DNA Sequencing and Data Analysis

Prof Noam Shomron

Hadas Volkov

Lecture 8, December 23, 2022

DNA Sequencing and Data Analysis

Friday 8:45 AM to 11:15 AM

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DNA Sequencing and Data Analysis

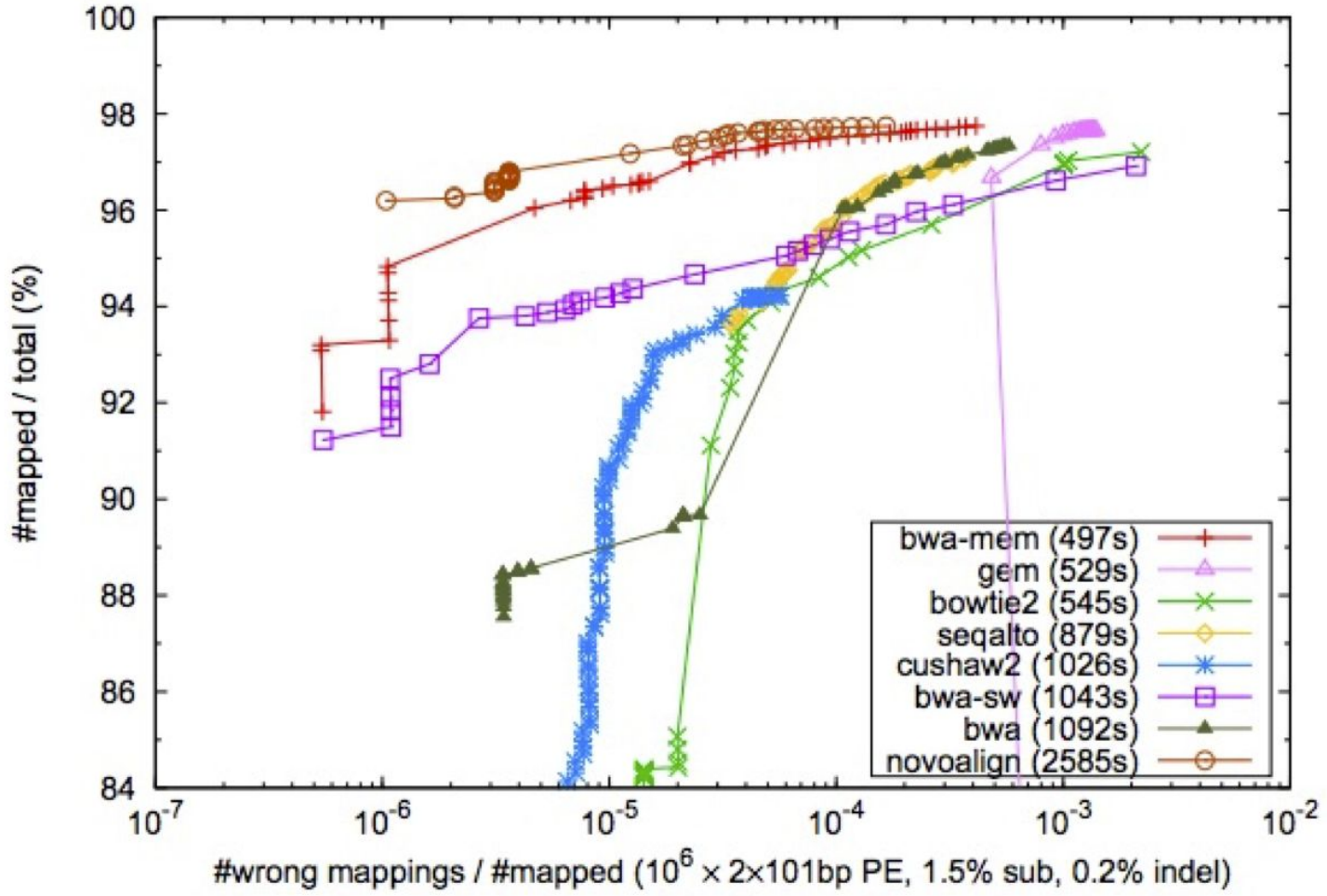
Variant Calling
SAM & VCF File Formats
IGV

Class	Title	Content/assignments	Activity, location
1, 4.11	Introduction to Cells and DNA	Basic knowledge of biology	In the lecture hall, Noam
2, 11.11	DNA Sequencing past and present	Basic knowledge of molecular DNA	In the lecture hall, Noam
3, 18.11	Genomics technologies	DNA, RNA, technologies	In the lecture hall, Noam
4, 25.11	Introduction to Bioinformatics challenges in reading DNA	Focus on three methods: WES/WGS, RNA-seq, cell-free DNA	In the lecture hall, Noam
5, 2.12	Modern DNA Sequencing, 2nd wave File Formats, tools.	Analysis approaches for WES/WGS, RNA-seq, cell-free DNA	In the lecture hall, Hadas and Noam
6, 9.12	De novo Shotgun Assembly	The algorithms and methods behind the assembly problem	In computer class, Hadas and Noam
7, 16.12	Sequence Mapping and Alignment	The algorithms behind mapping and alignment, fast and heuristics	In computer class, Hadas and Noam
8, 23.12	**Variant Calling and Somatic Variant Analysis**	**The bioinformatics behind discovery of novel mutations in cancer**	**In computer class, Hadas and Noam**
9, 30.12	Nanopore data analysis introduction and class activity	The bioinformatics behind Nanopore analysis, activity on computers	In computer class, Hadas and Noam
10, 6.1	Practice molecular biology techniques	Pipetting, transferring small amounts of fluids, running a dry Nanopore experiment	In biology class, Meitar and Noam
11, 13.1	Nanopore DNA sequencing	Nanopore DNA sequencing, experimental run	In biology class, Meitar, Hadas, Assaf
12, 20.1	Nanopore data analysis	Nanopore DNA analysis, experimental run	In computer class, Hadas and Noam
13, 27.1	Nanopore data analysis and presentations	Groups present their results	In the lecture hall, Hadas and Noam

Aligners Comparison

Aligner	Index	Applications	Availability
BWA-mem	Burrows-Wheeler	DNA, SE, PE	open-source
Bowtie2	Burrows-Wheeler	DNA, SE, PE	open-source
Novoalign	Hash-Based	DNA, SE, PE	propriety
TopHat	Burrows-Wheeler	RNA-seq	open-source
STAR	Hash-Based (reads)	RNA-seq	open-source
GSNAP	Hash-Based (reads)	RNA-seq	open-source

Aligners Comparison



https://arxiv.org/pdf/1303.3997v2.pdf

BWA-MEM Workflow

This takes a long time, but you do it <u>once</u>

Create BWT of reference genome.

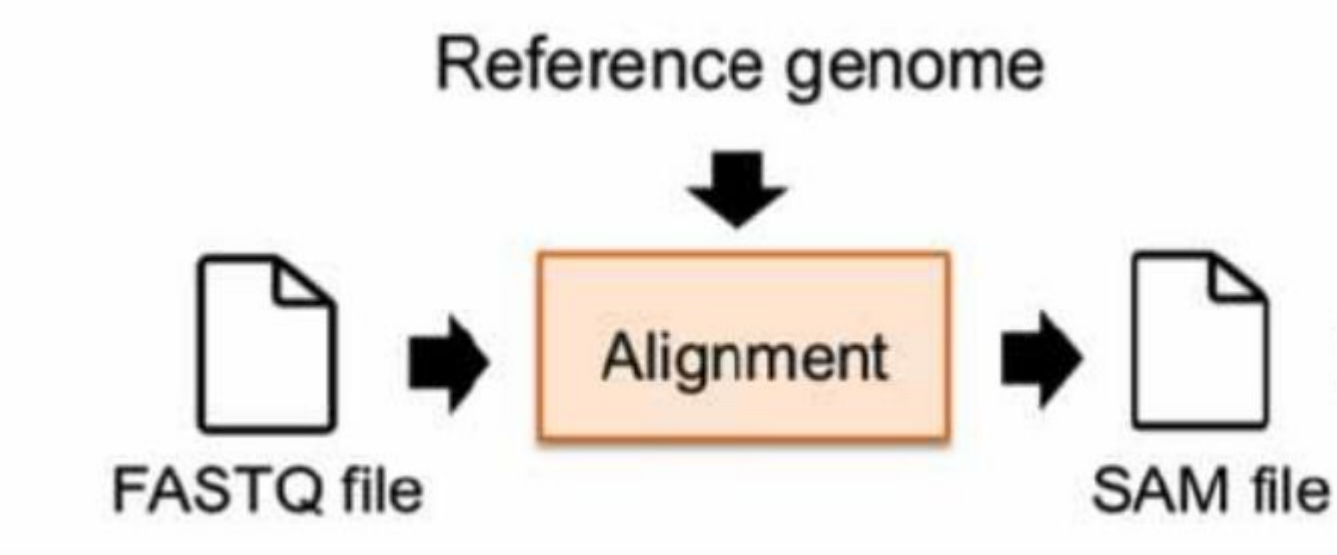
```
$ bwa index grch38.fa
```

Output is in SAM format.
Use multiple threads if you have a computer with multiple CPUs.

Align paired-end FASTQ to BWT index.

```
$ bwa mem -t 16 grch38.fa 1.fq 2.fq > sample.sam
```

FASTQ to SAM



Sequence Alignment and Mapping (SAM)

BIOINFORMATICS **APPLICATIONS NOTE** *Vol. 25 no. 16 2009, pages 2078–2079*
doi:10.1093/bioinformatics/btp352

Sequence analysis

The Sequence Alignment/Map format and SAMtools

Heng Li[1,†], Bob Handsaker[2,†], Alec Wysoker[2], Tim Fennell[2], Jue Ruan[3], Nils Homer[4], Gabor Marth[5], Goncalo Abecasis[6], Richard Durbin[1,*] and 1000 Genome Project Data Processing Subgroup[7]

[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK, [2]Broad Institute of MIT and Harvard, Cambridge, MA 02141, USA, [3]Beijing Institute of Genomics, Chinese Academy of Science, Beijing 100029, China, [4]Department of Computer Science, University of California Los Angeles, Los Angeles, CA 90095, [5]Department of Biology, Boston College, Chestnut Hill, MA 02467, [6]Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109, USA and [7]http://1000genomes.org

Received on April 28, 2009; revised on May 26, 2009; accepted on May 30, 2009

Advance Access publication June 8, 2009

Associate Editor: Alfonso Valencia

Table 1. Mandatory fields in the SAM format

No.	Name	Description
1	`QNAME`	Query NAME of the read or the read pair
2	`FLAG`	Bitwise FLAG (pairing, strand, mate strand, etc.)
3	`RNAME`	Reference sequence NAME
4	`POS`	1-Based leftmost POSition of clipped alignment
5	`MAPQ`	MAPping Quality (Phred-scaled)
6	`CIGAR`	Extended CIGAR string (operations: `MIDNSHP`)
7	`MRNM`	Mate Reference NaMe ('=' if same as `RNAME`)
8	`MPOS`	1-Based leftmost Mate POSition
9	`ISIZE`	Inferred Insert SIZE
10	`SEQ`	Query SEQuence on the same strand as the reference
11	`QUAL`	Query QUALity (ASCII-33=Phred base quality)

Sequence Alignment and Mapping (SAM)

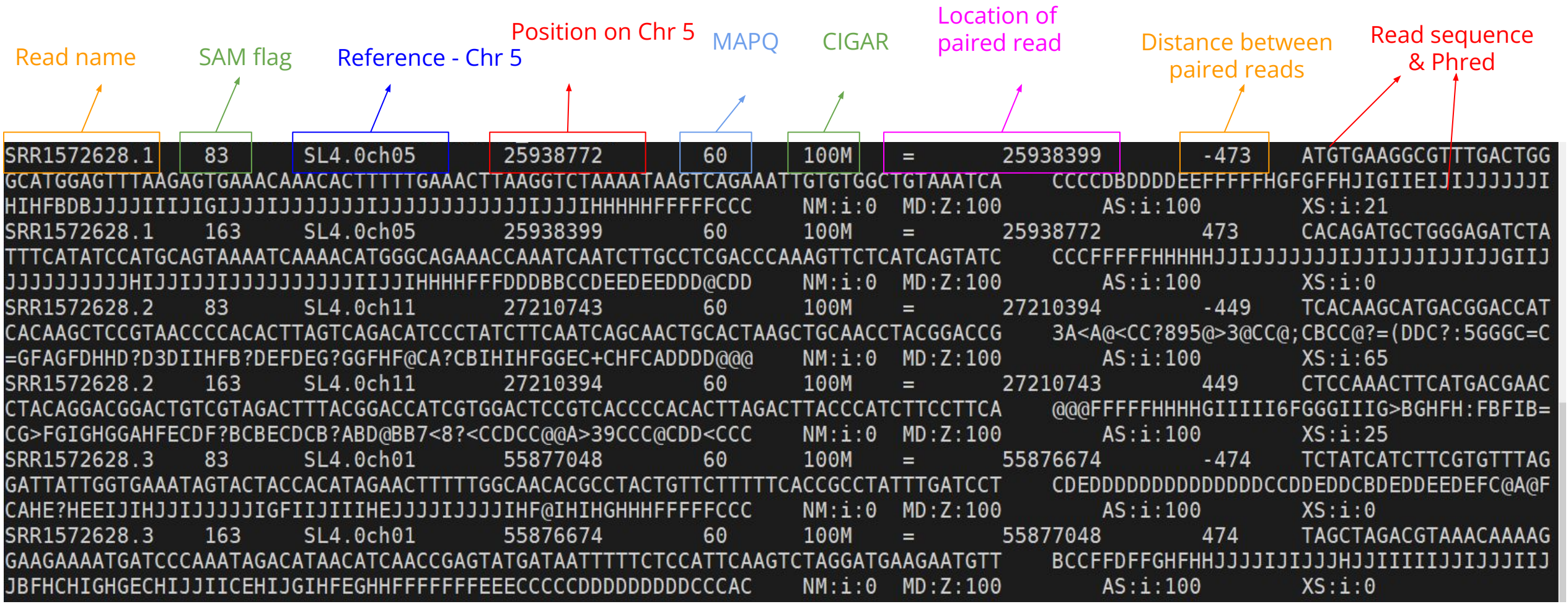
What critical information do we need for sequence alignments?

