Inference from NGS data

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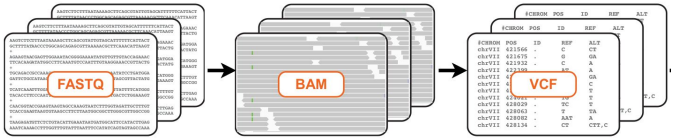
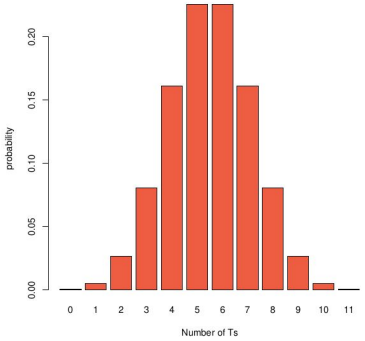
This session

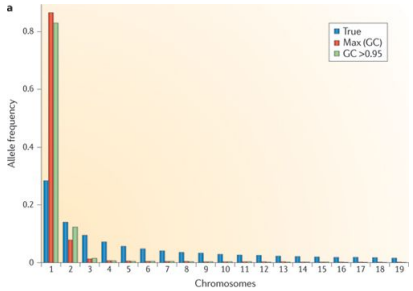
This morning

- 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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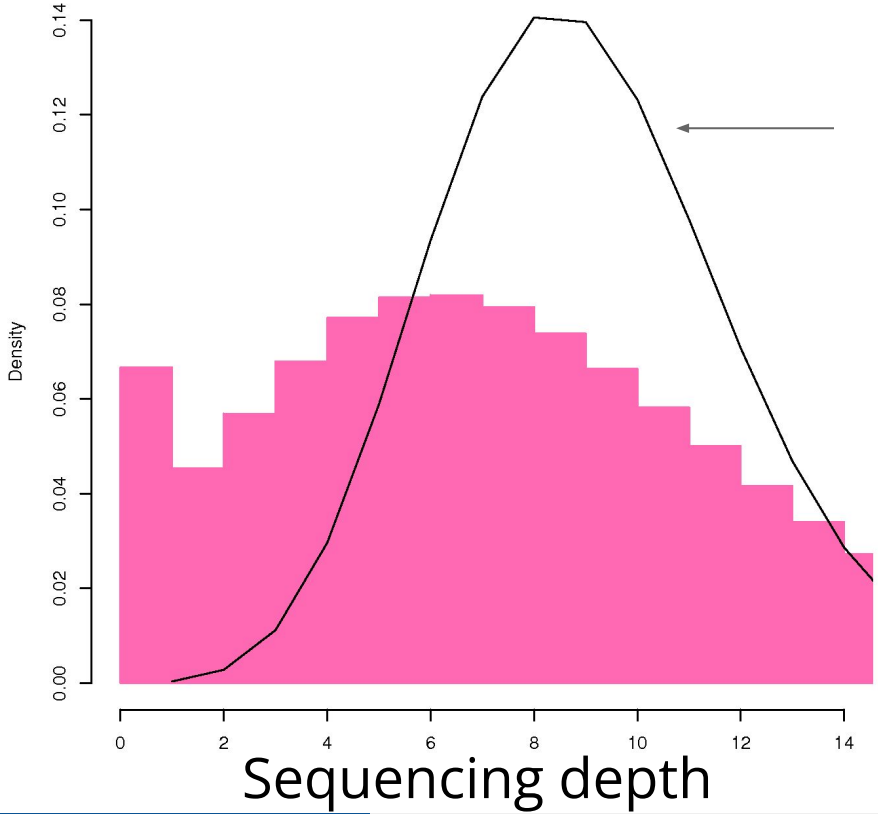
Sequencing Depth

Sequencing depth is the number of reads covering a position
Average depth is often written as X e.g. **15X** sequencing
Coverage = depth or the fraction of genome with data

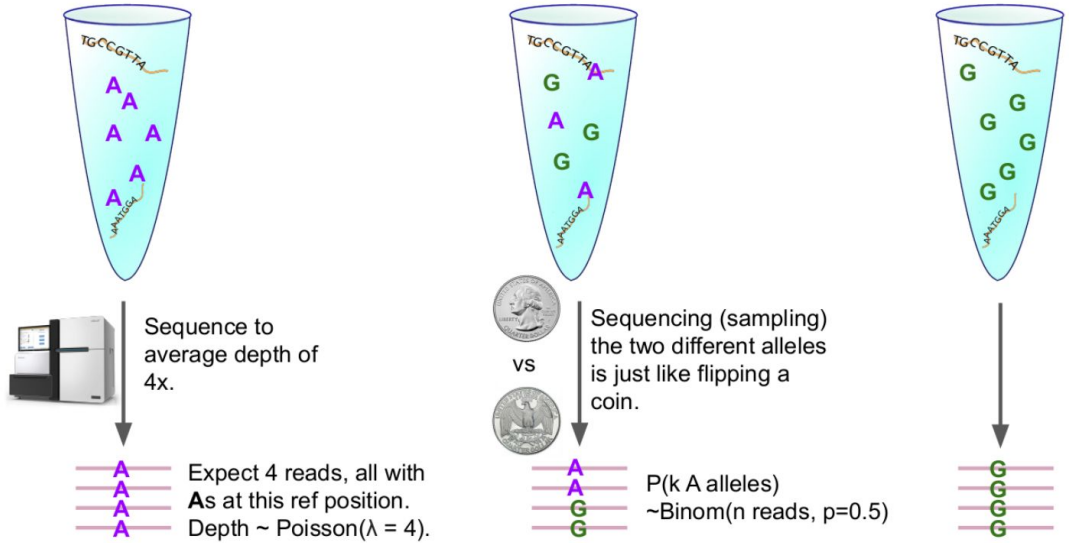
Depth distribution



Theoretical distribution (Poisson) for 8X avg. depth if reads mapped perfectly and there was no bias

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?

slido

Is the individual heterozygous or homozygous

ⓘ Click **Present with Slido** or install our Chrome extension to activate this poll while presenting.

