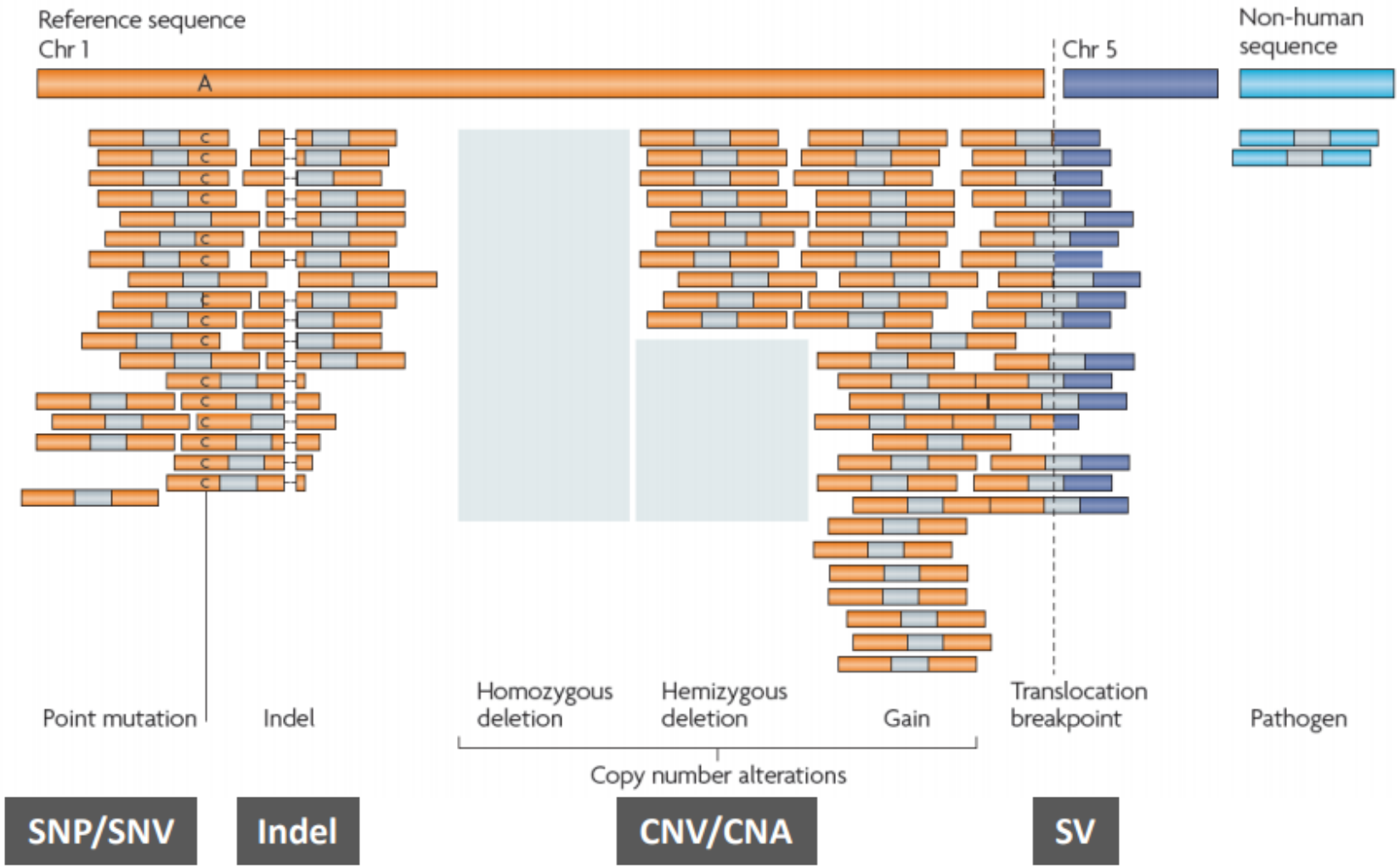
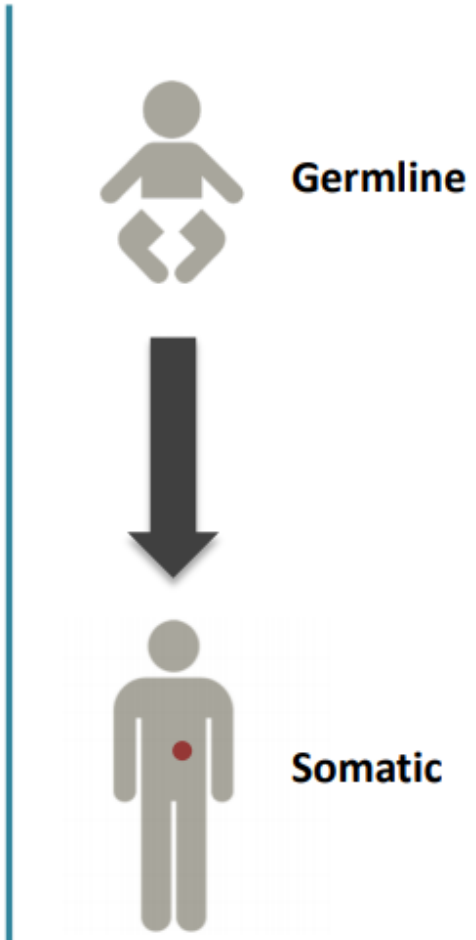
Somatic Variant Calling

Different types of variants

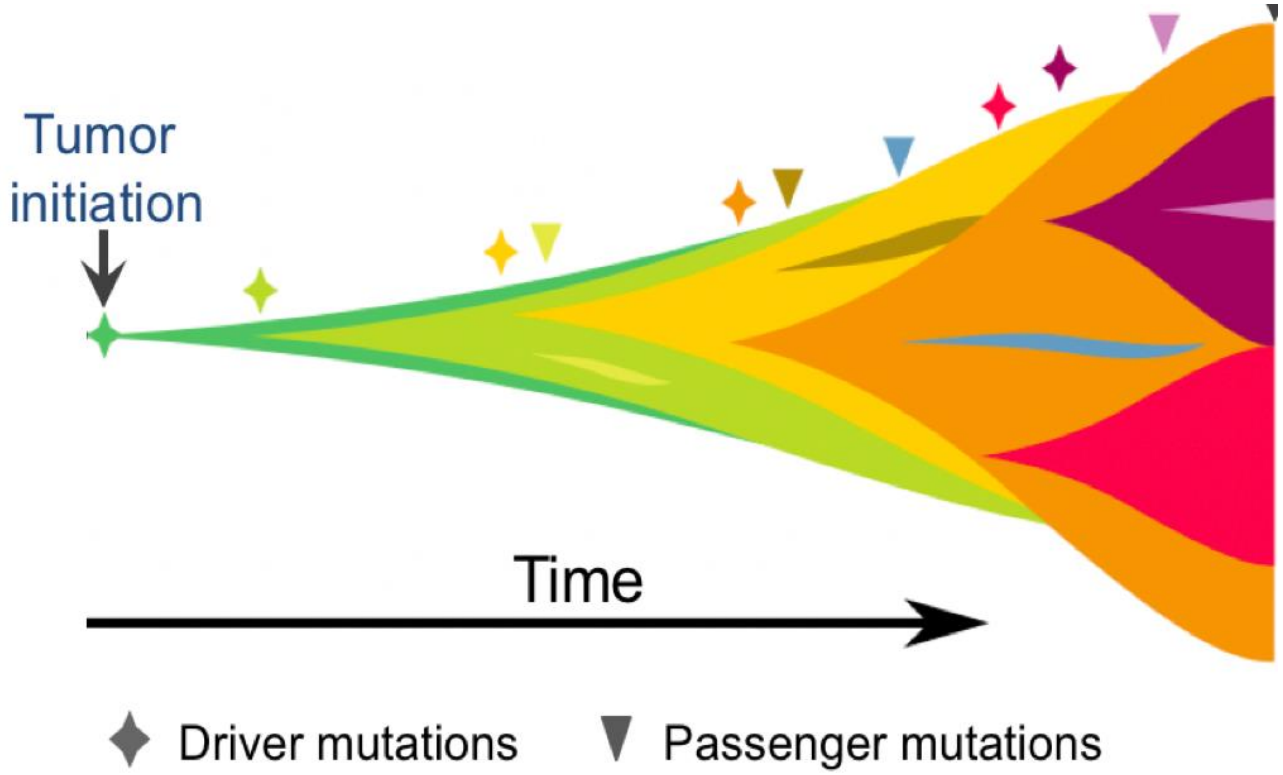




from Introduction to Somatic Variant Discovery, GATK Best Practices for Variant Discovery

