EIMD 2012
Case Study

RESEARCH ARTICLE

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Development of Genomic Resources for a thraustochytrid Pathogen and Investigation of Temperature Influences on Gene Expression

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Genomic Characterization

Assembly Parameter	Value
Number of contigs	21,280
N50 contig length	5.6 kb
Total contig length	34.7 Mb
Average contig length	1629 bp
G+C content (%)	33.4

Table 1. Characteristics of QPX genomic assembly.

CSV Download CSV

Genomic Characterization

Table_S1.txt

Dataset S1: Putative SNPs from genomic DNA sequencing

Contig ID	Position (bp)	Allele Variations		Allele Fr
QPX_v015_contig_6	1521	A/G	52.8/47.2	75/67	1
QPX_v015_contig_6	118	G/A	57.8/42.2	85/62	1
QPX_v015_contig_6	287	T/C	60.1/39.9	89/59	1
QPX_v015_contig_6	307	A/C	58.0/42.0	87/63	1
QPX_v015_contig_6	686	C/T	57.8/42.2	89/65	1
QPX_v015_contig_6	296	A/C	62.2/37.8	97/59	1
QPX_v015_contig_6	107	T/G	55.7/44.3	88/70	1
QPX_v015_contig_6	130	G/C	55.1/44.9	87/71	1
QPX_v015_contig_6	1745	A/C	61.4/38.6	97/61	1
QPX_v015_contig_6	360	G/T	55.6/44.4	89/71	1
QPX_v015_contig_6	304	G/A	57.1/42.9	92/69	1
QPX_v015_contig_6	1290	G/A	53.4/46.6	86/75	1
QPX_v015_contig_6	401	G/A	51.2/48.8	85/81	1
QPX_v015_contig_6	671	C/A	54.8/45.2	91/75	1
QPX_v015_contig_6	534	T/C	56.2/43.8	95/74	1
QPX_v015_contig_6	405	A/T	50.9/49.1	88/85	1
QPX_v015_contig_6	555	A/G	54.6/45.4	95/79	1
QPX_v015_contig_6	1717	G/C	62.6/37.4	112/67	1
QPX_v015_contig_6	598	A/G	59.1/40.9	107/74	1
QPX_v015_contig_6	870	C/T	56.2/43.8	104/81	1
QPX_v015_contig_6	1590	C/T	59.5/40.5	110/75	1

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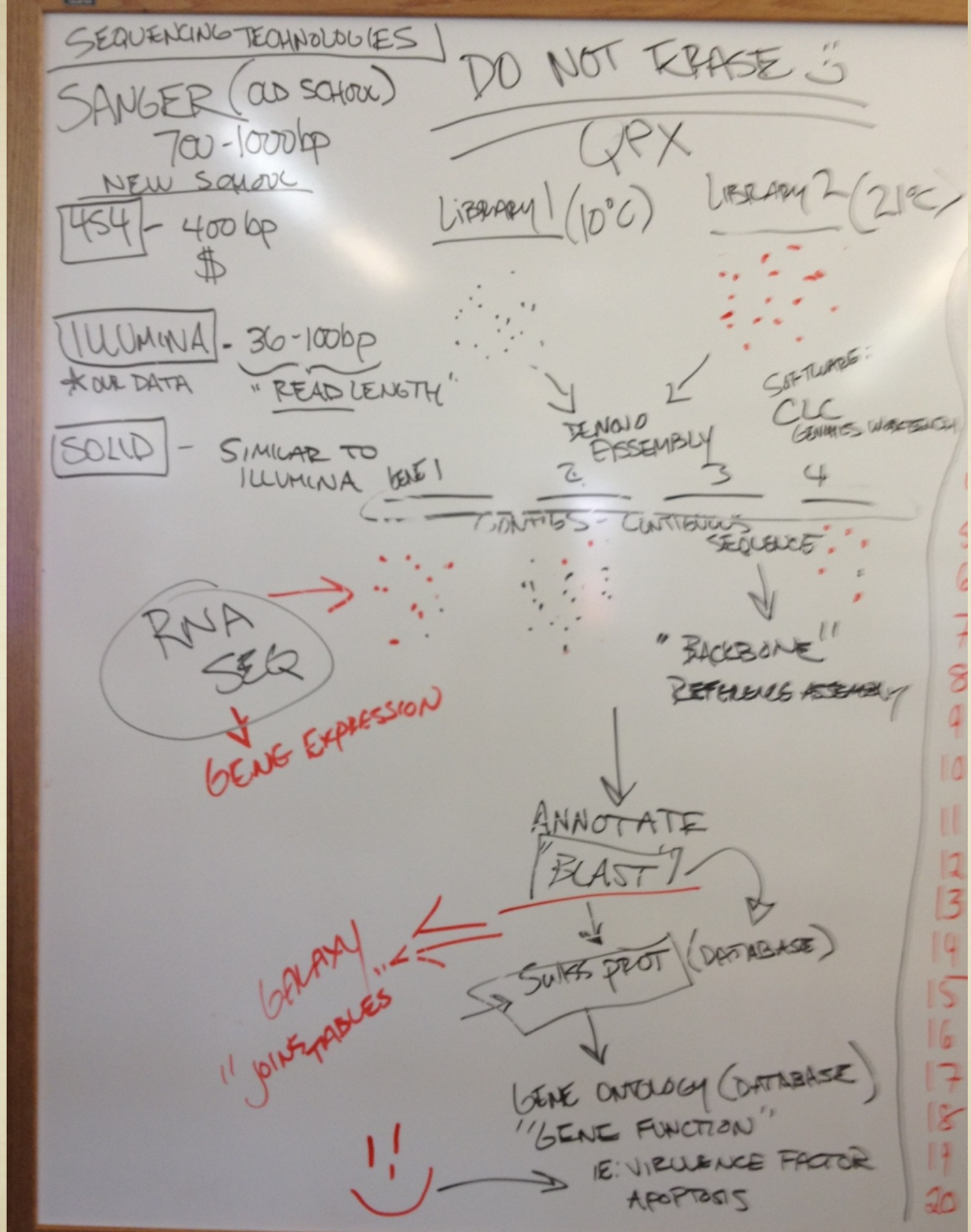
Putative single nucleotide polymorphisms (SNPs) from genomic DNA sequencing.

