Lecture 7: The RNA quantification problem

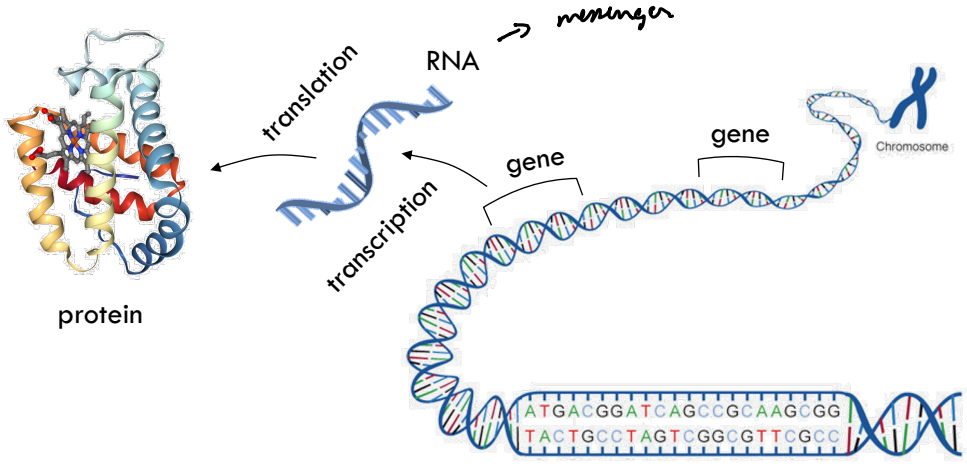
ECE 365

Announcements:

- Lab 3 deadline moved to Friday 03/26
- Lab 3 session moved to Thursday 03/25
- Quiz 1 tonight at 7pm
  - Material includes everything up to (and including) GWAS
  - You are allowed one double-sided sheet of handwritten notes
  - You are allowed to use a calculator

