CS4330: Combinatorial Methods in Bioinformatics

Genome characteristics estimation using K-mers

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Acknowledgement: This set of slides were adapted from Ken Sung's

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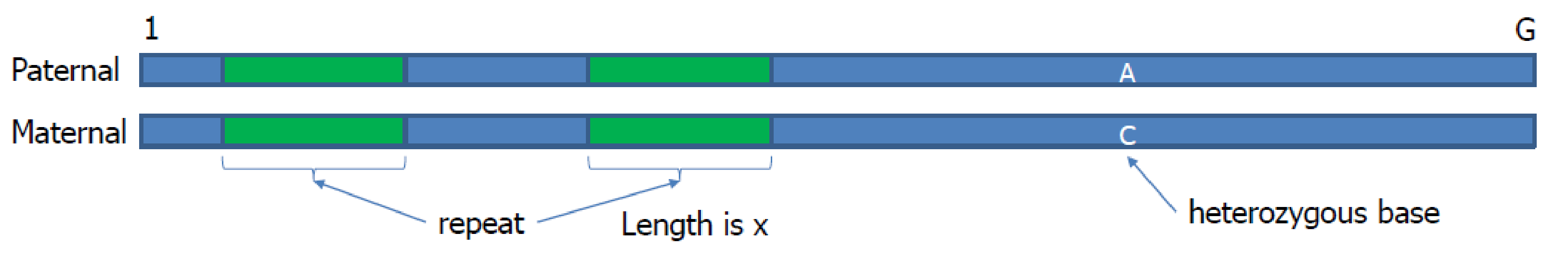
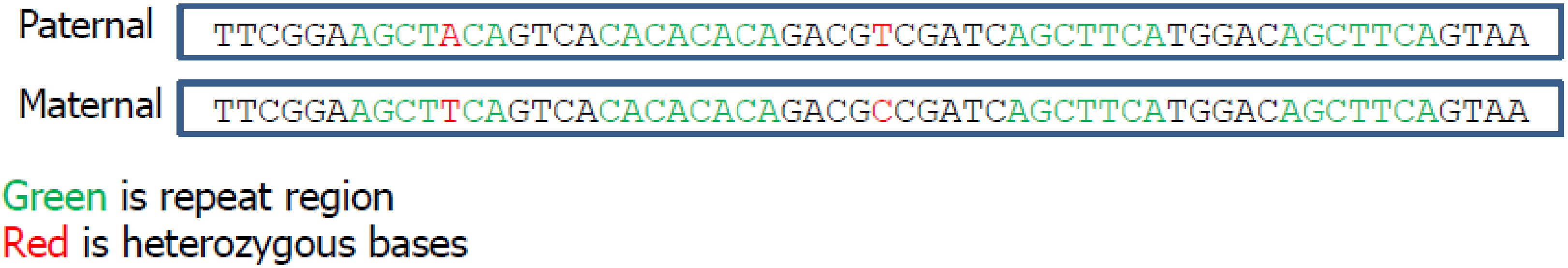
Genome characteristics



$$\text{Percentage of Repeat Content} = \left(\frac{\text{Total Length of Repeats}}{\text{Total Genome Length}} \right) \times 100$$

$$\text{Heterozygous Rate} = \left(\frac{\text{Number of Heterozygous Positions}}{\text{Total Number of Analyzed Positions}} \right) \times 100$$

Exercise



Paternal TTCGGAAGCTACAGTCACACACACAGACGTCGATCAGCTTCATGGACAGCTTCAGTAA

Maternal TTCGGAAGCTTCAGTCACACACACAGACGCCGATCAGCTTCATGGACAGCTTCAGTAA

Green is repeat region

Red is heterozygous bases

Compute the followings:

Genome size

Percentage of repeat content

Heterozygous rate

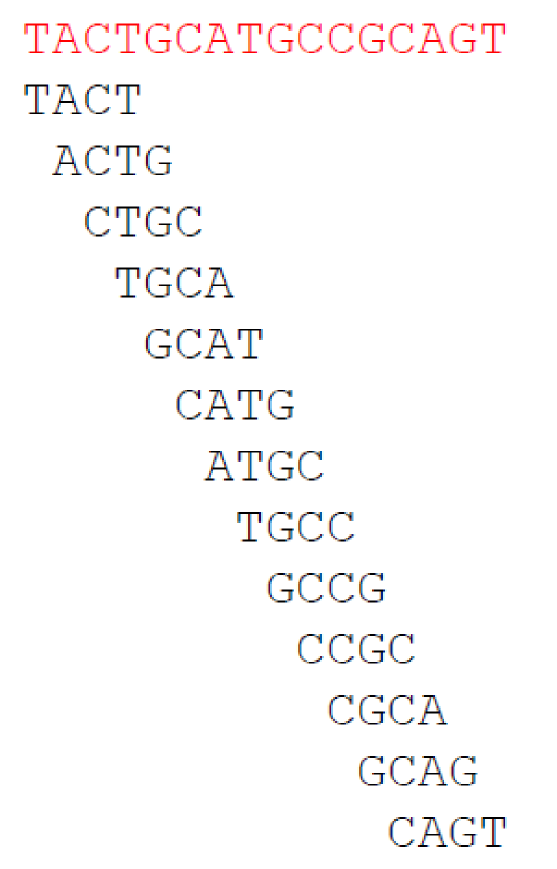
Homozygous repeat-free genome

In a homozygous repeat-free genome, most K-mers occurring in it have similar counts

Unique K-mers of a genome are K-mers occurring exactly once in the genome / in each read

Example

In this genome, all 4-mers are “unique”



```
TACTGCATGCCGCAGT
TACT
 ACTG
  CTGC
   TGCA
    GCAT
     CATG
      ATGC
       TGCC
        GCCG
         CCGC
          CGCA
           GCAG
            CAGT
```

Genome size estimation

Let G = genome size, i.e. length of the haploid genome

Let L = mean read length

Let N = # of reads

If C = sequencing coverage is known, then $G \approx N L / C$

However, estimating C is resource demanding as the reads have to be aligned and then get the average number of reads aligned to each position in the consensus genome

Genome size estimation by K-mers

Let G = size of a *homozygous repeat-free genome*

Let L = mean read length

Let N = # of reads

Why?

Let μ = mean K-mer count in the reads covering a base

= mean # of reads a K-mer occurs in, as K-mers are unique

$\therefore \mu G \approx$ Total K-mer counts in the reads $= N (L - K + 1)$

$\Rightarrow G \approx N (L - K + 1) / \mu$

Estimating genome size was easy for homozygous repeat-free genomes

Real genomes are hardly ever homozygous repeat-free

Needs modelling … using K-mer spectrum

K-mer spectrum

K-mer spectrum shows how frequently each K-mer occurs in a given set of DNA sequences

```
ACGTC
 CGTCA
  GTCAA
   TCAAG
    CAAGT
```

Reads

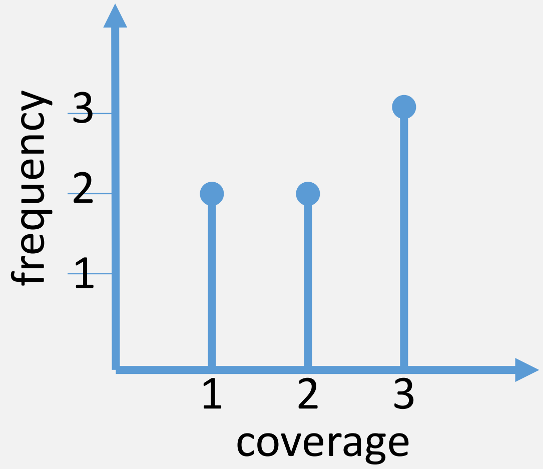
k-mer	count
ACG	1
CGT	2
GTC	3
TCA	3
CAA	3
AAG	2
AGT	1