Experiment

QPX cultures were transferred to seawater and grown at either 10°C or 21°C for 72 hours.

Exp


SEQUENCING TECHNOLOGIES
SANGER (OLD SCHOOL)
700-1000bp
NEW SCHOOL
454 - 400bp
$
ILLUMINA - 36-100bp
* OUR DATA
"READ LENGTH"
SOLID - SIMILAR TO ILLUMINA
DO NOT ERASE :)
QPX
LIBRARY 1 (10°C)
LIBRARY 2 (21°C)
1
DENOVO ASSEMBLY
2
3
SOFTWARE:
CLC GENOMICS WORKBENCH
4
CONTIGS - CONTIGUOUS SEQUENCE
"BACKBONE"
REFERENCE ASSEMBLY
RNA SEQ
GENE EXPRESSION
ANNOTATE
"BLAST"
GALAXY
"JOIN TABLES"
SWISS PROT (DATABASE)
GENE ONTOLOGY (DATABASE)
"GENE FUNCTION"
IE: VIRULENCE FACTOR
APOPTOSIS

Transcriptome Annotation

Table_S3.txt

Table S3: QPX transcriptome gene ontology information

Contig ID	SwissProt ID	Gene description	e-value	Gen
QPX_transcriptome_v1_Contig_2	P52712	Serine carboxypeptidase-lik		
QPX_transcriptome_v1_Contig_2	P52712	Serine carboxypeptidase-lik		
QPX_transcriptome_v1_Contig_2	P52712	Serine carboxypeptidase-lik		
QPX_transcriptome_v1_Contig_3	P55737	Heat shock protein 90-2	0	
QPX_transcriptome_v1_Contig_3	P55737	Heat shock protein 90-2	0	
QPX_transcriptome_v1_Contig_3	P55737	Heat shock protein 90-2	0	
QPX_transcriptome_v1_Contig_4	Q54PV7	Eukaryotic translation init		
QPX_transcriptome_v1_Contig_4	Q54PV7	Eukaryotic translation init		
QPX_transcriptome_v1_Contig_4	Q54PV7	Eukaryotic translation init		
QPX_transcriptome_v1_Contig_6	Q943E7	16.9 kDa class I heat shock		
QPX_transcriptome_v1_Contig_6	Q943E7	16.9 kDa class I heat shock		
QPX_transcriptome_v1_Contig_8	P42824	DnaJ protein homolog 2	5.0	
QPX_transcriptome_v1_Contig_8	P42824	DnaJ protein homolog 2	5.0	
QPX_transcriptome_v1_Contig_8	P42824	DnaJ protein homolog 2	5.0	
QPX_transcriptome_v1_Contig_9	Q6NCX7	2,3-bisphosphoglycerate-ind		
QPX_transcriptome_v1_Contig_9	Q6NCX7	2,3-bisphosphoglycerate-ind		
QPX_transcriptome_v1_Contig_9	Q6NCX7	2,3-bisphosphoglycerate-ind		
QPX_transcriptome_v1_Contig_9	Q6NCX7	2,3-bisphosphoglycerate-ind		
QPX_transcriptome_v1_Contig_10	Q17770	Protein disulfide-isomerase		
QPX_transcriptome_v1_Contig_10	Q17770	Protein disulfide-isomerase		

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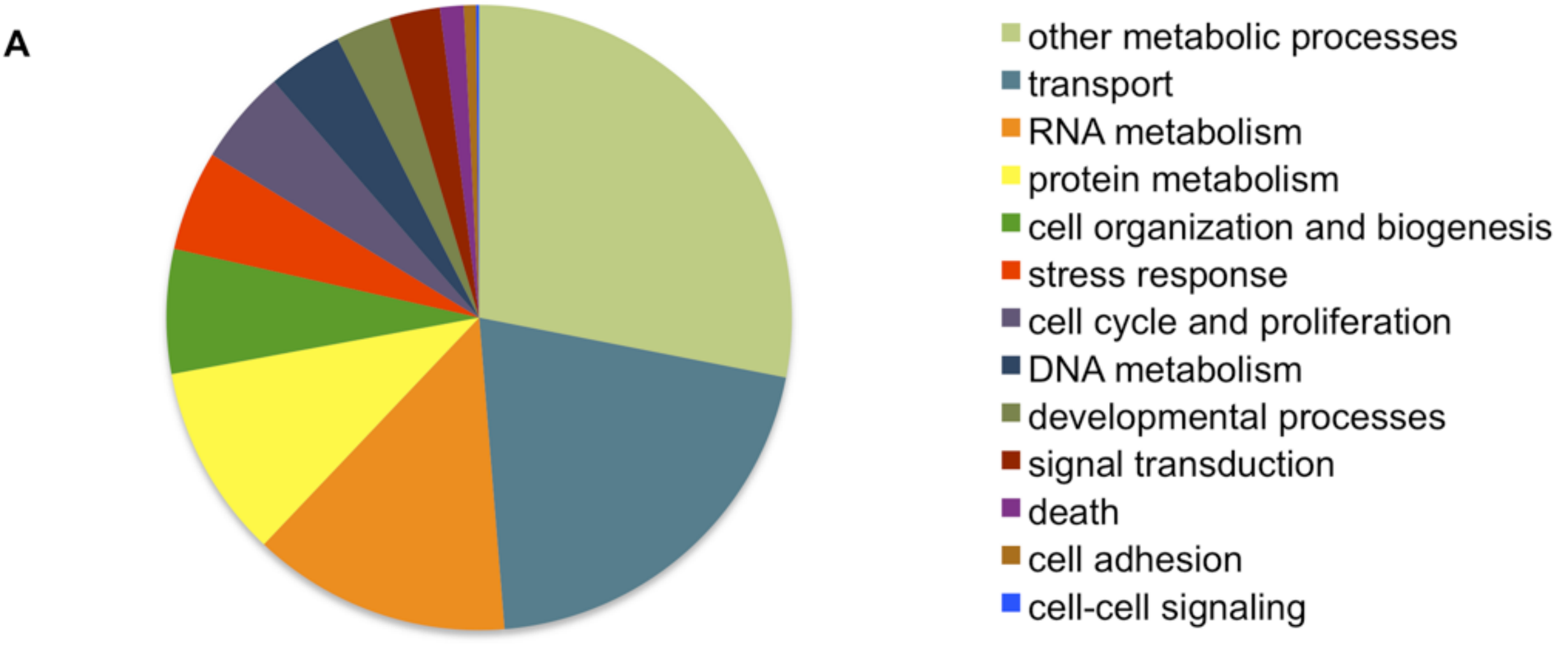
QPX transcriptome Gene Ontology (GO) information. GO and GO Slim classifications

