BIOL 343
Applied Bioinformatics I
FASTQ files

Dr. Nic Wheeler

Learning Objectives

You will be able to:

1.

Stages of sequencing data analysis

Primary, secondary, and tertiary

Primary

Occurs on the machine in real-time

Provides base calls and quality scores

Illumina Connected Analytics

Secondary

Demultiplexing and compression (DRAGEN)

Alignment

Assembly

Specialized tools selected by the end user

Tertiary

Questions of biological relevance

Differential expression, variant calling, etc

Specialized tools selected by the end user

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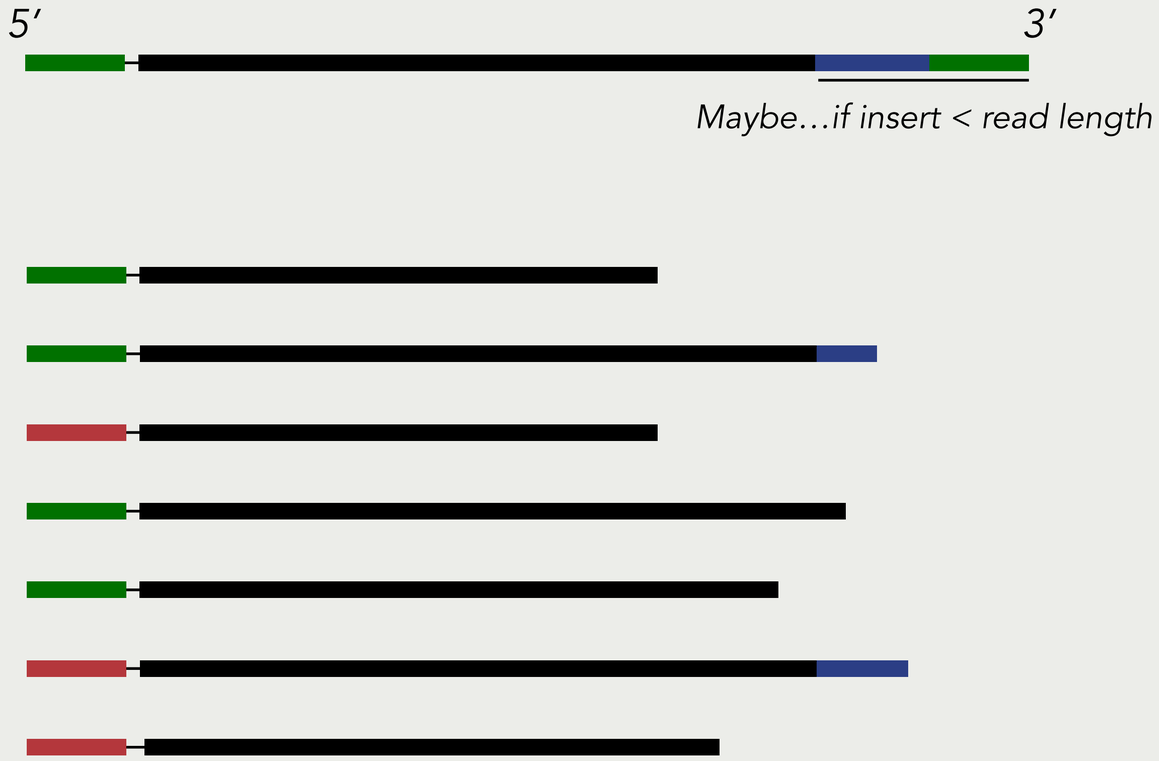
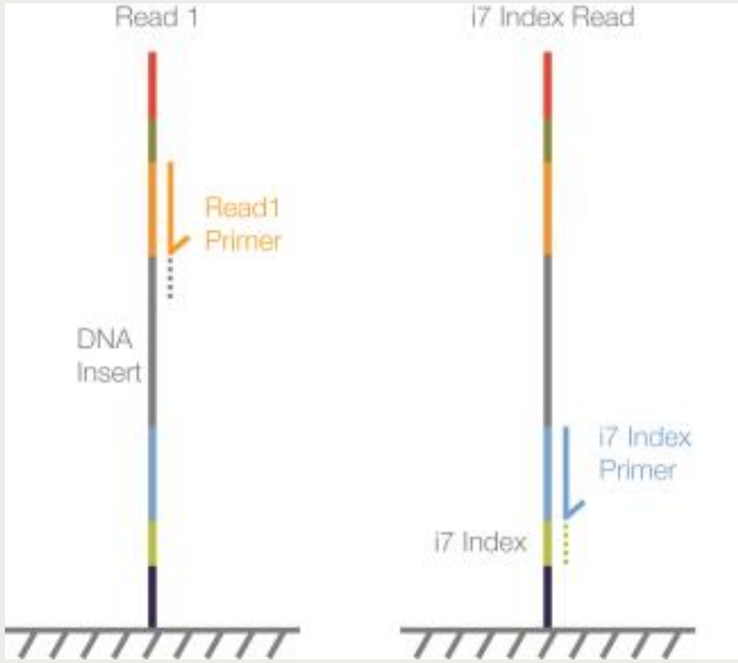
Produces FASTQ files

