Intro to NGS
Data Processing and Formats

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Copenhagen Popgen Summer course 2022

With some poached materials from Anders Albrechtsen

Some motivations for Next Generation Sequencing, NGS.

- More data!
- Efficient for detecting and characterizing **structural variation**.
- Genome-wide association studies (**GWAS**).
- **Landscape of genetic variability** E.g. How important are average levels of genetic diversity to population viability compared to how it's distributed along the genome.
- **Demography** Ability to infer different genealogical histories along the genome (e.g. PSMC) and summarize variation across the entire genome (e.g. SFS) has greatly enhanced our ability to reconstruct population histories.
- **Role of hybridization in evolution**. NGS has greatly enhanced our view of how species engage one another to influence biodiversity and evolution.
- **Genetic monitoring** through eDNA and metabarcoding.
- Effective for sequencing degraded **historic and ancient DNA**.

What’s your favorite flavor?

- Whole genome sequencing (WGS)
  - Short read (~100-150 bp reads)
  - Long read (>10 kb long reads)

- Reduced representation targets a fraction of the genome.
  - Targeted sequence capture
  - Restriction-associated digest (RAD)
    - single-digest
    - double-digest
    - “Rapture”

Whole genome sequencing (WGS)

Pros:

- Good for discovering both single nucleotide polymorphisms (SNP) and structural variation.
- Better ability to finely identify causal variants in association studies and divergence scans.
- Minimal design and library preparation is easier.
- More contiguity (more LD-based methods on the table).

Cons:

- Require’s a reference genome (for most analyses), which can be difficult to generate depending on the organism.
- More sequencing (i.e. cost) to achieve a desired depth-of-coverage.

Reduced representation

Pros:

- Can increase sample sizes at a fixed budget and depth-of-coverage, which can provide more statistical power and accurate inference.

- *de novo* reference assembly and mapping can be easier since the data has often been pre-screened for “well behaved” regions of the genome.

Cons:

- Gamble on being able to identify particular loci of interest through linkage-disequilibrium (LD).

- Capture kits can be expensive (7,000 USD for a 4 reaction kit), *but* still may be cheaper than additional sequencing required for WGS.

- Can require design.

- RAD is anonymous (you may not be able to find from where in the genome your data are derived).

A typical NGS roadmap

Phase 1. Wetlab data generation.

1) DNA extraction
2) library construction
3) Sequencing

Phase 2. Mapping / Alignment

1) FASTQ quality control (FastQC, cutadapt, trimmomatic, Trim Galore, PEAR, FLASH, super_deduper).
2) Assembly
3) Mapping (bwa, Bowtie, Novoalign).

Phase 3. Variant discovery

1) Site-level quality control
2) Call genotypes and SNPs/INDELS (ANGSD, bcftools, GATK, freeBayes)

Phase 4. Characterize genetic variation and make biological inference

1) Estimate allele frequencies (ANGSD)
2) Population structure, demography, selection.

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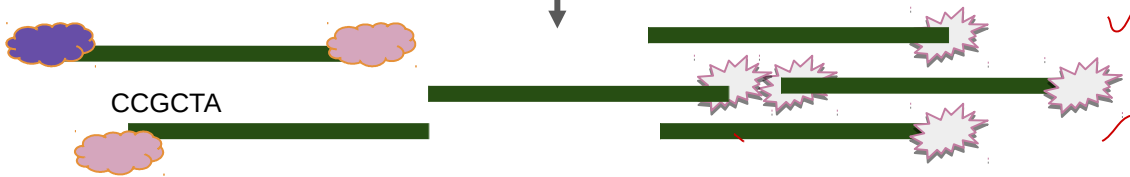
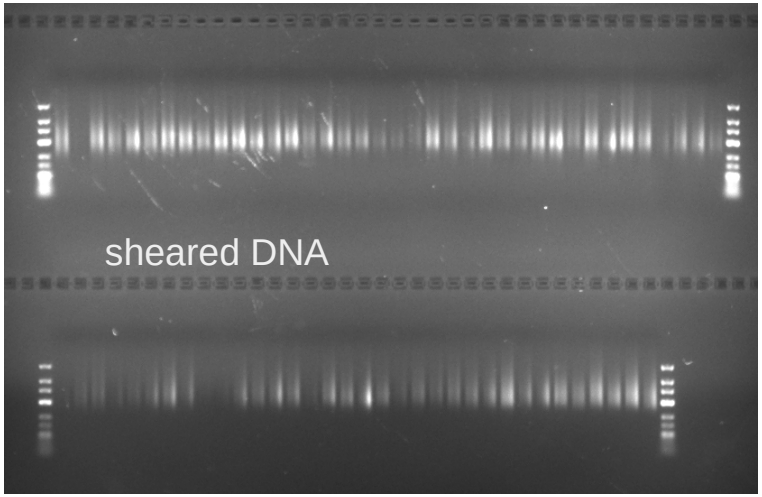
Extracted DNA strand

Break up the genomic DNA somehow: sonic shearing, restriction enzymes, mother nature.

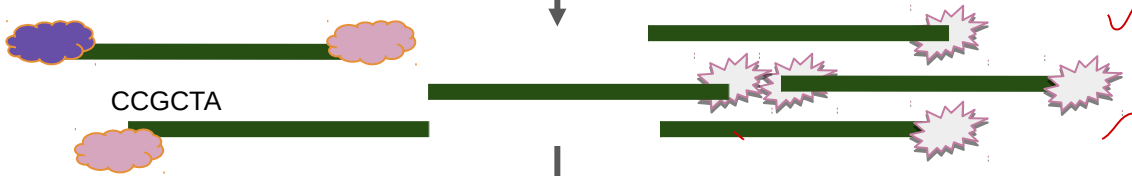




Phase 1: Library preparation

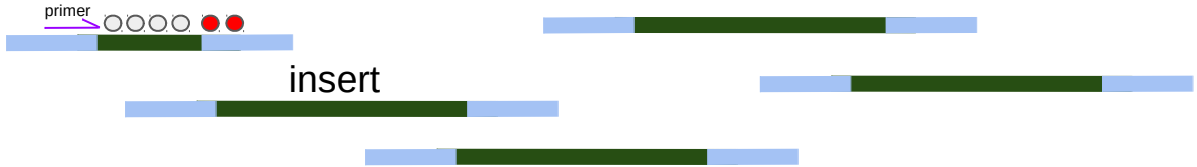
Extracted DNA strand

Break up the genomic DNA somehow: ultrasonic waves, restriction enzymes, mother nature.



Sequencing into the adapter = adapter contamination!!!