SAM Format

Col #	Name	Meaning	Example
1	QNAME	Read or Pair name	HWI:ST156_1:278:1:1058:4544:0
2	FLAG	Bitwise FLAG	***soon!***
3	RNAME	Reference sequence name	chr1
4	POS	1-based alignment start coordinate	8,724,005
5	MAPQ	Mapping quality	***soon!***
6	CIGAR	Extended CIGAR string	***soon!***
7	MRNM	If paired, the mate's reference seq.	chr1
8	MPOS	If paired, the mate's alignment start	8,724,505
9	ISIZE	If paired, the insert size	562
10	SEQ	The sequence of the query/mate	ACAAATTCAG...
11	QUAL	The quality string for the query/mate	HHH$^^%$$$...
12	OPT	Optional Tags	XA:i:2, MD:Z:0T34G15

http://samtools.sourceforge.net/samtools.shtml

SAM Format


Read name
SAM flag
Reference - Chr 5
Position on Chr 5
MAPQ
CIGAR
Location of paired read
Distance between paired reads
Read sequence & Phred
SRR1572628.1 83 SL4.0ch05 25938772 60 100M = 25938399 -473 ATGTGAAGGCGTTTGACTGG
GCATGGAGTTTAAGAGTGAAACAAACACTTTTTGAAACTTAAGGTCTAAAATAAGTCAGAAATTGTGTGGCTGTAAATCA CCCCDBDDDDEEFFFFFHGFGFFHJIGIIEIJIJJJJJJI
HIHFBDBJJJJIIIJIGIJJJIJJJJJJJIJJJJJJJJJJJJIJJJIHHHHHFFFFFCCC NM:i:0 MD:Z:100 AS:i:100 XS:i:21
SRR1572628.1 163 SL4.0ch05 25938399 60 100M = 25938772 473 CACAGATGCTGGGAGATCTA
TTTCATATCCATGCAGTAAAATCAAAACATGGGCAGAAACCAAATCAATCTTGCCTCGACCCAAAGTTCTCATCAGTATC CCCFFFFFHHHHHJJIJJJJJJJIJJIJJJIJJIJJGIIJ
JJJJJJJJJJHIJJIJJIJJJJJJJJJJIIJJIHHHHFFFDDDBBCCDEEDEEDDD@CDD NM:i:0 MD:Z:100 AS:i:100 XS:i:0
SRR1572628.2 83 SL4.0ch11 27210743 60 100M = 27210394 -449 TCACAAGCATGACGGACCAT
CACAAGCTCCGTAACCCCACACTTAGTCAGACATCCCTATCTTCAATCAGCAACTGCACTAAGCTGCAACCTACGGACCG 3A<A@<CC?895@>3@CC@;CBCC@?=(DDC?:5GGGC=C
=GFAGFDHHD?D3DIIHFB?DEFDEG?GGFHF@CA?CBIHIHFGGEC+CHFCADDDD@@@ NM:i:0 MD:Z:100 AS:i:100 XS:i:65
SRR1572628.2 163 SL4.0ch11 27210394 60 100M = 27210743 449 CTCCAAACTTCATGACGAAC
CTACAGGACGGACTGTCGTAGACTTTACGGACCATCGTGGACTCCGTCACCCCACACTTAGACTTACCCATCTTCCTTCA @@@FFFFFHHHHGIIIII6FGGGIIIG>BGHFH:FBFIB=
CG>FGIGHGGAHFECDF?BCBECDCB?ABD@BB7<8?<CCDCC@@A>39CCC@CDD<CCC NM:i:0 MD:Z:100 AS:i:100 XS:i:25
SRR1572628.3 83 SL4.0ch01 55877048 60 100M = 55876674 -474 TCTATCATCTTCGTGTTTAG
GATTATTGGTGAAATAGTACTACCACATAGAACTTTTTGGCAACACGCCTACTGTTCTTTTTCACCGCCTATTTGATCCT CDEDDDDDDDDDDDDDCCDDEDDCBDEDDEEDEFC@A@F
CAHE?HEEIJIHJJIJJJJJIGFIIJIIIHEJJJJIJJJJIHF@IHIHGHHHFFFFFCCC NM:i:0 MD:Z:100 AS:i:100 XS:i:0
SRR1572628.3 163 SL4.0ch01 55876674 60 100M = 55877048 474 TAGCTAGACGTAAACAAAAG
GAAGAAAATGATCCCAAATAGACATAACATCAACCGAGTATGATAATTTTTCTCCATTCAAGTCTAGGATGAAGAATGTT BCCFFDFFGHFHHJJJJIJIJJJHJJIIIIIJJIJJJIIJ
JBFHCHIGHGECHIJJIICEHIJGIHFEGHHFFFFFFFEEECCCCCDDDDDDDDDCCCAC NM:i:0 MD:Z:100 AS:i:100 XS:i:0

MAPQ

MAPQ - mapping quality

Definition: $-10 \log_{10} \Pr\{\text{mapping position is wrong}\}$

The higher - the better

Usually between 0 and 60

Calculation of MAPQ is differ between aligners

It considers alignment score, Phred score and alternative mappings

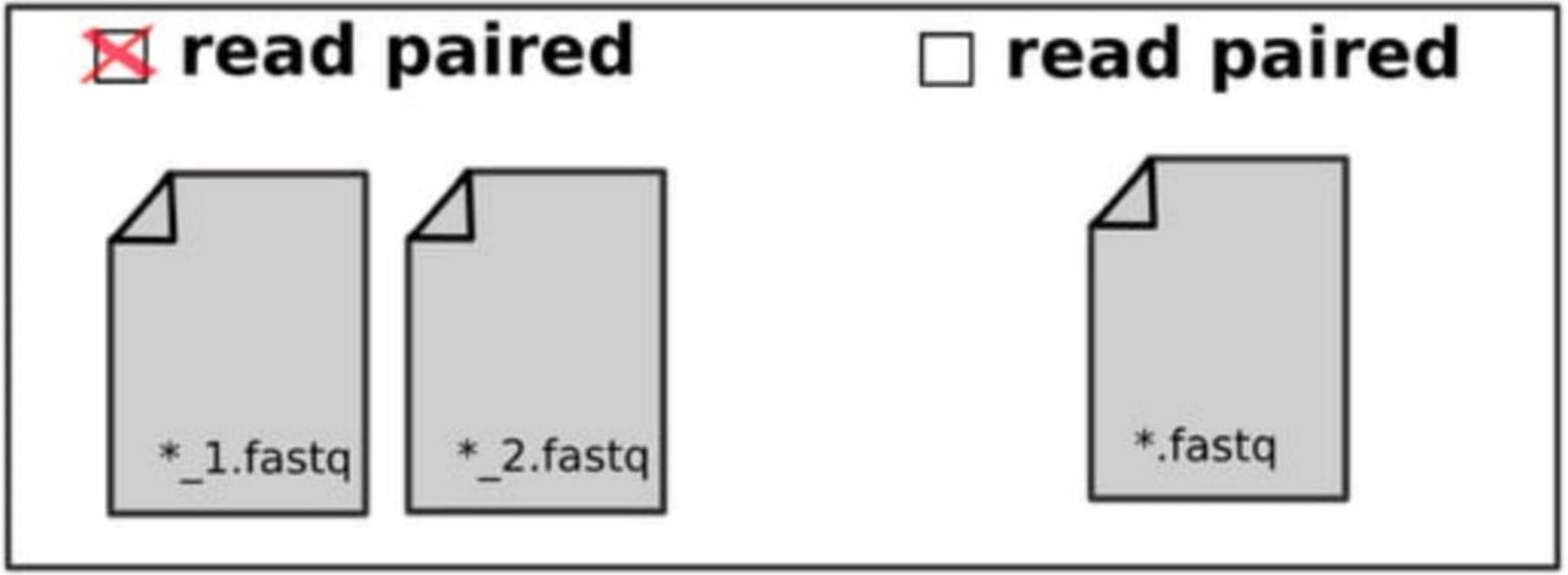
As a rule of thumb:

- MAPQ > 30 is considered a good mapping
- MAPQ 0 usually means ambiguous mapping

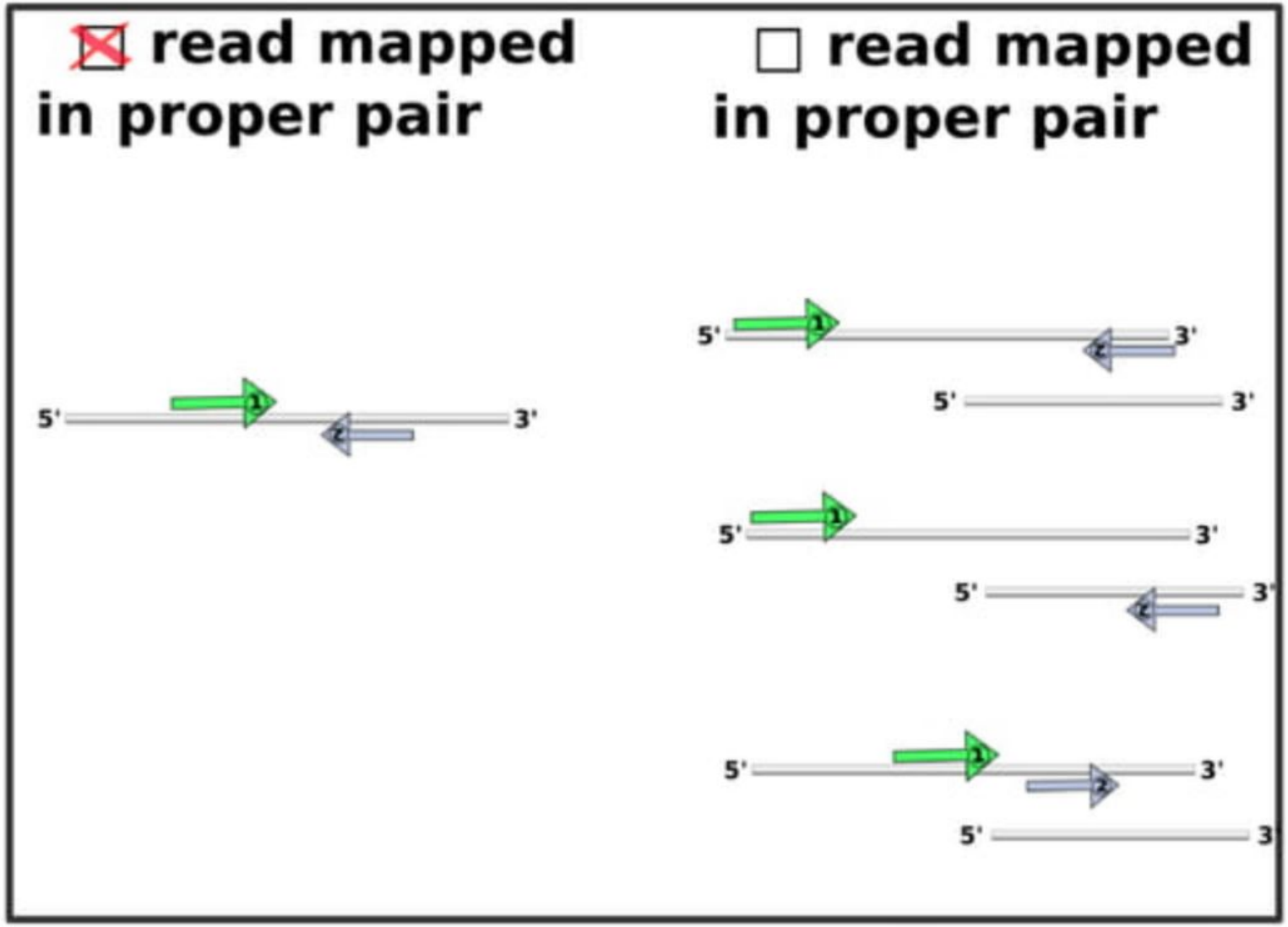
SAM Flag

base2	base10	base16	Meaning	Applies to:
00000000001	1	0x0001	The read originated from a paired sequencing molecule	Both
00000000010	2	0x0002	The read is mapped in a **proper** pair	Pairs only
00000000100	4	0x0004	The query sequence itself is unmapped	Both
00000001000	8	0x0008	The query's mate is unmapped	Pairs only
00000010000	16	0x0010	Strand of the query (0 for forward; 1 for reverse strand)	Both
00000100000	32	0x0020	Strand of the query's mate	Pairs only
00001000000	64	0x0040	The query is the first read in the pair	Pairs only
00010000000	128	0x0080	The read is the second read in the pair	Pairs only
00100000000	256	0x0100	The alignment is not primary	Both
01000000000	512	0x0200	The read fails platform/vendor quality checks	Both
10000000000	1024	0x0400	The read is either a PCR duplicate or an optical duplicate	Both

SAM Flag

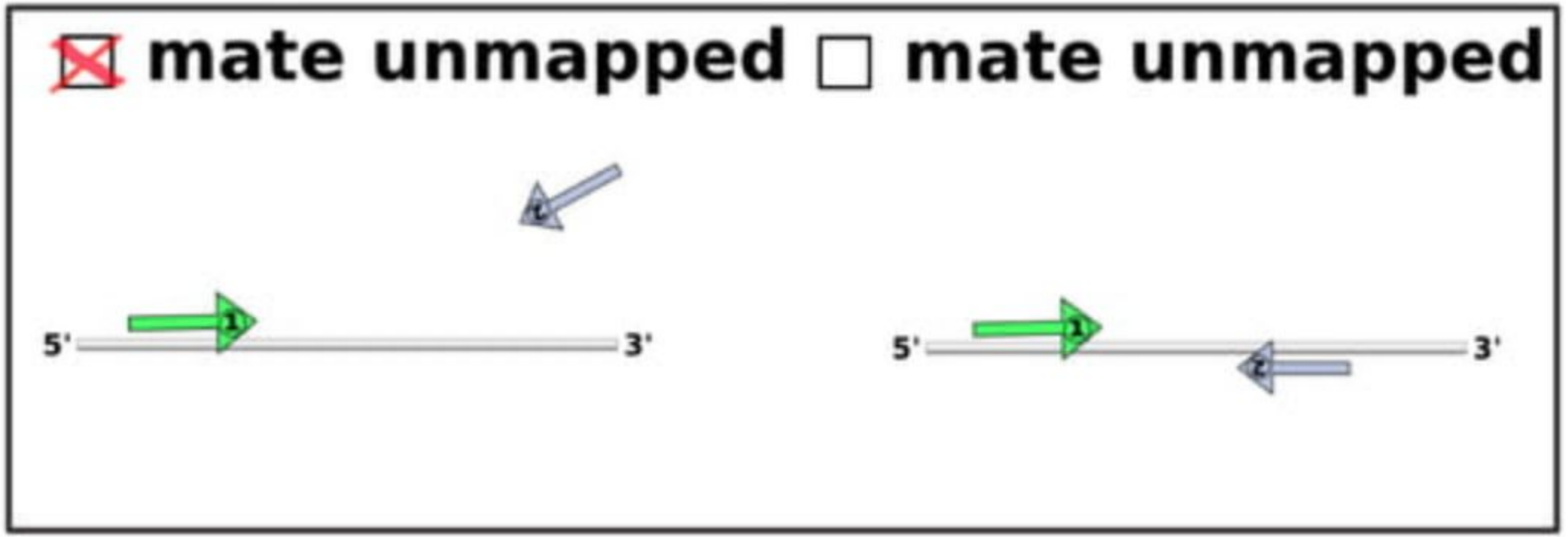


SAM Flag

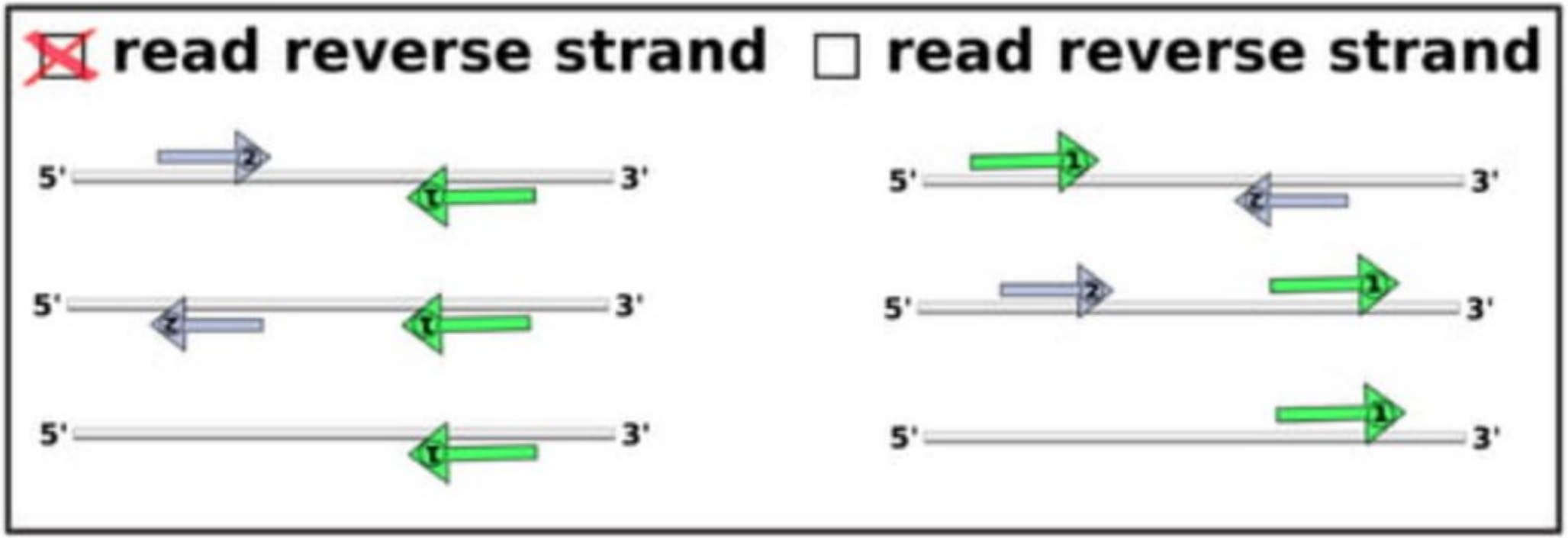

read mapped in proper pair
5'
3'
read mapped in proper pair
5'
3'
5'
3'
5'
3'
5'
3'
5'
3'
5'
3

