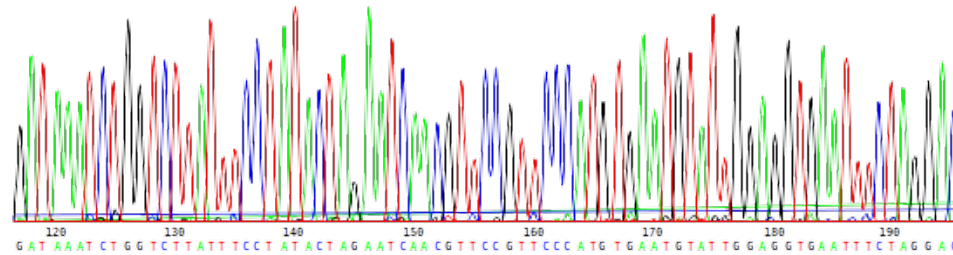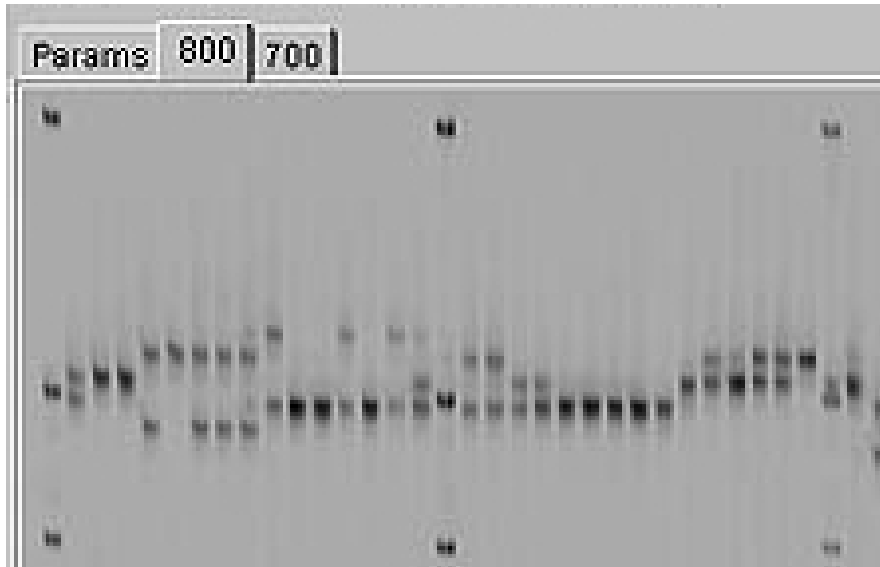# Genotype likelihoods, allele frequencies, and SNP calling from NGS data
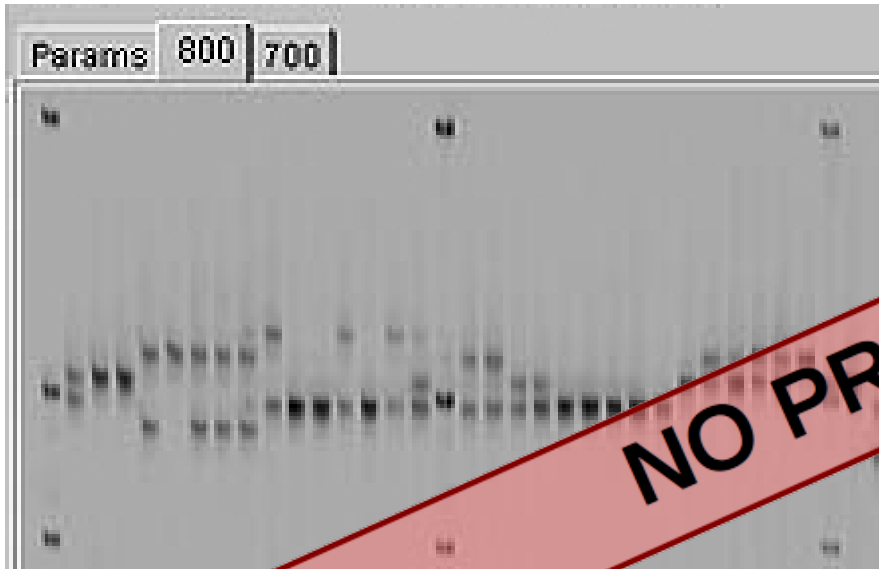
Tyler Linderoth
Physalia lcWGS course 2023

Why Probabilistic Methods?

