Genetic Colony Management

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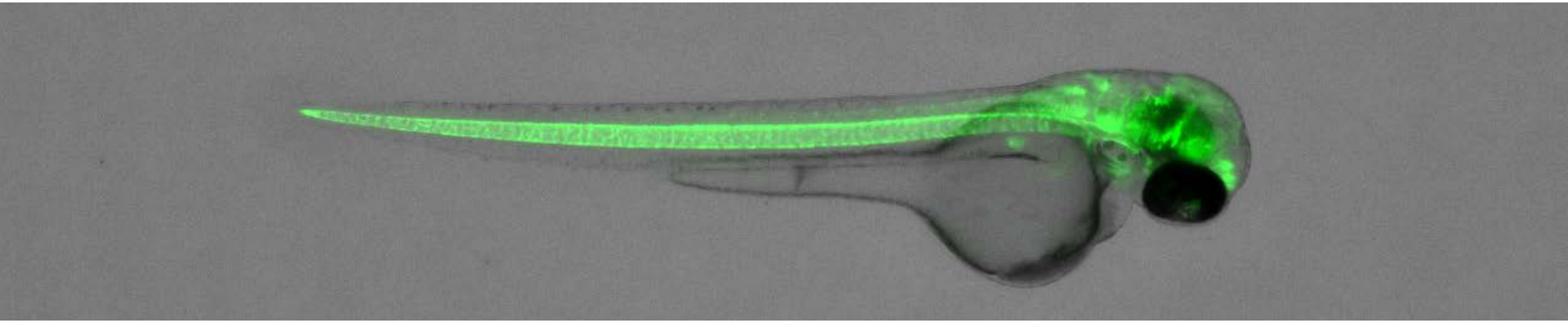


5th Annual International Zebrafish Husbandry Course
Buguggiate, Italy 2016

Outline

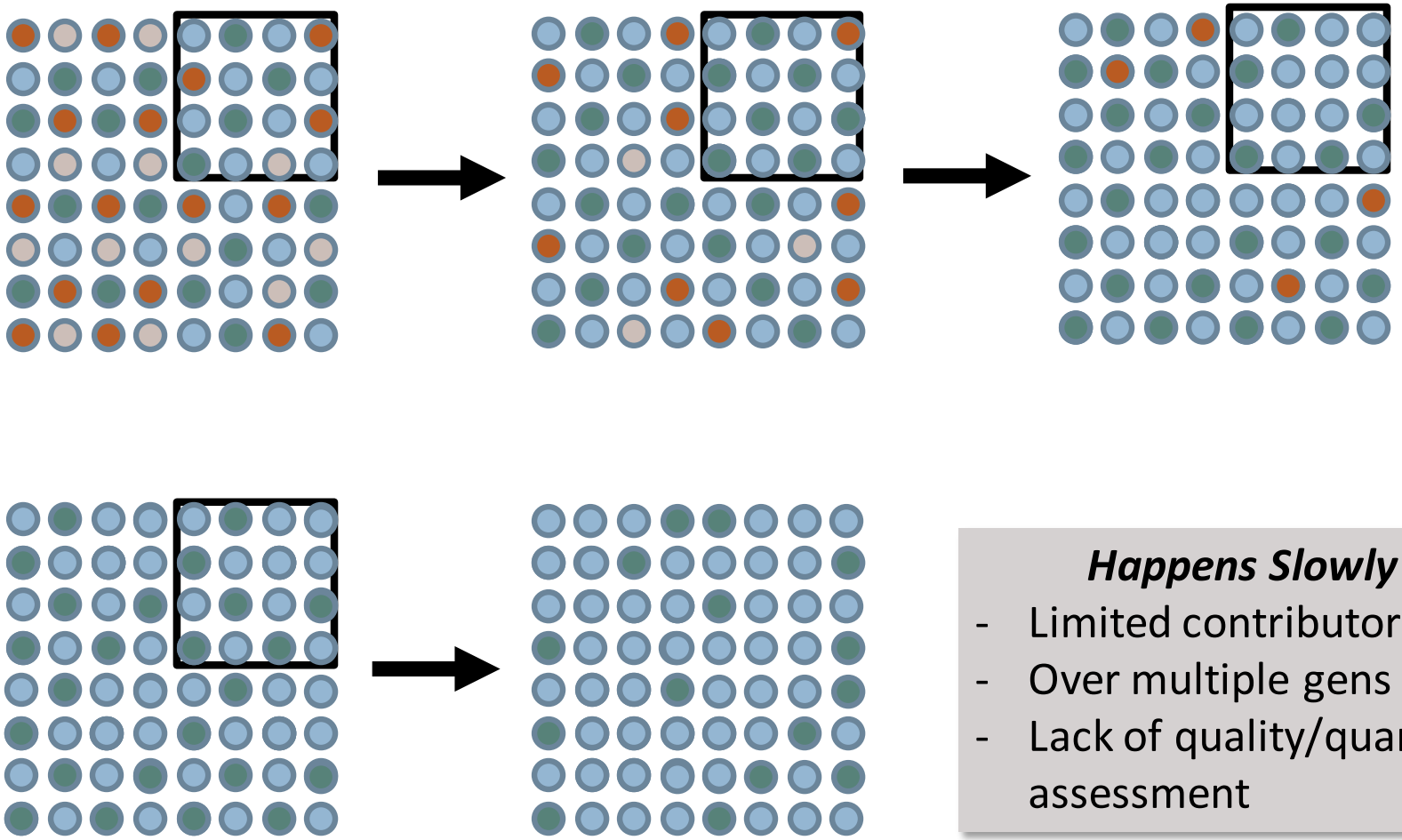
- Inbreeding vs. Bottlenecking
- Negative effects of inbreeding
- In crossing (IX) and out crossing (OX)
- Line maintenance strategies
  - Wild type, transgenic, mutant, CRISPR
- Importing lines
- General recommendations

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”.

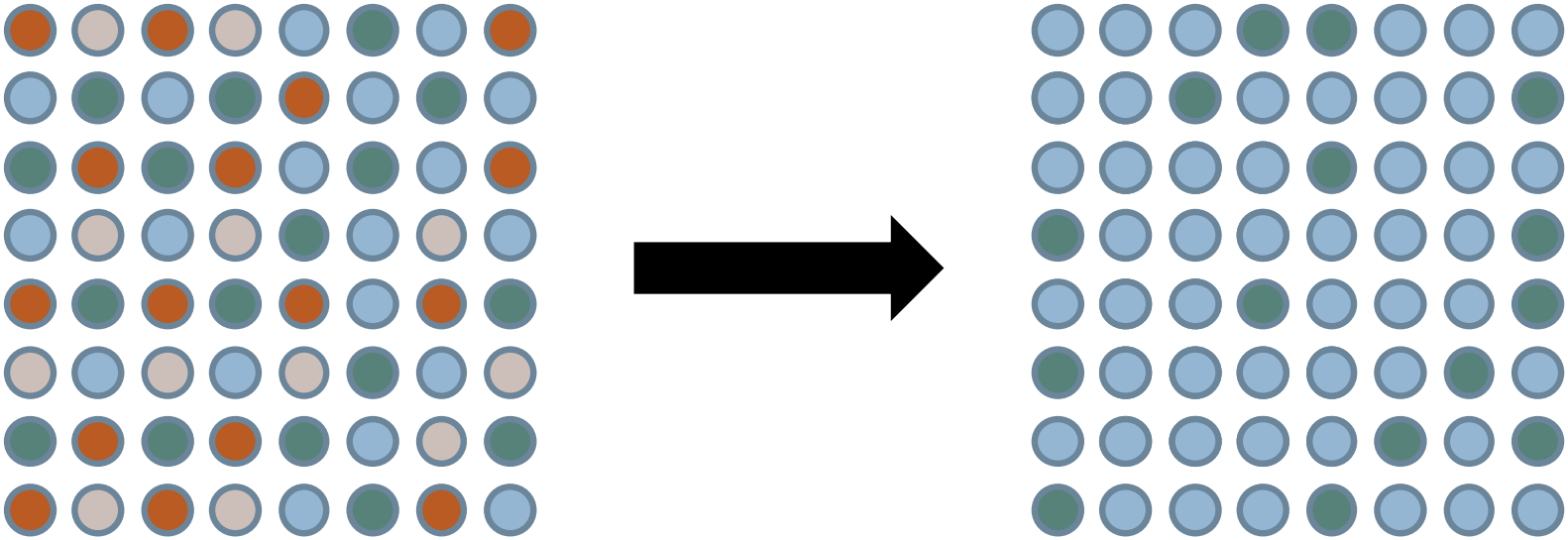
Inbreeding Depression



Happens Slowly

- Limited contributors
- Over multiple gens
- Lack of quality/quantity assessment