Stages of sequencing data analysis

Primary analysis produces FASTQ data and demultiplexes it

Read 1

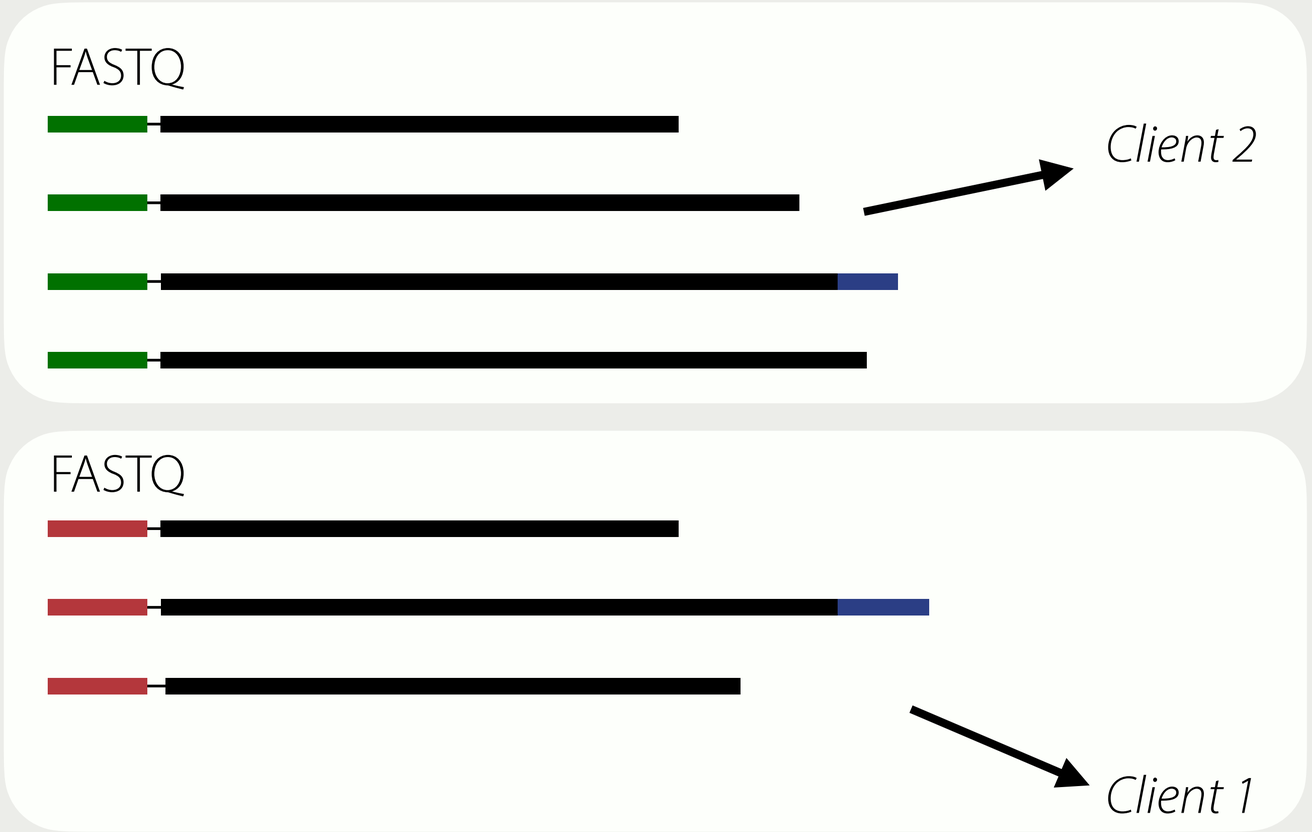


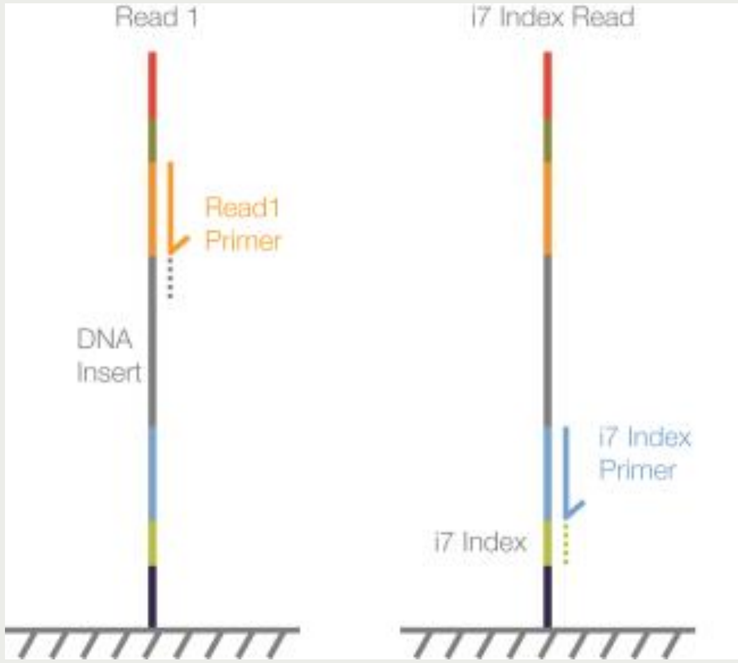


Stages of sequencing data analysis

Primary analysis produces FASTQ data and demultiplexes it

Read 1





Types of sequence storage files

FASTA and FASTQ

FASTA

- Used for storing generic primary sequence data
- Nucleotide or amino acids (ASCII format)
- Usually KB to MB in size
- Header defined by >
  - Sequence name and attributes
  - Spaces discouraged
- Sequence in the line immediately after
  - Can have new line characters (\n)

```
>Smp_104210.1 RHO cdna:protein_coding
TACTTCATTTATCATTCTGGTAAGTAATGAGTTAACTAAA
TCTGTTCATTTGTTTCACTAAATTTTAAATCAGAAATTTC
TTTTTTTTAACACTATTTCTAAACTGTTAAATGCACATTT
ATTTTTCAATTTCGTTTAATATCTAGTAGAGTAATCAGTC
TATGTTATTTTAATGAGAATCCTCATTAAAATACATTTCA
GATACTTGTTGAGTTCAATTGAAAAACATTCTCAGAAGGG
GTTTTGTGGAGATTTCAGTATTTTCATAGTTGAAATCATG
AGTCATTTGAAGCTAAACCCCCATGGAAAACCTAGGAGCA
ATGGACGGCCGTCTCGTTGTATTGTGAGACTCCTCAGCAG
TACCCATCCACGATCCCGCCTCGTGAGATTCGAACCCAGG
ATCTACCAGTCTCGCGCCAGAGCGCTTAACCACTAGATAT
TCTTTTATTATGTTAGGAAGTAATAAAAGTTTTCTTGAG
```

