Fred Hutch
Cancer Center

CANCER GENOMICS

Lecture 2: Probabilistic Methods for Mutation Detection

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Outline: Probabilistic Methods for Mutation Detection

1. **Primer on statistical modeling (cont'd)**
   - Mixture models, inference and parameter estimation using the EM algorithm
2. **Detecting Mutations in Cancer Genomes**
   - Visualizing somatic vs germline SNVs
   - Sequencing read count data
3. **Mixture Models for SNV Detection**
   - SNV genotyping strategy
   - SNVMix probabilistic model and EM inference
   - Predicting somatic SNVs in cancer

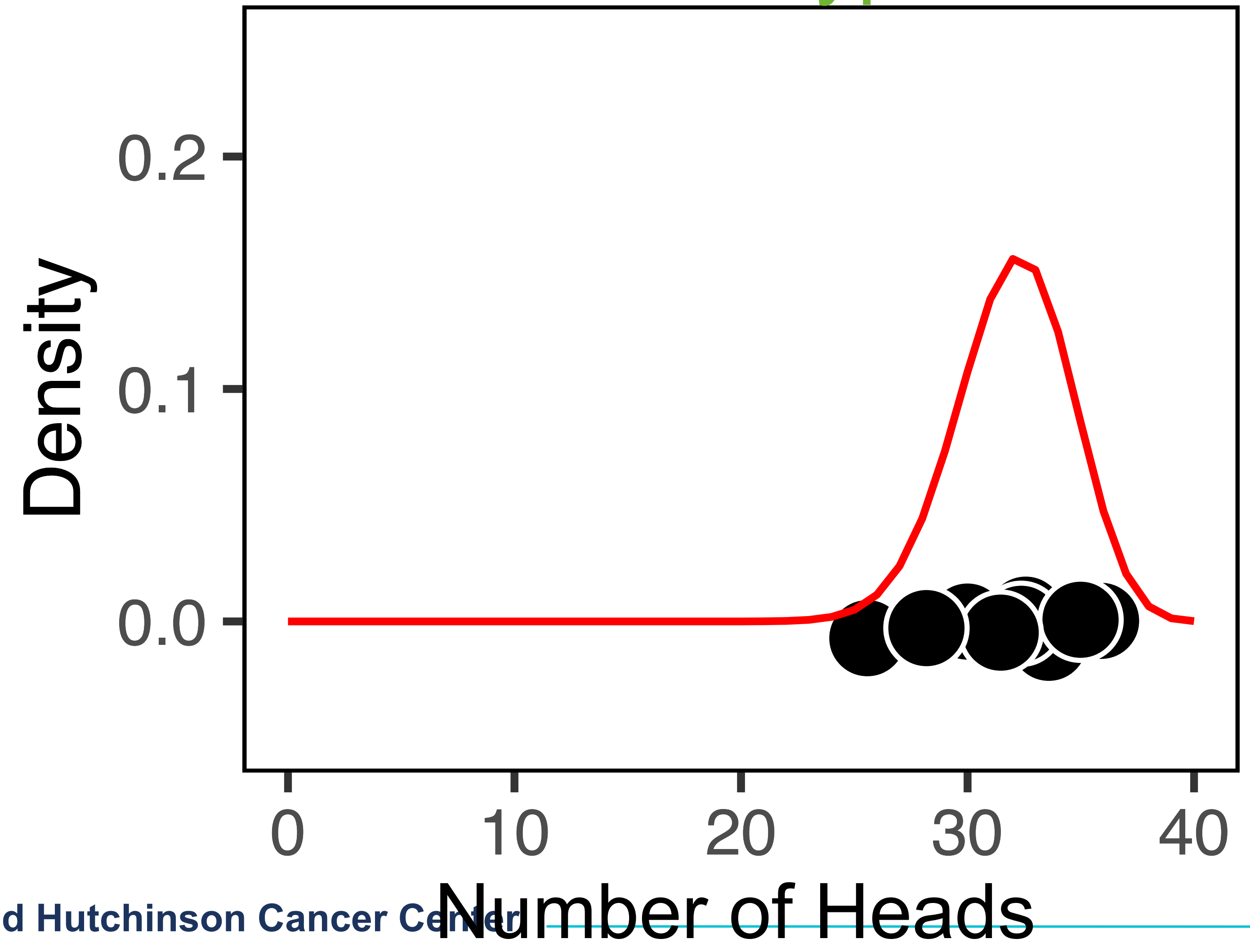
1. Primer on statistical modeling (cont'd)

- Probability
  - Unsupervised learning, probability rules & Bayes' theorem
  - Binomial distribution, Bayesian statistics
  - Beta-binomial model example
- **Mixture models, EM inference & parameter learning**
- References:
  - Murphy, K. (2012). Machine Learning: A Probabilistic Perspective. MIT Press. ISBN: 9780262018029
  - Bishop, C. M. (2006). Pattern Recognition and Machine Learning (Information Science and Statistics). Springer. ISBN: 0387310738

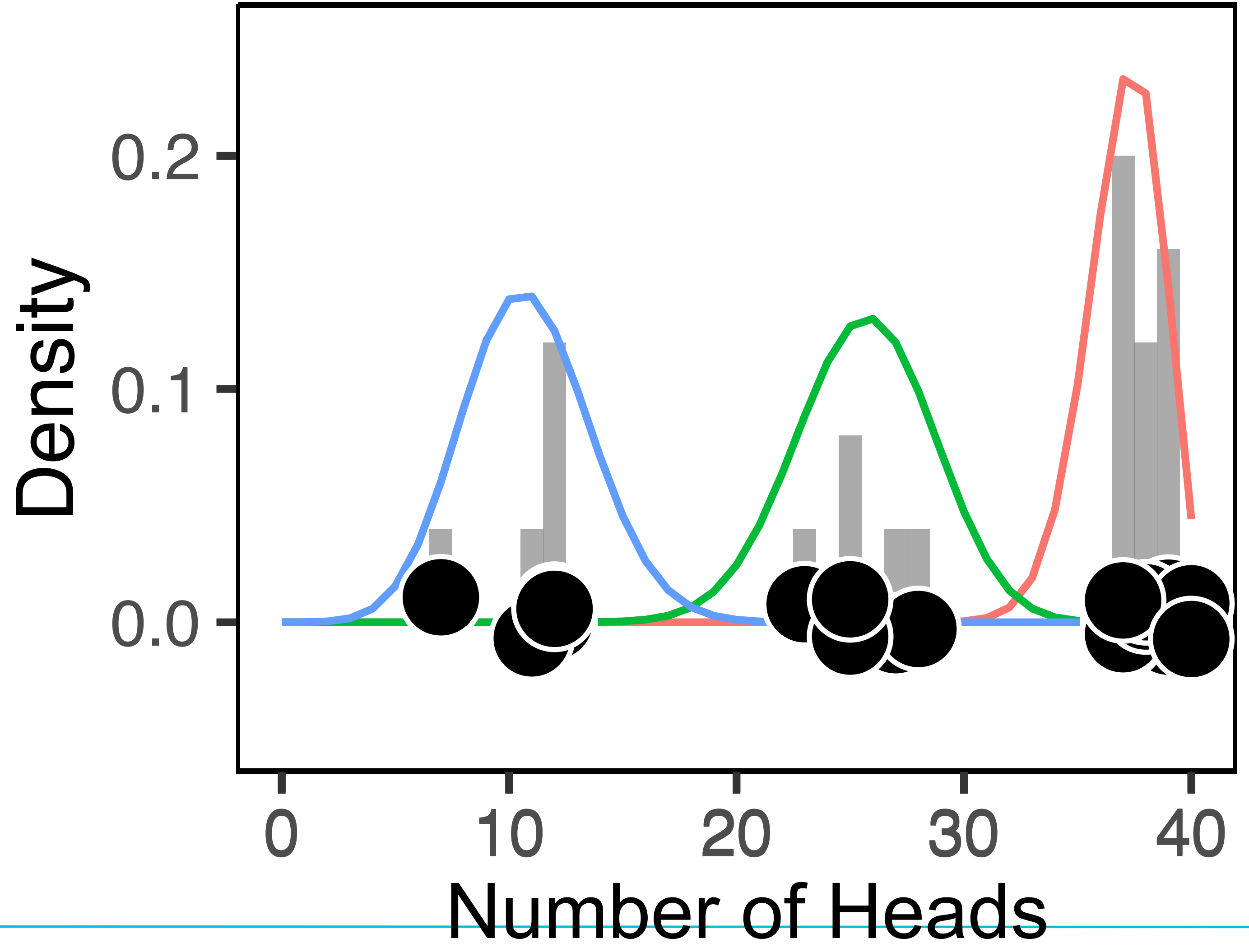
Mixture Model: Referee example with multiple coins

- Recall: There are T different referees who tossed the *same* coin $N = \{1,\ldots,N_T\}$ times and came up with counts of heads $\boldsymbol{x} = \{1,\ldots,x_T\}$.
- Now suppose there are **3 types of coins**: (1) probably fair, (2) unfairly favors heads, (3) unfairly favors tails denoted as $\{fair, heads, tails\}$.
- Each referee **draws one coin** (with replacement) from a hat containing these coin types mixed together.

1 coin type



3 coin types



Mixture Model: Referee example with multiple coins

- Recall: There are T different referees who tossed the *same* coin $N = \{1, \ldots, N_T\}$ times and came up with counts of heads $\boldsymbol{x} = \{1, \ldots, x_T\}$.
- Now suppose there are **3 types of coins**: (1) probably fair, (2) unfairly favors heads, (3) unfairly favors tails denoted as $\{fair, heads, tails\}$.
- Each referee **draws one coin from a hat** that contains a bunch of these coin types mixed together.

1. We don't know the proportion of each coin type in the hat.

2. We don’t know which coin each referee drew from the hat.

3. We don't know the fairness (probability of heads) for each type of coin.

Referee	# of tosses (*N*)	# of heads (*x*)	Prop. of heads	Type of coin used?
Referee 1	40	25	0.63	?
Referee 2	42	35	0.83	?
Referee 3	39	27	0.69	?
Referee 4	x_T	N_T	x_T/N_T	?

Coin Type	Proportion in hat	Prob. of heads
“Fair”	?	?
“Heads”	?	?
“Tails	?	?

Mixture Model: Latent state model

1. What is the proportion of each coin type in the hat?

Find the probability for drawing a coin type.

- π_k is the probability of drawing coin type $k \in \{fair, heads, tails\}$
- $\boldsymbol{\pi} = (\pi_{fair}, \pi_{heads}, \pi_{tails})$ are the *mixture weights* where $\sum_{k=1}^{K} \pi_k = 1$

Coin Type	Proportion in hat	Prob. of heads
“Fair”	π_{fair}	?
“Heads”	π_{heads}	?
“Tails	π_{tails}	?

2. Which coin did each referee draw?

Define the latent variables.

- Let $Z_i = k$ be the type of coin that referee i draws
- Z_i is called a ***latent variable*** and follows a *Categorical* distribution with parameter $\boldsymbol{\pi}$

$$p(Z_i = k \mid \pi_{1:K}) = Cat(Z_i = k \mid \pi_{1:K})$$

$$= \begin{cases} \pi_{fair} & \text{if } k = fair \\ \pi_{heads} & \text{if } k = heads \\ \pi_{tails} & \text{if } k = tails \end{cases}$$

- The proportions $\pi_{1:K}$ of the coin types follows a Dirichlet distribution (conjugate prior)

$$p(\pi_{1:K} \mid \delta_{1:K}) = Dirichlet(\pi_{1:K} \mid \delta_{1:K})$$

Referee	Type of coin used?
Referee 1	Z_1
Referee 2	Z_2
Referee 3	Z_3
Referee T	Z_T

Chapter 9 in Bishop (2006). Pattern Recognition and Machine Learning

Section 11.2 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

Mixture Model: Likelihood as a mixture of binomials

3. What is the fairness (prob. of heads) for each type of coin?

Find the probability of heads for each coin type.

