# DNANEXUS

**DNAnexus Development** Environment **Bioinformaticists & Developers** 

- Introduction dx-toolkit
- Supported languages bash, python, docker
- Supported resources
- App/Applet building experience Peter FitzGerald (bash) Carl McIntosh (Python) Skyler Kuhn (Docker)
- Open Discussion



### DNAnexus provides a simplified, structured and managed access to Amazon Web Services (AWS) and Microsofts (Azure)





## **DNAnexus Applet System Requirements** Default: mem1\_ssd1\_x4

#### Common AWS instance types:

Name	Memory_GB	Storage_GB	CPU_Cores
mem1_ssd1_x2	3.8	32	2
mem1_ssd1_x4	7.5	80	4
mem1_ssd1_x8	15.0	160	8
mem1_ssd1_x16	30.0	320	16
mem1_ssd1_x32	60.0	640	32
mem2_ssd1_x2	7.5	32	2
mem2_ssd1_x4	15.0	80	4
mem2_ssd1_x8	30.0	160	8
mem3_ssd1_x2	15.0	32	2
mem3_ssd1_x4	30.5	80	4
mem3_ssd1_x8	61.0	160	8
mem3_ssd1_x16	122.0	320	16
mem3_ssd1_x32	244.0	640	32
$mem1_sd2_x2$	3.8	160	2
$mem1_sd2_x4$	7.5	320	4
$mem1_sd2_x8$	15	640	8
$mem1_sd2_x16$	30	1280	16
$mem1_sd2_x36$	60	2880	36

### • Memory:

- Storage:
- Harddrive:

### https://wiki.dnanexus.com/API-Specification-v1.0.0/Instance-Types

_	_		

• AWS: 3.8 - 244 GB

• Azure: 3.9 - 448 GB

• AWS: 32 - 2,880 GB

• Azure: 32 - 1,024 GB

• Standard Drive

Solid-State Drive

• Number of Cores:

• AWS: 2-36

• Azure: 2-32

Common Azure instance types:

Name	Memory_GB	Storage_GB	CPU_C
azure:mem1_ssd1_x2	3.9	32	2
azure:mem1_ssd1_x4	7.8	64	4
azure:mem1_ssd1_x8	15.7	128	8
azure:mem1_ssd1_x16	31.4	256	16
azure:mem2_ssd1_x1	3.5	128	1
azure:mem2_ssd1_x2	7.0	128	2
azure:mem2_ssd1_x4	14.0	128	4
azure:mem2_ssd1_x8	28.0	256	8
azure:mem2_ssd1_x16	56.0	512	16
azure:mem3_ssd1_x2	14.0	128	2
azure:mem3_ssd1_x4	28.0	128	4
azure:mem3_ssd1_x8	56.0	256	8
azure:mem3_ssd1_x16	112.0	512	16
azure:mem3_ssd1_x20	140.0	640	20
azure:mem4_ssd1_x2	28.0	128	2
azure:mem4_ssd1_x4	56.0	128	4
azure:mem4_ssd1_x8	112.0	256	8
azure:mem4_ssd1_x16	224	512	16
azure:mem4_ssd1_x32	448	1024	32



## Command-Line

### **DX-toolkit**

The DNAnexus SDK (dx-toolkit) helps users utilize the DNAnexus platform to its full potential. It provides commandline tools to run Apps/applets from a remote command-line/ script. Additionally, it provides the environment for App/ applet development

- The dx-toolkit is Installed on Helix/Biowulf in the module system and can be run with the following command (Note: can only be run on bioiwulf interactive nodes)
  - module load DNAnexus

