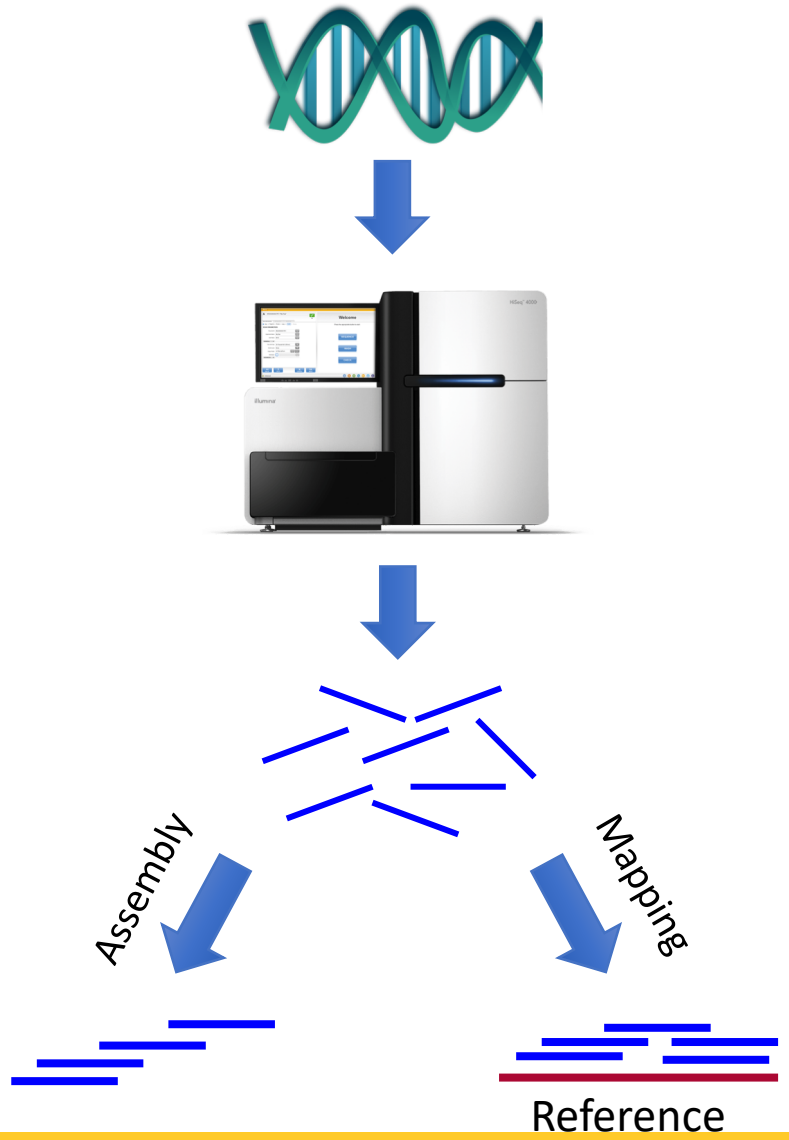
Mapping

Aligning sequencing reads to a reference

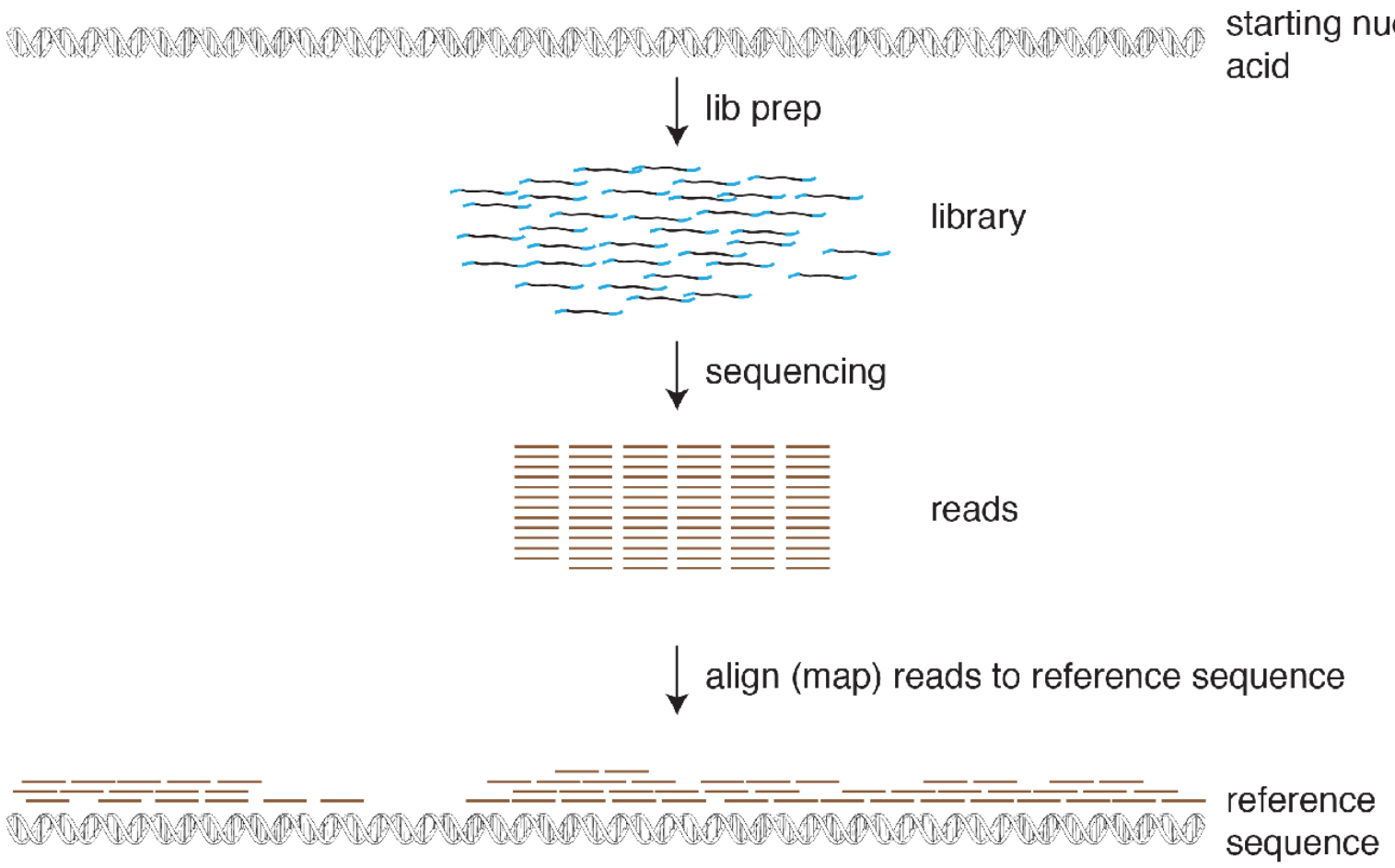
So you got some sequences… now what?



