

BIOL 343

Applied Bioinformatics I

RNA extraction

Dr. Nic Wheeler

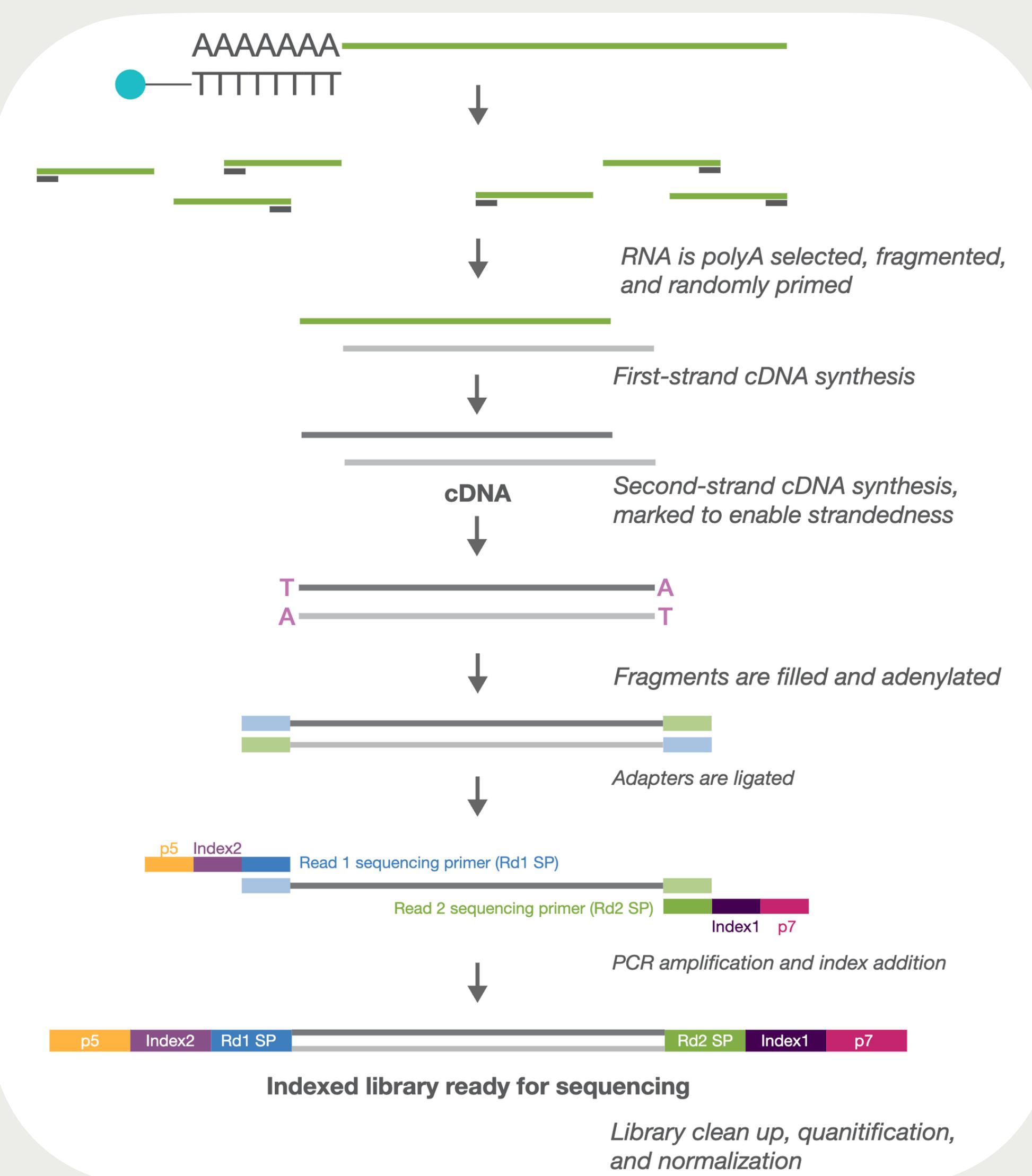
Learning Objectives

You will be able to:

1. Explain the biology and chemistry underlying popular RNA extraction protocols
2. Describe post-extraction protocols and their place in the RNA sequencing pipeline
3. Interpret RNA quality control metrics and predict reasons for poor results

Steps to preparing a library for RNA sequencing

From extraction to library preparation



What's in a cell?

And how do we separate RNA from everything else?