## **Development Environment**

### The main controlling module of an App/applet can be written in either of the following"

- Bash
- Python
- Docker

## Helpful Web Pages

- https://wiki.dnanexus.com/Developer-Portal

https://wiki.dnanexus.com/Command-Line-Client/Quickstart

## **DNAnexus External Resources**

- Packgage Mangers
  - Advanced Packaging Tool (APT)
    - Libraries, Samtools, Bedtools, etc.
  - Python Package Index (PyPI)
  - Comprehensive Perl Archive Network (Perl)
  - Ruby Gems (gem)
  - Comprehensive R Archive Network (CRAN)

DNAnexus How-to https://wiki.dnanexus.com/Execution-Environment-Reference

https://wiki.dnanexus.com/List-of-packages-available-in-the-Execution-Environment

## **Applet Design Process**

- Sketch out Workflow (OMNIGraffle for example)
- Required Resources (Asset Bundle vs Applet Resource) -> Detailed web link
- Applet Model (standard, parallelize, SPG) -> Detailed web link
- Input Elements -> Detailed web link
- Output
  - Properties and Tags
  - Directory Structure
- Final Touches
  - Documentation (MacDown) -> Detailed web link
  - Script.sh
  - Versions

- From *Terminal* on Biowulf/Helix
  - module load DNAnexus
  - dx-app-wizard
- From *Terminal* on Local Computer
  - downloads
  - dx-app-wizard



Download and Install DNAnexus Platform SDK https://wiki.dnanexus.com/

## dx-app-wizard Applet Parameters

- Timeout policy[48h] (m | h | d)
- Applet Programming language: (Python | bash), but supports other languages
- Applet Access to Internet [N]:
- Applet Access to Parent Project: [N]
- Ubuntu 14.04
- Compute Nodes



## dx-app-wizard Applet Parameters

### Input Specification

You will now be prompted for each input parameter to your app. Each parameter should have a **unique name** that uses only the **underscore** "\_" and **alphanumeric** characters, and **does not start with a number**.

### **1st input name (<ENTER> to finish): parameter**

Label (optional human-readable name) []:

### Your input parameter must be of one of the following classes:

applet array:applet array:boolean

array:file array:float array:int

array:record array:string boolean

### **Output Specification**

The same

file	int
float	record
hash	string

# DNAnexus dx-app-wizardBasicParallelizedScatter-Process-Gather



dx-app-wizard --template (basic | parallelized | scatter-process-gather)

Figures and a tutorial at DNAnexus: https://wiki.dnanexus.com/Developer-Tutorials/Parallelize-Your-App



## **Cloud Workstation**

DNAnexus features a Cloud Workstation App that sets up a cloud workstation as an interactive computer node.

### What are typical use cases for this app?

This app can be used as a workstation inside of the DNAnexus cloud platform. By running the app with --ssh or --allow-ssh, users can login to a machine inside of the DNAnexus cloud platform. From there, users can upload/download data to/from the project in which the app is run, perform data analysis, and install additional packages from sources such as apt, cran, pip, github, etc.

It's good for debugging, since you can do so interactively on the node as its running.

dx run app-cloud\_workstation --ssh

unset DX\_WORKSPACE\_ID dx cd \$DX\_PROJECT\_CONTEXT\_ID:

dx download file.txt ## download to workstation from parent project dx upload file.txt ## upload to parent project from workstaion

dx terminate \$DX\_JOB\_ID #terminate the session

https://wiki.dnanexus.com/Developer-Tutorials/Cloud-Workstations

## Genome Analysis Unit (GAU) DNAnexus Applet Development

Custom Work Flows developed by Carl McIntosh and Peter FitzGerald (GAU)



Using DNAnexus to make the ADAP program readily available to a naive audience. The program was originally written, many years ago, and has had several interface iterations (Web App, Standalone Mac/PC program). The program takes a DNA fasta file and recodes the sequence using alternate AA codons, to generate a new sequence As Different As Possible from the original, yet codes the same protein. This approach is useful in the over expression of proteins.

