1. Secondary DNA analysis.
2. Variant calling.
3. Cancer analysis.

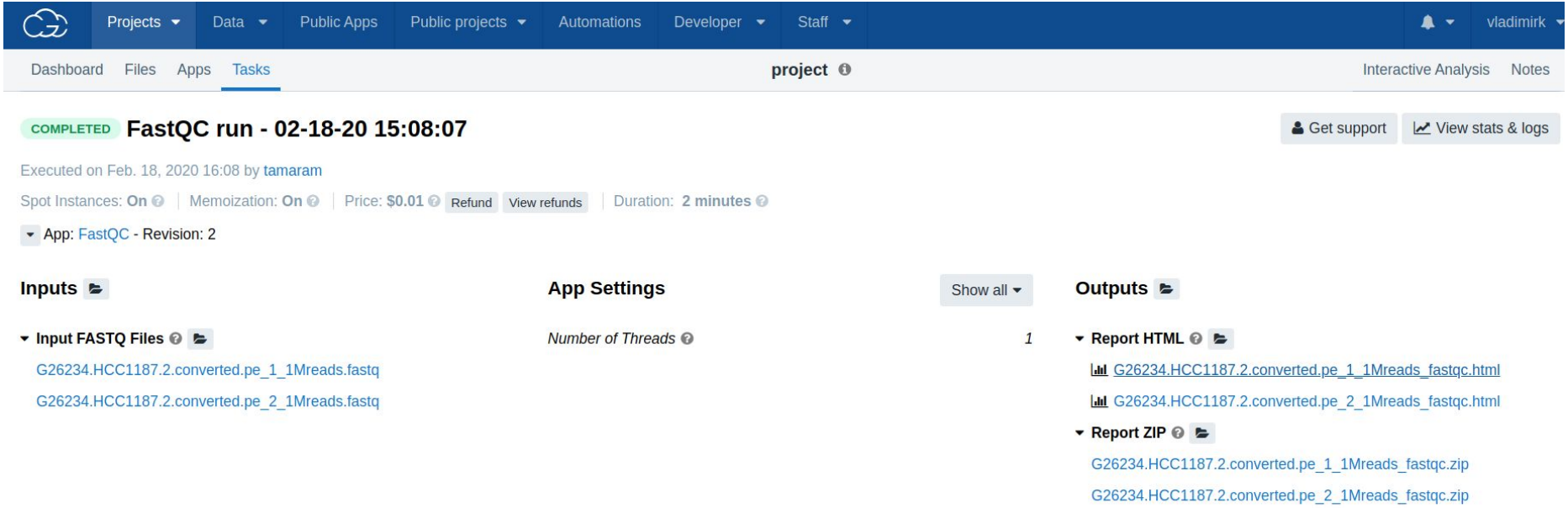
Lesson 03

Announcement - lecture next week

Wednesday, 4th of March, 18:00, room 55

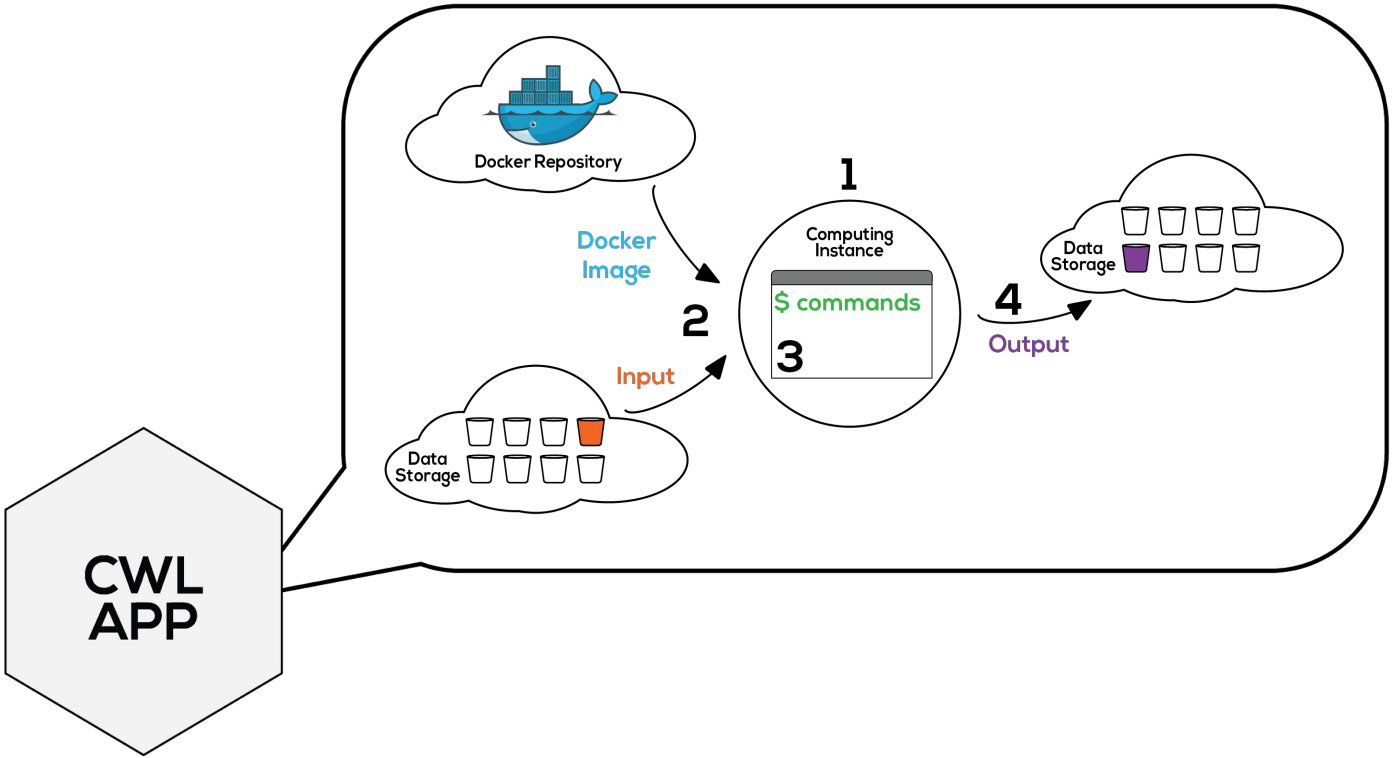
Other lectures in regular time slot

Exercise 1


Projects
Data
Public Apps
Public projects
Automations
Developer
Staff
vladimirk
Dashboard
Files
Apps
Tasks
project
Interactive Analysis
Notes
COMPLETED
FastQC run - 02-18-20 15:08:07
Get support
View stats & logs
Executed on Feb. 18, 2020 16:08 by tamaram
Spot Instances: On
Memoization: On
Price: $0.01
Refund
View refunds
Duration: 2 minutes
App: FastQC - Revision: 2
Inputs
Input FASTQ Files
G26234.HCC1187.2.converted.pe_1_1Mreads.fastq
G26234.HCC1187.2.converted.pe_2_1Mreads.fastq
App Settings
Show all
Number of Threads
1
Outputs
Report HTML
G26234.HCC1187.2.converted.pe_1_1Mreads_fastqc.html
G26234.HCC1187.2.converted.pe_2_1Mreads_fastqc.html
Report ZIP
G26234.HCC1187.2.converted.pe_1_1Mreads_fastqc.zip
G26234.HCC1187.2.converted.pe_2_1Mreads_fastqc.zip

CWL - Recap

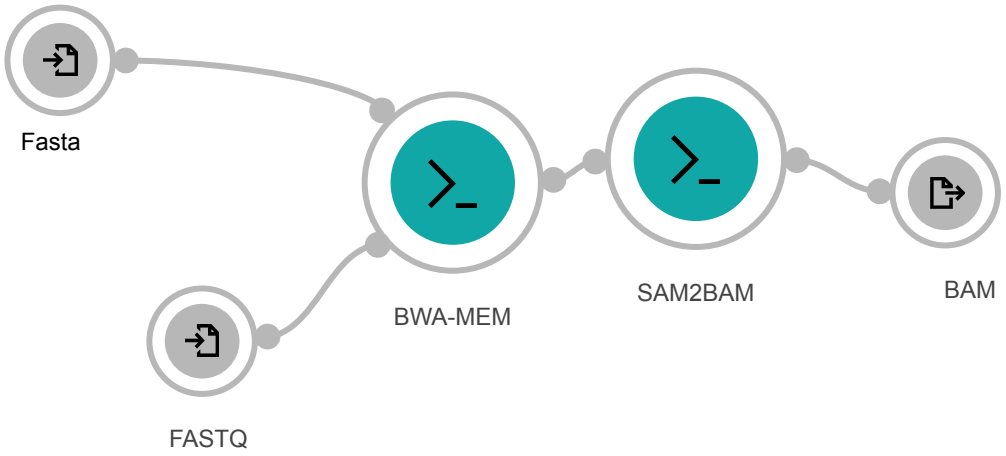
COMMON WORKFLOW LANGUAGE



What is a workflow? - Recap

- Acyclic graph of tools connected to perform some analysis
- Workflow's nodes are:
  - Inputs (file or parameter)
  - Tools
  - Outputs
  - Workflow

```
bwa mem ref.fa read1.fq read2.fq >
aln.sam
sam2bam aln.sam > aln.bam
```



1. Secondary DNA analysis.

Secondary DNA analysis

Base calling
(vendor tool)

FASTQ file:
raw HTS reads

Alignment
or assembly

SAM/BAM file:
aligned HTS reads

Variant calling

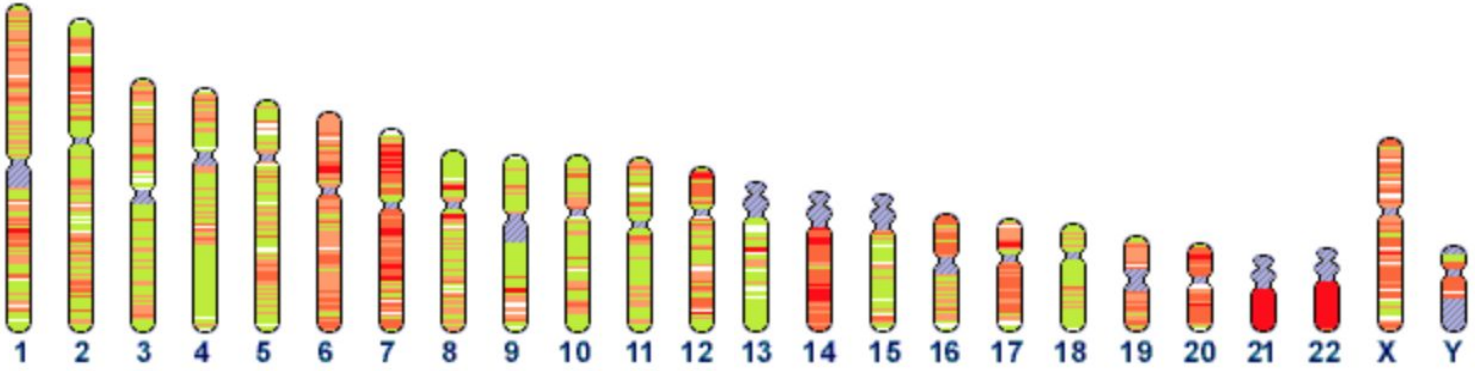
VCF file:
genomic variation

.sam file: Uncompressed text file
.bam file: Compressed and indexed file

VCF Files can be block-compressed and indexed.

Human genome stats

- The human genome has 23 pairs of chromosomes:
  - Males have 22 autosomes, one X and one Y chromosome
  - Females have 22 autosomes and two X chromosomes
- There is also a separate Mitochondrial DNA contig
- Total length is ~3 Gigabases (3B basepairs)
- About 20 000 genes covering about 30 Megabases



Human genome HG38

- Current version of the chromosomes is *HG38 (well HG39 is almost out)* (though 37 is still widely used)
- Released December 24th 2013
- Additional sequences are added to the genome:
  - Unplaced sequences (some genomes contain them, somewhere)
  - Unlocalized sequences (chromosome known, but coordinates are not)
  - Alternate sequences (some genomes contain them, instead of something parts)
  - Human Herpesvirus 4 type 1
- Patches are commonly added
- http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/

FASTA file format

- A simple format for storing reference files
- Two “types” of lines:
  - Description lines, start with ‘>’, contain sequence name and optional description
  - Sequence lines follow a description line and contain the actual nucleotide sequence
- Sequences are represented by a single description line,
  followed by one or more sequence lines (usually 70 bases or less in a line)

```
>chr 1
ACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCT
ACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCT
ACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCGTACCT
ACTGCCCTCCGTACCTACTGCCCTCCGTACCTACTGCCCTCCG
```

Pysam - Python fasta interface

- **`Pysam`** - a Python toolkit for working with genomic files
- pysam.Fastafile:
  - Create a fasta file parser: `fasta = pysam.Fastafile(path_to_file)`
  - Get the sequence names in the file: `fasta.references`
  - Get the lengths of the sequences: `fasta.lengths`
  - Retrieve a (part of) sequence: `fasta.(sequence_name, [start], [stop])`
- Fasta coordinates in pysam are zero-based
  - Not true for all file types in pysam!
- Other fasta interfaces for Python exist
  - pyfasta - works only on fasta files
  - biopython - a much larger toolkit that complement sequences, etc.
  - We are describing pysam, as it covers all the file types covered in the course

Pysam

Pysam can be used to process BAM files
pysam.AlignmentFile

- AlignmentFile(path_to_file)
- Iteration through reads in BAM file:

```
for read in AlignmentFile(path_to_file):
```

Reads are wrapped in AlignedSegment objects

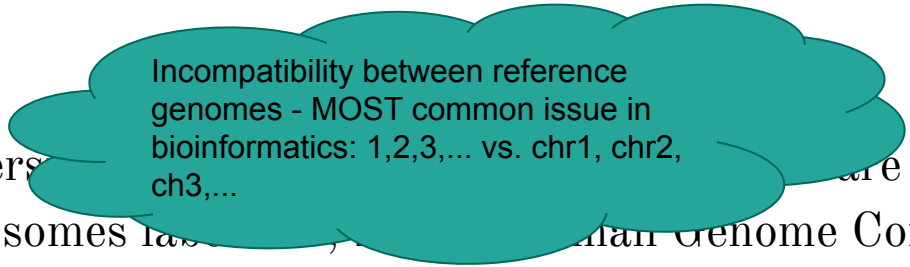
- AlignedSegment provides access to read fields and helpers

pysam.AlignmentFile supports fetching regions

- The BAM file needs to be sorted and BAI indexed!