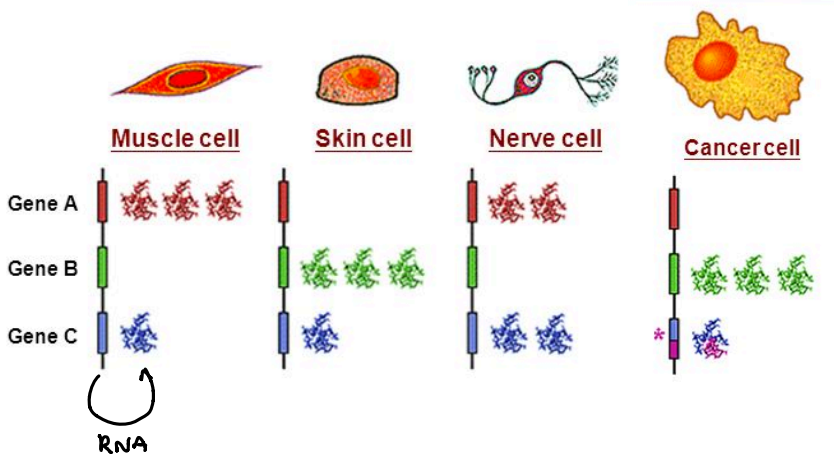
The central dogma of molecular biology

- DNA → RNA → protein



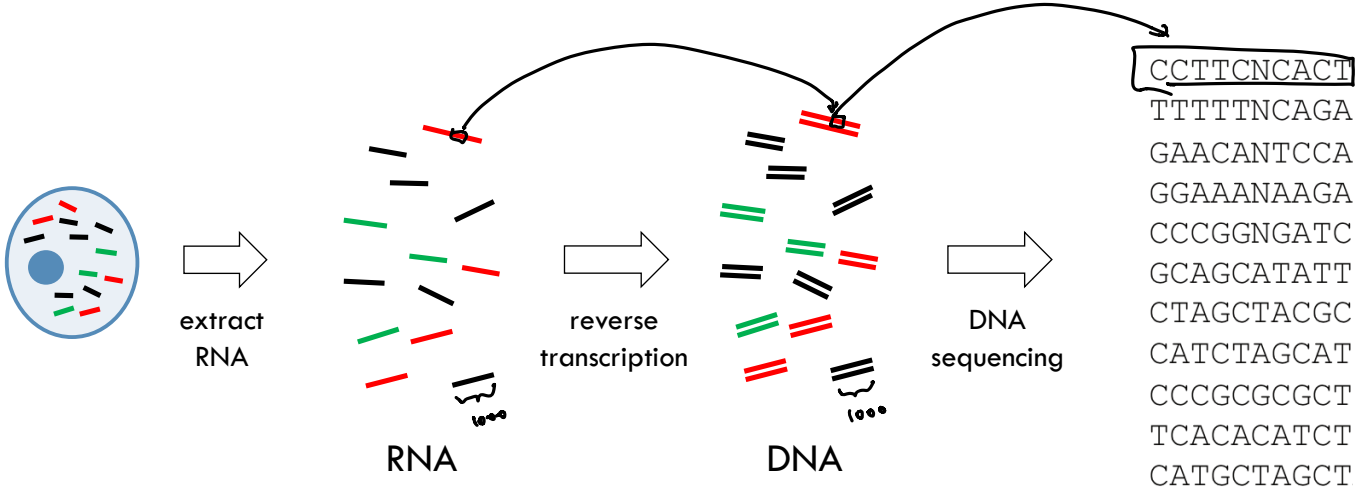
Why do we care about RNA?

- Different genes are *expressed* in different cell types



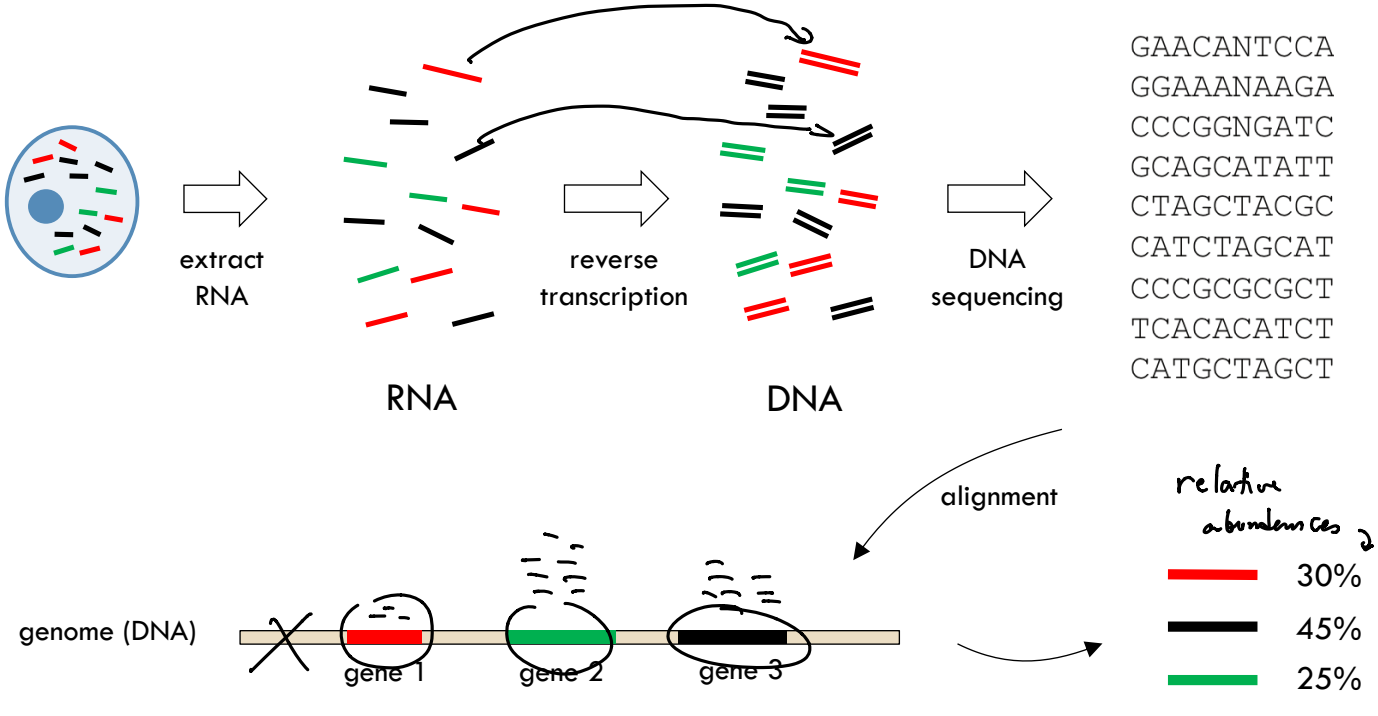
- RNA is an "intermediate step" between genes and proteins
- RNA levels in a cell can tell us which genes are "on/off"

How do we sequence RNA?