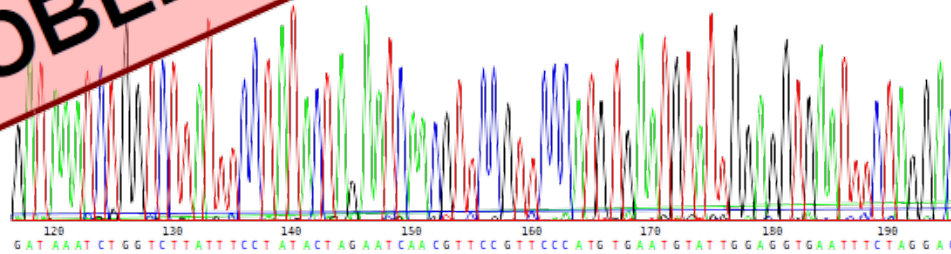# Why Probabilistic Methods?

NO PROBLEM!

http://www.licor.com/bio/products/software/saga_gt/details.html

Why Probabilistic Methods?
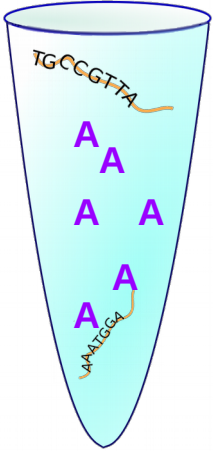
The library for an individual homozygous for the **A** allele will consist only of **A**s.
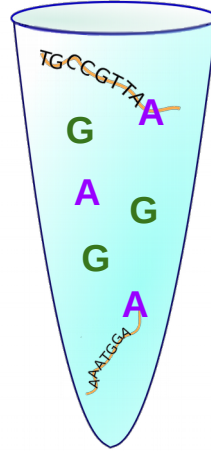
The library for a heterozygous individual at a site contains both **A**s and **G**s.
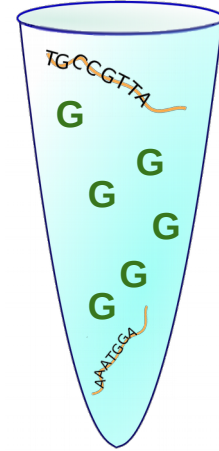
The library for an individual homozygous for the **G** allele will consist only of **G**s.

Why Probabilistic Methods?
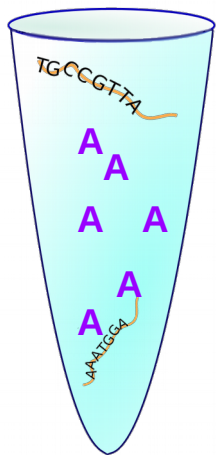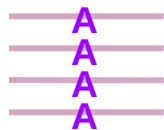
Sequence to average depth of 4x.

Expect 4 reads, all with **A**s at this ref position.
Depth ~ Poisson($\lambda = 4$).
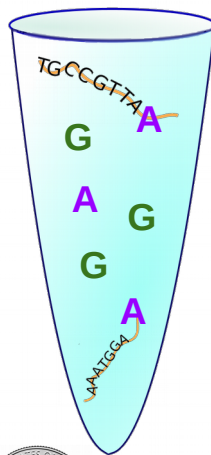
vs

Sequencing (sampling) the two different alleles is just like flipping a coin.

P(k A alleles)
~Binom(n reads, p=0.5)

Why Probabilistic Methods?

Sequence to average depth of 4x.

vs
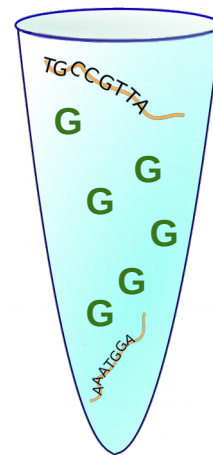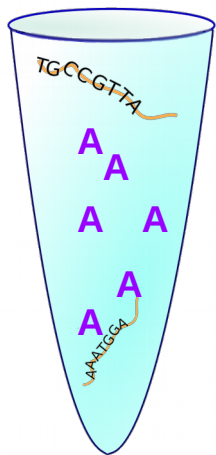
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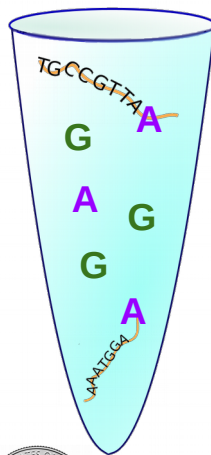
P(k A alleles) ~Binom(n reads, p=0.5)
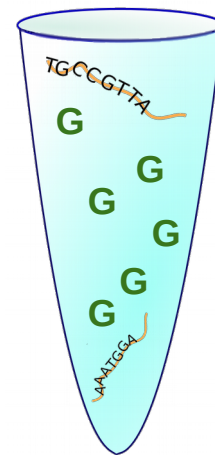
Why Probabilistic Methods?
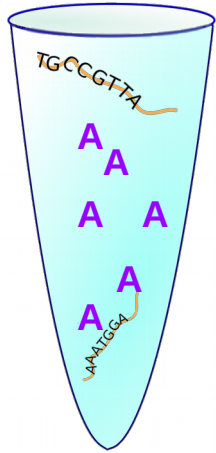
A sequencing error occurs. This can occur at rates of around 0.1% in Illumina data.