FASTA File IUPAC Codes (and contig names)

- A, C, T, G, U - Nucleotides (Adenine, Cytosine, Guanine, Thymine, Uracil)
- Ambiguous bases:
  - R - A or G
  - Y - C, T, or U
  - N - Any base
  - K, M, S, W, B, D, H, V, X, - https://en.wikipedia.org/wiki/FASTA_format



- Often two vers… …are found
  - Chromosomes la… …man Genome Consortium style)
  - Chromosomes labeled chr1, chr2, chr3... (UCSC style)

FASTA index (fai)

- Some tools build or require indices of the fasta file
  - Needs to be in the same folder, and have the same name as the fasta + .fai extension
- Fai file structure:

1. The name of the sequence
2. The length of the sequence
3. The offset of the first base in the file
4. The number of bases in each fasta line
5. The number of bytes in each fasta line

Genes in HG38 (UCSC Genome Browser)

- A FASTA file contains raw sequences of nucleotide from a genome
- Some sections of these sequences have biological meanings attached
- Examples:
  - Chromosome 17, 43.04 Mb - 43.13 Mb is the BRCA1 Gene, implicated in breast cancer
  - Chromosome X, 73.82 Mb - 73.85 Mb is the XIST lncRNA, implicated in X inactivation
  - Chromosome 1, 121.1 Mb - 124.3 Mb is the Chromosome 1 centromere
- USCS Genome browser is a place where different annotation tracks can be explored in depth
- USCS Genome browser is located at https://genome.ucsc.edu/

Annotations file formats

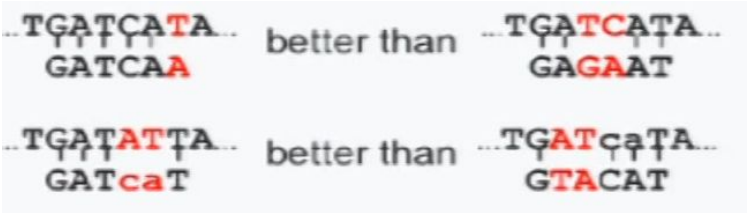
- Annotations are stored in few (similar) file types
  - **BED**
  - GTF
  - GFF
  - ...
- **BED file format** (tab-separated):

CHROM START END NAME score ticks blocks

- Chrom, start and end are required
- Start is 0-based
- End is open interval (not included)

Short Read Alignment

- For Reference genome and set of reads: Report at least one “good” alignment for each read
  - Where in the reference genome did read originate?
- What is “good”? For now, we concentrate on:
  - Fewer mismatches are better
  - Failing to align a low-quality base is better than failing to align a high-quality base



Penalty (score) - based alignment

- Some of the reads will match perfectly to the reference
- Many reads will not:
  - Genomic variations (‘mutations’, SNP, Indel, etc.)
  - Sequencing errors
- Aligners commonly take a score-based approach:
  - Calculate a score, related to the distance between the read and the local reference sequence
  - Place the read so that the score in maximised
- Many algorithms exist, there is a speed/precision tradeoff

Two-step aligners

- Finding precise alignments can be expensive
  (for a human genome ~1 billion reads)
- Most modern aligners take a two-step approach
  (also called **“seed and extend”**)
- First find “coarse” alignments or seeds
- Than do fine grain alignments in the vicinity of the seeds
- Choose the best scoring fine grain alignment

Coarse alignment step (seeding)

- Find a set of possible coordinates
- Many false-positives, but very is usually fast
- Several common approaches:
  - K-mer hashing-based approaches
  - Radix-tree based approaches
  - FM index-based approaches (Burrows-Wheeler)
- Most require an index-building step
- A common tradeoff is speed vs RAM footprint

A simple coarse aligner

- Example: a simple hash-based scheme
- Create a hash-table which holds the positions, where each kmer in the genome occurs
- For each kmer in the read find the list of locations
- Regions with hits from many different kmers are hits
- Some kmers will have no hits, some many
- Indices quickly grow too large for whole genomes

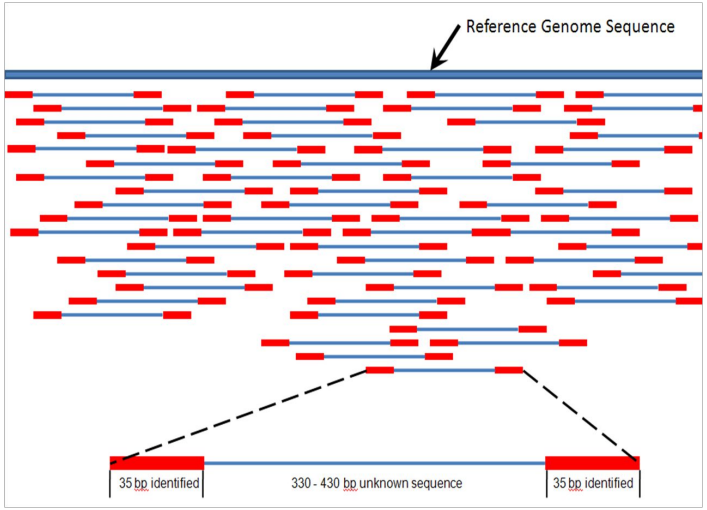
Fine-grain alignment step (extending)

- Calculates a precise sequences match score
- Algorithms slower than coarse grain aligners
- Often use a lot of RAM (related to sequence size)
- The extend step also needs to produce the information on *how* the sequences match
- Mostly based on dynamic programming

BWA-MEM usage

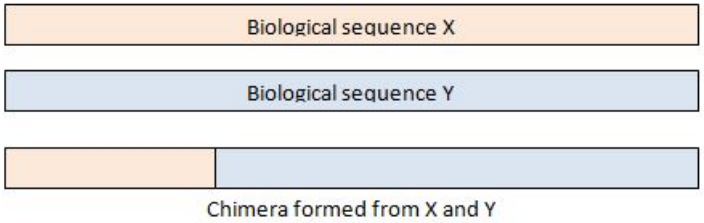
```
bwa mem ref.fa read1.fq read2.fq > aln.sam
```

- Reference genome index must exist
- Paired-end reads
- Primary and secondary alignment (random)



BWA-MEM usage

- Chimeric reads, Supplementary alignment
  - Hard and soft clipped reads
  - 10H10M10H - 10 bases long
  - 10S10M10S - 30 bases long
- BWA-MEM program arguments:
  - `-R '@RG\tID:foo\tSM:bar\tPL:Illumina'`
  - `-Y use soft clipping for supplementary alignments (hard clipping is default)`
  - `-a Maximum insert size for a read pair to be considered being mapped properly [500]`



SAM file format - BWA-MEM output

Line from SAM file:

```
SRR035022.2621862 163 16 59999 37 22S54M = 60102 179 CCAACCCAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCGACCCTCACCCTCACCC
>AAA=>?AA>@@B@B?AABAB?AABAB?AAC@B?@AB@A?A>A@A?AAAAB??ABAB?79A?AAB;B?@?@<=8:8 XT:A:M XN:i:2 SM:i:37 AM:i:37 XM:i:0 XO:i:0 XG:i:0 RG:Z:SRR035022 NM:i:2
MD:Z:0N0N52 OQ:Z:CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBCCCCCCBBCC@CCCCCCCCCCACCCCC;CCCBBC?CCCACCACA@
```

QNAME	SRR035022.2621862
FLAG	163
RNAME	16
POS	59999
MAQ	37
CIGAR	22S54M
MRNM	=
MPOS	60102
ISIZE	179
SEQ	CCAACCCAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCGACCCTCACCCTCACCC
QUAL	>AAA=>?AA>@@B@B?AABAB?AABAB?AAC@B?@AB@A?A>A@A?AAAAB??ABAB?79A?AAB;B?@?@<=8:8
TAG	XT:A:M
TAG	XN:i:2
TAG	SM:i:37
TAG	AM:i:37
TAG	XM:i:0
TAG	XO:i:0
TAG	XG:i:0
TAG	RG:Z:SRR035022
TAG	NM:i:2
TAG	MD:Z:0N0N52
TAG	OQ:Z:CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBCCCCCCBBCC@CCCCCCCCCCACCCCC;CCCBBC?CCCACCACA

1	the read is paired in sequencing, no matter whether it is mapped in a pair
1	the read is mapped in a proper pair
0	not unmapped
0	mate is not unmapped
0	forward strand
1	mate strand is negative
0	the read is not the first read in a pair
1	the read is the second read in a pair

Only Read group ID is present in every read

SAM file format

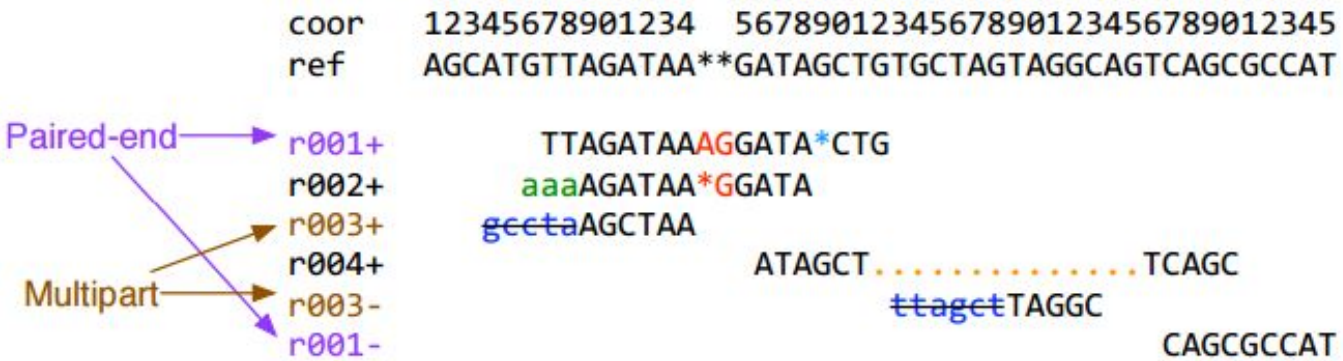
Flag	Description
0x0001	the read is paired in sequencing, no matter whether it is mapped in a pair
0x0002	the read is mapped in a proper pair (depends on the protocol, normally inferred during alignment) [1]
0x0004	the query sequence itself is unmapped
0x0008	the mate is unmapped [1]
0x0010	strand of the query (0 for forward; 1 for reverse strand)
0x0020	strand of the mate [1]
0x0040	the read is the first read in a pair [1,2]
0x0080	the read is the second read in a pair [1,2]
0x0100	the alignment is not primary (a read having split hits may have multiple primary alignment records)
0x0200	the read fails platform/vendor quality checks
0x0400	the read is either a PCR duplicate or an optical duplicate

op	Description
M	Alignment match (can be a sequence match or mismatch
I	Insertion to the reference
D	Deletion from the reference
N	Skipped region from the reference
S	Soft clip on the read (clipped sequence present in <seq>)
H	Hard clip on the read (clipped sequence NOT present in <seq>)
P	Padding (silent deletion from the padded reference sequence)

Field	Regular expression	Range	Description
QNAME	`[^ \t\n\r]+`		Query pair NAME if paired; or Query NAME if unpaired [2]
FLAG	`[0-9]+`	[0,2^{16}-1]	bitwise FLAG (Section 2.2.2)
RNAME	`[^ \t\n\r@=]+`		Reference sequence NAME [3]
POS	`[0-9]+`	[0,2^{29}-1]	1-based leftmost POSition/coordinate of the clipped sequence
MAPQ	`[0-9]+`	[0,2^{8}-1]	MAPping Quality (phred-scaled posterior probability that the mapping position of this read is incorrect) [4]
CIGAR	`([0-9]+[MIDNSHP])+|\*`		extended CIGAR string
MRNM	`[^ \t\n\r@]+`		Mate Reference sequence NaMe; "=" if the same as <RNAME> [3]
MPOS	`[0-9]+`	[0,2^{29}-1]	1-based leftmost Mate POSition of the clipped sequence
ISIZE	`-?[0-9]+`	[-2^{29},2^{29}]	inferred Insert SIZE [5]
SEQ	`[acgtnACGTN.=]+|\*`		query SEQuence; "=" for a match to the reference; n/N/. for ambiguity; cases are not maintained [6,7]
QUAL	`[!-~]+|\*`	[0,93]	query QUALity; `ASCII-33` gives the Phred base quality [6,7]
TAG	`[A-Z][A-Z0-9]`		TAG
VTYPE	`[AifZH]`		Value TYPE
VALUE	`[^\t\n\r]+`		match <VTYPE> (space allowed)

NM	i	Edit distance to the reference, including ambiguous bases but excluding clipping
OQ	Z	Original base quality (usually before recalibration). Same encoding as QUAL.
OP	i	Original mapping position (usually before realignment)
OC	Z	Original CIGAR (usually before realignment)
PG	Z	Program. Value matches the header PG-ID tag if @PG is present.
PQ	i	Phred likelihood of the template, conditional on both the mapping being correct
PU	Z	Platform unit. Value to be consistent with the header RG-PU tag if @RG is present.
Q2	Z	Phred quality of the mate/next fragment. Same encoding as QUAL.
R2	Z	Sequence of the mate/next fragment in the template.
RG	Z	Read group. Value matches the header RG-ID tag if @RG is present in the header.

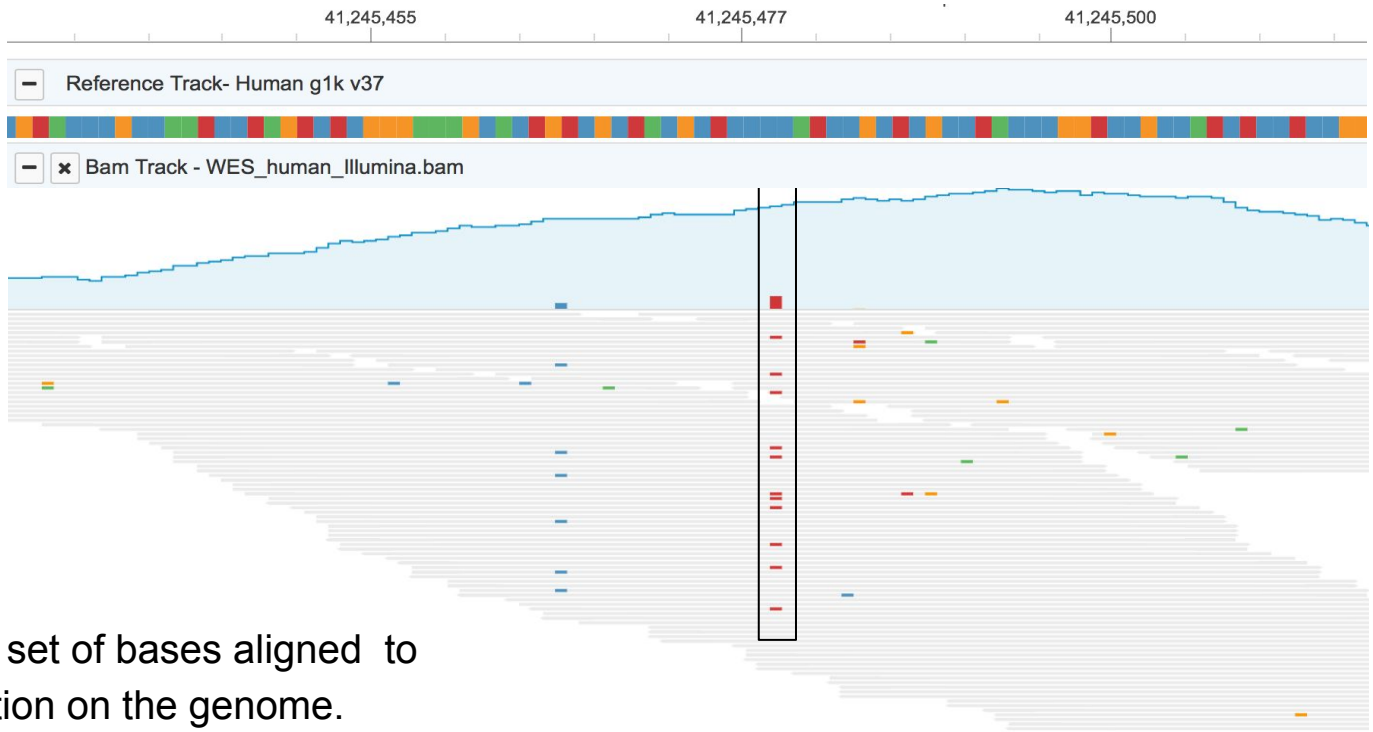
SAM file format - BWA-MEM output



Ins & padding
Soft clipping
Splicing
Hard clipping

```
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTA *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA    *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       *  0   0 TAGGC     *   NM:i:0
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT         *
```

What is a pileup?



Pileup is the set of bases aligned to a single position on the genome.

