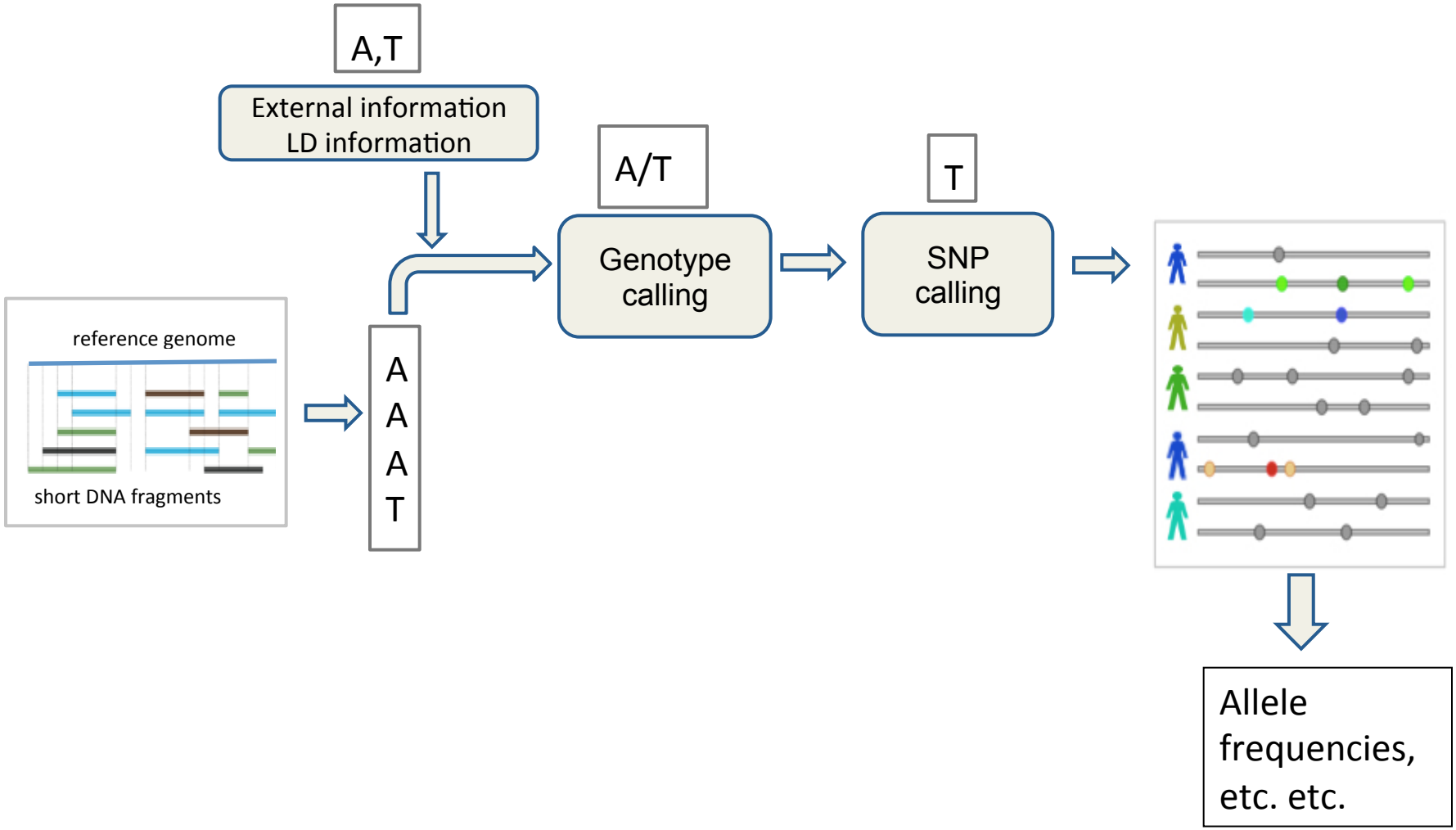
Population genetics from low-depth data

Matteo Fumagalli

Next Generation Sequencing data processing



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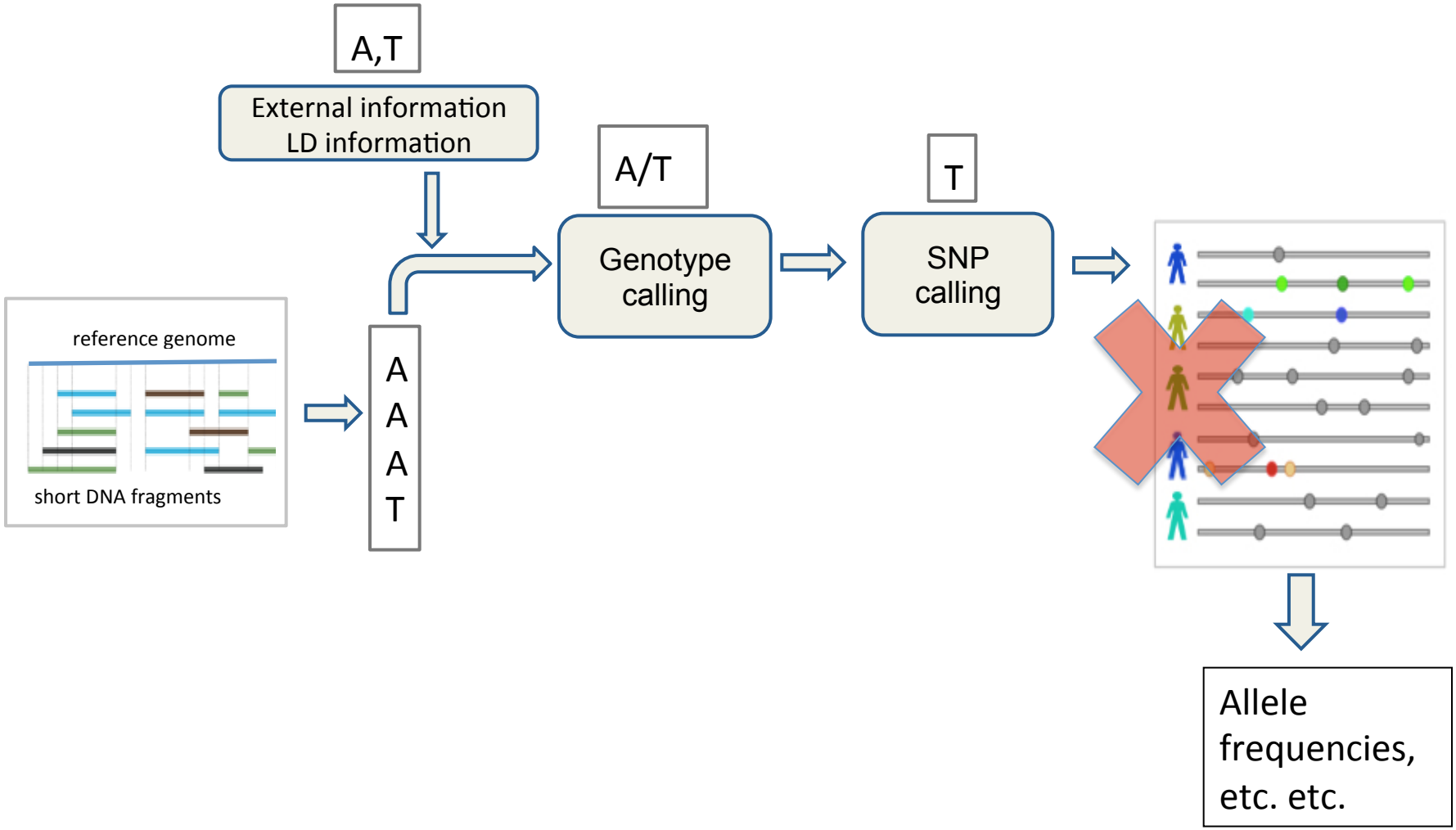
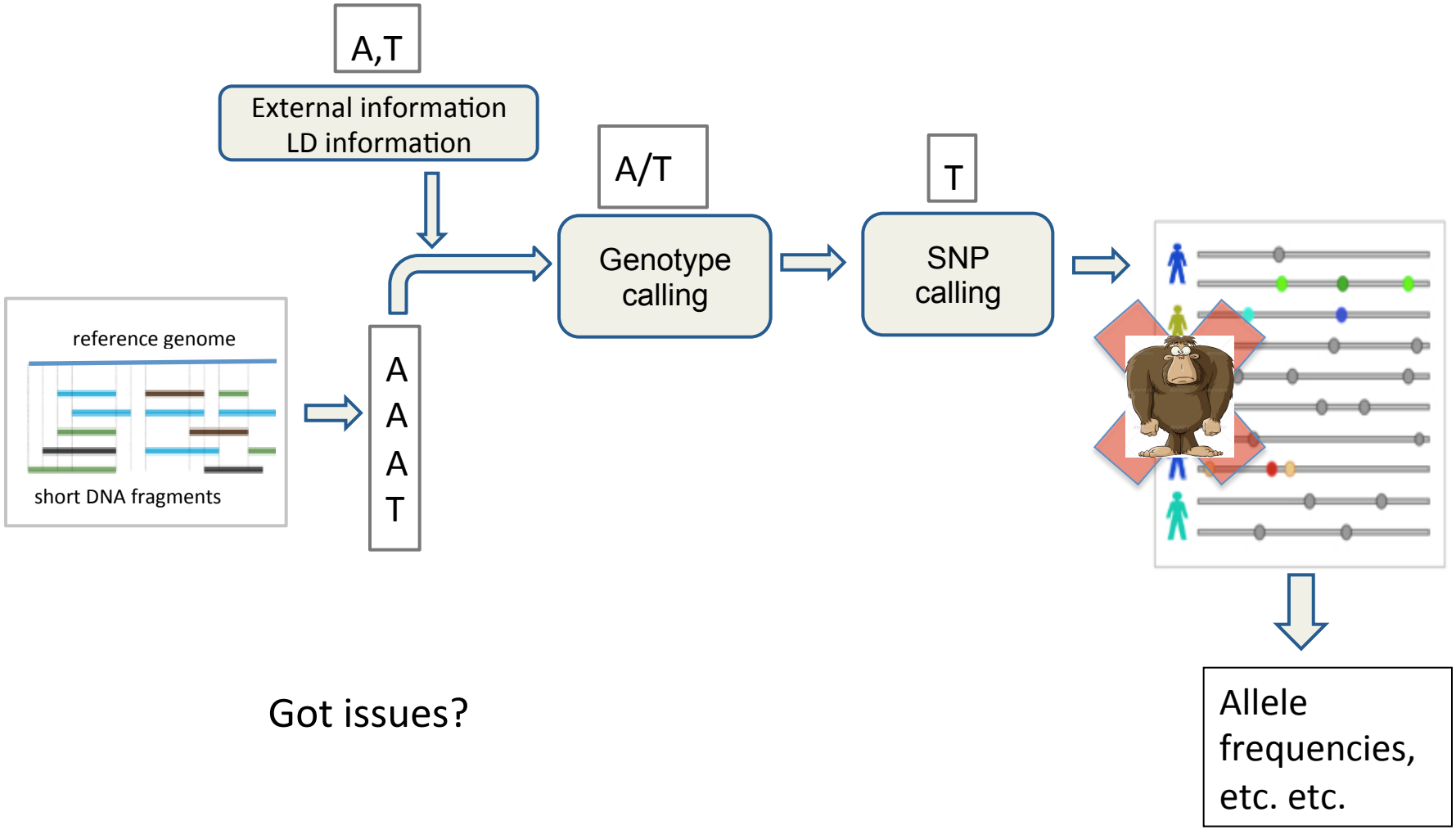


