# 3000788 Intro to Comp Molec Biol

## Lecture 5: Processing of DNA sequencing data

August 31, 2023


**Sira Sriswasdi, PhD**

- Research Affairs
- Center of Excellence in Computational Molecular Biology (CMB)
- Center for Artificial Intelligence in Medicine (CU-AIM)

# Quality control

# FASTQ format

```
@ERR000589.41 EAS139_45:5:1:2:111/1
CTTTCCTCCCTGCTTTCCTGGCCCCACCATTTCCAGGGAACATCTTGTCAT
+
3IIIIIIIIIIIII>1IIIFF9BG08E00I%IG+&?(4)%00646.C1#&(
@ERR000589.42 EAS139_45:5:1:2:1293/1
AGTTGTTAAAATCCAAGCCAATTAAGATAGTCTTATCTTTTTAAAAGAAAT
+
IIIIIGII.AIIII=?I9G-/II=+I=4?761BA2C9I+5A711+&>1$/I
```

- Header: Location of cluster on Illumina's flow cell
- Sequence
- Quality score

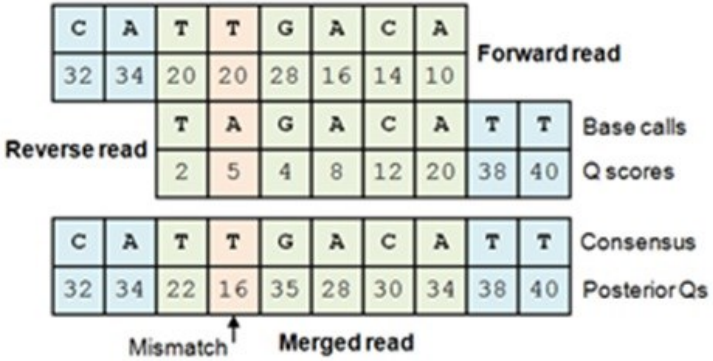
# FASTQ for paired-end sequencing

Same order

```
1  @ERR000589.41 EAS139_45:5:1:2:111/1
2  CTTTCCTCCCTGCTTTCCTGGCCCCACCATTTCCAGGGAACATCTTGTCAT
3  +
4  3IIIIIIIIIIIII>1IIIFF9BG08E00I%IG+&?(4)%00646.C1#&(
5  @ERR000589.42 EAS139_45:5:1:2:1293/1
6  AGTTGTTAAAATCCAAGCCAATTAAGATAGTCTTATCTTTTTAAAAGAAAT
7  +
8  IIIIIGII.AIIII=?I9G-/II=+I=4?761BA2C9I+5A711+&>1$/I
```

```
1  @ERR000589.41 EAS139_45:5:1:2:111/1
2  CTTTCCTCCCTGCTTTCCTGGCCCCACCATTTCCAGGGAACATCTTGTCAT
3  +
4  3IIIIIIIIIIIII>1IIIFF9BG08E00I%IG+&?(4)%00646.C1#&(
5  @ERR000589.42 EAS139_45:5:1:2:1293/1
6  AGTTGTTAAAATCCAAGCCAATTAAGATAGTCTTATCTTTTTAAAAGAAAT
7  +
8  IIIIIGII.AIIII=?I9G-/II=+I=4?761BA2C9I+5A711+&>1$/I
```

- Two FASTQ files
  - SAMPLE1_R1.fastq / SAMPLE1_R2.fastq
- Merged into a single FASTQ files
  - Forward & reverse reads must overlap
  - 300bp paired-end 16S rRNA sequencing




https://drive5.com/usearch/manual8.1/merge_pair.html

# Phred score

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

| Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.00000 | 33 ! | 11 | 0.07943 | 44 , | 22 | 0.00631 | 55 7 | 33 | 0.00050 | 66 B |
| 1 | 0.79433 | 34 " | 12 | 0.06310 | 45 - | 23 | 0.00501 | 56 8 | 34 | 0.00040 | 67 C |
| 2 | 0.63096 | 35 # | 13 | 0.05012 | 46 . | 24 | 0.00398 | 57 9 | 35 | 0.00032 | 68 D |
| 3 | 0.50119 | 36 $ | 14 | 0.03981 | 47 / | 25 | 0.00316 | 58 : | 36 | 0.00025 | 69 E |
| 4 | 0.39811 | 37 % | 15 | 0.03162 | 48 0 | 26 | 0.00251 | 59 ; | 37 | 0.00020 | 70 F |
| 5 | 0.31623 | 38 & | 16 | 0.02512 | 49 1 | 27 | 0.00200 | 60 < | 38 | 0.00016 | 71 G |
| 6 | 0.25119 | 39 ' | 17 | 0.01995 | 50 2 | 28 | 0.00158 | 61 = | 39 | 0.00013 | 72 H |
| 7 | 0.19953 | 40 ( | 18 | 0.01585 | 51 3 | 29 | 0.00126 | 62 > | 40 | 0.00010 | 73 I |
| 8 | 0.15849 | 41 ) | 19 | 0.01259 | 52 4 | 30 | 0.00100 | 63 ? | 41 | 0.00008 | 74 J |
| 9 | 0.12589 | 42 * | 20 | 0.01000 | 53 5 | 31 | 0.00079 | 64 @ | 42 | 0.00006 | 75 K |
| 10 | 0.10000 | 43 + | 21 | 0.00794 | 54 6 | 32 | 0.00063 | 65 A | | | |

https://www.drive5.com/usearch/manual/quality_score.html

- Q score = – 10 x $\text{Log}_{10}$ (base call error rate)
- Base call error of 10% → Q score = ?
- Base call error of 0.0001 → Q score = ?

# Expected error at the ends of read

```
@ERR000589.41 EAS139_45:5:1:2:111/1
CTTTCCTCCCTGCTTTCCTGGCCCCACCATTTCCAGGGAACATCTTGTCAT
+
3IIIIIIIIIIIII>1IIIFF9BG08E00I%IG+&?(4)%00646.C1#&(
```

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

| Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.00000 | 33 ! | 11 | 0.07943 | 44 , | 22 | 0.00631 | 55 7 | 33 | 0.00050 | 66 B |
| 1 | 0.79433 | 34 " | 12 | 0.06310 | 45 - | 23 | 0.00501 | 56 8 | 34 | 0.00040 | 67 C |
| 2 | 0.63096 | 35 # | 13 | 0.05012 | 46 . | 24 | 0.00398 | 57 9 | 35 | 0.00032 | 68 D |
| 3 | 0.50119 | 36 $ | 14 | 0.03981 | 47 / | 25 | 0.00316 | 58 : | 36 | 0.00025 | 69 E |
| 4 | 0.39811 | 37 % | 15 | 0.03162 | 48 0 | 26 | 0.00251 | 59 ; | 37 | 0.00020 | 70 F |
| 5 | 0.31623 | 38 & | 16 | 0.02512 | 49 1 | 27 | 0.00200 | 60 < | 38 | 0.00016 | 71 G |
| 6 | 0.25119 | 39 ' | 17 | 0.01995 | 50 2 | 28 | 0.00158 | 61 = | 39 | 0.00013 | 72 H |
| 7 | 0.19953 | 40 ( | 18 | 0.01585 | 51 3 | 29 | 0.00126 | 62 > | 40 | 0.00010 | 73 I |
| 8 | 0.15849 | 41 ) | 19 | 0.01259 | 52 4 | 30 | 0.00100 | 63 ? | 41 | 0.00008 | 74 J |
| 9 | 0.12589 | 42 * | 20 | 0.01000 | 53 5 | 31 | 0.00079 | 64 @ | 42 | 0.00006 | 75 K |
| 10 | 0.10000 | 43 + | 21 | 0.00794 | 54 6 | 32 | 0.00063 | 65 A | | | |

# FastQC tool

## Basic Statistics

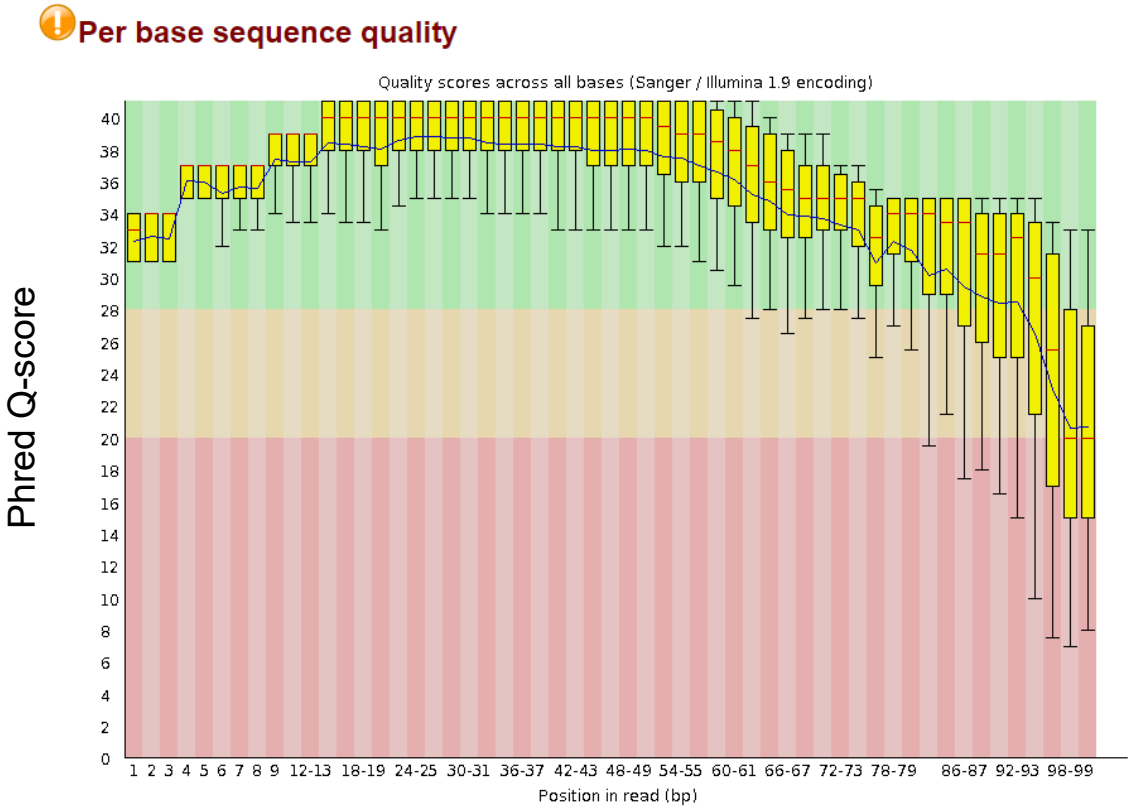
| Measure | Value |
|---|---|
| Filename | small_rna.fastq.gz |
| File type | Conventional base calls |
| Encoding | Sanger / Illumina 1.9 |
| Total Sequences | 250000 |
| Sequences flagged as poor quality | 0 |
| Sequence length | 100 |
| %GC | 45 |

