Data Wrangling and Processing for Genomics

Elizabeth Everman

Postdoctoral Fellow, Macdonald Lab, Molecular Biosciences

Goals for today:

- Project Organization
- Background and metadata
- Assessing sequencing read quality
- Trimming and filtering reads based on read quality
- Variant calling workflow
- Automating a variant calling workflow

Monday:

- Finish up what we don’t get to today
- Introduce cloud computing for genomics

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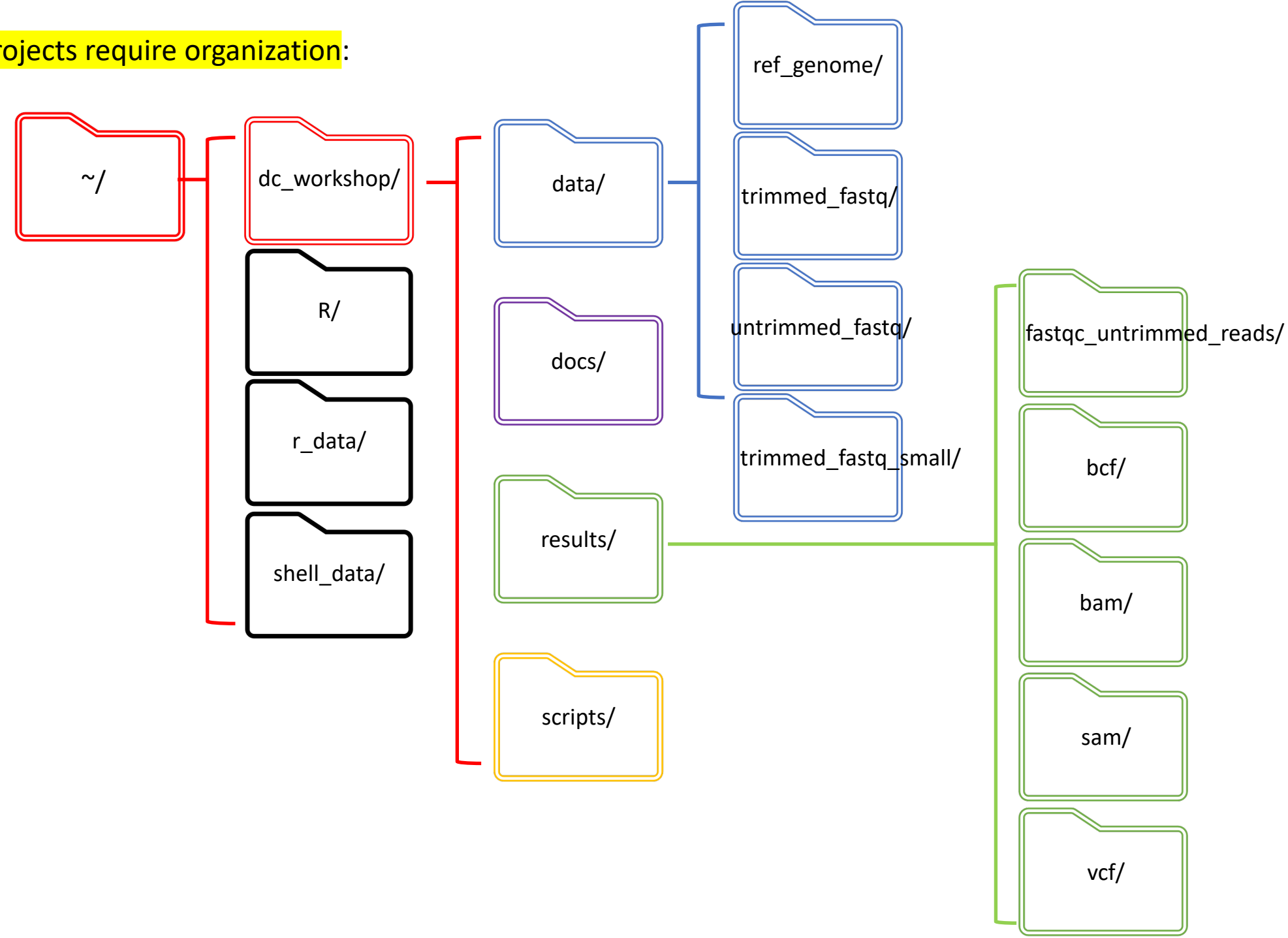
Objectives:

- Create an organized file system for a bioinformatics project
- Identify which types of files should be stored in different directories
- Learn tools to document your work

Bioinformatics projects require organization:



Exercise: Make the following directories in your home directory

dc_workshop
dc_workshop/docs
dc_workshop/data
dc_workshop/results
dc_workshop/scripts

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Improve efficiency and reproducibility:

- 2 tools:
  - nano
  - Shell script or custom program

```
# Bioinformatics project template

# Instructions:
# CHANGE the PROJECT_DIR directory path in the script below:

PROJECT_DIR=/home/dcuser/dc_workshop/

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# SCRIPTS:
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Create each directory and subdirectory using the VARIABLE names

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Customize these sections to fit your project organization needs and preferences by adding additional directories

Downloading data from your AMI:

Windows:

1. If you haven't already done so, download pscp from http://the.earth.li/~sgtatham/putty/latest/x86/pscp.exe
2. Make sure that the PSCP program is in your downloads folder
3. Open PowerShell:
   - Start menu → Search 'cmd' → Open PowerShell
4. Change directory to the location you want to store the file (e.g. Downloads, Desktop)
   - cd DIRECTORY
5. Download the ProjectTemplate.sh script:

```
C:\USER\DIRECTORY\> pscp.exe dcuser@ecX-XX-XX-XXX-XX.compute-1.amazonaws.com:/home/dcuser/ProjectTemplate.sh .
```

Replace with your information

Mac:

1. Open a new tab on your terminal (command t)
2. Confirm that you are using the bash interactive shell:
   ```
   ps –p $$
   ```
3. Change directory to the location you want to store the file (e.g. Downloads, Desktop)
   - cd DIRECTORY
4. Download the ProjectTemplate.sh script:

```
scp dcuser@ecX-XX-XX-XXX-XX.compute-1.amazonaws.com:/home/dcuser/ProjectTemplate.sh .
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Replace with your information

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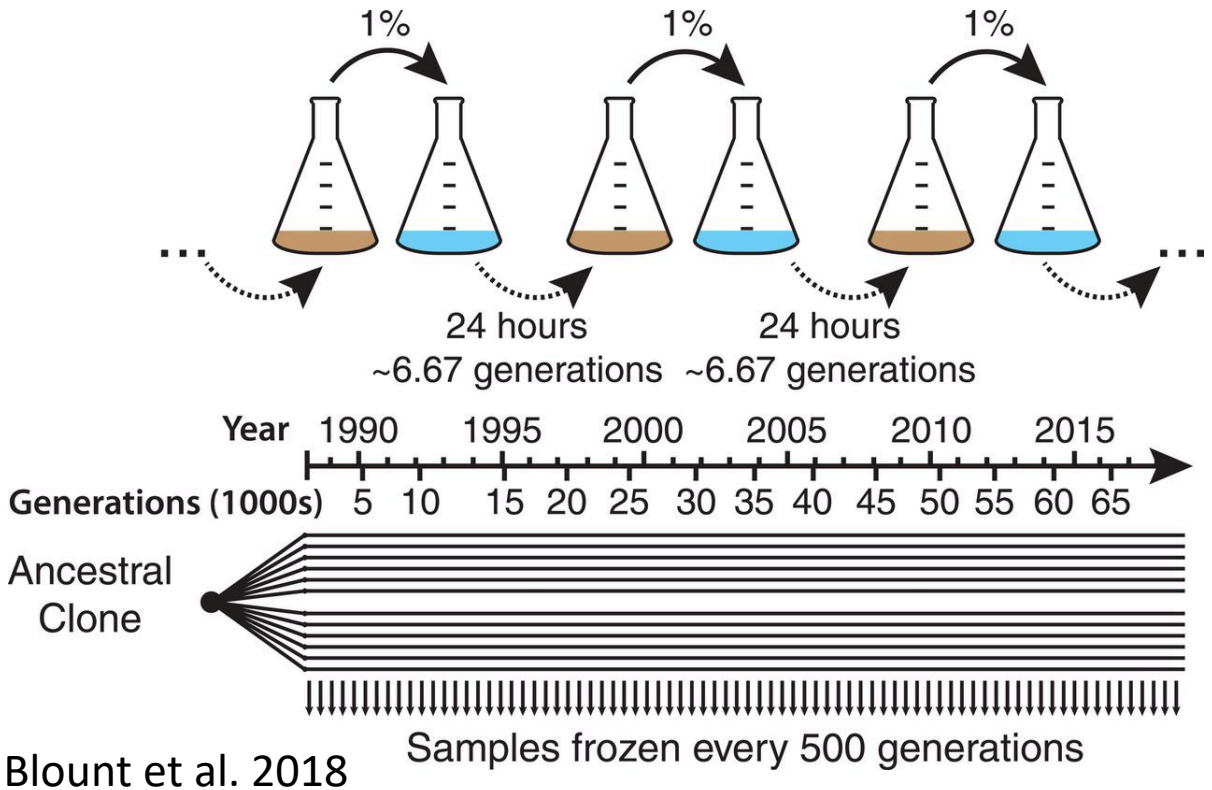
Escherichia coli:

- Single-celled
- *Fast* generation time (doubles every 20 minutes)
- Low genetic complexity (comparatively)
- Plethora of genetic tools and protocols are available to study adaptation

Data from:
https://science.sciencemag.org/content/362/6415/eaam5979/tab-pdf

Long-Term Evolution Experiment with *E. coli:*



Blount et al. 2018

- Glucose-limited media
- High concentration of citrate
  - Normally not usable under aerobic conditions.
  - Between generations 31,000 and 31,500, a spontaneous citrate using mutant appeared
  - Certain regions of the genome became hypermutable

Our data:

- Subset of the larger experiment
- We will work with three sample events of the Ara-3 strain
  - Generations 5,000, 15,000, and 50,000
- This population changed substantially through the experiment
- OUR GOAL:
  - Identify and characterize adaptive changes using a variant calling workflow

- Step 1: Download and examine the metadata associated with this project.

Metadata:

- **strain** = strain name
- **generation** = generation when sample was frozen
- **clade** = based on parsimony-based tree
- **reference** = study the samples were originally sequenced for
- **population** = ancestral population group
- **mutator** = hypermutability mutant status
- **facility** = facility samples were sequenced at
- **run** = sequence read archive sample ID
- **read_type** = library type of reads
- **read_length** = length of reads in sample
- **sequencing_depth** = depth of sequencing
- **cit** = citrate-using mutant status

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Based on the metadata, answer the following questions:

1. How many different generations exist in the data?
2. How many rows and how many columns are in this data?
3. How many citrate+ mutants have been recorded in Ara-3?
4. How many hypermutable mutants have been recorded in Ara-3?

Questions? Time for a break?

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Objectives:

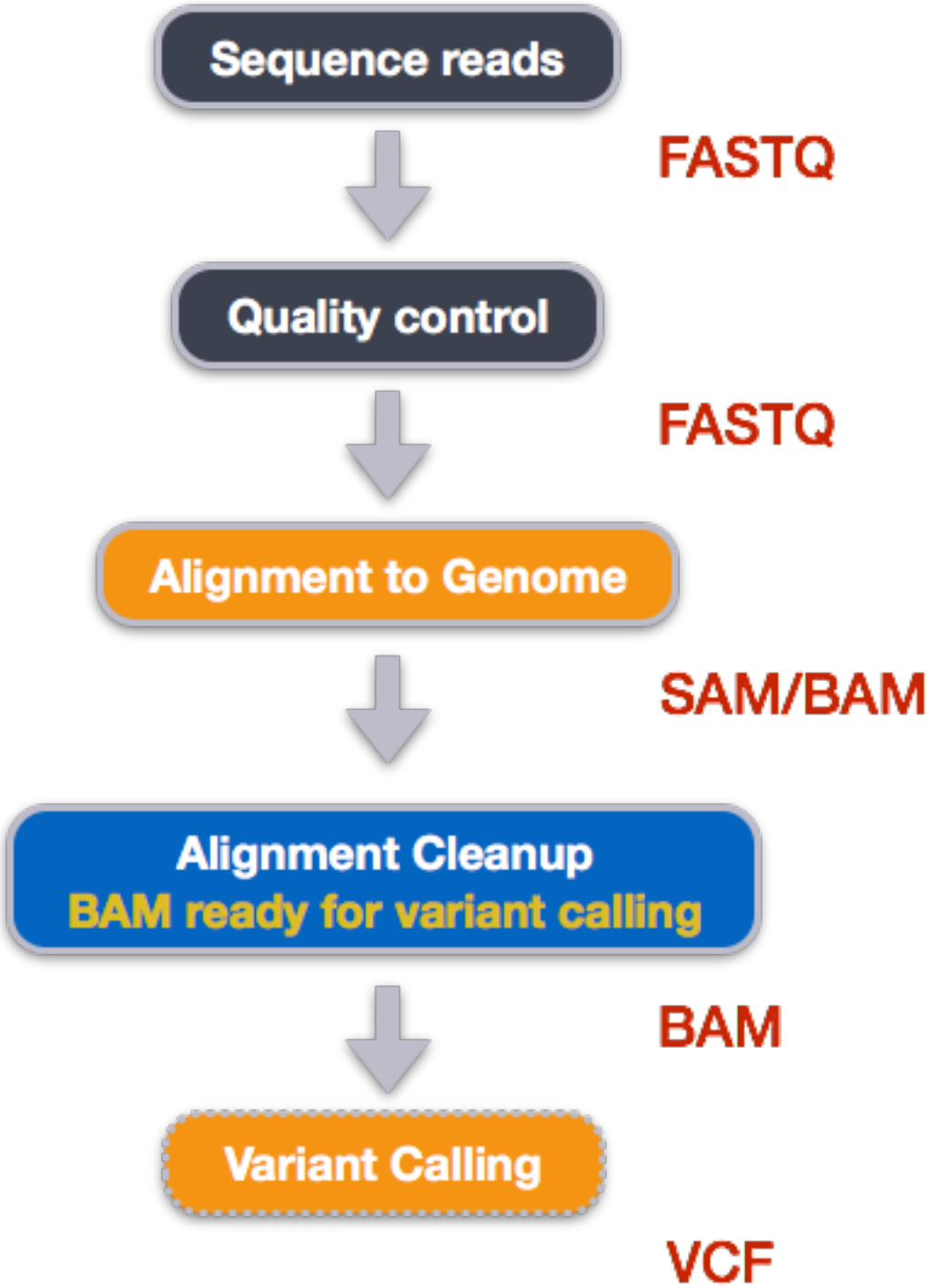
- Understand how a FASTQ file encodes per-base quality scores.
- Interpret a FastQC plot summarizing per-base quality across all reads.
- Use for loops to automate operations on multiple files.

