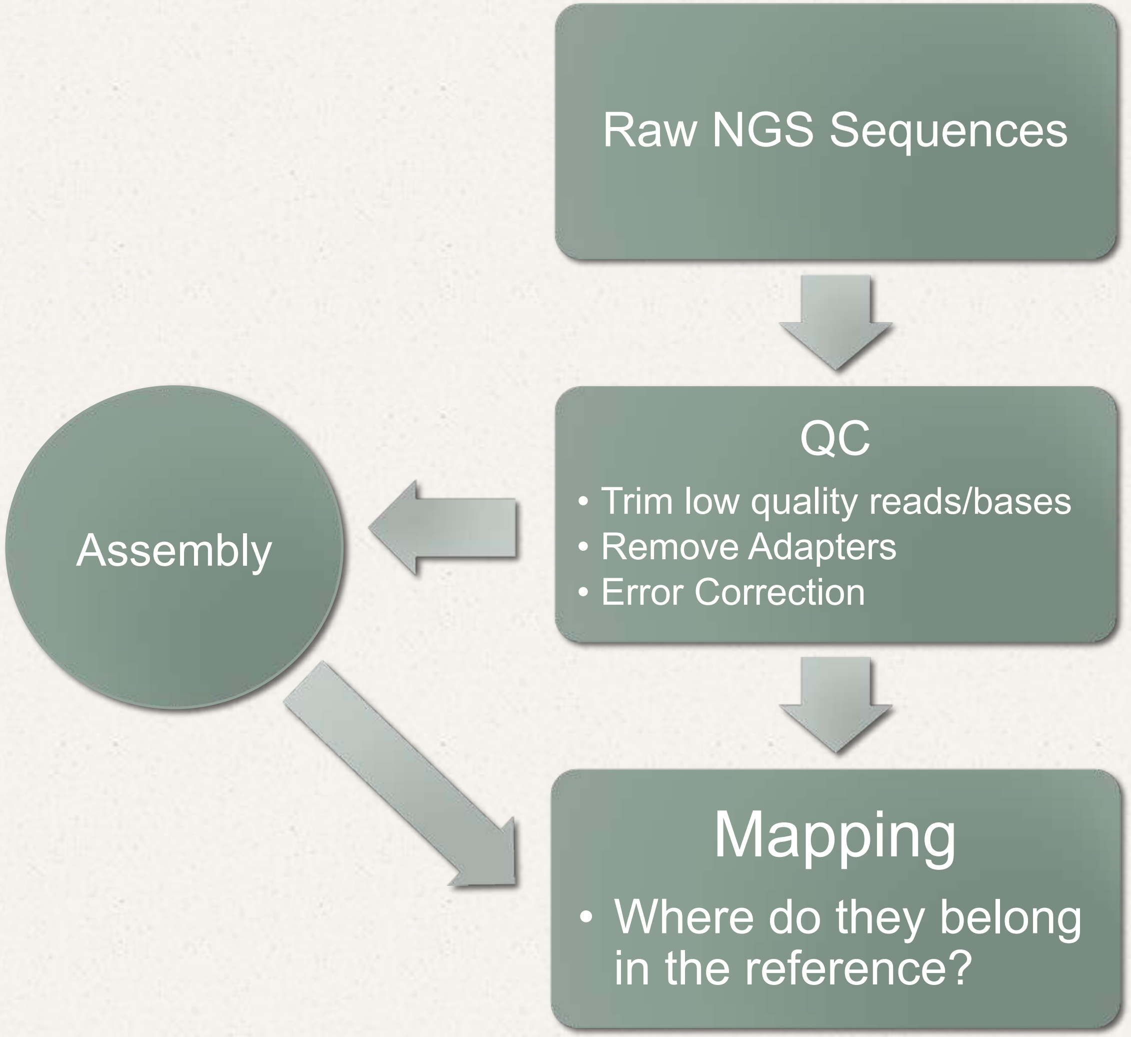
MAPPING

Aligning sequencing reads to a reference

Where are we?



Raw NGS Sequences

QC

- Trim low quality reads/bases
- Remove Adapters
- Error Correction

Assembly

Mapping

- Where do they belong in the reference?

Why do we map reads?

