Genotype-specific immune response in cold water fish

(Transcriptomic analysis of immune response in wild, domesticated, and growth hormone transgenic salmon)

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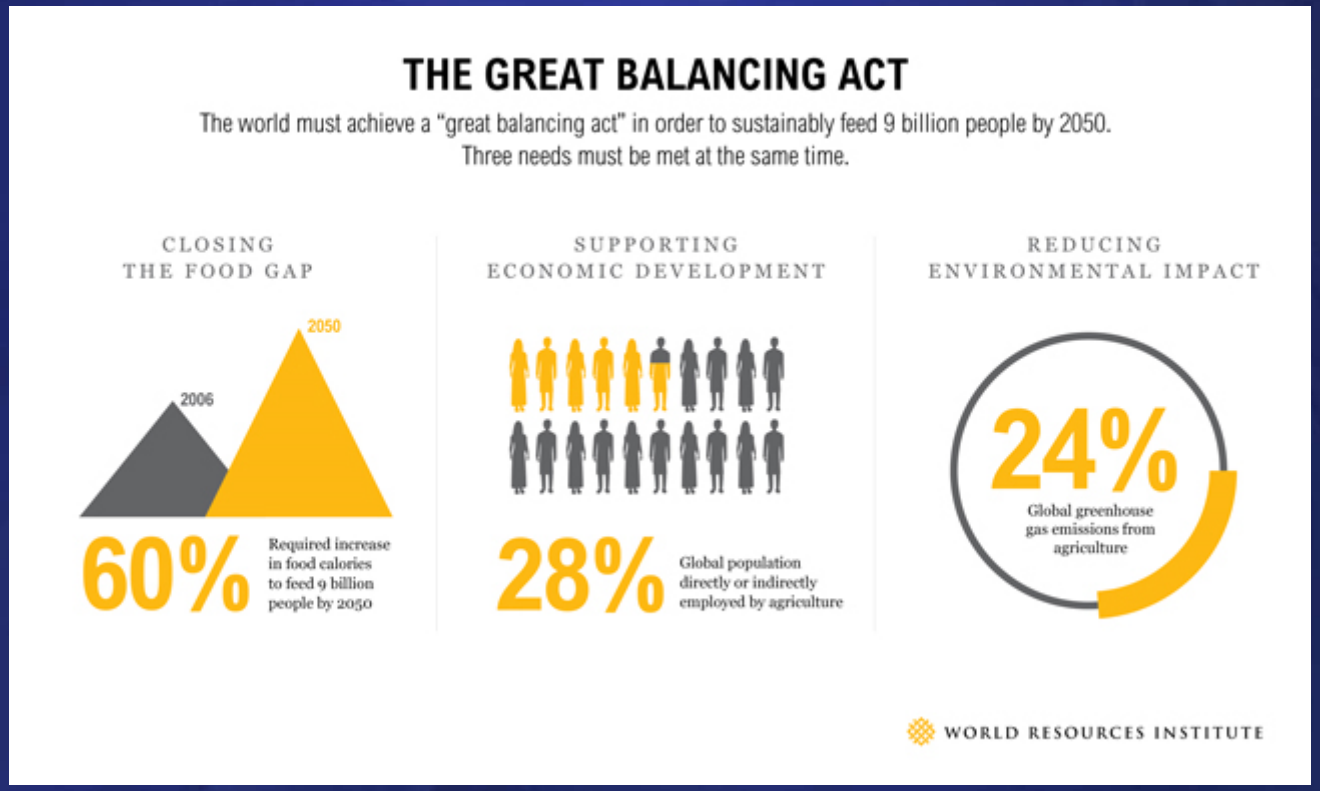
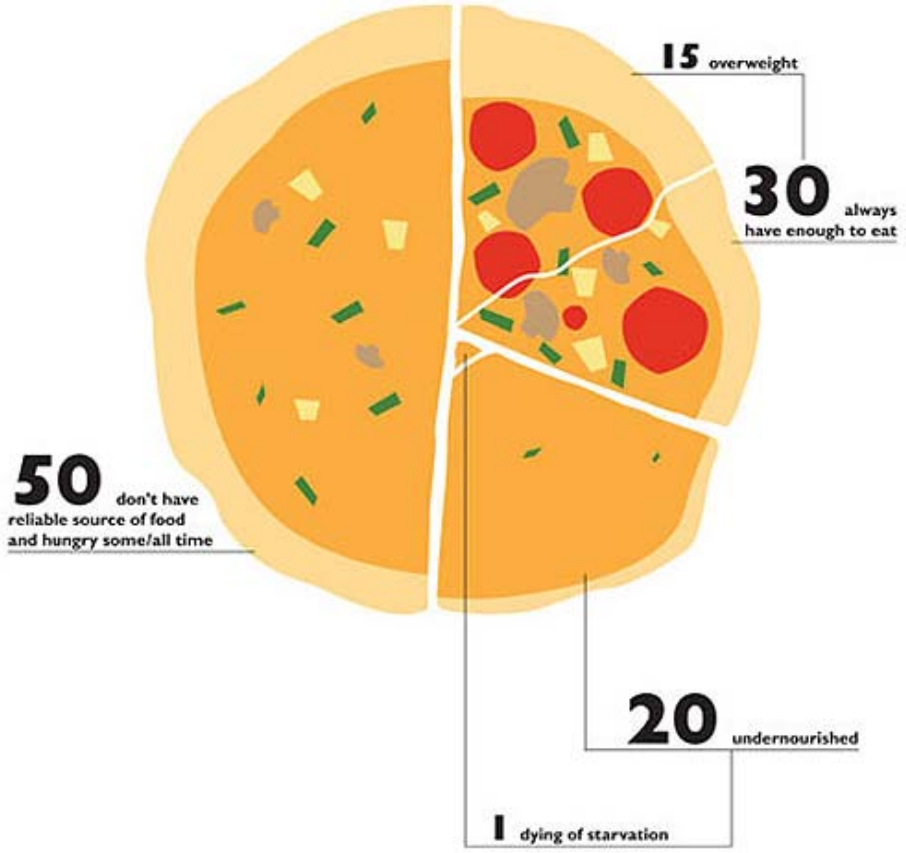
Research Backgrounds

Global Challenges



If the world were a village of 100 people

FOOD



Aquaculture technolegy as a solution

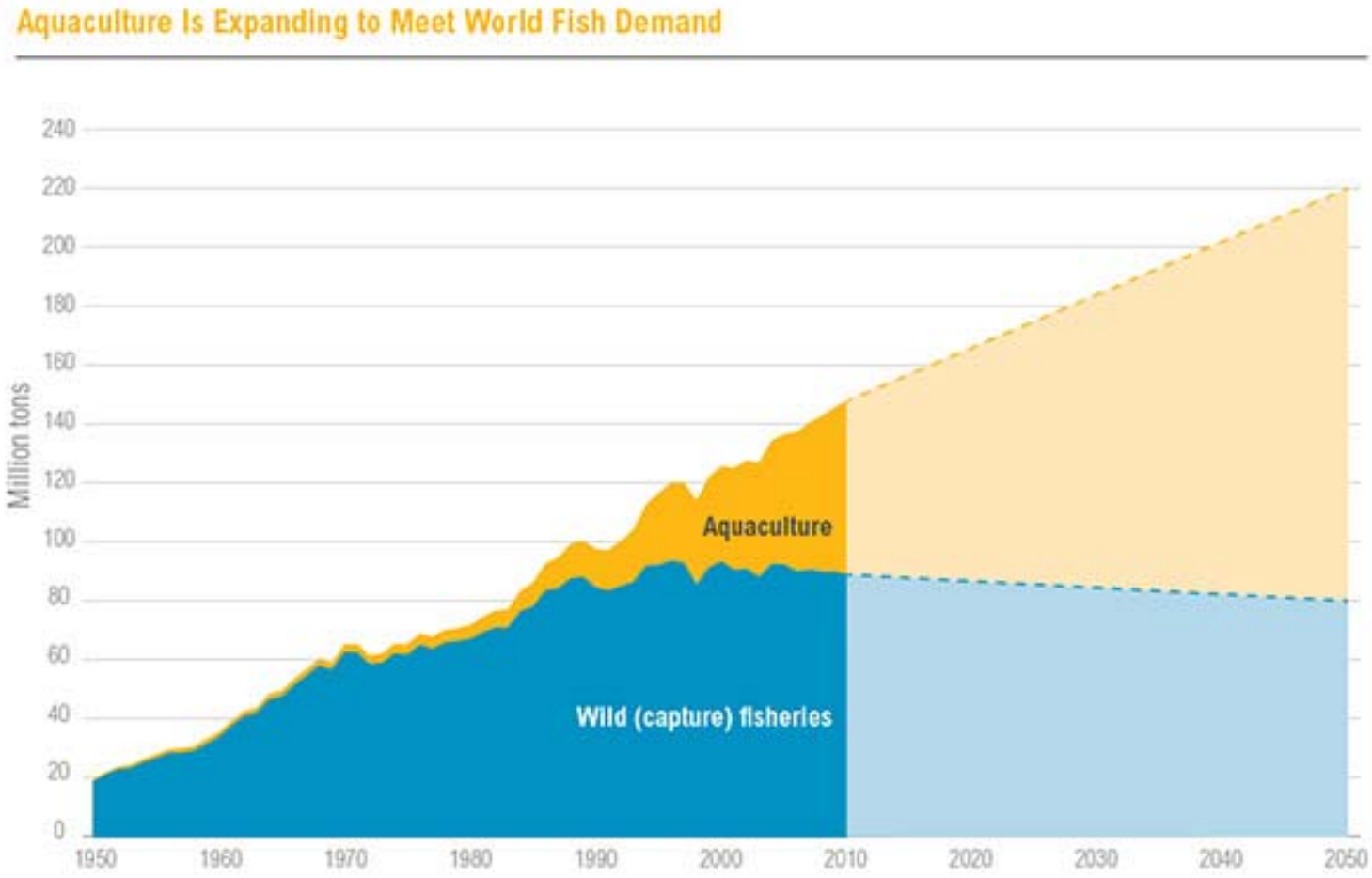
Conserving Wild Fish Populations

Aquaculture feed relies on wild fish to deliver the Omega-3 fatty acids that make fish so healthy to eat. Salmon is already one of the world's most efficient protein producers. Our AquAdvantage® Salmon grows to market-size using 25 percent less feed than traditional Atlantic salmon on the market today. This makes an already efficient protein producer even better because it requires less wild fish to be converted into salmon feed.

				
Carbon footprint kg CO2/kg edible meat	2.9 kg	3.4 kg	5.9 kg	30 kg
Water consumption litre/kg edible meat	1,400 litre[(1)]	4,300 litre	6,000 litre	15,400 litre

Note: 1) The figure reflects traditional smolt production in plants with water flow through. Recirculation plants, which are being implemented to an increasing extent, requires significantly less fresh water (up to 99% of the fresh water is recycled).

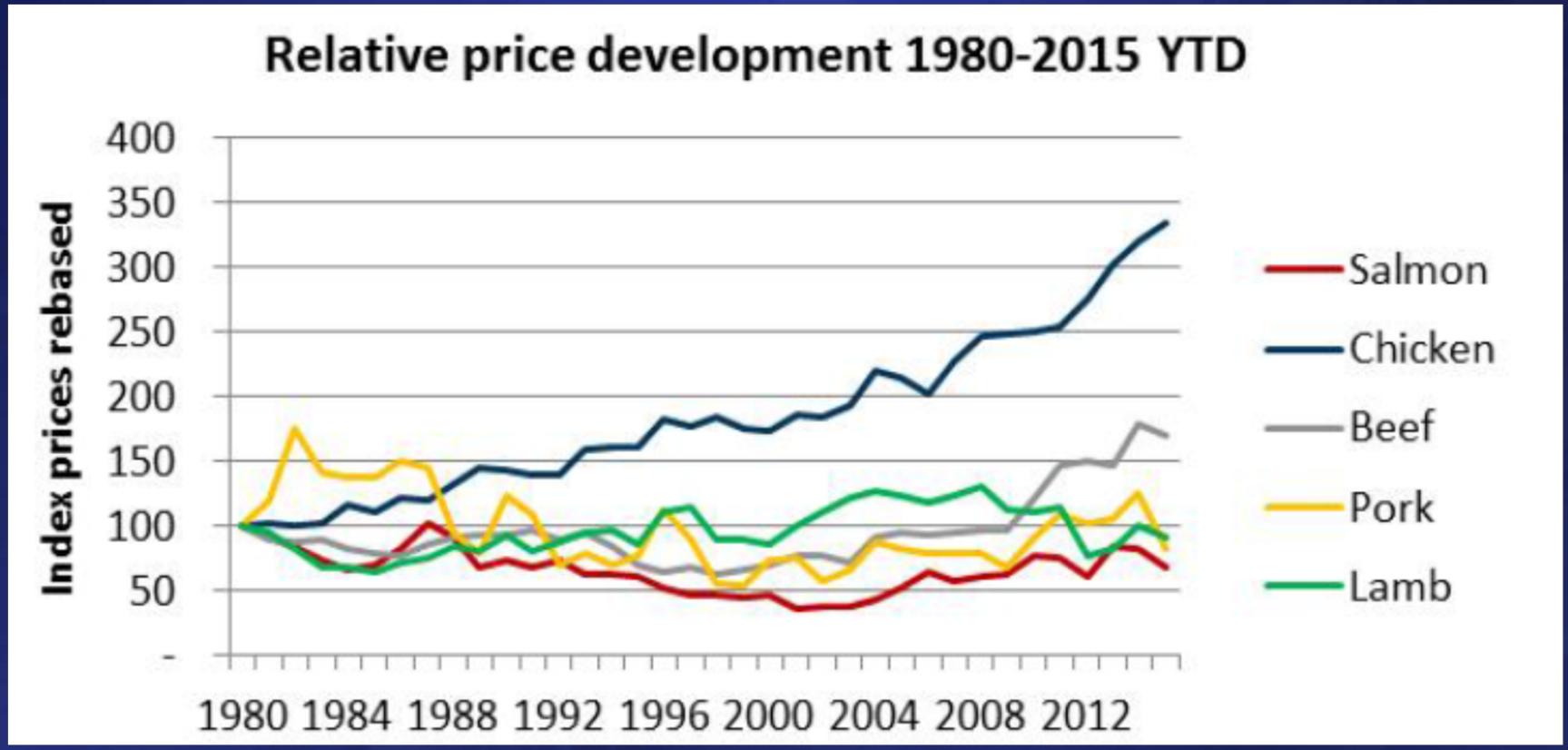
World Fish Production (in million tons)



Source: Historical data 1950–2010: FAO. 2014. "FishStatJ." Rome: FAO. Projections 2011–2050: Calculated at WRI, assumes 10 percent reduction in wild fish catch between 2010 and 2050, and linear growth of aquaculture production at an additional 2 million tons per year between 2010 and 2050.

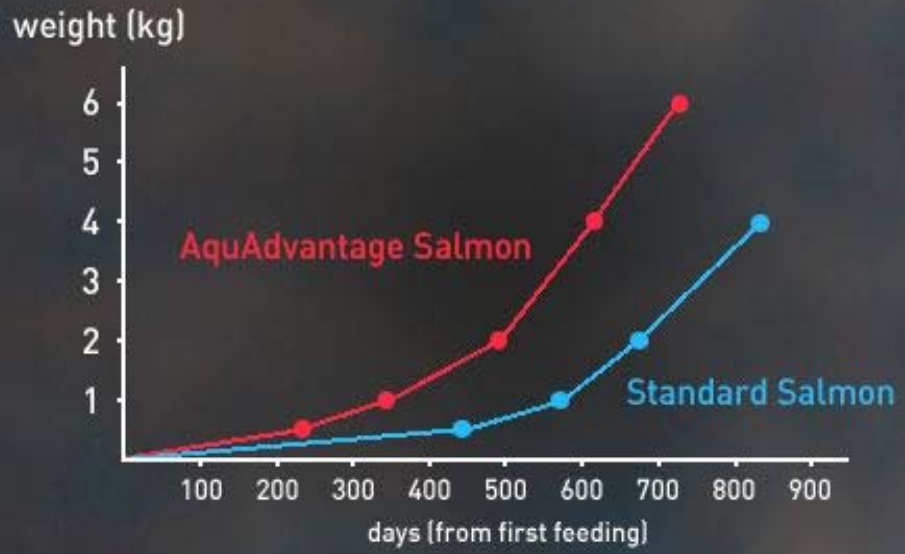
See www.wri.org/publication/improving-aquaculture for full paper.

