

Collaborative Genomic Data Analyses in the Cloud

Steven B. Roberts

Associate Professor

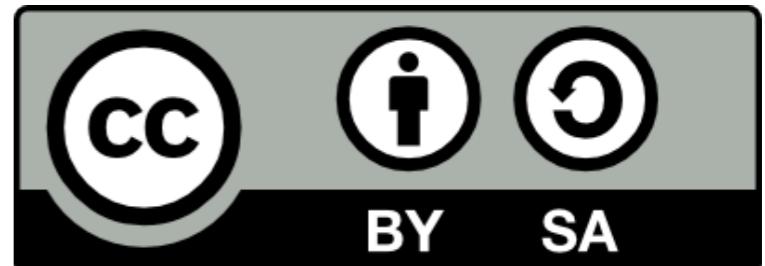
School of Aquatic and Fishery Sciences

University of Washington

robertslab.info

Open Science

- You are free to Share!
- Our lab practices open notebook science



IP[y]: IPython
Interactive Computing

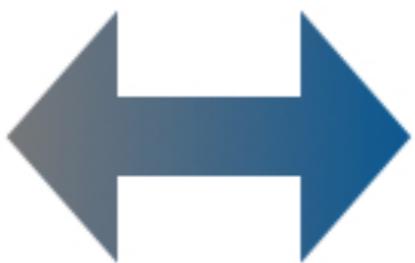
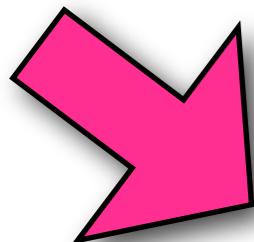


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sr320@uw.edu







SQLSHARE

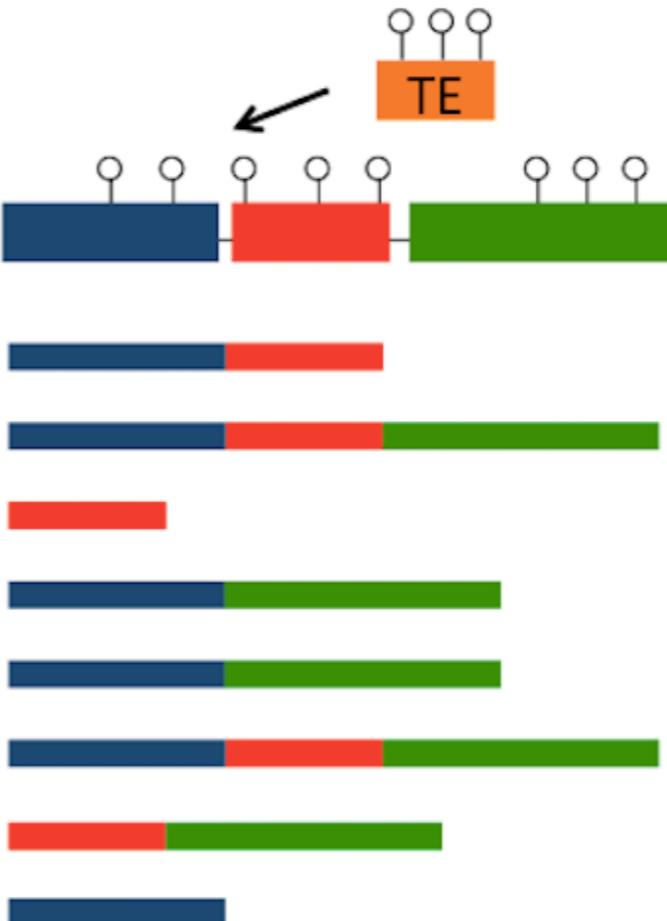
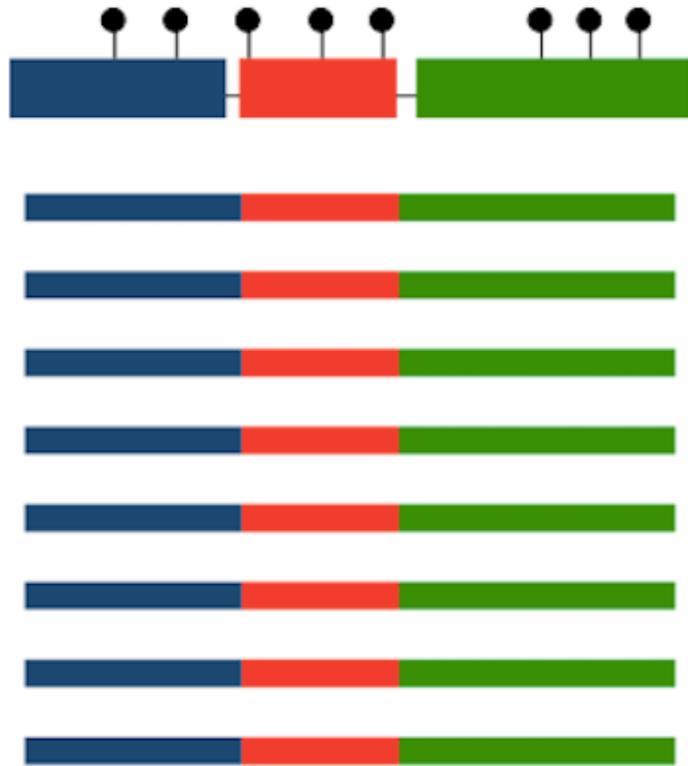
Galaxy

