Bulk RNA-Seq Workshop Manual

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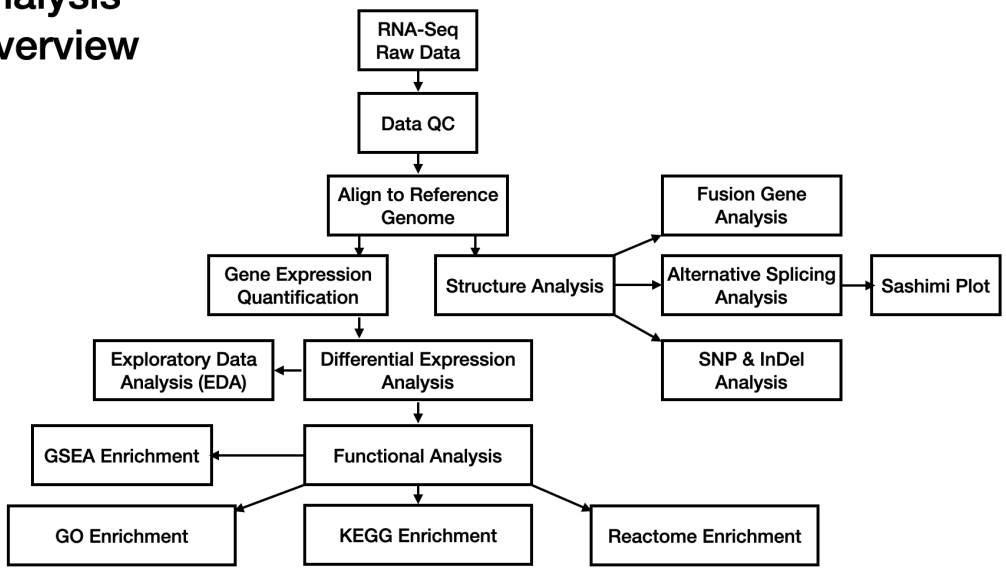
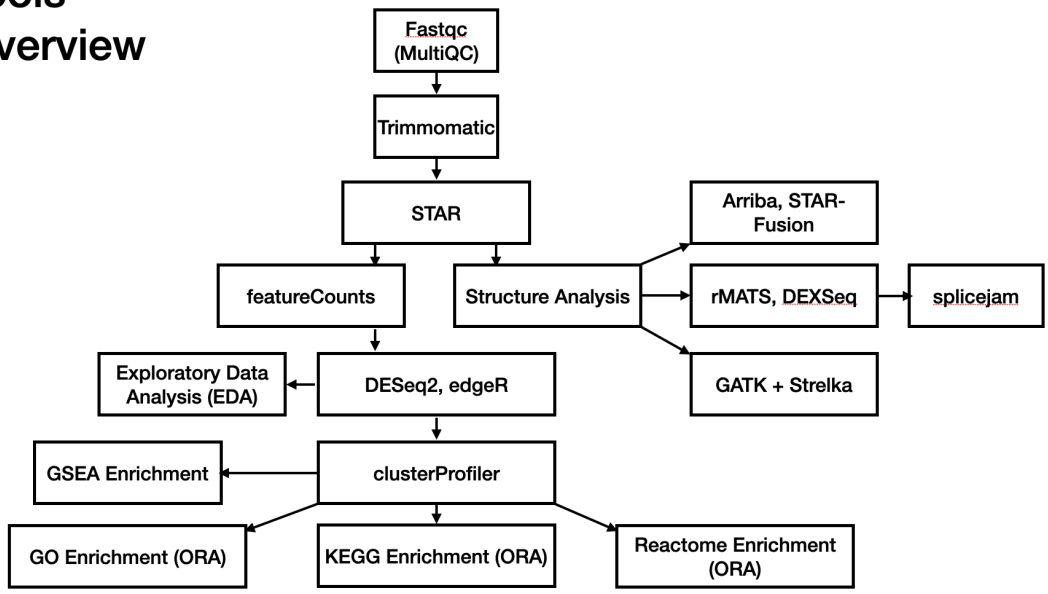
https://github.com/elinorv21/RNA-Seq_workshop/

Analysis Overview



Tools Overview



Data analysis workflow:

On Savio (Scratch directory storage limit: Unlimited; Home directory storage limit: 10GB), your computer, or Galaxy (Storage limit: 250GB):

