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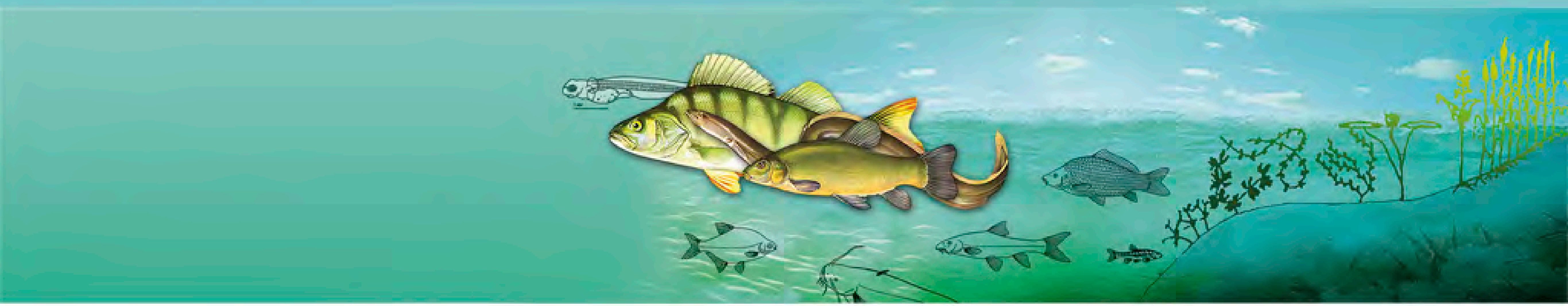
Surrogate production technology in fish



Martin Pšenička, Taiju Saito

www.frov.jcu.cz

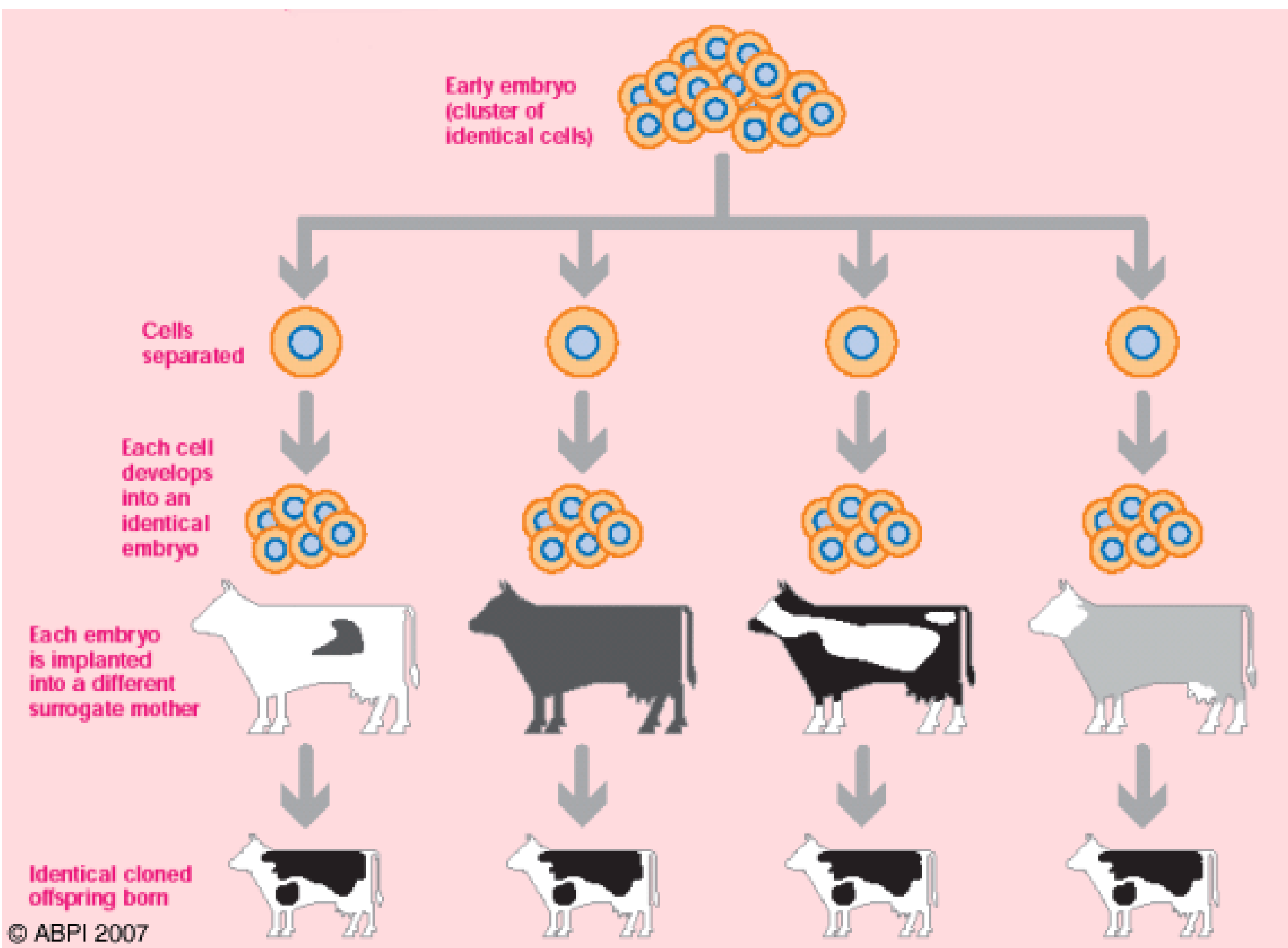
Content of presentation

- Introduction to a new biotechnological technique, “surrogate production” in fish.

- The surrogate production in fish using:
  - PGCs
  - spermatogonia and oogonia

In mammals…

The "Surrogate production" means "embryo transfer" into the uterus of a host mother. Its purpose is to produce many offspring (cow) or carry the baby for couples who cannot have a baby themselves (human).



Surrogate mother

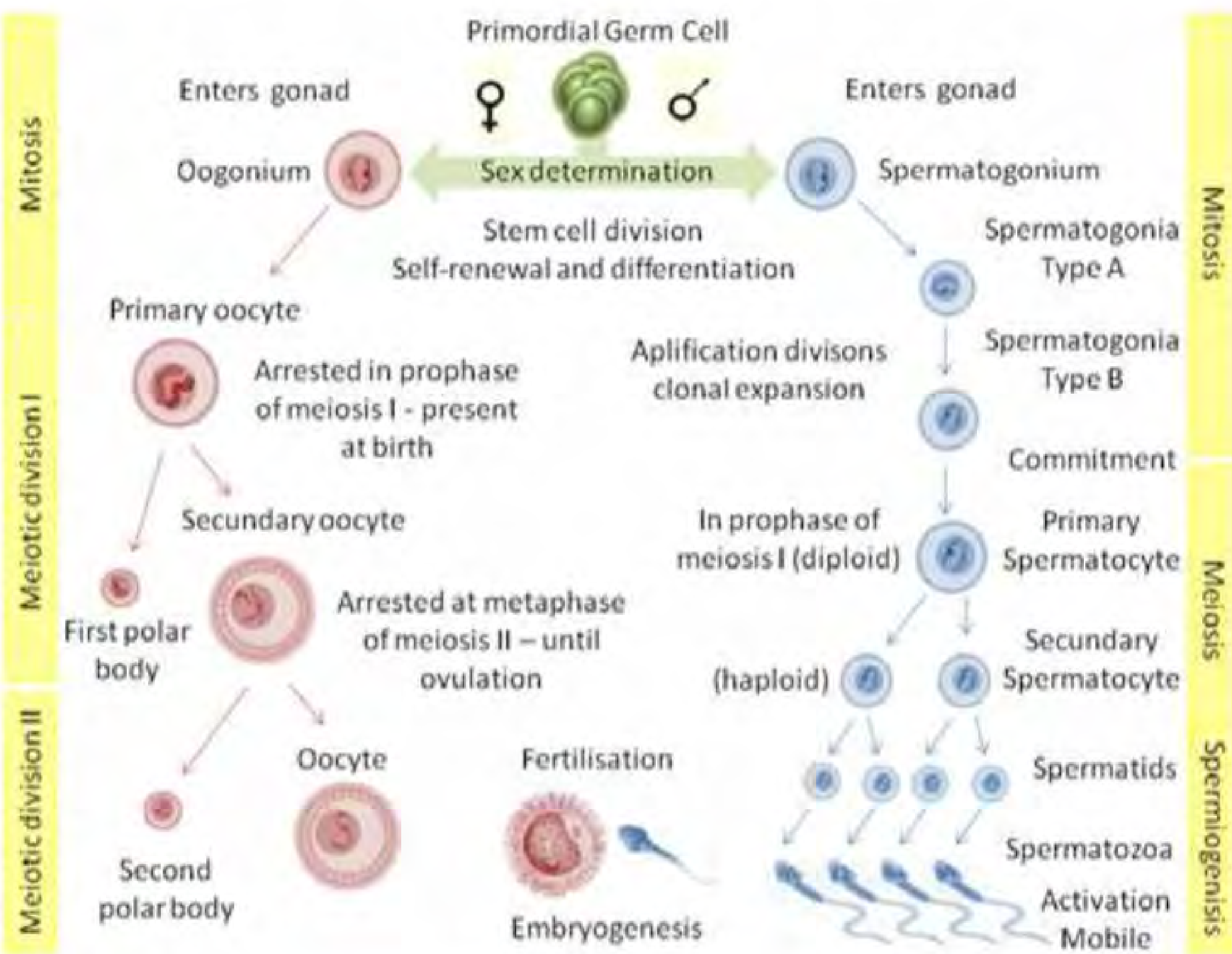
But in fish…

Usually a lot of embryos develop outside of the parent's body.

In fish…

Surrogate production means “germline stem cells” transplantation into a host individual.

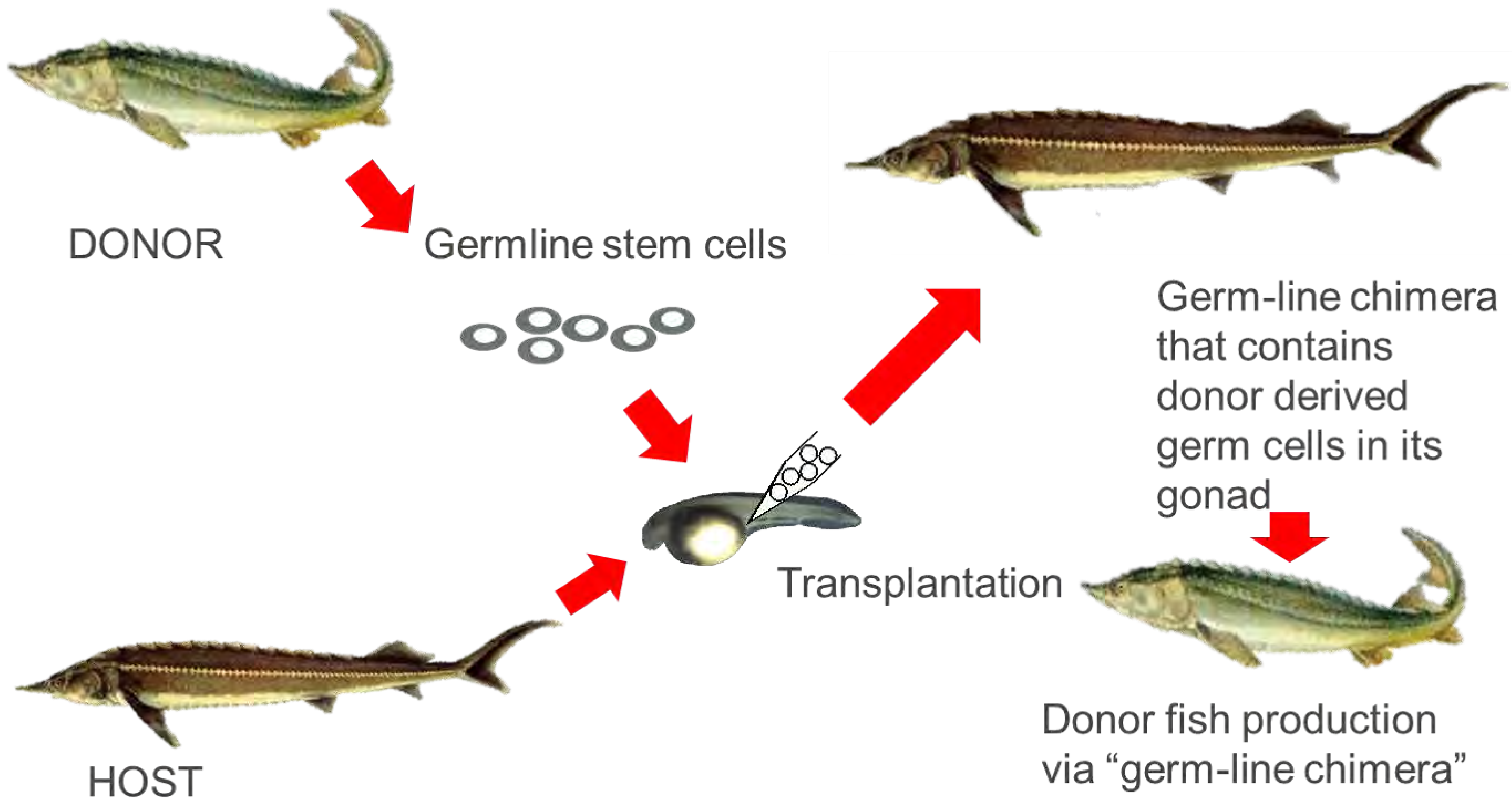
Germline stem cells are the origins of all germ cells and gametes.



