Fred Hutch
Cancer Center

CANCER GENOMICS

Lecture 4: Tumor heterogeneity, Mutation power analysis, Structural variation in cancer

GENOME 541 Spring 2023
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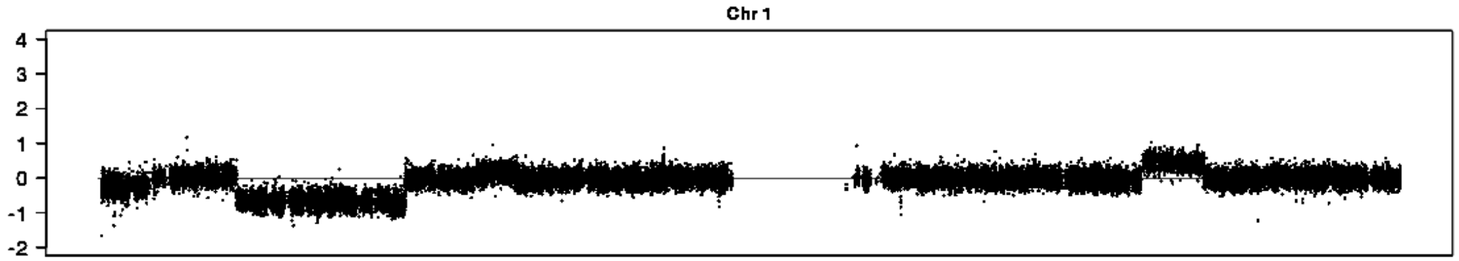
Outline: Probabilistic Methods for Mutation Detection

1. **Additional Copy Number Analysis Features**
   - Allelic copy number analysis
2. **Estimating tumor heterogeneity**
   - Modeling tumor-normal admixture
   - Modeling tumor clonality and heterogeneity
3. **Assessing Statistical Power for Variant Discovery**
   - Power calculation
   - Calibrating sequencing depth for variant discovery
4. **Structural Rearrangement Analysis in Cancer Genomes**
   - Structural variant types predicted from sequencing analysis
   - Complex genomic structural rearrangement patterns

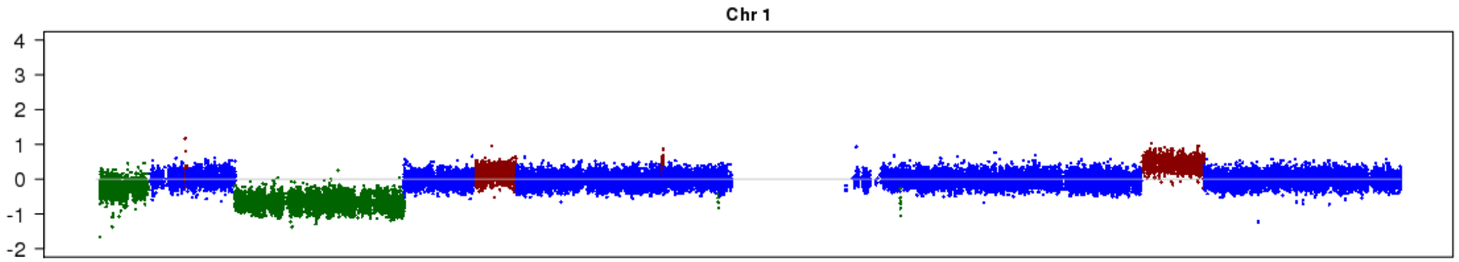
Allele-based Copy Number Analysis

Corrected log2 ratio



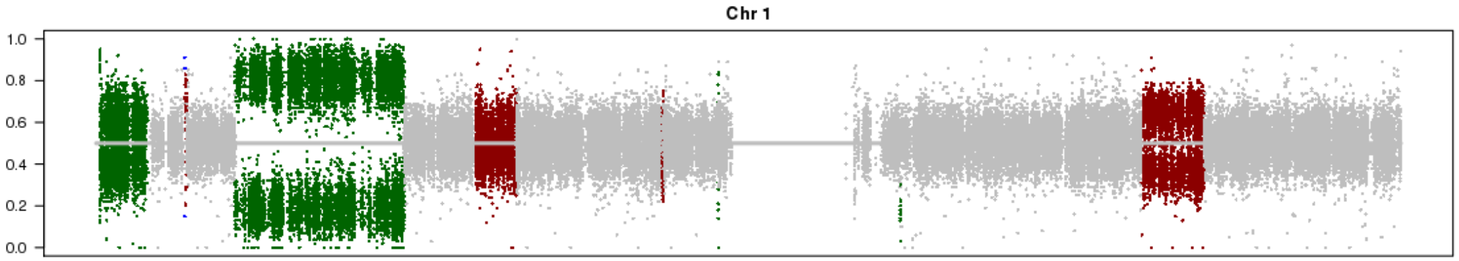
Data normalization

Copy Number (log2 ratio)



Total Copy Number Only

Allele fraction (ref/depth)



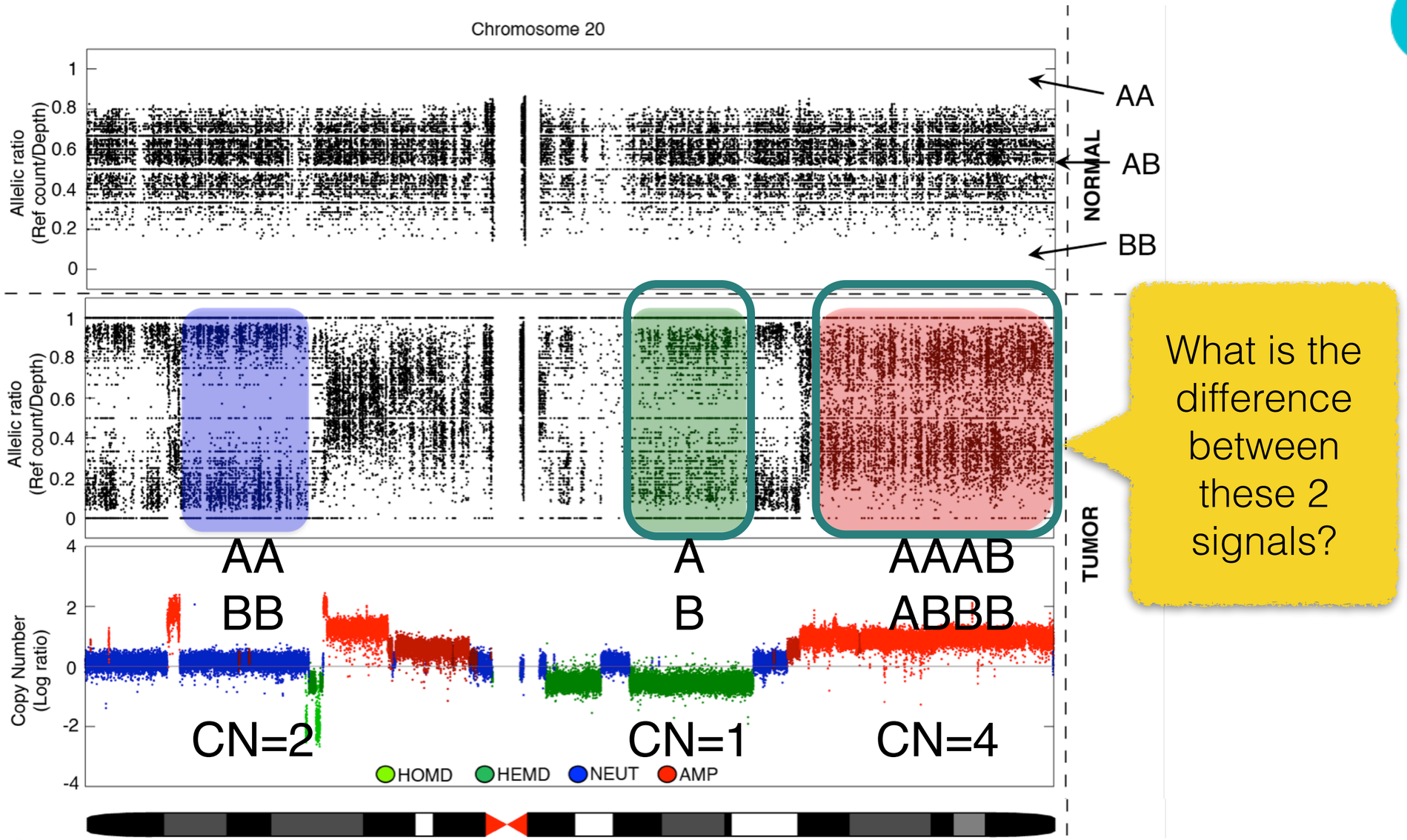
Allelic Copy Number

Copy Number Analysis: Allelic Features

Normal (ref/depth)

Tumor (ref/depth)

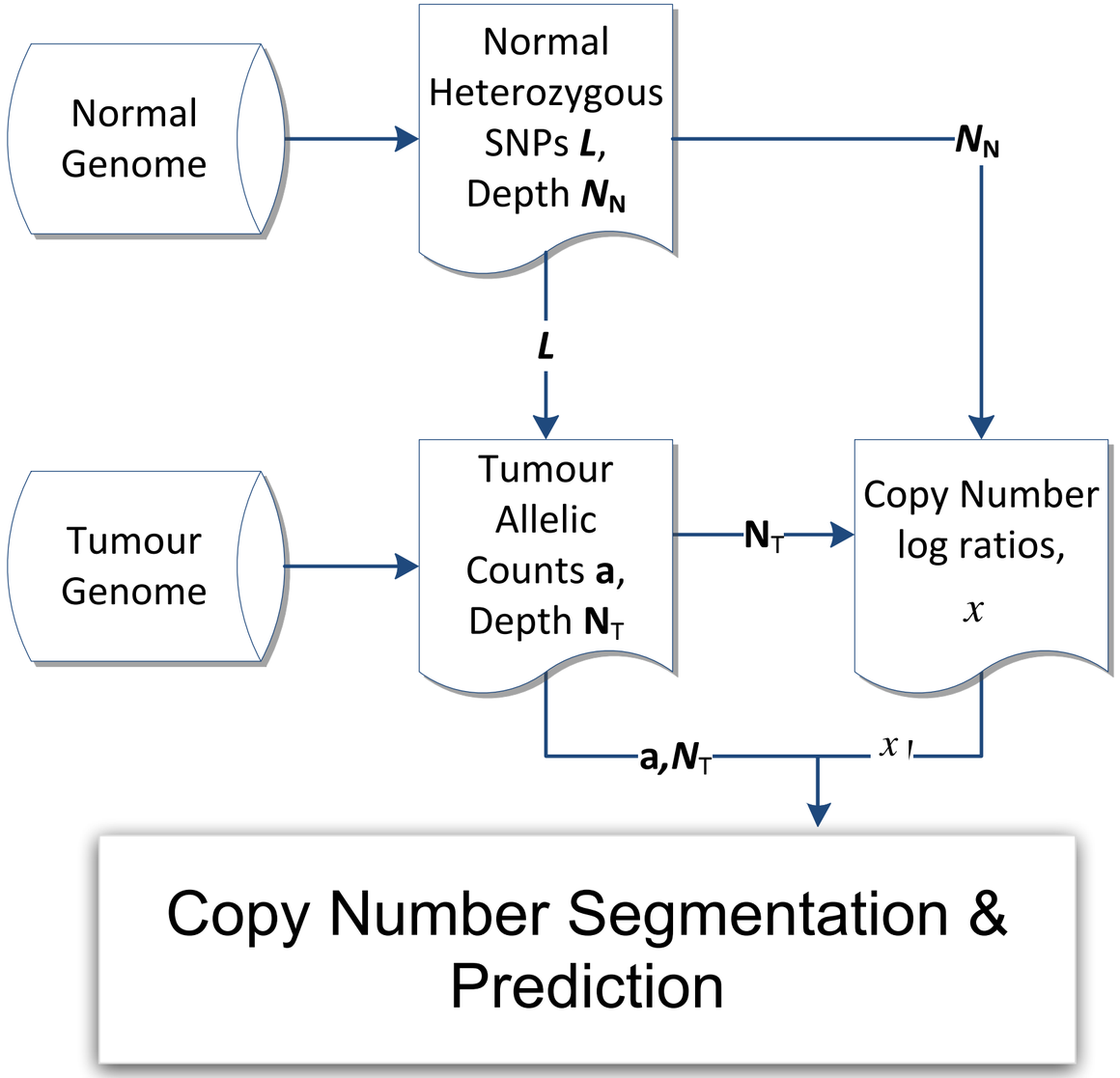
Tumor



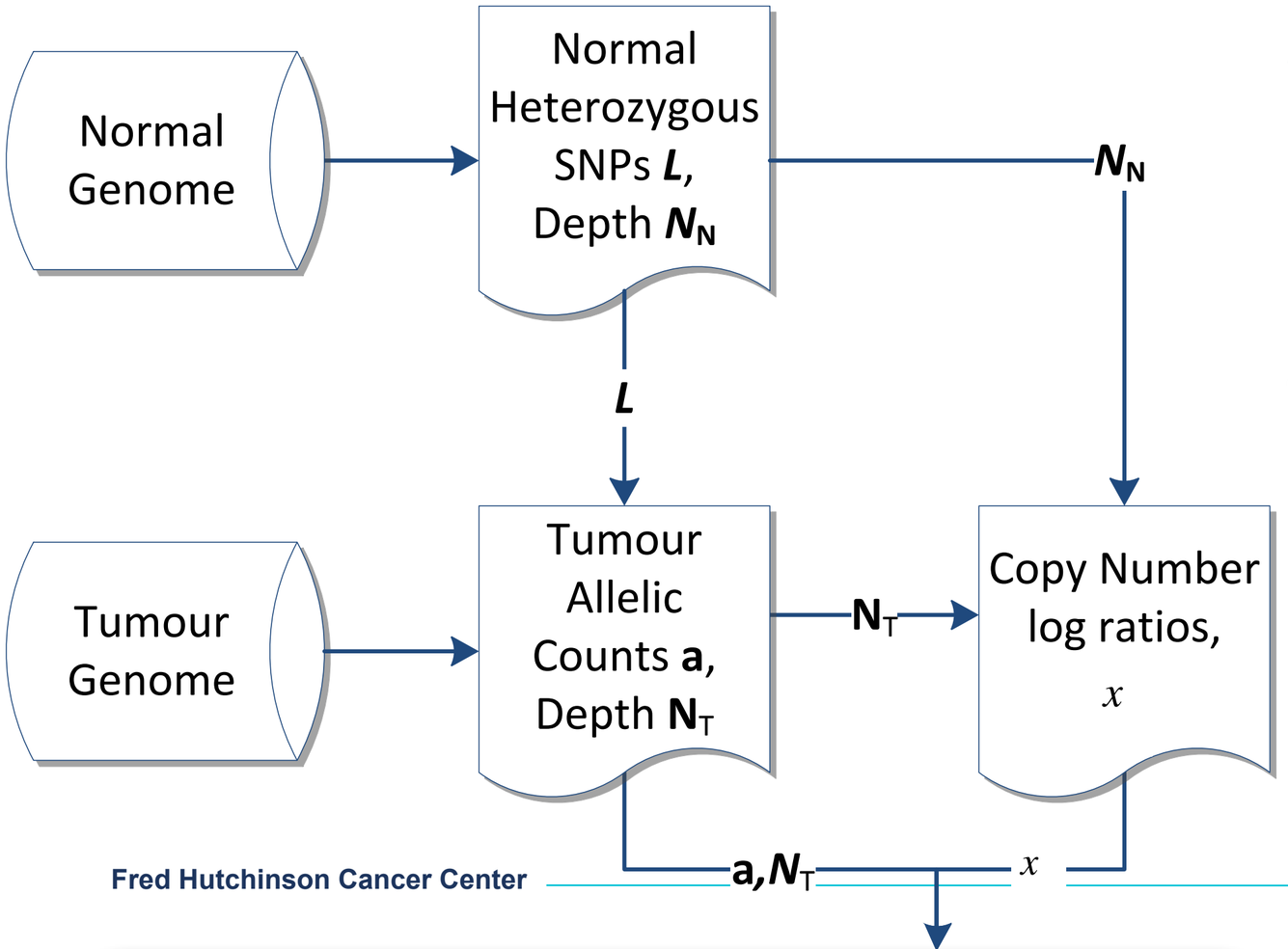
What is the difference between these 2 signals?

