# Variant calling and more...

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GDA
International Course on
Genomic Data Analysis

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

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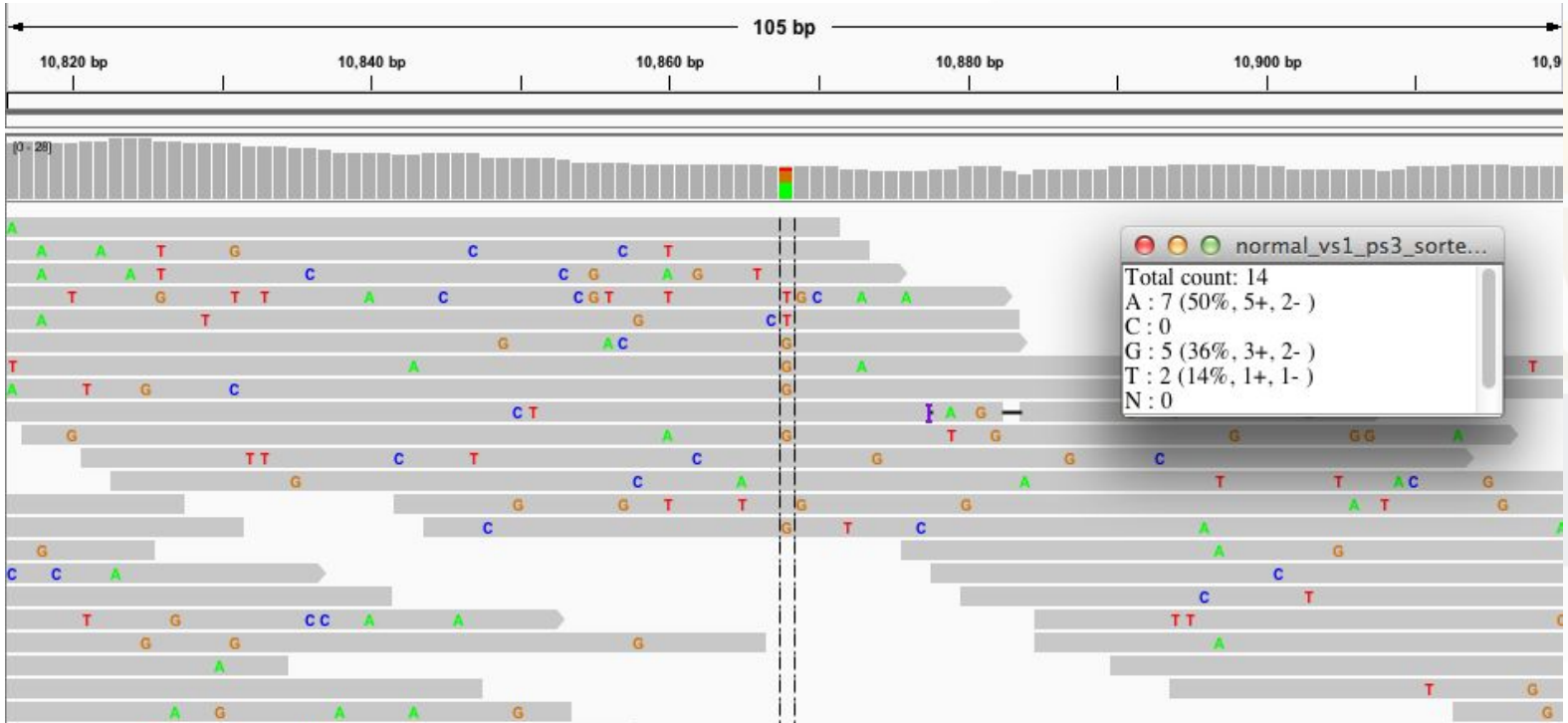
# 1 Introduction

## What is variant calling?

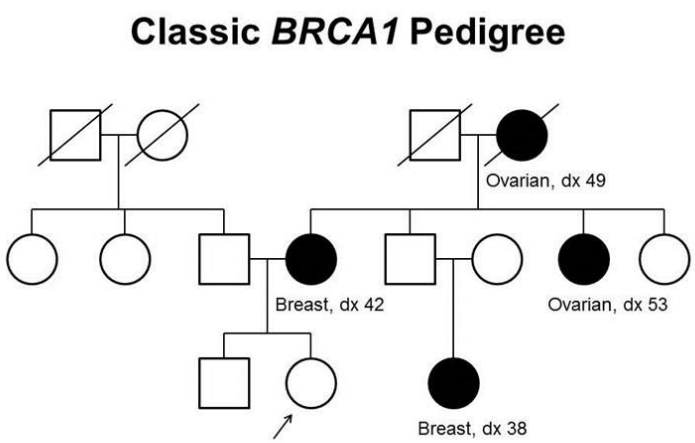
Find (robust) differences between sample genome and reference genome.

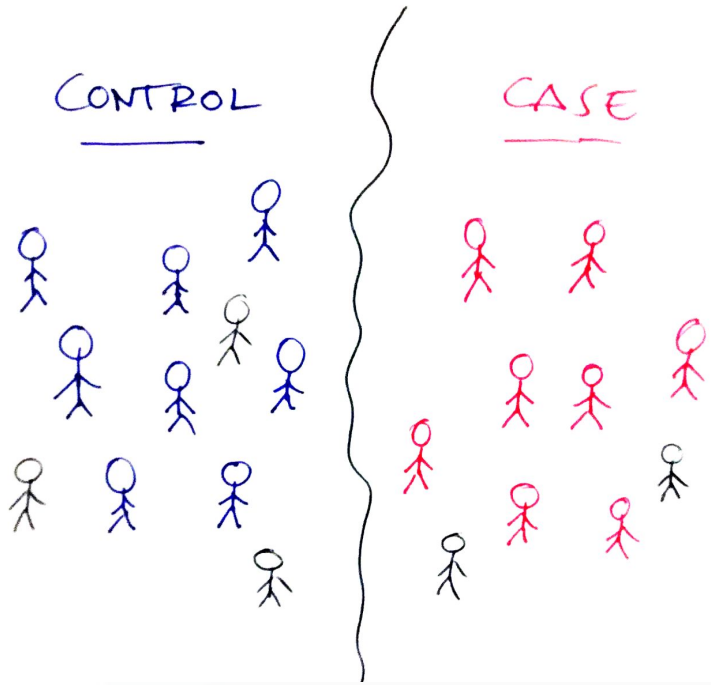


# 1 Introduction

## Why we study variants?

### Variants can explain group or phenotypic differences

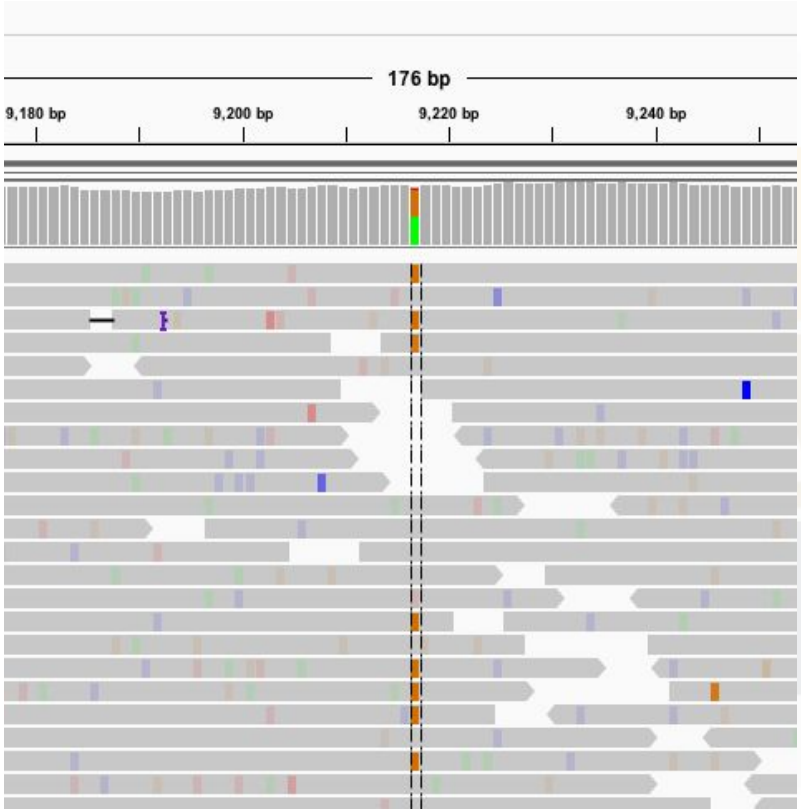


# 1 Introduction

## Dealing with uncertainty

Inherent noise in NGS makes us to face the problem from a **statistical point of view**