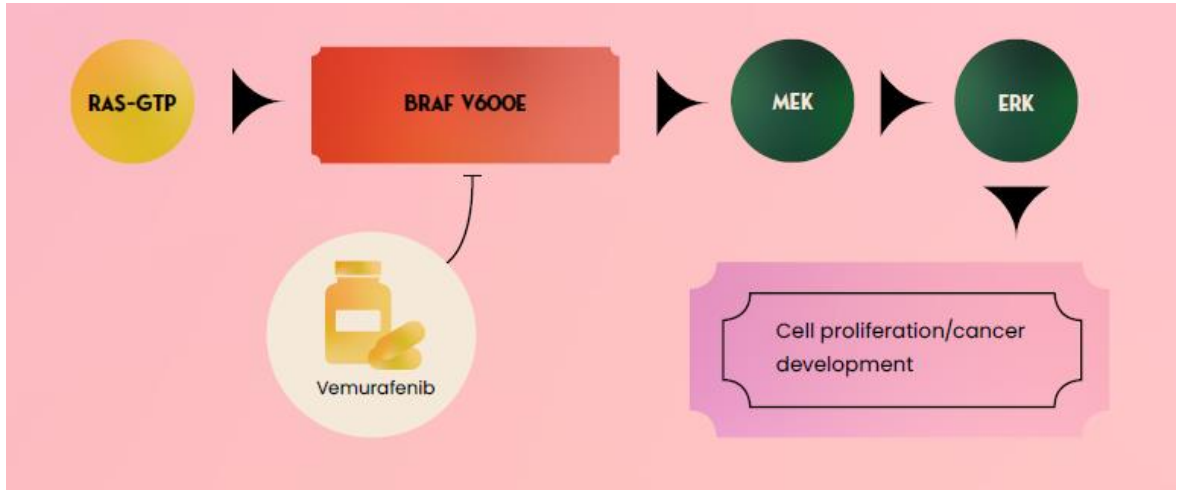
Role of somatic mutation events in tumor progression

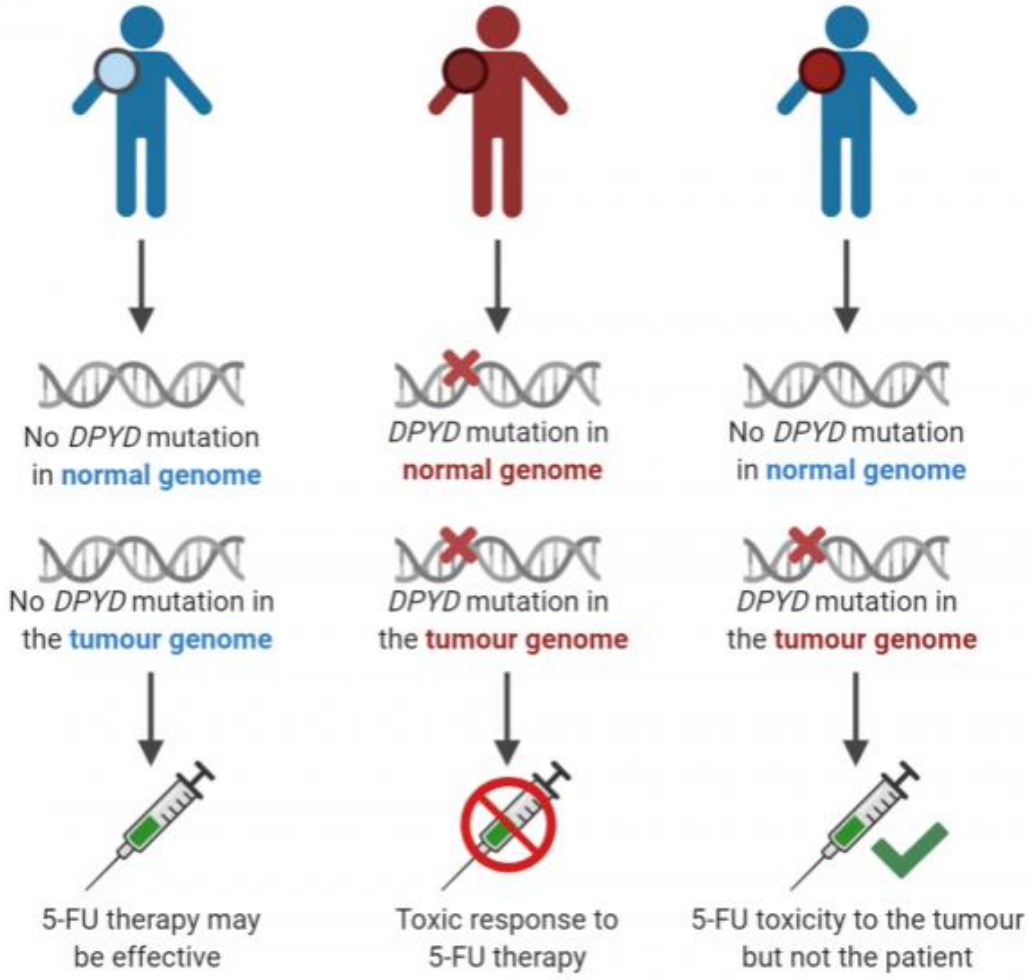
Increasing tumor heterogeneity as genomic instability increases



https://www.zhenghulab.org/project/computationalmodeling/featured.png

Cancer sequencing helps guide and prioritize cancer treatment options





https://www.technologynetworks.com/cancer-research/infographics/the-genetic-basis-of-cancer-382057

https://www.bcgsc.ca/news/genome-sequencing-helps-prioritize-cancer-treatment-options

Why is SNV calling in cancer samples more complicated ?