Exercise 2: Pysam - CGC Interactive analysis

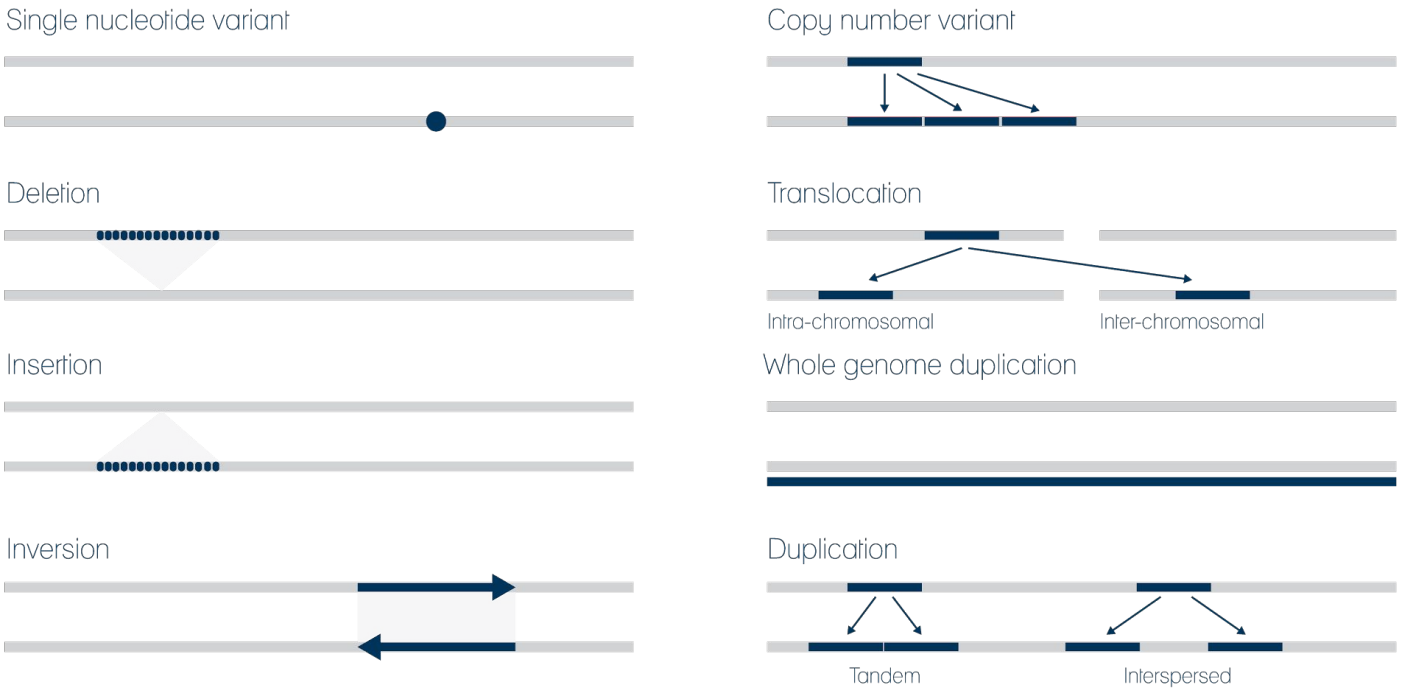
- Create an `AlignmentFile` object for “merged-tumor.bam” from Public files gallery
  - Take the first read from the `AlignmentFile`
  - Inspect the fields in the `AlignedSegment`
  - Inspect the flag field
- Check out the flag for some reads
- Calculate:
  - How many unmapped reads are in the file?
  - Total number of reads
  - Number of reads with mapping quality 0
  - Average mapping quality for all the reads
  - Average mapping quality if reads with 0 mapp quality are filtered out
- Send link to Github repo with Jupyter Notebook of executed analysis to vladimir.kovacevic@sbgenomics.com by 5th of March

Variant Calling

Introduction to Variant calling

- Variant calling is the process of finding differences between reference genome and observed sample
- We need aligned reads to the reference genome so we can find – “call” variants
- Different types of genomic variants

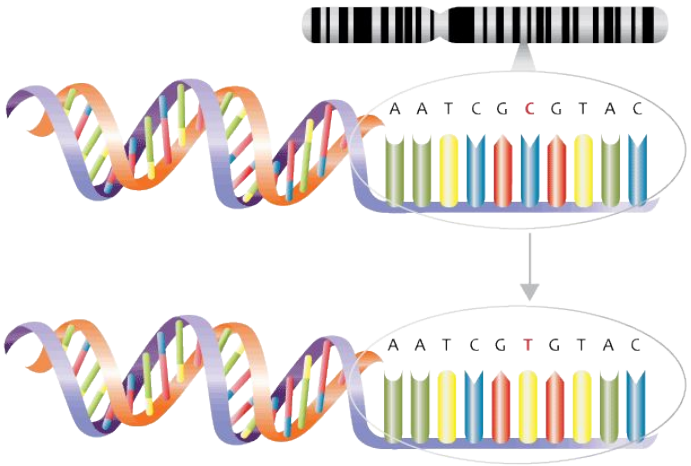
Genomic Variants



Genomic Variants

- SNV (Single Nucleotide Variant)

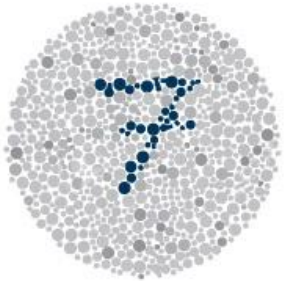
  Simple ones - not a big change on the first look, but...



Genomic Variants

Each of those characteristics causes one SNV

LONGER EYELASHES



DALTONISM

LESS SLEEPING

SUPER STRENGTH

Genomic Variants

Breast Cancer

BRCA2 gene (TS)

SNV id : rs1799954

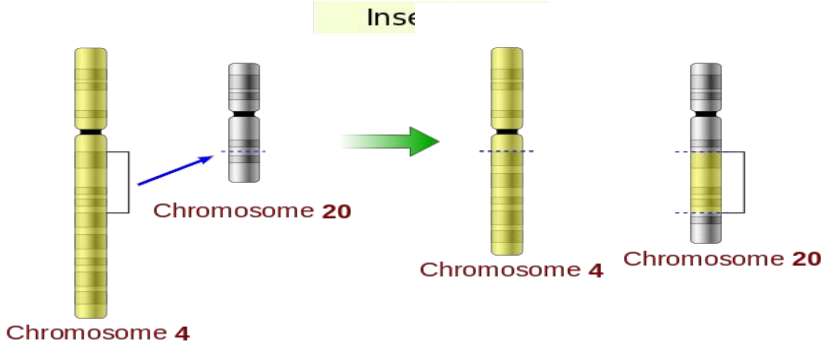
Chromosome 13
Position 32,340,455

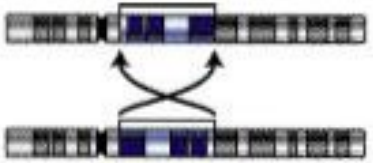
Cancer genotypes: **CC, CT and TT**

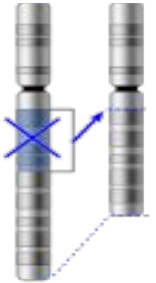
http://www.eupedia.com/genetics/cancer_related_snp.shtml
https://www.snpedia.com/index.php/Rs1799954

Genomic Variants

Deletions, Insertions, Translocations, Inversions, and some others...



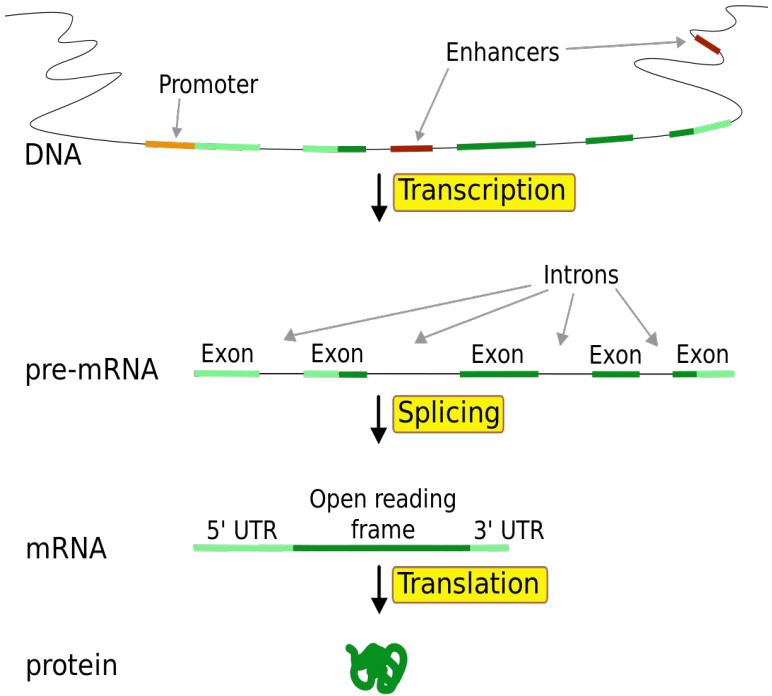




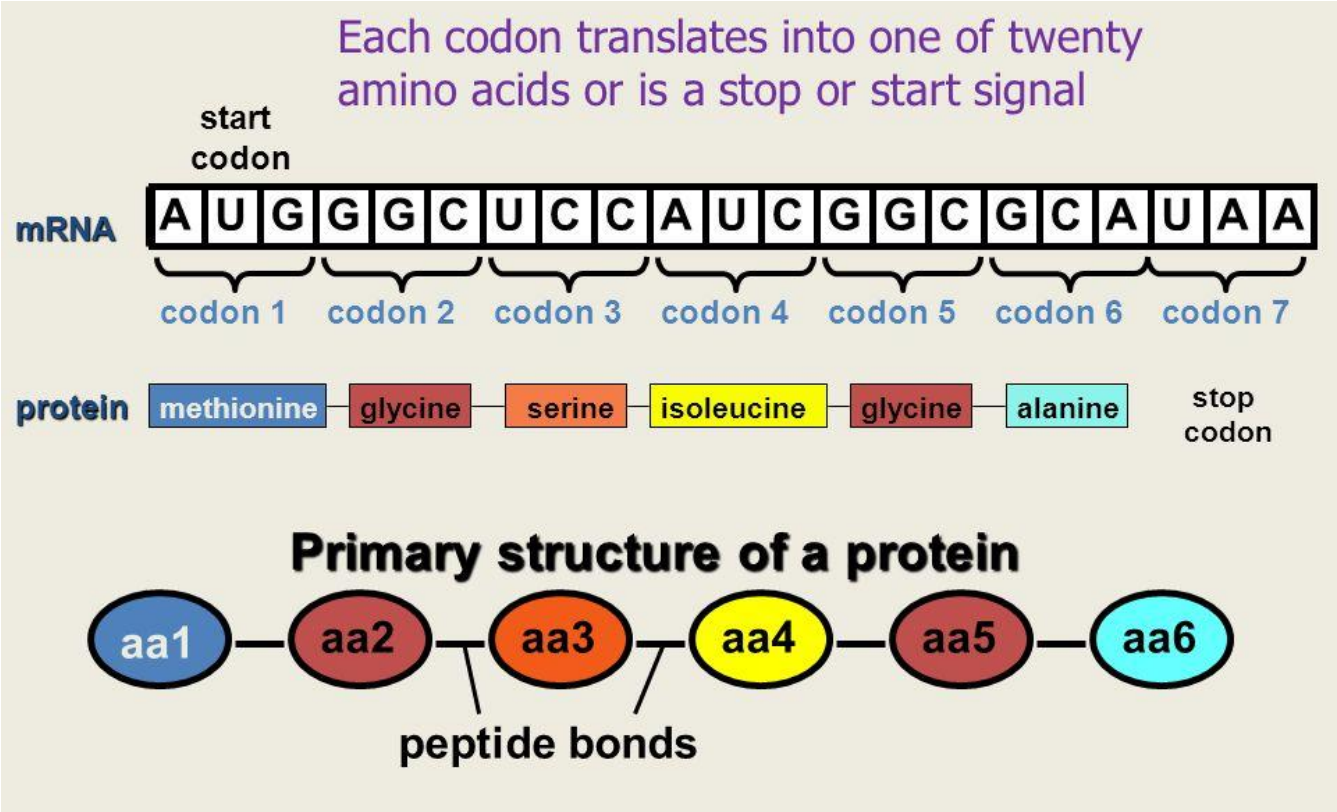
Genomic Variants

Based on the variant location, we can predict if mutation will have impact.

- Central dogma



RNA to Protein

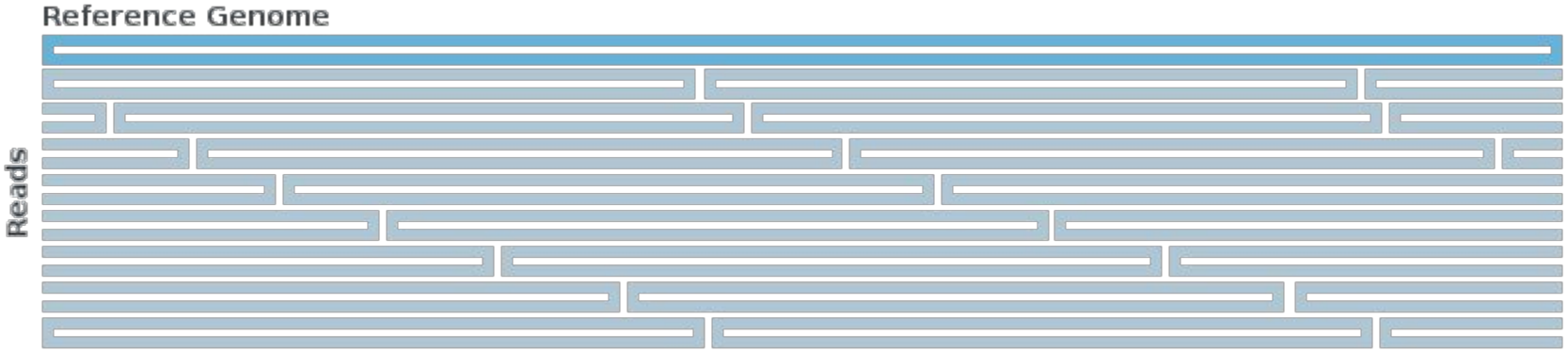


Genomic Variants

- Variants can have different impact on human cells and organism
- Single Nucleotide Variants(**SNV**):
  - Harmless
    - **Silent** – Usually no effect
  - Harmful:
    - **Missense** – Amino acid change
    - **Nonsense**(Start/Stop Gain/Lost) – AUG / UAG, UAA, UGA
  - Depends on the location
    - **Noncoding regions** (Promoter, Enhancer, lncRNA, miRNA...)
- Insertions/Deletions – **INDELS**
  - **In frame**
  - **Out of frame (Frameshift)**

