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RNA Seq Walkthrough

Introduction

Purpose: provide a step-by-step, end-to-end RNA Seq analysis walkthrough.

Environment:

- All commands and scripts are made to run on the UCSC `courtyard` server, but should work in any bash environment.
  ```
  OS:      Red Hat 4.8.5-39
  Python3: 3.6.8
  R:       3.6.0
  ```
- If running on Windows try the Linux Subsystem for Windows or a Docker instance. Either of these can be used to create Linux environments on Windows (albeit with some drawbacks). I suspect any of the recent Ubuntu releases (16.04, 18.04, 20.04) should work.
- If using `courtyard` (or any remote server) it's **highly recommended** that you start commands in a `screen` environment. This is a very simple step and provides at least two substantial advantages:
  1. A disruption to your `ssh` connection will not result in termination of whatever you're running. (Execution can take many hours to days so this is a likely occurence)
  2. You can open multiple windows in the same terminal and switch between them, running different commands in each.

In the examples below I will always be working in a `screen` window, however, I will only demonstrate how to start one in section `0.0`.
More on using screens.

The Data: I will be using paired-ended 151 bp Illumina sequence data starting in fastq.gz format.

Step 0: Getting the Data

In cases where it seems to make sense I will include the generic command followed by the command I am using for actual data as an example. Otherwise I will just show the command I am using.

0.1 From an SFTP Server

0. Connect to courtyard, start a `screen` and navigate to your directory

   ```
   $ ssh <username>@courtyard.gi.ucsc.edu
   $ ssh emalekos@courtyard.gi.ucsc.edu
   # enter password at prompt

   $ screen
   ```

```
$ cd <directory/to/transfer/data/to>
$ cd /public/groups/carpenterlab/people/emalekos/
```

1. Connecting to data storage on remote server

```
$ sftp <username>@<hostname>
$ sftp emalekos@sftp.genewiz.com
# enter password at prompt
```

2. We are now in an `sftp` environment (`$` -> `sftp`). Navigate to data folder, use `ls` to list available directories

```
sftp> ls
30-422456969

# Now I navigate to the folder I found above
sftp> cd/30-422456969
sftp> ls
00_fastq

# Found another folder, I'll navigate into that.
# This time I find the data files.
sftp> cd 00_fastq/
sftp> ls -lh
```

If you are in the data folder you should see something like:

```
    -rwxr--r--    ? 0        0            2.8G Jan  6 14:04 1M-Ctl-
AM_R1_001.fastq.gz
    -rwxr--r--    ? 0        0            3.0G Jan  6 14:04 1M-Ctl-
AM_R2_001.fastq.gz
    -rwxr--r--    ? 0        0            3.0G Jan  6 14:04 1M-LPS-
AM_R1_001.fastq.gz
    -rwxr--r--    ? 0        0            3.1G Jan  6 14:04 1M-LPS-
AM_R2_001.fastq.gz
    -rwxr--r--    ? 0        0            3.0G Jan  6 14:04 1W-Ctl-
AM_R1_001.fastq.gz
    -rwxr--r--    ? 0        0            3.3G Jan  6 14:13 1W-Ctl-
AM_R2_001.fastq.gz
    -rwxr--r--    ? 0        0            3.0G Jan  6 14:13 1W-LPS-
AM_R1_001.fastq.gz
    -rwxr--r--    ? 0        0            3.2G Jan  6 14:13 1W-LPS-
AM_R2_001.fastq.gz
    -rwxr--r--    ? 0        0            2.1G Jan  6 14:14 2M-CSE-
AM_R1_001.fastq.gz
    -rwxr--r--    ? 0        0            2.3G Jan  6 14:14 2M-CSE-
AM_R2_001.fastq.gz
```

1. To copy the files from this server to your workspace use the `get` command. Here I give some examples

```
 # To copy a single file
 sftp> get 1M-Ctl-AM_R1_001.fastq.gz

 # To copy all files
 sftp> get *

 # To copy all files ending in 'fastq.gz'
 sftp> get *fastq.gz

 # To copy all files starting with 'LPS' somewhere in the middle
 sftp> get *LPS*

 # To copy all files starting with 'LPS' and with '_R1' somewhere
before the end
 sftp> get LPS*_R1*
```

NOTE: '`*`' performs wildcard expansion - it can fill in for any characters and is useful for pattern matching. When you use it it's good to check which patterns it's actually matching by double tapping the `Tab` key. This will list everything that matches. If you see the files you want press `Enter` to execute.