- Identify Variants
  - substitutions (fixed difference)
  - polymorphisms (SNPs)
  - structural
- Quantification (RNA-seq expression levels)
- Remove sequences of specific origins
  - Contamination
  - Parasites
  - organellar DNA)

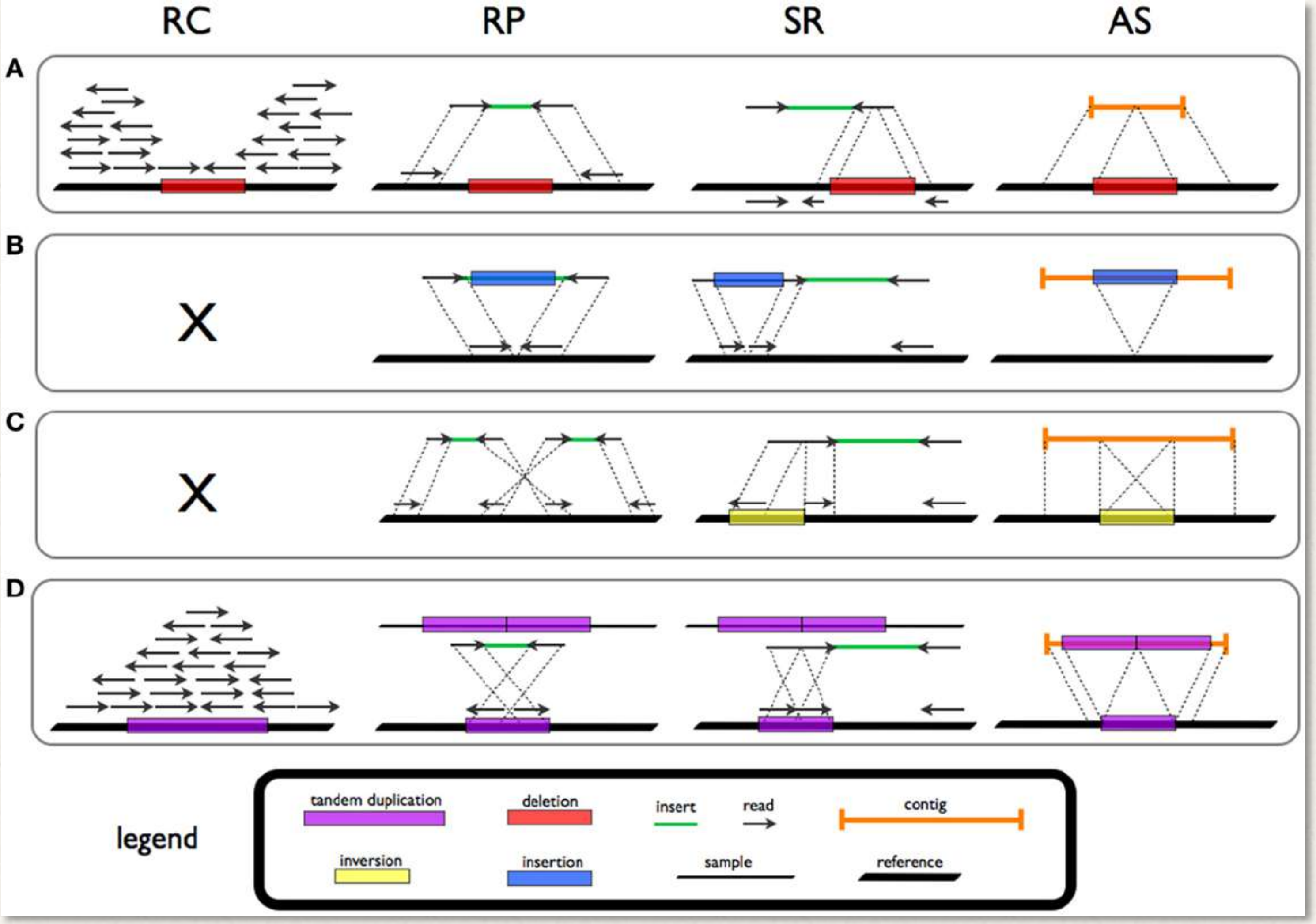
Structural Variants

Deletion

Insertion

Inversion

Duplication



Tattini et al. (2015) Front. Bioeng. Biotechnol

DIY time!

Map the Reads!

