

CANCER GENOMICS

Lecture 1: Introduction to Cancer Genome Analysis

GENOME 541
Spring 2020



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Human Biology Division



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GavinHaLab.org

Overview of Cancer Genomics Module

- 1. Introduction to Cancer Genome Analysis**

- 2. Probabilistic Methods for Mutation Detection**

- 3. Probabilistic Methods for Profiling Copy Number Alteration**

- 4. Additional Topics: Tumor Heterogeneity, Mutation Detection Power, Structural Variation**

Homework Assignments and Office Hours

TA for Module: Anna-Lisa Doebley (adoebley@uw.edu)

Homework #5

Due: May 8th

Office Hours

- Monday, May 4, 2-3pm
- Wednesday, May 6, 2-3pm

Homework #6

Due: May 15th

Office Hours

- Monday, May 11, 2-3pm
- Wednesday, May 13, 2-3pm

Outline: Introduction to Cancer Genome Analysis

1. Background on Cancer Genome Alterations

- Genomic alterations in cancer: drivers vs passengers, somatic vs germline
- Tumor evolution and heterogeneity

2. Overview of Cancer Genome Analysis

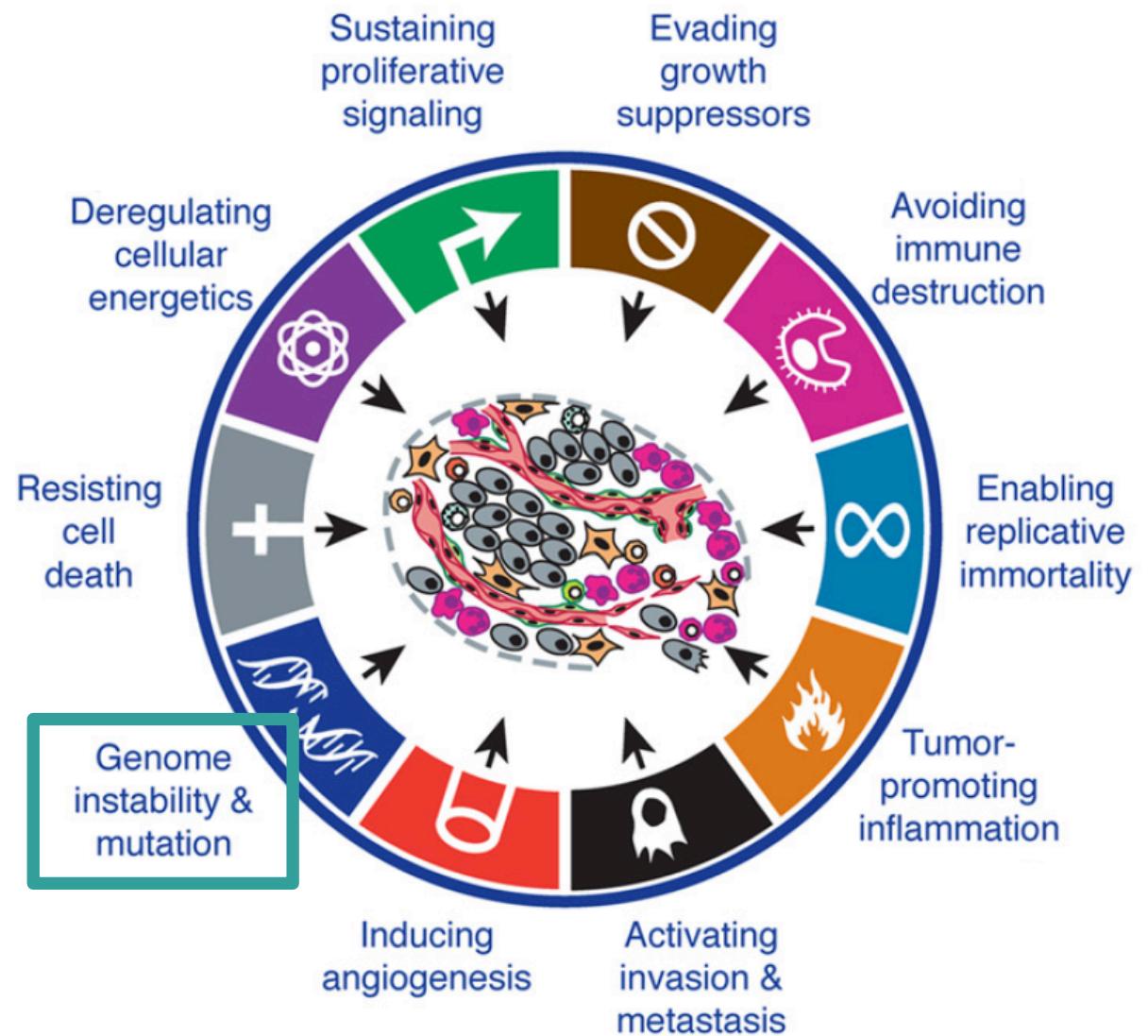
- Computational strategy and workflow
- Tumor DNA Sequencing
- Types of genomic alterations predicted from tumor sequencing
- Methods/tools/algorithms in following lectures

3. Primer on statistical modeling

- Probability distribution, Bayesian statistics, inference

The hallmarks of cancer

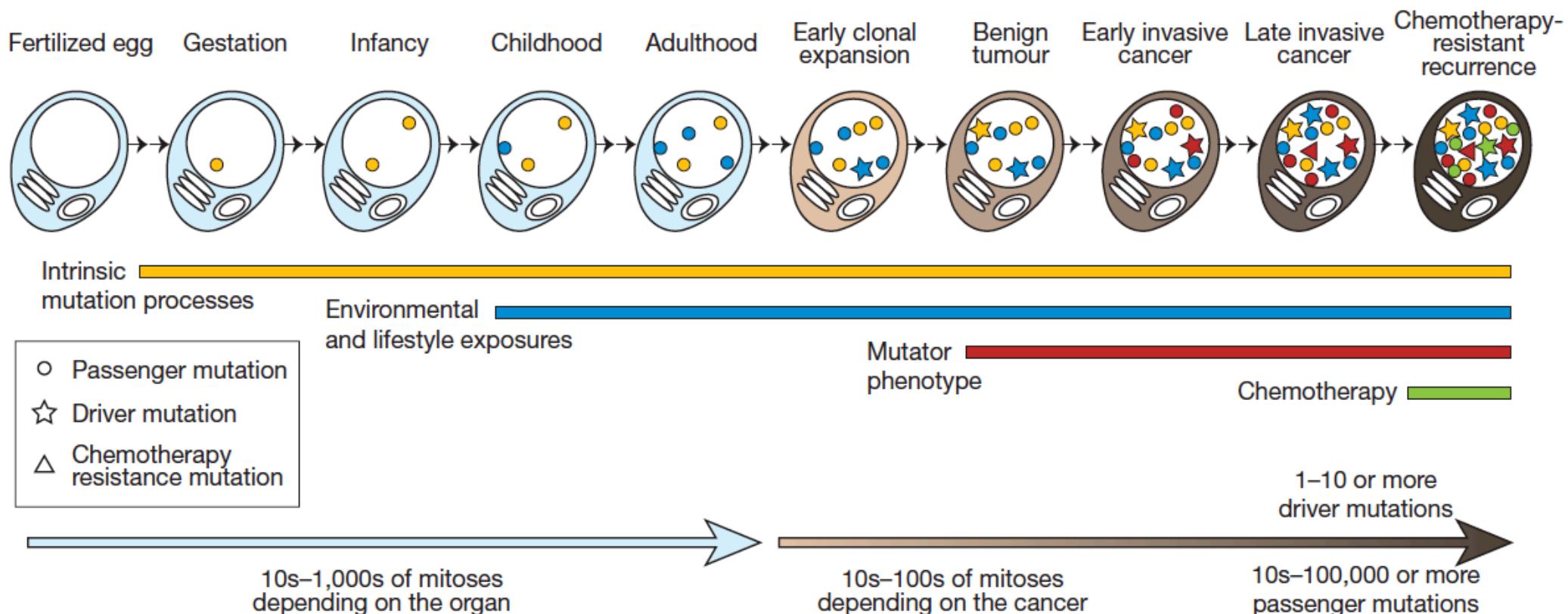
- All cancers exhibit many of these hallmarks that lead to tumor growth
- **Genome instability & mutation** is an enabling characteristic that can result in multiple hallmarks



Cancer is a disease of the genome

Cancer progression results from **mutations** acquired throughout lifetime

- Few **driver** mutations, many **passenger** mutations
- Mutational process can be intrinsic and from environmental mutagens



Genomic Variation: Somatic and Germline

Variant or Mutation or Alteration or Polymorphism

- Changes in the genome sequence of a sample compared to a reference sequence

Germline Variant

- Chromosomes: 22 autosomal pairs + 1 sex pair
 - Each set inherited from maternal and paternal germline cells
- Variant inherited from one or both parental chromosomes
- Source of genetic differences between ancestral populations and individuals
- Polymorphism: >1% frequency in a population

Somatic Variant

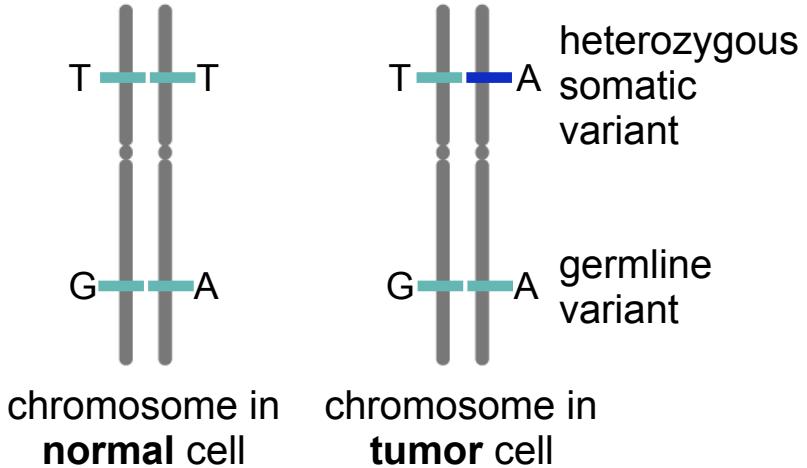
- Mutation acquired during individual's lifetime
- Important to identify in sporadic cancers and other non-familial diseases

Types of Genomic Variation: Small/Short mutations

1. Single nucleotide base substitutions

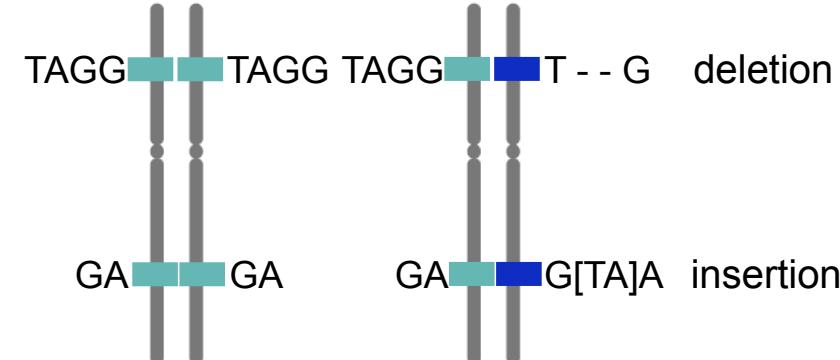
- Germline single nucleotide polymorphism (SNP)
- Somatic single nucleotide variant (SNV)

