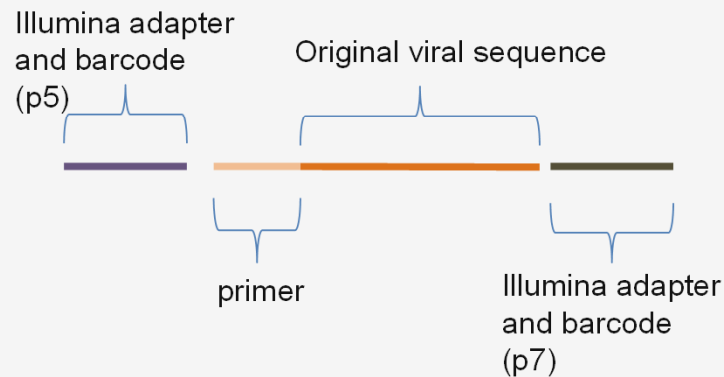


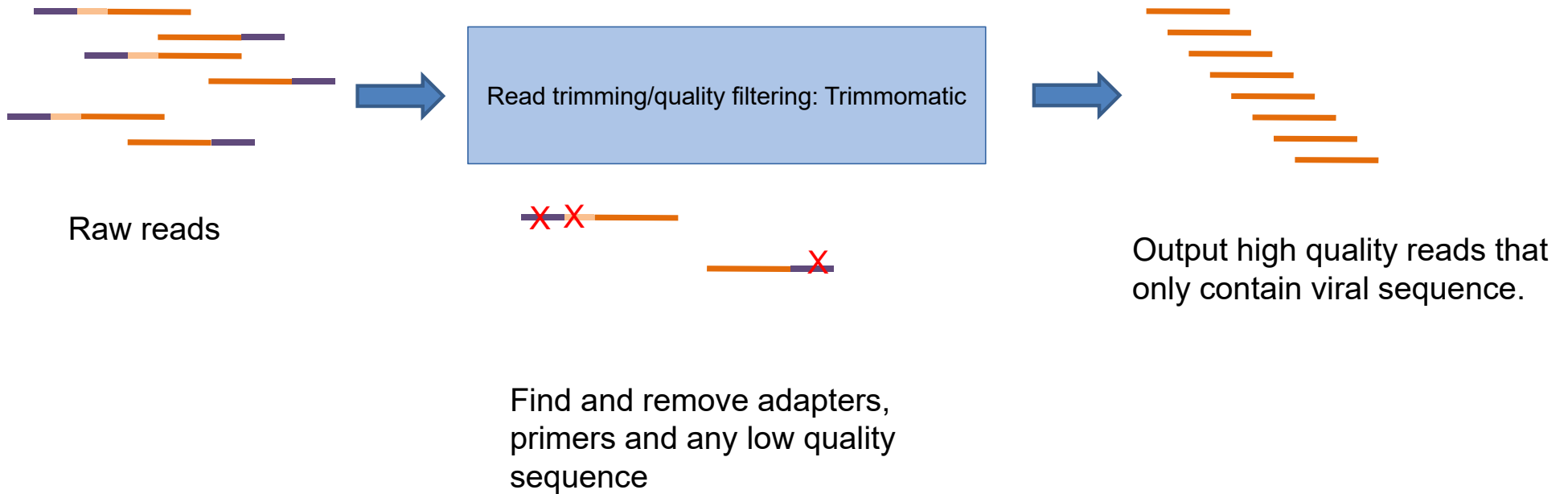
# Library Preparation



Add Illumina adapters, which are DNA sequences necessary for sequencing on the Miseq.

- **Remember that sequences were added during sample and library preparations that are not part of the original viral sequence.**
- **We need to remove those sequences now.**

# Read trimming/quality filtering



Read1  
.fastq.  
gz

Read2  
.fastq.  
gz

adapters\_  
primers.fa  
sta

- Take raw reads and list of sequences added during library prep.
- Remove those sequences, and any sequence of low quality

java program to run

Paired-end  
mode

Save log file.

Raw read files

Indicates the line below is a continuation  
of this line, and not a new command.