FastQC Report

## Summary

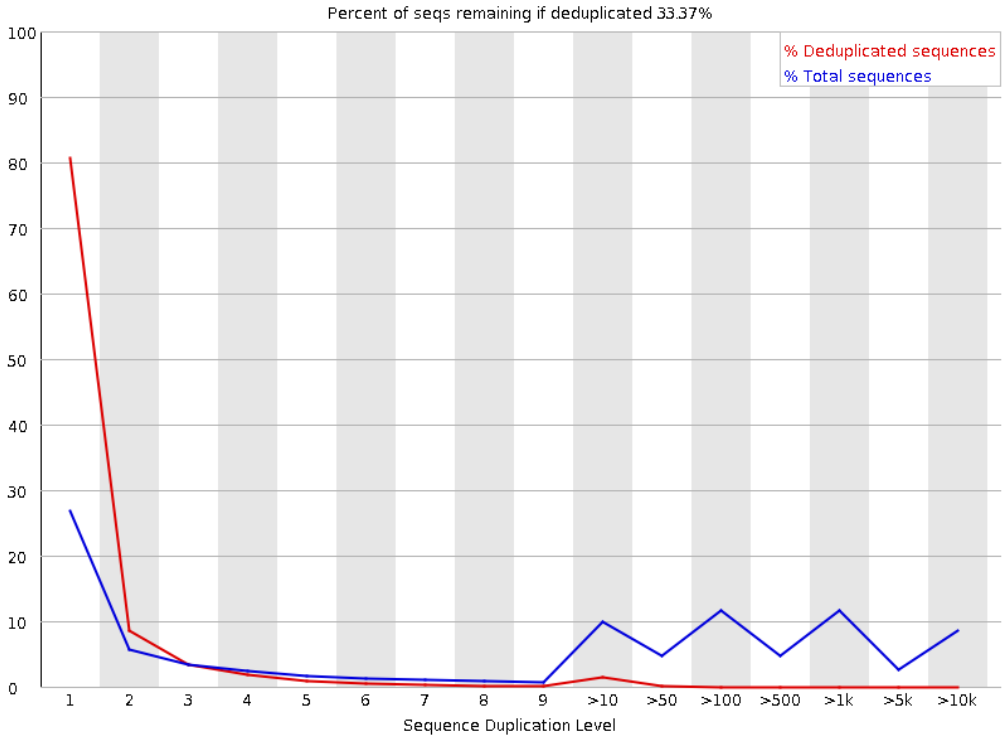
- Basic Statistics
- Per base sequence quality
- Per tile sequence quality
- Per sequence quality scores
- Per base sequence content
- Per sequence GC content
- Per base N content
- Sequence Length Distribution
- Sequence Duplication Levels
- Overrepresented sequences
- Adapter Content

# Base calling quality



# Duplicated reads

## Sequence Duplication Levels



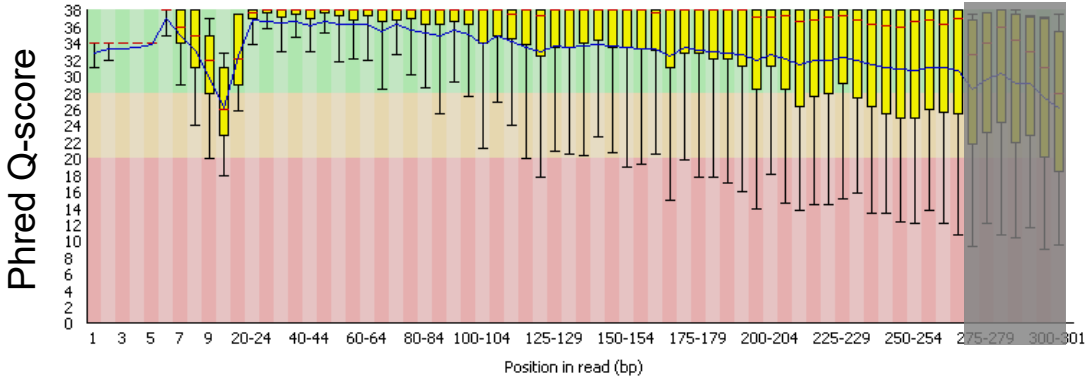
# Possible adapter read-through

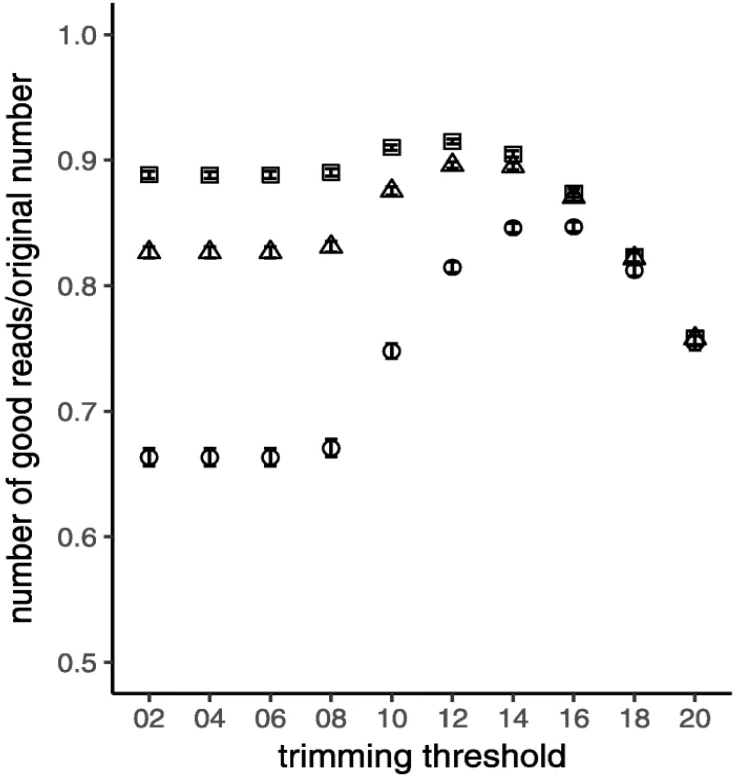
## Overrepresented sequences

| Sequence | Count | Percentage | Possible Source |
|---|---|---|---|
| TGAGGTAGTAGATTGTATAGTTAGATCGGAAGAGCACACGTCTGAACTCC | 10865 | 4.346 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| TAGCTTATCAGACTGATGTTGACAGATCGGAAGAGCACACGTCTGAACTC | 10845 | 4.338 | Illumina Multiplexing PCR Primer 2.01 (100% over 27bp) |
| TCTTTGGTTATCTAGCTGTATGAGATCGGAAGAGCACACGTCTGAACTCC | 7062 | 2.8247999999999998 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| TCTTTGGTTATCTAGCTGTATGAAGATCGGAAGAGCACACGTCTGAACTC | 4056 | 1.6223999999999998 | Illumina Multiplexing PCR Primer 2.01 (100% over 27bp) |
| TGAGGTAGTAGTTTGTGCTGTTAGATCGGAAGAGCACACGTCTGAACTCC | 3737 | 1.4948 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| TGAGGTAGTAGTTTGTACAGTTAGATCGGAAGAGCACACGTCTGAACTCC | 3549 | 1.4196 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| TGAGGTAGTAGGTTGTATGGTTAGATCGGAAGAGCACACGTCTGAACTCC | 2931 | 1.1724 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| AACCCGTAGATCCGATCTTGTAGATCGGAAGAGCACACGTCTGAACTCCA | 1910 | 0.764 | Illumina Multiplexing PCR Primer 2.01 (100% over 29bp) |
| CGCGACCTCAGATCAGACGTAGATCGGAAGAGCACACGTCTGAACTCCAG | 1749 | 0.6996 | Illumina Multiplexing PCR Primer 2.01 (100% over 30bp) |
| TGAGGTAGTAGGTTGTATAGTTAGATCGGAAGAGCACACGTCTGAACTCC | 1647 | 0.6588 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| TCTTTGGTTATCTAGCTGTATAGATCGGAAGAGCACACGTCTGAACTCCA | 1622 | 0.6487999999999999 | Illumina Multiplexing PCR Primer 2.01 (100% over 29bp) |
| TAGCTTATCAGACTGATGTTGATAGATCGGAAGAGCACACGTCTGAACTC | 1328 | 0.5312 | Illumina Multiplexing PCR Primer 2.01 (100% over 27bp) |
| TTCAAGTAATCCAGGATAGGCTAGATCGGAAGAGCACACGTCTGAACTCC | 1248 | 0.4992 | Illumina Multiplexing PCR Primer 2.01 (100% over 28bp) |
| AGCAGCATTGTACAGGGCTATGAAGATCGGAAGAGCACACGTCTGAACTC | 1248 | 0.4992 | Illumina Multiplexing PCR Primer 2.01 (100% over 27bp) |

