

# Recherche de variants génomiques en oncologie clinique

Avec des diapos, données & scripts R de:  
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# Technologies de recherche de variants

- Sanger
  - Toujours utilisé en consultation de génétique
- SNP arrays
  - ~1M SNP (recherche GWAS ou « 23&Me »)
- Panel de gènes
  - Une série d'exons d'intérêt (gènes de cancer= 100kb)
- WES (Exome)
  - Tous les exons du génome (30 Mb)
- WGS
  - Whole genome (3 Gb)

NGS

# Génétique constitutionnelle

At hospital



Blood sample

Sequence  
gene panel



Look for  
specific  
alteration  
(BRCA)

Research



Genotype  
or  
Sequence



Compare  
disease and  
healthy  
cohorts

GWAS studies, 1000 Genome Project...

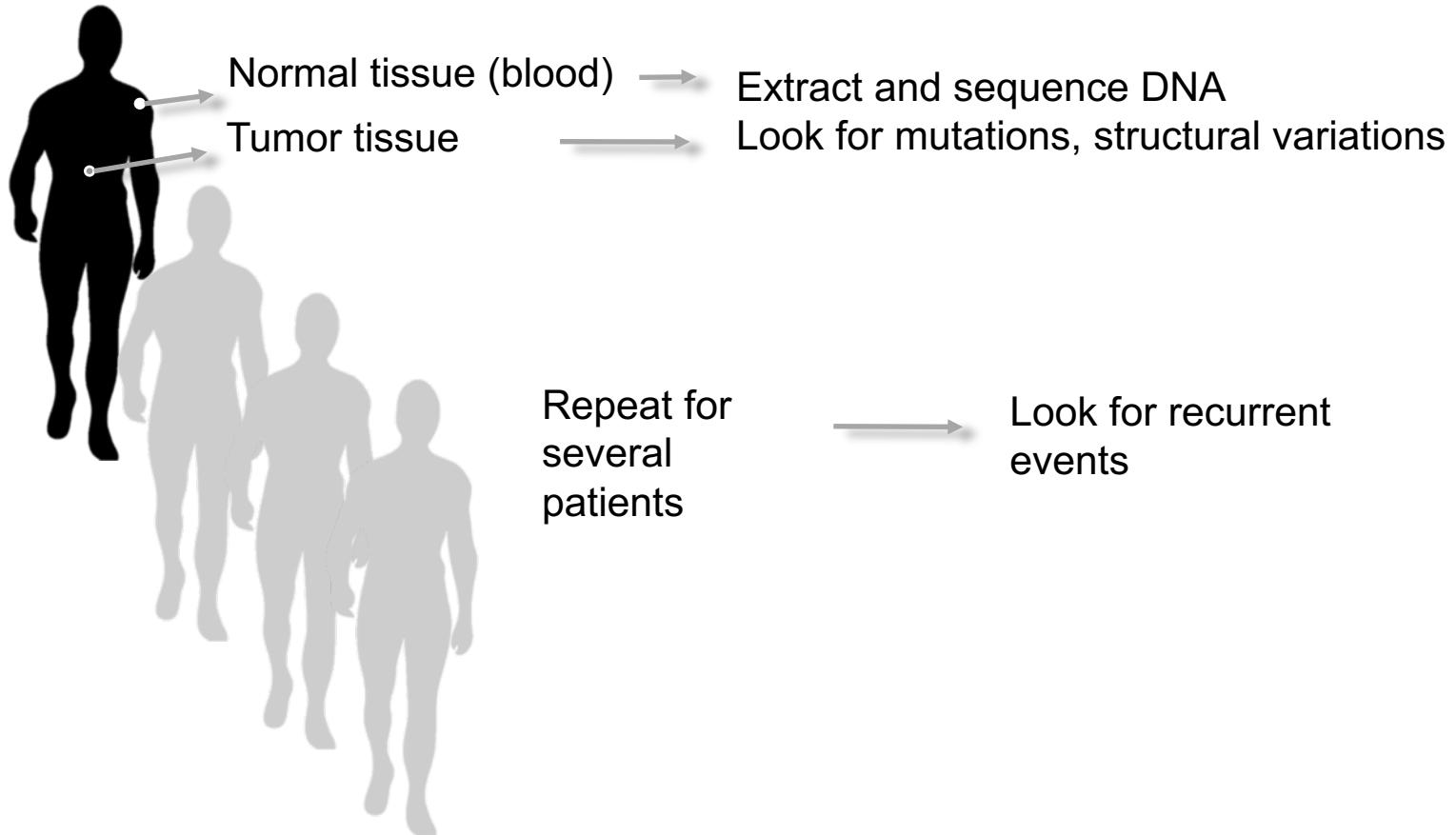
# NGS dans le diagnostic de génétique familiale

Panel de gènes pour cibler:

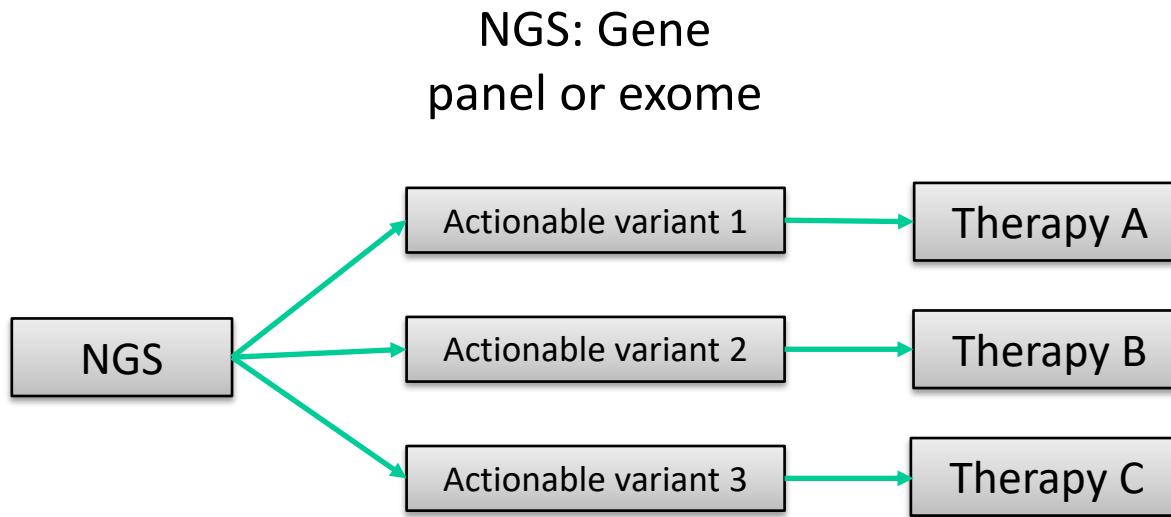
- BRCA1/2 (breast/ovary cancer)
- XPC, XPV.. (melanoma)
- ERCC1 (colorectal cancer)

# En génétique somatique

Finding somatic mutations in the tumor genome

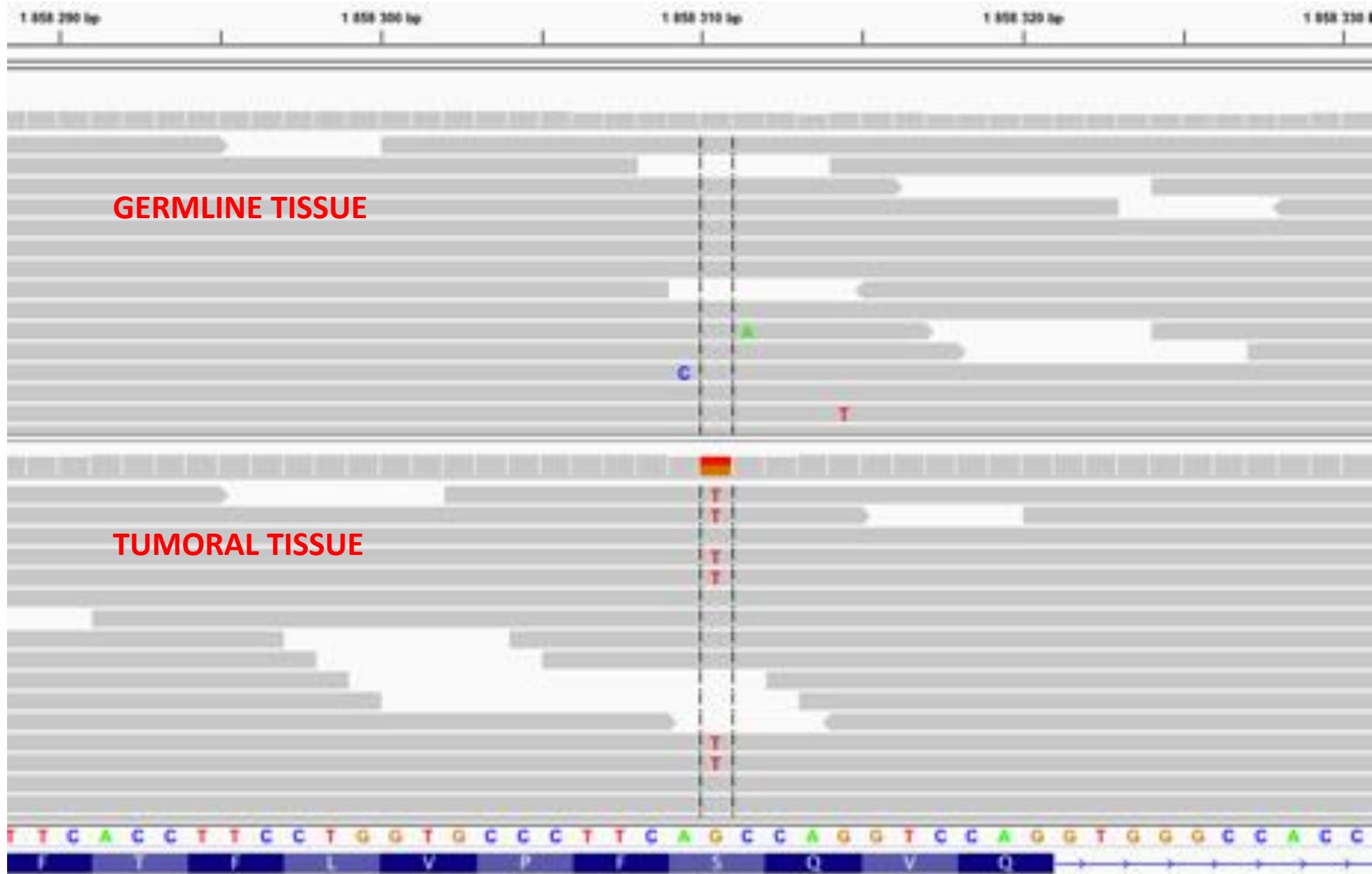


# NGS for precision medicine

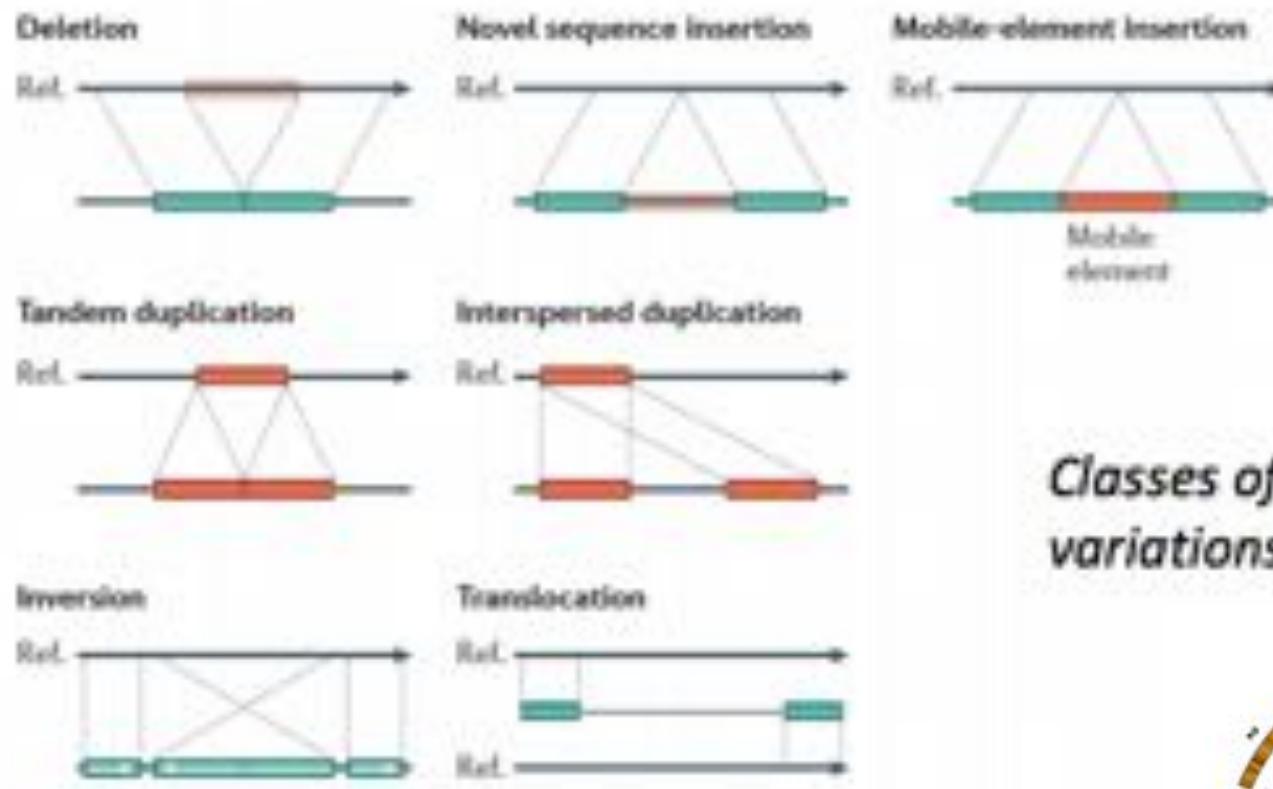


- Clinical trials: MOSCATO (GR), SAFIR (GR), SHIVA (Curie), ...
- Ipilimumab (anti-CTLA4), Nivolumab (anti-PD1), Trastuzumab (anti-HER2), Cetuximab (anti-EGFR)

# Les mutations somatiques

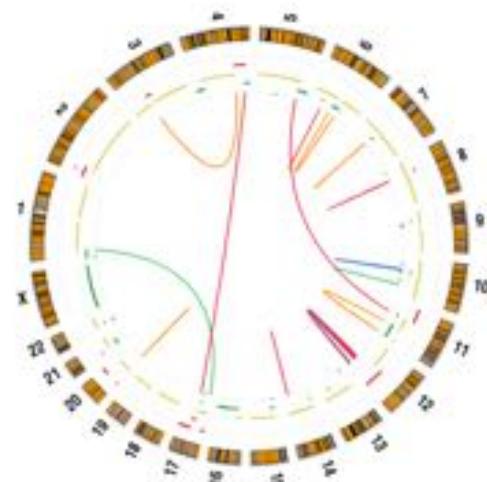


# Variants structuraux



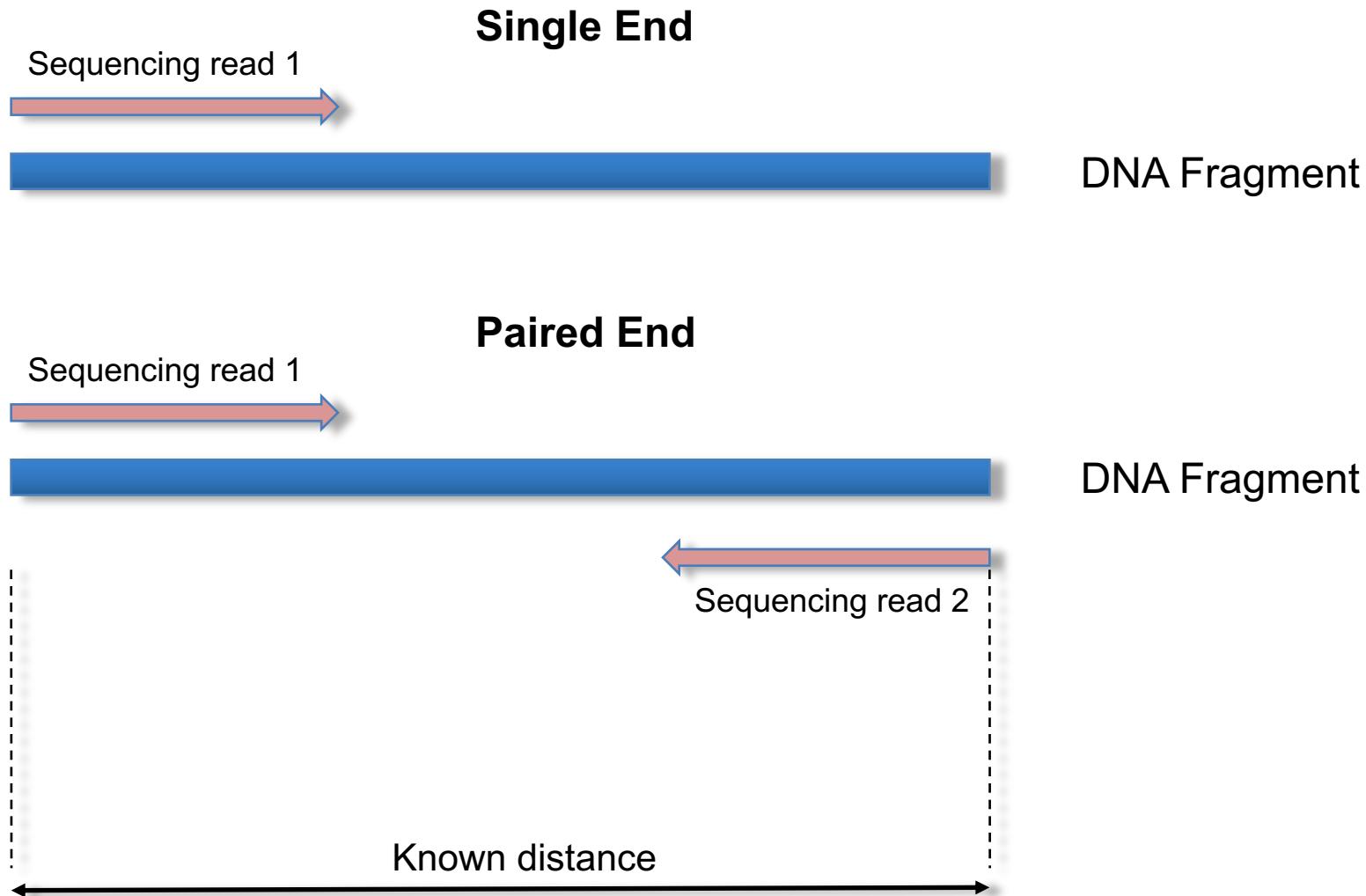
*Classes of structural variations*

Alkan et al 2012



# Intérêt du séquençage paired-end:

## Résolution des repeats et des variants structuraux



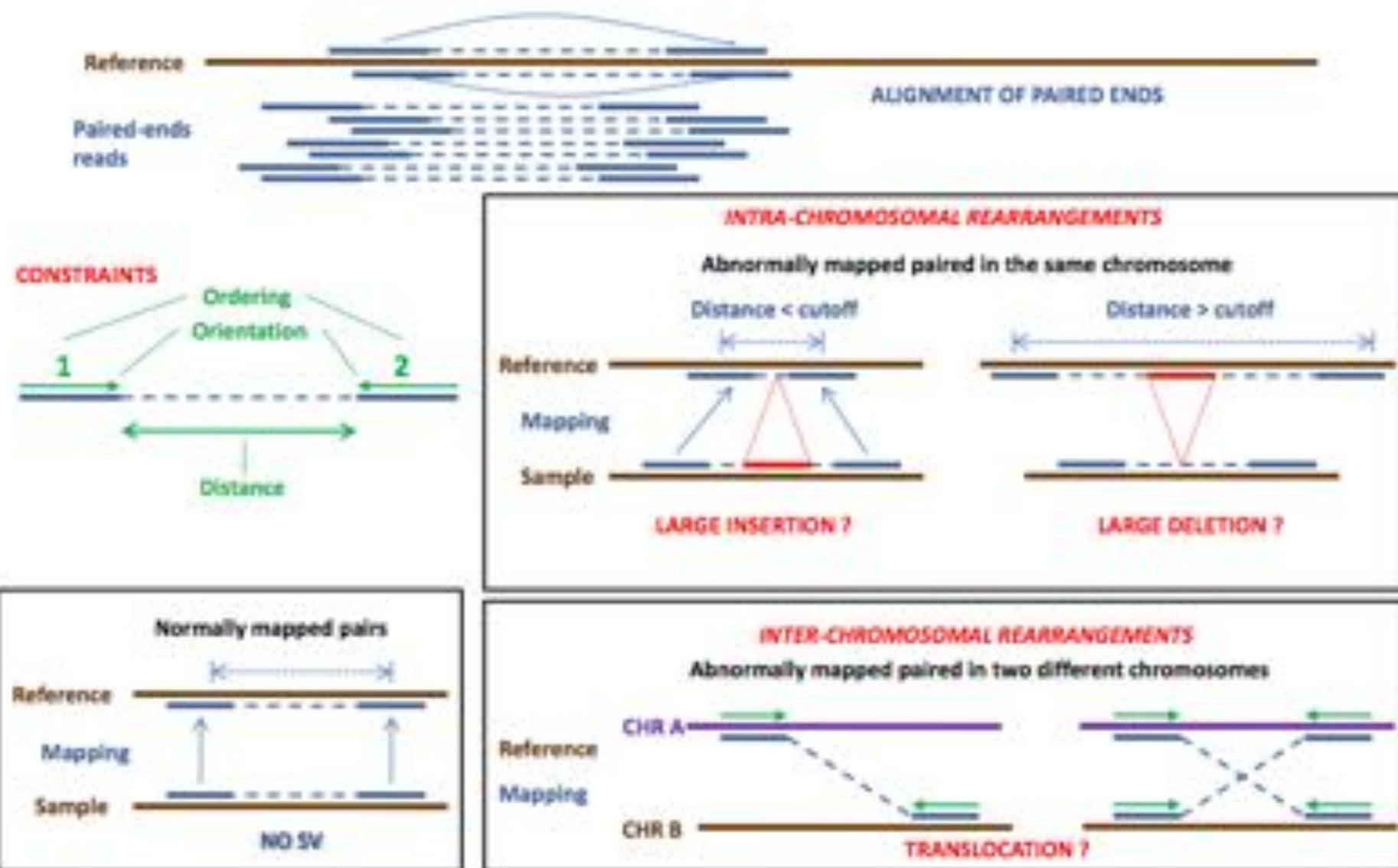
# Exemple

- Single-end alignment – repeated sequence

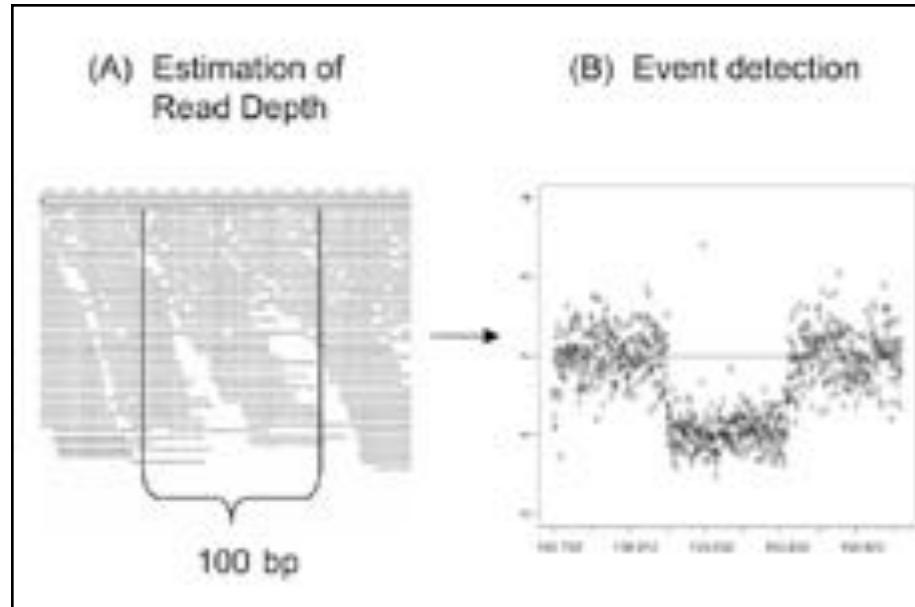
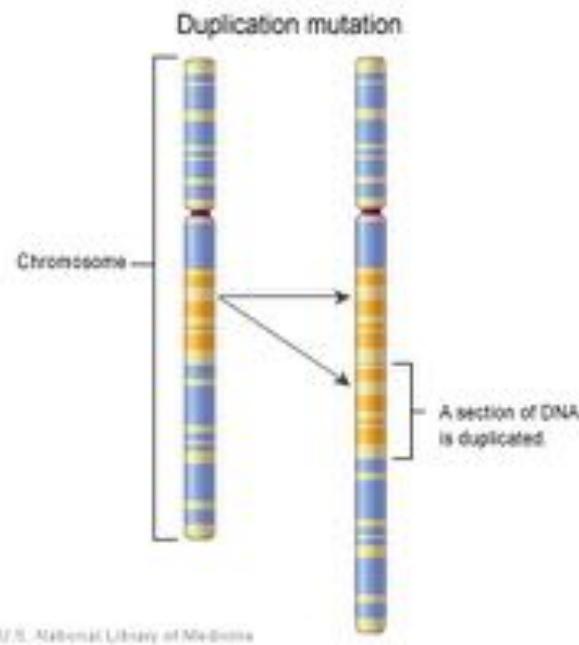


