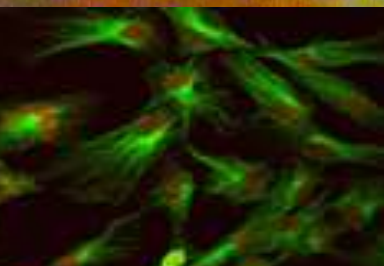


# Somatic cells as an additional resource for genome preservation: the promises and the difficulties.

Catherine Labbé,  
Fish Physiology and Genomics, INRA Rennes, France



**AQUAGAMETE**



# Diploid Cell Nuclear Transfer, a tool in genome conservation

Haploid cells  
(half the genome)

*Spermatozoa*



*Oocytes*

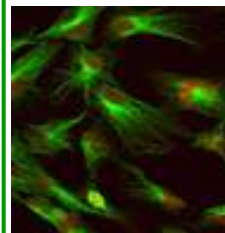
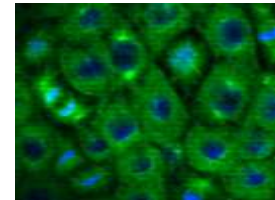


Diploid cells  
(biparental genome)

*Embryos*



*Embryonic cells, PGCs, SSC ...*



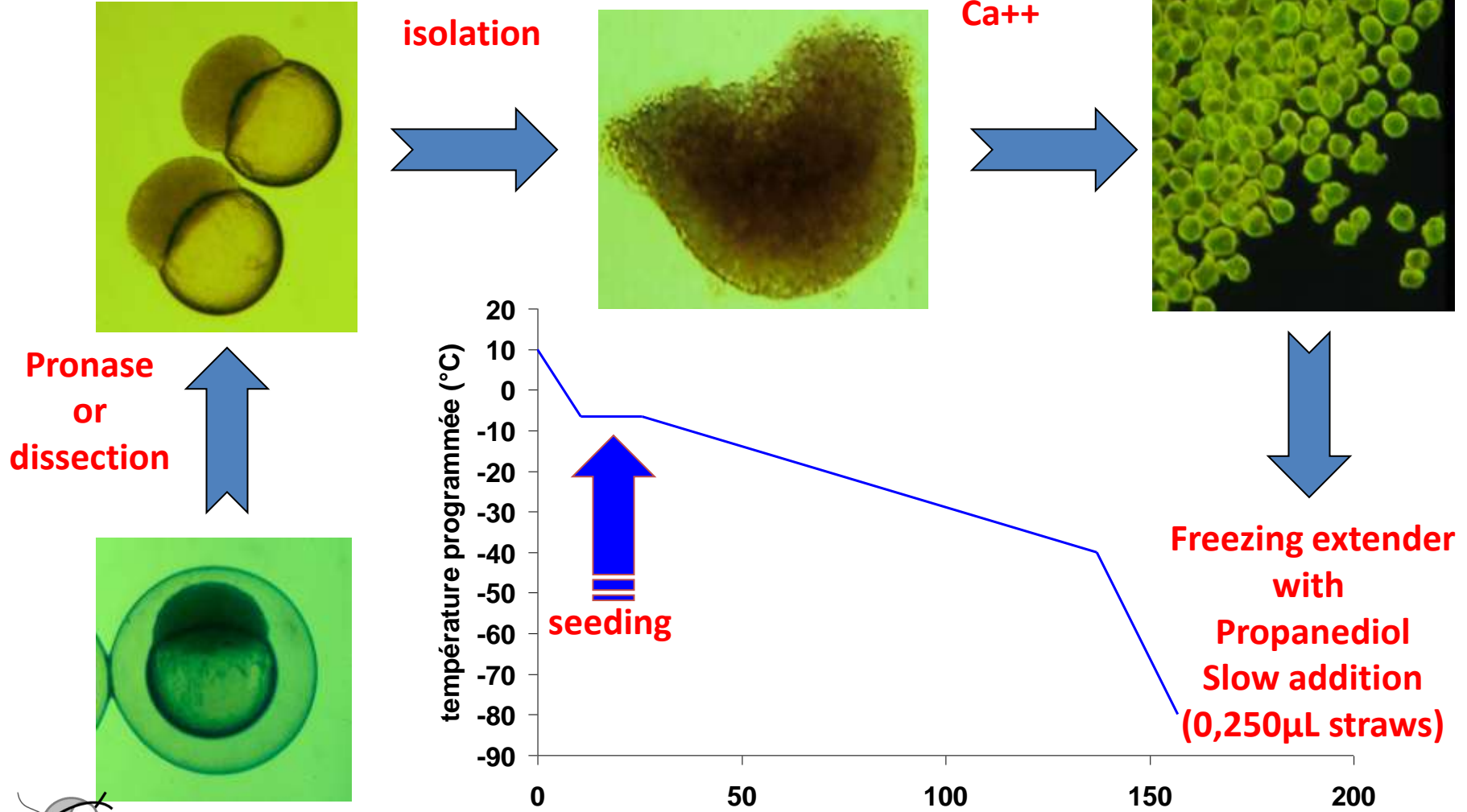
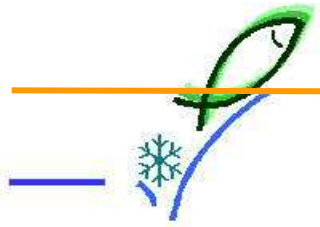
*Fin somatic cells*

- Undetermined fish OK as donor
- Fins easy to collect
- Extensive cryobanking of a diploid genome

- ⇒ Cryopreservation of diploid cells
- ⇒ Fish reconstruction: **NUCLEAR TRANSFER**



# Cryopreservation of embryonic cells



Pronase  
or  
dissection

isolation

Medium  
without  
Ca<sup>++</sup>

température programmée (°C)

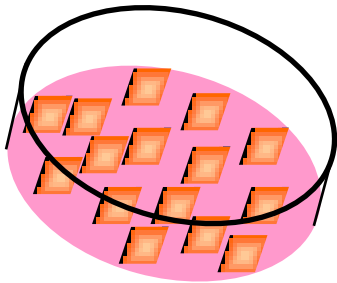
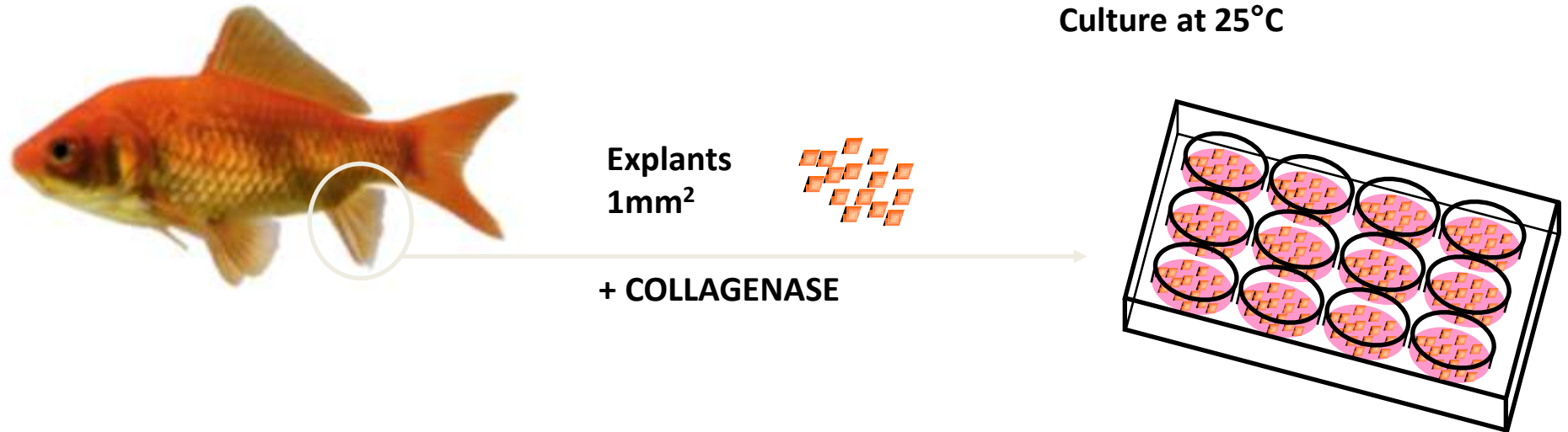
seeding