Ha et al. *Genome Research* **22**:1995-2007 (2012)

Cancer Genome Copy Number Analysis Workflow

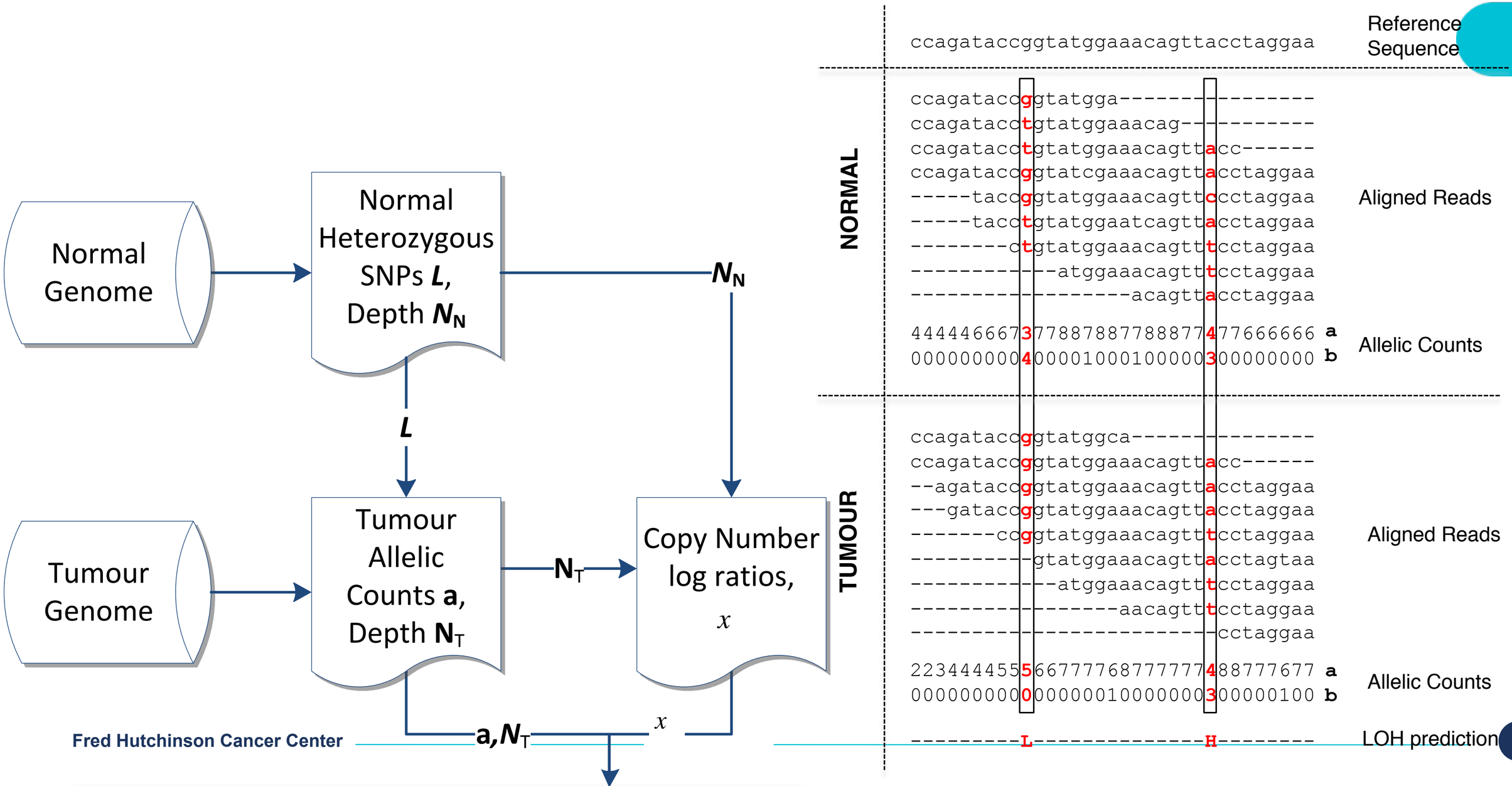


Copy Number Analysis Workflow: Allele Features



1. Correct GC/mappability biases for tumor read depth
2. **Identify germline heterozygous SNP sites from normal**
3. **Extract read counts at SNPs from tumor**
4. Perform segmentation and copy number prediction

Copy Number Analysis Workflow: Allele Features



Probabilistic Model for *Allelic* Copy Number Analysis

Input Data: T different genomic loci

- log ratio data $x_{1:T}$
- reference counts $a_{1:T}$ and read depth $N_{1:T}$ for SNP data

Latent State Model: copy number states

There are 8 possible joint copy number state and allele genotype states.

K	Genotype	CN
1	A/B	1
2	AA/BB	2
3	AB	2
4	AAA/BBB	3
5	AAB/ABB	3
6	AAAA/BBB	4
7	AAAB/ABBB	4
8	AA/BB	4

Transition Model

The transition model is similar to before for matrix $\boldsymbol{A} \in \mathbb{R}^{K \times K}$

Emission Model: joint likelihood for log ratio and allele data

The **emission model** is a mixture of the joint distributions (multivariate)

$$p(x_t, a_t \mid Z_i = k, N_t, \boldsymbol{\mu^c}, \boldsymbol{\sigma^2}, \boldsymbol{\mu^a}) = \mathcal{N}(x_t \mid \mu_k^c, \sigma_k^2) \times Bin(a_t \mid N_t, \mu_k^a)$$

Prior Model

$$p(\boldsymbol{\pi} \mid \boldsymbol{\delta^\pi}) = Dirichlet(\boldsymbol{\pi} \mid \boldsymbol{\delta^\pi})$$

$$p(\mu_k^c \mid m_k, s_k) = \mathcal{N}(\mu_k^c \mid m_k, s_k)$$

$$p(\sigma_k^2 \mid \alpha_k, \beta_k) = InvGamma(\sigma_k^2 \mid \alpha_k^c, \beta_k^c)$$

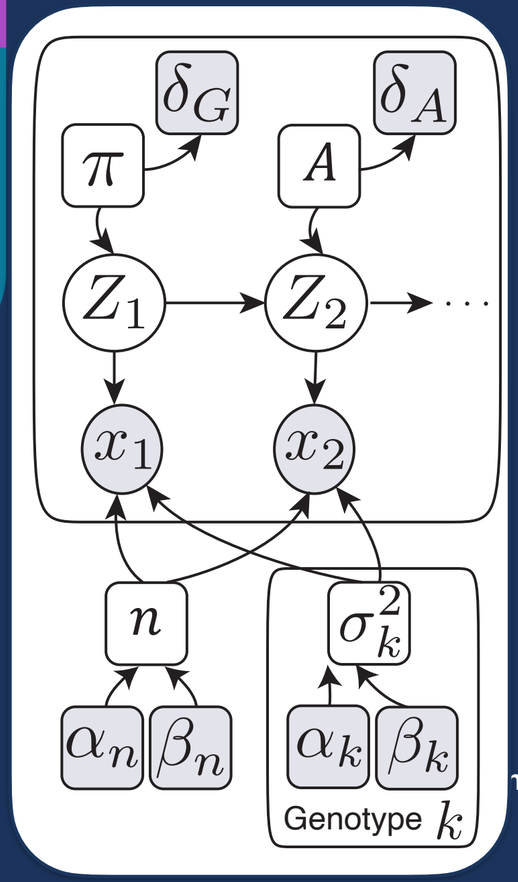
$$p(\mu_k^a \mid \alpha_k, \beta_k) = Beta(\mu_k^a \mid \alpha_k^a, \beta_k^a)$$

$$p(\boldsymbol{A_{k,1:K}} \mid \boldsymbol{\delta^A}) = Dirichlet(\boldsymbol{A_{k,1:K}} \mid \boldsymbol{\delta_k^A})$$

Ha et al. *Genome Research* **22**:1995-2007 (2012).
Adalsteinsson*, Ha* Freeman* et al. *Nat Commun* **8**:1324 (2017)

2. Estimating tumor heterogeneity

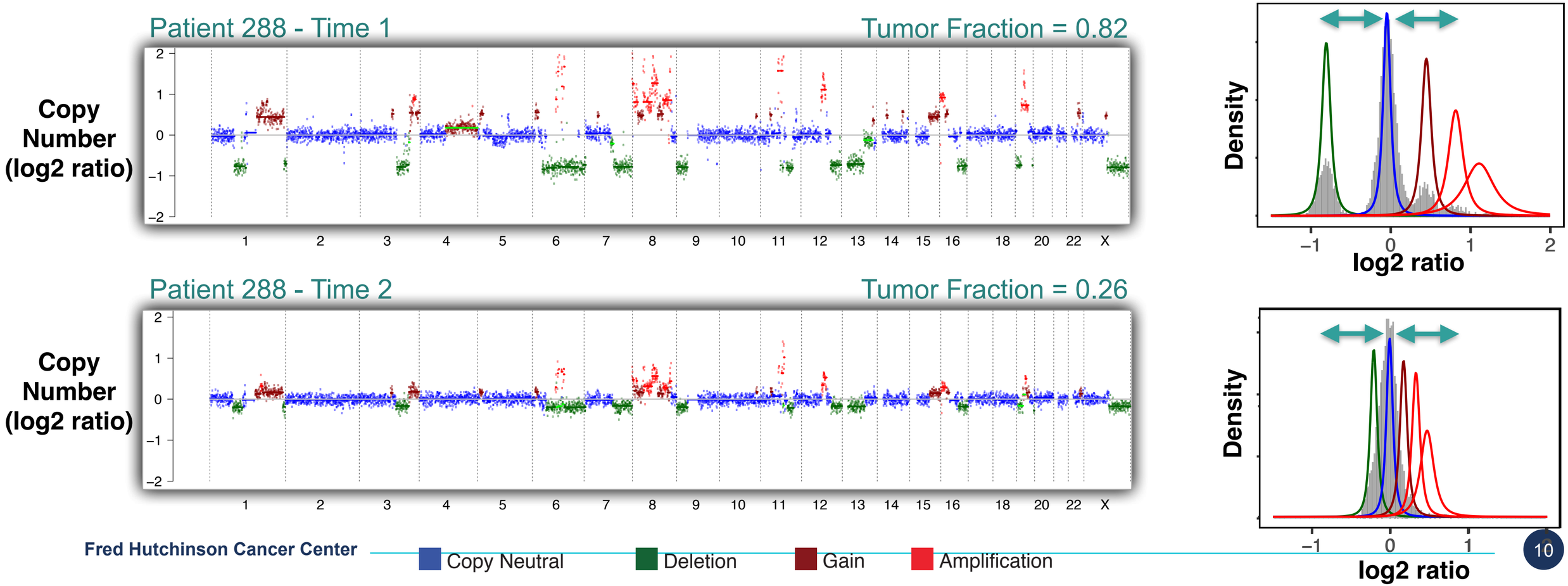
- Estimating tumor heterogeneity from copy number analysis
- References:
  - **ichorCNA** - Adalsteinsson*, Ha* Freeman* et al. *Nature Communications* **8**:1324 (2017).
  - **HMMcopy** - Ha et al. *Genome Research* **22**:1995-2007 (2012).
  - **TitanCNA** - Ha et al. TITAN: inference of copy number architectures in clonal cell populations from tumor whole-genome sequencing data. *Genome Research* **24**:1881-1893 (2014).
  - 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



Modeling tumor-normal admixture

Why estimate the model parameters $\boldsymbol{\mu} = \{\mu_0, \ldots, \mu_5\}$ and $\boldsymbol{\sigma^2} = \{\sigma_0^2, \ldots, \sigma_5^2\}$?

- Data variability due to sequencing depth (technical) and *tumor heterogeneity* (biological)



Modeling tumor-normal admixture

The mean ($\boldsymbol{\mu}$) of the copy number state mixture components can inform the tumor fraction.

- Recall: the log ratio input data is computed as

$$x_t = \log_2\left(\frac{\hat{N}_t^{Tumor}}{\hat{N}_t^{Normal}}\right)$$

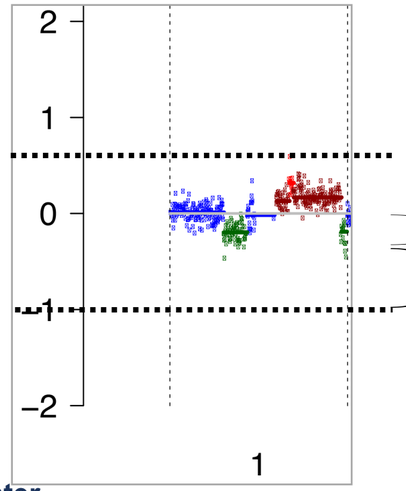
- For number $c_k \in \{1, 2, 3, 4, 5\}$, a pure tumor with 1.0 tumor fraction copy will have log ratios $\bar{\mu}_{1:K}$

$$\bar{\mu}_{1:K} = \left\{\log_2\left(\frac{c_{1:K}}{2}\right)\right\} =$$

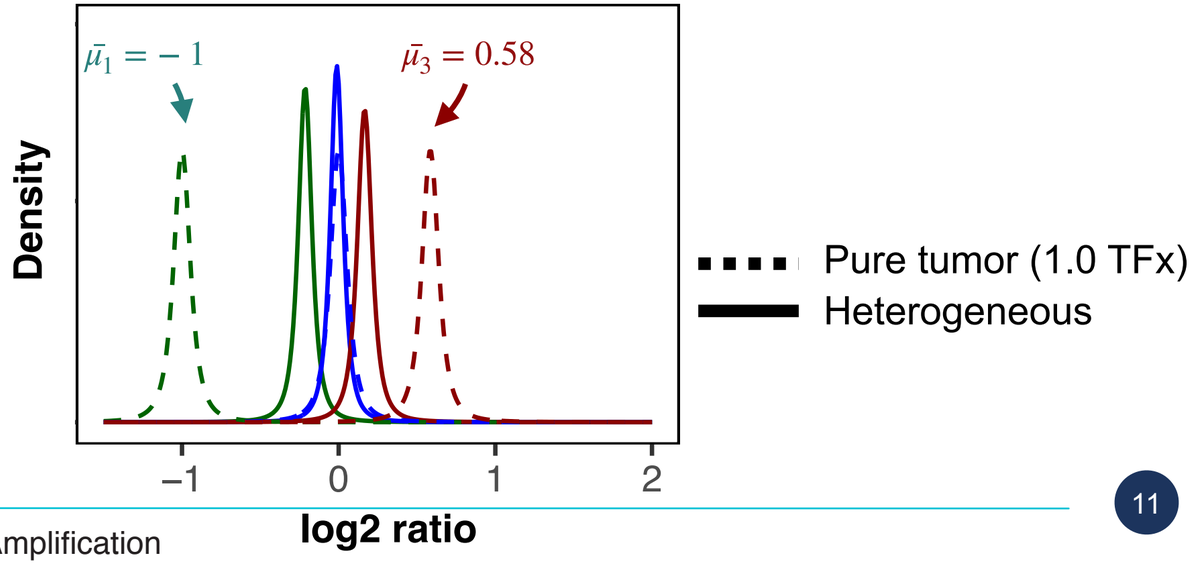
Patient 288 - Time 2
Tumor Fraction = 0.26

Gain (1.0 TFx)

Deletion (1.0 TFx)



Tumor
Normal
?



