# Bioinformatics Lessons Schedule

- RNA-seq
- single cell RNA-seq
- RRBS

| Date | Subject |
|---|---|
| 12-24 | Christmas break |
| 12-31 | Christmas break |
| 01-07 | Process RNA-seq |
| 01-14 | Process RNA-seq, continued |
| 01-21 | Process RNA-seq, continued |
| 01-28 | Analyze RNA-seq |
| 01-28 | Analyze RNA-seq, continued |

# Trim Command

```
for i in *R1.fastq.gz; do trim_galore
--paired --fastqc --illumina --output analysis/01_trim/
--retain_unpaired -q 30 $i ${i/R1/R2}; done
```

# Trim Command

```
for i in *R1.fastq.gz; do trim_galore
--paired --fastqc --illumina --output analysis/01_trim/
--retain_unpaired –q 30 $i ${i/R1/R2}; done
```

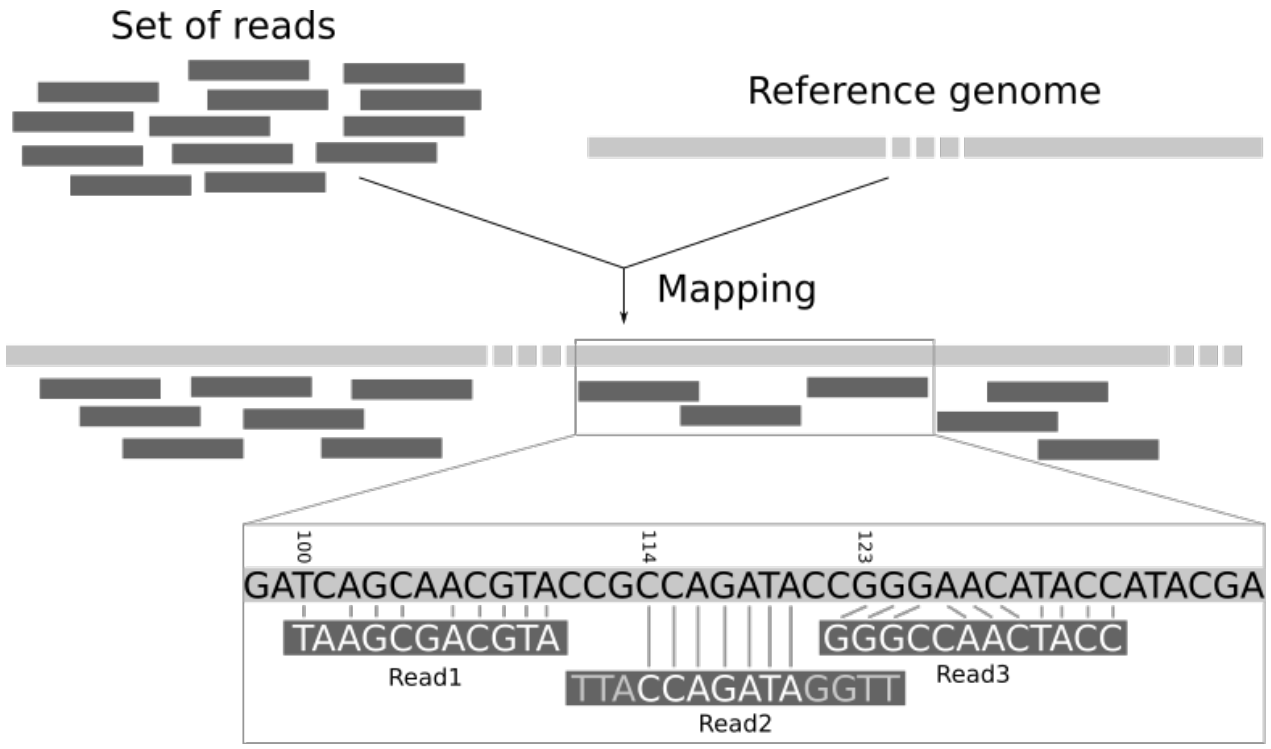
**Look at the results!**

# Align

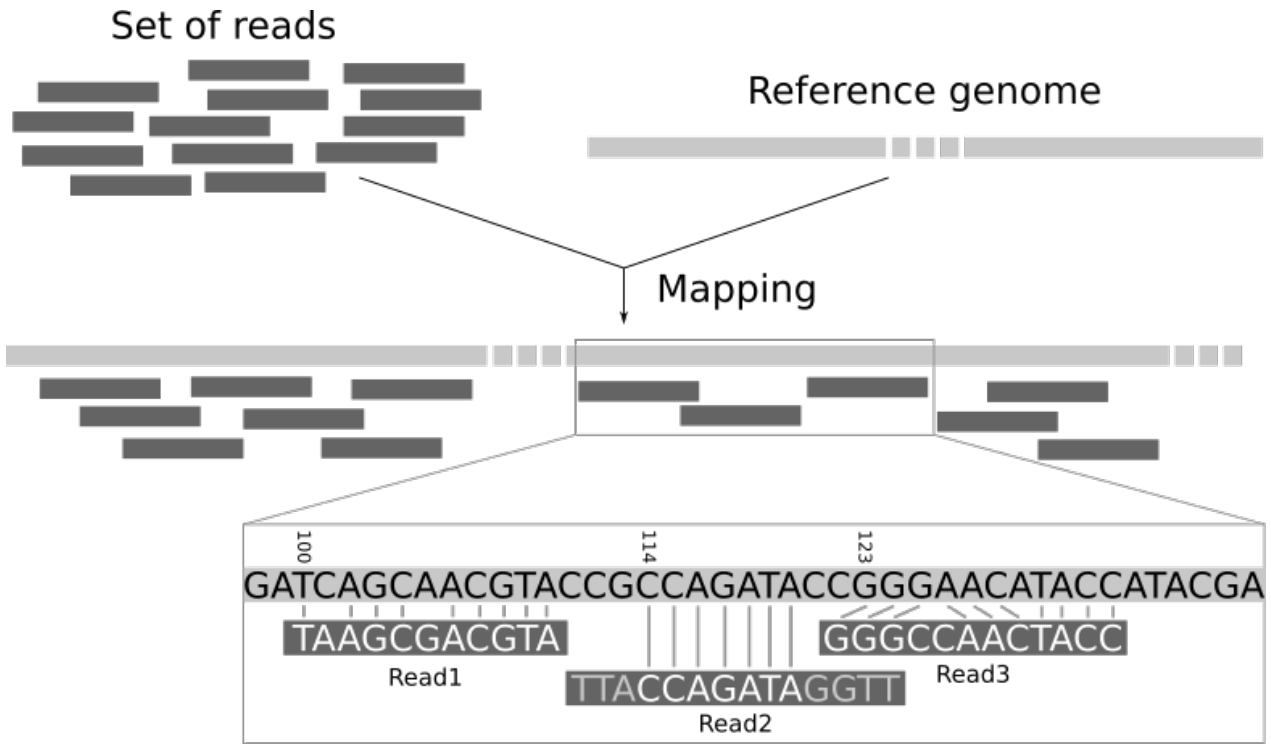
# What is aligning and why do it?