- Quality Control
  - Trim low quality reads/bases
  - Remove Adapters
  - Error Correction
- Mapping
  - Comparison to a reference sequence (genome, transcriptome, etc)
- Assembly
  - Generate a new consensus sequence (genome, transcriptome, etc)

Mapping

- The process by which sequencing reads are aligned (matched) to a region of the genome from which they derive (*reference*)



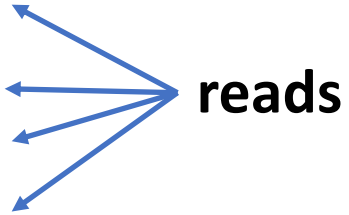
Why Mapping?

- Identify variants
  - *Substitutions* (fixed difference from reference)
  - *Polymorphisms* (multiple alleles, heterozygous)
    - Single nucleotide polymorphisms = *SNPs*
  - *Structural variants* (insertion-deletion events, duplications, etc)

Variants

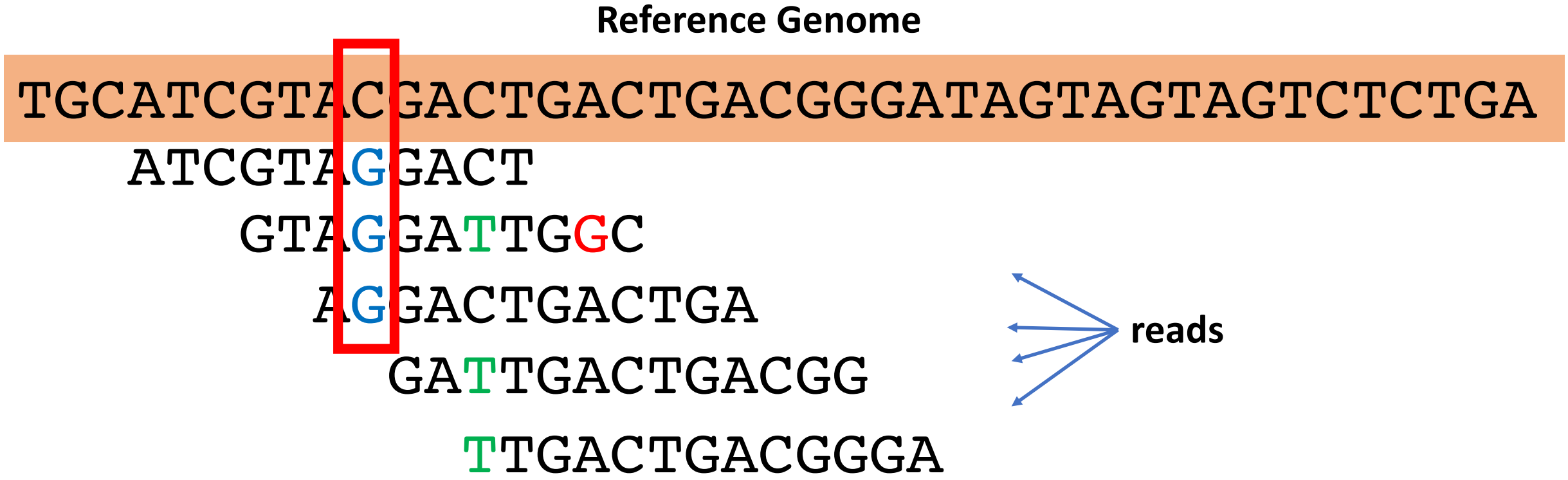
Reference Genome

```
TGCATCGTACGACTGACTGACGGGATAGTAGTAGTCTCTGA
    ATCGTAGGACT
       GTAGGATTGGC
         AGGACTGACTGA
           GATTGACTGACGG
             TTGACTGACGGGA
```



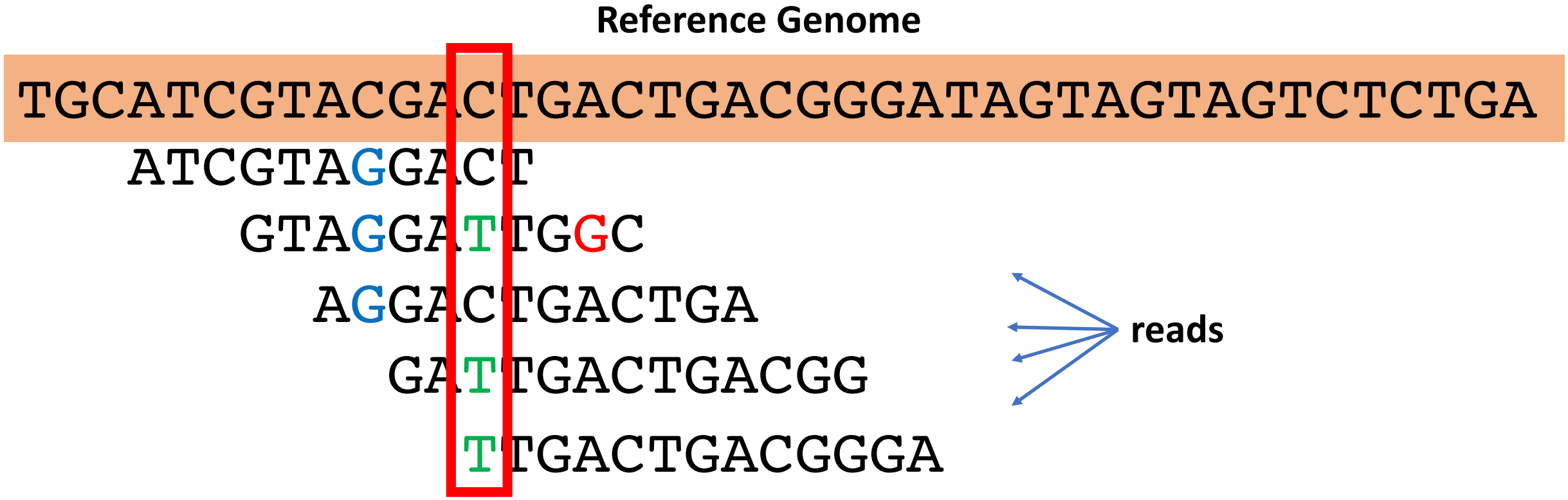
G = substitution T = polymorphism G = sequencing error

