Pathogen Genomics: Introduction to genome sequencing and analysis

Adam Reid & Steve Doyle

Wellcome Sanger Institute/LSHTM

LSHTM Pathogen Genomics

Summary

- What is the point of a genome sequence?
- Genome sequencing technologies
- Sequence data files
- Viewing genomes
- Computer practical 1: Viewing genome sequences
- Computer practical 2: Analysis of sequence variants

Why do genome sequencing?

- Reference for molecular biology
  - *Tropheryma whipplei* causes the potentially fatal Whipple's disease. Could not easily be grown. Genome revealed it had lost genes involved in producing amino acids.
- Identify all the genes that determine the function of the organism
  - *Neisseria meningitidis*, a major cause of meningitis. The first vaccine for a particular form of meningitis for identified by looking for candidates in its genome.
  - *Rickettsia prowazekii* is the cause of epidemic typhus, which killed millions in the early 20th century. It cannot reproduce outside of these cells. It was found to have just over 800 genes.
- Examine evolution by comparative genomics
- Track spread of pathogens
- Identify antimicrobial/drug resistance genes and drug targets
  - Mtb researchers made bacteria resistant to a new drug. Genome sequencing identified the gene involved in resistance.
- Basis for other omics technologies – RNA-seq, ChIP-seq, Methylome etc.

Why do genome sequencing? - Video of Wellcome Sanger Institute researchers

Technology overview

- Sanger sequencing produces ~500bp reads
  - Pros: Highly accurate
  - Cons: Expensive, laborious
  - Uses: High quality reference genomes
- Illumina's sequencing-by-synthesis 75-250bp
  - Pros: cheap, lots of reads (e.g. 500 million per run)
  - Cons: short reads
  - Uses: Resequencing, draft genomes, RNA-seq
- Pacific Biosciences Single-Molecule Real Time (SMRT) reads of 5000bp-40000bp
  - Pros: long reads
  - Cons: Fewer reads than Illumina - ~1 million, low accuracy
  - Uses: Reference genomes

Genome sequencing technologies – Interview with Mike Quail

Sequence data

Fasta

```
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Fastq

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EMBL

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SV   AF115338.1
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DT   23-AUG-1999 (Rel. 60, Last updated, Version 2)
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KW   .
OS   Pseudomonas fluorescens
OC   Bacteria; Proteobacteria; gamma subdivision; Pseudomonadaceae; Pseudomonas.
RN   [1]
RP   1-591
RX   MEDLINE; 99369842.
RA   Brinkman F.S., Schoofs G., Hancock R.E., De Mot R.;
RT   "Influence of a putative ECF sigma factor on expression of the major outer
RT   membrane protein, OprF, in Pseudomonas aeruginosa and Pseudomonas
RT   fluorescens";
RL   J. Bacteriol. 181(16):4746-4754(1999).
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RA   De Mot R.;
RT   ;
RL   Submitted (04-DEC-1998) to the EMBL/GenBank/DDBJ databases.
RL   F.A. Janssens Laboratory of Genetics, Applied Plant Sciences, K.
RL   Mercierlaan 92, Heverlee B-3001, Belgium
DR   SPTREMBL; Q9X4L7; Q9X4L7.
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     cggcgttacc agcgacatt atttaacgtt tgtgcgagat atcttgggaa cgatcgcgac        180
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     tatcggaagg aacggcgaaa gcgtcgcttg atggacgcat tgagtcttga ccccctcgag        360
```

EMBL Flat File

- **Header**
  - Title
  - Taxonomy
  - Citation
- **Features (AA seq)**
- **DNA Sequence**

