Genetic Diversity and Gene Pool Workshop in Yellow Sea

Cloning and identification of the genes involved in immune response from scallop

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Content

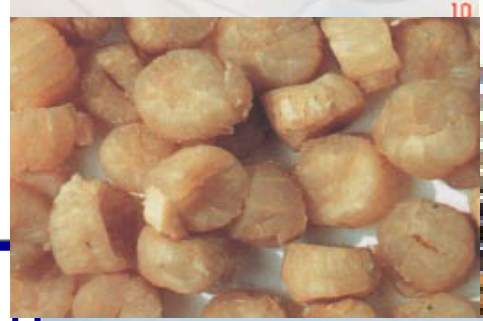
- Cloning and characterization of the genes involved in immune defense.
- Genetic diversity of some species in yellow sea

1. Cloning and characterization of the functional genes

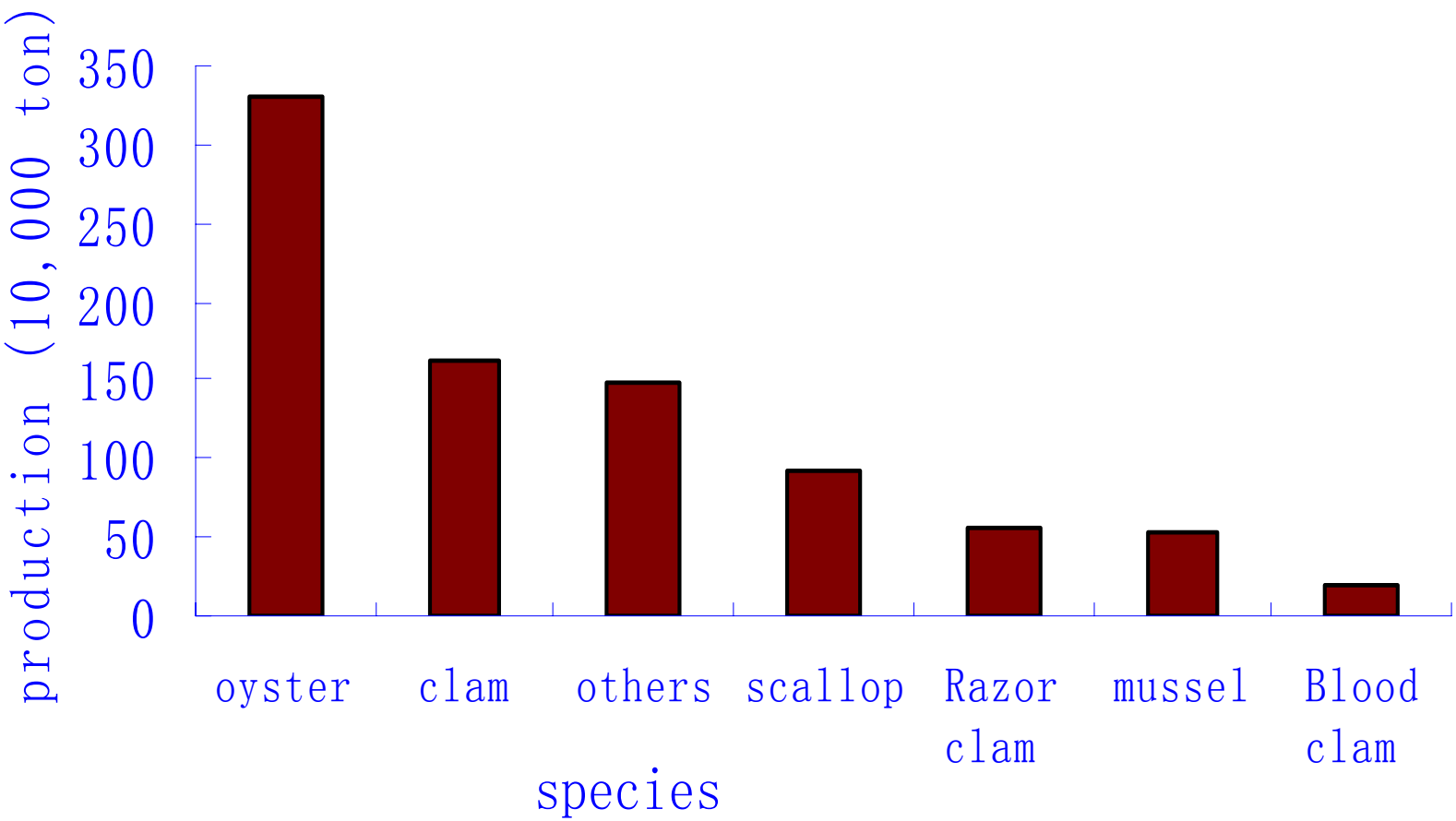
- Scallop aquaculture in China.
- cDNA libraries and EST analysis.
- Immune recognition.
- Signal modulation and amplification.
- Signal transduction.
- Transcription activation.
- Pathogen elimination.

Scallop aquaculture in China

Scallop aquaculture in China has been growing rapidly in the past decades.



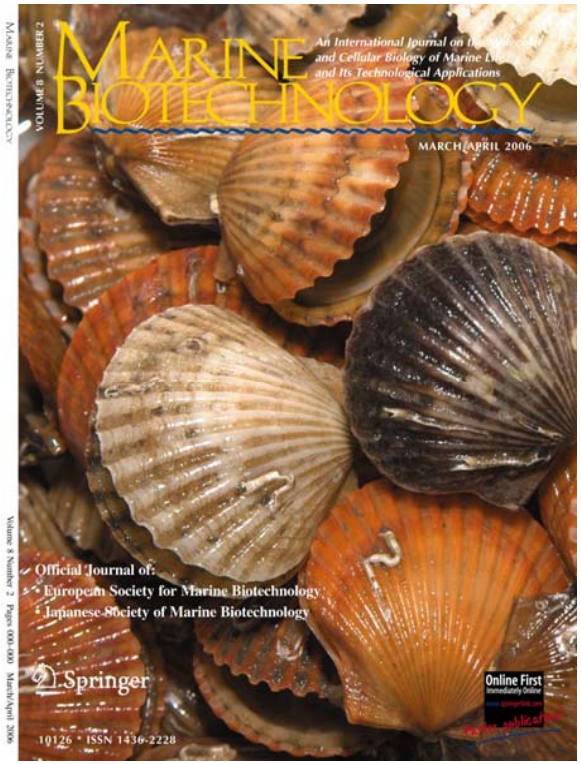
Aquaculture production of molluscan in China in 2000



The production of molluscan quaculture was 8.60 million tons, accounting 81.8% of the total mariculture production in China in 2000.

Chlamys farreri and *Argopecten irradians* are the two major cultured species

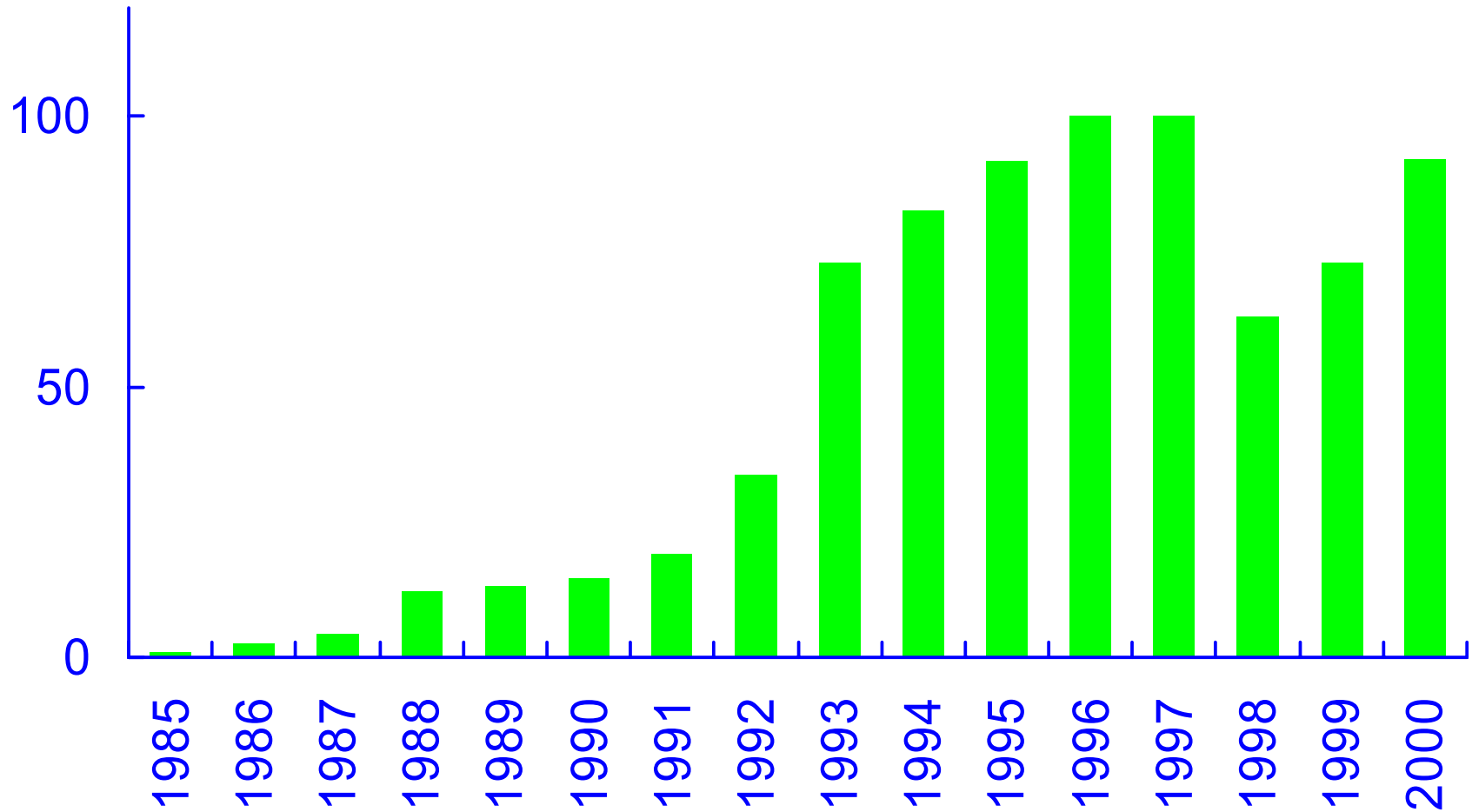
Zhikong scallop
Chlamys farreri



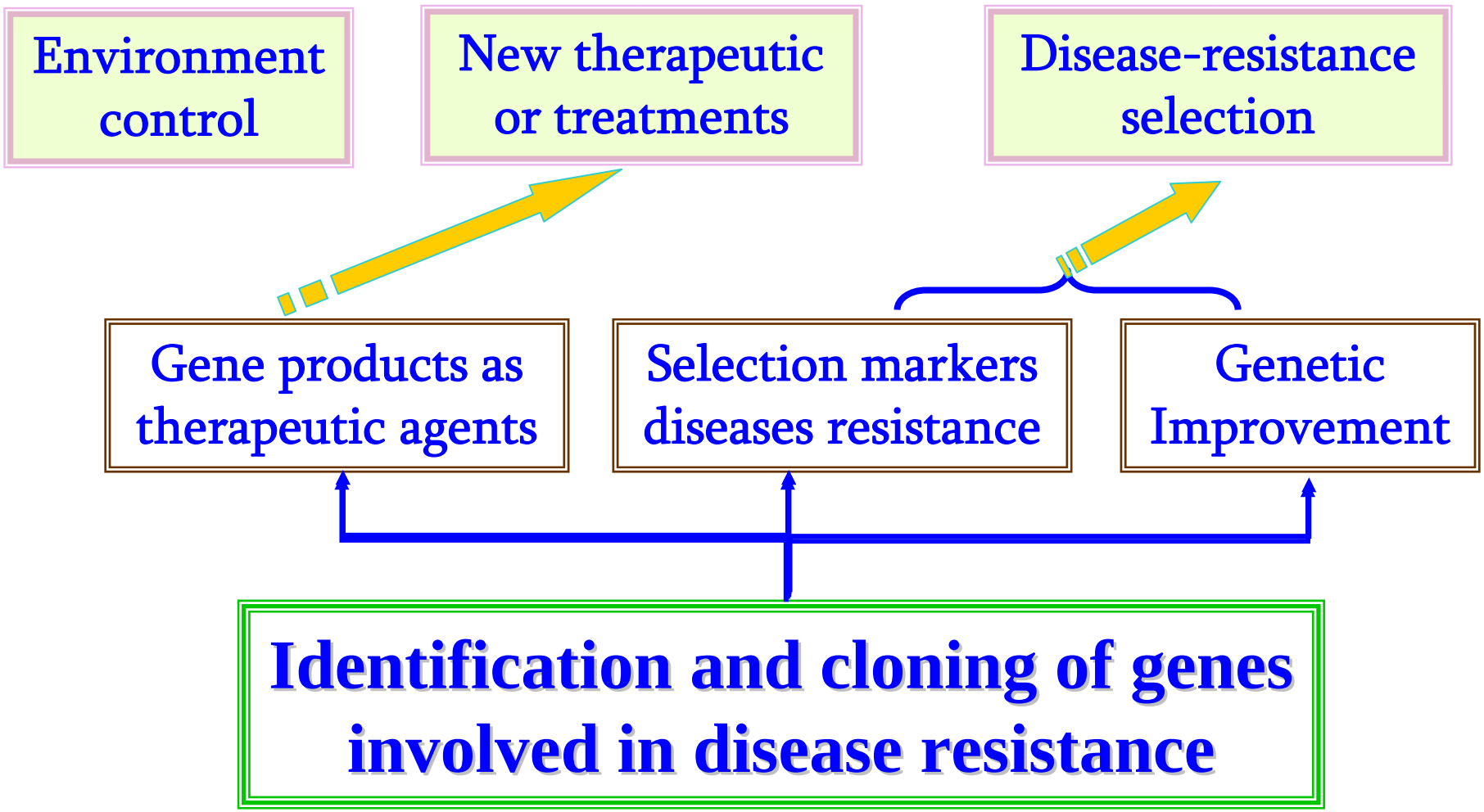
Bay scallop
Argopecten irradians

Prof. Fusui Zhang

Aquaculture production of scallop in China from 1985 to 2000



Approaches to control diseases



Invertebrate immunity system is comprised of two branches:

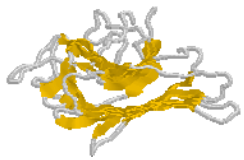
Humoral response:

Antimicrobial peptides.

Macrokines.

Clotting system

proPO activating system



lectins

Cellular response:

Phagocytosis

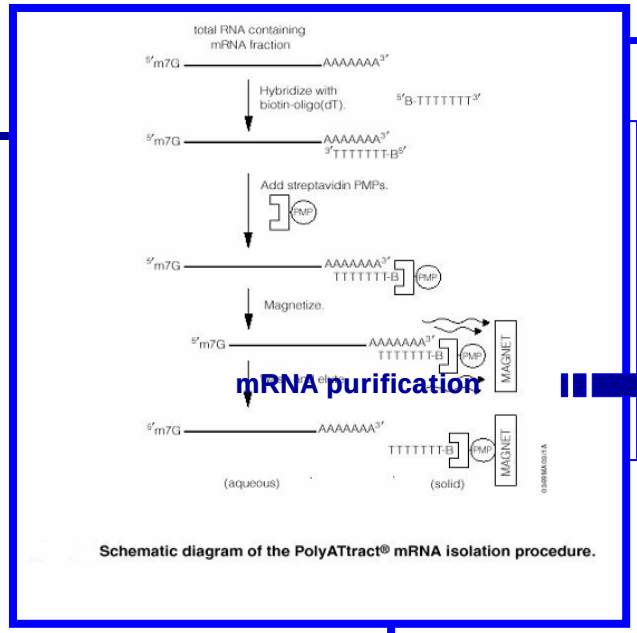
Encapsulation

Nodulation

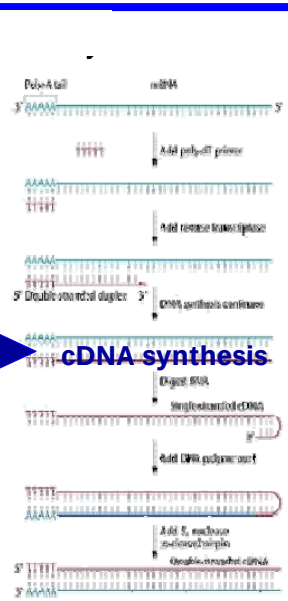
The process of humoral response

1. Immune recognition
2. Signal modulation and amplification
3. Signal transduction
4. Transcription activation
5. Eliminating invaders

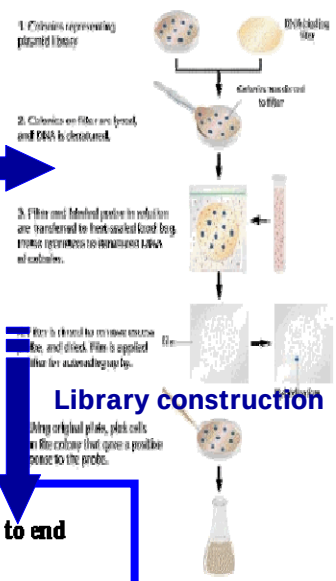
cDNA library construction and EST analysis



mRNA purification

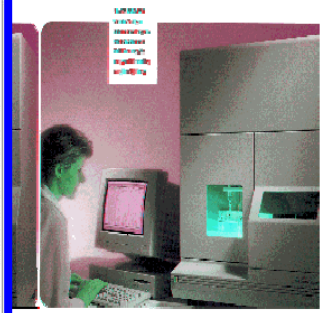


cDNA synthesis

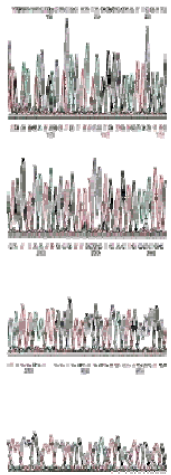


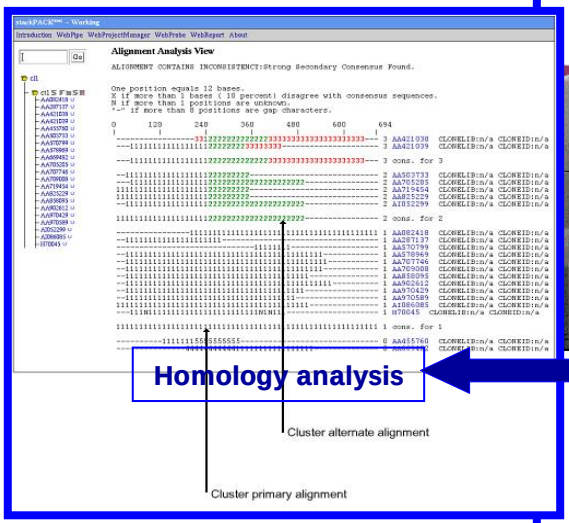
Library construction

Automated DNA sequencing used fluorescent dyes to end label newly synthesized strands of DNA