Single nucleotide variant



2. Small insertions or deletions

- Germline or somatic insertion or deletion (INDEL)
- Small indels: 1 bp - 20 bps
- Large indels: 20 - 10,000 bps



Insertion-Deletion (INDEL)

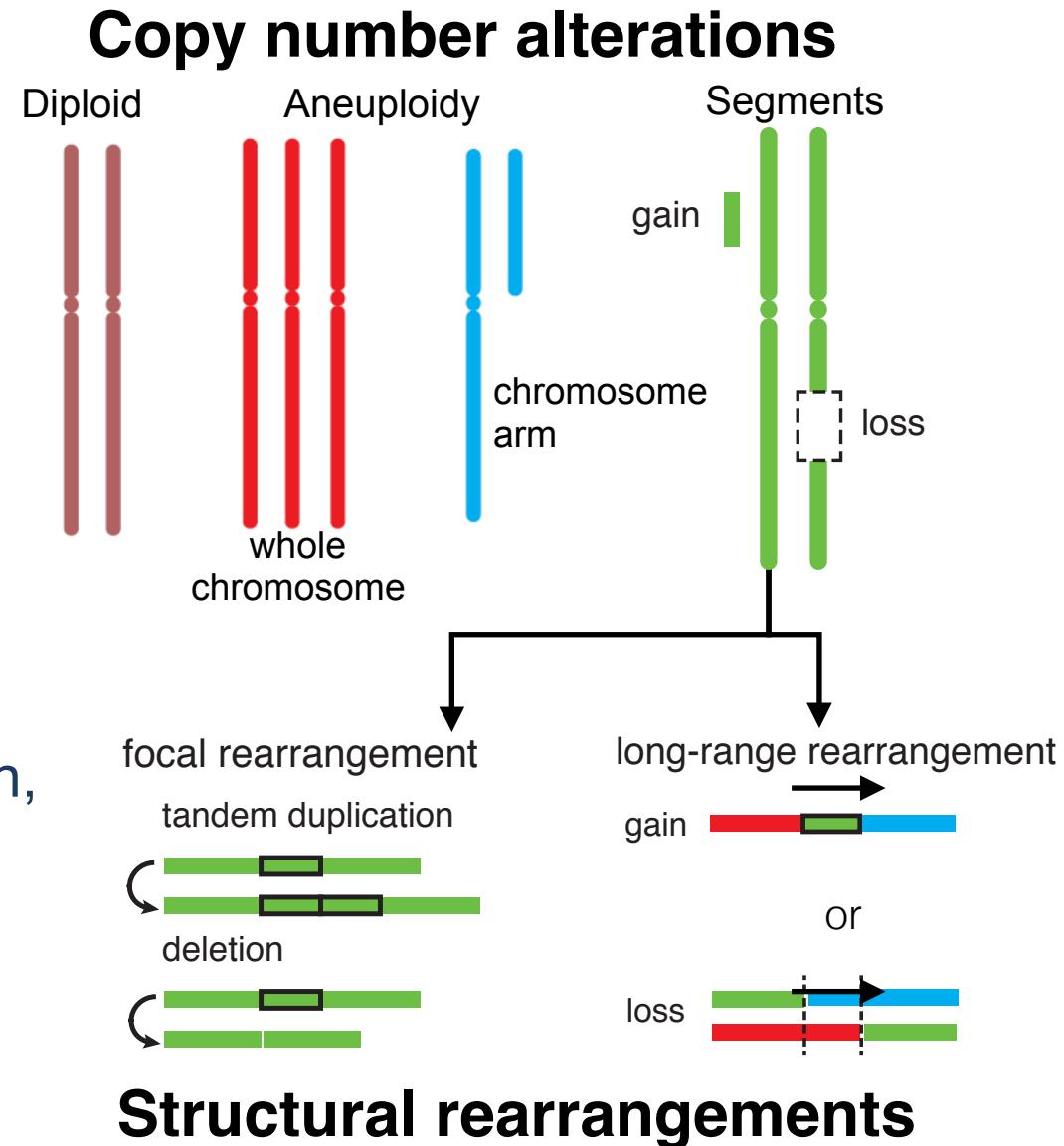
Types of Genomic Variation: Large alterations

3. Copy number changes

- Germline copy number variant (CNV) or polymorphism (CNP)
- Somatic copy number variant (CNV) or alterations (CNA)
- Size > 1 kbps, typically mega-bases (depending on resolution)

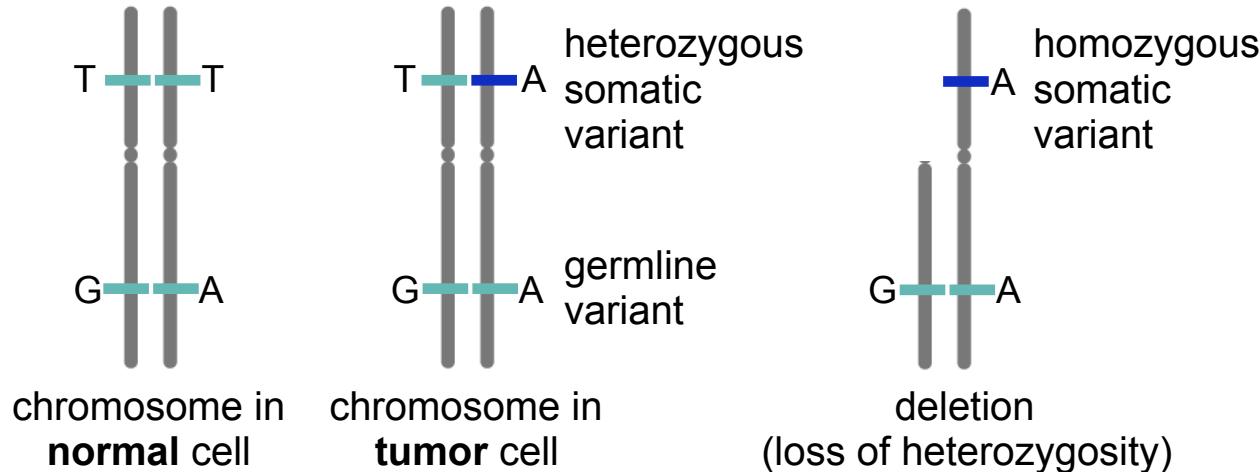
4. Structural rearrangements

- Germline or Somatic structural variant (SV)
- Simple events: deletion, duplication, inversion, translocation
- Single nucleotide resolution for breakpoints
- Size > 20 bps, typically kilo-bases to mega-bases



Types of Genomic Variation in Cancer

Single nucleotide variant

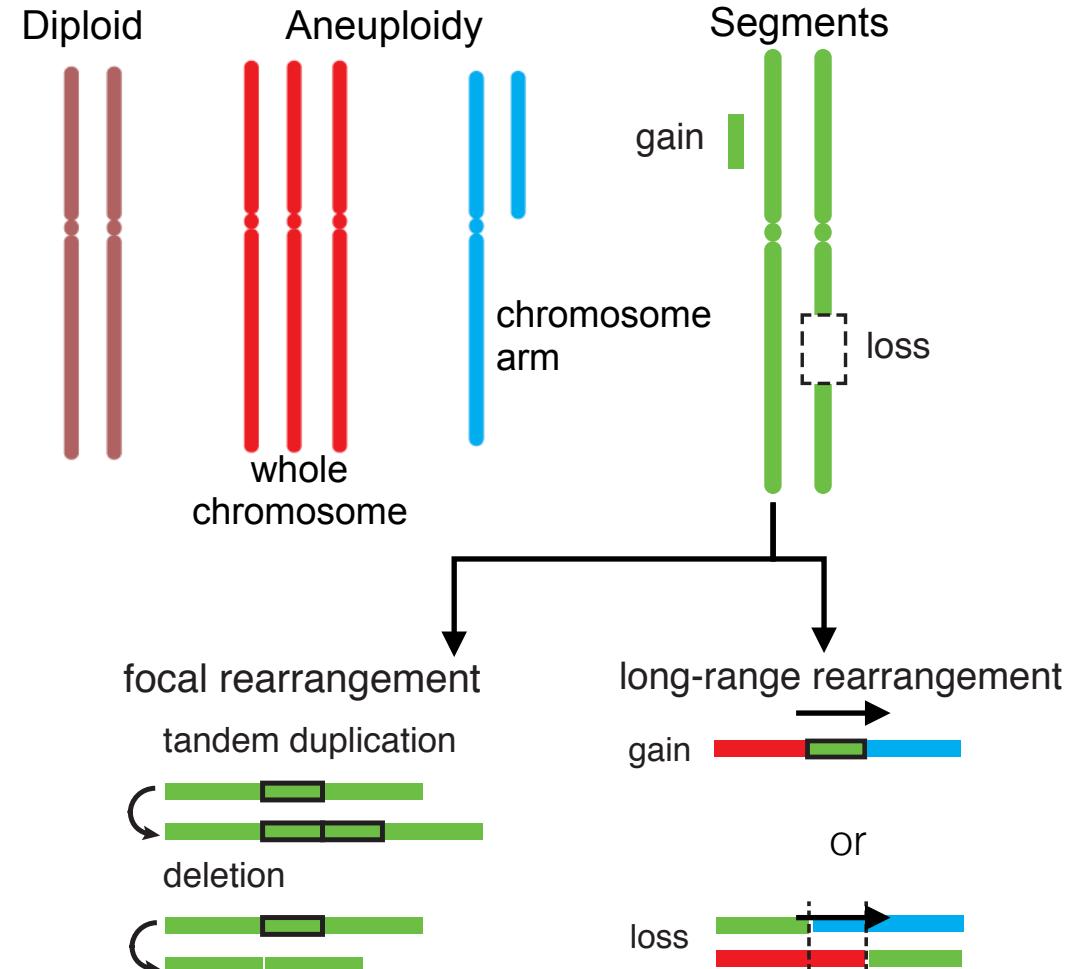


TAGG TAGG TAGG T - - G deletion

GA GA GA G[TA]A insertion

Insertion-Deletion (INDEL)