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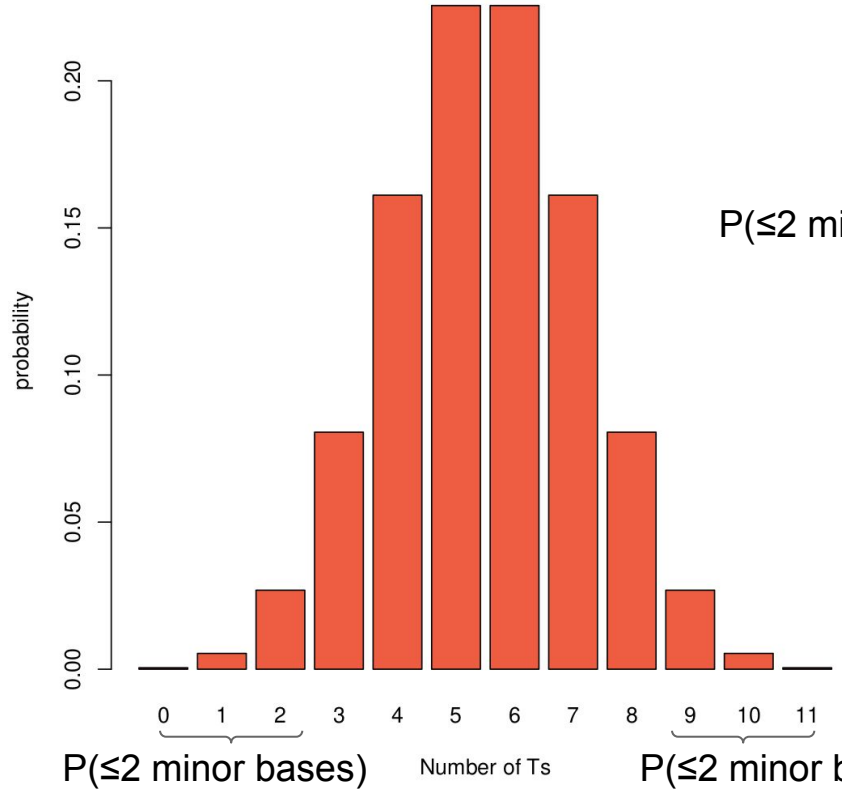
Assuming heterozygous (C/T)



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) = 0.0052 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?

Why don’t we observe genotype

P(≥2 errors | T/T) = 0.0052 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?

Approximate / rough calculations

P(T/T | data) = p(data|T/T)*p(T/T)/p(data)
≈ p(data|T/T)*p(T/T)/(p(data|T/T)*p(T/T) +p(data|T/C)*p(T/C))
≈ p(error≥2|T/T)p(Hom)/(p(≥2errors|T/T)p(Hom)+p(error≤2|T/C)p(Het))
= 0.0052*0.999/(0.0052*0.999+0.065*0.001)
=0.987=98.7%

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})

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 :

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

Data (X_{ij})

bases (b):
TCCTTTTTTTT
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data X_{ij}

- D is the depth (number of read at position j individual i)
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Simple genotype likelihoods

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

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

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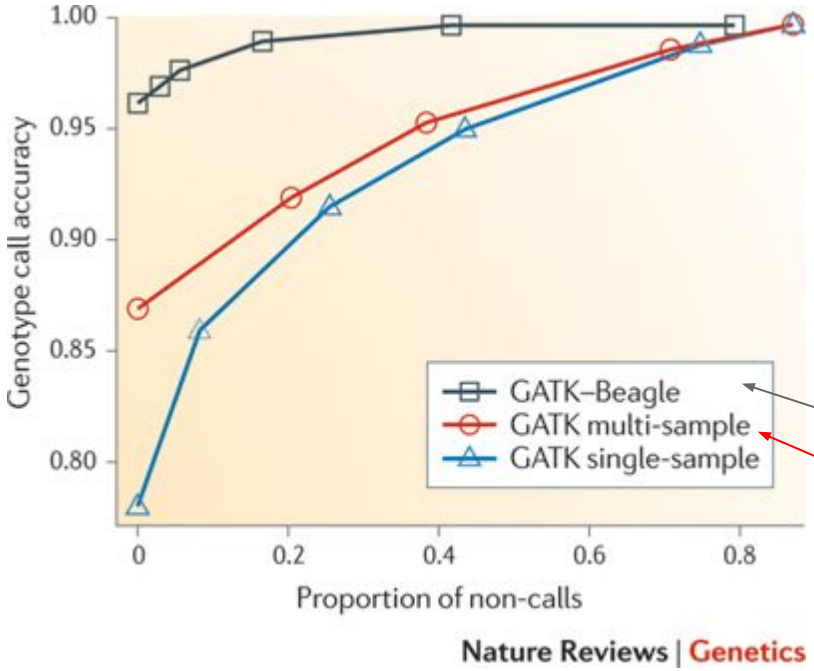
Which genotype caller is best



- 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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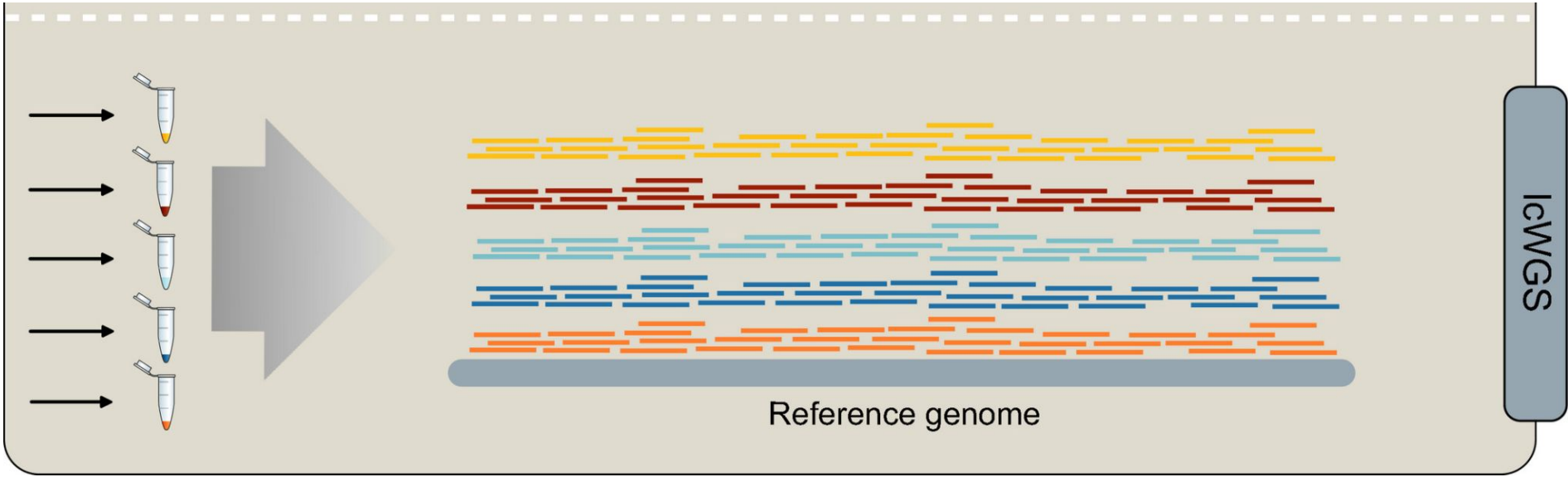
How to do inference from low depth