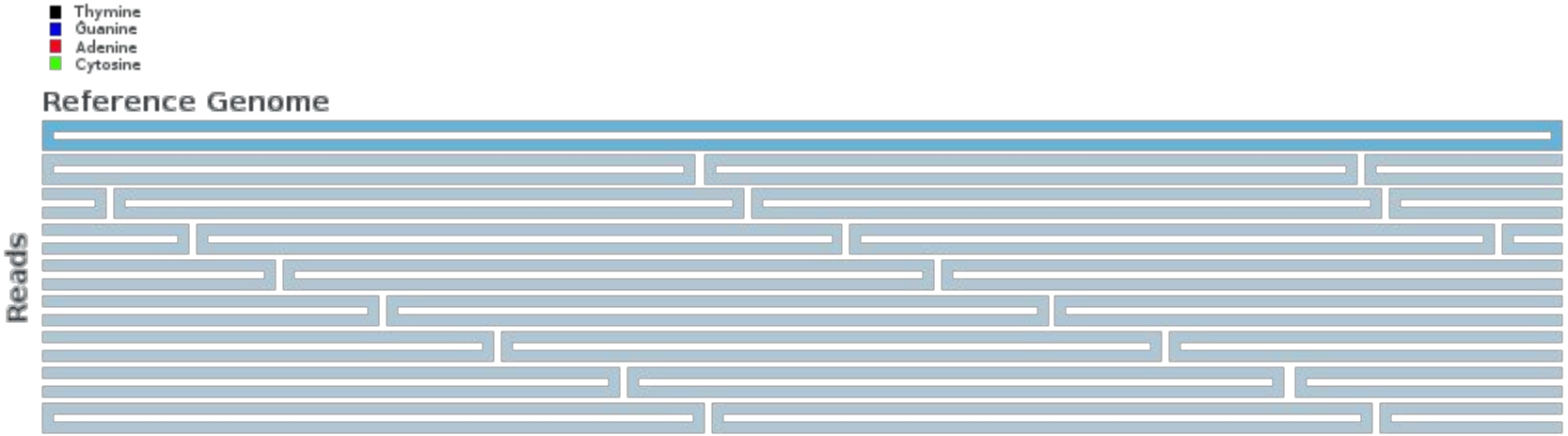
Ideal Variant Calling

Ideal Variant Calling

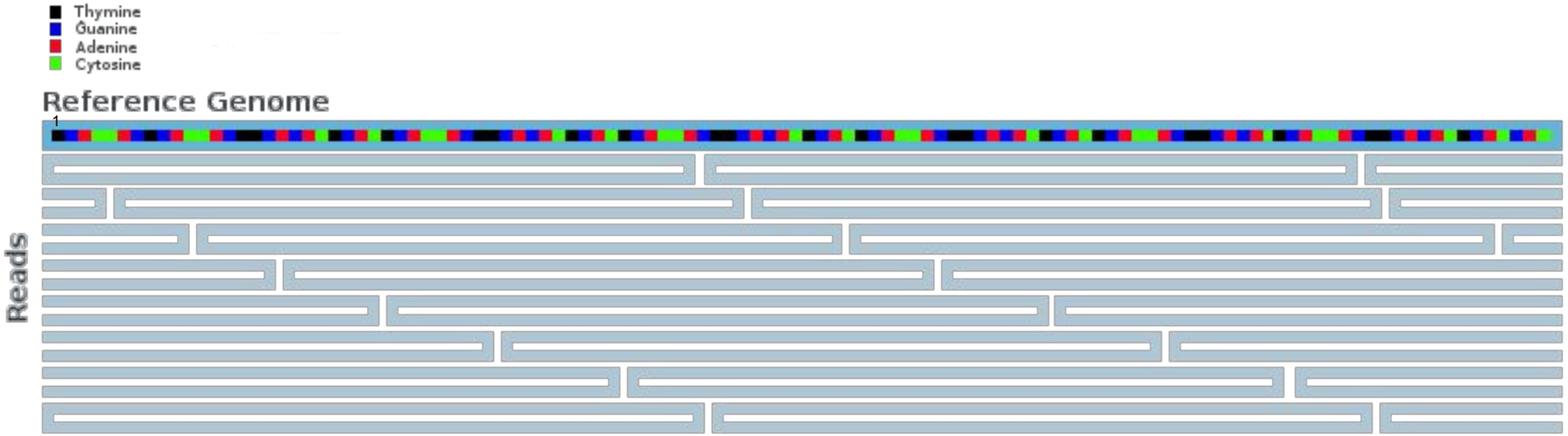


Ideally we will have uniform distribution of reads.

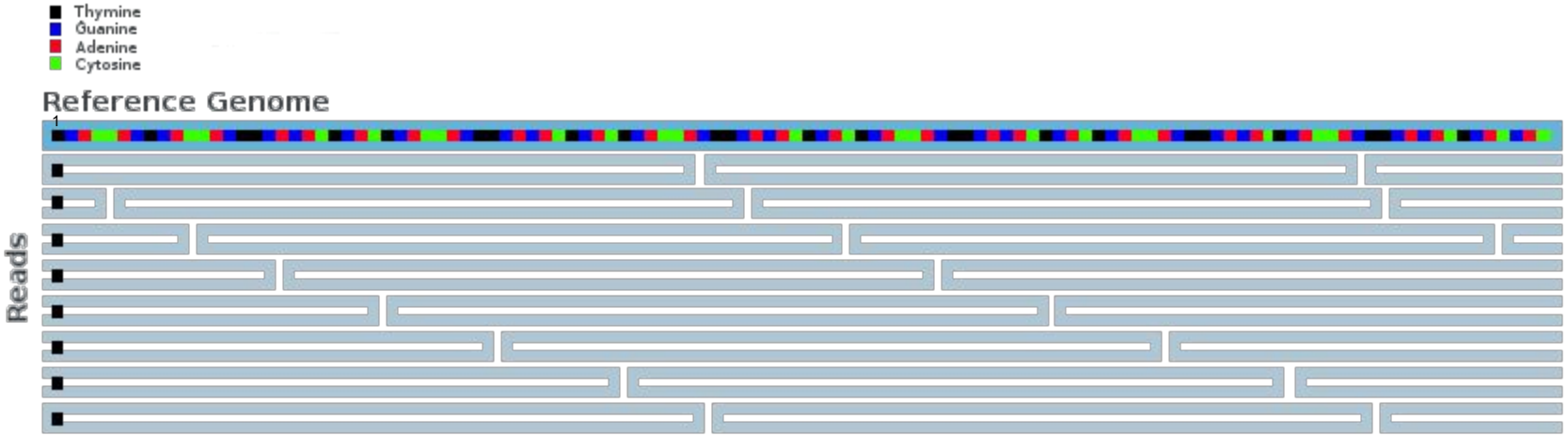
Ideal Variant Calling



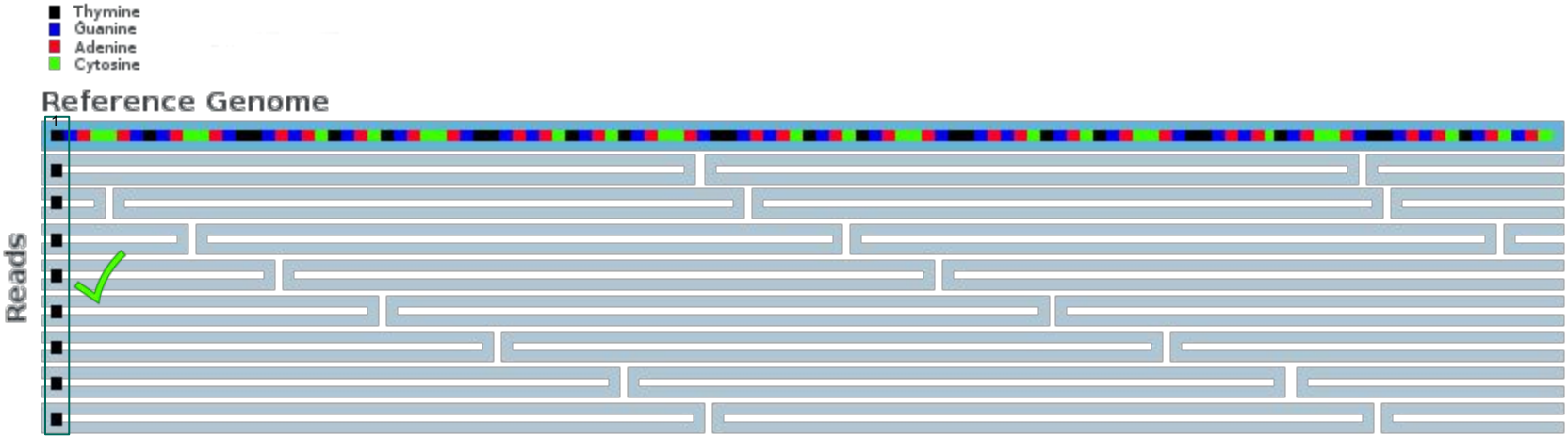
Ideal Variant Calling

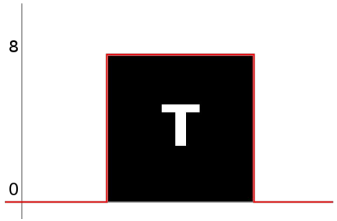


Ideal Variant Calling



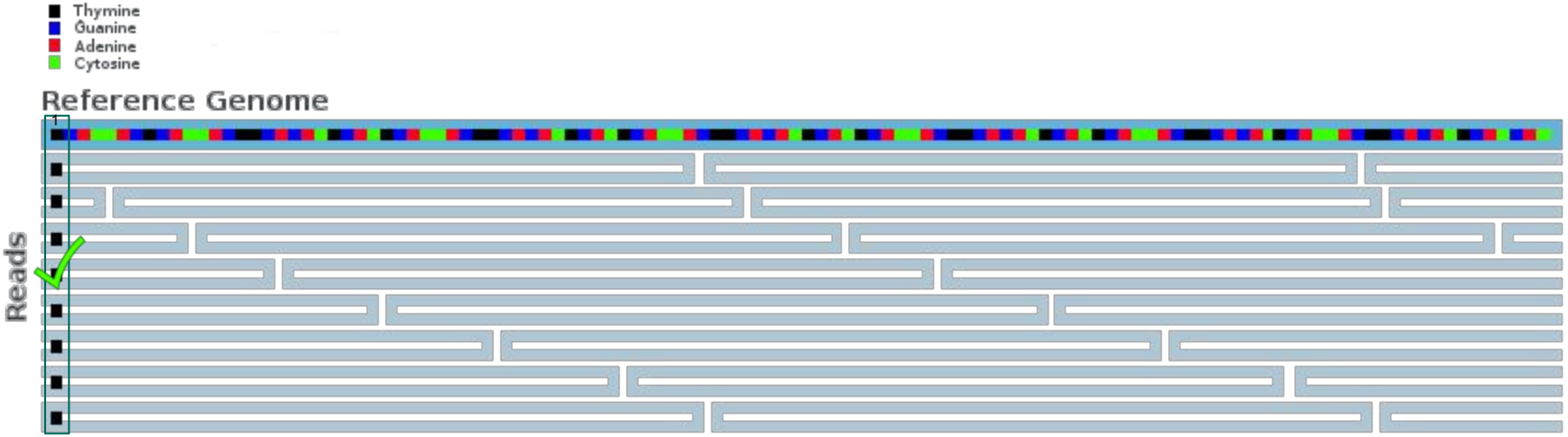
Ideal Variant Calling

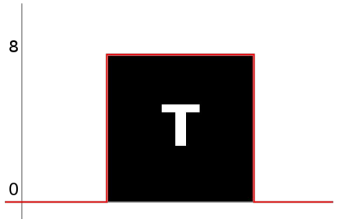




- We have “T” in the all reads covering that position

Ideal Variant Calling

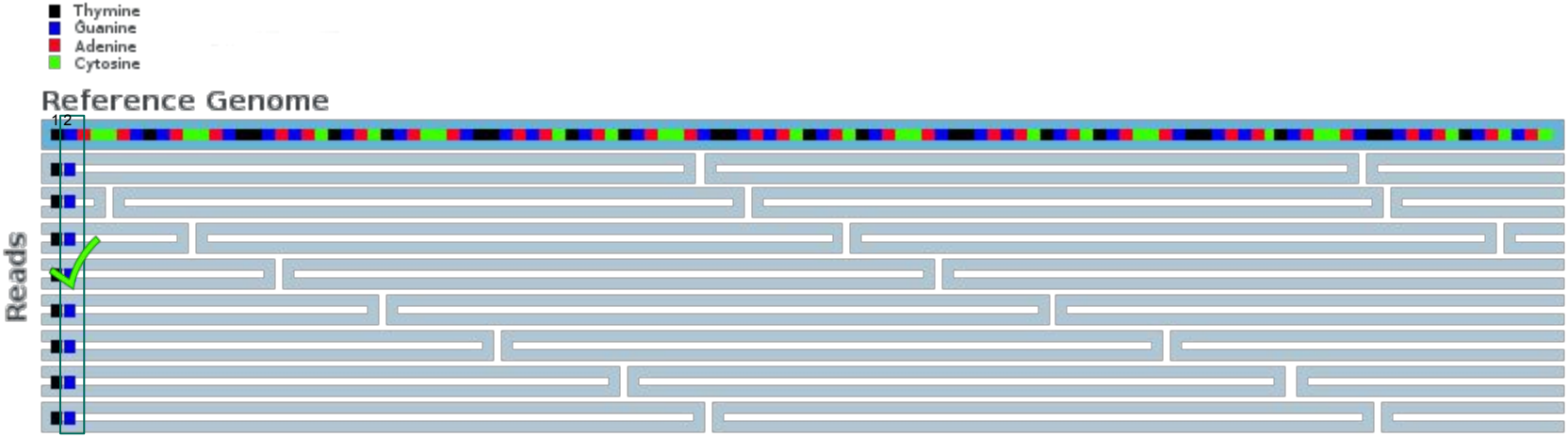


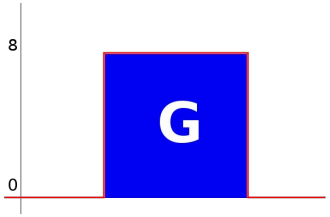


How can we represent what we have observed?

