European Commission

5th Framework program for Research, Technology and Development

A functional genomic approach to measuring stress in fish aquaculture.

« STRESSGENES »

November 2001 – January 2005

Participants

Participants:

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Introduction

*** *Applied research:* Marker-assisted selection in rainbow trout: Identification of genes reponsible for genetic variation in response to stress and for further development of a sufficient number of molecular markers.**

*** *Fundamental research:* Physiological and genetic information on stress responses in fish.**

Objectives and expected achievement.

♦To apply a functional genomic approach using microarray technology to identification and study of genes of which expression is regulated by stress in trout.

♦<u>Selected stress situations :</u>

- ***confinement**
- ***salinity**
- ***hypoxia**
- ***température**
- ***exposition to pathogens.**

<u>Target tissues which will be analysed:</u>

Kidney, anterior head-kidney (interrenal), leucocytes, spleen, gill, skin, liver, brain, pituitary.

- Validate these marker genes as *potential genetic markers* for a particular stress response phenotype: use of trout families selected for their response to acute confinement stress, for growth or for their ability to adapt to hyperosmotic environment.

To carry out this large analysis of gene expression profiles analysis in trout exposed to stressors:

→Construction of a large trout cDNA collections enriched in gene regulated during stress response.

→ Construction of a common « Stressgenes » microarray containing trout genes regulated by stress.

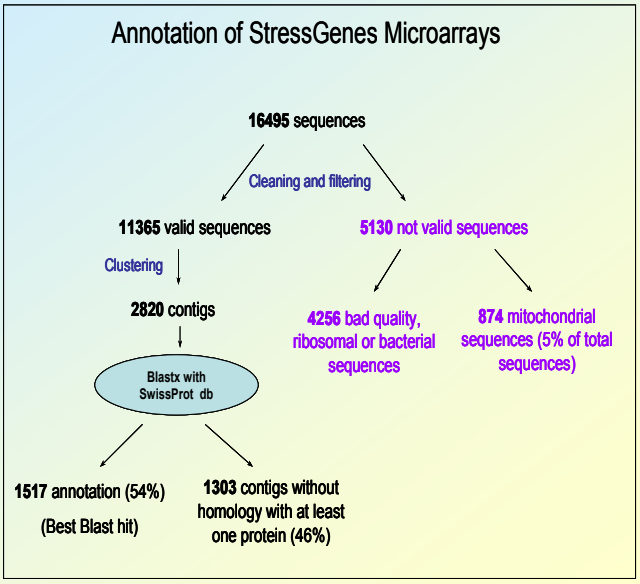
A Common strategy to generate trout subtracted cDNA libraries:

→ Suppression Subtractive Hybridization (SSH)

→ Subtraction between cDNA from stress/control fish (forward or reverse libraries)

→ For each stressors, several tissues have been used.

	Number of clones
Liver (L)	2254
Brain (B)	1608
Muscle (M)	363
Gill (G)	768
Skin (N)	205
Intestine (I)	872
Head Kidney (Y)	855
Spleen (S)	47
Pituitary (P)	932
Mixed Tissues(X)	197
Total	8101



Biological process level 4	percentage
Macromolecule metabolism	28,33%
Cell growth and/or maintenance	23,55%
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	14,42%
biosynthesis	13,66%
catabolism	9,81%
Signal transduction	7,63%
Response to biotic stimulus	7,29%
Regulation of metabolism	6,71%
Response to stress	5,70%
Immune response	5,70%

Main biological process categories.

Biological process level 4
Macromolecule metabolism
Response to extracellular stimulus
Blood coagulation
biosynthesis
catabolism
circulation
Response to biotic stimulus
Regulation of body fluids
Response to stress
Immune response
Regulation of organismal physiological process
Response to external stimulus

Categories of biological process overexpressed

⇒**Stressgenes collection is enriched in genes involved in various aspects of responses to stressors.**

Gene profil analysis in gill of trout transferred from FW to SW

* FW-acclimated trout directly transferred to SW (30 ppm).
* Sampling times: 2h, 8h, 24h, 3 days, 21 days
* At each time points: 6 control (FW) and 6 stress (SW) fish.