 **iPlant
Collaborative™**

Hyank



Stochastic Variation

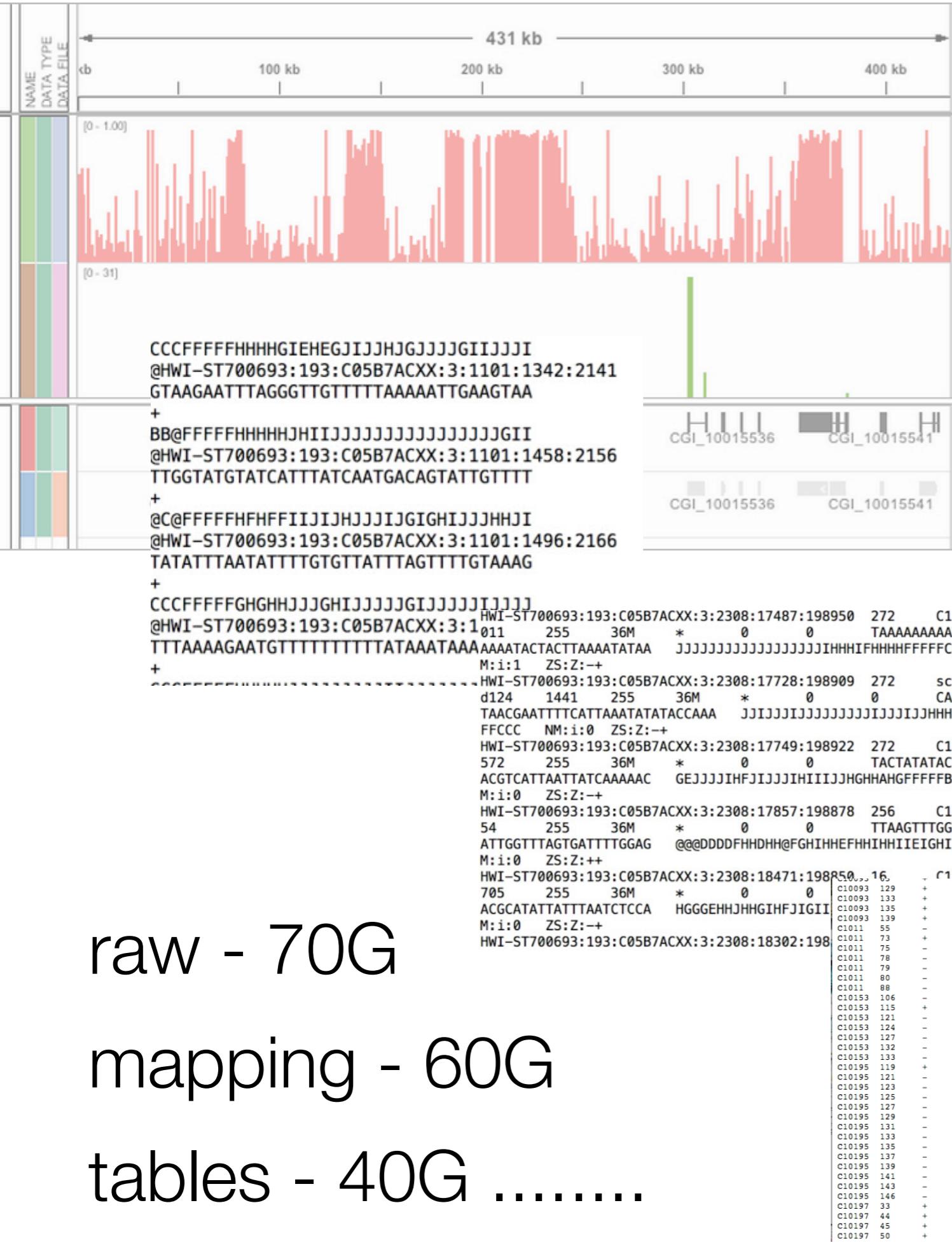


**A context dependent role for
DNA methylation in bivalves**

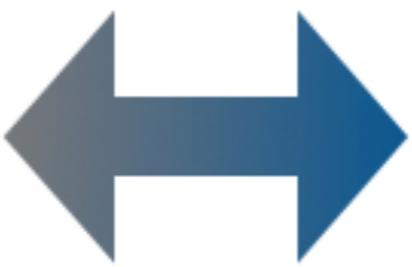
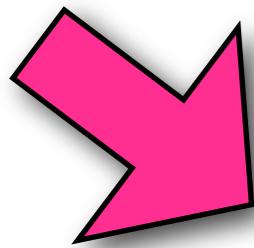
Mackenzie R. Gavery and Steven B. Roberts

