Genetic Colony Management

Carrie Barton

Sinnhuber Aquatic Research Laboratory

Oregon State University

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What are the goals?

- Understand how to detect common problems and prevent them
- Develop line maintenance strategies that:

    1) encourage positive attributes and

    2) discourage negative attributes

- Prevent contamination – Are your lines what you say they are?
- Robust colony that can reliably support research

Outline

Identify the common problems:

Inbreeding depression
founder effect
Bottleneck
genetic drift
contamination

Symptoms:

Health
Fecundity
Growth Rates
Survival

Mitigation Strategies:

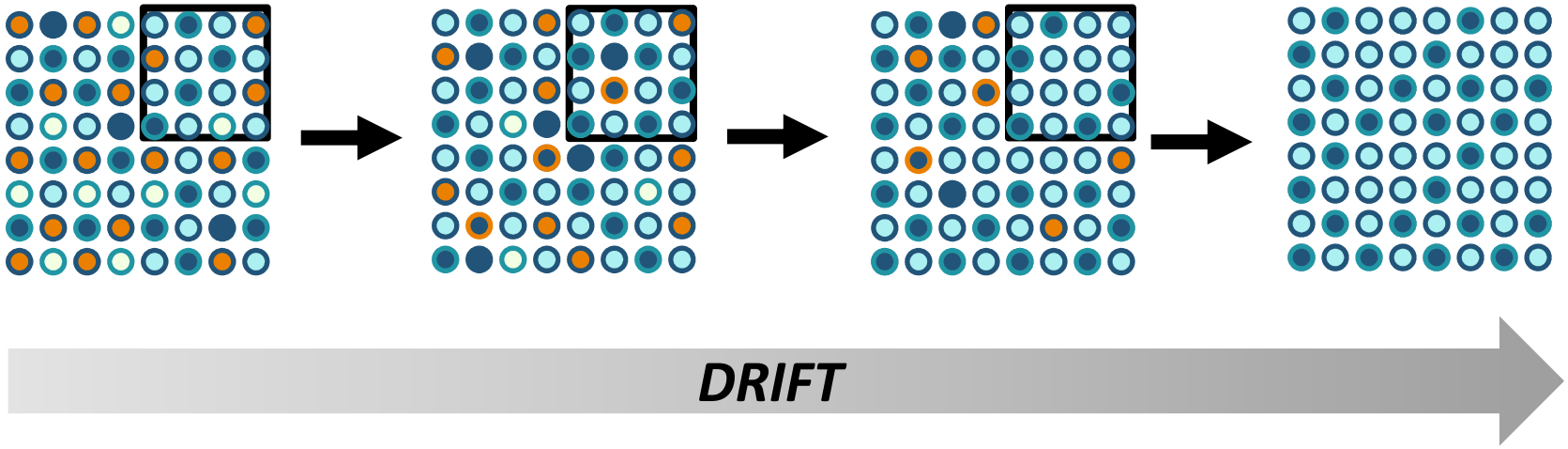
Wild type maintenance strategies
Mutant maintenance strategies
3 to 1 rule
Reducing generation turn over (f#)
Reducing sibling pairings

Inbreeding Depression

Inbreeding depression is the reduced biological fitness in a given population as a result of **inbreeding** - ie., breeding of related individuals. *Biological fitness refers to its ability to survive and reproduce itself.*

Sometimes referred to as “bottlenecking”.

Inbreeding Depression



Happens Slowly

- Limited contributors over multiple gens
- Lack of quality assessment
- Results in drastic reduction in diversity

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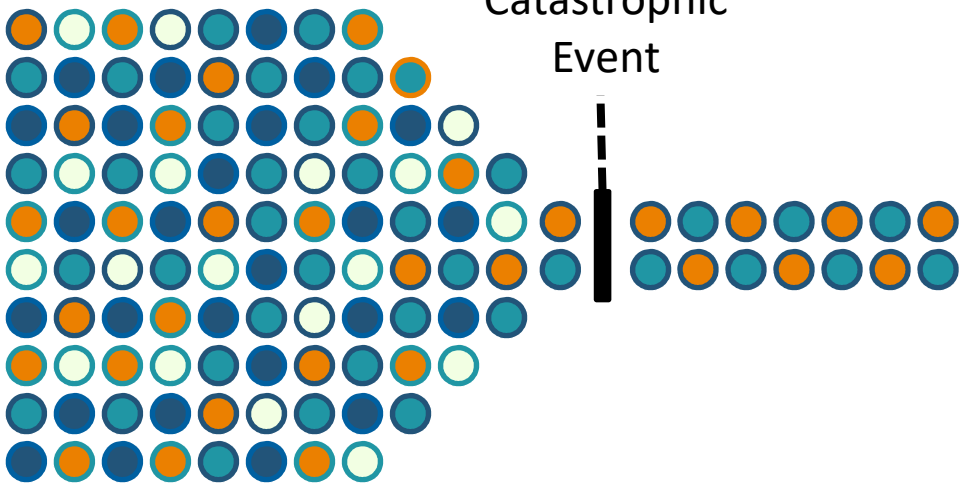
- Lack of quality assessment
- Results in drastic reduction in diversity

Inbreeding Depression - Considerations

- This is far easier to address and prevent with mutant/transgenic/ko lines
- Long term maintenance of wild type lines is typically where this occurs and more difficult to address
- Incorporating mitigation strategies before a problem is apparent is ideal

Population Bottleneck

Diverse Population



Reduced genetic contribution in subsequent generations

Happens Rapidly

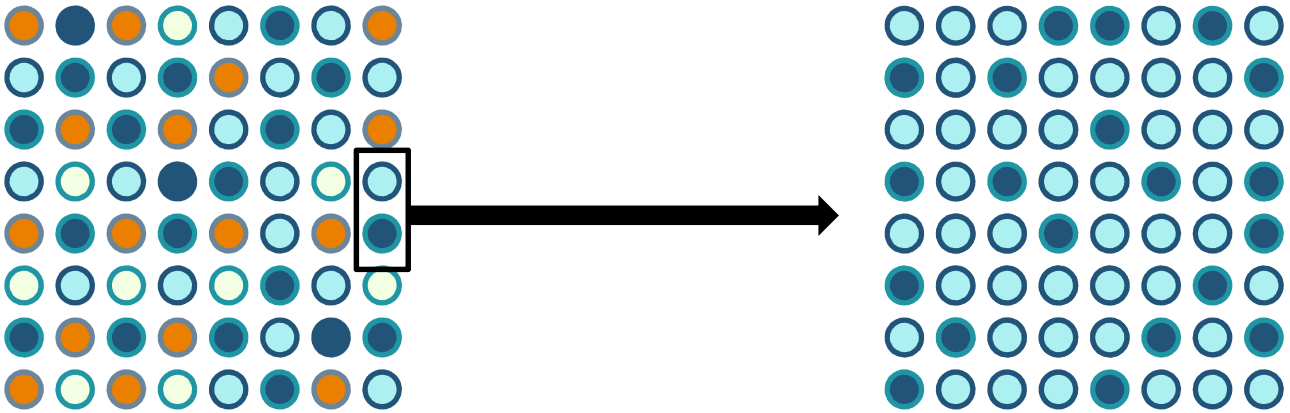
- Nursery survival
- Equipment malfunction
- Die off

Population Bottleneck – Considerations

- Set minimum survivor threshold
    - if overall stock survival drops below a certain percentage, don't use to propagate the line
- Determine the cause of the event
    - address the problem to avoid impact on other stocks/populations
- Redo the stock if possible
    - This saves work in the long run

Founder Effect

The loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population.



