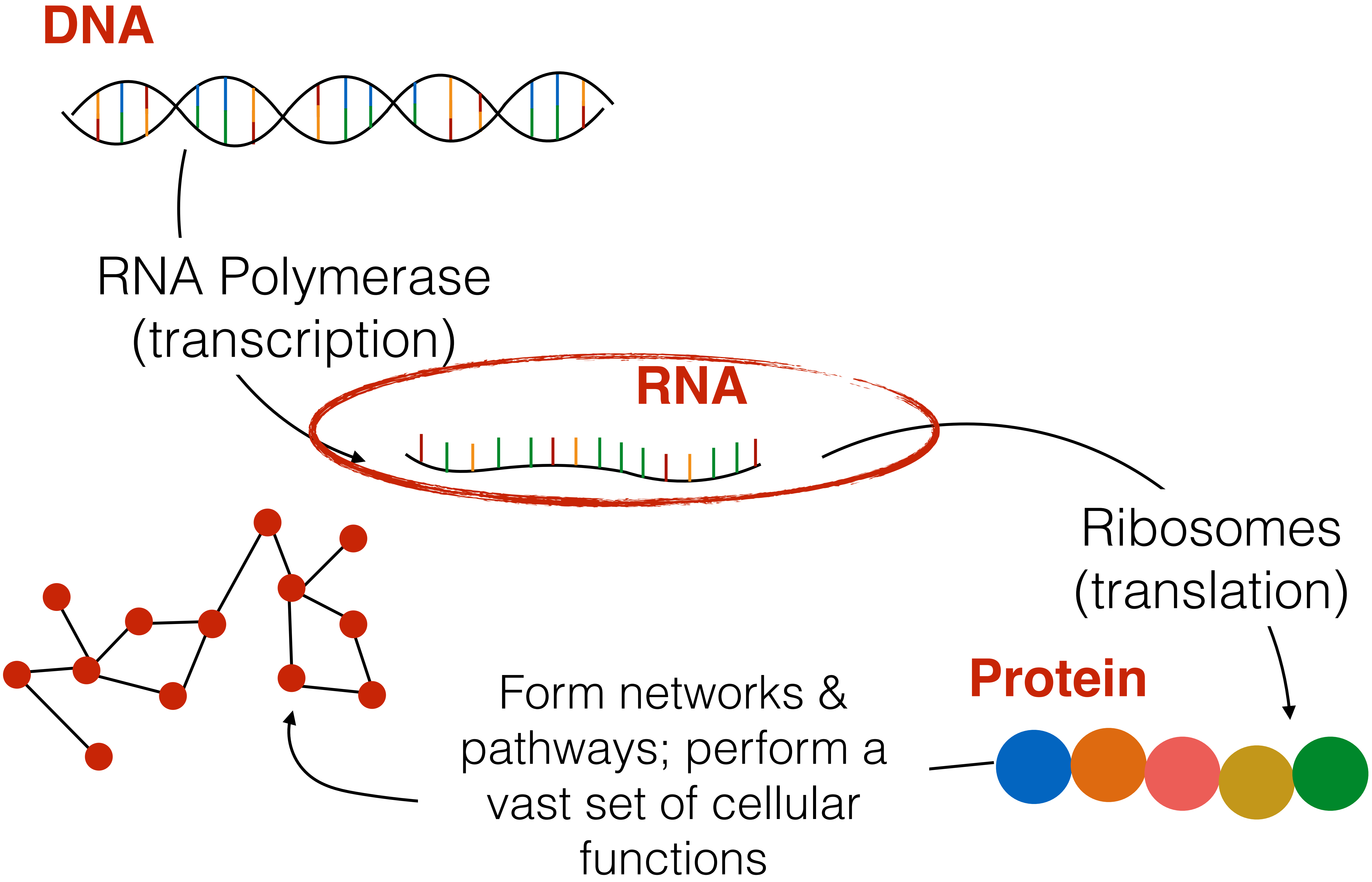
Analyzing gene and transcript expression using RNA-seq

UNIVERSITY OF MARYLAND

“Flow” of information in the cell



DNA

RNA Polymerase
(transcription)

RNA

Ribosomes
(translation)

Protein

Form networks &
pathways; perform a
vast set of cellular
functions

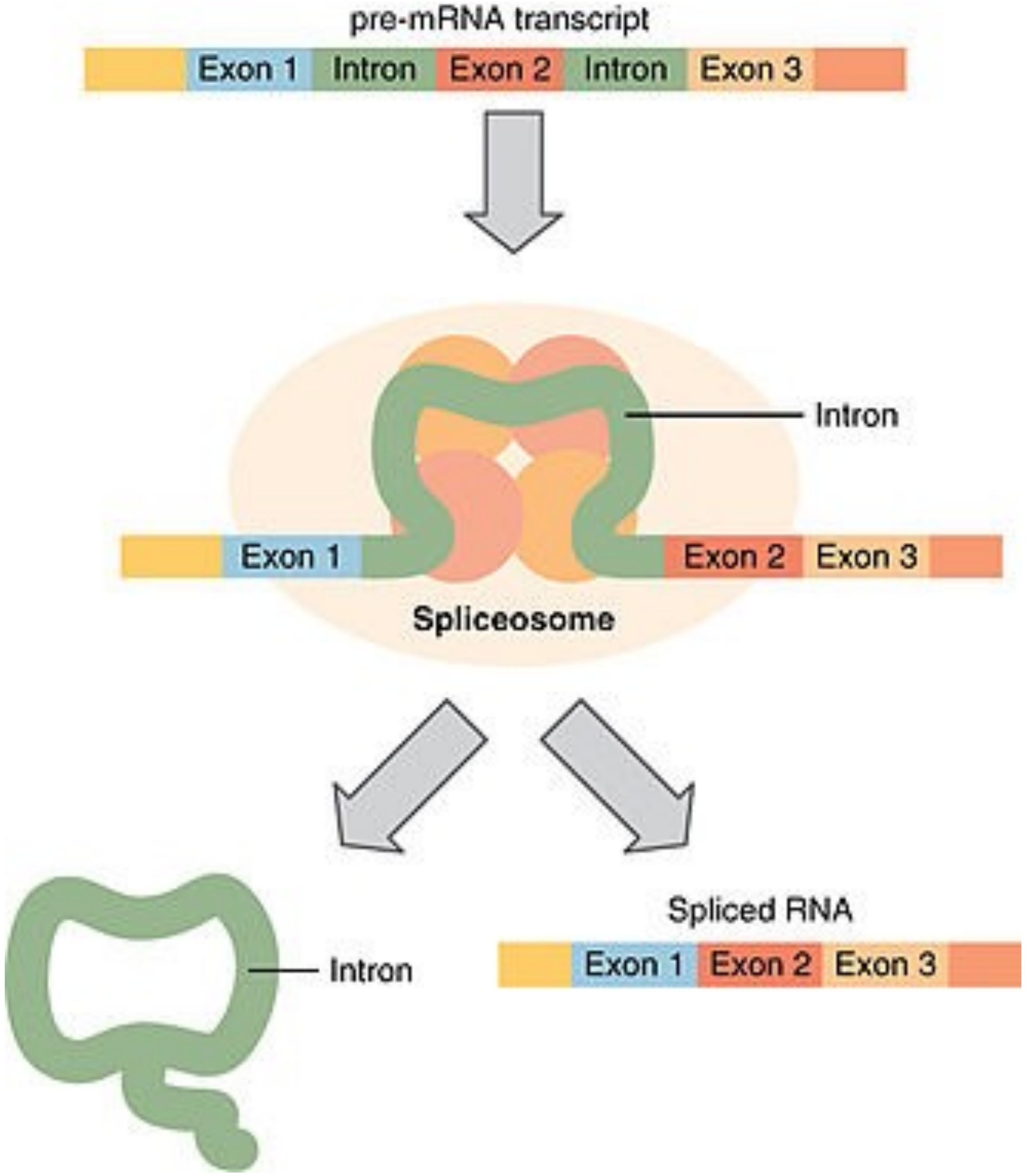
RNA Splicing

DNA transcribed into pre-mRNA

Some “processing occurs”
capping & **polyadenylation**

Introns removed from pre-mRNA

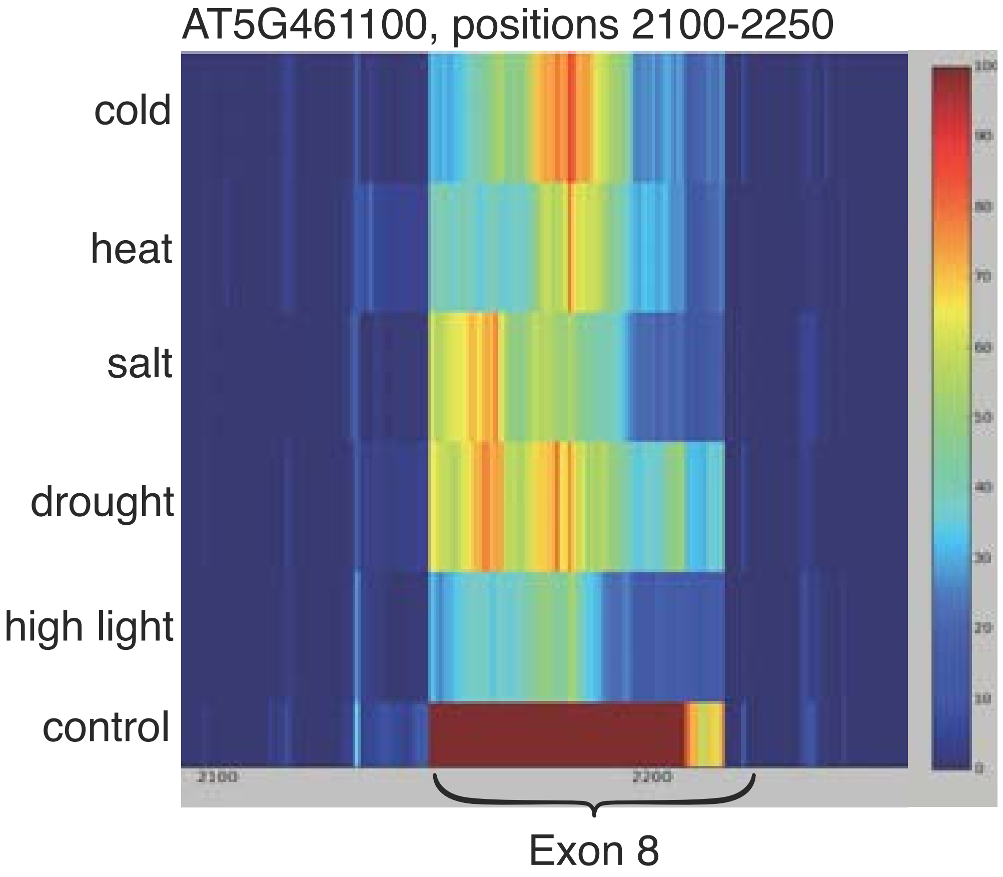
Introns removed resulting in
mature mRNA

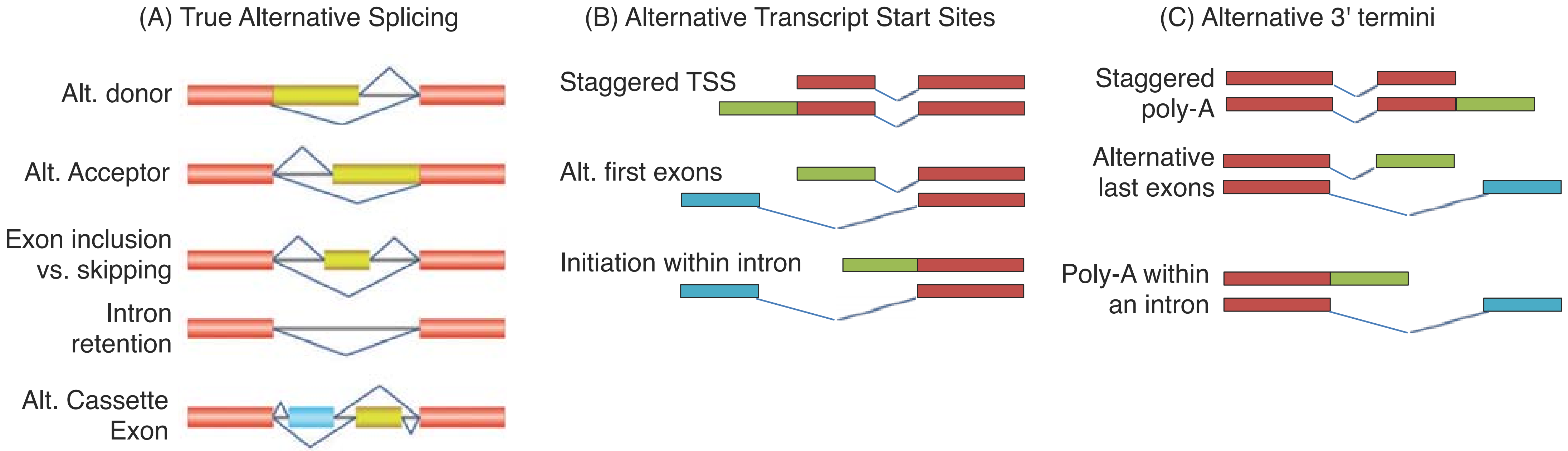


en.wikipedia.org

Alternative Splicing & Isoform Expression

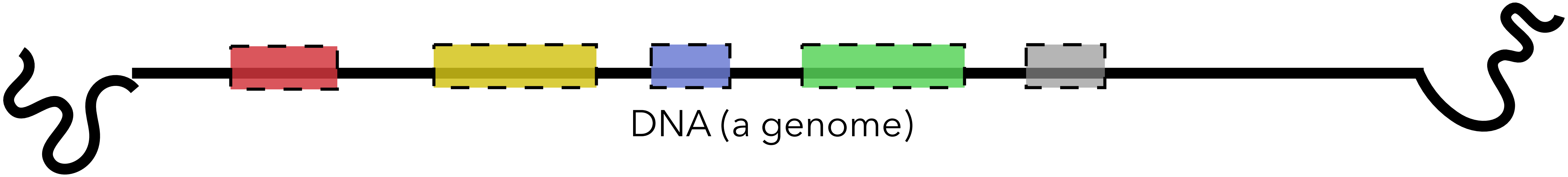
- Expression of genes can be measured via RNA-seq (sequencing transcripts)
- Sequencing gives you short (35-300bp length reads)





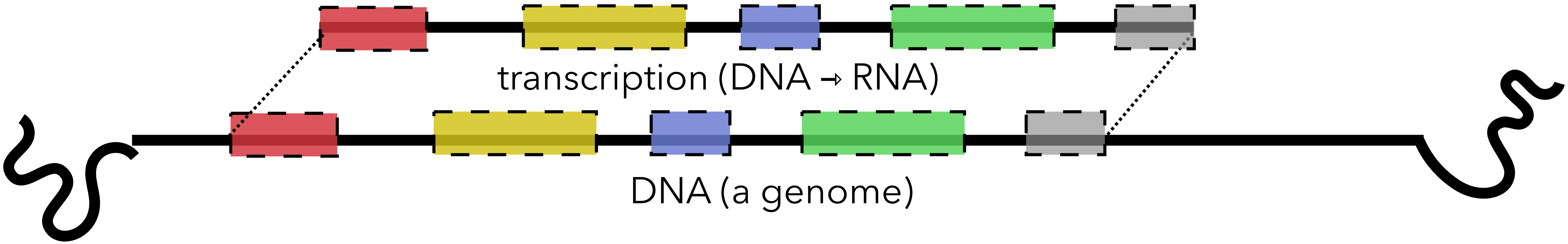
slide courtesy of Carl Kingsford

What is RNA sequencing



* most protocols actually sequence complementary DNA (cDNA), not RNA directly

What is RNA sequencing



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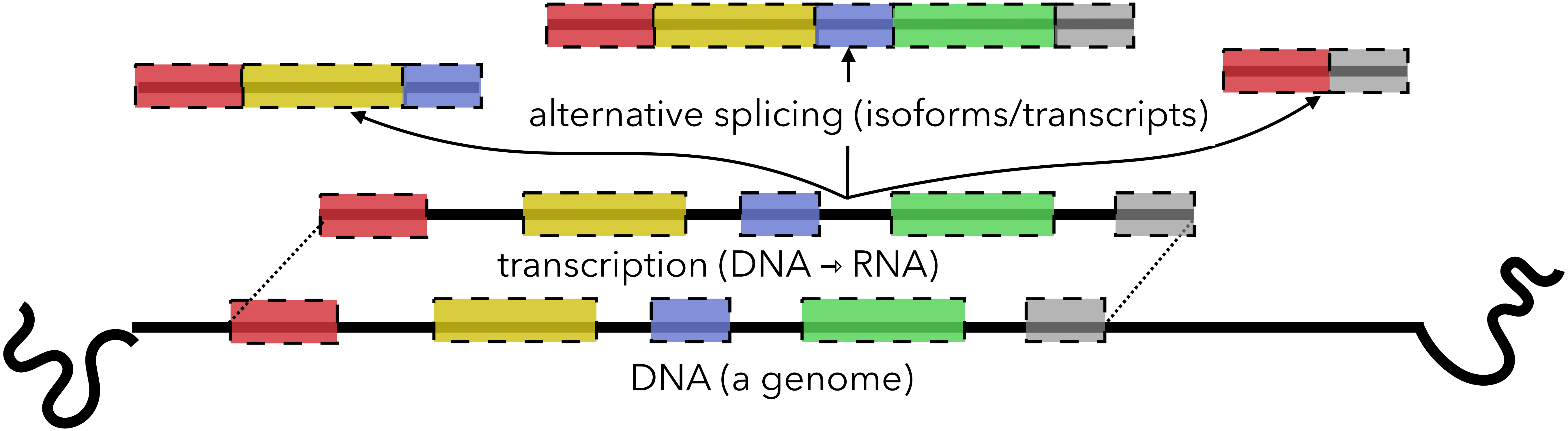
alternative splicing (isoforms/transcripts)
transcription (DNA → RNA)
DNA (a genome)

* most protocols actually sequence complementary DNA (cDNA), not RNA directly

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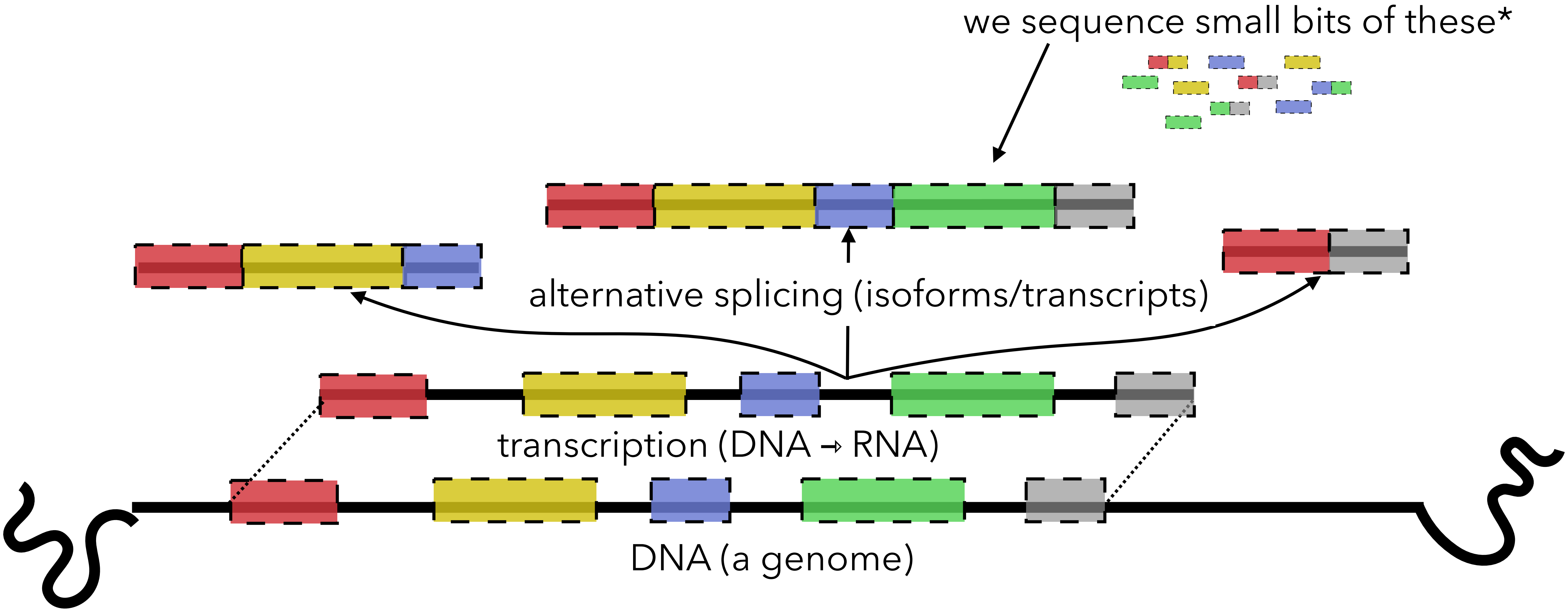


we sequence small bits of these*

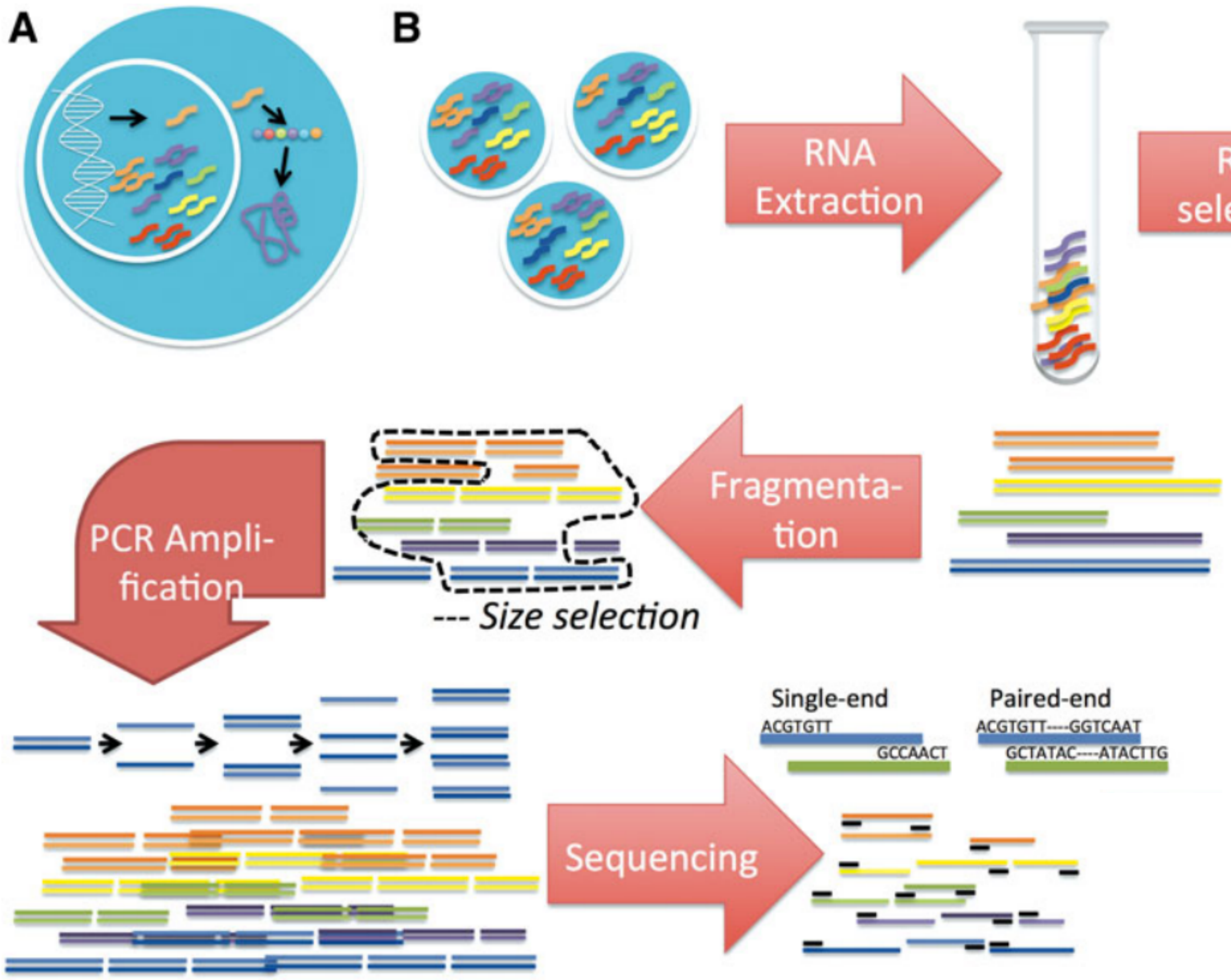
alternative splicing (isoforms/transcripts)

transcription (DNA → RNA)

DNA (a genome)

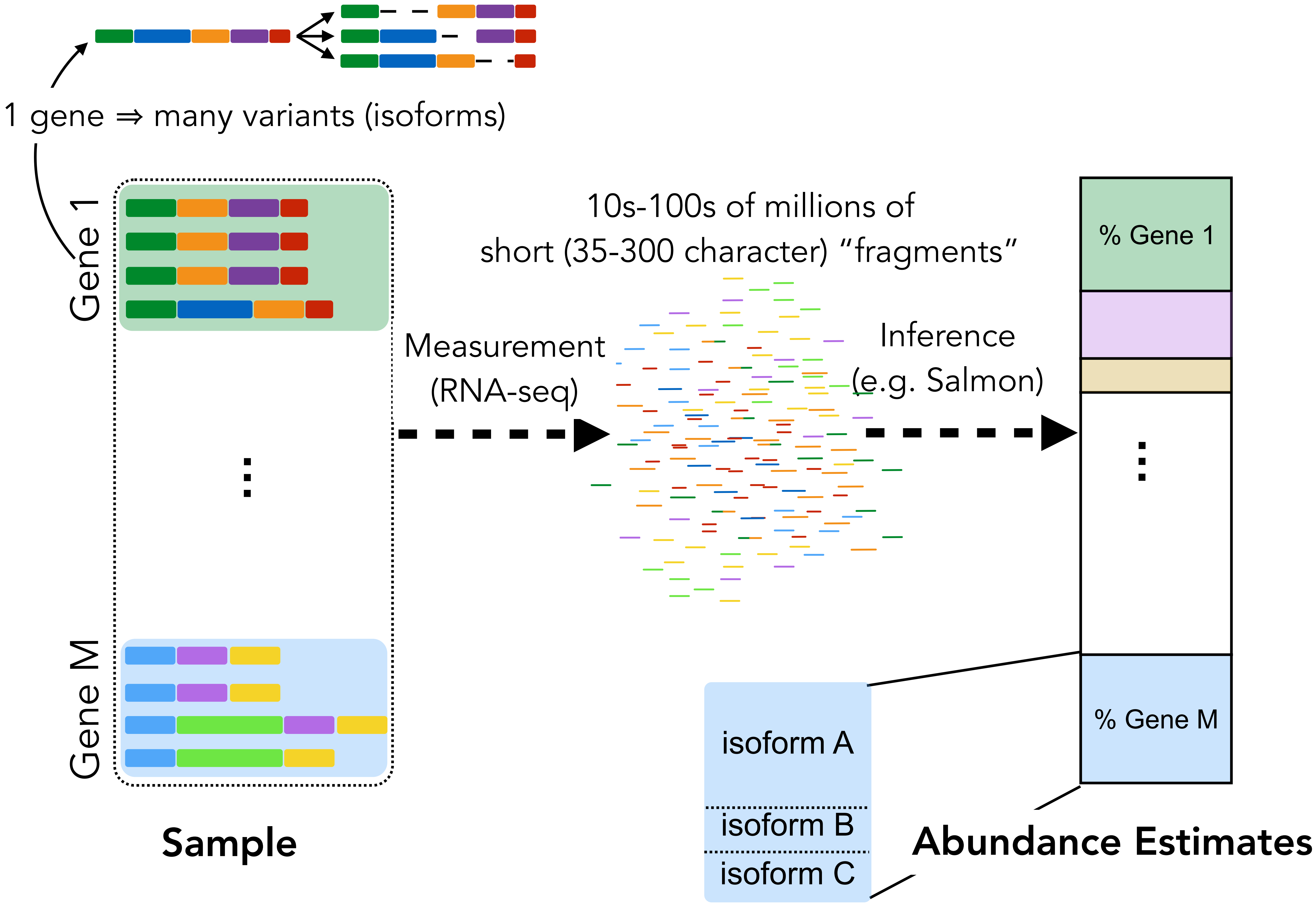
* most protocols actually sequence complementary DNA (cDNA), not RNA directly

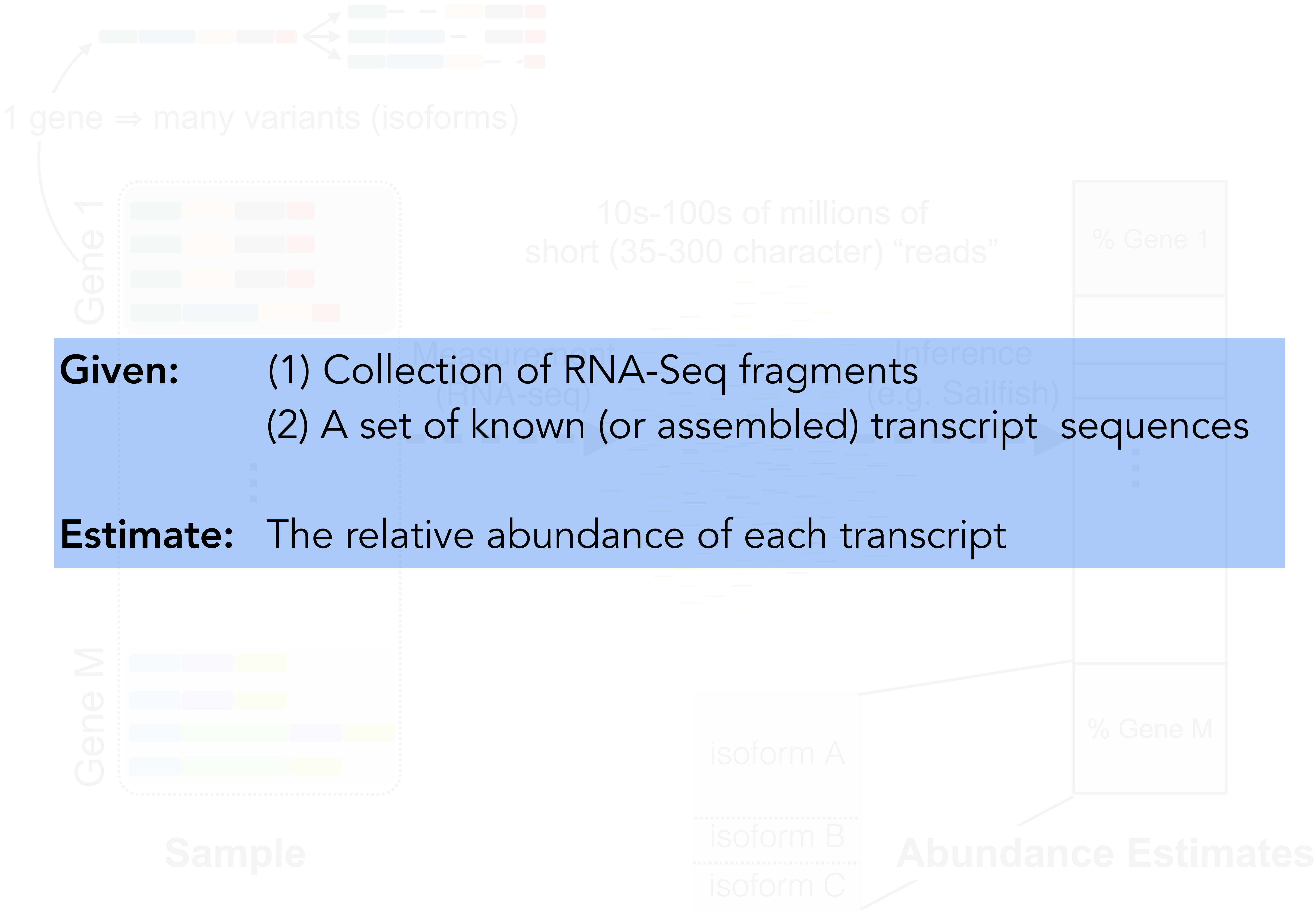
Actual protocols are much more involved



Prakash, Celine, and Arndt Von Haeseler. "An Enumerative Combinatorics Model for Fragmentation Patterns in RNA Sequencing Provides Insights into Nonuniformity of the Expected Fragment Starting-Point and Coverage Profile." *Journal of Computational Biology* 24.3 (2017): 200-212.

