Pathogen Genomics: Introduction to genome sequencing and analysis

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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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DT   23-AUG-1999 (Rel. 60, Last updated, Version 2)
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OC   Bacteria; Proteobacteria; gamma subdivision; Pseudomonadaceae; Pseudomonas.
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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
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EMBL Flat File

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

SAM/BAM

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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
```
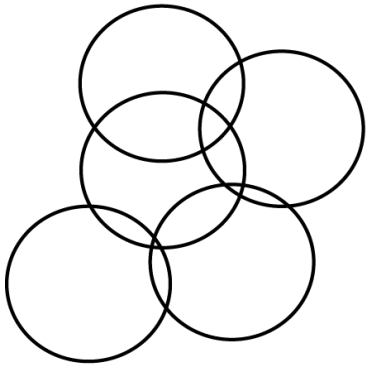
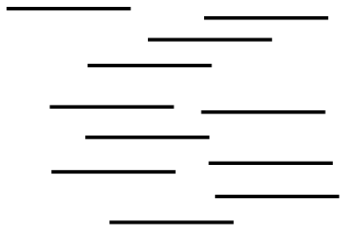

What do we do with these data?



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

```
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        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?

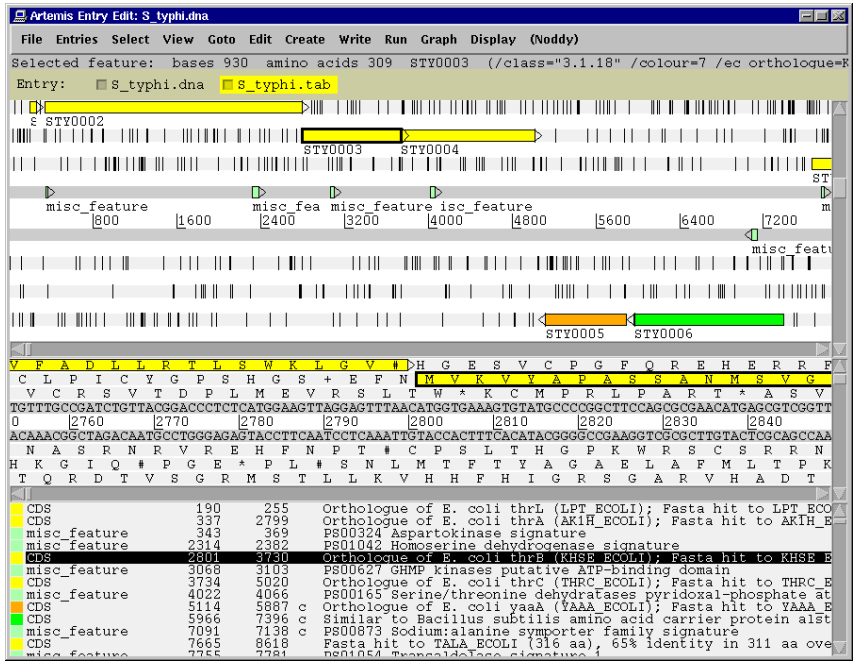
- Virtual machine with Linux
- Artemis for viewing genomes
- Various command line tools for mapping, assembling etc.
- Web-based applications

What will we do in the practicals?