- Paired-end alignment – unique sequence





# Recherche de CNV (copy number variations)



- Attempts to infer variations in copy number from the **local read depth**.
- A strong GC% debiasing is required

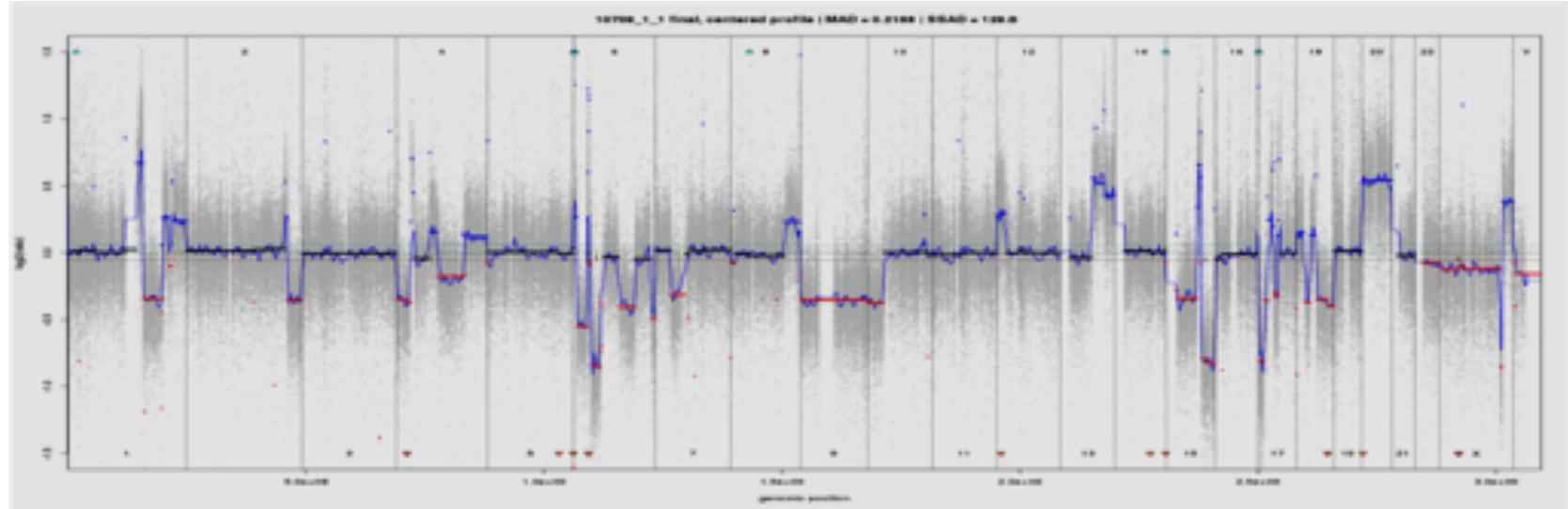
Voir cours Bastien Job

*Yoon, 2009*

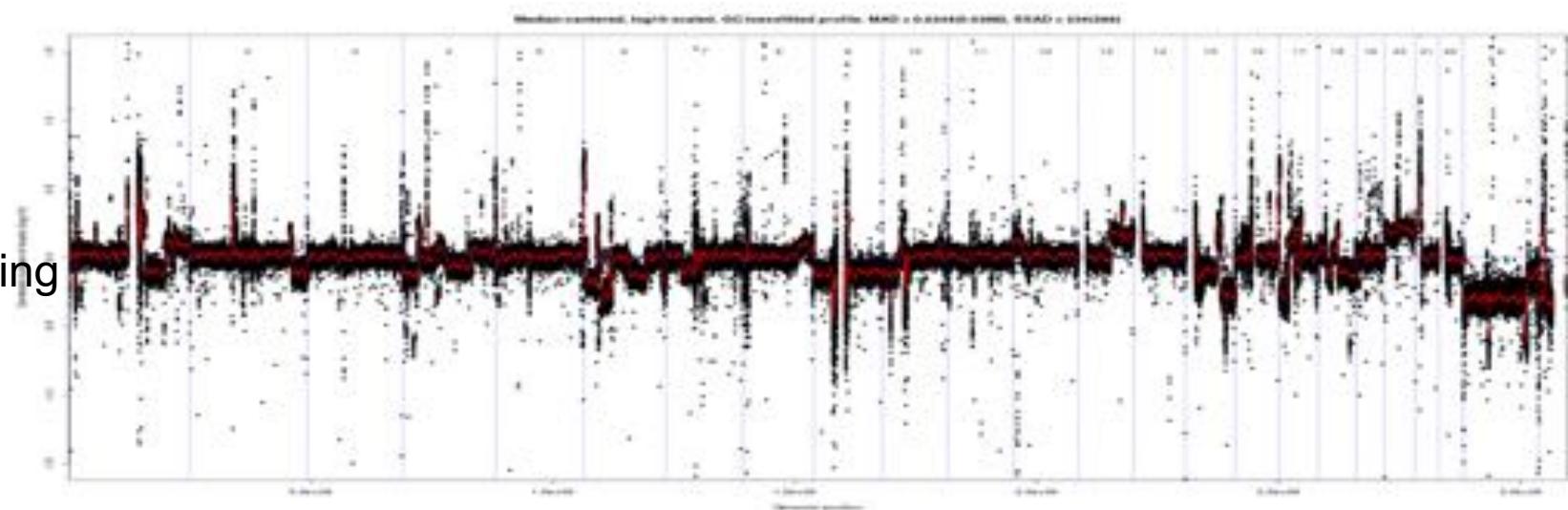
# NGS vs CGH

CGH

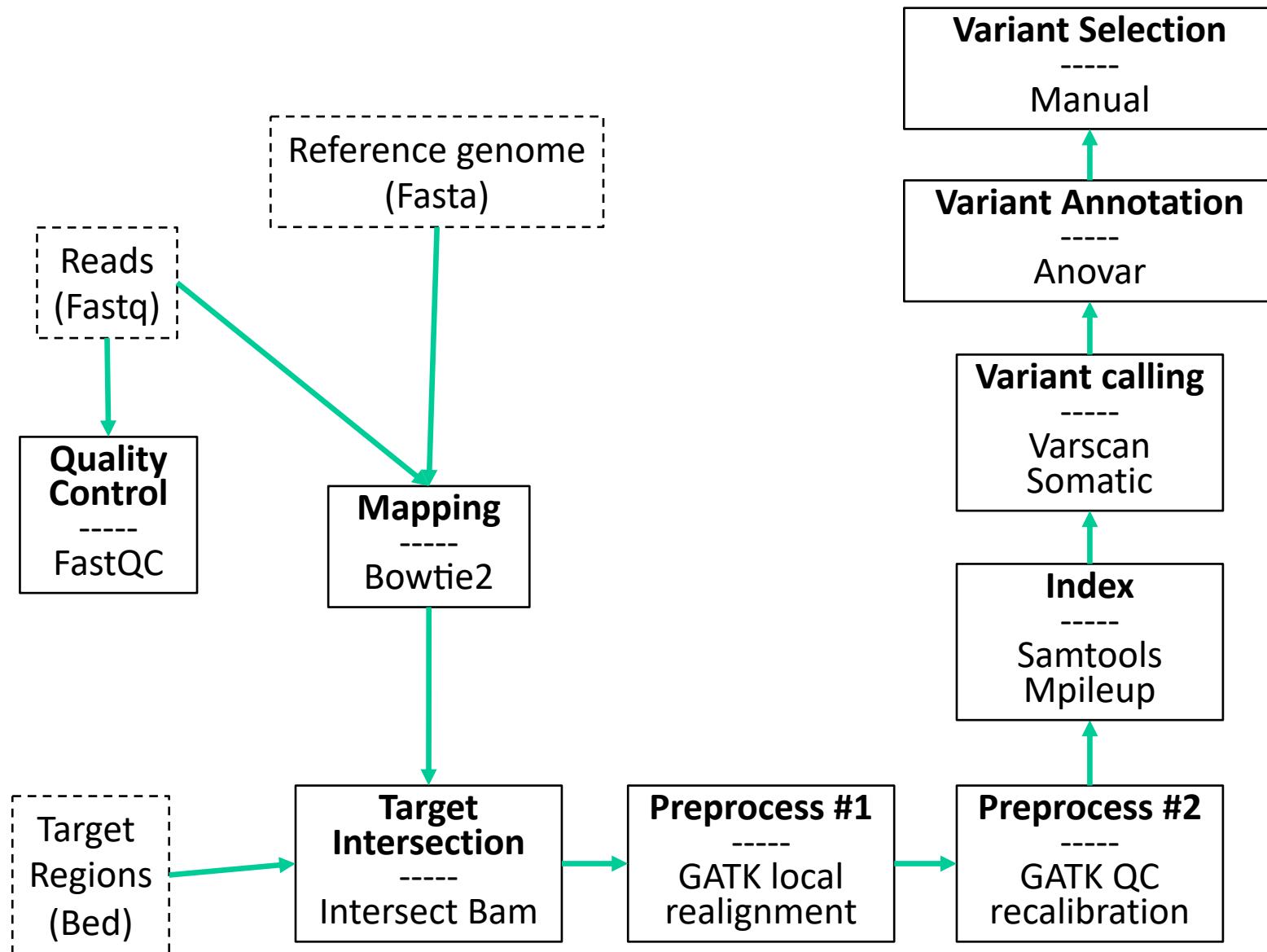
comparative  
genome  
hybridization



Sequencing



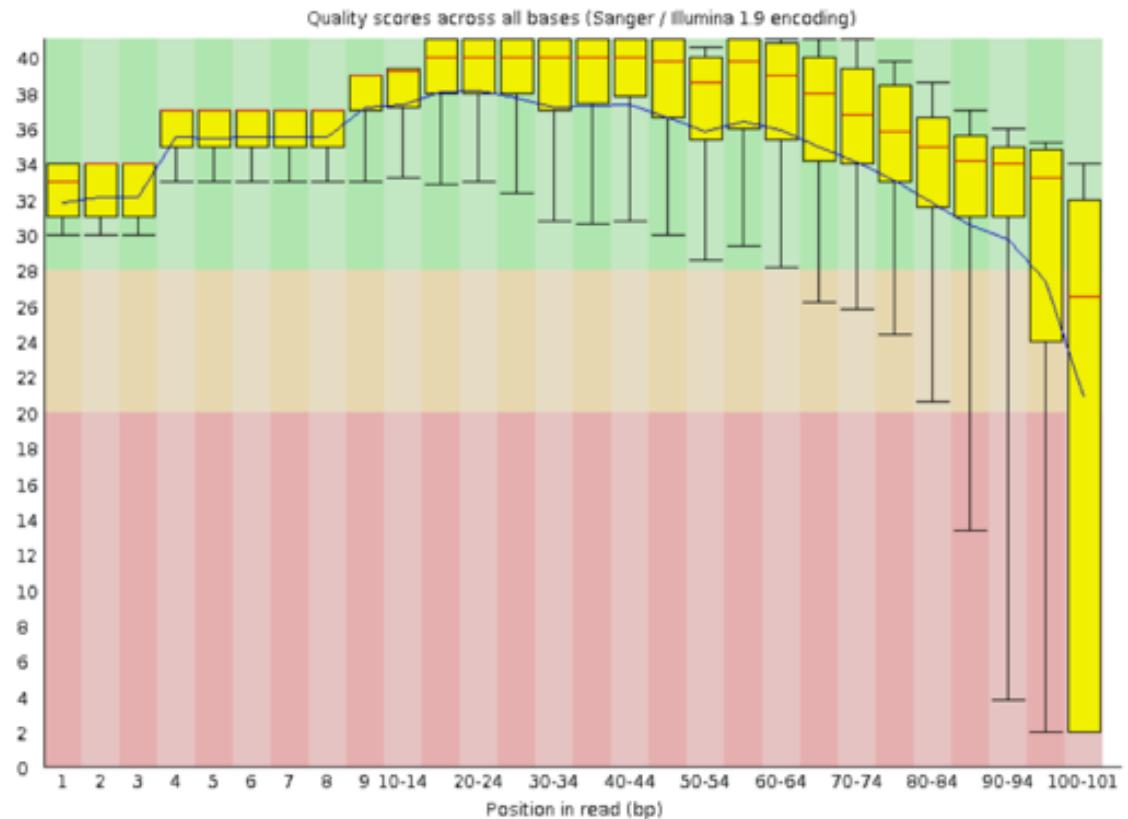
# Un pipeline « variants »



# FastQC Metrics

- Look at the different metrics for both reads
- **Problem:** the per base sequence quality of the Read2 are quite low towards the end

✖ Per base sequence quality



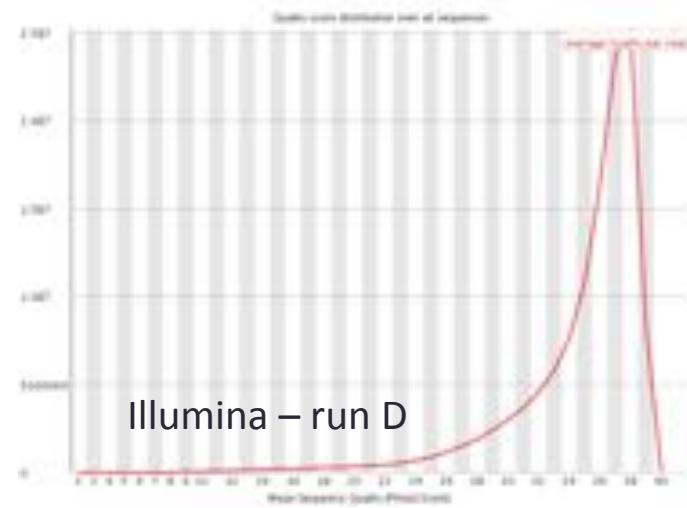
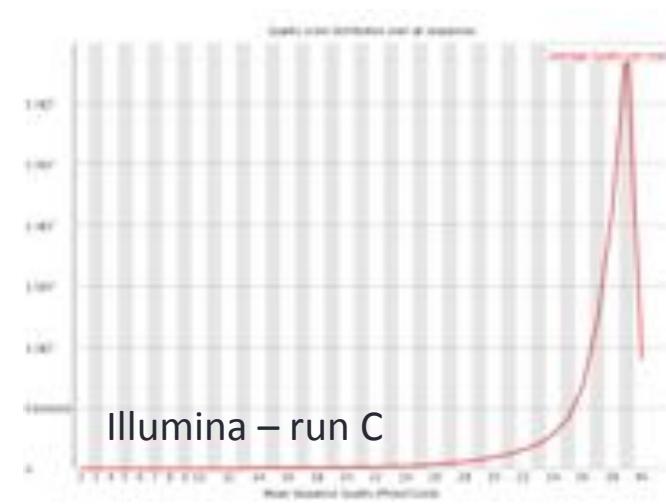
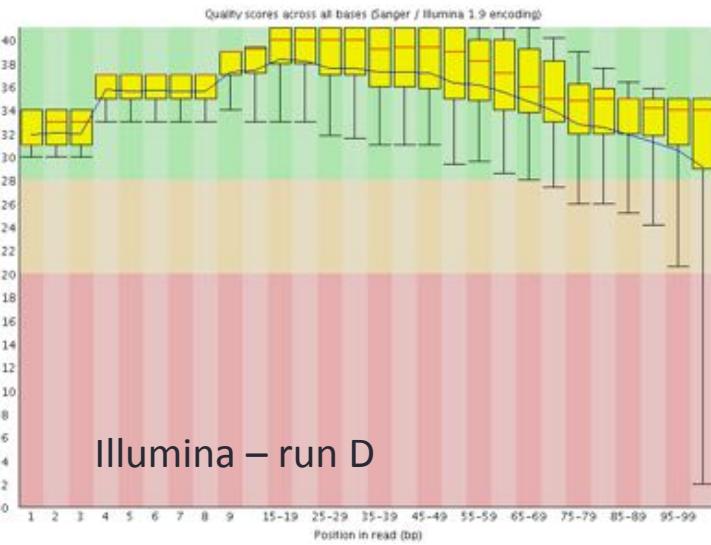
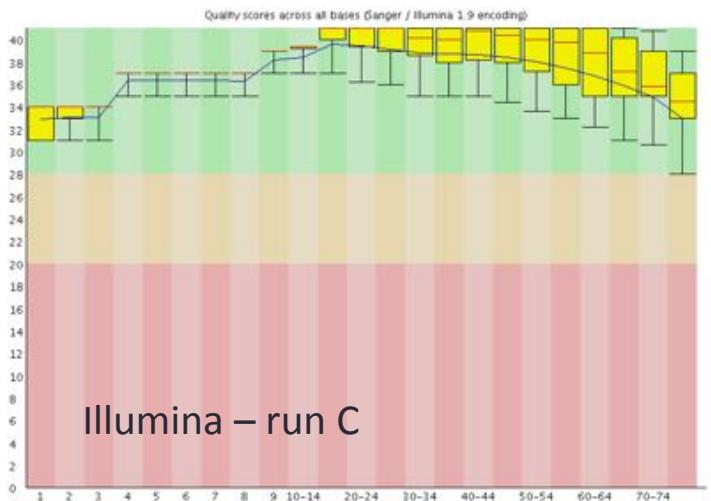
## Solution:

Trim low quality bases from the 3' end of the reads

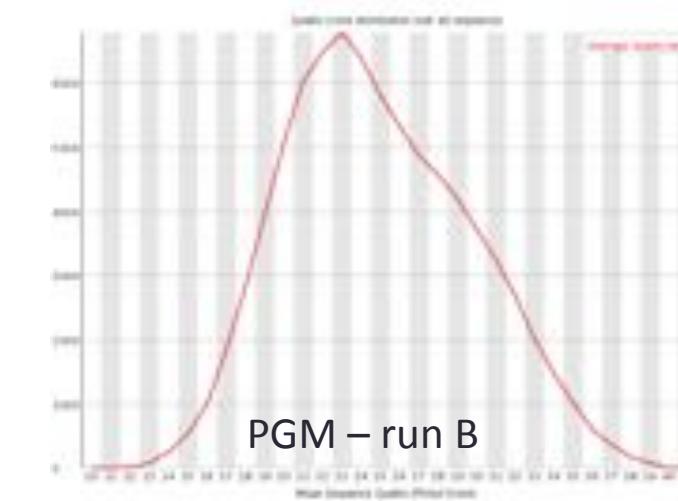
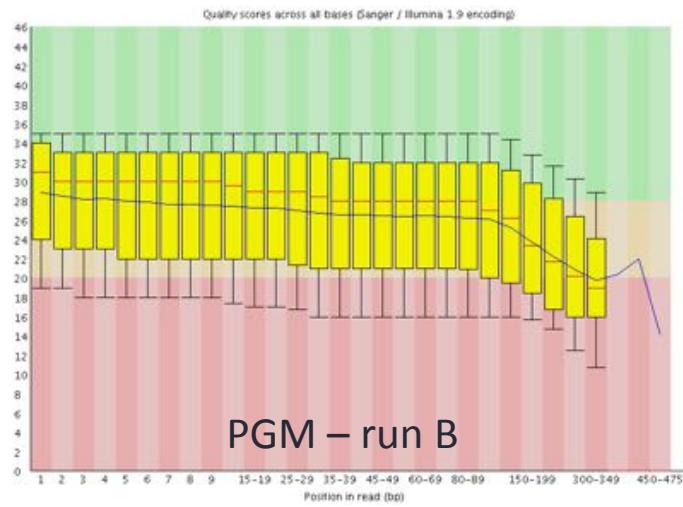
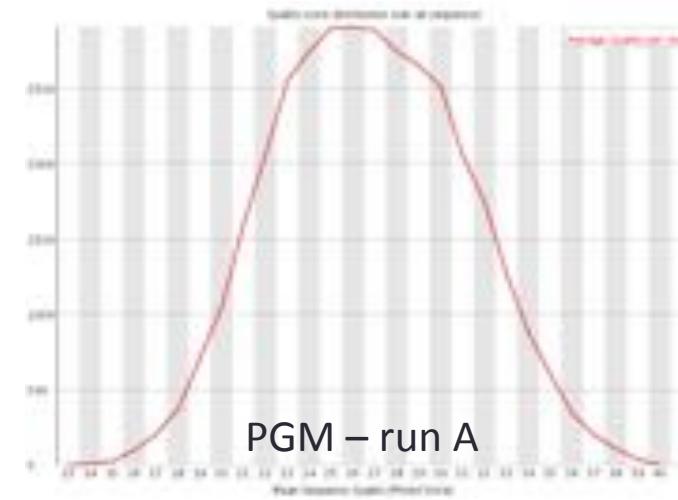
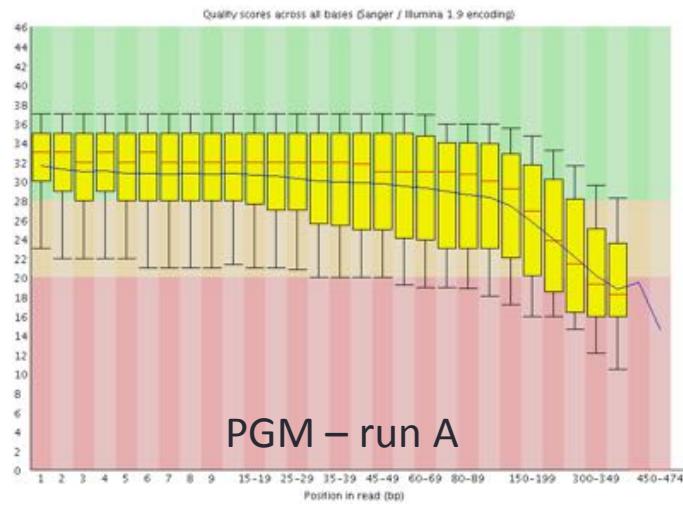
➤ Higher confidence in the sequenced information