Copy number alterations



Structural rearrangements

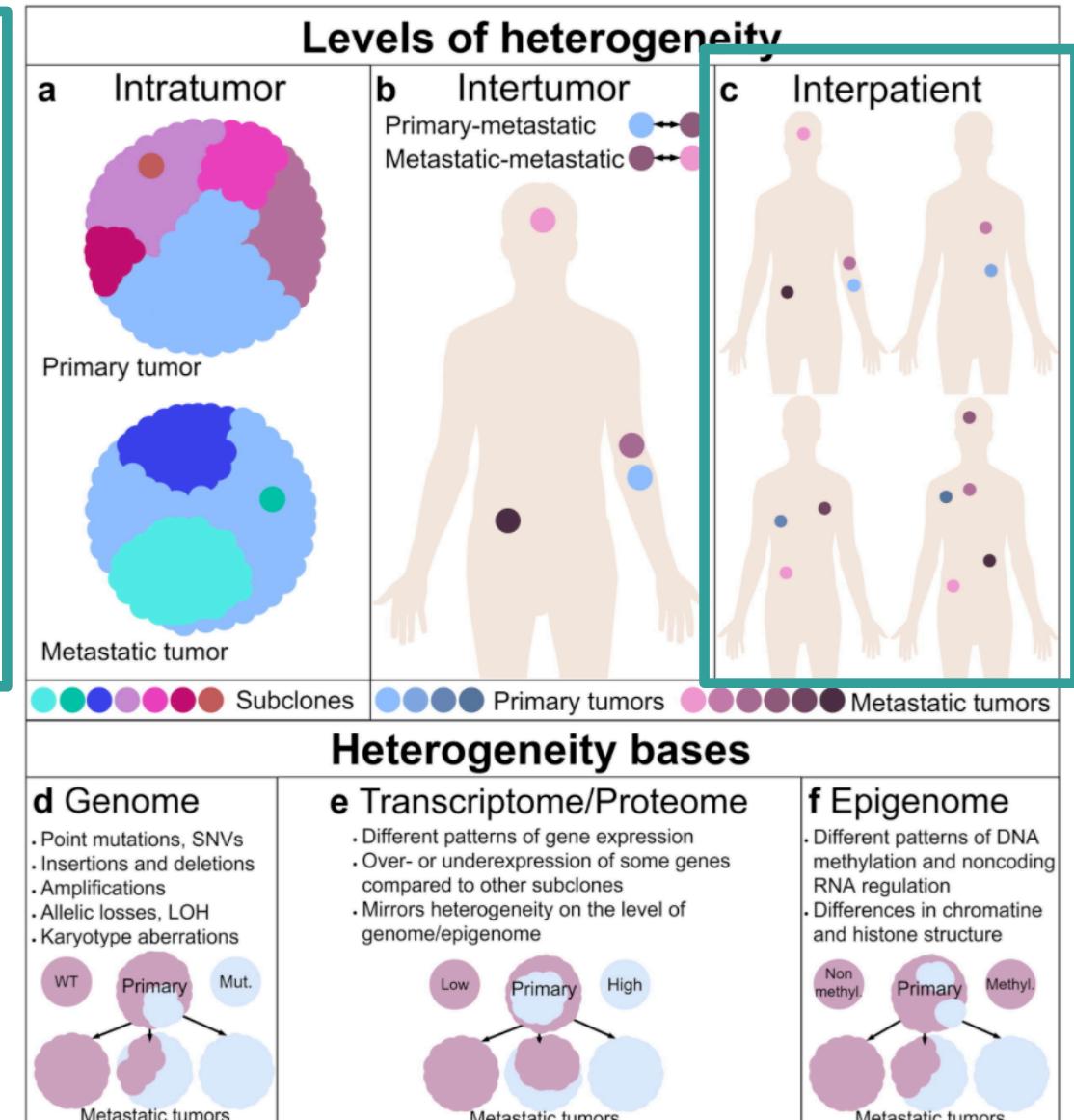
Tumors exhibit different levels of heterogeneity

Across patient populations:

1. **Cancer types:** between primary tumors of different organs or tissue-of-origin (eg. Breast and lung cancers)
2. **Same-subtype:** between tumors from different patients
3. **Tumor subtypes:** between subset of patients with tumors having similar molecular features (e.g. ER+ and ER- breast cancers)

Within an individual patient:

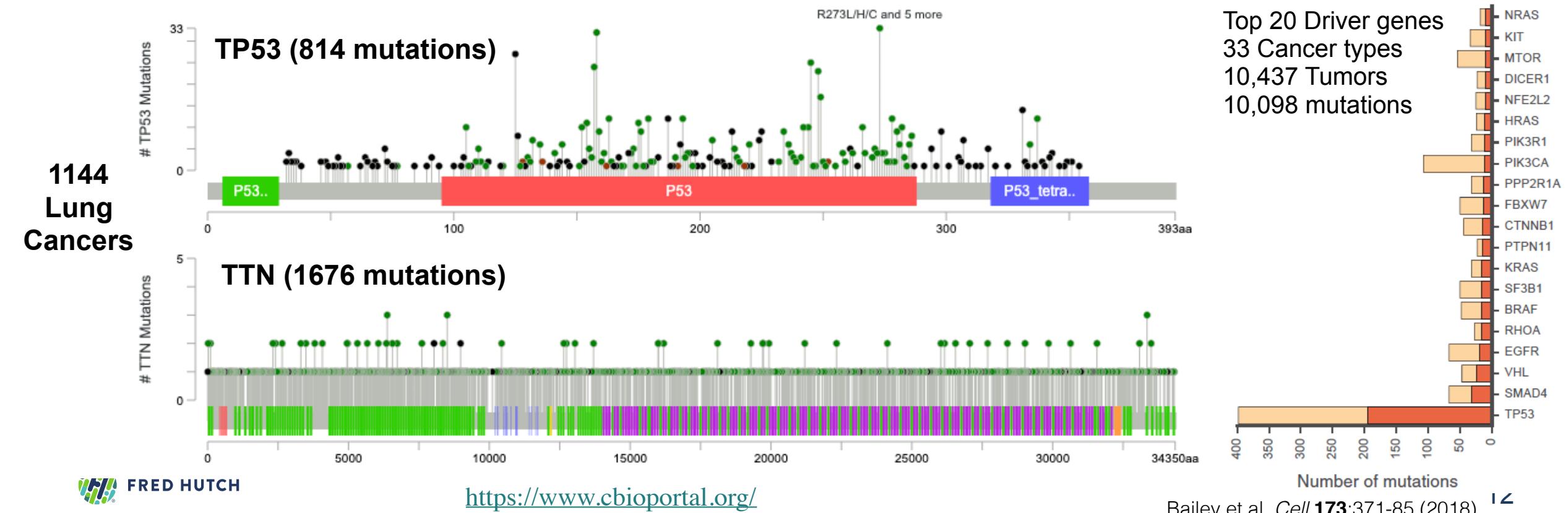
4. **Inter-tumor:** between tumors within a patient
5. **Intra-tumor heterogeneity:** between cells within a tumor lesion (e.g. tumor clones, stromal cells, infiltrating lymphocytes)



Cancer Genes: Driver vs Passenger Genomic Alterations

How do we find the mutated genes that *drive* cancer?

- **Significantly Mutated Genes:** recurrently mutated genes in patient cohorts
- Account for covariates (e.g. gene length, expression, replication timing)



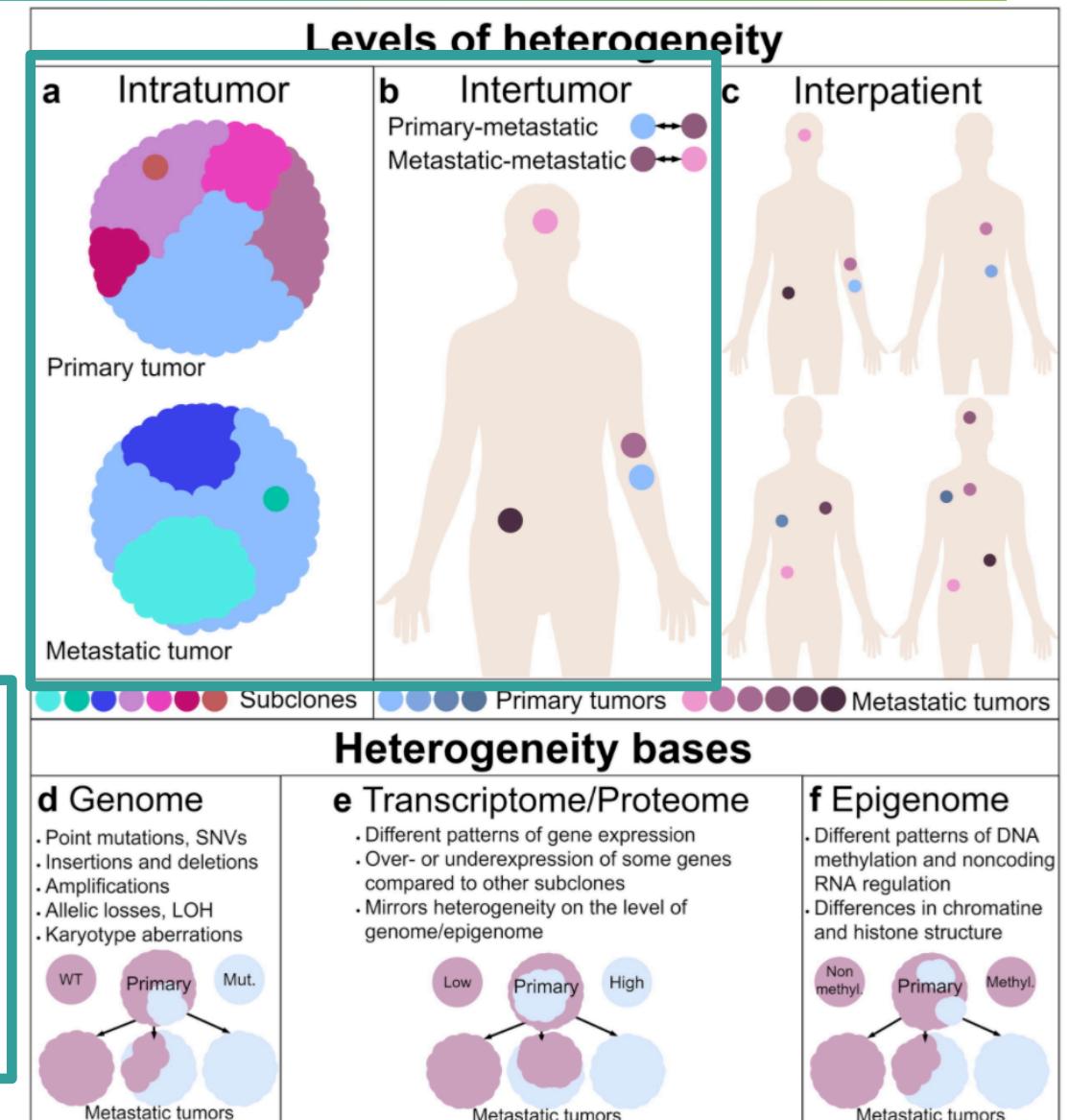
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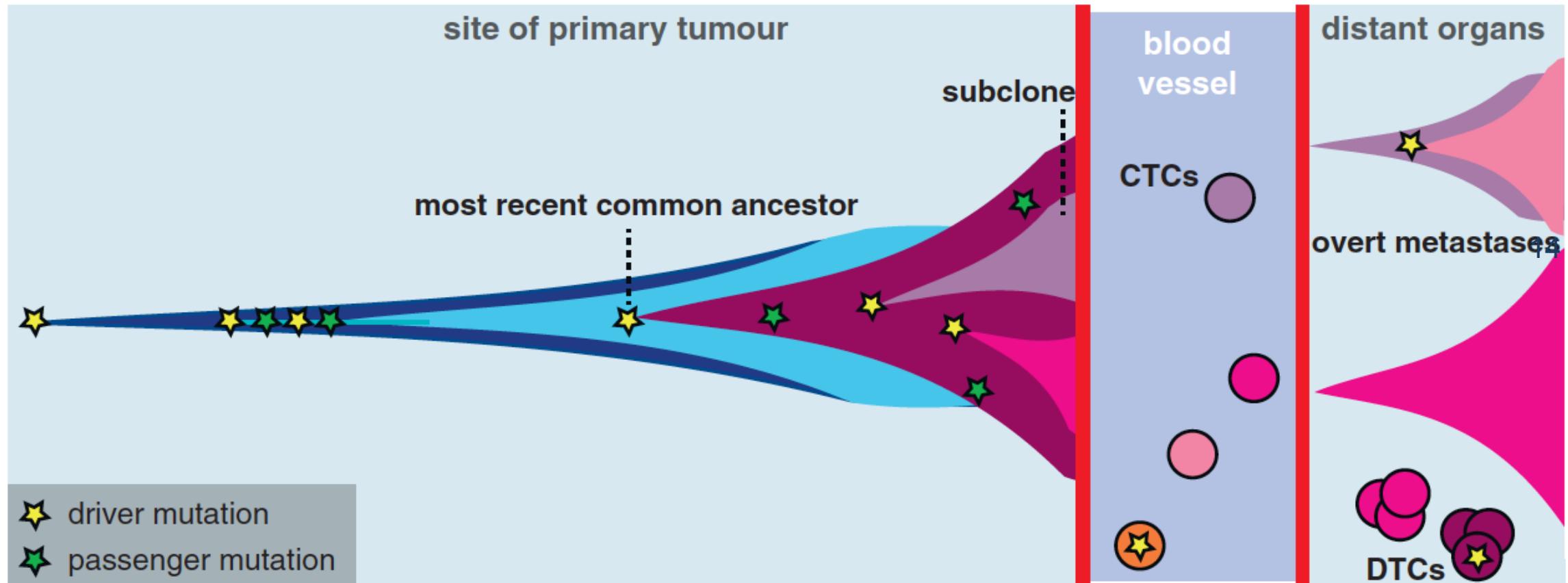
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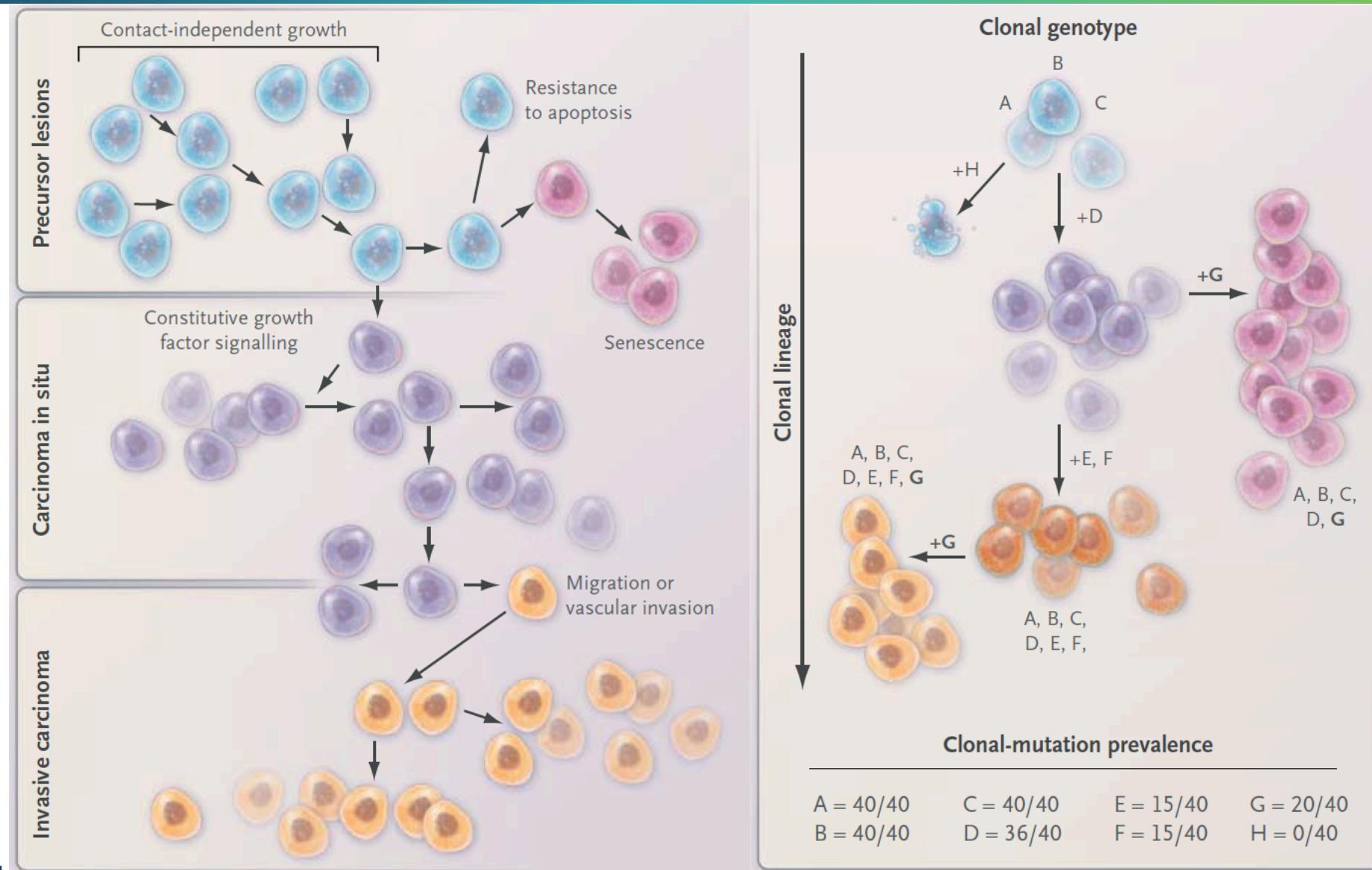