10.1093/bfgp/elt054
10.6084/m9.figshare.880763





Why cloud?
big,
big,
compute
intensive,
education



SQLSHARE

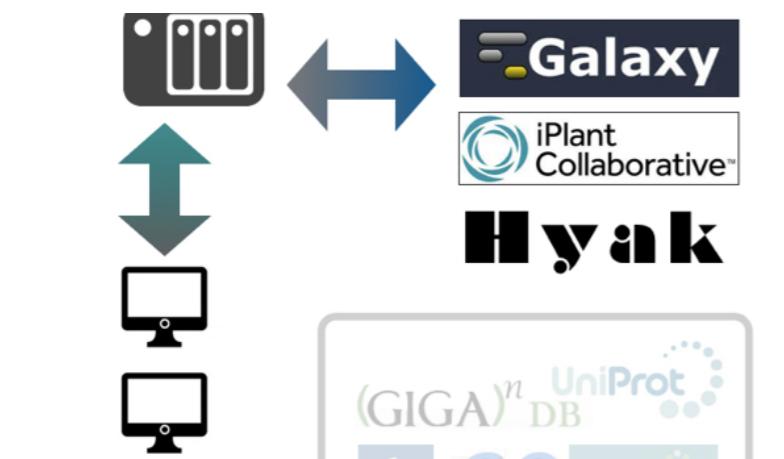
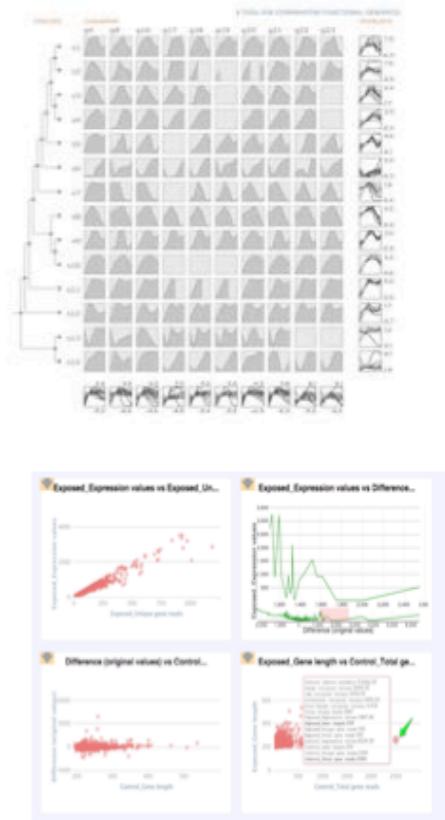
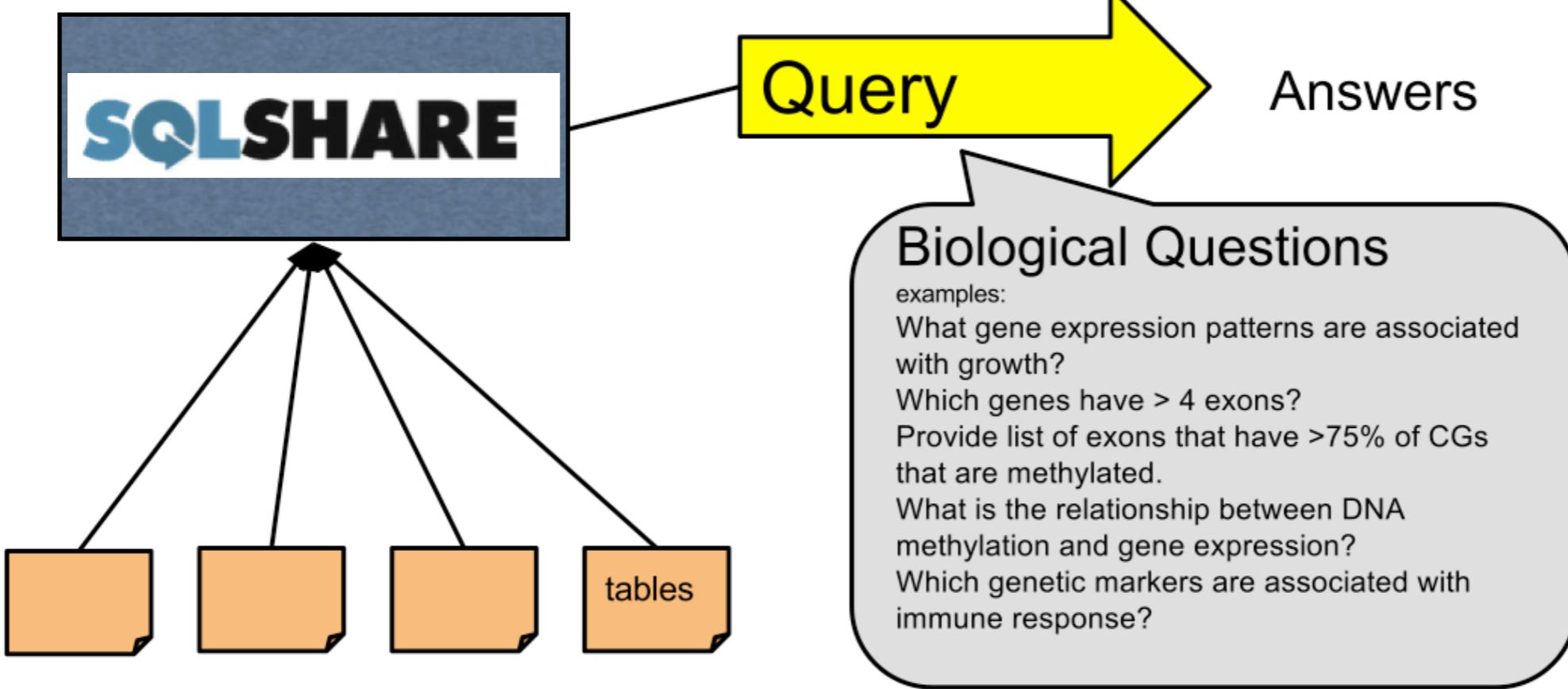
Galaxy