Coin Type	Proportion in hat	Prob. of heads
"Fair"	π_{fair}	μ_{fair}
"Heads"	π_{heads}	μ_{heads}
"Tails"	π_{tails}	μ_{tails}

- Recall: for a single coin, $p(x_i \mid N_i, \mu) = Bin(x_i \mid N_i, \mu)$
- Define the likelihood for a ***3-component mixture of binomials*** with 3 parameters, $\mu_{fair}, \mu_{heads}, \mu_{tails}$, one for each type of coin

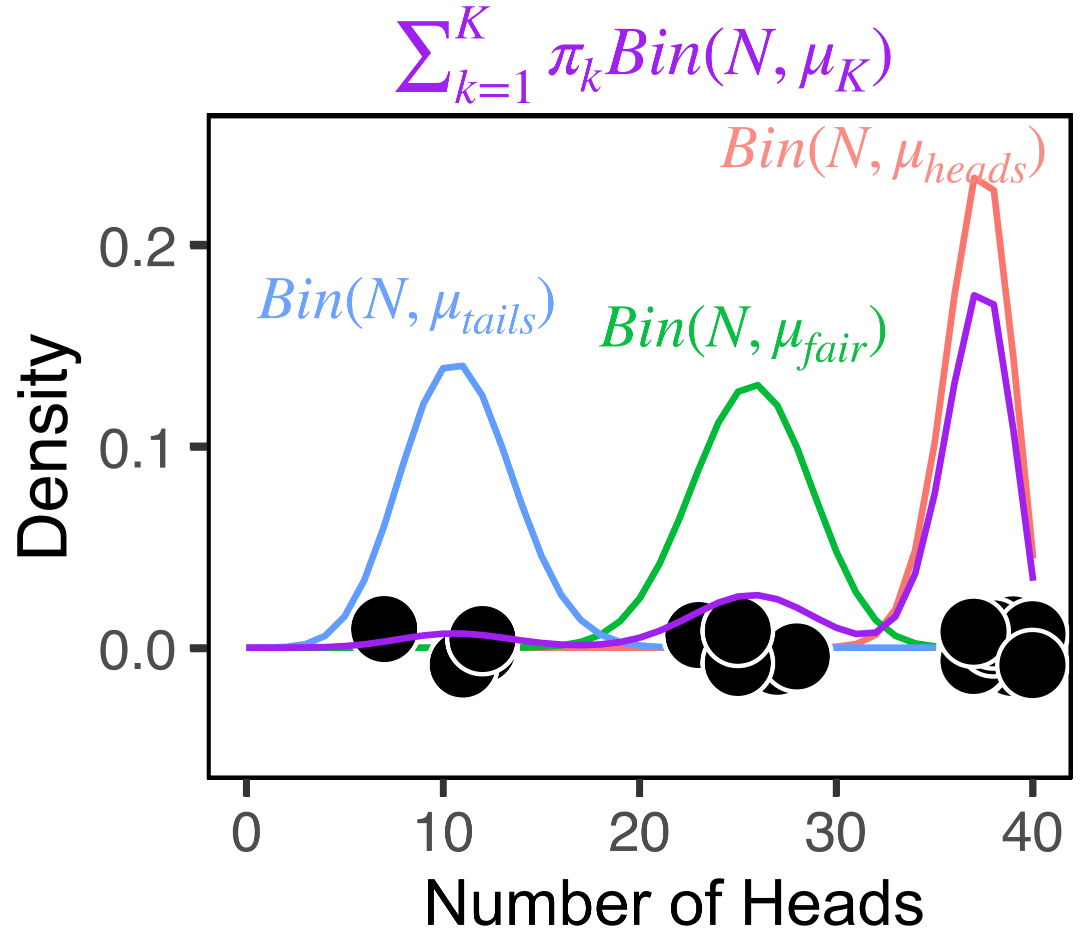
$$p(x_i \mid Z_i = k, N_i, \mu_{1:K}) = Bin(x_i \mid N_i, \mu_k)$$

Observed likelihood

$$p(x_i \mid N_i, \mu_{1:K}, \pi_{1:K}) = \sum_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k)$$

Mixture model

- Beta prior distribution $p(\mu_k \mid \alpha_k, \beta_k) = Beta(\mu_k \mid \alpha_k, \beta_k)$



Log Likelihood Function of the Model

$$L(x_{1:T}, N_{1:T} \mid \mu_{1:K}, \pi_{1:K}) = \prod_{i=1}^{T} \sum_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k)$$

Likelihood function

$$\ell = \sum_{i=1}^{T} \log \left(\sum_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k) \right)$$

Log likelihood

Chapter 9 in Bishop (2006). Pattern Recognition and Machine Learning. Springer

Section 3.3, 3.4, 11.2 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

Mixture Model: Inference & parameter estimation using EM (1)

Expectation-Maximization

Initialize parameters: $\pi_{1:K}$ and $\mu_{1:K}$

E-Step: compute “responsibilities” (inference)

1. Which coin did each referee draw? (Posterior of the latent states $\gamma(Z_{1:T})$)

- **Soft-clustering:** Referee i has a probability for using each of the coins.
- **Responsibilities:** “coin that is responsible for generating observation x_i”

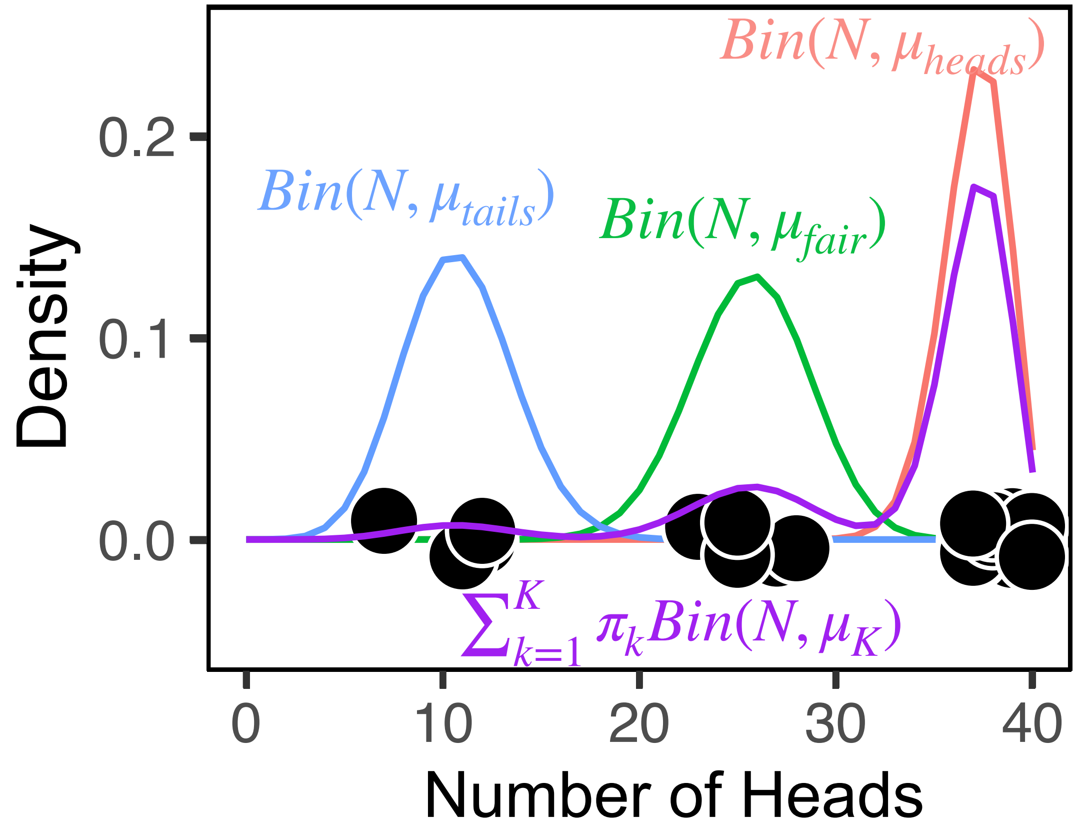
M-Step: Update parameters (learning)

2. What is the proportion of each coin type in the hat? $\pi_{1:K}$
3. What is the fairness (prob. of heads) for each coin type? $\mu_{1:K}$

Iterate between **E-Step** and **M-Step**,

- check when log-posterior stops increasing.

	Responsibilities		
Referee	Fair Coin	Heads Coin	Tails Coin
1	$\gamma(Z_1 = F)$	$\gamma(Z_1 = H)$	$\gamma(Z_1 = T)$
2	$\gamma(Z_2 = F)$	$\gamma(Z_2 = H)$	$\gamma(Z_2 = T)$
3	$\gamma(Z_3 = F)$	$\gamma(Z_3 = H)$	$\gamma(Z_3 = T)$
T	$\gamma(Z_T = F)$	$\gamma(Z_T = H)$	$\gamma(Z_T = T)$



Chapter 9 in Bishop (2006). Pattern Recognition and Machine Learning. Springer

Section 3.3, 3.4, 11.2 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

Mixture Model: Inference & parameter estimation using EM (2)

E-Step: compute responsibilities (inference)

1. What is the probability for a referee to draw each coin type? (Posterior of the latent states $Z_{1:T}$)

- Find the responsibilities given the current parameters

$$
\begin{aligned}
p(Z_i = k \,|\, x_i, N_i, \pi_{1:K}, \mu_{1:K}) &= \frac{p(x_i \,|\, Z_i = k) p(Z_i = k)}{p(x_i)} \\
&= \frac{Bin(x_i \,|\, N_i, \mu_k)\pi_k}{\sum_{k'=1}^{K} Bin(x_i \,|\, N_i, \mu_k{}')\pi_k{}'} \\
&= \gamma(Z_i = k)
\end{aligned}
$$

Bayes' Rule
Posterior distribution of the latent variables

Responsibilities
Matrix $T \times K$

Responsibilities = "coin that is responsible for generating observation x_i"

- Soft-clustering: Referee i has a probability for using each of the coins.
- $\gamma(Z_{1:T})$ is a matrix of probabilities with dimensions $T \times K$

Mixture Model: Inference & parameter estimation using EM (3)

M-Step: Update parameters (learning)

2. What is the proportion of each coin type in the hat?

$$\hat{\pi}_k = \frac{\sum_{i=1}^{T} \gamma(Z_i = k) + \delta(k) - 1}{\sum_{j=1}^{K} \sum_{i=1}^{T} \left\{ \gamma(Z_i = j) + \delta(j) - 1 \right\}}$$

MAP for π

3. What is the fairness (prob. of heads) for each coin type?

$$\hat{\mu}_k = \frac{\sum_{i=1}^{T} \gamma(Z_i = k) x_i + \alpha_k - 1}{\sum_{i=1}^{T} \gamma(Z_i = k) N_i + \alpha_k + \beta_k - 2}$$

MAP for μ

Evaluate the log likelihood and log posterior: use updated parameters

$$\log \mathbb{P} = \underbrace{\sum_{i=1}^{T} \log \left(\sum_{k=1}^{K} \hat{\pi}_k Bin(x_i \mid N_i, \hat{\mu}_k) \right)}_{\text{Log likelihood}} + \underbrace{\log Dir(\hat{\boldsymbol{\pi}} \mid \boldsymbol{\delta}) + \sum_{k=1}^{K} \log Beta(\hat{\mu}_k \mid \alpha_k, \beta_k)}_{\text{Log priors}}$$

Log posterior

Iterate between E-Step and M-Step:

- Stop EM when new $\log \mathbb{P}$ changes less than ϵ compared to previous EM iteration.

Chapter 9 in Bishop (2006). Pattern Recognition and Machine Learning. Springer

Section 3.3, 3.4, 11.2 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

Recall: MAP estimate

Beta-Binomial Model: Posterior distribution

$$p(\mu \mid x_i) \propto Bin(x_i \mid N_i, \mu) \times Beta(\mu \mid \alpha, \beta) = Beta(\mu \mid \overset{\alpha'}{x_i + \alpha}, \overset{\beta'}{N_i - x_i + \beta})$$

Posterior

- Then, what is the probability of heads, μ, of this coin given the **evidence** and the **prior**?

Maximum a posteriori (MAP) estimate

- From the posterior, we can estimate the parameter using the ***maximum a posteriori (MAP)***, $\hat{\mu}_{MAP}$
- MAP refers to the mode of the posterior distribution and the mode of a Beta is $\dfrac{\alpha - 1}{\alpha + \beta - 2}$
- Since the posterior has the form of a Beta distribution, then the MAP is $\dfrac{\alpha' - 1}{\alpha' + \beta' - 2}$