1) Primordial germ cells (PGC) – embryonic cells

2) Oogonial and spermatogonial stem cell – in testes or ovary

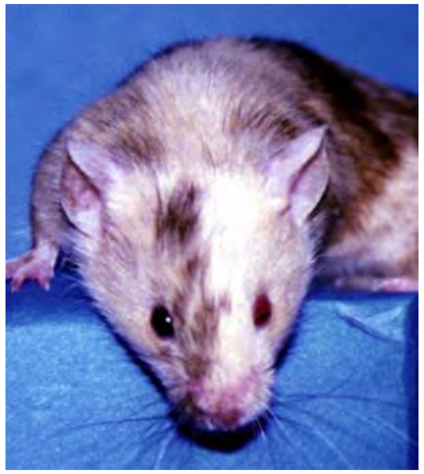
What is the surrogate production in fish?



Surrogate production is the strategy to obtain the gametes of target species via host species. Production of “germ-line chimera” is a KEY for the surrogate production in fish.

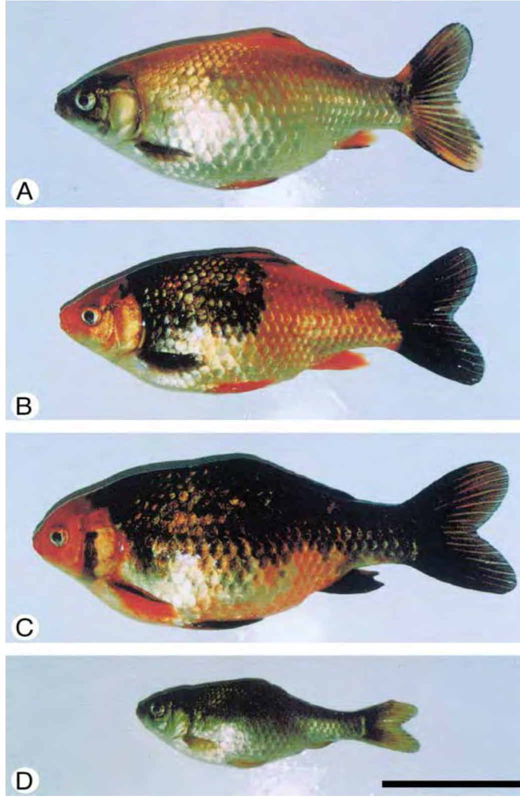
What is chimera?

- Mythology: creature compound of different animals



- Science: individual compound of genetically different population of cells

- Germ line chimera – individual carring germ cells of different individual

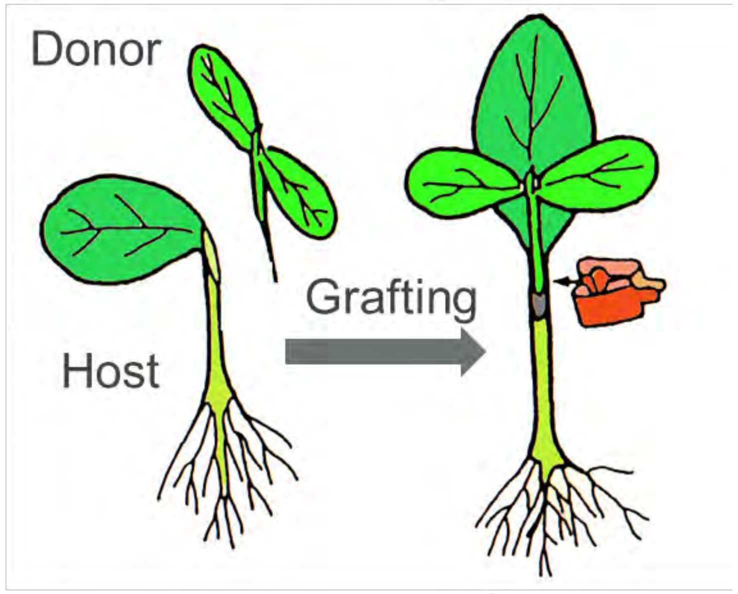


The idea about germ cell transplantation technology seems like a dream…

but the idea came from plant,

producing CHIMERA is in practice, NOT in talking.

Grafting in plants was in use by the Chinese 2000 BC, and it was well established by ancient Greeks. They used this technic for grapes, lemon tree, and so on.




Natural fusion of trees.

Benefits

Precocity:
Reduction of the time for fruit production

Dwarfing:
Making it easy to harvest fruit for farmers.

Ease of propagation:
As seen in Sakura trees

Disease tolerance:
Host part provide tolerance to disease from soil.

Hardiness:
Host part provide tolerance to difficult soil conditions


Almost all sakura trees are produced by “Grafting” –chimerism

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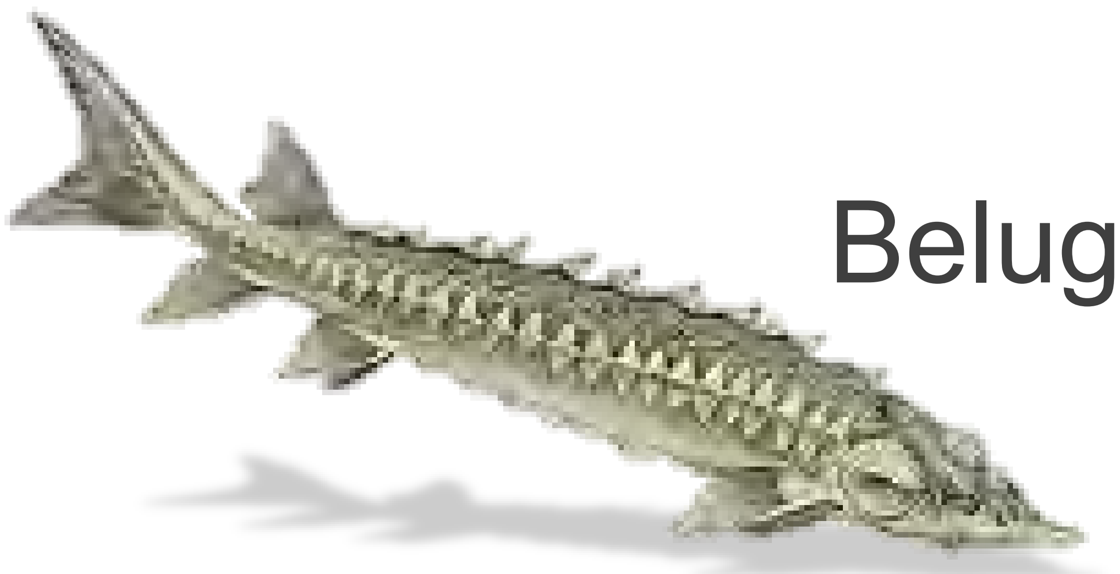
Benefits of the surrogate production in fish

1. Control of generation cycle

- Between fish with short and long generation cycle



Beluga sturgeon (18-20 years)

About 15 years reduction for reproduction

Sterlet sturgeon (4-5 years)

2. Reduction of the space for keeping fish

- Between large and small size species

Tuna

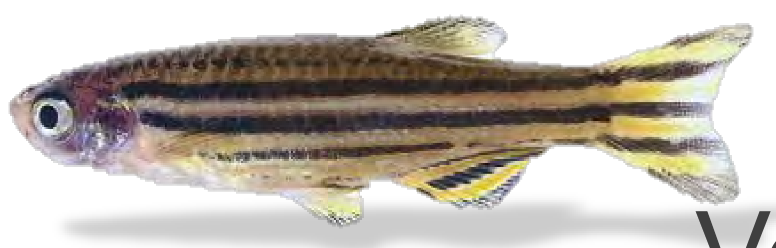
Weight 300 kg

Reproducing big fish in a small aquarium

Mackerel

Weight 300 g

3. Control of total egg/sperm production - Between the species which have large and small number of gametes



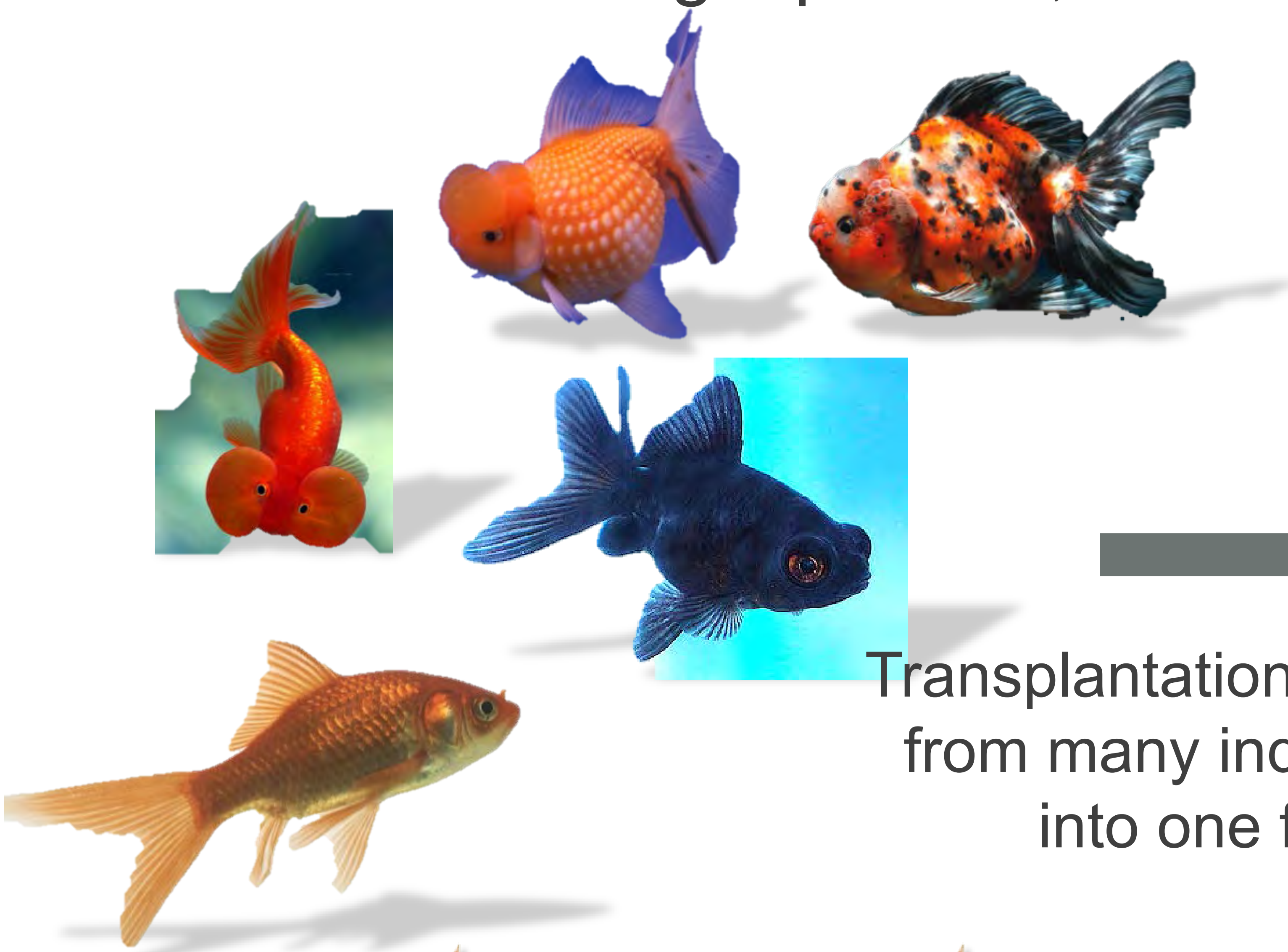
Number of eggs: 300
Volume of sperm: up to 1 ul

Boosting gametes production

Number of eggs: several thousands
Volume of sperm: more than 50 ul

4. Preservation of genetic diversity

- Host: single parents, Donor: PGCs with many diversity

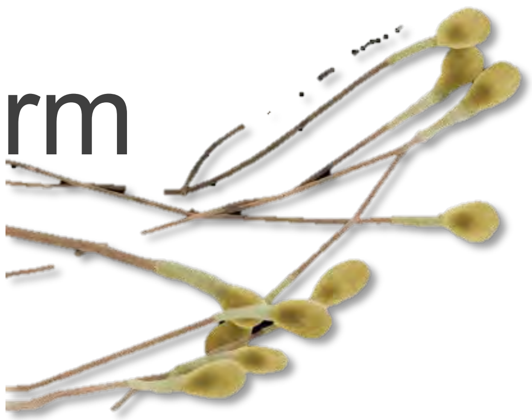


Transplantation of PGCs from many individuals into one fish

One time crossing produce many combination of gene cocktail

5. Preservation of genetic resources in Liquid Nitrogen

Sperm

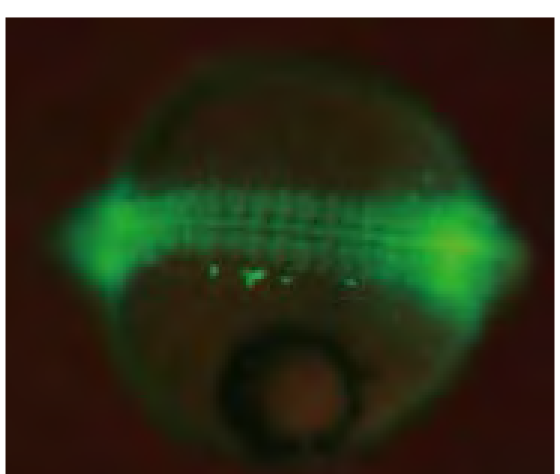


Technology for cryopreservation of sperm is well developed, however, maternal genes and mitochondria cannot be stored.

