

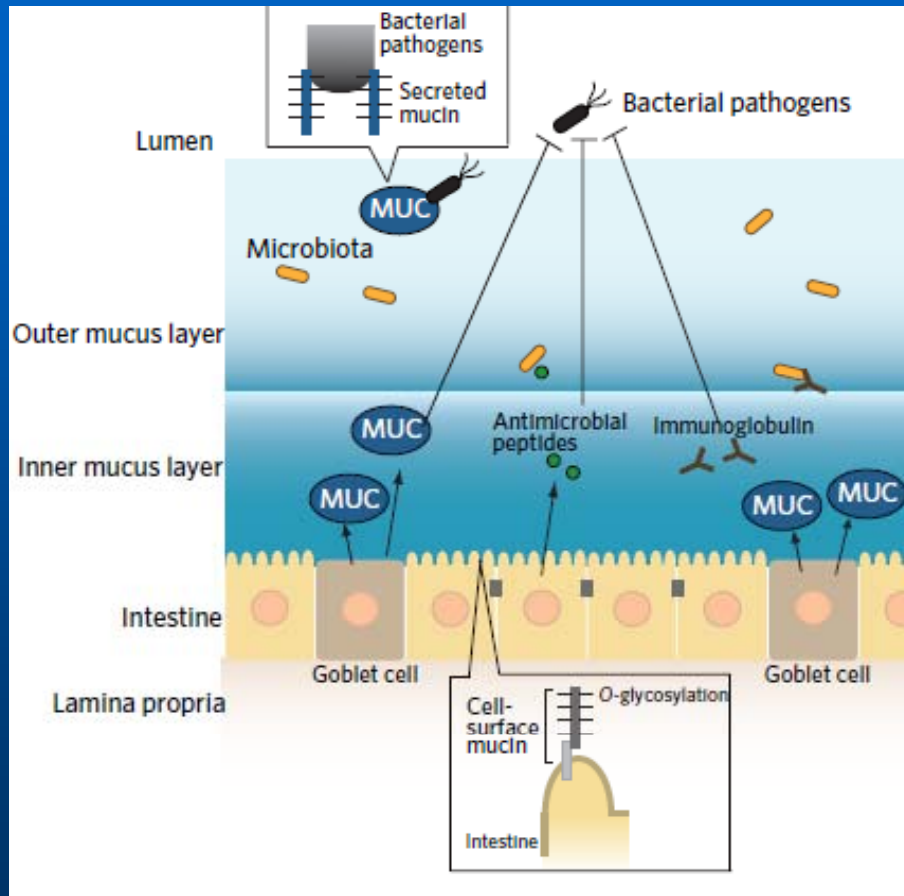
# Genomic Signatures of Intestinal Barrier Disruption & Pathogen Entry after Bacterial Infection