Next Generation Sequencing data processing in the non-model world



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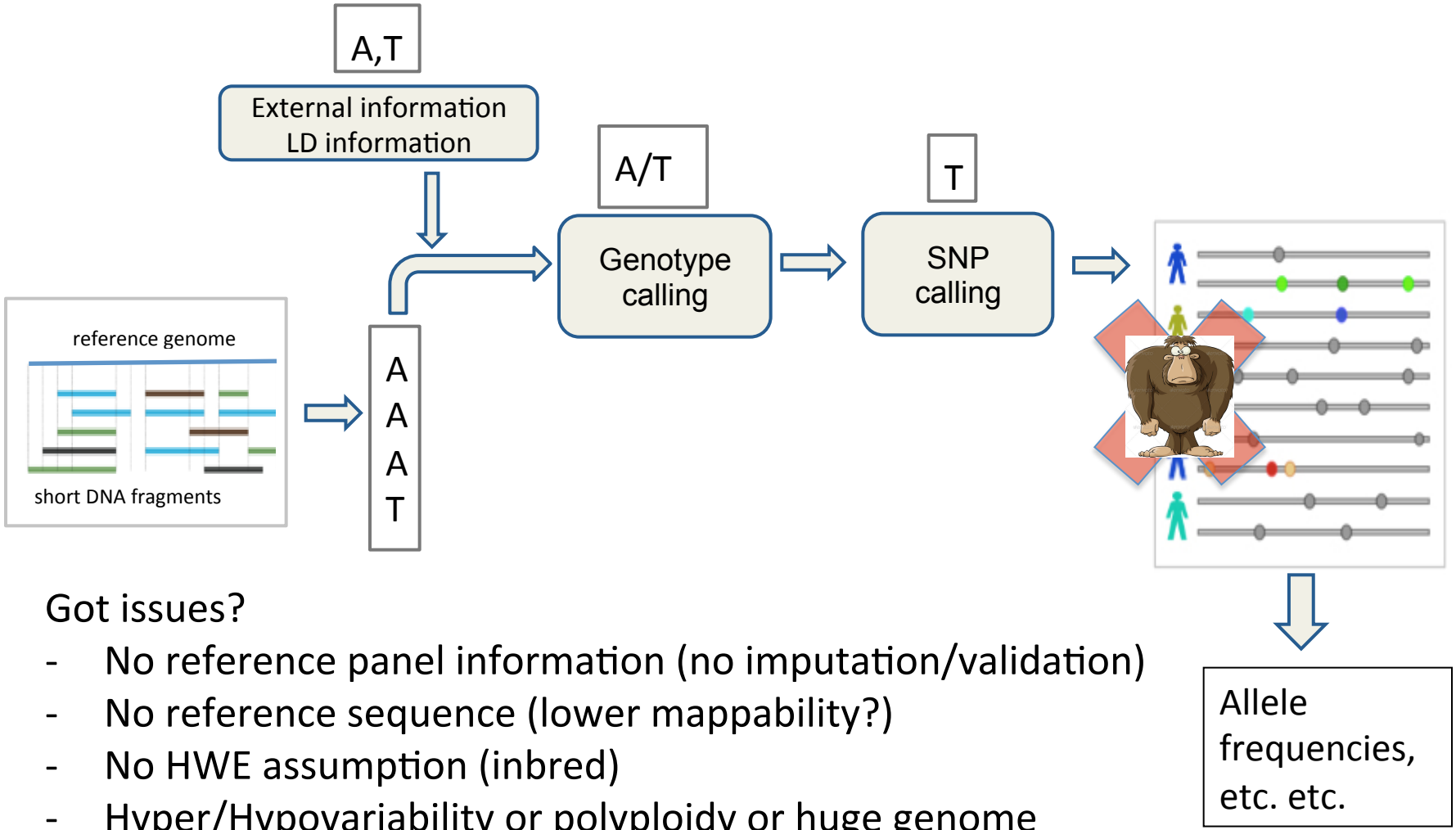

Got issues?

- 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 (?)
- …

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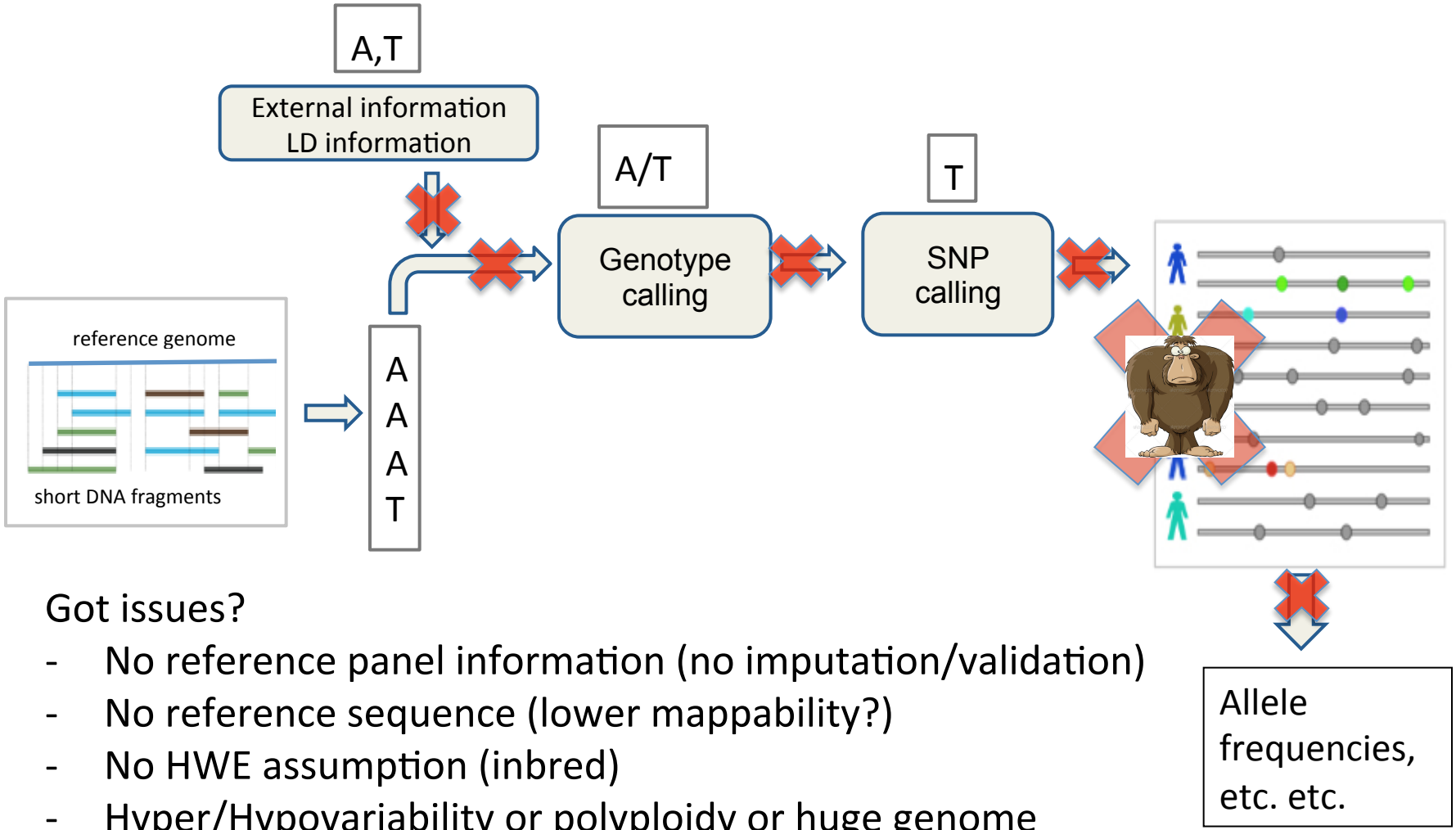

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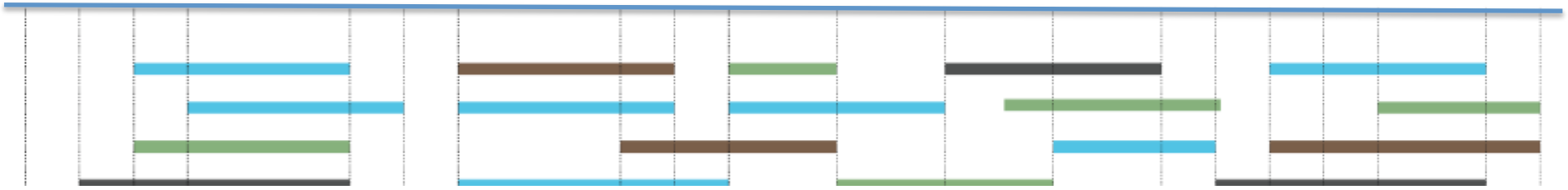
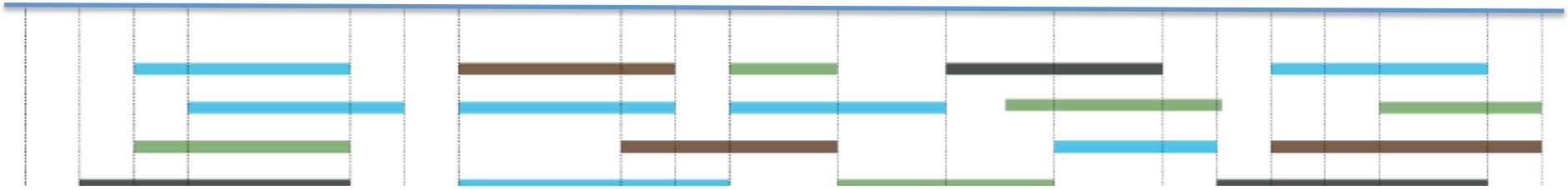
- No reference panel information (no imputation/validation)
- No reference sequence (lower mappability?)
- No HWE assumption (inbred)
- Hyper/Hypovariability or polyploidy or huge genome
- No money (?)
- **Your inferences will be wrong!**

