School of Engineering
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Dual dynamic staining electrophoretic detection and characterization of dsRNA contaminants in mRNA vaccines

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Motivation – Analytical Landscape

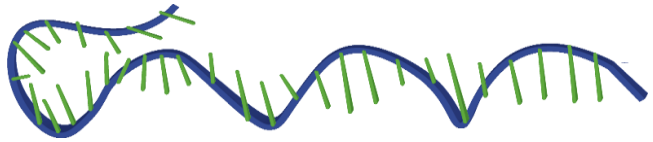
mRNA vaccines are at the forefront of the vaccine industry

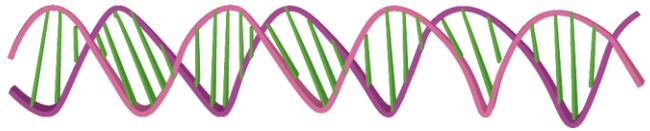
- Safety and efficacy
- Shortened time between pathogen discovery and vaccine development

Problem: the *in vitro* transcription (IVT) of mRNA vaccines results in several by-products, and despite purification efforts, small concentrations of dsRNA can remain

- dsRNA poses a length-dependent risk to humans

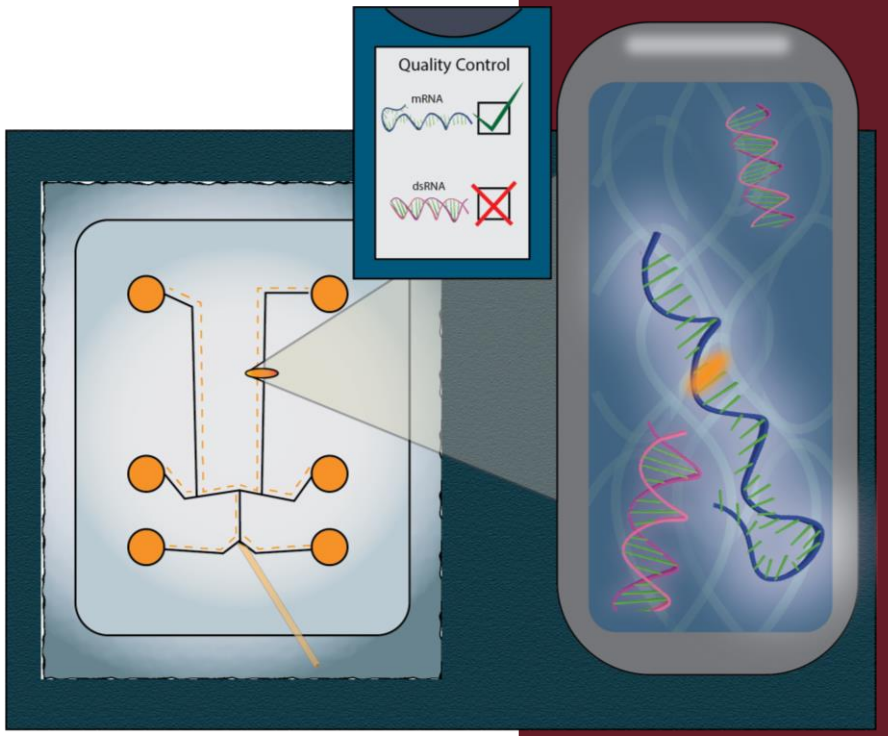
Current Analytical Technique





Method	Sensitivity	Resolution	Turnaround time	Throughput	Sample requirements
ELISA/Dot blot	Highest (pg/µL)	**n/a**	2-4 h/plate	High	Lowest

Goal: Develop a microfluidic electrophoresis **high throughput** and **high resolution** analytical method for the detection and characterization of dsRNA contaminants in mRNA vaccines.



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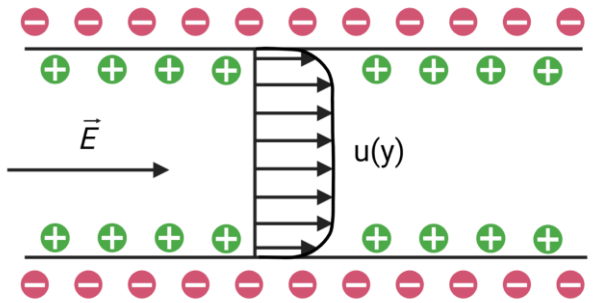
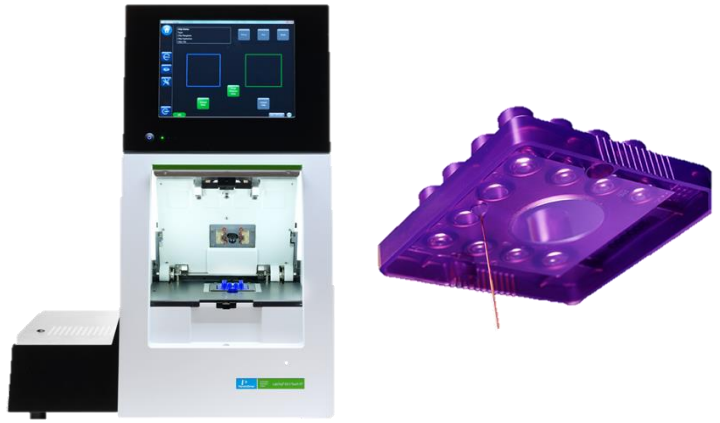
Analytical Platform