iPlant
Collaborative™



Hyank





eScience Institute

SQLSHARE

SQLShare is an easier way to store and share your data. Get answers to your research questions right now.

The SQLShare application interface is shown in a browser window. It features a sidebar with navigation links like 'All queries', 'Favorites', 'Recently Viewed >', 'Shared with you >', 'Upload new data', 'Create new query', and 'Tags' (with entries 'test', 'database', 'blah', 'safefield', 'folder', 'tigrfam'). The main area displays two datasets: 'TIGRFamAnnotation_surface_copy_12081977' and 'Table02_100032_copy_of_this'. Each dataset has a preview table, an 'EDIT QUERY' button, and a 'RUN QUERY' button. Below the tables are download options ('DOWNLOAD', 'VISUALIZE', 'COPY', 'QUERY TABLE') and a 'DELETE THIS DATASET' link.

Log in using your account:

UNIVERSITY of WASHINGTON

Google

Don't have an account?

Create a [Google Account](#) and start using SQLShare quickly.

One can view (and contribute) using the links you with a screenshot of the data in the table in a new webpage.

Upload

Upload any tabular data and start analyzing instantly. No need to install, configure, or design a database.

Modify

Exercise the full power of SQL even with zero programming experience: joins, subqueries, set operations.

Share

Analyze and compare your data collaboratively. Derive new datasets and share them with your colleagues.

```
https://sqlshare.es
https://sqlshare.es
seq
https://sqlshare.es
seq
```

Your datasets	
All datasets	View
Shared datasets	View
Recent activity... 18	View
Recently viewed »	View
Upload dataset	Upload
New query	New query

Your Datasets

Filter dataset by keyword:

Name	Description	Sharing / Owner	Modified
qdod_proteome_blast_mouse		sr320@washington.edu	Jan 2018
qDOD_v9_gene	GFF format file of oyster genes ~28k gene	sr320@washington.edu	Nov 2017
_qdod_goslim_graphtest		sr320@washington.edu	Oct 2017
SNP_RNAseqLibary_SB_BiGill	SNP table from RNA-seq library - SB gill tissue pool (BiGill complement)	sr320@washington.edu	Oct 2017
BiGill_meth_Zhang_exp	Gene-centric data including length, CG, percent methylation (gill) and tissue specific RPKM data from Zhang et al.	sr320@washington.edu	Oct 2017
qDOD_Cgigas_gene_fasta	Tabular format of Cgigas gene sequence fasta file Derived using Dataset: Genomic data from the Pacific oyster	sr320@washington.edu	Oct 2017
qDOD_Cgigas_Gene Descriptions (Swiss-prot)	Description and evalutes associated with Cgigas 28K genes Derived using Dataset: Genomic data from the Pacific oyster	sr320@washington.edu	Oct 2017

Use Cases

- Joining on Annotations
- File Conversion
- Querying Gene Tables

Secondary stress: proteomics

Original input file had some peptides of charge state >2, so had to redo everything with fixed input file.