SAM/BAM

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:-10    XN:i:0  XM:i:2  XO:i:0  XG:i:0  NM:i:2  MD:Z:24G39T10    YT:Z:UP XS:A:-  NH:i:1
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:-12    ZS:i:-12        XN:i:0  XM:i:0  XO:i:0  XG:i:0  NM:i:0  MD:Z:75 YT:Z:UP XS:A:-  NH:i:3
HS34_24228:8:1209:16347:84469   99      Pf3D7_01_v3     144     255     66M9S   =       242     173     ACCCTAACCCTAAACCCTAAACCTAAAACCCTGAAC
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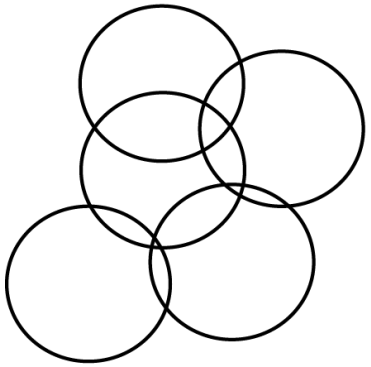
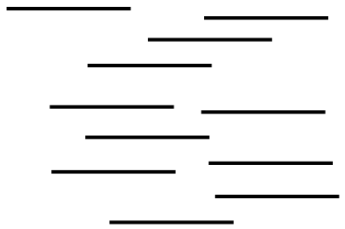
:0      XO:i:0  XG:i:0  NM:i:0  MD:Z:75 YS:i:-9 YT:Z:CP XS:A:-  NH:i:1
```

What do we do with these data?



1
Break many copies of the chromosome into small pieces



2
Sequence the pieces to find out the order of DNA letters in each one

```
ACTGTCTTCGCG
        TACGACTACGAC

    GATAGCATACGA
CTTCGCGCGCAG

        TCTTCGCGCGCA
            ATAGCATACGAC
CGCAGATAGCAT
```

3a
Find overlaps between these sequences to make a reference genome

```
                CGCAGATAGCAT
    TCTTCGCGCGCA ATAGCATACGAC
     CTTCGCGCGCAG       TACGACTACGAC
ACTGTCTTCGCG     GATAGCATACGA
```

```
ACTGTCTTCGCGCGCAGATAGCATACGACTACGAC
```

Annotation (find the genes)

3b
Map these sequences to a reference genome for resequencing to find differences

