Development of a novel luminescent assay for sensitive and specific quantitation of double-stranded RNA

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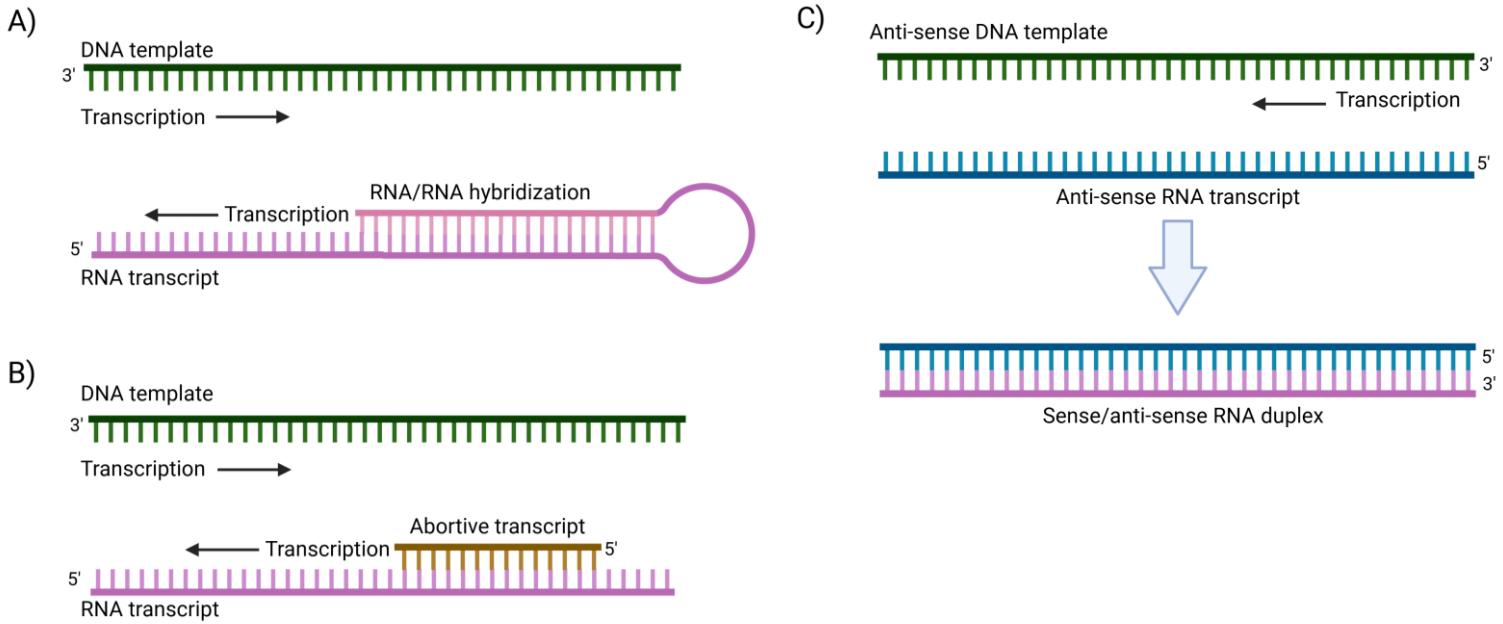
USP mRNA Virtual Summit, 2025