Copy Neutral Deletion Gain Amplification

Modeling tumor fraction as a parameter

- A tumor biopsy contains both tumor and normal cells

$$tumor\ signal \approx \left[(1-n) \times tumor\ CN\right] + \left[n \times normal\ CN\right]$$

  - n is the fraction of non-cancer cells
  - $(1-n)$ is the fraction of cancer cells
  - Typically $normal\ CN = 2$

- Then, the expected log ratio can be written as

$$\bar{\mu}_k = \log_2\left(\frac{c_k}{2}\right)$$

Pure tumor

Normal **Tumor**

$$\mu_k = \log_2\left(\frac{2n + (1-n)c_k}{2}\right)$$

Tumor-normal admixture (Heterogeneous)

where $c_k \in \{1, 2, 3, 4, 5\}$ is the tumor copy number for state k

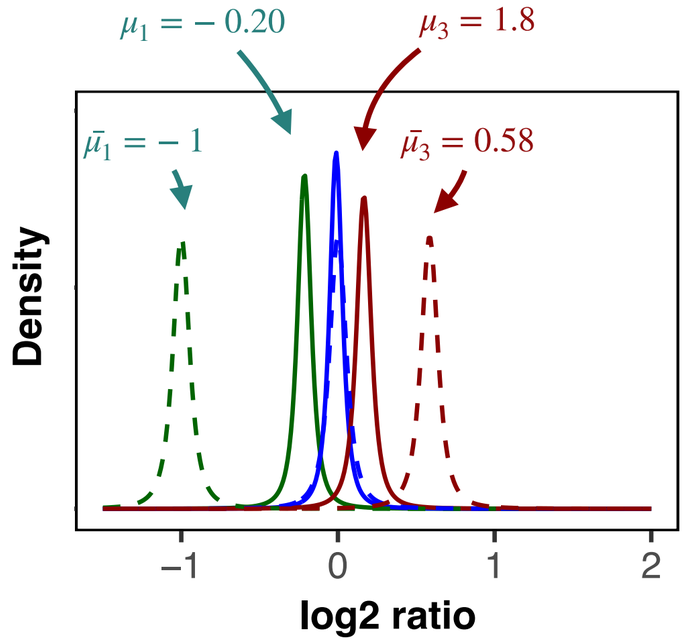
- Let's use some examples of *deletions* (CN=1) from the Slide 11:

$\bar{\mu}_1 =$ [ ]

Pure tumor ($n = 0$)

[ ]

Tumor-normal admixture ($n = 0.74$)



- Note that this formulation does not account for genome doubling in the tumor which would involve a tumor ploidy parameter ϕ and denominator of the ratio would be $2n + (1-n)\phi$ instead of just 2

Adalsteinsson*, Ha* Freeman* et al. *Nature Communications* **8**:1324 (2017)

Modeling tumor fraction as a parameter

- The expected log ratio for copy number state k is

$$\mu_k = \log_2\left(\frac{2n + (1-n)c_k}{2}\right), \text{ where } c_k \in \{1, 2, 3, 4, 5\}$$

- Recall the likelihood model:

$$p(x_i \mid Z_i = k, \boldsymbol{\mu}, \boldsymbol{\sigma^2}) = \mathcal{N}(x_i \mid \mu_k, \sigma_k^2)$$

- Since μ_k is now a function of n, we no longer need to estimate μ_k.
- **However, the non-cancer proportion n is what we want to estimate to obtain the tumor fraction $(1-n)$.**

~~$p(\mu_k \mid m_k, s_k) = \mathcal{N}(\mu_k \mid m_k, s_k)$~~

$$p(n \mid \alpha_n, \beta_n) = Beta(n \mid \alpha_n, \beta_n)$$ **Prior for n**

Log Posterior (with n terms)

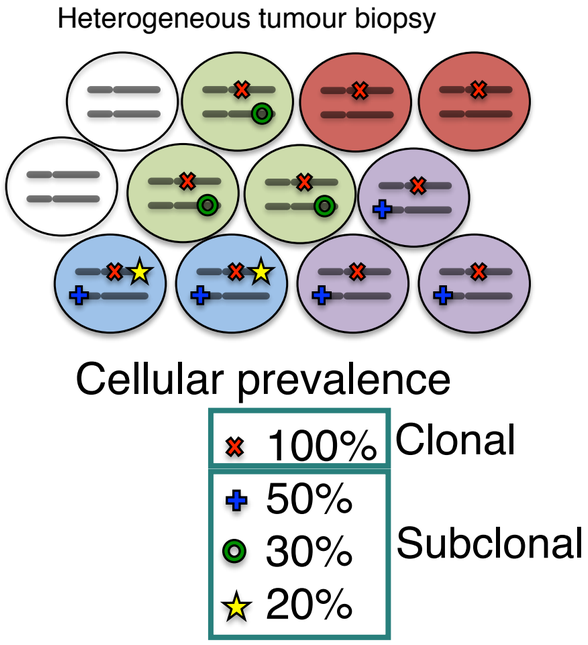
$$\log \mathbb{P}(n) \propto \sum_{t=1}^{T}\sum_{k=1}^{K} \gamma(Z_t = k)\log \mathcal{N}(x_t \mid \mu_k, \sigma_k^2) + \sum_{k=1}^{K} \log Beta(\mu_k \mid \alpha_n, \beta_n)$$

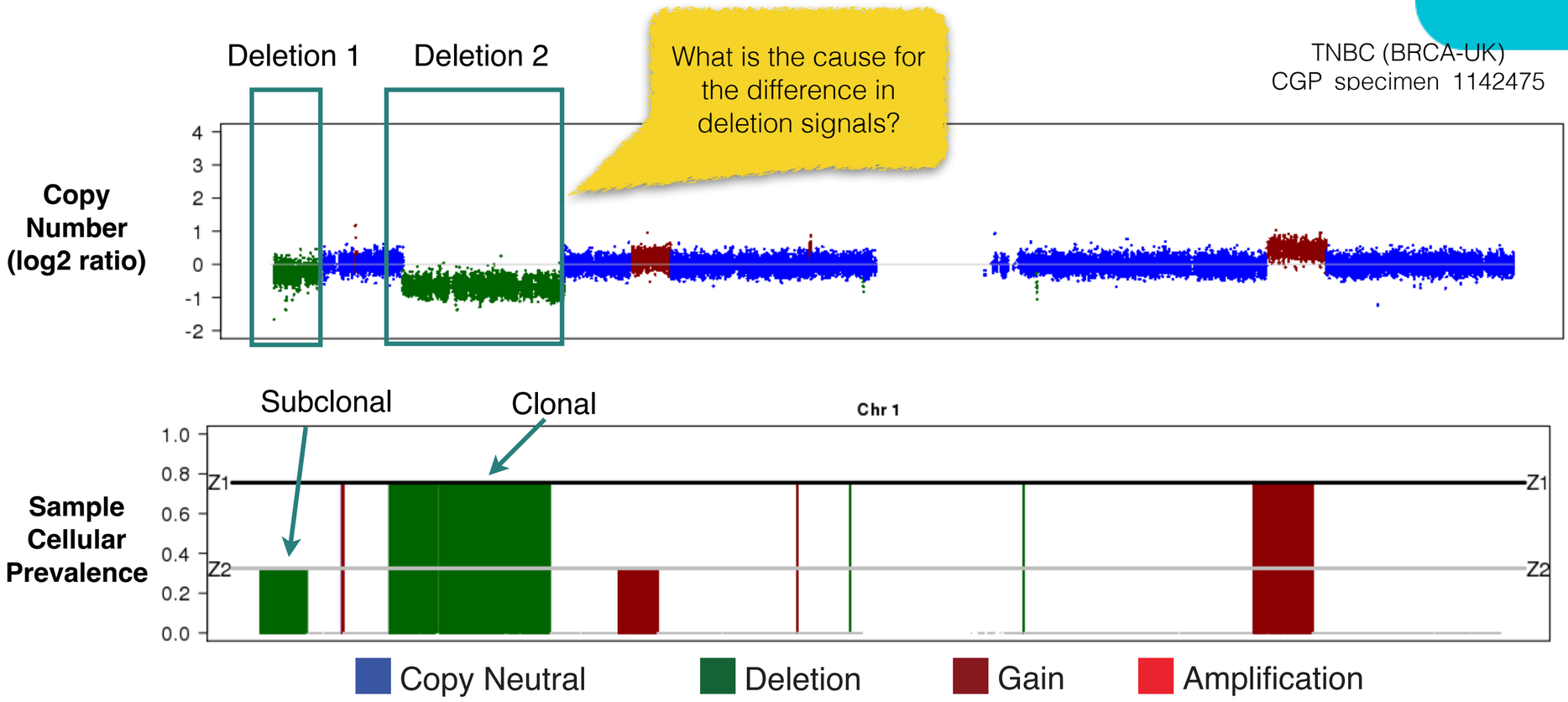
1. Take the derivative wrt to n
2. Equate to 0
3. Find the roots to estimate n

$$\frac{\partial(\log \mathbb{P}(n))}{\partial \boldsymbol{\mu}} \times \frac{\partial \boldsymbol{\mu}}{\partial n} = \frac{\partial(\log \mathbb{P}(n))}{\partial n} = 0 \text{ , then find } n$$

Since the Beta distribution is not conjugate with the Gaussian, we can use numerical optimization to find $\hat{n}$ that maximizes the $\log \mathbb{P}$

Copy Number Analysis of Subclonal Heterogeneity





- **Subclonal** CNA events have weaker signals compared to clonal CNAs because of contribution from *cancer cells* without the CNA event

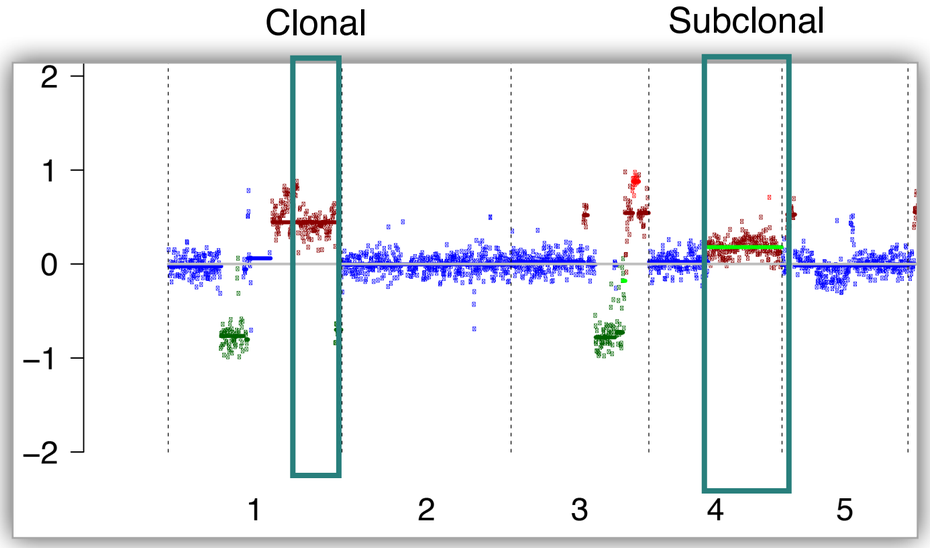
Ha et al. *Genome Research* **24**:1881-1893 (2014)

Modeling subclonal copy number

- Add two additional states for subclonal deletion and subclonal gain, $K_{sc} = \{1, 3\}$ and $K = \{0, 1, 2, 3, 4, 5, K_{sc}\}$
- The expected log ratio for subclonal copy number state $k_{sc} \in \{1, 3\}$ is

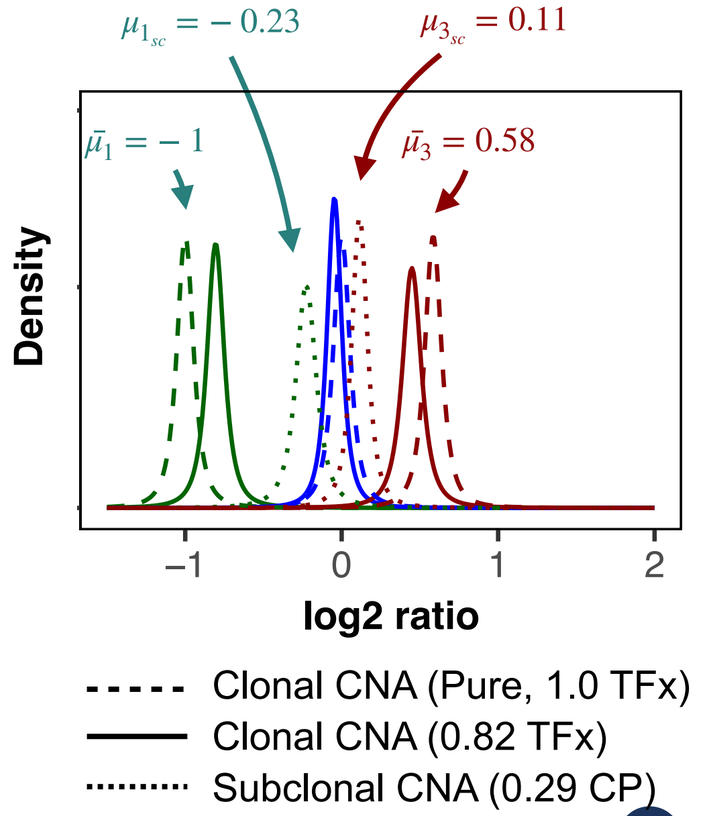
$$\mu_{k_{sc}} = \log_2\left(\frac{\overbrace{2n}^{\text{Normal}} + \overbrace{2(1-n)s}^{\text{Tumor w/o event}} + \overbrace{(1-n)(1-s)c_{k_{sc}}}^{\text{Tumor w/ event}}}{2}\right)$$

  - s is the fraction of **cancer cells without** CNA event
  - $(1 - s)$ is the fraction of **cancer cells with** CNA event (aka tumor cellular prevalence)



Tumor Fraction = 0.82

Cellular Prevalence = 0.29



Adalsteinsson*, Ha* Freeman* et al. *Nature Communications* **8**:1324 (2017)

3. Assessing Statistical Power for Variant Discovery

- Power calculation
- Calibrating sequencing depth for variant discovery
- References:
  - Cibulskis et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature Biotechnology* **31**:213-19 (2013)
  - Adalsteinsson et al. *Nature Communications* **8**:1324 (2017). DOI: 10.1038/s41467-017-00965-y

Sensitivity of Mutation Calling is Subject to Heterogeneity

- Tumor biopsy samples may exhibit intra-tumor heterogeneity
  - The tumor fraction (aka tumor content) influences our ability to detect an SNV at a specific locus

- Here are some questions that warrant statistical considerations:
  - What is our power (sensitivity) to detect an SNV given the read depth?
  - What read depth is required to detect an SNV at a specific power?
  - If we do not detect a mutation, is it because (1) there is no mutation? Or (2) we do not have sufficient power to make a confident call?

- Answering these questions with theoretical power calculations can help to calibrate the required sequencing depth and the expectation to detect mutations.

