STSM Scientific Report

COST STSM Reference Number: COST-STSM-FA1205-13175

COST Action: FA1205

STSM Applicant: M.Sc. Zuzana Linhartova, University of South Bohemia, Faculty of Fisheries and Protection of Waters, Vodnany (CZ), linhartova@frov.jcu.cz

STSM Topic: Improvement of nuclear transfer technology via germ-line chimera

The purpose of this STSM had as main aims:

- To dedicate to nuclear transfer experiments in zebrafish and goldfish and to identify the primordial germ cells (PGCs) in the reconstructed embryos. The whole project was aimed at allowing genetic resources preservation of fish by way of somatic cells, and to provide regeneration technologies to reconstruct the fish from those cryopreserved cells.
- To connect the collaborative project between Ing. Martin Pšenička, Ph.D (Head of the Laboratory Reproductive Physiology, Faculty of Fisheries and Protection of Waters (FFPW), Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic) and Dr. Catherine Labbe (Head of the Cryopreservation and Regeneration group, Laboratory of Fish Physiology and Genomics (LPGP), INRA , Rennes, France

Description of the work and of the main results carried out during the STSM:

During my stay in the Laboratory of Fish Physiology and Genomics (LPGP), INRA, in Rennes I was able to introduce me into the project: **Production of nuclear-cytoplasmic hybrid between goldfish/goldfish and goldfish/zebrafish via germ-line chimera.** The main steps and results were:

1) We dissociated somatic cells from fin of transgenic zebrafish from FFPW (with tagged B-actin-promotor: by green fluorescent protein (GFP) and vasa-promotor: by red fluorescent protein (RFP)) and somatic cells from fin of goldfish (cryopreserved in LPGP, INRA). These somatic cells of zebrafish/goldfish were used as donor of nucleus.

2) We did nuclear transfer by transplantation of somatic cells of zebrafish or goldfish (donors) into non-activated eggs of goldfish (host).

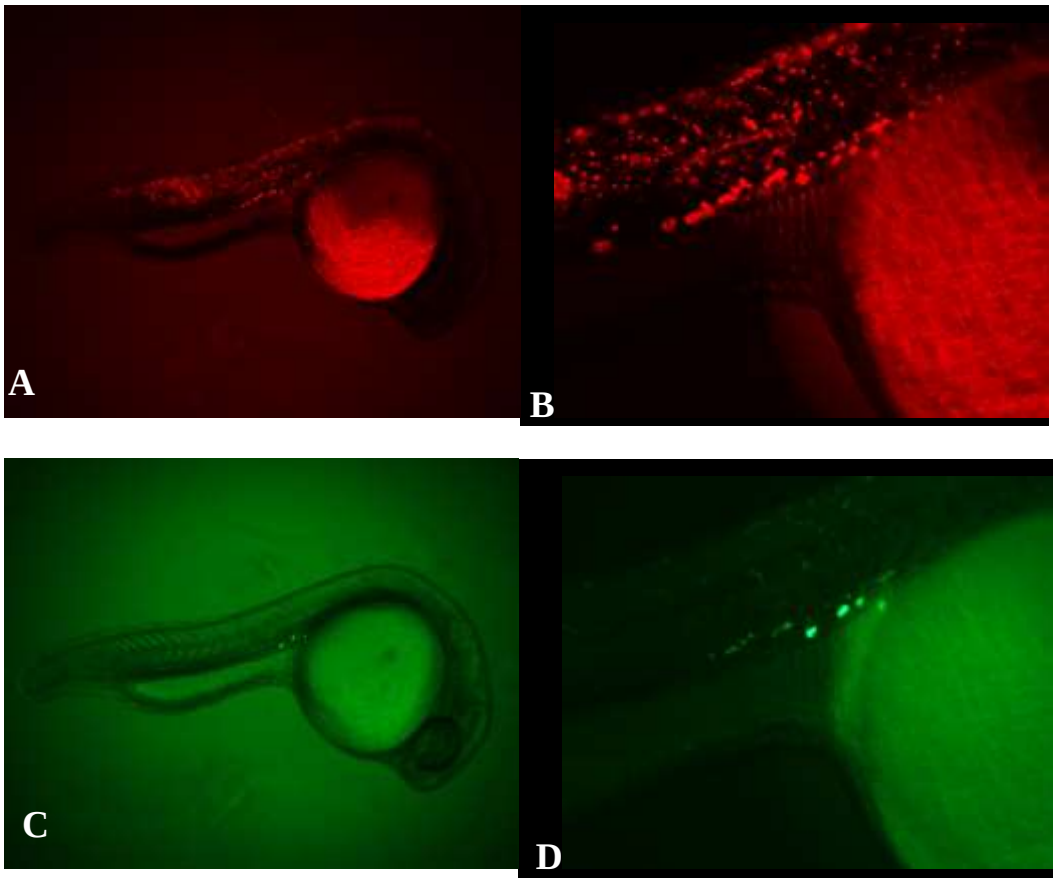
3) To visualize the PGCs of the host (goldfish) we injected our artificially synthetized mRNA combining Green Fluorescent Protein: GFP-nanos1 3'UTR mRNA at the stage of 2-8 cells stage of cleaving embryos.