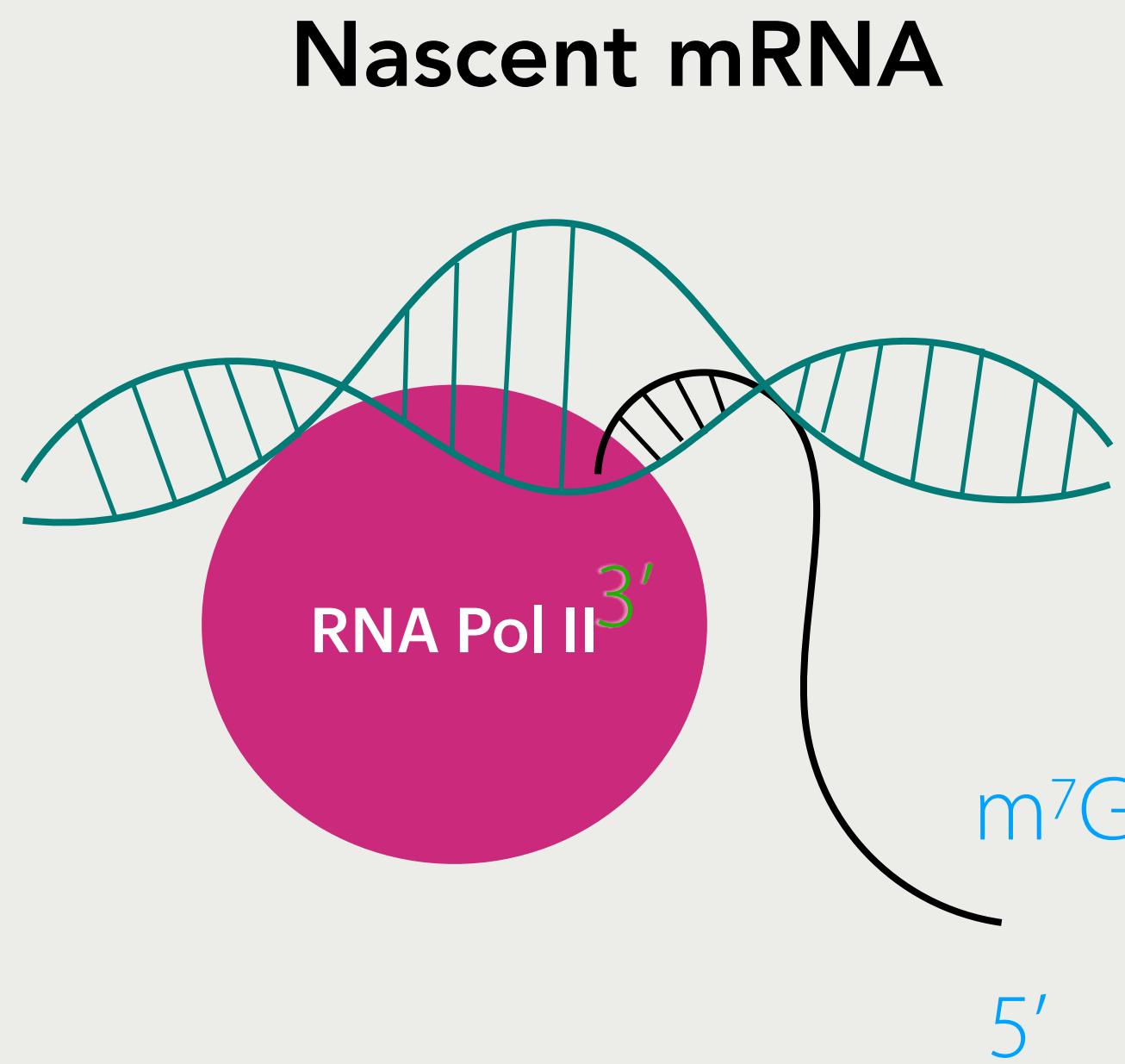
Organic molecule

Physiochemical properties

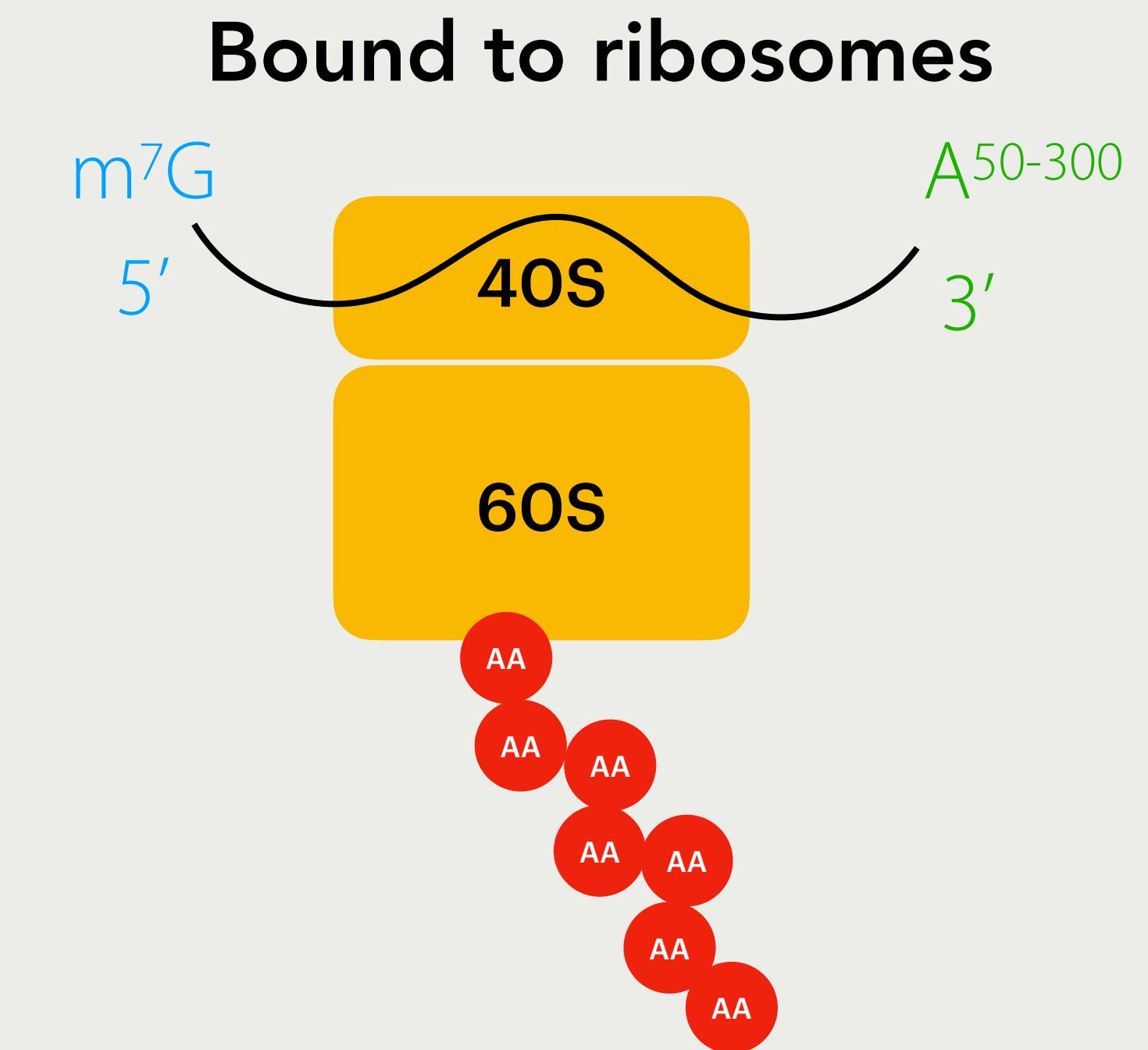
Amount

Many sources of mRNA

Free and attached to proteins or DNA