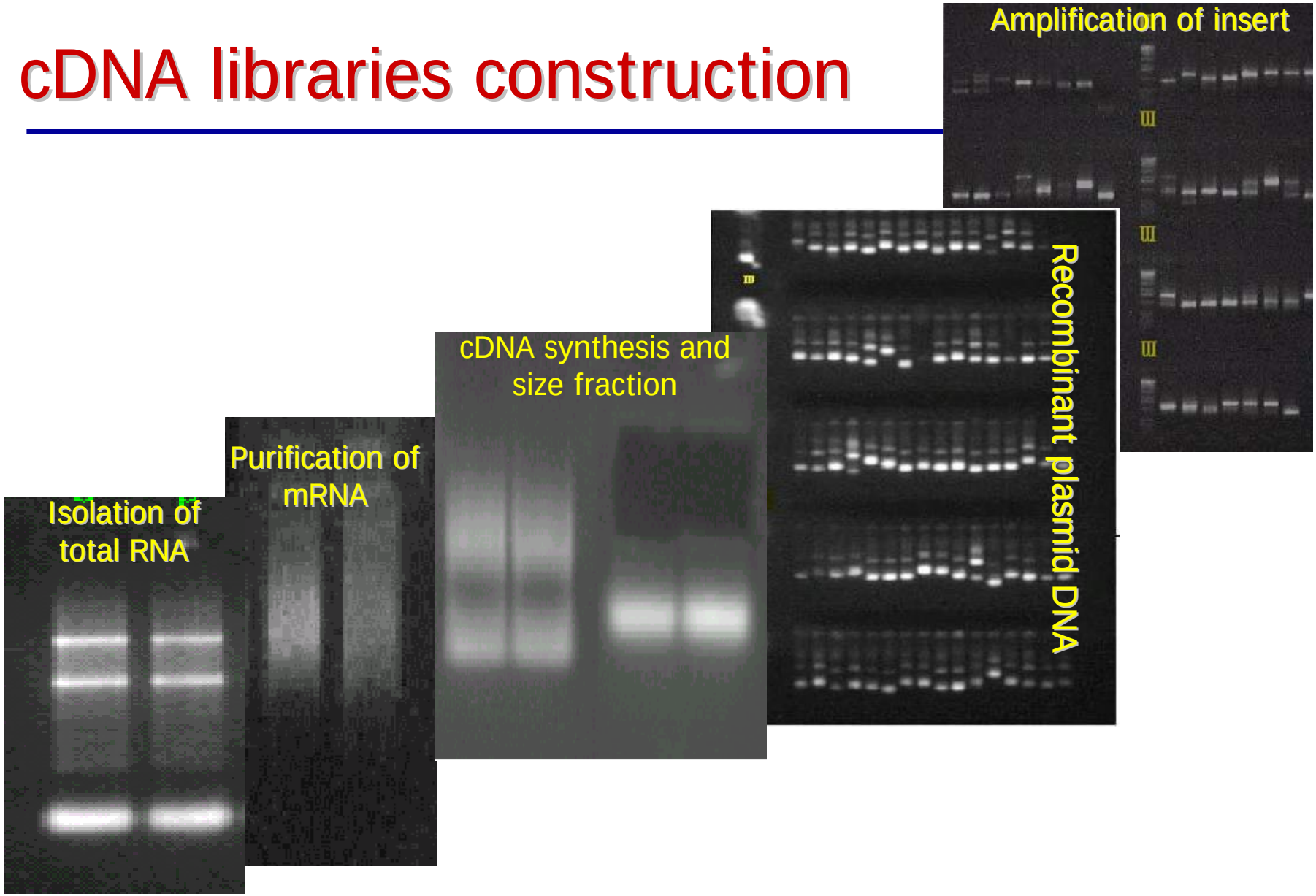
DNA sequencing



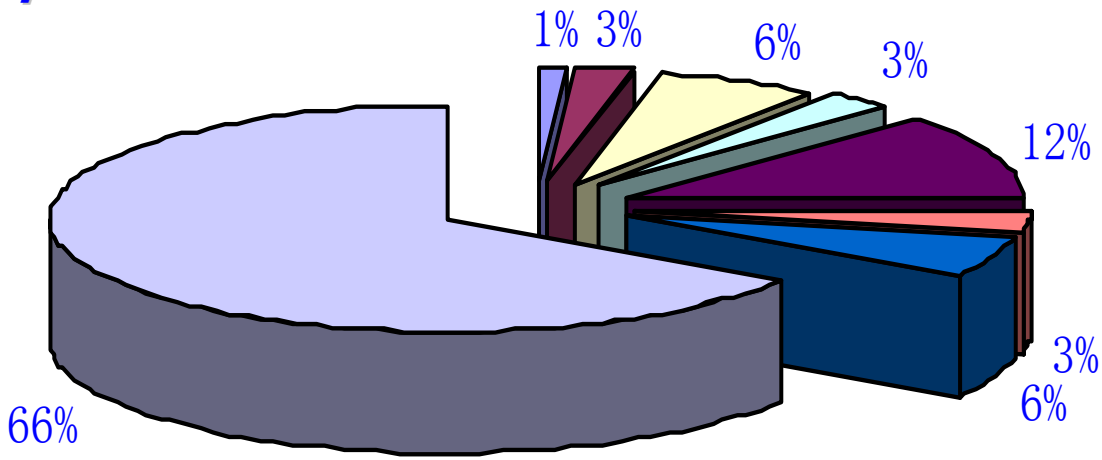


Homology analysis

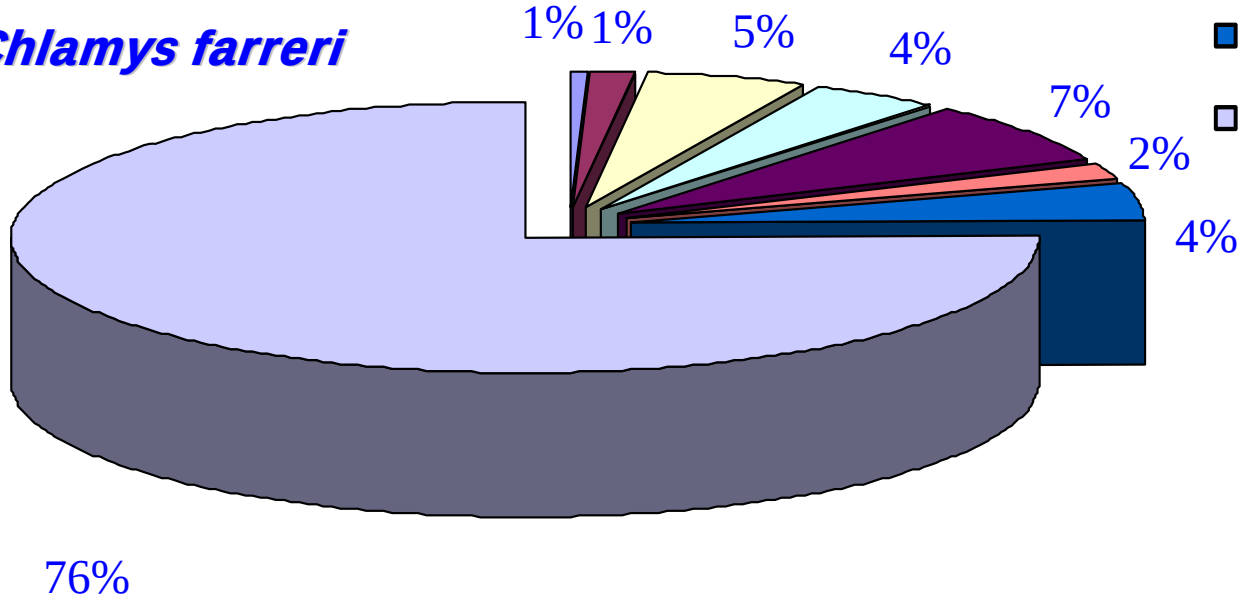
cDNA libraries construction



Argopecten irradians

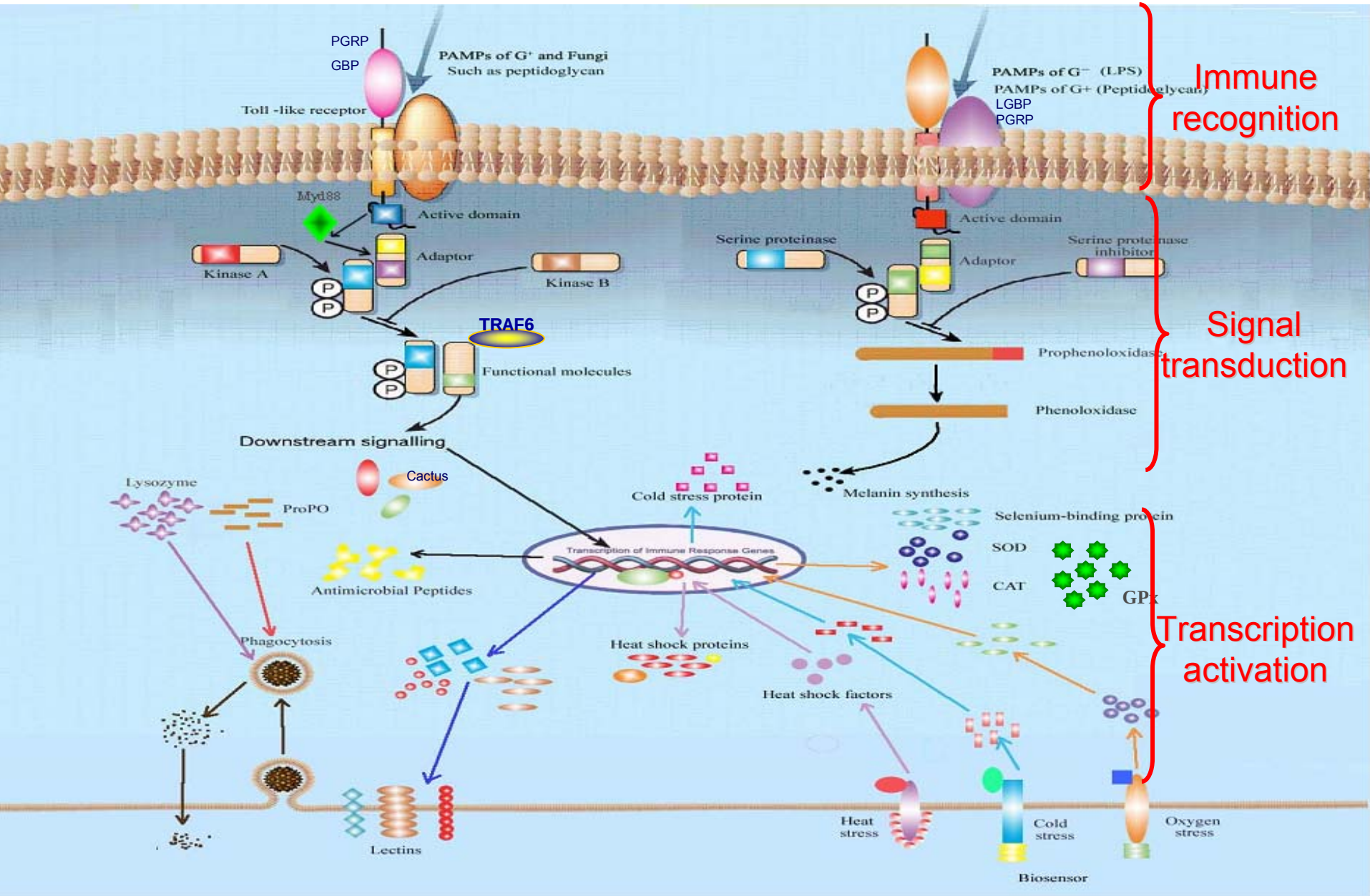


Chlamys farreri



- cell division
- cell signalling/communicati
- cell structure/motility
- cell/organism defense
- gene/protein expression
- metabolism
- unknown
- no match

Gene cloning and characterization



(1) Genes involved in immune recognition (LGBP, PGRP, Lectins, TLRs)

Jianguo Su et al., 2004, Aquaculture

Duojiao Ni et al, 2007, Dev. Com. Immuonol

Jianguo Su et al., 2007, Fish & shellfish Immunol

Hao Wang et al, 2007, Mol. Immunol

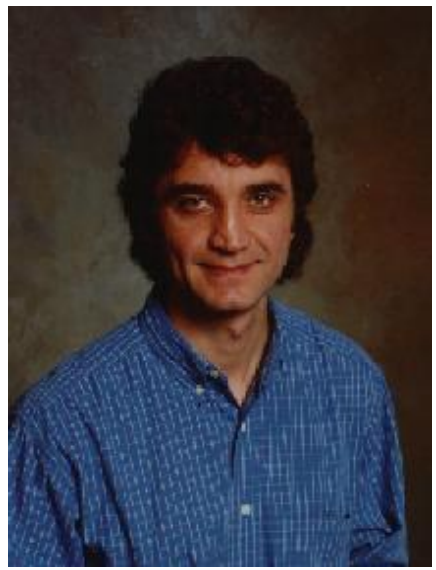
Limei Qiu et al., 2007, Fish & shellfish Immunol

Pattern recognition

Pathogen-associated molecular pattern, PAMP

Pattern recognition receptor, PRR

Charles Janeway



Ruslan Medzhitov

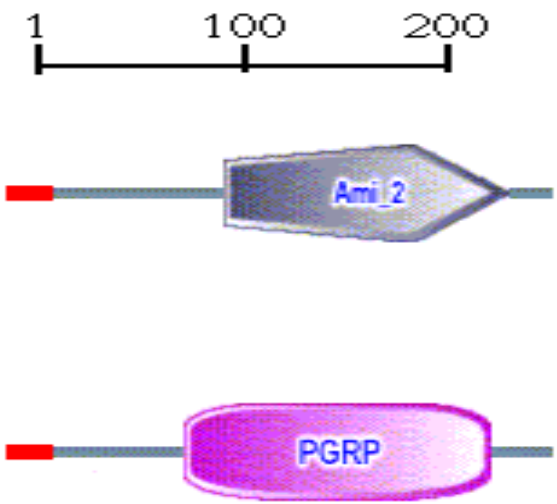
The genes involved in immune recognition (14)

Gene name	Function	Full-length/ ORF (bp)	Accession No.	species
AiPGRP	recognition/G^+	1018/615	AY437875	*A. irradians*
CfPGRP		1073/759	AY987008	*C. farreri*
CfLGBP	recognition/G^- or Fungi	1876/1320	AY259542	*C. farreri*
CfCTL-A	Recognition the virus carbohydrate/glycoprotein	1038/684	AY676311	*C. farreri*
CfCTL-1		708/171	DQ209289	*C. farreri*
CfCTL-2		1772/221	DQ209290	*C. farreri*
CfCTL-3		2257/524	DQ209291	*C. farreri*
CfCTL-4a		2086/633	DQ209292	*C. farreri*
CfCTL-4b		1897/633	DQ2092893	*C. farreri*
TLR-4	recognition/G^-	4307/3594	--	*C. farreri*
TLRs(1,5,8)	recognition	1038-3085	--	*C. farreri*
SR	recognition	partial/1439	--	*C. farreri*

✧ Cloning and mRNA expression of LGBP and PGRP gene from scallops

- LGBP (Lipopolysaccharide-and beta-1,3-glucan-binding protein) and PGRP (Peptidoglycan recognition protein) play a crucial role in the innate immune response as a pattern recognition protein.
- They can recognize and bind lipopolysaccharide in the G^- bacteria and glucan in fungi, or peptidoglycan in the G^+ bacteria to trigger the responses such as phagocytosis, nodule formation, encapsulation, activation of proteinase cascades, and synthesis of antimicrobial peptides.

The predicated structure of PGRP

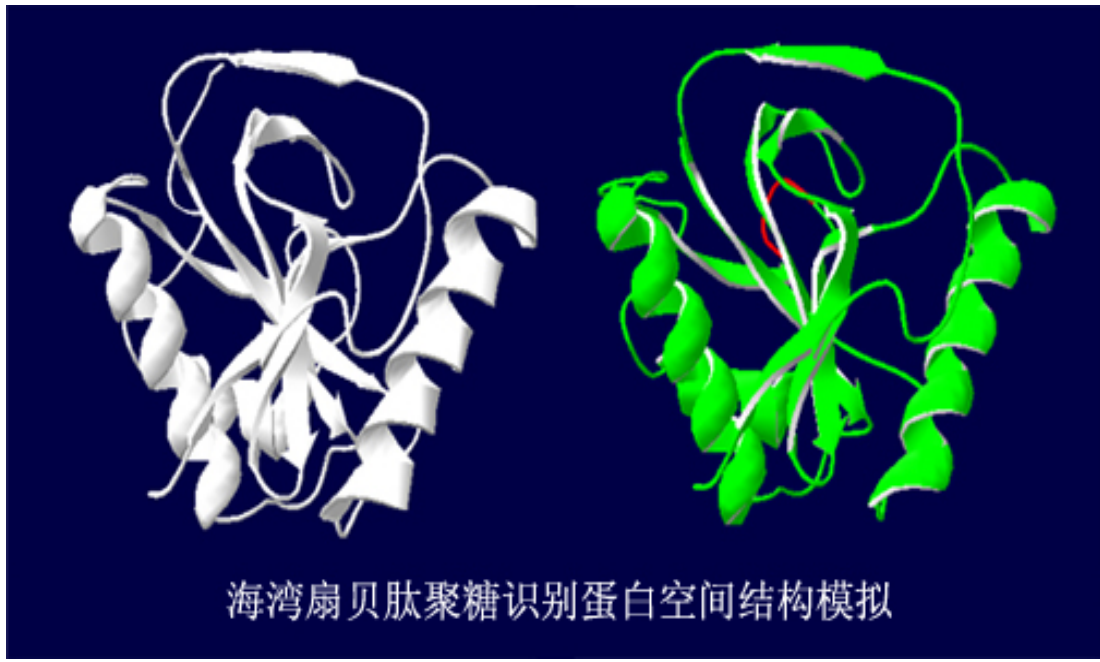


Signal peptide: 1-22 Aa；

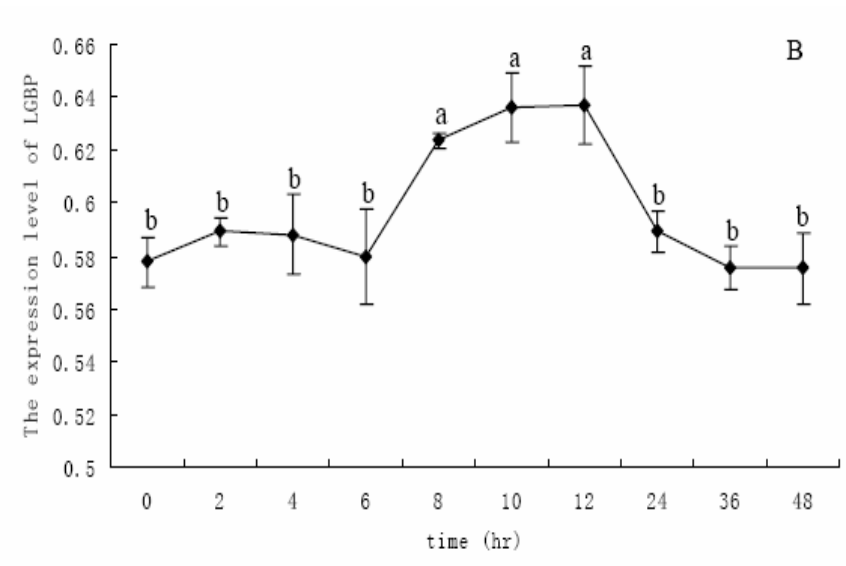
PGRP domain: 83-225；

Ami_2 domain: 100-231

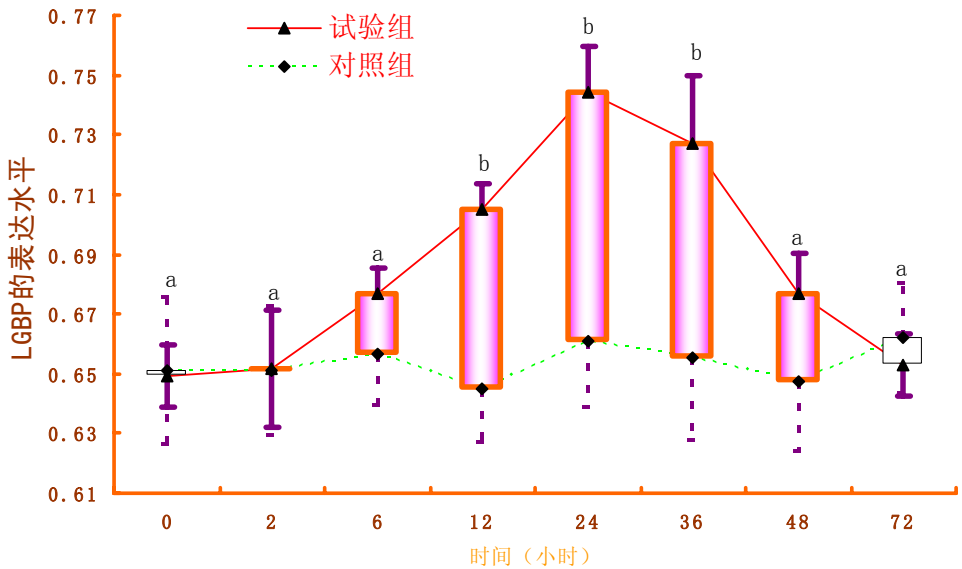
Human Scallop



The expression of LGBP in scallop challenged by bacteria or yeast



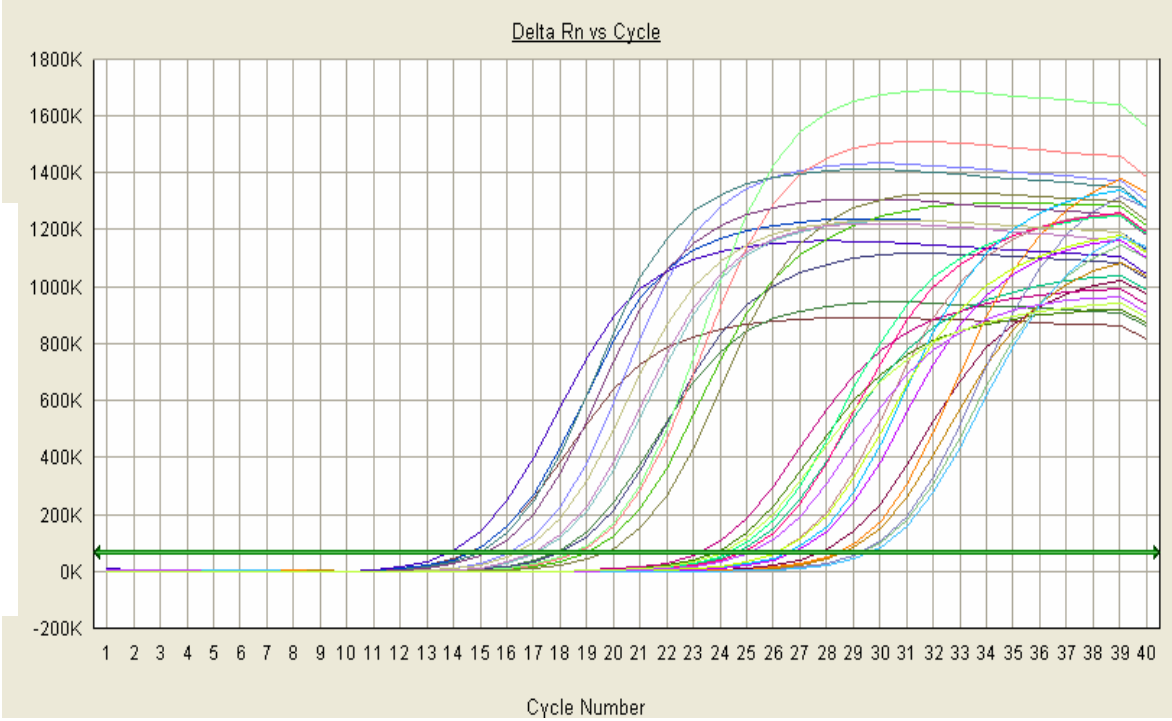
Vibro

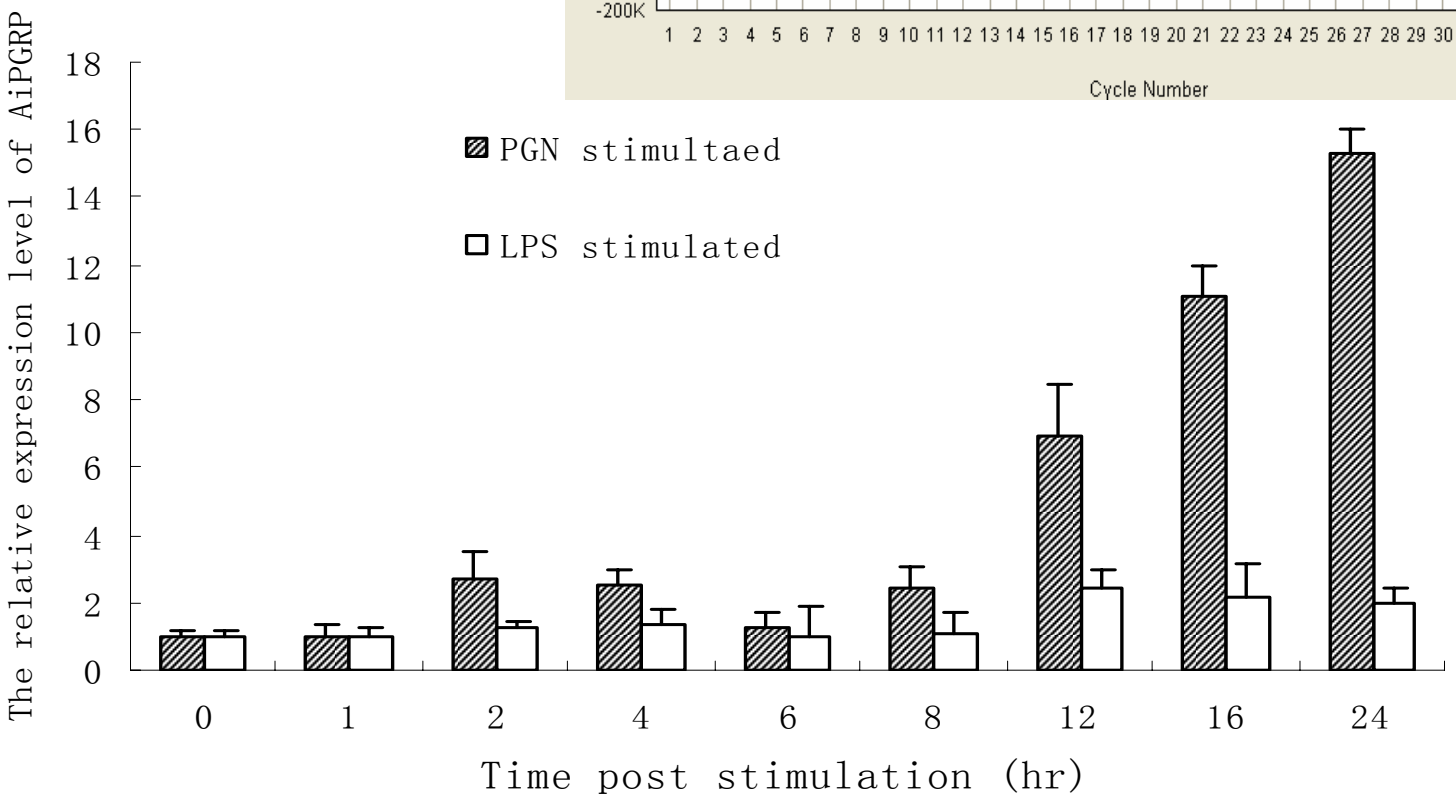


yeast

Data plotted are mean + SD of three replicates. Data that are significantly different are indicated by different letters above the bars(1-way ANOVA, p<0.05)

The expression of PGRP in the pre culture of hemocytes stimulated by LPS andPGN.





✦ C type lectin genes cloned from scallop *Chlamys farreri* (5)

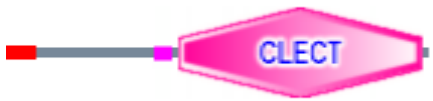
Name	Full length	A.a	CLECT domains
CfCTL-A	708	171	1
CfCTL-B	1772	221	1
CfCTL-C	2257	524	3
CfCTL-D1	2086	633	4
CfCTL-D2	1897	633	4

Lectin is a family of sugar-binding proteins of non-immune origin that agglutinates cells or precipitates glycoconjugates.

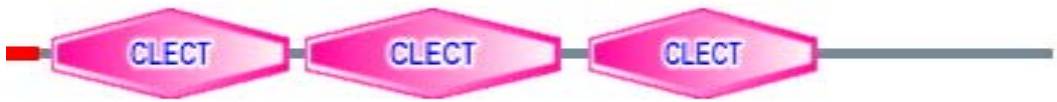
The predicated structure of scallop C-lectins

CfCTL-A

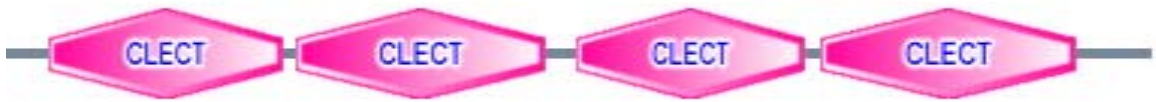
CfCTL-B



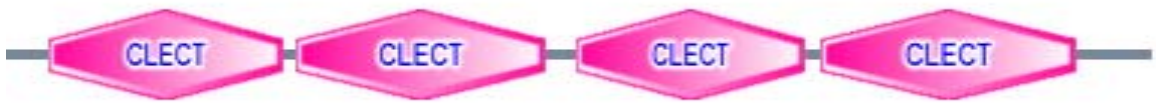
CfCTL-C



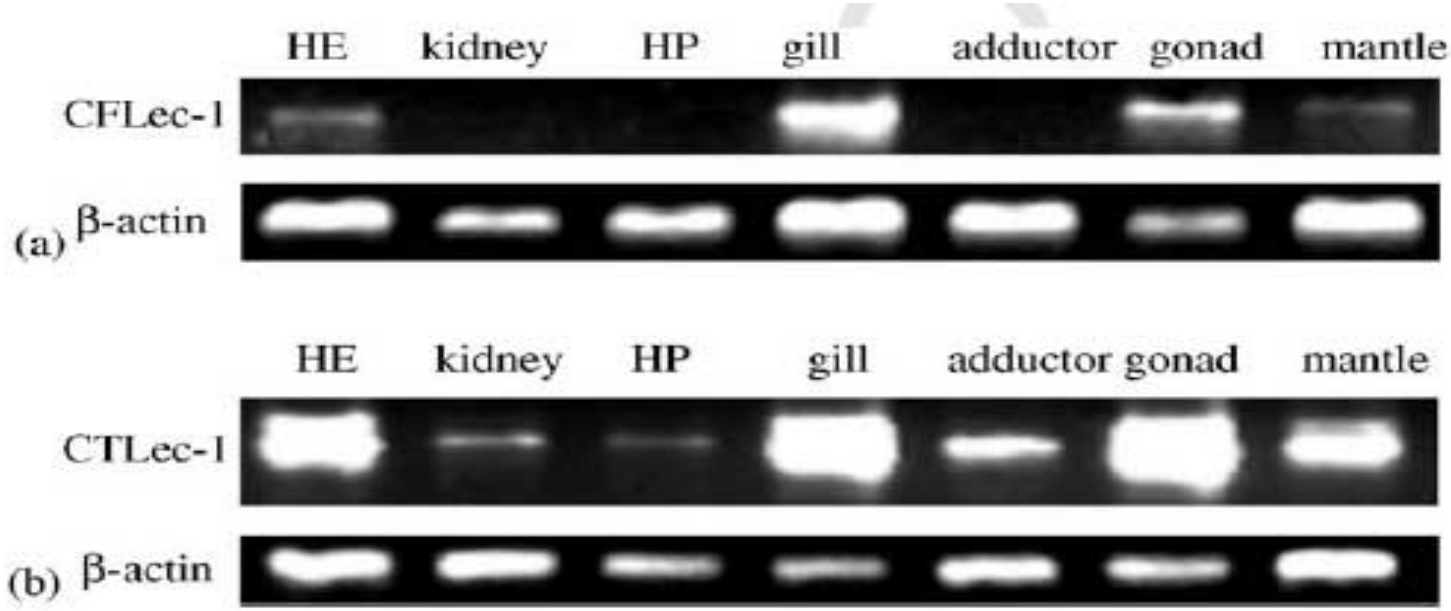
CfCTL-D1



CfCTL-D2



mRNA expression of CFLec-1 in different tissues

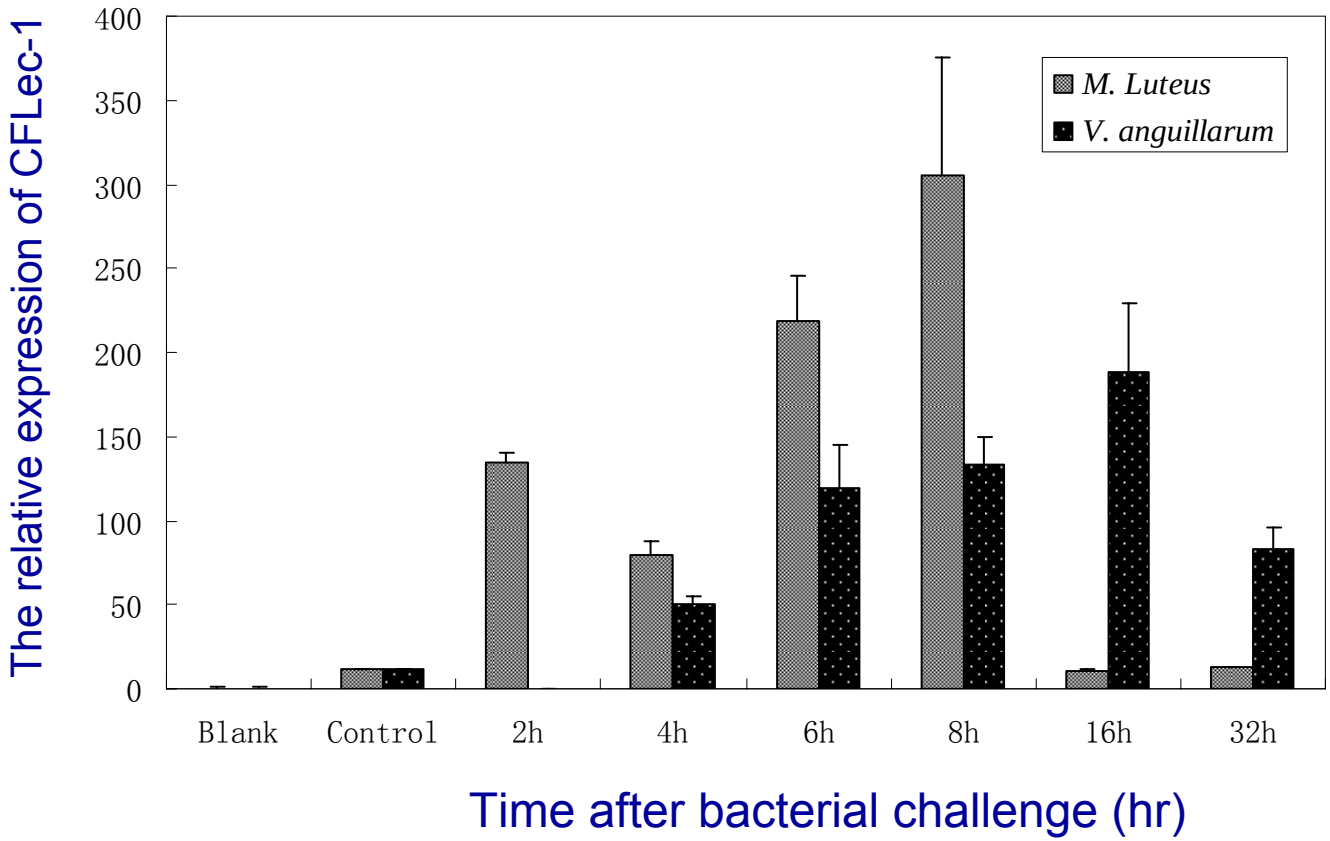


(a): Healthy scallop; (b): Scallops challenged by bacteria.