- Reference in gray:
- “*It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief…*”
- Reads are in blue, differences are shown in red. Spaces count!
- http://lyorn.idyll.org/~t/assembly-exercise/index.cgi

Things to Consider:

- Coverage?
- Error rate?
- How many variants (SNPs) can you find?
- Extra Credit: Book title and Author?
  - No Googling!

DIY time!

Map the Reads!

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- http://lyorn.idyll.org/~t/assembly-exercise/index.cgi

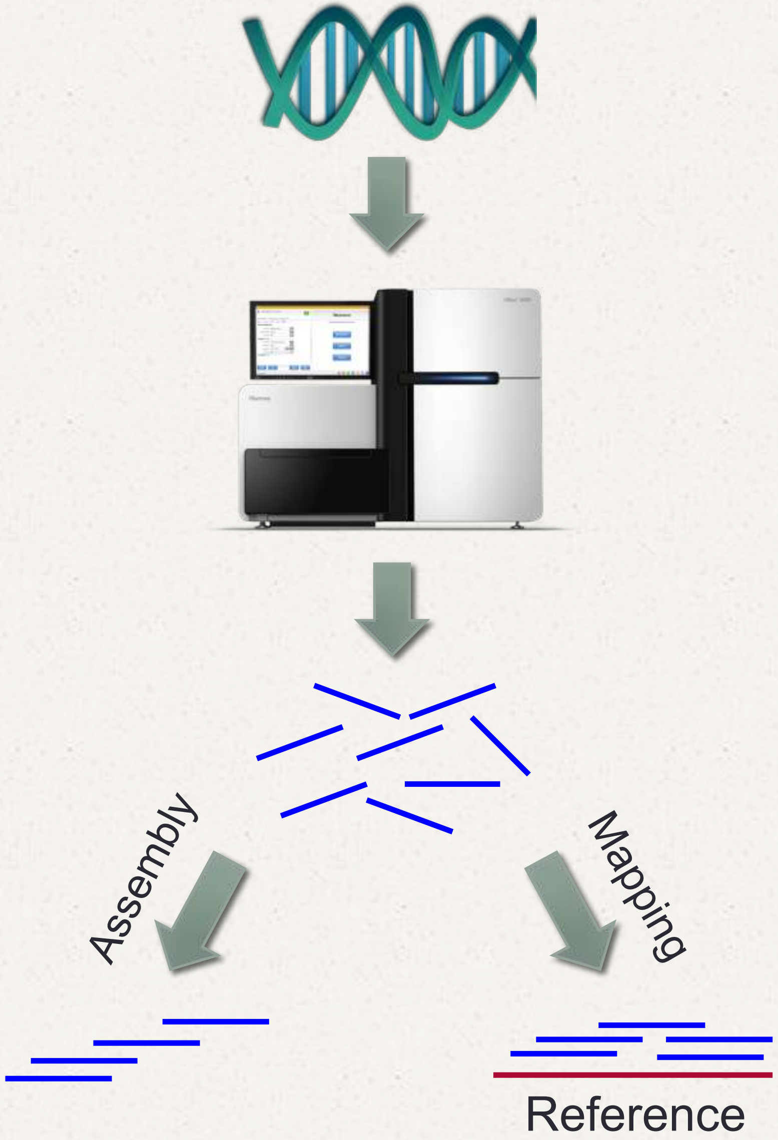
Things to Consider:

- Coverage?
  - 7X
- Error rate?
  - 10%
- How many variants (SNPs) can you find?
  - 2? 3? – tim[i/e]s wa[s/k] ep[o/r]ch
- Extra Credit: Book title and Author?
  - No Googling!

Just a pairwise alignment, right?

Yes.
x 400 million (or more)