Small imports - Line development – Limited availability of fish

Founder Effect - Considerations

- Reliance on adult pairs to establish new lines can exacerbate this problem
  - Adult pairs are often limited in quantity
  - Often the best option for quickest path through quarantine

- Strategies to satisfy both the research goal and the health of line must be incorporated

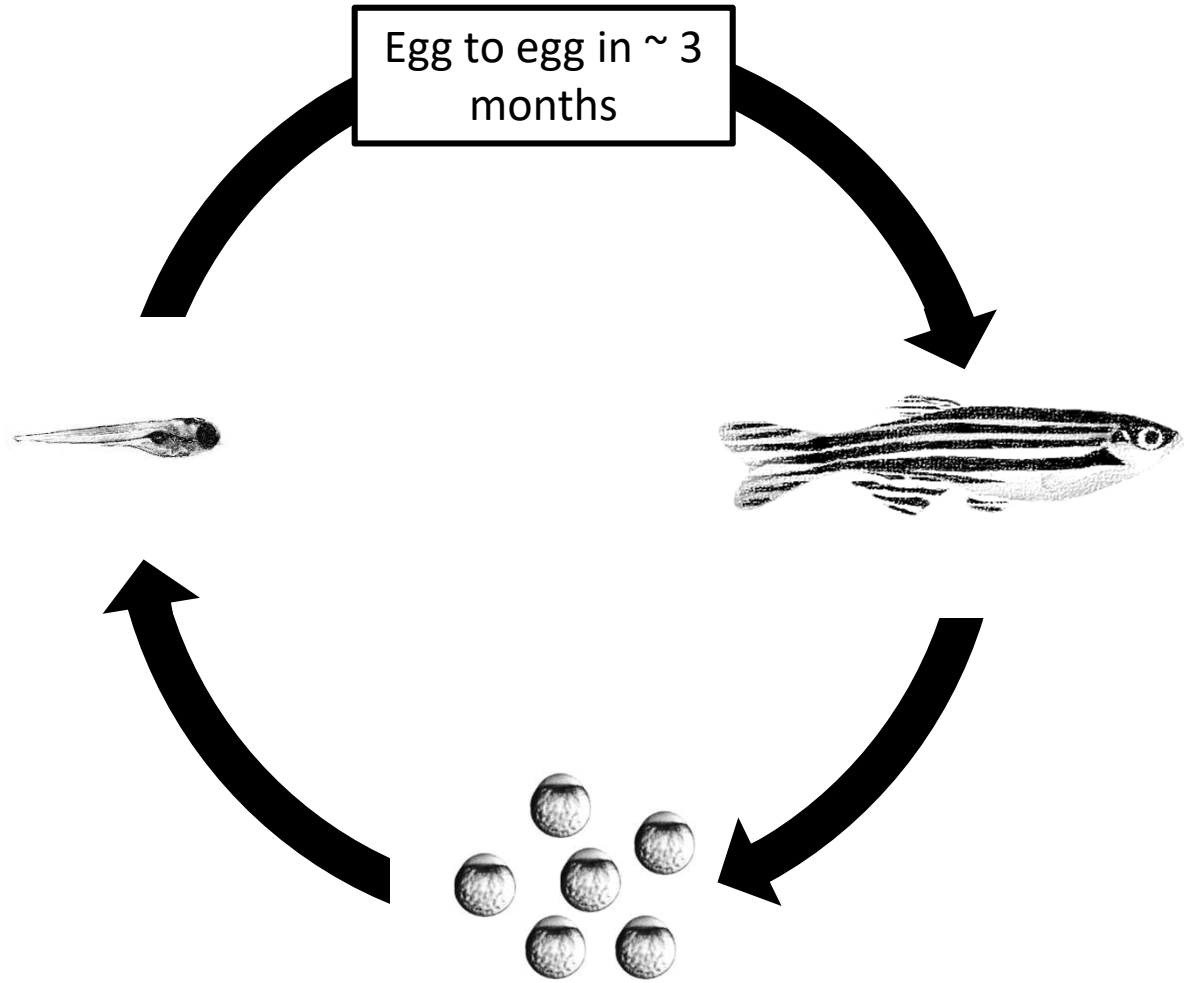
Negative Outcomes and Symptoms

Reduced Growth Rates

Reduced Fecundity

Increased Mortality

Decreased Health



Negative Outcomes and Symptoms

Reduced Growth Rates

Reduced Fecundity

Increased Mortality

Decreased Health

VS

- Decline in successful pairings
- Decline in number of embryos per fish
- Decline in viable embryos

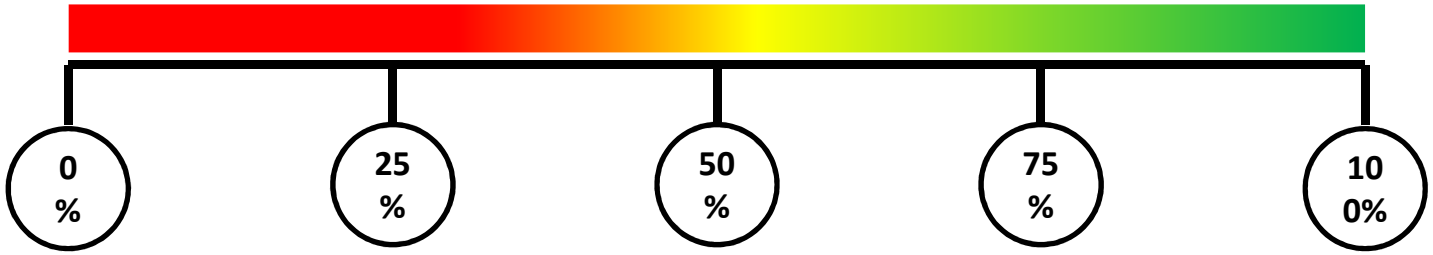
Negative Outcomes and Symptoms

Reduced Growth Rates

Reduced Fecundity

Increased Mortality

Decreased Health



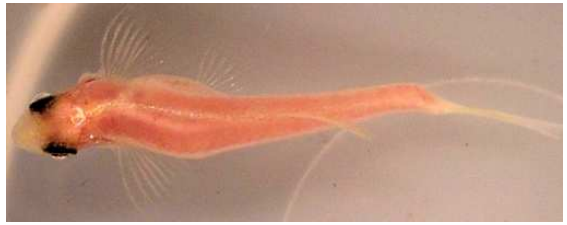
- Survival at early embryonic and larval life stages can be impacted
- Background mutations can arise, many are lethal
- Subtle process

Negative Outcomes and Symptoms

Reduced Growth Rates
Reduced Fecundity

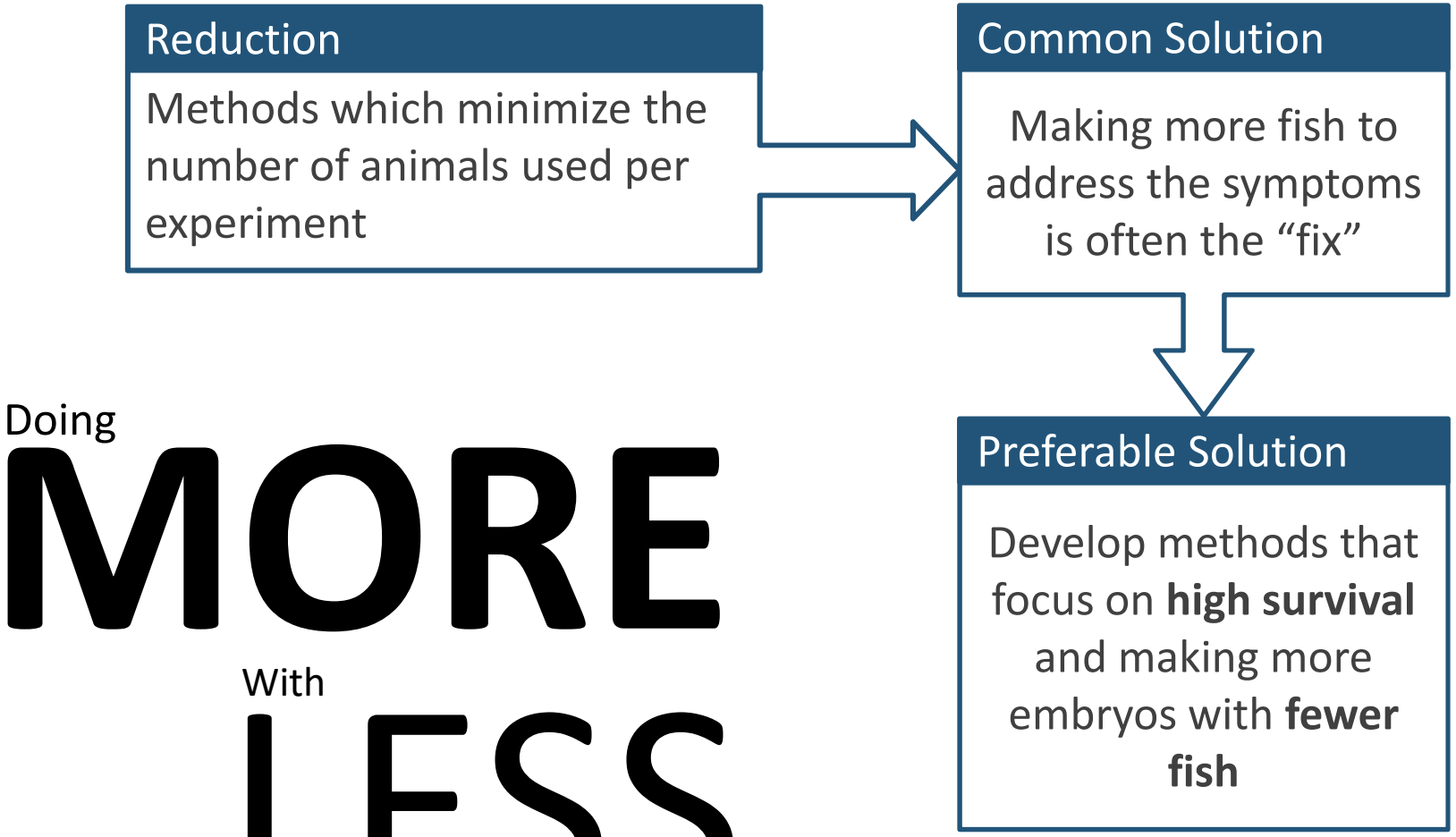
Increased Mortality
Decreased Health

Disease or inbreeding related problem?



