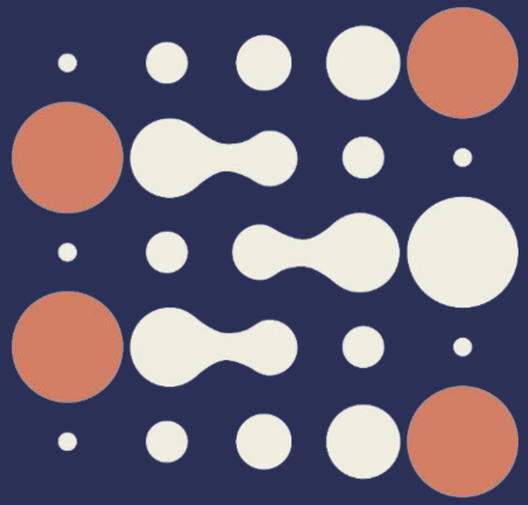
Introduction to medical genomics

Matthieu Foll
follm@iarc.who.int
Nov. 24th 2025



International Agency
for Research on Cancer
World Health
Organization

Agenda

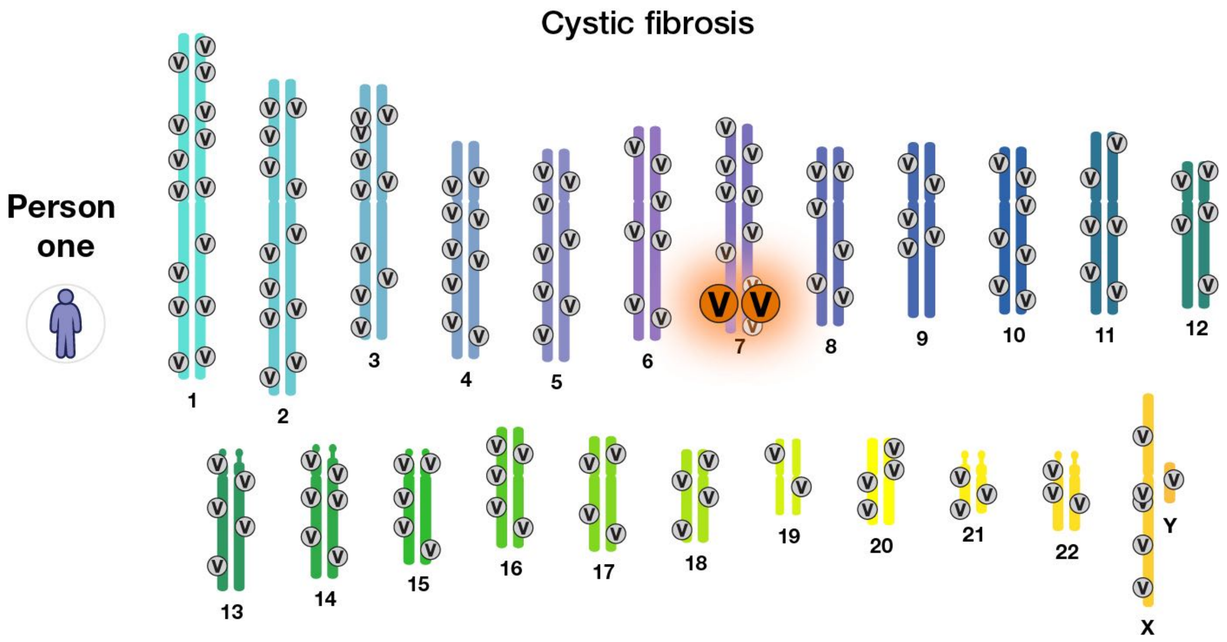
1. Genomic architecture of diseases and cancer genomics
2. Genomic databases
3. Identify genomic alterations

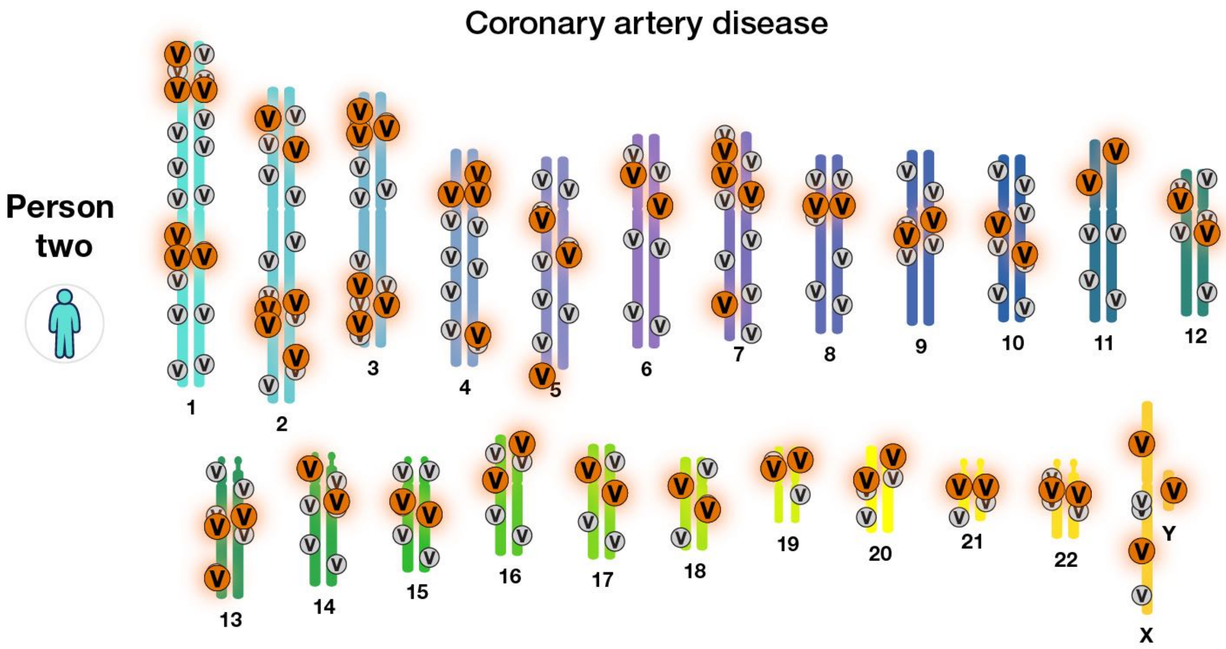
Personalized/Precision Medicine

- Discover genetic mechanisms and risk factors for diseases (inherited conditions, cancer, autoimmune disorders etc.)
- Provide DNA-based predictions of individual susceptibility to future diseases.
- Use precision diagnostics to accurately detect and classify existing conditions.
- Tailor treatments and prevention strategies to individual genetic profiles

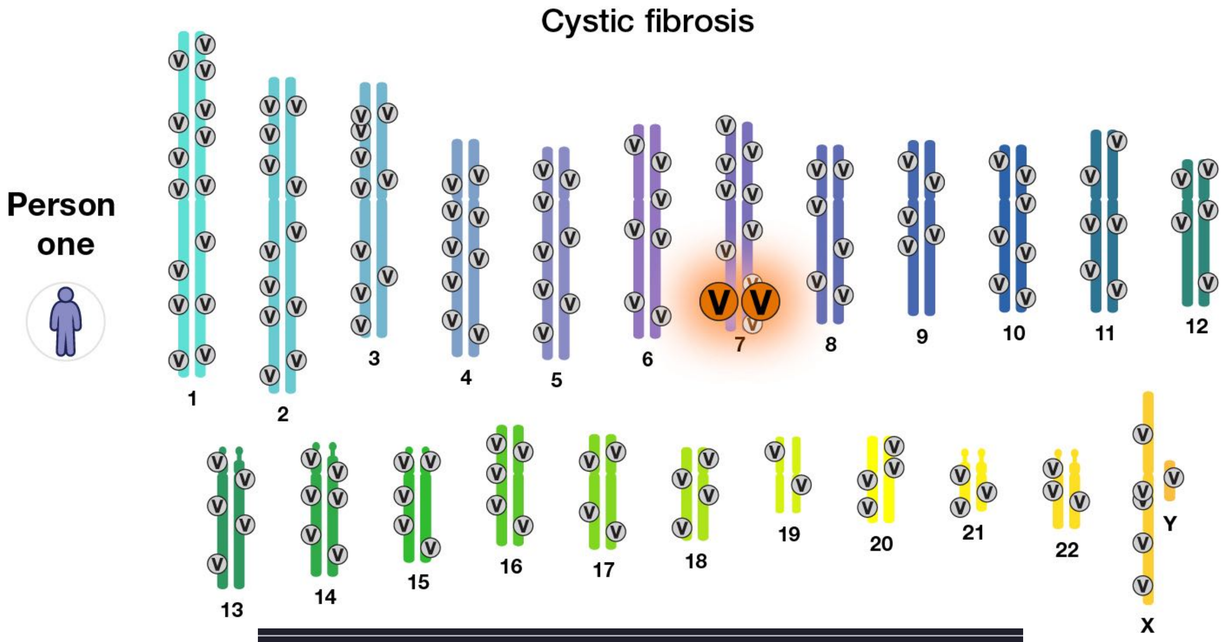
1. Genomic architecture of diseases and cancer genomics

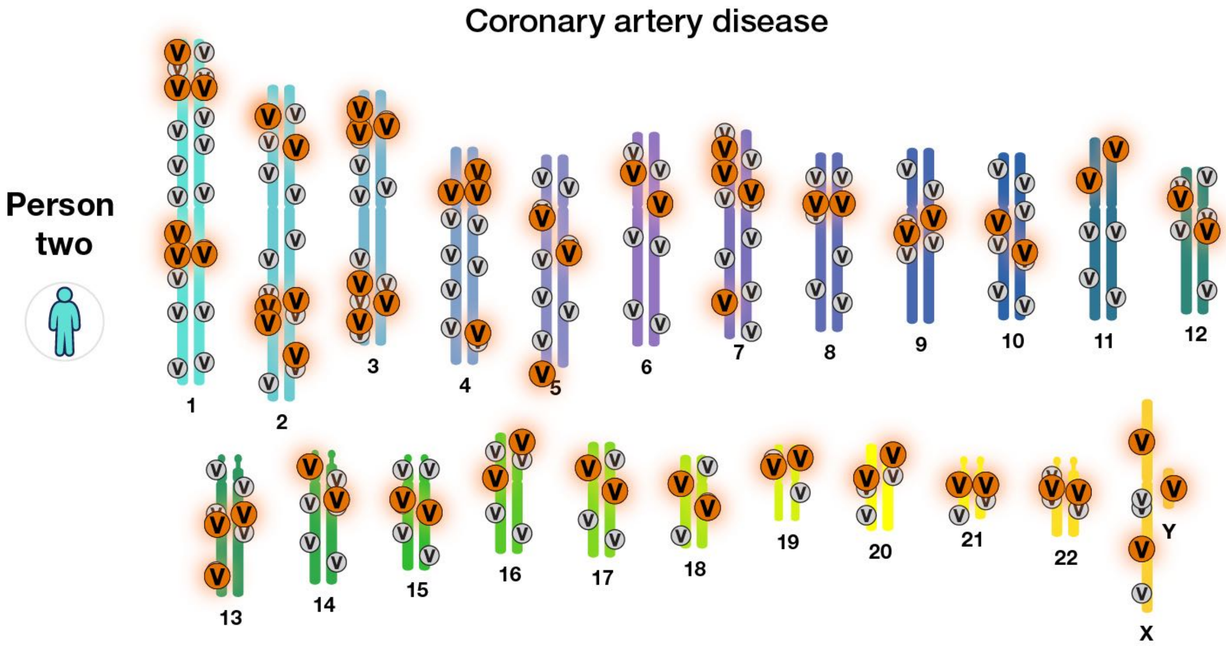
Architecture of complex traits

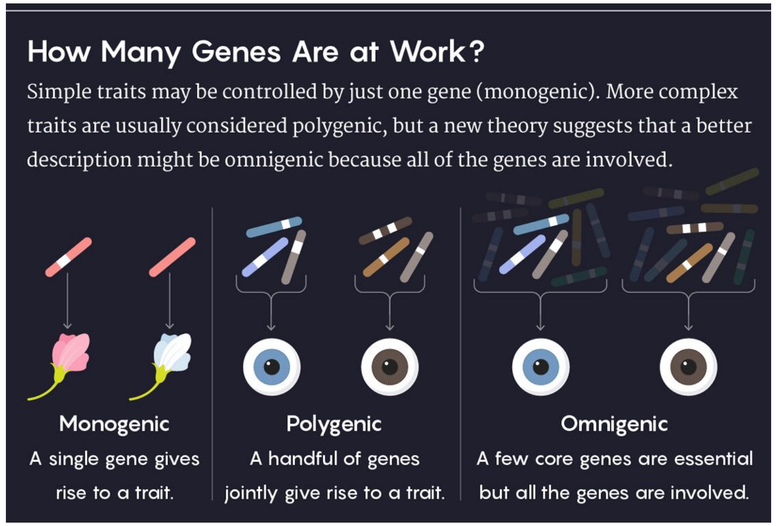




Architecture of complex traits

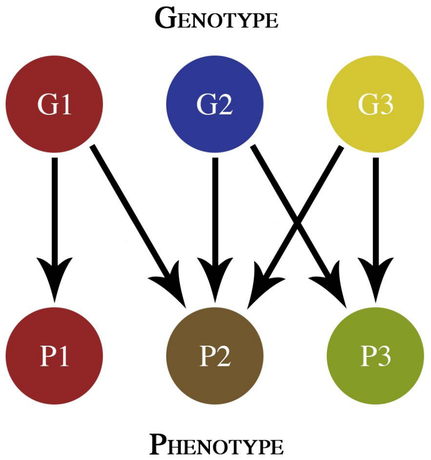




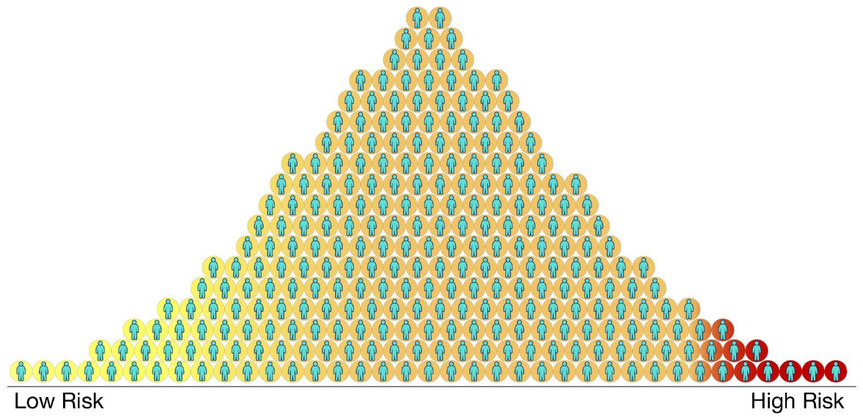


Lucy Reading-Ikkanda/Quanta Magazine

Pleiotropy



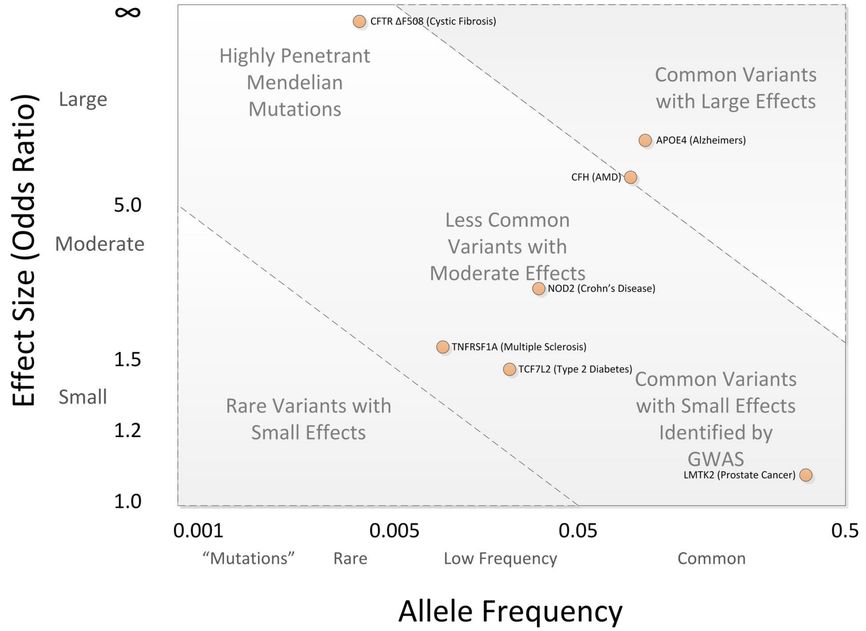
Polygenic Risk Score



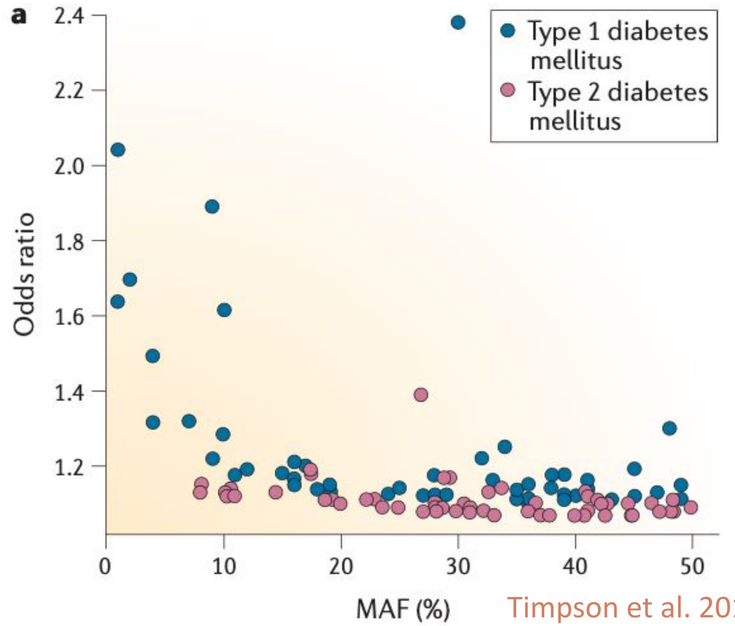
https://www.genome.gov/Health/Genomics-and-Medicine/Polygenic-risk-scores

Frequency and effect size

- **Rare variants tend to have larger effects:**
  Highly penetrant mutations (eg cystic fibrosis): rare but strong impact (large odds ratios).
- **Common variants usually have smaller effects:**
  eg type 2 diabetes: several known variants, frequent but modest odds ratios.
- **Continuum from rare, high-impact variants to common, low-impact variants shaped by evolutionary forces:**
  - **Rare, high-impact mutations under strong negative selection**
  - **Some common variants (eg associated with type 2 diabetes) may have conferred an advantage in the past (fat storage and efficient energy use during scarce food availability periods).**



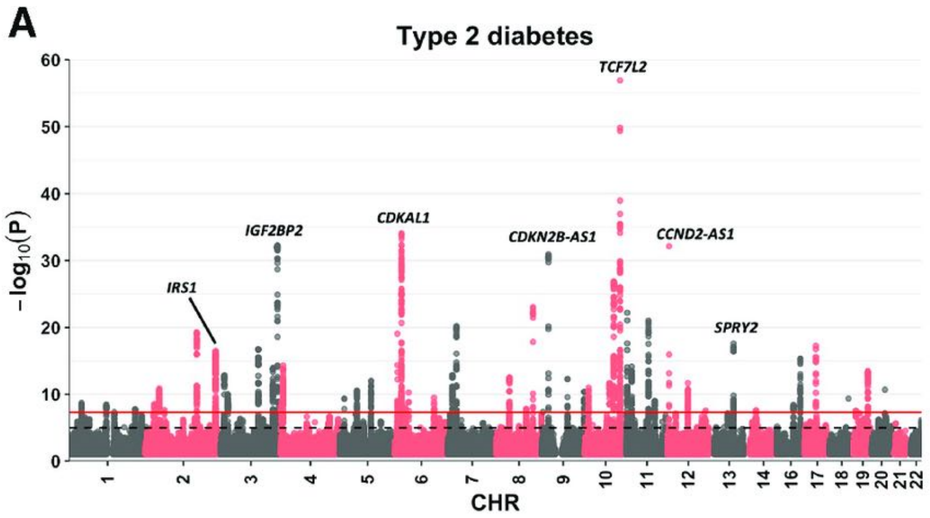
Bush and Moore 2012



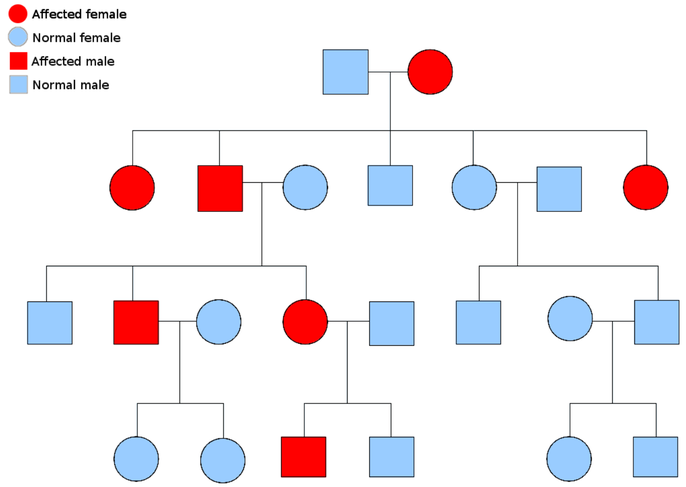
Timpson et al. 2018

How Are Genetic Variants Discovered?