Challenges

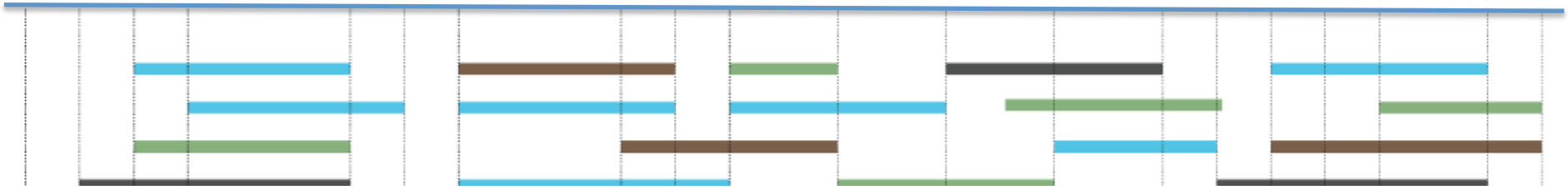


Challenges



- Variable and low depth
- High sequencing and mapping errors

Challenges

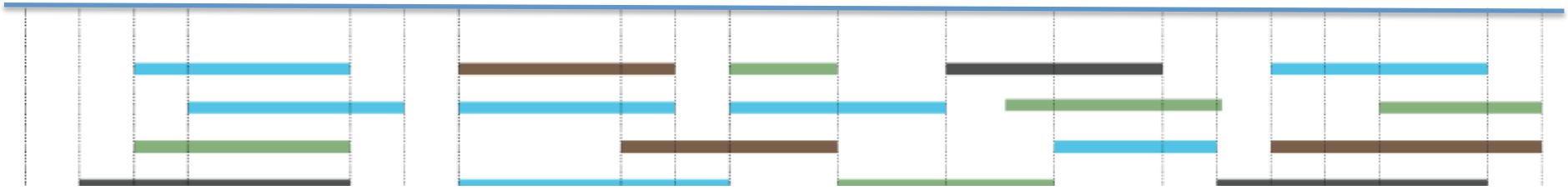


- Variable and low depth

- High sequencing and mapping errors

Quality control filters

Data filtering



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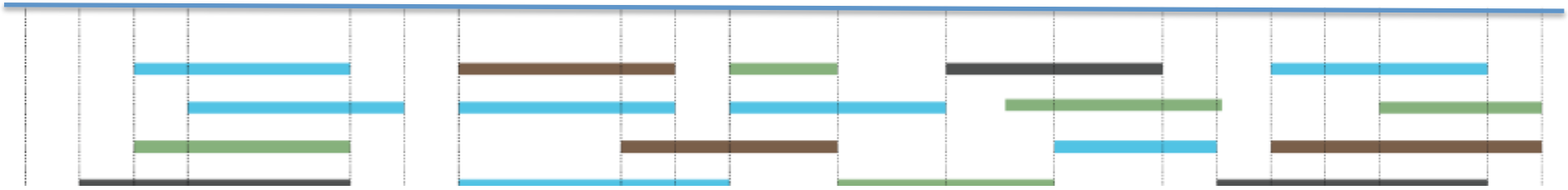
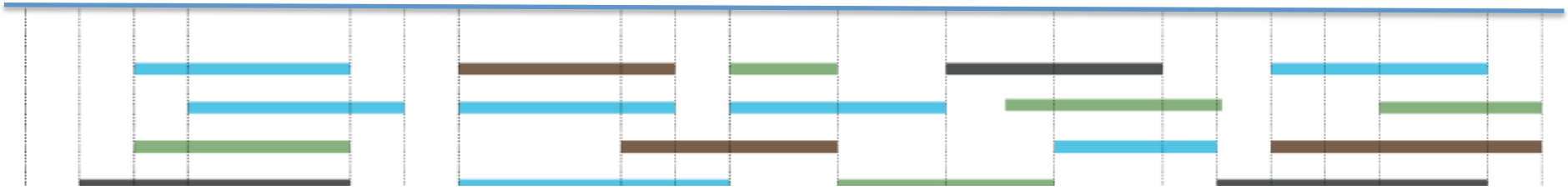
Data filtering



- Variable and low depth

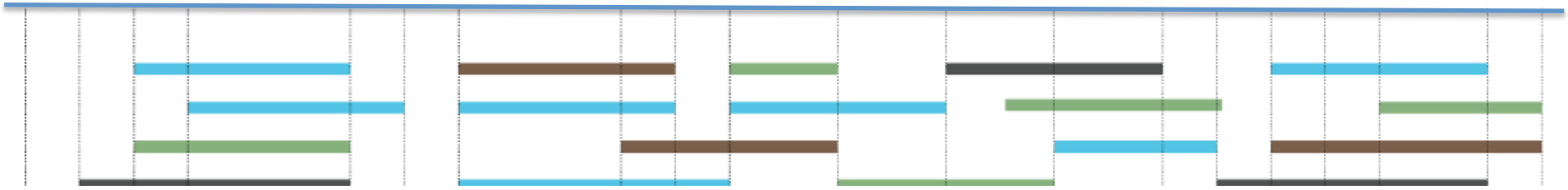
Minimum depth
Maximum depth
Even depth across samples
…

Data filtering



- Sequencing and mapping errors

Data filtering



- Sequencing and mapping errors

Minimum base quality
Minimum mapping quality
Base quality bias
…

Site Frequency Spectrum (SFS)

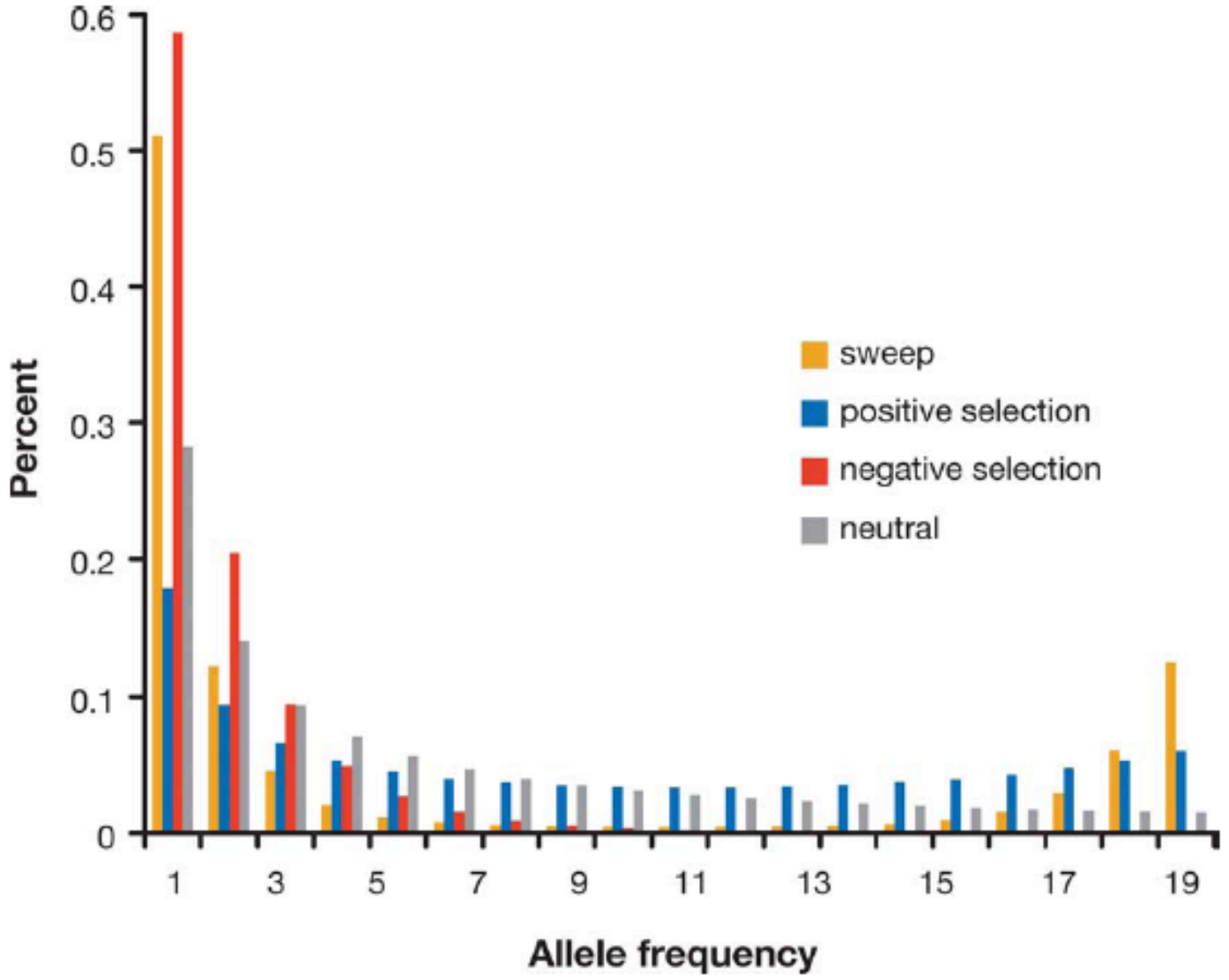
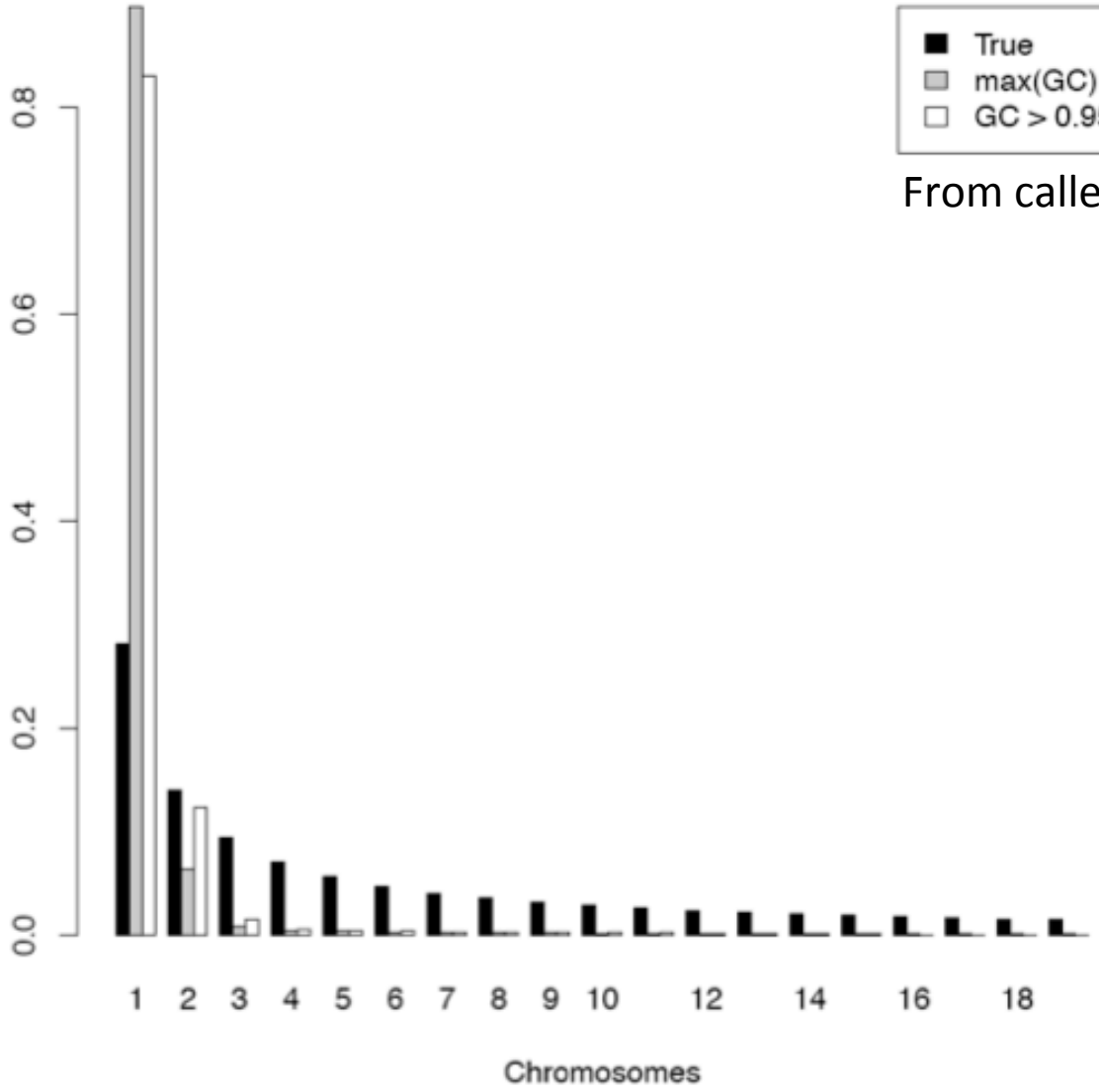