# Trimming

# Quality trimming

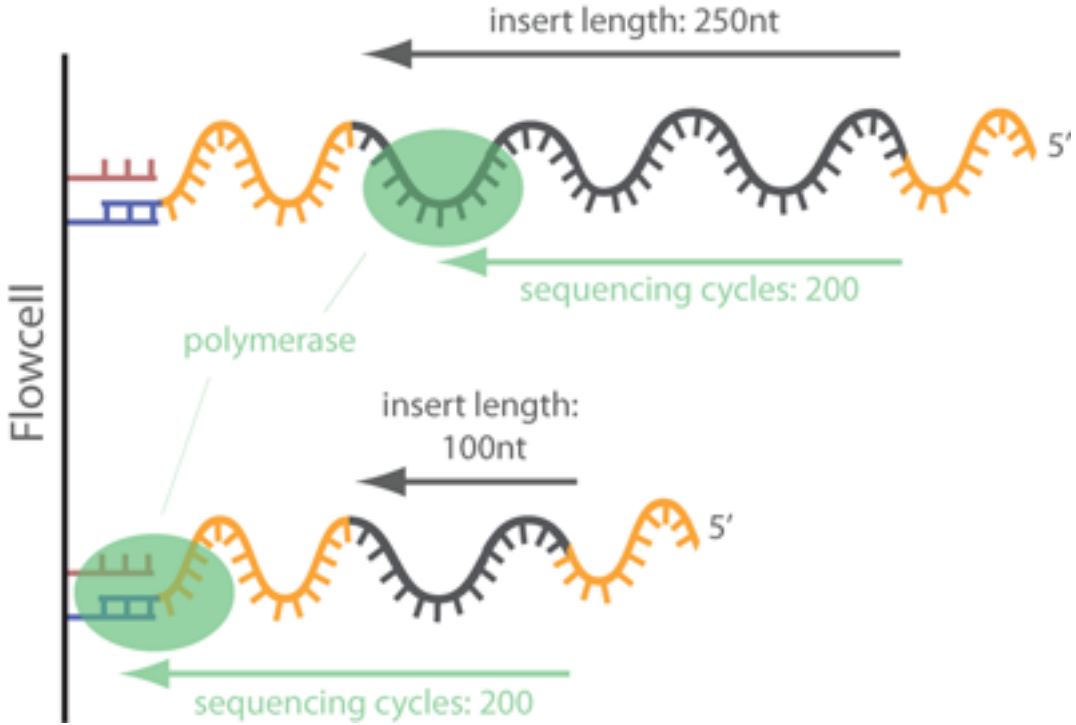



- Remove bases from the end until a minimum quality is reached
- May lose reads but lead to better results in downstream analysis

Mohsen, A. et al. BMC Bioinformatics 20:581 (2019)

# Adapter trimming




main / Trimmomatic / adapters /

TonyBolger Parallel Compression

..

- NexteraPE-PE.fa
- TruSeq2-PE.fa
- TruSeq2-SE.fa
- TruSeq3-PE-2.fa
- TruSeq3-PE.fa
- TruSeq3-SE.fa

https://www.ecseq.com/support/ngs/trimming-adapter-sequences-is-it-necessary

# Trimmomatic code

```
trimmomatic PE -threads 4 SRR_1056_1.fastq SRR_1056_2.fastq  \
            SRR_1056_1.trimmed.fastq SRR_1056_1un.trimmed.fastq \
            SRR_1056_2.trimmed.fastq SRR_1056_2un.trimmed.fastq \
            ILLUMINACLIP:SRR_adapters.fa SLIDINGWINDOW:4:20
```

header
sequence
header
sequence

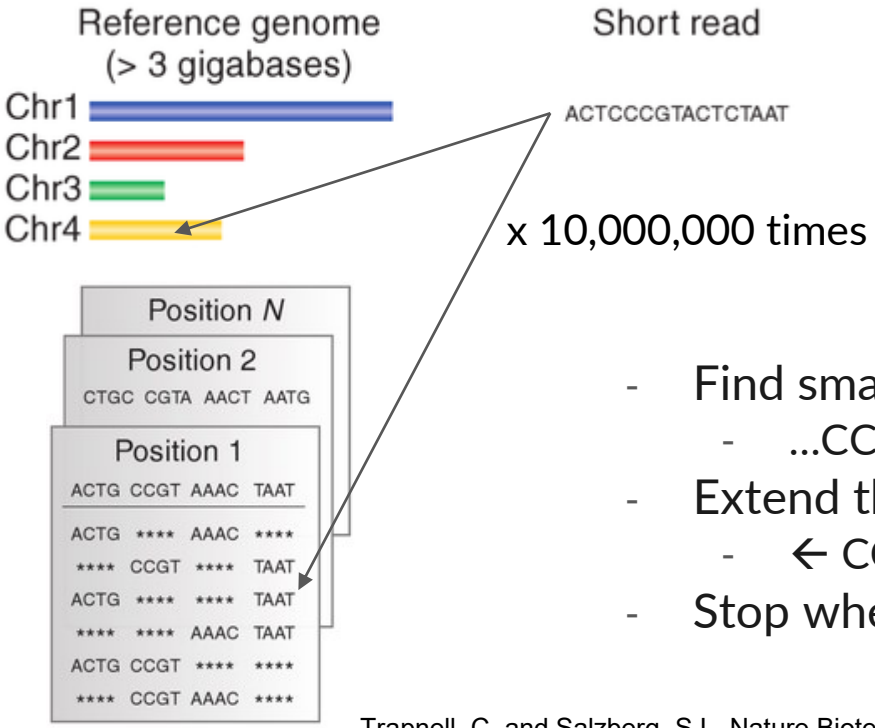
```
>PrefixNX/1
AGATGTGTATAAGAGACAG
>PrefixNX/2
AGATGTGTATAAGAGACAG
>Trans1
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
>Trans1_rc
CTGTCTCTTATACACATCTGACGCTGCCGACGA
>Trans2
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
>Trans2_rc
CTGTCTCTTATACACATCTCCGAGCCCACGAGAC
```

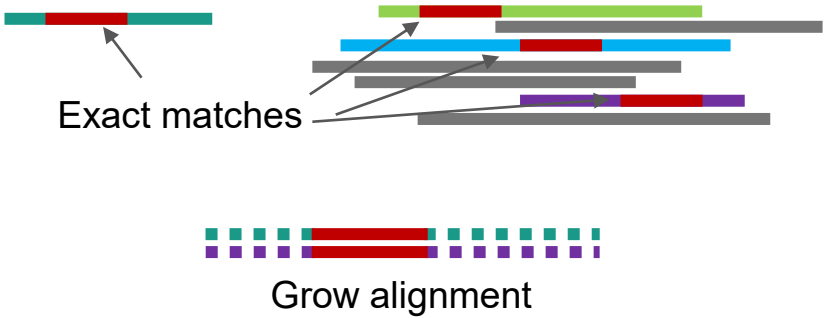
FASTA format

- Using 4 CPU threads
- Process 3 sets of paired end FASTQ
- Remove adapters listed in SRR_adapters.fa
- Check quality in a sliding window

# Alignment

# Sequence alignment is a form of search




- Find small segments with exact matches
  - ...CCCGTA...
- Extend the alignment to both sides
  - ← CCCGTA →
- Stop when the number of mismatches is too high

Trapnell, C. and Salzberg, S.L. Nature Biotechnology 27:455-7 (2009)

# Searching with suffix array

Reference Sequence

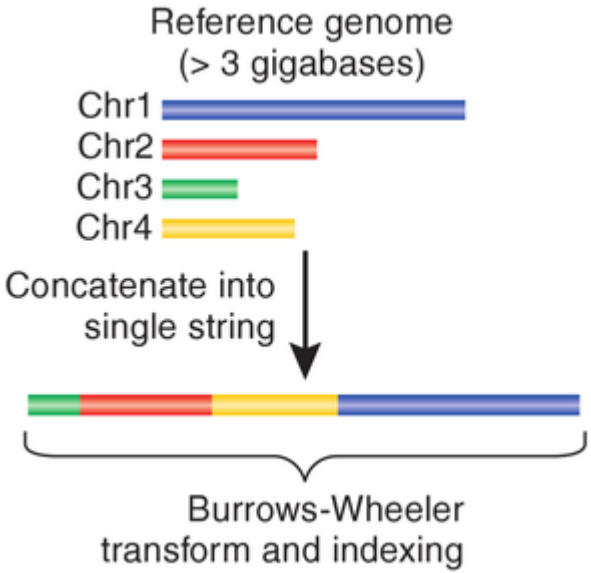
ATTGCAGTCCG

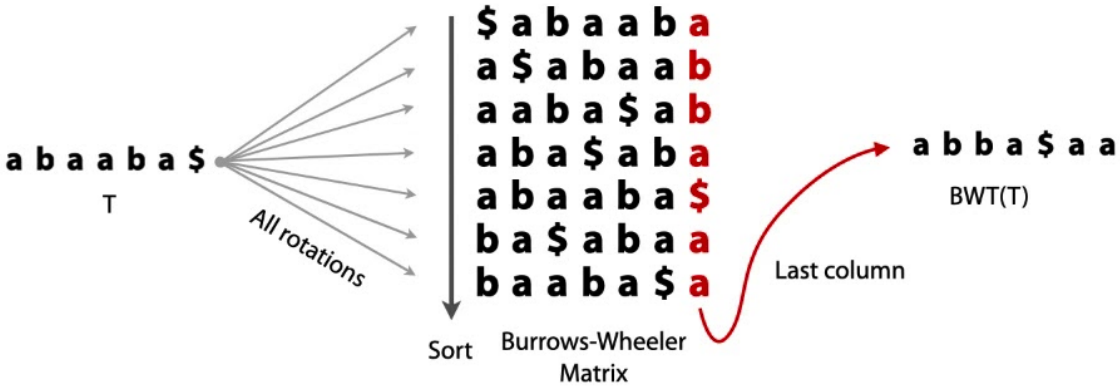
| | |
|---|---|
| AGTCCG | 6 |
| ATTGCAGTCCG | 1 |
| CAGTCCG | 5 |
| CCG | 9 |
| CG | 10 |
| G | 11 |
| GCAGTCCG | 4 |
| GTCCG | 7 |
| TCCG | 8 |
| TGCAGTCCG | 3 |
| TTGCAGTCCG | 2 |