Highly inbred populations will likely see increase in background malformations

General health and fitness can decrease = less robust fish



Doing

MORE

With

LESS

Wild Type Maintenance

Strategy should include:

- methods to maximize contributors
- quality assessment step

Example of Long Term Strategy to Maintain AB

Minimum Contribution = 25 male/25 female

-25 pair crosses OR

-25 small group crosses

Clutches screened for:

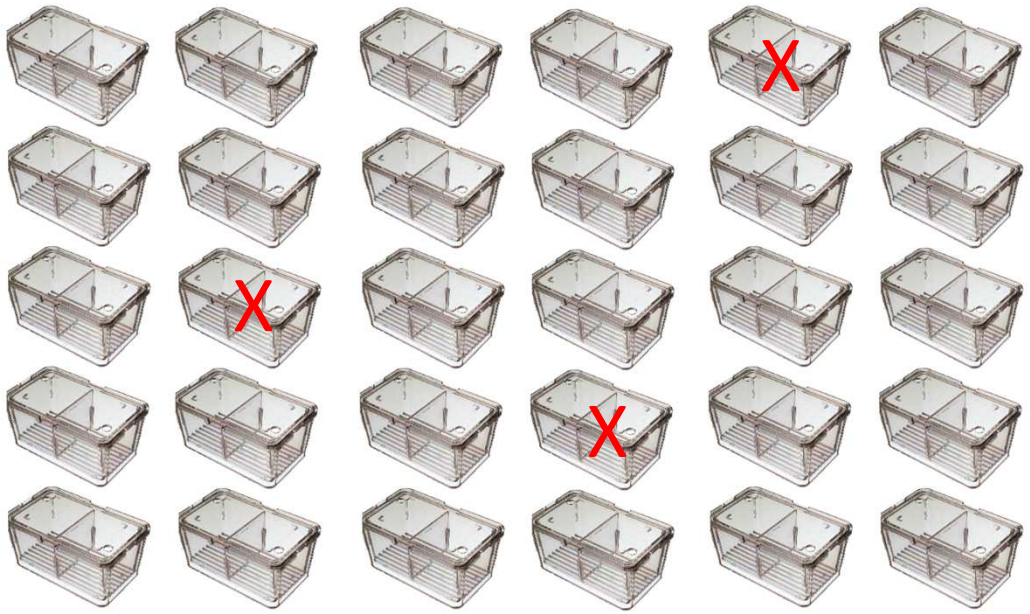
-size

-quality

-fertility rate

Long Term Wild Type Maintenance

Long Term Wild Type Maintenance



- Keep clutches separate to screen for low fertility, low success, or malformations
- Score clutches and eliminate those that do not pass

Quality Assessment – Clutch Scoring

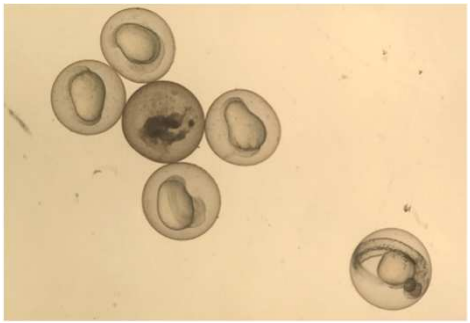
Factors to consider

Number of embryos produced: Is the # embryos per female good?

Fertility: For the number produced, is the fertility rate appropriate?

Necrotic embryos: Are there necrotic or bad embryos present?

Malformations: Are there malformations present?

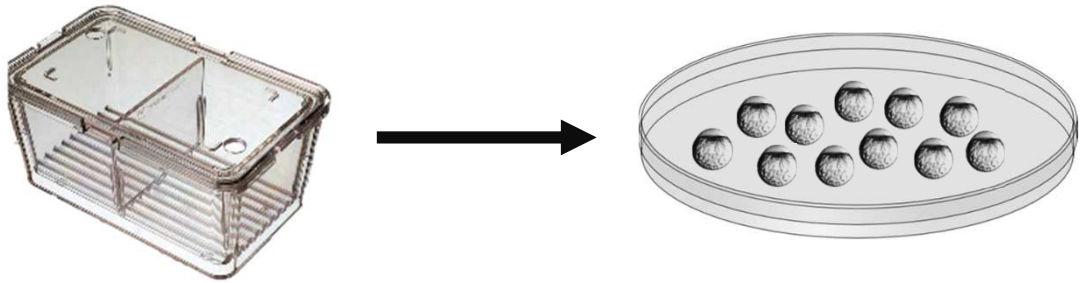


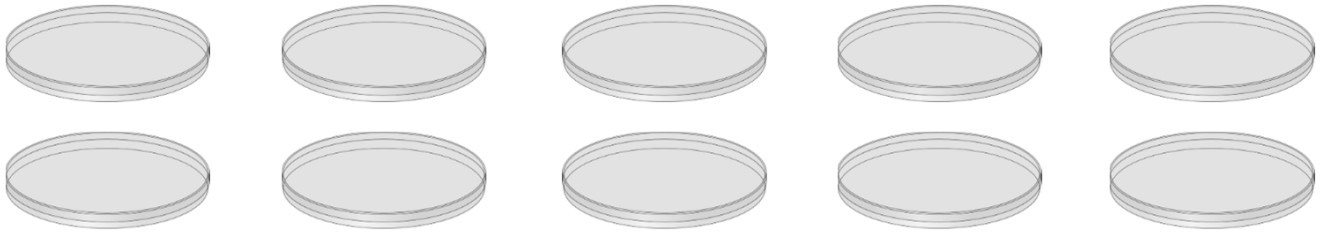
Scoring Rubric

A scoring rubric can standardize how people are assessing the quality of a clutch

Wild Type Next Generation Clutch Scoring			
Scoring system used to pass or fail a clutch of embryos used to create an AB next gen. Pass = 11 points			
Parameters	**Above Average – *3 points***	**Average - *2 points***	**Below Average - *0 points***
Number of embryos per clutch	More than 100 per female	100 per female	Less than 100 per female
Infertile	Less than 5% present	5-10% present	More than 10% present
Necrotic	Less than 5% present	5-10% present	More than 10% present
Malformations	None present	Less than 5% present	More than 5% present
25 clutch minimum is required for an AB next gen. *This can be either pair crosses or small group crosses (2 females/3 males)* *Expect to set up at least 50 pairs/small group crosses to achieve minimum contribution.*			

Reducing Direct Sibling Mating

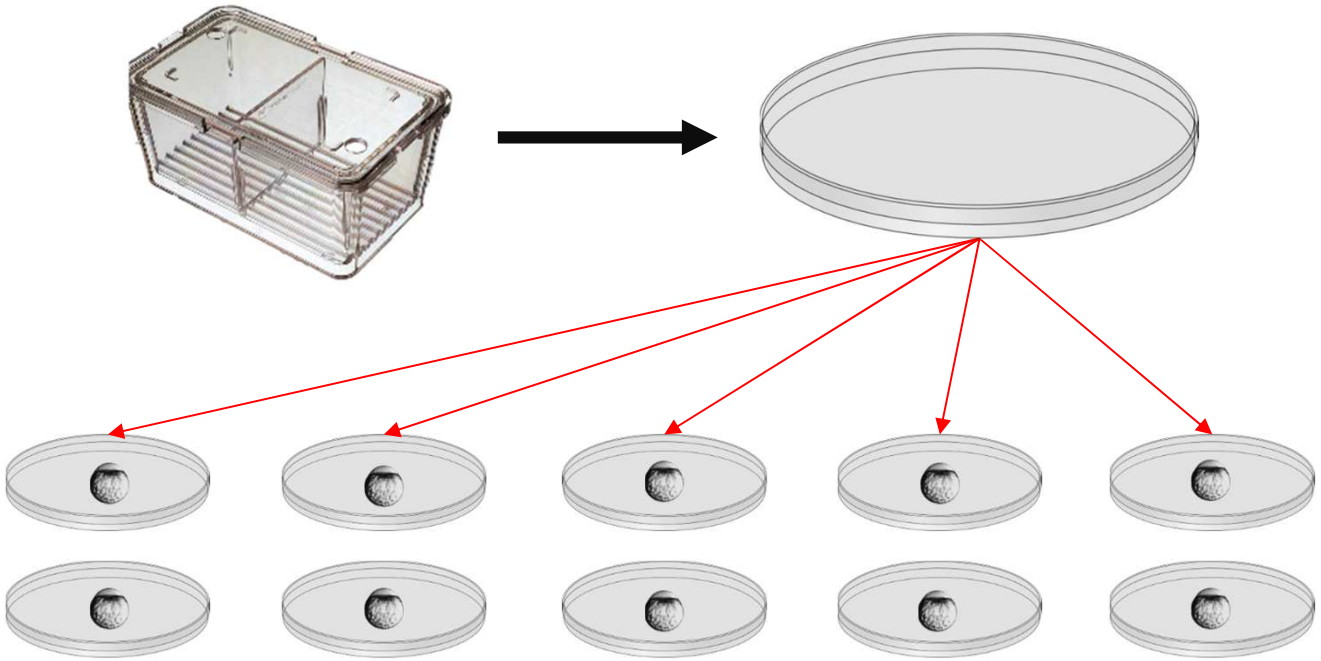




Each individual clutch passing the score, can then be distributed between the desired number of tanks.

