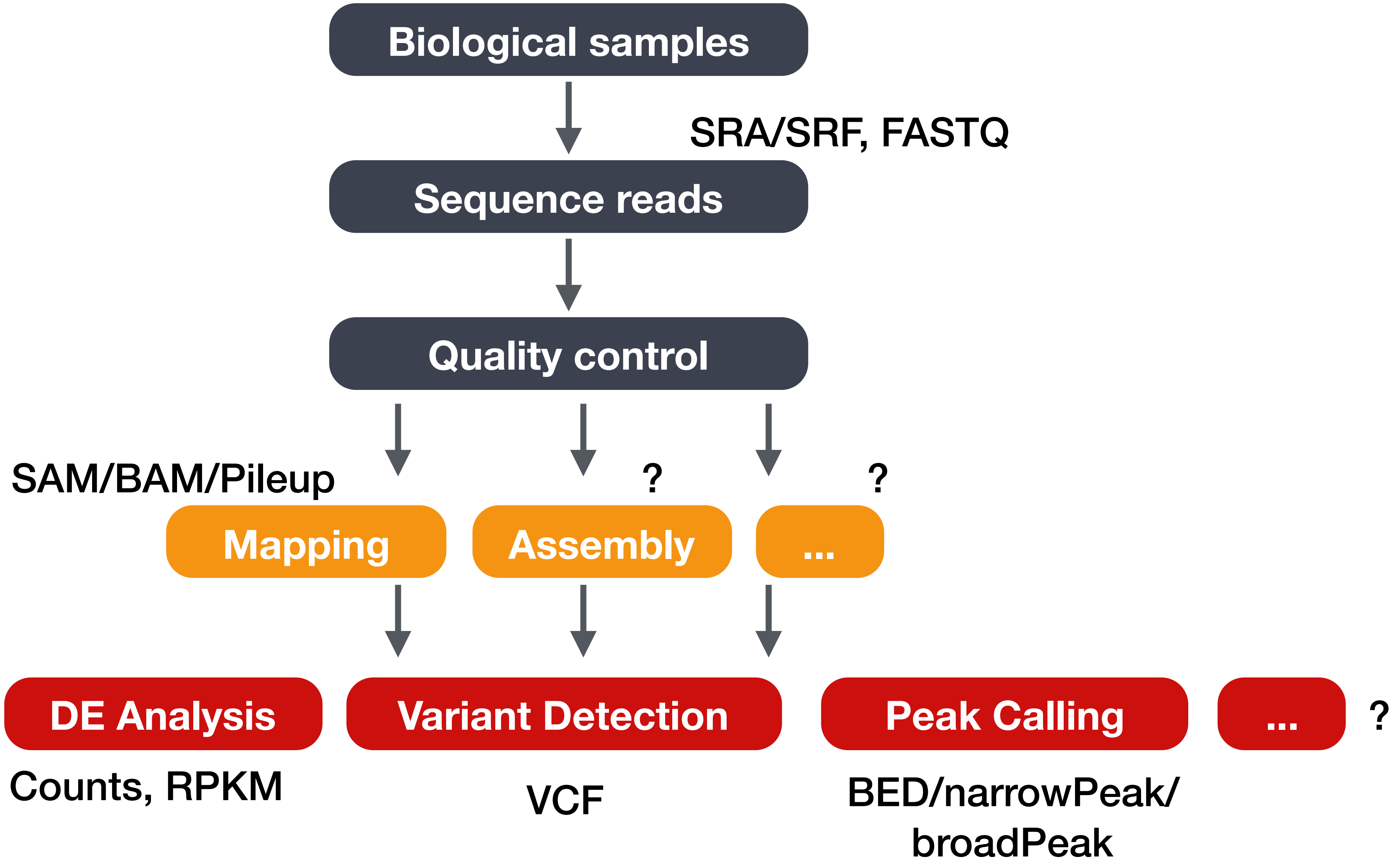
Workflow and
data standards



Biological samples

SRA/SRF, FASTQ

Sequence reads

Quality control

SAM/BAM/Pileup

?

?

Mapping

Assembly

...

