Dissection and Fixation for Histology

Materials Needed:

- **Euthanasia supplies and protocol**
- Live fish
- Razor blade or scalpel
- Micro dissecting scissors
- Forceps
- Fixative
- Rocker
- Vials or sealable tubes
- Parafilm

Tricaine-S (MS222)

~13 mls 3X buffered tricaine per 100mls fish water

Shallow container w/ fish water
-spawning baskets work well

Dissection and Fixation for Histology

Materials Needed:

- Euthanasia supplies and protocol
- **Live fish**
- Razor blade or scalpel
- Micro dissecting scissors
- Forceps
- Fixative
- Rocker
- Vials or sealable tubes
- Parafilm

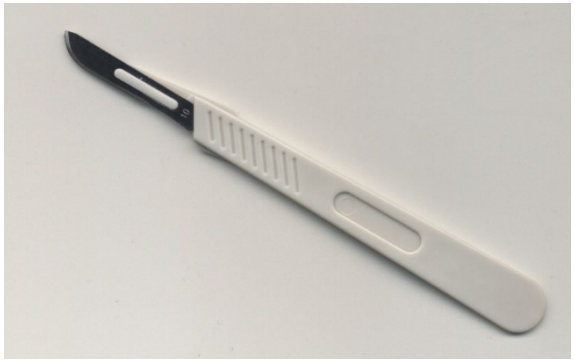
http://zdmsociety.org/zebrafish-and-human-disease/

Fish must be freshly euthanized prior to fixation. Fish found dead are not appropriate samples for histology as the process of autolysis has already begun.

Dissection and Fixation for Histology

Materials Needed:

- Euthanasia supplies and protocol
- Live fish
- **Razor blade or scalpel**
- **Micro dissecting scissors**
- **Forceps**
- Fixative
- Rocker
- Vials or sealable tubes
- Parafilm



Dissection and Fixation for Histology

Materials Needed:

- Euthanasia supplies and protocol
- Live fish
- Razor blade or scalpel
- Micro dissecting scissors
- Forceps
- **Fixative**
- Rocker
- Vials or sealable tubes
- Parafilm

Dietrich's Solution

- 30 ml Ethanol (95%)
- 10ml Formalin

(Formaldehyde 37% solution, histological grade, contains 10-15% methanol)

- 2 ml Glacial Acetic Acid
- 58 ml Distilled water

- OR -

10% Formalin

This will likely depend on the service provider

Dissection and Fixation for Histology

Materials Needed:

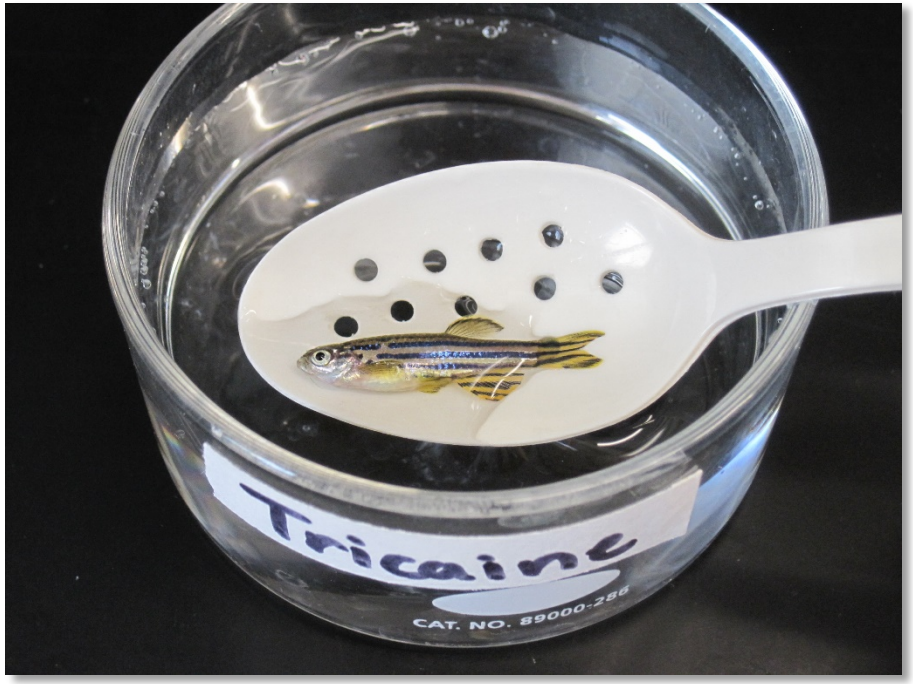
- Euthanasia supplies and protocol
- Live fish
- Razor blade or scalpel
- Micro dissecting scissors
- Forceps
- Fixative
- **Rocker**
- **Vials or sealable tubes**
- **Parafilm**

*cut into strips

Dissection and Fixation for Histology

1. Euthanatize fish using an overdose of MS-222 (Tricaine)

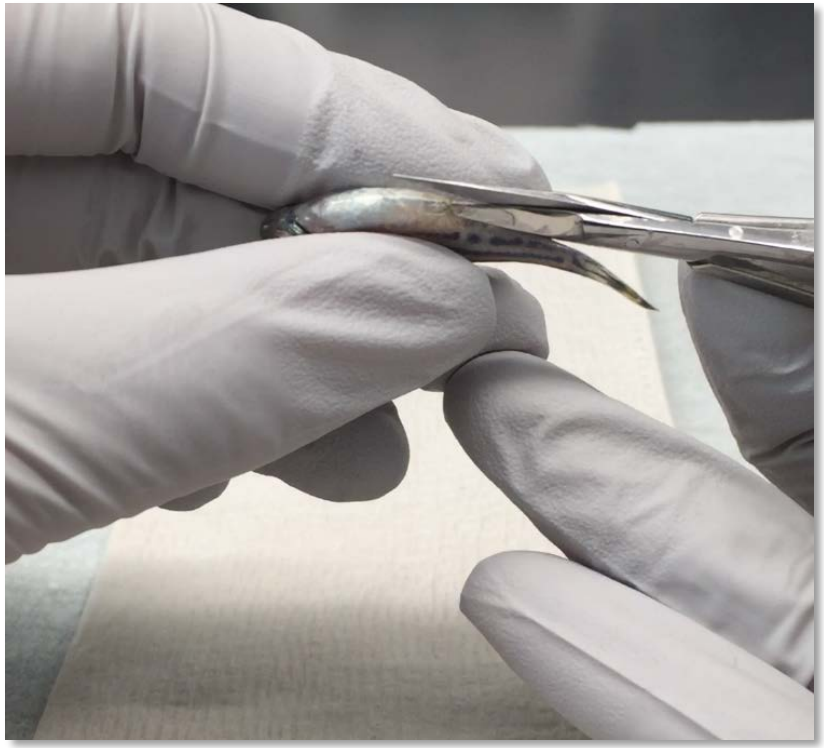
2. Remove fish from tricaine and blot excess water away.