Introduction

- dsRNA is a byproduct and contaminant of in vitro transcription (IVT) products



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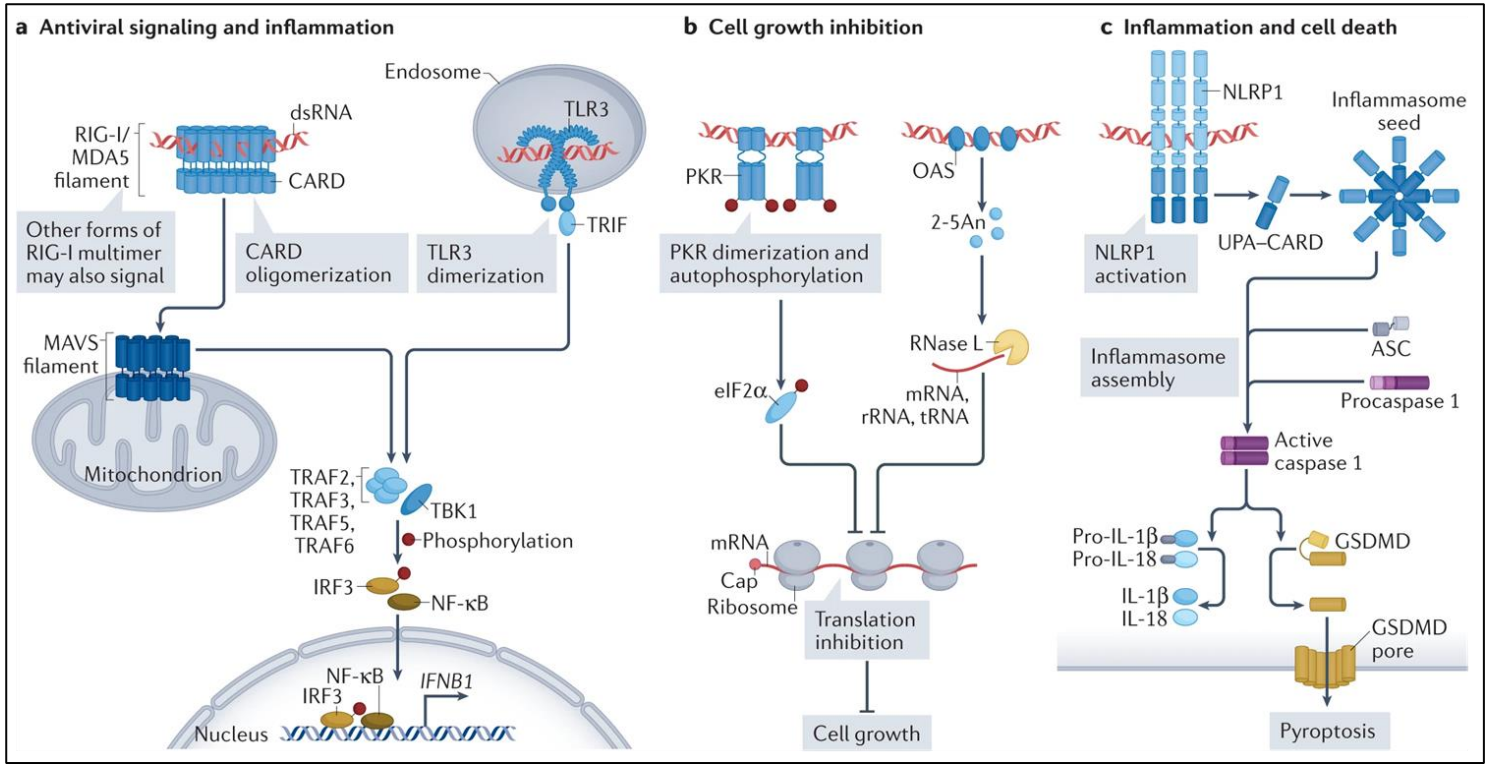
- dsRNA is highly immunogenic and can be detected by several intracellular or endosomal sensors, leading to inflammation, translation inhibition, and cell death



