Follow Up from Yesterday

- Cyberduck

```
scp ~/.ssh/id_rsa.pub kkeith@10.1.105.13:.ssh/authorized_keys
```

- GitHub
  - I put the repository in the wrong folder
  - Walk through it again slowly

Processing RNA-seq Data

2020-07-23

Trim Bad Quality Sequences

What is trimming and why do it?

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- Why is that important?
  - Helps prevent incorrect base calls by removing poor quality information
  - Increases speed and accuracy of alignment by removing artificial sequences and low quality sequences

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  - Helps prevent incorrect base calls by removing poor quality information
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- Trimming does two complementary things:
  1. Removes any sequence information that comes from library preparation or sequencing
  2. Removes low quality bases / low quality reads

Trim Sequences

1. Go back up to the rnaseq directory
2. Make a folder to put the analysis results in, `analysis`
3. Make a folder inside the analysis folder to put the trimmed reads in, `analysis/01_trim`

Trim Sequences

```
for i in rnaseq_data/*R1.fastq.gz;
     do trim_galore
          --paired
          --fastqc
          --illumina
          --output analysis/01_trim/
          --retain_unpaired
          $i
          ${i/R1/R2};
done
```

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call the program

reads are paired-end

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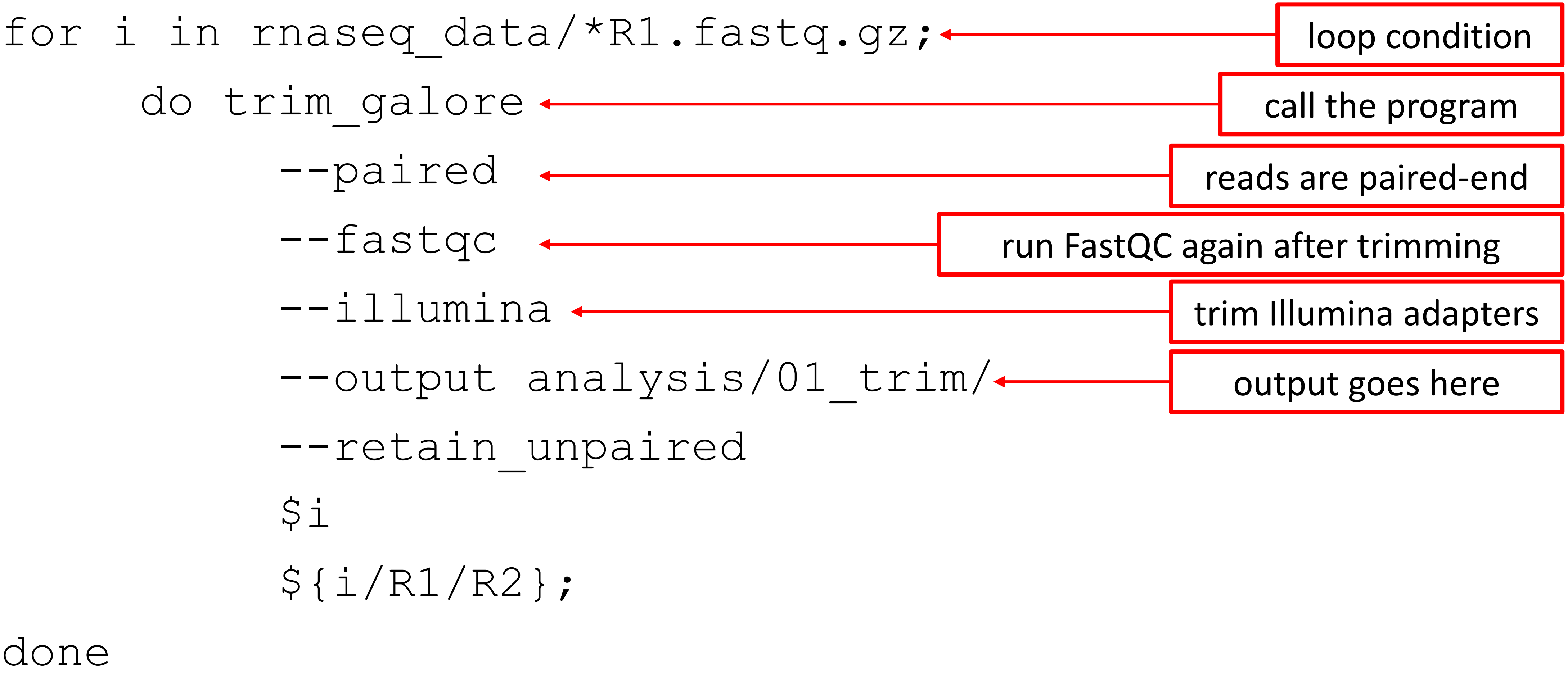
trim Illumina adapters

