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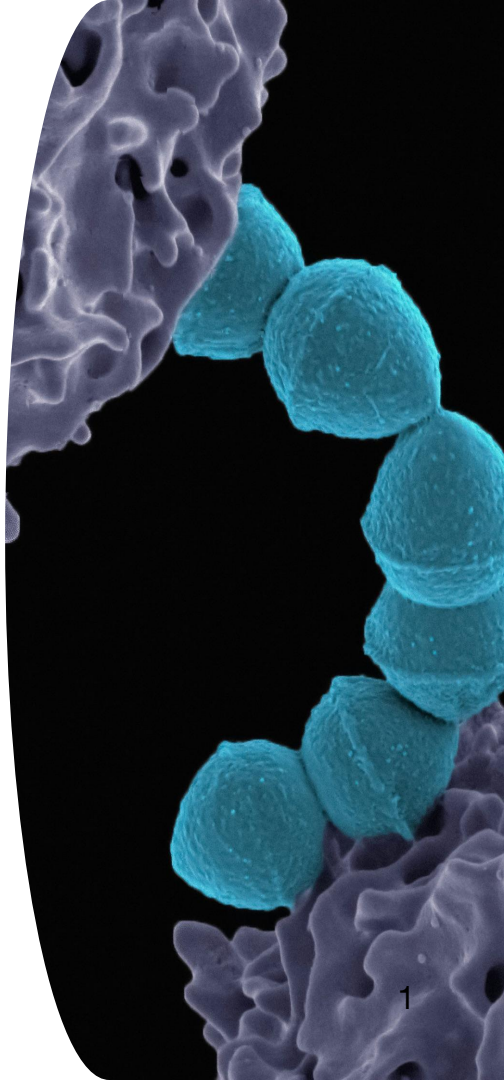
ACORN

Bash, File formats and Quality Control

Lecture: Introduction to Linux systems, command line and file formats



Instructor:
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AMR Virtual course (Africa and Asia)

21 May 2024

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

SAPPHGenE

Learning outcome

- Learn how to use the Unix command
- Understand the different file formats

Overview

- Background: Operating systems, Unix
- Basic Unix commands
- Introduction to colab notebooks (demo)
- Introduction to the basic file formats and their functions in genomics

Background: Operating systems

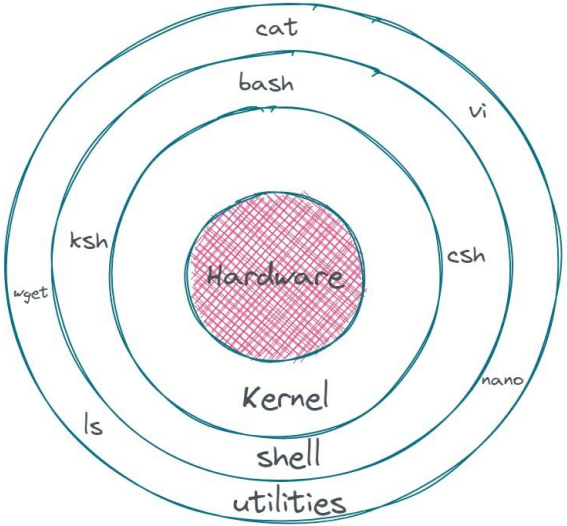
- An operating system (OS) is the software that functions as an interface between the computer user and its hardware
- The software enables and controls the set of programs that we install in our machines
- Some functions include: allocation of storage, processor management, security, error detection, file management
- Examples of most used OS include: Windows, Linux, Unix, MAC OS, Android, etc.

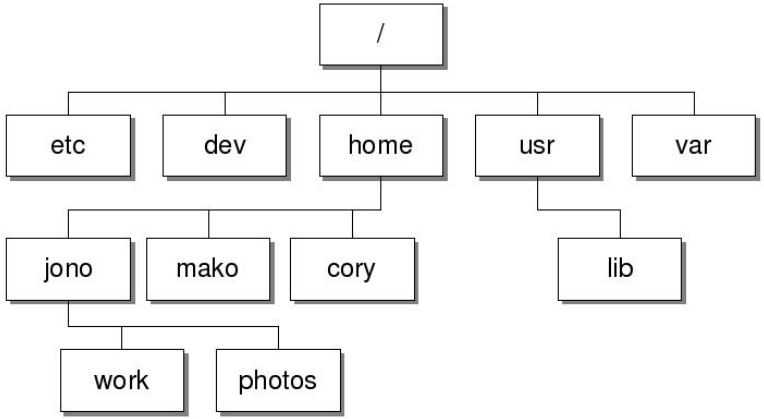
Background: Unix

- Unix was originally called AT&T Unix, and was first developed in 1969 at the Bell Lab research center
- A family of OS's that allow for multiple users and allow for multitasking
- Beneficial for working with large text files (or genomic data files) and accommodates several powerful yet flexible commands
- Ability to combine these multiple commands for different objectives