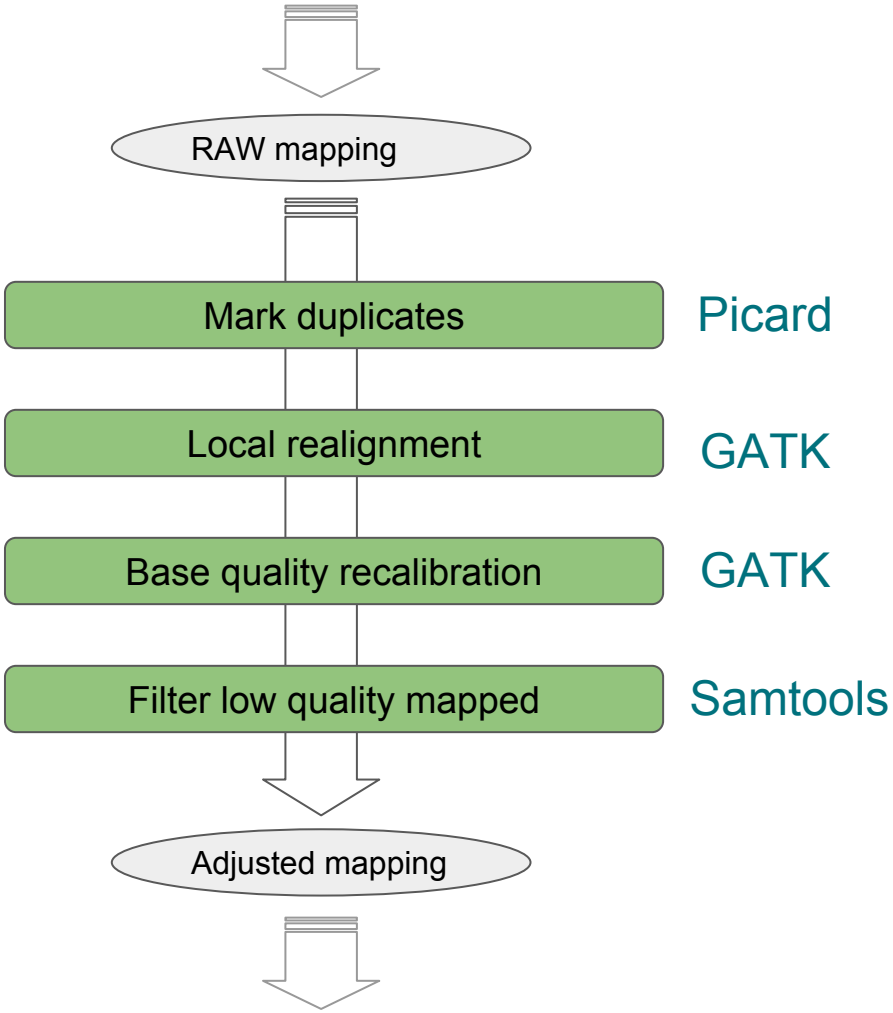



## Where it comes from?

- Random noise
- Sequencer bias
- Primary processing
- Batch effects

# 2 Tailoring your mapping file

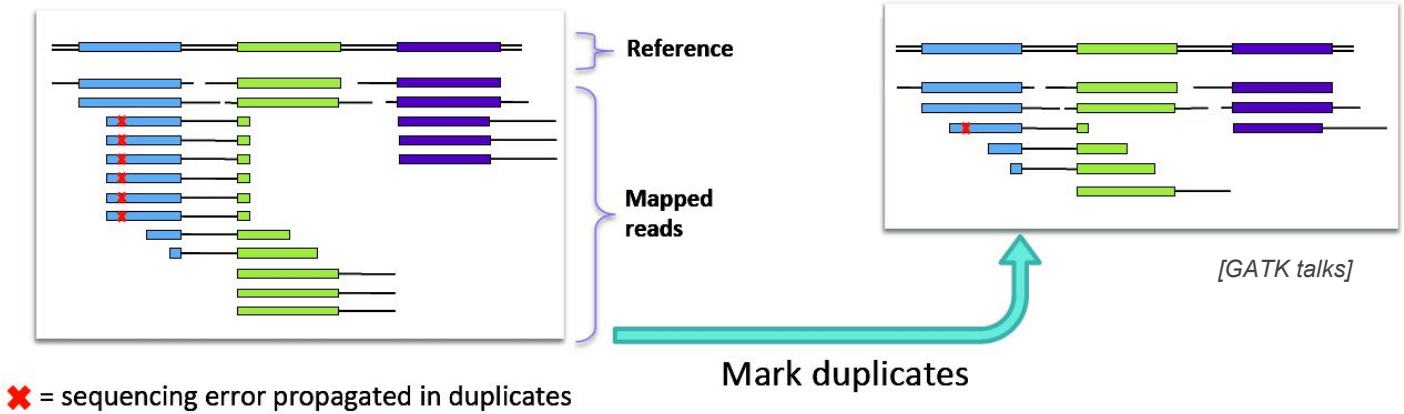
## General view



# 2 Tailoring your mapping file

## Marking PCR duplicates

- Reads from the same DNA template



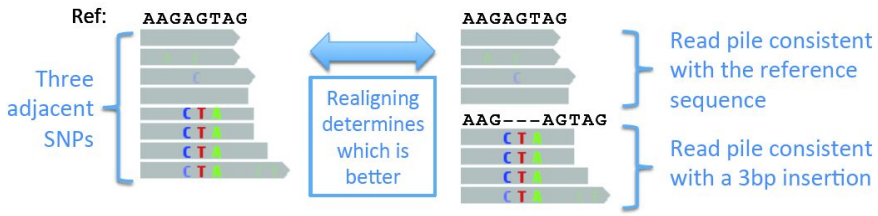

## Implications

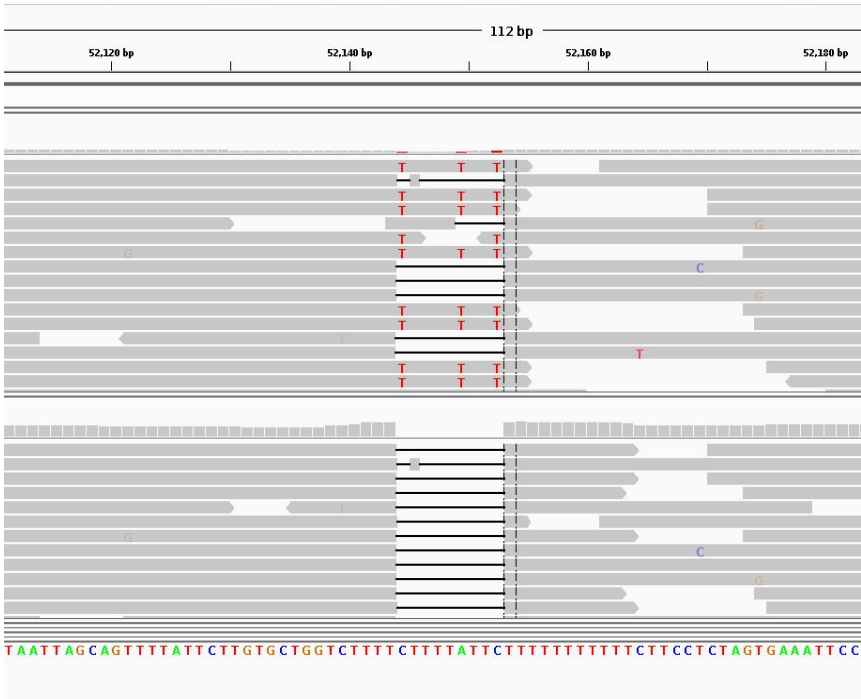
- Allele imbalance
- Common artifacts are supported by several reads

# 2 Tailoring your mapping file

## Local realignment

- Indels can confuse mappers
- Indels + Homopolymers produce ambiguity (several valid solutions)
- FPs are removed when a plausible indel is inserted



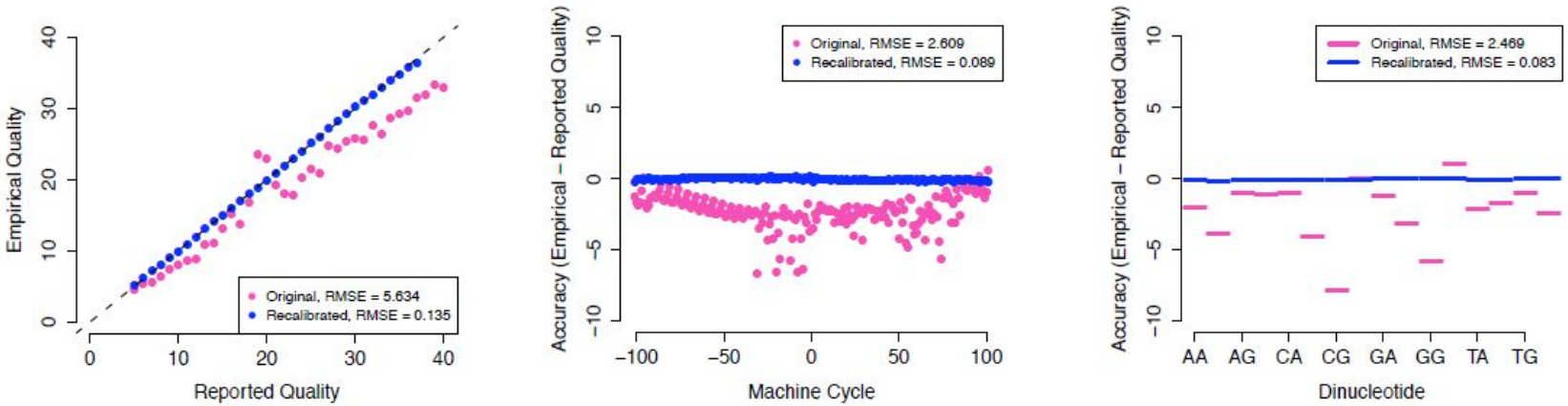

[GATK talks]

