Inference from NGS data

Anders Albrechtsen

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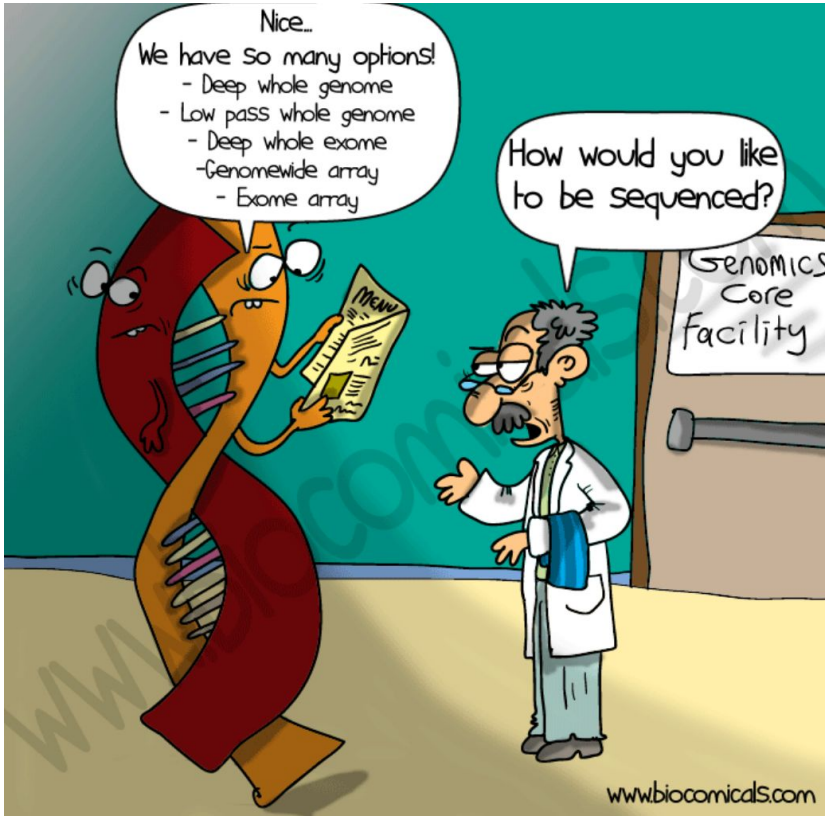
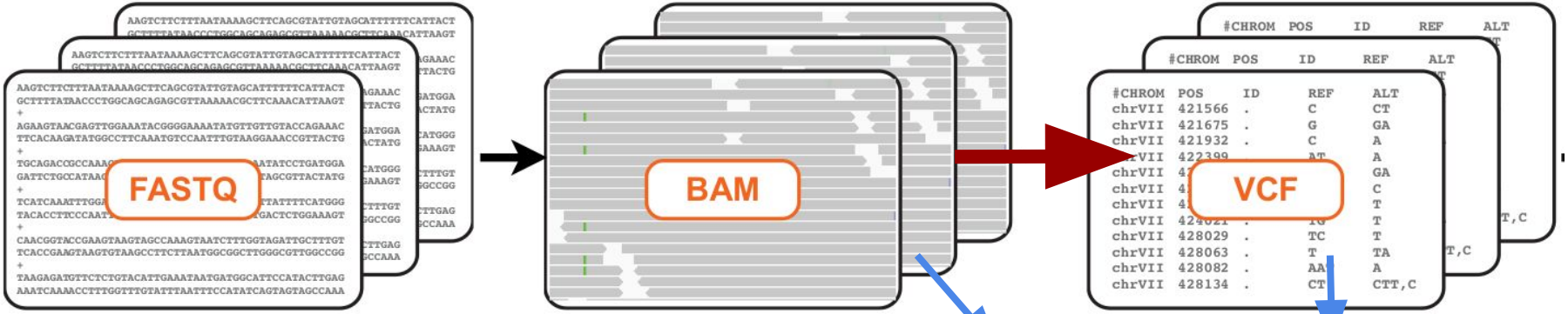
Many type of sequencing



This session

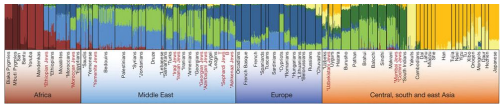


This morning

- Genotype likelihoods (GL)
- Estimate allele frequencies
- Calling variable sites
- Calling genotype

Later

- Perform analysis from Genotypes or GLs



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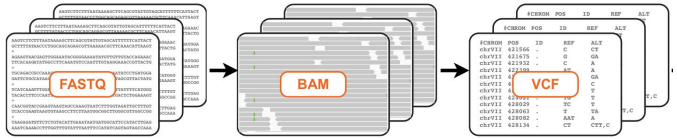
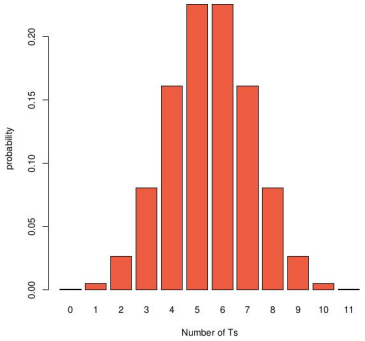
This session

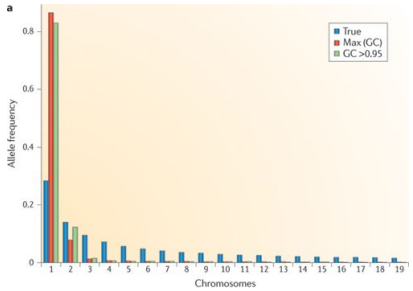
This morning

- File formats and NGS data
- Discuss the issues of genotype calling from read data
- Calculate genotype likelihoods (GL)
- Use GLs as the basis for
  - Genotype calling
  - Variant calling
  - Allele frequency estimation
  - Site frequency estimation
- Exercises



$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|G_1) + \frac{1}{2} P(b_d|G_2) \right)$$





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Data formats

Genome (FASTA)

```
>ARPM2ref|NC_000001.10|:2938046-2939467 Homo sapiens chromosome 1, GRCh37 primary
reference assembly
TGGAAGAGGCCTCAGCAGGCCCAGGCCACCTGGAGGGAGAGCAGACCTGCGGCTGAGGATGCAGGGCTCC
CGGGCACGGTGCTAGCCCTGCCTTGAGACACCCCGAGAGCTGTGGGAAGAGCTGTGGGATCCCCTATTGC
ATCACAAAGCGGCCCTGGAGGGCTGGTCTTTATTTTGATGAGGCTGAGAAGGGAAGGCTGCGGGCATGTT
TAATCCGCACGCTTTAGACTCCCCGGCTGTGATTTTTGACAATGGCTCGGGGTTCTGCAAAGCGGGCCTG
TCTGGGGAGTTTGGACCCCGGCACATGGTCAGCTCCATCGTGGGGCACCTGAAATTCCAGGCTCCCTCAG
```

Reads (FASTQ)

```
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
```

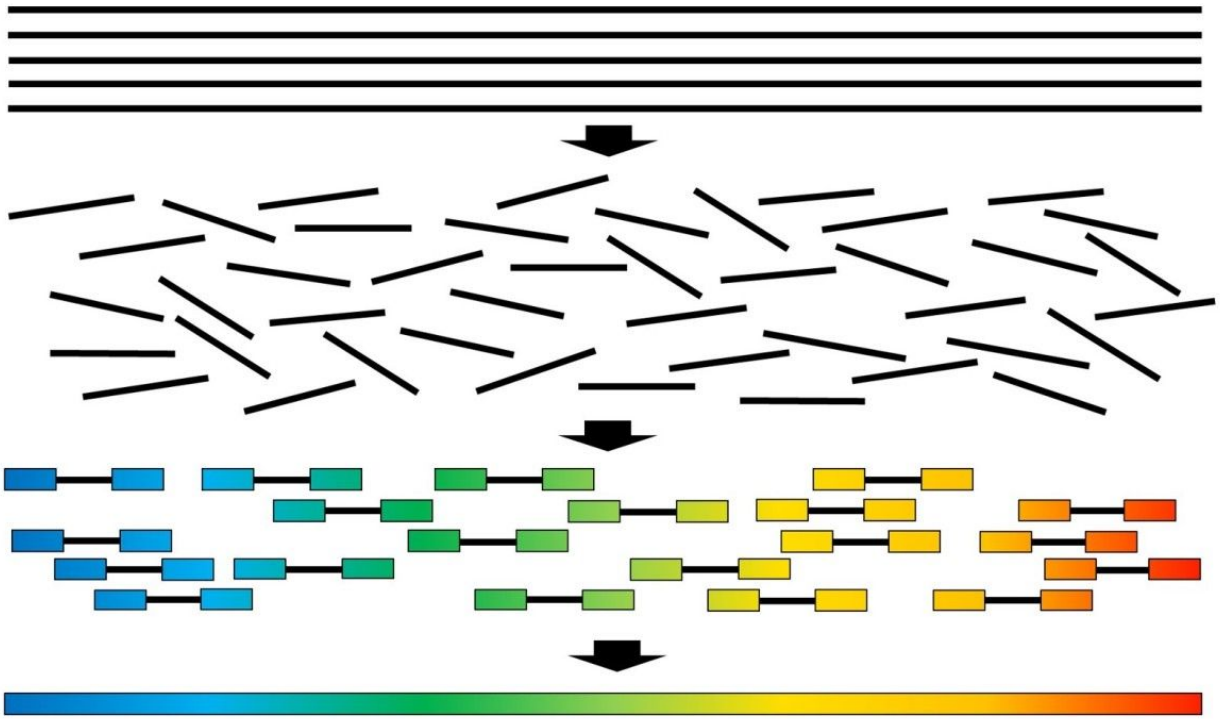
Mapped Reads (mpileup, BAM)

```
seq1 272 T 24  ,.$.....,.,.,.,.,.,.,..^+. <<<+;<<<<<<<<<<=<;<;7<&
seq1 273 T 23  .....,.,.,.,.,.,.,.,.A <<<;<<<<<<<<<3<=<<<;<<+
seq1 274 T 23  ,.$.....,.,.,.,.,.,.,.     7<7;<;<<<<<<<<<=<;<;<<&
seq1 275 A 23  ,$.....,.,.,.,.,.,.,.,...^l.  <+;9*<<<<<<<<<=<<:;<<<<
seq1 276 G 22  ...T,,.,.,.,.,.,.,.,.,.  33;+<<7=7<<7<&<<1;<<6<
seq1 277 T 22  ....,.,.,.,.C.,.,.,.,.G.  +7<;<<<<<<<&<=<<:;<<&<
seq1 278 G 23  .....,.,.,.,.,.,.,.,.,.,.^k.   %38*<<;<7<<7<=<<<;<<<<<
seq1 279 C 23  A..T,,.,.,.,.,.,.,.,.,.,.,. ;75&<<<<<<<<<=<<<9<<:<<
```

Variants (VCF)

```
##fileformat=VCFv4.1
##fileDate=20140930
##source=23andme2vcf.pl https://github.com/arrogantrobot/23andme2vcf
##reference=file:///23andme_v3_hg19_ref.txt.gz
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
#CHROM  POS     ID        REF     ALT     QUAL    FILTER  INFO    FORMAT  GENOTYPE
chr1    82154   rs4477212       a       .       .       .       .       GT      0
/0
chr1    752566  rs3094315       g       A       .       .       .       GT      1
/1
chr1    752721  rs3131972       A       G       .       .       .       GT      1
/1
chr1    798959  rs11240777      g       .       .       .       .       GT      0
/0
chr1    800007  rs6681049       T       C       .       .       .       GT      1
/1
```

Next generation sequencing



Multiple versions of the same genome (DNA from many cells)

Genome fractured into small fragments

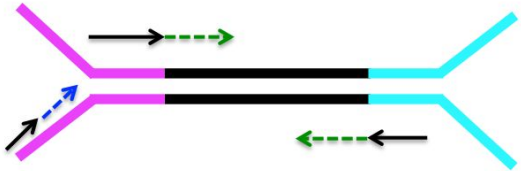
Fragments sequenced and ordered according to position on the genome

Fragment library (input DNA sample)