SAM Flag

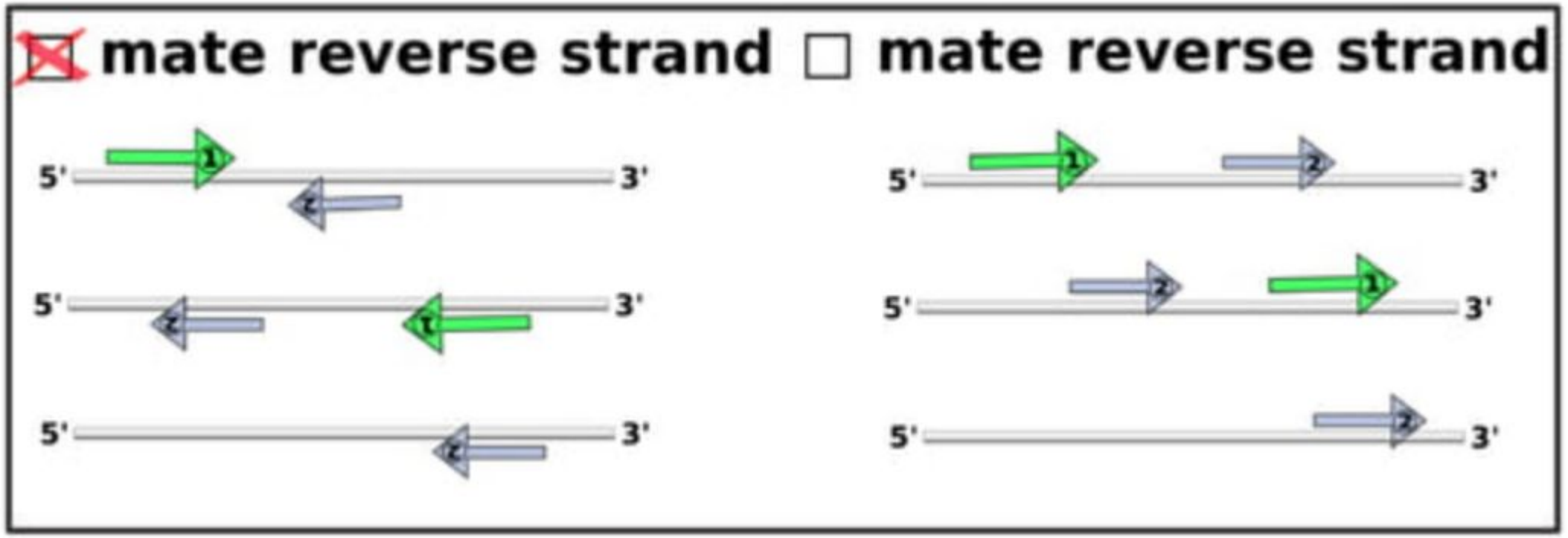
SAM Flag



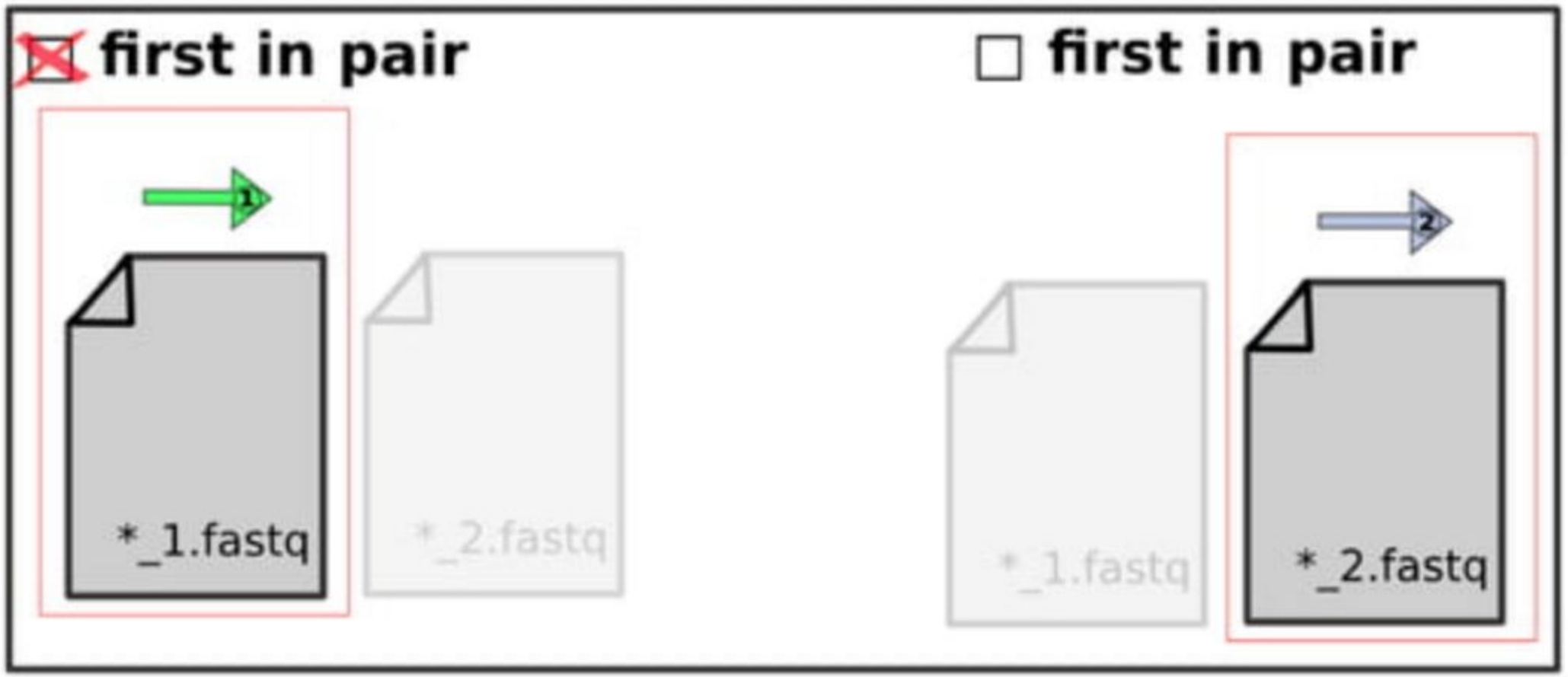
SAM Flag



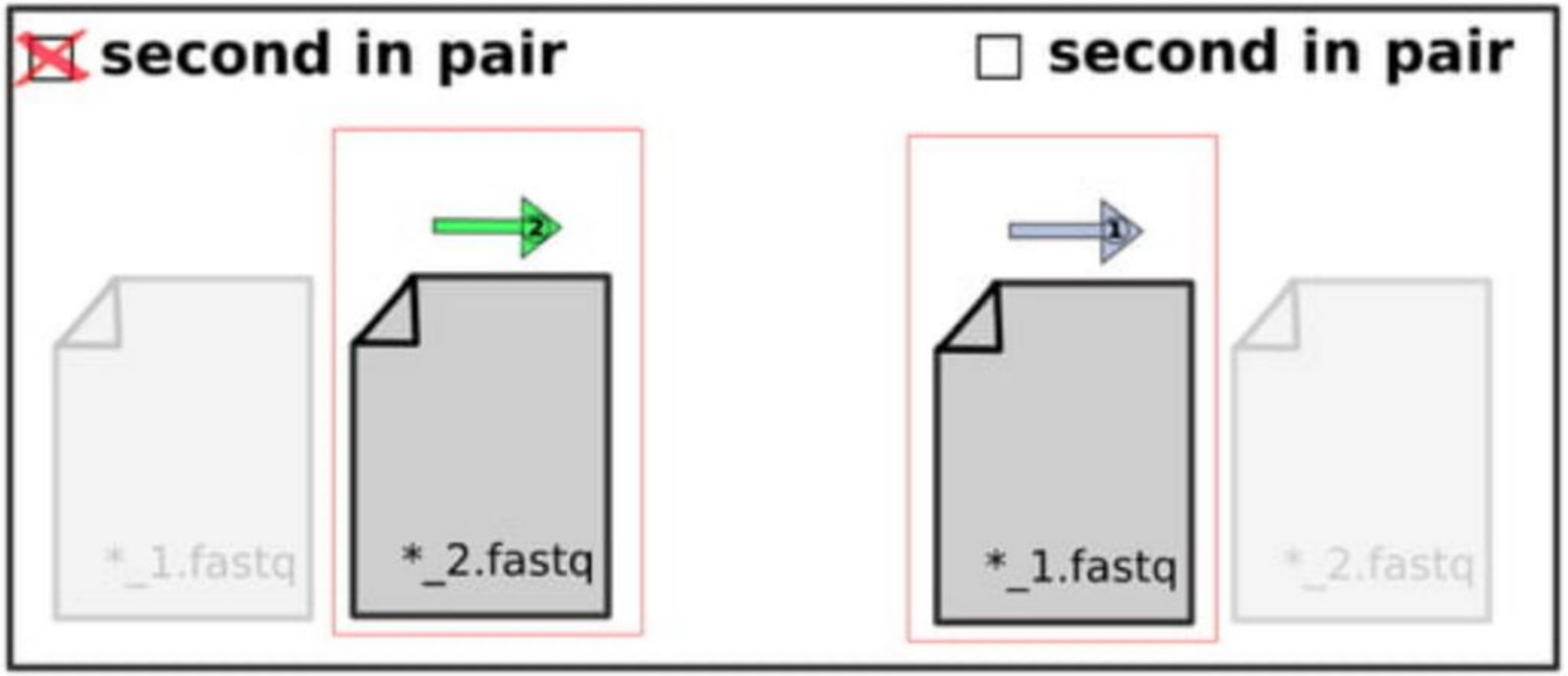
SAM Flag



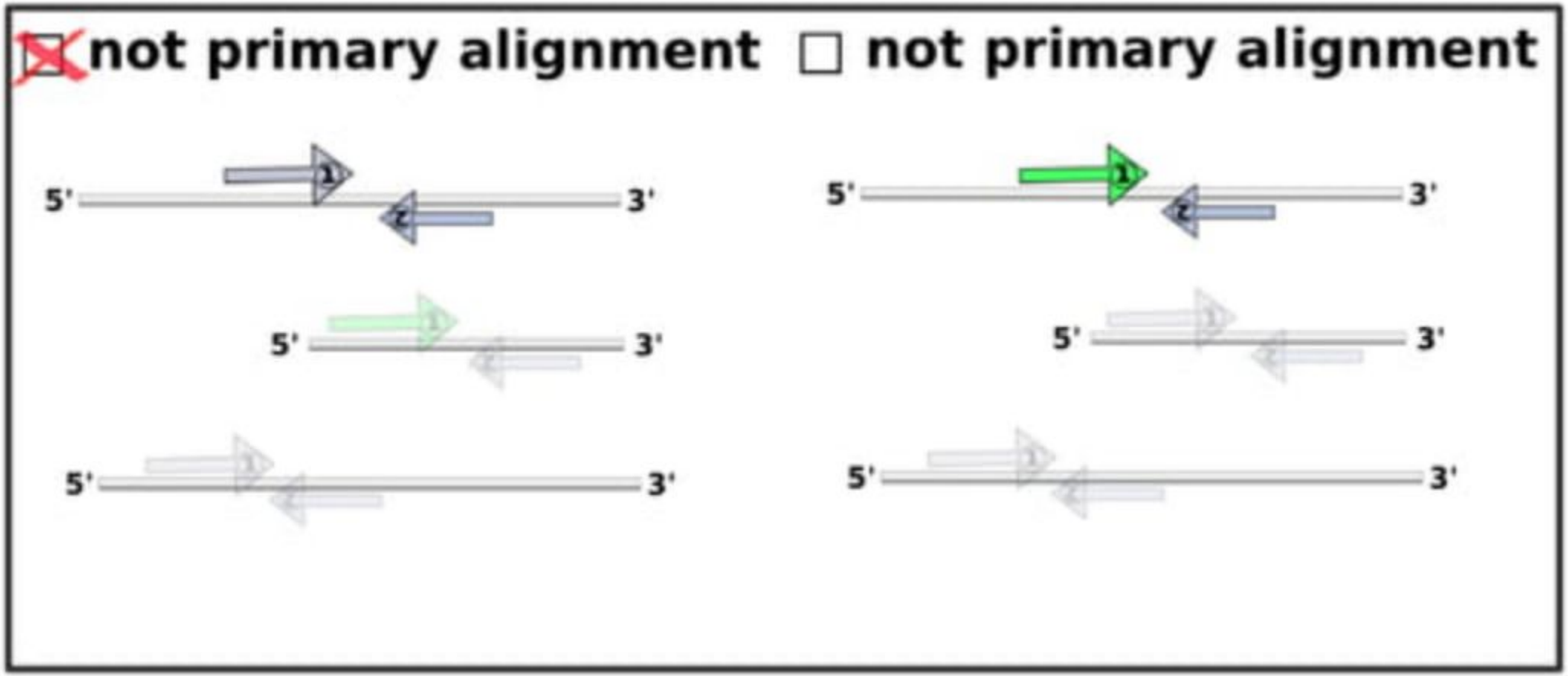
SAM Flag



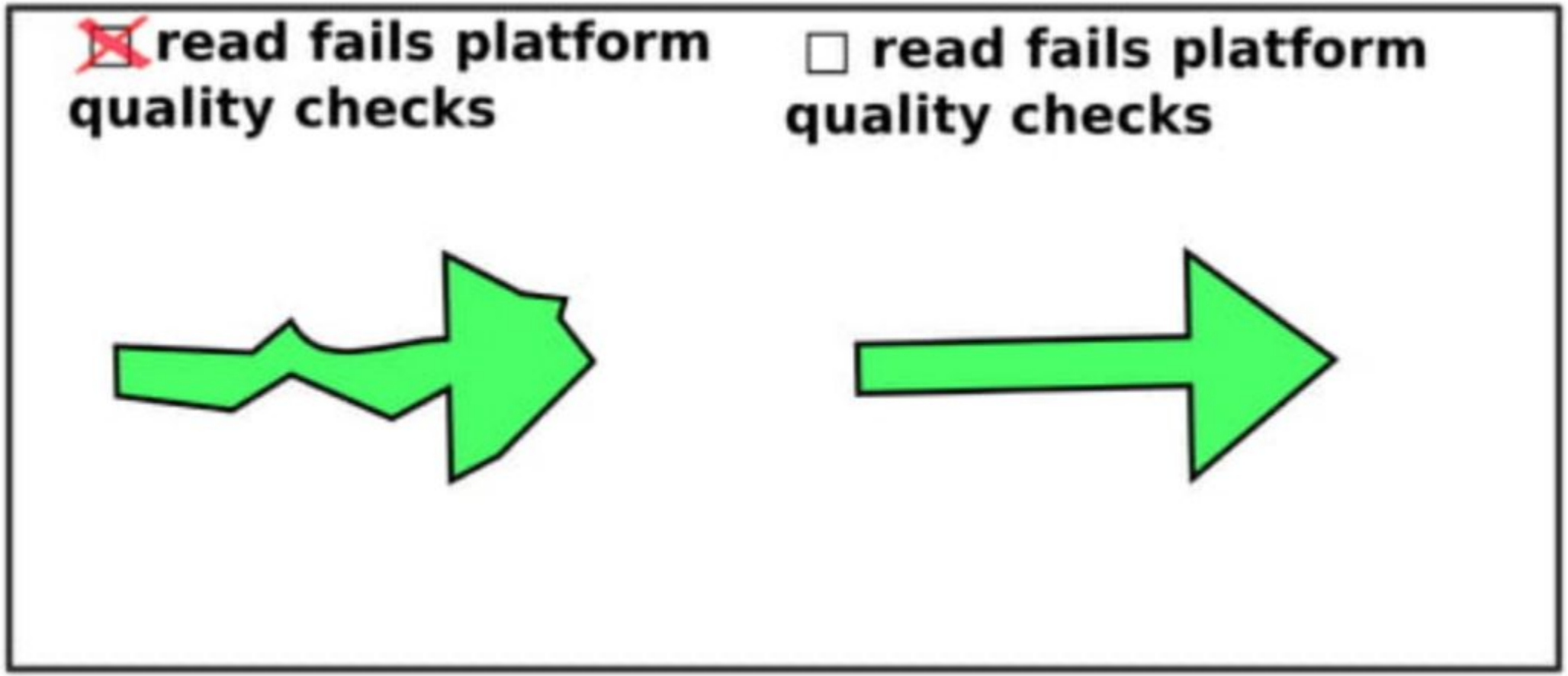
SAM Flag



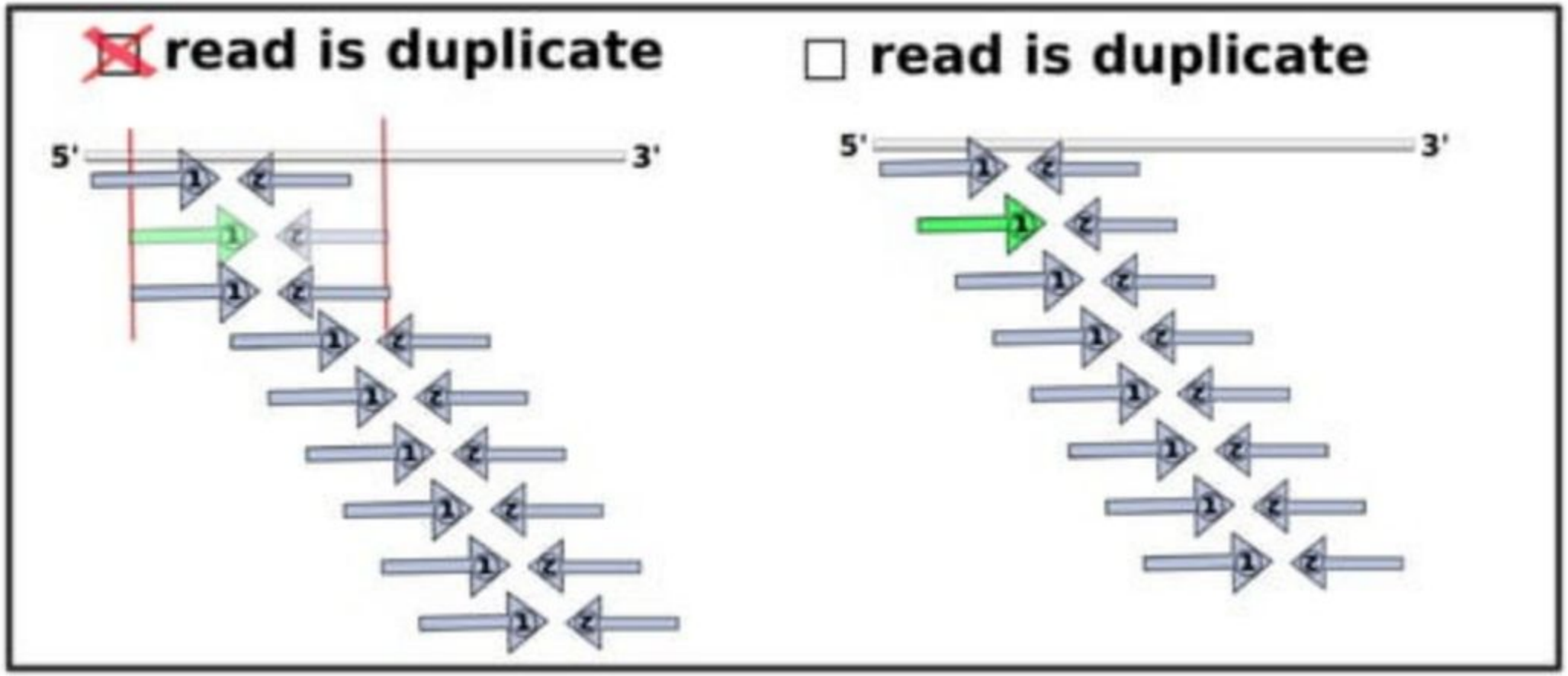
SAM Flag


not primary alignment
not primary alignment
5'
3'
5'
3'
5'
3'
5'
3'
5'
3'
5'
3'

SAM Flag


☒ read fails platform quality checks
☐ read fails platform quality checks

SAM Flag



ST-E00223:32:H5J57CCXX:4:1220:14651:8868 99 1 10086

base2	base10	base16	Meaning	Applies to:
00000000001	1	0x0001	The read originated from a paired sequencing molecule	Both
00000000010	2	0x0002	The read is mapped in a **proper** pair	Pairs only
00000000100	4	0x0004	The query sequence itself is unmapped	Both
00000001000	8	0x0008	The query's mate is unmapped	Pairs only
00000010000	16	0x0010	Strand of the query (0 for forward; 1 for reverse strand)	Both
00000100000	32	0x0020	Strand of the query's mate	Pairs only
00001000000	64	0x0040	The query is the first read in the pair	Pairs only
00010000000	128	0x0080	The read is the second read in the pair	Pairs only
00100000000	256	0x0100	The alignment is not primary	Both
01000000000	512	0x0200	The read fails platform/vendor quality checks	Both
10000000000	1024	0x0400	The read is either a PCR duplicate or an optical duplicate	Both

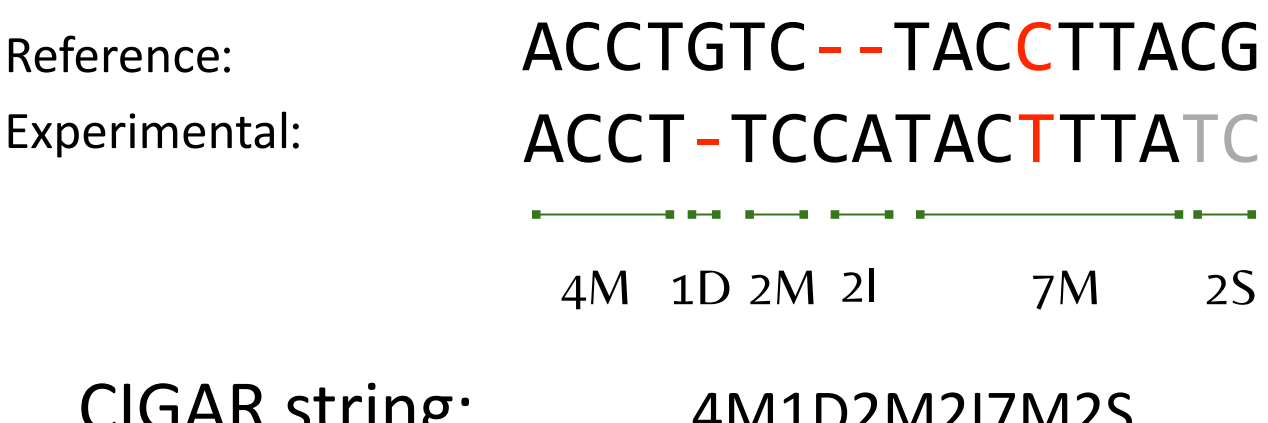
00001100011

$2^6+2^5+2^1+2^0 = 64+32+2+1 = 99$

Concise Idiosyncratic Gapped Alignment Report (CIGAR)

Encoding the details of the alignment

Operation	Meaning
M	Match*
D	Deletion w.r.t. reference
I	Insertion w.r.t. reference
N	Split or spliced alignment
S	Soft-clipping
H	Hard-clipping
P	Padding

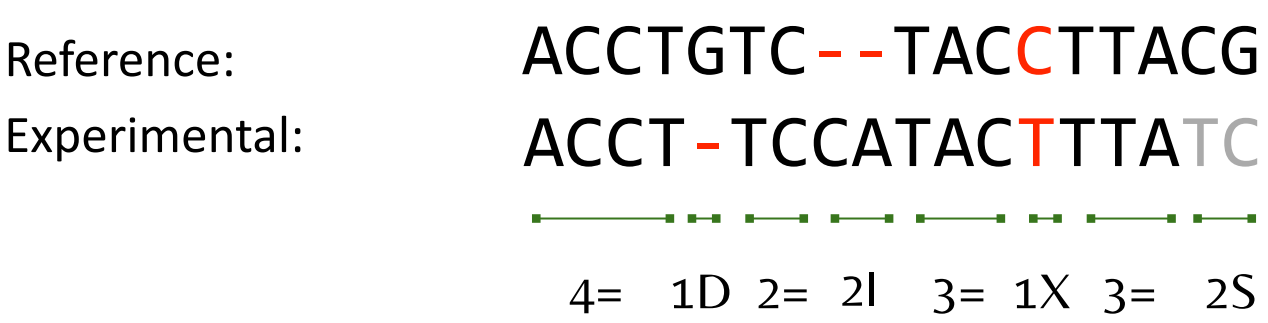


