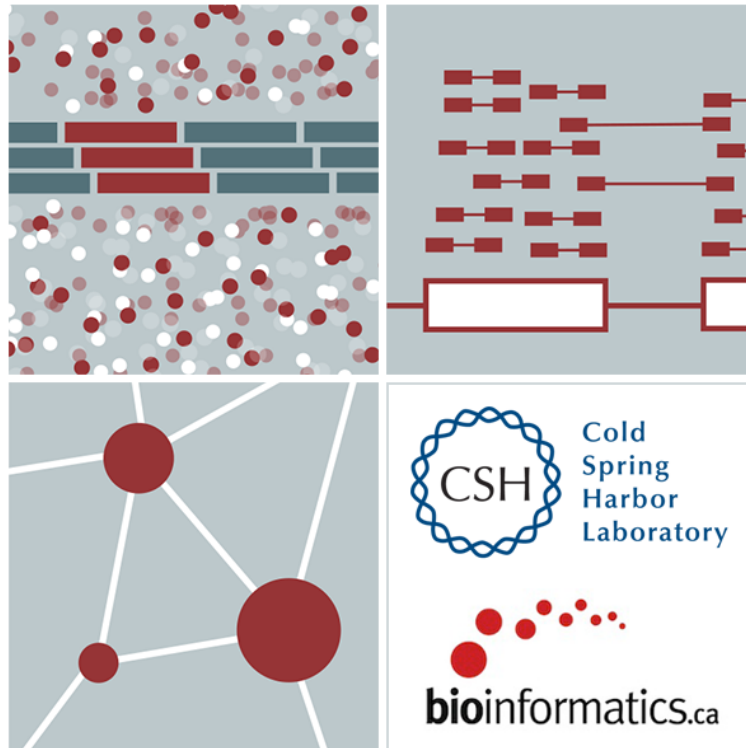


# RNA-Seq Module 2

## FASTA/FASTQ/GTF formats

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# Fasta – format for representing nucleic acid or amino acid sequences

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

```
ATATTAGGTTTTTACCTACCCAGGAAAAGCCAACCAACCTCGATCTCTTGTAGATCTGTTCTCTAAACGA  
ACTTTAAAATCTGTGTAGCTGTTCGCTCGGCTGCATGCCTAGTGCACCTACGCAGTATAAACAATAATAAA  
TTTTACTGTCGTTGACAAGAAACGAGTAACTCGTCCCTCTTCTGCAGACTGCTTACGGTTTCGTCCGTGT  
TGCAGTCGATCATCAGCATACTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTTTC  
TTGGTGTCAACGAGAAAACACACGTCCAACCTCAGTTTGCCTGTCCTTCAGGTTAGAGACGTGCTAGTGCG  
TGGCTTCGGGGACTCTGTGGAAGAGGCCCTATCGGAGGCACGTGAACACCTCAAAAATGGCACTTGTGGT  
...
```

```
>FJ882960.1 SARS coronavirus ExoN1 isolate P3pp34, complete genome  
CGATCTCTTGTAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTAGCTGTTCGCTCGGCTGCATGCCTA  
GTGCACCTACGCAGTATAAACAATAATAAATTTTTACTGTCGTTGACAAGAAACGAGTAACTCGTCCCTCT  
TCTGCAGACTGCTTACGGTTTCGTCCGTGTTGCAGTCGATCATCAGCATACTAGGTTTCGTCCGGGTGT  
...
```

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
```

```
CTTTTTTATTTTGTCTGACTGGGTTGATTCAAAA
```

```
+
```

```
CCCFHHHHGJHIJHIHIIIFHIJJJIJJGIBBFGE
```

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

Sequence line

Spacer line

Quality values

```
@HWUSI-EAS100R:6:2303:11793:37095#0/1
```

```
ATGAATTATAGGGCTGTATTTTAATTTTGCATTTTAA
```

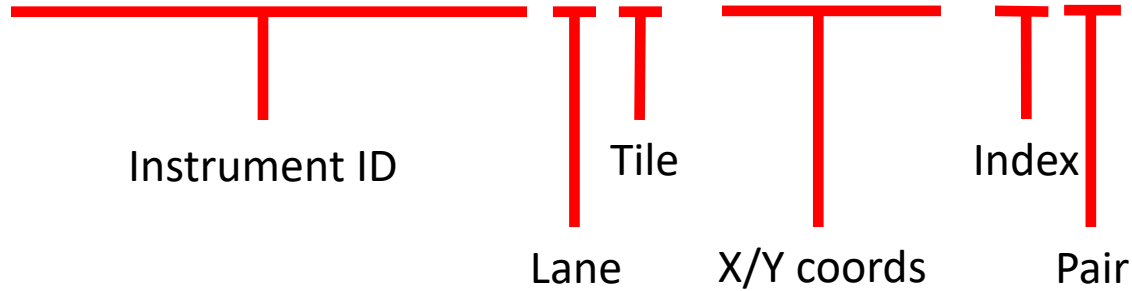
```
+
```