WORLD RESOURCES INSTITUTE


Relative price development 1980-2015 YTD
Index prices rebased
400
350
300
250
200
150
100
50
-
1980 1984 1988 1992 1996 2000 2004 2008 2012
Salmon
Chicken
Beef
Pork
Lamb

BREAKING NEWS

FDA Approves GMO Salmon



AquAdvantage salmon are Atlantic salmon with a growth hormone gene from chinook salmon, to accelerate growth, and a fragment of DNA from ocean pout, to help activate the chinook gene.

Source: Images, video and chart data courtesy of AquaBounty Technologies


Alaska
US
Arctic Circle
(Grønland)
DK
Cercle Arctique
Gulf of Alaska
Golfe d'Alaska
Labrador Sea
Mer du Labrador
ATLANTIC OCEAN
Hudson Bay
Baie d'Hudson
PACIFIC OCEAN
Canada
FR
OCÉAN ATLANTIQUE
United States of America
États-Unis d'Amérique
GB
Tropic of Cancer
Tropique du cancer
OCÉAN PACIFIQUE
Mexico
Mexique
Gulf of Mexico
Golfe du Mexique
Bahamas
Cuba
DO
AG
KN
DM
BB
LC
VC
GD
TT
Haiti
Haïti
Jamaica
Jamaïque
Mer des Caraïbes
Caribbean Sea
Belize
Guatemala
Honduras
HN
NL
MX
NORTH AMERICA
AMÉRIQUE DU NORD
0 700 1400 km
El Salvador
Nicaragua
Costa Rica
Panama
Colombia
Venezuela
GY
Brazil
Brésil
SOUTH AMERICA
AMÉRIQUE DU SUD
CR
Equator
Équateur
© 2001. Her Majesty the Queen in Right of Canada, Natural Resources Canada.
Sa Majesté la Reine du chef du Canada, Ressources naturelles Canada.

U.S. Food and Drug Administration

AquAdvantage® Salmon

Environmental Assessment

In support of an approval of

a New Animal Drug Application related to AquAdvantage Salmon,

which are triploid, hemizygous, all-female Atlantic salmon (*Salmo salar*)

bearing a single copy of the α-form of the *opAFP-GHc2* recombinant DNA construct

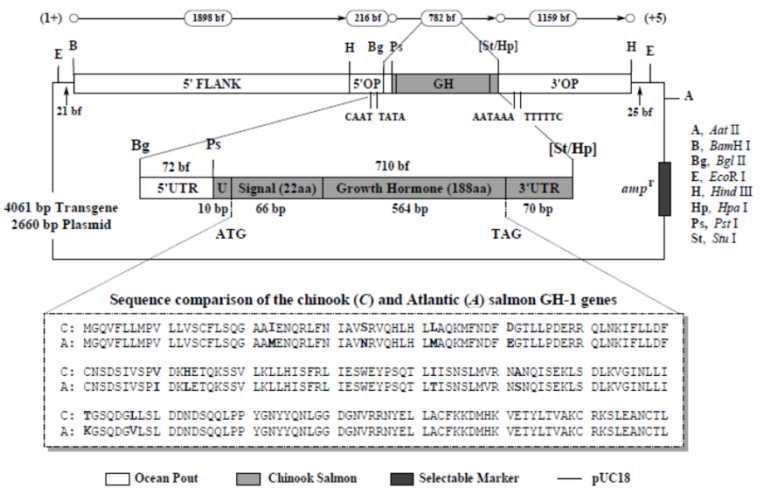
at the α-locus in the EO-1α lineage

November 12, 2015

Prepared by

Center for Veterinary Medicine
United States Food and Drug Administration
Department of Health and Human Services

Figure E.1. Physical Description of the AquAdvantage Construct, opAFP-GHc2 *



* ***bp*** length is used in the narrative and figures in reference to the physical size of a DNA in fully-duplexed form; base fragment (***bf***) length is used in reference to the number of bases between, and inclusive of, the 5′- and 3′-nucleotides comprising the restricted recognition sequences on the boundaries of the + strand. ***amp***^{r}, *bla* gene providing ampicillin resistance.

Figure 4. Reproductive Biology of AquAdvantage Broodstock and Eyed-Egg Production

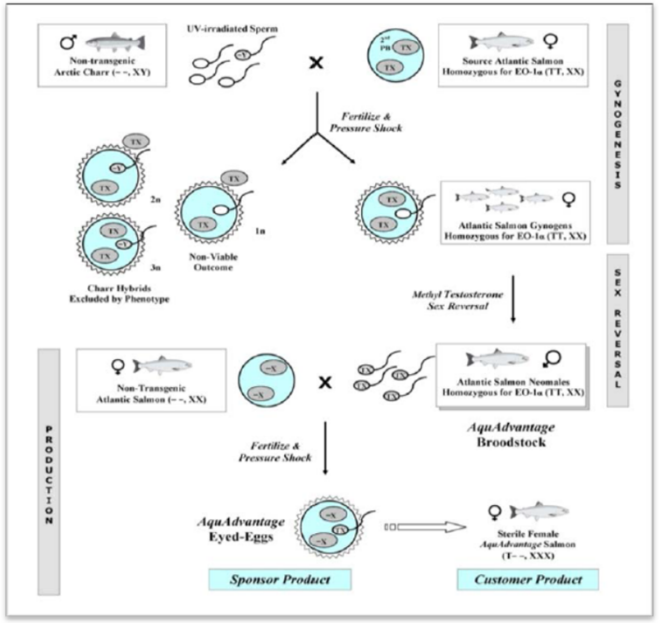


Figure 5. Technical Details & Logistics of Commercial Production *

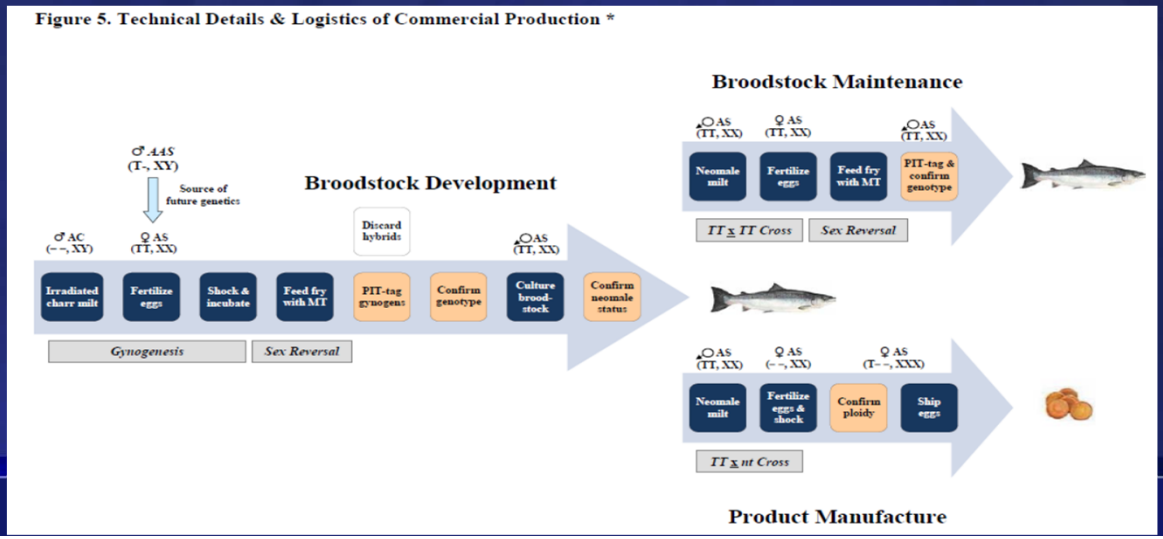


Figure 2. Conceptual Model for Risk Assessment

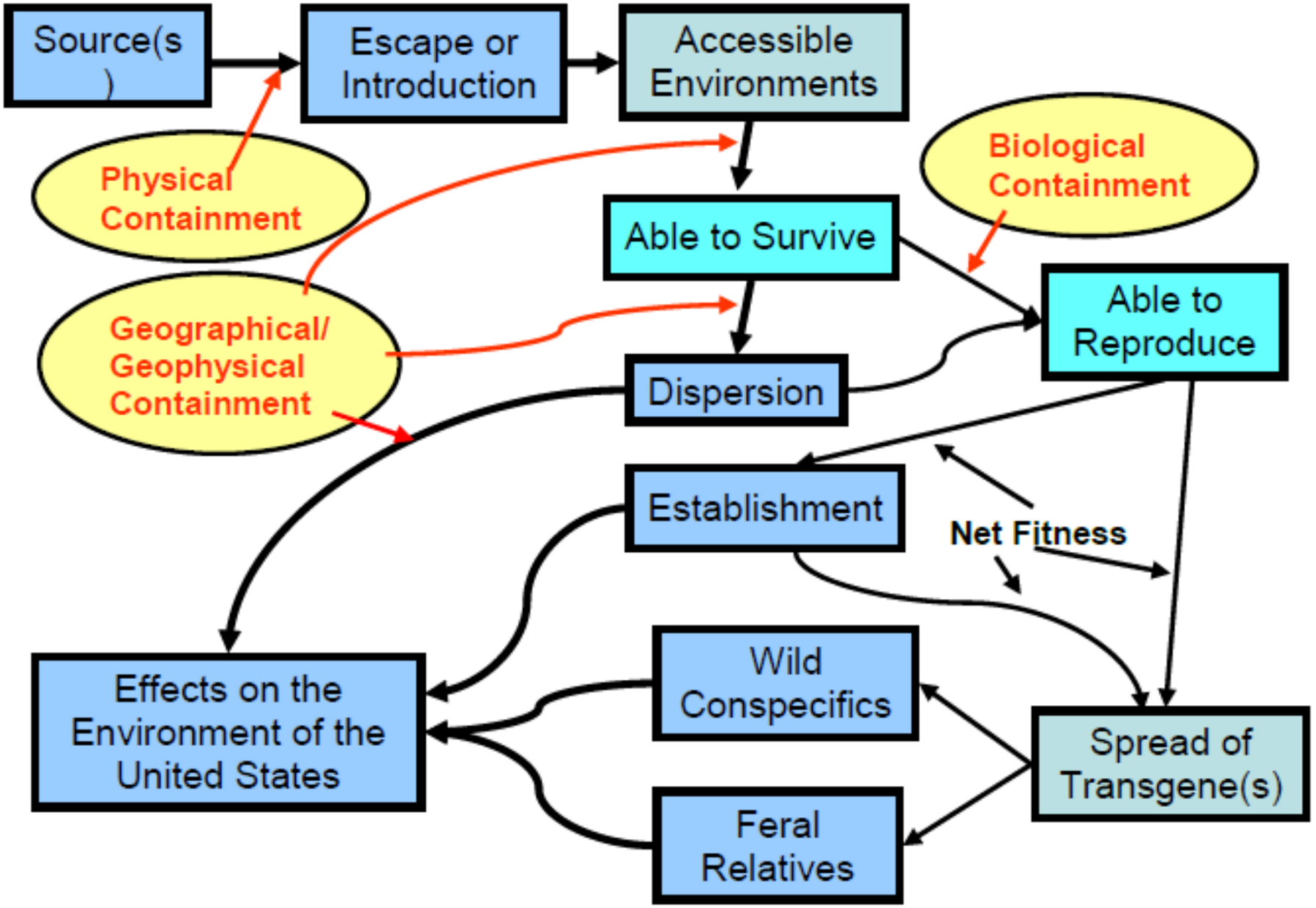
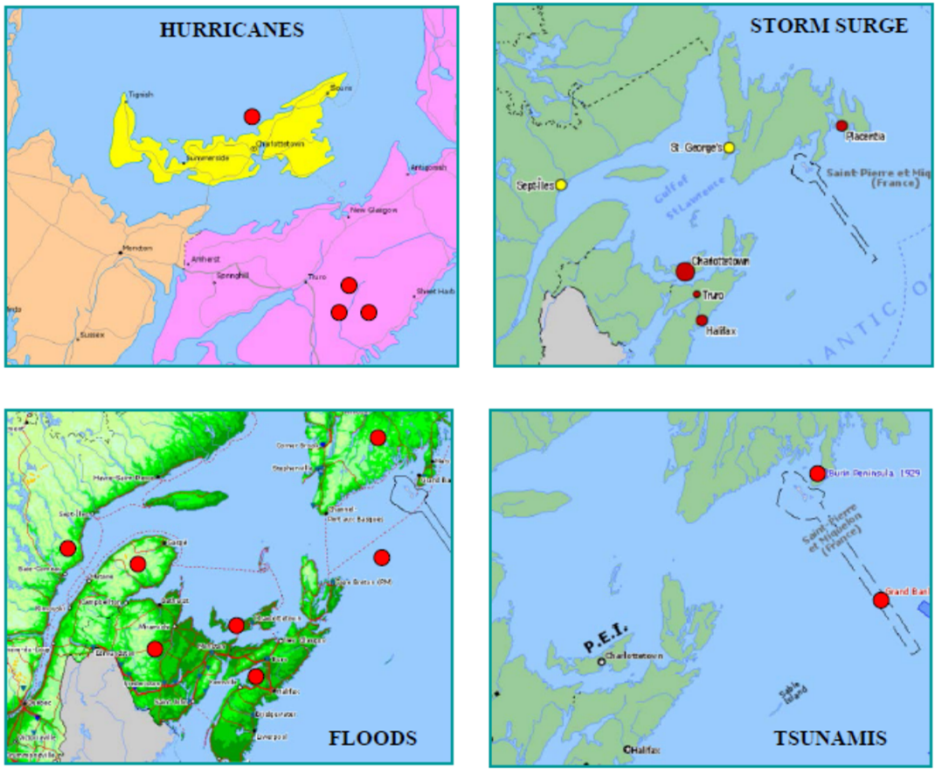
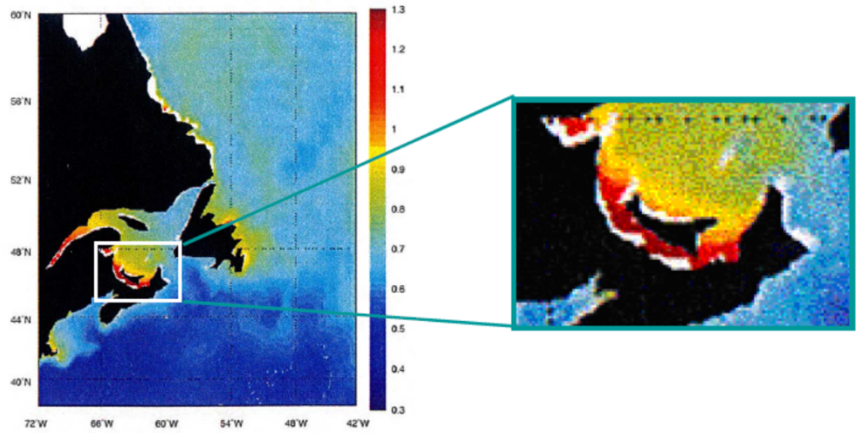


Figure 8. Occurrence of Natural Hazards in Proximity to PEI *



* With the exception of *Storm Surge*, where circle size is indicative of frequency (small, medium, large = low, medium, high) and circle color is indicative of severity (green, yellow, red = low, medium, high), all other circles are location indicators for single events reported by National Resources Canada through 1999. **Note:** The red dots indicating location of weather-related events have been significantly increased in size for ease of identification; their exact locations may differ slightly from those in the original graphic on the National Resources Canada website.