Mapping



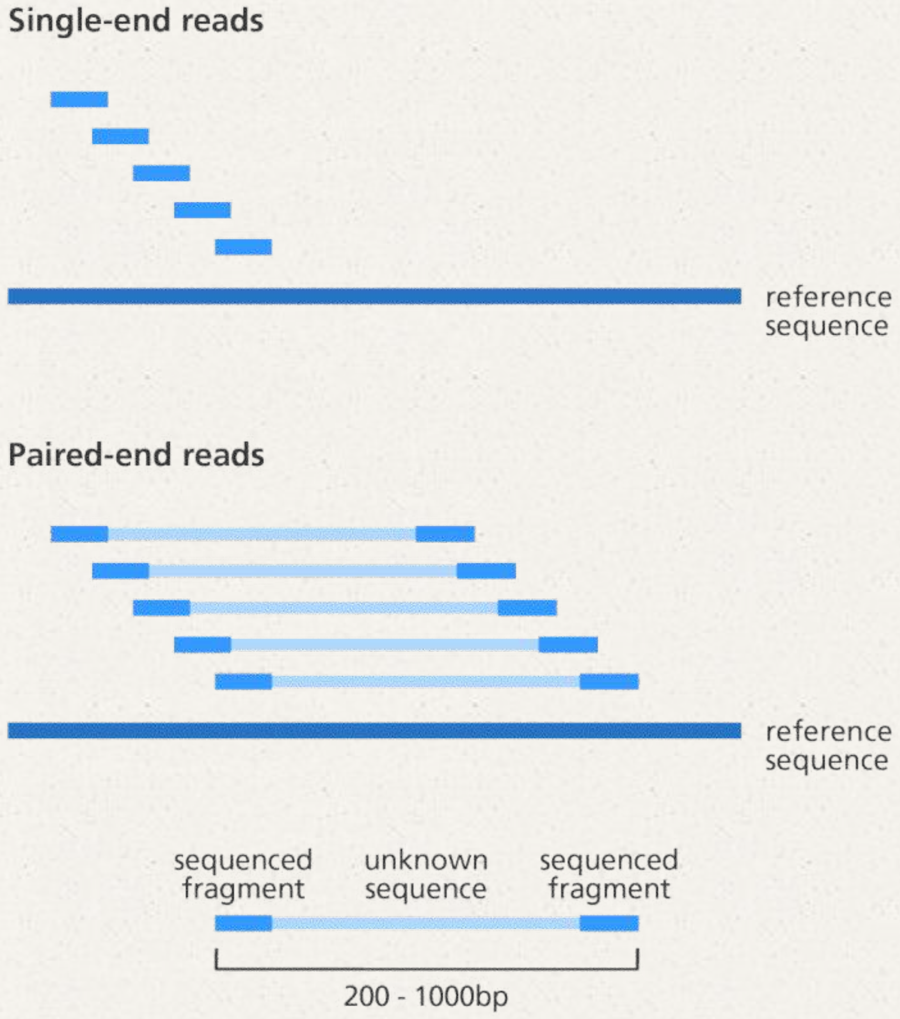
Challenges

- Large numbers
- Short length
- Sequencing errors
- Repeats
- Indels
- Variants

What is mapping?

Which Software?