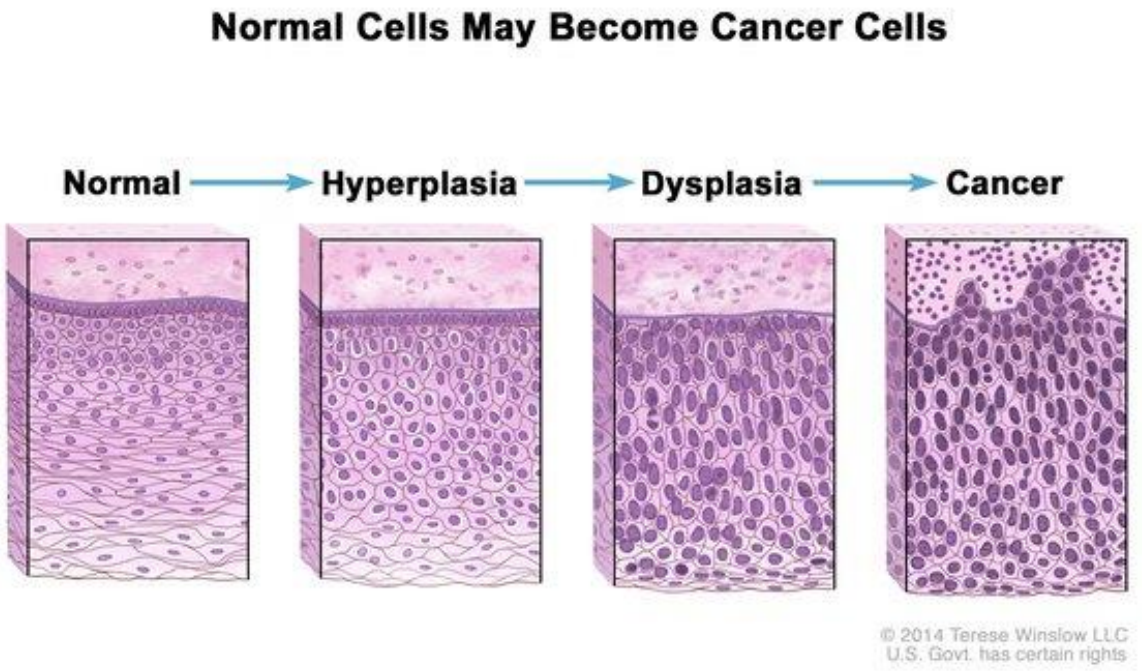
FOR MANY REASONS

Why is SNV calling in cancer samples more complicated ?

Low tumor cellularity (tumour DNA content)

Tumor samples may have lower DNA content

→ need sensitivity in variant calling +++



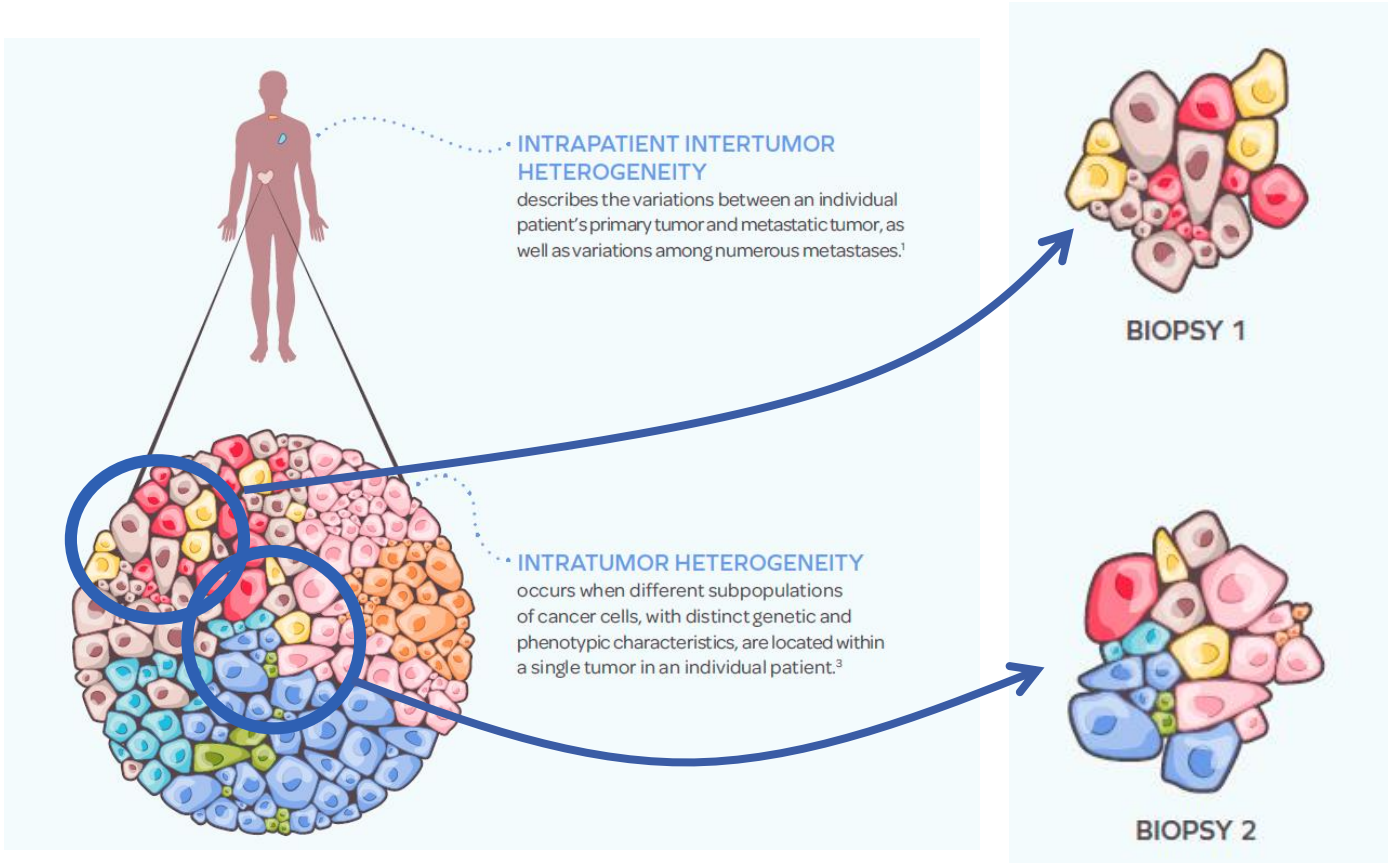
https://visualsonline.cancer.gov/details.cfm?imageid=9752

Why is SNV calling in cancer samples more complicated ?

Intra-tumour heterogeneity in which multiple tumour cell populations (subclones) exist

Multiple subclonal populations that are constantly evolving.

→ variants can be present in only one subclonal population



https://go.technologynetworks.com/tumor-heterogeneity

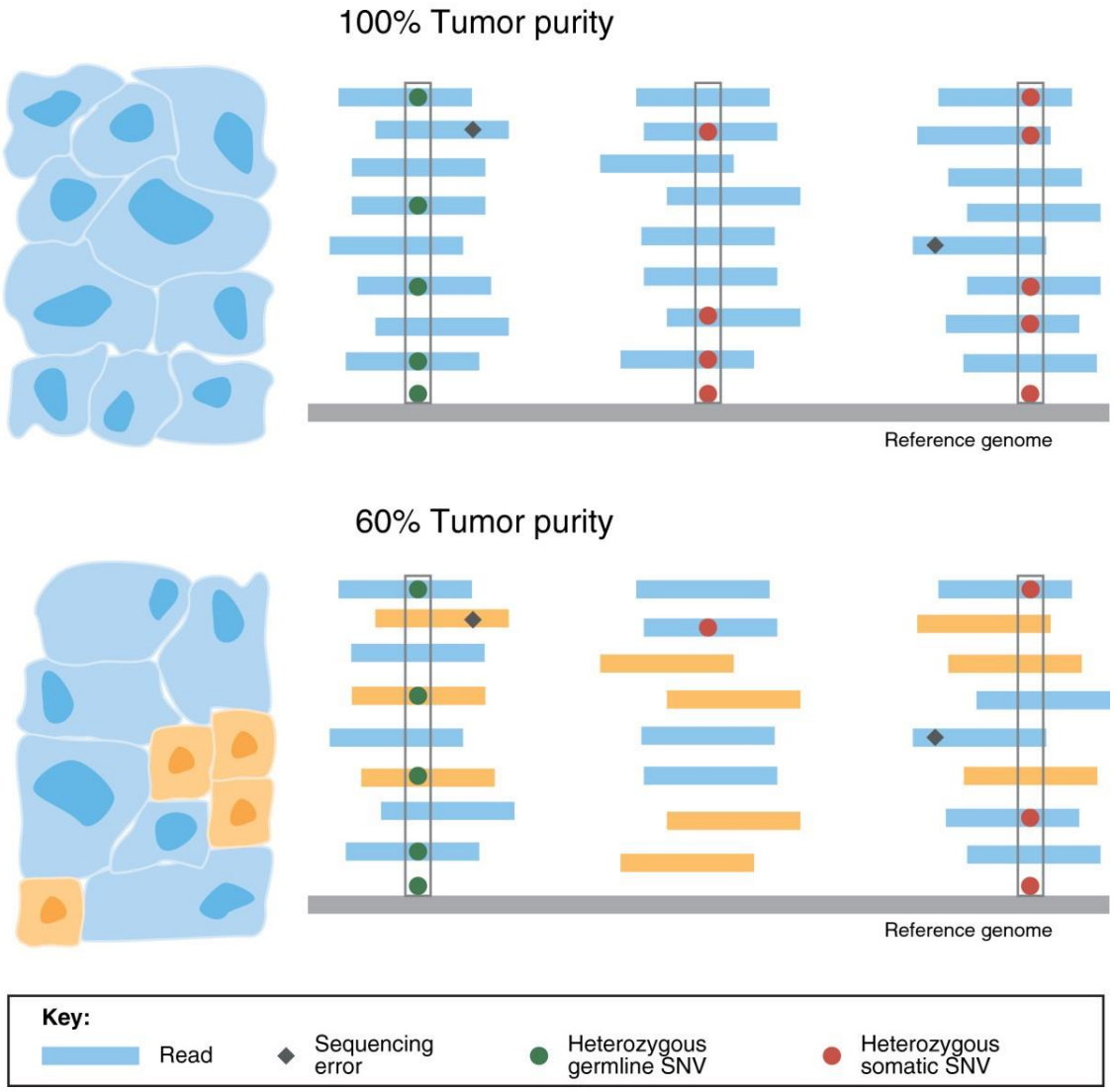
Why is SNV calling in cancer samples more complicated ?

