

Overview on molecular tools in bivalve shellfish aquaculture

Pierre Boudry

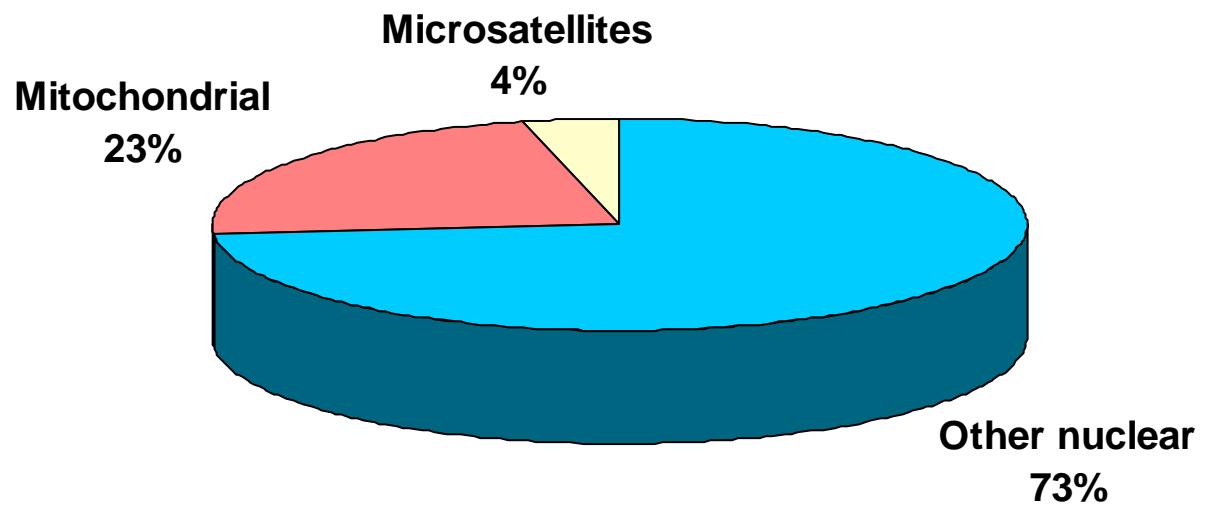
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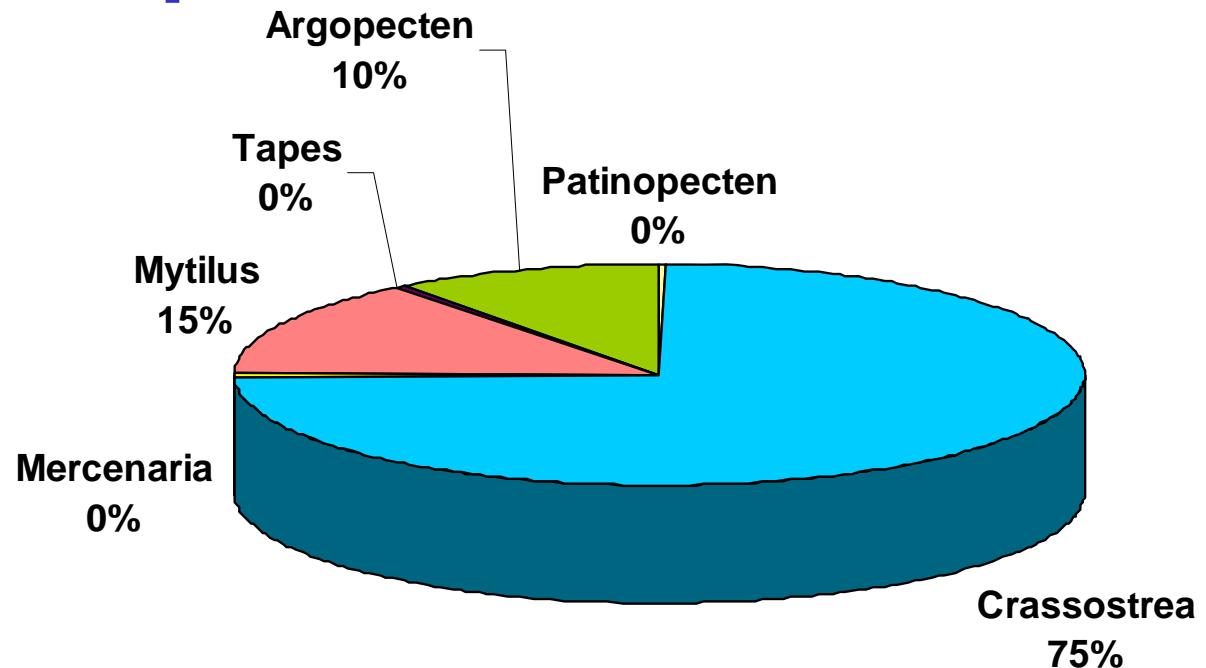


Bivalves in GenBank



Bivalve Nuclear Sequences in GenBank

(excluding microsatellites)



Nuclear Sequences from “single-gene” research

Immunity and disease (oyster, mussel) :

- Antimicrobial peptides

Byssus formation (mussel) :

- Foot adhesive proteins

Digestion (mussel, oyster) :

- Amylases, cellulases

Pollution and stress (mussel, oyster) :

- Metallothioneins, HSP70

Shell formation (pearl oyster)

Development (mussel, oyster)