Tumors undergo genome evolution and clonal expansion

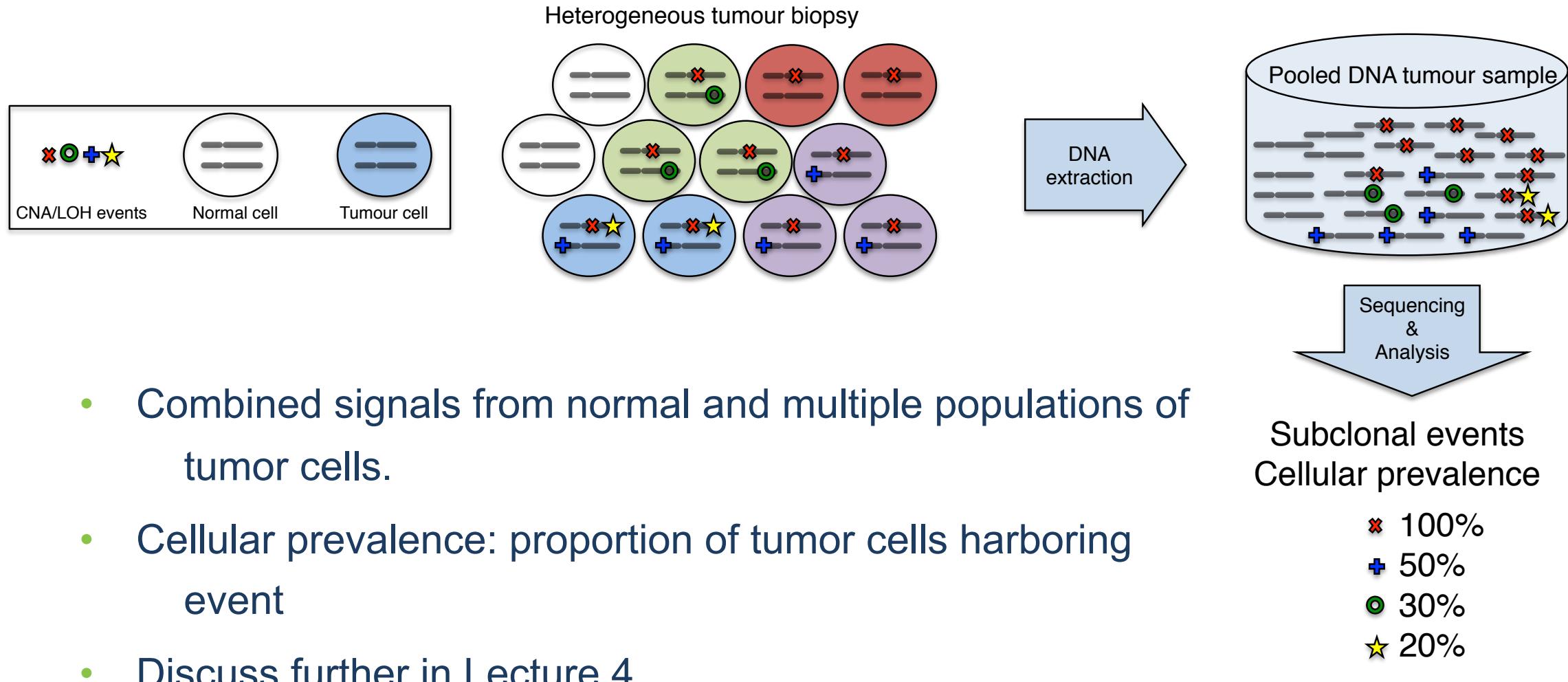
- Clonal diversity may have implications for treatment resistance
- Dynamics of clones can change in the blood and metastases



