

A transcriptomic approach in search of disease resistance in endangered black abalone

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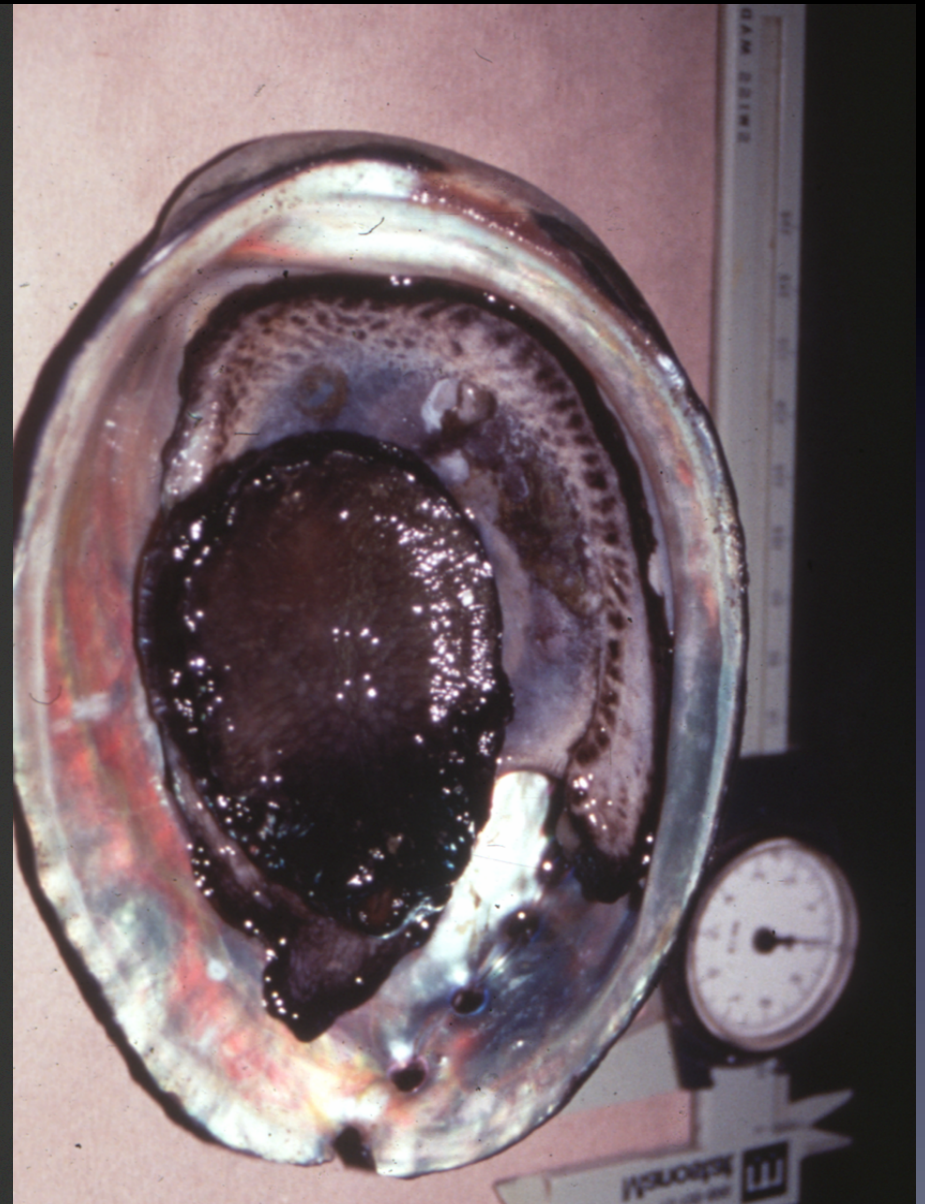
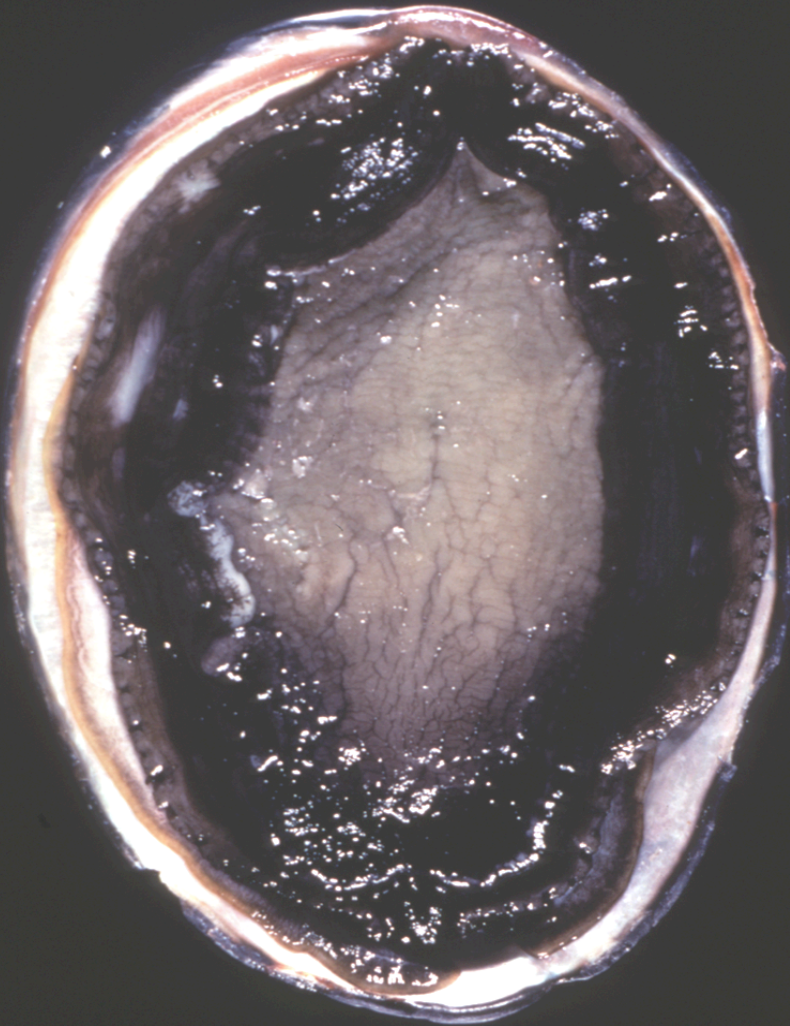