Chen & Hur, Nat Rev MCB, 2022

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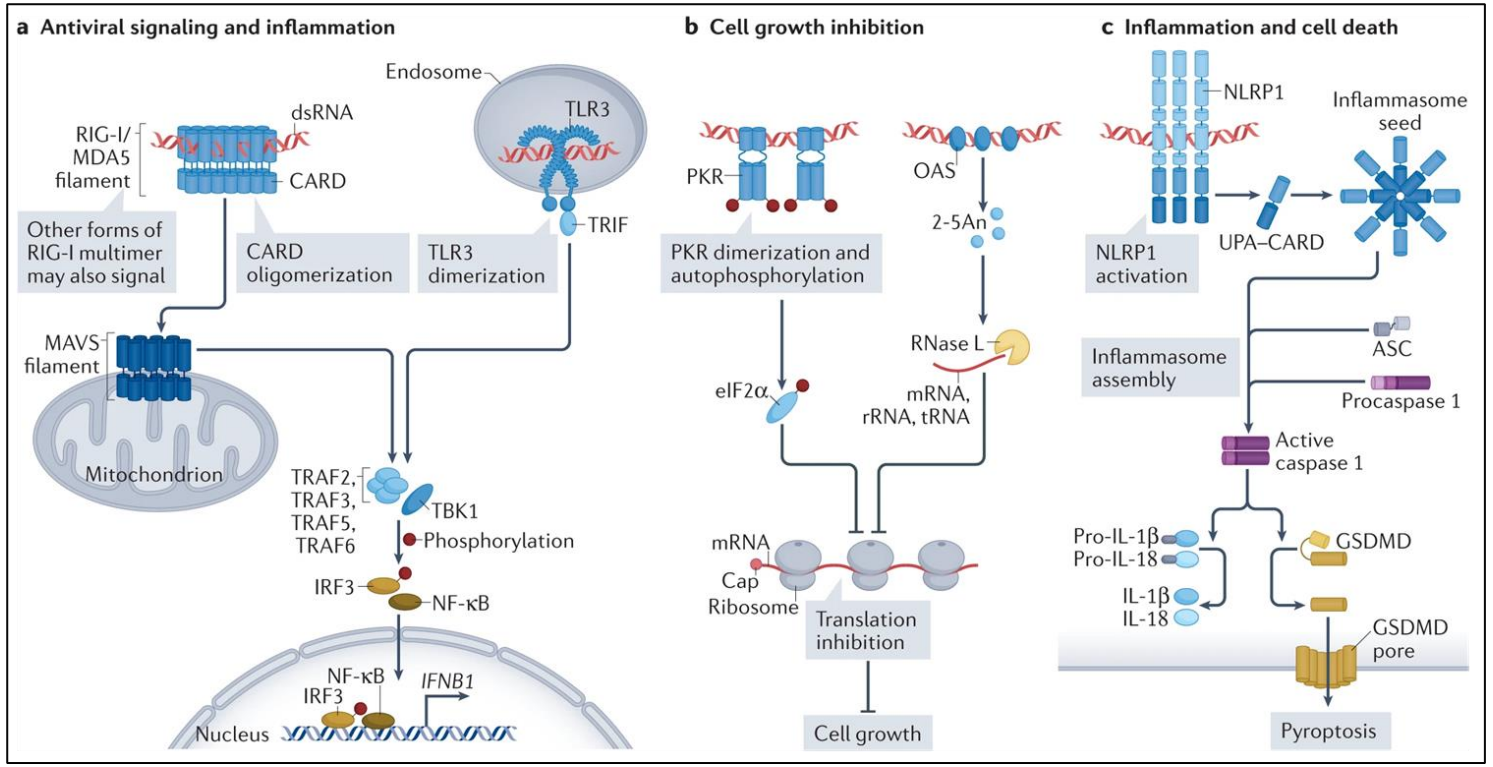
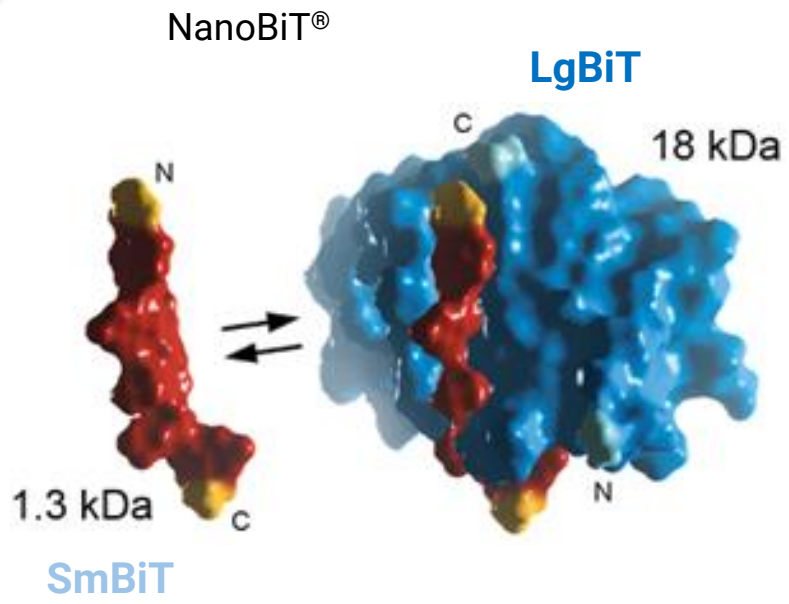