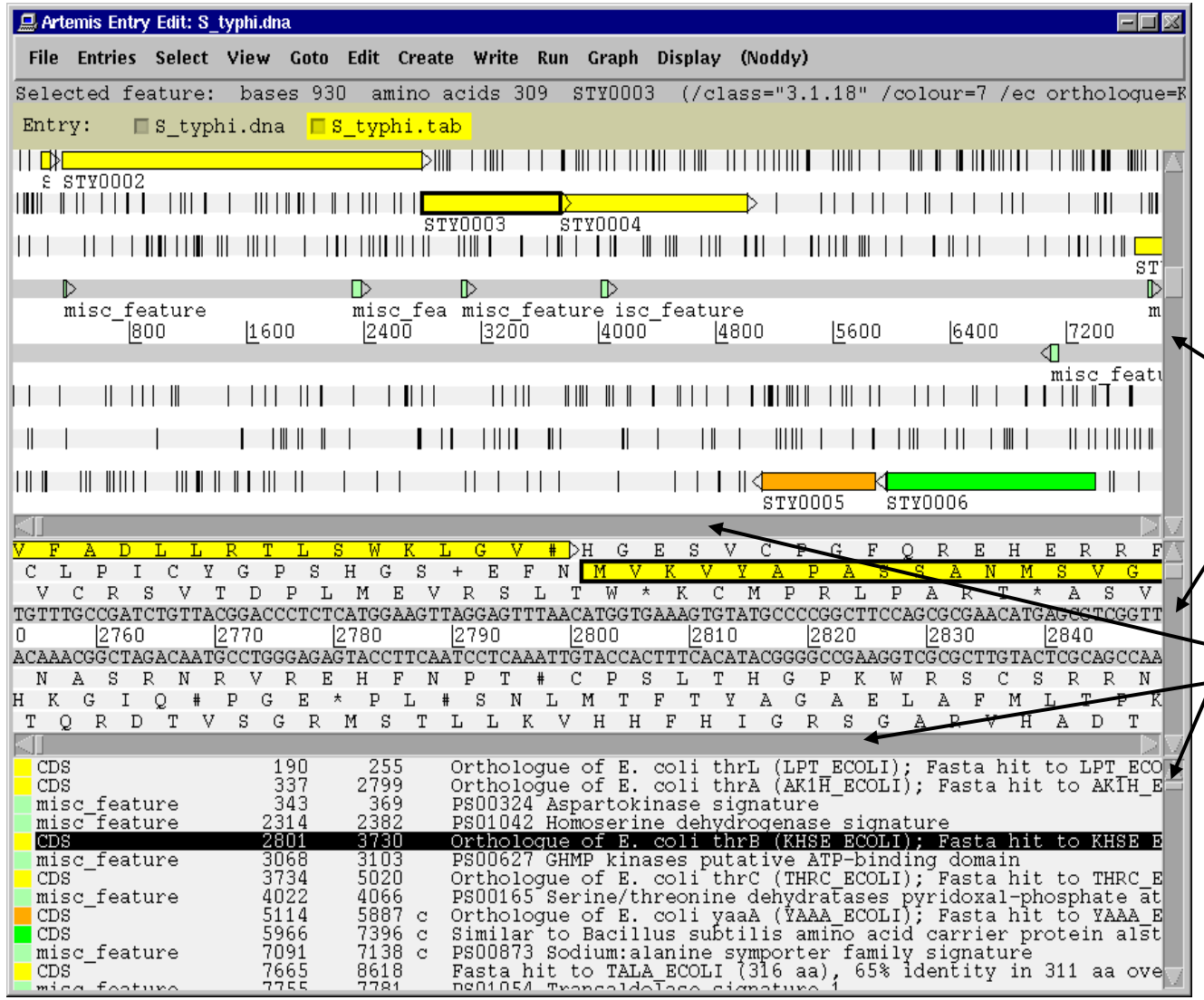
- Get familiar with the Virtual Machine
- Computer practical 1: Use Artemis to get familiar with looking at genomes (morning)
- Computer practical 2: Map short-read genome sequencing data to identify differences between closely related bacteria (afternoon)