CIGAR string: 4M1D2M2I7M2S

LENGTH/OPERATION

CIGAR Extended

Operation	Meaning
=	Exact match
X	Mismatch
D	Deletion w.r.t. reference
I	Insertion w.r.t. reference
N	Split or spliced alignment
S	Soft-clipping
H	Hard-clipping
P	Padding



CIGAR string: 4=1D2=2I3=1X3=2S

SAM to BAM

Do it once

Create BWT of reference genome.

```
$ bwa index grch38.fa
```

Output is in SAM format

Align paired-end FASTQ to BWT index.

```
$ bwa mem -t 16 grch38.fa 1.fq 2.fq > sample.sam
```

Output is in BAM format.
Unsorted!
random genomic order as reads are randomly placed in FASTQ by sequencer.

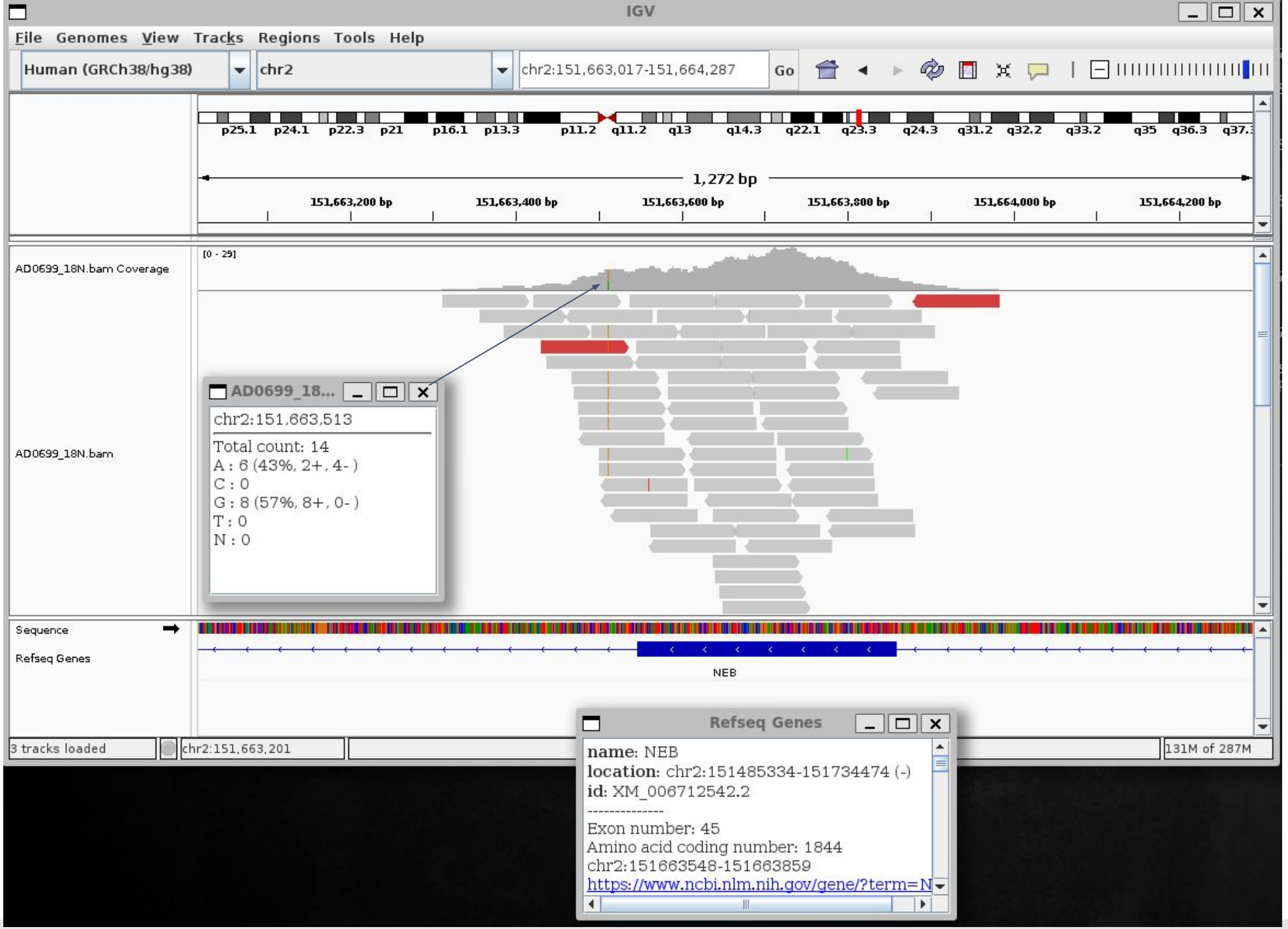
Convert SAM to BAM

```
$ samtools view -b sample.sam > sample.bam
```

Integrative Genomics Viewer (IGV)

Visualization tool for exploring and analyzing genomic data

https://software.broadinstitute.org/software/igv/


IGV
File Genomes View Tracks Regions Tools Help
Human (GRCh38/hg38)
chr2
chr2:151,663,017-151,664,287
Go
p25.1 p24.1 p22.3 p21 p16.1 p13.3 p11.2 q11.2 q13 q14.3 q22.1 q23.3 q24.3 q31.2 q32.2 q33.2 q35 q36.3 q37.3
1,272 bp
151,663,200 bp
151,663,400 bp
151,663,600 bp
151,663,800 bp
151,664,000 bp
151,664,200 bp
AD0699_18N.bam Coverage
[0 - 29]
AD0699_18N.bam
AD0699_18...
chr2:151,663,513
Total count: 14
A : 6 (43%, 2+, 4-)
C : 0
G : 8 (57%, 8+, 0-)
T : 0
N : 0
Sequence
Refseq Genes
NEB
Refseq Genes
name: NEB
location: chr2:151485334-151734474 (-)
id: XM_006712542.2
Exon number: 45
Amino acid coding number: 1844
chr2:151663548-151663859
https://www.ncbi.nlm.nih.gov/gene/?term=N
3 tracks loaded
chr2:151,663,201
131M of 287M

Genetic Variation

Differences in DNA content or structure among individuals

- Any two individuals have ~99.8% identical DNA.

The human genome is big - a set of 23 chromosomes has 3.1 billion nucleotides.