Transcript Quantification: An Overview





Given: (1) Collection of RNA-Seq fragments

(2) A set of known (or assembled) transcript sequences

Estimate: The relative abundance of each transcript

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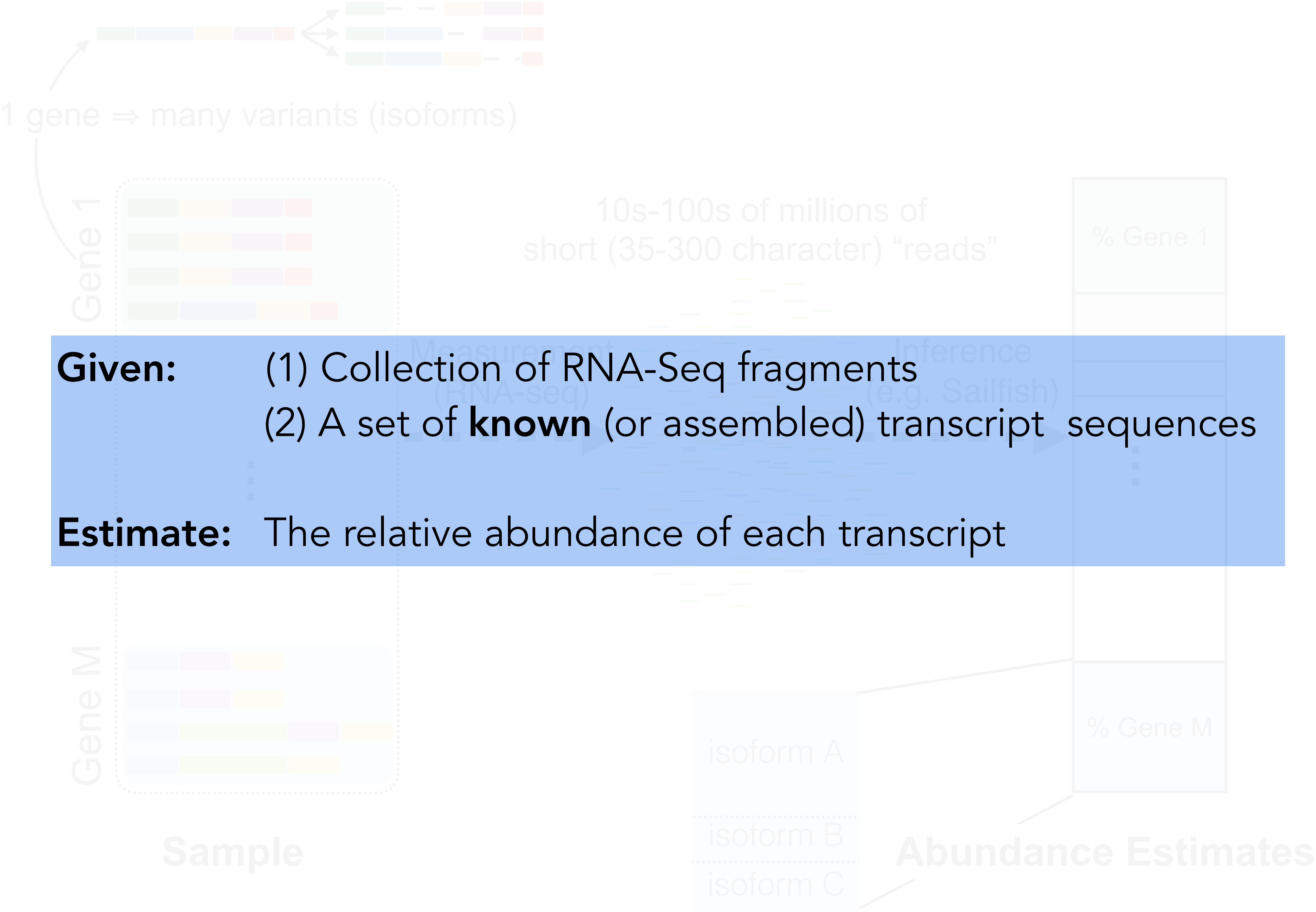


Why not simply “count” reads

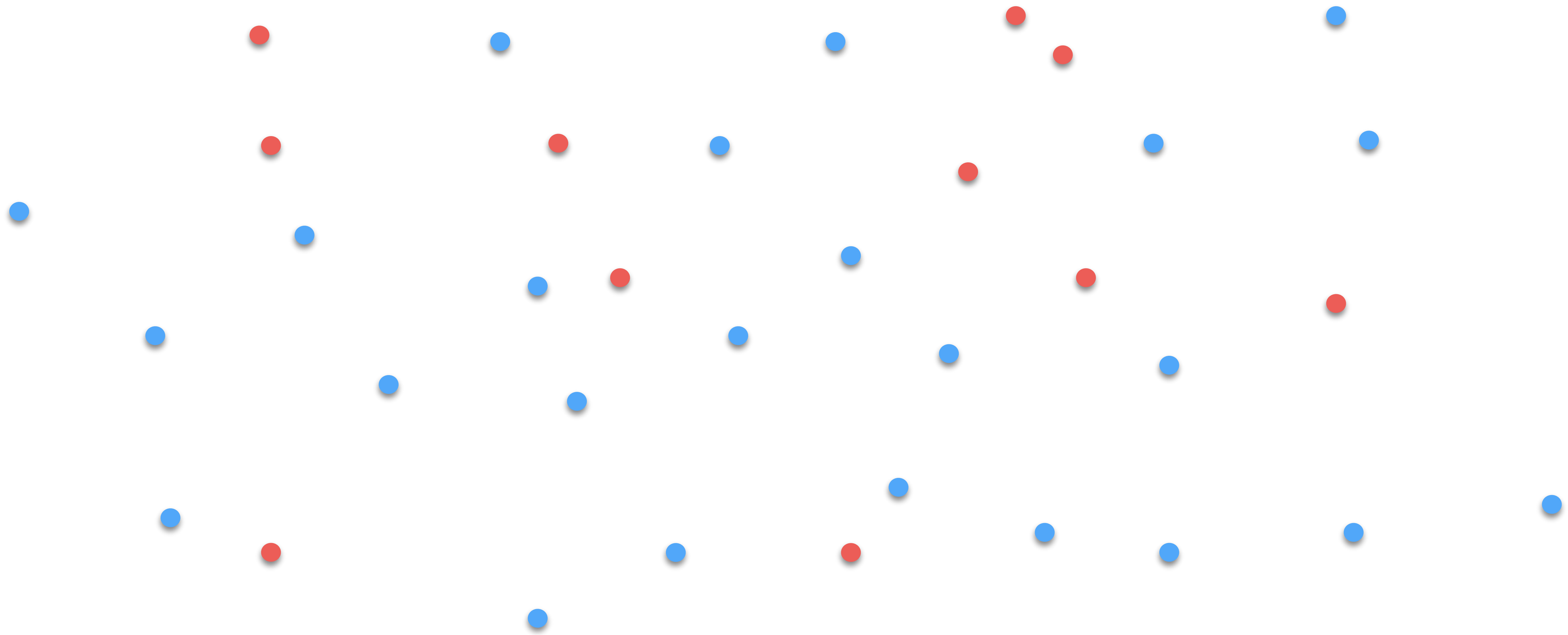
The RNA-seq reads are drawn from transcripts, and our (spliced) aligners let us map them back to the transcripts on the genome from which they originate.

Problem: How do you handle reads that align equally-well to multiple isoforms / or multiple genes?

- Discarding multi-mapping reads leads to incorrect and biased quantification
- Even at the gene-level, the transcriptional output of a gene should depend on what isoforms it is expressing.

First, consider this non-Biological example

Imagine I have two colors of circle, red and blue. I want to estimate the **fraction of circles** that are red and blue. I'll *sample* from them by tossing down darts.



Here, a dot of a color means I hit a circle of that color.

What type of circle is more prevalent?

What is the fraction of red / blue circles?

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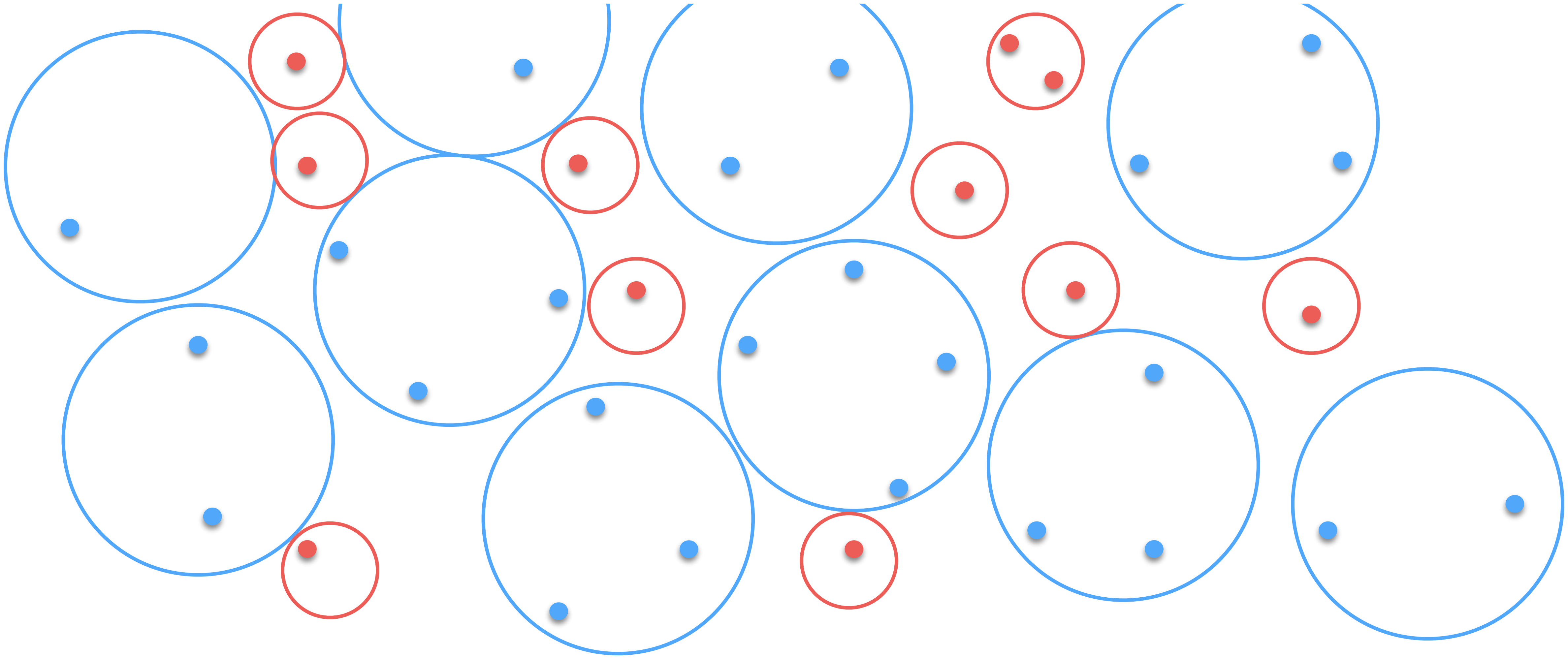

You're missing a **crucial piece of information!**

The areas!

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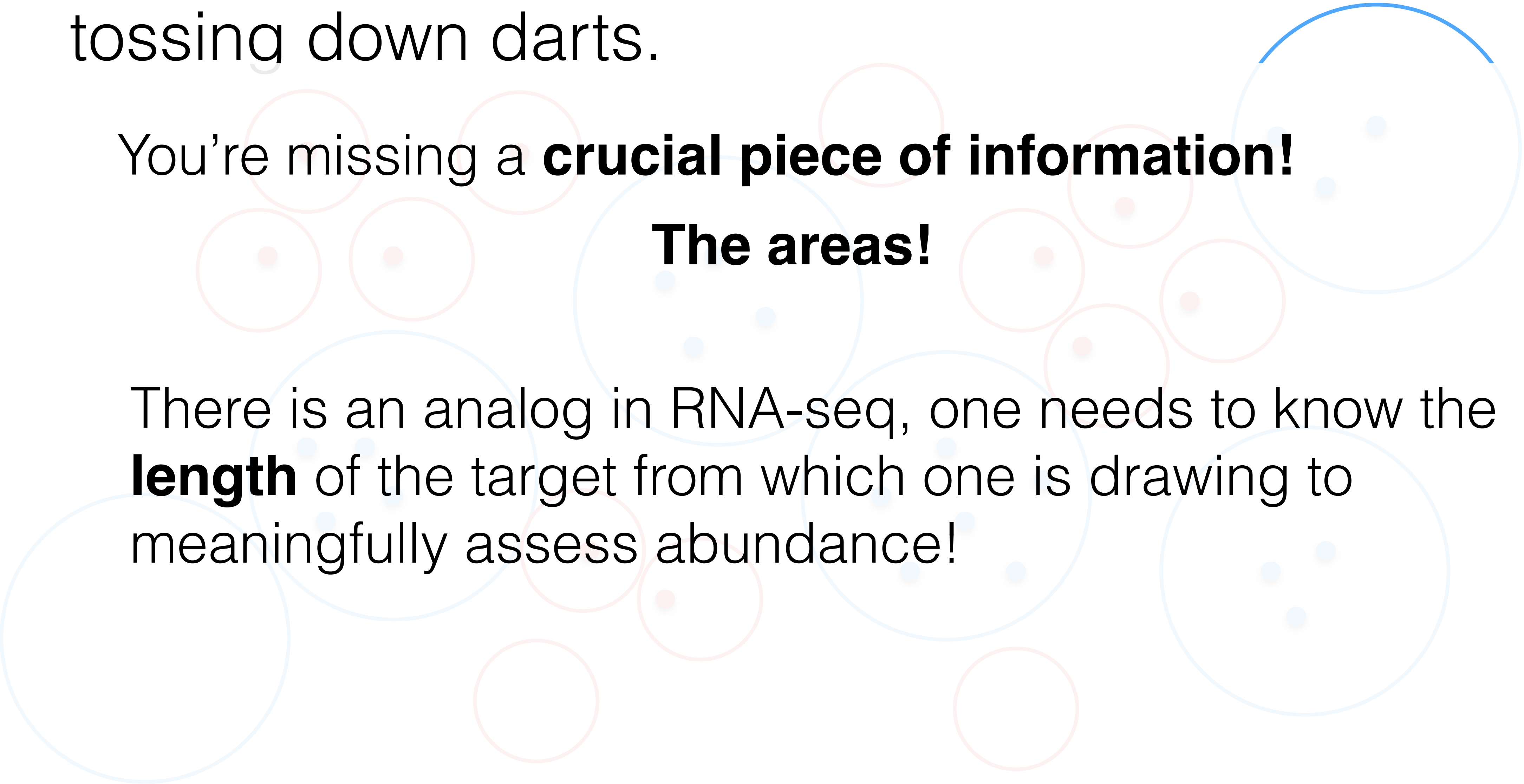


You're missing a **crucial piece of information!**

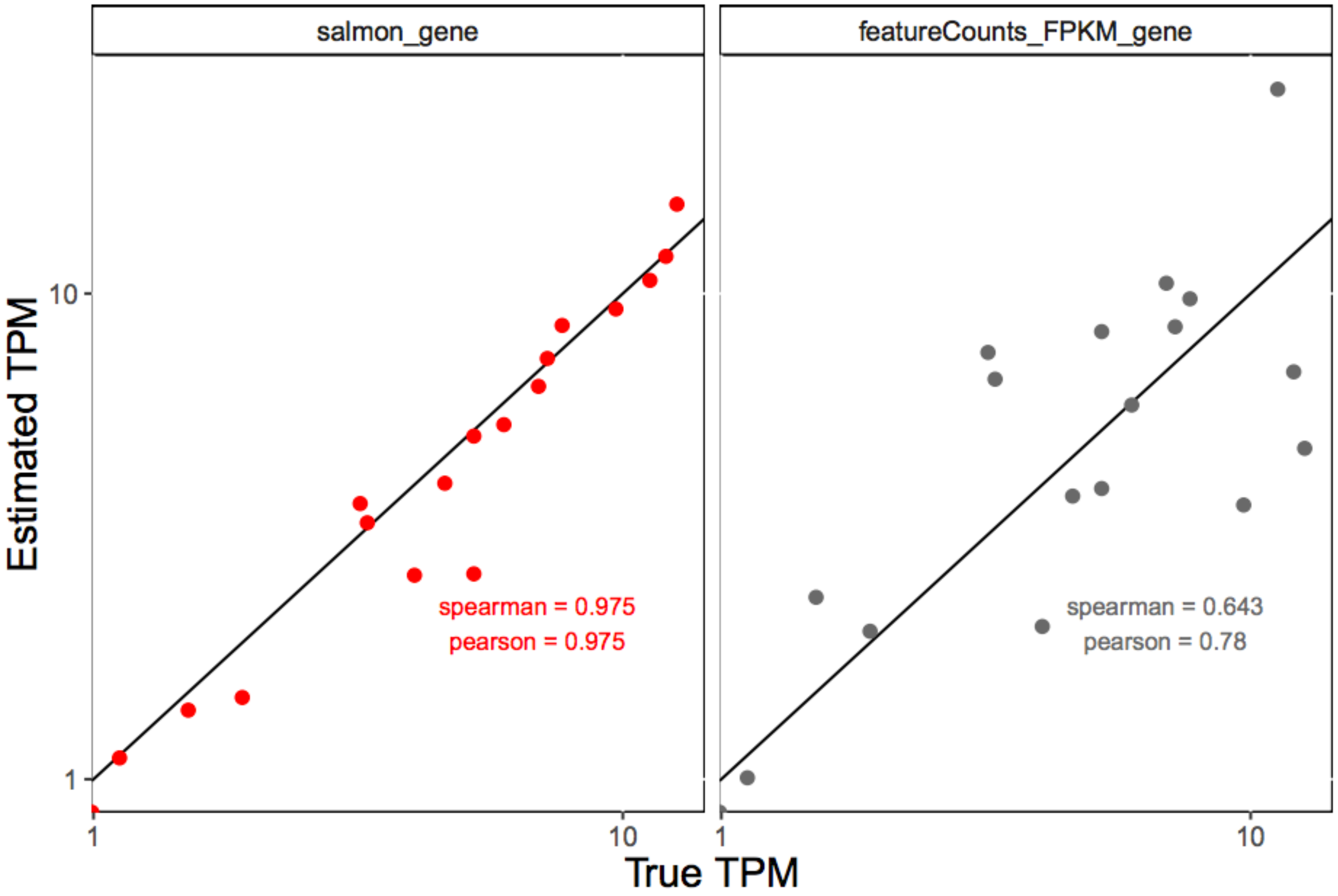
The areas!

There is an analog in RNA-seq, one needs to know the **length** of the target from which one is drawing to meaningfully assess abundance!

Resolving multi-mapping is fundamental to quantification

Can even affect abundance estimation in **absence** of alternative-splicing (e.g. paralogous genes)

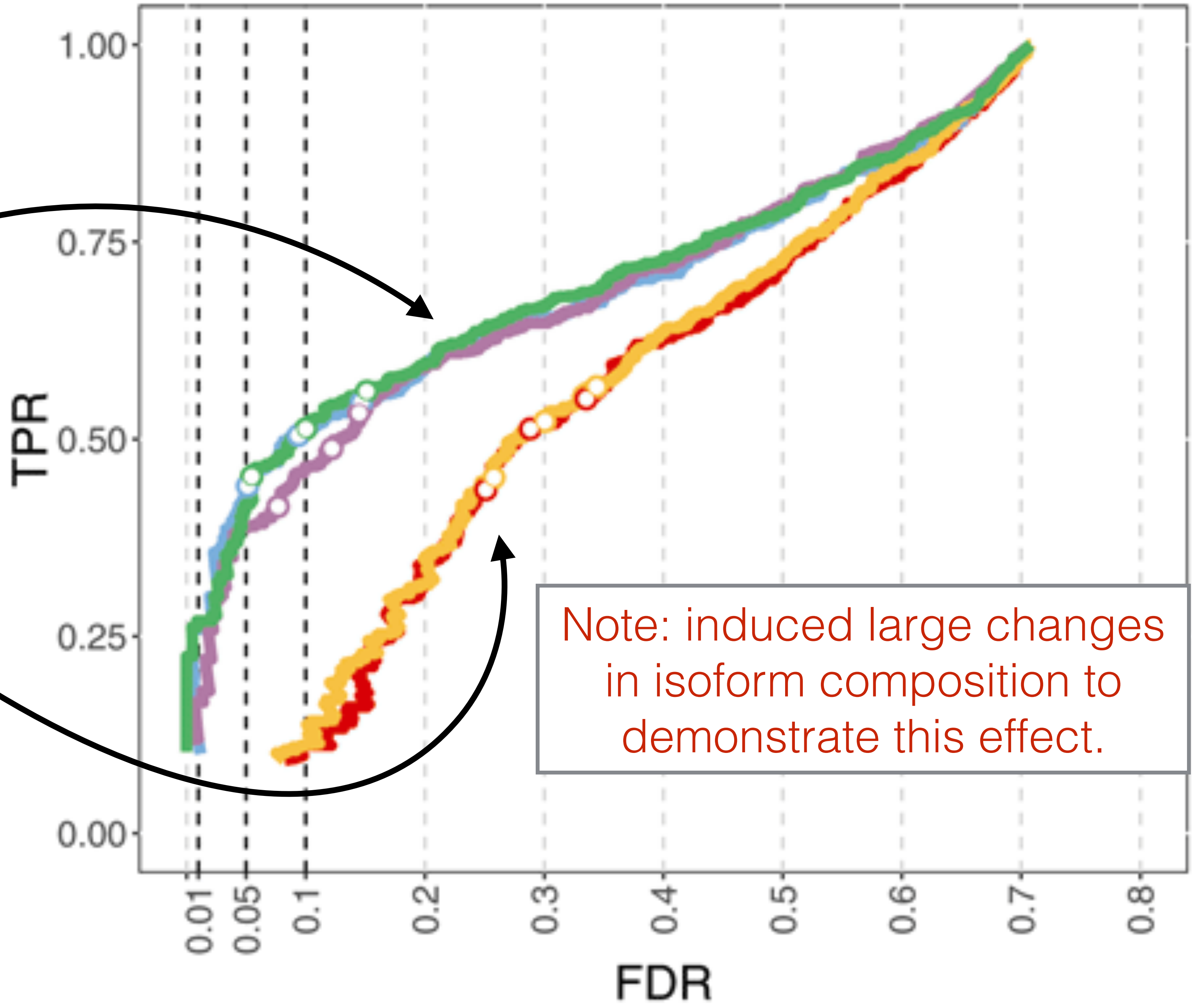
Paralogs of ENSG00000090612



From: Soneson C, Love MI and Robinson MD 2016 [version 2; referees: 2 approved] F1000Research 2016, 4:1521 (doi: 10.12688/f1000research.7563.2)

Resolving multi-mapping is fundamental to quantification

These errors can affect DGE calls



Variants of Salmon

Variants of “counting”

Note: induced large changes in isoform composition to demonstrate this effect.

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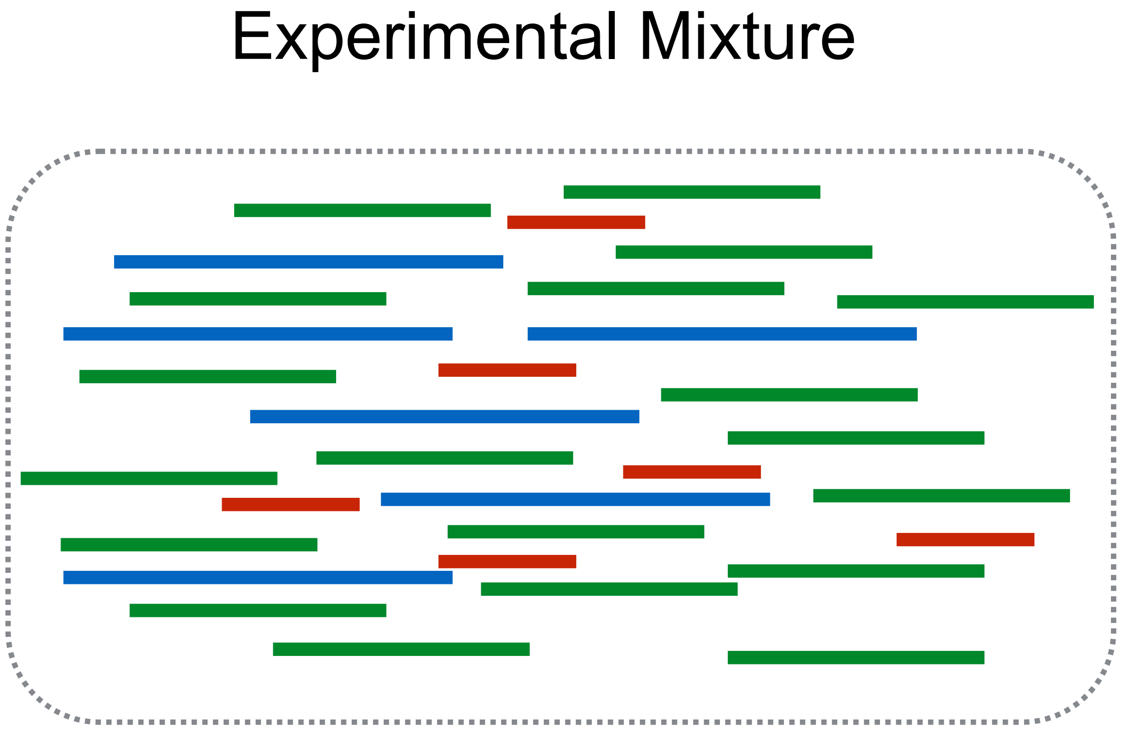
How can we perform inference from sequenced fragments?



In an unbiased experiment, sampling fragments depends on:

- # of copies of each txp type
- length of each txp type

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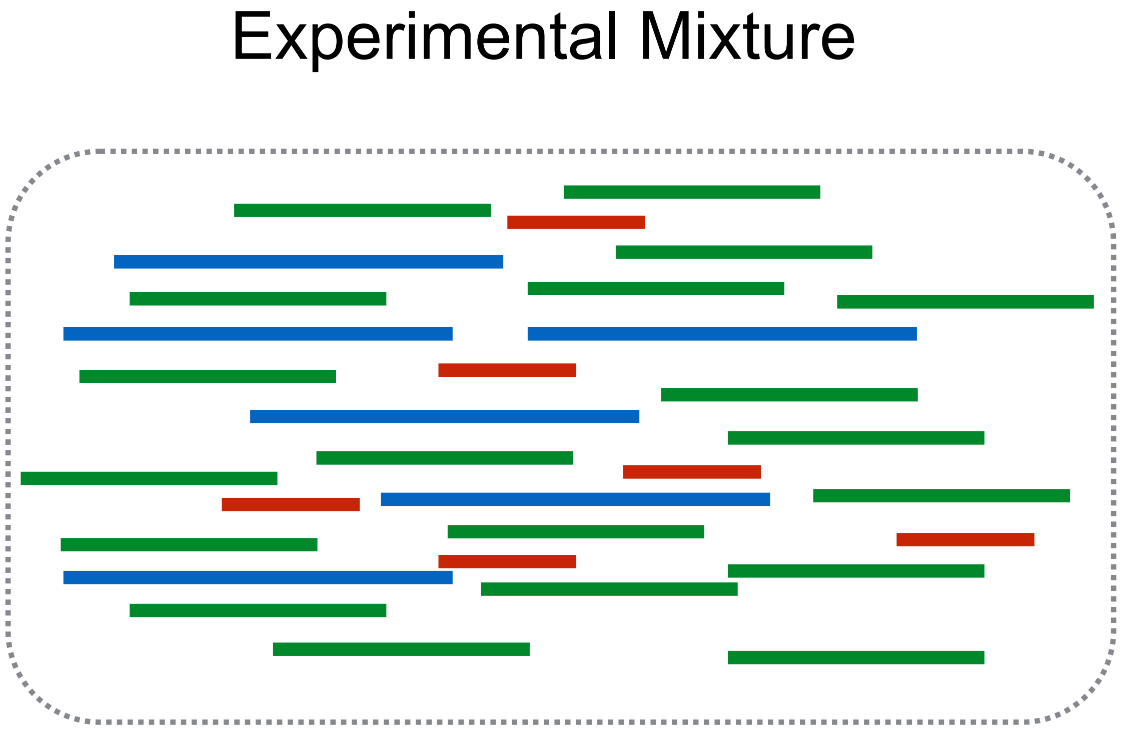

length(▬) = 100

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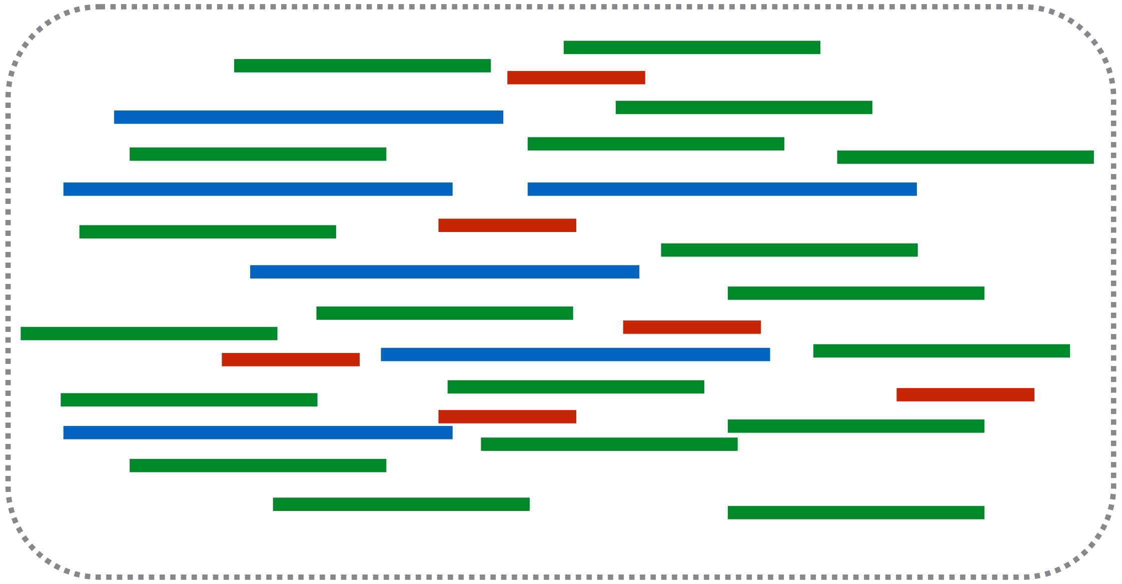
How can we perform inference from sequenced fragments?

Experimental Mixture



In an unbiased experiment, sampling fragments depends on:

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length(━━━━) = 100 x 6 copies

How can we perform inference from sequenced fragments?