SR discovered that for some proteins, a peptide was sequenced multiple times and so had multiple expression values. From the unique protein associations file in SQLshare, I summed the expression values for all identical peptides.

```
SELECT [peptide sequence], SUM([2_01 TotalArea]) AS CG2_01, SUM([2_02 TotalArea]) AS CG2_02, SUM([2_03
TotalArea]) AS CG2_03, SUM([5_01 TotalArea]) AS CG5_01, SUM([5_02 TotalArea]) AS CG5_02, SUM([5_03
TotalArea]) AS CG5_03, SUM([8_01 TotalArea]) AS CG8_01, SUM([8_02 TotalArea]) AS CG8_02, SUM([8_03
TotalArea]) AS CG8_03, SUM([11_01 TotalArea]) AS CG11_01, SUM([11_02 TotalArea]) AS CG11_02, SUM([11_03
TotalArea]) AS CG11_03, SUM([26_01 TotalArea]) AS CG26_01, SUM([26_02 TotalArea]) AS CG26_02, SUM([26_03
TotalArea]) AS CG26_03, SUM([29_01 TotalArea]) AS CG29_01, SUM([29_02 TotalArea]) AS CG29_02, SUM([29_03
TotalArea]) AS CG29_03, SUM([32_01 TotalArea]) AS CG32_01, SUM([32_02 TotalArea]) AS CG32_02, SUM([32_03
TotalArea]) AS CG32_03, SUM([35_01 TotalArea]) AS CG35_01, SUM([35_02 TotalArea]) AS CG35_02, SUM([35_03
TotalArea]) AS CG35_03, SUM([221_01 TotalArea]) AS CG221_01, SUM([221_02 TotalArea]) AS CG221_02,
SUM([221_03 TotalArea]) AS CG221_03, SUM([224_01 TotalArea]) AS CG224_01, SUM([224_02 TotalArea]) AS
CG224_02, SUM([224_03 TotalArea]) AS CG224_03, SUM([227_01 TotalArea]) AS CG227_01, SUM([227_02
TotalArea]) AS CG227_02, SUM([227_03 TotalArea]) AS CG227_03, SUM([230_01 TotalArea]) AS CG230_01,
SUM([230_02 TotalArea]) AS CG230_02, SUM([230_03 TotalArea]) AS CG230_03,
SUM([242_01 TotalArea]) AS CG242_01, SUM([242_02 TotalArea]) AS CG242_02, SUM([242_03 TotalArea]) AS
CG242_03, SUM([245_01 TotalArea]) AS CG245_01, SUM([245_02 TotalArea]) AS CG245_02, SUM([245_03
TotalArea]) AS CG245_03, SUM([248_01 TotalArea]) AS CG248_01, SUM([248_02 TotalArea]) AS CG248_02,
SUM([248_03 TotalArea]) AS CG248_03, SUM([251_01 TotalArea]) AS CG251_01, SUM([251_02 TotalArea]) AS
CG251_02, SUM([251_03 TotalArea]) AS CG251_03
```

Your datasets
All datasets
Shared datasets
Recent activity... 18
Recently viewed »
Upload dataset
New query