Trim Sequences



```
for i in rnaseq_data/*R1.fastq.gz;
     do trim_galore
          --paired
          --fastqc
          --illumina
          --output analysis/01_trim/
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          ${i/R1/R2};
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```

loop condition

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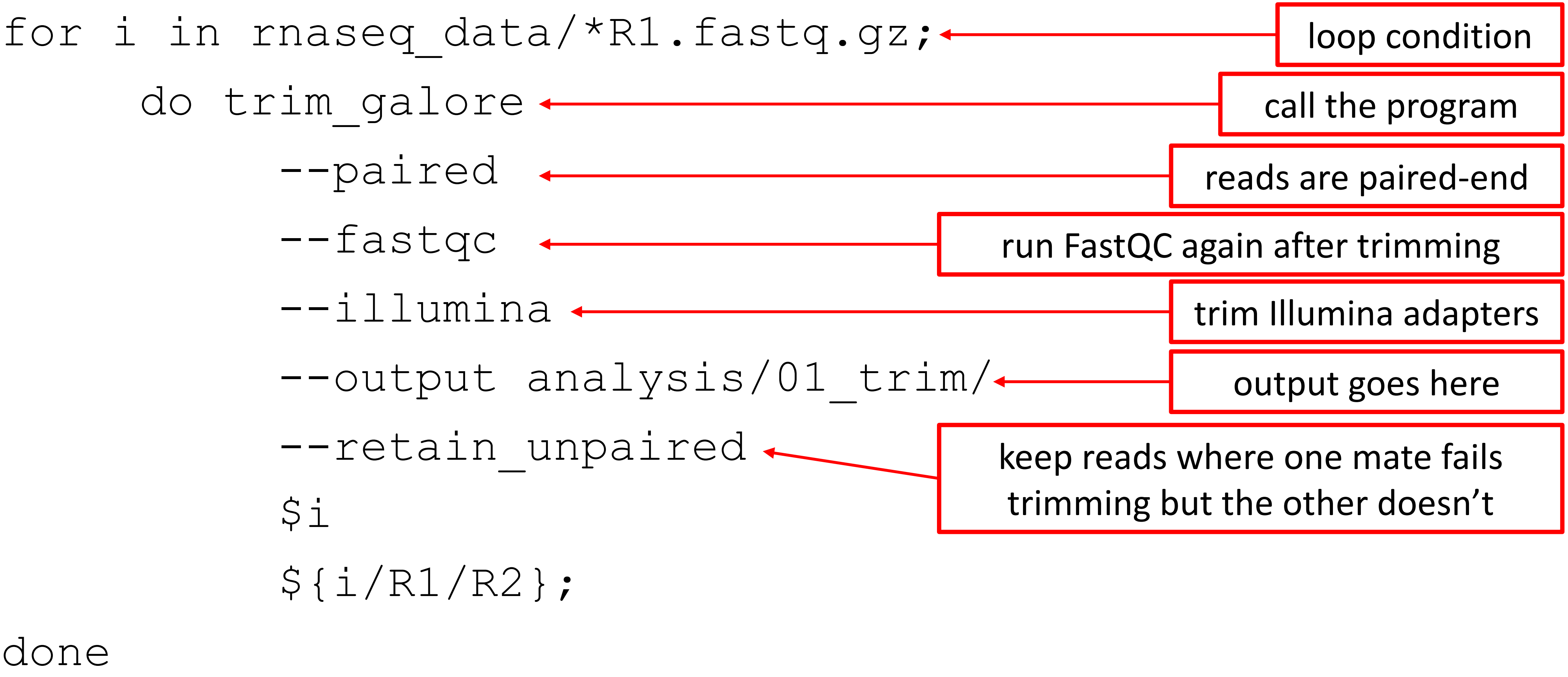
trim Illumina adapters

output goes here

Trim Sequences