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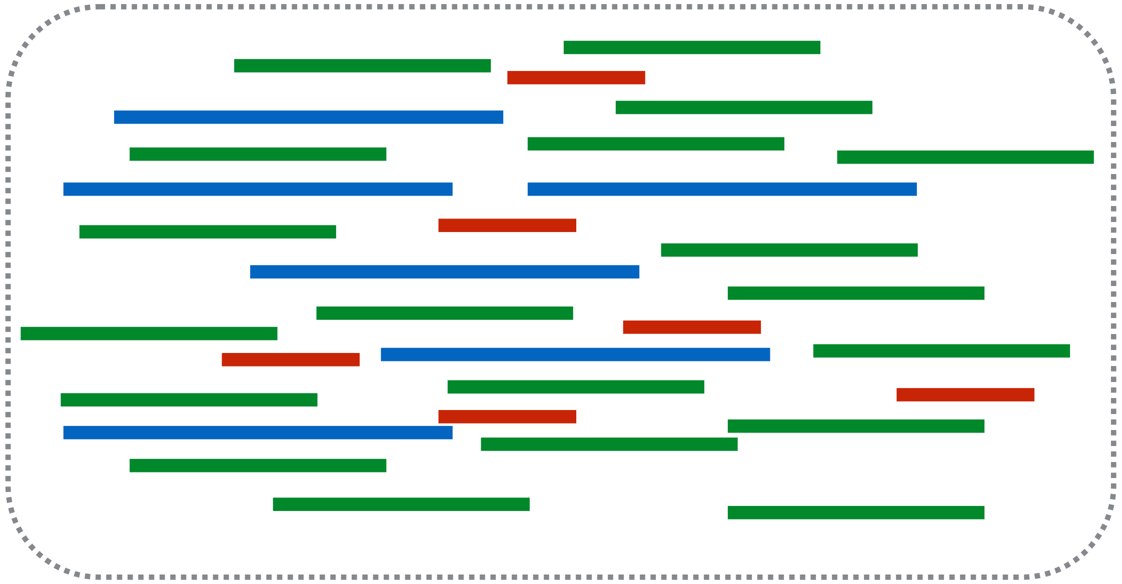

In an unbiased experiment, sampling fragments depends on:

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length(▬) = 100 x 6 copies = 600 nt

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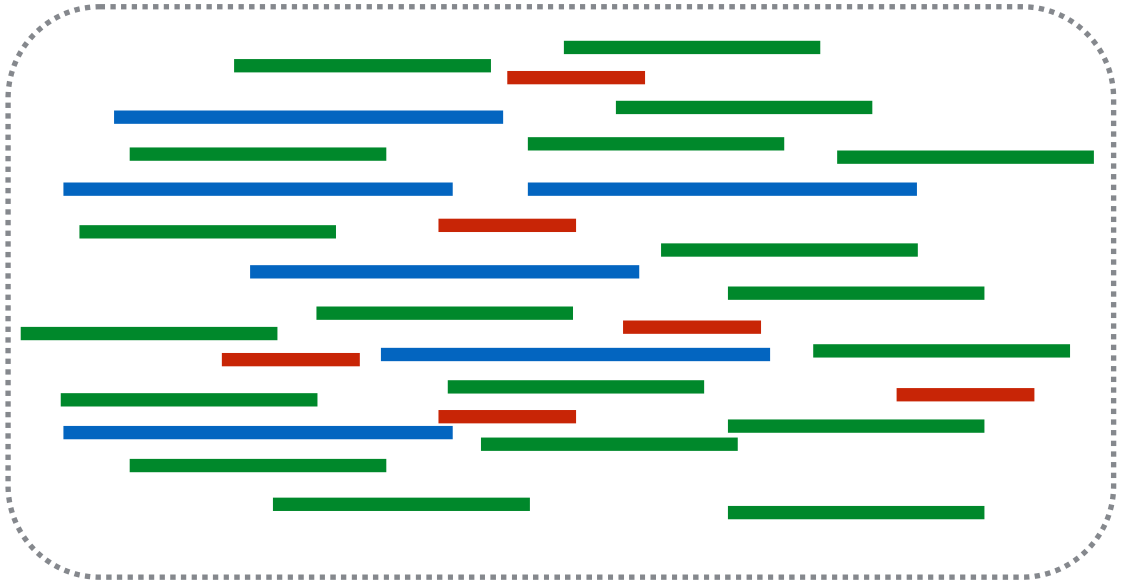

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length(🟦) = 100 x 6 copies = 600 nt

length(🟩) = 66 x 19 copies = 1254 nt

length(🟥) = 33 x 6 copies = 198 nt

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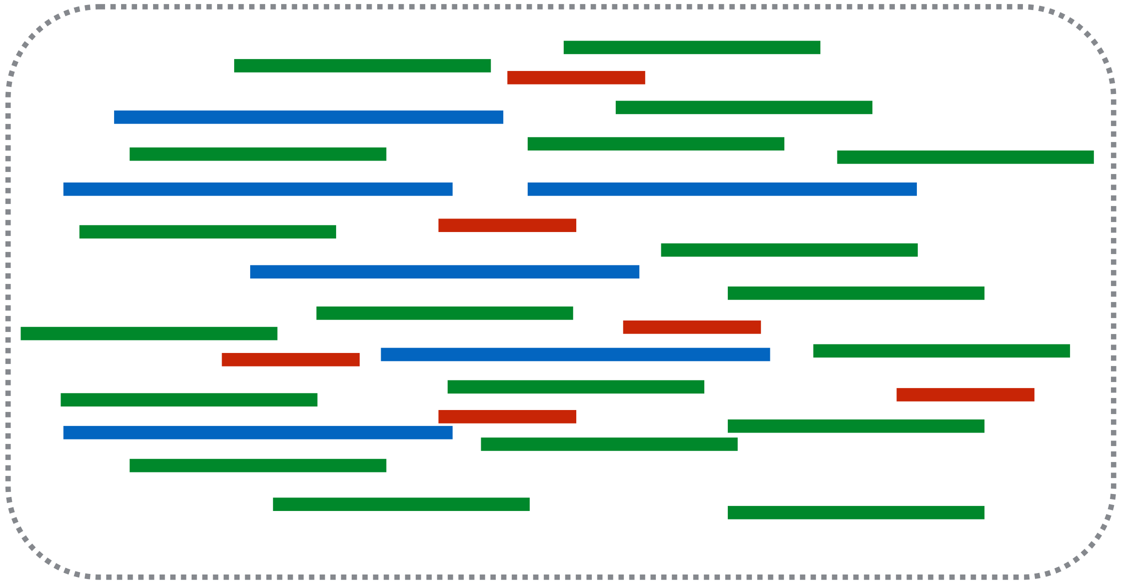

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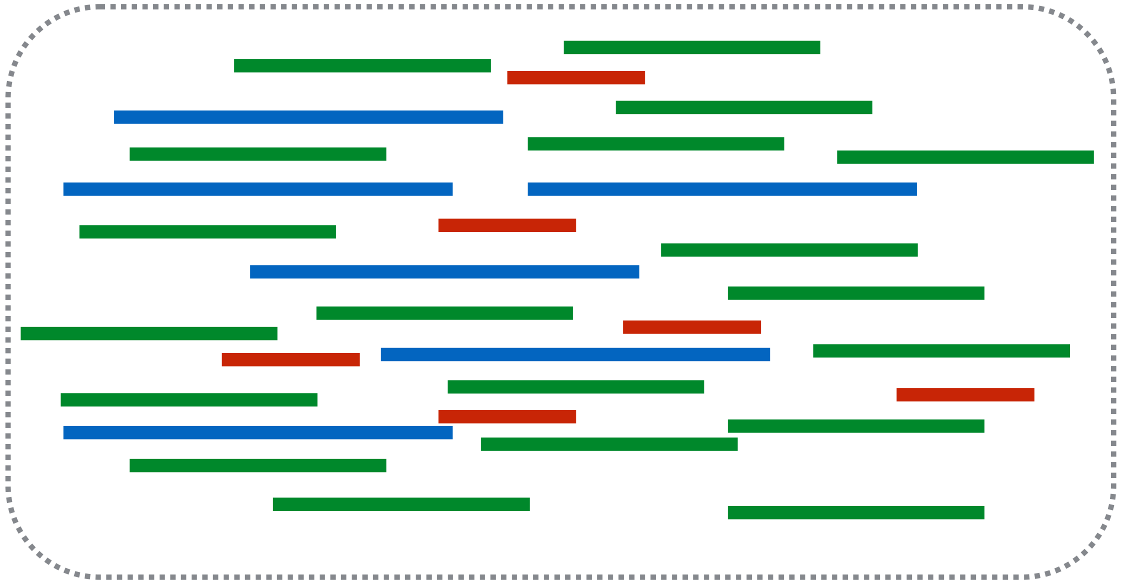

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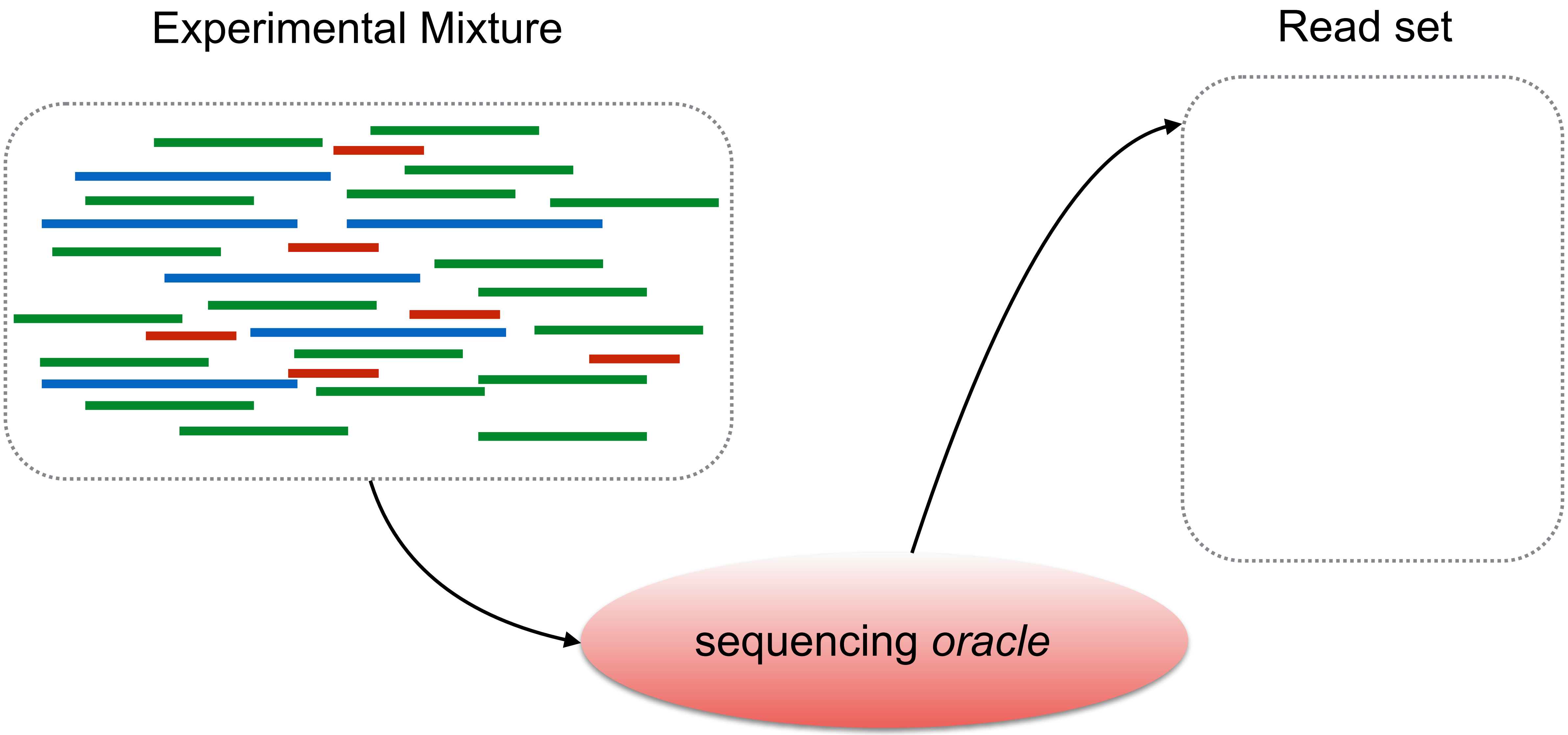
↑

We call these values η = [0.3, 0.6, 0.1] the nucleotide fractions, they become the primary quantity of interest

How can we perform inference from sequenced fragments?

Think about the "ideal" RNA-seq experiment . . .



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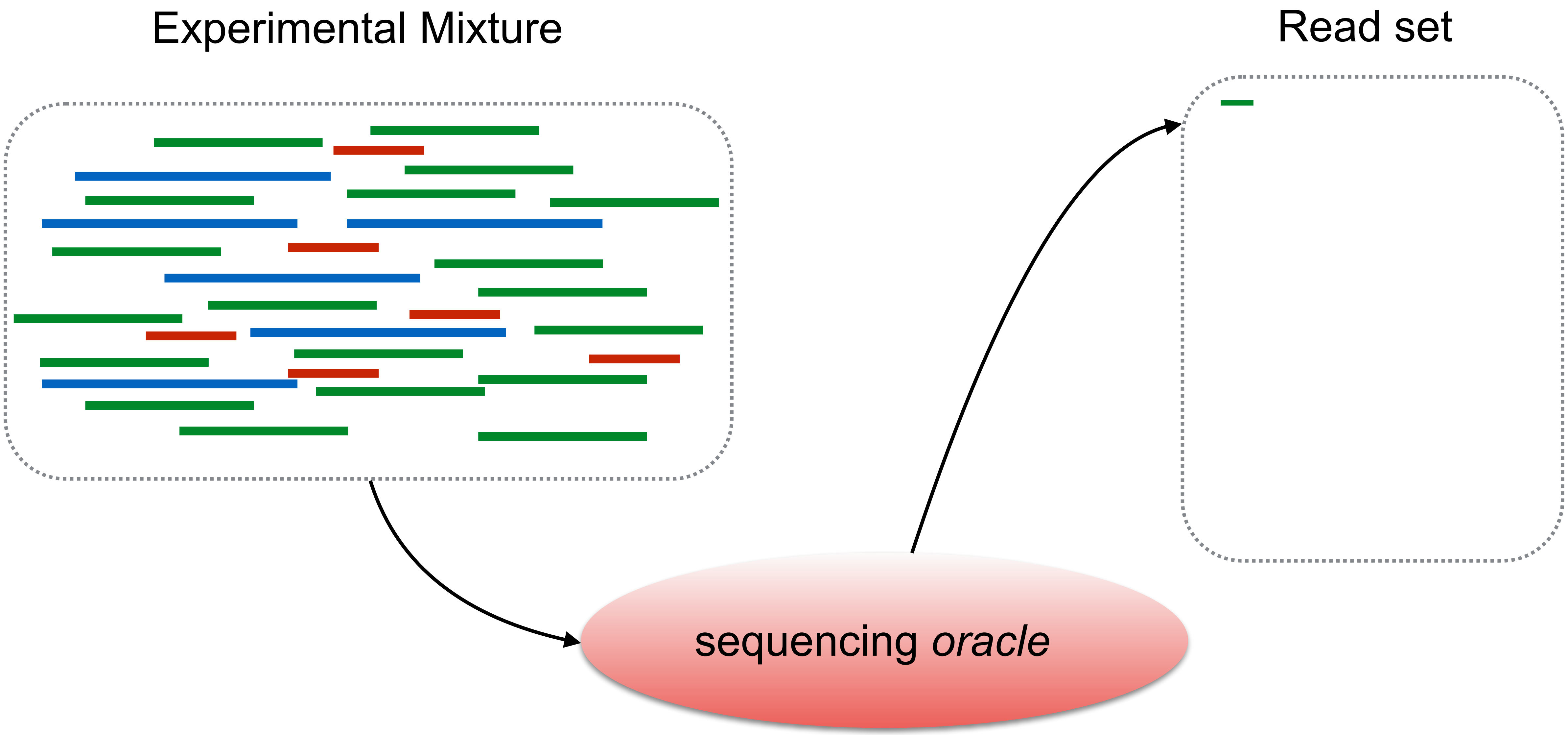

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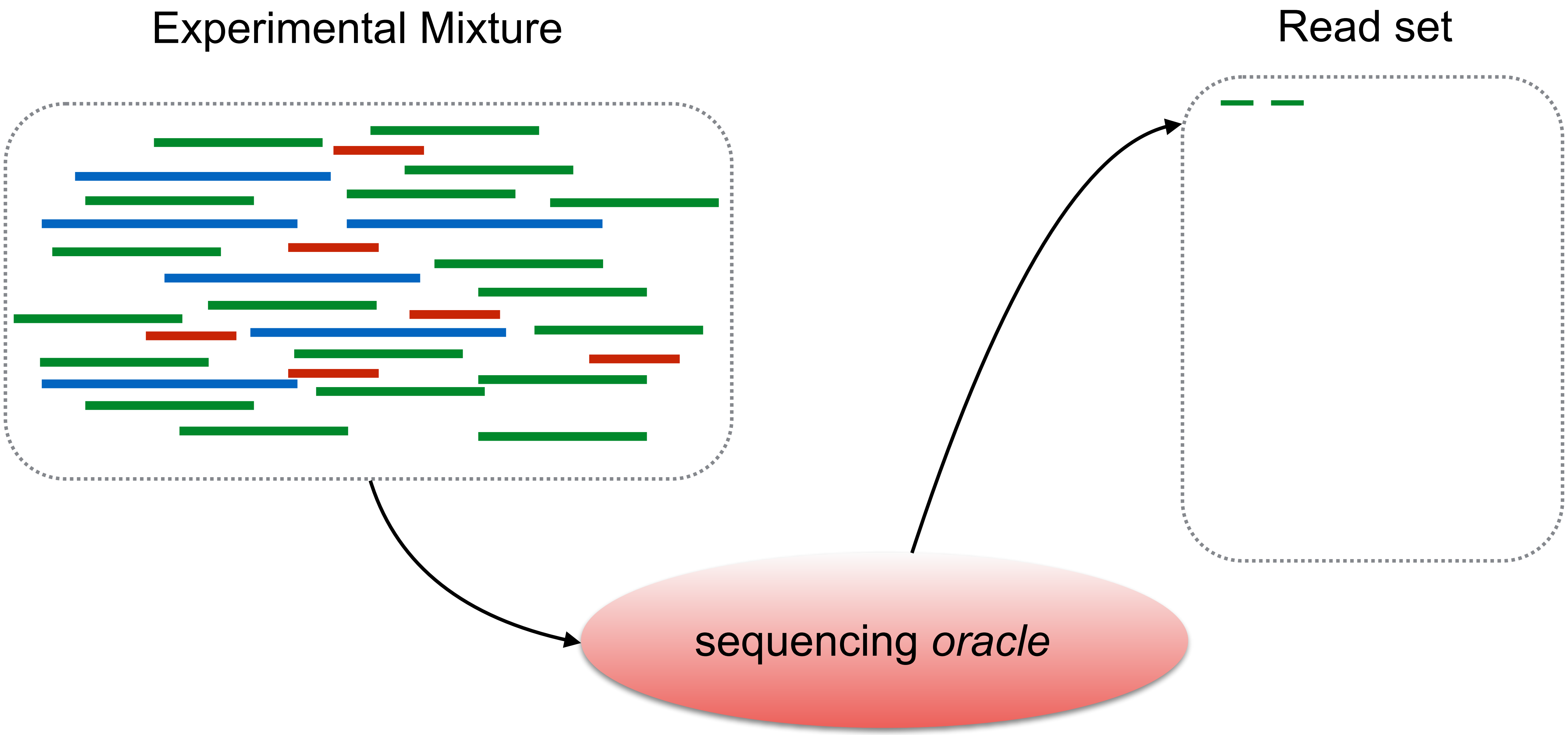

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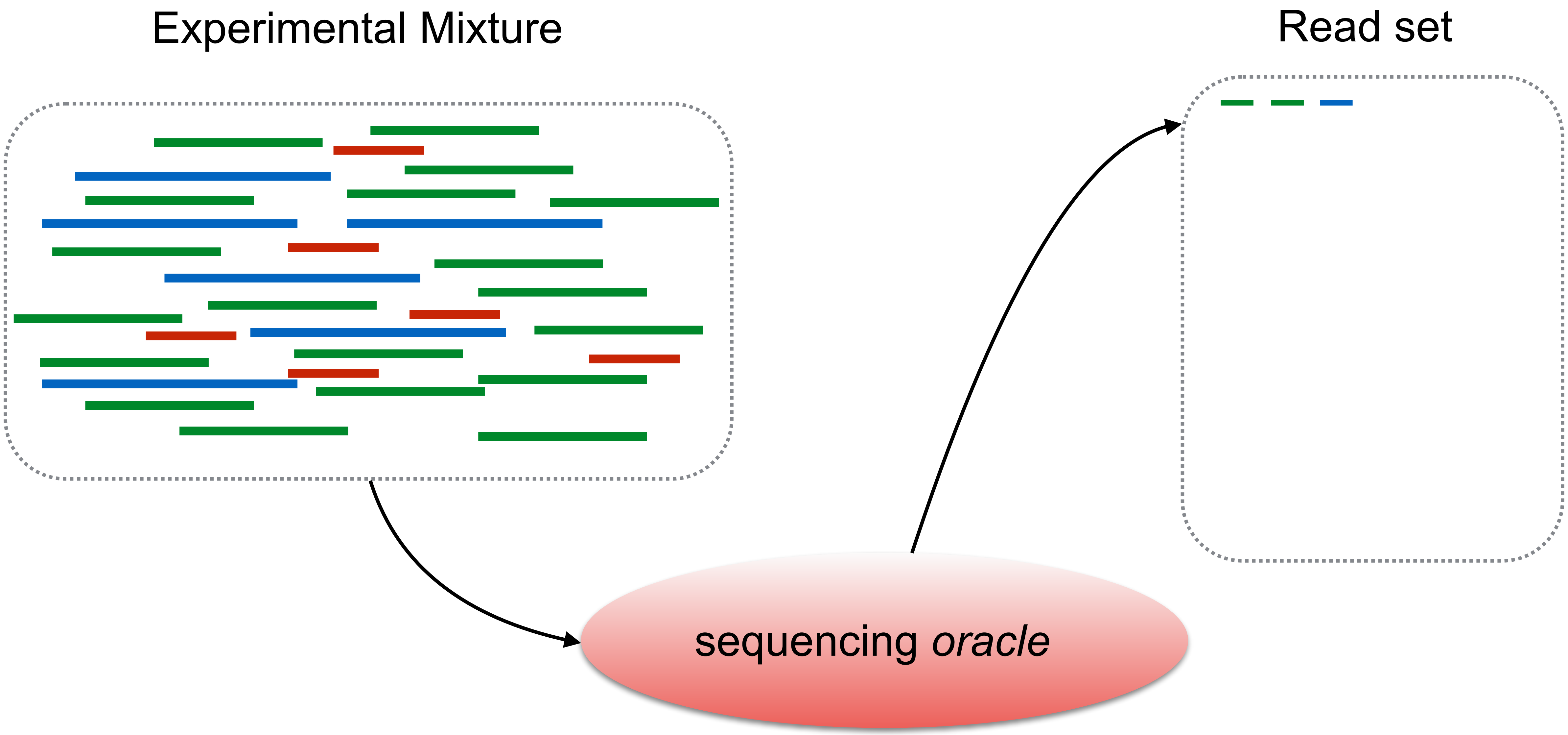

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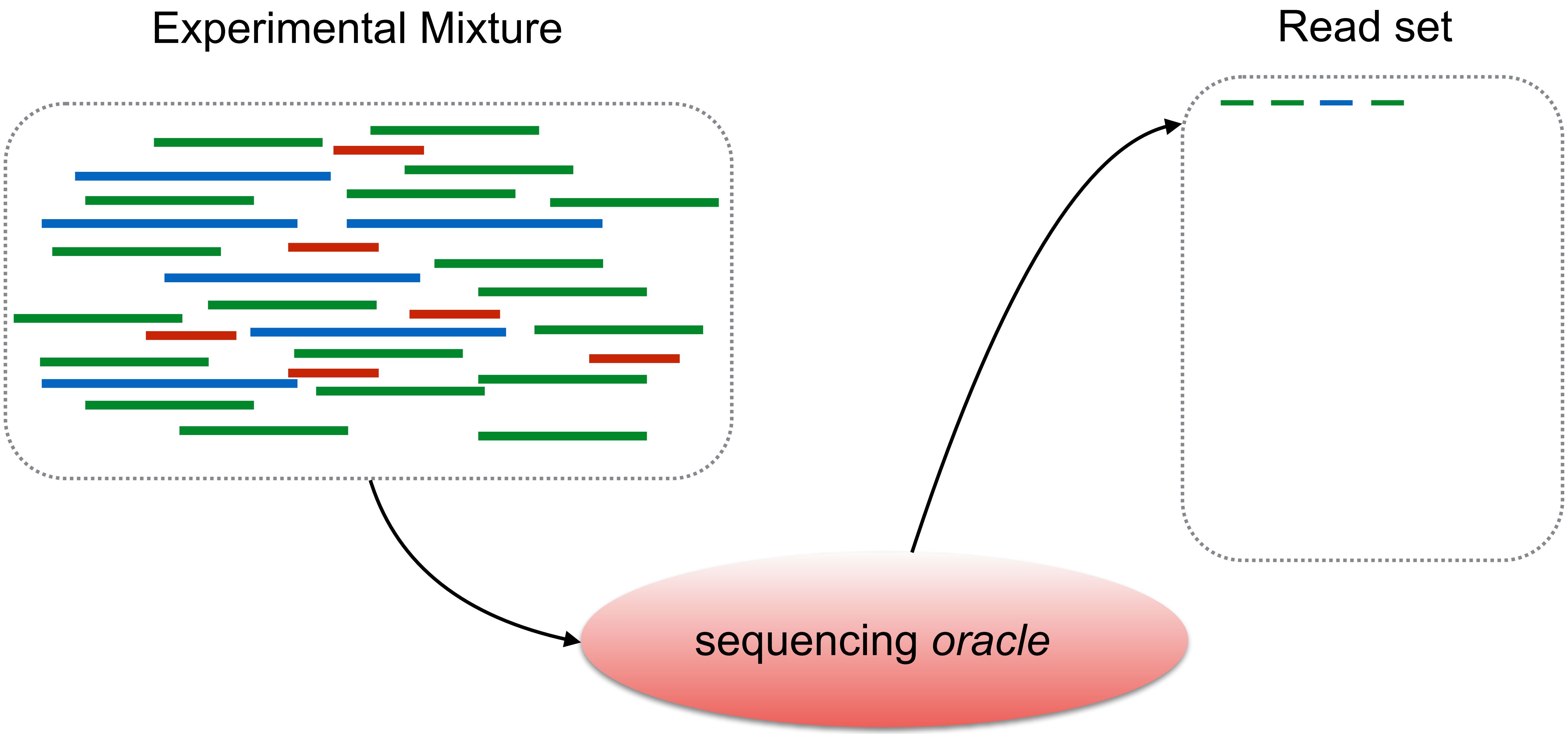

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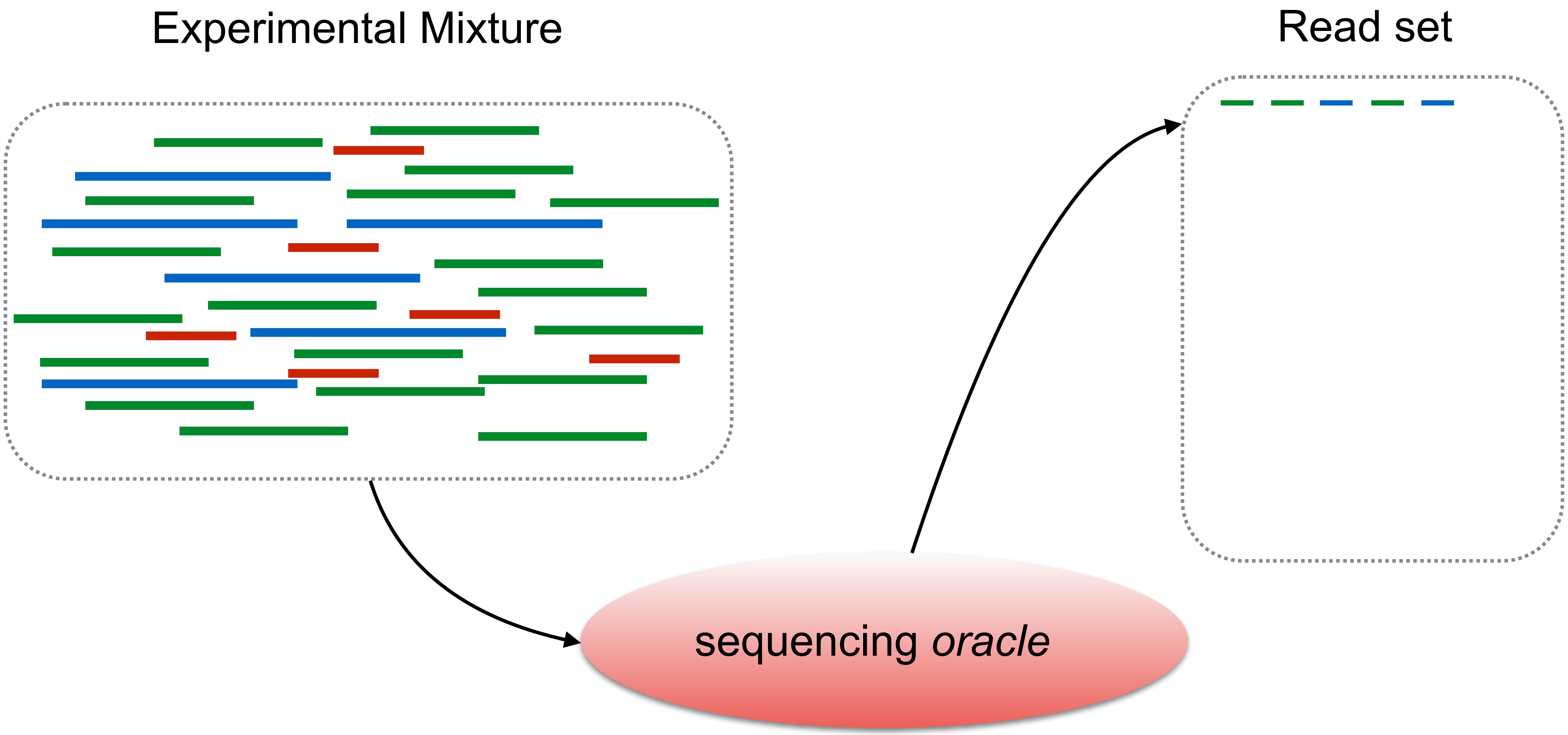

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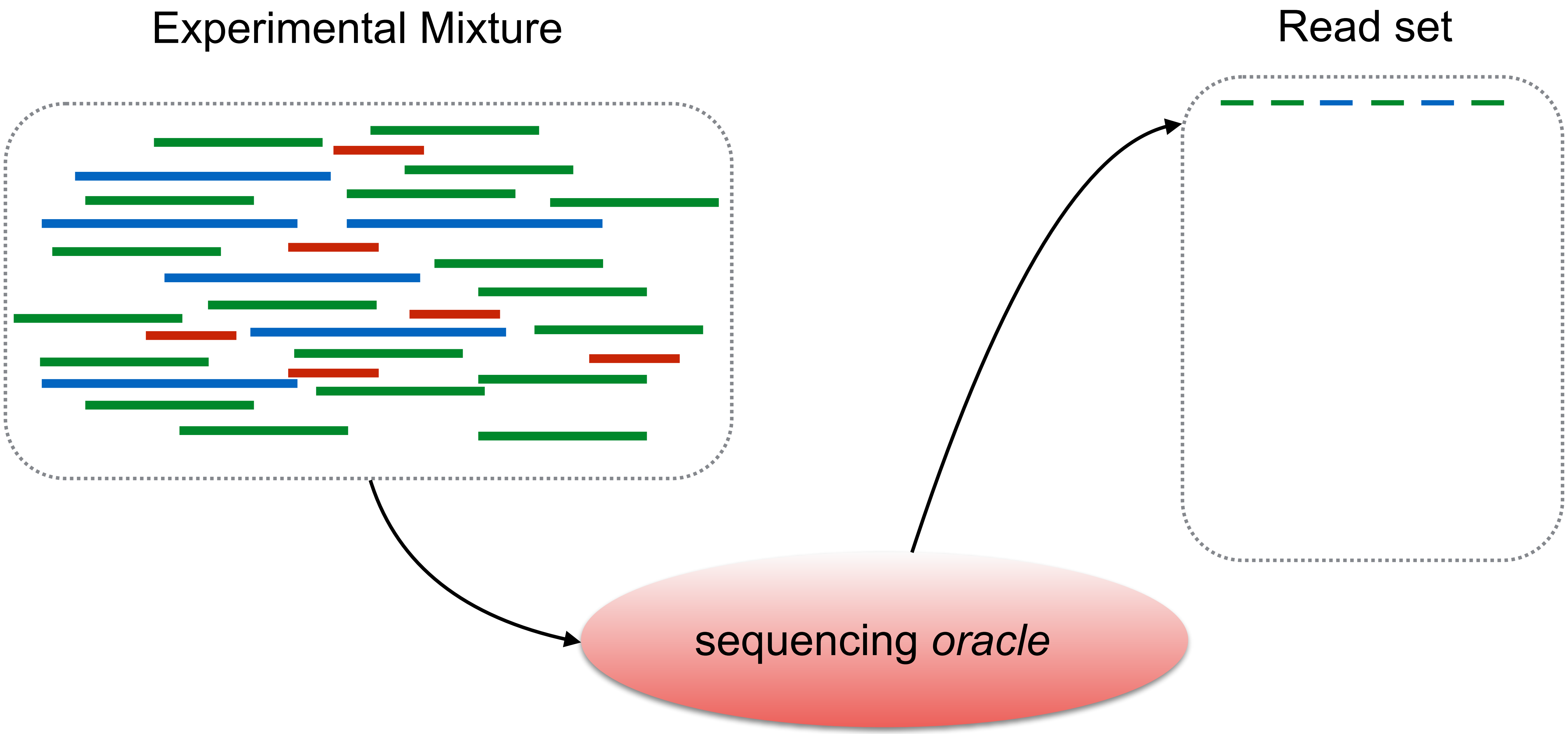