- Microfluidics: systems that manipulate small amounts of fluids (10^{-9} to 10^{-18} L)
- Usually coupled with a driving force to generate movement, here electric fields
- Electric fields were used to generate particle movement due to the ability to generate linear velocity profiles



Benefits



- System = high throughput
- Scale = rapid
- Plug analysis = resolution

Work conducted in collaboration with Revvity

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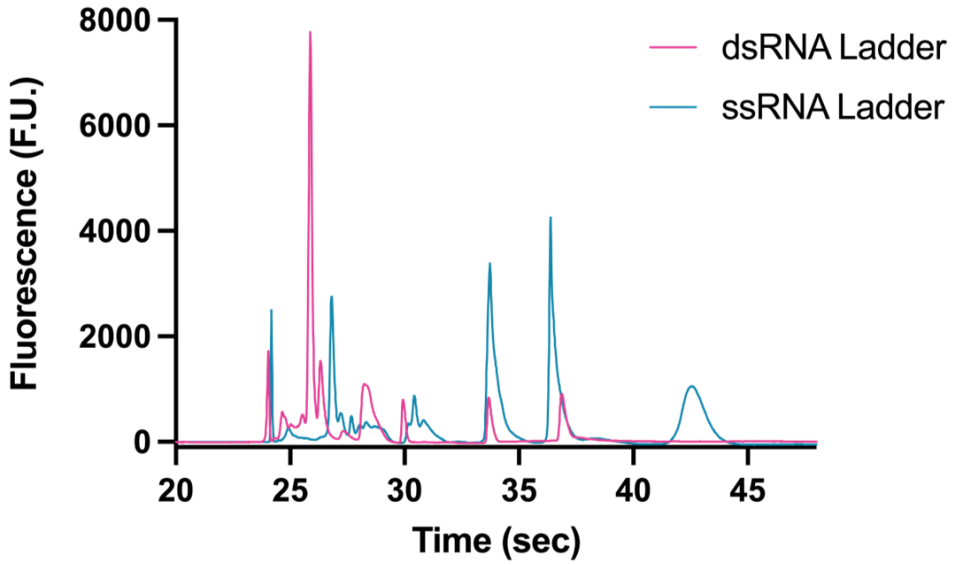
Analysis of dsRNA and ssRNA ladders

A.