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# Mucosal Immune Responses



*Hiroshi Ashida et al. 2012*

Enteric pathogens

Bacterial stratagems

*Salmonella spp.*

Breach cell-cell junction

*Shigella spp.*

Disruption of mucus layer

*Vibrio cholerae*

Disruption of mucus layer

EPEC

Epithelial cell death and shedding

EHEC

Disruption of mucus layer



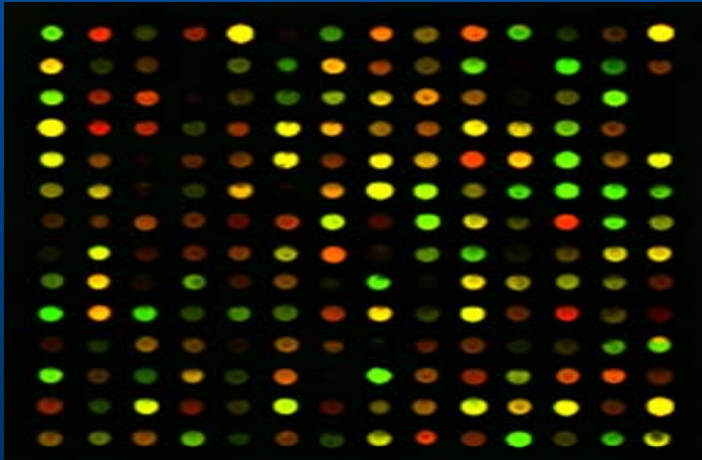
# ESC



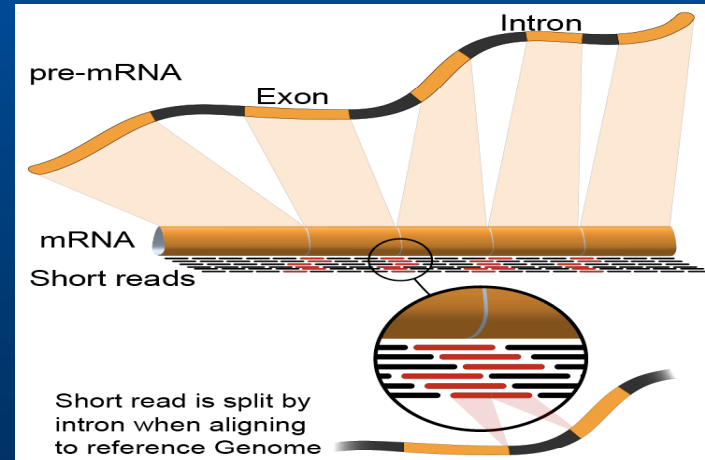
[http://cnre.vt.edu/fisheries/afs/fisheries\\_techniques/Chapter14/Enteric%20septicemia%20on%20a%20c atfish.jpg](http://cnre.vt.edu/fisheries/afs/fisheries_techniques/Chapter14/Enteric%20septicemia%20on%20a%20c atfish.jpg)