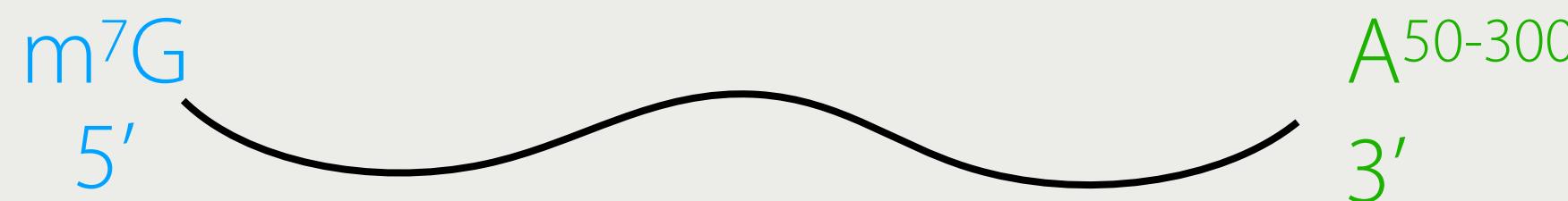


**Challenge: isolate
all source of mRNA
without
degradation**



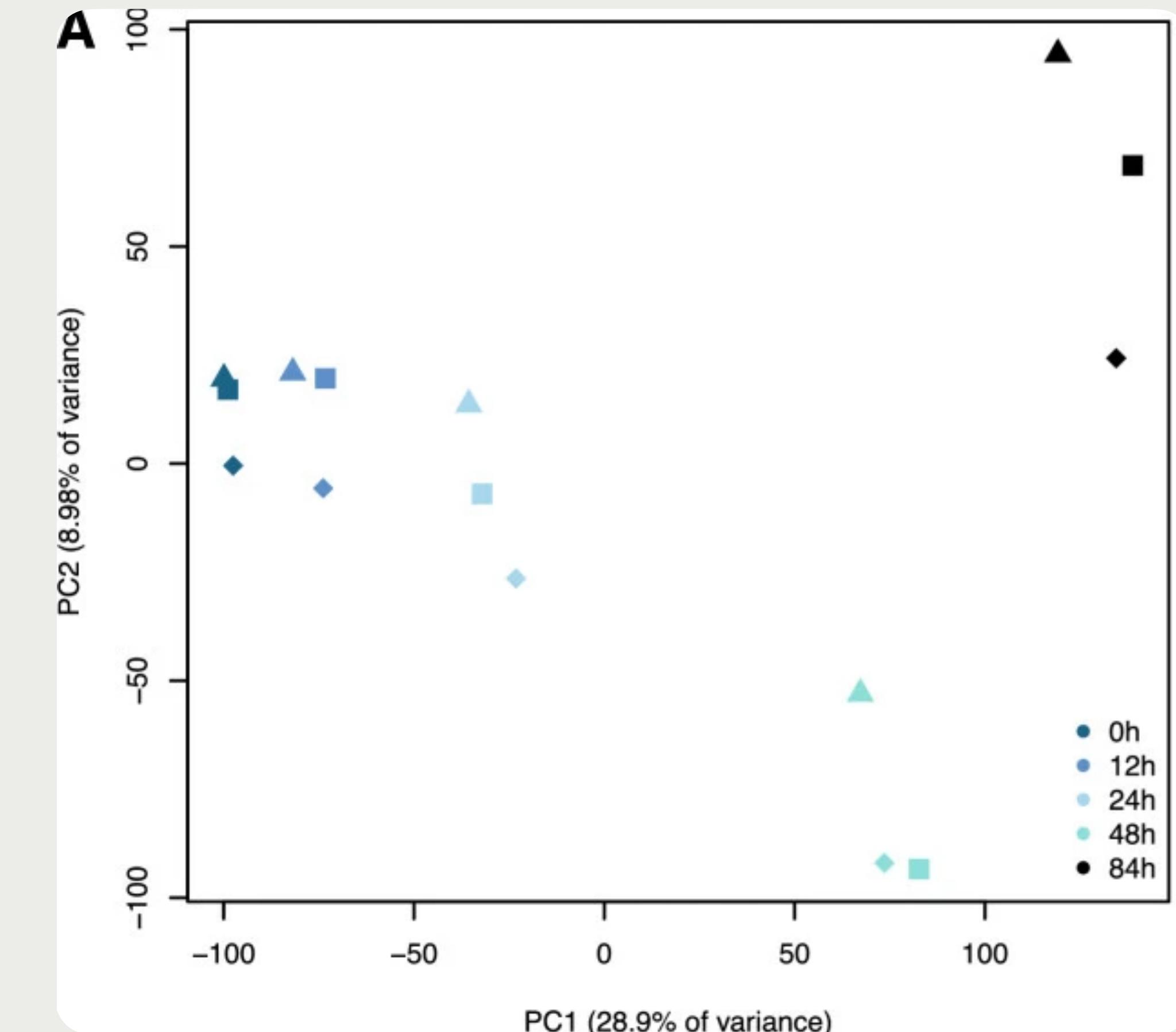
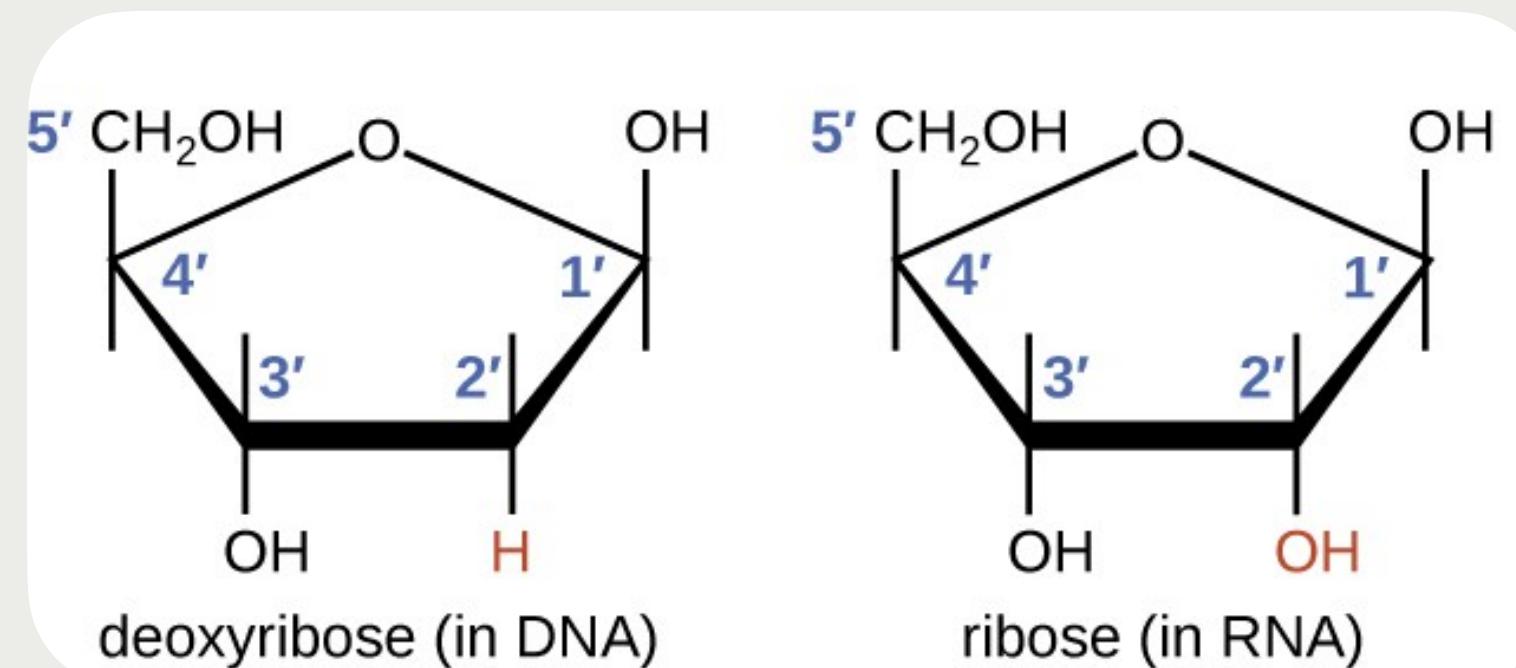
Let's talk about degradation and decay