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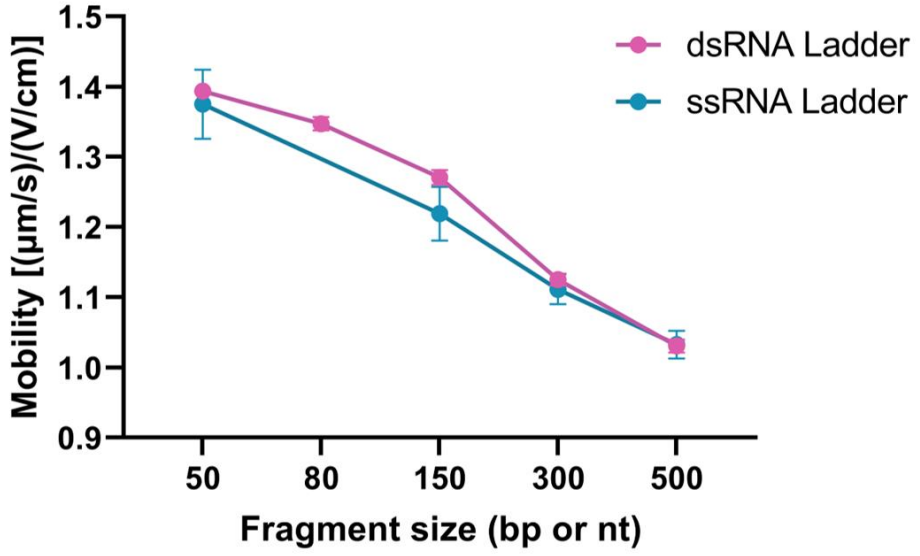
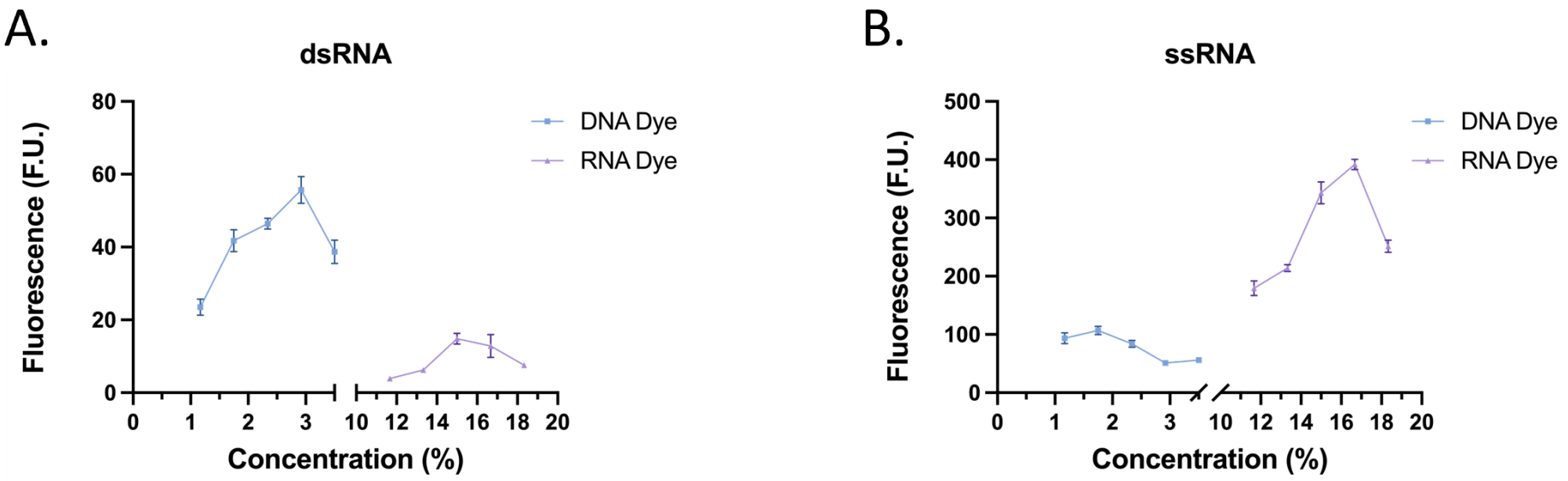


No mobility difference in the 50-500 bp or nt range – cannot differentiate based on mobility

Coll De Peña et al., *Analyst* (2023)

