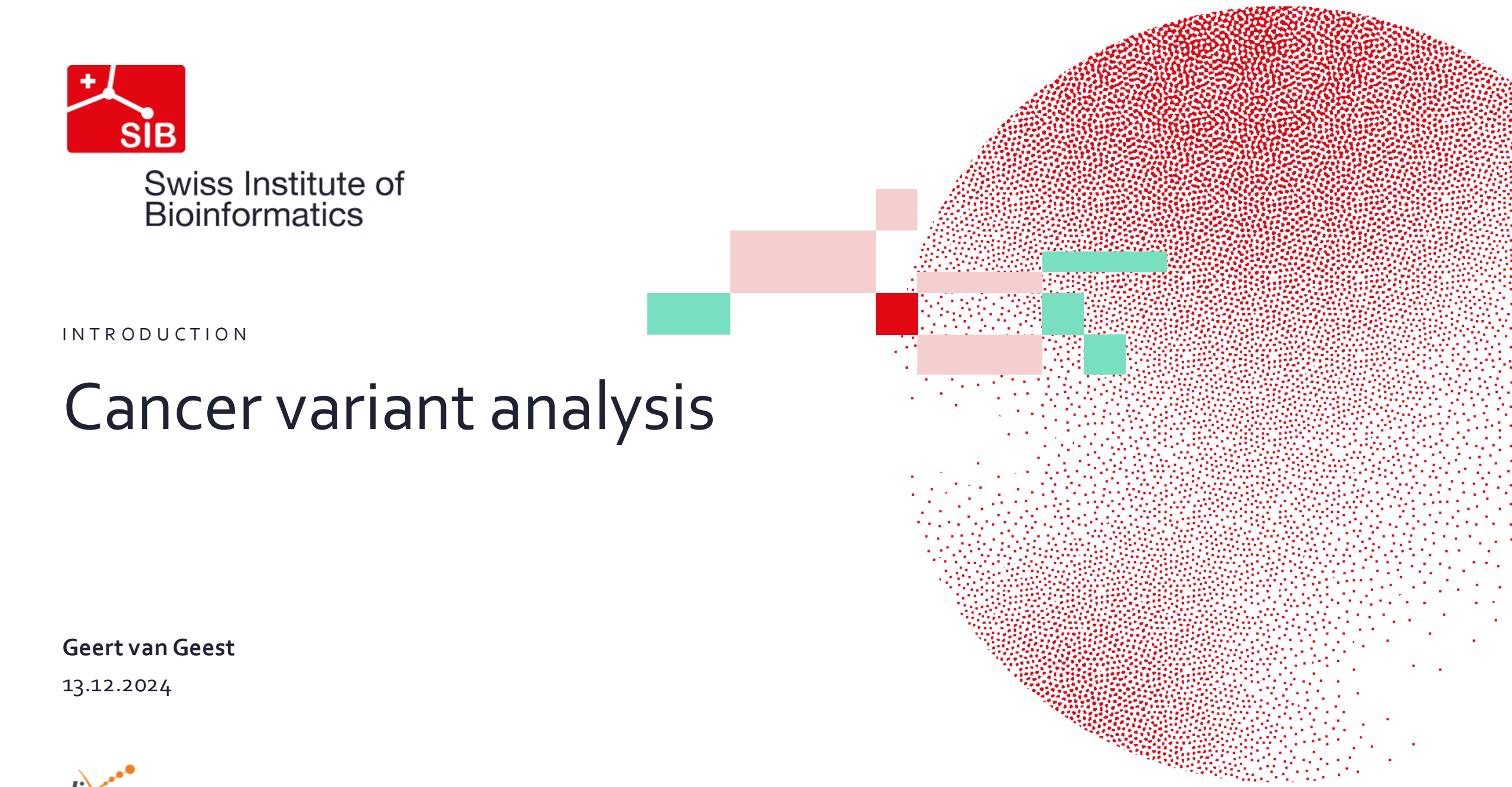

Swiss Institute of Bioinformatics

INTRODUCTION

Cancer variant analysis

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13.12.2024

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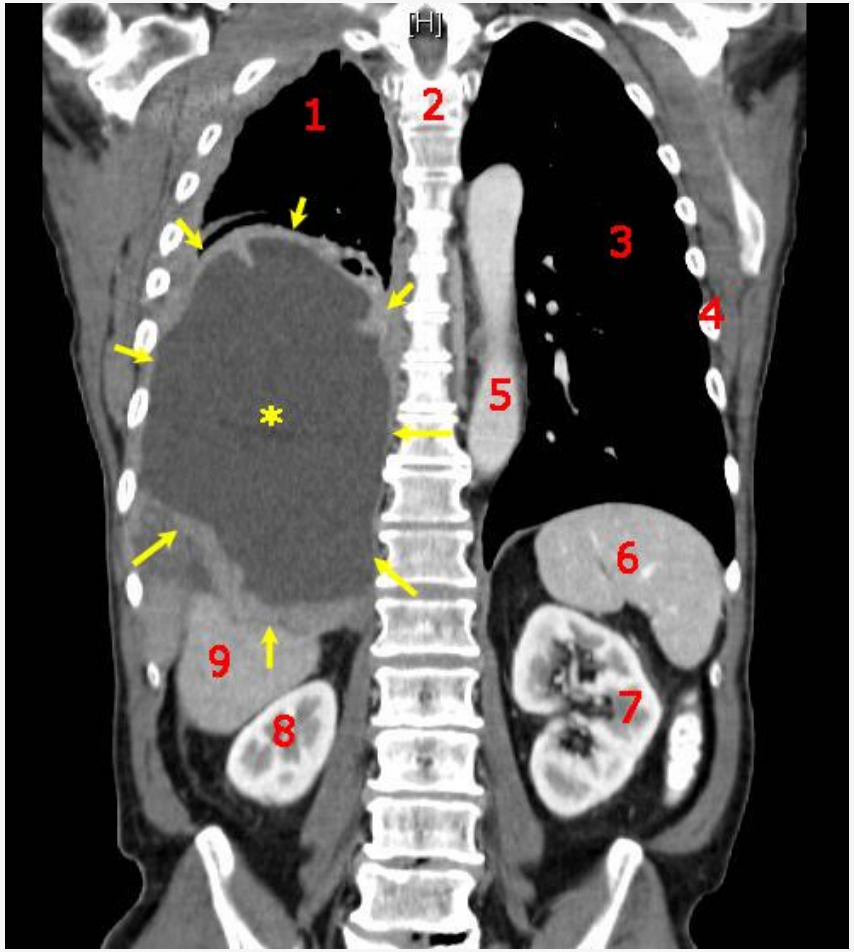
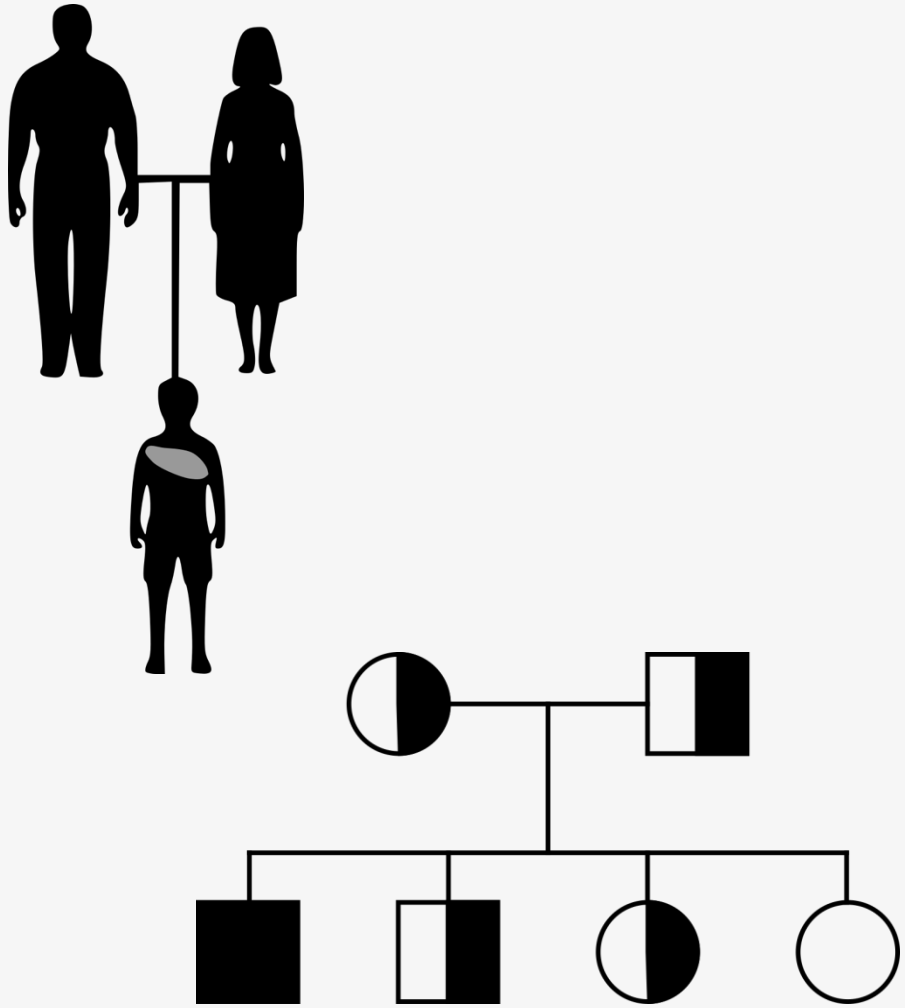
Cancer is a disease of the genome

- Abnormal cell growth
- Potential to invade or spread
- Mostly initiated by **acquired** genomic **mutations** affecting **genes** regulating **cell growth**
- Mutations occur **by chance**, but their rate can be affected by **environmental factors**