Things that can affect read quality:

- The length of the read
  - Sequencing errors
  - Quality typically decreases toward the end of the read
- Upstream sample processing
  - Low quality DNA or very small amounts

Bioinformatic Pipeline:

- Well-designed pipelines have the advantage of producing the correct input files for the next step in the pipeline
  - Files are generated according to a common standard format
- Pipelines can be fully automated once the bugs are worked out
- The backbone of the pipeline we will use can serve as a starting point for other bioinformatics pipelines
- There are other tools available for checking quality and performing alignments
  - Keep in mind you should always assess the suitability of each tool for your analysis



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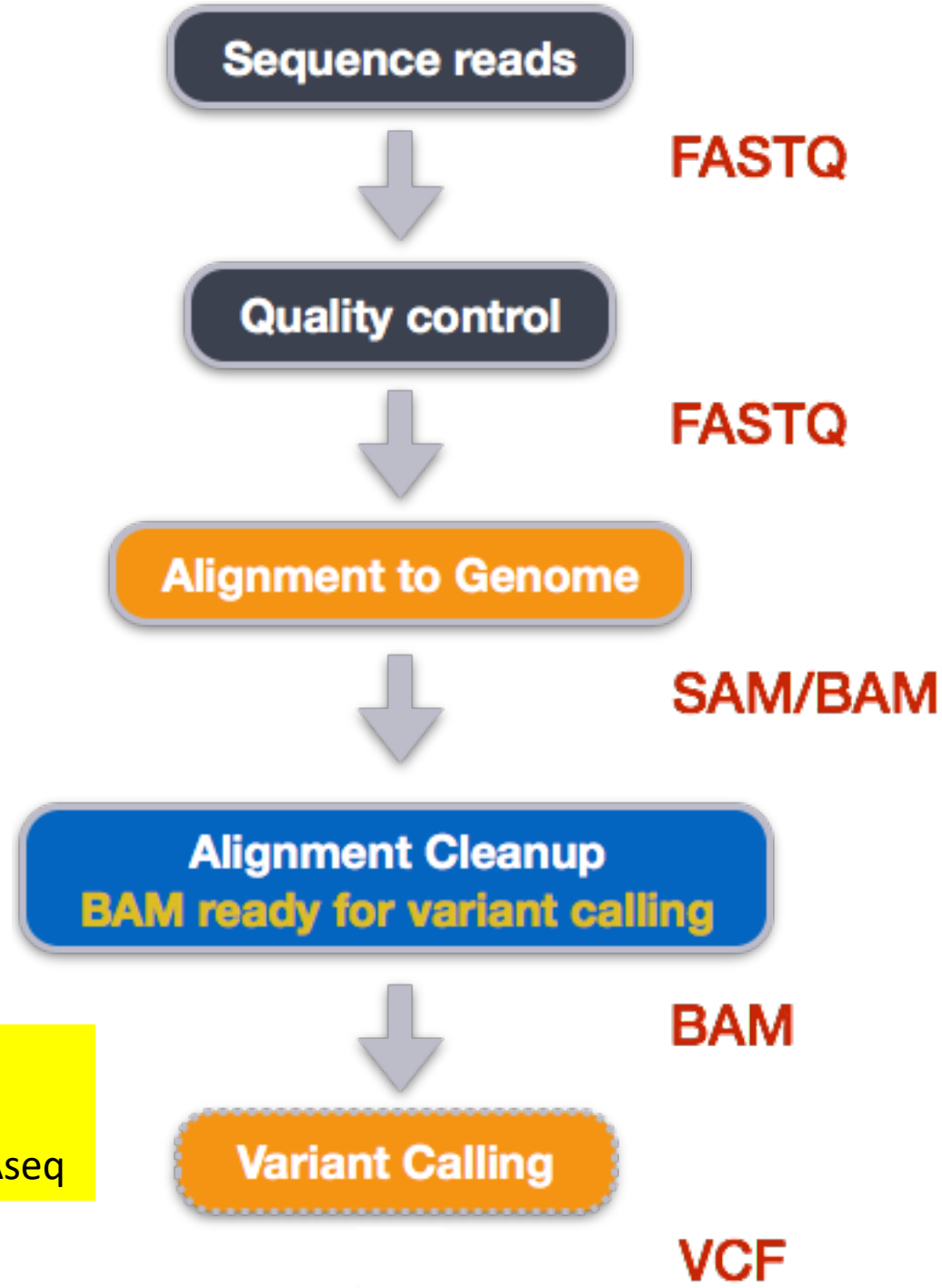
Pipelines can be highly versatile
- Our example is WGS
- Steps (especially quality and alignment) are similar for RNAseq



Our data files:

- We will work with 3 samples from the LTEE *E. coli* experiment
  - Generations 5000, 15000, and 50000
  - Expect that the population has evolved over this time
- Data are paired-end, so there will be 2 files for every sample

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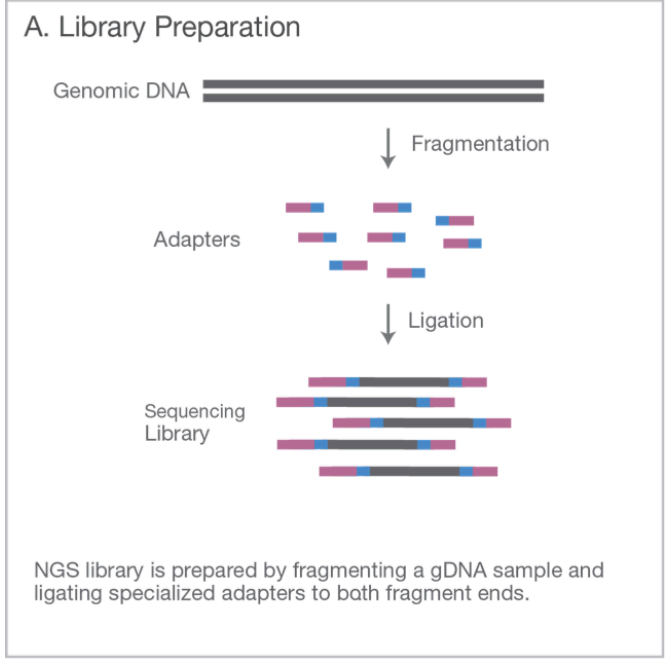

https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

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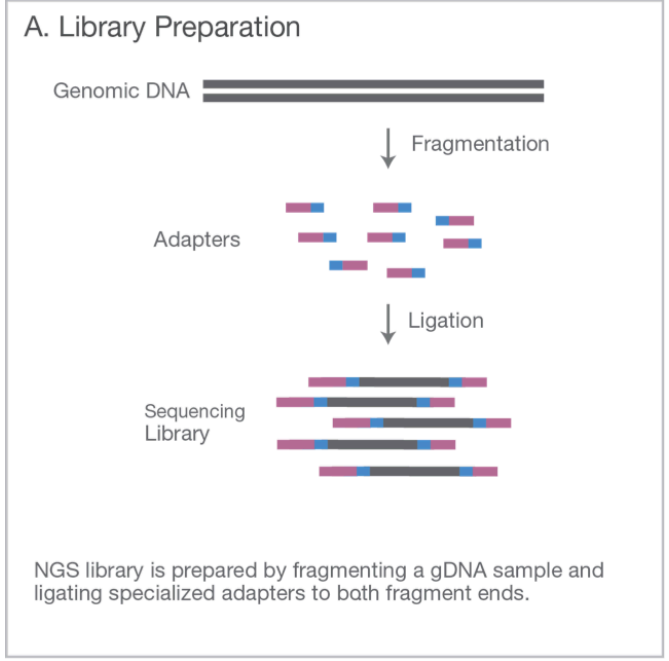

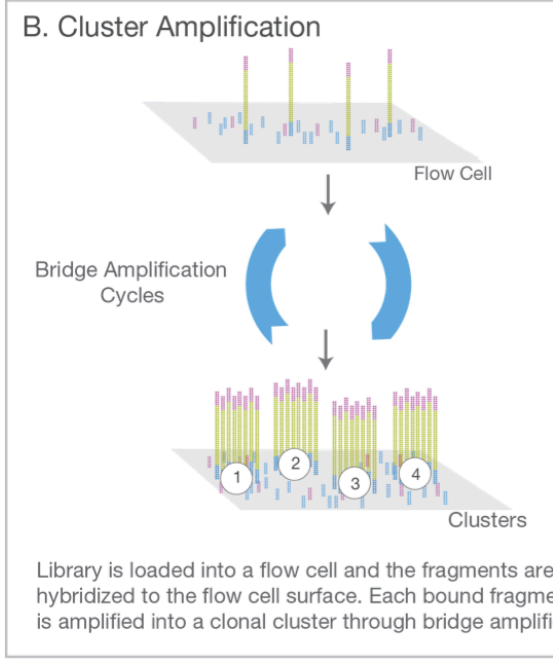

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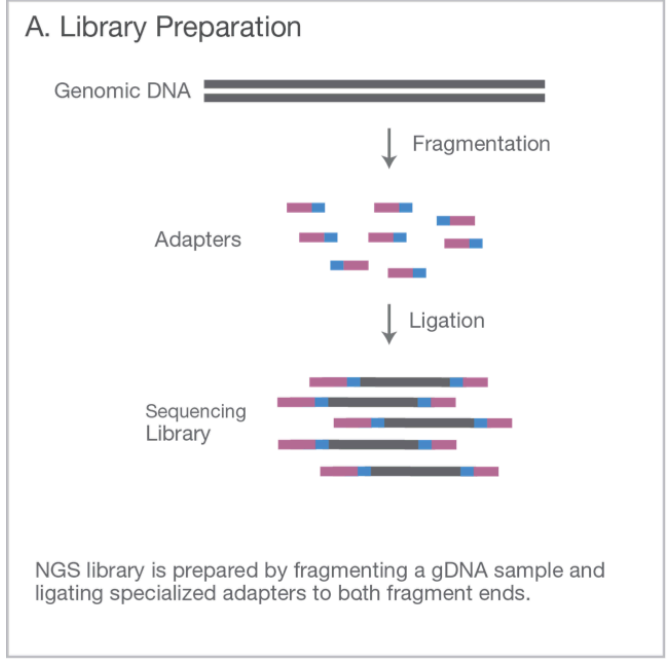
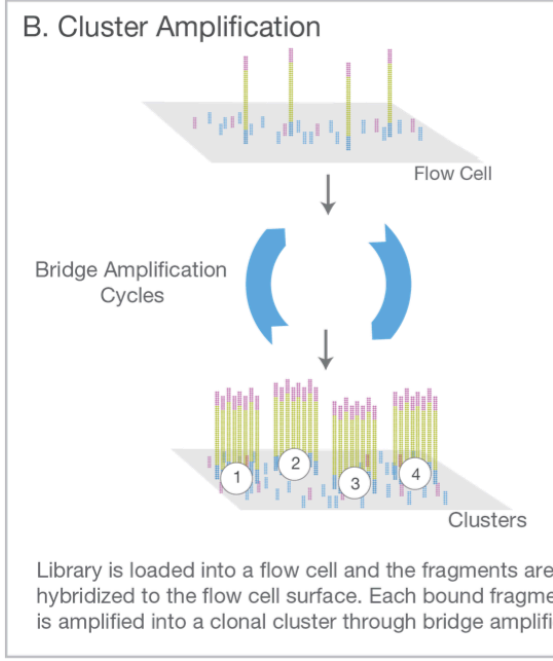
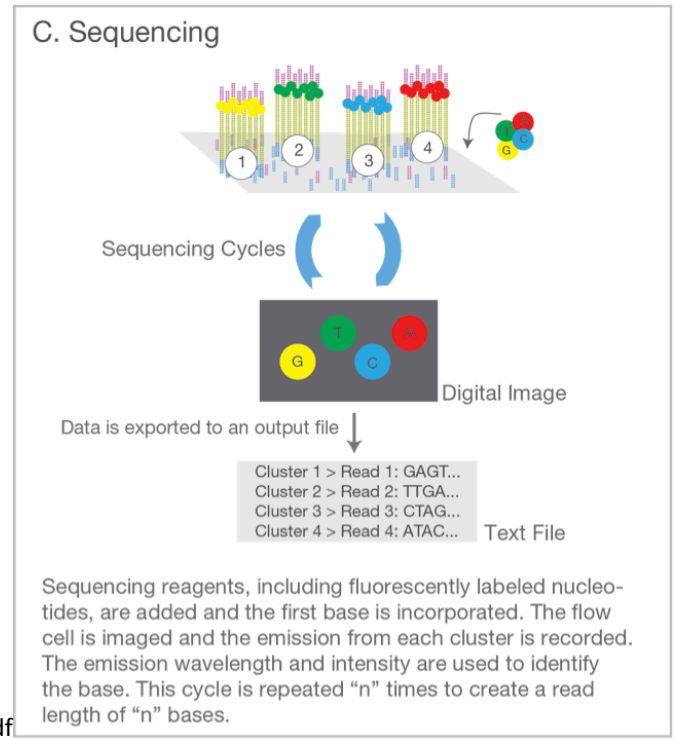
  B. Library is loaded into a flow cell and the fragments bind to short sequences that are complementary to the adapters. Bridge amplification generates clusters of primed templates across the flow cell.
  C. Sequencing by synthesis occurs as bases are added to the sequencing primer in a series of cycles. Bases are fluorescently labeled and when they are incorporated, they emit a base-specific wavelength that is decoded in an output file. One base is incorporated each cycle. The number of cycles determines the length of the read.







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  - The ENA "provides a comprehensive record of the world's nucleotide sequencing information, covering raw sequencing data, sequence assembly information and functional annotation."
  - Data are provided in FASTQ format

Download the data:

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Quality Control:

- FASTQ files have a specific format:
  - LINE 1 @ followed by information about the read
  - LINE 2 DNA sequence
  - LINE 3 + and sometimes same info as line 1
  - LINE 4 Quality scores for each base

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Output

```
Quality encoding: !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJ
                   |         |         |         |         |
Quality score:    01........11........21........31........41
```

https://medium.com/@robertopreste/phred-quality-score-2837415f0af

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Quality score to accuracy probability:

Q = -10 log10 P

Search this file...