Library prep

Sequencing library
Double-stranded or Y- **adaptors** added

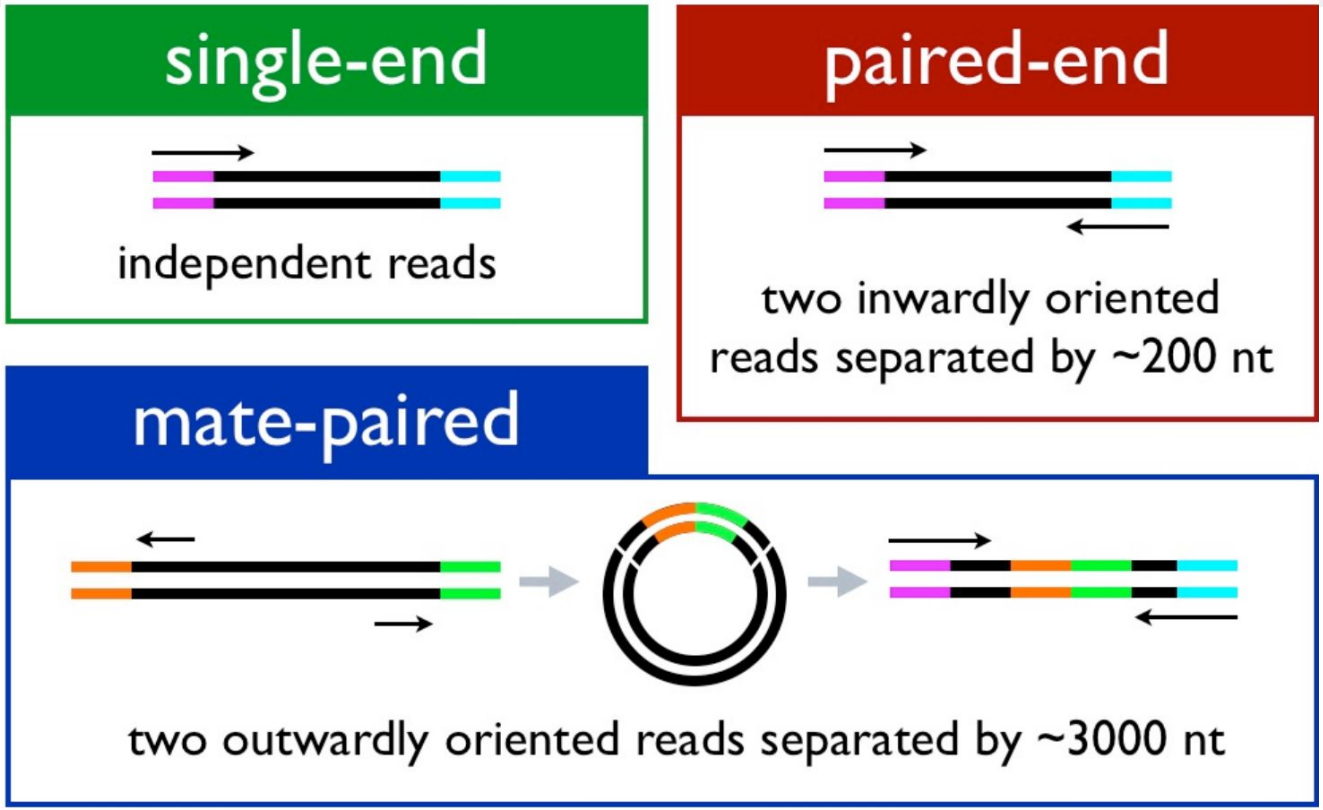
DNA sequencing



Barcode (6–12 bases) – so many samples can be run in one physical space (lane). Data is **demultiplexed**.

Primers **Reads** (36–1000+ bases)

Single or pair of fq files.



fastQ (.fq.gz)


```
a‘X_\Va\J‘KaYJHG^]b\a^BBBBBBBBBBBB    <-- quality score
@FC42BF1AAXX:6:1:5:732#0/1            <-- read ID
TGATTCTCTCGATATCCAGTCCTTAGTGNCATAGN   <-- read (bases)
+
a^_aaaa‘aa‘_aaa_aaa‘__‘‘_‘VBBBBBBBB
@FC42BF1AAXX:6:1:5:492#0/1
AACAGTGGGAGGCTGCAGCAGGAGGATTNCTGAAN
+
ababb_abbbZbabaab^‘aaTaabbaBBBBBBBB
@FC42BF1AAXX:6:1:5:480#0/1
ACCTCCTCAGAGTTCTCGAGCTCGAGAANTCTGGN
```

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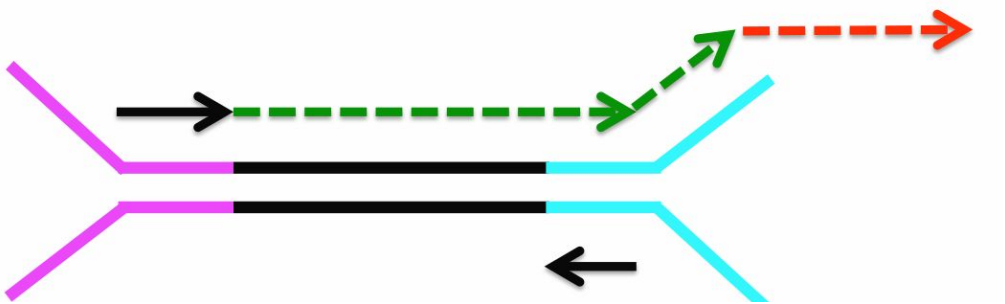
Selected issues with sequencing data

- **Adapter contamination of data**
  - If the DNA is too short we will sequence the adapters

Adapter contamination

If the DNA fragment is too small you will sequencing into the adapter+junk



Solution: Identify the problem using fastQC and trim the 3' end of the read to remove the **adapter** + **junk (AAA...)** if needed

Selected issues with sequencing data

- Adapter contamination of data
  - If the DNA is too short we will sequence the adapters
- **Sequencing errors**
  - The reads by have errors

Sequencing error

```
FastQ file
a‘X_\Va\J‘KaYJHG^]b\a^BBBBBBBBBBBB    <-- quality score
@FC42BF1AAXX:6:1:5:732#0/1            <-- read ID
TGATTCTCTCGATATCCAGTCCTTAGTGNCATAGN   <-- read (bases)
TGACTCTCTCGATATCAAGTCCTTAGTGNCATAGN   <-- sequenced DNA fragment
```

Solution: Translate the quality score to error rates

Identify the scale of the problem using fastQC

Use the error rates when calling genotypes

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Table 1 ASCII Characters Encoding Q-scores 0–40

Symbol	ASCII Code	Q-Score	Symbol	ASCII Code	Q-Score	Symbol	ASCII Code	Q-Score
!	33	0	/	47	14	=	61	28
"	34	1	0	48	15	>	62	29
#	35	2	1	49	16	?	63	30
$	36	3	2	50	17	@	64	31
%	37	4	3	51	18	A	65	32
&	38	5	4	52	19	B	66	33
'	39	6	5	53	20	C	67	34
(	40	7	6	54	21	D	68	35
)	41	8	7	55	22	E	69	36
*	42	9	8	56	23	F	70	37
+	43	10	9	57	24	G	71	38
,	44	11	:	58	25	H	72	39
-	45	12	;	59	26	I	73	40
.	46	13	<	60	27			

quality scores/Phred scores

```
a‘X_\Va\J‘KaYJHG^]b\a^BBBBBBBBBBBB    <-- quality score
```

Ascii	Dec	Qscore (Dec -33)	Error (ϵ)
+	43	10	10%
5	53	20	1%
?	63	30	0.1%
I	73	40	0.01%

Convert Qscores to seuquencing error rates

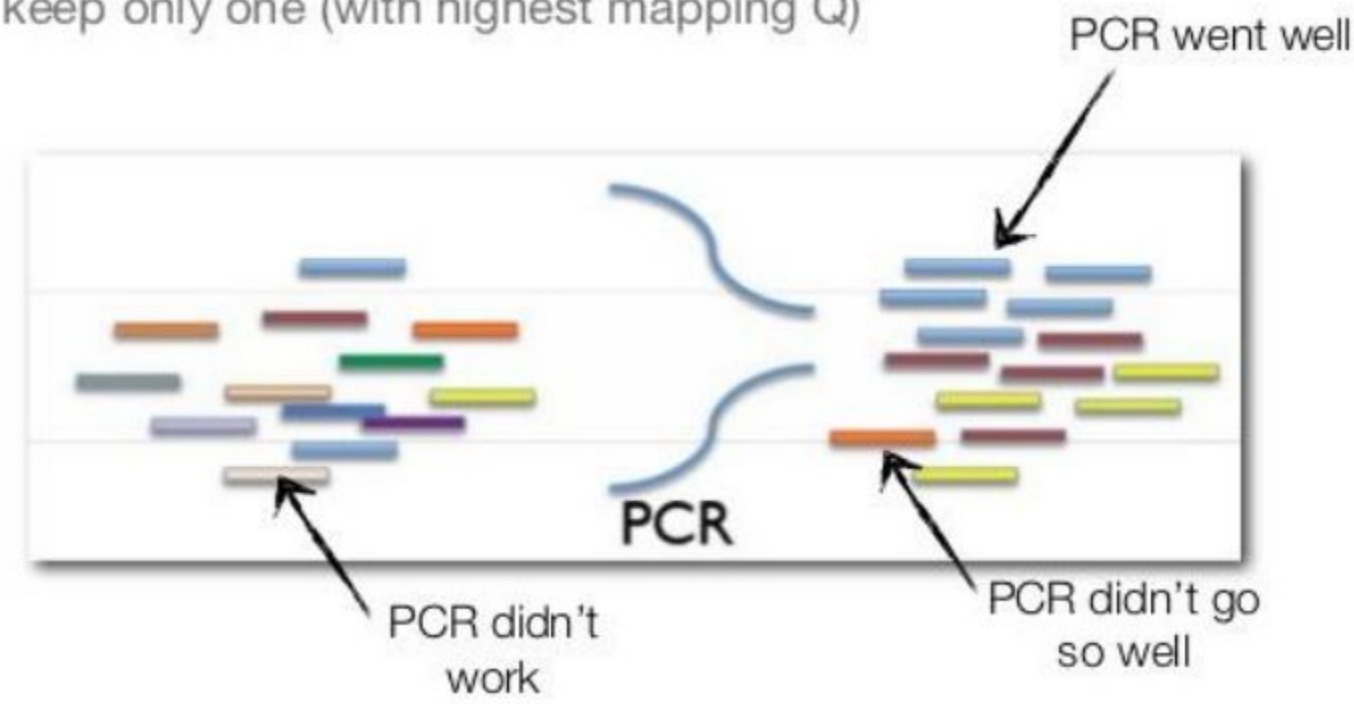
$$\text{Qscore} = -10\log_{10}(\epsilon) \quad \Leftrightarrow \quad \epsilon = 10^{-Q/10}$$

Selected issues with sequencing data

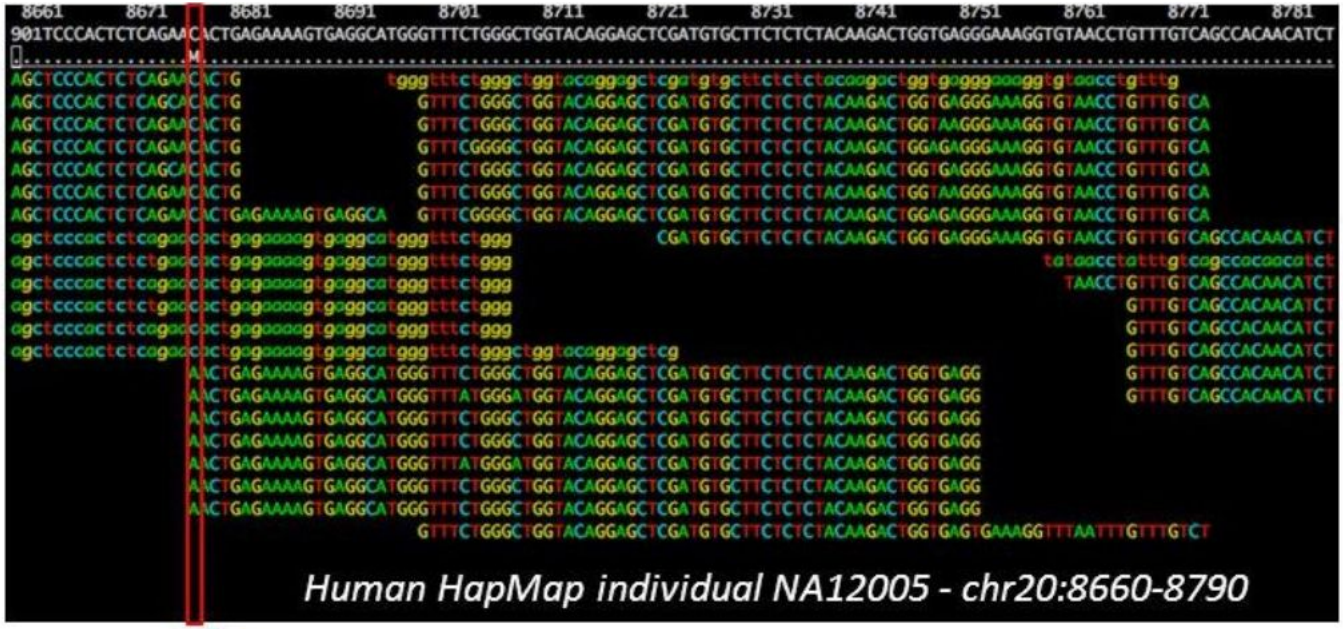
- Adapter contamination of data
  - If the DNA is too short we will sequence the adapters
- Sequencing errors
  - The reads by have errors
- **PCR or optical duplicates**
  - Reads can be duplicated ether from PCR or from the chip