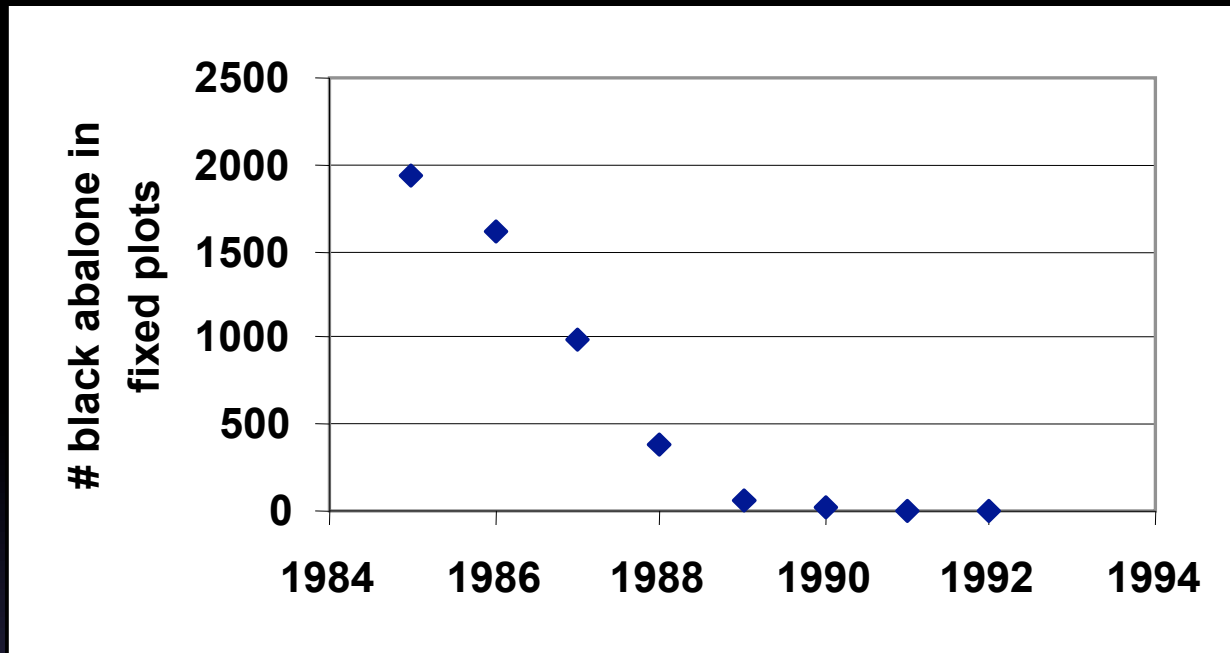
Outline

- Abalone/rickettsia interaction
- Abalone disease challenge
- Next Generation Sequencing
- Preliminary results
- Future work



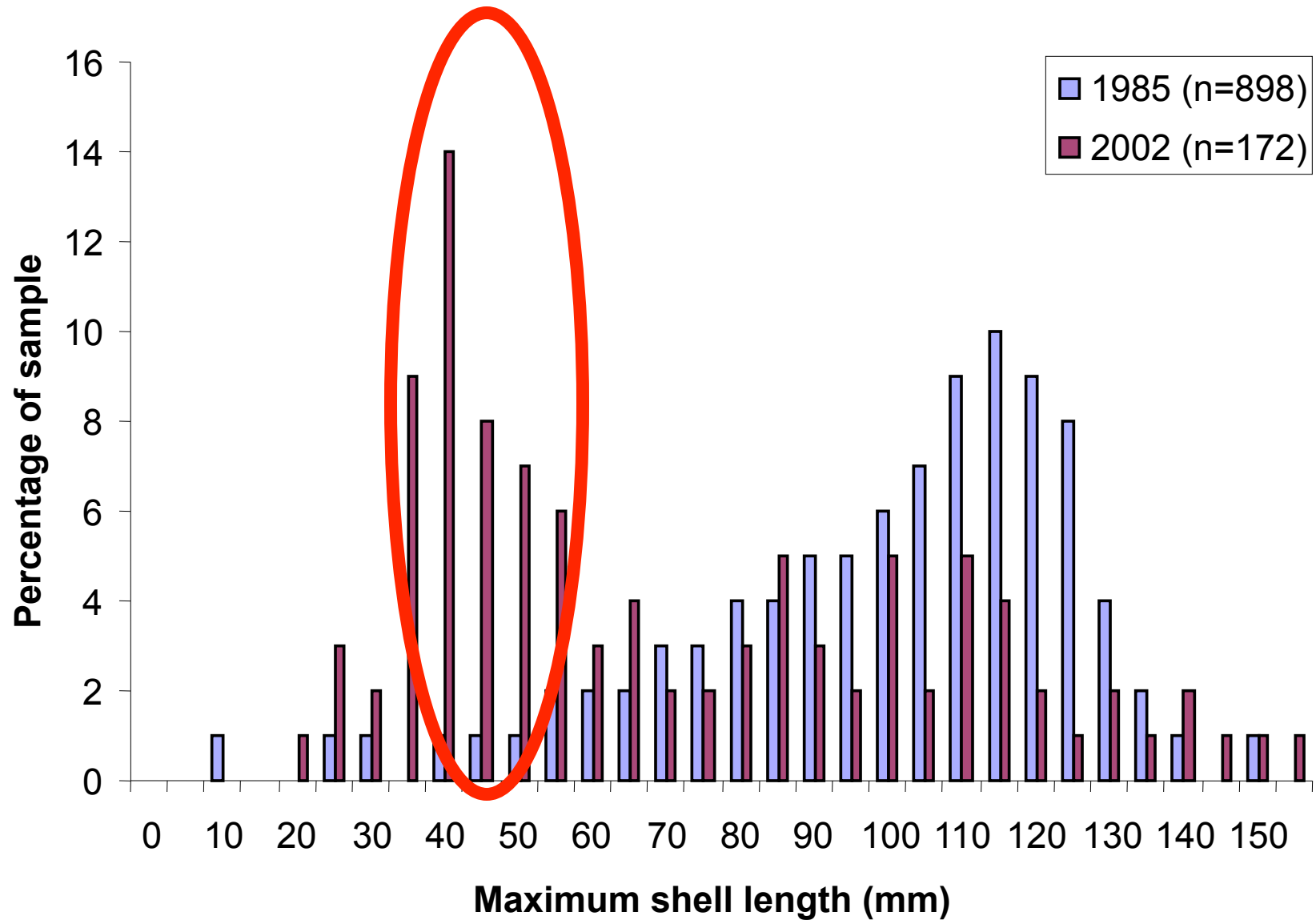
Withering Syndrome (WS)





- Affects multiple species of wild and cultured abalone
 - 99% mortality in wild black abalone
 - Federally listed as “ENDANGERED” in 2009
- Threatens abalone restoration programs
 - Current culture within endemic zone