- Natural **selection** of **malignant** cells – mutations build up



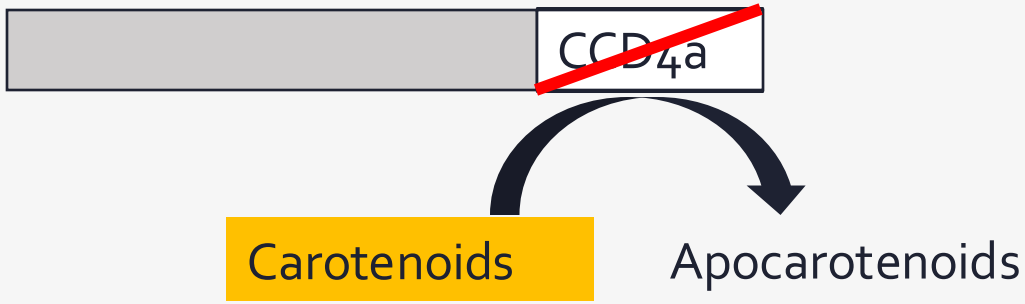
Variants – some definitions

- **Variant**: a difference between DNA
- Caused by a **mutation**: the process of a change in DNA
- **Somatic variant**: occurs only in a part of an organism
- **Germline variant**: can be passed on the next generation
- **Polymorphism**: variant that is common in a population



Variant – an example

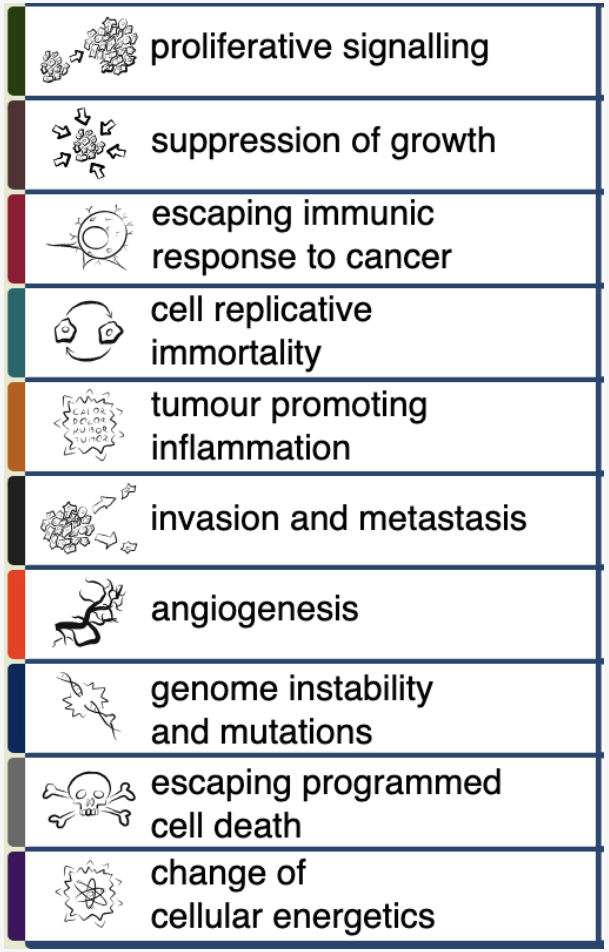
- **Mutation**: the change in DNA that caused the petals to turn yellow
- **Variant**: the difference between the DNA in the yellow petals and the wild type