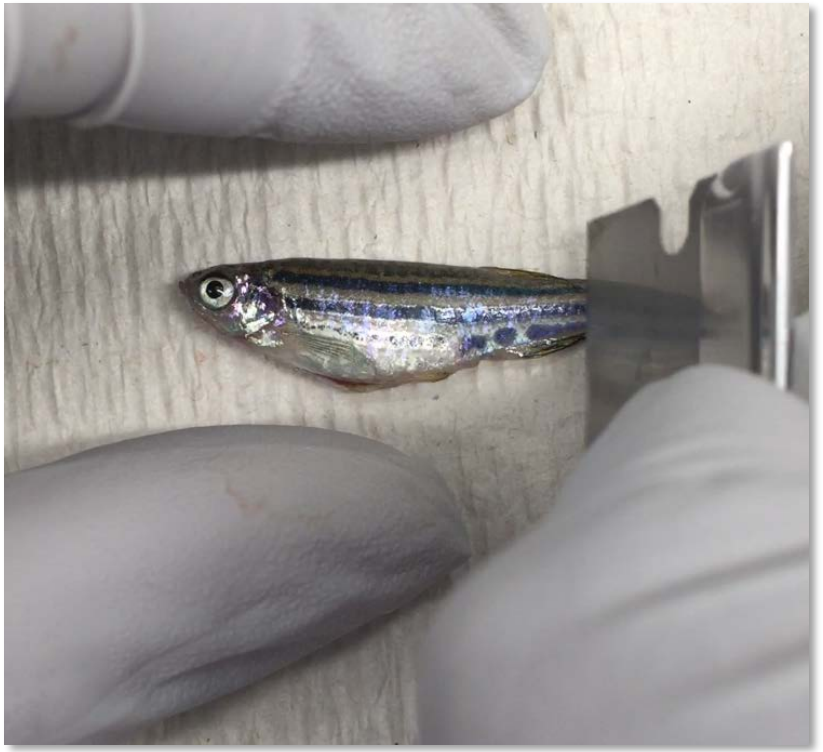


Dissection and Fixation for Histology

3. Using micro scissors, cut open the coelomic cavity.

4. Using razor blade, cut tail off at the caudal peduncle region.

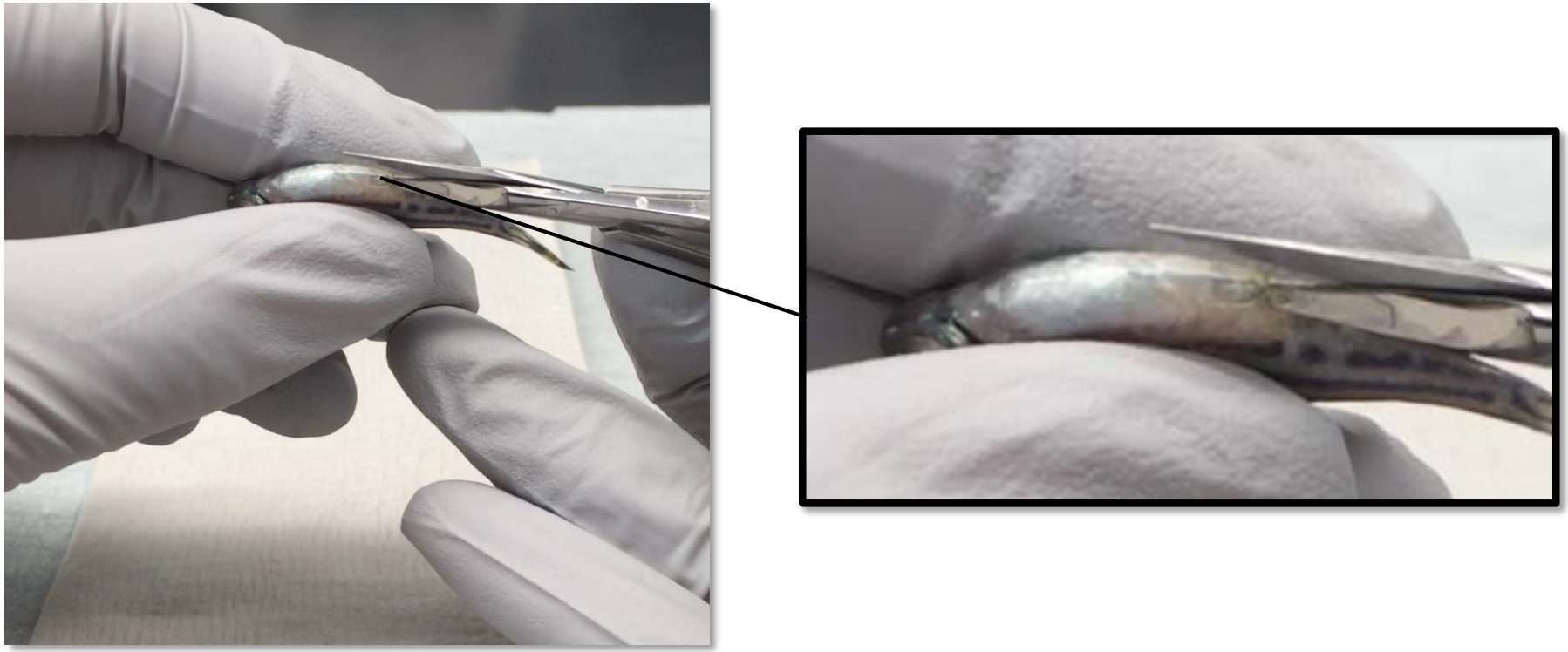




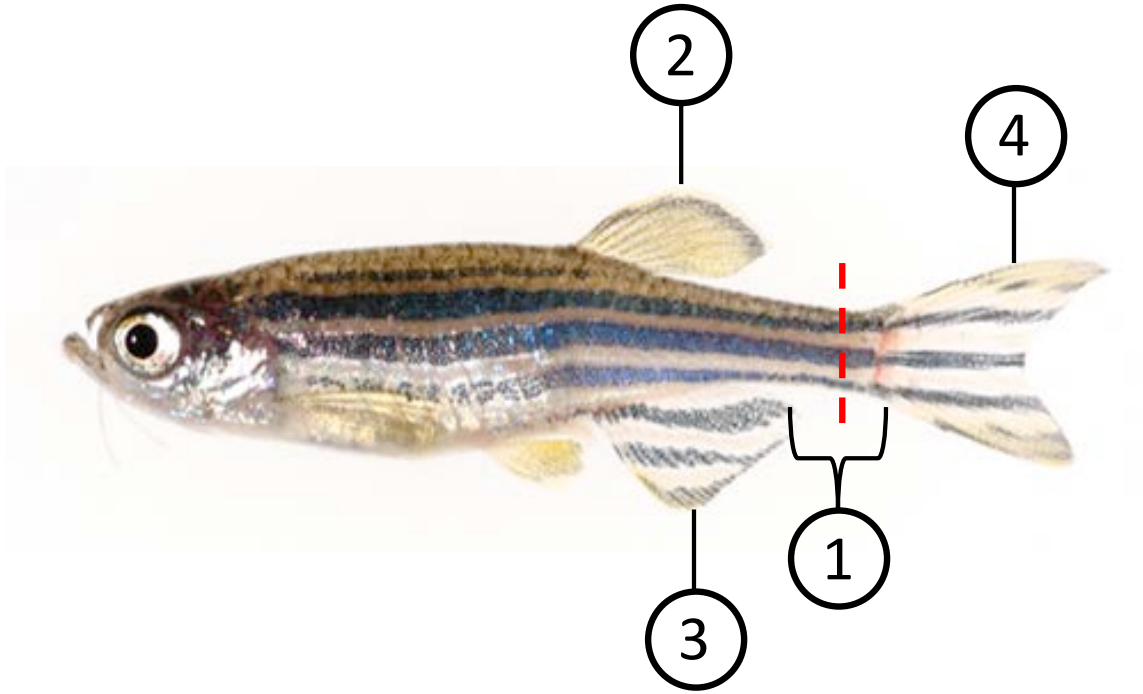
Dissection and Fixation for Histology

