Cold Spring Harbor Laboratory

RNA-Seq Module 1: **FASTA/FASTQ/GTF**

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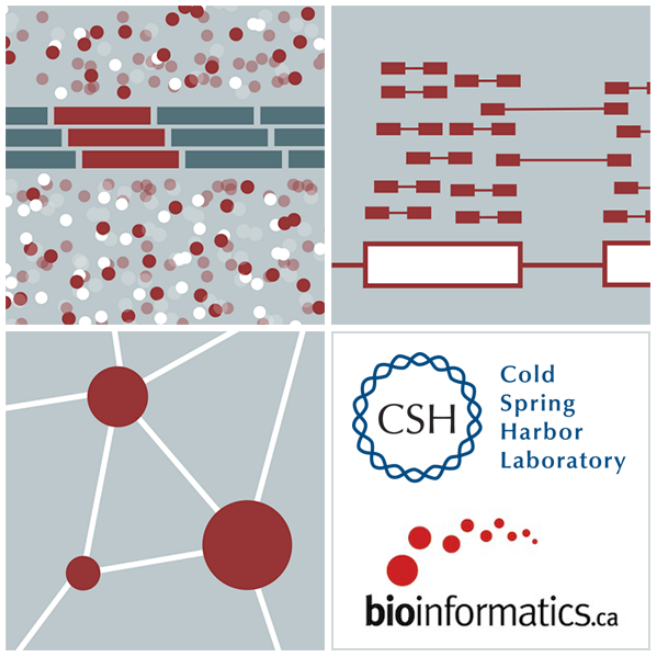
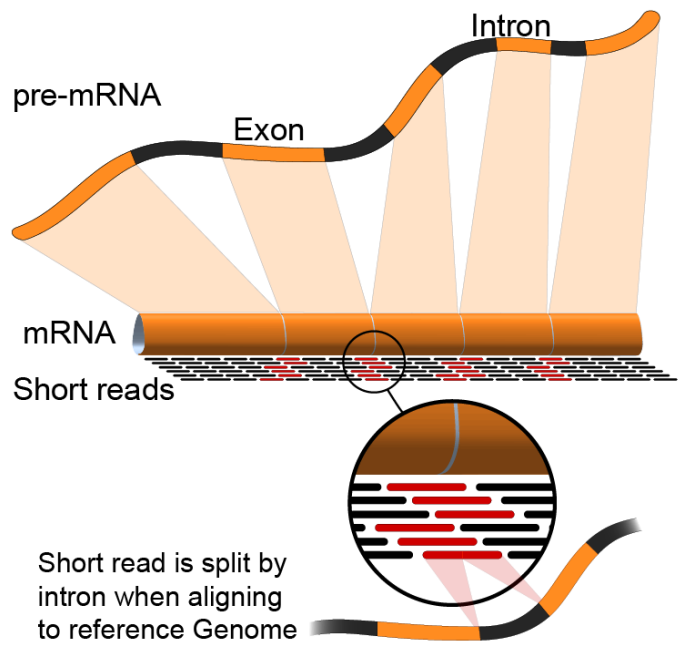




Washington University in St. Louis
School of Medicine

Fasta – format for representing nucleic acid or amino acid sequences

```
>AY274119.3 Severe acute respiratory syndrome-related coronavirus isolate
Tor2, complete genome


ATATTAGGTTTTTACCTACCCAGGAAAAGCCAACCAACCTCGATCTCTTGTAGATCTGTTCTCTAAACGA

ACTTTAAAATCTGTGTAGCTGTCGCTCGGCTGCATGCCTAGTGCACCTACGCAGTATAAACAATAATAAA

TTTTACTGTCGTTGACAAGAAACGAGTAACTCGTCCCTCTTCTGCAGACTGCTTACGGTTTCGTCCGTGT

TGCAGTCGATCATCAGCATACCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTTC

TTGGTGTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTCCTTCAGGTTAGAGACGTGCTAGTGCG

TGGCTTCGGGGACTCTGTGGAAGAGGCCCTATCGGAGGCACGTGAACACCTCAAAAATGGCACTTGTGGT

…


>FJ882960.1 SARS coronavirus ExoN1 isolate P3pp34, complete genome

CGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTAGCTGTCGCTCGGCTGCATGCCTA

GTGCACCTACGCAGTATAAACAATAATAAATTTTACTGTCGTTGACAAGAAACGAGTAACTCGTCCCTCT

TCTGCAGACTGCTTACGGTTTCGTCCGTGTTGCAGTCGATCATCAGCATACCTAGGTTTCGTCCGGGTGT

…
```

First line starts with “>” header or ”Comment”; used as a summary/description, often starting with unique accession/identifier

