STSM Scientific Report

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STSM Topic: Establishment of nuclear transfer technology via germ-line chimera documented by time-lapsing

The purpose of this STSM had as main aims:

To establish the nuclear transfer technology between two fish species: zebrafish and goldfish, create germ-line chimeras within nuclear-cytoplasmic hybrids (donors) and goldfish (hosts) and document the success of nuclear incorporation by standard PCR, qPCR, and fluorescent stereomicroscopy.

During my stay in the Laboratory of Fish Physiology and Genomics (LPGP), INRA, in Rennes I was able to introduce me into the project: Establishment of nuclear transfer technology via germ-line chimera.

The main methods and preliminary results were:

Methodology:

In this study, we used as donor of nucleus our transgenic zebrafish from Faculty of Fisheries and Protection of Waters (FFPW) (with tagged B-actin-promotor: by green fluorescent protein (GFP) – stains the somatic cells, and vasa-promotor: by red fluorescent protein (RFP) - stains the primordial germ cells (PGCs). The nuclear transfer was done by transplantation of somatic cells of zebrafish (donor) into non-activated eggs of goldfish (host) from Laboratory of Fish Physiology and Genomics.

1) We dissociated somatic cells from fin or skin of transgenic zebrafish in PBS with 0.05% trypsin/EDTA, stopped enzymatic activation after 1h, filtered and kept on ice until transplantation. These zebrafish somatic cells were used as donors of nuclei.

2) We transplanted per 1 somatic cell of zebrafish (donor) into non-activated egg of goldfish (host) = nuclear transfer (creation of clones), we activated the clones by water after 30 min, dechorionated with Pronase and incubated in Ringer´s solution with 1.6% eggs´ white at 26°C.

3) To be able to obtain germ-line chimera producing donor gametes only, we sterilized the hosts for clones transplantation (goldfish embryos at 2-cell stage) by injecting anti-dnd morpholino oligonucleotide (MO) together with our artificially synthetized mRNA combining

Green Fluorescent Protein: GFP-nanos1 3'UTR mRNA as a control if MO functions well. If the MO functions, no PGCs will be labeled by GFP.

4) We transplanted the blastomeres (marginal layer) of clones (containing PGCs) with transferred donor nucleus into the sterilized host - goldfish embryos at midblastula transition (MBT) stage.

5) To exactly define the expression of germ cell genes of generated nuclear-cytoplasmic hybrids in time, we documented the migration routes of PGCs in the new created germ-line chimeras under fluorescent stereomicroscope with camera by pictures and time-laps. If the transplanted nuclei of transgenic zebrafish successfully incorporated to embryos of goldfish, then the PGCs of goldfish will be labeled with RFP (red expression) and somatic cells with GFP (green expression).

6) To validate if the created clones accepted transplanted nucleus and if it proliferated in clones and also chimeras, we analyzed the samples by using molecular analysis PCR and qPCR with species specific primers for zebrafish and goldfish.

7) PCR: A) Firstly we extracted genome DNA of clones, chimeras and controls (eggs of zebrafish/goldfish).

B) We used for each fish specie 1 "specie" specific primer for its DNA: for zebrafish called as ZF J.J. (bp length 1kb); goldfish: G2 (microsatellite; 250 bp)

C) Samples: 1) positive and negative controls of samples of zebrafish, n=8 (numbers are numbers of eggs used for DNA extraction): 1Z, 5Z, 10Z, Czf (control DNA from LPGP) tested with zebrafish primer (ZF J.J.) = positive controls and goldfish primer (G2) = negative controls (Fig. 4.)

2) positive and negative controls of samples of goldfish (6 samples): G1C, G20, Cg (control DNA from LPGP) tested with goldfish primer (G2) = positive controls and zebrafish primer (ZF J.J.) = negative controls (Fig. 4.)

3) negative controls for PCR amplification with no template (Czf- and Cg-) (Fig. 4.)

4) 3 clones and 9 chimeras (triplicates from each clone): 12 samples tested with zebrafish primer (Fig. 5.) and goldfish primers (Fig. 6.)

8) qPCR: the specifity and sensitivity of designed primers for gonads of both species: Vasa and for housekeeping genes: B-actin (both species) and ubiquitin (zebrafish) were tested on cDNA of same samples used for PCR (in STSM report of Ksenia Pocherniaievaia).

Preliminary results:

1) Transplantation experiments and creating germ-line chimeras:

Figures show transplanted somatic cells (green cells in the tail of embryo in Fig. 1.) and PGCs (red cells in Fig. 2.) in goldfish/zebrafish gem-line chimera after transplantation of blastomeres with transferred donor (zebrafish) nucleus. Control GFP-MO group, where no PGCs should be labeled (Fig. 3.). Unfortunately the MO did not completely sterilized the hosts (goldfish) – GFP also labeled the PGCs (green cells) of host (Fig. 3), therefore we have to repeat these experiments. Also we found out that the somatic cell of zebrafish cannot proliferate in goldfish, therefore we will repeat this part of nuclear transfer within one fish specie (clones of one specie - goldfish somatic cell in goldfish embryo).