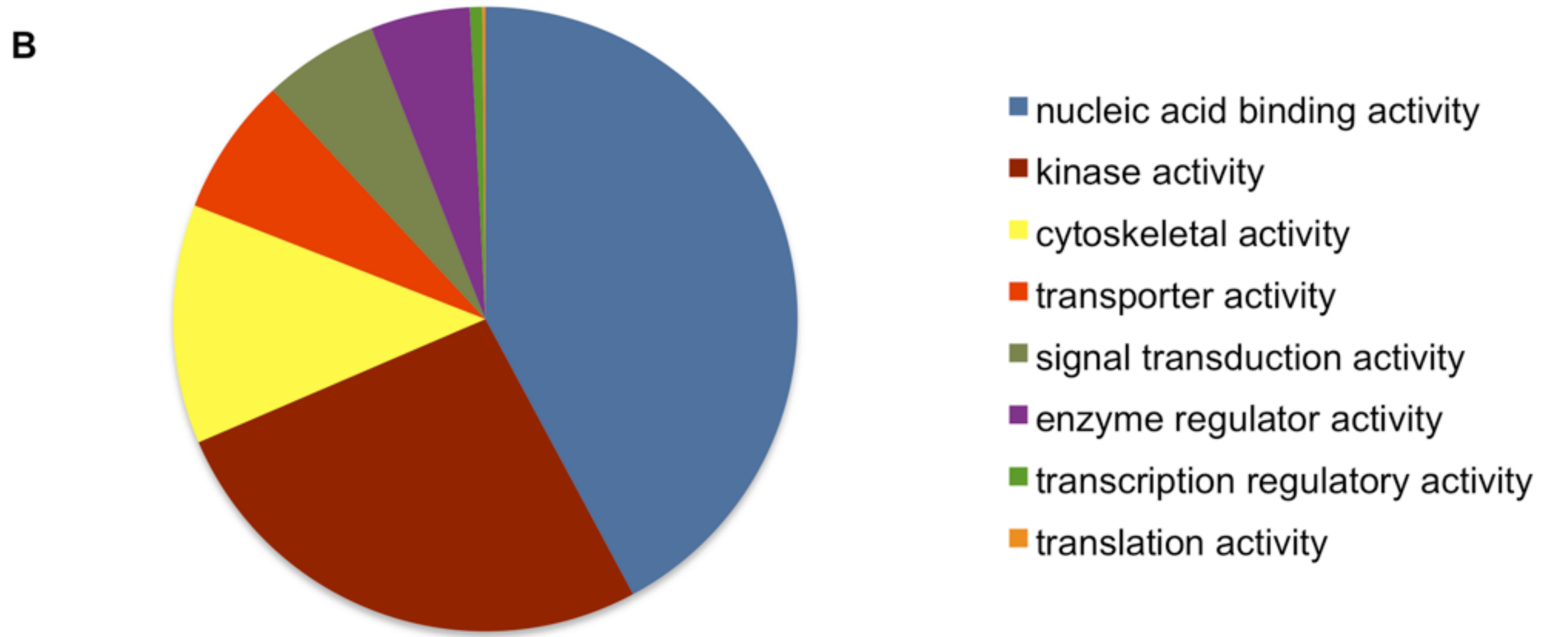
Transcriptome Annotation

GO Slim



Transcriptome Annotation

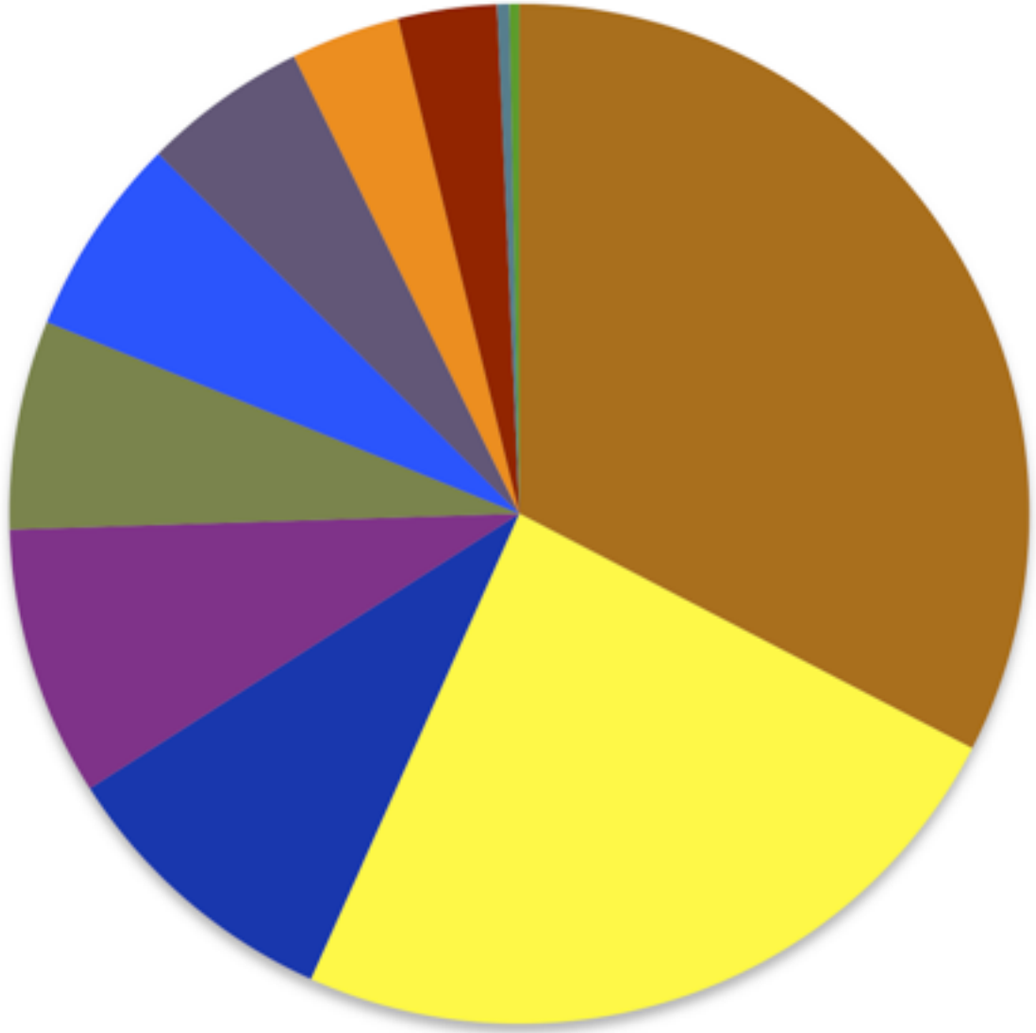
GO Slim

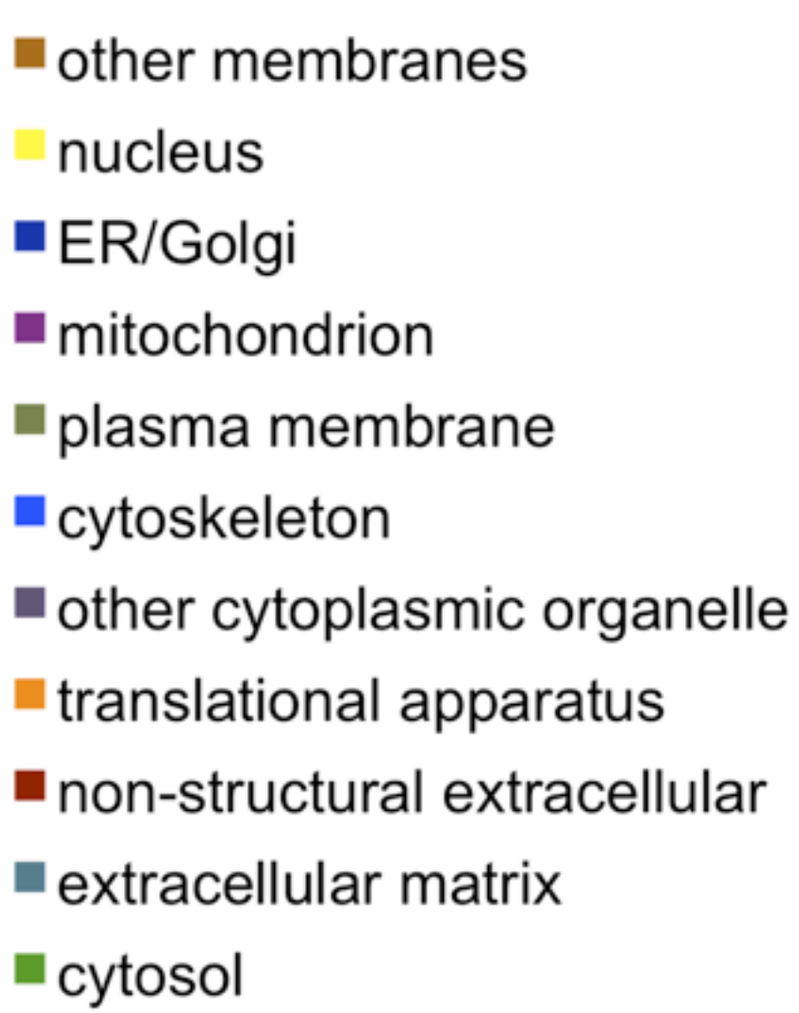


Transcriptome Annotation