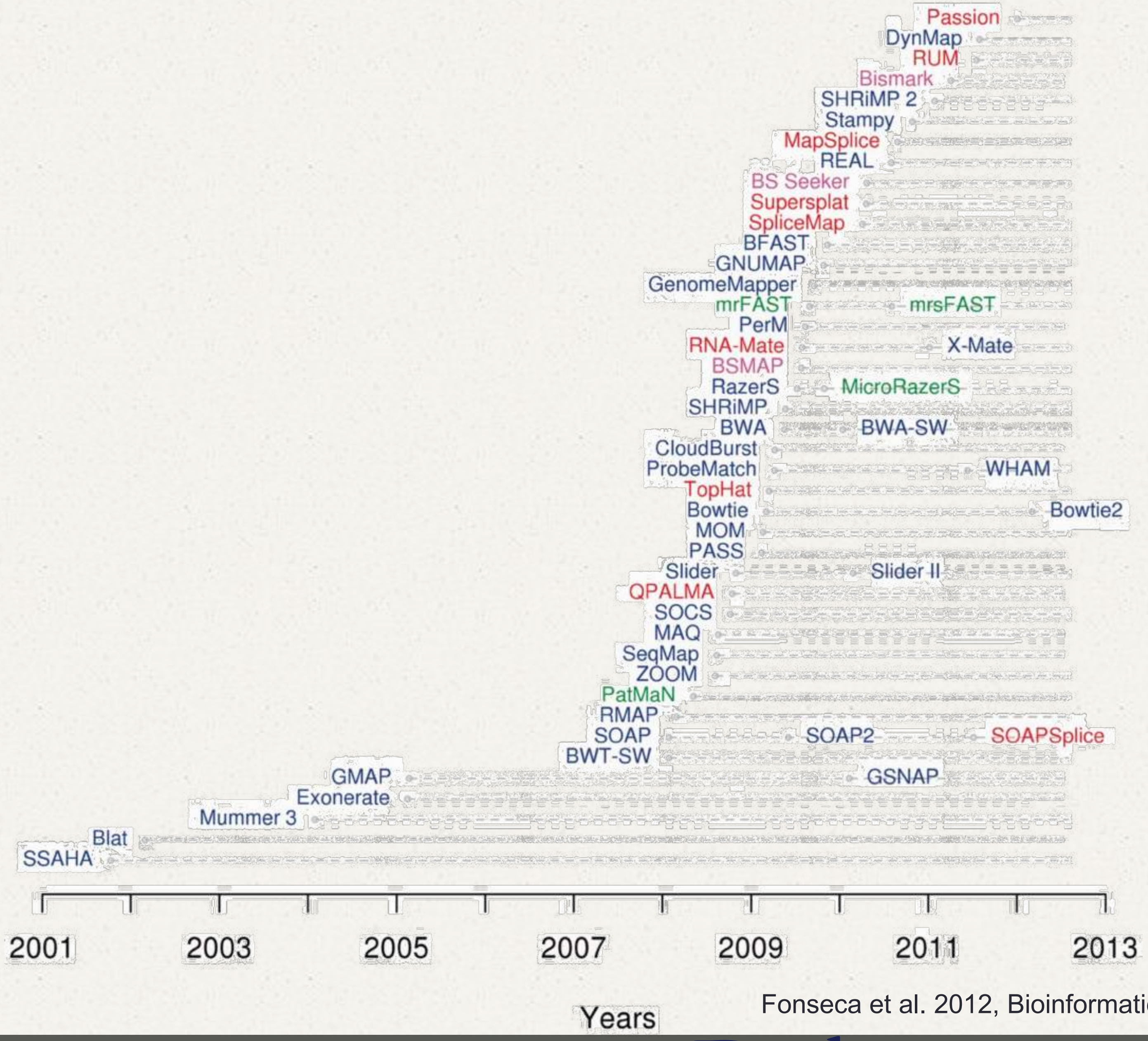
- >70 published programs
- Input data type
- Reference
- Speed vs sensitivity
- Memory



www.yourgenome.org

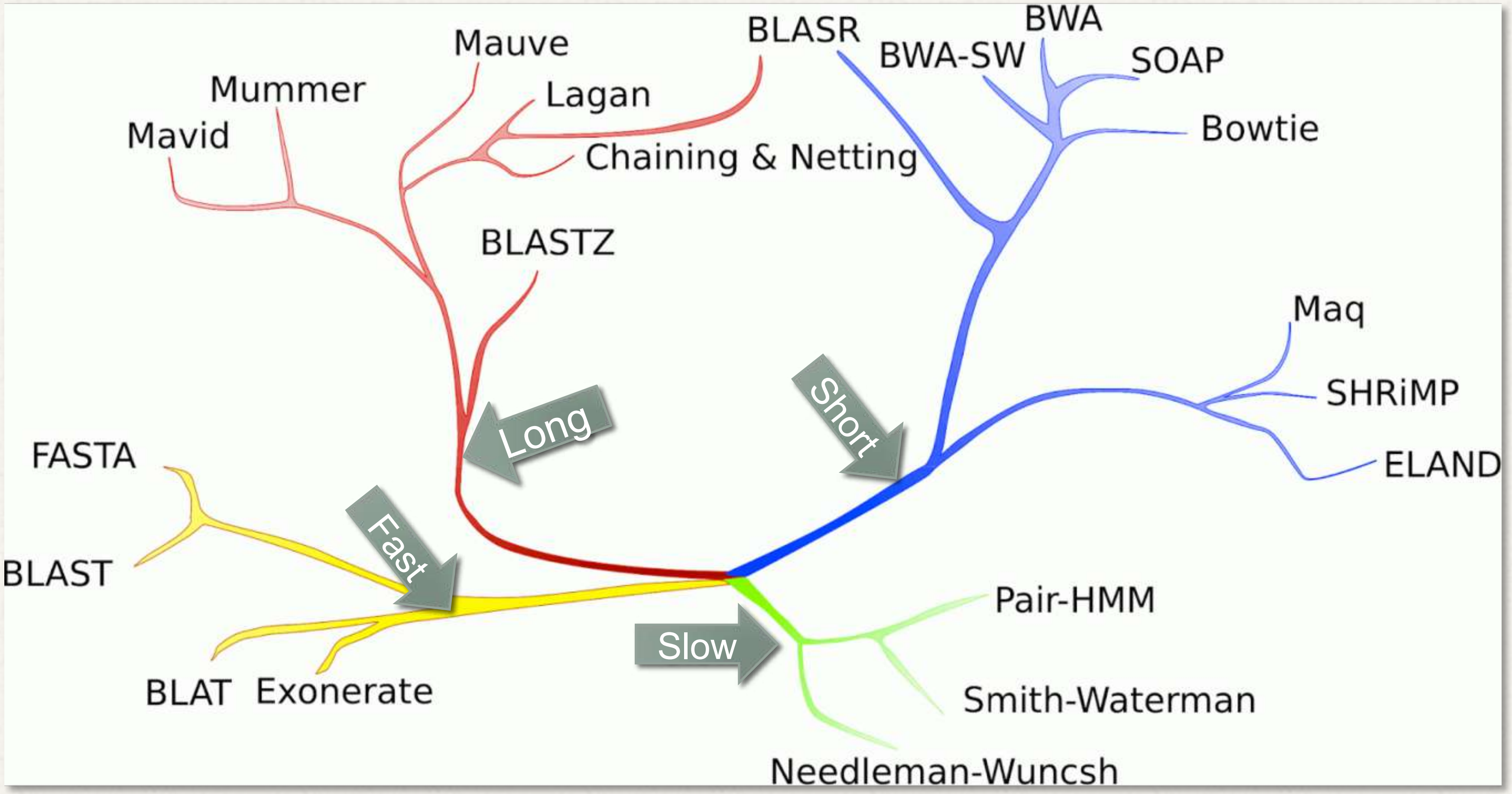
What is mapping?