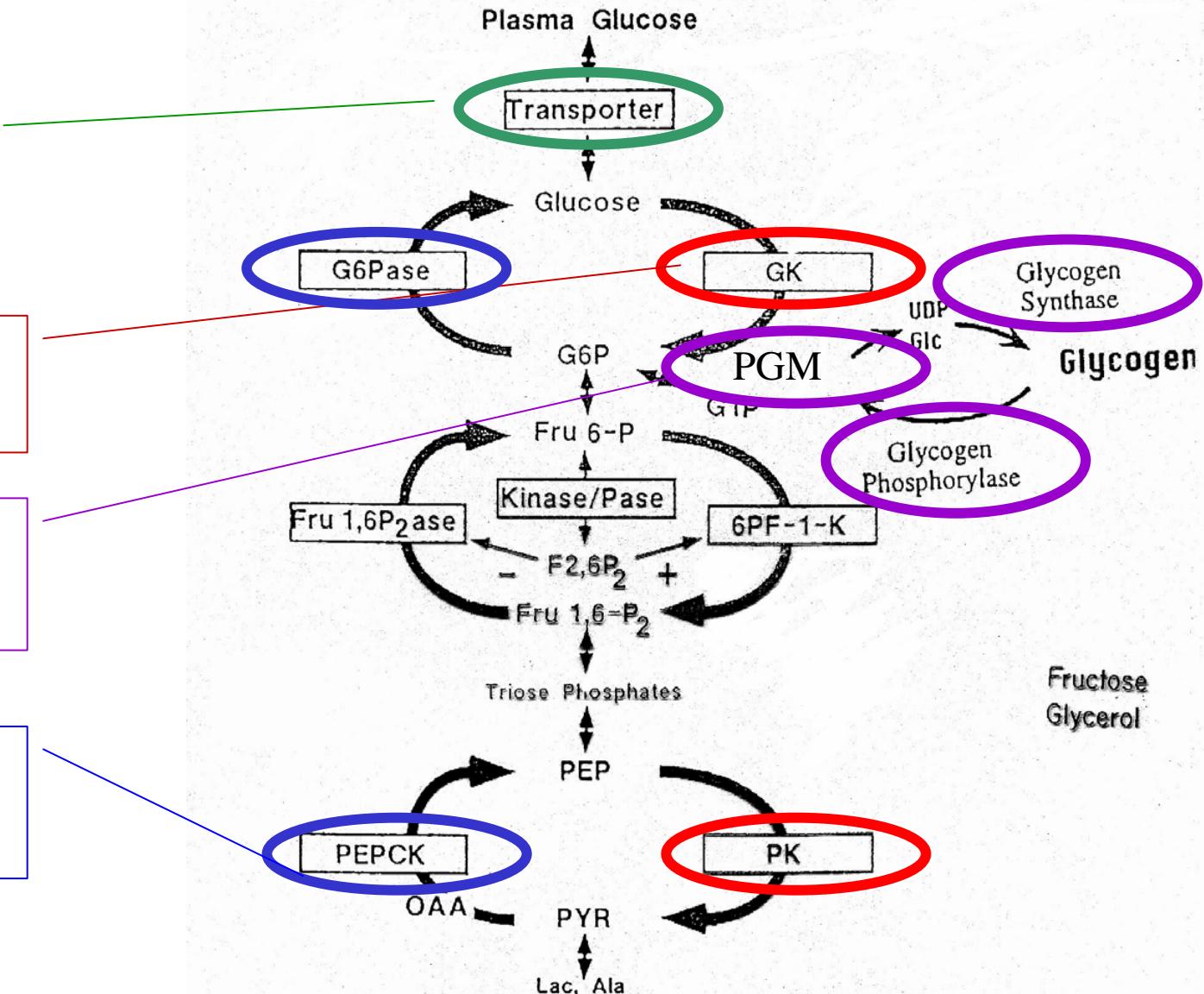
Enzymes in glucose pathway: characterized cDNAs (*C. gigas*)

Glucose transporter
(SGLT)

Glycolysis
(PK, hexokinase)

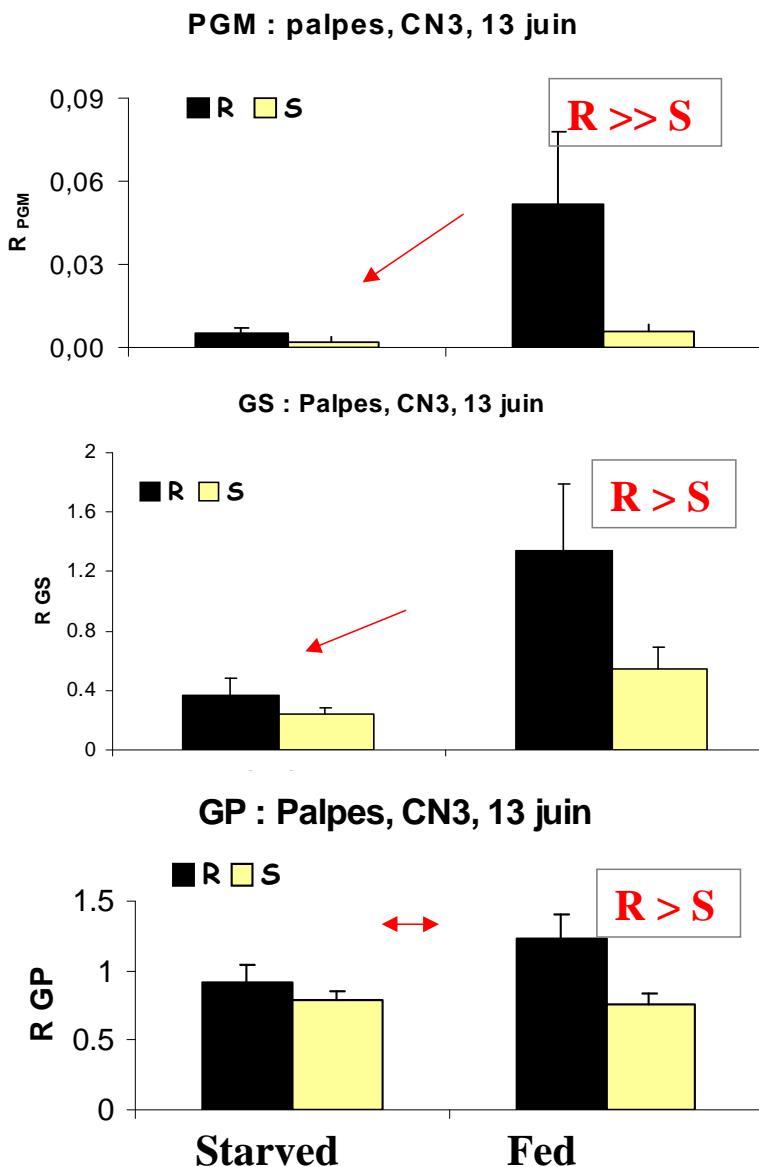
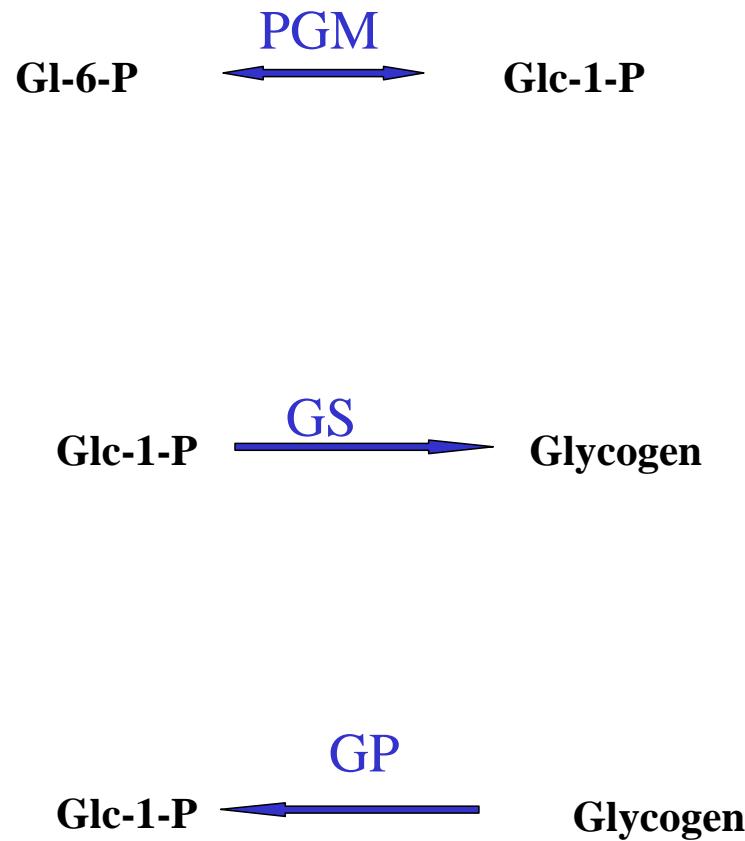
Glycogen metabolism
(GS, GP, PGM)