Chen & Hur, Nat Rev MCB, 2022

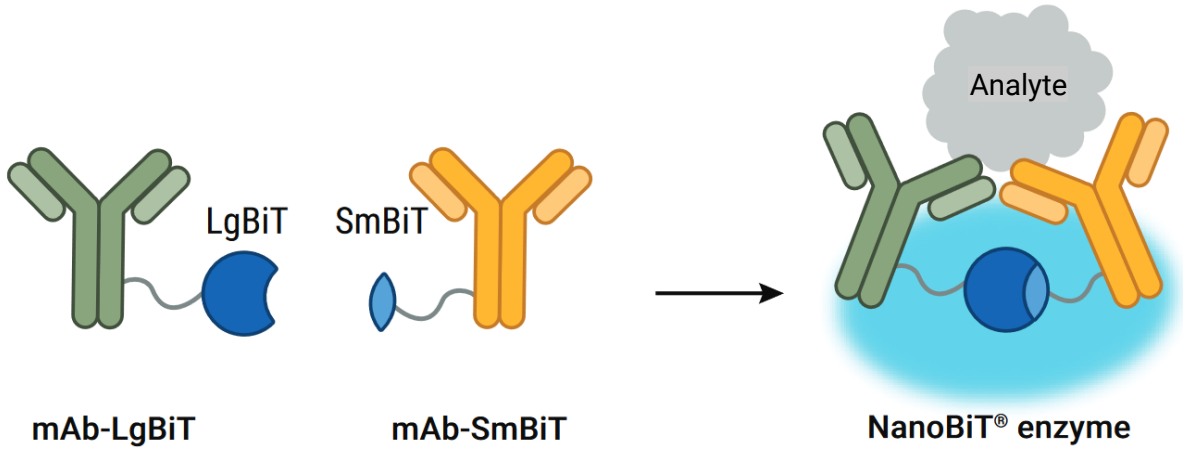
- Existing methods to detect dsRNA in mixed solutions lack quantitation and sensitivity
- **We have developed a novel assay system for dsRNA detection using bioluminescence**
  - dsRNA detection using NanoBiT® technology

dsRNA detection using Nanoluciferase Binary Technology (NanoBiT®)

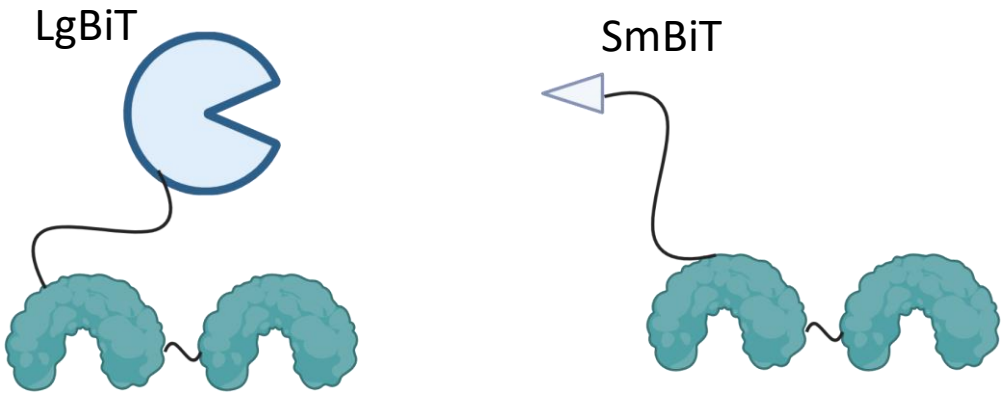


- Nanoluciferase is split into two subunits: SmBiT and LgBiT
- Complementation of SmBiT and LgBiT generates a functional luciferase and the generation of light in the presence of substrate
- SmBiT and LgBiT have very low affinity in solution