```
for i in rnaseq_data/*R1.fastq.gz;
    do trim_galore
        --paired
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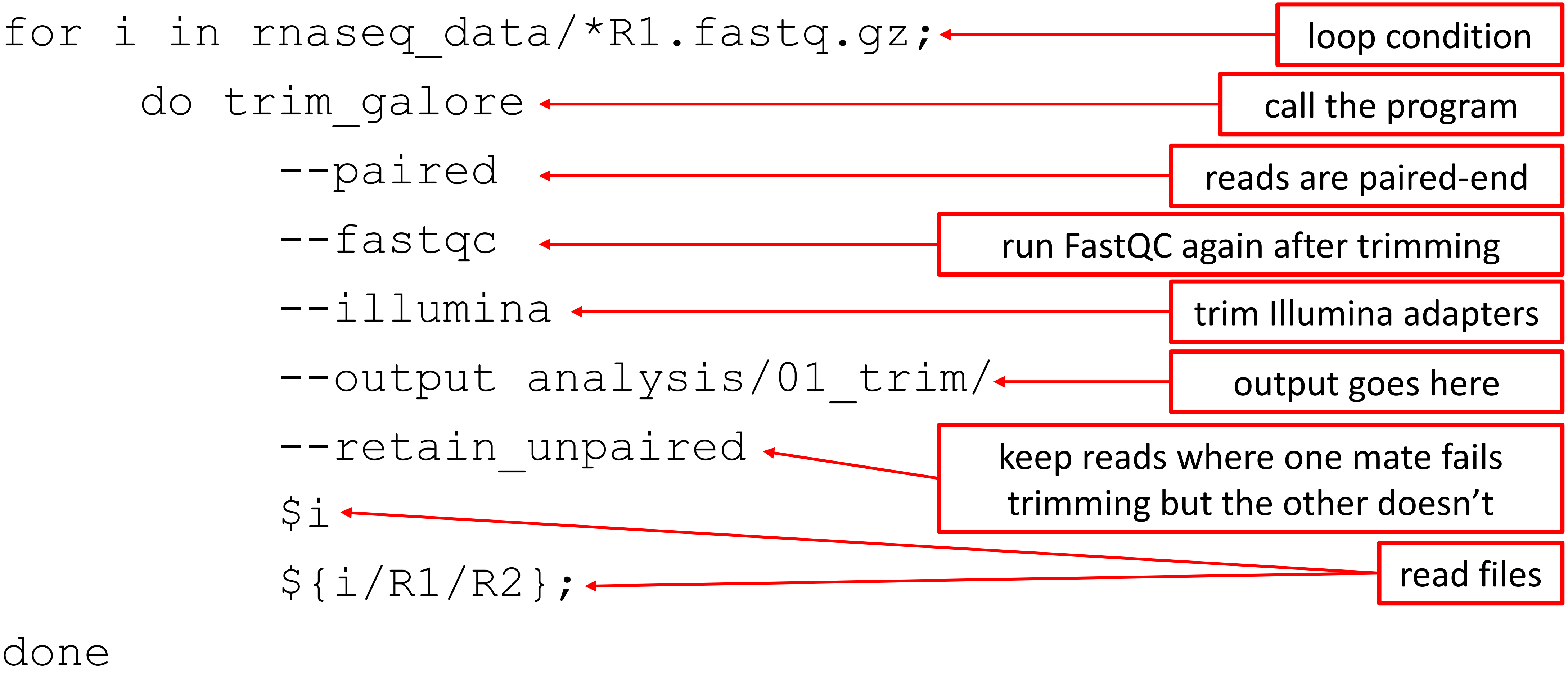
output goes here