(Trimmomatic)

# Illumina

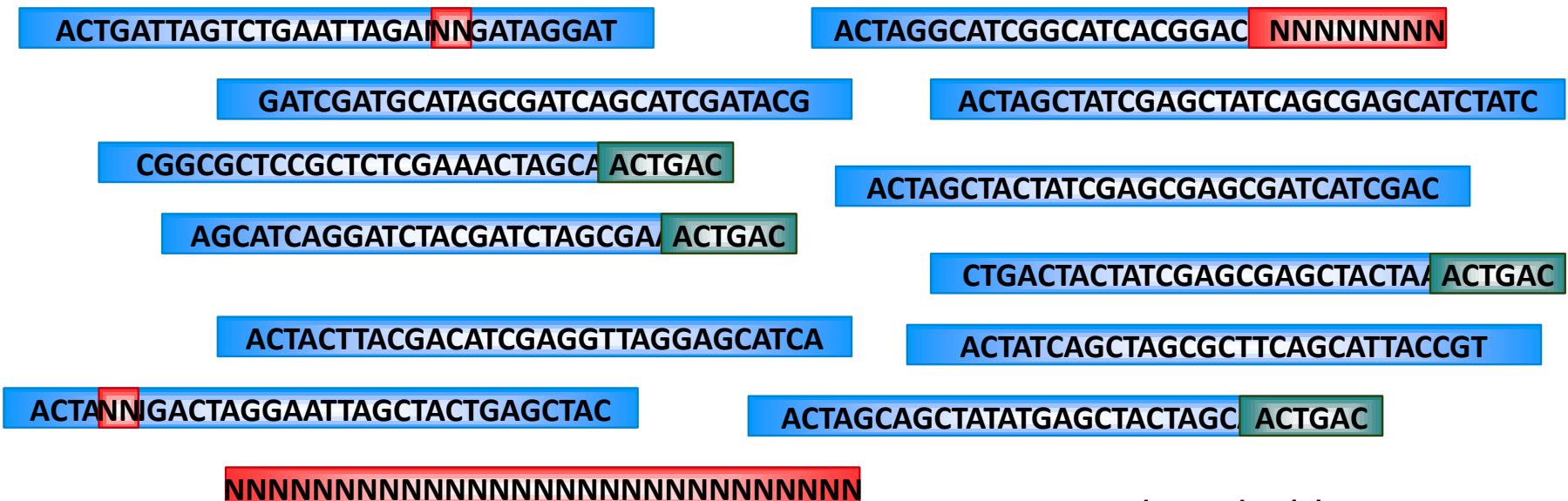


# PGM

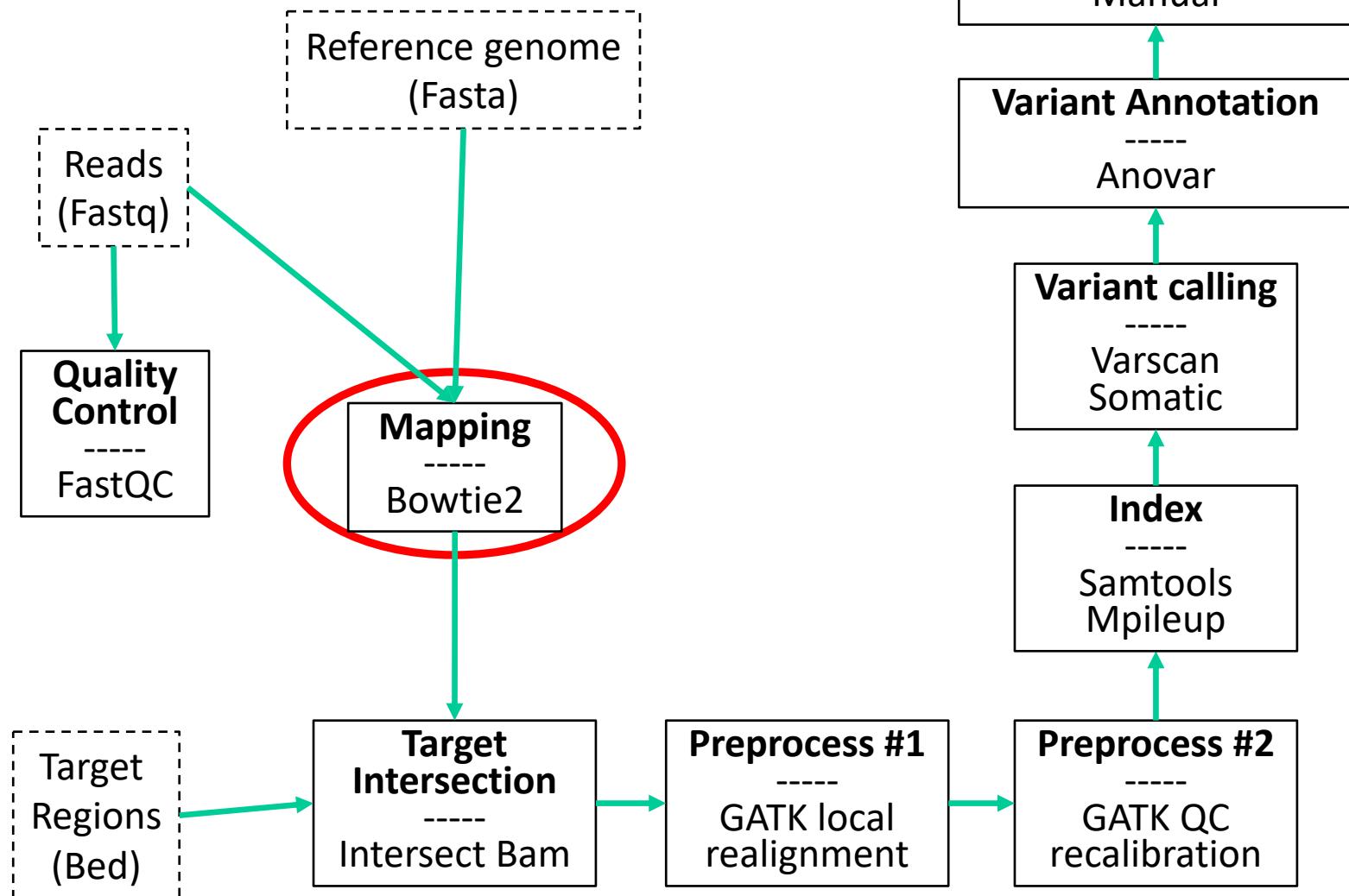


# Trimming and discarding low quality reads...

A first Quality Control of raw reads is mandatory and can be established according to the application ('N', adapter sequences, barcode, contamination, etc.)



Processed reads: blue parts are to be kept, green and red parts to be removed



# Alignment des reads sur le génome de référence



# Algorithme en $O(mn)$

ACGTTACCGAATCGATCAAGTCGA  
TAC



OK pour 1 read:  $O(3.10^9 \times 100)$   
Mais pour  $1^8$  reads???

« supercalifragilis-ticexpialidocious »

←   →

Préfixe                                      Suffixe

## Suffix array

“GOOGOL”

Tableau trié de tous les suffixes  
d'une chaîne de caractères

0 GOOGOL\$	6 \$	
1 OOGOL\$	3 GOL\$	
2 OGOL\$	0 GOOGOL\$	
3 GOL\$	5 L\$	→ (6,3,0,5,2,4,1)
4 OL\$	2 OGOL\$	
5 L\$	4 OL\$	
6 \$	1 OOGOL\$	

Propriété: toutes les occurrences d'une même chaîne sont regroupées.

# Suffix arrays

Exemple: trouver la chaîne **GO**

0 GOOGOL\$		6 \$
1 OOGOL\$		3 <b>G</b> O L\$
2 OGOL\$		0 <b>GO</b> OGOL\$
3 GOL\$	→	5 L\$
4 OL\$		2 OGOL\$
5 L\$		4 OL\$
6 \$		1 OOGOL\$

# Most popular aligners for variant analysis

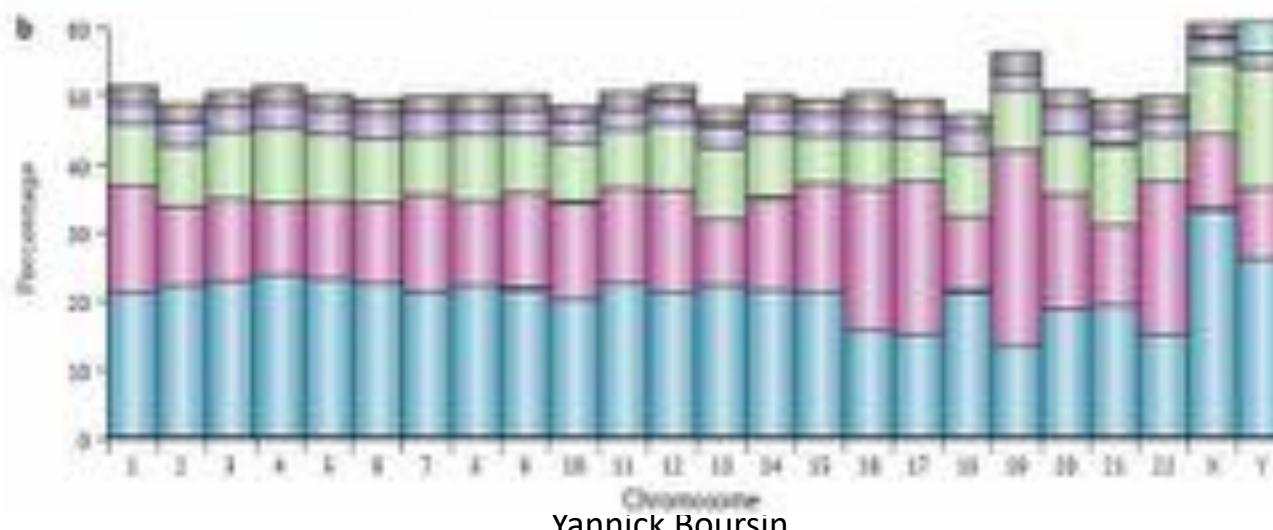
(support mismatched, gapped, paired-end alignment)

- BWA
  - Li H. and Durbin R. (2009)
- Bowtie2
  - Langmead B, Salzberg S (2012)

# Alignment key parameters - Repeats

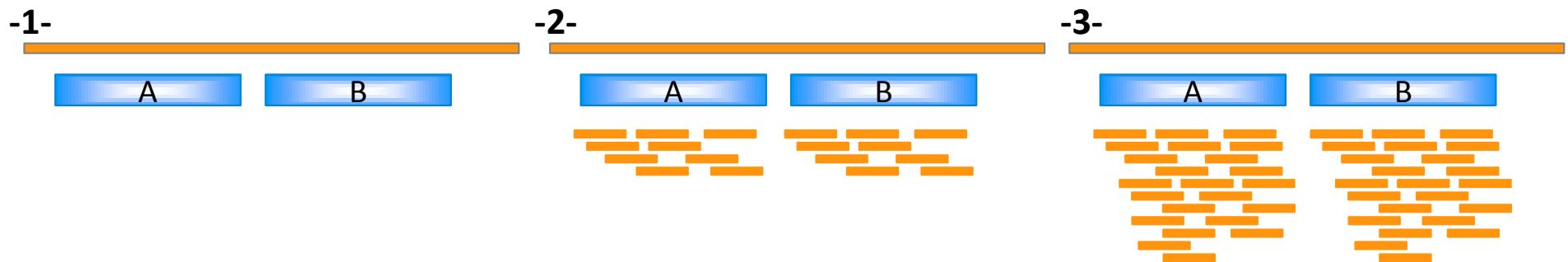
Approximately **50%** of the human genome is comprised of repeats

Repeat class	Repeat type	Number (bp/chr)	Ctg.	Length (bp)
Microsatellite, minisatellite or nanosatellite	Tandem	425,518	1%	2-100
LINE	Interspersed	1,747,375	11%	100-1000
Alu	Interspersed	463,778	2%	200-2000
LTR retrotransposon	Interspersed	718,125	2%	300-5,000
LINE	Interspersed	1,508,345	21%	500-10,000
rDNA (18S, 28S, 5.8S and 25S)	Tandem	601	0.01%	1,000-41,000
Segmental duplications and other elements	Tandem or interspersed	2,779	0.2%	1,000-100,000



# Alignment key parameters – Repeats – 3 strategies

- 1- Report only unique alignment
- 2- Report best alignments and randomly assign reads across equally good loci
- 3- Report all (best) alignments



Treangen T.J. and Salzberg S.L. 2012. Nature review Genetics 13, 36-46

# Reads alignés: le format BAM/SAM



# Format BAM/SAM

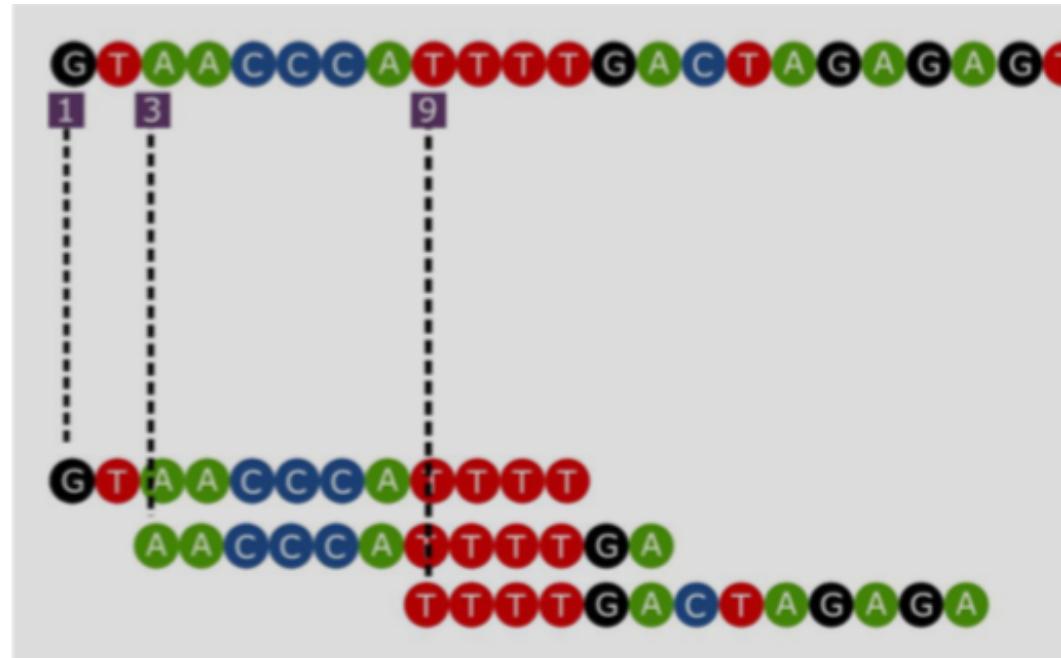
Contient les séquences alignées sur le génome

Concept:

chr7 1324324 ACGTGCCTTCGCGT

chr8 1424324 GCGTGATGCGTAAG

chr8 1724354 GTATGTTATATGTA

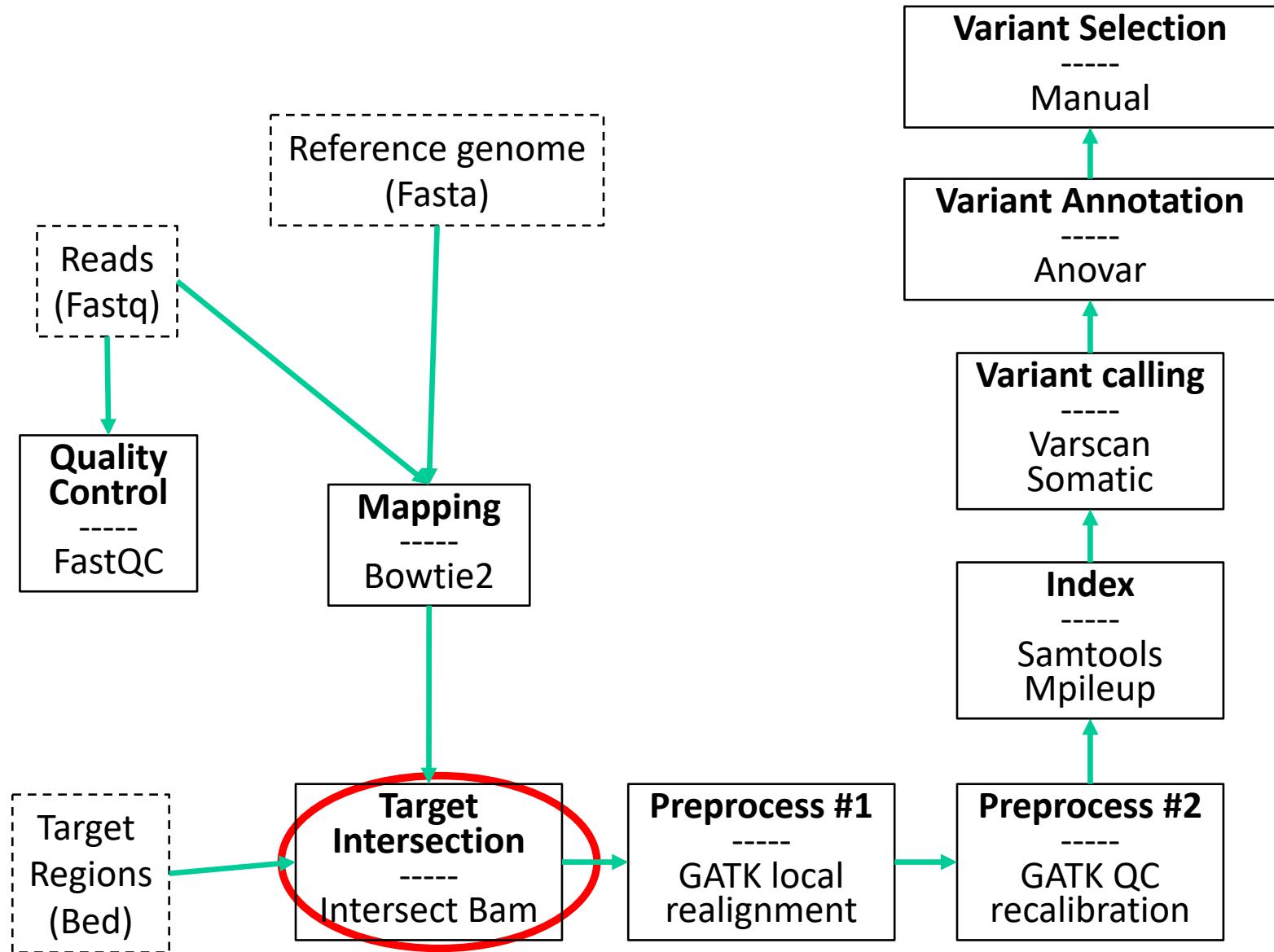


# Format SAM/BAM

- A real SAM:

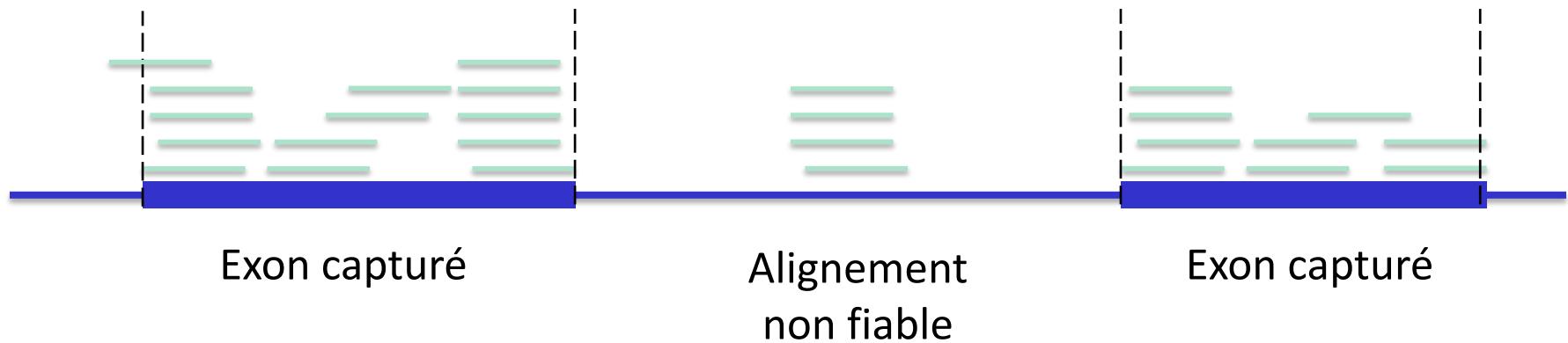
```
@RG  ID:group1  SM:1425_CD34  PL:ILLUMINA  LB:lib1 PU:unit1
@PG  ID:bwa  PN:bwa  VN:0.7.12-r1039 CL:bwa mem -M -t 2 -A 2 -E 1 -R @RG\tID:group1\tSM:1425_CD34\tPL:ILLUMINA\tLB:lib1\tPU:unit1 /root/myd
ERR166338.13782800  83  chr13  32890449  60  101M  =  32890343  -207  GGGACTGAATTAGAACAAATTTCAGCGCTT
ERR166338.13782800  163  chr13  32890343  60  75M  =  32890449  207  CACTAGCCACGTTCGAGTGCTTAATGTGGCTAGTGGC
ERR166338.26716588  99  chr13  32890406  60  101M  =  32890553  222  AATGTTCCCACCTCACAGTAAGCTGTTACCGTTCCAG
ERR166338.26716588  147  chr13  32890553  60  75M  =  32890406  -222  TTGCAGACTTACCAAGCATTGGAGGAATATCGTA
ERR166338.27259961  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.27259961  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
ERR166338.63037998  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.63037998  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
```