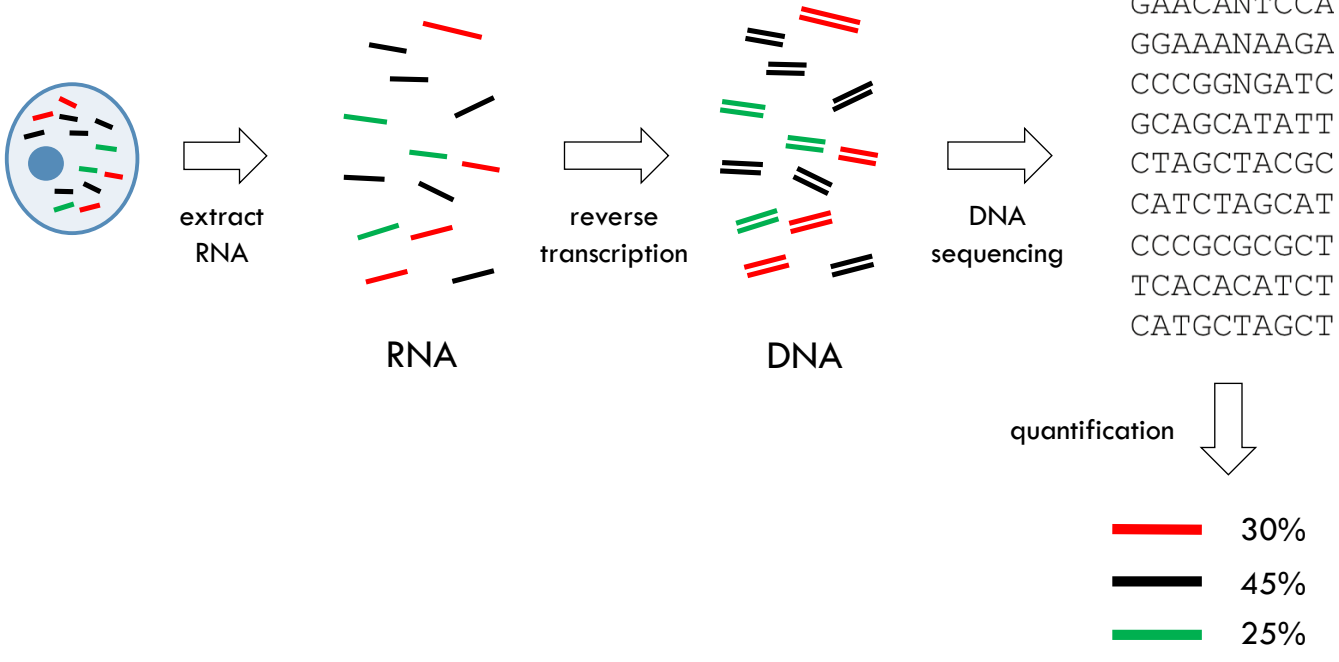


RNA-seq

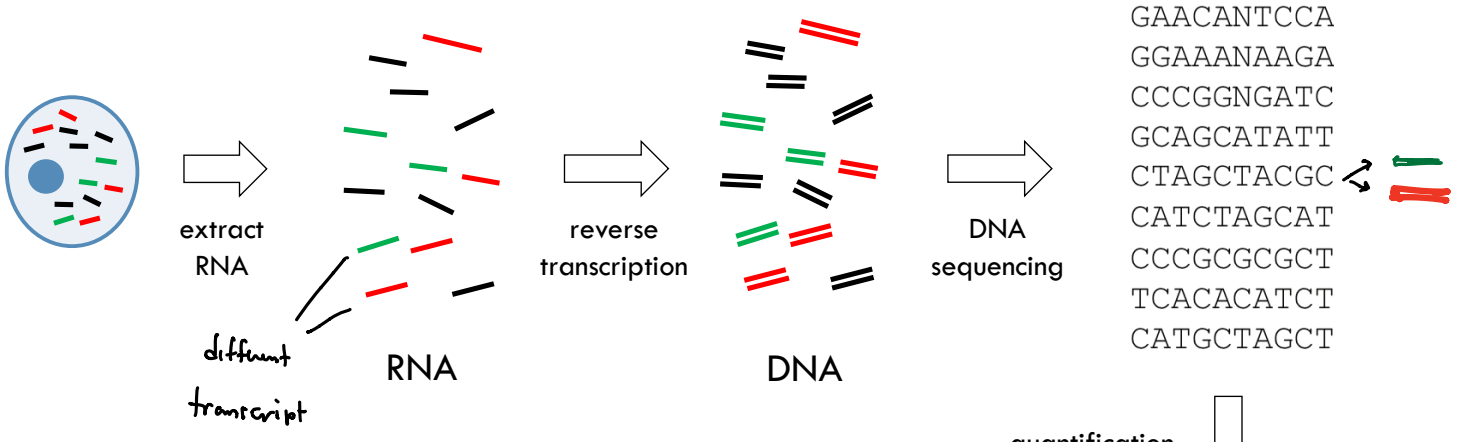
Our first goal: RNA quantification



Our first goal: RNA quantification