Inbreeding Depression

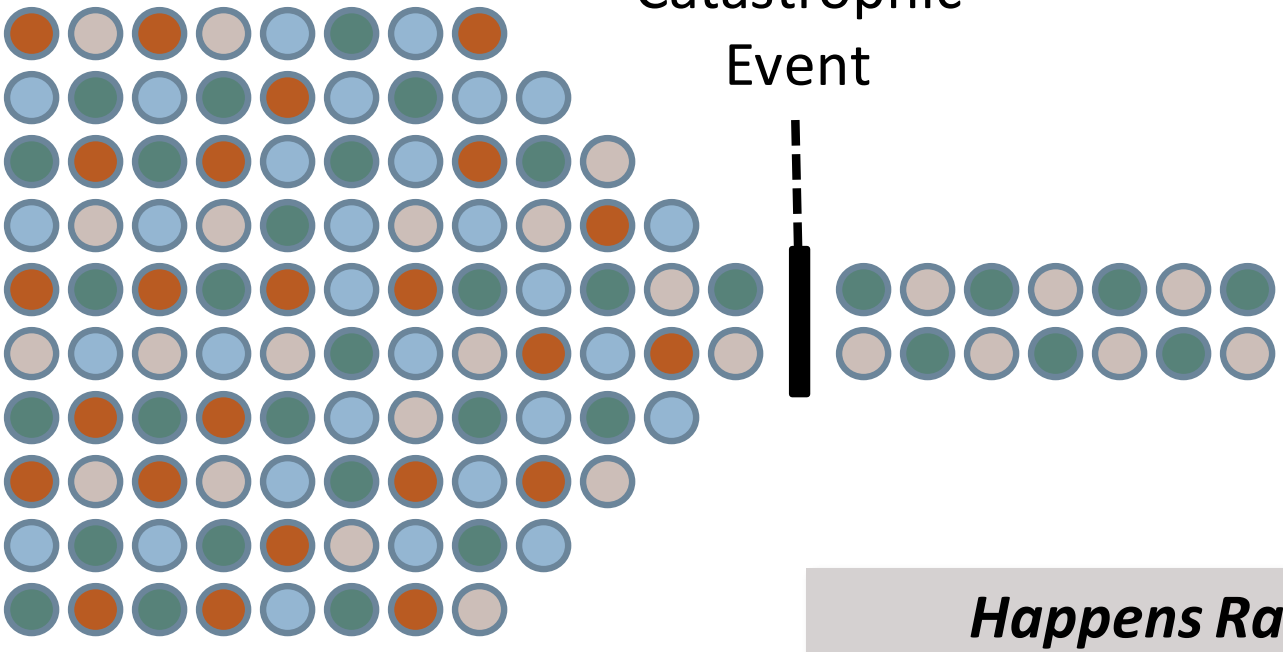


Because inbreeding depression happens slowly, and over many generations, it is difficult to know if your population is effected before you start seeing problems.

Genetic Bottlenecking

Diverse Population

Catastrophic Event



Reduced genetic contribution in subsequent generations

Happens Rapidly

- Nursery survival
- Equipment malfunction
- Small imports

Negative Effects of Excessive Inbreeding

- **Reduced growth rates**
  - ***Longer time from egg to egg***
- Reduced fecundity
  - *no eggs = no research = no grant money*
- Increased mortality at early age stages – Embryos and juveniles
  - *Less fish survive = less fish for supporting research*
- Impact on Health
  - *Inbreed fish are not healthy fish*

Egg to egg in ~ 3 months

Negative Effects of Excessive Inbreeding

- Reduced growth rates
  - *Longer time from egg to egg*
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VS

Negative Effects of Excessive Inbreeding

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  - *Longer time from egg to egg*
- Reduced fecundity
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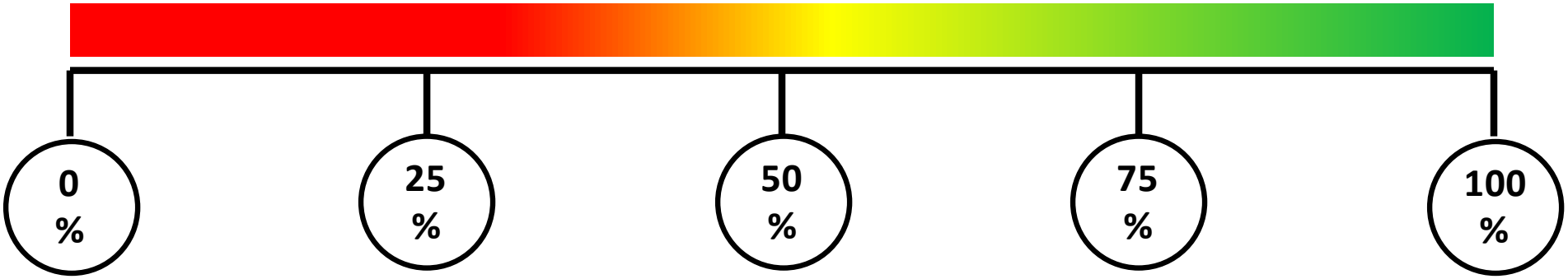

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- Increased mortality at early age stages – Embryos and juveniles
  - *Less fish survive = less fish for supporting research*
- **Impact on Health**
  - ***Inbreed fish are not healthy fish***

www.zebrafish.org

Disease or excessive inbreeding??

Positive Effects of Good Genetics

- Better survival and growth
  - *Egg to egg easier and faster*
  - *Improved mutants (transgenic)*
- Better embryo production
  - Fewer fish can produce more embryos
- Improved reproducibility of research outcomes
  - *Improves the validity of the model*
- Healthier fish
  - *Frequently out crossed fish are healthier*

Positive Effects of Good Genetics

3Rs of Research

- Better Survival
- Higher embryo production with fewer fish

- Healthier Fish
- Better Survival
- Better Growth

Definition of Terms

In Crossing

Reproduction of offspring from the breeding of organisms that are closely related genetically.

Crossing fish from the same line/or same sibling group

Tg(fli1:EGFP)y1

X

Tg(fli1:EGFP)y1

Out crossing

The practice of introducing unrelated genetic material into a breeding line. It increase genetic diversity.

Crossing fish from one line or sibling group with fish from another

WT (AB)

X

Tg(fli1:EGFP)y1

Definition of Terms

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

Consequences of Excessive Inbreeding

General health and function of a line or population will usually decrease

Strategy for Wild Type Maintenance - Outline

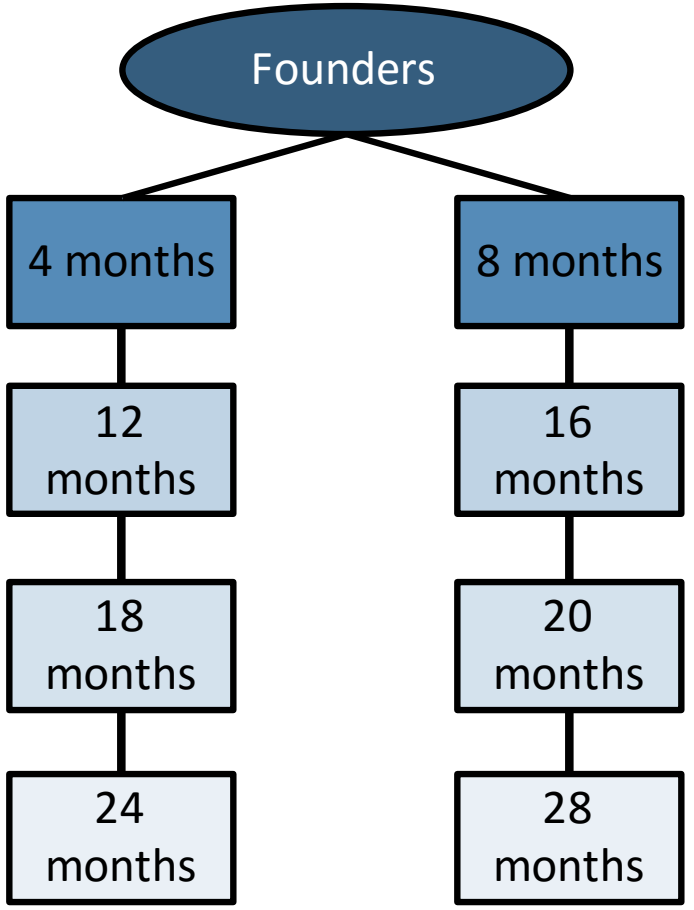
- Next generation frequency
- Reducing sibling pairings
- Minimum contributors
- Assessing quality/spawn success of contributors (grading clutches-size, fertility, malformations)