A Collaboration with Christopher Buck & Diana Pastrana (Laboratory of Cellular Oncology, NCI/CCR)

#!/bin/bash # The following line causes bash to exit at any point if there is any error # and to output each line as it is executed -- useful for debugging set -e -x

# Inputs dx download "\$input" -o input.fasta — no-progress # make a directory for the output mkdir -p out/results

# Processing diana -f input.fasta -c /usr/lib/diana.codes > out/results/adap.log

# Outputs - upload the out/results directory

dx-upload-all-outputs

The simplest of applets - simple bash script (5 lines!), and a single binary from C code compiled on biowulf

## **GV Session Maker**

- launching from a custom built HTML page it provides a stable record of what is represented in the view.
- Applet consistis of a singe bash script, and used dx commands and variables

## Mon Apr 8 21:00:54 UTC 2019 **Ecoli Genome**

**IGV\_Session\_Maker generated output** Date Genome This page contains a link to an IGV session file. This file will allow the specified data to be streamed to IGV without the need to explicitly download the data. **IMPORTANT - IGV must be running on your local machine** *before* you click the link

Description: This session contain the file type sample 04/09/2019

The following files are include in the Session file

- /PAUSING/1096-no-chase\_S1\_L001\_R1\_001\_aligner/1096-no-chase\_S1\_L001\_R1\_001\_m14\_M30\_uniq.bam
- /PAUSING/TSS/1096-no-chase S1\_L001\_R1\_001\_m14\_M30\_finder/1096-no-chase\_S1\_L001\_R1\_001\_m14\_M30\_50\_51\_100\_MG1655\_median.bw

Click on the button below

Launch IGV with relevant BAM files

This link will work for 100 days from its date of generation

• Designed to be a helper applet that allows easy visualization of large files (bam,vcf,big-wig) by a locally running copy of IGV, without the need to download the entire files. It can be run standalone or incorporated into an workflow. By

### **Descriptive phrase**

### List of files

• /PAUSING/TSS/1096-no-chase\_S1\_L001\_R1\_001\_m14\_M30\_finder/1096-no-chase\_S1\_L001\_R1\_001\_m14\_M30\_50\_51\_100\_MG1655\_median\_peaks.bw





ABOUT US

**OUR RESEARCH** 

NIH CLINICAL CENTER

Search Principal Investigators





## Mikhail Kashlev, Ph.D. **Senior Investigator**

RNA Biology Laboratory

NCI/CCR



## Pausing Peak Tools



Building 560, Room 11-85A Frederick, MD 21702-1201

301-846-1798

kashlevm@mail.nih.gov



Biological system: Various microbial organisms Primary goal was to identify RNA pausing sites, from netSeq data, and correlate with: genome postion gene expression transcription start sites (TSS) specific sequence motifs protected read length Additionally, we needed the ability to compare the effect of different gene deletions.

## Pausing Peak Tool



## Pausing\_Peak\_Aligner

- Remove sequencing primers/adapters using cutadapt (java)
- Use molecular bar code to identify and remove duplicate molecules with BBmap (bin)
- Remove molecular bar code with cutadapt (java)
- Align vs genome with bowtie (bin)
- Get gene expression read count with Salmon (bin)

## Pausing Peak Tool

## Pausing\_Peak\_Finder

- Identify pause peaks from bam file modified samtools (bin)
- Generate big-wig files for location of 3' ends of reads
- Annotate peaks with info relative to genes or TSS from solite DB
- Generate Interactive web pages using DateTable and Plotly (javascript)