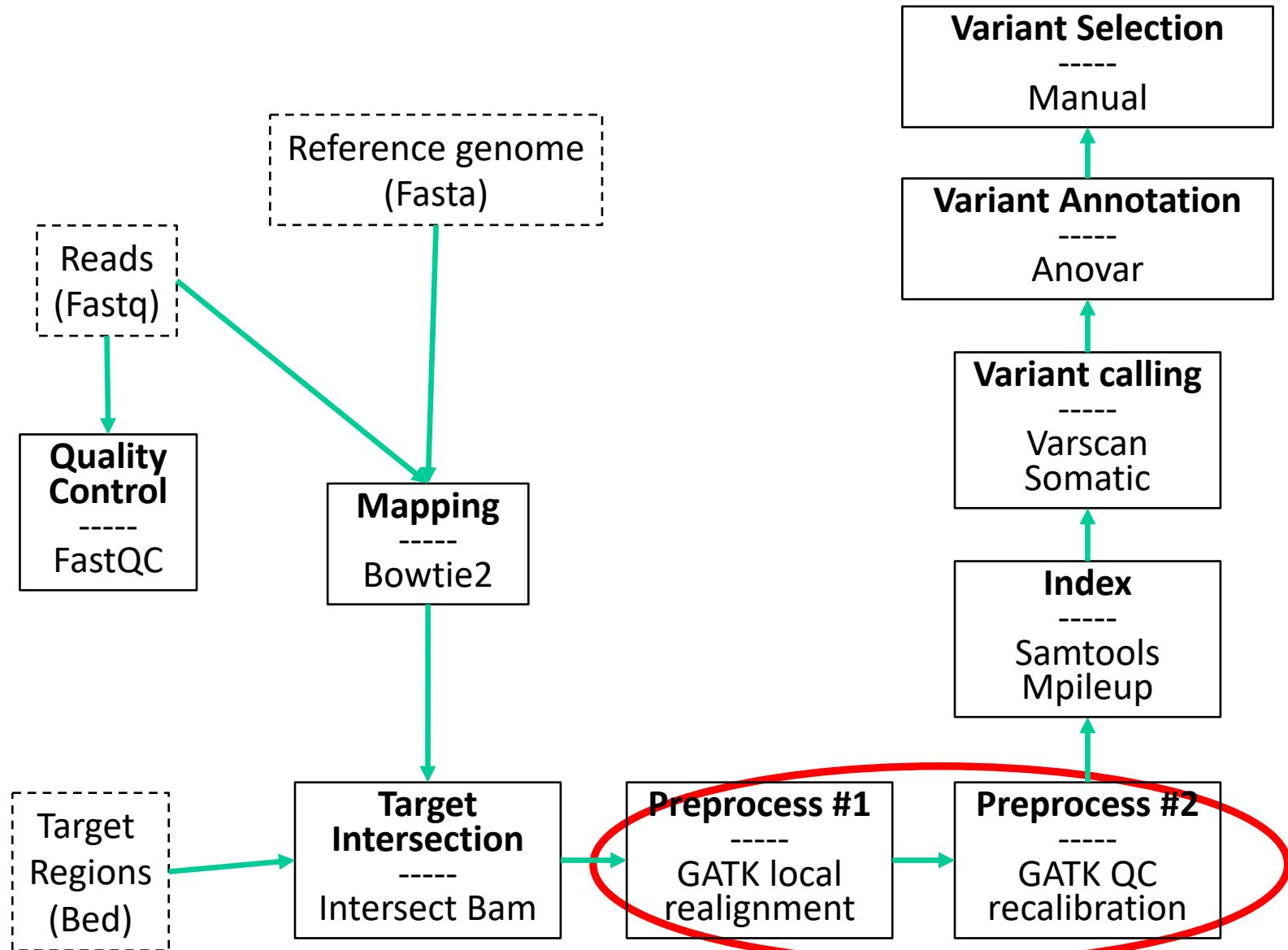
↑  
read ID  
↑  
flag  
↑  
position  
↑  
CIGAR  
↑  
mapping qual.  
↑  
mate info



# Target intersection

- Comparer l'alignement obtenu à la liste des positions visées par le protocole de capture





# Why realign around indels ?

- Small Insertion/deletion (Indels) in reads (especially near the ends) can trick the mappers into wrong alignments
  - Alignment scoring – cheaper to introduce multiple Single Nucleotide Variants (SNVs) than an indel: induce a lot of false positive SNVs
- ➔ artifactual mismatches
- **Realignment around indels helps improve the downstream processing steps**

# Wrong alignment near indels

Genome

CTACGAAGTAAAAAAAAGAGAGAGTTACT

CTACGAAGT - -AAAAAAAAGAGAGAGTTACT

CTACGAAGTAAAAAAAAGAGAGAG**TTACT**

*Cost for 2 indels < 4 mismatches*

CTACGAAGT - -AAAAAAAAGAGAGA

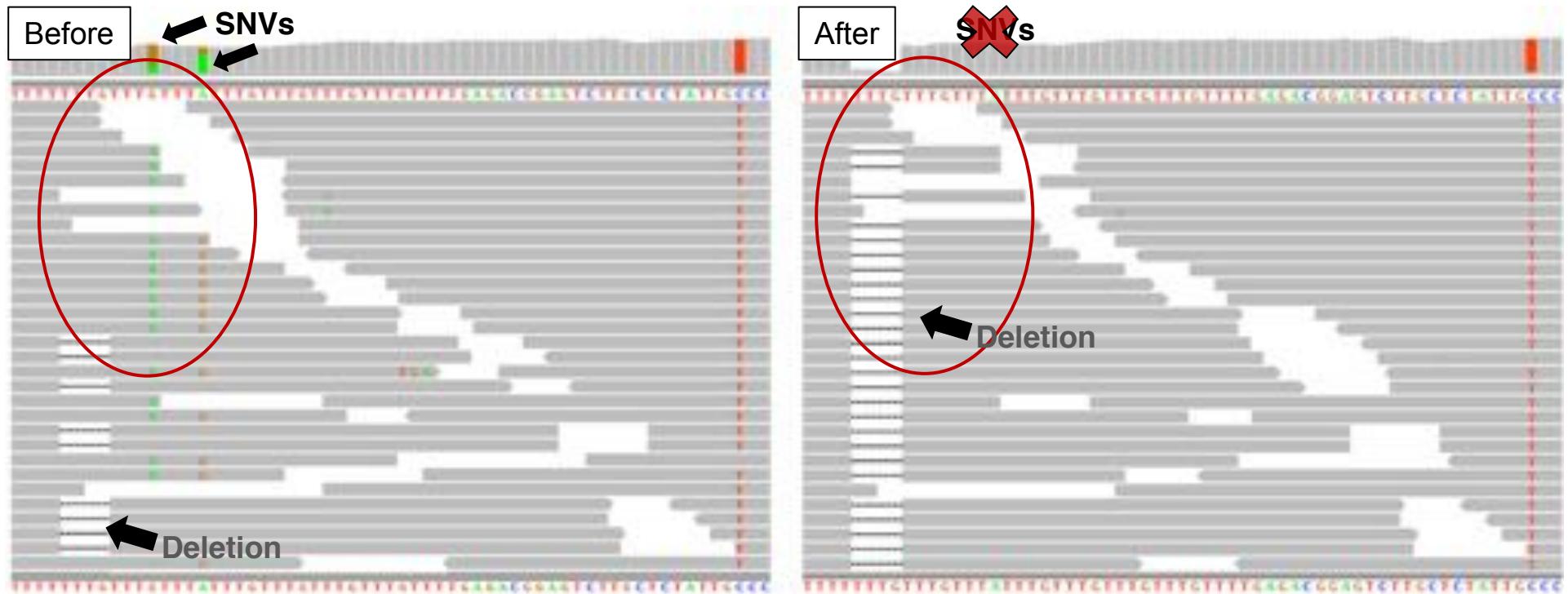
CTACGAAGTAAAAAAAAG**GAGAGA**

*Cost for 2 indels > 1 mismatch*

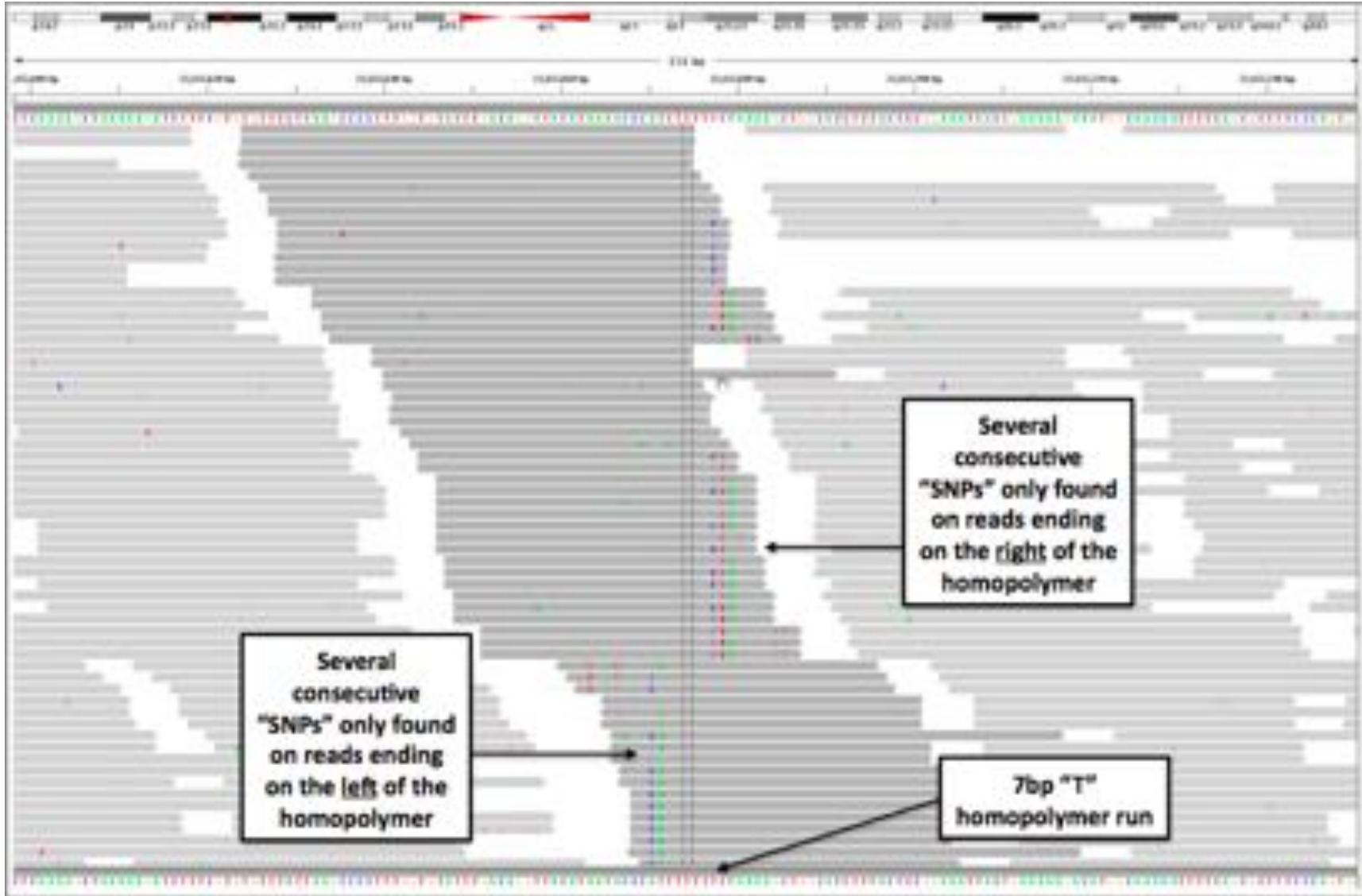
Read 1: 2 deletions

Read 2: 2 deletions

# Local realignment around indels



# Local realignment around indels



# Local realignment around indels



# Indel realignment in 2 steps

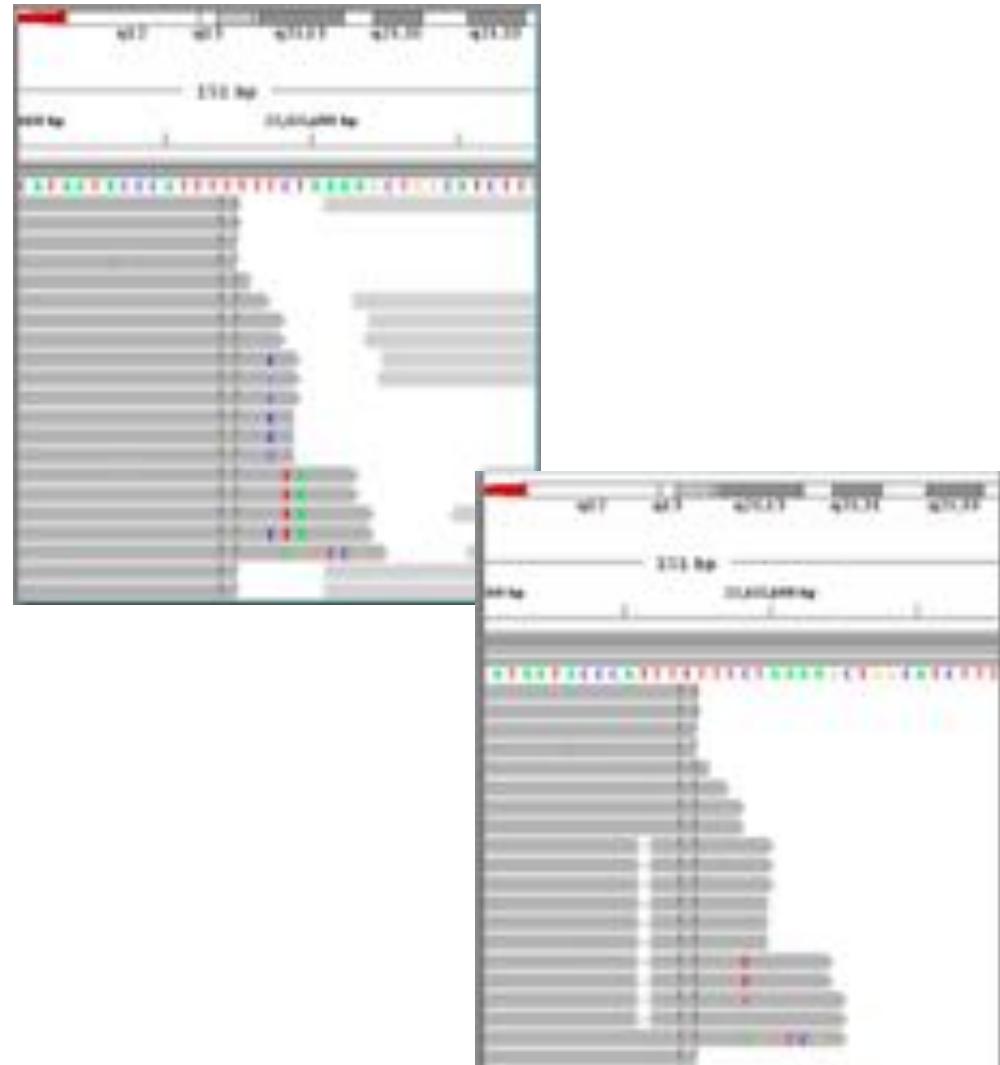
1. Identify what regions need to be realigned

- RealignerTargetCreator + known sites

Intervals  
↓

2. Perform the actual realignment (BAM output)

- IndelRealigner

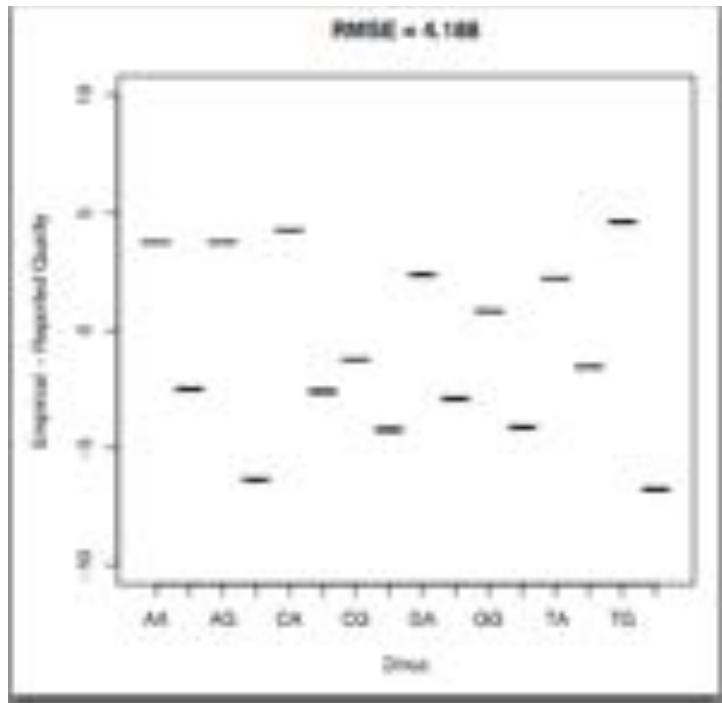


# Types of realignment targets

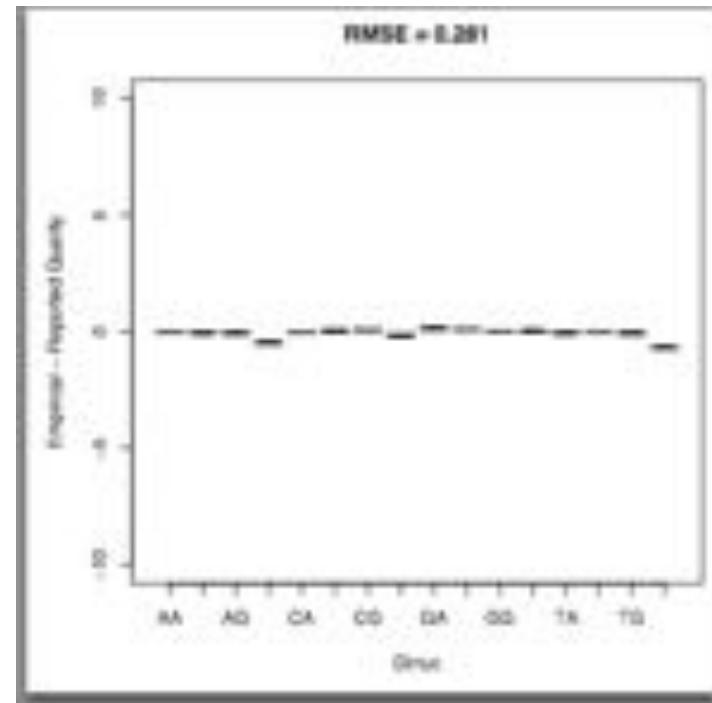
1. Indels seen in original alignments (in CIGAR, indicated by I for Insertion or D for Deletion)
2. Sites where evidences suggest a hidden indel (SNV abundance)
3. Known sites:
  - Common polymorphisms: dbSNP, 1000Genomes

# The quality scores issued by sequencers are biased

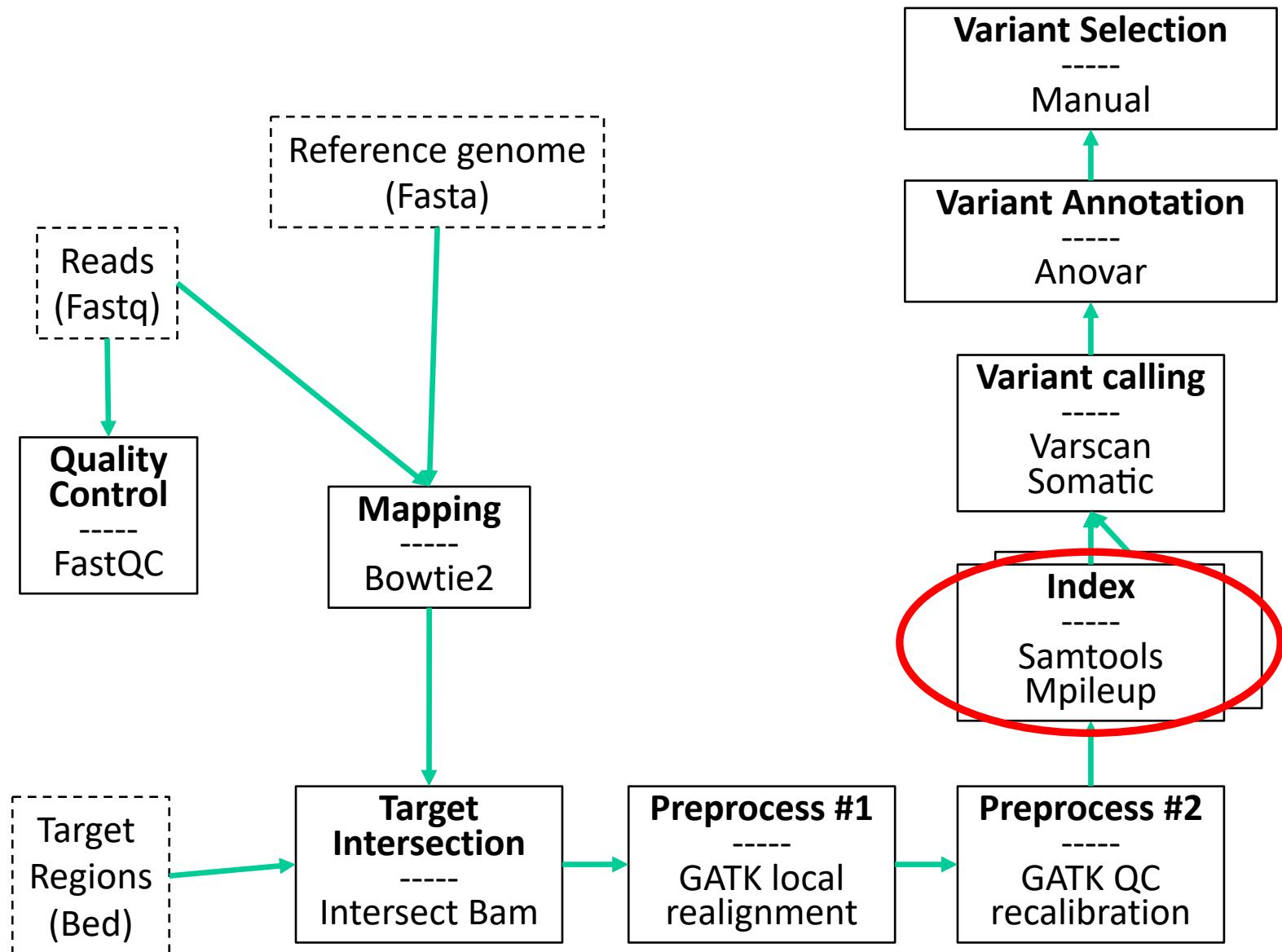
- Quality scores are critical for all downstream analysis
- Systematic biases are a major contributor to bad calls
- Example of sequence context bias in the reported qualities:



before



after



# PileUp:

# Pourquoi un nouveau format de fichier?

Rappel BAM:

```
@RG  ID:group1  SM:1425_CD34  PL:ILLUMINA  LB:lib1 PU:unit1
@PG  ID:bwa  PN:bwa  VN:0.7.12-r1039 CL:bwa mem -M -t 2 -A 2 -E 1 -R @RG\tID:group1\tSM:1425_CD34\tPL:ILLUMINA\tLB:lib1\tPU:unit1 /root/myd
ERR166338.13782800  83  chr13  32890449  60  101M  =  32890343  -207  GGGACTGAATTAGAACAAATTTCAGCGCTT
ERR166338.13782800  163  chr13  32890343  60  75M  =  32890449  207  CACTAGCCACGTTCGAGTGCTTAATGTGGCTAGTGGC
ERR166338.26716588  99  chr13  32890406  60  101M  =  32890553  222  AATGTTCCCACCTCACAGTAAGCTGTTACCGTTCCAG
ERR166338.26716588  147  chr13  32890553  60  75M  =  32890406  -222  TTGCAGACTTACCAAGCATTGGAGGAATATCGTAA
ERR166338.27259961  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.27259961  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
ERR166338.63037998  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.63037998  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
```