DE Analysis

Variant Detection

Peak Calling

...

?

Counts, RPKM

VCF

BED/narrowPeak/
broadPeak

NGS analysis workflows

Common data types and file formats

- You will encounter 3 major types of data, with several associated file formats:
    - Sequence data
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- Some file formats are not human-readable (***binary***).
- Many are human readable, but extremely large; never use Word or Excel to open these!

Simple sequence formats

- FASTA (simple representation of sequence data: protein & nucleotide)
- FASTQ (complex, includes data quality information: raw sequencing)

FASTA

```
>SRR014849.1 EIXKN4201CFU84 length=93
GGGGGGGGGGGGGGGGCTTTTTTTGTTTGGAACCGAAAGGGTTTTGAATTTCAAACCCTTTTCGGTTTCCAACCTTCCAAAGCAATGCC
AATA
```

```
>gi|340780744|ref|NC_015850.1| Acidithiobacillus caldus SM-1 chromosome, complete genome
ATGAGTAGTCATTCAGCGCCGACAGCGTTGCAAGATGGAGCCGCGCTGTGGTCCGCCCTATGCGTCCAACTGGAGCTCGTCACGAG
TCCGCAGCAGTTCAATACCTGGCTGCGGCCCCTGCGTGGCGAATTGCAGGGTCATGAGCTGCGCCTGCTCGCCCCCAATCCCTTCG
TCCGCGACTGGGTGCGTGAACGCATGGCCGAACTCGTCAAGGAACAGCTGCAGCGGATCGCTCCGGGTTTTGAGCTGGTCTTCGCT
CTGGACGAAGAGGCAGCAGCGGCGACATCGGCACCGACCGCGAGCATTGCGCCCGAGCGCAGCAGCGCACCCGGTGGTCACCGCCT
CAACCCAGCCTTCAACTTCCAGTCCTACGTCGAAGGGAAGTCCAATCAGCTCGCCCTGGCGGCAGCCCGCCAGGTTGCCCAGCATC
CAGGCAAATCCTACAACCCACTGTACATTTATGGTGGTGTGGGCCTCGGCAAGACGCACCTCATGCAGGCCGTGGGCAACGATATC
CTGCAGCGGCAACCCGAGGCCAAGGTGCTCTATATCAGCTCCGAAGGCTTCATCATGGATATGGTGCGCTCGCTGCAACACAATAC
CATCAACGACTTCAAACAGCGTTATCGCAAGCTGGACGCCCTGCTCATCGACGACATCCAGTTCTTTGCGGGCAAGGACCGCACCC
```

```
>gi|129295|sp|P01013|OVAX_CHICK GENE X PROTEIN (OVALBUMIN-RELATED)
QIKDLLVSSSTDLDTTLVLVNAIYFKGMWKTAFNAEDTREMPFHVTKQESKPVQMMCMNNSFNVATLPAE
```

FASTQ: FASTA with Quality scores

```
@SRR014849.1 EIXKN4201CFU84 length=93
GGGGGGGGGGGGGGGGCTTTTTTTGTTTGGAACCGAAAGGGTTTTGAATTTCAAACCCTTTTCGGTTTCCAACCTTCCAAAGCAATGCCAATA
+SRR014849.1 EIXKN4201CFU84 length=93
3+&$#"""""""""""7F@71,’";C?,B;?6B;:EA1EA1EA5’9B:?:#9EA0D@2EA5’:>5?:%A;A8A;?9B;D@/=<?7=9<2A8==
```

Line	Description
1	Always begins with '@' and then information about the read
2	The actual DNA sequence
3	Always begins with a '+' and sometimes the same info in line 1
4	Has a string of characters which represent the quality score

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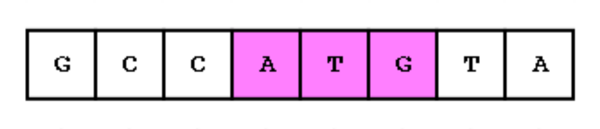
Genomic coordinates can be represented in 2 ways

Where is base 1 and where is base 8?