HE: Haemocytes; HP: hepatopancreas

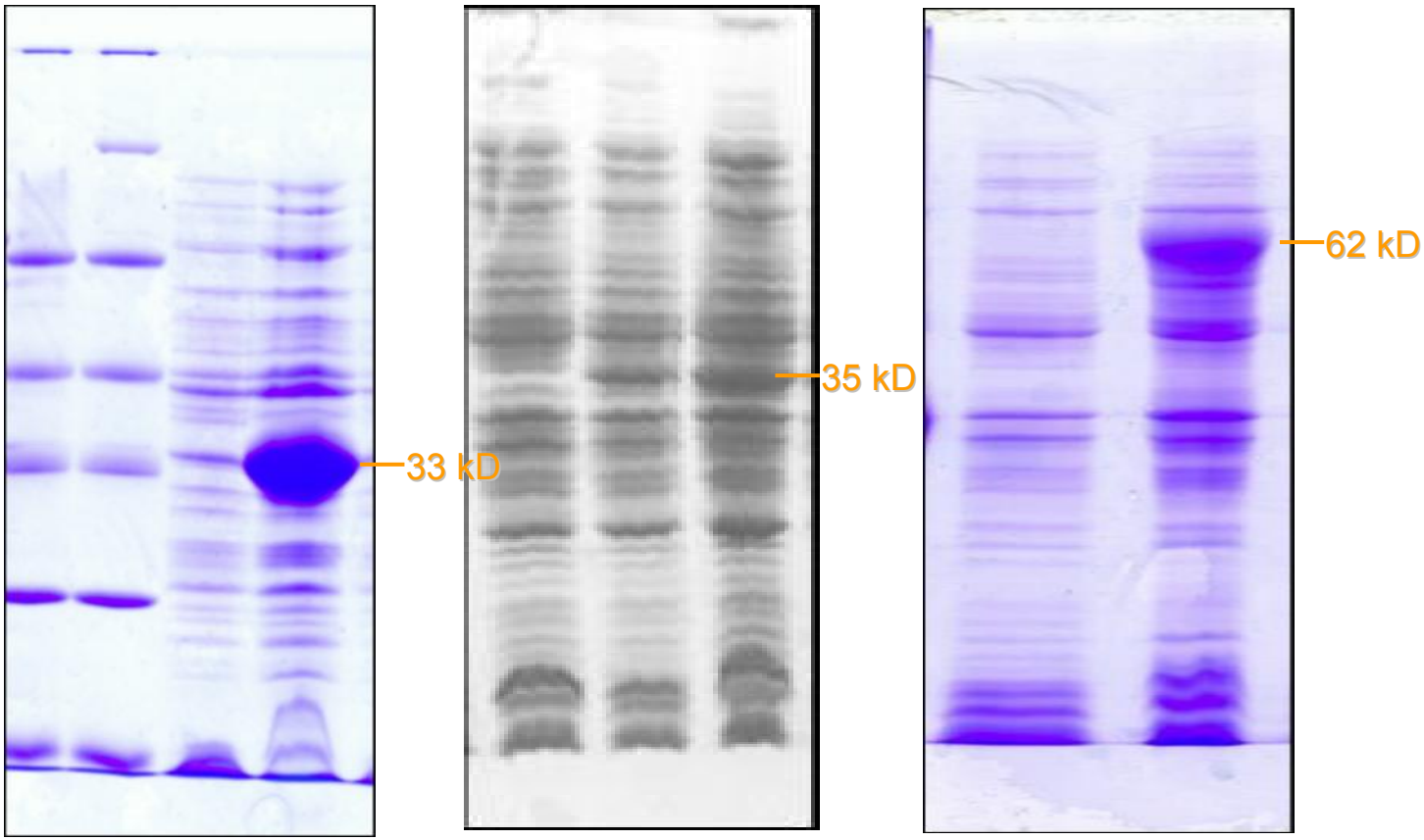
CFLec-1 is predominate expressed in haemocytes, gills, and gonad.

The temporal expression of CFLec-1 after bacteria challenges

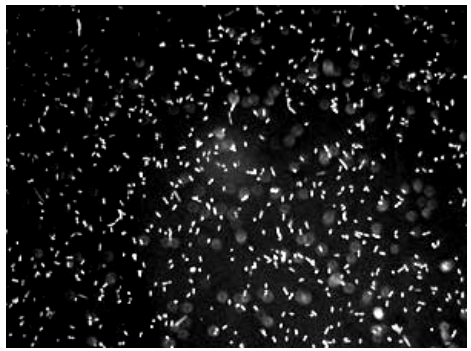


Bacteria (G^+, G^-) challenge up-regulate the expression of CfLec-1.

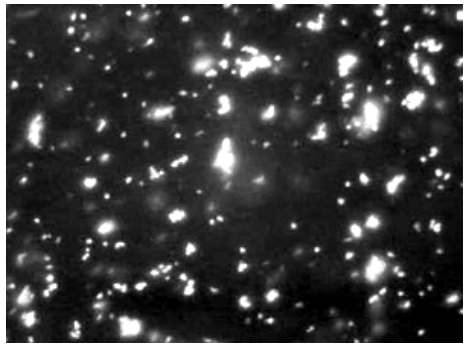
Recombination and expression of C-Lectins



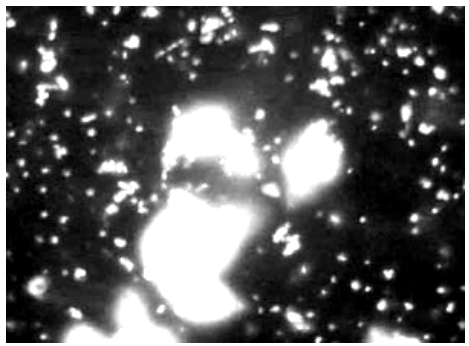
The bioassay of recombinant Lectin



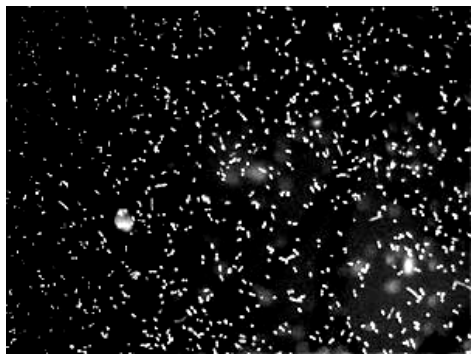
BSA (1mg mL^{-1})



rCFLec-1 (~ 20 ug mL^{-1})



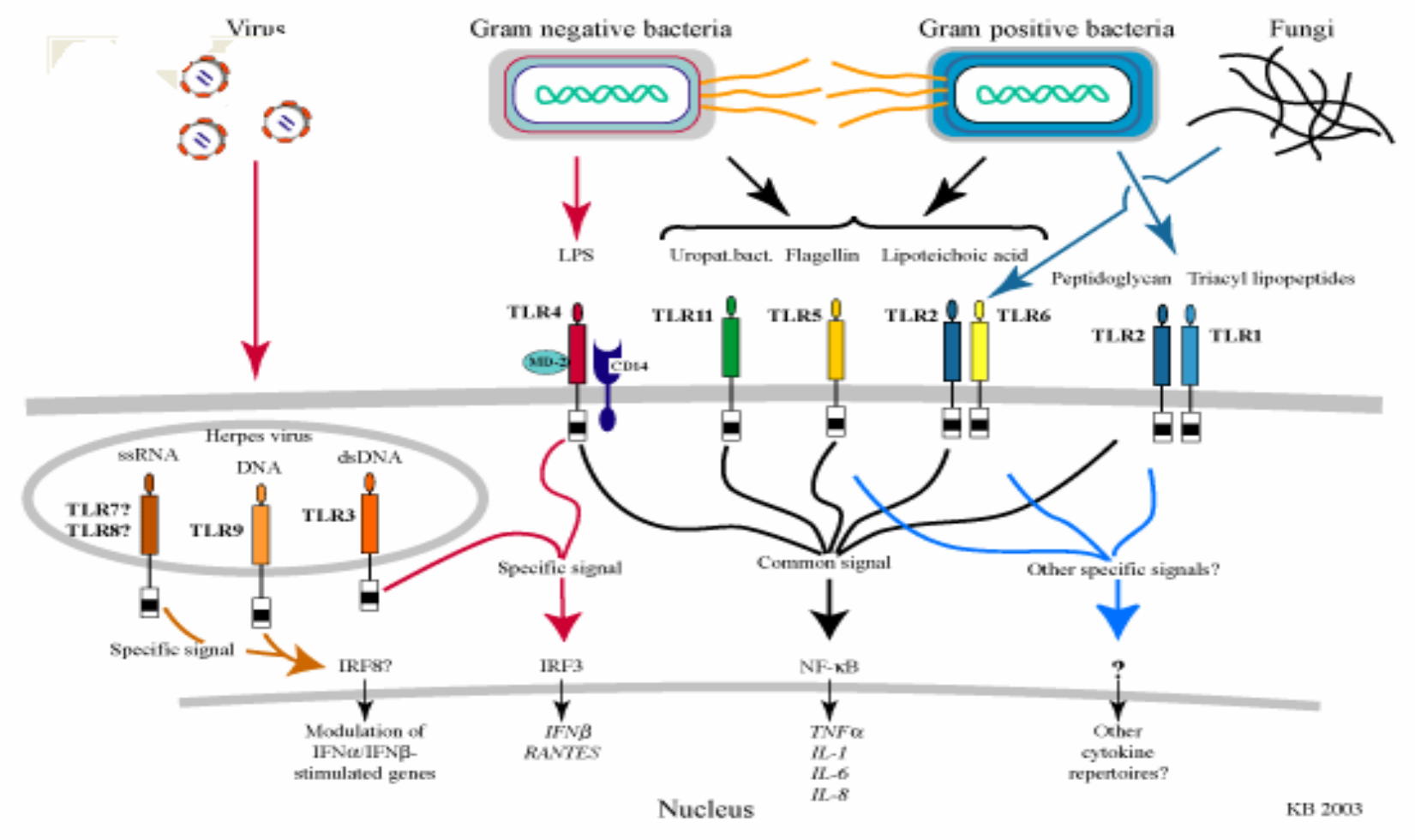
rCFLec-1 (~ 80 ug mL^{-1})



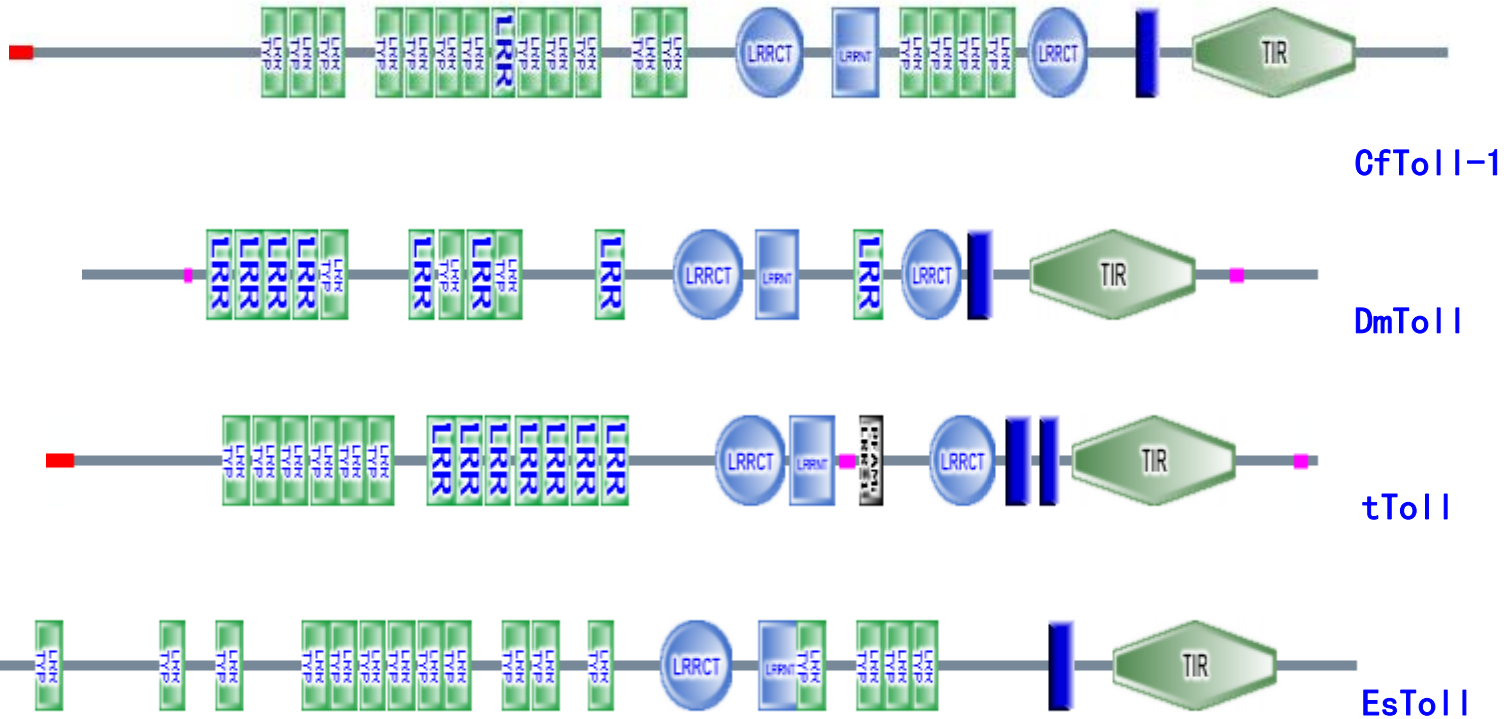
rCFLec-1 (~80 umg mL^{-1})
in TBS-EDTA

The recombinant Lectin displayed strong activity to agglutinate bacteria.

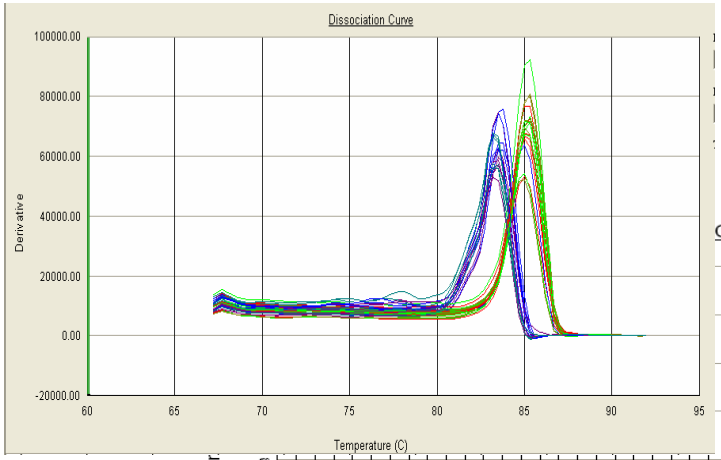
- TLR is a family of PRRs which can recognize and bind different PAMPs and plays a crucial role in the innate immune response.

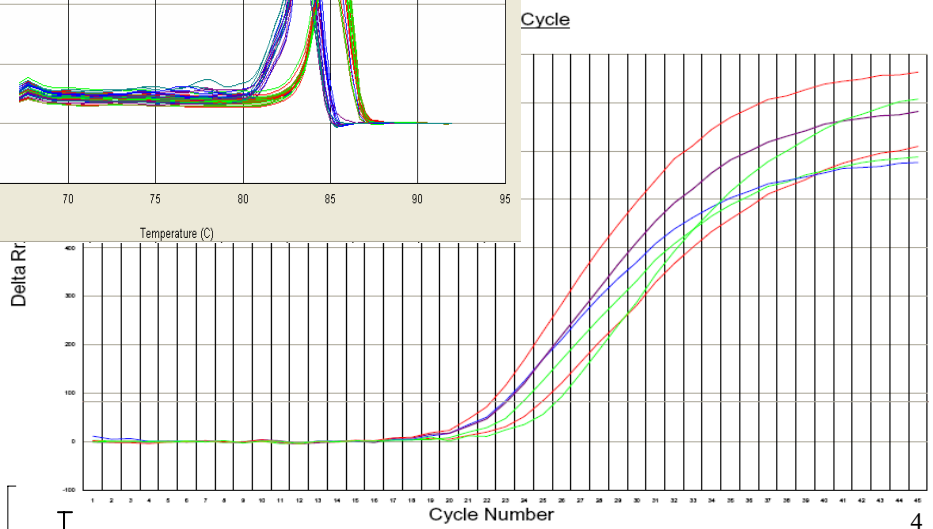


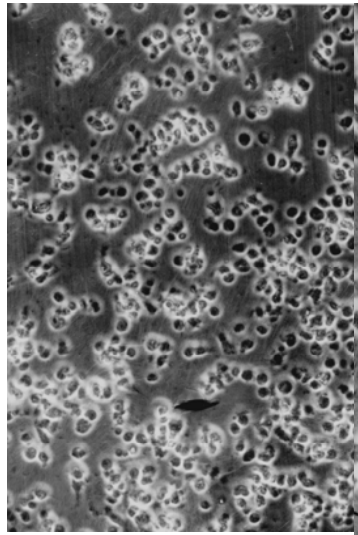
The predicated structure of scallop CfToll-1



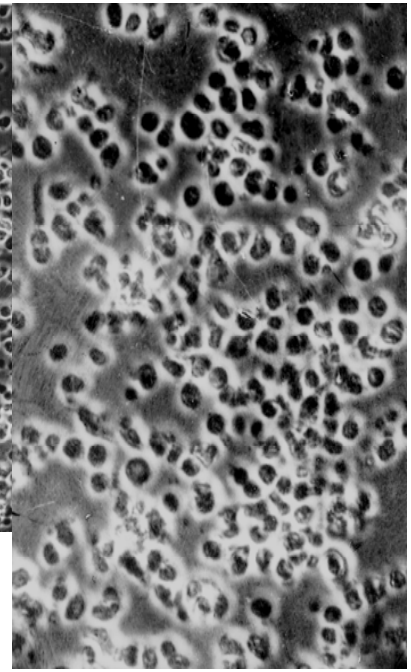
Limei Qiu et al., 2007, Fish & shellfish Immunol



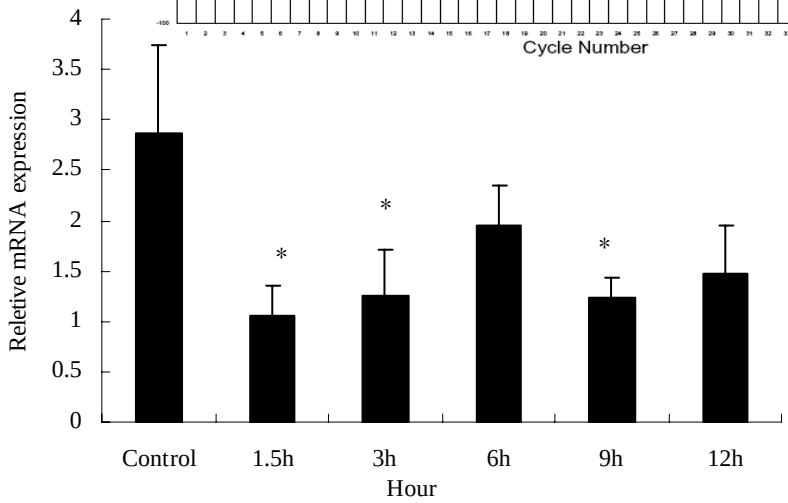




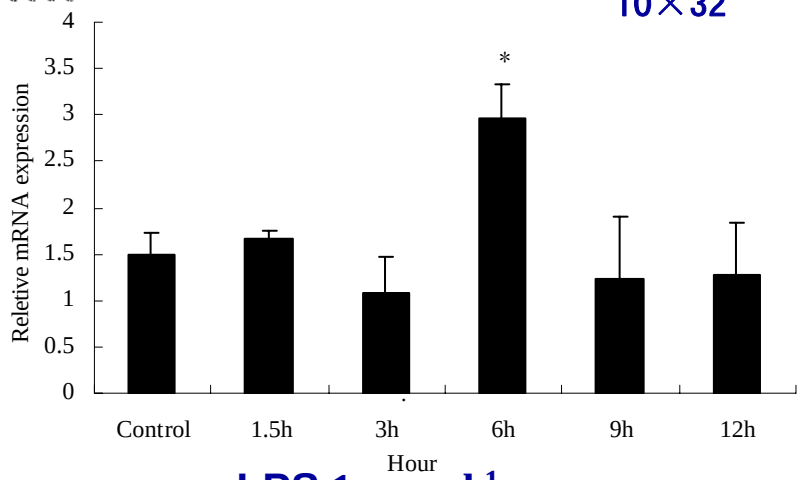
10×20



10×32



LPS 100ng.ml^{-1}



LPS 1 μ g.ml^{-1}

The expression of CfToll-1 in the heamocytes after LPS stimulation

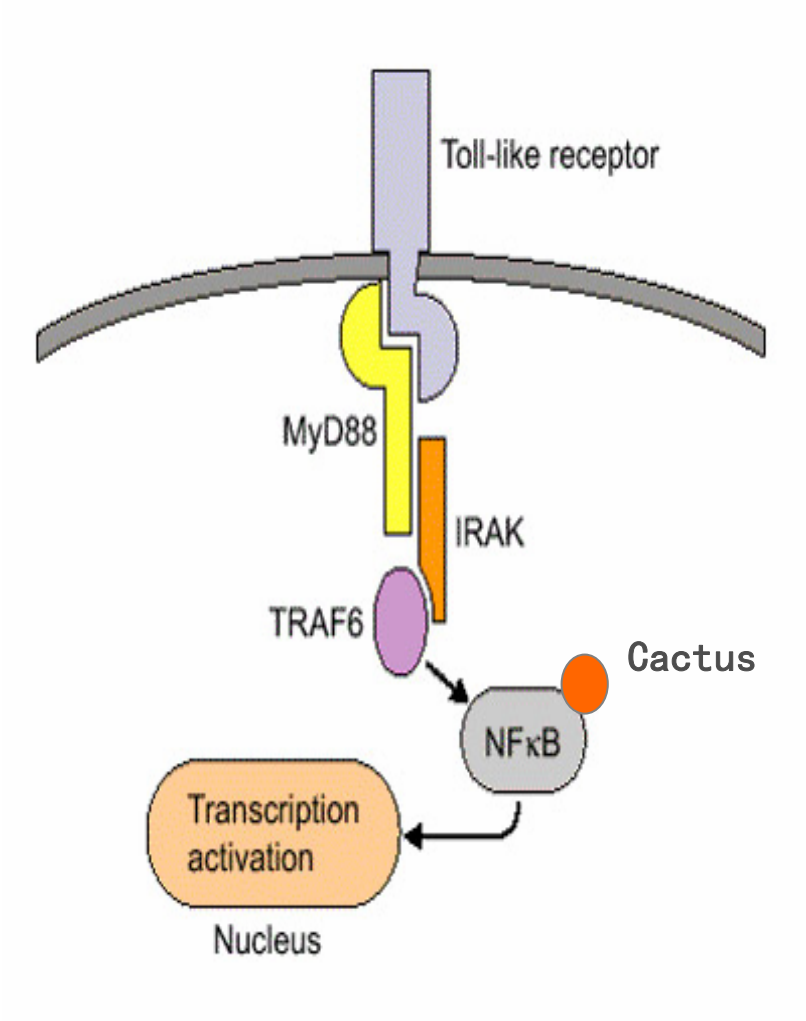
(2) Genes involved in Signal modulation, amplification and transduction

(Myd 88, TRAF6, Cactus, SERPINs)