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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Main Sequence View Panel
Magnified Sequence View Panel
Feature Menu
Sliders
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2840
CDS 190 255 Orthologue of E. coli thrL (LPT_ECOLI); Fasta hit to LPT_ECO
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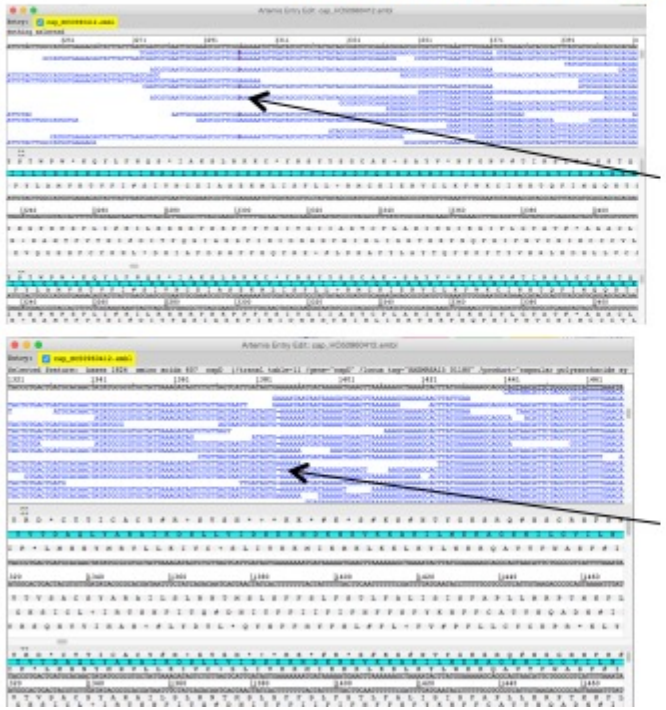
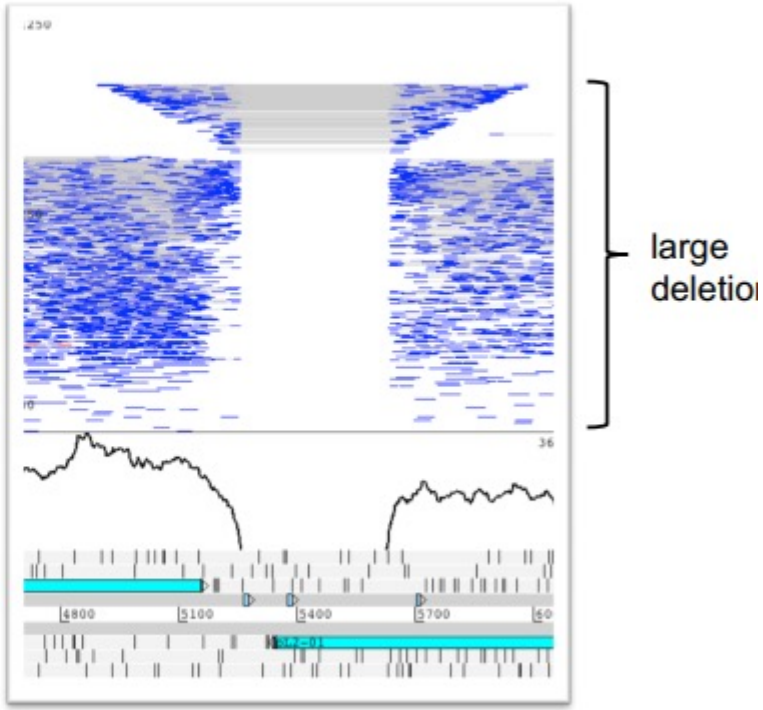
CDS 5966 7396 c Similar to Bacillus subtilis amino acid carrier protein alst
misc_feature 7091 7138 c PS00873 Sodium:alanine symporter family signature
CDS 7665 8618 Fasta hit to TALA_ECOLI (316 aa), 65% identity in 311 aa ove

Viewing mapped reads in Artemis

- Illumina data (bam files)
  - identification of indels

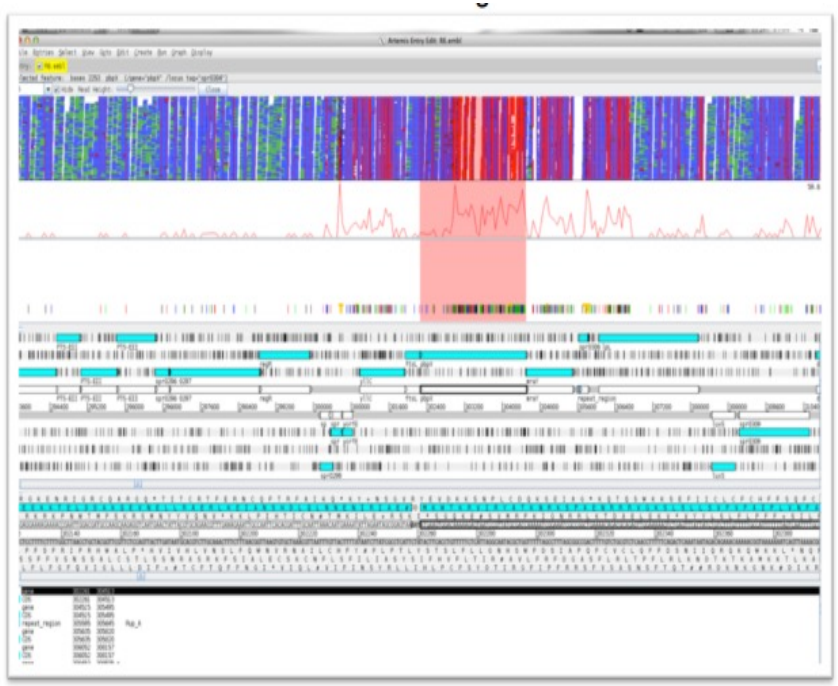




- we will see it on Module 4

Viewing mapped reads in Artemis

Single nucleotide variants (SNVs)