<http://namufigu.tripod.com/pictures-of-septicemia.html>



<http://www.imbb.forth.gr/people/poirazi/researchEP.html>

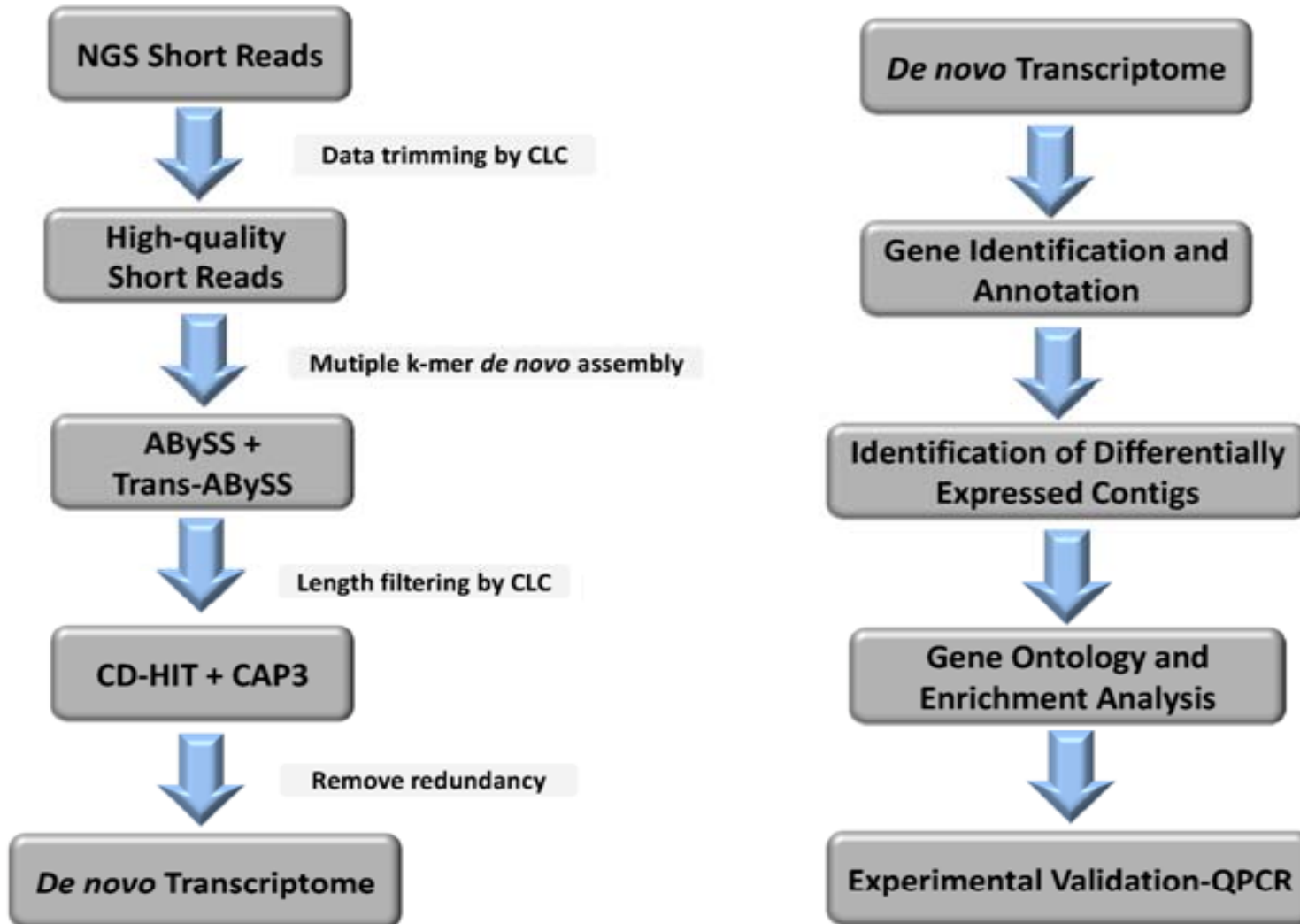


<http://en.wikipedia.org/wiki/File:RNA-Seq-alignment.png>

# Research Goals

- **RNA-seq to characterize differentially expressed genes after *ESC disease* challenge**
- **Understand the elements of catfish mucosal immune responses**