Freezing extender  
with  
Propanediol  
Slow addition  
(0,250µL straws)

0 50 100 150 200

minutes

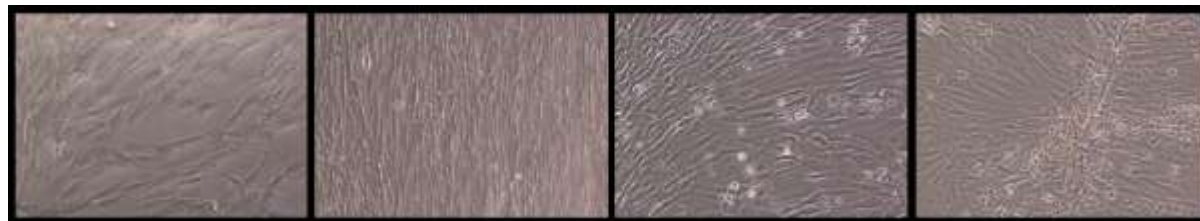
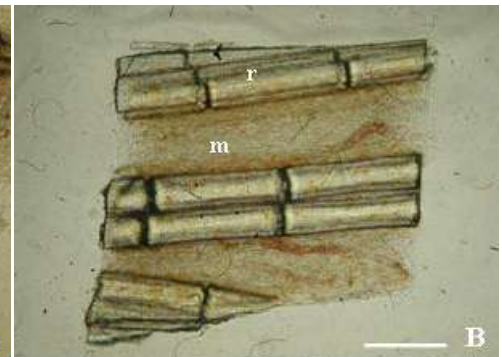
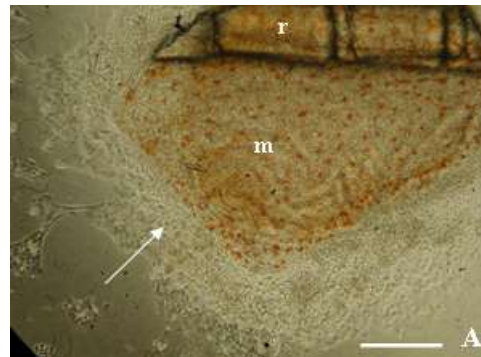
# Culture of somatic cells



Adhering

+

Non-adhering



# Culture of somatic cells

## Fresh Explant

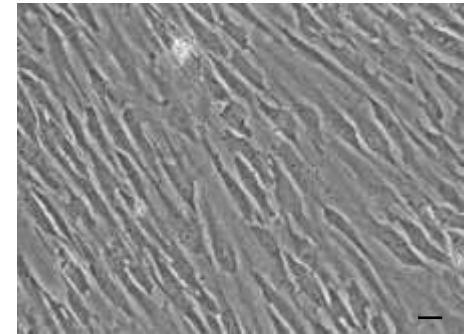
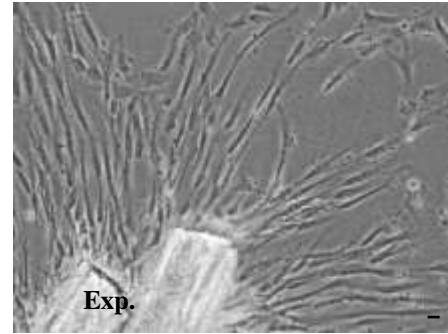
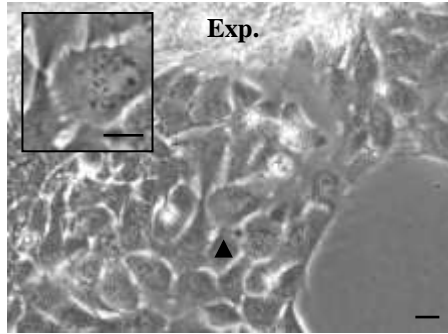
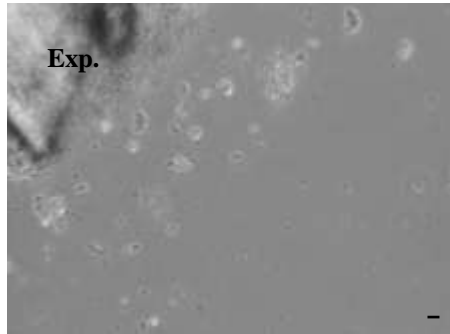
## Recycled Explant

d1

d3

d1

d3

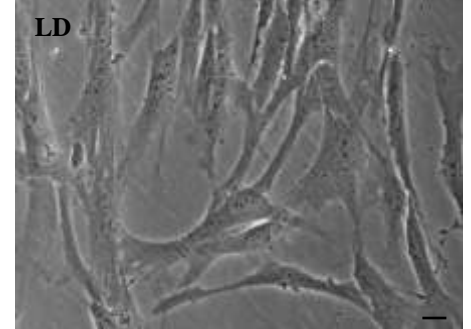
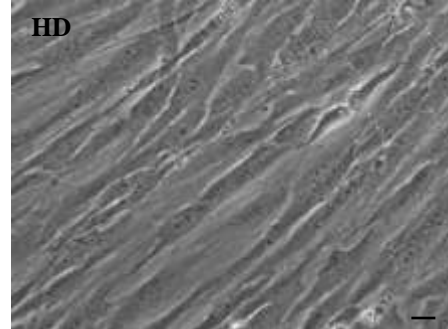
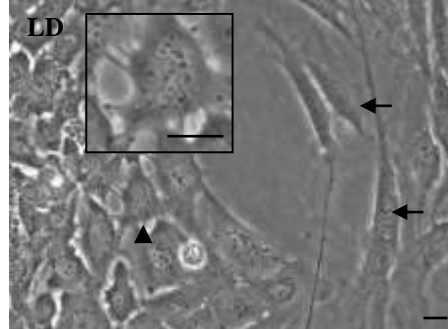
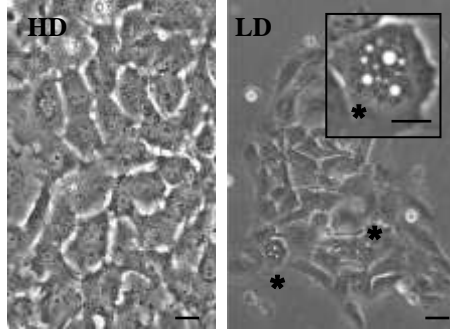


d7

d7

d7

d7

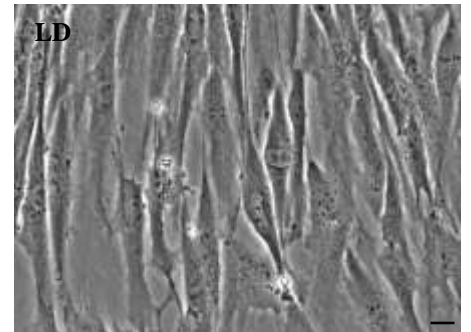
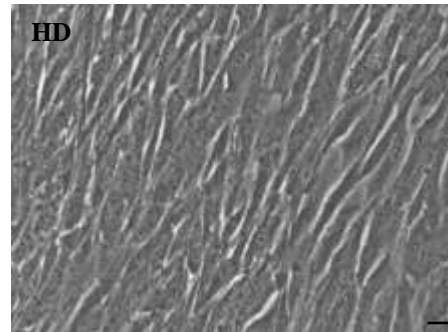
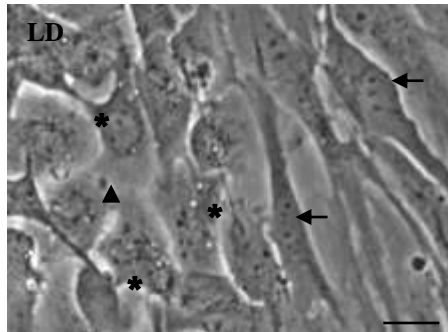
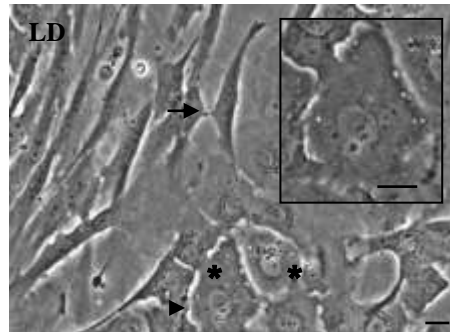