- Suffix = ending part of a string
- Organize suffixes in an easily searchable data structure
- Also record the start positions

Christley, S. et al. BMC Bioinformatics 9:15 (2008)

# Burrows-Wheeler transform



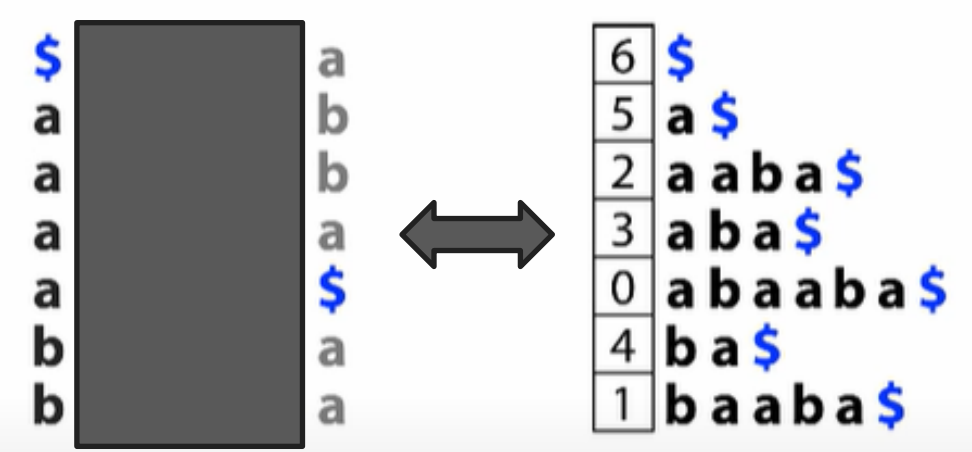




Burrows, M. and Wheeler, D.J. A block sorting lossless data compression algorithm. 1994

- BWT is easy to describe: **a1b2a1$a2**
- BWT contains the same information as the original string

Trapnell, C. and Salzberg, S.L. Nature Biotechnology 27:455-7 (2009)

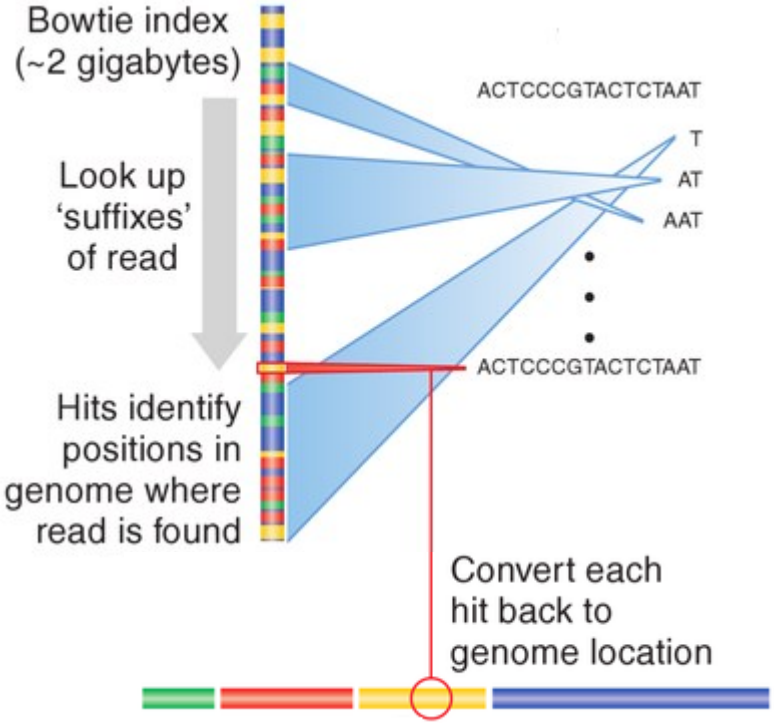
# FM indexing




FM index by Ben Langmead

- Making BWT searchable like suffix array
- There are alternative indexing & searching algorithms
- Still being improved nowadays!

# Genome-scale indexing and searching




- BWT + algorithm provides information on all short segments of the genome
- 20x smaller memory than straightforward indexing for human genome
- 30x faster search speed

Trapnell, C. and Salzberg, S.L. Nature Biotechnology 27:455-7 (2009)

# Dynamic programming for sequence alignment

Dynamic programming matrix:

j → (sequence **y**)

| i (sequence **x**) | | 0 | 1 **T** | 2 **G** | 3 **C** | 4 **T** | 5 **C** | 6 **G** | 7 **T** | 8 = N **A** |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | 0 | −6 | −12 | −18 | −24 | −30 | −36 | −42 | −48 |
| 1 | **T** | −6 | 5 | −1 | −7 | −13 | −19 | −25 | −31 | −37 |
| 2 | **T** | −12 | −1 | 3 | −3 | −2 | −8 | −14 | −20 | −26 |
| 3 | **C** | −18 | −7 | −3 | 8 | 2 | 3 | −3 | −9 | −15 |
| 4 | **A** | −24 | −13 | −9 | 2 | 6 | 0 | 1 | −5 | −4 |
| 5 | **T** | −30 | −19 | −15 | −4 | 7 | 4 | −2 | 6 | 0 |
| M = 6 | **A** | −36 | −25 | −21 | −10 | 1 | 5 | 2 | 0 | 11 |

Optimum alignment scores 11:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **T** | **–** | **–** | **T** | **C** | **A** | **T** | **A** |
| **T** | **G** | **C** | **T** | **C** | **G** | **T** | **A** |
| +5 | −6 | −6 | +5 | +5 | −2 | +5 | +5 |

- The best alignments for long sequences depend on the best alignments of shorter sequences
- The best alignment for TTCATA vs TGCTCGTA is either
  - T/T + best alignment for TCATA vs GCTCGTA
  - T/– + best alignment for TCATA vs TGCTCGTA
  - –/T + best alignment for TTCATA vs GCTCGTA

Eddy, S.R. Nature Biotechnology 22:909-10 (2004)

# Bowtie2 command

Usage:

```
bowtie-build [options]* <reference_in> <ebwt_base>
```

Usage:

```
bowtie [options]* -x <ebwt> {-1 <m1> -2 <m2> | --12 <r> | --interleaved <i> | <s>} [<hit>]
```

- Use bowtie-build to index a genome database (FASTA file)
  - `bowtie-build GRCh38_v1.fasta GRCh38_v1`
- Use bowtie to perform alignment
  - `bowtie -x GRCh38_v1 -1 sample1_R1.fastq -2 sample1_R2.fastq -sam --threads 8 sample1.sam`

# SAM/BAM manipulation

# Sequence Alignment Map (SAM)

Sort Order = by genomic coordinate

SN = reference sequence's name (FASTA header)

LN = reference sequence's length

```
@HD VN:1.6 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
```

- r001 = read name (from sequencing FASTQ)
- ref = reference sequence name (from genomic FASTA)
- 7 = first position on the reference sequence
- 30 = Mapping quality score = $-10 \times \text{Log}_{10}(\text{error})$
- 8M2I4M1D3M = CIGAR string = matches, insertion, deletion information

# SAM manipulation with samtools

## Samtools

Samtools is a suite of programs for interacting with high-throughput sequencing data. It consists of three separate repositories:

| | |
|---|---|
| **Samtools** | Reading/writing/editing/indexing/viewing SAM/BAM/CRAM format |
| **BCFtools** | Reading/writing BCF2/VCF/gVCF files and calling/filtering/summarising SNP and short indel sequence variants |
| **HTSlib** | A C library for reading/writing high-throughput sequencing data |

- By default, SAM output from aligner is unsorted
- Also, SAM is large

- BAM is a zipped version of SAM
- Use samtools to convert SAM→BAM and sort

# Example of SAM manipulation

- ```
  bowtie -x GRCh38_v1 -1 sample1_R1.fastq -2 sample1_R2.fastq -sam
  --threads 8 | samtools view -Sb - | samtools sort >
  sample1_sorted.bam
  ```