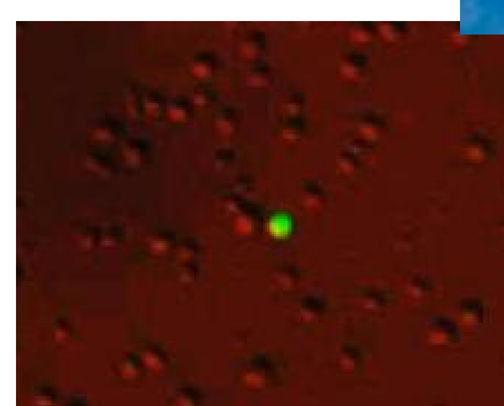
Embryo

It is impossible to cryopreserve a whole embryo.

Germ stem cells



Cryopreservation →



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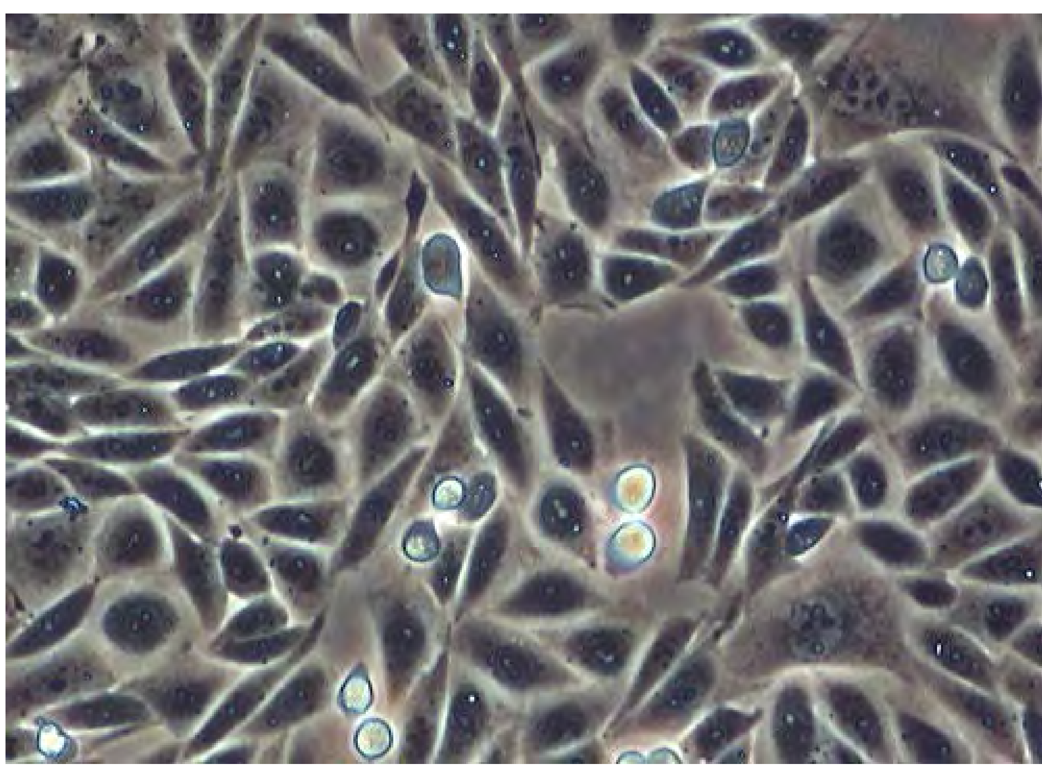
6. Application of cell culture technology for breeding of target species

Cell culture applications.
(i.e. gene targeting, gene transfer, induction of a point mutation like "ZFNs")

PGCs/spermatogonia/
Oogonia

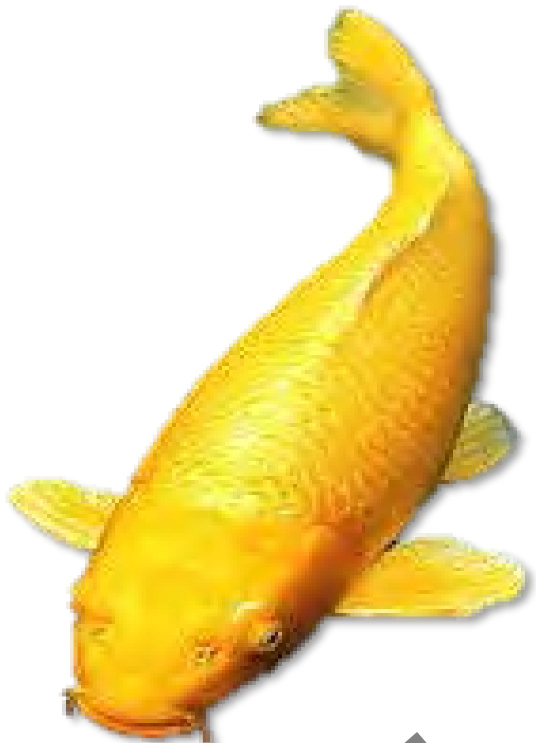
Cultivation



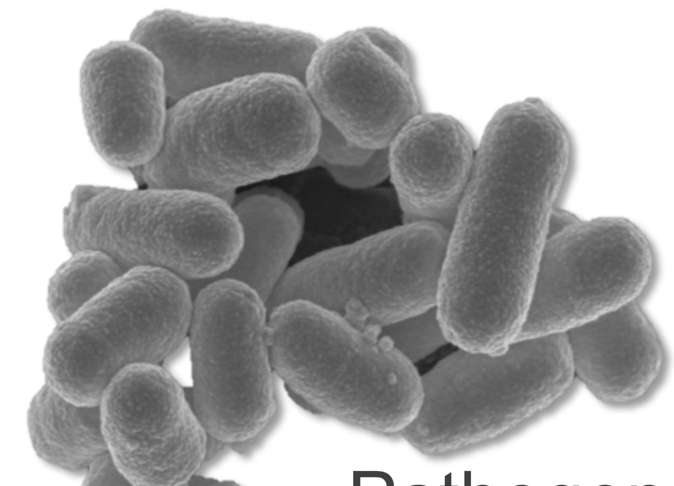
Transplantation

7. Gene stocks saving from fish disease

- Host: resistant strain. Donor; susceptible strain



Infection



Pathogens (KHV)

A strain, which has useful characteristics, such as good growth rate, good meet production, beautiful colors, and so on… BUT no tolerance for disease.

NO infection

Transplantation of Germ cells

Disease tolerant strain

8. Wide range adaptation to water

- Between marine and fresh-water fish

Marine flounder

Transplantation

Fresh-water flounder

How can we produce germline chimeras?

In fish, some methods have been developed by using "germline stem cells".

1. Primordial germ cells (PGCs) transplantation
2. Spermatogonia or oogonia transplantation

Primordial germ cells transplantation during embryonic stage

a) blastomeres containing PGCs
b) single PGCs

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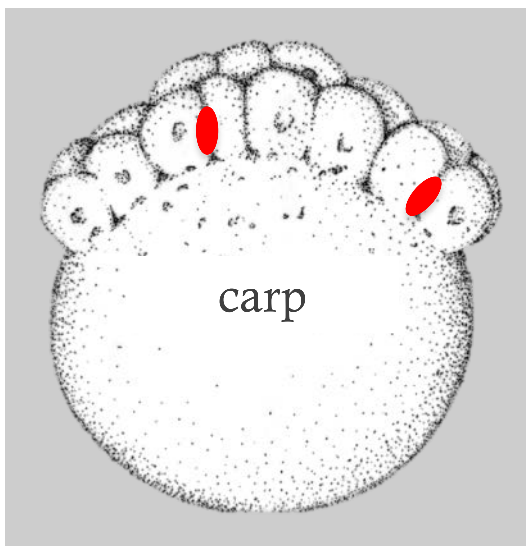
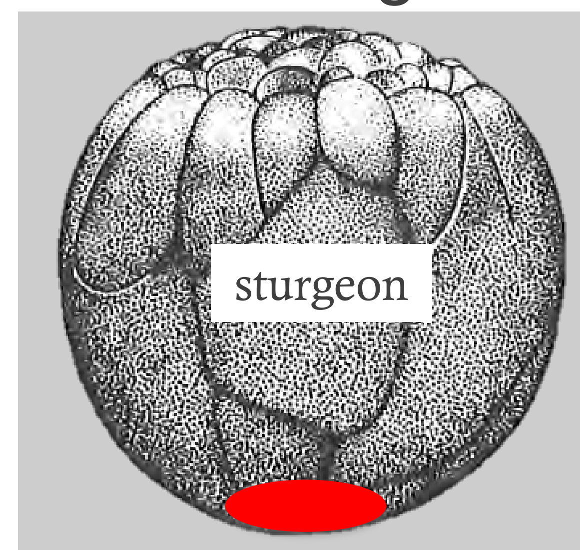
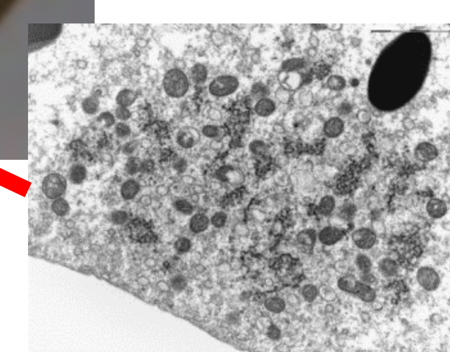
PGCs origin – determined by maternal determinants (germ plasm)

Meroblastic cleavage



Holoblastic cleavage





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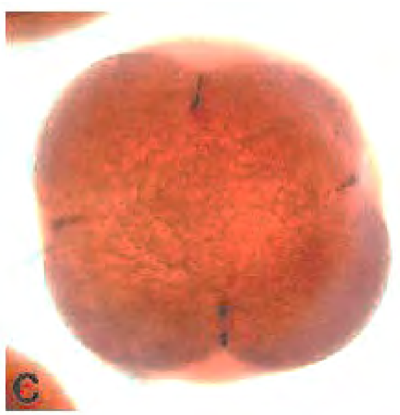
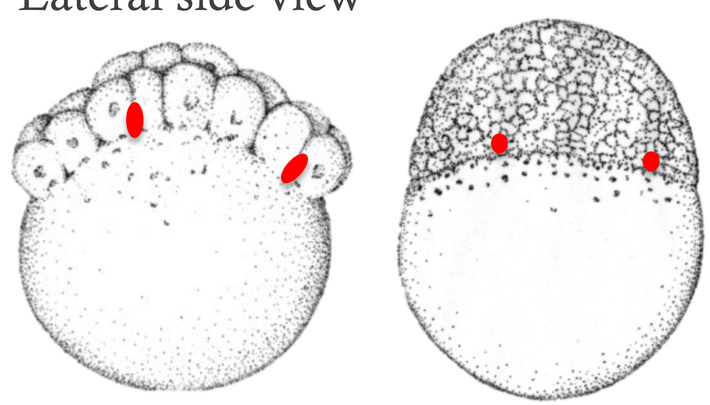
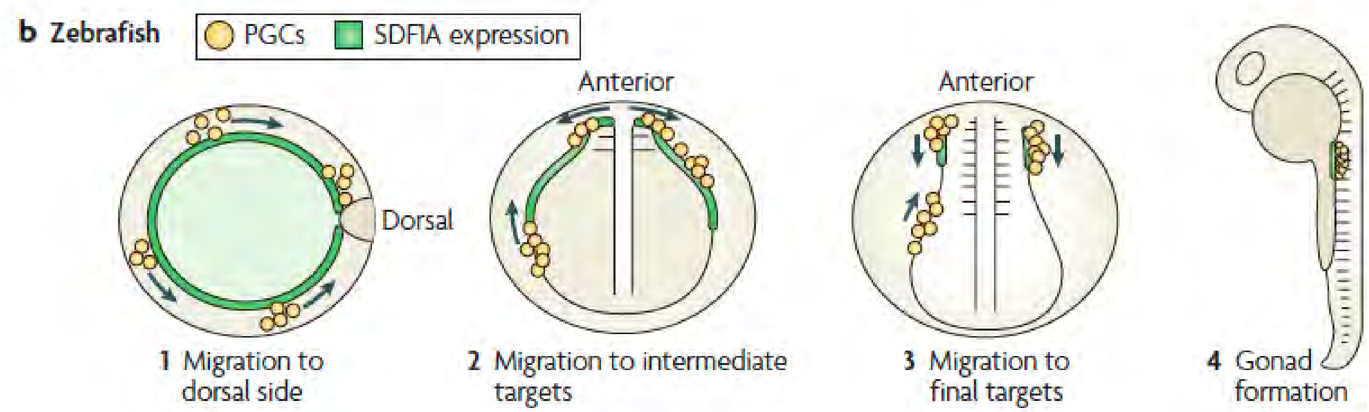
In fish, PGCs are formed at random positions in embryo and migrate to the gonadal region during development.