d15

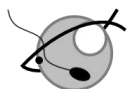
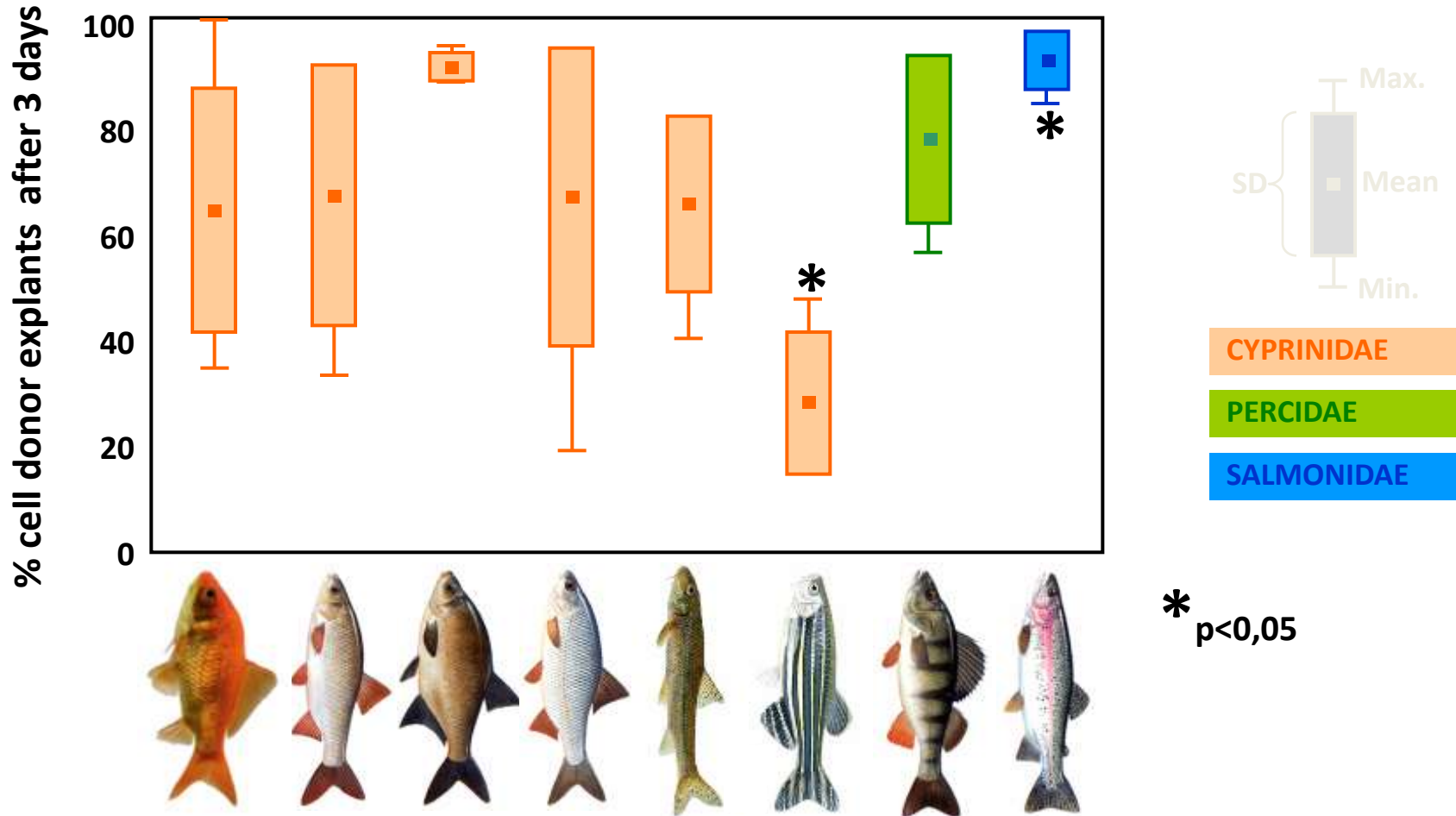
d15

d15

d15

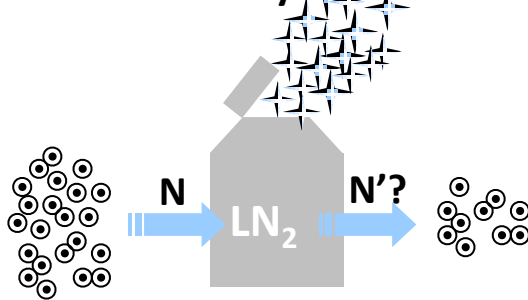


# Culture of somatic cells

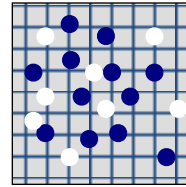


# Cryopreservation of somatic cells

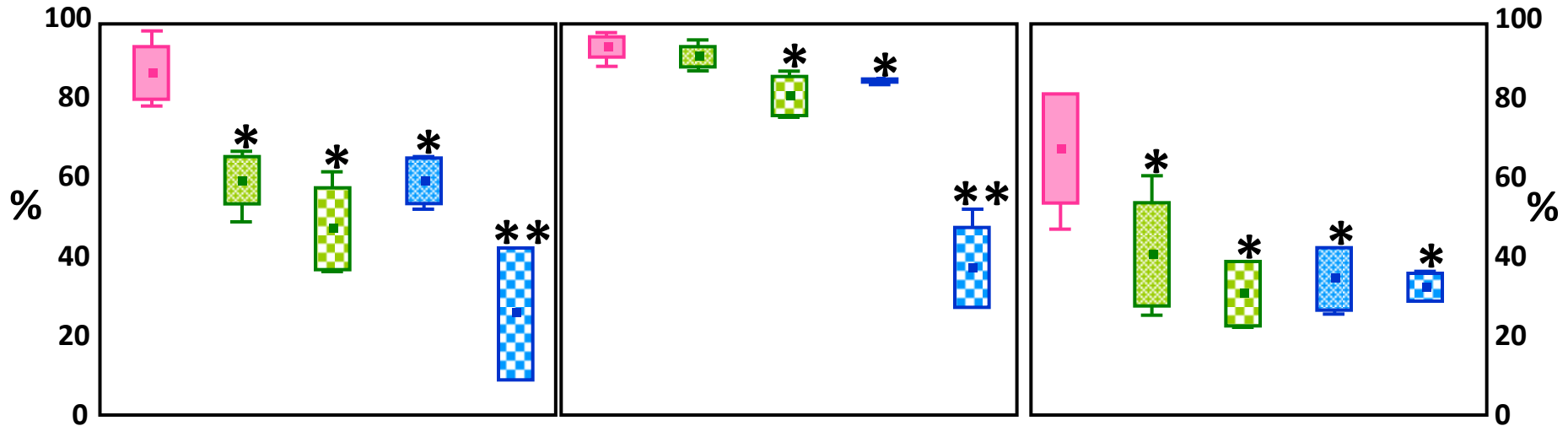
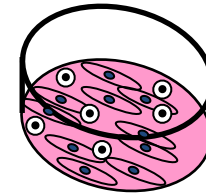
1. Recovery %



2. Viability (trypan blue)



3. Culture ability



L-15

Fresh cells

DMSO

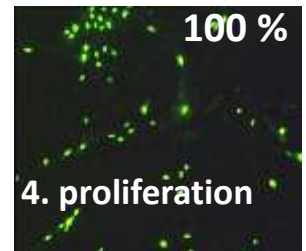
PrOH

+ sucrose + serum (10%)