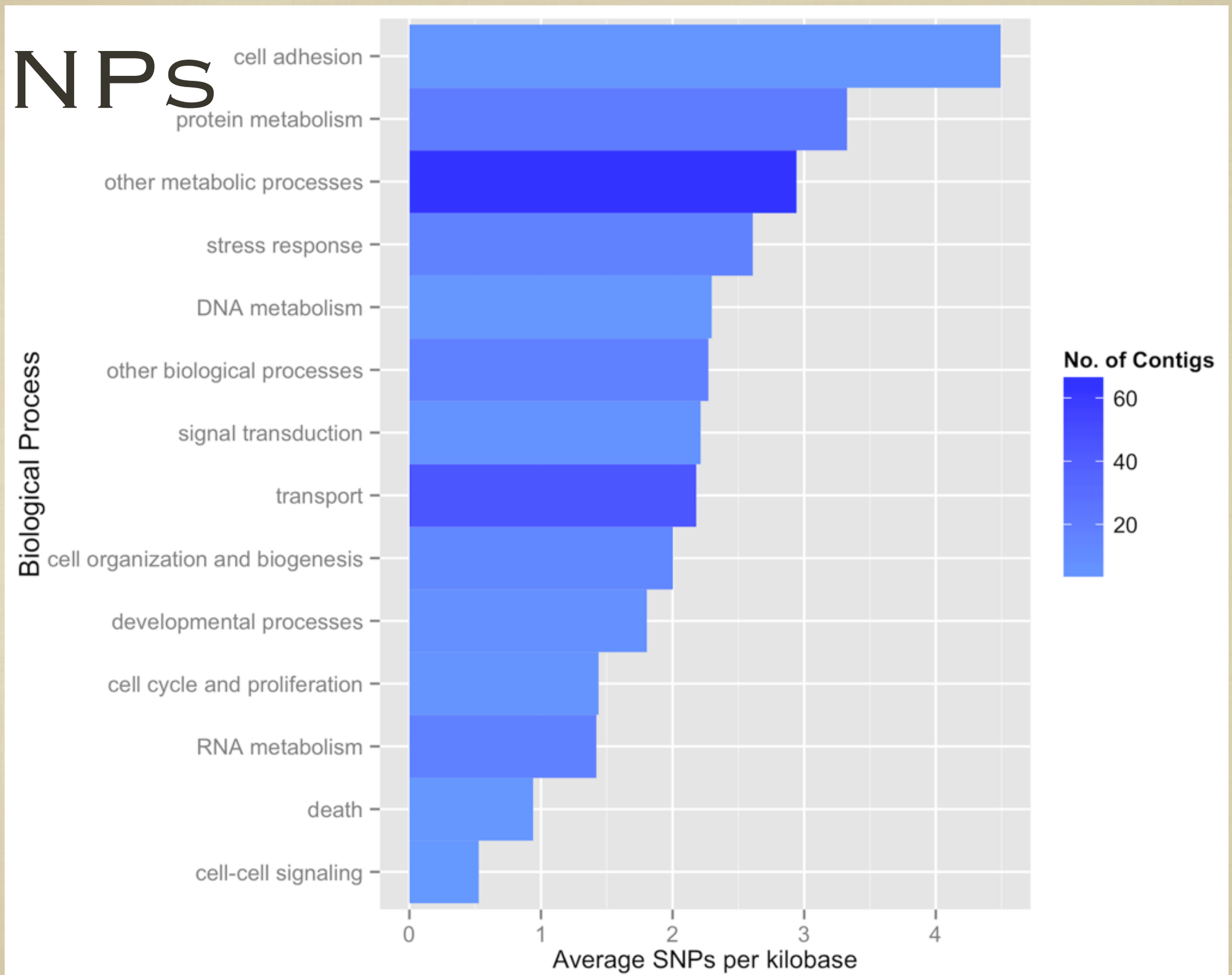
GO Slim

C

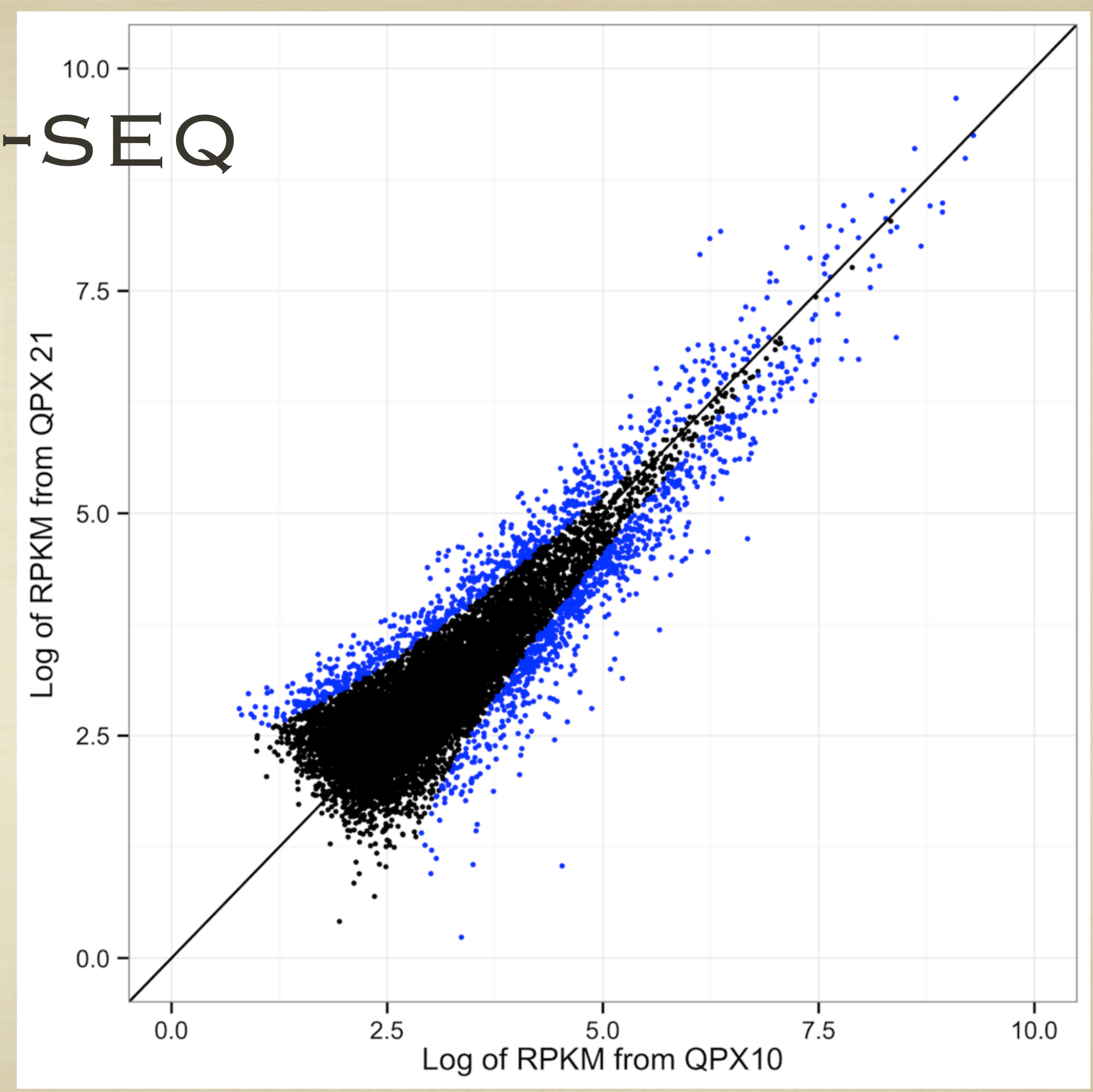




SNPs


cell adhesion
protein metabolism
other metabolic processes
stress response
DNA metabolism
other biological processes
signal transduction
transport
cell organization and biogenesis
developmental processes
cell cycle and proliferation
RNA metabolism
death
cell-cell signaling
Biological Process
0
1
2
3
4
Average SNPs per kilobase
No. of Contigs
60
40
20