Thus creating several individual tanks that don’t share to same parents.

Reducing Direct Sibling Mating



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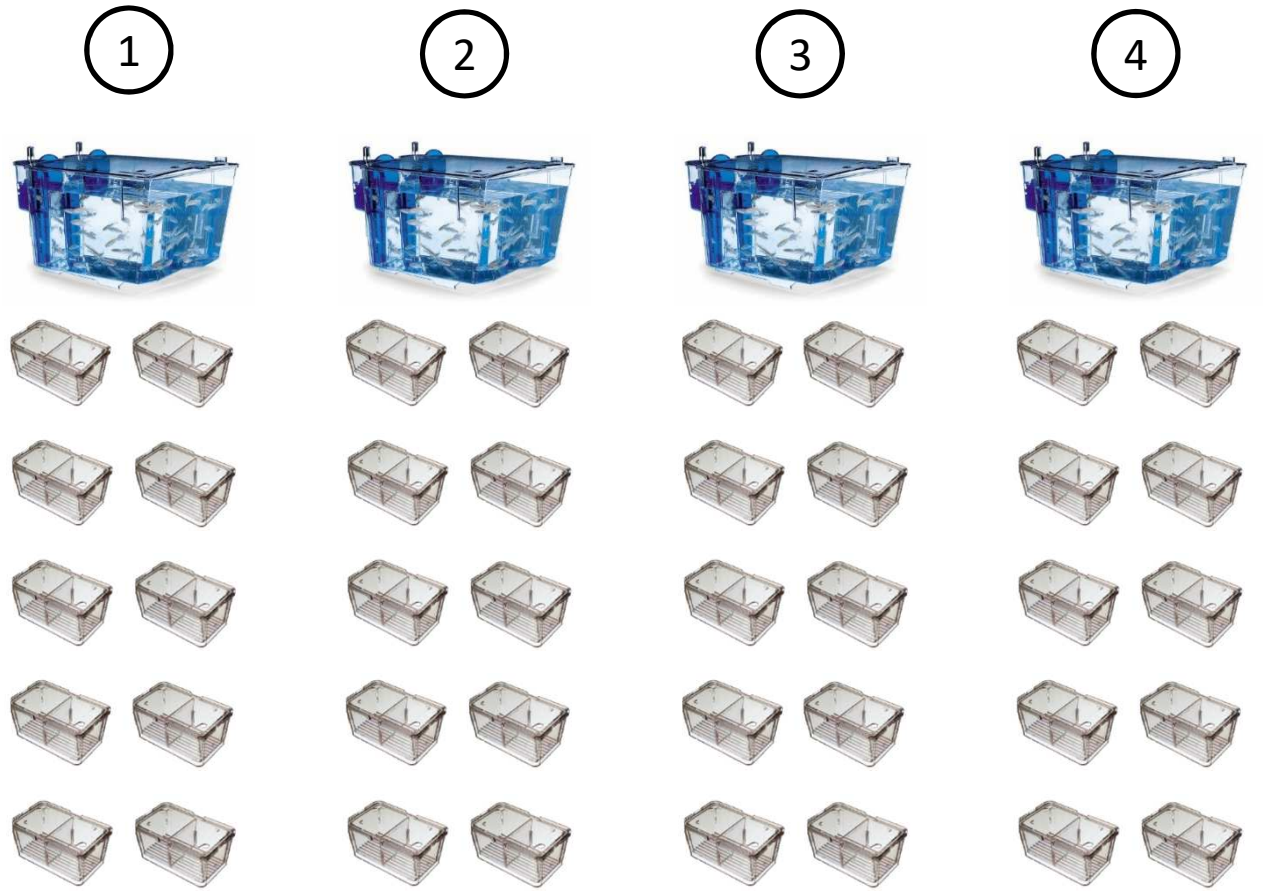
Reducing Direct Sibling Mating – Alt Method



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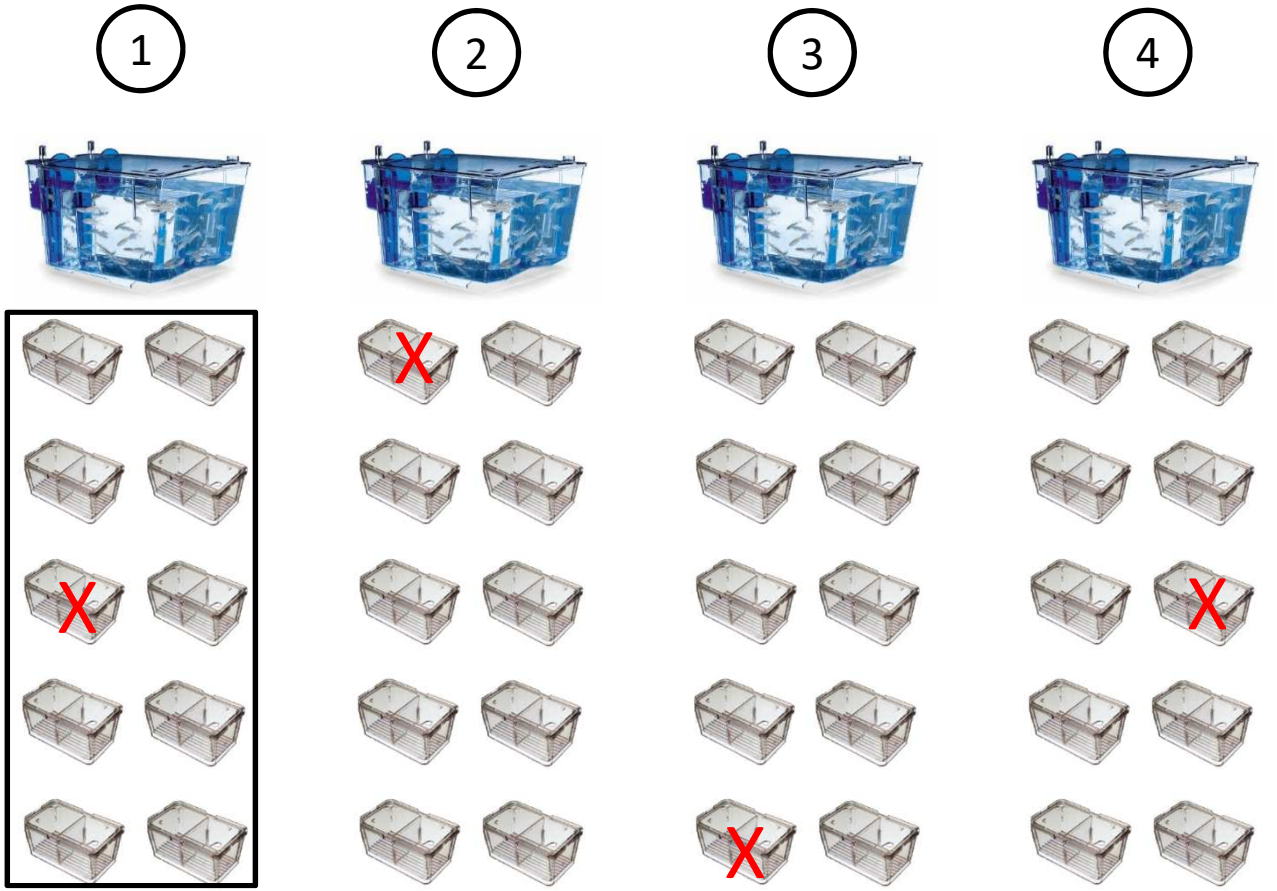