Normal contamination

Normal cells can “contaminate” the tumor biopsy.

$$Tumor\ purity = \frac{tumor\ cells}{(normal + tumor\ cells)}$$

Tumor sample

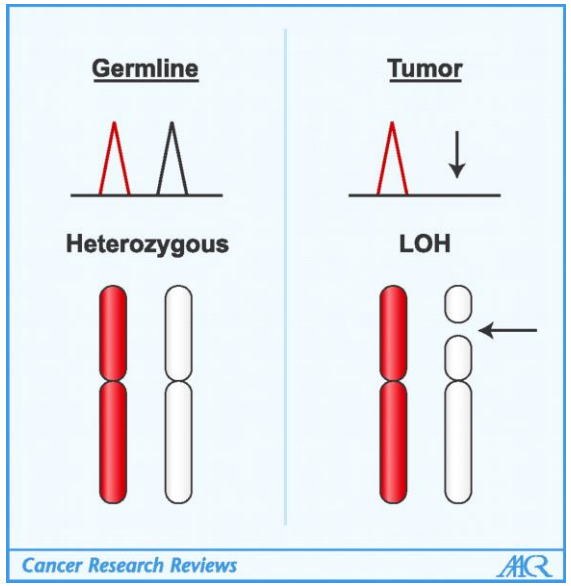


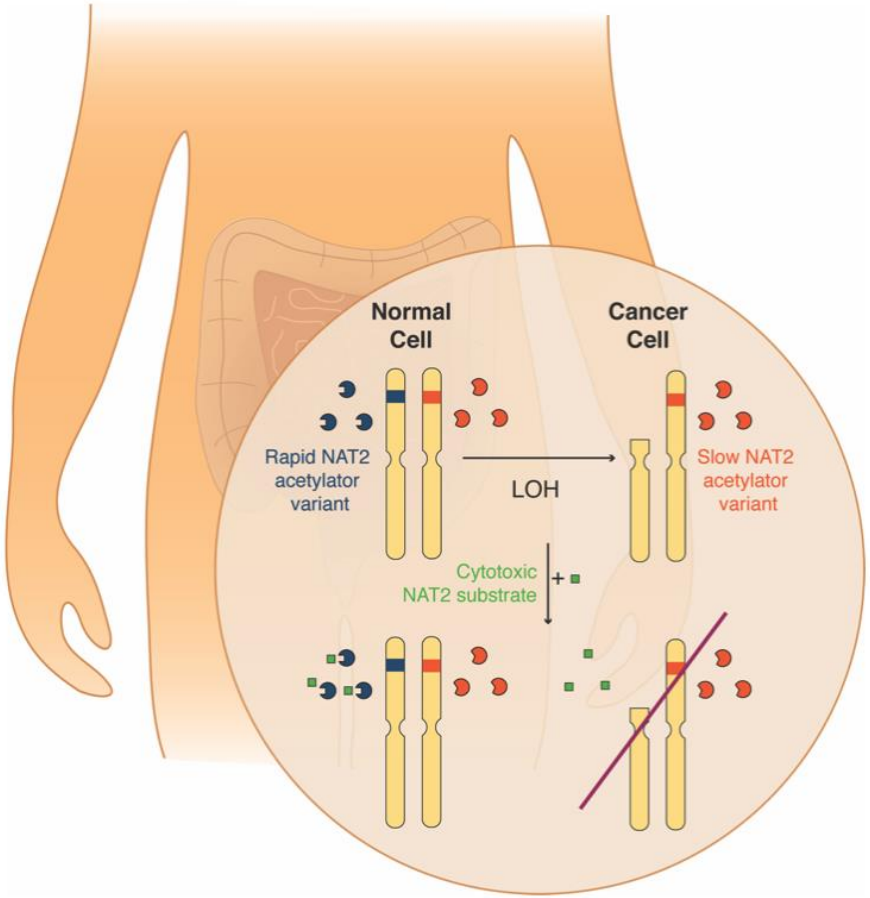
Raphael, Ben *et al*. (2014). Identifying driver mutations in sequenced cancer genomes: Computational approaches to enable precision medicine. Genome medicine

Why is SNV calling in cancer samples more complicated?

Unbalanced structural variations (deletions, duplications, etc.)

→ need to detect LOH events





https://cancerres.aacrjournals.org/content/69/17/6759

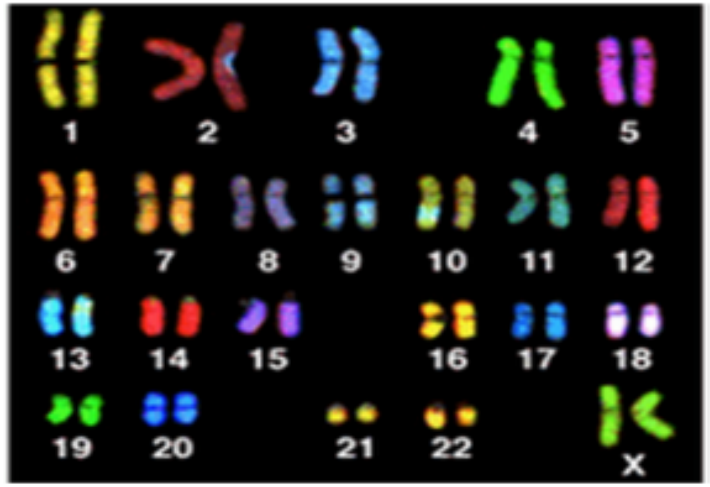
https://cancercommunity.nature.com/posts/61527-a-tumor-s-loss-of-heterozygosity-is-our-gain

Why is SNV calling in cancer samples more complicated?