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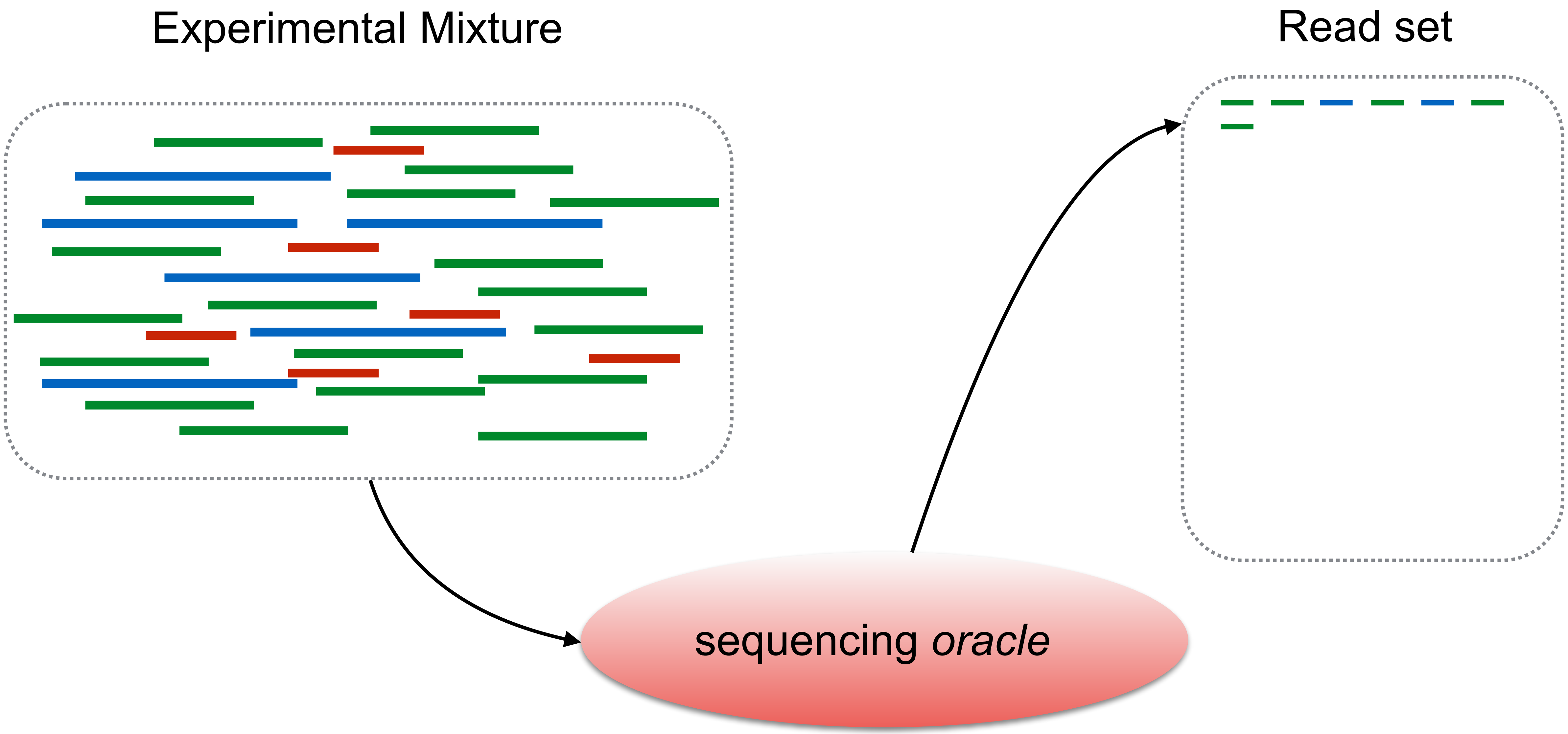

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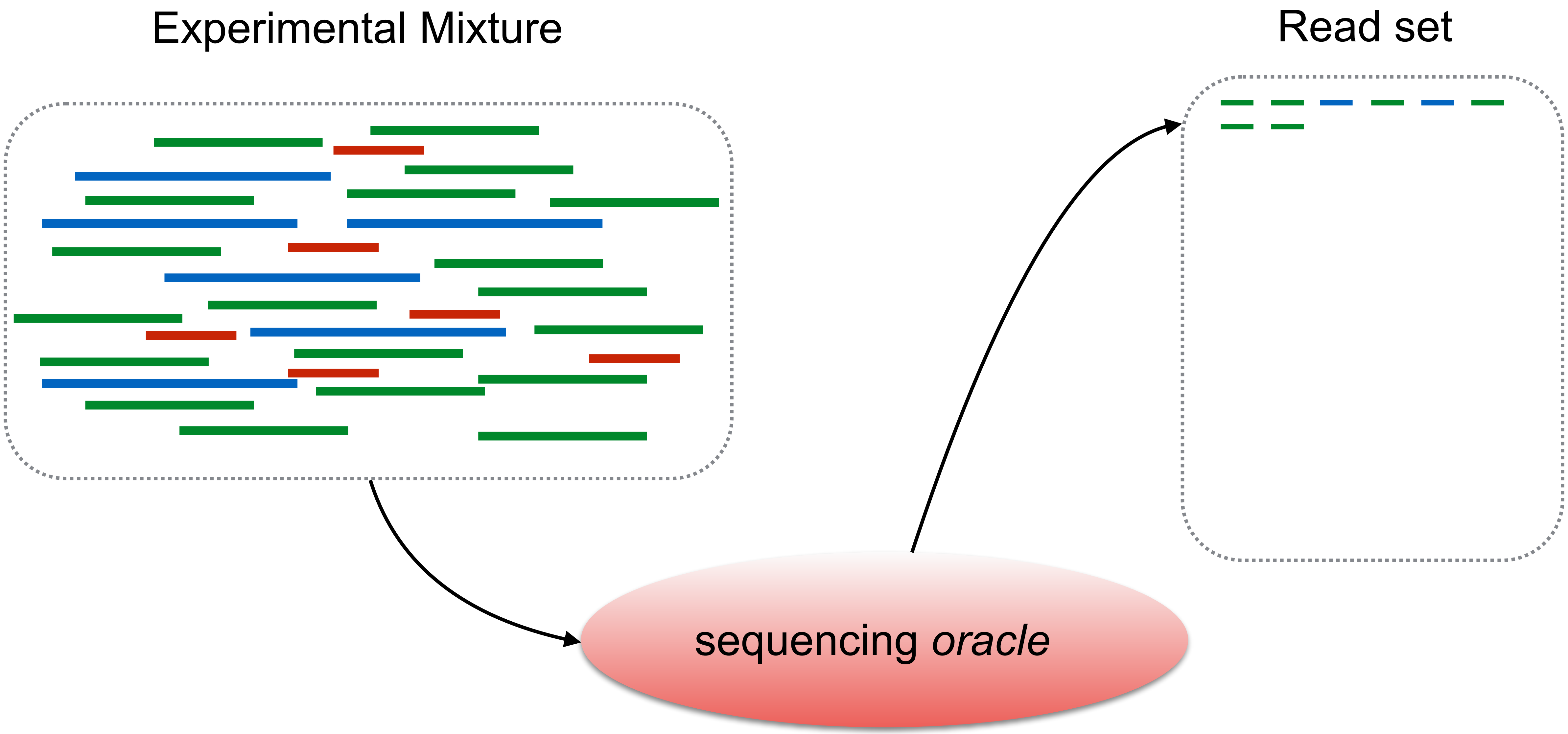

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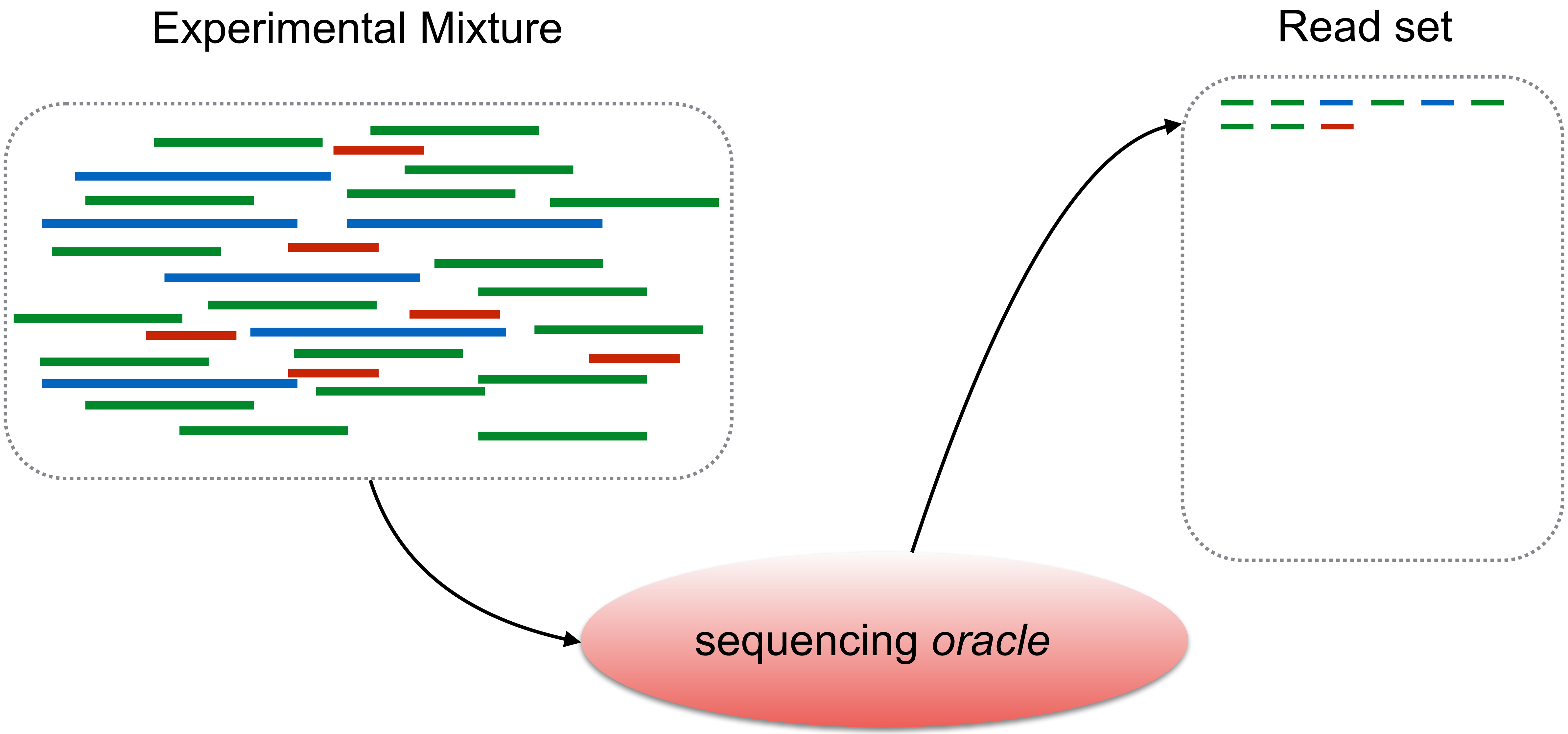

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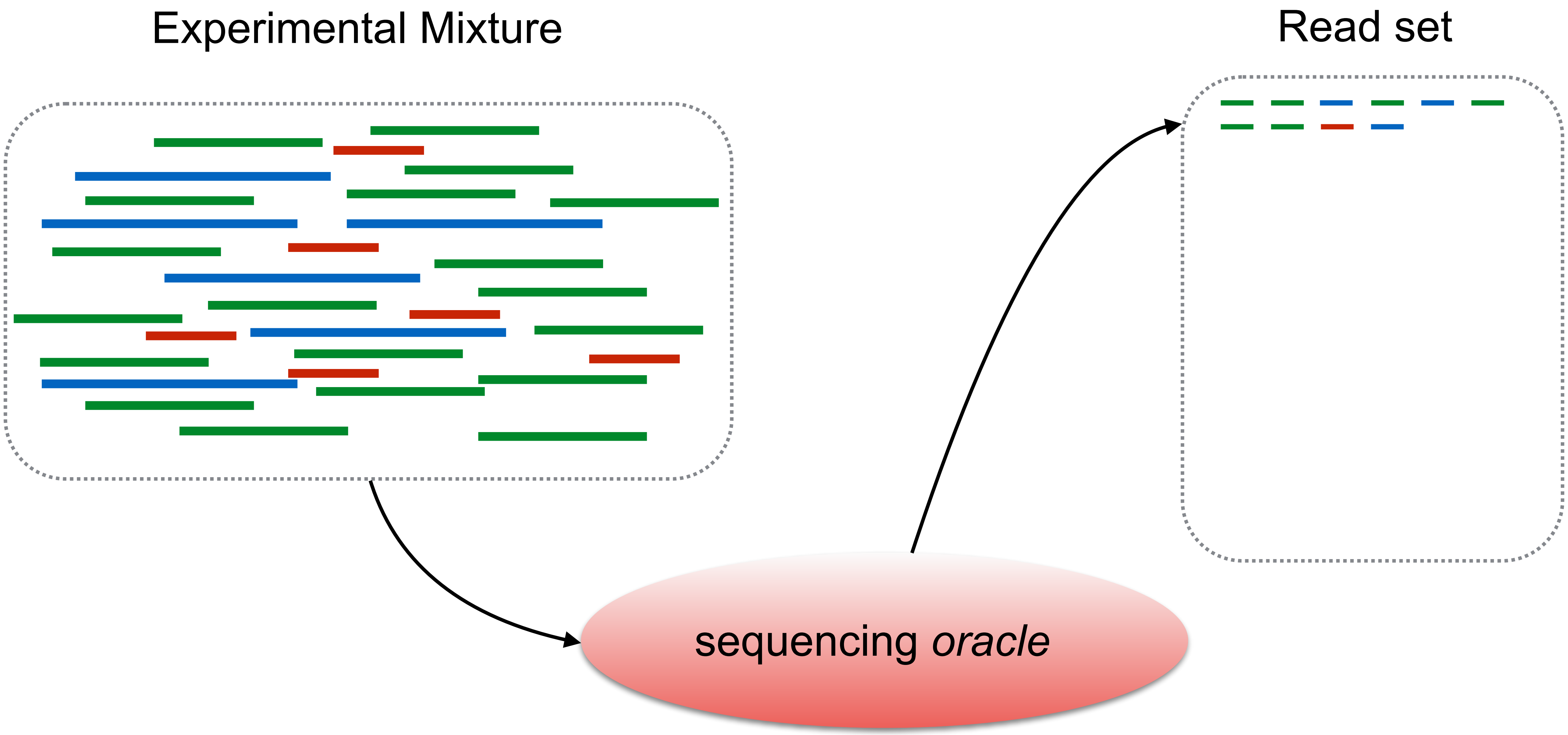

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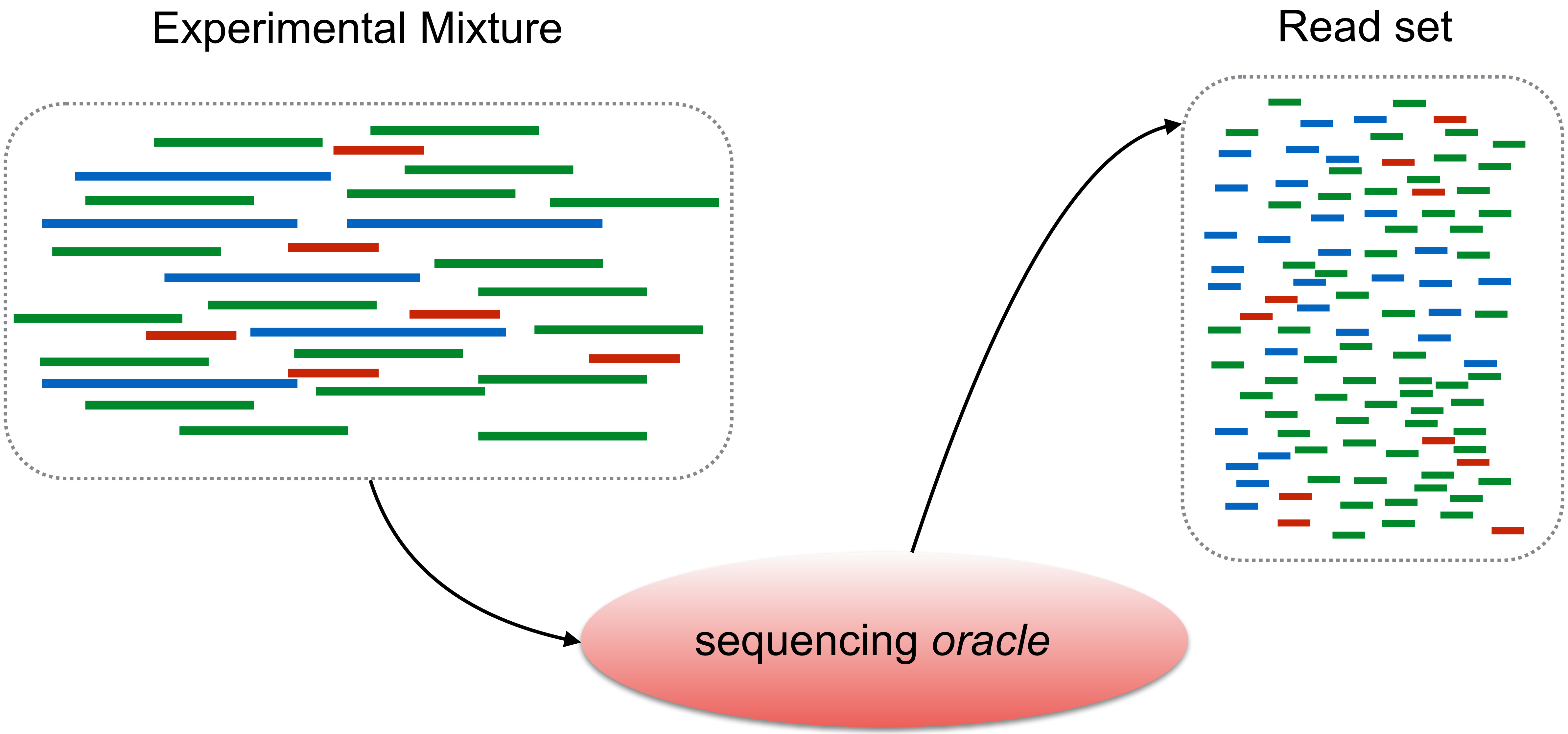

Experimental Mixture

Read set

sequencing *oracle*

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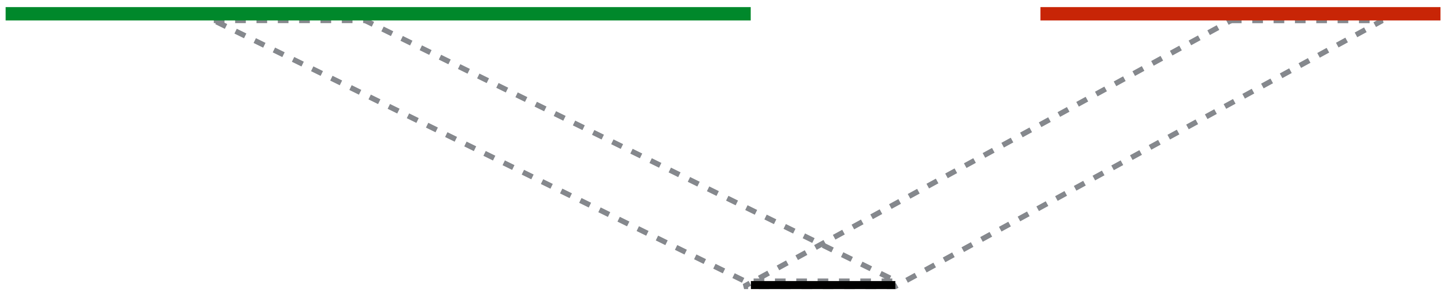
Resolving a single multi-mapping read



Say we *knew* the η, and observed a *single* read that mapped ambiguously, as shown above.

What is the probability that it truly originated from G or R?

$$\Pr\{r \text{ from } G\} = \frac{\frac{\eta_G}{\text{length}(G)}}{\frac{\eta_G}{\text{length}(G)} + \frac{\eta_R}{\text{length}(R)}} = \frac{\frac{0.6}{66}}{\frac{0.6}{66} + \frac{0.1}{33}} = 0.75$$

$$\Pr\{r \text{ from } R\} = \frac{\frac{\eta_R}{\text{length}(R)}}{\frac{\eta_G}{\text{length}(G)} + \frac{\eta_R}{\text{length}(R)}} = \frac{\frac{0.1}{33}}{\frac{0.6}{66} + \frac{0.1}{33}} = 0.25$$

normalization factor

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Units for Relative Abundance

TPM (Transcripts Per Million)

$$\mathrm{TPM}_i = \rho_i \times 10^6 \text{ where } 0 \leq \rho_i \leq 1 \text{ and } \sum_i \rho_i = 1$$

$$\rho_i = \frac{\frac{X_i}{\ell_i}}{\sum_j \frac{X_j}{\ell_j}}$$

Reads coming from transcript i

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abundance of i
as fraction of all
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Reads coming from transcript i

Length of transcript i

Aside: Maximum Likelihood Est. and the EM Algorithm

The following slides on MLE & EM are taken from the UW CSE 312 Web*

Parameter Estimation

Assuming sample $x_1, x_2, ..., x_n$ is from a parametric distribution $f(x|\theta)$, estimate θ.

E.g.: Given sample HHTTTTTHTHTTTHH of (possibly biased) coin flips, estimate

$$\theta = \text{probability of Heads}$$

$f(x|\theta)$ is the Bernoulli probability mass function with parameter θ

Likelihood

$P(x \mid \theta)$: Probability of event x given *model* θ

Viewed as a function of x (fixed θ), it's a *probability*

- E.g., $\Sigma_x P(x \mid \theta) = 1$

Viewed as a function of θ (fixed x), it's a *likelihood*

- E.g., $\Sigma_\theta P(x \mid \theta)$ can be anything; *relative* values of interest.
- E.g., if θ = prob of heads in a sequence of coin flips then
  $P(HHTHH \mid .6) > P(HHTHH \mid .5)$,
- I.e., event HHTHH is *more likely* when $\theta = .6$ than $\theta = .5$
- And what θ make HHTHH *most* likely?

Likelihood

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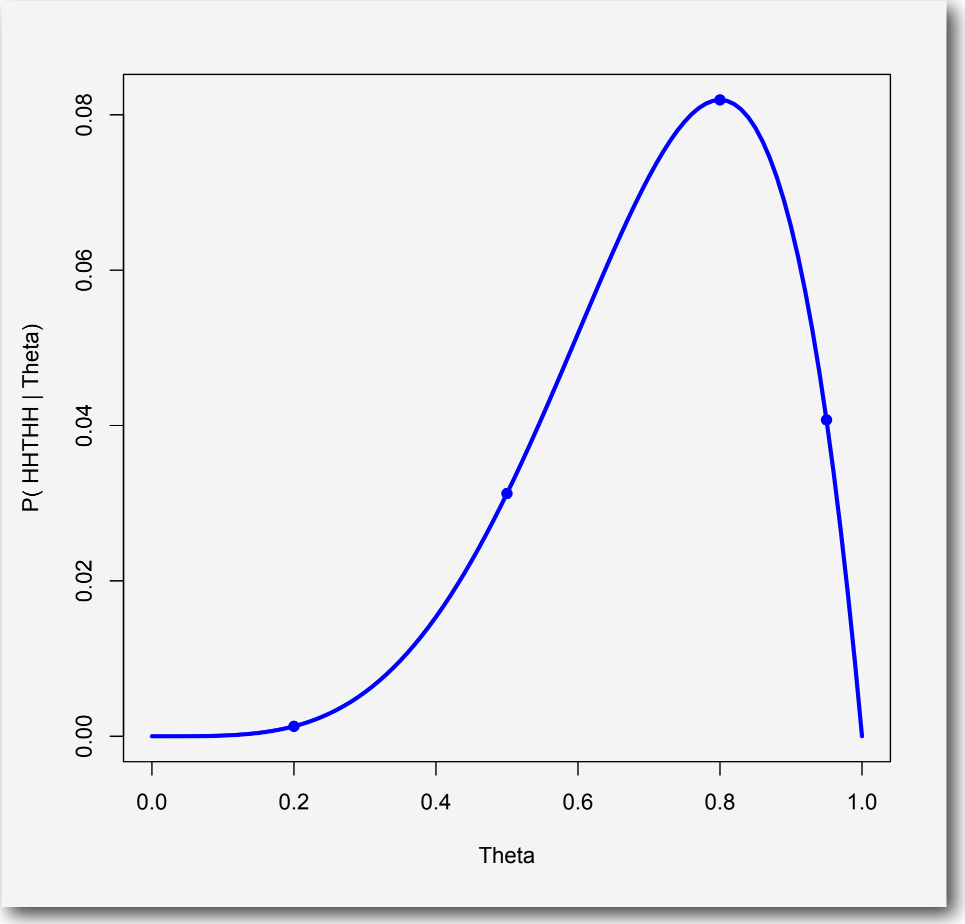
I.e., event HHTHH is *more likely* when $\theta = .6$ than $\theta = .5$

And what θ make HHTHH *most* likely?

Likelihood Function

Probability of HHTHH, given P(H) = θ:

θ	$\theta^4(1-\theta)$
0.2	0.0013
0.5	0.0313
0.8	0.0819
0.95	0.0407



Maximum Likelihood Parameter Estimation

One (of many) approaches to param. est.

Likelihood of (indp) observations $x_1, x_2, ..., x_n$

$$L(x_1, x_2, \ldots, x_n \mid \theta) = \prod_{i=1}^{n} f(x_i \mid \theta)$$

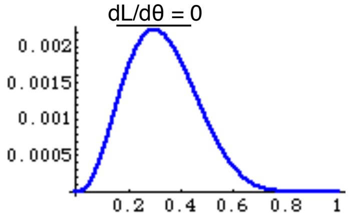
As a function of θ, what θ maximizes the likelihood of the data actually observed

Typical approach: $\frac{\partial}{\partial\theta} L(\vec{x} \mid \theta) = 0$ or $\frac{\partial}{\partial\theta} \log L(\vec{x} \mid \theta) = 0$

Example 1

n coin flips, $x_1, x_2, ..., x_n$; n_0 tails, n_1 heads, $n_0 + n_1 = n$;

θ = probability of heads



$$
\begin{aligned}
L(x_1, x_2, \ldots, x_n \mid \theta) &= (1-\theta)^{n_0}\theta^{n_1} \\
\log L(x_1, x_2, \ldots, x_n \mid \theta) &= n_0 \log(1-\theta) + n_1 \log\theta \\
\tfrac{\partial}{\partial\theta} \log L(x_1, x_2, \ldots, x_n \mid \theta) &= \tfrac{-n_0}{1-\theta} + \tfrac{n_1}{\theta}
\end{aligned}
$$

Setting to zero and solving:

$$\boxed{\hat{\theta} = \frac{n_1}{n}}$$

Observed fraction of successes in sample is MLE of success probability in population

(Also verify it's max, not min, & not better on boundary)

Bias

A desirable property: An estimator Y of a parameter θ is an *unbiased* estimator if

$$E[Y] = \theta$$

For coin ex. above, MLE is unbiased:

Y = fraction of heads = $(\Sigma_{1 \le i \le n} X_i)/n$,

(X_i = indicator for heads in ith trial) so

$$E[Y] = (\Sigma_{1 \le i \le n} E[X_i])/n = n\,\theta/n = \theta$$

Aside: are all unbiased estimators equally good?

- No!
- E.g., “Ignore all but 1st flip; if it was H, let Y’ = 1; else Y’ = 0”
- Exercise: show this is unbiased
- Exercise: if observed data has at least one H and at least one T, what is the likelihood of the data given the model with θ = Y’ ?

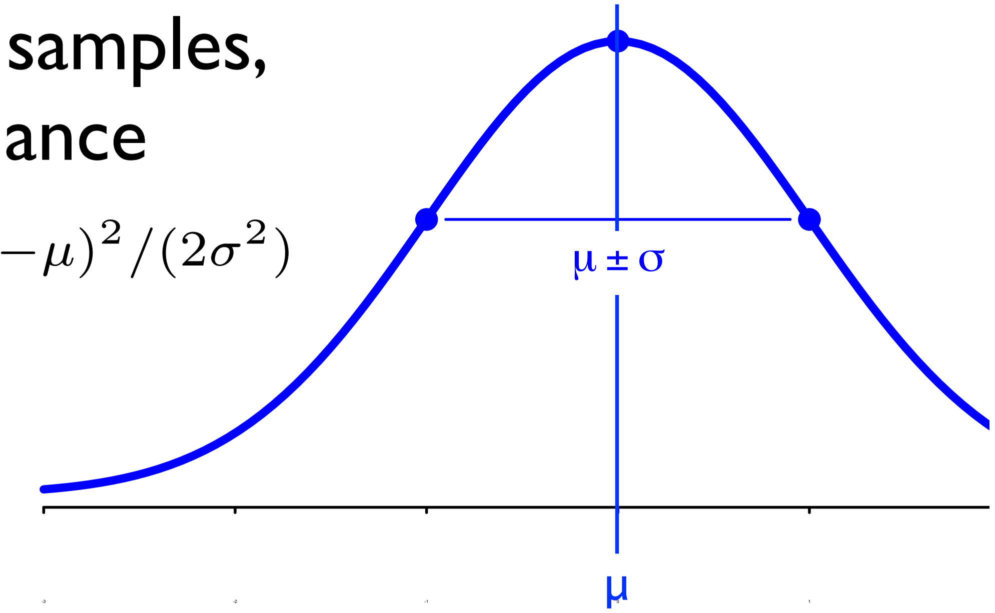
Parameter Estimation

Assuming sample $x_1, x_2, ..., x_n$ is from a parametric distribution $f(x|\theta)$, estimate θ.