```
                CGCATATAGCAT
    TCTTCGCGCGCA ATAGCATAGGAC
     CTTCGCGCGCAT       TAGGACTACGAC
ACTGTCTTCGCG     TATAGCATAGGA
ACTGTCTTCGCGCGCAGATAGCATACGACTACGAC
```

- Look for interesting differences
- Build a tree

How are we going to do our bioinformatics?

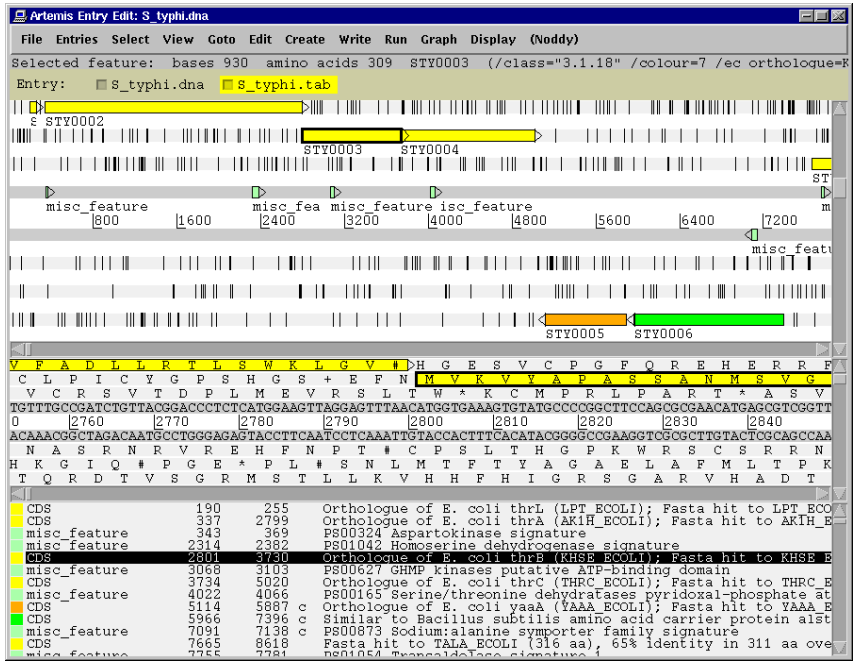
- Virtual machine with Linux
- Artemis for viewing genomes
- Various command line tools for mapping, assembling etc.
- Web-based applications

What will we do in the practicals?

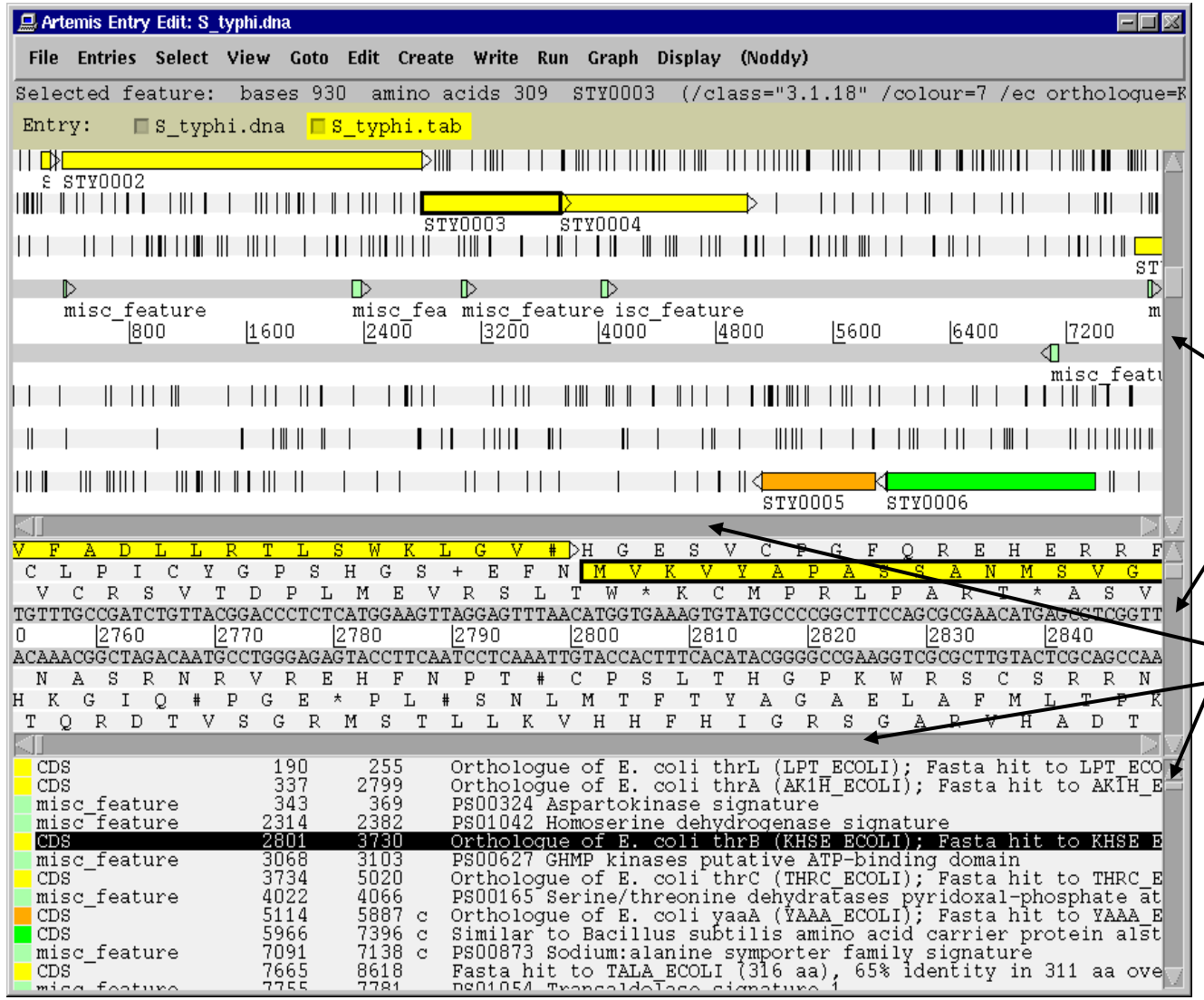
- Get familiar with the Virtual Machine
- Computer practical 1: Use Artemis to get familiar with looking at genomes (morning)