Black abalone size frequencies, San Nicolas Island



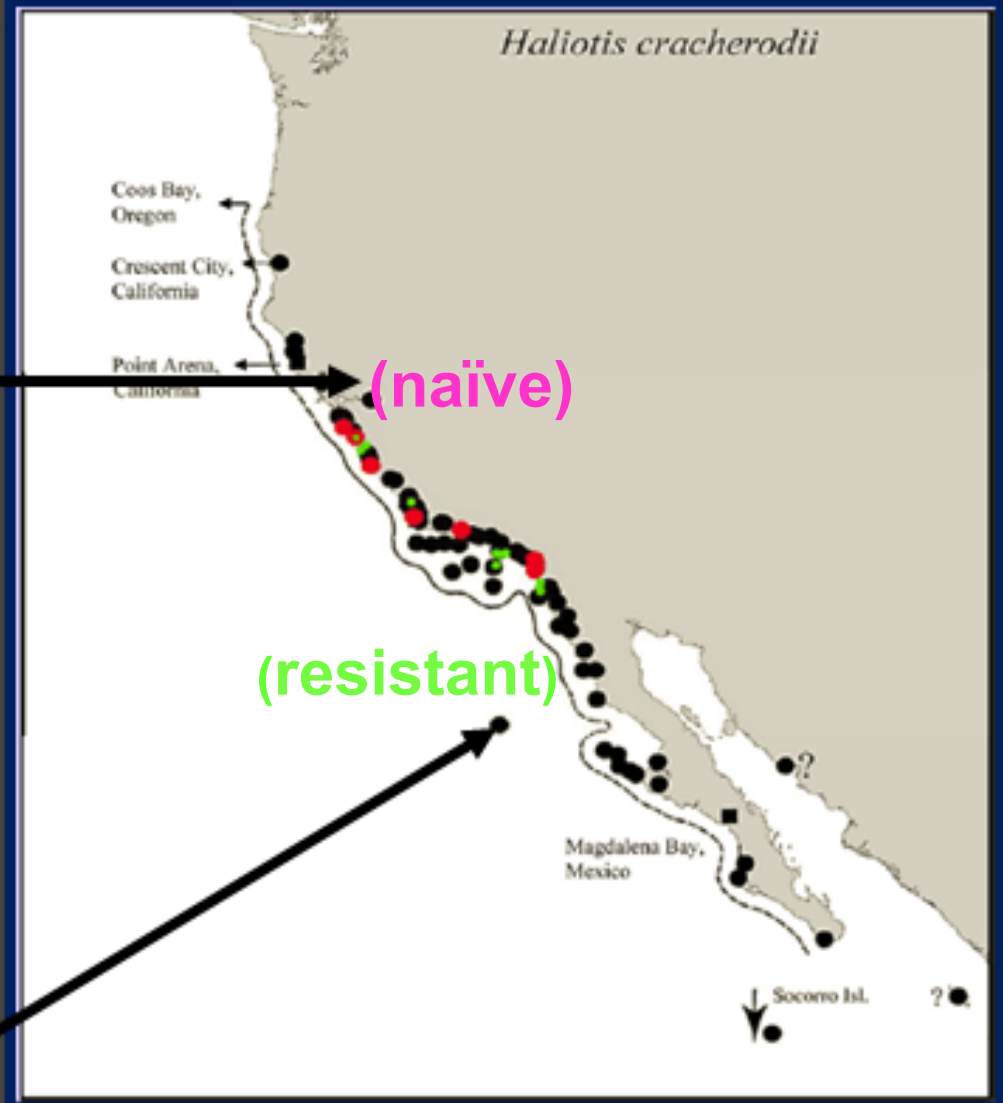
Are progeny of the surviving black abalone more resistant to WS than are naïve animals?

2006-2007 Study

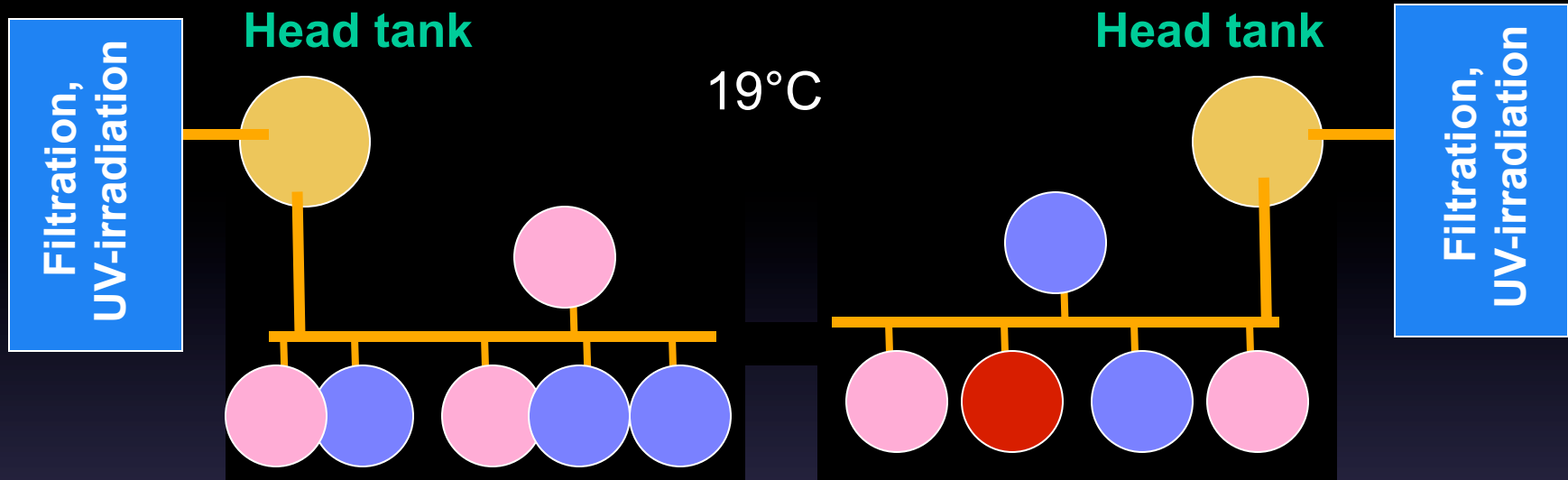
Carmel Point



San Nicolas Island



Recirculating Systems



Experimental System

1. RLO infected red abalone added to head tanks for 2 mo to expose the 5 SNI (violet) and 5 Carmel (pink) tanks of 8 abs each (n=40 each)
2. After removal of infected abalone from head tanks, a tank of uninfected red abalone was added to assess tank independence

Control System – Same except head tanks with uninfected reds

What we know:

- Naïve animals excrete more RLO gene copies
- Naïve animals have increase mortality over a shorter time
- Resistant animals do not undergo as much metaplasia or become as infected

Million \$ Question: WHY AND HOW??