RNAseq data

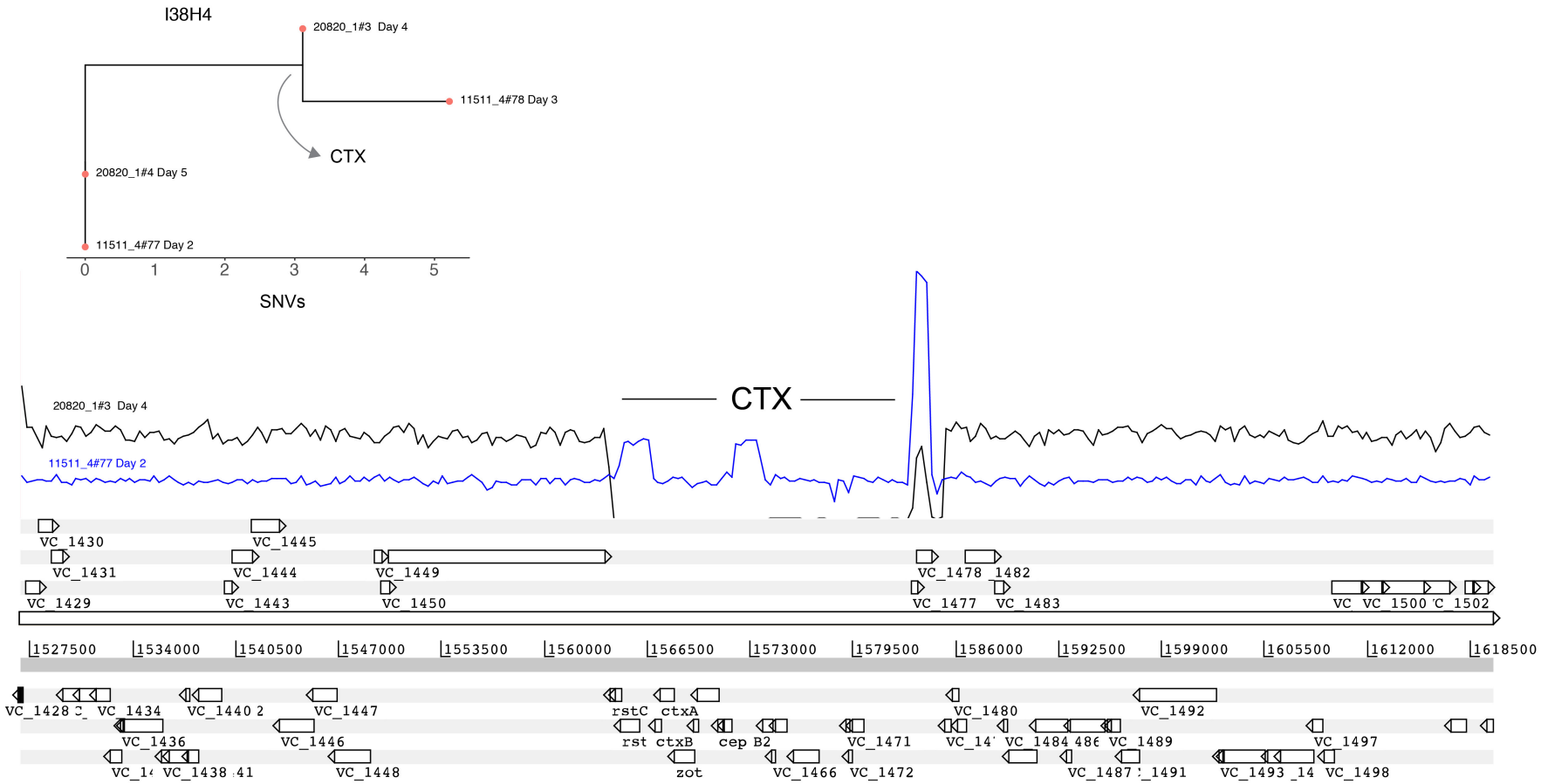
Illumina data (bam files)