Maybe the single biggest enemy to good RNA-seq experiments



Sources of degradation:

1. Time
2. Heat
- 3. Ribonucleases (RNases)**



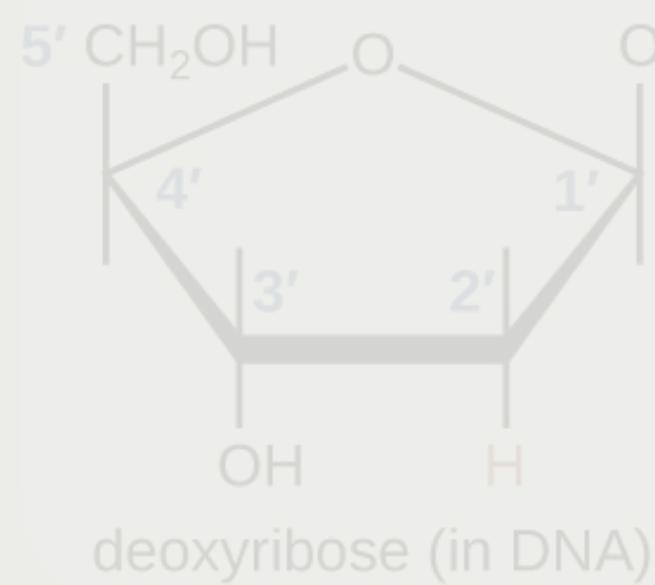
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Maybe the single biggest enemy to good RNA-seq experiments

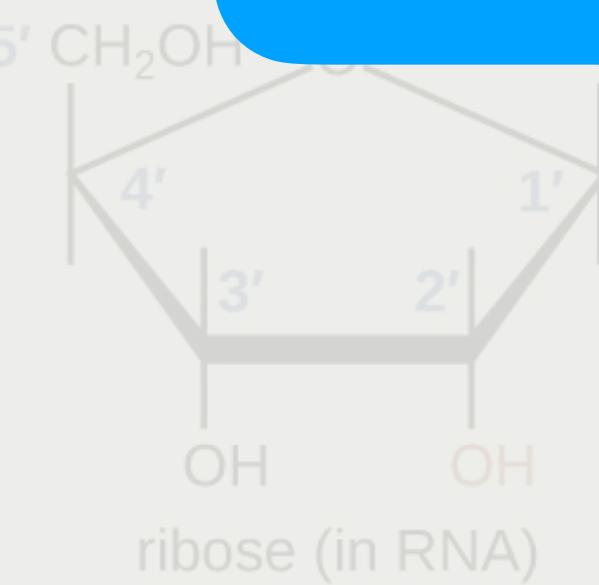


Sources of degradation

1. Time
2. Heat
3. Ribonucleases

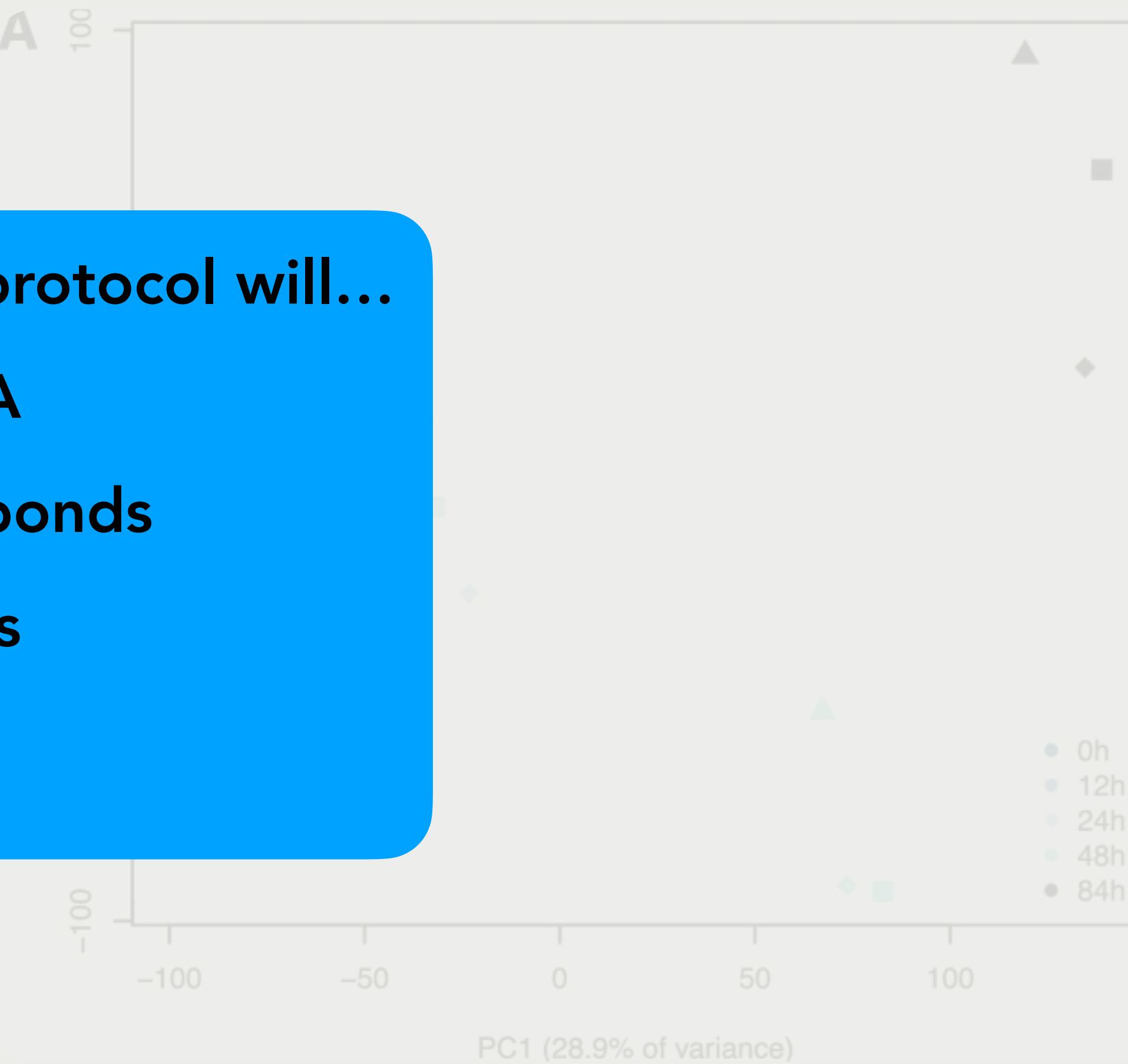


deoxyribose (in DNA)



ribose (in RNA)