Tumor genome evolution selects for cellular phenotypes

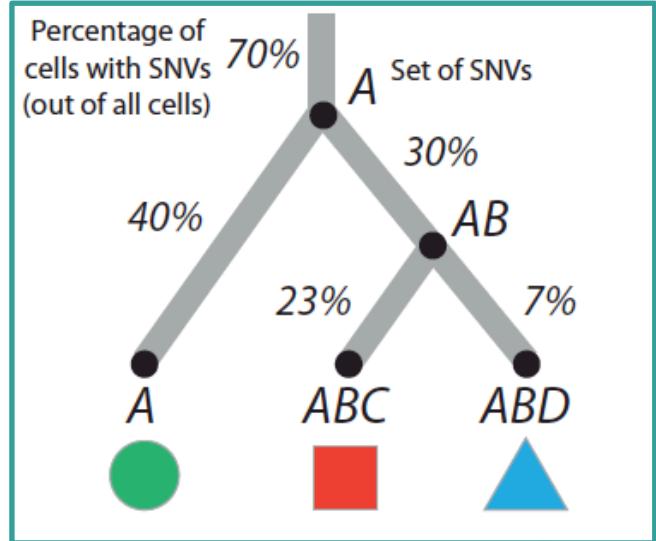


Inferring intra-tumor genomic heterogeneity from sequencing

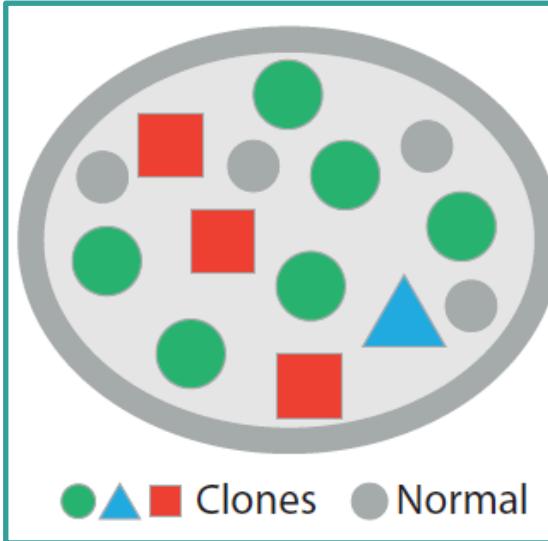


Inferring evolutionary history of a tumor from sequencing

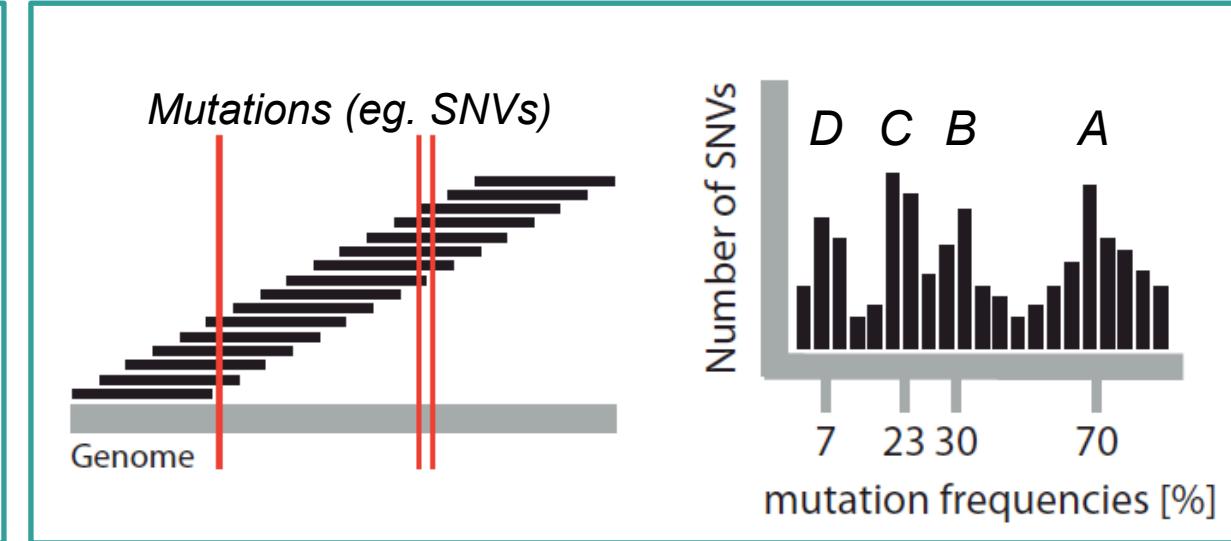
Evolutionary History



Clonal Cell Populations



Sequencing Data



3. Infer evolutionary (phylogenetic) tree

2. Infer clonal prevalence

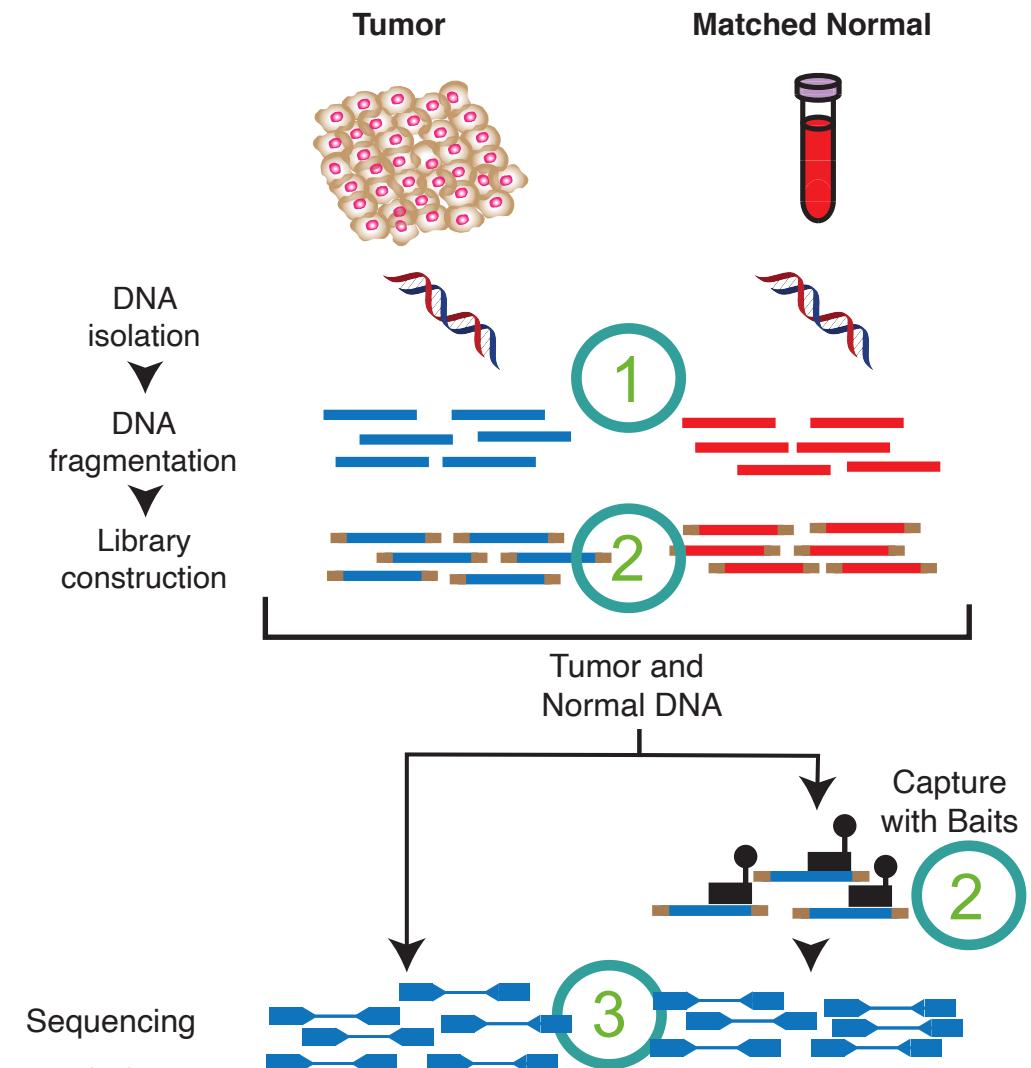
1. Mutation Calling & Analysis

2. Overview of Cancer Genome Analysis

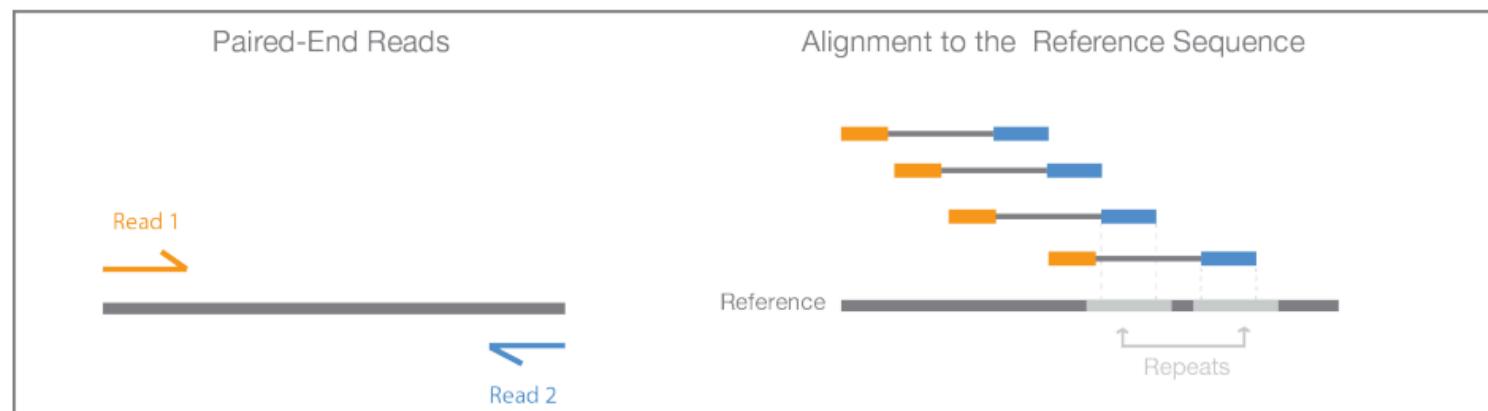
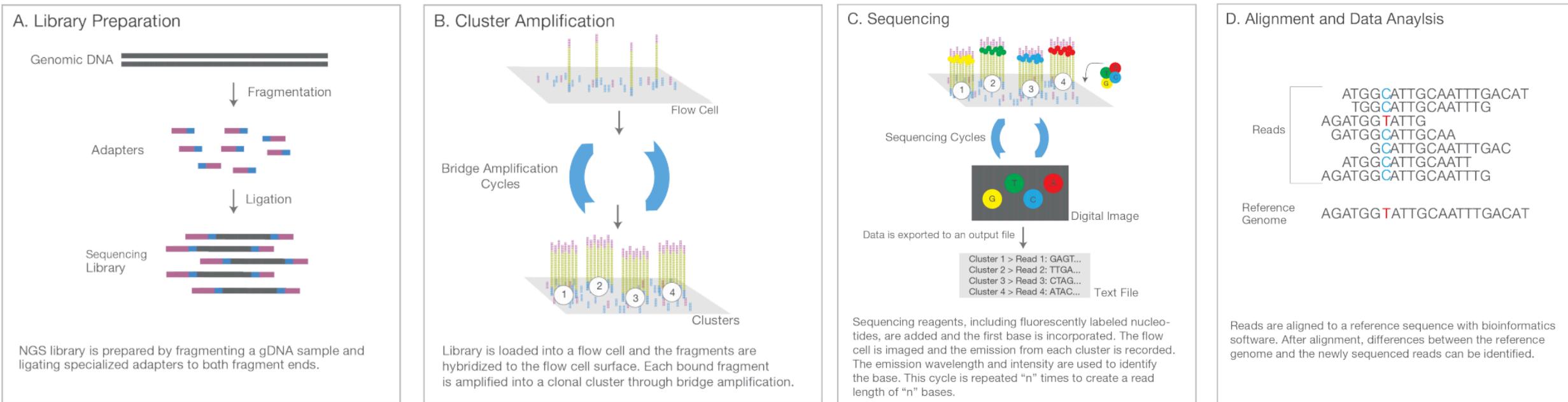
- Computational strategy and workflow
- Tumor DNA sequencing
- Whole genome vs whole exome vs targeted sequencing
- Types of genomic alterations predicted from tumor sequencing
- Methods/tools/algorithms in following lectures

General Workflow of Tumor Genome Sequencing (1)