Variants



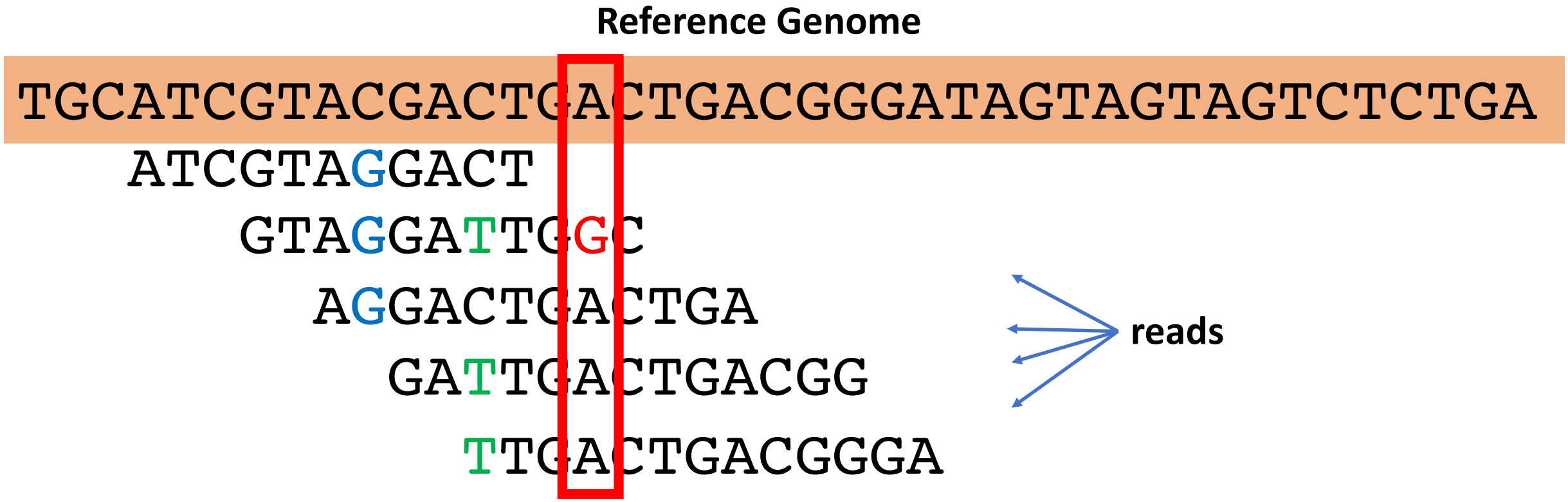
G = substitution T = polymorphism G = sequencing error