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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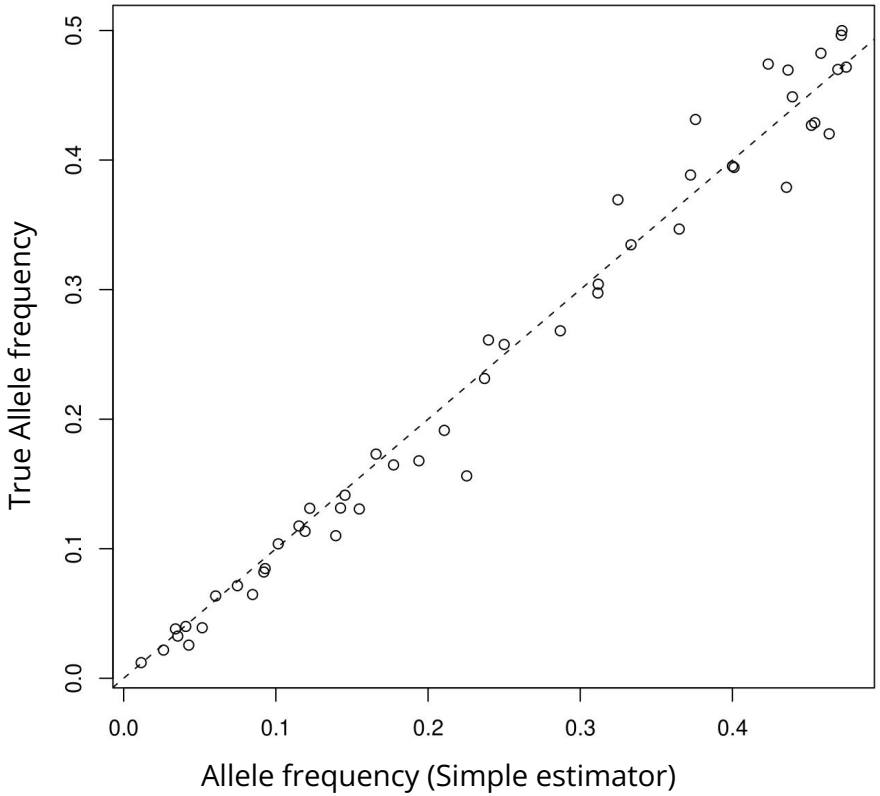
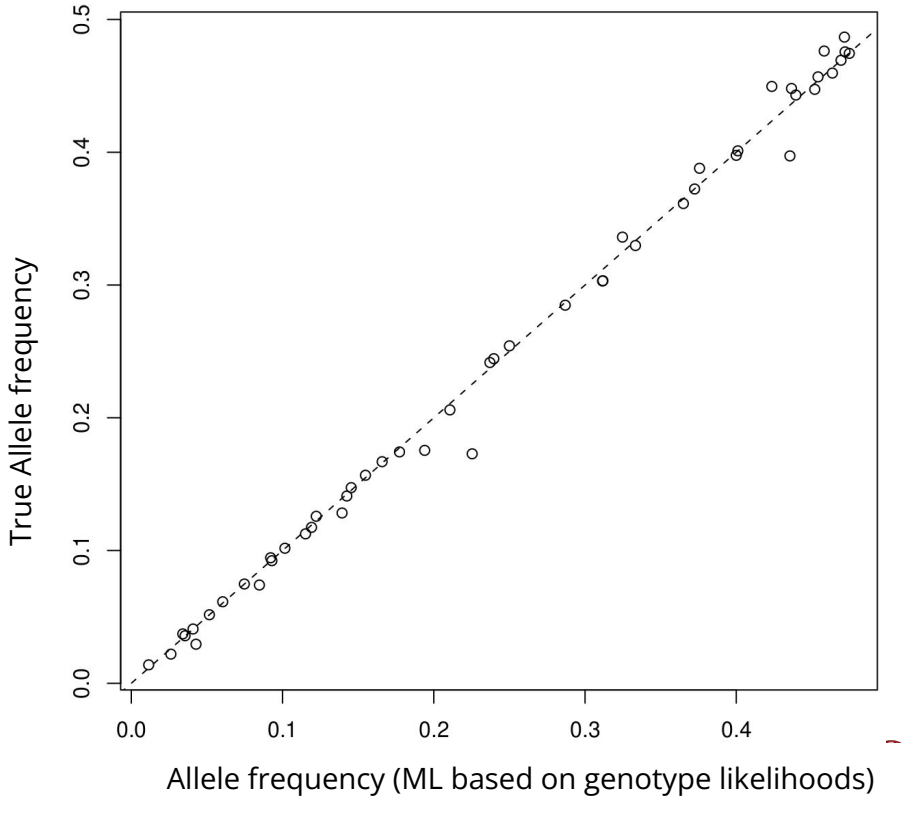

True Allele frequency
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.1
0.2
0.3
0.4
Allele frequency (Simple estimator)

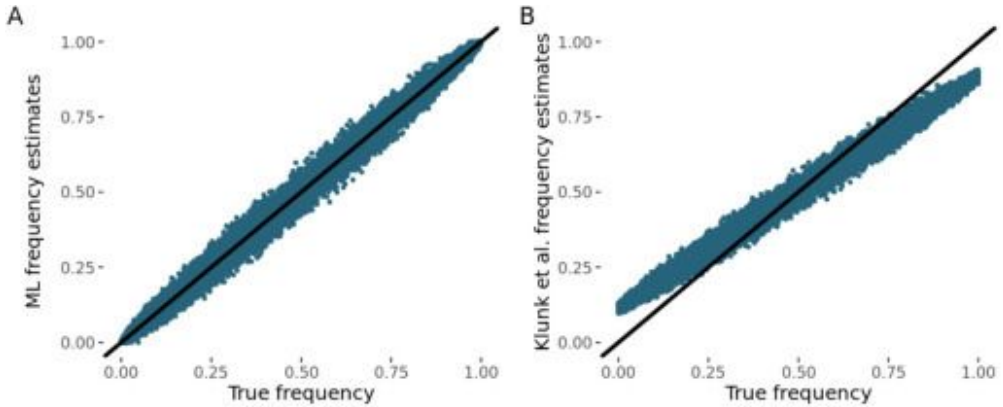

True Allele frequency
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.1
0.2
0.3
0.4
Allele frequency (ML based on genotype likelihoods)

Important to use GL correctly

nature > articles > article

Article | Published: 19 October 2022

Evolution of immune genes is associated with the Black Death



Insufficient evidence for natural selection associated with the Black Death

Alison R. Barton, Cindy G. Santander, Pontus Skoglund, Ida Moltke, David Reich, Iain Mathieson

doi: https://doi.org/10.1101/2023.03.14.532615

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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)