Utilize **next generation sequencing** to characterize/
compare the transcriptomes of:

- RLO exposed vs. control
- Resistant (SNI) vs. naïve (Carmel)

Elucidate mechanisms associated with ↑ disease tolerance

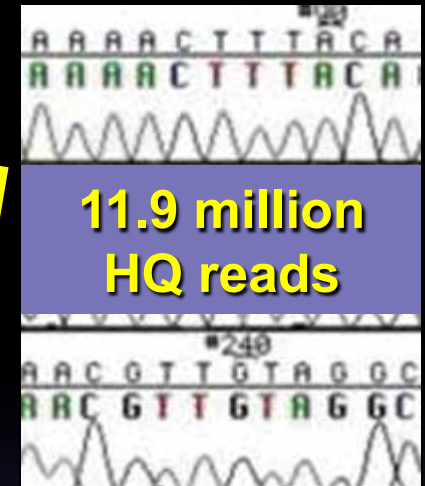
Current Research Goals

1. Discover genes actively expressed in black abalone
2. Characterize genes specifically involved in abalone innate immunity

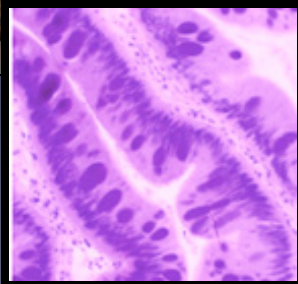
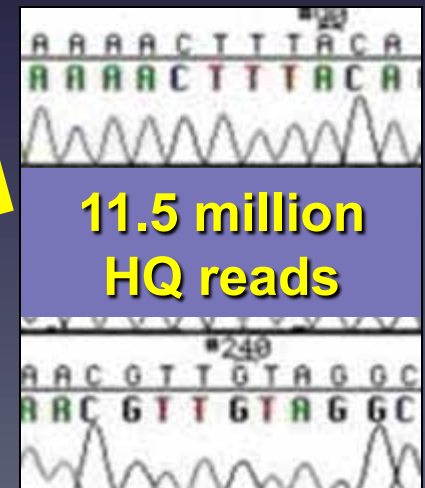




TRANSCRIPTOMICS:
reflects genes actively
expressed



**Next Generation
Sequencing**



Challenge with Abalone

- No annotated reference genome or transcriptome 😞
- Only 85 black abalone sequence submissions



NGS Solution 😊

1. WS-Resistant
Exposed

2. WS-Resistant
Control

3. Naïve
Exposed

4. Naïve
Control

De novo assemble (homemade
reference backbone) &
annotate (BLAST / Swiss-Prot)

RNA-Seq (proportion of reads mapping
back to reference)

Join annotation & GO - Galaxy

Enrichment Analysis - DAVID

Visualize data - REVIGO



Results - RLO exposed vs. control



Number of reads	Avg.length	Number of reads after trim	Percentage trimmed	Avg.length after trim
32,011,762	49.6	11,540,752	36.05%	40.1



Number of reads	Avg.length	Number of reads after trim	Percentage trimmed	Avg.length after trim
34,887,723	49.6	11,946,848	34.24%	39.7