- **Genome-Wide Association Studies (GWAS):**
  - Compares genetic variants across large populations to identify statistical associations with trait.
  - Requires thousands to millions of participants to detect small effect sizes.
  - Results visualized as a Manhattan plot, showing the strength of associations across the genome.
- **Family-Based Studies and Linkage Analysis:**
  - Focuses on identifying genetic variants shared among affected family members.
  - Effective for discovering rare, high-penetrance mutations associated with Mendelian diseases.
  - Relies on mapping genetic markers inherited along with the disease trait in families.

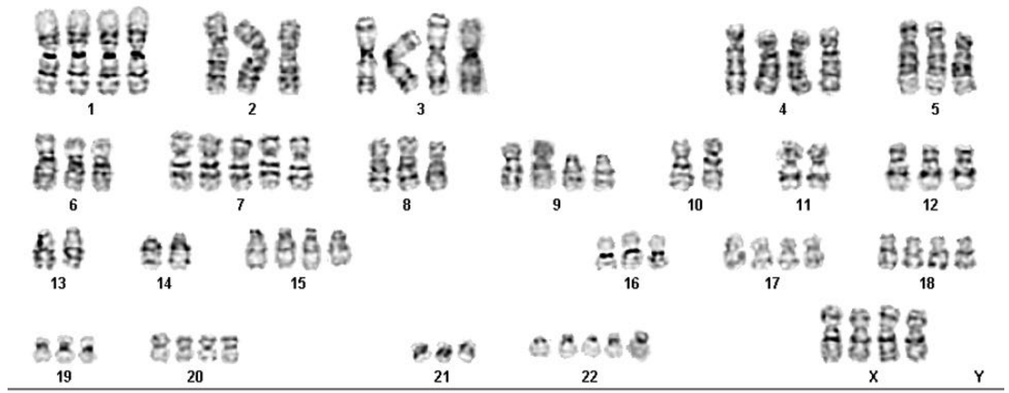


Maina *et al.* 2023

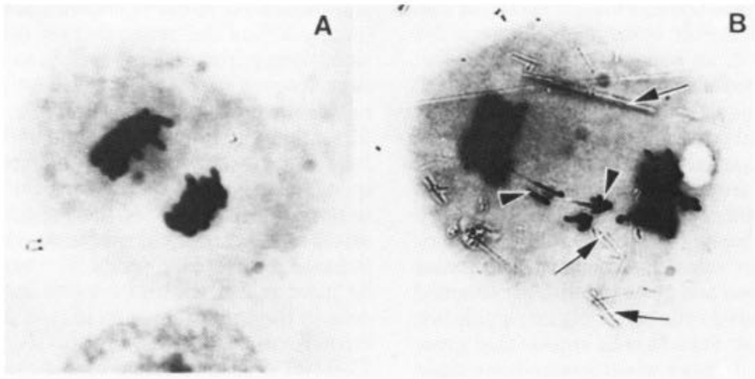


Cancer is a disease of the genome

- **Somatic Mutations:**
  - Cancer arises from acquired mutations in cells during a person's lifetime.
  - These mutations drive uncontrolled cell growth and survival.
  - Not heritable: not passed from parents to offspring.
  - Unlike genetic diseases, occur in somatic cells, not germline cells.
- **Cancer Susceptibility Genes:**
  - Some hereditary mutations (e.g., BRCA1, BRCA2) increase the risk of cancer.
  - These predispositions interact with somatic mutations to initiate cancer.
- **Focus of Cancer Genomics:**
  - Understanding somatic mutations and their role in tumor biology.
  - Identifying actionable targets for therapy and precision medicine.

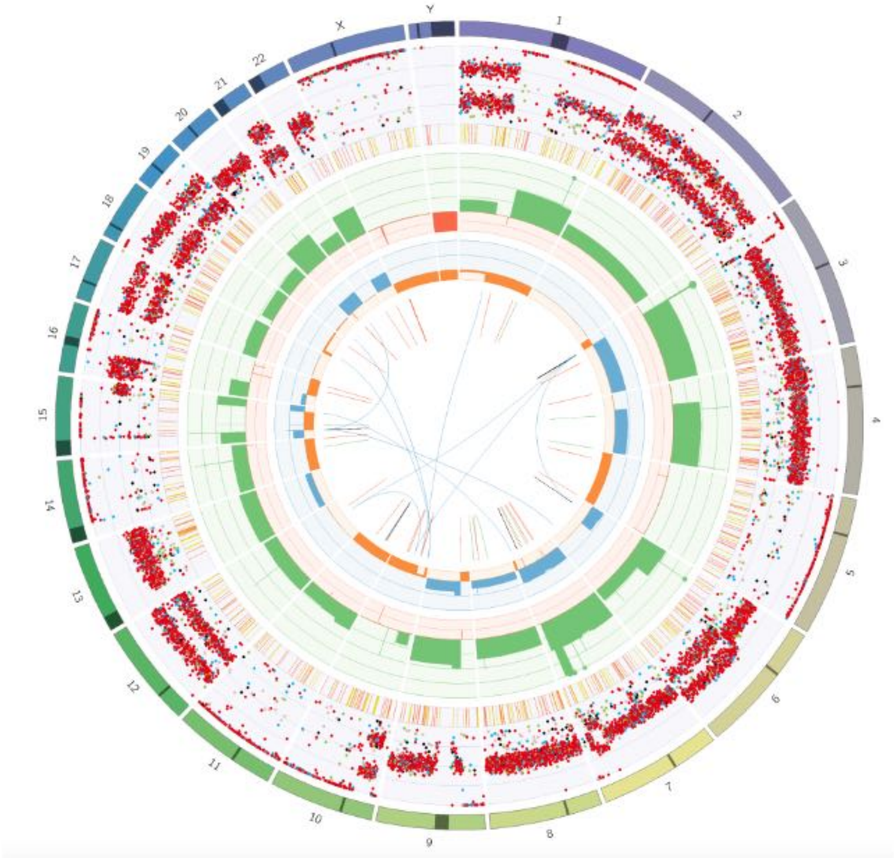


Glioma karyogram (GTG-banding). 78,<4n>,XXXX,-2,-5,-6,del(6)(q21q23)x2,+7,-8,del(8)(q22q24.1),del(9)(p10)x2,-10,-10,-11, -11,-12,-13,-13,-14,-14,-16,-19,del(19)(p10),-21,+22

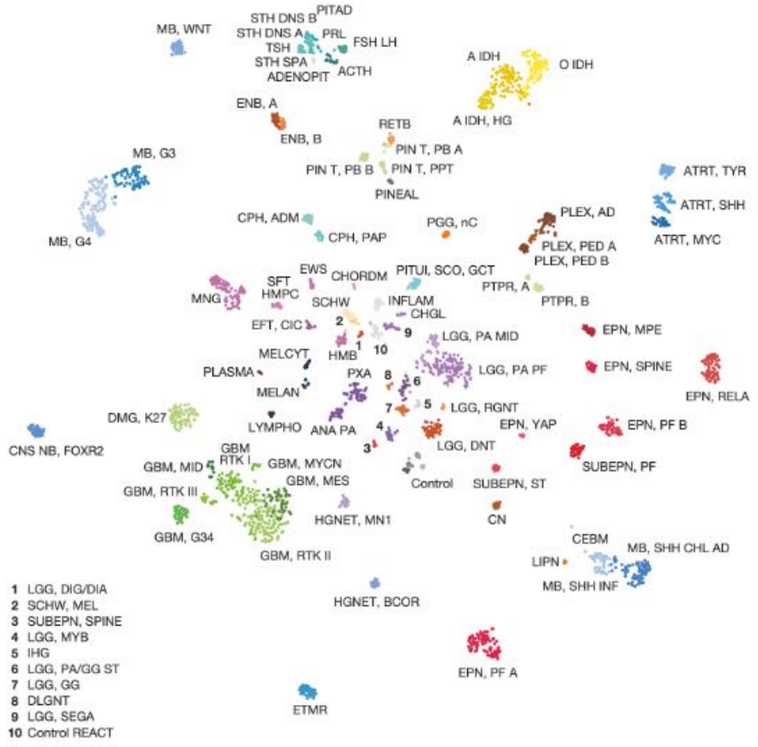


Normal (A) and abnormal (B) anaphase Hesterberg and Barrett 1985

Cancer is a disease of the (epi)genome



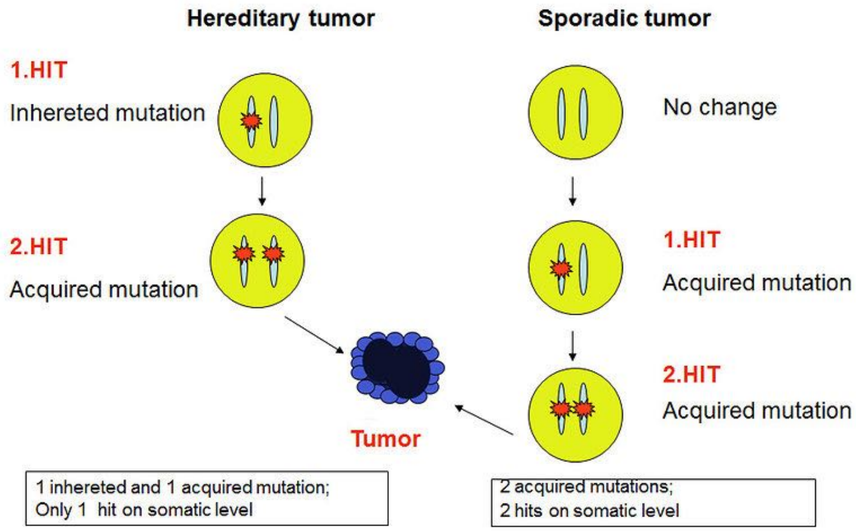
From PURPLE (Hartwig Medical Foundation)



Capper *et al.* 2018 Nature

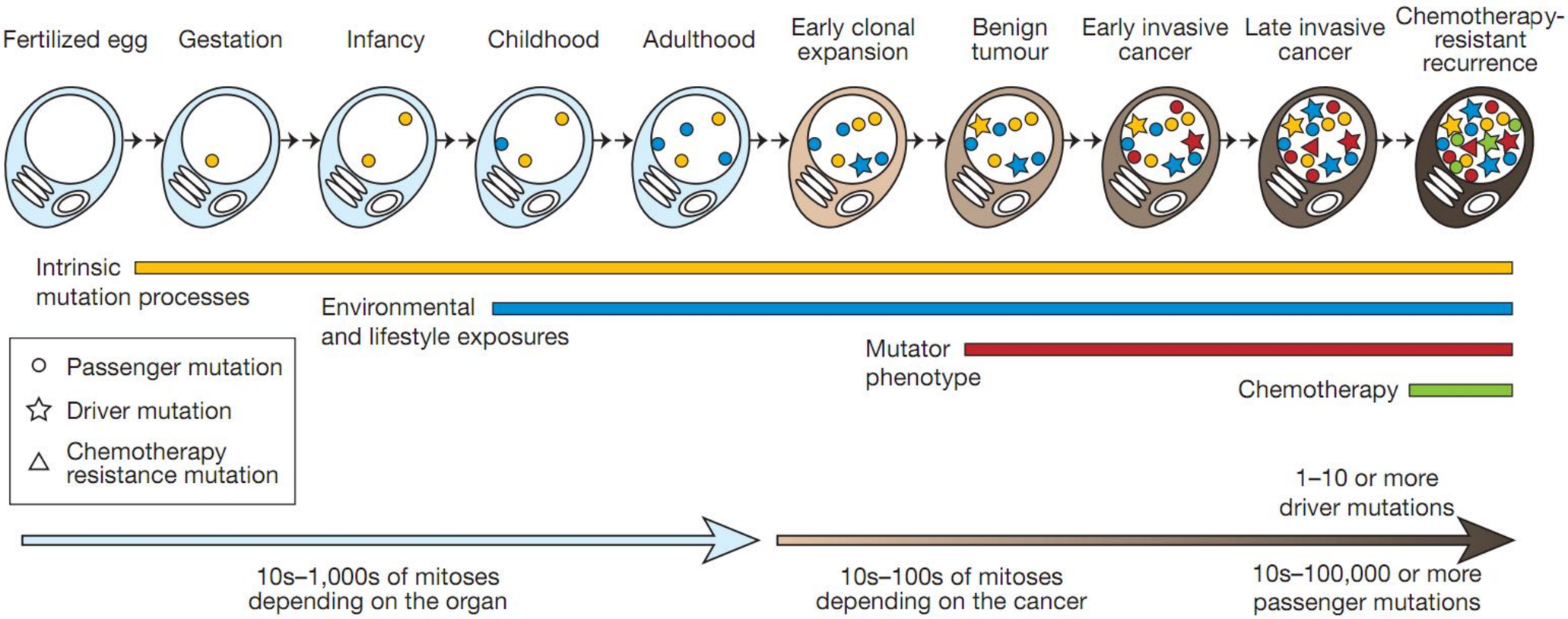
Genetics of cancer

- **Tumor Suppressor Genes**
  - **Protect cells from uncontrolled growth. Function lost when both alleles are inactivated (recessive loss).**
  - **Examples: *RB1*, *BRCA1*.**
- **Oncogenes**
  - **Promote cell growth and division, become cancerous when mutated or overactivated (dominant gain).**
  - **Examples: *KRAS*, *EGFR*.**
- **Knudson's Two-Hit Hypothesis**
  - **Explains how tumor suppressor genes are inactivated in cancer.**
  - **First "hit": Inherited or acquired mutation in one allele.**
  - **Second "hit": Mutation or loss of the second allele in somatic cells.**
  - **Often seen in hereditary cancers (e.g., retinoblastoma).**