Limei Qiu et al., 2007, Fish & shellfish Immunol

Ling zhu et al, 2006,200,7 Fish & shellfish Immunol

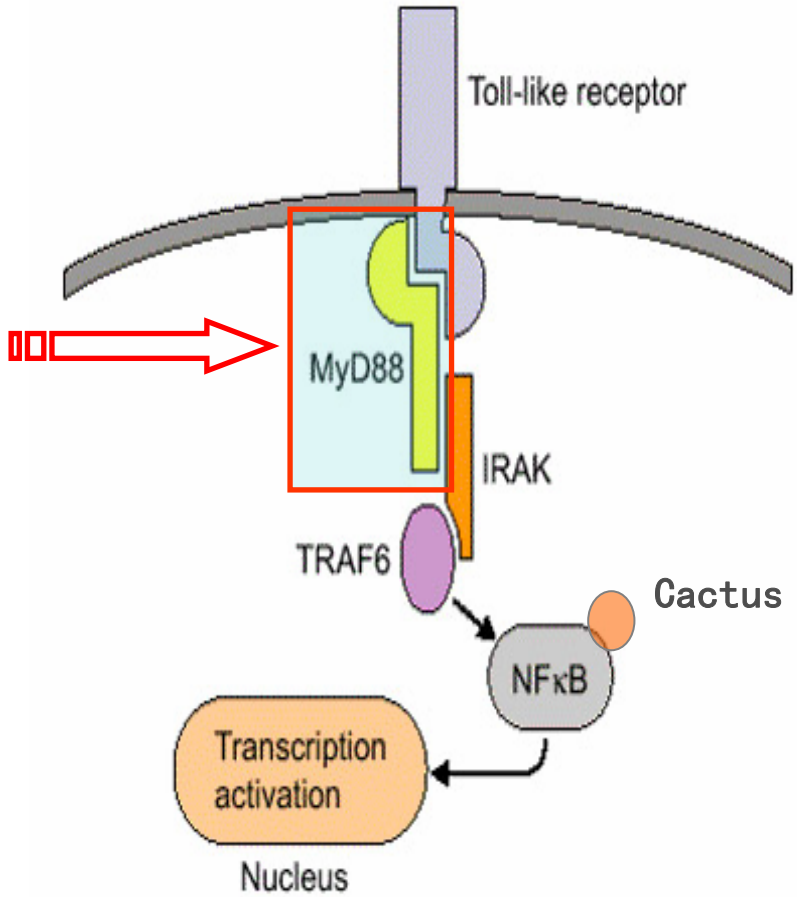
Toll signaling pathway



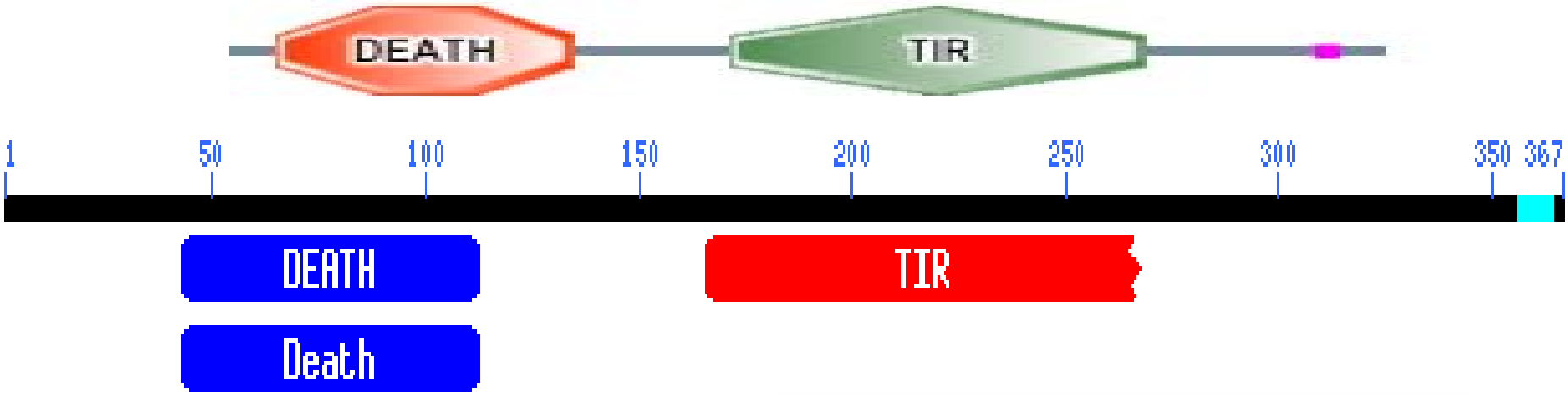
The genes involved in immune signal modulation, amplification and transduction (13)

Gene name	Function	Full-length/ ORF (bp)	Accession No.	species
CfMyd88	Adaptor and transduction	1564 /1101	DQ249918	*C. farreri*
TRAF6		2510 /1965	--	*C. farreri*
Cactus	transduction	2488/827		*C. farreri*
CfSP-1		1121/1062	DQ186670	*C. farreri*
CfSP-2		1119/1008	--	*C. farreri*
CfSP-3		922/798	--	*C. farreri*
CfSERPIN -1	PPO and PO cascade	1841 /1524	QD236243	*C. farreri*
CfSERPIN -2		1358 /1041	QD236244	*C. farreri*
CfSERPIN -3		1187 /1092		*C. farreri*
CfSERPIN -4		1064 /969		*C. farreri*
CfSERPIN -5		632 /279	--	*C. farreri*
AiSERPIN -1		1020 /834	AY830445	*A. irradians*
AiSERPIN -2		714 /897	QD236241	*A. irradians*
AiSERPIN -3		642 /459	QD236242	*A. irradians*

✦ Cloning and expression of Myeloid differentiation factor 88 (Myd88) from *Chlamys farreri*



The structure of *Chlamys farreri* Myd 88

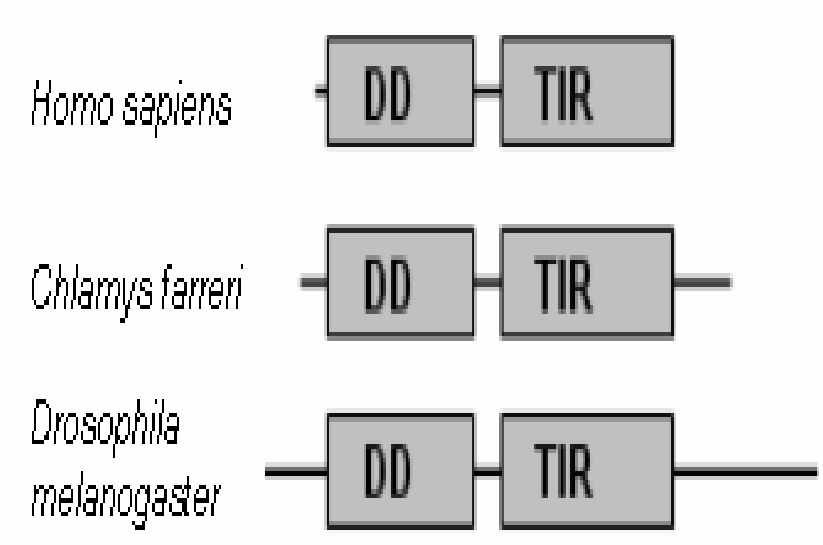


Full length:1554bp;

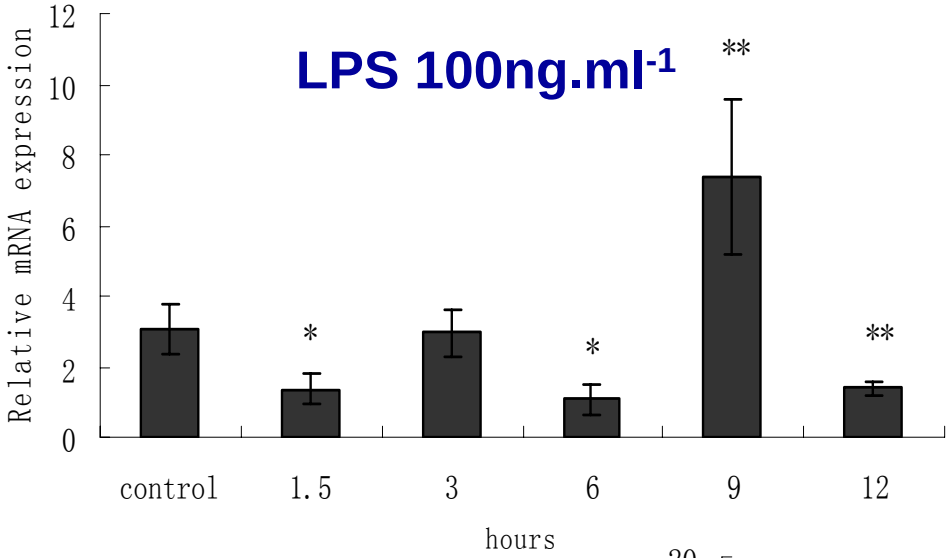
Protein: 367Aa

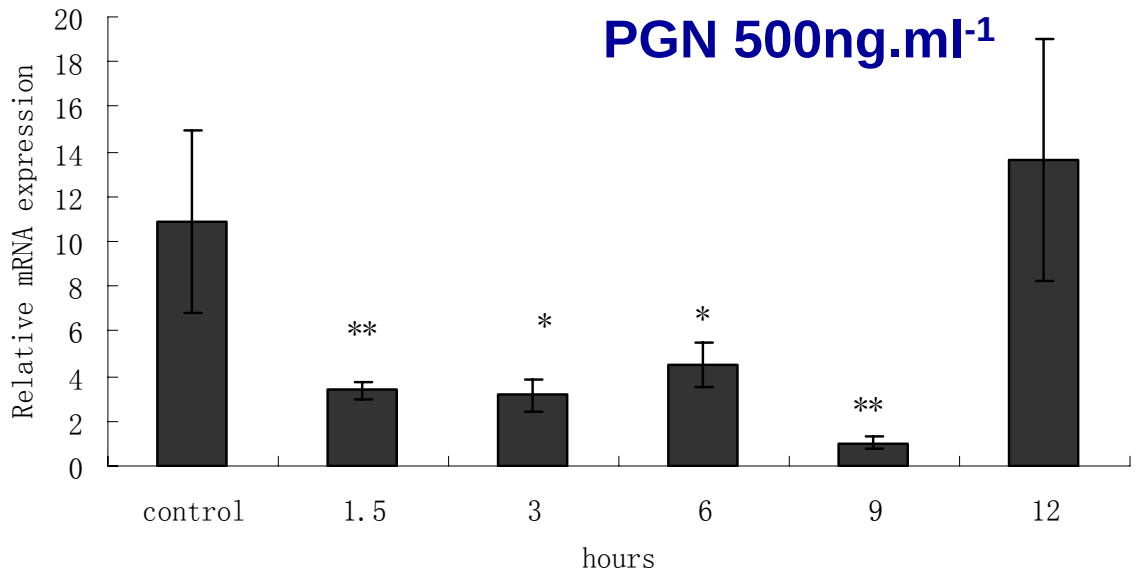
death domain (DD);

TIR domain

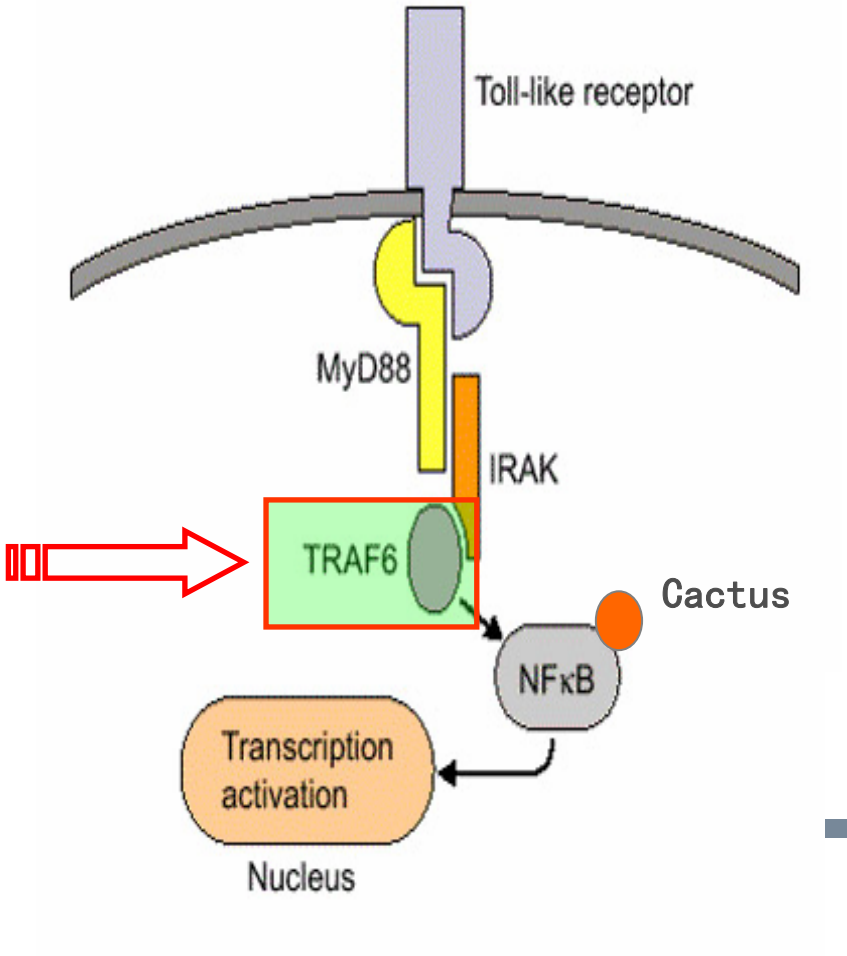


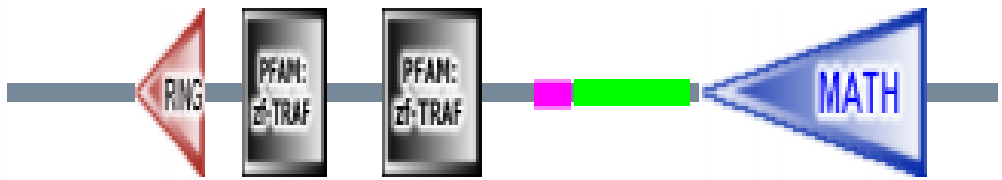
The mRNA expression of Myd88 in the primary culture of heamocytes stimulated by LPS and PGN



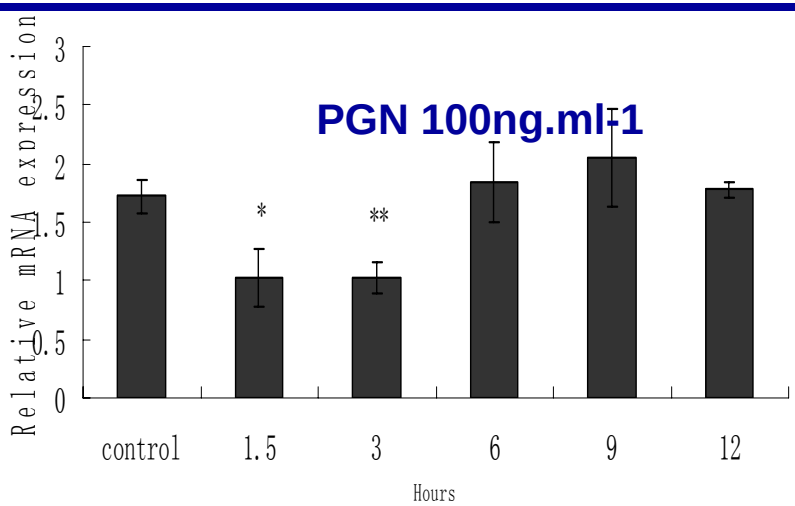


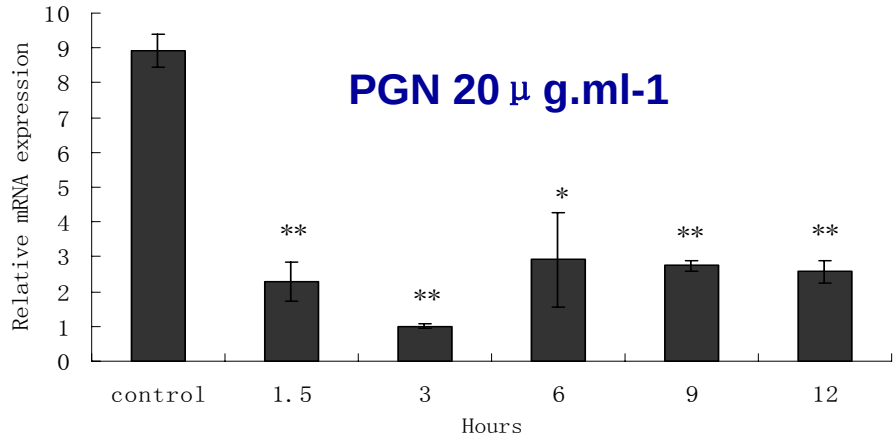
TNFR-associated factor 6 (TRAF6)

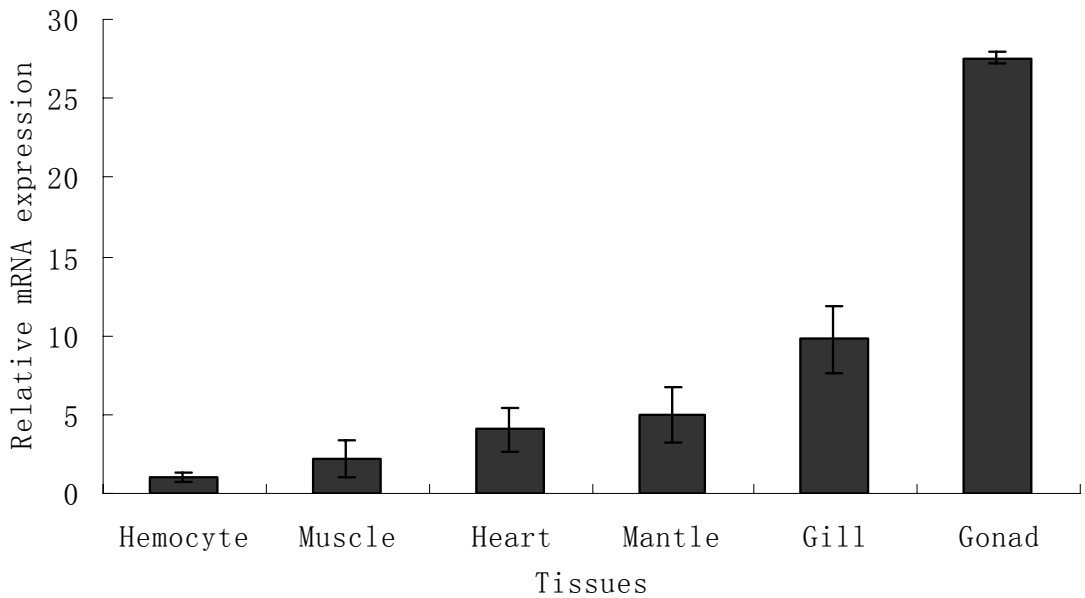




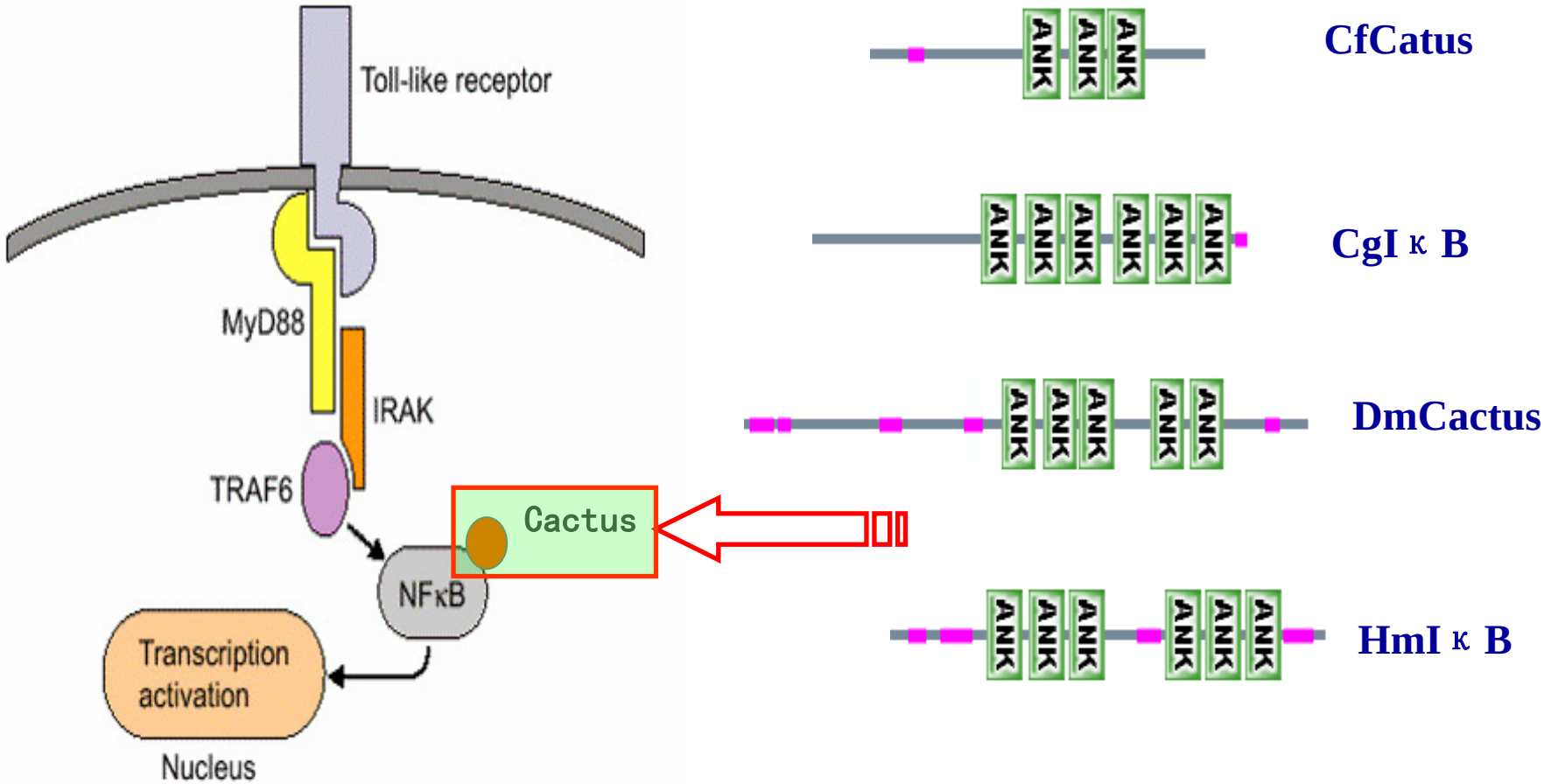
mRNA expression of CfTRAF6



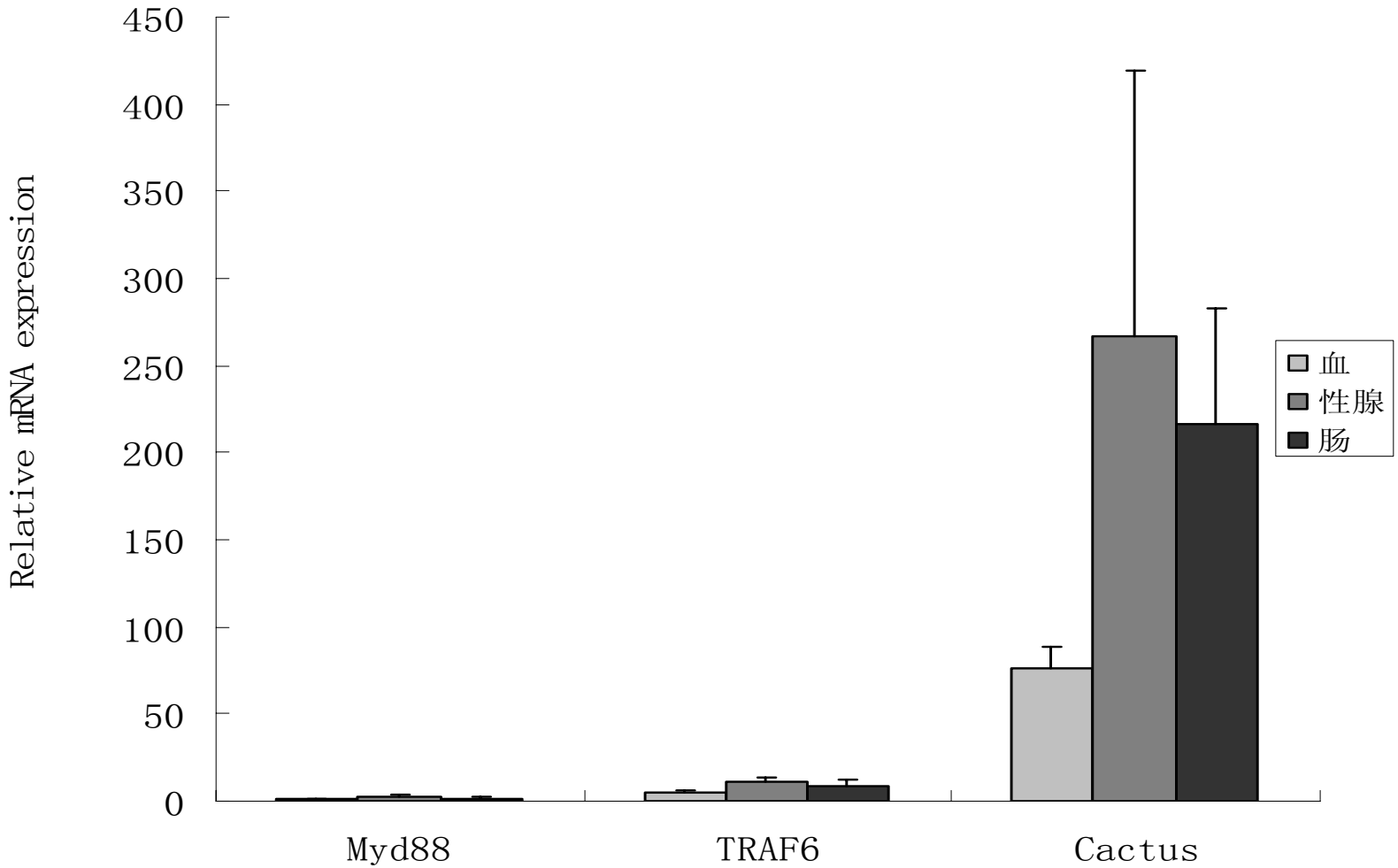




Cactus


Toll-like receptor
MyD88
IRAK
TRAF6
Cactus
NFκB
Transcription activation
Nucleus
ANK
CfCatus
CgIκB
DmCactus
HmIκB