Duplicated reads

> keep only one (with highest mapping Q)



Duplicated reads can cause



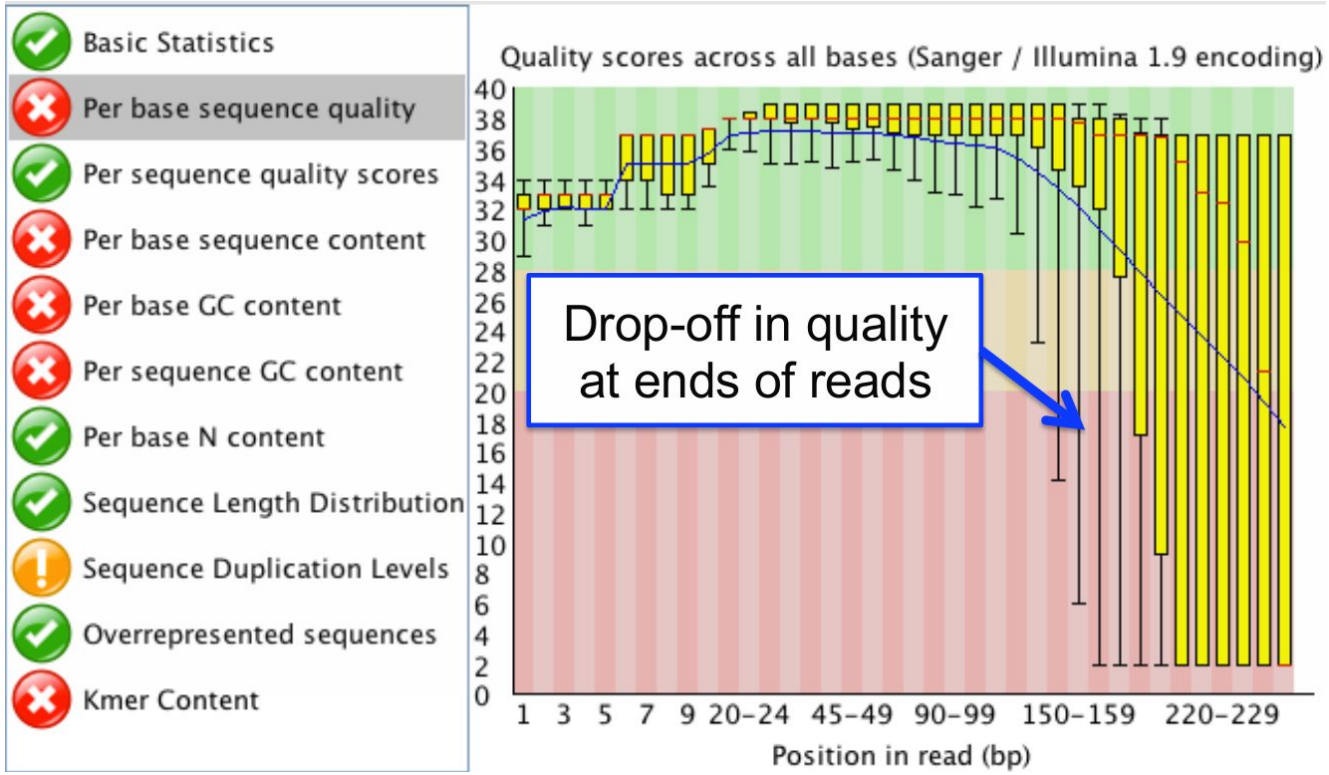
Solution: Identify the problem using fastQC

Identify the duplicated reads and remove or mark them

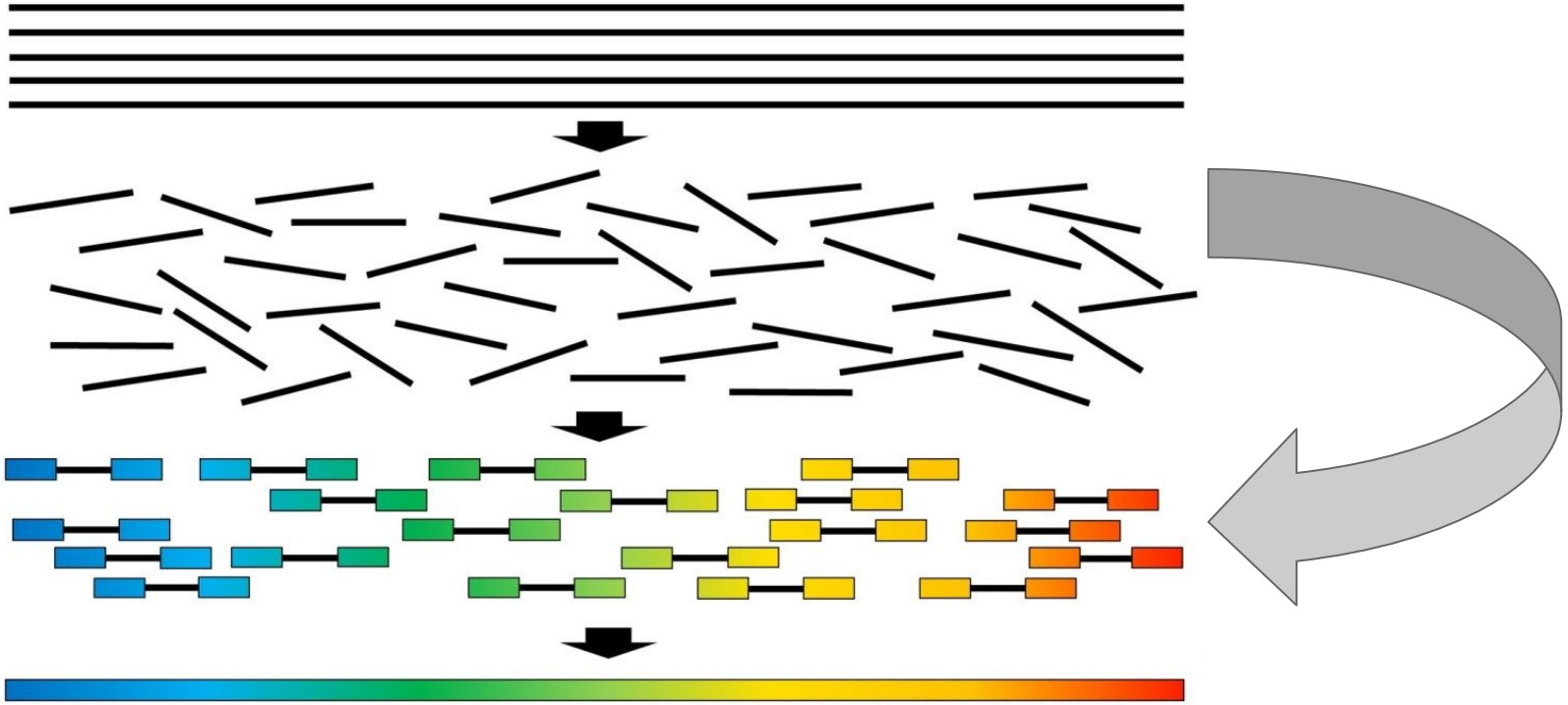
FAST QC

Easy to use tool for evaluating the qualify of your data (fastQ or Bam files)

Quality for each cycle

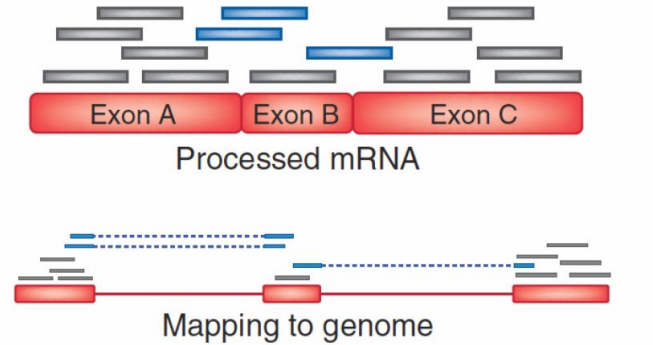


Mapping - alignment of reads



Alignment file (.bam)

reads	TTTGTTCTTTCTTTCTCTCTAGTCTTCTT ...
Qscore	NVFVN]^]'^_]^^U]]'][_VS[_^Z]_ ...
Position	chr4 53351385
Mismatch	2
strand	+
mapQ	30
Mate	mapped chr4 53351145
Alt map	chr2 15331145 with 2 mismatch



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Mapping quality

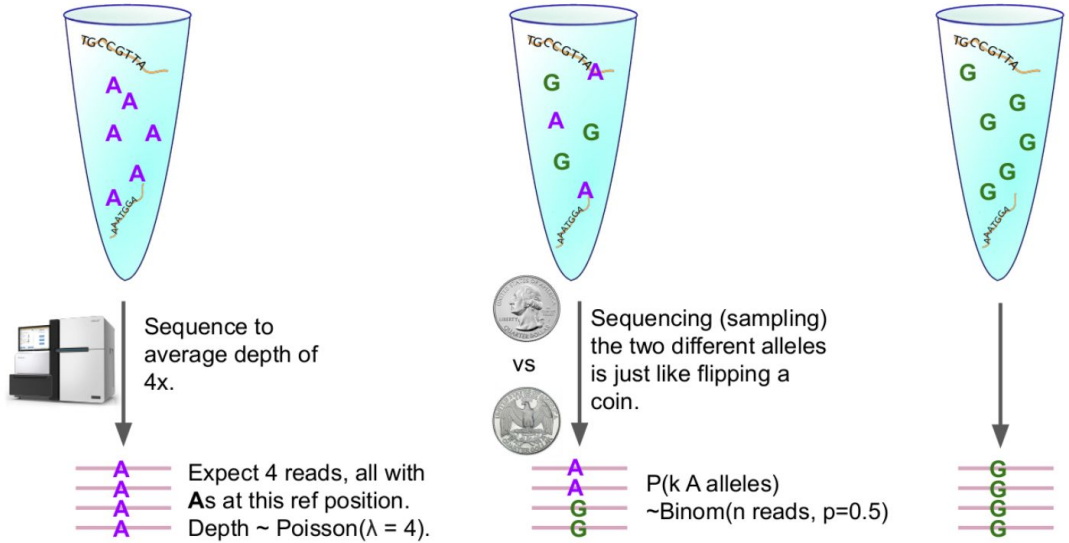
Mapping quality – what is the probability that the read is correctly mapped to this location in the reference genome?

Read 1 can be mapped two places on the genome while **Read 2** only maps to one

- **Which of the two reads has the highest mapping quality?**

Why don’t we observe genotype

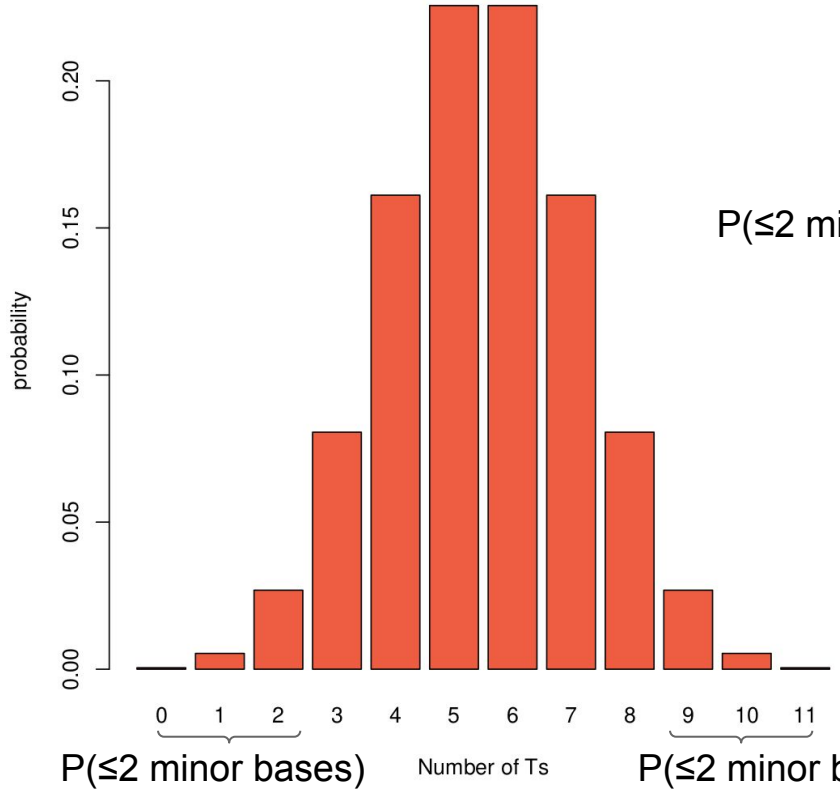
Each allele is sequenced separately and alleles are sampled with replacement



Why don’t we observe genotype

Question: Assuming an error rate of 1%
Is the individual heterozygous C/T?