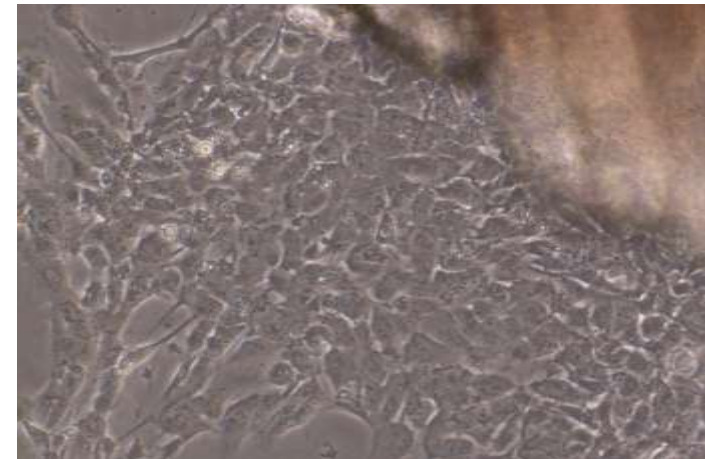
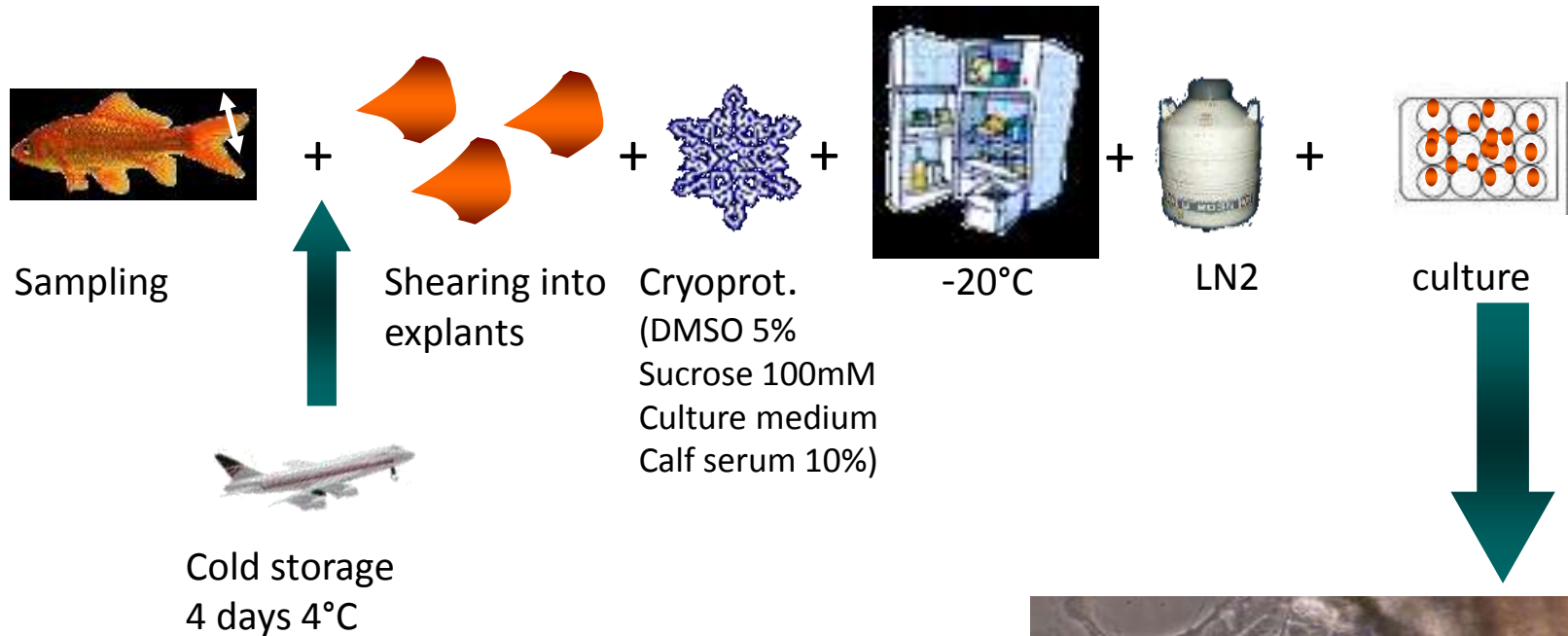
Slow (cryovials)

Fast (straws)

2 freezing rates



# Cryopreservation of fin pieces: Freeze first, think later

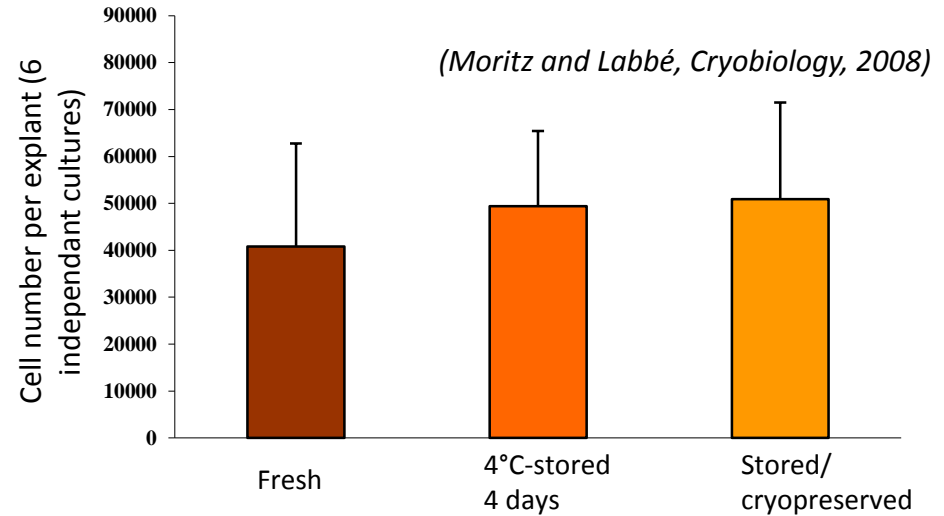
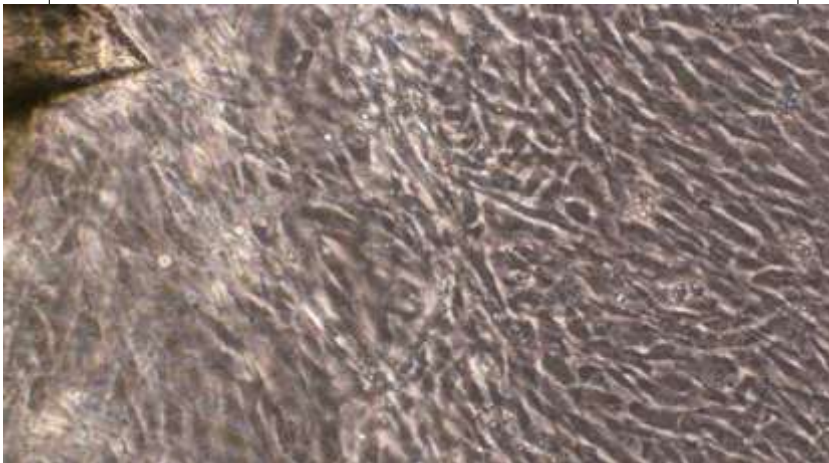
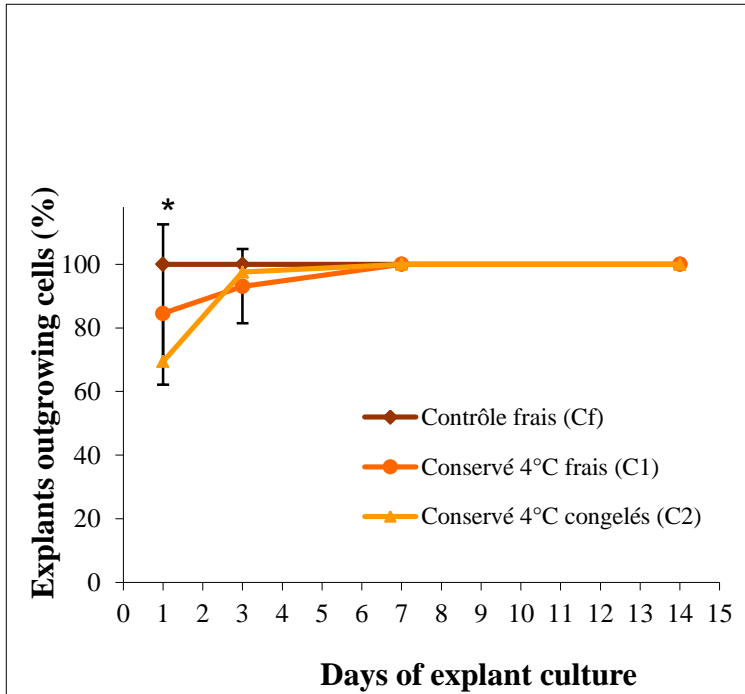