0.2 From Google Drive

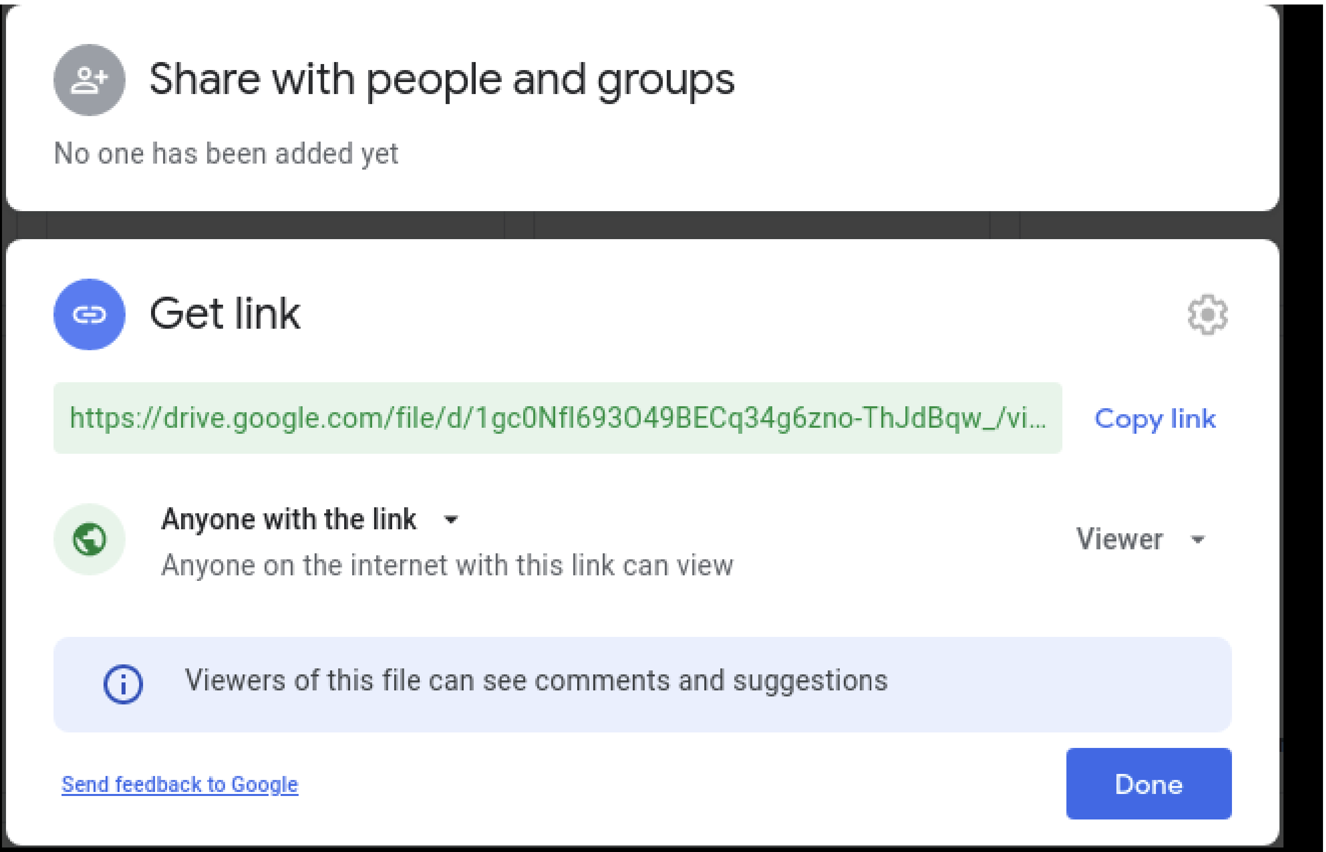
You can use this method to transfer data files from your Google Drive account to `courtyard`. It works but is somewhat clunky, and there may be a better way. For instance this method transfers one file at a time, if all relevant files could be zipped together only one command would be required.

1. We will use the python package `gdown`. The first time you use this you will have to install it

```
 pip3 install gdown --user
```

2. Get the share link for your Gdrive file

   - **IMPORTANT** - make sure the the file is accessible to "Anyone with the link"
   - The relevant part of the link is the string of characters between `/d/` and `/view/`
   - In the example below this is what we want: `1gc0Nfl693O49BECq34g6zno-ThJdBqw_`



Share with people and groups

No one has been added yet

Get link

https://drive.google.com/file/d/1gc0Nfl693O49BECq34g6zno-ThJdBqw_/vi...

Anyone with the link

Anyone on the internet with this link can view

Viewer

Viewers of this file can see comments and suggestions

3. Run with python3

   - Now you can start a python3 session and copy the files over

```
  $ python3

  # Now in python shell
  >>> import gdown
  >>> url="https://drive.google.com/uc?
id=1gc0Nfl693O49BECq34g6zno-ThJdBqw_"
  >>> output="desired_filename.fastq.gz"
  >>> gdown.download(url, output, quiet=False)
```

   - Alternatively you can open a text editor and write a script like this one.

```
  import gdown
  url_prefix = "https://drive.google.com/uc?id="
  suffix = ".fastq.gz"
  filedict = {"file_1_1" : "1gc0Nfl693O49BECq34g6zno-ThJdBqw_",
              "file_1_2" : "###################",
              "file_2_1" : "###################",
              "file_2_2" : "###################"}

  for key, value in filedict.items():
      gdown.download(url_prefix + value, key + suffix,
quiet=False)
```

After changing the entries in `filedict` to your desired filenames and corresponding links, run with:

```
python3 file.py
```

0.3 Make Smaller Files for Pipeline Practice (OPTIONAL)

You may want to practice running through the pipeline with a reduced dataset. This would allow you to troubleshoot much more quickly than if you tried with all of your data at once. We can make some reduced, but still functional `fastq.gz` files with the following command

```
zcat <file.in> | head -n <# of lines> | gzip > <file.out>

zcat full_file.fastq.gz | head -n 10000000 | gzip > reqduced_file.gz
```

This example takes the first `10000000` lines of the input file (or the first `2500000` fastq entries). The resulting gzipped files is ~200 MB. Adjust `<# of lines>` as you see fit, but make it divisible by 4 to avoid cutting off fastq entries.