Minimizing Number of Generations

Linear Generations

VS

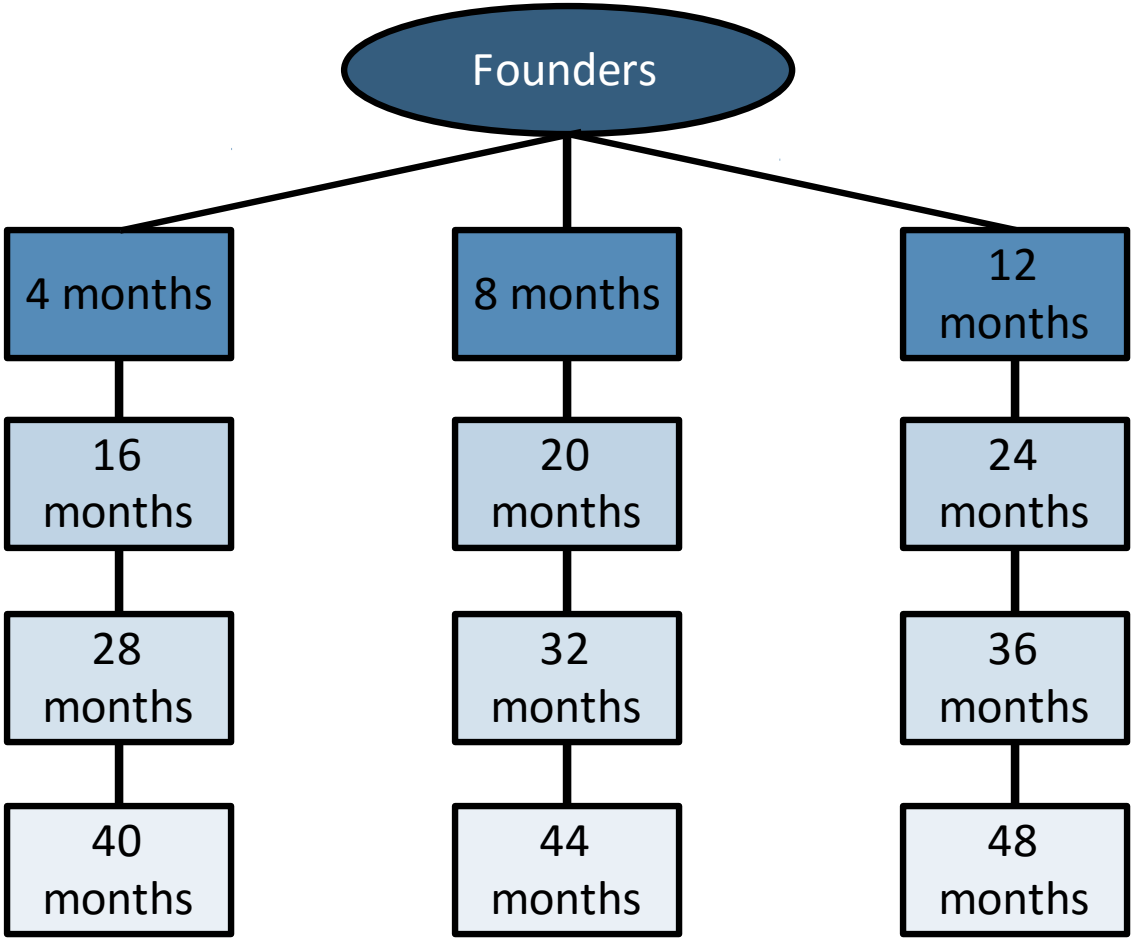
Sub Families



Goal should always be to minimize # generations away from founder population

Minimizing Number of Generations

Sub Families



Long Term Wild Type Maintenance

In House Method

Each generation must be comprised of no

Less than 25 small group or pair crosses

To ensure at least 25 males and 25 females

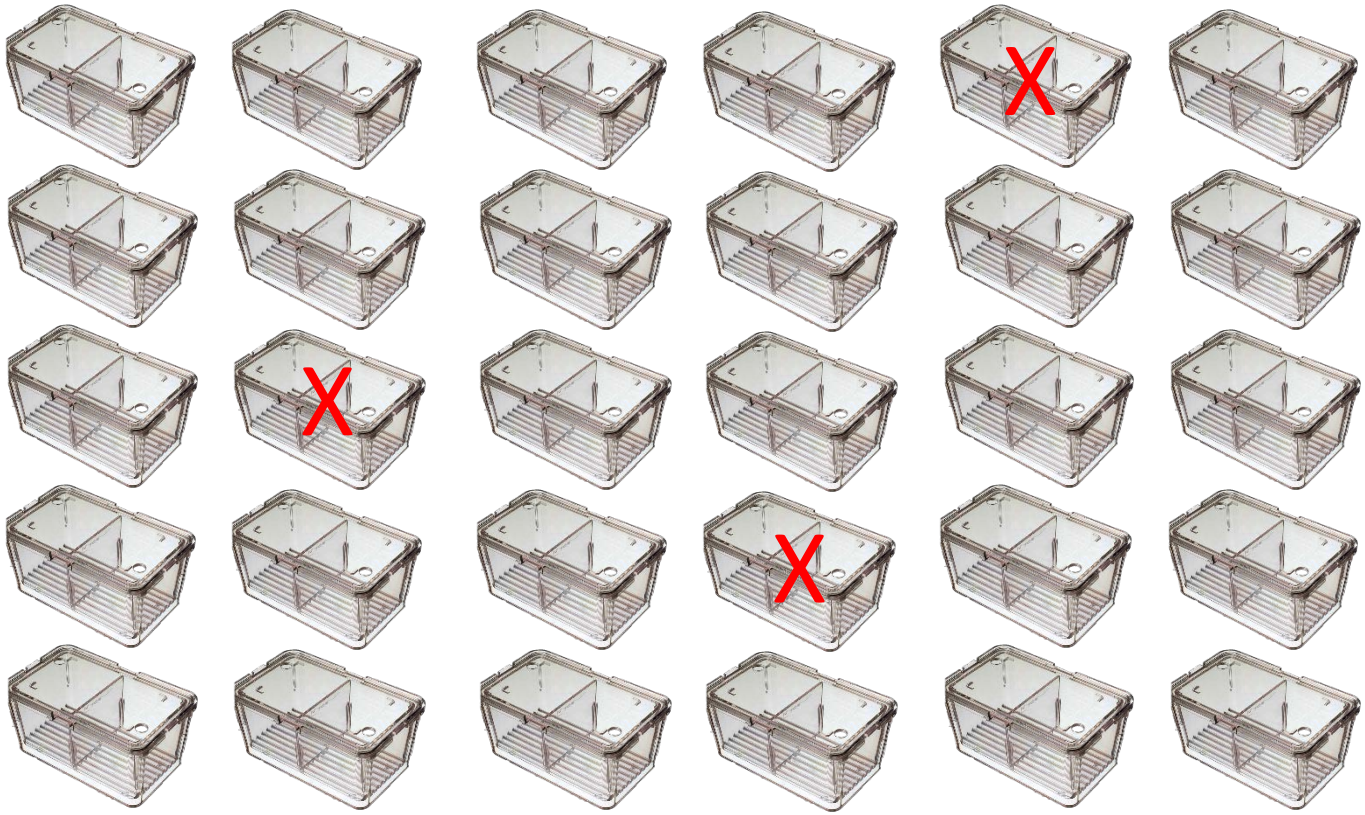
Contributed to the population.

Each clutch is kept separate and screened

For a number of factors such as quality, quantity, and fertility.