- samtools view –Sb converts SAM to BAM
- samtools sort sorts the BAM file

- | is the pipe command which passes output from one software to another
- > is the redirect command which writes output of a software to a file
  ```

# Pileup format

A
A
A
G
G
A
G
A
---
A

| Sequence | Position | Reference Base | Read Count | Read Results | Quality |
|---|---|---|---|---|---|
| seq1 | 272 | T | 24 | ,.$....,,.,.,...,,,.,..^+. | <<<+;<<<<<<<<<<=<;<;7<& |
| seq1 | 273 | T | 23 | ,.....,.,.,...,,,.,..A | <<<;<<<<<<<<<3<=<<<;<<+ |
| seq1 | 274 | T | 23 | ,.$....,,.,.,...,,,.,... | 7<7;<;<<<<<<<<<=<;<;<<6 |
| seq1 | 275 | A | 23 | ,$....,,.,.,...,,,.,...^l. | <+;9*<<<<<<<<<=<<:;<<<< |
| seq1 | 276 | G | 22 | ...T,,.,.,...,,,.,.... | 33;+<<7=7<<7<&<<1;<<6< |
| seq1 | 277 | T | 22 | ....,,.,.,.C.,,,.,..G. | +7<;<<<<<<<&<=<<:;<<&< |
| seq1 | 278 | G | 23 | ....,,.,.,...,,,.,....^k. | %38*<<;<7<<7<=<<<;<<<<< |
| seq1 | 279 | C | 23 | A..T,,.,.,...,,,.,..... | 75&<<<<<<<<<=<<<9<<:<<< |

Image from wikipedia

- Focus on each reference base pair position
  - Whether each read matches the reference or not

# Sequence assembly

# *De novo* assembly

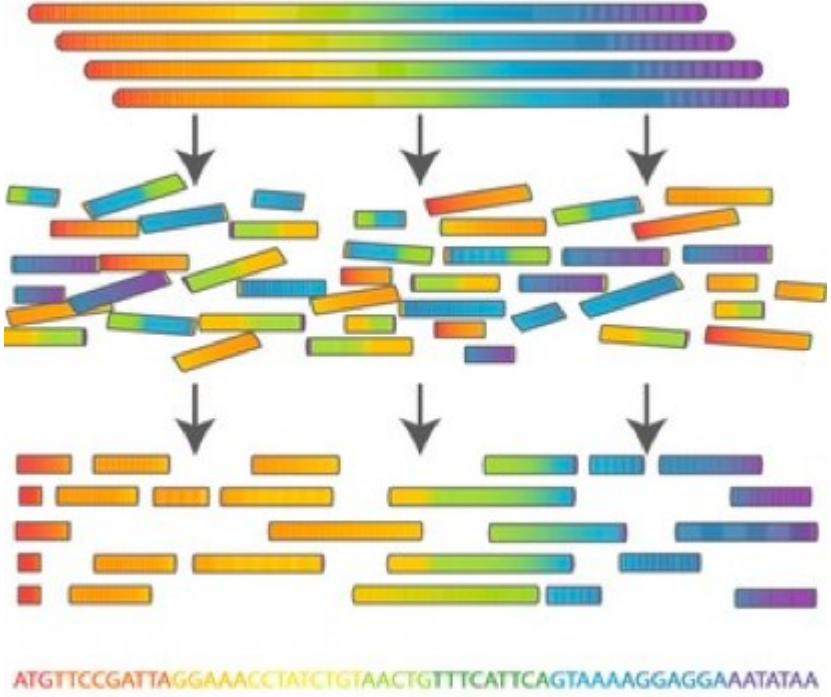
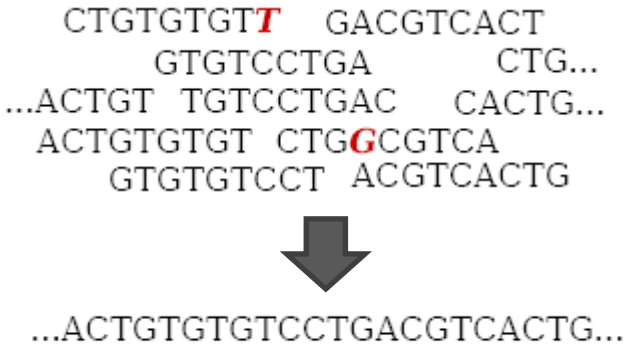


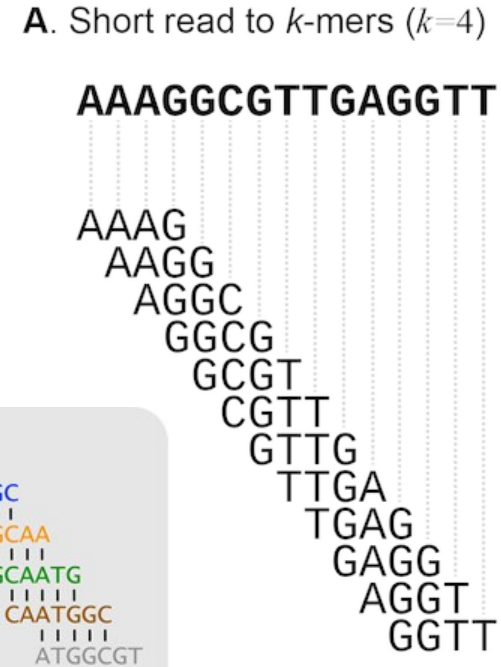

Image from wikipedia

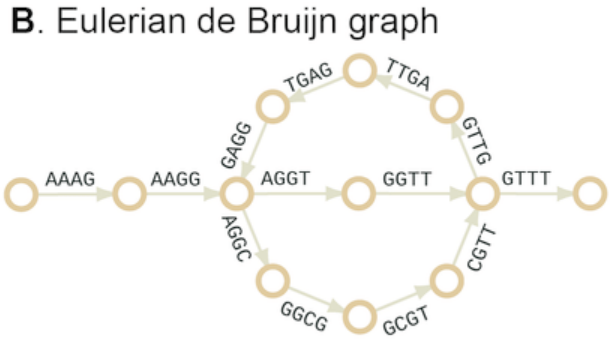


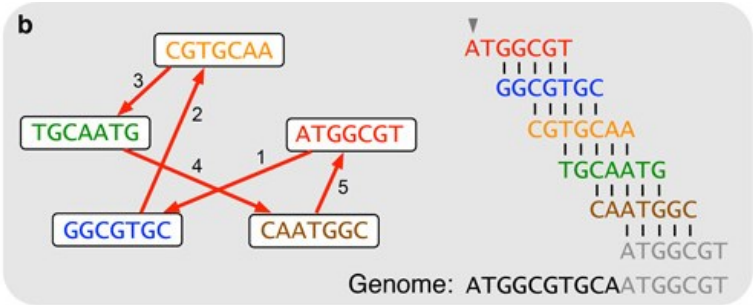

Chandra Varma Bogaraju, S. Int J Embed Syst 9:74 (2017)

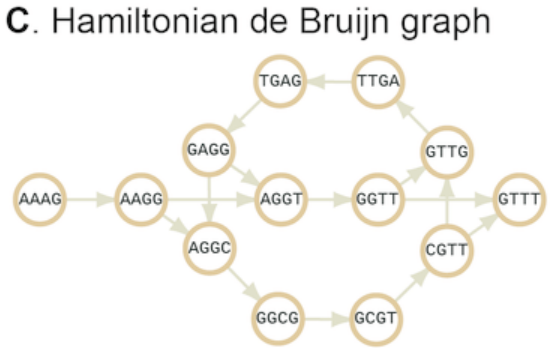
# Assembly via de Bruijn graph

- Each directed path in a de Bruijn graph represents a possible contiguous segment of the genome



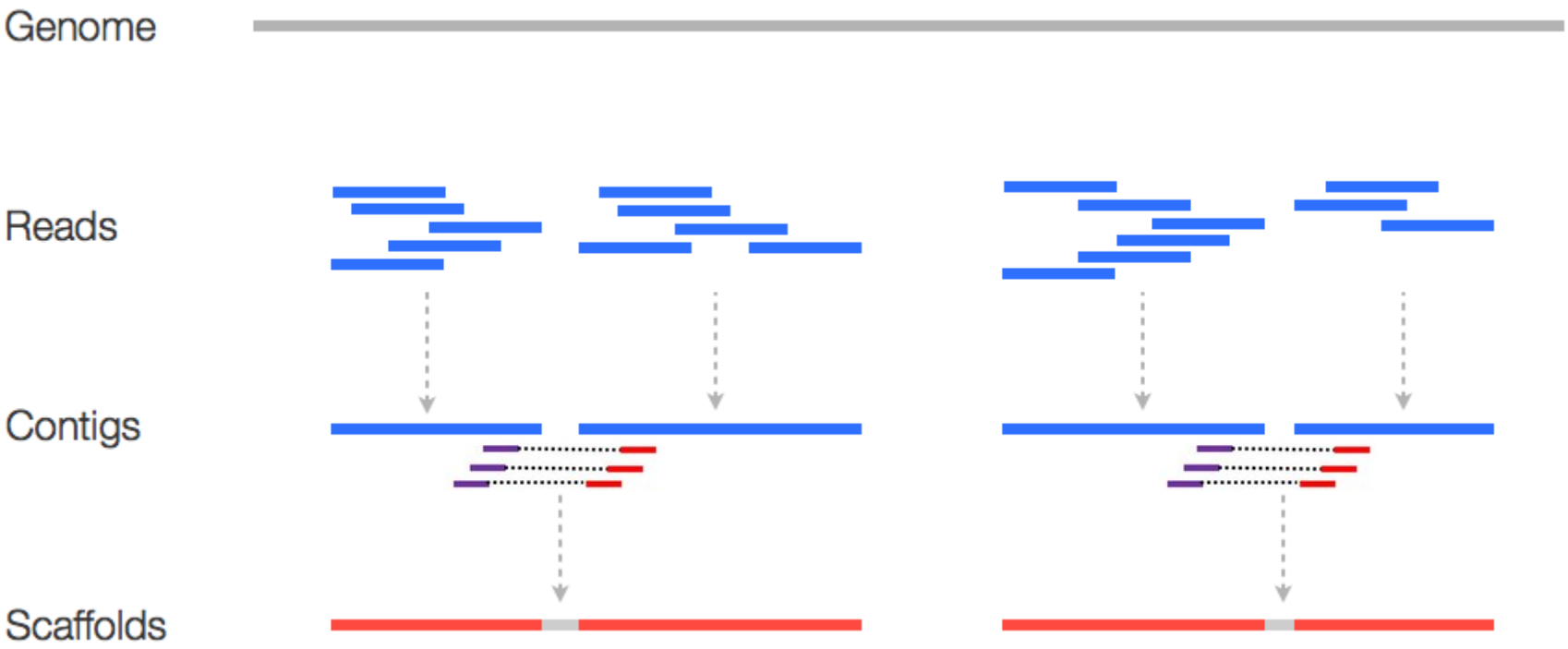




Compeau, P.E. et al. Nature Biotechnology 29:987-991 (2011)

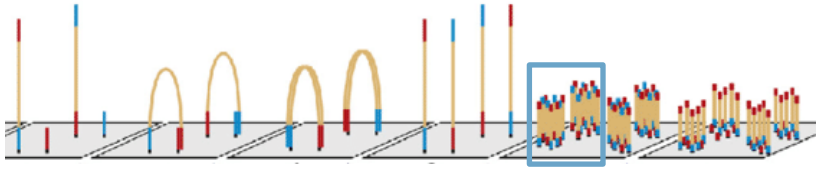
Sohn, J.-I. and Nam, J.-W. Briefings in Bioinformatics 19:bbw096 (2016)

# Contig and scaffold

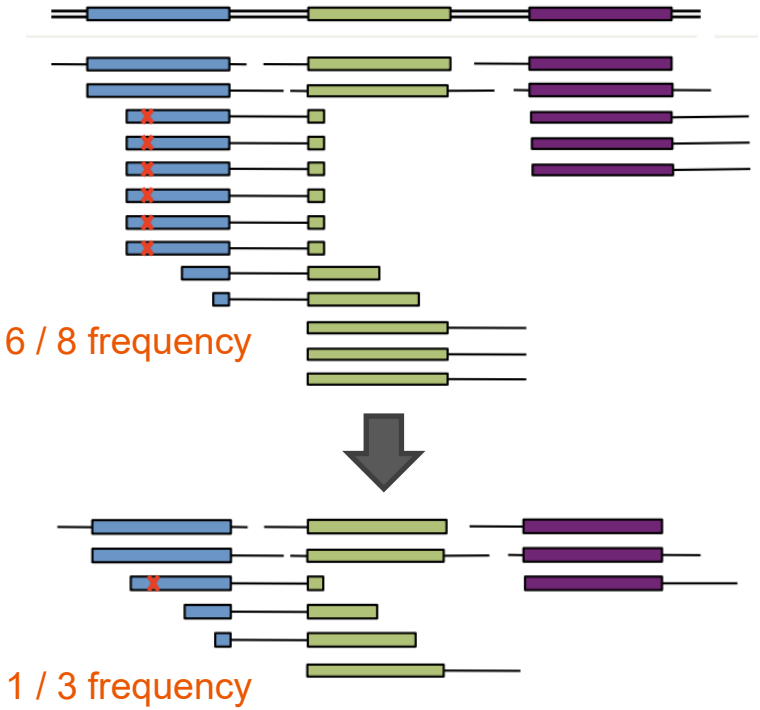


# Deduplication

# Duplicated = derived from the same molecule



- Similar sequences coming from nearby coordinates in Illumina flow cells
- Reads with the same start and end
  - Highly unlikely to generate the exact same DNA molecules by chance
- Lead to incorrect frequency estimates




Images from qcb.ucla.edu

# Mark duplicate command examples