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



Fonseca et al. 2012, Bioinformatics

The phylogeny of pairwise alignment



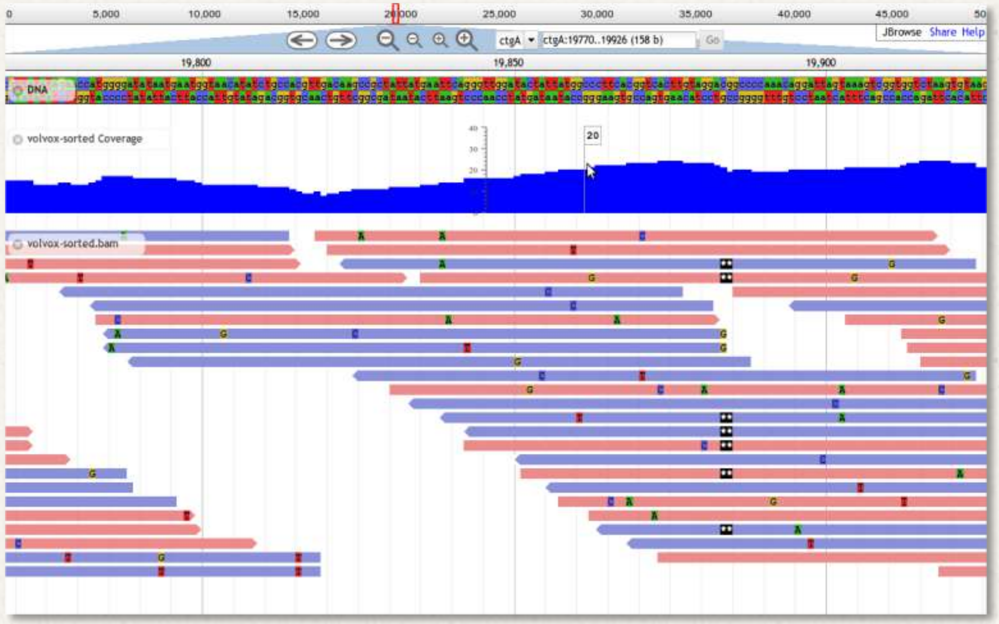
Chaisson & Tesler 2012, *BMC Bioinformatics*