$$\alpha' = x_i + \alpha$$

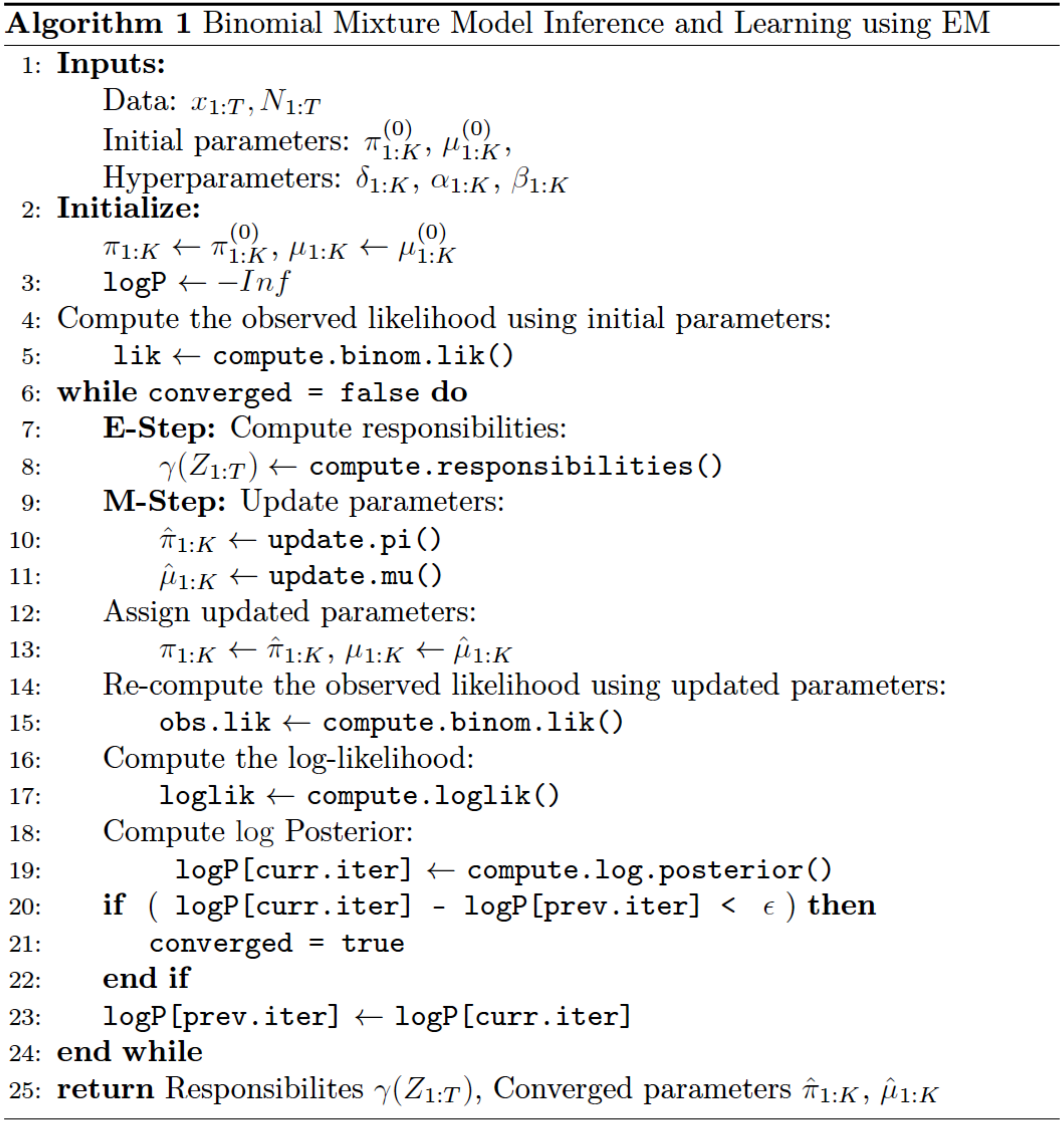
$$\beta' = (N_i - x_i) + \beta$$

$$\hat{\mu}_{MAP} = \frac{x_i + \alpha - 1}{N_i + \alpha + \beta - 2}$$

MAP

1. Take log of the posterior
2. Take the derivative wrt μ
3. Equate to 0
4. Solve for μ

Section 3.3 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press



Algorithm 1 Binomial Mixture Model Inference and Learning using EM

```
Inputs:
    Data: $x_{1:T}, N_{1:T}$
    Initial parameters: $\pi^{(0)}_{1:K}$, $\mu^{(0)}_{1:K}$,
    Hyperparameters: $\delta_{1:K}$, $\alpha_{1:K}$, $\beta_{1:K}$
Initialize:
    $\pi_{1:K} \leftarrow \pi^{(0)}_{1:K}$, $\mu_{1:K} \leftarrow \mu^{(0)}_{1:K}$
    logP $\leftarrow -Inf$
Compute the observed likelihood using initial parameters:
     lik $\leftarrow$ compute.binom.lik()
while converged = false do
    E-Step: Compute responsibilities:
        $\gamma(Z_{1:T}) \leftarrow$ compute.responsibilities()
    M-Step: Update parameters:
        $\hat{\pi}_{1:K} \leftarrow$ update.pi()
        $\hat{\mu}_{1:K} \leftarrow$ update.mu()
    Assign updated parameters:
        $\pi_{1:K} \leftarrow \hat{\pi}_{1:K}$, $\mu_{1:K} \leftarrow \hat{\mu}_{1:K}$
    Re-compute the observed likelihood using updated parameters:
        obs.lik $\leftarrow$ compute.binom.lik()
    Compute the log-likelihood:
        loglik $\leftarrow$ compute.loglik()
    Compute log Posterior:
         logP[curr.iter] $\leftarrow$ compute.log.posterior()
    if ( logP[curr.iter] - logP[prev.iter] < $\epsilon$ ) then
        converged = true
    end if
    logP[prev.iter] $\leftarrow$ logP[curr.iter]
end while
return Responsibilites $\gamma(Z_{1:T})$, Converged parameters $\hat{\pi}_{1:K}$, $\hat{\mu}_{1:K}$
```

Mixture Model: Inference & parameter estimation using EM (extra slide 1)

Incomplete data log likelihood

$$L(x_{1:T}, N_{1:T} \mid \mu_{1:K}, \pi_{1:K}) = \prod_{i=1}^{T} \sum_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k)$$

- The incomplete data log likelihood (plus the priors) is used to monitor EM convergence

Expected complete data log likelihood

Complete data likelihood

$$L(\mu_{1:K}, \pi_{1:K} \mid x_{1:T}, Z_{1:T}, N_{1:T}) = \prod_{i=1}^{T} \prod_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k)^{\mathbb{I}(Z_i = k)}$$

Complete data log likelihood

$$\ell(\mu_{1:K}, \pi_{1:K} \mid x_{1:T}, Z_{1:T}, N_{1:T}) = \sum_{i=1}^{T} \sum_{k=1}^{K} \mathbb{I}(Z_i = k) \left\{ \log \pi_k + \log Bin(x_i \mid N_i, \mu_k) \right\}$$

Expected complete data log likelihood »

$$Q = \mathbb{E}\left[\ell(\mu_{1:K}, \pi_{1:K} \mid x_{1:T}, Z_{1:T}, N_{1:T}) \right] = \sum_{i=1}^{T} \sum_{k=1}^{K} \mathbb{E}\left[\mathbb{I}(Z_i = k) \right] \left\{ \log \pi_k + \log Bin(x_i \mid N_i, \mu_k) \right\}$$

$$= \sum_{i=1}^{T} \sum_{k=1}^{K} \gamma(Z_i = k) \left\{ \log \pi_k + \log Bin(x_i \mid N_i, \mu_k) \right\}$$

- The **expected complete data log likelihood** in the **M-Step** is used when updating parameters.

Additional definitions for your reference

Mixture Model: Inference & parameter estimation using EM (extra slide 2)

M-Step: Update the parameters given the responsibilities

$$p(\pi_{1:K}, \mu_{1:K}) = Dir(\boldsymbol{\pi} \,|\, \boldsymbol{\delta}) \prod_{k=1}^{K} Beta(\mu_k \,|\, \alpha, \beta)$$ **Priors**

$$\mathcal{O} = Q + \log p(\pi_{1:K}, \mu_{1:K})$$ **Complete data log likelihood + log priors**

- The object function $\mathcal{O}$ is used to obtain the update equations for $\pi_{1:K}$ and $\mu_{1:K}$

$$\frac{\partial \mathcal{O}}{\partial \mu_k} = 0, \text{ find } \hat{\mu}_k \text{ and } \frac{\partial \mathcal{O}}{\partial \pi_k} = 0, \text{ find } \hat{\pi}_k$$

EM Convergence: after each iteration, monitor the log posterior

$$\ell = \sum_{i=1}^{T} \log \left(\sum_{k=1}^{K} \pi_k Bin(x_i \,|\, \mu_k, N_i) \right)$$ **Incomplete Data Log likelihood**

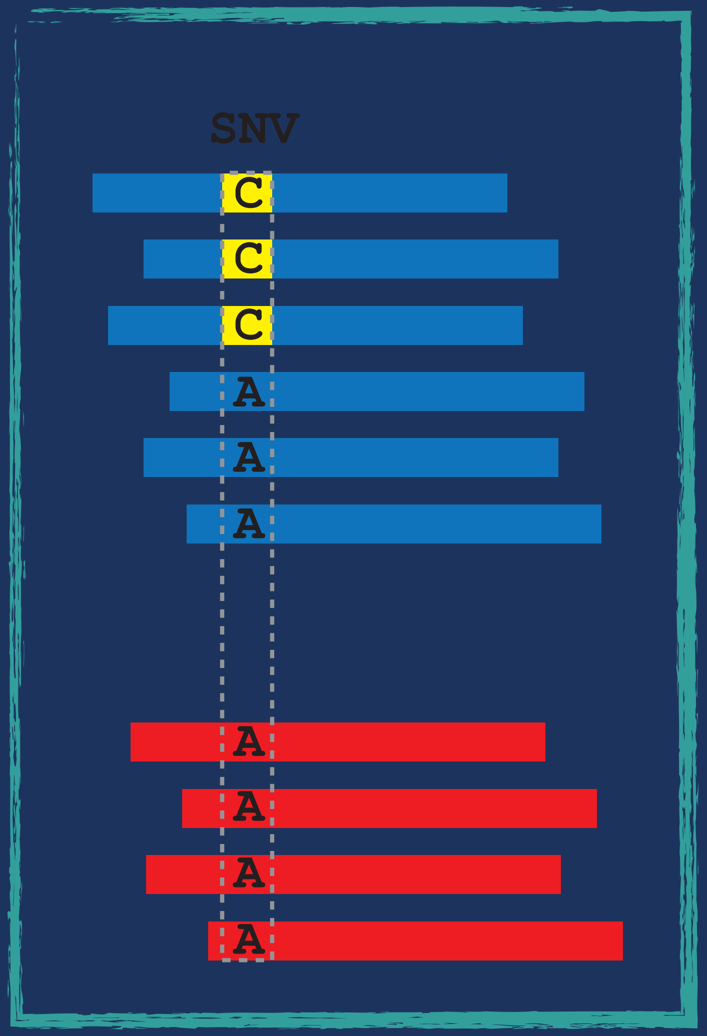
$$\log \mathbb{P}(\pi_{1:K}, \mu_{1:K} \,|\, x_{1:T}) = \ell + \log p(\pi_{1:K}, \mu_{1:K})$$ **Log posterior**

- If the log posterior, $\log \mathbb{P}(\pi_{1:K}, \mu_{1:K} \,|\, x_{1:T})$, stops increasing by ϵ, then EM is converged.
- If not using a Bayesian framework, then use the log likelihood, ℓ, to monitor convergence.

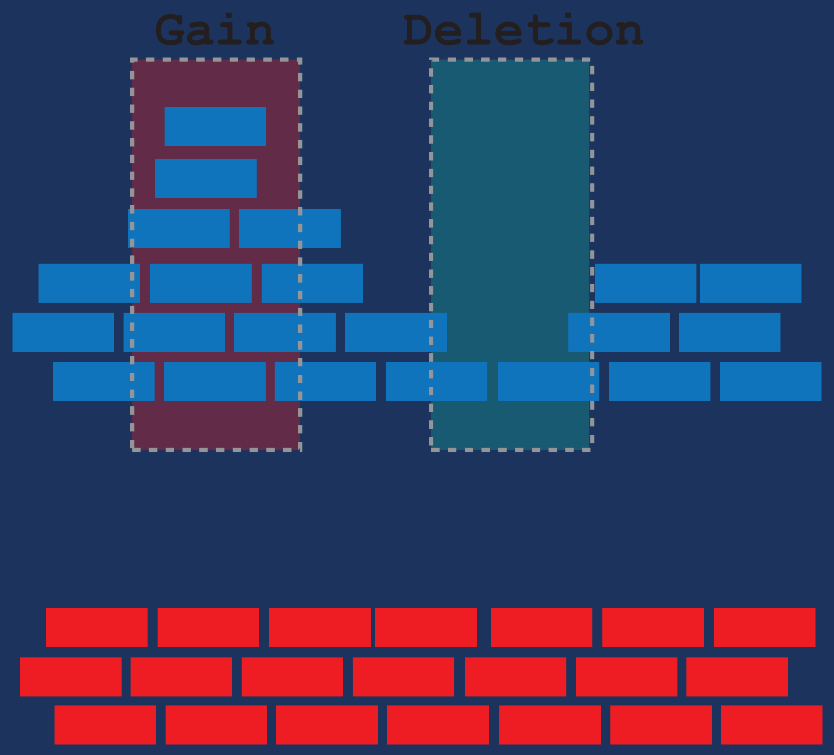
Additional definitions for your reference

2. Detecting Mutations in Cancer Genomes

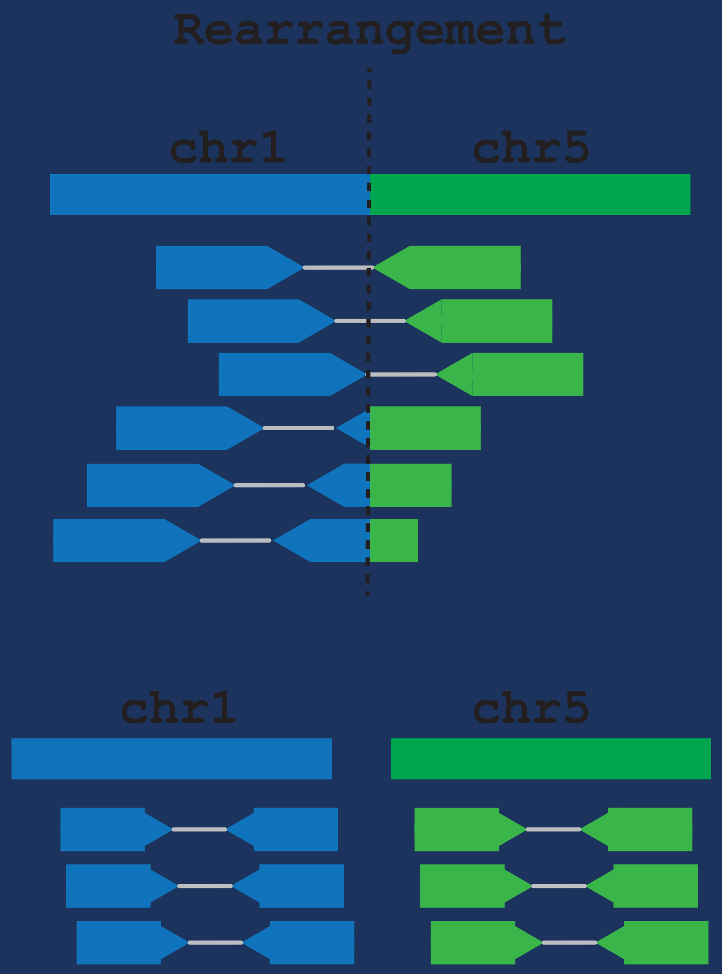
Mutations (SNV, INDEL)