Basic Unix: Basic terms

- Shell
  Command-line interpreter
- Terminal
  Tool used for shell commands
- Command prompt
  (base) ubuntu@student-1:~$
- Directories
  - Equivalent to folders with files
- Root
  - The mother directory where all directories stem from
- Syntax
  - Command [options] [arguments]
  - Different programs will have their own syntax with a number of options
  - Commands are case sensitive





Basic Unix: Commands

File system navigation commands
- pwd – print working directory
- ls – list directories and file
- cd – change directory
- mkdir – make new directory
- touch – create and empty file
- rm – remove
- mv – move
- cp – copy
- wget – downloads a file with a provided URL

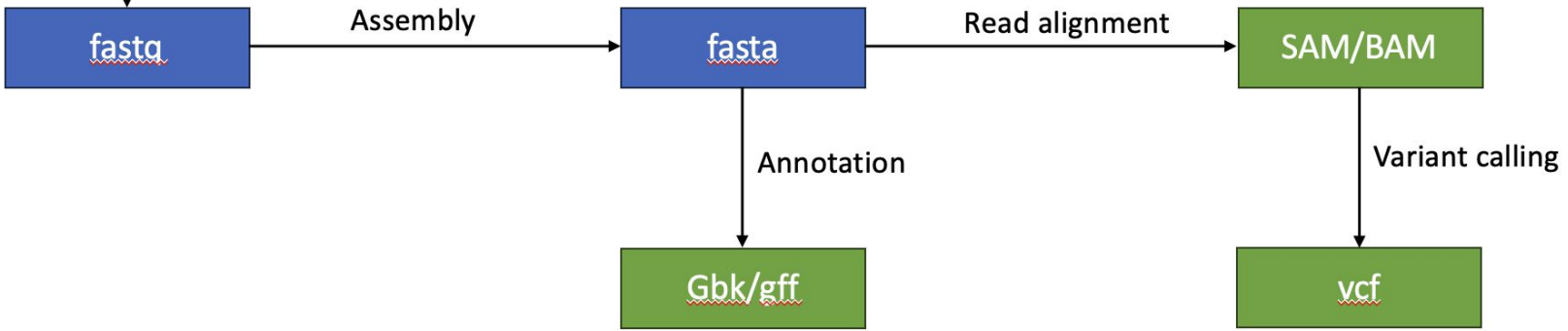
Tricks and other commands
- man – prints command manual
- Tab complete
- Wildcard
- Arrows
- history – prints your command history trail
- Pipes (|)
- kill command – terminates job

Text management
- nano/less – view file
- cat – concatenate and pint file
- head – print first lines from a file
- tail – print last file from a file
- cut – retrieves data from selected columns in a tab-delimited file
- grep – prints line matching a pattern
- wc – counts lines and words

https://cdn.hostinger.com/tutorials/pdf/Linux-Commands-Cheat-Sheet.pdf?_gl=1*1qpygsr*_gcl_au*MTc3ODMzODAwMi4xNzE0OTM3MDUx&_ga=2.258160322.999754537.1714937051-998924758.1714937051

SEQUENCING FILE FORMATS

Introduction to the basic file formats - Data transformation



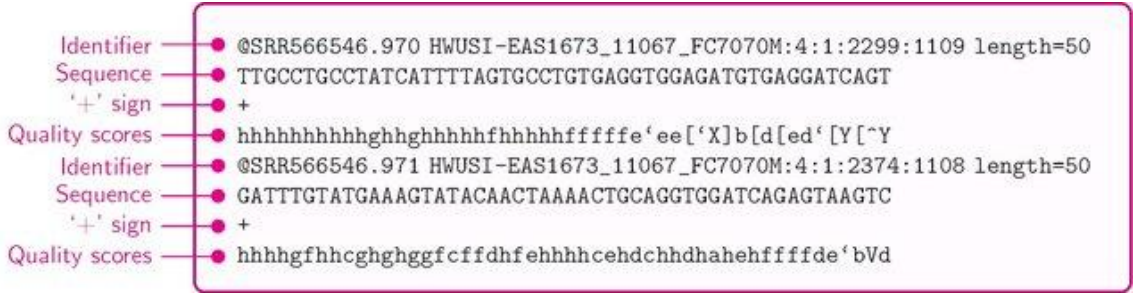
Introduction to the basic file formats

- Data is information contained in a file
- Different file formats carry data in a specific design and optimization for some programs to be able to read and display the information in an understandable way
- The basic progression of sequencing data and formats involved:
  - **Fastq** – raw sequence reads
  - **Fasta** – genome assembly
  - **.gff/.gbk** – annotation files
  - **SAM/BAM** – read alignment
  - **VCF** – variant calling