Figure 2

Effect of errors on SFS



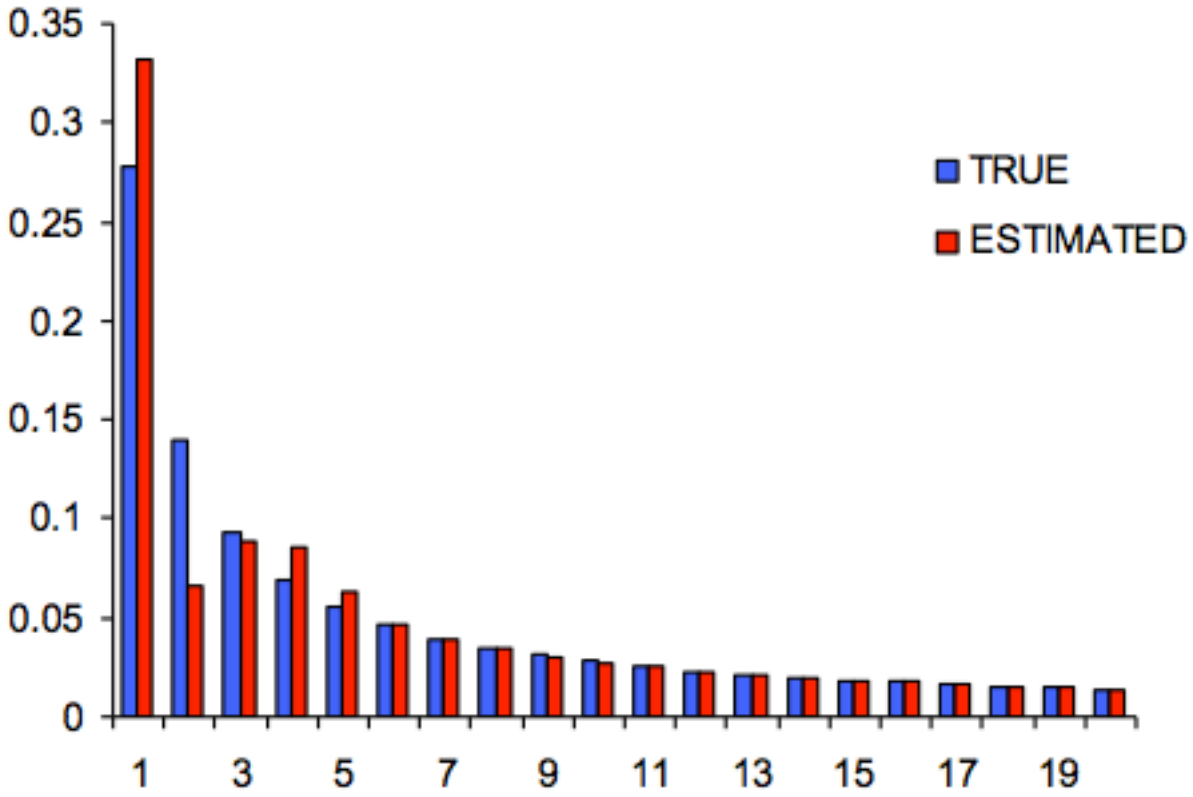
5X

From called genotypes

Slide by R. Nielsen

Effect of errors on SFS

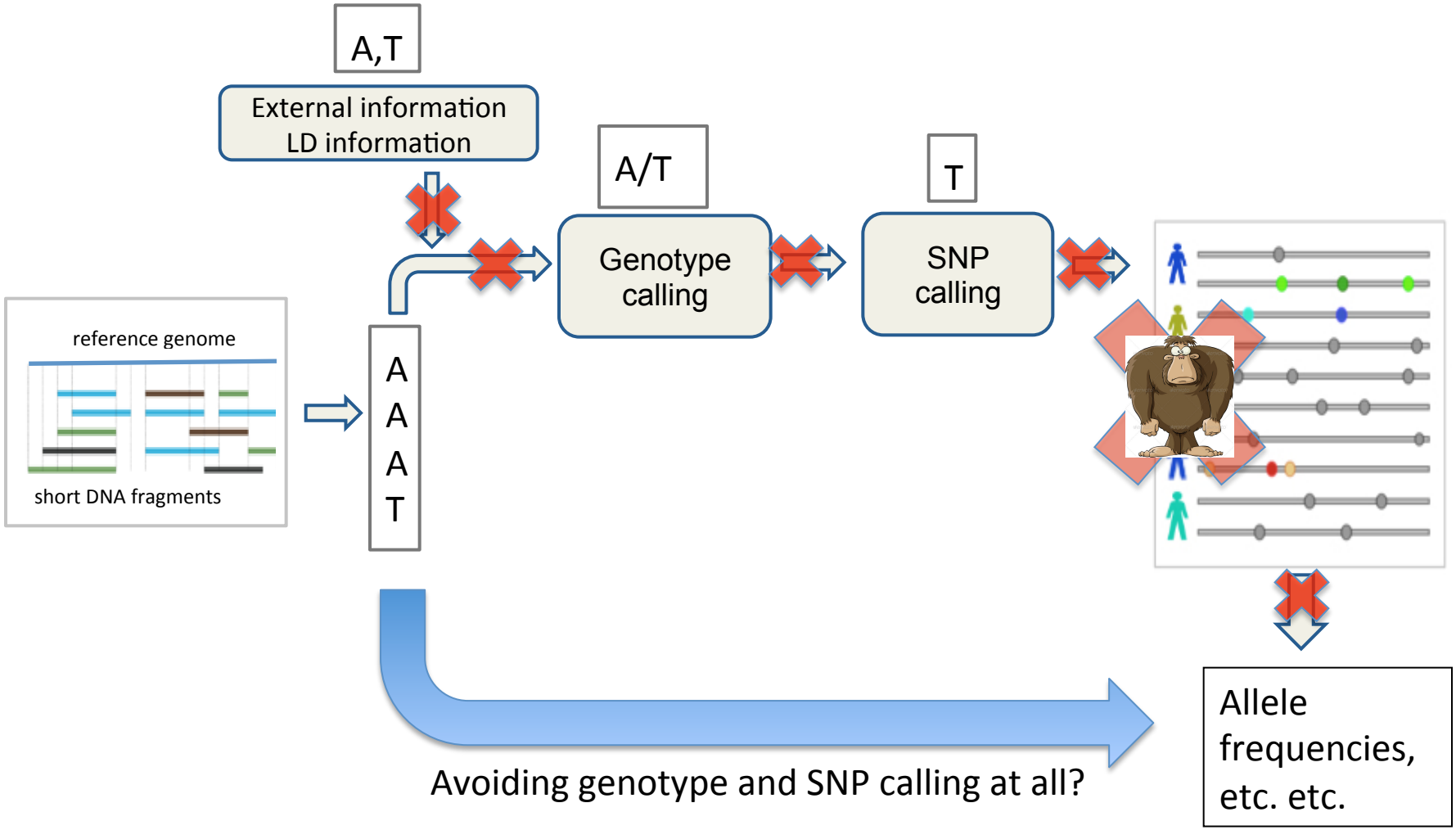
Using an ad hoc fixed cutoff for SNP calling…



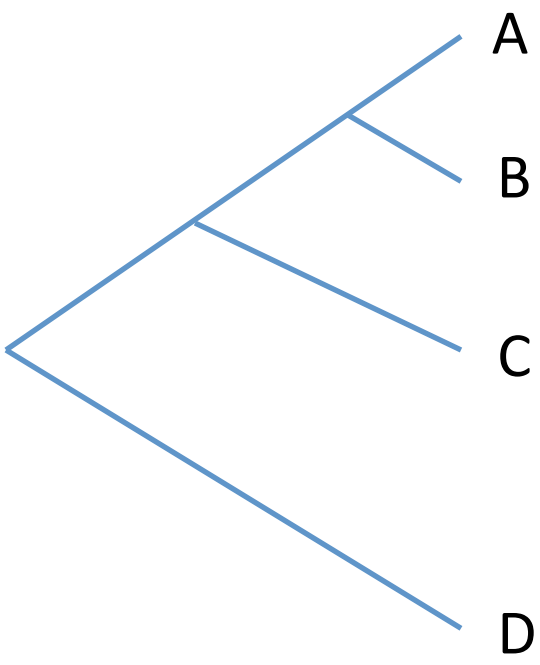
can never produce unbiased estimates.

Slide by R. Nielsen

Next Generation Sequencing data processing in the non-model world

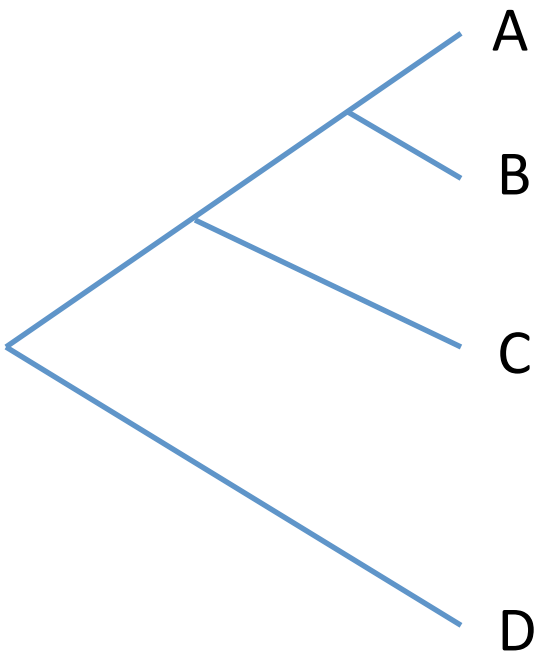


Genetic distances



Genotype 1	Genotype 2	Distance
aa	aa	0
aa	aA	1
aa	AA	2
aA	aa	1
aA	aA	0
aA	AA	2
…	…	…

Genetic distances



Genotypes are {aa, aA, AA} as {0, 1 ,2}

For individuals i and j and N sites:

$$d(i,j) = -\log\left(1 - \frac{1}{N}\sum_{s=1}^{N}\frac{|g(i,s) - g(j,s)|}{2}\right)$$

genotype of *i* at site *s*

e.g. G(i=A,s=1)=0 and G(j=B,s=1)=1 then d(i,j)=1

Genetic distances from known genotypes

Genotypes are {aa, aA, AA} as {0, 1 ,2}
For individuals i and j and N sites:

$$d(i,j) = -\log\left(1 - \frac{1}{N}\sum_{s=1}^{N}\frac{|g(i,s) - g(j,s)|}{2}\right)$$

d(i,j) = 1*1.00 = 1.00/2

B

A

	0	**1**	**2**
0	0	1	0
1	0	0	0
2	0	0	0

Expected value

- The expected value of a discrete random variable is the probability-weighted average of all possible values

- Average value if you perform the same experiment many times

Genetic distances from (un)known genotypes

Genotypes are {aa, aA, AA} as {0, 1 ,2}
For individuals i and j and N sites:

$$d(i,j) = -\log\left(1 - \frac{1}{N}\sum_{s=1}^{N}\frac{|g(i,s) - g(j,s)|}{2}\right)$$

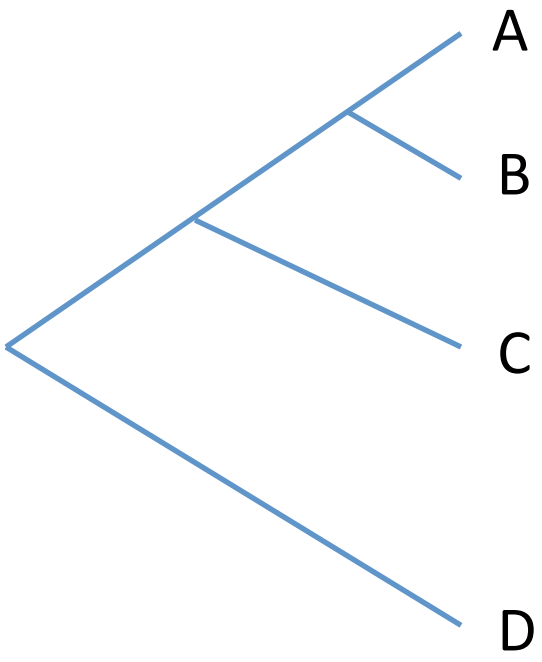
E[d(i,j)] = 0*0.30 + 1*0.50 + 2*0.10 + 1*0.10 + … = 0.80/2