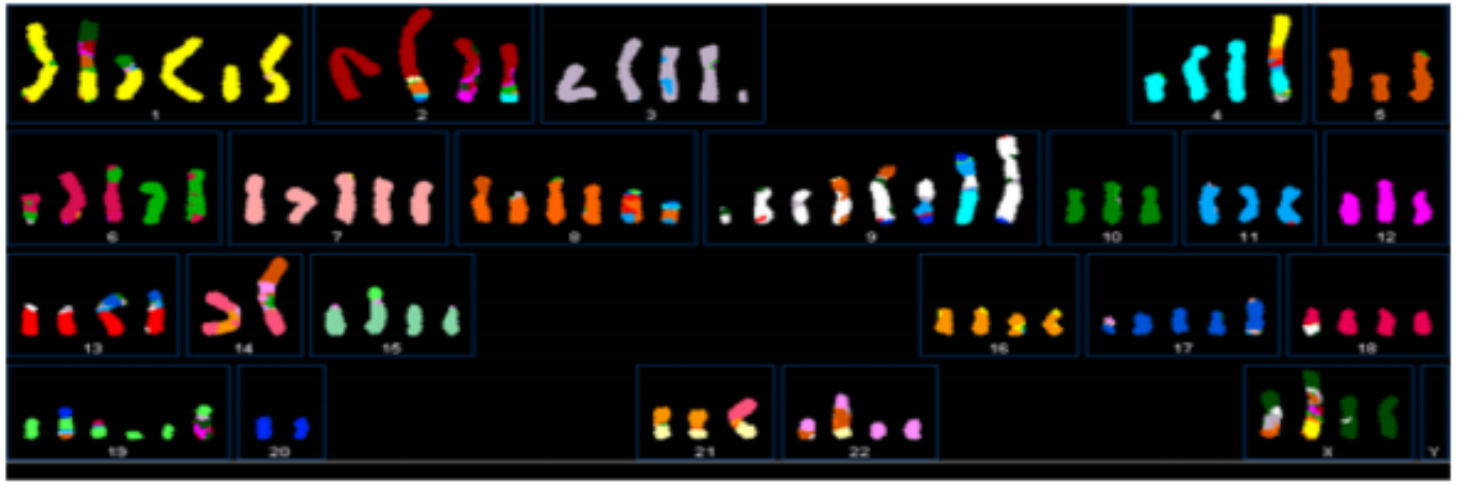
Aneuploidy → need for variant calling algorithm with no assumption on ploidy

Somatic alterations can be dramatic

Normal Cell



Cancer Cell Line HCC1954

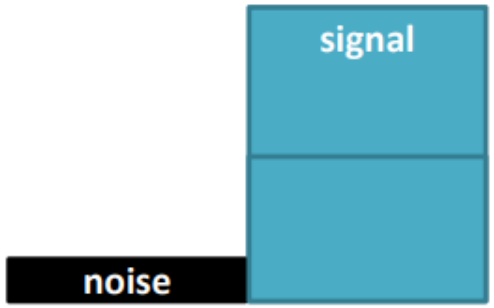


from Introduction to Somatic Variant Discovery, GATK Best Practices for Variant Discovery

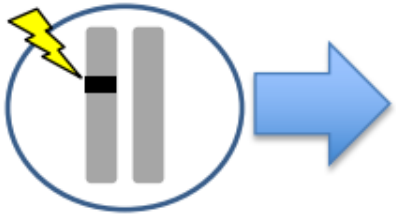
Why is SNV calling in cancer samples more complicated?

Amount of signal may be comparable to noise

Expectation for germline variants



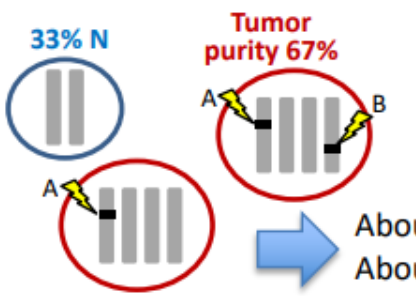
+ AF expected to follow ploidy



About 50% of reads will support the alternate allele

Expectation for somatic variants

+ no reliance on ploidy for AF



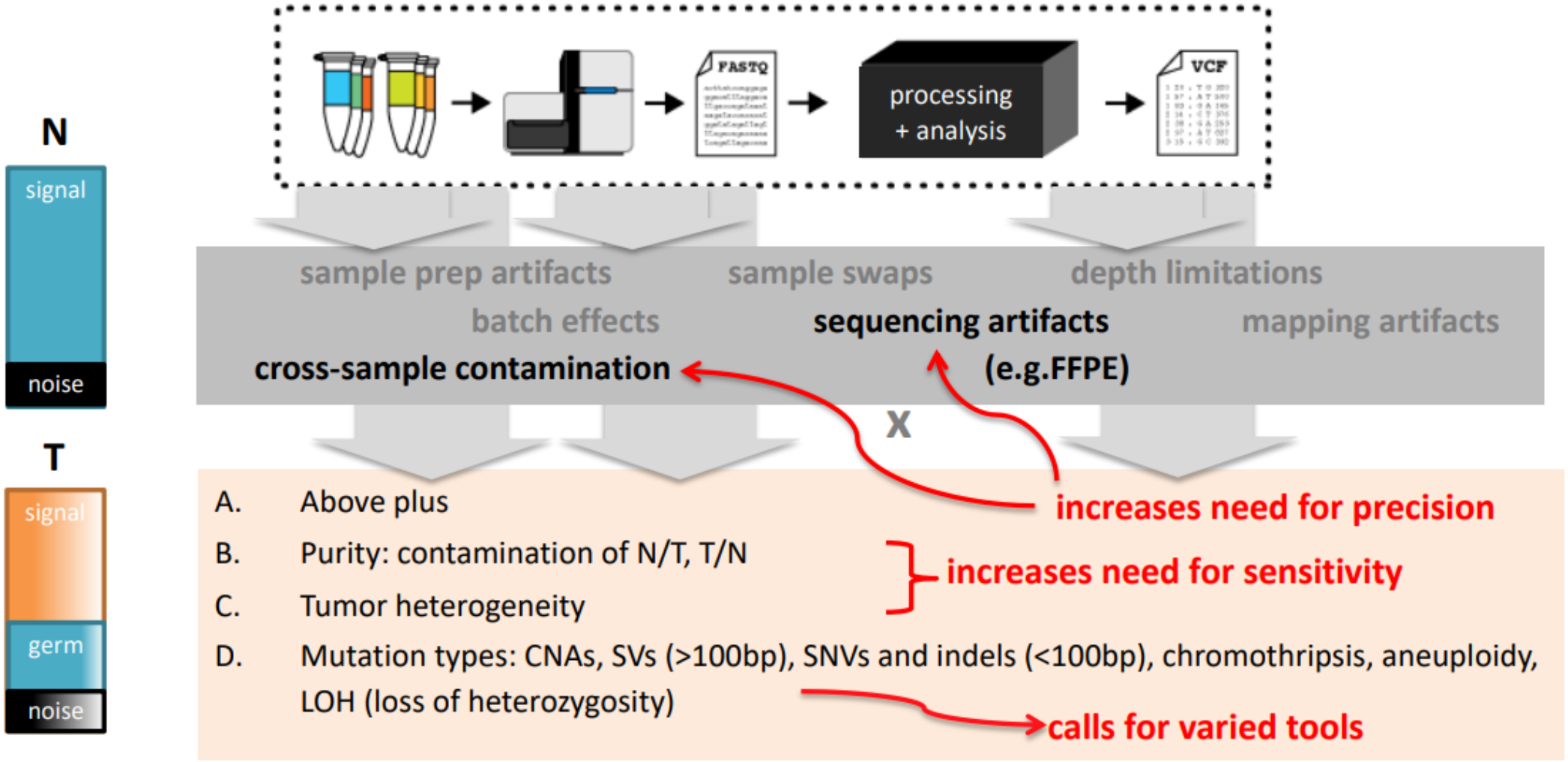
Example tumor sample:

- Clonal SNV (A)
- Clonal copy number duplication
- Subclone of 50% of cancer cells (heterogeneity)
- Subclonal SNV (B)