Expect 4 reads, all with **A**s at this ref position.
Depth ~ Poisson($\lambda = 4$).
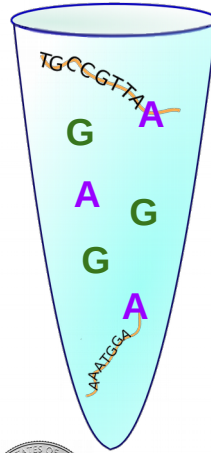
vs

Sequencing (sampling) the two different alleles is just like flipping a coin.

P(k A alleles)
~Binom(n reads, p=0.5)
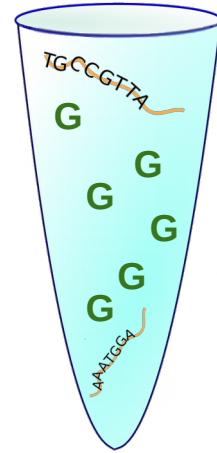
# A basic model for a diploid individual's genotype

paternal allele

maternal allele

Product over all reads covering a site for an individual

$$P(X|G=bh) = \prod_{i=1}^{r} \left(\frac{L_b^{(i)}}{2} + \frac{L_h^{(i)}}{2} \right)$$

$$b, h \in \{A, C, G, T\}$$

Example for an individual with observed sequencing reads **AAAG** at a site.

$$P(X|G=bh)=\prod_{i=1}^{r}\left(\frac{L_b^{(i)}}{2}+\frac{L_h^{(i)}}{2}\right)$$

10 potential genotypes for a diploid individual

AA

AC

AG

AT

CC
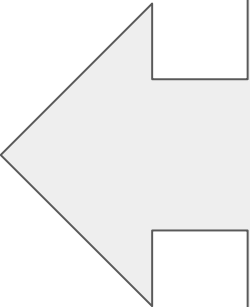
CG

CT

GG

GT

TT

For every genotype, we could calculate it's likelihood by iterating over all of the observed reads: AAAG.

Let's calculate the likelihood for the AC genotype.

Example for an individual with observed sequencing reads **AAAG** at a site.

$$P(X|G=bh)=\prod_{i=1}^{r}(\frac{L_b^{(i)}}{2}+\frac{L_h^{(i)}}{2})$$

A,A,A,G

$$P(X|G=AC)=(\frac{L_A^{(1)}}{2}+\frac{L_C^{(1)}}{2})*(\frac{L_A^{(2)}}{2}+\frac{L_C^{(2)}}{2})*(\frac{L_A^{(3)}}{2}+\frac{L_C^{(3)}}{2})*(\frac{L_A^{(4)}}{2}+\frac{L_C^{(4)}}{2})$$

=
A
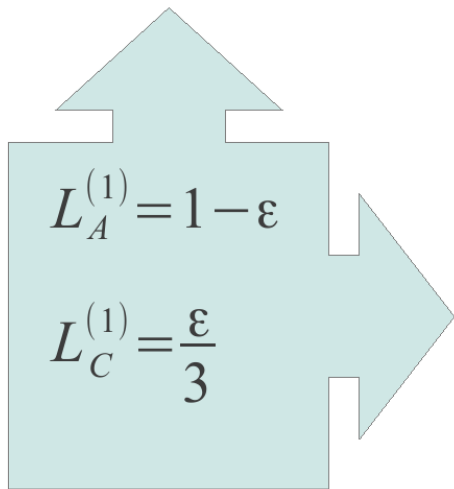
ε = Probability of an error

There are 3 potential erroneous reads for a error to turn into, hence the 1/3 * ε

$$L_A^{(1)}=1-\varepsilon$$

$$L_C^{(1)}=\frac{\varepsilon}{3}$$

$$P(X=A|G=AC)=\frac{1-\varepsilon}{2}+\frac{\varepsilon}{6}$$

Example for an individual with observed sequencing reads **AAAG** at a site.