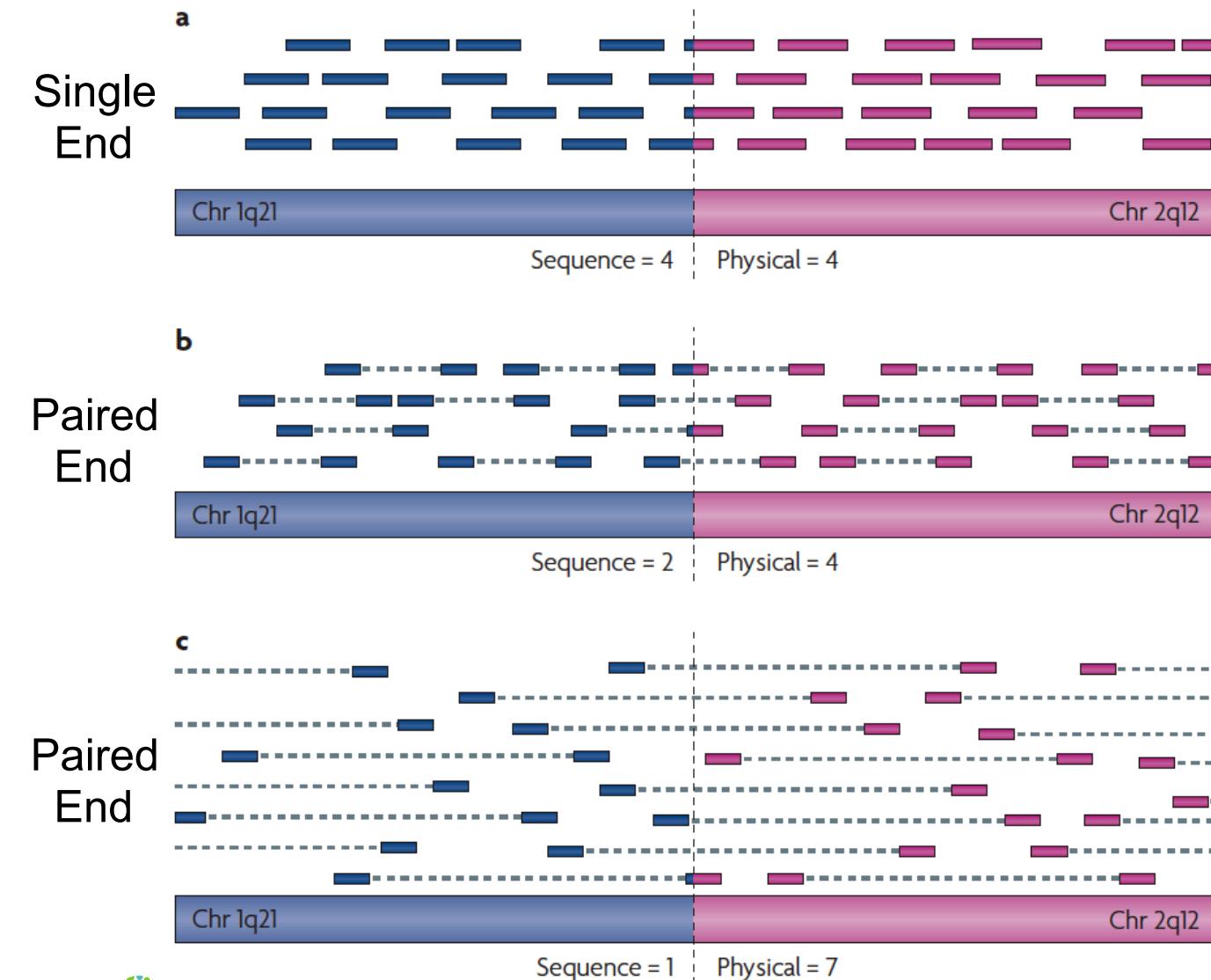
- Tumor and Normal pairing
 - Distinguish somatic and germline alterations
- Capture baits can be used to select regions
 - e.g. whole exome or targeted gene panels
- Potential sources of error can arise
 1. Cytosine deamination (8-oxoG)
 2. PCR errors and GC content bias
 3. Sequencing errors



Genome Sequencing: Massively Parallel Sequencing



Genome Sequencing: Sequence vs Physical Coverage

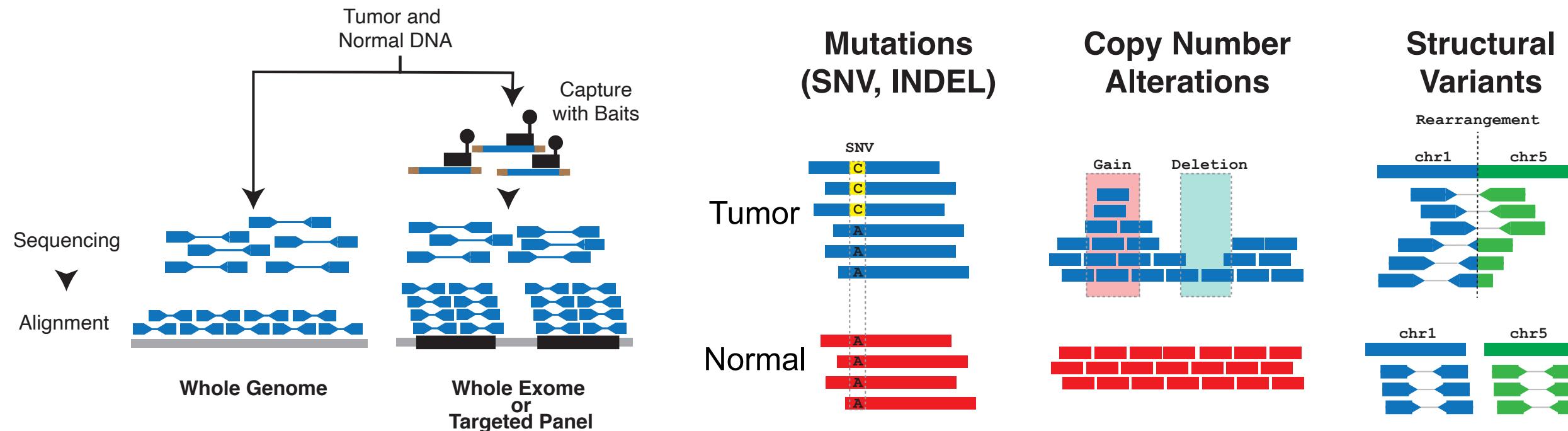


Sequence Coverage = number of sequenced reads spanning locus

Physical Coverage = number of DNA fragments spanning locus

- Mutation detection rely on sequence coverage
- Rearrangement detection rely on both

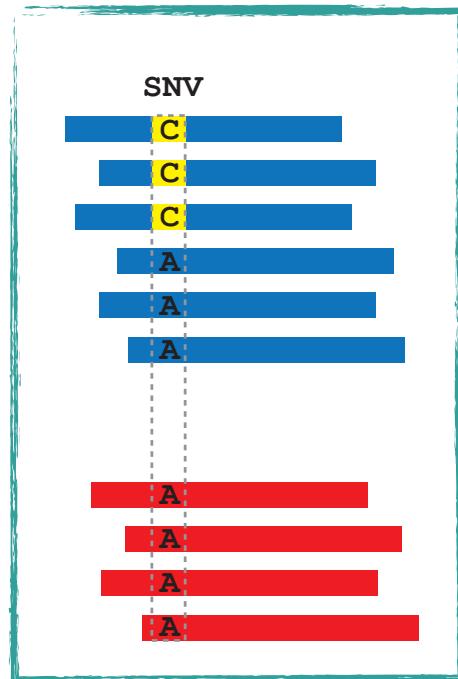
General Workflow of Tumor Genome Sequencing (2)



Whole Genome Sequencing	Whole Exome Sequencing	Targeted Gene Sequencing
<ul style="list-style-type: none"> • Genome-wide (unbiased) • 0.1-100x genome coverage 	<ul style="list-style-type: none"> • Exons (2% of genome) • 50-500x target coverage 	<ul style="list-style-type: none"> • Target regions (1-5Mb) • 100-25000x target coverage
<ul style="list-style-type: none"> • More sequencing required • Expensive 	<ul style="list-style-type: none"> • Less sequencing required • Cost-effective 	<ul style="list-style-type: none"> • Least sequencing required • Panel design costs
<ul style="list-style-type: none"> • Coding/Non-coding mutations • Copy number alterations • Structural variation 	<ul style="list-style-type: none"> • Coding mutations (all genes) • Copy number alterations • Gene fusions rearrangements 	<ul style="list-style-type: none"> • Coding mutations (selected) • Targeted rearrangements

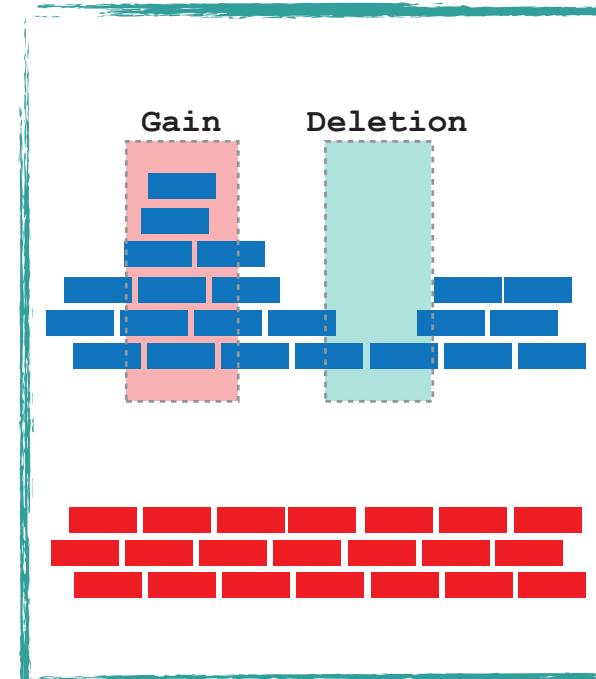
Types of Genomic Alterations Predicted from Sequencing

Mutations (SNV, INDEL)



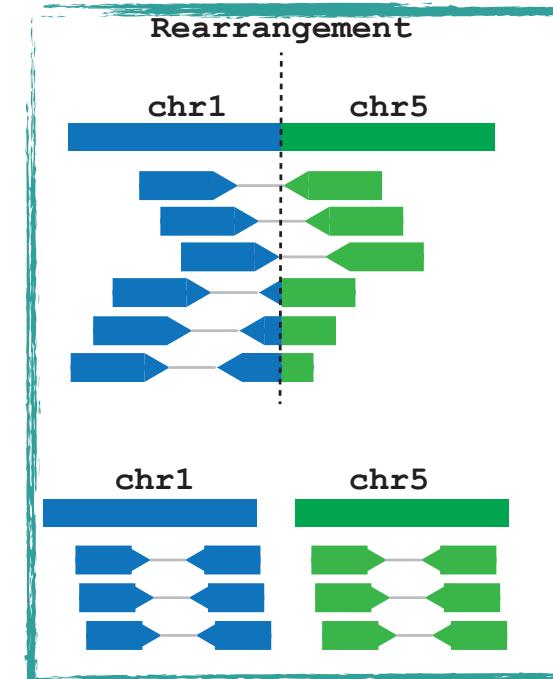
Lecture 2

Copy Number Alterations



Lecture 3

Structural Variants



Lecture 4?

Genome Sequencing: International Consortia & Projects

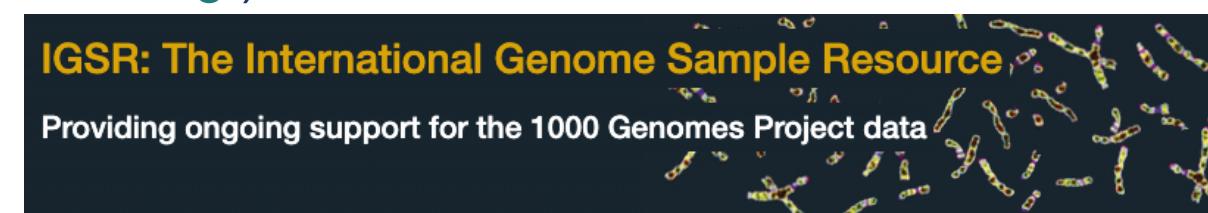
1000 Genomes Project (<https://www.internationalgenome.org/>)

UK10K (<https://www.uk10k.org/>)

The 100,000 Genomes Project

(<https://www.genomicsengland.co.uk/>)

- Rare disease, cancer, infectious disease



UK10K

Rare Genetic Variants in Health and Disease



#100kThankYous

Genome 10K Project (<https://genome10k.soe.ucsc.edu/>)

- Genomic “zoo” of 16,000 vertebrate species



Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>)

Genome Aggregation Database (gnomAD) (<https://gnomad.broadinstitute.org/>)

The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>)

International Cancer Genome Consortium (ICGC) (<https://icgc.org>)



Cancer Genome Sequence Data: Databases & Online Resources

NATIONAL CANCER INSTITUTE
GDC Data Portal

Home Projects Exploration Analysis Repository

Quick Search Manage Sets Login Cart 0 GDC Apps

Harmonized Cancer Datasets

Genomic Data Commons Data Portal

Get Started by Exploring:

Projects Exploration Analysis Repository

e.g. BRAF, Breast, TCGA-BLCA, TCGA-A5-A0G2

Data Portal Summary

Data Release 22.0 - January 16, 2020

PROJECTS	PRIMARY SITES	CASES
64	67	83,709

FILES	GENES	MUTATIONS
526,931	22,872	3,142,246



Cases by Major Primary Site

Primary Site	Cases (in 1000s)
Adrenal Gland	0.1
Bile Duct	0.1
Bladder	1.5
Blood	0.5
Bone	0.5
Bone Marrow	9.0
Brain	1.2
Breast	8.5
Cervix	0.5
Colorectal	7.8
Esophagus	1.2
Eye	0.2
Head and Neck	1.8
Kidney	3.2
Liver	1.2
Lung	11.2
Lymph Nodes	0.5
Nervous System	3.5
Ovary	3.2
Pancreas	2.5
Pleura	0.5
Prostate	2.5
Skin	2.5
Soft Tissue	1.2
Stomach	1.2
Testis	0.5
Thymus	0.5
Thyroid	1.2
Uterus	2.5

Cancer Genome Sequence Data: Databases & Online Resources



Cancer genomics data sets visualization, analysis and download.