Animal pole view



Lateral side view





Active migration of PGCs

Visualization of PGCs in fish embryos

Synthesized mRNA

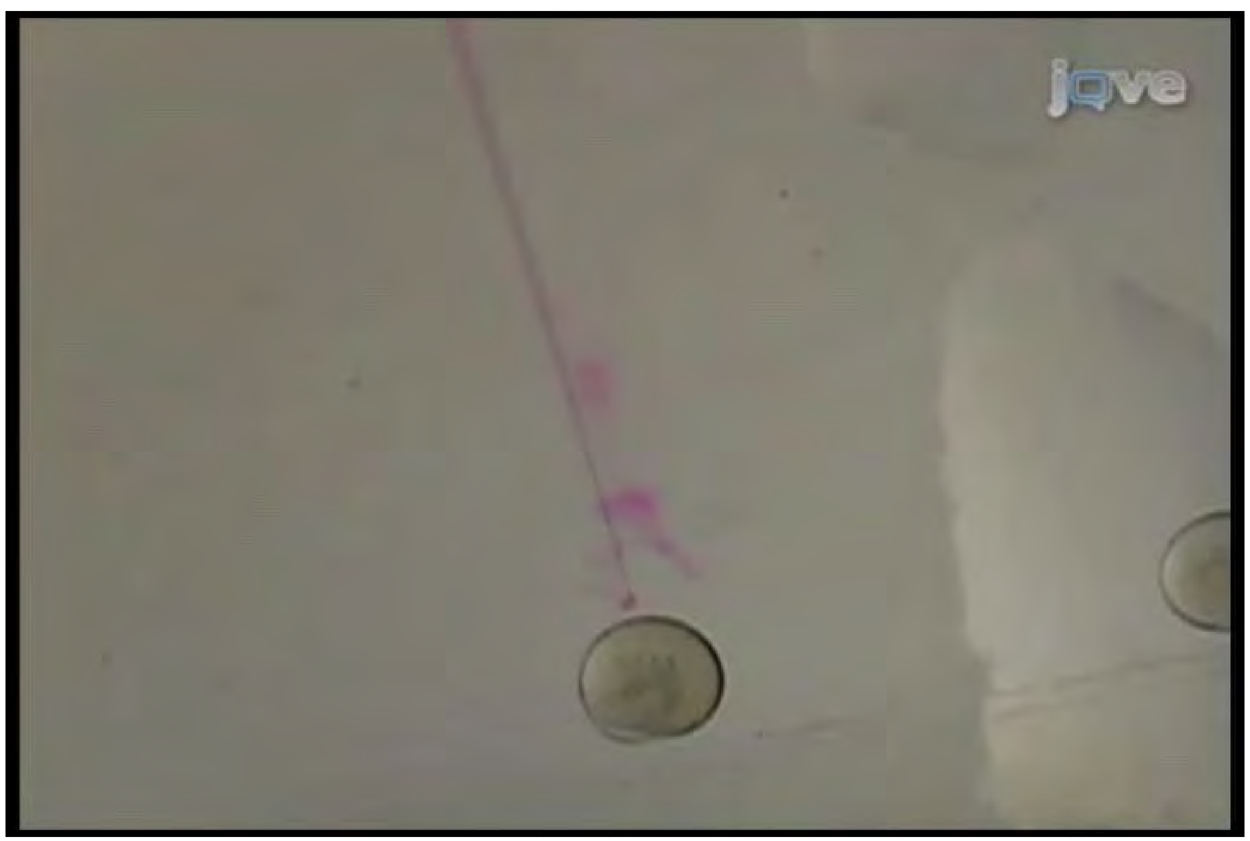
GFP | Zf *nos*1 3' UTR

↓ microinjection

Function of the nos1 3'UTR:

Enrichment of the mRNA in PGCs

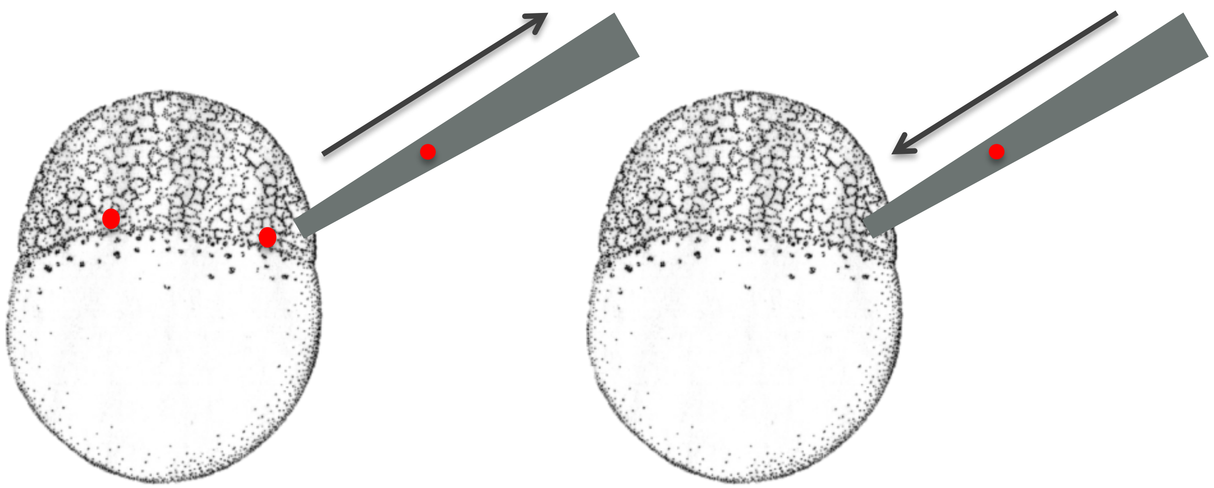
Degradation of the mRNA in somatic cells

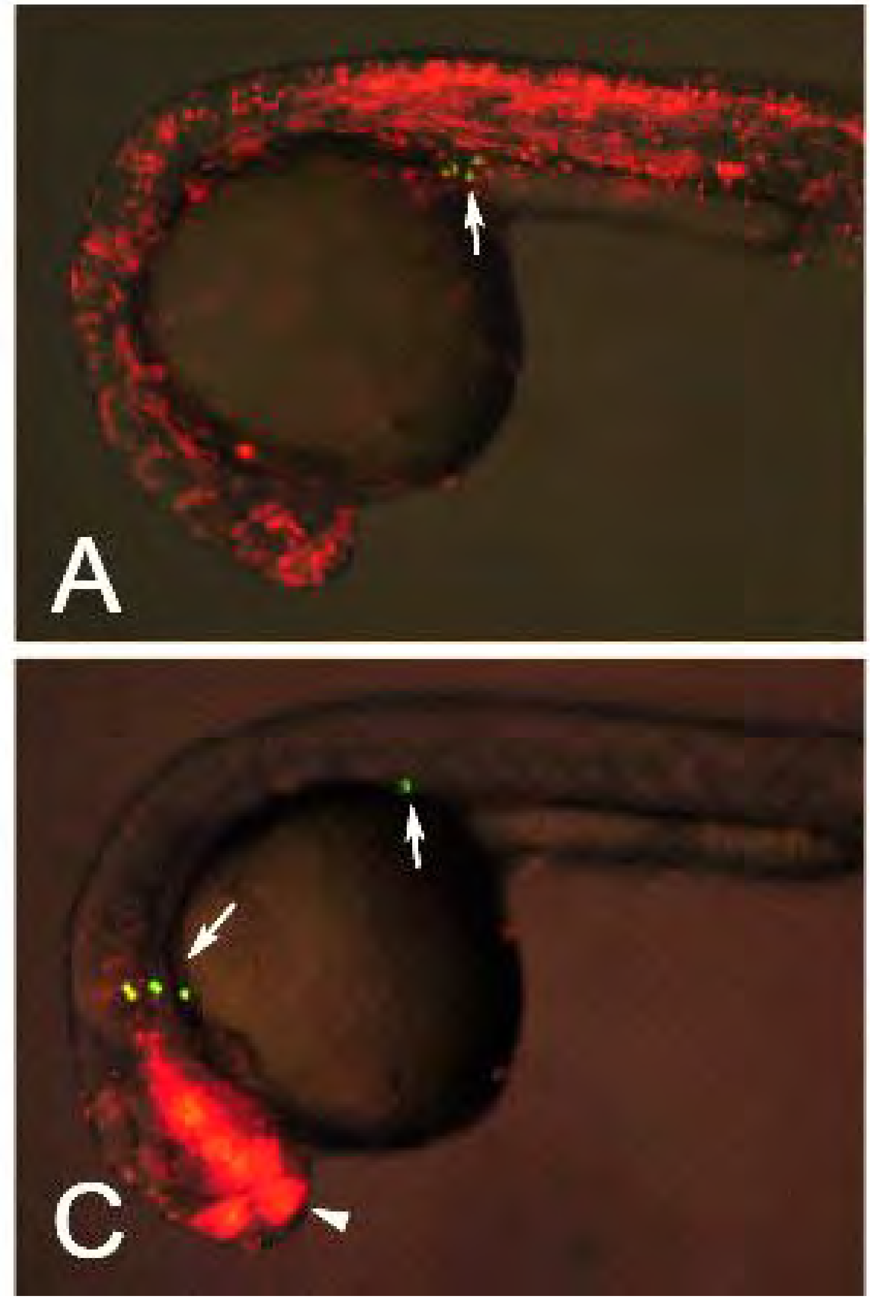


Köprunner et al., 2001

Blastomeres transplantation at the blastula stage

PGCs are located around the marginal region of the blastoderm





A: zebrafish blastomeres -> zebrafish

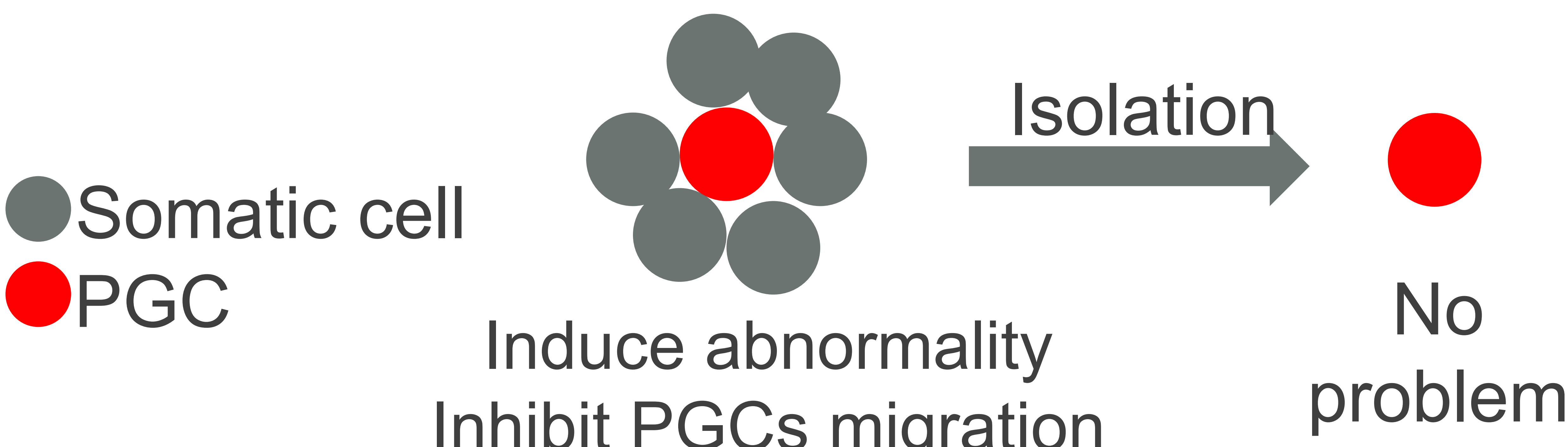
C: goldfish blastomeres -> zebrafish

This technique doesn’t work between different species!!!

In blastomeres transplantation methods at the blastula stage, germline chimera could be produced between same species.

However, somatic cells disturb the embryonic development and PGCs migration, in case of the combination of different species.

It is needed to isolate PGCs!



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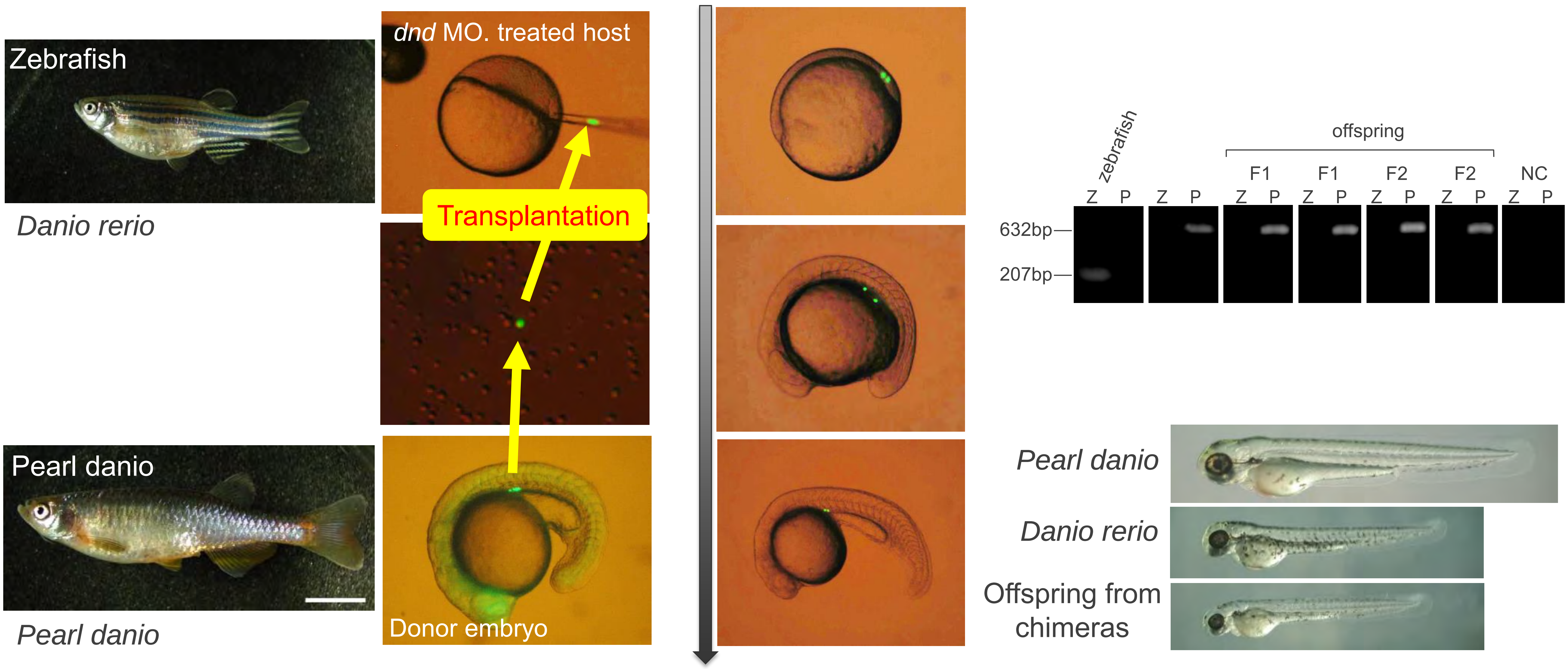
A single PGC transplantation between different species Saito et al. 2008 (BoR)



Efficiency of PGCs transplantation

	Total no. of transplanted embryos	Survived embryos at 2-dpf (%)	No. of successful PGC transfer	Efficiency (%)
Exp.	212	160 (75.5)	73	45.0
Cont.	164	120 (73.2)		

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Can PGC of far related fish species migrate to the gonadal region of host embryo?