Gluconeogenesis
(PEPCK, G6Pase)



The substrate cycles and enzymes involved in hepatic glucose metabolism. PEP, phosphoenolpyruvate; PYR, pyruvate; OAA, oxaloacetate; Kinase/Pase, 6-PF-2K/Fru-2,6-P₂ase; Lac, lactate.

Real-time PCR studies of genes involved in glycogen pathway



cDNA libraries in Bivalves

<i>Species</i>	<i>Tissue</i>	<i>Author (lab)</i>
Oyster CG	Hemocytes Mantle Larvae Gill	Bachere, Escoubas (Montpellier) Cunningham (Bergen) Favrel (Caen) Rafferty (Galway) Moraga (Brest)
Oyster CV	Hemocytes, embryos Gonad	Chapman (Charleston, SC) Liu (Auburn, AL)
Mussel MG	Whole body Sperm	Venier (Padova) Cornudella (Barcelona)
Scallop AI	Spat	Roberts (Woods Hole MBL)

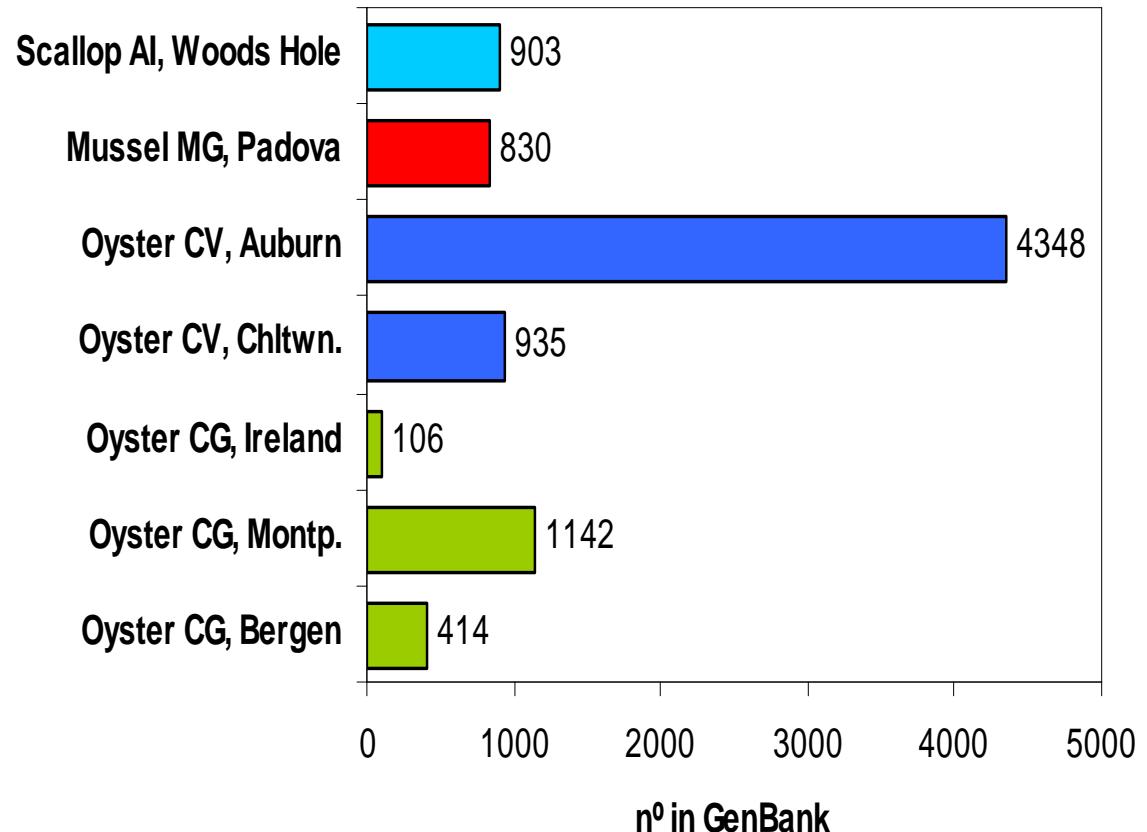
ESTs in Bivalves

- EST characterization of genes involved in relevant biological processes

Oyster CV – stress response
(Chapman, SC) – 649 ESTs, 29 potential biomarkers

Oyster CG – immune function
(Bachere et al., Montpellier) – 1200 ESTs, 20 immunity genes
– **Stress response**
(Moraga, et al., Samain et al., Brest)