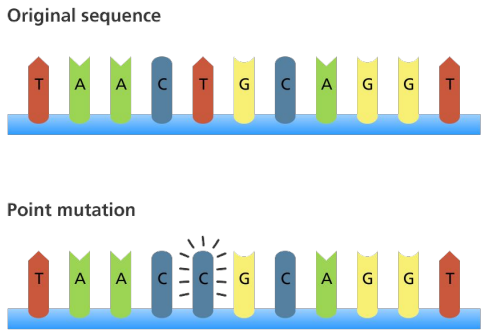
There are >100,000,000 know genetic variants in the human genome

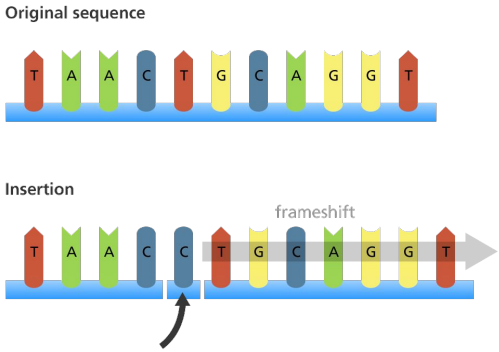
~99.8% identical DNA
(differ at 1/ 620 - 1/750 bp)

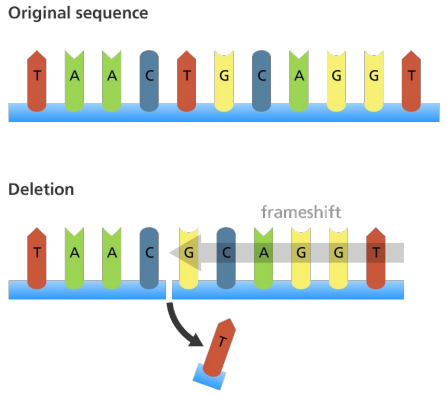
99% identical DNA

Types of Genetic Variation

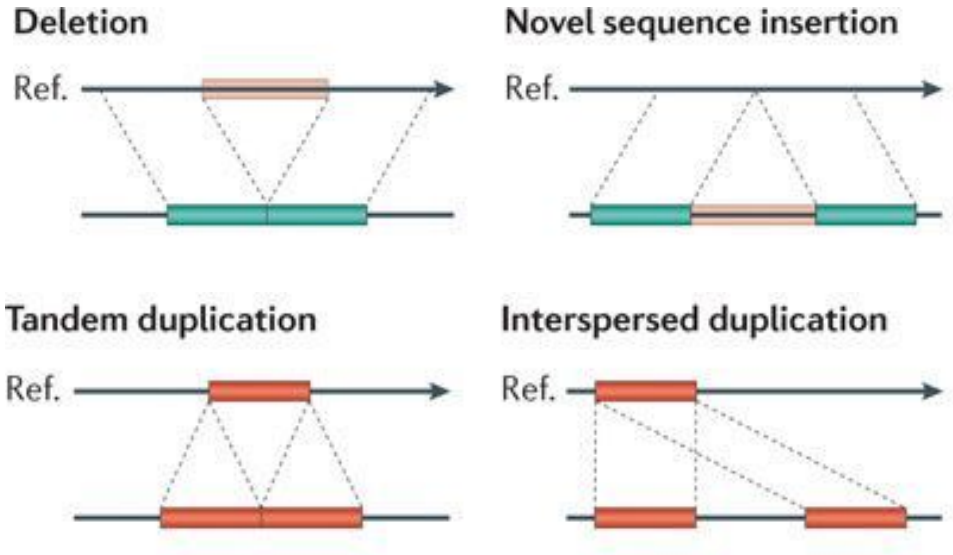
Small-scale

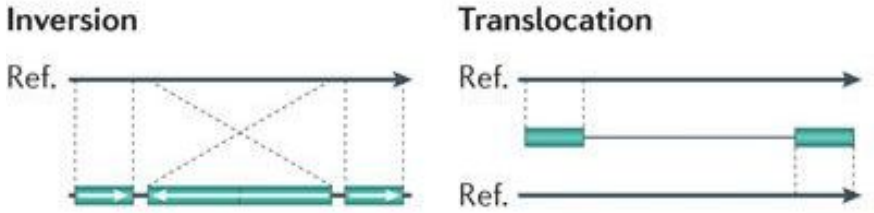






Large scale (Structural)





A Normal Human

"We find that a typical [human] genome differs from the reference human genome at **4.1 million to 5.0 million sites**. Although >**99.9% of variants consist of SNPs and short indels**, structural variants affect more bases: the typical genome contains an estimated **2,100 to 2,500 structural variants** (~1,000 large deletions, ~160 copy-number variants, ~915 Alu insertions, ~128 L1 insertions, ~51 SVA insertions, ~4 NUMTs, and ~10 inversions), **affecting ~20 million bases of sequence.**

A global reference for human genetic variation

The 1000 Genomes Project Consortium*

The 1000 Genomes Project set out to provide a comprehensive description of common human genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations. Here we report completion of the project, having reconstructed the genomes of 2,504 individuals from 26 populations using a combination of low-coverage whole-genome sequencing, deep exome sequencing, and dense microarray genotyping. We characterized a broad spectrum of genetic variation, in total over 88 million variants (84.7 million single nucleotide polymorphisms (SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants), all phased onto high-quality haplotypes. This resource includes >99% of SNP variants with a frequency of >1% for a variety of ancestries. We describe the distribution of genetic variation across the global sample, and discuss the implications for common disease studies.

Mutation != Polymorphism (or SNP)

Mutations

acctccgagta acctccgagta

acctccgagta acctccgagta

acctccgagta acctccgagta

acctccgagta acctccgagta

acctccgagta acctccgagta

a toy population of 10 identical chromosomes

Mutations

Mutation creates genetic diversity

acctccgagta	acctccgagta
acctccgagta	acctccgagta
acctccgagta	acctccgagta
acctccgagta	acctccgagta
acctccgagta	acctcc**T**gagta

mutation:
private to this chromosome / individual

Mutations

From mutation to polymorphism

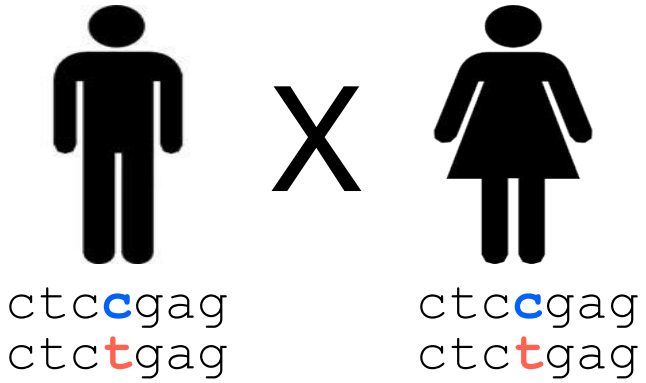
```
acctccgagta
acctccgagta
acctccgagta
acctcTgagta
acctccgagta
```

```
acctcTgagta
acctccgagta
acctcTgagta
acctccgagta
acctcTgagta
```

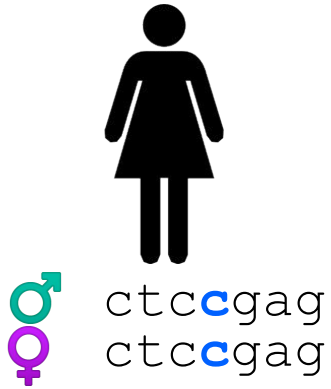
Diploid Genomes

Our genome is comprised of a paternal and a maternal "haplotype".
Together, they form our "genotype"

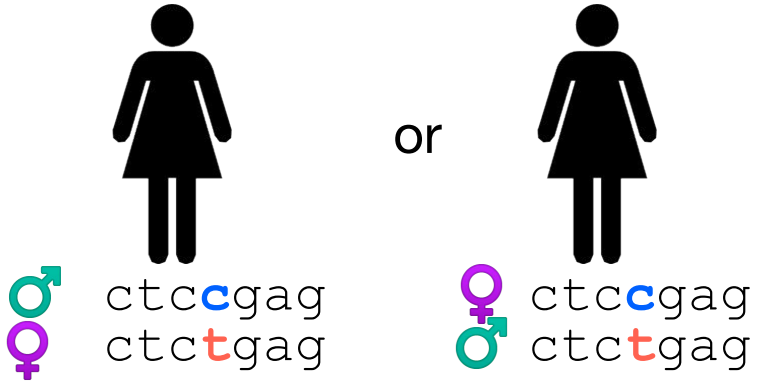
Inherited Germline Variation



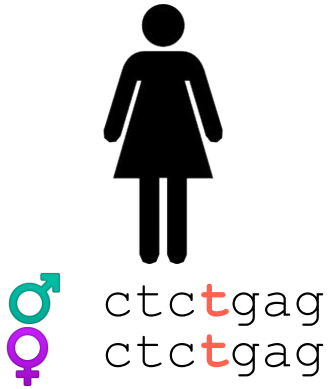
Example: Mom and dad are heterozygous; that is, the zygote from which they developed was comprised of a sperm and egg with two different alleles



Kid is homozygous (C/C)

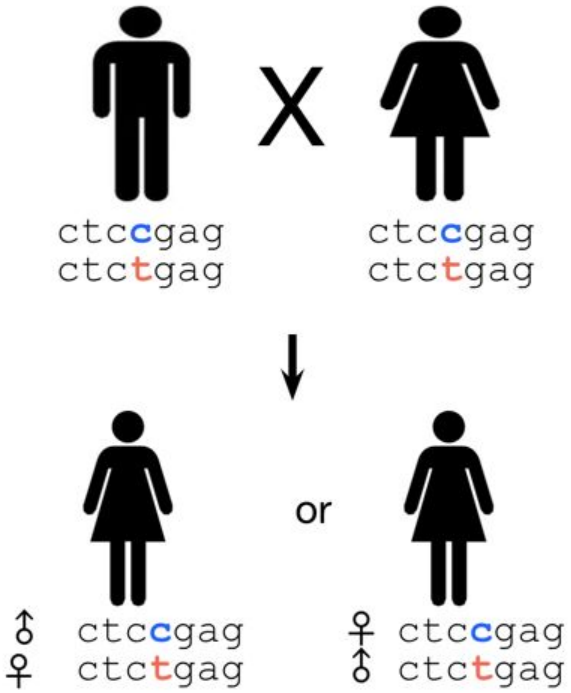


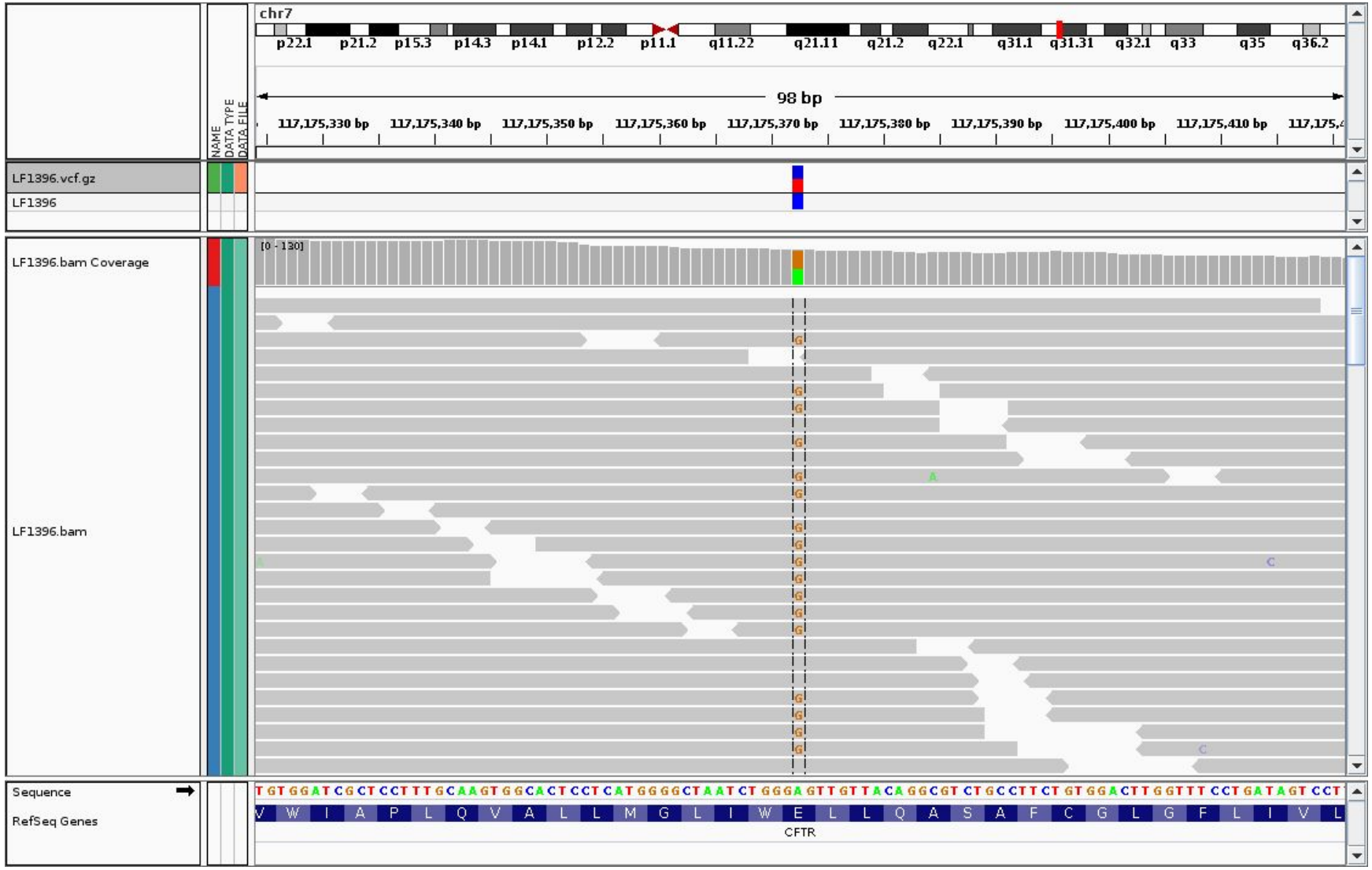
Kid is heterozygous (C/T)



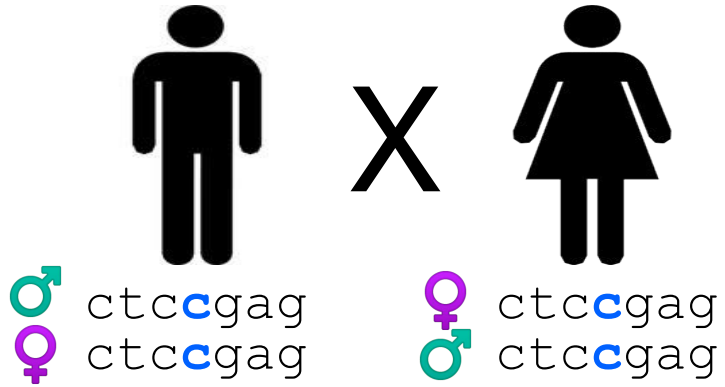
Kid is homozygous (T/T)

Heterozygous Variation





De novo Mutation

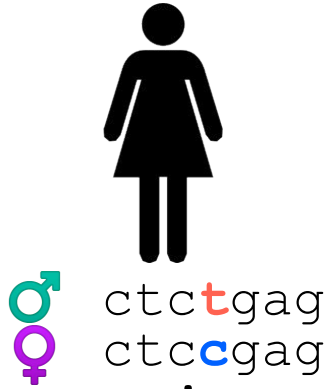


Example: Mom and dad are homozygous for the same alleles.