Assuming heterozygous (C/T)



P(≤2 minor bases | T/A) = 0.065

Assuming homozygous (T/T)

P(≥2 errors | T/T) = 0.0052

Why don’t we observe genotype

P(≥2 errors | T/T) = 0.0052 P(≤2 minor bases | T/C) = 0.065

Question: Assuming an error rate of 1%
Is the individual heterozygous C/T?

Why don’t we observe genotype

P(≥2 errors | T/T) = 0052 P(≤2 minor bases | T/C) = 0.065 Heterozygosity is 0.1%

Question: Assuming an error rate of 1%
Is the individual heterozygous C/T?

Genotype likelihoods

how to quantify genotype uncertainty

Likelihood of the data

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

$\rightarrowtail$

10 possible genotypes

	A	C	G	T
A	1	2	3	4
C		5	6	7
G			8	9
T				10

The likelihood for the data for site j in indi

$$P(X_{ij}|G = \{G_1, G_2\}) = P(X_{ij}|G) \quad \text{where} \quad G \in \{A, C, G, T\}^2$$

Simple genotype likelihoods

GATK: McKenna et al 2010

Simple calculation of genotype likelihoods :

$P(X_{ij}|G)$

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

data X_{ij}

- D is the depth (number of read at position j individual i)
- b is the bases: e.g "TCCTTTTTTTT" (i,j omitted for simplicity)

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Simple genotype likelihoods

GATK: McKenna et al 2010

Simple calculation of genotype likelihoods :

$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2)$$

Data (X_{ij})

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Simple genotype likelihoods

GATK: McKenna et al 2010

Simple calculation of genotype likelihoods :

$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|G_1) + \frac{1}{2} P(b_d|G_2) \right)$$

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

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$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|G_1) + \frac{1}{2} P(b_d|G_2) \right)$$

where $P(b_d|G_l) = \begin{cases} \frac{\epsilon_d}{3} & b_d \neq G_l \\ 1 - \epsilon_d & b_d = G_l \end{cases}$,

where $G = \{G_1, G_2\}$, b_d is the observed base and ϵ_d is the probability of error from the quality score.

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

data X_{ij}

- D is the depth (number of read at position j individual i)
- b is the bases: e.g "TCCTTTTTTTT" (i,j omitted for simplicity)

Simple genotype likelihoods

GATK: McKenna et al 2010

Simple calculation of genotype likelihoods :

$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|G_1) + \frac{1}{2} P(b_d|G_2) \right)$$

where $P(b_d|G_I) = \begin{cases} \frac{\epsilon_d}{3} & b_d \neq G_I \\ 1 - \epsilon_d & b_d = G_I \end{cases}$,

where $G = \{G_1, G_2\}$, b_d is the observed base and ϵ_d is the probability of error from the quality score.

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Question: Assume you observe a read with base "T" and the ascii base quality score of "5"

- What is the $P(b_d$="T"$|G_1$="C")?

Ascii "5" is equal to a score of 20 = 1% error rate

Simple genotype likelihoods

GATK: McKenna et al 2010

Simple calculation of genotype likelihoods :

$$P(X_{ij}|G) \propto \prod_{d=1}^{D} P(b_d|G_1, G_2) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|G_1) + \frac{1}{2} P(b_d|G_2) \right)$$

where $P(b_d|G_I) = \begin{cases} \frac{\epsilon_d}{3} & b_d \neq G_I \\ 1 - \epsilon_d & b_d = G_I \end{cases}$,

where $G = \{G_1, G_2\}$, b_d is the observed base and ϵ_d is the probability of error from the quality score.

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Question: Assume you observe a read with base “C” and the ascii base quality score of “5”

- What is the $P(b_d$=”C”$|G_1$=”C”)?

Ascii “5” is equal to a score of 20 = 1% error rate

b	Q_{ascii}	Q_{score}	ϵ_d	$p(b_d\|T)$	$p(b_d\|C)$	$p(b_d\|G/A)$
T	+	10	0.1	0.9	0.033	0.033
C	,	11	0.079	0.026	0.92	0.026
C	7	22	0.0063	0.0021	0.99	0.0021
T	7	22	0.0063	0.99	0.0021	0.0021
T	&	5	0.32	0.68	0.11	0.11
T	&	5	0.32	0.68	0.11	0.11
T	8	23	0.005	0.99	0.0017	0.0017
T	8	23	0.005	0.99	0.0017	0.0017
T	8	23	0.005	0.99	0.0017	0.0017
T	8	23	0.005	0.99	0.0017	0.0017
T	+	10	0.1	0.9	0.033	0.033

$$P(X|G = TC) \propto \prod_{d=1}^{D} P(b_d|T, C) = \prod_{d=1}^{D} \left(\frac{1}{2} P(b_d|T) + \frac{1}{2} P(b_d|C) \right)$$

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

All 10 possible genotype likelihoods

log Likelihood $P(D|G)$

	A	C	G	T
A	-52.84	-44.49	-52.84	-16.66
C		-43.13	-44.49	-8.31
G			-52.84	-16.66
T				-10.79

likelihood (normal scale)

	A	C	G	T
A	0	0	0	0
C		0	0	0.00025
G			0	0
T				0.00002

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Many genotype likelihood models

GATK haplotype caller

GATK unified genotyper caller

Samtools/bcftools

freeBayes

ATLAS (for ancient DNA)

Genotype calling

log Likelihood $P(D|G)$

	A	C	G	T
A	-52.84	-44.49	-52.84	-16.66
C		-43.13	-44.49	-8.31
G			-52.84	-16.66
T				-10.79

likelihood (normal scale)

	A	C	G	T
A	0	0	0	0
C		0	0	0.00025
G			0	0
T				0.00002

Data (X_{ij})

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

simple genotype callers - Maximum likelihood

ML I Choose the genotype with the largest likelihood $\arg\max_G P(X|G)$

ML II only call a genotype if the likelihood with much better than the second best e.g. a likelihood ratio $>$ 2

Genotype calling: bayesian

Data X_{ij}

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Likelihood

$p(X_{ij} | G)$

	A	C	G	T
A	0	0	0	0
C		0	0	0.00025
G			0	0
T				0.00002

Prior

uniform:
p(A/A)=1/10
p(A/C)=1/10
....
p(T/T)=1/10

Posterior probability

	A	C	G	T
A	0	0	0	0
C		0	0	0.922
G			0	0
T				0.077

Bayes formula

$$p(G|X_{ij}) = \frac{p(X_{ij}|G)p(G)}{p(X_{ij})}$$

Modified slide from
matteo Fumagalli

Genotype calling: bayesian

Data X_{ij}

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Likelihood

$p(X_{ij} | G)$

	A	C	G	T
A	0	0	0	0
C		0	0	0.00025
G			0	0
T				0.00002

Prior

f_A=0
f_C=0.01
f_G=0
f_T=0.99
Assume HWE

Bayes formula

$$p(G|X_{ij}, f) = \frac{p(X_{ij}|G)p(G|f)}{p(X_{ij}|f)}$$

Posterior probability

	A	C	G	T
A	0.0	0	0.0	0.0
C		0	0	0.20
G			0	0
T				0.80

HWE assumption: $p(C/C)=f^2_C$,$p(C/T)=2f_Cf_T$,$p(T/T)=f^2_T$

Genotype calling: empirical bayes

Data X_{ij}

bases (b):
TCCTTTTTTTT
quality scores (Q):
+,77&&8888+

Likelihood

$p(X_{ij} | G)$

	A	C	G	T
A	0	0	0	0
C		0	0	0.00025
G			0	0
T				0.00002

Use all of your samples to estimate allele frequencies

Prior

f_A=0
f_C=0.05
f_G=0
f_T=0.95
Assume HWE

Bayes formula

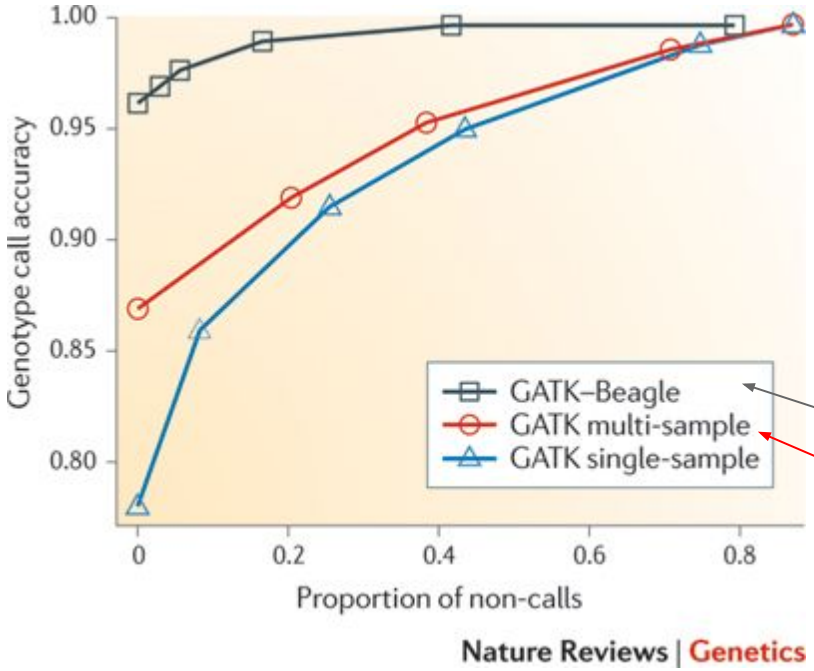
$$p(G|X_{ij}, f) = \frac{p(X_{ij}|G)p(G|f)}{p(X_{ij}|f)}$$

Posterior probability

	A	C	G	T
A	0.0	0.0	0.0	0.0
C		0.0	0.0	0.56
G			0.0	0
T				0.44

HWE assumption: $p(C/C)=f^2_C$,$p(C/T)=2f_Cf_T$,$p(T/T)=f^2_T$

Which genotype caller is best



- Data 1000 genotypes
- Low depth ~avg 3.5X
- Common (MAF>5%) SNPs

Empirical bayes using haplotype frequencies

Empirical bayes using allele frequencies

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Simple allele frequency estimator

Simple frequency estimator

- assume only two allele types exits
- let n_1^i and n_2^i be the counts of observed alleles in individual i.
- $f = \frac{\sum n_1^i}{\sum (n_1^i + n_2^i)}$

Heterozygous with one m allele and one M allele

Example

i	1	2	3	4	5	6	Total
True Geno	MM	MM	Mm	Mm	mm	mm	
#M Reads	7	25	5	4	0	0	41
#m reads	0	1	3	4	2	4	14

$$f = \frac{41}{41+14} = 0.75$$

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Maximum likelihood estimator

ML frequency estimator

- assume only two alleles type exits
- $P(X^i|f) = \sum_{g \in \{0,1,2\}} P(X|G=g)P(G=g|f)$
- assume HWE e.g. $P(G = A_1A_1|f) = f^2$
- $\hat{f_{ML}} = argmax_p \prod_i P(X^i|f)$