CONTIG	POS	REF	ALT	GT
X	1	T	-	0/0

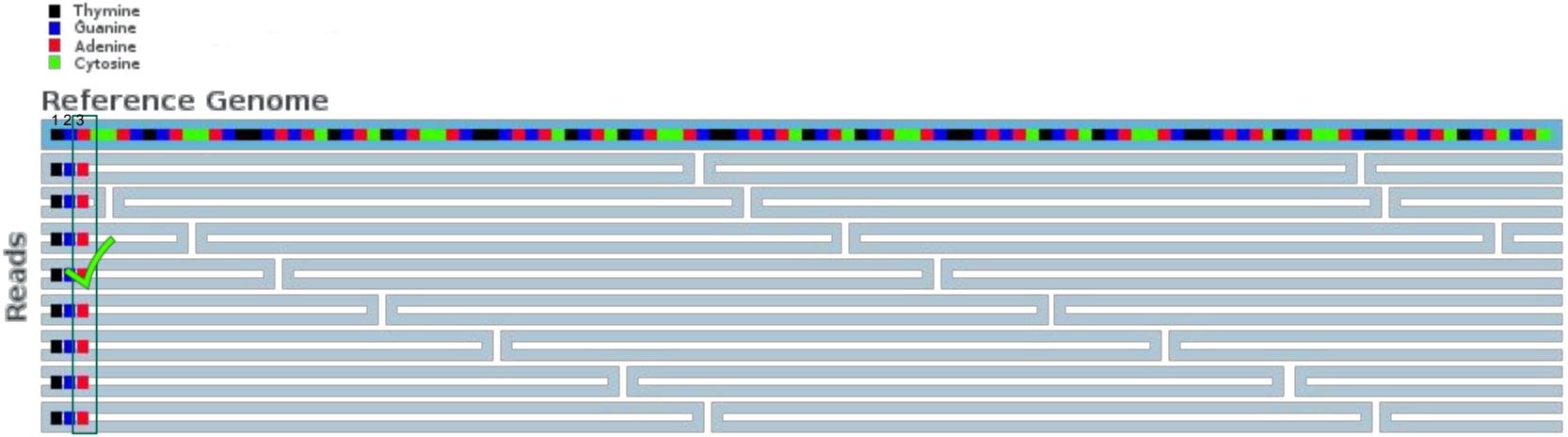
Ideal Variant Calling

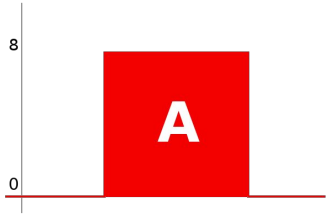




CONTIG	POS	REF	ALT	GT
X	1	T	-	0/0
X	2	G	-	0/0

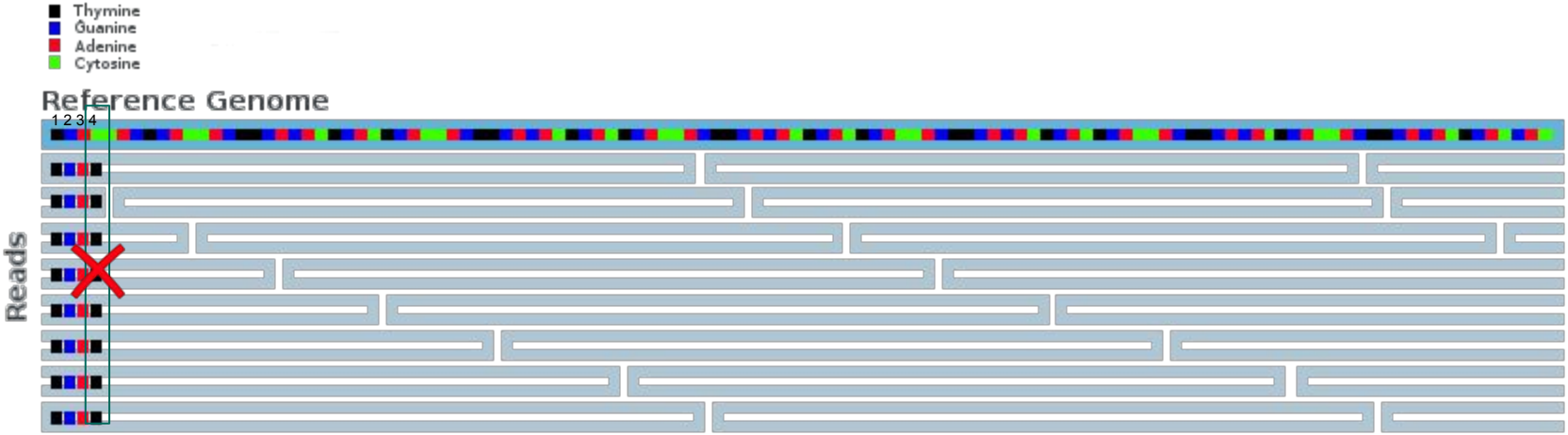
Ideal Variant Calling

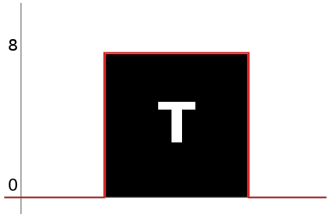




CONTIG	POS	REF	ALT	GT
X	1	T	-	0/0
X	2	G	-	0/0
X	3	A	-	0/0

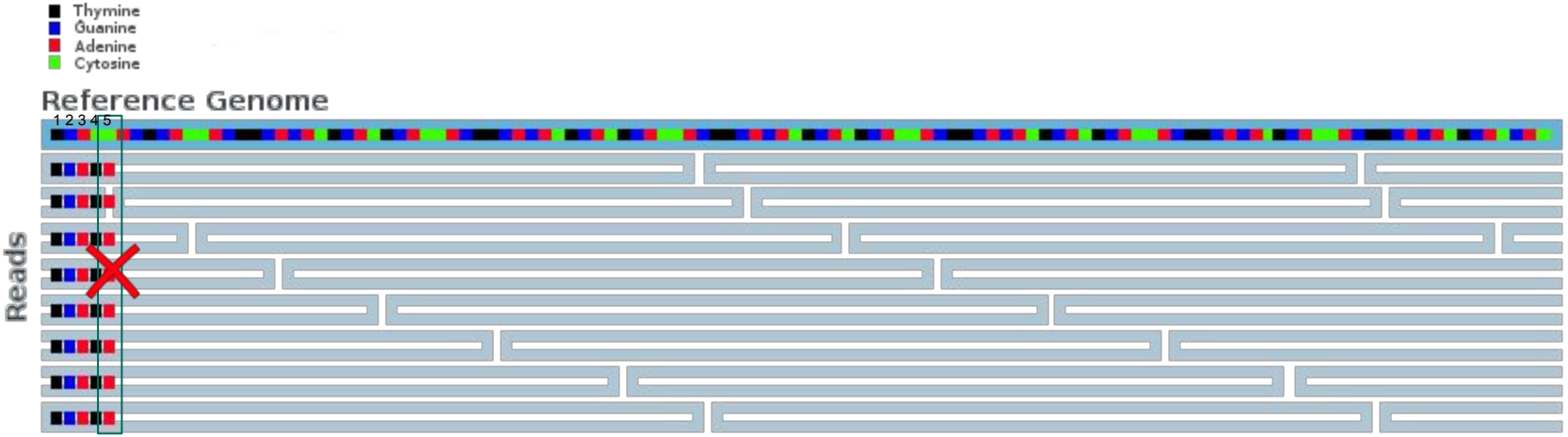
Ideal Variant Calling

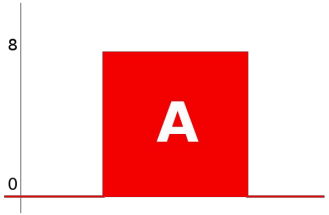




CONTIG	POS	REF	ALT	GT
X	2	G	-	0/0
X	3	A	-	0/0
X	4	**C**	**T**	**1/1**

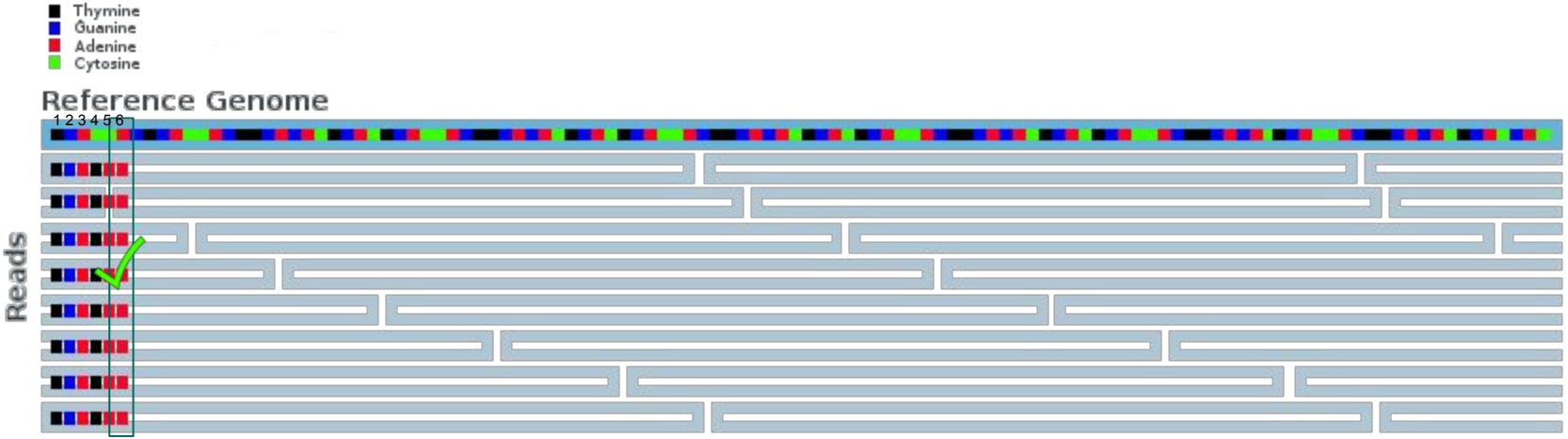
Ideal Variant Calling

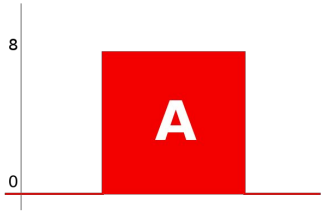




CONTIG	POS	REF	ALT	GT
X	3	A	-	0/0
X	4	**C**	**T**	**1/1**
X	5	**C**	**A**	**1/1**

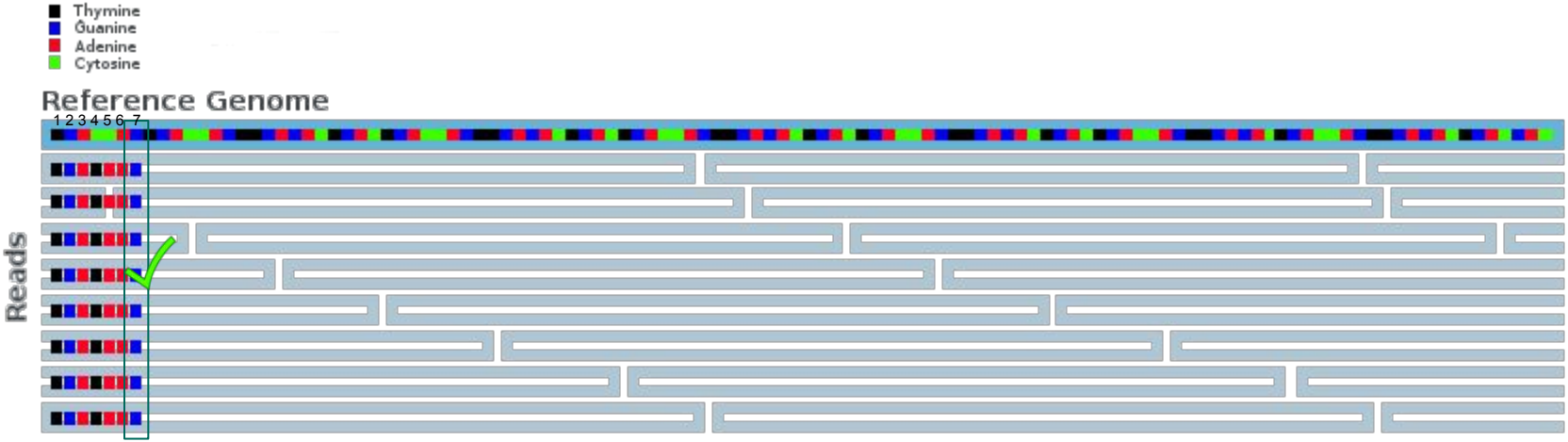
Ideal Variant Calling

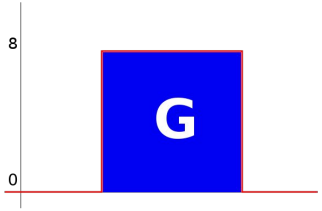




CONTIG	POS	REF	ALT	GT
X	4	**C**	**T**	**1/1**
X	5	**C**	**A**	**1/1**
X	6	A	-	0/0

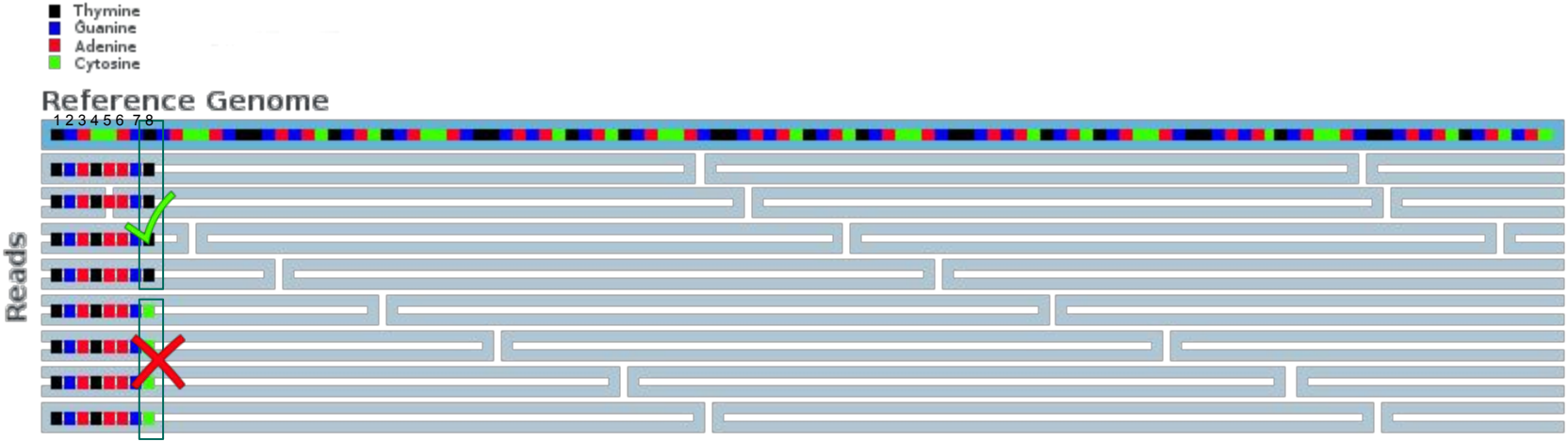
Ideal Variant Calling

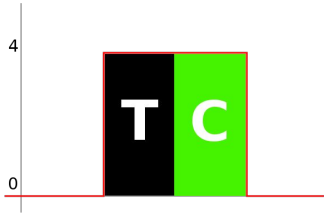




CONTIG	POS	REF	ALT	GT
X	5	**C**	**A**	**1/1**
X	6	A	-	0/0
X	7	G	-	0/0

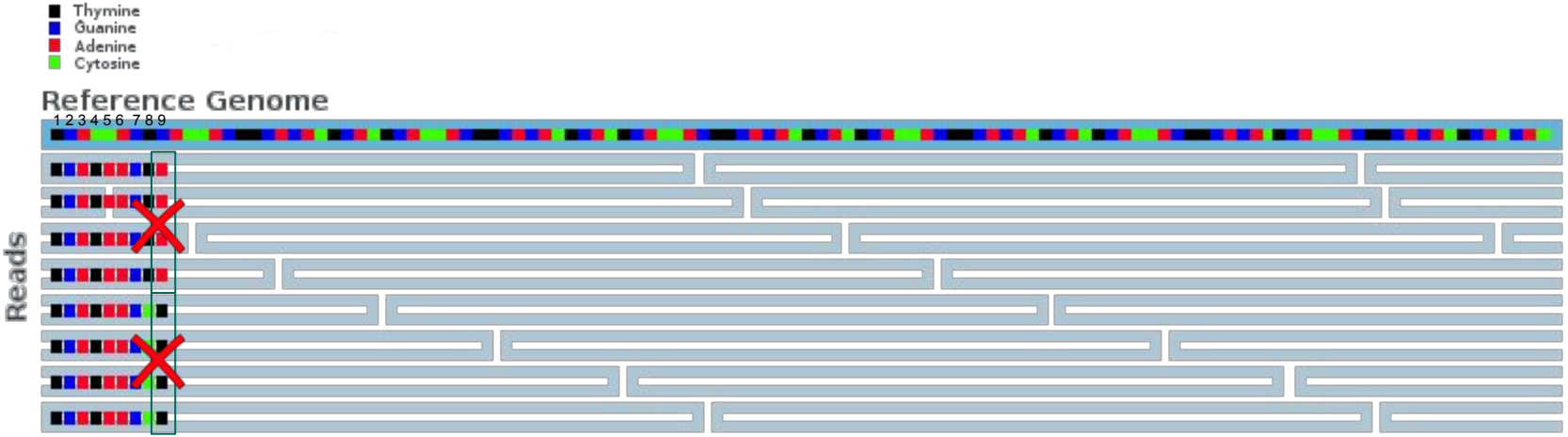
Ideal Variant Calling

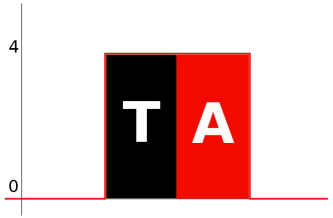




CONTIG	POS	REF	ALT	GT
X	6	A	-	0/0
X	7	G	-	0/0
X	8	**T**	**C**	**0/1**

Ideal Variant Calling





CONTIG	POS	REF	ALT	GT
X	7	G	-	0/0
X	8	**T**	**C**	**0/1**
X	9	**G**	**A,T**	**1/2**

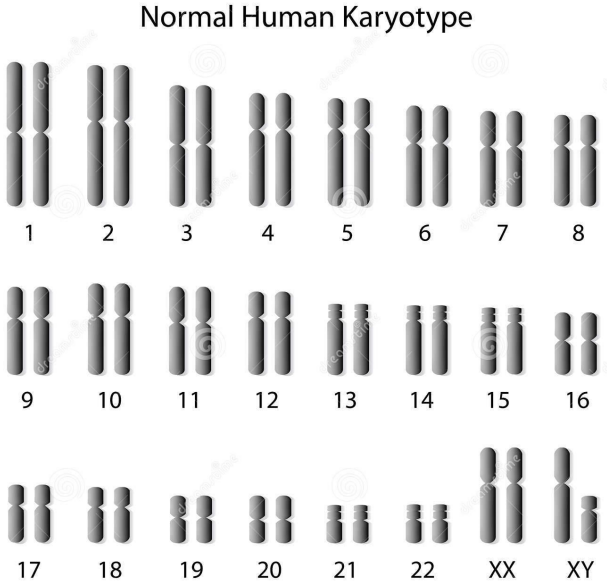
Variant Calling

Two possible cases:

1. All of the bases in pileup are the same nucleotide [A,T,C,G]

2. Different nucleotides exist in the pileup

- In the simplest case, assume diploidy.There can be only two alleles at a site
- If there are more than two different letters in the pileup we will only consider the most common two (assume others are errors and discard them)

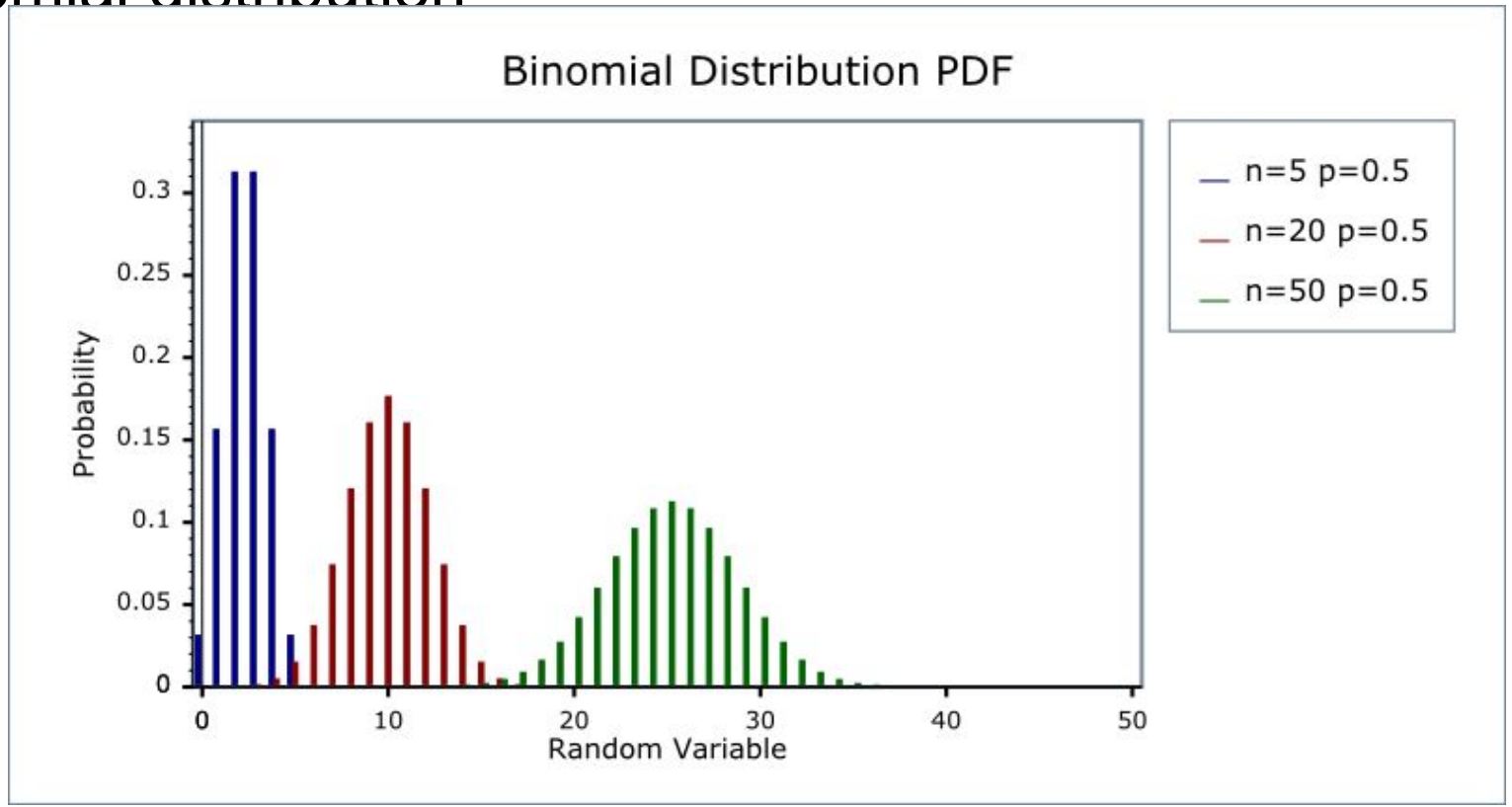


Binomial distribution

- Models the number of successes in a sequence of yes/no experiments
- Parameters:
  - n - number of trials
  - p - probability of a success in a single trial

$$f(k;n,p) = \Pr(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

Binomial distribution



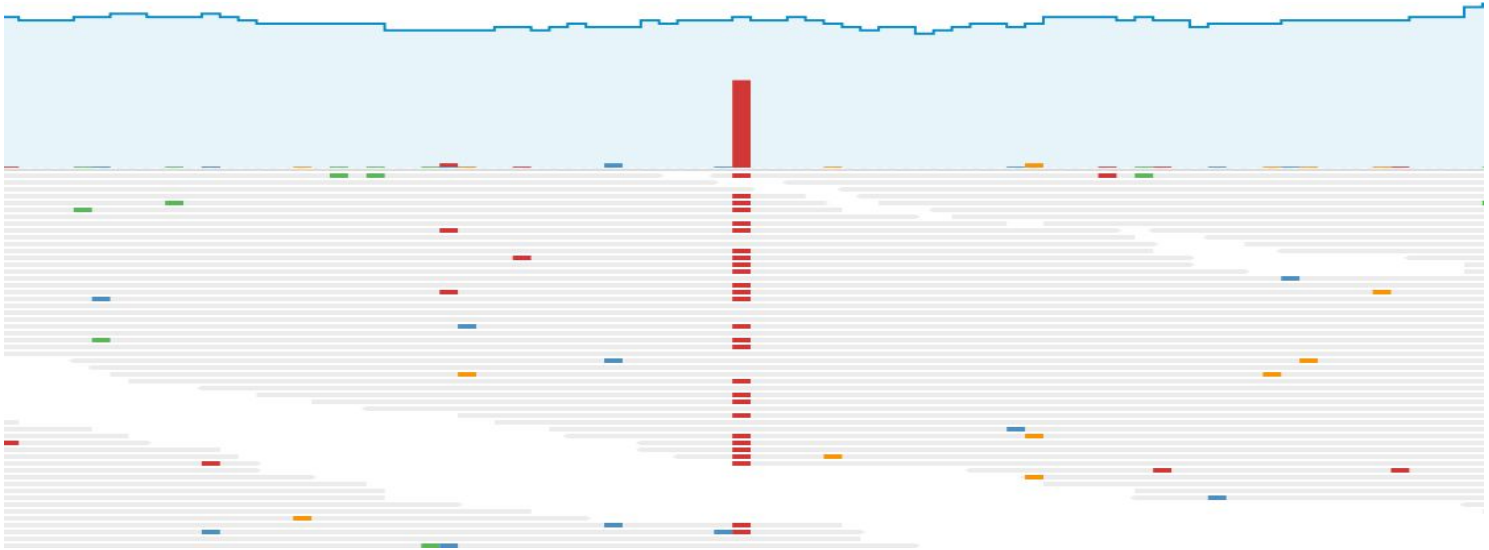
Variant Calling

- So, when we have two letters in the pileup, what should we call?
  - Let's call the two "letters" **b** and **b'** *(b, b' ∈ [A, C, T, G])*
  - Let **n** be the total number of bases, and **k** number of b' bases
  - Three possible explanations for the pileup:
    - Genotype is bb; k bases are errors, n-k are correct
    - Genotype is b'b'; n-k bases are errors, k are correct
    - Genotype is bb'; all n bases are correct
  - Now we need to find the probabilities of these three cases
    Will pick the most probable one!

Variant Calling – advance

- We assumed a flat error rate
  - But we have Base qualities from the sequencer
  - Machine-specific error profiles
- We can look at mapping qualities
  - Mapping errors are a big source of errors
- We can look at haplotypes
  - Errors don’t segregate nicely
- Population-based methods
  - Separate variant calling from genotyping

Variant calling results – check out BAM file



CHR	POS	REF	ALT	FORMAT	NA12877
1	14125	T	C	GT, VAF	0/1, 0.6

Variant calling results

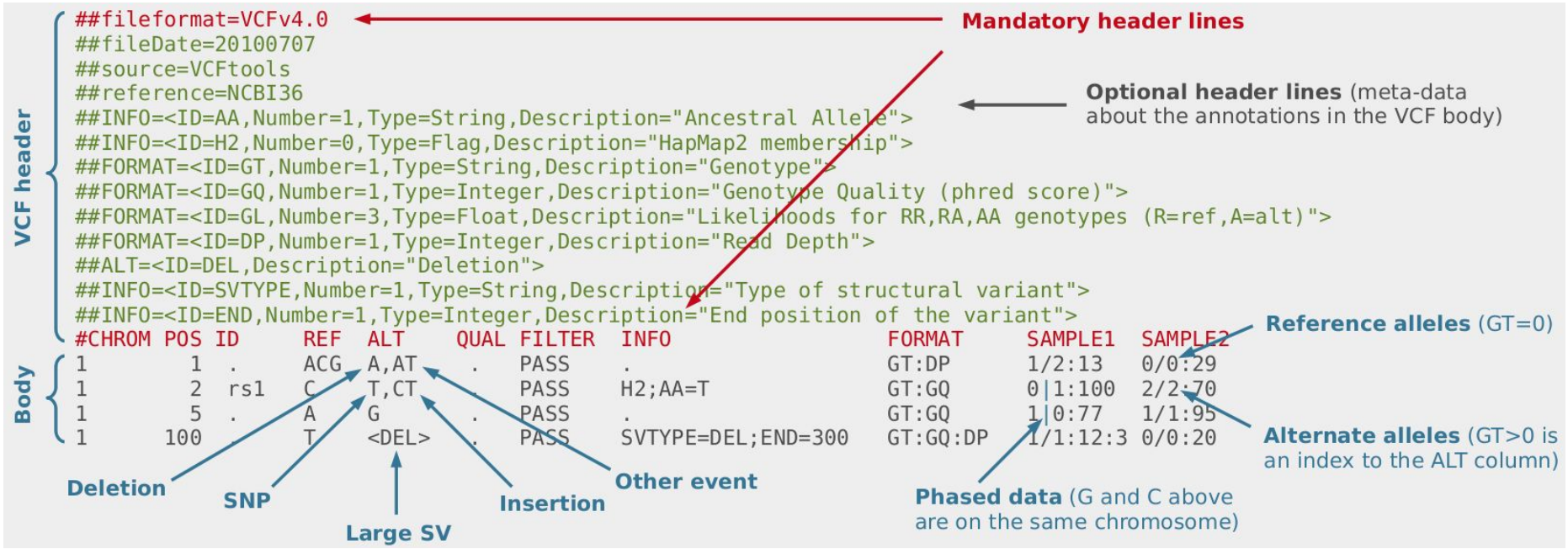
- The result of Variant Calling is a file in VCF format, which contains mutations
- A plain text file format for storing variant data
- A number of line starting with ## -the header
- Main header line:
  #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT SAMPLE1
- This is followed by the actual variant data, one entry per line
  22 10001 . A C 40 PASS DP=14 GT 0/1
- More than one sample can be in one line
- For details: http://samtools.github.io/hts-specs/VCFv4.2.pdf

Variant calling results

- Example of VCF format
- Each row represents one mutation

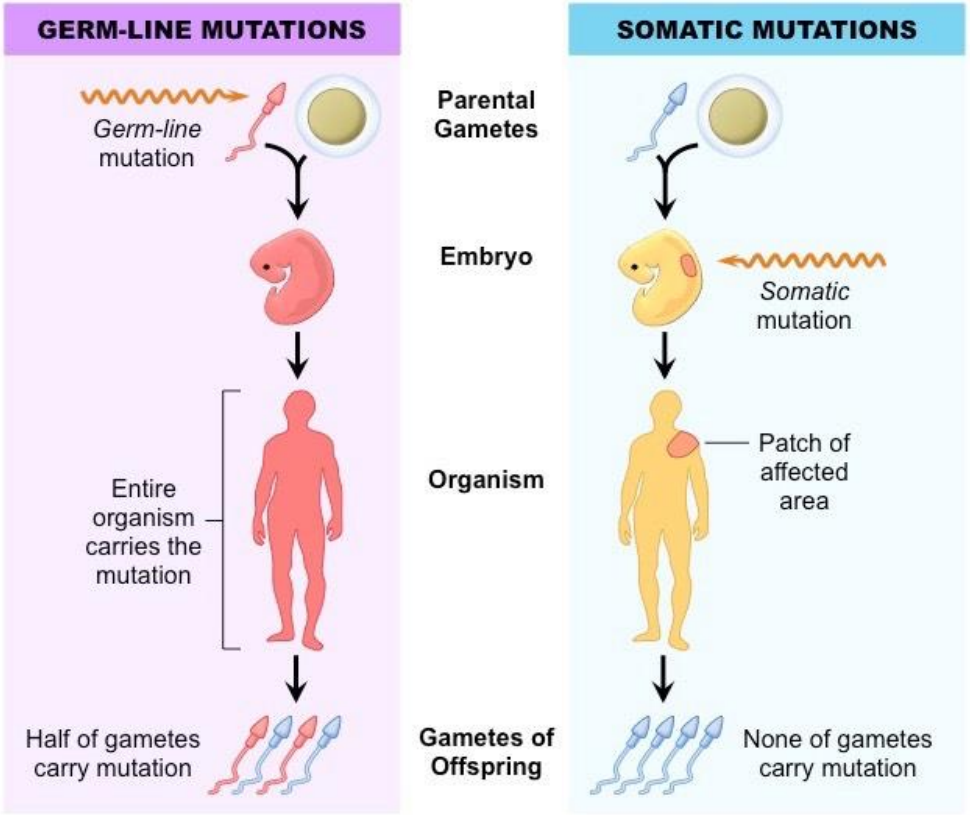
CHR	POS	REF	ALT	FORMAT	NA12878
1	14300	A	G	GT, VAF	0/1, 0.4
2	15367	A	C	GT, VAF	1/1, 0.9
3	25612	C	G,A	GT, VAF	1/2, ?
5	5632	TA	T	GT, VAF	0/1, 0.5
7	7824	T	TA	GT, VAF	1/1, 0.8

Variant Calling Format File

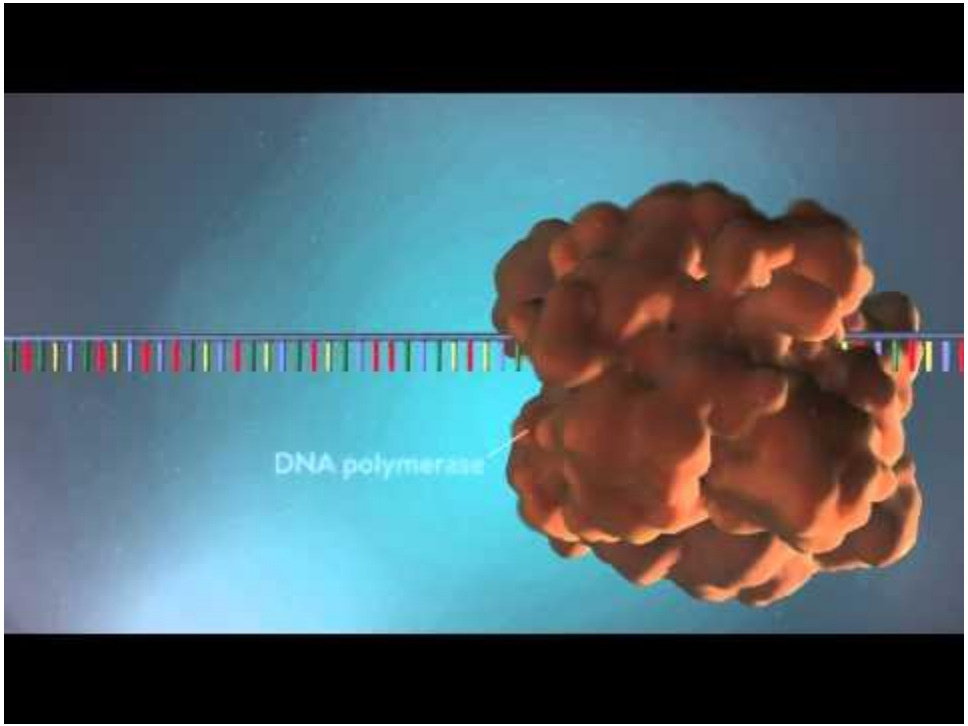


Computational Cancer Analysis

Variants?