Question

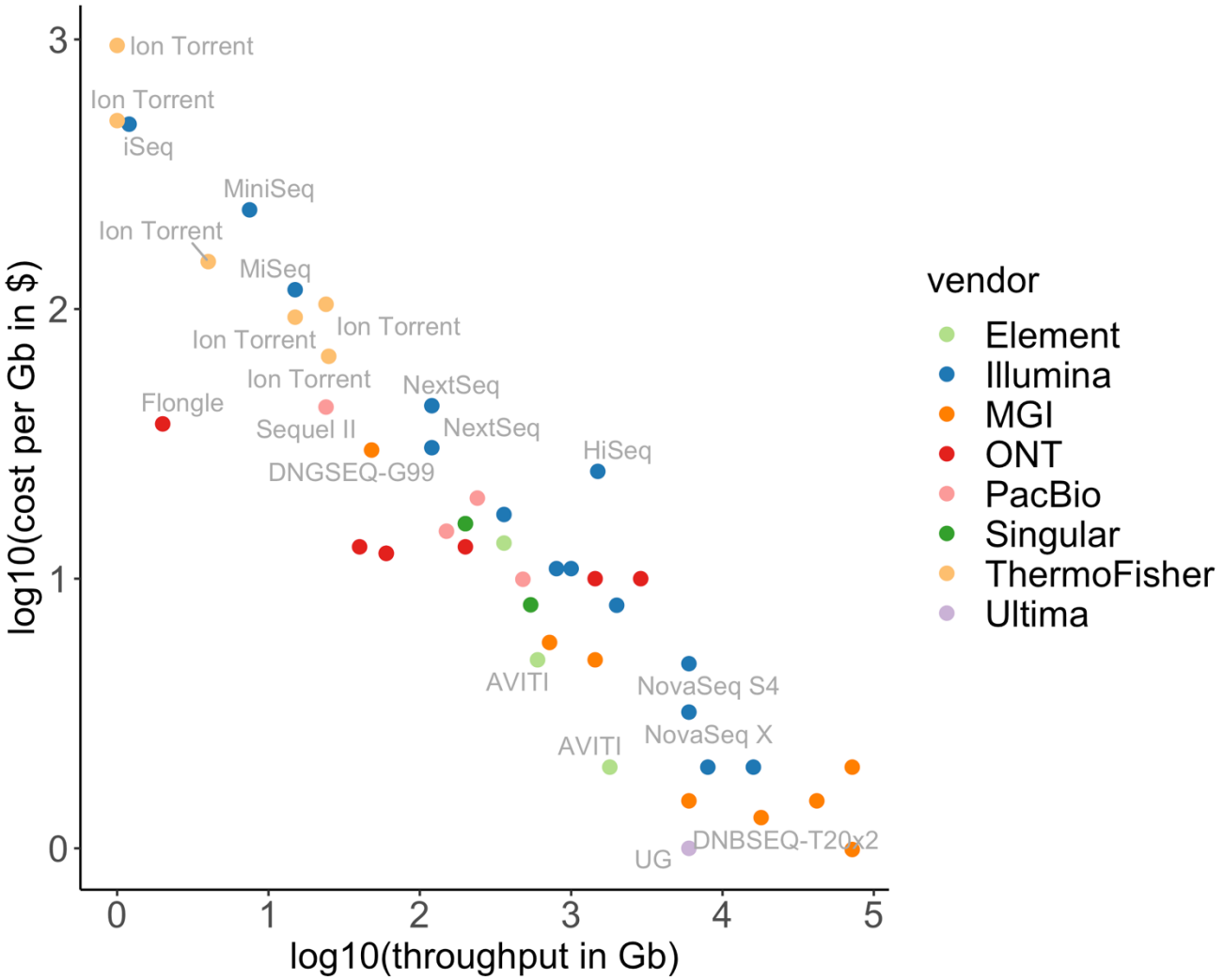
Types of mutations in cancer

- **Small mutations** (SNVs INDELs)
- **Copy Number Variation** (CNV)
  - Loss of heterozygosity (LOH)
- **Structural variation**
  - Fusion transcripts



Mutation detection - sequencing

- Whole genome sequencing
- Bait capture
  - Whole exome sequencing
  - Custom panels
- Sequencing methods:
  - Short reads (Illumina, MGI, Element)
  - Long reads (PacBio, ONT)