B

A

	0	**1**	**2**
0	0.30	0.50	0.10
1	0.10	0	0
2	0	0	0

Genetic distances from unknown genotypes



Genotypes are {aa, aA, AA} as {0, 1 ,2}

For individuals i and j and N sites:

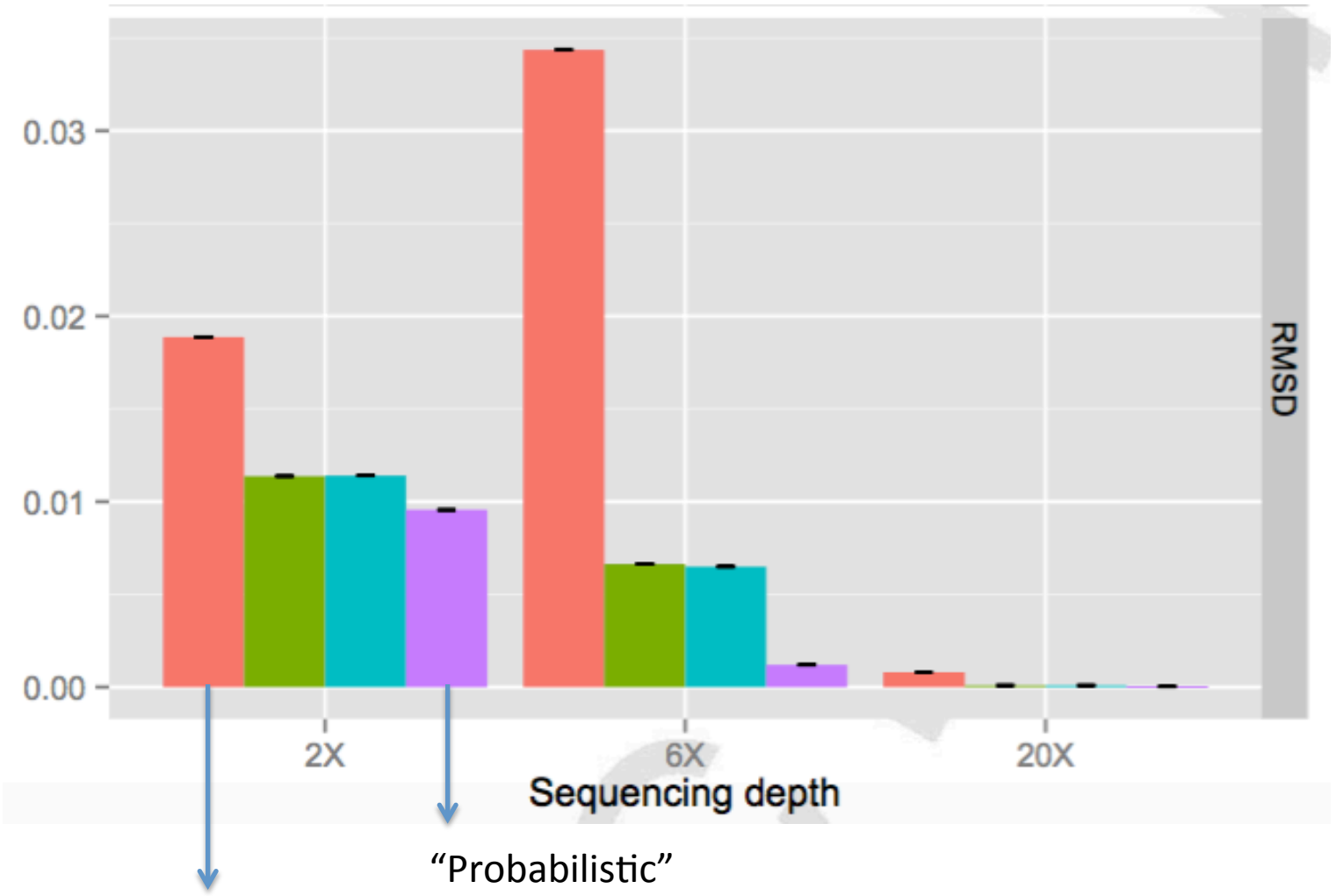
$$d(i,j) = -\log\left(1 - \frac{1}{N}\sum_{s=1}^{N}\frac{|g(i,s) - g(j,s)|}{2}\right)$$

Iterate across all possible genotypes

Genotypes probability

$$d(i,j) = -\log\left(1 - \frac{1}{N}\sum_{s=1}^{N}\sum_{g(i,s)=0}^{2}\sum_{g(j,s)=0}^{2}\frac{|g(i,s) - g(j,s)|}{2} * P(g(i,s), g(j,s))\right)$$

Genetic distances from unknown genotypes

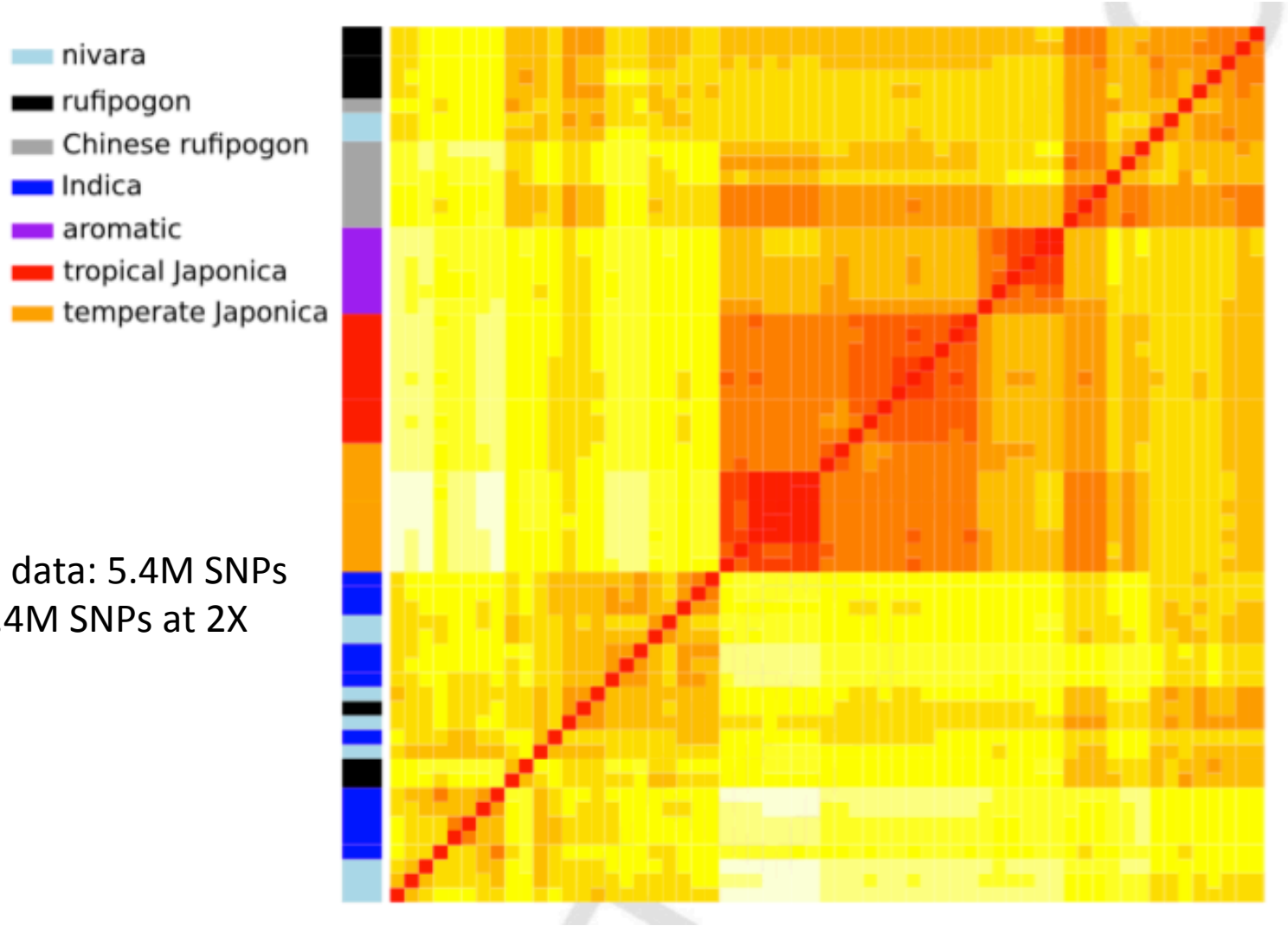


"Probabilistic"

Genotype calling (no prior)

Vieira et al. BJLS 2016

Clustering



Original data: 5.4M SNPs
Here: 5.4M SNPs at 2X

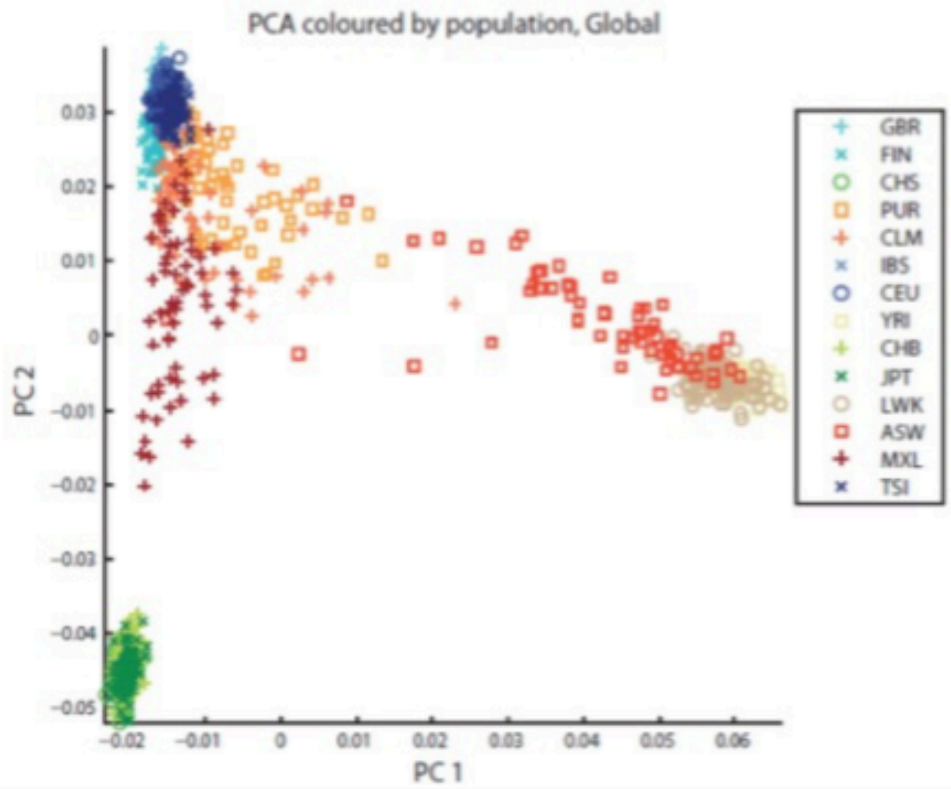
https://github.com/fgvieira/ngsDist

Vieira et al. BJLS 2016

Population structure

Principal Component Analysis (PCA) is a **data reduction** method for

- **visualization**,
- correction for population stratification,
- population history and differentiation.