# 2 Tailoring your mapping file

## Base quality recalibration score

- Sequencers do not provide confident qualities (they are biased and innacurate)
  - Reported quality score
  - Position within the read (machine cycle)
  - Adjacent nucleotides

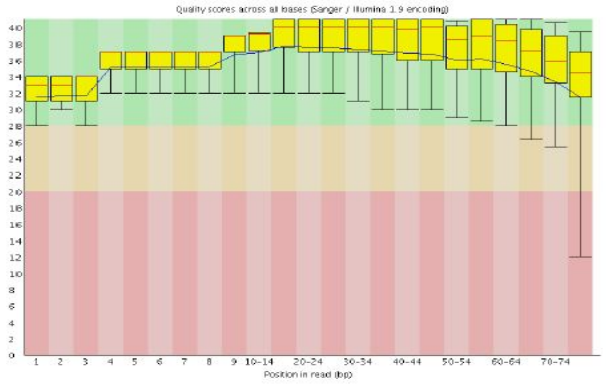
*[GATK talks]*



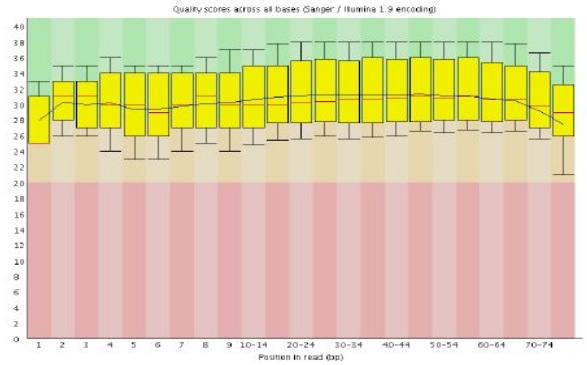
# Tailoring your mapping file

## Base quality recalibration score (BQRS)

Before

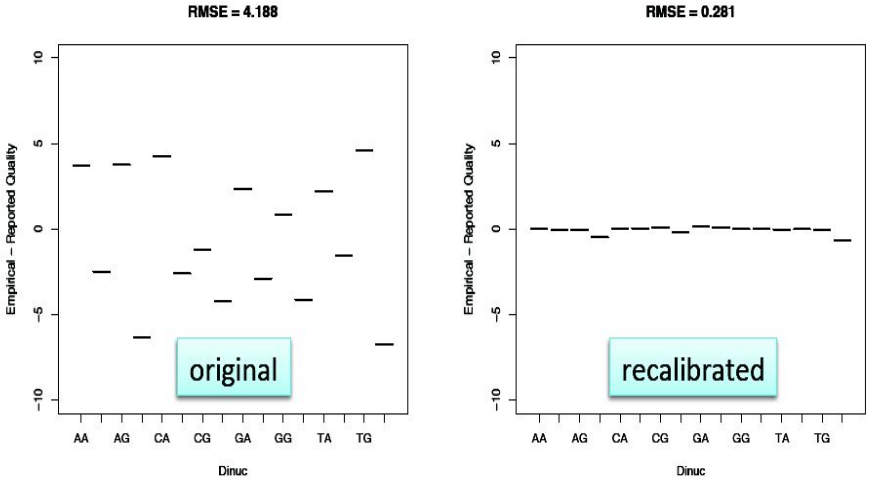



After




*[GATK talks]*

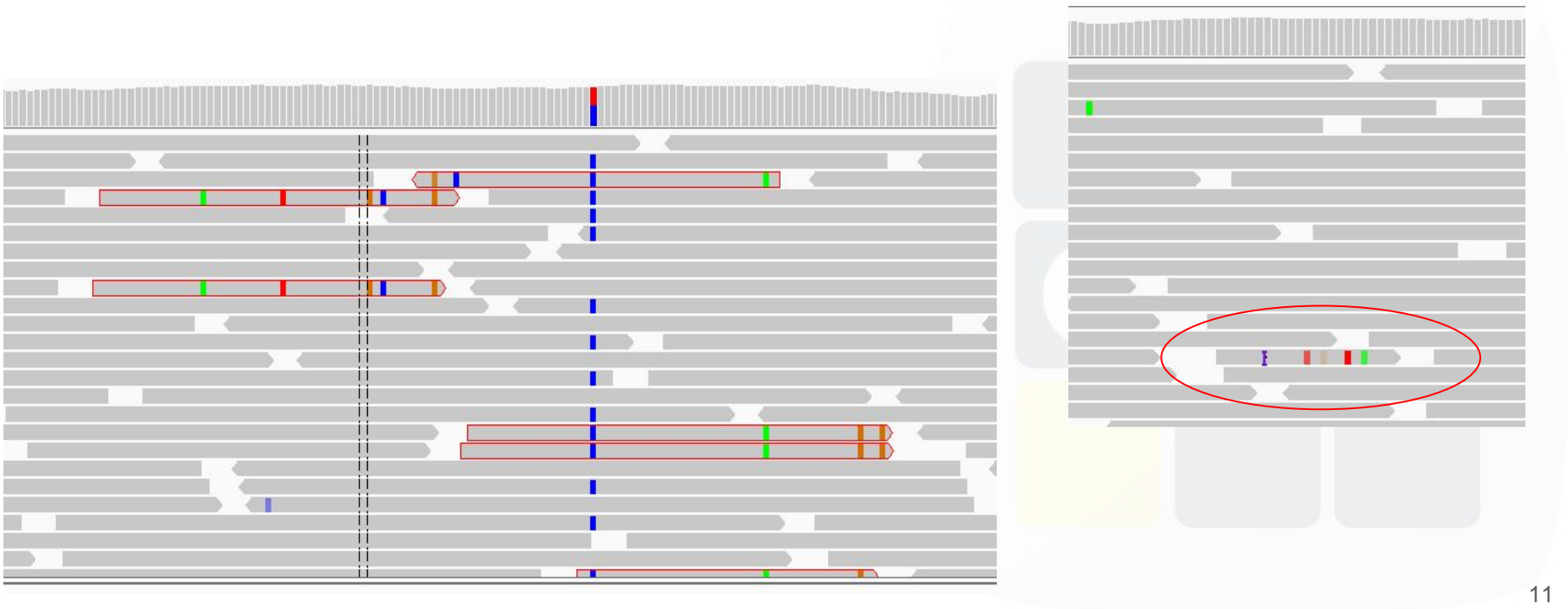
Example of bias: qualities reported depending on nucleotide context



# 2 Tailoring your mapping file

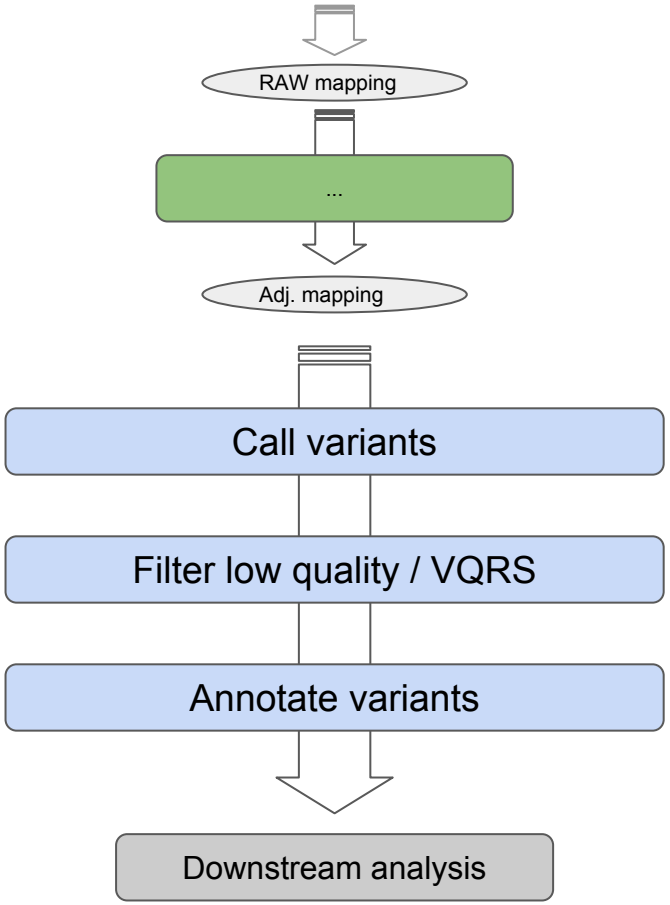
Filter low quality mapped reads

- MQ threshold ~ 0-10 (conditioned to mapper!)
- # of trimmed bases



# 3 Predicting variants

## General view



# 3 Predicting variants

## Several tools

- MAQ
- GATK
- Samtools
- Freebayes
- …

Resource

**Mapping short DNA sequencing reads and calling variants using mapping quality scores**

Heng Li,[1] Jue Ruan,[2] and Richard Durbin[1,3]