(Moritz and Labbé, Cryobiology, 2008)





# Culture of fin pieces after cryopreservation



- Ability of the fins to outgrow somatic cells after storage and freezing
- All tested species outgrew fin cells: salmonids, cyprinids, sturgeons
- Thick fins not as suitable toward cryopreservation
- Cryopreservation of cells AFTER explant culture possible for several species



# Regeneration of fish from fin cells



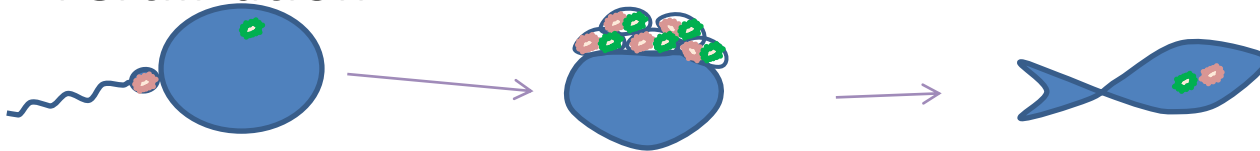
© 2001 MIKE GETTERS—DAYTON DAILY NEWS-TRIBUNE MEDIA SERVICE



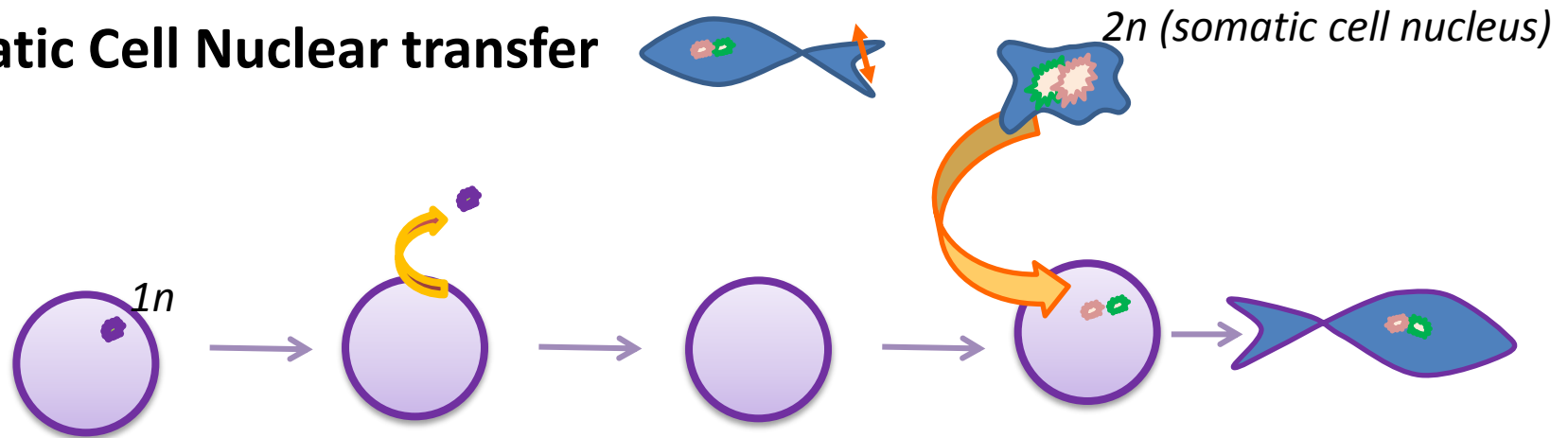
# The principle of Somatic Cell Nuclear Transfer in fish

= restoring fish from frozen-thawed explants/cells

## Fertilization



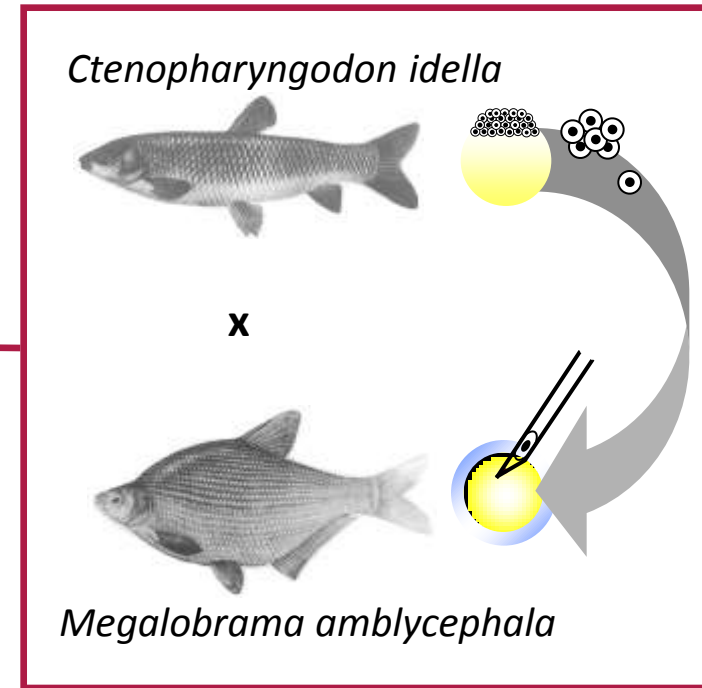
## Somatic Cell Nuclear transfer



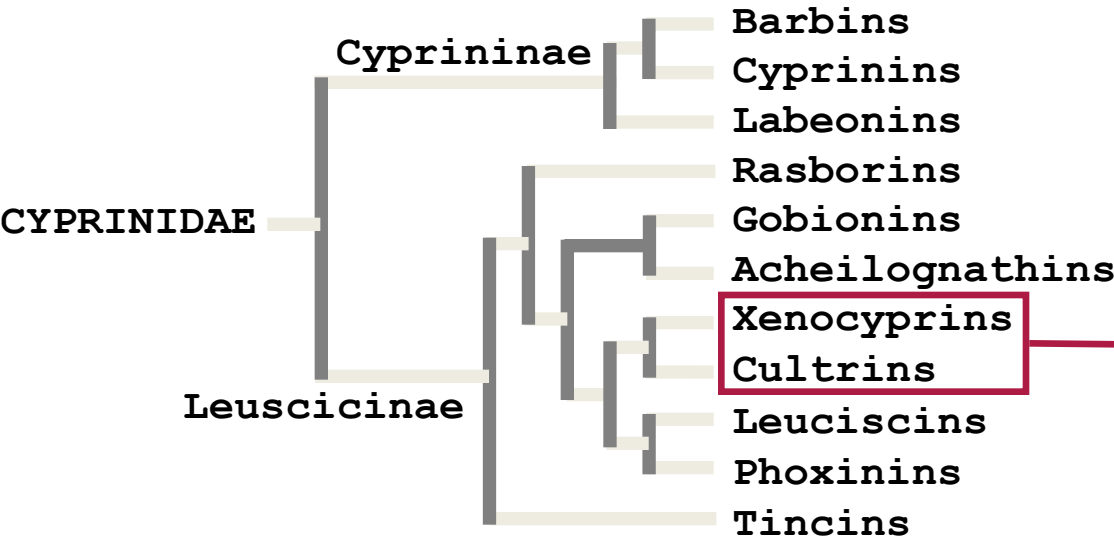
# Recipient egg: same or different species ?