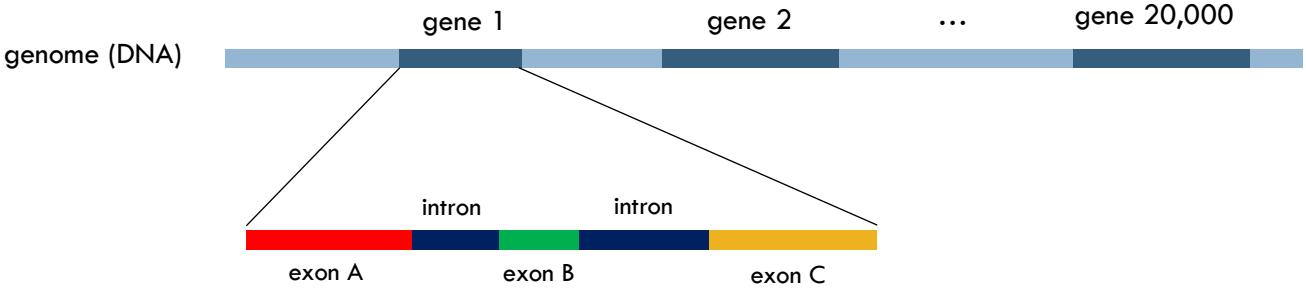


Our first goal: RNA quantification

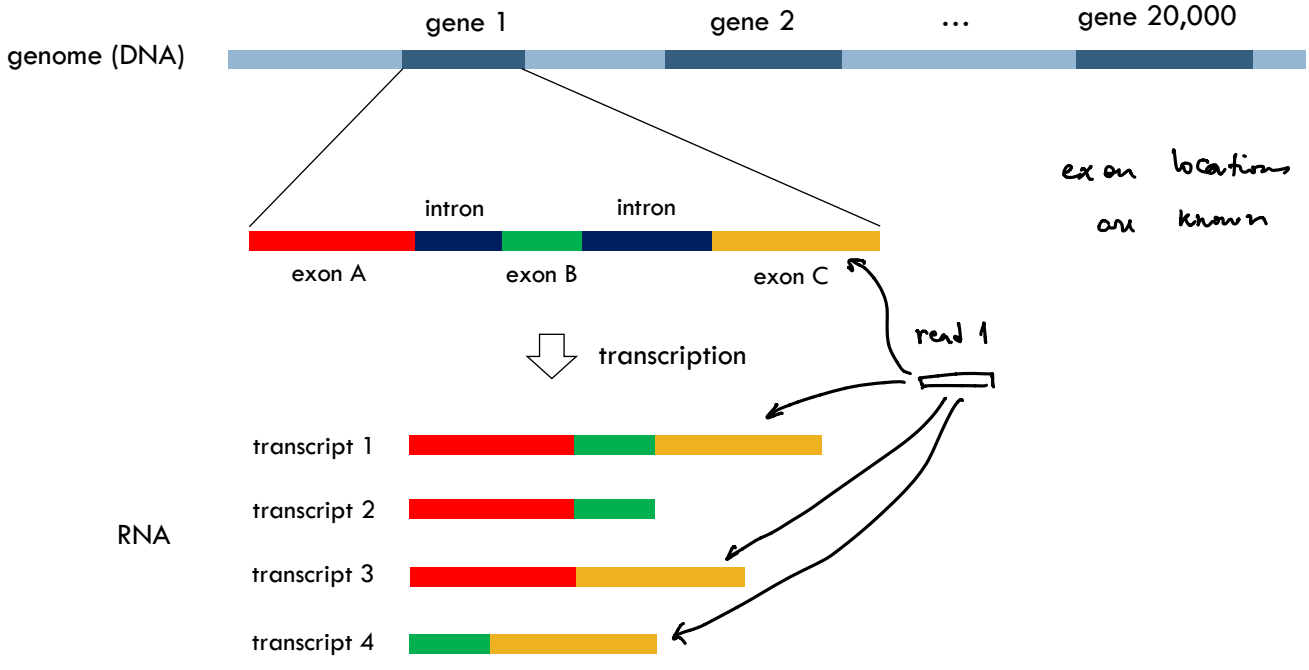


Why is this hard? Because transcripts may "look alike"