keep reads where one mate fails trimming but the other doesn’t

Trim Sequences



```
for i in rnaseq_data/*R1.fastq.gz;
     do trim_galore
          --paired
          --fastqc
          --illumina
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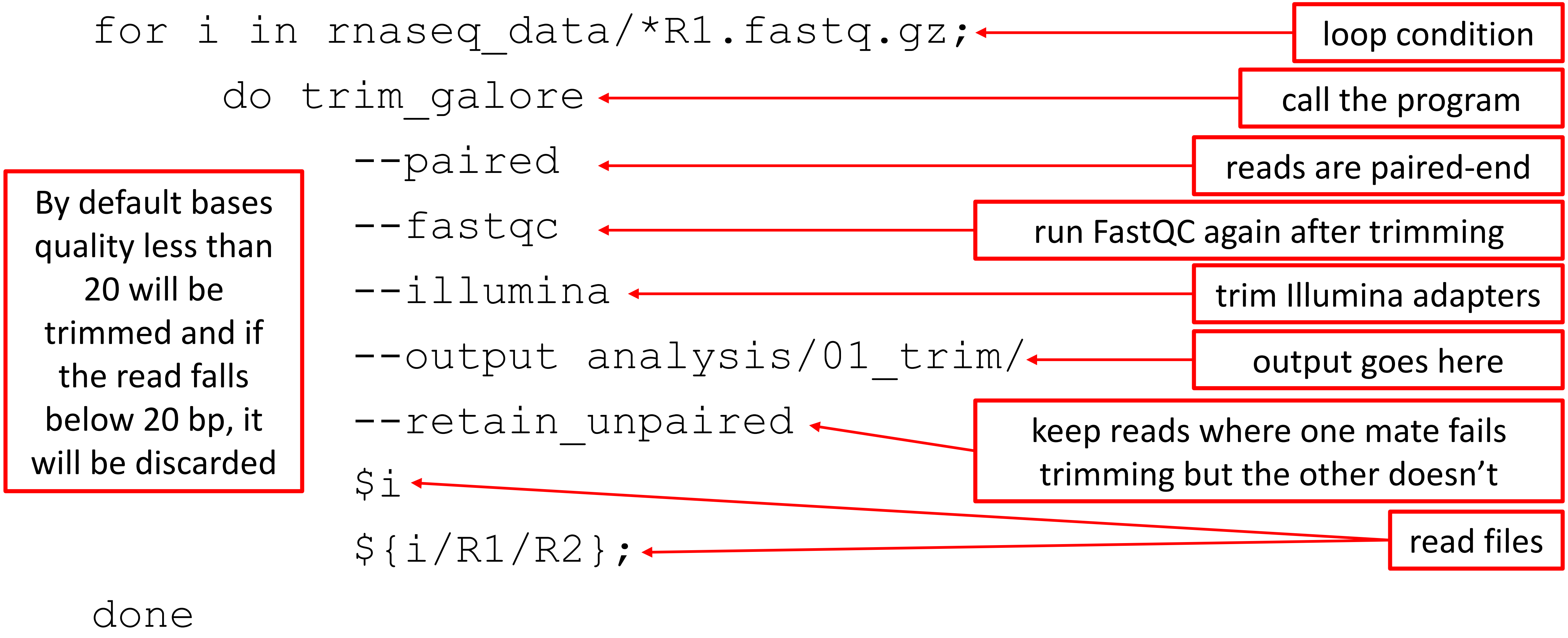
read files

Trim Sequences



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for i in rnaseq_data/*R1.fastq.gz;
        do trim_galore
                --paired
                --fastqc
                --illumina
                --output analysis/01_trim/
                --retain_unpaired
                $i
                ${i/R1/R2};
done
```

loop condition

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output goes here

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read files

By default bases quality less than 20 will be trimmed and if the read falls below 20 bp, it will be discarded

Trim Sequences

```
for i in rnaseq_data/*R1.fastq.gz; do trim_galore
--paired --fastqc --illumina --output analysis/01_trim/
--retain_unpaired $i ${i/R1/R2}; done
```

Align

How does aligning work?