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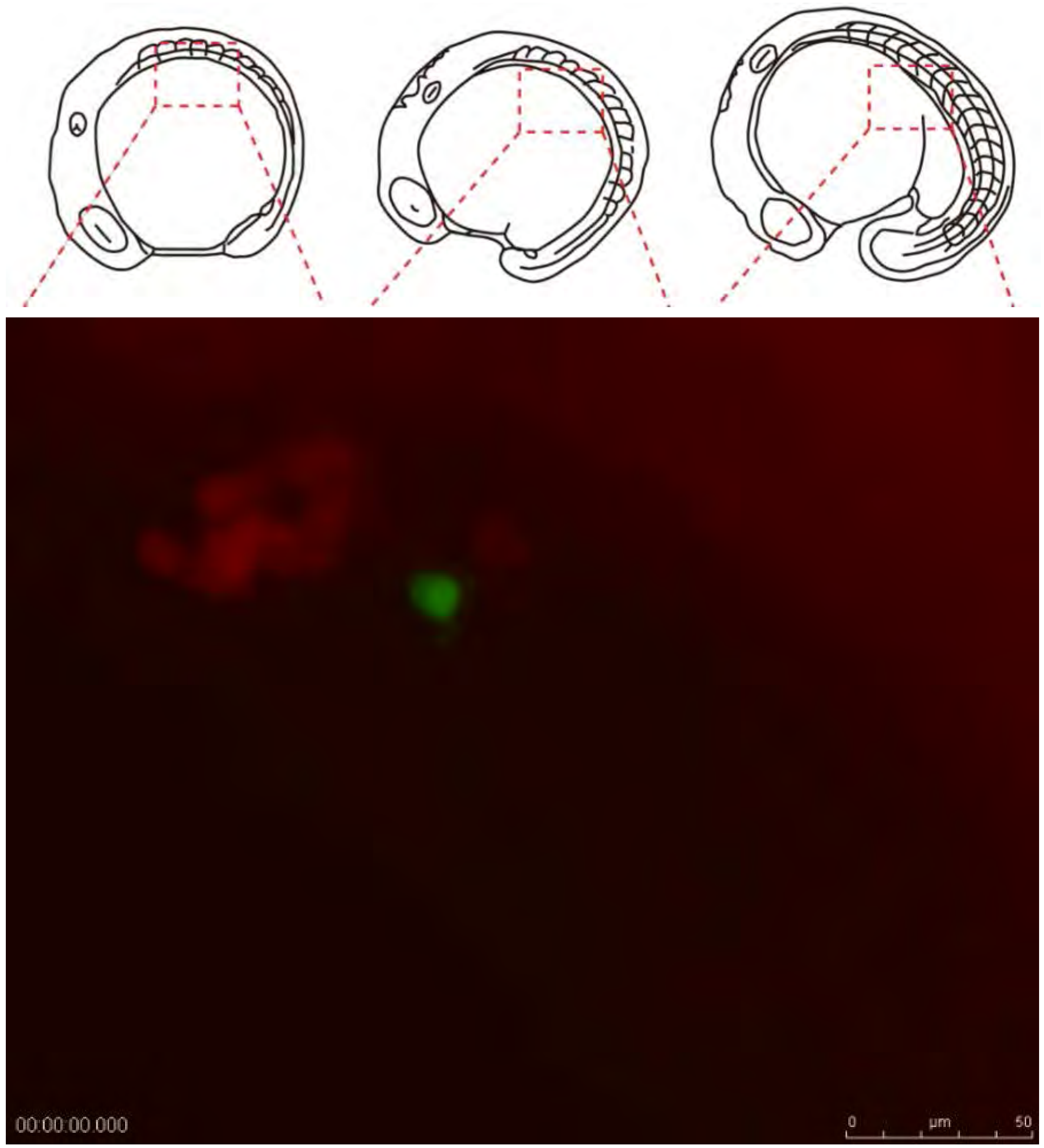
Japanese eel's PGC can migrate to the gonadal region of zebrafish embryo



Zebrafish PGCs: RFP
Eel PGC: GFP

However, transplanted PGC disappeared during its gonadal development.

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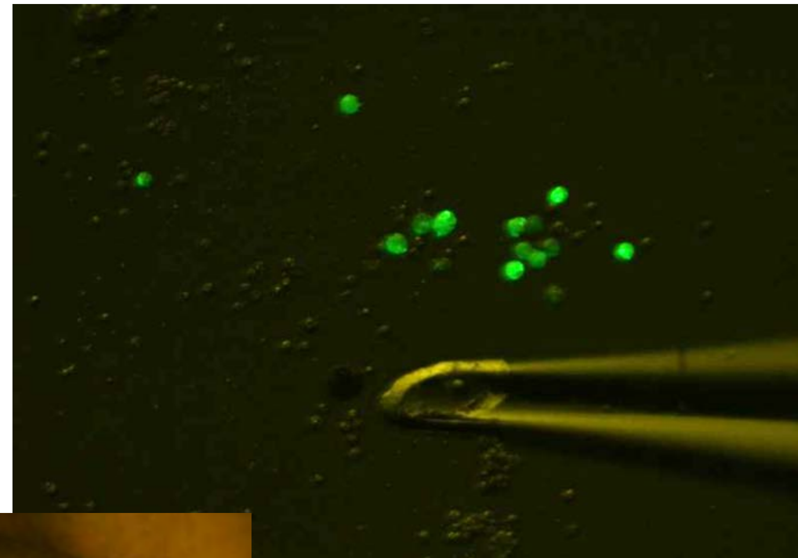
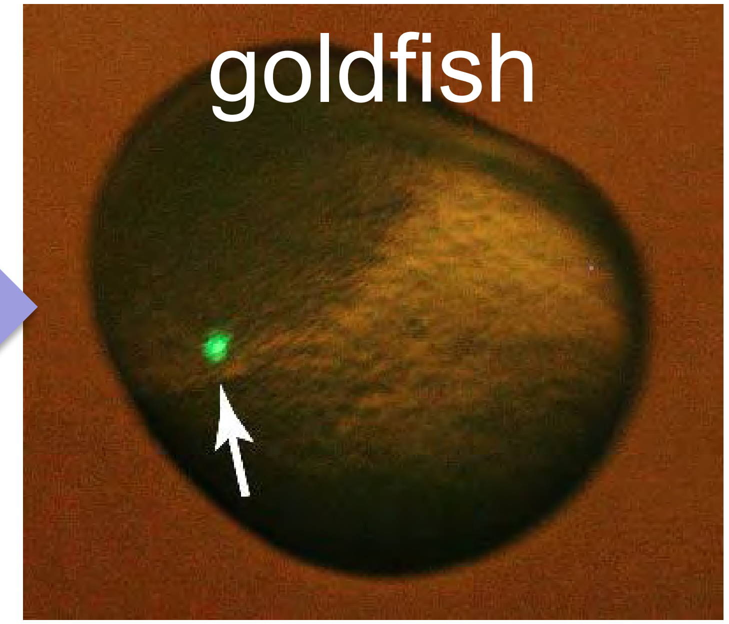
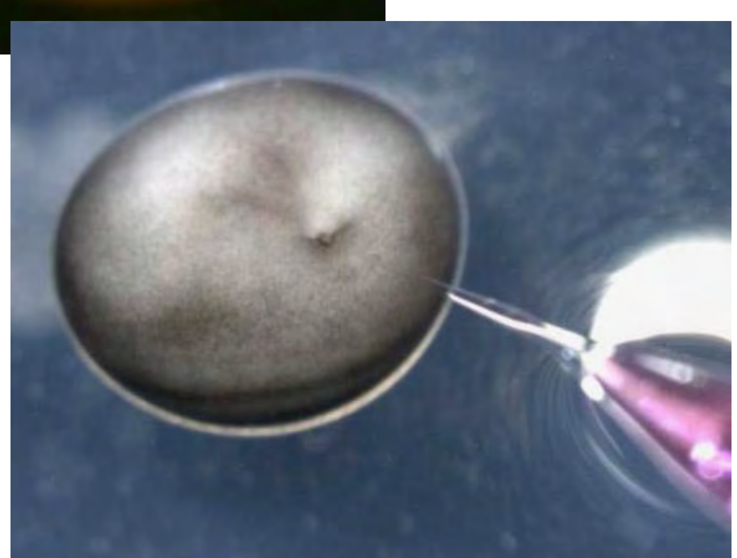
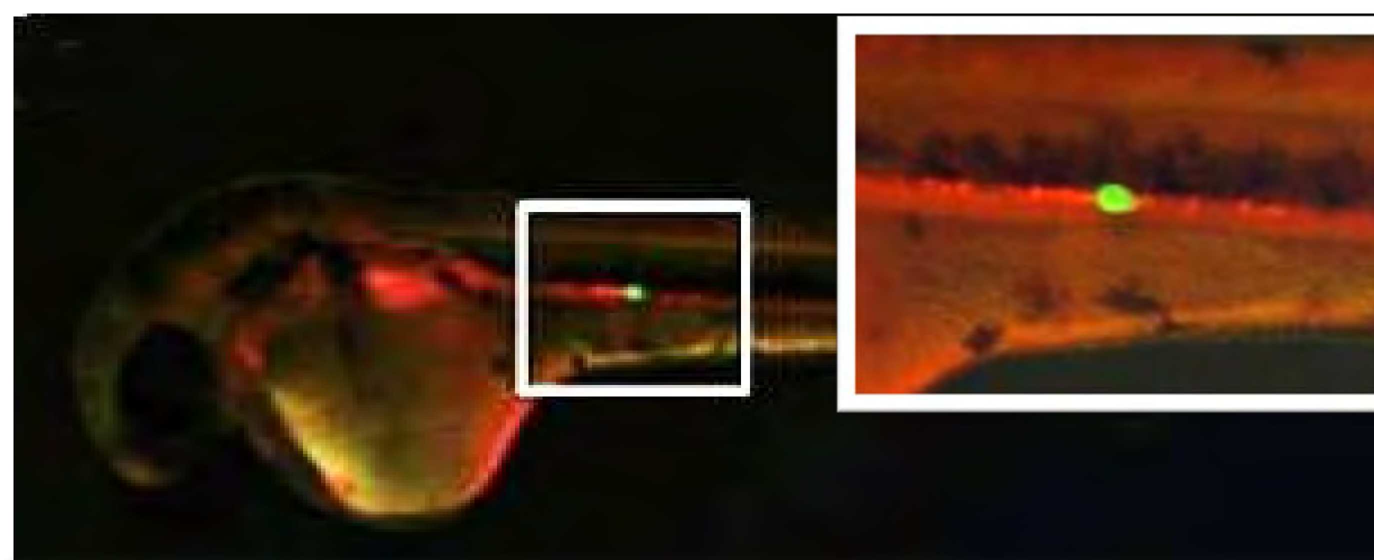
Transplantation of sturgeon PGC
Saito et al., 2014, Plos One









Sturgeon PGC in goldfish 6 days later

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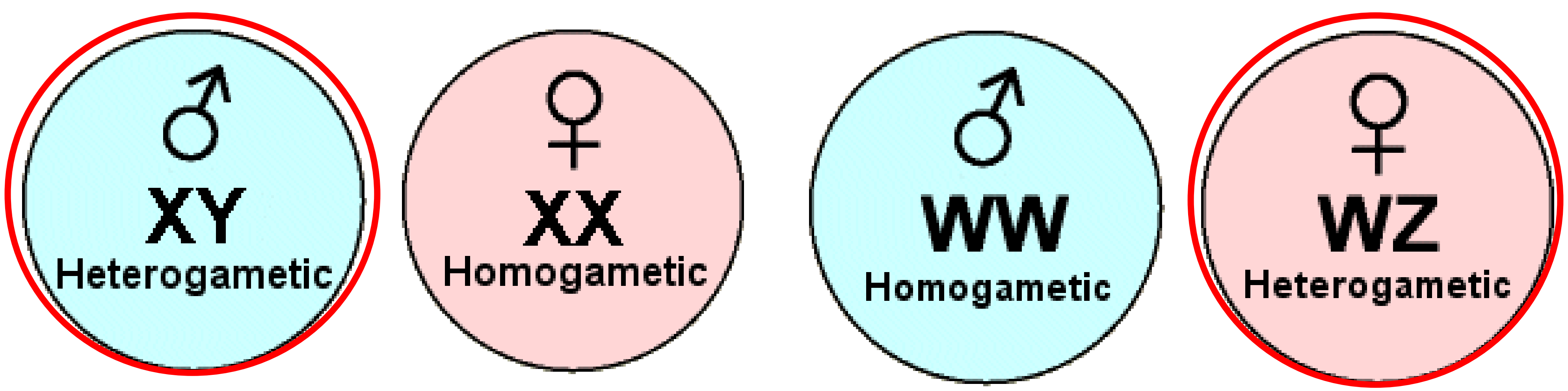
Summary of xenogeneic germ line chimera with zebrafish as host