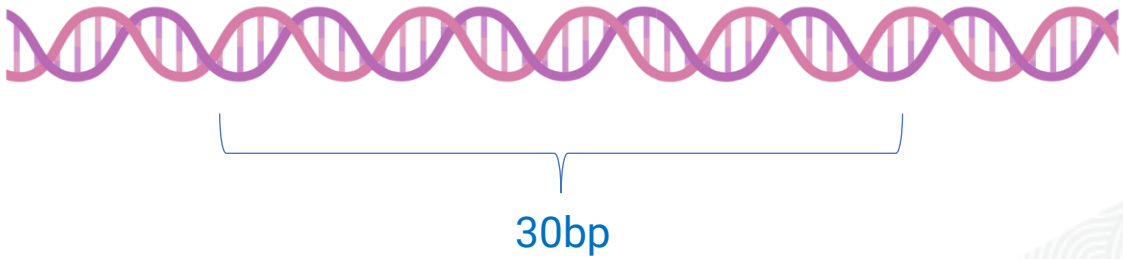
- Forced complementation of the NanoBiT® luciferase is achieved by fusion of the BiTs to binding partners
- When used to detect analytes, this technology is called **Lumit™**



Lumit® dsRNA Sensors



- SmBiT and LgBiT are genetically fused to dsRNA binding domains
- Specific binding to dsRNA induces complementation of NanoBiT® luciferase and generation of light
- Specificity for dsRNA results from:
  - Binding to 2’-OH (RNA only)
  - Binding to both strands (dsRNA only)
- Binding is independent of internal base pair sequence