```
java -jar picard.jar MarkDuplicates \
      I=input.bam \
      O=marked_duplicates.bam \
      M=marked_dup_metrics.txt
```

```
samtools markdup positionsort.bam markdup.bam
```

```
@SIM:1:FCX:1:15:6329:1045:GATTACT+GTCTTAAC 1:N:0:ATCCGA
TCGCACTCAACGCCCTGCATATGACAAGACAGAATC
+
<>;##=><9=AAAAAAAAAA9#:<#<;<<<????#=
```

- Require just the sorted alignment output
  - Illumina flow cell (x, y) coordinate is in the read name
  - Mapped chromosome position is in the SAM/BAM

# Variant calling

# Type of variants

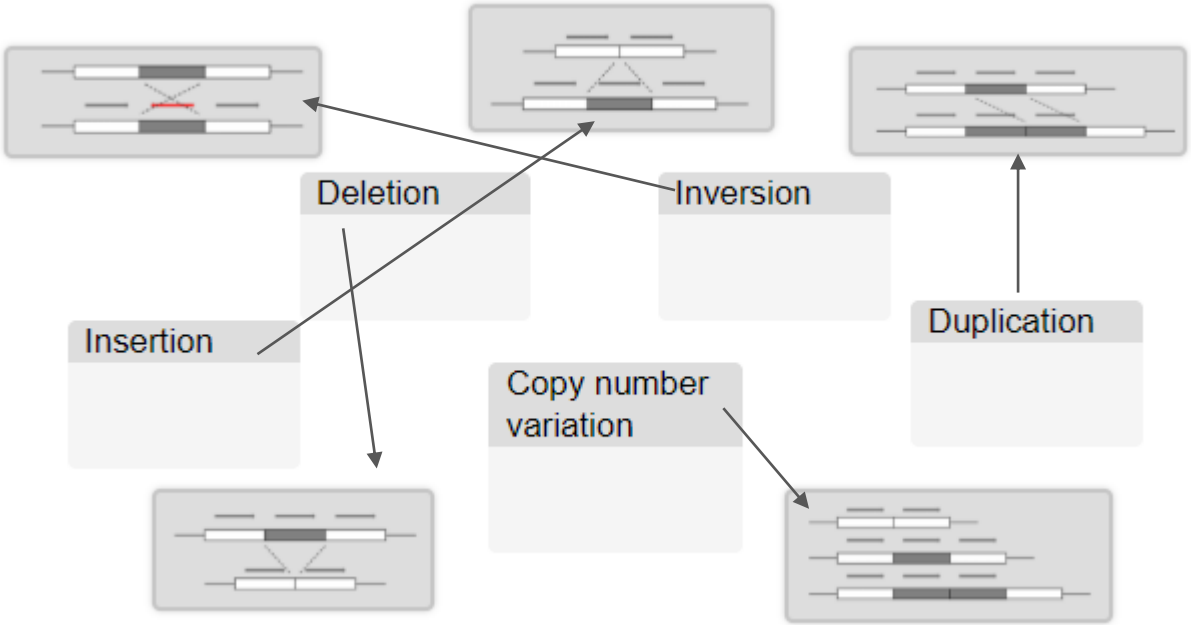



Image from EBI

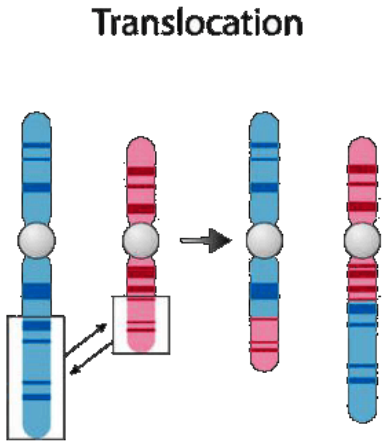
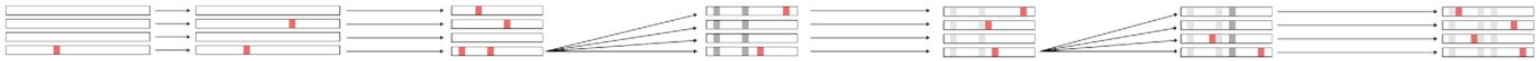



Image from wikipedia

# Not all differences are true variants



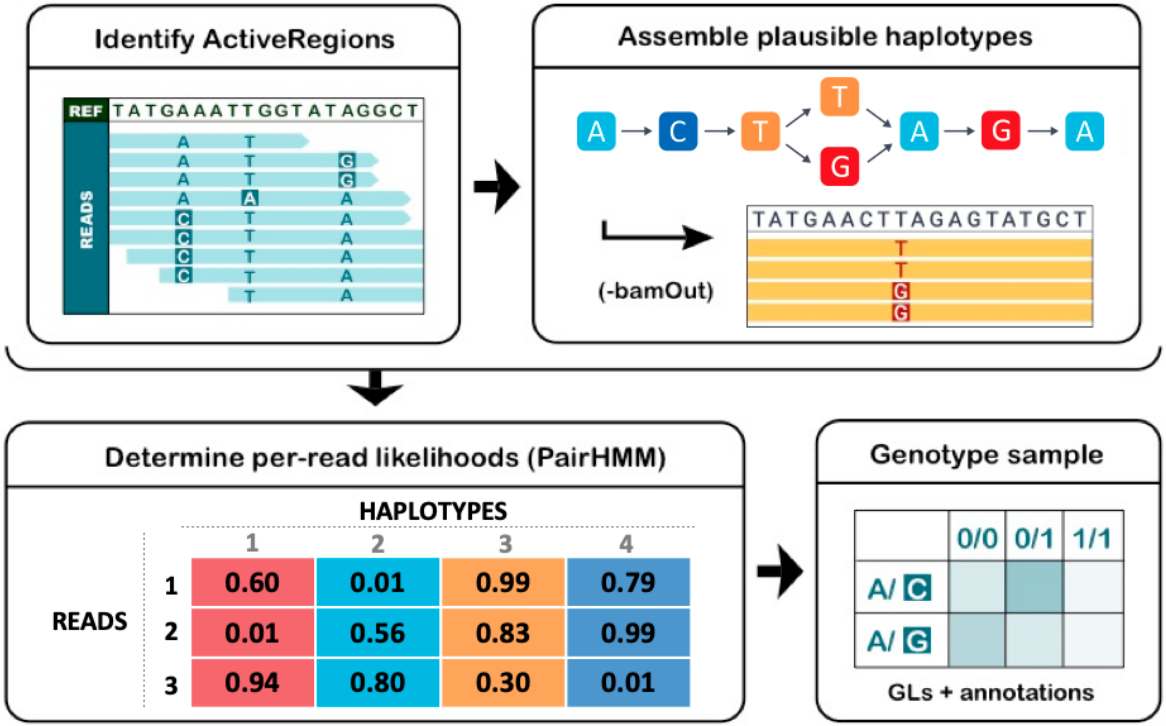
| Step | Sample → | DNA extraction → | Fragmentation → | PCR amplification → | Flow cell hybridization → | Cluster generation → | Sequencing by Synthesis |
|---|---|---|---|---|---|---|---|
| Error source | Mutagenesis | Oxidative damage | Oxidative damage | Polymerase mistakes | | Cluster PCR errors | Phasing, Fluorophore crosstalk |
| Error category | Biological variants | DNA damage | DNA damage | PCR errors | | Sequencing errors | Sequencing errors |
| Detection | | | | | ← Ignored | Called → | |

- Errors are random
  - High depth of sequencing can provide the correct consensus
  - Filter using read depth + allele frequency

# Variant calling strategy

- Small-scale variants, such as SNV and short indel
  - Compare to reference to identify differences and assess significance
- Copy number variations
  - Look for loci with high or low frequencies compared to others
- Chromosomal translocation and inversion
  - Consider reads with forward and reverse mapped to different regions
  - *De novo* assembly

# Small-scaled variant calling




P(haplotype | reads)

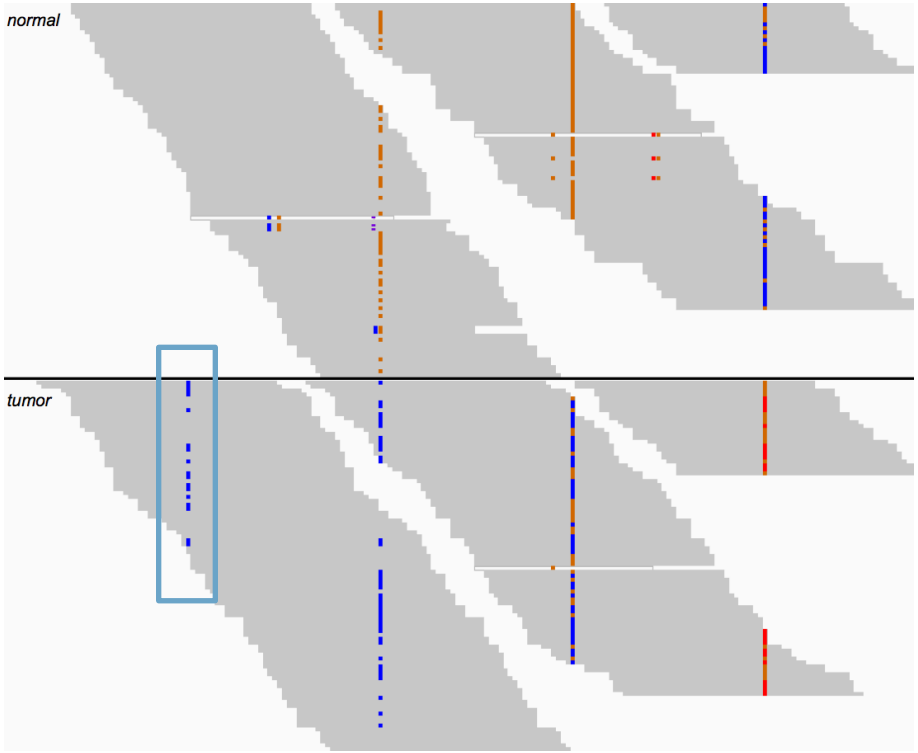
https://gatk.broadinstitute.org/hc/en-us/articles/360035531412-HaplotypeCaller-in-a-nutshell

# Germline vs somatic variants

- Germline mutations = inherited and appear in every cell of the offspring
  - Compare DNA from normal tissues to reference genomes
  - Fixed ploidy

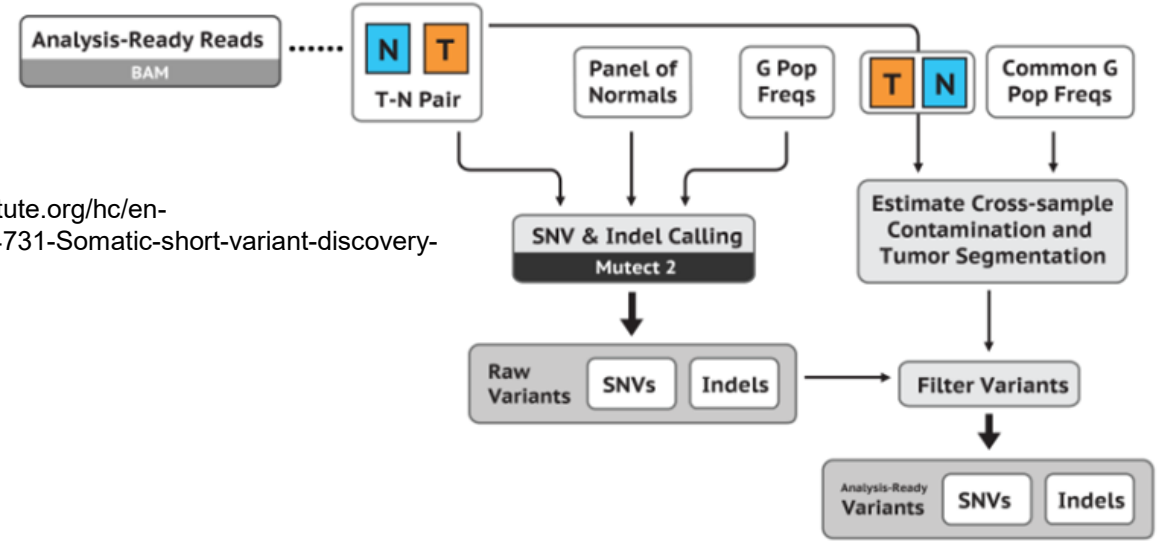
