A) Short scientific report

Abstract

The identification of *vasa* as the first marker in germ line in zebrafish (*Danio rerio*) involved, from the molecular point of view, a significant improvement for fish germ line evaluation. As consequence of this, vasa mRNAs and proteins, have been used as germ cell molecular markers in fish surrogate production being a valuable tool to study germ cell development during xenotransplantation assays. These techniques could be transferred to large pelagic species (e.g. meagre). Seed production from large fish with long generation times is expensive, because it requires extensive rearing space and is labour-intensive.

Purpose of the STSM

The aim of this STSM was the characterization of vasa mRNA in meagre by cloning and design specific primers for qPCR, and to test in situ hybridation technique.

Description of the work carried out during the STSM

Cloning

Meagre *vasa* molecular marker were isolated using total RNA from the gonads of 24 months juveniles (Fig 1) using a commercial kits. The degenerated oligos were obtained from aligning *vasa* homologue sequences from different teleosts (GenBank) (Fig 2). In all cases, the PCR products were extracted from 1 % agarose gel. Purified PCR products were cloned into pCRII vector and sent for sequenciation.

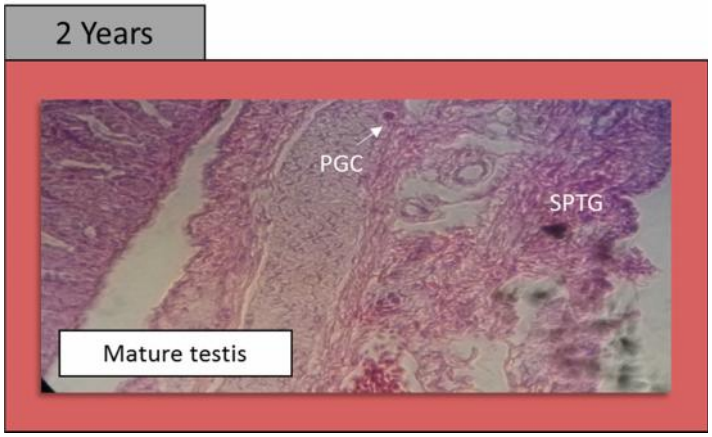

Fig1. Histological section staining with H-E of gonads of 24 months meagre juveniles.

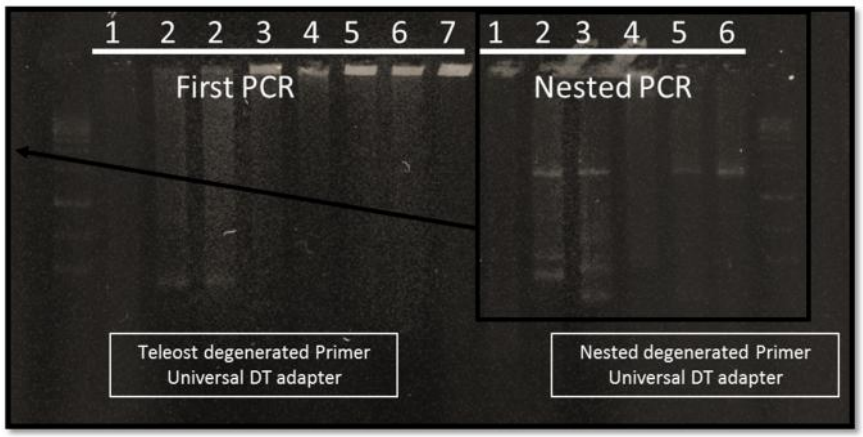

Fig2. PCR and nested PCR with degenerated oligos were obtained from aligning vasa homologue sequences from different teleosts (GenBank).

In situ Hybridation

Histological sections, previously obtained in the last STSMs, were hybridized with the sense and antisense probes. The hybridization signal was detected by staining the anti-DIG antibody conjugated alkaline phosphatase with NBT and BCIP as chromogenic substrate.

Sequence analysis in Sole: Molecular markers

Moreover, new molecular markers as *dnd, cxcr4b* and *sox 2* were found in Solea senegalensis by RNA Seq Assembly. A couple of oligos were designed in order to test the sequences. This analysis provide an important tool for the spermatogonia stem cell identification and isolation (by sorter).

Elsa Cabrita

(Researcher at CCMAR)

(The Supervisor)

Marta F Riesco

(The Applicant)