[1]The Wellcome Trust Sanger Institute, Hinxton CB10 1SA, United Kingdom; [2]Beijing Genomics Institute, Chinese Academy of Science, Beijing 100029, China

BIOINFORMATICS ORIGINAL PAPER Vol. 27 no. 21 2011, pages 2987–2993 doi:10.1093/bioinformatics/btr509

Sequence analysis Advance Access publication September 8, 2011

**A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data**

Heng Li

Medical Population Genetics Program, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, USA

Associate Editor: Jeffrey Barrett

Resource

**The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data**

Aaron McKenna,[1] Matthew Hanna,[1] Eric Banks,[1] Andrey Sivachenko,[1] Kristian Cibulskis,[1] Andrew Kernytsky,[1] Kiran Garimella,[1] David Altshuler,[1,2] Stacey Gabriel,[1] Mark Daly,[1,2] and Mark A. DePristo[1,3]

[1]Program in Medical and Population Genetics, The Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142, USA; [2]Center for Human Genetic Research, Massachusetts General Hospital, Richard B. Simches Research Center, Boston, Massachusetts 02114, USA

**Haplotype-based variant detection from short-read sequencing**

Erik Garrison and Gabor Marth

July 24, 2012

**Abstract**

The direct detection of haplotypes from short-read DNA sequencing data requires changes to existing small-variant detection methods. Here, we develop a Bayesian statistical framework which is capable of modeling multiallelic loci in sets of individuals with non-uniform copy number. We then describe our implementation of this framework in a haplotype-based variant detector, FreeBayes.

# 3 Predicting variants

gatk

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The Genome Analysis Toolkit or GATK is a software package for analysis of high-throughput sequencing data, developed by the Data Science and Data Engineering group at the Broad Institute. The toolkit offers a wide variety of tools, with a primary focus on variant discovery and genotyping as well as strong emphasis on data quality assurance. Its robust architecture, powerful processing engine and high-performance computing features make it capable of taking on projects of any size.

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# 3 Predicting variants

## GATK

- SNV and Indel calling are of special interest

Bayesian model

$$\Pr\{G|D\} = \frac{\overbrace{\Pr\{G\}}^{\text{Prior of the genotype}}\ \overbrace{\Pr\{D|G\}}^{\text{Likelihood of the genotype}}}{\Sigma_i \Pr\{G_i\}\Pr\{D|G_i\}}, \text{ [Bayes' rule]}$$

$$\Pr\{D|G\} = \prod_j \left(\frac{\Pr\{D_j|H_1\}}{2} + \frac{\Pr\{D_j|H_2\}}{2}\right) \text{ where } \overbrace{G = H_1 H_2}^{\text{Diploid assumption}}$$

$$\Pr\{D|H\} \text{ is the haploid likelihood function}$$

# 3 Predicting variants

## GATK

- But, it is a framework (with tons of tools) to manage VCF and BAM files

**1. PRE-PROCESSING**

Pre-processing starts from raw sequence data, either in FASTQ or uBAM format, and produces analysis-ready BAM files. Processing steps include alignment to a reference genome as well as some data cleanup operations to correct for technical biases and make the data suitable for analysis.

**2. VARIANT DISCOVERY**

Variant Discovery starts from analysis-ready BAM files and produces a callset in VCF format. Processing involves identifying sites where one or more individuals display possible genomic variation, and applying filtering methods appropriate to the experimental design.

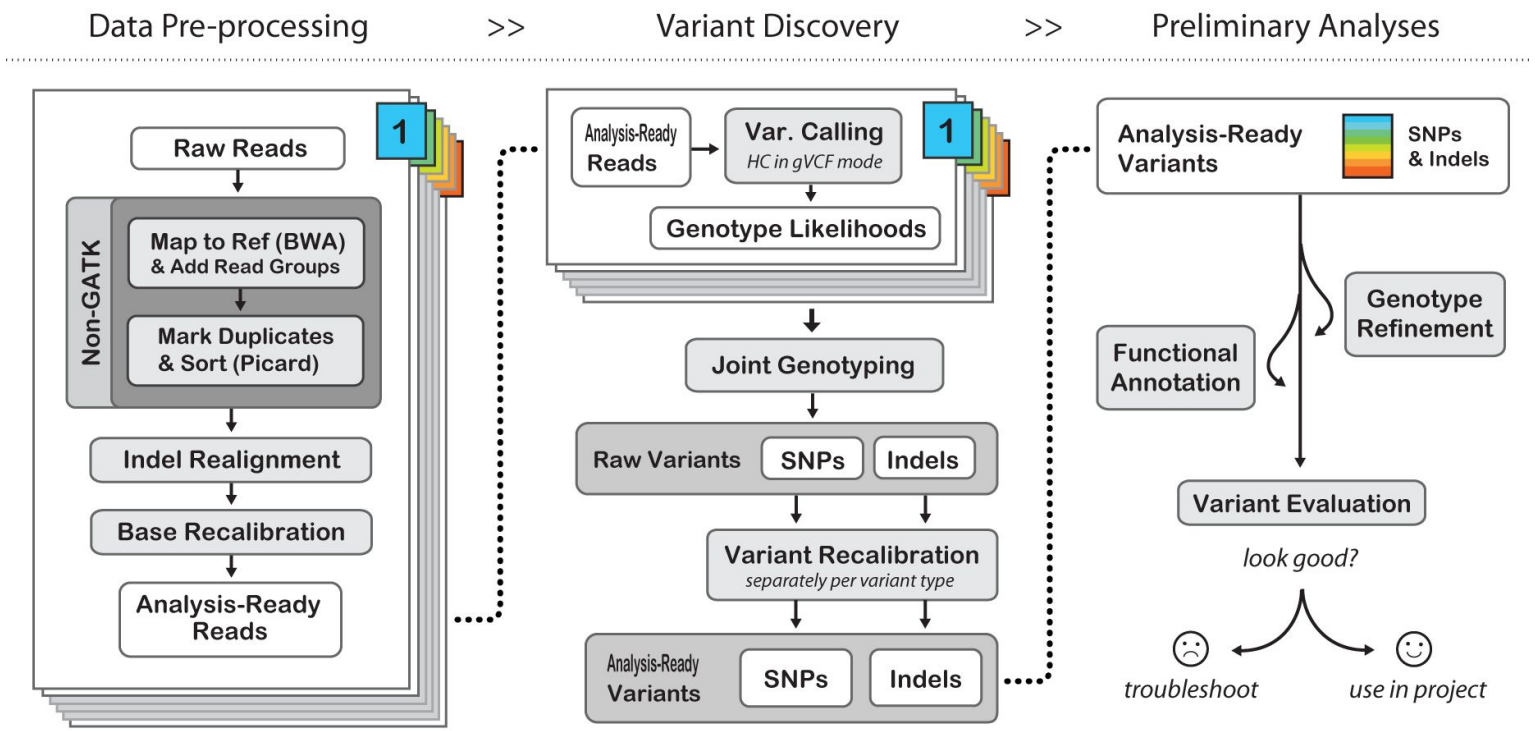
**3. CALLSET REFINEMENT**

Callset Refinement starts and ends with a VCF callset. Processing involves using meta-data to assess and improve genotyping accuracy, attach additional information and evaluate the overall quality of the callset.