GERM-LINE MUTATIONS
SOMATIC MUTATIONS
Parental Gametes
Embryo
Organism
Gametes of Offspring
Germ-line mutation
Somatic mutation
Entire organism carries the mutation
Patch of affected area
Half of gametes carry mutation
None of gametes carry mutation

DNA replication



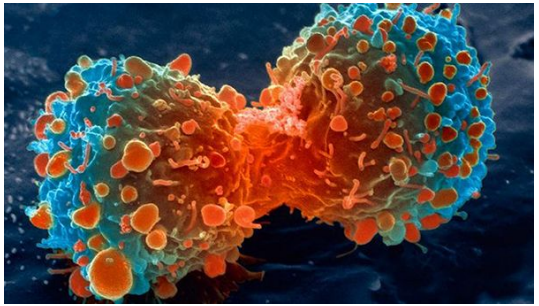
What is cancer?

Mutation during DNA replication can fall to:

1. Intron (no change)
2. Important gene (cell dies, organism lives)
3. Gene that stops cell division (cell lives, organism…)

What causes cancer (increases probability of mutation)?

1. EM radiation
2. Chemical agents
3. Free radicals
4. Genetic factors
5. Infections (viruses)



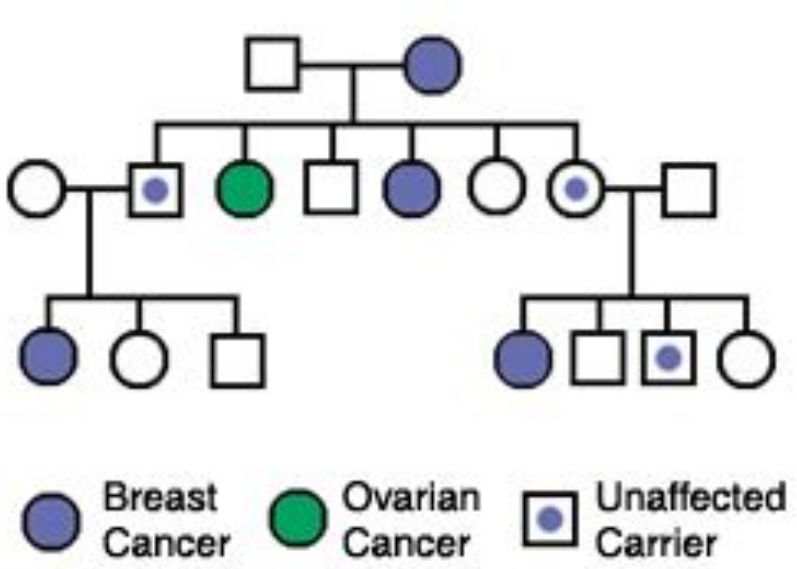
A dividing lung cancer cell.
Credit: National Institutes of Health

Genetic factors

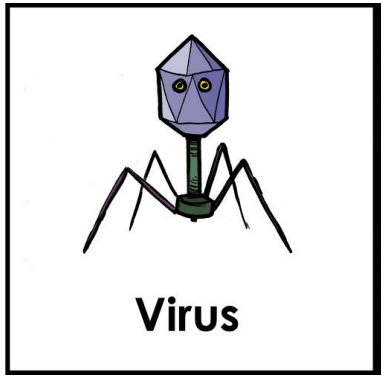
A pedigree from a family with a mutation in the BRCA1 (tumor suppressor) gene

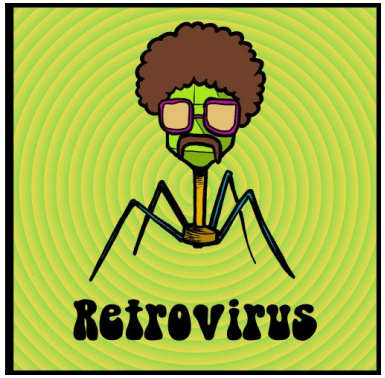
Fathers can be carriers and pass the mutation onto offspring

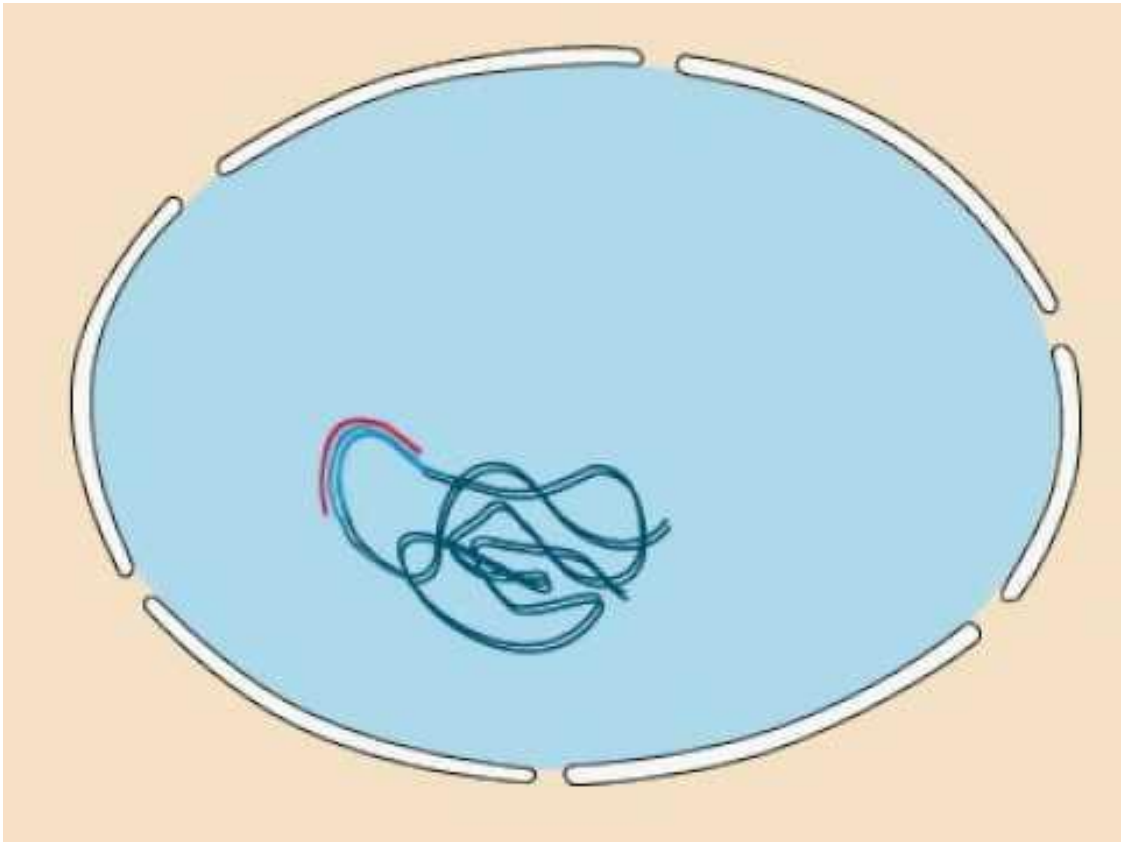
Not all people who inherit the mutation develop the disease, thus patterns of transmission are not always obvious



Viruses and retroviruses

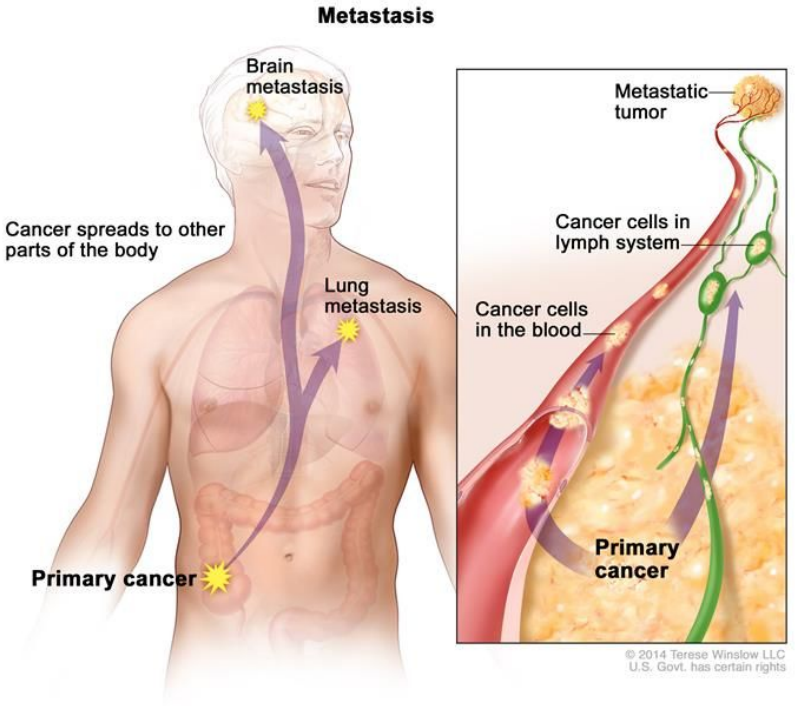




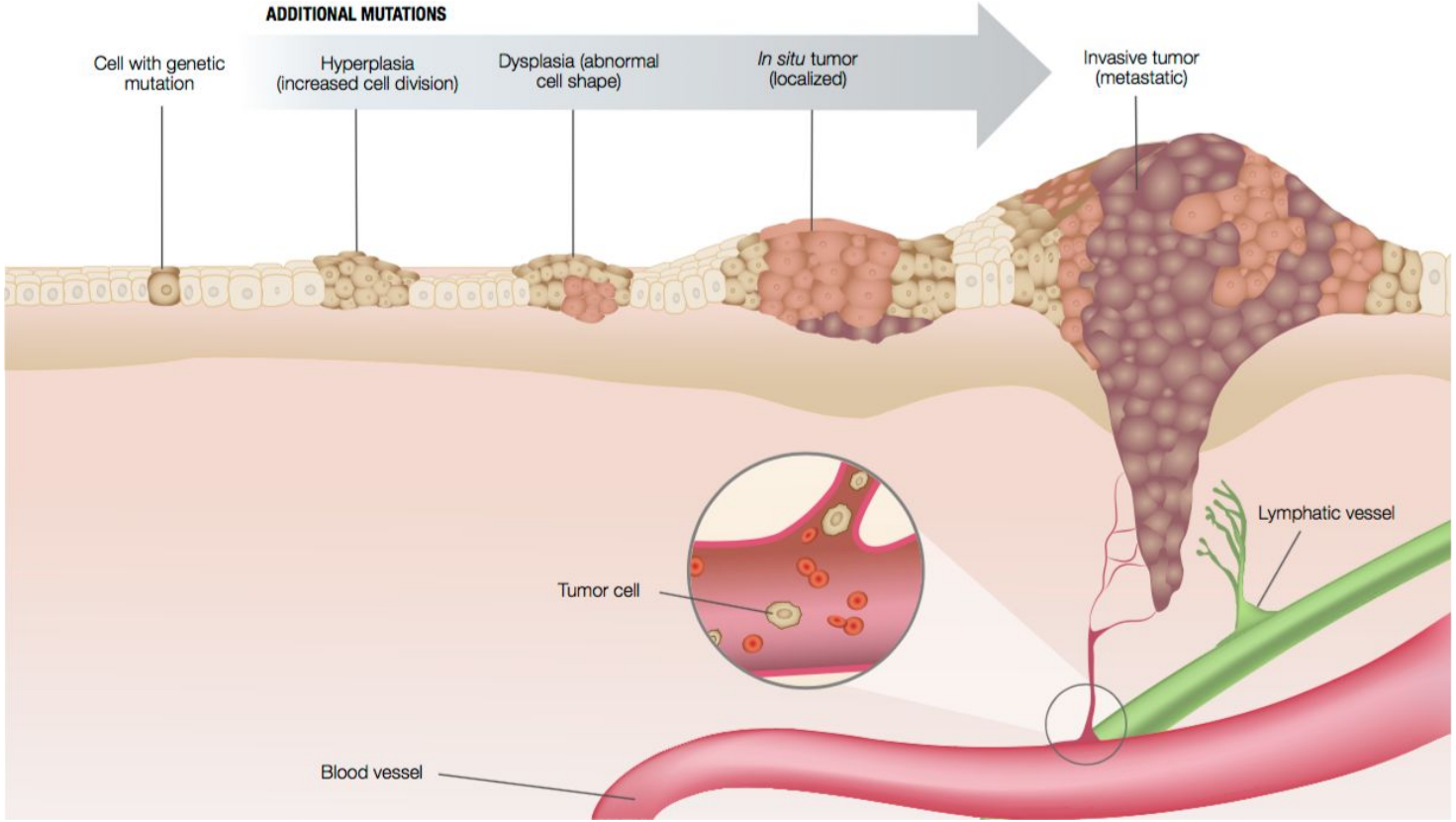


What is metastasis?