Your Datasets

Name
qdod_proteome_blast_mouse
qDOD_v9_gene GFF format file of oyster genes ~28k gene
_qdod_goslim_graphtest
SNP_RNAseqLibary_SB_BiGill SNP table from RNA-seq library - SB gill tissue pool (BiGill complement) qdod2
BiGill_meth_Zhang_exp Gene-centric data including length, CG, percent methylation (gill) and tissue specific RPKM data from Zhang et al a qdod2
qDOD_Cgigas_gene.fasta Tabular format of Cgigas gene sequence fasta file Derived using Dataset: Genomic data from the Pacific oyster qdod2
qDOD Cgigas Gene Descriptions (Swiss-prot) Description and evals associated with Cgigas 28k genes Derived using Dataset: Genomic blast

```

SELECT cgslim.CGI_ID,Description,evalue,SPID,GOID,term,GOSlim_bin,sequence
  FROM [sr320@washington.edu].[qDOD_Cgigas_GO_Goslim] cgslim
LEFT JOIN [sr320@washington.edu].[qDOD_Cgigas_gene_fasta] cfg
    ON cgslim.CGI_ID = cfg.CGI_ID
   WHERE term LIKE '%methyl%'
      OR
      term LIKE '%histone%'

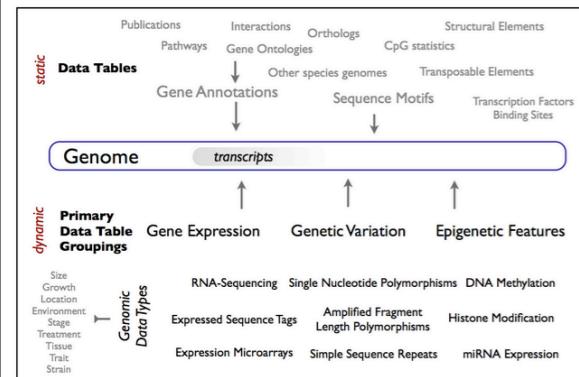
SELECT [peptide sequence], SUM([2_01 TotalArea]) AS CG2_01, SUM([2_02 TotalArea]) AS CG2_02, SUM([2_03
TotalArea]) AS CG2_03, SUM([5_01 TotalArea]) AS CG5_01, SUM([5_02 TotalArea]) AS CG5_02, SUM([5_03
TotalArea]) AS CG5_03, SUM([8_01 TotalArea]) AS CG8_01, SUM([8_02 TotalArea]) AS CG8_02, SUM([8_03
TotalArea]) AS CG8_03, SUM([11_01 TotalArea]) AS CG11_01, SUM([11_02 TotalArea]) AS CG11_02, SUM([11_03
TotalArea]) AS CG11_03, SUM([26_01 TotalArea]) AS CG26_01, SUM([26_02 TotalArea]) AS CG26_02, SUM([26_03
TotalArea]) AS CG26_03, SUM([29_01 TotalArea]) AS CG29_01, SUM([29_02 TotalArea]) AS CG29_02, SUM([29_03
TotalArea]) AS CG29_03, SUM([32_01 TotalArea]) AS CG32_01, SUM([32_02 TotalArea]) AS CG32_02, SUM([32_03
TotalArea]) AS CG32_03, SUM([35_01 TotalArea]) AS CG35_01, SUM([35_02 TotalArea]) AS CG35_02, SUM([35_03
TotalArea]) AS CG35_03, SUM([221_01 TotalArea]) AS CG221_01, SUM([221_02 TotalArea]) AS CG221_02,
SUM([221_03 TotalArea]) AS CG221_03, SUM([224_01 TotalArea]) AS CG224_01, SUM([224_02 TotalArea]) AS
CG224_02, SUM([224_03 TotalArea]) AS CG224_03, SUM([227_01 TotalArea]) AS CG227_01, SUM([227_02
TotalArea]) AS CG227_02, SUM([227_03 TotalArea]) AS CG227_03, SUM([230_01 TotalArea]) AS CG230_01,
SUM([230_02 TotalArea]) AS CG230_02, SUM([230_03 TotalArea]) AS CG230_03,
SUM([242_01 TotalArea]) AS CG242_01, SUM([242_02 TotalArea]) AS CG242_02, SUM([242_03 TotalArea]) AS
CG242_03, SUM([245_01 TotalArea]) AS CG245_01, SUM([245_02 TotalArea]) AS CG245_02, SUM([245_03
TotalArea]) AS CG245_03, SUM([248_01 TotalArea]) AS CG248_01, SUM([248_02 TotalArea]) AS CG248_02,
SUM([248_03 TotalArea]) AS CG248_03, SUM([251_01 TotalArea]) AS CG251_01, SUM([251_02 TotalArea]) AS
CG251_02, SUM([251_03 TotalArea]) AS CG251_03

```

qdod: Querying Disparate Oyster Datasets

This repository provides access to genomic data and workflows (IPython notebooks) that are being integrated as part of effort to increase efficiency of biological discovery. The [wiki](#) associated with this repository will serve as the *primary means for documentation*. Currently the documentation is focused on 1) **describing current datasets** and 2) **providing workflow tutorials**.

In brief, data in the form of delimited text files is aggregated into [SQLShare](#) where they can be easily queried. Below is schematic representation of the different types of datasets.



During the initial phases the focus is on the Pacific oyster and primary data from the [Roberts Lab](#).

Select IPython Notebooks

- [Static Data Tables - Universal](#)
- [Static Data Tables - Annotations](#)

Currently the documentation is focused on 1) **describing current datasets** and 2) **providing workflow tutorials**.

A. Raw Data

- [Select NGS Data via Roberts Lab](#)

B. Datasets in SQLShare

- [Universal](#)
- [Generic Oyster Datasets](#)
- [Tissue Specific Oyster Datasets](#)

C. Tutorials

- [Simple Gene Search](#)
- [Standard SQLShare Queries](#)
- [Annotating Genes](#)
- [File Format Conversions](#)

D. Genome Browser Feature Tracks

- [Canonical Tracks](#)
- [Bisulfite sequencing \(gill tissue\)](#)
- [Reference Genome Files](#)

Please use [GitHub's Issue feature](#) to ask question, report problems, or suggest features.

Last edited by sr320, 9 days ago



Galaxy

Analyze Data Workflow Shared Data Visualization Cloud Help User Using 13%

Published Workflows | rna-seq-helin-group | RNA-seq differential analysis replicates)

Annotation: Workflow based on Tophat and cuffdiff. Inputs: 4 fastq files (ex. Outputs: bam, bigwig, xls, and html reports with quality controls.

Step

Step 1: Input dataset
Gene Annotations
select at runtime

Step 2: Input dataset
Group1 R1
select at runtime

Step 3: Input dataset
Group1 R2
select at runtime

Step 4: Input dataset
Group2 R1
select at runtime

Step 5: Input dataset
Group2 R2
select at runtime

Step 6: Input dataset
Pseudo reads BAM file
select at runtime

Step 7: Tophat for Illumina
RNA-Seq FASTQ file
Output dataset 'output' from step 2
Use a built in reference genome or own from your history
Use a built in genome
<https://usegalaxy.org/library/index>

Data Lib Access published resources (ditions, 2 replicates) + About this Workflow

Data Libraries Beta

Published Histories
Published Workflows
Published Visualizations
Published Pages

Annotation

reads, 2 conditions, 2 reads, 1 gtf file (annotations).