Adapter ligation & indexing



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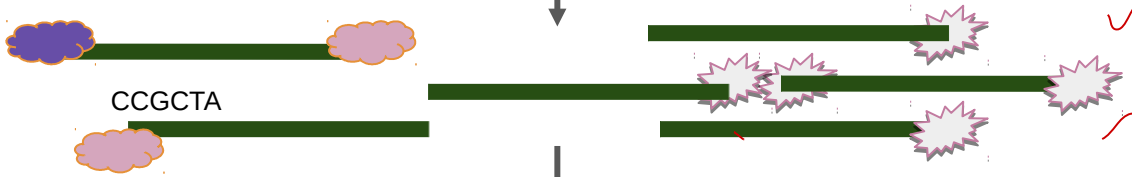

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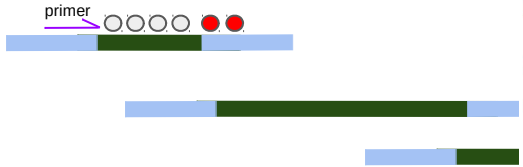

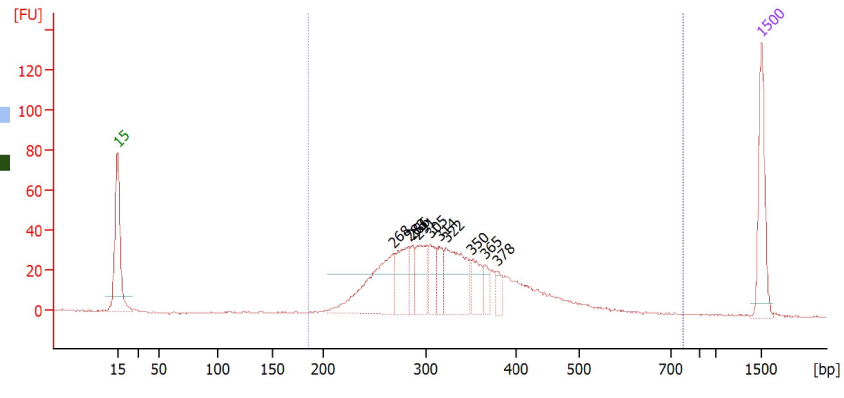

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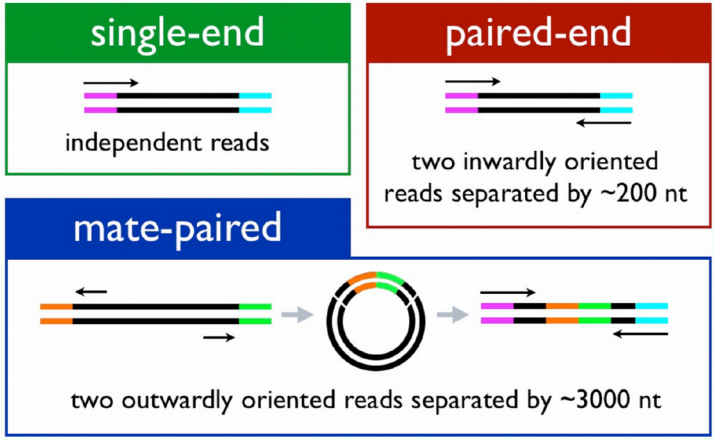
Break up the genomic DNA somehow: ultrasonic waves, restriction enzymes, mother nature.

Adapter ligation

Sequencing into the adapter = adapter contamination

sequencing



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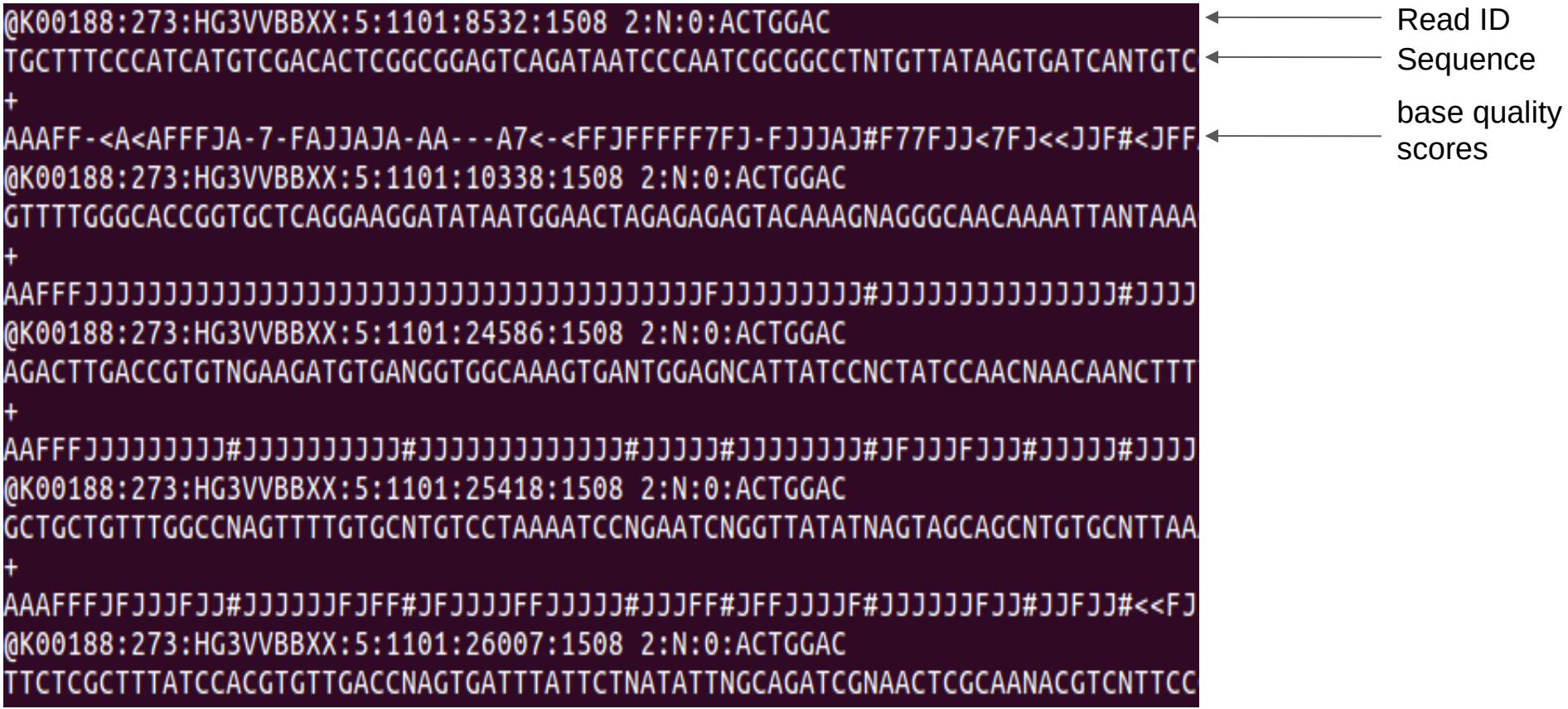
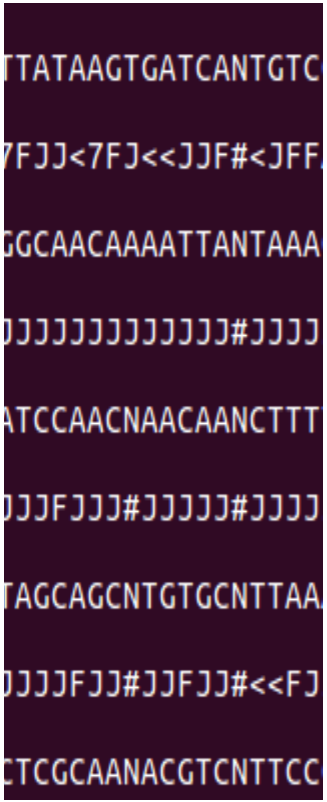
Raw sequencing reads in FASTQ format