Variants



G = substitution T = polymorphism G = sequencing error

Variants

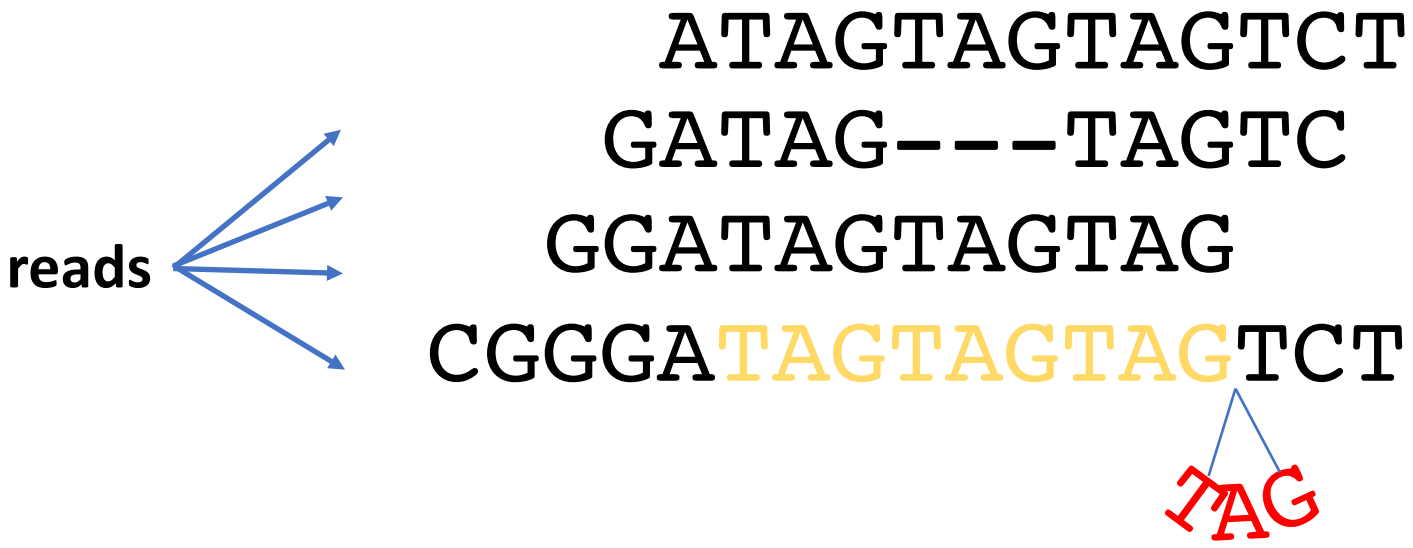


G = substitution T = polymorphism G = sequencing error

Variants

Reference Genome

TGCATCGTACGACTGACTGACGGGATAGTAGTAGTCTCTGA

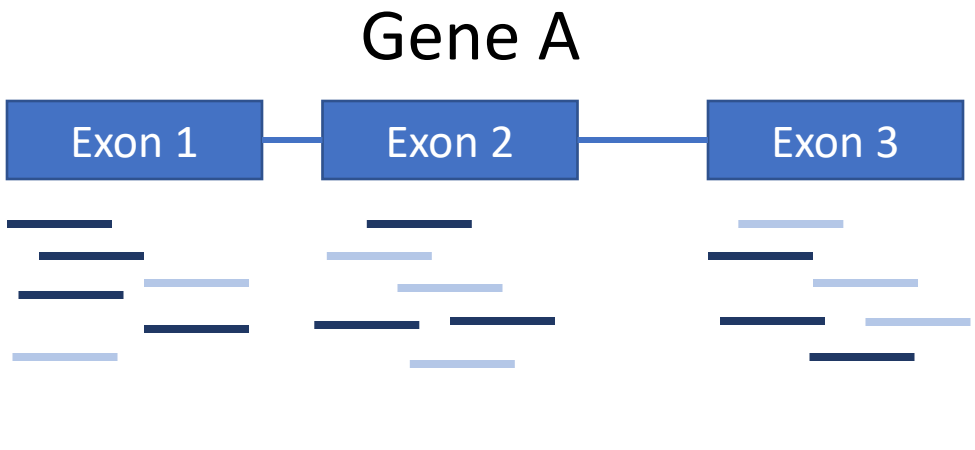


--- = deletion from reference; TAG = insertion relative to reference

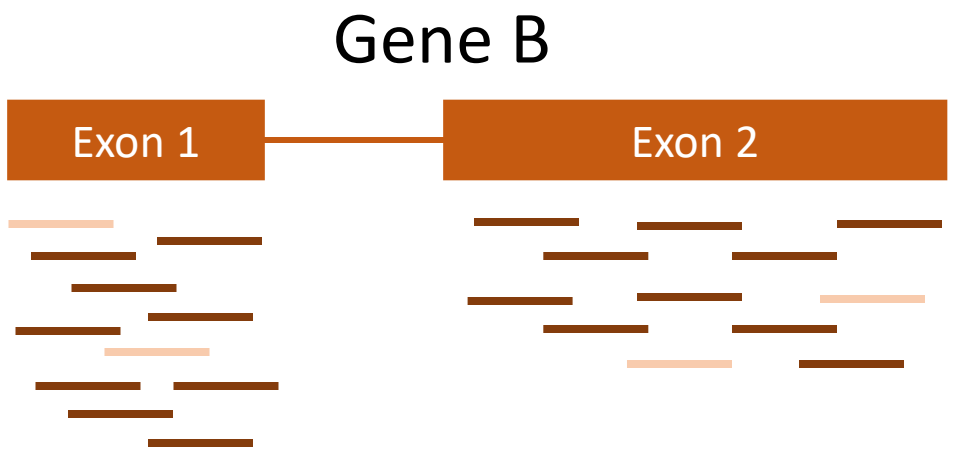
Why Mapping?

- Identify variants
  - *Substitutions* (fixed difference from reference)
  - *Polymorphisms* (multiple alleles, heterozygous)
    - Single nucleotide polymorphisms = *SNPs*
  - *Structural variants* (insertion-deletion events, duplications, etc)
- Quantification (counting)
  - Expression level of genes in a transcriptome (RNA-seq)

Quantification



= Control
= Treatment

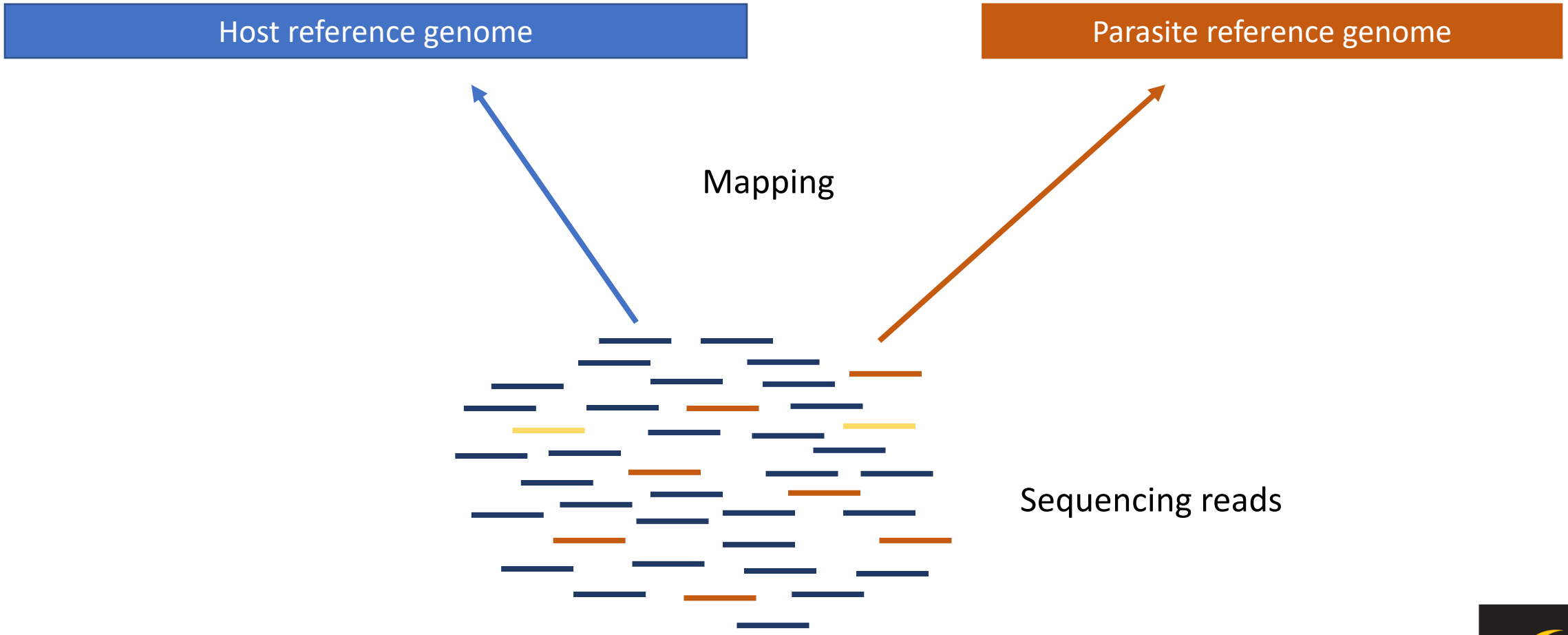


= Control
= Treatment

Why Mapping?