3. Using micro scissors, slit open the coelomic cavity.

4. Using razor blade, cut tail off at the caudal peduncle region.



Dissection and Fixation for Histology



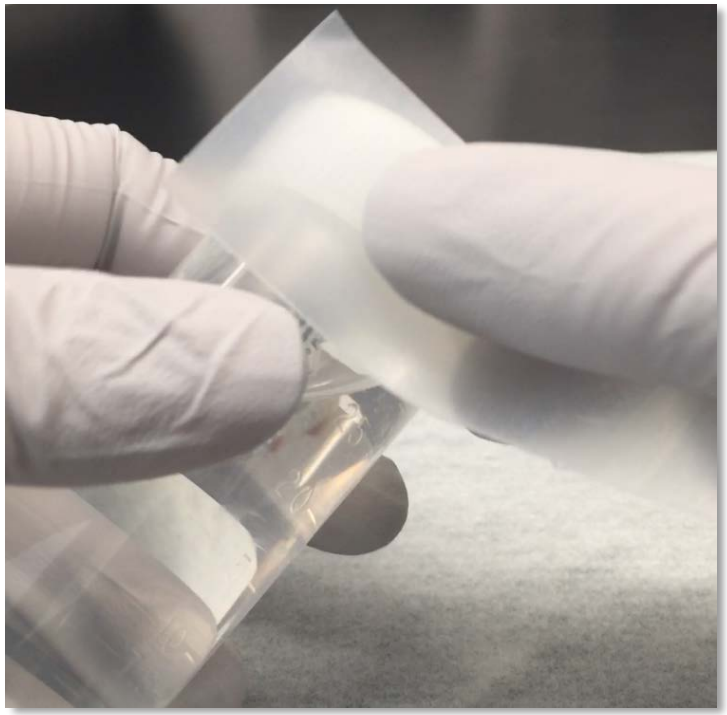
The 1) caudal peduncle is the tapered region behind the 2) dorsal and 3) anal fin where the 4) caudal fin attaches to the body.