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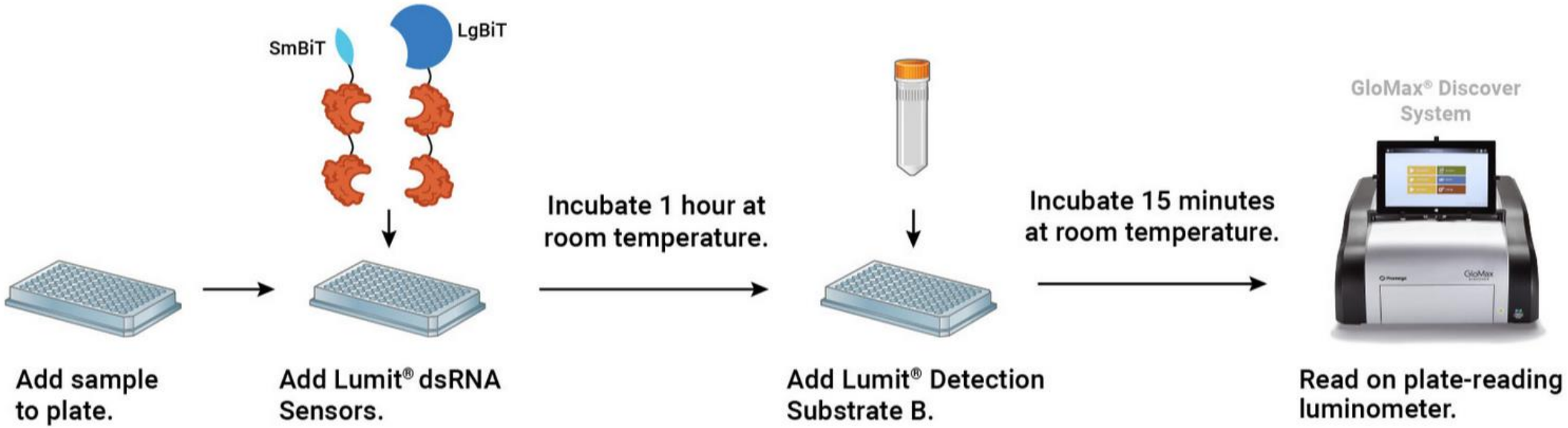
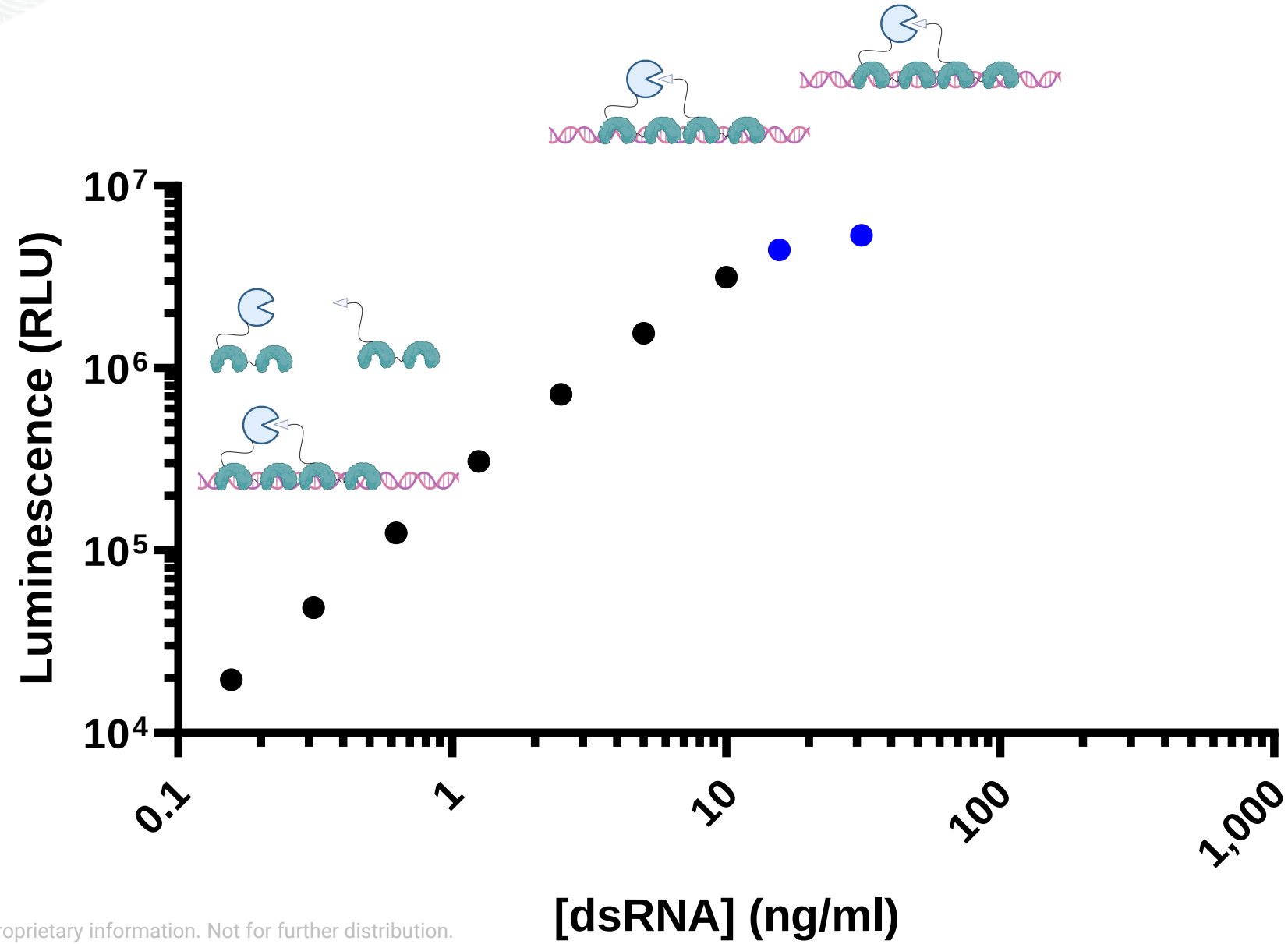
Lumit® dsRNA Detection Assay Protocol