- Somatic mutations = occur during lifetime
  - Compare DNA from disease tissues to normal tissues
  - Also compare to DNA from other healthy individuals
  - Allow variation in ploidy (different disease cells can have different mutations)

# Germline vs somatic variants




https://gatk.broadinstitute.org/hc/en-us/articles/360035890491

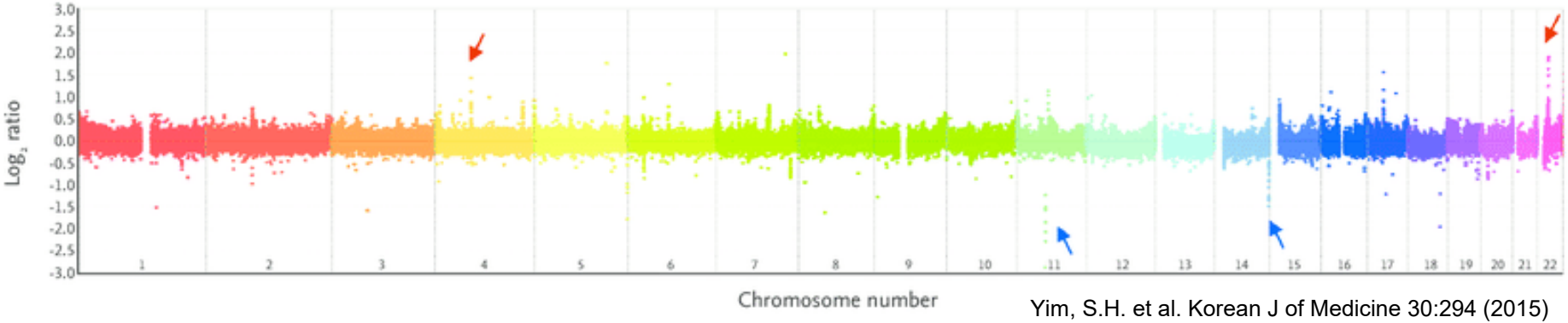
# Genome Analysis Toolkit (GATK) somatic workflow




https://gatk.broadinstitute.org/hc/en-us/articles/360035894731-Somatic-short-variant-discovery-SNVs-Indels-

- Inclusion of matched normal (N), panels of healthy individual (Panels of Normals), and allele frequency in the general population (G Pop Freqs)
- Also estimate contamination = normal cells in disease sample

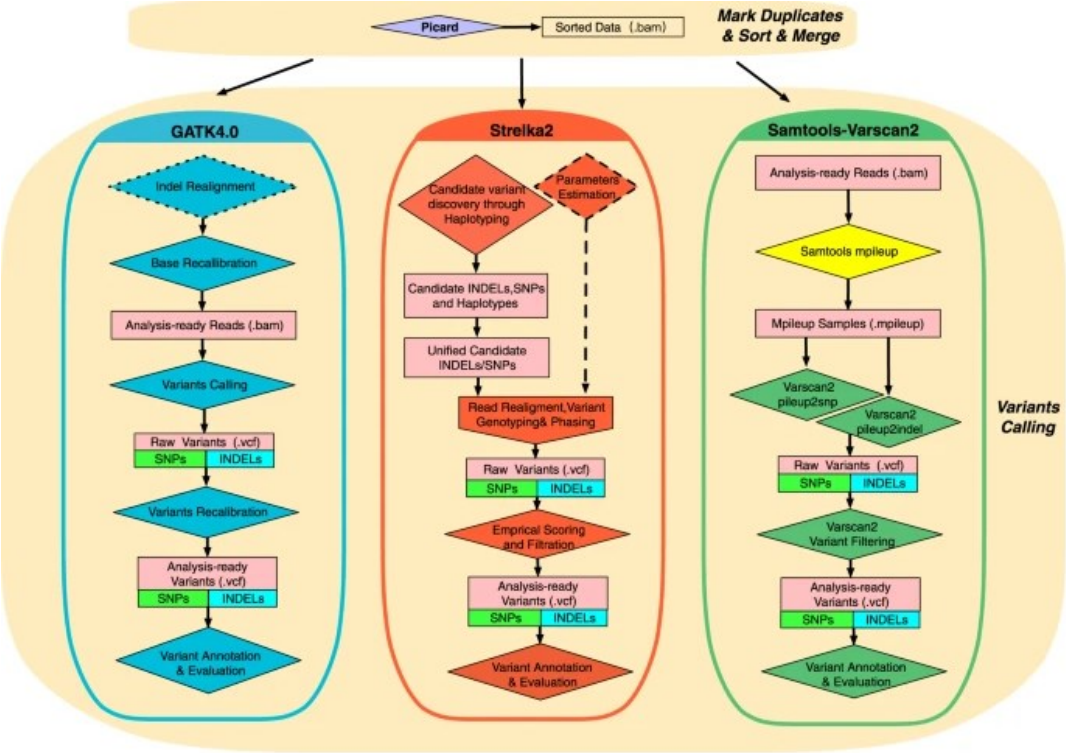
# Copy number variations




Yim, S.H. et al. Korean J of Medicine 30:294 (2015)

- Look for loci with high or low read frequencies compared to others

# Utilizing multiple callers




Chen, J. et al. Scientific Reports 9:9345 (2019)

# Variant Call Format (VCF)

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF    ALT     QUAL FILTER INFO                              FORMAT      NA00001        NA00002
20    14370   rs6054257 G      A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51
20    17330   .         T      A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3
20    1110696 rs6040355 A      G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2
20    1230237 .         T      .       47   PASS   NS=3;DP=13;AA=T                  GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51
20    1234567 microsat1 GTC    G,GTCT  50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2
```

| = phased

/ = unphased

# Variant filtering

```
gatk VariantFiltration \
    -V snps.vcf.gz \
    -filter "QD < 2.0" --filter-name "QD2" \
    -filter "QUAL < 30.0" --filter-name "QUAL30" \
    -filter "SOR > 3.0" --filter-name "SOR3" \
    -filter "FS > 60.0" --filter-name "FS60" \
    -filter "MQ < 40.0" --filter-name "MQ40" \
    -filter "MQRankSum < -12.5" --filter-name "MQRankSum-12.5" \
    -filter "ReadPosRankSum < -8.0" --filter-name "ReadPosRankSum-8" \
    -O snps_filtered.vcf.gz
```