Mussel MG – genes expressed in normal life conditions vs. polluted – 524 ESTs (Venier; Padova)



http://www.ifremer.fr/GigasBase

BLAST - Netscape

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Signtex Adresse : http://www.ifremer.fr/GigasBase/Contig/affichage_blast.php

BLAST FILE on Contig 53

Ifremer

!SEQUENCE_LIST 1.0
BLASTX 2.2.1 [Aug-1-2001]

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acid Res. 25:3389-3402.

Query= /tmp_mnt/home1/iletudy/www/htdocs/gigasBase/Contig/Projet1/Blas t/.../Temp/contig53.seq
(734 letters)

Database: genpept
1,064,824 sequences; 332,857,153 total letters

Color Key for Alignment Scores:
<40 40-50 50-60 60-70 70-80 80-90 90-100 >100

NCBI Sequence Viewer - Netscape

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Signtex Adresse : http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=Protein&term=AAG42824&doptcmdl=GenPept

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□ 1: AAG42824, tissue inhibitor ...[gi:11993634]

LOCUS AAG42824 221 aa linear INV 30-JUL-2001

DEFINITION tissue inhibitor of metalloproteinase TIMP [Crassostrea gigas].

ACCESSION AAG42824

VERSION AAG42824.1 GI:11993634

DBSOURCE locus AF321279 accession AF321279.1

KEYWORDS .

SOURCE Crassostrea gigas (Pacific oyster)

ORGANISM Crassostrea gigas

Eukaryota; Metazoa; Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreidae; Ostreidae; Crassostrea.

REFERENCE 1 (residues 1 to 221)

AUTHORS Montagnani,C., Le Roux,F., Berthe,F. and Escoubas,J.M.

TITLE Cg-TIMP, an inducible tissue inhibitor of metalloproteinase from the Pacific oyster Crassostrea gigas with a potential role in wound healing and defense mechanisms(1)

JOURNAL FEBS Lett. 500 (1-2), 64-70 (2001)

NEEDLINE 21329024

PUBLISHER 11434928

REFERENCE 2 (residues 1 to 221)

AUTHORS Montagnani,C. and Escoubas,J.M.

TITLE Direct Submission

JOURNAL Submitted (14-NOV-2000) DRIM, CNRS-IFREMER-UMII, 2 Place E. Bataillon, Montpellier 34095, France

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Signtex Adresse : http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed&cmd=Retrieve&list_uids=21329024&dopt=Citation

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□ 1: FEBS Lett. 2001 Jun 29,500(1-2):64-70.

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ElSIEVIER SCIENCE FULL-TEXT ARTICLE

Cg-TIMP, an inducible tissue inhibitor of metalloproteinase from the Pacific oyster Crassostrea gigas with a potential role in wound healing and defense mechanisms(1).

Montagnani C, Le Roux F, Berthe F, Escoubas JM.

Laboratoire de Defense et Resistance chez les Invertébrés Marins (DRIM), Université de Montpellier II, place Eugène Bataillon, CC80, 30495 Montpellier, France.

We have cloned and characterized a cDNA encoding Cg-TIMP, the first tissue inhibitor of metalloproteinase identified in mollusks. The isolated cDNA encodes a protein of 221 residues that has a domain organization similar to that of vertebrate TIMPs including a signal sequence, and the 12 cysteines characteristic of the TIMP signature. Analysis of Cg-TIMP expression in adult oyster tissues, by Northern blot and in situ hybridization, indicates that Cg-TIMP was only expressed in hemocytes which are the key components of defense mechanisms in mollusks. We also observed that Cg-TIMP mRNA accumulated during shell damage and bacterial challenge. This pattern of expression suggests that Cg-TIMP may be an important factor in wound healing and defense mechanisms.

MeSH Terms:

- Amino Acid Sequence

InterPro: Tissue inhibitor of metalloproteinase - Netscape

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Signtex Adresse : http://www.ebi.ac.uk/interpro/entry?acc=IPR001820

EMBL-EBI European Bioinformatics Institute

Ost Nucleotide sequences for Site search

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InterPro home Text Search Sequence Search Databases Documentation FTP site Browser FAQ

Search: Search Entries Search InterPro

IPR001820 Matches: 61 proteins View matches: Overview|... sorted by Name|Detailed view|Table view

Name Tissue inhibitor of metalloproteinase

Signature PF00965.TIMP (61 proteins)
PS00288.TIMP (44 proteins)
SM00206.NTR (53 proteins)

