Pathogen Genomics: Introduction to genome sequencing and analysis

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Wellcome Sanger Institute/LSHTM

LSHTM Pathogen Genomics 2021

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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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
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RL   J. Bacteriol. 181(16):4746-4754(1999).
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EMBL Flat File

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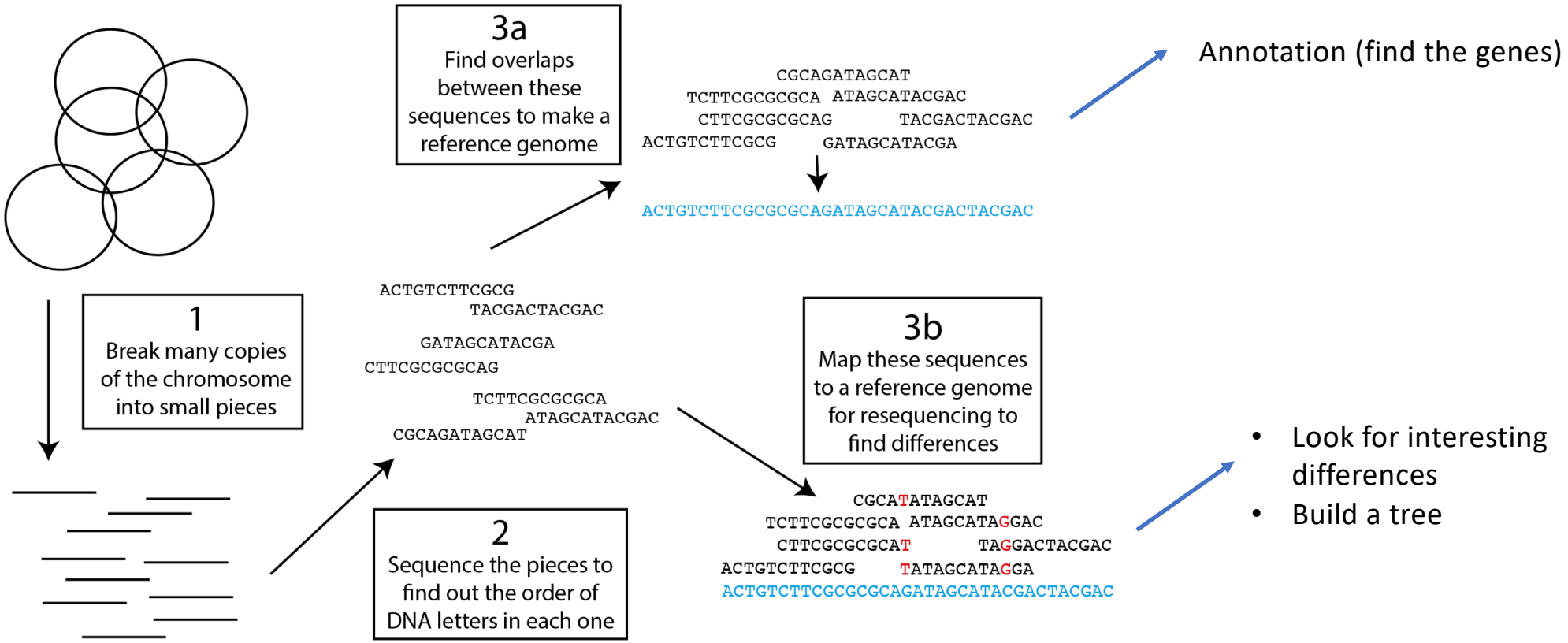
  - •Taxonomy
  - •Citation
- Features (AA seq)
- DNA Sequence

What do we do with these data?



How are we going to do our bioinformatics?