4) To be able to obtain germ-line chimera producing donor gametes only, we sterilized the host for blastomeres transplantation (goldfish embryos at 2-cell stage) by injecting anti-dnd morpholino oligonucleotide.

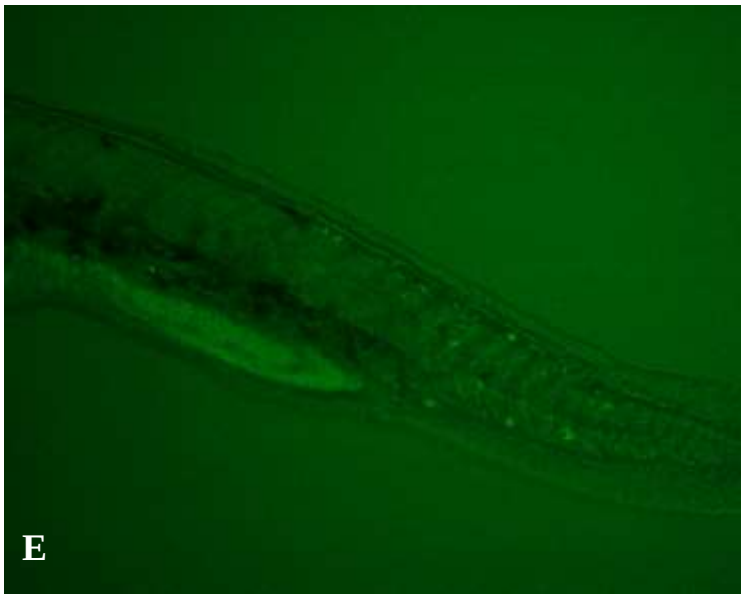
5) We transplanted the blastomeres (containing PGCs) with transferred donor nucleus into the sterilized host (goldfish embryos at midblastula transition (MBT) stage).

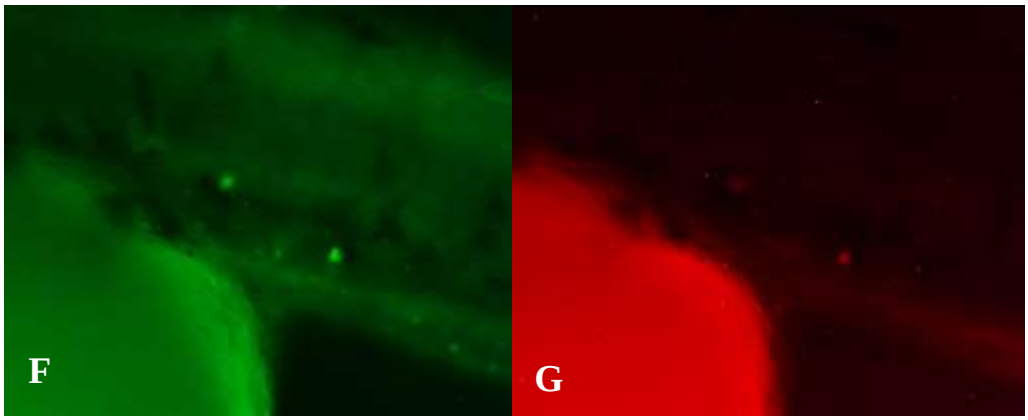
6) We documented the migration routes of PGCs in the new created germ-line chimeras and counted the efficiency of survived individuals.

Preliminary result 1: Transplanted somatic and germ (all) cells (red cells in Fig. A, the detail in Fig. B) and PGCs (green cells in Fig. C, the detail in Fig. D) in goldfish gem-line chimera after transplantation of blastomeres with transferred donor (goldfish) nucleus.



Preliminary result 2: Transplanted somatic and germ (all) cells (green cells in the tail of embryo in Fig. E, detail in Fig. F) and PGCs (red cellsint Fig. G) in goldfish/zebrafish gem-line chimera after transplantation of blastomeres with transferred donor (zebrafish) nucleus.





The future result that we would like to obtain would be offspring that could be transgenic zebra/goldfish nuclear-cytoplasmic hybrid.