- Identify variants
  - *Substitutions* (fixed difference from reference)
  - *Polymorphisms* (multiple alleles, heterozygous)
    - Single nucleotide polymorphisms = *SNPs*
  - *Structural variants* (insertion-deletion events, duplications, etc)
- Quantification
  - Expression level of genes in a transcriptome (RNA-seq)
- Identify or remove sequences of specific origins
  - Contamination
  - Parasites, microbiome, pathogens
  - Organellar DNA (mtDNA, cpDNA)

Identifying/Removing sequences from mixed origin



DIY Exercise!!

Map the reads to the reference!

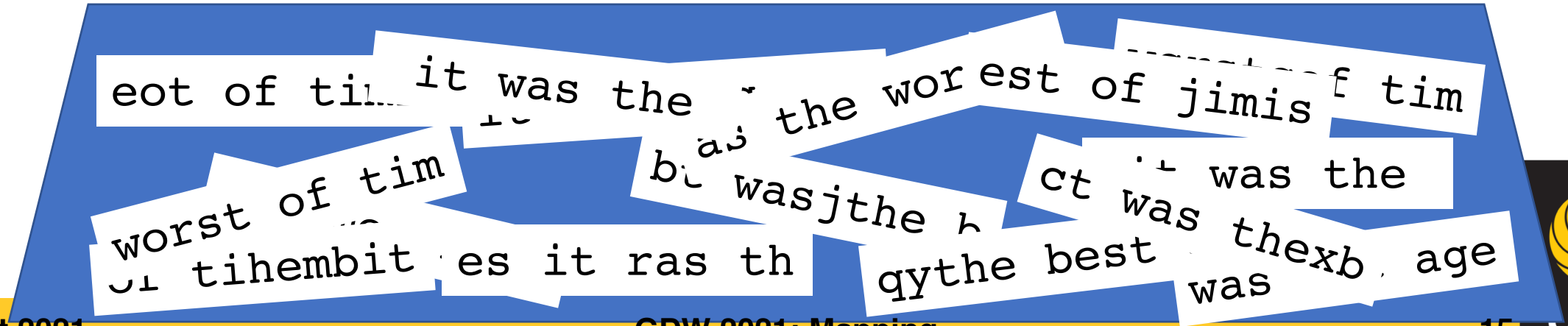
http://ivory.idyll.org/blog/the-assembly-exercise.html

it was the best of times it was the worst of times it was the age

Reference “Genome”

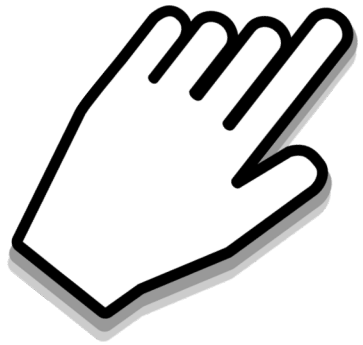
Read “Library”

(shotgun sequenced pieces of the reference genome)

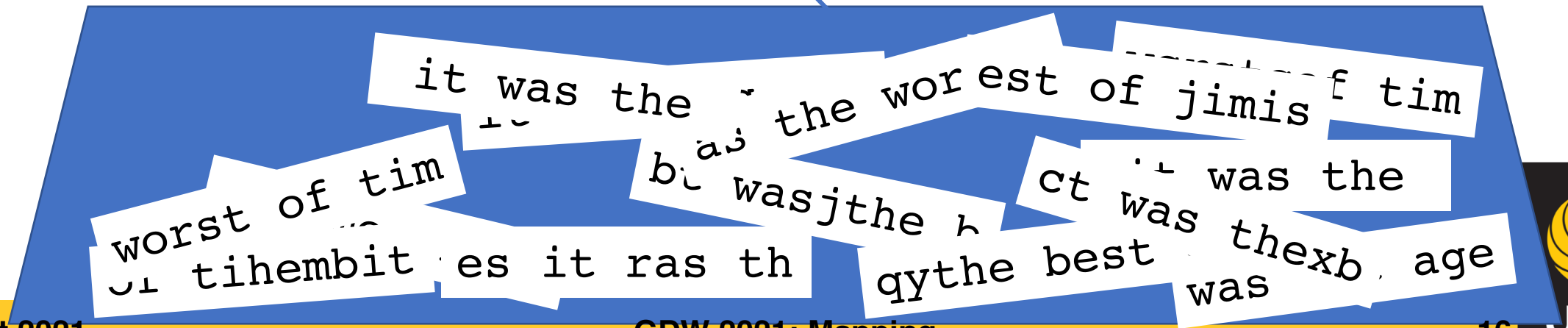


it was the best of times it was the worst of times it was the age

eot of timis



Drag and drop the “reads” from the library and align them to the reference genome.



DIY Exercise!!

- Report the:
  - Coverage
  - Error rate
  - How many variants (SNPs)?
  - Mapping rate (reads/sec)?
- Extra credit: Name the book and author
  - No Googling!

http://ivory.idyll.org/blog/the-assembly-exercise.html

DIY Exercise!!

- Report the:
  - Coverage = 7X
  - Error rate = 10%
  - How many variants (SNPs)? = 2? 3? – tim[i/e]s wa[s/k] ep[o/r]ch
  - Mapping rate (reads/sec)?
- Extra credit: Name the book and author
  - Tale of Two Cities