Raw sequencing reads in FASTQ format

Dec	Char	Dec	Char	Dec	Char	Dec	Char
0	NUL (null)	32	SPACE	64	@	96	`
1	SOH (start of heading)	33	!	65	A	97	a
2	STX (start of text)	34	"	66	B	98	b
3	ETX (end of text)	35	#	67	C	99	c
4	EOT (end of transmission)	36	$	68	D	100	d
5	ENQ (enquiry)	37	%	69	E	101	e
6	ACK (acknowledge)	38	&	70	F	102	f
7	BEL (bell)	39	'	71	G	103	g
8	BS (backspace)	40	(	72	H	104	h
9	TAB (horizontal tab)	41	)	73	I	105	i
10	LF (NL line feed, new line)	42	*	74	J	106	j
11	VT (vertical tab)	43	+	75	K	107	k
12	FF (NP form feed, new page)	44	,	76	L	108	l
13	CR (carriage return)	45	-	77	M	109	m
14	SO (shift out)	46	.	78	N	110	n
15	SI (shift in)	47	/	79	O	111	o
16	DLE (data link escape)	48	0	80	P	112	p
17	DC1 (device control 1)	49	1	81	Q	113	q
18	DC2 (device control 2)	50	2	82	R	114	r
19	DC3 (device control 3)	51	3	83	S	115	s
20	DC4 (device control 4)	52	4	84	T	116	t
21	NAK (negative acknowledge)	53	5	85	U	117	u
22	SYN (synchronous idle)	54	6	86	V	118	v
23	ETB (end of trans. block)	55	7	87	W	119	w
24	CAN (cancel)	56	8	88	X	120	x
25	EM (end of medium)	57	9	89	Y	121	y
26	SUB (substitute)	58	:	90	Z	122	z
27	ESC (escape)	59	;	91	[	123	{
28	FS (file separator)	60	<	92	\	124	|
29	GS (group separator)	61	=	93	]	125	}
30	RS (record separator)	62	>	94	^	126	~
31	US (unit separator)	63	?	95	_	127	DEL



The quality scores are in ASCII encoding and can be interpreted as the probability of an error, ϵ.

They are in Phred scale: $Qscore = -10*\log_{10}(\epsilon)$

Given the quality score then, $\epsilon = 10^{-Q/10}$

Example:

Raw decimal values are normally offset by 33 (64 on some older platforms). So a Qscore of '5' is 53-33 = 20.

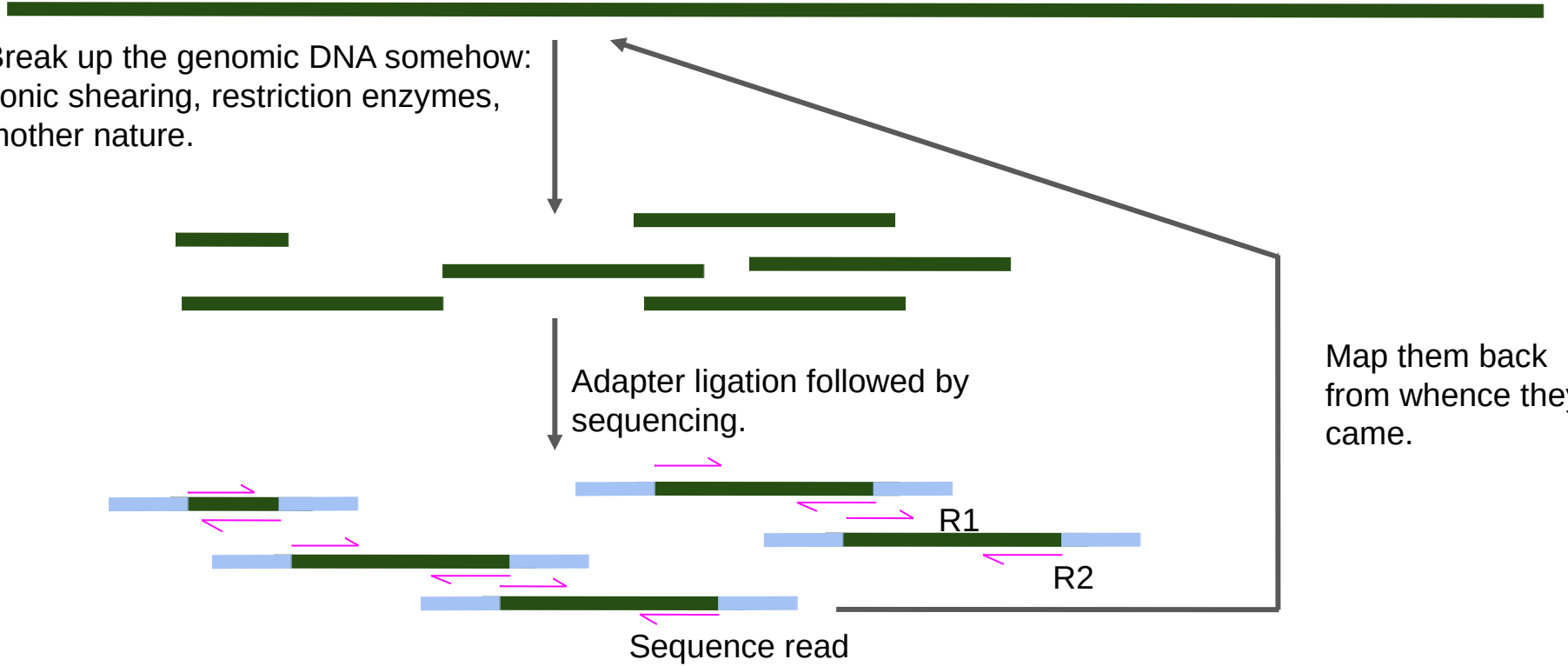
$10^{-20/10} = 1\%$.