Type Family

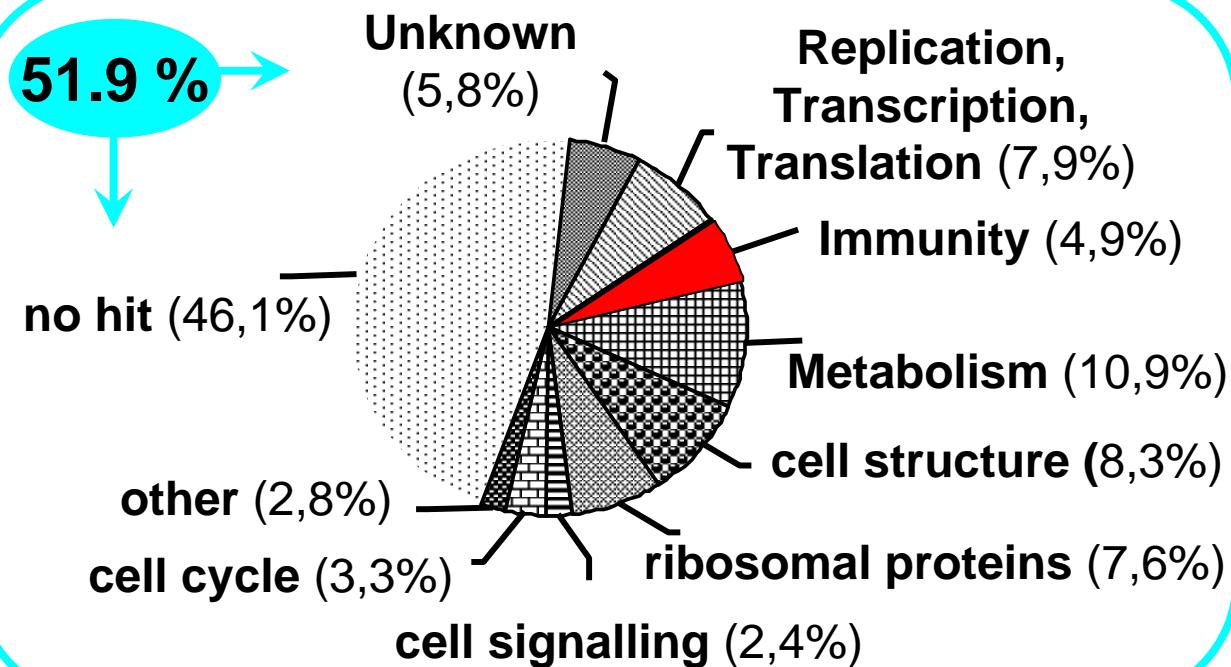
Dates 1999-10-08 17:25:0 (created)
2001-11-02 18:35:13.0 (modified)

Function metalloendopeptidase inhibitor activity (GO:0008191)

Component extracellular matrix (GO:0005578)

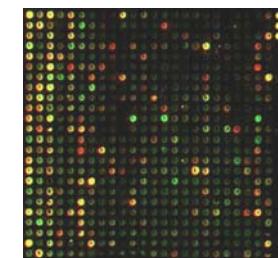
Abstract Tissue inhibitors of metalloproteinases (TIMP) are a family of proteins [1, 2, 3] that can form complexes with extracellular matrix metalloproteinases (such as collagenases) and irreversibly inactivate them. Members of this family are common in extracellular regions of vertebrate species [4]. TIMPs are proteins of about 200 amino acid residues, 12 of which are cysteines involved in disulfide bonds [5]. The basic structure of such a type of inhibitor is shown in the following schematic representation:





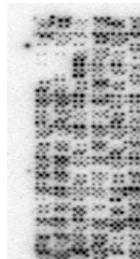
**cDNA macroarray
Real time PCR**

cDNA microarray



**Oyster array
(M.U.S.C.)**

**cDNA macroarray (SSH)
(2304 clones)**



Oyster array C.g

→ **Real time PCR**

Studies of Genome Function

➤ cDNA Microarray development

Oyster CV – Environmental genomics – development of gene chips for monitoring environmental pollution (mercury) (Auburn, US)

Oyster CG – For monitoring response to pathogens, to environmental parameters, resistance to summer mortality (France)

Mussel MG - similar approach for hydrocarbon pollution (L. Cornudella; Barcelona)

➤ MSSP (massively parallel signature sequencing)

Oyster CG – Heterosis - patterns of expression in inbred and hybrid larvae (D. Hedgecock, Lynx Therapeutics; CA, USA)

➤ Proteomics

Mussel MG and ME – proteome characterization (G. Alvarez, Spain)



BAC libraries in Bivalves

- Only available in oysters (CG and CV)
- Clemson University Genomics Institute (J. Tomkins)
- Coverage 10X (CG) and 12X (CV)
- Average insert size 134 Kb (CG) and 150 Kb (CV)
- Available on line (\$2304.00 /library)



orders A small icon of a white microplate with a grid of wells.



Mitochondrial Genomes in Bivalves

➤ Special mtDNA inheritance – DUI

2 genomes F and M, transmitted through females and males, respectively

Demonstrated in marine Mussels (Mytilidae), freshwater mussels (Unionidae) and clams (Veneridae)

➤ Complete mt Genomes sequenced

Mussel F and M (Brown, USA; Lunt, Hull; Zouros, Greece)

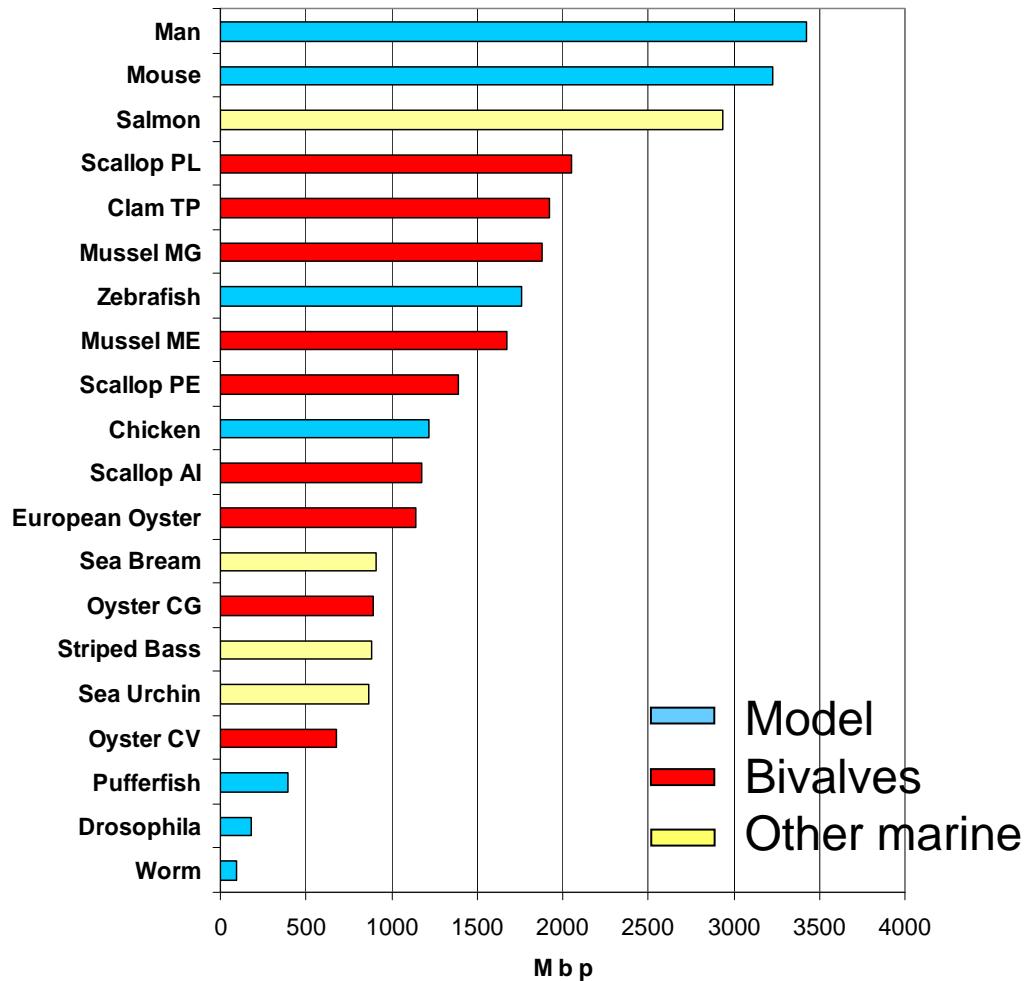
Clam F and M (Okazaki, Tokyo)

