Mapping and SNP

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IRD

TP goal

- Quality control of NGS data
- Learn to manipulate NGS data
- Having a critical look on *Mapping*
- Learn to launch a *Calling* and having a critical look

The data

Diploid Asian Rice, *Oryza sativa*

From Wikimedia

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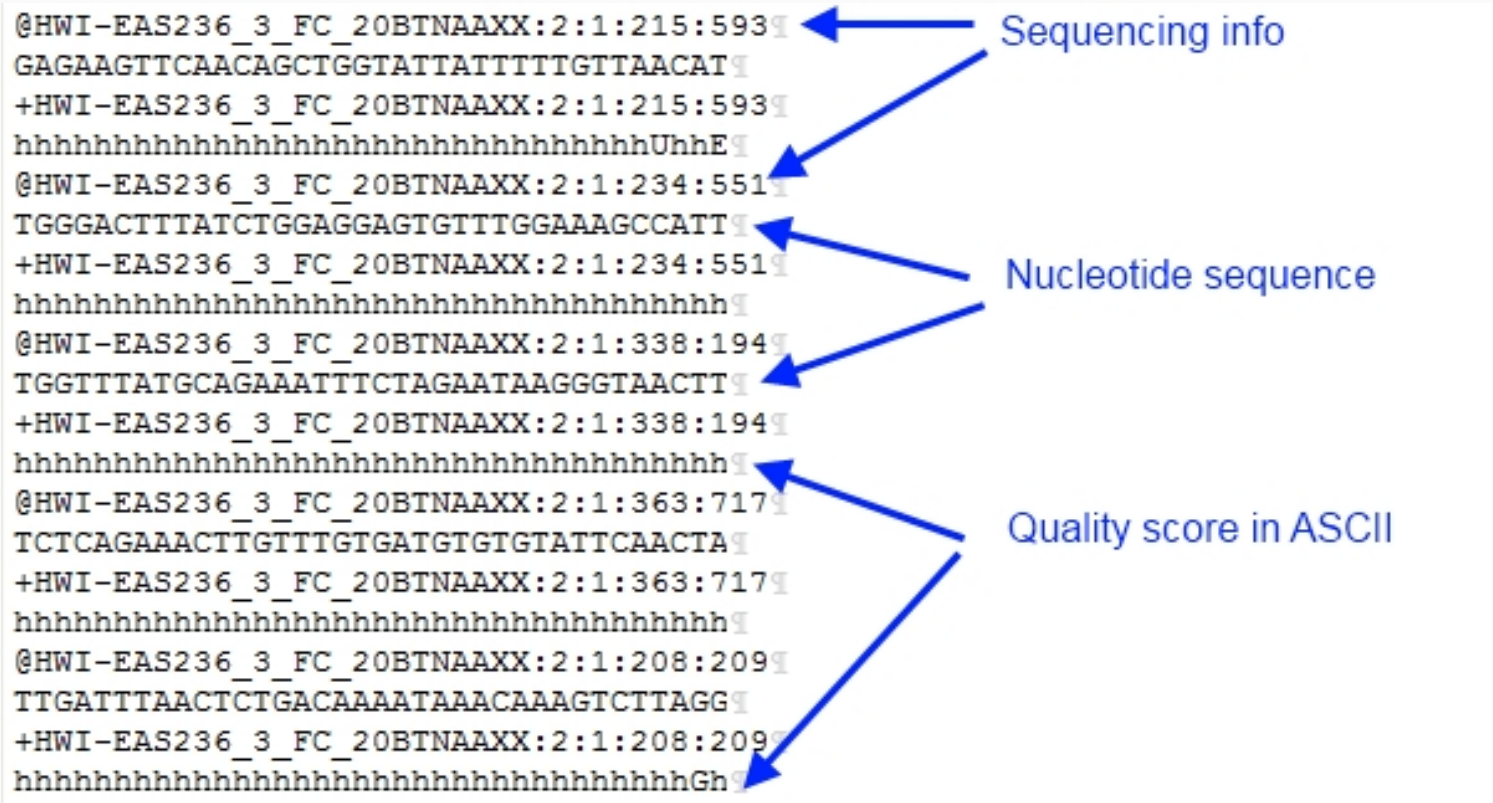
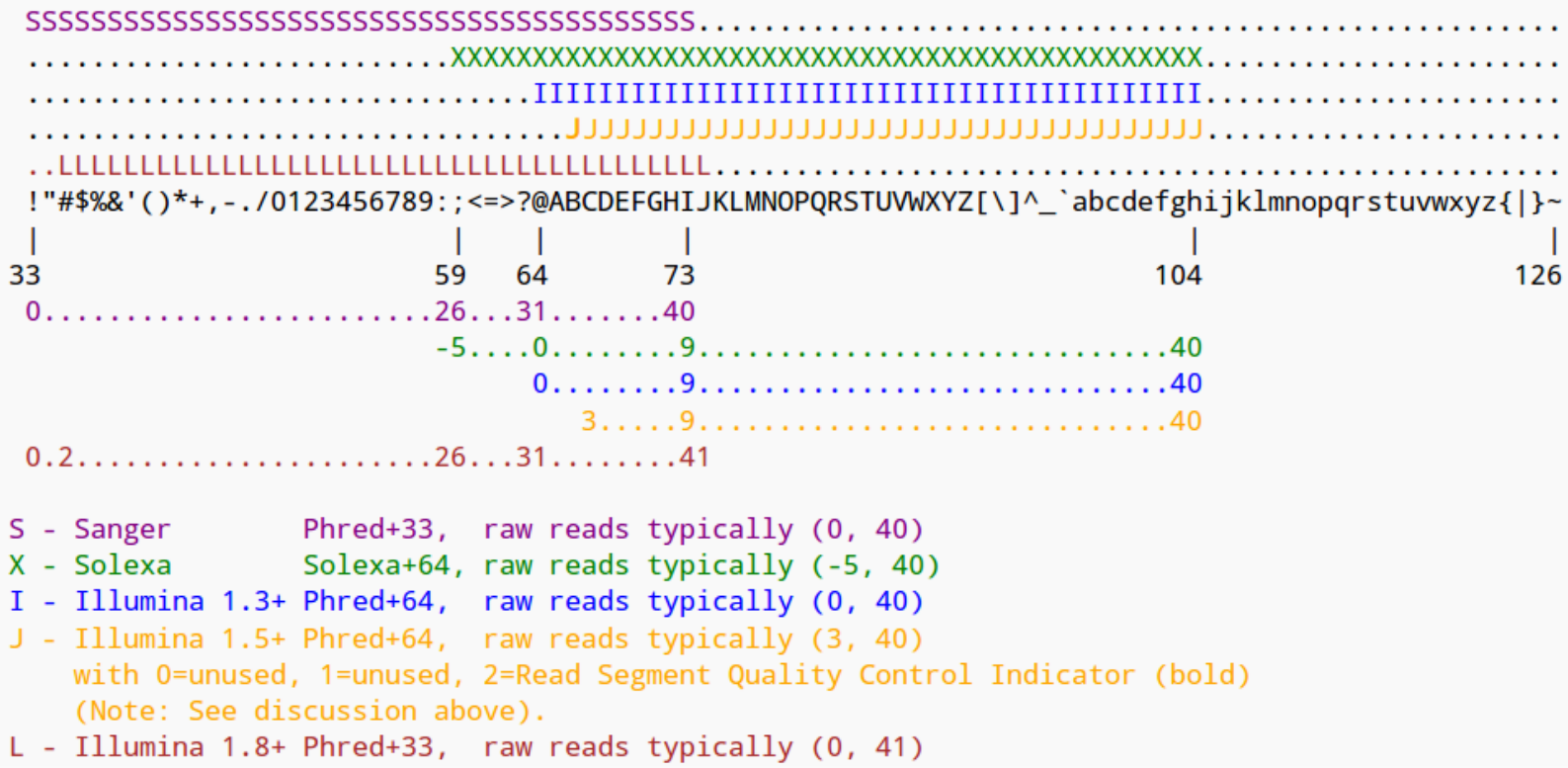
4. Generate short & long reads for each clone...
5. ~~Torturing students with these data~~