Figure 9. Variability of Storm Surge for the Atlantic Coast of Canada



* Left-most figure of Atlantic Canada abstracted from Lemmen *et al.*, 2008, p. 132.

VS

GMOs in public and media

Historically, anti-GMOs movements are the most successful activity


WHO IS RESPONSIBLE FOR GMO SAFETY TESTING?
MONSANTO
USDA
FDA

Thus, in order to diminish the gap between public perception and truth, need for objective scientific data to allow informed discussion by the public, policy makers, NGOs, and regulators.

Our study is not about generating transgenic strains, but to provide scientific support to regulatory process.

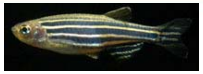
Transgenic organism in science

Transgenic organisms (GMOs) have received sequences of DNA by artificial means, followed by integration of one or more of the novel sequences into their chromosomal DNA.

Macleanand Laight. 2000. Transgenic fish: an evaluation of the risks and benefits. Fish and Fisheries 1:146-172. Fish and Fisheries 1:146-172

Transgenic Fish

Carassius auratus



Danio rerio

Misgurnus anguillicaudatus

Oreochromis urolepis

Salvelinus alpinus alpinus

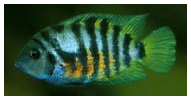
Catla catla

Esox lucius

Misgurnus fossilis

Oreochromis mossambicus

Sander vitreus



Cichlasoma nigrofasciatum

Fundulus heteroclitus

Misgurnus mizolepis

Oreochromis niloticus

Sparus aurata

Cirrihinus mrigala

Ictalurus punctatus

Oncorhynchus clarkii

Oryzias latipes

Xiphophorus sp.

Clarias gariepinus

Labeo rohita

Oncorhynchus kisutch

Poeciliopsis lucida

Mulinia lateralis

Ctenopharyngodon idellus

Leucopsarion petersii

Oncorhynchus mykiss

Salmo salar

Cyprinus carpio

Megalobrama amblycephala

Oncorhynchus tshawytscha

Salvelinus fontinalis

Growth of GH overexpression

Atlantic salmon

Rohu

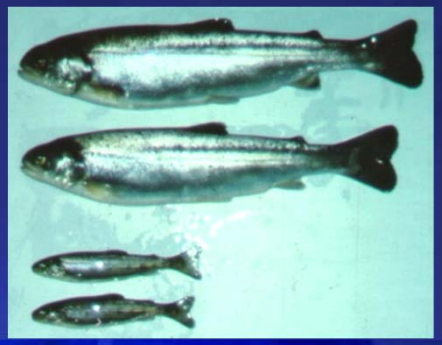
Tilapia

Carp

Mud loach

Channel catfish

Rainbow trout



Arctic charr

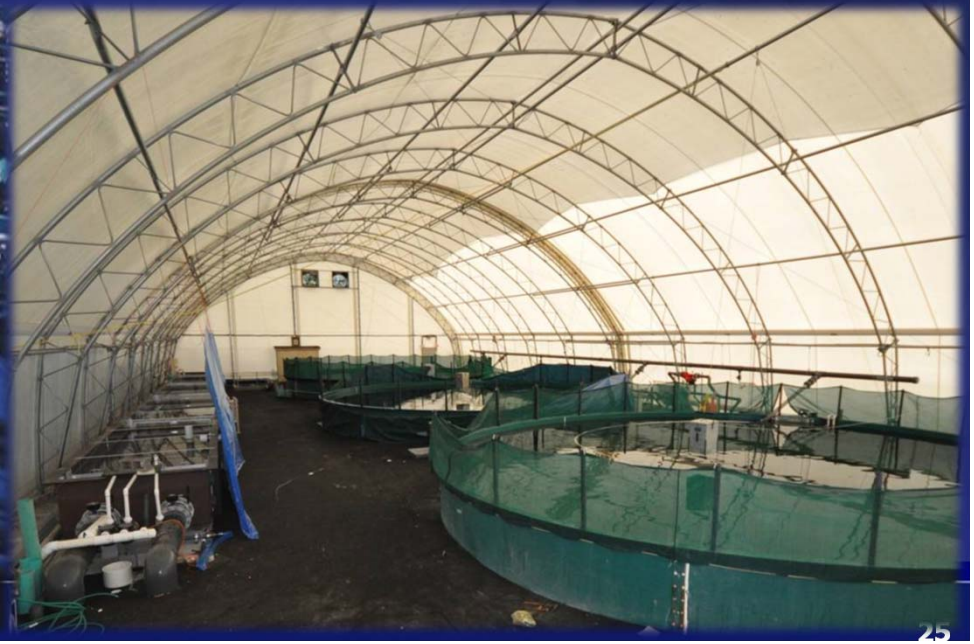
Chinook salmon

Benefits & Risks

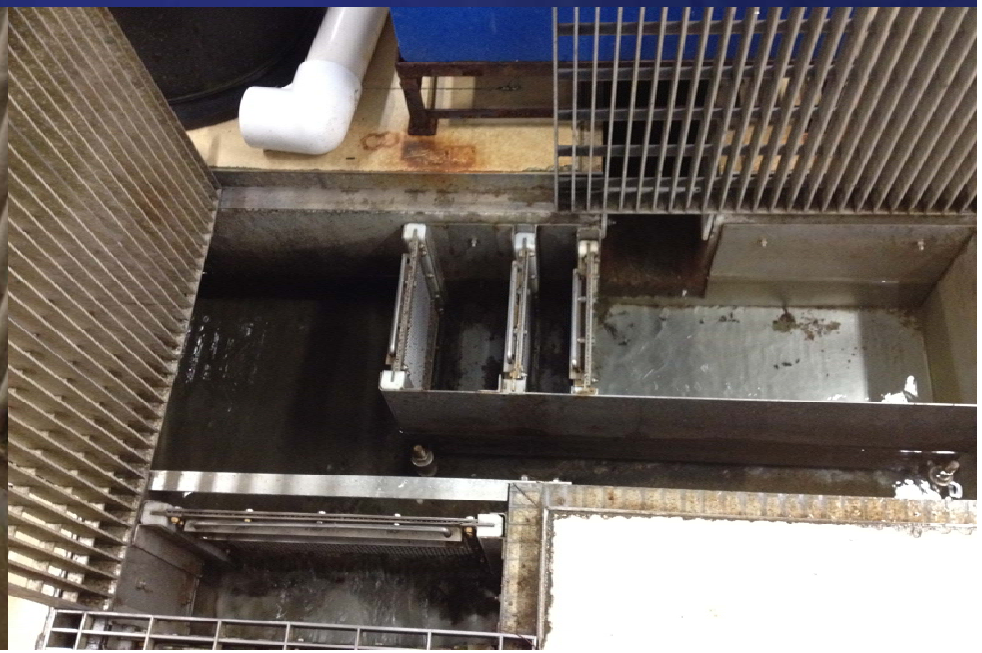
	Content	Probability
Benefits	Low cost, fast growing	High
	Cheaper and greener diet	Medium in long term
	Disease resistance.	Low in short term
	Cold and salinity tolerance	Low in short term
	Sterilization	Not perfect
	Pharmaceutical proteins	High
Risks	No advantage	Law
	Harmful to health	Law
	Affect to other species.	? Requires control
	Interbreed with wild fish	? Requires control
	Die out quickly	? Requires control

Materials and Methods

Centre for Aquaculture and Environmental Research

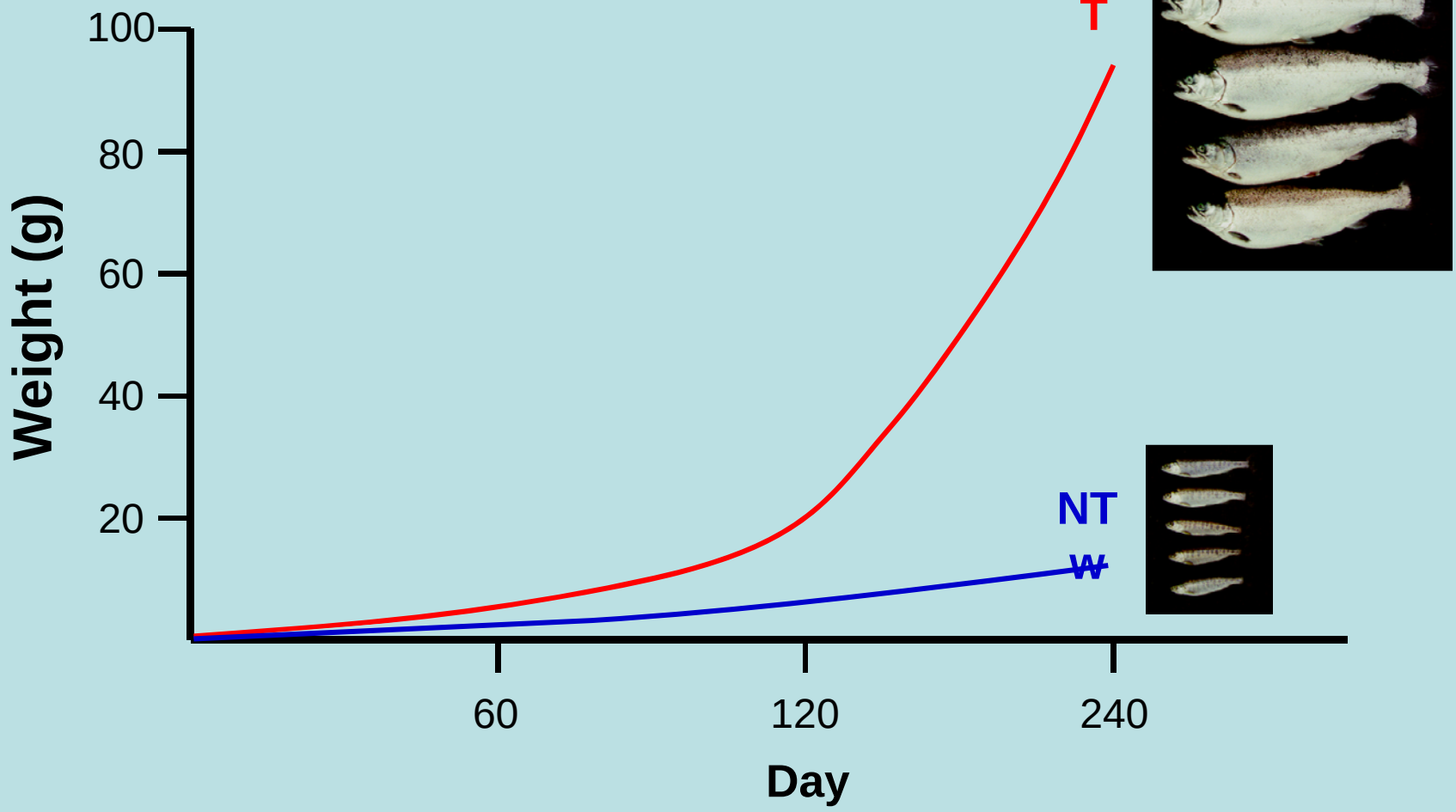


Containment facilities



Non-transgenic Transgenic

Growth



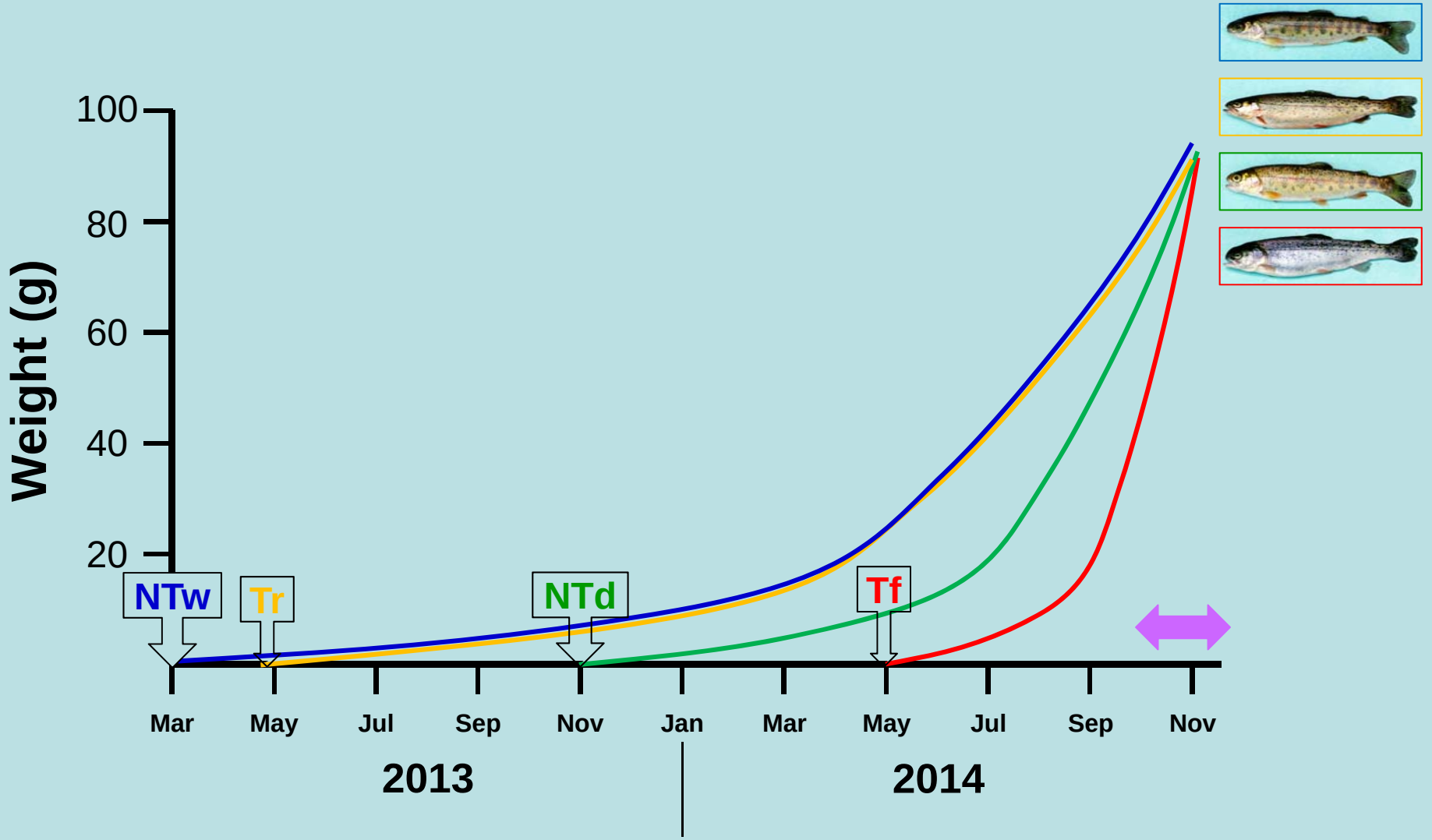
Devlin et al.
2003 Nature

Fish genotypes

Four size matched fish strains

- Non-T
  - Wild — Wild-type strain on full ration; **NTw**
  - Domesticated — Fast-growing domesticated strain on full ration; **NTd**
- T
  - Fully fed — GH transgenic strain on full ration; **Tf**
  - Ration restricted — GH transgenic strain on wild-type ration; **Tr**

How to get size-matched fishes ?