# Research Methods



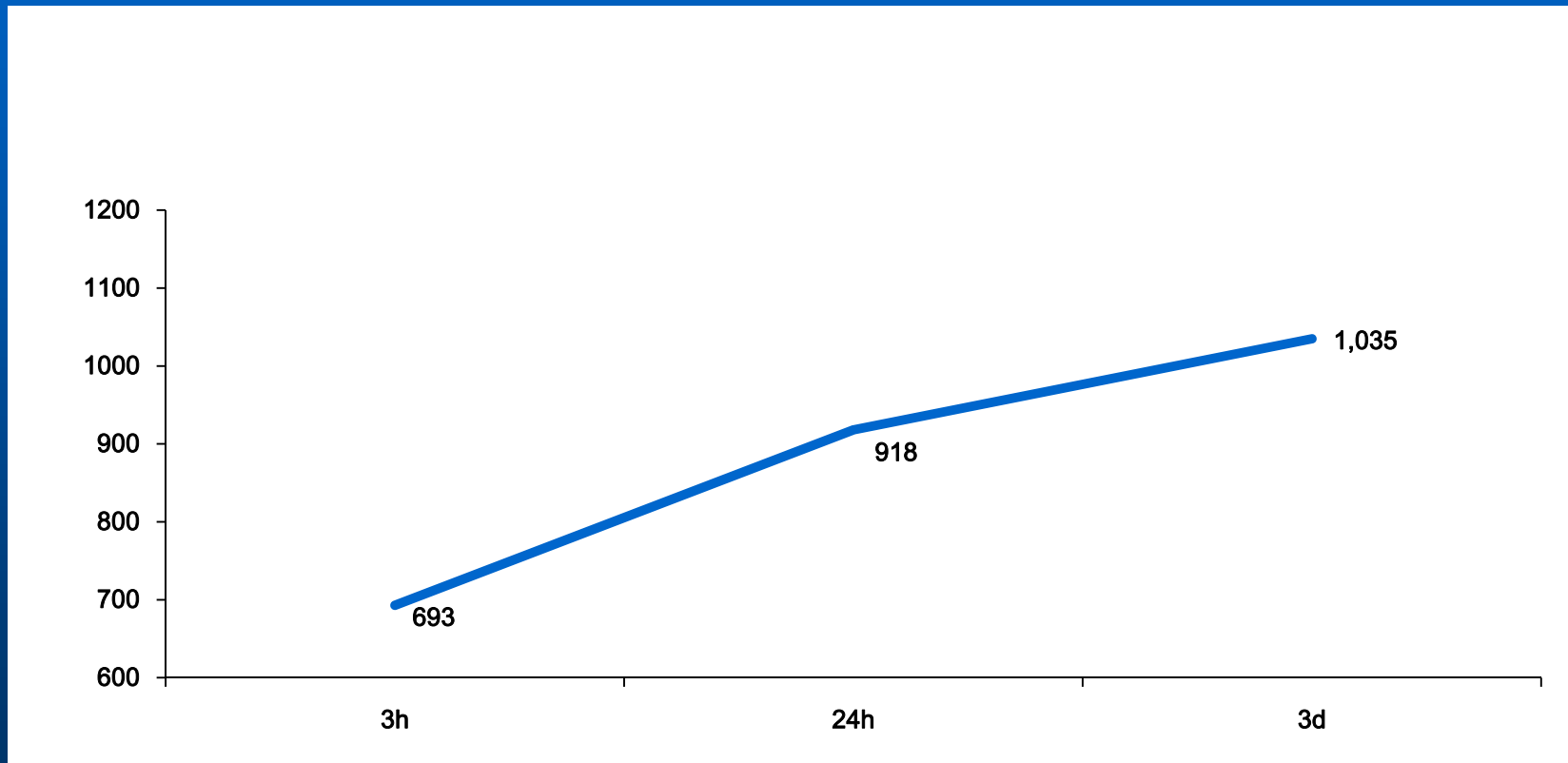
# Assembly Results

	Trans-ABYSS
Contigs( $\geq 100$ bp)	630,209
Large contigs ( $\geq 1000$ bp)	140,357
Maximum length (bp)	17,585
Average length (bp)	725.1
N50 (bp)	1,676
Contigs after length filtering( $\geq 200$ bp)	376,005
Percentage contigs kept after length filtering	59.66%
Average contig length after length filtering (bp)	1,120
Contigs (After CD-HIT-EST+ CAP3)	176,481
Average length (bp) (After CD-HIT-EST+ CAP3)	893.7
Reads mapped to final reference (%)	88.42%

# Annotation Results

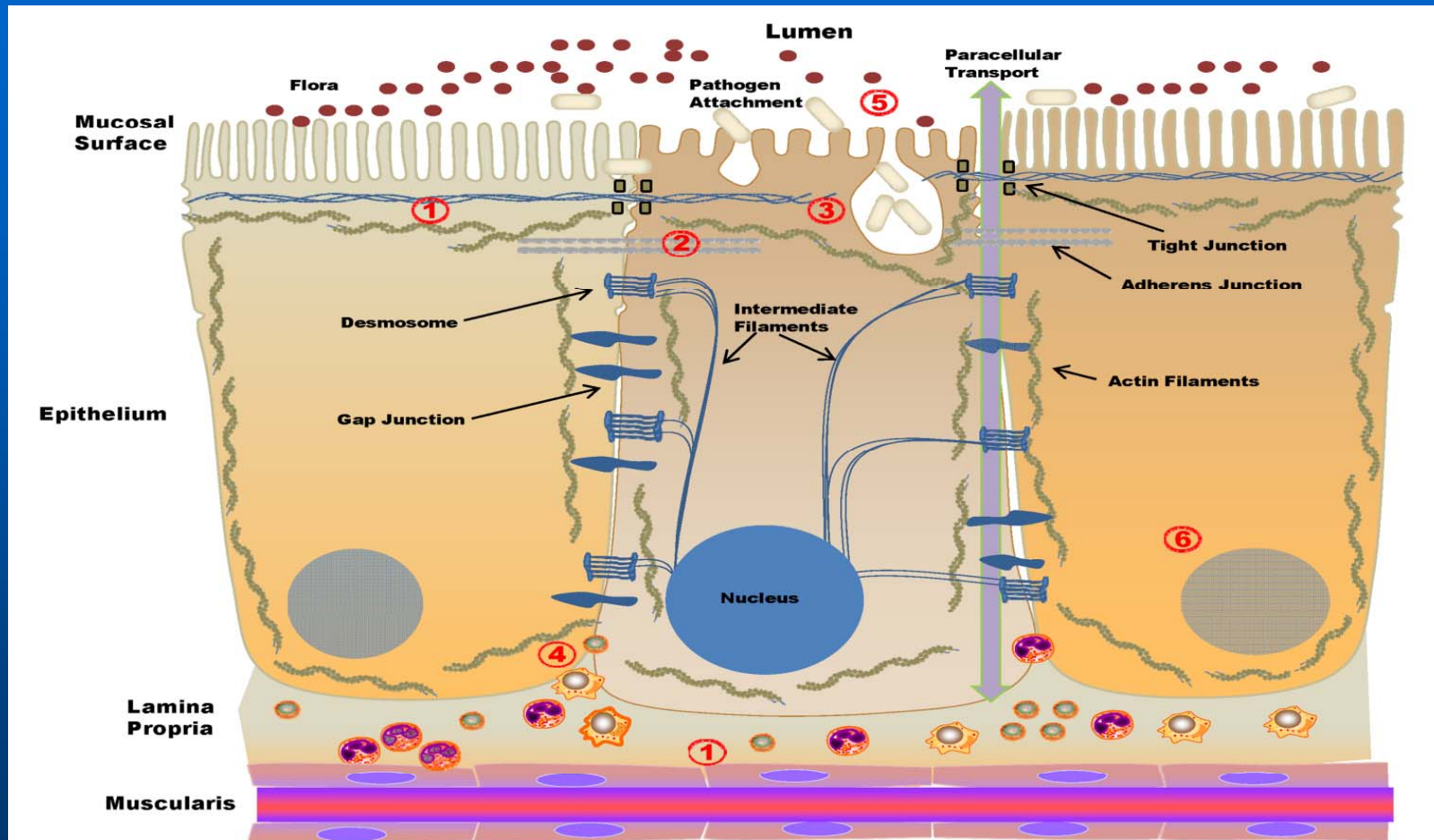
	Contigs with putative gene matches	Annotated contigs $\geq 500\text{bp}$	Annotated contigs $\geq 1000\text{bp}$	Unigene matches	Quality Unigene matches
Zebrafish	73,330	37,500	14,868	15,640	14,457
UniProt	55,551	42,524	31,136	17,476	15,786
NR	77,577	51,215	34,742	23,205	19,960