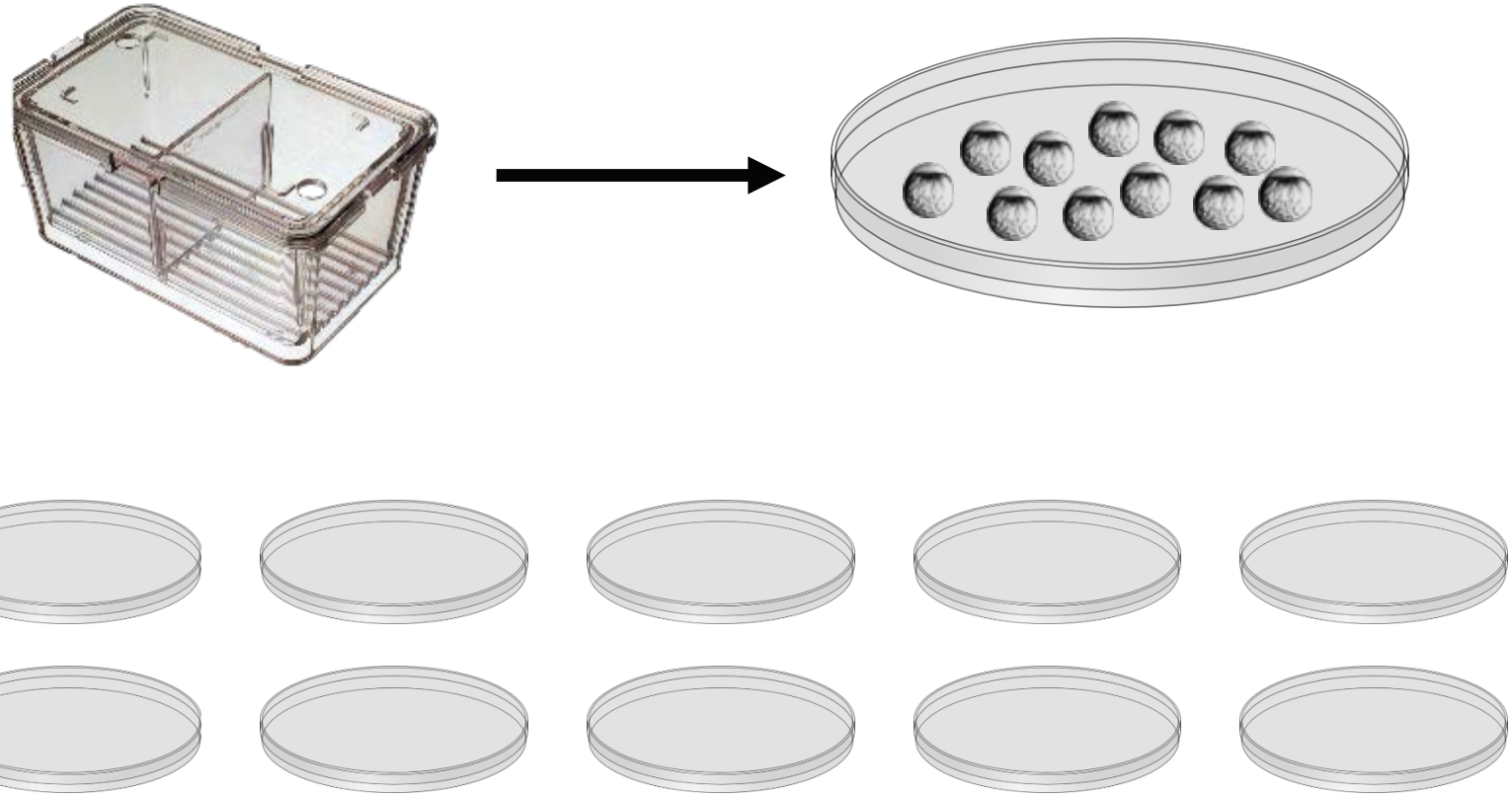
Long Term Wild Type Maintenance

Long Term Wild Type Maintenance



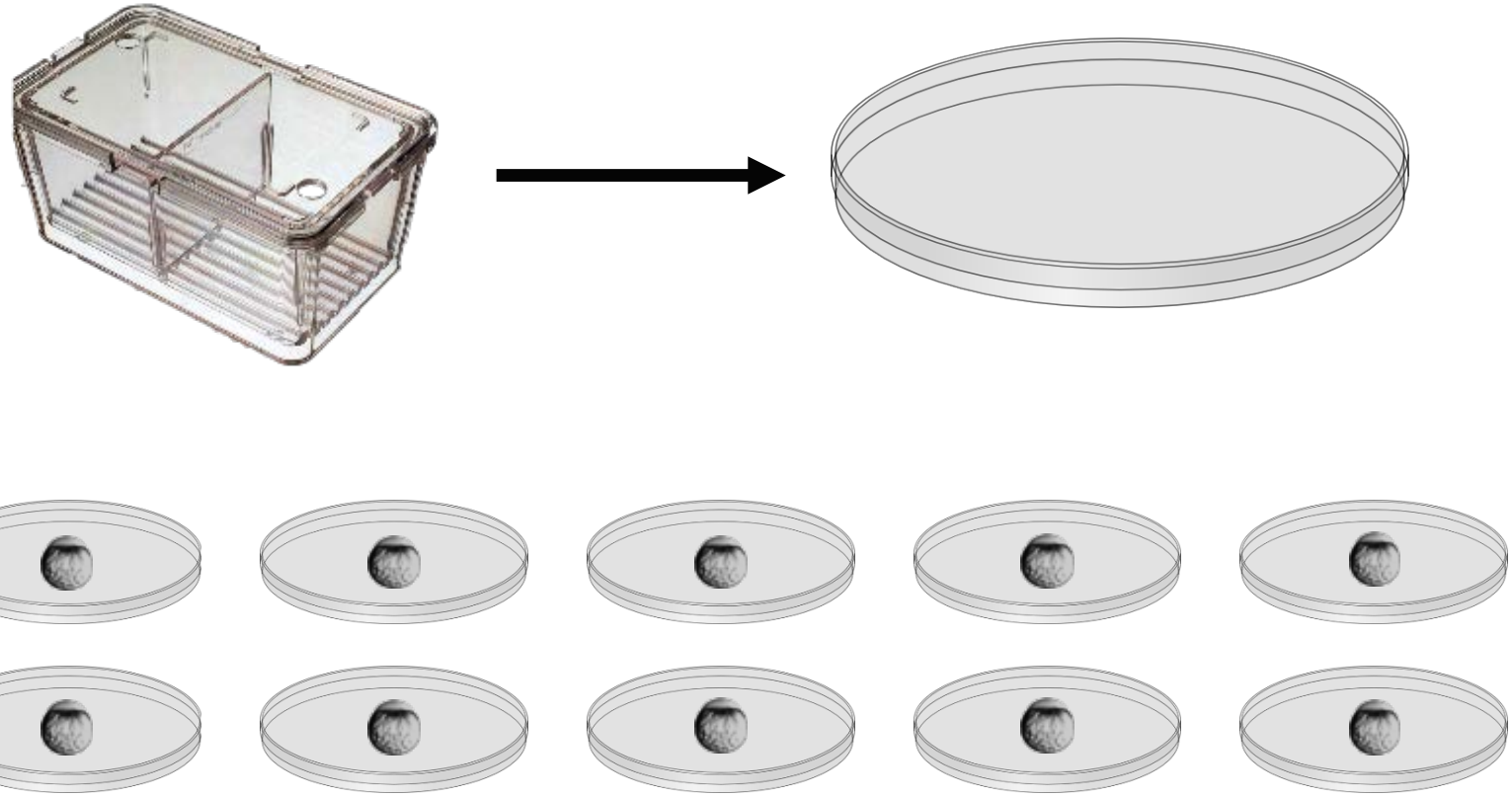
- Keep clutches separate to screen for low fertility, low success, or malformations
- Score clutches and eliminate those that do not pass

Incorporating methods to limit sibling mating



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

Reducing Direct Sibling Mating



This will create multiple stock tanks that are comprised of non-sibling animals

Scoring Clutches

Factors to consider

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

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

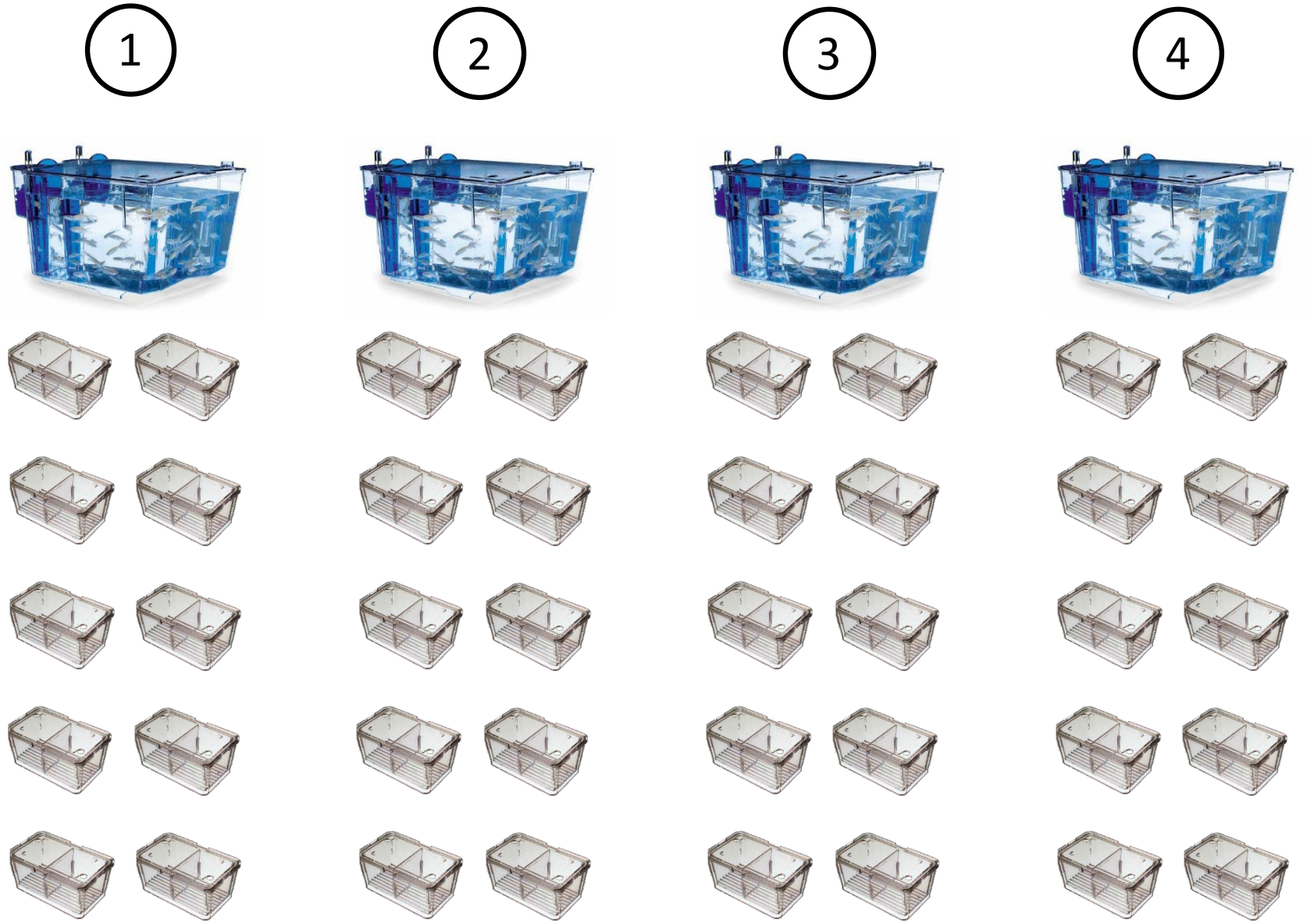
Necrotic embryos: Are there necrotic or bad embryos present?

Malformations: Are there malformations present?

Scoring System

Create a points based scoring system that determines if clutches can be used for a next generation. One point per category. Clutches with malformations should never be used to propagate lines.