From genes to transcripts



From genes to transcripts



RNA (transcript) quantification problem

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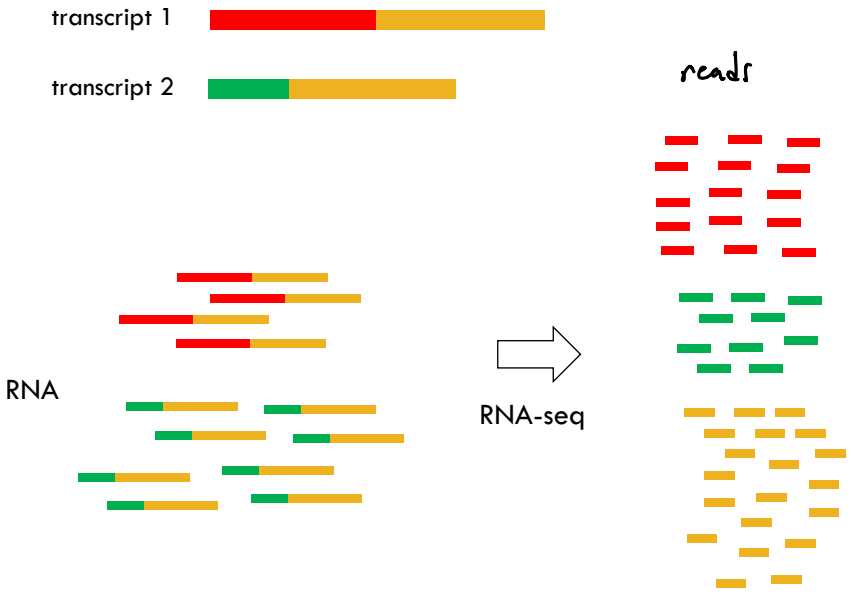
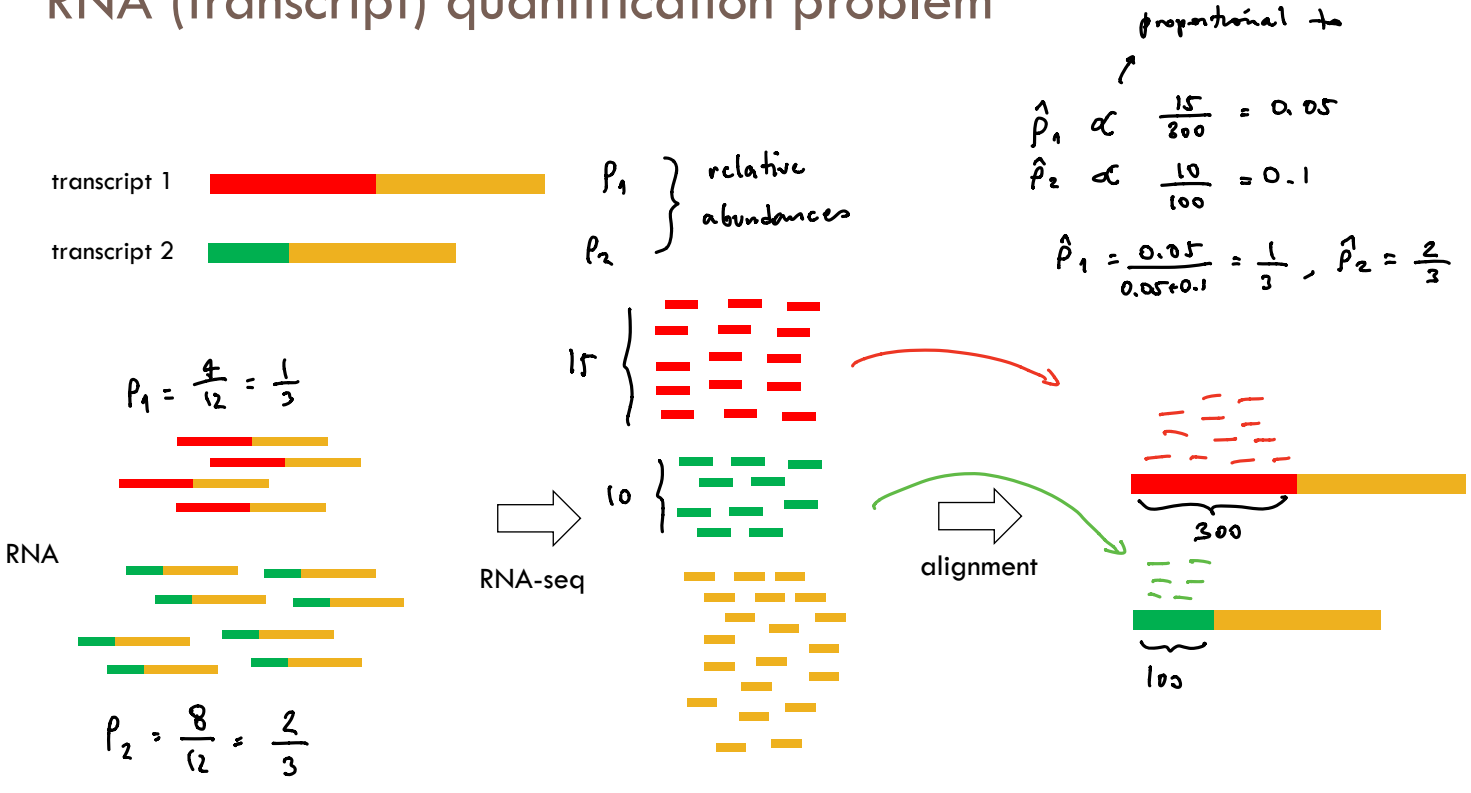
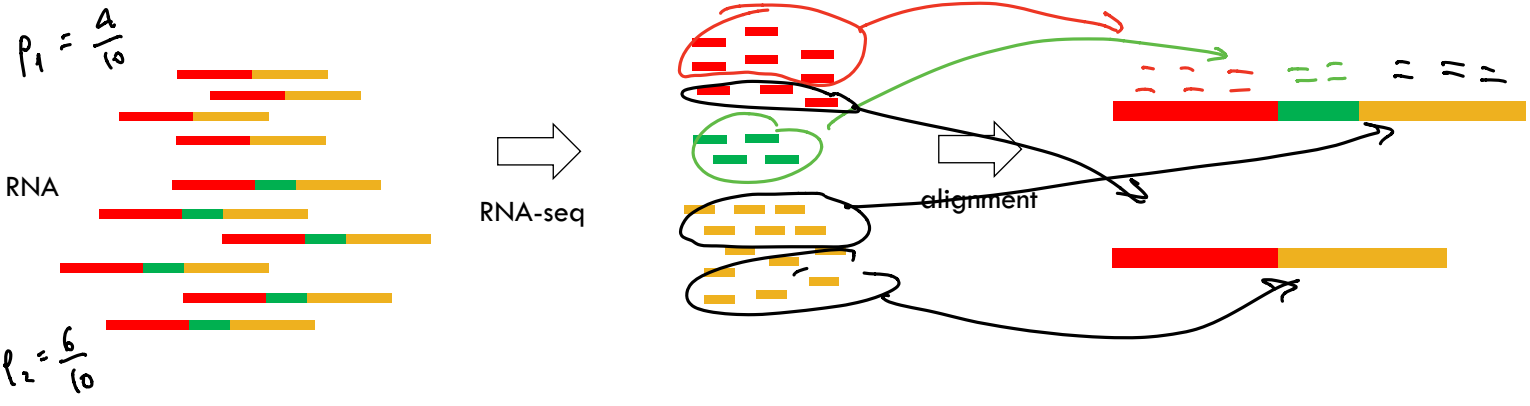