Genotype	Likelihood (log10)
AA	-2.49
AC	**-3.38**
AG	-1.22
AT	-3.38
CC	-9.91
CG	-7.74
CT	-9.91
GG	-7.44
GT	-7.74
TT	-9.91

A

A

A

G

$\epsilon = 0.01$

Genotype calling

Genotype	Likelihood (log10)
AA	-2.49
AC	-3.38
AG	**-1.22**
AT	-3.38
CC	-9.91
CG	-7.74
CT	-9.91
GG	-7.44
GT	-7.74
TT	-9.91

AAAG & $\epsilon = 0.01$
What is the genotype?
AG.

Maximum Likelihood

The simplest genotype caller: choose the genotype with the highest likelihood.

This is essentially what you are doing in ANGSD when you choose a uniform prior probability distribution for the genotypes.

Major and minor alleles

Likelihood function

$$\log P(D|G = A) = \sum_{i=1}^{R} \log L_{A_j,i}$$

AAAG & $\epsilon = 0.01$

Allele	Likelihood
A	**-2.49**
C	-3.38
G	**-1.22**
T	-3.38

We can reduce the genotype space to 3 entries (from 10, for diploids).

Can we somehow use other information present in our data to further increase our genotype calling accuracy?

```
6  .....,         DEGEGG    9  ,.,,.,,.,      DABGIIIII  5  ,,.,.  >AB/A  6  .,,...  FGG
6  .....,         DEGEGG    9  ,.,,.,,.,      DABGIIIII  5  ,,.,.  >ABDA  6  .,,...  3GGBGB
6  .....,         DEGEGG    9  ,.,,.,,.,      DABGIIIII  5  ,,.,.  >ABDA  6  .,,...  3GGBGB
6  .....,         DEGEGG   10  ,.,,.,,.,^].   DABGIIIIIE  5  ,,.,.  >AB/A  6  .,,...  3GGBGB
6  .....,         DEGEGG   10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >AB/A  6  .,,...  3GGBGB
6  .....,         DEGEGG   10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >ABDA  6  .,,...  BGGBGB
6  .....,         DEGEGG   10  ,.,,.,.,,.     DABGIIIIII  5  ,,.,.  >ABDA  6  C,,...  5G/BGB
7  .....,^].      DEGEGGE  10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >ABDA  6  .,,...  5GIBGB
7  .....,.        DEGEGGG  10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >ABDA  6  .,,...  5GIBGB
8  .....,.^],     DEGEGGGB 10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >ABDA  6  .,,...  5GIBGB
8  .....,.,       DEGEGGGB 10  ,.,,.,.,,.     DABGIIIIII  5  ,,.,.  >AB/A  6  .,,...  DGIBGB
8  .....,.,       DEGEGGGB 10  ,.,,.,.,,.     DABGIIIIII  5  ,,.,.  >ABAA  6  .,,...  DGIBGB
8  .....,.,       DEGEGGGB 10  ,.,,.,,.,.     DABGIIIIII  5  ,,.,.  >/BAA  6  .,,...  DGIBGB
9  .....,.,^],    DEGEGGGBE 10  ,.,,.,,.,.    DABGIIIIII  5  ,,.,.  >BBAA  6  .,,...  DGIBGB
9  .G...gG,,       DEGEGGGBG 10  ,.,,.,,.,.   D3BGIIIIII  5  ,,.,C  >BBAA  6  .,,...  DGIBGB
9  .....,.,,      DEGEGGGBG 10  ,.,,.,,.,.    D3BGIIIIII  5  ,,.,.  >BBAA  6  .,,...  /GIBGB
```
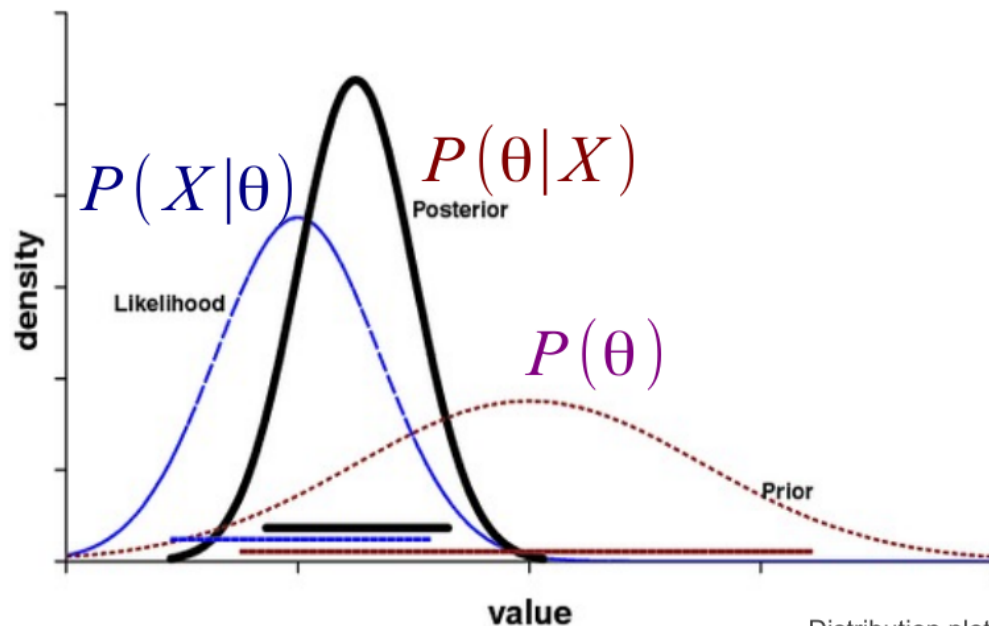
Wouldn't it be awesome if you knew what the frequency of C was in the rest of the sample or population.