1000 Genomes Consortium 2012

Covariance matrix

Genotype (0,1,2)

Allele frequency

$$cov(i,j) = \frac{1}{(m-1)} \frac{\sum_{s=1}^{m} (G_s^{(i)} - 2\hat{p}_s)(G_s^{(j)} - 2\hat{p}_s)}{\sqrt{\hat{p}_s(1-\hat{p}_s)}}$$

Covariance matrix

Genotype (0,1,2)

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$$cov(i,j) = \frac{1}{(m-1)} \frac{\sum_{s=1}^{m} (G_s^{(i)} - 2\hat{p}_s)(G_s^{(j)} - 2\hat{p}_s)}{\sqrt{\hat{p}_s(1-\hat{p}_s)}}$$

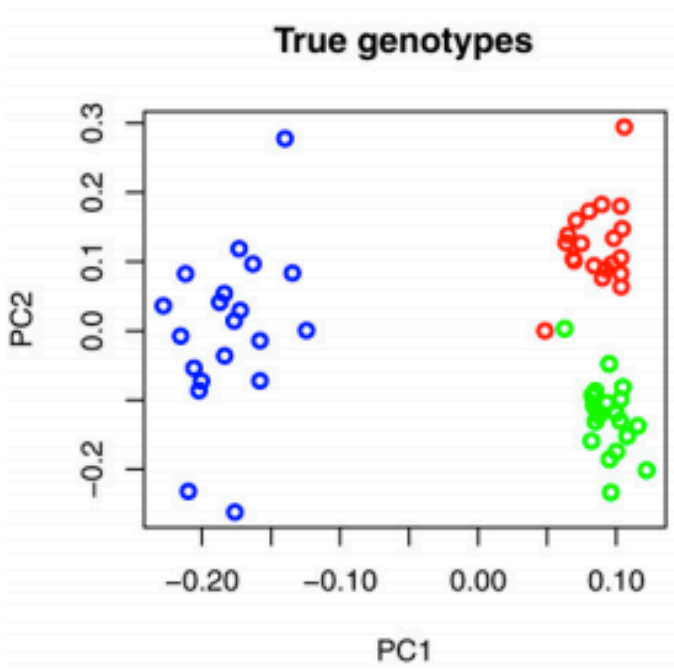
Iterate across all genotypes

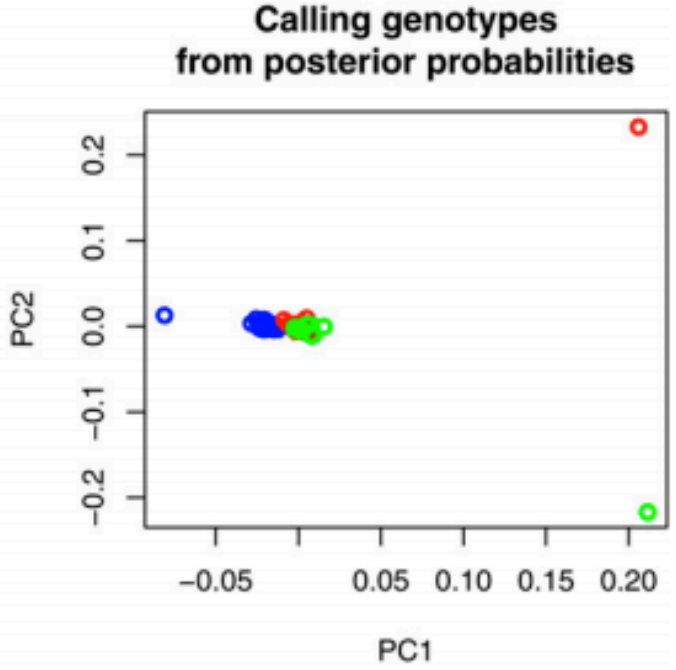
Weight by their probability

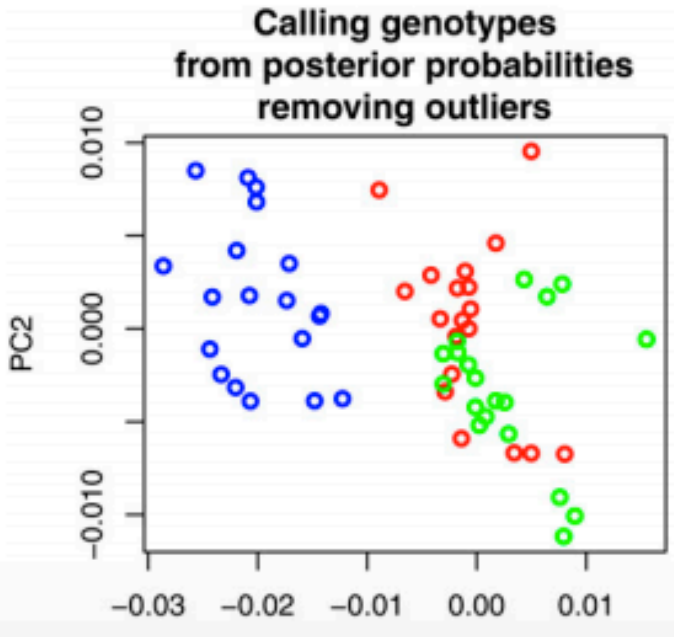
$$\hat{cov}_{(i,j)} := \frac{1}{(\sum_{s=1}^{m} P_{var,s}) - 1} \frac{\sum_{s=1}^{m} \left(\sum_{G_s^{(i)}=0}^{2} \sum_{G_s^{(j)}=0}^{2} (G_s^{(i)} - 2\hat{p}_s)(G_s^{(j)} - 2\hat{p}_s) P(G_s^{(i)} | X_s^{(i)}) P(G_s^{(j)} | X_s^{(j)}) \right) P_{var,s}}{\sqrt{\hat{p}_s(1-\hat{p}_s)}}$$

Probability of the site being variable
(to avoid SNP calling)

Depth=2X

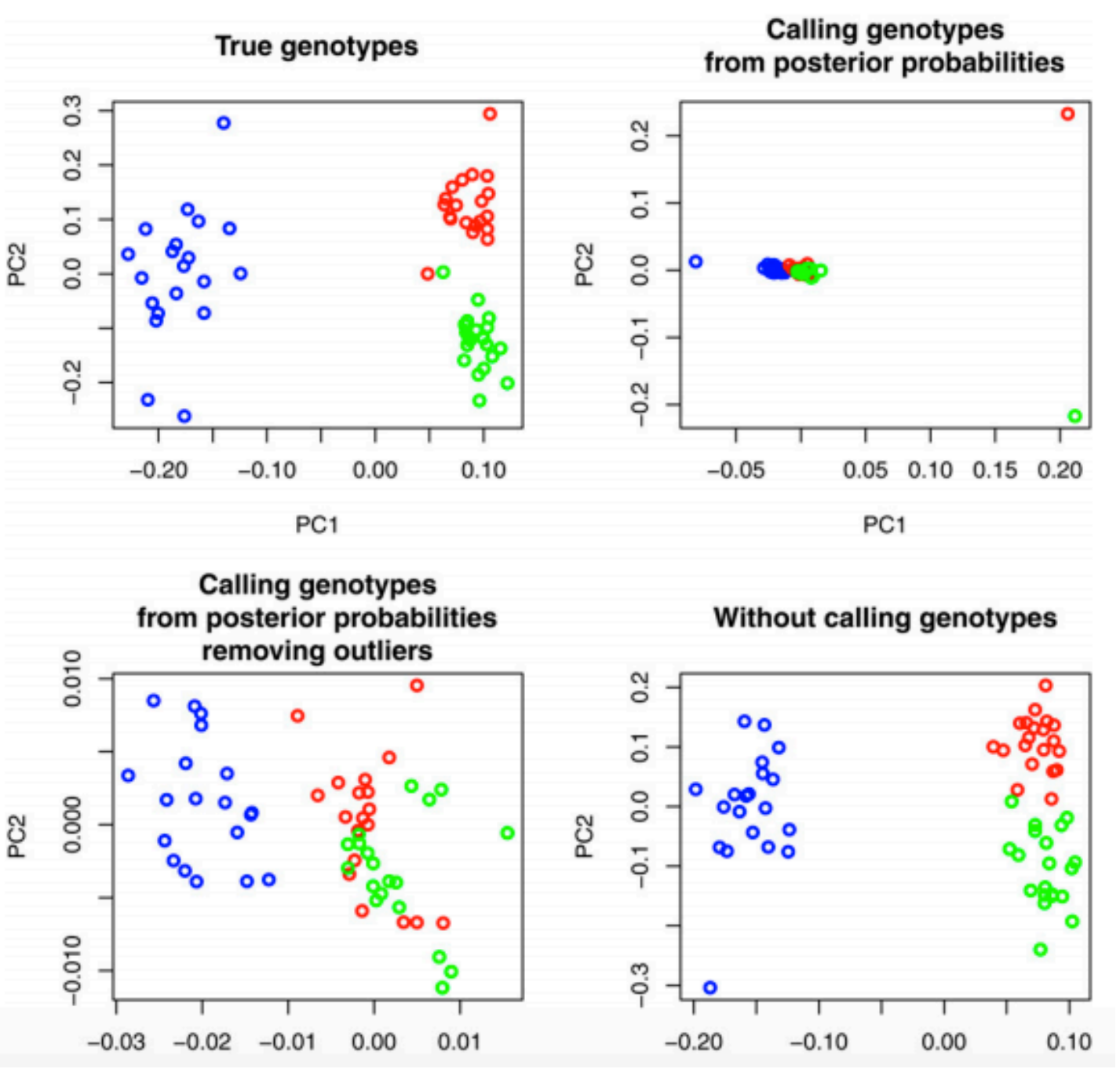

True genotypes
PC2
PC1
0.3
0.2
0.1
0.0
−0.2
−0.20
−0.10
0.00
0.10


Calling genotypes
from posterior probabilities
PC2
PC1
0.2
0.1
0.0
−0.1
−0.2
−0.05
0.05
0.10
0.15
0.20


Calling genotypes
from posterior probabilities
removing outliers
PC2
0.010
0.000
−0.010
−0.03
−0.02
−0.01
0.00
0.01

Without calling genotypes

Depth=2X


True genotypes
PC2
PC1
Calling genotypes
from posterior probabilities
PC2
PC1
Calling genotypes
from posterior probabilities
removing outliers
PC2
Without calling genotypes
PC2

Sample allele frequency

- *With k* diploid individuals, how many possible sample allele frequencies can I observe?