Example

i	1	2	3	4	5	6	Total
True Geno	MM	MM	Mm	Mm	mm	mm	
#M Reads	7	25	5	4	0	0	41
#m reads	0	1	3	4	2	4	14

$$\hat{f}_{ML} = 0.46$$

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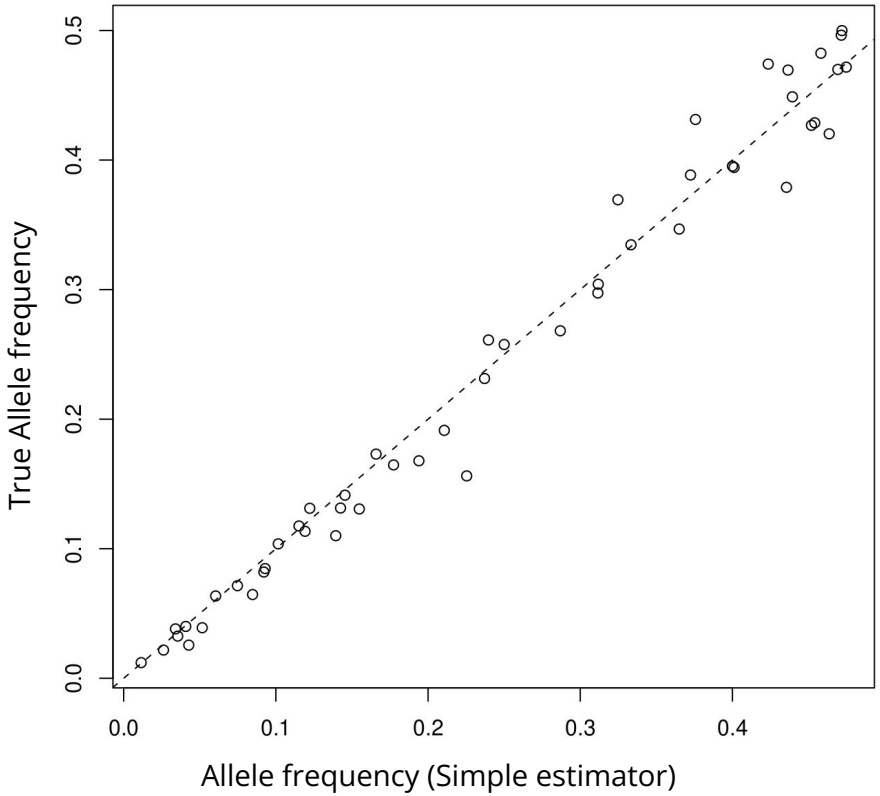
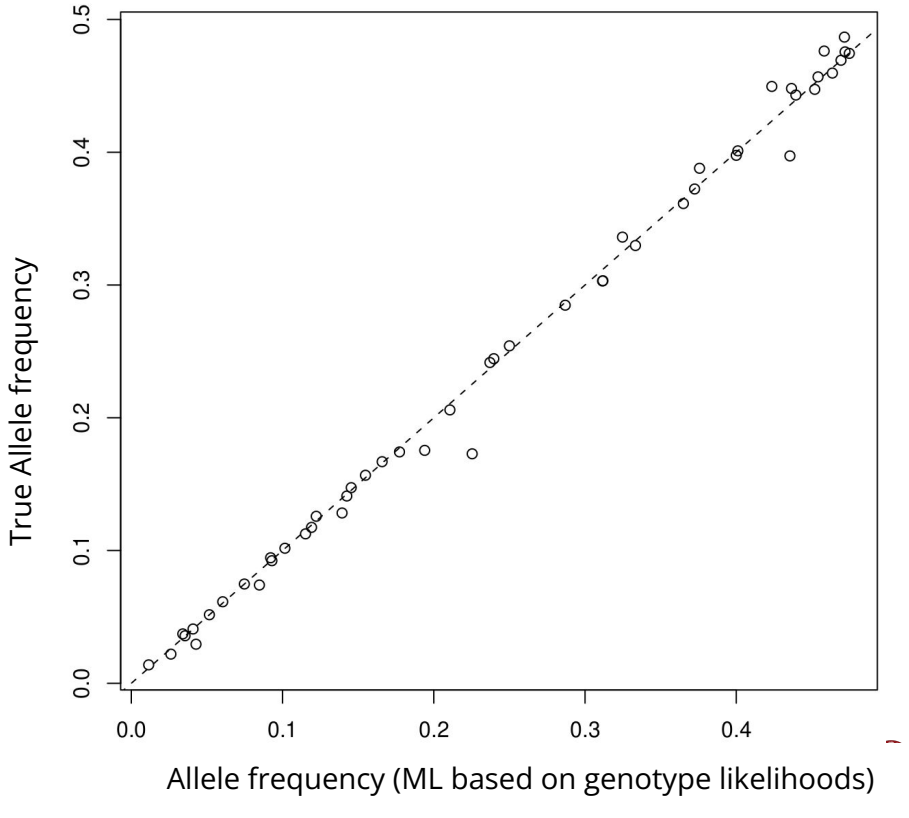

True Allele frequency
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.1
0.2
0.3
0.4
Allele frequency (Simple estimator)


True Allele frequency
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.1
0.2
0.3
0.4
Allele frequency (ML based on genotype likelihoods)

Other use of frequencies from GL

SNP calling

Null model: $f_A=0, f_C=0, f_G=0, f_T=1$

$L_{null} \propto p(X_j | f_A=0, f_C=0, f_G=0, f_T=1)$

Alt model: $f_A=0, f_C=0.05, f_G=0, f_T=0.95$

$L_{alt} \propto p(X_j | f_A=0, f_C=0.05, f_G=0, f_T=0.95)$

Likelihood ratio test

$2\log(L_{alt}/L_{null}) \sim \chi^2_1$

Test difference in frequency

Null model: $f_A= f_G=0, f_C=0.05, f_T=0.95$

$L_{null} \propto p(X_j | f_A= f_G=0, f_C=0.05, f_T=0.95)$

Alt model:

group1: $f_A= f_G=0, f_C=0.02, f_T=0.98)$

group2: $f_A= f_G=0, f_C=0.07, f_T=0.93)$

$L_{alt} \propto p(X_{j1} | f_A=f_G=0, f_C=0.02, f_T=0.98) * p(X_{j2} | f_A=f_G=0, f_C=0.07, f_T=0.93)$

Likelihood ratio test

$2\log(L_{alt}/L_{null}) \sim \chi^2_1$

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Software for variant & genotype calling

GATK

samtools/bcftools

freeBayes

- Assembly-based caller (as in GATK)

Local re-alignment around putative variants; better resolution for INDELs detection.

- Haplotype-based caller (as in freebayes)

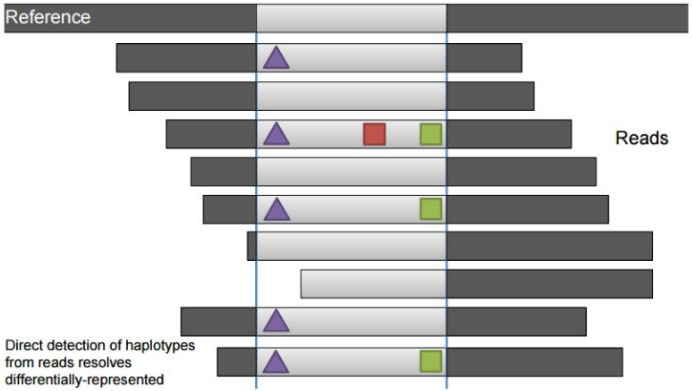

Figure from Erik Garrison

VCF files

Metadata

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##fileformat=VCFv4.1
##fileDate=20120630
##source=freeBayes version 0.9.6
##reference=W303.fasta
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of samples with data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Total number of alternate alleles in called genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=AF,Number=A,Type=Float,Description="Estimated allele frequency in the range (0,1]">
##INFO=<ID=RO,Number=1,Type=Integer,Description="Reference allele observations">
##INFO=<ID=AO,Number=A,Type=Integer,Description="Alternate allele observations">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality">
```

Body

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	BYB1_G07-0
chrVIII	255912	.	GA	G	1408.89	.	AO=18;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	263821	.	G	A	6257.23	.	AO=201;...	GT:GQ:DP:...	1/1:50000:...
chrVIII	275478	.	TGGCCAG	TGCCAA	5885.31	.	AO=185;...	GT:GQ:DP:...	1/1:50000:...
chrVIII	276438	.	CA	C	63.5434	.	AO=3;...	GT:GQ:DP:...	0/1:63.5064:...
chrVIII	290238	.	TA	T	12.4555	.	AO=5;...	GT:GQ:DP:...	0/1:12.4555:...
chrVIII	298817	.	CT	C	482.635	.	AO=13;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	314728	.	CAT	C	101.007	.	AO=8;...	GT:GQ:DP:...	0/1:101.007:...
chrVIII	317567	.	T	G,A	160.186	.	AO=37;...	GT:GQ:DP:...	0/1:160.126:...
chrVIII	323237	.	G	GA	99.7114	.	AO=9;...	GT:GQ:DP:...	0/1:95.3368:...
chrVIII	340061	.	TTA	T	360.562	.	AO=5;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	361913	.	AT	A	1237.88	.	AO=17;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	368029	.	T	A,G	1630.61	.	AO=35,31;...	GT:GQ:DP:...	1/2:50000:...

G A	SNP
G GA	insertion
CT C	deletion
TTA T	2 bp deletion
TGGCCAG TGCCAA	complex mutation
T A,G	multiple alternate alleles

VCF files

Metadata

```
##fileformat=VCFv4.1
##fileDate=20120630
##source=freeBayes version 0.9.6
##reference=W303.fasta
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of samples with data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Total number of alternate alleles in called genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=AF,Number=A,Type=Float,Description="Estimated allele frequency in the range (0,1]">
##INFO=<ID=RO,Number=1,Type=Integer,Description="Reference allele observations">
##INFO=<ID=AO,Number=A,Type=Integer,Description="Alternate allele observations">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality">
```

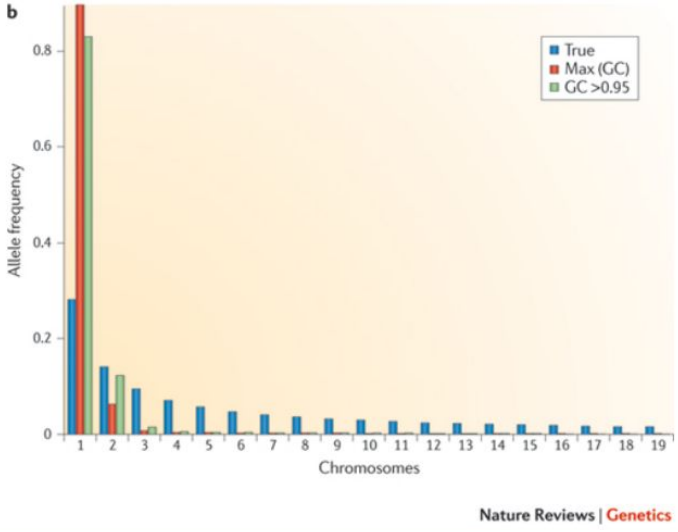
Body

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	BYB1_G07-0
chrVIII	255912	.	GA	G	1408.89	.	AO=18;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	263821	.	G	A	6257.23	.	AO=201;...	GT:GQ:DP:...	1/1:50000:...
chrVIII	275478	.	TGGCCAG	TGCCAA	5885.31	.	AO=185;...	GT:GQ:DP:...	1/1:50000:...
chrVIII	276438	.	CA	C	63.5434	.	AO=3;...	GT:GQ:DP:...	0/1:63.5064:...
chrVIII	290238	.	TA	T	12.4555	.	AO=5;...	GT:GQ:DP:...	0/1:12.4555:...
chrVIII	298817	.	CT	C	482.635	.	AO=13;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	314728	.	CAT	C	101.007	.	AO=8;...	GT:GQ:DP:...	0/1:101.007:...
chrVIII	317567	.	T	G,A	160.186	.	AO=37;...	GT:GQ:DP:...	0/1:160.126:...
chrVIII	323237	.	G	GA	99.7114	.	AO=9;...	GT:GQ:DP:...	0/1:95.3368:...
chrVIII	340061	.	TTA	T	360.562	.	AO=5;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	361913	.	AT	A	1237.88	.	AO=17;...	GT:GQ:DP:...	0/1:50000:...
chrVIII	368029	.	T	A,G	1630.61	.	AO=35,31;...	GT:GQ:DP:...	1/2:50000:...