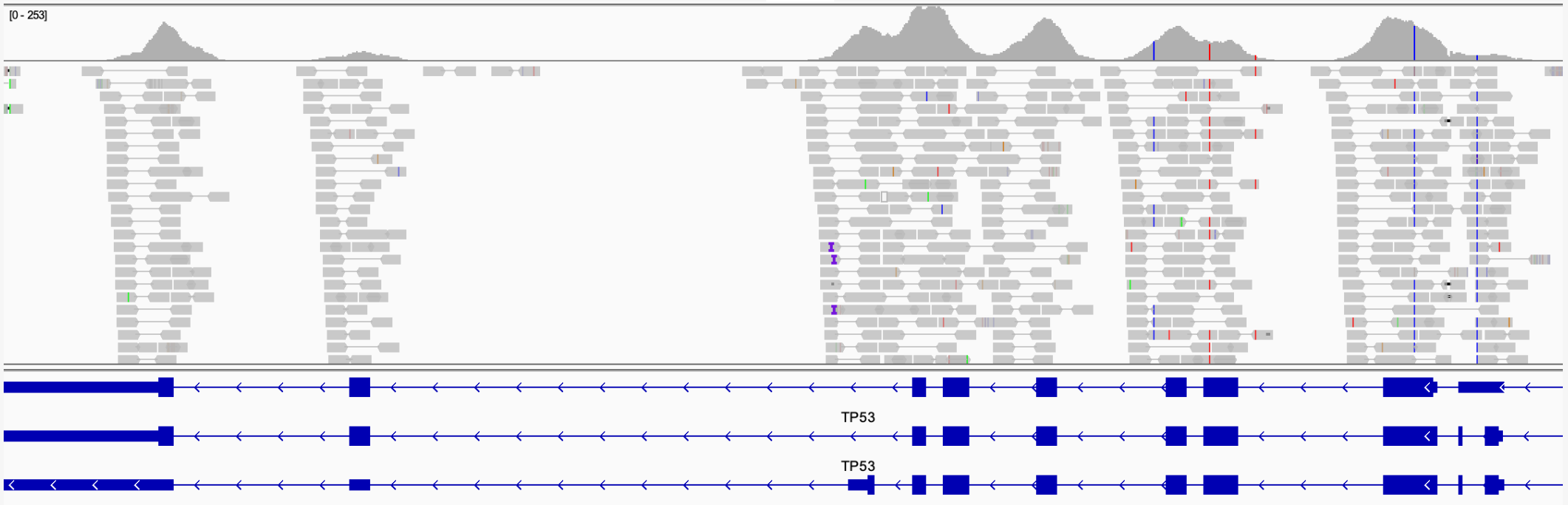


2 questions on NGS

Bait capture

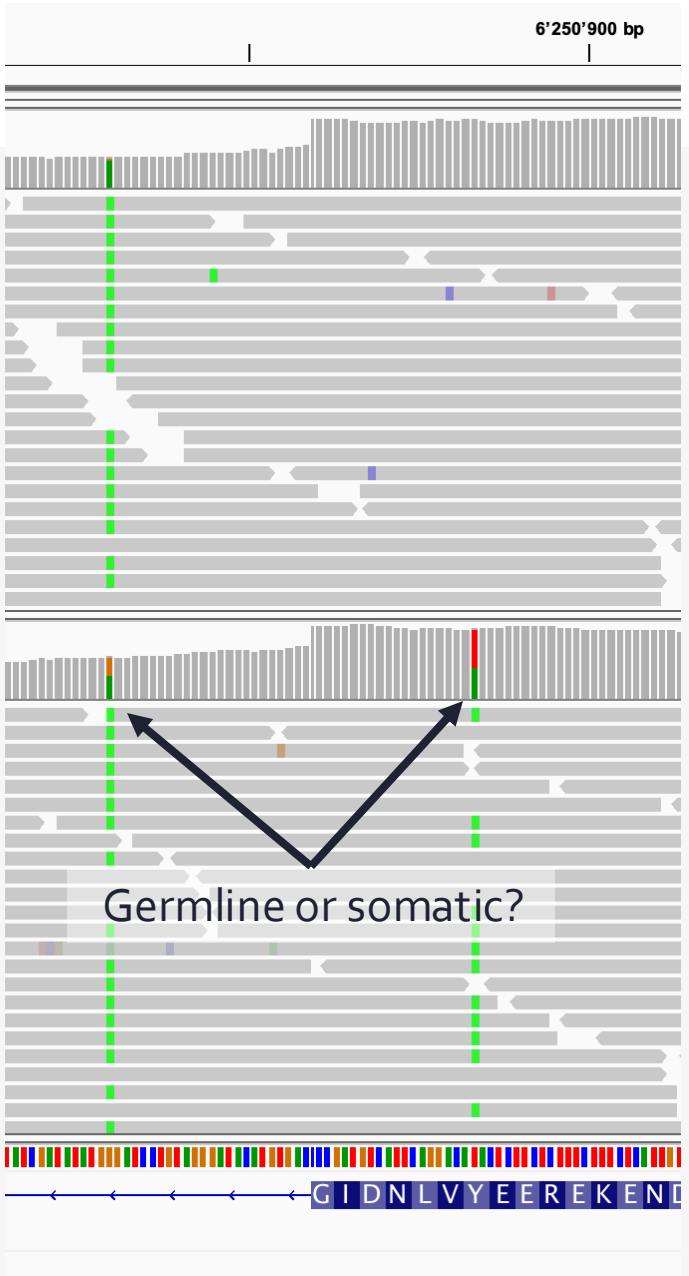
- RNA/DNA baits are designed for regions of interest
- Library prep as usual
- Capture with biotinylated baits

- (Much) lower sequencing and processing costs :
  - WES 100x: 25 M 2 x 100 bp
  - WGS 30x: 450 M 2 x 100 bp
- Not sequenced regions can contain valuable information, e.g. structural variation



Experimental design

- Tumor tissue is often not purely ‘tumor’. It contains e.g. immune cells.
- How to discriminate between germline and somatic variation?
- Per patient two samples:
  - Tumor
  - Normal (e.g. blood)



Downstream processing

- **Adapter removal**
  - e.g. fastp
- **Alignment**
  - bwa-mem or dragen
- **Adding read groups**
  - during alignment or e.g. samtools addreplacerg

- **Base quality score recalibration**
  - GATK framework
- **Deduplication**
  - gatk MarkDuplicates
- **Coverage/enrichment evaluation**
  - e.g. mosdepth or gatk CollectHsMetrics

Marking duplicates

- Variant caller assumes each fragment is **unique representation** of the genome
- PCR/optical **duplicates** are **not**
- **Marking** PCR/optical duplicates **improves** variant calling **accuracy**
- Adding Unique Molecular Identifiers (**UMI**) strongly improves **duplicate marking accuracy**