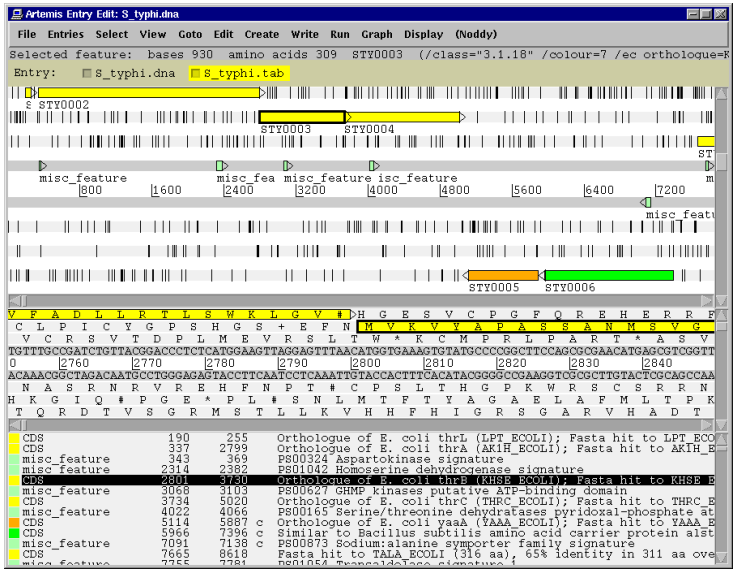
- Virtual machine with Linux
- Artemis for viewing genomes
- Various command line tools for mapping, assembling etc.
- Web-based applications