http://ivory.idyll.org/blog/the-assembly-exercise.html

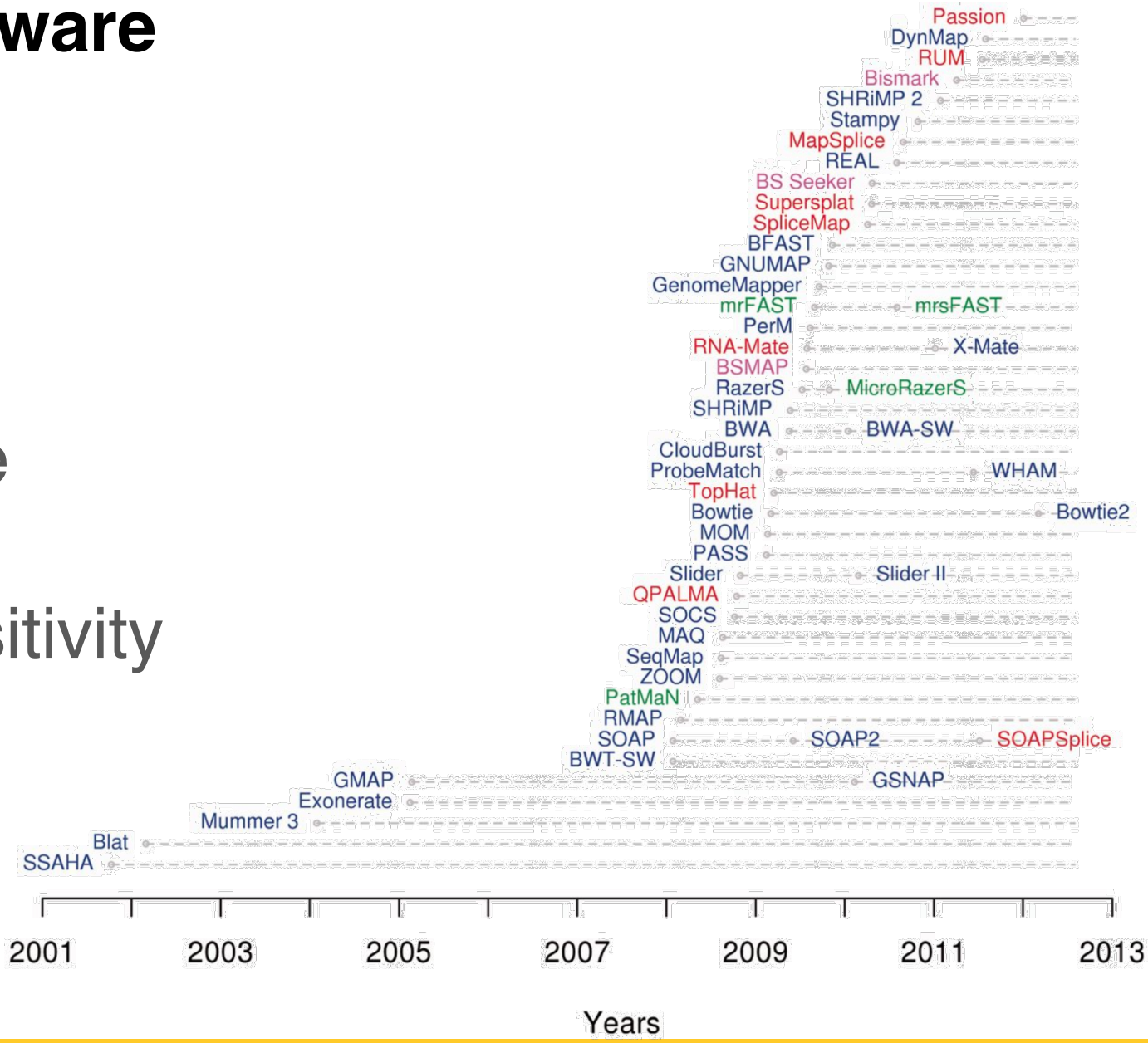
Just a pairwise alignment, right?

Yes.

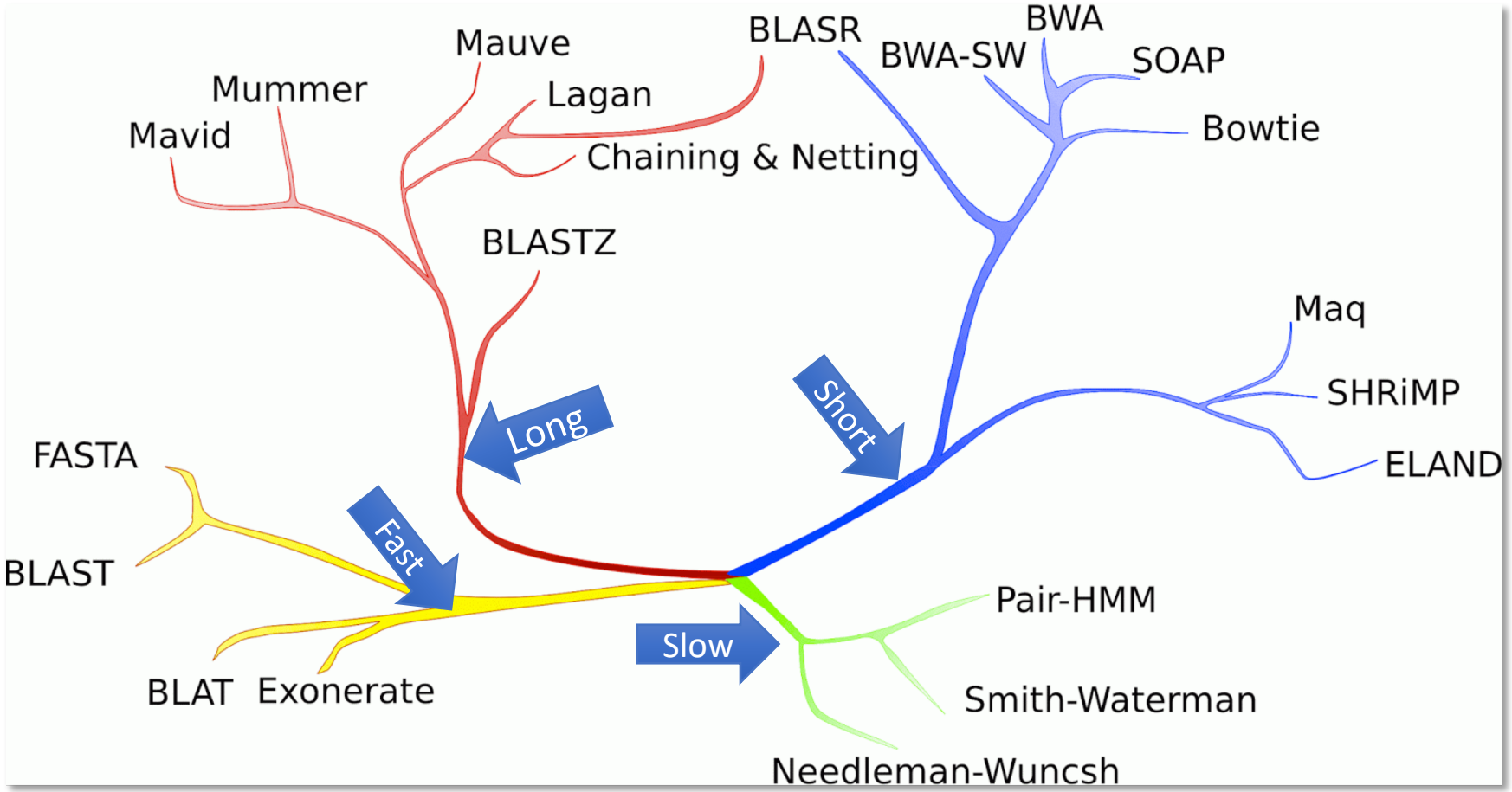
x 400 million (or more)