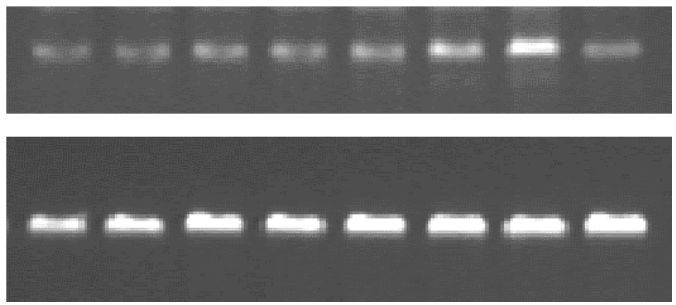
The cascade of Myd88-TRAF6-Cactus in different tissue



✦ cDNA characteristics of scallop serine proteinase inhibitor (SERPIN) genes

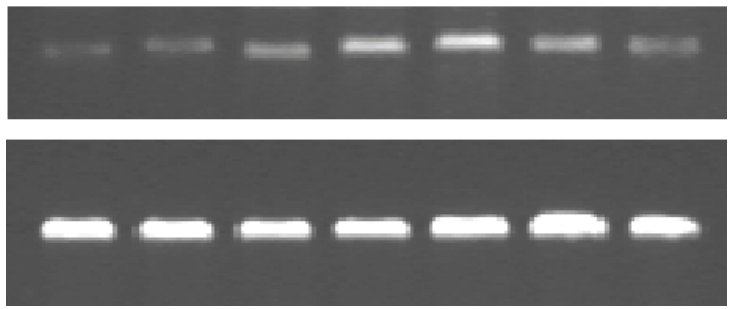
Gene Name	CfSERPIN—1	CfSERPIN—2	AiSERPIN—1	AiSERPIN—2
Full Length (bp)	1070	1193	907	980
5'-UTR (bp)	80	80	54	39
3'-UTR (bp)	42	39	141	144
ORF (bp)	948	1071	711	834
Aa coded	316	357	237	278
MW (Kd)	30.04	38.04	25.87	30.17

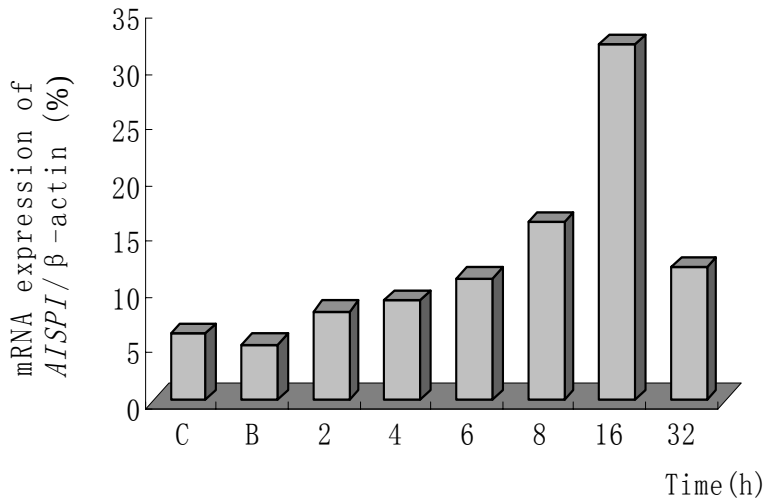
The expression patterns of bay scallop SERIP-1 gene

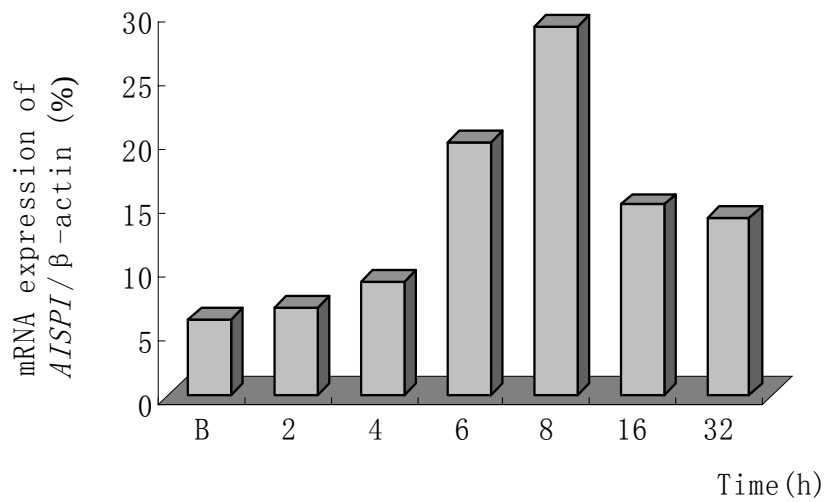


AISPI

Actin







mRNA expression of scallop *AISPI*-1 at different time point after bacterial challenge

mRNA expression of scallop *AISPI-1* at different time point after adductor muscle injury

(3) Immune effecter genes

(Lysozyme, defensin)

Jianmin Zhao et al., 2007, Mol. Immunol

Jianmin Zhao et al., 2007, Mol. Immunol

The functions of Lysozyme

- Lysozymes are characterized by their ability to hydrolyze preferentially the β-1,4 glucosidic linkages between N-acetylmuramic acid and N-acetylglucosamine which occur in the mucopeptide cell wall structure of certain microorganisms.
- Thus it was suggested that these enzymes play a role in the body defense against infection.

Lysozyme categories

- Lysozyme is of widespread distribution in animals and plants.
- Several types of lysozymes have been described:

  c (chicken);

  g (goose);

  i (invertebrate) ;

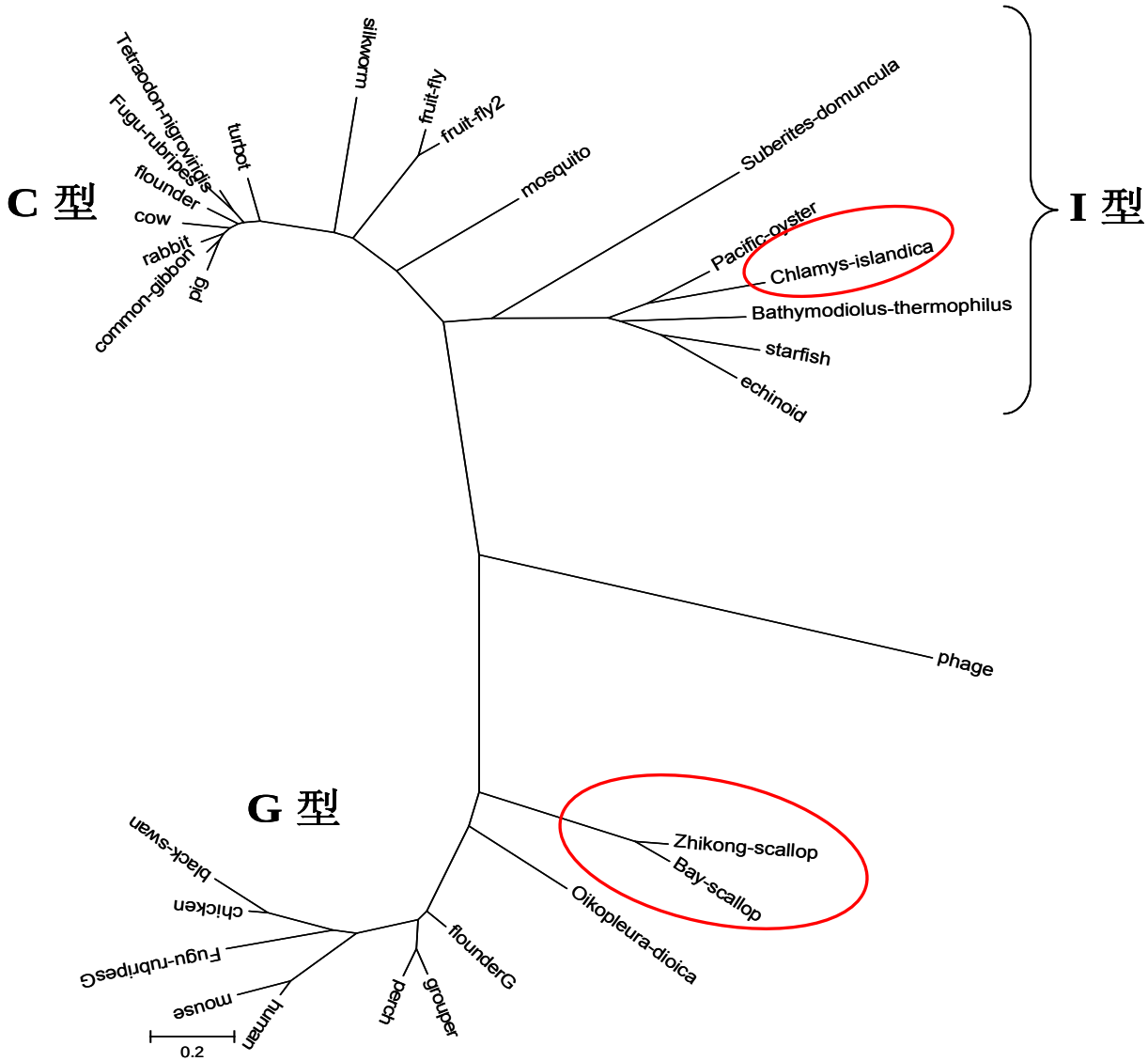
  phage, bacteria;

  plant.

G-Lysozyme from scallop

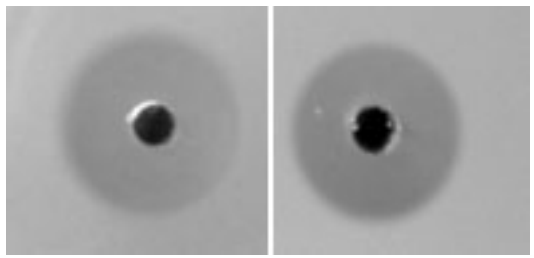
Gene Name	CfLyz-g	AiLyz-g
Full Length (bp)	829	659
5’-UTR (bp)	21	18
3’-UTR (bp)	218	41
ORF (bp)	588	600
Aa coded	196	200
MW (Kd)	22.39	21.99

The phylogenic tree constructed based on amino acid sequences of lysozyme

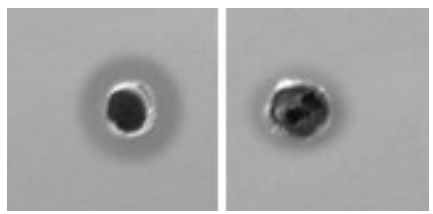


Recombinant expression and bioassay of AiLyz-g

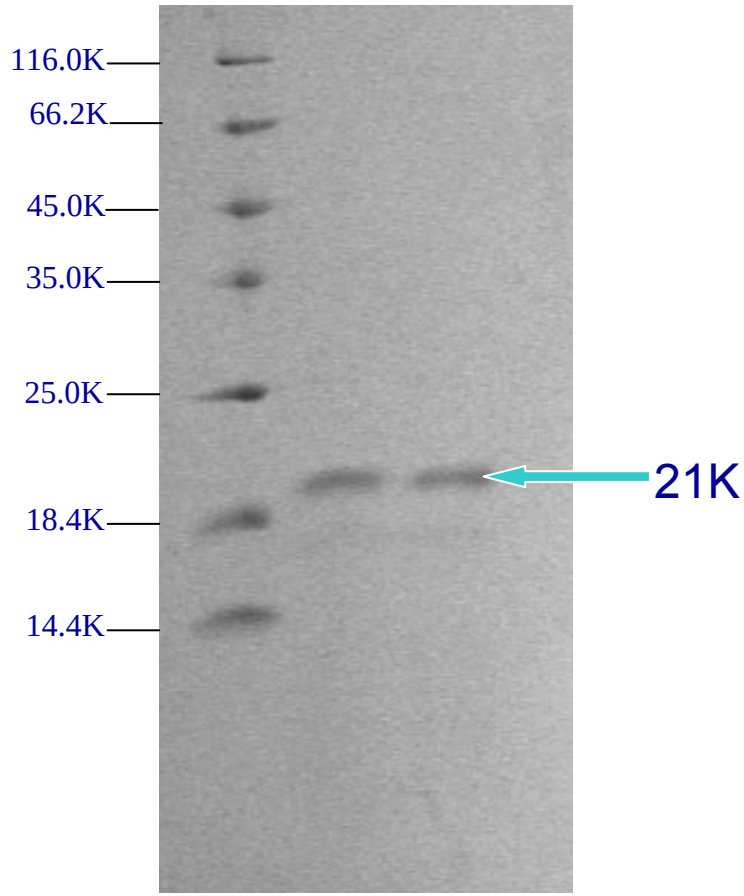
Bioassay of re-lysozyme



rAiLyz-g against G^+ bacteria



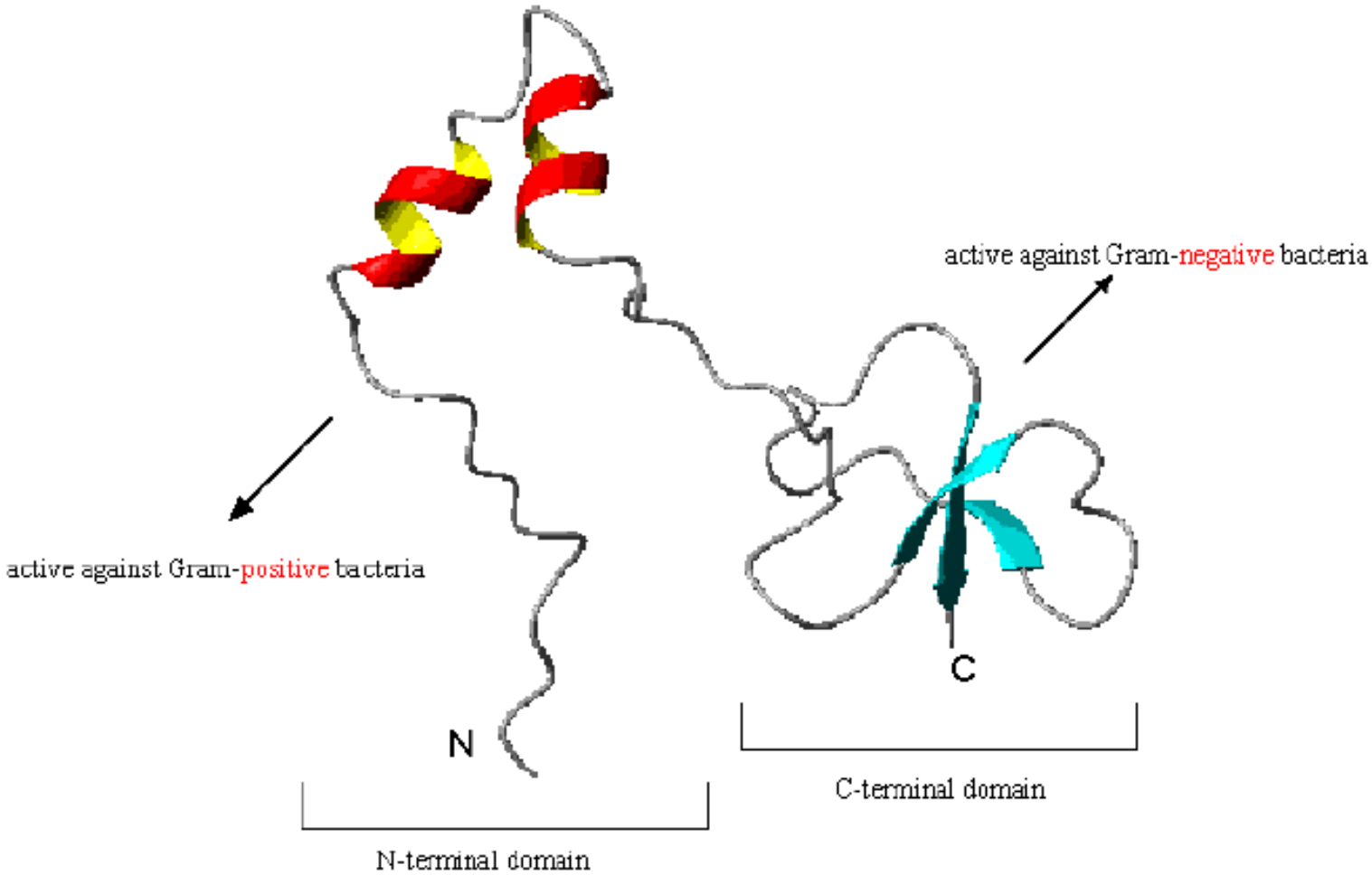
rAiLyz-g against G^- bacteria



Defensin

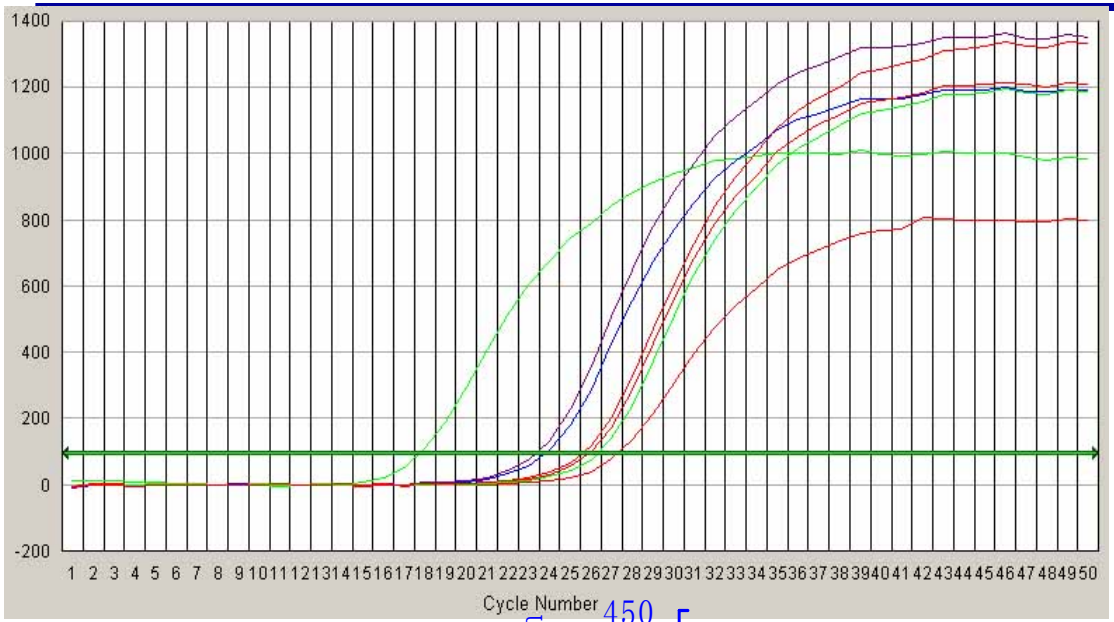
A family of potent antibiotics made within the body by neutrophils and macrophages. They are small peptides unusually rich in the amino acid cysteine (Cys).

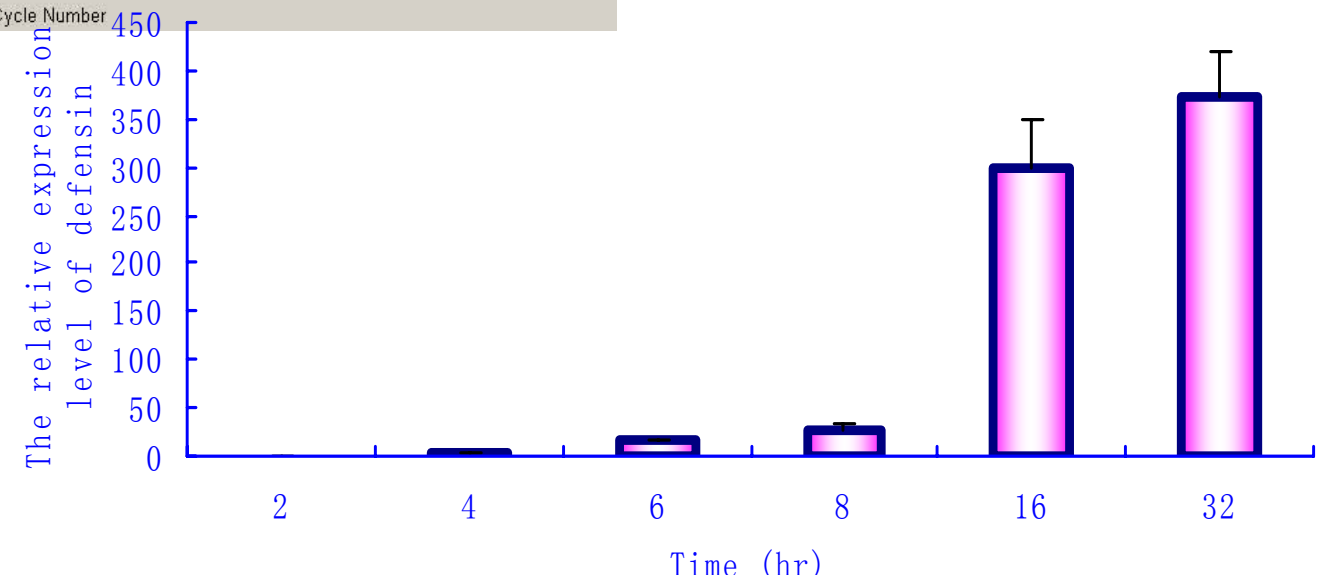
The defensins play important roles against invading microbes. They act against bacteria, fungi and viruses by binding to their membranes and increasing membrane permeability.



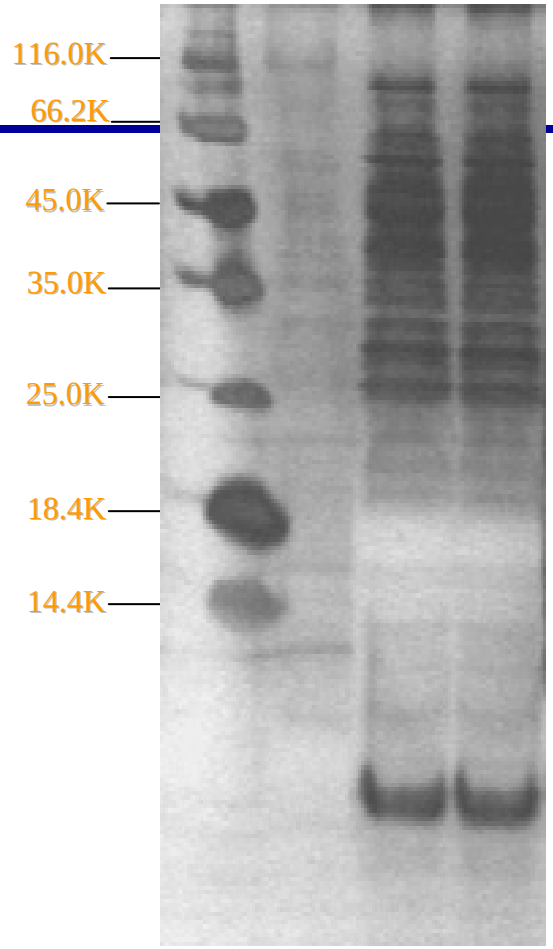
The predicated structure of bay scallop defensin

mRNA expression of scallop defensin after bacteria challenge

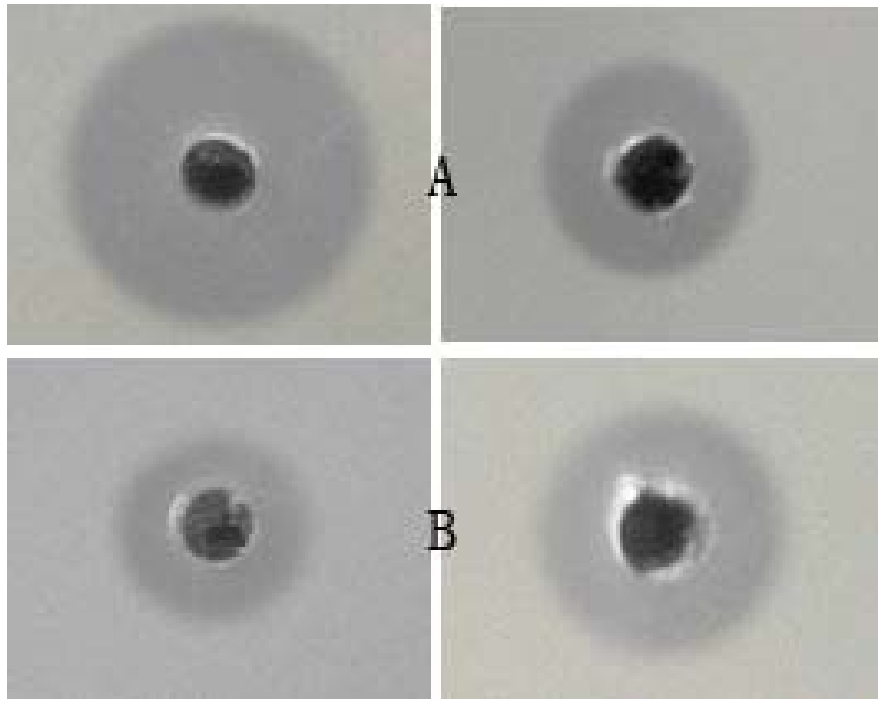




Bioassay of re-defensin



The recombinant expression of scallop defensin in yeast



A: G^+ bacteria

B: G^- bacteria

(4) The genes involved in the oxidation/reduction

(CAT, Prxs, SOD, GPx, GST , Trx， SeBP)