Subsequent lines contain sequence

- Interleaved: sequence broken into multiple lines of characters
- Sequential: entire sequence on a single line

Multiple sequence FASTA obtained by simply concatenating multiple FASTA records together

Fastq – format for representing raw sequence – base calls and quality values

```
@HWUSI-EAS100R:6:73:941:1973#0/1
CTTTTTTATTTTTGTCTGACTGGGTTGATTCAAAA
+
CCCFFFFFHHHHGJHIIJHIHIIIFHIJJJJIJJGIBBFGE

@HWUSI-EAS100R:6:2303:11793:37095#0/1
ATGAATTATAGGGCTGTATTTTAATTTTGCATTTTAA
+
@@??BDDFFF<FHEGFFGGIEBGHIIIIIBEHIIGIH<FHE
```

First line starts with “@” header or ”Comment”; followed by sequence identifier and optional description

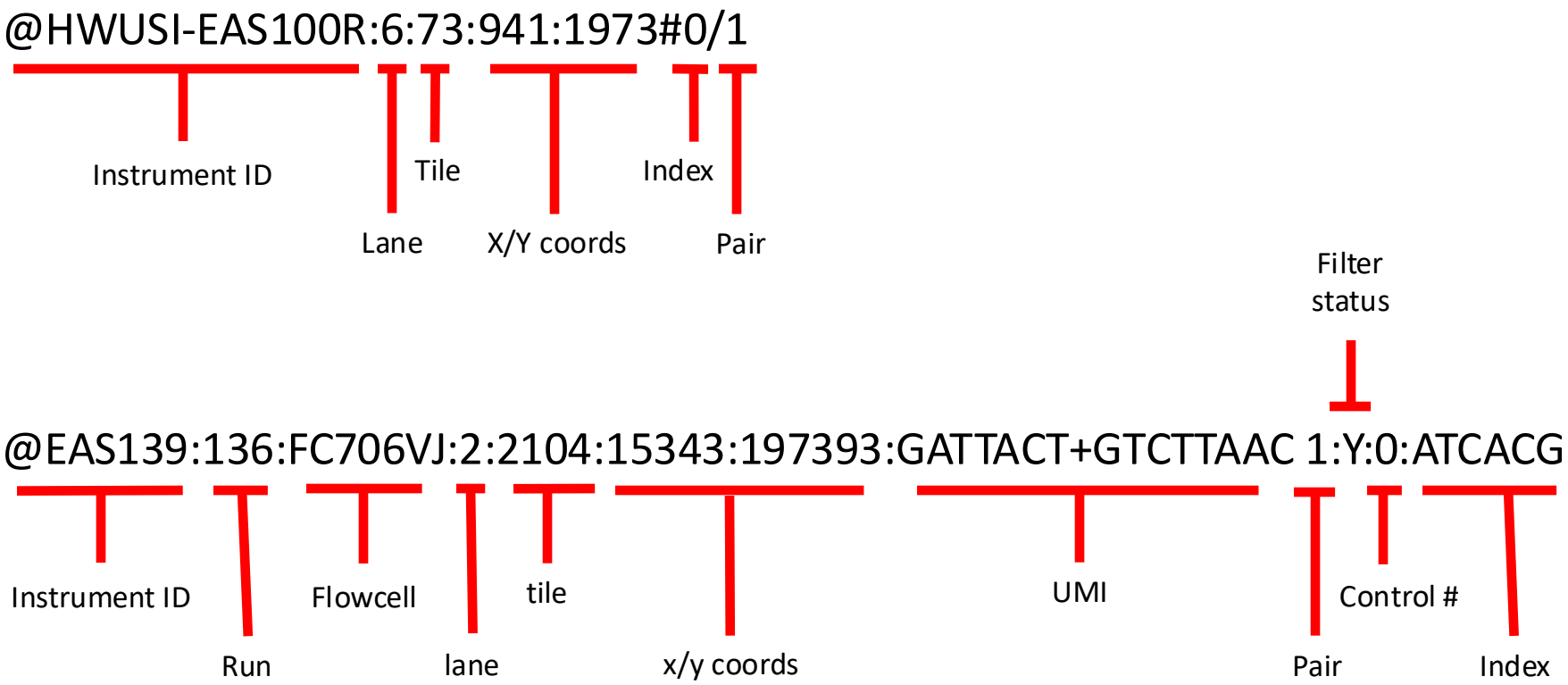
Sequence line

Spacer line

Quality values

Next sequence record

Read naming conventions



Quality values - Phred scores and ASCII glyphs

Phred Q	Probability (P) of Wrong Base	Base Call Accuracy	Sanger "Q + 33" Shift	Sanger "Q + 33" Shift ASCII glyph
0	1	0	33	!
1	0.794	0.206	34	"
2	0.631	0.369	35	#
10	0.1	0.9	43	+
20	0.01	0.99	53	5
30	0.001	0.999	63	?