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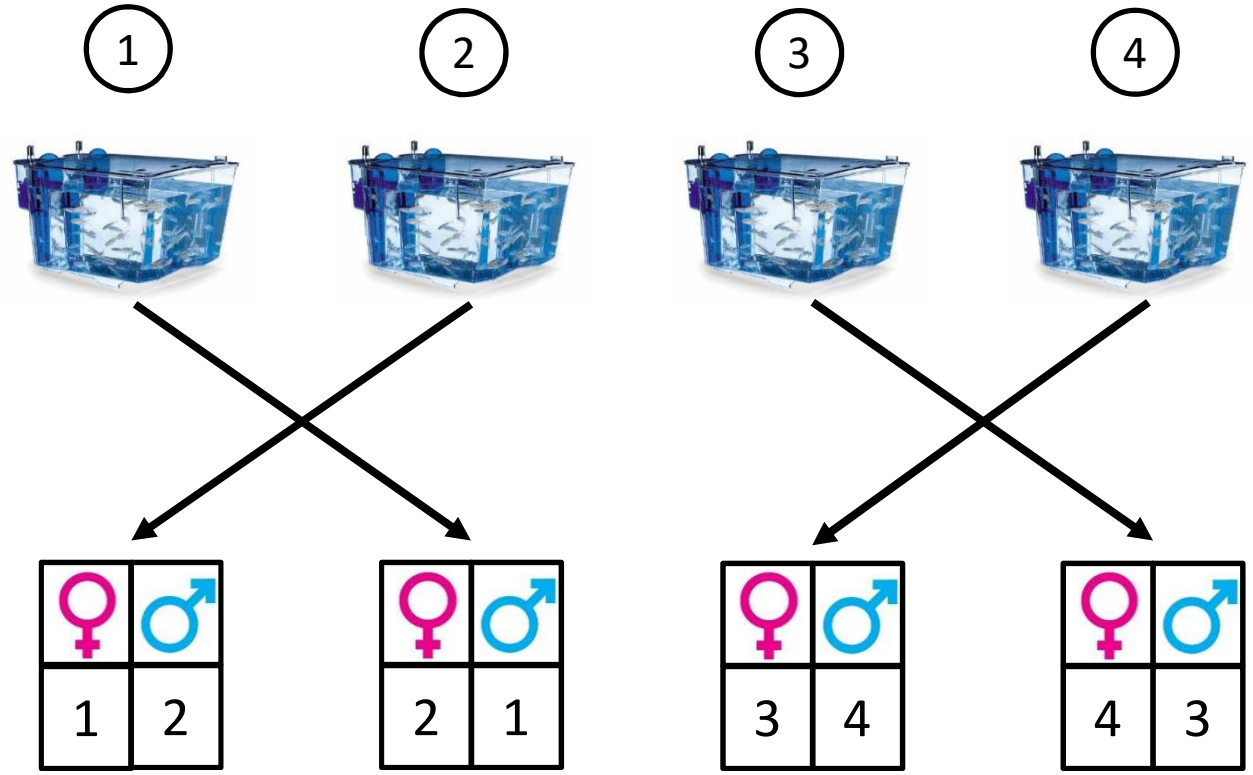


Alternate Strategies for Wild Type Maintenance

Import Method

- Introduce new genetic material
- Reduce potential for inbreeding depression
- Mitigate bottlenecks
- Ensure ability to support research

This is ideal for smaller facilities or facilities That don't have space or staffing to maintain Large volumes of wild type animals.

What is a Reputable Supplier??

Any source for research zebrafish that can provide you the following information:

Disease monitoring and sentinel testing results as well as full disclosure of any background health concerns.

fish	S-SYS-ZF311-pool 3	S-SYS-ZF311-pool 4
Mycobacterium abscessus	-	-
Mycobacterium chelonae	-	-
Mycobacterium fortuitum	-	-
Mycobacterium haemophilum	-	-
Mycobacterium marinum	-	-
Mycobacterium peregrinum	-	-
Pseudoloma neurophilia	-	-

fish	S-SYS-ZF311-pool 5	S-SYS-ZF311-pool 6
Mycobacterium spp.	-	-
Mycobacterium abscessus	-	-
Mycobacterium chelonae	-	-
Mycobacterium fortuitum	-	-
Mycobacterium haemophilum	-	-
Mycobacterium marinum	-	-
Mycobacterium peregrinum	-	-
Pseudoloma neurophilia	-	-

Oversight veterinarian endorsed health certificate.

AQUATIC ANIMAL INTERNATIONAL HEALTH CERTIFICATE

Identification
Contents of Shipment: Live fish adults and embryos
Species: *Danio rerio*
Common name: Zebrafish
Description: This is a tropical, freshwater aquarium species commonly used as a model organism for genetic and biomedical research.
Age: Adults (2 years)
Number: Adults (6)

Place of Production
Aquaculture Facility: Weill Cornell Medical College
Address: 510 East 70th Street
New York, NY 10021
United States of America

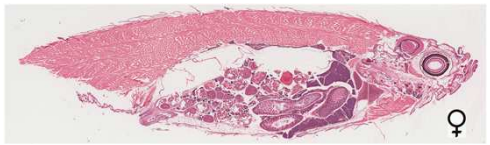
Destination
Name: Aristedes Arrenberg, Ph.D.
Address: Abteilung Entwicklungsbiologie
Institut für Biologie 1
Universität Freiburg
Hauptstrasse 1
Freiburg, D-79104 Germany
Phone (office): 49-761-203-8343
Importing Country: Germany
Shipping Company: World Courier

Declaration
I, the undersigned, certify that the live fish in the present consignment have as their place of production a scientific aquaculture facility that maintains *Danio rerio* exclusively, accepts additions only from other scientific institutions, has been subjected to continual fish health surveillance, and are free from all reportable pathogens causing the diseases listed in the World Organization for Animal Health (OIE) 2007 *Aquatic Animal Health Code*, 10th Ed. [Epizootic haematopoietic necrosis, Infectious haematopoietic necrosis, *Oncorhynchus masou* virus disease, Spring viraemia of carp, Viral haemorrhagic septicemia, Channel catfish virus disease, Viral encephalopathy and retinopathy, Infectious pancreatic necrosis, Infectious salmon anaemia, Epizootic ulcerative syndrome, Bacterial kidney disease (*Renibacterium salmoninarum*), Enteric septicaemia of catfish (*Edwardsiella ictaluri*), Piscirickettsiosis (*Piscirickettsia salmonis*), Gyrodactylosis (*Gyrodactylus salaris*), Red sea bream iridoviral disease, White Sturgeon iridoviral disease, and Koi herpesvirus disease]. The zebrafish were born and bred for laboratory testing only.

Exporting Country: United States of America
Date of Issue: 2/19/13
Certifying Official: Christine Liggi; DVM, DACLAM, [illegible] Accredited Veterinarian (NY) # 001220
Address: 1275 York Ave, Box 370, NY, NY, 10065

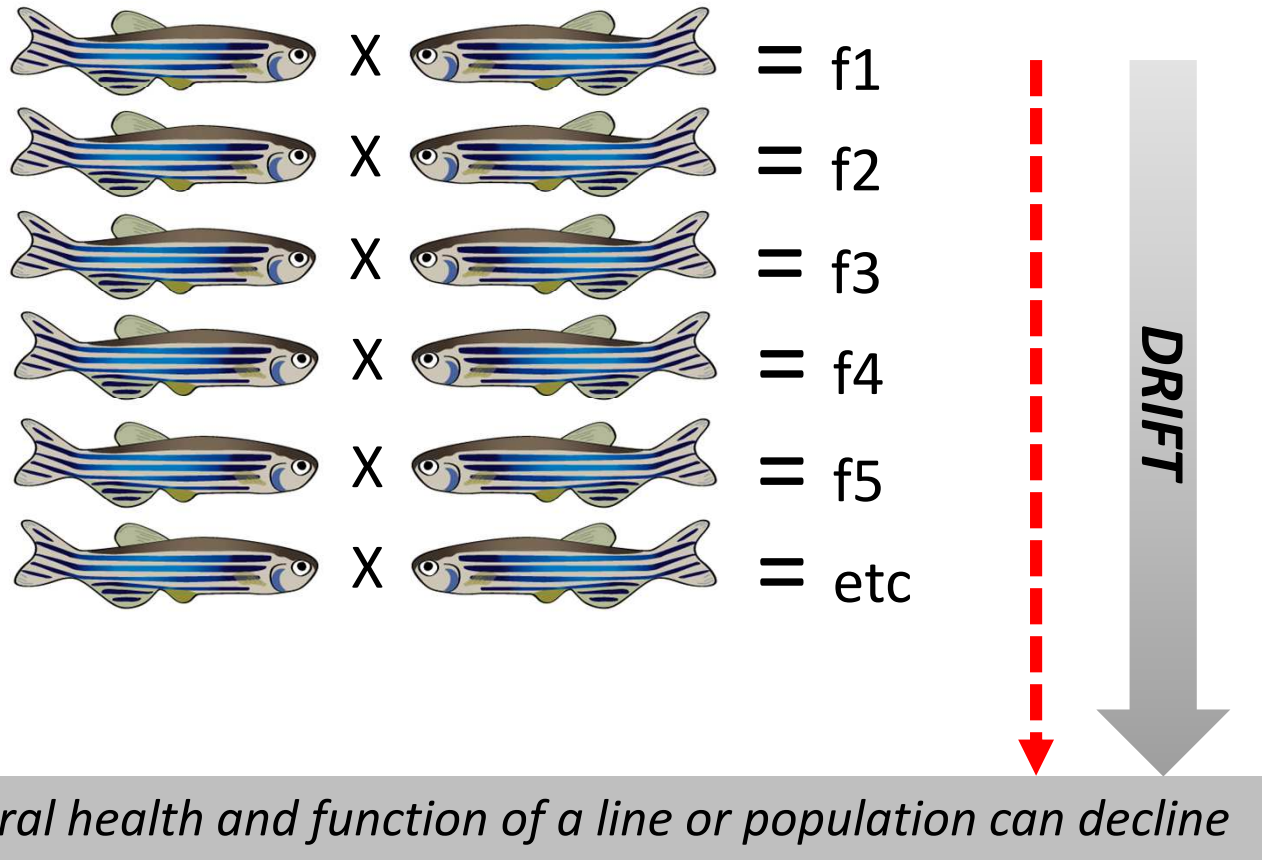
Signature: _____ Date: 2/19/13

Information about propagation techniques used to ensure genetic integrity.