The sam/bam file format

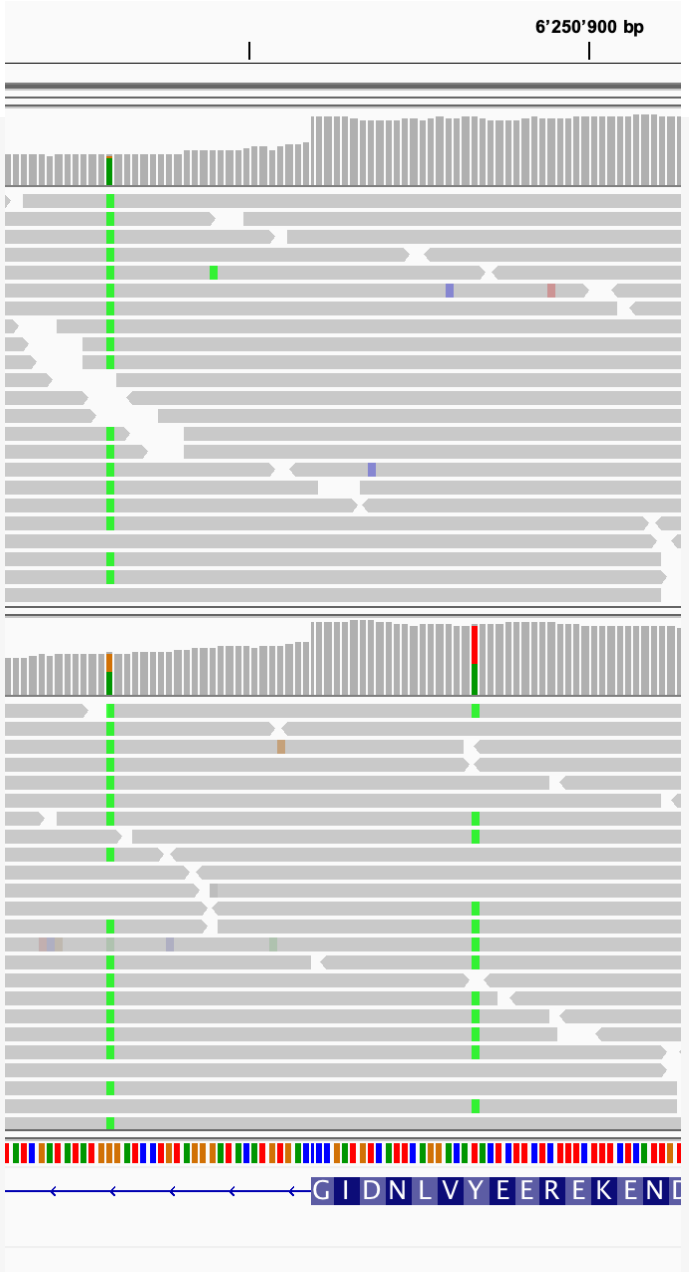
- Usage: store alignments
- bam = compressed sam
- Important format to understand!


```
@HD    VN:1.6  SO:coordinate
@SQ    SN:chr6 LN:170805979
@SQ    SN:chr17        LN:83257441
@PG    ID:bwa  PN:bwa  VN:0.7.17-r1188 CL:bwa mem ref_genome.fa normal_R1.fastq.gz normal_R2.fastq.gz
@PG    ID:samtools     PN:samtools     PP:bwa  VN:1.21 CL:samtools sort

HWI-ST466:135068617:C1TD1ACXX:7:1114:9733:82689 163     chr6    60001   60      100M    =       60106   205     GATCTTATATAACTGTGAGATTAATCTCAGATAATGACACAA
CCCFFFFFHHHHHJJIJHIJJEIJIJJJJJIJJIJJJJIJJ     NM:i:0  MD:Z:100 MC:Z:100M AS:i:100 XS:i:0

HWI-ST466:135068617:C1TD1ACXX:7:1303:2021:90688 99      chr6    60001   60      100M    =       60104   203     GATCTTATATAACTGTGAGATTAATCTCAGATAATGACACAA
@CCFFFFFFGGHHIIFHGIEGHJICEHIJJIIJIJEGHIIJJ     NM:i:0  MD:Z:100 MC:Z:100M AS:i:100 XS:i:0

HWI-ST466:135068617:C1TD1ACXX:7:2304:7514:30978 113     chr6    60001   60      2S98M   =       61252   1194    TAGATCTTATATAACTGTGAGATTAATCTCAGATAATGACAC
DEDDCEEFEEEEFFFFFFFHHHHHHIJJJHIGJJJJIHHFGGJ     NM:i:0  MD:Z:98 MC:Z:60S40M AS:i:98 XS:i:0
```

More about the bam file format at the course [NGS – Quality Control, Alignment, Visualization]

Small variants – different assumptions

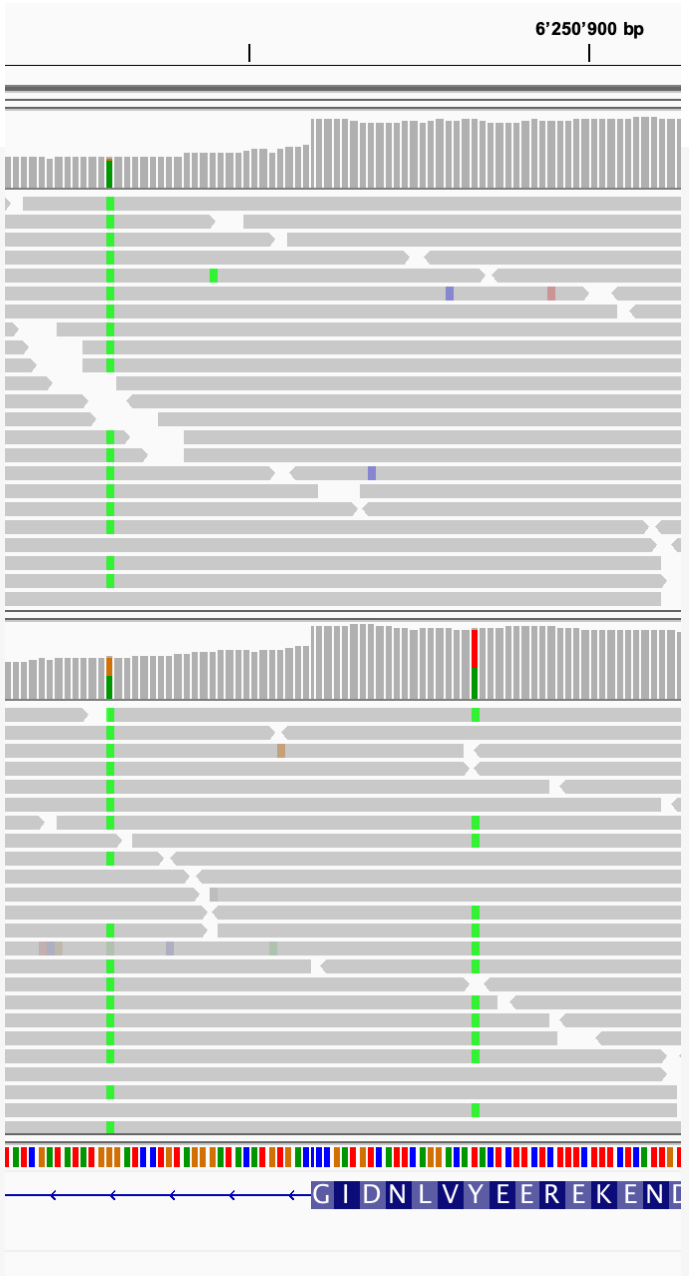
- Germline variants have assumed variant allele frequencies:
  - 100% if homozogous alternative
  - 50% if heterozygous
- These do not hold for somatic variants – heavily relies on tumor purity and heterogeneity
- Biased error strongly influences accuracy