About 20% of reads will support A
About 10% of reads will support B

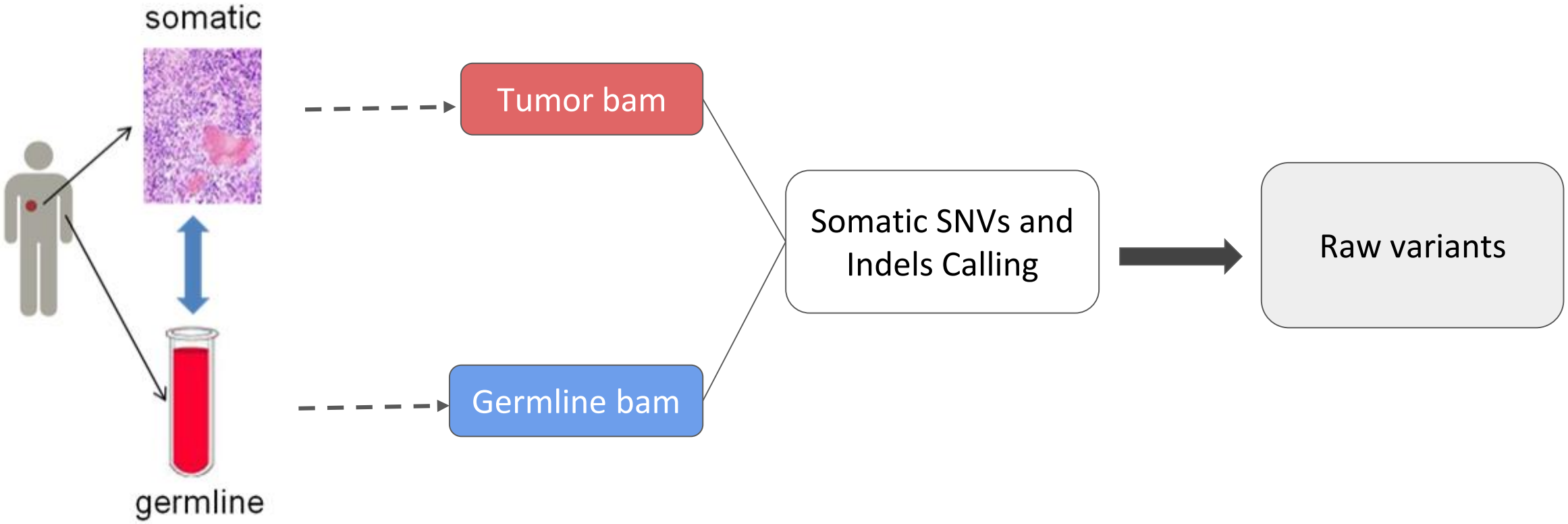
from Introduction to Somatic Variant Discovery, GATK Best Practices for Variant Discovery

Cancer-specific challenges confound analyses



from Introduction to Somatic Variant Discovery, GATK Best Practices for Variant Discovery

Tumor - Normal pair analysis workflow

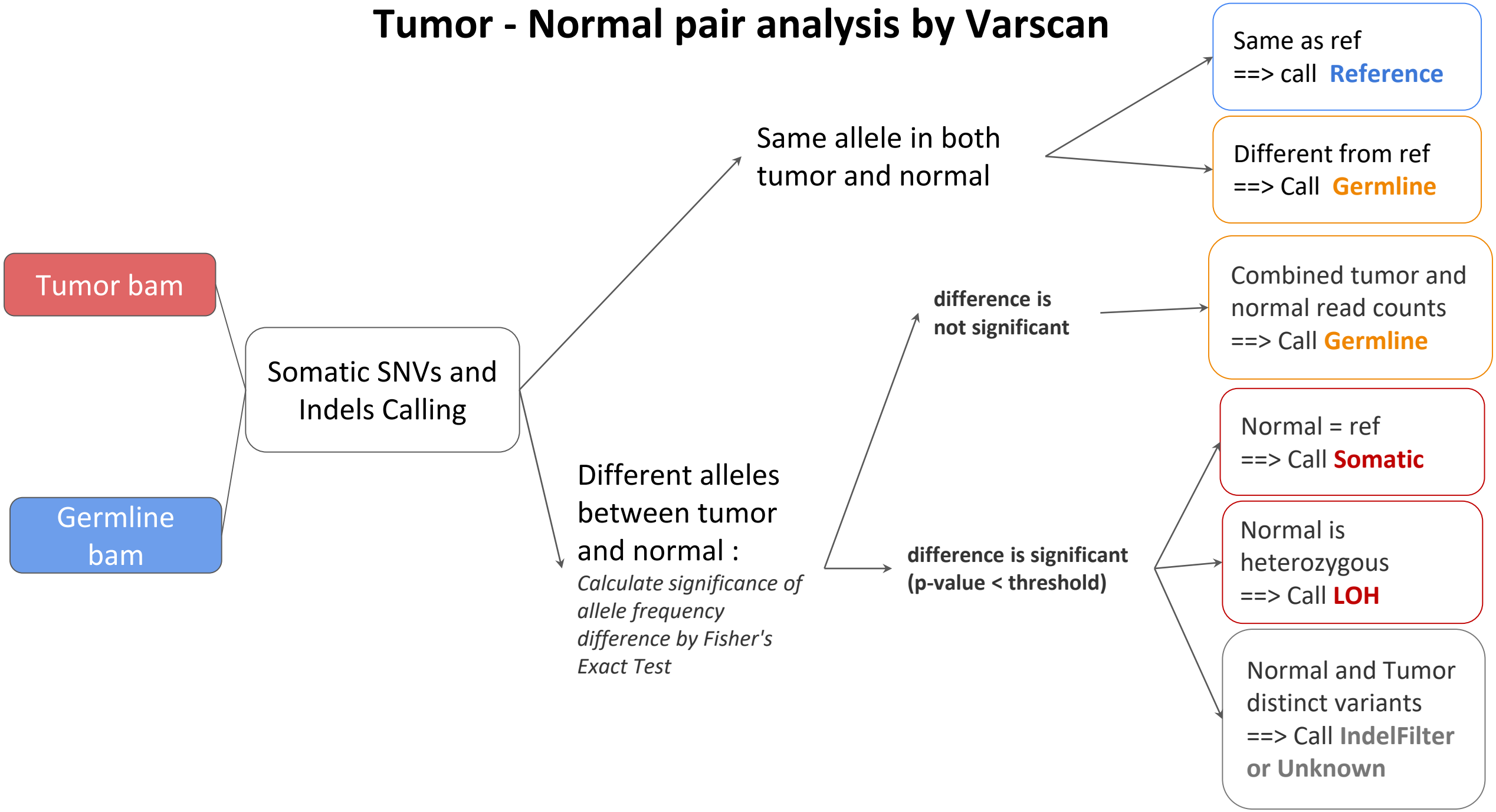


Logic of the Tumor-Normal workflow

Comparison to matched normal -> subtraction of germline background

from Introduction to Somatic Variant Discovery, GATK Best Practices for Variant Discovery

