May 3rd, 2010

For tomorrow's class, you need to complete these preliminary steps.
To simplify this task, all the raw files or links are present here:
http://physiology.med.cornell.edu/faculty/mason/lab/data/NGS/

1. **Download the ENSEMBL FTF Annotation file.**

   Follow the link for the GTF file from the Mason Lab page:
   ftp://ftp.ensembl.org/pub/current_gtf/homo_sapiens/Homo_sapiens.GRCh37.57.gtf.gz
   Once downloaded, you must unzip the file:
   ```
   ]gunzip Homo_sapiens.GRCh37.57.gtf.gz
   ```

2. **Download the Bowtie BWT genome**
   Follow the link for the Bowtie genome, located here:
   ftp://ftp.cbcb.umd.edu/pub/data/bowtie_indexes/hg19.ebwt.zip
   Once downloaded, you must unzip the file
   ```
   ]unzip hg19.ebwt.zip
   ```

   You should see:
   ```
   Archive:  hg19.ebwt.zip
     inflating: hg19.1.ebwt
     inflating: hg19.2.ebwt
     inflating: hg19.3.ebwt
     inflating: hg19.4.ebwt
     inflating: hg19.rev.1.ebwt
     inflating: hg19.rev.2.ebwt
     inflating: make_hg19.sh
   ```

3. **Download Bowtie and add to your PATH.**
   Go to: http://sourceforge.net/projects/bowtie-bio/files/
   Find the appropriate file for your operating system (if 64-bit, choose the 64-bit files. If you are in Linux, choose Linux).

   Unzip the Bowtie executable
   ```
   ]unzip bowtie-0.12.5-macos-10.5-x86_64.zip
   ```

   Add Bowtie to your PATH, so that TopHat can use it
   ```
   ]export PATH=$PATH:/Users/chm2042/Desktop/bowtie-0.12.5/
   ```

4. **Download and compile TopHat**
   Download this file: http://tophat.cbcb.umd.edu/downloads/tophat-1.0.13.tar.gz

   Unzip and untar your file:
   ```
   ]gunzip tophat-1.0.13.tar.gz
   ]tar –xvf tophat-1.0.13.tar
   ```

Go into the directory and compile TopHat

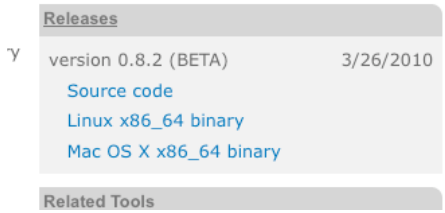
```
]cd tophat-1.0.13
]./configure
]make
]make install
```

5. **Download the example data file**

   Download some example RNA-Seq data:
   http://physiology.med.cornell.edu/faculty/mason/lab/data/NGS/burge_liver.fq
   http://physiology.med.cornell.edu/faculty/mason/lab/data/NGS/burge_brain.fq

6. **Download the cufflinks binary files**
   Go to:
   http://cufflinks.cbcb.umd.edu/tutorial.html

   Select the appropriate operating system binary file and download the file:



   Double-click the file to unzip it.

If you have a 32-bit Mac, you will need to compile your own cufflinks (to create files for #6). See here:

1. **Download and compile Boost Libraries**
   Download the appropriate files from here:
   http://sourceforge.net/projects/boost/files/boost/1.42.0/

   For example, most users will use:
   http://sourceforge.net/projects/boost/files/boost/1.42.0/boost_1_42_0.tar.gz/download

   Unzip and untar your boost libraries file:

```
]gunzip boost_1_42_0.tar.gz
]tar –xvf boost_1_42_0.tar
```

   Go into the directory and compile the boost libraries

```
]cd boost_142_0/
```

```
]./bootstrap.sh --
prefix=/Users/chm2042/Desktop/boost_1_42_0/booster
]./bjam install
```

2. **Download and compile Cufflinks with Boost**
   Download the Cufflinks
   http://cufflinks.cbcb.umd.edu/downloads/cufflinks-0.8.1.tar.gz

   Unzip and compile Cufflinks with Boost Libraries

```
]gunzip cufflinks-0.8.2.tar.gz
]cd cufflinks-0.8.2/
]./configure –-prefix=/Users/chm2042/Desktop/cufflinks-0.8.2/  --
with-boost=/Users/chm2042/Desktop/boost_1_42_0/booster/

]make
]make install
```

If you have any questions, feel free to email me at chm2042@med.cornell.edu