Small variants - likelihood

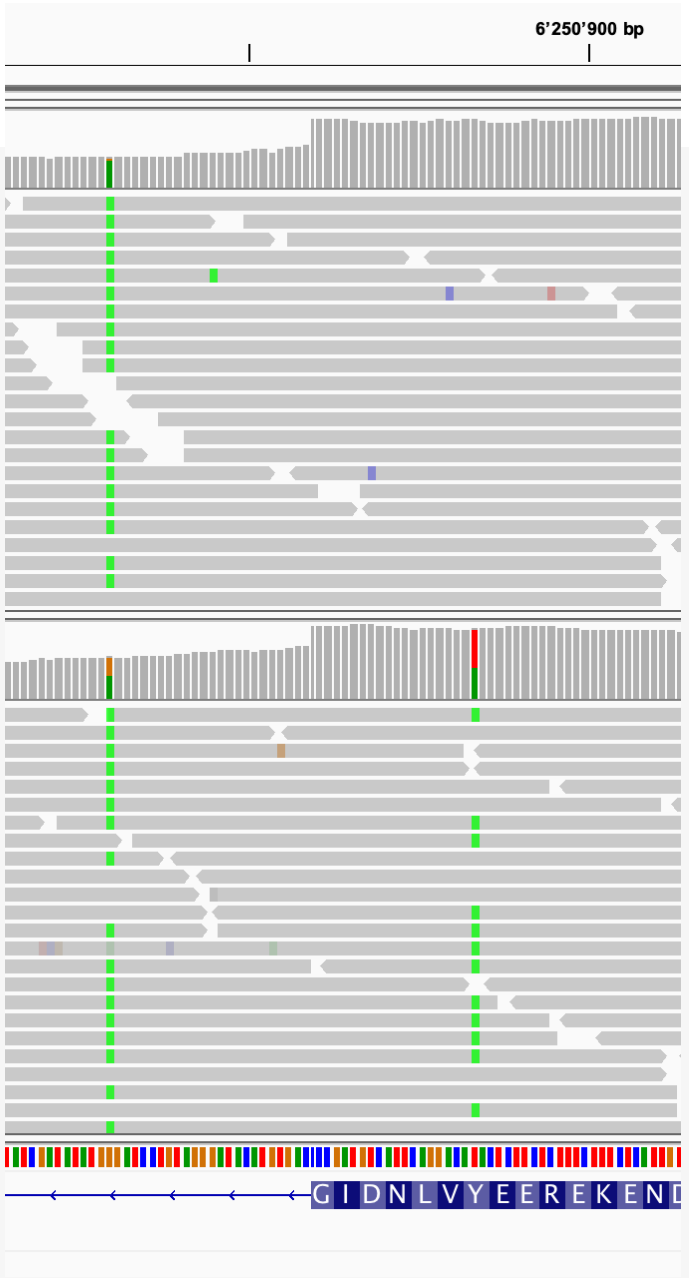
Estimating likelihood and filtering can be based on:

- **Sequencing error**: base qualities and variant allele frequency
- **Technical artifacts**: 'panel of normals' – information about expected non-random errors/artifacts
- Possibility of being a **germline variant**:
  - Germline call of normal sample
  - Databases of known germline variants, e.g. gnomAD



Small variants – GATK workflow

- Mutect2 - variant calling
- FilterMutectCalls – filtering variants based on:
  - Sequencing error
  - Technical artifacts
  - Germline variants