Quality score normalized by read depth

Quality score

Strand bias scores

Mapping quality scores

- Mostly follow guideline from software developer or publications
  - But take note of the thresholds just in case of unusual outputs

# Variant annotation

# Understanding the importance of a variant

- Impact on sequence
  - Non-synonymous, splice site, frameshift, regulatory element

- Is it known?
  - Genome Aggregation Database: gnomAD
  - dbSNP

- Clinical implication: observed in patients, treatment response, drug target
  - ClinVar, COSMIC, PharmGKB

- Variant effect predictor (VEP)
- Funcotator/Oncotator

# ClinVar

## NM_007294.3(BRCA1):c.*6207C>T

| | |
|---|---|
| **Interpretation:** | **Benign** |
| **Review status:** | ★★★☆ reviewed by expert panel |
| **Submissions:** | 1 |
| **First in ClinVar:** | Sep 29, 2015 |
| **Most recent Submission:** | Sep 29, 2015 |
| **Last evaluated:** | Jan 12, 2015 |
| **Accession:** | VCV000209219.3 |
| **Variation ID:** | 209219 |
| **Description:** | single nucleotide variant |

**NM_007294.3(BRCA1):c.*6207C>T**

| | |
|---|---|
| **Allele ID:** | 206177 |
| **Variant type:** | single nucleotide variant |
| **Variant length:** | 1 bp |
| **Cytogenetic location:** | 17q21.31 |
| **Genomic location:** | 17: 43039471 (GRCh38) GRCh38 UCSC<br>17: 41191488 (GRCh37) GRCh37 UCSC |

**HGVS:**

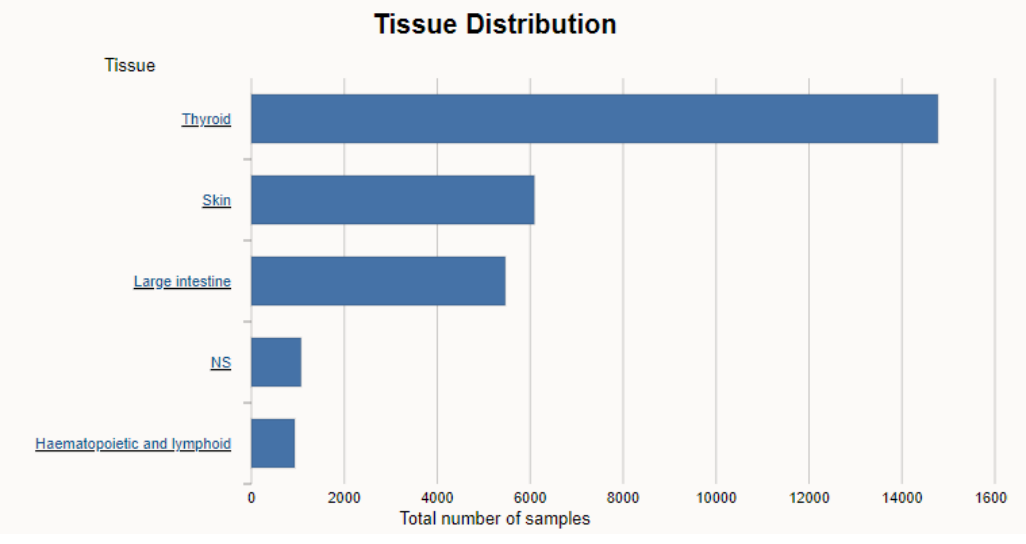
| Nucleotide | Protein | Molecular consequence |
|---|---|---|
| NC_000017.11:g.43039471G>A | | |
| NC_000017.10:g.41191488G>A | | |
| NG_005905.2:g.178513C>T | | |

... more HGVS

| | |
|---|---|
| **Protein change:** | - |
| **Other names:** | 11918 C>T |
| **Canonical SPDI:** | NC_000017.11:43039470:G:A |
| **Functional consequence:** | - |
| **Global minor allele frequency (GMAF):** | 0.00679 (A) |
| **Allele frequency:** | Trans-Omics for Precision Medicine (TOPMed) 0.00211 |

# Catalog of Somatic Mutations in Cancer (COSMIC)

**Mutation**
**COSV56056643**




| Sample name | Gene name | Transcript | Primary Tissue | Tissue Subtype 1 | Primary Histology | Histology Subtype 1 | Pubmed ID |
|---|---|---|---|---|---|---|---|
| 1011-mel | BRAF | ENST00000646891.1 | NS | NS | Malignant melanoma | NS | 15467732 |
| 1022043 | BRAF | ENST00000646891.1 | NS | NS | Malignant melanoma | NS | 16007203 |

# Pharmocogenomics knowledgebase

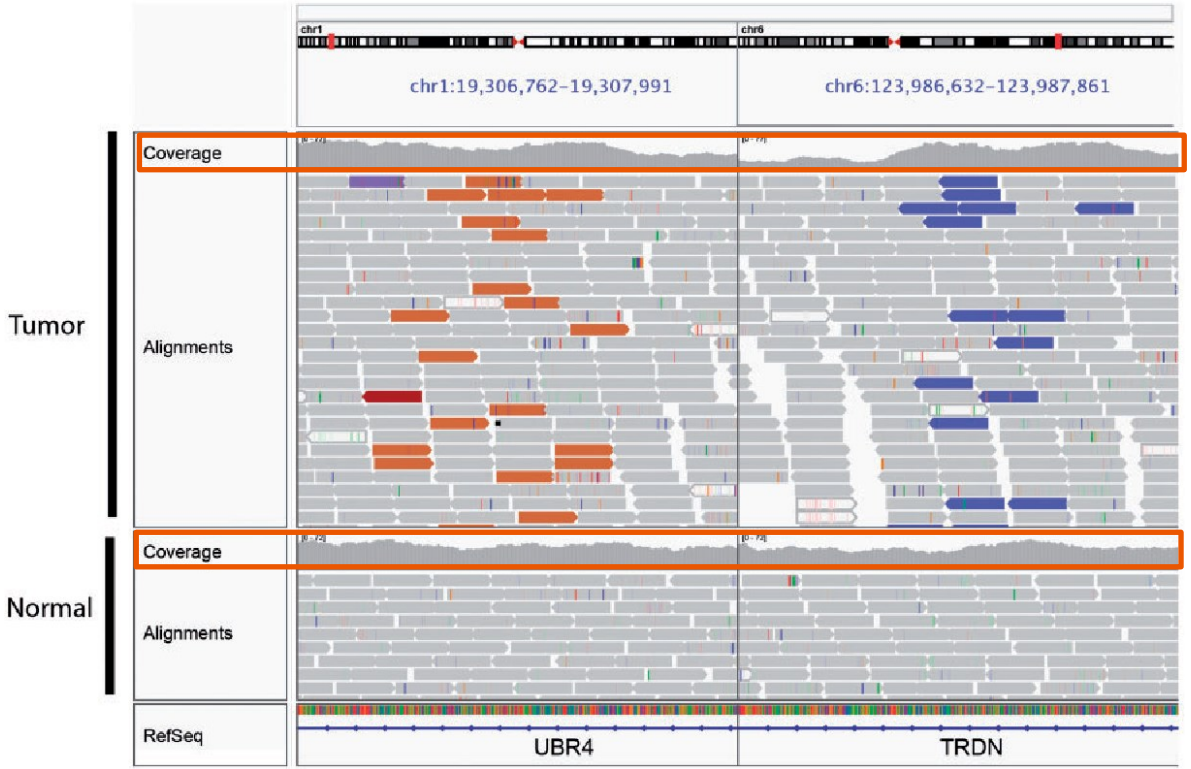
PHARMGKB

| VARIANT | LITERATURE | DRUGS | GENES | ASSOCIATION |
|---|---|---|---|---|
| rs2069502 | PMCID:PMC3959225 | somatropin recombinant | CDK4 | Genotype CC is associated with decreased respc to somatropin recombinant in children with Tur Syndrome as compared to genotypes CT + TT. |

| VARIANT | SIGNIFICANCE | P-VALUE | # OF CASES | # OF CONTROLS | BIOGEOGRAPHICAL GROUPS | PHENOTYPE CATEGORIES |
|---|---|---|---|---|---|---|
| rs2069502 | yes | < 0.05 | 147 | 0 | Unknown | • Efficacy |

# Visualization

# Integrated Genomics Viewer (IGV)




Thorvaldsdottir, H. et al. Briefings in Bioinformatics (2012)

# Genomics data processing workflow summary

- Check quality of FASTQ files
- Trimming

- Alignment and/or assembly
- Deduplicate

- Variant calling
  - Germline or Somatic
  - Copy number variation
  - Translocation

- Variant filtering
- Variant annotation

# Any question?

- See you on September 4