# How does aligning work?

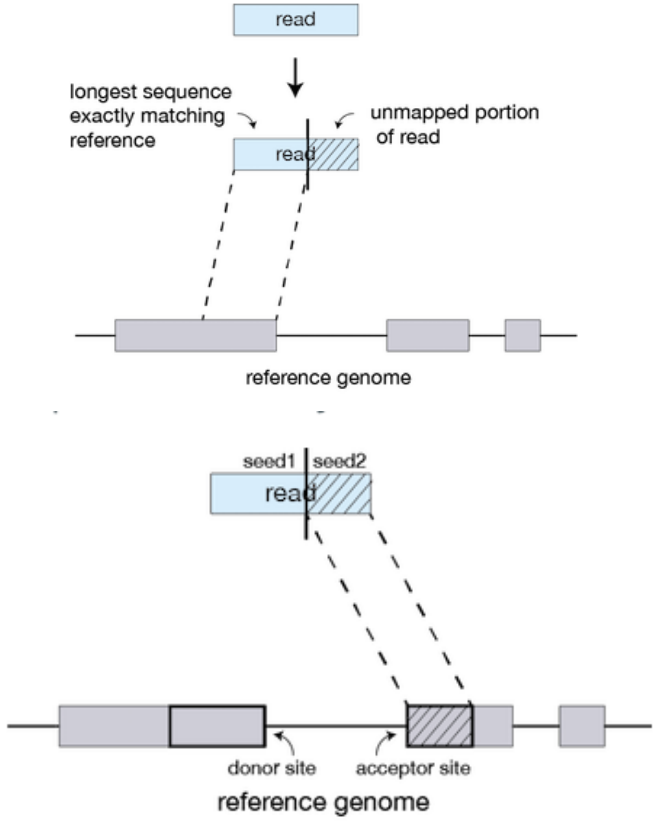
# How does aligning work?



# How does aligning work?




## STAR (Spliced Transcripts Alignment to a Reference)



# 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
```

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     do STAR
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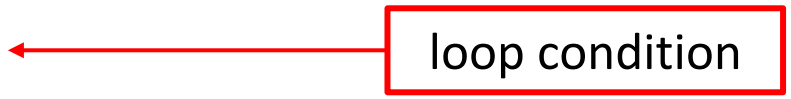

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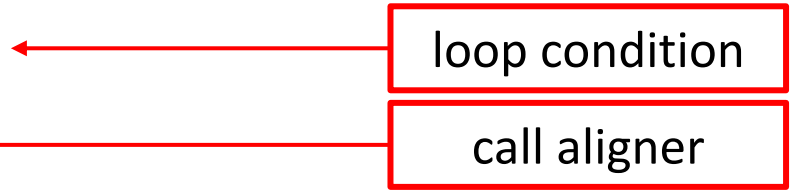

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```

loop condition

call aligner

path to reference genome

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trimmed read files

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loop condition

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trimmed read files

zipped files

write the files here

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        --readFilesCommand zcat
        --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

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 Command

```
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/ \
             homo_sapiens_hg38_chr21.gtf
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             counts.txt}
          -R BAM
          $i;
done
```

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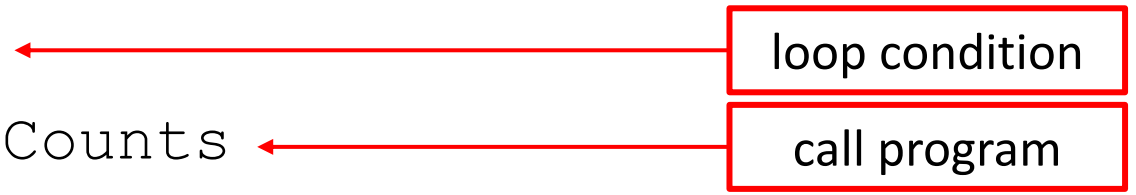
          -o ../03_count/${i/ \
             Aligned.sortedByCoord.out.bam/ \
             counts.txt}
          -R BAM
          $i;
done
```



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            -a /mnt/data/gdata/human/hg38/chr21/ \
               homo_sapiens_hg38_chr21.gtf
            -o ../03_count/${i/ \
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               counts.txt}
            -R BAM
            $i;
done
```

loop condition

call program

path to genome annotation file

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where to write the output

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           Aligned.sortedByCoord.out.bam/ \
           counts.txt}
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        $i;
done
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loop condition

call program

path to genome annotation file

where to write the output

input files are BAM

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           Aligned.sortedByCoord.out.bam/ \
           counts.txt}
        -R BAM
        $i;
done
```

loop condition

call program

path to genome annotation file

where to write the output

input files are BAM

input file

# Count Features

```
for i in *.bam; do featureCounts -a
/mnt/data/gdata/human/hg38/chr21/homo_sapiens_hg
38_chr21.gtf -o
../03_count/${i/Aligned.sortedByCoord.out.bam/co
unts.txt} -R BAM $i; done
```

# General Steps

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