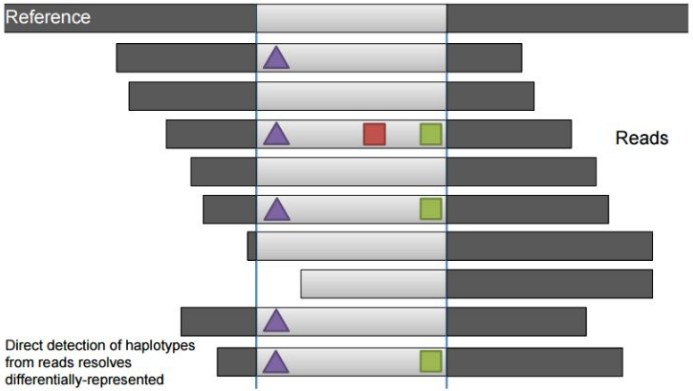

Figure from Erik Garrison

VCF files

Metadata

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##fileDate=20120630
##source=freeBayes version 0.9.6
##reference=W303.fasta
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##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">
```

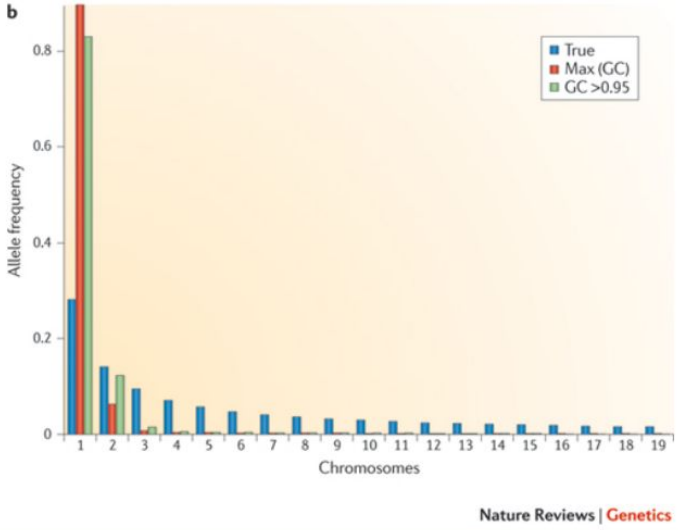
Body

#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
```