Types of sequence storage files

FASTA and FASTQ

FASTQ

- Used for storing sequencing data and quality
- ASCII format
- Usually MB to GB in size
- Four lines per sequence:
    1. Header (@ instead of >)
    2. Sequence
    3. + (sometimes the seq id)
    4. Phred quality scores

```
@SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
TATAAGCACAATCTGCTATCACAAGAAAACCAACGAGATATTTGCTTGTAACTCTTCTATTTAGAAACG
+SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEAEEEEEEEEEE/EEEEEEEEEEEEEAEEE/EEAEEA
@SRR26691082.2 NB501229:521:H5HFKBGXG:1:11101:14456:1055_ATTATN length=69
TATACAAACTAAGACAGATATTTTTTTTAGATTTAGCTATCTTCAACTTTCATTCCATTATTGTAATTA
+SRR26691082.2 NB501229:521:H5HFKBGXG:1:11101:14456:1055_ATTATN length=69
EEEEEEEEEEEEEEEEEE6EEEEEEEEEEEEEEEEEAEEEEEE/EEEEEEEEEEAEEEEEEEEEEEA
@SRR26691082.3 NB501229:521:H5HFKBGXG:1:11101:20997:1055_ATCGCN length=69
TATATGATAATTGGTGGTTCCAGACGTGCAGCTTGGAAGCGCCTCAATACTGTAGAGATGCGACGAAAG
```

- Regex for the block: `@<seqname>\n<seq>\n+[<seqname>]\n<qual>\n`

Types of sequence storage files

FASTA and FASTQ

FASTQ Header

- @SRR26691082.1 - SRR ID and read #
- NB501229 - instrument name
- 521 - Run ID
- H5HFKBGXG - flow cell ID
- 1 - flow cell lane
- 11101 - flow cell tile #
- 4638 - x coordinate in the tile
- 1052 - y coordinate in the tile
- TAACAN - index sequence

```
@SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
TATAAGCACAATCTGCTATCACAAGAAAACCAACGAGATATTTGCTTGTAACTCTTCTATTTAGAAACG
+SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEAEEEEEEEEEE/EEEEEEEEEEEEEAEEE/EEAEEA
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TATACAAACTAAGACAGATATTTTTTTTAGATTTAGCTATCTTCAACTTTCATTCCATTATTGTAATTA
+SRR26691082.2 NB501229:521:H5HFKBGXG:1:11101:14456:1055_ATTATN length=69
EEEEEEEEEEEEEEEEEEE6EEEEEEEEEEEEEEEEEEAEEEEEE/EEEEEEEEEEAEEEEEEEEEEEA
@SRR26691082.3 NB501229:521:H5HFKBGXG:1:11101:20997:1055_ATCGCN length=69
TATATGATAATTGGTGGTTCCAGACGTGCAGCTTGGAAGCGCCTCAATACTGTAGAGATGCGACGAAAG
```

Types of sequence storage files

FASTA and FASTQ

Phred quality

- String of the same length as <seq>
- Each character represents the Phred quality of the corresponding nt
- Represents the likelihood that the base call was correct

$$Q = -10\log_{10}(e)$$

Where $e is the error probability

- If the quality of a base call is 30, the probability that it is wrong is 0.001. In other words, given 1000 base calls with Q=30, one of them is wrong in average.
- Minimum of 0 (!) and maximum of 42 (K)

```
@SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
TATAAGCACAATCTGCTATCACAAGAAAACCAACGAGATATTTGCTTGTAACTCTTCTATTTAGAAACG
+SRR26691082.1 NB501229:521:H5HFKBGXG:1:11101:4638:1052_TAACAN length=69
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEAEEEEEEEEEE/EEEEEEEEEEEEEAEEE/EEAEEA
@SRR26691082.2 NB501229:521:H5HFKBGXG:1:11101:14456:1055_ATTATN length=69
TATACAAACTAAGACAGATATTTTTTTTAGATTTAGCTATCTTCAACTTTCATTCCATTATTGTAATTA
+SRR26691082.2 NB501229:521:H5HFKBGXG:1:11101:14456:1055_ATTATN length=69
EEEEEEEEEEEEEEEEEEE6EEEEEEEEEEEEEEEEEAEEEEEE/EEEEEEEEEEAEEEEEEEEEEA
@SRR26691082.3 NB501229:521:H5HFKBGXG:1:11101:20997:1055_ATCGCN length=69
TATATGATAATTGGTGGTTCCAGACGTGCAGCTTGGAAGCGCCTCAATACTGTAGAGATGCGACGAAAG
```