1. Upload RNA-seq Data (Illumina platform) to Savio HPC server - Fastq files
2. Quality Control of Reads (**Software: FastQC, MultiQC**)
3. Trimming and Filtering Reads (**Software and bash code: Trimmomatic, slurm files**)
4. Aligning/Mapping Reads to a Reference Genome (**Software and bash code: STAR, slurm file**)
5. Calculating Expression levels: Quantification (**Software and bash code: featureCounts, slurm file**)
6. Differential Expression (Comparing Gene Expression Between Conditions) (**Software and R code: DESeq2, edgeR, mouse_dge.R**)
7. Functional Analysis (**Software and R code: clusterProfiler, mouse_cluster.R**)
8. Alternative Splicing Analysis (**Software and R code: rMATS, DEXSeq, splicejam, ggsashimi; mouse_rmats.R**)
9. Visualization of Data (**R Code: mouse_dge.R, mouse_cluster.R, mouse_rmats.R, splicejam, ggsashimi**)

Setting Up Your System

1. Logging On
2. Learning How to Edit Your Files on the Terminal
3. Setting up Conda
4. Creating a Conda Environment and Activating It
5. Uploading Files
6. Learning About Bash
7. Learning About SLURM files

1. Logging On

HPC computing on Savio:
To obtain a Savio account, you need access to at least one project. A user account will be created for you automatically when you are granted access to a project. It usually takes a few days to get your account.

Instructions for obtaining HPC Savio account:
https://docs-research-it.berkeley.edu/services/high-performance-computing/getting-account/

A. Quick Instructions for Logging On

("$" is the terminal prompt; don't type $)

Log into Savio from local machine terminal window using a bash command:
Bash:

$ ssh username@hpc.brc.berkeley.edu (password is your pin+authenticator digits)

To logout of Savio using a bash command:

$ logout

For information regarding logging into Savio, see:

https://docs-research-it.berkeley.edu/services/high-performance-computing/user-guide/logging-brc-clusters/

After you've logged onto Savio, move to Scratch:

$ cd /global/scratch/users/your-username

B. Step-By-Step Instructions for Logging On

Logging onto Savio (Berkeley's HPC cluster) from a terminal on a local computer:

$ ssh username@hpc.brc.berkeley.edu

Password: Your-pin + Google Authenticator (phone app) #

Example: Google Authenticator says: "105 149" and your pin is "9876", so your password is: 9876105149

If successful, you'll see:

Last login: Tue Jun 10 16:27:25 2025 from "IP address"
[your-username@ln003 ~]$

The "~" means that you are located in your home directory. Switch immediately to your "scratch" directory:

$ cd /global/scratch/users/your-username

You'll see:

[your-username@ln003 your-username]$

On Savio, the scratch directory is called "your-username" - that's why you now see "your-username" vs "~".

2. Learning How to Edit Your Files on the Terminal

Creating and Editing Files on the Terminal: The Nano Editor

To create/open a file for editing:
$ nano .condrc (the cursor is the "$"; the file's name is ".condarc")

To edit:
Just start typing and use up/down/sideways arrows to move around

To save the file:
Press "control" and o

To close the file:
Press "control" and x

Creating and Editing Files on the terminal: The Vim Editor
To create/open a file for editing:
$ vi .condarc (the cursor is the "$"; the file's name is ".condarc")

To edit:
Type "i": Enters editing mode (from the left). Now you can type your script.

Hit "esc": Exits editing mode. You can use up/down/sideways arrows to move around.
Now, type "dd": Deletes an entire row wherever the cursor is.
Type "x": Deletes a single character where the cursor is.

Type "i" again (Enter editing mode from the left) or type "a" (Enter editing mode on the right).

Hit "esc": Exits editing mode.
Type "ZZ": Saves and closes the file.

3. Setting up Conda

Move to your home directory:

$ cd

Obtain Miniconda:

$ wget https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86_64.sh

Install Miniconda:

$ bash Miniconda3-latest-Linux-x86_64.sh

Activate Miniconda:

$ source ~/.bashrc

Verify that Miniconda works:

$ conda --version

Update and install Conda channels:

$ conda update -n base -c defaults conda
$ conda config --add channels defaults
$ conda config --add channels bioconda
$ conda config --add channels conda-forge

Set-up your .condarc file (this directs your installations to scratch first, then to home if needed)**:**

Step 1. You need to first make some directories because you are installing software in your scratch directory not your home directory (remember to replace “your-username” with your actual username):

$ mkdir -p /global/scratch/users/your-username/conda/pkgs/
$ mkdir -p /global/scratch/users/your-username/conda/envs/

Next, move to your home directory unless you are already there:
$ cd /global/home/users/your-username/

Step 2. Use nano or vim to create/edit your .condarc file (I’m using vim) in your terminal (see above for nano and vim description):

$ vi .condarc (if you use nano, the command is: $ nano .condarc; remember “$” is the prompt)