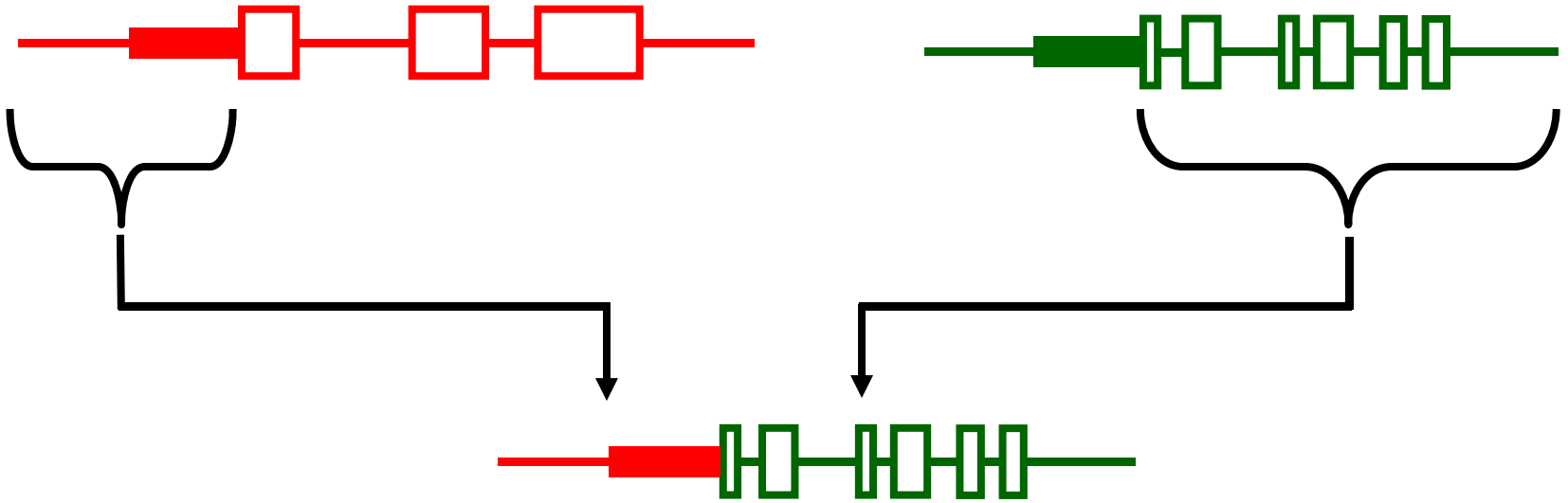


Metallothionein Gene

-Expression in many tissues
-Moderate constitutive expression
-Inducible

Growth Hormone Gene

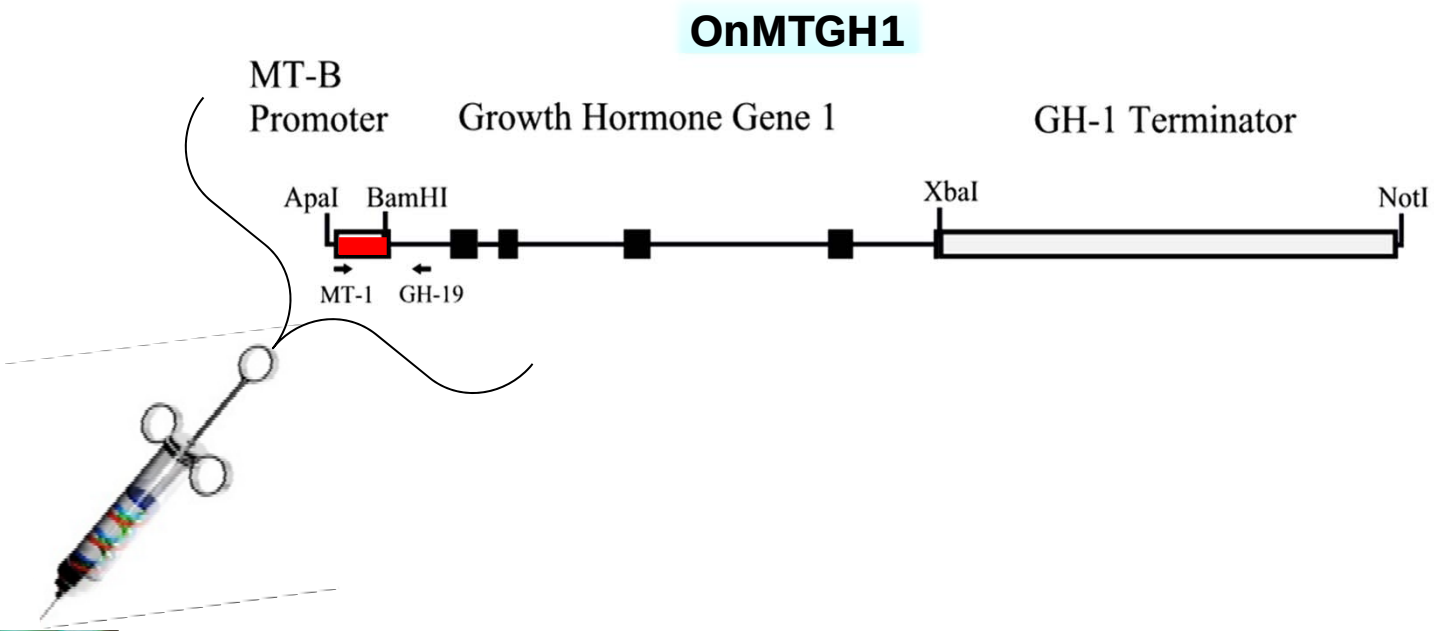
-Expression in pituitary gland
-Seasonal regulation
-Negative feedback regulation by GH/IGF-I

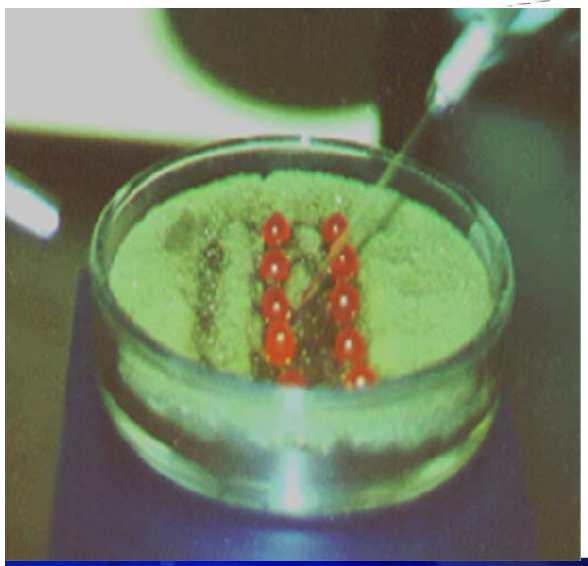


Mt/GH Gene Construct

-Expression of GH in many tissues
-Insensitive to feedback regulation
-Seasonally uncoupled
-Inducible

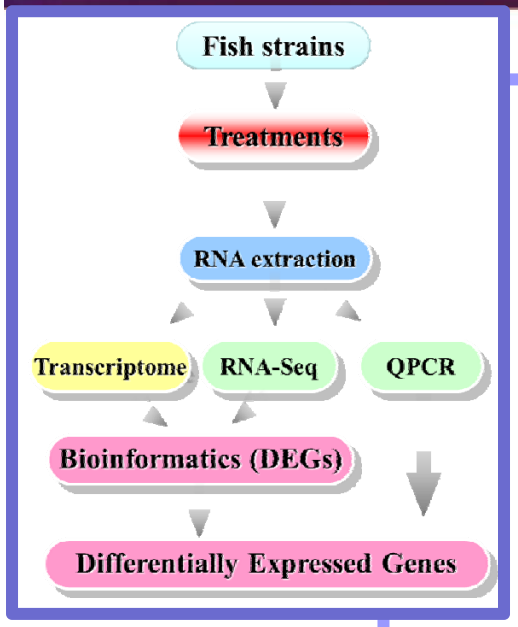
How to deliver of trans GH-gene into fish?





- Introduced into fertilized eggs by micro-injection.
- Insert into genome at a frequency of 1%
- Stable Mendelian transmission

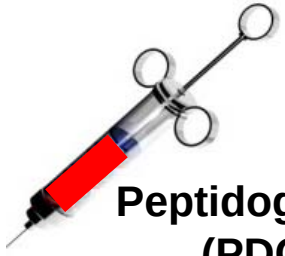
Experimental design



Control

PBS

Bacterial mimic



Peptidoglycan (PDG)

Viral mimic

Poly I:C (PIC)

NTw

NTd

Tf

Tr

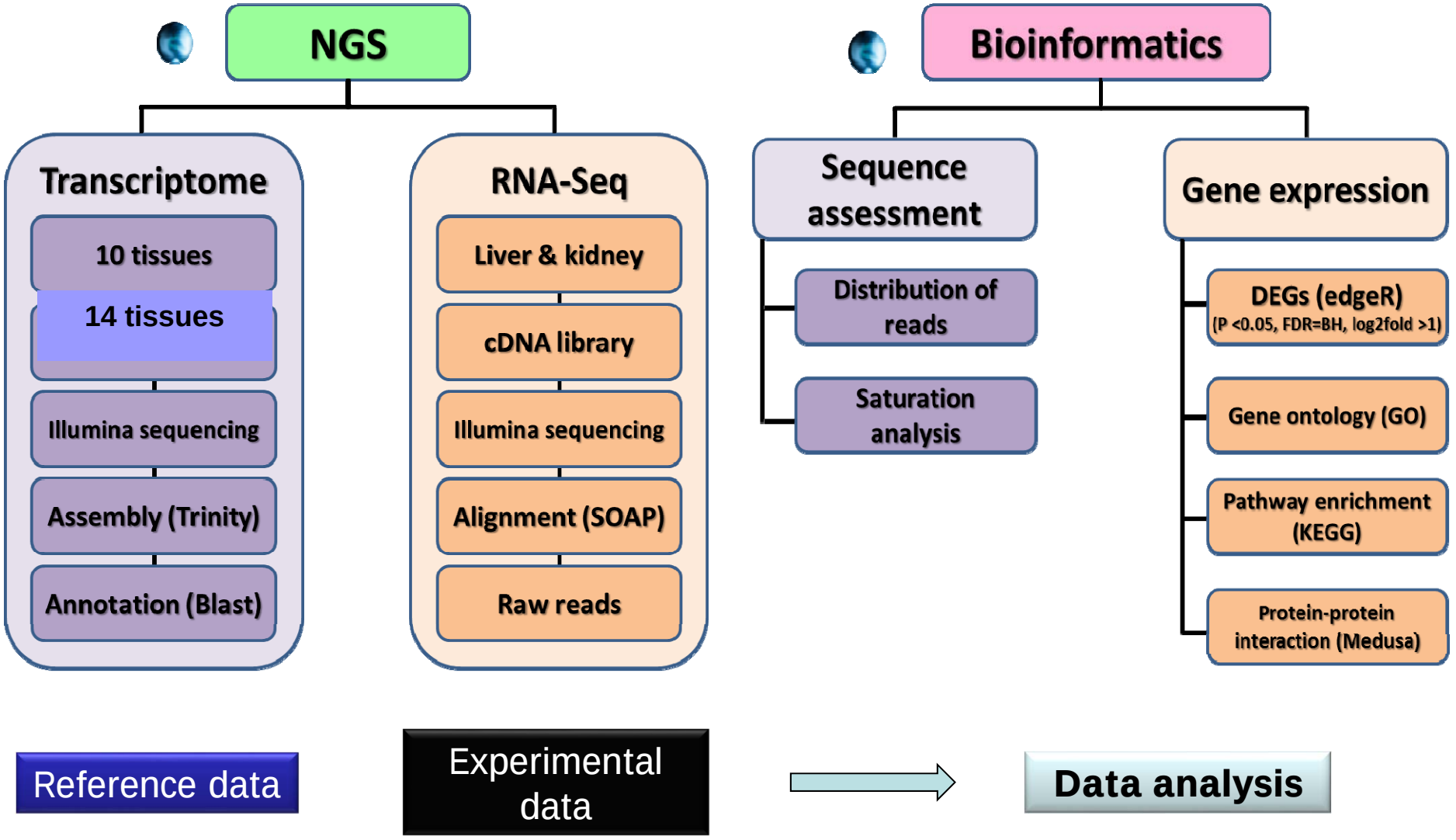
After 24 hour

Liver Head kidney

RNA extraction

Sequencing (RNASeq)