Fig. 1. and 2.: Goldfish/zebrafish gem-line chimera after transplantation of blastomeres with transferred donor (zebrafish) nucleus.

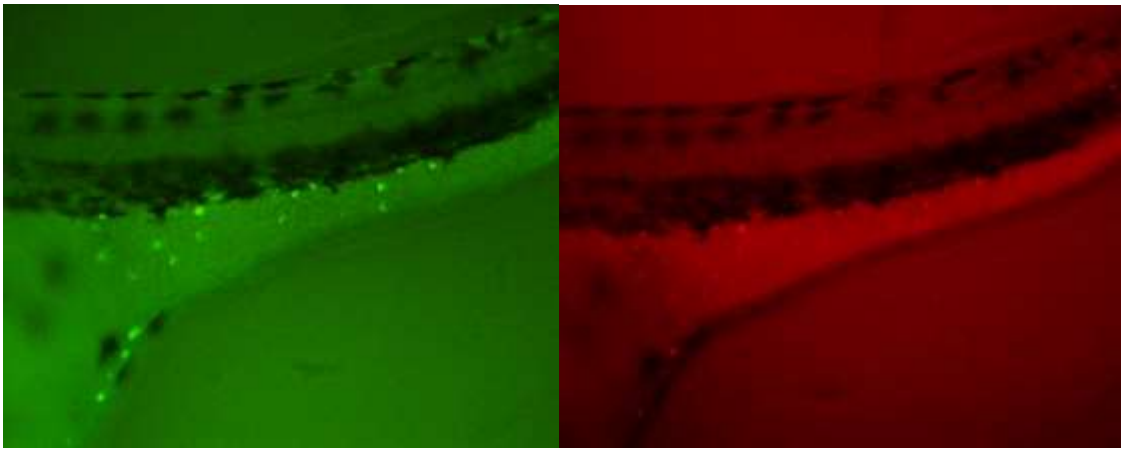
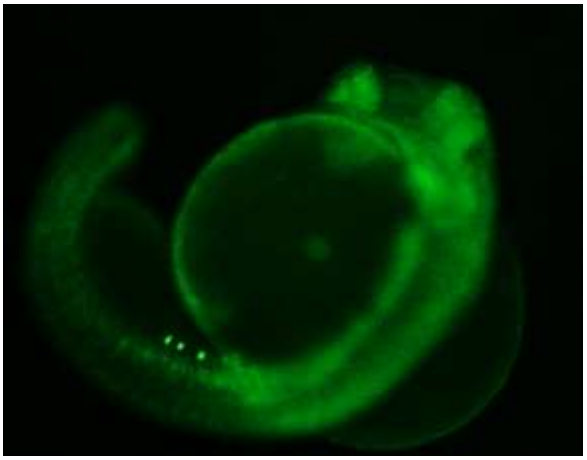


Fig. 3.: GFP-MO injected goldfish embryo (host).



2) PCR analysis

Results are shown on 3 gels (Fig. 4.-6.). Z1B (DNA from 1 zebrafish egg), 5Z (5 zebrafish eggs , 10Z (10 zebrafish eggs), Czf (control zebrafish DNA from LPGP), G1C (DNA from 1 goldfish egg), 20G (20 goldfish eggs), Cg (control goldfish DNA from LPGP), Czf- and Cg- (negative controls for PCR); CL1,1, CL2,1 and CL2,2 (3 clones); CH1,1A, CH1,1B and CH1,1C (chimeras created from clone CL1,1); CH2,1A, CH2,1B and CH2,1C (chimeras created from clone CL2,1); CH2,2A, CH2,2B and CH2,2C (chimeras created from clone CL2,2);

We obtained nice results in Fig. 4. - our primers were specie specific, Fig. 5. shows that from 3 clones one clone CL1_1 was positive with incorporation of zebrafish DNA, Fig. 6. confirmed that chimeras are goldfish as was expected.

Fig. 4.: Positive and negative controls of zebrafish and goldfish.