Phred Quality Score	Incorrect base call prob	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%

phred_quality_score_1.csv hosted with ♥ by GitHub view raw

https://medium.com/@robertopreste/phred-quality-score-2837415f0af

Quality Control:

- The tool we will use to interpret and report the quality scores in a more human-readable output is FASTQC
- Verify that you have FASTQC installed

```
SYNOPSIS

        fastqc seqfile1 seqfile2 .. seqfileN

    fastqc [-o output dir] [--(no)extract] [-f fastq|bam|sam]
           [-c contaminant file] seqfile1 .. seqfileN

DESCRIPTION

    FastQC reads a set of sequence files and produces from each one a quality
    control report consisting of a number of different modules, each one of
    which will help to identify a different potential type of problem in your
    data.

    If no files to process are specified on the command line then the program
    will start as an interactive graphical application.  If files are provided
    on the command line then the program will run with no user interaction
    required.  In this mode it is suitable for inclusion into a standardised
    analysis pipeline.

    The options for the program as as follows:

    -h --help       Print this help file and exit

    -v --version    Print the version of the program and exit

    -o --outdir     Create all output files in the specified output directory.
                    Please note that this directory must exist as the program
                    will not create it.  If this option is not set then the
                    output file for each sequence file is created in the same
                    directory as the sequence file which was processed.
```

Basic Syntax

Syntax with options

Quality Control:

- The tool we will use to interpret and report the quality scores in a more human-readable output is FASTQC
- Verify that you have FASTQC installed
- Assessing quality with FASTQC:
  - Gives a quick impression of problems with data so that you can take them into consideration in subsequent steps
  - Summarizes quality information by SAMPLE rather than by READ
  - We will access the summary using an output of FASTQC

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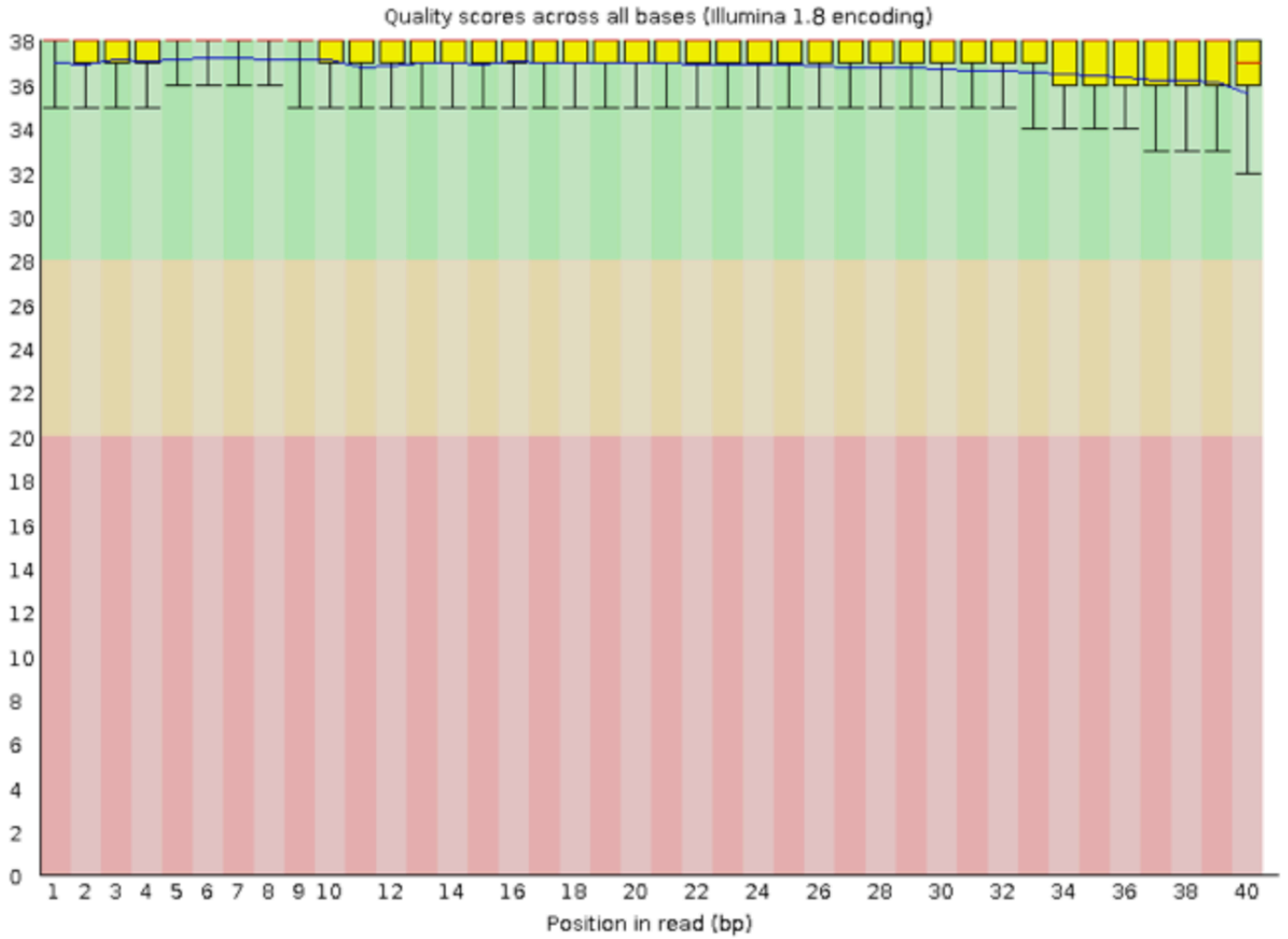
High Quality Sample:

- 40 bp read
- Each position is summarized with a box and whisker plot
  - 50% of reads have a quality score that falls within the range of the yellow box at that position

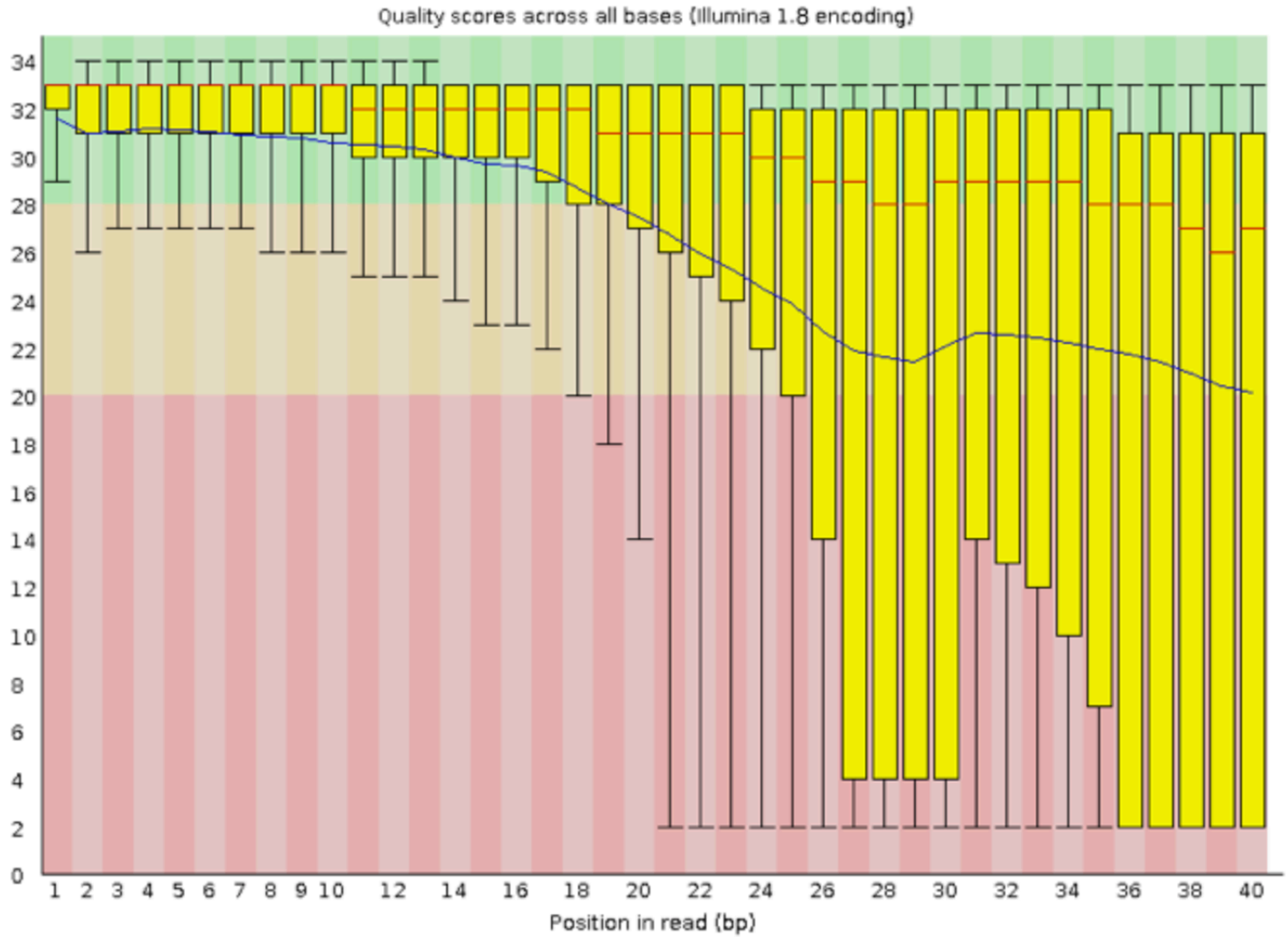


Quality Control:

- The tool we will use to interpret and report the quality scores in a more human-readable output is FASTQC
- Verify that you have FASTQC installed
- Assessing quality with FASTQC:
  - Gives a quick impression of problems with data so that you can take them into consideration in subsequent steps
  - Summarizes quality information by SAMPLE rather than by READ
  - We will access the summary using an output FASTQC

Low Quality Sample:

- 40 bp read
- Quality at each position is more variable
- Quality scores drop very low, particularly at the end of reads.



Running FASTQC:

```
SYNOPSIS

     fastqc seqfile1 seqfile2 .. seqfileN

     fastqc [-o output dir] [--(no)extract] [-f fastq|bam|sam]
            [-c contaminant file] seqfile1 .. seqfileN

DESCRIPTION

     FastQC reads a set of sequence files and produces from each one a quality
     control report consisting of a number of different modules, each one of
     which will help to identify a different potential type of problem in your
     data.

     If no files to process are specified on the command line then the program
     will start as an interactive graphical application.  If files are provided
     on the command line then the program will run with no user interaction
     required.  In this mode it is suitable for inclusion into a standardised
     analysis pipeline.

     The options for the program as as follows:

     -h --help       Print this help file and exit

     -v --version    Print the version of the program and exit

     -o --outdir     Create all output files in the specified output directory.
                     Please note that this directory must exist as the program
                     will not create it.  If this option is not set then the
                     output file for each sequence file is created in the same
                     directory as the sequence file which was processed.
```

Basic Syntax

Syntax with options

For loop syntax:

```
for OBJECT in LIST_OF_OBJECTS
do
OPERATION $OBJECT
done
```

- For loops use an indexing system to move through each element in a list

For loop syntax:

```
for OBJECT in LIST_OF_OBJECTS
do
OPERATION $OBJECT
done
```

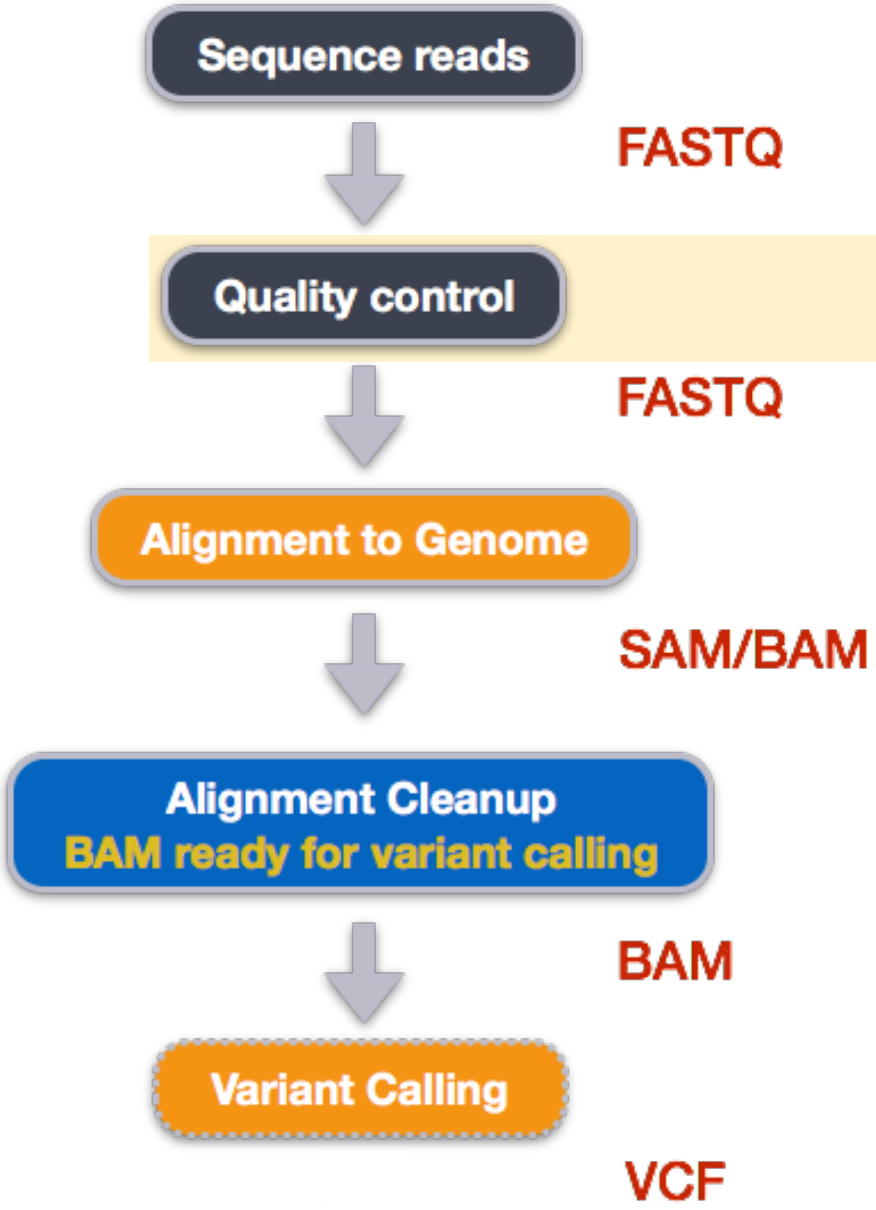
- For loops use an indexing system to move through each element in a list

For loop syntax:

```
for filename in *.zip
do
unzip $filename
done
```

- We have 6 .zip files.
- The first time the loop iterates, the variable filename is assigned as SRR2584863_1_fastqc.zip and the file is unzipped
- For the second iteration, the variable filename is assigned as SRR2584863_2_fastqc.zip

Building our Pipeline:




```
# STEP 1: FASTQC raw data

fastqc *.fastq*                 # Run FASTQC

for filename in *.zip           # unzip fastqc .zip files
do
unzip $filename
done

cat */summary.txt > fastqc_summaries.txt          # obtain fastqc summary
grep FAIL fastqc_summaries.txt > fastqc_FAIL.txt  # identify problem samples
```

Goals for today:

- Project Organization
- Background and metadata
- Assessing sequencing read quality
- Trimming and filtering reads based on read quality
- Variant calling workflow
- Automating a variant calling workflow

Monday:

- Finish up what we don’t get to today
- Introduce cloud computing for genomics

Goals for today:

- Project Organization
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- Assessing sequencing read quality
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Objectives:

- Remove sequence data that doesn't meet quality standards
- Select and set multiple options for command-line bioinformatics tools
- Write for loops with two variables

Cleaning reads:

- FASTQC revealed there is variability in sequencing quality
- We want to base downstream analyses on data that has high quality and high confidence in accuracy
  - Low confidence base calls can inflate false positive and negatives
  - Variant calling is sensitive to low confidence in base calls.

Cleaning reads:

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  - Variant calling is sensitive to low confidence in base calls.