Which Mapping Software

- >70 published programs
  - Input data type
  - Reference
  - Speed vs sensitivity
  - Memory



Phylogeny of Pairwise Alignment



Chaisson & Tesler 2012, *BMC Bioinformatics*

Comparison (10 million human reads, 40 bp)

Software	Algorithm	Mismatches	Memory (GB)	Time (min)
BWA	BWT	yes	2.2	73
Bowtie	BWT	yes	7.4	166
BFAST	Spaced seeds	yes	9.7	902
MPScan	Suffix tree	no	2.7	80
PerM	Spaced seeds	yes	13.8	785

Schbath et al. 2012 *J Comput Biol*

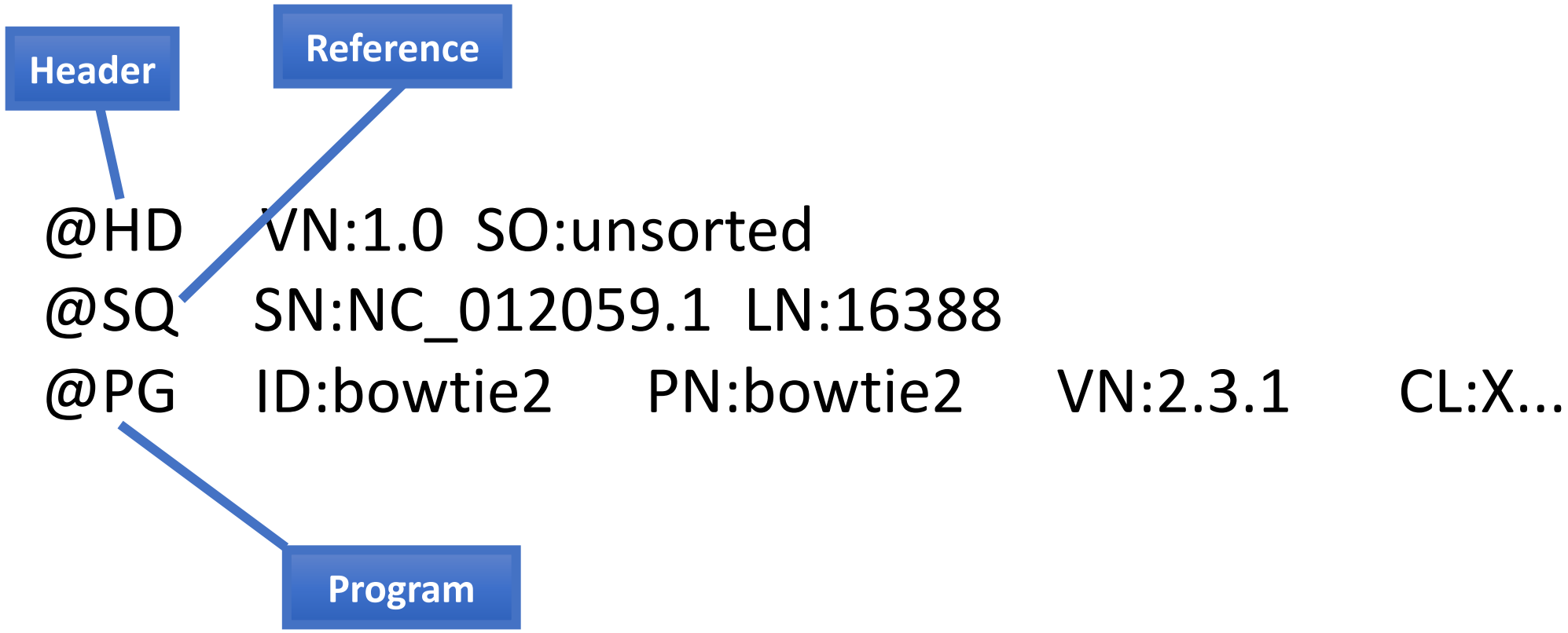
Storing Read Alignments

Sequence Alignment (SAM/BAM) Format

- Universal Standard
- SAM (readable)
- BAM (binary, compressed form)
- Specifications:
  - https://samtools.github.io/hts-specs/SAMv1.pdf

- **Structure**
  - Header: programs, version, reference info, sort order, sample info, etc.
  - Read alignment records
    - One record per line

SAM: Header



X =bowtie2-align-s --wrapper basic-0 -q --phred33 --very-sensitive -t -p 1 -x NC_012059.1 -1 ERR1938563_1.fq -2 ERR1938563_2.fq

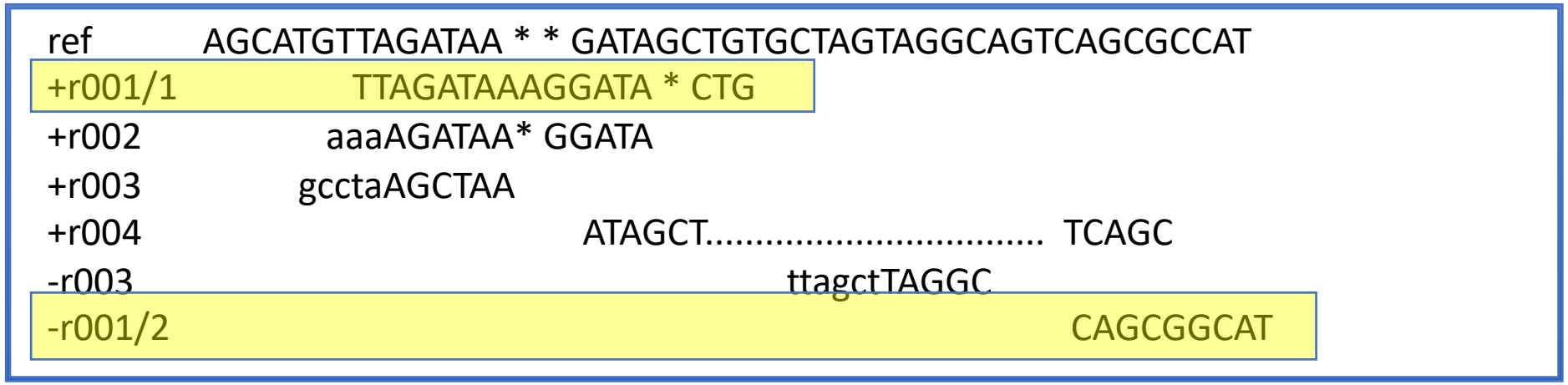
SAM: Alignments

```
ref          AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1            TTAGATAAAGGATA * CTG
+r002            aaaAGATAA* GGATA
+r003          gcctaAGCTAA
+r004                              ATAGCT.....  TCAGC
-r003                                      ttagctTAGGC
-r001/2                                                CAGCGGCAT
```