dsRNA and ssRNA Fluorescence Profiles

Dye concentration



Efficiency of each stain is different for dsRNA and ssRNA

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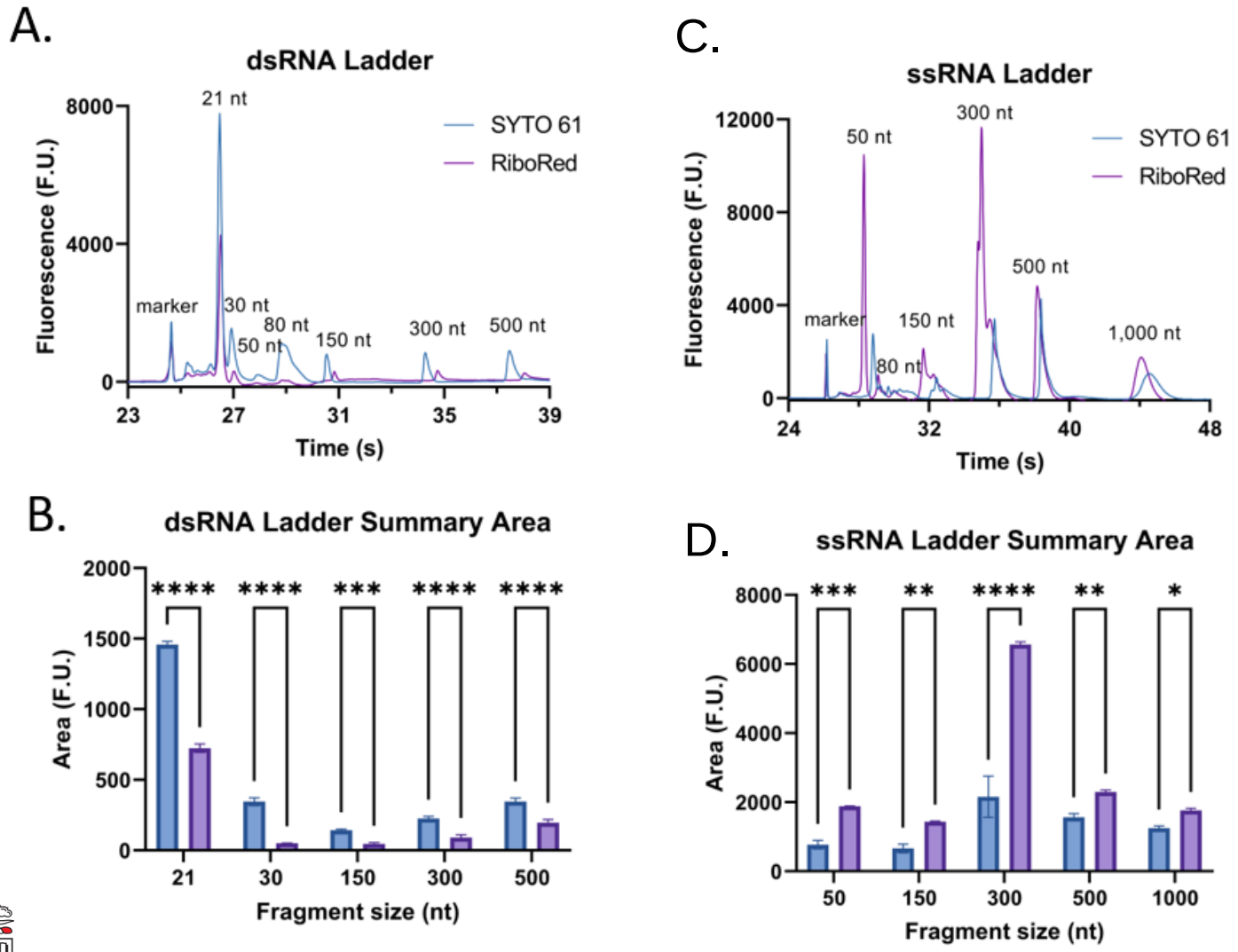
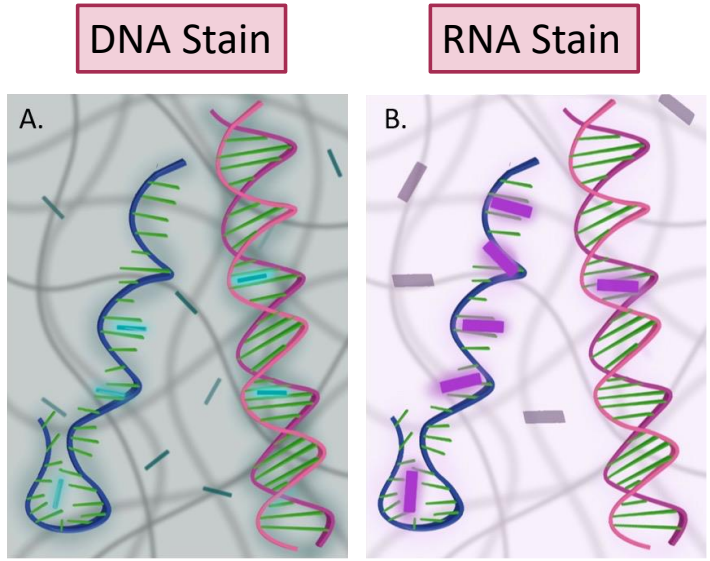
Coll De Peña et al., *Analyst* (2023)