Dissection and Fixation for Histology

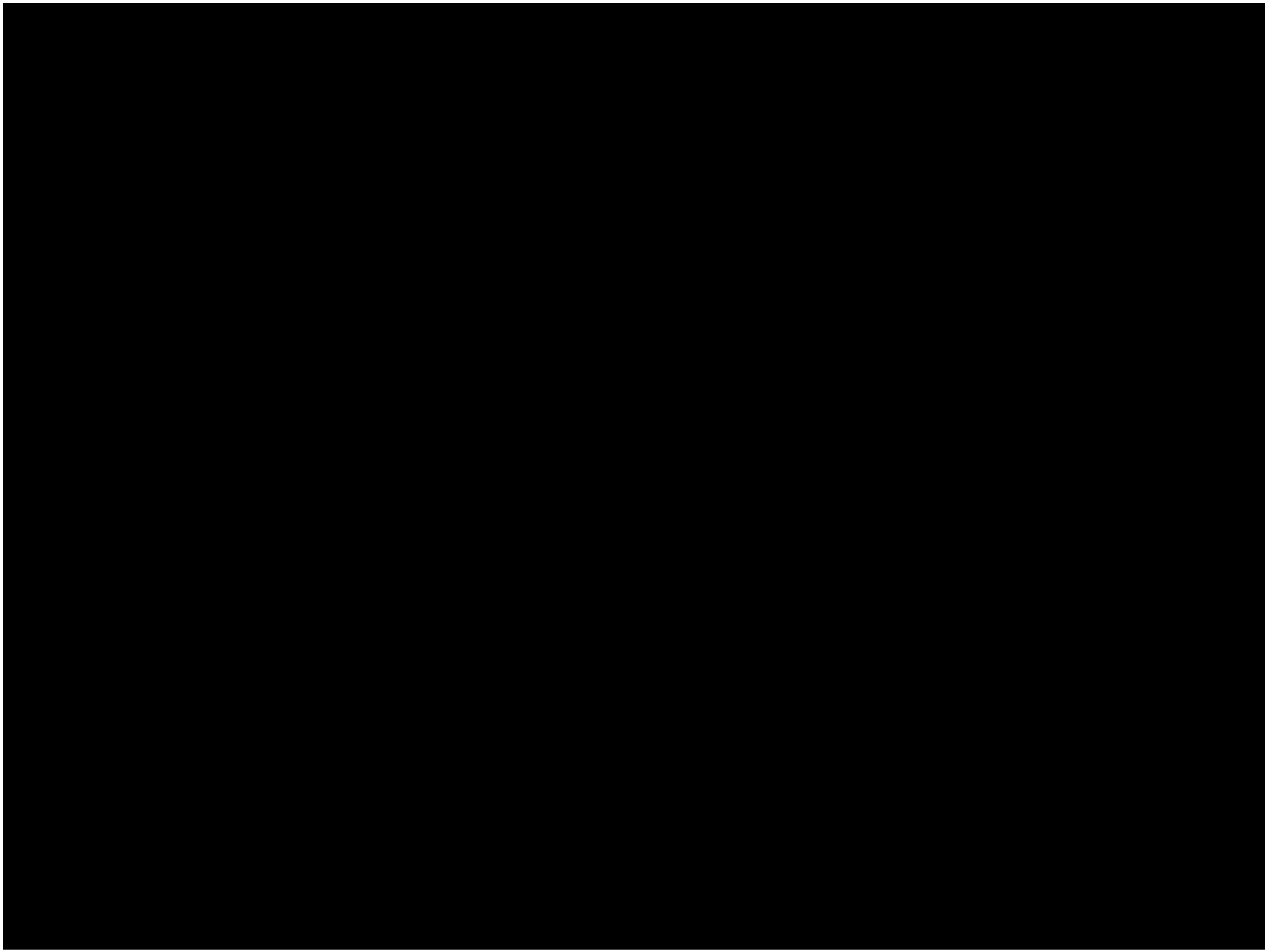
5. Using forceps, drop fish into sealable container of fixative of choice.
   -volume is ~10-15mls of fixative per 1-2 fish

6. Strips of parafilm can be used to seal caps of tubes as a precautionary measure.

7. Place container of fixative and fish on rocker for 24 hours post fixation.
   -Fish can remain in Dietrich's for several weeks.



Dissection for Histology - Video



Dechorionation - Supplies

Materials Needed:

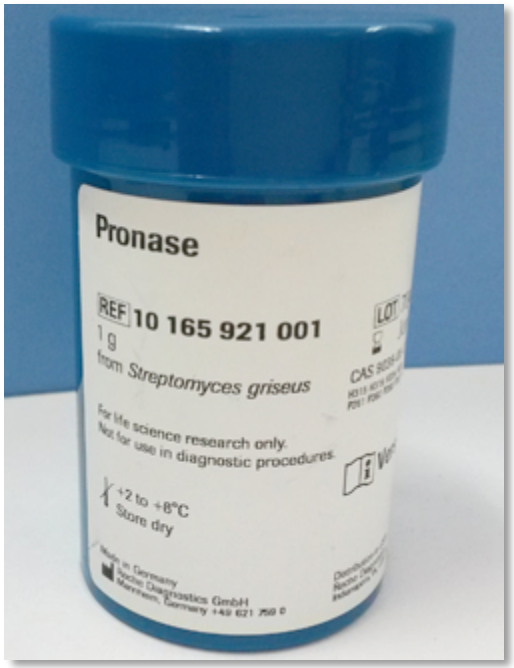
- **60mm glass petri dishes**
- **Timer**
- **Fish water squirt bottle**
- Pronase

Glass dishes are essential. Newly dechorionated embryos can stick to plastic and be damaged

Dechorionation - Supplies

Materials Needed:

- 60mm glass petri dishes
- Timer
- Fish water squirt bottle
- **Pronase**



50µL pronase per dish of embryos to be dechorionated

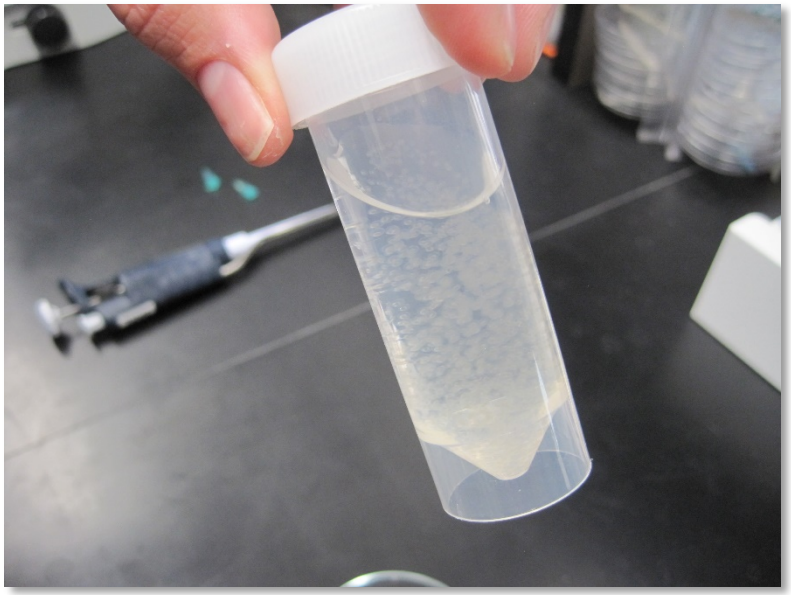
41mg/ml concentration

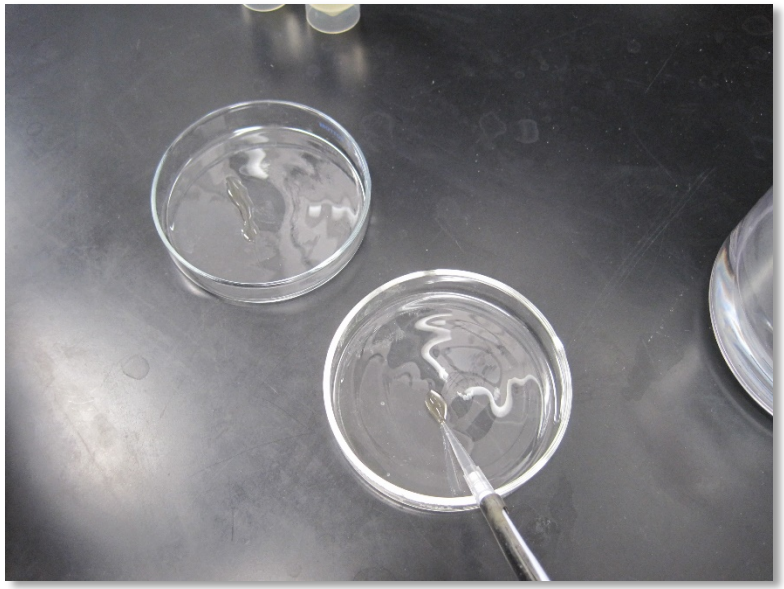
50µL aliquots can be stored in freezer for up to 4 months
-thaw immediately before use

Dechorionation - Procedure

1. Add up to 1500 embryos to 25ml fish water

2. Pipette 50µL 41mg/ml concentration pronase into a 60mm glass petri





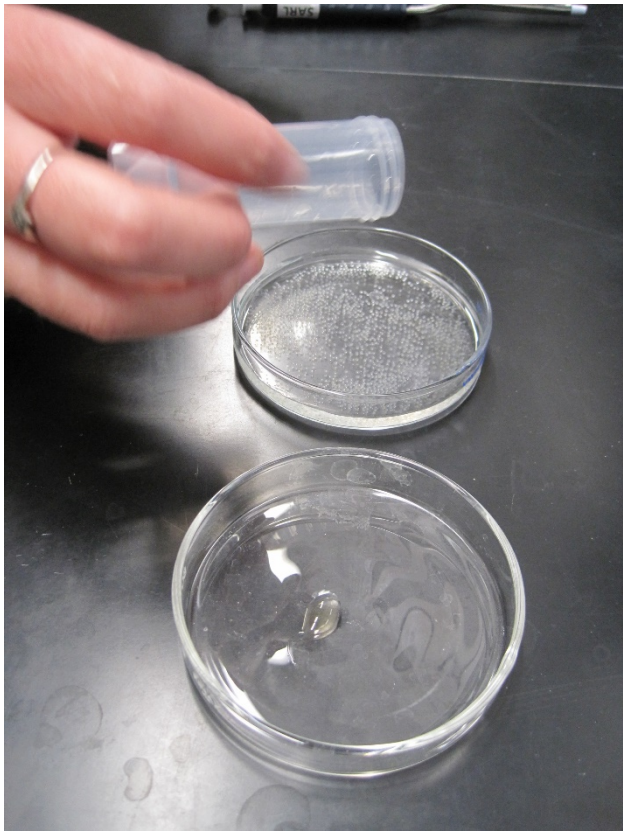
Dechorionation - Procedure

3. Pour the embryos and fish water into the petri dish

4. Start time and begin swirling embryos

Exposure time to pronase depends on age of embryos.