Quick Search Search

e.g. BRAF, KRAS G12D, DO35100, MU7870, Fl998, apoptosis, Cancer Gene Census, imatinib, GO:0016049

Advanced Search

By donors By genes By mutations

Data Release 28

March 27th, 2019

Cancer projects	86
Cancer primary sites	22
Donor with molecular data in DCC	22,330
Total Donors	24,289
Simple somatic mutations	81,782,588

Download Release

3. Primer on statistical modeling

- Probability
 - Unsupervised learning, probability rules & Bayes' theorem
 - Binomial distribution, Bayesian statistics
 - Beta-binomial model example
- Mixture models, EM inference
- 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
 - <https://www.cs.ubc.ca/~murphyk/Teaching/CS340-Fall06/reading/bernoulli.pdf>

Sequencing Data Analysis Requires Probabilistic Models

- Sequencing data contain uncertainty due to
 - Technical noise from imperfect measurements & errors
 - Biological features in the signal measurements
- How do we predict genomic alterations accounting for these features and noise?
 - Need probabilistic approaches...

Types of machine learning:

- Supervised: output data y , input data x , and training set D , $p(y|x, D)$
 - Classification (y are labels), Regression (y is continuous)
- Unsupervised: Only given output data y , $p(x)$
 - E.g. clustering input data x into K clusters by estimating their assignments z

Primer: Probability Theory

Let X be a random variable. The probability for the event $X = x$ for some value x is $p(X = x)$ or $p(x)$ for short. Let Y be another random variable.

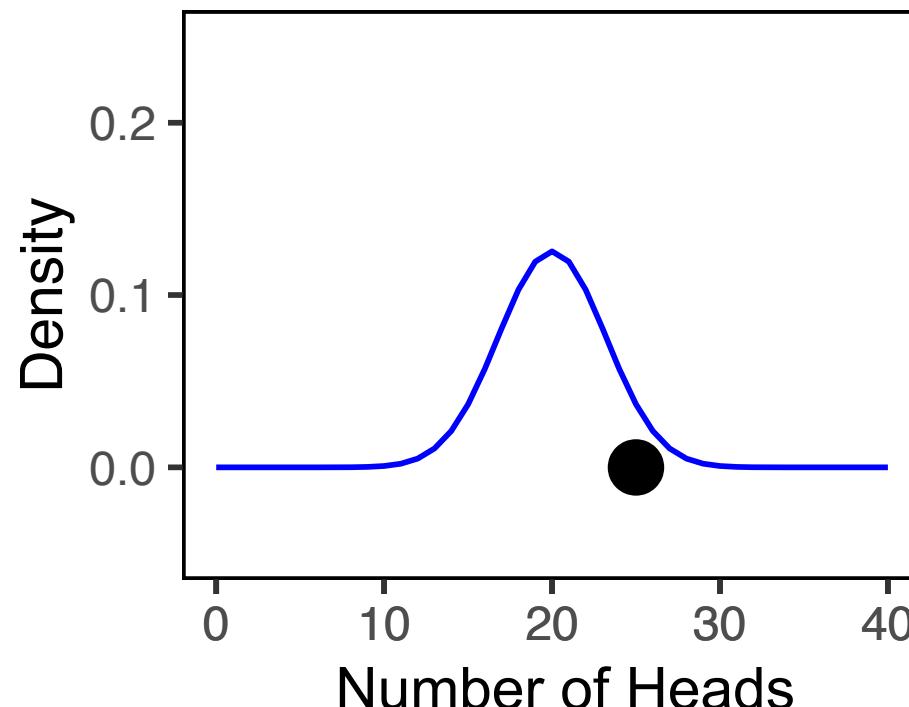
Probability Rules

- **Sum rule:** $p(X) = \sum_y P(X, Y = y)$
- **Product rule:** $p(X, Y) = p(Y|X)p(X)$ and $p(Y, X) = p(X|Y)p(Y)$
- Joint Probabilities: $p(X) = \sum_y p(Y, X) = \sum_y p(X|Y = y)p(Y = y)$
- Conditional Probabilities: $p(Y|X) = \frac{p(X, Y)}{p(X)}$
- **Bayes' Theorem (rule):**
$$p(Y = y | X = x) = \frac{p(X = x, Y = y)}{p(X = x)} = \frac{p(X = x | Y = y)p(Y = y)}{\sum_{y'} p(X = x | Y = y')p(Y = y')}$$

Probability distribution: Binomial

Binomial Distribution: Referee Coin Toss Example

- A referee has a coin that he uses to decide which team gets first possession. She tossed the coin N times last season, once per game. We assume this coin was fair and had a probability $\mu = 0.5$ for showing a head. We kept track of the number of heads x that appeared.
- What is the probability of seeing a specific number of heads? e.g. $x = 25$ out of $N = 40$ tosses



Probability distribution: Binomial

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Probability mass function

- Let X be the random variable representing the number of heads. If the probability of heads is μ , then X has a binomial distribution, $X \sim Bin(N, \mu)$ or $p(X = x | N, \mu) = Bin(x | N, \mu)$ where

$$Bin(x | N, \mu) = \binom{N}{x} \mu^x (1 - \mu)^{N-x}$$

$$\binom{N}{k}$$

number of ways the 25 heads
is observed among the sequence of
40 tosses.

- My coin-toss example: for $x = 25$ out of $N = 40$ and a fair coin $\mu = 0.5$

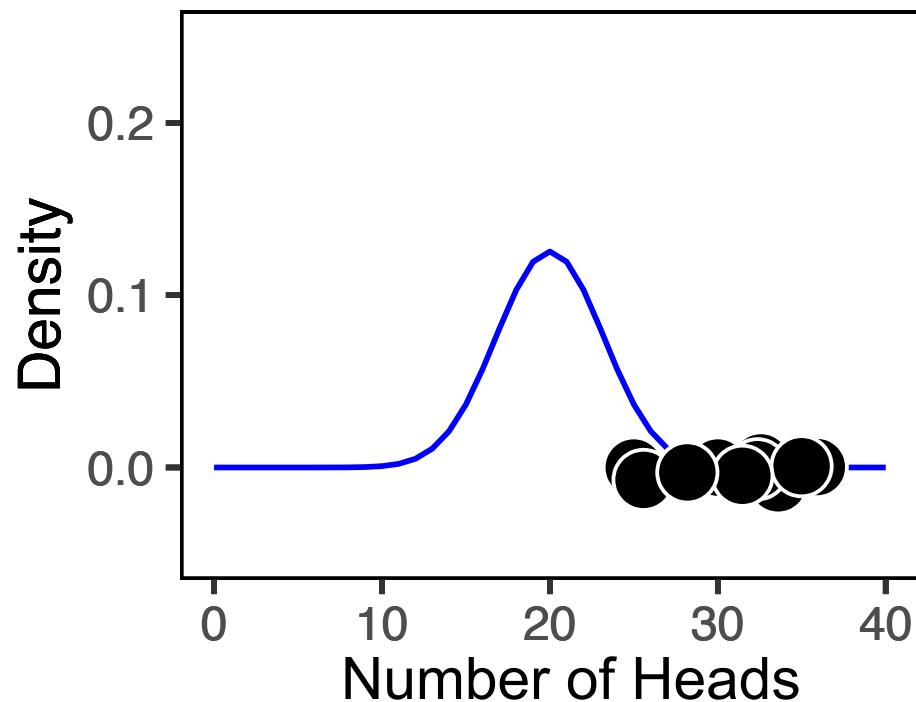
$$p(X = 25 | N = 40, \mu = 0.5) = Bin(25 | 40, 0.5) = \binom{40}{25} 0.5^{25} (1 - 0.5)^{40-25}$$

Binomial likelihood model

- Suppose there are T different referees who toss the same coin $N = \{1, \dots, N_T\}$ times and come up with head counts $x = \{1, \dots, x_T\}$.
- Assuming the referees' tosses are *independent* and *identically distributed (iid)*, what is the probability of observing the head counts given the coin (e.g. $\mu = 0.5$)?

$$p(x_{1:T} | N_{1:T}, \mu) = \prod_{i=1}^T \text{Bin}(x_i | N_i, \mu) \quad \text{Likelihood}$$

- What if the coin wasn't fair and the probability of heads, μ , might not be 0.5?



	# of tosses (N)	# of heads (x)
Referee 1	40	25
Referee 2	42	35
Referee 3	39	27
Referee T	x_T	N_T

Maximum likelihood estimation (MLE)

- What is the probability of heads, μ , of this coin given the evidence?
- We can estimate this model parameter using
maximum likelihood estimation

$$p(x_{1:T} | N_{1:T}, \mu) = \prod_{i=1}^T \text{Bin}(x_i | N_i, \mu)$$

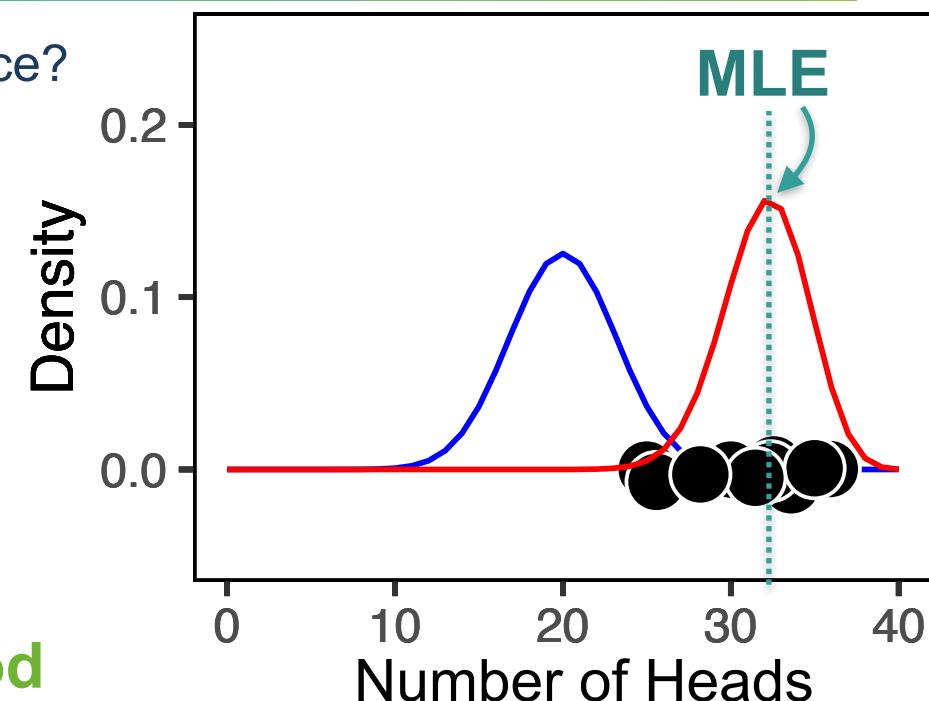
Likelihood

$$\log p(x_{1:T} | N_{1:T}, \mu) = \sum_{i=1}^T \log \text{Bin}(x_i | N_i, \mu)$$

Log-likelihood

$$\hat{\mu} = \frac{\sum_{i=1}^T x_i}{\sum_{i=1}^T N_i}$$

MLE



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

Bayesian Statistics: Prior distribution for model parameters

Likelihood for Binomial Model

$$p(x_{1:T} | N_{1:T}, \mu) = \prod_{i=1}^T \text{Bin}(x_i | N_i, \mu)$$

Likelihood

- MLE uses the evidence to estimate parameter $\hat{\mu}$ but our sample size is small and MLE may **overfit**
- **Zero count or sparse data problem:** If you have a bad sports statistician who only tallies coin tosses from referees who never toss heads, then does that mean the concept of heads on a coin does not exist at all?
- Can we capture a more natural expectation of how a coin might behave? Also, what if we have some knowledge that the coin might be biased?