- A good extraction protocol will...
- Not degrade RNA
 - Break hydrogen bonds
 - Denature proteins
 - Inhibit RNases



RNA extraction requirements

Maintain RNA integrity while separating it from proteins

Recall phenol-chloroform extraction of DNA:

1. Lyse and homogenize
2. Deproteinize
3. Precipitate

RNA extraction requirements

Maintain RNA integrity while separating it from proteins

Acid guanidinium thiocyanate-phenol ("Trizol") chloroform extraction of RNA:

1. Lyse and homogenize
2. Deproteinize
3. Precipitate

Key differences:

- N-Laurylsarcosine - detergent that is soluble along with guanidinium (unlike SDS)
- Guanidinium thiocyanate - inactivates RNases
- Beta-mercaptoethanol - kills RNases
- Lysis, homogenization, and deproteinization happens in the same solution

Key similarities:

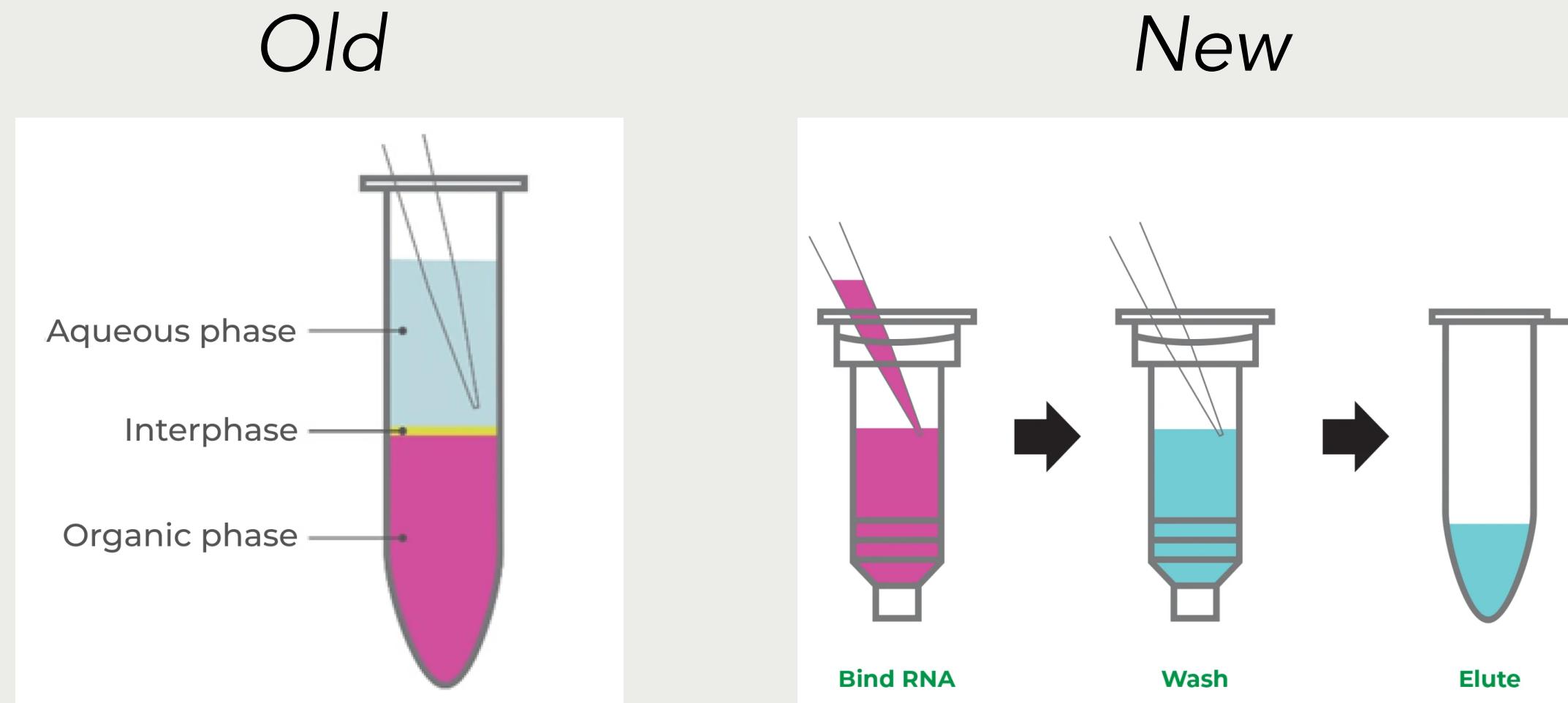
- Phenol as the deproteinizer
- Sodium acetate as the precipitator

RNA extraction updates

Silica-column binding instead of phase separation

Acid guanidinium thiocyanate-phenol ("Trizol") chloroform extraction of RNA:

1. Lyse and homogenize
2. Deproteinate using a silica-column
3. Elute



Direct Method (Direct-zol™)

7 Minutes

- Lyse Sample ≥ 1 min.
- Bind to Spin Column 1 min.
- Washes 3 min.
- Elute 1 min.

Non-Biased, Total RNA

- ✓ No Phase Separation
- ✓ NGS-Grade
- ✓ Small RNA Recovery
- ✓ DNA-free

Phenol-Chloroform Method with Phase Separation

≥ 100 Minutes

- Lyse Sample ≥ 1 min.
- Add Chloroform
- Centrifugation
- Transfer Aqueous Phase
- Isopropanol Precipitation
- Ethanol Washes ≥ 15 min.
- Air Dry 15 min.
- Resuspend RNA Pellet 5 min.

Biased RNA (miRNA loss)