# Differentially Expressed Genes



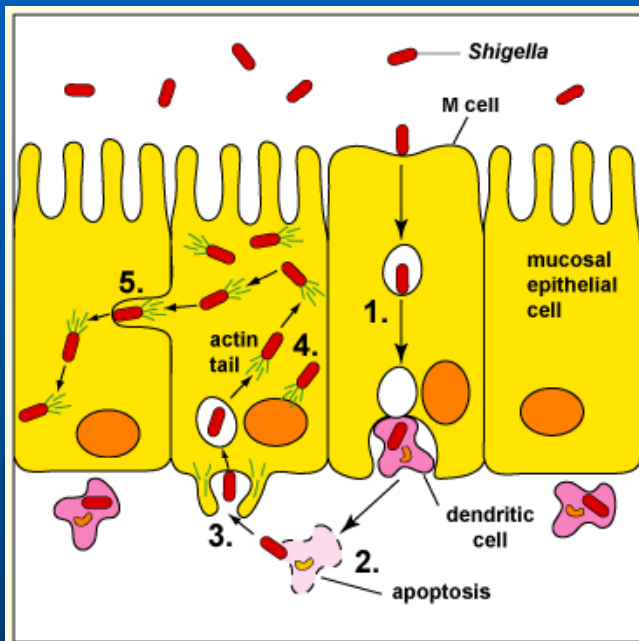


# Intestinal mucosal barrier



- ① Cytoskeletal and muscle fiber dynamics; ② Junctional modification and disruption;  
③ Lysosome/phagosome responses; ④ Immune activation and inflammation;  
⑤ Attachment and pathogen recognition; ⑥ Endocrine/growth disruption.

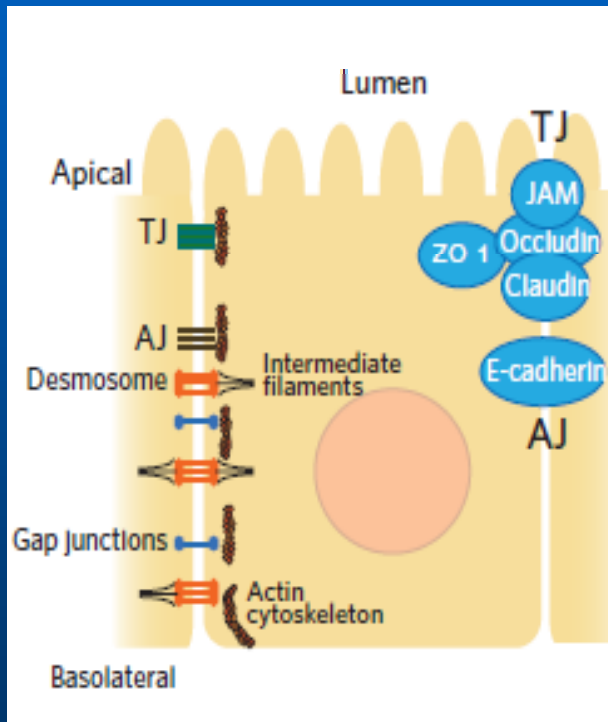
# Cytoskeletal/Muscle Fiber Dynamics



[http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit2/bacpath/Shig\\_invas\\_mcell.html](http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit2/bacpath/Shig_invas_mcell.html)

Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Cytoskeletal/ Muscle Fiber Dynamics	AHNAK nucleoprotein	Contig22801	<u>7.27</u>	<u>9.02</u>	<u>8.73</u>
	Arp2/3	Contig17702	-1.62	-1.98	-2.17
	CDC42SE2	Contig11032	-5.54	-1.37	-1.22
	Ezrin like	Contig20491	1.21	-4.2	-2.76
	Filamin A, alpha	Contig476	1.98	2.18	2.31
	Gelsolin-like (CAPG)	k70_862348	<u>6.93</u>	<u>5.78</u>	<u>7.3</u>
	Myosin heavy chain	Contig14966	<u>-72.4</u>	<u>-21.66</u>	<u>-43.6</u>
	Small GTPase RhoA	Contig4251	-1.43	-1.73	-3.23
	Supervillin-like, partial	Contig12472	4.82	2.9	4.18

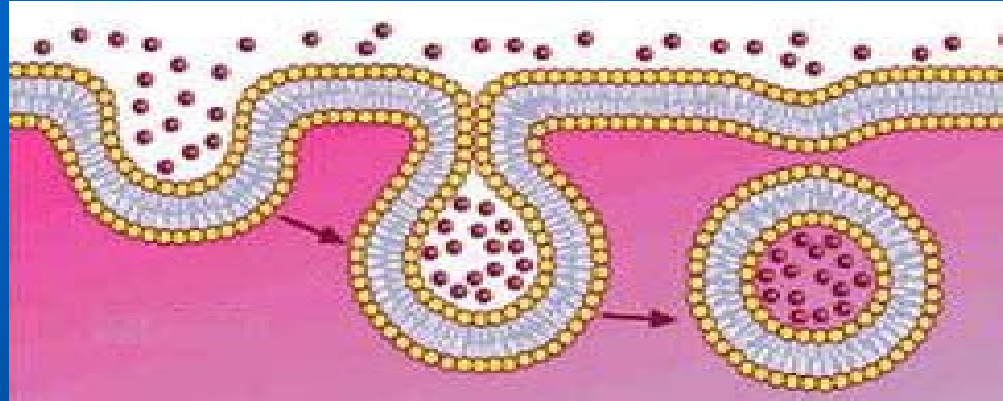
# Junctional Modification/Disruption



Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Junctional Modification/Disruption	Aquaporin 8	Contig14861	1.35	3.02	6.42
	Claudin 15a	k65_774609	<u>-3.28</u>	<u>-3.09</u>	-1.26
	Claudin-9	Contig16617	<u>-2.31</u>	-1.24	-1.11
	Desmocollin 2	Contig26678	3.39	3.47	3.67
	Desmoglein-2	Contig6653	2.18	2.83	1.95
	Desmoplakin	Contig14264	1.62	2.16	2.36
	Occludin-like	Contig1340	1.48	1.86	1.32
	Plakophilin 3	Contig27202	1.45	1.6	1.38
	Zonadhesin	Contig19706	2.39	1.3	1.59