Power Calculation for Mutation Detection

- Let μ be the expected probability of observing a variant read at a locus
- Tumor fraction α, copy number c, and multiplicity M

$$\mu = \frac{\alpha M}{\alpha c + 2(1-\alpha)}$$

average tumor copies

average normal copies

"average # of chromosomes with the variant tumor cells in the sample"

"average # of chromosomes from all cells in sample"

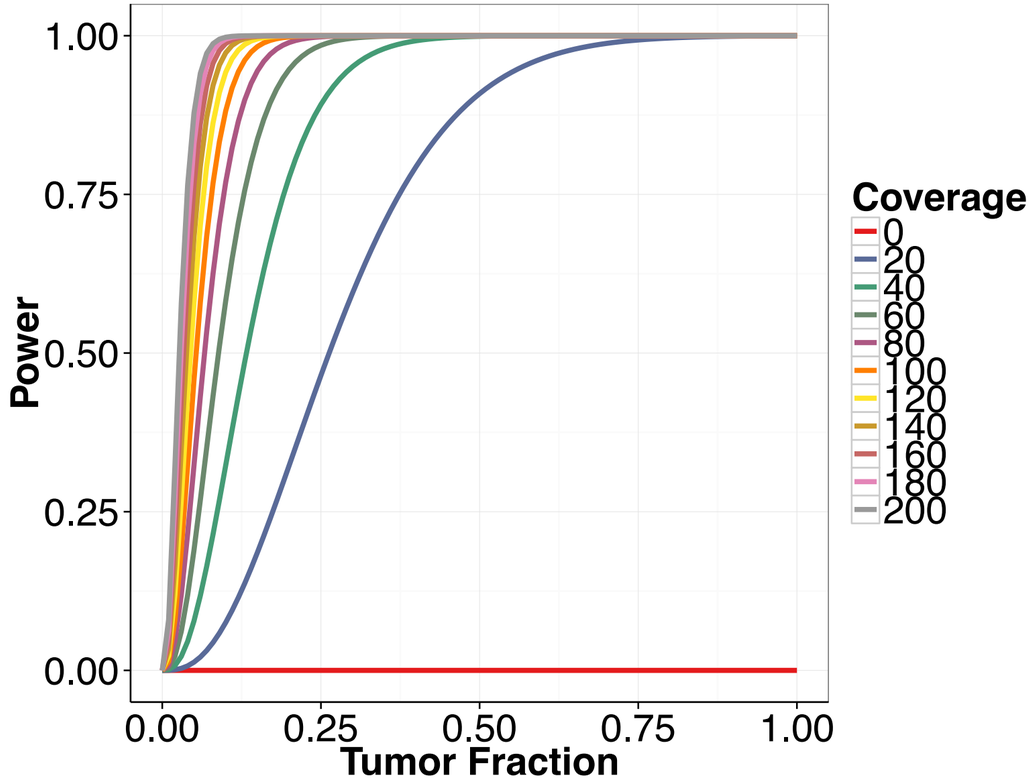
- $\mu = \frac{\alpha}{2}$ for tumor copy number $c = 2$ and multiplicity $M = 1$ (for heterozygous SNV, e.g. AB)
- The power to detect ≥ 3 variant reads at locus i with N_i total read depth is estimated using a binomial exact test

$$p(X \geq 3) = \sum_{k=3}^{N} Bin(k \mid N, \mu)$$

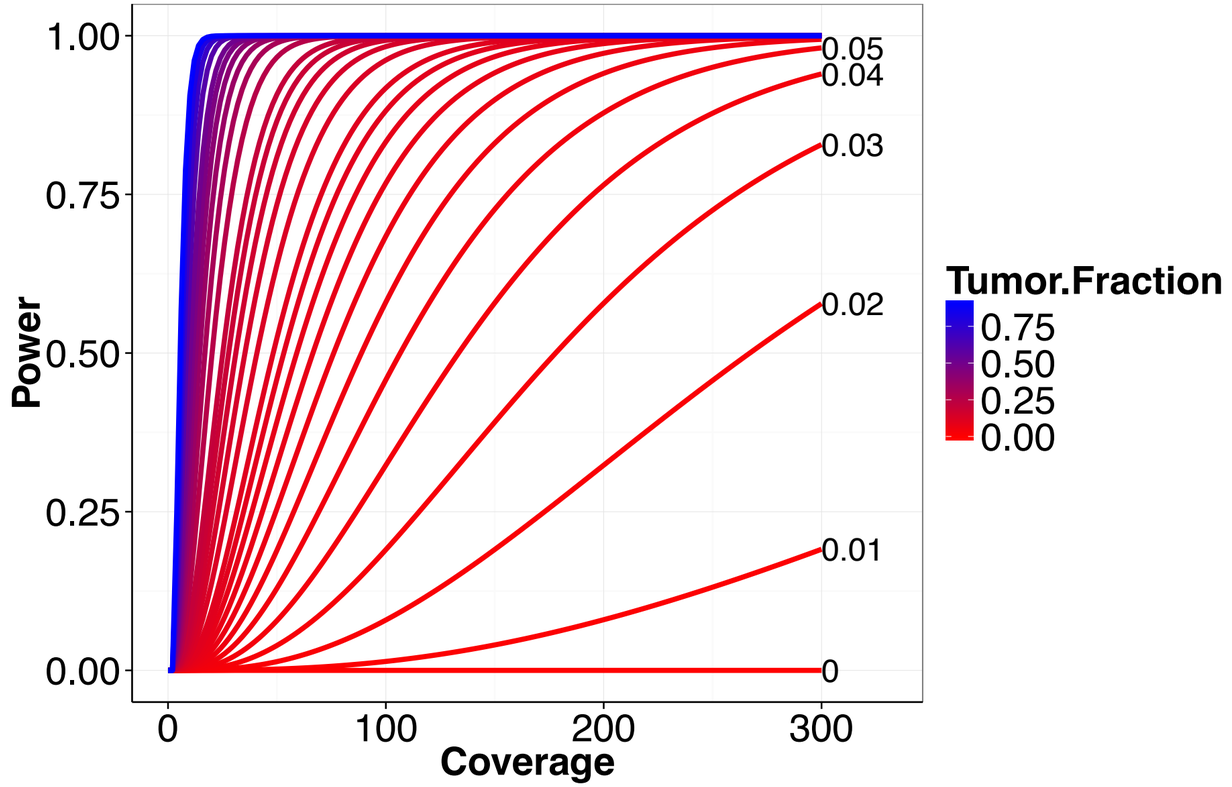
$$p(X \geq 3) = 1 - \left[Bin(0 \mid N, \mu) + Bin(1 \mid N, \mu) + Bin(2 \mid N, \mu)\right]$$

Power Calculation for Mutation Detection

What is our power (sensitivity) to detect an SNV at a specific tumor fraction?

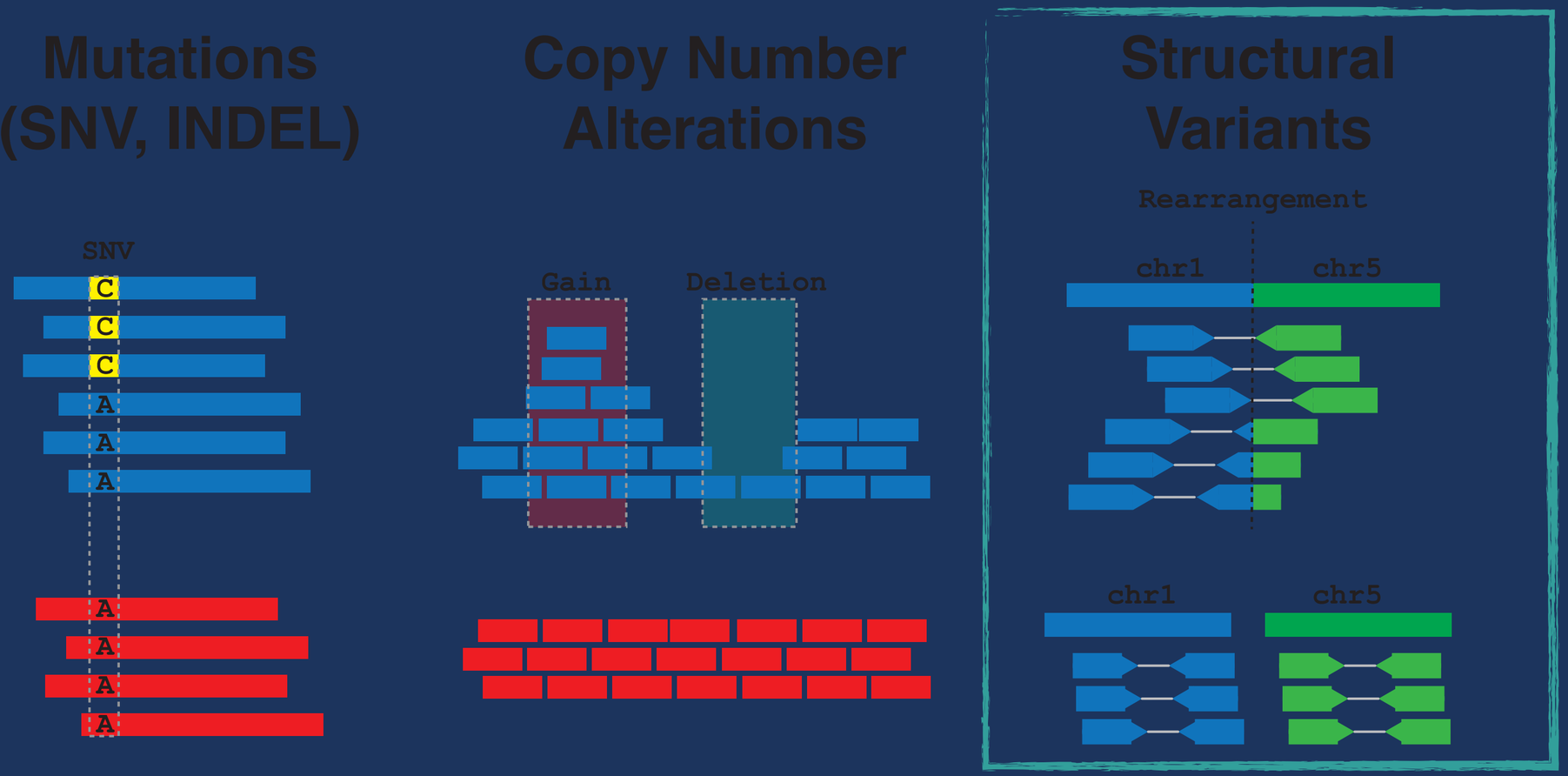


What read depth is required to detect an SNV at a specific power?



Adalsteinsson*, Ha*, Freeman* et al. *Nat Commun* **8**:1324 (2017)

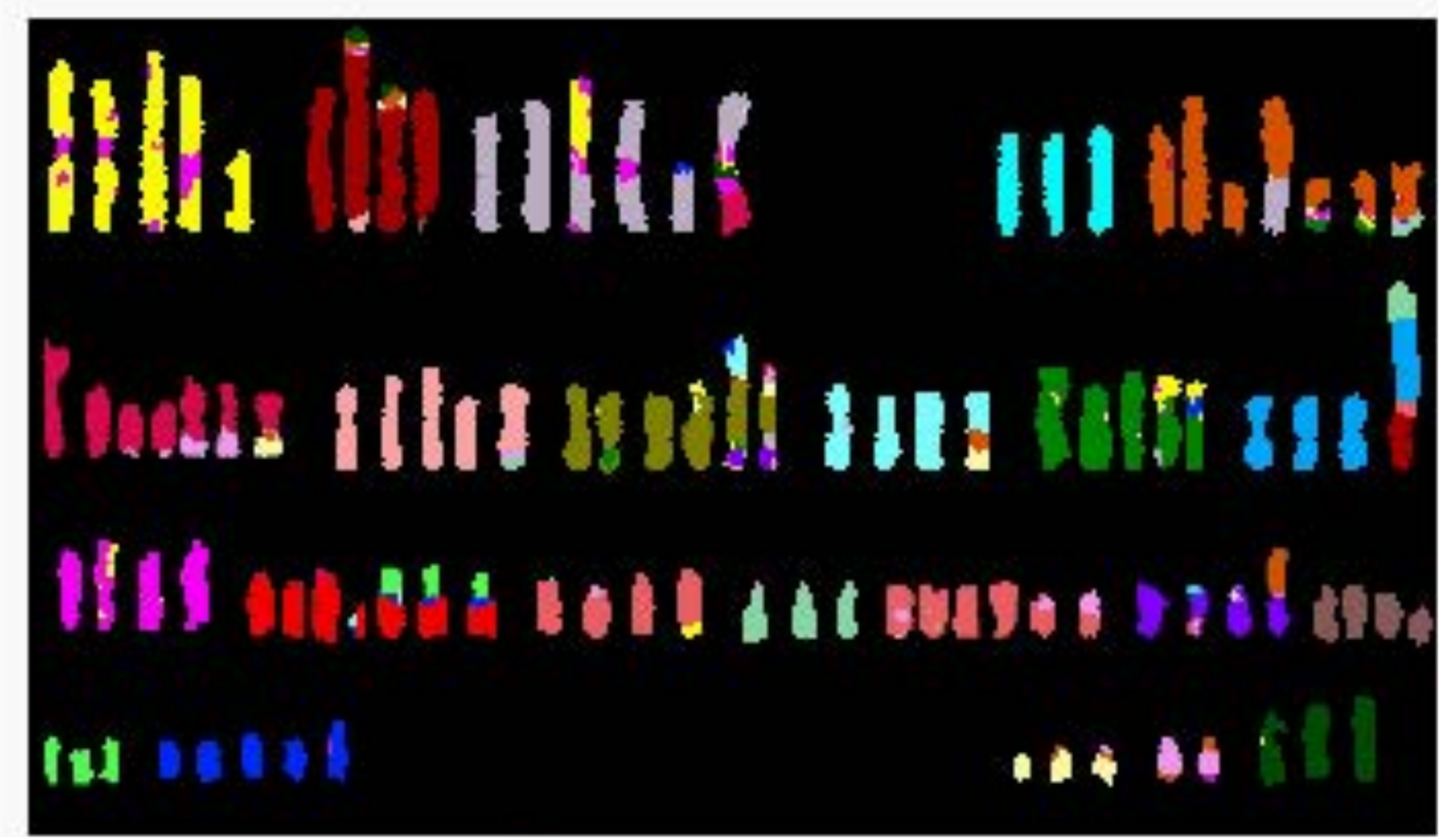
4. Structural Rearrangement Analysis of Cancer Genomes