Specify whether data are Paired-end or Single-end reads

```
$ trimmomatic
Usage:
       PE [-version] [-threads <threads>] [-phred33|-phred64] [-trimlog <trimLogFile>] [-summary
<statsSummaryFile>] [-quiet] [-validatePairs] [-basein <inputBase> | <inputFile1> <inputFile2>] [-baseout
<outputBase> | <outputFile1P> <outputFile1U> <outputFile2P> <outputFile2U>] <trimmer1>...
   or:
       SE [-version] [-threads <threads>] [-phred33|-phred64] [-trimlog <trimLogFile>] [-summary
<statsSummaryFile>] [-quiet] <inputFile> <outputFile> <trimmer1>...
   or:
       -version
```

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

Cleaning reads:

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  - Low confidence base calls can inflate false positive and negatives
  - Variant calling is sensitive to low confidence in base calls.

Specify whether data are Paired-end or Single-end reads

Specify the two files that have the paired data for each sample

```
$ trimmomatic
Usage:
       PE [-version] [-threads <threads>] [-phred33|-phred64] [-trimlog <trimLogFile>] [-summary
<statsSummaryFile>] [-quiet] [-validatePairs] [-basein <inputBase> | <inputFile1> <inputFile2>] [-baseout
<outputBase> | <outputFile1P> <outputFile1U> <outputFile2P> <outputFile2U>] <trimmer1>...
   or:
       SE [-version] [-threads <threads>] [-phred33|-phred64] [-trimlog <trimLogFile>] [-summary
<statsSummaryFile>] [-quiet] <inputFile> <outputFile> <trimmer1>...
   or:
       -version
```

Name the output files. Two types:
Output file with surviving read pairs (1/2P)
Output file with orphaned reads (1/2/U)

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

Cleaning reads:

- FASTQC revealed there is variability in sequencing quality
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  - Variant calling is sensitive to low confidence in base calls.

step	meaning
`ILLUMINACLIP`	Perform adapter removal.
`SLIDINGWINDOW`	Perform sliding window trimming, cutting once the average quality within the window falls below a threshold.
`LEADING`	Cut bases off the start of a read, if below a threshold quality.
`TRAILING`	Cut bases off the end of a read, if below a threshold quality.
`CROP`	Cut the read to a specified length.
`HEADCROP`	Cut the specified number of bases from the start of the read.
`MINLEN`	Drop an entire read if it is below a specified length.
`TOPHRED33`	Convert quality scores to Phred-33.
`TOPHRED64`	Convert quality scores to Phred-64.

Long commands can be separated across lines with \

```
$ trimmomatic PE \
              -threads 4 \
              Input_1.fastq Input_2.fastq \
              Output_1.trimmed.fastq Output_1un.trimmed.fastq Output_2.trimmed.fastq Output_2un.trimmed.fastq \
              ILLUMINACLIP:SRR_adapters.fa \  # clip Illumina adapters from the input file
              SLIDINGWINDOW:4:20              # use a sliding window size of 4 bases, remove bases
                                              #  with phred score < 20
```

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

Long commands can be separated across lines with \

```
$ trimmomatic PE \
              -threads 4 \
              Input_1.fastq Input_2.fastq \
              Output_1.trimmed.fastq Output_1un.trimmed.fastq Output_2.trimmed.fastq Output_2un.trimmed.fastq \
              ILLUMINACLIP:SRR_adapters.fa \  # clip Illumina adapters from the input file
              SLIDINGWINDOW:4:20              # use a sliding window size of 4 bases, remove bases
                                              #  with phred score < 20
```

```
trimmomatic PE \
> SRR2589044_1.fastq.gz SRR2589044_2.fastq.gz \
> SRR2589044_1.trim.fastq.gz SRR2589044_1un.trim.fastq.gz \
> SRR2589044_2.trim.fastq.gz SRR2589044_2un.trim.fastq.gz \
> SLIDINGWINDOW:4:20 \        # sliding window size = 4, remove bases with phred score < 20
> MINLEN:25 \                 # discard reads with < 25 bases after trimming
> ILLUMINACLIP:NexteraPE-PE.fa:2:40:15      # Usage <adapters.fa>:<seed matches>:
                                              <palindrome clip threshold>:<simple clip threshold>
```

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

```
for infile in *_1.fastq.gz
do
  base=$(basename ${infile} _1.fastq.gz)
  trimmomatic PE ${infile} ${base}_2.fastq.gz \
               ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \
               ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \
               SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
done
```

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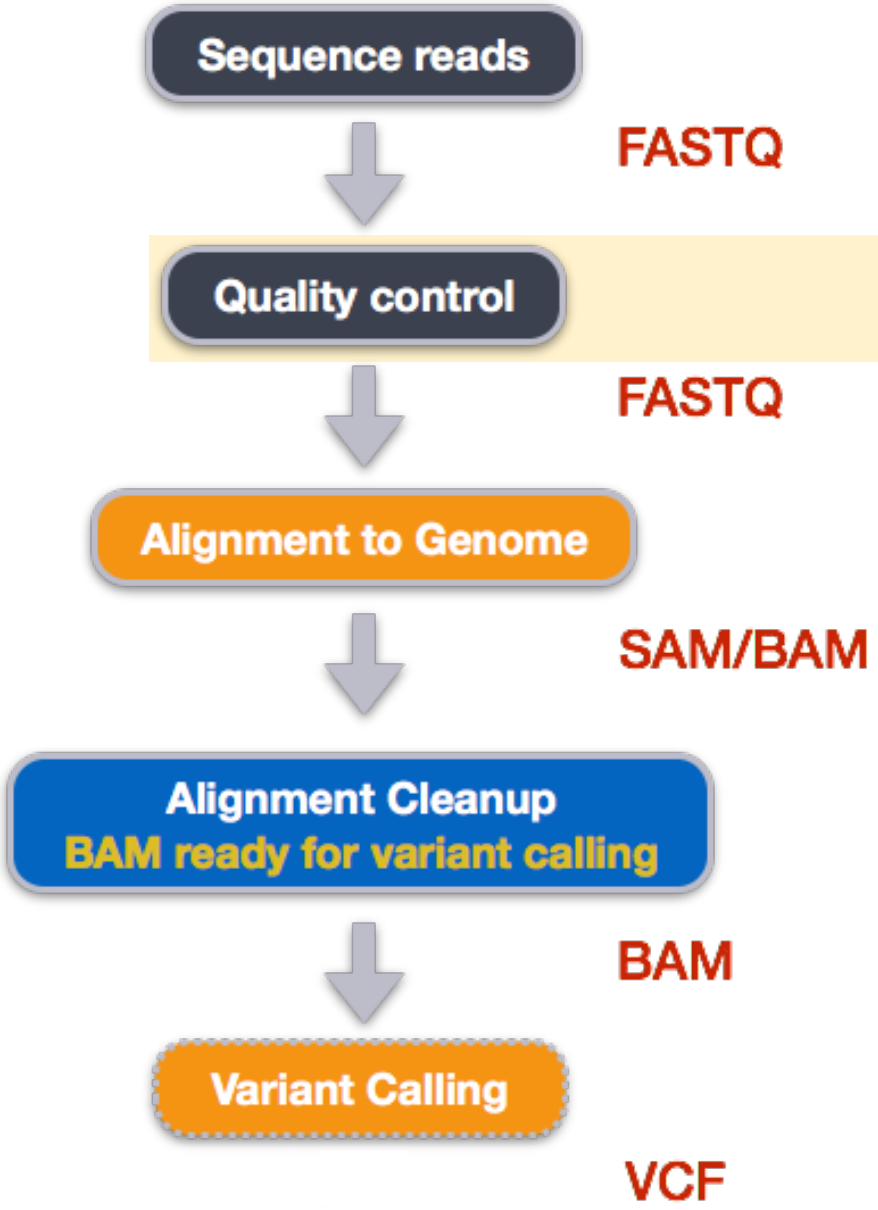



```
# STEP 1: FASTQC raw data

fastqc *.fastq*                 # Run FASTQC

for filename in *.zip           # unzip fastqc .zip files
do
unzip $filename
done

cat */summary.txt > fastqc_summaries.txt          # obtain fastqc summary
grep FAIL fastqc_summaries.txt > fastqc_FAIL.txt  # identify problem samples

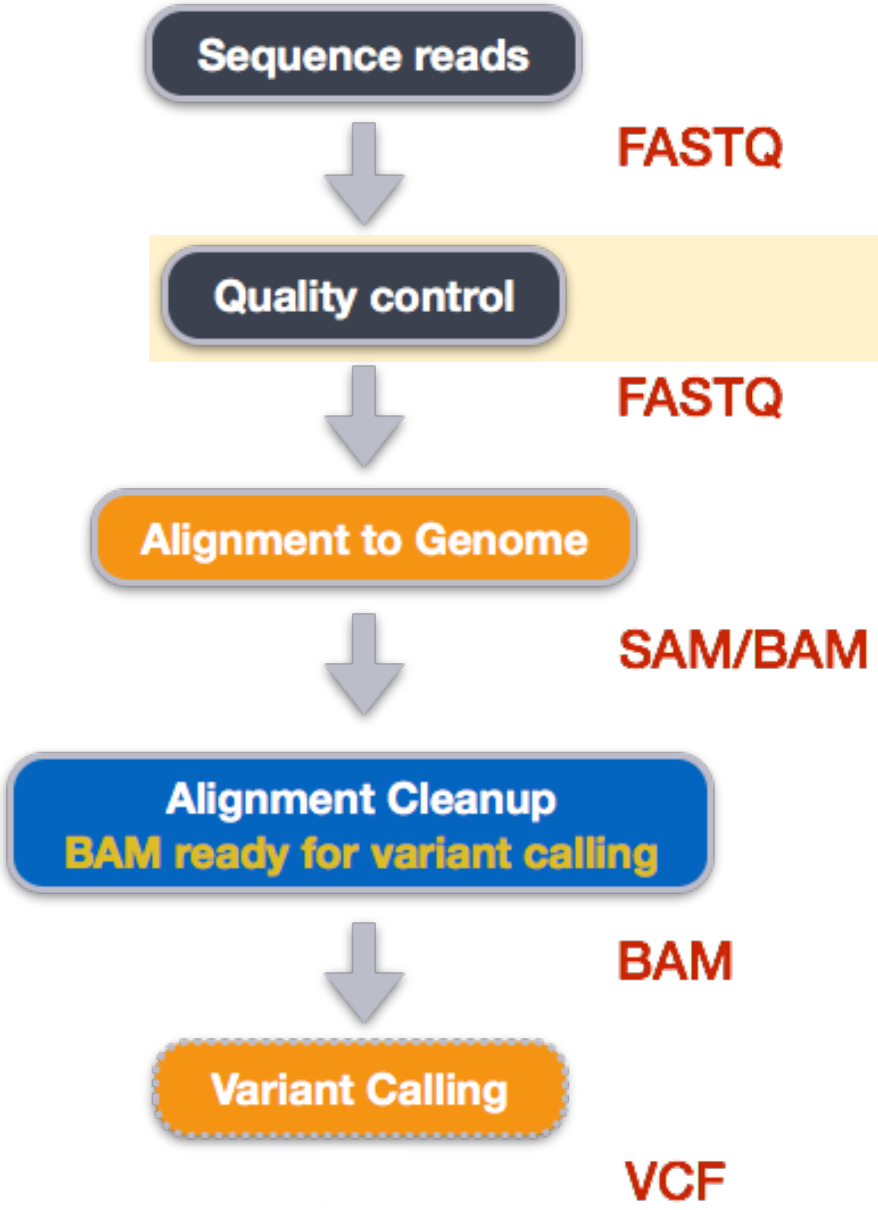
# STEP 2: TRIM and FILTER

for infile in *_1.fastq.gz              # Run trimmomatic
do
  base=$(basename ${infile} _1.fastq.gz)
  trimmomatic PE ${infile} ${base}_2.fastq.gz \
                ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \
                ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \
                SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
done

# STEP 3: FASTQC filtered data

fastqc ~/dc_workshop/data/trimmed_fastq*.fastq*             # Run FASTQC
```

Building our Pipeline:

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for infile in *_1.fastq.gz              # Run trimmomatic
do
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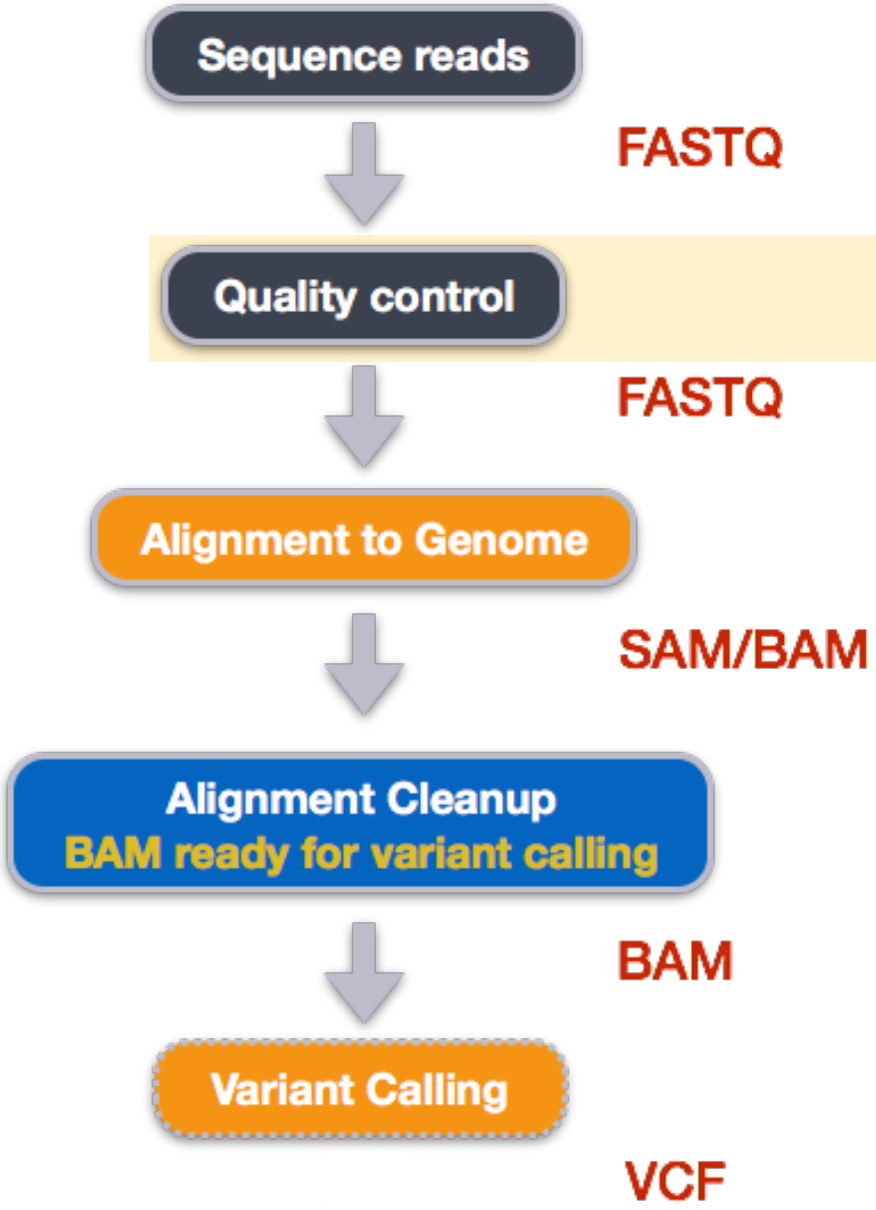
  trimmomatic PE ${infile} ${base}_2.fastq.gz \
                ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \
                ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \
                SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
done

# STEP 3: FASTQC filtered data

fastqc ~/dc_workshop/data/trimmed_fastq*.fastq*             # Run FASTQC
```

Questions?

Building our Pipeline:

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# STEP 2: TRIM and FILTER

for infile in *_1.fastq.gz              # Run trimmomatic
do
  base=$(basename ${infile} _1.fastq.gz)
  trimmomatic PE ${infile} ${base}_2.fastq.gz \
                ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \
                ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \
                SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
done

# STEP 3: FASTQC filtered data

fastqc ~/dc_workshop/data/trimmed_fastq*.fastq*             # Run FASTQC
```

Questions?