## Pausing Peak Tool

#### Escherichia coli - MG1655

#### **Pause Sites - Median Calculation Method**

Clear Column Searches Single Select - Graph Plot Log of Primary Data for Selected Ro Show 10 rows Copy CSV Excel Showing 1 to 10 of 407 entries          Peak       Locus       Gene       TSS       TSS_Offset       Gene         Yppe to x       Type to x       Type to x       From To       From         GeneA: (IGV - if alreay open)       GeneA: (EcoCyc)       GeneA: (EcoCyc)       Iocus         Bubmitt to Vienna       Submitt to RNA Structure       930.00         3429795       b3282       tsaC       3429701       -94       930.00         342978       b3994       folog       4082837       -141       2183.00         3219875       b2405       xapR       2519890       -15       260.00	se_S1_L001_R1_001_	01_R1_001_m14_M30 -r 50 -c 51 -w 100							
	Clear Colu Show 10 ro owing 1 to	mn Searches Sows Copy	Single Select	Excel	ot Log of Prima	ary Data for Sele	ected Row	end to WebL	.ogo
	-	Peak Type to x	Locus	Gene	TSS From To	TSS_Offset	Gene_Offset	Sense Type to x	Expr From
		Peak	Locus	Gene	TSS	TSS_Offset	Gene_Offset	Sense	Expr
	٢	3705690	b3544	dppA	3705893	-203	1569.00	SENSE	35.8
		GeneA: (IGV - if a	alreay open)						
		GeneA: (EcoCyc)							
		Locus:							
		Product:							
		Logo DNA:							
		Submitt to Vienna	Submitt to m	Fold Submitt to R	NA Structure				
	٢	3429795	b3282	tsaC	3429701	-94	930.00	SENSE	18.9
	٢	2170861	b2092	gatC	2171205	-344	-84.00	SENSE	47.4
	0	4082978	b3894	fdoG	4082837	-141	2183.00	SENSE	43.0
1	٢	2519875	b2405	xapR	2519890	-15	260.00	SENSE	3.14
		Peak	Locus	Gene	TSS	TSS_Offset	Gene_Offset	Sense	Expr
Sho	owing 1 to	o 10 of 407 entries							
P	Plot Primai	ry Data for Selecte	d Row						
									I
	0								

#### ŝ -200 Sal Peak -400 Raw -600 20 40 0

Copy/exp selected subsets o data -

### Data filtering and sorting

- Text
- Value Rar

### Expanding -Annotation

## Pausing Peak Tool



(based on selected row)

CREATED BY	Peter Fitzgerald (by running pausing_pe Aligner Method 3 )	ak_aligner_m3 in t
CREATED	Apr 9, 2019 3:35 PM	Using ta informat
MODIFIED	Apr 9, 2019 3:35 PM	and proc
TAGS	Escherichia x coli x NC_000913.	2 x
	Build	NC_000913.2
	Genus	Escherichia
	Species	coli
	stats01 Aligner	Bowtie 1.2.2
	stats02 Reads in this bam	3375586
	stats03 FASTQ Reads Input	3585806
PROPERTIES	stats04 Cutadapt Reads Output	3528248
	stats05 Clumpify Reads Output	3398154
	stats06 Reads Input	3398154
	stats07 Reads Unmapped	22568 (0.66%)
	stats09 Mapped	3375586 (99.34%

### ags to surface tion about files cesses (\*.bam)

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## **Tumor Mutational Burden**

### NATIONAL CANCER INSTITUTE **Center for Cancer Research**

#### CLINICAL TRIALS RESEARCH TRAINING

Home » Thoracic Surgery Branch » Haobin Chen, M.D., Ph.D.

## Haobin Chen, M.D., Ph.D.



Assistant Clinical Investigator Thoracic Surgery Branch

Dr. Chen's research focuses on developing novel epigenetic therapies for small cell lung cancer. He is board certified in internal medicine and board certified in medical oncology.