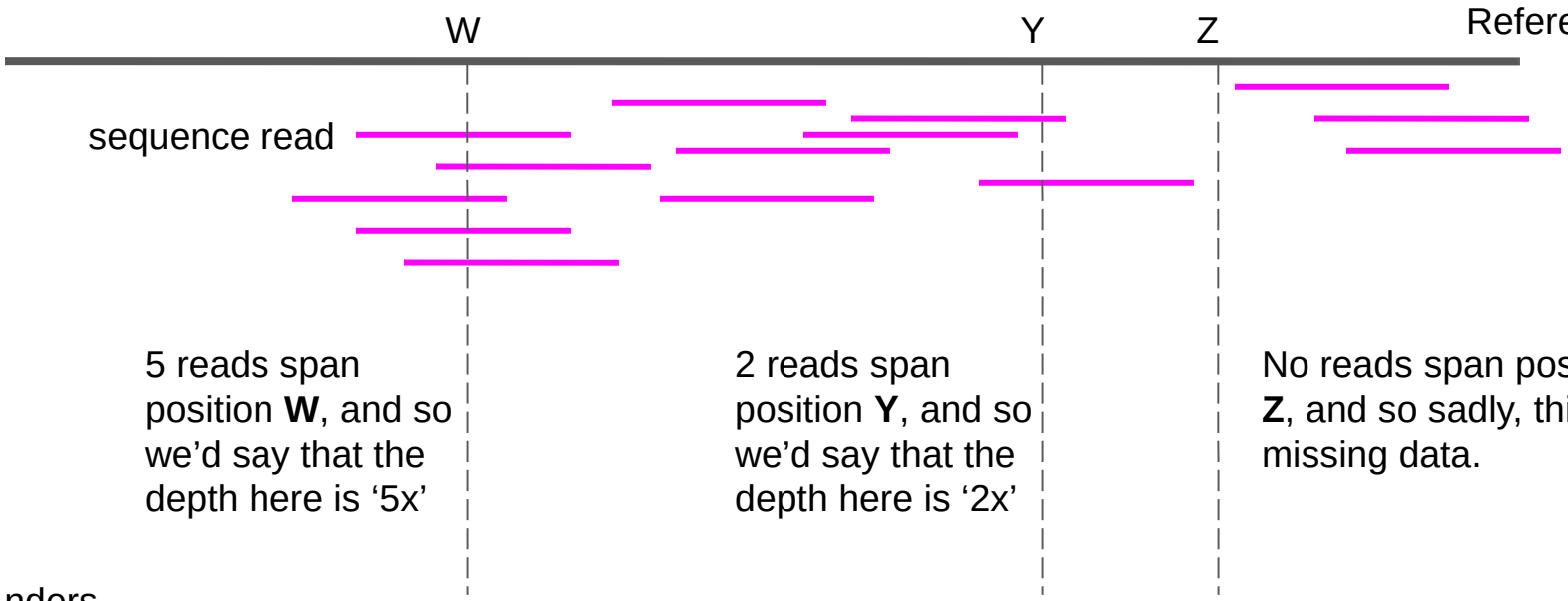
There is a 1% probability that a base with Qscore '5' is an error.

Some things to watch out for in your FASTQ files

- Adapter contamination
- Low quality bases (usually at the ends of reads and can be soft-clipped)
- Low complexity reads
- Excess missing bases (‘N’)
- Overlapping reads

DNA strand (sequence represented in reference assembly)





Anders

My definitions (The literature is not consistent)

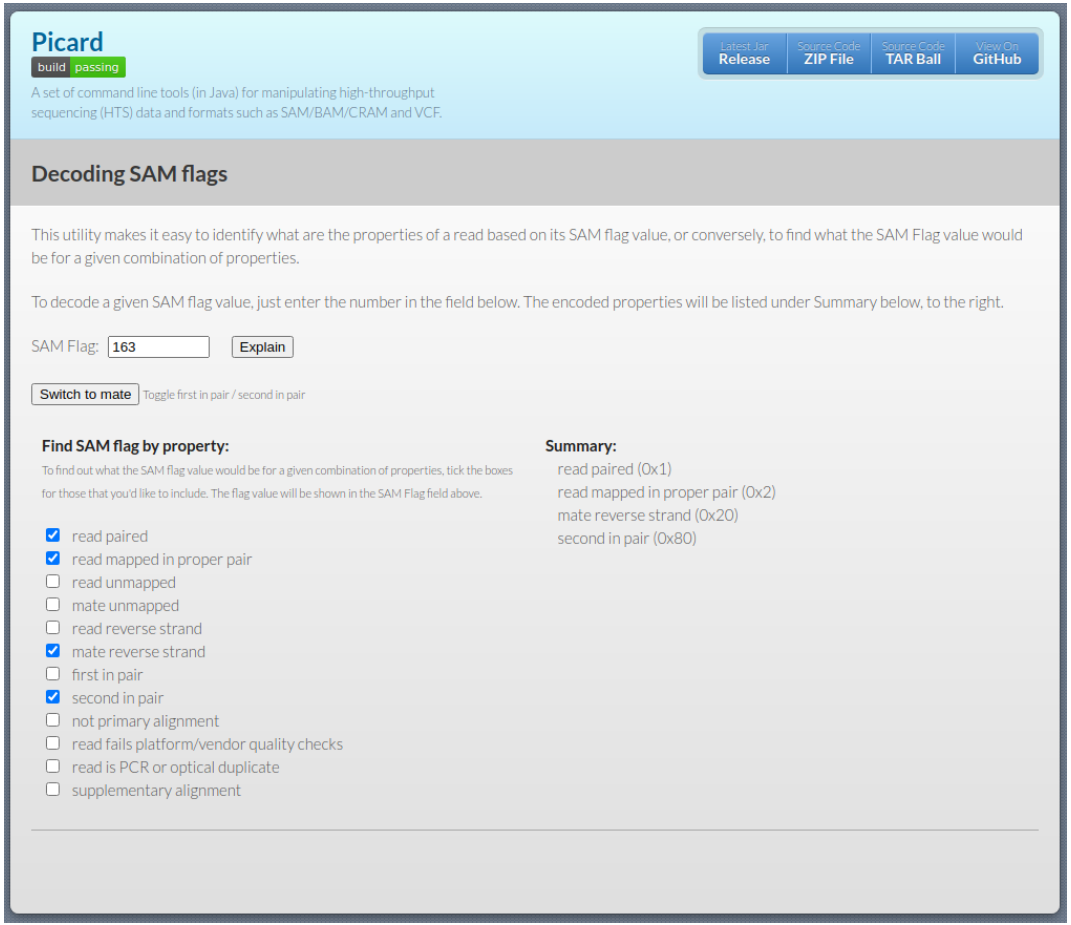
Depth	The number of reads that maps to a position
Coverage	The fraction of the genome (region) with data
Depth-of-coverage	Average depth for sites that are covered