The FASTQ Format



The QPHRED Scale



List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...

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Between 8 and 15 different commands...

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5.
6.

SAM file format for Sequence Alignment Map

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

Type	Tag	Description
HD - header	VN*	File format version.
	SO	Sort order. Valid values are: *unsorted*, *queryname* or *coordinate*.
	GO	Group order (full sorting is not imposed in a group). Valid values are: *none*, *query* or *reference*.
SQ - Sequence dictionary	SN*	Sequence name. Unique among all sequence records in the file. The value of this field is used in alignment records.
	LN*	Sequence length.
	AS	Genome assembly identifier. Refers to the reference genome assembly in an unambiguous form. Example: HG18.
	M5	MD5 checksum of the sequence in the uppercase (gaps and space are removed)
	UR	URI of the sequence
	SP	Species.
RG - read group	ID*	Unique read group identifier. The value of the ID field is used in the RG tags of alignment records.
	SM*	Sample (use pool name where a pool is being sequenced)
	LB	Library
	DS	Description
	PU	Platform unit (e.g. lane for Illumina or slide for SOLiD); should be a full, unambiguous identifier
	PI	Predicted median insert size (maybe different from the actual median insert size)
	CN	Name of sequencing center producing the read.
	DT	Date the run was produced (ISO 8601 date or date/time).
	PL	Platform/technology used to produce the read.
PG - Program	ID*	Program name
	VN	Program version
	CL	Command line
CO - comment		One-line text comments