# 3 Predicting variants

## GATK

- They promoted “Best practices in NGS processing”



# 4 Understanding VC output

## VCF format

Misc

```
##fileformat=VCFv4.1
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
```

Info

```
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
```

Filter

```
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
```

Format

```
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
```

| #CHROM | POS | ID | REF | ALT | QUAL | FILTER | INFO | FORMAT | NA00001 | NA00002 |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 14370 | rs6054257 | G | A | 29 | PASS | NS=3;DP=14;AF=0.5;DB;H2 | GT:GQ:DP:HQ | 0\|0:48:1:51,51 | 1\|0:48:8:51,51 |
| 20 | 17330 | . | T | A | 3 | q10 | NS=3;DP=11;AF=0.017 | GT:GQ:DP:HQ | 0\|0:49:3:58,50 | 0\|1:3:5:65,3 |
| 20 | 1110696 | rs6040355 | A | G,T | 67 | PASS | NS=2;DP=10;AF=0.333,0.667;AA=T;DB | GT:GQ:DP:HQ | 1\|2:21:6:23,27 | 2\|1:2:0:18,2 |
| 20 | 1230237 | . | T | . | 47 | PASS | NS=3;DP=13;AA=T | GT:GQ:DP:HQ | 0\|0:54:7:56,60 | 0\|0:48:4:51,51 |
| 20 | 1234567 | microsat1 | GTC | G,GTCT | 50 | PASS | NS=3;DP=9;AA=G | GT:GQ:DP | 0/1:35:4 | 0/2:17:2 |

variant summary

summary of quality

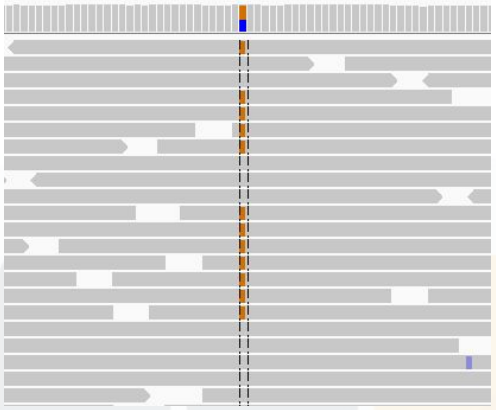
Sample summary and tool dependent inferred parameters

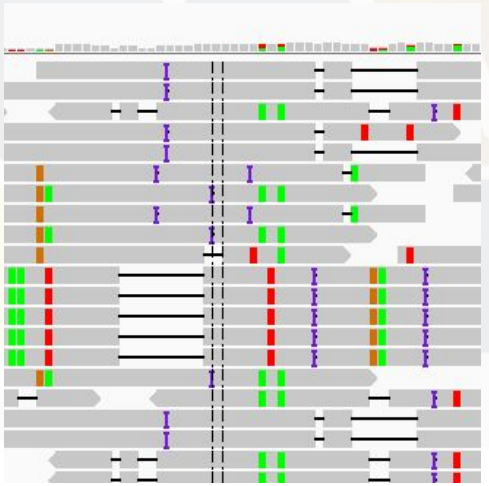
Sample detail

# 4 Understanding VC output

## How to (hard) filter variants for SNV/Indels

- Depth (DP) ?/?
- QualByDepth (QD) < 2/2
- RMSMappingQuality (MQ) < 40.0/-
- FisherStrand (FS) > 60.0/200.0
- HaplotypeScore > 13.0/-
- MQRankSumTest < -12.5/-
- ReadPosRankSumTest < -8.0/-20.0



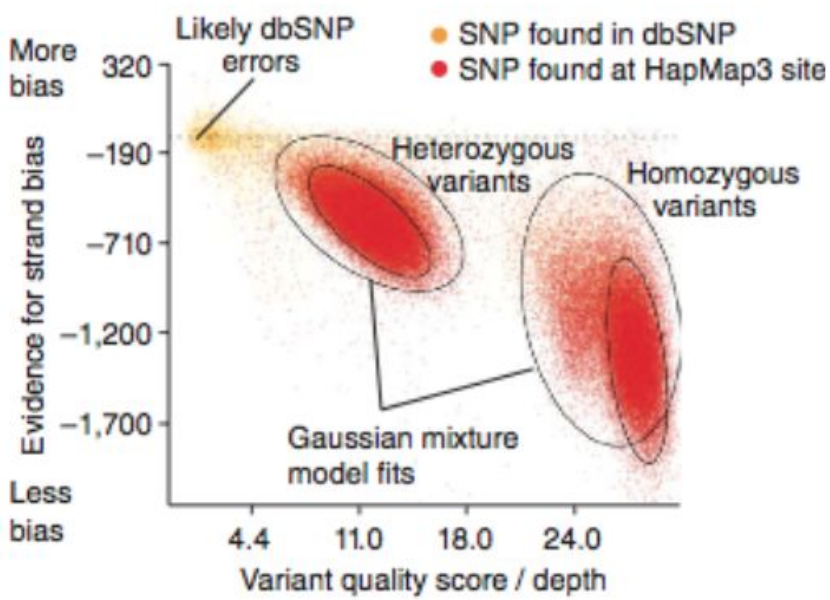


# 4 Understanding VC output

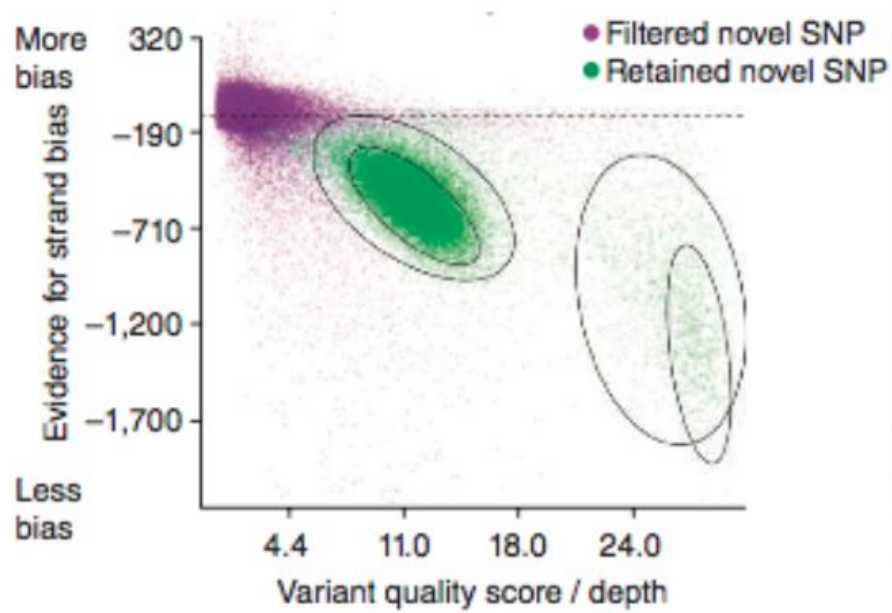
## Variant Quality Recalibration Score (VQRS)

- We can use together quality scores in a model
- We need a set of true (known) variants (e.g. dbSNP)

(1) Train model using HapMap




(2) Apply model to callset



# 5 Variant annotation

## How we enrich our prediction?

- Mutation effect
  - Annovar
  - SnpEff

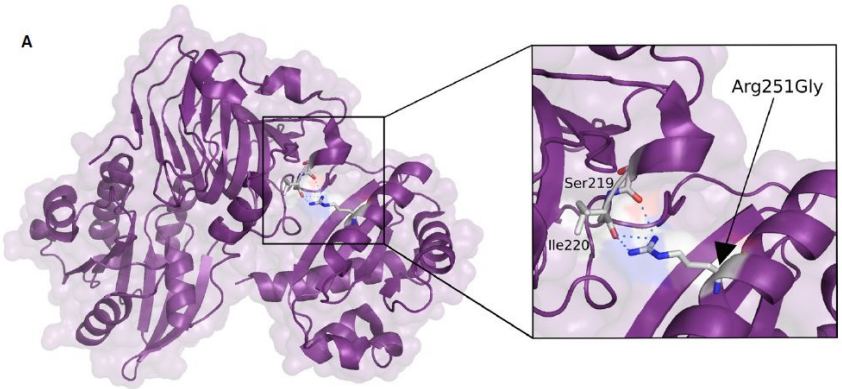
**Type**

| Type (alphabetical order) | Count | Percent |
|---|---|---|
| DOWNSTREAM | 2,093 | 1.766% |
| INTERGENIC | 26,314 | 22.204% |
| INTRAGENIC | 78 | 0.066% |
| INTRON | 54,238 | 45.767% |
| NON_SYNONYMOUS_CODING | 237 | 0.2% |
| NON_SYNONYMOUS_START | 1 | 0.001% |
| SPLICE_SITE_DONOR | 4 | 0.003% |
| START_GAINED | 57 | 0.048% |
| STOP_GAINED | 3 | 0.003% |
| STOP_LOST | 1 | 0.001% |
| SYNONYMOUS_CODING | 378 | 0.319% |
| TRANSCRIPT | 32,163 | 27.14% |
| UPSTREAM | 2,102 | 1.774% |
| UTR_3_PRIME | 690 | 0.582% |
| UTR_5_PRIME | 149 | 0.126% |

**Region**

| Type (alphabetical order) | Count | Percent |
|---|---|---|
| DOWNSTREAM | 2,093 | 1.766% |
| EXON | 620 | 0.523% |
| INTERGENIC | 26,314 | 22.204% |
| INTRON | 54,238 | 45.767% |
| NONE | 32,241 | 27.206% |
| SPLICE_SITE_DONOR | 4 | 0.003% |
| UPSTREAM | 2,102 | 1.774% |
| UTR_3_PRIME | 690 | 0.582% |
| UTR_5_PRIME | 206 | 0.174% |