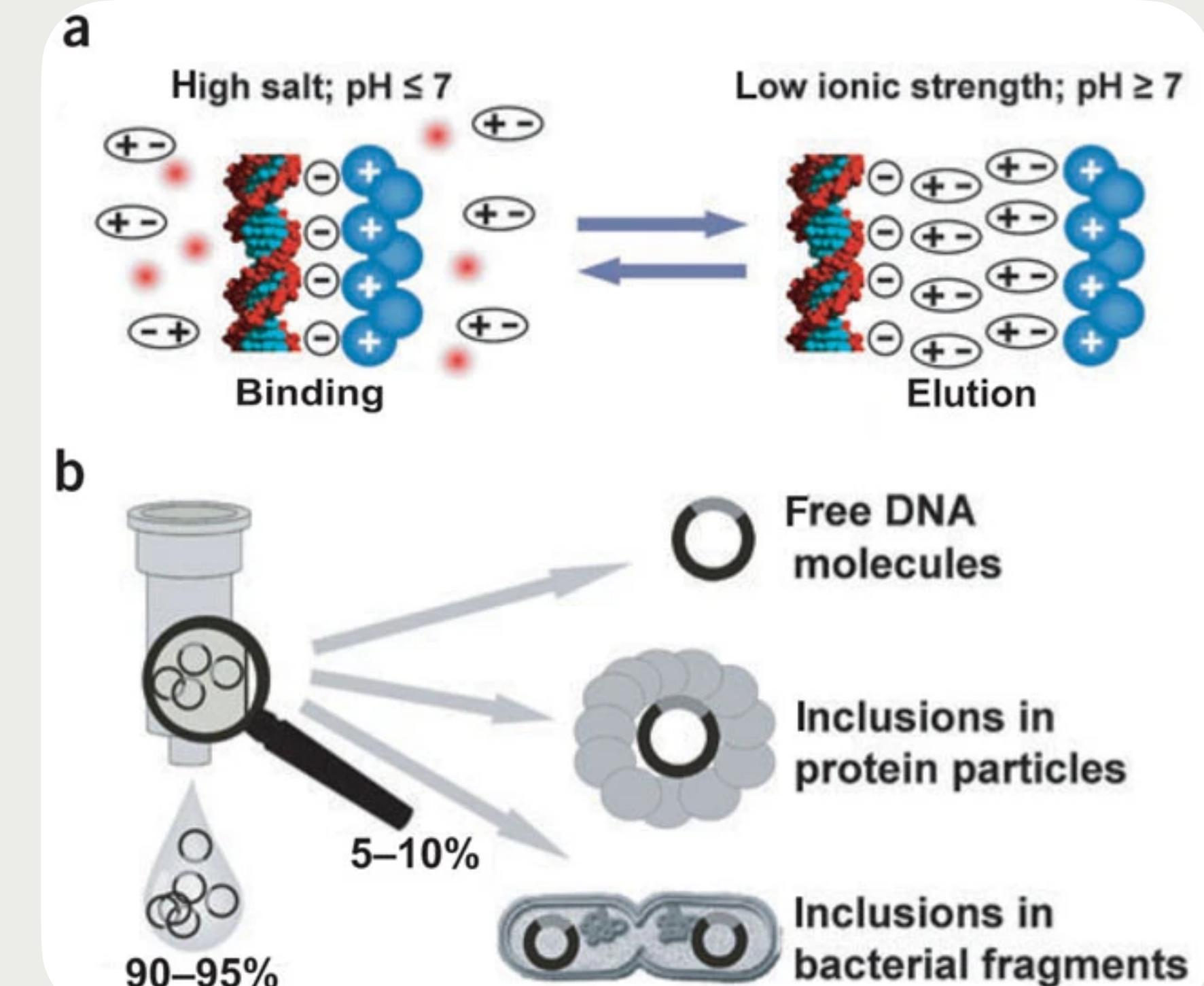
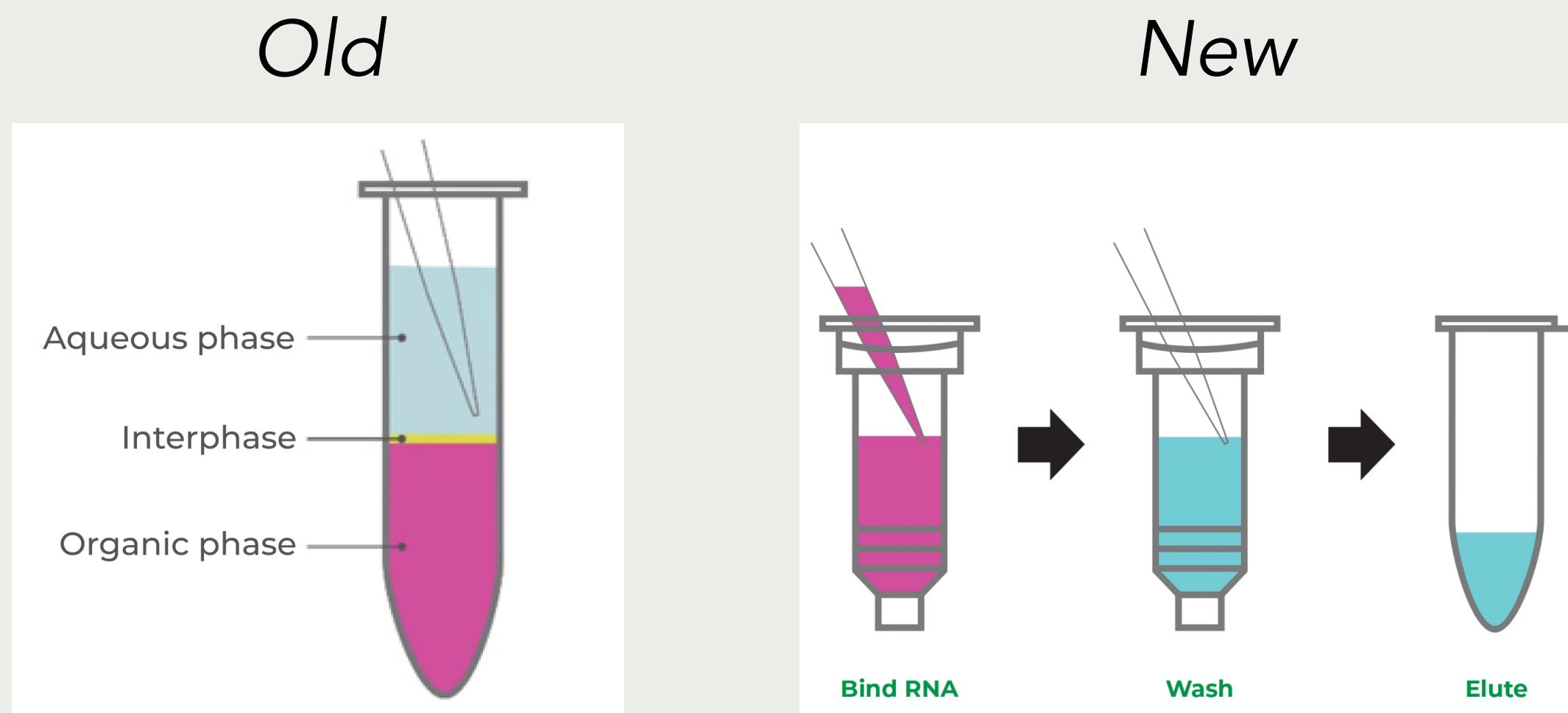
- ✗ Laborious Process
- ✗ Not NGS-Grade
- ✗ Small/microRNA Loss
- ✗ Risk of DNA Contamination

RNA extraction updates

Silica-column binding instead of phase separation

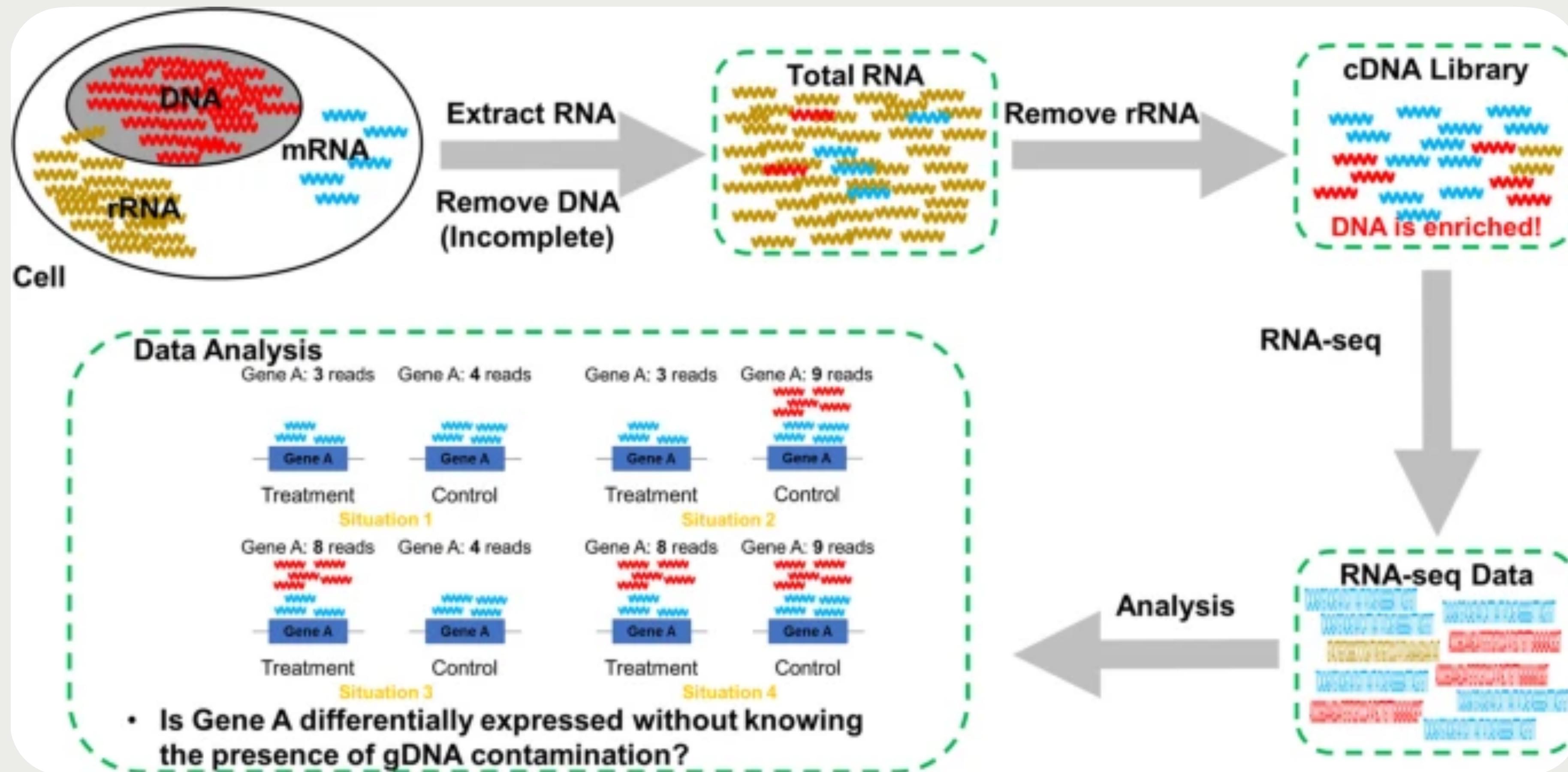
Acid guanidinium thiocyanate-phenol ("Trizol") chloroform extraction of RNA:

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Post-extraction cleanup

No extraction is perfect



Post-extraction cleanup

No extraction is perfect

DNA contamination

Caused by imperfect separation
of RNA/DNA

Wastes reads during sequencing

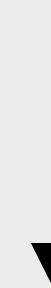
Can cause mistakes in DEG
identification

rRNA contamination

Much more cellular rRNA than
mRNA

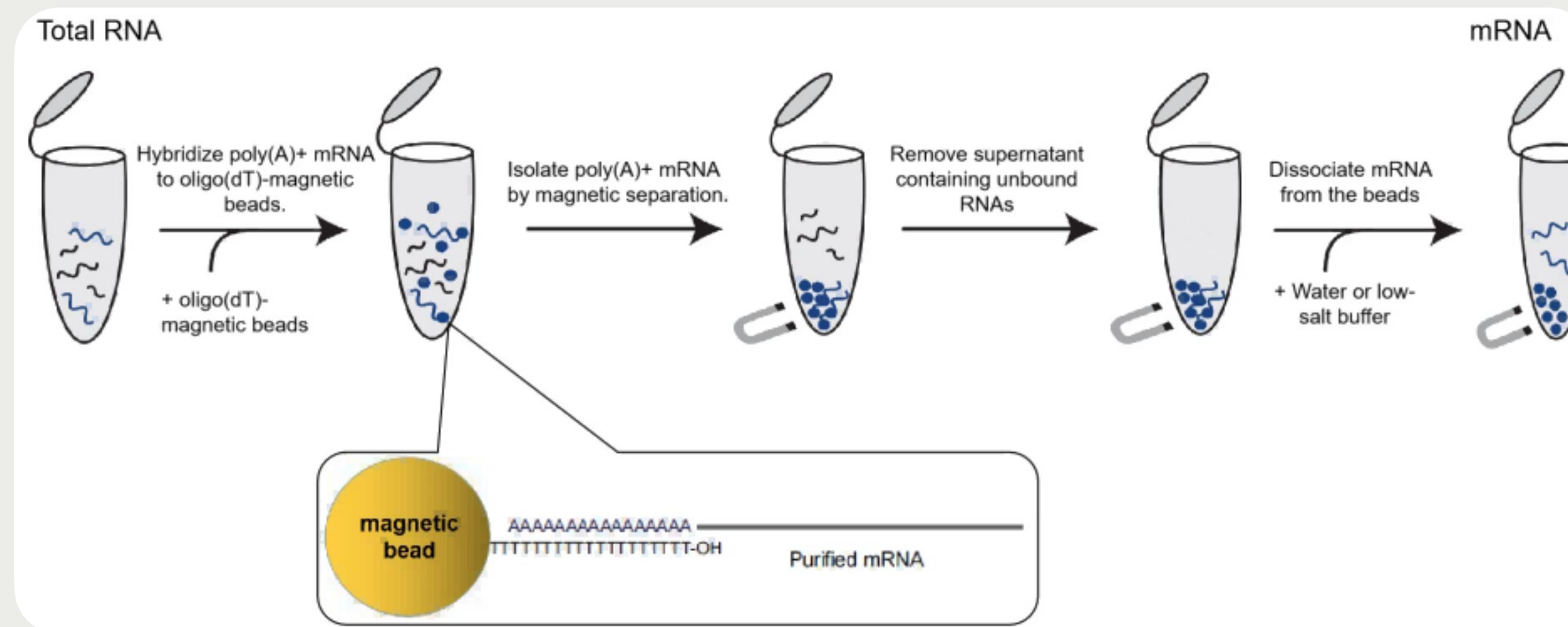
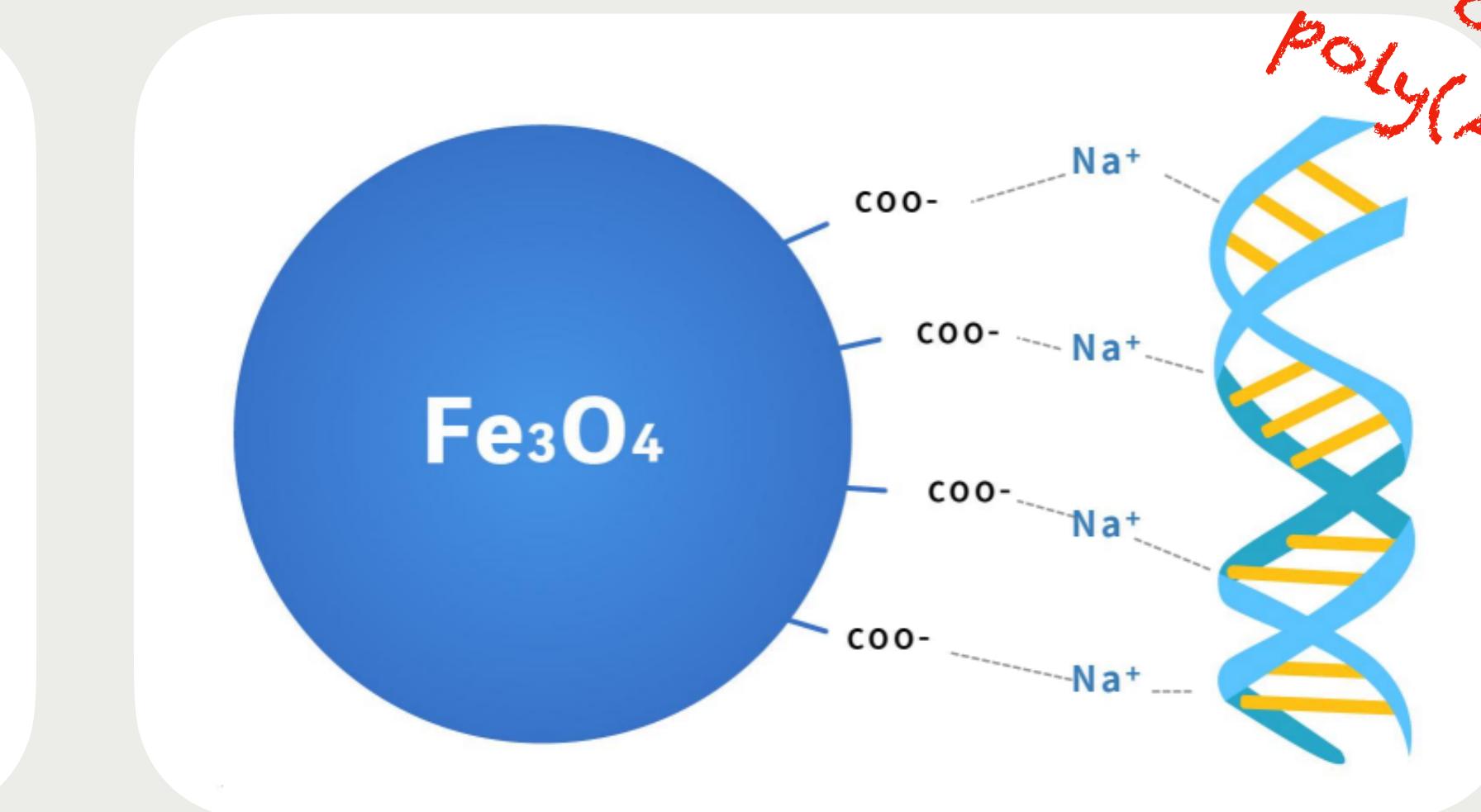
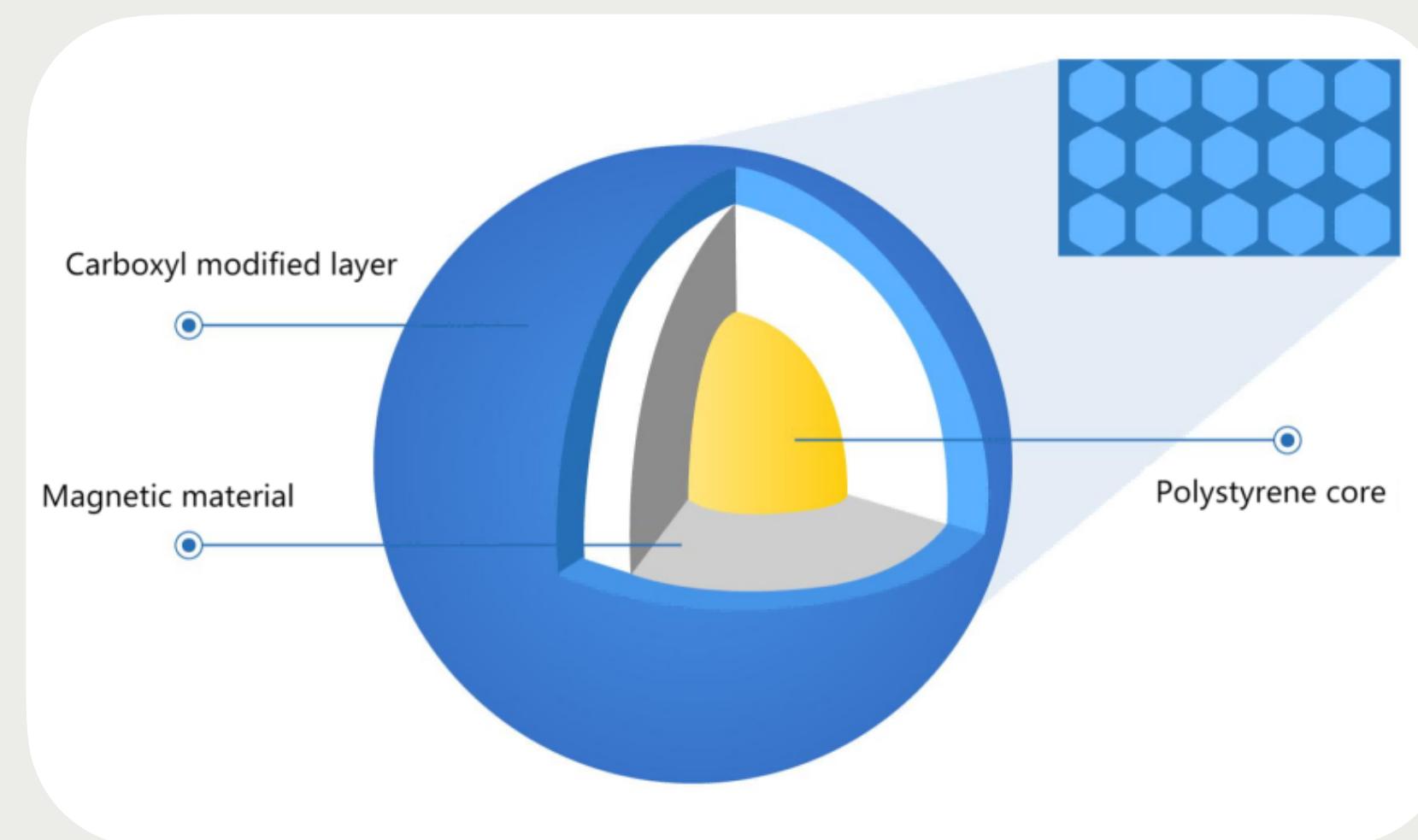
Wastes many reads during
sequencing (up to 90%)