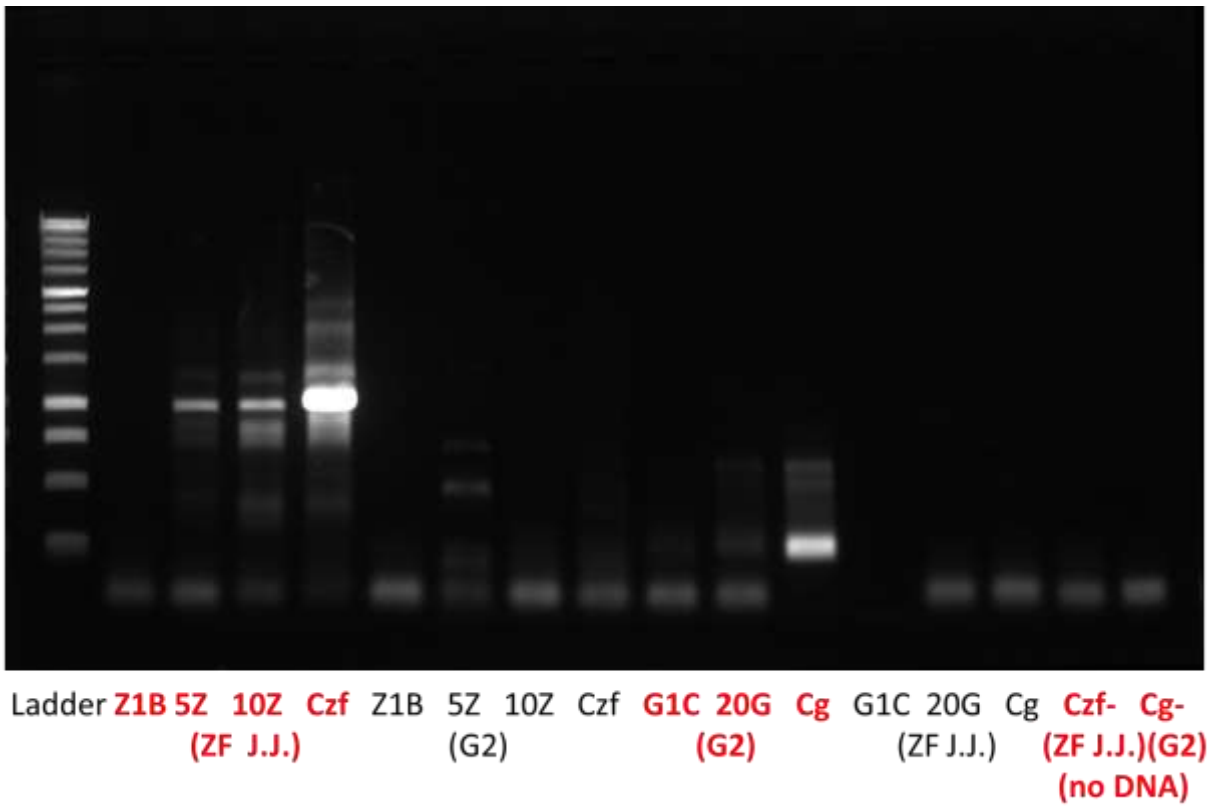


Fig. 5.: Clones (CL), chimeras (CH) with zebrafish primer and zebrafish control (Czf).

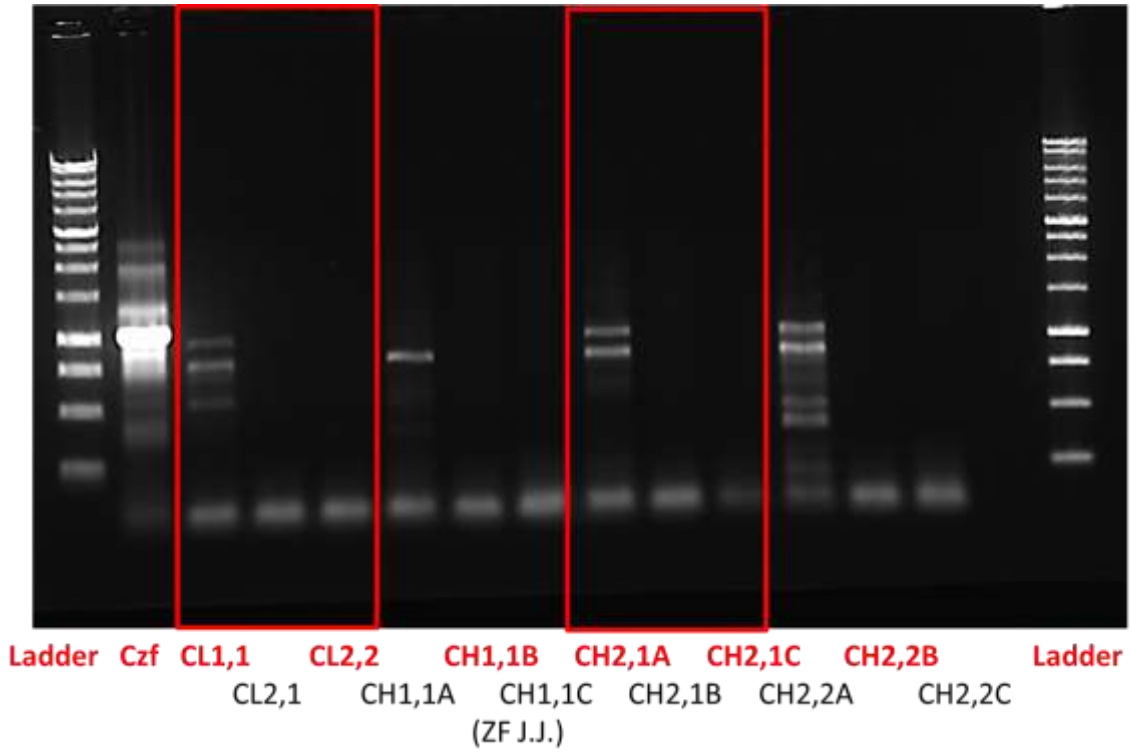


Fig. 6.: Clones (CL) and chimeras (CH) with goldfish primer.