Reducing Direct Sibling Mating – Large Stock Methods



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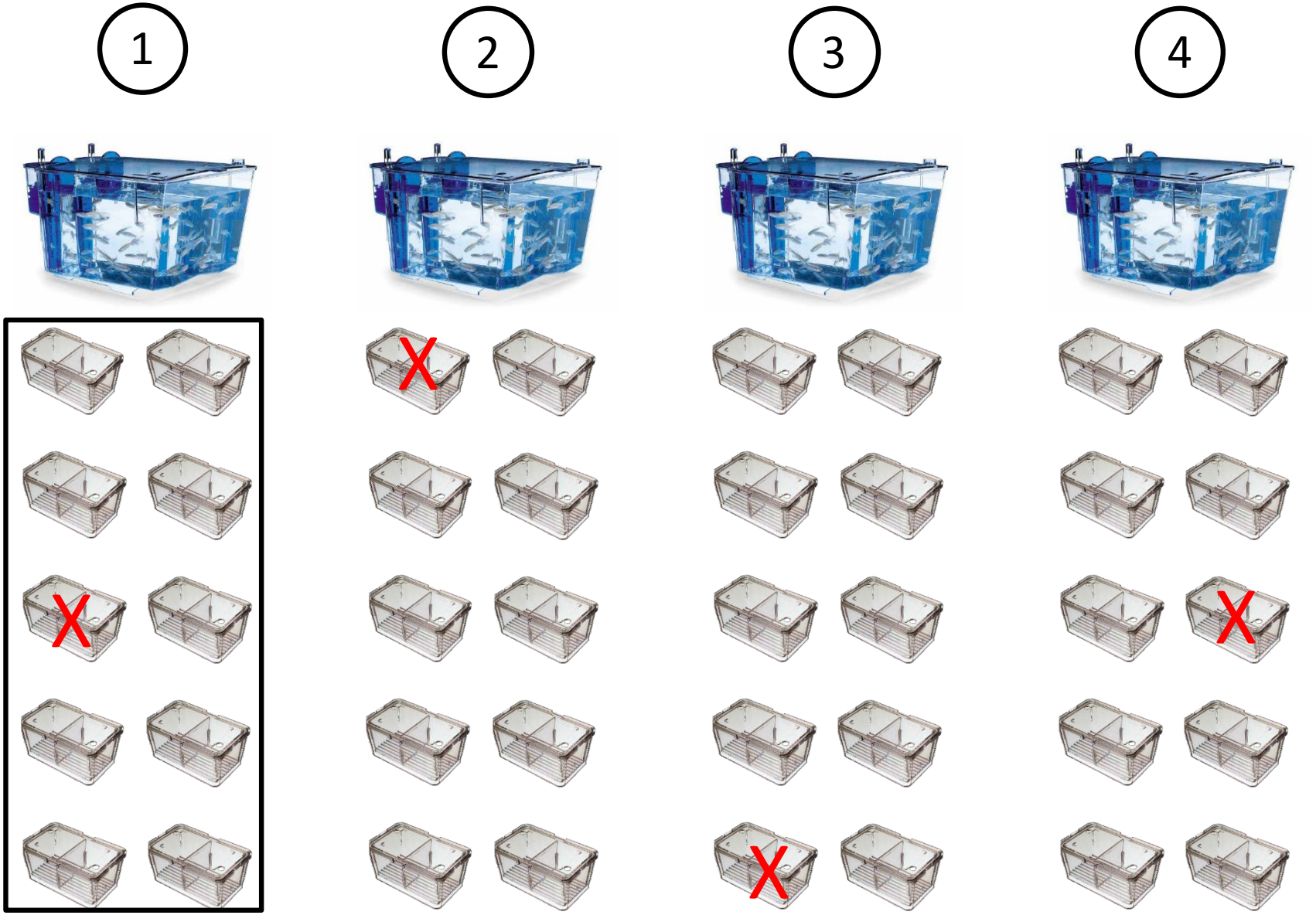

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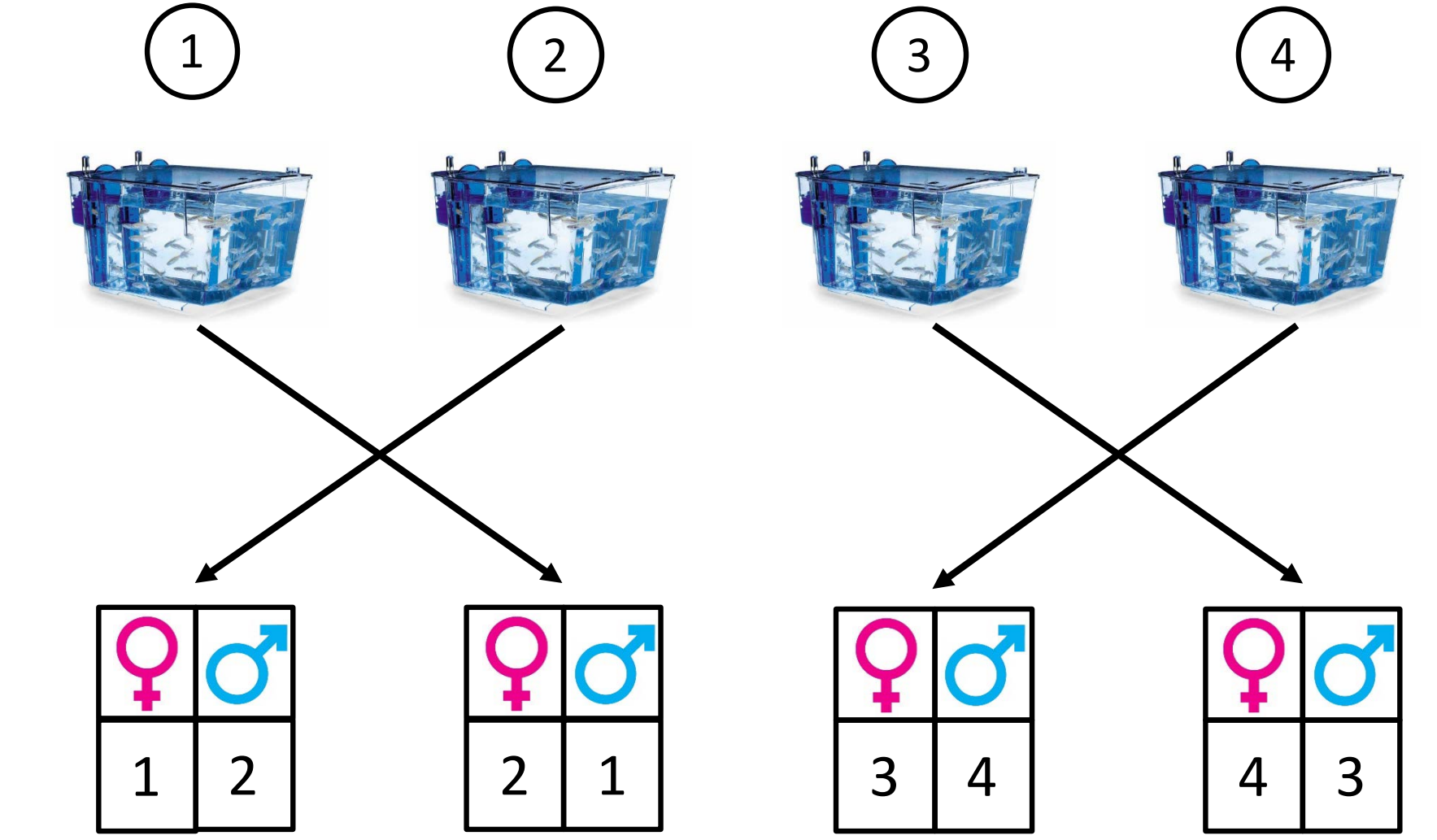


Alternate Strategy for Wild Type Maintenance

Import Method

Purchase fresh brood stock/wild type animals from a reputable zebrafish (for research) supplier as needed to maintain colony function.

- 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.

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.

Oversight veterinarian endorsed health certificate.

Information about propagation techniques used to ensure genetic integrity.

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	-	-

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)

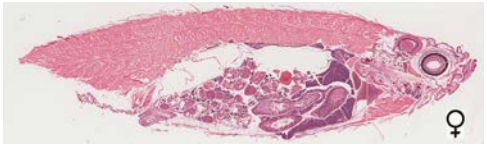
Place of Production
Aquaculture Facility: Weill Cornell Medical College
Address: 510 East 70th Street
New York, NY 10021
United States of America

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 Liegi, DVM, DACLAM, Attending Laboratory Veterinarian (NY) #: 001220
Address: 1275 York Ave, Box 370, NY, NY, 10065