Prior Distribution for binomial parameter, ρ

- The proportion of heads is between 0 and 1 ($\mu \in [0,1]$) and can be sampled from a distribution itself
- μ can be drawn from a Beta distribution, which is in the interval $[0,1]$, with **hyper-parameters** α and β

$$\mu \sim \text{Beta}(\alpha, \beta)$$

$$p(\mu) = \text{Beta}(\mu | \alpha, \beta)$$

Prior

	# of tosses (N)	# of heads (x)	Prop. of heads
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

Bayesian statistics: Posterior for Beta-Binomial Model (1)

Binomial likelihood and Beta prior

- T different head counts $x = \{1, \dots, x_T\}$ for $N = \{1, \dots, N_T\}$ sets of tosses and a **prior** distribution on μ (prob. of heads)

$$p(x_{1:T} | N_{1:T}, \mu) = \prod_{i=1}^T \text{Bin}(x_i | N_i, \mu) \quad \text{Likelihood}$$
$$p(\mu) = \text{Beta}(\mu | \alpha, \beta) \quad \text{Prior}$$

- To estimate parameter μ in a Bayesian framework
 - We need the **posterior**, $p(\mu | x)$, but only have $p(x | \mu)$ and $p(\mu)$
- Recall Bayes' Theorem:
$$\frac{p(Y = y | X = x)}{\text{Posterior}} = \frac{p(X = x | Y = y)p(Y = y)}{\sum_{y'} p(X = x | Y = y')p(Y = y')} \propto \frac{p(X = x | Y = y)p(Y = y)}{\text{Likelihood}} \frac{p(\mu)}{\text{Prior}}$$
- The **posterior** is our **belief state** by combining evidence from observations and our prior beliefs.

Bayesian statistics: Posterior for Beta-Binomial Model (2)

Beta-Binomial Model: Posterior distribution

- To estimate the model parameter μ in a Bayesian framework, we compute the *posterior*, $p(\mu | x)$

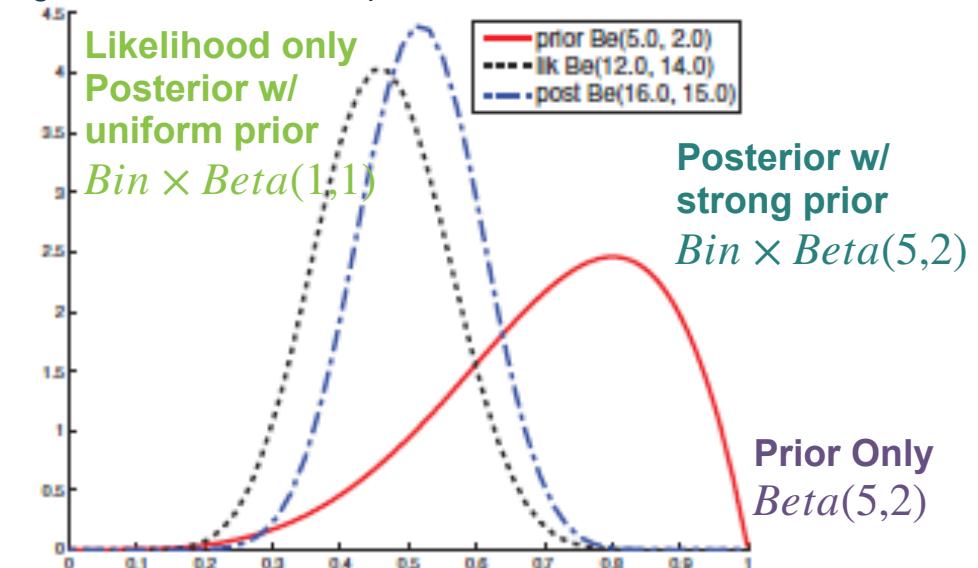
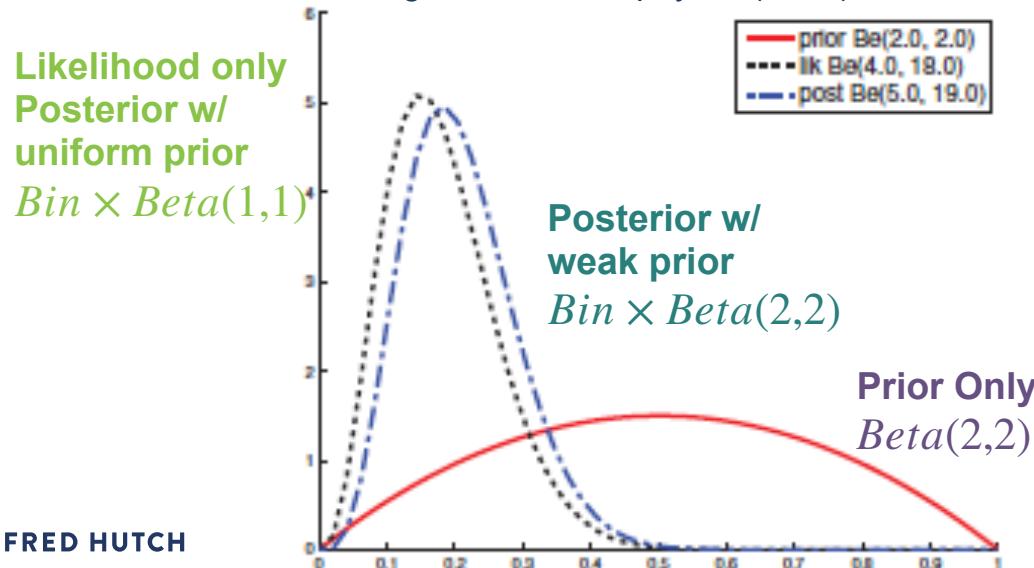
$$p(\mu | x) \propto \prod_i^T Bin(x_i | N_i, \mu) \times Beta(\mu | \alpha, \beta)$$

- Beta is a *conjugate prior* for the binomial; *the product of binomial and Beta has the form of a Beta*

$$p(\mu | x) \propto \prod_i^T Bin(x_i | N_i, \rho) \times Beta(\mu | \alpha, \beta) = \prod_i^T Beta(\mu | x_i + \alpha, N_i - x_i + \beta)$$

Likelihood Prior Posterior

Figure 3.6 in Murphy, K. (2012). Machine Learning: A Probabilistic Perspective. MIT Press



Bayesian statistics: MAP estimate

Beta-Binomial Model: Posterior distribution

$$p(\mu | \mathbf{x}) \propto \prod_i^T Bin(x_i | N_i, \rho) \times Beta(\mu | \alpha, \beta) = \prod_i^T Beta(\mu | x_i + \alpha, N_i - x_i + \beta)$$

Posterior

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

Maximum a posteriori (MAP) estimate

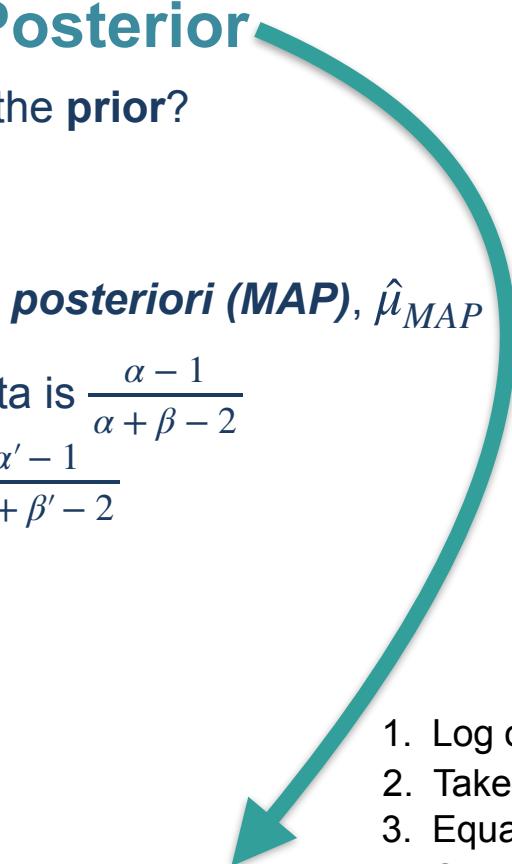
- From the posterior, we can estimate the parameter using the **maximum a posteriori (MAP)**, $\hat{\mu}_{MAP}$
- MAP refers to the mode of the posterior distribution and the mode of a Beta is $\frac{\alpha - 1}{\alpha + \beta - 2}$
- Since the posterior has the form of a Beta distribution, then the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

$$\alpha' = \sum_i^T x_i + \alpha$$

$$\beta' = \sum_i^T (N_i - x_i) + \beta$$

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

MAP

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

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

Mapping the Referee Example to Mutation Calling

Referee Coin Toss Example

Data

Referees $1, \dots, T$

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: π_{fair} , π_{heads} , π_{tails}

Probability of heads for 3 types of coins

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

Responsibilities

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

Mutation Calling from Sequencing Data

Data

Genomic loci $1, \dots, T$

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: π_{AA} , π_{AB} , π_{BB}

Probability of reference base for 3 genotypes:

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

Responsibilities

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

Mixture Models: Online Tutorial and Resource

fiveMinuteStats (<https://stephens999.github.io/fiveMinuteStats/>)

by **Dr. Matthew Stephens**, Professor in Statistics & Human Genetics at University of Chicago

1. Introduction to mixture models with probabilistic derivations and R code

- Examples with Bernoulli and Gaussian models
- https://stephens999.github.io/fiveMinuteStats/intro_to_mixture_models.html

2. Introduction to EM with Gaussian Mixture Model example and R code

- https://stephens999.github.io/fiveMinuteStats/intro_to_em.html

Homework #5: 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 8th

Office Hours with Anna-Lisa Doebley (adoebley@uw.edu)

- Monday, May 4, 2-3pm
- Wednesday, May 6, 2-3pm