4. Structural Rearrangement Analysis of Cancer Genomes

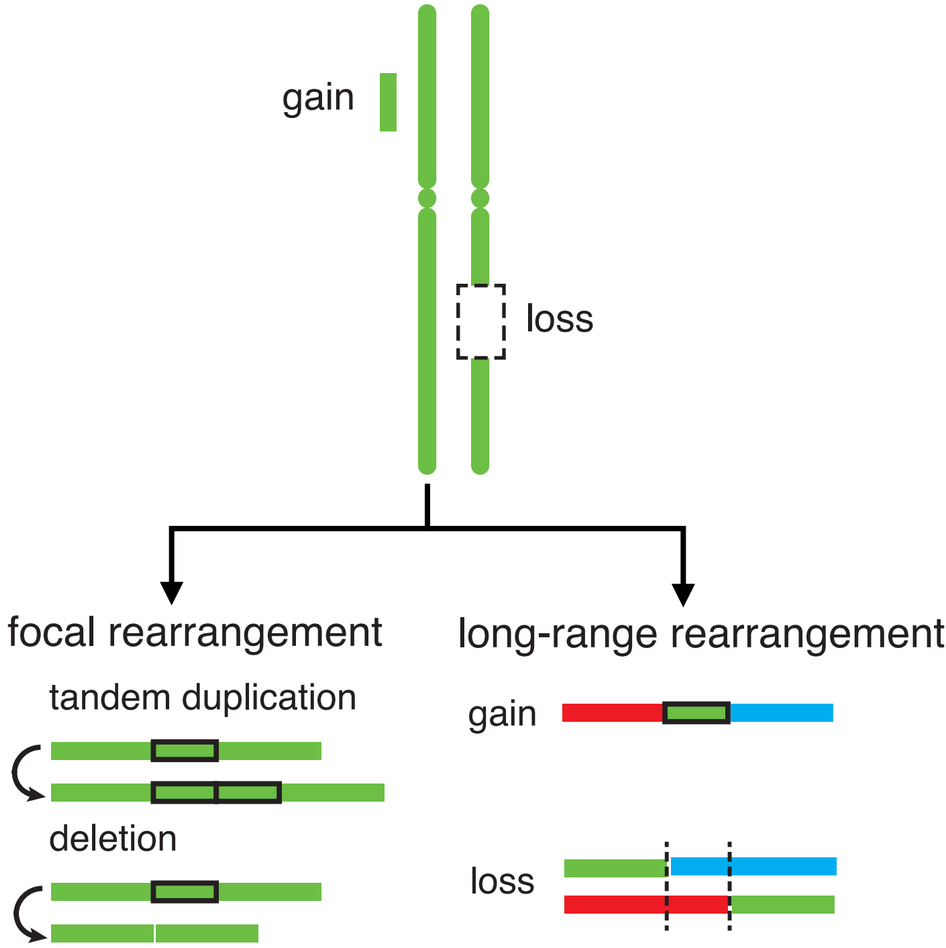
- Structural variant types predicted from sequencing analysis
- Complex genomic structural rearrangement patterns
- Brief overview of software tools

Abnormal chromosomal rearrangements are prevalent in cancer



David Huntsman, BC Cancer Agency

Copy number alterations
(amplitude/dosage)



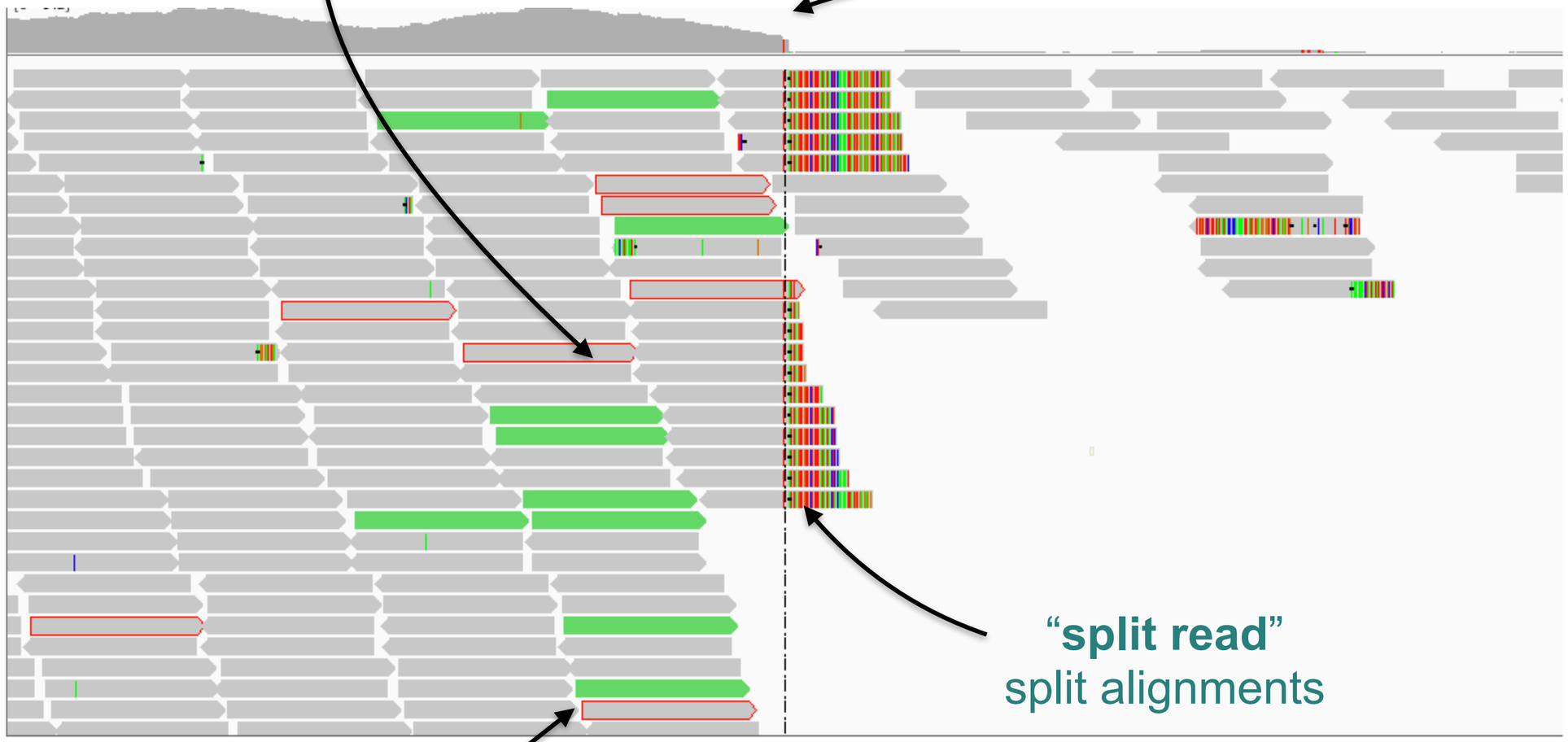
Structural rearrangements
(location/configuration)

Zhang and Pellman. *CSH Symp Quant Biol.* **80**:117-37 (2016)