Signature: _____ Date: 2/19/13

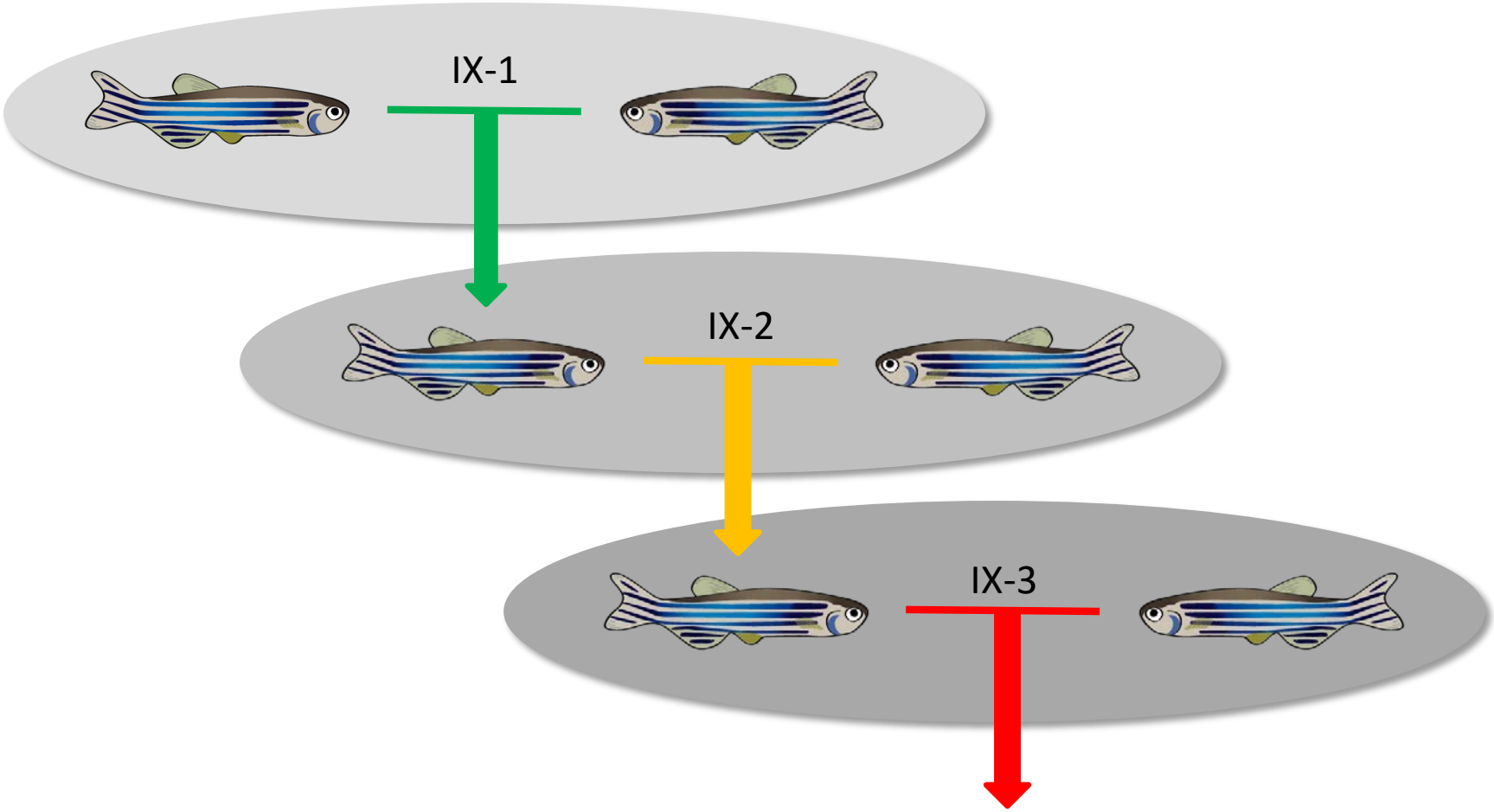


http://zebrafish.org/health/index.php#

Strategies for Mutant and Transgenic Lines

- Maintenance schemes and frequency
- Minimum contributors
- Assessing quality/spawn success of contributors
  - presence of correct mutations
  - bright transgenic signal
  - minimal background mutations

3 to 1 Rule

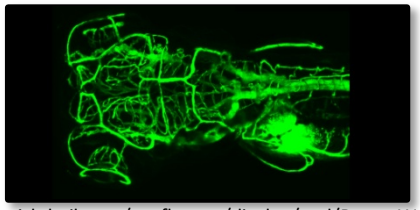


Outcross this population to WT

Mutant Maintenance Scheme

3 to 1 Rule:

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

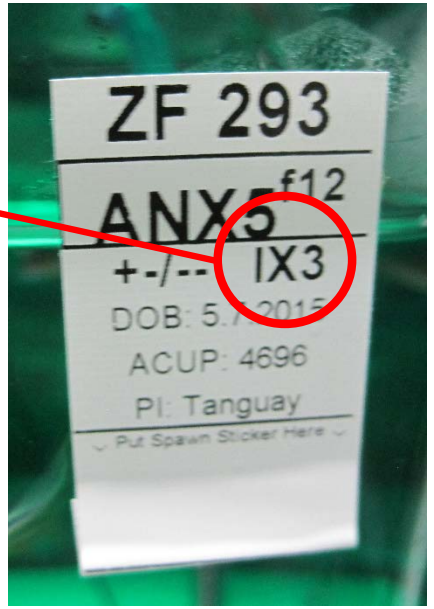


https://science.nichd.nih.gov/confluence/display/pgd/Brant+Weinstein+Lab

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

Next gens should be made ~6 months



Database can be a tool to help ensure genetic integrity

Commercial database systems are not readily available for zebrafish colony management

Beta or in house options

Danio Data

http://www.daniodata.com/research.php

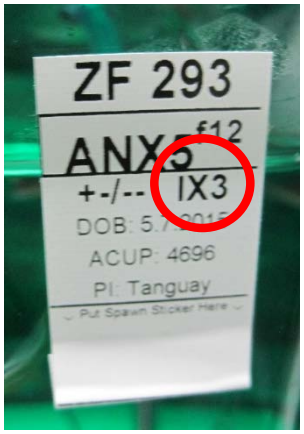
Zbase

http://zebase.bio.purdue.edu/

GofishDB

http://www.davidtulga.com/fish.htm

Filemaker Pro



Strategies for Mutant and Transgenic Lines

Guidelines for maintaining functional mutant lines:

- **Set standards for minimum contributors for each next generation**
- Screen offspring for robust mutation and minimal background problems
  - Eliminate clutches/embryos that fall outside the set standard

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

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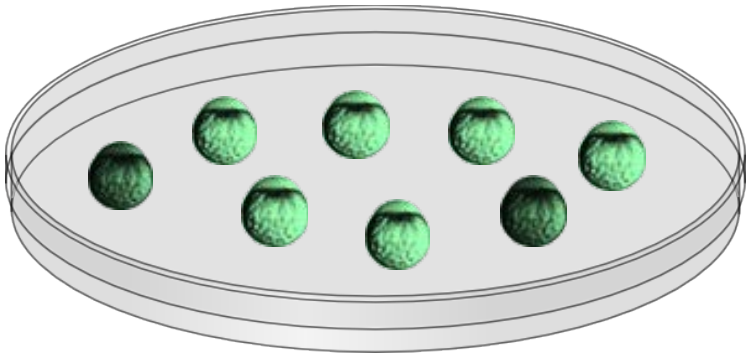

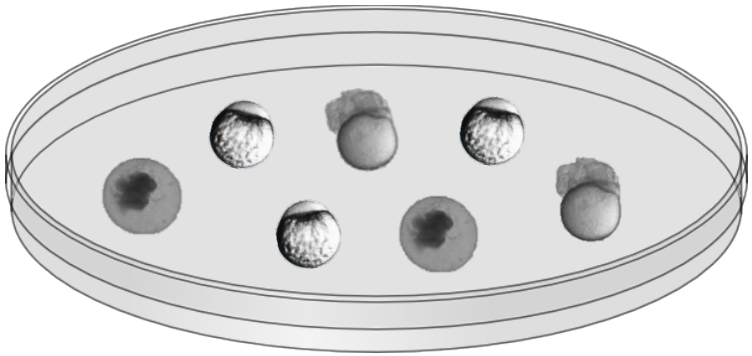

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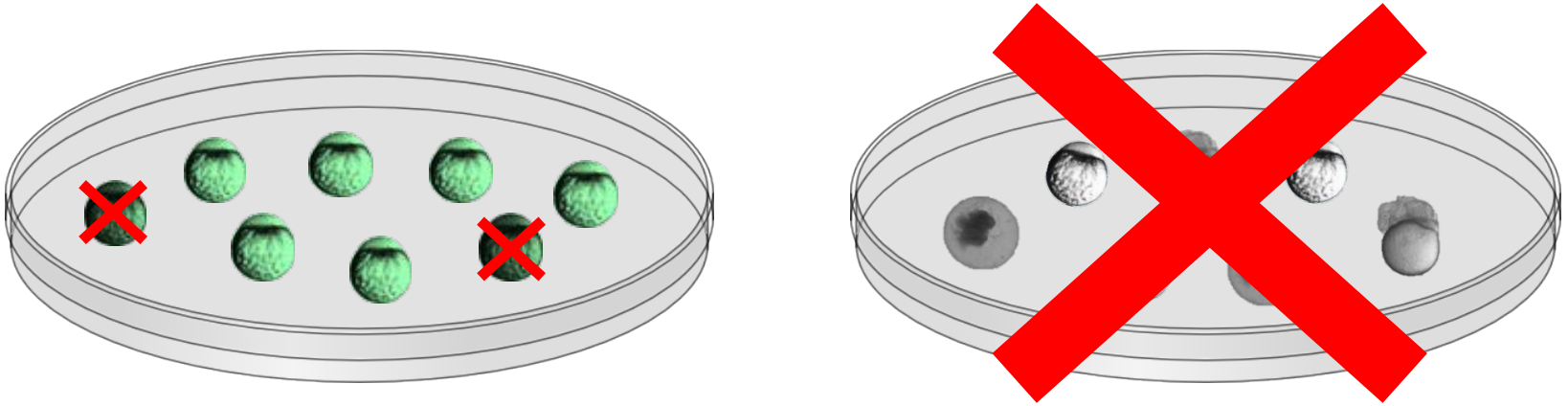
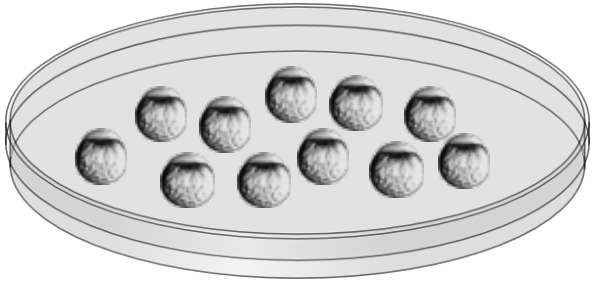


Newly Developed Mutants – Propagation

CRISPR/Cas9: *Clustered regularly interspaced short palindromic repeats. Used to efficiently mutate specific loci in zebrafish (Danio rerio) and screen for genes involved in vertebrate biological processes.*



Best carriers ID'd via fin clip or paired OX

This can be very few fish!