```
AB=0; ABP=0; AC=2; AF=1; AN=2; AO=185; CIGAR=1M1D4M1X; DP=192; DPRA=0; EPP=4.99397; EPPR=9.52472; HWE=-0;
LEN=6; MEANALT=5; MQM=57.1243; MQMR=43; NS=1; NUMALT=1; ODDS=94.868; PAIRED=0.972973; PAIREDR=1; RO=3;
RPP=10.3464; RPPR=9.52472; RUN=1; SAP=8.18662; SRP=9.52472; TYPE=complex; XAI=0.0102812; XAM=0.0122725;
XAS=0.00199131; XRI=0; XRM=0; XRS=0; BVAR
```

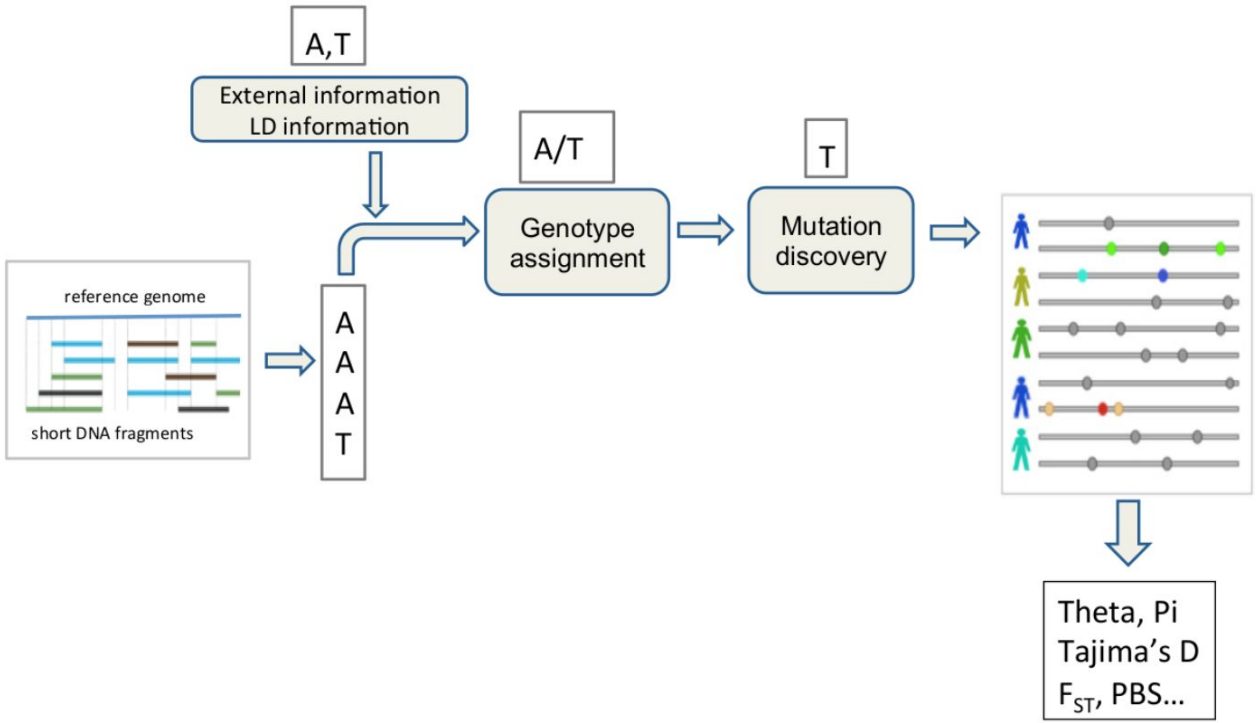
Should we always call genotypes

Site frequency spectrum

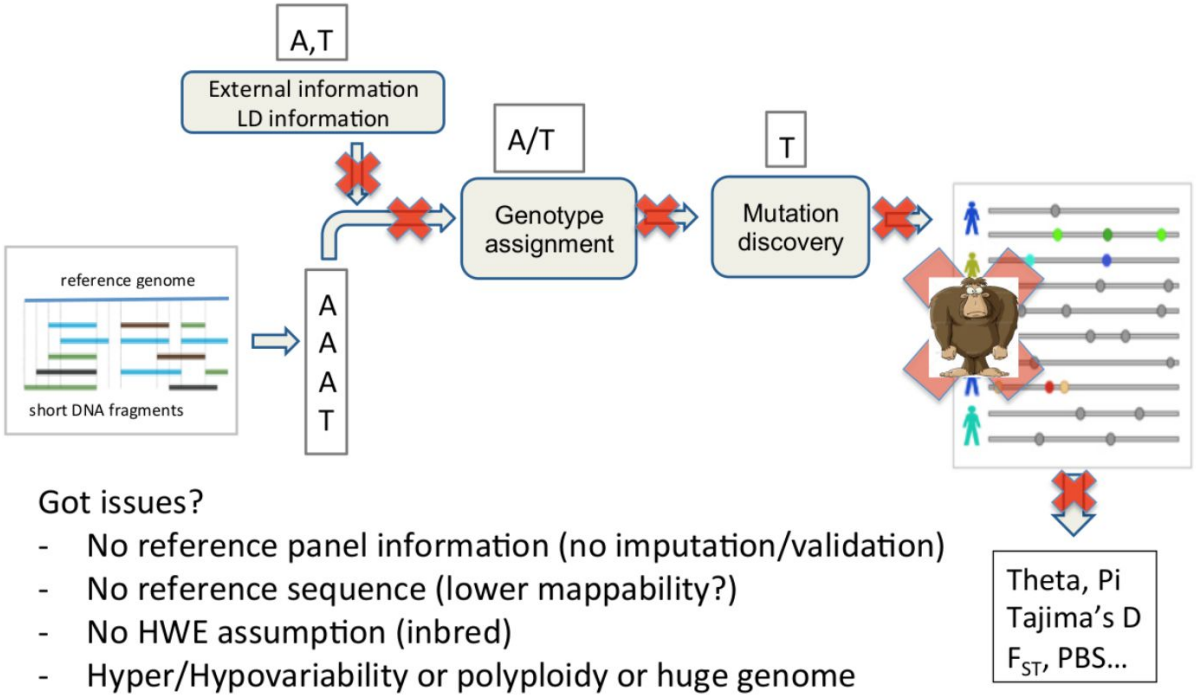


For low depth there are no filter or haplotype imputation approach that will work

High quality data



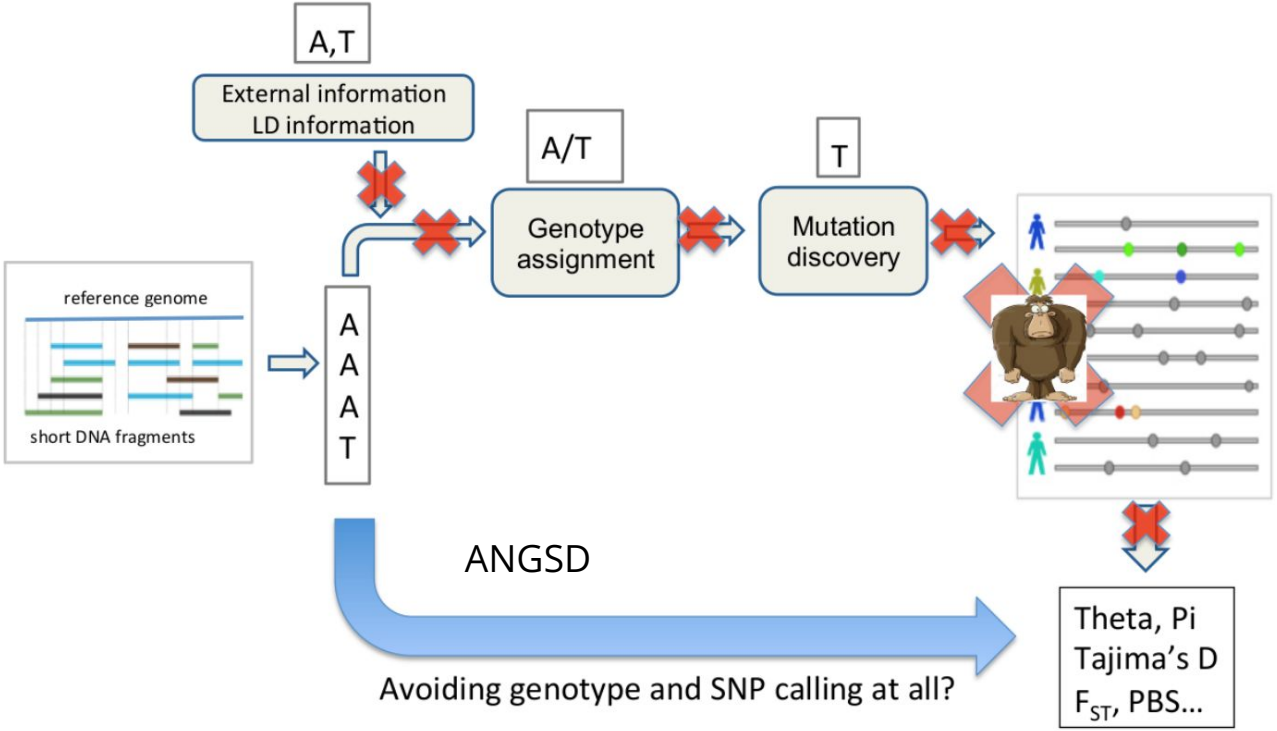
If you don’t have high quality data



Got issues?

- No reference panel information (no imputation/validation)
- No reference sequence (lower mappability?)
- No HWE assumption (inbred)
- Hyper/Hypovariability or polyploidy or huge genome
- No money (?)
- **Your inferences will be wrong!**

Alternative is to not call genotypes



Time for exercises

Go to

tinyurl.com/kilifiNGS

The likelihood

$$\underbrace{p(X \mid \theta)}_{1} = \underbrace{\prod_{i=1}^{N} p(X_i|\theta)}_{2} = \underbrace{\prod_{i=1}^{N} \sum_{z} p(X_i|Z_i = z)p(Z_i = z|\theta)}_{3}$$

1. Probability of data given parameters
2. Assumes individuals are independent
3. Introduce latent variable using law of total probability

Input
Genotype likelihoods

i	P(X|Z=MM)	P(X|Z=mM)	P(X|Z=mm)
1	0.93	0.0078	1.0×10^{-14}
2	0.0078	1.5×10^{-8}	9.9×10^{-51}
3	9.5×10^{-7}	3.9×10^{-3}	9.7×10^{-11}
4	9.6×10^{-9}	3.9×10^{-3}	9.6×10^{-9}
5	1.0×10^{-8}	0.25	0.98
6	1.0×10^{-8}	0.062	0.96

Notation

X is all of the data for a single site
X_i is the data for individual i
θ is the frequency of the two alleles
$\theta = (\theta_M, \theta_m)$
N is the number of individuals

Latent variable

z is latent state of the genotype
Z_i is the genotype for individual i
$Z_i \in \{MM, Mm, mm\}$

Binomial (HWE)

$$p(Z_i = MM|\theta) = \theta_M \theta_M$$

$$p(Z_i = Mm|\theta) = 2\theta_M \theta_m$$

$$p(Z_i = mm|\theta) = \theta_m \theta_m$$

First iteration in EM algorithm

$$p(Z_i|X_i,\theta^{(0)}) = \frac{p(X_i|Z_i)p(Z_i|\theta^{(0)})}{p(X_i|\theta^{(0)})}$$

Unobserved Genotypes

G
MM
MM
mM
mM
m
m

Input Genotype likelihoods

i	P(X|Z=MM)	P(X|Z=mM)	P(X|Z=mm)
1	0.93	0.0075	4.6×10^{-18}
2	0.0026	1.3×10^{-8}	1.2×10^{-62}
3	3.5×10^{-8}	3.7×10^{-3}	4.0×10^{-13}
4	1.2×10^{-10}	3.7×10^{-3}	1.2×10^{-10}
5	1.1×10^{-5}	0.25	0.98
6	1.2×10^{-10}	0.061	0.96

i	P(Z=MM|X)	P(Z=mM|X)	P(|Z=mm|X)
1	1.00	0.00	0.00
2	1.00	0.00	0.00
3	0.00	1.00	0.00
4	0.00	1.00	0.00
5	0.00	0.67	0.33
6	0.00	0.34	0.66
Σ	2.00	3.01	0.99

Expected genotypes

$$\theta^{(0)} = (\theta_M^{(0)}\theta_m^{(0)}) = (0.2, 0.8)$$

$$\theta_m^{(1)} = \frac{3.01 + 2 \times 0.99}{2 \times 2.00 + 2 \times 3.01 + 2 \times 0.99}$$