# Pileup format

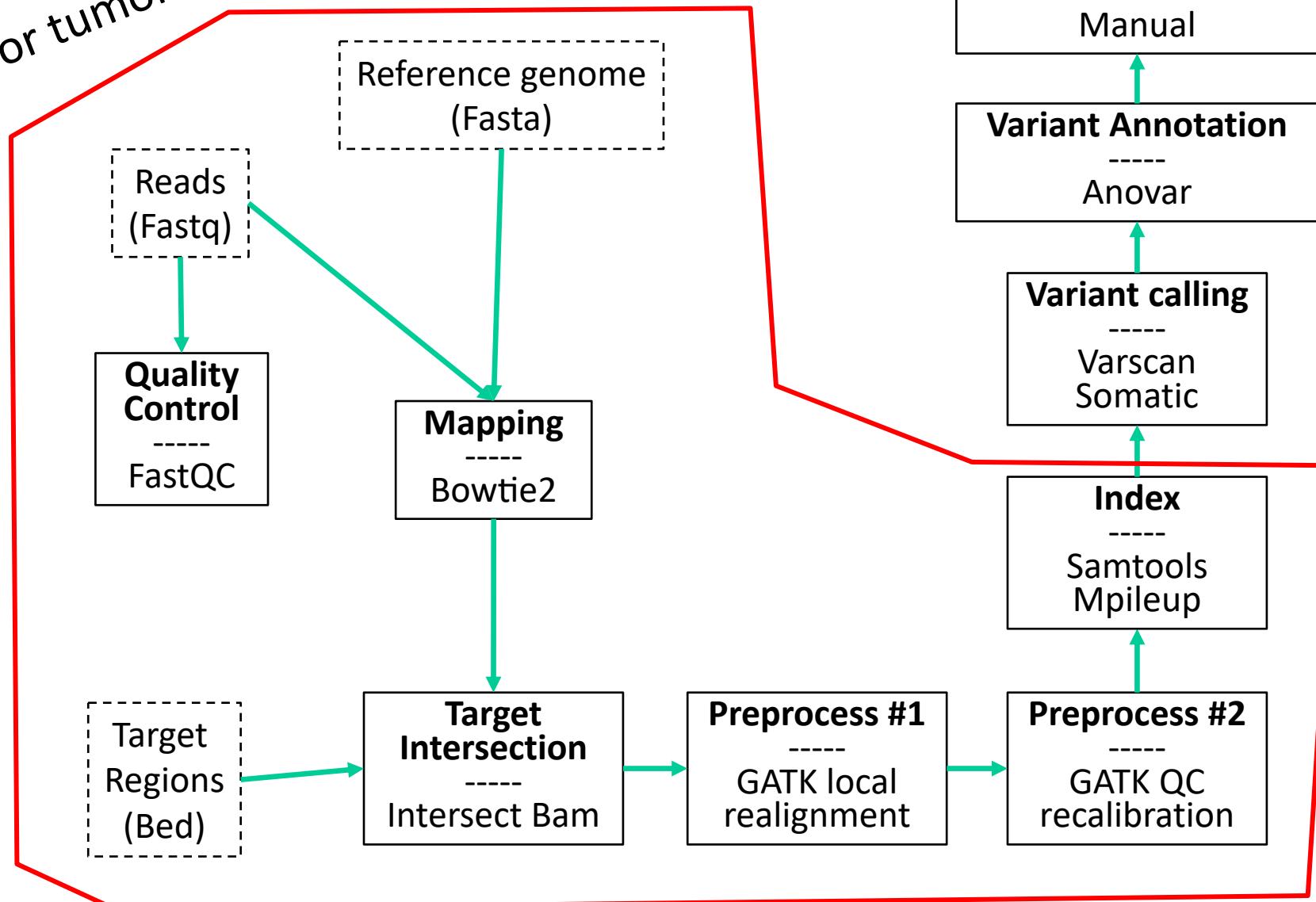
Chr11	26692809	T	65	,S,S,S.....	@C@CCFCFHNGHIGJIIJJDA7Ge@fJHGJ3WIFIJS
chr11	26692810	A	62	,S,.....	HFFCNGHHHFG<J1,2GDCH3dEe3JF3k2FH1,j3qS
chr11	26692811	C	62	,g6gg,99,999999,6,g,6,..9,99,9,..g6gg,999,..9,999,..9,..gg,6	CC>HHHHHG?JIIIJ29CGI'@e3JIIIJEElkIpI?J:IBJS
chr11	26692812	A	64	.....	CC@DHHHGD<J3JLj@CGjaIgJJIJ1J0Ij1JqS
chr11	26692813	A	62	,S,S,.....	CCCDFHHHHHb-HHHGJAFEJb6fJ1J1Jk2Hm_IoS
chr11	26692814	A	62	.....	CDFFFFFHGCHHHHHCHIUBFJJIJnJEDkFInHFS
chr11	26692815	G	61	,S,.....	C=FFFFFFH:HHHHHC?EHOGeJTLJkICCmGnEBjII1GBIIS
chr11	26692816	A	62	.....	B0FFFHD2HFHHHDADH2B9gI1JHkGEFmgjhMaFChIDG1DBIS
chr11	26692817	C	62	.....	7FFFFD0=FHHHDHC<JPGfJ3J2JjIDCkgj@CA2fID19JMS
chr11	26692818	A	68	.....	7BDDFD0?DAFC=7H EaH2H1lHDE FHnAF [I?GHJ3H95
chr11	26692819	C	63	,S,.....	?gCCCFDBFFFEPD0FH3HgJ3J1JmJ0JmJyJn999S
chr11	26692820	A	62	,S,.....	@C@CCD?FFFDFD?BH1G1J2GJmJ0J1iJnDHkckJFH:IDJJS
chr11	26692821	T	61	,S,S,S	C@CCFDFFFBCACDFnHFHUJ2JjICHUhInEE2JLJS
chr11	26692822	A	58	,S,.....	BC?FFF:FCDDFNHfHJ3J1J3C1JhJn9A=JJEJEI:JJJFIIIS
chr11	26692823	T	57	.....	C;CCCCFEDDFcFFHJ3JkJDmJjJnCHgjEJFJ<J2GDHHS
chr11	26692824	T	57	,S,S,.....	C8C@C@CCB0DgBHHHIJk1EBmJjM9E<J>G?J0HIIJIIIFIS
chr11	26692825	A	55	,S,S,S	BgggCCg0FdFjHHHJ3KJFFnLJ@HGH1J3D@GJ1JJHJgJJJS
chr11	26692826	G	52	,S,S,.....	@BA7g8]D1FH0HjIggnLJnnHfgfJ3C3gJFHJ3GJBIJ3JH97F:EEFJHS
chr11	26692827	C	58	,S,S,.....	9?7@JB1FHfH1uHCEmJjgIBAeI<JB1ADJ3JH6J3JHHEC>FEFcJC
chr11	26692828	A	48	,S,S,.....	?g^D1FHGH1HDfInJjUHg<J3H0HJUDFIJ1EJ1J3JHHEE;FCFnJg
chr11	26692829	A	46	.....	g81FFHH1HDH1kJmCFT1J<JF3A1CJ1JFJ1J3JHEEH;DDFnJ>
chr11	26692830	G	46	.....	G;HFFFH1HD0kkJ14HAcJ3J8JH1HJ1J1J3J3JGFEF?ADEmJC
chr11	26692831	A	46	,S,S,.....	I7kCF0FFGDH1mJmFG9hJF3gJ0JIEHIIJ3JGHPHEFAEnJE
chr11	26692832	T	46	.....	eCFDFFFDHeeJj<FigJA3gJFIEJ>EIGI33333J@HAAECmJED
chr11	26692833	A	46	,S,S,..,S	hCFDFFFDGmJkCDH1J2J8FGJHIC1J3J1J1J1Gc=@HmJCD0
chr11	26692834	T	43	.....	CDFFFDrnHn3AGLJ9I:IBIGJEJ0J1J3JHGG=HHHmJEB0
chr11	26692835	C	45	.....	CCFFg0mneGLFE<gJ>ICJ>JF3J3H0J1J1J661E3E6H0HnJCAD
chr11	26692836	C	45	,S,.....	CBCCFD@nnnIk<@EbjCJ7IEJ1JFIDgJgJJJDGDg@C1HnJgCD
chr11	26692837	T	44	,S,.....	@CCDFmneHnD>0hJ<I:FB3J3JFJ3J3J>GE=:I@J3mJCCC
chr11	26692838	T	42	,S,.....	CCDFmneH4FAeJ:H7JGJ1J3T1J1J1C166HDJInJFDC
chr11	26692839	G	42	,S,.....	CDg1JFm?PAkJF3?J3H3J3J1J3J3J3GGICEEGJFnJFDD
chr11	26692840	A	41	,S,.....	CBm1Fm=A7kJBJ@IJJJJGJ1JJJC1G0HAAH@JImJDEE
chr11	26692841	T	48	,S,.....	8H1FnDFOKJCGIGHIII6JGJ1J1H6D0HFCEGJ1LJ800
chr11	26692842	G	39	,S,.....	1kfjAD=j12IAGFHG1HfJ1H1J6Gifg@=FIIGnJADD
chr11	26692843	T	39	.....	UDkDD=HgQHAEHAFGHCfHNFfICGF7g#FHGFmI=CC
chr11	26692844	A	36	.....	J@1D8dFG2FFBEE6EcI0GIII=F67.BFICKIAAC
chr11	26692845	C	39	.....	HC]=4JH2J3J3J3J3J3J3J3J3J3H0HBCJ3J3J3HEE
chr11	26692846	T	38	,S,S,.....	K817DdH:G<IIGIIIGGEJ3J3J3JGGJHHEAJ1nJH0D
chr11	26692847	C	38	,S,S,.....	J@J8fHDH=J3J3J3J3J3J3J3J3J3J3HJ3J3J3J3J3J3J3CE
chr11	26692848	C	38	.....	81dHD0DJ3J3J3HJ3J3J3J3J3J3HJ3J3J3J3J3J3J3EEC
chr11	26692849	A	39	,S,S,.....	?1ffBFPGJF3J3M3J3M3J3D3J3J3HfDGJ1qJmCEEC
chr11	26692850	A	37	.....	JFDHFHH@IIJF3IC3J3J3J3G6IIICBCJJqJG7FEA
chr11	26692851	A	37	.....	efBFCHH@IJ3J3J3J3J3G6HID=C=J1qJG=FFD

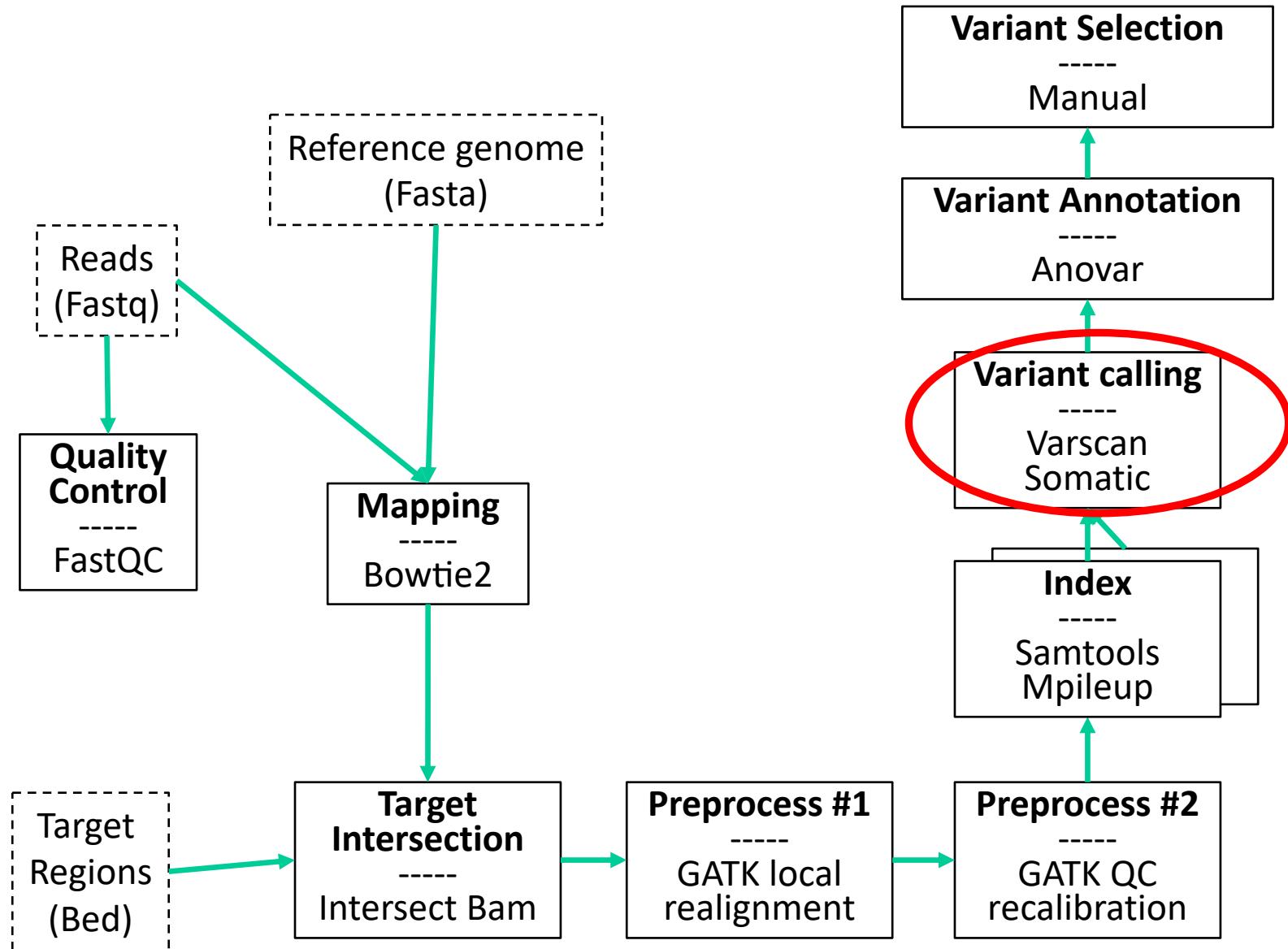
# Pileup format

Describes base-pair information at each position

		Reference base	Base qualities
chr12	112888238	A 108	=4 =??????@??@@@ @=@ ??@ ? @? ? ??< ? ??@??????? ? @??? ? ??@?? A???@ @ @@@???AB????= ? @ @@??@ @?@ A 00
chr12	112888239	C 108	.\$t,,,..T,tT.,,T.,..t.tTtt.tTT,t.T,tTt.tT,T.,.t TtTttt.,,Tt.ttt.,T,..tT,,T,T.,tT,,t,TttTtT,T. 936 78??6??6 45<875? ??? ?@6 @6????<?=6 66= ? ??? 6=7??=???<8@7=7=? ,tt,,T,T,tttt^F.^F, 77?7?8 ??78?????? 8 <8??88 9?8 ?0048
Number of reads covering the site (total depth)		Read bases:	. / , = match on forward/reverse strand ACGTN / acgtn = mismatch on forward/reverse strand `-\+[0-9]+\[ACGTNacgtn]+\` indicates an indel

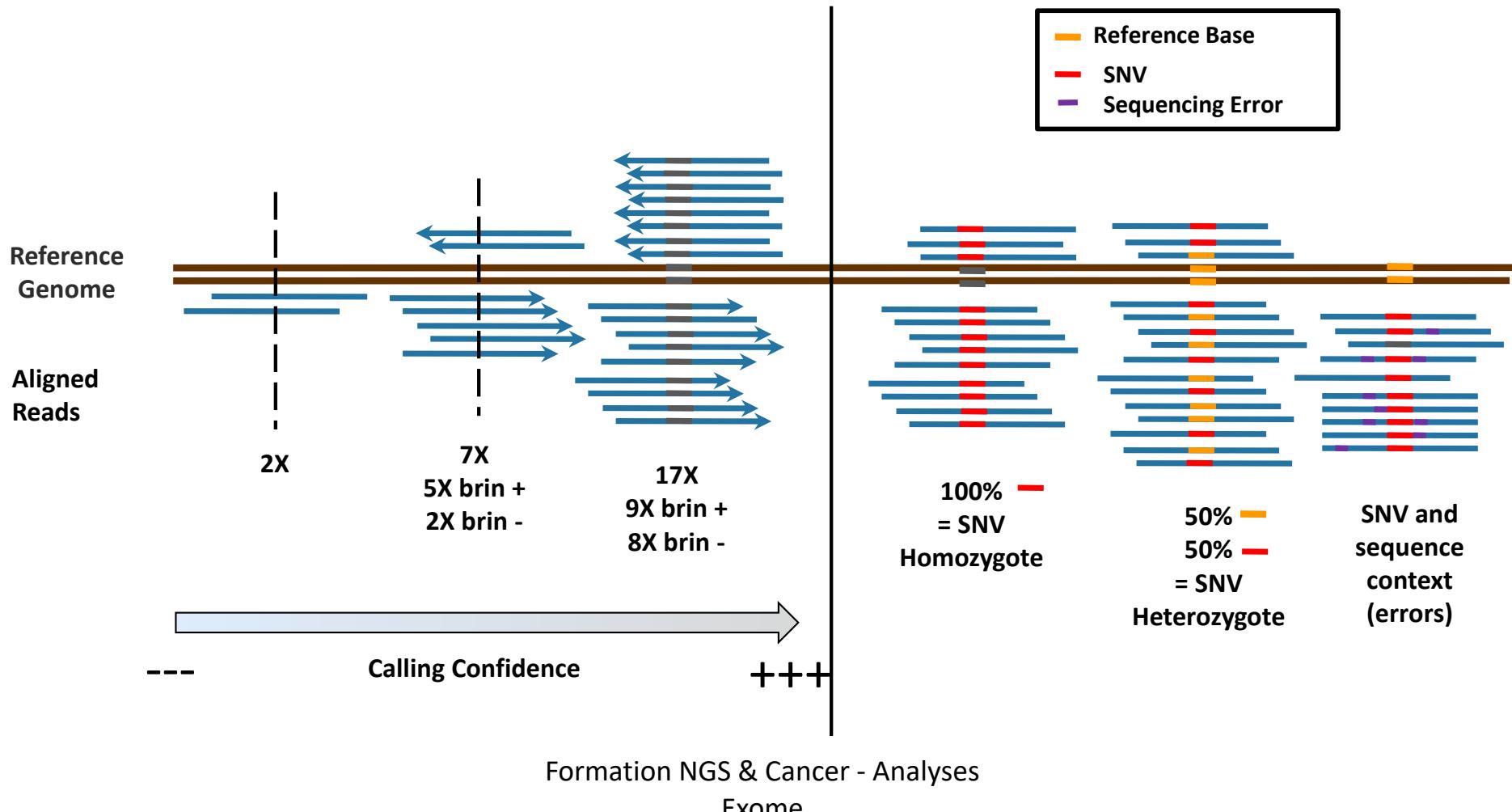
X2 for tumor & normal





# Variant calling criteria

Depth of Coverage = number of reads supporting one position  
ex: 1X, 5X, 100X... >1000X



# Factors to consider for Variant Calling

- Calling a SNV:
  - Base call qualities of each supporting base (base quality)
  - Proximity to small indels, or homopolymer run
  - Mapping qualities of the reads supporting the SNP
  - Sequencing **depth**: >=30x for constit ; >=100 for tumor
  - SNVs position within the reads: Higher error rate at the reads ends
  - Look at strand bias (SNVs supported by only one strand are more likely to be artifactual)
  - **Allelic frequency**: Tumor cellularity will reduce the % of an heterozygous variant
- Calling an indel:
  - Higher stringency (and Sanger validation often needed)

# VarScan2

- Mutation caller written in **Java** (portable)
- Works with **Pileup files** of Targeted, Exome, and Whole-Genome sequencing data (DNAseq or RNAseq)
- **Multi-platforms:** Illumina, SOLiD, Life/PGM, Roche/454
- Germline mode:
  - Variants in individual samples
  - Multi-sample variants **shared or private** in multi-sample datasets
- Somatic Mode: **Tumor/Normal pairs:**
  - Somatic, germline, LOH events
  - Somatic copy number alterations (CNAs)

# VarScan2 Performance

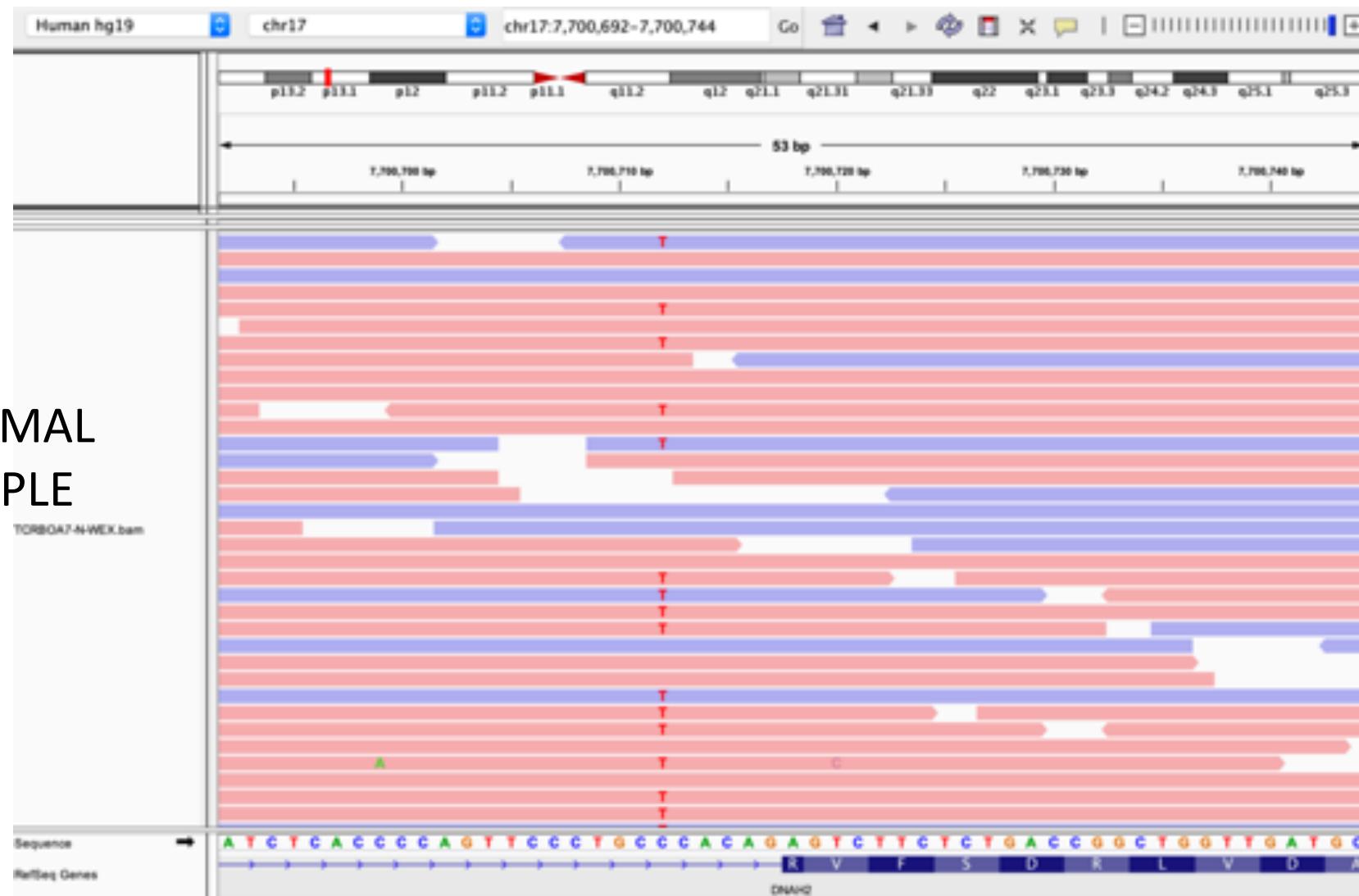
- VarScan uses a robust **heuristic/statistic** approach to call variants that meet desired thresholds for read depth, base quality, variant allele frequency, and statistical significance
- Stead *et al.* (2013) compared 3 different **somatic callers** : MuTect, Strelka, VarScan2
  - **VarScan2 performed best** overall with sequencing depths of 100x, 250x, 500x and 1000x required to accurately identify variants present at 10%, 5%, 2.5% and 1% respectively
- Other widely used tool: **GATK**