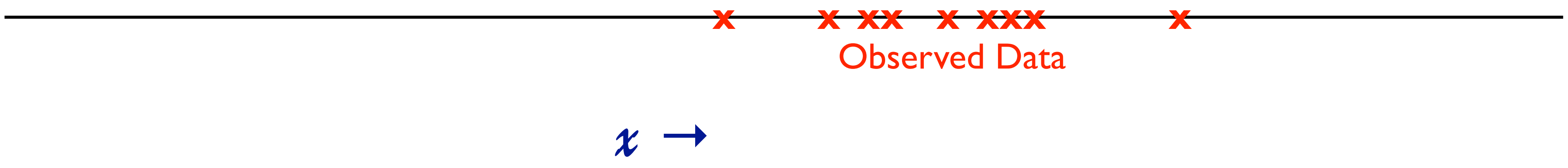
E.g.: Given *n* normal samples, estimate mean & variance

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-(x-\mu)^2/(2\sigma^2)}$$

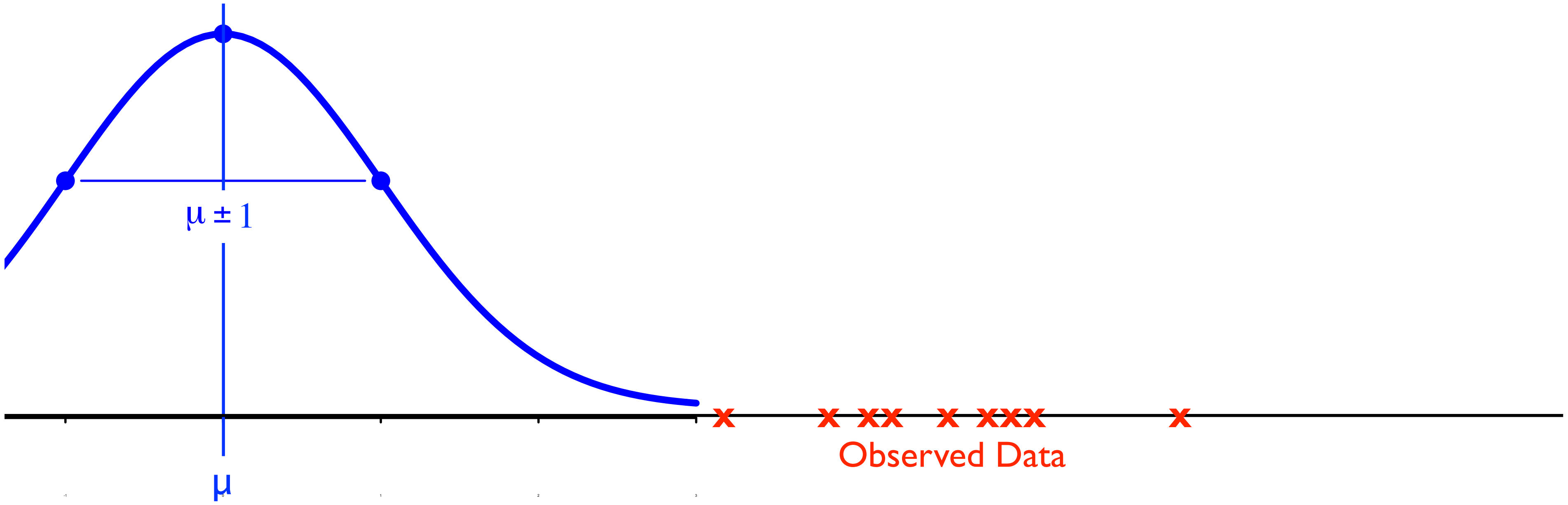
$$\theta = (\mu, \sigma^2)$$



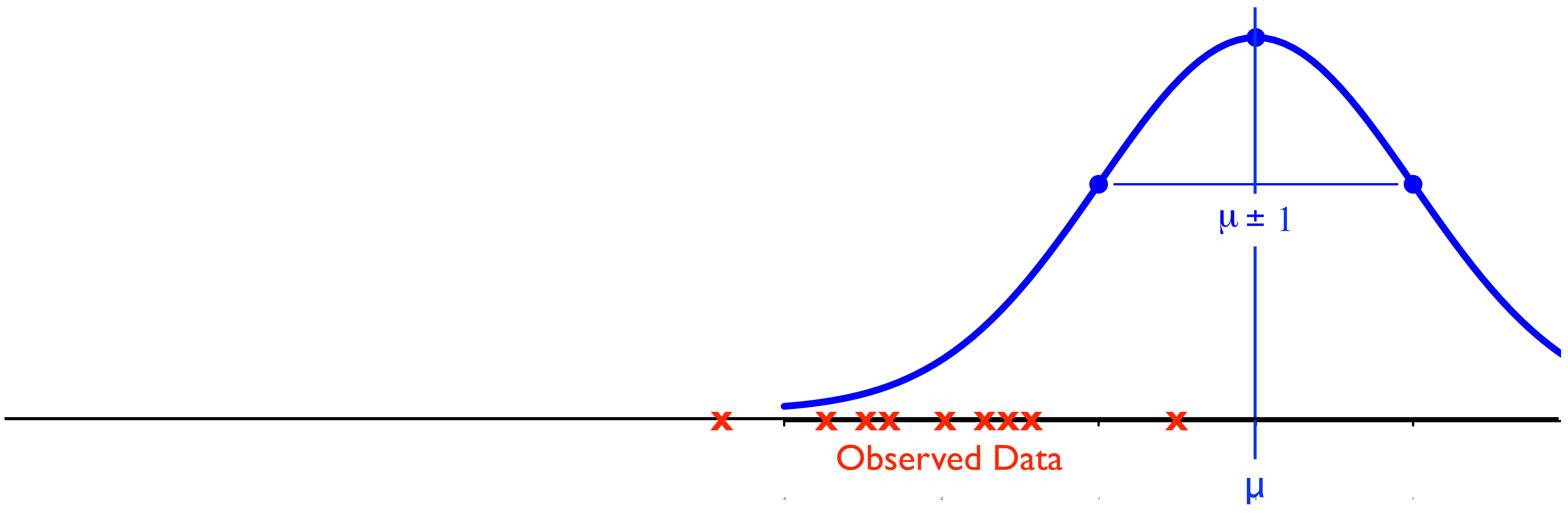
Ex2: I got data; a little birdie tells me it's normal, and promises $\sigma^2 = 1$



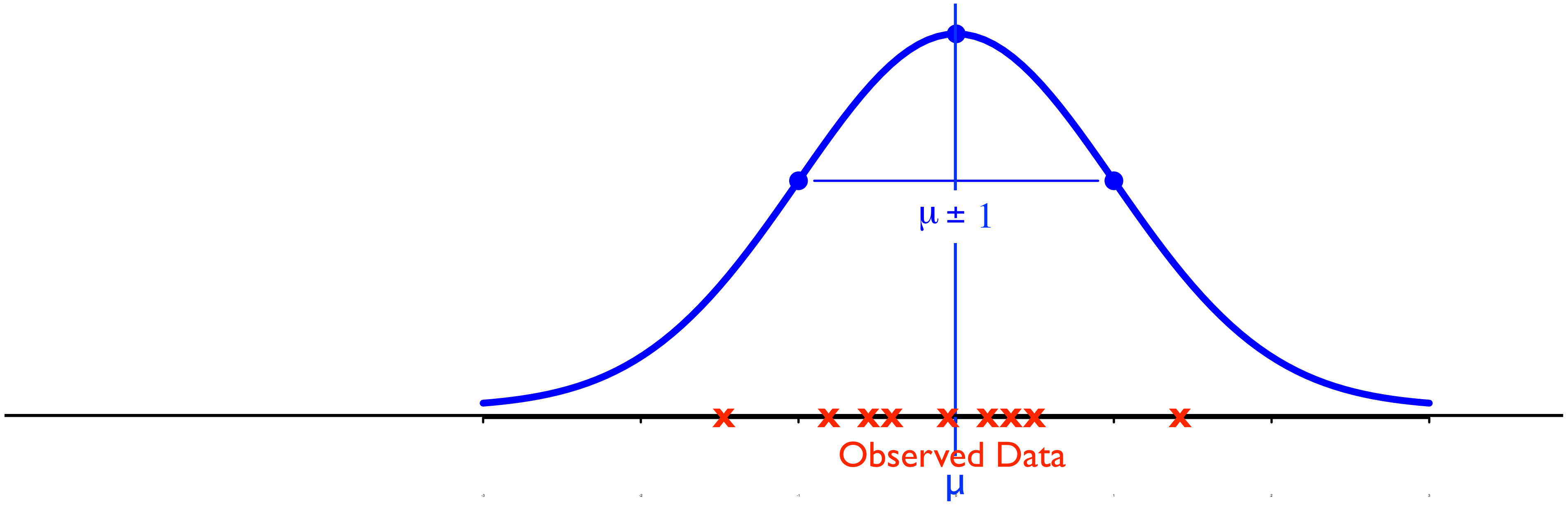
Which is more likely: (a) this?



Which is more likely: (b) or this?

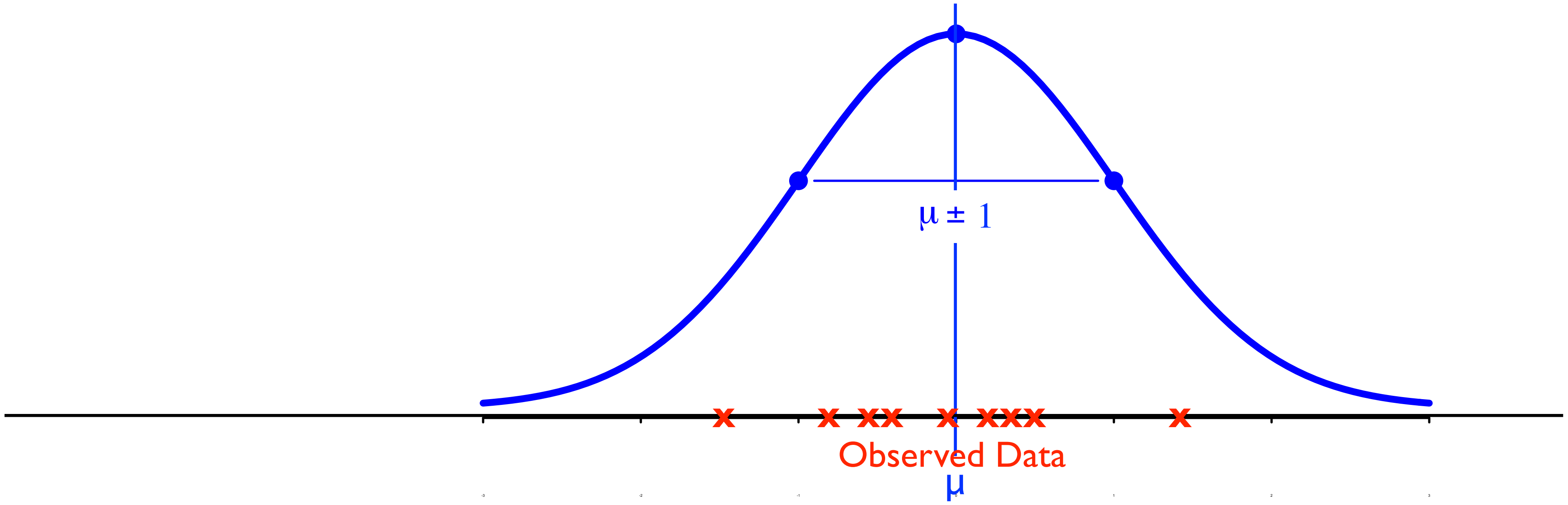


Which is more likely: (c) or *this*?



Which is more likely: (c) or *this*?

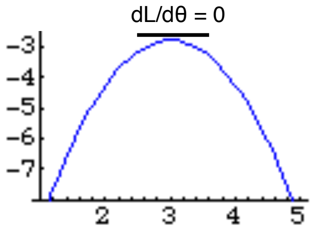
Looks good by eye, but how do I optimize my estimate of μ ?



Ex. 2: $x_i \sim N(\mu, \sigma^2),\ \sigma^2 = 1,\ \mu \text{ unknown}$

$$\begin{aligned}
L(x_1, x_2, \ldots, x_n|\theta) &= \prod_{1\le i\le n} \frac{1}{\sqrt{2\pi}} e^{-(x_i-\theta)^2/2} \\
\ln L(x_1, x_2, \ldots, x_n|\theta) &= \sum_{1\le i\le n} -\frac{1}{2}\ln 2\pi - \frac{(x_i-\theta)^2}{2} \\
\tfrac{d}{d\theta} \ln L(x_1, x_2, \ldots, x_n|\theta) &= \sum_{1\le i\le n} (x_i - \theta) \\
&= \left(\textstyle\sum_{1\le i\le n} x_i\right) - n\theta \;=\; 0
\end{aligned}$$

And verify it's max, not min & not better on boundary



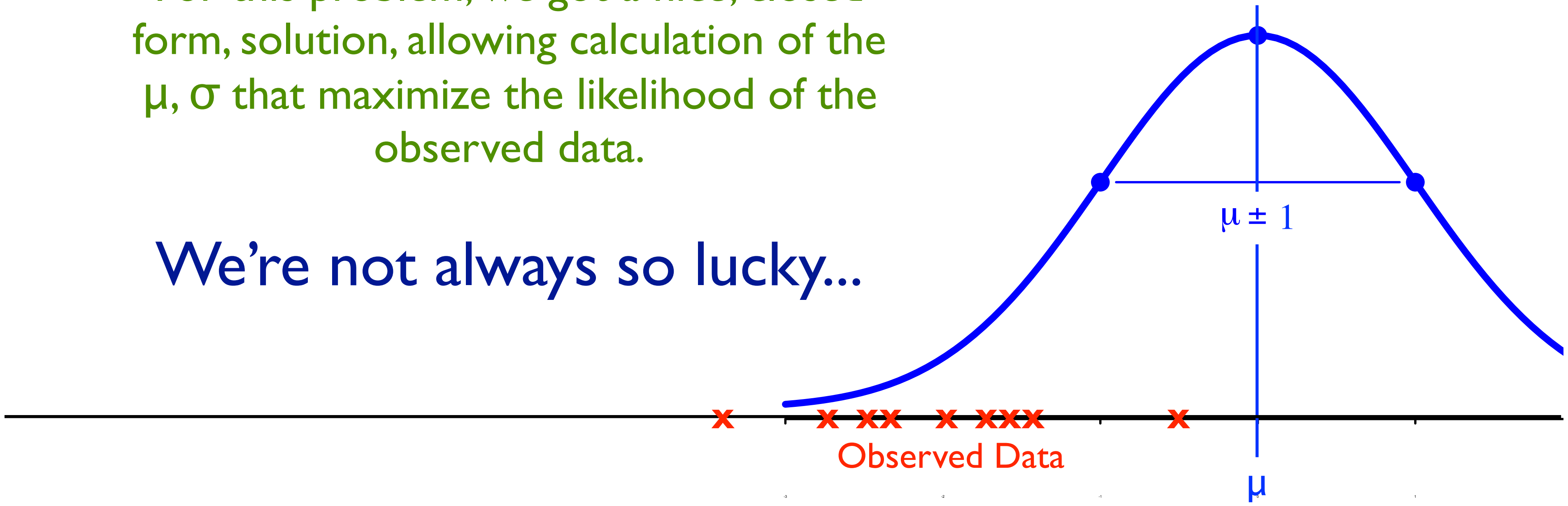
$$\boxed{\hat{\theta} \;=\; \left(\textstyle\sum_{1\le i\le n} x_i\right)/n \;=\; \bar{x}}$$

Sample mean is MLE of population mean

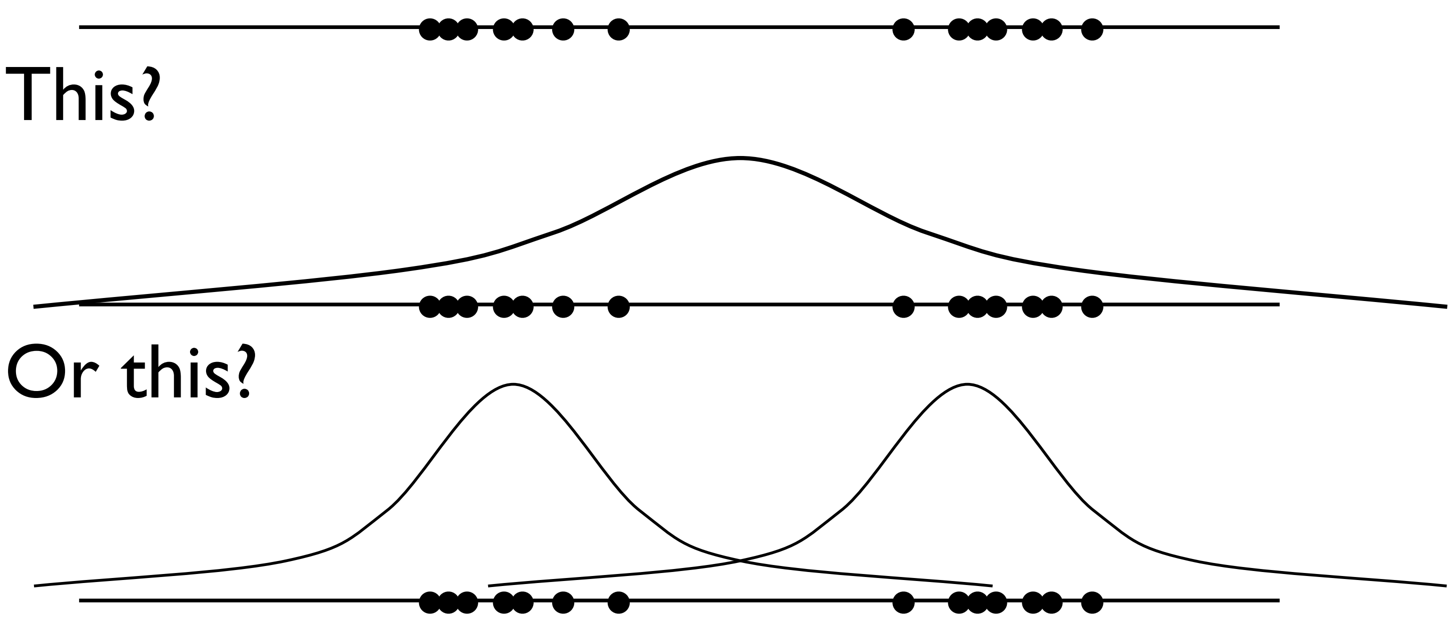
Last lecture:
How to estimate μ given data

For this problem, we got a nice, closed form, solution, allowing calculation of the μ, σ that maximize the likelihood of the observed data.

We're not always so lucky...



More Complex Example



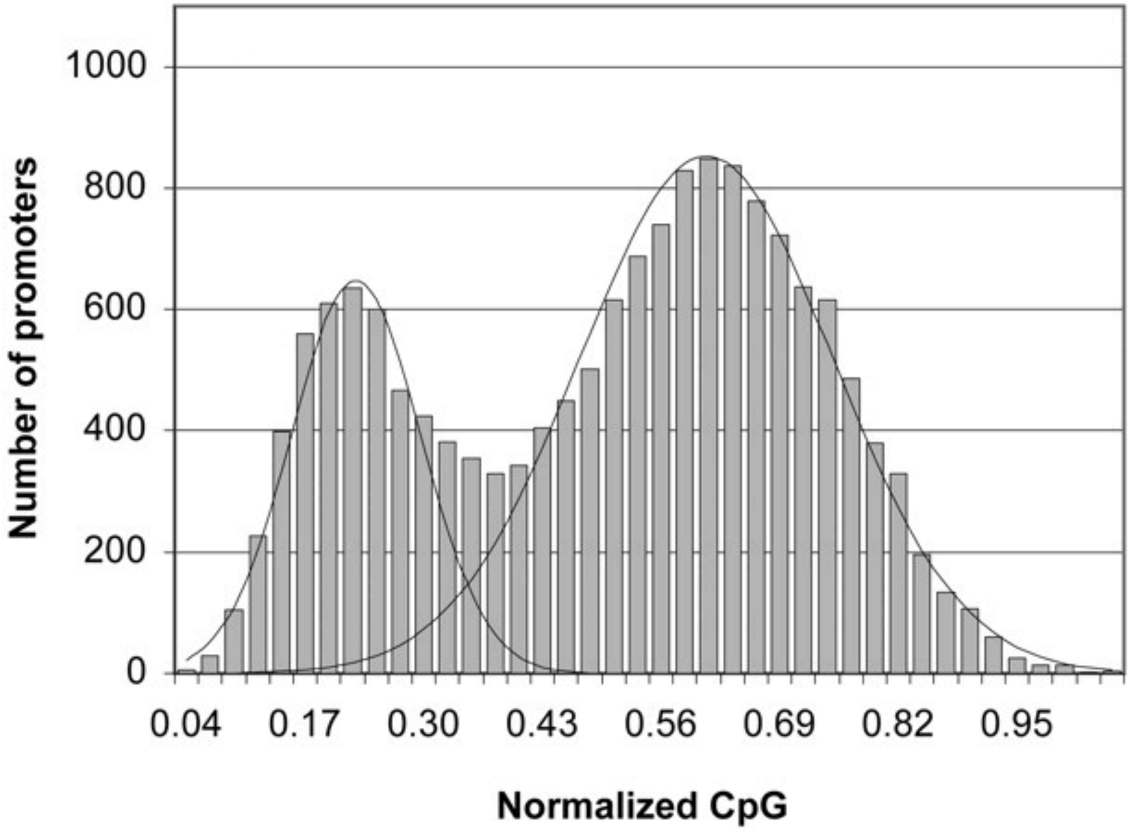
This?

Or this?

(A modeling decision, not a math problem...,
but if later, what math?)

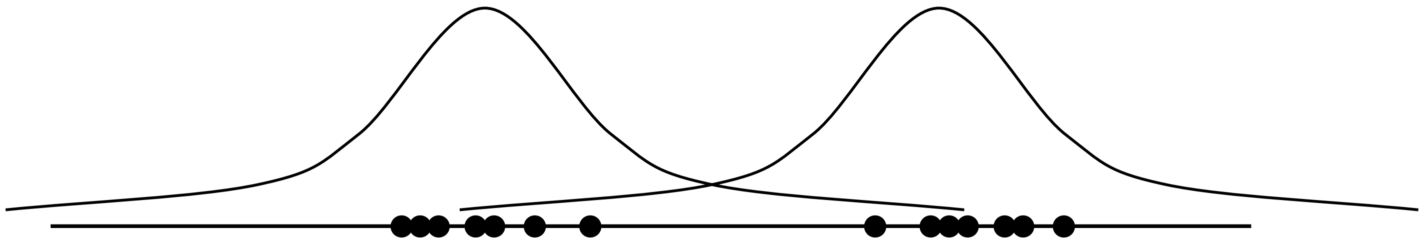
A Real Example:

CpG content of human gene promoters



"A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters" Saxonov, Berg, and Brutlag, PNAS 2006;103:1412-1417

Gaussian Mixture Models / Model-based Clustering



Parameters θ

means	μ_1	μ_2
variances	σ_1^2	σ_2^2
mixing parameters	τ_1	$\tau_2 = 1 - \tau_1$
P.D.F.	$f(x\|\mu_1, \sigma_1^2)$	$f(x\|\mu_2, \sigma_2^2)$

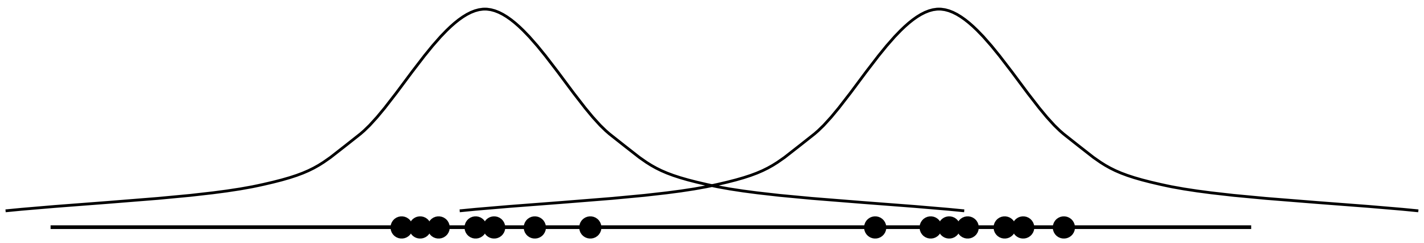
Likelihood

$$L(x_1, x_2, \ldots, x_n | \mu_1, \mu_2, \sigma_1^2, \sigma_2^2, \tau_1, \tau_2)$$

$$= \prod_{i=1}^{n} \sum_{j=1}^{2} \tau_j f(x_i | \mu_j, \sigma_j^2)$$

No closed-form max

Gaussian Mixture Models / Model-based Clustering



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mixing parameters	τ_1	$\tau_2 = 1 - \tau_1$

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Likelihood

$$L(x_1, x_2, \ldots, x_n | \mu_1, \mu_2, \sigma_1^2, \sigma_2^2, \tau_1, \tau_2)$$

$$= \prod_{i=1}^{n} \sum_{j=1}^{2} \tau_j f(x_i | \mu_j, \sigma_j^2)$$

Mixing proportion

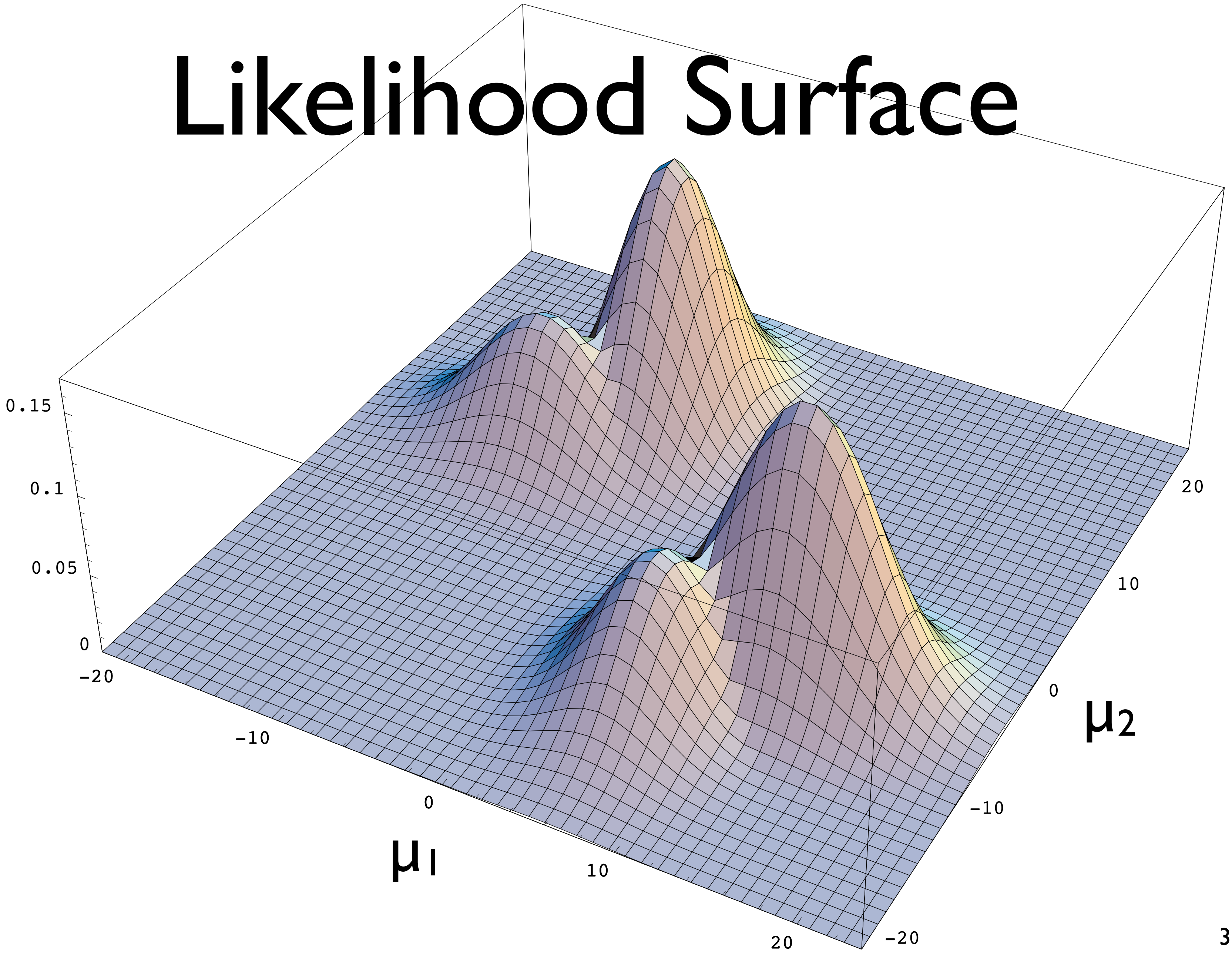
No closed-form max

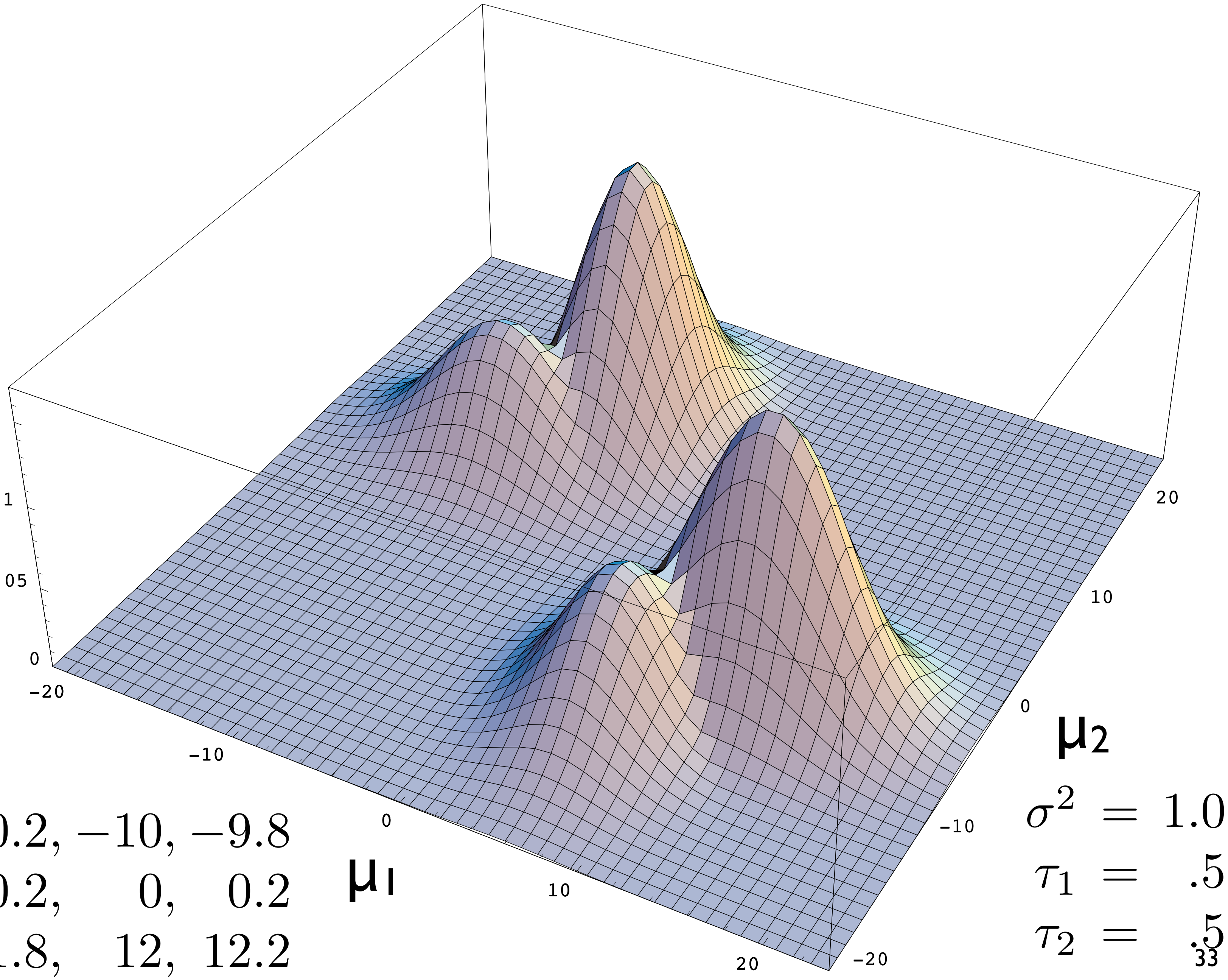
Product over data points (assumed independent)

Sum over possible distribution of origin

Likelihood of data point given this distribution

Likelihood Surface





x_i =

$-10.2, -10, -9.8$

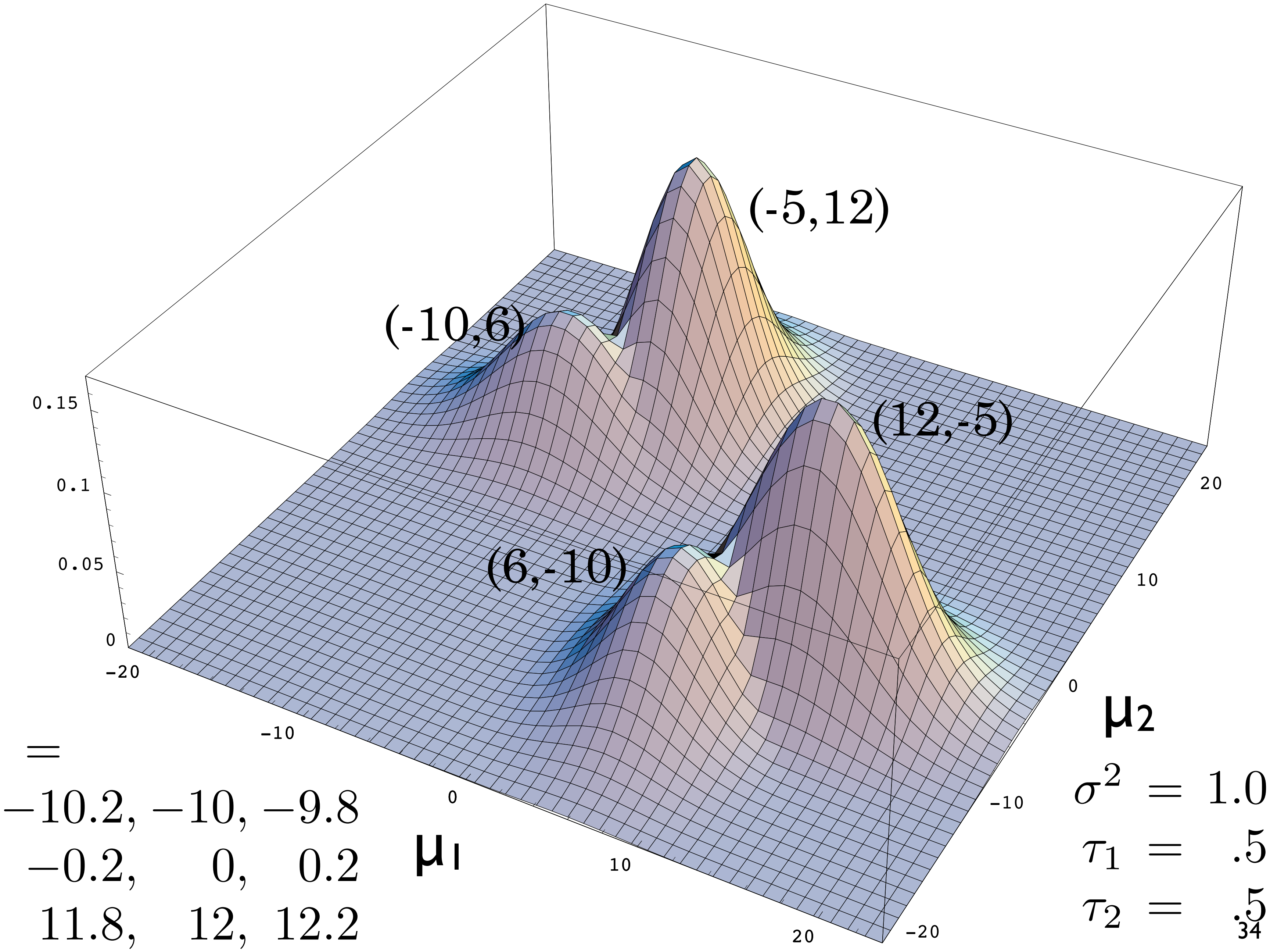
$-0.2, \quad 0, \quad 0.2$

$11.8, \quad 12, \quad 12.2$

$\sigma^2 = 1.0$

$\tau_1 = \ .5$

$\tau_2 = \ .5$



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$-0.2, 0, 0.2$

$11.8, 12, 12.2$

$\sigma^2 = 1.0$

$\tau_1 = .5$

$\tau_2 = .5$

A What-If Puzzle

Likelihood

$$L(x_1, x_2, \ldots, x_n | \overbrace{\mu_1, \mu_2, \sigma_1^2, \sigma_2^2, \tau_1, \tau_2}^{\theta})$$

$$= \prod_{i=1}^{n} \sum_{j=1}^{2} \tau_j f(x_i | \mu_j, \sigma_j^2)$$

Messy: no closed form solution known for finding θ maximizing L

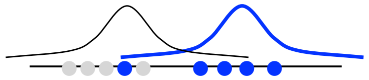
But *what if* we knew the *hidden data*?

$$z_{ij} = \begin{cases} 1 & \text{if } x_i \text{ drawn from } f_j \\ 0 & \text{otherwise} \end{cases}$$

EM as Egg vs Chicken

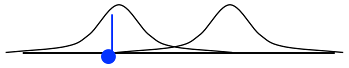
IF z_{ij} known, could estimate parameters θ

E.g., only points in cluster 2 influence μ_2, σ_2



IF parameters θ known, could estimate z_{ij}

E.g., if $|x_i - \mu_1|/\sigma_1 << |x_i - \mu_2|/\sigma_2$, then $z_{i1} >> z_{i2}$



But we know neither; (optimistically) iterate:

E: calculate expected z_{ij}, given parameters

M: calc "MLE" of parameters, given $E(z_{ij})$

Overall, a clever "hill-climbing" strategy

Simple Version: “Classification EM”

If z_{ij} < .5, pretend it’s 0; z_{ij} > .5, pretend it’s 1

I.e., *classify* points as component 0 or 1

Now recalc θ, assuming that partition

Then recalc z_{ij} , assuming that θ

Then re-recalc θ, assuming new z_{ij}, etc., etc.