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

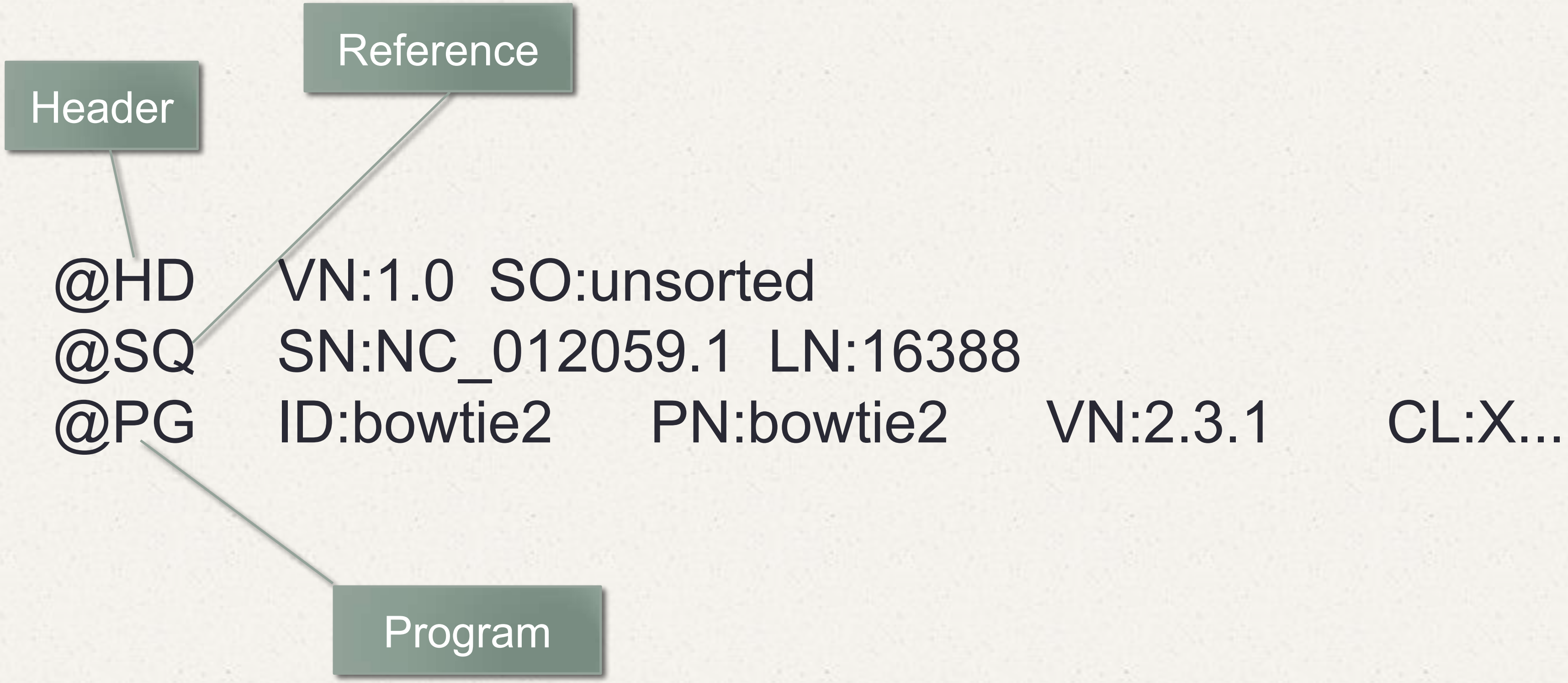


Sequence Alignment (SAM/BAM) Format

- 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



Reference

Header

```
@HD    VN:1.0  SO:unsorted
@SQ    SN:NC_012059.1  LN:16388
@PG    ID:bowtie2    PN:bowtie2    VN:2.3.1    CL:X...
```