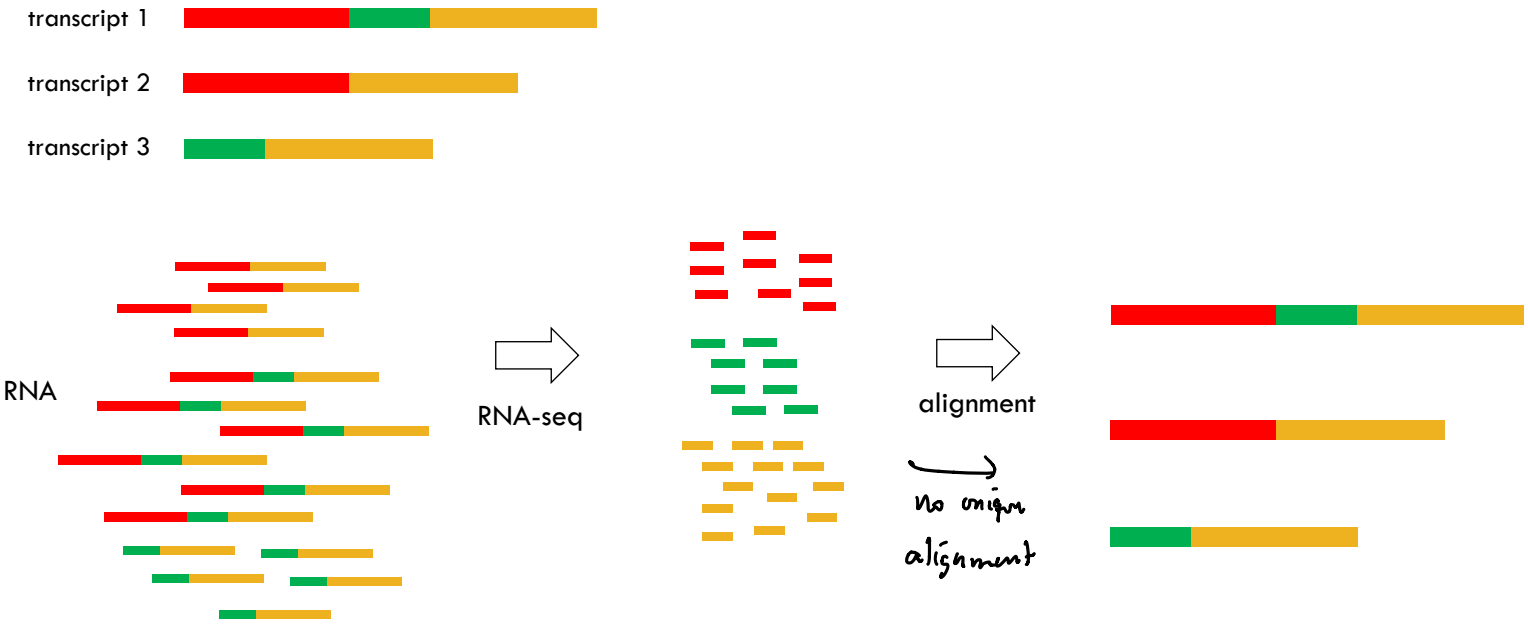
RNA (transcript) quantification problem



RNA (transcript) quantification problem



RNA (transcript) quantification problem



General RNA quantification problem

- Input: **aligned read data**

relative abundances

	tr. 1	tr 2	⋯		tr. K
read 1	0	1	1	0	0	0
read 2	0	0	0	1	0	0
	0	1	1	0	0	0
⋮	0	0	0	0	1	0
	1	0	0	0	0	0
	1	0	0	0	1	0
read N	0	1	0	0	0	1