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

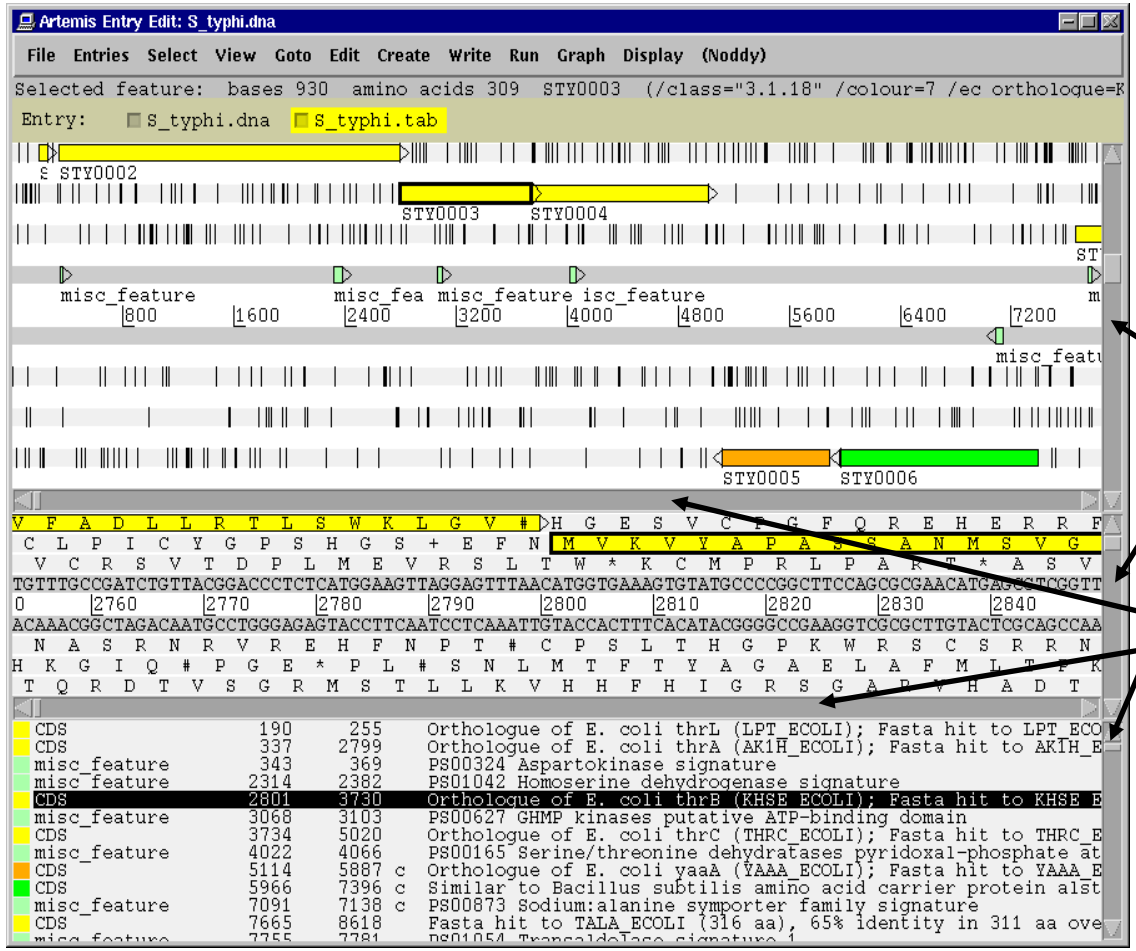
Drop Down Menus

Entry Button Line

Main Sequence View Panel

Magnified Sequence View Panel

Feature Menu