Fluorescent Staining Response





dsRNA: SYTO 61 signal > RiboRed signal
ssRNA: SYTO 61 signal < RiboRed signal

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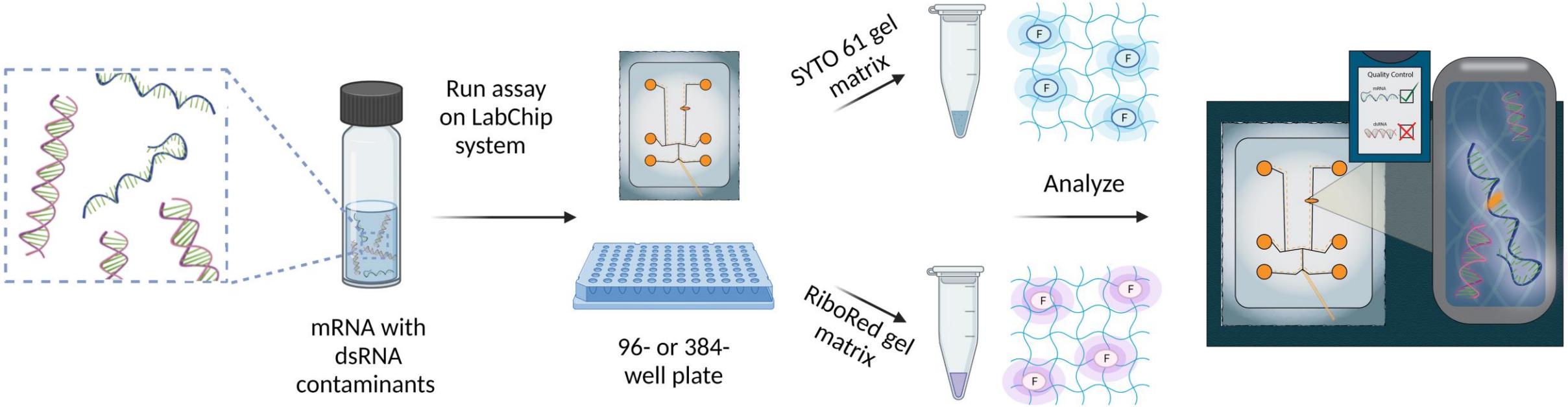
Identification Workflow



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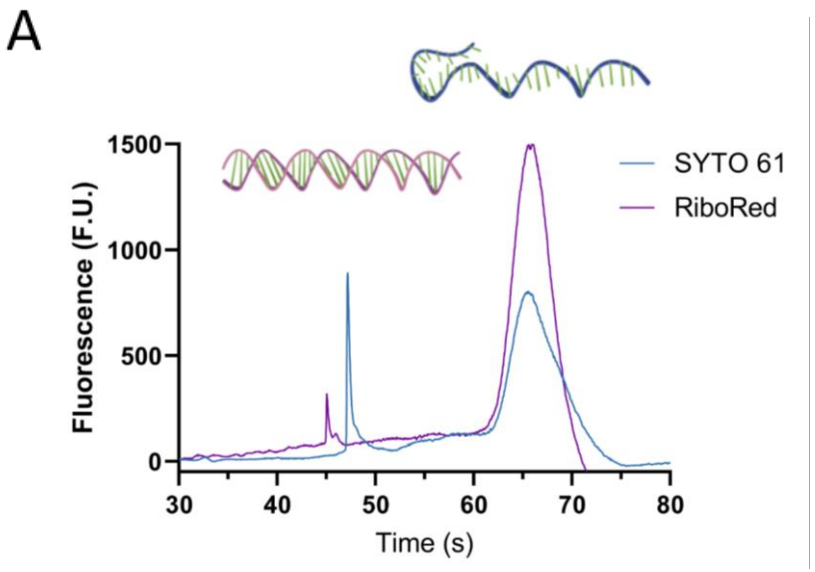
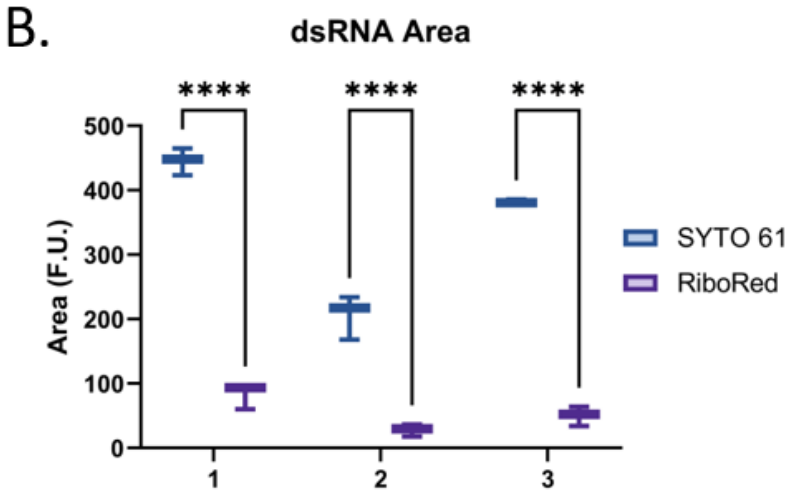
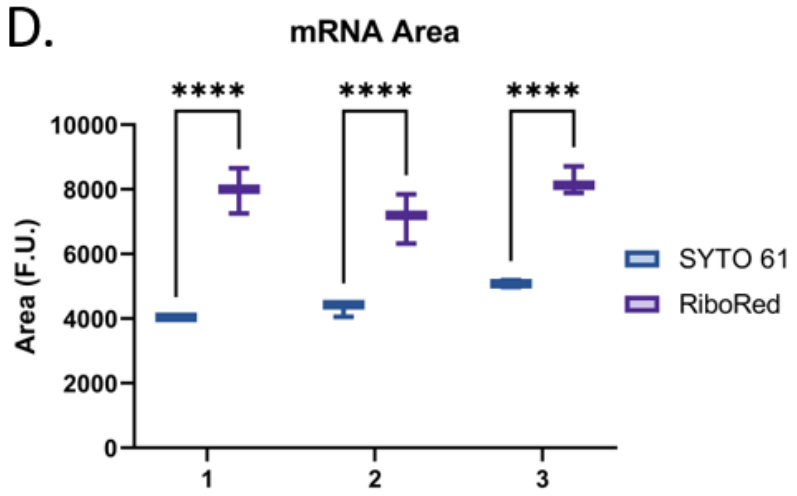
Application: dsRNA and mRNA mixture