Oyster CG (Kim, S. Korea)

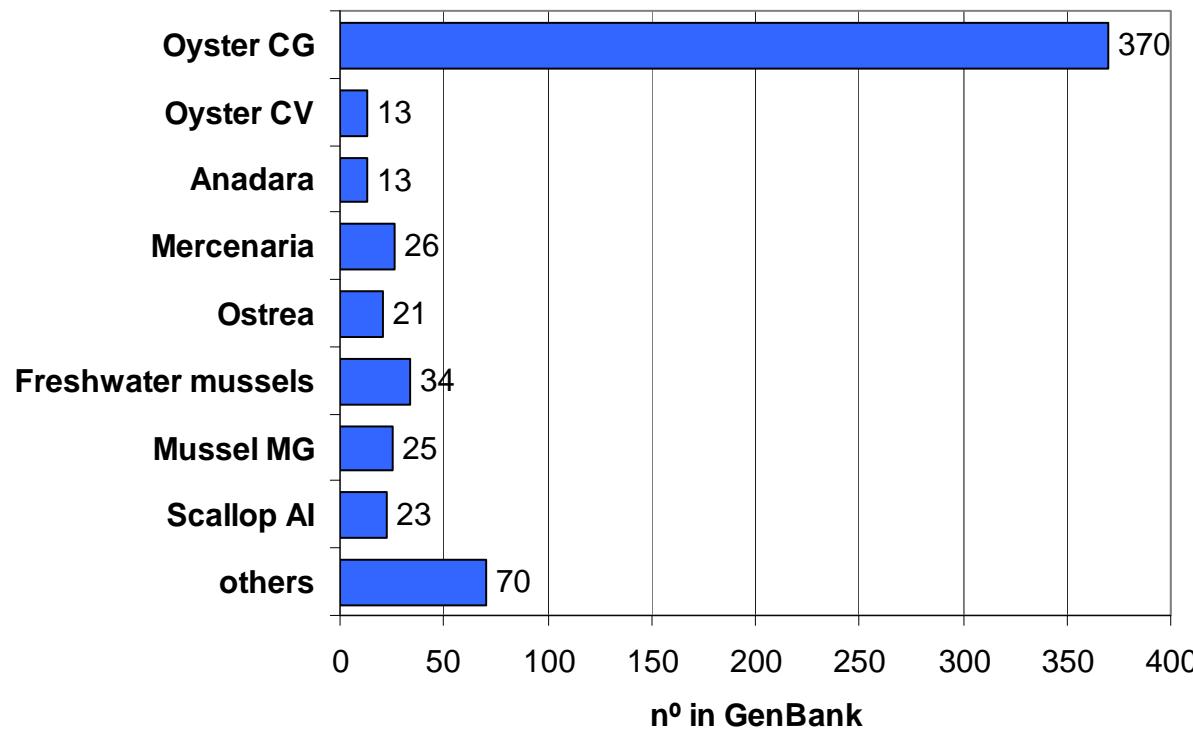
The Oyster Genome Consortium

- Constituted in 2003 PAG meeting
- Coordination: Pr. Dennis Hedegeock
- 70 member laboratories
- 10 Countries: 6 European, Australia, Canada, China, Japan, USA