Introduction to the basic file formats

Fastq

- Standard format directly from the sequencing instrument



Fasta

- Following genome assembly
- fasta format is much simpler
- Contains only an identifier and the sequence

```
>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6)
ACCCTCTTTTCTTATCATTGACATTTAAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT
GCCACTTCCCCTGCCGAGCGGCGGTGAGAAGTGTGGGAACCGGCGCTGCCAGGCTCACCTGCCTCCCCGC
CCTCCGCTCCCAGGTAACCGCCCGGGCTCCGGCCCCGGCCCGGCTCGGGGCCCGCGGGGCCTCTCCGCTG
CCAGCGACTGCTGTCCCCAAATCAAAGCCCGCCCCAAGTGGCCCCGGGGCTTGATTTTTGCTTTTAAAAG
GAGGCATACAAAGATGGAAGCGAGTTACTGAGGGAGGGATAGGAAGGGGGGTGGAGGAGGGACTTGTCTT
TGCCGAGTGTGCTCTTCTGCAAAAGTAGCAAAATGTTCCACTCCTAAGAGTGGACTTCCAGTCCGGCCCT
GAGCTGGGAGTAGGGGGCGGGAGTCTGCTGCTGCTGTCTGCTAAAGCCACTCGCGACCGCGAAAAATGCA
GGAGGTGGGGACGCACTTTGCATCCAGACCTCCTCTGCATCGCAGTTCACGACATCCACGCTTGGGAAAG
TCCGTACCCGCGCCTGGAGCGCTTAAAGACACCCTGCCGCGGGTCGGGCGAGGTGCAGCAGAAGTTTCCC
GCGGTTGCAAAGTGCAGATGGCTGGACCGCAACAAAGTCTAGAGATGGGGTTCGTTTCTCAGAAAGACGC
```

Introduction to the basic file formats

Multifasta

- Concatenated fasta files usually following multisequence alignment

Genbank (gbk)

- Genome annotation output
- Combination of computer and human-readable information
- Still need another application to visualize annotations

```
LOCUS       NC_002516            6264404 bp    dna     circular UNK
DEFINITION  Pseudomonas aeruginosa PAO1 chromosome, complete genome.
ACCESSION   NC_002516
FEATURES             Location/Qualifiers
     source          1..6264404
                     /mol_type="genomic DNA"
                     /db_xref="taxon:208964"
                     /strain="PAO1"
                     /organism="Pseudomonas aeruginosa PAO1 (Reference)"
     gene            483..2027
                     /locus_tag="PA0001"
                     /db_xref="GeneID:878417"
                     /name="dnaA"
     CDS             483..2027
                     /locus_tag="PA0001"
                     /db_xref="GeneID:878417"
                     /translation="MSVELWQQCVDLLRDELPSQQFNTWIRPLQVEAEGDELRVYAPN
                     RFVLDWVNEKYLGRLLELLGERGEGQLPALSLLIGSKRSRTPRAAIVPSQTHVAPPPP
                     VAPPPAPVQPVSAAPVVVPREELPPVTTAPSVSSDPYEPEEPSIDPLAAAMPAGAAPA
                     VRTERNVQVEGALKHTSYLNRTFTFENFVEGKSNQLARAAAWQVADNLKHGYNPLFLY
                     GGVGLGKTHLMHAVGNHLLKKNPNAKVVYLHSERFVADMVKALQLNAINEFKRFYRSV
                     DALLIDDIQFFARKERSQEEFFHTFNALLEGGQQVILTSDRYPKEIEGLEERLKSRFG
                     WGLTVAVEPPELETRVAILMKKAEQAKIELPHDAAFFIAQRIRSNVRELEGALKRVIA
                     HSHFMGRPITIELIRESLKDLLALQDKLVSIDNIQRTVAEYYKIKISDLLSKRRSRSV
                     ARPRQVAMALSKELTNHSLPEIGVAFGGRDHTTVLHACRKIAQLRESDADIREDYKNL
                     LRTLTT"
                     /product="chromosomal replication initiator protein DnaA"
```

Introduction to the basic file formats

Sequence alignment map (SAM)