SAM/BAM/CRAM format (for mapped reads)

```
HS36_21523:8:1214:17897:31562   163     chr7    16      23      125M    =       503     612     CGAGAGTGCAGCAGTTTGGTGAAAGGAACAGGTGAAGAAGAGGTGAGCATCCAAGAGACGAGCAAGAGGACAGTTTCAAAGCAGACATTATGGCACAATAAAAGCTGT
AAGAGCAGAGATTCATT   EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE   AS:i:120        XS:i:125        MQ:i:23 MC:Z:125M
ms:i:4587       MD:Z:108G16     NM:i:1  RG:Z:21523_8#1
HS36_21523:8:2114:12416:4261    99      chr7    35      0       125M    =       446     533     TGAAAGGAACAGGTGAAGAAGAGGTGAGCATCCAAGAGACGAGCAAGAGGACAGTTTCAAAGCAGACATTATGGCACAATAAAAGCTGTAAGAGCAGAGATTCATTCA
TATCTTTACTATTTTTC   AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA   AS:i:120        XS:i:125        XA:Z:chr8,-23982542,125M
,0;chr16,-2795556,125M,1;chr18,+31933268,125M,1;chr18,+30035788,125M,2;chr8,-23993448,125M,3;   BC:Z:GAGGATGGTCTTTCCC   QT:Z:BBBBB<FF/BBBBBFF   MQ:i:0  MC:Z:122M3S     ms:i:3656       MD:Z:89G35      NM:i
:1  RG:Z:21523_8#1
HS36_21523:8:1103:15663:2731    99      chr7    45      47      125M    =       593     673     AGGTGAAGAAGAGGTGAGCATCCAAGAGACGAGCAAGAGGACAGTTTCAAAGCAGACATTATGGCACAATAAAAGCTGTGAGAGCAGAGATTCATTCATATCTTTACT
ATTTTTCTTTAGCTTCT   BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB   AS:i:125        XS:i:120        XA:Z:chr8,-23982532,125M
,1;chr16,-2795546,125M,2;chr18,+31933278,125M,2;chr8,-23993438,125M,3;chr18,+30035798,125M,3;   BC:Z:GAGGATGGTCTTTCCC   QT:Z:BBBBBFFFBBBBBFFF   MQ:i:48 MC:Z:125M       ms:i:3343       MD:Z:125        NM:i
:0  RG:Z:21523_8#1
```

<table>
<tr><th>Col</th><th>Field</th><th>Type</th><th>Regexp/Range</th><th>Brief description</th></tr>
<tr><td>1</td><td>QNAME</td><td>String</td><td><code>[!-?A-~]{1,254}</code></td><td>Query template NAME (read ID)</td></tr>
<tr><td>2</td><td>FLAG</td><td>Int</td><td>$[0, 2^{16} - 1]$</td><td>bitwise FLAG</td></tr>
<tr><td>3</td><td>RNAME</td><td>String</td><td><code>*|[:rname:^*=][:rname:]*</code></td><td>Reference sequence NAME[11]</td></tr>
<tr><td>4</td><td>POS</td><td>Int</td><td>$[0, 2^{31} - 1]$</td><td>1-based leftmost mapping POSition</td></tr>
<tr><td>5</td><td>MAPQ</td><td>Int</td><td>$[0, 2^{8} - 1]$</td><td>MAPping Quality</td></tr>
<tr><td>6</td><td>CIGAR</td><td>String</td><td><code>*|([0-9]+[MIDNSHPX=])+</code></td><td>CIGAR string</td></tr>
<tr><td>7</td><td>RNEXT</td><td>String</td><td><code>*|=|[:rname:^*=][:rname:]*</code></td><td>Reference name of the mate/next read</td></tr>
<tr><td>8</td><td>PNEXT</td><td>Int</td><td>$[0, 2^{31} - 1]$</td><td>Position of the mate/next read</td></tr>
<tr><td>9</td><td>TLEN</td><td>Int</td><td>$[-2^{31} + 1, 2^{31} - 1]$</td><td>observed Template LENgth</td></tr>
<tr><td>10</td><td>SEQ</td><td>String</td><td><code>*|[A-Za-z=.]+</code></td><td>segment SEQuence</td></tr>
<tr><td>11</td><td>QUAL</td><td>String</td><td><code>[!-~]+</code></td><td>ASCII of Phred-scaled base QUALity+33</td></tr>
</table>

https://broadinstitute.github.io/picard/explain-flags.html



Can use this tool from the Broad to interpret the bitwise flags.