- Computer practical 2: Map short-read genome sequencing data to identify differences between closely related bacteria (afternoon)

Genome browser and annotation tool

- visualization of sequence
  - DNA
  - six frame translation
  - Panoramic and sequence view
- Annotation
  - Features
  - Mapped and listed
  - Editable
  - In layers (entry)
- perform and view analysis
  - basic analysis
  - Basic stats & index can be plotted
  - import and view the results of other searches/analysis
  - Different lines of evidence can be seen together



Artemis


Drop Down Menus
Entry Button Line
Main Sequence View Panel
Magnified Sequence View Panel
Feature Menu
Sliders
Sliders
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Selected feature: bases 930 amino acids 309 STY0003 (/class="3.1.18" /colour=7 /ec orthologue=K
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STY0002
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3200
4000
4800
5600
6400
7200
2760
2770
2780
2790
2800
2810
2820
2830
2840
CDS 190 255 Orthologue of E. coli thrL (LPT_ECOLI); Fasta hit to LPT_ECO
CDS 337 2799 Orthologue of E. coli thrA (AK1H_ECOLI); Fasta hit to AK1H_E
misc_feature 343 369 PS00324 Aspartokinase signature
misc_feature 2314 2382 PS01042 Homoserine dehydrogenase signature
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CDS 5114 5887 c Orthologue of E. coli yaaA (YAAA_ECOLI); Fasta hit to YAAA_E
CDS 5966 7396 c Similar to Bacillus subtilis amino acid carrier protein alst
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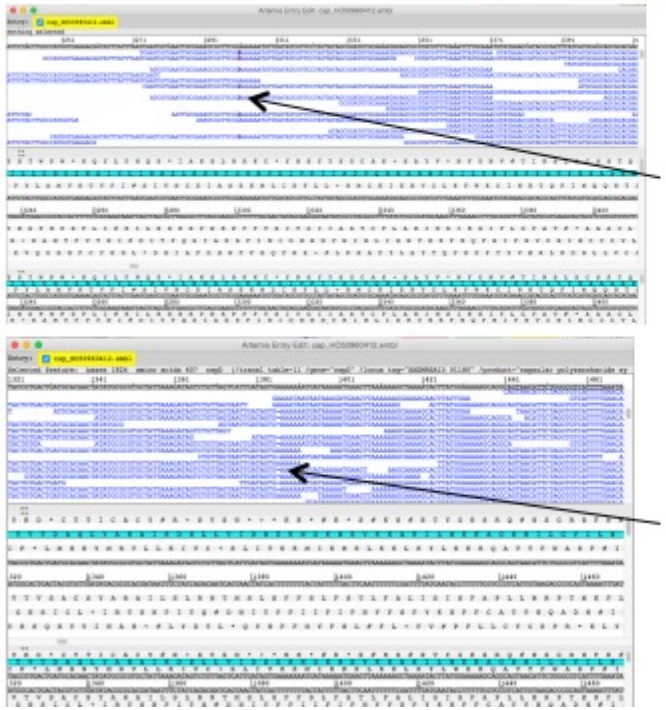
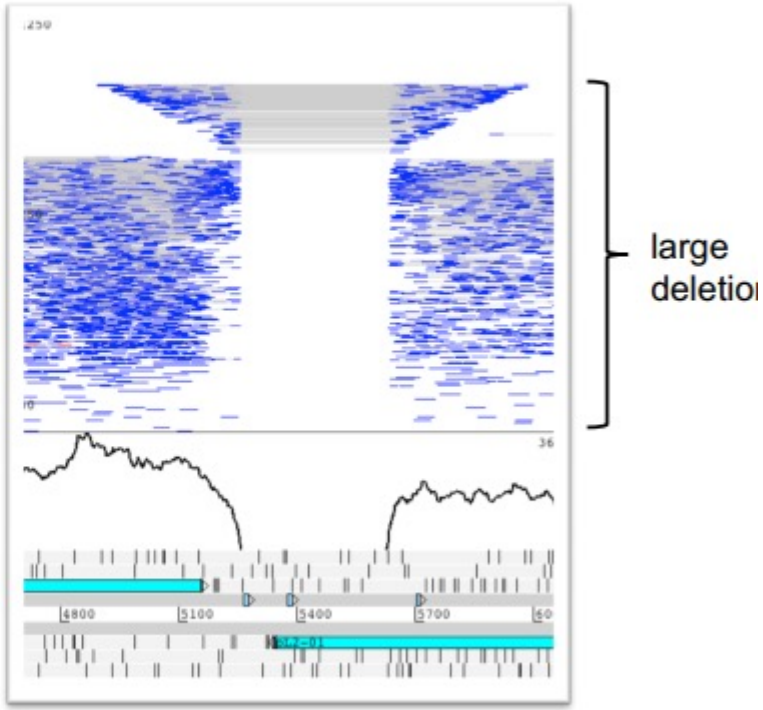
Viewing mapped reads in Artemis

- Illumina data (bam files)
  - identification of indels

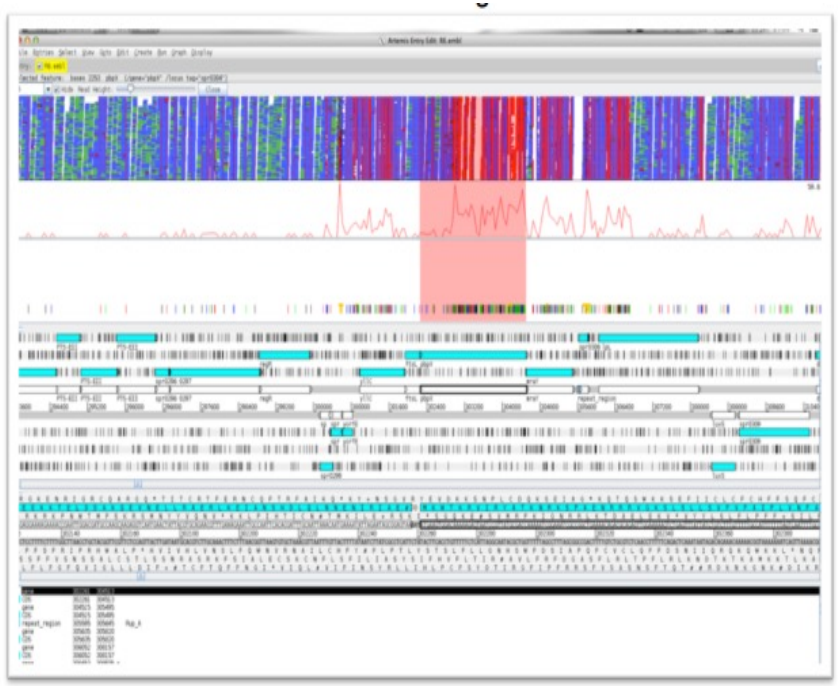




- we will see it on Module 4

Viewing mapped reads in Artemis

Single nucleotide variants (SNVs)



RNAseq data

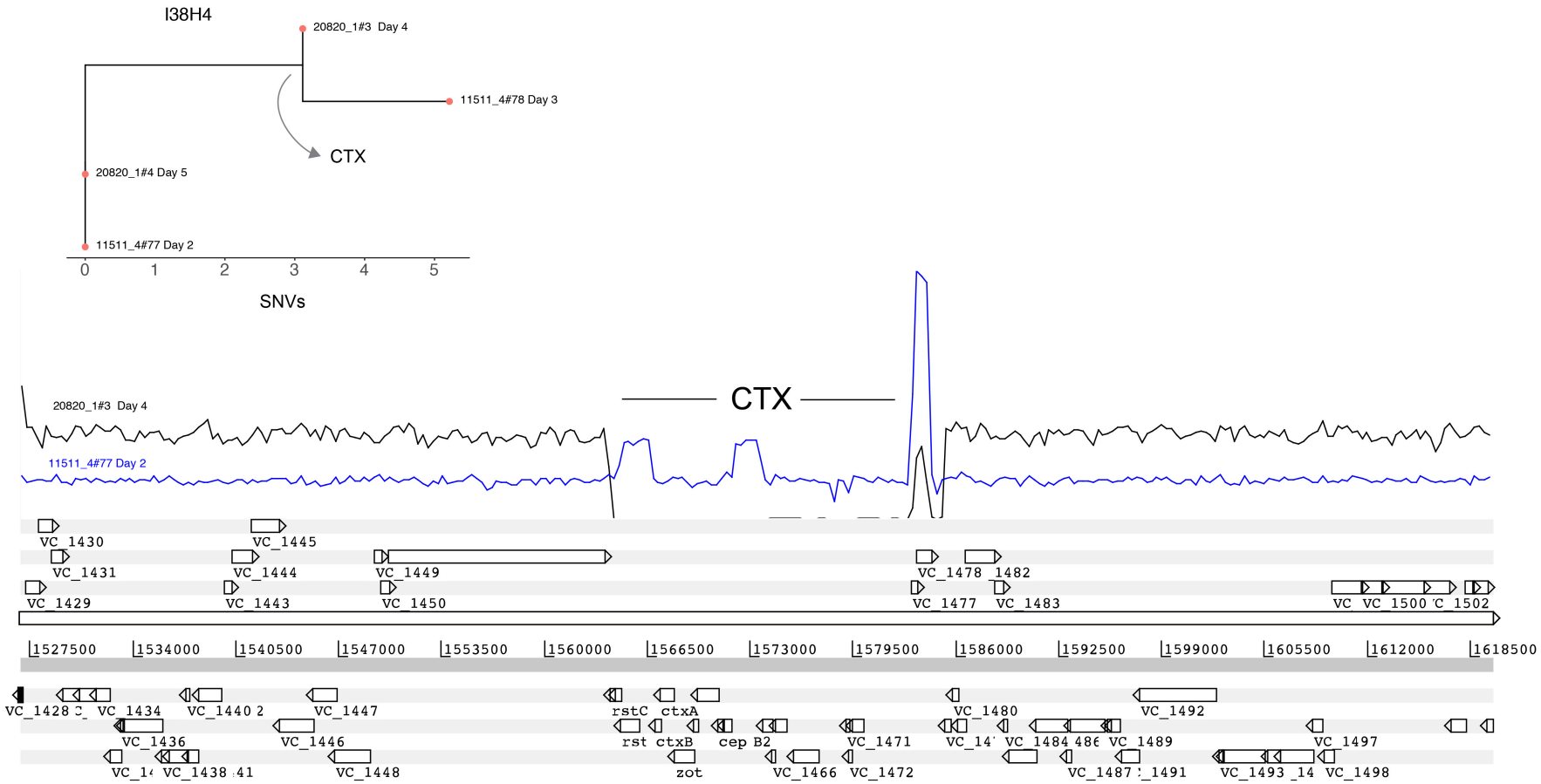
Illumina data (bam files)