K-mer spectrum

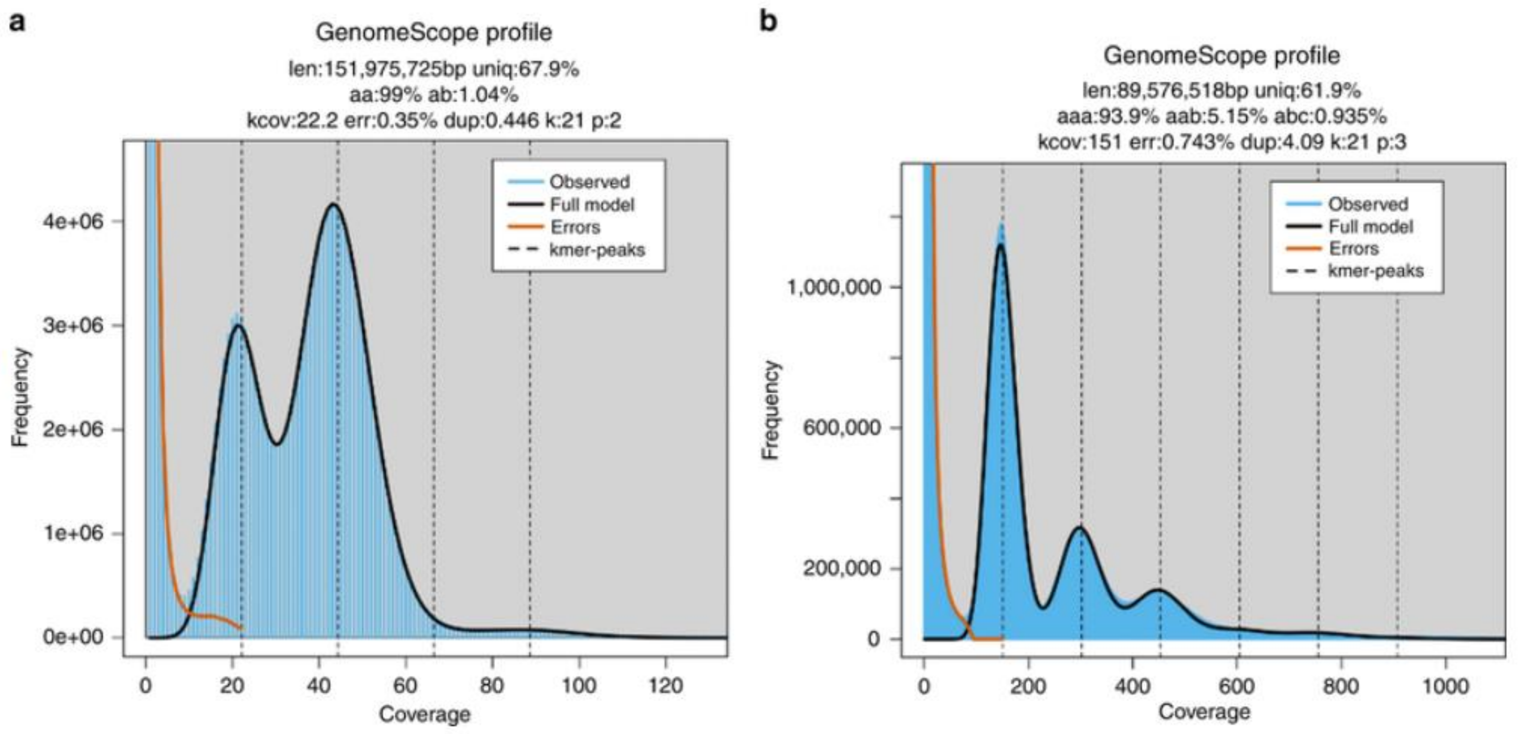
K-mer spectrum is often visualized as a histogram

x-axis = counts of diff K-mers

y-axis = # of K-mers with a specific count



K-mer spectra of heterozygous diploid & triploid genomes

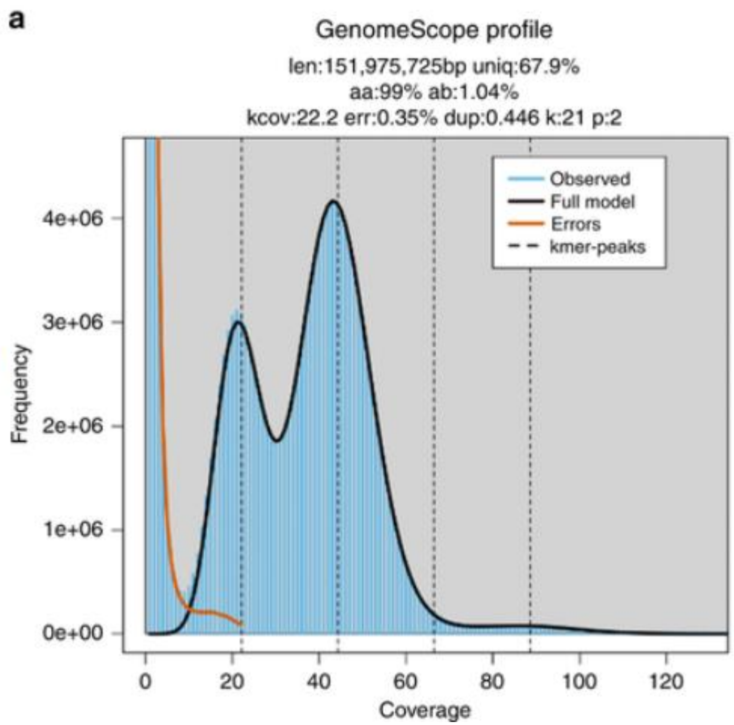


GenomeScope plots for heterozygous species K-mer spectra and fitted models for (a) diploid Arabidopsis thaliana and (b) triploid Meloidogyne enterolobii. Note that the diploid plot has two major peaks, while the triploid plot has three major peaks. Both also have high frequency putative error k-mers with coverage near 1.

Ranallo-Benavidez et al., “GenomeScope 2.0”, *Nature Comm* 11:1432, 2020

Exercise

Given this K-mer spectrum for a diploid genome

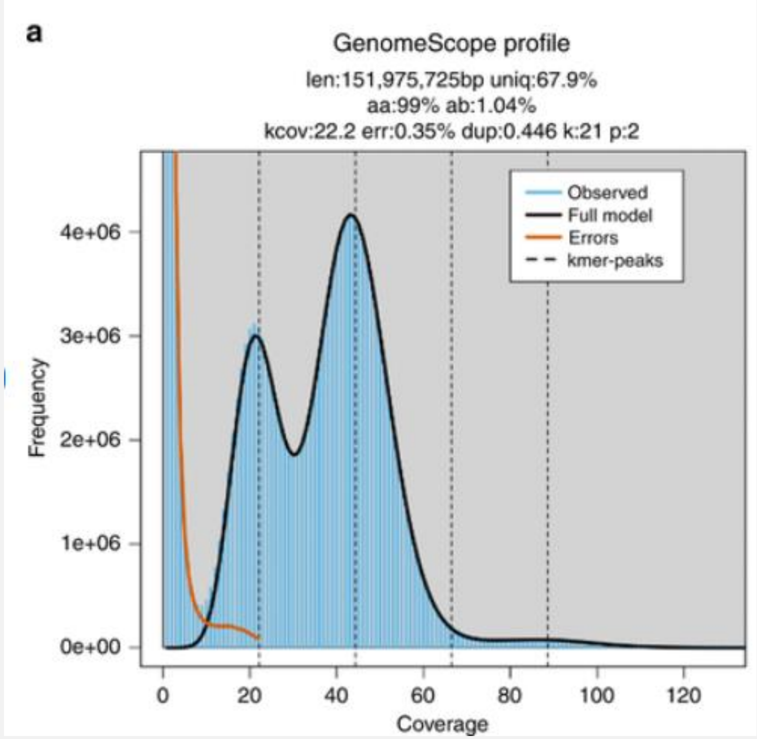


Which peak corresponds to K-mers covering homozygous bases?

Which peak corresponds to K-mers covering heterozygous bases?

What is the sequencing coverage?