Phase 2: Mapping and alignment

```
HS36_21523:8:1214:17897:31562   163     chr7    16      23      125M    =       503     612     CGAGAGTGCAGCAGTTTGGTGAAAGGAACAGGTGAAGAAGAGGTGAGCATCCAAGAGACGAGCAAGAGGACAGTTTCAAAGCAGACATTATGGCACAATAAAAGCTGT
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,0;chr16,-2795556,125M,1;chr18,+31933268,125M,1;chr18,+30035788,125M,2;chr8,-23993448,125M,3;   BC:Z:GAGGATGGTCTTTCCC   QT:Z:BBBBB<FF/BBBBBFF   MQ:i:0  MC:Z:122M3S     ms:i:3656       MD:Z:89G35      NM:i
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,1;chr16,-2795546,125M,2;chr18,+31933278,125M,2;chr8,-23993438,125M,3;chr18,+30035798,125M,3;   BC:Z:GAGGATGGTCTTTCCC   QT:Z:BBBBBFFFBBBBBFFF   MQ:i:48 MC:Z:125M       ms:i:3343       MD:Z:125        NM:i
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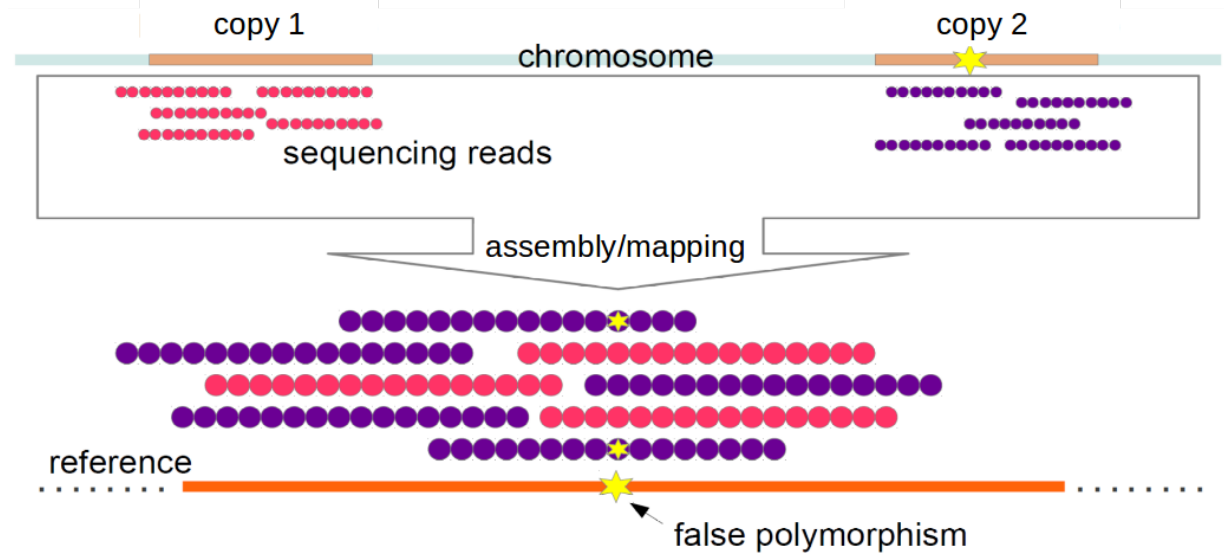
Read HS36_21523:8:1214:17897:31562 is mapped in a properly mapped pair. Its leftmost coordinate is at chr7:16 on the reference and was identical to the reference along all 125 of its bases. The mapping quality is 23 and so could be mismapped with ~0.5% probability.

The problem of paralogs (or any other type of highly similar sequences repeated throughout the genome)



- Sequence depth proportional to the number of copies.
- Tendency to inflate estimates of heterozygosity .

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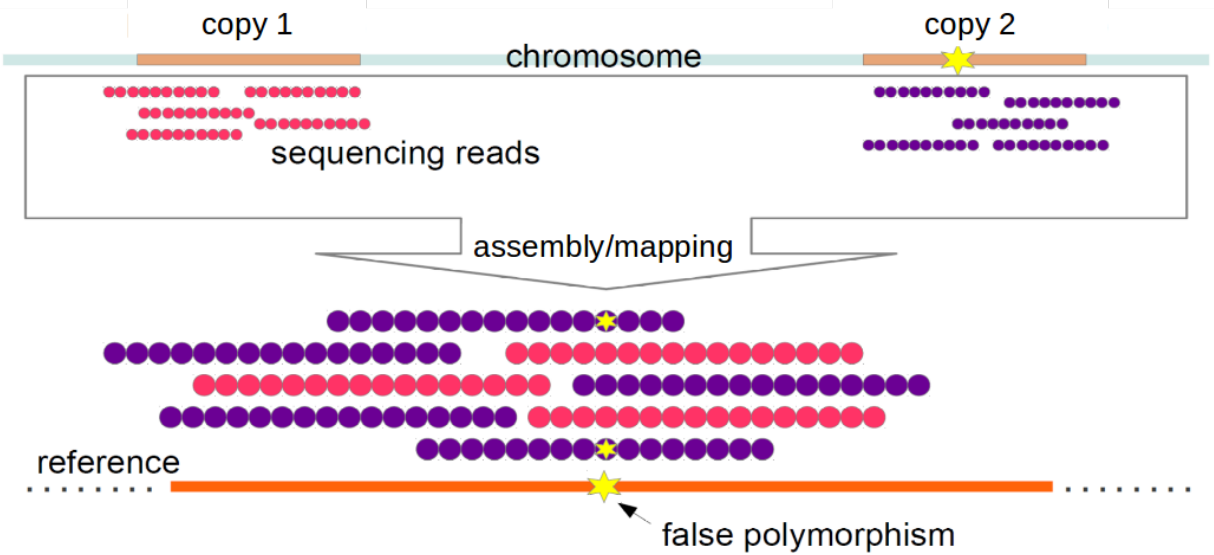


- Sequence depth proportional to the number of copies.
- Tendency to inflate estimates of heterozygosity.
- Can generate improperly mapped reads

Pileup format (generated with SAMtools)

chr7	10000	T	2	.,	EB	7	.,,,,,^],	>IIBGGE	6	.....,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10001	C	3	.,^].	EBB	7	.,,,,,,	>IIBGGI	6	.....,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10002	G	3	.,.	EBB	7	.,,,,,,	>IIBGGI	6	.....,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10003	G	3	.,.	EBB	7	.,,,,,,	>IIBGGI	6	.....,	DEGEGG	10	,.,,.,,.,^].	DABGIIIIIE
chr7	10004	A	3	.,.	EBB	8	.,,,,,,^].	>IIBGGIE	6	.....,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10005	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	6	.....,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10006	A	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	6	.....,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10007	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	7	.....,^].	DEGEGGE	10	,.,,.,,.,.	DABGIIIIII
chr7	10008	C	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	7	.....,.	DEGEGGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10009	A	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.^],	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10010	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10011	C	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10012	T	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10013	T	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	9	.....,.,^],	DEGEGGGBE	10	,.,,.,,.,.	DABGIIIIII
chr7	10014	A	4	.,.^].	EBBE	8	.,,,,,,.	>IIBGGIG	9	.G...gG,,	DEGEGGGBG	10	,.,,.,,.,.	D3BGIIIIII
chr7	10015	G	4	.,..	EBBI	8	.,,,,,,.	>IIBGGIG	9	.....,.,,	DEGEGGGBG	10	,.,,.,,.,.	D3BGIIIIII

Left-most fields:

(1) Reference sequence
(2) Position
(3) Reference base

Each individual has 3 fields:

(1) Depth
(2) read bases
(3) quality scores

Base codes:

. = reference match on forward strand
, = reference match on revere strand
[ACGTacgt] = alternate allele (forward strand = uppercase, reverse strand = lowercase)

Pileup format (generated with SAMtools)

chr7	10000	T	2	.,	EB	7	.,,,,,^],	>IIBGGE	6	.....,,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10001	C	3	.,^].	EBB	7	.,,,,,,	>IIBGGI	6	.....,,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10002	G	3	.,.	EBB	7	.,,,,,,	>IIBGGI	6	.....,,	DEGEGG	9	,.,,.,,.,	DABGIIIII
chr7	10003	G	3	.,.	EBB	7	.,,,,,,	>IIBGGI	6	.....,,	DEGEGG	10	,.,,.,,.,^].	DABGIIIIIE
chr7	10004	A	3	.,.	EBB	8	.,,,,,,^].	>IIBGGIE	6	.....,,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10005	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	6	.....,,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10006	A	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	6	.....,,	DEGEGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10007	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	7	.....,^].	DEGEGGE	10	,.,,.,,.,.	DABGIIIIII
chr7	10008	C	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	7	.....,.	DEGEGGG	10	,.,,.,,.,.	DABGIIIIII
chr7	10009	A	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.^],	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10010	G	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10011	C	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10012	T	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	8	.....,.,	DEGEGGGB	10	,.,,.,,.,.	DABGIIIIII
chr7	10013	T	3	.,.	EBB	8	.,,,,,,.	>IIBGGIG	9	.....,.,^],	DEGEGGGBE	10	,.,,.,,.,.	DABGIIIIII
chr7	10014	A	4	.,.^].	EBBE	8	.,,,,,,.	>IIBGGIG	9	.G...gG,,	DEGEGGGBG	10	,.,,.,,.,.	D3BGIIIIII
chr7	10015	G	4	.,..	EBBI	8	.,,,,,,.	>IIBGGIG	9	.....,.,,	DEGEGGGBG	10	,.,,.,,.,.	D3BGIIIIII

Start of read with ASCII MQ ‘]’

Other characters that show up:
$ = end of read
* = missing base
</> = reference skip

A typical NGS roadmap

Phase 1. Wetlab data generation.

1) DNA extraction
2) library construction
3) Sequencing

Phase 2. Mapping / Alignment

1) FASTQ quality control (FastQC, cutadapt, trimmomatic, Trim Galore, PEAR, FLASH, super_deduper).
2) Assembly
3) Mapping (bwa, Bowtie, Novoalign).

Phase 3. Variant discovery

1) Site-level quality control
2) Call genotypes and SNPs/INDELS (ANGSD, bcftools, GATK, freeBayes)

Phase 4. Characterize genetic variation and make biological inference

1) Estimate allele frequencies (ANGSD)
2) Population structure, demography, selection.

Variant Call Format (VCF)