4hpf embryos = 6 minutes
24hpf embryos = 3 minutes



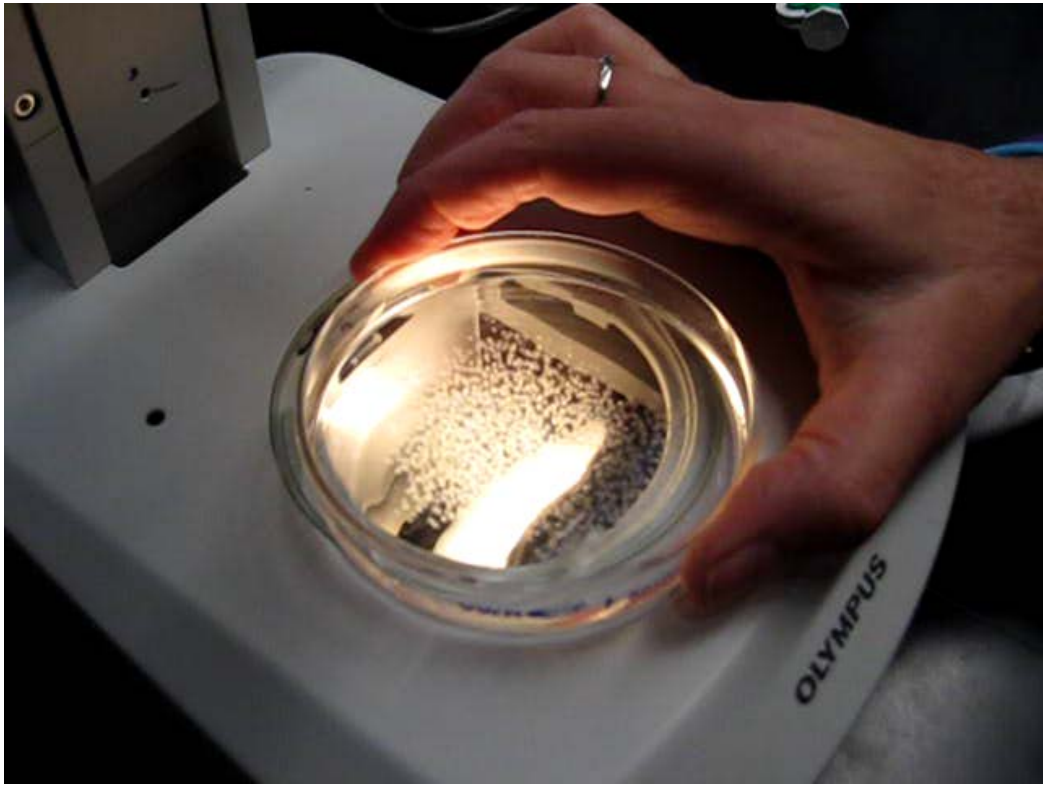
Dechorionation - Procedure

5. Continue to swirl the embryos for 6 minutes.

6. Continually observe embryos under microscope for signs up digestion.

Signs of digestion include:

- **Deflated embryos**
- **Chorion pieces**
- **Embryos outside of chorions**

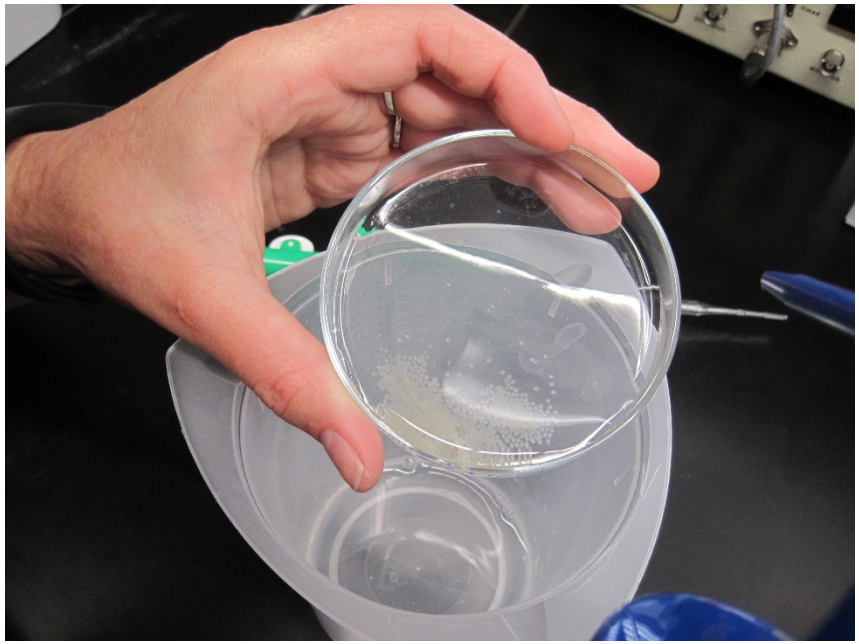


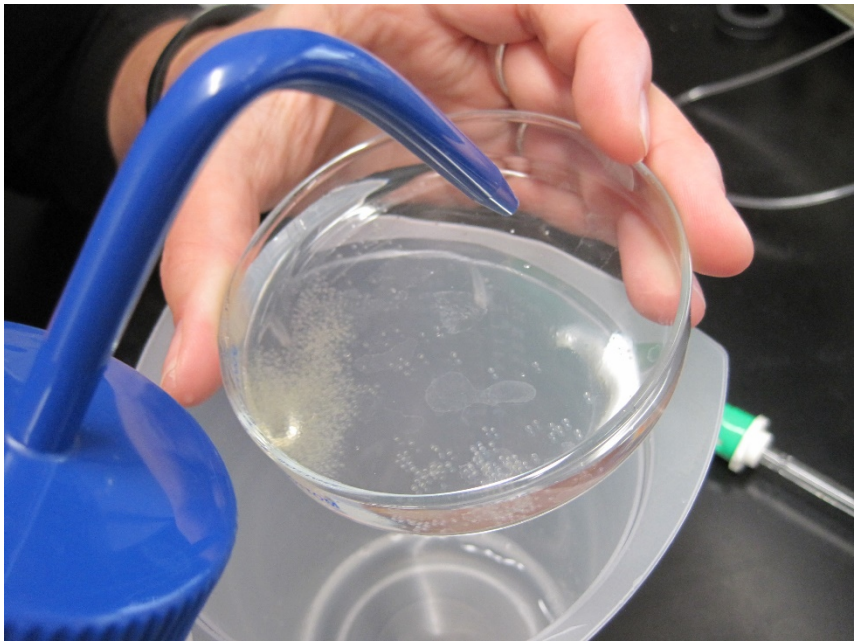
Dechorionation - Procedure

7. When 3 or 6 minutes have passed, or when a significant portion of embryos are showing signs of digestion, begin rinse process.

8. Pour pronase solution out of dish, and start rinsing with fish water. Embryos are delicate so introduce water above embryos and not directly on them.