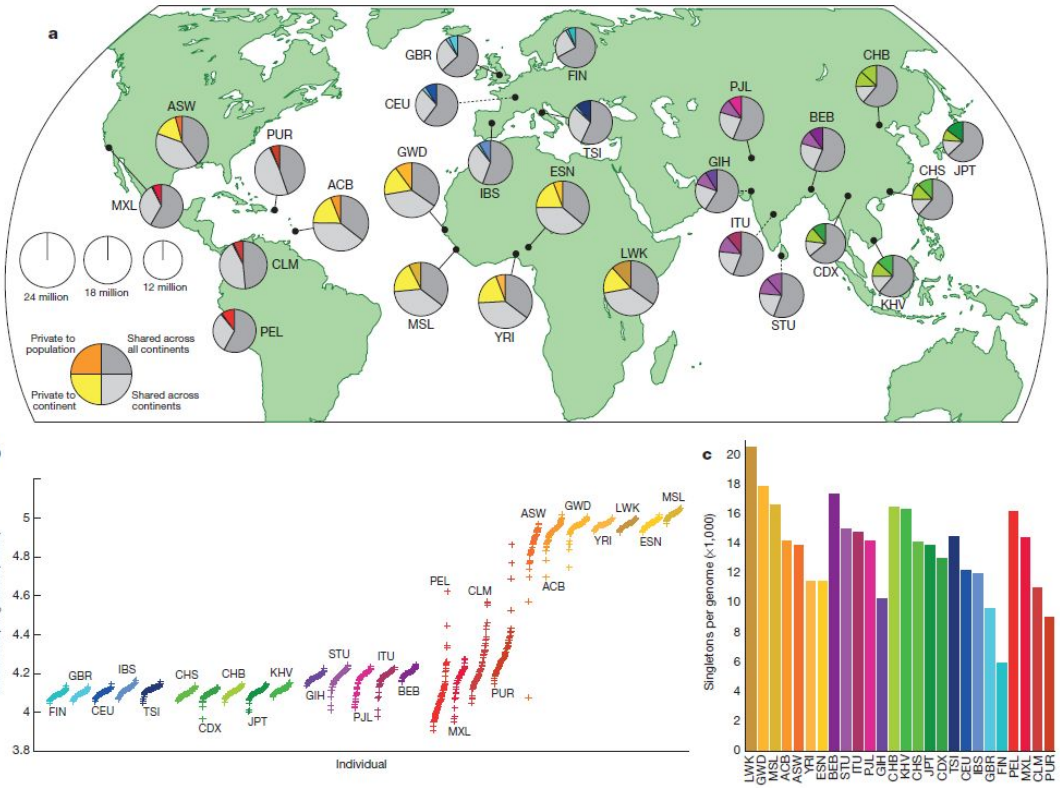
- Loss/Gain of function in protein?
  - SIFT
  - Polyphen
  - CAAD



# 5 Variant annotation

How we enrich our prediction?

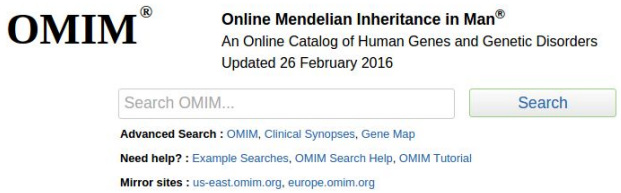
- Allele frequency in (almost) healthy populations
  - 1000K
  - dbSNP
  - ExAC

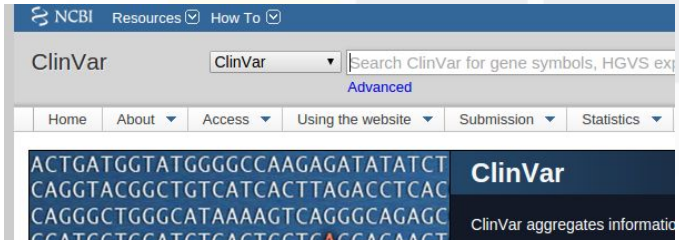


# 5 Variant annotation

## How we enrich our prediction?

- Variant allele in pathological mutation DBs
  - COSMIC (Catalogue of somatic mutations in cancer)
    - http://cancer.sanger.ac.uk/cosmic
  - HGMD (Human Gene Mutation Database)
    - http://www.hgmd.cf.ac.uk/
  - OMIM (Online Mendelian inheritance in men)
    - http://www.omim.org/
  - ClinVar (NCBI clinical variants repository)
    - http://www.ncbi.nlm.nih.gov/clinvar/

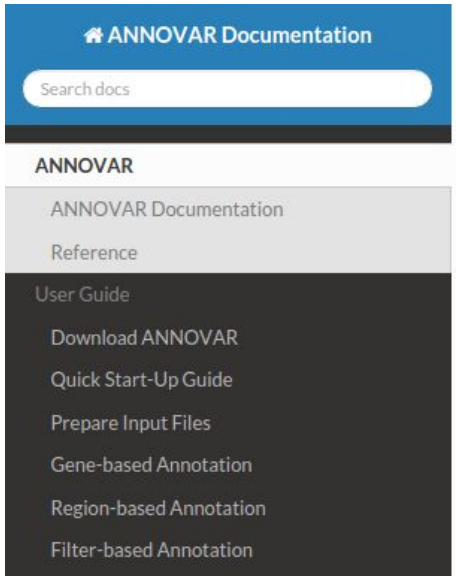


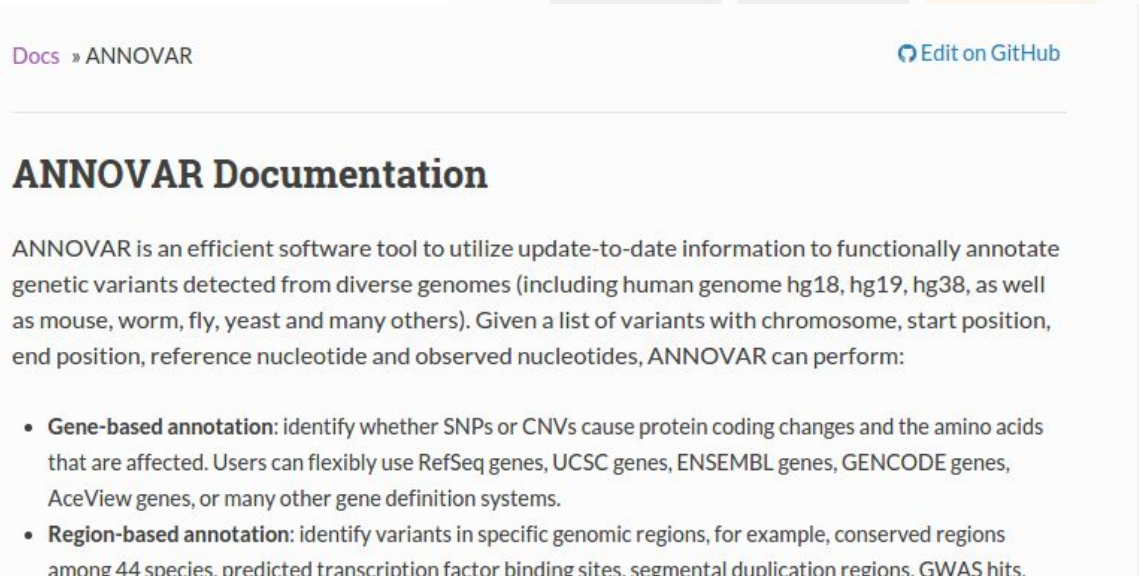



# 5 Variant annotation

## Command line tools

- Annovar
  - Region-based annotator
  - Highly flexible (precomputed scores for almost anything you want to annotate to a variant)