SAM: Alignments

```
ref          AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1            TTAGATAAAGGATA * CTG
+r002            aaaAGATAA* GGATA
+r003          gcctaAGCTAA
+r004                              ATAGCT.....  TCAGC
-r003                                      ttagctTAGGC
-r001/2                                                CAGCGGCAT
```

SAM: Alignments



SAM: Alignments

```
ref        AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1          TTAGATAAAGGATA * CTG
+r002          aaaAGATAA* GGATA
+r003        gcctaAGCTAA
+r004                    ATAGCT.....  TCAGC
-r003                                ttagctTAGGC
-r001/2                                          CAGCGGCAT
```

SAM: Alignments

```
ref          AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1            TTAGATAAAGGATA * CTG
+r002            aaaAGATAA* GGATA
+r003          gcctaAGCTAA
+r004                              ATAGCT.....  TCAGC
-r003                                      ttagctTAGGC
-r001/2                                                CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

SAM: Alignments

```
ref         AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1               TTAGATAAAGGATA * CTG
+r002               aaaAGATAA* GGATA
+r003             gcctaAGCTAA
+r004                                 ATAGCT.....................  TCAGC
-r003                                             ttagctTAGGC
-r001/2                                                          CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Read name

SAM: Alignments

```
ref        AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1          TTAGATAAAGGATA * CTG
+r002          aaaAGATAA* GGATA
+r003        gcctaAGCTAA
+r004                          ATAGCT.....................  TCAGC
-r003                                    ttagctTAGGC
-r001/2                                                   CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Flag: pair information, orientation, mapped, etc.

SAM: Alignments

```
ref          AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1            TTAGATAAAGGATA * CTG
+r002            aaaAGATAA* GGATA
+r003          gcctaAGCTAA
+r004                              ATAGCT.....  TCAGC
-r003                                    ttagctTAGGC
-r001/2                                                CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Reference sequence name & position

SAM: Alignments

```
ref        AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1          TTAGATAAAGGATA * CTG
+r002          aaaAGATAA* GGATA
+r003        gcctaAGCTAA
+r004                          ATAGCT.....................  TCAGC
-r003                                    ttagctTAGGC
-r001/2                                                    CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Mapping Quality (MQ): -10 * $\log_{10}$(pr[wrongly mapped])

SAM: Alignments

```
ref         AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1               TTAGATAAAGGATA * CTG
+r002               aaaAGATAA* GGATA
+r003             gcctaAGCTAA
+r004                                 ATAGCT.....................  TCAGC
-r003                                             ttagctTAGGC
-r001/2                                                          CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

CIGAR string

CIGAR String: Compact Idiosyncratic Gapped Alignment Report

```
REF   ACGATACATAC
READ  ACGA-ACATAC

CIGAR: 4M1D6M
```

[4 Matches + 1 Deletion + 6 Matches]

```
REF     GACA-AACC
READ atGTCATAACC

CIGAR: 2S4M1I4M
```

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

CIGAR String: Compact Idiosyncratic Gapped Alignment Report

```
REF   ACGATACATAC
READ  ACGA-ACATAC
```

CIGAR: 4M1D6M

[4 Matches + 1 Deletion + 6 Matches]

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READ atGTCATAACC
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[4 Matches + 1 Deletion + 6 Matches]

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READ atGTCATAACC
```

CIGAR: 2S4M1I4M

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

CIGAR String: Compact Idiosyncratic Gapped Alignment Report

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REF   ACGATACATAC
READ  ACGA-ACATAC
```

CIGAR: 4M1D6M

[4 Matches + 1 Deletion + 6 Matches]

```
REF     GACA-AACC
READ atGTCATAACC
```

CIGAR: 2S4M1I4M

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

CIGAR String: Compact Idiosyncratic Gapped Alignment Report

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REF   ACGATACATAC
READ  ACGA-ACATAC
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CIGAR: 4M1D6M

[4 Matches + 1 Deletion + 6 Matches]

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REF     GACA-AACC
READ atGTCATAACC
```

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[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

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REF   ACGATACATAC
READ  ACGA-ACATAC
```

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[4 Matches + 1 Deletion + 6 Matches]

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REF     GACA-AACC
READ atGTCATAACC
```

CIGAR: 2S4M1I4M

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

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READ  ACGA-ACATAC
```

CIGAR: 4M1D6M

[4 Matches + 1 Deletion + 6 Matches]

```
REF     GACA-AACC
READ atGTCATAACC
```

CIGAR: 2S4M1I4M

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

CIGAR String: Compact Idiosyncratic Gapped Alignment Report

```
REF   ACGATACATAC
READ  ACGA-ACATAC
```

CIGAR: 4M1D6M

[4 Matches + 1 Deletion + 6 Matches]

```
REF     GACA-AACC
READ atGTCATAACC
```

CIGAR: 2S4M1I4M

[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]

SAM: Alignments

```
ref          AGCATGTTAGATAA  * *GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1            TTAGATAAAGGATA * CTG
+r002             aaaAGATAA* GGATA
+r003           gcctaAGCTAA
+r004                              ATAGCT.....................  TCAGC
-r003                                          ttagctTAGGC
-r001/2                                                       CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Mate sequence, location, insert size

SAM: Alignments

```
ref         AGCATGTTAGATAA  * *GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1               TTAGATAAAGGATA * CTG
+r002                aaaAGATAA* GGATA
+r003              gcctaAGCTAA
+r004                                ATAGCT.....................  TCAGC
-r003                                        ttagctTAGGC
-r001/2                                                          CAGCGGCAT
```

The corresponding SAM format is:

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
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
```

Read sequence & quality (* = no quality stored)

Now for when you DON’T have a reference…

Mark Stenglein