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EM algorithm assuming HWE

Log likelihood

$$log(L(\theta)) = log(p(X|\theta)) = \sum_i log\left(\sum_z p(X_i, Z_i = z|\theta)\right) = \sum_i log\left(\sum_z p(X_i|Z_i = z)p(Z_i = z|\theta)\right)$$

$$\theta_m + \theta_M = 1 \quad \text{and} \quad P(X|Z,\theta) = P(X|Z) \quad \text{and} \quad P(Z_i|\theta) = B(Z_i; n = 2, \theta)$$

E step (Q)

$$Q_i(Z_i = z) = p(Z_i = z|X_i, \theta^{(n)}) = \frac{p(X_i|Z_i = z, \theta^{(n)})p(Z_i = z|\theta^{(n)})}{\sum_{z'} p(X_i|Z_i = z', \theta^{(n)})p(Z_i = z'|\theta^{(n)})}$$

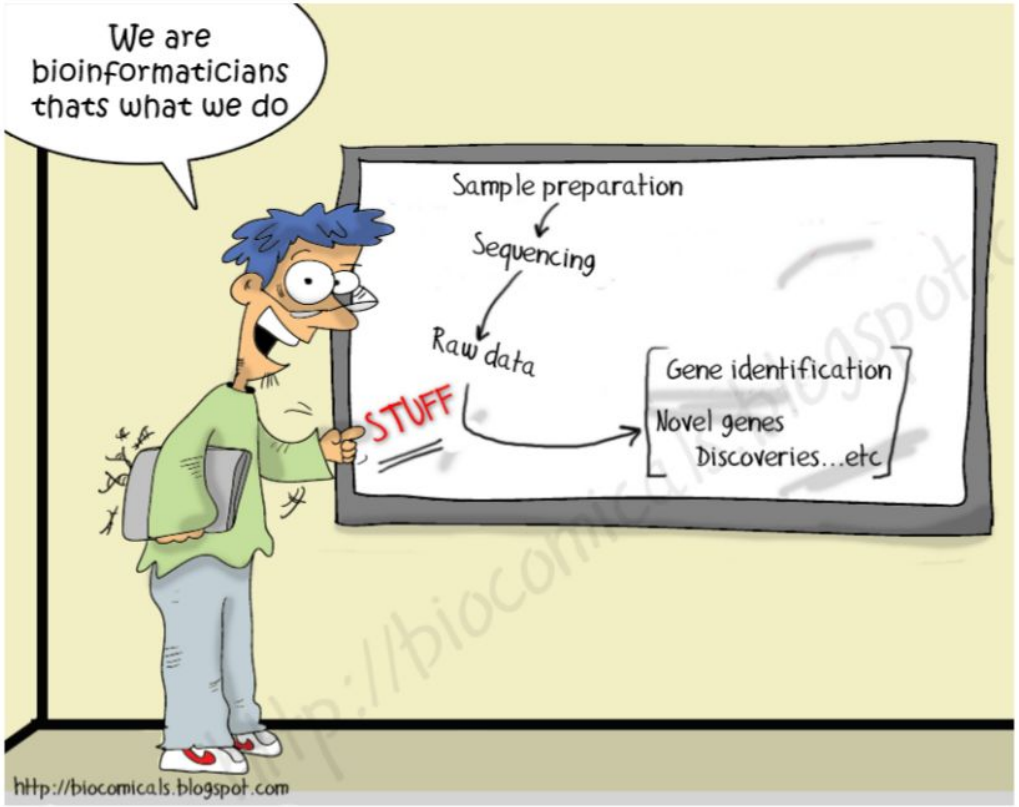
M step

$$\theta_m^{(n+1)} = \frac{\sum_i (0 \times Q_i(Z = MM) + 1 \times Q_i(Z = mM) + 2 \times Q_i(Z = mm))}{\sum_i \sum_z Q_i(Z_i = z)}$$

X is all of the data for a single site
X_i is the data for individual i
θ is the frequency of the two alleles
$\theta = (\theta_M, \theta_m)$
N is the number of individuals

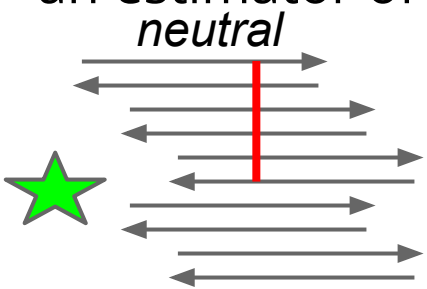
z is latent state of the genotype
Z_i is the genotype for individual i
$Z_i \in \{MM, Mm, mm\}$

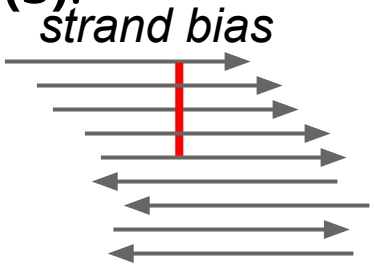
Recap: Analysis of NGS data

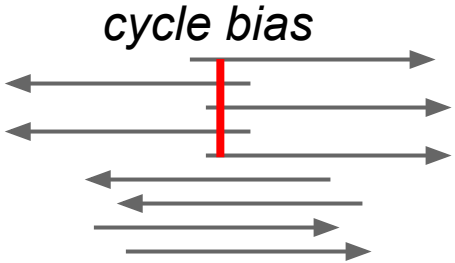


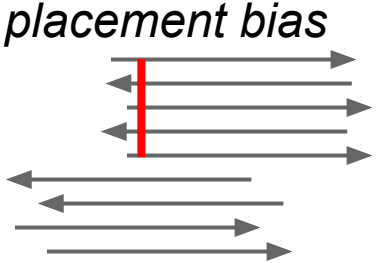
Are our locus and alleles sequenceable?

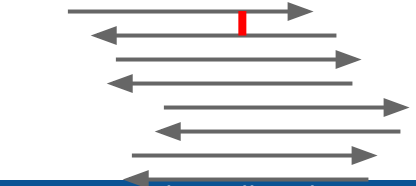
In WGS, biases in the way we observe an allele (placement, position, strand, cycle, or balance in heterozygotes) are often correlated with error. We include this in our posterior **P(G,S|R)**, and to do so we need an estimator of **P(S)**.





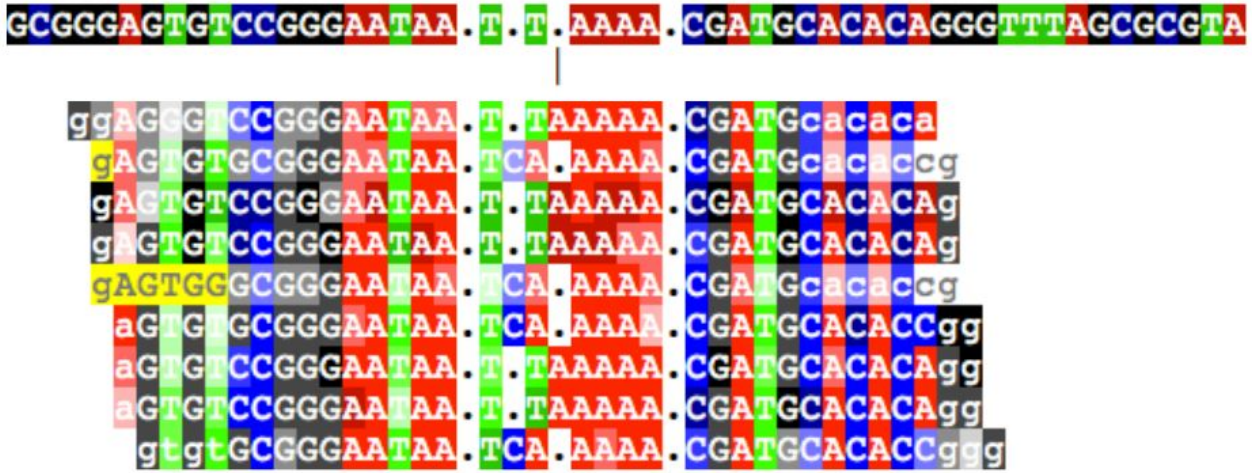


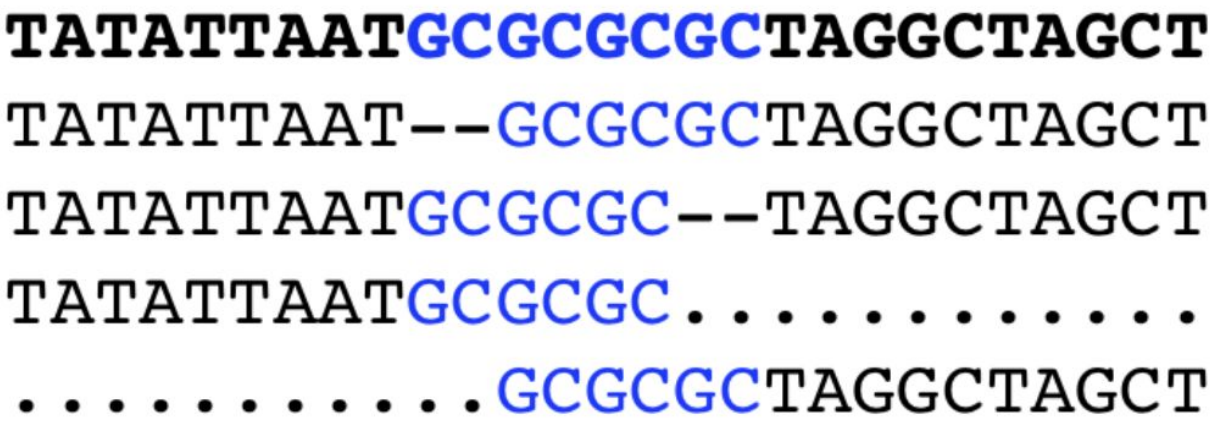




$$P(S) \propto \times \prod_{\forall b \in \{B\}} \begin{array}{l} multinom([|R \equiv b| \forall b_1, \ldots, b_K]; |\{R\}|, f_i, \ldots, f_K) \\ binom(|forwardStrand(\{R \equiv b\})|; |\{R \equiv b\}|, 1/2) \\ \times binom(|placedLeft(\{R \equiv b\})|; |\{R \equiv b\}|, 1/2) \\ \times binom(|placedRight(\{R \equiv b\})|; |\{R \equiv b\}|, 1/2) \end{array}$$

Mapper does not know our data e.g. it does not know whether it is haploid/diploid/pooled individual ect.

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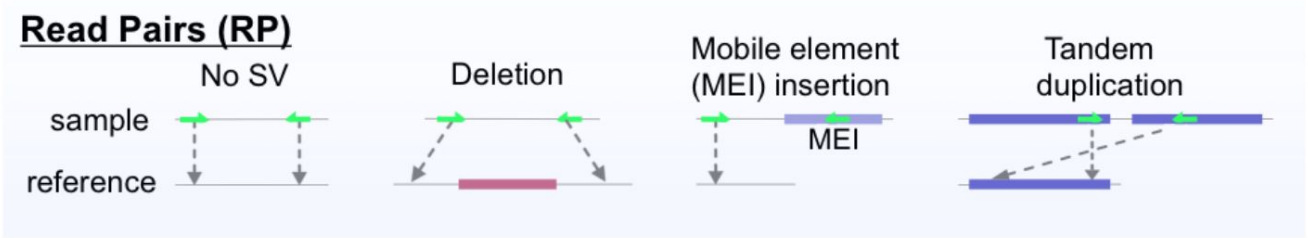
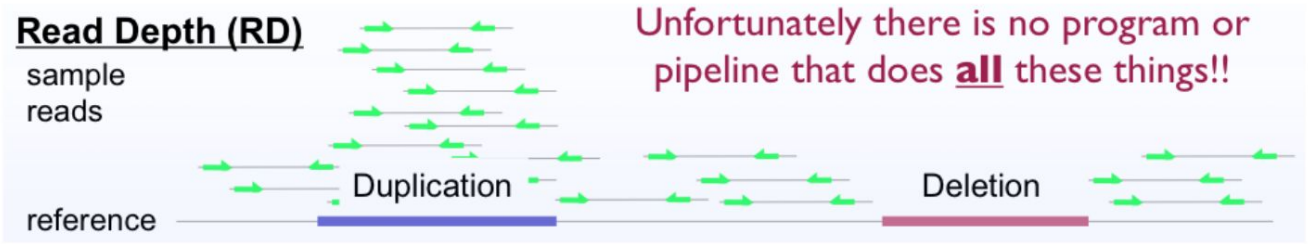
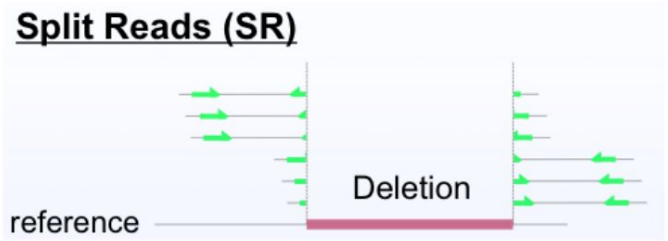
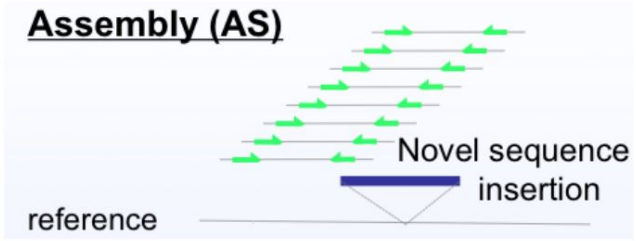
TATATTAAT--GCGCGCTAGGCTAGCT
TATATTAATGCGCGC--TAGGCTAGCT
TATATTAATGCGCGC.............
............GCGCGCTAGGCTAGCT

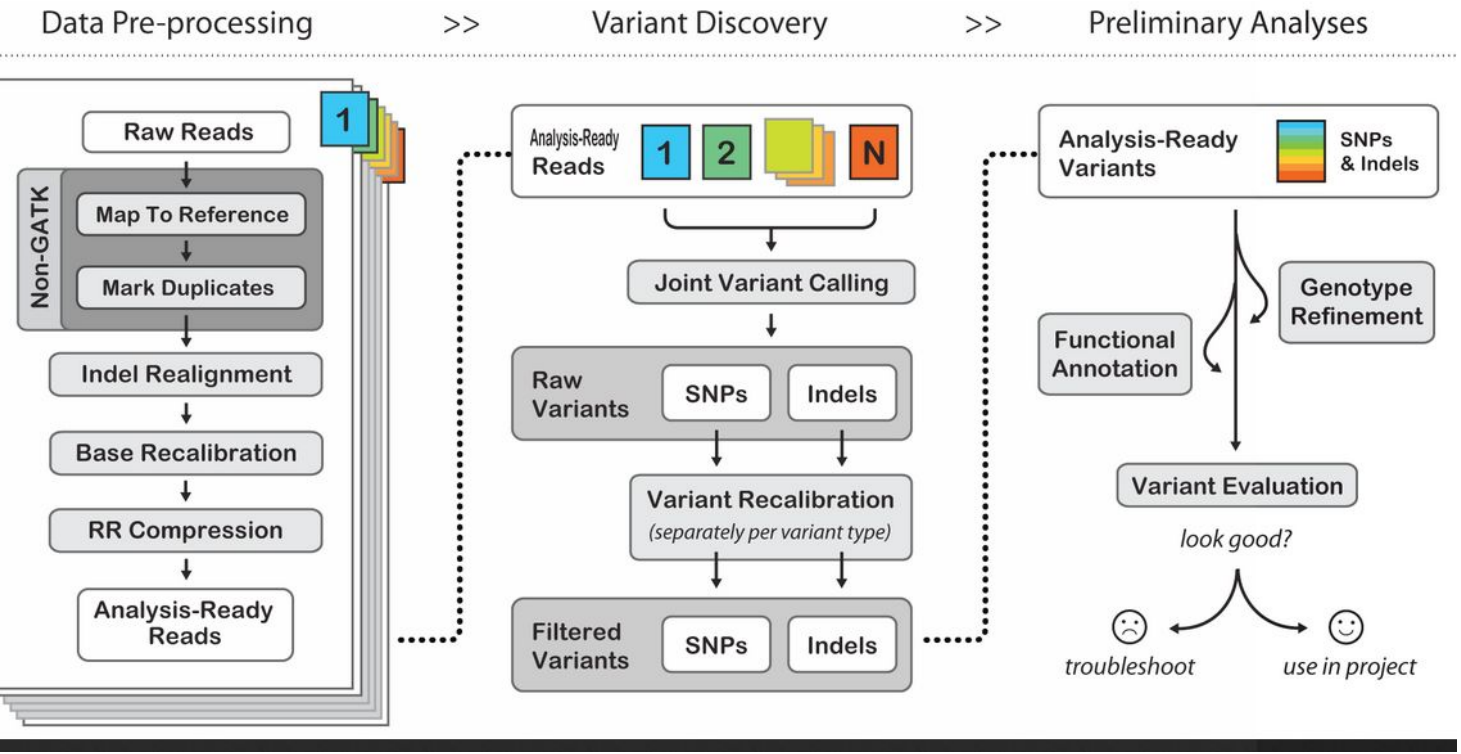


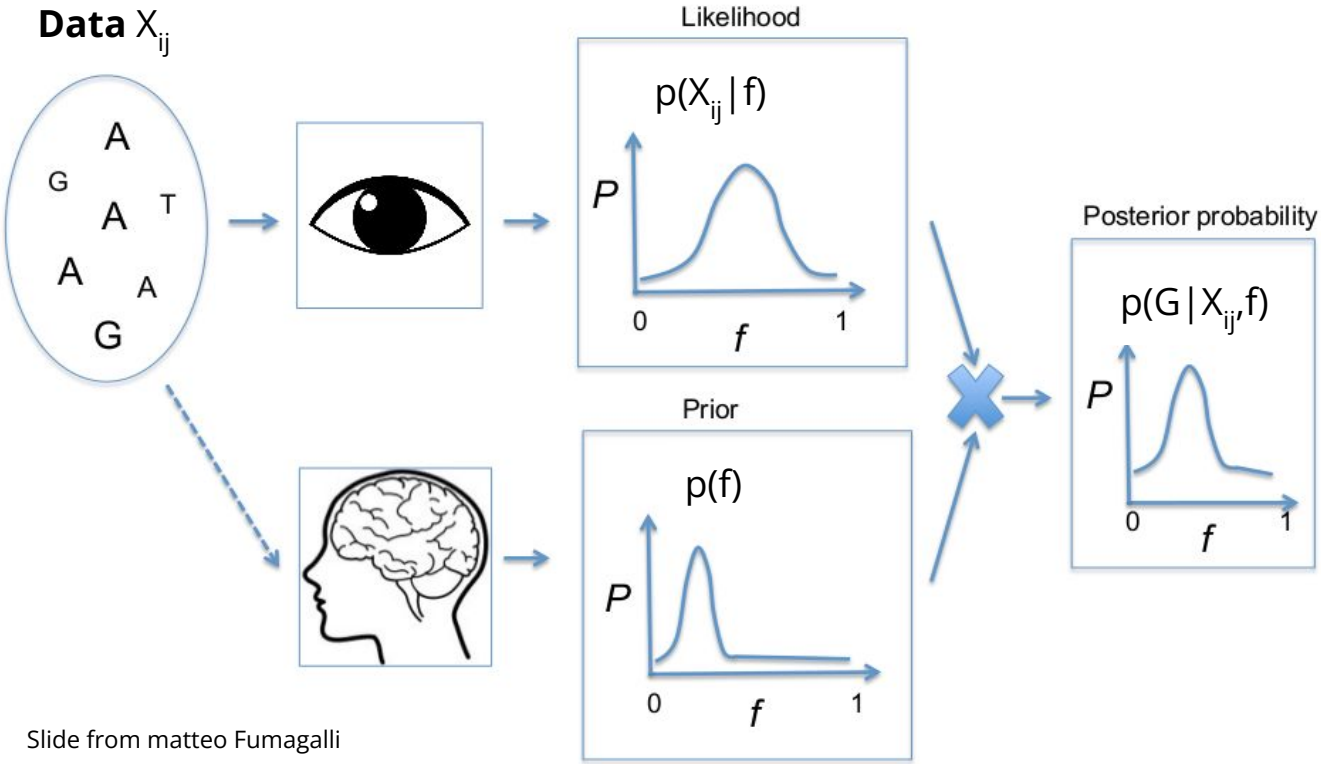


Unfortunately there is no program or pipeline that does **all** these things!!






Data Pre-processing
>>
Variant Discovery
>>
Preliminary Analyses
Raw Reads
1
Non-GATK
Map To Reference
Mark Duplicates
Indel Realignment
Base Recalibration
RR Compression
Analysis-Ready Reads
Analysis-Ready Reads
1
2
N
Joint Variant Calling