**Warning! The next command is lengthy and contains many options.**  
**It is written on several lines for ease of viewing.**

```
java -jar trimomatic-0.35.jar PE-trimlog trim.log Read1.fastq.gz Read2.fastq.gz  
trimmedR1_paird.fastq trimmedR1_unpaired.fastq trimmedR2_paird.fastq trimmedR2_unpaired.fastq \  
ILLUMINACLIP:adapters_primers.fasta:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:30
```

Remove these sequences from reads

Trim reads based on quality

Output files

trimmed  
R1\_pair  
ed.fastq

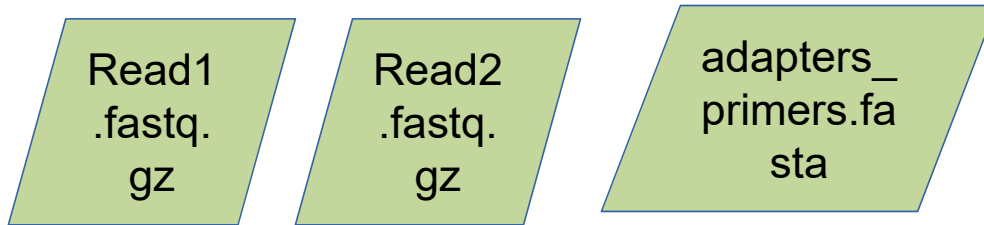
trimmed  
R2\_pair  
ed.fastq

Reference  
Based  
Assembly

~~trimmed  
R1\_unpa  
ired.fastq~~

~~trimmed  
R2\_unpa  
ired.fas  
tq~~

Discard for  
this  
analysis



- Take raw reads and list of sequences added during library prep.
- Remove those sequences, and any sequence of low quality

java program to run

Paired-end  
mode

Save log file.

Raw read files

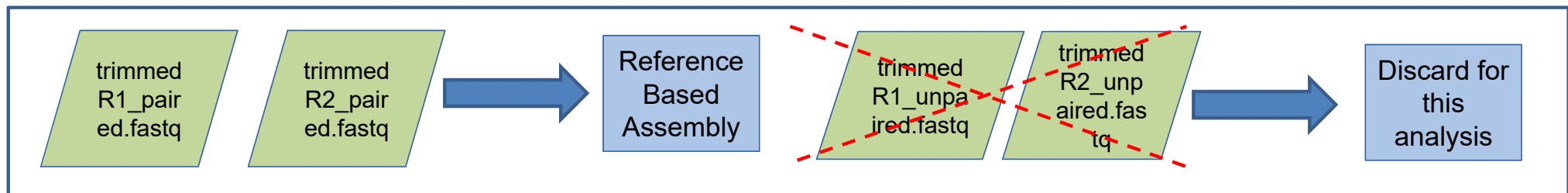
Indicates the line below is a continuation  
of this line, and not a new command.

```
java -jar trimmomatic-0.33.jar PE-trimlog trim.log Read1.fastq.gz Read2.fastq.gz \  
trimmedR1_pair ed.fastq trimmedR1_unpaired.fastq trimmedR2_pair ed.fastq trimmedR2_unpaired.fastq \  
ILLUMINACLIP:adapters_primers.fasta:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:30
```

Remove these sequences from reads

Trim reads based on quality

Output files



Read1  
.fastq.  
gz

Read2  
.fastq.  
gz

adapters\_  
primers.fa  
sta

- Take raw reads and list of sequences added during library prep.
- Remove those sequences, and any sequence of low quality

java program to run

Paired-end  
mode

Save log file.

Raw read files

Indicates the line below is a continuation  
of this line, and not a separate command.

Note that this command requires outputting 4 files,  
but we will only use two in the subsequent steps.

```
java -jar trimmomatic.jar -SE -P -I -L adapters_primers.fasta:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:30  
trimmedR1_pair ed.fastq trimmedR1_unpaired.fastq trimmedR2_pair ed.fastq trimmedR2_unpaired.fastq \
```

Remove these sequences from reads

Trim reads based on quality

Output files

trimmed  
R1\_pair  
ed.fastq

trimmed  
R2\_pair  
ed.fastq

Reference  
Based  
Assembly

~~trimmed  
R1\_unpa  
ired.fastq~~

~~trimmed  
R2\_unp  
aired.fas  
tq~~

Discard for  
this  
analysis