Pipeline for sequencing

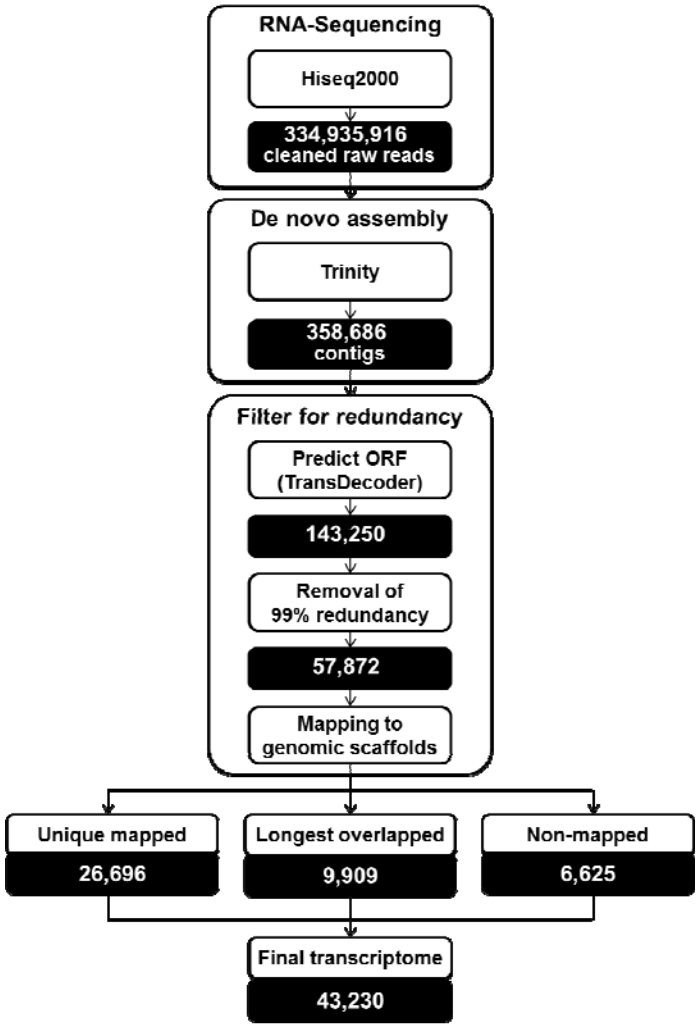


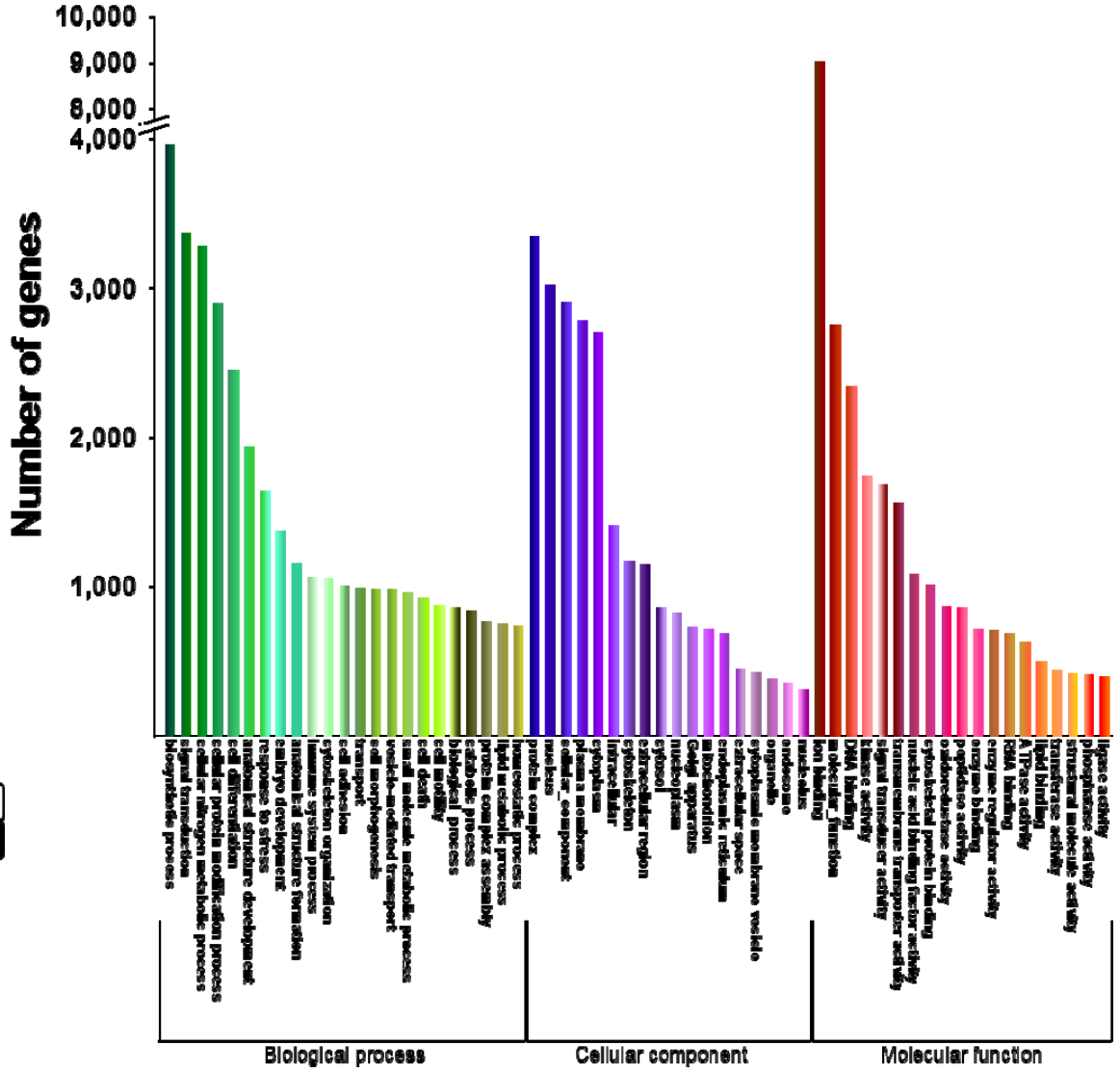
Statistic analysis by Two-way ANOVA

Genotype / Treatment	NTw	NTd	Tf	Tr
Pathogen	Main effect + Interaction effect			
Control				

Results & Discussion

Construction of transcriptome





Differentially Expressed Genes (DEGs)

Tissue -specific

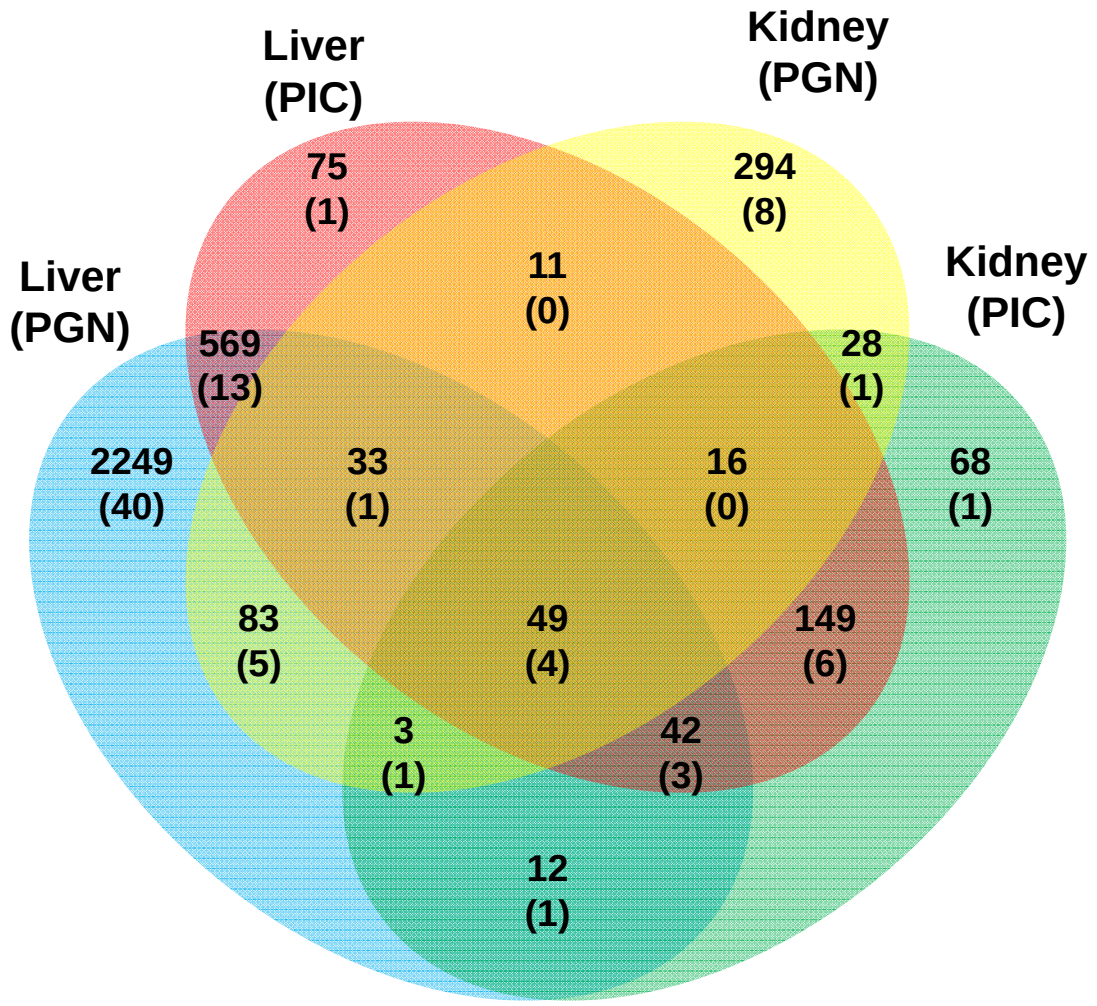
Liver (PIC)

Kidney (PGN)

		Liver		Kidney	
Gene	Annotation	Peptidoglycan	Poly I:C	Peptidoglycan	Poly I:C
unigene22407215	**Proto-oncogene c-fos-like**	**1.1**		**1.5**	
Unigene22398575	**Marcks-related**	**1.4**		**1.9**	
Unigene22394700	**Tumor necrosis factor alpha-2 precursor**	**4.7**		**3.9**	
Unigene22393447	**Interleukin-1 beta**	**5.4**		**4.2**	
Unigene22398574	**Marcks-related**	**1.6**		**1.9**	
unigene22402141	**Interferon regulatory factor 7**		**2.9**		**2.1**
Unigene22392264	**Signal transducer and activator of transcription 2 isoform x1**		**2.3**		**1.5**
Unigene22411959	**Sub-family b atp-binding cassette transporter 2**		**2.6**		**1.5**
Unigene22420866	**E3 ubiquitin ISG15 ligase trim25**		**2.0**		**1.4**
Unigene22392028	**Probable atp-dependent rna helicase dhx58**		**3.6**		**2.4**
Unigene22405102	**Platelet basic protein precursor**		**3.9**		**2.8**
Unigene22419730	**Heat shock 7 kda protein 4-like**	**3.0**	**2.2**		
Unigene22391490	**Tumor necrosis factor receptor superfamily member 5 precursor**	**5.4**	**2.1**		
Unigene22419732	**Heat shock 7 kda protein 4-like**	**3.4**	**2.6**		
Unigene22404762	**Claudin 5b**	**1.1**	**1.2**		
Unigene22420793	**Stat3_chick ame: full=signal transducer and activator of transcription 3**	**1.4**	**1.7**		
Unigene22414431	**Mitogen-activated protein kinase kinase kinase 8**	**1.7**	**1.5**		
Unigene22401110	**Lipid phosphate phosphohydrolase 1-like**	**3.9**	**2.2**		
Unigene22418403	**Dna-directed rna polymerase iii subunit rpc5**	**1.5**	**1.1**		
Unigene22407565	**Interferon-gamma receptor alpha chain**	**2.3**	**1.8**		
Unigene22404345	**High affinity immunoglobulin gamma fc receptor i precursor**	**2.0**	**1.2**		
Unigene22400526	**Nf-kappa-b inhibitor epsilon**	**3.2**	**1.7**		

		Liver		Kidney	
Gene	Annotation	Peptidoglycan	Poly I:C	Peptidoglycan	Poly I:C
Unigene22406581	**C-C motif chemokine 19 precursor**	**2.8**	**3.0**	**2.6**	**2.6**
unigene22422936	**Matrix metalloproteinase-9**	**3.0**	**1.5**	**1.2**	**1.1**
unigene22418378	**Growth-regulated alpha**	**1.9**	**2.0**	**1.1**	**1.1**
unigene22426238	**---NA---**	**3.5**	**2.5**	**1.9**	**1.8**

Differentially Expressed Genes (DEGs)



Differentially Expressed Genes (DEGs)

a) Liver on PGN **b) Liver on PIC**

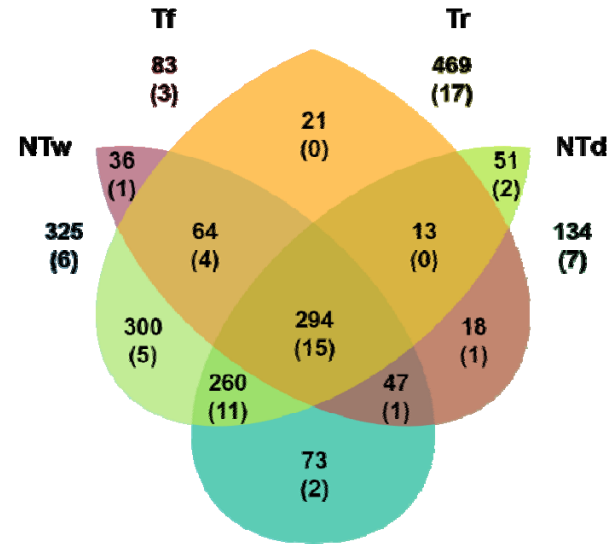
	Tf	Tr		Tf	Tr		
Tissue	**Treatment**	**Gene**	**Annotation**	**NTw**	**NTd**	**Tf**	**Tr**
Liver	Peptidoglycan	unigene22407215	**Proto-oncogene c-fos-like**	**2.1**	**2.3**	0.1	-0.1
		unigene22424575	**Guanylate cyclase soluble subunit beta-1**	**-1.8**	**-1.9**	-1.4	-1.7
	Poly I:C	unigene22418378	**Growth-regulated alpha**	**3.1**	**3.2**	-0.2	1.7
		unigene22426238	**Unknown**	**3.9**	**3.9**	-0.6	2.7
Kidney	Peptidoglycan	unigene22394700	**TNF alpha-2**	**4.5**	**4.0**	2.3	4.8
		unigene22413649	**Actin-related protein 2 complex subunit 1a-like**	**-1.4**	**-1.5**	-1.0	-1.0
		unigene22419733	**Heat shock 70 kda protein 4-like**	**1.3**	**1.2**	0.7	1.1

Tissue	Treatment	Gene	Annotation	NTw	NTd	Tf	Tr
Liver	Peptidoglycan	unigene22385939	Claudin-14-like isoform X1	-0.7	-0.5	-0.1	-1.2
		unigene22386649	Transforming growth factor beta-1	0.3	0.2	1.3	2.4
		unigene22393807	Caspase	1.0	0.9	0.5	1.7
		unigene22397333	Serine threonine-protein kinase TBK1	0.2	0.2	0.4	1.3
		unigene22398557	Tir domain-containing adapter molecule 1-like	0.7	0.4	-0.2	1.1
		unigene22398574	MARCKS-related	1.1	1.3	1.8	2.3
		unigene22399760	Plasminogen activator inhibitor 1	1.4	0.8	1.7	2.5
		unigene22401602	Caspase-8-like isoform X3	0.6	1.2	0.4	2.0
		unigene22401732	5-azacytidine-induced protein 2	0.9	0.5	0.4	1.4

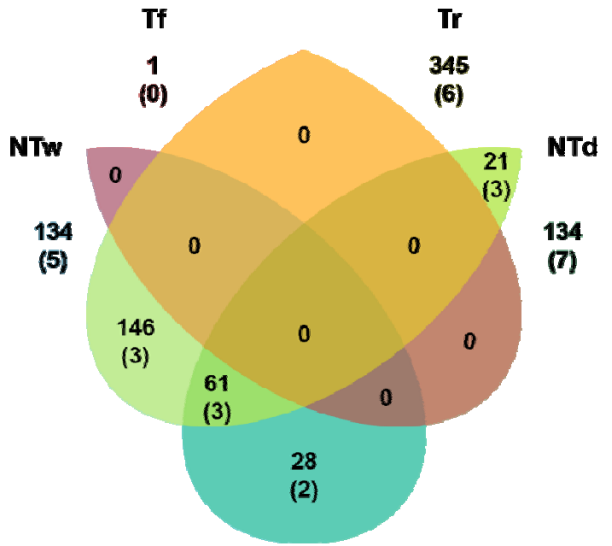
Tissue	Treatment	Gene	Annotation	NTw	NTd	Tf	Tr
Liver	Peptidoglycan	unigene22391490	TNF receptor superfamily member 5	**6.0**	**5.5**	**4.0**	**6.0**
		unigene22391703	Complement component C7-like	**2.7**	**2.8**	**2.9**	**2.9**
		unigene22392836	Toll-like receptor 5	**4.0**	**4.1**	**4.6**	**3.9**
		unigene22396127	Clusterin	**1.7**	**2.3**	**2.8**	**2.1**
		unigene22400525	NF-kb inhibitor epsilon	**3.2**	**3.3**	**3.6**	**2.6**
		unigene22400526	NF-kb inhibitor epsilon	**2.6**	**3.2**	**3.3**	**3.8**
		unigene22401110	Lipid phosphate phosphohydrolase 1-like	**5.0**	**4.1**	**3.3**	**3.2**
		unigene22401681	Mucosa-associated lymphoid tissue lymphoma translocation protein 1-like	**1.1**	**1.1**	**1.4**	**1.1**
		unigene22401878	SHC-transforming protein 2 isoform X1	**-2.0**	**-2.1**	**-1.8**	**-2.0**
		unigene22404345	High affinity immunoglobulin gamma FC receptor I	**1.4**	**2.7**	**1.7**	**2.0**
		unigene22416115	Junctional adhesion molecule B isoform X1	**2.0**	**1.9**	**2.5**	**2.6**
		unigene22418403	DNA-directed RNA polymerase III subunit RPC5	**1.7**	**1.2**	**1.2**	**1.8**
		unigene22419732	Heat shock 70 kda protein 4-like	**3.8**	**3.8**	**2.6**	**3.2**
		unigene22422936	Matrix metalloproteinase-9	**3.4**	**3.2**	**2.5**	**3.1**
		unigene22423393	Ubiquitin carboxyl-terminal hydrolase CYLD	**1.5**	**1.3**	**1.3**	**1.5**