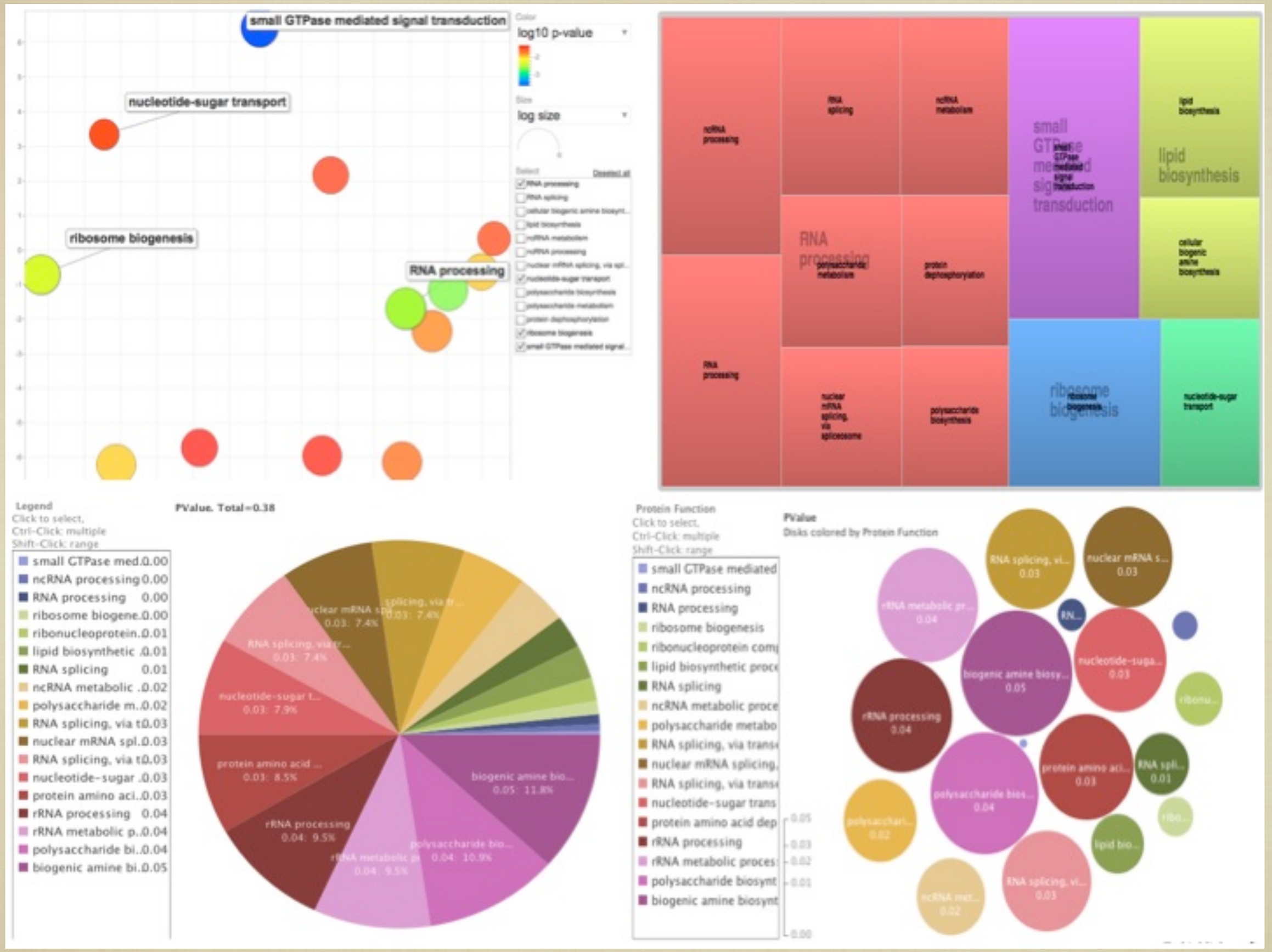
RNA-SEQ


10.0
7.5
5.0
2.5
0.0
Log of RPKM from QPX 21
0.0
2.5
5.0
7.5
10.0
Log of RPKM from QPX10

Enrichment

For those genes expressed at an elevated level in the QPX10 library, 26 biological processes were enriched.
For those genes expressed at an elevated level in the QPX21 library, 60 biological processes were enriched.

Enrichment analysis based on gene ontology revealed that enriched biological processes include those associated primarily with **translation, response to heat, cellular transport and metabolism.**