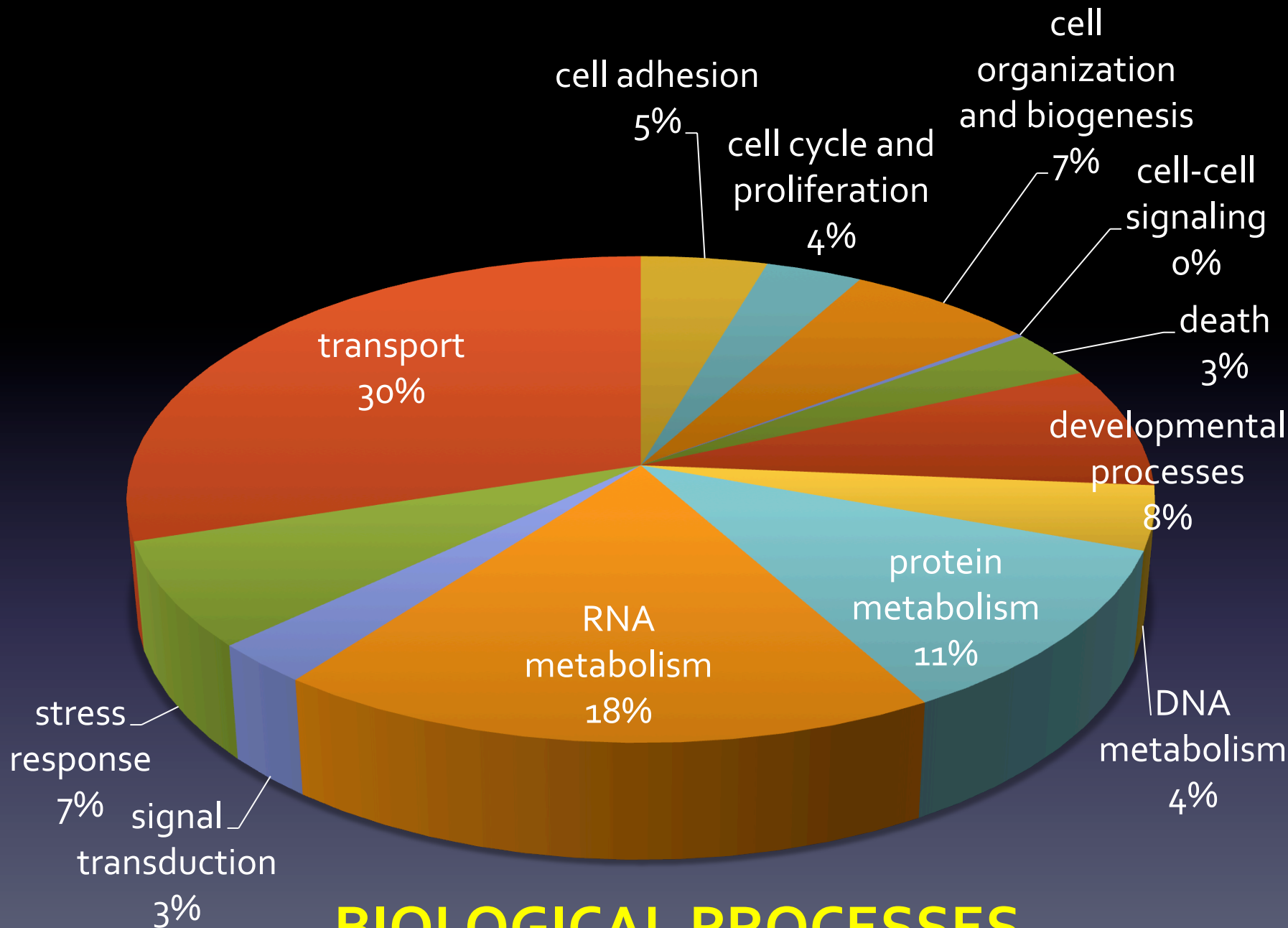
Nonatime	gi 122003207.1 SP P03670.2 PDBA_DANTE	00.00	45	3	0	1	51	1	45	4.7	36.7
Nonatime	gi 182112887.1 sp Q9D0T5.1 SP15TH_DANRE	77.78 <td>18</td> <td>4<td>0<td>2</td><td>55</td><td>148</td><td>165</td><td>0.040</td><td>36.2</td></td></td>	18	4 <td>0<td>2</td><td>55</td><td>148</td><td>165</td><td>0.040</td><td>36.2</td></td>	0 <td>2</td> <td>55</td> <td>148</td> <td>165</td> <td>0.040</td> <td>36.2</td>	2	55	148	165	0.040	36.2
Nonatime	gi 18202949.1 sp Q9H9P2.1 CHOLD_HUMAN	47.83	23	12	0	88	164	186	1.8	31.2	
Nonatime	gi 74997256.1 sp Q54258.1 SP2_DIC	48.00	25	13	0	53	127	15	39	5.2	29.6
Nonatime	gi 2548136.1 sp Q9H9G6.2 SP5_CAE	45.00	30	11	0	74	15	286	225	6.8	29.3
Nonatime	gi 640432.1 sp Q6A5Y1.1 KL	47.13	36	24	0	410	273	146	191	1e-06	51.6
Nonatime	gi 126393.1 sp Q9VW31.1 8	92.06	1	0	20	61	2	15	0.55	32.7	
Nonatime	gi 485233.1 sp Q9VW31.1 8	92.06	1	0	20	61	2	15	0.55	32.7	
Nonatime	gi 29891669.1 sp A1ZAU8.1 SSPA_DROME	33.33	39	26	0	164	48	45	83	2.3	30.8
Nonatime	gi 57812950.1 sp Q8BUU3.1 NARGA_MOUSE	48.54	37	28	1	15	119	794	830	5.2	29.6
Nonatime	gi 14983464.1 sp Q9H9G6.2 SP5_CAE	45.00	30	11	0	74	15	286	225	6.8	29.3
Nonatime	gi 68033195.1 sp F518P7.1 SP17_PAN	49.25	49	25	2	20	6	254	301	3.0	38.4
Nonatime	gi 224471853.1 sp Q9VW31.1 8	92.06	1	0	20	61	2	15	0.55	32.7	
Nonatime	gi 123629974.1 sp Q9VW31.1 8	92.06	1	0	20	61	2	15	0.55	32.7	
Nonatime	gi 71648714.1 sp Q6AE59.1 MUR1_LEIXD	51.85	27	13	0	96	16	40	66	1.1	32.0
Nonatime	gi 11368799.1 sp Q5718P.1 FL1R_BUCAT	28.36	67	48	1	291	1	128	191	0.12	35.5

Swiss-Prot description

GO Terms

I <3 GALAXY

ID	splice	Symbol	Taxon	Qualifier	GO ID	GO Name Reference	Evidence	With	Aspect	Date	Source
UniProtKB/Swiss-Prot		AOA183	-	LCE6A	9606	-	GO:0031424	keratinization	GO_REF:0000004	IEA	SP_KW:KW-0417 Process
UniProtKB/Swiss-Prot		AOA1F3	-	LDHA	30521	-	GO:0016491	oxidoreductase activity	GO_REF:0000004	IEA	SP_KW:KW-0560
UniProtKB/Swiss-Prot		AOA1F3	-	LDHA	30521	-	GO:0055114	oxidation reduction	GO_REF:0000004	IEA	SP_KW:KW-0560
UniProtKB/Swiss-Prot		AOA1F3	-	LDHA	30521	-	GO:0006096	glycolysis	GO_REF:0000004	IEA	SP_KW:KW-0324 Process
UniProtKB/Swiss-Prot		AOA1F3	-	LDHA	30521	-	GO:0005737	cytoplasm	GO_REF:0000004	IEA	SP_KW:KW-0963 Component
UniProtKB/Swiss-Prot		AOA1F4	-	eys	7227	-	GO:0005576	extracellular region	GO_REF:0000004	IEA	SP_KW:KW-0964
UniProtKB/Swiss-Prot		AOA1F4	-	eys	7227	-	GO:0016020	membrane	GO_REF:0000004	IEA	SP_KW:KW-0472 Component
UniProtKB/Swiss-Prot		AOA1F4	-	eys	7227	-	GO:0005711	integral to membrane	GO_REF:0000004	IEA	SP_KW:KW-0812
UniProtKB/Swiss-Prot		AOA1F4	-	eys	7227	-	GO:0005555	protein binding	PMID:19672878	IPI	UniProtKB:O97148
UniProtKB/Swiss-Prot		AOA1F4	-	eys	7227	-	GO:0005509	calcium ion binding	GO_REF:0000004	IEA	SP_KW:KW-0106
UniProtKB/Swiss-Prot		AOA314	-	rps12-A	13443	-	GO:0009536	plastid	GO_REF:0000004	IEA	SP_KW:KW-0934 Component
UniProtKB/Swiss-Prot		AOA314	-	rps12-A	13443	-	GO:0009507	chloroplast	GO_REF:0000004	IEA	SP_KW:KW-0150 Component
UniProtKB/Swiss-Prot		AOA314	-	rps12-A	13443	-	GO:0019843	rRNA binding	GO_REF:0000004	IEA	SP_KW:KW-0699 Function
UniProtKB/Swiss-Prot		AOA314	-	rps12-A	13443	-	GO:0003723	RNA binding	GO_REF:0000004	IEA	SP_KW:KW-0694 Function
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UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0015979	photosynthesis	GO_REF:0000004	IEA	SP_KW:KW-0602 Process
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0046872	metal ion binding	GO_REF:0000004	IEA	SP_KW:KW-0479
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0016021	integral to membrane	GO_REF:0000004	IEA	SP_KW:KW-0812
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0016020	membrane	GO_REF:0000004	IEA	SP_KW:KW-0472 Component
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0009507	chloroplast	GO_REF:0000004	IEA	SP_KW:KW-0150 Component
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0022900	electron transport chain	GO_REF:0000004	IEA	SP_KW:KW-0408
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0005506	iron ion binding	GO_REF:0000004	IEA	SP_KW:KW-0359
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0009635	response to herbicide	GO_REF:0000004	IEA	SP_KW:KW-0359
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0006810	transport	GO_REF:0000004	IEA	SP_KW:KW-0813 Process
UniProtKB/Swiss-Prot		AOA315	-	psbA	13443	-	GO:0005233	starch catabolism	GO_REF:0000004	IEA	SP_KW:KW-0644 Component