Total rinse time should equal 10 minutes or a total of 1000ml fish water per dish





Dechorionation - Procedure

Dechorionated embryos require a rest period:

- 4hpf must rest post-dechor for at least 30 minutes
- 24hpf must rest post-dechor for at least 15 minutes

Fish Shipping - Supplies

Materials Needed:

- **ThermoSafe box**
- **Secondary containment bag**
- Cubitainer
- Absorbent bench towels
- fish water
- Ammonia binder
- Heat packs
- Rubber bands or zip tie
- Live animal labels
- Appropriate documentation

Fish Shipping - Supplies

Materials Needed:

- ThermoSafe box
- Secondary containment bag
- **Cubitainer**
- **Absorbent bench towels**
- fish water
- Ammonia binder
- Heat packs
- Rubber bands or zip tie
- Live animal labels
- Appropriate documentation

Fish Shipping - Supplies

Materials Needed:

- ThermoSafe box
- Secondary containment bag
- Cubitainer
- Absorbent bench towels
- **fish water**
- **Ammonia binder**
- Heat packs
- Rubber bands or zip tie
- Live animal labels
- Appropriate documentation

Appropriate density for shipping is ~5 fish per liter

Fish Shipping - Supplies

Materials Needed:

- ThermoSafe box
- Secondary containment bag
- Cubitainer
- Absorbent bench towels
- fish water
- Ammonia binder
- **Heat packs**
- **Rubber bands or zip tie**
- Live animal labels
- Appropriate documentation

Fish Shipping - Supplies

Materials Needed:

- ThermoSafe box
- Secondary containment bag
- Cubitainer
- Absorbent bench towels
- fish water
- Ammonia binder
- Heat packs
- Rubber bands or zip tie
- **Live animal labels**
- **Appropriate documentation**

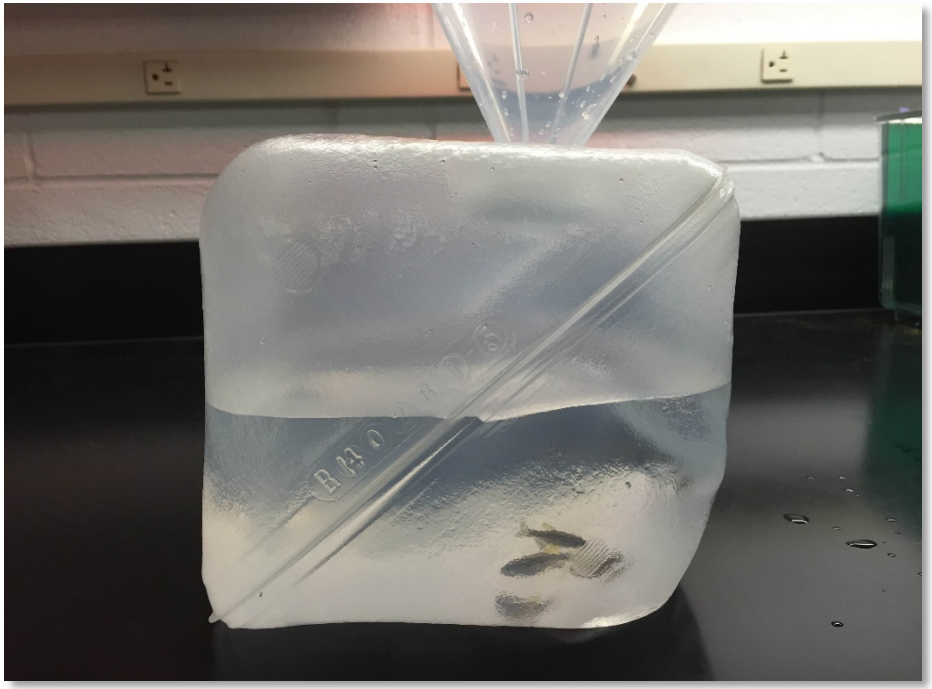
This almost entirely depends on the country of origin and destination

WORLD COURIER®
A SERVICE NO ONE ELSE CAN DELIVER

Fish Shipping - Procedure

1. Add ammonia binder to fish water
2. Fill the cubitainer no more than 1/3 full using funnel
3. Add fish and seal lid tightly

1 part water to 2 parts air space!!