# Somatic variant calling

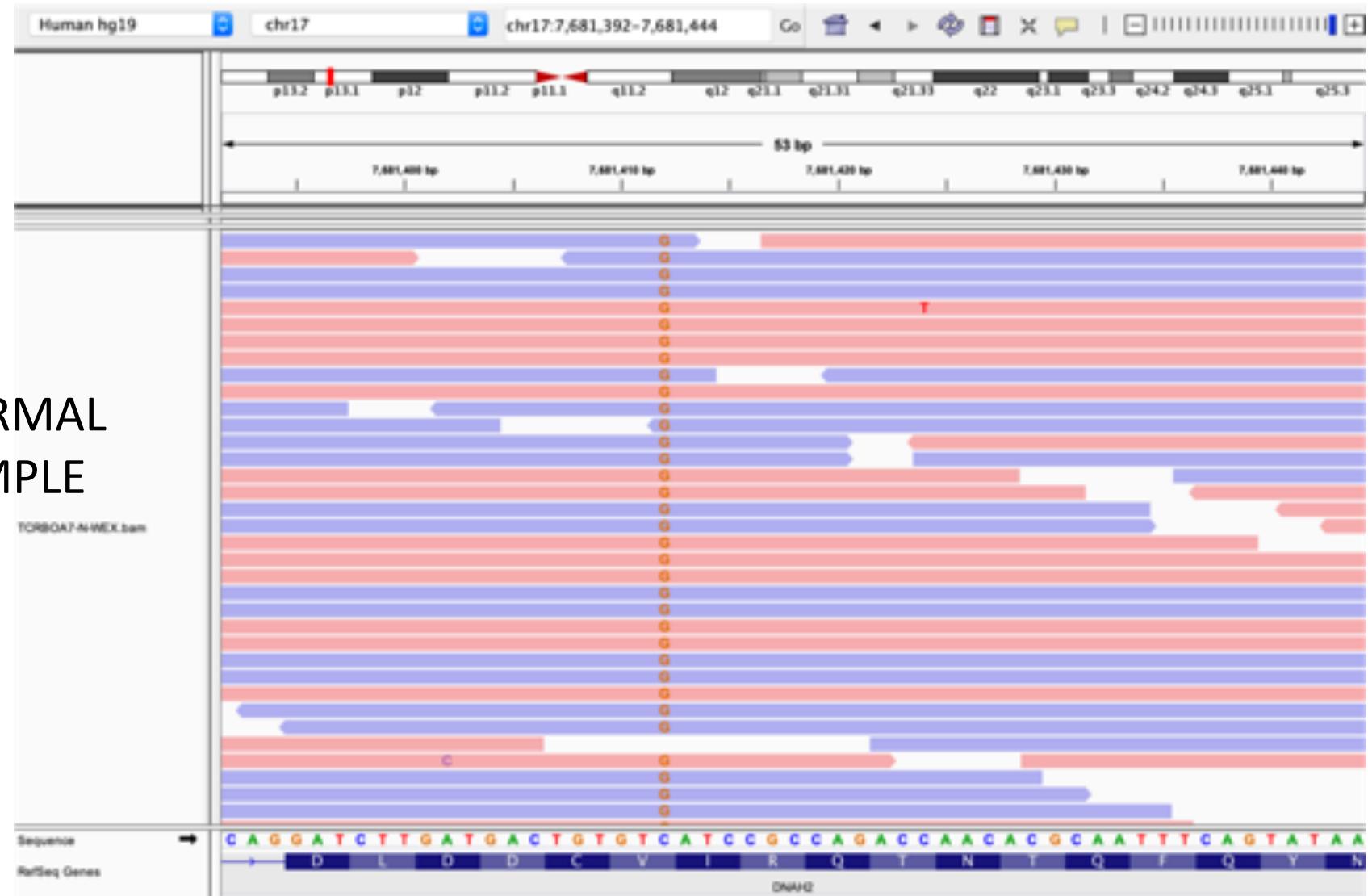
# Fréquence/fraction allélique

- Termes
  - Germline/population: Minor Allele frequency (**MAF**). Par ex. dans données 1000Genomes.
  - Somatic: Variant Allele frequency (**VAF**) ou B-Allele Frequency (**BAF**) (or Allelic Fraction)
- Où trouver l'info?
  - Colonne info#AF dans VCF

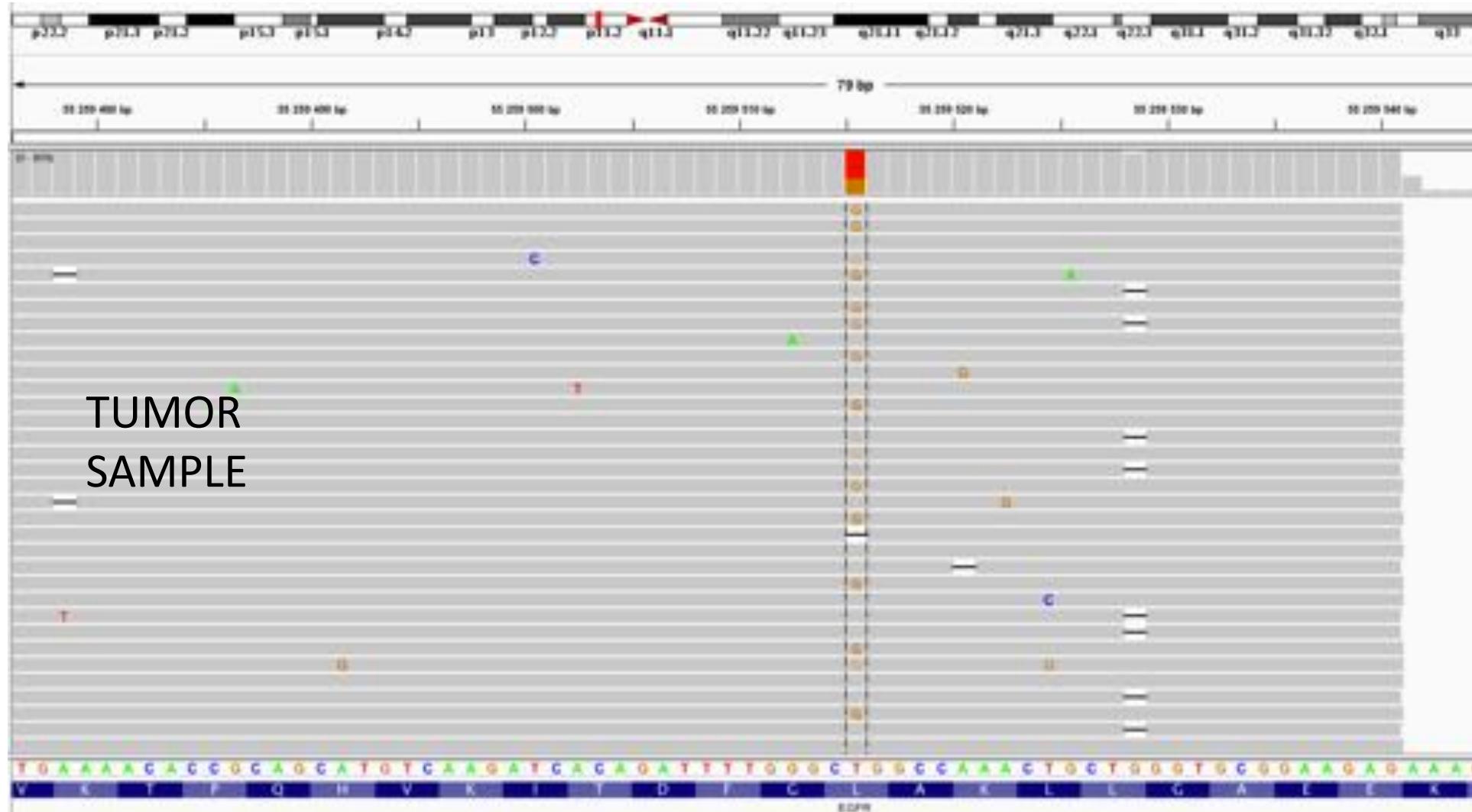
# Un polymorphisme (SNP) hétérozygote



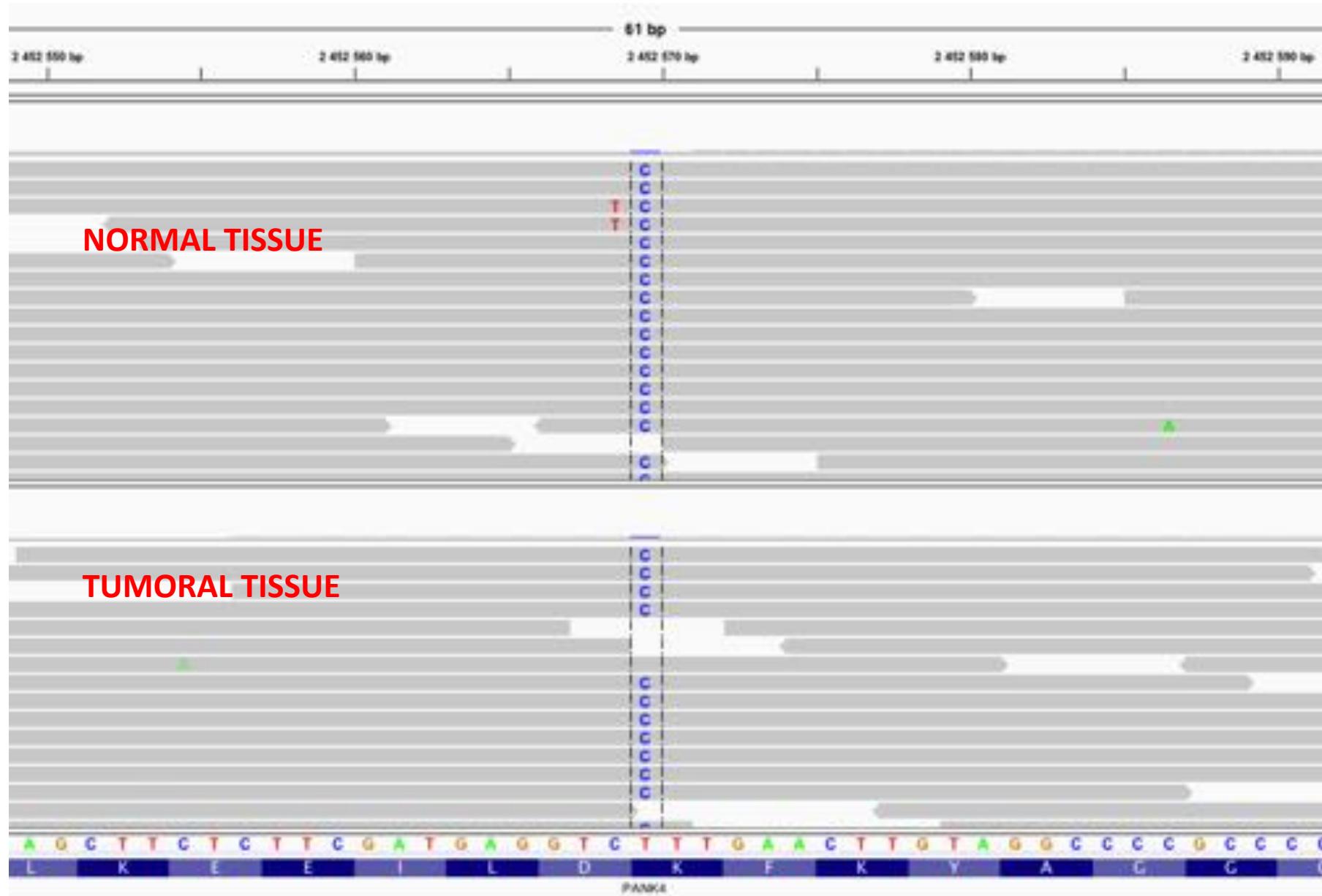
# Un polymorphisme (SNP) homozygote



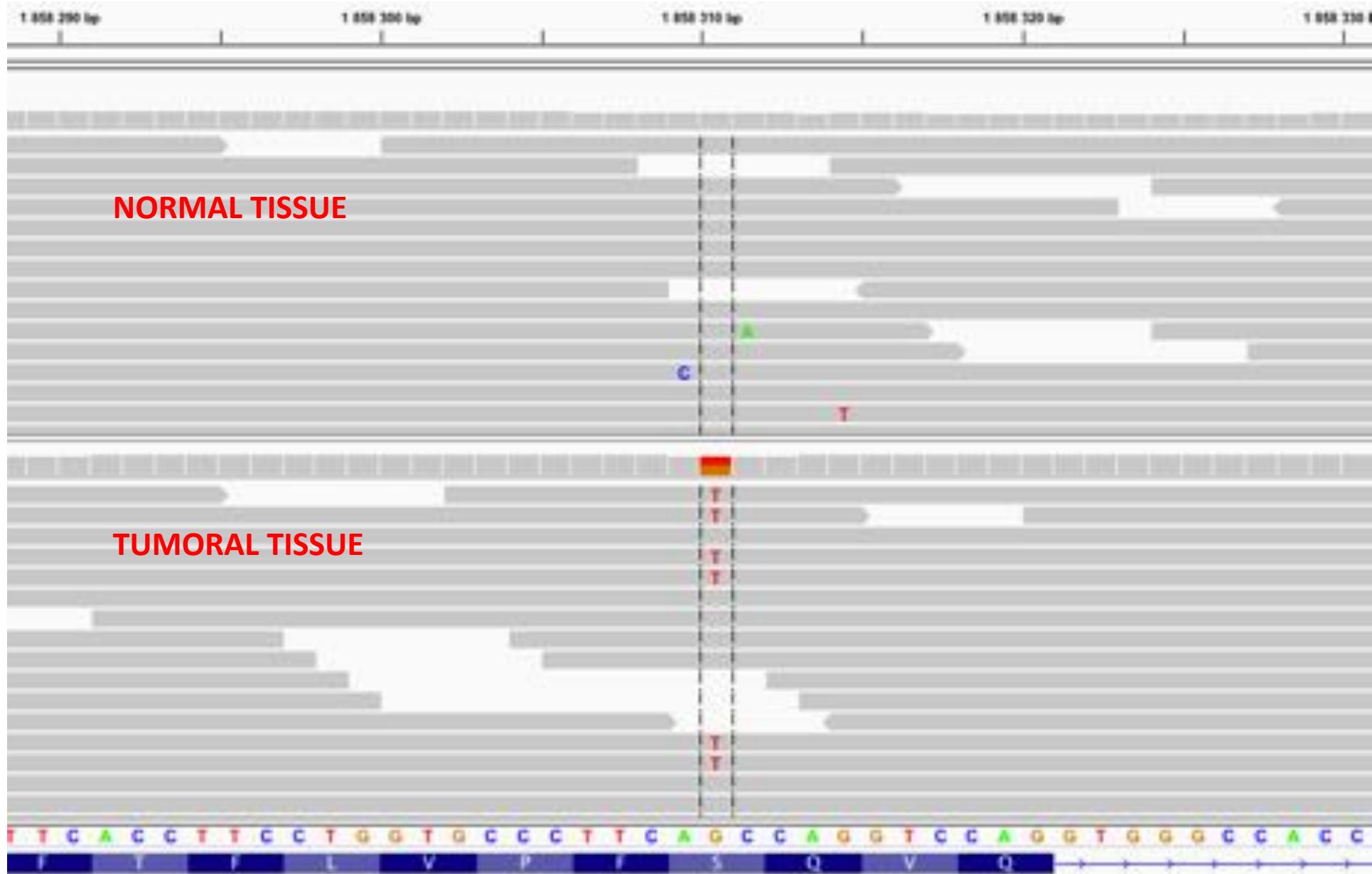
# Un variant tumoral: polymorphisme ou mutation?



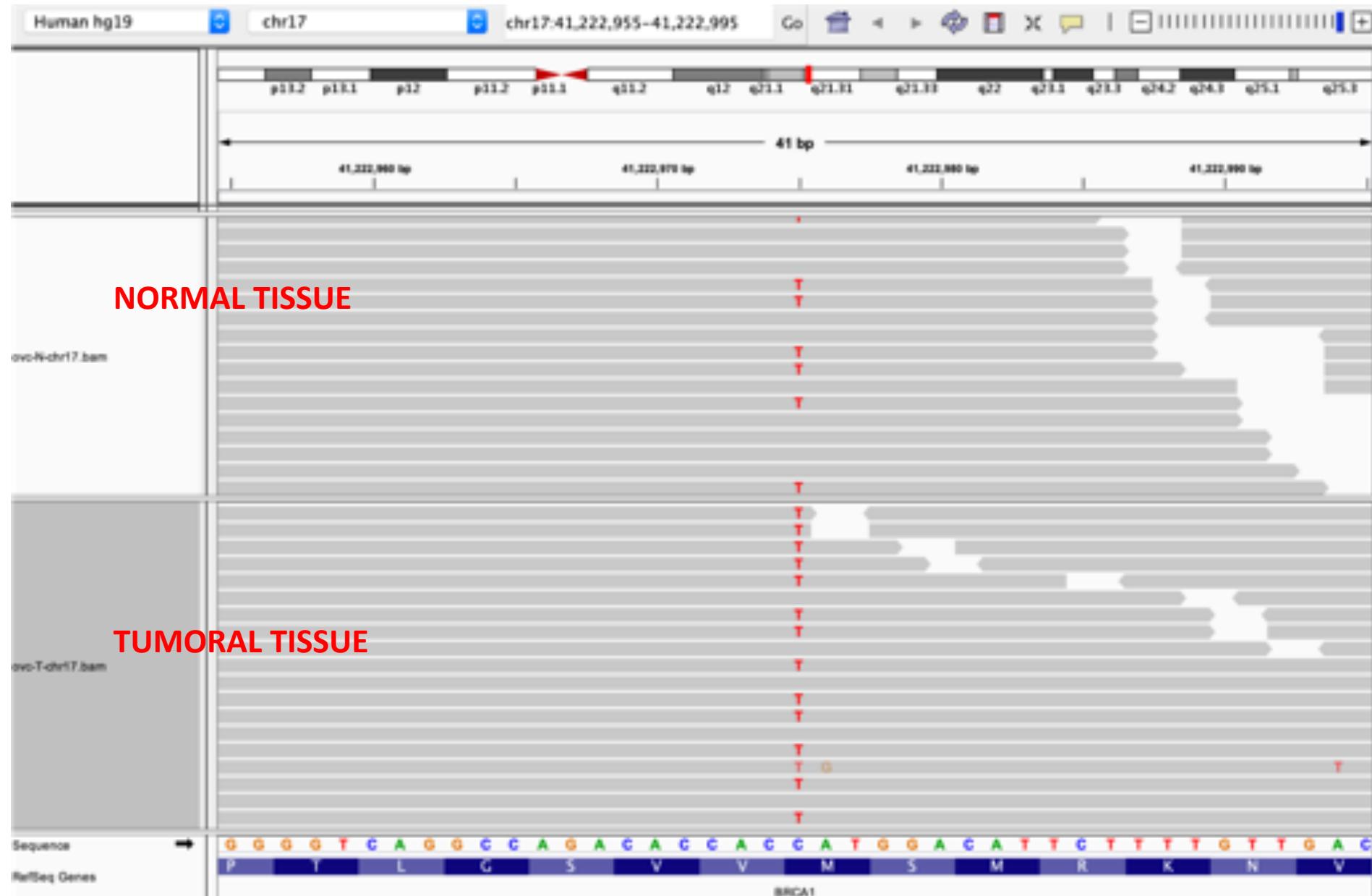
# Un polymorphisme vu dans N et T



# Une mutation somatique



# Une LOH (loss of heterozygosity)



# Varscan's Somatic P-value

## Variant Calling and Comparison

At every position where both normal and tumor have sufficient coverage, a comparison is made. First, normal and tumor are called independently using the germline consensus calling functionality. Then, their genotypes are compared by the following algorithm:

Calculate significance of allele frequency difference by Fisher's Exact Test

**If difference is significant (p-value < threshold):**

If normal matches reference

==> Call Somatic

Else If normal is heterozygous

==> Call LOH

Else normal and tumor are variant, but different

==> Call IndelFilter or Unknown

**If difference is not significant:**

==> Call Germline

The diagram illustrates the logic for determining if a variant is somatic or LOH based on allele counts in normal (N) and tumor (T) samples.

**Alleles**

	Ref	Var
N	8	0
T	6	7

**Somatic Call:** A red arrow points from the table to the text "Somatic".

	Ref	Var
N	4	4
T	8	1

**LOH Call:** A red arrow points from the table to the text "LOH".

# Format VCF

```
##fileformat=VCFv4.2
##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=<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=1,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=<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
20  14370  rs6064257 G       A       29      PASS    NS=3;DP=14;AF=0.5;DB;H2
20  17330  .        T       A       3       q10    NS=3;DP=11;AF=0.017
20  1110696 rs6040355 A,G,T   67      PASS    NS=2;DP=10;AF=0.333,0.667;AA=T;DB
20  1230237 .        T       .       47      PASS    NS=3;DP=13;AA=T
20  1234567 microsat1 GTC   G,GTCT  50      PASS    NS=3;DP=9;AA=G
```

mandatory

Optional header: meta-data about available annotation

samples

FORMAT	NA00001	NA00002	NA00003
GT:GQ:DP:HQ	0 0:48:1:51,51	1 0:48:8:51,51	1/1:43:5:...
GT:GQ:DP:HQ	0 0:49:3:58,50	0 1:3:5:65,3	0/0:41:3
GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2	2/2:36:4
GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:51,51	0/0:61:2
GT:GQ:DP	0/1:35:4	0/2:17:2	1/1:40:3

deletion

Insertion  
(2 events here)

NS: number of samples with data  
DP: combined depth  
AF: allelic fraction  
AA: ancestral allele

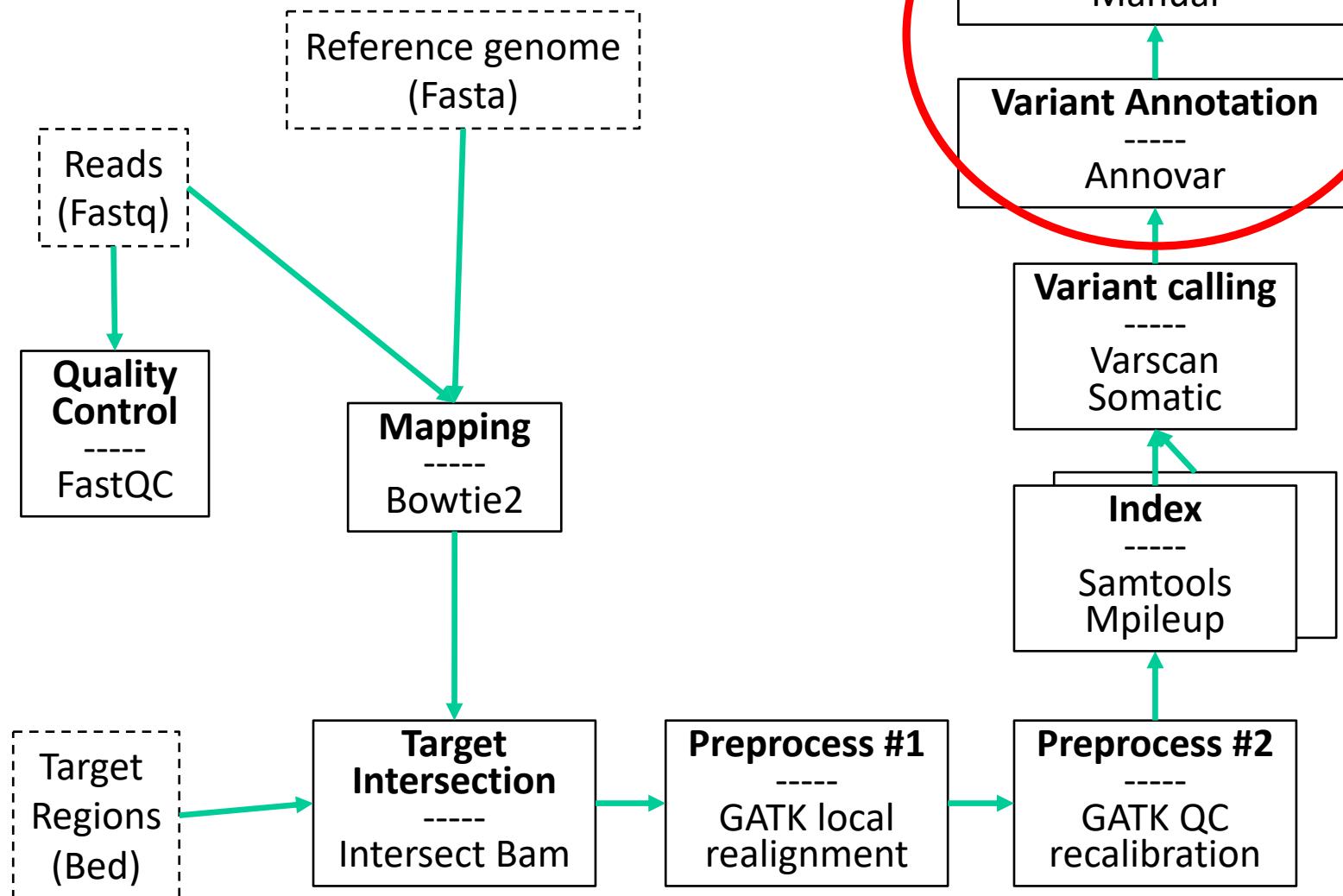
GT: genotype (0=ref, 1=alt)  
GQ: genotype quality  
DP: read depth  
HQ: haplotype quality (phased samples)