“Full EM” is a bit more involved, but this is the crux.

Full EM

x_i's are known; θ unknown. Goal is to find MLE θ of:

$$L(x_1, \ldots, x_n \mid \theta)$$ (hidden data likelihood)

Would be easy *if* z_{ij}'s were known, i.e., consider:

$$L(x_1, \ldots, x_n, z_{11}, z_{12}, \ldots, z_{n2} \mid \theta)$$ (complete data likelihood)

But z_{ij}'s aren't known.

Instead, maximize *expected* likelihood of visible data

$$E(L(x_1, \ldots, x_n, z_{11}, z_{12}, \ldots, z_{n2} \mid \theta)),$$

where expectation is over distribution of hidden data (z_{ij}'s)

The E-step:

Find E(Z_{ij}), i.e. P(Z_{ij}=1)

Assume θ known & fixed

A (B): the event that x_i was drawn from f_1 (f_2)

D: the observed datum x_i

Expected value of z_{i1} is P(A|D)

$E = 0 \cdot P(0) + 1 \cdot P(1)$

$$\boxed{P(A|D) = \frac{P(D|A)P(A)}{P(D)}}$$

$$P(D) = P(D|A)P(A) + P(D|B)P(B)$$

$$= f_1(x_i|\theta_1)\,\tau_1 + f_2(x_i|\theta_2)\,\tau_2$$

Repeat for each x_i

Complete Data Likelihood

Recall:

$$z_{1j} \;=\; \begin{cases} 1 & \text{if } x_1 \text{ drawn from } f_j \\ 0 & \text{otherwise} \end{cases}$$

so, correspondingly,

$$L(x_1, z_{1j} \mid \theta) \;=\; \begin{cases} \tau_1 f_1(x_1 \mid \theta) & \text{if } z_{11} = 1 \\ \tau_2 f_2(x_1 \mid \theta) & \text{otherwise} \end{cases}$$

Formulas with “if’s” are messy; can we blend more smoothly?
Yes, many possibilities. Idea 1:

$$L(x_1, z_{1j} \mid \theta) \;=\; z_{11} \cdot \tau_1 f_1(x_1 \mid \theta) + z_{12} \cdot \tau_2 f_2(x_1 \mid \theta)$$

Idea 2 (Better):

$$L(x_1, z_{1j} \mid \theta) \;=\; (\tau_1 f_1(x_1 \mid \theta))^{z_{11}} \cdot (\tau_2 f_2(x_1 \mid \theta))^{z_{12}}$$

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Why is this better? How will this behave differently when we take the log?

M-step:

Find θ maximizing E(log(Likelihood))

(For simplicity, assume $\sigma_1 = \sigma_2 = \sigma; \tau_1 = \tau_2 = .5 = \tau$)

$$L(\vec{x}, \vec{z} \mid \theta) = \prod_{1 \le i \le n} \frac{\tau}{\sqrt{2\pi\sigma^2}} \exp\left(- \sum_{1 \le j \le 2} z_{ij} \frac{(x_i - \mu_j)^2}{2\sigma^2} \right)$$

$$E[\log L(\vec{x}, \vec{z} \mid \theta)] = E\left[\sum_{1 \le i \le n} \left(\log \tau - \frac{1}{2} \log 2\pi\sigma^2 - \sum_{1 \le j \le 2} z_{ij} \frac{(x_i - \mu_j)^2}{2\sigma^2} \right) \right]$$

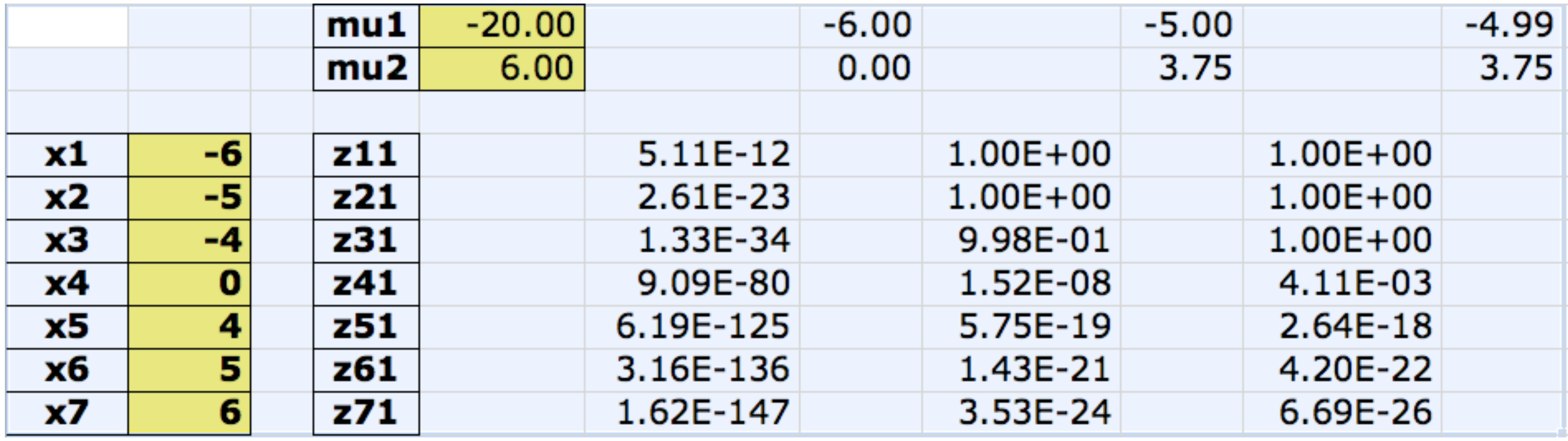
$$= \sum_{1 \le i \le n} \left(\log \tau - \frac{1}{2} \log 2\pi\sigma^2 - \sum_{1 \le j \le 2} E[z_{ij}] \frac{(x_i - \mu_j)^2}{2\sigma^2} \right)$$

Find θ maximizing this as before, using $E[z_{ij}]$ found in E-step. Result:

$\boxed{\mu_j = \sum_{i=1}^{n} E[z_{ij}] x_i / \sum_{i=1}^{n} E[z_{ij}]}$ (intuit: avg, weighted by subpop prob)

2 Component Mixture

$\sigma_1 = \sigma_2 = 1; \ \tau = 0.5$



		mu1	-20.00		-6.00		-5.00		-4.99
		mu2	6.00		0.00		3.75		3.75
x1	-6	z11		5.11E-12		1.00E+00		1.00E+00	
x2	-5	z21		2.61E-23		1.00E+00		1.00E+00	
x3	-4	z31		1.33E-34		9.98E-01		1.00E+00	
x4	0	z41		9.09E-80		1.52E-08		4.11E-03	
x5	4	z51		6.19E-125		5.75E-19		2.64E-18	
x6	5	z61		3.16E-136		1.43E-21		4.20E-22	
x7	6	z71		1.62E-147		3.53E-24		6.69E-26	

Essentially converged in 2 iterations

Applications

Clustering is a remarkably successful exploratory data analysis tool

- Web-search, information retrieval, gene-expression, ...
- Model-based approach above is one of the leading ways to do it

Gaussian mixture models widely used

- With many components, empirically match arbitrary distribution
- Often well-justified, due to "hidden parameters" driving the visible data

EM is extremely widely used for "hidden-data" problems

- Hidden Markov Models

EM Summary

Fundamentally a maximum likelihood parameter estimation problem

Useful if hidden data, and if analysis is more tractable when 0/1 hidden data z known

Iterate:

E-step: estimate E(z) for each z, given θ

M-step: estimate θ maximizing E(log likelihood) given E(z) [where "E(logL)" is wrt random z ~ E(z) = p(z=1)]

EM Issues

Under mild assumptions, EM is guaranteed to increase likelihood with every E-M iteration, hence will *converge*.

But it may converge to a *local*, not global, max. (Recall the 4-bump surface...)

Issue is intrinsic (probably), since EM is often applied to problems (including clustering, above) that are *NP-hard*

Nevertheless, widely used, often effective

Aside: Maximum Likelihood Est. and the EM Algorithm

End of slides on MLE & EM taken from the UW CSE 312 Web*

A probabilistic view of RNA-Seq quantification

nucleotide fractions

known transcriptome

assumes independence of fragments

$$\Pr\{\mathcal{F} \mid \boldsymbol{\eta}, \mathcal{T}\} = \prod_{j=1}^{N} \Pr\{f_j \mid \boldsymbol{\eta}, \mathcal{T}\}$$

observed fragments (reads)

$$= \prod_{j=1}^{N} \sum_{i=1}^{M} \Pr\{t_i \mid \boldsymbol{\eta}\} \cdot \Pr\{f_j \mid t_i, \boldsymbol{z}_{ji} = 1\}$$

Prob. of selecting t_i *given* **η**

Prob. of generating fragment f_j *given* that it originates from t_i

Depends on abundance estimate

Independent of abundance estimate

We want to find the values of **η** that ***maximize*** this probability. We can do this (at least locally) using the EM algorithm.

*Li, Bo, and Colin N. Dewey. "RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome." BMC bioinformatics 12.1 (2011): 1.

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observed fragments (reads)

We can safely truncate Pr{t_i | **η**} to 0 for transcripts where a fragment doesn't map/align.

$$= \prod_{j=1}^{N} \boxed{\sum_{i=1}^{M} \Pr\{t_i \mid \boldsymbol{\eta}\} \cdot \boxed{\Pr\{f_j \mid t_i, \boldsymbol{z}_{ji} = 1\}}}$$

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A probabilistic view of RNA-Seq quantification

E-step: (what is the “soft assignment” of each read to the transcripts where it aligns)

$$E_{Z|\mathscr{F},\eta^{(t)}}[Z_{nij}] = P(Z_{nij} = 1 \mid \mathscr{F}, \eta^{(t)}) = \frac{(\eta_i^{(t)}/\ell_i)P(f_n \,|\, Z_{nij} = 1)}{\sum_{i',j'} (\eta_{i'}^{(t)}/\ell_i')P(f_n \,|\, Z_{ni'j'} = 1)}$$

M-step: Given these soft assignments, how abundant is each transcript?

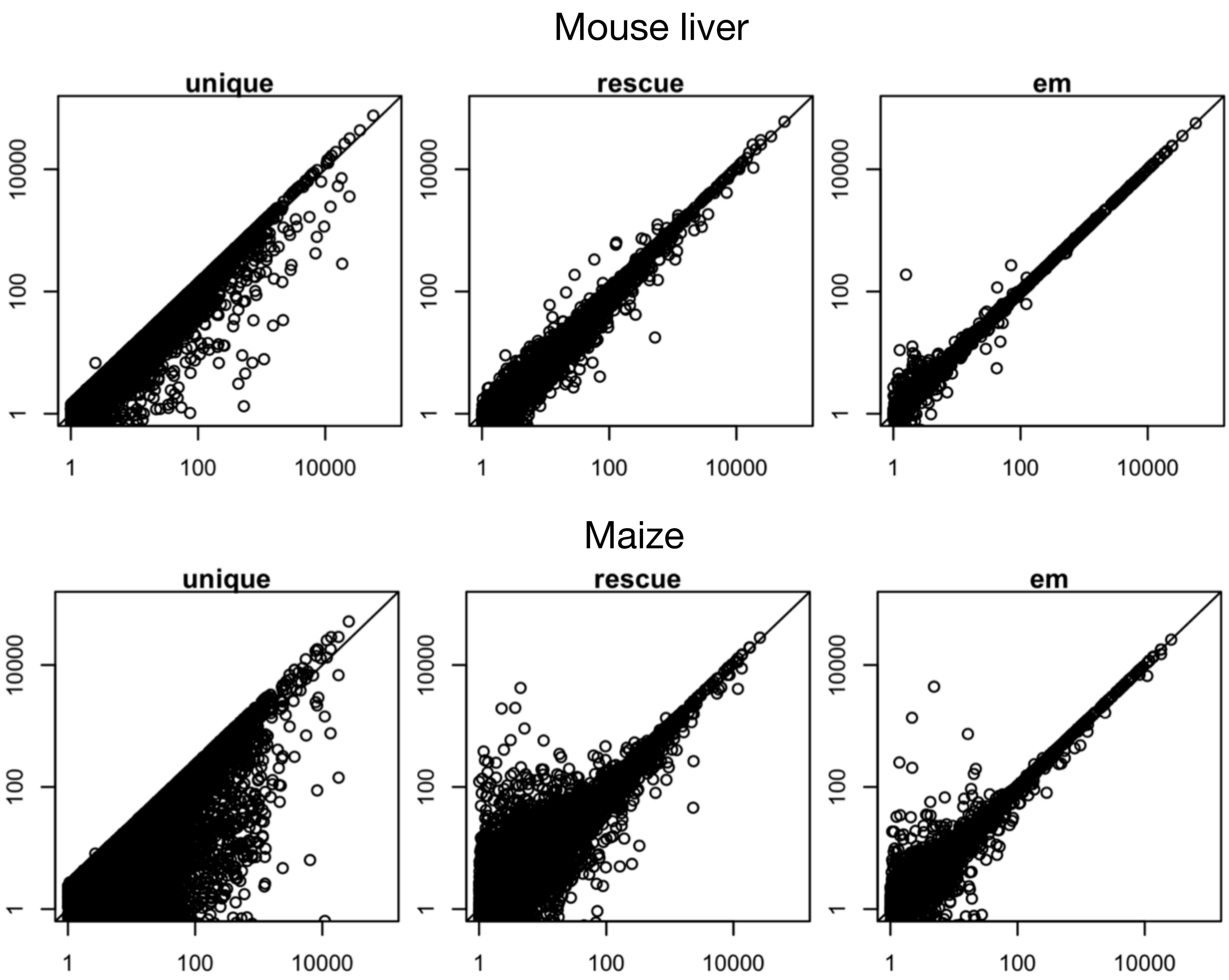
$$\eta_i^{(t+1)} = \frac{E_{Z|\mathscr{F},\eta^{(t)}}\left[C_i\right]}{N},$$

$$\text{where } C_i = \sum_{n,j} Z_{nij}$$

This approach is quite effective. Unfortunately, it’s also quite slow.

Equations adapted from: Bo Li, Victor Ruotti, Ron M. Stewart, James A. Thomson, Colin N. Dewey; RNA-Seq gene expression estimation with read mapping uncertainty, *Bioinformatics*, Volume 26, Issue 4, 15 February 2010, Pages 493–500, https://doi.org/10.1093/bioinformatics/btp692

Gene expression estimation accuracy in simulated data



From supplementary material of : Bo Li, Victor Ruotti, Ron M. Stewart, James A. Thomson, Colin N. Dewey; RNA-Seq gene expression estimation with read mapping uncertainty, *Bioinformatics*, Volume 26, Issue 4, 15 February 2010, Pages 493–500, https://doi.org/10.1093/bioinformatics/btp692

A probabilistic view of RNA-Seq quantification

We want to find the values of **η** that ***maximize*** this probability. We can do this (at least locally) using the EM algorithm.

but

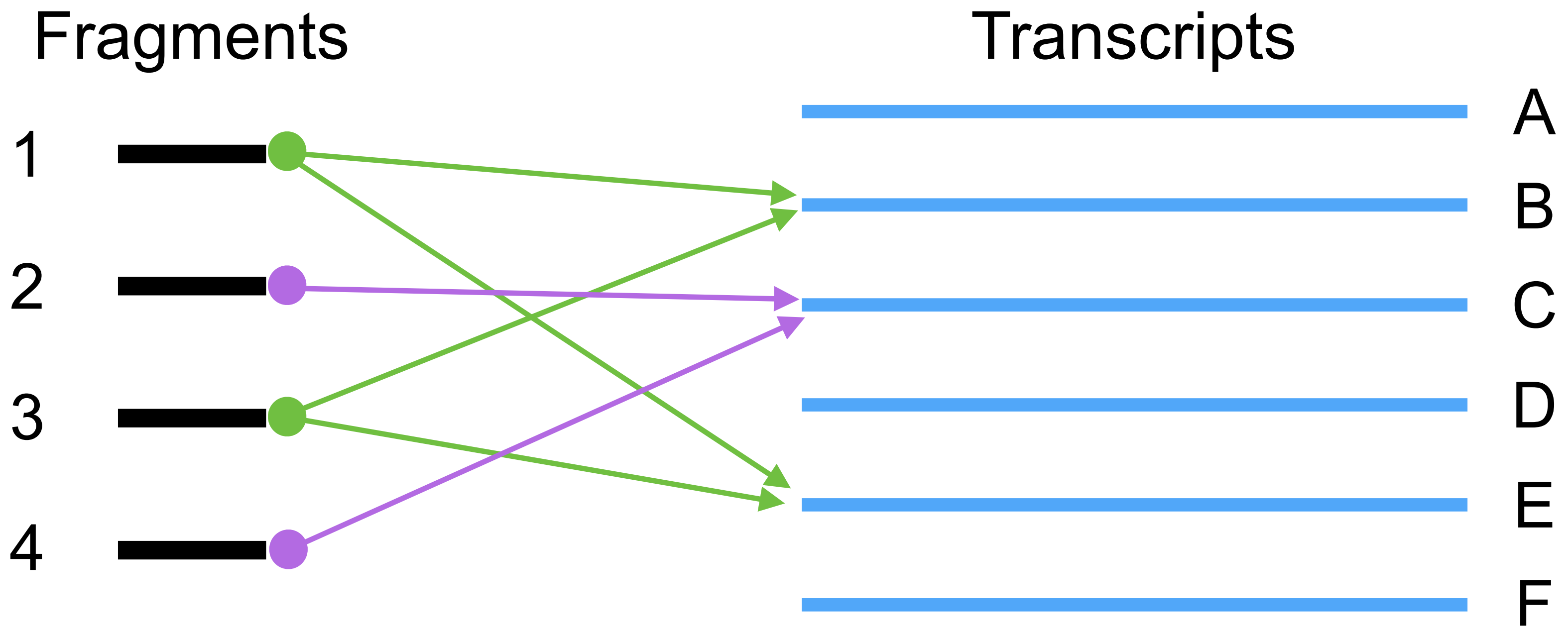
This leads to an iterative EM algorithm where *each iteration* scales in the total number of **alignments** in the sample (typically on the order of 10^7 — 10^8), and typically 10^2—10^3 **iterations**

$$\mathcal{L}(\boldsymbol{\eta}; \mathcal{F}, \mathcal{T}) = \boxed{\prod_{f \in \mathcal{F}} \sum_{t_i \in \Omega(f)}} \Pr(t_i \mid \boldsymbol{\eta}) \Pr(f \mid t_i)$$

Set of transcripts where f maps/aligns

*Li, Bo, and Colin N. Dewey. "RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome." BMC bioinformatics 12.1 (2011): 1.

Fragment Equivalence Classes



Reads 1 & 3 both map to transcripts B & E
Reads 2 & 4 both map to transcript C

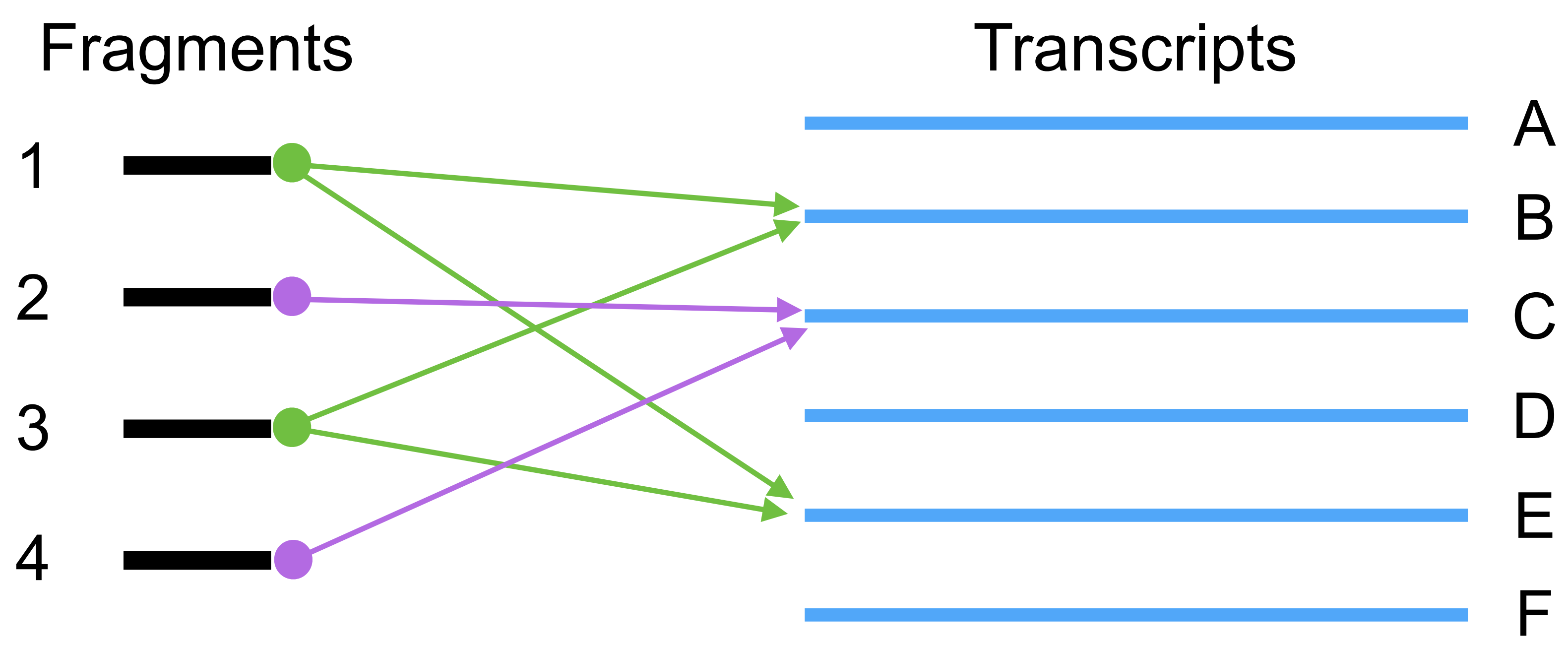
We have 4 reads, but only 2 eq. classes of reads

eq. Label	Count	Aux weights
{B,E}	2	$w^{\{B,E\}}_B, w^{\{B,E\}}_E$
{C}	2	$w^{\{C\}}_C$

This idea goes quite far back in the RNA-seq literature; at least to MMSeq (Turro et al. 2011)

Turro, Ernest, et al. "Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads." *Genome biology* 12.2 (2011): R13.

Fragment Equivalence Classes



Reads 1 & 3 both map to transcripts B & E
Reads 2 & 4 both map to transcript C

w^j_i encodes the "affinity" of class *j* to transcript *i* according to the model. This is $P\{f_j \mid t_i\}$, aggregated for all fragments in a class.

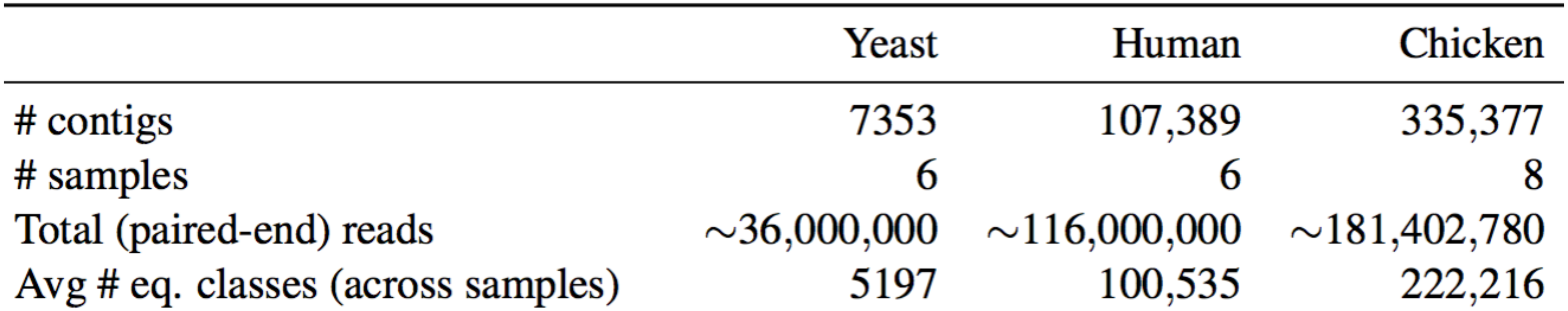
We have 4 reads, but only 2 eq. classes of reads

eq. Label	Count	Aux weights
{B,E}	2	$w^{\{B,E\}}_B, w^{\{B,E\}}_E$
{C}	2	$w^{\{C\}}_C$

This idea goes quite far back in the RNA-seq literature; at least to MMSeq (Turro et al. 2011)

Turro, Ernest, et al. "Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads." *Genome biology* 12.2 (2011): R13.

The number of equivalence classes is small



	Yeast	Human	Chicken
# contigs	7353	107,389	335,377
# samples	6	6	8
Total (paired-end) reads	∼36,000,000	∼116,000,000	∼181,402,780
Avg # eq. classes (across samples)	5197	100,535	222,216

The **# of equivalence classes grows with the complexity of the transcriptome** — independent of the # of sequence fragments.

Typically, ***two or more orders of magnitude*** fewer equivalence classes than sequenced fragments.

The offline **inference** algorithm **scales in # of fragment equivalence classes**.

This naturally handles different types of multi-mapping *without* having to rely on the annotation

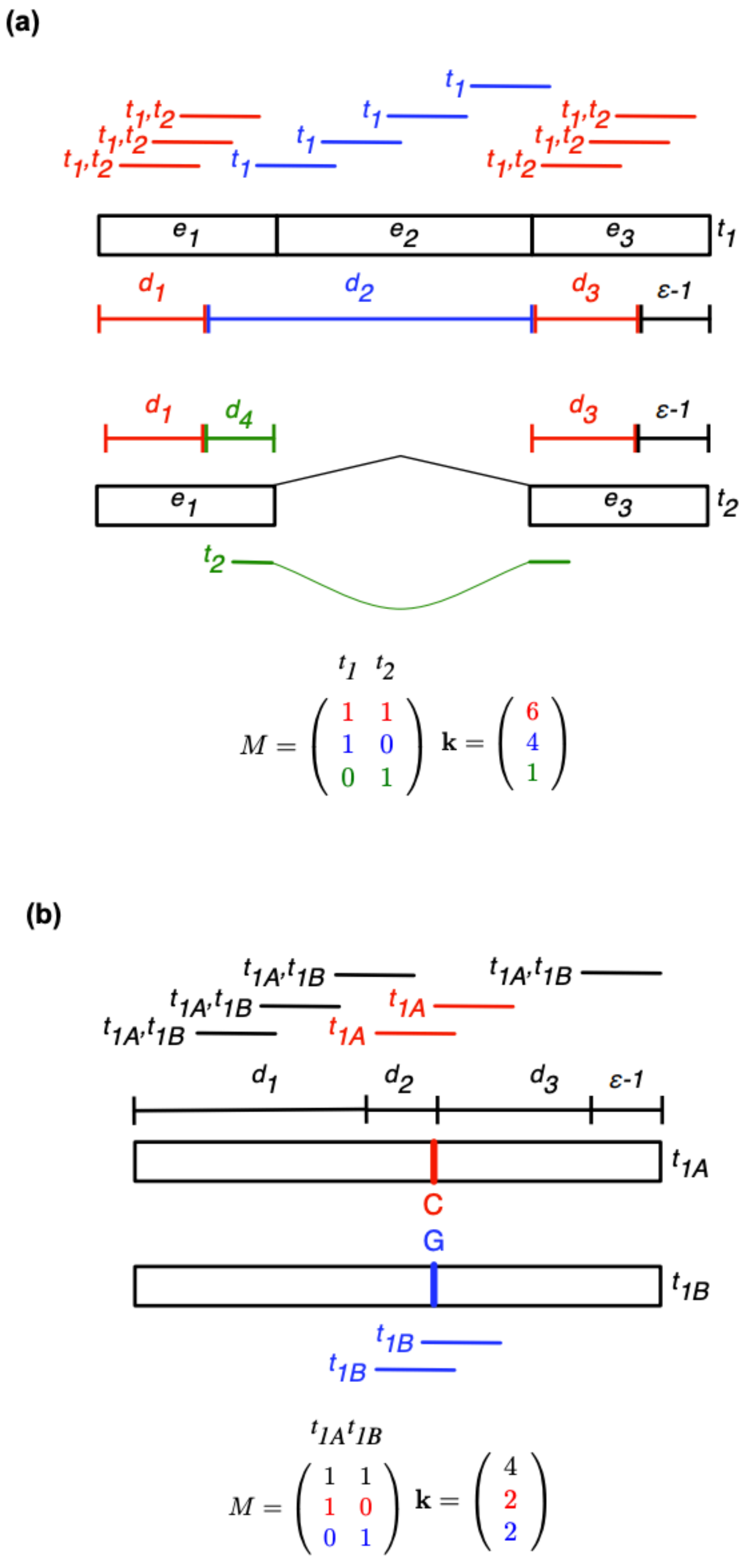

(a)
t1,t2
t1
t1
t1
t1
e1
e2
e3
t1
d1
d2
d3
ε-1
d1
d4
d3
ε-1
e1
e3
t2
t2
$M = \begin{array}{c} \\ \\ \end{array}\begin{array}{cc} t_1 & t_2 \\ \end{array}$
$M = \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} \quad \mathbf{k} = \begin{pmatrix} 6 \\ 4 \\ 1 \end{pmatrix}$
(b)
t1A,t1B
t1A
d1
d2
d3
ε-1
t1A
C
G
t1B
t1B
$t_{1A} \; t_{1B}$
$M = \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} \quad \mathbf{k} = \begin{pmatrix} 4 \\ 2 \\ 2 \end{pmatrix}$

Figure 2 from Turro, Ernest, et al. "Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads." *Genome biology* 12.2 (2011): R13.