Structural Variants: Sequence Features

“**discordant read pair**”
read pairs with aberrant inferred fragment length

“**copy number change**”
abrupt change in read coverage

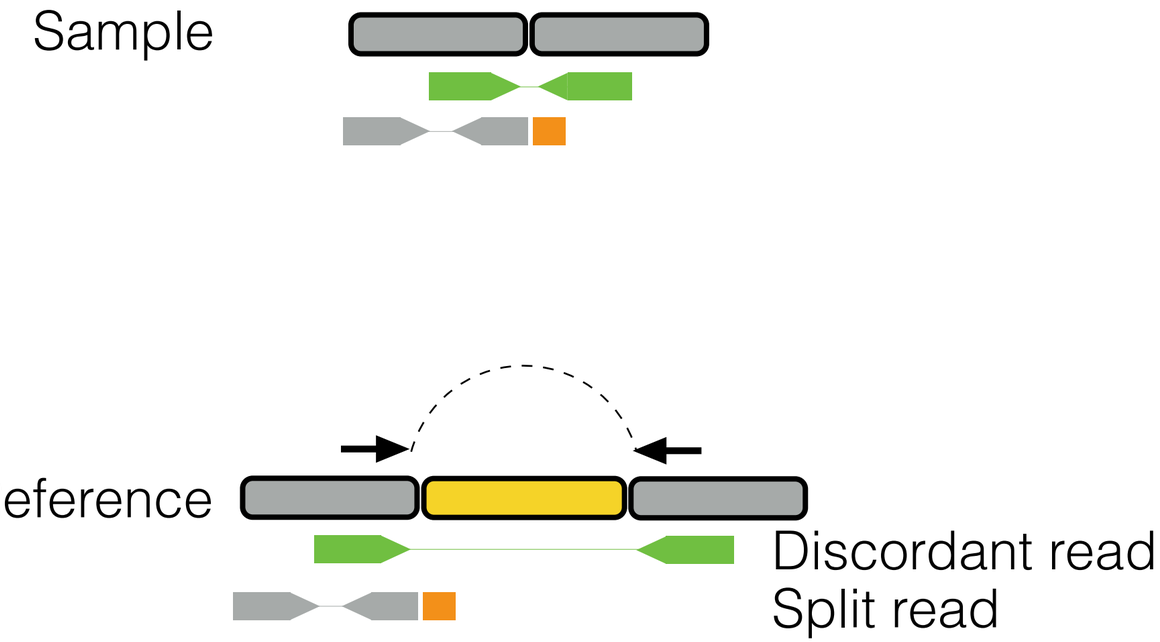


“**split read**”
split alignments

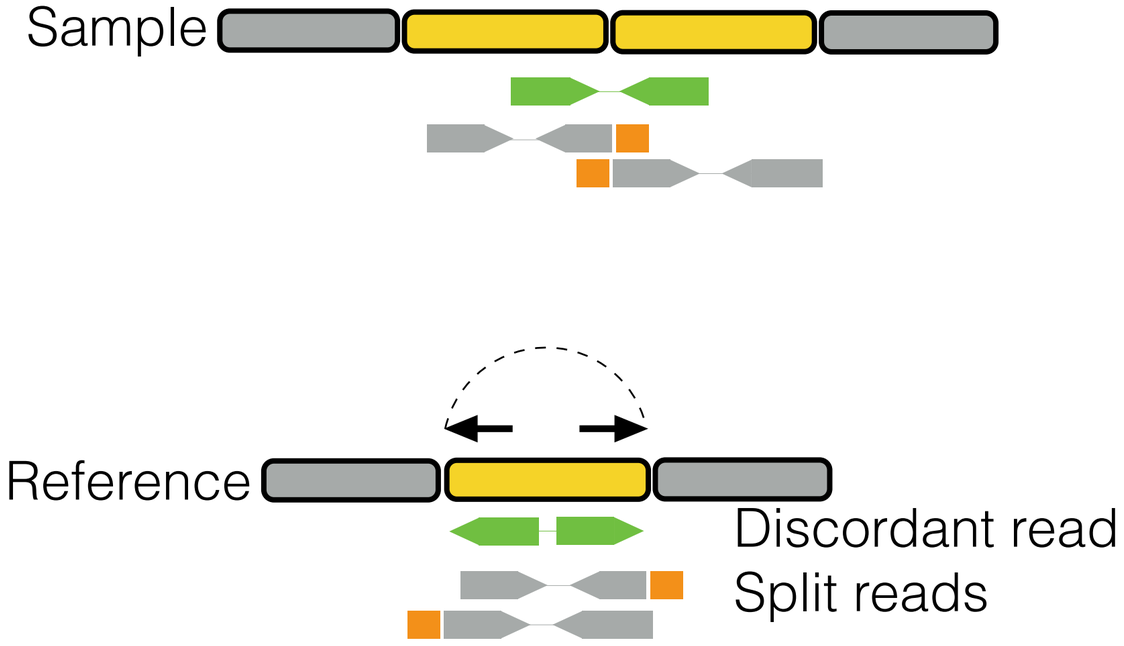
pair-mates unmapped

Simple Structural Variants: Deletion & Tandem Duplications

Deletion

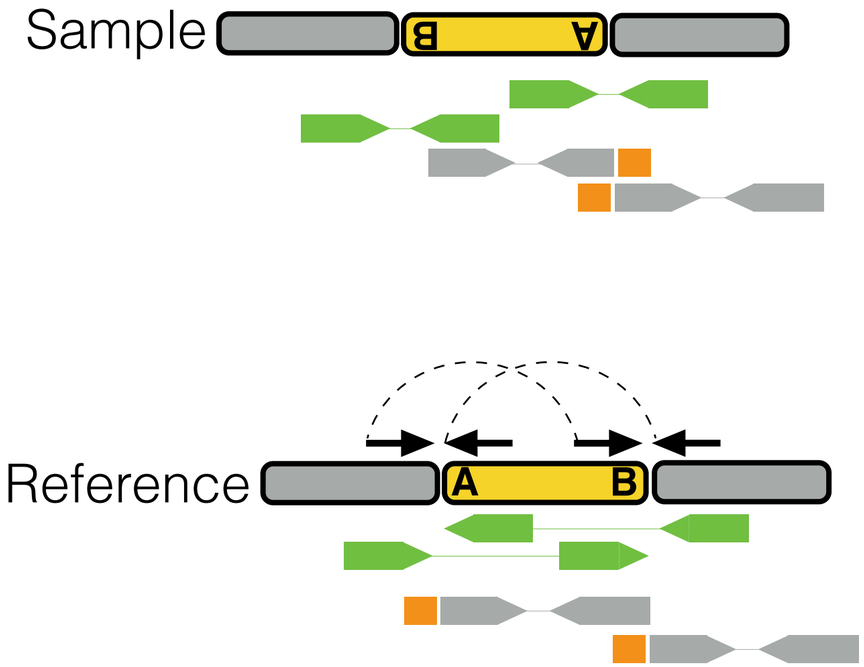


Tandem Duplication

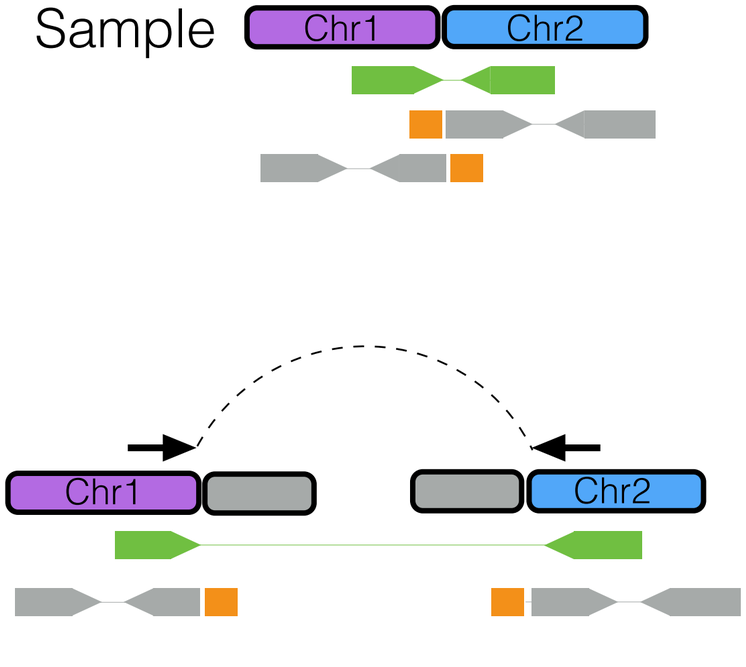


Simple Structural Variants: Inversions & Translocations

Inversion



Translocation

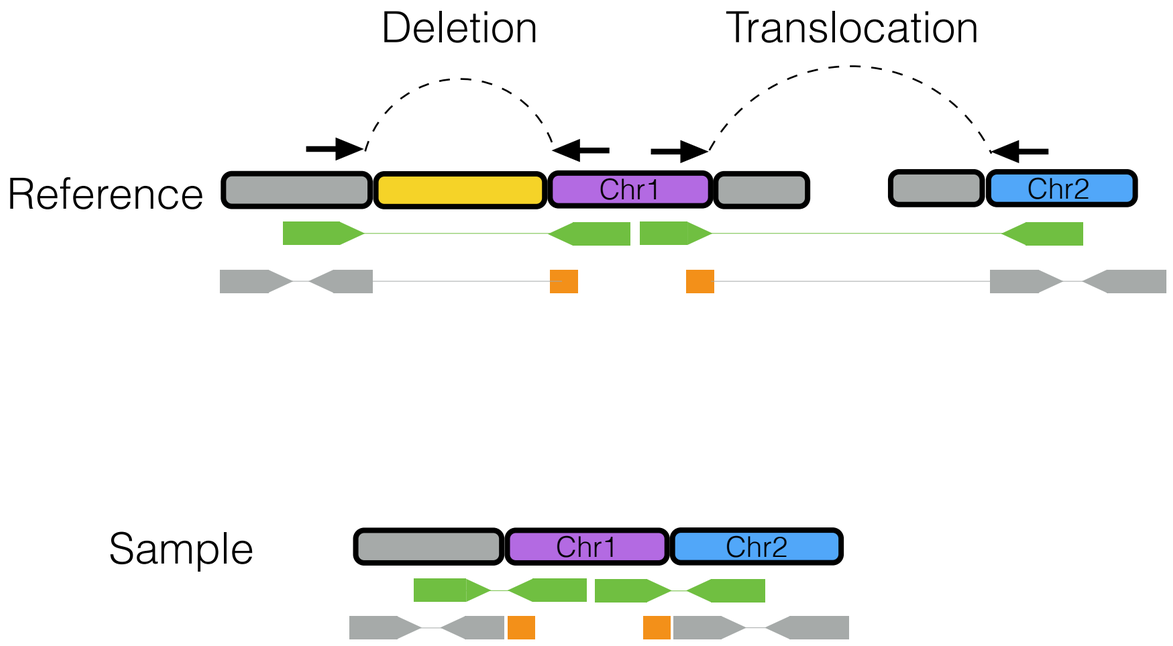


Discordant read
Split reads

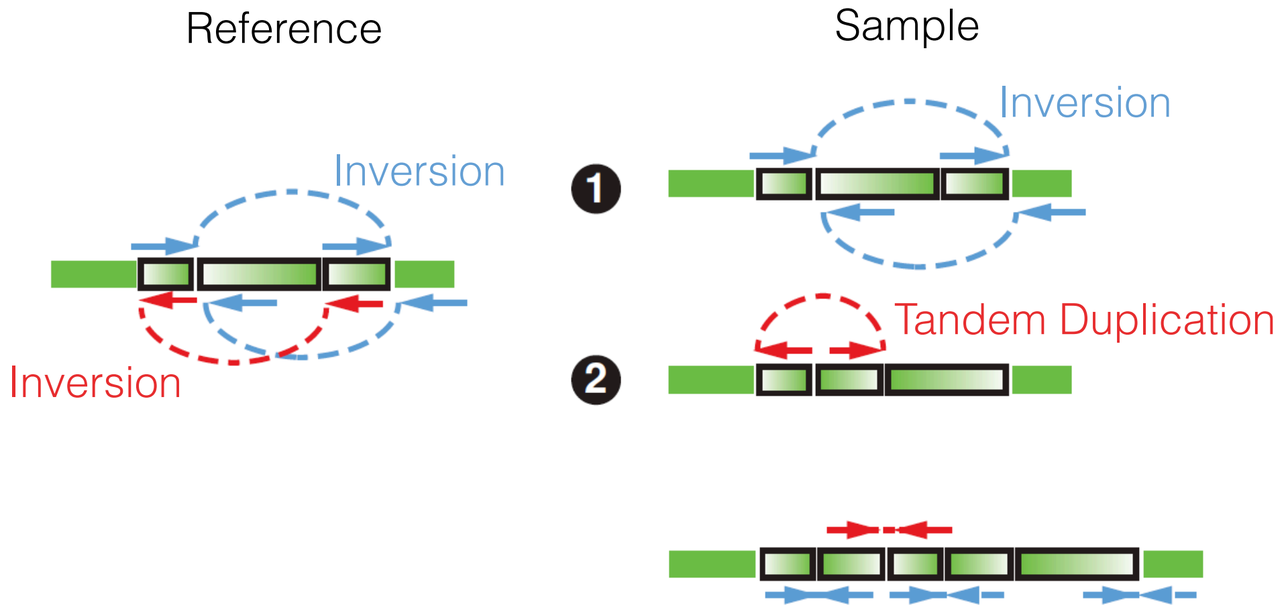
Reference
Discordant read
Split read

Complex Structural Variants of 2+ events

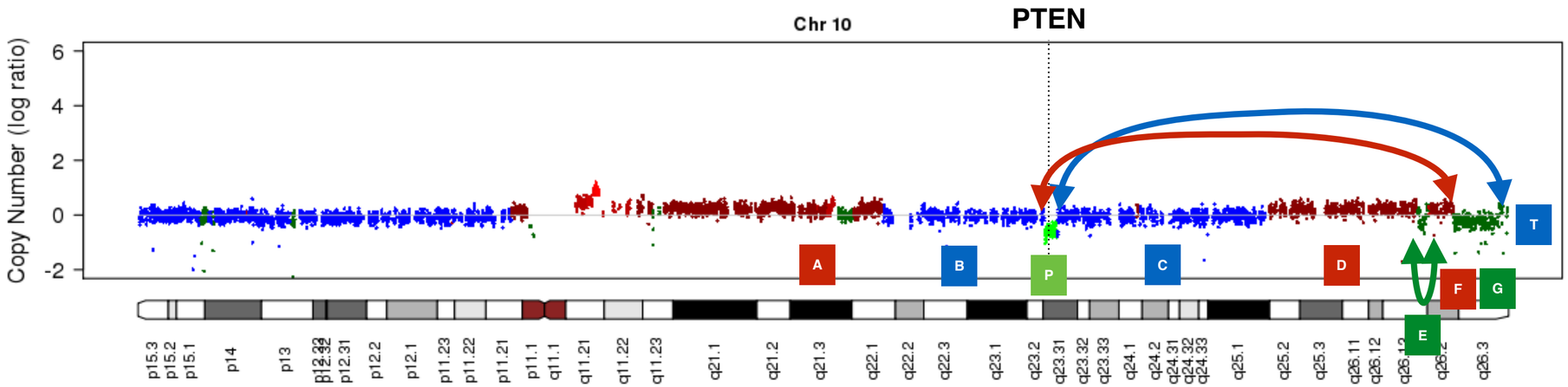
Complex Event (non-overlapping)

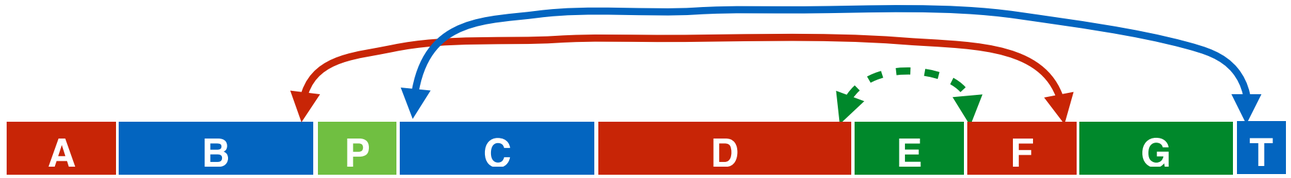


Complex Event (overlapping)

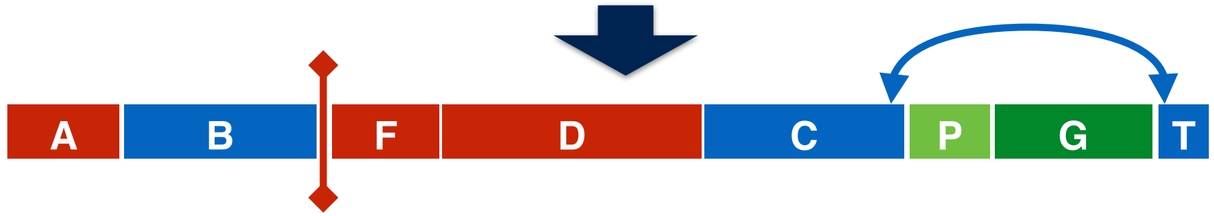


Complex Structural Variant: Example of PTEN deletion

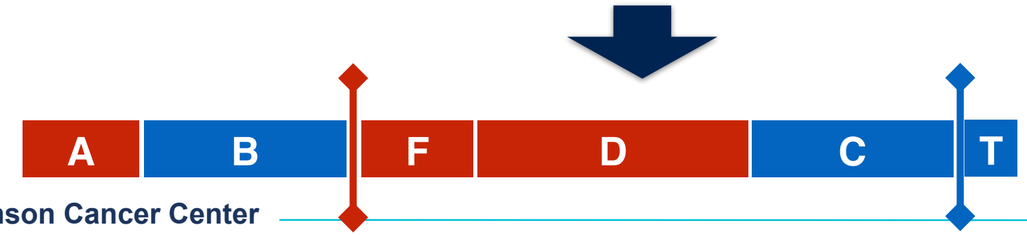




INVERSIONS

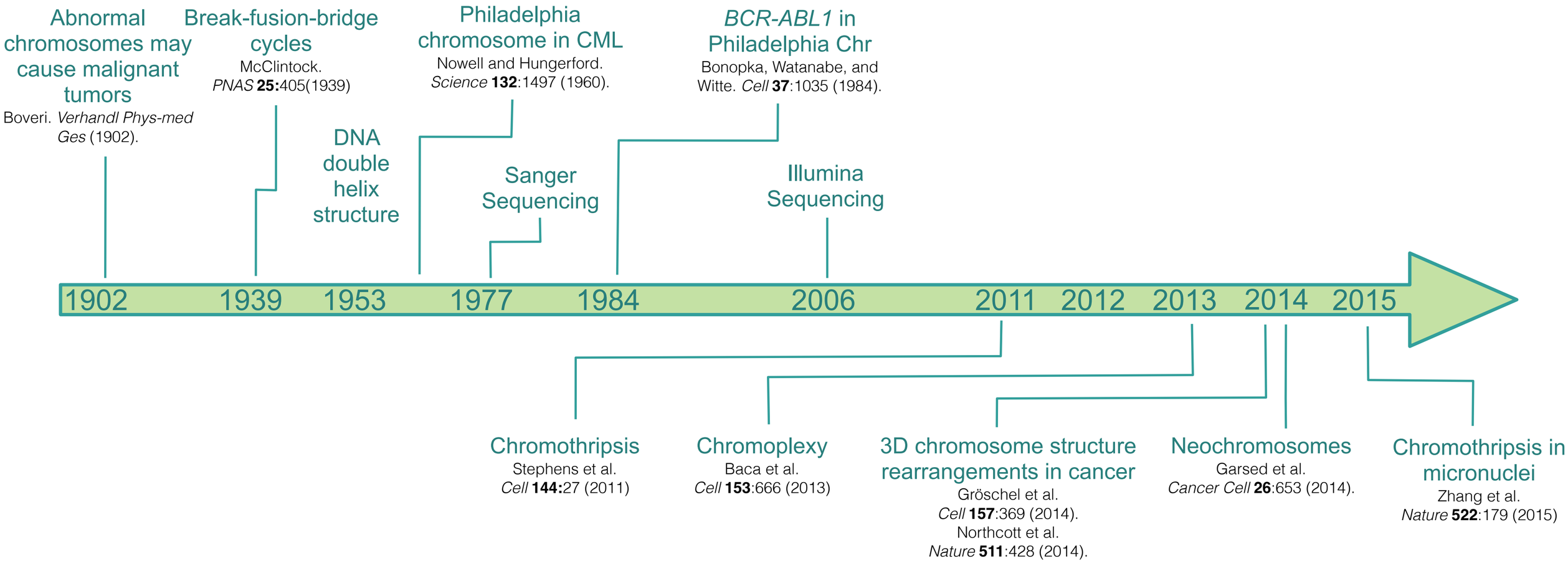


DELETION



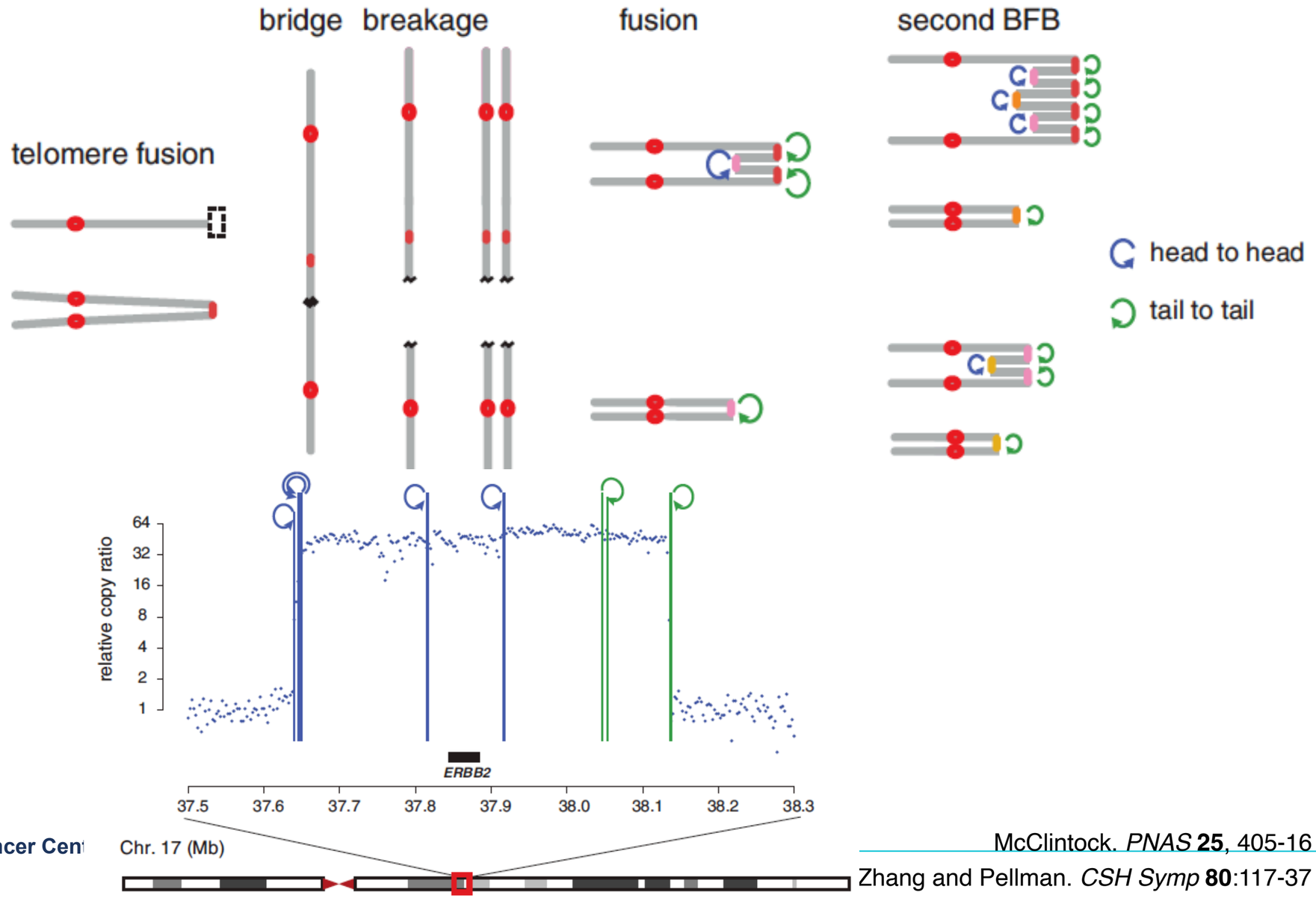
Viswanathan*, Ha*, Hoff*, et al. *Cell* **174**:433-447 (2018)