*Hiroshi Ashida et al. 2012*

# Lysosome/Phagosome Patterns



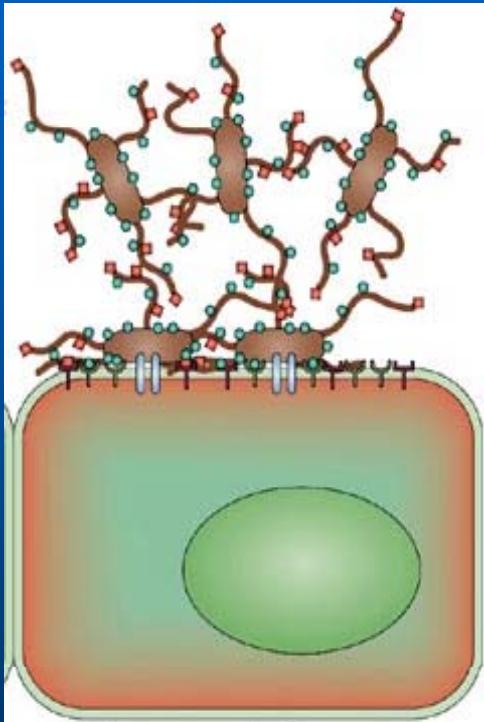
<http://www.cuh.org.uk/addenbrookes/services/clinical/lysosomal/disorders/gaucher.html>

Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Lysosome/ Phagosome	Cathepsin Z	Contig3027	9.78	6.36	15.49
	CD209 antigen (DC-SIGN)	Contig3473	-1.64	-2.84	-2.43
	Lysosomal glycoprotein 2	Contig18023	2.95	2.21	1.01
	Lysosomal protective protein	Contig3023	2.21	1.58	1.53
	NADPH oxidase 1	Contig38009	-2.98	-4.55	-3.21
	NOXO1	Contig4675	-2.68	-7.35	-6.47

# Immune Activation/Inflammation

Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Immune/ Activation Inflammation	C1Q component-binding protein	k66_875137	-9.95	-1.81	-1.45
	CC chemokine SCYA102	Contig10839	1.37	-3.27	-1.28
	CC chemokine SCYA104-like	Contig37889	1.02	-4.19	-3.8
	Chemokine CXCL12	Contig160	2.55	2.51	2.41
	Chemokine CXCL2	Contig22481	1.23	1.45	3.53
	Jun D proto-oncogene-like	k70_513305	6.6	4.59	2.3
	LEAP-2	Contig14731	-2.1	-2.3	-1.44
	Macrophage MIF	Contig40086	3.96	9.6	4.4
	Nattectin precursor	Contig16280	<u>-30.08</u>	<u>-12.82</u>	<u>-7.71</u>
	Neurotoxin/C59/Ly-6-like protein	k71_580318	-1.58	-3.86	-5.96
	NF-kappa-B p100 subunit	Contig2282	2.61	2.2	3.71
	Novel immune-type receptor 7	Contig39943	4.17	3.83	4.56
	Prostaglandin E synthase 3	k50_966012u	5.18	5.39	4
	Serum amyloid P component-like 2	Contig32897	-3.07	-2.42	-3.42
	Suppressor of cytokine signaling 1	Contig38899	3.08	-1.95	-1.85
	TNF receptor member 1a	k78_684621	7.09	4.71	7.89
	Tumor protein p53 protein 1	Contig15021	<u>1.7</u>	<u>-10.06</u>	<u>-5.06</u>