Modelling observed K-mer spectrum



GenomeScope fits a theoretical model (black curve) to the observed K-mer spectrum (blue histogram)

Genome size (~152B), heterozygous rate (1.04%), etc. are then extracted from parameters of the fitted model

Let’s see how this is done…

K-mer spectrum of a homozygous repeat-free genome

Assume the followings:

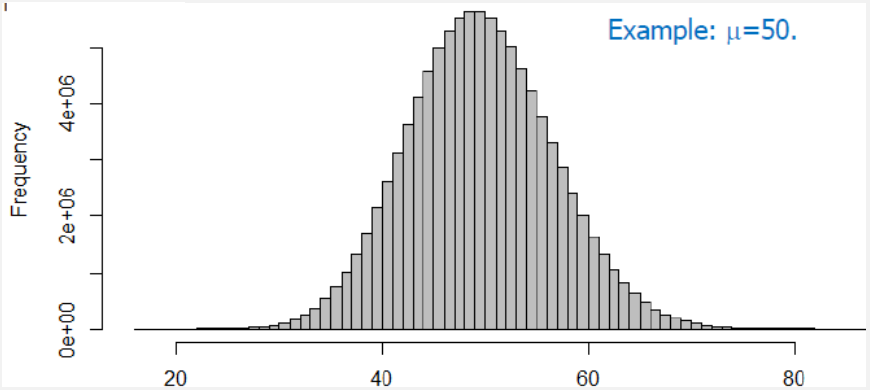
No sequencing error, no heterozygosity, no repeat

K-mers are randomly extracted from the genome

Lander & Waterman, "Genomic mapping by fingerprinting random clones: a mathematical analysis", *Genomics* 2(3):231-239, 1988

Then:

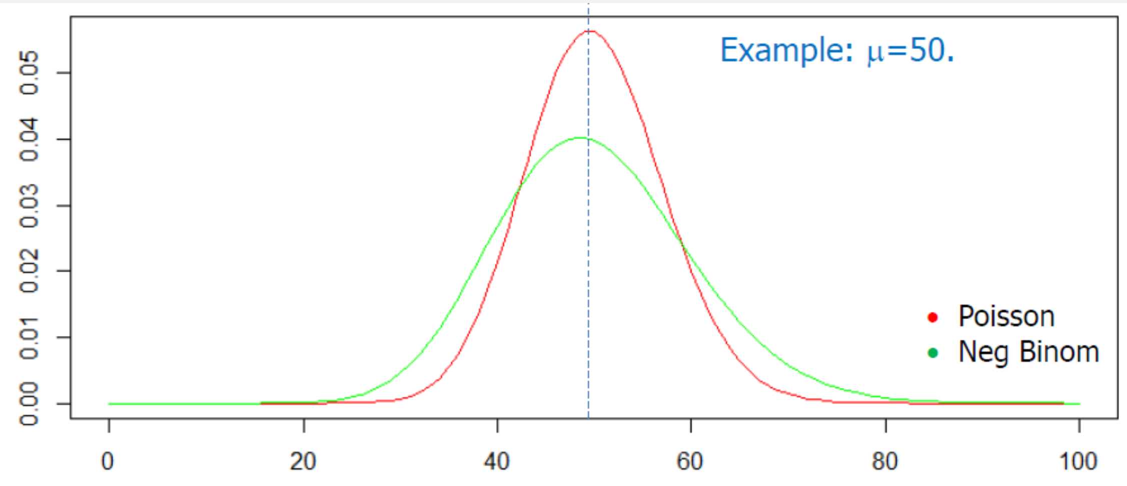
K-mer spectrum is a Poisson distribution having μ = the mean K-mer count



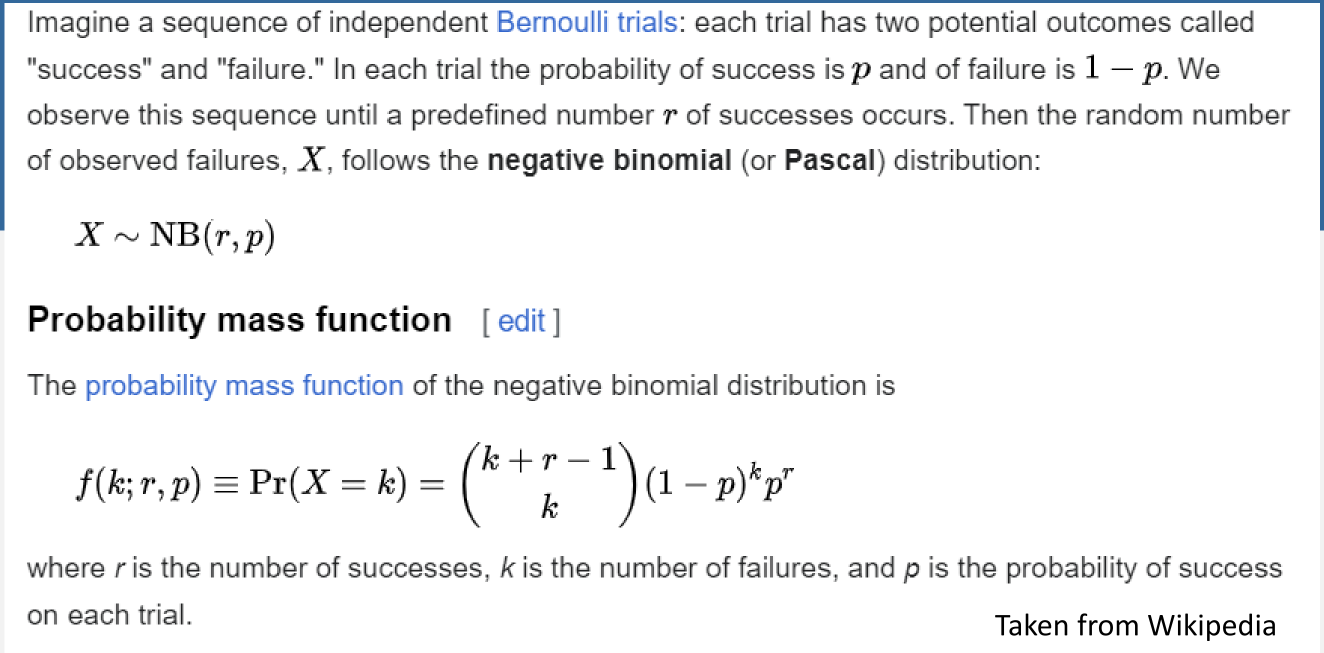
Sometimes, Poisson(μ) does not fit well…

Real sequencing data is a bit over-dispersed compared to Poisson

Negative binomial NB(μ, μ / ρ) is used instead, where ρ is a variant parameter that controls over-dispersion



Negative binomial



Imagine a sequence of independent Bernoulli trials: each trial has two potential outcomes called "success" and "failure." In each trial the probability of success is p and of failure is $1 - p$. We observe this sequence until a predefined number r of successes occurs. Then the random number of observed failures, X, follows the **negative binomial** (or **Pascal**) distribution:

$$X \sim \mathrm{NB}(r, p)$$

Probability mass function [edit]

The probability mass function of the negative binomial distribution is

$$f(k; r, p) \equiv \Pr(X = k) = \binom{k + r - 1}{k} (1 - p)^k p^r$$

where *r* is the number of successes, *k* is the number of failures, and *p* is the probability of success on each trial.

Taken from Wikipedia

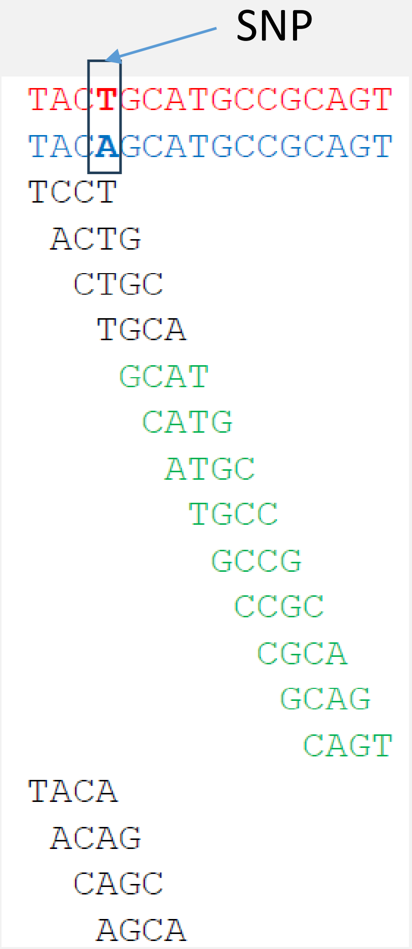
Use the pmf, f(c, μ, μ / ρ), of a negative binomial to model the prob of a random K-mer having coverage c, where μ is the observed mean K-mer coverage and ρ a fitted parameter