This lets us approximate the likelihood efficiently

Approximate this:

sum over all alignments of fragment

$$\mathcal{L}\left(\boldsymbol{\eta};\mathcal{F}\right) = \prod_{f_j\in\mathcal{F}} \sum_{i=1}^{M} \Pr\left(t_i \mid \boldsymbol{\eta}\right) \Pr\left(f_j \mid t_i\right)$$

product over all fragments

with this:

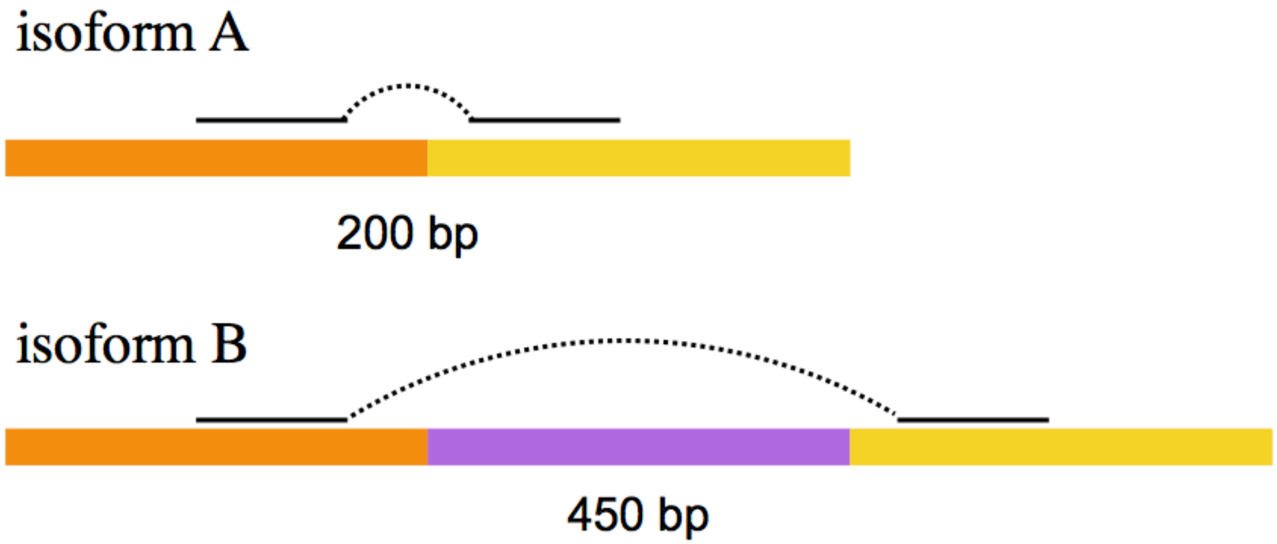
$$\mathcal{L}\left(\boldsymbol{\eta};\mathcal{F}\right) \approx \prod_{\mathcal{F}^q\in\boldsymbol{\mathcal{C}}} \left(\sum_{\langle i,t_i\rangle\in\Omega(\mathcal{F}^q)} \Pr\left(t_i \mid \boldsymbol{\eta}\right) \cdot \Pr\left(f \mid \mathcal{F}^q, t_i\right)\right)^{N^q}$$

sum over all transcripts labeling this eq. class

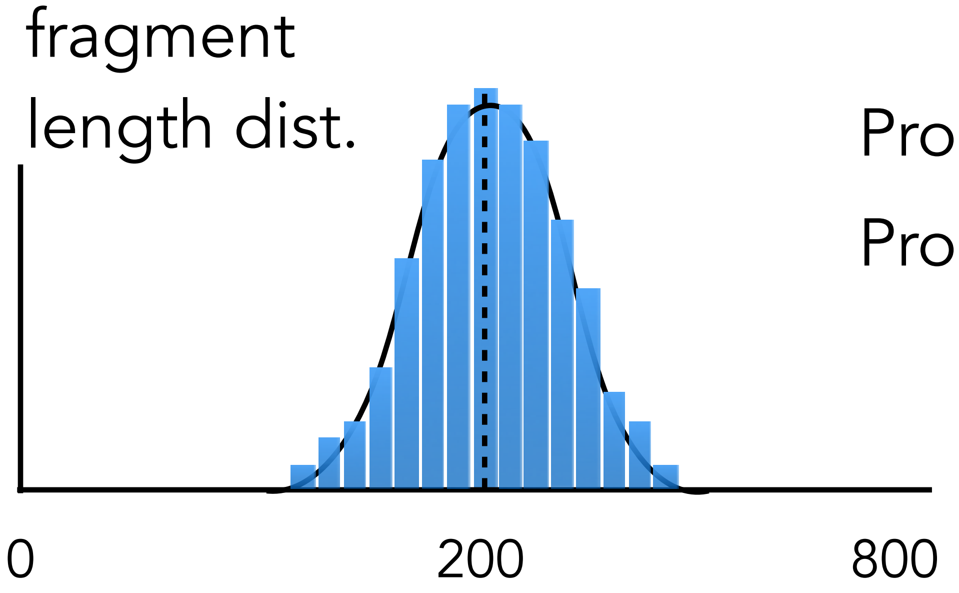
product over all equivalence classes

Why might $\Pr(f_j \mid t_i)$ matter?

Consider the following scenario:



Conditional probabilities can provide valuable information about origin of a fragment! ***Potentially different for each transcript/fragment pair.***



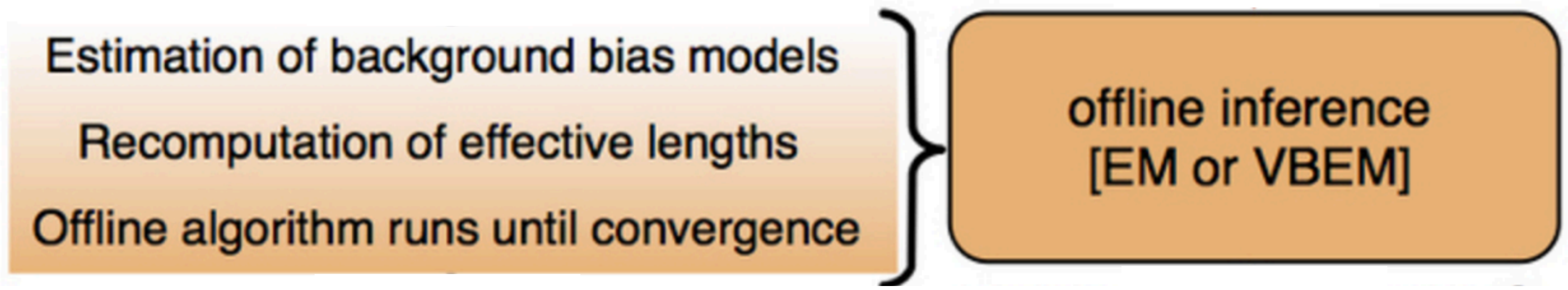
Prob of observing a fragment of size ~200 is **large**

Prob of observing a fragment of size ~450 is **small**

Many terms can be considered in a general "fragment-transcript agreement" model[1]. e.g. position, orientation, alignment path etc.

[1] "Salmon provides fast and bias-aware quantification of transcript expression", Nature Methods 2017

Optimizing the objective



Estimation of background bias models

Recomputation of effective lengths

Offline algorithm runs until convergence

offline inference
[EM or VBEM]

our ML objective has a simple, **closed-form update rule** in terms of our eq. classes

count of eq.
class j

$$\alpha_i^{u+1} = \sum_{\mathcal{F}^q \in \mathcal{C}} N^q \left(\frac{\alpha_i^u w_i^q}{\sum_{\langle k, t_k \rangle \in \Omega(\mathcal{F}^q)} \alpha_k^u w_k^q} \right)$$

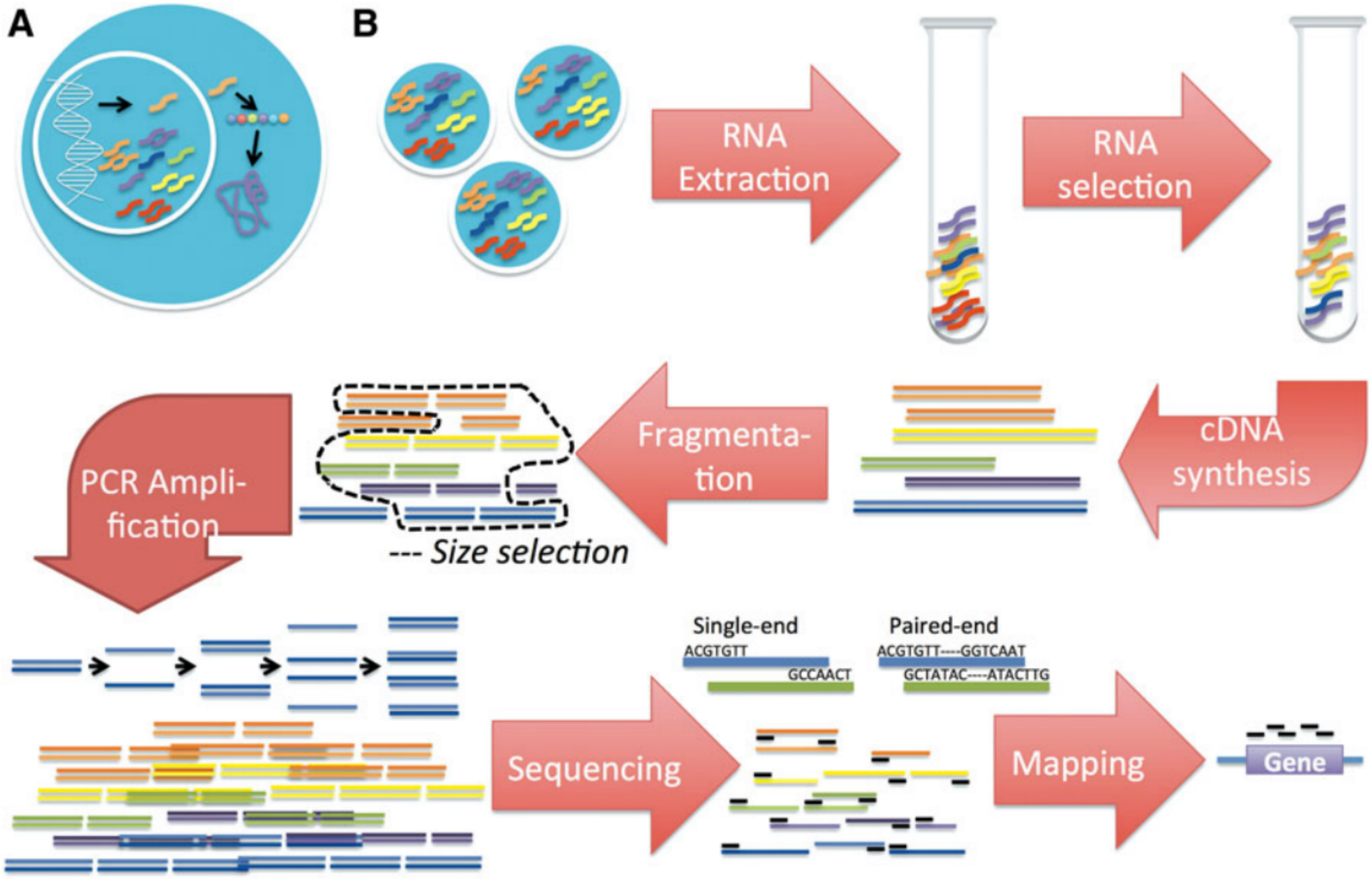
weight of t_i in eq.
class q

estimated read count from transcript i
at iteration u+1

$$\hat{\eta}_i = \frac{\alpha_i}{\sum_j \alpha_j}$$

we also provide the *option* to use a **variational Bayesian** objective instead

Actual RNA-seq protocols are a bit more “involved”

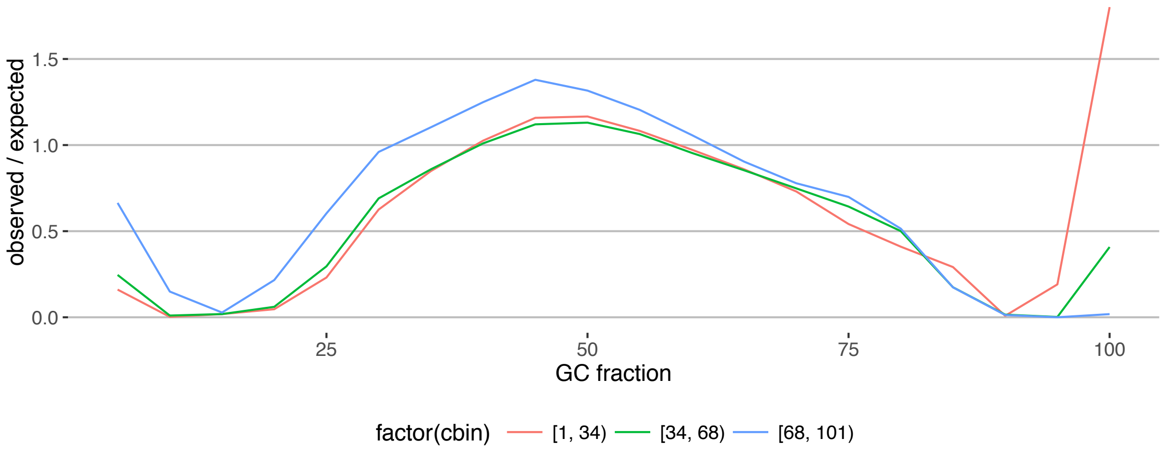


There is **substantial** potential for biases and deviations from the *basic* model — indeed, we see quite a few.

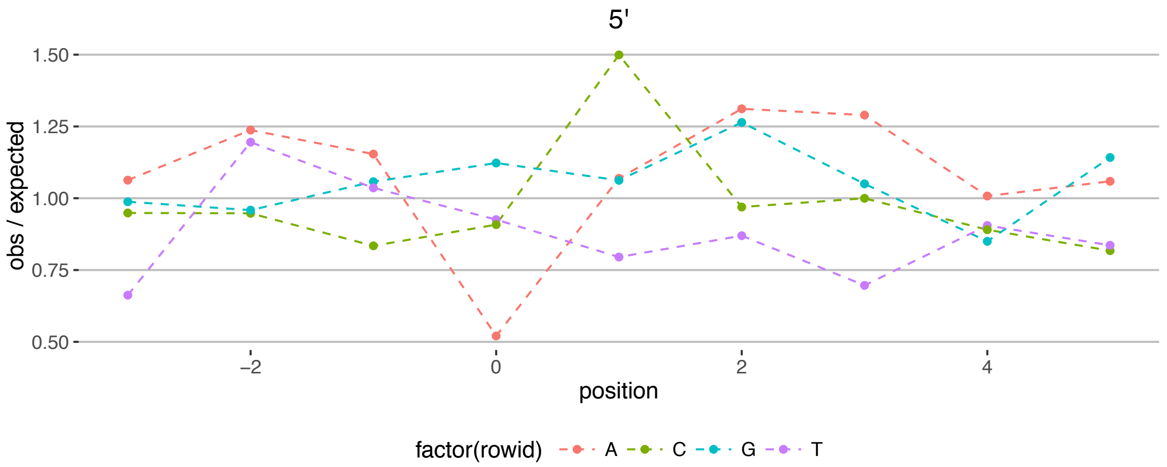
Prakash, Celine, and Arndt Von Haeseler. "An Enumerative Combinatorics Model for Fragmentation Patterns in RNA Sequencing Provides Insights into Nonuniformity of the Expected Fragment Starting-Point and Coverage Profile." *Journal of Computational Biology* 24.3 (2017): 200-212.

Biases abound in RNA-seq data

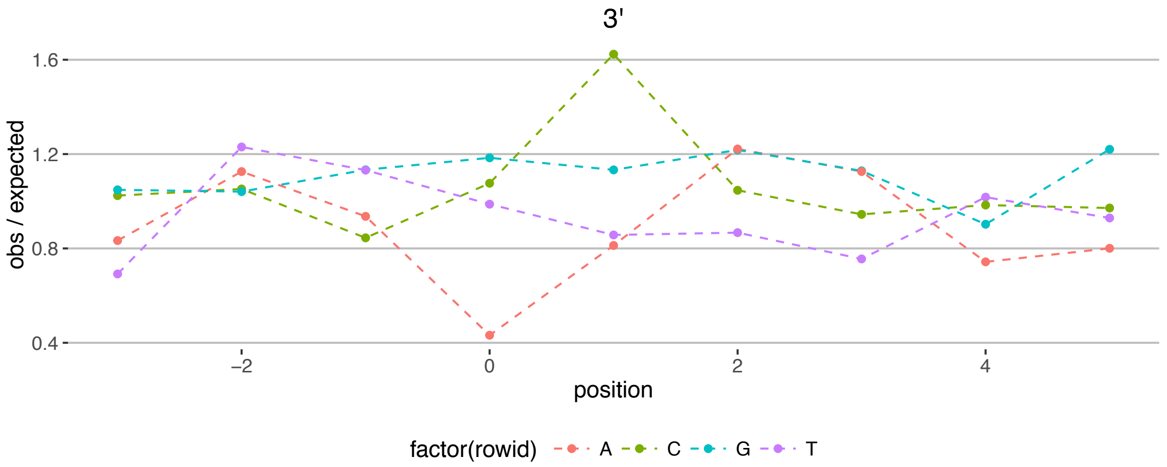
Biases in prep & sequencing can have a significant effect on the fragments we see:



Fragment gc-bias[1]—
The GC-content of the fragment affects the likelihood of sequencing



Sequence-specific bias[2]—
sequences surrounding fragment affect the likelihood of sequencing

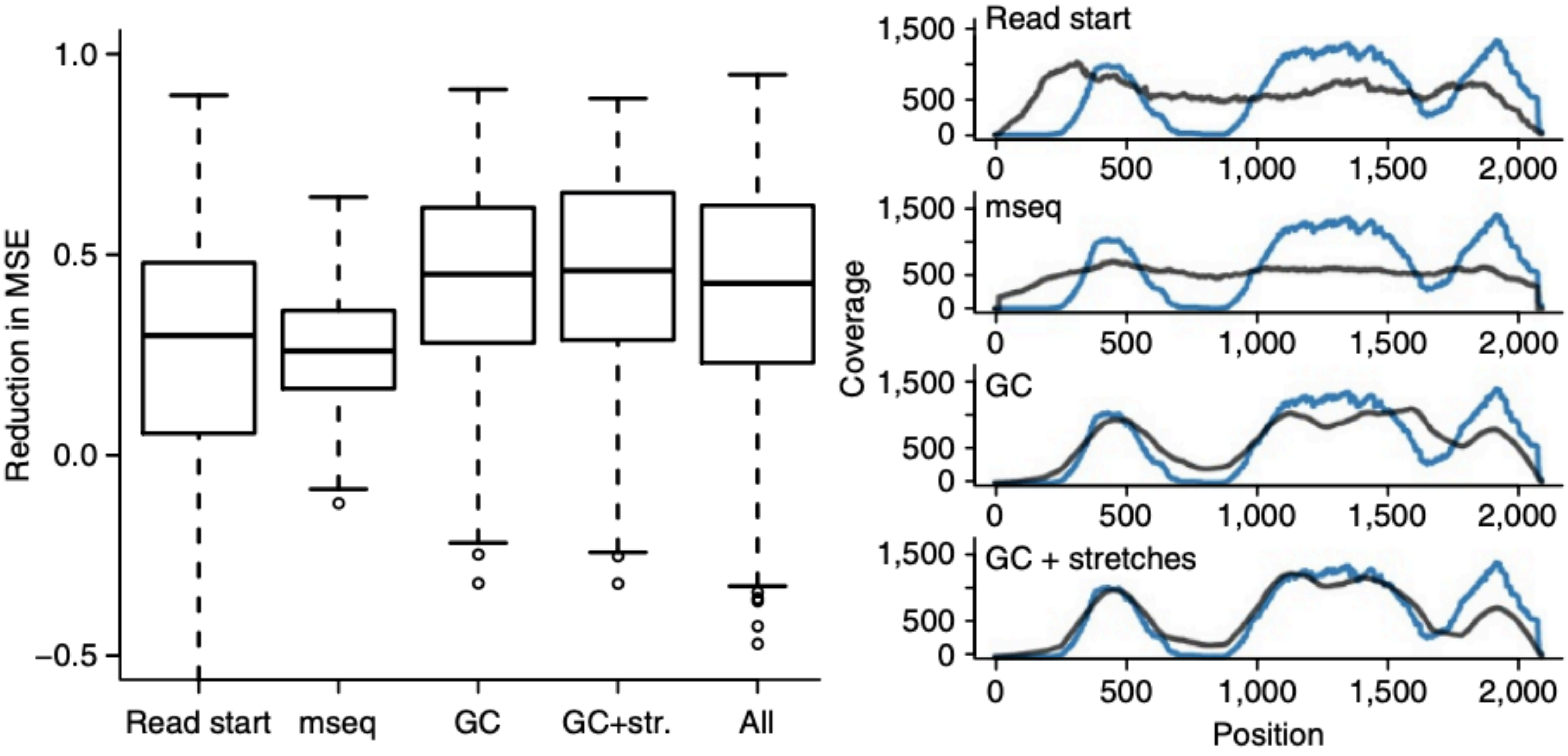


Positional bias[2]—
fragments sequenced non-uniformly across the body of a transcript

[1] Love, Michael I., John B. Hogenesch, and Rafael A. Irizarry. "Modeling of RNA-seq fragment sequence bias reduces systematic errors in transcript abundance estimation." bioRxiv (2015): 025767.

[2] Roberts, Adam, et al. "Improving RNA-Seq expression estimates by correcting for fragment bias." Genome biology 12.3 (2011): 1.

Biases abound in RNA-seq data



Fragment GC-bias is often the most extreme

Love, M. I., Hogenesch, J. B., & Irizarry, R. A. (2016). Modeling of RNA-seq fragment sequence bias reduces systematic errors in transcript abundance estimation. *Nature biotechnology*, *34*(12), 1287.

Basic idea (1): Modify the “effective length” of a transcript to account for changes in the sampling probability. This leads to changes in soft-assignment in EM -> changes in TPM.

Basic idea (2):The effective length of a transcript is the sum of the bias terms at each position across a transcript. The bias term at a given position is simply the (observed / expected) sampling probability.

The trick is how to define “expected” given only biased data.

Bias Modeling

Bias correction works by adjusting the effective lengths of the transcripts: The effective length becomes the sum of the per-base biases

$$\tilde{\ell}'_i = \sum_{j=1}^{j \leq \ell_i} \sum_{k=1}^{k \leq f_i(j,L)} \frac{b_{gc^+}(t_i, j, j+k)}{b_{gc^-}(t_i, j, j+k)} \cdot \frac{b^{5'}_{s+}(t_i, j)}{b^{5'}_{s-}(t_i, j)} \cdot \frac{b^{3'}_{s+}(t_i, j+k)}{b^{3'}_{s-}(t_i, j+k)} \cdot \frac{b^{5'}_{p+}(t_i, j+k)}{b^{5'}_{p-}(t_i, j+k)} \cdot \frac{b^{3'}_{p+}(t_i, j+k)}{b^{3'}_{p-}(t_i, j+k)} \cdot \Pr\{X = j\}$$

Fragment GC bias model:

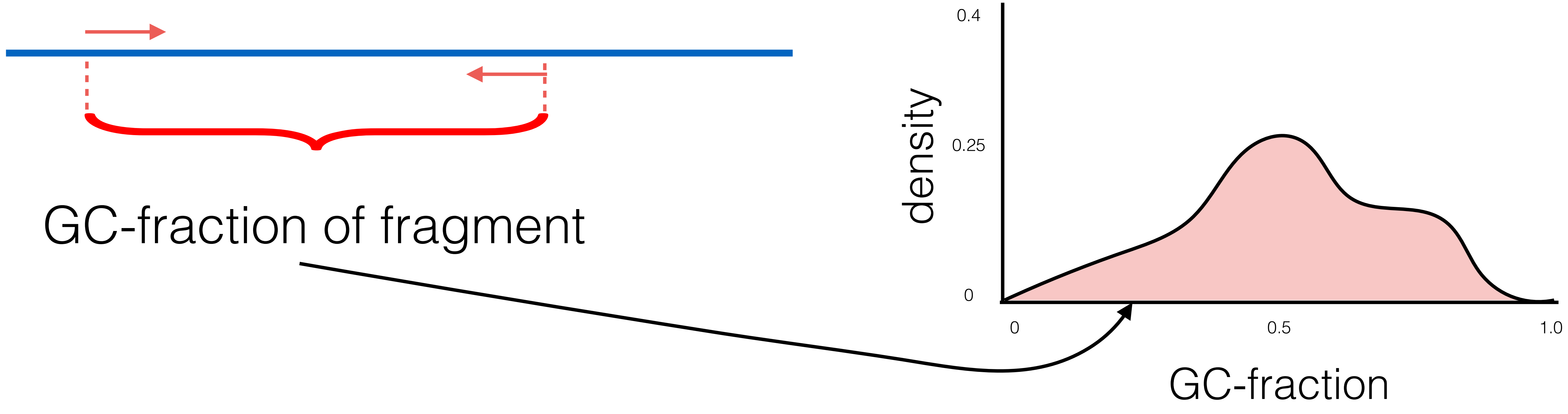
Density of fragments with specific GC content, **conditioned** on GC fraction at read start/end

Foreground:

Observed

Background:

Expected given est. abundances



First explored in Love, Michael I., John B. Hogenesch, and Rafael A. Irizarry. "Modeling of RNA-seq fragment sequence bias reduces systematic errors in transcript abundance estimation." *Nature biotechnology* 34.12 (2016): 1287.

Bias Modeling

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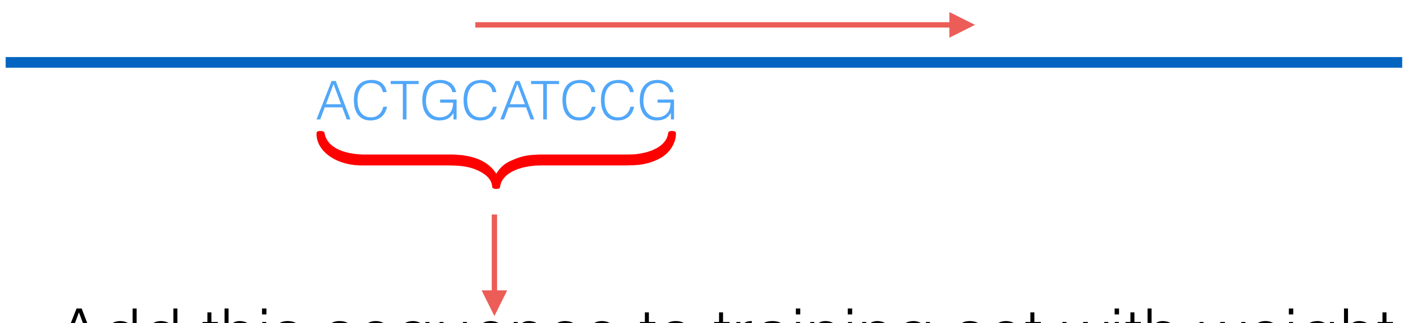
Seq-specific bias model*:

VLMM for the 10bp window surrounding the 5' read start site and the 3' read start site



Add this sequence to training set with weight = P{f | t$_i$}

Foreground:

Observed

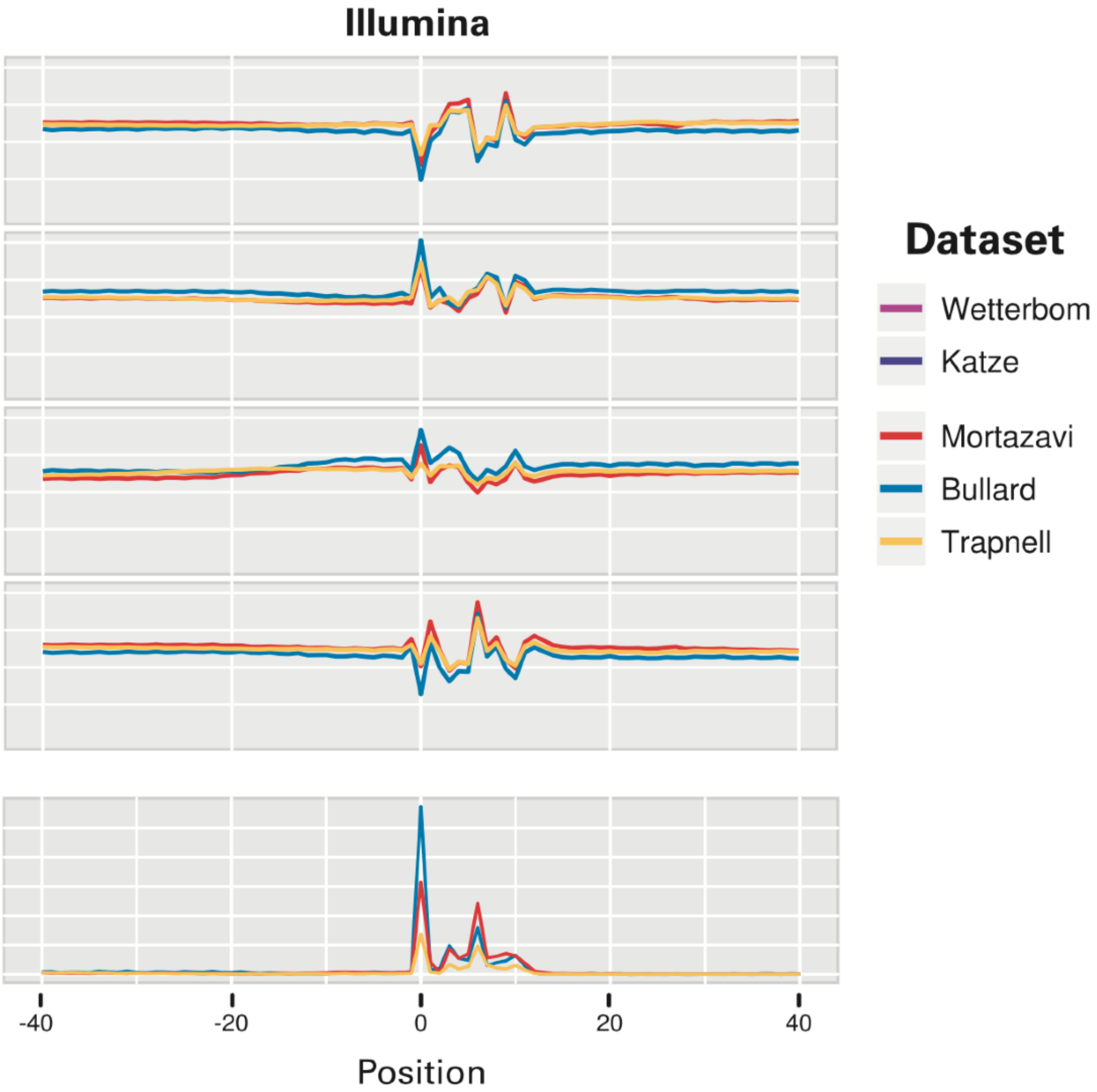
Background:

Expected given est. abundances

Same, but independent model for 3' end

*Roberts, Adam, et al. "Improving RNA-Seq expression estimates by correcting for fragment bias." Genome biology 12.3 (2011): 1.

Priming bias is sample & sequence-specific



Jones, Daniel C., et al. "A new approach to bias correction in RNA-Seq." *Bioinformatics* 28.7 (2012): 921-928.