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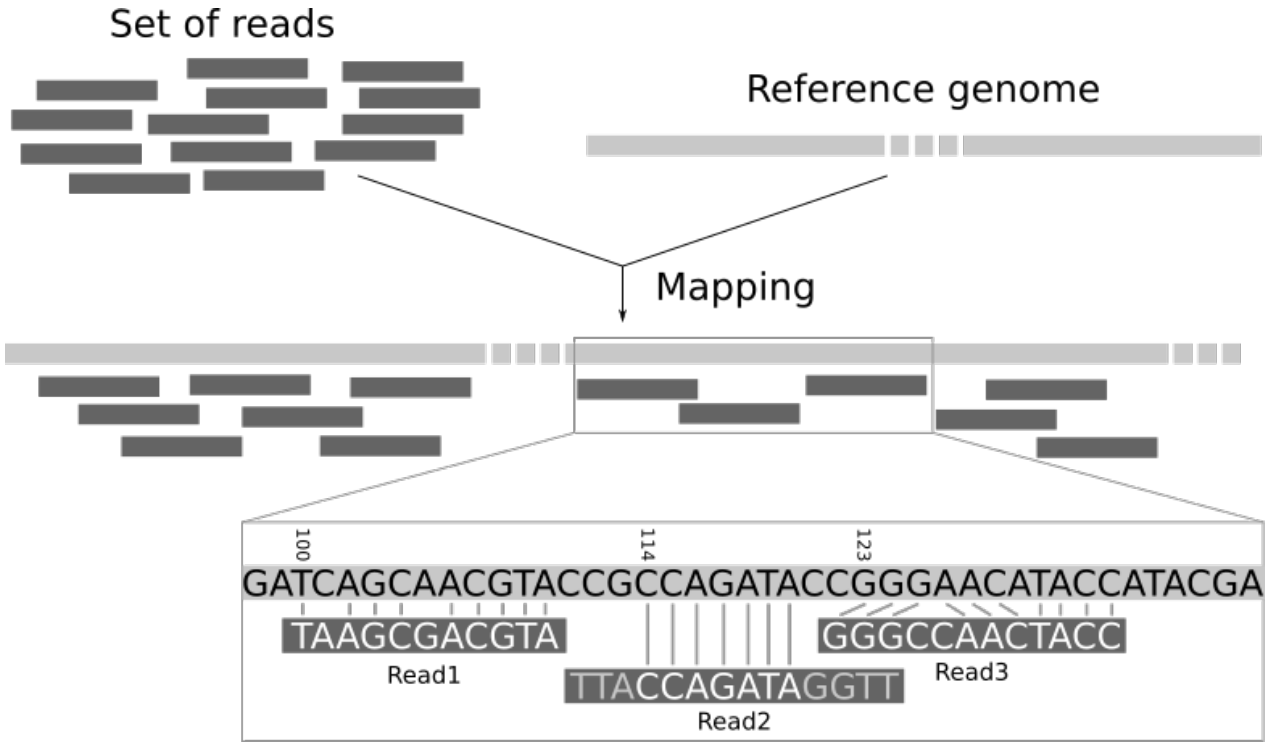
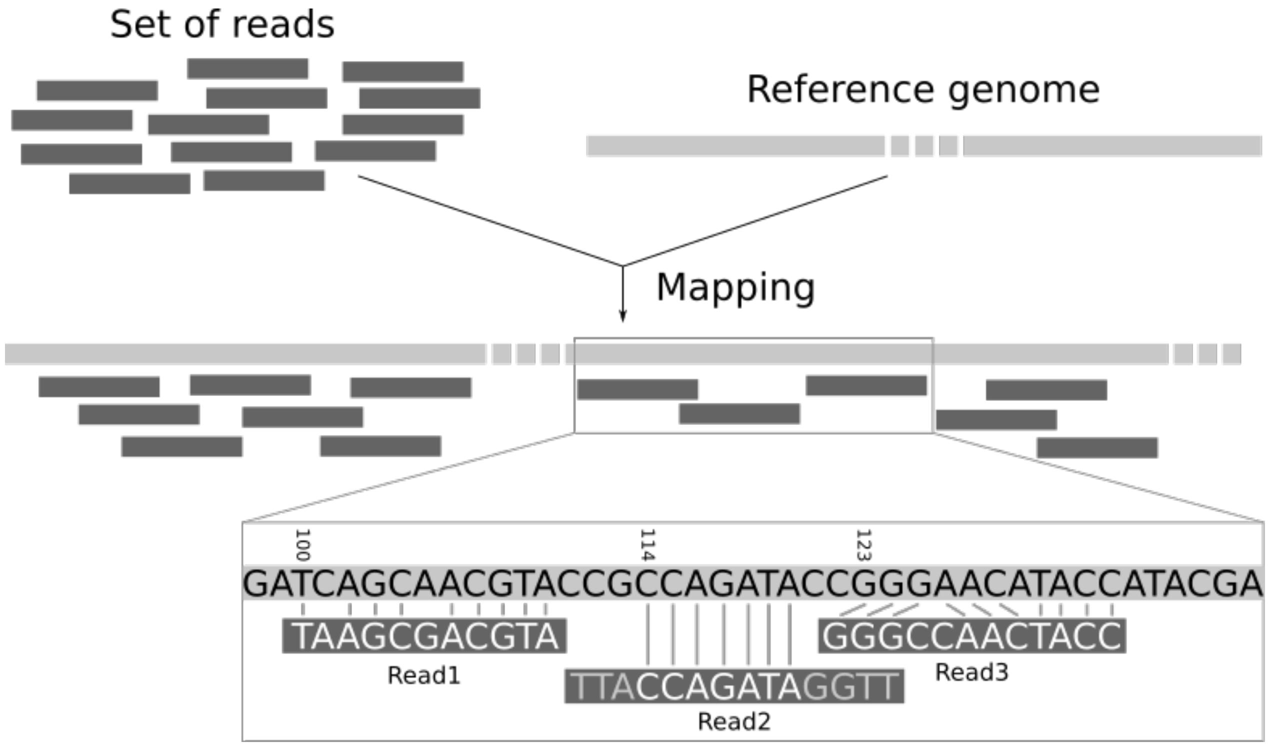
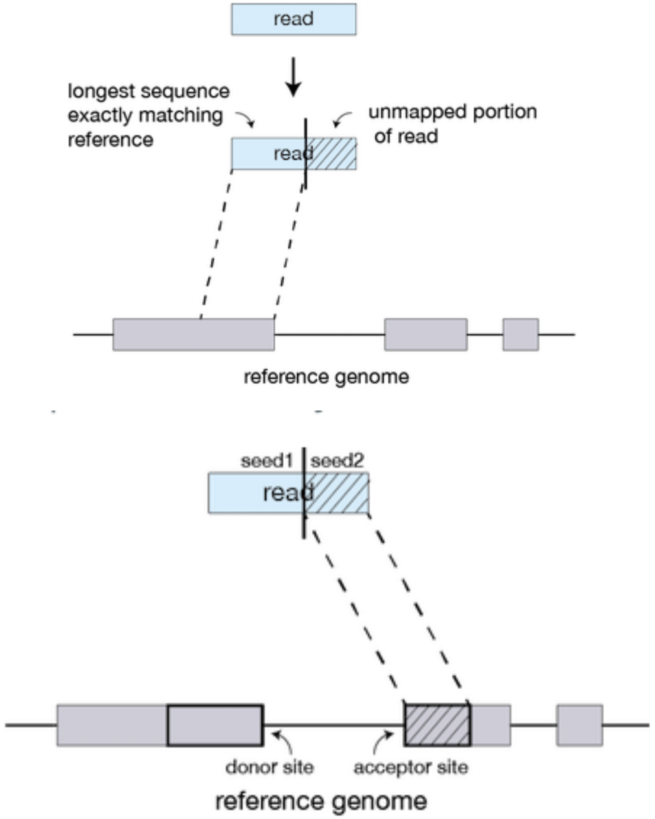