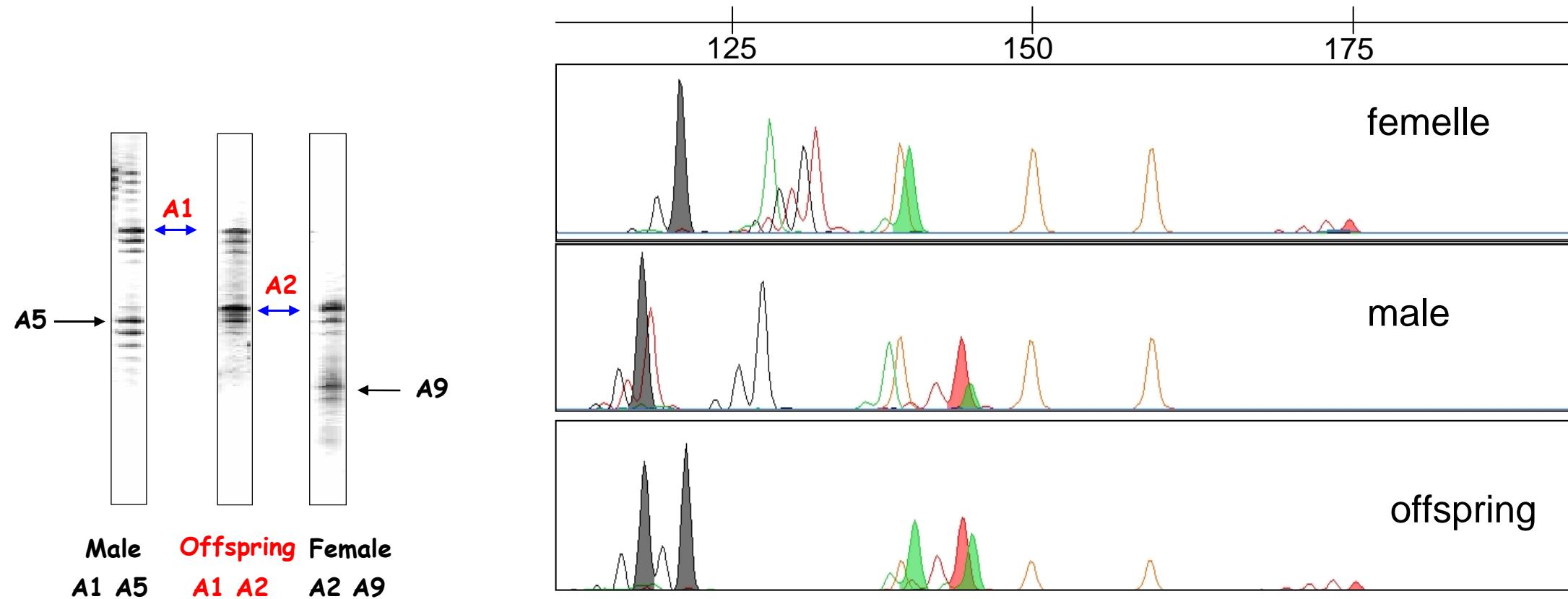
- OGC requested JGI (DOE Join Genome Institute) to sequence *C. gigas* genome.
- This first request was rejected because *C. gigas* genome is too large



Microsatellites in Bivalves



Mixed-family approach using microsatellite-based parentage analysis

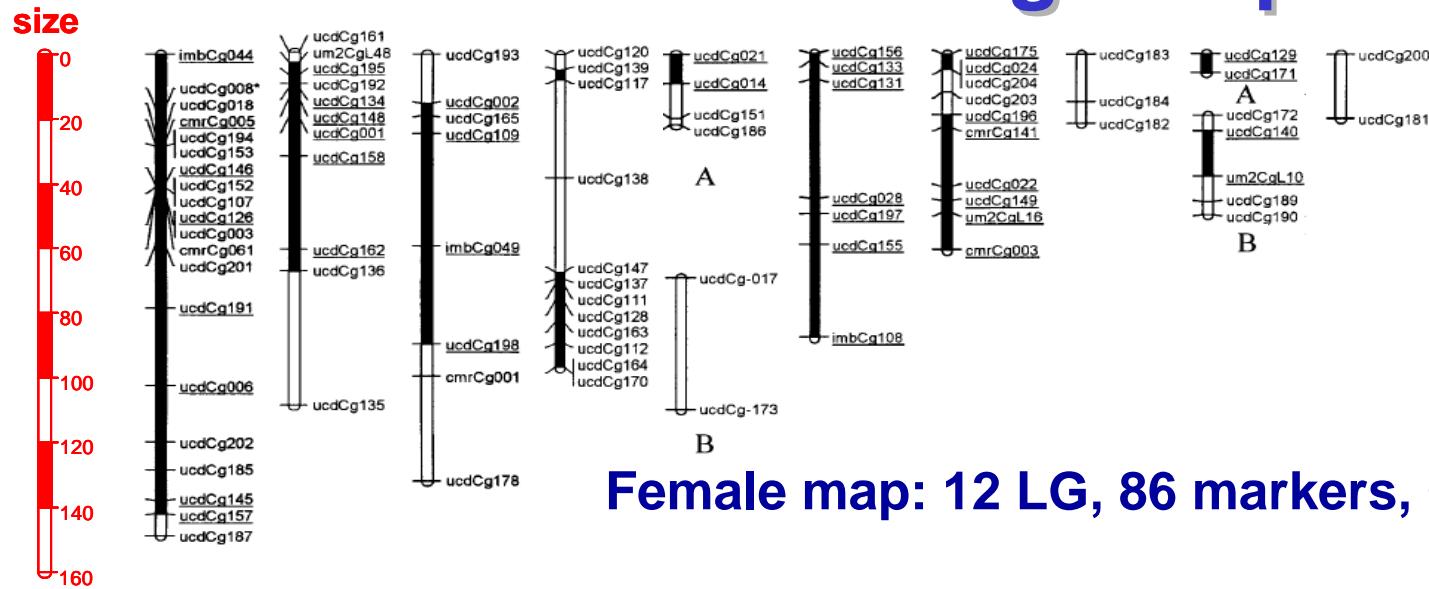


PCR-multiplexed loci (*Taris et al., in press*)