Interspecific nuclear transfer: when females of the donor strain are not available

Yan *et al.*, 1985 :

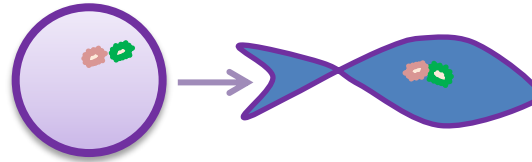


**Mammals** : *Bos taurus* x *Bos gaurus* (Lanza *et al.*, 2000)



# Genetic considerations

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## The mitochondrial DNA

**37 genes (rRNA, tRNA, 13 proteins)**

**transcription controlled by nuclear DNA factors**

**The egg material: mRNA, proteins, mitochondria**

**Roots of the compatibility between species**

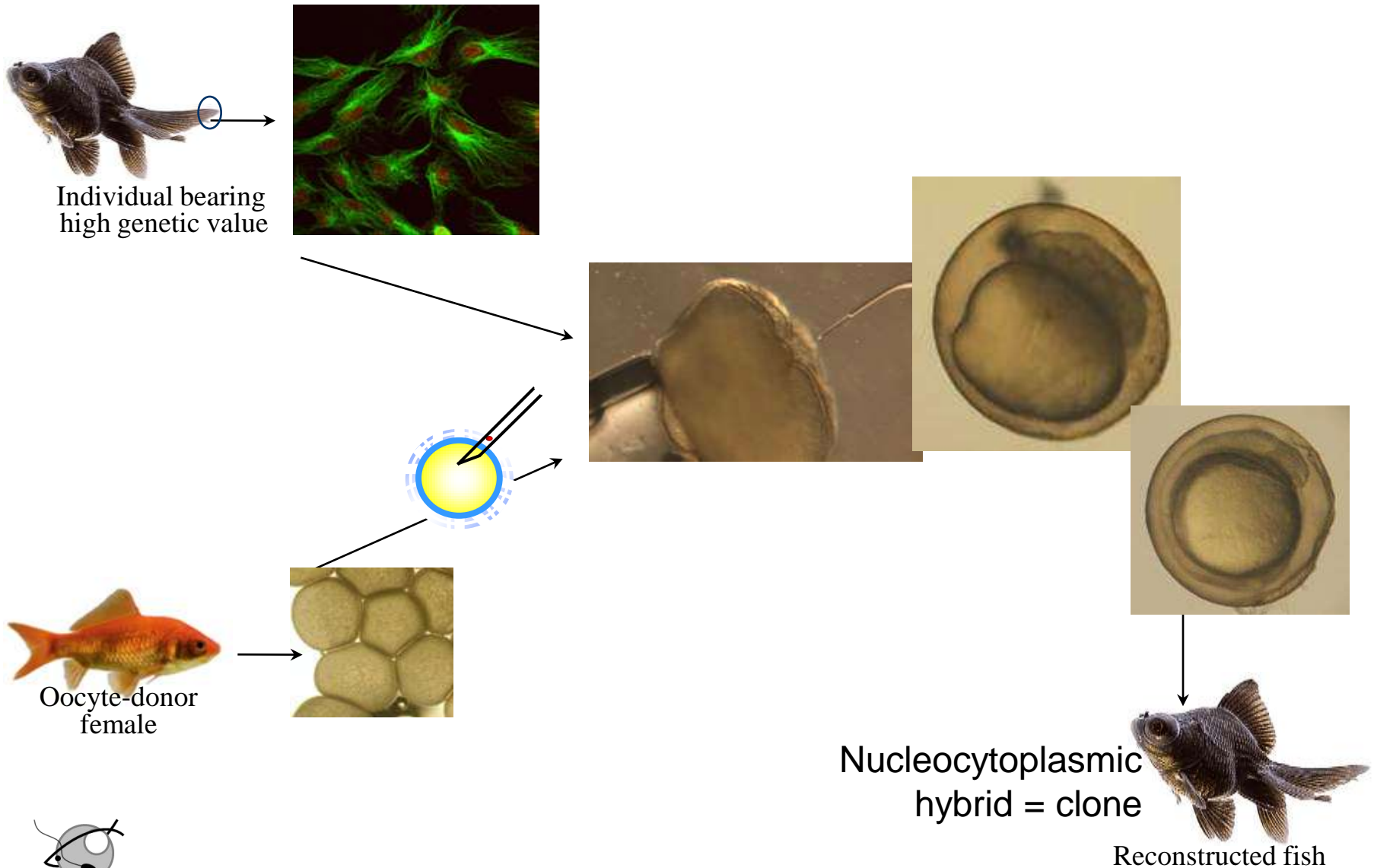