- A tab-delimited, line oriented text format of information on alignments
- Header section with metadata in each column
- Alignment section with corresponding information on the alignment

```
HD VN:1.5 SD:coordinate
SD SN:ref LN:45
```

HEADER section

```
r001    99   ref    7   30   8M2I4M1D3M  =    37     39   TTAGATAAAGGATACTG *
r002     0   ref    9   30   3S6M1P1I4M  *     0      0   AAAAGATAAGGATA    *
r003     0   ref    9   30   5S6M        *     0      0   GCCTAAGCTAA       *   SA:Z:ref,29,-,6H5M,17,0;
r004     0   ref   16   30   6M14N5M     *     0      0   ATAGCTTCAGC       *
r003  2064   ref   29   17   6H5M        *     0      0   TAGGC             *   SA:Z:ref,9,+,5S6M,30,1;
r001   147   ref   37   30   9M          =     7    -39   CAGCGGCAT         *   NM:i:1
```

ALIGNMENT section

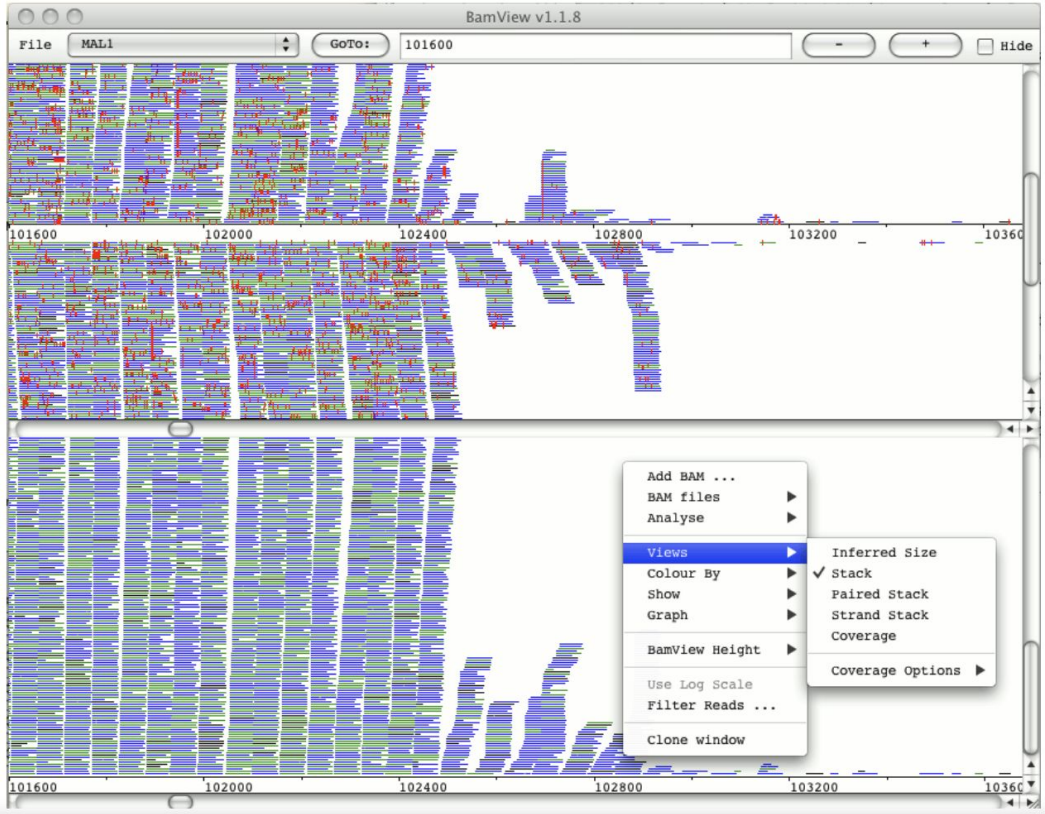
QNAME FLAG RNAME POS MAPQ CIGAR RNEXT PNEXT TLEN SEQ QUAL

https://samtools.github.io/hts-specs/SAMv1.pdf

Introduction to the basic file formats

Binary alignment map (BAM)