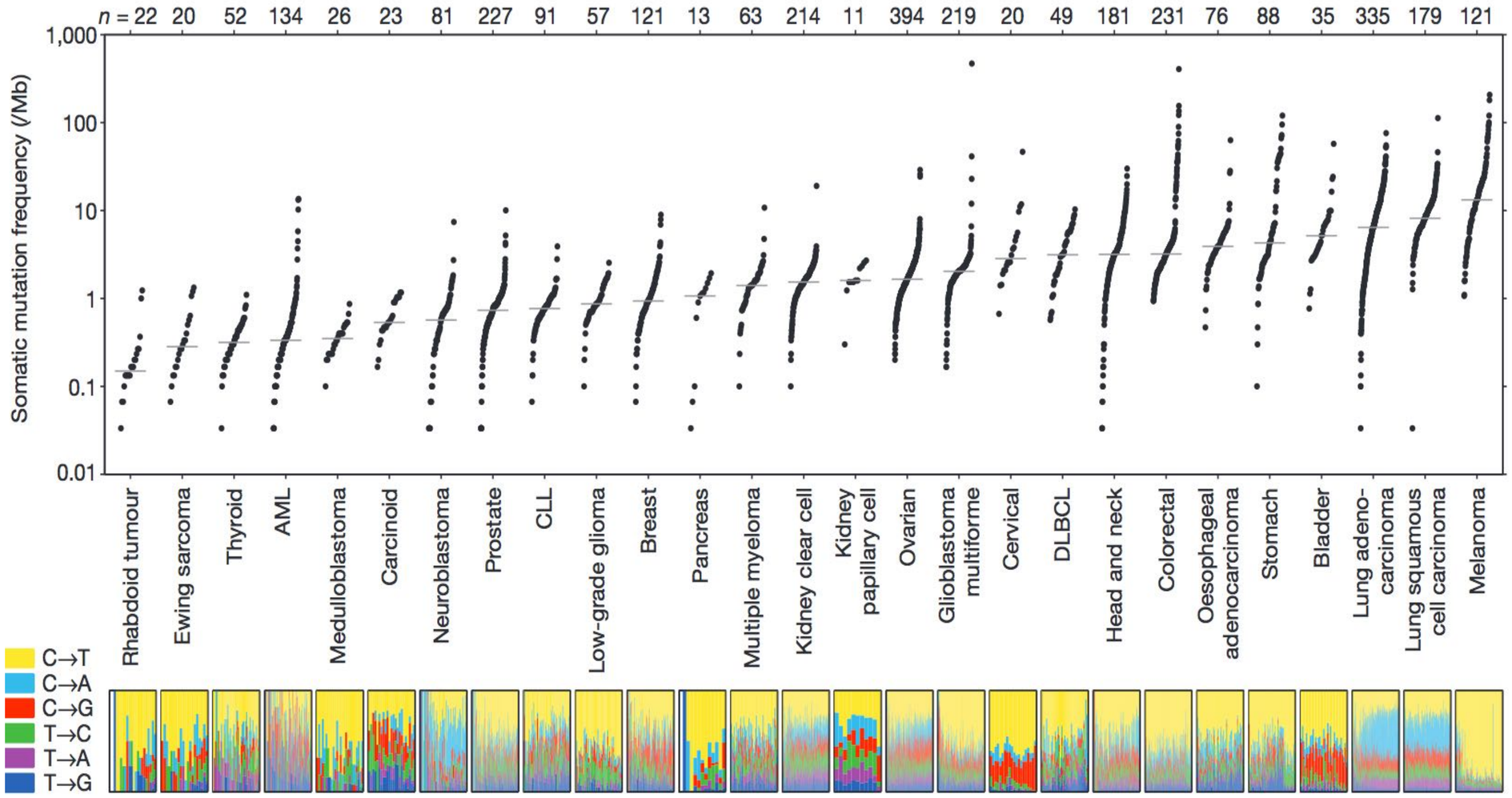
Bajčiová 2015

Cancer cells accumulate mutations over time



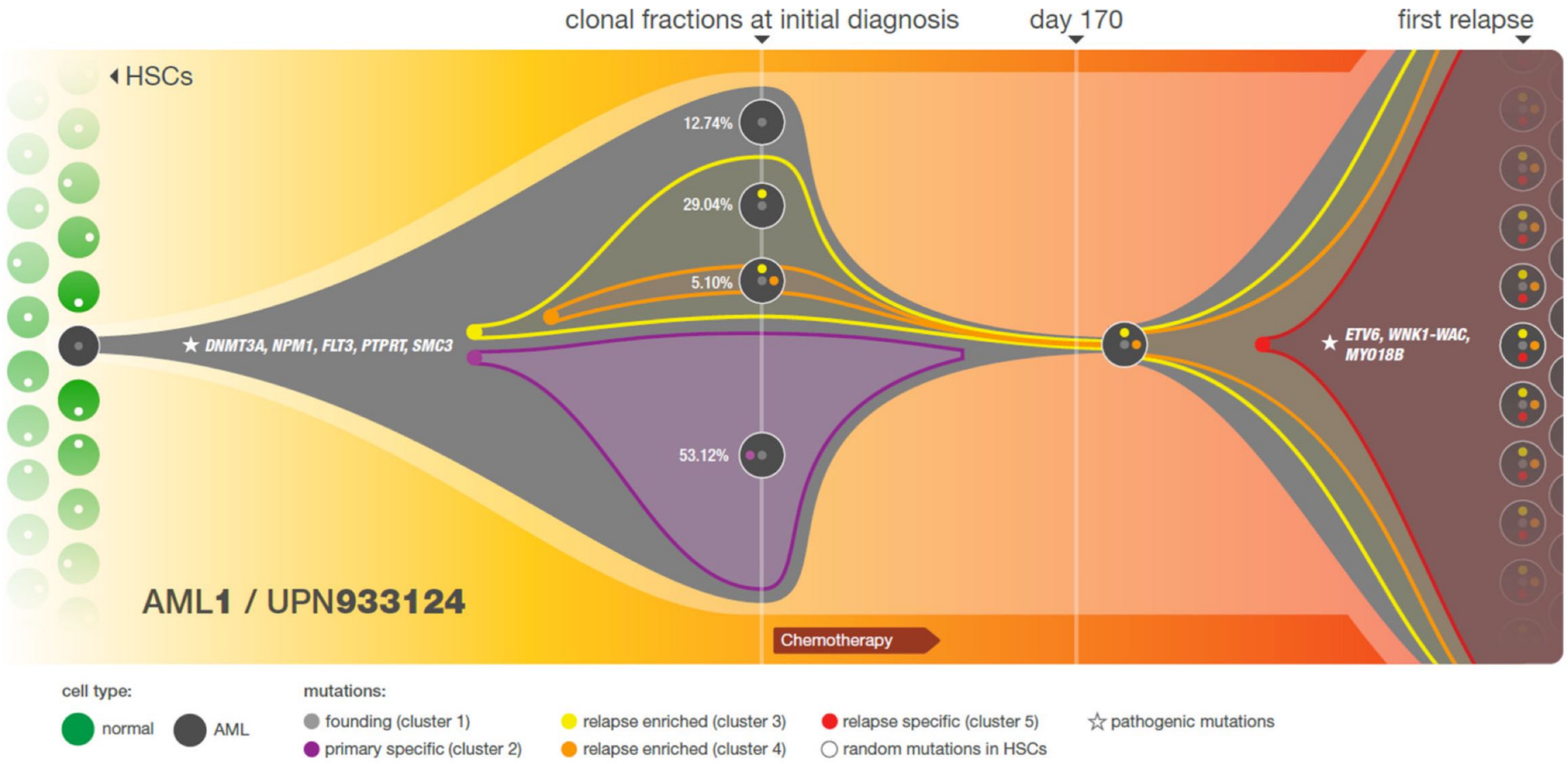
Stratton MR, Campbell PJ, Futreal PA. *Nature*. 2009

Mutation burden varies by cancer type, exposure, age of onset, & DNA repair ability



Lawrence et al. Nature 2013 Jul 11;499(7457):214-8.

Cancers are a mix of subclones



Ding et al. Nature. 2012 Jan 11;481(7382):506-10.

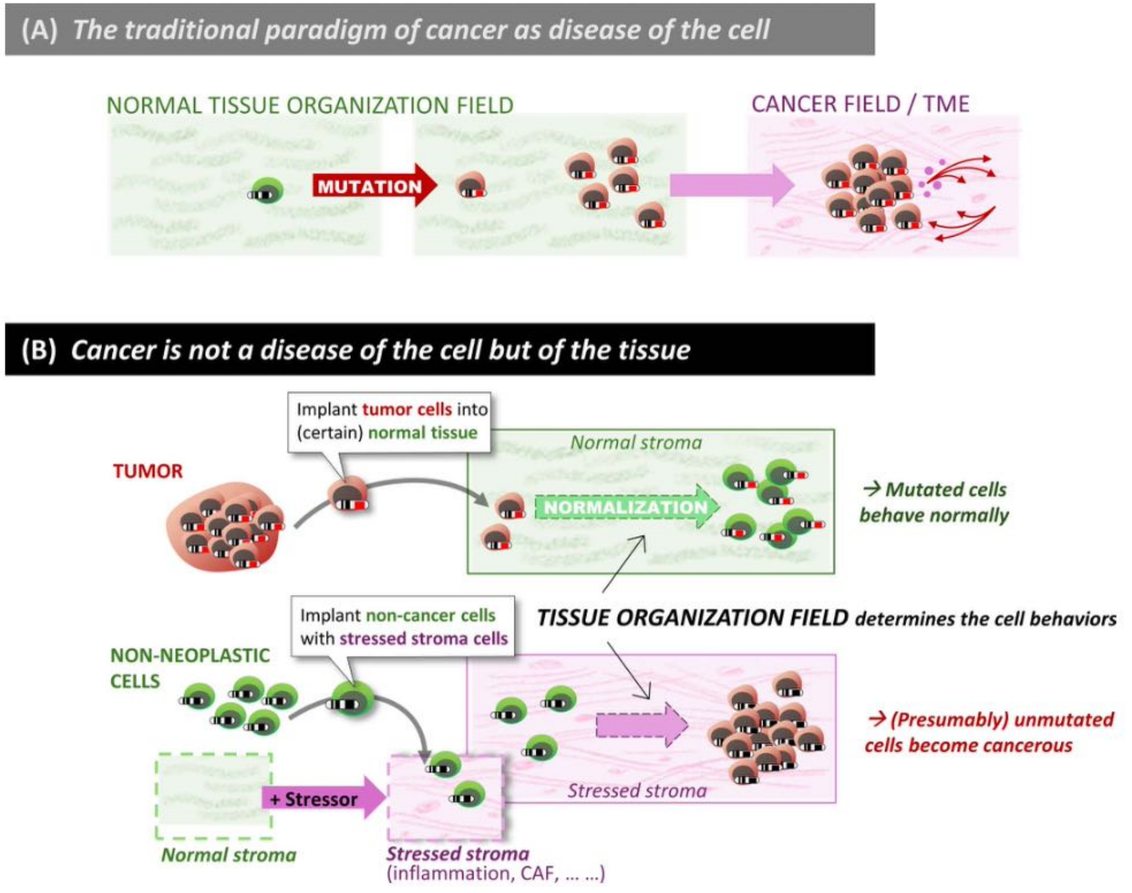
ESSAY

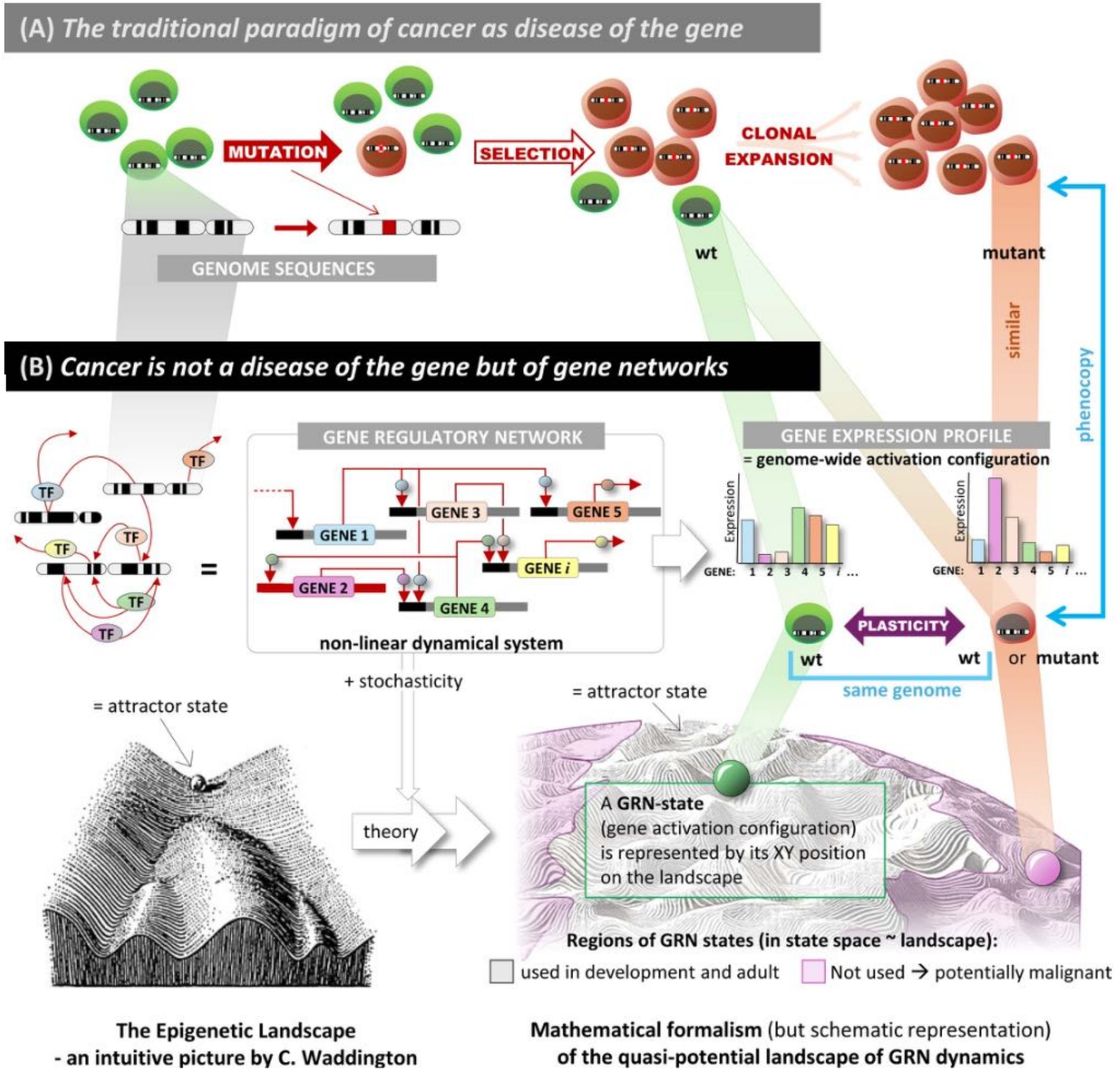
The end of the genetic paradigm of cancer

Sui Huang[1*], **Ana M. Soto**[2,3*], **Carlos Sonnenschein**[2,3*]

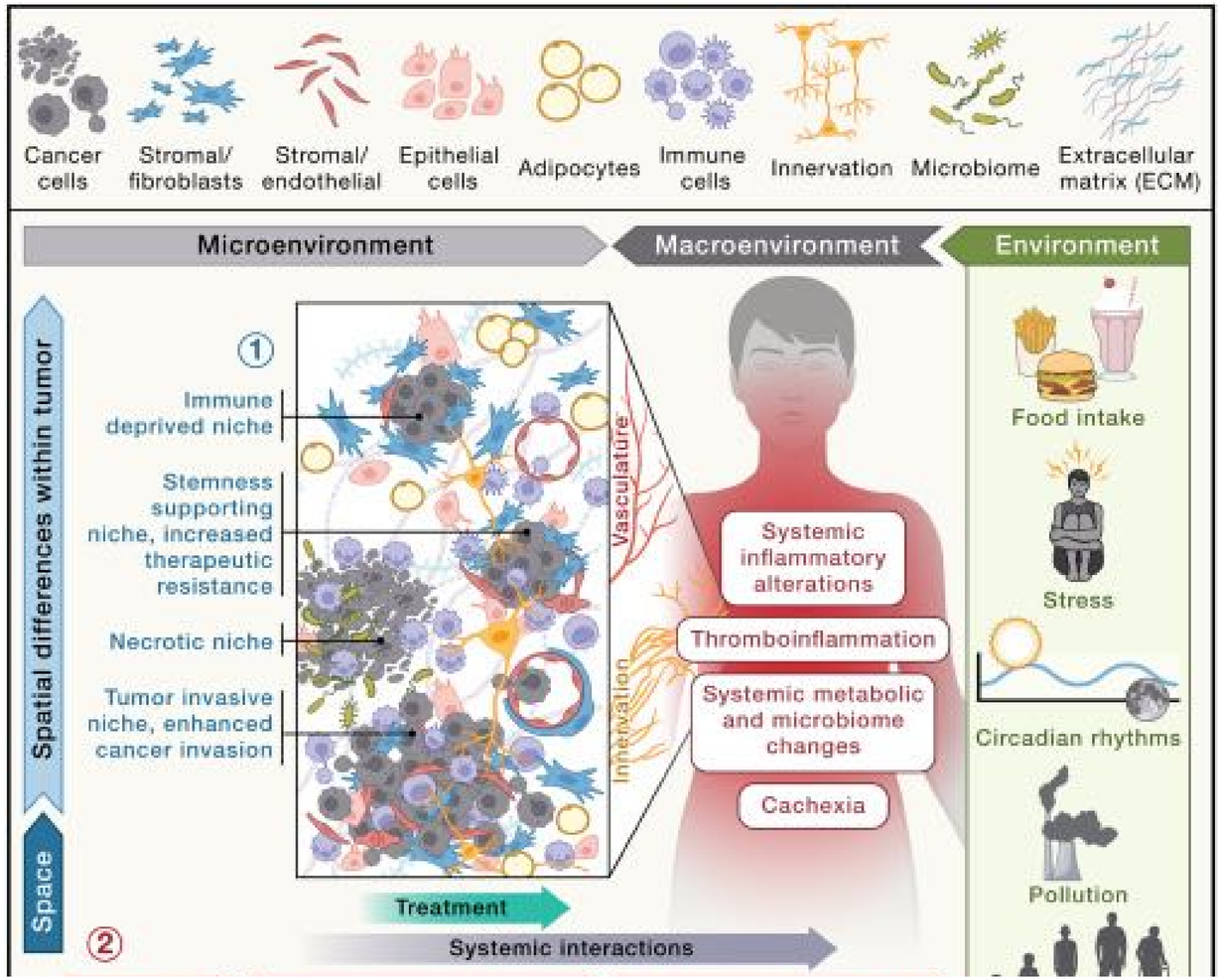
1 Institute for Systems Biology, Seattle, Washington, United States of America, **2** Tufts University Schoo of Medicine, Immunology, Boston, Massachusetts, United States of America, **3** Centre Cavaillès, Ecole Normale Superieure, Paris, France

* sui.huang@isbscience.org (SH); ana.soto@tufts.edu (AMS); carlos.sonnenschein@tufts.edu (CS)





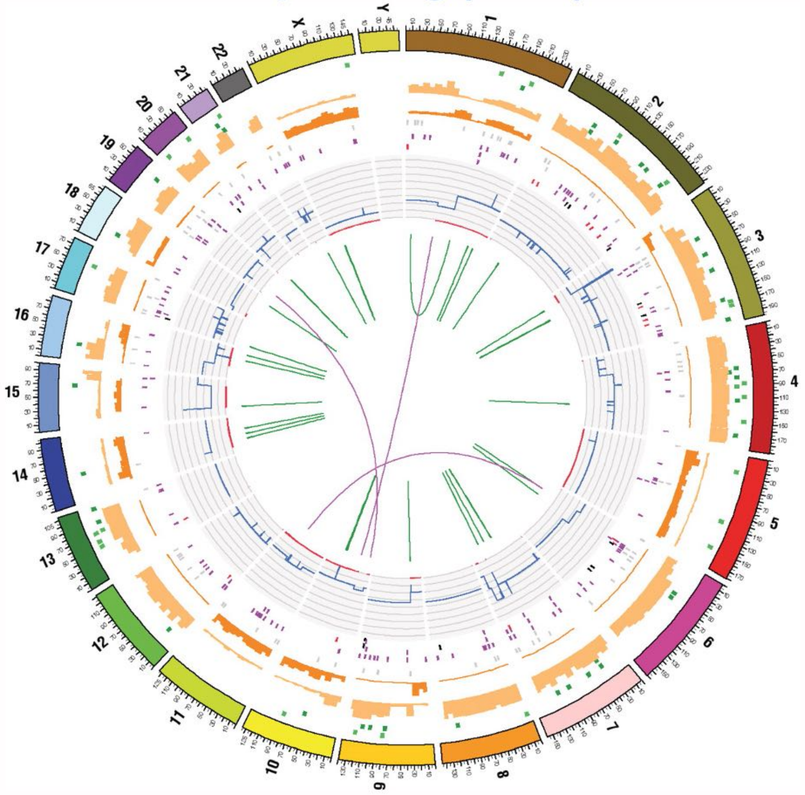
The cancer ecosystem



Swanton *et al. Cell* 2024.