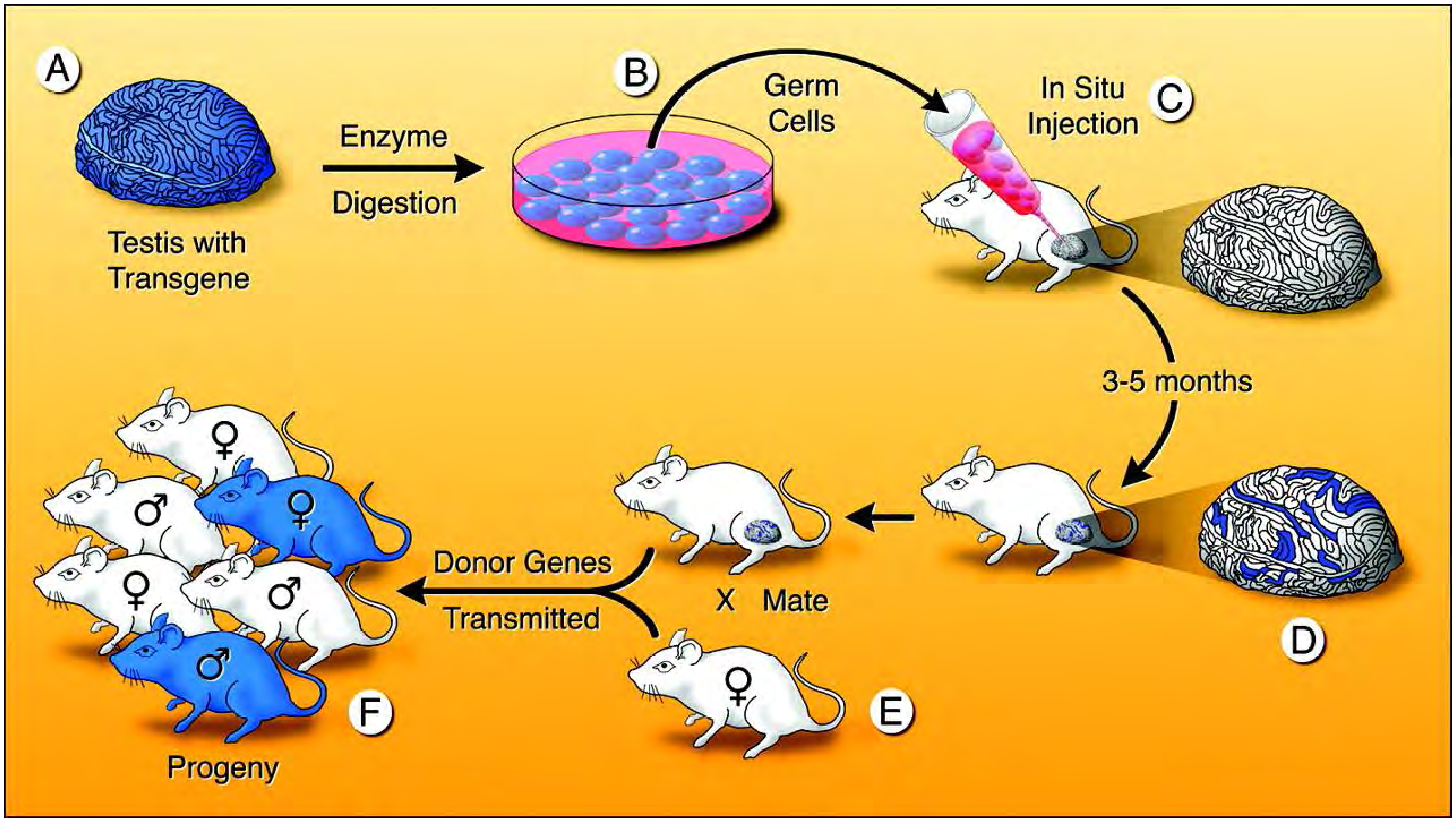
	Donor species	PGCs migration	Spermato-genesis	oogenesis
	Zebrafish (same species: *Danio rerio*)	○	○	○
	Pearl danio (same genus: *Danio*)	○	○	○
	Goldfish (same sub-family: Cyprininae)	○	○	X
	Loach (same sub-order: Cypriniforms)	○	○	X
	Medaka (different order: Beloniformes)	○	X	X
	Eel (different order: Anguilliforms)	○	X	X

Generation of germ line chimeras by transplantion of:

1. Primordial germ cells (PGCs)
2. Spermatogonia or oogonia



This technique was originated from mammalian's knowledge (Brinster et al. 1994)

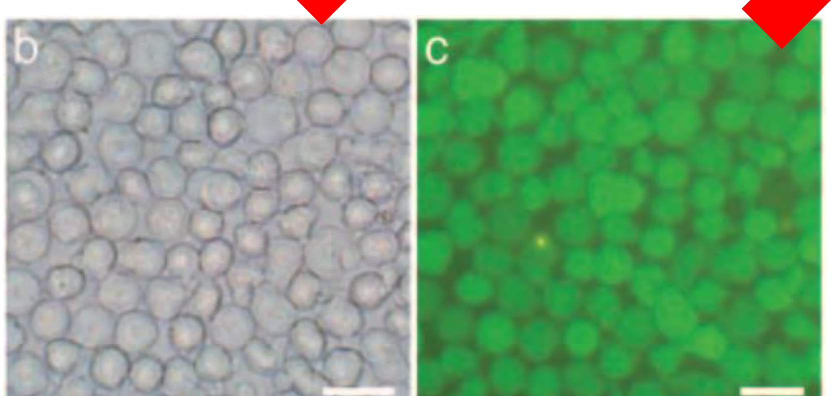


Isolation of spermatogonia and oogonia in fish

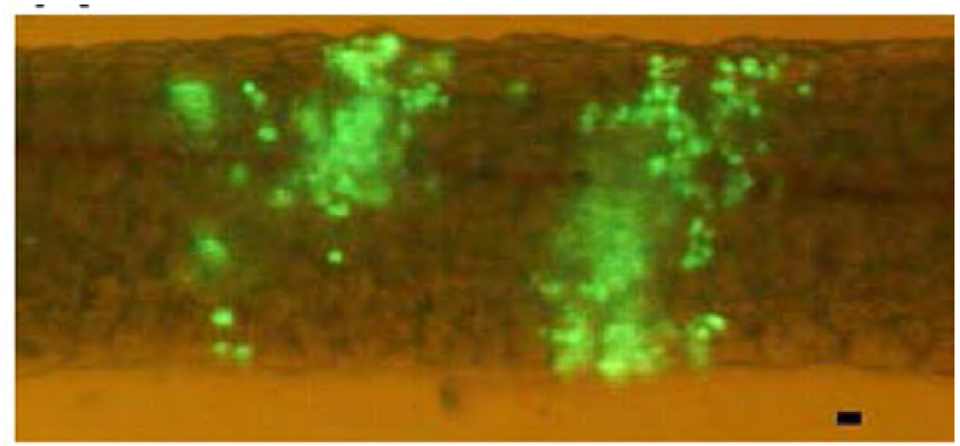
Generally testes or ovary is:

1) dissected
2) minced
3) dissociated by trypsin or collagenase
4) filtered
5) sorted (by percoll gradient, FACS, magnetic sorting, etc.)
6) transferred into host body

Spermatogonia transplantation into the body cavity hatched fry in salmonid species Okutsu et al. 2006.



Isolation and purification of spermatogonia



In the host gonad, transplanted spermatogonia proliferated!

Spermatogonia transplantation into the body cavity hatched fry in salmonid species Okutsu et al. 2007.