```
@@??BDDFFF<FHEGFFGGIEBGHIIIIIBEHIIGIH<FHE
```

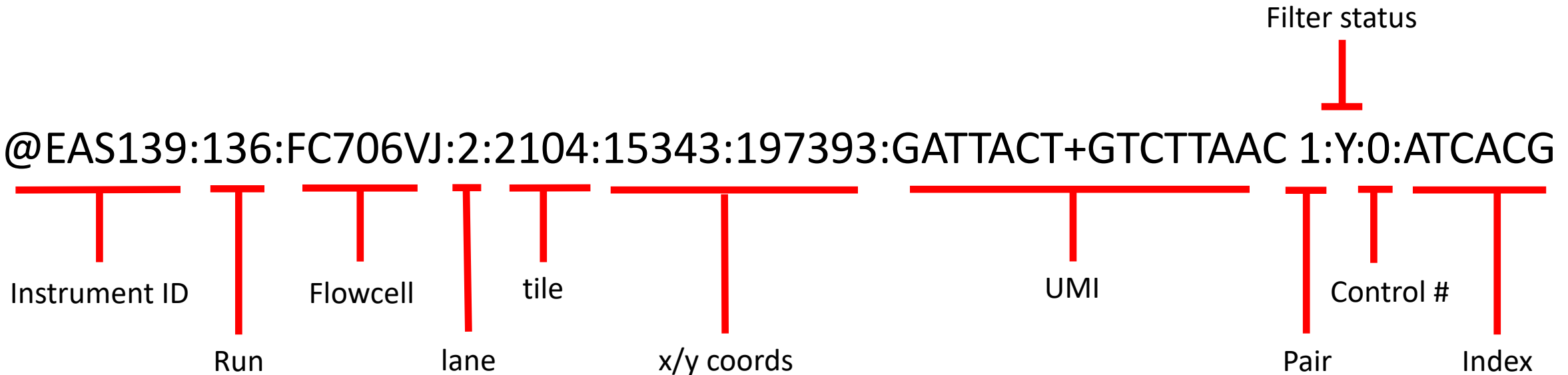
Next sequence record

# Read naming conventions

@HWUSI-EAS100R:6:73:941:1973#0/1



@EAS139:136:FC706VJ:2:2104:15343:197393:GATTACT+GTCTTAAC 1:Y:0:ATCACG



# 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-separated files with 9 fields
  - Differing content in 9<sup>th</sup> column

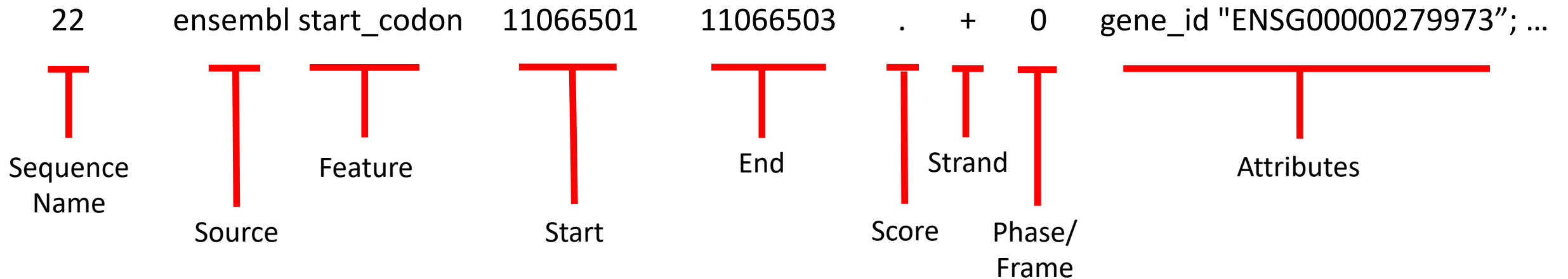
# GFF/GTF – general structure

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. <a href="#">Augustus</a> or <a href="#">RepeatMasker</a> ) or an organization (like <a href="#">TAIR</a> ).
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 <a href="#">standards released by the Sequence Ontology Project</a> .
4	start	Genomic start of the feature, with a <b>1-base offset</b> . This is in contrast with other 0-offset half-open sequence formats, like <a href="#">BED files</a> .
5	end	Genomic end of the feature, with a <b>1-base offset</b> . This is the same end coordinate as it is in 0-offset half-open sequence formats, like <a href="#">BED files</a> . <sup>[<i>citation needed</i>]</sup>
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 <b>Sense (molecular biology) strand</b> 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](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)