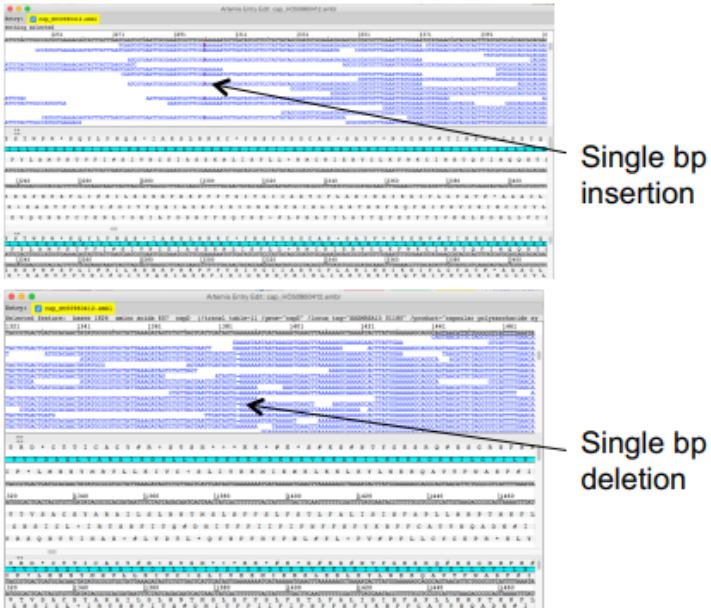


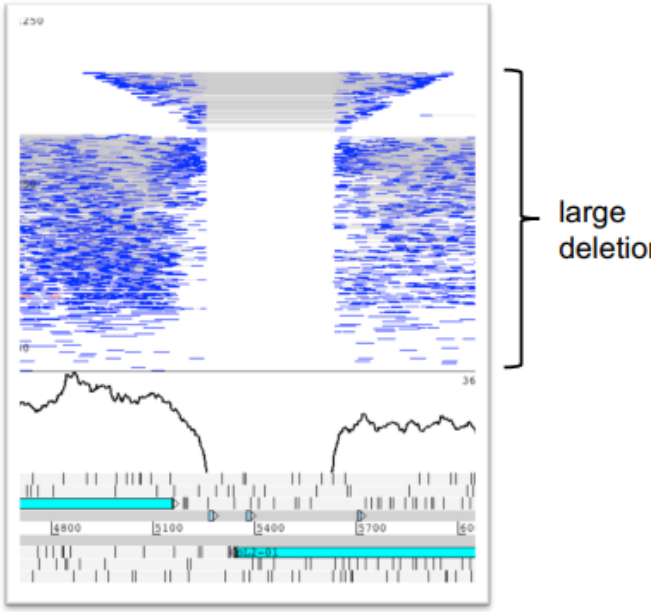
Sliders

Sliders

Viewing mapped reads in Artemis

- Illumina data (bam files)
  - identification of indels