G	C	C	A	T	G	T	A

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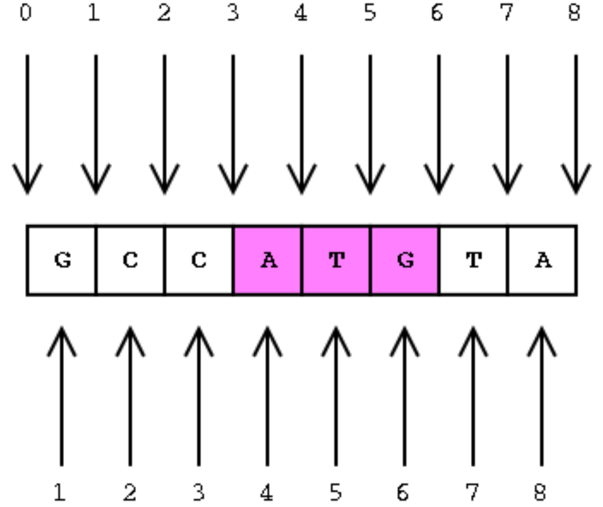

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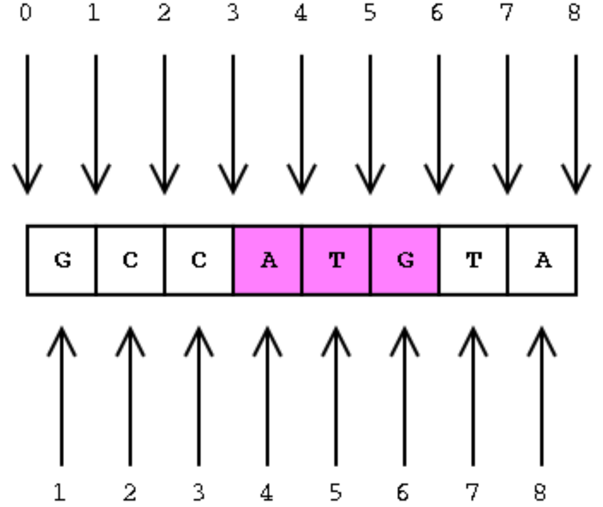
Coords ***Where is ATG?*** ***Length***



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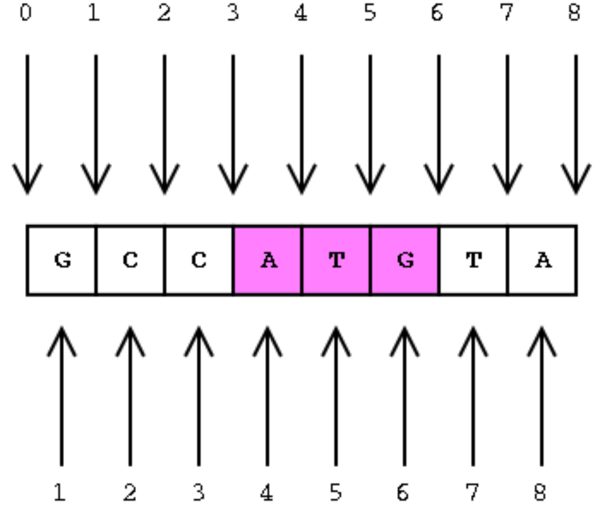
Coords	***Where is ATG?***	***Length***
0-based (half-open) *preferred by programmers*	`( 3, 6 ]`	Len = end - start



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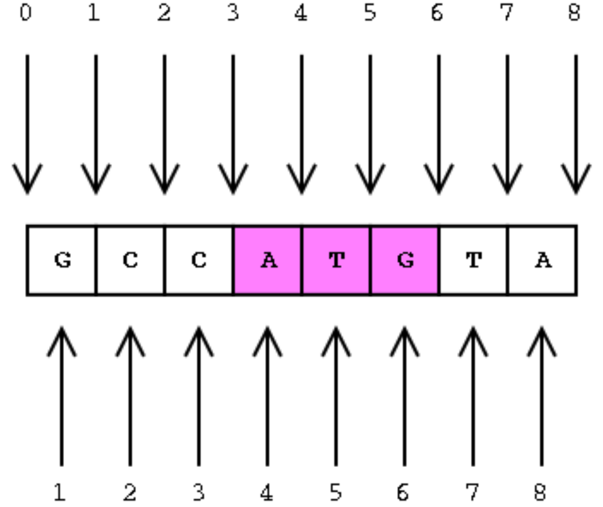 0 1 2 3 4 5 6 7 8 G C C A T G T A 1 2 3 4 5 6 7 8		
1-based (closed) *preferred by biologists*	[4, 6]	Len = end – start + 1