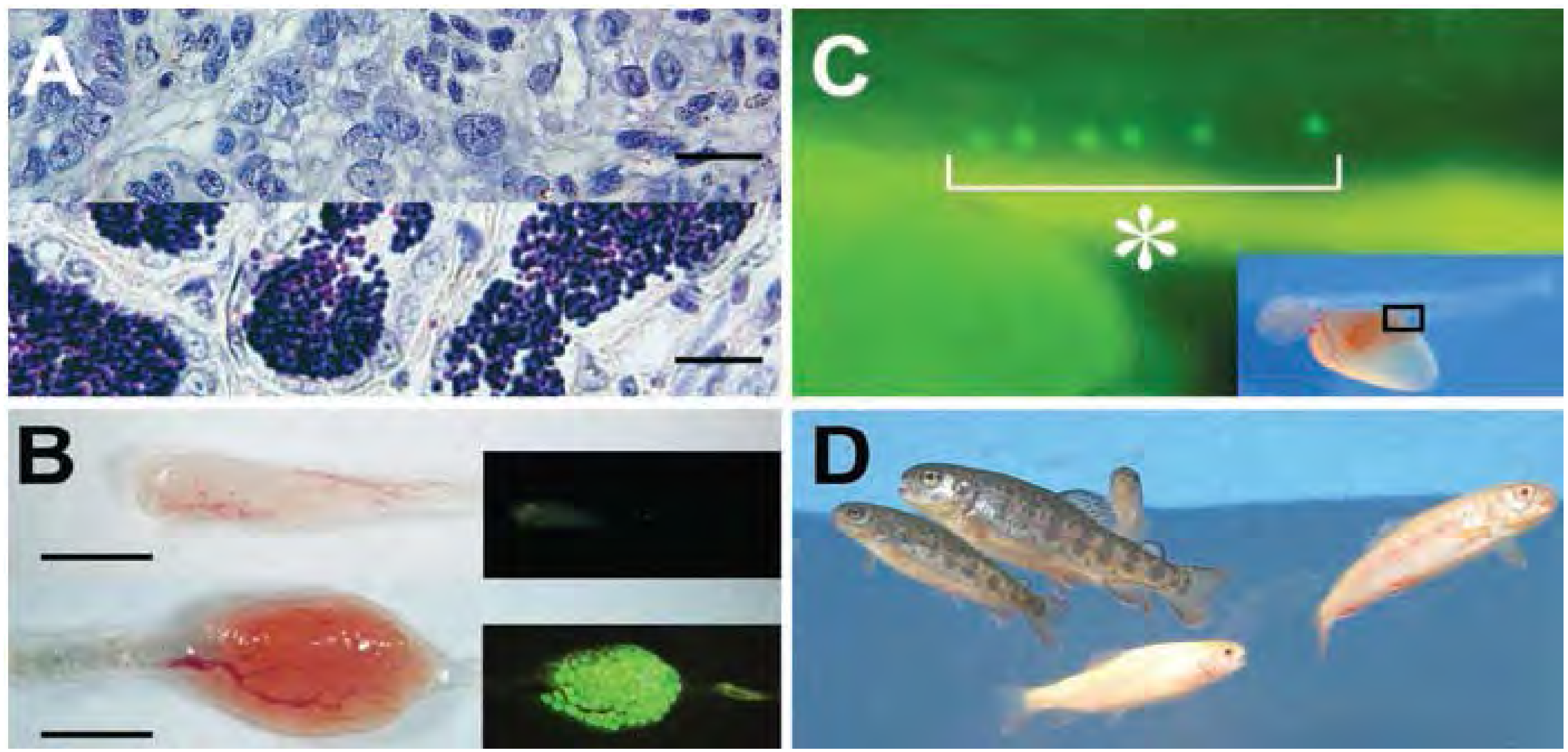
Chimera

Triploid

Chimera

Triploid

Chimera



Parents – salmon
Offspring - trout

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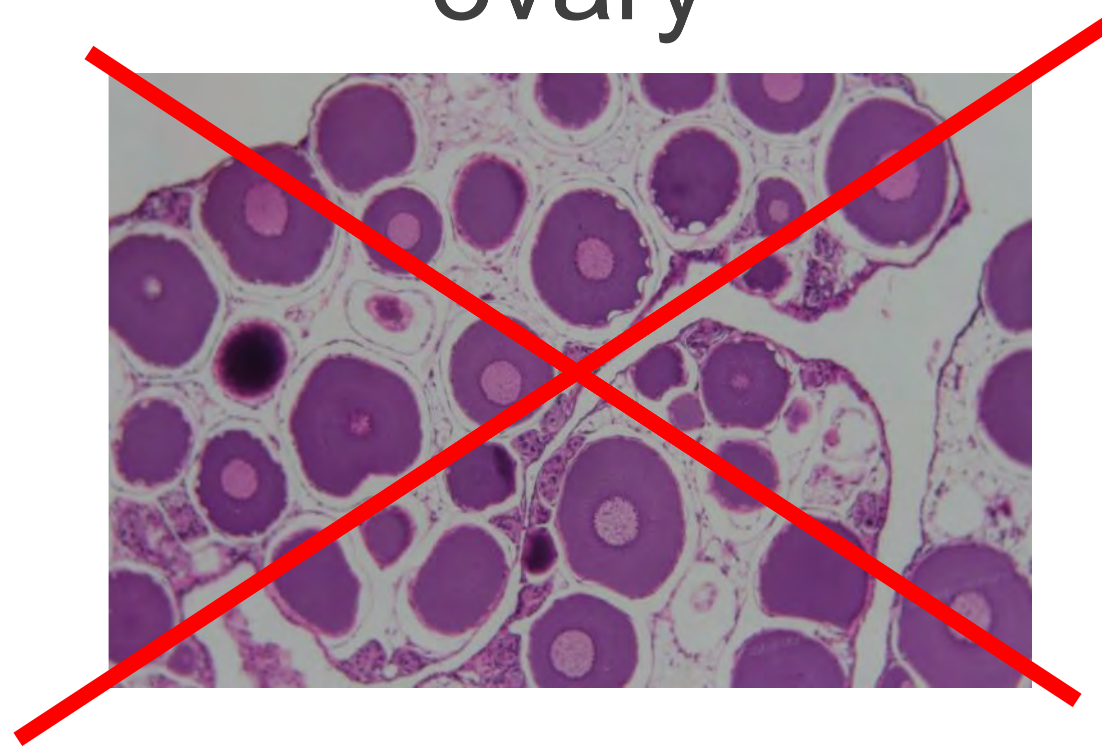
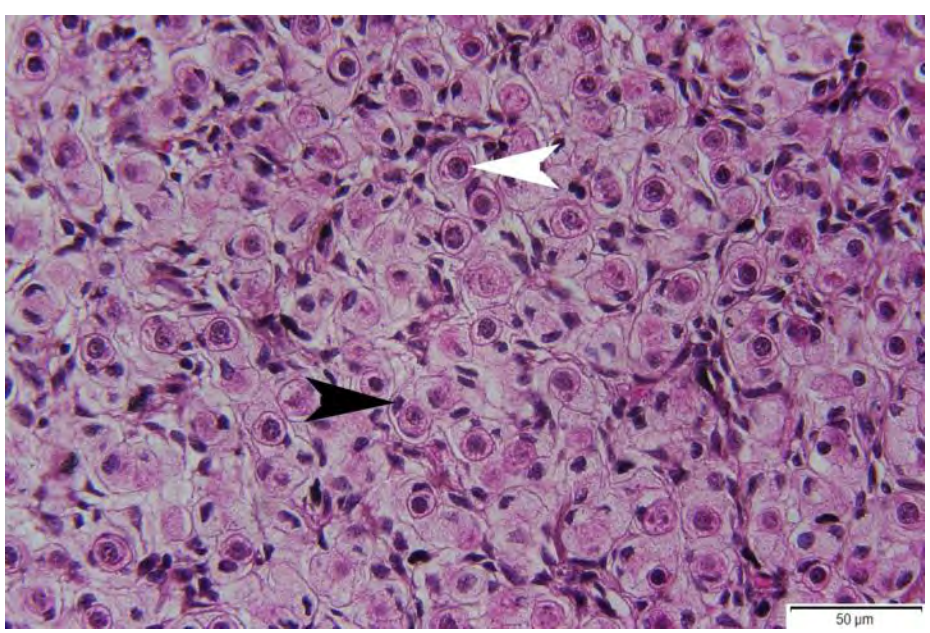
??? DONOR STAGE ???

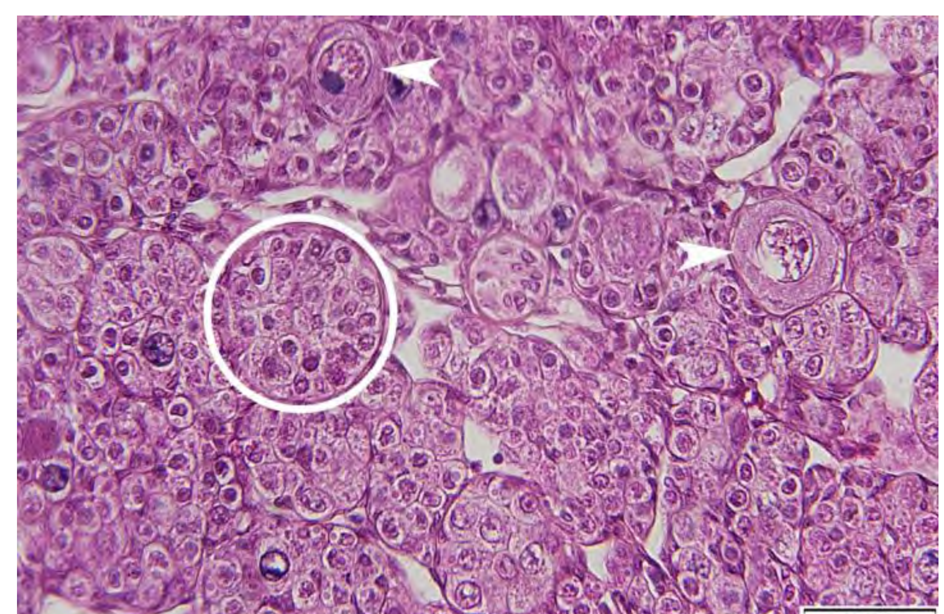
Stage of sturgeon donor

testes

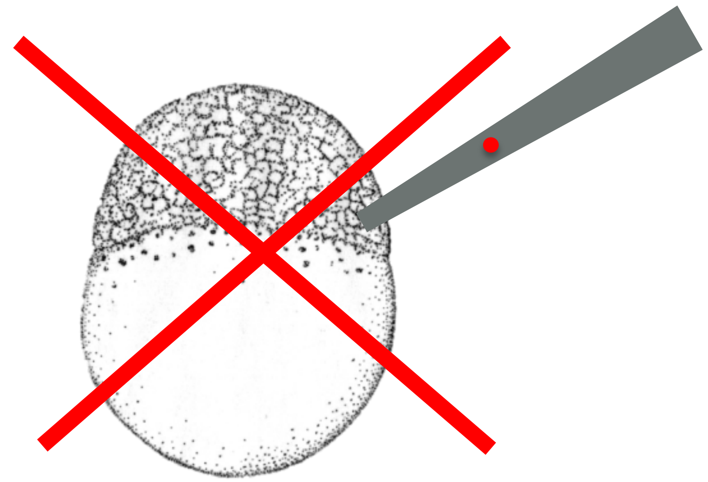
ovary



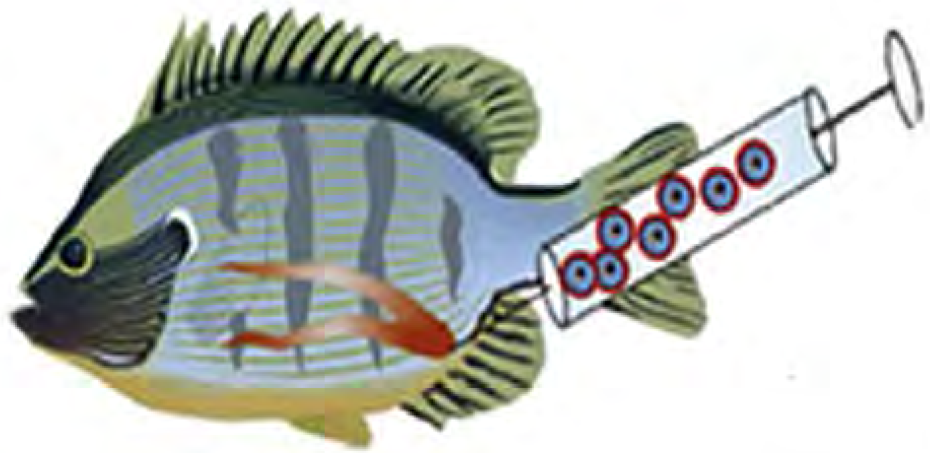




??? HOST STAGE ???



Okutsu et al. 2006



Spermatogonial transplantation in adult Nile tilapia

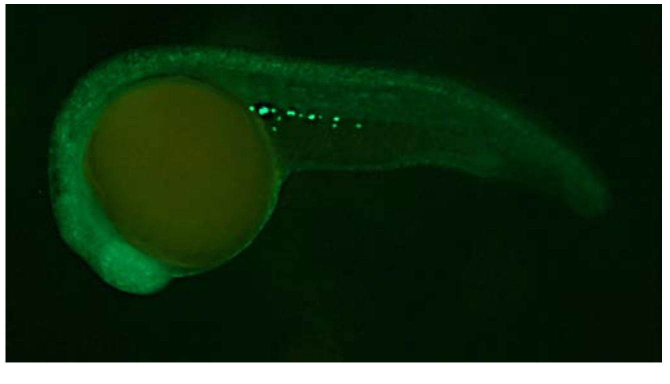
Lacerda et al. 2013

Advantages of spermatogonia/oogonia transplantation

You can obtain a lot of germline cells from a small piece of gonad.

From one 4-year-old Siberian sturgeon (gonad/body weight 4.3/1015) can be isolated approx. 1 mil. Spermatogonia/oogonia

X 5000



From one embryo can be transplanted up to 10 PGCs

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Sterilization of host

to produce only donor derived gametes

- Hybridization
- Triploidization
- Thermo-chemical treatment (busulfan)
- Knock-down of maternal mRNA

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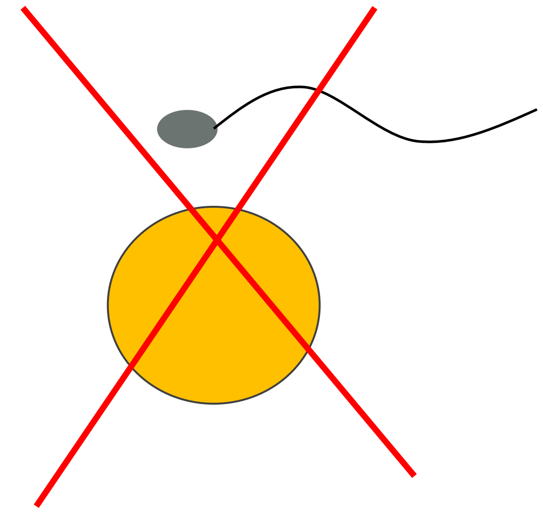
Hybridization

X

=



X

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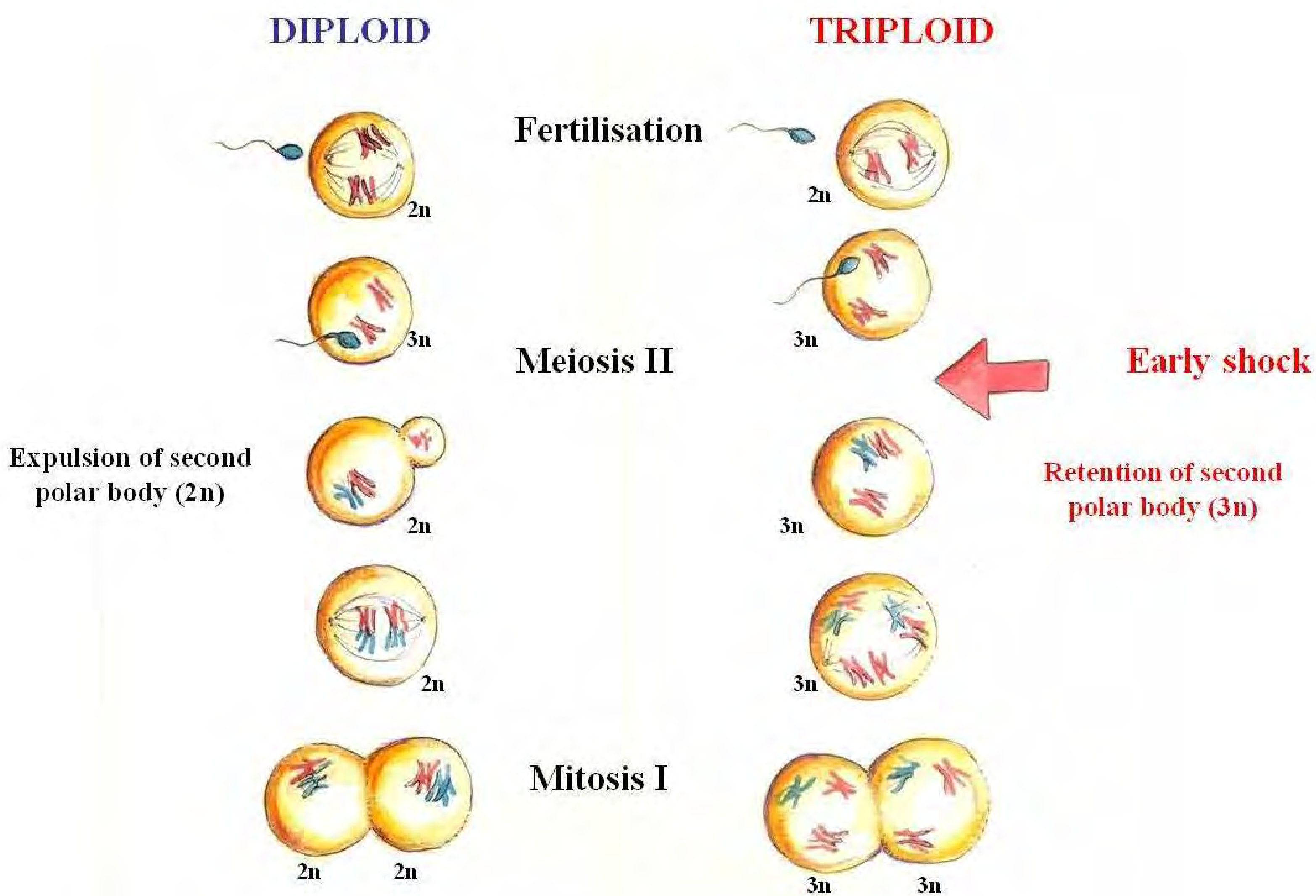
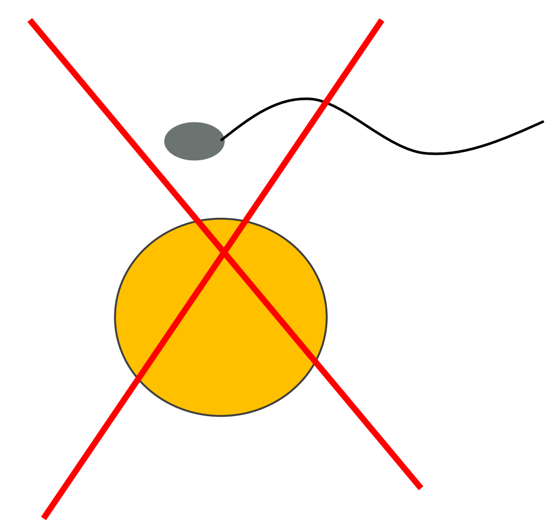
Triploidization