New mutation occurs in father's or mother's germ cell

♂ ctccgag → ♂ ctctgag

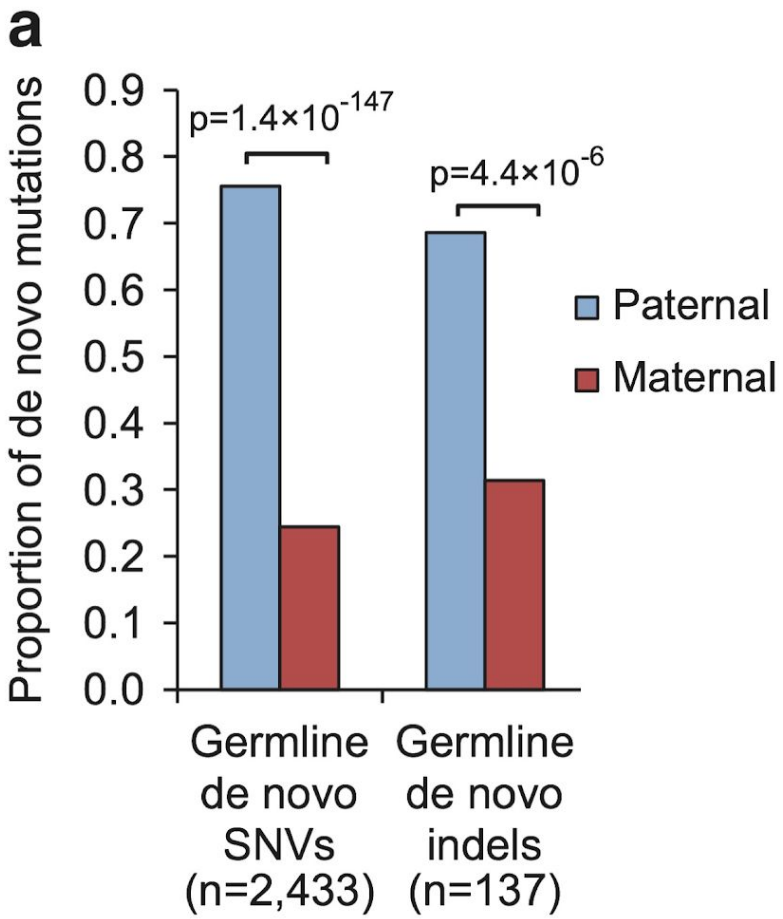
Note: This is a derivative chromosome of the one the father inherited from His parents



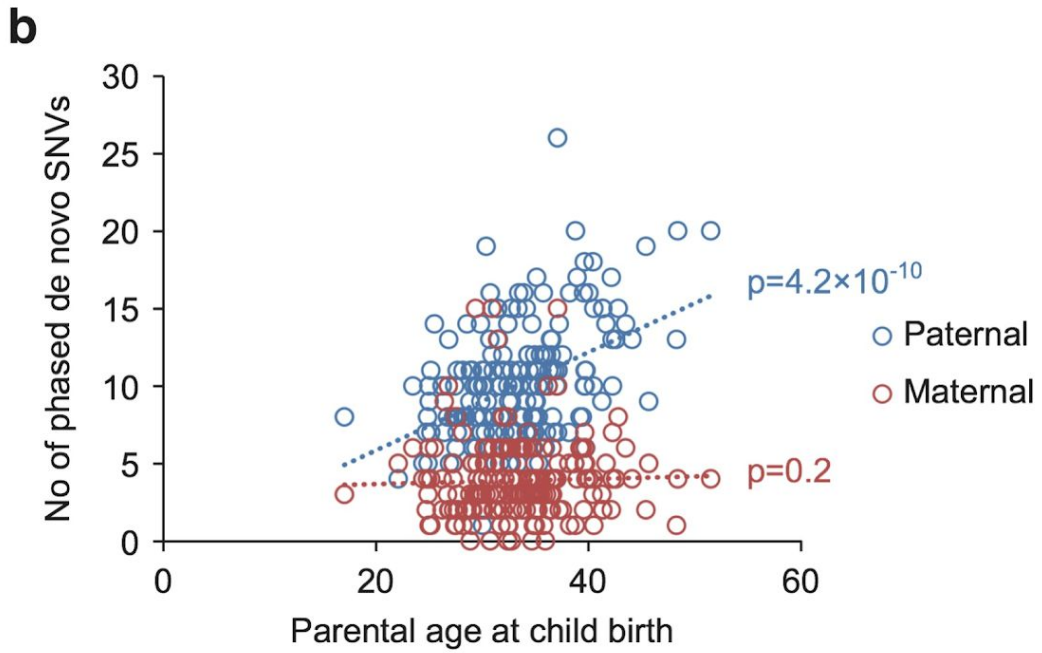
Kid is heterozygous owing to *de novo mutation.*

(C/T)

DNMs Frequency



2 new DNMs per year of paternal age (Kong et al. 2012, *Nature*)



(data from 200 ASD trios)

Yuen et al. (2016) *Nature Genomic Medicine*

Tom Sasani

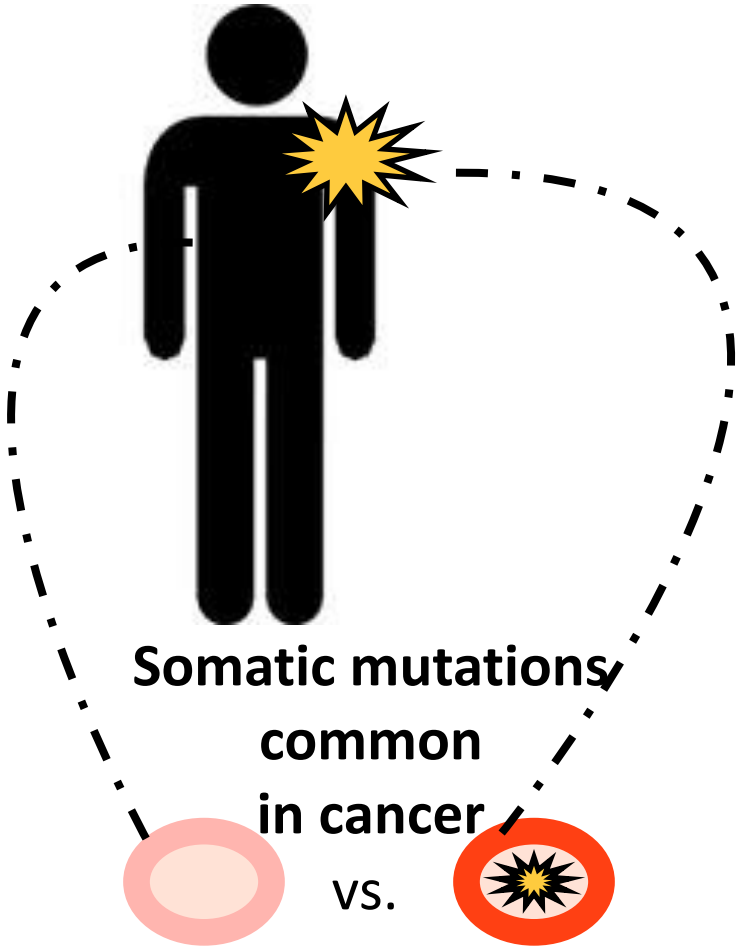
Somatic Mutations

Germline mutation

- occur in sperm or egg.
- are heritable

Somatic mutation

- non-germline tissues.
- **<u>are not heritable</u>**



compare DNA from cancer cells to healthy cells from same individual

The 1000 (2504) Genome Project

ARTICLE

OPEN

doi:10.1038/nature15393

A global reference for human genetic variation

The 1000 Genomes Project Consortium*

The 1000 Genomes Project set out to provide a comprehensive description of common human genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations. Here we report completion of the project, having reconstructed the genomes of 2,504 individuals from 26 populations using a combination of low-coverage whole-genome sequencing, deep exome sequencing, and dense microarray genotyping. We characterized a broad spectrum of genetic variation, in total over 88 million variants (84.7 million single nucleotide polymorphisms (SNPs), 3.6 million short insertions/deletions (indels), and 60,000 structural variants), all phased onto high-quality haplotypes. This resource includes >99% of SNP variants with a frequency of >1% for a variety of ancestries. We describe the distribution of genetic variation across the global sample, and discuss the implications for common disease studies.

The 1000 (2504) Genome Project

2,504 individuals from diverse ancestries

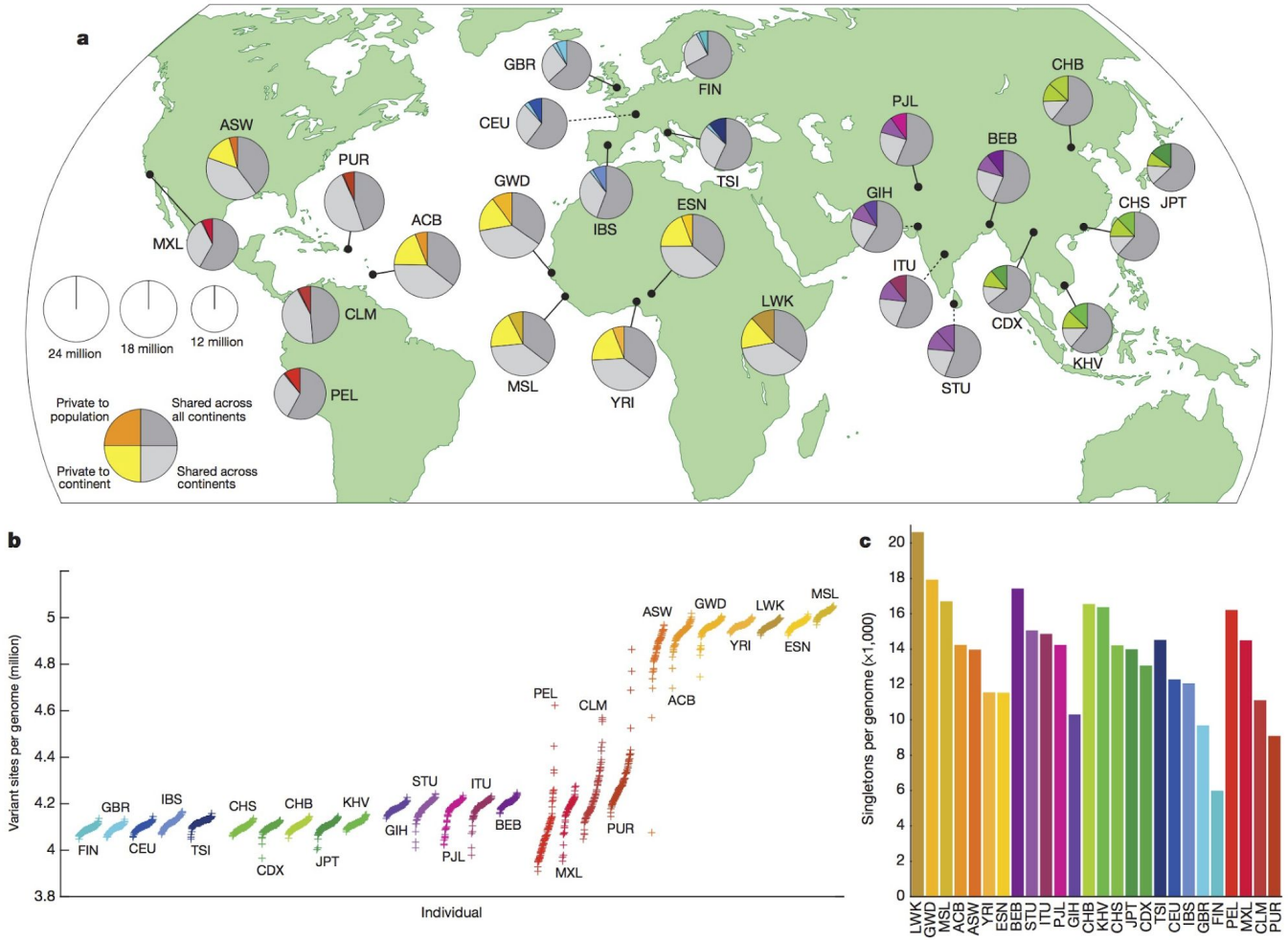


Figure 1 | Population sampling. **a**, Polymorphic variants within sampled populations. The area of each pie is proportional to the number of polymorphisms within a population. Pies are divided into four slices, representing variants private to a population (darker colour unique to population), private to a continental area (lighter colour shared across continental group), shared across continental areas (light grey), and shared across all continents (dark grey). Dashed lines indicate populations sampled outside of their ancestral continental region. **b**, The number of variant sites per genome. **c**, The average number of singletons per genome.

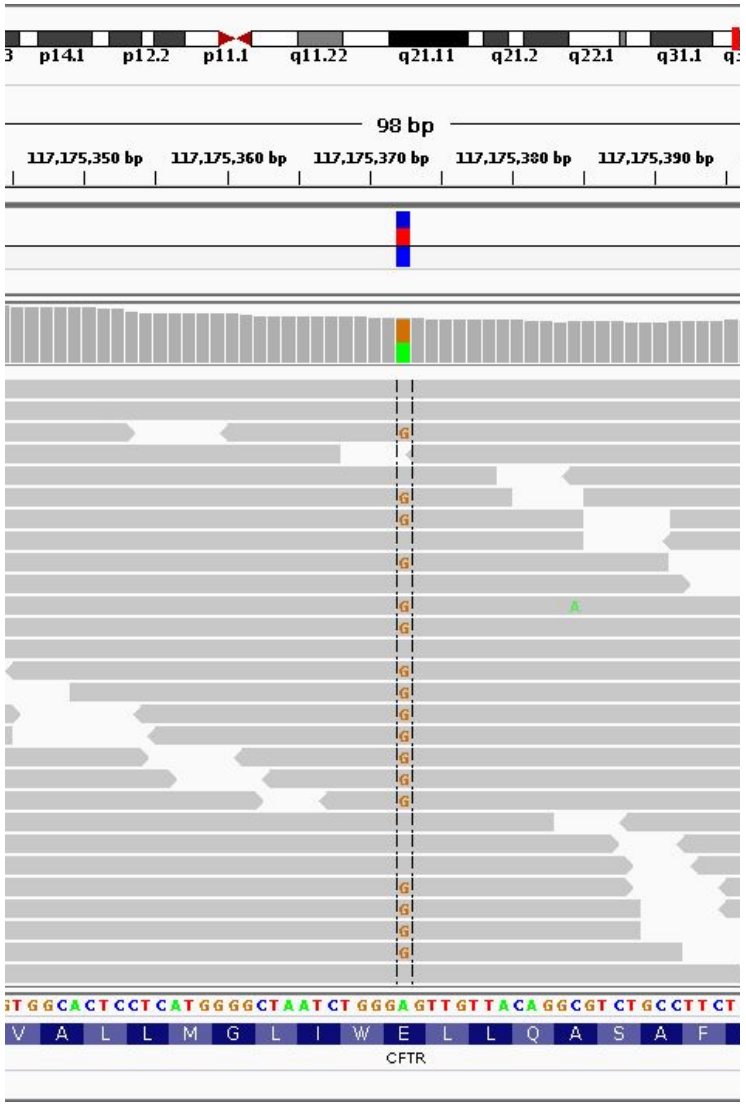
The extent of genetic variation by subpopulation

Table 1 | Median autosomal variant sites per genome

	AFR		AMR		EAS		EUR		SAS	
Samples	661		347		504		503		489	
Mean coverage	8.2		7.6		7.7		7.4		8.0	
	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons
SNPs	4.31M	14.5k	3.64M	12.0k	3.55M	14.8k	3.53M	11.4k	3.60M	14.4k
Indels	625k	-	557k	-	546k	-	546k	-	556k	-
Large deletions	1.1k	5	949	5	940	7	939	5	947	5
CNVs	170	1	153	1	158	1	157	1	165	1
MEI (Alu)	1.03k	0	845	0	899	1	919	0	889	0
MEI (L1)	138	0	118	0	130	0	123	0	123	0
MEI (SVA)	52	0	44	0	56	0	53	0	44	0
MEI (MT)	5	0	5	0	4	0	4	0	4	0
Inversions	12	0	9	0	10	0	9	0	11	0
Nonsynon	12.2k	139	10.4k	121	10.2k	144	10.2k	116	10.3k	144
Synon	13.8k	78	11.4k	67	11.2k	79	11.2k	59	11.4k	78
Intron	2.06M	7.33k	1.72M	6.12k	1.68M	7.39k	1.68M	5.68k	1.72M	7.20k
UTR	37.2k	168	30.8k	136	30.0k	169	30.0k	129	30.7k	168
Promoter	102k	430	84.3k	332	81.6k	425	82.2k	336	84.0k	430
Insulator	70.9k	248	59.0k	199	57.7k	252	57.7k	189	59.1k	243
Enhancer	354k	1.32k	295k	1.05k	289k	1.34k	288k	1.02k	295k	1.31k
TFBSs	927	4	759	3	748	4	749	3	765	3
Filtered LoF	182	4	152	3	153	4	149	3	151	3
HGMD-DM	20	0	18	0	16	1	18	2	16	0
GWAS	2.00k	0	2.07k	0	1.99k	0	2.08k	0	2.06k	0
ClinVar	28	0	30	1	24	0	29	1	27	1

See Supplementary Table 1 for continental population groupings. CNVs, copy-number variants; HGMD-DM, Human Gene Mutation Database disease mutations; k, thousand; LoF, loss-of-function; M, million; MEI, mobile element insertions.