Copy Number Alterations



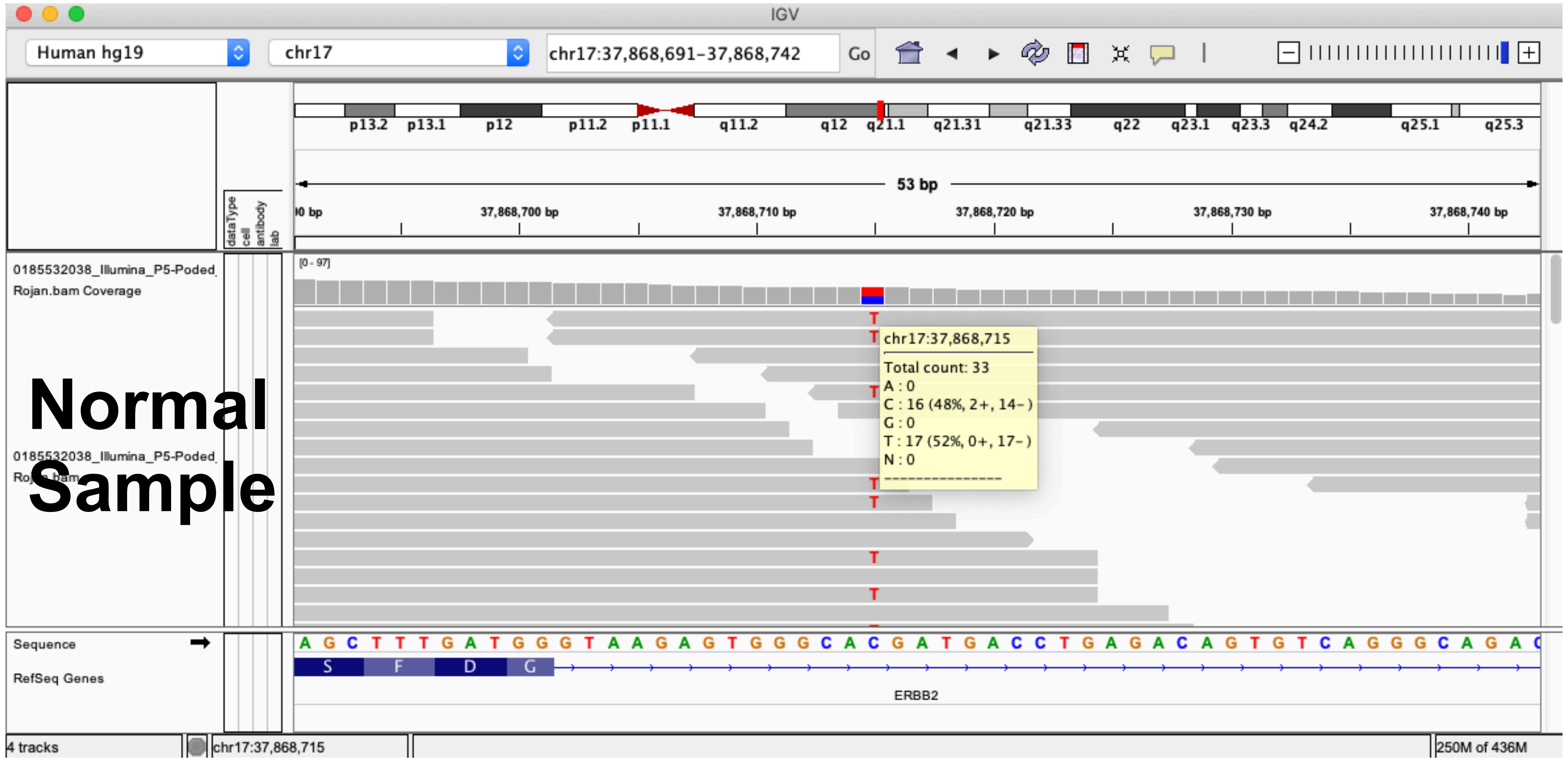
Structural Variants



Visual inspection using IGV: Germline SNVs

Integrative Genomics Viewer (https://software.broadinstitute.org/software/igv)

- ~1.5 to 2 million **SNPs** per individual
- Identify SNPs from normal peripheral blood mononuclear cells (PBMC)



```
chr17:37,868,715
________________
Total count: 33
A : 0
C : 16 (48%, 2+, 14- )
G : 0
T : 17 (52%, 0+, 17- )
N : 0
---------------
```

Normal Sample

Heterozygous SNP with 17 reads containing the variant and having depth 33 reads

17/33 (48%) variant allele fraction (VAF)

Visual inspection using IGV: Germline SNVs

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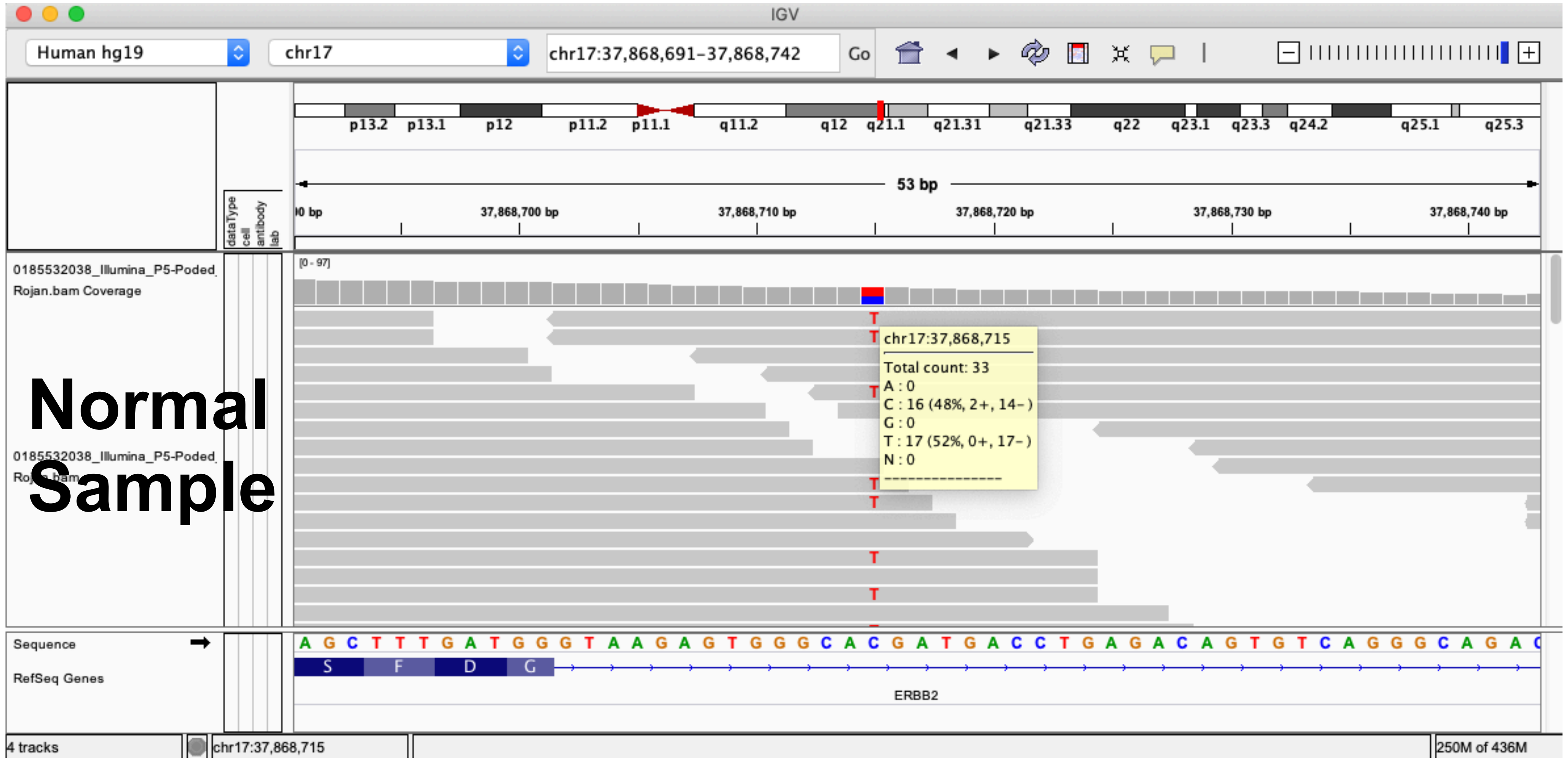

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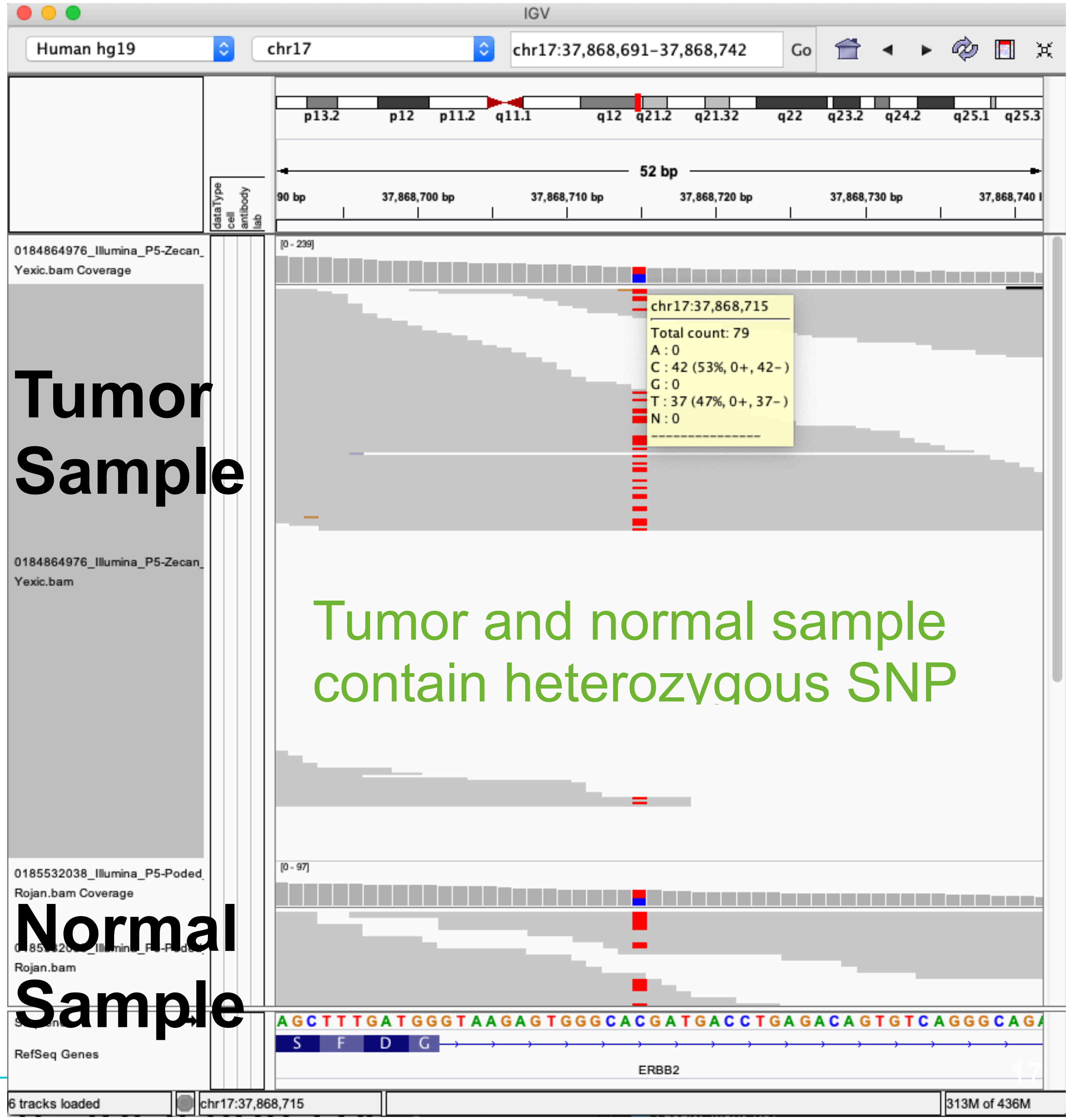

IGV
Human hg19
chr17
chr17:37,868,691-37,868,742
Go
p13.2
p12
p11.2
q11.1
q12
q21.2
q21.32
q22
q23.2
q24.2
q25.1
q25.3
52 bp
90 bp
37,868,700 bp
37,868,710 bp
37,868,720 bp
37,868,730 bp
37,868,740 bp
dataType
cell
antibody
lab
[0 - 239]
0184864976_Illumina_P5-Zecan_Yexic.bam Coverage
0184864976_Illumina_P5-Zecan_Yexic.bam
[0 - 97]
0185532038_Illumina_P5-Poded_Rojan.bam Coverage
0185532038_Illumina_P5-Poded_Rojan.bam
RefSeq Genes
A G C T T T G A T G G G T A A G A G T G G G C A C G A T G A C C T G A G A C A G T G T C A G G G C A G A
S
F
D
G
ERBB2
6 tracks loaded
chr17:37,868,715
313M of 436M