If unfolded, *2k+1* entries

p_0	p_1	p_2	p_3	…	p_{2k}

If folded, *k+1* entries

p_0	p_1	p_2	…	p_k

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e.g. A is ancestral, G is derived (alternate)
AA AA AG AA AG AA AA AA AA

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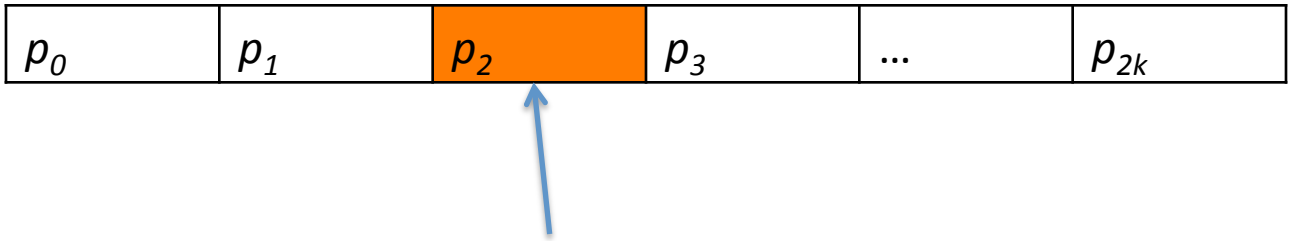

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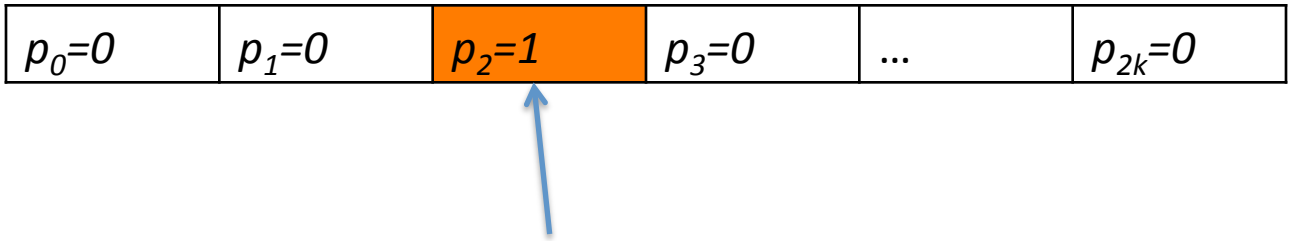

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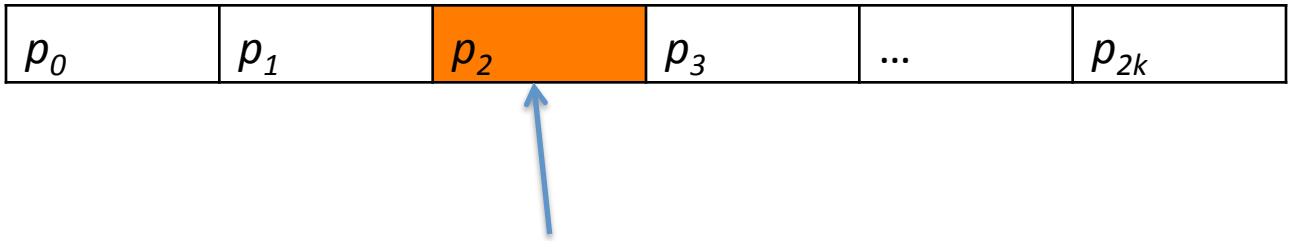
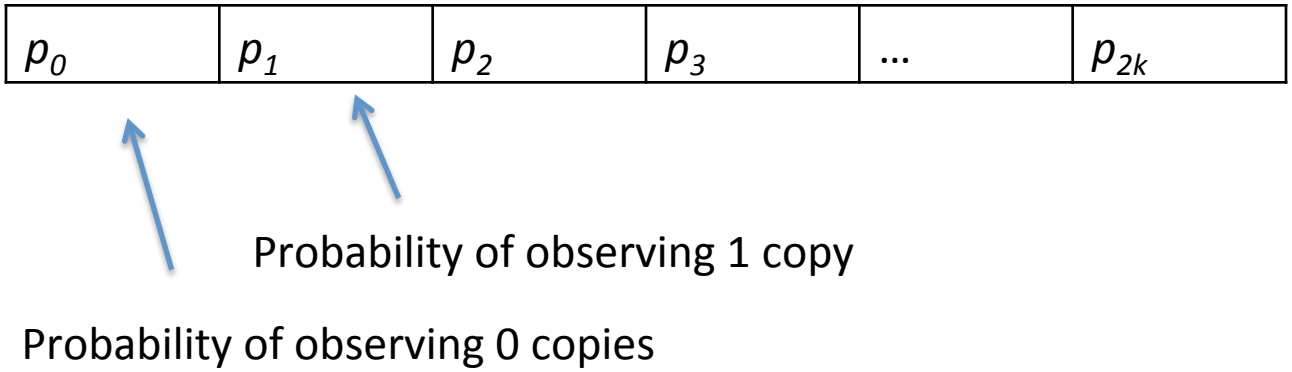


e.g. A is ancestral, G is derived (alternate)
If genotypes are unknown? Counting is not possible?

Sample allele frequency

- *With k* diploid individuals, how many possible sample allele frequencies can I observe?

If unfolded, *2k+1* entries



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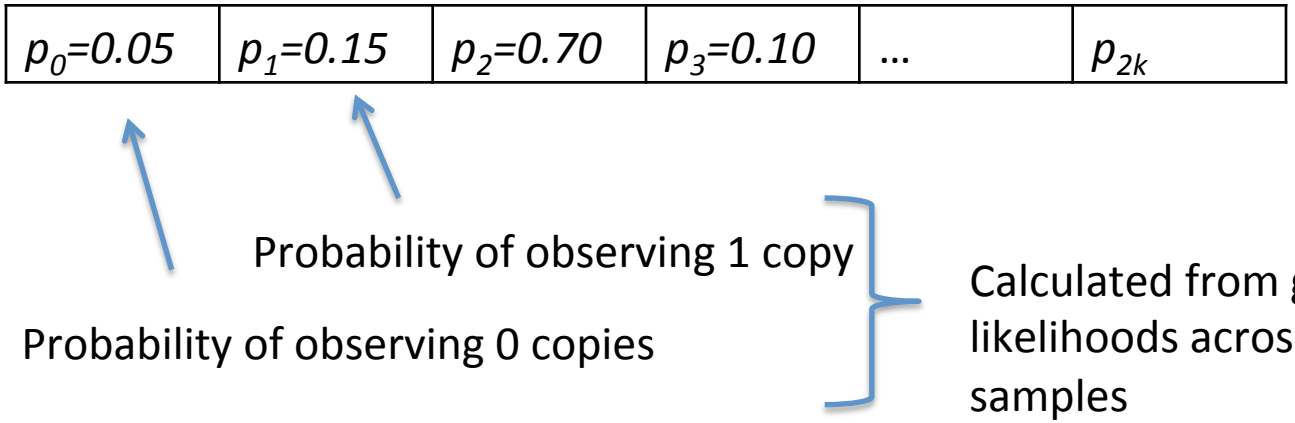

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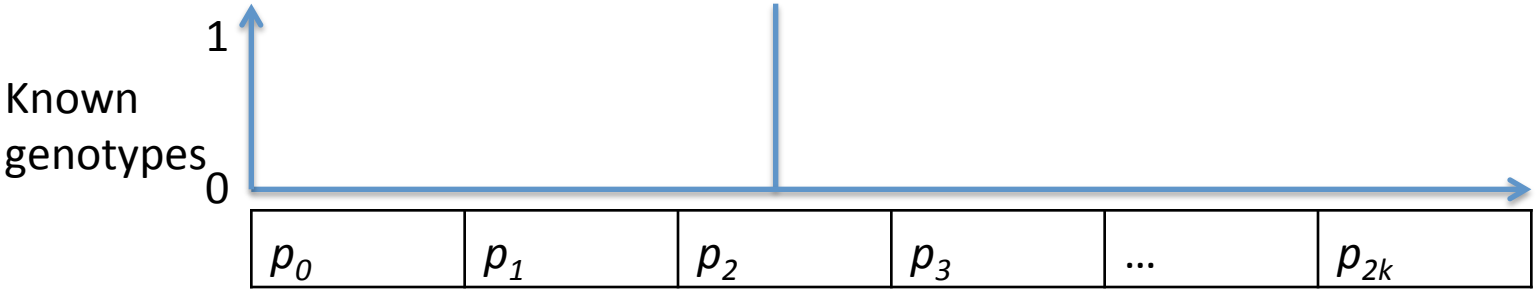
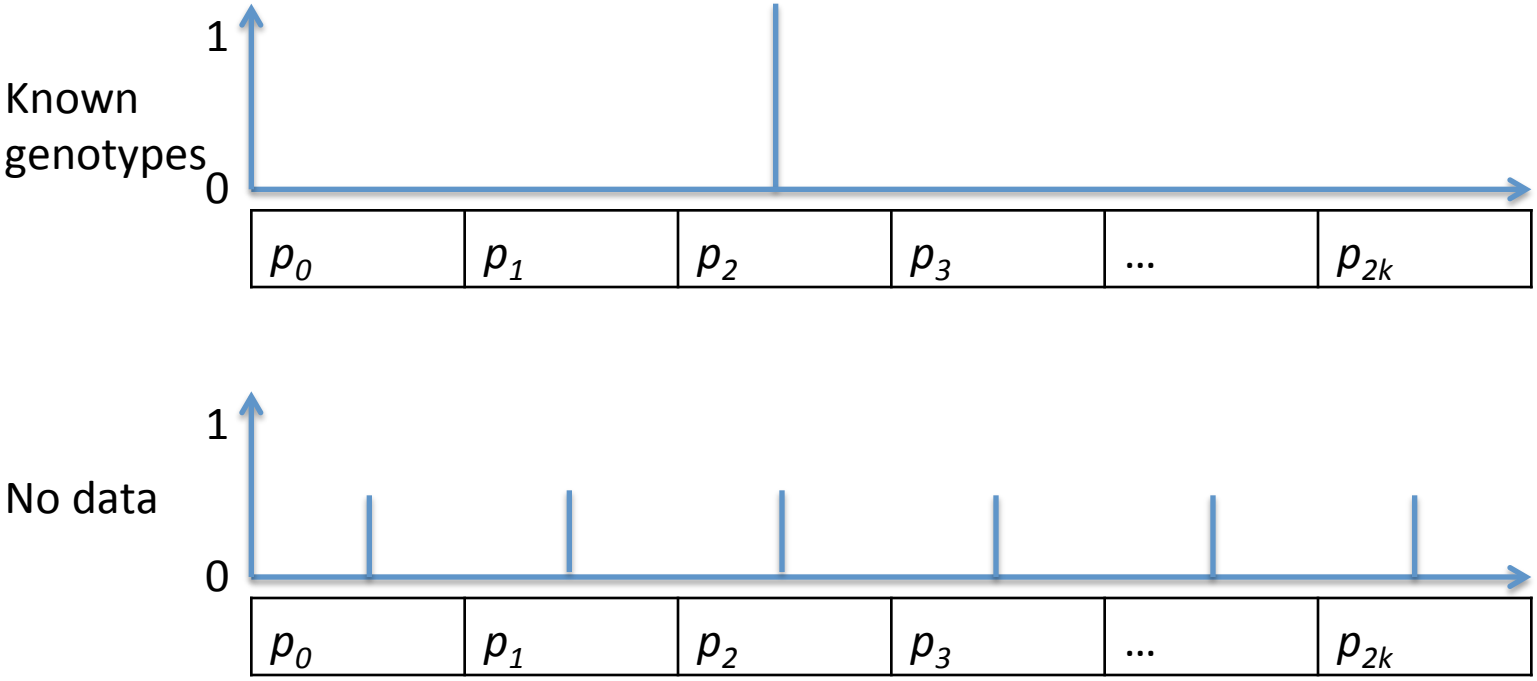
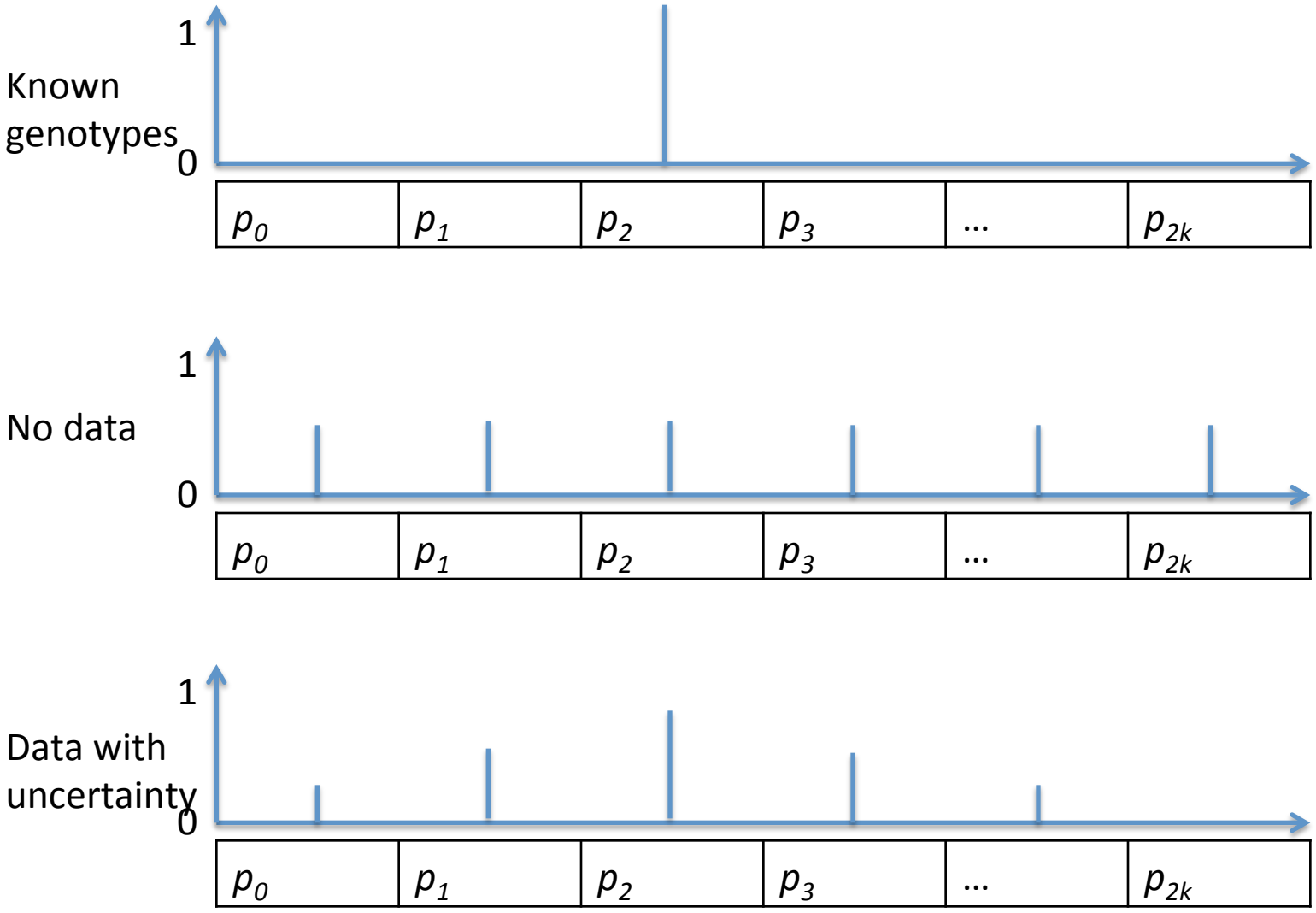
Sample allele frequency probabilities