# Alternative: VarScan Tabulated Format

Chrom	Position	Ref	Cons	Reads1	Reads2	VarFreq	Strands 1	Strands 2	Qual1	Qual2	Pvalue	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	113348849	C	Y	31	30	49.18%	2	2	27	27	0.98	1	1	19	12	25	5	T
chr12	113354329	G	R	72	2	2.70%	2	2	31	26	0.98	1	1	48	24	1	1	A
chr12	113357193	G	A	2	72	97.30%	1	2	28	24	0.98	1	1	2	0	45	27	A
chr12	113357209	G	A	0	77	100%	0	2	0	29	0.98	0	1	0	0	51	26	A

**Cons** : Consensus Genotype of Variant Called (IUPAC code):

M -> A or C	Y -> C or T	D -> A or G or T	W -> A or T	V -> A or C or G
R -> A or G	K -> G or T	B -> C or G or T	S -> C or G	H -> A or C or T



# Different types of SNVs

- SNVs and short indels are the most frequent events:
  - Intergenic
  - Intronic
  - *cis*-regulatory
  - splice sites
  - frameshift or not
  - synonymous or not
  - benign or damaging etc...
- Example of SNV one want to pinpoint:
  - non-synonymous + highly deleterious + somatically acquired

# Resources dedicated to human genetic variation

- dbSNP and 1000-genomes
  - Population-scale DNA polymorphisms
- COSMIC
  - Catalogue Of Somatic Mutations In Cancer
- Non synonymous SNVs predictions
  - SIFT, Polyphen2 (damaging impact)... PhyloP, GERP++ (conservation)

# Annovar

« Annovar » annotates SNVs and Indels

Takes Multi sample VCF (Tumor &+normal samples)

- RefGene: Gene & Function & AminoAcid Change (HGVS format:  
c.A155G ; p.Lys45Arg)
- 1000g2012apr\_all: Minor Allele Frequency for all ethnies
- ESP6500: Exome Sequencing Project
- Ljb\_all : predictions (**SIFT**, **Polyphen2**, LRT, MutationTaster, PhyloP, GERP++)

❖ Tabulated file

Chr	Start	End	Ref	Alt	Func.refGene	Gene.refGene	ExonicFunc.refGene	AAChange.refGene	1000g2012apr_all	snp137	cosmic68	esp6500_all	LJB_PhylоП	LJB_PhylоП_Pre
chr1	160251792	160251792	A	G	intronic		PEX19		NA		NA			
chr1	167082869	167082869	G	A	intronic		DUSP27		NA		NA			
chr1	167095163	167095163	G	C	exonic		DUSP27		nonsynonymous SNV	DUSP27:NM_001080426:exon5:c.G795C:p.E265D				
chr1	167095881	167095881	G	A	exonic		DUSP27		nonsynonymous SNV	DUSP27:NM_001080426:exon5:c.G1513A:p.A505T				
chr1	167097739	167097739	C	A	exonic		DUSP27		nonsynonymous SNV	DUSP27:NM_001080426:exon5:c.C3371A:p.T1124N				
chr1	214803969	214803969	G	C	exonic		CENPF		nonsynonymous SNV	CENPF:NM_016343:exon9:c.G1287C:p.K429N				

Formation NGS & Cancer - Analyses  
Exome

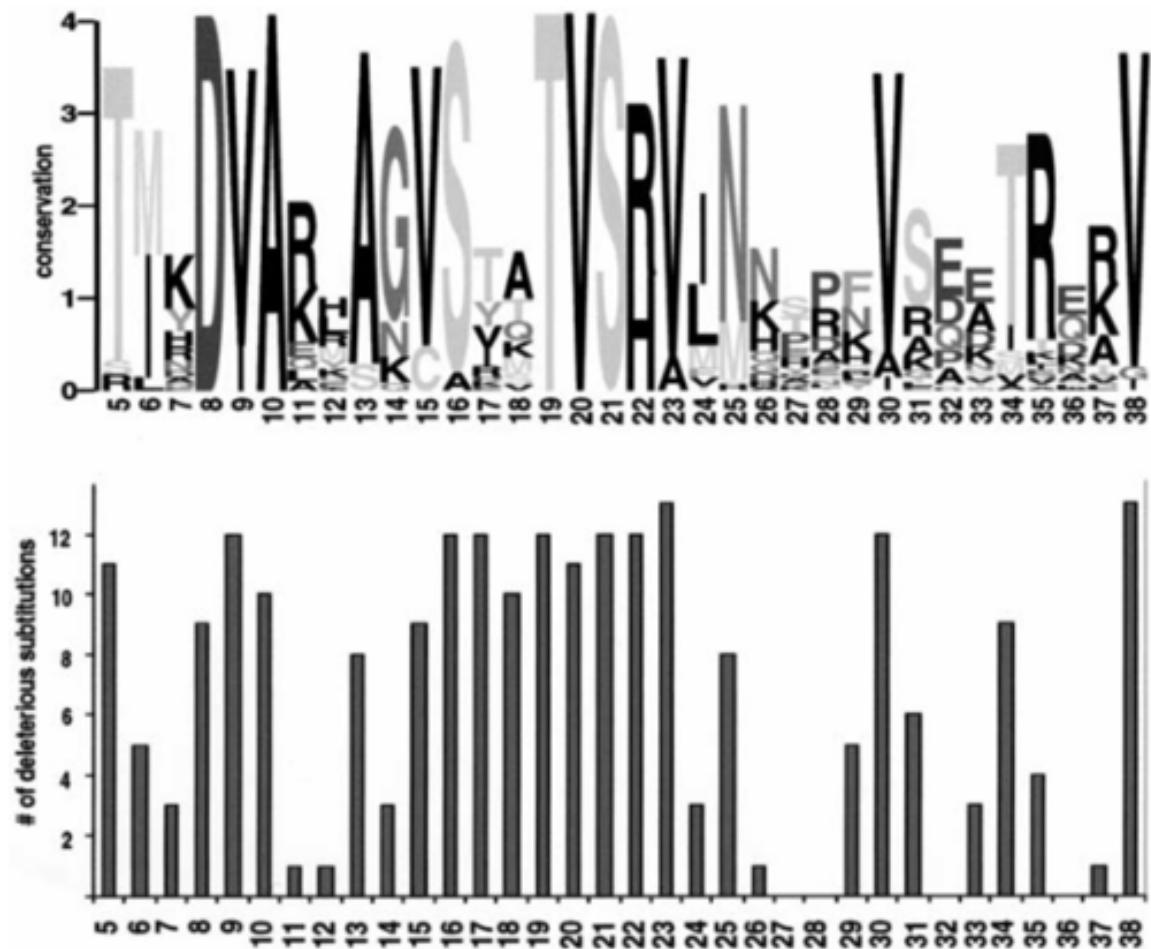


# Sorting Intolerant From Tolerant

Ng & Henikoff,  
Genome Res. 2001

Utilise la  
conservation des  
domaines protéiques  
comme indication du  
caractère délétère  
d'une substitution

Classe en  
**D**(eleterious),  
**T**(olerant),  
. (unknown)



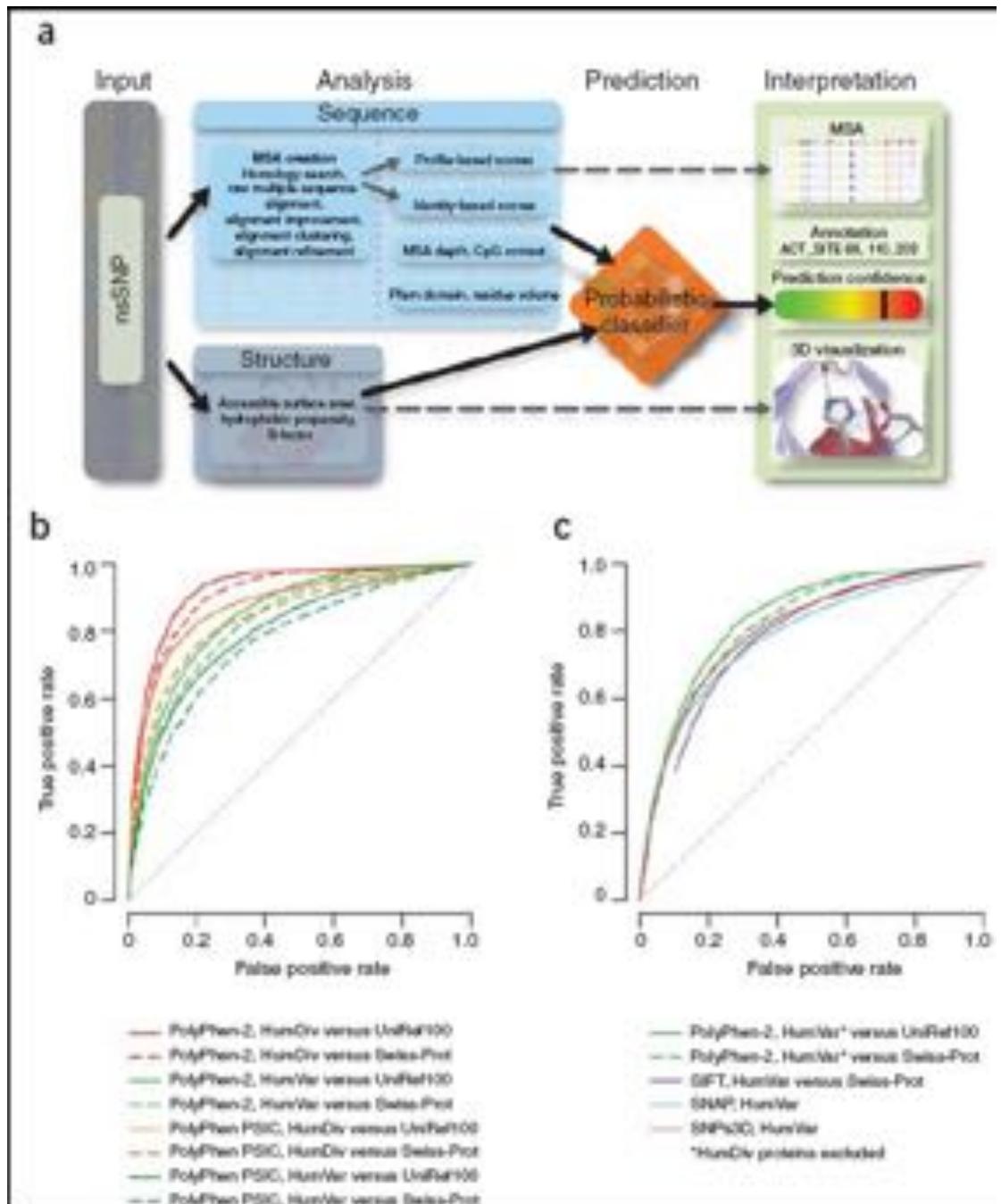
effet réel: LacI

# PolyPhen2

Adzhubei et al. *Nature Methods* 2010.

**Probabilistic classifier:**  
Estimates the probability of the missense mutation being damaging based on a combination of seq+struct properties.

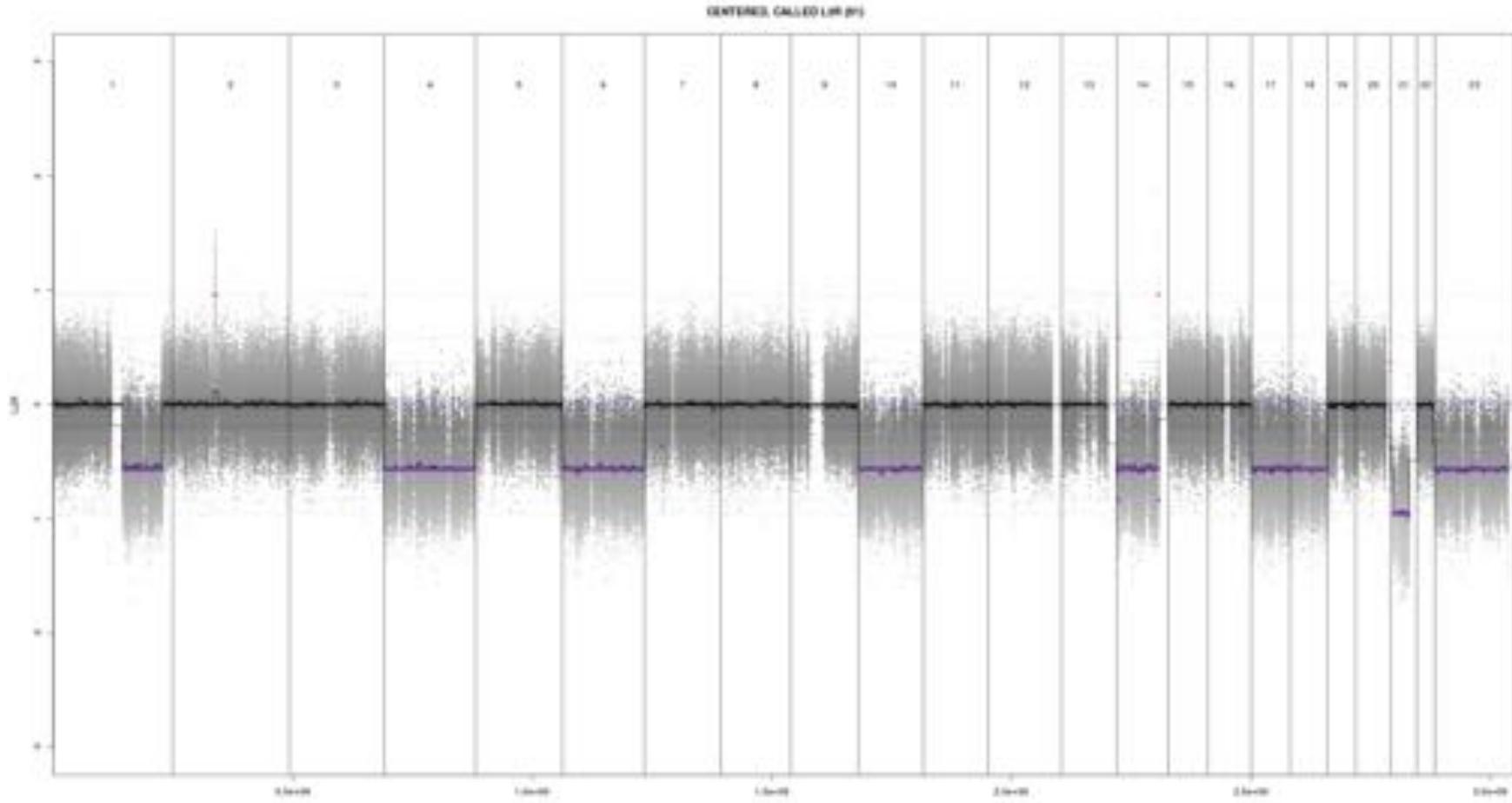
Classe en: **Benign**,  
**Possibly damaging**, or  
probably **Damaging**



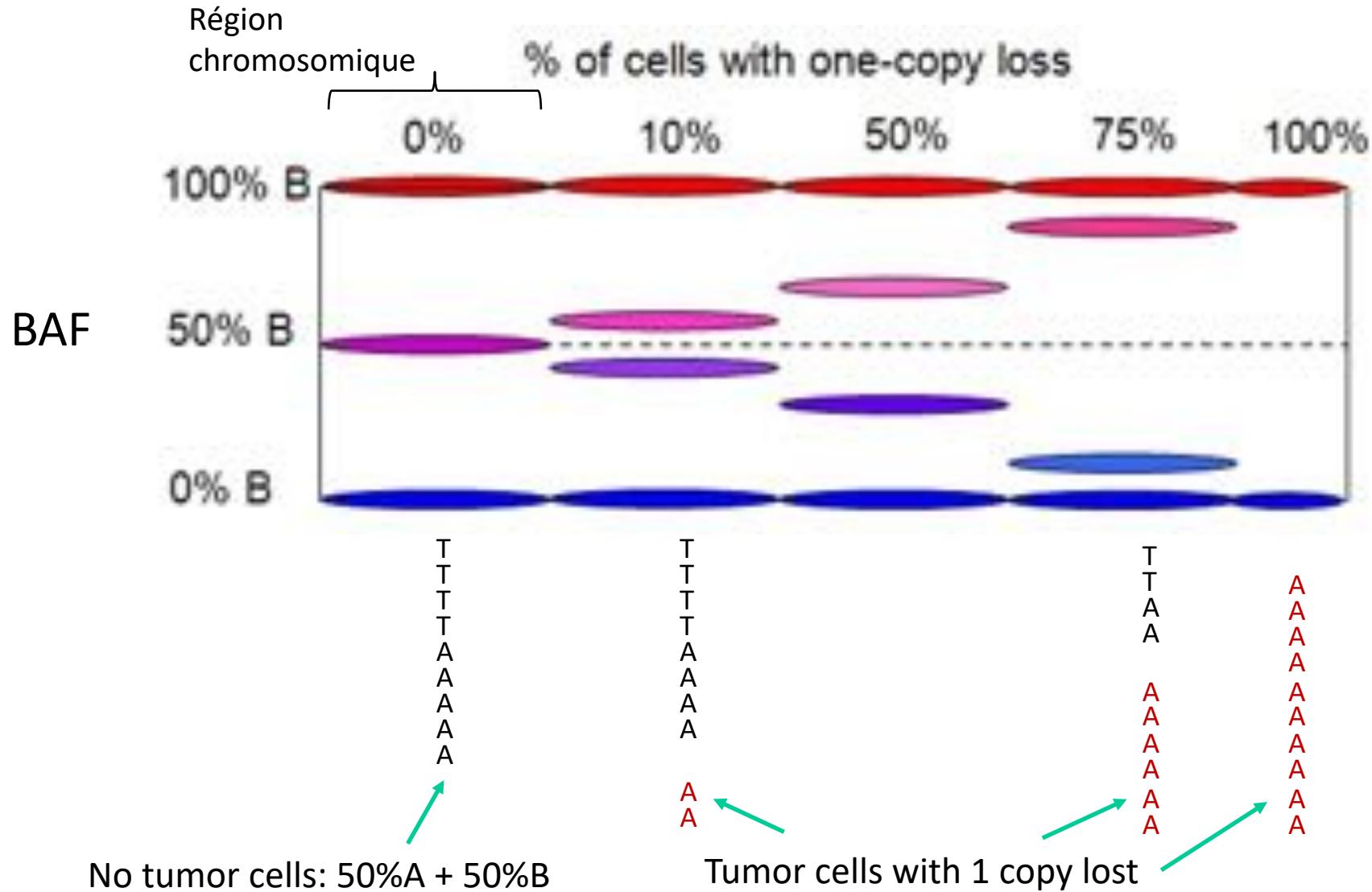
# Coverage & Allelic Frequencies For CNV detection

# Detection of copy-number variations

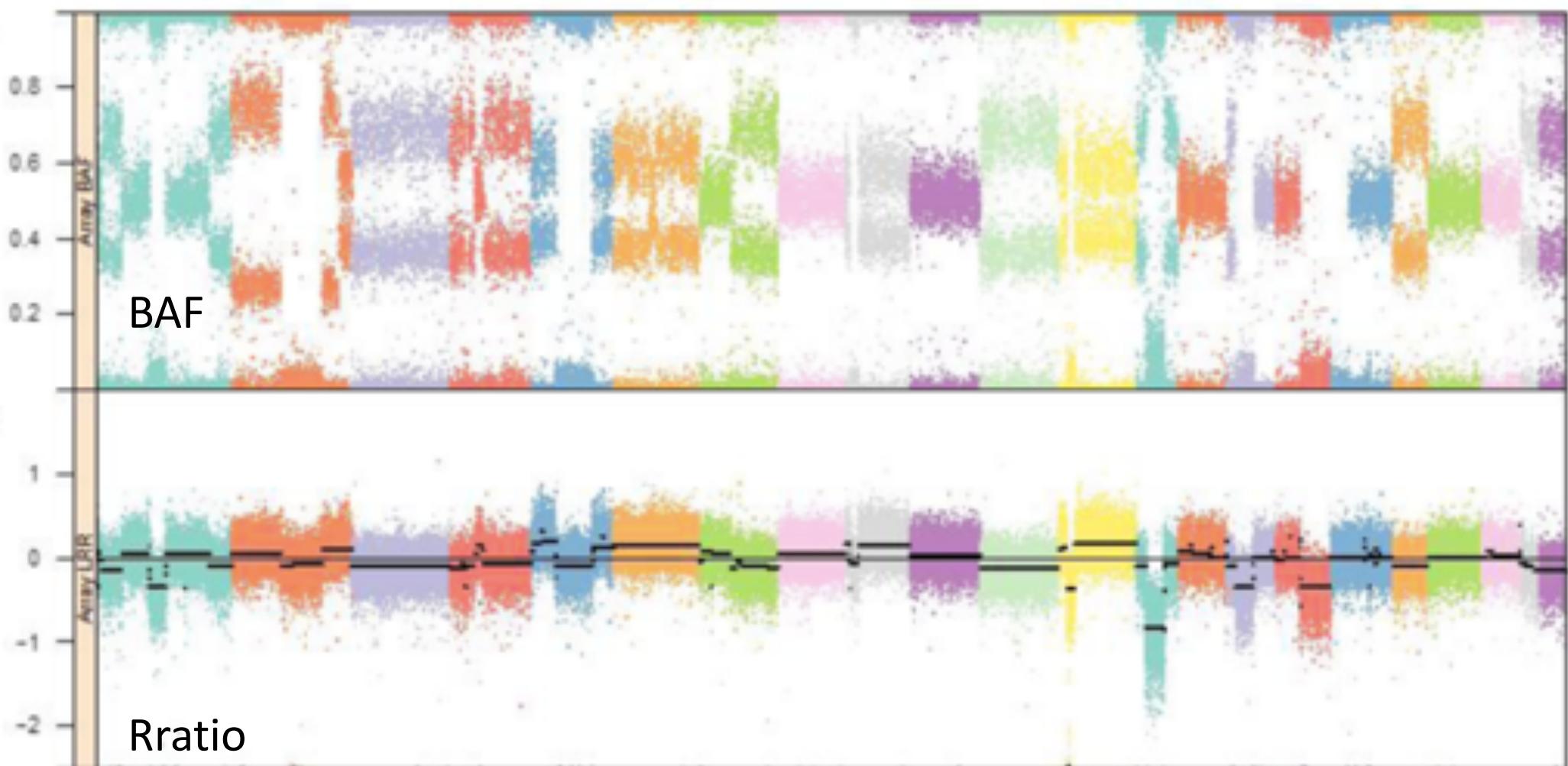
Are there any copy-number alteration (gain or loss of chromosomal regions, amplifications ...) that could explain tumorigenesis ?