Tumor Sample

chr17:37,868,715

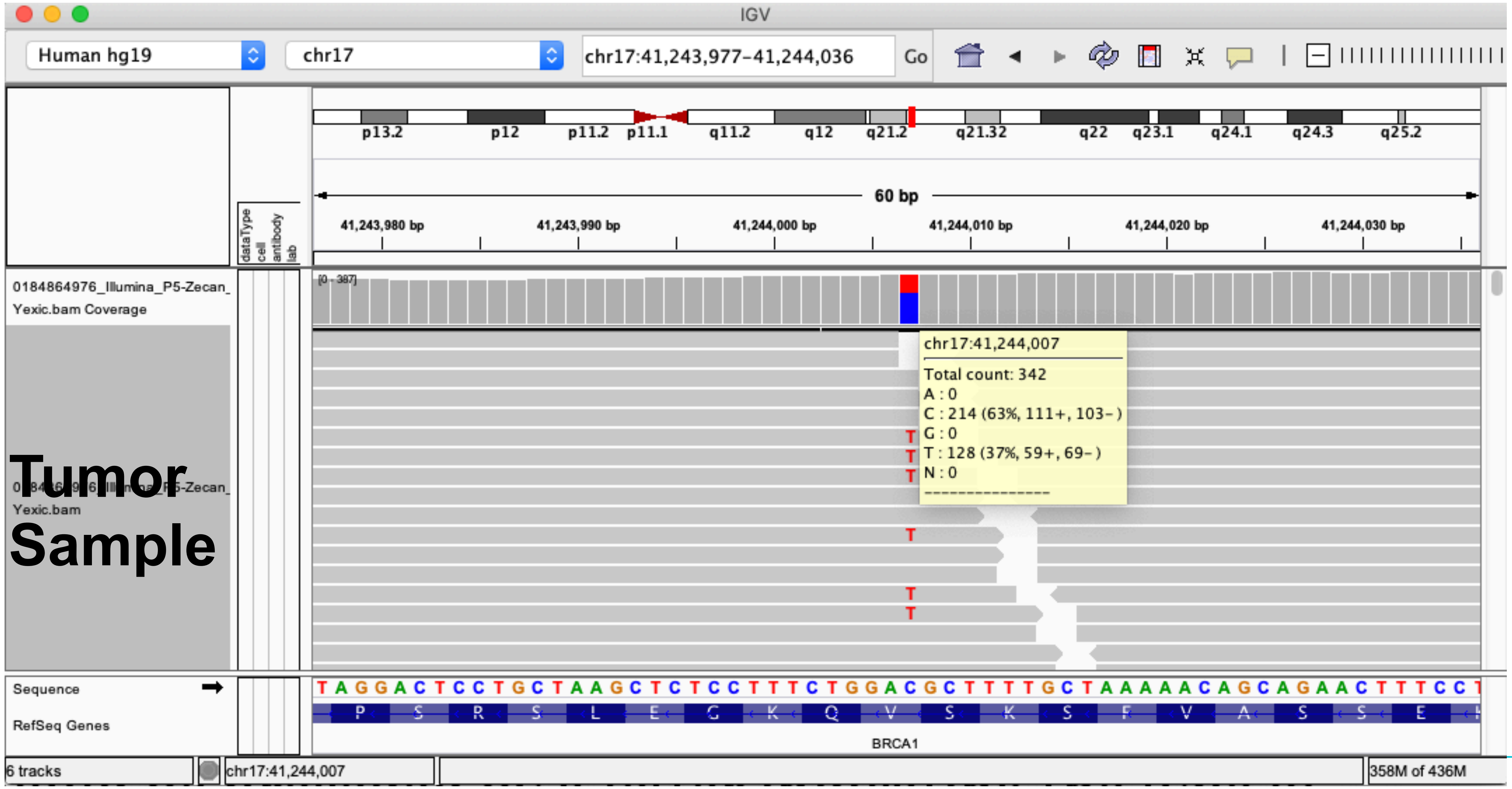
Total count: 79
A : 0
C : 42 (53%, 0+, 42-)
G : 0
T : 37 (47%, 0+, 37-)
N : 0

Tumor and normal sample contain heterozygous SNP

Normal Sample

Visual inspection using IGV: Somatic SNVs

- Somatic **SNV** requires comparing case (tumor) with control (PBMC)
- On the order of 10 to 10^4 number of mutations



IGV

Human hg19 | chr17 | chr17:41,243,977-41,244,036 | Go

```
chr17:41,244,007
Total count: 342
A : 0
C : 214 (63%, 111+, 103- )
G : 0
T : 128 (37%, 59+, 69- )
N : 0
---------------
```

0184864976_Illumina_P5-Zecan_Yexic.bam Coverage

0184864976_Illumina_P5-Zecan_Yexic.bam

Tumor Sample

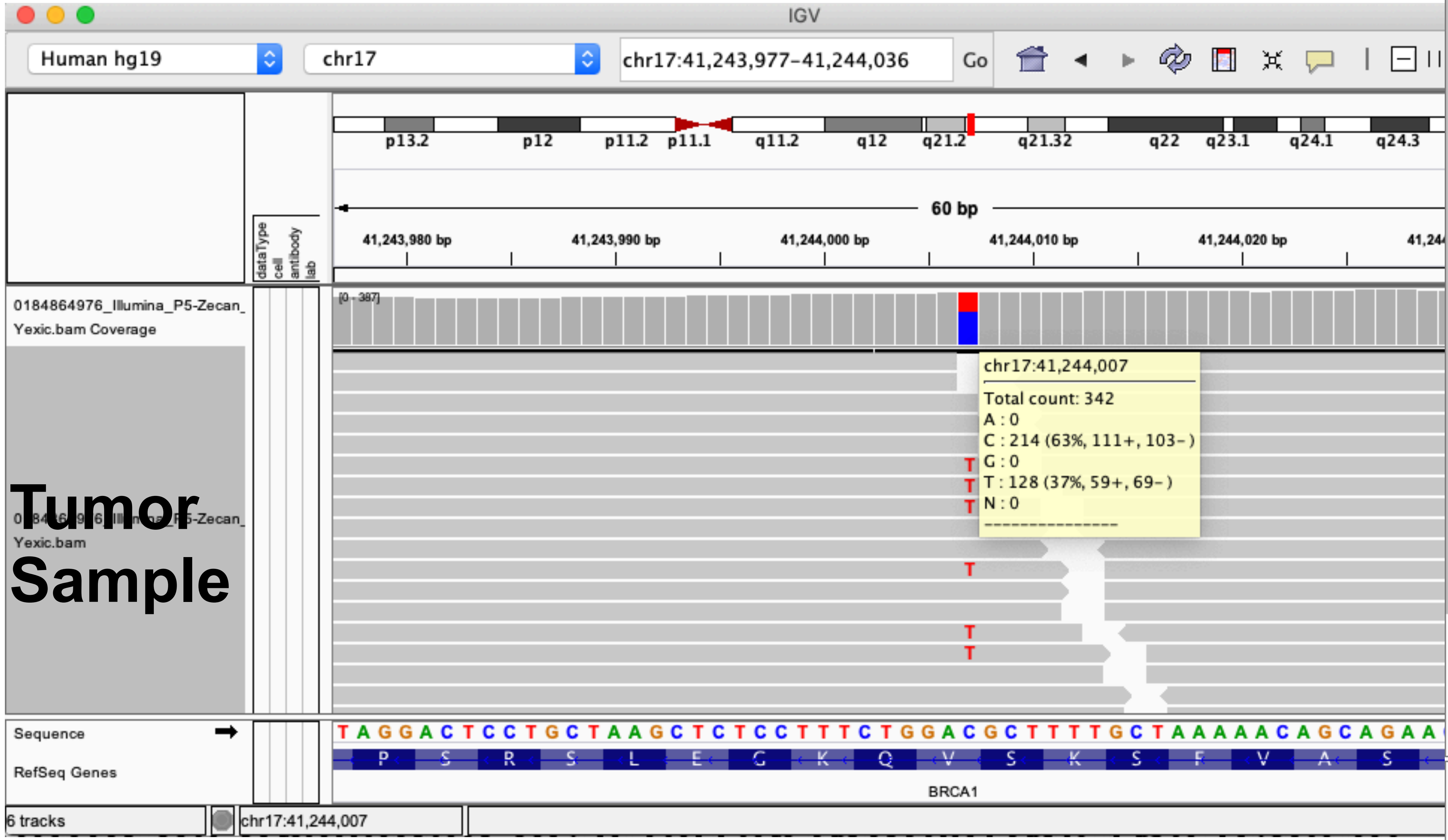
6 tracks | chr17:41,244,007 | 358M of 436M

Potential SNV with 128/342 (37%) VAF

p.V1181I

Visual inspection using IGV: Somatic SNVs

- Somatic **SNV** requires comparing case (tumor) with control (PBMC)
- On the order of 10 to 10^4 number of mutations

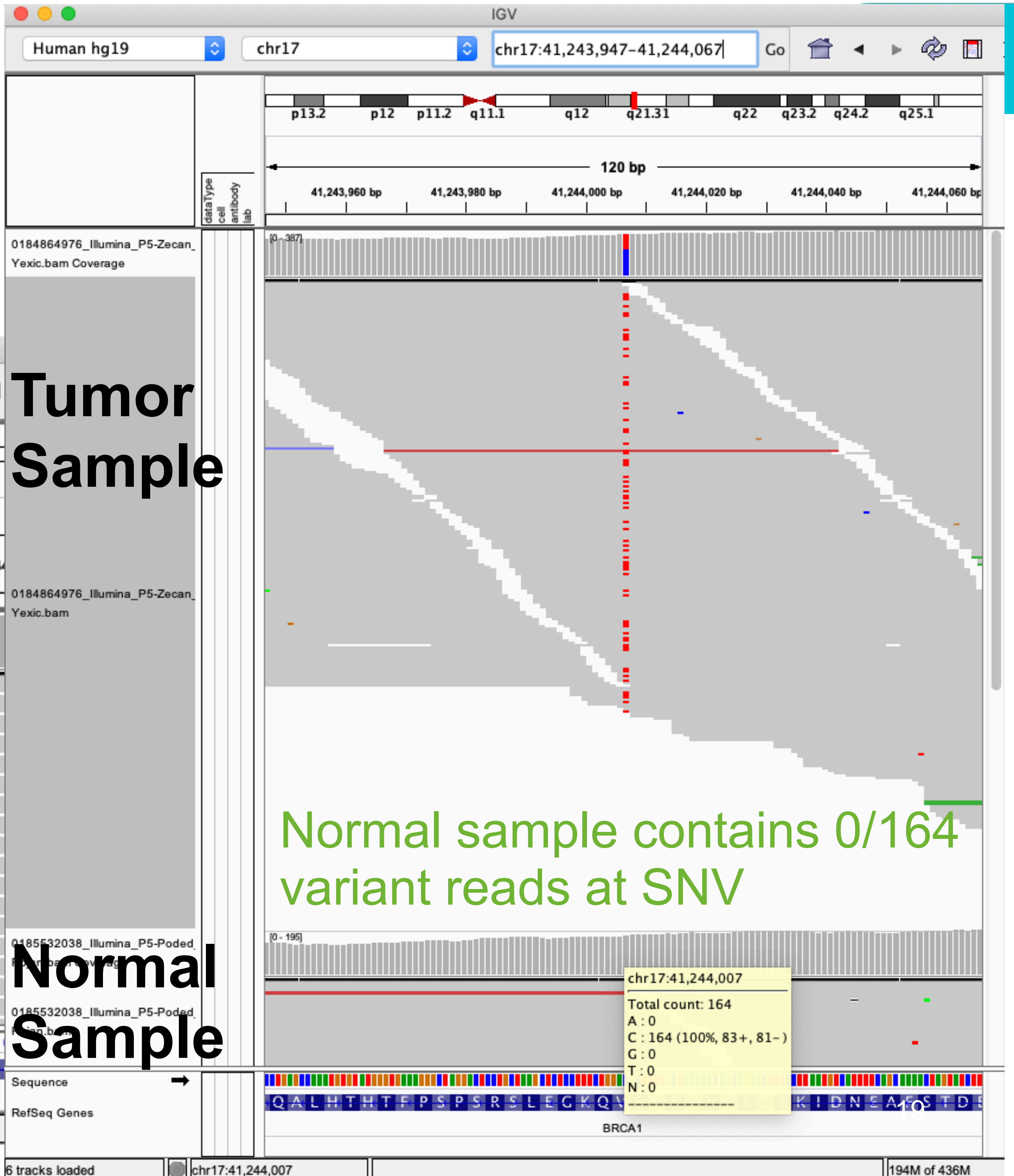


IGV
Human hg19
chr17
chr17:41,243,977-41,244,036
Go
p13.2
p12
p11.2
p11.1
q11.2
q12
q21.2
q21.32
q22
q23.1
q24.1
q24.3
60 bp
41,243,980 bp
41,243,990 bp
41,244,000 bp
41,244,010 bp
41,244,020 bp
41,24
dataType
cell
antibody
lab
0184864976_Illumina_P5-Zecan_Yexic.bam Coverage
[0 - 387]
0184864976_Illumina_P5-Zecan_Yexic.bam
T
T
T
T
T
T
Sequence
RefSeq Genes
T A G G A C T C C T G C T A A G C T C T C C T T T C T G G A C G C T T T T G C T A A A A A C A G C A G A A
P S R S L E G K Q V S K S F V A S
BRCA1
6 tracks
chr17:41,244,007

chr17:41,244,007

Total count: 342
A : 0
C : 214 (63%, 111+, 103-)
G : 0
T : 128 (37%, 59+, 69-)
N : 0