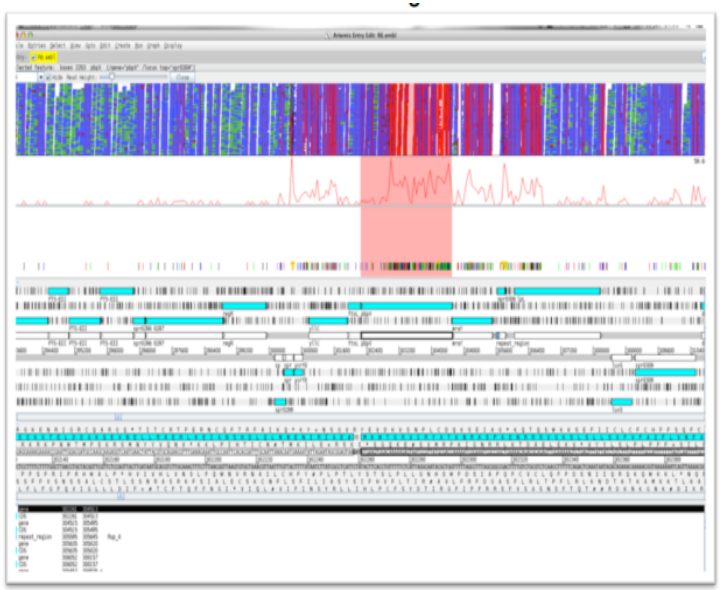




- we will see it on Module 4

Viewing mapped reads in Artemis

Single nucleotide variants (SNVs)



RNAseq data

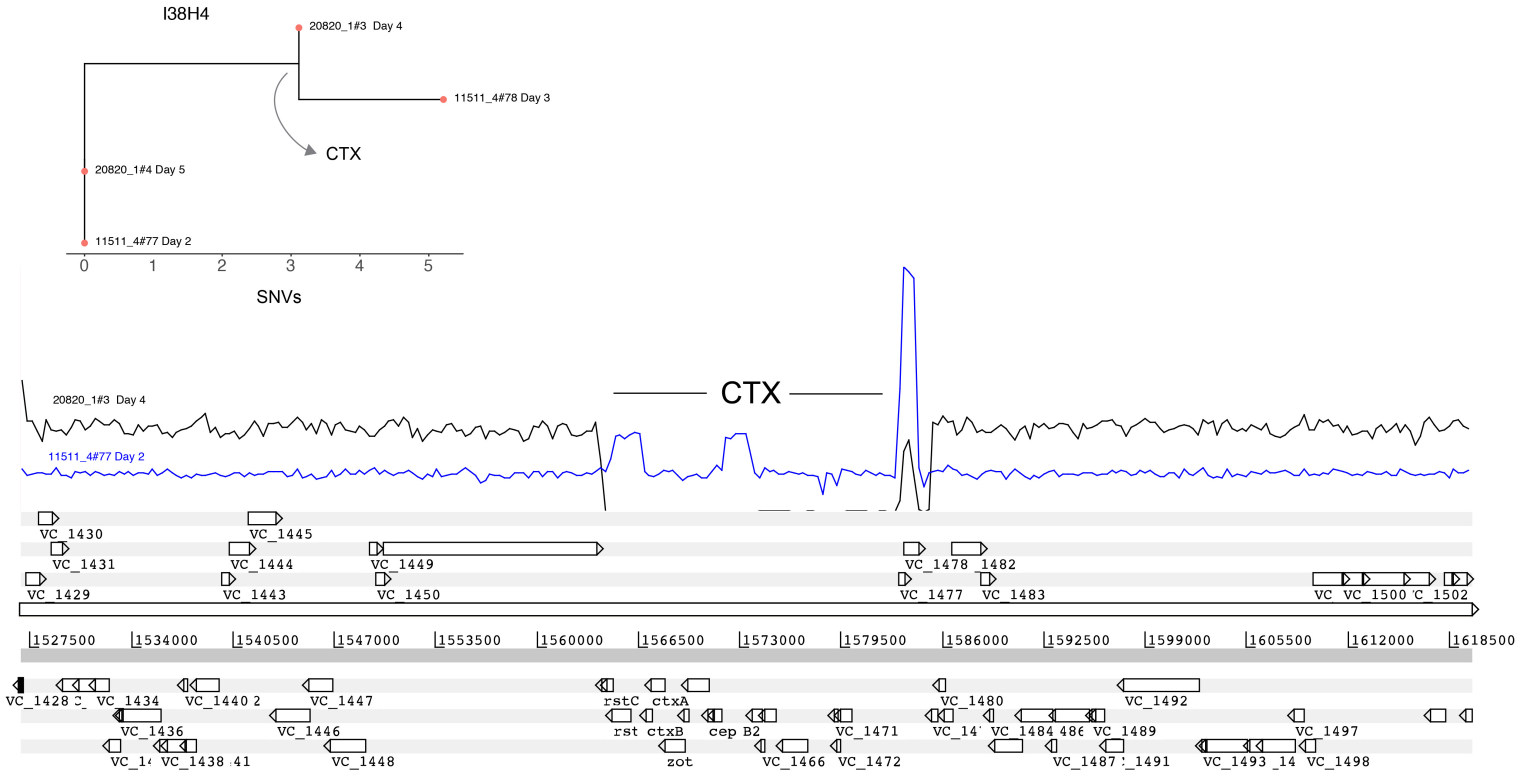
Illumina data (bam files)