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Col	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	NRNM	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 reference
11	QUAL	query QUALity (ASCII-33

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA   *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       *  0   0 TAGGC    *   NM:i:0
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT         *
```

SAM format: FLAG field

numeric	binary	description
1	00000001	template has multiple fragments in sequencing
2	00000010	each fragment properly mapped according to aligner
4	00000100	fragment is unmapped
8	00001000	mate is unmapped
16	00010000	sequence is reverse complemented
32	00100000	sequence of mate is reversed
64	01000000	is first fragment in template
128	10000000	is second fragment in template

From J. Aerts, in SlideShare

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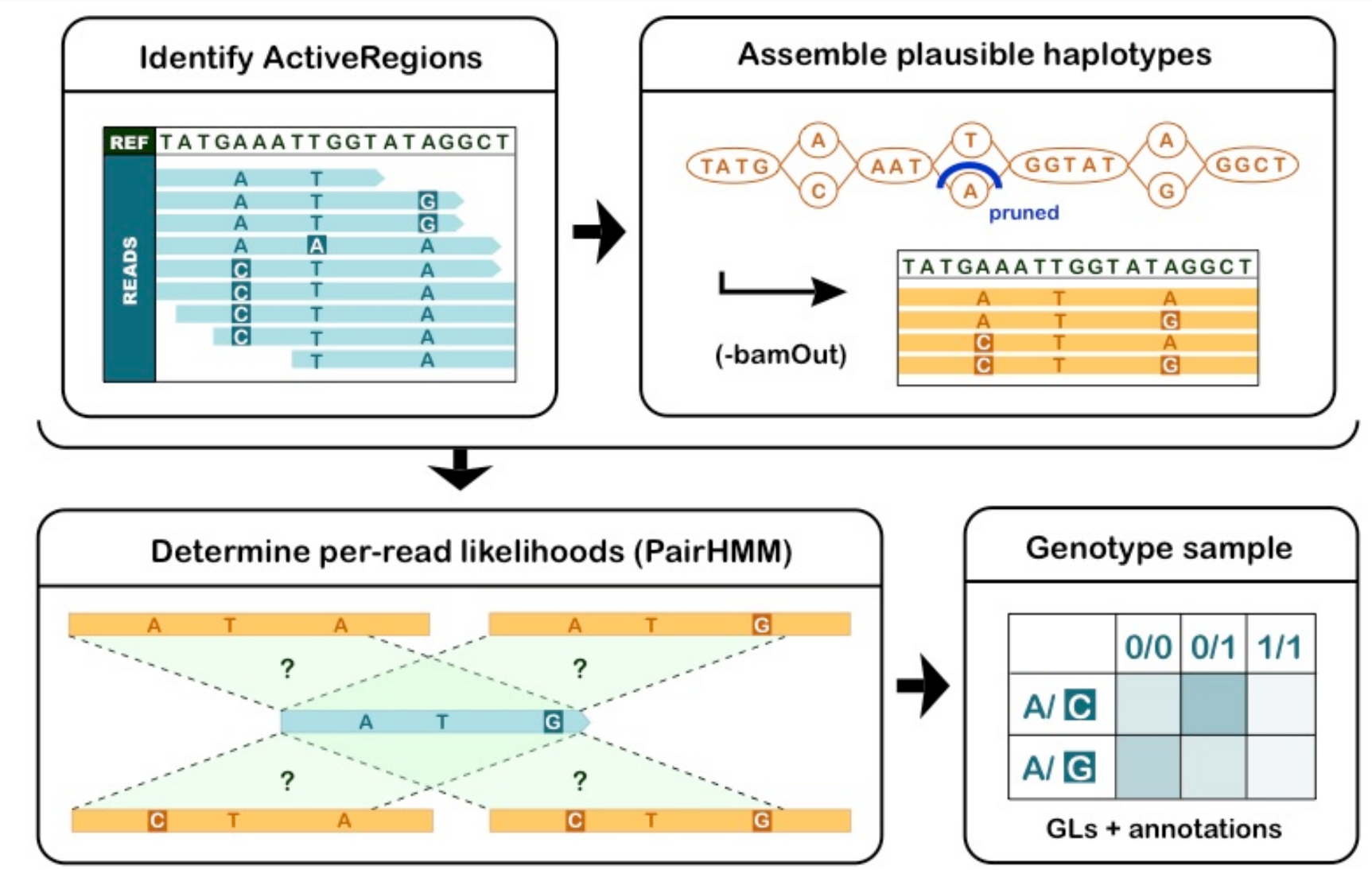
5. Mark the duplicates
6. Call SNP on this individual

