The trout transcriptome project of the National Agronomical Research Institute of France

Florence Le Gac, Marina Govoroun, Yann Guiguen, Benoit Fauconneau

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THE TROUT TRANSCRIPTOME PROJECT OF THE NATIONAL AGRONOMICAL RESEARCH INSTITUTE OF FRANCE

F. Le Gac, M. Govorun, Y. Guiguen and B. Fauconneau.
INRA-HYFS, Beaulieu, 35042-Rennes Fr.

This program is part of a project of farm animal genomics by INRA, named AGENA. Our objective is to perform systematic expression studies on large numbers of genes potentially involved in physiological functions, based on the production and use of microarrays supporting important collections of partially sequenced cDNAs (~ 40,000 ESTs per species). Furthermore, we will use this information together with mapping information to select traits of agronomical interest.
Collaborations with other research Institutes are anticipated and encouraged.

Step 1: Construction of a trout (*Oncorhynchus mykiss)* cDNA library: RNA messengers have been collected from various tissues at different physiological stages. A multi-tissue complementary DNA library is being constructed in recombinant bacteria and normalized (equalisation of the representation of each RNA transcript) following the method of B. SOARES (Bonaldo et al 1996).

Step 2: Systematic Sequencing of 40 to 50,000 gene ESTs (Expressed Sequence Tag) by sequencing 500 bp at 3' and 5' ends of cDNA. Organisation of unique clones in microtitration plates and storage at -80°C will be done at the Resource Centre located in INRA, Jouy en Josas, near Paris.

Step 3: High-density filter and microarray production in our Resource Center: Deposit density: 10 to 100 bacterial clones / cm^2 of nylon membrane for medium density filters; 10,000 / cm^2 for microarrays (nylon or glass). Microarray production will also take place in the Resource Centre of Paris.

Step 4: Use of microarrays and EST sequences to study trout physiology, nutrition and genetics - INRA intends to explore genes: involved in muscular development and control of meat texture; implicated in the sexual differentiation of gonads; important in responses to infection; involved in the response to stress; specific to the different stages of gametogenesis and involved in gamete quality; responsible for efficient utilisation of nutrients.
The location of the ESTs or genes on the genetic map and information on gene polymorphism will be used for selection of specific characters (QTL).

INRA initiates the " Trout Transcriptome Project "

Systematic analysis of transcripts for regulation studies

Florence Le Gac, Marina Govoroun, Yann Guiguen and Benoît Fauconneau
Département Hydrobiologie et Faune Sauvage / SCRIBE Campus de Beaulieu 35042 Rennes France

Aims and objectives

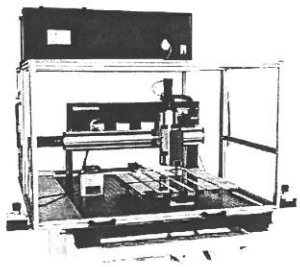
The French National Institute for Agricultural Research (INRA) has initiated a vast project of animal genomics (AGENA), for the comprehensive analysis of the main biological functions in 4 domestic species: cattle, pig, poultry and trout.
Numerous INRA laboratories are involved in the " Trout Transcriptome Project " and a large number of collaborations with other external research laboratories are anticipated.

Systematic study of the genes implicated in the regulation of physiological functions or in traits of agronomical interest.
Investigation of the expression of several thousand transcripts by exploring large collections of partially sequenced cDNA clones (30-40,000 sequenced EST per species in 2001).

The 4 steps of the project

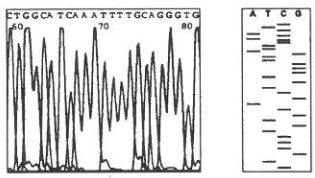
Step 1: Construction of the trout cDNA library

- RNA messengers from different tissues at different physiological stages.
- Complementary DNA bank in recombinant bacteria (mean insert length 1200 bp).
- Normalization of the library: Equalisation of the representation of each RNA transcript following the method of Bento Soares.
- Organisation of 150,000 clones in microtitration plates.
- Storage at -80°C in a ressource center.



Step 2: Systematic sequencing of ESTs (Expressed Sequence Tag)

- Sequences of 500 bp at 3' and 5' ends of each cDNA.
- Objective : 40,000 partially sequenced cDNA (EST)s at the end of 2001.

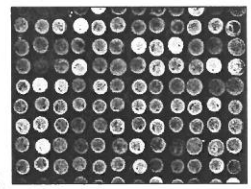


Step 3: High density filters and microarrays

Large cDNA collections amplified by PCR and spotted individually on nylon filters or on glass slides by robot.
Deposit density: 10 cDNA / cm² for high density filters,
1000 cDNA / cm² for microarrays.

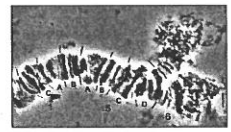
Step 4: Exploitations

Analysis of gene expression



Use of filters and microarrays to analyse expression of genes implicated in the functions studied.
E.g.: genes differentially expressed in response to a viral infection.
Two complex labeled probes will be constructed from both healthy and infected sample RNA and will be hybridized with the microarrays containing the bank.)

Gene mapping



Locate on chromosomes or on a " genomic map " put together from the positioning of microsatellites.

Genetic variability

The location of the genes and information on gene polymorphism will be used for the selection of specific characters linked to Quantitative Trait Loci (QTL).

Examples of functions that INRA intends to explore in fish

Genes involved in the influence of temperature on muscle development

Genes implicated in the sexual differentiation of gonads

Genes expressed in response to an infection

Genes specific to the different stages of gametogenesis

Genes responsible for efficient nutrient utilisation

Genes involved in the response to chronic stress