The VCF file format

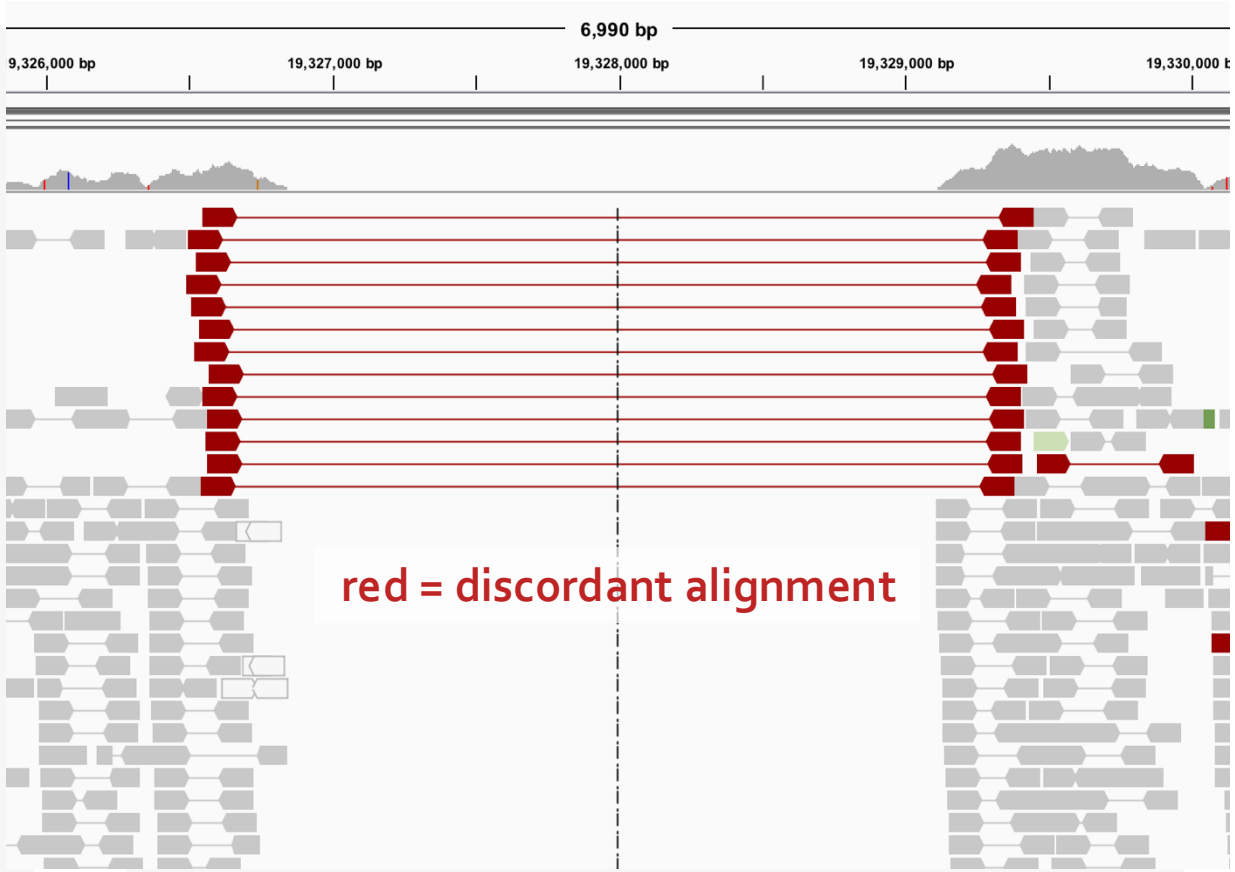
```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,>
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##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=2;DP=9;AF=0.25	GT:GQ:DP:HQ	0|0:48:1:51,51	1|0:48:8:51,51
20	17330	.	T	A	3	q10	NS=2;DP=8;AF=0.25	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=6;AF=0.5,0.5	GT:GQ:DP:HQ	1|2:21:6:23,27	2|1:2:0:18,2
20	1230237	.	T	.	47	PASS	NS=2;DP=11	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=2;DP=6	GT:GQ:DP	0/1:35:4	0/2:17:2

Question

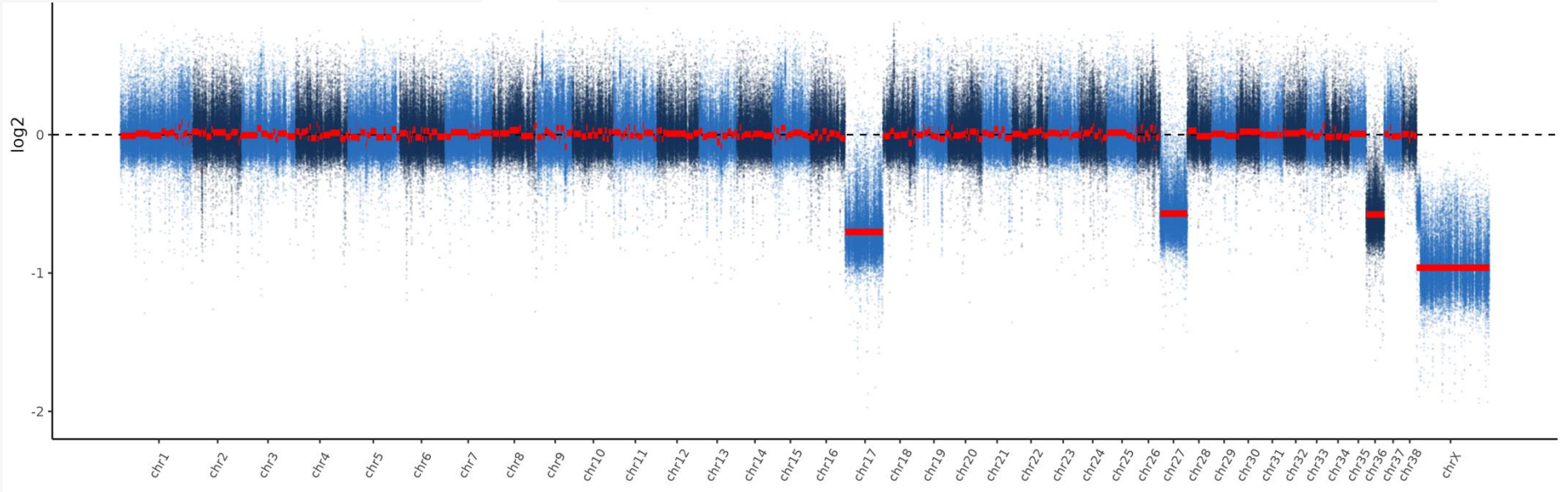
Structural variation

- Large INDELs
- Translocations
- Inversions