```
##INFO=<ID=MQ,Number=1,Type=Integer,Description="Average mapping quality">
##INFO=<ID=PV4,Number=4,Type=Float,Description="P-values for strand bias, baseQ bias, mapQ bias and tail distance bias">
##bcftools_callVersion=1.13-3-g89a566b+htslib-1.13-3-gd16bed5
##bcftools_callCommand=call --ploidy 2 -a PV4,GQ,GP -m -P 0.001 -O u; Date=Sun Jul 25 00:26:03 2021
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of samples with data">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele frequency">
##INFO=<ID=ExcHet,Number=A,Type=Float,Description="Test excess heterozygosity; 1=good, 0=bad">
##bcftools_pluginVersion=1.13-3-g89a566b+htslib-1.13-3-gd16bed5
##bcftools_pluginCommand=plugin fill-tags -O b -o /home/tyler/ngs_intro/output/calmas_allsites.bcf.gz -- -tAF,NS,ExcHet; Date=Sun Jul 25 00:26:04 2021
##bcftools_viewVersion=1.3.1-98-ga6a7829+htslib-1.3.1-64-g74bcfd7
##bcftools_viewCommand=view -r chr7:10000-10200 calmas_allsites.bcf.gz; Date=Mon Jul 26 11:44:41 2021
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  CMASS6607982    CMASS6607991    CMASS6389725    CMASS6608065    CMASS6169461    CMASS6169452    CMASS6169443    CMASS63
chr7    10000   .       T       .       4752.11 .       DP=165;AD=165;SCR=21;MQSBZ=0.639526;FS=0;MQ0F=0;AN=78;DP4=89,76,0,0;MQ=56;NS=39 GT:DP:AD:SCR:QS 0/0:2:2:0:69    0/0:7:7:1:258   0/0:6:6
chr7    10001   .       C       .       4795.11 .       DP=166;AD=166;SCR=21;MQSBZ=0.609616;FS=0;MQ0F=0;AN=78;DP4=90,76,0,0;MQ=56;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:7:7:1:258   0/0:6:6
chr7    10002   .       G       .       4874.11 .       DP=167;AD=167;SCR=21;MQSBZ=0.580082;FS=0;MQ0F=0;AN=78;DP4=91,76,0,0;MQ=57;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:7:7:1:258   0/0:6:6
chr7    10003   .       G       .       4920.11 .       DP=170;AD=170;SCR=21;MQSBZ=0.493647;FS=0;MQ0F=0;AN=78;DP4=94,76,0,0;MQ=57;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:7:7:1:258   0/0:6:6
chr7    10004   .       A       .       23.6449 .       DP=172;AD=171;SCR=21;SGB=-0.626698;RPBZ=1.6921;MQBZ=-2.46491;MQSBZ=0.499982;BQBZ=-1.67883;SCBZ=3.32672;FS=0;MQ0F=0;AN=78;DP4=94,77,1,0;
chr7    10005   .       G       .       5018.12 .       DP=173;AD=173;SCR=21;MQSBZ=0.472176;FS=0;MQ0F=0;AN=78;DP4=96,77,0,0;MQ=57;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296   0/0:6:6
chr7    10006   .       A       .       24.4501 .       DP=174;AD=172;SCR=21;VDB=0.14;SGB=-2.84764;RPBZ=2.23109;MQBZ=-3.51912;MQSBZ=0.506159;BQBZ=-2.38185;SCBZ=4.42789;FS=0;MQ0F=0;AN=78;DP4=9
chr7    10007   .       G       .       5087.12 .       DP=176;AD=176;SCR=21;MQSBZ=0.451479;FS=0;MQ0F=0;AN=78;DP4=98,78,0,0;MQ=57;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296   0/0:7:7
chr7    10008   .       C       .       5066.12 .       DP=176;AD=176;SCR=21;MQSBZ=0.451479;FS=0;MQ0F=0;AN=78;DP4=98,78,0,0;MQ=57;NS=39 GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296   0/0:7:7