Do this separately for each kind of K-mers: homozygous, heterozygous, 2-copy repeats, 3-copy repeats

Repeat-free diploid genome

This is a diploid genome where all K-mers are unique

One heterozygous base gives 2K heterozygous K-mers



SNP

```
TACTGCATGCCGCAGT
TACAGCATGCCGCAGT
TCCT
 ACTG
  CTGC
   TGCA
    GCAT
     CATG
      ATGC
       TGCC
        GCCG
         CCGC
          CGCA
           GCAG
            CAGT
TACA
 ACAG
  CAGC
   AGCA
```

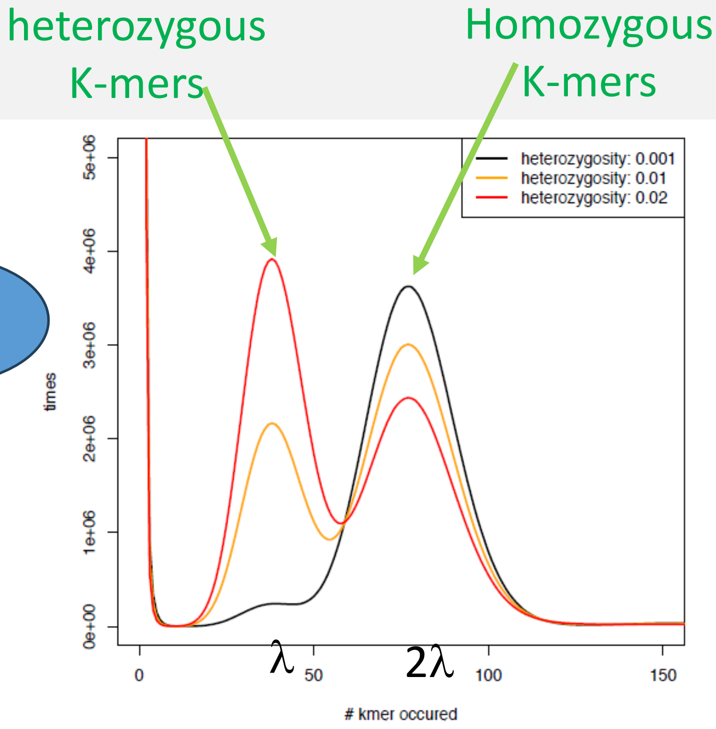
K = 4
The SNP creates 8 (= 2K) 4-mers

K-mer spectrum of repeat-free diploid genome

If a genome is heterozygous and repeat-free, there are two peaks at K-mer coverage λ and 2λ

Why?

As one heterozygous base creates 2K heterozygous K-mers, the heterozygous peak grows fast



Supplementary Figure 1. Impact of heterozygosity on the *k-mer* profile. *K-mer* profiles were draw from 100x sequencing coverage of simulated reads with 0.1%, 1% and 2% heterozygosity embedded into the *D. melanogaster* reference genome.

Vurture et al., "GenomeScope", *Bioinformatics* 33(14):2202-2204, 2017

Homozygous vs heterozygous K-mers

Consider a repeat-free diploid genome

Let r = heterozygosity rate

Then,

$(1 - r)^K$ = prob that a random K-mer is homozygous

$1 - (1 - r)^K$ = prob that a random K-mer is heterozygous

Homozygous vs heterozygous K-mers

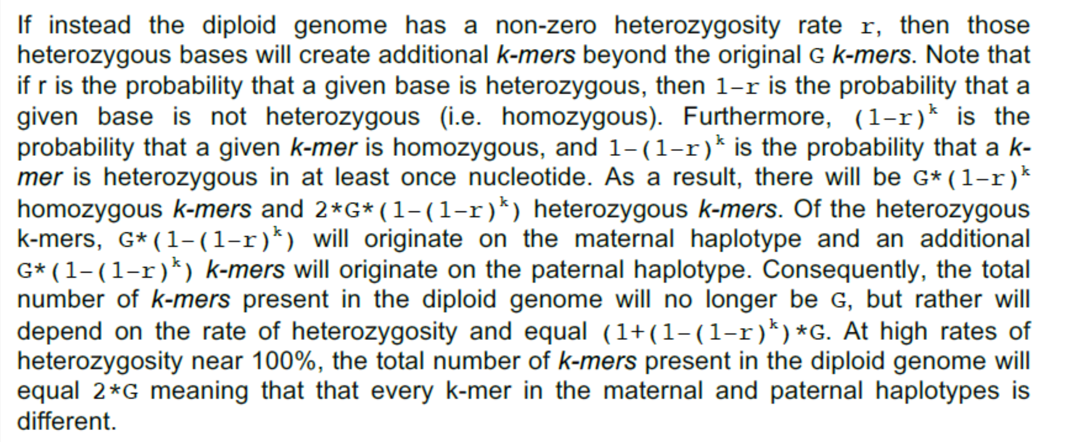
Let α = proportion of heterozygous K-mers wrt genome size

Let β = proportion of homozygous K-mers wrt genome size

Then,

$\alpha = 2\,(1 – (1 – r)^K)$

$\beta\ = (1 – r)^K$



If instead the diploid genome has a non-zero heterozygosity rate r, then those heterozygous bases will create additional *k-mers* beyond the original G *k-mers*. Note that if r is the probability that a given base is heterozygous, then $1-r$ is the probability that a given base is not heterozygous (i.e. homozygous). Furthermore, $(1-r)^k$ is the probability that a given *k-mer* is homozygous, and $1-(1-r)^k$ is the probability that a *k-mer* is heterozygous in at least once nucleotide. As a result, there will be $G*(1-r)^k$ homozygous *k-mers* and $2*G*(1-(1-r)^k)$ heterozygous *k-mers*. Of the heterozygous k-mers, $G*(1-(1-r)^k)$ will originate on the maternal haplotype and an additional $G*(1-(1-r)^k)$ *k-mers* will originate on the paternal haplotype. Consequently, the total number of *k-mers* present in the diploid genome will no longer be G, but rather will depend on the rate of heterozygosity and equal $(1+(1-(1-r)^k)*G$. At high rates of heterozygosity near 100%, the total number of *k-mers* present in the diploid genome will equal $2*G$ meaning that that every k-mer in the maternal and paternal haplotypes is different.

Vurture et al., “GenomeScope”, *Bioinformatics* 33(14):2202-2204, 2017

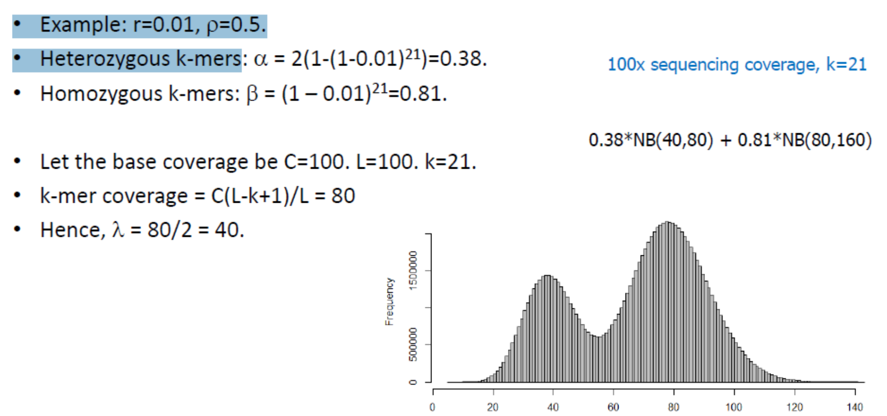
A model of K-mer spectrum for repeat-free diploid genome

$$F(X) = \alpha\ NB(X; \lambda, \lambda / \rho) +$$
$$\beta\ NB(X; 2\lambda, 2\lambda / \rho)$$

X coverage values

λ mean heterozygous K-mer coverage

ρ dispersion parameter

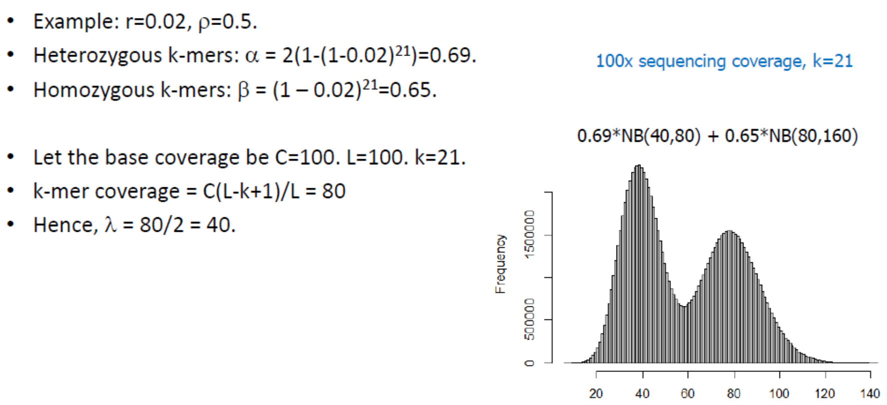


- Example: r=0.01, ρ=0.5.
- Heterozygous k-mers: $\alpha = 2(1-(1-0.01)^{21})=0.38$.
- Homozygous k-mers: $\beta = (1 – 0.01)^{21}=0.81$.