#### Areas of Expertise

1) lung cancer 2) epigenetics 3) molecular biology

**CCR** Central

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#### **CONTACT INFO**

Haobin Chen, M.D., Ph.D. Center for Cancer Research National Cancer Institute Building 10-CRC, Room 3-5848 Bethesda, MD 20892 240-760-6177 Ph: haobin.chen@nih.gov

PERMALINK



## **Tumor Mutational Burden**

### Cancer Cell Article

### Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in Combination with Ipilimumab in Small-Cell Lung Cancer

Matthew D. Hellmann,<sup>1,11,12,\*</sup> Margaret K. Callahan,<sup>1,11</sup> Mark M. Awad,<sup>2</sup> Emiliano Calvo,<sup>3</sup> Paolo A. Ascierto,<sup>4</sup> Akin Atmaca,<sup>5</sup> Naiyer A. Rizvi,<sup>6</sup> Fred R. Hirsch,<sup>7</sup> Giovanni Selvaggi,<sup>8</sup> Joseph D. Szustakowski,<sup>9</sup> Ariella Sasson,<sup>9</sup> Ryan Golhar,<sup>9</sup> Patrik Vitazka,<sup>9</sup> Han Chang,<sup>9</sup> William J. Geese,<sup>9</sup> and Scott J. Antonia<sup>10</sup>

Cancer Cell. 2018 May 14;33(5):853-861.e4. doi: 10.1016/j.ccell.2018.04.001. Epub 2018 May 3.



## **Tumor Mutation Burden Workflow**



## **Tumor Mutational Burden Workflow**



## **Tumor Mutational Burden**

- Annotation withsnpEff and snpSift runs on TNsnv VCF and Strelka2 VCF
  - Cosmic67 Catalogue of Mutations In Cancer, Welcome Sanger Institute
  - ExACv03 Exome Aggregation Consortium, Broad Institute
  - 1000 Genomes The International Genome Sample Resource
  - dbSNPv138 Single Nucleotide Polymorphism Database
- Filter Process
  - Missense mutations AND Cosmic67, OR
  - Missense mutations AND NOT in 3 dbs (ExACv03, 1000 Genomes, dbSNPv138)
- Filter Process Count represents Tumor Mutational Burden Value

# Salmon RNAseq

## Salmon – Don't count . . . quantify! **Overview**

Salmon is a tool for quantifying the expression of transcripts using RNA-seq data. Salmon uses new algorithms (specifically, coupling the concept of quasi-mapping with a two-phase inference procedure) to provide accurate expression estimates very quickly (i.e. wicked-fast) and while using little memory. Salmon performs its inference using an expressive and realistic model of RNA-seq data that takes into account experimental attributes and biases commonly observed in real RNA-seq data.

### **Workflow - Three Separate Applets**

- stage designed to run with separate node for each sample.
- Given a set of "read count files" (quant.sf) generate a combined count matrix or both transcripts and genes.
- bootstrap distribution.
- Hand off DEG and other tertiary analyses to Shiny Apps iDEP and/or Biojupies.

• Use Salmon to "align" to Gencode transcriptome and generate both quant files (Read Count and TPM per transcript) for both transcripts and genes. Additionally, gather data from optional bootstrap for subsequent use in Sleuth DEG program. This

• Generate interactive HtML pages for each sample or combined samples for gene count, with graphic representaion of





## Salmon RNAseq Elements

- Two Assets
  - salmon\_0.12.0\_asset
  - r\_base\_3.5.2\_asset
- **Cloud Workstation**

https://wiki.dnanexus.com/developer-tutorials/cloud-workstations

About this Applet About GAU About BTEP

#### Usefull Links

See a complete summary at DNAnexus Job Monitor.

#### Input Transcript Quant File Summary

- 1. brain\_rep2\_quant.sf
- 2. brain rep3 quant.sf
- brain\_rep1\_quant.sf
- muscle\_rep2\_quant.sf
- 5. muscle\_rep3\_quant.sf
- muscle\_rep1\_quant.sf

#### Input Gene Quant File Summary

- brain\_rep2\_quant\_genes.sf
- 2. brain\_rep3\_quant\_genes.sf
- 3. brain rep1 quant genes.sf
- 4. muscle\_rep2\_quant\_genes.sf
- 5. muscle\_rep3\_quant\_genes.sf
- muscle rep1 quant genes.sf