Take a break or complete Step 3

Goals for today:

- Project Organization
- Background and metadata
- Assessing sequencing read quality
- Trimming and filtering reads based on read quality
- Variant calling workflow
- Automating a variant calling workflow

Monday:

- Finish up what we don’t get to today
- Introduce cloud computing for genomics

Goals for today:

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Monday:

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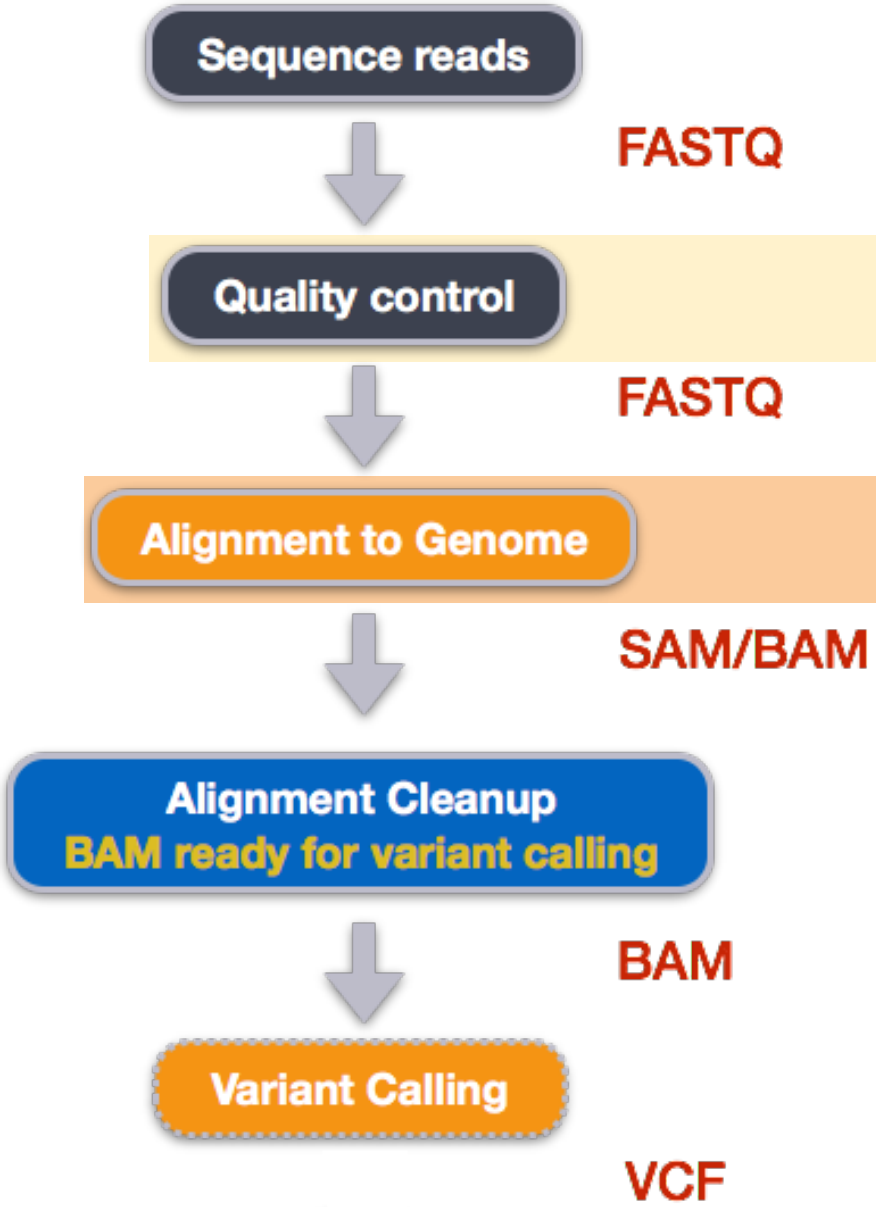
- Introduce cloud computing for genomics

Objectives:

- Find sequence variants between samples and reference genome
- Describe types of data formats encountered during variant calling
- Use command line tools to perform variant calling

Building our Pipeline:




```
# STEP 1: FASTQC raw data

# STEP 2: TRIM and FILTER

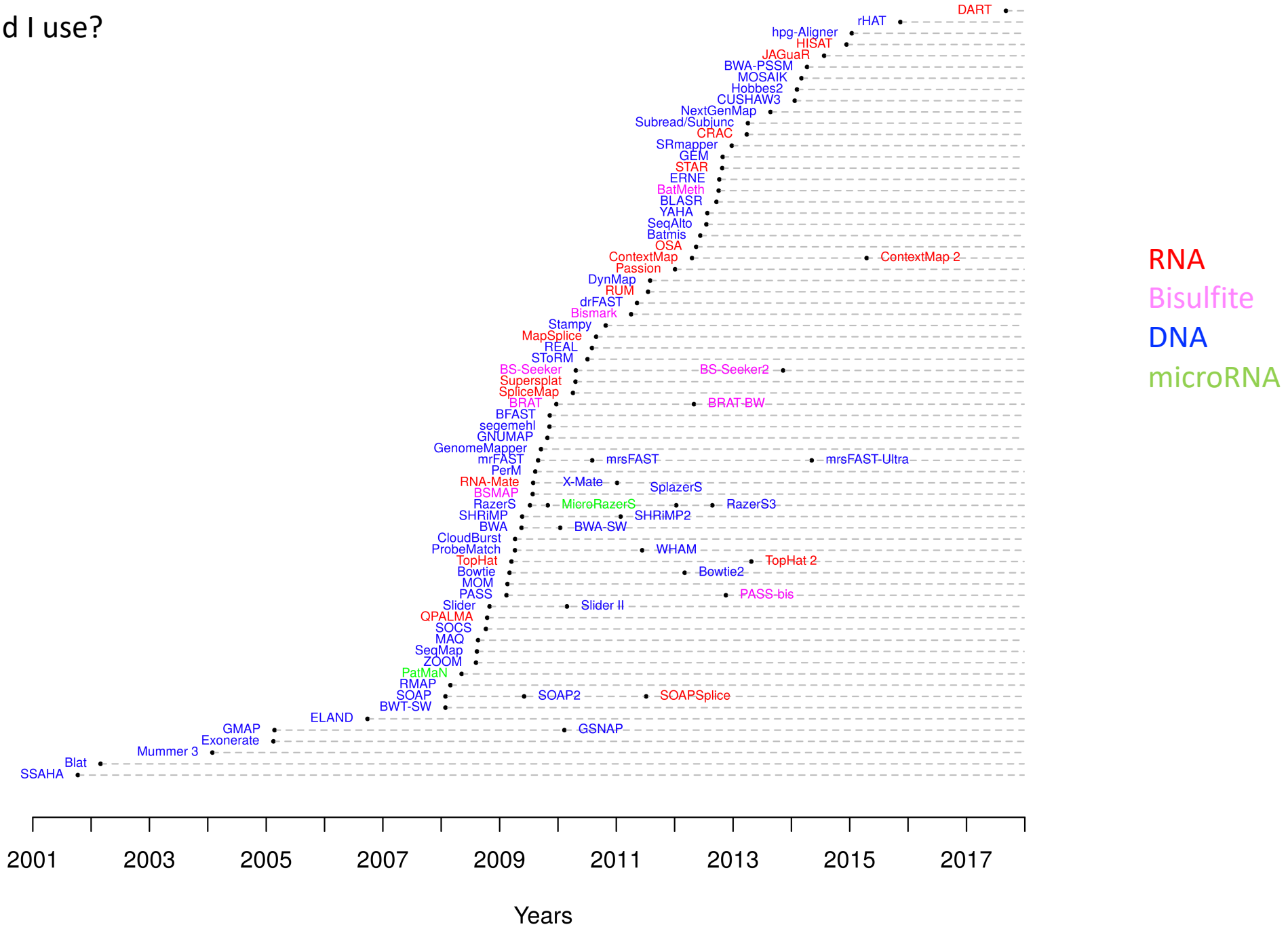
# STEP 3: FASTQC filtered data

# STEP 4: Index reference genome

# STEP 5: Align trimmed reads
```

- Alignment requires a reference genome
  - Our LTEE population samples are derived from the REL606 strain
  - The REL606 genome will be our reference genome
- Many tools are available
  - Application and sequencing technology are important considerations
- Burros Wheeler Aligner (BWA)
  - http://bio-bwa.sourceforge.net
  - 2 step process

Which aligner should I use?

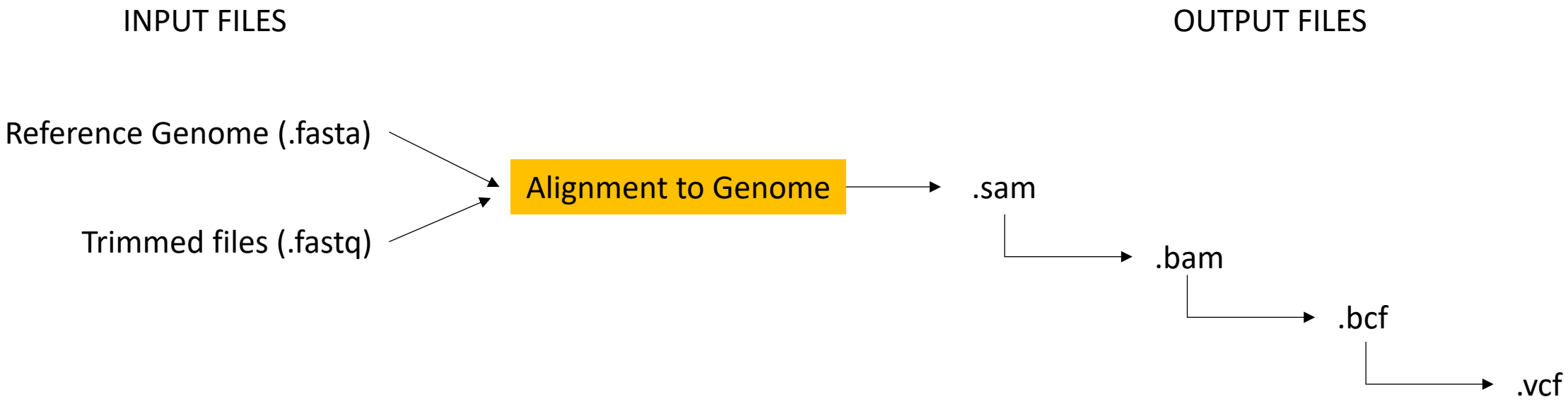


RNA

Bisulfite

DNA

microRNA



- To help with organization, we will make directories ahead of time to hold each file type.

Indexing the reference genome:

- Indexing allows the aligner to quickly find potential alignment sites for query sequences
- Indexing varies by aligner tool. This method is specific to BWA
- Indexing only needs to be run once
- Like an index in a book:
  - Indexing allows the aligner to narrow down the genome to a smaller region before trying to match reads to genome

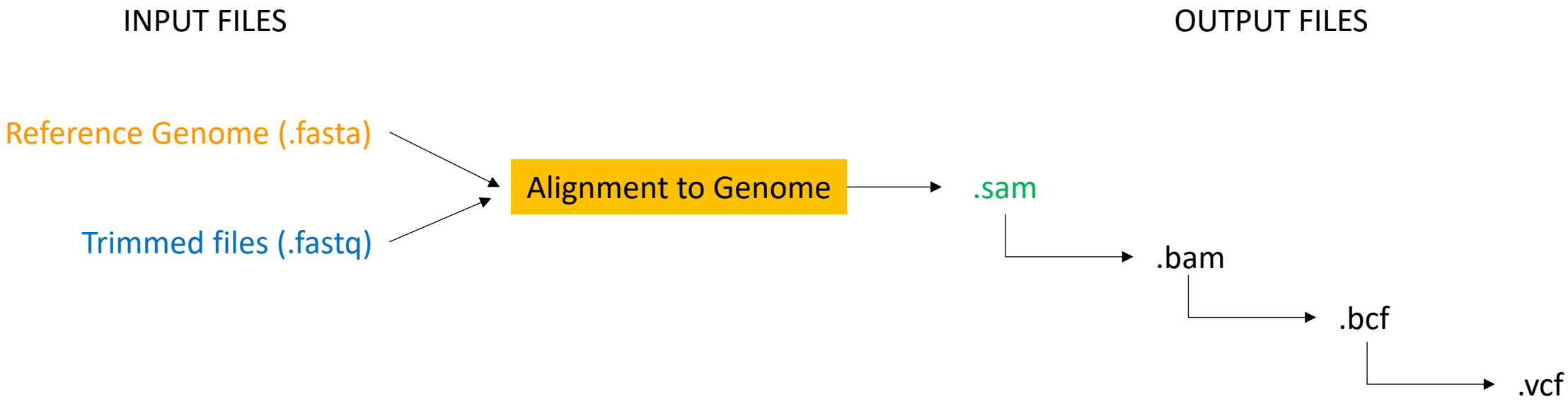
- Alignment is about fitting individual pieces (reads) into the correct part of the puzzle
- The human genome project gave us the picture on the box cover (the reference genome)
- Imperfections in how the pieces fit can indicate changes to a copy of the picture

Reference: AGCCTGAGACCGTAAAAAAGTCAAG

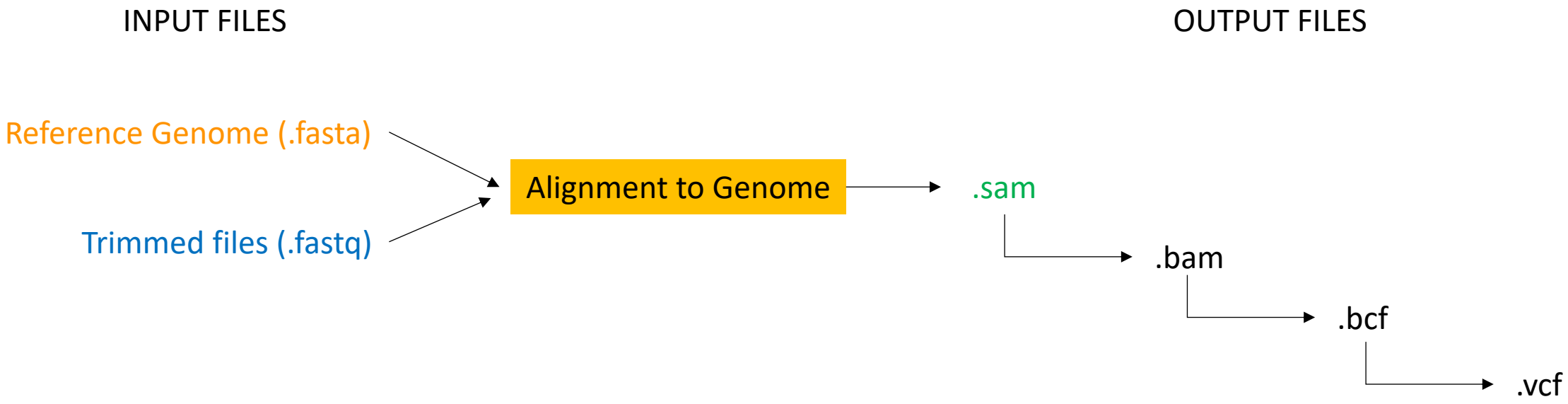
A read sequence: GAGACCGTAAAAACGTC

A variant!