OX to wild type

Heterozygotes ID'd & IX'd

ID HMZ for Research

Focus is often:
-fastest path to homozygote animals
-repeated IX events

For stability of the line, and to differentiate between off target effects vs gene related dis-function, additional efforts should be placed on outcrossing and proper line propagation.

Newly Developed Mutants - Fact Finding

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

Questions to ask:

-What preliminary experiments have been done?

-What were the outcomes?

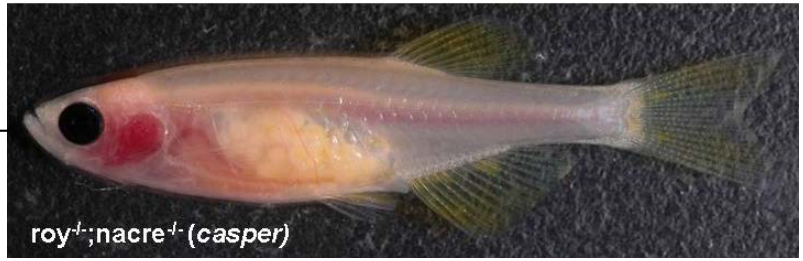
-Is the disrupted gene known to cause problems with reproduction or survival in other model organisms.

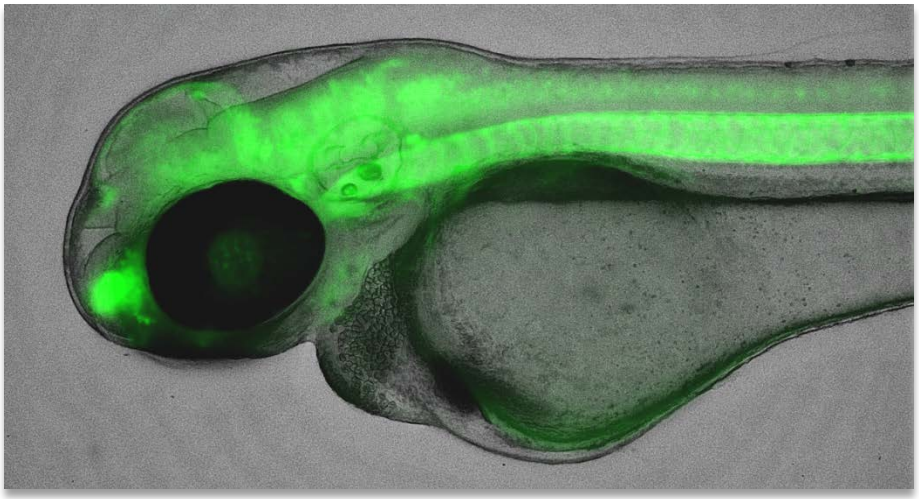
-Is manipulating this gene lethal in other model organisms?

-What is the intended use of the line?

Color Mutant Background Lines

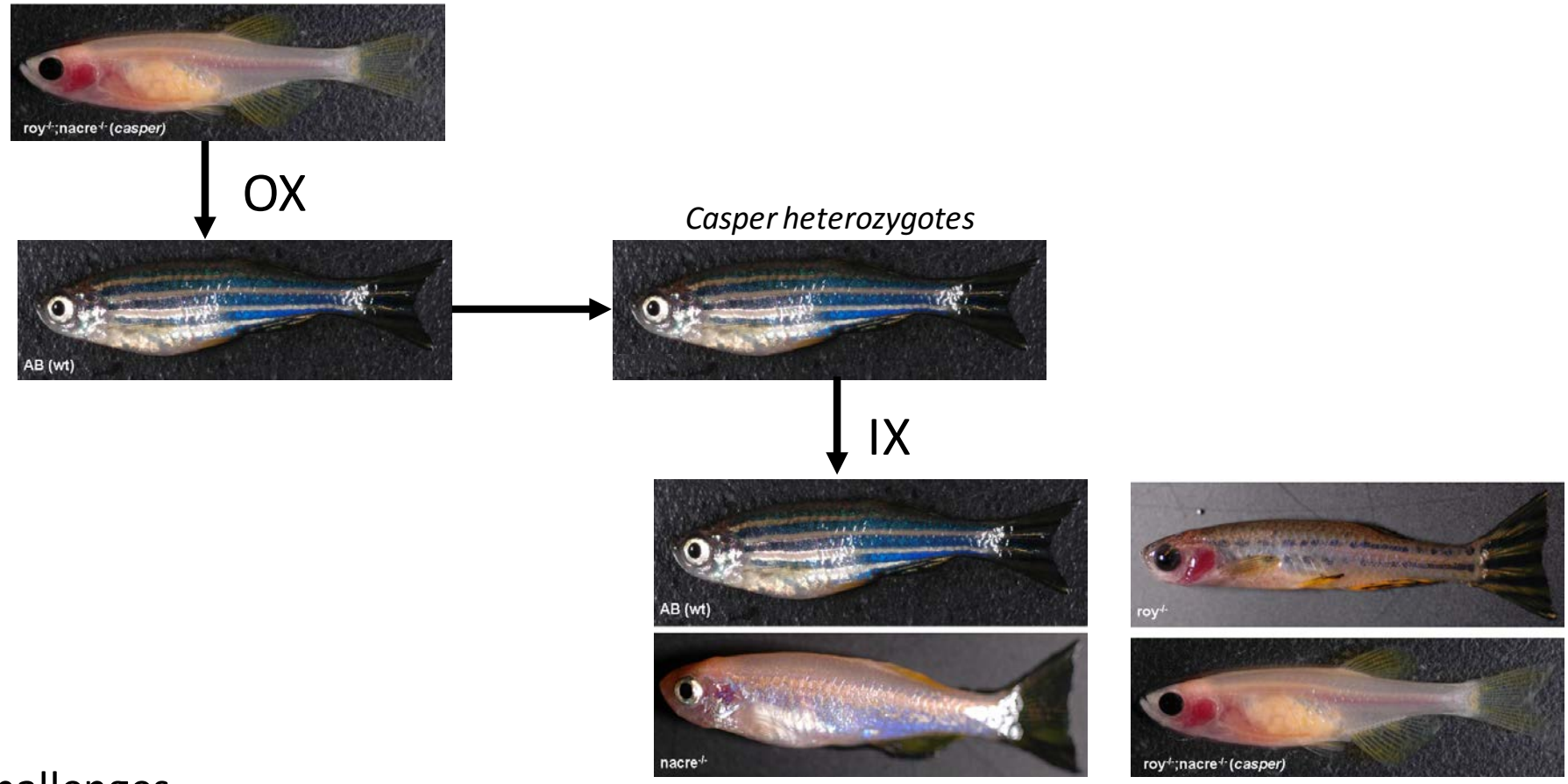
An increasing number of transgenic lines are being maintained on casper and other color mutant backgrounds due to the increased imaging capabilities of transparent lines.





Transgenic photos courtesy of PHD candidate Gloria Garcia

Color Mutant Background Lines

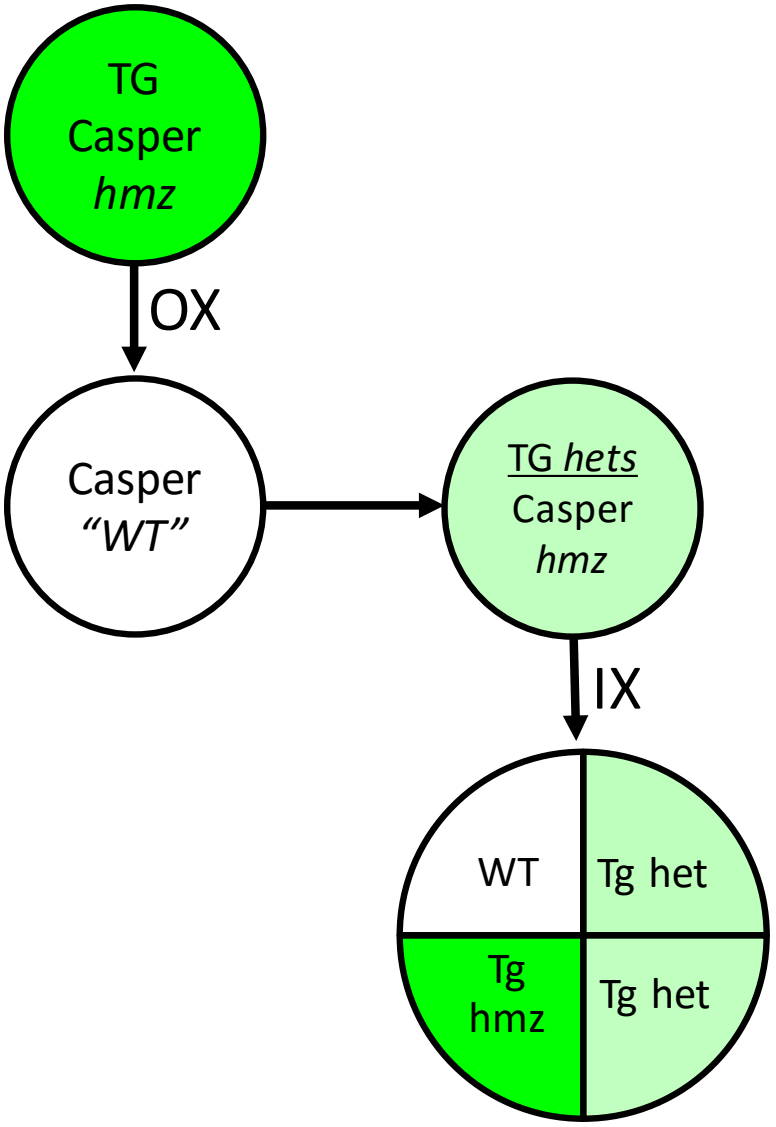


Challenges

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

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

Color Mutant Background Lines



All animals are casper

Strategies for Mutant and Transgenic Lines

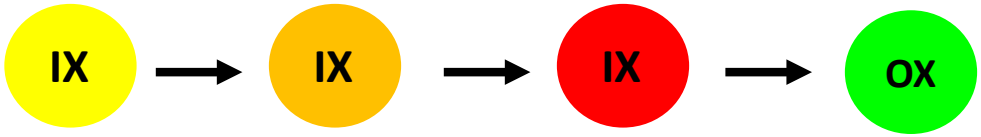
What are the most common reasons given for not outcrossing mutant and transgenic lines?