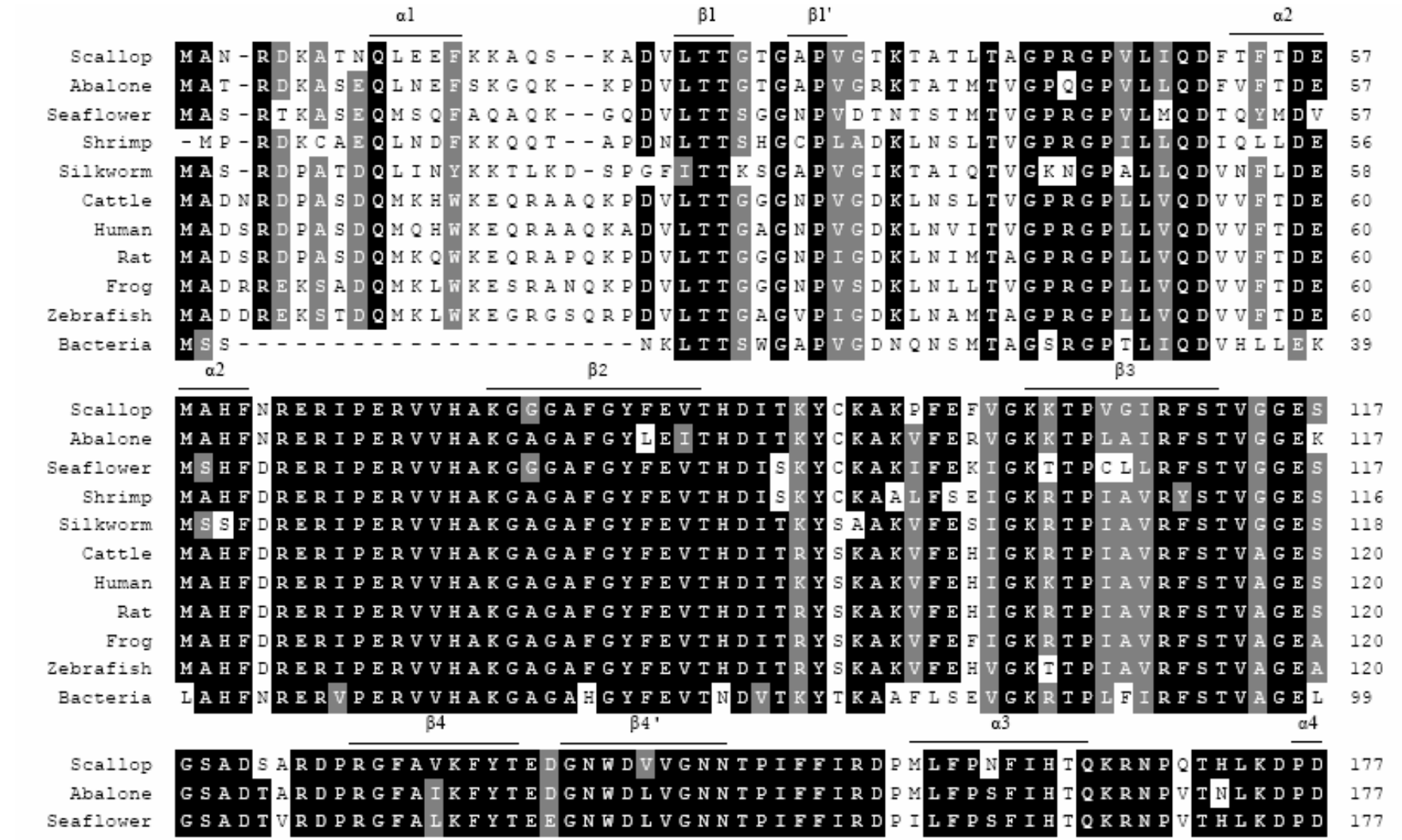
Huibin Zou et al., 2006, Dev. Com. Immunol

Duojiao Ni et al., 2007, Fish & shellfish Immunol

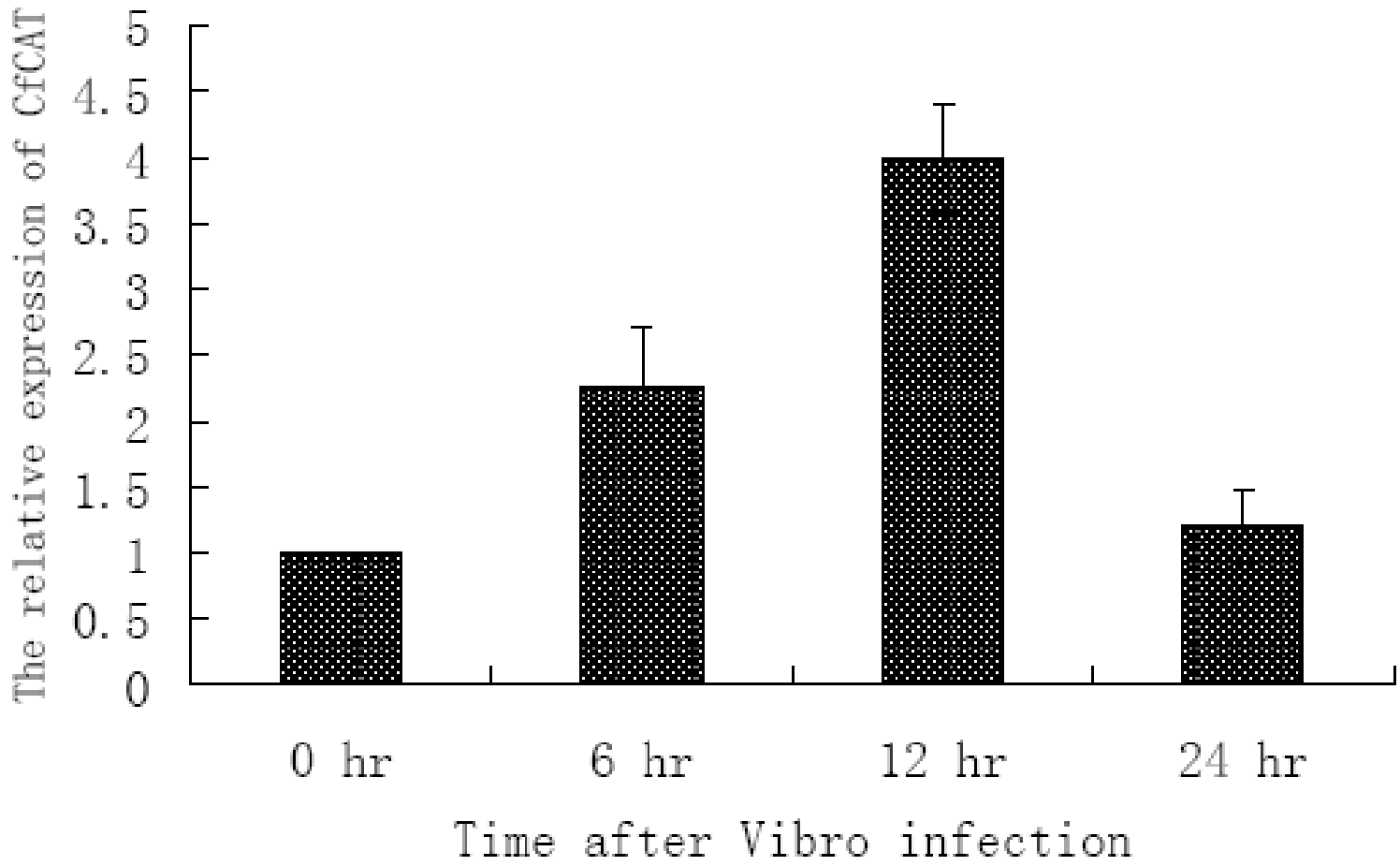
The genes involved in oxidation/reduction

Name	Function	Full-length/ ORF (bp)	Accession No.	species
CfSeBP	Anti-oxidation	1664 /1440	AY835660	*C. farreri*
CfGPX-1	Anti-oxidation	1194 /651	--	*C. farreri*
CfGPX-2		1290/705	--	*C. farreri*
CfSOD	Anti-oxidation	1022/459	DQ400349	*C. farreri*
CfGST-1	Glutathione-necessary	1483 /717	--	*C. farreri*
CfGST-2		954 /672	--	*C. farreri*
CfGST-3		1135/609	--	*C. farreri*
CfGST-4		1387/615	--	*C. farreri*
CAT	H_2O_2 reduce	3144 /975	DQ862859	*C. farreri*
Thioredoxin, Trx	Oxidation /reduction	1494 /324	--	*C. farreri*
tyrosinase, TYR	PO-like	1711 /1458	--	*C. farreri*

The alignment of CfCAT with selected CATs from other species



Temporal expression of the CfCAT transcript in haemocytes after *Vibrio anguilarum* infection



(5) The acute phase protein genes

name	founction	Full-length/ ORF (bp)	Accession No.	species
CfHSP90	Molecular chaperon; Stress response; Heavy metal, ROS binding and clearance; Infection.	2710 /2181	AY362761	*C. farreri*
CfHSP70		2573 /1968	AY206871	*C. farreri*
CfHSP22		849 /576	AY362760	*C. farreri*
AiHSP70		2651 /1980	AY485261	*A. irradians*
MyHSP70		2641 /1974	AY485262	*M. yessoensis*
AiMT-1	Heavy metal binding, anti-oxidation	787 /438	--	*A. irradians*
AiMT-2		664/ 333	--	*A. irradians*
AiMT-3		582 /273	--	*A. irradians*

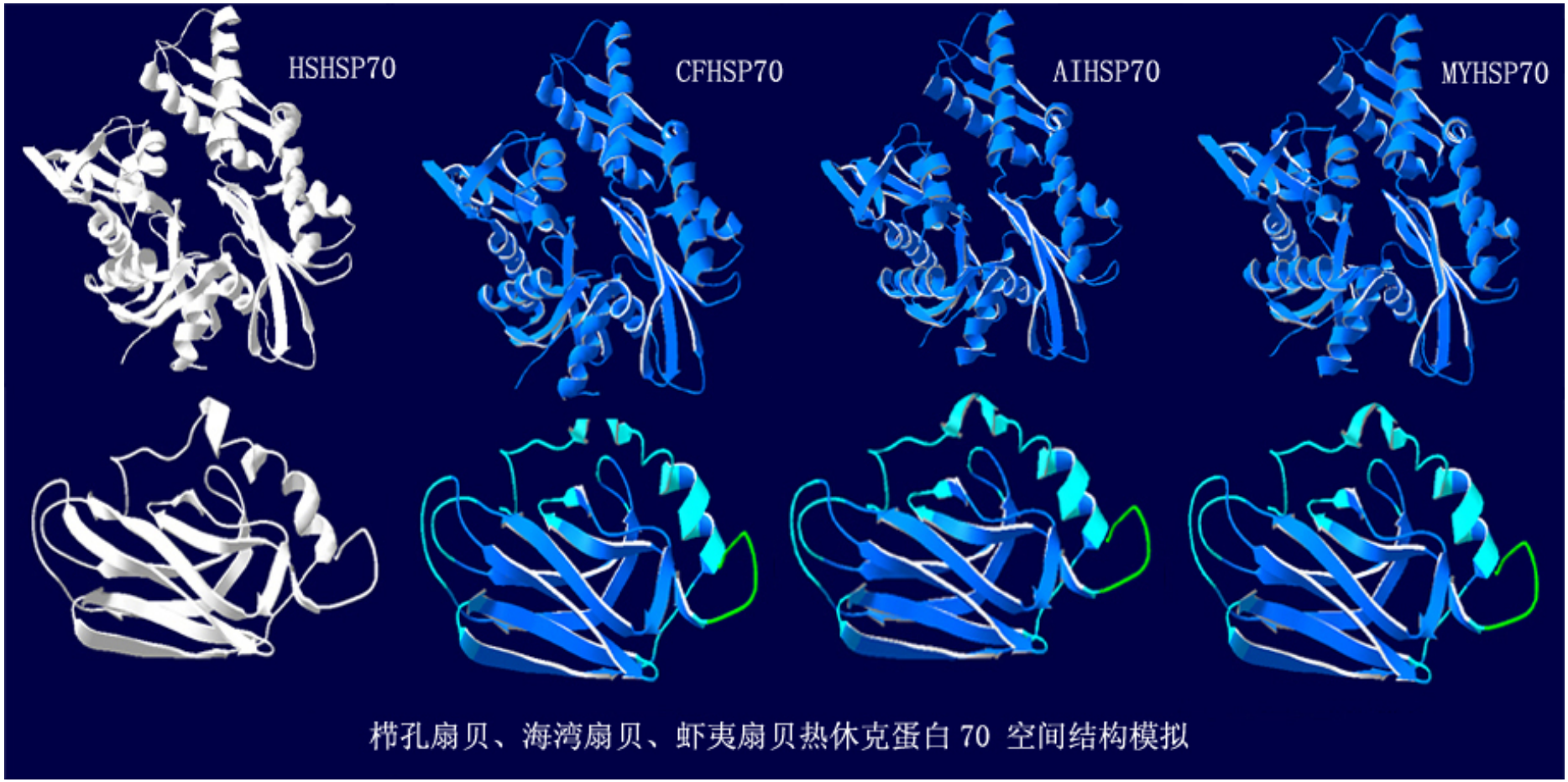
Qiang Gao et al., 2007, CPB

Linsheng Song et al., 2006, Fish & shellfish Immunol

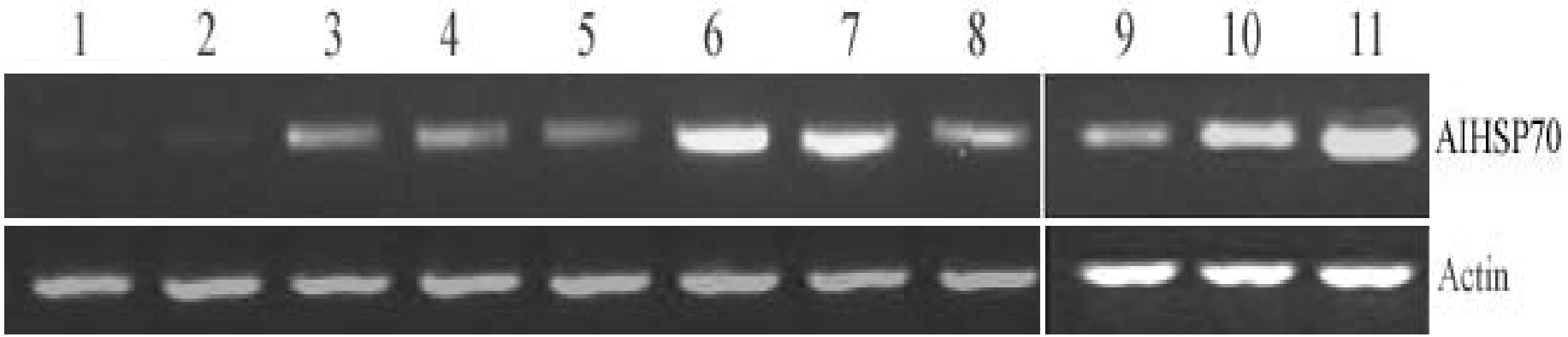
吴龙涛等，2003，高技术通讯

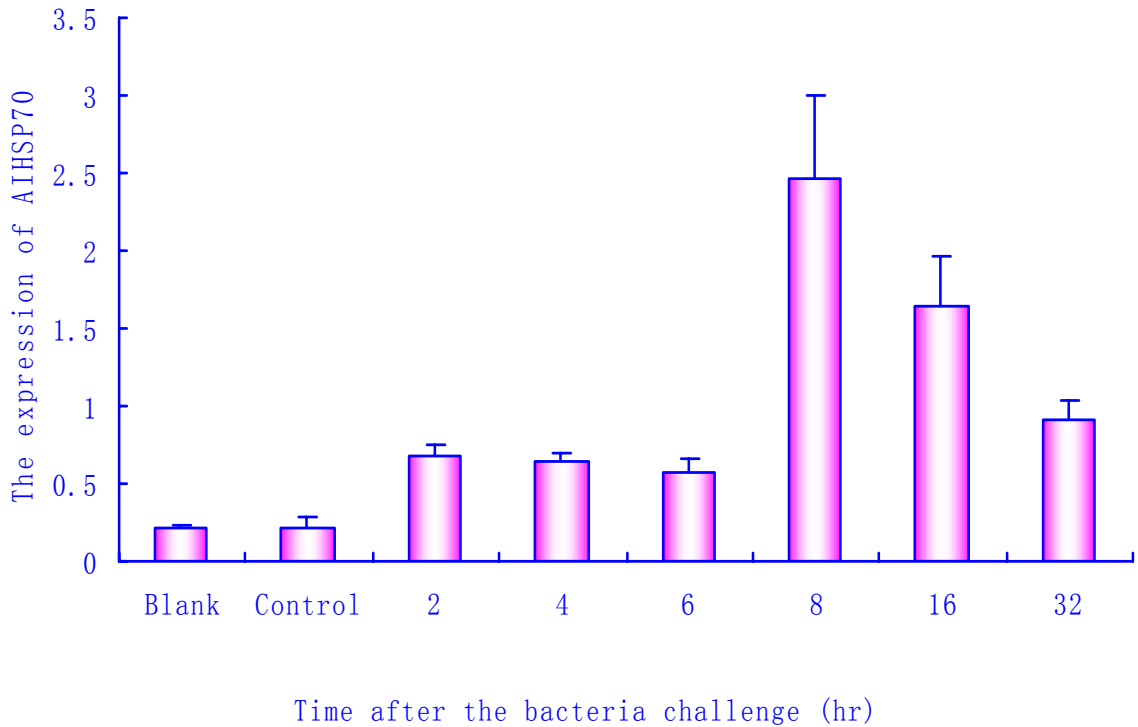
刘维青等，2006， 海洋与湖沼007

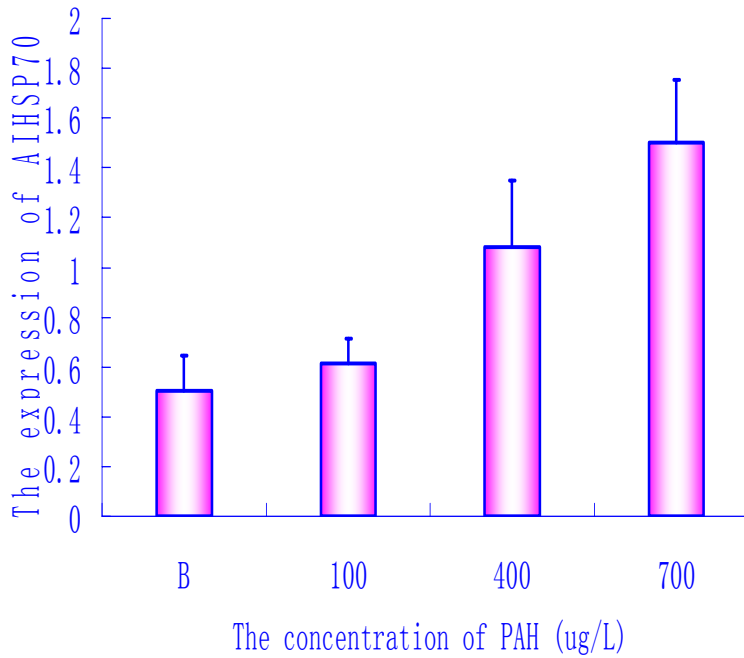
Heat shock protein 70



The expression of AiHSP70 after bacteria challenge and PAH stimulation

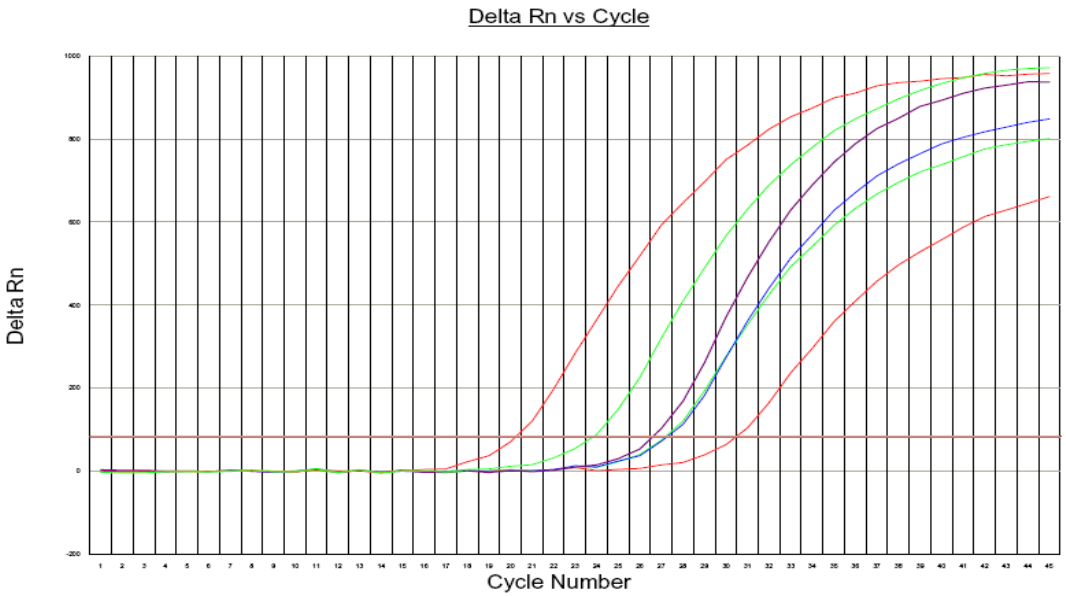




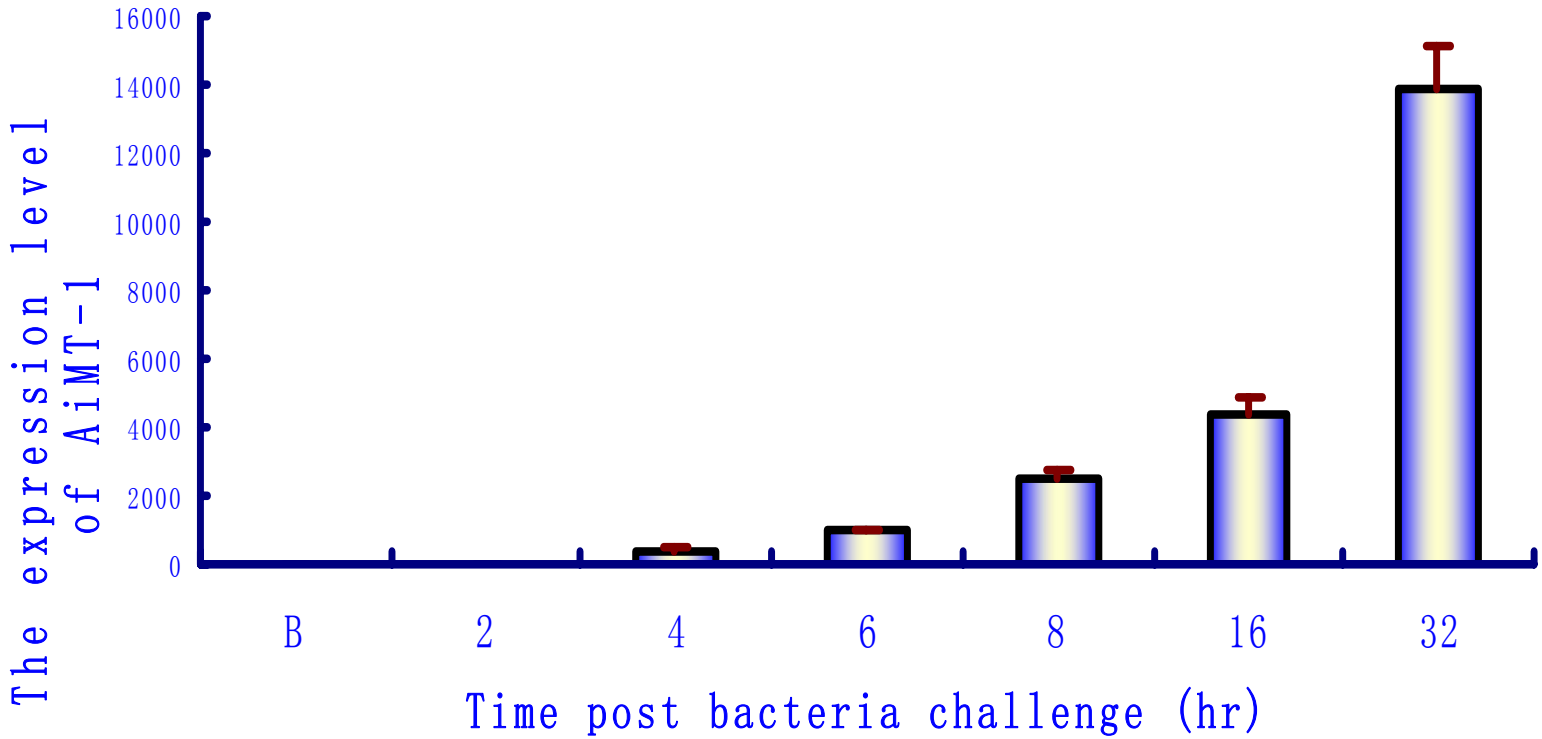


The Metallothionein genes cloned from bay scallop

Gene name	AiMT-1	AiMT-2	AiMT-3
cDNA full length	787	664	582
Aa	145	110	90
Cys	40	28	28
C-C	2	2	2
C-X-C	8	8	10
C-X-X-C	6	6	2
C-X-X-X-C	5	5	6
CKCXXXCXCX	1	1	1



The expression of AiMT-1 after bacteria challenge



Other genes

cyclophilin A (AY362759),

C1q

Serine proteinase ,

TNF receptor,

Defender Against Apopototic Cell Death 1 (DAD1)

……

SUMMARY

- Over 10,000 scallop ESTs were submitted to GenBank.
- Over 100 genes involved in immune response were cloned from scallop.
- The expression patterns of some genes under the challenges of microorganisms or stress were examined.
- Myd88 dependent signaling pathway in scallop.
- G-lysozyme in invertebrate.
- Defensin, lysozyme and lectin were expressed in *E. coli* and yeast.