BIOLOGICAL PROCESSES

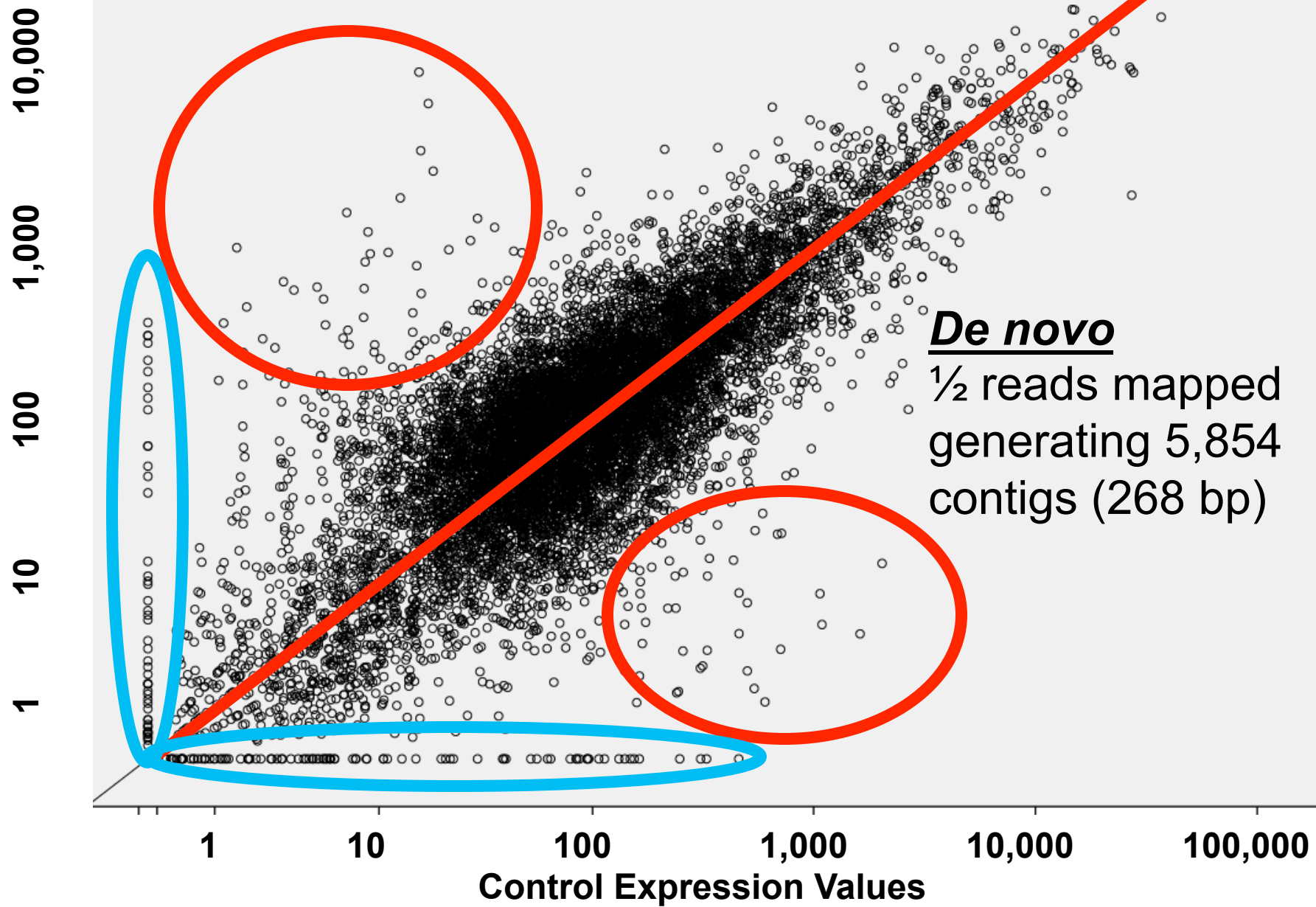
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RNA-Seq