- Same trend
- Areas vary







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Peak classification/identification

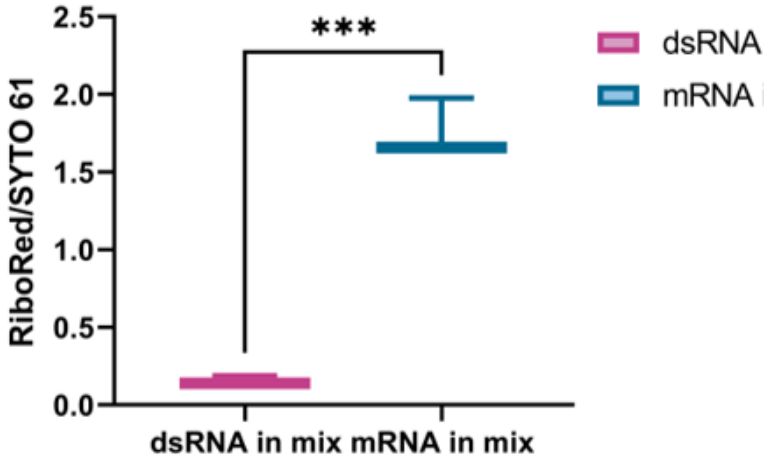
Since the total area can vary, we decided to look at the ratios instead

if:

$$\frac{Peak\ Area_{RiboRed}}{Peak\ Area_{SYTO\ 61}} < 1$$, then the peak producing molecule is dsRNA

Or if:

$$\frac{Peak\ Area_{RiboRed}}{Peak\ Area_{SYTO\ 61}} > 1$$, then the peak producing molecule is mRNA (or ssRNA)