Differentially Expressed Genes (DEGs)

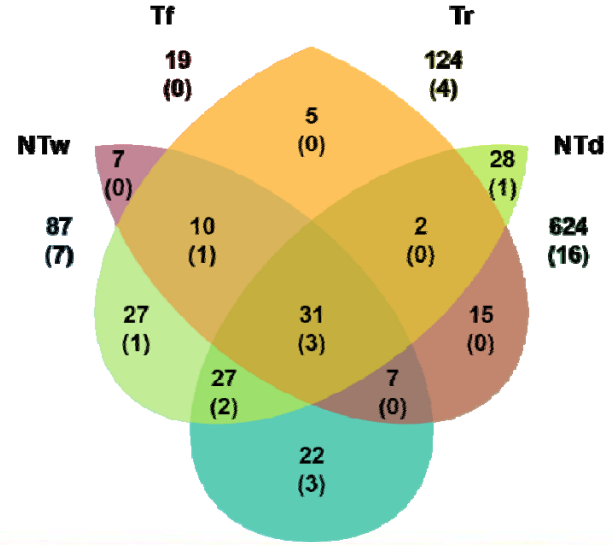
a) Liver on PGN



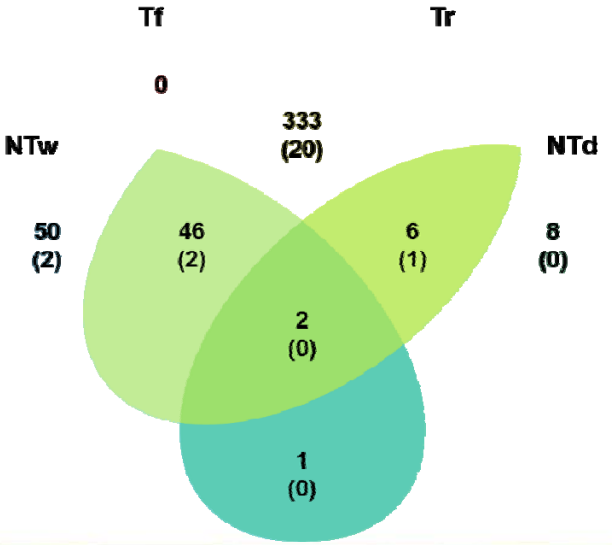
b) Liver on PIC



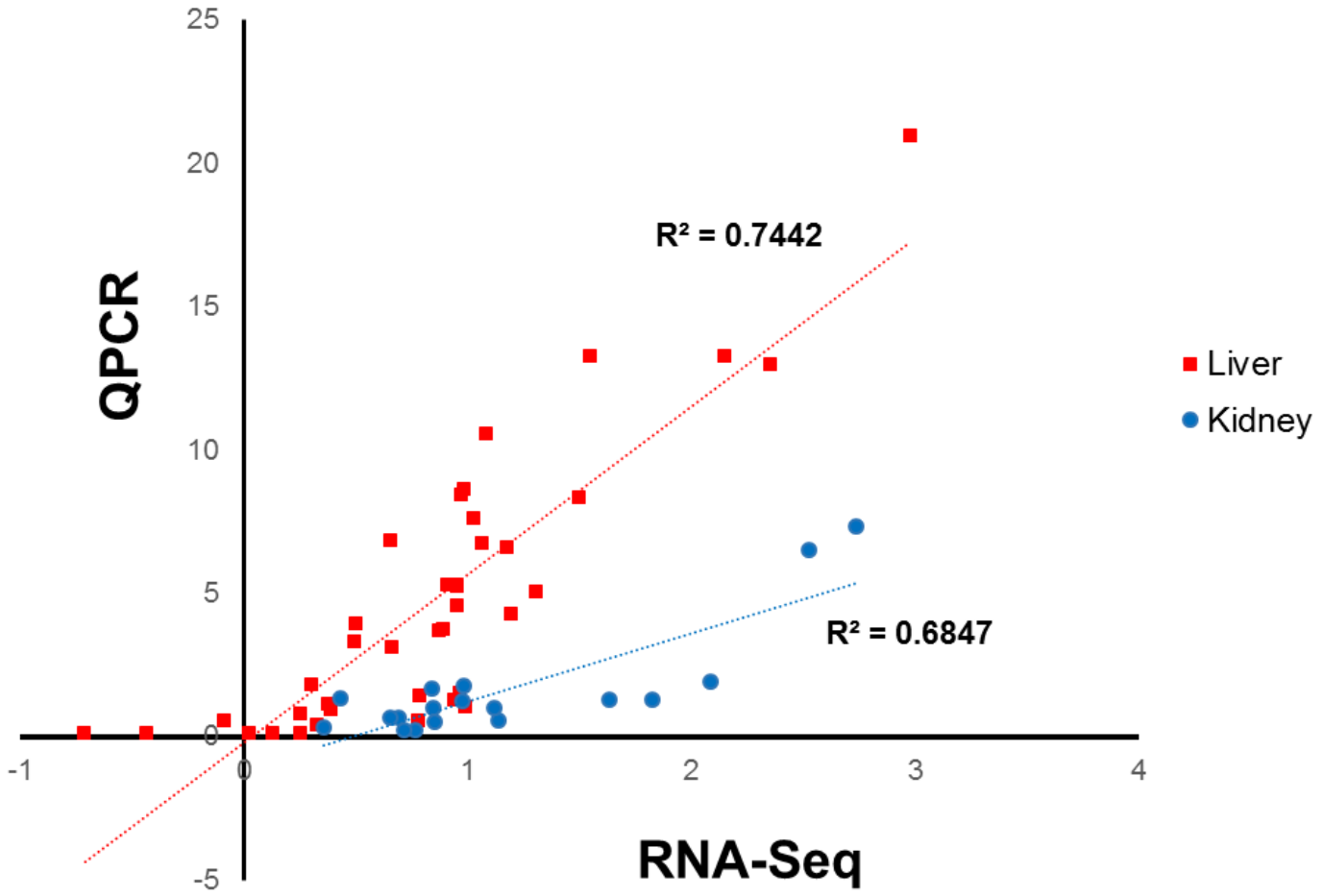
c) Kidney on PGN



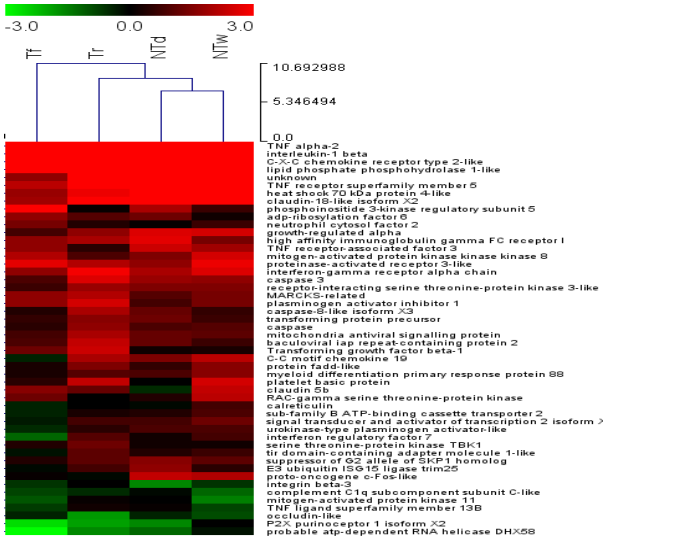
d) Kidney on PIC



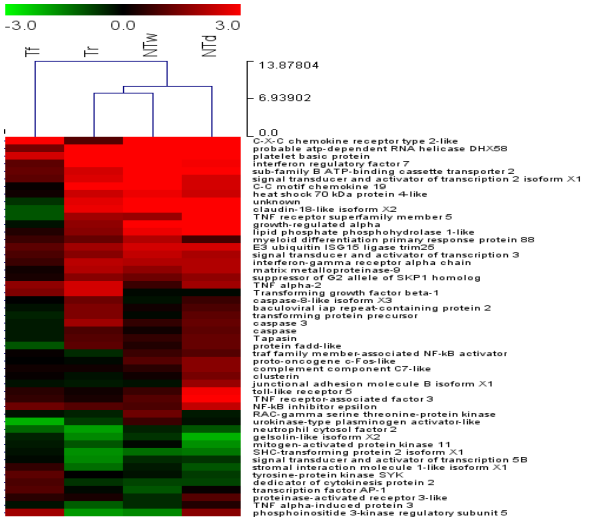
QPCR validation of RNA-Seq



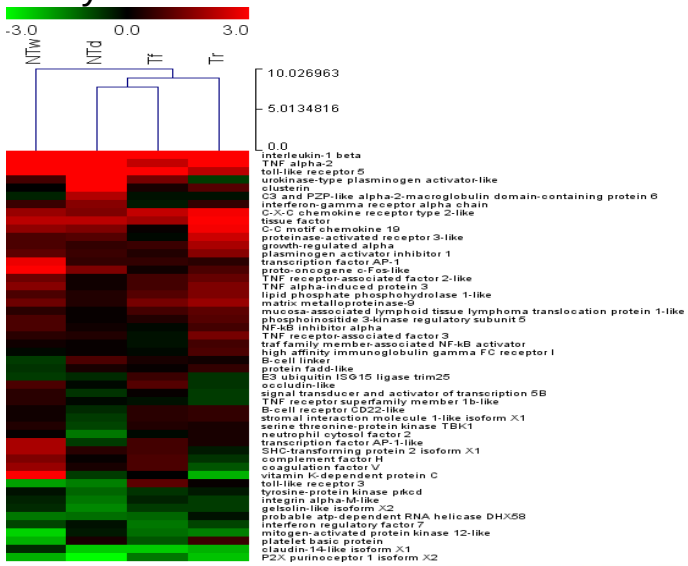
Liver on PGN



Liver on PIC



Kidney on PGN



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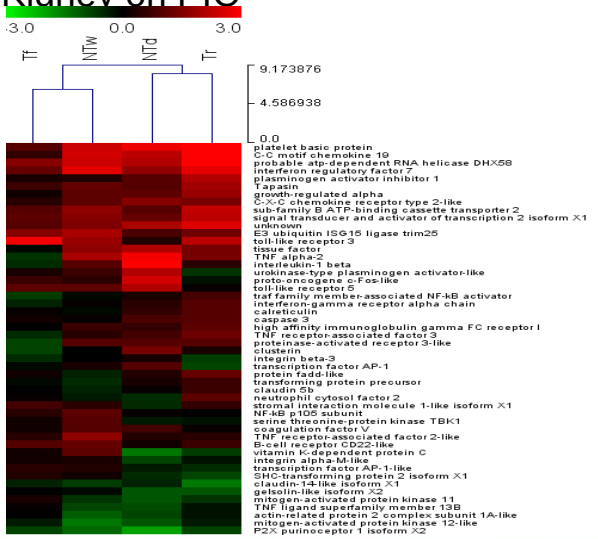
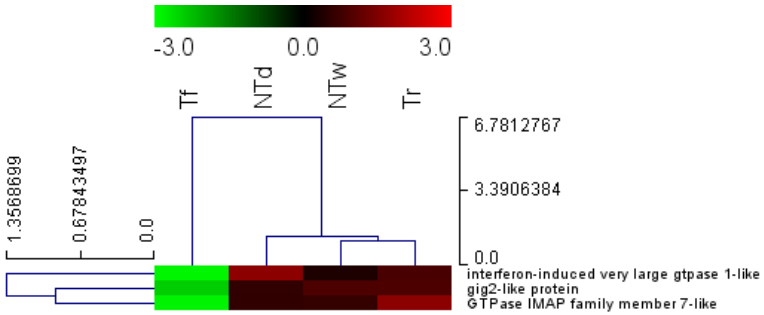
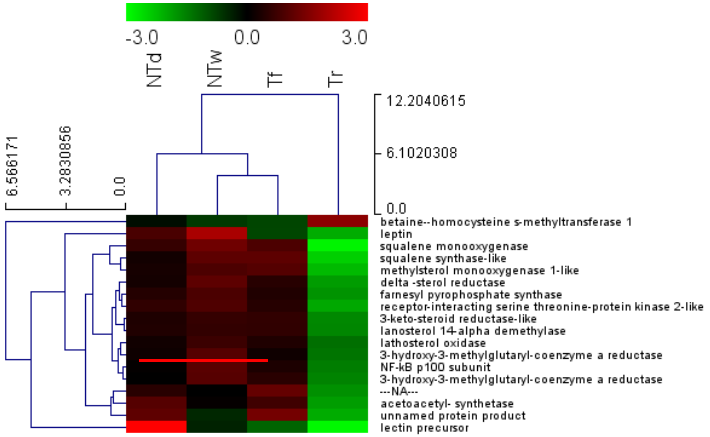