**genetic distance?**

**yolk size?**

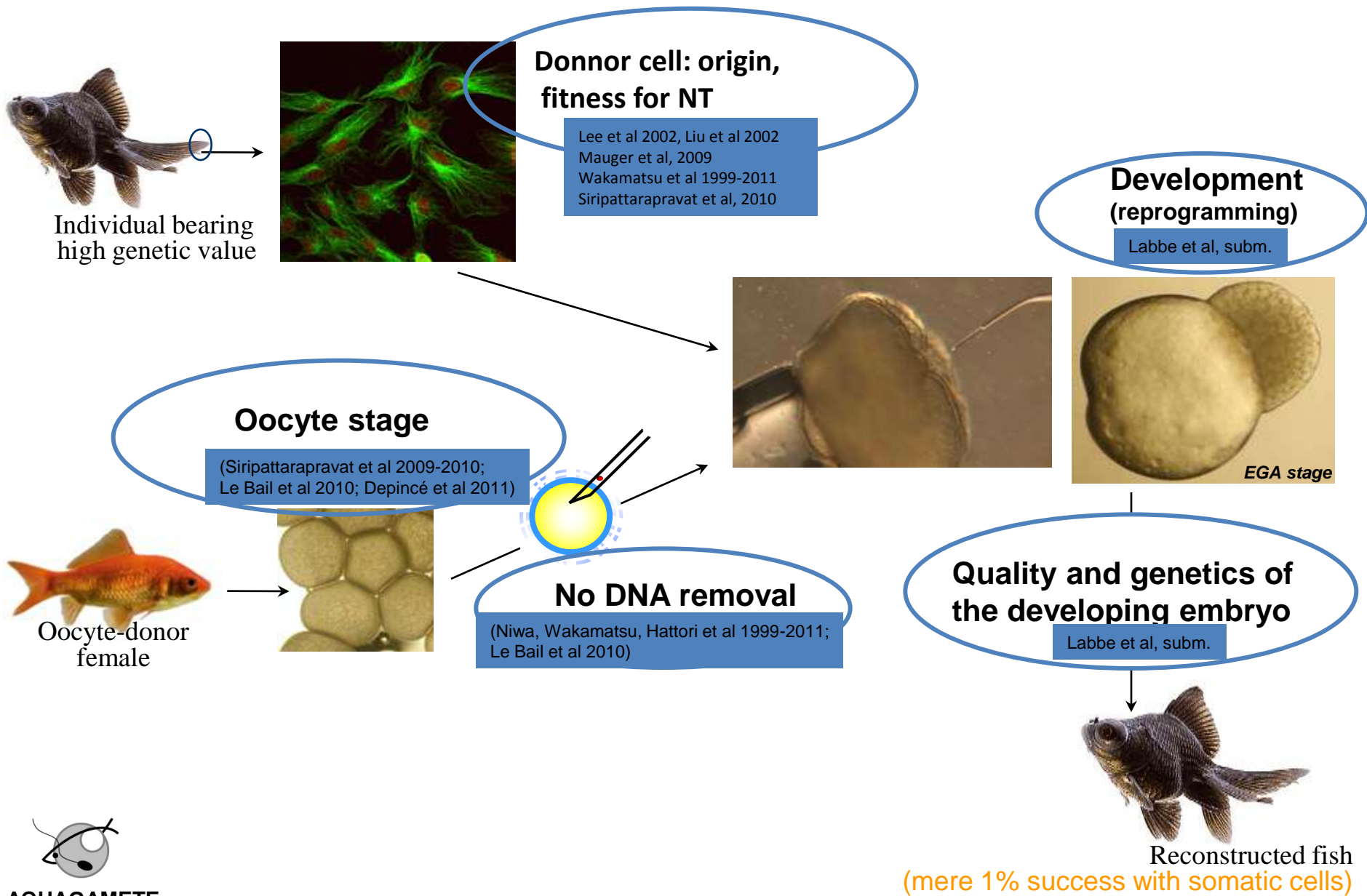
**development speed?**



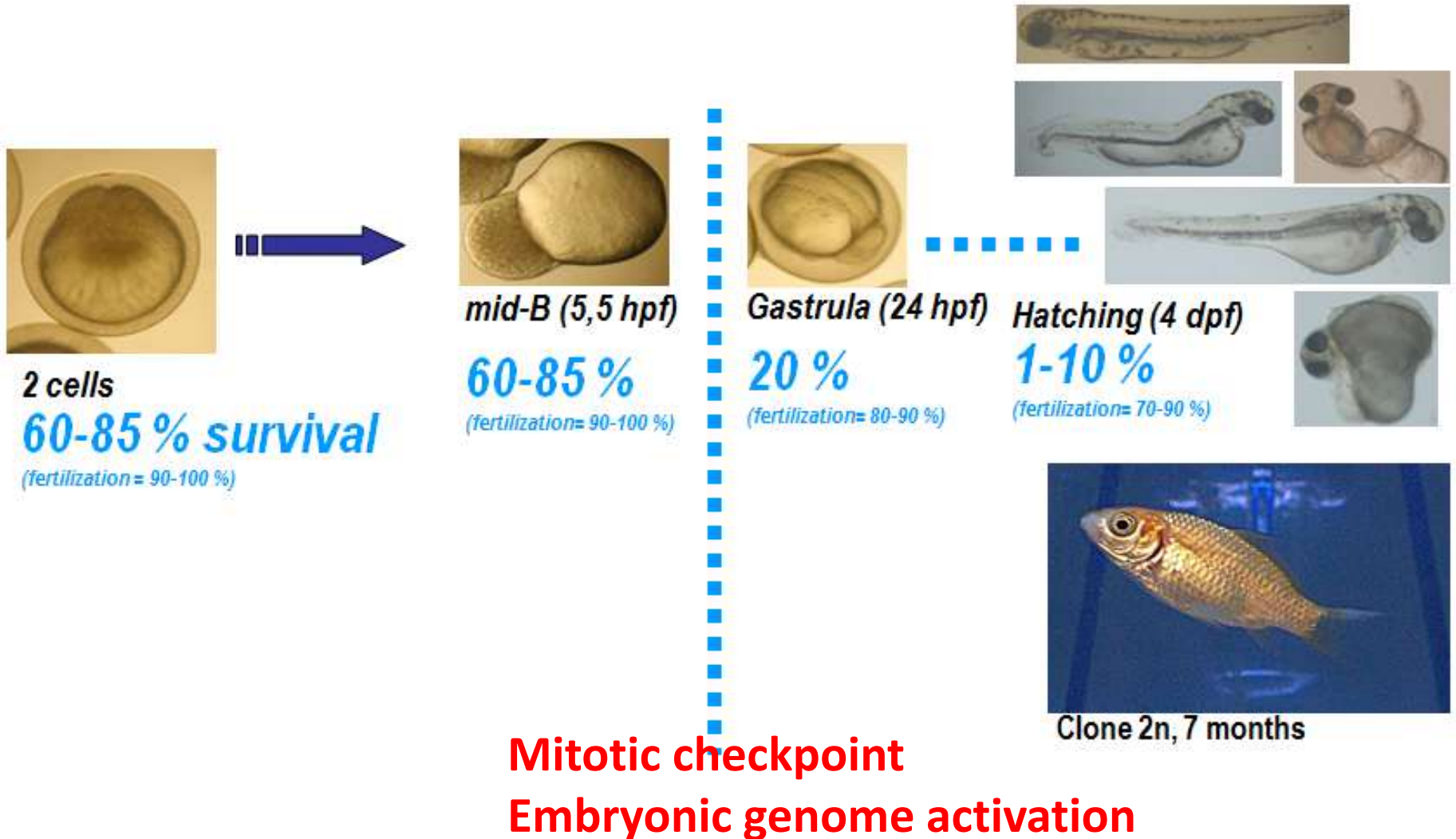
# Somatic Cell Nuclear Transfer in fish



# Somatic Cell Nuclear Transfer in fish



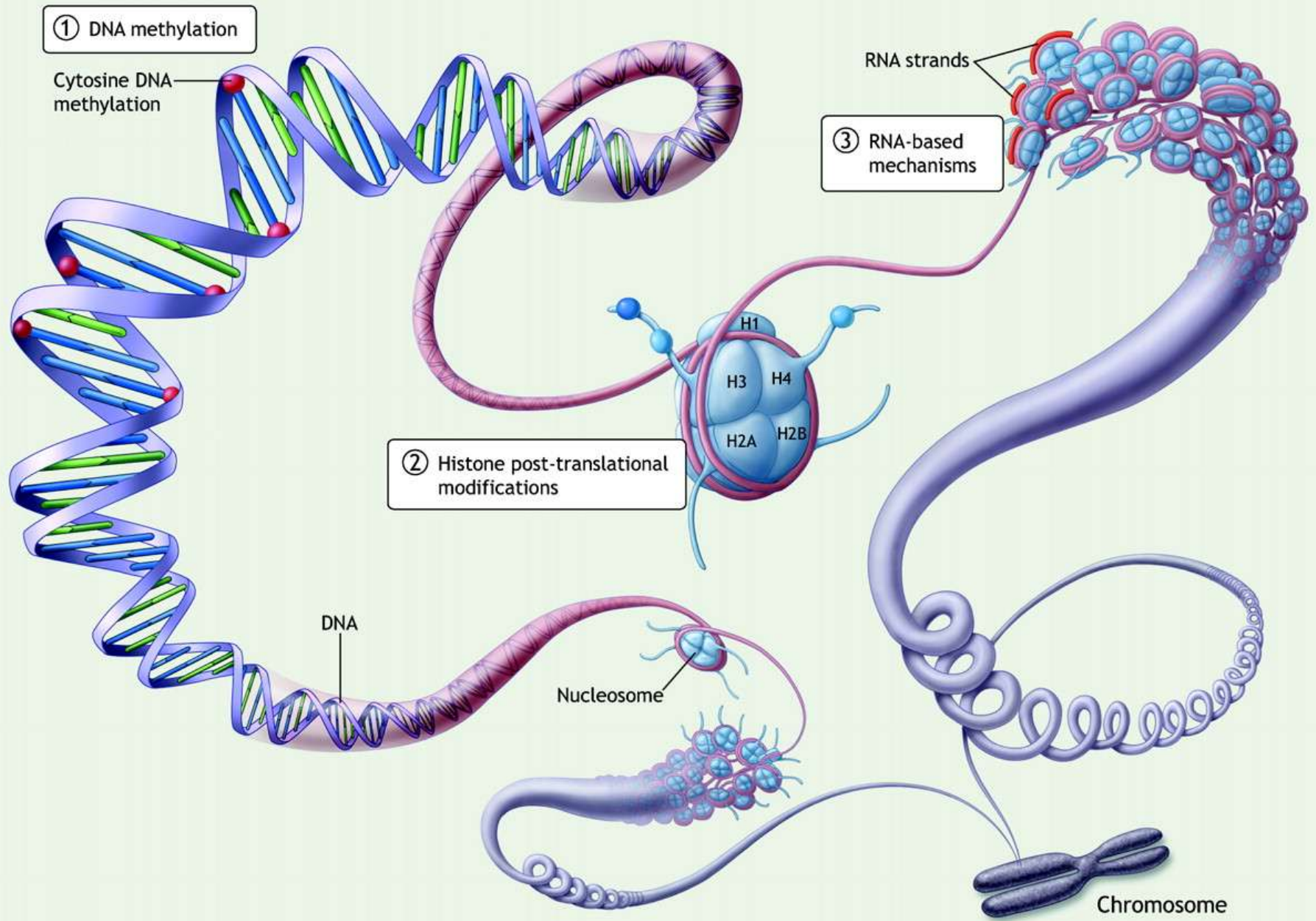
# Somatic Cell Nuclear Transfer in fish



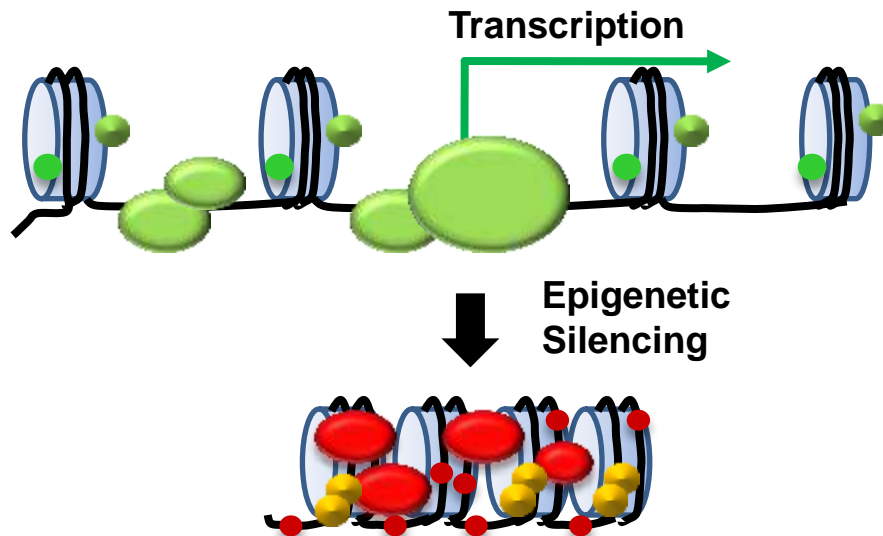
Altered gene expression: wrong genome resetting after « fertilization » ?