Fish Shipping - Procedure

4. Place absorbent bench towel inside containment bag within the box

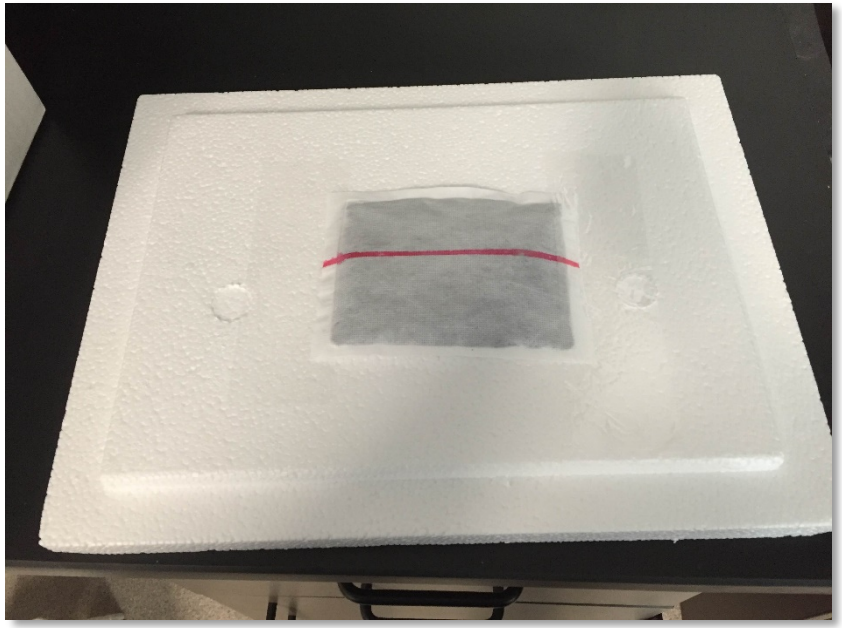
5. Nest cubitainer inside bag

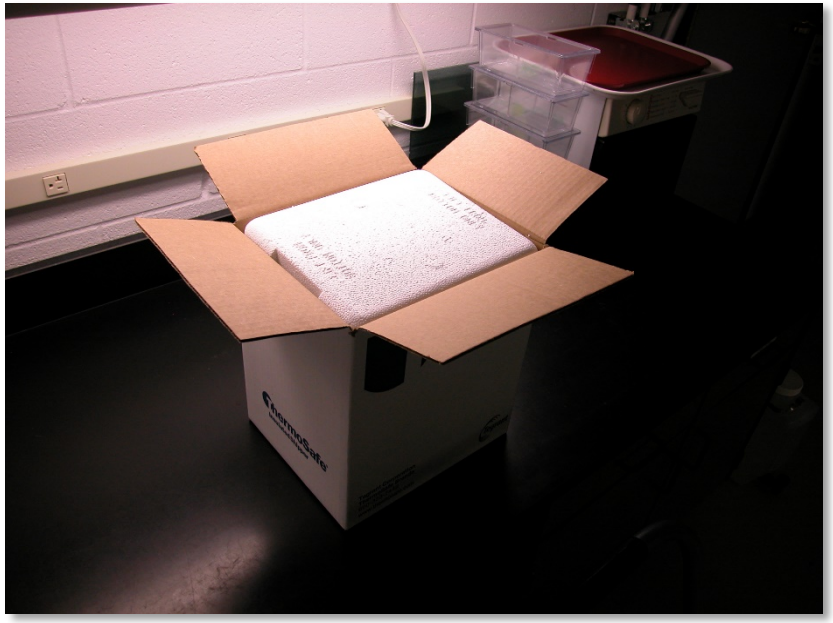
Fish Shipping - Procedure

6. Twist the top of bag, knot, and fasten with zip tie
7. Fill any additional space in box with non-absorbent packing material or bagged/cubitainers of water

Fish Shipping - Procedure

8. Affix heat pack to lid, making sure it can't come in contact with water
9. Fit Styrofoam lid on to box, place copy of documents inside and seal





Fish Shipping – Procedure

Notes

- Separate fish to be shipped for several days prior to shipment for observation
- Withholding feed the morning of shipment can help maintain water quality
- Some countries require a second or third set of documents to be fastened to the outside of the box in addition to the copy within the carton to be delivered to various agencies during transit
- Always label and declare that you are shipping live animals!

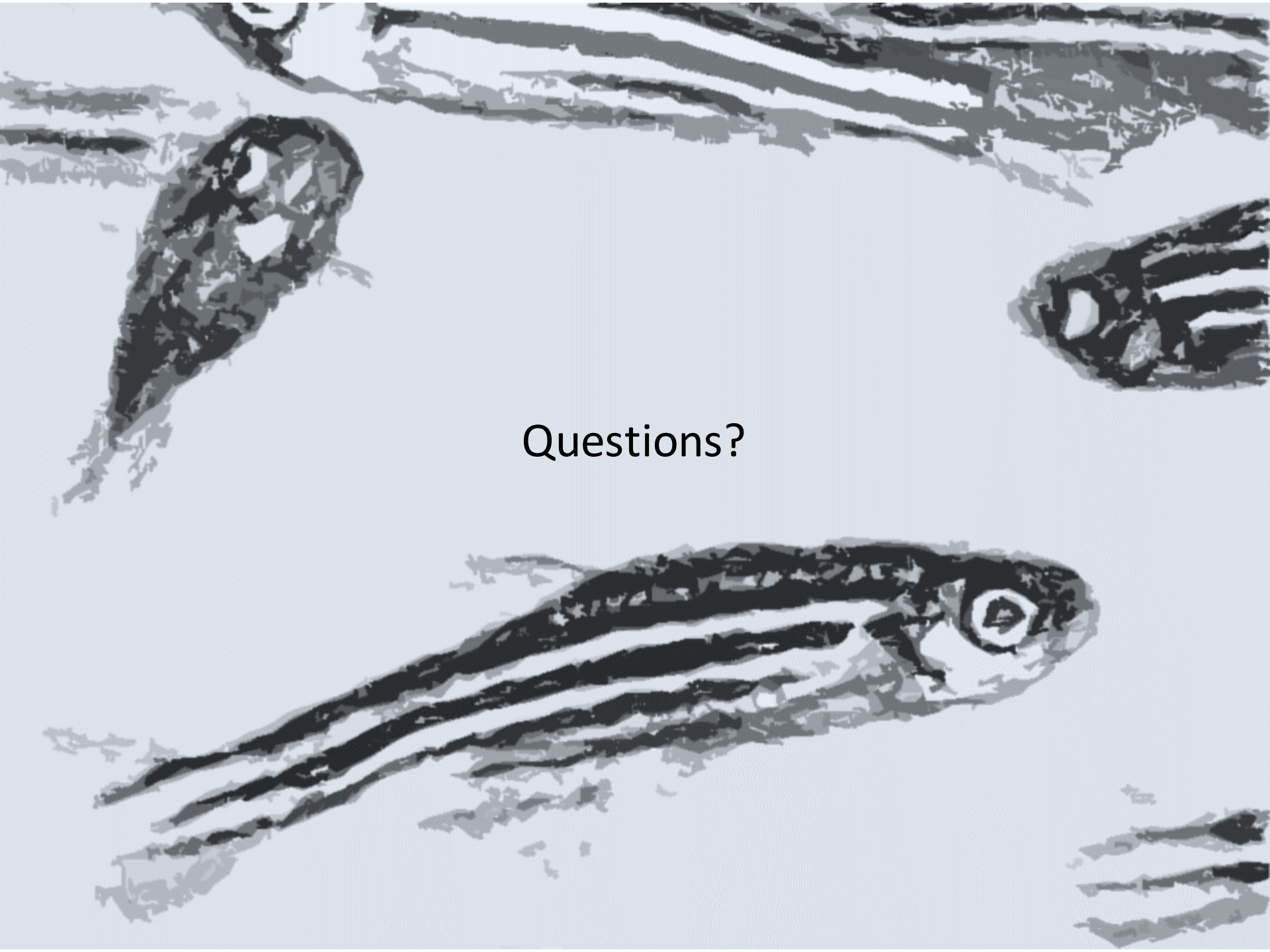
Embryo Shipping – Procedure

The procedure for shipping embryos remains the same, but the containers and solutions are different.

In place of fish water and ammonia binder, use sterile fish water or embryo media w/methylene blue

In place of cubitainers, use 250 ml tissue culture flasks

- Fill flasks w/150-200ml EM
- Pipette up to 100 embryos per flask
- It is recommended that embryos be bleached


Questions?