Slide credit: CHSL, O. and M. Griffith



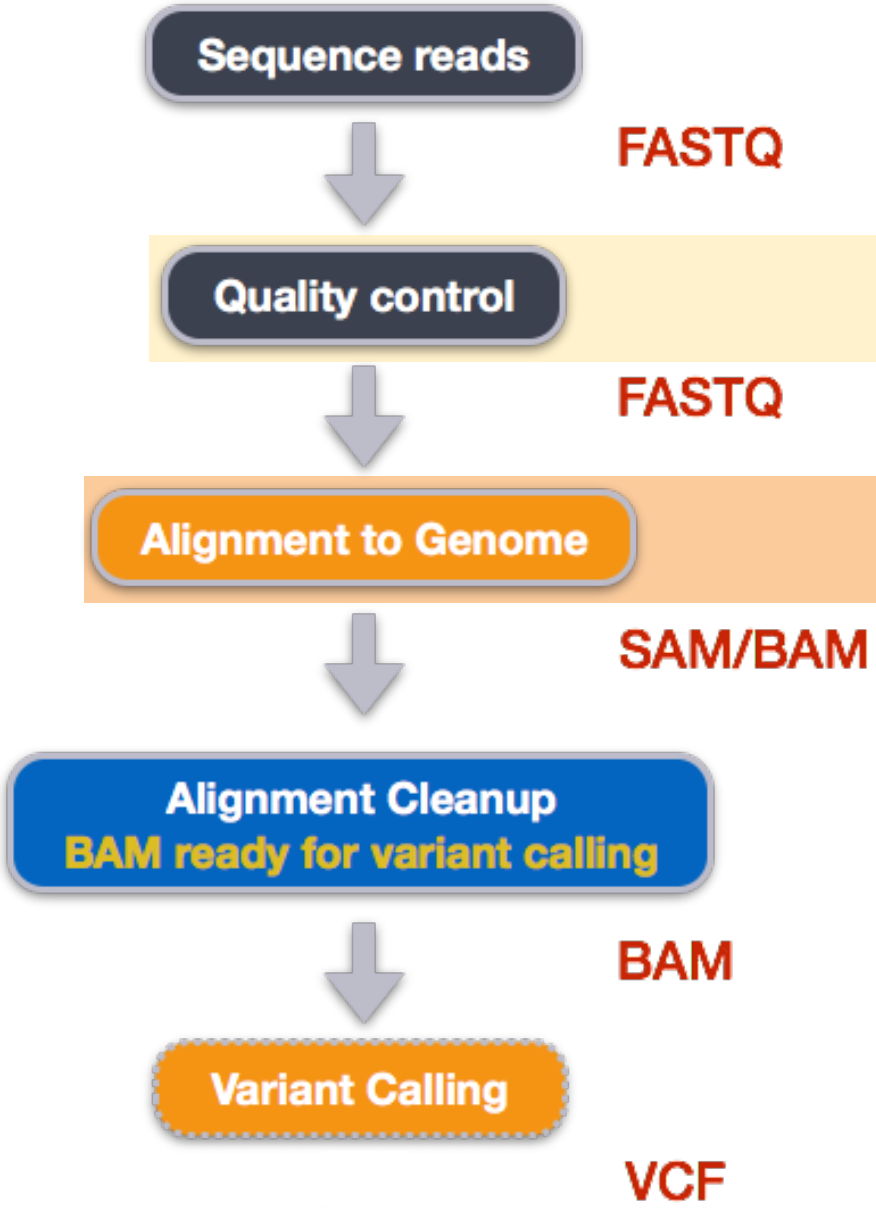

```
$ bwa mem ref_genome.fasta input_file_R1.fastq input_file_R2.fastq > output.sam
```




```
$ bwa mem ref_genome.fasta input_file_R1.fastq input_file_R2.fastq > output.sam
```

- Our input and output files are organized across directories, so we will include the paths in our command

Building our Pipeline:




```
# STEP 1: FASTQC raw data

# STEP 2: TRIM and FILTER

# STEP 3: FASTQC filtered data

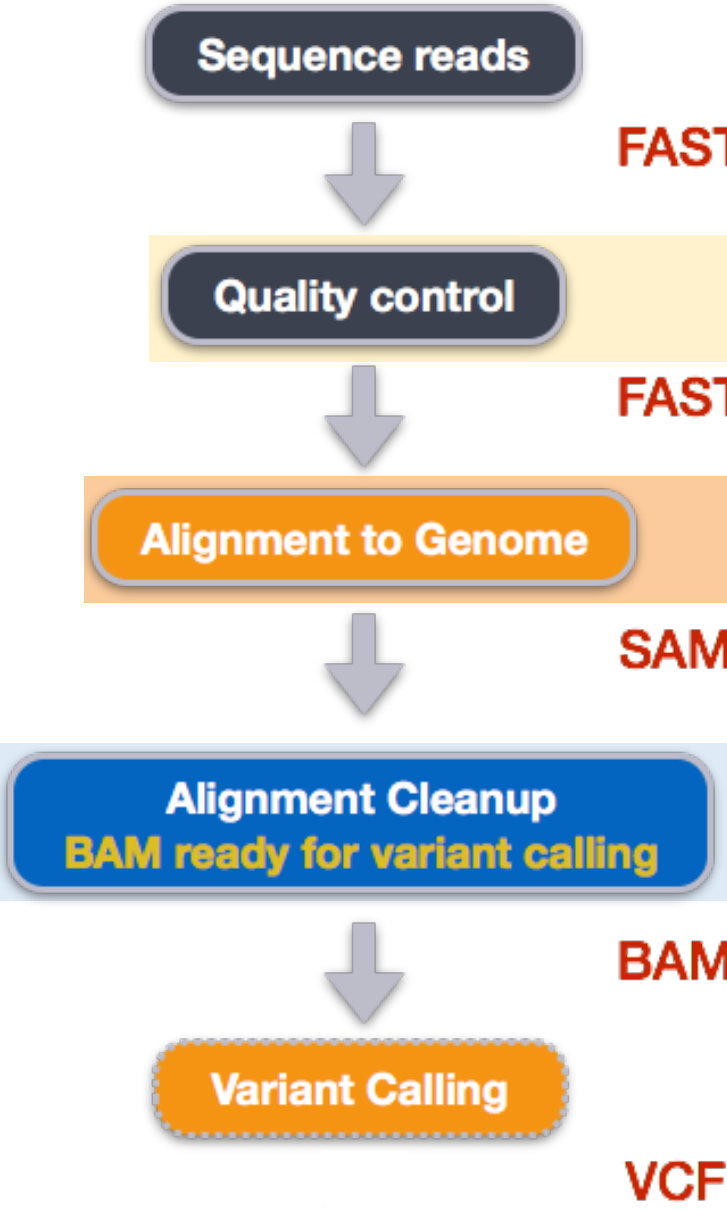
# STEP 4: Index reference genome

bwa index PATH/ref_genome/ref_genome.fasta

# STEP 5: Align trimmed reads

bwa mem ref_genome.fasta input_file_R1.fastq input_file_R2.fastq > output.sam
```

Building our Pipeline:

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# STEP 1: FASTQC raw data

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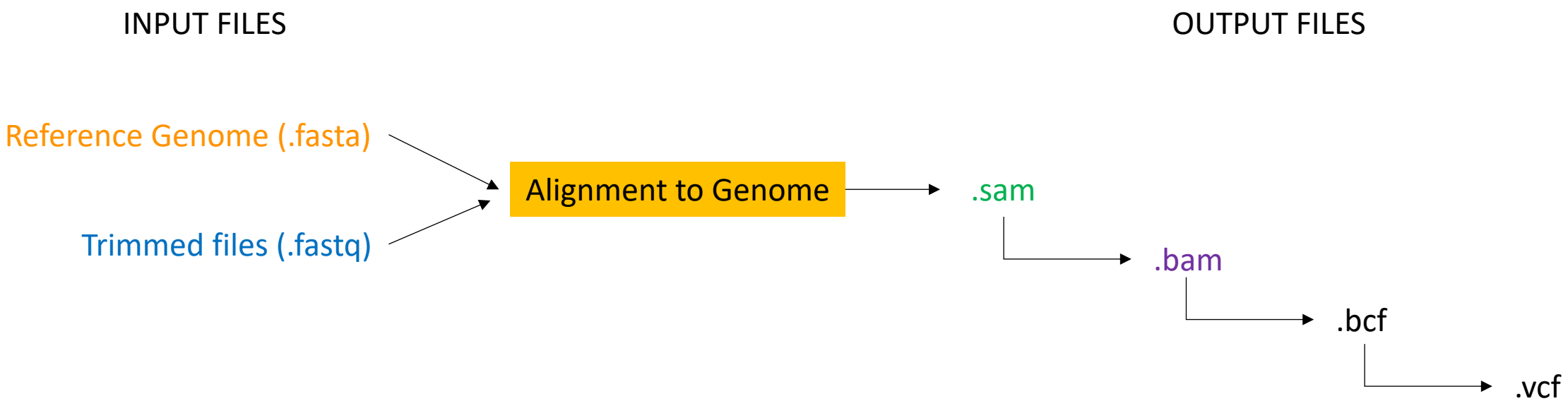
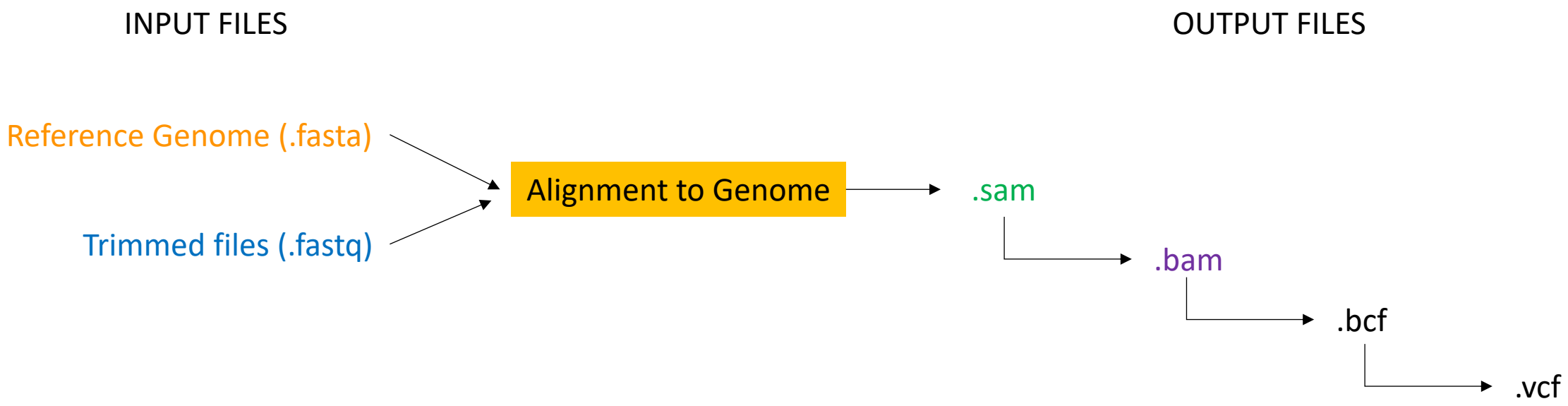
# STEP 6: Convert from SAM to BAM

# STEP 7: Sort BAM file
```

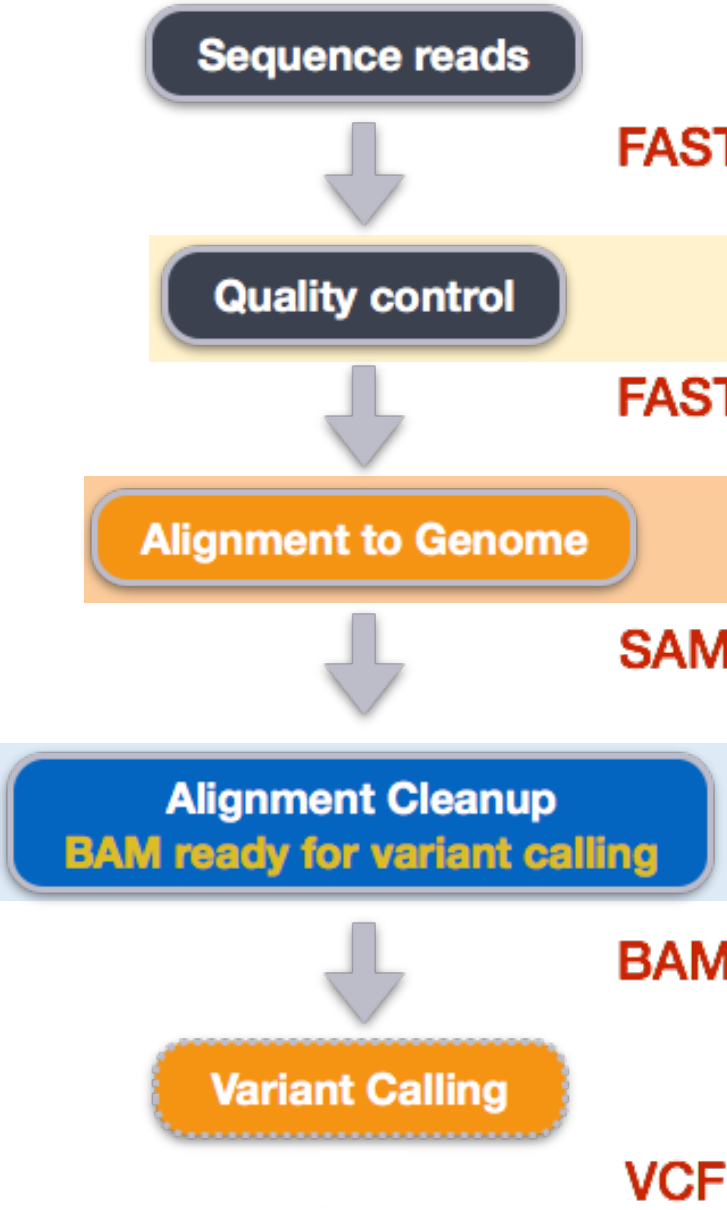



```
$ samtools view -S -b PATH/sam/output.sam > PATH/bam/output.bam
```




```
samtools sort -o PATH/bam/output.sorted.bam PATH/bam/output.bam
```

Building our Pipeline:




```
# STEP 1: FASTQC raw data

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# STEP 3: FASTQC filtered data