- Let the base coverage be C=100. L=100. k=21.
- k-mer coverage = C(L-k+1)/L = 80
- Hence, λ = 80/2 = 40.

100x sequencing coverage, k=21

0.38*NB(40,80) + 0.81*NB(80,160)



- Example: r=0.02, ρ=0.5.
- Heterozygous k-mers: $\alpha = 2(1-(1-0.02)^{21})=0.69$.
- Homozygous k-mers: $\beta = (1 – 0.02)^{21}=0.65$.

- Let the base coverage be C=100. L=100. k=21.
- k-mer coverage = C(L-k+1)/L = 80
- Hence, λ = 80/2 = 40.

100x sequencing coverage, k=21

0.69*NB(40,80) + 0.65*NB(80,160)

Estimating genome characteristics

Once the model is fitted to the observed K-mer spectrum

Heterozygous rate is obtained as the value of r used in defining α and β

Genome size is obtained by summing total # of K-mers and dividing by 2λ, the estimated mean coverage of homozygous K-mers

Why?

GenomeScope

In general, a genome may have repeats

GenomeScope fits a mixture of four evenly spaced negative binomial distributions to the K-mer spectrum to model the relative abundances of heterozygous, homozygous, and two-copy repeats of various types

GenomeScope only models 2-copy repeats

For non-repeats:

α = proportion of unique heterozygous K-mers

Each K-mer has 1 copy

β = proportion of unique homozygous K-mers

Each K-mer has 2 copies

r = heterozygosity rate

For 2-copy repeats:

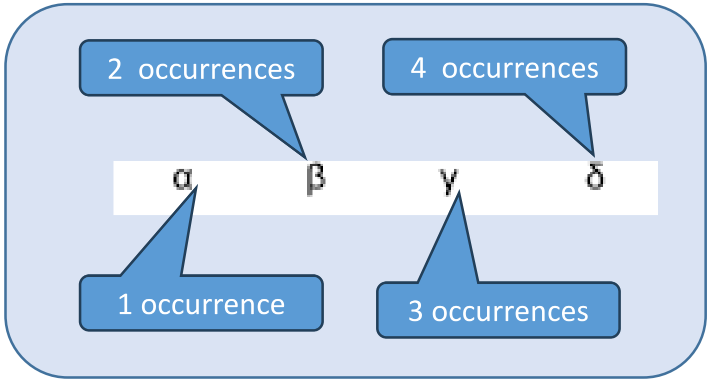
γ = proportion of duplicated heterozygous K-mers

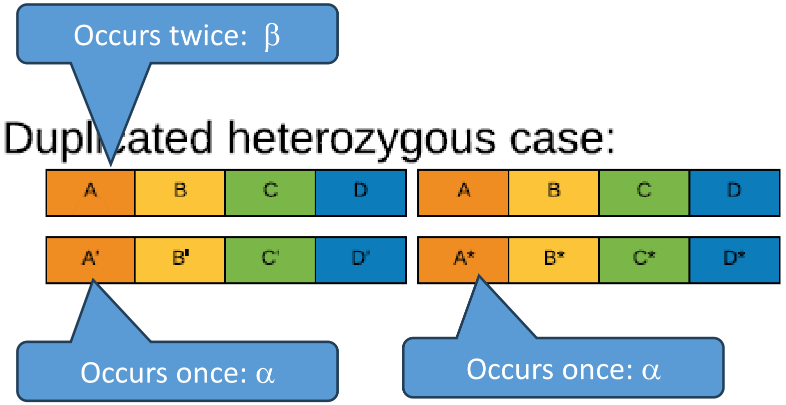
Each K-mer has 3 copies

δ = proportion of duplicated homozygous K-mers

Each K-mer has 4 copies

d = proportion of repeat regions in the genome



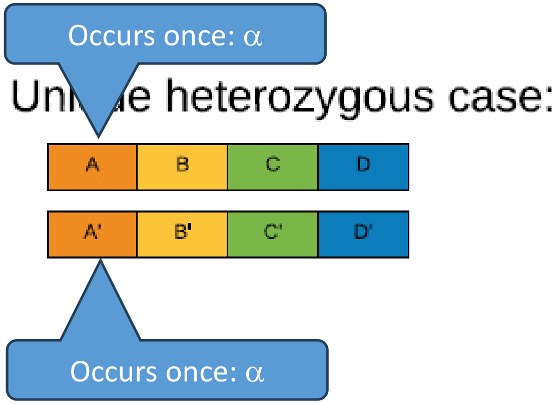


Occurs twice: β

Duplicated heterozygous case:

Occurs once: α

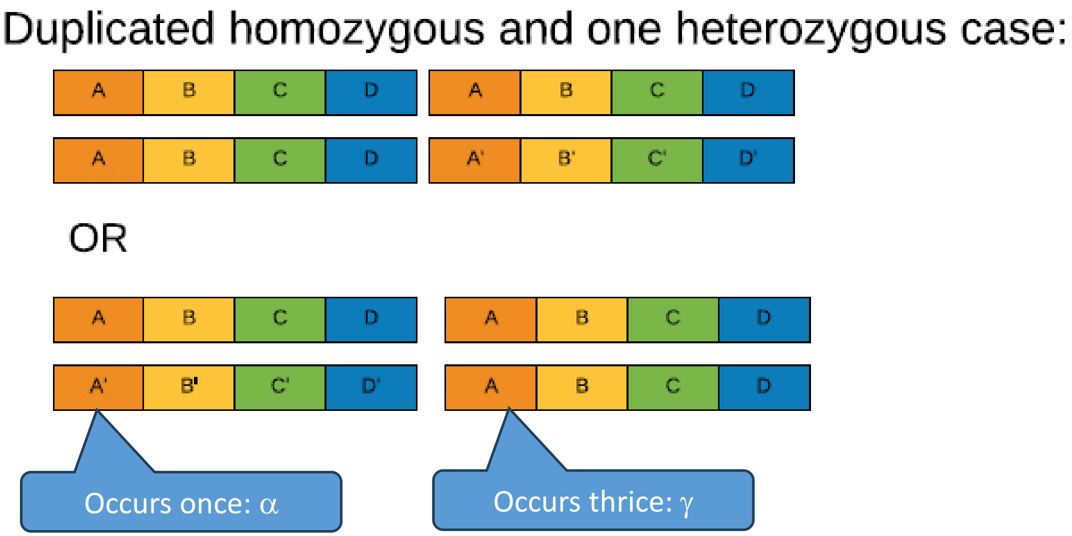
Occurs once: α



Occurs once: α

Unique heterozygous case:

Occurs once: α

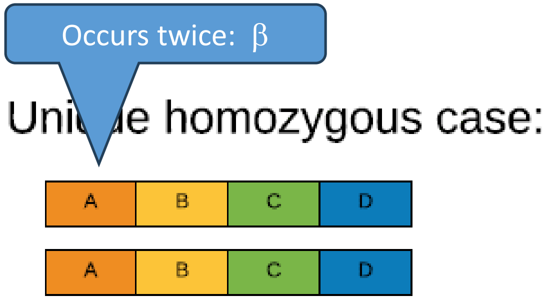


Duplicated homozygous and one heterozygous case:

OR

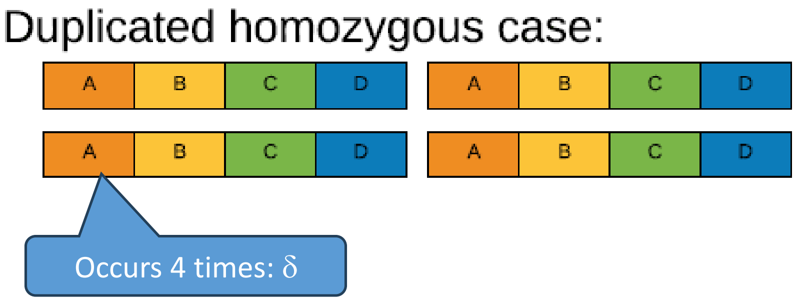
Occurs once: α

Occurs thrice: γ



Occurs twice: β

Unique homozygous case:

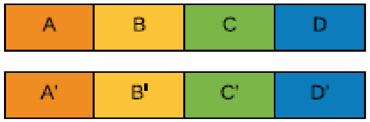


Duplicated homozygous case:

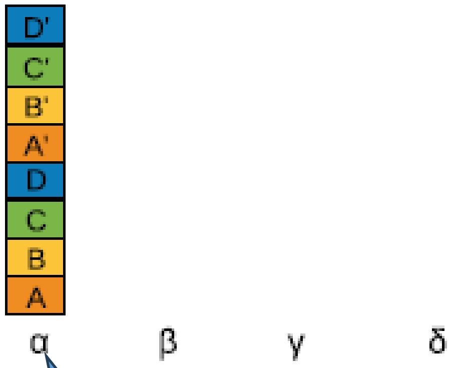
Occurs 4 times: δ

Unique heterozygous K-mers

Unique heterozygous case:



total contribution to α peak: 2(1-d)(1-(1-r)^k)

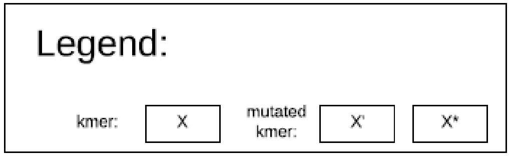


1 occurrence

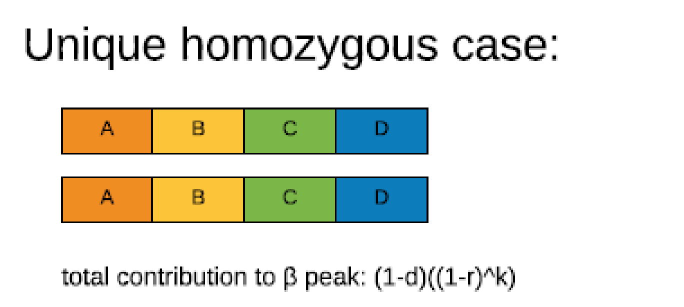
$$\alpha = 2\,(1 - d)\,(1 - (1 - r)^K) + \ldots$$

Non-repeat

Heterozygous

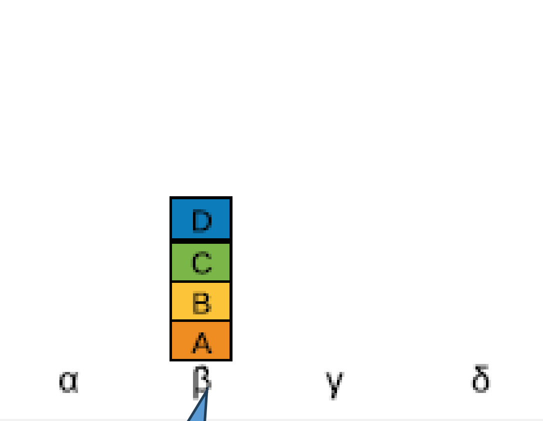


Unique homozygous K-mers



Unique homozygous case:

total contribution to β peak: (1-d)((1-r)^k)

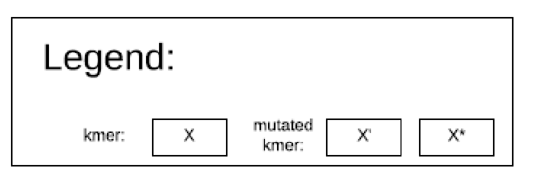


2 occurrences

$$\beta = (1 - d)\ (1 - r)^K + \ldots$$

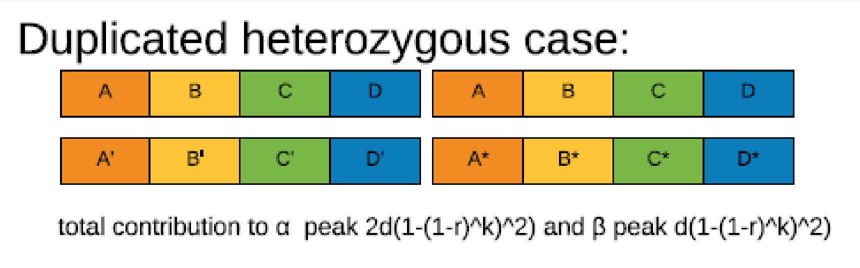
Non-repeat

Homozygous



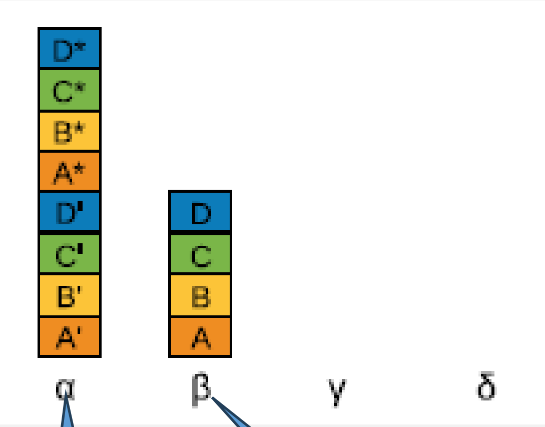
Legend:

Duplicated heterozygous K-mers



Duplicated heterozygous case:

total contribution to α peak 2d(1-(1-r)^k)^2) and β peak d(1-(1-r)^k)^2)



1 occ.

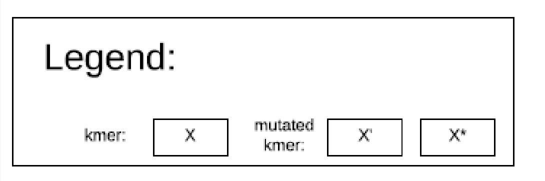
2 occ.

$$\alpha = 2\, d\, (1 - (1 - r)^K)^2 + \ldots$$

$$\beta = d\, (1 - (1 - r)^K)^2 + \ldots$$

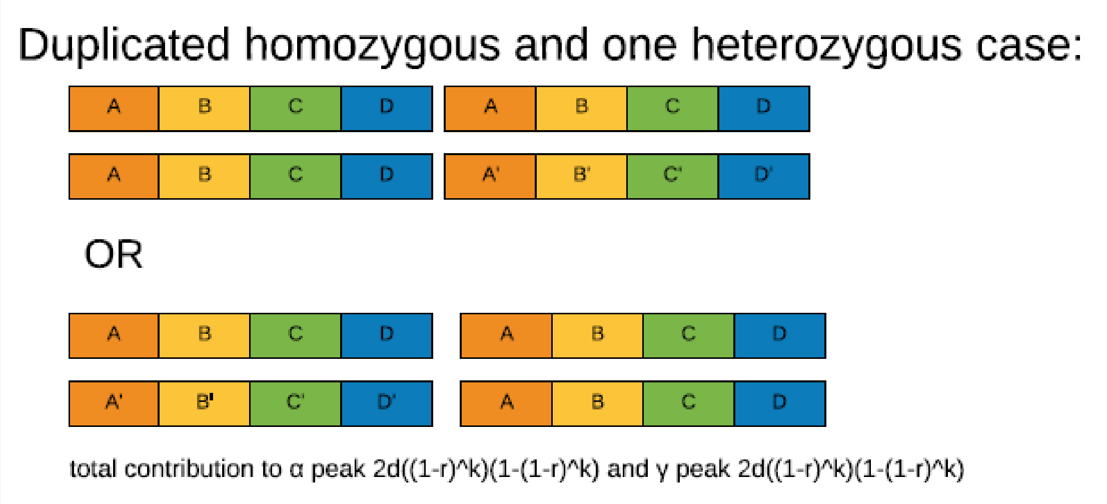
Repeat

Heterozygous



Legend:

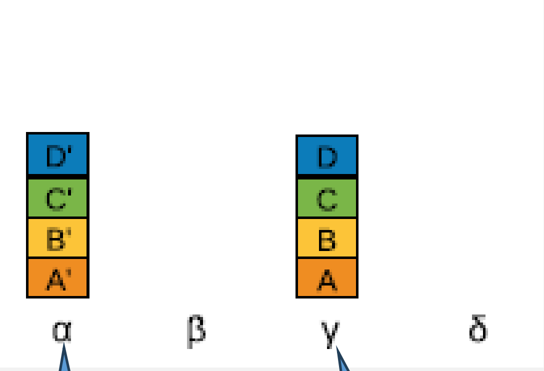
Duplicated mixed homozygous heterozygous K-mers



Duplicated homozygous and one heterozygous case:

OR

total contribution to α peak 2d((1-r)^k)(1-(1-r)^k) and γ peak 2d((1-r)^k)(1-(1-r)^k)



1 occ.

3 occ.

$$\alpha = 2\ d\ (1 - r)^K\ (1 - (1 - r)^K) + \ldots$$

$$\gamma = 2\ d\ (1 - r)^K\ (1 -(1 - r)^K) + \ldots$$

Repeat

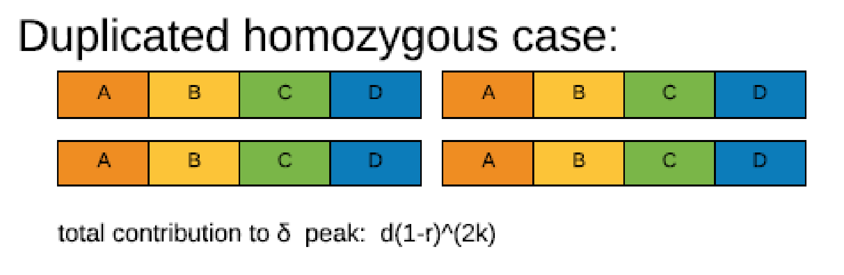
Homozygous

Heterozygous

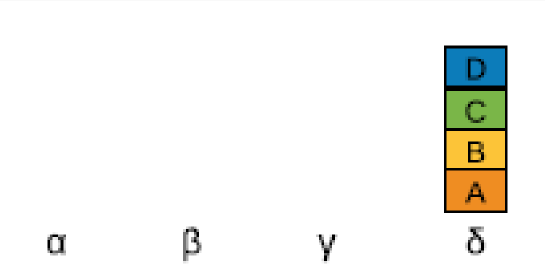
Legend:

kmer:	X	mutated kmer:	X'	X*

Duplicated homozygous K-mers


Duplicated homozygous case:

total contribution to δ peak: d(1-r)^(2k)

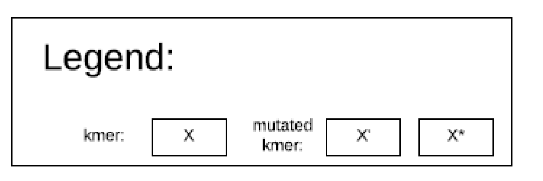


4 occurrences

$$\delta = d\ (1 - r)^{2K} + \ldots$$

Repeat

Homozygous


Legend:

In summary

GenomeScope fits the K-mer spectrum by a mixture of four negative binomials spaced at λ, 2λ, 3λ, and 4λ:

$$F(X) = G * (\alpha\, NB(X; \lambda , \lambda / \rho) + \beta\, NB(X; \lambda , 2\lambda / \rho) + \gamma\, NB(X; \lambda , 3\lambda / \rho) + \delta\, NB(X; \lambda , 4\lambda / \rho))$$

G is scaling parameter corresponding to genome size

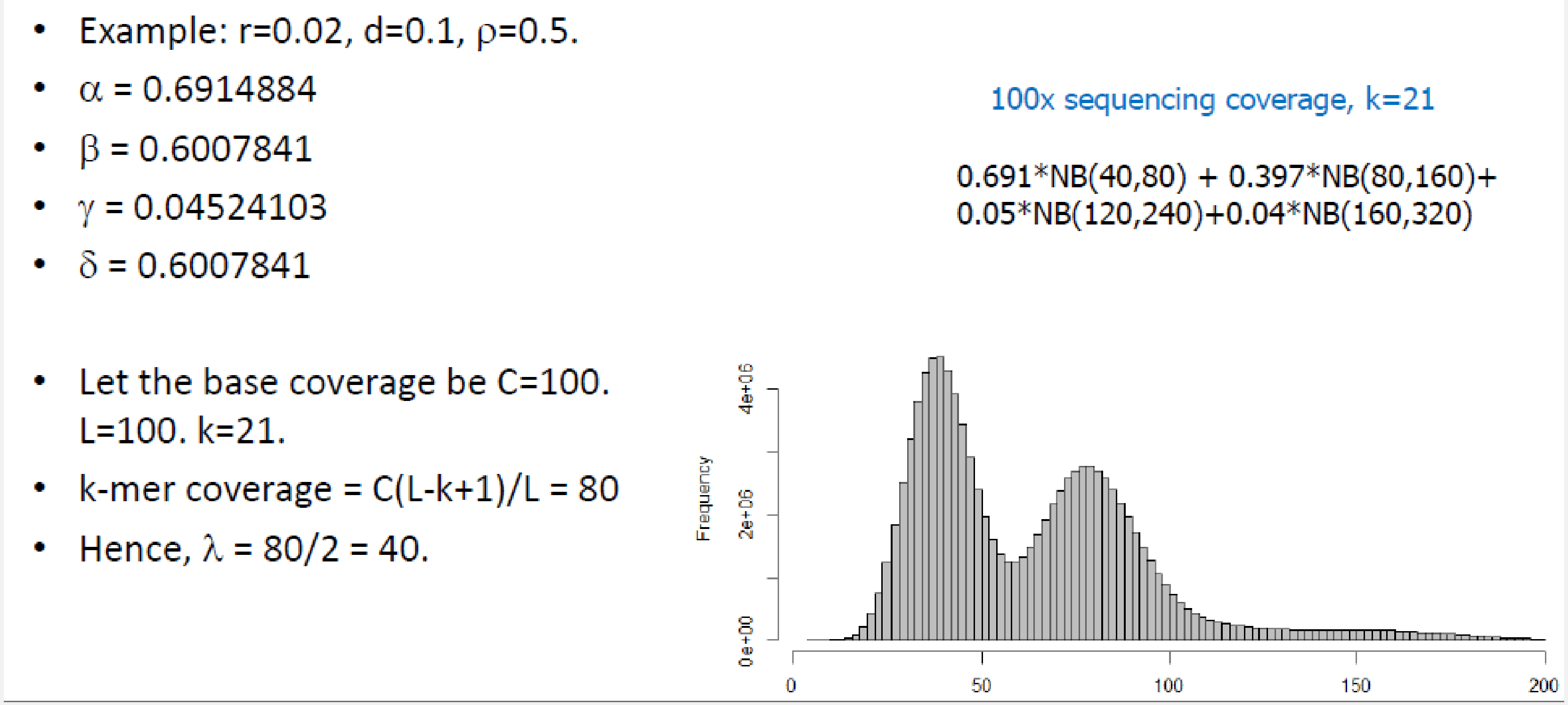
$\alpha = 2(1–d)(1–(1–r)^K) + 2d(1–(1–r)^K)^2 + 2d(1–r)^K(1–(1–r)^K)$

$\beta = (1 – d)(1 – r)^K + d (1 – (1 – r)^K)^2$

$\gamma = 2 d (1 – r)^K (1 – (1 – r)^K)$

$\delta =d (1 – r)^{2K}$

Example



- Example: r=0.02, d=0.1, ρ=0.5.
- α = 0.6914884
- β = 0.6007841
- γ = 0.04524103
- δ = 0.6007841