Body's cells begin to divide without stopping and spread into surrounding tissues
Cancer cells - ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or **apoptosis**, which the body uses to get rid of unneeded cells



Increased epitel cell division leading to metastasis



"Drivers" of Cancer

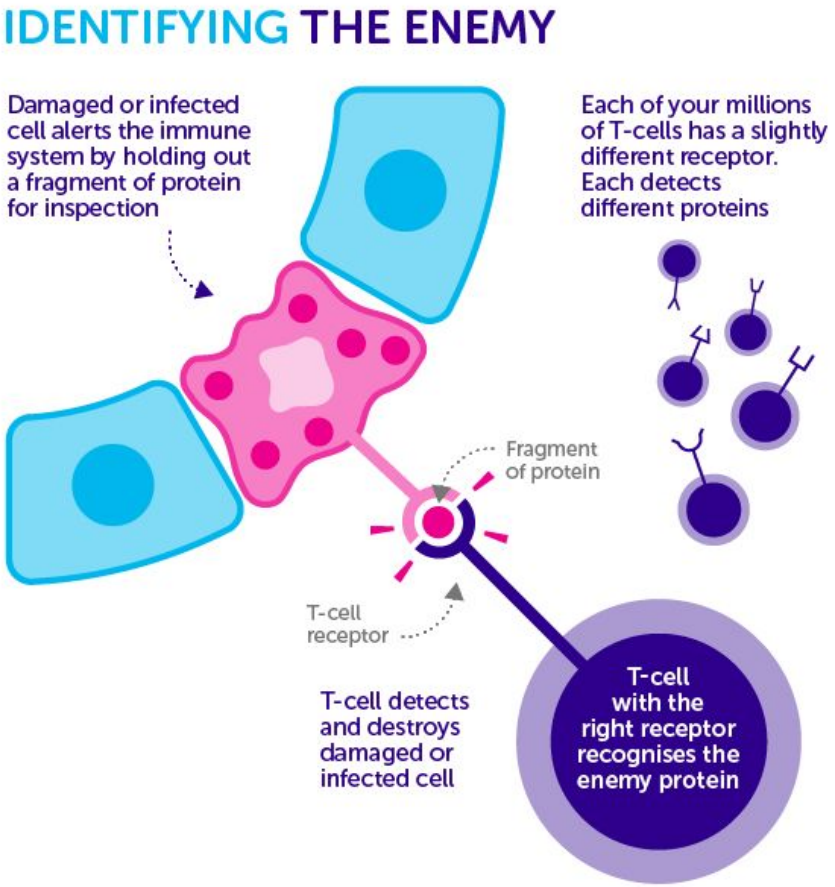
Cancer is a genetic disease that is caused by changes to genes which control the way our cells function, especially how they grow and divide:

1. **Abnormal growth (proto-oncogenes)** Cellular growth mechanism is damaged and cell starts to multiply uncontrollably
2. **Damaged control mechanism** (tumor suppressors) - Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner (TP53 - Apoptosis)
3. **Damaged DNA repair mechanism** (Accumulated errors in this group of genes can lead to uncontrollable proliferation)

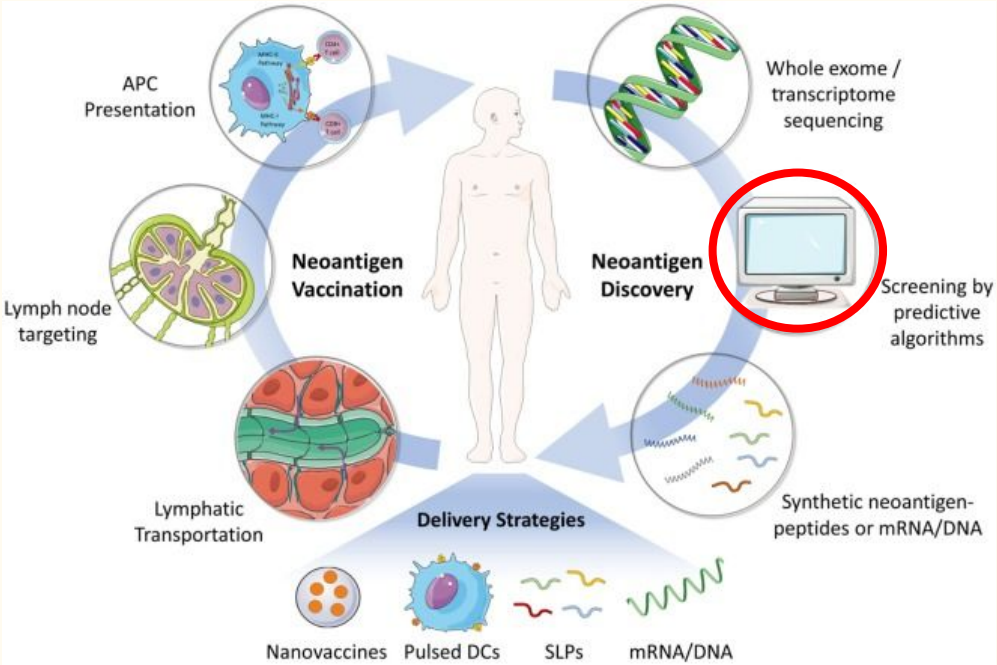
Cancer cells

Our body develops thousands cancer cells every day.

OMG! OMG! OMG!



Neoantigens

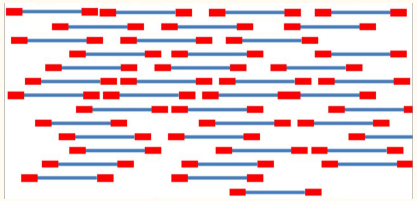


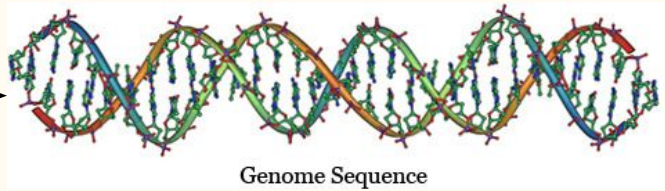
- Neoantigens - proteins presented only by cancer cells
- When neoantigen is known -> immune T-cells can be “programmed” to destroy cancer cells
- These unique cancer markers could be a key to developing a new generation of personalized, targeted cancer immunotherapies

Yugang Guo et all. Neoantigen Vaccine Delivery for Personalized Anticancer Immunotherapy

How can we discover neoantigens?

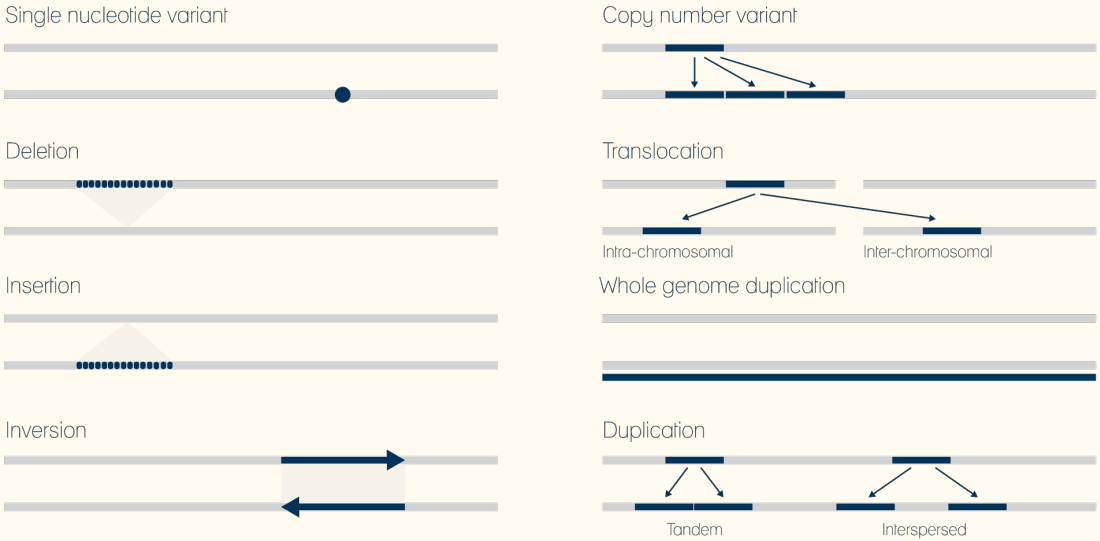
1. Reconstruct DNA of tumor and normal tissue



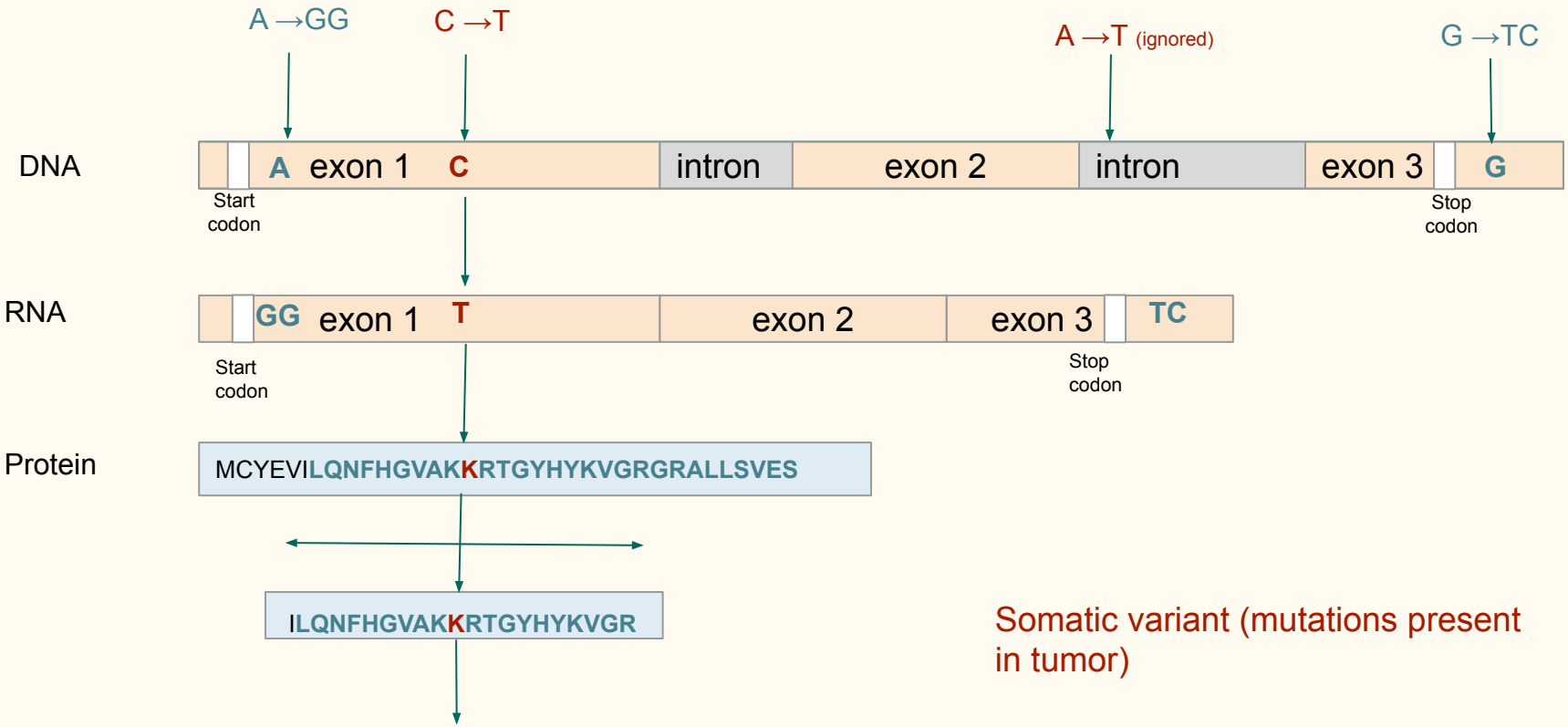


How can we discover neoantigens?

2. Compare DNA from Tumor and Normal tissue



How can we discover neoantigens?



Somatic variant (mutations present in tumor)

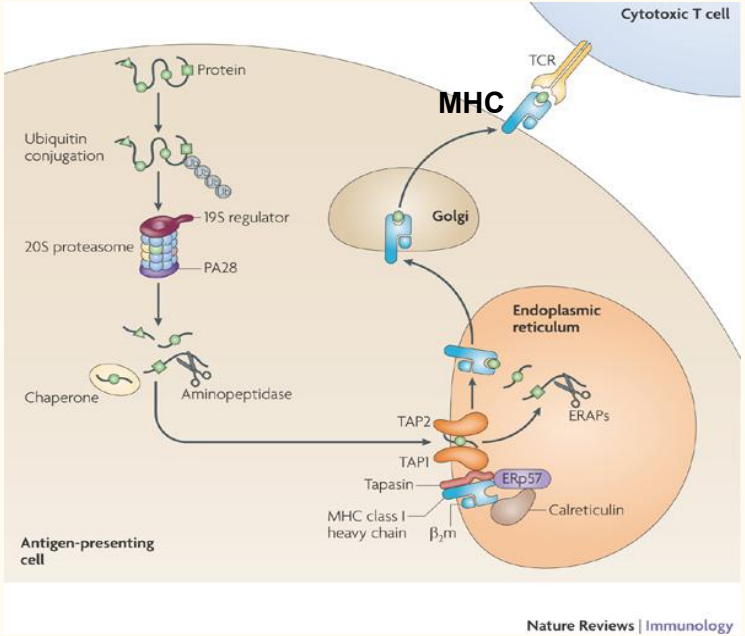
3. Protein extraction

How can we discover neoantigens?

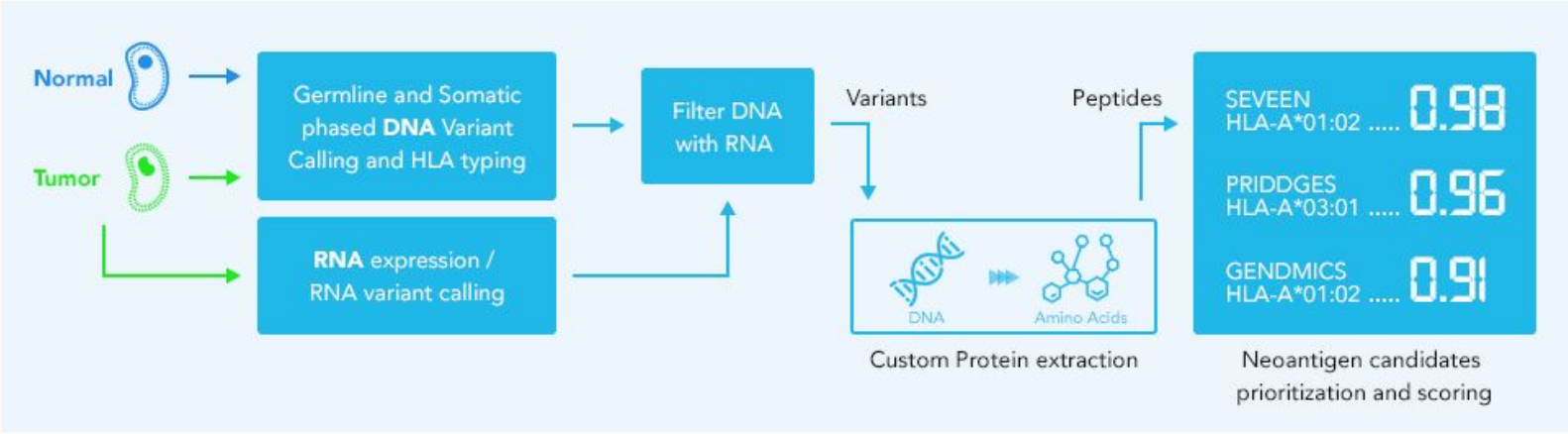
4. Discover HLA type from genome (translates to MHC molecule)

5. Perform scoring of protein-HLA sets (IEDB processing tools)

Mutation	HLA type	peptide	NetMHC score	Pickpocket score	NetCTLPan score	RNA expression
1_111957245_C_A	HLA-A*02:01	MMLSSSPV	0.881	0.633	1.09815	11.5
8_144392368_T_C	HLA-A*02:01	WLLEKLEQL	0.828	1.097	1.06133	12.5
17_28537638_C_T	HLA-A*02:01	VLDEFPHV	0.836	0.374	1.06015	23



Neoantigen workflow



References

How the Immune System Works - Lauren Sompayrac

Seven Bridges