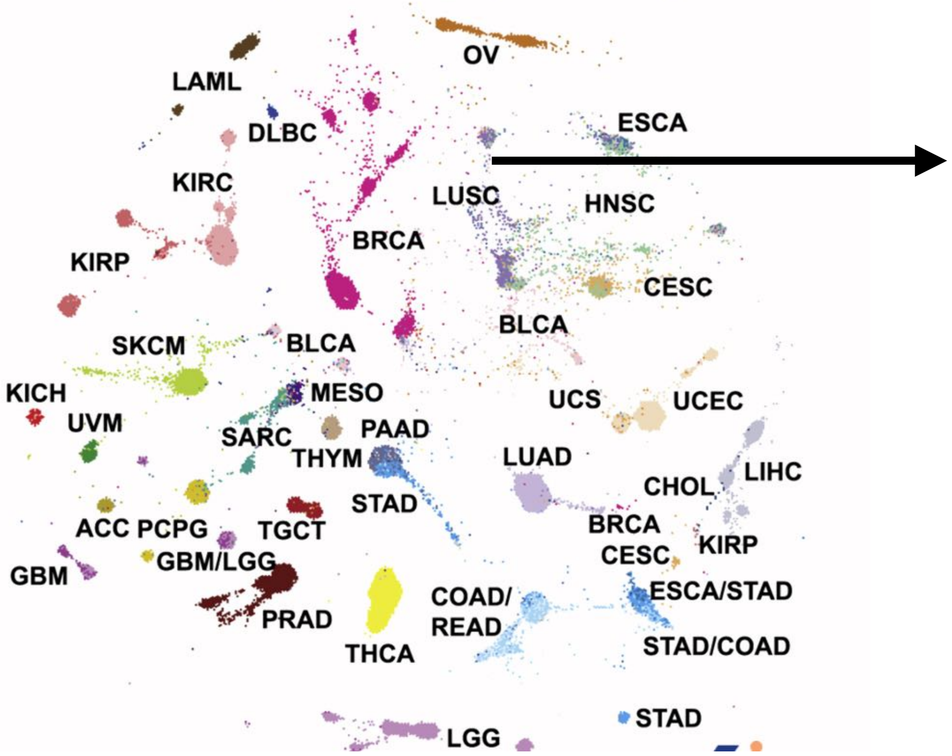
Cancer genotype and phenotype

A single cancer somatic mutation landscape from whole genome sequencing (WGS)



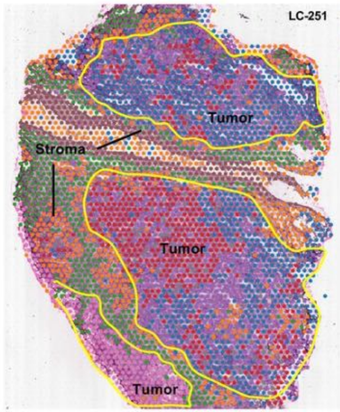
Forbes *et al.* 2011

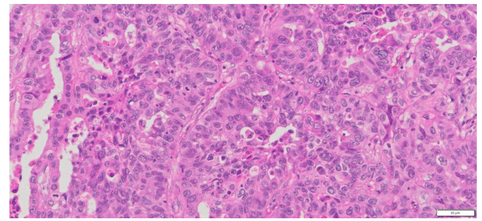
Average expression profiles of 10’000 cancers from transcriptome sequencing (RNAseq)



Hoadley *et al.* 2018

A single cancer single-cell and spatial transcriptome and scanned image

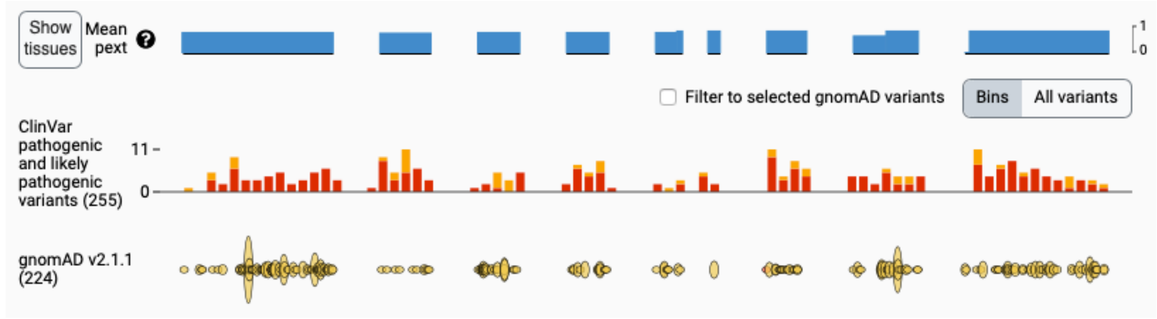


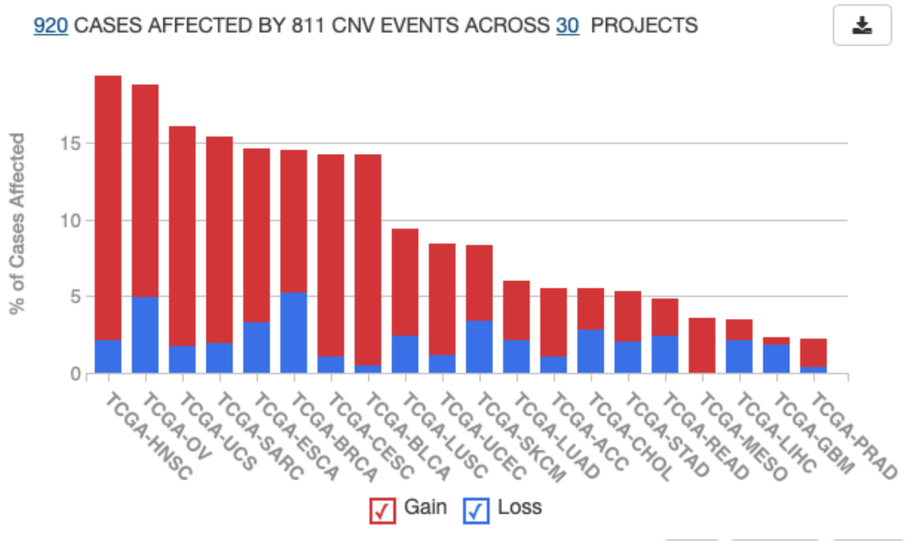


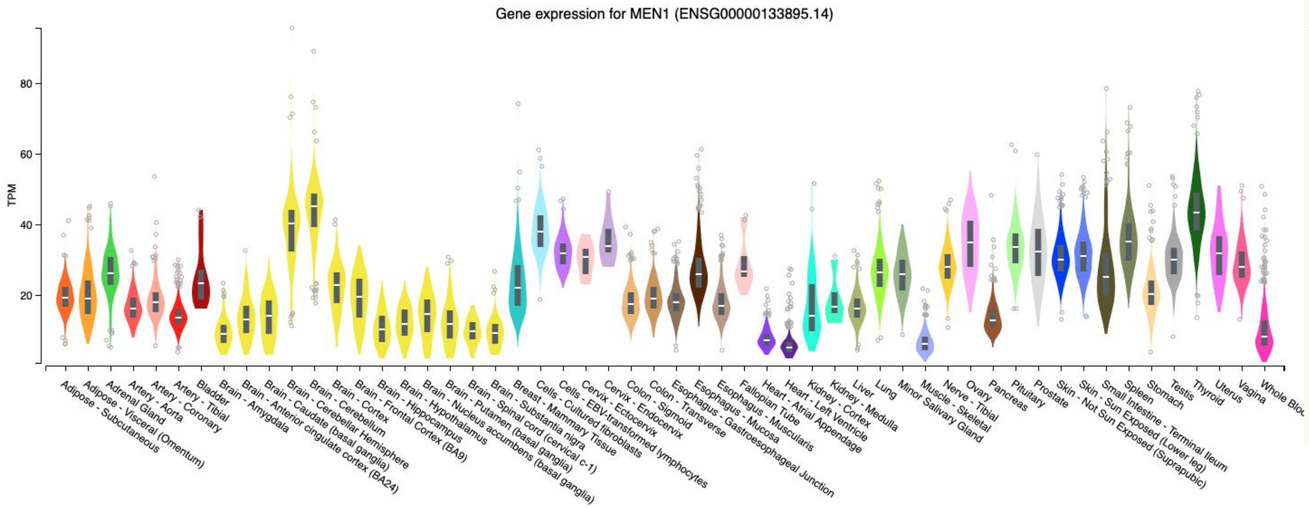
2. Genomic databases

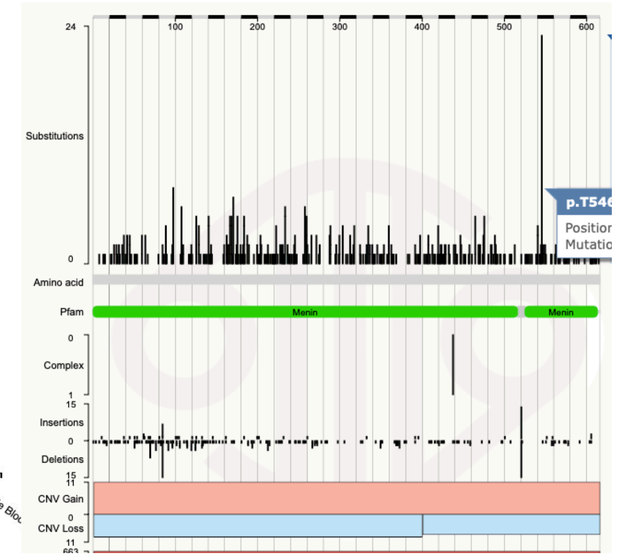
Human genomics databases

- Genome Reference Consortium (GRC)
- RefSeq: Reference sequences for genomes, transcripts, proteins. NCBI gene web portal.
- dbSNP: Single Nucleotide Polymorphisms
- ClinVar: clinical significance of variants
- COSMIC: Catalogue Of Somatic Mutations In Cancer
- OMIM: Online Mendelian Inheritance in Man
- Flagship datasets: HapMap, 1000G, HGDP, UK Biobank, Genomics England 100,000 Genomes Project, TCGA, ICGC, GTEx, gnomAD
- Database of publicly available datasets: SRA, GEO, dbGaP, EGA









*613733
Table of Contents

- Title
- Gene-Phenotype Relationships
- Text
  - Description
  - Cloning and Expression
  - Gene Structure
  - Mapping
  - Gene Function
  - Biochemical Features
  - Molecular Genetics
  - Animal Model
- Allelic Variants
  - Table View
- References
- Contributors
- Creation Date
- Edit History

* 613733

MENIN 1 ; MEN1

Alternative titles; symbols

MEN1 GENE
MENIN

HGNC Approved Gene Symbol: MEN1

Cytogenetic location: 11q13.1 ***Genomic coordi***

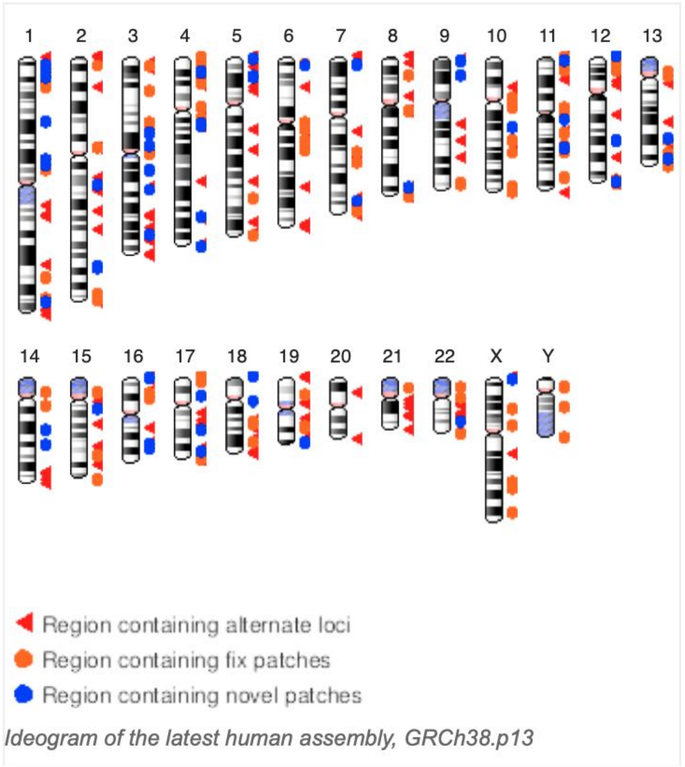
Gene-Phenotype Relationships

Location	Phenotype
11q13.1	Adrenal adenoma, somatic
	Angiofibroma, somatic
	Carcinoid tumor of lung
	Lipoma, somatic
	Multiple endocrine neoplasia 1
	Parathyroid adenoma, somatic

PheneGene Graphics

Reference genome

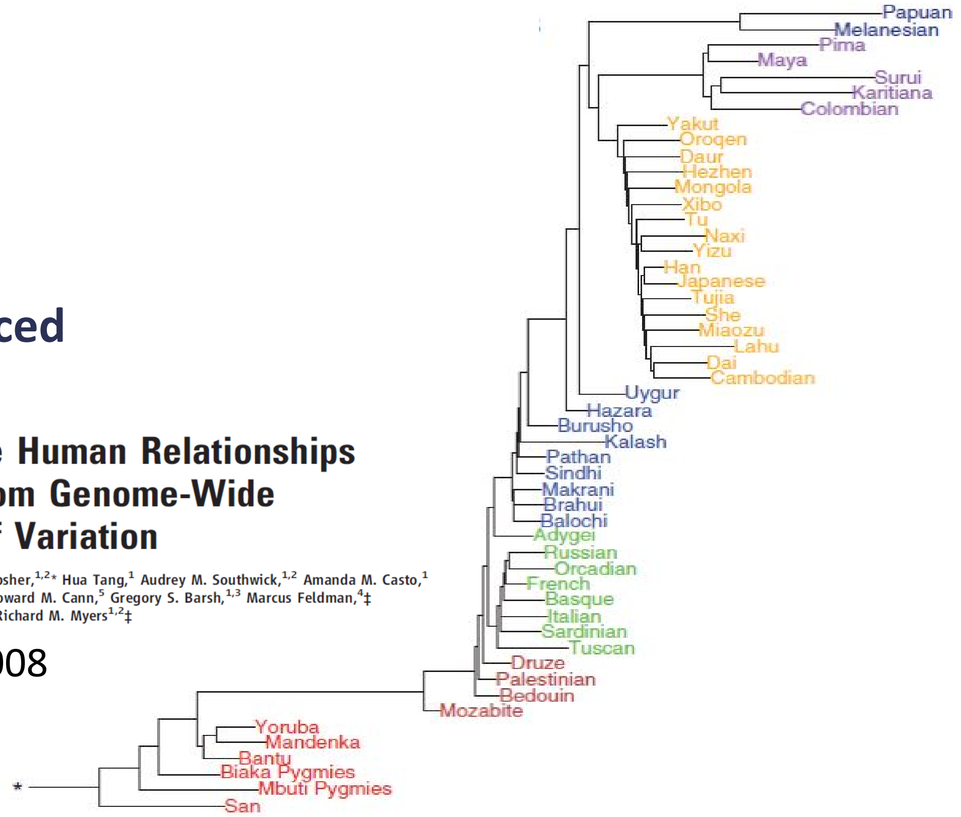
- The “reference” human genome is maintained by the Genome Reference Consortium
- 70% from a single male from Buffalo, NY
- There are several versions, current is GRCh38 (2013)
- Big FASTA file (~3GB)
- Europeans differ from the reference in ≈4M sites



Ideogram of the latest human assembly, GRCh38.p13

Human Genome Diversity Project (HGDP)

- Was a major effort to collect DNA from in order to capture the most genetic diversity worldwide
  - 2002: 1056 individuals, 52 populations, 377 STRs
  - 2008: 938 individuals, 51 populations, 660k SNPs
  - 2020: 929 individuals, 54 populations, whole genome sequenced
- Important dataset for population genetics
  - Data freely available



Worldwide Human Relationships Inferred from Genome-Wide Patterns of Variation

Jun Z. Li,[1,2]*† Devin M. Absher,[1,2]* Hua Tang,[1] Audrey M. Southwick,[1,2] Amanda M. Casto,[1] Sohini Ramachandran,[4] Howard M. Cann,[5] Gregory S. Barsh,[1,3] Marcus Feldman,[4]‡ Luigi L. Cavalli-Sforza,[1]‡ Richard M. Myers[1,2]‡

Science 2008

Genetic Structure of Human Populations

Noah A. Rosenberg,[1]* Jonathan K. Pritchard,[2] James L. Weber,[3] Howard M. Cann,[4] Kenneth K. Kidd,[5] Lev A. Zhivotovsky,[6] Marcus W. Feldman[7]

Science 2002

Insights into human genetic variation and population history from 929 diverse genomes

ANDERS BERGSTRÖM, SHANE A. MCCARTHY, RUOYUN HUI, MOHAMED A. ALMARRI, QASIM AYUB, PETR DANECEK, YUAN CHEN, SABINE FELKEL, PILLE HALLAST, JACK KAMM, HÉLÈNE BLANCHÉ, JEAN-FRANÇOIS DELEUZE, HOWARD CANN, SWAPAN MALLICK, DAVID REICH, MANJINDER S. SANDHU, PONTUS SKOGLUND, AYLWYN SCALLY, YALI XUE, RICHARD DURBIN, AND CHRIS TYLER-SMITH fewer Authors Info &

Science 2020

The 1000 Genomes Project

- Started in 2008, at the onset of NGS
- Based on relatively low-coverage sequencing (≈7x)
- Total: 2504 individuals from 26 population

ARTICLE 2010, Pilot phase

doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

ARTICLE 2012, Phase 1

doi:10.1038/nature11632

An integrated map of genetic variation from 1,092 human genomes

The 1000 Genomes Project Consortium*

ARTICLE 2015, Phase 3 and final

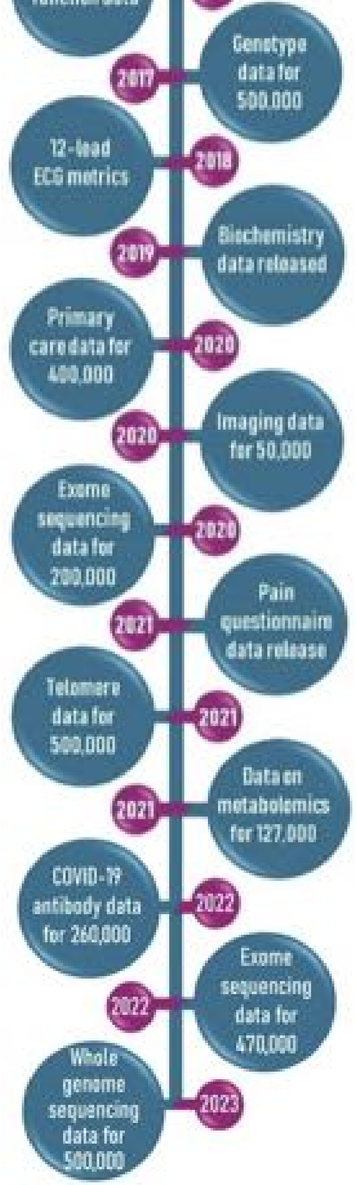
OPEN
doi:10.1038/nature15393

A global reference for human genetic variation

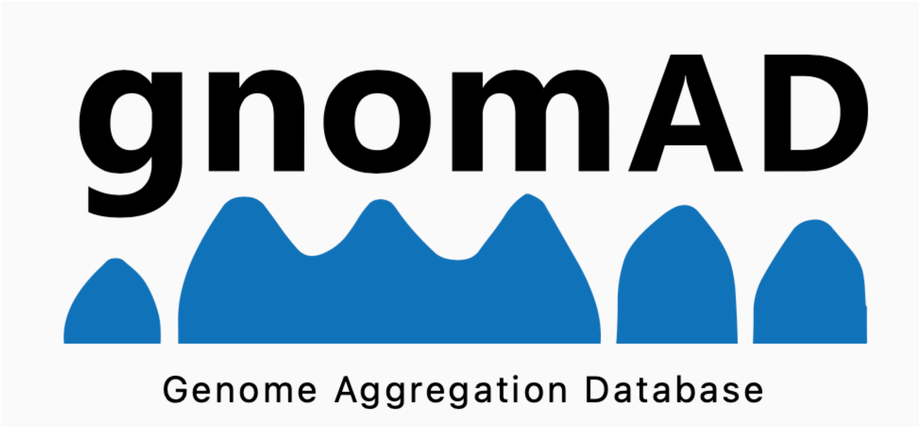
The 1000 Genomes Project Consortium*

The UK BioBank

- **A study of 500,000 volunteers of ages 40-69**
  - Recruitment started in 2006, follow up expected for 30 years
  - Questionnaires and interviews on lifestyle, medical history, nutritional habits, etc.
  - Height, weight, blood pressure, cognitive ability, visual acuity, etc. were measured
  - Blood and urine samples collected, blood biomarkers available
  - Additional medical data is continuously collected
  - Now Whole Genome Sequencing available for all participants
  - Protected access, with a fee on a cloud-based platform