Tumor Sample



Tumor Sample

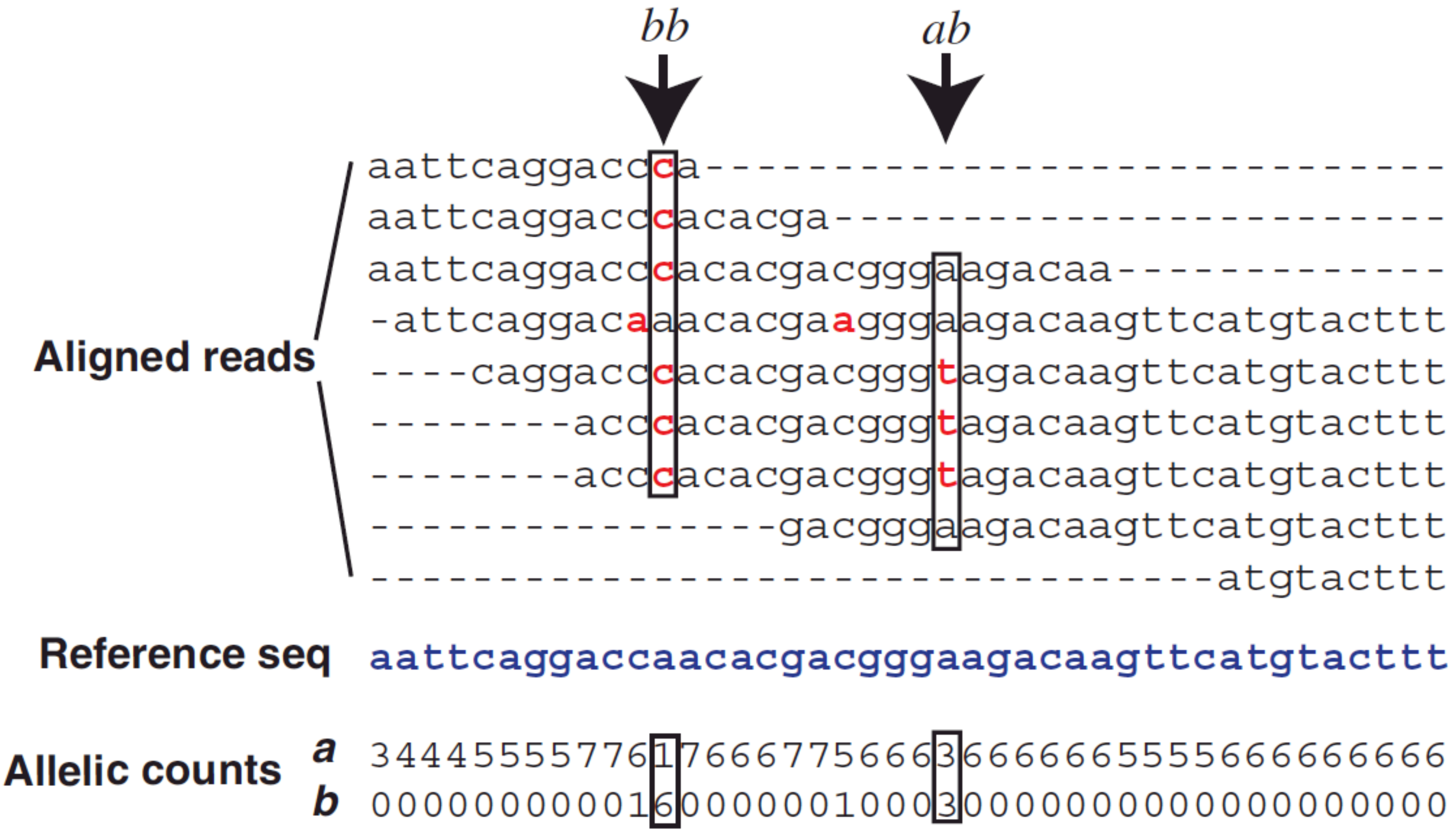
Normal sample contains 0/164 variant reads at SNV

chr17:41,244,007

Total count: 164
A : 0
C : 164 (100%, 83+, 81-)
G : 0
T : 0
N : 0

Normal Sample

Single Nucleotide Variant (SNV) Calling: Single Sample



```
                    aattcaggaccca-----------------------------
                    aattcaggacccacacga------------------------
                    aattcaggacccacacgacgggaagacaa-------------
Aligned reads       -attcaggacaaacacgaagggaagacaagttcatgtacttt
                    ----caggacccacacgacgggtagacaagttcatgtacttt
                    --------acccacacgacgggtagacaagttcatgtacttt
                    --------acccacacgacgggtagacaagttcatgtacttt
                    ----------------gacgggaagacaagttcatgtacttt
                    ---------------------------------atgtacttt

Reference seq       aattcaggaccaacacgacgggaagacaagttcatgtacttt

Allelic counts   a  344455557761766677566636666665555666666666
                 b  000000000016000000100030000000000000000000
```

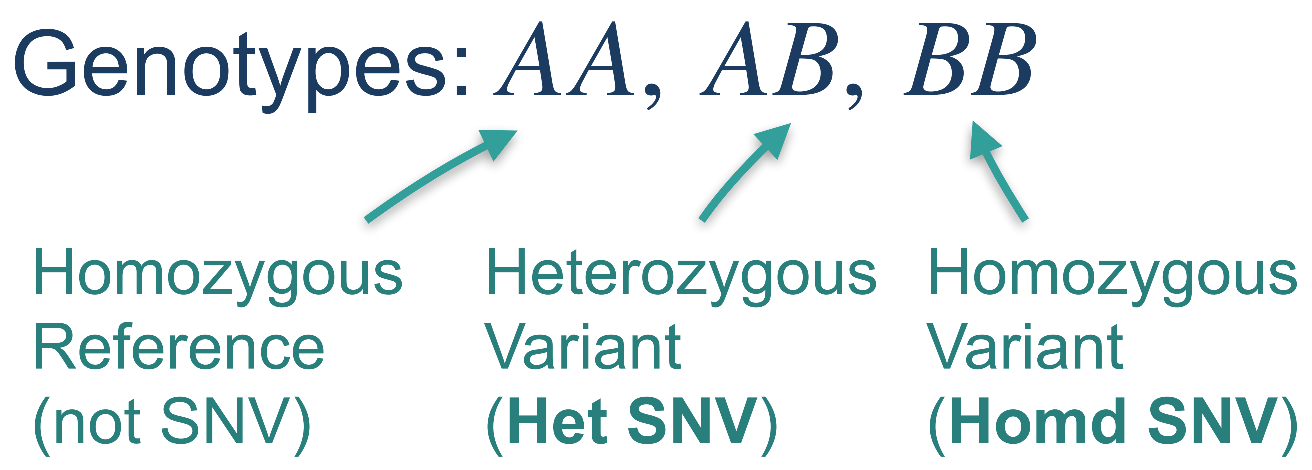
Reference Counts

Non-reference Counts

Goya et al. *Bioinformatics* **26**:703-36 (2010)

SNV Variant Allele Fraction and Genotypes

Variant Allele Fraction (VAF) Analysis



Genotypes: $AA,\ AB,\ BB$

Homozygous Reference (not SNV)

Heterozygous Variant (**Het SNV**)

Homozygous Variant (**Homd SNV**)

Genotype	**AA**	**AB**	**BB**
Allelic Fraction	~1.0	~0.5	~0

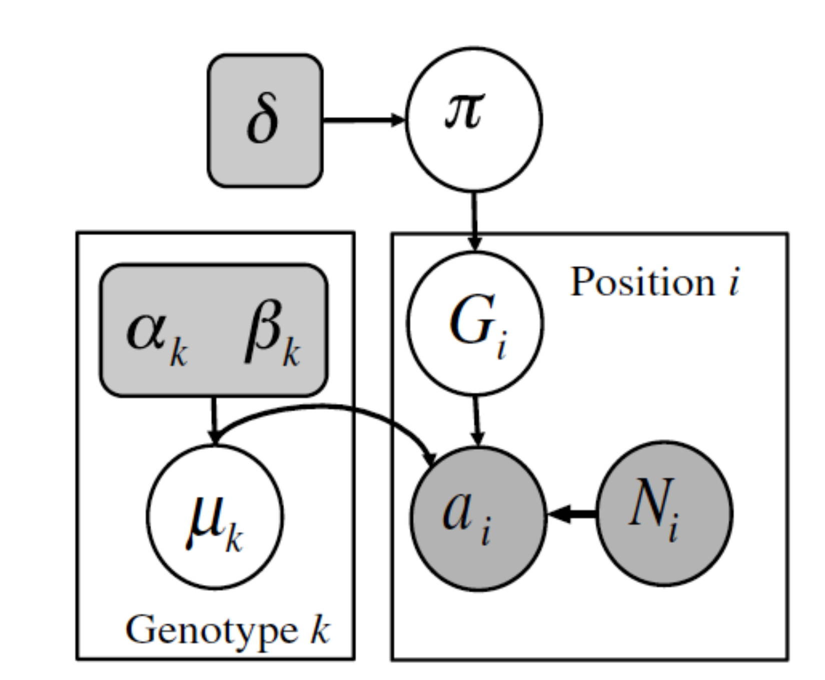
- **Allelic Fraction** is defined as the fraction of reference reads, $\frac{A}{N}$, where depth $N = A + B$
- Values in the table are the *expected* proportions of *reference reads* for each genotype
- Why might the observed allelic fractions be different than the expected values?

3. Mixture Model for SNV Detection

- SNVMix probabilistic model and EM inference
- Predicting somatic SNVs in cancer

References:

- Goya et al. **SNVMix**: predicting single nucleotide variants from next-generation sequencing of tumors. *Bioinformatics* **26**:730-36 (2010)
- Roth et al. **JointSNVMix**: a probabilistic model for accurate detection of somatic mutations in normal/ tumour paired next-generation sequencing data. *Bioinformatics* **28**:907-13 (2012)



SNVMix1 model

Mapping the Referee Example to Mutation Calling

Referee Coin Toss Example

Data

Referees $1, \ldots, T$

For each Referee i

- Coin Tosses: N_i
- Count of heads: x_i
- Count of tails: $N_i - x_i$

Parameters

Probability to draw coins: $\pi_{fair}, \pi_{heads}, \pi_{tails}$

Probability of heads for 3 types of coins

$$\mu_{fair}, \mu_{heads}, \mu_{tails}$$

Responsibilities

Probability that Referee i used coin k: $\gamma(Z_i = k)$

Mutation Calling from Sequencing Data

Data

Genomic loci $1, \ldots, T$

For each locus i

- Depth (total reads): N_i
- Count of reference reads: x_i
- Count of variant reads: $N_i - x_i$

Parameters

Probability of genotypes: $\pi_{AA}, \pi_{AB}, \pi_{BB}$

Probability of reference base for 3 genotypes:

$$\mu_{AA}, \mu_{AB}, \mu_{BB}$$

Responsibilities

Probability that locus i has genotype k: $\gamma(Z_i = k)$

SNVMix: Probabilistic Model

Sequence Data

There are T different genomic loci with read depths $N = \{1,\ldots,N_T\}$ and reference base counts $\boldsymbol{x} = \{1,\ldots,x_T\}$.
There are $K = 3$ different possible genotypes $AA,\ AB,\ BB$

Mixture Model Setup

1. The **probabilities for the genotypes** are $\pi_{AA},\ \pi_{AB},\ \pi_{BB}$
2. Thus, a specific genotype $k \in AA,\ AB,\ BB$ can be assigned to the **latent state** Z_i at locus i with these probabilities