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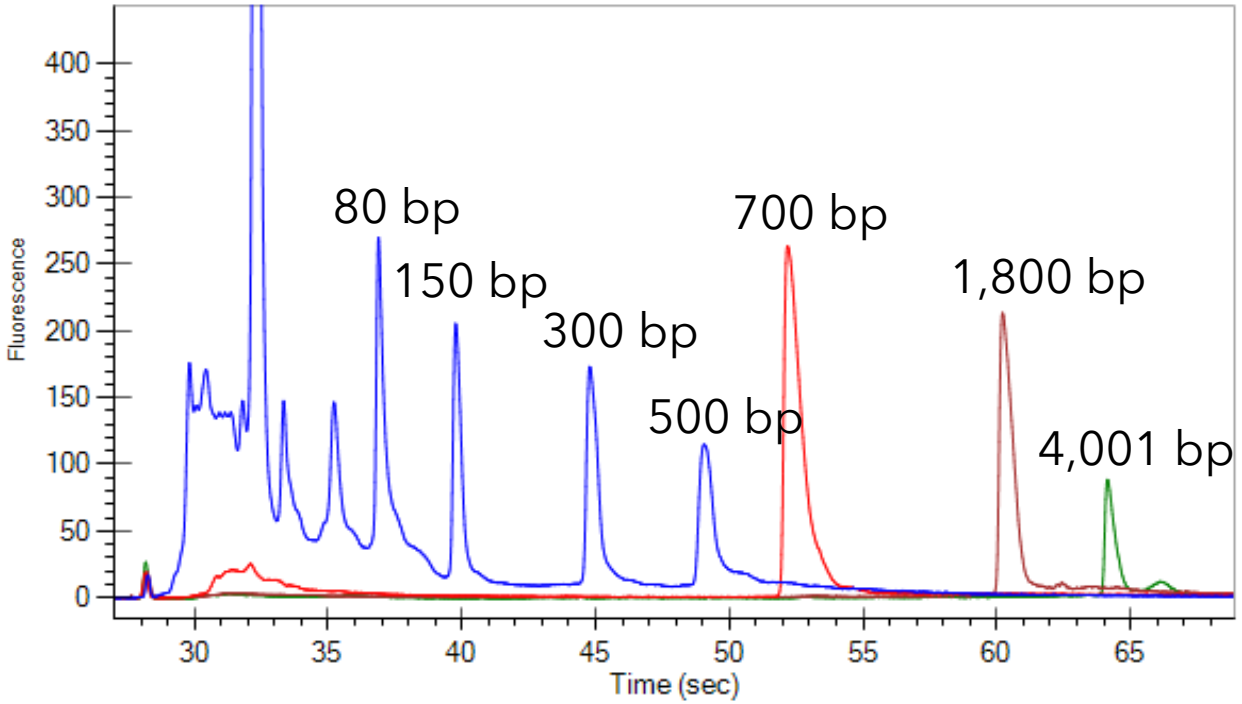
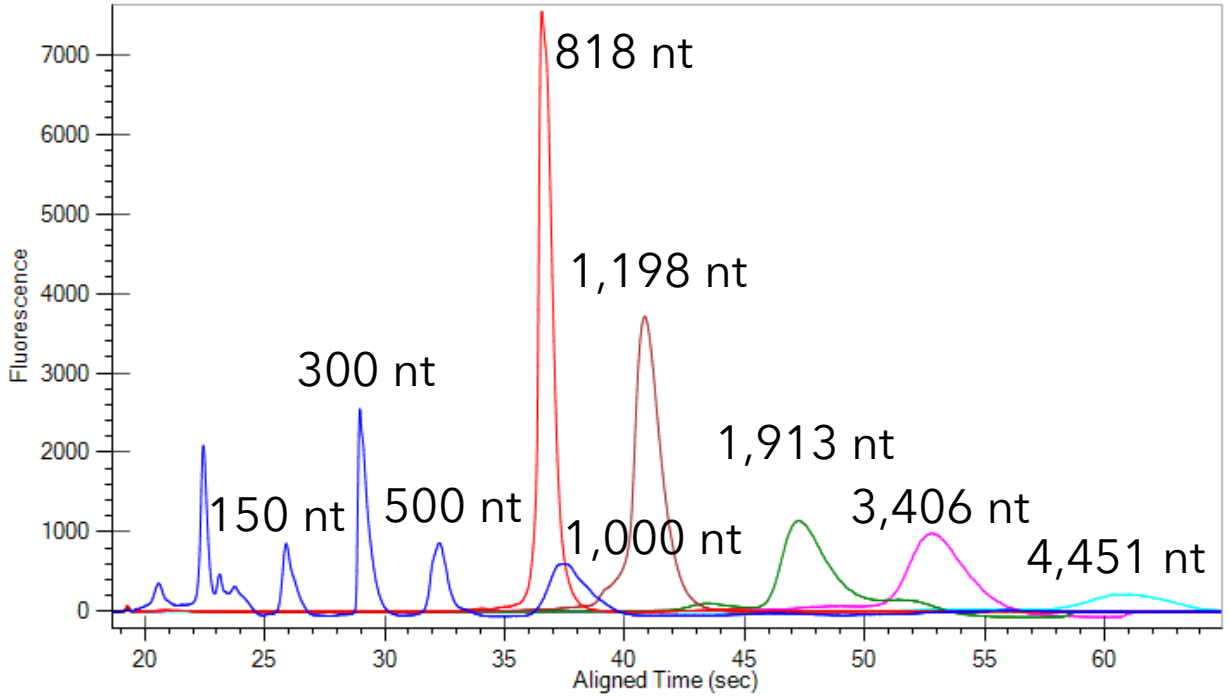
Size Calibration