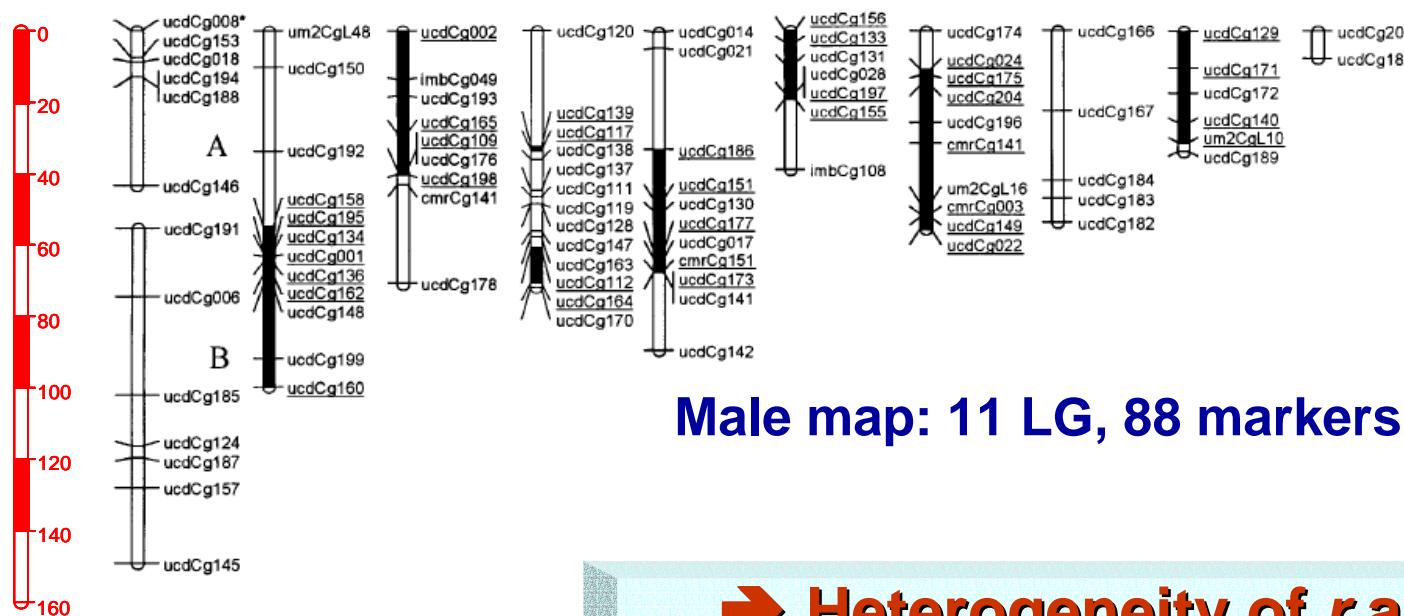
Linkage maps in Bivalves

- Only available in Oyster (n=10)
- *C. virginica* – X. Guo (Haskin SRL, Rutgers)
 - 282 markers: 298 AFLP, 5 microsats, 2 EST
 - 12 linkage groups
 - Physical mapping (FISH) in progress -
- *C. gigas* – D. Hedgecock (USC, CA)
 - 100 microsats, >1100 AFLP
 - 10 linkage groups
 - X. Guo
 - 96 AFLP

Female & Male Linkage Maps of *C. gigas*



Female map: 12 LG, 86 markers, GL≈1020 cM

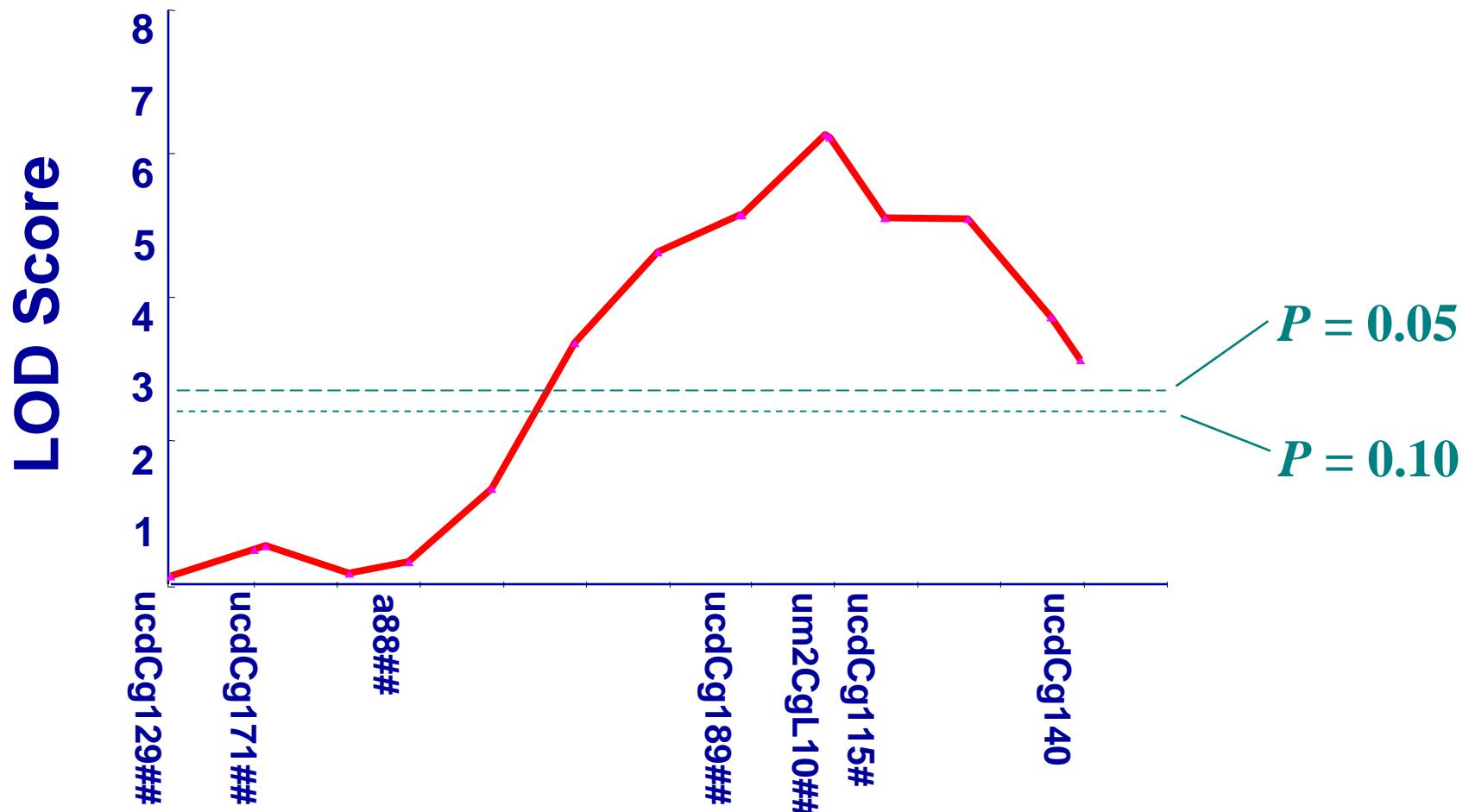


→ Heterogeneity of r and gene order!

Tagged F₂: Largest & Smallest



Size QTL on LG IX for 35x51 F₂



Same QTL observed in samples of both indoor- and outdoor-reared, individually tagged juveniles