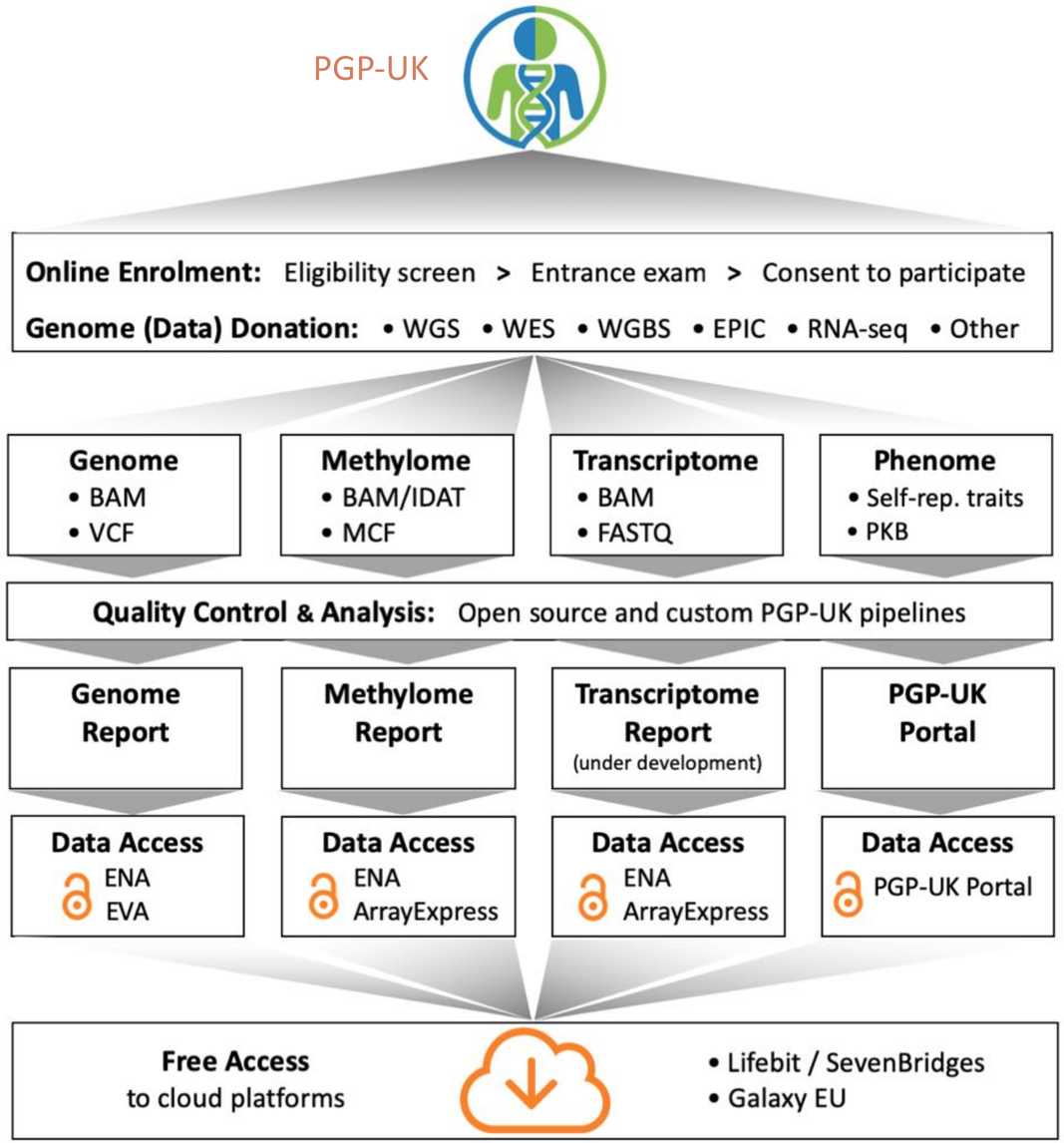
biobank uk
Improving the health of future generations



	ExAC	gnomAD v2	gnomAD v3	gnomAD v4*		
	#	#	#	#	%	Fold increase from v2
Admixed American	5,789	17,720	7,647	30,019	3.72%	1.7x
African	5,203	12,487	20,744	37,545	4.65%	3x
Ashkenazi Jewish	-	5,185	1,736	14,804	1.83%	2.9x
East Asian	4,327	9,977	2,604	22,448	2.78%	2.3x
European^	36,667	77,165	39,345	622,057	77.07%	8.1x
Middle Eastern	-	-	158	3,031	0.38%	New
Remaining Individuals^	454	3,614	1,503	31,172	3.93%	8.8x
South Asian	8,256	15,308	2,419	45,546	5.64%	3x
Total	**60,706**	**141,456**	**76,156**	**-**	**807,162**	**-**

*v4 includes all v3 samples
^ Due to small sample sizes Finnish was included in European and Amish was included in Remaining Individuals

Personal Genome Project



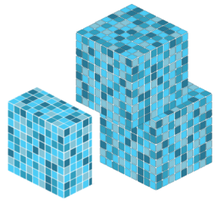
The Cancer Genome Atlas (TCGA)

NATIONAL CANCER INSTITUTE
THE CANCER GENOME ATLAS

TCGA BY THE NUMBERS

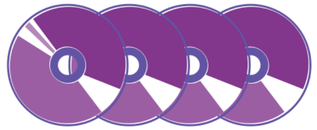
TCGA produced over

2.5 PETABYTES of data



To put this into perspective, **1 petabyte** of data is equal to

212,000 DVDs



TCGA data describes

33 DIFFERENT TUMOR TYPES

...including

10 RARE CANCERS

...based on paired tumor and normal tissue sets collected from

11,000 PATIENTS

...using

7 DIFFERENT DATA TYPES

TCGA RESULTS & FINDINGS

MOLECULAR BASIS OF CANCER	Improved our understanding of the genomic underpinnings of cancer	For example, a TCGA study found the basal-like subtype of breast cancer to be similar to the serous subtype of ovarian cancer on a molecular level, suggesting that despite arising from different tissues in the body, these subtypes may share a common path of development and respond to similar therapeutic strategies.
TUMOR SUBTYPES	Revolutionized how cancer is classified	TCGA revolutionized how cancer is classified by identifying tumor subtypes with distinct sets of genomic alterations.*
THERAPEUTIC TARGETS	Identified genomic characteristics of tumors that can be targeted with currently available therapies or used to help with drug development	TCGA's identification of targetable genomic alterations in lung squamous cell carcinoma led to NCI's Lung-MAP Trial, which will treat patients based on the specific genomic changes in their tumor.

THE TEAM

20 COLLABORATING INSTITUTIONS across the United States and Canada

WHAT'S NEXT?

The Genomic Data Commons (GDC) houses TCGA and other NCI-generated data sets for scientists to access from anywhere. The GDC also has many expanded capabilities that will allow researchers to answer more clinically relevant questions with increased ease.

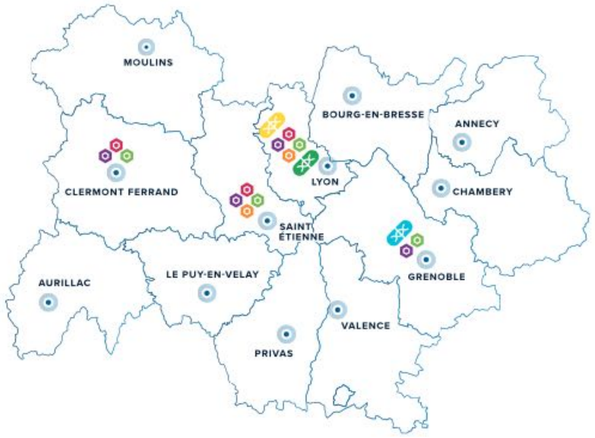
*TCGA's analysis of stomach cancer revealed that it is not a single disease, but a disease composed of four subtypes, including a new subtype characterized by infection with Epstein-Barr virus.

www.cancer.gov/ccg

FRANCE MÉDECINE GÉNOMIQUE 2025

>200’000 genomes per year in 2025

La plateforme AURAGEN



La plateforme AURAGEN propose une offre de séquençage à très haut débit aux acteurs impliqués en cancérologie et dans la prise en charge des maladies rares sur la région Auvergne Rhône-Alpes.

EN SAVOIR PLUS

CONSORTIUM

- Hôpitaux : Clermont-Ferrand, Grenoble, Lyon, Saint-Etienne
- CLCC : Centre L.Bérard, Centre J.Perrin, Inst. Cancérologie Loire
- Universités : Clermont-Ferrand, Grenoble, Lyon, Saint-Etienne
- Fondation Synergie Lyon Cancer, Mines de Saint-Etienne

LBMMS (Laboratoire de Biologie Médicale Multi-Sites)

- Site HCL : séquençage
- Site CHUGA : curation maladies rares
- Site CLB : curation génome tumoral

3. Identify genomic alterations

General DNA sequencing workflow

Genomic DNA or RNA

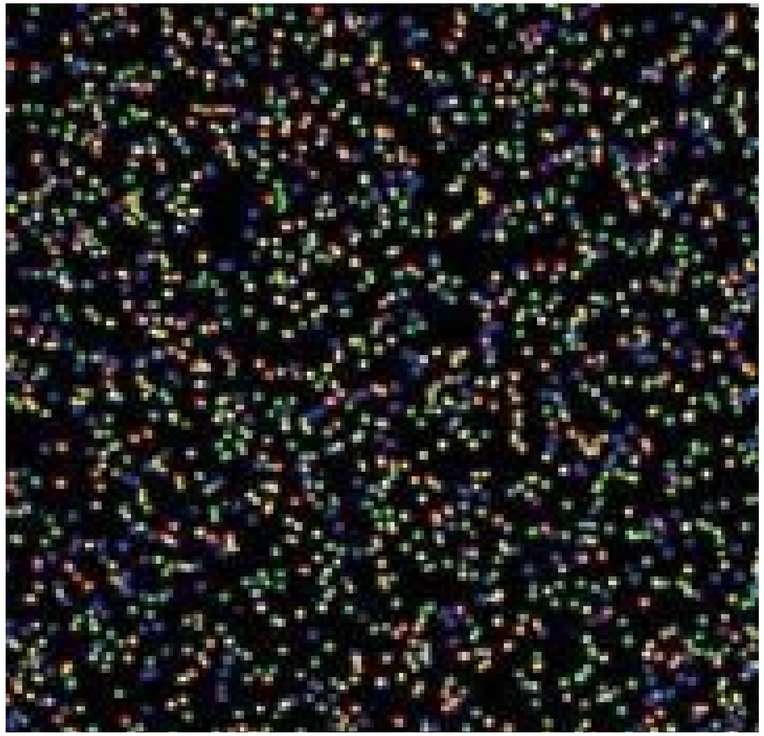
Library of DNA fragments

Sequencing device

Computational analysis

Basecalling

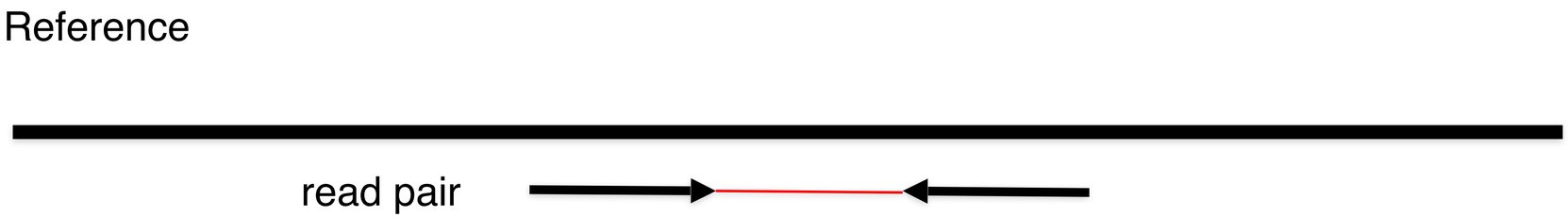
- **Prediction of the DNA sequence from the images**



FASTQ format

```
@HWI-ST791:134804979:D1VCPACXX:2:1212:13180:45201/1
CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC
+
CCCFFFFFHHHHHJJJJJJJJIJJJJJJIJJIJIGIGBFGGIJJJCFCHIG
@HWI-ST791:134804979:D1VCPACXX:2:2210:18677:62252/2
CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC
+
@@@DDDDDHFHHHHGAGHBEHH?EFHGHIICB?8BFIH=2?;?D2(9)8C@
@HWI-ST791:134804979:D1VCPACXX:2:2213:9376:11010/2
CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC
+
CCCFFFFFHHHHHJJJJJJJIJJJJIJJJJJJJJJIJJIIJIJJIGJIJF
@HWI-ST791:134804979:D1VCPACXX:2:2305:18281:53137/2
CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC
+
CCCFFFFFHHHHHJJJJJJJJJIJJJJJIJJIHHGHJJHGCGEEGGHIIB
@HWI-ST791:134804979:D1VCPACXX:2:2316:14351:64252/1
CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC
+
CCCFFFFFG;CDFIIBDACGGFAGIGCEBGIIGDEGEGHIGIG?F8;82BG
```

Paired-end sequencing



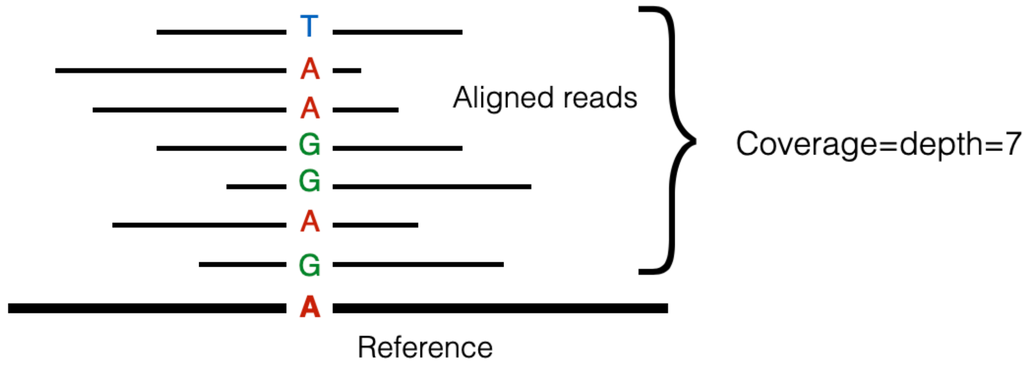
- Expected orientation:
  - one read on the forward strand, one read on the reverse strand

Reference Mapping

- **Why do we map reads to the reference?**
  - **By comparing the reads from a sequenced individual to a reference genome we can identify variants like SNPs, and rearrangements**
  - **To do this we need to identify where in the reference genome that a read might have come from**

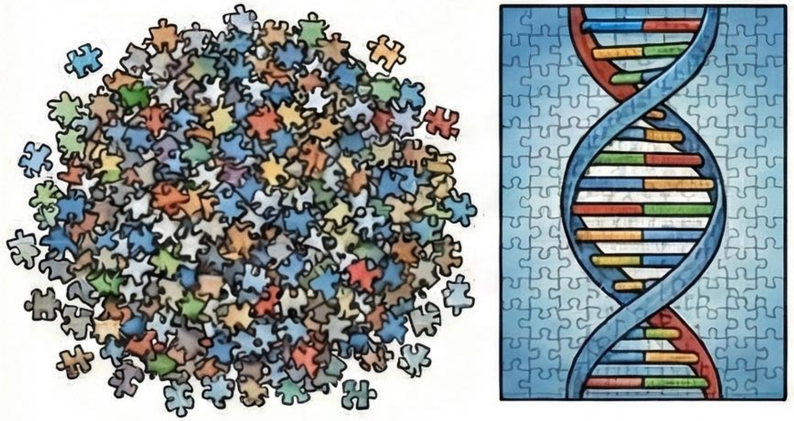
FASTA format

```
>yegR
ACTAACGGCTGCCACCGATAAATTTCAAAAAAGAGCATATACCTAATATTCAACTAAACA
GTGGCATCTTCAATATAATATATTAAAGCCCCCATGGAGTTACCCTGAAGGGCCTCAATG
TCCGTAATTCCTACTTATGTAGGAAATGTTGTACAGAACATTTATTATAATCCTATTCAA
TTATAATAATCATGCCATTATTATATTTAAACACTAGAGAGTGTCGTTGGTATTTAATGG
GGGAAGGTGAGATGAAAAAGATAGCTGCTATATCATTAATTAGTATTTTTATTATGTCTG
G
>emrK
AAATCAGGGATTGTACCGATGATTTATAGTTTCAAGTTGGCACTATAAGTCTTCTTACTA
ATCCTACAGGCGTAAGAATTGTATTGCAAAAGCCACGGTTTAGTCCTCTGTTGTTTTTTT
TGCACCTCATTTAAATTAGGCCTCCAACGTTCCTGGGATAATGTGCAACACATGCACTGT
GTTTGATATGAAGAATGAATGCTCTTTTCATTCAATTCATAAATTTCATCTATGAGAAAT
GAGAGATAATAGTGGAACAGATTAATTCAAATAAAAAACATTCTAACAGAAGAAAATACT
T
>evgA
AATACAATTCTTACGCCTGTAGGATTAGTAAGAAGACTTATAGTGCCAACTTGAAACTAT
AAATCATCGGTACAATCCCTGATTTTATTGTTGACATTTCATTTATGCCGACTATTTATA
TGGTATACTTGTCGAATTATCTTAAAGGAAGCTCAGATTTTCTTATTTTTATTGAGAAAA
TGAGATGACGCCTTATGTCTGTATTACTACAGGGAGAAGGGAGATGCTTCATTGCAAAGG
GAATAATCTATGAACGCAATAATTATTGATGACCATCCTCTTGCTATCGCAGCAATTCGT
>yfdX
TGGCTGTATTTACATTTAATTAATCAGTATTTACATCGATATAATAAATGACATCTCTTT
GTGGTATATAAGAATAGTTCTCTGCGACAGGAAGCATATTCCTACAATTGTAAGACTAAA
ATACTTCTTGCGATAATAACTACAACTGTAAGATAACCCTTTCAAAATGACCGTTGCTCT
CTGATTTCTCATTTCATGCTCACCCAATATGATGGCGGCGTTTTCTAAAACTGTTAAAGA
ATGAGGTAAGTATGAAACGTTTAATTATGGCCACGATGGTCACAGCAATTCTGGCATCTT
C
```