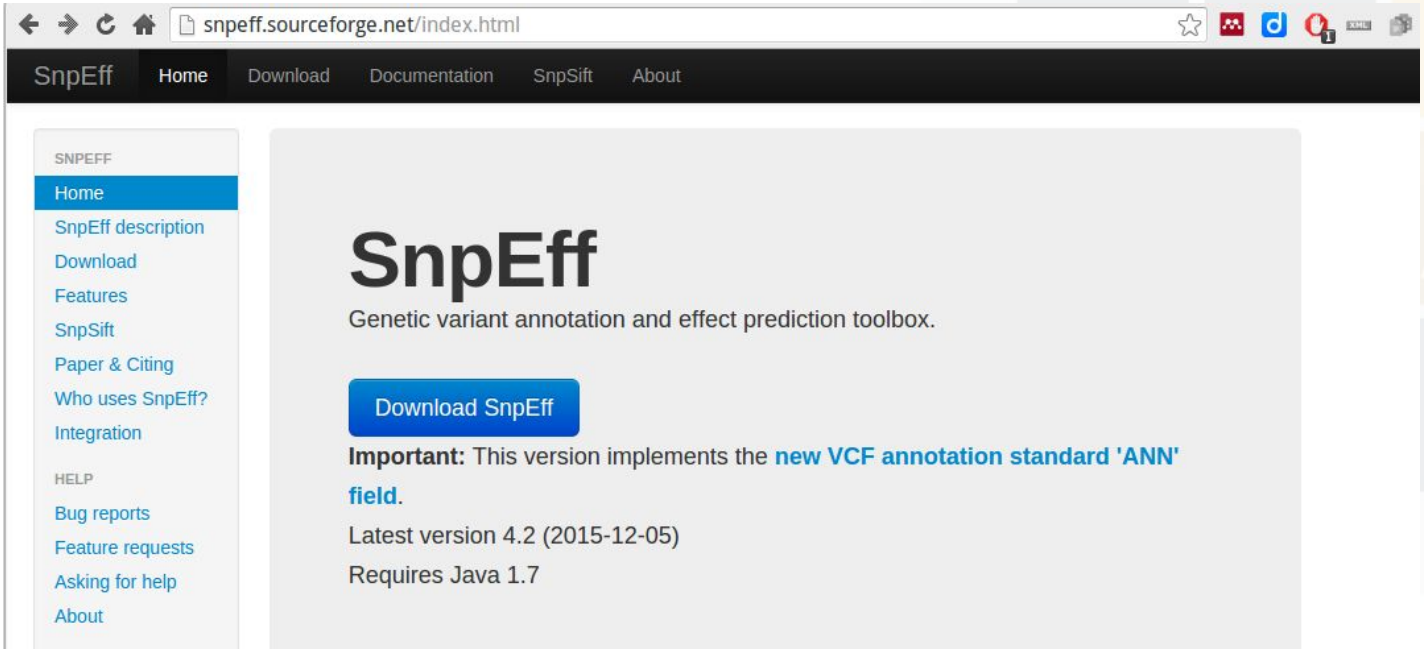


# 5 Variant annotation

## Command line tools

- SnpEff
  - Less flexible than Annovar
  - But faster than Annovar

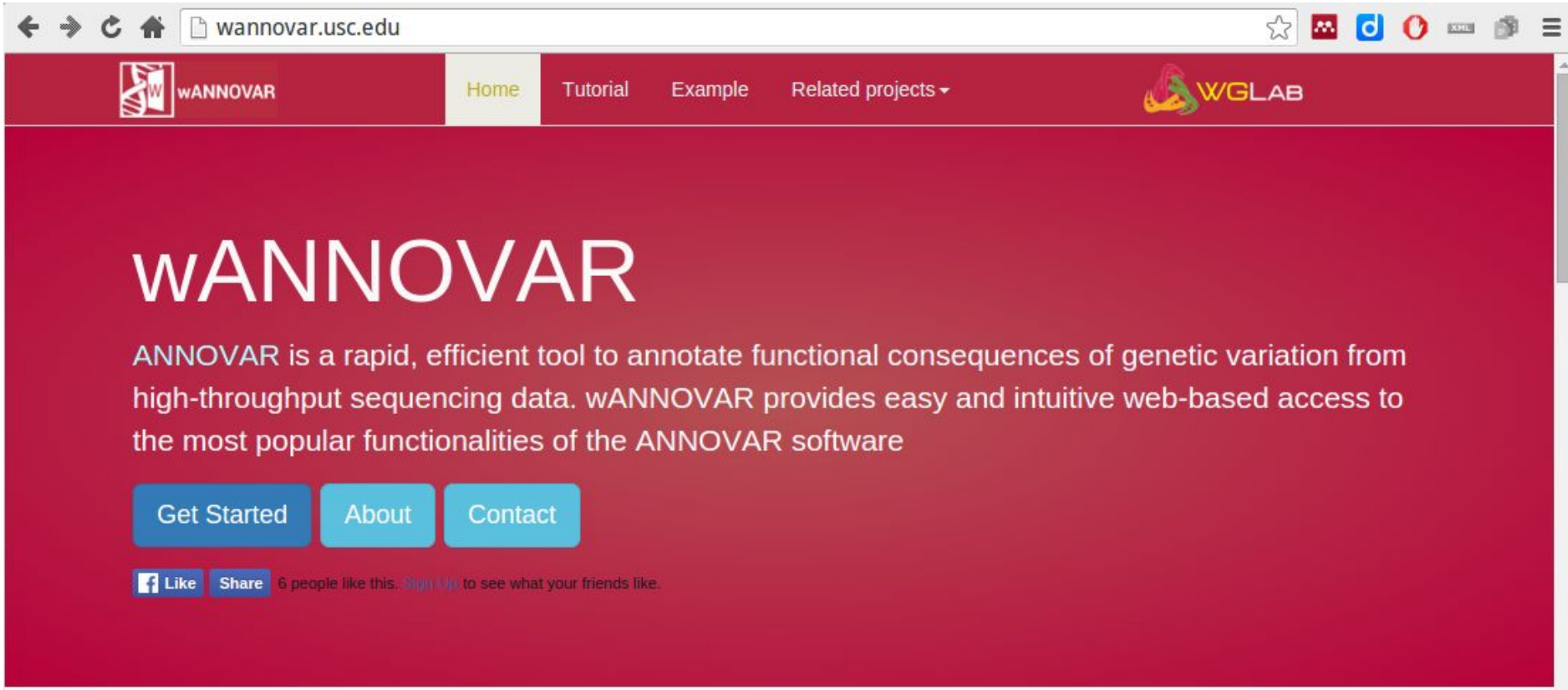


# 5 Variant annotation

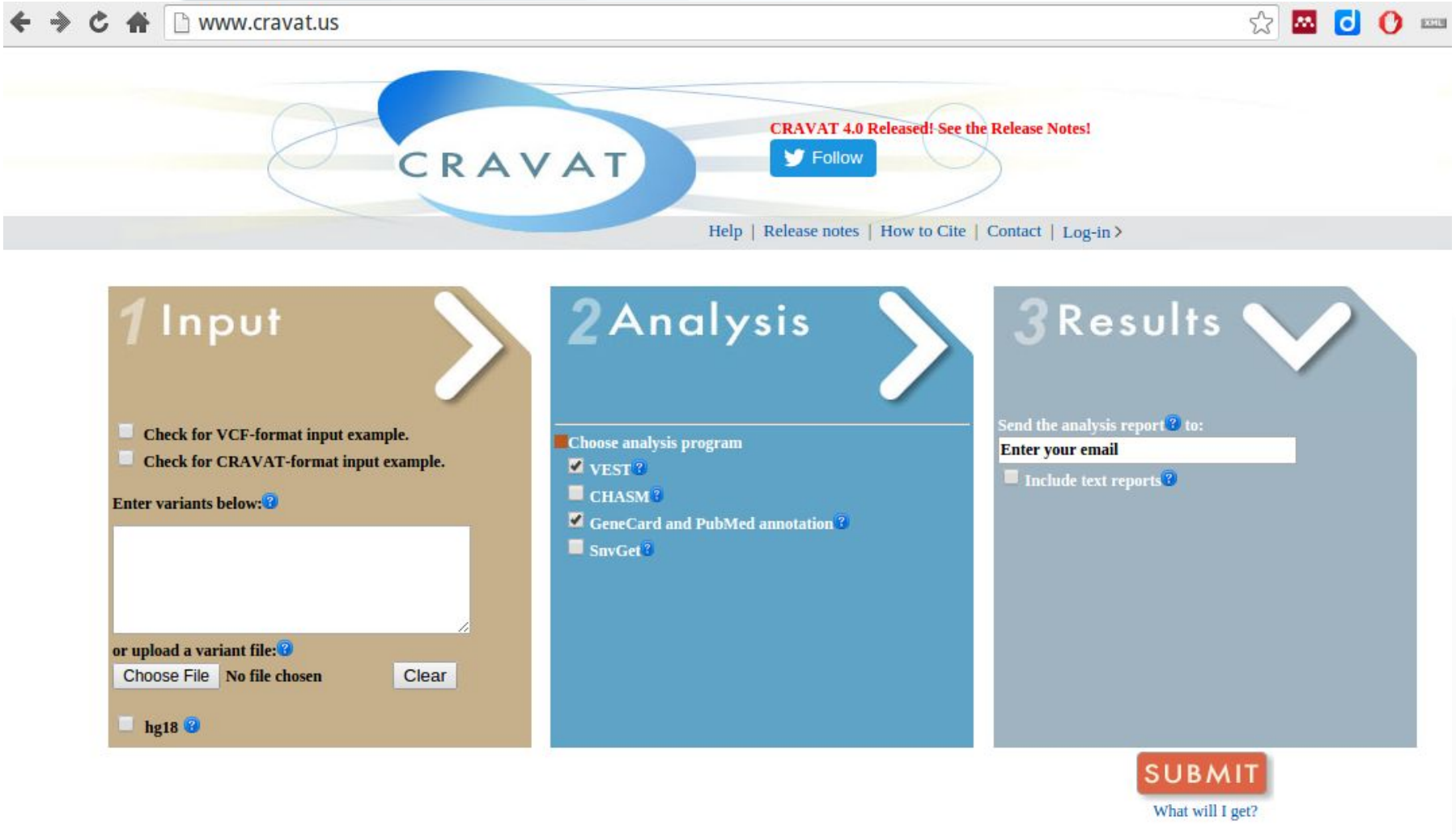
## Webtools

- Easy to use
- Useful for beginners in informatics
- But not so useful for (relatively) large VCFs
- Small number of candidates
- They cannot be include in a bioinformatics pipeline

# 5 Variant annotation



# 5 Variant annotation



# 5 Variant annotation

https://www.broadinstitute.org/oncotator/

Oncotator

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## What is Oncotator?