Bias Modeling

Bias correction works by adjusting the effective lengths of the transcripts: The effective length becomes the sum of the per-base biases

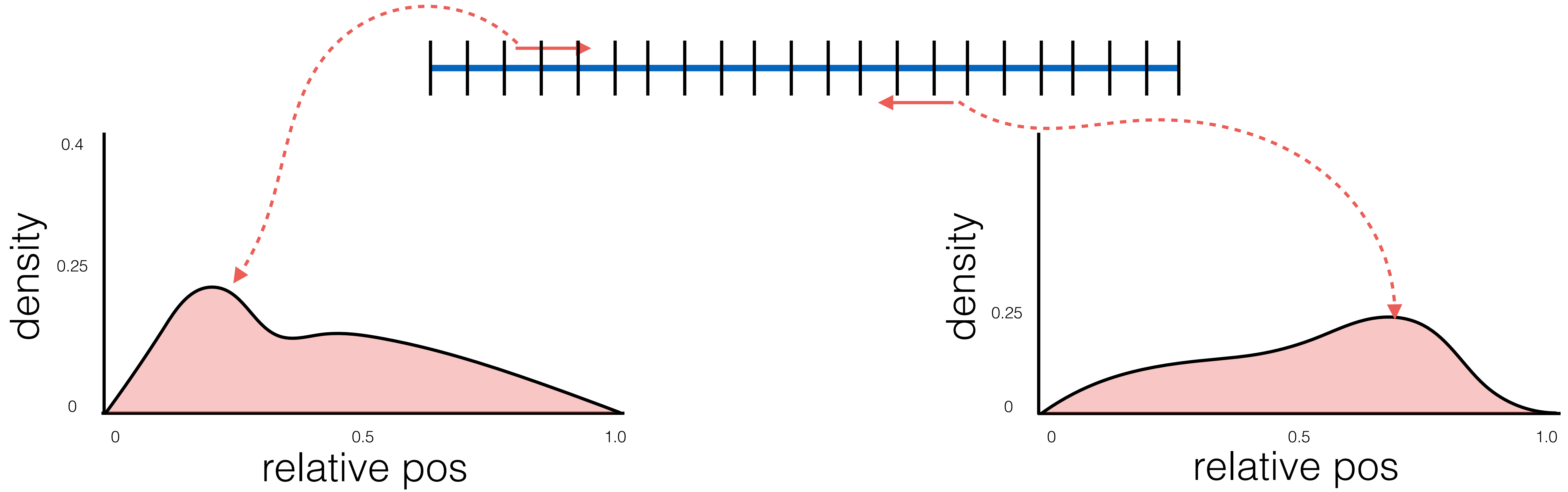
$$\tilde{\ell}'_i = \sum_{j=1}^{j\leq\ell_i} \sum_{k=1}^{k\leq f_i(j,L)} \frac{b_{gc^+}\left(t_i, j, j+k\right)}{b_{gc^-}\left(t_i, j, j+k\right)} \cdot \frac{b^{5'}_{s+}\left(t_i, j\right)}{b^{5'}_{s-}\left(t_i, j\right)} \cdot \frac{b^{3'}_{s+}\left(t_i, j+k\right)}{b^{3'}_{s-}\left(t_i, j+k\right)} \cdot \boxed{\frac{b^{5'}_{p+}\left(t_i, j+k\right)}{b^{5'}_{p-}\left(t_i, j+k\right)} \cdot \frac{b^{3'}_{p+}\left(t_i, j+k\right)}{b^{3'}_{p-}\left(t_i, j+k\right)}} \cdot \Pr\left\{X = j\right\}$$

Position bias model*:

Foreground:
Observed

Density of 5’ and 3’ read start positions — different models for transcripts of different length

Background:
Expected given est. abundances



*Roberts, Adam, et al. "Improving RNA-Seq expression estimates by correcting for fragment bias." Genome biology 12.3 (2011): 1.

Estimating Posterior Uncertainty

One “issue” with maximum likelihood (ML)

The generative statistical model is a principled and elegant way to represent the RNA-seq process.

It can be optimized efficiently using e.g. the EM / VBEM algorithm.

but, these efficient optimization algorithms return “point estimates” of the abundances. That is, there is no notion of how *certain* we are in the computed abundance of transcript.

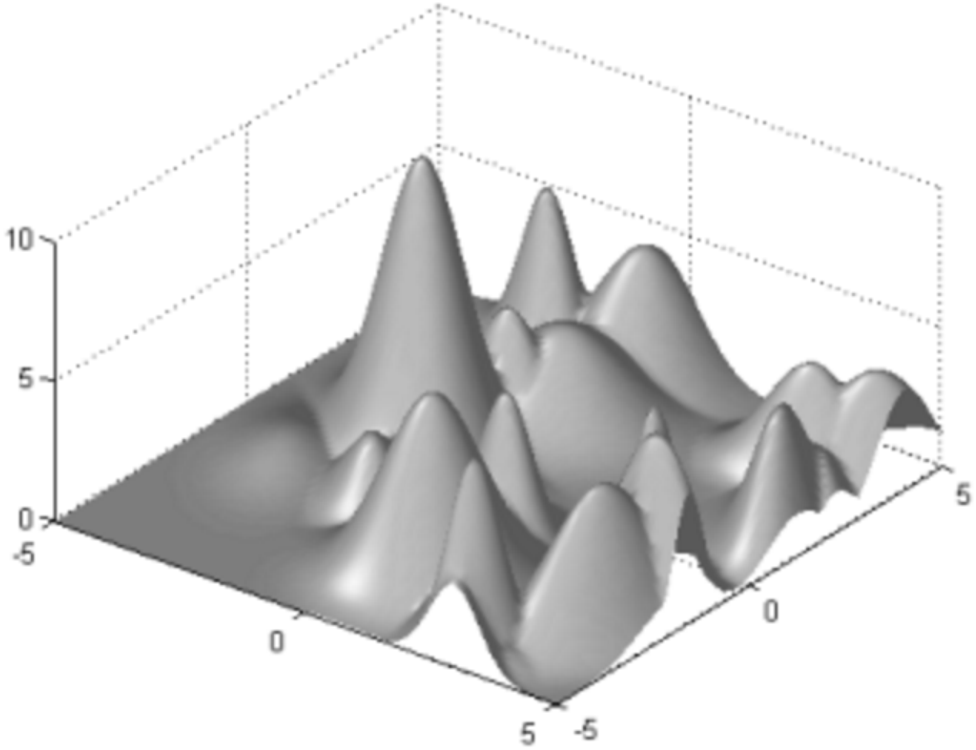
One “issue” with maximum likelihood (ML)

There are multiple sources of uncertainty e.g.

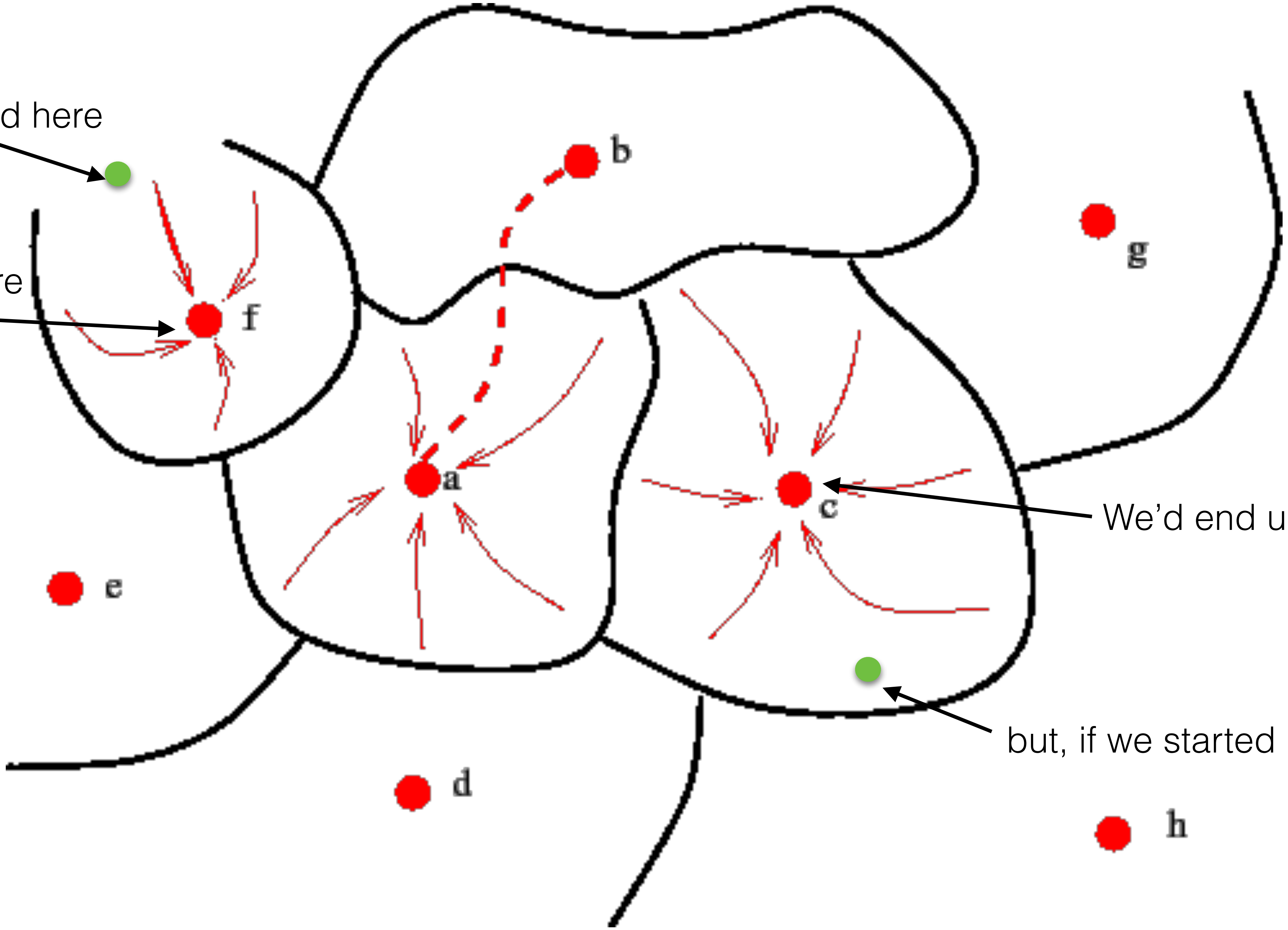
- Technical variance : If we sequenced the *exact* same sample again, we’d get a different set of fragments, and, potentially a different solution.

- Uncertainty in inference: We are almost never guaranteed to find a unique, globally optimal result. If we started our algorithm with different initialization parameters, we might get a different result.

We’re trying to find the *best* parameters in a space with 10s to 100s of thousands of dimensions!



One “issue” with maximum likelihood (ML)



If we started here

We’d end up here

We’d end up here

but, if we started here

https://commons.wikimedia.org/wiki/File:Local_search_attraction_basins.png (CC BY-SA 3.0)

Assessing Uncertainty

There are a few ways to address this “issue”

Do a fully Bayesian inference[1]:

Infer the entire posterior distribution of parameters, not just a ML estimate (e.g. using MCMC) — too slow!

✓ Posterior Gibbs Sampling[2,3]:

Starting from our ML estimate, do MCMC sampling to explore how parameters vary — if our ML estimate is good, this can be made *quite fast.*

✓ Bootstrap Sampling[4]:

Resample (from range-factorized equivalence class counts) with replacement, and re-run the ML estimate for each sample. This can be made reasonably fast.

[1]: BitSeq (with MCMC) actually does this. It’s very accurate, but very slow. [Glaus, Peter, Antti Honkela, and Magnus Rattray. "Identifying differentially expressed transcripts from RNA-seq data with biological variation." Bioinformatics 28.13 (2012): 1721-1728.]

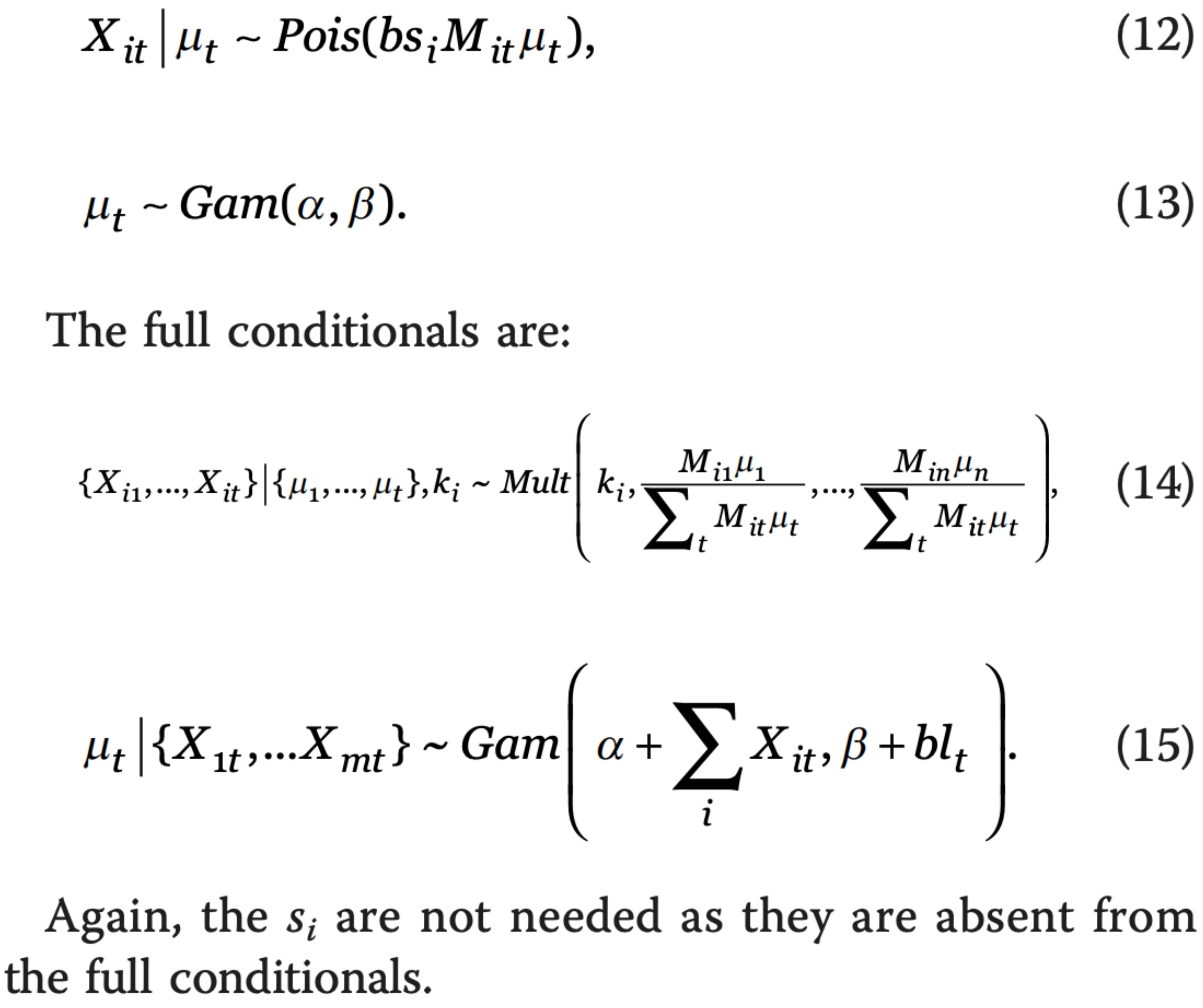
[2]: RSEM has the ability to do this, and it seems to work well, but each sample scales in the # of reads. [Li, Bo, and Colin N. Dewey. "RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome." BMC bioinformatics 12.1 (2011): 1.]

[3]: MMSEQ can perform Gibbs sampling over shared variables (i.e. equiv classes), producing estimates from the mean of the posterior dist.Turro, Ernest, et al. "Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads." Genome biology 12.2 (2011): 1.

[4]: IsoDE introduced the idea of bootstrapping counts to assess quantification uncertainty. [Al Seesi, Sahar, et al. "Bootstrap-based differential gene expression analysis for RNA-Seq data with and without replicates." BMC genomics 15.8 (2014): 1.], but it was first made practical / fast in kallisto by doing the bootstrapping over equivalence classes.

A few ways to implement Gibbs Sampling for this problem

The model of MMSeq



$$X_{it} \mid \mu_t \sim Pois(bs_i M_{it} \mu_t), \tag{12}$$

$$\mu_t \sim Gam(\alpha, \beta). \tag{13}$$

The full conditionals are:

$$\{X_{i1},...,X_{it}\} \mid \{\mu_1,...,\mu_t\}, k_i \sim Mult\left(k_i, \frac{M_{i1}\mu_1}{\sum_t M_{it}\mu_t}, ..., \frac{M_{in}\mu_n}{\sum_t M_{it}\mu_t} \right), \tag{14}$$

$$\mu_t \mid \{X_{1t},...X_{mt}\} \sim Gam\left(\alpha + \sum_i X_{it}, \beta + bl_t \right). \tag{15}$$

Again, the s_i are not needed as they are absent from the full conditionals.

Turro, Ernest, et al. "Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads." Genome biology 12.2 (2011): 1.

A few ways to implement Gibbs Sampling for this problem

The model of BitSeq

$$
\begin{aligned}
P(I_n|\boldsymbol{\theta}, \theta^{act}, R) &= \mathrm{Cat}(I_n|\boldsymbol{\phi_n}), && (10)\\
\phi_{n0} &= P(r_n|\mathrm{noise})(1-\theta^{act})/Z_n^{(\phi)}, &&\\
m \neq 0; \phi_{nm} &= P(r_n|I_n)\theta_m\theta^{act}/Z_{\boldsymbol{n}}^{(\phi)}, &&\\
P(\boldsymbol{\theta}|\boldsymbol{I}, \theta^{act}, R) &= \mathrm{Dir}(\boldsymbol{\theta}|(\alpha^{dir}+C_1, \ldots, \alpha^{dir}+C_M)), && (11)\\
P(\theta^{act}|\boldsymbol{I}, \boldsymbol{\theta}, R) &= \mathrm{Beta}(\theta^{act}|\alpha^{act}+N-C_0, \beta^{act}+C_0), && (12)\\
C_m &= \textstyle\sum_{n=1}^{N}\delta(I_n = m). &&
\end{aligned}
$$

[Glaus, Peter, Antti Honkela, and Magnus Rattray. "Identifying differentially expressed transcripts from RNA-seq data with biological variation." Bioinformatics 28.13 (2012): 1721-1728.]

A few ways to implement Gibbs Sampling for this problem

The model of BitSeq (collapsed sampler)

$$
\begin{aligned}
P(I_n|I^{(-n)}, R) &= \mathrm{Cat}(I_n|\boldsymbol{\phi_n^*}), \qquad (9)\\
\phi_{n0}^* &= P(r_n|\mathrm{noise})(\beta^{act} + C_0^{(-n)})/Z_n^{(\phi^*)},\\
m \neq 0; \phi_{nm}^* &= P(r_n|I_n)(\alpha^{act} + C_+^{(-n)})\tfrac{(\alpha^{dir}+C_m^{(-n)})}{(M\alpha^{dir}+C_+^{(-n)})}/Z_n^{(\phi^*)},\\
C_m^{(-n)} &= \textstyle\sum_{i\neq n}\delta(I_i = m),\\
C_+^{(-n)} &= \textstyle\sum_{i\neq n}\delta(I_i > 0)\ ,
\end{aligned}
$$

with $Z_n^{(\phi^*)}$ being a constant normalising $\boldsymbol{\phi_n}^*$ to sum up to 1, and $\alpha^{dir} = 1, \alpha^{act} = 2, \beta^{act} = 2.$

[Glaus, Peter, Antti Honkela, and Magnus Rattray. "Identifying differentially expressed transcripts from RNA-seq data with biological variation." Bioinformatics 28.13 (2012): 1721-1728.]

This uncertainty matters

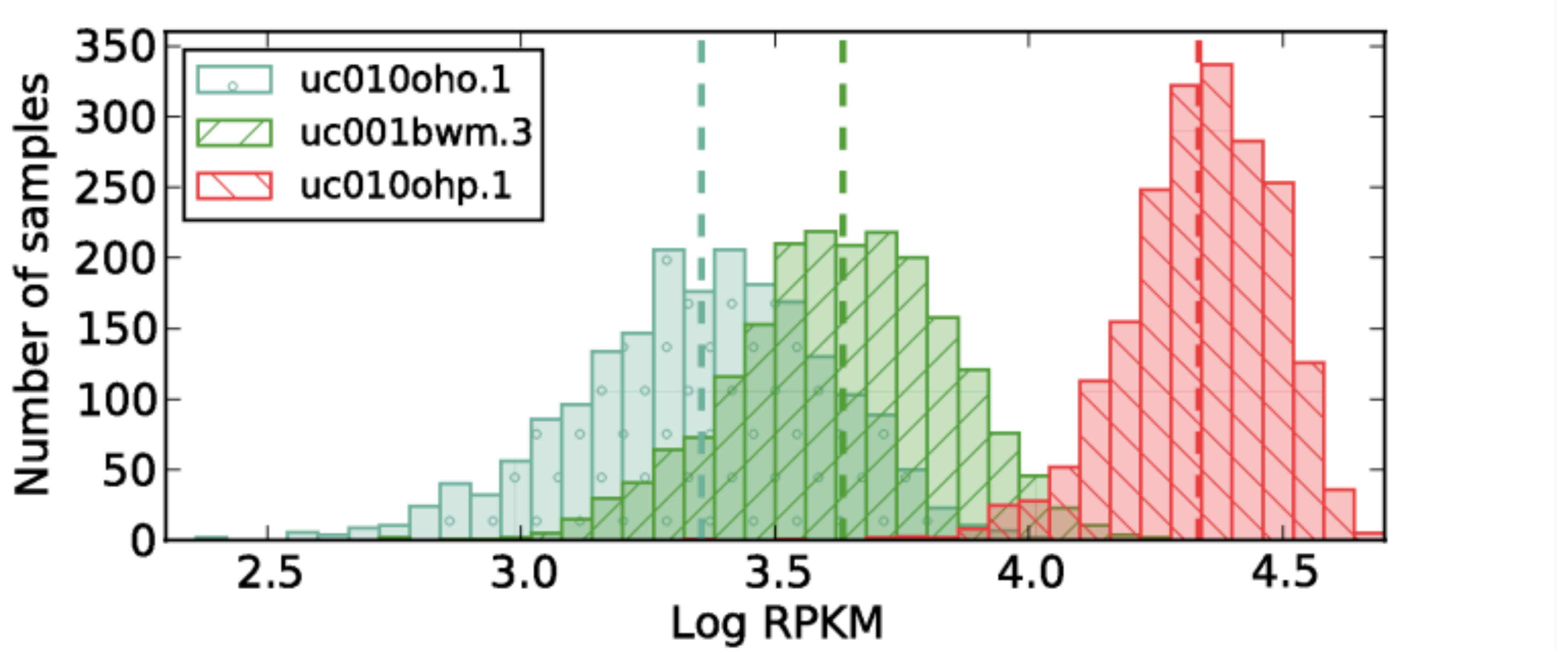
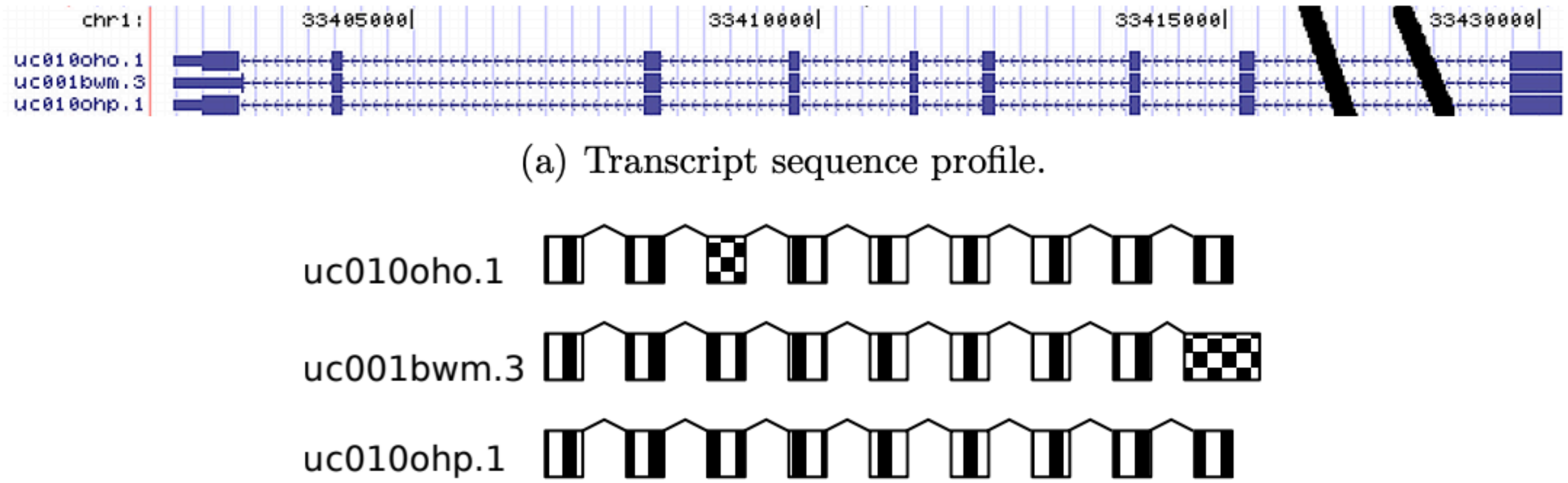


Figure 2.10: **Posterior distribution of expression levels of three transcripts of gene Q6ZMZ0.** The posterior distribution is represented in form of a histogram of expression samples converted into Log RPKM expression measure. The dashed lines mark the mean expression for each transcript.

*Glaus, Peter. *Bayesian Methods for Gene Expression Analysis from High-throughput Sequencing Data*. Diss. University of Manchester, 2014.

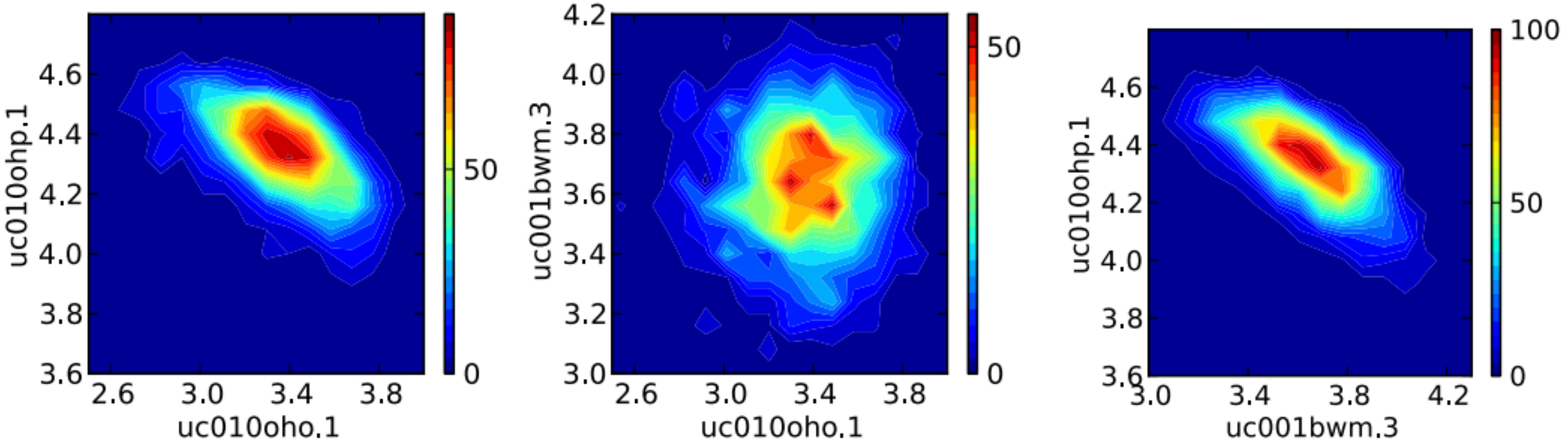
This uncertainty matters



(a) Transcript sequence profile.

(b) Splice variant model.

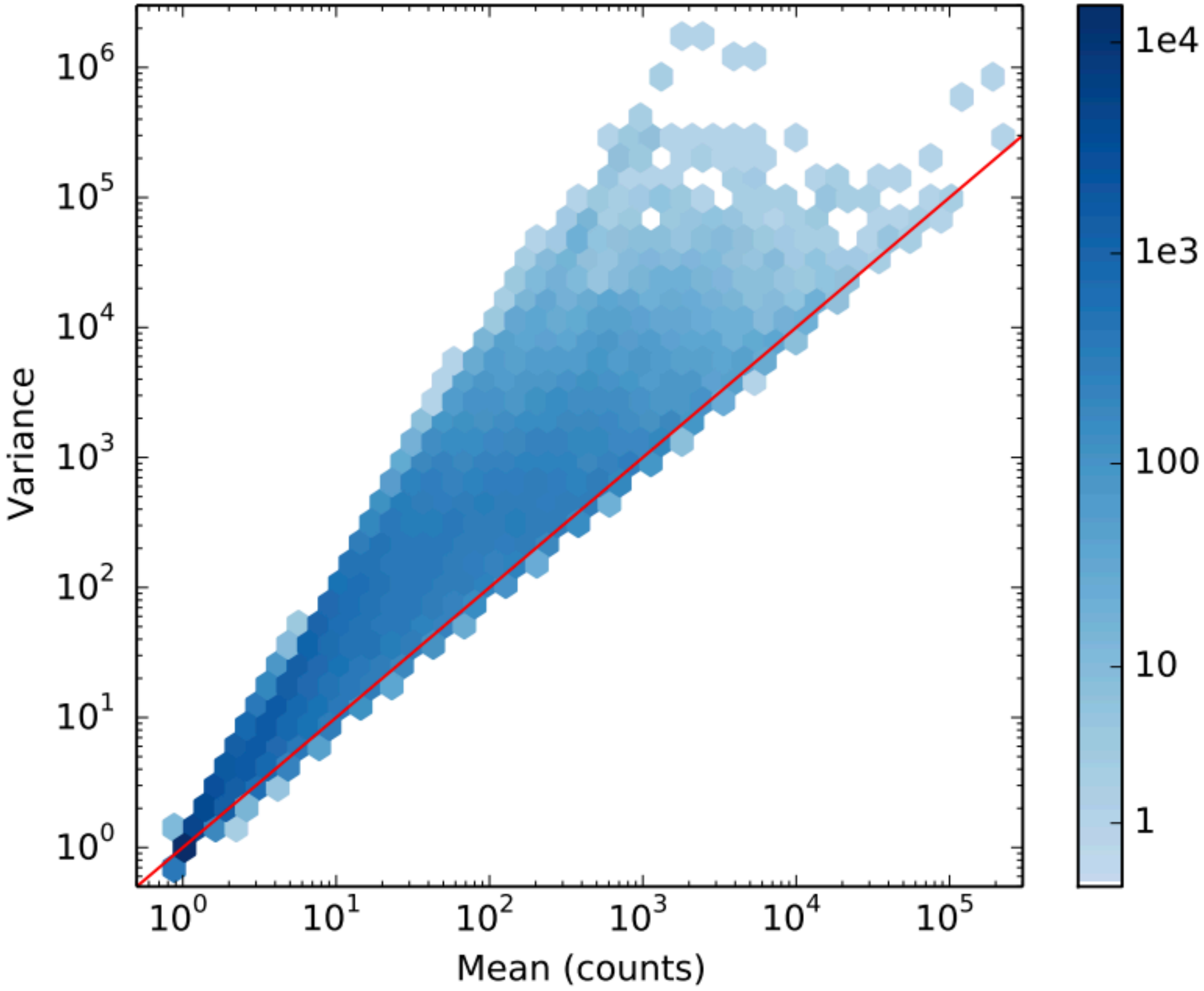
Figure 2.12: **Exon model of transcripts of gene Q6ZMZ0.** (a) transcript sequence profile obtained from the UCSC genome browser (Kuhn et al., 2013). In this annotation, transcript uc001bwm.3 has different 3' untranslated region and transcript uc010oho.1 has extra nucleotides at the end of second exon. As the second change cannot be distinguished in the UCSC genome browser diagram, we provide schematic splice variant model highlighting the differences (b).



*Glaus, Peter. *Bayesian Methods for Gene Expression Analysis from High-throughput Sequencing Data*. Diss. University of Manchester, 2014.

This uncertainty matters

We observe considerably increased variance due to read mapping ambiguity



If we know this increased uncertainty, we can propagate it & use it in downstream analysis (differential expression)!

*Glaus, Peter. *Bayesian Methods for Gene Expression Analysis from High-throughput Sequencing Data*. Diss. University of Manchester, 2014.