Sample allele frequency probabilities



Sample allele frequency probabilities



Sample allele frequency posterior probabilities

$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

- Estimating allele frequency

$$\hat{f} =$$

Sample allele frequency posterior probabilities

$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

- Estimating allele frequency

$$\hat{f} = \sum_{i=0}^{2k} \left(\frac{i}{2k} \right) p(S = i)$$

Sample allele frequency posterior probabilities

With 6 chromosomes (3 diploids)

p_0=0.10	p_1=0.15	p_2=0.50	p_3=0.15	p_4=0.05	p_5=0.05	p_6=0.00

- SNP calling

$$p_{\text{var}} = \ ?$$

$$p_{\text{var}} > t$$

with *t* being 0.95, 0.99, 0.999 and so on.

Sample allele frequency posterior probabilities

p_0=0.10	p_1=0.15	p_2=0.50	p_3=0.15	p_4=0.05	p_5=0.05	p_6=0.00

- SNP calling

$$p_{\text{var}} = 1 - p(S=0) - p(S=2k) \quad = 0.90$$

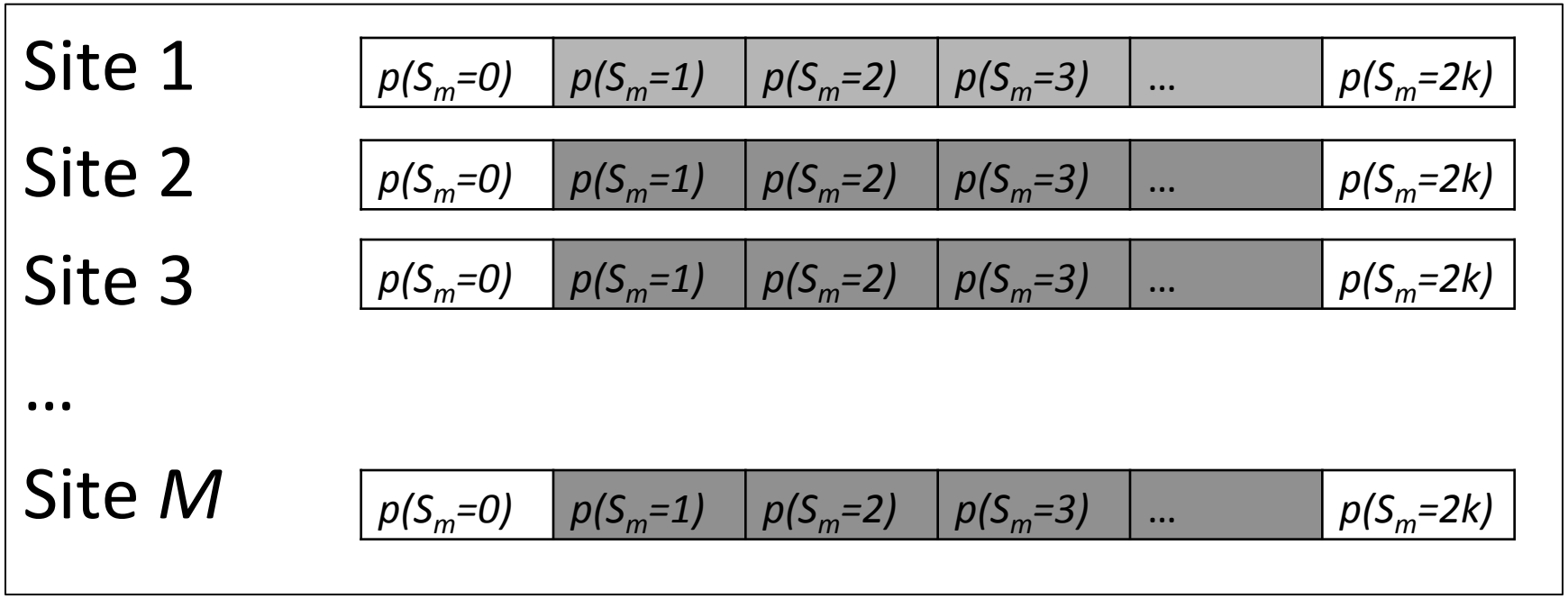
$$p_{\text{var}} > t$$

with *t* being 0.95, 0.99, 0.999 and so on.

Nr of segregating sites

Site 1	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 2	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 3	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
…						
Site *M*	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

Nr of segregating sites



Nr of segregating sites

Site 1	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 2	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 3	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
…						
Site *M*	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

$$E[S] = \sum_{m=1}^{M} p_{\text{var}}^{(m)} = \sum_{m=1}^{M} (1 - p(S_m = 0) - p(S_m = 2k))$$

Nucleotide diversity

Site 1	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 2	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 3	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
…						
Site *M*	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

$$D = 2f(1-f)$$

$$E[D] =$$

Nucleotide diversity

Site 1	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 2	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
Site 3	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$
…						
Site *M*	$p(S_m=0)$	$p(S_m=1)$	$p(S_m=2)$	$p(S_m=3)$	…	$p(S_m=2k)$

$$E[D] = \sum_{m=1}^{M} \sum_{j=0}^{2k} 2 \left(\frac{i}{2k} \right) \left(\frac{2k-i}{2k} \right) p(S_m = i)$$

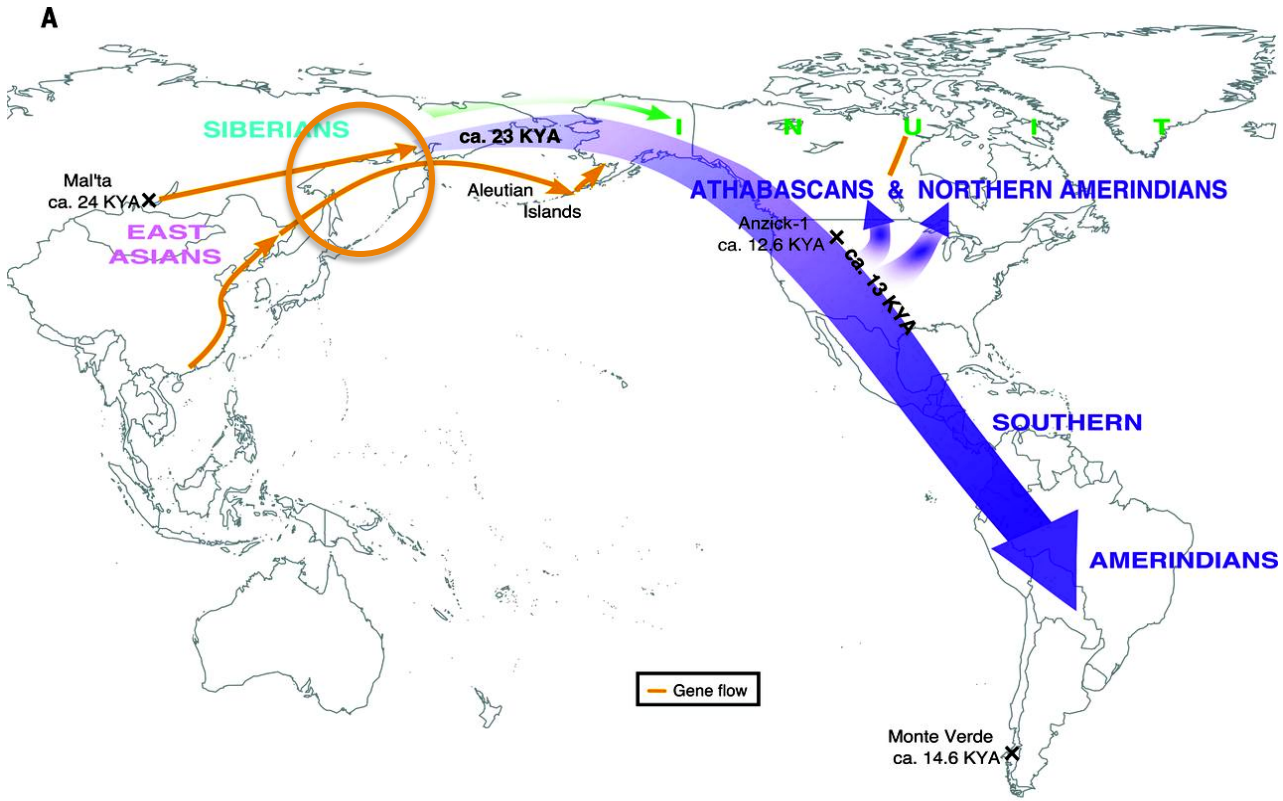
Applications

...

- Model and non-model species
- Plants
- Ancient genomes
- …

Practical

- Basic filtering
- Estimation of allele frequencies and SNP calling
- Genotype calling
- Advanced methods to estimate SFS



Raghavan et al. 2015 Science