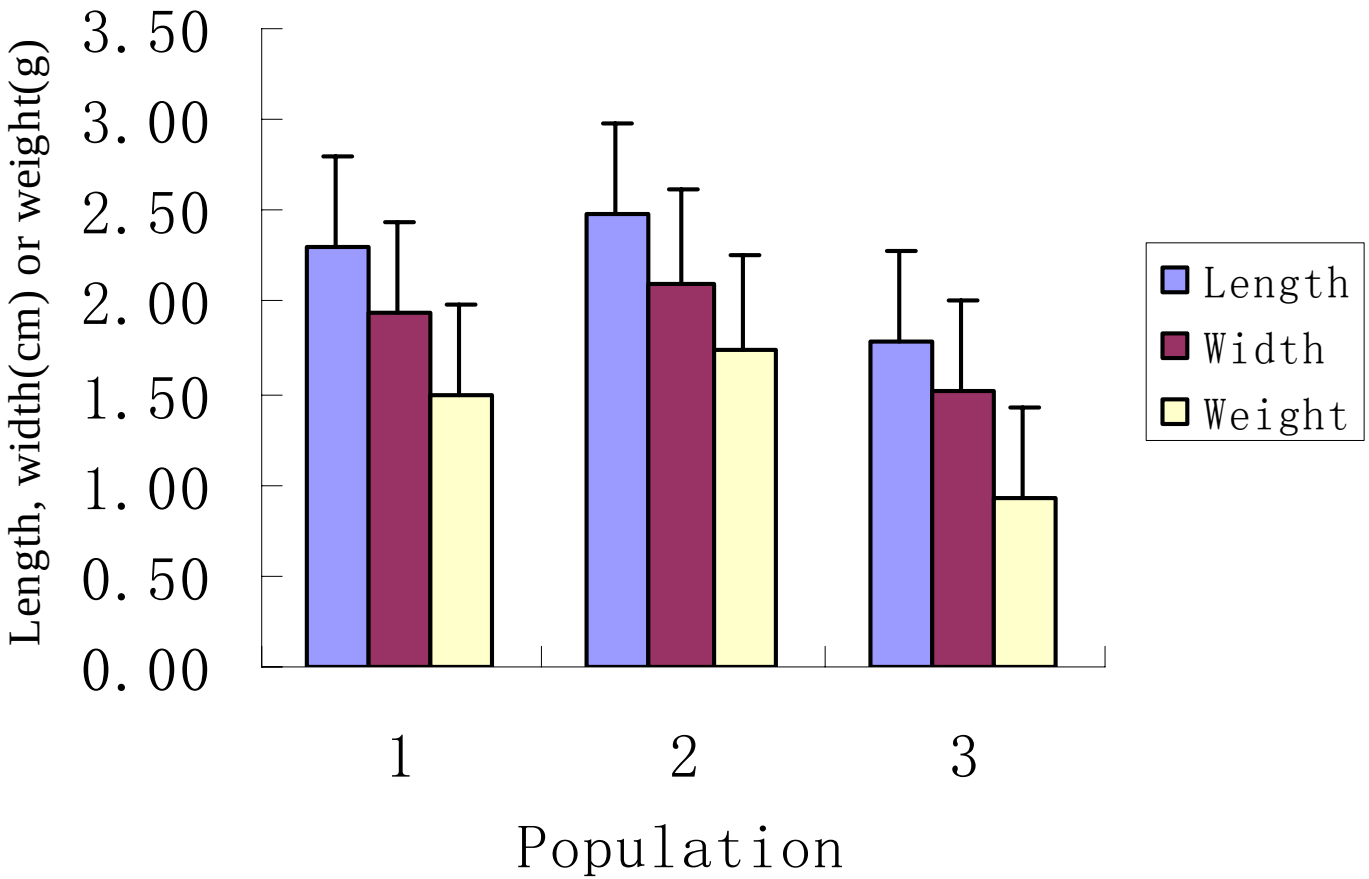
2. Genetic diversity of some species in yellow sea

The genetic diversity and heterosis in scallop *C. farreri*

- Genetic differentiation occurred between the different geographic populations of *C. farreri*.
- The heterosis (growth performance, immunity) existed in the different population of *C. farreri*.
- The heterosis may be related to the genetic differentiation of parents.

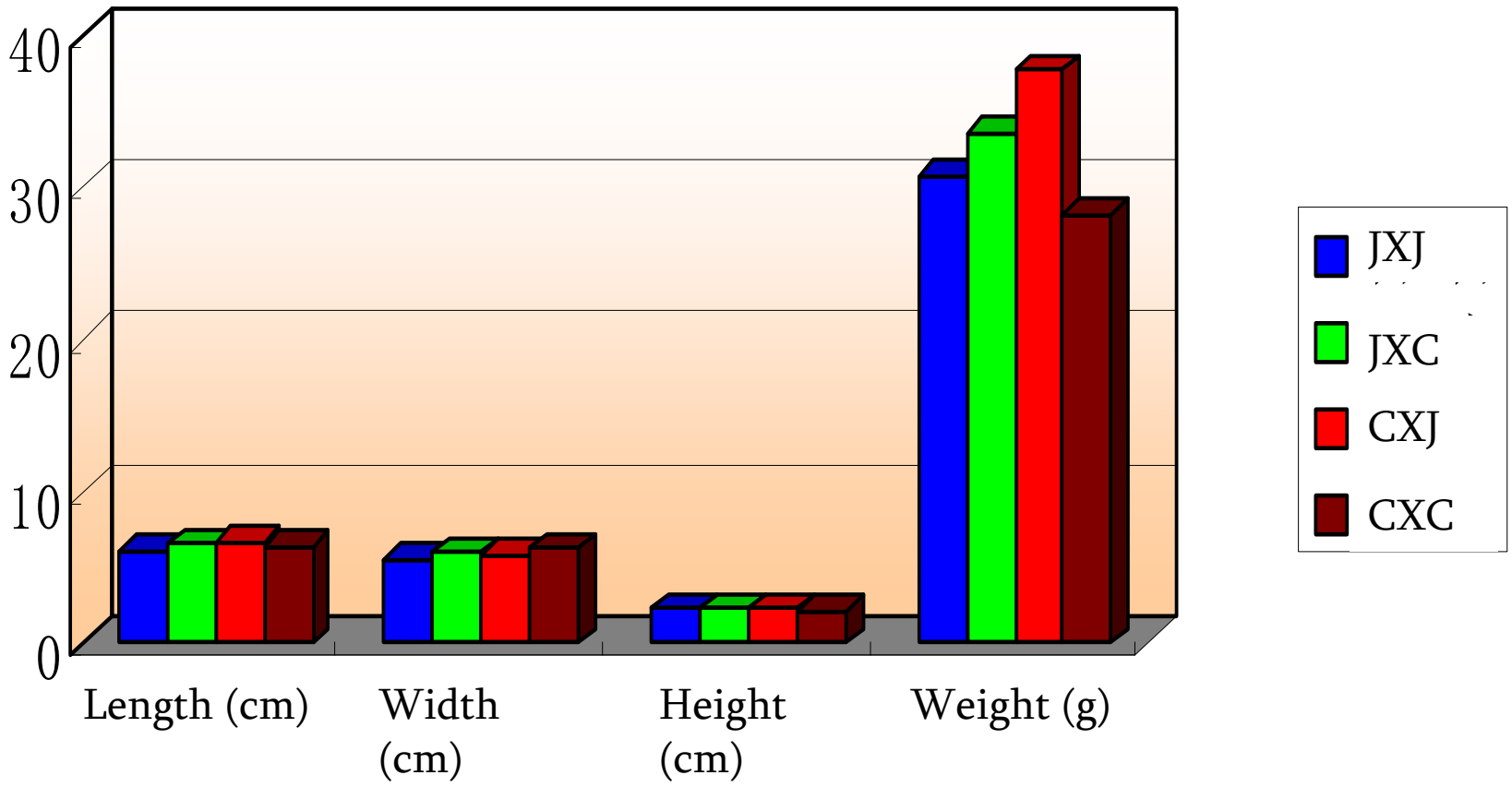
Honglei Li et al., 2001

Comparison of the growth performance index among three F1 progeny populations

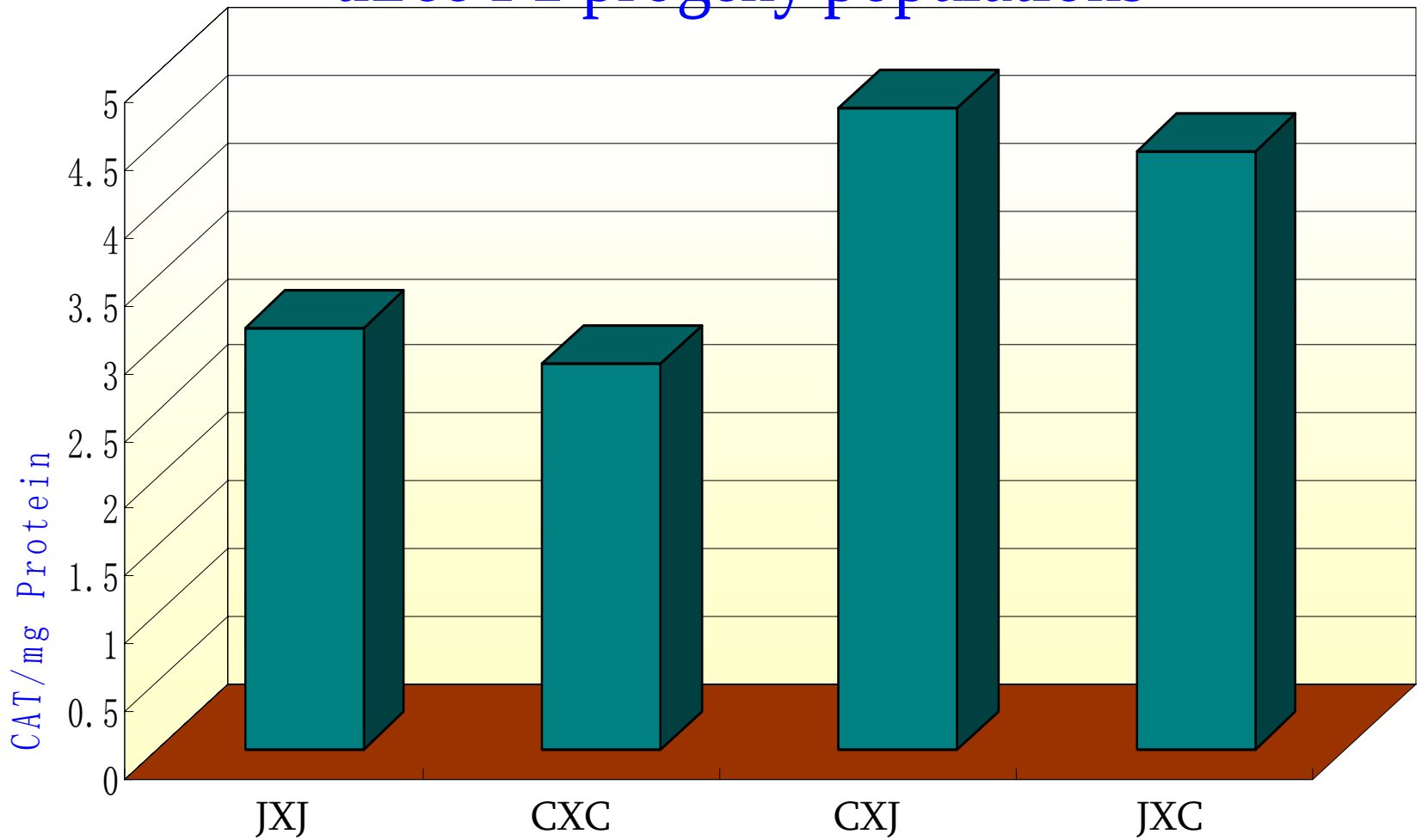


1: Korea wild popualtion; 2: Hybrid F1 progeny between Korea wild popualtion and China culture stock; 3: China culture stock.

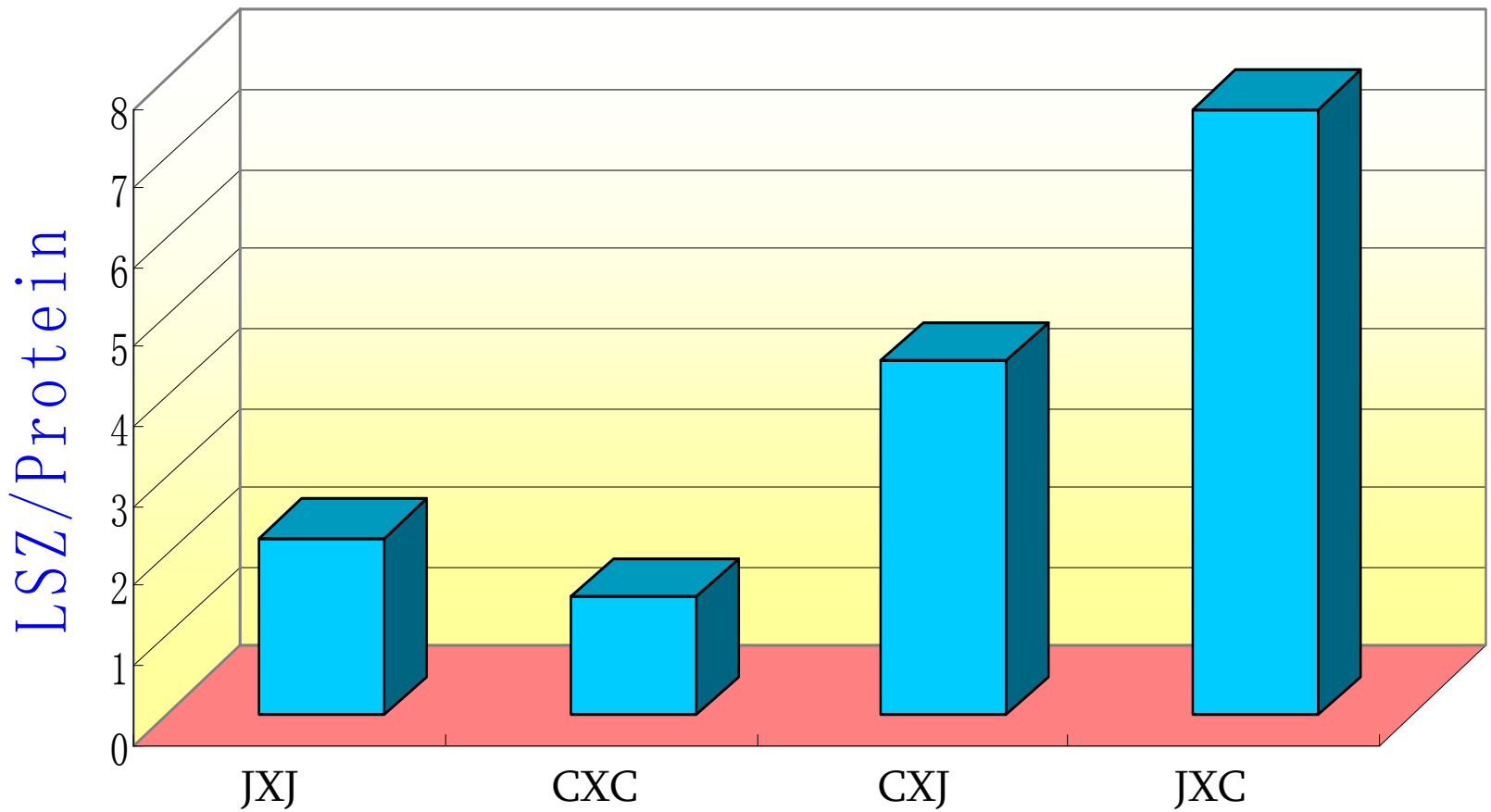
Comparison of the growth performance index among three F1 progeny populations



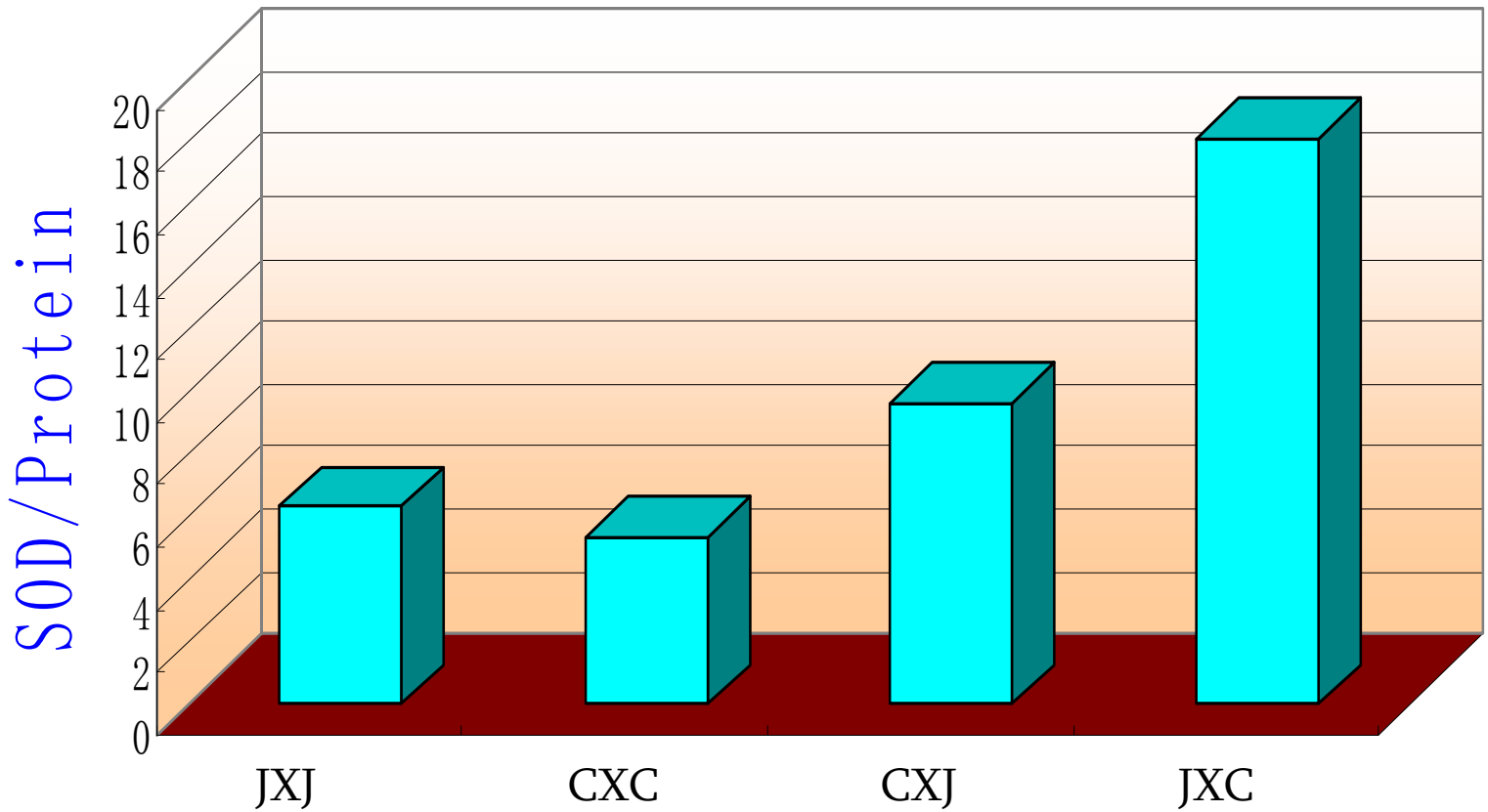
Comparison of the CAT activity among three F1 progeny populations



Comparison of the LSZ activity among three F1 progeny populations



Comparison of the SOD activity among three F1 progeny populations



Assessment of genetic diversity in the shrimp *Penaeus chinensis* using the complete mitochondrial control region

- An important penaeid species in fisheries and aquaculture in China and Korea.
- Distributed in Bohai Sea and Yellow Sea

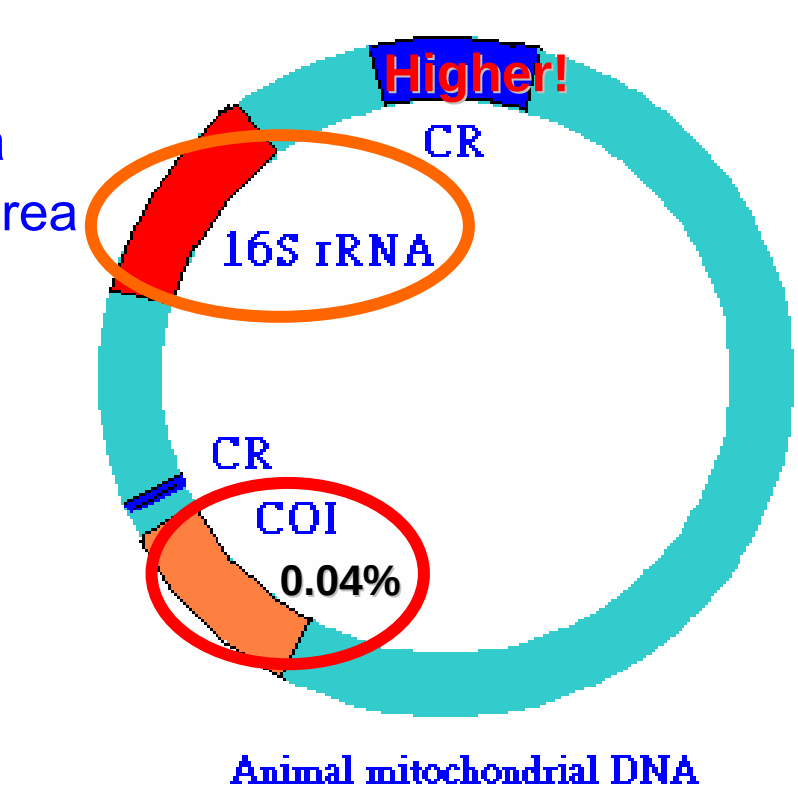
Cui et al., 2005

Mitochondrial DNA

16SrRNA: 0.00% nucleotide diversity

COI: 0.04% nucleotide diversity

Between Bohai Sea and Yellow Sea population of China and west coast of Korea population (Quan et al., 2001).



control region (CR)