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  - E.g. human chromosome 1
    - **UCSC – ‘chr1’** versus **Ensembl/NCBI – ‘1’**

- Best practice: get feature format files from the same source (i.e UCSC, Ensembl, NCBI) as the reference genome

Alignment file: SAM

- SAM – Sequence Alignment/Map format
- SAM file format stores alignment information, including read name, alignment coordinates, mismatches, etc.
- Plain text
- **1-based coordinates**
- Files can be very large: Many 100’s of GB or more

Alignment file: BAM

- BAM – BGZF compressed SAM format
- Binary (compressed) version of SAM and is therefore not human readable
- **0-based coordinates**
- Makes the alignment information easily accessible to downstream applications (SAM format is mostly useless for downstream analyses)
- Files are typically very large: ~ 1/5 of SAM, but still very large

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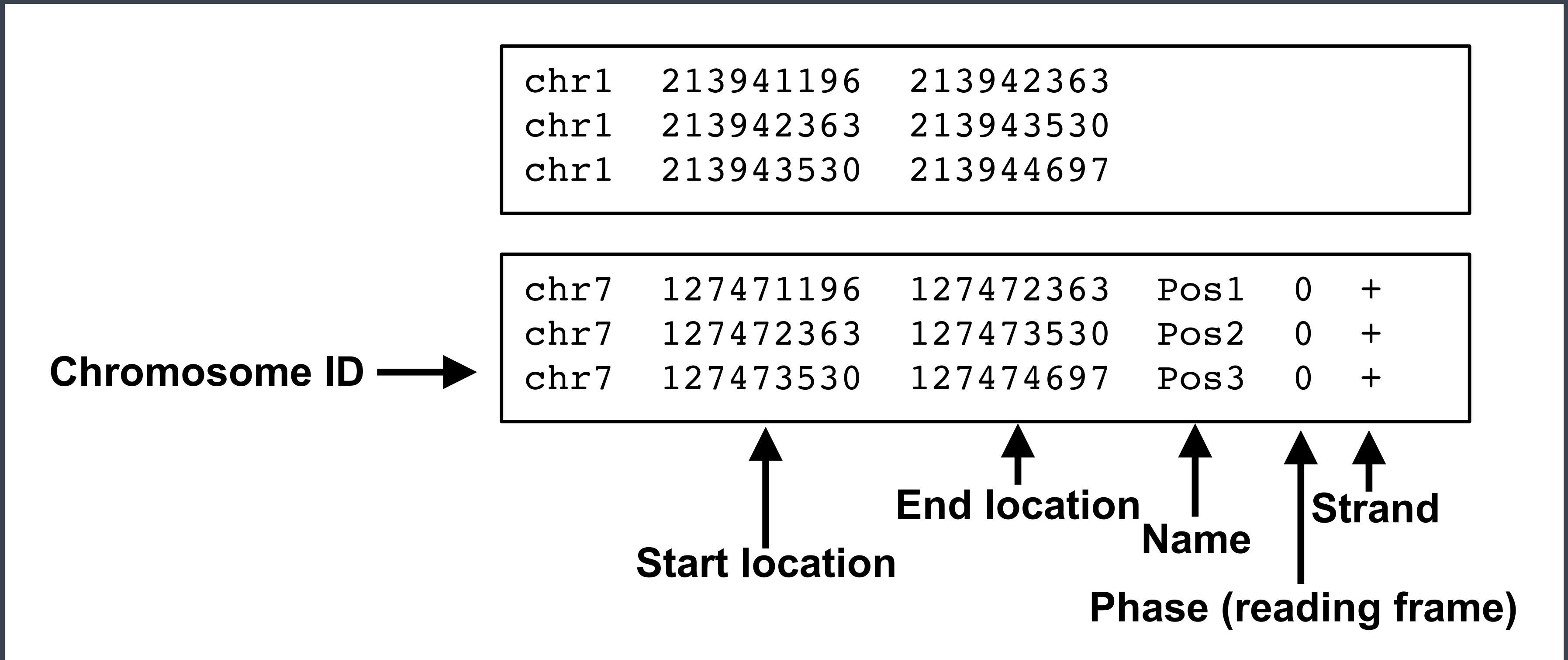

```
chr1  213941196  213942363
chr1  213942363  213943530
chr1  213943530  213944697
```

```
chr7  127471196  127472363  Pos1  0  +
chr7  127472363  127473530  Pos2  0  +
chr7  127473530  127474697  Pos3  0  +
```