Exposed Expression Values

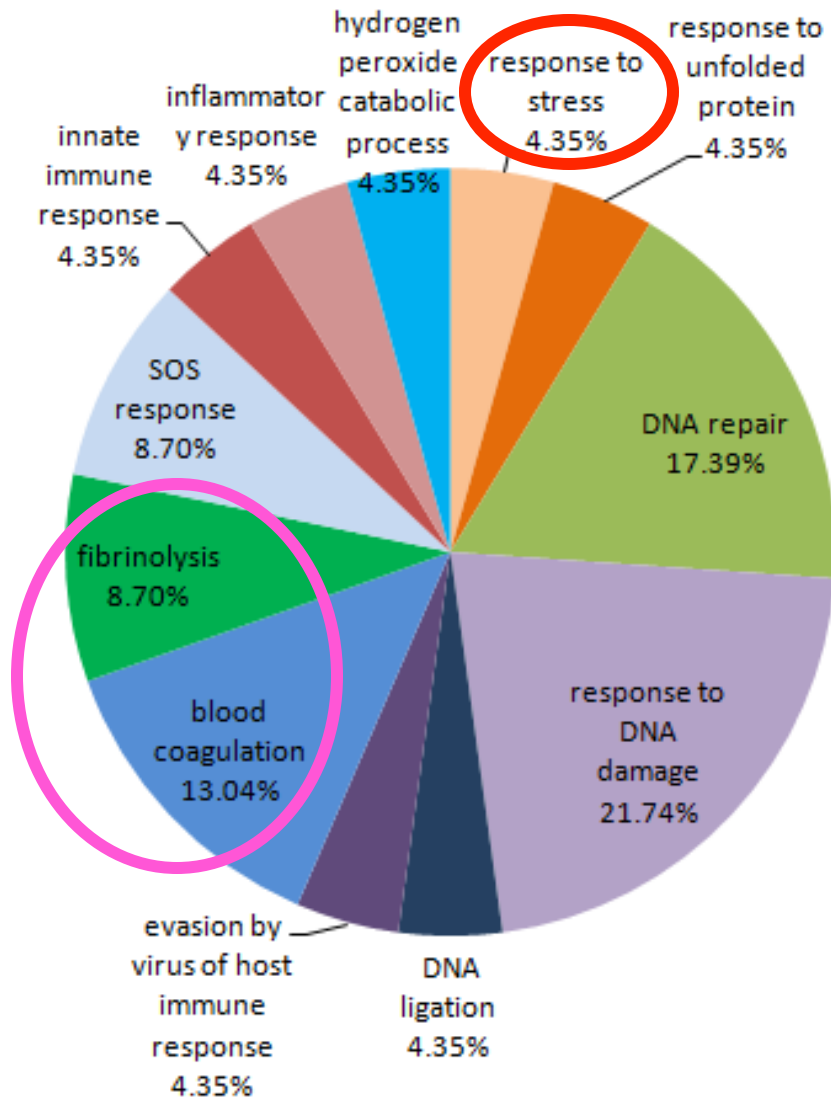


RNA-Seq Results

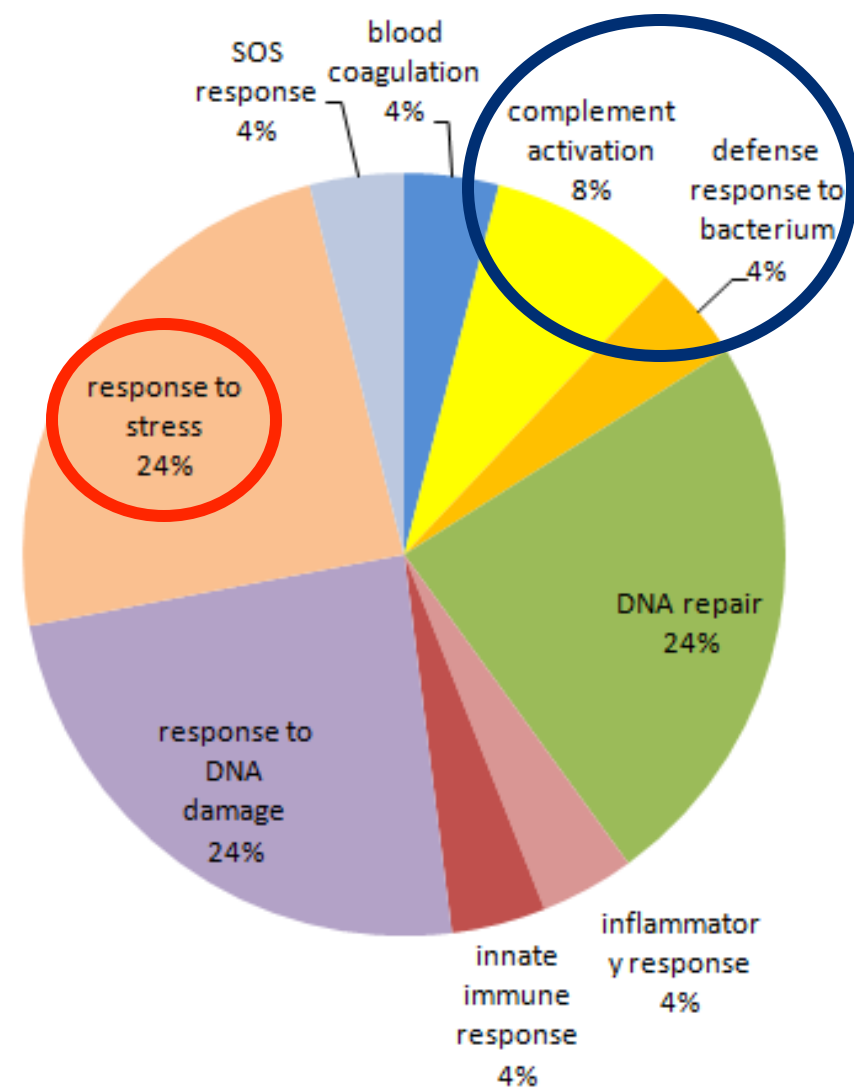
- 386 genes differentially expressed (≥ 4 fold)
 - 183 genes up regulated in RLO exposed
 - 203 genes up regulated in control