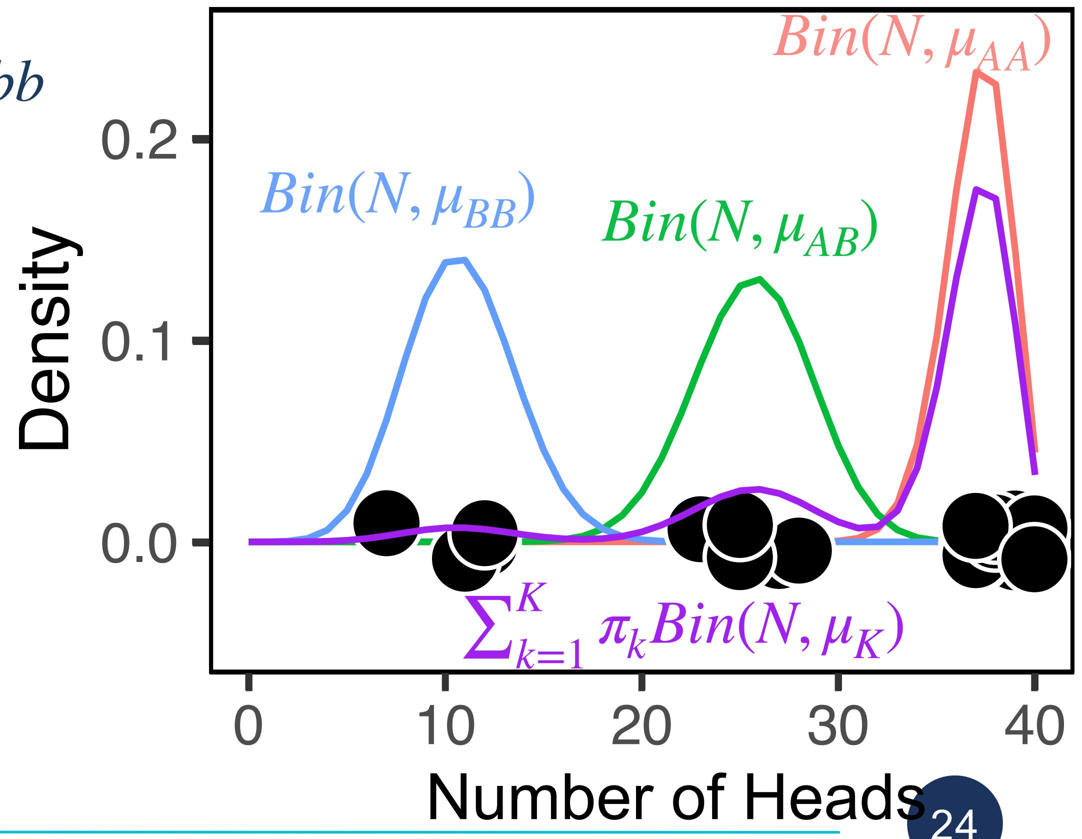
$$p(Z_i = k \mid \pi_{1:K}) = \begin{cases} \pi_{AA} \text{ if } k = AA \\ \pi_{AB} \text{ if } k = AB \\ \pi_{BB} \text{ if } k = BB \end{cases}$$

3. The **probability of observing a reference base** for the genotypes are $\mu_{aa},\ \mu_{ab},\ \mu_{bb}$
4. The likelihood is a ***3-component mixture of binomials***

$$p(x_i \mid N_i, \mu_{1:K}, \pi_{1:K}) = \sum_{k=1}^{K} \pi_k Bin(x_i \mid N_i, \mu_k)$$

5. The **priors** for genotype $k \in \{aa,\ ab,\ bb\}$in the model are

$$p(\pi_{1:K} \mid \delta_{1:K}) = Dirichlet(\pi_{1:K} \mid \delta_{1:K})$$
$$p(\mu_k \mid \alpha_k, \beta_k) = Beta(\mu_k \mid \alpha_k, \beta_k)$$



Goya et al. *Bioinformatics* **26**:703-36 (2010)

SNVMix: Inference & parameter estimation using EM (revisited)

E-Step: compute responsibilities

1. What is the probability of locus i having genotype k?

$$\gamma(Z_i = k) = \frac{\pi_k Bin(x_i \mid N_i, \mu_k)}{\sum_{j=1}^{K} \pi_j Bin(x_i \mid N_i, \mu_j)}$$

Responsibilities

Matrix $T \times K$

M-Step: update parameters

2. What is the probability of genotype k?

$$\hat{\pi}_k = \frac{\sum_{i=1}^{T} \gamma(Z_i = k) + \delta(k) - 1}{\sum_{j=1}^{K} \left\{ \sum_{i=1}^{T} \gamma(Z_i = j) + \delta(j) - 1 \right\}}$$

MAP for π

3. What is the probability of observing a reference base for genotype k?

$$\hat{\mu}_k = \frac{\sum_{i=1}^{T} \gamma(Z_i = k) x_i + \alpha_k - 1}{\sum_{i=1}^{T} \gamma(Z_i = k) N_i + \alpha_k + \beta_k - 2}$$

MAP for μ

Evaluate the log likelihood and log posterior: use updated parameters

$$\log \mathbb{P} = \sum_{i=1}^{T} \log \left(\sum_{k=1}^{K} \hat{\pi}_k Bin(x_i \mid \hat{\mu}_k, N_i) \right) + \log Dir(\hat{\pi}_k \mid \delta_k) + \sum_{k=1}^{K} \log Beta(\hat{\mu}_k \mid \alpha_k, \beta_k)$$

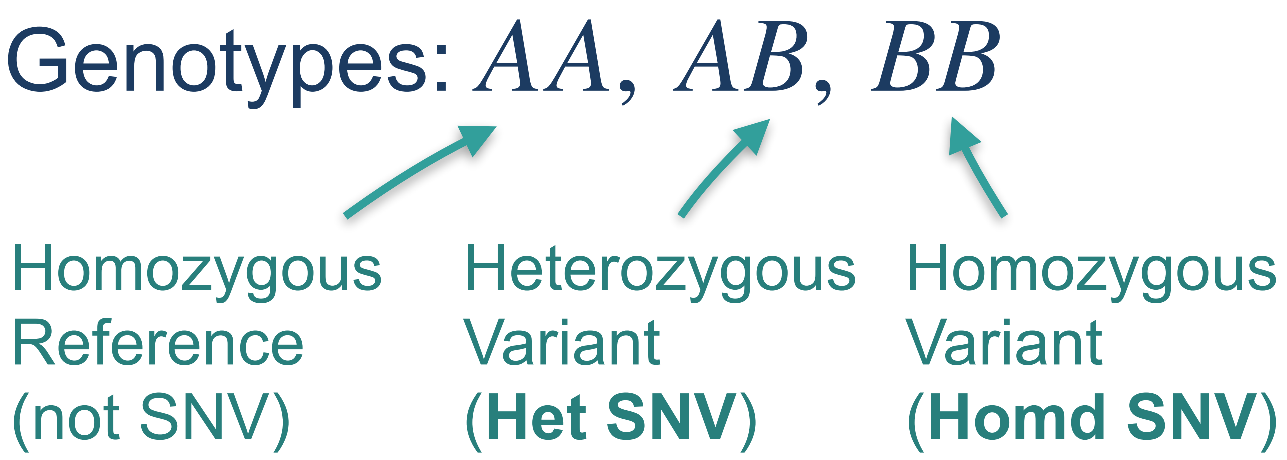
Log posterior

Iterate between E-Step and M-Step: stop when $\log \mathbb{P}$ changes less than ϵ compared to previous EM iteration.

Chapter 9 in Bishop (2006). Pattern Recognition and Machine Learning. Springer

Section 3.3, 3.4, 11.2 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

SNVMix: Calling somatic SNVs from genotype inference



Genotypes: AA, AB, BB

Homozygous Reference (not SNV)

Heterozygous Variant (**Het SNV**)

Homozygous Variant (**Homd SNV**)

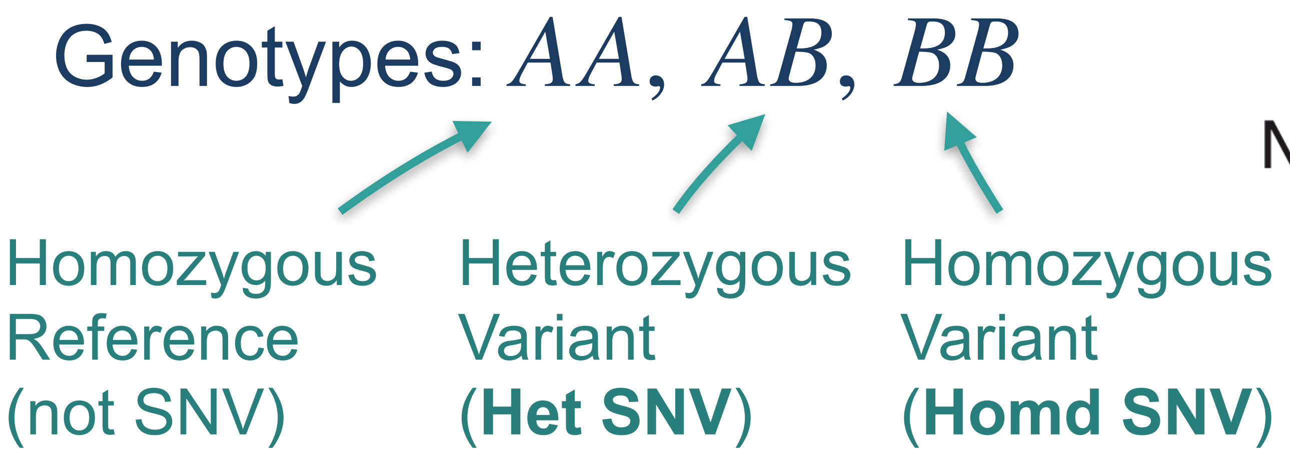
	Responsibilities		
Locus	AA	AB	BB
1	$\gamma(Z_1 = AA)$	$\gamma(Z_1 = AB)$	$\gamma(Z_1 = BB)$
2	$\gamma(Z_2 = AA)$	$\gamma(Z_2 = AB)$	$\gamma(Z_2 = BB)$
3	$\gamma(Z_3 = AA)$	$\gamma(Z_3 = AB)$	$\gamma(Z_3 = BB)$
T	$\gamma(Z_T = AA)$	$\gamma(Z_T = AB)$	$\gamma(Z_T = BB)$

- To call a variant for each locus i, we can apply a threshold on the responsibilities $\gamma(Z_i)$
- Sum $\gamma(Z_i = AB)$ and $\gamma(Z_i = BB)$ to get the overall probability (either genotype AB or BB) that locus i is a variant containing the non-reference allele (B)
- Additional steps required for filtering and determining if variant is somatic vs germline
  - Minimum 3 variant reads $(N_i - x_i)$ is typically required
  - Account for mapping and base qualities of sequenced reads (i.e. SNVMix2)
  - Compare locus i in tumor sample to (1) matched normal sample, (2) germline databases

SNV Genotyping Callers

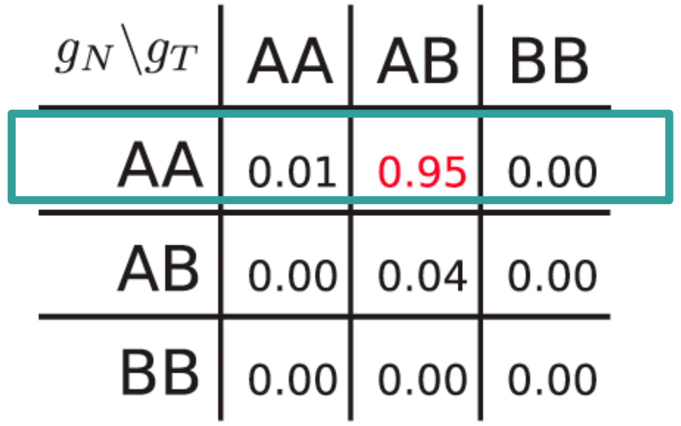
Variant Allele Fraction Analysis

- **Single sample**


Genotypes: AA, AB, BB
Homozygous Reference (not SNV)
Heterozygous Variant (**Het SNV**)
Homozygous Variant (**Homd SNV**)

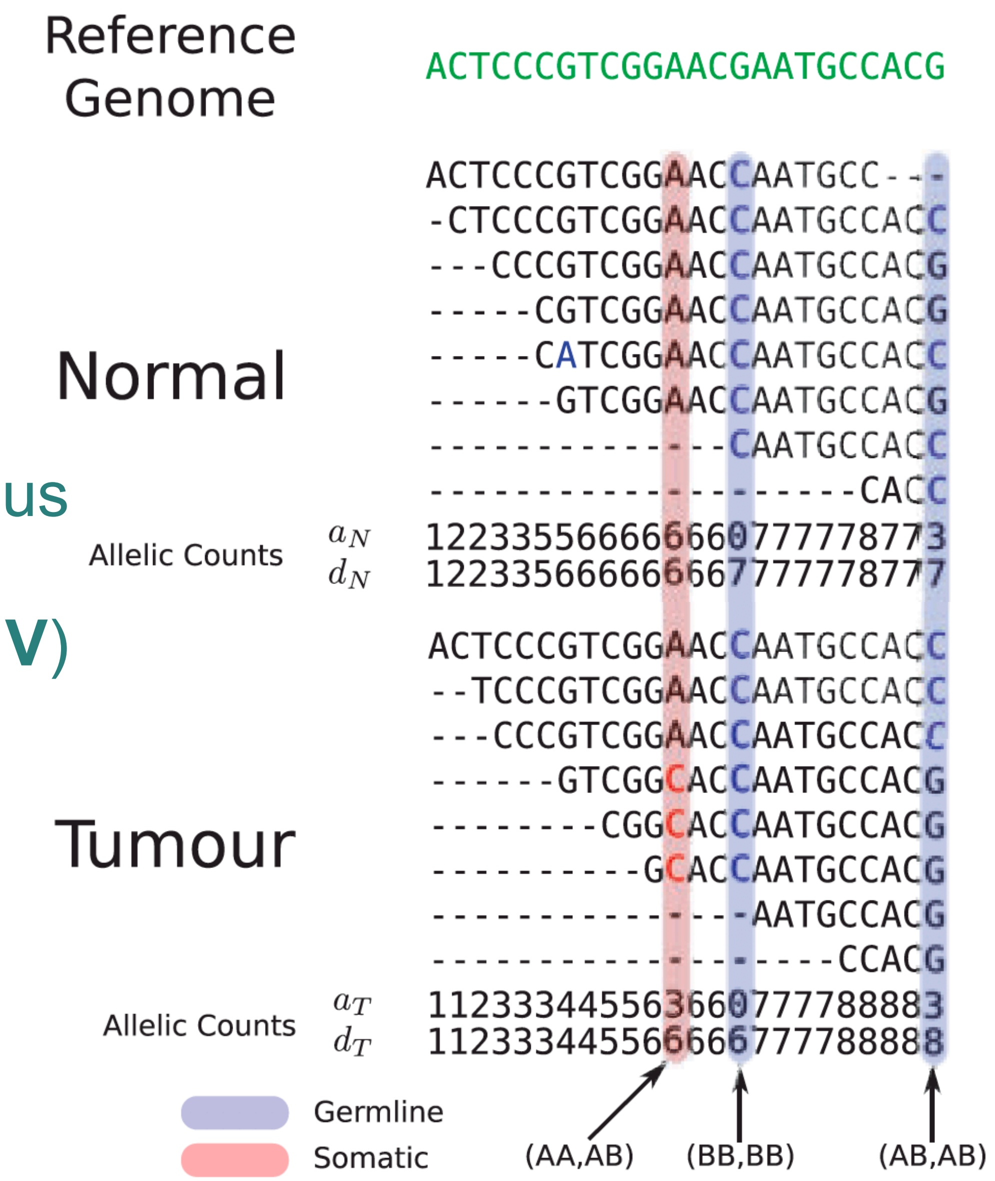
- **Joint tumor-normal**

Joint Genotypes:

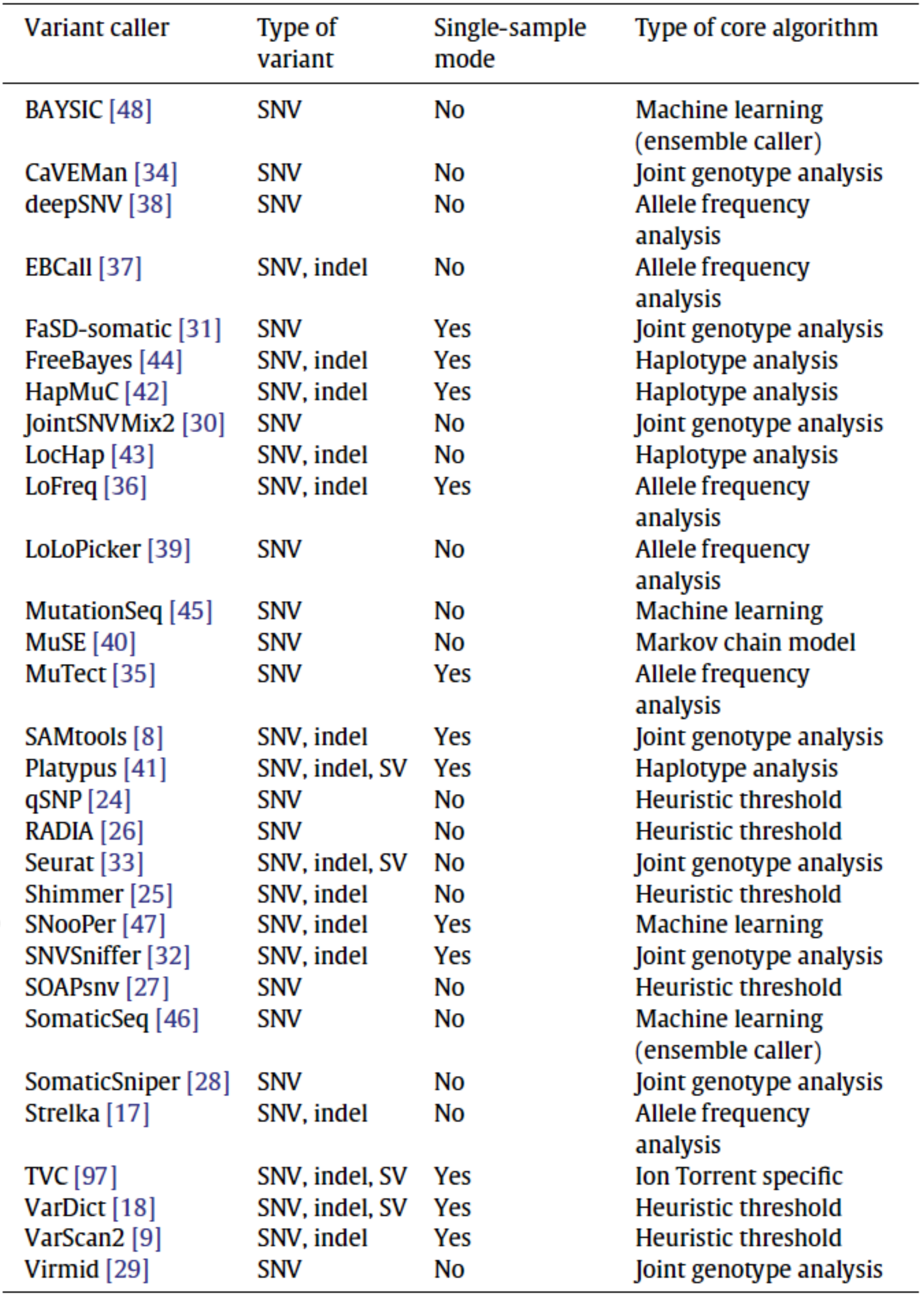


$g_N \backslash g_T$	AA	AB	BB
AA	0.01	0.95	0.00
AB	0.00	0.04	0.00
BB	0.00	0.00	0.00

- **Cohort level or panel:** Machine Learning (supervised)



JointSNVMix. Roth et al. *Bioinformatics* **28**:907-13 (2012)



Variant caller	Type of variant	Single-sample mode	Type of core algorithm
BAYSIC [48]	SNV	No	Machine learning (ensemble caller)
CaVEMan [34]	SNV	No	Joint genotype analysis
deepSNV [38]	SNV	No	Allele frequency analysis
EBCall [37]	SNV, indel	No	Allele frequency analysis
FaSD-somatic [31]	SNV	Yes	Joint genotype analysis
FreeBayes [44]	SNV, indel	Yes	Haplotype analysis
HapMuC [42]	SNV, indel	Yes	Haplotype analysis
JointSNVMix2 [30]	SNV	No	Joint genotype analysis
LocHap [43]	SNV, indel	No	Haplotype analysis
LoFreq [36]	SNV, indel	Yes	Allele frequency analysis
LoLoPicker [39]	SNV	No	Allele frequency analysis
MutationSeq [45]	SNV	No	Machine learning
MuSE [40]	SNV	No	Markov chain model
MuTect [35]	SNV	Yes	Allele frequency analysis
SAMtools [8]	SNV, indel	Yes	Joint genotype analysis
Platypus [41]	SNV, indel, SV	Yes	Haplotype analysis
qSNP [24]	SNV	No	Heuristic threshold
RADIA [26]	SNV	No	Heuristic threshold
Seurat [33]	SNV, indel, SV	No	Joint genotype analysis
Shimmer [25]	SNV, indel	No	Heuristic threshold
SNooPer [47]	SNV, indel	Yes	Machine learning
SNVSniffer [32]	SNV, indel	Yes	Joint genotype analysis
SOAPsnv [27]	SNV	No	Heuristic threshold
SomaticSeq [46]	SNV	No	Machine learning (ensemble caller)
SomaticSniper [28]	SNV	No	Joint genotype analysis
Strelka [17]	SNV, indel	No	Allele frequency analysis
TVC [97]	SNV, indel, SV	Yes	Ion Torrent specific
VarDict [18]	SNV, indel, SV	Yes	Heuristic threshold
VarScan2 [9]	SNV, indel	Yes	Heuristic threshold
Virmid [29]	SNV	No	Joint genotype analysis

Xu. *Comput Struct Biotechnol.* **16**:15-24 (2018)

Somatic SNV Detection using Joint Inference from Tumor-Normal Pairs

1.Latent variable state space

- 9 genotype pairs $\left(k_n,\ k_t\right)$
- $n, t \in \{AA, AB, BB\}$

		Tumor, t		
	$k_n \backslash k_t$	AA	AB	BB
Normal, n	AA	0.01	0.95	0.00
	AB	0.00	0.04	0.00
	BB	0.00	0.00	0.00

2.Probability of the genotypes

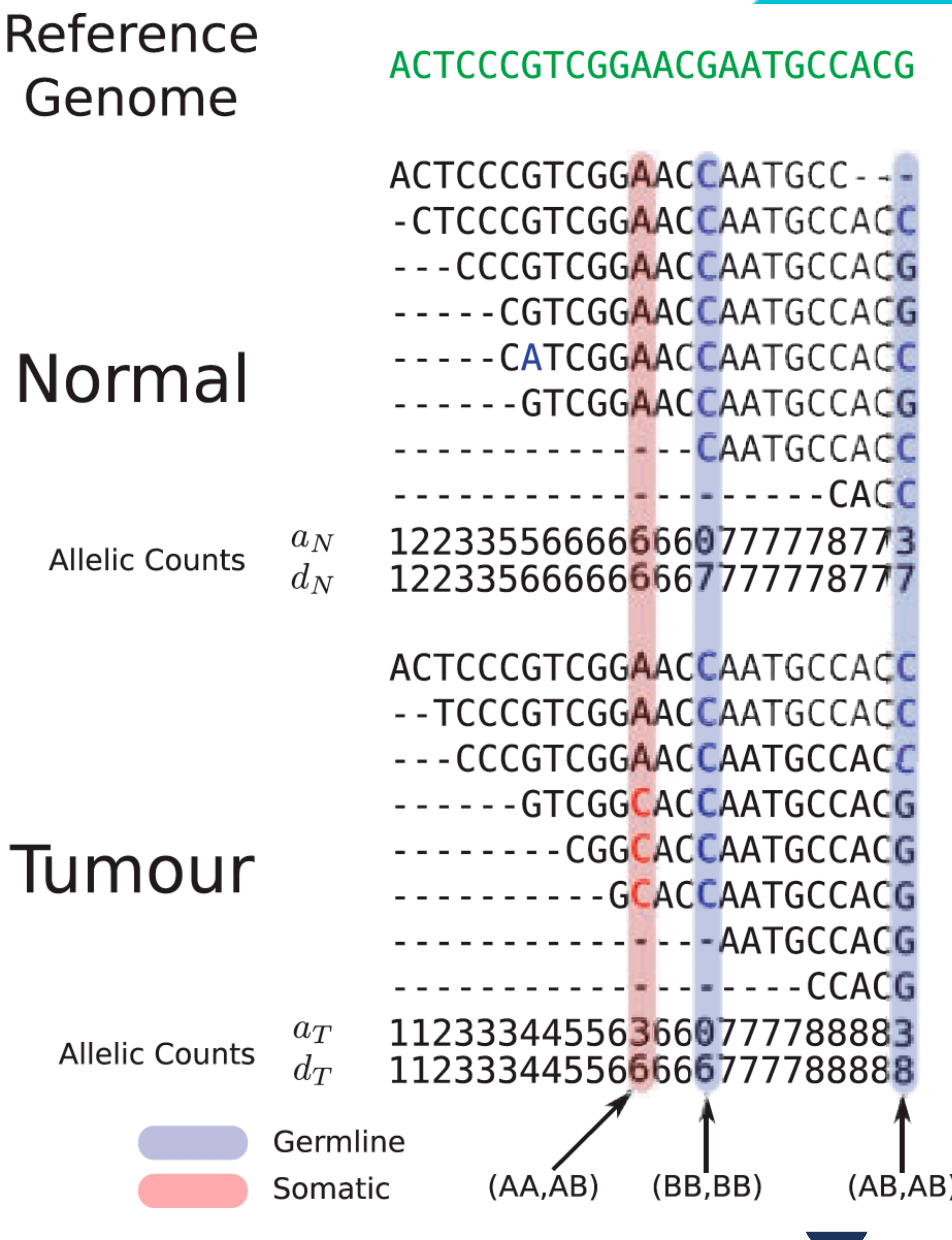
- 9 mixture weights $\pi_{(k_n,k_t)}$

3.Joint binomial mixture model

- 9-component mixture model

$$p(x_i^n, x_i^t \,|\, N_i^n, N_i^t, \mu_{1:K}^n, \mu_{1:K}^t) = \sum_{k_n=1}^{K} \sum_{k_t=1}^{K} \pi_{(k_n,k_t)} Bin(x_i^n \,|\, N_i^n, \mu_{k_n}^n) Bin(x_i^t \,|\, N_i^t, \mu_{k_t}^t)$$

- with 9 parameter tuples (μ^n, μ^t)



Roth et al. *Bioinformatics* **28**:907-13 (2012)

Homework #7: Single-nucleotide Genotype Caller

Implement a standard binomial mixture model described in Lecture 2.

- Learn the parameters and infer the genotypes
- Annotate the mutation status for a set of genomic loci.
- Expected outputs for each question will be provided so that you can check your code.
- RStudio Markdown and Python Jupyter Notebook templates provided.

Due: May 19th, 2023