Chromosome ID →

Start location

End location

Name

Phase (reading frame)

Strand

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- Often used for displaying density or coverage information

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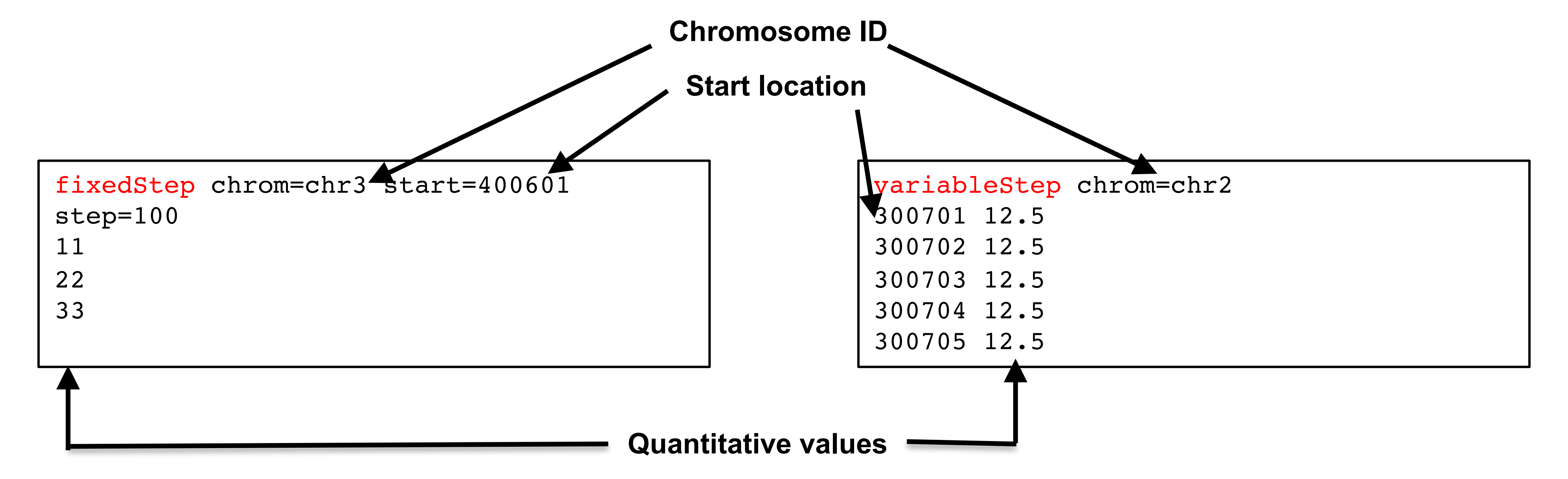

Chromosome ID

Start location

```
fixedStep chrom=chr3 start=400601
step=100
11
22
33
```

```
variableStep chrom=chr2
300701 12.5
300702 12.5
300703 12.5
300704 12.5
300705 12.5
```