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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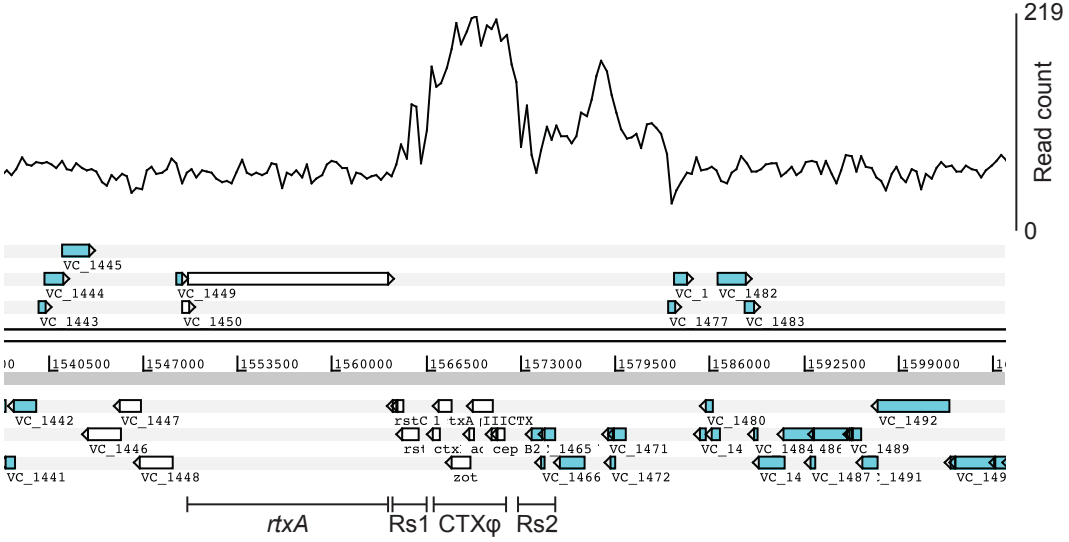
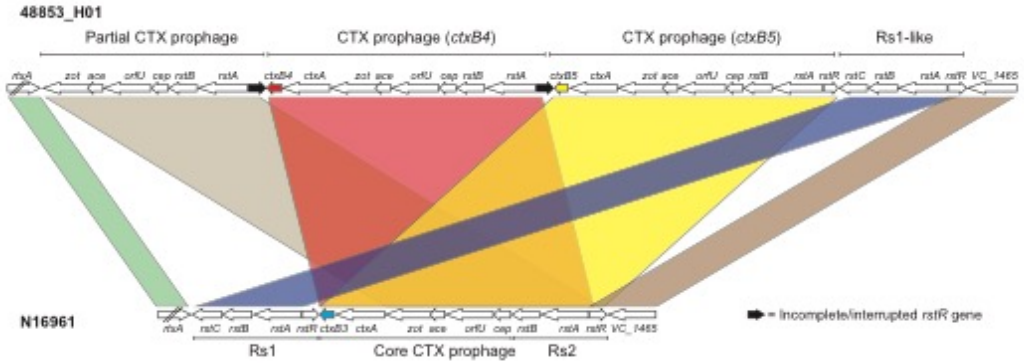
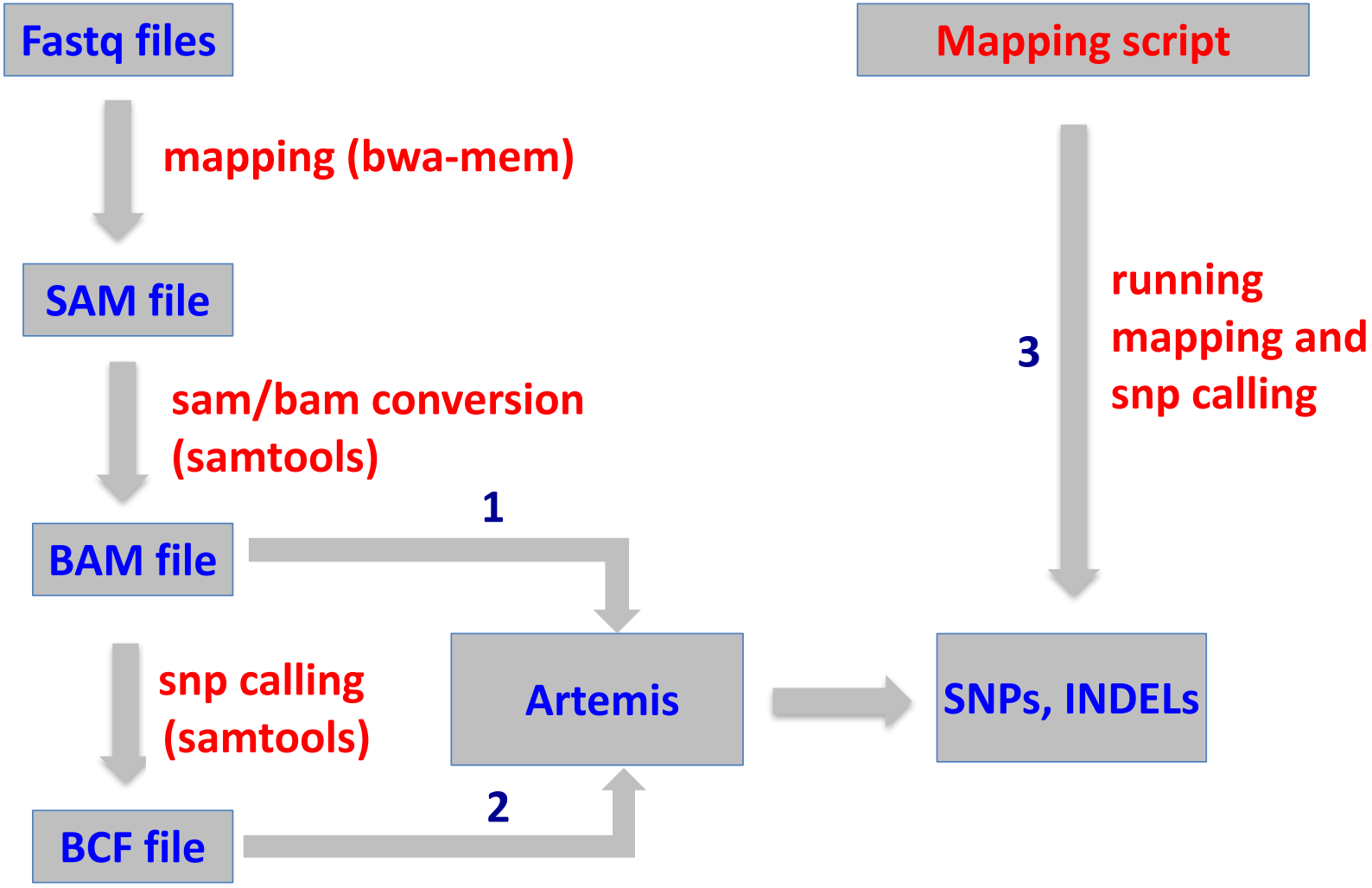




Mapping sequencing reads-Workflow



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