Brief History of Genome Rearrangement Discoveries in Cancer



Complex Cancer Genome Rearrangement Patterns

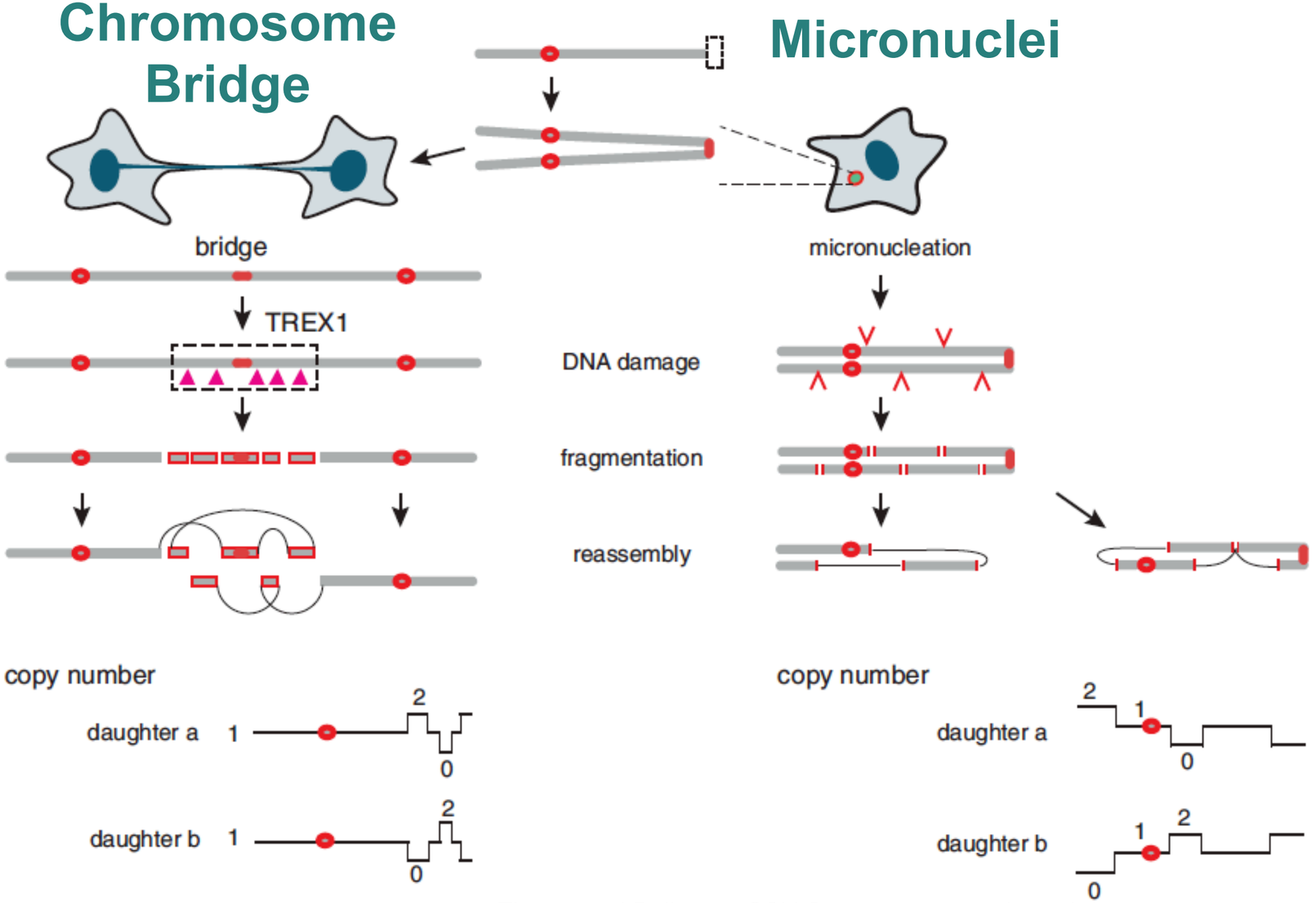
Breakage-Fusion-Bridge (BFB) Cycles



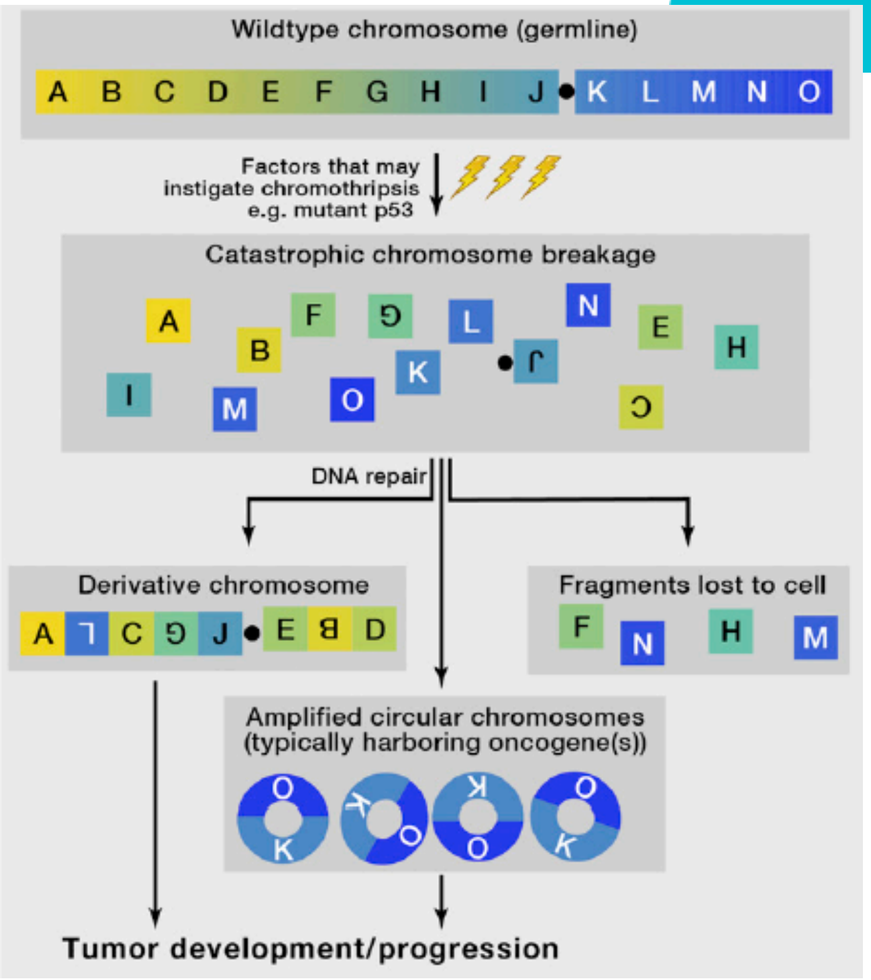
McClintock. *PNAS* **25**, 405-16 (1939)

Zhang and Pellman. *CSH Symp* **80**:117-37 (2016)

Chromothripsis: Catastrophic DNA shattering

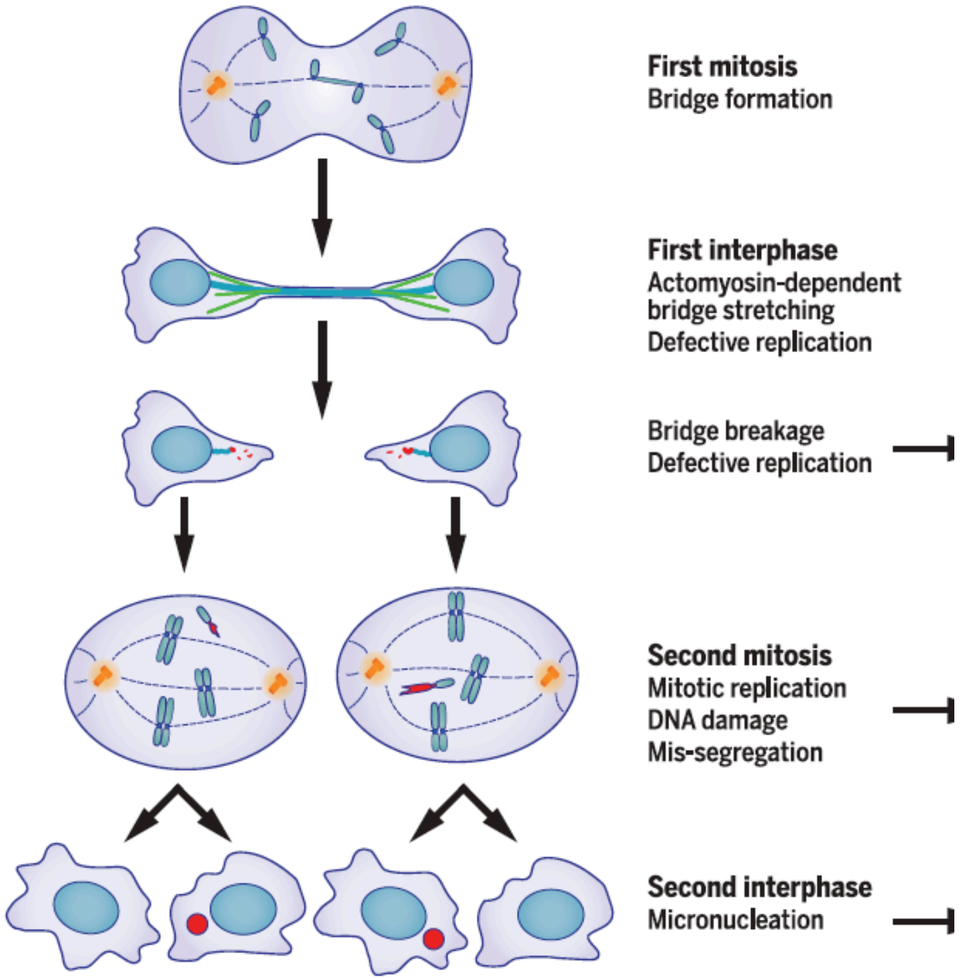


Zhang and Pellman. *CSH Symp* **80**:117-37 (2016)

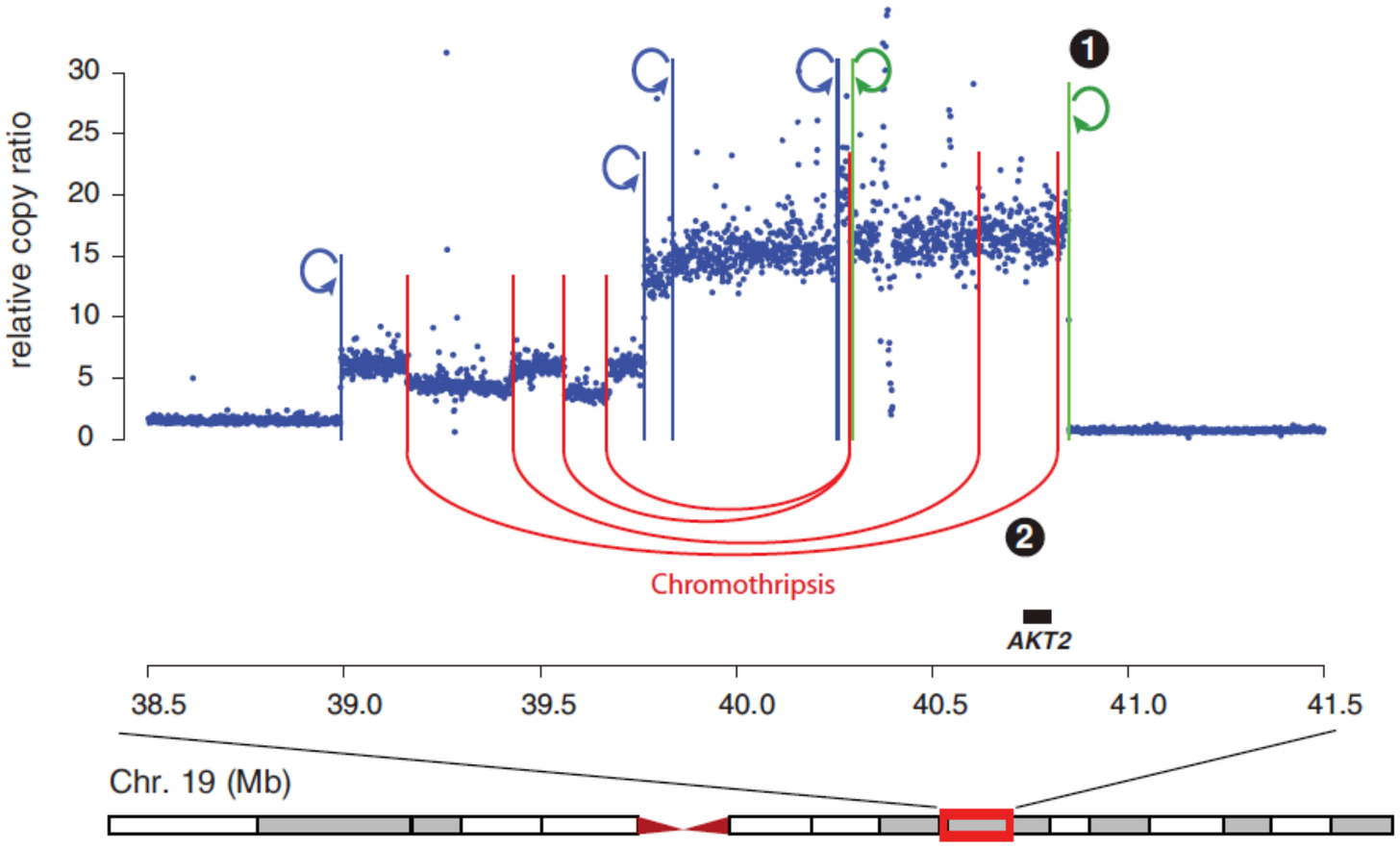


Stephens et al. *Cell* **144:**27-40 (2011)
Korbel and Campbell. *Cell* **152**:1226-36 (2013)

Concurrent Breakage-Fusion-Bridge & Chromothripsis

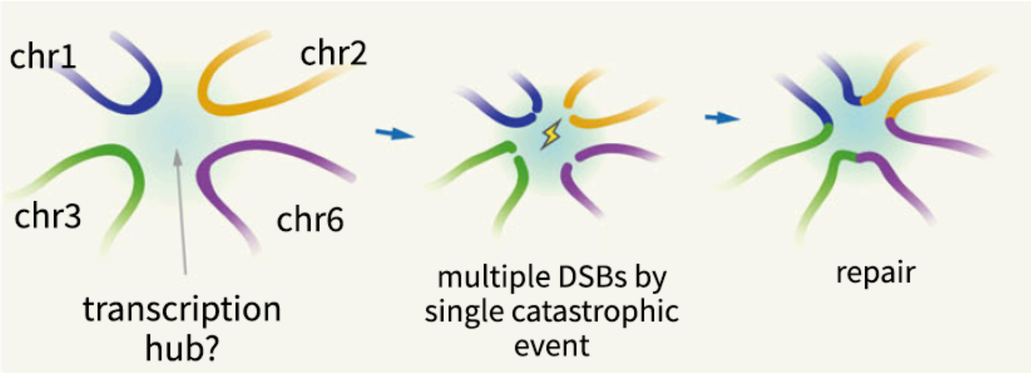


Umbreit et al. *Science* **368**:282 (2020)