Encoding History:

- Sanger Format (shown above): Q of 0 to 93 using ASCII 33 to 126
  - Sanger data, SAM format, Illumina 1.8+
- Solexa/Illumina 1.0: Q of -5 to 62 using ASCII 59 to 126
- Illumina 1.3 to 1.8: Q of 0 to 62 using ASCII 64 to 126
- Illumina 1.5 to 1.7: Phred scores 0 to 2 have a slightly different meaning
- Illumina 1.8+ -> Sanger Format

GFF/GTF - representing sequence features

- GFF – General/Generic Feature Format; Gene Finding Format
  - Two versions in wide use
    - GFF2 (see also GTF)
    - GFF3
      - Added formal support for multiple levels (and direction) of hierarchy (e.g., gene -> transcript -> exon)
- GTF – Gene Transfer Format
  - An extension of GFF2
- GFF2, GFF3 and GTF are all tab-separate files with 9 fields
  - Differing content in 9th column

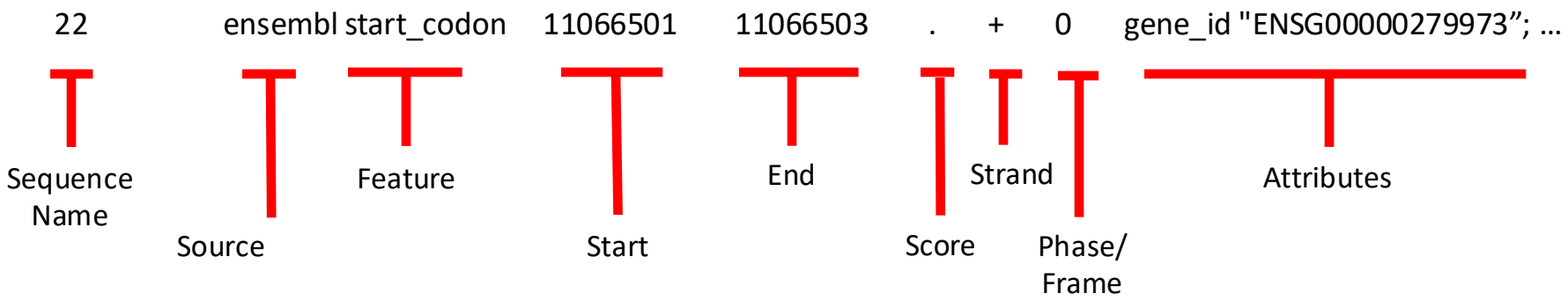
GFF/GTF – general structure

General GFF structure

Position index	Position name	Description
1	sequence	The name of the sequence where the feature is located.
2	source	Keyword identifying the source of the feature, like a program (e.g. Augustus or RepeatMasker) or an organization (like TAIR).
3	feature	The feature type name, like "gene" or "exon". In a well structured GFF file, all the children features always follow their parents in a single block (so all exons of a transcript are put after their parent "transcript" feature line and before any other parent transcript line). In GFF3, all features and their relationships should be compatible with the standards released by the Sequence Ontology Project.
4	start	Genomic start of the feature, with a **1-base offset**. This is in contrast with other 0-offset half-open sequence formats, like BED files.
5	end	Genomic end of the feature, with a **1-base offset**. This is the same end coordinate as it is in 0-offset half-open sequence formats, like BED files.[*citation needed*]
6	score	Numeric value that generally indicates the confidence of the source on the annotated feature. A value of "." (a dot) is used to define a null value.
7	strand	Single character that indicates the Sense (molecular biology) strand of the feature; it can assume the values of "+" (positive, or 5'->3'), "-", (negative, or 3'->5'), "." (undetermined).
8	phase	phase of CDS features; it can be either one of 0, 1, 2 (for CDS features) or "." (for everything else). See the section below for a detailed explanation.
9	Attributes.	All the other information pertaining to this feature. The format, structure and content of this field is the one which varies the most between the three competing file formats.

https://en.wikipedia.org/wiki/General_feature_format

Ensembl GTF example record



Example of attributes string:

gene_id "ENSG00000279973"; gene_version "1"; transcript_id "ENST00000624155"; transcript_version "1"; exon_number "1"; gene_name "BAGE5"; gene_source "ensembl"; gene_biotype "protein_coding"; transcript_name "BAGE5-201"; transcript_source "ensembl"; transcript_biotype "protein_coding"; tag "basic"; transcript_support_level "1";

Note: there will be many GTF records/rows per transcript per gene (UTRs, start_codon, exons, etc)

We are on a Coffee Break & Networking Session