http://zebrafish.org/health/index.php#

Minimizing Number of Generations



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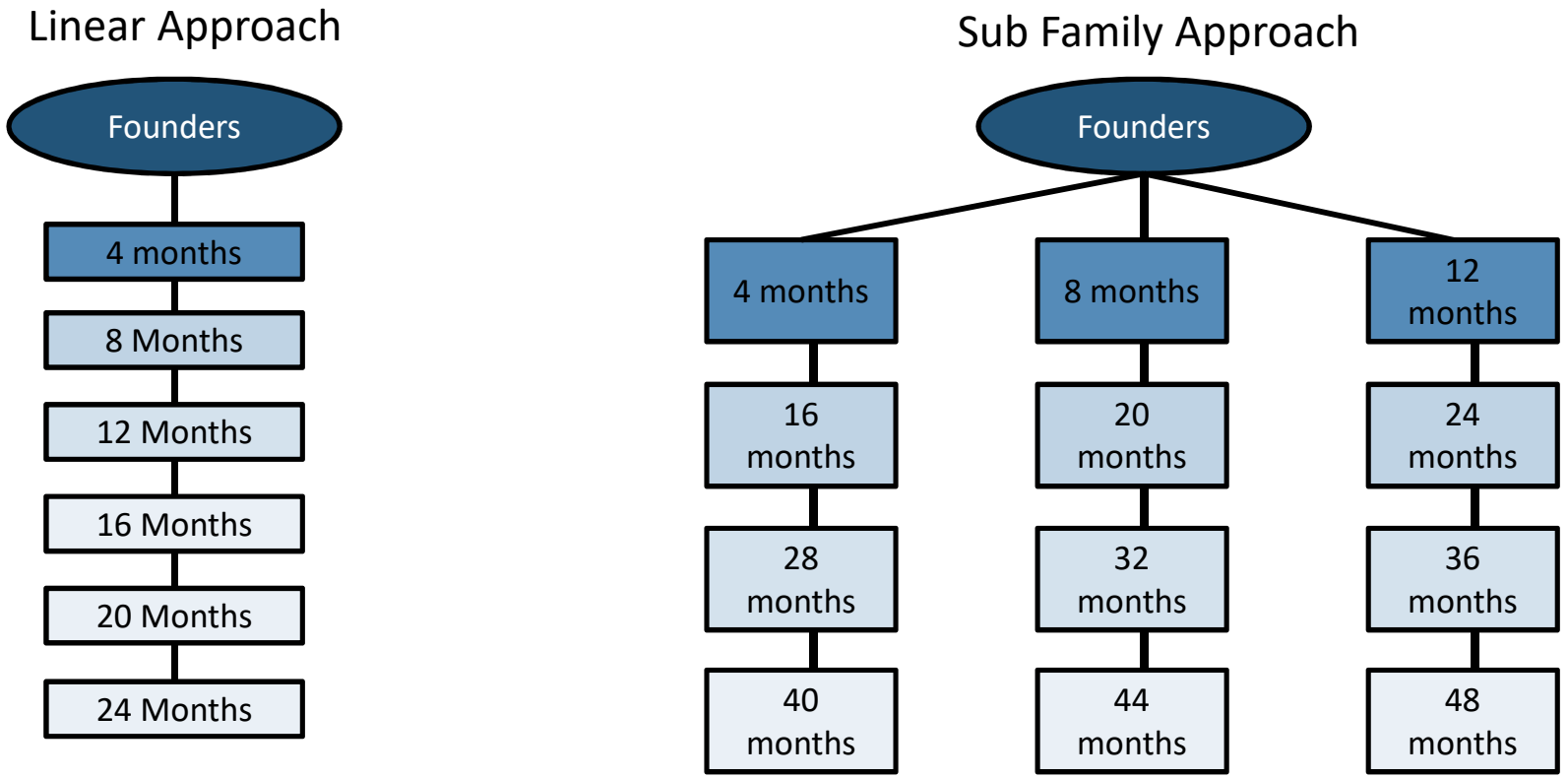
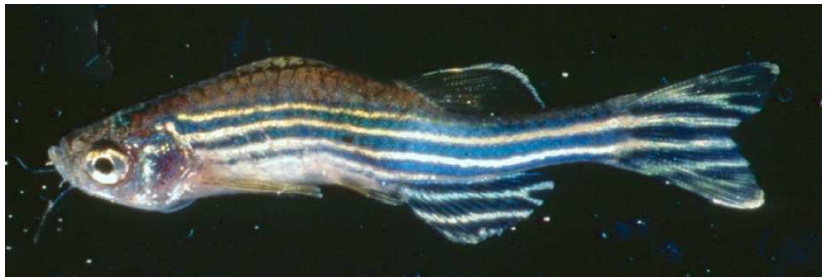


Out crossing

How important is routine outcrossing of lines?



www.zebrafish.org

Disease or excessive inbreeding??

- Laboratory reached out for help with a “disease” problem
- All animals from a certain line had developed overt spinal malformations
- Subset of all effected tanks were sampled and sent for histological analysis
- No disease process was found

3 to 1 Rule

Founder (F0)  X

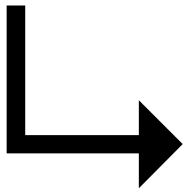
Offspring are 1st Incross

(F1)  X

Offspring are 2nd Incross

(F2)  X

Offspring are 3rd Incross



Out Cross and Rederive Homozygous

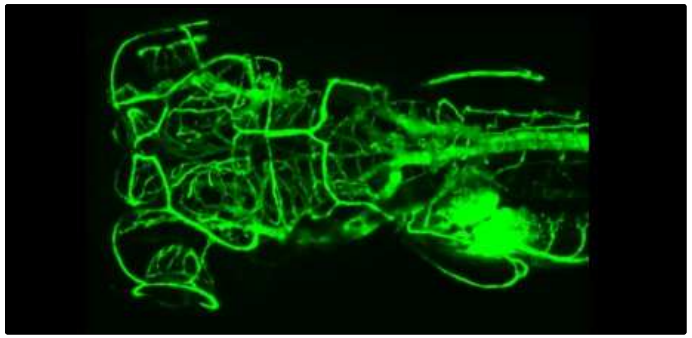
Strategies for Mutant and Transgenic Lines

3 to 1 Rule:

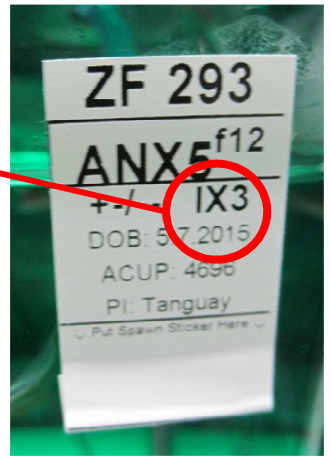
For every 3 in cross (IX) events, outcross (OX) line to wild type

OX requires staff to be 100% proficient in gender identification of zebrafish

IX and OX status can be tracked in a database and IX # can be incorporated into tank labels



https://science.nichd.nih.gov/confluence/display/pgd/Brant+Weinstein+Lab



A robust outcrossing program can yield better embryo production!!

Set standards for minimum contributors for each next generation

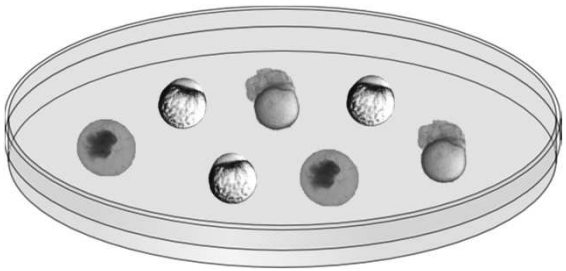
Minimum of 10 paired crosses or groups must contribute to next gen
but, the more the better!