#### Instructions

- 1. Download Expression Tables from DNAnexus
  - Download RAW Counts Table for Transcripts

  - Download RAW Counts Table for Genes
- Download <u>Design Table</u>
- 3. Select Analysis Site and Upload Expression Table



### **Gene Expression Analysis**

 Download TPM (Transcripts Per Million) Counts Table for Transcripts Download TPM (Transcripts Per Million) Counts Table for Genes

2. Download and Edit Design Table in Excel or Text Editor for use in iDEP

 Upload an Expression Table File to BioJupies Upload an Expression Table File to iDEP

Created by: Genome Analysis Unit

## Get Help for an Applet salmon\_spg\_wf

usage: dx run /SalmonWF/Applications/salmon\_spg\_wf [-iINPUT\_NAME=VALUE ...]

Applet: salmon\_spg\_wf

salmon\_spg\_wf

Inputs:

salmon\_idx\_file: -isalmon\_idx\_file=(file)

Bootstrap Value: [-ibootstrap\_value=(int, default=0)] Number of bootstraps to preform

Outputs:

Batch of quant\_sf files: quant\_sf\_s (array:file) Salmon quant.sf files.

Batch of quant\_genes\_sf files: quant\_genes\_sf\_s (array:file) Salmon quant.genes.sf files.

Batch of abundance\_h5 files: abundance\_h5\_s (array:file) Wasabi derived files need for Sleuth.

### Commands on Terminal

module load DNAnexus dx login dx select "DEMO\_Project" dx ls -I /SalmonWF/Applications/salmon\_spg\_wf dx run /SalmonWF/Applications/salmon\_spg\_wf -- help

fastq\_gz\_list: -ifastq\_gz\_list=(file) [-ifastq\_gz\_list=... [...]]

<u>Salmon Results Directories (tar.gz)</u>: quant\_sf\_files (array:file)

## Upload Data for salmon\_spg\_wf

Commands on Terminal to Upload Data

dx mkdir /demo\_data dx upload \*.fastq.gz --destination /demo\_data dx upload yeast\_S288C\_salmon\_idx.tar.gz --destination /demo\_data/yeast\_S288C\_salmon\_idx.tar.gz dx ls -l /demo data

Project: GAU\_Development (project-FVqKF6j0v1xv6fxK15Bzj9B2) Folder : /demo\_data Last modified Name (ID) Size State 2019-04-10 13:43:20 81.42 MB DST1\_G418\_B\_R1.fastq.gz (file-FXg2fG80v1xkp89Y9jz3xvzY) closed closed 2019-04-10 13:43:20 89.09 MB DST1\_G418\_B\_R2.fastq.gz (file-FXg2fJ80v1xx8p178Vv7B3XG) 2019-04-10 13:43:25 108.73 MB DST1\_G418\_C\_R1.fastq.gz (file-FXg2fJj0v1xq0189BbQpB6Fg) closed 2019-04-10 13:43:30 116.46 MB DST1\_G418\_C\_R2.fastq.gz (file-FXg2fK80v1xb8QJ47kG69jjG) closed 2019-04-10 14:06:42 135.66 MB yeast\_S288C\_salmon\_idx.tar.gz (file-FXg319Q0v1xkpVV6BvgfQy2G) closed





## Run salmon spg\_vf

Commands on Terminal to run and see results for salmon\_spg\_wf

```
dx run /SalmonWF/Applications/salmon_spg_wf \
-ifastq_gz_list=/demo_data/DST1_G418_B_R1.fastq.gz \
-ifastq_gz_list=/demo_data/DST1_G418_B_R2.fastq.gz \
-ifastq_gz_list=/demo_data/DST1_G418_C_R1.fastq.gz \
-ifastq_gz_list=/demo_data/DST1_G418_C_R2.fastq.gz \
-isalmon_idx_file=/demo_data/yeast_S288C_salmon_idx.tar.gz \
-ibootstrap_value=0 \
--destination /demo_result
dx ls -l /demo_result
```