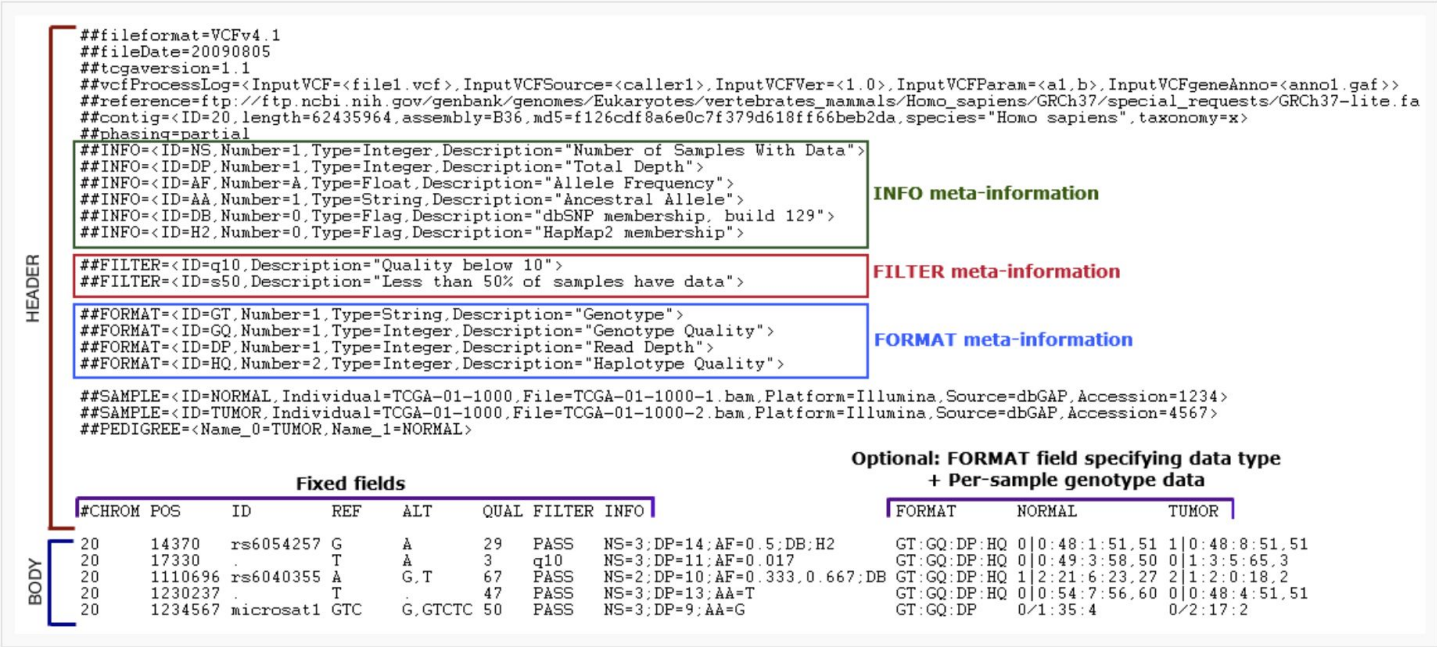
- Compressed version of SAM
- Not human-readable
- Can be recognized by certain programs that allow you to visualize the alignment (e.g IGV, Artemis)
- Ready for variant calling



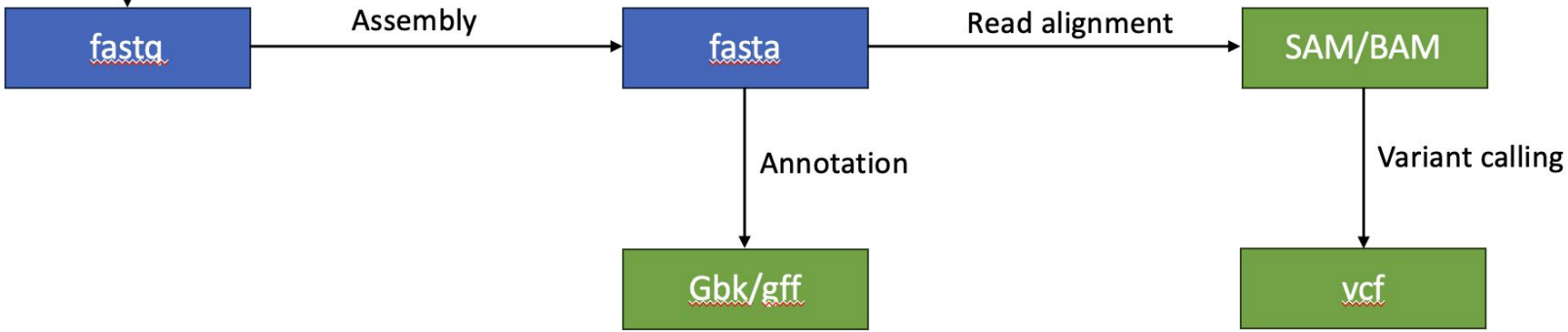
Introduction to the basic file formats

Variant call format (VCF)

- Output from extraction of variance/variations between the query sequence and the reference sequence directly from the BAM file.



Introduction to the basic file formats - Data transformation



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