Resequencing and mapping

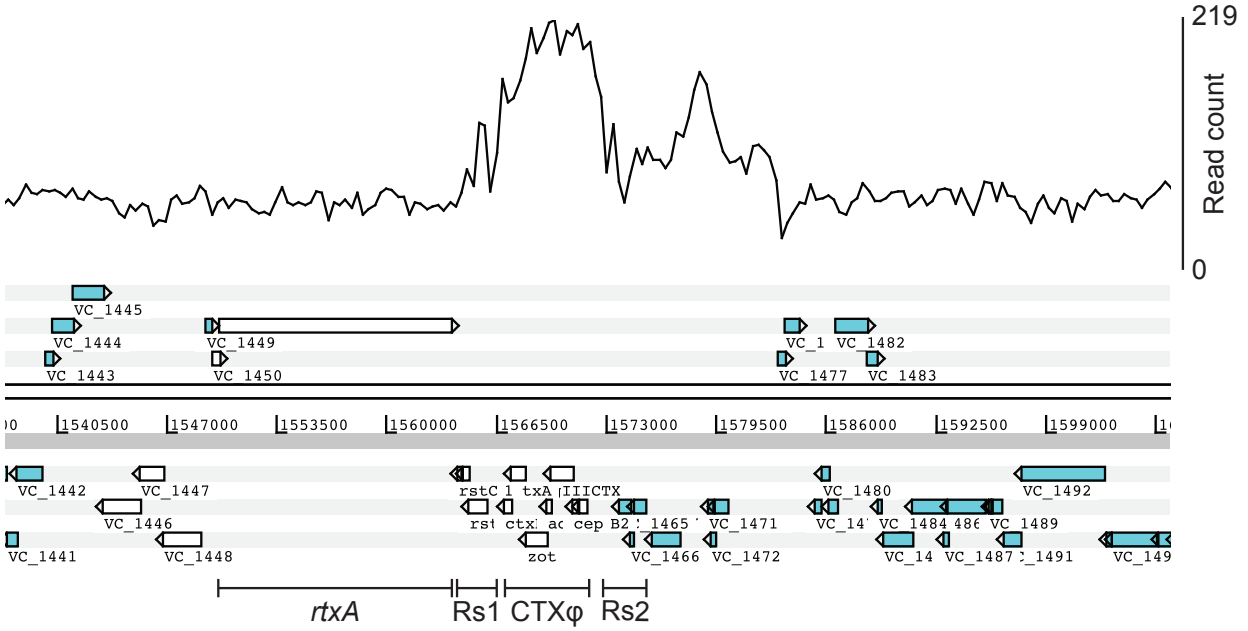
- Aims to capture information on:
  - Single Nucleotide Variants (SNVs/SNPs),
  - insertions and deletions (indels)
  - Copy Number Variants (CNVs) between individuals of a species.

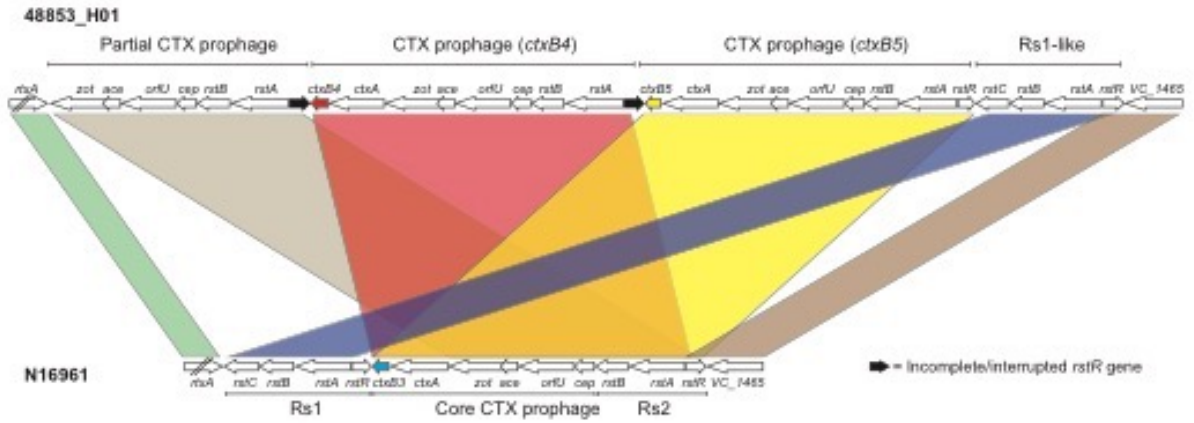
- As sequences diverge from the reference, mapping becomes progressively less effective

Gene presence / absence



Copy number variation





In this session, you will:

- Use Artemis to view genomes
- Understand genome data files
- Understand relationship between the sequence and annotation
- Understand what bacterial genomes look like and how they are arranged