Phred+33 encoding

- $Q + 33, take the corresponding ASCII character
- Use this chart to get $Q for E, /, and A

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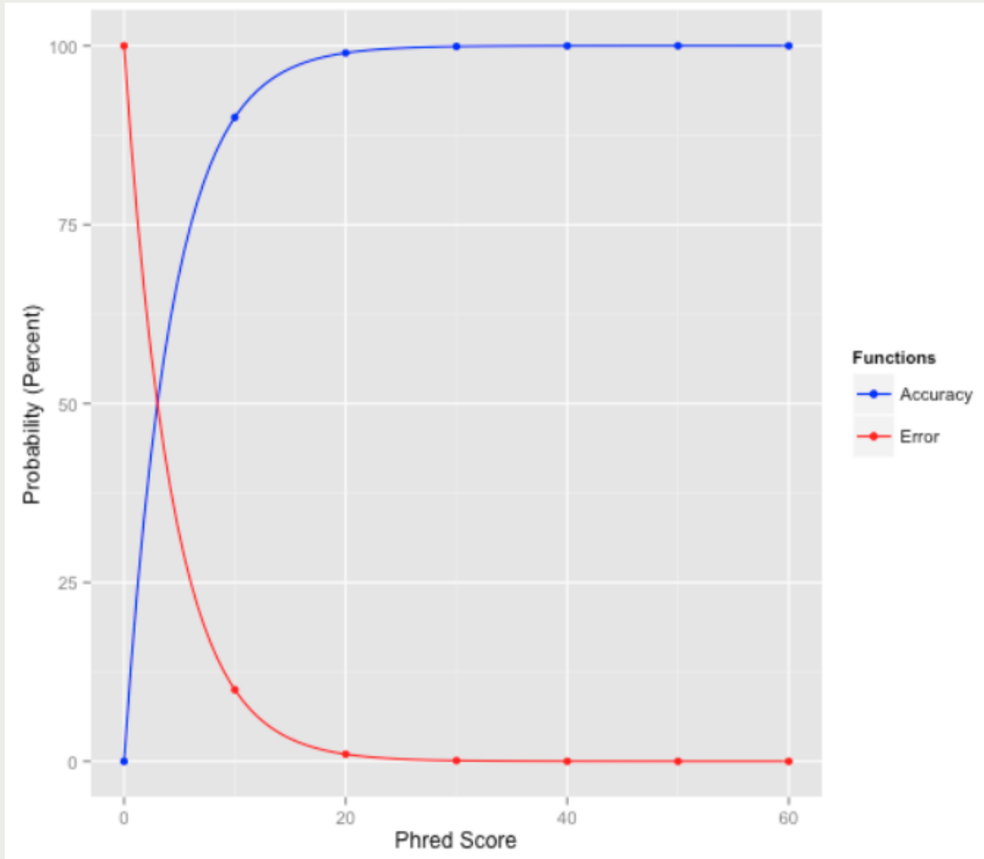

https://gatk.broadinstitute.org/hc/en-us/articles/360035531872-Phred

FASTQ databases

NCBI SRA & ENA

NCBI SRA

- https://www.ncbi.nlm.nih.gov/sra
- Sequence Read Archive
- Free database of FASTQ (or BAM) files
- Most public sequencing data can be found on SRA
  - Europeans post to ENA
- Dedicated software: SRA Toolkit (`conda install bioconda::sra-tools`)
  - Already installed in `biol343` environment