Author rna-seq-helin-group

Related Workflows

All published workflows
Published workflows by rna-seq-helin-group

Rating

Community (3 ratings, 5.0 average)

Yours

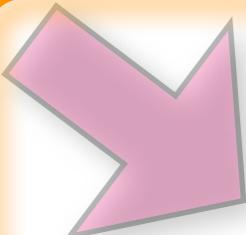
Tags

SQLSHARE



Sharing Collaboration*

Reads computationally generated from a unique sequence to avoid zero coverage genes.



Open Notebook Science

SQLSHARE

 Galaxy

 iPlant
Collaborative™

 Hyak



Reproducible

Collaboration

Open

Open Notebook Science

... there is a URL to a laboratory notebook that is freely available and indexed on common search engines. It does not necessarily have to look like a paper notebook but it is essential that all of the information available to the researchers to make their conclusions is equally available to the rest of the world.

—Jean-Claude Bradley

Open Notebook Science

 Roberts Lab Wiki

Wiki Home Projects Recent Changes Pages and Files Members Search

 OPEN Notebook Science

Katie's Notebook

February 4, 2014

This weekend, I downloaded Colony onto Thomas's computer (since it is Windows). I have been running the 2011 data on it since Saturday afternoon. As of this morning, it was making some pretty good progress. I will post the results on here when they are done! Tonight, I am going to download "HP-Rare" onto his computer so I can look at the rarefaction curve of this data. I am also working on organizing the south sound data from David Stick so that I can analyze that and compare it to the data I am working on now.

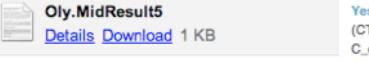
I made a Public Folder in my dropbox where I will keep all of the Microsat data so that there is a live file anyone can look at. Here is the link:
<https://www.dropbox.com/sh/5839mey4mgn2y28>

So far, there are the allele frequencies from David's relatedness values. I am working on Excel with this data and doing this through Colony when I get the other files thru.

I also started running the 2010 restoration grade.

January 30, 2014

In FSH 207 running Colony. I *think* I am now at


Oly.MidResult5
Details Download 1 KB

January 29, 2014

I am in FSH 207 trying to finish the first Colony

January 28, 2014

Filtering Larvae data

6 days ago Ran all files through BSMAP and methratio. Now need to filter so context= CG and a coverage cutoff of 5x or greater. Here is the query I am using to filter the data, and am changing the file

Bioinformatics 2014- Module 6

6 days ago See Module 1 for De novo assembly with Trinity using oyster gonad RNA-Seq data:
<https://www.evernote.com/shard/s232/sh/0596b21b-0000-4000-a000-000000000000>

Untitled Note

Last week For next figure look at this file:
[/Volumes/web/cnidarian/BiGo_gene_pmeth_expression.csv](http://Volumes/web/cnidarian/BiGo_gene_pmeth_expression.csv)
Porzio<- read.csv("Users/claireolson/Desktop/Porzio et al 2011")

Beautiful graphics in R- experimenting with plotly

Last week # Required packages from CRAN .pkgs = c("RJSONIO", "RCurl", "devtools") # Install required packages from CRAN (if not) .inst <- .pkgs %in% installed.packages()

Figure 1 for beautiful graphics in R

Last week With labels: Exon<- read.table("Volumes/web/Mollusk/174gm_analysis/Whole_Genome_Analyses/IntersectBedfiles_Steven")

Bioinformatics 2014- Module 4

Last week My file of p-values for all genes in gill v gonad: http://eagle.fish.washington.edu/Mollusk/FIS_H546_2014/DESeq%20results%20for%20Tissue%20


che625's notebook
Created: Feb 18, 2014 Modified: Feb 18, 2014
Larvae T1D5 correlation
file.list <- list('Volumes/web/Mollusk/bs_larvae_exp/methylkit_files/round2/CgLarv_T1D5_nov.txt',
'Volumes/web/Mollusk/bs_larvae_exp/methylkit_files/round2/CgLarv_T1D5_sept.txt')

myobj<-read(file.list,pipeline=list(fraction=TRUE,header=T, chr.col=1,start.col=2,end.col=3,strand.col=4, coverage.col=freqT.col=7),
 sample.id=list("T1D5_nov", "T1D5_sept"),assembly="v9",treatment=c(1,0))

meth<-unite(myobj,destrand=FALSE)

head(meth)

nrow(meth)

getCorrelation(meth,nInt=T)

IP[y]: Notebook BiGo_methratio Last Checkpoint: Sep 13 09:53 (autosaved)

File Edit View Insert Cell Kernel Help

Cell Toolbar: None

DNA methylation of Oyster Sperm based on Genomic Features

methratio file in SQLShare https://sqlshare.escience.washington.edu/sqlshare#/s=query/sr320%2540washington.edu/clean_BiGo_methratio_v1