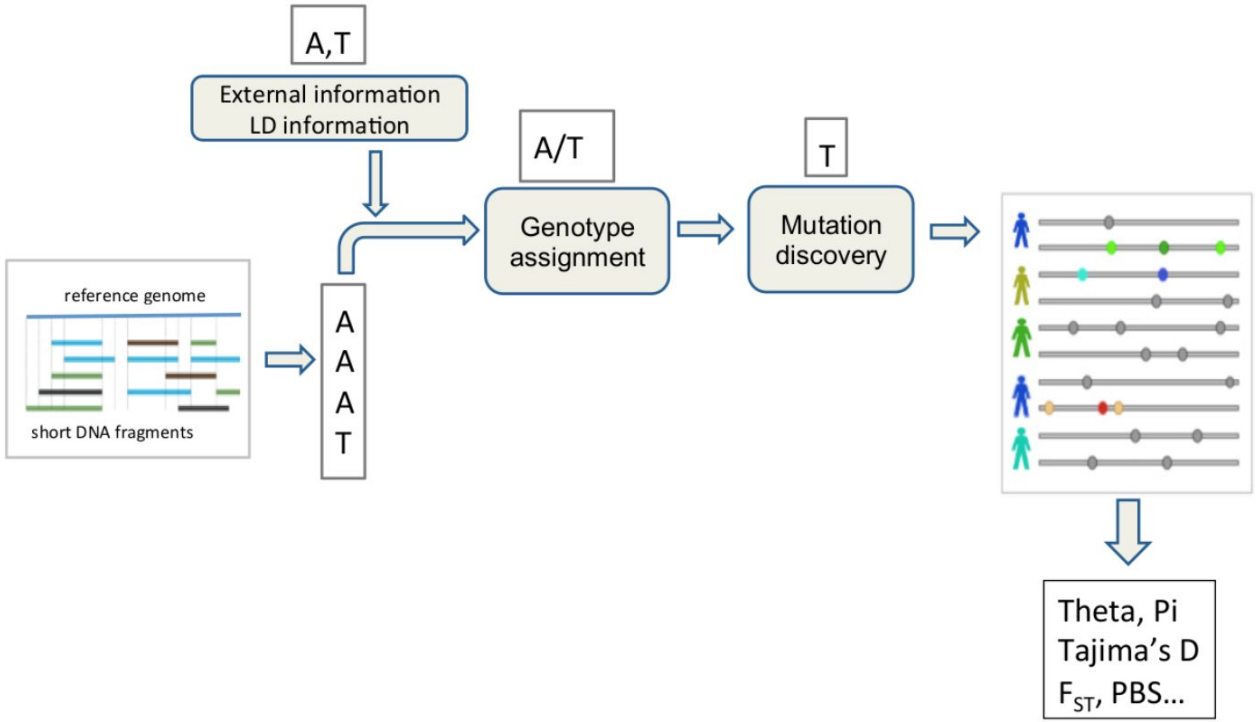
Should we always call genotypes

Site frequency spectrum

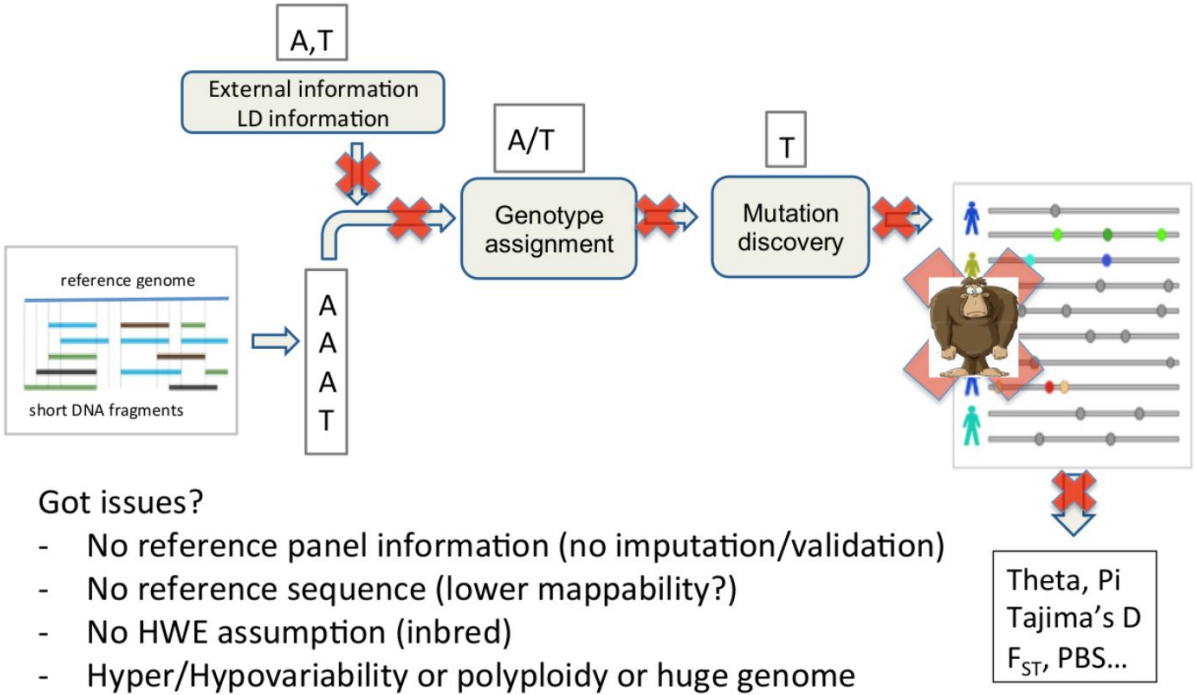


For low depth there are no filter or haplotype imputation approach that will work

High quality data



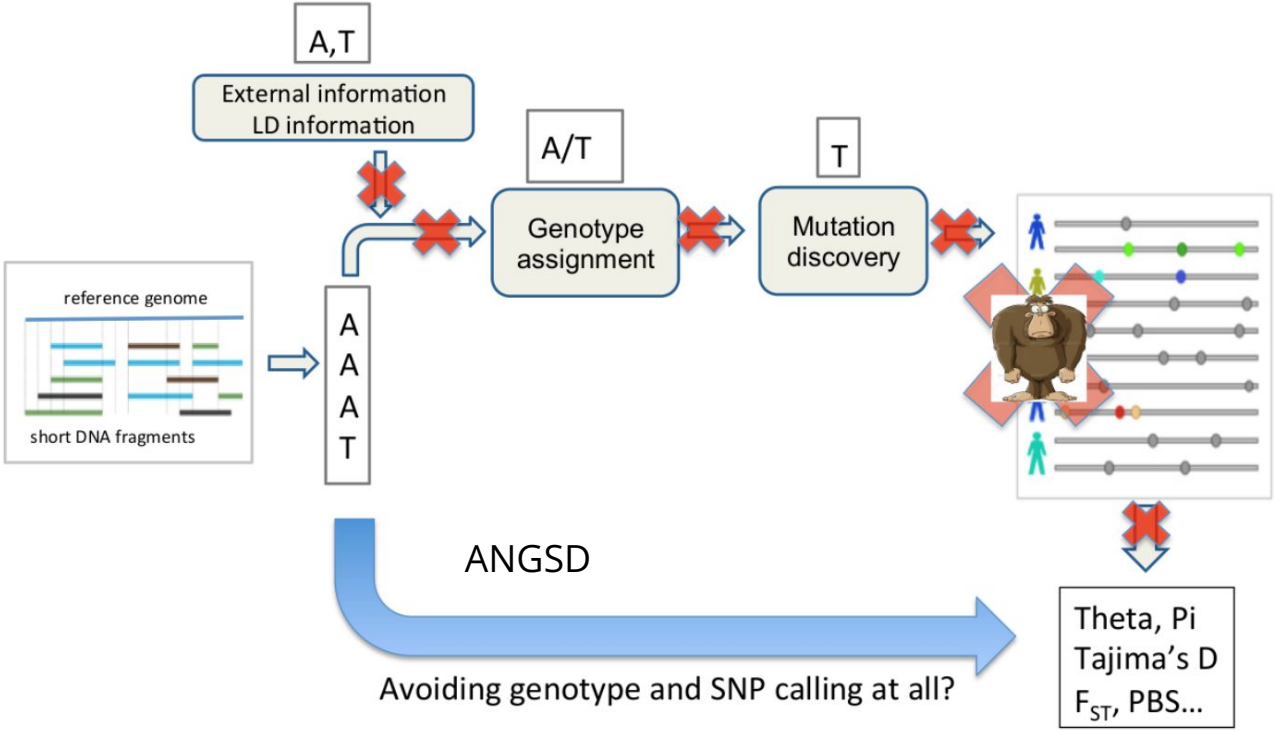
If you don’t have high quality data



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



Low depth vs high depth

Genotype call based

- plink -- freq
- plink --hardy
- plink --genome
- plink --assoc
- plink --pca / smartPCA
- plink --ibs
- plink --fst
- ADMIXTURE
- ADMIXTURE project
- PCrelate
- admixtools

GL based

- angsd -doMaf
- angsd -dohet (PCangsd –inbreeding)
- angsd -GL -> ngsRelate (NGSremix)
- angsd -doAssoc
- angsd -GL -> PCAngsd
- angsd -doIBS
- angsd -doSAF -> winSFS
- NGSadmix
- fastNGSadmix
- PCAngsd –kinship
- angsd -doAbbababa

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DOI: 10.1111/mec.16077

SPECIAL ISSUE

MOLECULAR ECOLOGY WILEY

A beginner's guide to low-coverage whole genome sequencing for population genomics

Runyang Nicolas Lou[1] | Arne Jacobs[1] | Aryn P. Wilder[2] | Nina Overgaard Therkildsen[1]

https://onlinelibrary.wiley.com/doi/10.1111/mec.16077#

Time for exercises

Go to

popgen.dk/popgen24github

Download NGS intro ipynb

Go to emily server

Upload ipynb (press upload again)

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)