small GTPase mediated signal transduction
nucleotide-sugar transport
ribosome biogenesis
RNA processing
Color
log10 p-value
Size
log size
Select
Deselect all
RNA processing
RNA splicing
lipid biosynthesis
ncRNA metabolism
ncRNA processing
nucleotide-sugar transport
polysaccharide biosynthesis
polysaccharide metabolism
protein dephosphorylation
ribosome biogenesis
small GTPase mediated signal...
ncRNA processing
RNA splicing
ncRNA metabolism
small GTPase mediated signal transduction
lipid biosynthesis
RNA processing
polysaccharide metabolism
protein dephosphorylation
cellular biogenic amine biosynthesis
RNA processing
nuclear mRNA splicing, via spliceosome
polysaccharide biosynthesis
ribosome biogenesis
nucleotide-sugar transport
Legend
Click to select,
Ctrl-Click: multiple
Shift-Click: range
small GTPase med..0.00
ncRNA processing 0.00
RNA processing 0.00
ribosome biogene..0.00
ribonucleoprotein..0.01
lipid biosynthetic ..0.01
RNA splicing 0.01
ncRNA metabolic ..0.02
polysaccharide m..0.02
RNA splicing, via t..0.03
nuclear mRNA spl..0.03
RNA splicing, via t..0.03
nucleotide-sugar ..0.03
protein amino aci..0.03
rRNA processing 0.04
rRNA metabolic p..0.04
polysaccharide bi..0.04
biogenic amine bi..0.05
PValue. Total=0.38
splicing, via tr...
0.03: 7.4%
RNA splicing, via tr...
0.03: 7.4%
nucleotide-sugar t...
0.03: 7.9%
protein amino acid ...
0.03: 8.5%
rRNA processing
0.04: 9.5%
rRNA metabolic pr...
0.04: 9.5%
polysaccharide bio...
0.04: 10.9%
biogenic amine bio...
0.05: 11.8%
Protein Function
Click to select,
Ctrl-Click: multiple
Shift-Click: range
small GTPase mediated
ncRNA processing
RNA processing
ribosome biogenesis
ribonucleoprotein com
lipid biosynthetic proce
RNA splicing
ncRNA metabolic proce
polysaccharide metabo
RNA splicing, via trans
nuclear mRNA splicing,
RNA splicing, via trans
nucleotide-sugar trans
protein amino acid dep
rRNA processing
rRNA metabolic proces
polysaccharide biosynt
biogenic amine biosynt
PValue
Disks colored by Protein Function
RNA splicing, vi...
0.03
nuclear mRNA s...
0.03
rRNA metabolic pr...
0.04
RN...
biogenic amine biosy...
0.05
nucleotide-supa...
0.03
ribonu...
rRNA processing
0.04
protein amino aci...
0.03
RNA spli...
0.01
polysaccharide bios...
0.04
polysacchari...
0.02
ribo...
lipid bio...
ncRNA met...
0.02
RNA splicing, vi...
0.03
0.05
0.03
0.02
0.01
0.00

Temperature: HSPs

Interestingly, most of the HSPs that were associated with enriched biological process were expressed at higher levels at 10°C relative to 21°C

Temperature: HSPs

One explanation for this pattern is that translational activity is in fact elevated at 21°C relative to 10°C, resulting in the depletion of transcripts at 21°C.

In other words, protein expression might be increased at 21°C and an **increased rate of translation could deplete the relative transcript abundance.**

Temperature: HSPs

Alternatively, higher transcript levels observed at 10°C could reflect a thermal response in which cooler temperature induced increased gene expression.

QPX cultures were maintained at 21°C prior to the experimental trial, and the shift to 10°C could represent **an acute environmental stress** which triggered a general stress response, as has been observed in yeast.

Temperature: BE

Beta enolase is a glycolytic enzyme that can localize to the cell surface and concentrate plasminogen, a proenzyme of the protein-degrading serine protease plasmin.

Enolase production has been suggested as a mechanism of tissue invasion in bacterial and fungal pathogens

ZINC METALLOPROTEASES

These contigs have the greatest sequence similarity to proteases identified in **snake venom**, shown to inhibit cell proliferation and platelet aggregation in cultured cells

PROTEASES MAY BE VIRULENCE FACTORS AT HIGHER TEMPERATURES

ANTIBIOTIC BIOSYNTHESIS

Tyrocidine and linear gramicidin work in concert to regulate the process of **sporulation** and are associated with heat-tolerance in spores. Moreover, gramicidin and tyrocidine are both associated with the **release of extracellular proteases**, and gramicidin D was found to be a potent **molluscicide** in zebra mussels.

Conclusions

Previous studies have hypothesized that host thermal stress contributes to field observations of increased mortality in infected M. mercenaria at higher environmental temperatures.

However, upregulation of several potential virulence factors at higher experimental temperatures suggests that increased pathogen virulence may also play a role

QPX Genome Browser Feature Tracks

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QPX_igv_session_v_rsmol.xml	preview	download
QPX_SNPtable_genome.bed	preview	download
QPX_v017.fasta	preview	download
QPX_v017.fasta.fai	preview	download
QPXtrans_v1_Genome_blastn.gff	preview	download
SP_QPXv017_tblastn.gff	preview	download
QPX_genomeCov_21_bam.bedgraph	preview	download