→ **High evolutionary rate**

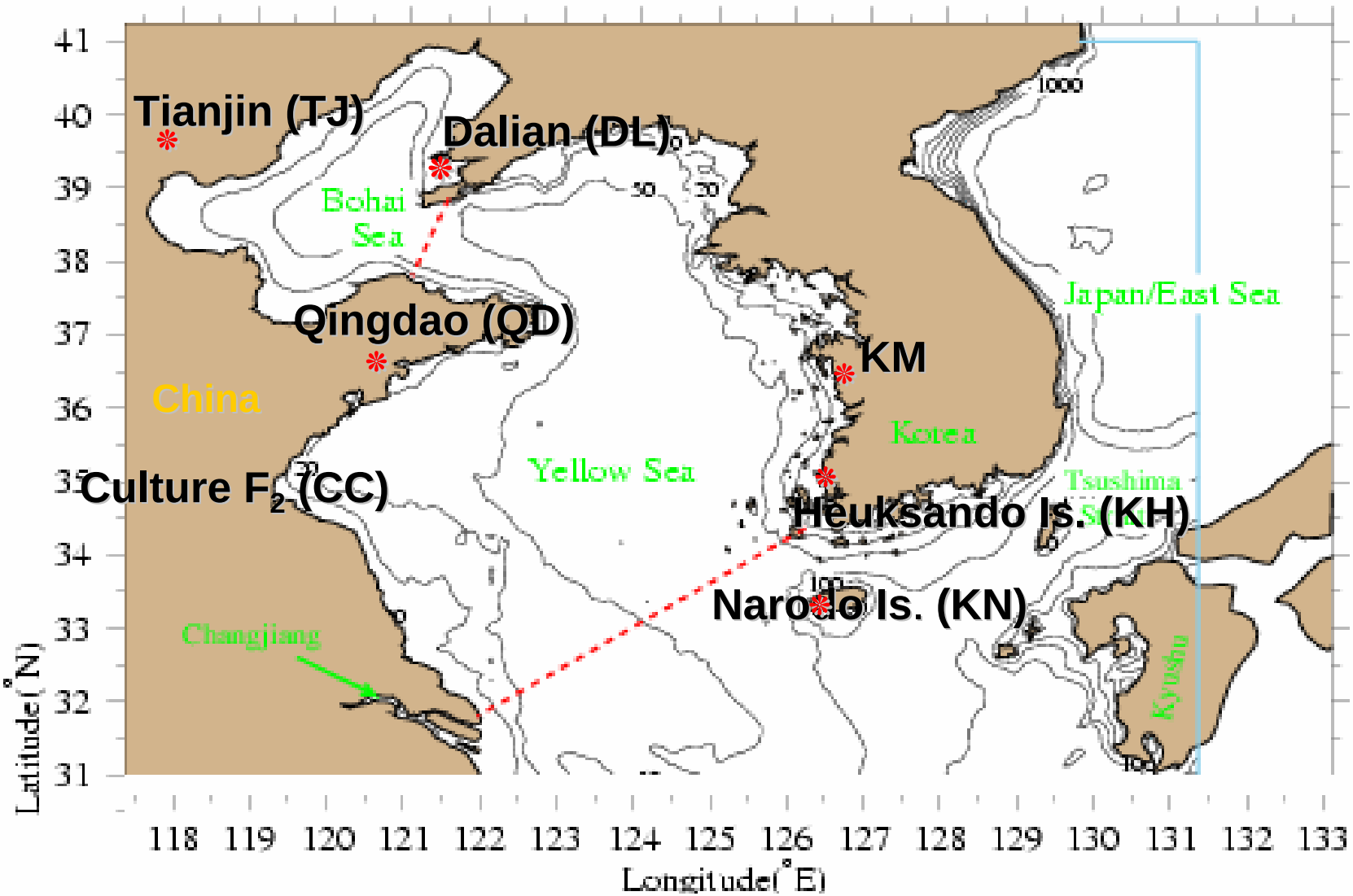
→ **High resolution in DNA variation**

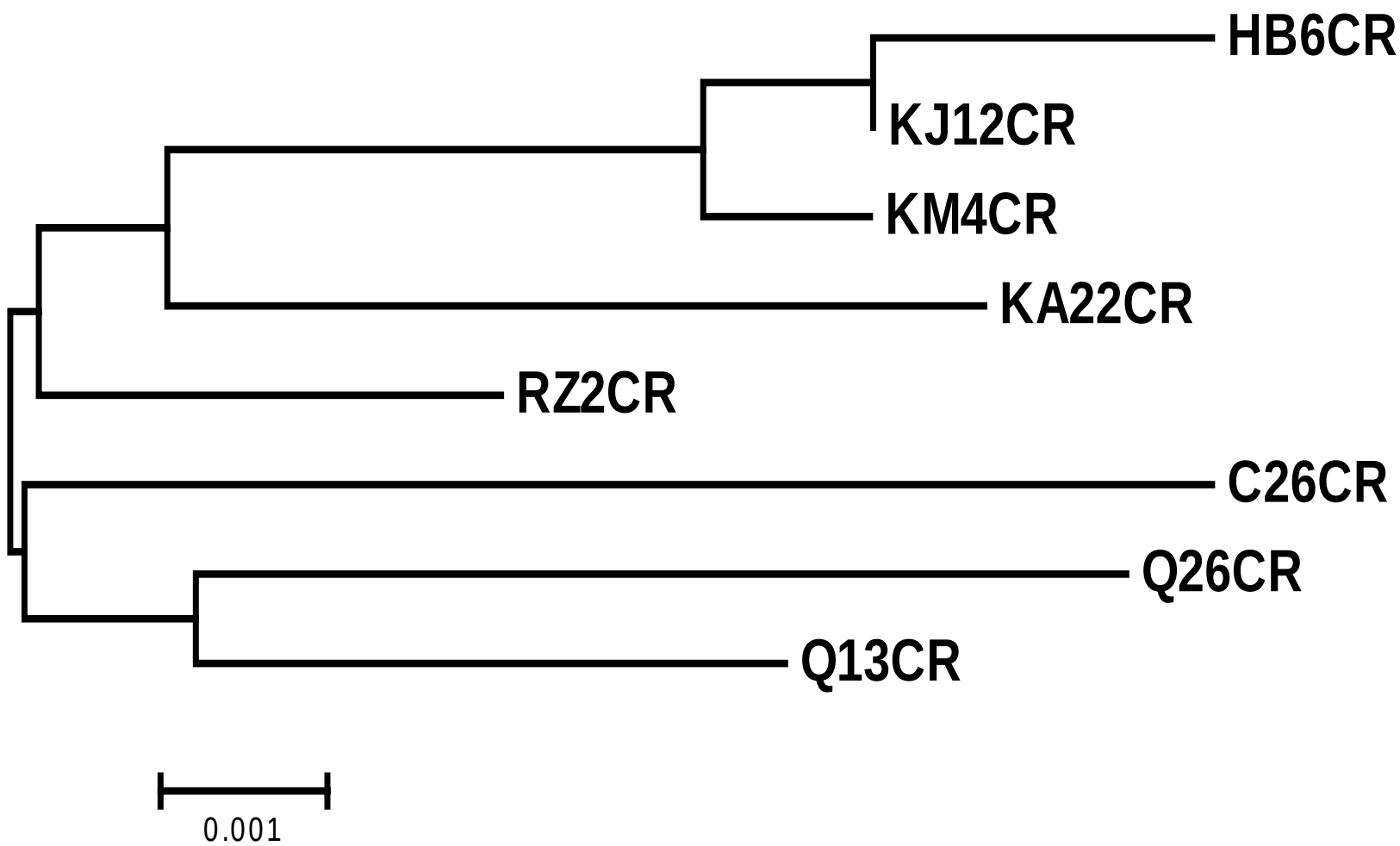
Method

***Direct sequencing**

***Restriction Fragment Length Polymorphism (RFLP)**

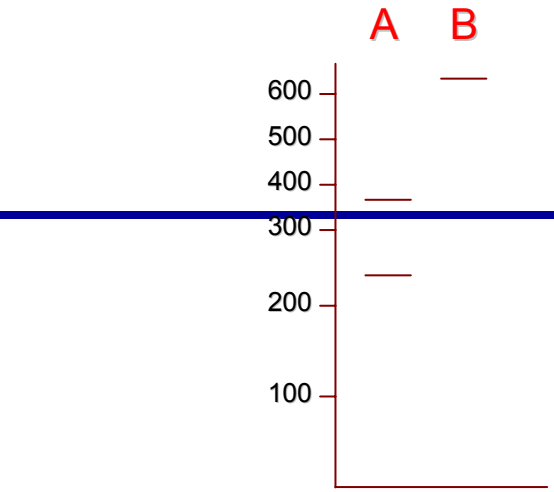
Sampling location



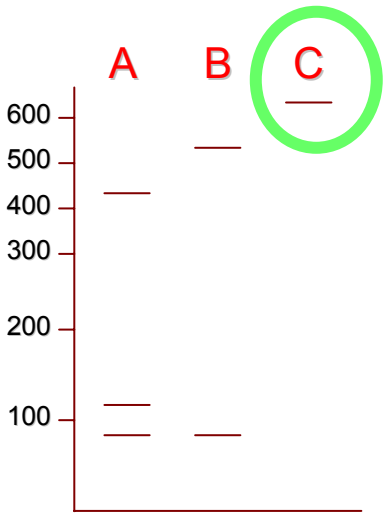


Neighbor-Joining tree

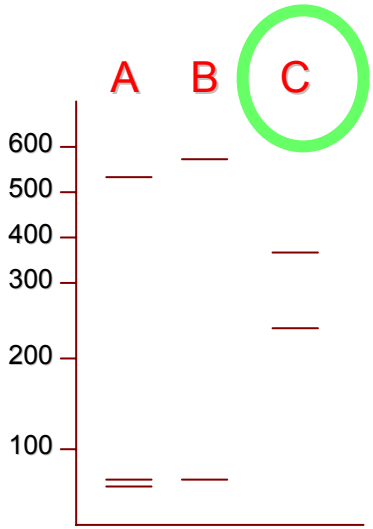
Neighbor-Joining tree



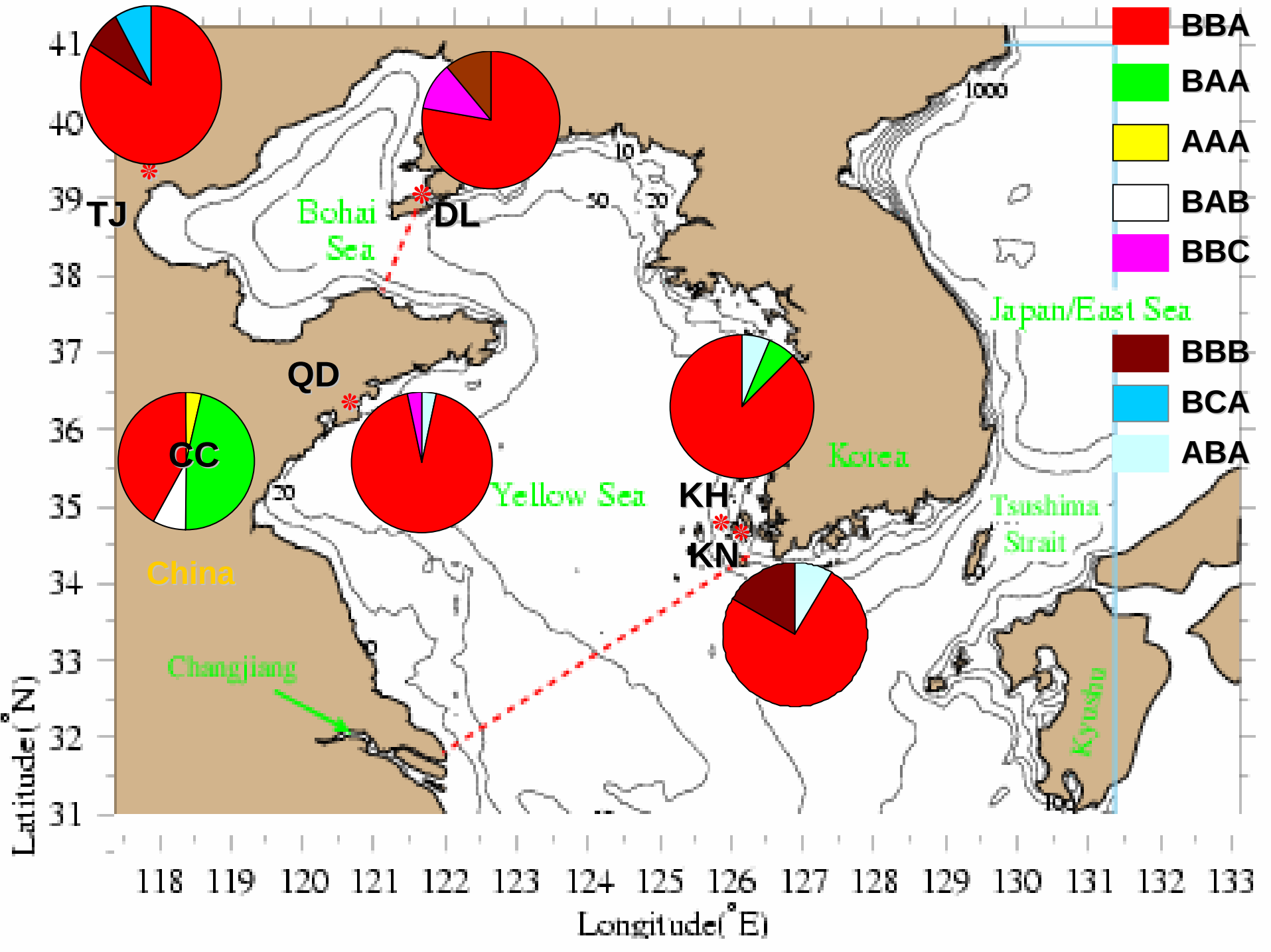
AccI **GT▼(A/C)(T/G)AC**



NlaIII **CATG▼**



RsaI **GT▼AC**

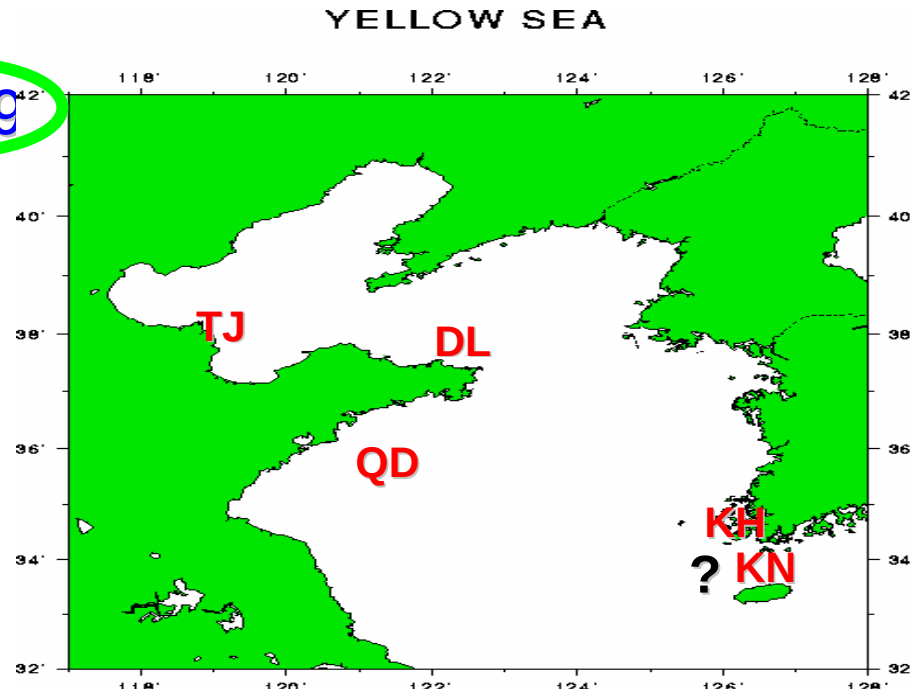

BBA
BAA
AAA
BAB
BBC
BBB
BCA
ABA
TJ
DL
QD
CC
KH
KN
Bohai Sea
Yellow Sea
China
Korea
Japan/East Sea
Tsushima Strait
Kyushu
Changjiang
10
30
50
100
1000
41
40
39
38
37
36
35
34
33
32
31
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
Latitude(°N)
Longitude(°E)

	Gene diversity H^	Mean number of pairwise differerces π ^
QD	0.136±0.085	0.276 ±0.307
DL	0.392 ±0.133	0.784 ±0.597
TJ	0.318 ±0.164	0.333 ±0.356
KH	0.242 ±0.135	0.250 ±0.300
KN	0.439 ±0.158	0.470 ±0.439
CC	**0.625 ±0.054**	**0.732 ±0.563**

F-statistics of haplotype frequency –

Average number of pairwise differences between populations

	QD	DL	TJ	KH
DL	0.272			
TJ	0.224	0.343		
KH	0.183	0.319	0.271	
KN	0.299	0.398	0.361	0.339



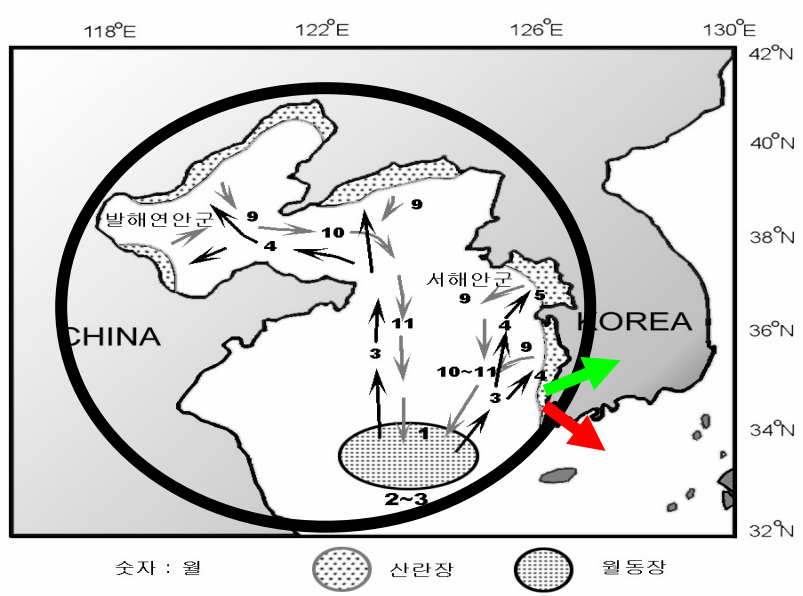
Summary

Populations of *P. chinensis* are genetically homogeneous in Bohai Sea and Yellow Sea of China and Korea.

The Narodo Is. population appears to be genetically distinct from the Heuksando Is. Population in Korea.

There is no loss in genetic diversity in the F2 culture stock in China. Interestingly, some haplotypes found in this stock are not found in the wild populations.

Culture stock



Phylogeny and species identification of puffer fish

Takifugu rubripes

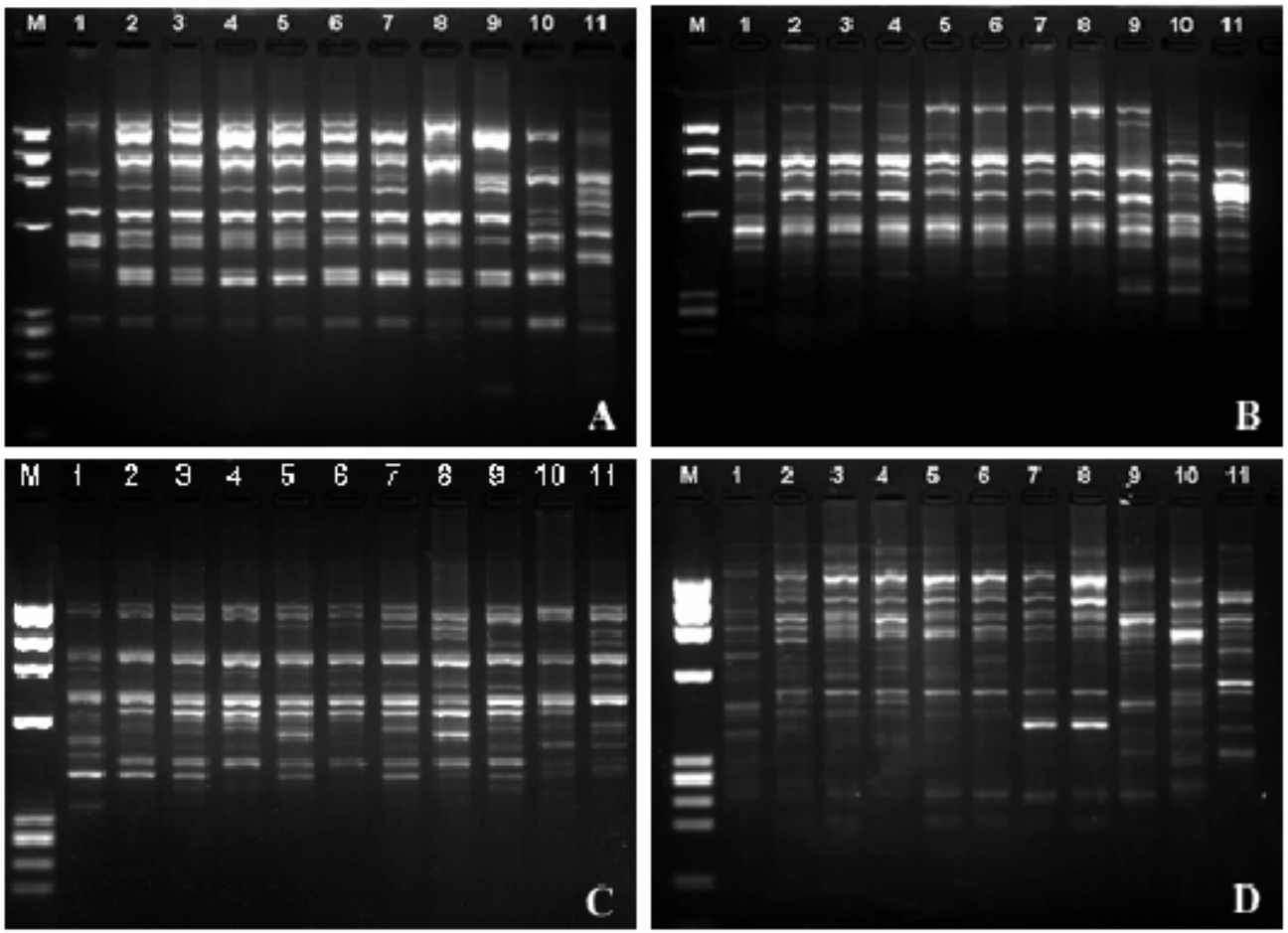
Takifugu pseudommus

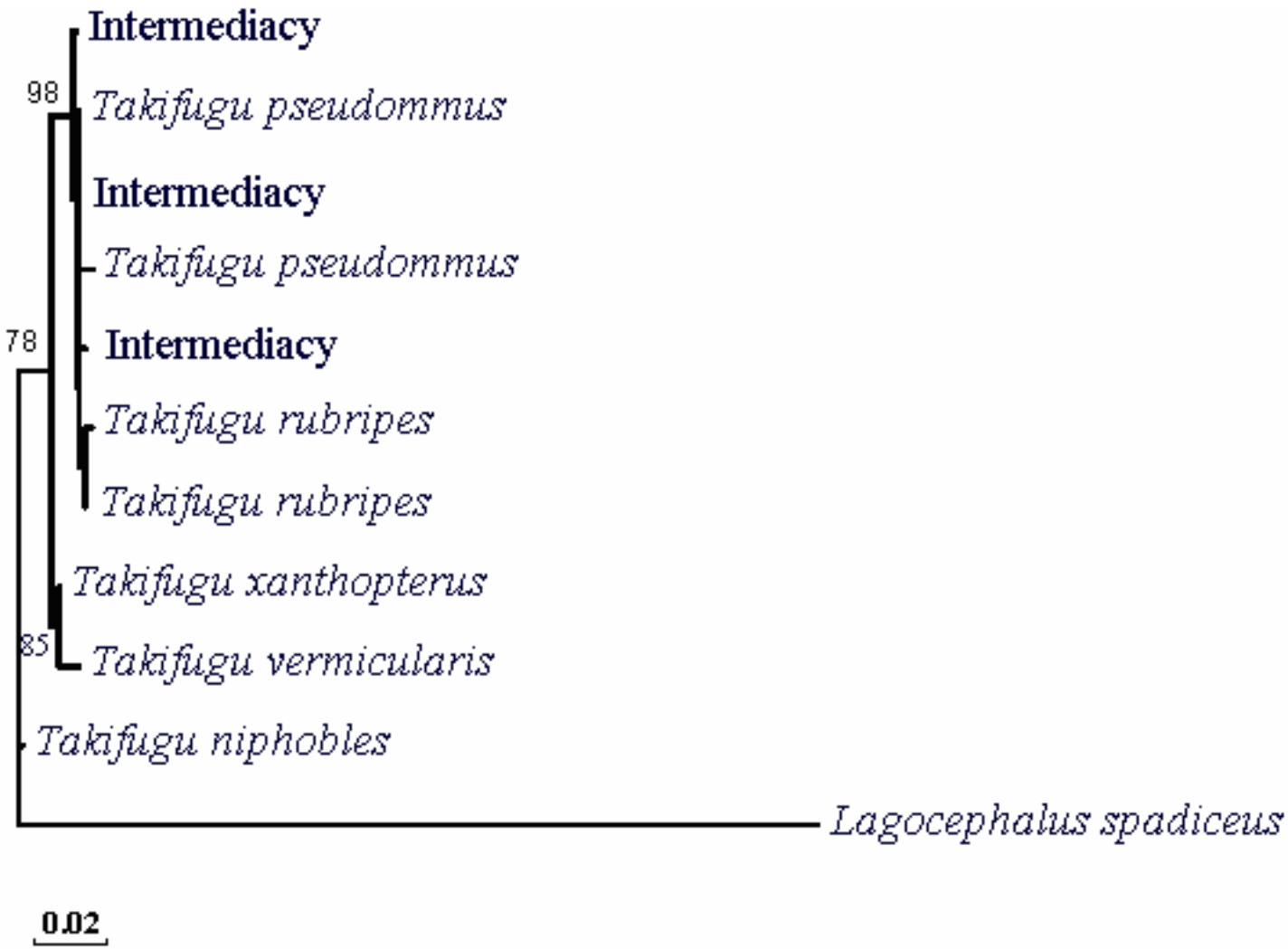
Takifugu xanthopterus

Takifugu vermicularis

Takifugu niphobles

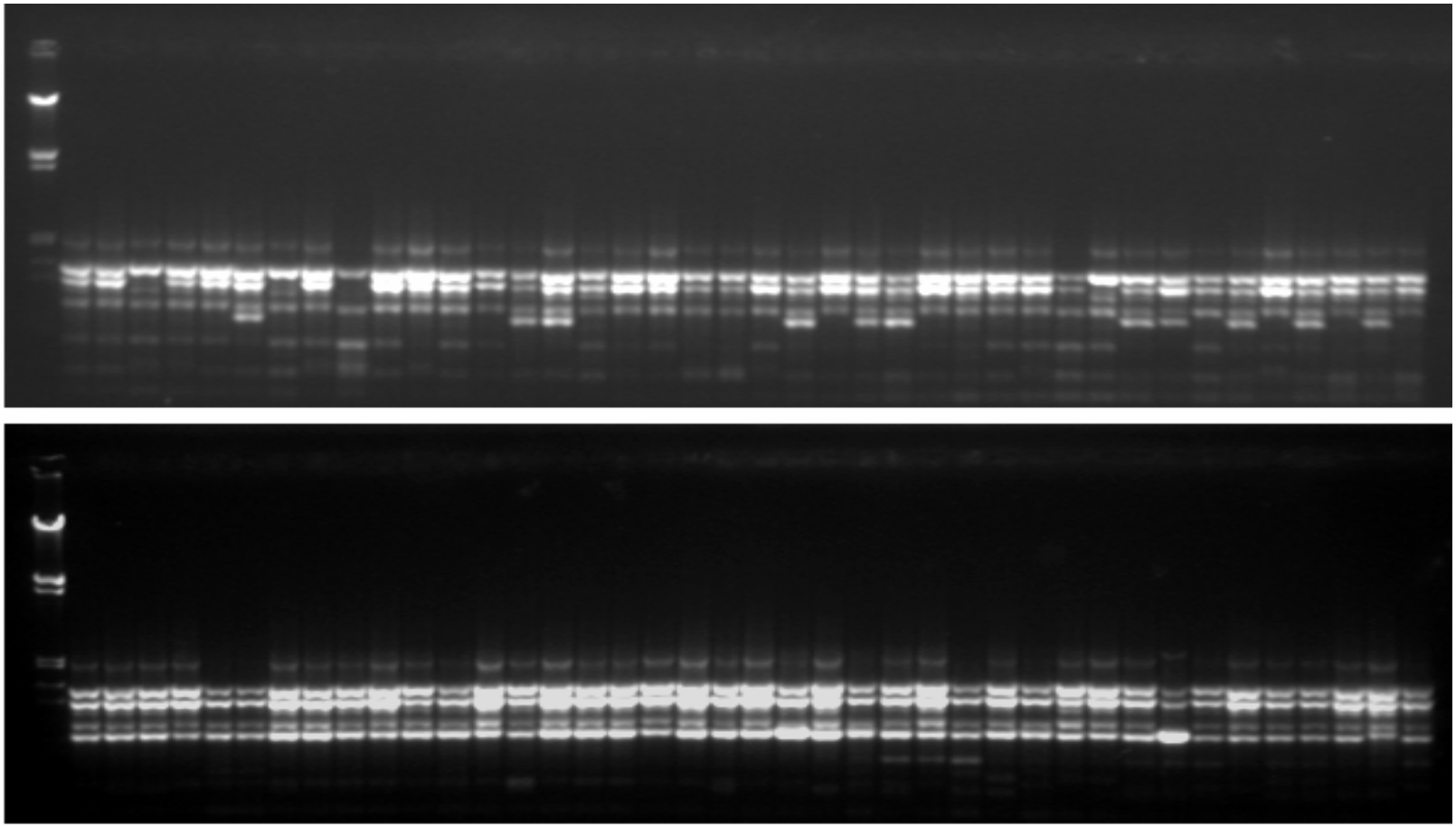
(Linsheng Song, 1999)


M 1 2 3 4 5 6 7 8 9 10 11
A
M 1 2 3 4 5 6 7 8 9 10 11
B
M 1 2 3 4 5 6 7 8 9 10 11
C
M 1 2 3 4 5 6 7 8 9 10 11
D



NJ distance tree for the 16S rDNA sequence data of pufferfish. The numbers on the tree correspond to the percentage of bootstrap replicates where the particular clade was found.

Population genetics and genetic differentiation of *T. rubripes and T. pseudommus*



The electrophoresis patterns of RAPD amplified with primers UBC-110. A: *T. rubripes*-TRJ population (left 20 lanes) and *T. rubripes*-TRC population (right 20 lanes). B: *T. pseudommus* population

Genetic diversity information among the populations

Population	TRC	TRJ	TRCJ	*T. pseudommus*
Total bands	120	120	120	120
Polymorphic bands	38	40	42	47
% polymorphism	31.7	33.3	35.0	39.2
Expected hetrozygosity	0.116	0.125	0.126	0.159

[a])TRC: *T. rubripes* population collected from China coast; TRJ: *T. rubripes* population collected from Japan coast; TRCJ: *T. rubripes* population including TRC and TRJ; *T. pseudommus*: *T. pseudommus* population.

Estimates for *T. pseudommus* and *T. rubripes* of *Fst* and θ (or *Nm*)

	Wright's *Fst*/*Nm*	θ/*Nm*	*Lynch & Milligan's* Fst/*Nm*
T. rubripes (China +Japan)	0.020/12.0	0.019/13.0	0.016/15.5
T. rubripes +*T. pseudommus*	0.024/10.2	0.022/11.1	0.017/14.7

Acknowledgement

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Thanks for your attention!