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



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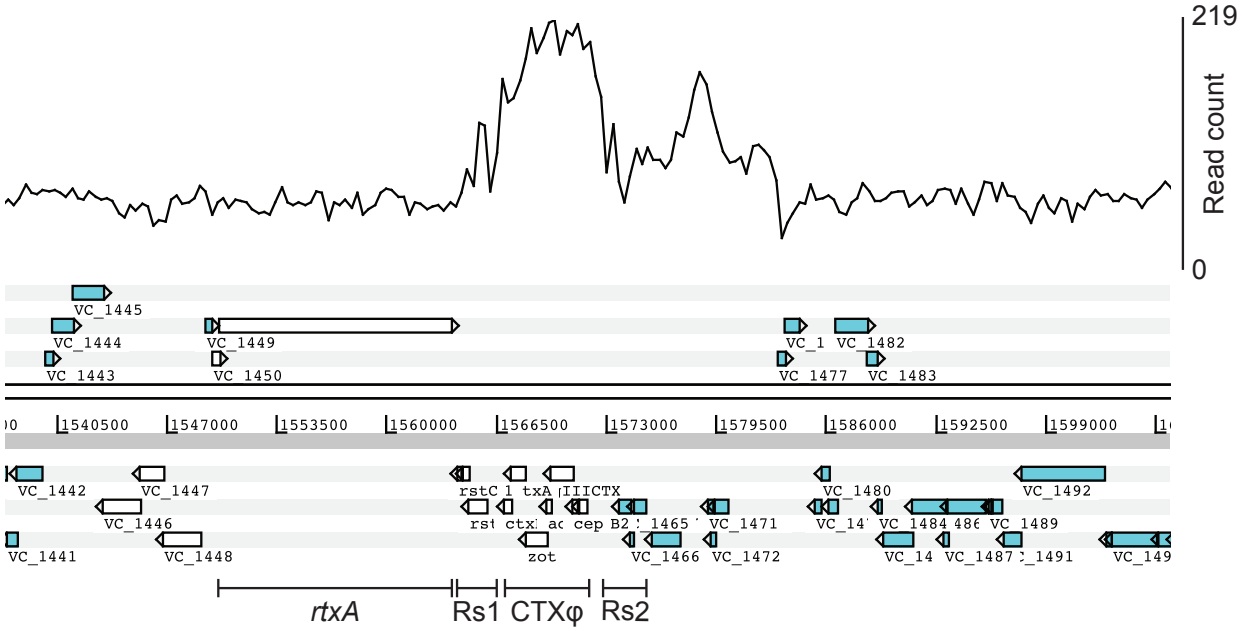
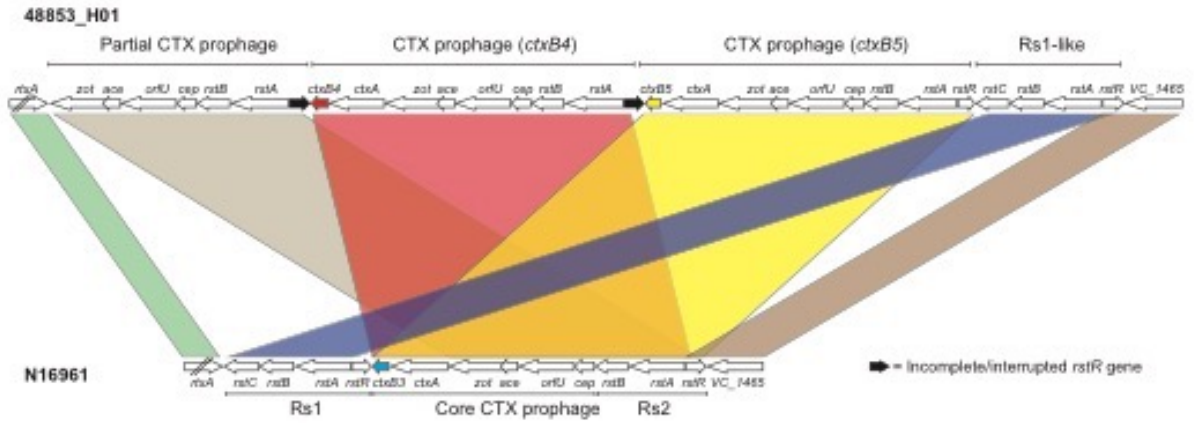
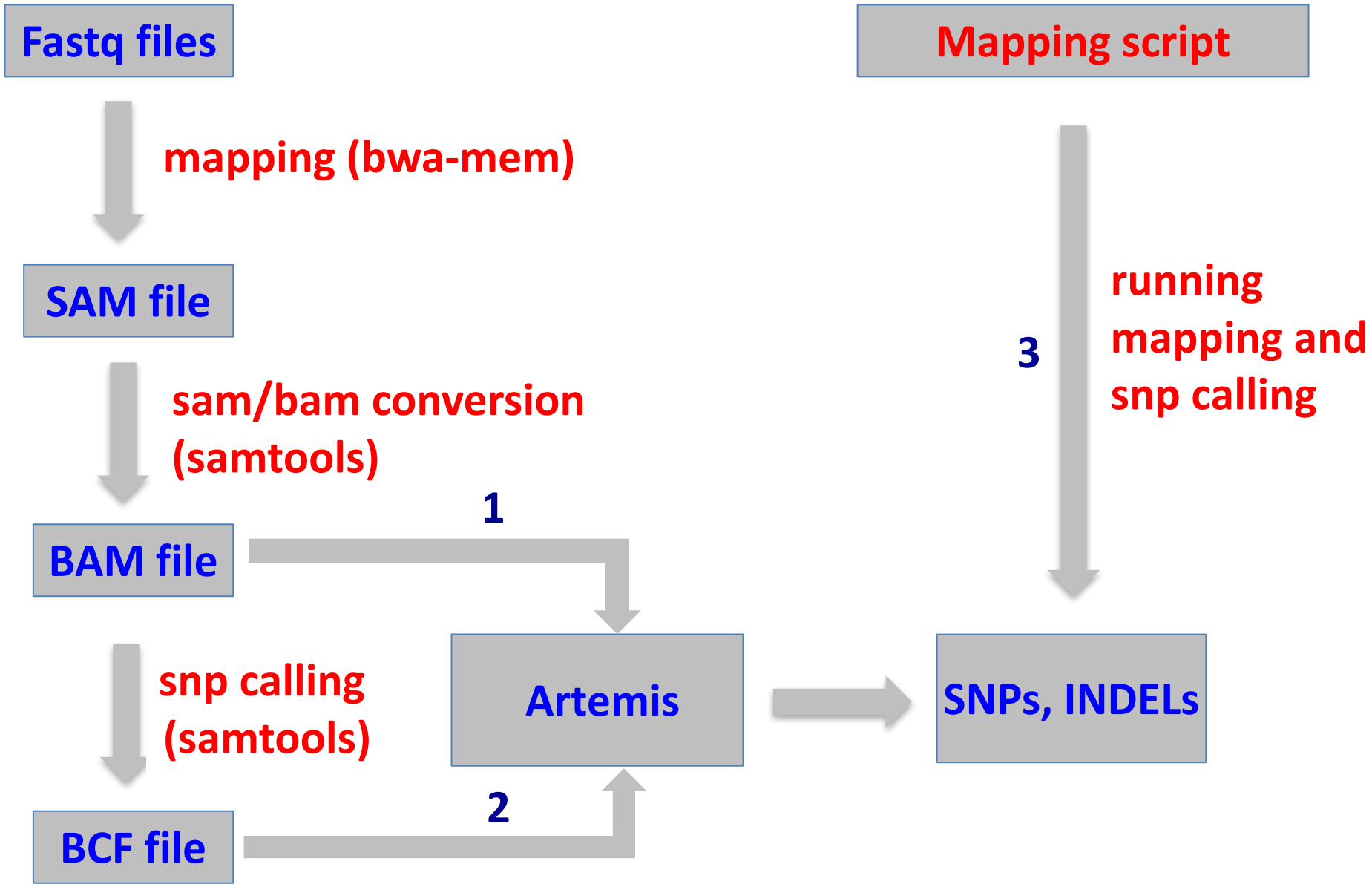




Mapping sequencing reads-Workflow



The Swedish Story

- Prior to 2006, C. *trachomatis* in Sweden was following the same pattern as in the UK
- In 2006, across Sweden there was a reported drop in cases

The Swedish story

- It was noticed that counties using the NAATs (Abbott / Roche) diagnostic system showed a drop in *C. trachomatis* cases in 2006
- Counties using other diagnostic methods (BecktonDickinson) still showed an increase in cases, in line with that of previous years
- The obvious conclusion was that the NAATs was missing a subset of infections

- Why?
  - Let’s find out using the awesome power of genomic sequencing!!!!

Summary

- Computer practical 1
  - 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
- Computer practical 2
  - Practice short read alignment
  - View mapped reads in Artemis
  - Call and view SNPs
  - Uncover why the PCR test failed for new variant Chlamydia