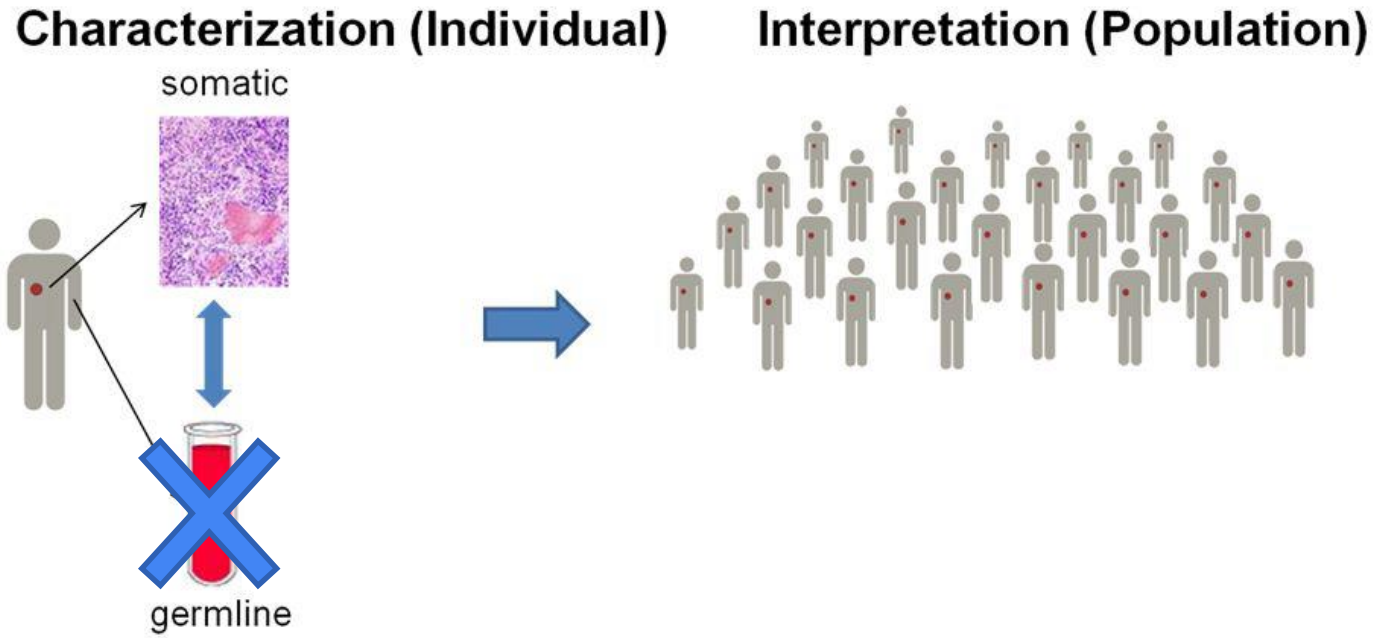
Tumor - Normal pair analysis by Varscan



Tumor-only analysis

If matched normal sample NOT available → Tumor only analysis

Pool of normals (PON) : used to eliminate common germline variation



Somatic variant filtering

Somatic variant callers output specific informations on variant :

➔ Somatic likelihood like score as phred-scale somatic p-value

➔ Status : - Germline,
- Somatic,
- LOH

⇒ first metrics to filter variants on

Somatic variant annotation

Somatic Annotation Databases

Databases of variant-disease and gene-disease associations

- Cancer HotSpots

  Single residue and in-frame indel mutation hotspots identified in 24,592 tumor samples.

- COSMIC

  COSMIC (Catalogue of Somatic Mutations in Cancer) is a data resource that is designed to store and display somatic mutation information and related details and contains information relating to human cancers.

  Data in COSMIC is curated from known Cancer Genes Literature and Systematic Screens.

- CIViC

  CIViC (Clinical Interpretation of Variant in Cancer) is a an open access, open source, community-driven web resource for Clinical Interpretation of Variants in Cancer. The goal is to enable precision medicine by providing an educational forum for dissemination of knowledge and active discussion of the clinical significance of cancer genome alterations.

Somatic Annotation Databases

Databases of variant-disease and gene-disease associations

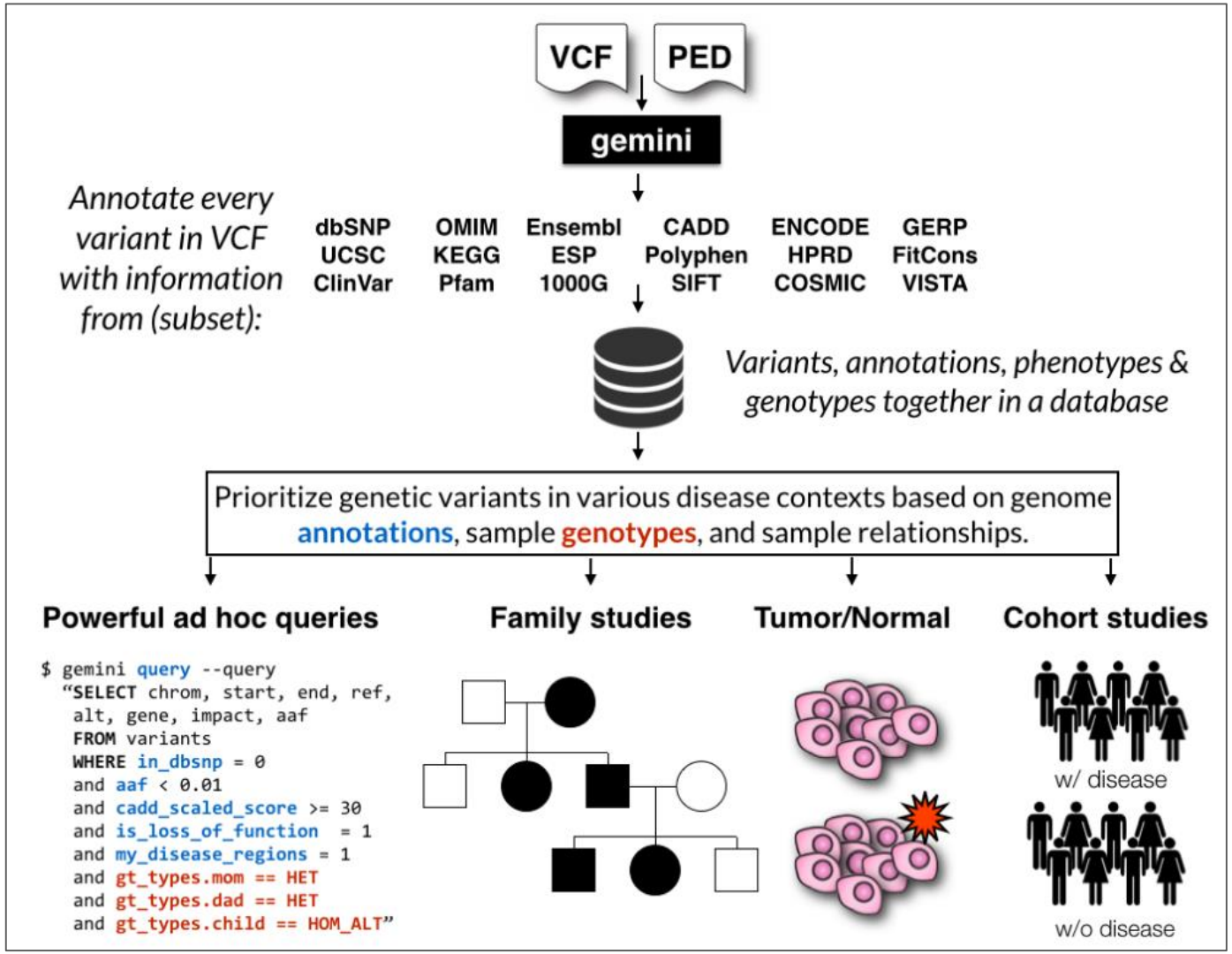
- Cancer Genome Interpreter (CGI)

  Cancer Genome Interpreter (CGI) is designed to support the identification of tumor alterations that drive the disease and detect those that may be therapeutically actionable. CGI relies on existing knowledge collected from several resources and on computational methods that annotate the alterations in a tumor according to distinct levels of evidence.
  It contains : a Cancer Biomarkers database, a Catalog of Validated Oncogenic Mutations and a Catalog of Validated Oncogenic Mutations