Here are the contents of the .condarc file:

```
channels:
  - https://software.repos.intel.com/python/conda
  - conda-forge
  - bioconda
  - defaults

pkgs_dirs:
  - /global/scratch/users/your-username/conda/pkgs/
  - /global/home/users/your-username/miniconda3/pkgs/

envs_dirs:
  - /global/scratch/users/your-username/conda/envs/
  - /global/home/users/your-username/miniconda3/envs

auto_activate_base: false

solver: libmamba
```

4. Creating a Conda Virtual Environment and Activating It

Check your current Python and R versions:

$ python --version (gives “Python 3.12.2”, for example)
$ R --version (gives “R 4.4.3”, for example)

Create a Conda virtual environment with a specific Python and R version:

$ conda create -n mouse_env python=3.12.2 r-base=4.4.* -c conda-forge -c bioconda

(“*” means the latest of the 4.4 versions)
Or, you can type (“$” and “>” are prompts; be sure to type the “\” when shown):

```
$ conda create -n mouse_env \
>  python=3.12.2 \
>  r-base=4.4.*      \
```

> -c conda-forge -c bioconda

Activate environment:

$ conda activate mouse_env

To deactivate environment:

$ conda deactivate

Installing software in your virtual environment with Conda:

Example: Install the software "samtools" into mouse_env:
$ conda activate mouse_env
$ conda install -c bioconda samtools (You only need to install once)

Other ways of installing software in your virtual environment:
1. Try using pip:
$ pip install samtools

2. Create a new conda environment for the specific software:

$ conda create -n samtools_env -c conda-forge -c bioconda samtools
(This creates a conda environment with the samtools software package)

5. Uploading/Downloading Files onto/from Savio

Method A: Use Open OnDemand:
Use Open OnDemand (upload/download files from local computer to Savio cluster) by clicking on the following website and then logging into your Open OnDemand account using your UC Berkeley credentials:

https://ood.brc.berkeley.edu

Method B: Use Globus:

https://docs-research-it.berkeley.edu/services/high-performance-computing/user-guide/data/transferring-data/using-globus-connect-savio/

Method C: Downloading files from online using wget:

Download file from a sequencing center's website:

Substitute actual portal for "novogene_data_portal"
wget https://novogene_data_portal.novogene.com/myfile.fastq.gz
("gz" means gunzipped file)

Download file from NCBI:

Example:

wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR102/004/SRR10207204/SRR10207204_1.fastq.gz

6. Learning About Bash: Common Bash Commands for the Terminal

Change to the Scratch directory from the Home directory, from a limited space (backed up) to an “unlimited” space (not backed up):

$ cd /global/scratch/users/your-username

Show your location:
$ pwd

Move backwards to previous directory:
$ cd .. (yes, you type two dots)

Move forwards to a directory within current directory (nested directories):
$ cd directory-name

Example:
$ pwd (gives us: toy_data/star_results/bam_files)
$ cd .. (moves us back to toy_data/star_results)
$ cd .. (moves us back to toy_data)

Then,
$ cd star_results (moves us forward to star_results folder), or
$ cd star_results/bam_files (moves us to bam_files)

Make a folder/directory:
$ mkdir folder-name

Copy a file:
$ cp original-file new-file

Remove a file:
$ rm file-name

Remove a directory:
$ rmdir directory-name

If folder is not empty, we need to empty it:
$ cd directory-name
$ rm *
$ cd ..
$ rmdir directory-name

Move a file to a new location/directory:
$ mv file-name new-location

List your files in a directory:
$ ls
or
$ ls -la

List the contents of a file:
cat file-name

Example:
cat hello.txt

7. Learning About SLURM files

Run a SLURM job:
Typically, you can run the SLURM file in your scratch base directory (in case, you are using relative paths):
$ cd /global/scratch/users/your-username

$ sbatch my-file.sh (using the file "my-file.sh"; .sh means it is a bash or SLURM file)

Savio replies with your job number

Check to see if your job finished:

$ squeue -u your-user-name

Checking on the status of your job:

$ ls <— you'll see *.err and *.out files

$ cat *.err (or, cat *.out) or (if multiple .err and .out files),

$ cat job-name-job-number.err
$ cat job-name-job-number.out

These commands give information on what the computer has accomplished so far or any errors.

The difference between sbatch and srun:

Use sbatch for SLURM files you already wrote, software like trimmomatic or rMATS with many files, and srun when debugging code:

sbatch trim.sh (using a bash code file and the trimmomatic software)
srun test.R (debugging a file)

To use srun:
$ srun --pty -A Savio-project-you-joined -p savio2 (or another) -t 01:00:00 (time=1 hour) bash -i