# STEP 4: Index reference genome

# STEP 5: Align trimmed reads

# STEP 6: Convert from SAM to BAM

samtools view -S -b PATH/sam/output.sam > PATH/bam/output.bam

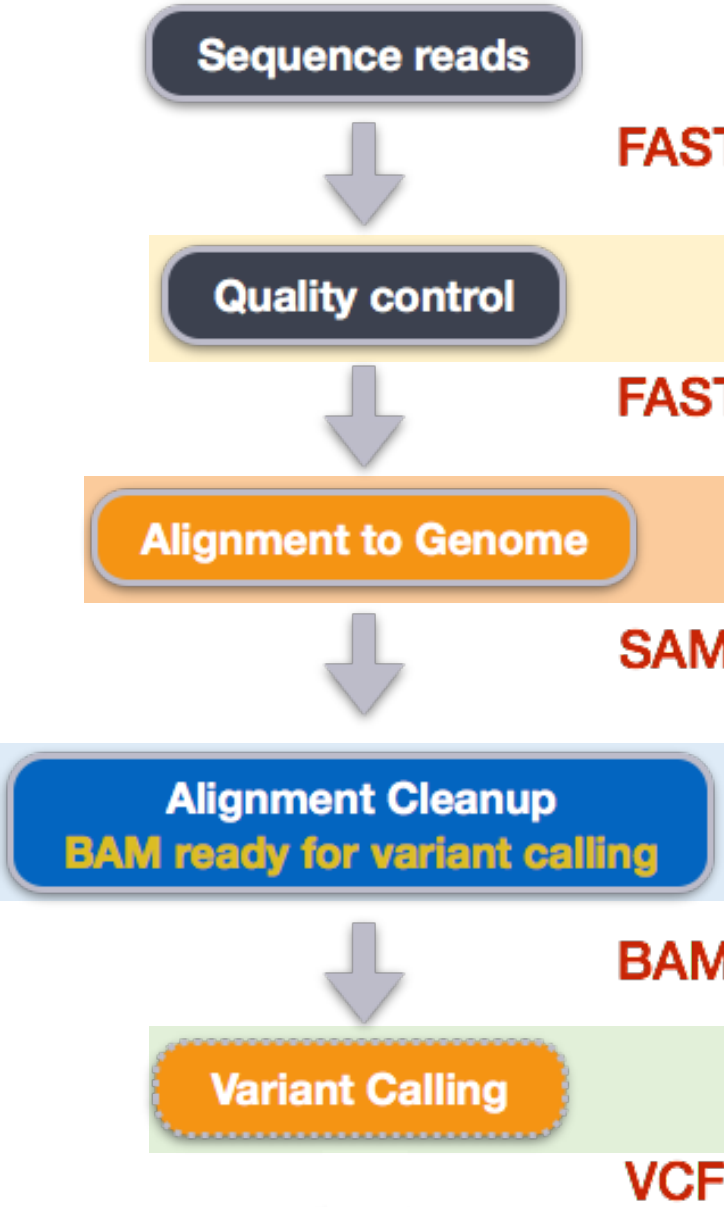
# STEP 7: Sort BAM file

samtools sort -o PATH/bam/output.sorted.bam PATH/bam/output.bam
```

Variant Calling:

- A variant is a different nucleotide at a given position compared to the reference
- Typically accompanied by an estimate of variant frequency and confidence

Building our Pipeline:




```
# STEP 1: FASTQC raw data

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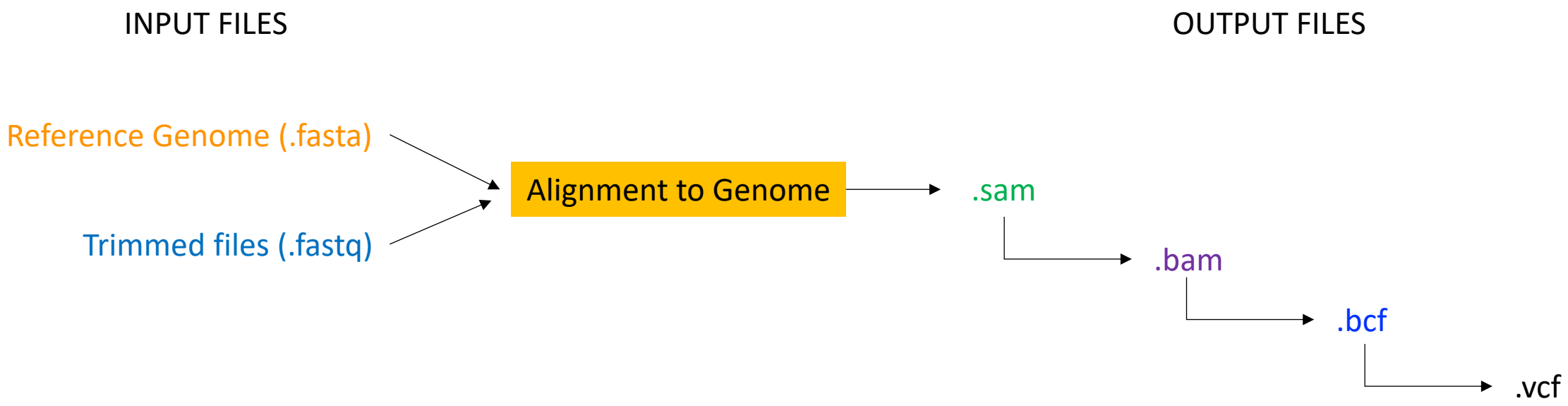
# STEP 6: Convert from SAM to BAM

# STEP 7: Sort BAM file

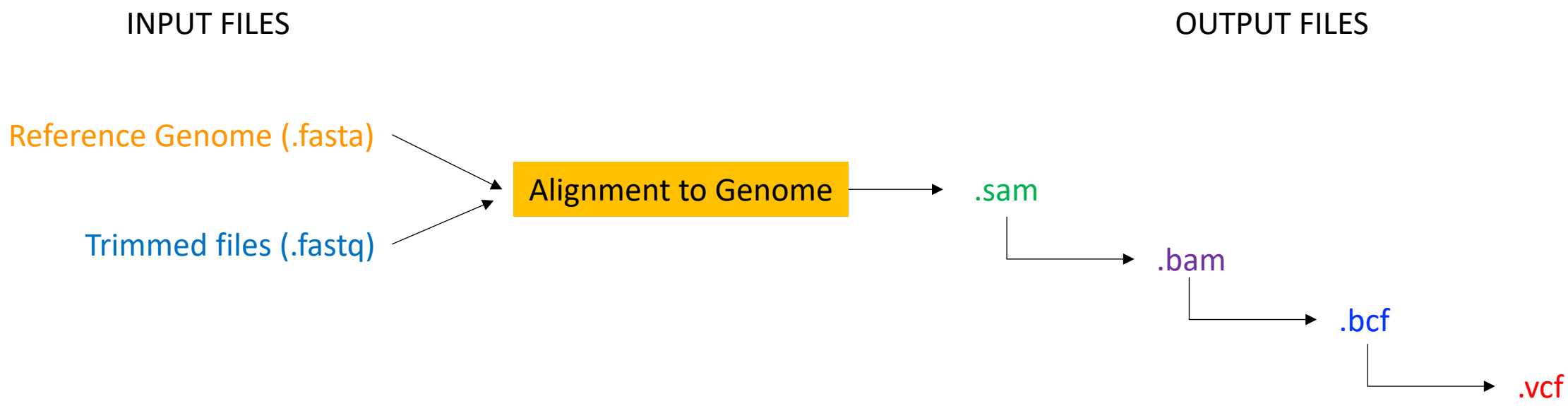
# STEP 8: Calculate read coverage of positions in genome

# STEP 7: Detect SNPs

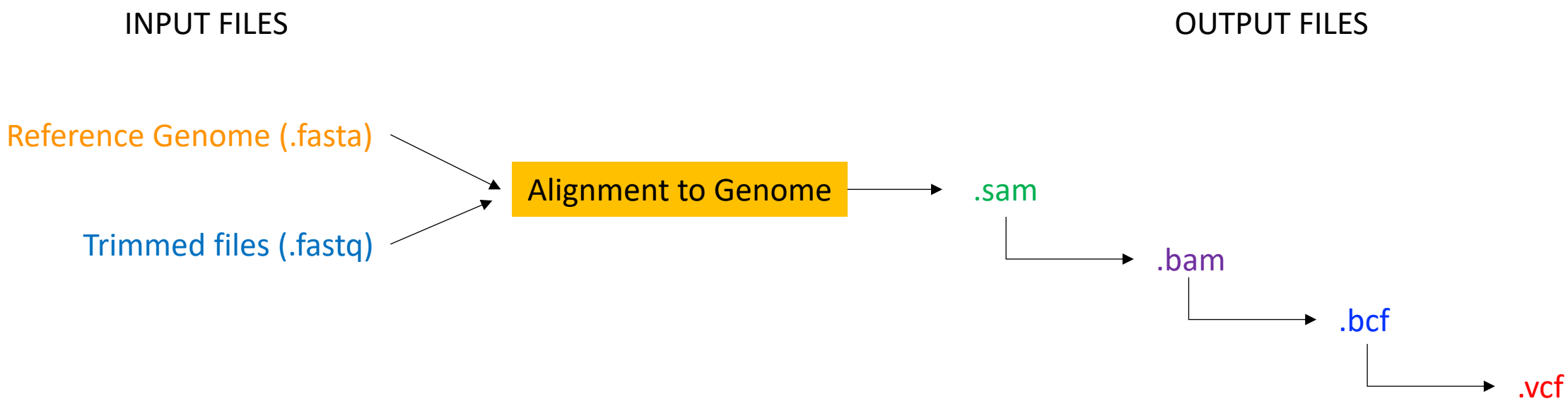
# STEP 9: Filter and report SNPs in VFC
```




```
bcftools mpileup -O b -o PATH/bcf/SAMPLE_raw.bcf
-f PATH/ref_genome/ref_genome.fasta PATH/bam/output.sorted.bam
```




```
bcftools call --ploidy 1 -m -v -o PATH/bcf/SAMPLE_variants.vcf PATH/bcf/SAMPLE_raw.bcf
```




```
vcfutils.pl varFilter PATH/bcf/SAMPLE_variants.vcf  > PATH/vcf/SAMPLE_final_variants.vcf
```

VCF decoding:

Contig location of variant

Position of variant

. until annotated

Reference genotype

Sample genotype

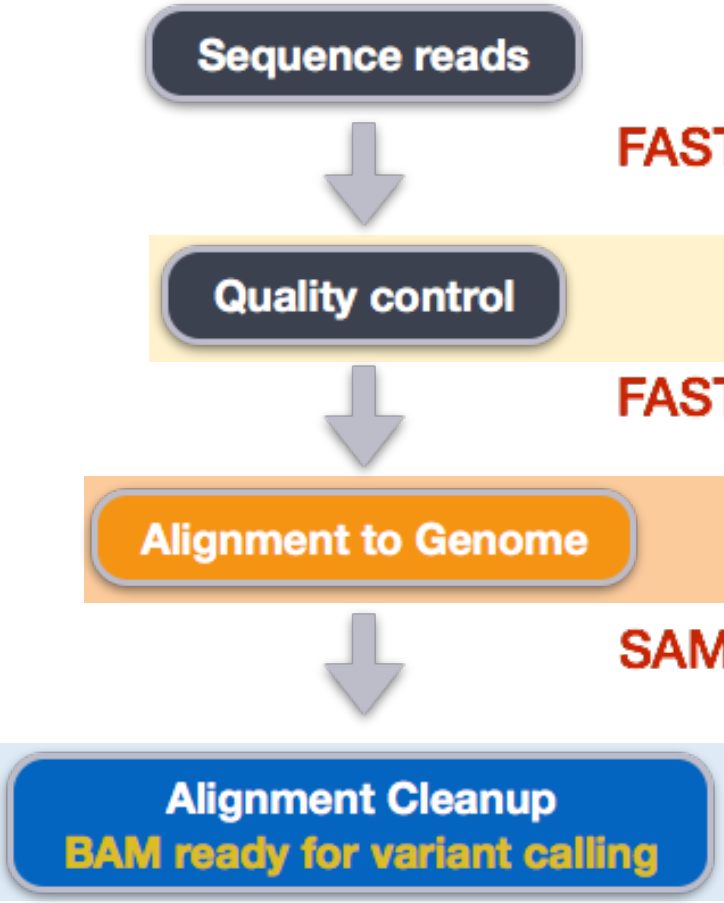
Phred-scaled probability that the observed variant exists

```
#CHROM      POS   ID  REF  ALT  QUAL  FILTER  INFO    FORMAT  results/bam/SRR2584866.aligned.sorted.bam
CP000819.1  1521  .   C    T    207   .       DP=9;VDB=0.993024;SGB=-0.662043;MQSB=0.974597;MQ0F=0;AC=1;AN=1;DP4=0,0,4,5;MQ=60          GT:PL  1:237,0
CP000819.1  1612  .   A    G    225   .       DP=13;VDB=0.52194;SGB=-0.676189;MQSB=0.950952;MQ0F=0;AC=1;AN=1;DP4=0,0,6,5;MQ=60         GT:PL  1:255,0
CP000819.1  9092  .   A    G    225   .       DP=14;VDB=0.717543;SGB=-0.670168;MQSB=0.916482;MQ0F=0;AC=1;AN=1;DP4=0,0,7,3;MQ=60        GT:PL  1:255,0
CP000819.1  9972  .   T    G    214   .       DP=10;VDB=0.022095;SGB=-0.670168;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,2,8;MQ=60       GT:PL  1:244,0
CP000819.1  10563 .   G    A    225   .       DP=11;VDB=0.958658;SGB=-0.670168;MQSB=0.952347;MQ0F=0;AC=1;AN=1;DP4=0,0,5,5;MQ=60         GT:PL  1:255,0
CP000819.1  22257 .   C    T    127   .       DP=5;VDB=0.0765947;SGB=-0.590765;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,2,3;MQ=60       GT:PL  1:157,0
CP000819.1  38971 .   A    G    225   .       DP=14;VDB=0.872139;SGB=-0.680642;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,4,8;MQ=60       GT:PL  1:255,0
CP000819.1  42306 .   A    G    225   .       DP=15;VDB=0.969686;SGB=-0.686358;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,5,9;MQ=60       GT:PL  1:255,0
CP000819.1  45277 .   A    G    225   .       DP=15;VDB=0.470998;SGB=-0.680642;MQSB=0.95494;MQ0F=0;AC=1;AN=1;DP4=0,0,7,5;MQ=60         GT:PL  1:255,0
CP000819.1  56613 .   C    G    183   .       DP=12;VDB=0.879703;SGB=-0.676189;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,8,3;MQ=60       GT:PL  1:213,0
CP000819.1  62118 .   A    G    225   .       DP=19;VDB=0.414981;SGB=-0.691153;MQSB=0.906029;MQ0F=0;AC=1;AN=1;DP4=0,0,8,10;MQ=59       GT:PL  1:255,0
CP000819.1  64042 .   G    A    225   .       DP=18;VDB=0.451328;SGB=-0.689466;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,7,9;MQ=60       GT:PL  1:255,0
CP000819.1  78808 .   C    T    225   .       DP=23;VDB=0.885435;SGB=-0.691153;MQSB=1;MQ0F=0;AC=1;AN=1;DP4=0,0,13,5;MQ=60      GT:PL  1:255,0
```

Depth per allele by sample and coverage

GT:PL:GQ = Genotype, likelihoods of genotypes, Phred-scaled confidence of genotype

Building our Pipeline:




```
# STEP 1: FASTQC raw data

# STEP 2: TRIM and FILTER

# STEP 3: FASTQC filtered data

# STEP 4: Index reference genome

# STEP 5: Align trimmed reads

# STEP 6: Convert from SAM to BAM

# STEP 7: Sort BAM file

# STEP 8: Calculate read coverage of positions in genome

bcftools mpileup -O b -o PATH/SAMPLE_raw.bcf -f PATH/ref_genome.fasta PATH/output.sorted.bam

# STEP 7: Detect SNPs

bcftools call --ploidy 1 -m -v -o PATH/bcf/SAMPLE_variants.vcf PATH/bcf/SAMPLE_raw.bcf

# STEP 9: Filter and report SNPs in VFC

vcfutils.pl varFilter PATH/bcf/SAMPLE_variants.vcf  > PATH/vcf/SAMPLE_final_variants.vcf
```

Goals for today:

- Project Organization
- Background and metadata
- Assessing sequencing read quality
- Trimming and filtering reads based on read quality
- Variant calling workflow
- Automating a variant calling workflow

Monday:

- Finish up what we don’t get to today
- Introduce cloud computing for genomics

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- Project Organization
- Background and metadata
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- Automating a variant calling workflow

Monday:

- Finish up what we don't get to today
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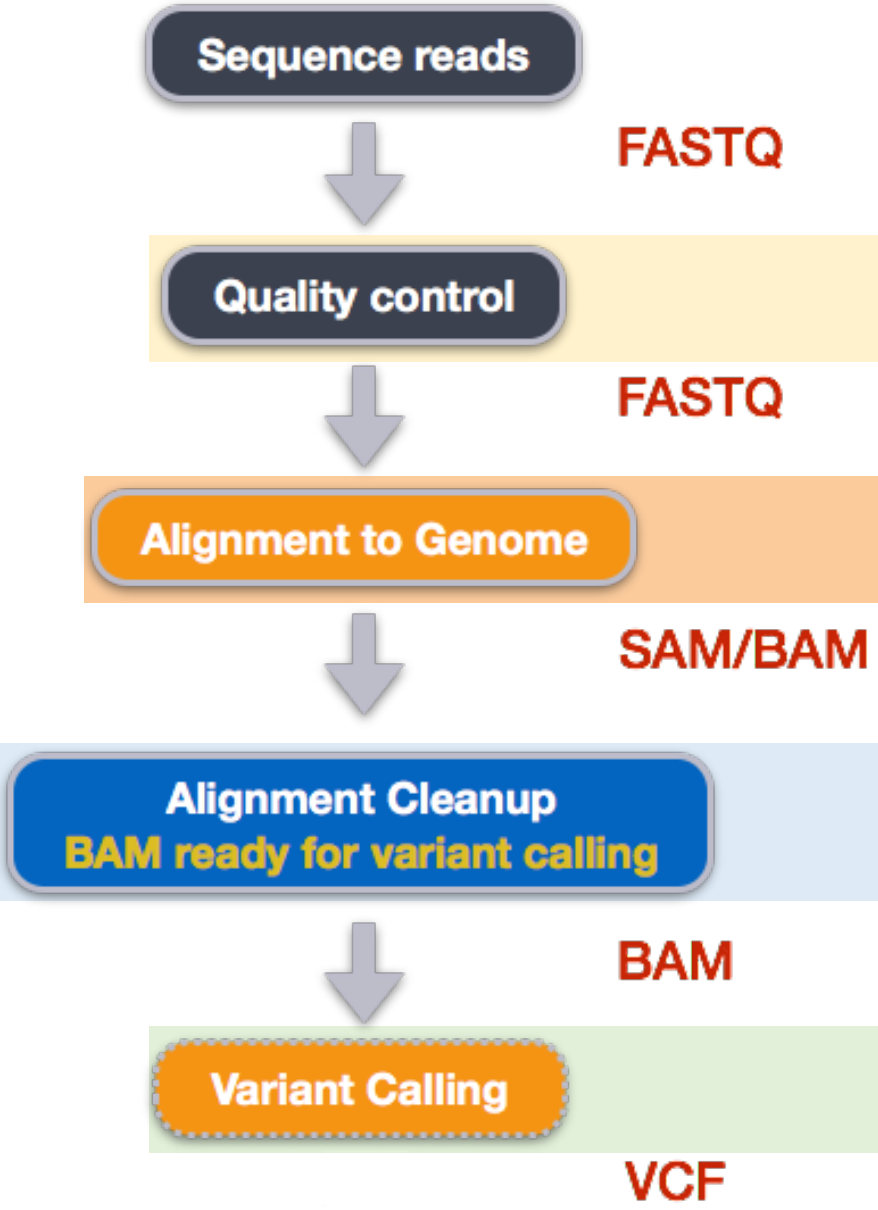
Objectives:

- Make a more efficient and less error-prone pipeline
- Write a shell script with multiple variables
- Incorporate a for loop into a shell script

Automating our Pipeline:




```
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# STEP 7: Detect SNPs
# STEP 9: Filter and report SNPs in VFC
```

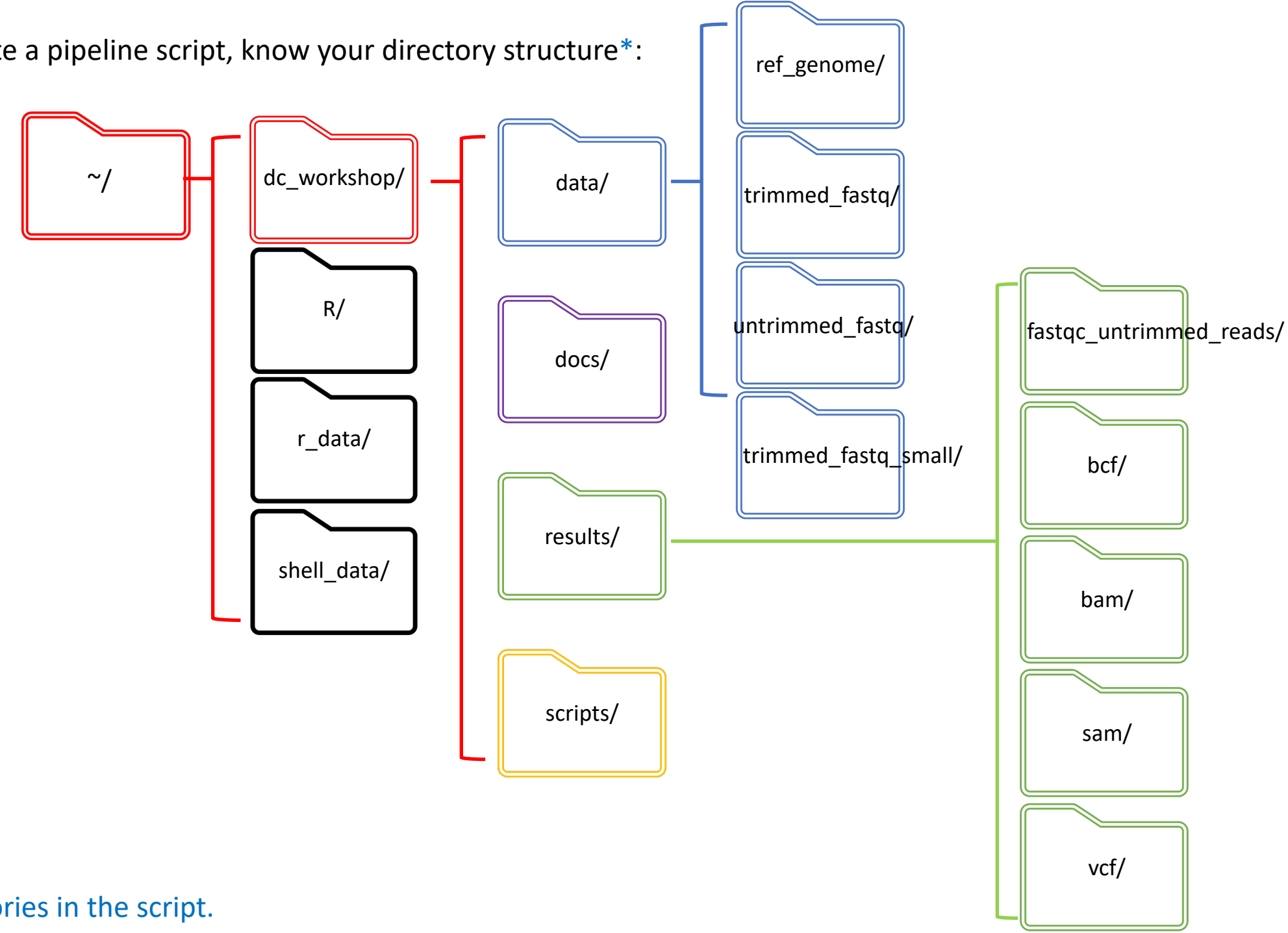
Analyzing Quality:

read_qc.sh

Align and Call Variants:

run_variant_calling.sh

BEFORE you write a pipeline script, know your directory structure*:



*or create directories in the script.

BEFORE you write a pipeline script, test elements of your script:

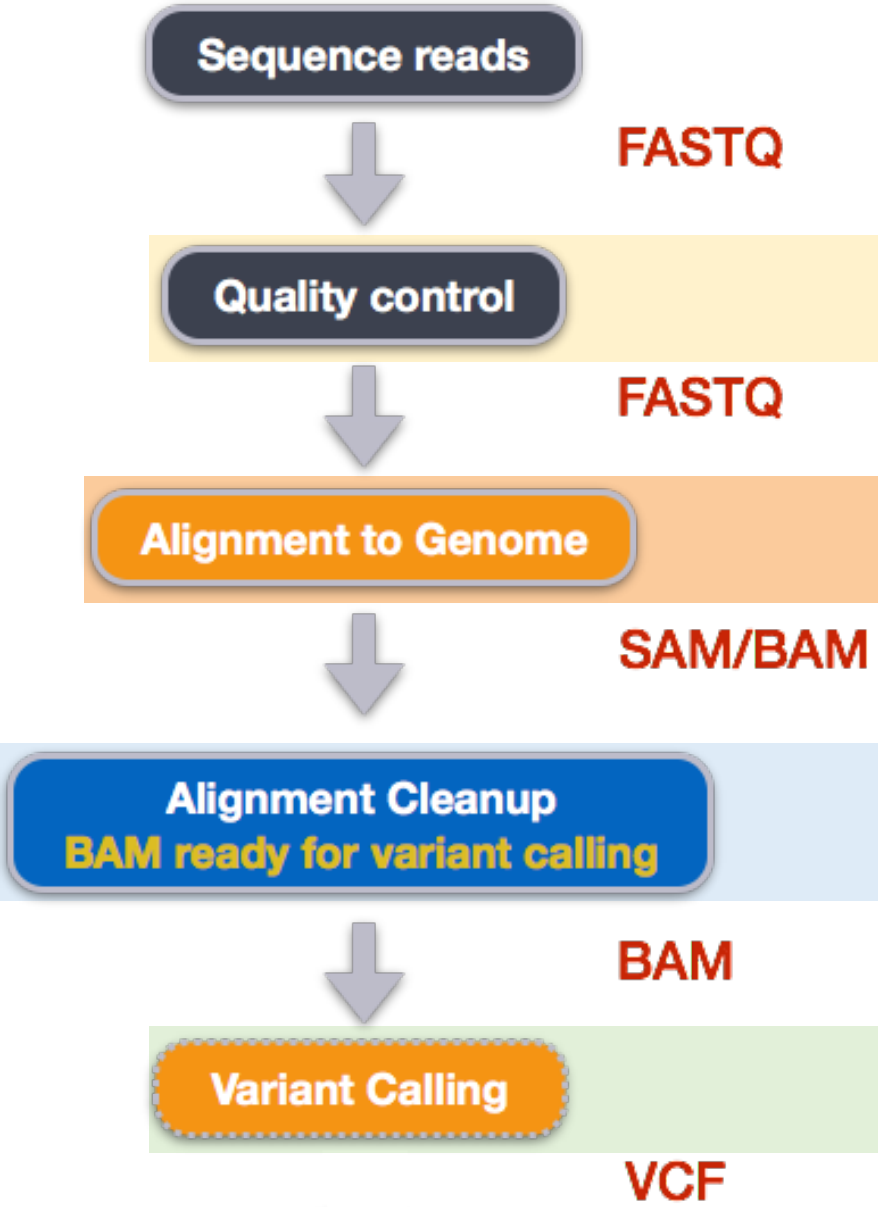
Unzipping .zip files:

```
for filename in *.zip
do
unzip $filename
done
```

Running Trimmomatic:

```
for infile in *_1.fastq.gz
do
  base=$(basename ${infile} _1.fastq.gz)
  trimmomatic PE ${infile} ${base}_2.fastq.gz \
               ${base}_1.trim.fastq.gz ${base}_1un.trim.fastq.gz \
               ${base}_2.trim.fastq.gz ${base}_2un.trim.fastq.gz \
               SLIDINGWINDOW:4:20 MINLEN:25 ILLUMINACLIP:NexteraPE-PE.fa:2:40:15
done
```

Automating our Pipeline:




```
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```

Analyzing Quality:

read_qc.sh

Align and Call Variants:

run_variant_calling.sh

```
set -e
cd ~/dc_workshop/results

genome=~/dc_workshop/data/ref_genome/ecoli_rel606.fasta

bwa index $genome

mkdir -p sam bam bcf vcf

for fq1 in ~/dc_workshop/data/trimmed_fastq_small/*_1.trim.sub.fastq
    do
    echo "working with file $fq1"

    base=$(basename $fq1 _1.trim.sub.fastq)
    echo "base name is $base"

    fq1=~/dc_workshop/data/trimmed_fastq_small/${base}_1.trim.sub.fastq
    fq2=~/dc_workshop/data/trimmed_fastq_small/${base}_2.trim.sub.fastq
    sam=~/dc_workshop/results/sam/${base}.aligned.sam
    bam=~/dc_workshop/results/bam/${base}.aligned.bam
    sorted_bam=~/dc_workshop/results/bam/${base}.aligned.sorted.bam
    raw_bcf=~/dc_workshop/results/bcf/${base}_raw.bcf
    variants=~/dc_workshop/results/bcf/${base}_variants.vcf
    final_variants=~/dc_workshop/results/vcf/${base}_final_variants.vcf

    bwa mem $genome $fq1 $fq2 > $sam
    samtools view -S -b $sam > $bam
    samtools sort -o $sorted_bam $bam
    samtools index $sorted_bam
    bcftools mpileup -O b -o $raw_bcf -f $genome $sorted_bam
    bcftools call --ploidy 1 -m -v -o $variants $raw_bcf
    vcfutils.pl varFilter $variants > $final_variants

    done
```

Define variables

Run alignment

Challenge/Homework:

- We were working with subsetted trimmed fastq data files for efficiency
- Re-run the alignment on the un-subsetted fastq files you generated earlier