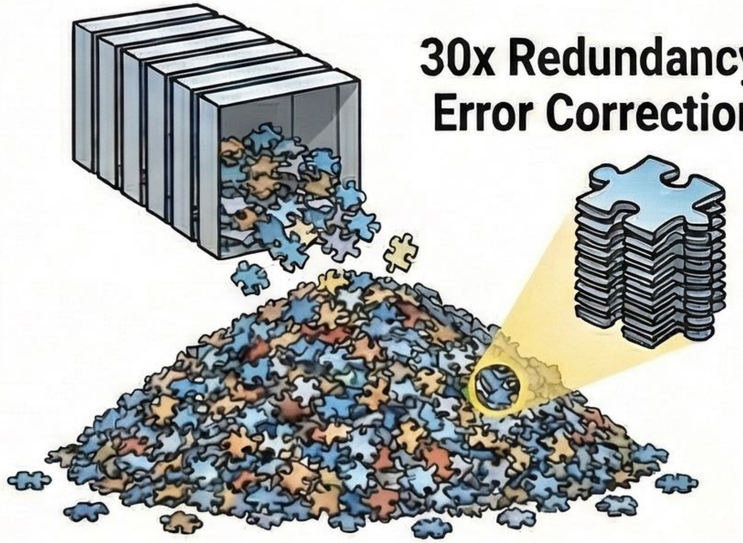
1. The Input (Reads)

Billions of pieces, unknown order



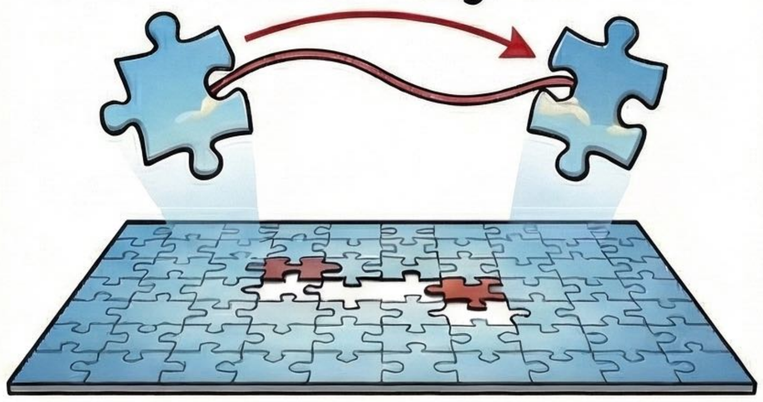
2. Coverage (Depth)

30x Redundancy: Error Correction



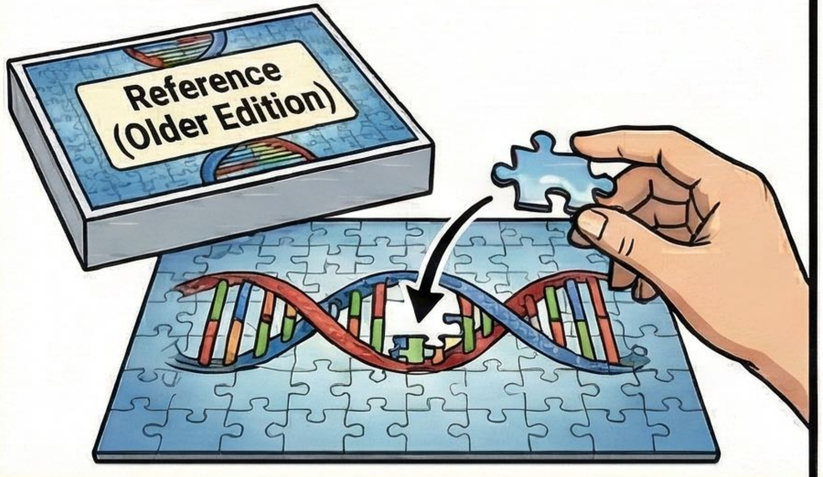
3. Paired-End

Connected pieces bridge gaps



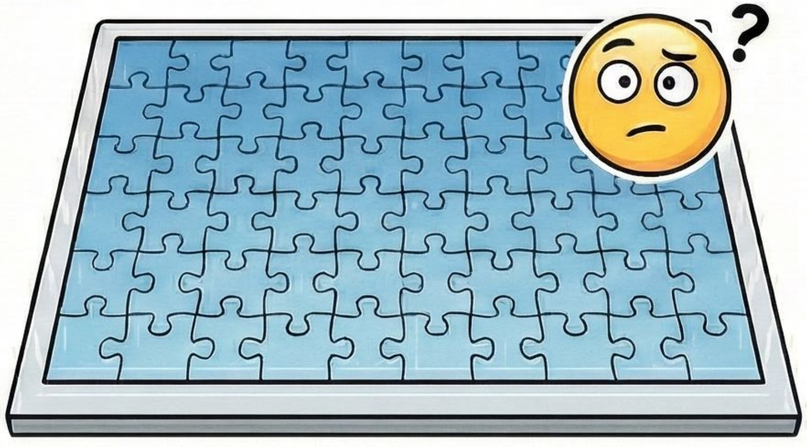
4. Alignment (Mapping)

Using the Box Lid as a Guide



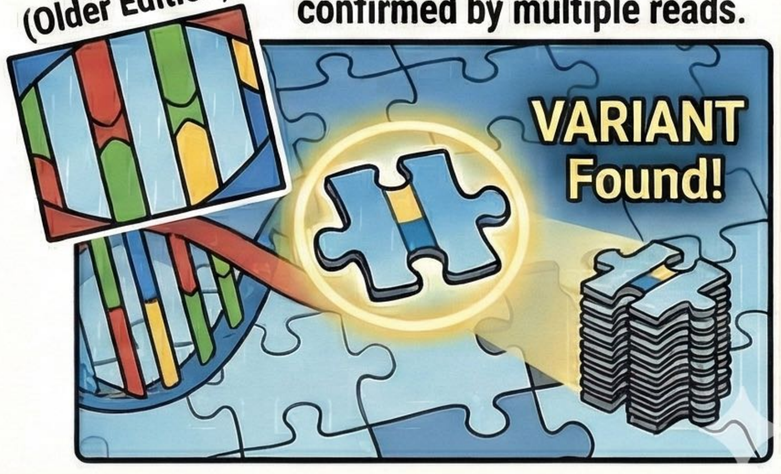
5. Repetitive Regions

The "Blue Sky" Problem



6. Variant Calling

Different base pair than the guide, confirmed by multiple reads.



What is a mutation

- **In NGS, a mutation is a position where we detected the presence of a non-reference allele**
- **Always relative to the reference genome**
  - **Not necessary unusual: sometimes the reference allele is extremely rare**
  - **Not necessarily matching the ethnicity of your samples**
  - **Latest version (hg38) contains some alternative contigs (ALT) for highly polymorphic regions of the genome (e.g. *HLA* genes on chromosome 6). Useful but adds extra step for the alignment.**

Types of variants

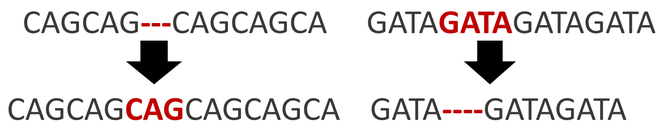
Single Nucleotide Variants/Substitutions (SNV)

$n_{\mathrm{SNV}} \approx 40 - 50$

Short insertions/deletions (indels; 1-20bp)

$n_{\mathrm{indel}} \approx 3$

Short Tandem Repeats (STR)

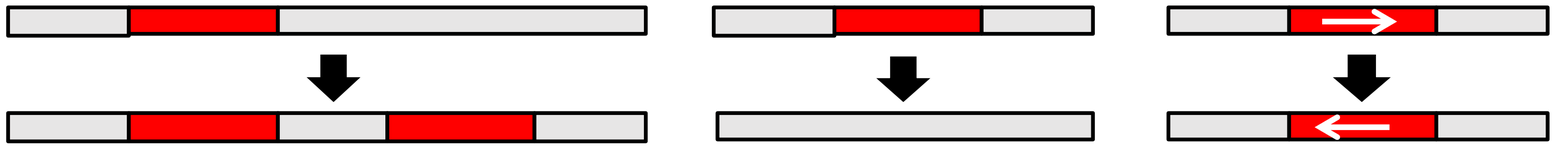


$n_{\mathrm{STR}} \approx 75$

Numbers are new variants per genome per generation:
"*de novo*" mutations

Types of variants

Structural variants (SV), copy number variants (CNV) (20bp to mega-bases)



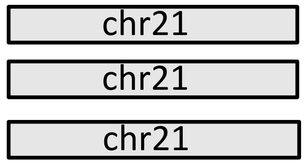
Duplication

Deletion

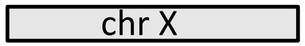
Inversion

$n_{\mathrm{SV}} \approx 0.2$

Aneuploidies



Down syndrome



$n_{\mathrm{ANE}} \approx 0.001$

Turner's syndrome

XXY: Klinefelter syndrome, XXX: Triple X …

Germline variants

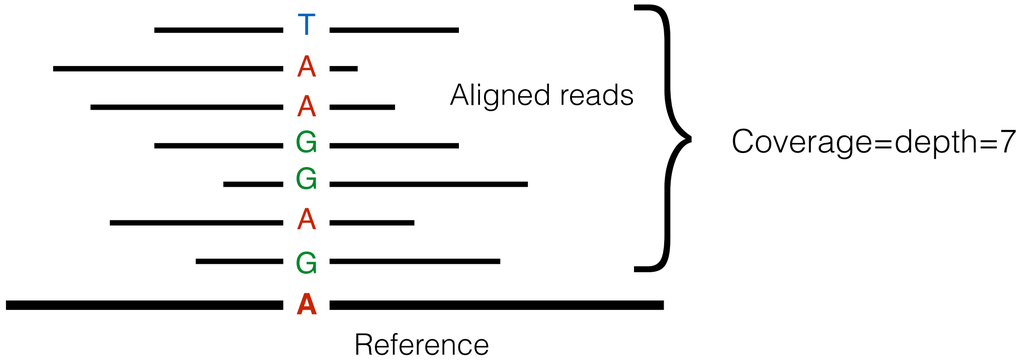
For European individuals, with respect to the reference:

- **3-4M single-nucleotide variants**
  - **Among them, 1.2M homozygous**
- **500k short insertions and deletions**
- **22k coding variants**
  - **Among them, 10k non-synonymous**
  - **200 loss of function**
- **~100s of copy number variants (total 5Mb)**
- **~1000s structural variations?**

Germline vs somatic variants

- **Germline are common: ~1/kb = 1000/Mb (compared to reference genome)**
  - **"De novo" germline (from germ cells of a parent) are very rare: ~100/genome**
- **Somatic are rare: ~1/Mb**
- **Exome ~ 50Mb (2% of the genome)**
  50 somatic and 50'000 germline
- **Genome ~ 3Gb**
  3'000 somatic and 3'000'000 germline
- **Remember: germline mutations are also present when sequencing tumors DNA**

NGS germline mutation calling



Germline variants: the easy case (with enough coverage)

- **10 possible genotypes: AG, AA, GG…**
- **Expected variant allelic fraction (VAF): 50%, 0% or 100%**
- **Binomial sampling + Sequencing errors**

GATK, Strelka

Cancer genomes have specific properties that warrant specialised analytical strategies

- **Tumor/normal admixture**
  - Tumour DNA is often contaminated with DNA from non-malignant cells
  - May dilute important biological signals
- **Intra-tumoural heterogeneity**
  - Cancer is often a mosaic of cellular populations that are genetically distinct
- **Genomic instability**
  - Copy number changes, loss of heterozygosity and genomic rearrangements will distort expected allelic distributions

- **Expected variant allelic fraction (VAF)?**
- **Sequencing errors vs somatic variant?**

“We conclude that somatic mutation calling remains an unsolved problem.”

A Comprehensive Assessment of Somatic Mutation Calling in Cancer Genomes

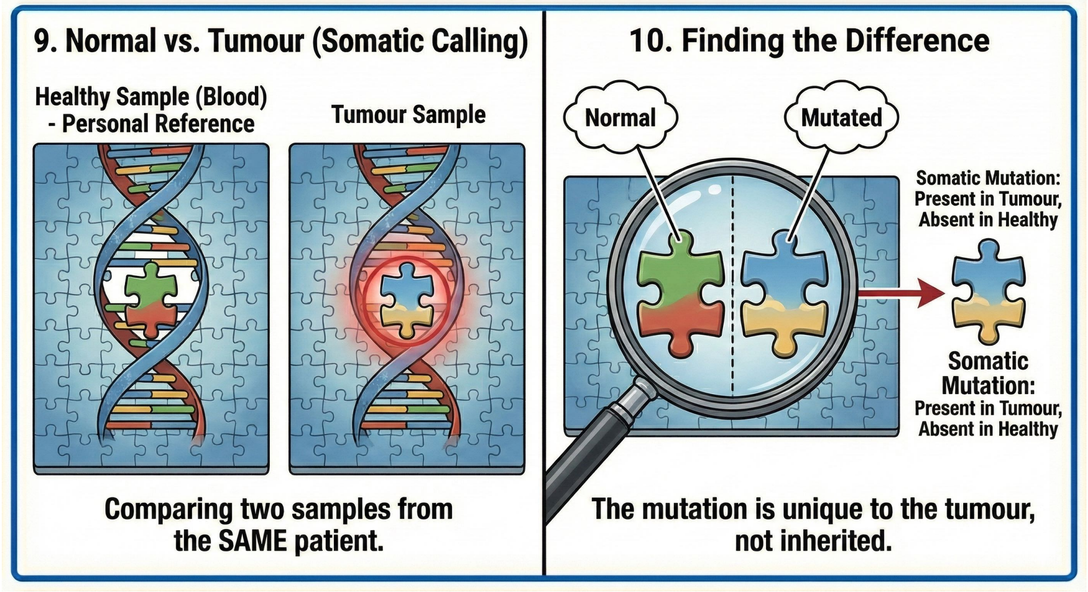
Tyler S Alioto, Sophia Derdak, Timothy A Beck, Paul C Boutros, Lawrence Bower, Ivo Buchhalter, Matthew D Eldridge, Nicholas J Harding, Lawrence Edward Heisler, Eivind Hovig, David T W Jones, Andrew G Lynch, Sigve Nakken, Paolo Ribeca, Anne-Sophie Sertier, Jared T Simpson, Paul Spellman, Patrick Tarpey, Laurie Tonon, Daniel Vodák, Takafumi N Yamaguchi, Sergi Beltran Agullo, Marc Dabad, Robert E Denroche, Philip Ginsbach, Simon C Heath, Emanuele Raineri, Charlotte L Anderson, Benedikt Brors, Ruben Drews, Roland Eils, Akihiro Fujimoto, Francesc Castro Giner, Minghui He, Pablo Hennings-Yeomans, Barbara Hutter, Natalie Jäger, Rolf Kabbe, Cyriac Kandoth, Semin Lee, Louis Létourneau, Singer Ma, Hidewaki Nakagawa, Nagarajan Paramasivam, Anne-Marie Patch, Myron Peto, Matthias Schlesner, Sahil Seth, David Torrents, David A Wheeler, Liu Xi, John Zhang, Daniela S Gerhard, Víctor Quesada, Rafael Valdés-Mas, Marta Gut, Peter J Campbell, Thomas J Hudson, John D McPherson, Xose S Puente, Ivo G Gut

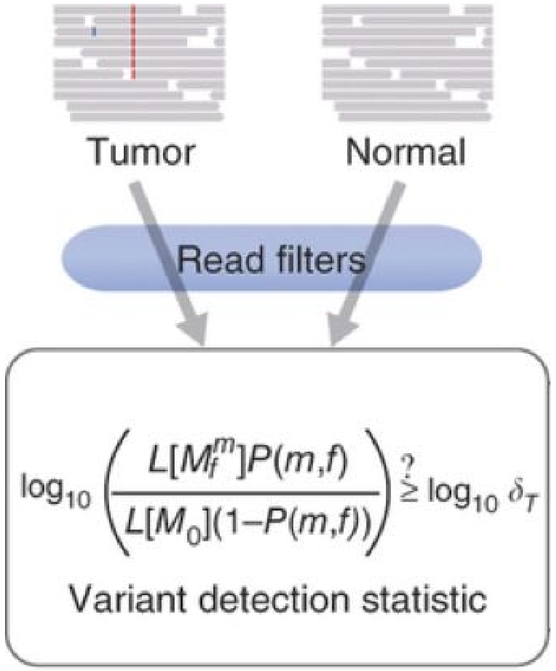
doi: http://dx.doi.org/10.1101/012997

2015, International Cancer Genome Consortium (ICGC)

Tumor-Normal pair

- Data are generated from a pair of DNA samples from the same patient: tumour and normal (preferably blood).
- Most common study design, routinely used. Works really well for AF>10%.
- Allows classification of variants in somatic/germline status.