Oncotator is a web application for annotating human genomic point mutations and indels with data relevant to cancer researchers. Annotations are aggregated from the following resources:

### Genomic Annotations

- Gene, transcript, and functional consequence annotations using GENCODE for hg19.
- Reference sequence around a variant.
- GC content around a variant.
- Human DNA Repair Gene annotations from Wood et al.

### Protein Annotations

- Site-specific protein annotations from UniProt.
- Functional impact predictions from dbNSFP.

### Cancer Variant Annotations

- Observed cancer mutation frequency annotations from COSMIC.
- Cancer gene and mutation annotations from the Cancer GenCensus.
- Overlapping mutations from the Cancer Cell Line Encyclopedia.
- Cancer gene annotations from the Familial Cancer Database.
- Cancer variant annotations from ClinVar.

### Non-Cancer Variant Annotations

- Common SNP annotations from dbSNP.
- Variant annotations from 1000 Genomes.
- Variant annotations from NHLBI GO Exome Sequencing Project (ESP).

## Get Started

Upload your input data below. Please note, only GRCh37/hg19 coordinates are supported and there is a maximum of 5,000 alterations per input file. If you need additional help, please visit our help page or feel free to contact us directly.

### Upload Mutations

Choose File No file chosen

Submit

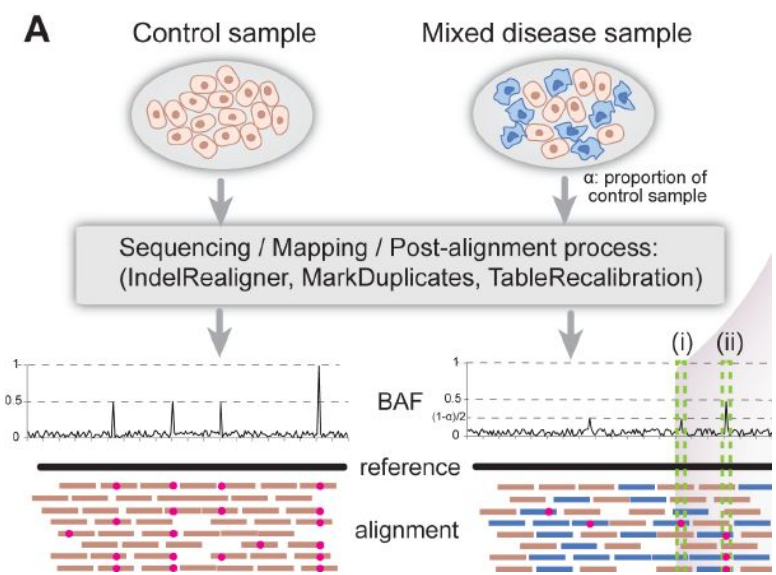
Upload TSV file of mutations in Oncotator format.

### Paste Mutations

# 6 Mosaicism and cellular heterogeneity

## Cancer genomics

- Huge amount of alterations
- Tumors are not homogeneous
- Germline models are not valid

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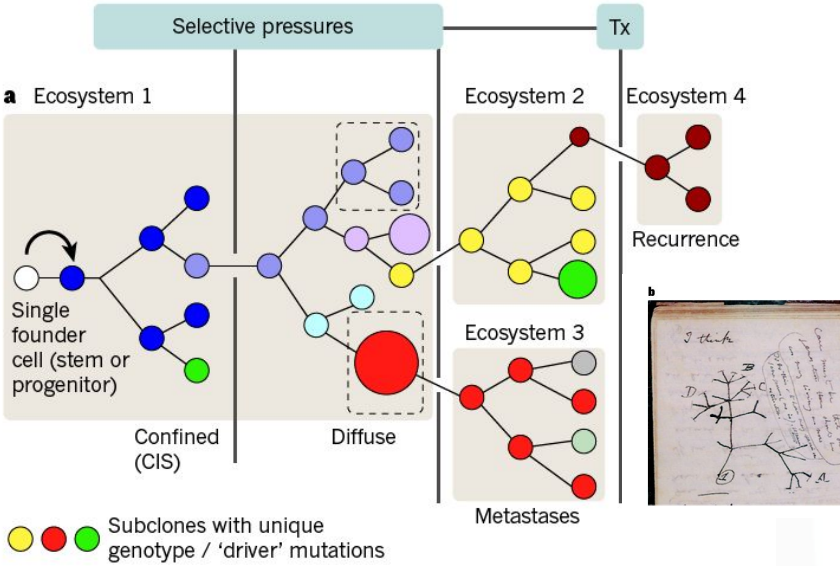


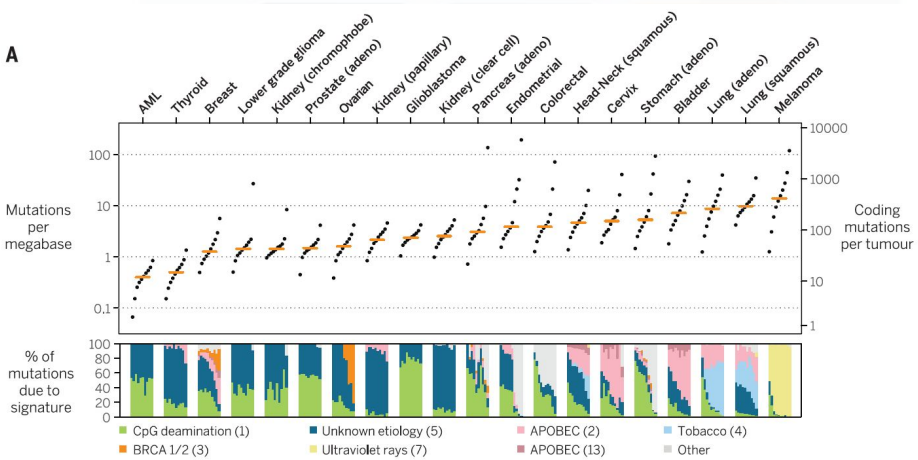


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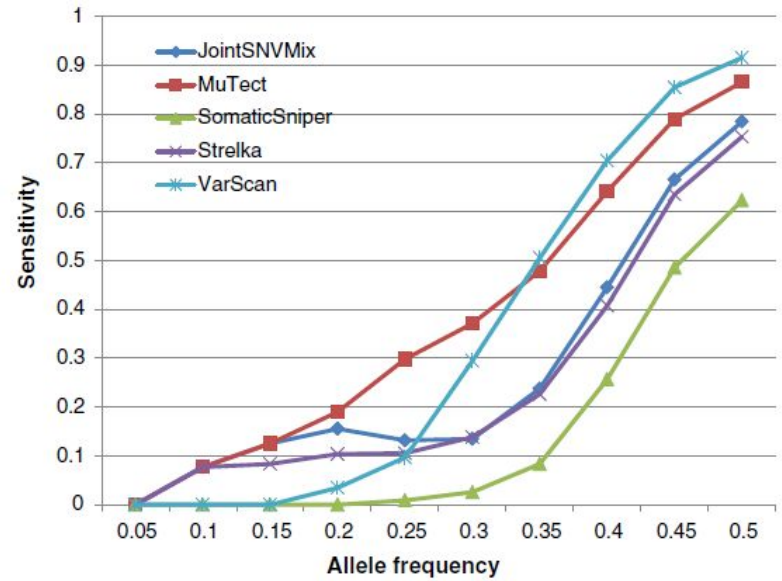
# 6 Mosaicism and cellular heterogeneity

## Cancer genomics

- Somatic variant calling
  - SNVmix
  - Varscan
  - **Mutect**
  - Virmid
  - Strelka

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Comparison of somatic mutation calling methods in amplicon and whole exome sequence data

Huilei Xu, John DiCarlo, Ravi Vijaya Satya, Quan Peng and Yexun Wang*

Kim and Speed BMC Bioinformatics 2013, 14:189
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RESEARCH ARTICLE Open Access

Comparing somatic mutation-callers: beyond Venn diagrams

Su Yeon Kim[1*] and Terence P Speed[1,2*]

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http://genomemedicine.com/content/5/10/91

Genome Medicine

RESEARCH Open Access

Detecting somatic point mutations in cancer genome sequencing data: a comparison of mutation callers

Qingguo Wang[1], Peilin Jia[1,2], Fei Li[3], Haiquan Chen[4,5], Hongbin Ji[3], Donald Hucks[6], Kimberly Brown Dahlman[6,7], William Pao[6,8*] and Zhongming Zhao[1,2,7,9*]

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## Some recommended papers


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# Now it’s time to practice!