

**Part 1:** Use fastq-dump, fastqc, and trimmomatic to process your short-read files

1. Convert your file from the SRA format to fastq format. At the end of this process, you should have two files, a forward-read file and a reverse-read file.
  - a. The module is `ncbi-sra`
  - b. The command is `fastq-dump`
  - c. The arguments you want are `--split-e` and `--split-spot`
2. Use fastqc to check the quality of your fastq files.
  - a. The module is `fastqc`
  - b. The command is `fastqc`
3. Use Trimmomatic to trim your files.
  - a. The module is `trimmomatic`
  - b. The command is `trimmomatic` followed by the arguments
  - c. See <http://www.usadellab.org/cms/?page=trimmomatic>
  - d. Do not type “java -jar” or “-0.39.jar”. In other words, the first argument after `trimmomatic` should be PE.
  - e. Make sure the `TruSeq3-PE-2.fa` file is in your folder and make sure you use this filename after `ILLUMINACLIP`. This tells `trimmomatic` that the adapter sequences are in this file.
  - f. You may want to direct the `trimmomatic` output to a file using “>”
  - g. You should write a script with your `trimmomatic` command so you have a record of it
4. Use fastqc to check the quality of your trimmed files.
  - a. Are there any remaining issues?
    - i. If so, discuss these with your group to decide whether you should trim the files more aggressively
5. Prepare to use `hisat2` and `stringtie` to map your reads against your genome (in class and homework).
  - a. Consult the `hisat2` manual online: <http://daehwankimlab.github.io/hisat2/>
    - i. Discuss with your group what `hisat2` does and why you need it
    - ii. Discuss with your group the commands you will need to use for `hisat2`
    - iii. If the manual by itself doesn't clear it up, use Google to find other sources of information – `hisat2` is very widely used
    - iv. What arguments will you need to provide `hisat2` for it to run successfully?
  - b. Consult the `stringtie` manual online:  
<https://ccb.jhu.edu/software/stringtie/index.shtml?t=manual>
    - i. Discuss with your group why you need the services of `stringtie`
    - ii. What commands do you need to run and what arguments do they take?
    - iii. Is there something you have to do to the `hisat2` output before you feed it into `stringtie`?
  - c. Homework: Everyone independently take the quiz on Canvas. This quiz is due Tuesday at 10 am.