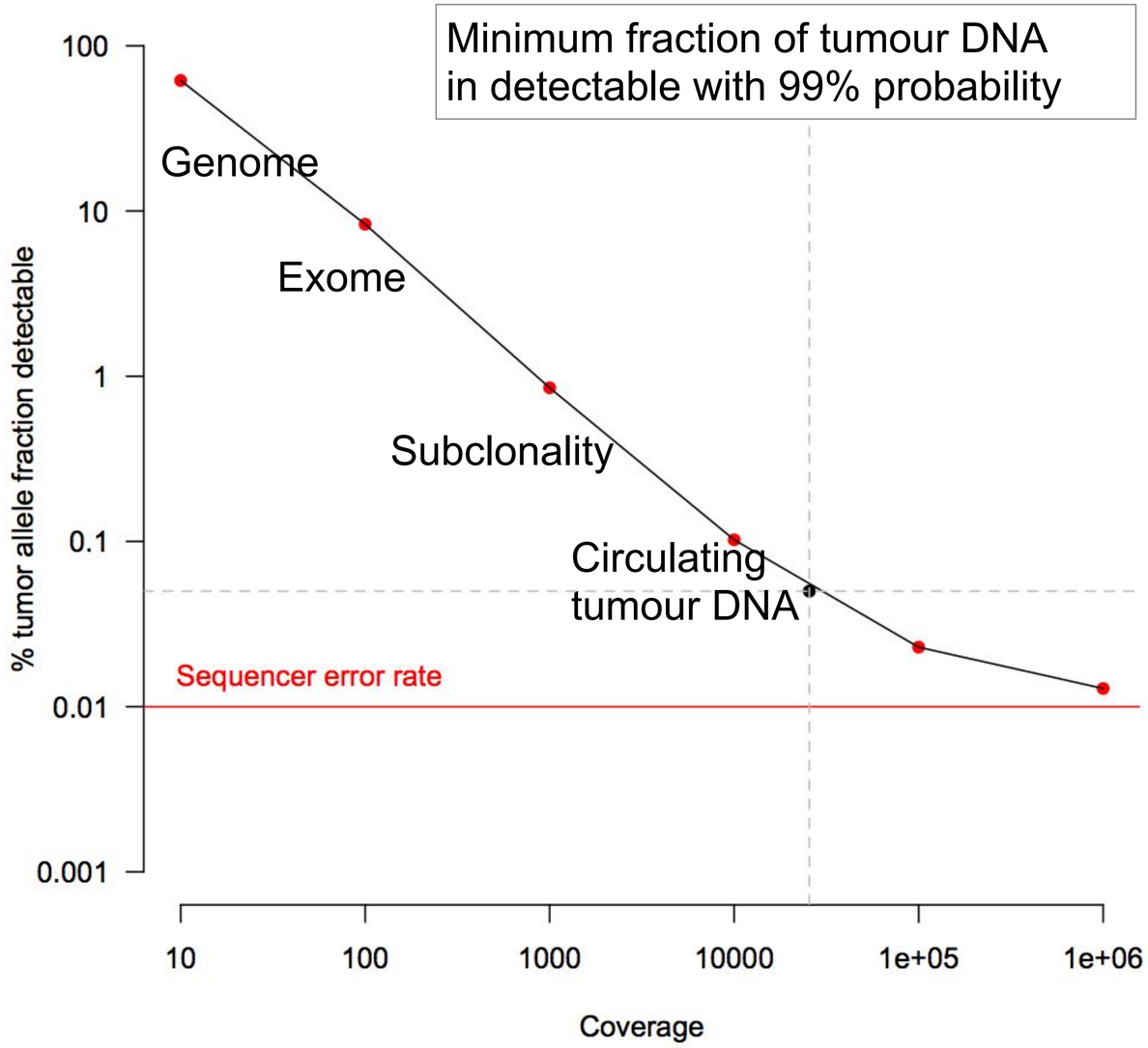


Software: MuTect, Strelka

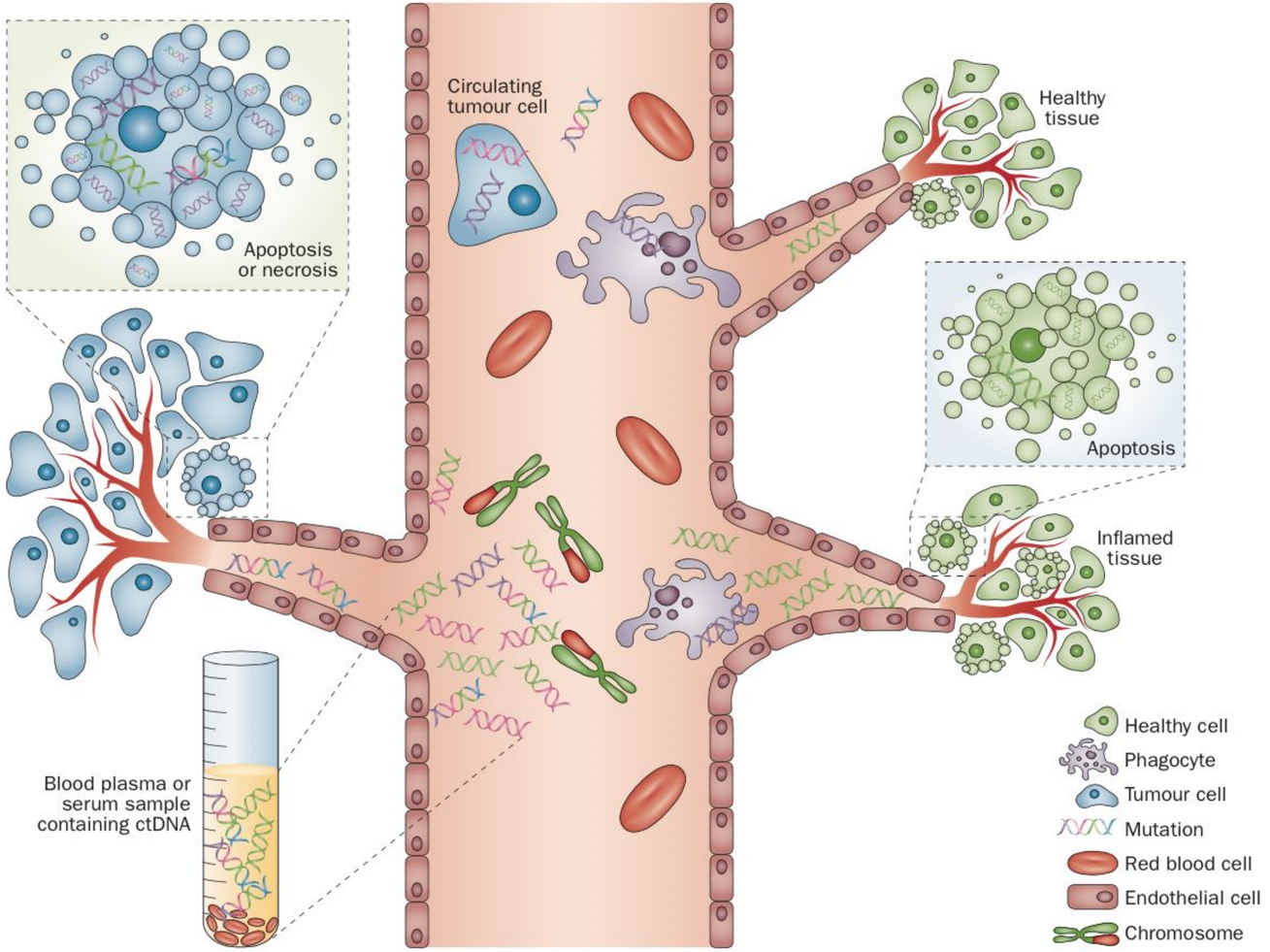
Statistical issue

- **When sequencing a tumor, 1/1000 variant found is actually somatic**
- **Need very high sensitivity to detect variants from matched germline DNA:**
  - **99.9% sensitivity → 1 missed /Mb**
    → 2 somatic detected /Mb
    → False Discovery Rate =50%

Mutation detection sensitivity is dictated by sequencing depth and is limited by sequencer & sequencing error

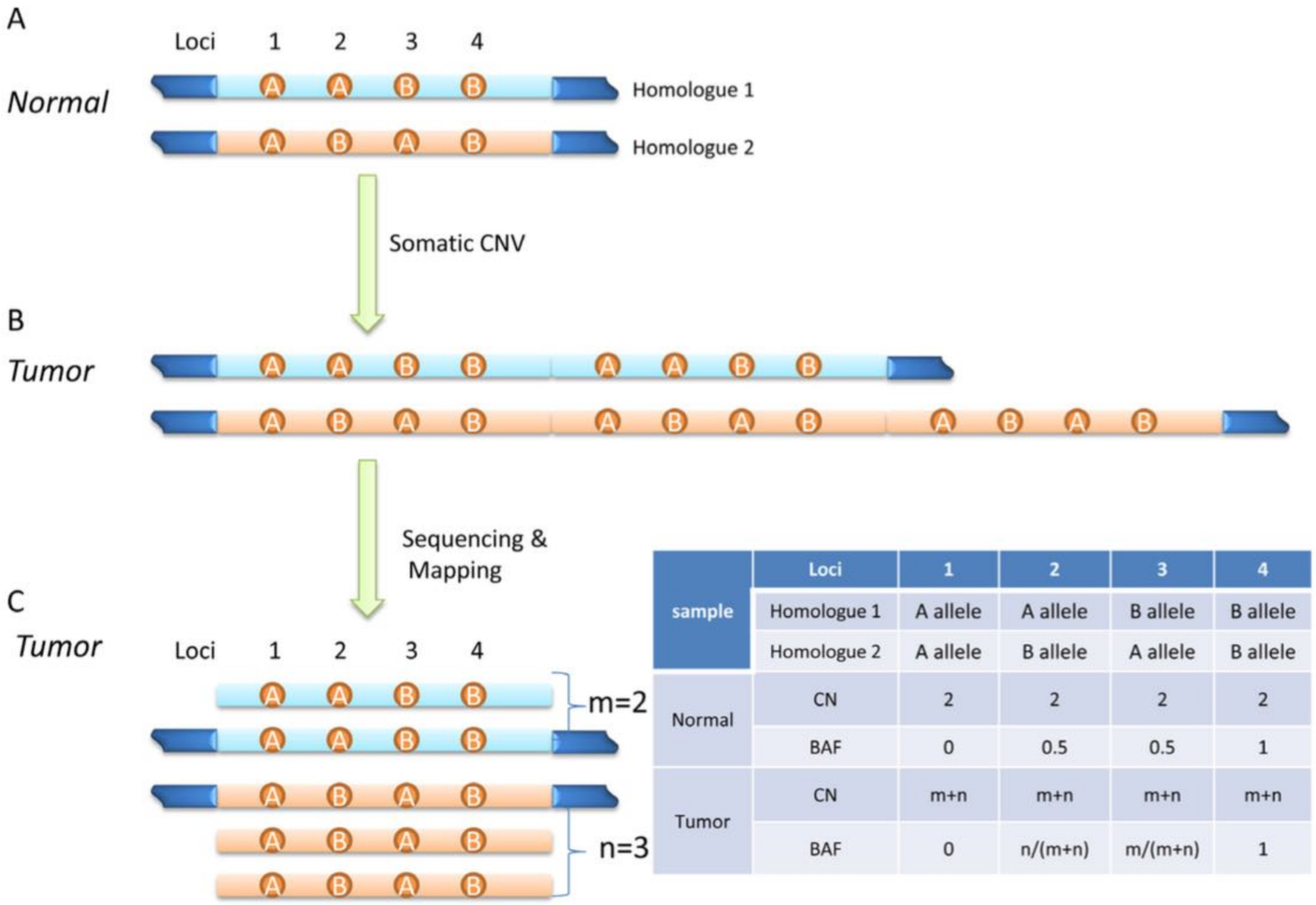


The ultimate complex mixture: Cell-free DNA dissolved in blood is derived from many normal cells and a few tumour cells



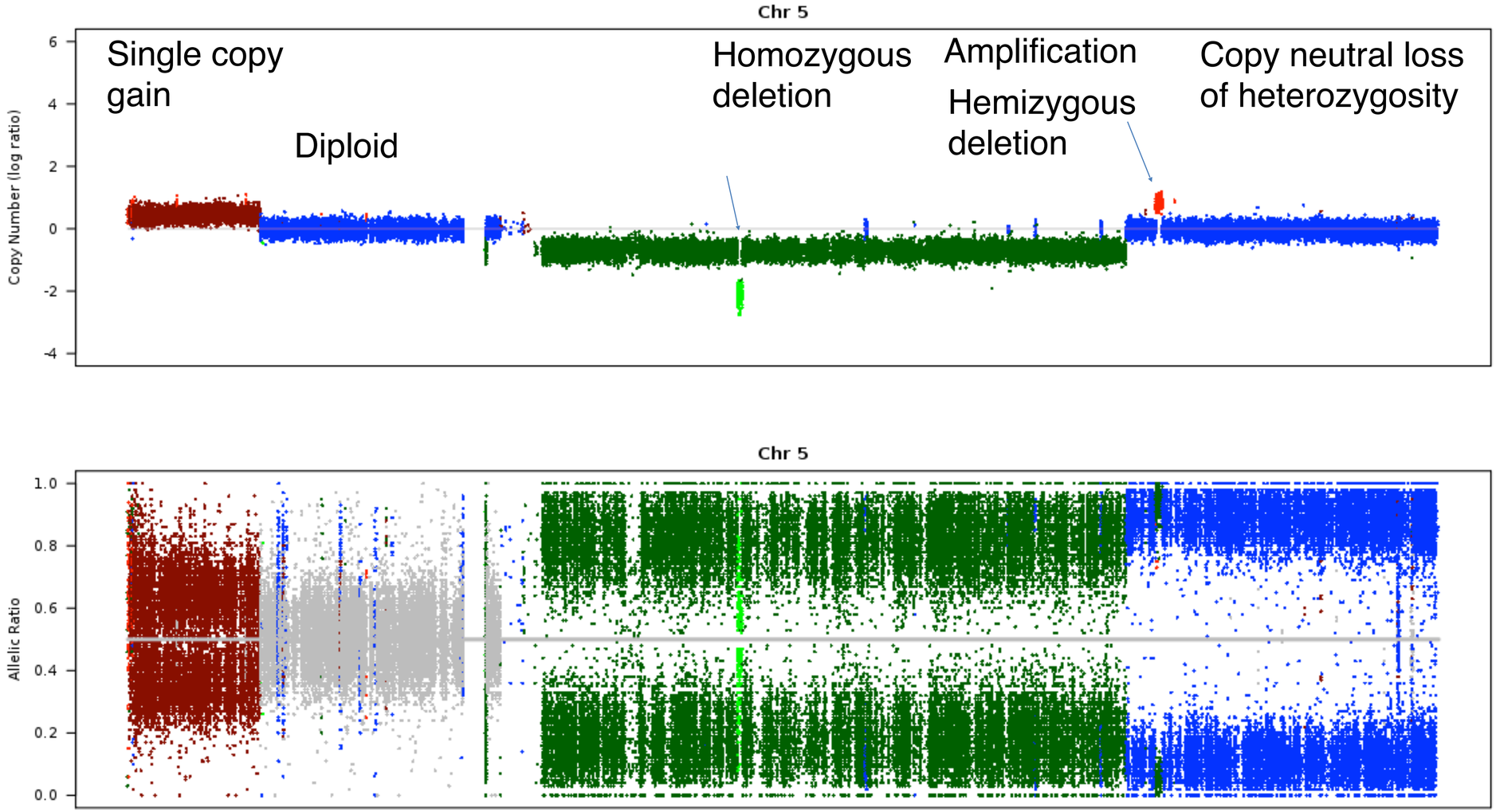
Crowley et al., *Nature Reviews Clinical Oncology*, 2013

Copy number variants (CNVs)

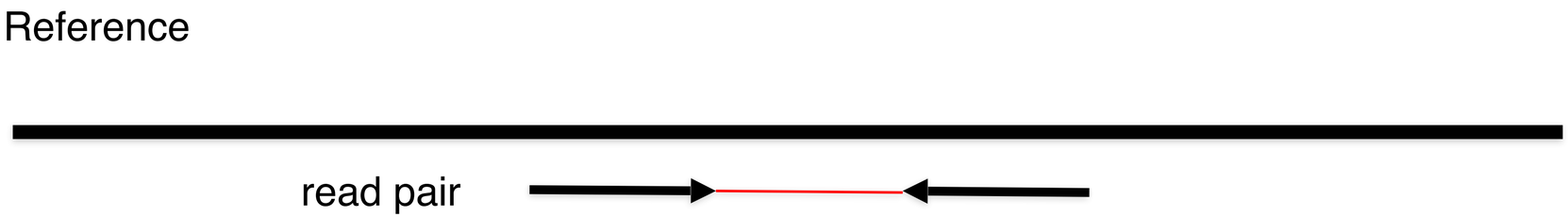


sample	Loci	1	2	3	4
	Homologue 1	A allele	A allele	B allele	B allele
	Homologue 2	A allele	B allele	A allele	B allele
Normal	CN	2	2	2	2
	BAF	0	0.5	0.5	1
Tumor	CN	m+n	m+n	m+n	m+n
	BAF	0	n/(m+n)	m/(m+n)	1

Classes of copy number alteration

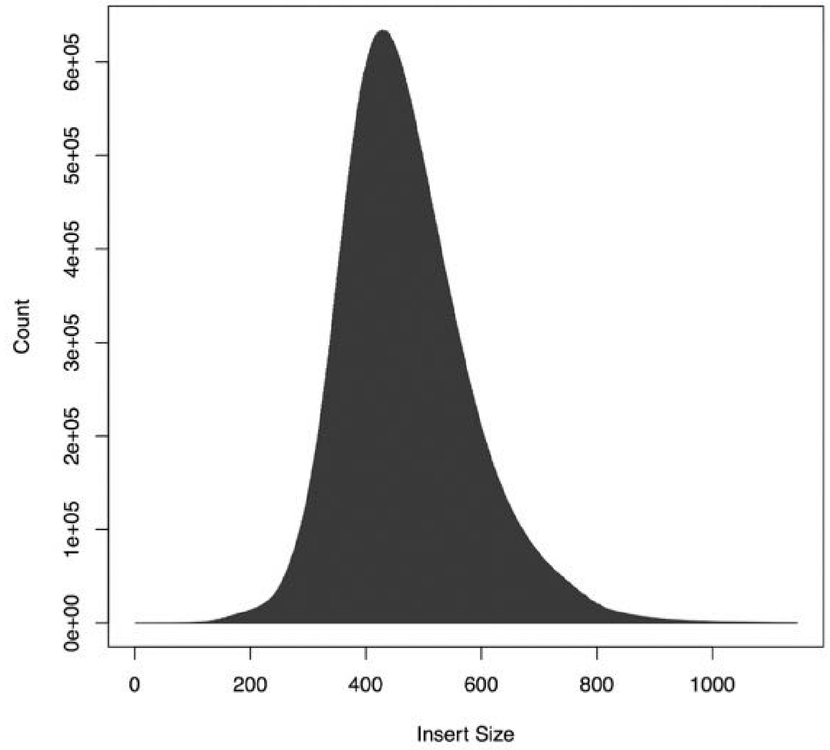


Read pair orientation and structural variants (SV)



- Expected orientation:
  - one read on the forward strand, one read on the reverse strand