- If this causes a loss or multiplication: **copy number variation** (CNV)
- Translocations/inversions can lead to **gene fusions**



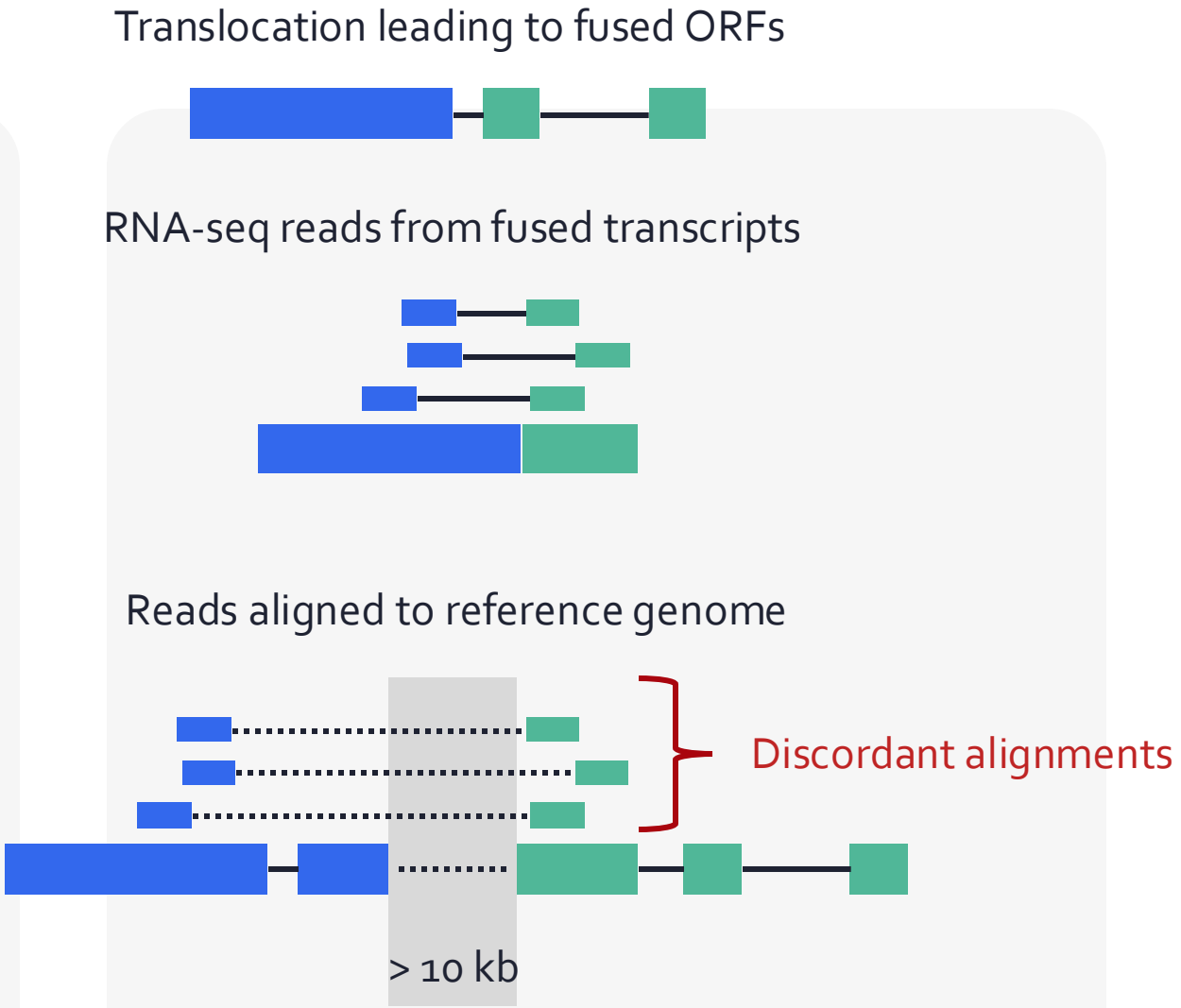
Copy number variation

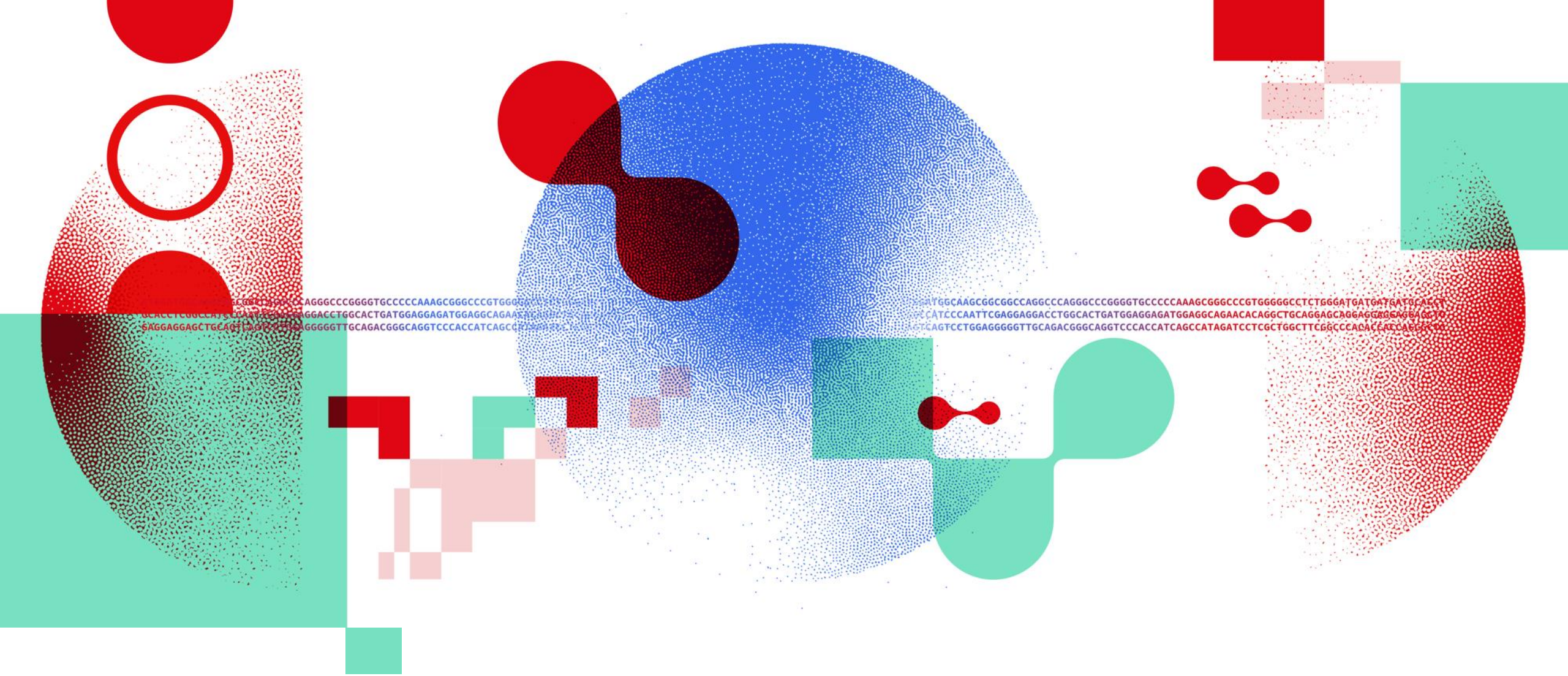
- Losses or gains in the genome - estimated by **variation** in **coverage**
- Coverage does not need to massive to estimate it
- Popular tool: cnvkit
- Loss of heterozygosity (LOH): loss of one allele (+ duplication of the other)



Gene fusion

- Fusion of ORF at the level of the genome
- Results in fused transcripts
- Can be estimated based on:
  - WGS
  - WES
  - RNA-seq
- Detection by discordant alignments





Time for exercises!

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