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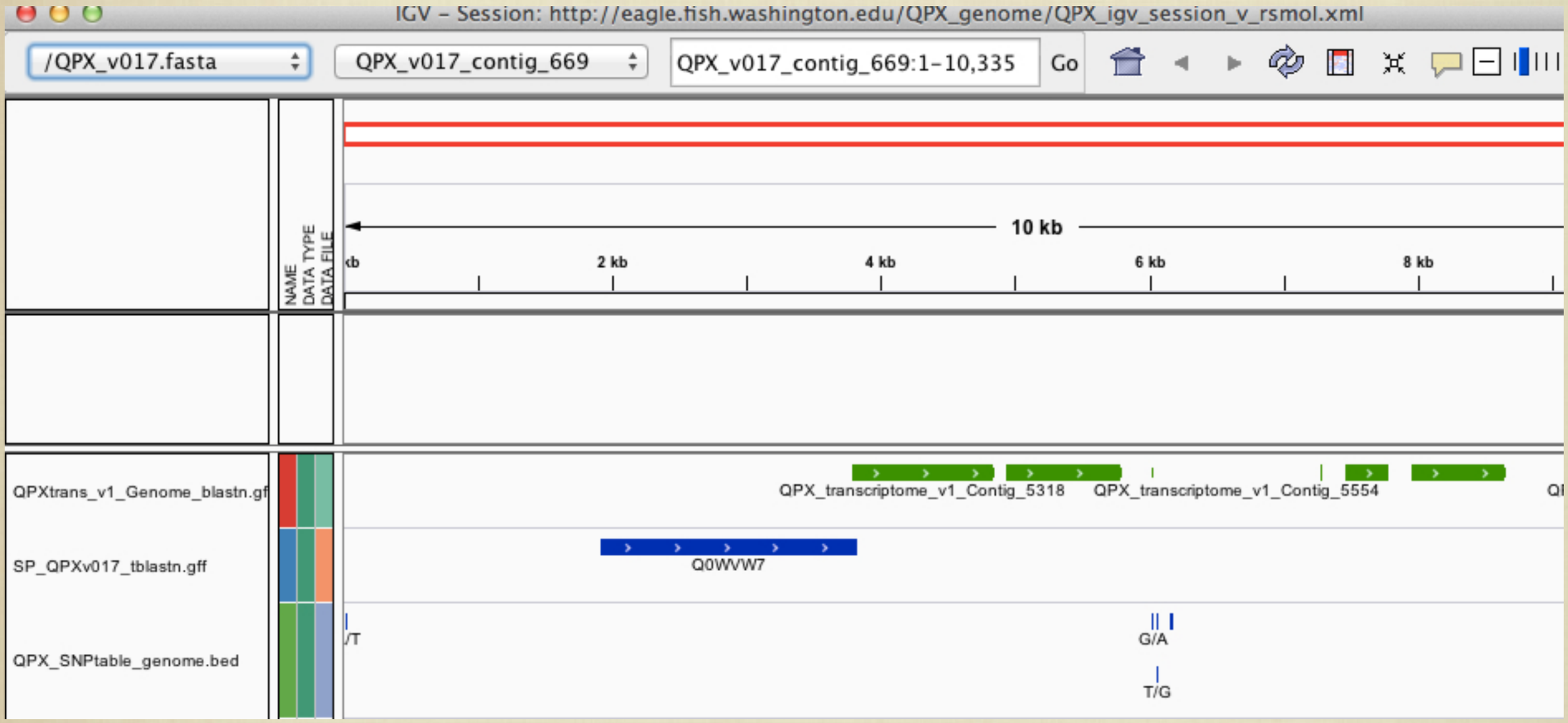
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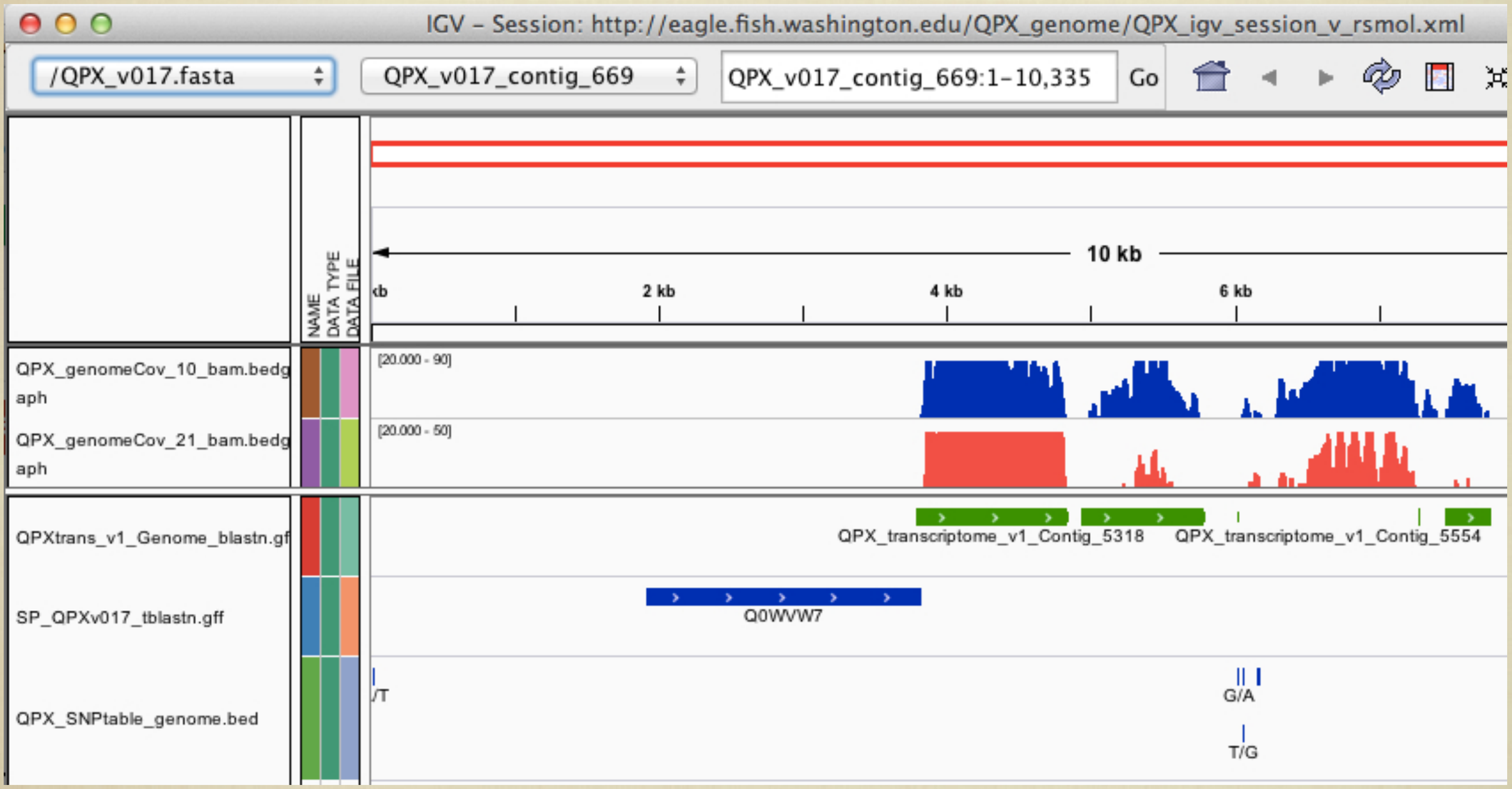
G/A
T/G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2 kb
4 kb
6 kb
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[20.000 - 90]
QPX_genomeCov_21_bam.bedgraph
[20.000 - 50]
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G/A
T/G