Bayesian Inference

$$P(\theta|X) = \frac{P(X|\theta)P(\theta)}{P(X)} = \frac{P(X|\theta)P(\theta)}{\sum_{\theta} P(X|\theta)P(\theta)}$$

Distribution plots from Bink 2008

From genotype likelihoods to posterior probabilities

Having an estimate of the allele frequency in the population would enable us to have prior knowledge about the probabilities of observing a particular genotype from principles like Hardy-Weinberg Equilibrium (HWE):

$P(\text{Genotype} = 0 \text{ minor alleles}) = (1-f_{minor})^2$

$P(\text{Genotype} = 1 \text{ minor allele}) = 2 * f_{minor} * (1-f_{minor})$

$P(\text{Genotype} = 2 \text{ minor alleles}) = (f_{minor})^2$

Things like inbreeding can easily be incorporated into these genotype probabilities. So, now we have to know how to estimate allele frequencies.

A simple model to estimate the population minor allele frequency, *f*, is given by

$$p(D_i | f) = \sum_{\mathbf{g} \in \{0,1,2\}} p(D_i | G_i = \mathbf{g}) p(G_i = \mathbf{g} | f)$$

These are the genotype likelihoods (D_i is the sequencing data for the *i*th individual) that we now know how to calculate.

And here is just the probability of the genotype given the minor allele frequency, which we can get through HWE.

$$\hat{f} = \arg\max_f \prod_i p(D_i | f) \longleftarrow$$

Figure out what minor allele frequency maximizes the above likelihood across all individuals in the sample, and you have a ML estimate of the minor allele frequency.

A simple model to estimate the population minor allele frequency, *f*, is given by

$$p(D_i|f) = \sum_{\mathbf{g} \in \{0,1,2\}} p(D_i|G_i = \mathbf{g})p(G_i = \mathbf{g}|f)$$

$$\hat{f} = \arg\max_f \prod_i p(D_i|f)$$

One thing to note here is that you can compare the likelihood for the ML minor allele frequency to the likelihood calculated from above with f set to zero:

$\lambda = -2*\log(L(f=0) - L(f=f_{ML}))$

$\lambda \sim$ Chi-square(1 d.f.)

Now you have a way to test whether the ML MAF is statistically nonzero, i.e. whether the site is a SNP.

Now, getting back to this individual with sequencing data AAAG at a site. If we estimate $f(A) = 0.7$ and we consider only the two most likely alleles (A and G) and ε is always 0.01 (Phred quality of 20, remember that?), then the genotype likelihoods are

Genotype	Likelihood
AA	-5.73
AG	-2.80
GG	-17.12

Apply Bayes' Theorem

Prior probability using $f(A) = 0.7$ and HWE

$$P(G|D) = \frac{P(D|G)\pi(G)}{\sum_{G \in \{0,1,2\}} P(D|G)\pi(G)}$$

And you get genotype posterior probabilities!

Genotype	Likelihood (log)	Prior	Posterior
AA	-5.73	0.49	0.06
AG	-2.80	0.42	0.94
GG	-17.12	0.09	0

Now, we can call the genotype as AG based on the max posterior probability (and we also have an estimate of how reliable this call is).