Suppression of meiosis II resulting in retention of the second polar body in fertilized eggs



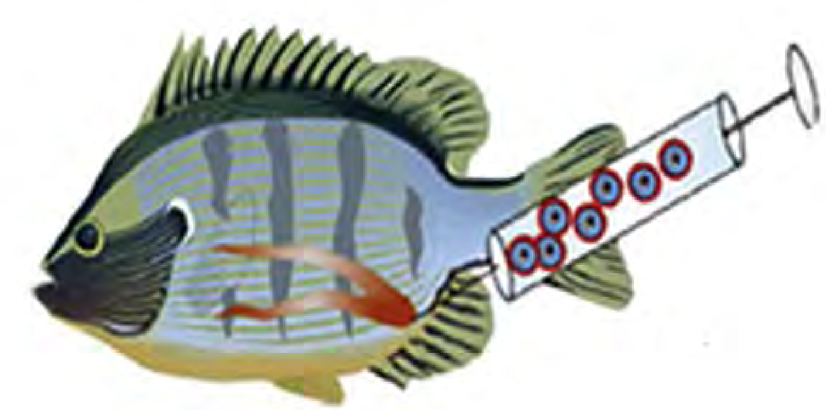
Three homologous chromosomal sets cannot correctly pair during the meiosis



Thermo-chemical treatment (busulfan)

Busulfan is used in cancer treatment. It affects faster proliferating cells.

Lacerda et al. 2013 treated telapia with higher temperature combined with busulfan, which cause temporal sterility.



Spermatogonial transplantation in adult Nile tilapia

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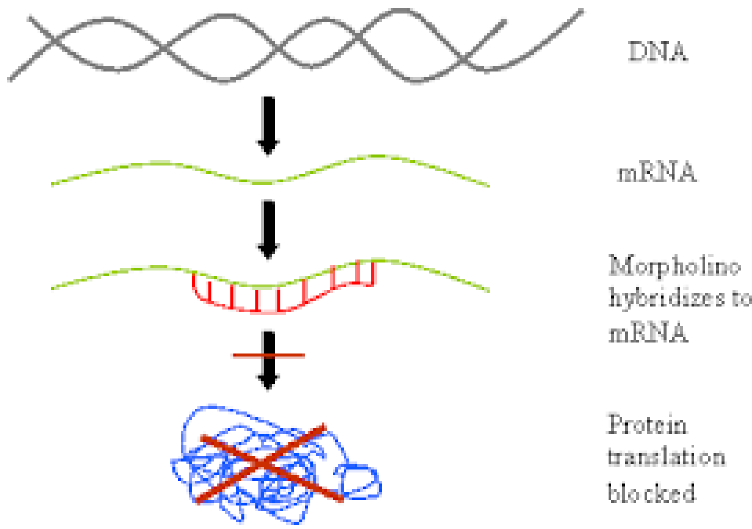
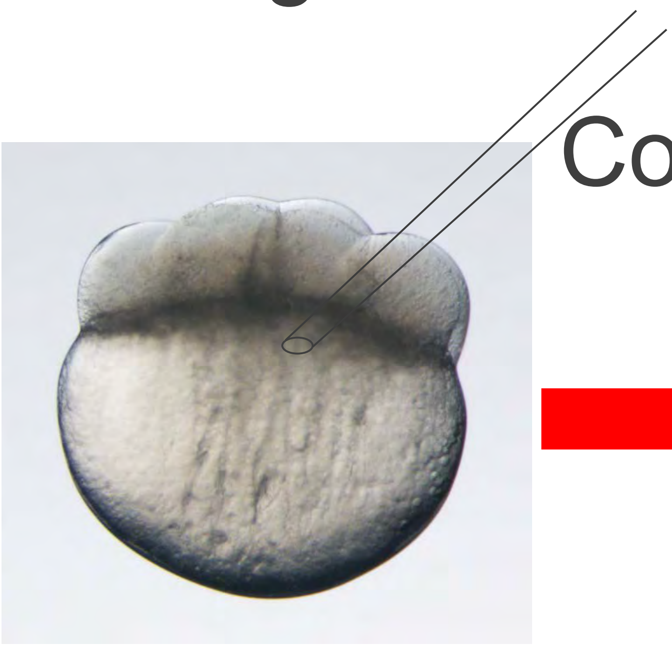
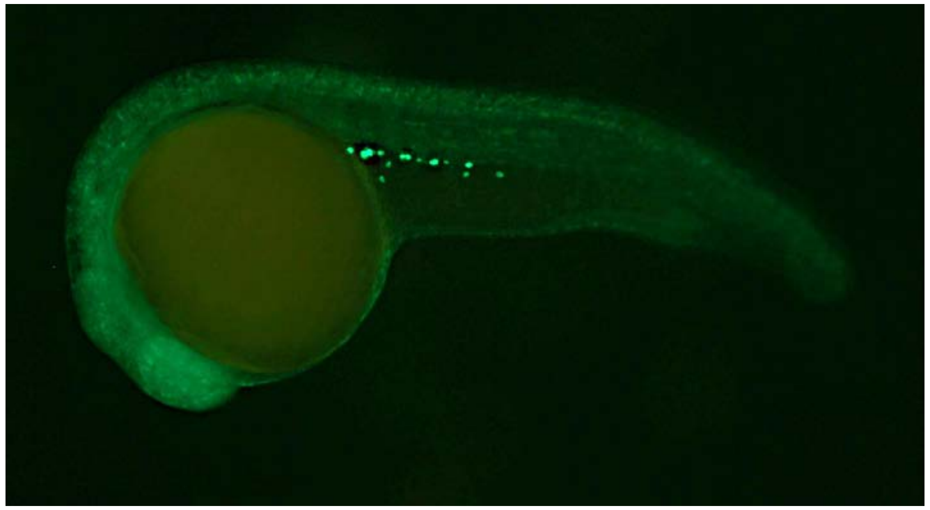
Knok-down of maternal mRNA

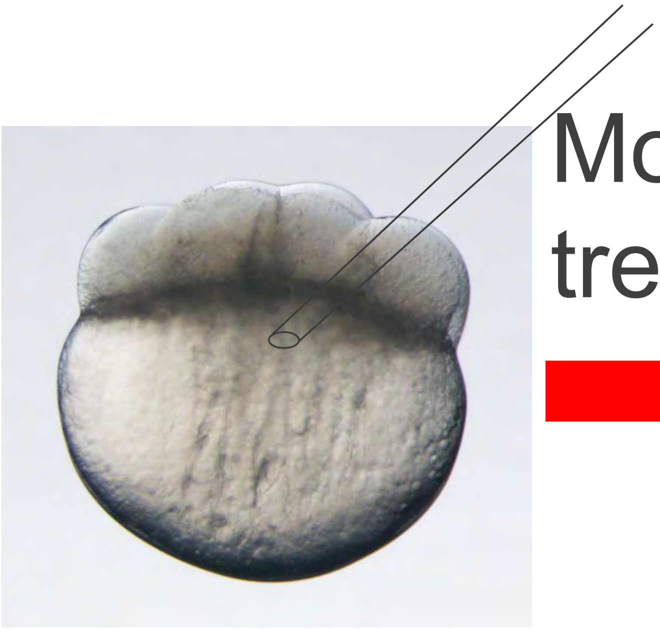


Inactivation of dead end (*dnd*) mRNA using antisense morpholino oligonucleotide, which inhibits gene translation.

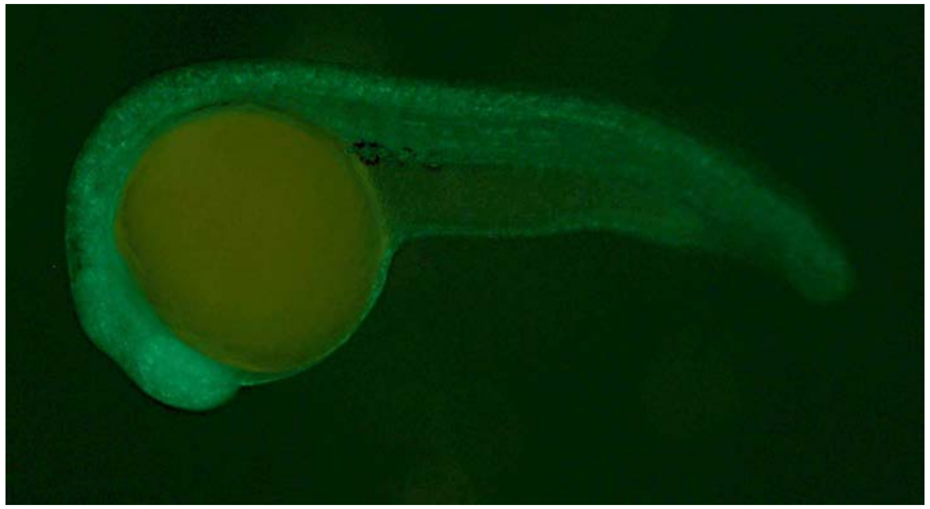


Control





Morpholino treatment



Concluding words

Biotechnology using germ stem cells has obviously high potential especially in fish having high fecundity throughout the life

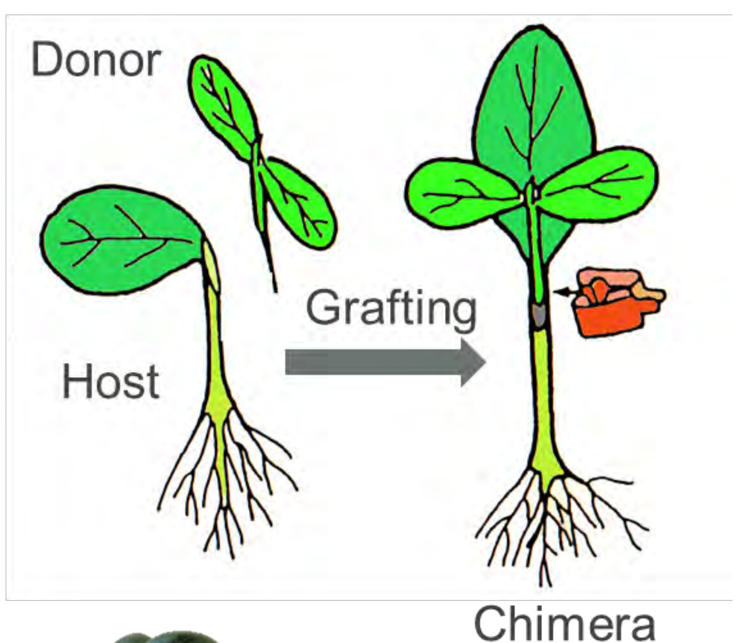
female – millions (10^6) / male – trillions (10^{12})

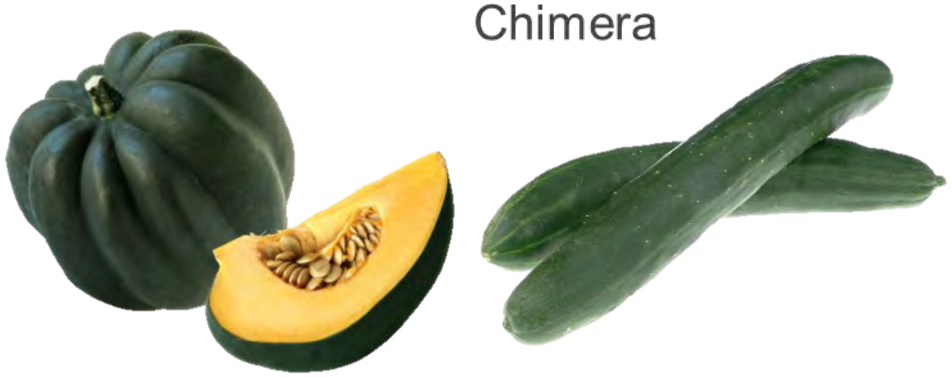
Cryopreservation and transplantation of spermatogonia and oogonia is quite easy and efficient approach.

Selection and preparation (sterilization) of donor is crucial.

Concluding words

Biotechnology using germ stem cells in practice is still sound of future, but profitable technologies are sooner or later introduced in practice and automated





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Acknowledgement

- COST Office (Food and Agriculture COST Action FA1205: *Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry*; AQUAGAMETE).

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Thank you for your attention