Raw Variants
SNPs
Indels
Variant Recalibration
(separately per variant type)
Filtered Variants
SNPs
Indels
Analysis-Ready Variants
SNPs & Indels
Genotype Refinement
Functional Annotation
Variant Evaluation
look good?
troubleshoot
use in project



Slide from matteo Fumagalli

The likelihood for allele frequencies

The likelihood

$$\underbrace{p(X \mid \theta)}_{1}$$

1. Probability of data given parameters

notation

X is all of the data for a single site
X_i is the data for individual i
θ is the frequency of the two alleles
$\theta = (\theta_M, \theta_m)$
N is the number of individuals

The likelihood

$$\underbrace{p(X \mid \theta)}_{1} = \underbrace{\prod_{i=1}^{N} p(X_i|\theta)}_{2}$$

1. Probability of data given parameters
2. Assumes individuals are independent

notation

X is all of the data for a single site
X_i is the data for individual i
θ is the frequency of the two alleles
$\theta = (\theta_M, \theta_m)$
N is the number of individuals

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The likelihood

$$\underbrace{p(X \mid \theta)}_{1} = \underbrace{\prod_{i=1}^{N} p(X_i|\theta)}_{2} = \underbrace{\prod_{i=1}^{N} \sum_{z} p(X_i|Z_i = z)p(Z_i = z|\theta)}_{3}$$

1. Probability of data given parameters
2. Assumes individuals are independent
3. Introduce latent variable using law of total probability

notation

X is all of the data for a single site
X_i is the data for individual i
θ is the frequency of the two alleles
$\theta = (\theta_A, \theta_a)$

notation

z is latent state of the genotype
Z_i is the genotype for individual i
$Z_i \in \{MM, Mm, mm\}$

The likelihood

$$\underbrace{p(X \mid \theta)}_{1} = \underbrace{\prod_{i=1}^{N} p(X_i|\theta)}_{2} = \underbrace{\prod_{i=1}^{N} \sum_{z} p(X_i|Z_i = z)p(Z_i = z|\theta)}_{3}$$

1. Probability of data given parameters
2. Assumes individuals are independent
3. Introduce latent variable using law of total probability

$p(X_i|\theta, Z_i = z) = p(X_i|Z_i = z)$

$p(X_i|Z_i = z)$ is the genotype likelihood

The likelihood

$$\underbrace{p(X \mid \theta)}_{1} = \underbrace{\prod_{i=1}^{N} p(X_i|\theta)}_{2} = \underbrace{\prod_{i=1}^{N} \sum_{z} p(X_i|Z_i = z)p(Z_i = z|\theta)}_{3}$$

1. Probability of data given parameters
2. Assumes individuals are independent
3. Introduce latent variable using law of total probability

$p(X_i|\theta, Z_i = z) = p(X_i|Z_i = z)$

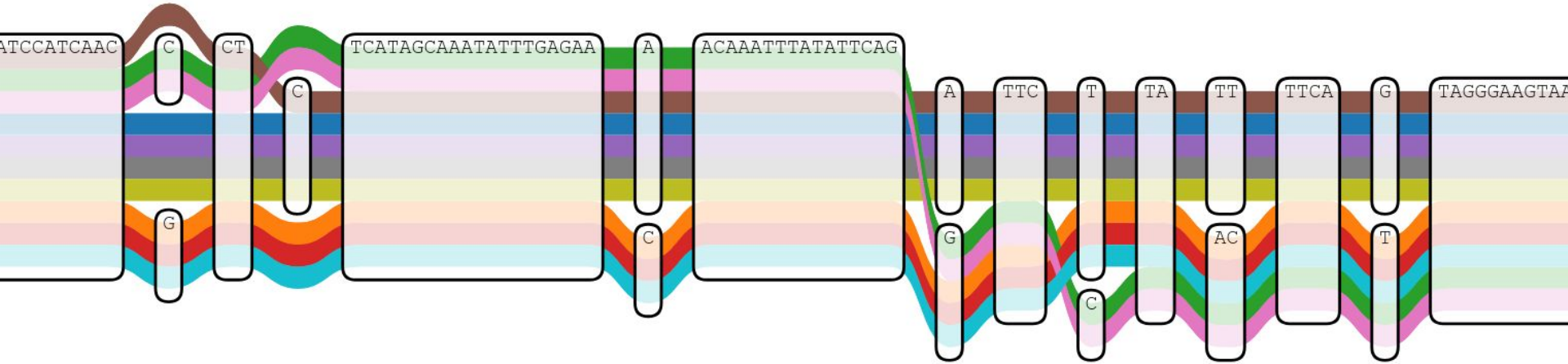
$p(X_i|Z_i = z)$ is the genotype likelhood

$p(Z_i = z|\theta)$

Binomial with parameters $n = 2, p = \theta_m, k = \#m)$

$$p(X_i|\theta_z) = B(k : n, p)$$

Hardy weinberg



Variation graph

https://vgteam.github.io/sequenceTubeMap/

Indel mappeding is not consistent

```
TATATTAATGCGCGCGCTAGGCTAGCT
TATATTAAT--GCGCGCTAGGCTAGCT
TATATTAATGCGCGC--TAGGCTAGCT
TATATTAATGCGCGC............
............GCGCGCTAGGCTAGCT
```