# Attachment/Pathogen Recognition



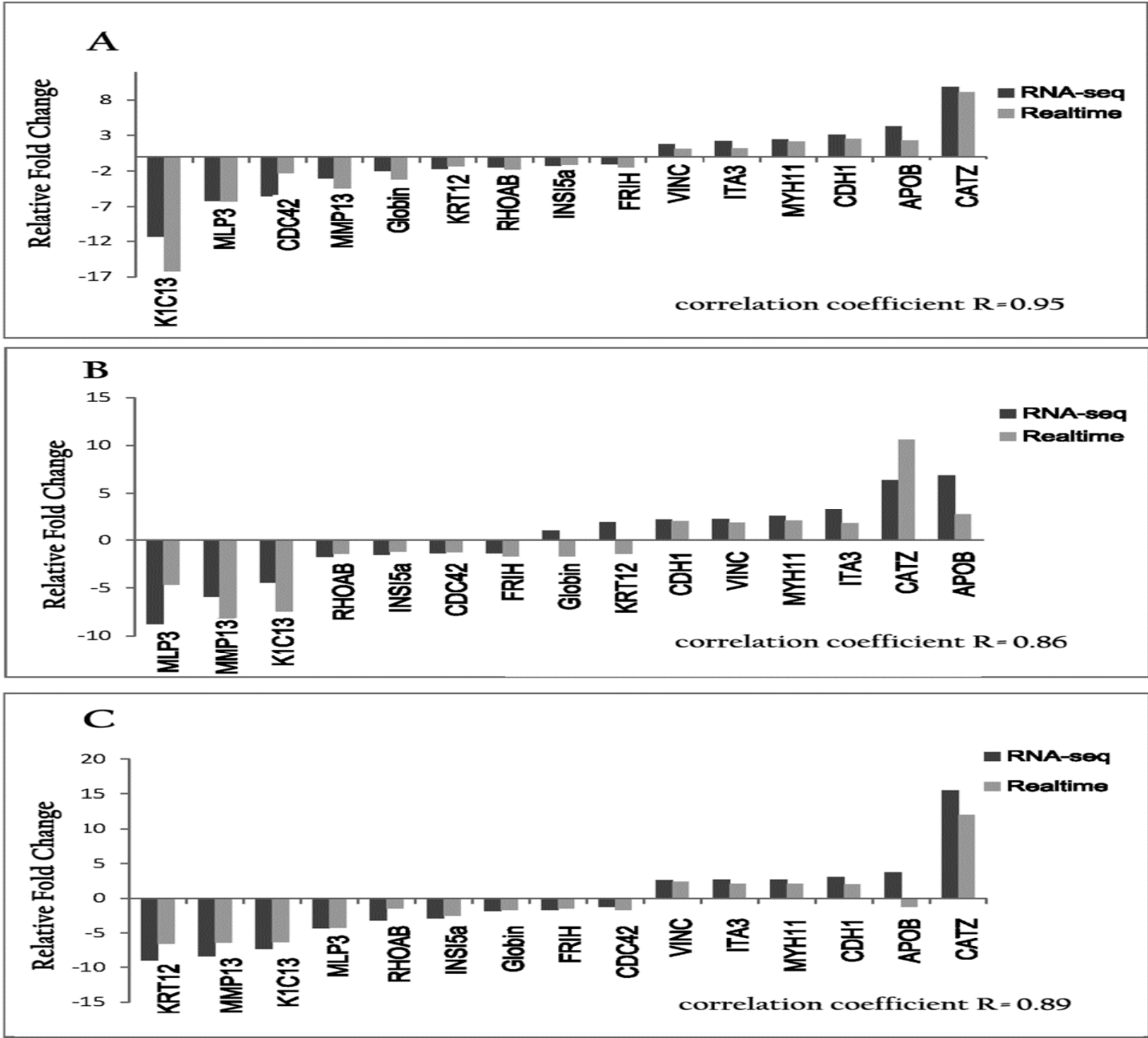
Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Attachment/ Pathogen Recognition	Integrin, beta 1b	k50_930983	2.19	2.17	2.32
	Mucin 2-like	Contig16584	4.42	5.67	5.17
	NLRC like-7	Contig14252	<u>4.24</u>	<u>7.2</u>	<u>9.36</u>
	NOD-3 like	k_93466446	2.5	2.28	2.32
	Podocan-like	Contig8588	13.42	6.6	3.53
	TLR 5	Contig14983	<u>1.06</u>	<u>1.51</u>	<u>2.06</u>

[http://www.nature.com/nrmicro/journal/v4/n7/fig\\_tab/nrmicro1443\\_F5.html](http://www.nature.com/nrmicro/journal/v4/n7/fig_tab/nrmicro1443_F5.html)

# Endocrine/Growth Disruption

Functional classification	Gene Name	Contig ID	3h fold change	24h fold change	3d fold change
Endocrine/ Growth Disruption	Ghrelin	Contig41751	<u>-3.19</u>	-1.42	1.06
	Igfbp7	Contig7012	2.24	-1.02	1.45
	Insulin receptor a	Contig11984	6.15	7.47	6.42
	Insulin-induced gene 1	Contig25801	1.84	-1.77	-3.08
	Peptide Y-like	k_83558074	<u>-5.11</u>	<u>-1.52</u>	<u>-2.5</u>
	Relaxin-3	Contig20841	-1.26	-1.52	-2.92

# qPCR Validation





# Conclusions

- **This is the first study of transcriptomic responses in the intestine after challenge with ESC**
- **A large number of 1,633 differentially expressed genes were identified**
- **Novel patterns of teleost mucosal gene expression were observed, which highlighted unexpected roles for some candidate genes and pathways that are often missed in previous studies**
- **This study sets the foundation for follow up studies of the genes involved in ESC immune response and disease resistance**

# Thanks

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