Program

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: Alignment Records

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

SAM: Alignment Records

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

SAM: Alignment Records

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

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

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@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: Alignment Records

```
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: Alignment Records

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

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@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

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```
ref    AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1      TTAGATAAAGGATA * CTG
+r002       aaaAGATAA* GGATA
+r003      gcctaAGCTAA
+r004                   ATAGCT.................................  TCAGC
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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;
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Mapping Quality (MQ): -10 * $\log_{10}$(pr[wrongly mapped])

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ref         AGCATGTTAGATAA * * GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1               TTAGATAAAGGATA * CTG
+r002                aaaAGATAA* GGATA
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r002      0  ref   9  30  3S6M1P1I4M  *   0   0  AAAAGATAAGGATA   *
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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
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[2 Skipped + 4 Matches + 1 Insertion + 4 Matches]
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+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

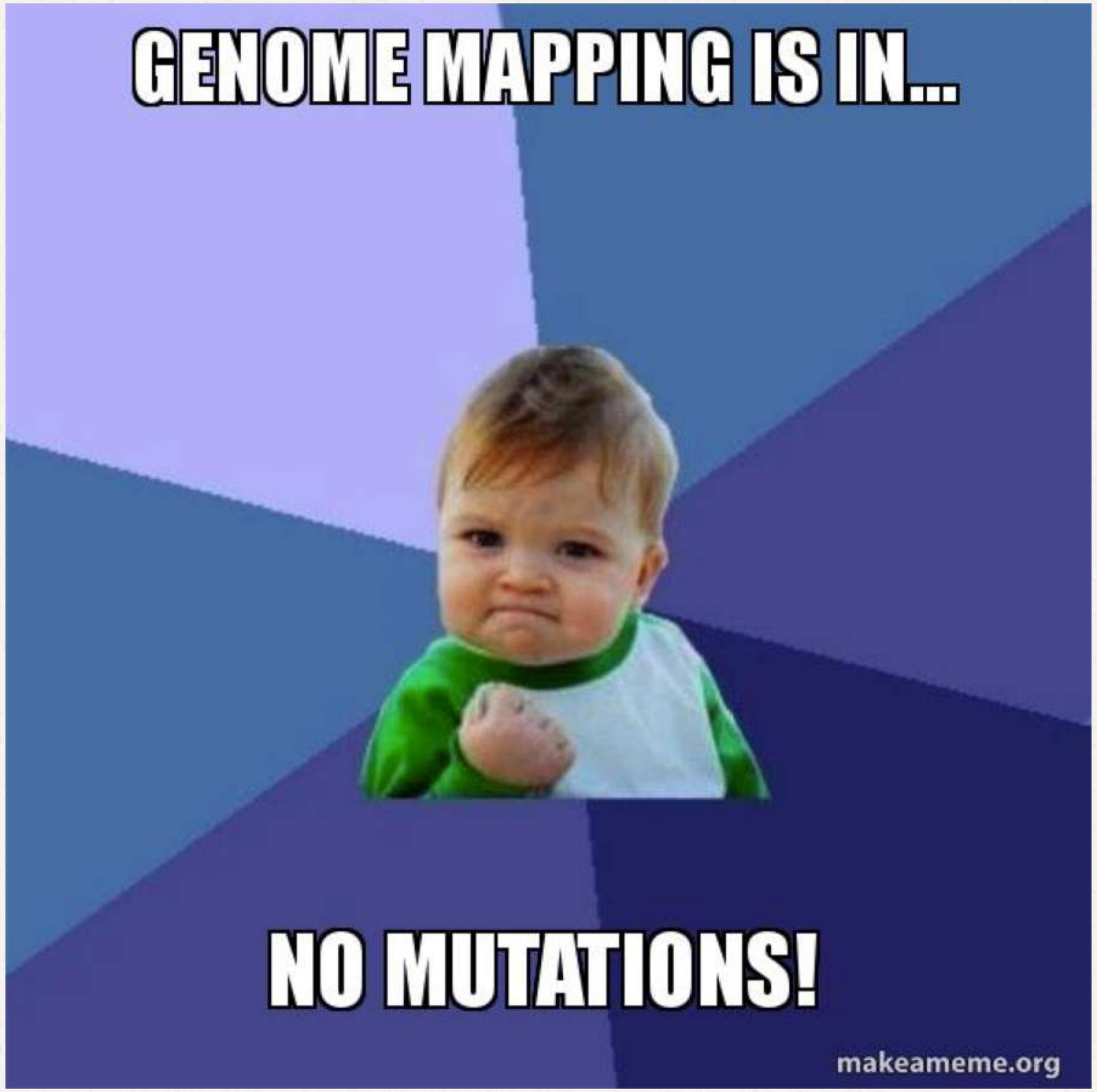
SAM: Alignment Records

```
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)


GENOME MAPPING IS IN...
NO MUTATIONS!
makeameme.org

NOW WHEN YOU DON'T HAVE A REFERENCE…

Mark Stenglein