DEGs for interaction effect

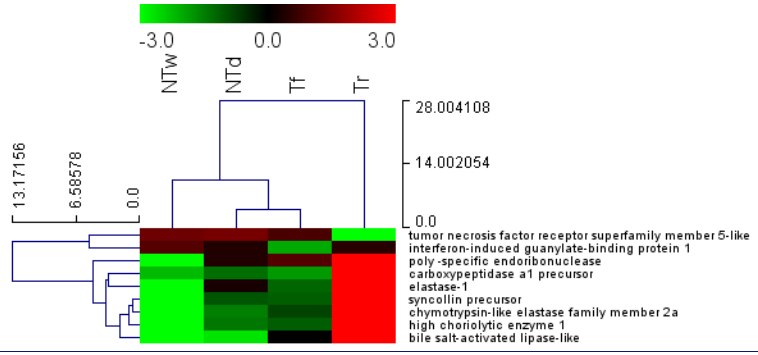
Liver on PGN



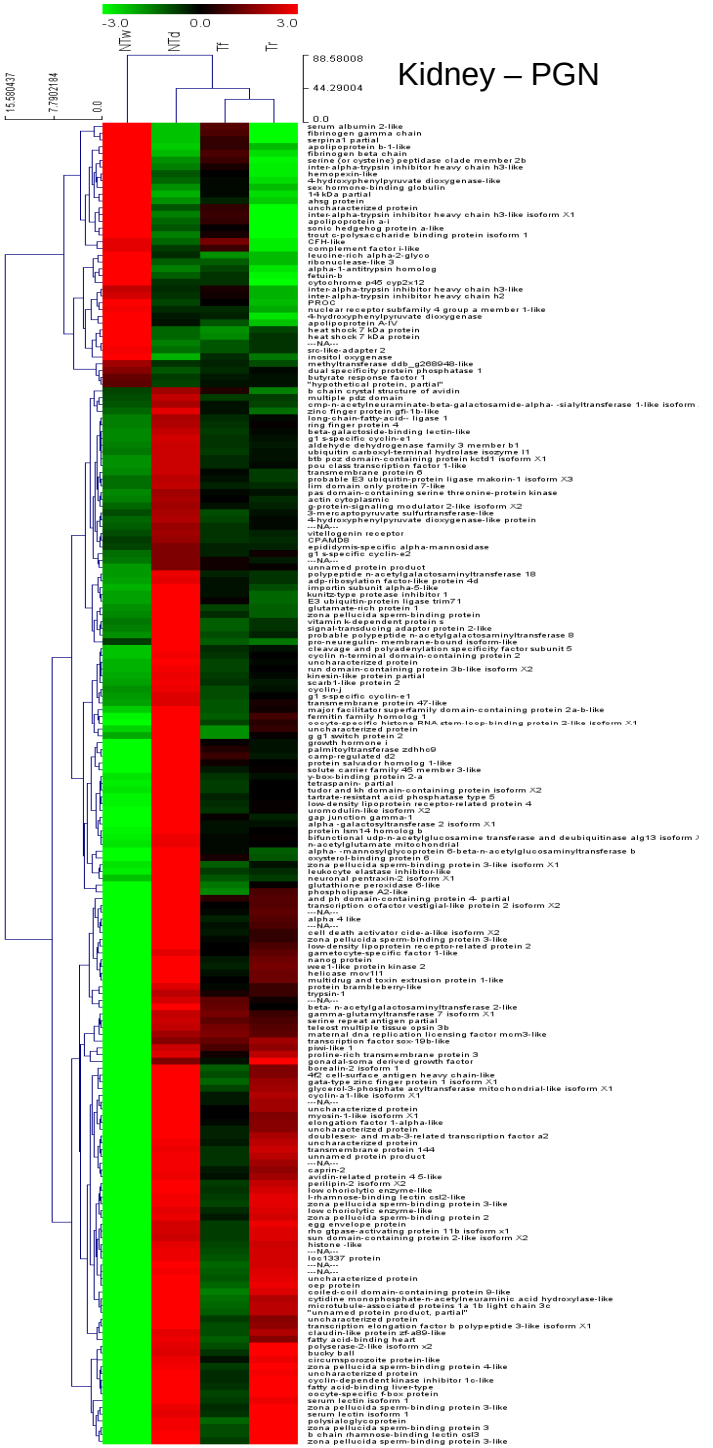
Liver on PIC



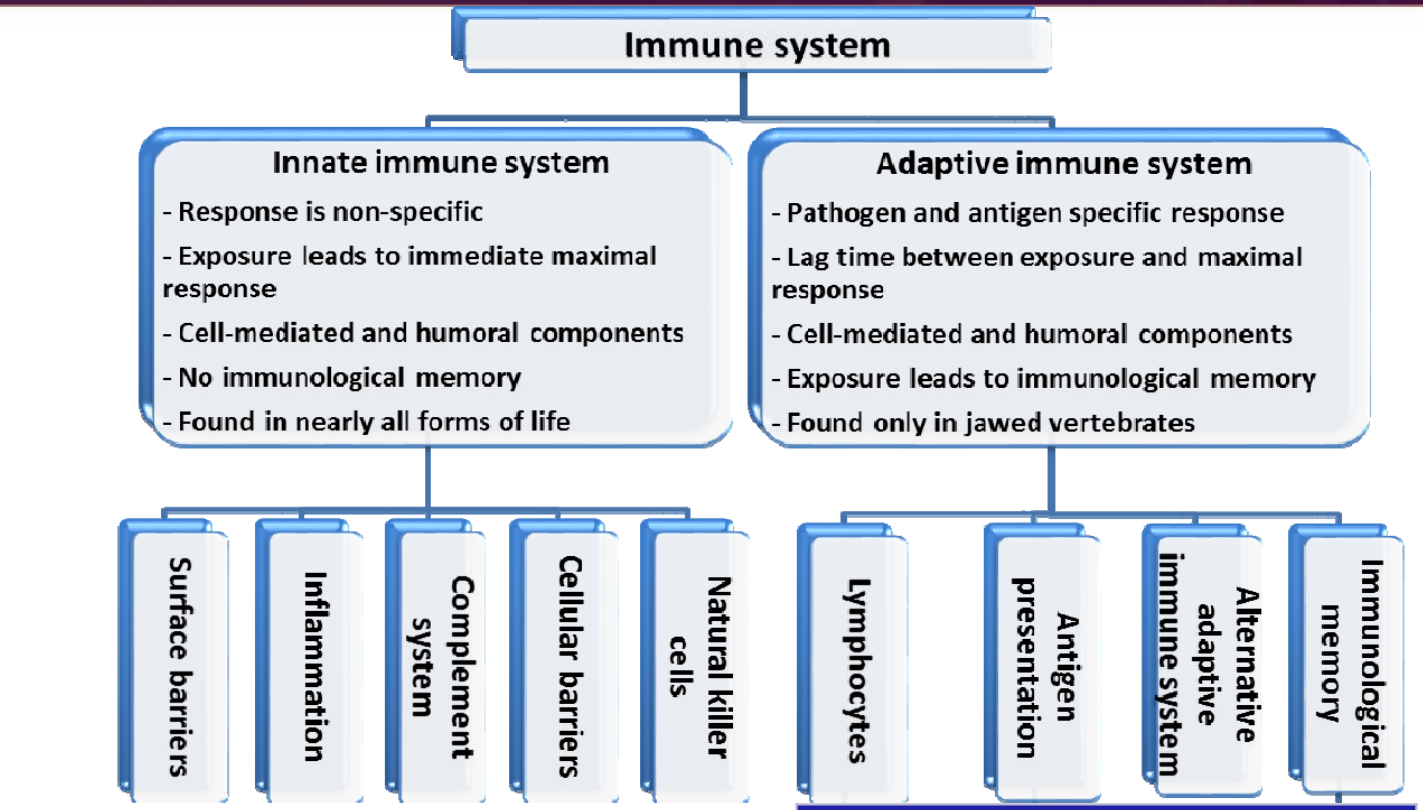
Kidney – PIC



Kidney – PGN



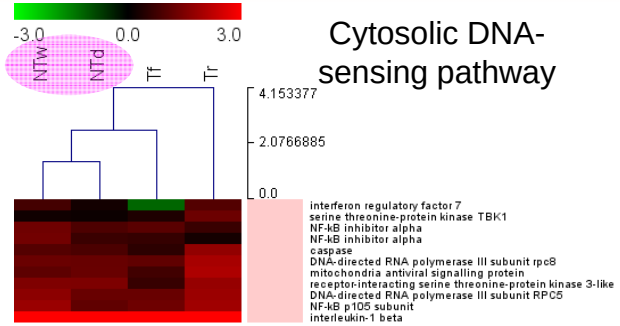
Components of the immune system



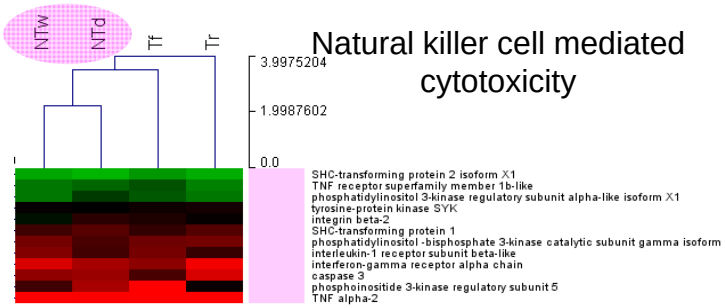
- ✓ Toll-like receptor signaling pathway
- ✓ Cytosolic DNA-sensing pathway
- ✓ Fc epsilon RI signaling pathway
- ✓ RIG-I-like receptor signaling pathway
- ✓ Hematopoietic cell lineage
- ✓ Complement and coagulation cascades
- ✓ Fc gamma R-mediated phagocytosis
- ✓ Intestinal immune network for IgA production
- ✓ Chemokine signaling pathway
- ✓ Natural killer cell mediated cytotoxicity
- ✓ NOD-like receptor signaling pathway

- ✓ Antigen processing and presentation
- ✓ T cell receptor signaling pathway
- ✓ B cell receptor signaling pathway
- ✓ Platelet activation
- ✓ Leukocyte transendothelial migration

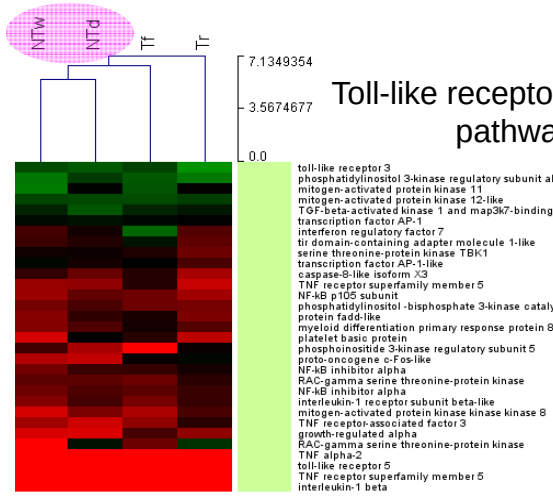
Genotype-specific KEGG pathway analysis



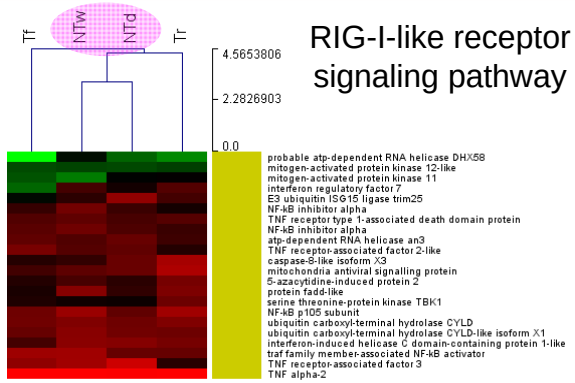
Cytosolic DNA-sensing pathway



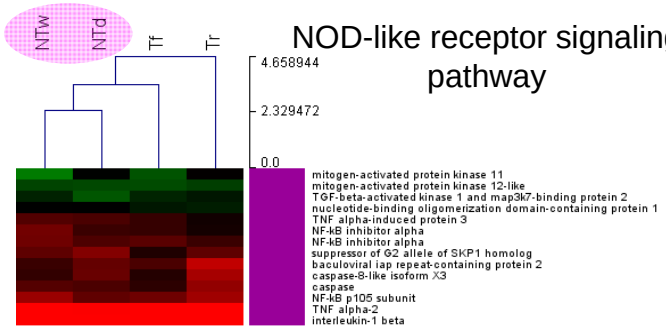
Natural killer cell mediated cytotoxicity



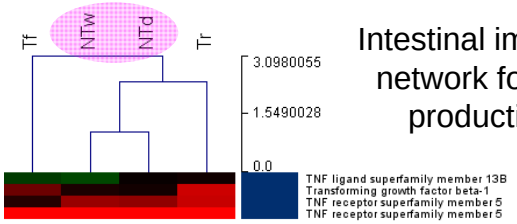
Toll-like receptor signaling pathway



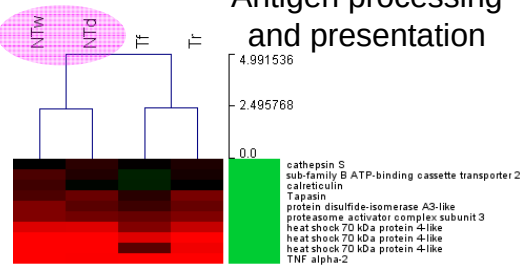
RIG-I-like receptor signaling pathway



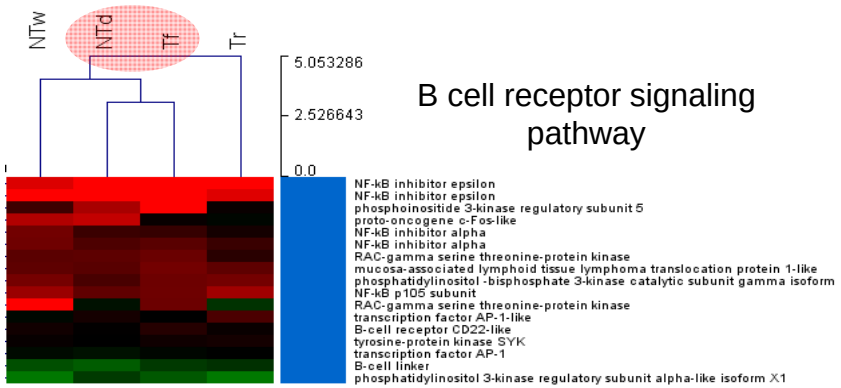
NOD-like receptor signaling pathway



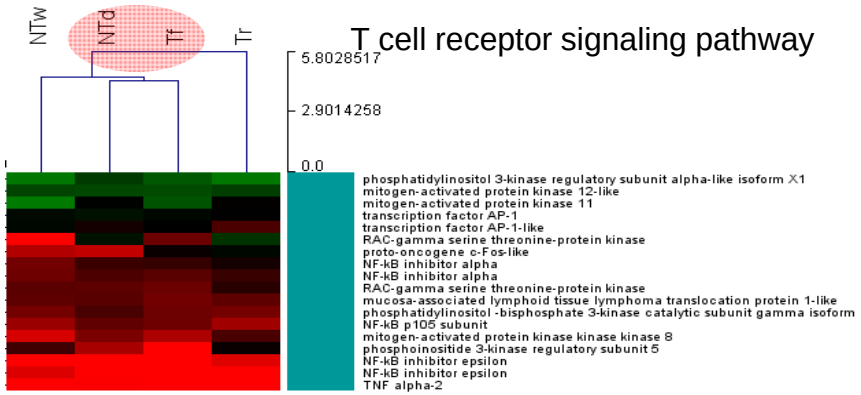
Intestinal immune network for IgA production