How does aligning work?



STAR (Spliced Transcripts Alignment to a Reference)



Align Sequences

1. Make a folder inside the analysis folder to put the aligned reads in, `analysis/02_align`
2. Change to the trimmed reads folder `analysis/01_trim`

Align Sequences

```
for i in *val_1.fq.gz;
     do STAR
         --genomeDir /mnt/data/gdata/human \
                      /hg38/chr21/STAR_index
         --readFilesIn $i ${i/R1_val_1/R2_val_2}
         --readFilesCommand zcat
         --outFileNamePrefix ../02_align/${i/R1*/}
         --outSAMtype BAM SortedByCoordinate;
done
```

Align Sequences

```
for i in *val_1.fq.gz;
     do STAR
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          --readFilesIn $i ${i/R1_val_1/R2_val_2}
          --readFilesCommand zcat
          --outFileNamePrefix ../02_align/${i/R1*/}
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done
```

loop condition

Align Sequences

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loop condition

call aligner

Align Sequences

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loop condition

call aligner

path to reference genome

Align Sequences

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trimmed read files

Align Sequences

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          --genomeDir /mnt/data/gdata/human \
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          --outFileNamePrefix ../02_align/${i/R1*/}
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for i in *val_1.fq.gz;
   do STAR
      --genomeDir /mnt/data/gdata/human \
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      --readFilesIn $i ${i/R1_val_1/R2_val_2}
      --readFilesCommand zcat
      --outFileNamePrefix ../02_align/${i/R1*/}
      --outSAMtype BAM SortedByCoordinate;
done
```

loop condition

call aligner

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write the files here

Align Sequences

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          --outFileNamePrefix ../02_align/${i/R1*/}
          --outSAMtype BAM SortedByCoordinate;
done
```