Variant Calling



What information is needed to decide if a variant exists?

- Depth of coverage at the locus
- Bases observed at the locus
- The base qualities of each allele
- The strand composition
- Mapping qualities
- Proper pairs?
- Expected polymorphism rate

Bayes' Theorem

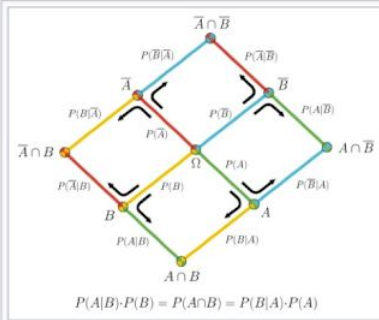
Statement of theorem [edit]

Bayes' theorem is stated mathematically as the following equation:[2]

$$P(A \mid B) = \frac{P(B \mid A)\,P(A)}{P(B)},$$

where A and B are events and $P(B) \neq 0$.

- $P(A)$ and $P(B)$ are the probabilities of observing A and B without regard to each other.
- $P(A \mid B)$, a conditional probability, is the probability of observing event A given that B is true.
- $P(B \mid A)$ is the probability of observing event B given that A is true.



Visualization of Bayes' theorem by the superposition of two decision trees

History [edit]

Bayes' theorem was named after the Reverend Thomas Bayes (1701–1761), who studied how to compute a distribution for the probability parameter of a binomial distribution (in modern terminology). Bayes' unpublished manuscript was significantly edited by Richard Price before it was posthumously read at the Royal Society. Price edited[3] Bayes' major work "An Essay towards solving a Problem in the Doctrine of Chances" (1763), which appeared in "Philosophical Transactions,"[4] and contains Bayes' Theorem. Price wrote an introduction to the paper which provides some of the philosophical basis of Bayesian statistics. In 1765 he was elected a Fellow of the Royal Society in recognition of his work on the legacy of Bayes.[5][6]

The French mathematician Pierre-Simon Laplace reproduced and extended Bayes' results in 1774, apparently quite unaware of Bayes' work.[7][8] The Bayesian interpretation of probability was developed mainly by Laplace.[9]

Stephen Stigler suggested in 1983 that Bayes' theorem was discovered by Nicholas Saunderson, a blind English mathematician, some time before Bayes;[10][11] that interpretation, however, has been disputed.[12] Martyn Hooper[13] and Sharon McGrayne[14] have argued that Richard Price's contribution was substantial:

> By modern standards, we should refer to the Bayes–Price rule. Price discovered Bayes' work, recognized its importance, corrected it, contributed to the article, and found a use for it. The modern convention of employing Bayes' name alone is unfair but so entrenched that anything else makes little sense.[14]

https://en.wikipedia.org/wiki/Thomas_Bayes

Bayes' Theorem Applications

Widely used in machine learning and finance.

Decision making in driverless cars

Email spam detection

Assess disease risk from test results

Voice recognition software

Text autocomplete…

Bayes' Theorem

$$P(A|B) = \frac{P(B|A) * P(A)}{P(B)}$$

Conditional probability. That is, the probability of A occurring, given that B has occurred.

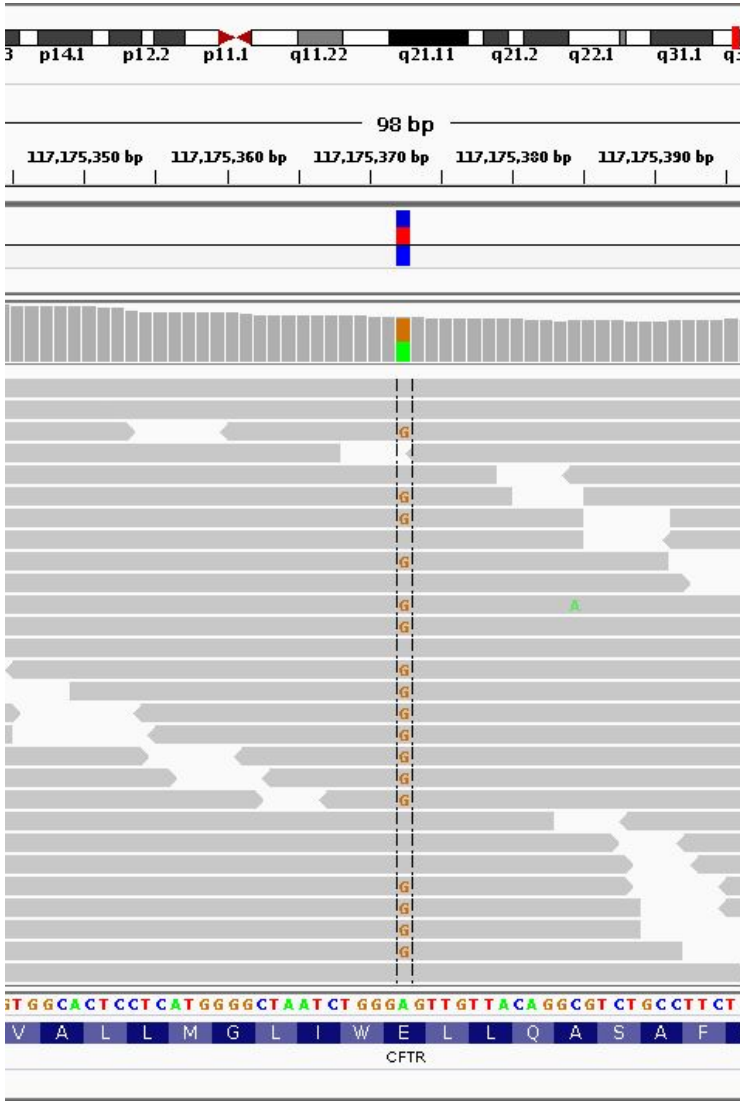
Bayes' Theorem

Posterior probability

Prior Probability Of A

$$P(A|B) = \frac{P(B|A) * P(A)}{P(B)}$$

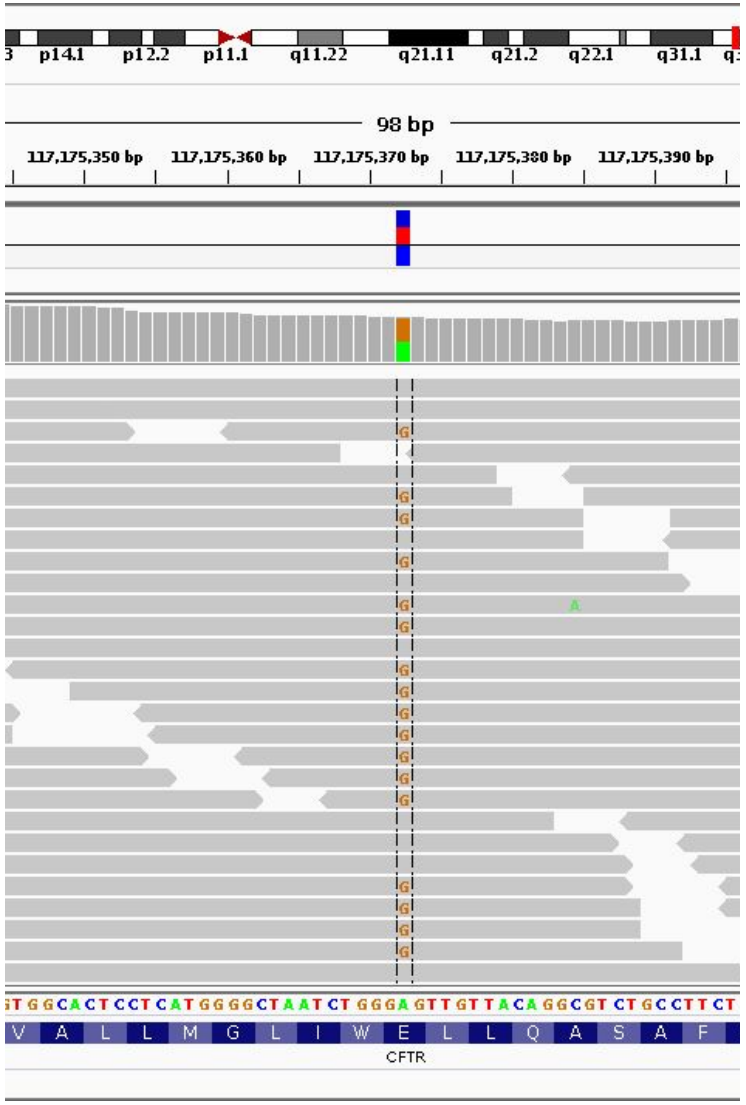
Bayesian SNP Calling



$$P(SNP|Data) = \frac{P(Data|SNP) * P(SNP)}{P(Data)}$$

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-10-r99

Bayesian SNP Calling



$$P(SNP|Data) = \frac{P(Data|SNP) * P(SNP)}{P(Data)}$$

- Depth of coverage at the locus
- Bases observed at the locus
- The base qualities of each allele
- The strand composition
- Mapping qualities
- Proper pairs?
- Expected polymorphism rate

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-10-r99

Bayesian SNP Calling

Bayesian posterior probability

Base call + Base quality

Expected (prior) polymorphism rate

$$P(SNP) = \sum_{all} \sum_{variable} S \frac{\frac{P(S_1 \mid R_1)}{P_{Prior}(S_1)} \cdot \ldots \cdot \frac{P(S_N \mid R_N)}{P_{Prior}(S_N)} \cdot P_{Prior}(S_1, \ldots, S_N)}{\sum_{S_{i_1} \in [A,C,G,T]} \ldots \sum_{S_{i_N} \in [A,C,G,T]} \frac{P(S_{i_1} \mid R_1)}{P_{Prior}(S_{i_1})} \cdot \ldots \cdot \frac{P(S_{i_N} \mid R_1)}{P_{Prior}(S_{i_N})} \cdot P_{Prior}(S_{i_1}, \ldots, S_{i_N})}$$

Probability of observed base composition (should model sequencing error rate)

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-10-r99

Genome Analysis Toolkit (GATK)

NATURE GENETICS | TECHNICAL REPORT

日本語要約

A framework for variation discovery and genotyping using next-generation DNA sequencing data

Mark A DePristo, Eric Banks, Ryan Poplin, Kiran V Garimella, Jared R Maguire, Christopher Hartl, Anthony A Philippakis, Guillermo del Angel, Manuel A Rivas, Matt Hanna, Aaron McKenna, Tim J Fennell, Andrew M Kernytsky, Andrey Y Sivachenko, Kristian Cibulskis, Stacey B Gabriel, David Altshuler & Mark J Daly

Affiliations | Contributions | Corresponding author

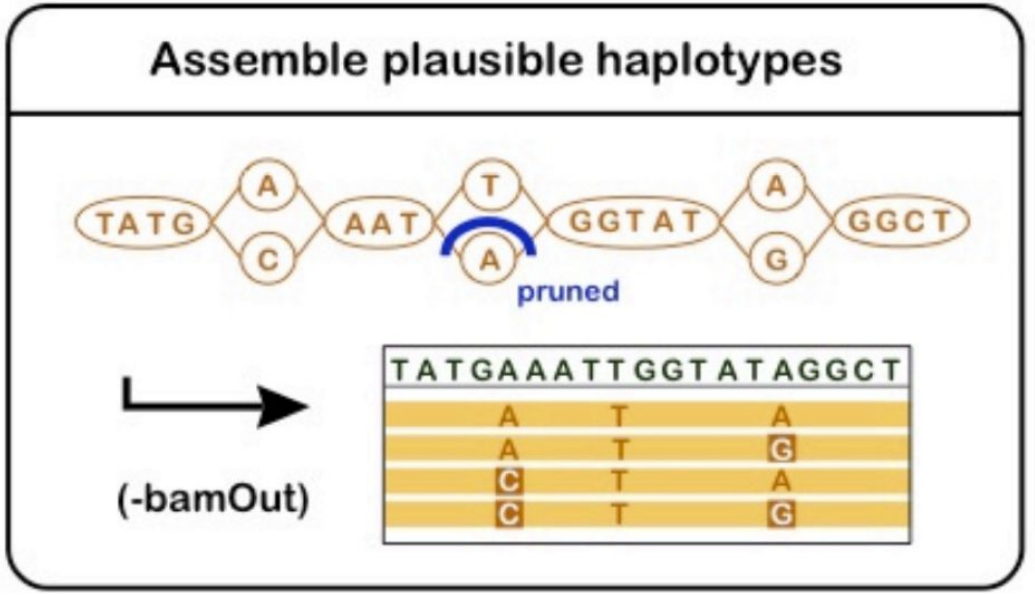
Nature Genetics **43**, 491–498 (2011) | doi:10.1038/ng.806