chr7    10009   .       A       .       5155.12 .       DP=179;AD=179;SCR=21;MQSBZ=0.431514;FS=0;MQ0F=0;AN=78;DP4=100,79,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296
chr7    10010   .       G       .       5128.12 .       DP=180;AD=180;SCR=21;MQSBZ=0.405226;FS=0;MQ0F=0;AN=78;DP4=101,79,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296
chr7    10011   .       C       .       5228.12 .       DP=183;AD=183;SCR=21;MQSBZ=0.444839;FS=0;MQ0F=0;AN=78;DP4=102,81,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296
chr7    10012   .       T       .       5332.12 .       DP=186;AD=186;SCR=21;MQSBZ=0.425788;FS=0;MQ0F=0;AN=78;DP4=104,82,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296
chr7    10013   .       T       .       5390.12 .       DP=188;AD=188;SCR=21;MQSBZ=0.432328;FS=0;MQ0F=0;AN=78;DP4=105,83,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:3:3:0:102   0/0:8:8:1:296
chr7    10014   .       A       G       259.035 .       DP=190;AD=177,12;SCR=21;VDB=0.311296;SGB=16.1258;RPBZ=-0.0261303;MQBZ=0.689528;MQSBZ=0.382678;BQBZ=0.848991;SCBZ=-1.16673;FS=0;MQ0F=0;A
chr7    10015   .       G       .       5441.12 .       DP=190;AD=190;SCR=21;MQSBZ=0.382678;FS=0;MQ0F=0;AN=78;DP4=107,83,0,0;MQ=57;NS=39       GT:DP:AD:SCR:QS 0/0:4:4:0:142   0/0:8:8:1:296
```

metadata

#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT CMASS6607982

chr7 10014 . A G 259.035 . DP=190;AD=177,12;SCR=21;VDB=0.311296;SGB=16.1258;RPBZ=-0.0261303;MQBZ=0.689528;;BQBZ=0.848991;SCBZ=-1.16673;FS=0;MQ0F=0;AC=3;AN=78;DP4=99,78,8,5;MQ=57;PV4=0.778225,1,1,1;NS=39;AF=0.0384615;ExcHet=0.961039 GT:PL:DP:AD:SCR:QS:GP:GQ 0/0:0,12,125:4:4,0:0:142,0:0.994672,0.00532842,5.66837e-16:22

Variant Call Format (VCF)

#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT CMASS6607982

chr7 10014 . A G 259.035 .
DP=190;AD=177,12;SCR=21;VDB=0.311296;SGB=16.1258;RPBZ=-0.0261303;MQBZ=0.689528;;BQBZ=0.848991;SCBZ=-1.16673;FS=0;MQ0F=0;AC=3;AN=78;DP4=99,78,8,5;MQ=57;PV4=0.778225,1,1,1;NS=39;AF=0.0384615;ExcHet=0.961039 GT:PL:DP:AD:SCR:QS:GP:GQ
0/0:0,12,125:4:4,0:0:142,0:0.994672,0.00532842,5.66837e-16:22

INFO: site-wide info

FORMAT: Format of the information for individuals

Information for the first individual

This is a lot of rich information that we can subset sites with using bcftools.

Site-level quality control

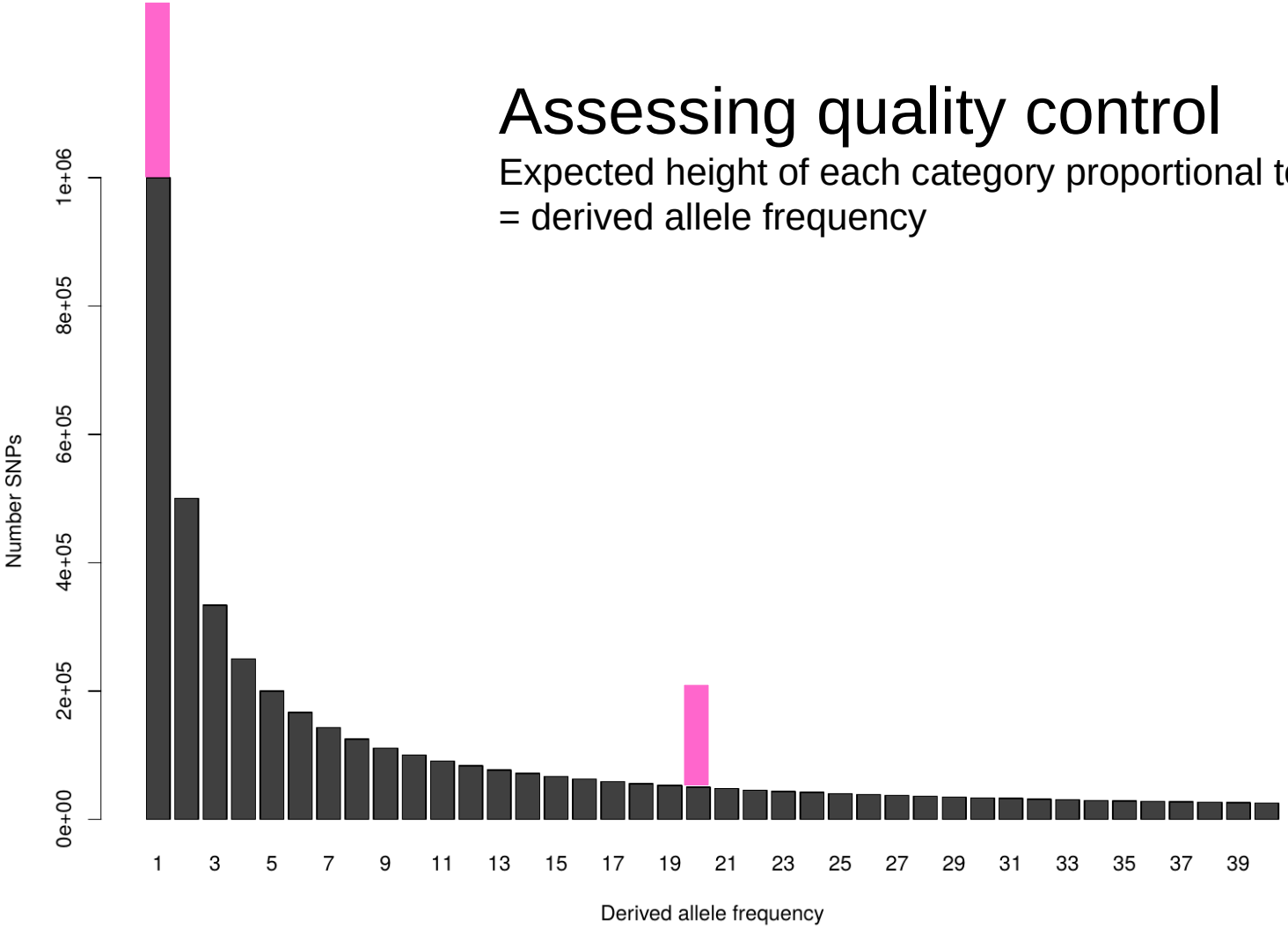
- Excessively low total site sequencing depth
- Excessively high total site sequencing depth
- Evenness of depth across individuals.
- Minimum number of individuals with called genotypes (sufficient high-confidence data)
- Mapping quality and excess mapping quality of zero
- Excess heterozygosity

Filters related to biases between reference and alternate alleles (we expect them to act the same):

- Map quality bias
- Base quality bias
- Read position bias
- Strand bias

Assessing quality control

Expected height of each category proportional to 1/x, x = derived allele frequency



Lets put it all into practice:

https://github.com/tplinderoth/Copenhagen-Popgen-Course/tree/main/ngs_intro_exercises