Fragment size distribution

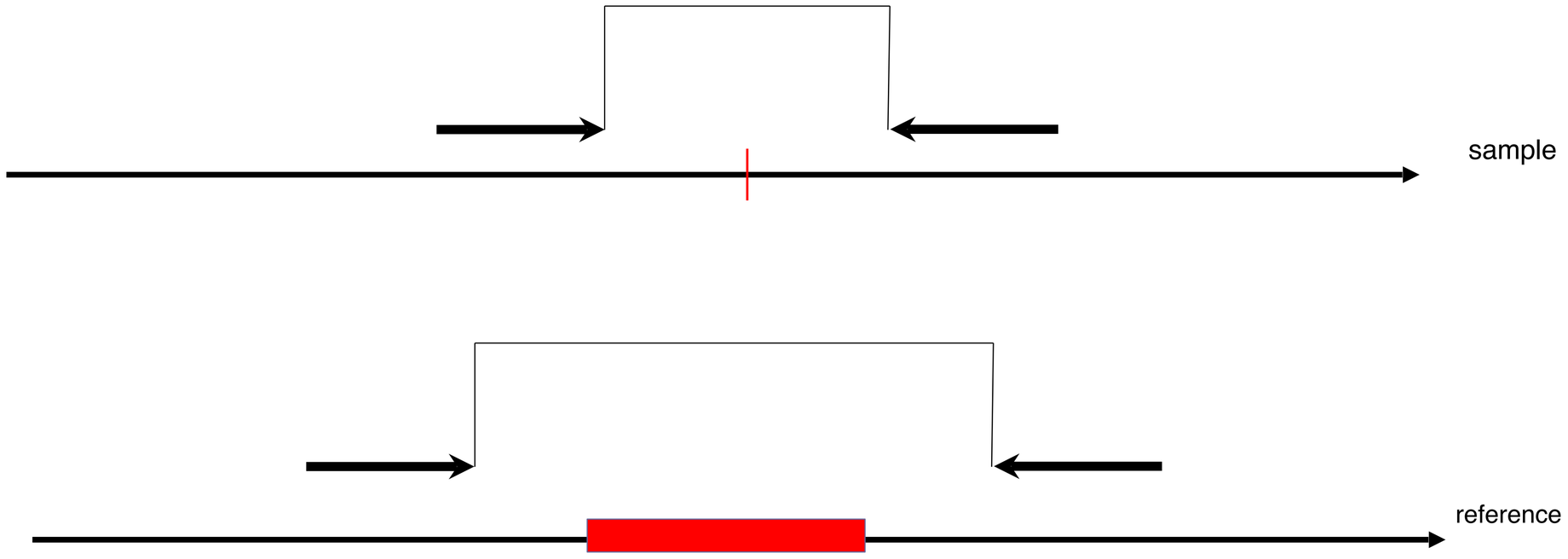


- Fragment/insert size is determined by library preparation
- Pairs that match the expected orientation and distance are called *concordant*
- *Discordant* read pairs give evidence of structural variation

from: doi:10.1038/ng.3121

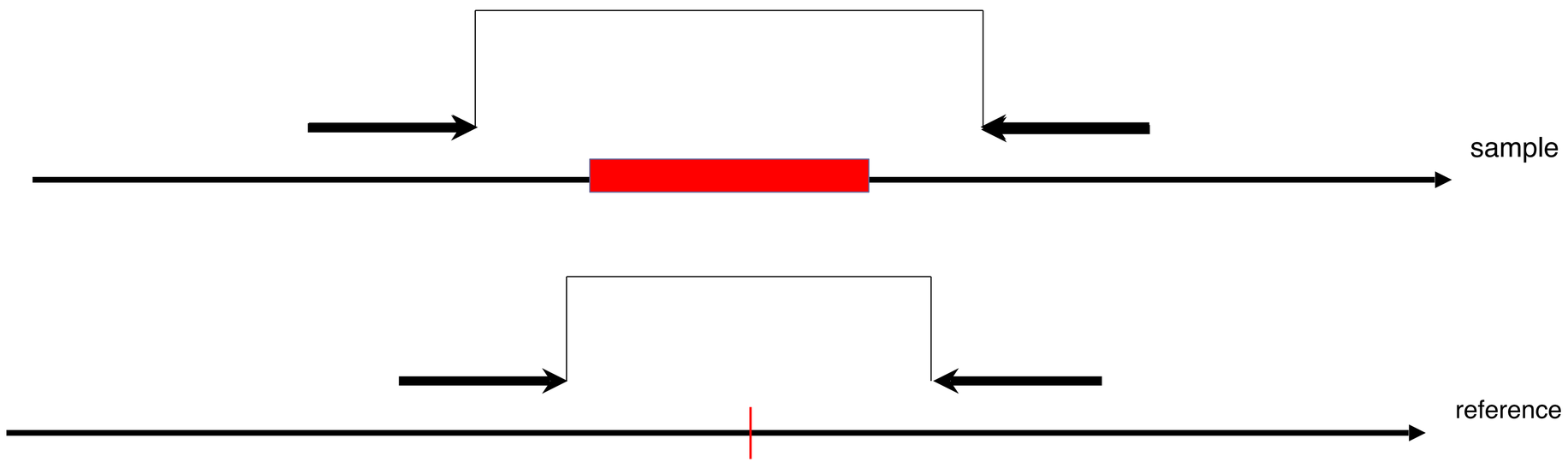
SV Signatures: Deletion

SV Signatures: Deletion



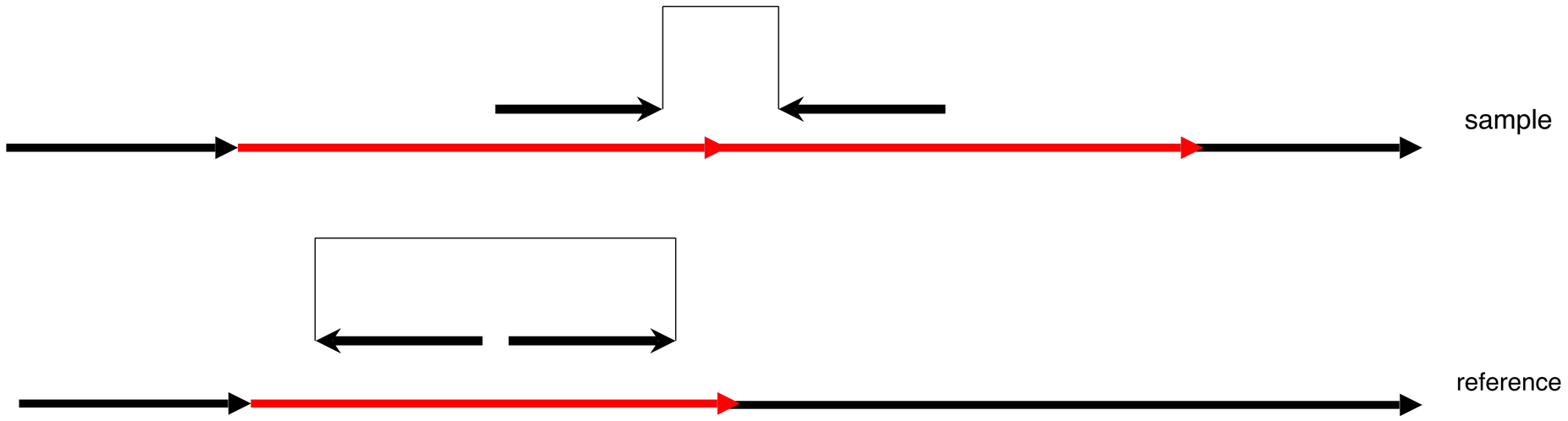
Signature: mapped insert size **larger** than expected

SV Signatures: Insertion



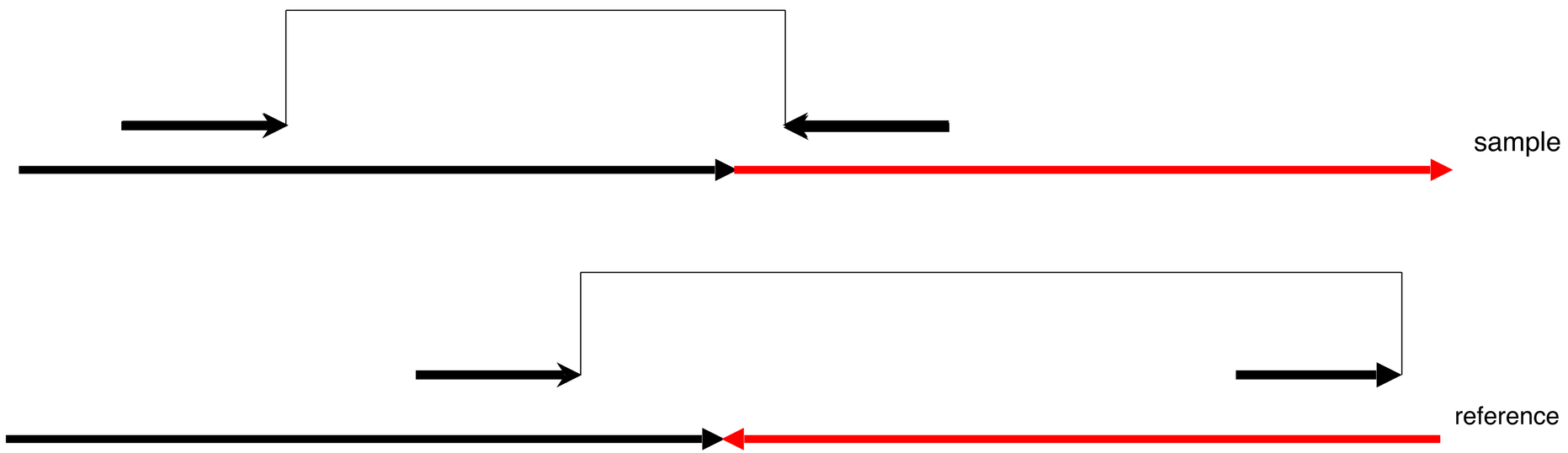
Signature: mapped insert size **smaller** than expected

SV Signatures: Tandem Duplication



Signature: wrong orientation

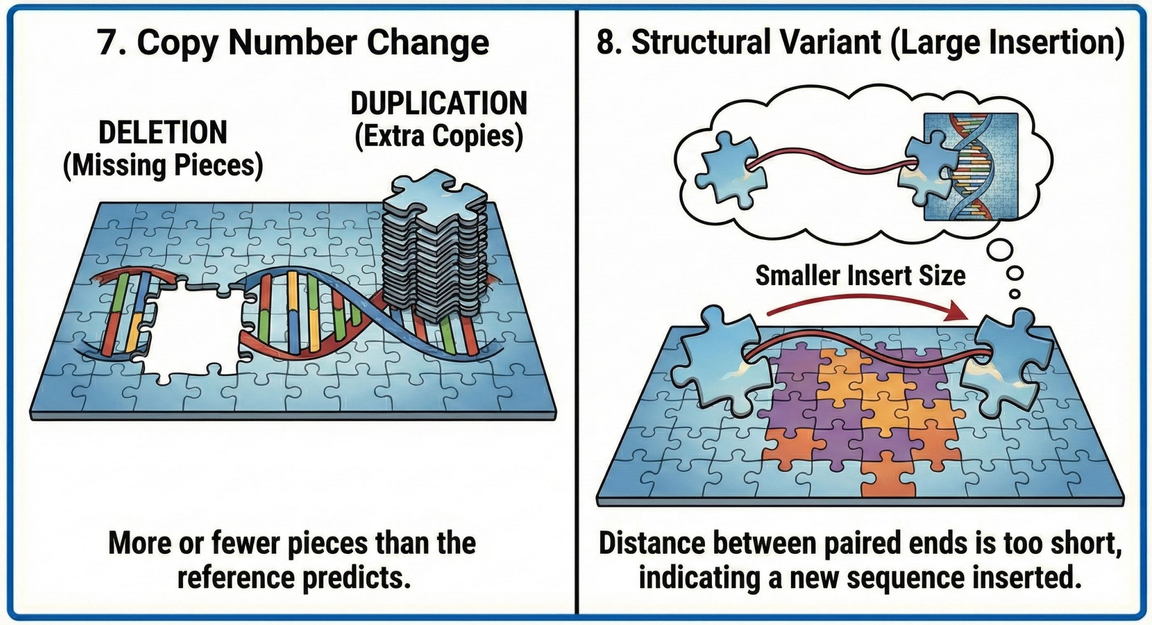
SV Signatures: Inversion



Signature: wrong orientation

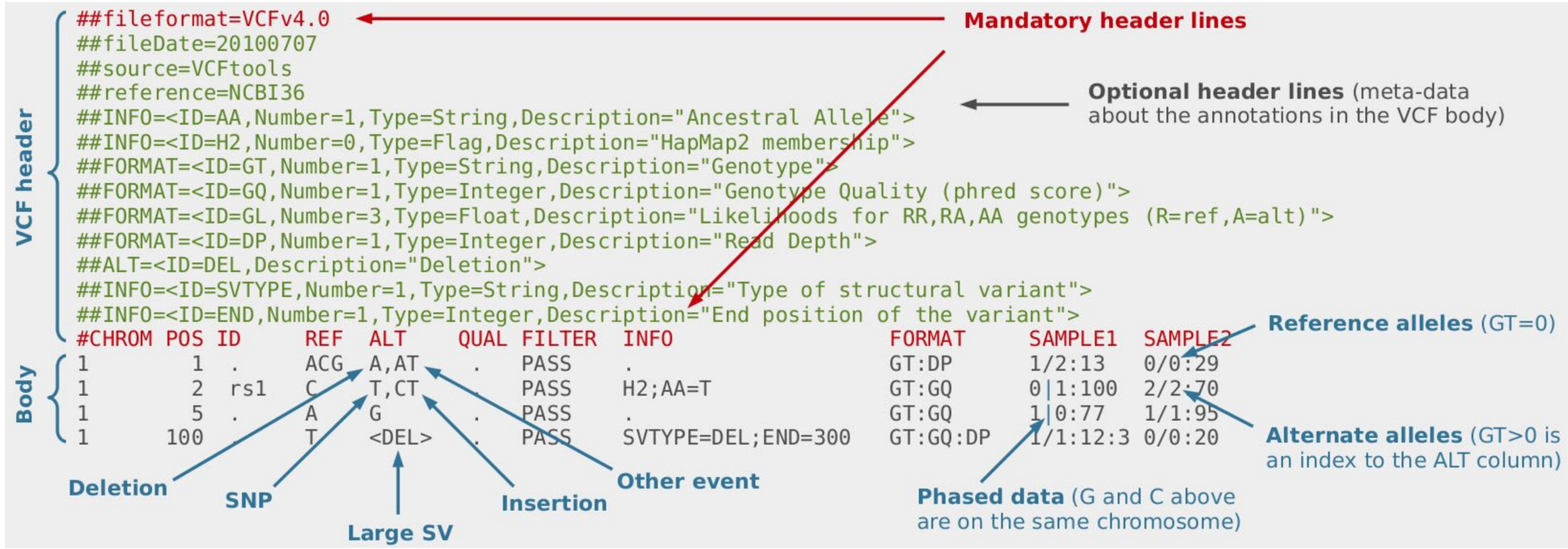
SV summary

Type	Mapped Distance	Orientation
Insertion	too small	correct
Deletion	too big	correct
Inversion	*	
Tandem duplication	*	
Interchromosomal	different chromosomes	N/A



VCF format for variant calling

- **Consists of Metadata and a precise definition of the variant in tab delimited fields**
  - **precise definitions: https://samtools.github.io/hts-specs/**

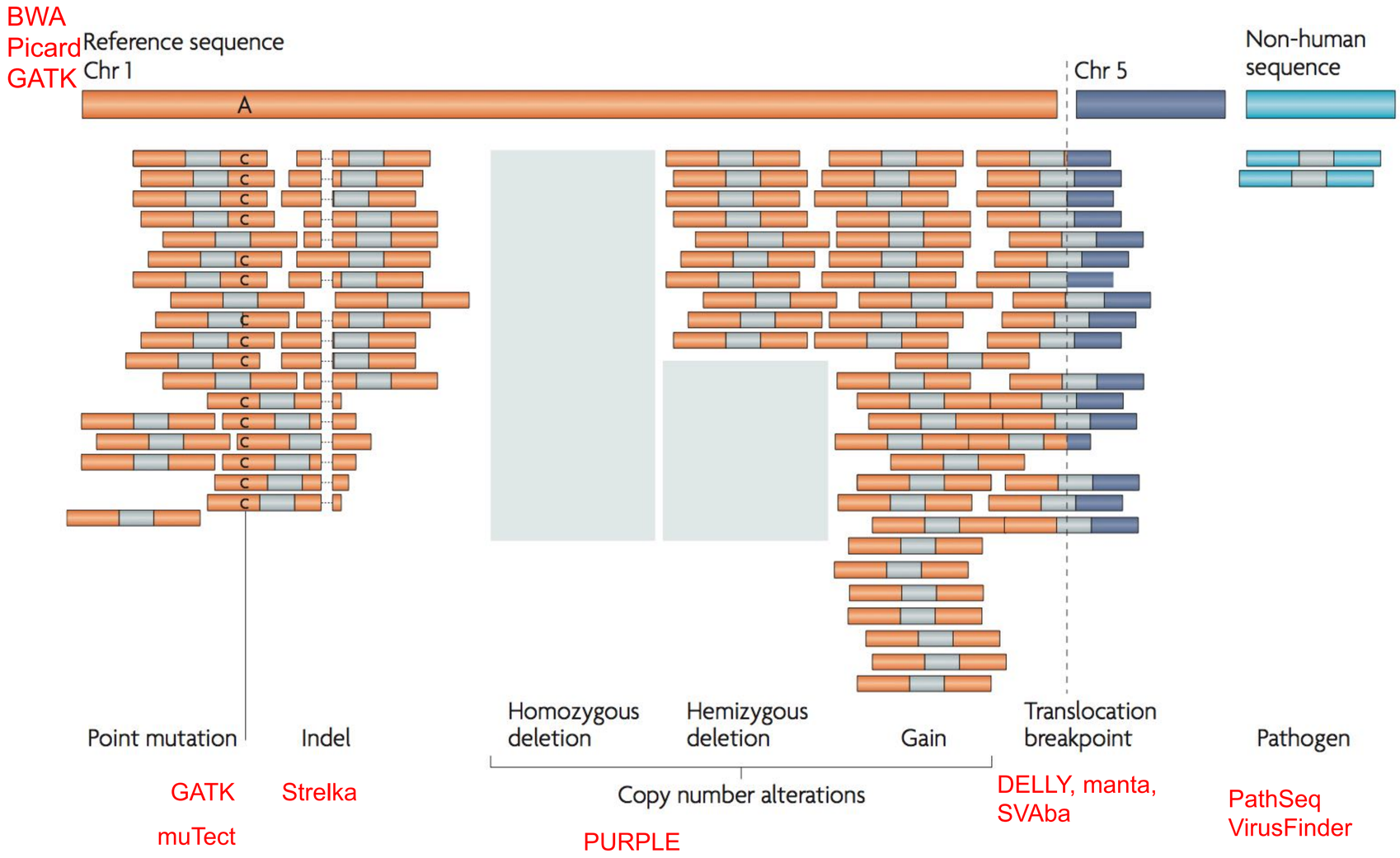


VCF file format

- Not a very human-readable format, but flexible a many tools exist to manage/ convert them:
  - bcftools: https://samtools.github.io/bcftools/
  - R VariantAnnotation package: bioconductor.org/packages/VariantAnnotation
- Generally a final annotation step also converts them in a TAB delimited text file (e.g. ANNOVAR)

Summary

Multiple types of cancer genome variation may be inferred from sequencing read alignments



Meyerson M, Gabriel S, Getz G. Nat Rev Genet. 2010