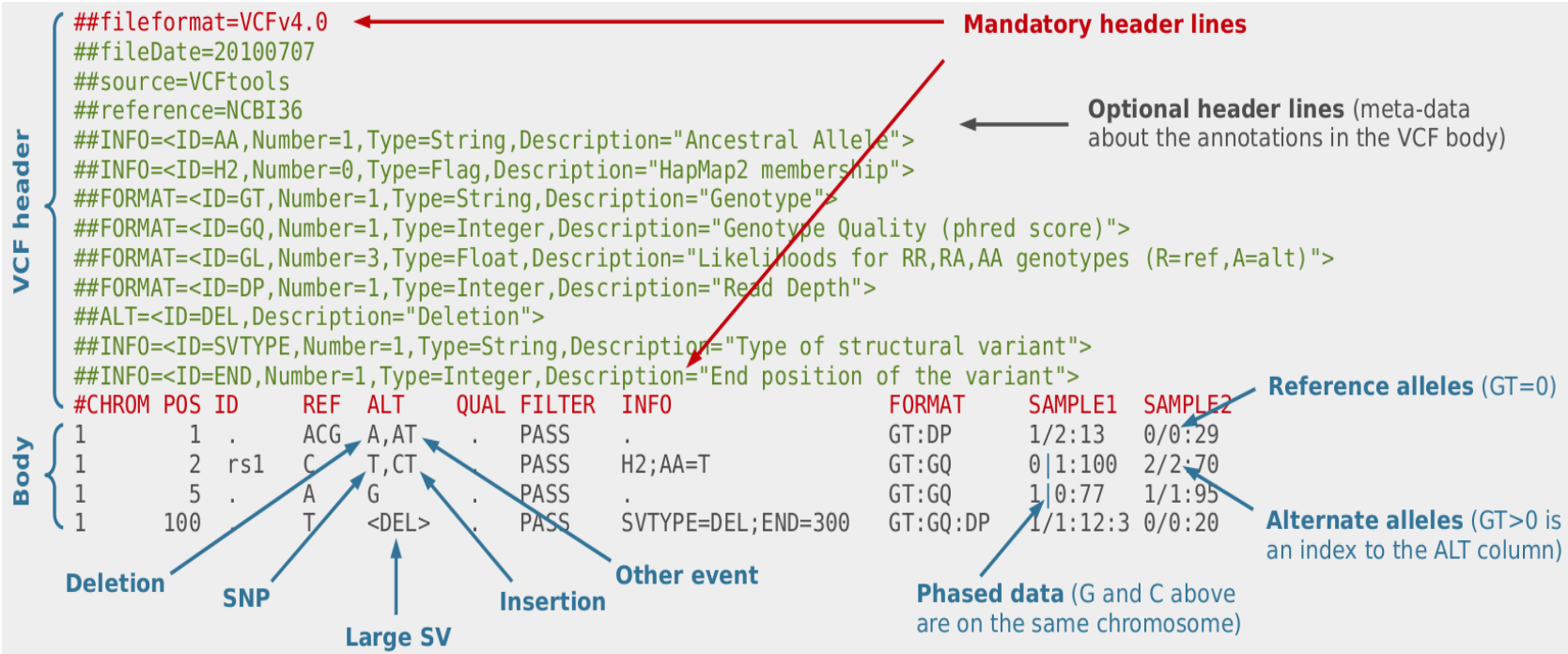
The HaplotypeCaller



From UniBe.ch Training

VCF file format for Variant Call Format

The Variant Call Format (VCF) used in bioinformatics for storing gene sequence variations



See you tomorrow…

A DEMAIN!!

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- Look at the mapping and try to find SNPs

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- QUAL filter:
  QUAL < 200
- SNPcluster filter:
  more than 3 SNP per 10b

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Basics Population Genomics using *sNMF*

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- R and Command-line version
- Much faster than ADMIXTURE or STRUCTURE, as efficient

Be “lazy” but clever

Problems with manual launches

- Long
- Fastidious
- Error prone
- Tracability and reproducibilty not ensured

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Solution $\Longrightarrow$ Workflow Manager (or scripts...): SnakeMake, TOGGLe, NextFlow

Thanks for your attention