Project: GAU\_Development (project-FVqKF6j0v1xv6fxK15Bzj9B2) Folder : /demo\_result logs/ Last modified Name (ID) State Size 2019-04-10 14:13:55 81.93 KB DST1\_G418\_B\_abundance.h5 (file-FXg33yj0bKxY49FpJvfKb0fX) closed 2019-04-10 14:13:55 218.73 KB DST1\_G418\_B\_quant.sf (file-FXg33y00bKxv4BbZ5X7k0k3V) closed 2019-04-10 14:13:55 179.09 KB DST1\_G418\_B\_quant\_genes.sf (file-FXg33yQ0bKxXG5PXJv13fBb5) closed 2019-04-10 14:13:55 613.44 KB DST1\_G418\_B\_salmon.tar.gz (file-FXg33x80bKxxBpp8JvVK4qBF) closed 2019-04-10 14:13:55 82.36 KB DST1\_G418\_C\_abundance.h5 (file-FXg34000bKxqZVvY9VQXJp6G) closed 2019-04-10 14:13:55 219.41 KB DST1\_G418\_C\_quant.sf (file-FXg33z80bKxVbfF3BF3gXY6K) closed 2019-04-10 14:13:55 179.46 KB DST1\_G418\_C\_quant\_genes.sf (file-FXg33zj0bKxVq47P9QxyQgXY) closed 2019-04-10 14:13:55 616.78 KB DST1\_G418\_C\_salmon.tar.gz (file-FXg33yj0bKxXG5PXJv13fBbJ) closed

## Markdown Documentation



### MacDown Markdown Editor for Mac OS https://macdown.uranusjr.com

### <!-- dx-header --> # salmon\\_spg\\_wf

This application takes

[Created by GAU](https://gau.ccr.cancer.gov)
<!-- /dx-header -->

<!-- Insert a description of your app here -->

# About Applet ...
Salmon Scatter-Process\_Gather Workflow

This applet process a batch of pair-end \_\*.fastq.gz\_ read files and runs [Salmon](https://salmon.readthedocs.io/en/latest/).

To use the developwer's words: > Salmon is a tool for **\*\*wicked-fast\*\*** transcript quantification from RNA-seq data. It requires a set of target transcripts (either from a reference or denovo assembly) to quantify. All you need to run Salmon is a FASTA file containing your reference transcripts and a (set of) FASTA/FASTQ file(s) containing your reads. Optionally, Salmon can make use of pre-computed alignments (in the form of a SAM/BAM file) to the transcripts rather than the raw reads.

Developed by: [Fitzgerald, Peter (NIH/NCI) [E]] (<fitzgepe@mail.nih.gov>) and [McIntosh, Carl (NIH/NCI) [E]] (<mcintoshc@mail.nih.gov>)

Group: [Genome Analysis Unit](https://gau.ccr.cancer.gov)

**##** Required Input Files

\*\*\_FASTQ Gzip Compressed Paired-end Files\_\*\* - A batch sample PE read files with the form \_\*\\_R1.fastq.gz\_ and \_\*\\_R2.fastq.gz\_.

\*\*\_Salmon Index tar.gz File\_\*\* - A Salmon Indexed genome files with the form
\_\*\\_salmon\_idx.tar.gz\_ .

**## Input Parameters** 

\*\*\_Output Folder\_\*\* - Provide an output directory name for result files.

\*\*\_Instance type\_\*\* - Asking for more computer resources will reduce run time
and will cost more.

#### salmon\_spg\_wf

This application takes

Created by GAU

#### About Applet ...

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Developed by: Fitzgerald, Peter (NIH/NCI) [E] and McIntosh, Carl (NIH/NCI) [E]

Group: Genome Analysis Unit

#### **Required Input Files**

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Salmon Index tar.gz File - A Salmon Indexed genome files with the form \*\_salmonidx.tar.gz\_.