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

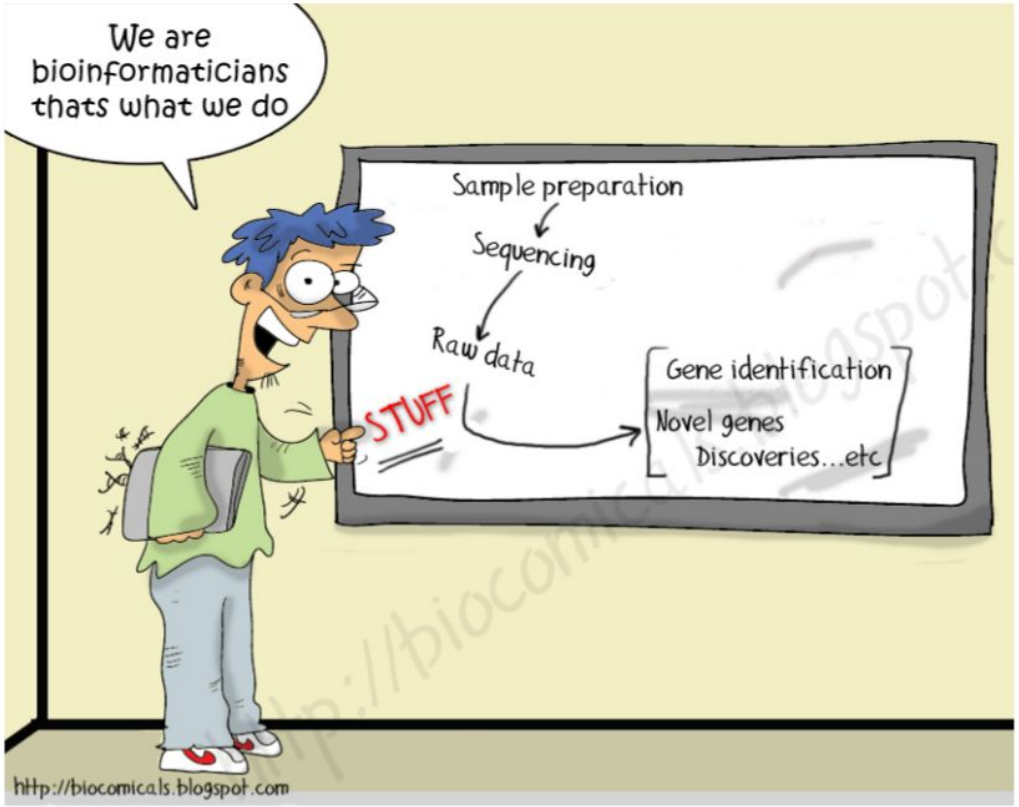
M step

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

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

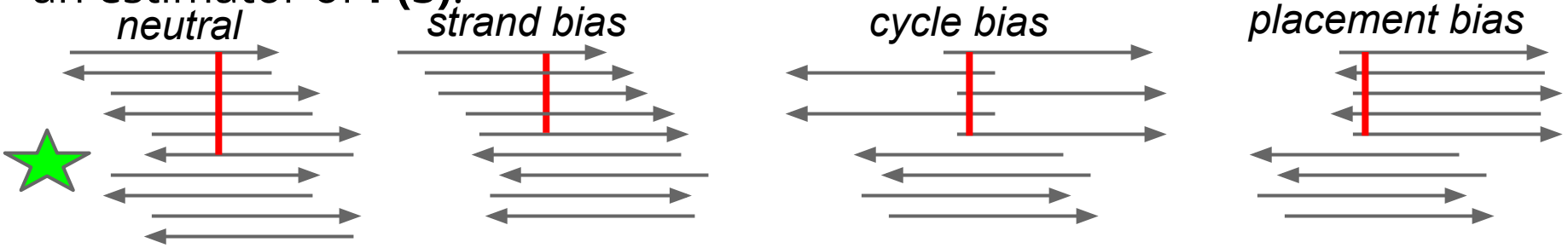
z is latent state of the genotype
Z_i is the genotype for individual i
$Z_i \in \{MM, Mm, mm\}$

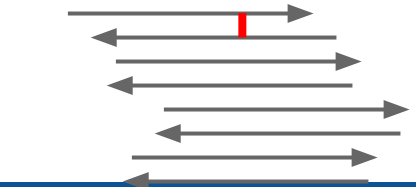
Recap: Analysis of NGS data



Are our locus and alleles sequenceable?

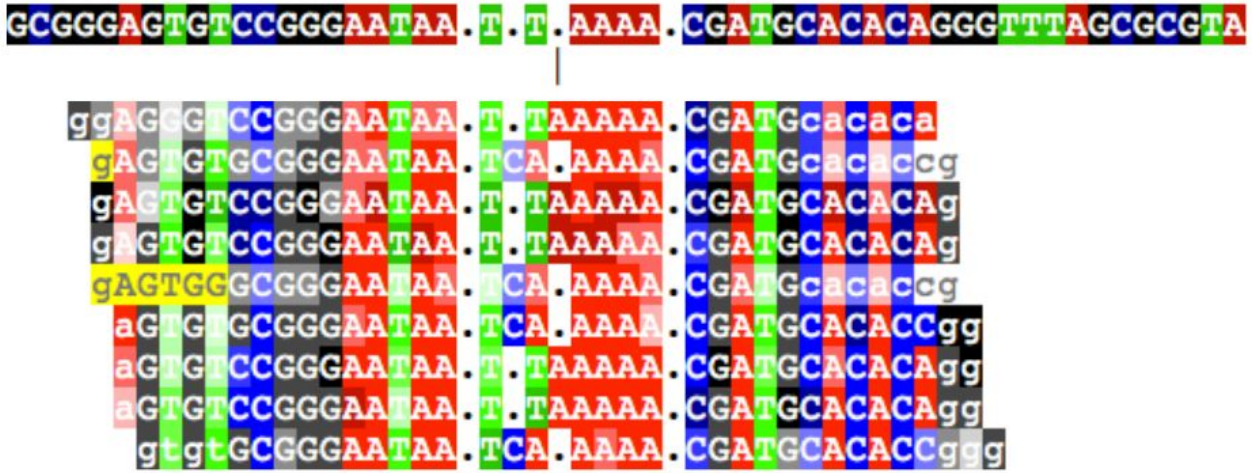
In WGS, biases in the way we observe an allele (placement, position, strand, cycle, or balance in heterozygotes) are often correlated with error. We include this in our posterior **P(G,S|R)**, and to do so we need an estimator of **P(S)**.