loop condition

call aligner

path to reference genome

trimmed read files

zipped files

write the files here

write a sorted BAM

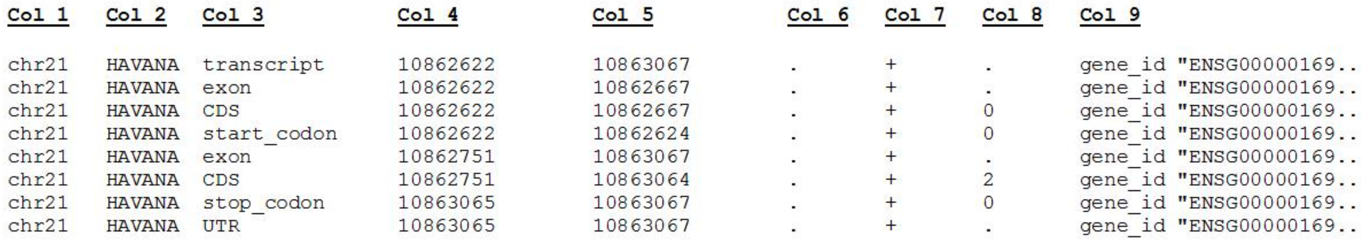
Align Sequences

```
for i in *val_1.fq.gz; do STAR --genomeDir
/mnt/data/gdata/human/hg38/chr21/STAR_index --
readFilesIn $i ${i/R1_val_1/R2_val_2} --
readFilesCommand zcat --outFileNamePrefix
../02_align/${i/R1*/} --outSAMtype BAM
SortedByCoordinate; done
```

Count Features

What do you mean by count features?

- We’re going to count genes, but you could also count:
    - transcripts
    - non-coding RNA
- Need an annotation file for whatever feature you want to count
- Going to use a gene transfer format (GTF) file for annotations



Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9
chr21	HAVANA	transcript	10862622	10863067	.	+	.	gene_id "ENSG00000169..
chr21	HAVANA	exon	10862622	10862667	.	+	.	gene_id "ENSG00000169..
chr21	HAVANA	CDS	10862622	10862667	.	+	0	gene_id "ENSG00000169..
chr21	HAVANA	start_codon	10862622	10862624	.	+	0	gene_id "ENSG00000169..
chr21	HAVANA	exon	10862751	10863067	.	+	.	gene_id "ENSG00000169..
chr21	HAVANA	CDS	10862751	10863064	.	+	2	gene_id "ENSG00000169..
chr21	HAVANA	stop_codon	10863065	10863067	.	+	0	gene_id "ENSG00000169..
chr21	HAVANA	UTR	10863065	10863067	.	+	.	gene_id "ENSG00000169..

Count Features

1. Make a folder inside the analysis folder to put the aligned reads in, `../03_count`
2. Change to the trimmed reads folder `../02_align/`

Count Features

```
for i in *.bam;
     do featureCounts
          -a /mnt/data/gdata/human/hg38/chr21/ \
             homo_sapiens_hg38_chr21.gtf
          -o ../03_count/${i/ \
             Aligned.sortedByCoord.out.bam/ \
             counts.txt}
          -R BAM
          $i;
done
```

Count Features

```
for i in *.bam;
     do featureCounts
          -a /mnt/data/gdata/human/hg38/chr21/ \
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          $i;
done
```

loop condition

Count Features

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          -R BAM
          $i;
done
```

loop condition

call program

Count Features

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          $i;
done
```

loop condition

call program

path to genome annotation file

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where to write the output

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input files are BAM

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input files are BAM

input file

Count Features

```
for i in *.bam; do featureCounts -a /mnt/data/gdata/human/hg38/chr21/homo_sapiens_hg38_chr21.gtf -o ../03_count/${i/Aligned.sortedByCoord.out.bam/counts.txt} -R BAM $i; done
```

General Steps

1. Check quality
2. Trim
3. Align
4. Count features
5. Statistics