Statistical analysis:

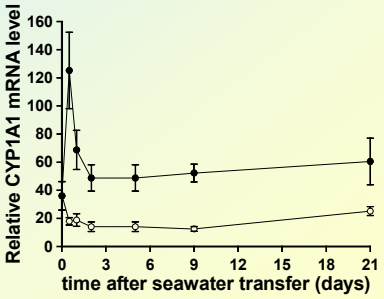
- Principal component analysis (PCA)
- SAM methods (estimation of a false discovery rate).
- Limma methods
- Student analysis

On the selected clones: Hierarchical analysis, annotation, Gene Ontology analysis.

Salinity stress and gene expression in gill

- **From the gill SSH cDNA collections (salinity stress), identification of several interesting genes**
  → Cytochrome P450 1A1, Cox 1…

- **Analysis of CYP 1A1 gene expression after salinity transfer:**



(Leguen, Nestor, Prunet, INRA)

Gene profil analysis in interrenal of trout exposed to confinement stress

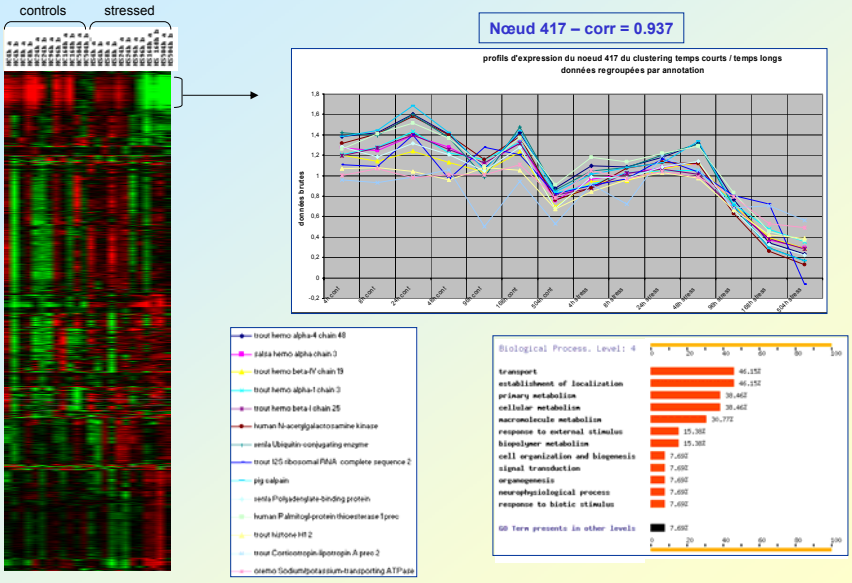
* Sampling times: 4h, 8h, 24h and 2, 4 , 7 , 21 days
* At each time points: 6 control (FW) and 6 stress (SW) fish.

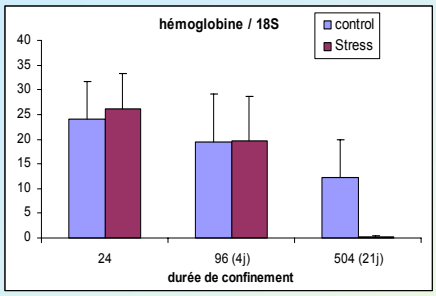
Statistical analysis:

- Principal component analysis (PCA)
- SAM methods
- ANOVA

On the selected clones: Hierarchical analysis, annotation, Gene Ontology analysis.

Time course analysis





Confirmation by real time PCR of the effect of chronic stress on hemoglobin transcript levels in rainbow trout head-kidney

Expected achievements

-Characterization of stress-responsive genes in trout:

- Commun to different stressors
- Specific to a given stress response

-Characterization of new molecular pathways involved in the stress response in fish.

Potential applications

-Potential candidate gene markers for marker assisted selection:

→ continuation of this approach within AQUAFIRST project.

- Potential candidate genes for diagnostic of stress situation.