dsRNA size calibration samples



ssRNA/mRNA size calibration samples



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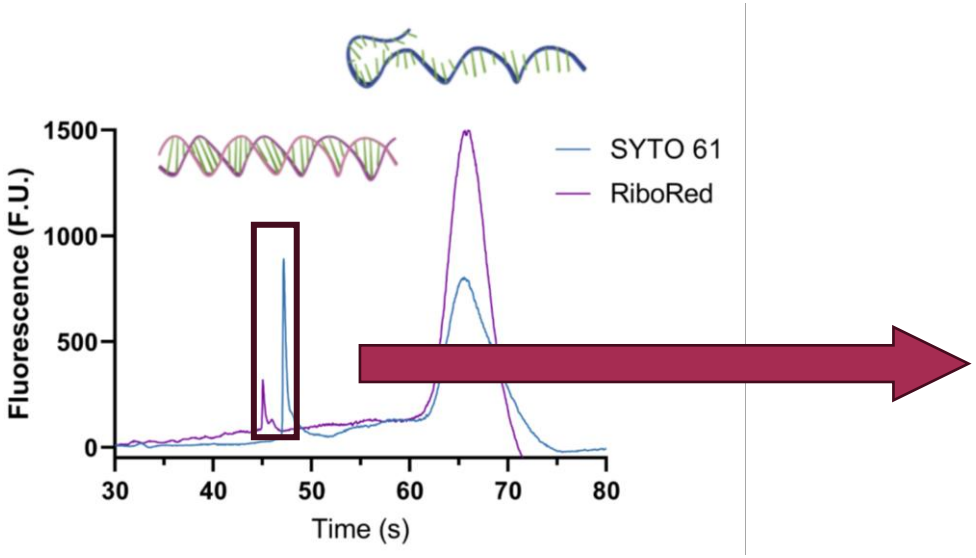
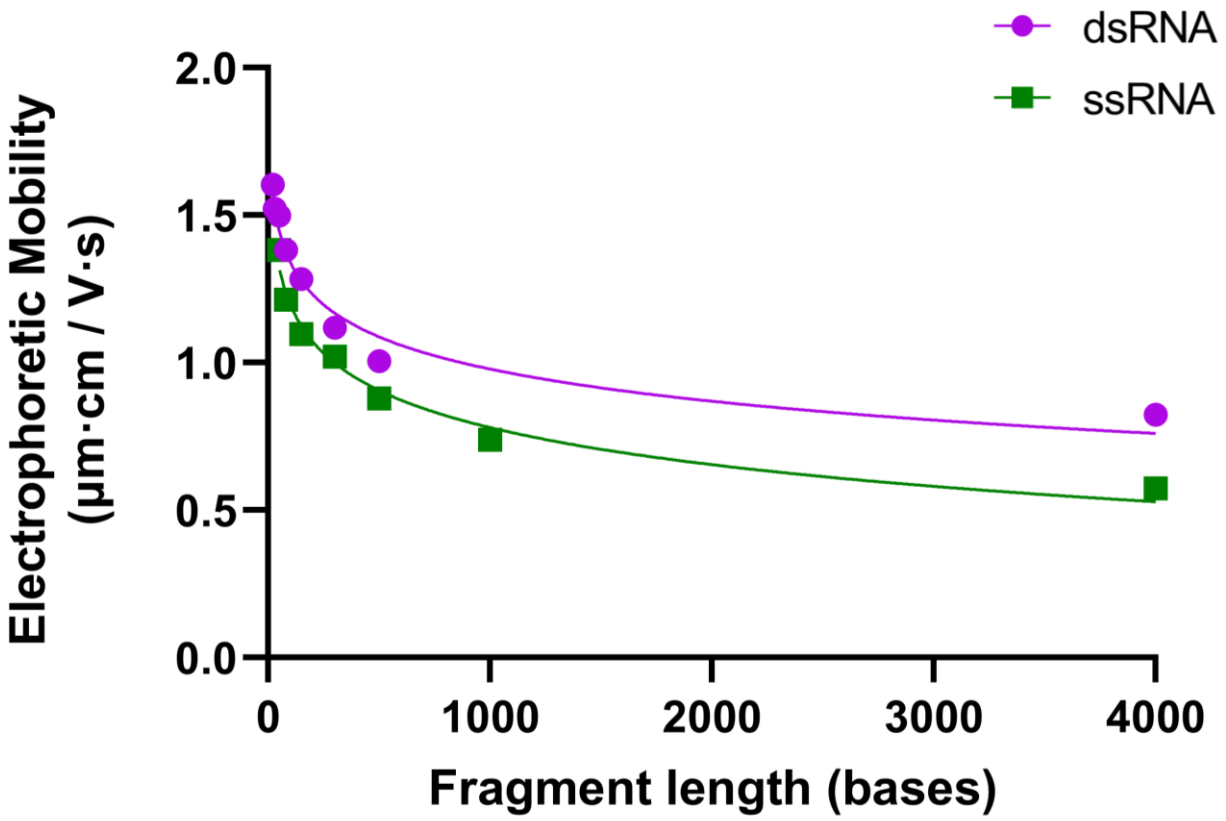
Application: Size determination

Mobility plot generated using calibration samples



Once the sample is identified, its mobility can be used to estimate the size of the contaminant.



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Summary

- dsRNA and mRNA mobilities may be different but cannot be used for differentiation
- Fluorescent staining response can serve to identify molecules prior to size determination
- dsRNA ladder can then be used to estimate contaminant length

Method	Sensitivity	Resolution	Turnaround time	Scalability	Sample requirements
ELISA	Highest	n/a	2-4 h/plate	High	Lowest
Microfluidics	High (relative)	High	2-3 h/plate	High	Low

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Acknowledgments

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Tripathi Lab

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DATA SCIENCE INITIATIVE

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Questions?