Quantitative values

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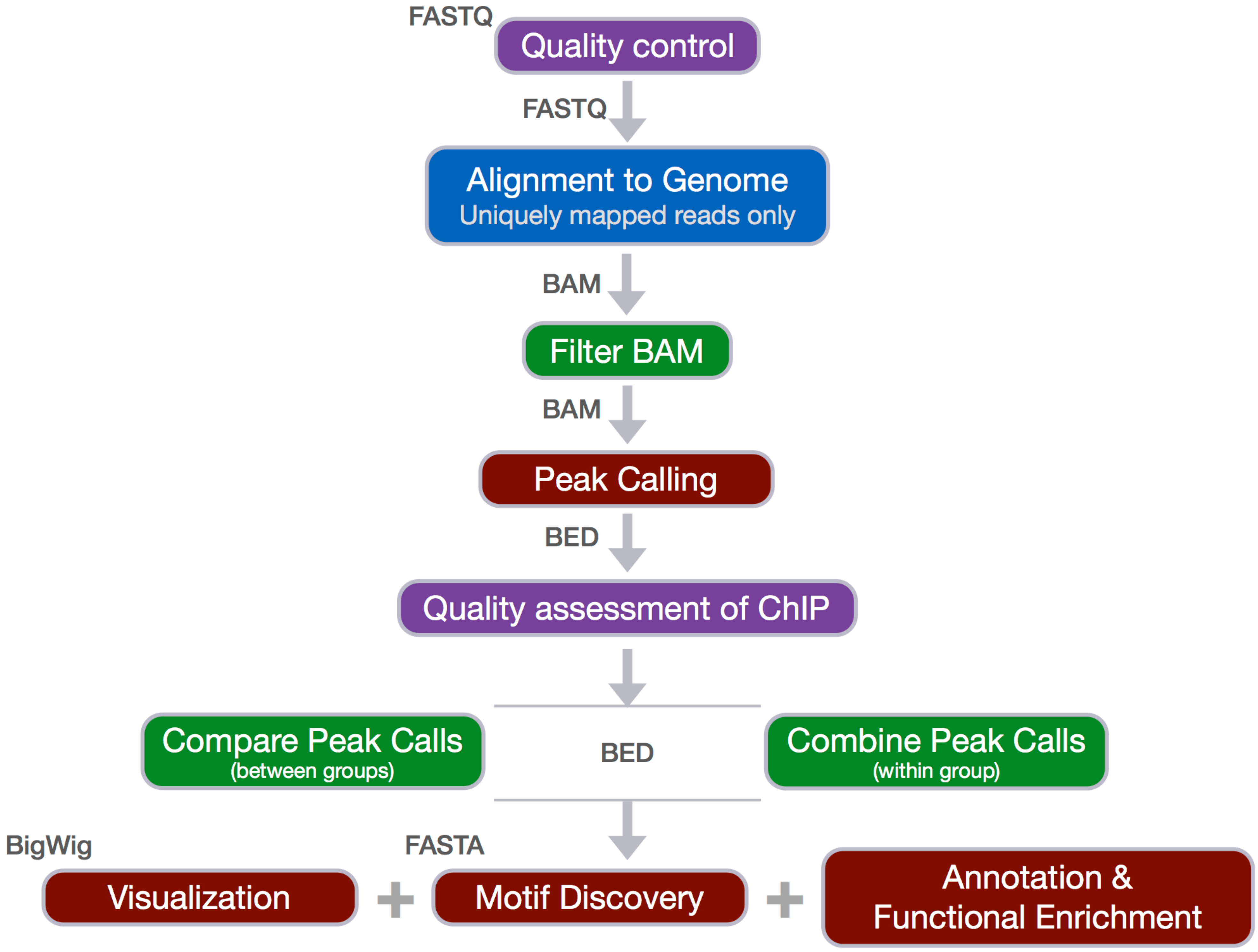
- **1-based coordinates**

Commonly used file formats

- FASTA
- FASTQ – Fasta with quality
- SAM – Sequence Alignment/Map format
- BAM – Binary Sequence Alignment/Map format
- Bed – Basic genome interval
- BedGraph
- Wiggle (wig, bigwig) – tab-limited format to represent continuous values
- *GFF3 – Gene feature format (genome interval ++)*
- *GTF – Gene transfer format (genome interval ++)*

http://genome.ucsc.edu/FAQ/FAQformat.html



ChIP-seq workflow