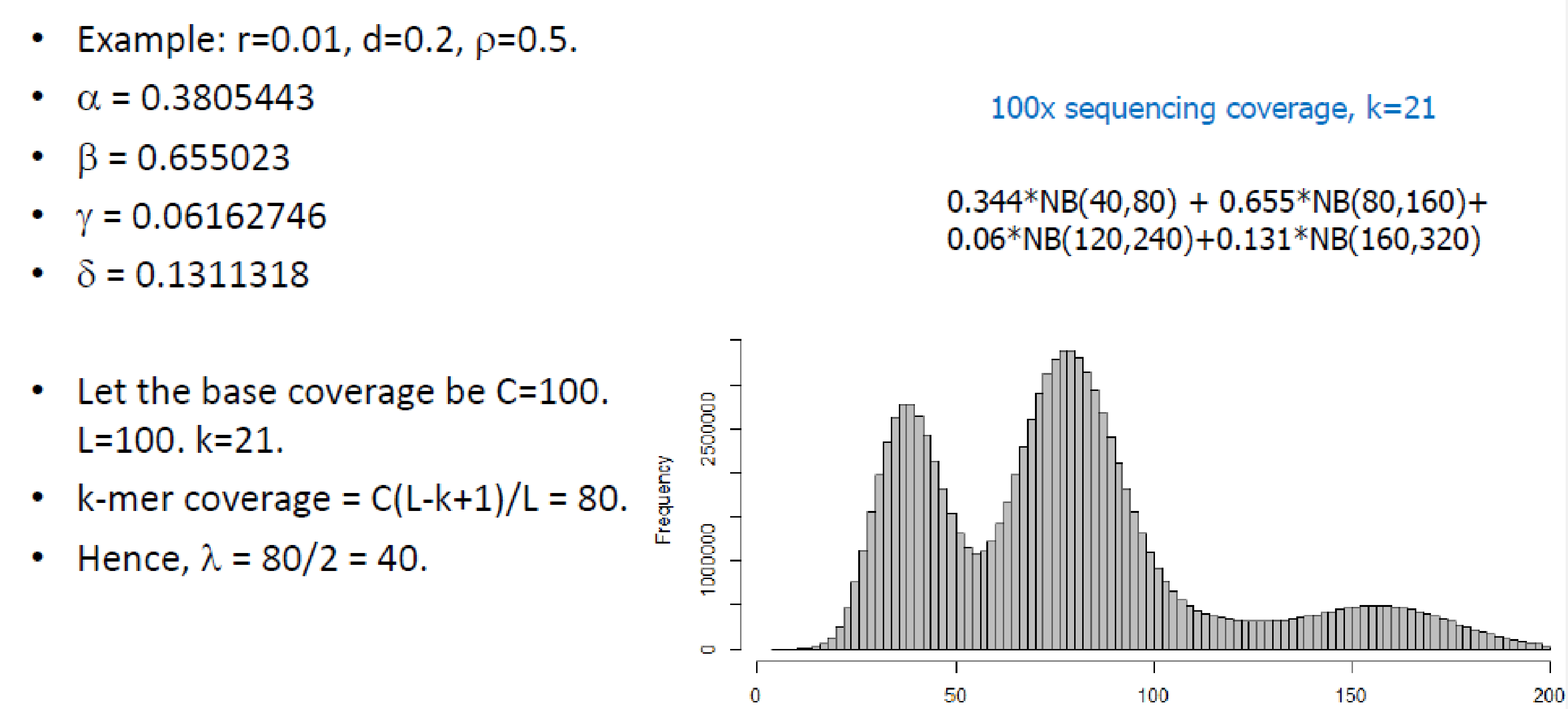
- Let the base coverage be C=100. L=100. k=21.
- k-mer coverage = C(L-k+1)/L = 80
- Hence, λ = 80/2 = 40.

100x sequencing coverage, k=21

0.691*NB(40,80) + 0.397*NB(80,160)+
0.05*NB(120,240)+0.04*NB(160,320)

Frequency
0e+00
2e+06
4e+06
0
50
100
150
200

Example



- Example: r=0.01, d=0.2, ρ=0.5.
- α = 0.3805443
- β = 0.655023
- γ = 0.06162746
- δ = 0.1311318

- Let the base coverage be C=100. L=100. k=21.
- k-mer coverage = C(L-k+1)/L = 80.
- Hence, λ = 80/2 = 40.

100x sequencing coverage, k=21

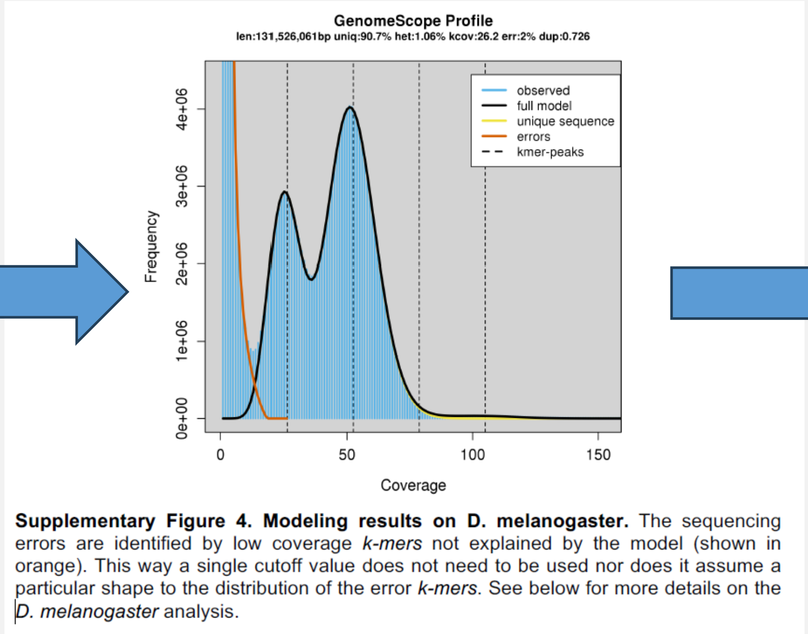
0.344*NB(40,80) + 0.655*NB(80,160)+
0.06*NB(120,240)+0.131*NB(160,320)

How genome characteristics are estimated

Perform K-mer counting to get empirical K-mer spectrum

Estimate d, r, λ, G to fit F(X) to the empirical distribution

Genome size=G.
Percentage of repeat content=r
Heterozygous rate=d
Coverage of haplotype= λ



Supplementary Figure 4. Modeling results on D. melanogaster. The sequencing errors are identified by low coverage *k-mers* not explained by the model (shown in orange). This way a single cutoff value does not need to be used nor does it assume a particular shape to the distribution of the error *k-mers*. See below for more details on the *D. melanogaster* analysis.

Vurture et al., *Bioinformatics* 33(14):2202-2204, 2017

G=131,526,061
(1-d)=90.7%
r=1.06%
λ=26.2

GenomeScope modelling results on *D. melanogaster*

Estimation of parameters

Initial model

$d = 0$, $r = 0$, $\rho = 0.5$, λ = estKmerCov, G = estGenomeSize

estKmerCov is coverage w/ max height in K-mer spectrum, after excluding low-coverage sequencing errors and K-mers with coverage > CovMax

estGenomeSize = # of observed K-mers / estKmerCov

Iterate

Based on previous model, remove low-coverage error K-mers & K-mer with coverage > CovMax

Minimize least square error to optimize d, r, ρ, λ

Set G = # of K-mers excluding errors / 2λ

Limitations of GenomeScope

Require decent sequencing coverage, > 25x

Require low error rate ⇒ cannot support long-read sequencing like ONT

Cannot support polyploid genomes (this is fixed in GenomeScope2.0)

Cannot support genomes having non-uniform copy number of their chromosomes (e.g. leukemia patients)

Must read

The GenomeScope paper, esp. its supplementary material

G. W. Vurture et al, “GenomeScope: Fast reference-free genome profiling from short reads”, *Bioinformatics* 33(14):2202-2204, 2017. https://doi.org/10.1093%2Fbioinformatics%2Fbtx153