Strategies for Mutant and Transgenic Lines

"Outcrossing events are a disruption to the availability of homozygous embryos."

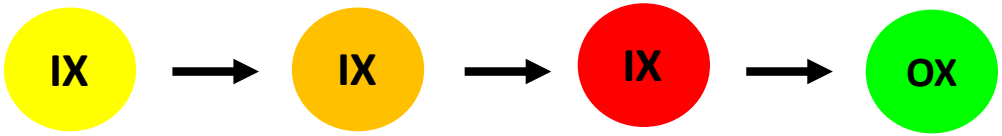
Strategies for Mutant and Transgenic Lines

Family A



ID

Family B



ID

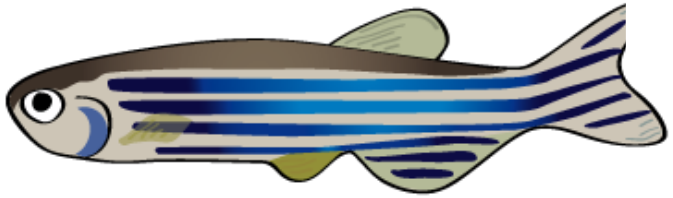
Utilize sub-families to eliminate disruption to availability of homozygous embryos

Strategies for Mutant and Transgenic Lines

"I can't trust (insert user group here) to properly ID males from females, and I'm afraid of contaminating my lines"

Strategies for Mutant and Transgenic Lines

- Develop better training methods to ensure proper gender identification
- Employ the use of line specific dedicated outcross tanks
- Utilize fin clipping to help train and build confidence in gender ID

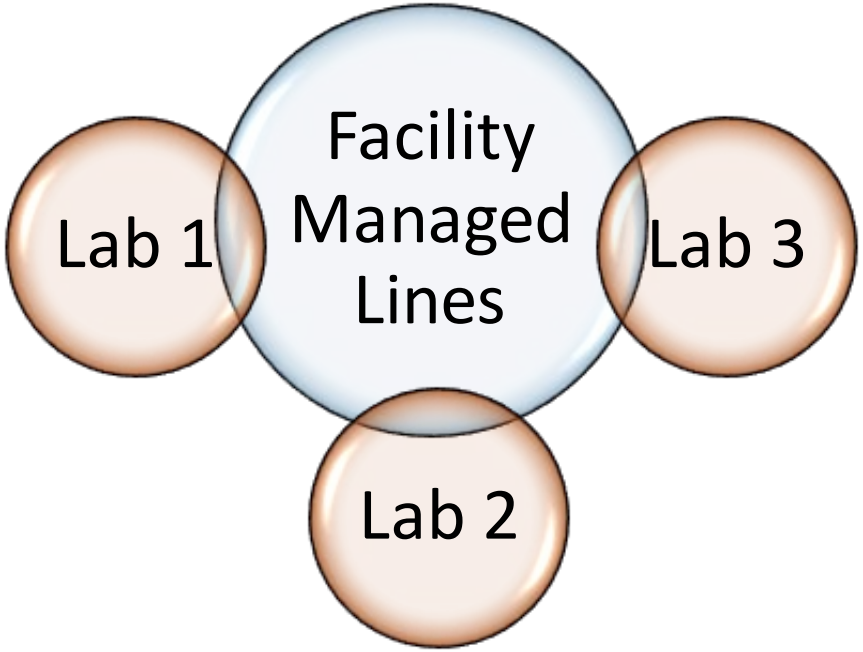


Strategies for Mutant and Transgenic Lines

"Outcrossing is a lot of extra work."

Considerations for all facilities

- **Husbandry staff should maintain the wild types and commonly used mutants to ensure care is taken to follow steps needed to maintain genetic health**
  - Dedicated OX and lab usage tanks should be used to reduce risk of line contamination
    - *especially for lines not propagated by facility staff*
  - Cryopreservation can be used to back up resources in the event of bottleneck event or inbreeding depression



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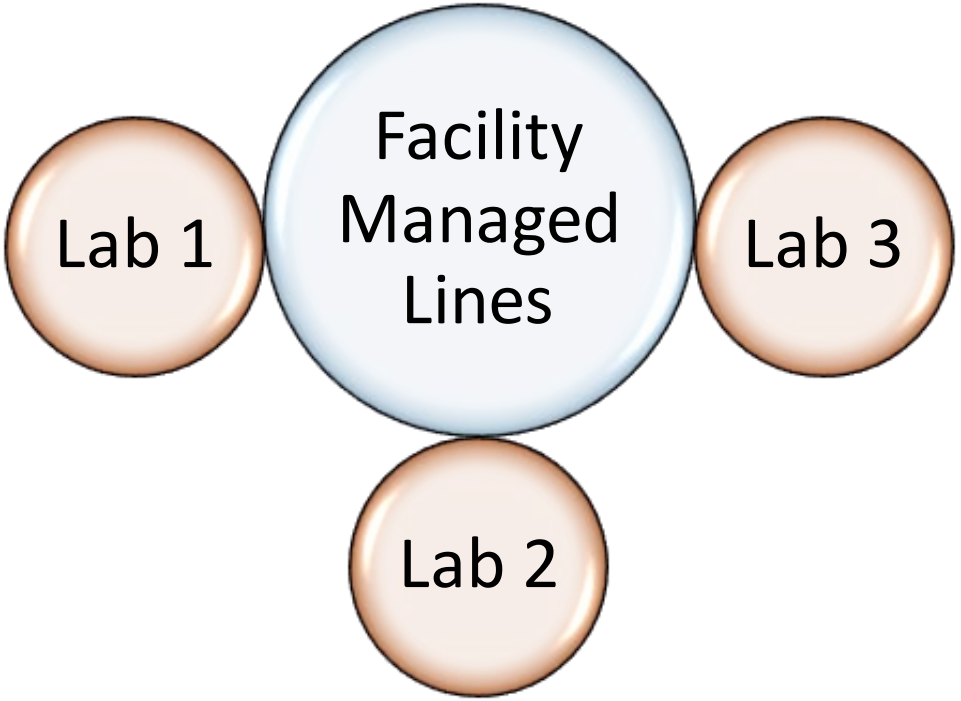

Other Considerations for all facilities

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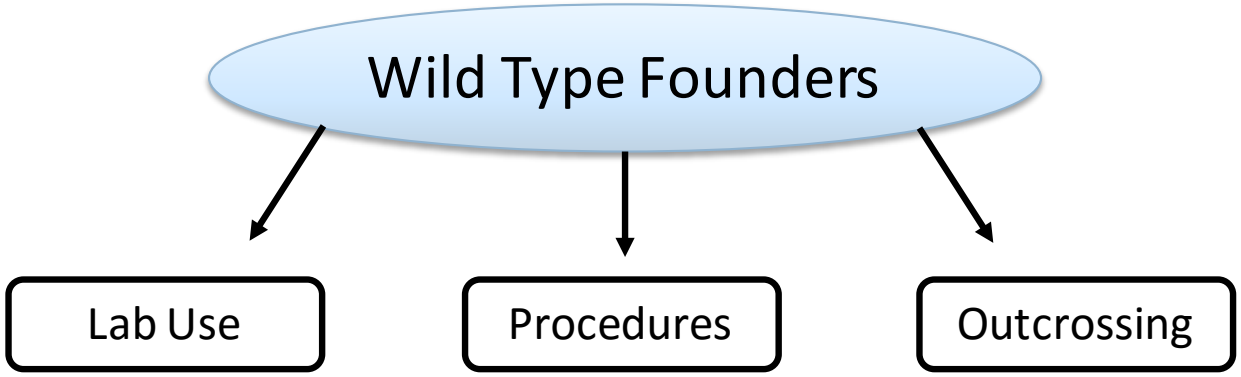

Other Considerations for all facilities

- Husbandry staff should maintain the wild types and commonly used mutants to ensure care is taken to follow steps needed to maintain genetic health
  - Dedicated OX and lab usage tanks should be used to reduce risk of line contamination
    - *especially for lines not propagated by facility staff*
- **Cryopreservation can be used to back up resources in the event of bottleneck event or inbreeding depression**

Questions?