Can make DEGs impossible to
identify



Two methods to mRNA enrichment

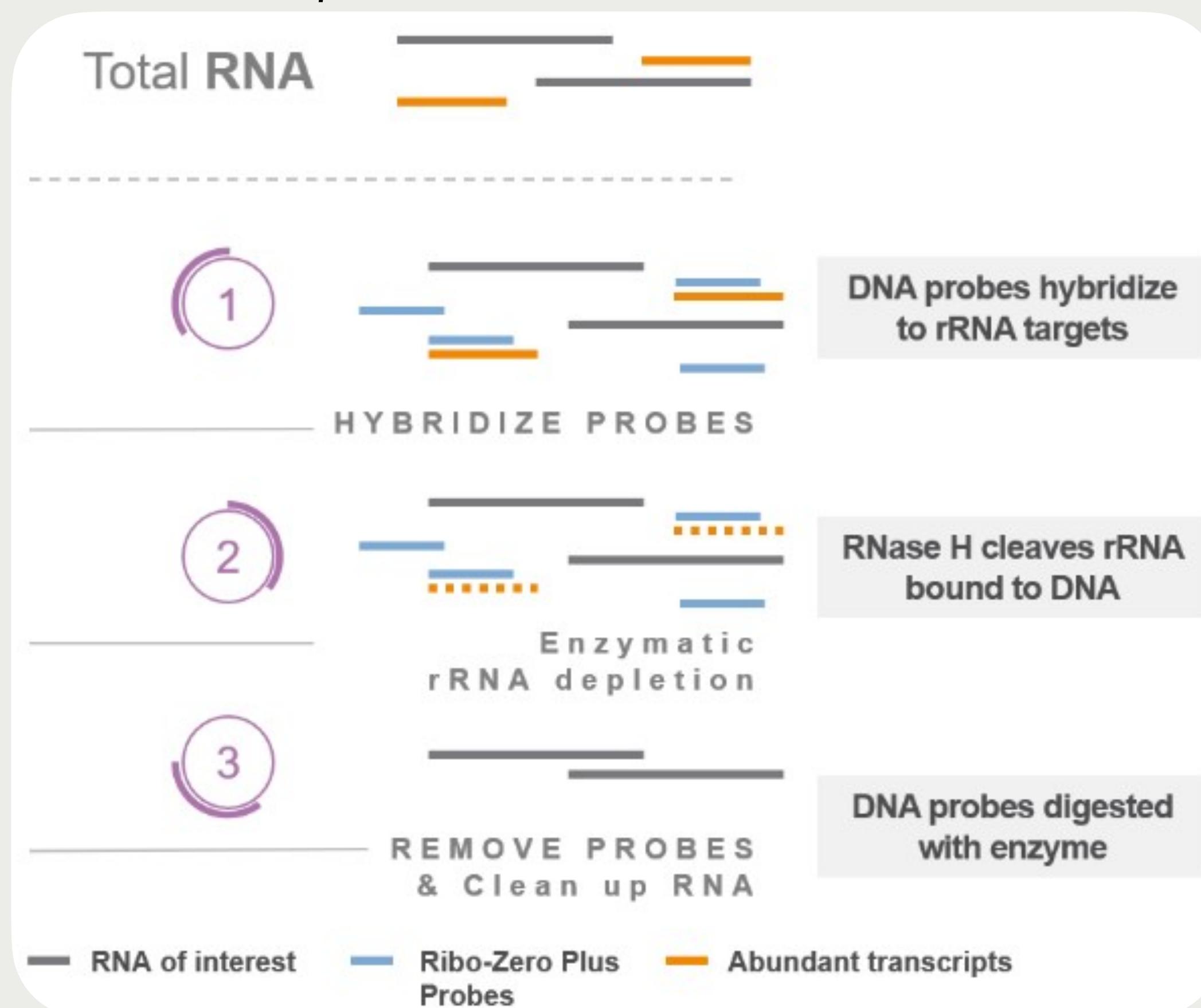
Poly(A) selection or rRNA depletion



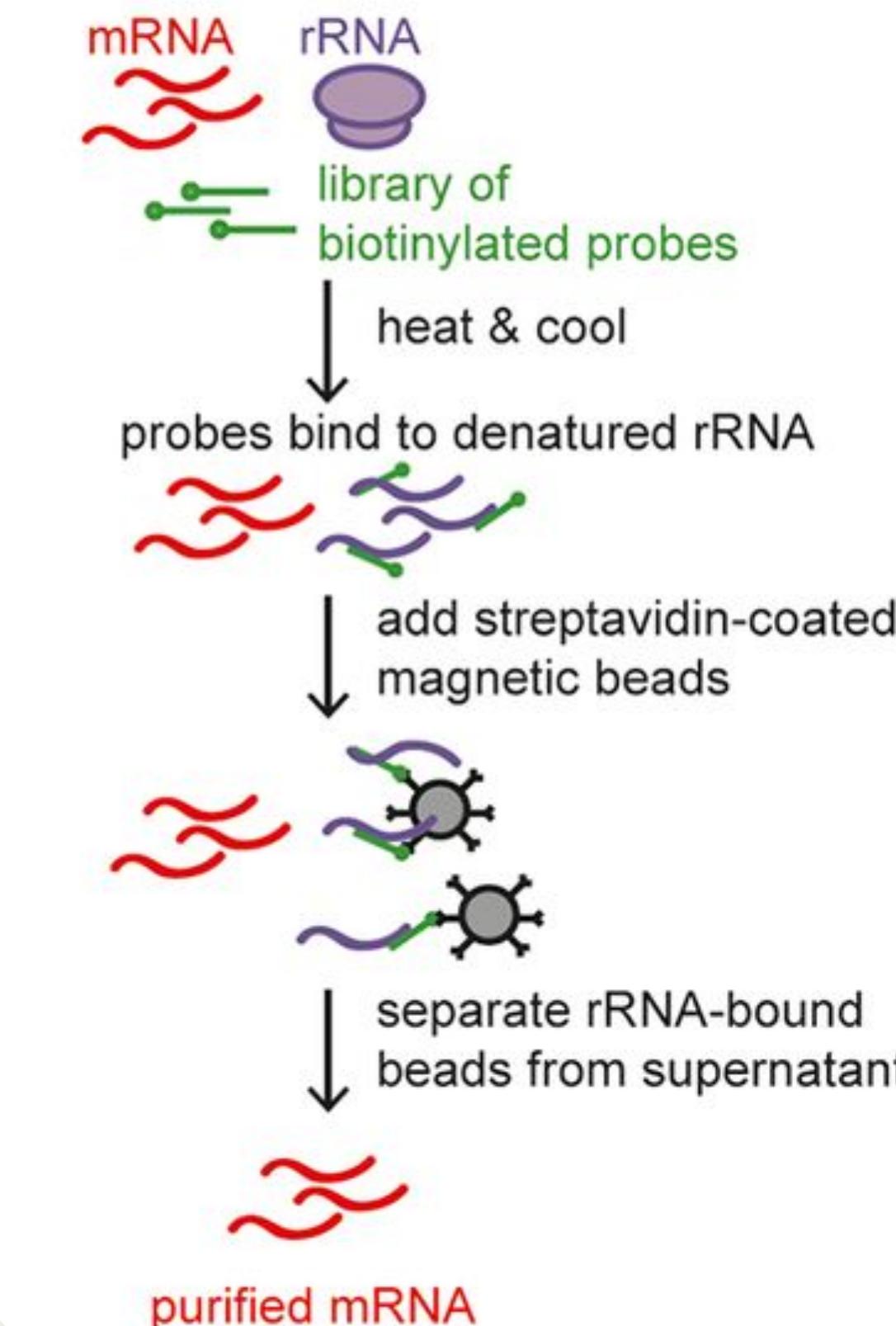
Two methods to mRNA enrichment

Poly(A) selection or rRNA depletion

Expensive (and better) way



DIY way

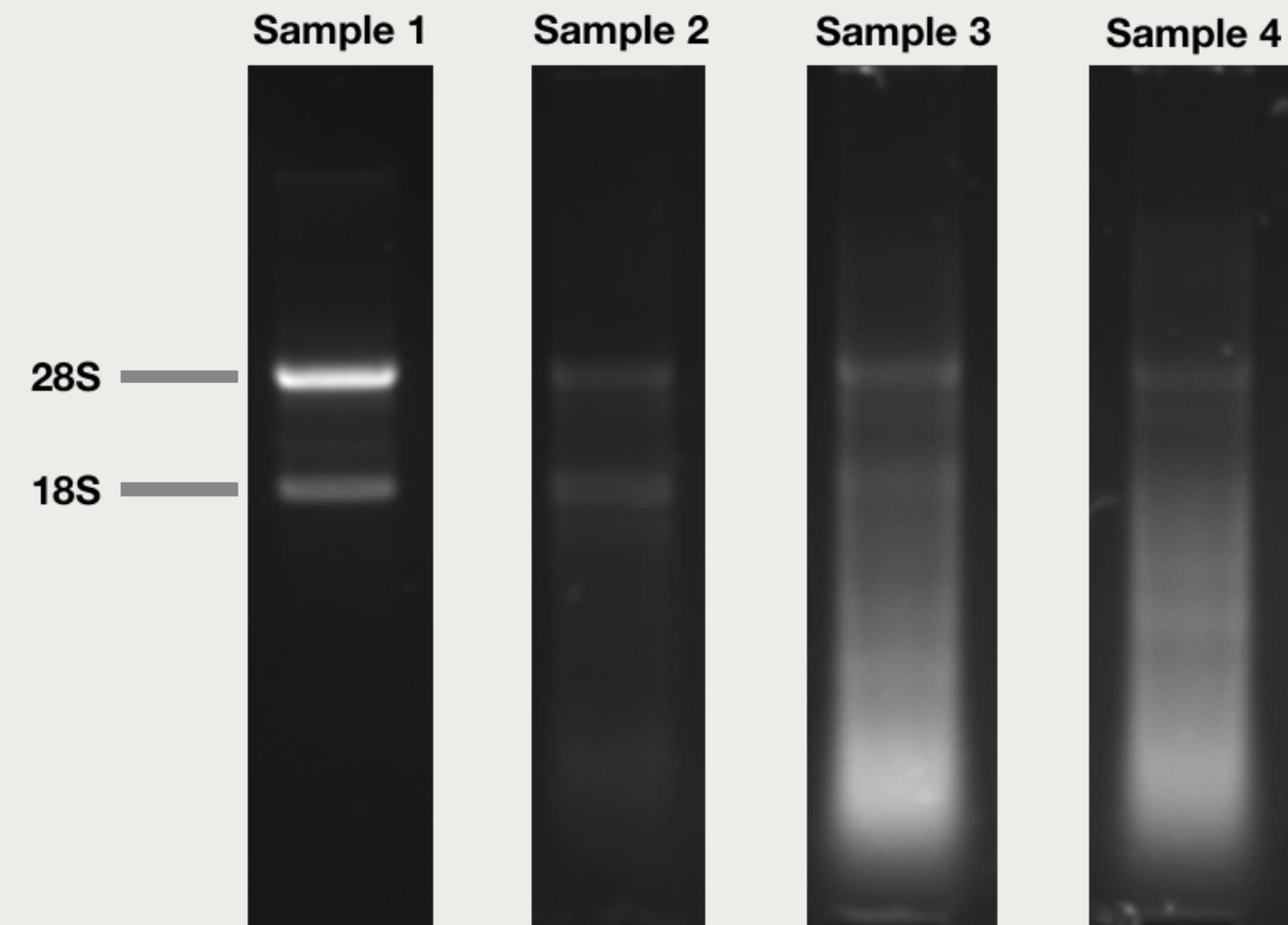


Putting it all together...

How do we know we have a RNA prep worth sequencing?

RNA quality control:

- RNA quality
 - Use rRNA quantity/ratio as proxy for mRNA degradation prior to selection/depletion
- Contamination (gDNA and rRNA)
 - Automated electrophoresis

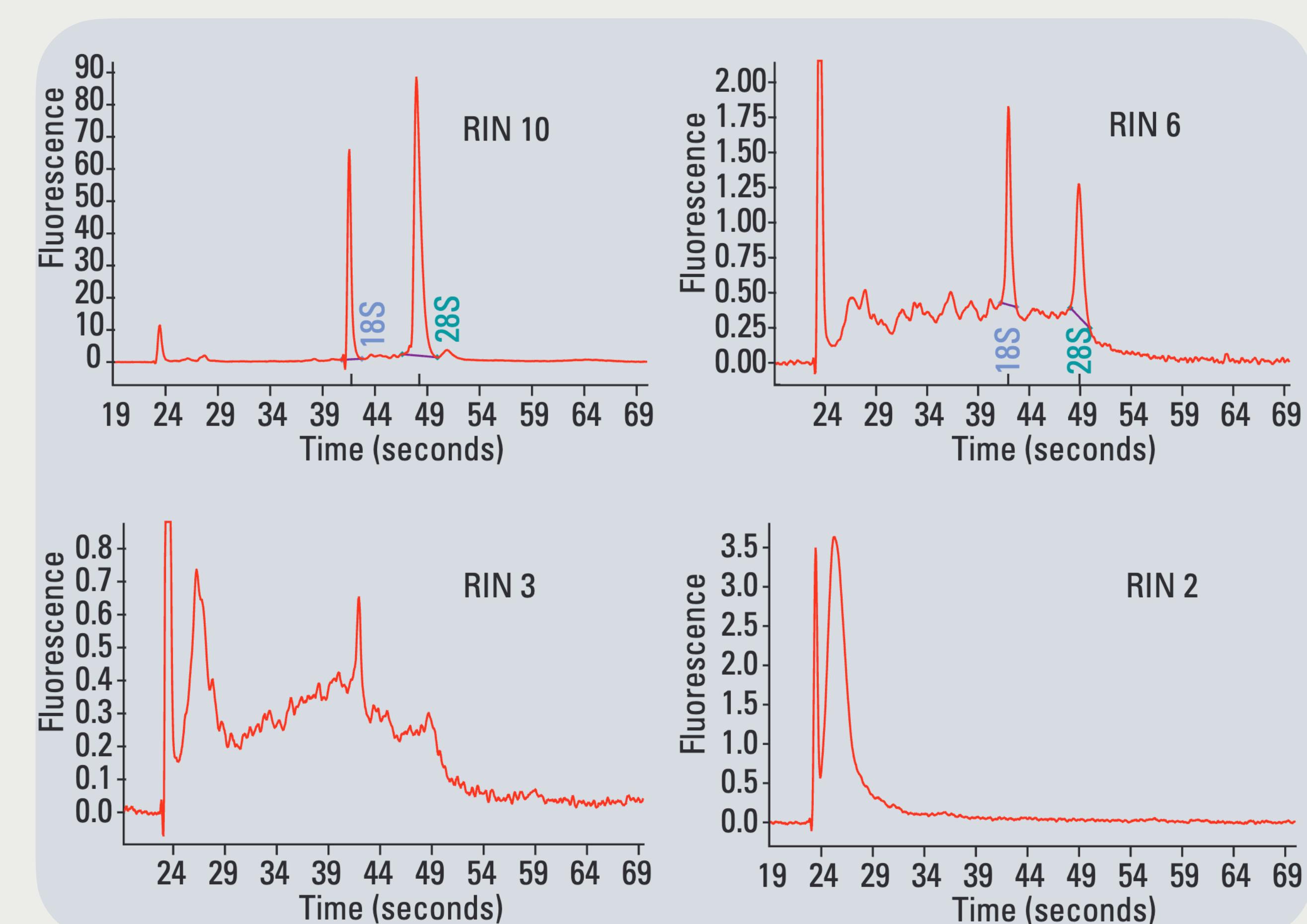


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