#### **Input Parameters**

Output Folder - Provide an output directory name for result files.

Instance type - Asking for more computer resources will reduce run time and will cost more.

#### **COMMON Input Parameters**

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#### CONFIGURE: SALMON\_SPG\_WF (APPLET)

✓ SSH is allowed for this app.

salmon\_spg\_wf

#### About Applet ...

Salmon Scatter-Process\_Gather Workflow

This applet process a batch of pair-end \*.*fastq.gz* read files and runs Salmon.

To use the developwer's words:

Salmon is a tool for **wicked-fast** transcript quantification from RNA-seq data. It requires a set of reference or de-novo assembly) to quantify. All you need to run Salmon is a FASTA file containing (set of) FASTA/FASTQ file(s) containing your reads. Optionally, Salmon can make use of pre-con SAM/BAM file) to the transcripts rather than the raw reads.

Developed by: [Fitzgerald, Peter (NIH/NCI) [E]] (fitzgepe@mail.nih.gov) and [McIntosh, Carl (NIH/NCI) [E]] (mcintoshc@mail.nih.gov) Group: Genome Analysis Unit

#### **Required Input Files**

*FASTQ Gzip Compressed Paired-end Files* - A batch sample PE read files with the form \*\_*R1.fastq.gz* and \*\_*R2.fastq.gz*. *Salmon Index tar.gz File* - A Salmon Indexed genome files with the form \*\_*salmon\_idx.tar.gz*.

#### Input Parameters

Output Folder - Provide an output directory name for result files.

Instance type - Asking for more computer resources will reduce run time and will cost more.

#### **COMMON** Input Parameters

Bootstrap Value (integer)

Salmon has the ability to optionally compute bootstrapped abundance estimates. This is done from the counts assigned to the fragment equivalence classes, and then re-running the optimize VBEM, for each such sample. The values of these different bootstraps allows us to assess tech estimates we produce. Such estimates can be useful for downstream (e.g. differential expression uncertainty estimates. This option takes a positive integer that dictates the number of bootstraps samples computed, the better the estimates of variance, but the more computation (and time)

#### **Output Files**

Per sample file, the following files are produced:

- Salmon Results Directory tar.gz File A file of form \_\_salmon.tar.gz\_\*. This is a directory that is tar.gz compressed and r
   xzf tarfile. These files are provided if you wish to do some custom analysis. Otherwise, it can be ignored.
- Salmon's Quant.sf File A file of form \_quant.sf\_\*. This file contains counts.
- Kallisto's abundance.h5 File A file of form \_\_abundance.h5\_\*. This files is transformed from Salmon's quant.sf file in a file is provied for downstream analysis using Sleuth.

#### Additional Files:

• Script File - This file is under development.

platform.dnanexus.com/projects/FVqKF6j0v1x

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	* Fields are required			-
	Name	salmon_spg_wf	*	
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by resampling (with replacement)				
inical variance in the main abundance				
o samples to compute. The more				
needs to be expanded using the command tar -				
Kallisto's HDF (Hierarchical Data Format) file. This				
			Reset to applet defaulte	



## **DNAnexus Developer Pages**

### https://wiki.dnanexus.com/Developer-Portal

https://wiki.dnanexus.com/Developer-Tutorials/Intro-to-Building-Apps

- DNAnexus CCR Pilot (https://gau.ccr.cancer.gov/dna-nexus-pilot-program/)
- Slack Channel for CCR\_DNAnexus Pilot (dnaxpilot.slack.com) (help, general, development)
- Creating Assets: <u>https://gau.ccr.cancer.gov/about-dnanexus-asset/</u>
- Building
- Example About Pages:
  - <u>https://gau.ccr.cancer.gov/rnaseg\_salmon/</u>
  - https://gau.ccr.cancer.gov/salmon spg wf/
  - <u>https://gau.ccr.cancer.gov/quant\_sf2express\_table/</u>

