

Program

Trimmomatic

Version

qc: 2019.01.29

trimmomatic: 0.39

Module

Trimmomatic requires the module qc. To load the module, use the following command **module load qc**

To check that the module was successfully loaded, use the command **module list**

If successfully loaded, you should see the following:

Currently Loaded Modulefiles:

1) slurm/slurm_cluster/19.05.7 2) fastp/0.20.0(default) 3) qc/2019.01.29(default)

Note, that if you are running trimmomatic with a script, you should include loading the module as one of the first steps. It then isn't necessary to load the module separately beforehand. If running trimmomatic straight on the command line, however, remember to load the module BEFORE running the command

Function

Trimmomatic runs on the command line to remove bad quality data and adaptor sequences

In this class, the workflow involving trimmomatic generally is as follows:

Download reads → run fastqc on reads determine quality → run trimmomatic to remove adaptor sequences/poor quality reads → use the trimmed sequences to assemble with Trinity

So be sure to run fastqc on the files beforehand to determine how much of the data to trim. After trimmomatic has finished running, you use Trinity to assemble the reads.

Input Files

Trimmomatic accepts FASTQ (.fastq or .fq) and zipped (.gz or .zip)

You can put in paired and unpaired reads or single reads

Flags

Trimmomatic has many different flags that you can include for more customized trims.

* There are some options that aren't included into this list, such as adjusting the Illumina adaptor, that won't be applicable to this class as we aren't sequencing data ourselves. If you need to change that, however, go to the sources and explore Trimmomatic's website (<http://www.usadellab.org/cms/?page=trimmomatic>)

- SLIDINGWINDOW:<windowSize>:<requiredQuality>
 - windowSize: specifies the number of bases to average across
 - requiredQuality: specifies the average quality required.
- LEADING:<quality>
 - quality: Specifies the minimum quality required to keep a base.
- TRAILING:<quality>
 - quality: Specifies the minimum quality required to keep a base.
- CROP:<length>
 - length: The number of bases to keep, from the start of the read.
- HEADCROP:<length>
 - length: The number of bases to remove from the start of the read.

- MINLEN:<length>
 - length: Specifies the minimum length of reads to be kept

Many times, it's not necessary to include all the possible flags

How to Run it on Bison Net

Example script for running on an interactive page: (to create a new script, use the command **nano** **<new_name_of_script>**). This script also runs fastqc at the end on the newly trimmed reads.

```
#!/bin/bash

#Description: runs trimmomatic to remove bad quality data

#Usage: run_trimmomatic.sh $1 $2

#loads module
module load qc

#runs trimmomatic on
trimmomatic PE $1 $2 $1_paired.fq $1_unpaired.fq $2_paired.fq $2_unpaired.fq
ILLUMINACLIP:/software/apps/Trimmomatic/current/adapters/TruSeq3-PE.fa:2:30:10:2:keepBothReads
HEADCROP:20 SLIDINGWINDOW:4:15 MINLEN:50

# cleans the paired files with fastqc
fastqc -o FASTQC_output2 $1 2$
```

When running the command, following the usage statement: **run_trimmomatic.sh \$1 \$2**

Here \$1 and \$2

Running things on the queue compared to on an interactive page mostly involves the same script. To run things on the queue, add a header at the top after the shebang, which looks like the following:

```
#SBATCH -p short
#SBATCH -N 1
#SBATCH -n 3
#SBATCH --mem-per-cpu=8192
#SBATCH --job-name="name_of_your_job"
#SBATCH -o slurm.%N.%j.stdout.txt
#SBATCH -e slurm.%N.%j.stderr.txt
#SBATCH --mail-user = your_username@bucknell.edu
#SBATCH --mail-type=ALL # mail events (NONE, BEGIN, END, FAIL, ALL)
```

The **blue** is where you change for your submission

When running the trimmomatic on the queue, the command to use **sbatch <name_of_script>**

While it on an interactive site, the command to use **sh <name_of_script>** or **./<name_of_script>**

Common Errors

Permission Denied:

If using `./name_of_script`, you may get the following error:

```
-bash: ./run_trinity_helppage.sh: Permission denied
```

The error is as described; you do not have permission to execute your script. To change this and to give yourself permission, use the command `chmod u+x name_of_script`. This script changes the mode of access on the script you specified; it gives the user (u) the additional (+) ability of executing (x) a certain script (name_of_script)

Additional Customization

In the functions section, I mentioned that the workflow of assembly involves running Trinity after trimmomatic. You could combine both tasks and run Trimmomatic and Trinity in the same script. While this more so leans into the Trinity than trimmomatic, you can add the following to Trinity scripts to have it run trimmomatic on the reads first.

```
--trimmomatic --quality_trimming_params "ADD SPECIFIC FLAGS"
```

Here's an example:

```
--trimmomatic --quality_trimming_params
```

```
"ILLUMINACLIP:/software/apps/Trimmomatic/current/adapters/TruSeq3-PE.fa:2:30:10:2:keepBothReads HEADCROP:20  
SLIDINGWINDOW:4:15 MINLEN:50"
```

Citations

<http://www.usadellab.org/cms/?page=trimmomatic>

http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic/TrimmomaticManual_V0.32.pdf