# Cellularité et Fréquence Allelique



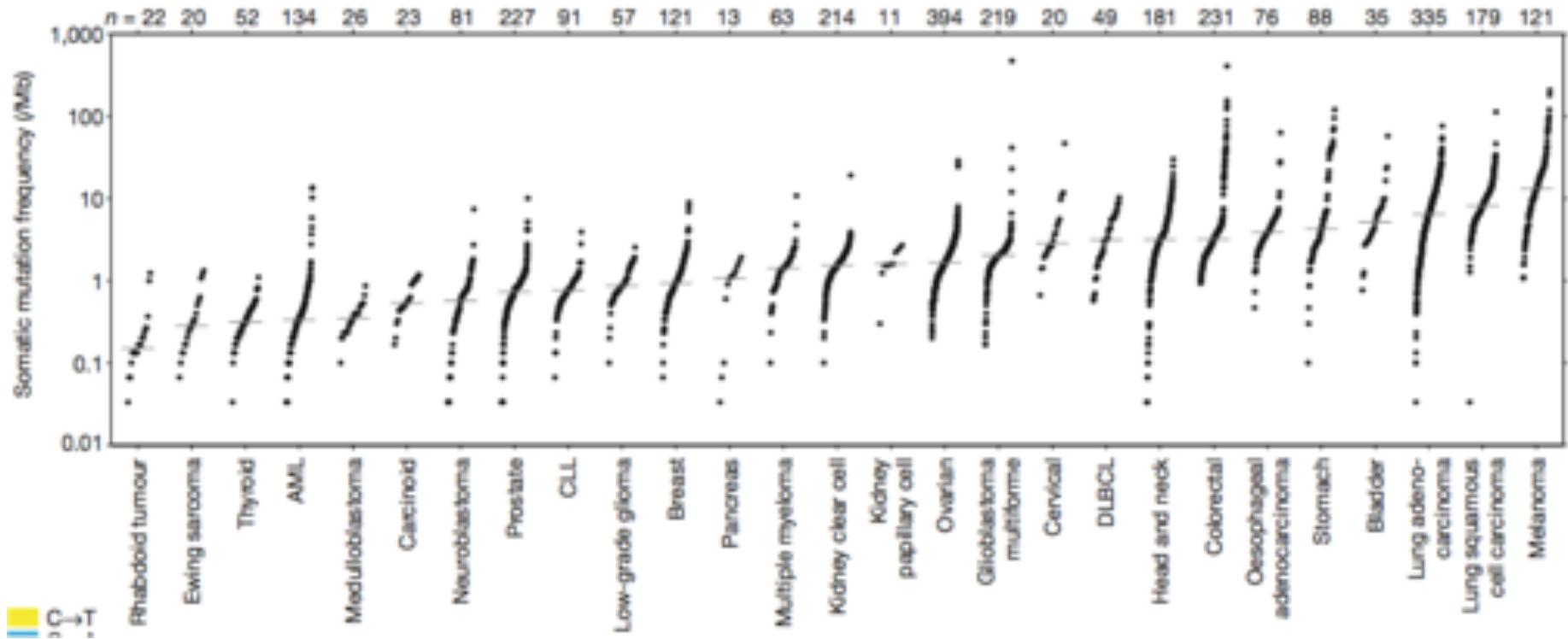
# Segmentation et fréquence allélique



R ratio=utilisé en CGH, =couverture en NGS

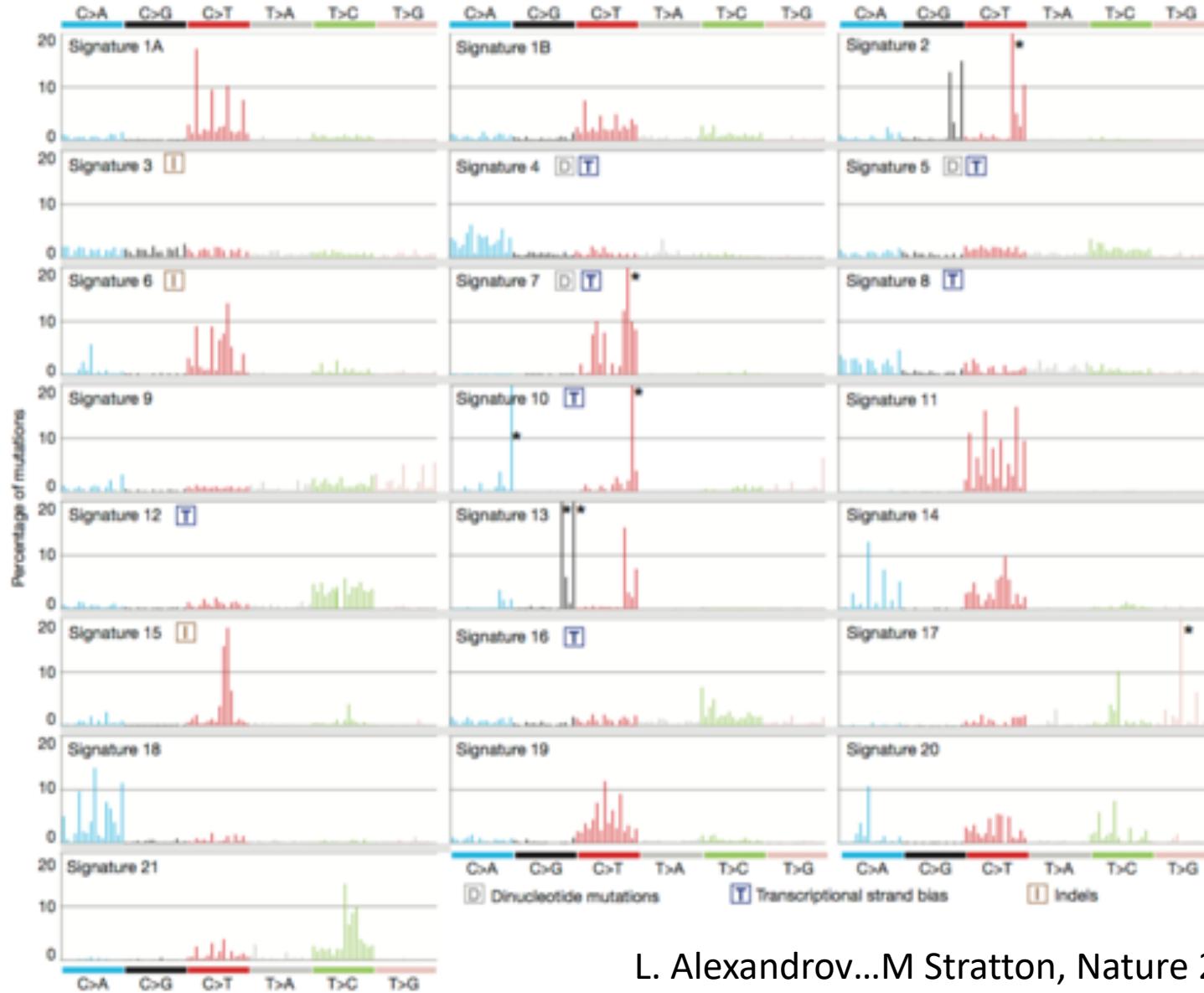
Scott et al. Gene 2014

# 2013: premières études pan-cancer WGS



Alexandrov...M Stratton, Nature 2013.  
Lawrence et al. Nature 2013.

# Les Signatures Mutationnelles



# Signatures et origine des tumeurs



# Annexes

- Formats de fichiers NGS

# Format fasta

**\*.fa , \*.fasta**

```
>identifiant1 commentaire libre
CAGCATCGATCGTCGGCGATGCATGCGGATGCTAGCTGATCACGATGC
CGCATGCTAGTCAGGCAGGGATATTATTAGCAGGTATCGGATGA
CAGCATTACGGCGGGAGTGCTATTATTATGAGCGCGAT
>identifiant2 commentaire libre
CAGGCAGGTTCTTATTATCGGCGGGCGGAGGCAGGATGCATC
CAGTGCAGTACGCTAGTCAGCGATGCATTATGACTGACTCAGTTT
CCCGCTAGCTATGCTATGCTATTGATCGATTGAGCTGATCTGGC
CAGCTATGCTTAGTA
```

# Format fastq

Descriptif du read (position sur la piste de séquençage, taille,...)

```
@SRR001666.1 071112_SLXA-EAS1_s_7:5:1:817:345 length=36
GGGTGATGGCCGCTGCCGATGGCGTCAAATCCCACC
+SRR001666.1 071112_SLXA-EAS1_s_7:5:1:817:345 length=36
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII9IG9IC
```

Qualité (probabilité que la base soit correcte) encodé par code ASCII

# Qualité dans le format fastq

$$\text{Qualité} = -10 \log_{10}(P_{\text{erreur}})$$

@SEQ\_ID1

GATTTGGGGT

+  
42,40,134,47,36 35 40 40 40



@SEQ\_ID1

GATTTGGGGT

+  
\*(ä/\$#((((



0	32	64	B	96	*	128	C	160	4	192	L	224	6
1	0	33	*	65	A	97	a	129	0	161	;	193	A
2	0	34	"	66	B	98	b	130	6	162	6	194	T
3	*	35	#	67	C	99	c	131	3	163	0	195	—
4	*	36	\$	68	D	100	d	132	4	164	8	196	—
5	*	37	X	69	E	101	e	133	2	165	R	197	+
6	*	38	B	70	F	102	F	134	8	166	9	198	W
7		39	*	71	G	103	g	135	6	167	8	199	A
8		40	<	72	H	104	h	136	2	168	L	200	E
9		41	>	73	I	105	i	137	8	169	0	201	U
10		42	*	74	J	106	j	138	4	170	+	202	0
11	6	43	*	75	K	107	k	139	1	171	%	203	U
12	9	44	-	76	L	108	l	140	1	172	8	204	2
13		45	-	77	M	109	m	141	1	173	4	205	=
14	R	46	-	78	N	110	n	142	8	174	c	206	U
15	*	47	/	79	O	111	o	143	8	175	0	207	*
16	▶	48	8	80	P	112	p	144	6	176	■	208	3
17	4	49	1	81	Q	113	q	145	8	177	■	209	0
18	*	50	2	82	R	114	r	146	8	178	■	210	E
19	!!	51	3	83	S	115	s	147	8	179	—	211	E
20	9	52	4	84	T	116	t	148	8	180	—	212	E
21	8	53	5	85	U	117	u	149	8	181	6	213	+
22	*	54	6	86	V	118	v	150	0	182	8	214	—
23		55	7	87	W	119	w	151	0	183	A	215	—
24	*	56	8	88	X	120	x	152	9	184	0	216	—
25	4	57	9	89	Y	121	y	153	0	185	3	217	—
26	*	58	:	90	Z	122	z	154	0	186	■	218	—
27	*	59	:	91	L	123	<	155	8	187	0	219	■
28	—	60	<	92	/	124	:	156	E	188	0	220	■
29	*	61	=	93	]	125	>	157	0	189	6	221	—
30	▲	62	>	94	—	126	?	158	x	190	Y	222	■
31	*	63	?	95	—	127	4	159	9	191	—	223	■
32	*	64	—	96	—	128	—	160	—	192	—	225	0

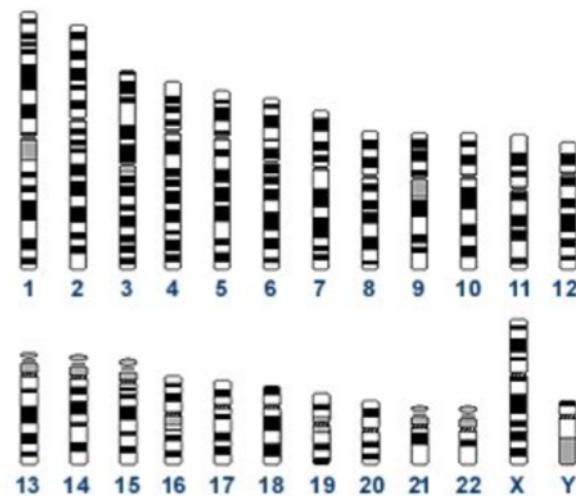
# Fichiers de régions

Coordonnées génomiques indiquant une région du génome

<chromosome>:<start>-<end>  
chr7:117465784-117715971

Formats de régions

- BED
- GTF/GFF: annotation de features dans le génome
- SAM/BAM: alignement de reads de séquence sur le génome
- VCF: variant calling file



# Format bed

obligatoire		<i>name</i>	<i>score</i>	<i>strand</i>	<i>Thick start</i>	<i>Thick end</i>	<i>color</i>	
chr7	127471196	127472363	Pos1	0	+	127471196	127472363	255,0,0
chr7	127472363	127473530	Pos2	0	+	127472363	127473530	255,0,0
chr7	127473530	127474697	Pos3	0	+	127473530	127474697	0,255,0
chr7	127474697	127475864	Pos4	0	+	127474697	127475864	255,0,255

## Attention

Le premier nucléotide est numéroté 0.

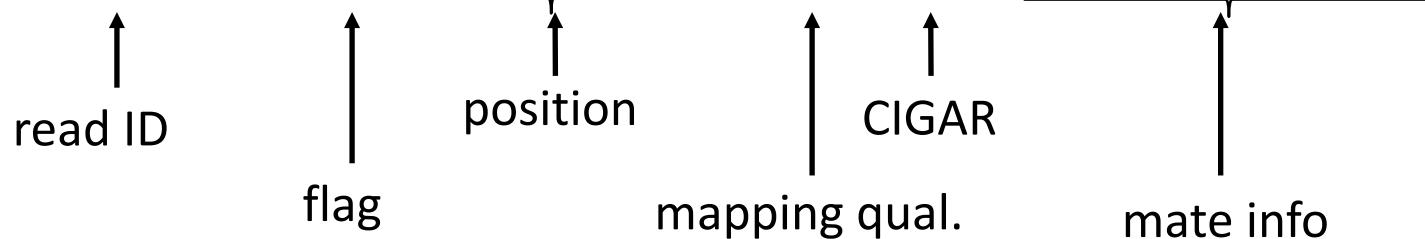
end - start = taille de la séquence



# Format SAM/BAM

Rappel BAM:

```
@RG  ID:group1  SM:1425_CD34  PL:ILLUMINA  LB:lib1 PU:unit1
@PG  ID:bwa  PN:bwa  VN:0.7.12-r1039 CL:bwa mem -M -t 2 -A 2 -E 1 -R @RG\tID:group1\tSM:1425_CD34\tPL:ILLUMINA\tLB:lib1\tPU:unit1 /root/myd
ERR166338.13782800  83  chr13  32890449  60  101M  =  32890343  -207  GGGACTGAATTAGAACAAATTTCAGCGCTT
ERR166338.13782800  163  chr13  32890343  60  75M  =  32890449  207  CACTAGCCACGTTCGAGTGCTTAATGTGGCTAGTGGC
ERR166338.26716588  99  chr13  32890406  60  101M  =  32890553  222  AATGTTCCCACCTCACAGTAAGCTGTTACCGTTCCAG
ERR166338.26716588  147  chr13  32890553  60  75M  =  32890406  -222  TTGCAGACTTACCAAGCATTGGAGGAATATCGTAA
ERR166338.27259961  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.27259961  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
ERR166338.63037998  99  chr13  32890496  60  101M  =  32890558  137  ACCTCAGTCACATAATAAGGAATGCATCCCTGTGTAAG
ERR166338.63037998  147  chr13  32890558  60  75M  =  32890496  -137  GACTTATTTACCAAGCATTGGAGGAATATCGTAGGTAA
```



# Le champ CIGAR

Example:

52M36890N45M3S

REF : chr20



## All Cigar operations

Op	BAM	Description
M	0	alignment match (can be a sequence match or mismatch)
I	1	insertion to the reference
D	2	deletion from the reference
N	3	skipped region from the reference
S	4	soft clipping (clipped sequences present in SEQ)
H	5	hard clipping (clipped sequences NOT present in SEQ)
P	6	padding (silent deletion from padded reference)
=	7	sequence match
X	8	sequence mismatch

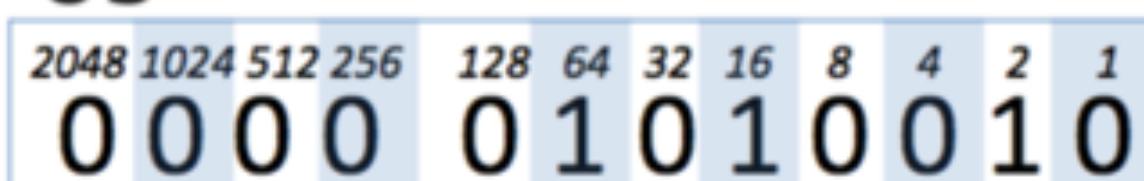
# Les Flags SAM

## Example:

- Decimal Flag Value

83

- Binary Flag Value



- To each bit corresponds a meaning

Bit	Description
1	0x1 template having multiple segments in sequencing
2	0x2 each segment properly aligned according to the aligner
4	0x4 segment unmapped
8	0x8 next segment in the template unmapped
16	0x10 SEQ being reverse complemented
32	0x20 SEQ of the next segment in the template being reverse complemented
64	0x40 the first segment in the template
128	0x80 the last segment in the template
256	0x100 secondary alignment
512	0x200 not passing filters, such as platform/vendor quality controls
1024	0x400 PCR or optical duplicate
2048	0x800 supplementary alignment

# Format VCF

```
##fileformat=VCFv4.2          mandatory
##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=<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=1,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=<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
20  14370  rs6064257 G      A      29     PASS   NS=3;DP=14;AF=0.5;DB;H2
20  17330  .       T      A      3      q10   NS=3;DP=11;AF=0.017
20  1110696 rs6040355 A      G,T    67     PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB
20  1230237 .       T      .      47     PASS   NS=3;DP=13;AA=T
20  1234567 microsat1 GTC   G,GTCT  50     PASS   NS=3;DP=9;AA=G
```

mandatory

Optional header: meta-data  
about available annotation

mandatory

samples

	NA00001	NA00002	NA00003
FORMAT	GT:GQ:DP:HQ	0 0:48:1:51,51	1 0:48:8:51,51
GT	0 0:49:3:58,50	0 1:3:5:65,3	0 0:41:3
GQ	GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2
DP	GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:51,51
HQ	GT:GQ:DP	0/1:35:4	0/2:17:2

deletion

Insertion  
(2 events  
here)

NS: number of samples with data  
DP: combined depth  
AF: allelic fraction  
AA: ancestral allele

GT: genotype (0=ref, 1=alt)  
GQ: genotype quality  
DP: read depth

# VarScan Tabulated Format

Chrom	Position	Ref	Cons	Reads1	Reads2	VarFreq	Strands 1	Strands 2	Qual1	Qual2	Pvalue	Map Qual1	Map Qual2	R1 +	R1 -	R2 +	Rs2 -	Alt
chr12	113348849	C	Y	31	30	49.18%	2	2	27	27	0.98	1	1	19	12	25	5	T
chr12	113354329	G	R	72	2	2.70%	2	2	31	26	0.98	1	1	48	24	1	1	A
chr12	113357193	G	A	2	72	97.30%	1	2	28	24	0.98	1	1	2	0	45	27	A
chr12	113357209	G	A	0	77	100%	0	2	0	29	0.98	0	1	0	0	51	26	A

**Cons** : Consensus Genotype of Variant Called (IUPAC code):

M -> A or C	Y -> C or T	D -> A or G or T	W -> A or T	V -> A or C or G
R -> A or G	K -> G or T	B -> C or G or T	S -> C or G	H -> A or C or T

# MAF format

Mutation Annotation Format (MAF) is a tab-delimited text file with aggregated mutation information from [VCF Files](#) and are generated on a project-level.

Column	Description
1 - Hugo_Symbol	<a href="#">HUGO</a> symbol for the gene (HUGO symbols are always in all caps). "Unknown" is used for regions that do not correspond to a gene
2 - Entrez_Gene_Id	<a href="#">Entrez gene</a> ID (an integer). "0" is used for regions that do not correspond to a gene region or Ensembl ID
3 - Center	One or more genome sequencing center reporting the variant
4 - NCBI_Build	The reference genome used for the alignment (GRCh38)
5 - Chromosome	The affected chromosome (chr1)
6 - Start_Position	Lowest numeric position of the reported variant on the genomic reference sequence. Mutation start coordinate
7 - End_Position	Highest numeric genomic position of the reported variant on the genomic reference sequence. Mutation end coordinate
8 - Strand	Genomic strand of the reported allele. Currently, all variants will report the positive strand: '+'
9 - Variant_Classification	Translational effect of variant allele
10 - Variant_Type	Type of mutation. TNP (tri-nucleotide polymorphism) is analogous to DNP (di-nucleotide polymorphism) but for three consecutive nucleotides. ONP (oligo-nucleotide polymorphism) is analogous to TNP but for consecutive runs of four or more (SNP, DNP, TNP, ONP, INS, DEL, or Consolidated)
11 - Reference_Allele	The plus strand reference allele at this position. Includes the deleted sequence for a deletion or "-" for an insertion
12 - Tumor_Seq_Allele1	Primary data genotype for tumor sequencing (discovery) allele 1. A "-" symbol for a deletion represents a variant. A "-" symbol for an insertion represents wild-type allele. Novel inserted sequence for insertion does not include flanking reference bases
13 - Tumor_Seq_Allele2	Tumor sequencing (discovery) allele 2
14 - dbSNP_RS	The rs-IDs from the <a href="#">dbSNP</a> database, "novel" if not found in any database used, or null if there is no dbSNP record, but it is found in other databases
15 - dbSNP_Val_Status	The dbSNP validation status is reported as a semicolon-separated list of statuses. The union of all rs-IDs is taken when there are multiple
16 - Tumor_Sample_Barcode	Aliquot barcode for the tumor sample
17 - Matched_Norm_Sample_Barcode	Aliquot barcode for the matched normal sample
18 - Match_Norm_Seq_Allele1	Primary data genotype. Matched normal sequencing allele 1. A "-" symbol for a deletion represents a variant. A "-" symbol for an insertion represents wild-type allele. Novel inserted sequence for insertion does not include flanking reference bases (cleared in somatic MAF)
19 - Match_Norm_Seq_Allele2	Matched normal sequencing allele 2
20 - Tumor_Validation_Allele1	Secondary data from orthogonal technology. Tumor genotyping (validation) for allele 1. A "-" symbol for a deletion represents a variant. A "-" symbol for an insertion represents wild-type allele. Novel inserted sequence for insertion does not include flanking reference bases
21 - Tumor_Validation_Allele2	Secondary data from orthogonal technology. Tumor genotyping (validation) for allele 2
22 - Match_Norm_Validation_Allele1	Secondary data from orthogonal technology. Matched normal genotyping (validation) for allele 1. A "-" symbol for a deletion represents a variant. A "-" symbol for an insertion represents wild-type allele. Novel inserted sequence for insertion does not include flanking reference bases (cleared in somatic MAF)