BiGO methratio GFF

```
SELECT
  chr AS seqname,
  'methratio' AS source,
  'CpG' AS feature,
  pos AS start,
  pos + 1 AS [end],
  ratio AS score,
  strand,
  '.' AS frame,
  '.' AS attribute
FROM [sr320@washington.edu].[clean_BiGo_methratio_v1]
WHERE
```

Open Notebook Science



Automating a Workflow: Beyond Blast - to GO Slim

The concept is that you can take a fasta file in a working directory and end up with GO slim information all within a single notebook that is automated. Currently this work by writing (and overwriting) a scratch file to SQLShare. Assumptions are that you are working in a directory with fasta file named `query.fa`. And blast algorithms are in PATH.

```
In [13]: #allows plots to be shown inline
%pylab inline

Populating the interactive namespace from numpy and matplotlib

In [4]: #Setting Working Directory
wd="/Volumes/web/whale/fish546/qpx_go_val"
#Setting directory of Blast Databases
dbd="/Volumes/Bay3/Software/ncbi-blast-2.2.29+/db/"
#Database name
dbn="uniprot_sprot_r2013_12"
#Blast algorithm
ba="blastx"
#Location of SQLShare python tools: you can empty ("") if tools are in PATH
spd="/Users/sr320/sqlshare-pythonclient/tools/"

In [5]: cd {wd}
/Volumes/web/whale/fish546/qpx_go_val

In [5]: !{ba} -query query.fa -db {dbd}{dbn} -out {dbn}_{ba}_out.tab -evalue 1E-50 -num_threads 4 -max_hsps_per_subject 1
BLAST Database error: No alias or index file found for protein database [/Volumes/Bay3/Software/ncbi-blast-2.2.29+
/db/uniprot_sprot_r2013_12] in search path [/Volumes/web/whale/fish546/pipeline_test_dir4::]

In [6]: !head -1 {dbn}_{ba}_out.tab
QPX_transcriptome_v1_Contig_2    sp|P52712|CBPX_ORYSJ    43.75   416    213    12    2095    869    6    40
7      3e-98     326

In [17]: #Translate pipes to tab so SPID is in separate column for Joining
!tr '|'"\t" <{dbn}_{ba}_out.tab> {dbn}_{ba}_out2.tab

In [18]: !head -1 {dbn}_{ba}_out2.tab

In [8]: #Uploads formatted blast table to SQLshare; currently has generic name and meant to be temporary: Warning will over
!python {spd}singleupload.py -d scratchblast_out {dbn}_{ba}_out2.tab
...
In [9]: !python {spd}fetchdata.py -s "SELECT * FROM [sr320@washington.edu].[scratchblast_out]blast Left Join [sr320@washing
In [10]: !head -2 {dbn}_join2goslim.txt
...
In [11]: !python {spd}singleupload.py -d scratchjoin.slim {dbn}_join2goslim.txt

processing chunk line 0 to 18037 (0.0718240737915 s elapsed)
pushing uniprot_sprot_r2013_12_join2goslim.txt...
parsing 9A18D989...
finished scratchjoin.slim

In [12]: #Sets GO aspect
!python {spd}fetchdata.py -s "SELECT Distinct Column1 as query, Column3 as SPID, GOSlim_bin FROM [sr320@washin
In [13]: !head justslim.txt
...
In [15]: from pandas import *
```

Set some variables

blast

convert file format

upload to SQLShare
(python client)

join in SQLShare -
download

read in pandas

matplotlib generates
graph of GOslim

Open Notebook Science

Comparison

Wiki - collaboration, versioning, search, publishing

Evernote - simple, multi-platform

IPython - executable, versioning*

no perfection solution

SQLSHARE

Galaxy



IP[y]: Notebook

File Edit View Insert Cell Kernel Help
Code Cell To



Wiki Home
Projects
Recent Changes
Pages and Files
Members
Search



Katie' ethyl kit.
Will keep
files listed in
February
d, cast
This week, as
Saturday at! as
Tonight, I a
organizing
I made a P
Here is the

che625's notebook
Created: Feb 18, 2014 Modified: Feb 18, 2014
Larvae T1D5 correlation
file.list <- list('/Volumes/web/Mollusk/bs_la
'/Volumes/web/Mollusk/bs_larvae_exp/me

myobj<-read(file.list,pipeline=list(fraction=
freqT.col=7),
sample.id=list("T1D5_nov", "T1D5

meth<-unite(myobj,destrand=FALSE)

head(meth)

nrow(meth)

getCorrelation(meth,plot=T)

**Challenges:
versioning, provenance,
collaboration, simple
sharing, discoverability**



**Reproducible
Open Science**

Acknowledgements

Mackenzie Gavery

Claire Olson

DNA methylation



EPA
STAR



Sam White

Brent Vadopalas

Jake Heare



Aquaculture Program

Bill Howe

Dan Halperin