Antigen processing and presentation

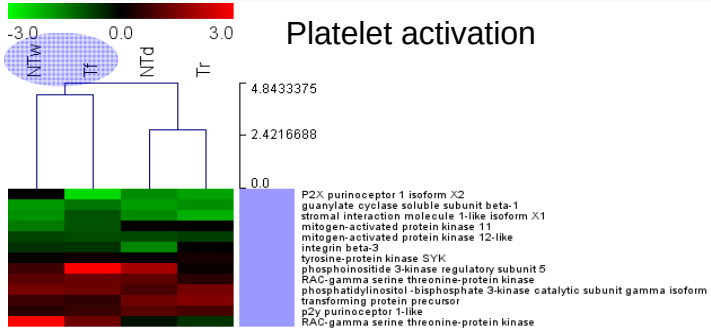


B cell receptor signaling pathway

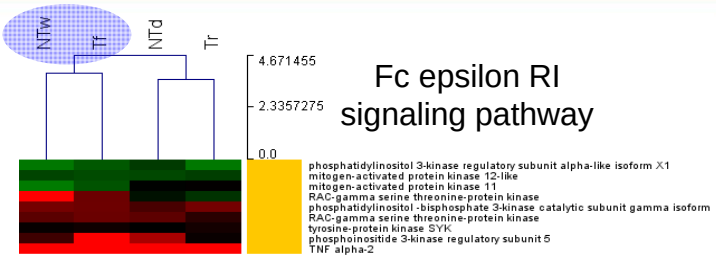


T cell receptor signaling pathway

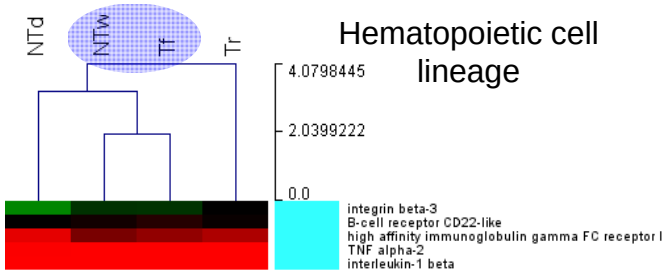
Platelet activation



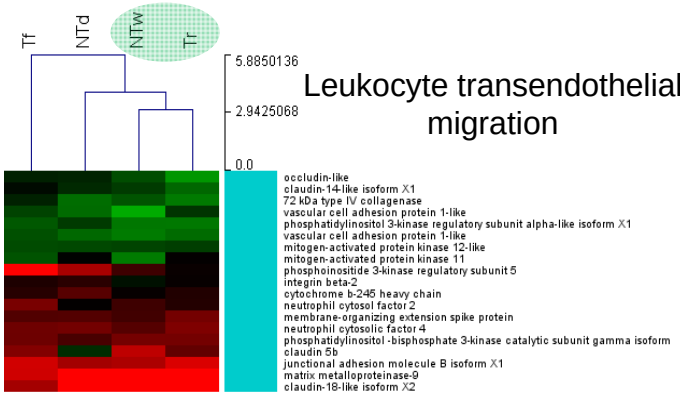
Fc epsilon RI signaling pathway



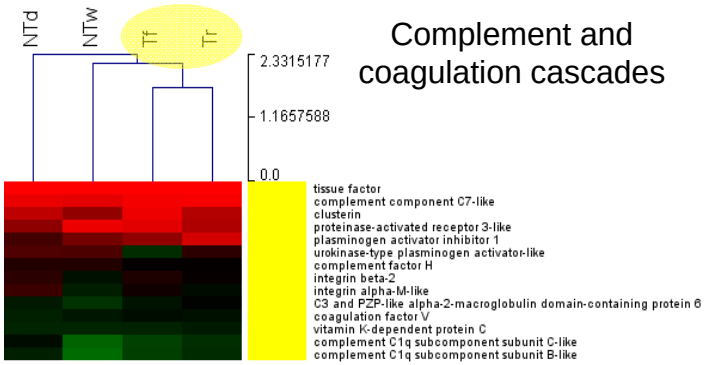
Hematopoietic cell lineage

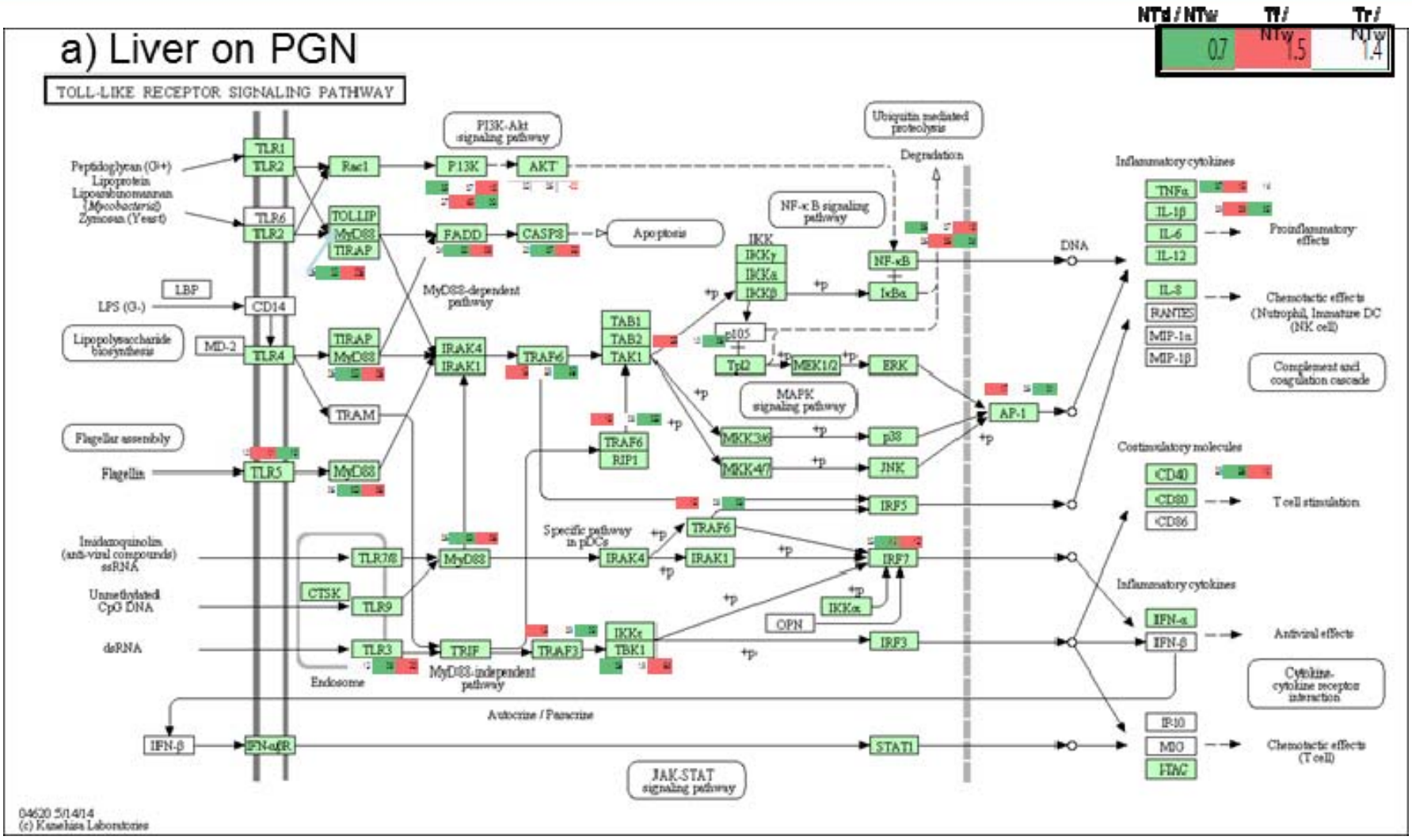


Leukocyte transendothelial migration

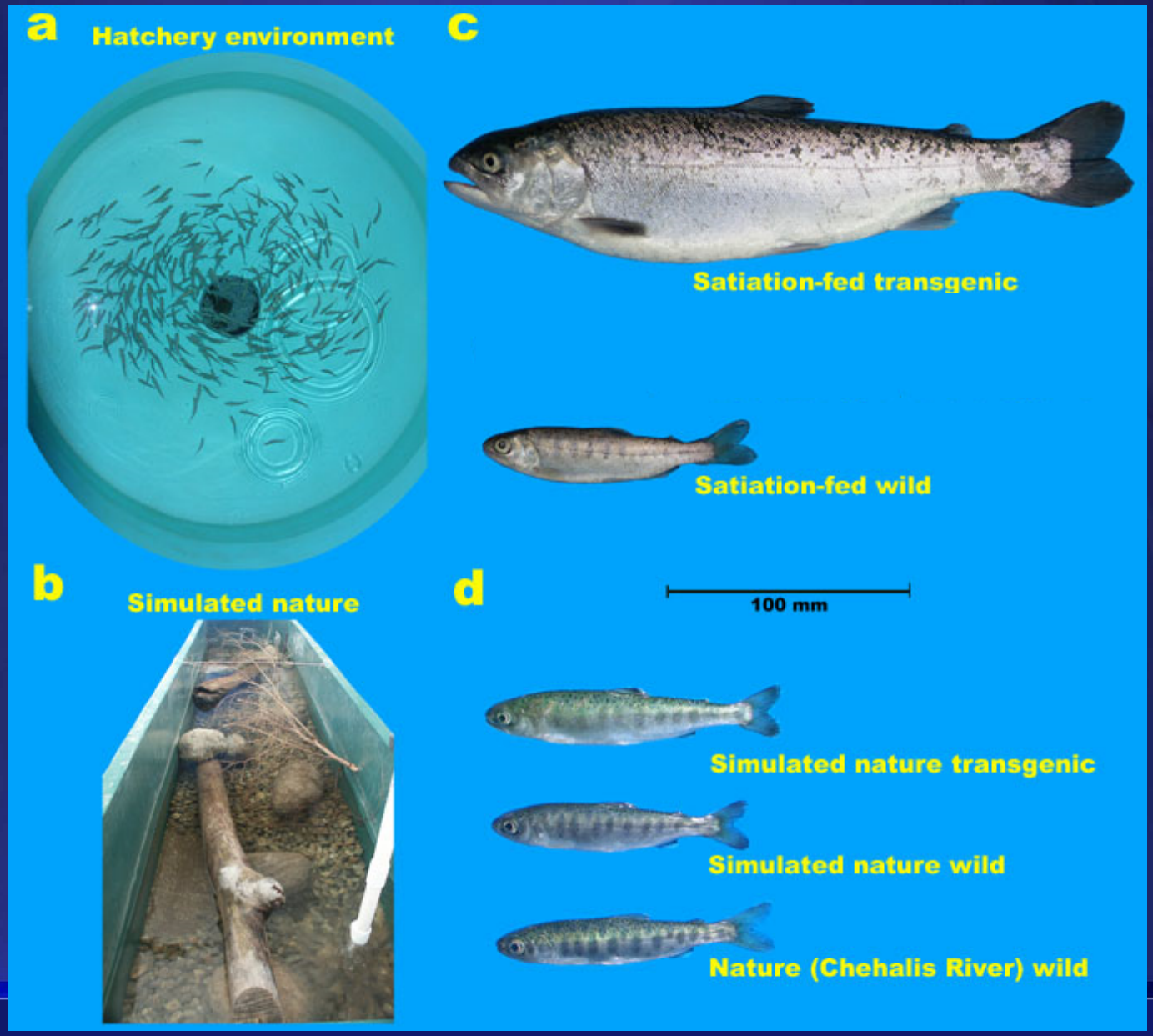


Complement and coagulation cascades

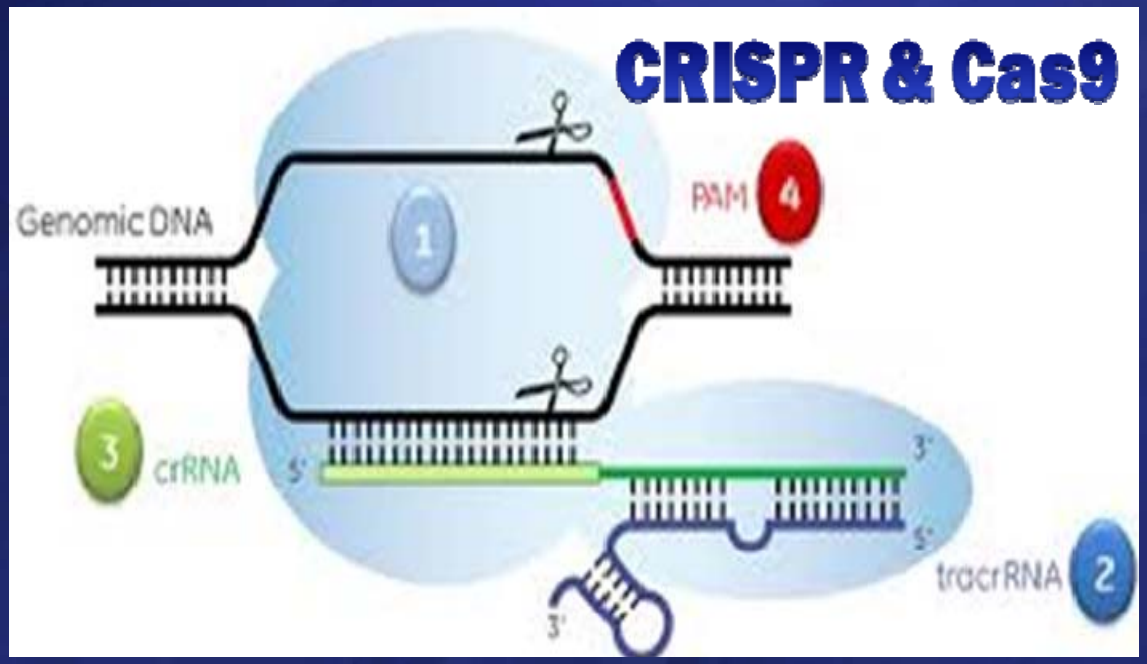



a) Liver on PGN
TOLL-LIKE RECEPTOR SIGNALING PATHWAY
NTd / NTw
Tf / NTw
Tr / NTw
0.7
1.5
1.4
Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan (Mycobacteria)
Zymosan (Yeast)
LPS (G-)
Lipopolysaccharide biosynthesis
Flagellar assembly
Flagellin
Imidazoquinolin (anti-viral compounds) ssRNA
Unmethylated CpG DNA
dsRNA
LBP
MD-2
CD14
TLR1
TLR2
TLR6
TLR4
TLR5
TLR7/8
TLR9
TLR3
TOLLIP
MyD88
TIRAP
TRAM
CTSK
Endosome
Rac1
PI3K
AKT
PI3K-Akt signaling pathway
FADD
CASP8
Apoptosis
MyD88-dependent pathway
IRAK4
IRAK1
TRAF6
TAB1
TAB2
TAK1
TRAF6
RIP1
TRIF
TRAF3
MyD88-independent pathway
Specific pathway in pDCs
IRAK4
IRAK1
TRAF6
IKKε
TBK1
IKKα
OPN
IRF7
IRF3
IRF5
Ubiquitin mediated proteolysis
Degradation
NF-κB signaling pathway
IKK
IKKγ
IKKα
IKKβ
NF-κB
IκBα
p105
Tpl2
MEK1/2
ERK
MAPK signaling pathway
MKK3/6
p38
MKK4/7
JNK
AP-1
DNA
Inflammatory cytokines
TNFα
IL-1β
IL-6
IL-12
Proinflammatory effects
IL-8
RANTES
MIP-1α
MIP-1β
Chemotactic effects (Neutrophil, Immature DC (NK cell)
Complement and coagulation cascade
Costimulatory molecules
CD40
CD80
CD86
T cell stimulation
Inflammatory cytokines
IFN-α
IFN-β
Antiviral effects
Cytokine-cytokine receptor interaction
IP10
MIG
ITAC
Chemotactic effects (T cell)
Autocrine / Paracrine
IFN-β
IFN-αβR
STAT1
JAK-STAT signaling pathway
04620 5/14/14
(c) Kanehisa Laboratories

Growth enhancement phenotype is reduced in naturalized vs. hatchery conditions



Study for biological containment

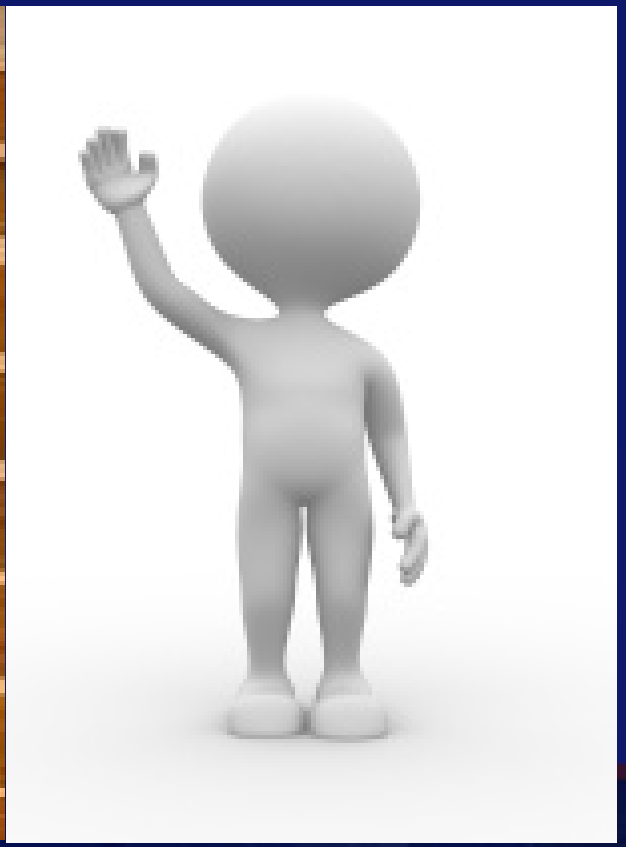


Sterilization for
Luteinizing hormone

Summary

- Transgenesis has the potential to dramatically alter the phenotype
- The intended immune responses were strongly activated. There was evidence for major differences in mRNA expression responses of different fish groups according to immune treatment (pathogen-associated molecular patterns - PAMPs).
- No significance of immune gene response by transgene effects
- The results can be used to inform us about the likely resistance of the groups to challenges with actual pathogens by helping to understanding of host-pathogen interactions.


WHO IS RESPONSIBLE
FOR GMO SAFETY
TESTING?
MONSANTO
USDA
FDA



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Thank You