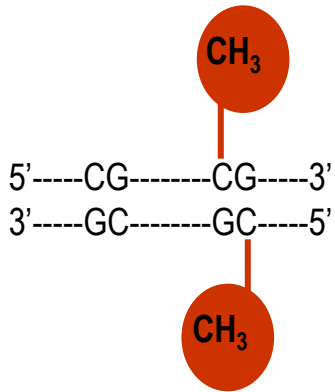


# Molecular basis of epigenetics

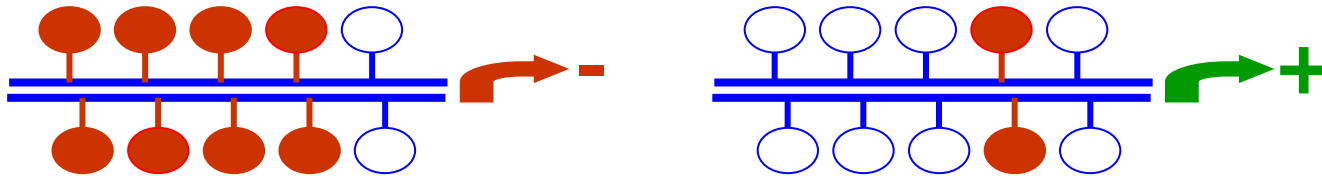


**Major role in chromatin organisation**

# DNA methylation, epigenetic lock against gene expression



Methylation of DNA at the cytosine site in a CpG dinucleotide = **epigenetic modification** found in promoter regions of genes whose transcription is repressed.



⇒ Provides a stable lock against gene expression of the methylation sensitive genes.

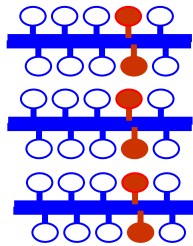
⇒ For these genes, removal of methylation provides a permissive state of gene expression





Differentiation during development

Embryo



Reprogramming

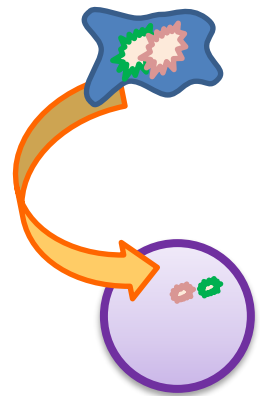
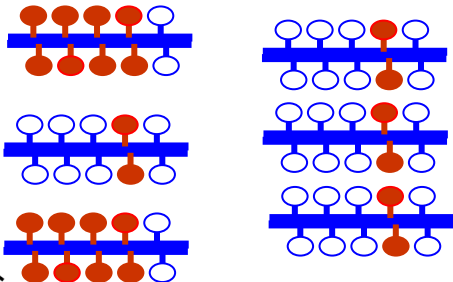
Differentiation



Somatic cell



Ova / spermatozoa



Is the somatic cell able to be reprogrammed ?

# Nuclear transfer and reprogramming ?

## Nanog methylation pattern

## Clones at 24h development (nicest to ugliest morphology)



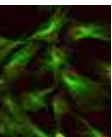
Sperm

Sperm 1 (3.5%)



24h fertilized embryos

24 hpf 1 (6.6%)  
 24 hpf 2 (6.4%)  
 24 hpf 3 (6.0%)  
 24 hpf 4 (5.1%)



Donor fin cells

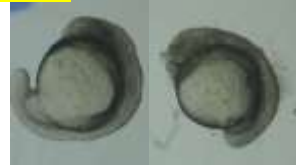
Cryo cell 1 (30.1%)  
 Cryo cell 2 (33.8%)

C24h-1 (13.9%)  
 C24h-6 (14.0%)  
 C24h-9 (3.9%)

C24h-12 (4.4%)  
 C24h-13 (15.4%)  
 C24h-15 (16.4%)  
 C24h-18 (42.3%)  
 C24h-19 (43.3%)

C24h-2 (23.6%)  
 C24h-3 (17.0%)  
 C24h-4 (29.7%)  
 C24h-5 (17.7%)  
 C24h-7 (15.8%)

C24h-8 (3.8%)  
 C24h-10 (14.1%)  
 C24h-11 (21.0%)  
 C24h-14 (24.3%)  
 C24h-16 (18.0%)  
 C24h-17 (34.1%)  
 C24h-20 (14.7%)  
 C24h-21 (8.9%)  
 C24h-22 (32.1%)



Zone de graphique

At 24h, the surviving clones still have an heterogeneous methylation pattern, close to that of the donor cell

# Summary



Fish diploid genome easily cryopreserved

- ⇒ Fin pieces (thin)
- ⇒ Fin cells

Suitability for high through-put cryobanking (fin pieces)



Embryonic/Somatic cell nuclear transfer

- ⇒ Micromanipulation possible (centralized facility)
- ⇒ Cell reprogramming into an embryonic pattern not fully achieved (random success and errors)
- ⇒ Cells have to be treated prior to nuclear transfer: culture conditions need to be optimized
- ⇒ Interspecies nuclear transfer: some biological challenges linked to the maternal factors, yolk size ...



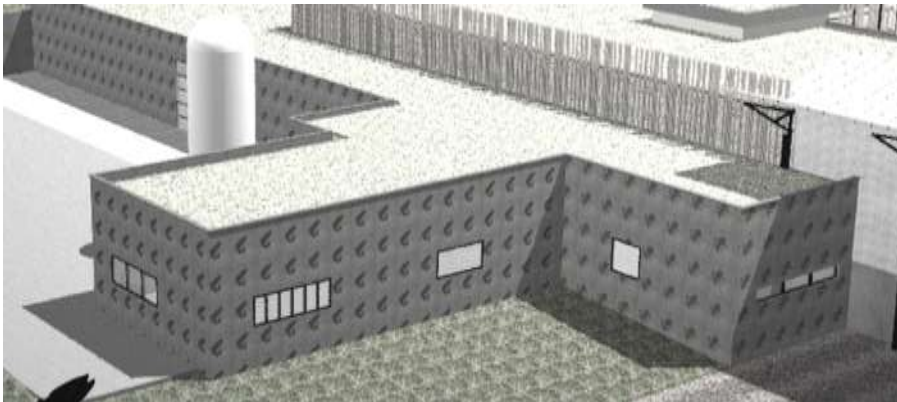
⇒ Making cryopreserved diploid material suitable for broodstock regeneration



# Preservation of genetic resources for aquatic species: the example of CryoAqua, the French centralized cryobank

## *How does it work?*

- Sperm / samples are collected in the farms / in the wild
- Samples are conditioned for shipping at 4°C (short-term preservation for 2-4 days)
- Samples are shipped to CryoAqua (with veterinary certificates),
- Staff at CryoAqua take care of the freezing (high through put or small samples) and of the storage into liquid nitrogen.
- Samples belong to the sender (farmer), but some resources can be donated to the French National Cryobank (public funding).



# Group for cell cryopreservation and fish regeneration



Catherine Labbé, Pierre-Yves Le Bail, Alexandra Depincé, Nathalie Chenais, Marie Conrard, Clémentine Roy (Gérard Maise).