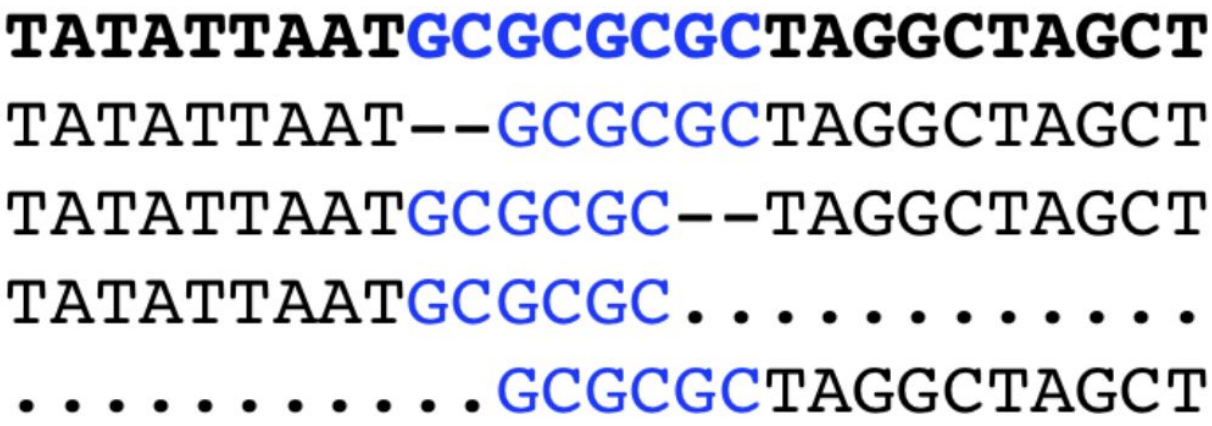


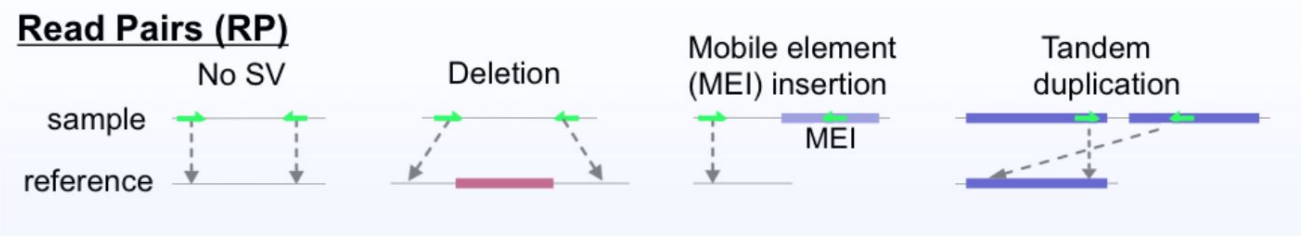


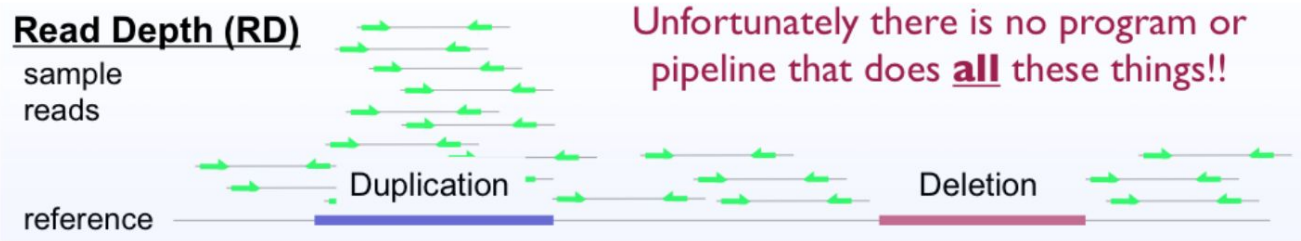
$$P(S) \propto \times \prod_{\forall b \in \{B\}} \begin{array}{l} multinom([|R \equiv b|\forall b_1, \ldots, b_K]; |\{R\}|, f_i, \ldots, f_K) \\ binom(|forwardStrand(\{R \equiv b\})|; |\{R \equiv b\}|, 1/2) \\ \times binom(|placedLeft(\{R \equiv b\})|; |\{R \equiv b\}|, 1/2) \\ \times binom(|placedRight(\{R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

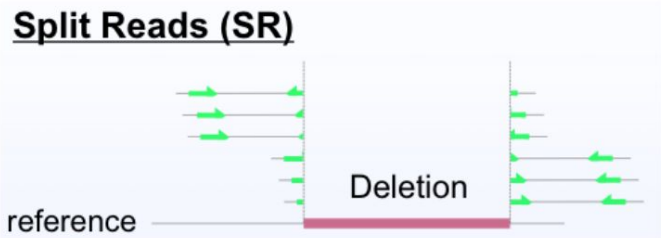
Mapper does not know our data e.g. it does not know whether it is haploid/diploid/pooled individual ect.

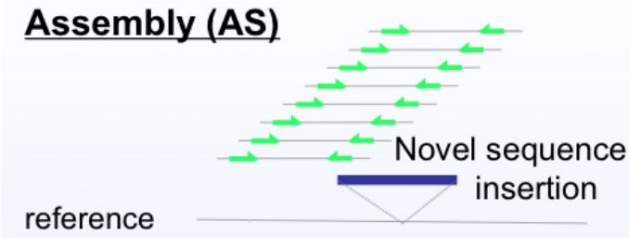


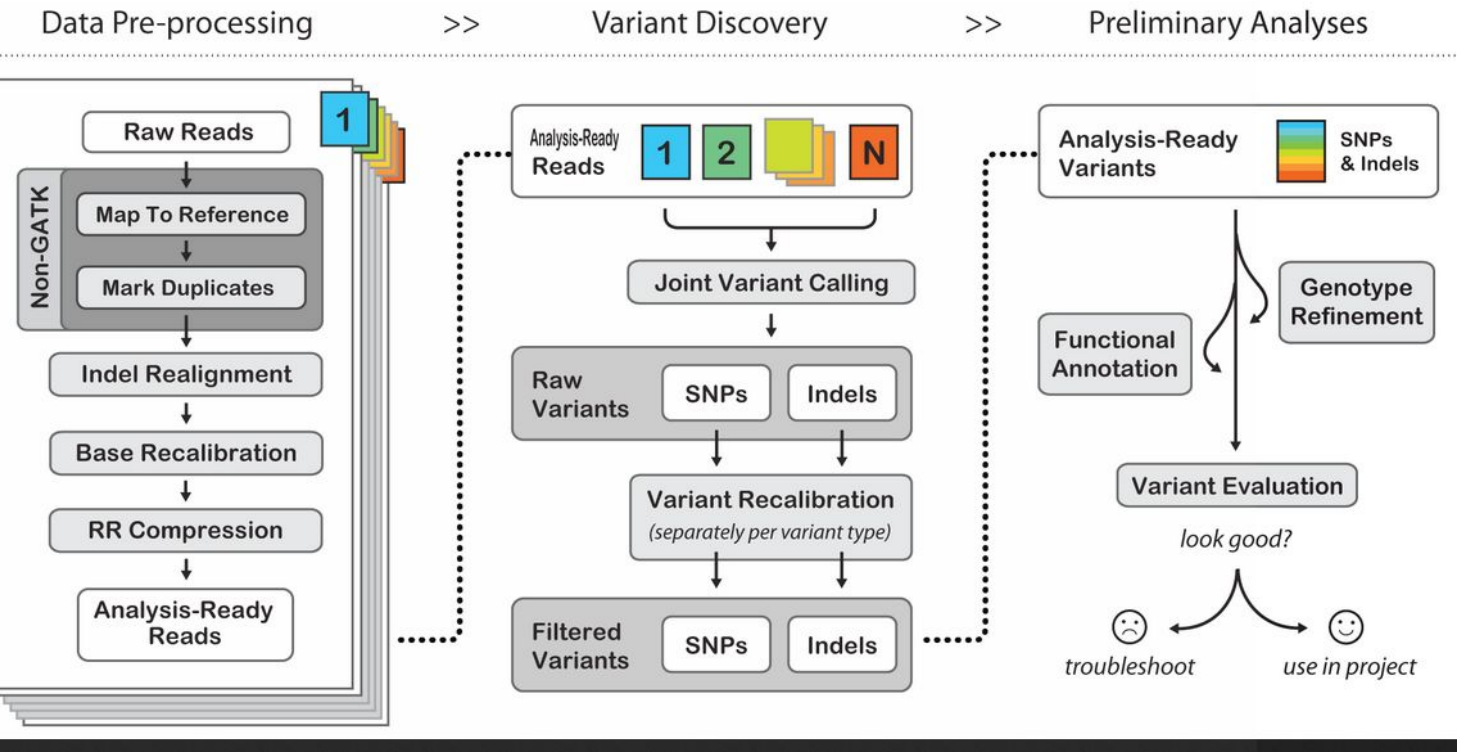

TATATTAATGCGCGCGCTAGGCTAGCT
TATATTAAT--GCGCGCTAGGCTAGCT
TATATTAATGCGCGC--TAGGCTAGCT
TATATTAATGCGCGC.............
............GCGCGCTAGGCTAGCT