Classification of stress related genes upon RLO exposure



DOWNREGULATED

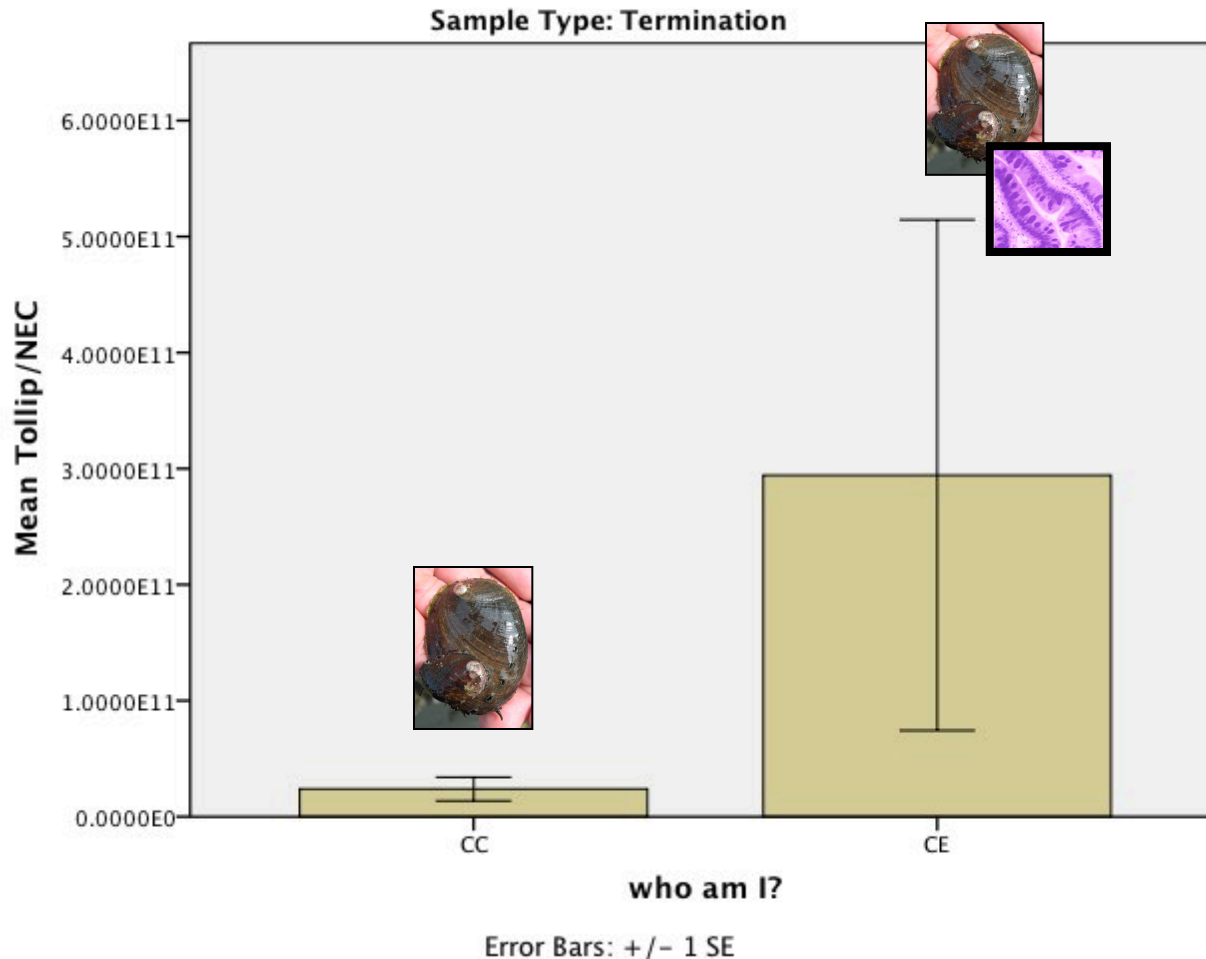


UPREGULATED

Stress related genes of interest

Fold Change	E-value	Description	Function
8.5	1.78E-04	Beta-1,3-glucan-binding protein	innate immune response; pattern recognition
13.6	2.09E-52	Toll-interacting protein (TOLLIP)	innate immune response; pattern recognition & inflammation
52.9	2.29E-91	Peroxidasin	functions in ECM; aids in phagocytosis & defense
117.7	3.13E-01	Complement C5	chemotaxis; membrane attack complex

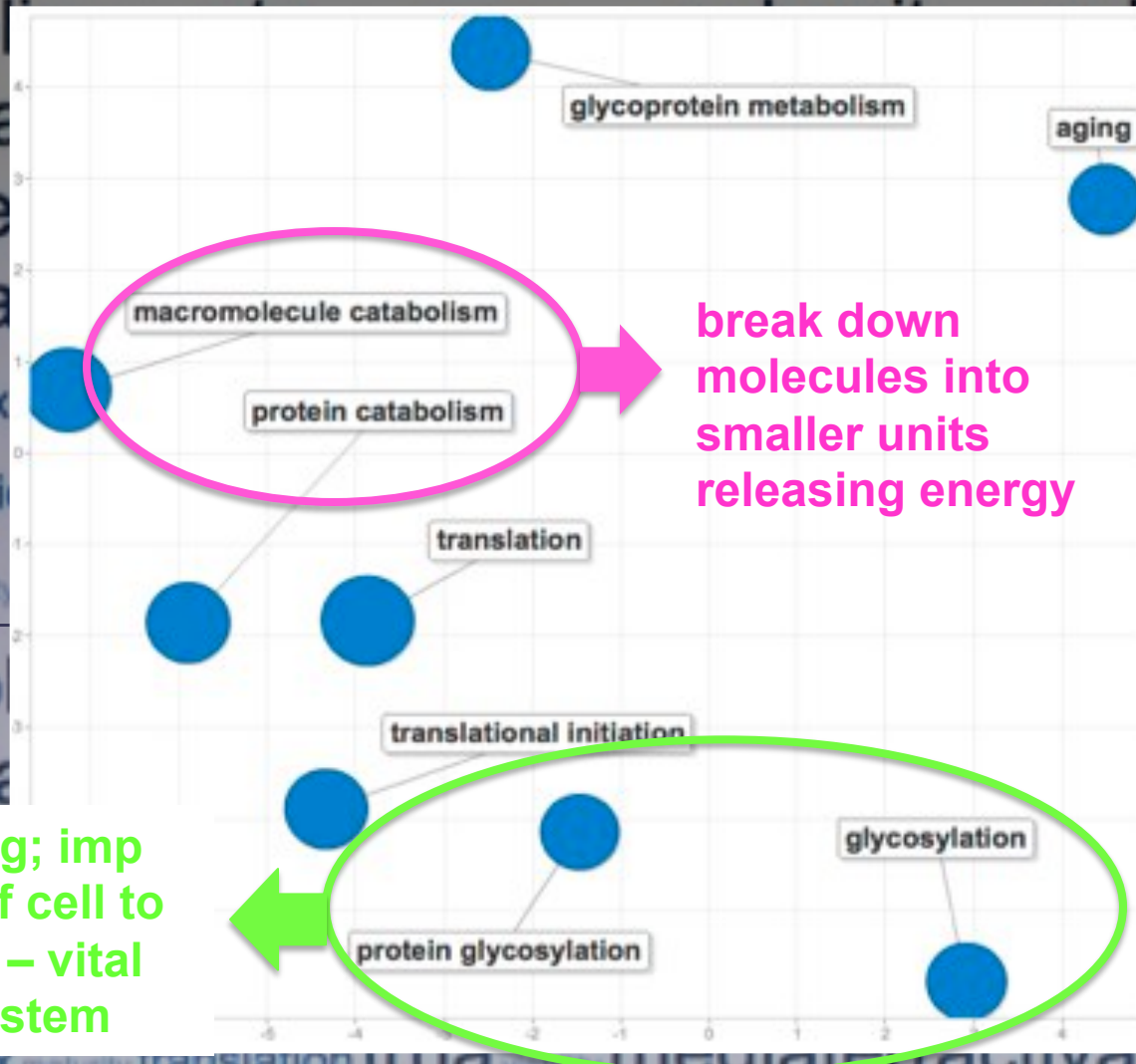
QPCR corroborates NGS



TOLLIP

- protein binding
- inflammatory response
- intracellular signaling cascades

Enrichment Analysis (DAVID) & Visualization (REViGO)



Conclusions

- Huge advance in gene discovery for abalones
- Important stress related genes up-regulated in exposed animals – potential candidates
- qPCR data corroborates NGS; Enrichment analysis syncs w/ disease progression

Future work

- Compare all 4 libraries to elucidate functional differences involved in disease resistance & ID specific genes involved
- Follow up with RAD-Seq (genotype / marker selection) & proteomics

Acknowledgements

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- US Navy
- NOAA Aquaculture
- NSA



Questions?