Received 27 August 2010 | Accepted 17 March 2011 | Published online 10 April 2011

http://www.nature.com/ng/journal/v43/n5/full/ng.806.html

GATK's HaplotypeCaller

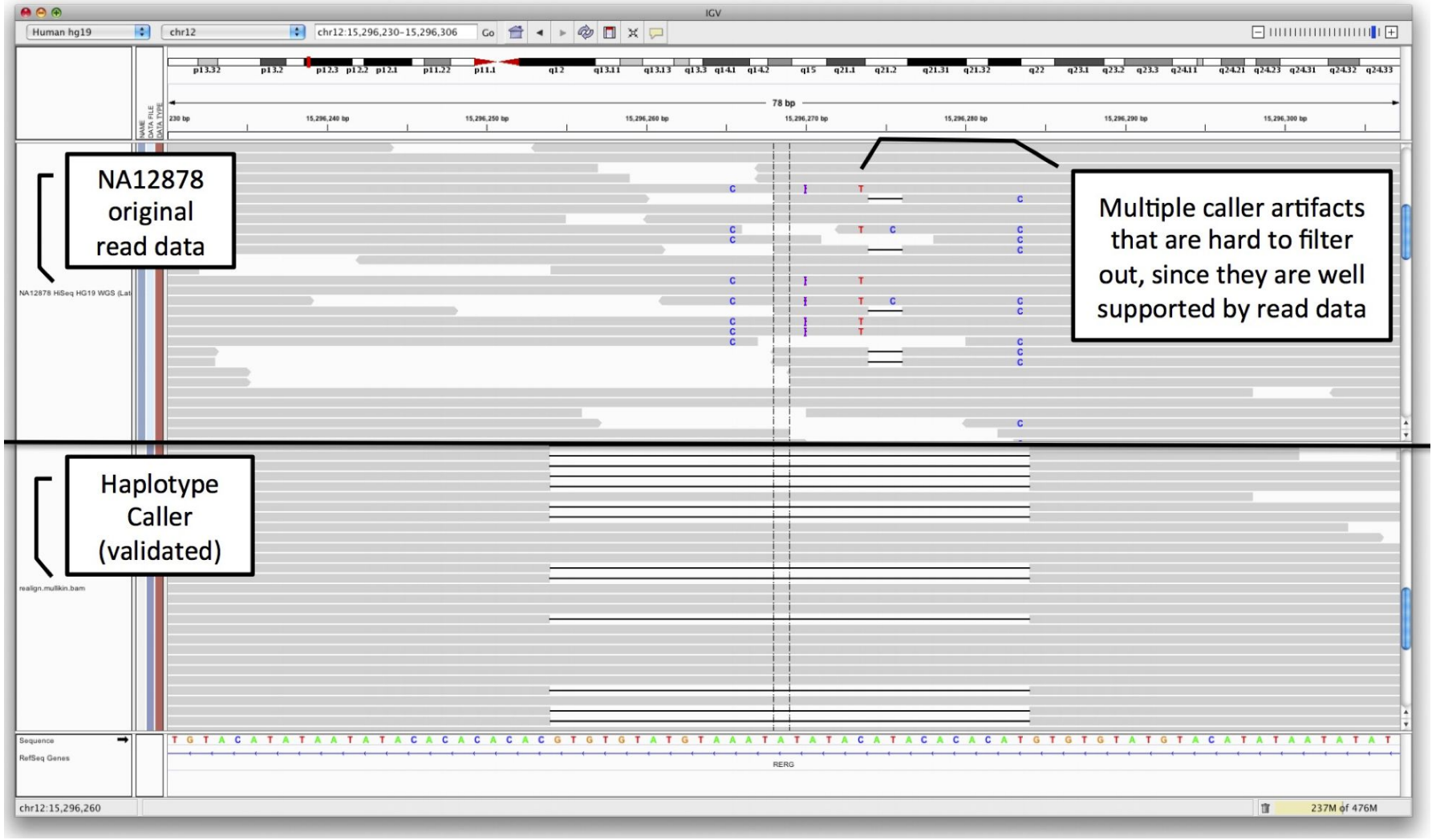
- Local re-assembly
- Traverse graph to collect most likely haplotypes
- Align haplotypes to ref using Smith-Waterman



Likely haplotypes + candidate variant sites

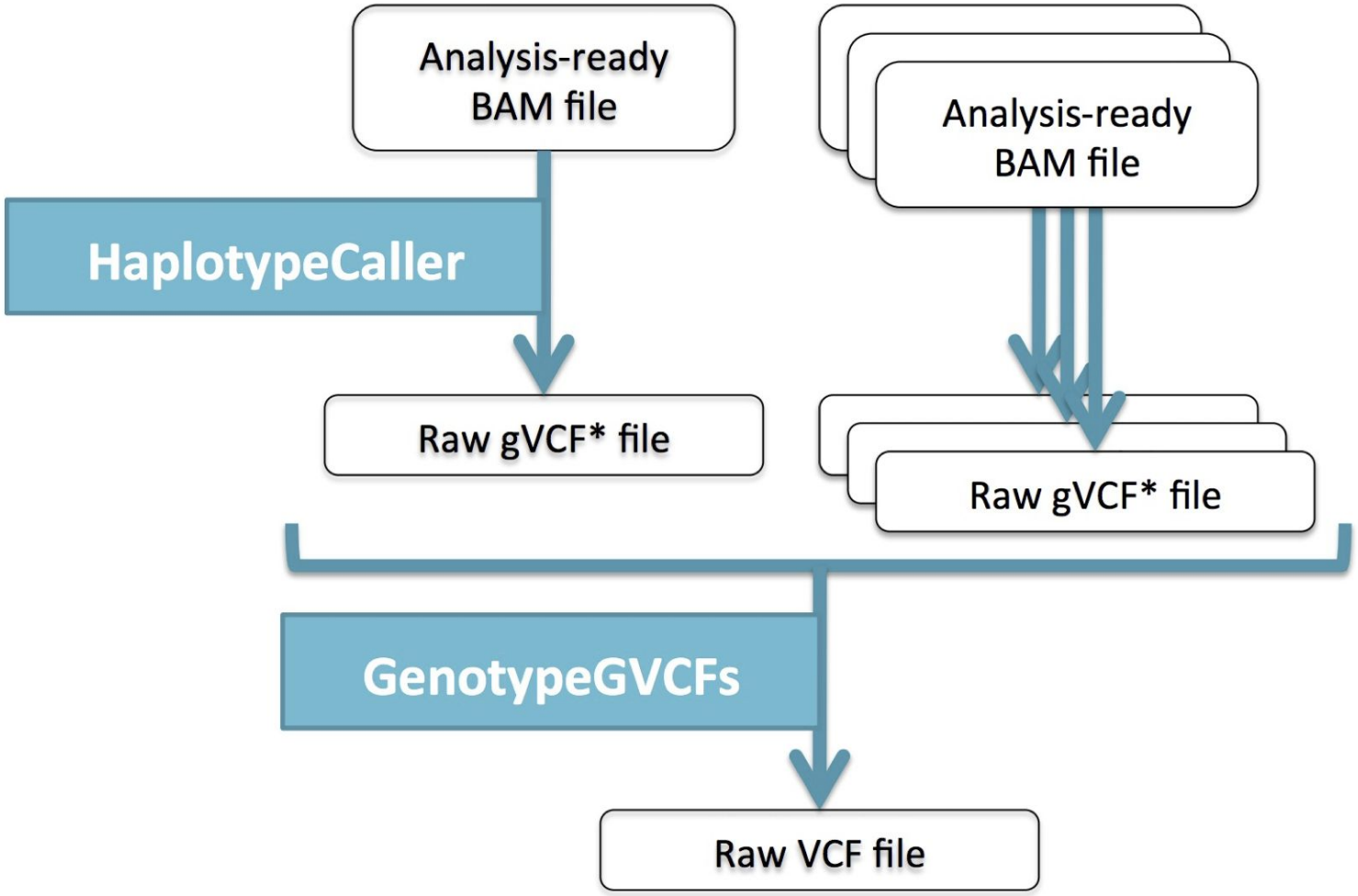
https://software.broadinstitute.org/gatk/events/slides/1506/GATKwr8-D-2-Variant_calling_and_joint_genotyping.pdf

GATK's HaplotypeCaller



https://software.broadinstitute.org/gatk/events/slides/1506/GATKwr8-D-2-Variant_calling_and_joint_genotyping.pdf

GATK's HaplotypeCaller



https://software.broadinstitute.org/gatk/events/slides/1506/GATKwr8-D-2-Variant_calling_and_joint_genotyping.pdf

Variant Calling

VCF Format

BIOINFORMATICS APPLICATIONS NOTE

Vol. 27 no. 15 2011, pages 2156–2158
doi:10.1093/bioinformatics/btr330

Sequence analysis

Advance Access publication June 7, 2011

The variant call format and VCFtools

Petr Danecek[1,†], Adam Auton[2,†], Goncalo Abecasis[3], Cornelis A. Albers[1], Eric Banks[4], Mark A. DePristo[4], Robert E. Handsaker[4], Gerton Lunter[2], Gabor T. Marth[5], Stephen T. Sherry[6], Gilean McVean[2,7], Richard Durbin[1,*] and 1000 Genomes Project Analysis Group[‡]

[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge CB10 1SA, [2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK, [3]Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109, [4]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA 02141, [5]Department of Biology, Boston College, MA 02467, [6]National Institutes of Health National Center for Biotechnology Information, MD 20894, USA and [7]Department of Statistics, University of Oxford, Oxford OX1 3TG, UK

Associate Editor: John Quackenbush

ABSTRACT

Summary: The variant call format (VCF) is a generic format for storing DNA polymorphism data such as SNPs, insertions, deletions and structural variants, together with rich annotations. VCF is usually stored in a compressed manner and can be indexed for fast data retrieval of variants from a range of positions on the reference genome. The format was developed for the 1000 Genomes Project, and has also been adopted by other projects such as UK10K, dbSNP and the NHLBI Exome Project. VCFtools is a software suite that implements various utilities for processing VCF files, including validation, merging, comparing and also provides a general Perl API.

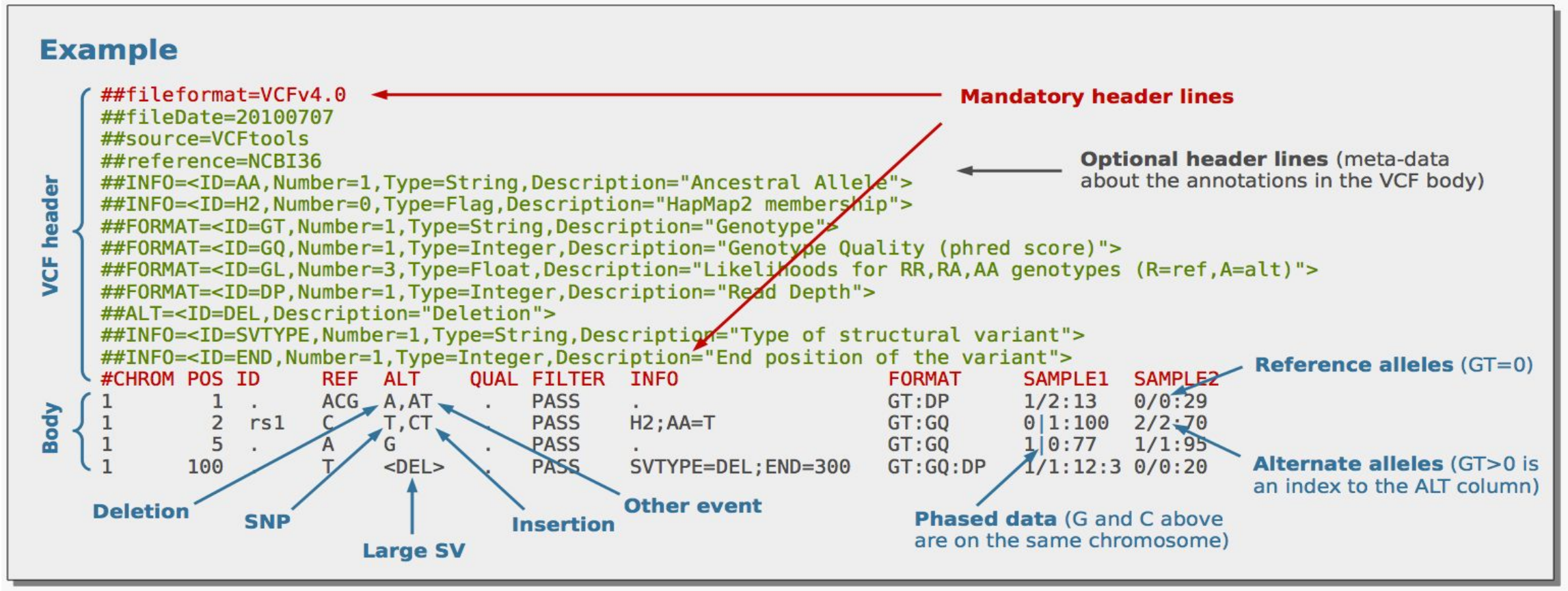
Availability: http://vcftools.sourceforge.net

Contact: rd@sanger.ac.uk

Although generic feature format (GFF) has recently been extended to standardize storage of variant information in genome variant format (GVF) (Reese *et al.*, 2010), this is not tailored for storing information across many samples. We have designed the VCF format to be scalable so as to encompass millions of sites with genotype data and annotations from thousands of samples. We have adopted a textual encoding, with complementary indexing, to allow easy generation of the files while maintaining fast data access. In this article, we present an overview of the VCF and briefly introduce the companion VCFtools software package. A detailed format specification and the complete documentation of VCFtools are available at the VCFtools web site.

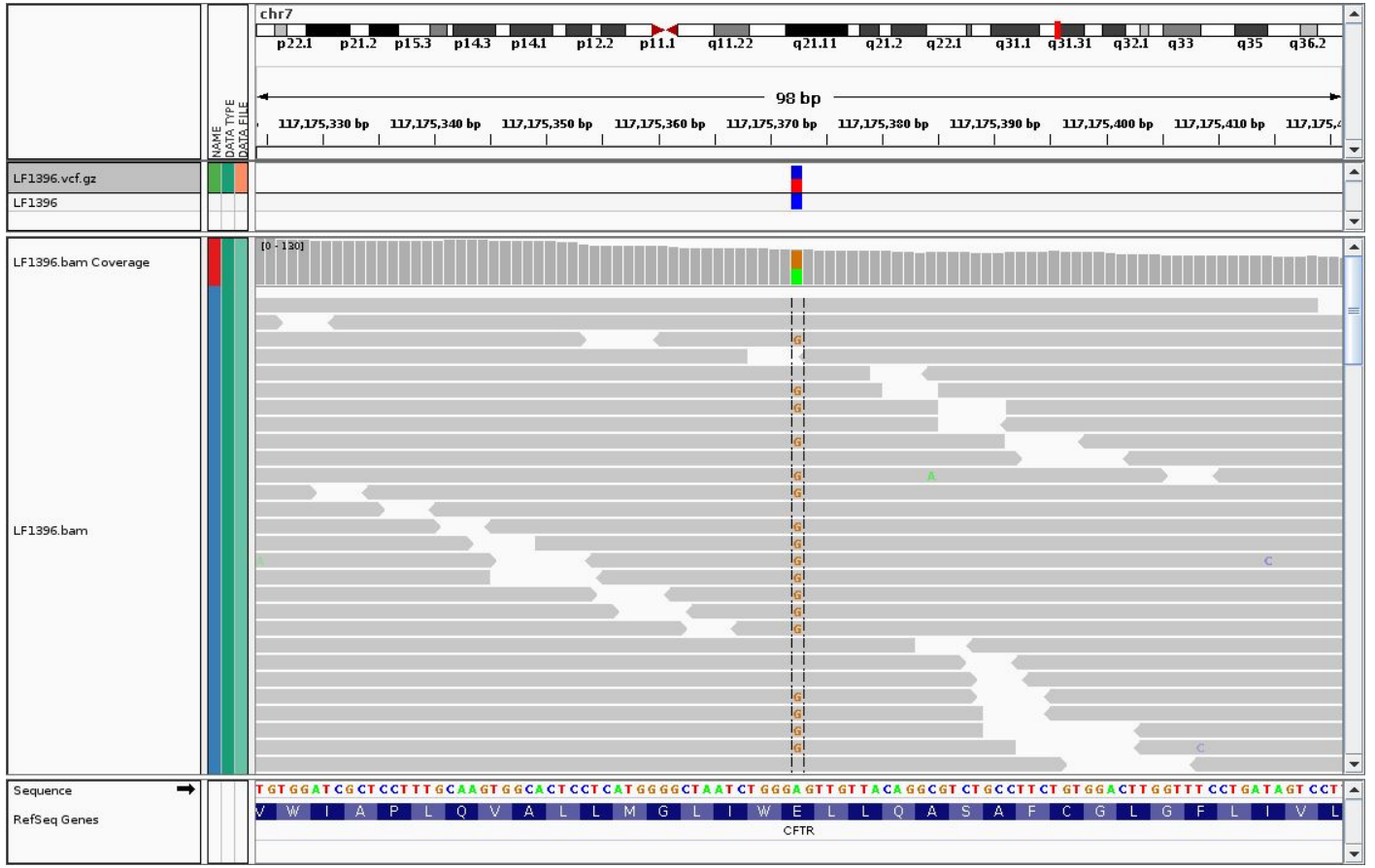
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3137218/pdf/btr330.pdf

VCF Format



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3137218/pdf/btr330.pdf

VCF Format



Heterozygous A/G. The REF allele is allele "0", ALT is allele "1"

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	LF1396
chr7	117175373	.	A	G	90	PASS	AF=0.5	GT	0/1