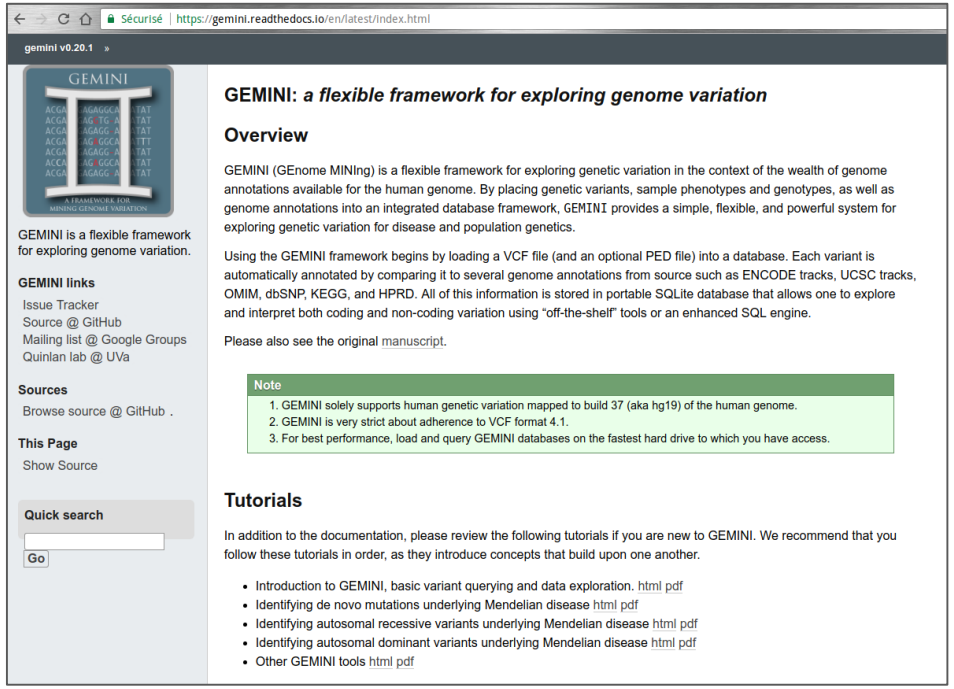
- The Cancer Genome Atlas (TCGA)

  The Cancer Genome Atlas (TCGA), a landmark cancer genomics program, molecularly characterized over 20,000 primary cancer and matched normal samples spanning 33 cancer types.TCGA generated over 2.5 petabytes of genomic, epigenomic, transcriptomic, and proteomic data.

GEMINI presentation



Documentation :
http://gemini.readthedocs.io
Tutorials :
https://speakerdeck.com/arq5x/



GEMINI database overview

The variants table

Gene information

gene
transcript
is_exonic
is_coding
is_lof
is_splicing
exon
codon_change
aa_change
aa_length
biotype
impact
impact_so
impact_severity
polyphen_pred
polyphen_score
sift_pred
sift_score
pfam_domain

Genotype information

gts	
gt_types	
gt_phases	
gt_depths	
gt_ref_depths	
gt_alt_depths	
gt_alt_freqs	
gt_quals	BLOB A compressed binary vector of the genc - Extracted from the VCF GQ genotype t

Variant and PopGen info

type	
sub_type	
call_rate	
num_hom_ref	
num_het	
num_hom_alt	
num_unknown	
aaf	
hwe	
inbreeding_coeff	FLOAT The inb
pi	FLOAT The co

Core VCF fields

column_name	
chrom	
start	
end	
vcf_id	
variant_id	
anno_id	
ref	
alt	
qual	
filter	

Population information

in_dbsnp
rs_ids
in_hm2
in_hm3
in_esp
in_1kg
aaf_esp_ea
aaf_esp_aa
aaf_esp_all
aaf_1kg_amr
aaf_1kg_eas
aaf_1kg_sas
aaf_1kg_afr
aaf_1kg_eur
aaf_1kg_all
in_exac
aaf_exac_all
aaf_adj_exac_all
aaf_adj_exac_afr
aaf_adj_exac_amr
aaf_adj_exac_eas

Disease phenotype info (from ClinVar).

in_omim	BOOL	0 : Absence of the variant in OMIM databas 1 : Presence of the variant in OMIM databa
clinvar_causal_allele	STRING	The allele(s) that are associated or causal f
clinvar_sig	STRING	The clinical significance scores for each of the variant according to ClinVar: *unknown*, *untested*, *non-pathogenic* *probable-non-pathogenic*, *probable-pathoge* *pathogenic*, *drug-response*, *histocompatibili* *other*
clinvar_disease_name	STRING	The name of the disease to which the varia
clinvar_dbsource	STRING	Variant Clinical Channel IDs
clinvar_dbsource_id	STRING	The record id in the above database
clinvar_origin	STRING	The type of variant. Any of: *unknown*, *germline*, *somatic*, *inherited*, *paternal*, *maternal*, *de-novo*, *biparental*, *uniparental*, *not-tested*, *tested-inconclusive*, *other*
clinvar_dsdb	STRING	Variant disease database name
clinvar_dsdbid	STRING	Variant disease database ID
clinvar_disease_acc	STRING	Variant Accession and Versions
clinvar_in_locus_spec_db	BOOL	Submitted from a locus-specific database?
clinvar_on_diag_assay	BOOL	Variation is interrogated in a clinical diagnostic assay?
clinvar_gene_phenotype	STRING	'|' delimited list of phenotypes associated with this gene (includes any variant in the same gene in clinvar not just the current variant).

The variant_impacts table

variant_id	INTEGER	PRIMARY_KEY (Foreign key to *variants* table)
anno_id	INTEGER	PRIMARY_KEY (Based on variant transcripts)
gene	STRING	The gene affected by the variant.
transcript	STRING	The transcript affected by the variant.
is_exonic	BOOL	Does the variant affect an exon for this transcript?
is_coding	BOOL	Does the variant fall in a coding region (excludes 3' & 5' UTR's of exons)?
is_lof	BOOL	Based on the value of the impact col, is the variant LOF?
exon	STRING	Exon information for the variants that are exonic
codon_change	STRING	What is the codon change?
aa_change	STRING	What is the amino acid change?
aa_length	STRING	The length of CDS in terms of number of amino acids (SnpEff only)
biotype	STRING	The type of transcript (e.g., protein-coding, pseudogene, rRNA etc.) (SnpEff only)
impact	STRING	Impacts due to variation (ref.impact category)
impact_so	STRING	The sequence ontology term for the impact
impact_severity	STRING	Severity of the impact based on the impact column value (ref.impact category)
polyphen_pred	STRING	Impact of the SNP as given by PolyPhen (VEP only) benign, possibly_damaging, probably_damaging, unknown
polyphen_scores	FLOAT	Polyphen score reflecting severity (higher the impact, *higher* the score) (VEP only)
sift_pred	STRING	Impact of the SNP as given by SIFT (VEP only) neutral, deleterious
sift_scores	FLOAT	SIFT prob. scores reflecting severity (Higher the impact, *lower* the score) (VEP only)

Etc.

Tables/fields descriptions :

http://gemini.readthedocs.io/en/latest/content/database_schema.html

GEMINI usages

```
SELECT column-names
FROM table-name
WHERE condition
ORDER BY sort-order
```

ad hoc data exploration

- ➔ Use SQL language to create queries and report data matching your requirements
- ➔ Can personalize your query to answer complex questions

`gemini query -q “SELECT gene, chrom, clinvar_gene_phenotype FROM variants”`

column_name	type
chrom	VARCHAR(20)
start	INTEGER
ref	TEXT
alt	TEXT
qual	FLOAT
filter	TEXT
in_omim	BOOLEAN
clinvar_sig	TEXT
clinvar_gene_phenotype	TEXT
gene	VARCHAR(60)

Table variants in Gemini database

https://gemini.readthedocs.io/en/latest/content/querying.html