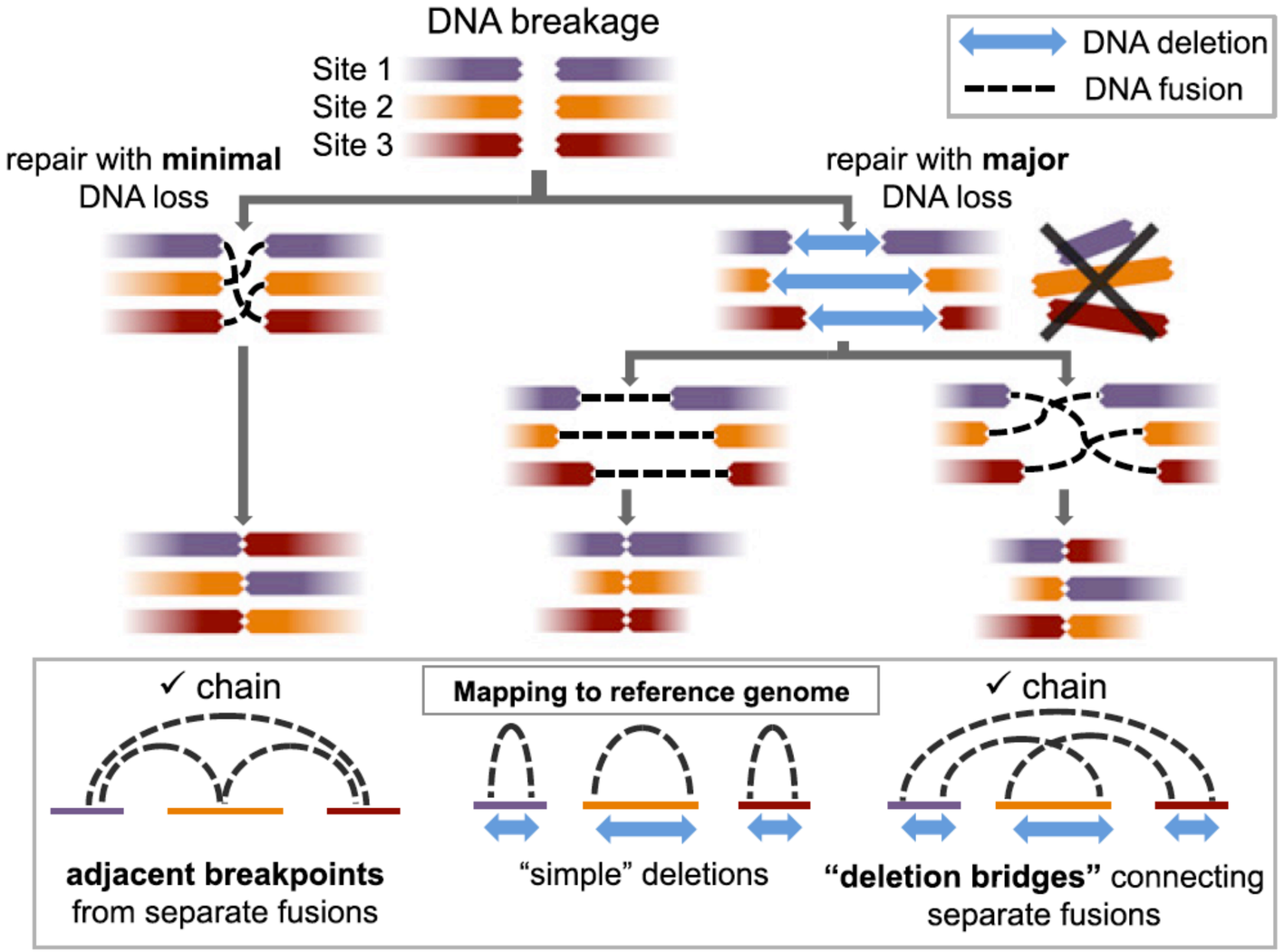


Zhang and Pellman. *CSH Symp* **80**:117-37 (2016)

Chromoplexy: Inter-dependent disruption of DNA within close spatial proximity



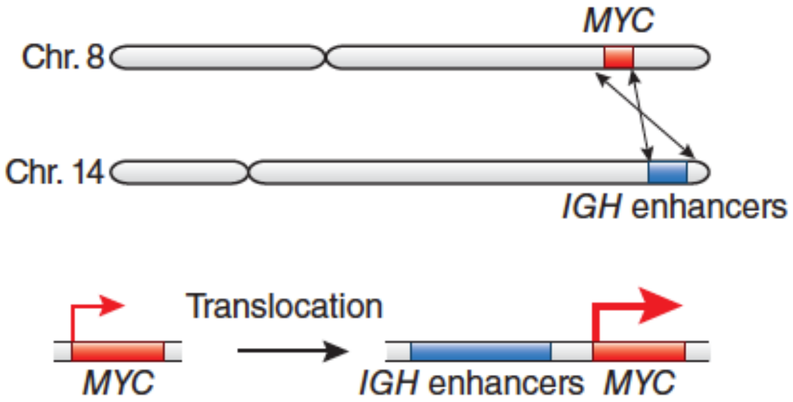
Yi and Ju. *Expt. Mol. Med.* **50**:98 (2014).



Baca et al. *Cell* **153**:666-77 (2013)

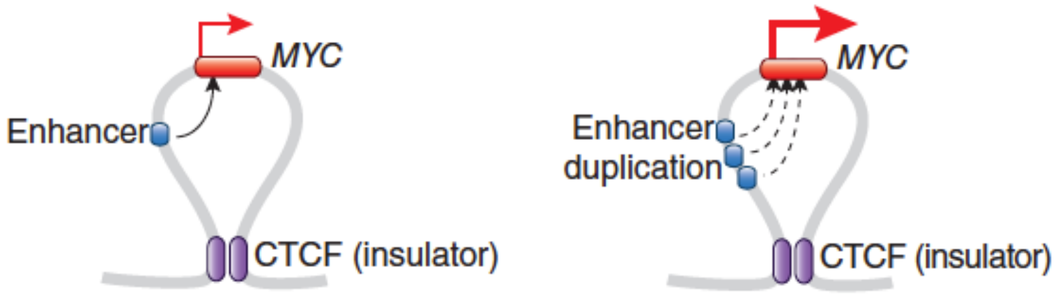
Alterations of oncogene regulation and genome topology

Translocation



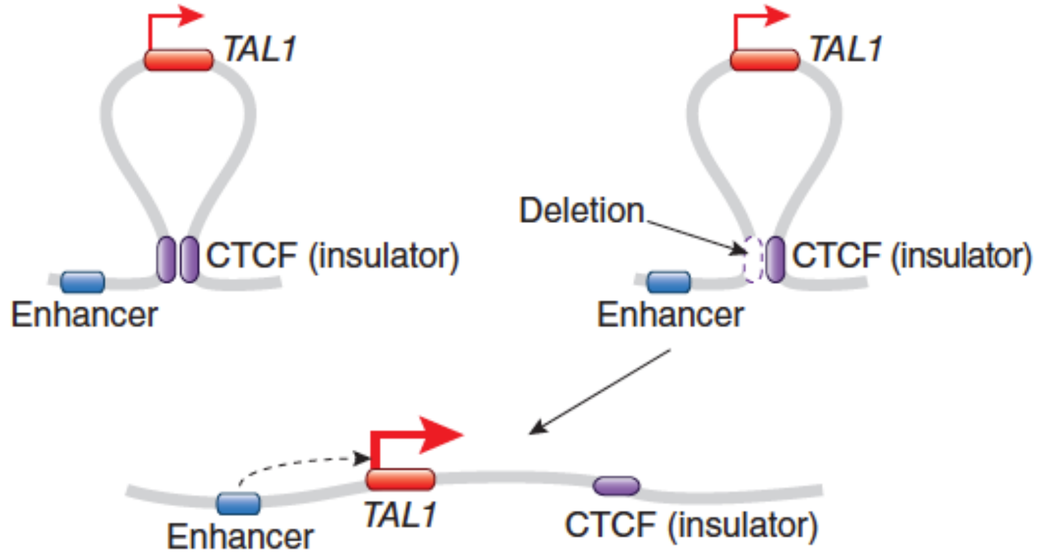
Battey et al. *Cell* **34**:779-87 (1983).

Duplication of Enhancer



Zhang et al. *Nat Genet* **48**:176-82 (2016).

Enhancer Hijacking



Beroukhim, Zhang, Meyerson. *Nat Genet* **49**:5-6 (2017).

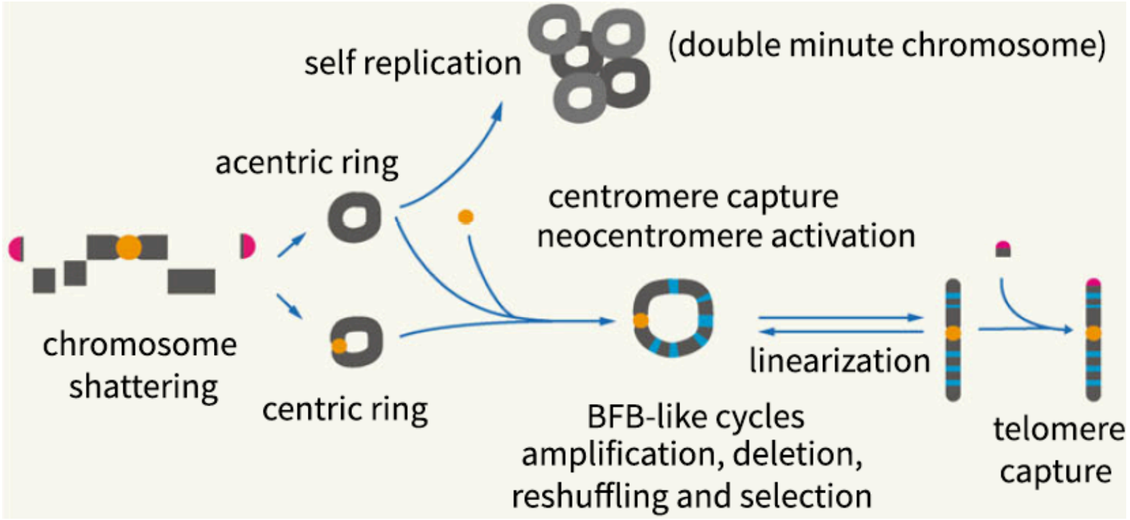
Gröschel et al. *Cell* **157**:369-81 (2014).

Northcott et al. *Nature* **511**:428-34 (2014).

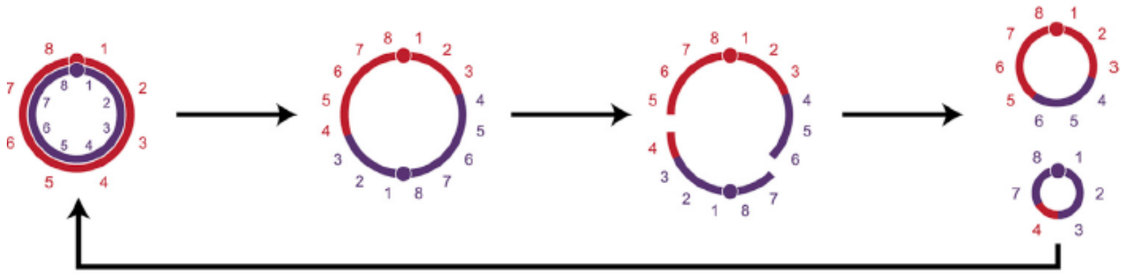
Hnisz et al. *Science* **351**:1454–58 (2016).

Weischenfeldt et al. *Nat Genet* **49**:65-74 (2017).

Extra-Chromosomal DNA: Double Minutes & Neo-chromosomes

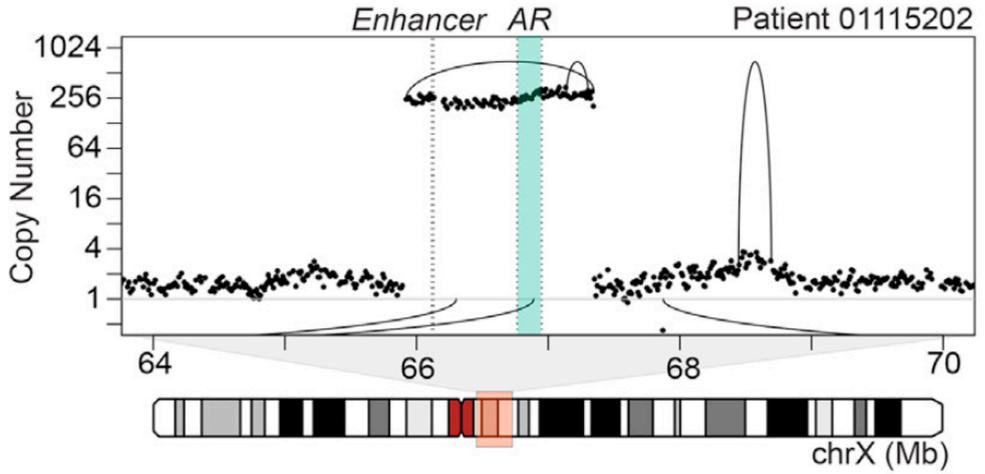


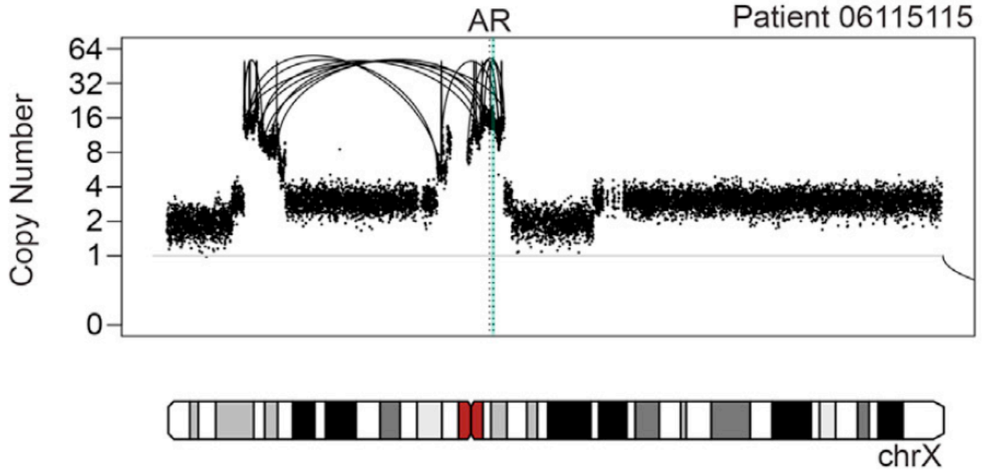
Yi and Ju. *Expt. Mol. Med.* **50**:98 (2014).



Garsed et al. *Cancer Cell* **26**:653-67 (2014).

Double Minute





Neo-Chromosomes

Viswanathan*, Ha*, Hoff*, et al. *Cell* **174**:433-447 (2018)

Structural Variation Tools for Cancer Genome Analysis

Popular SV Methods for Cancer Genomes

SV Breakpoint Methods	Discordant Reads	Split Reads	Assembly	Software	References
DELLY	✔	✔		https://github.com/dellytools/delly	Rausch et al. Genome Biol (2012)
LUMPY	✔	✔		https://github.com/arq5x/lumpy-sv	Layer et al. Genome Biol (2014)
GRIDSS	✔	✔	✔	https://github.com/PapenfussLab/gridss	Cameron et al. Genome Biol (2021)
SVABA	✔	✔	✔	https://github.com/walaj/svaba	Wala et al. Genome Res (2018)
BRASS	✔	✔	✔	https://github.com/cancerit/BRASS	Sanger Pipeline

Over 70 tools!

Complex Rearrangements	Methods	References
Chromothripsis	ShatterSeek ShatterProof	Cortés-Ciriano et al. Nat Genet (2020) Govind et al. BMC Bioinf (2014)
Chromoplexy	ChainFinder	Baca et al. Cell (2013)
Extra-chromosomal DNA	AmpliconArchitect	Deshpande et al. Nat Commun (2019)
SV clusters/footprints	ClusterSV GRIDSS	Li et al. Nature (2020) Cameron et al. Genome Res (2017)

Homework #8: Profiling copy number alterations

A. Implement a copy number alteration (CNA) caller described in Lecture 3

- Implement components of a continuous HMM in a Bayesian framework
- Learn the parameters and infer the genotypes using EM
- Predict the copy number alteration segments for a chromosome.
- Expected outputs for each question will be provided so that you can check your code.

B. Power calculations for mutation detection described in Lecture 4

Due: May 26th, 2023