$\Rightarrow$

$\hat{\rho}_1 = 0.01$

$\hat{\rho}_2 = 0.04$

⋮

$\hat{\rho}_K = 0.02$

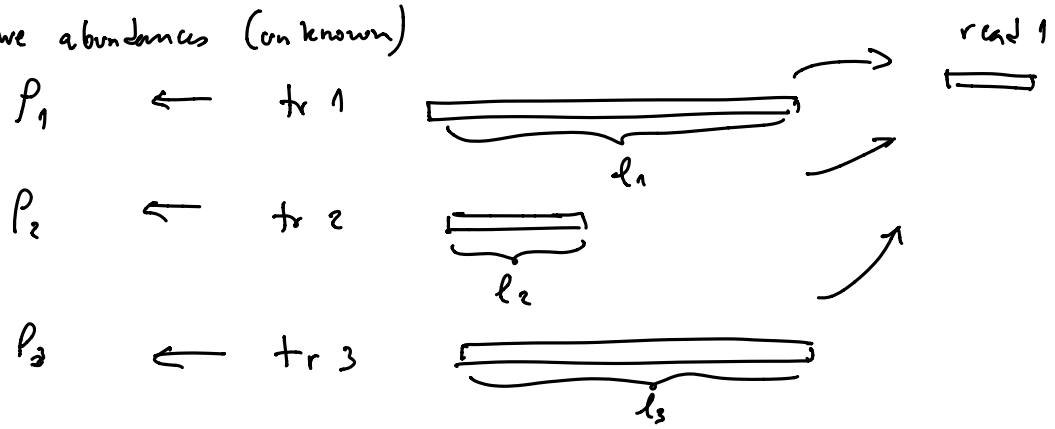
means: read N maps to tr. 2

General approach to solve this:

Expectation - Maximization Algorithm (EM)

Model for read data generation:

relative abundances (unknown)



$$P(\text{read 1 comes from tr. } k) = \frac{\rho_k \ell_k}{\sum_{j=1}^{K} \rho_j \ell_j}$$

True read origins/assignment (unknown)

$$Z_{ik} = \begin{cases} 1 & \text{if read } i \text{ comes from tr. } k \\ 0 & \text{otherwise} \end{cases}$$

↓ form a matrix

K transcripts

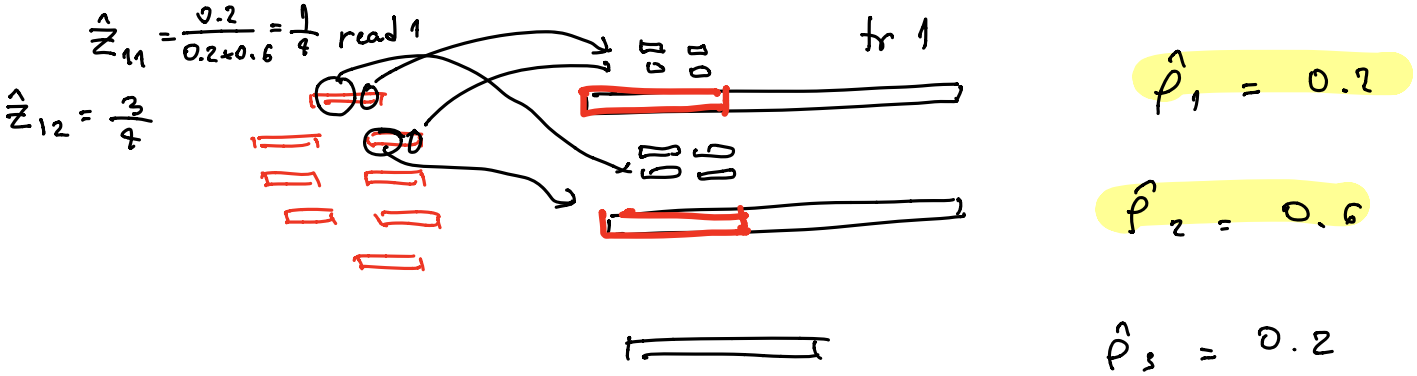
N reads $\left\{ \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \right.$ (one 1 per row)

→ "cleaned up" version of data matrix

EM strategy: Estimate $\hat{\rho}_k$ and $\hat{Z}_{ik}$ in an alternating fashion and recursively

Initialize : $\hat{\rho}_k = \frac{1}{K}$ for $k = 1, \ldots, K$

Based on $\hat{\rho}_k$'s, estimate $\hat{z}_{ik}$'s :



Let S_i be the set of transcripts read i maps to

$$\hat{z}_{ik} = \begin{cases} \dfrac{\hat{\rho}_k}{\sum_{j \in S_i} \hat{\rho}_j} & \text{if } k \in S_i \\ 0 \end{cases}$$