Strategies for Mutant and Transgenic Lines

Screen offspring for robust mutation and minimal background problems

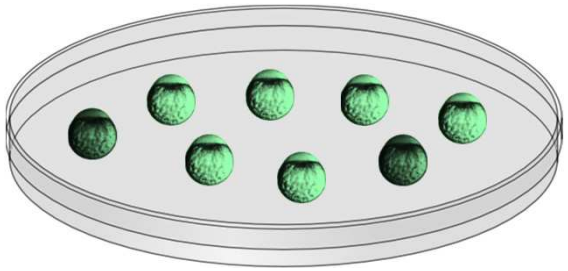
All Lines

Confirm General Quality
Eliminate clutches with problem



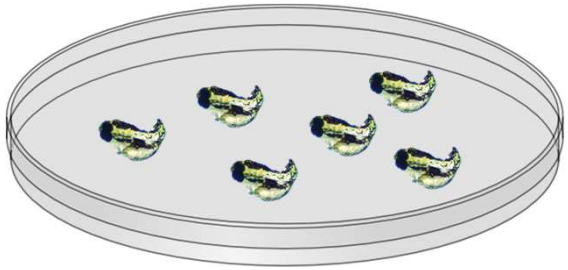
Transgenic Lines

Check for intensity of signal
Eliminate embryos with poor signal



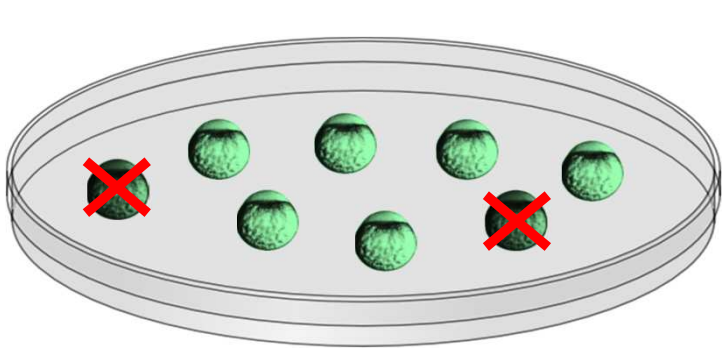
Mutations

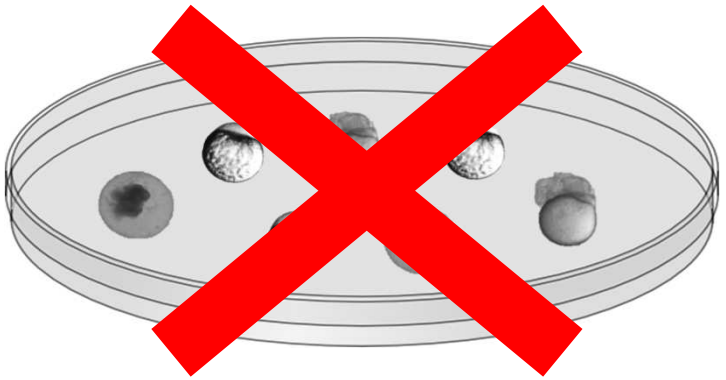
Confirm presence of correct mutation either visually or via PCR



Strategies for Mutant and Transgenic Lines

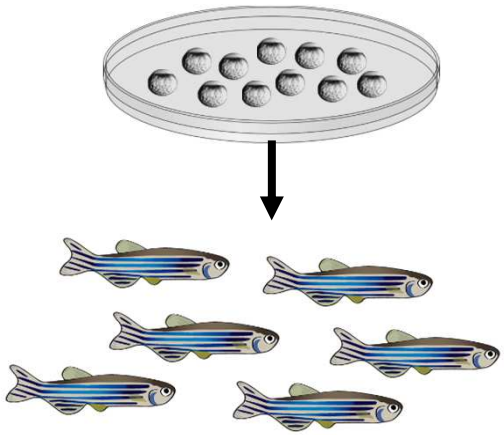
Always eliminate clutches/embryos that fall outside the set standard





Newly Developed Lines

Establishing a line via CRISPR



Best carrier is ID'd *This is often 1 fish!*

OX to wild type

Often 2-3 fish

Heterozygotes ID'd & IX'd

ID HMZ for Research

IX IX IX
IX IX IX
IX IX IX

The process inherently requires relying on less than ideal propagation methods.

-founder effect
-direct sibling mating
-repeated in cross events

Pool multiple spawn events

In addition to research support:
-plan early large scale OX event
-incorporate the use of non-sibling animals

Newly Developed Lines – Fact Finding

Information gathering prior to line creation can mitigate problems and eliminate wasted time.

<u>Questions to ask:</u>

-What preliminary experiments have been done?

-What were the outcomes?

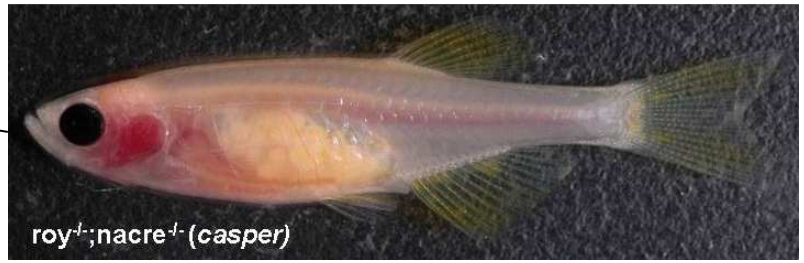
-Is the disrupted gene known to cause problems with reproduction or survival in other model organisms.

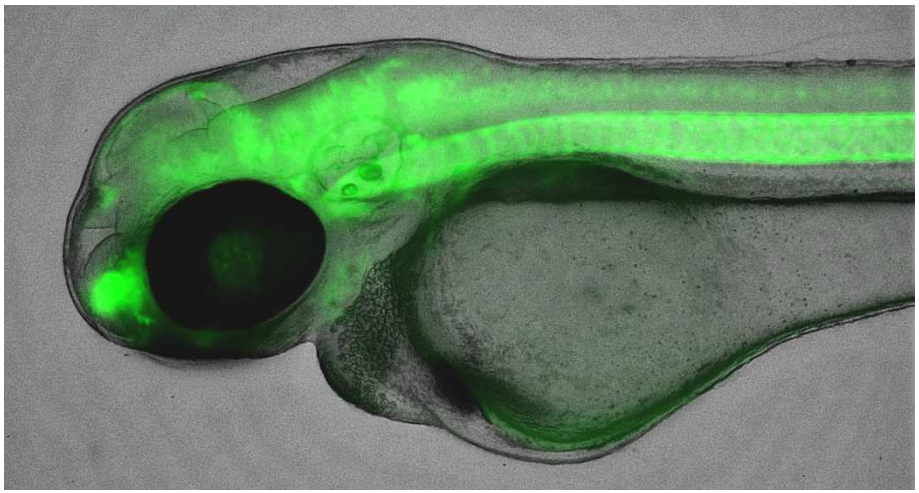
-Is manipulating this gene lethal in other model organisms?

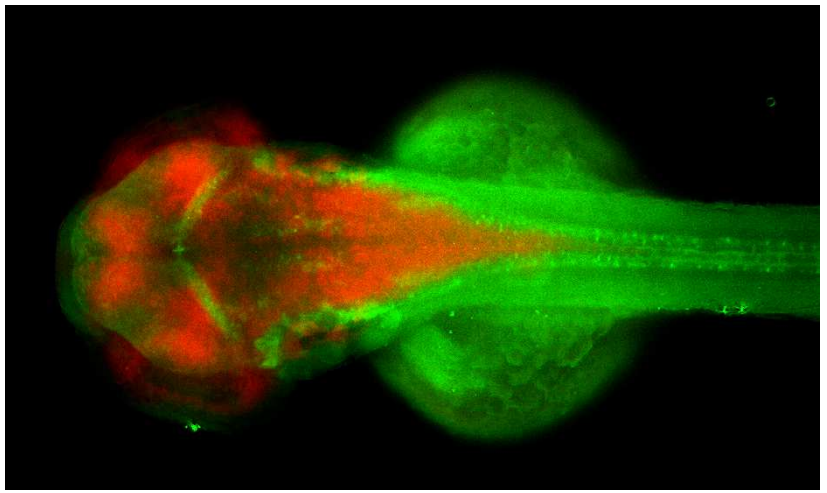
-What is the intended use of the line?

Color Mutant Background Lines

An increasing number of transgenic lines are being maintained on casper and other colorless backgrounds due to the increased imaging capabilities of transparent lines.