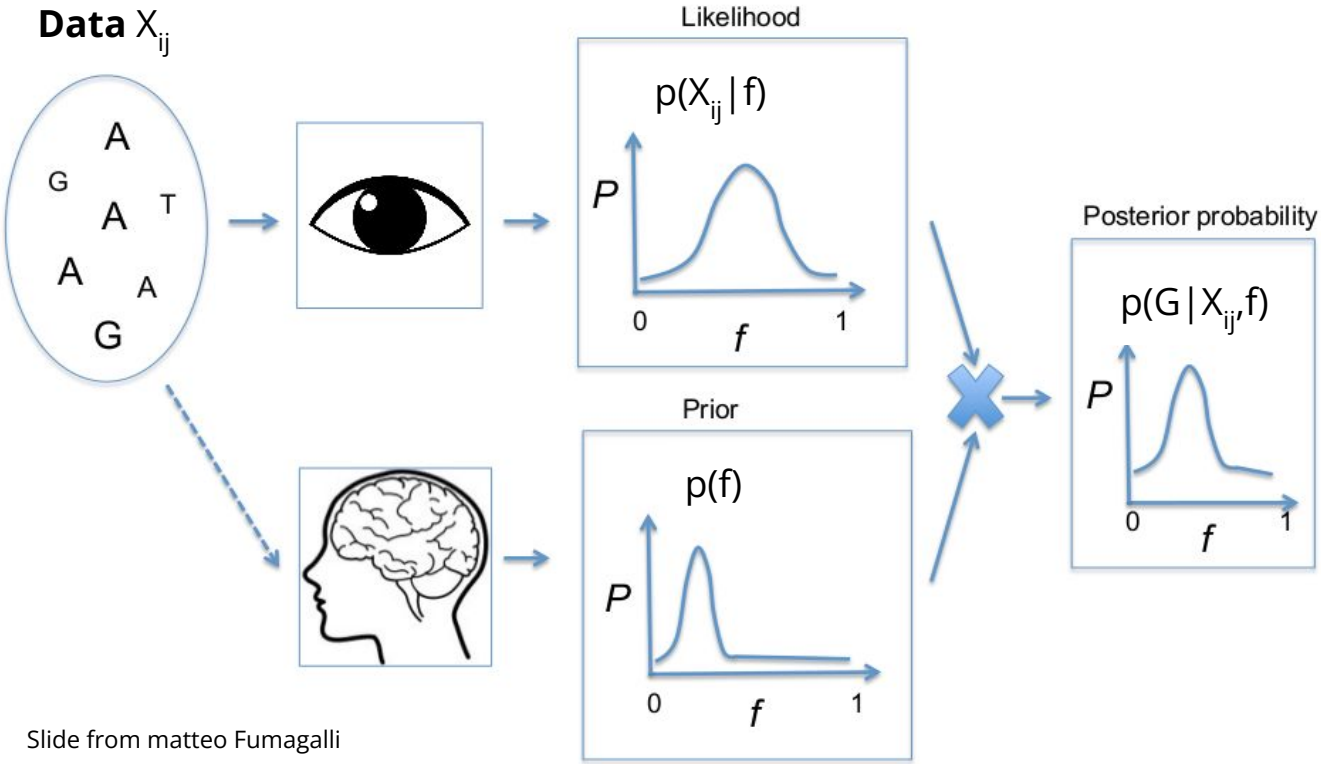

Read Pairs (RP)
No SV
Deletion
Mobile element (MEI) insertion
Tandem duplication
sample
reference
MEI


Read Depth (RD)
sample reads
Unfortunately there is no program or pipeline that does all these things!!
Duplication
Deletion
reference


Split Reads (SR)
Deletion
reference


Assembly (AS)
Novel sequence insertion
reference


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
$Z_i \in \{MM, Mm, mm\}$

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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

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

$p(X_i|Z_i = z)$ 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

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

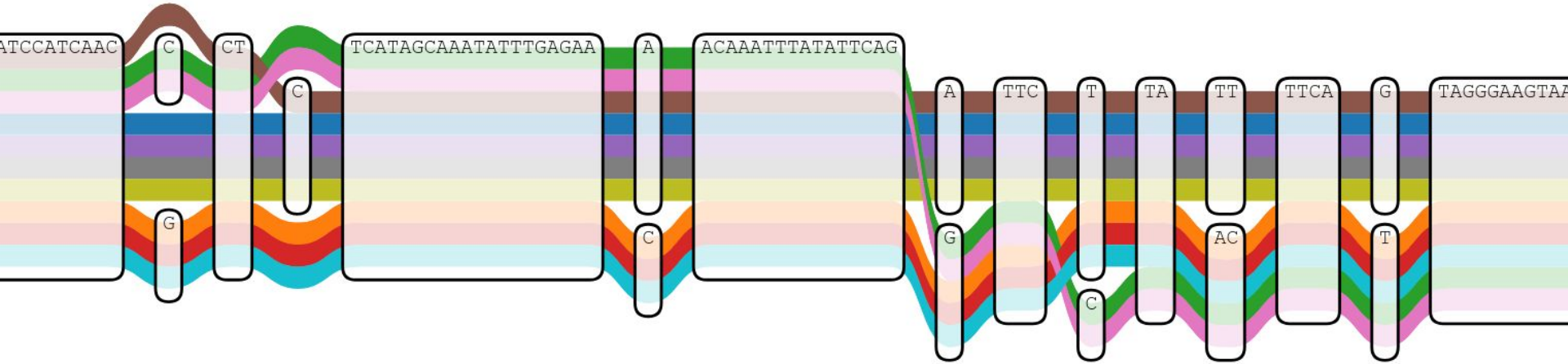
$p(X_i|Z_i = z)$ is the genotype likelhood

$p(Z_i = z|\theta)$

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
```