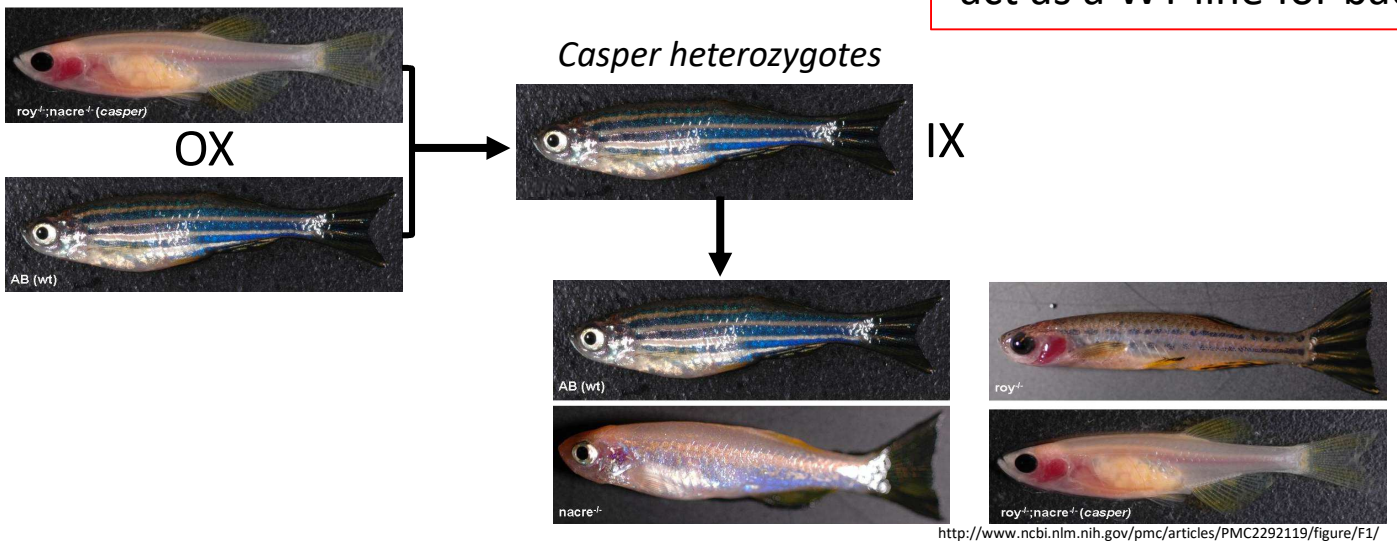
Transgenic photos courtesy of PHD candidate Gloria Garcia

Color Mutant Background Lines

Outcross mutants on casper background to a casper line!

A properly managed casper line can act as a WT line for back/outcrossing

Standard Casper Outcross Event



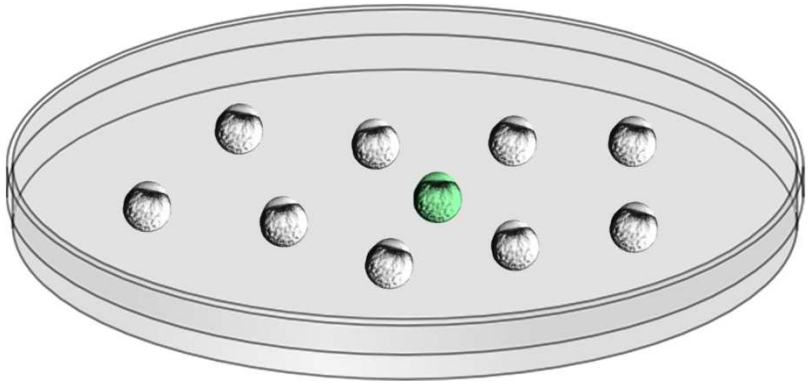
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2292119/figure/F1/

<u>Challenges of adding a transgene to the mix</u>

- Only ¼ of IX het offspring will be casper
- Not all will be transgenic

Contamination

Routinely check wild type lines to ensure no contamination has occurred

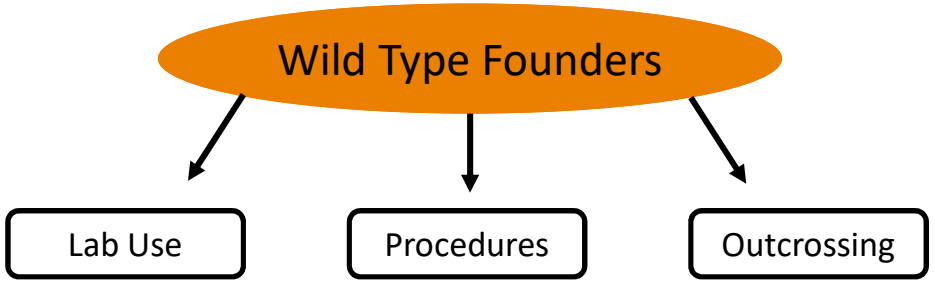


- Devastate research
- Create lack of confidence in the facility SOPs
- Cost time and money

Dedicated Outcross Fish

Employ dedicated outcross tanks to minimize fish to fish contact from mutant/transgenic lines to your wild type populations.

This can also be done for experiment or procedure practice fish



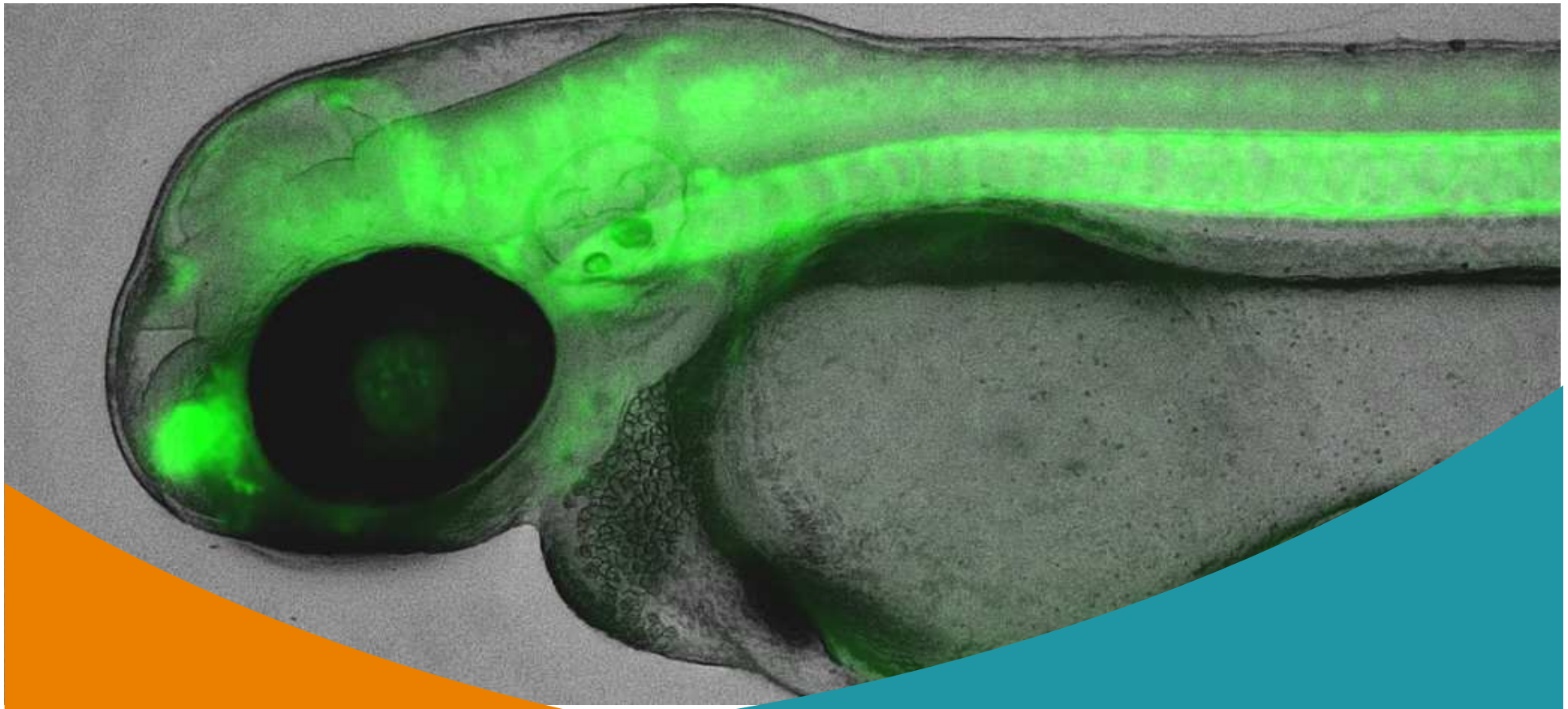
Strategies for All Lines

Cryopreservation

Can be used to back up resources in the event of bottleneck event or inbreeding depression

Reset the colony in the event of a disaster

Rescue lost diversity



Thank You

TANGUAY LAB
THINKING OUTSIDE THE TANK

Oregon State University

EHSQ
new discoveries