Luminescence is dependent on dsRNA concentration



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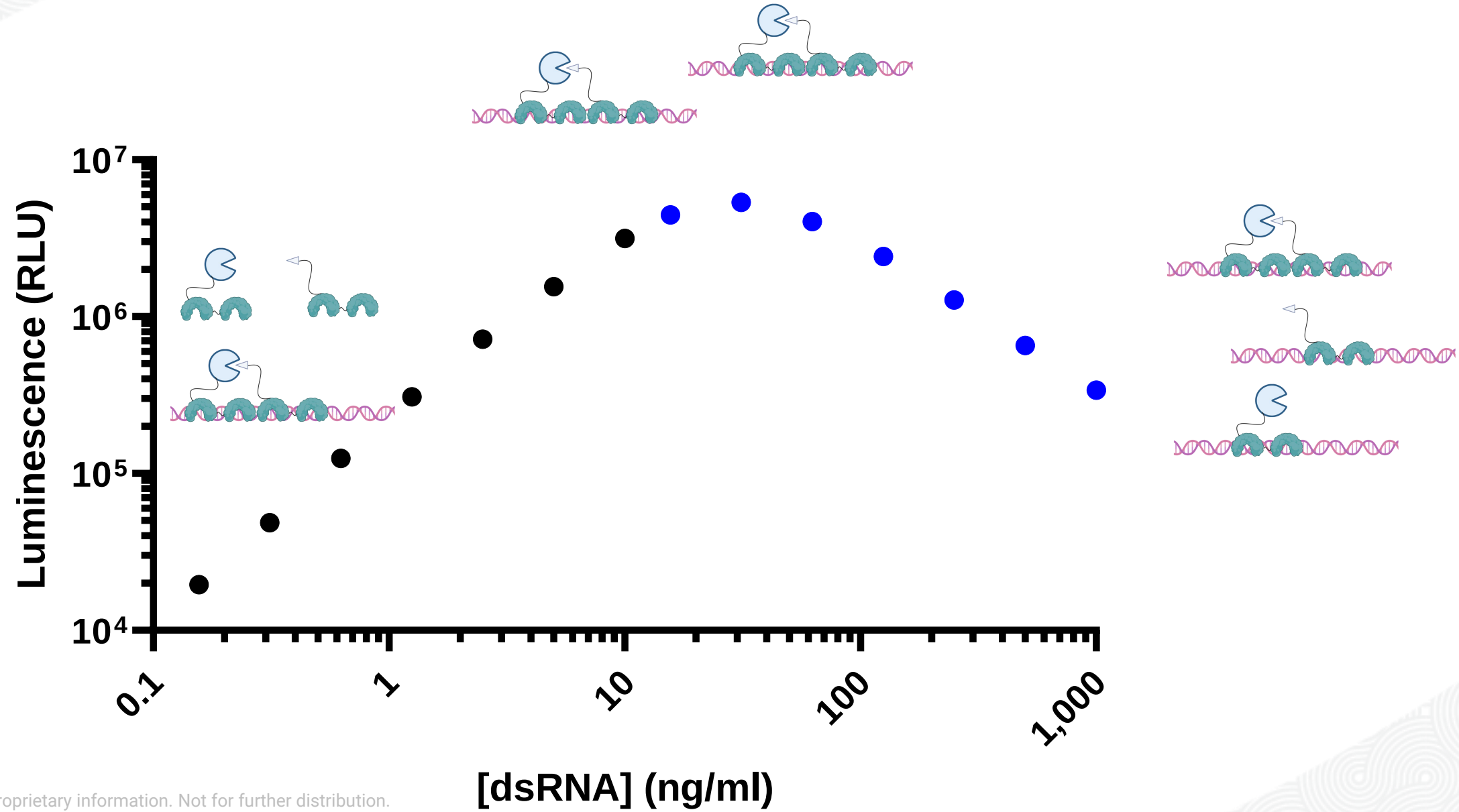

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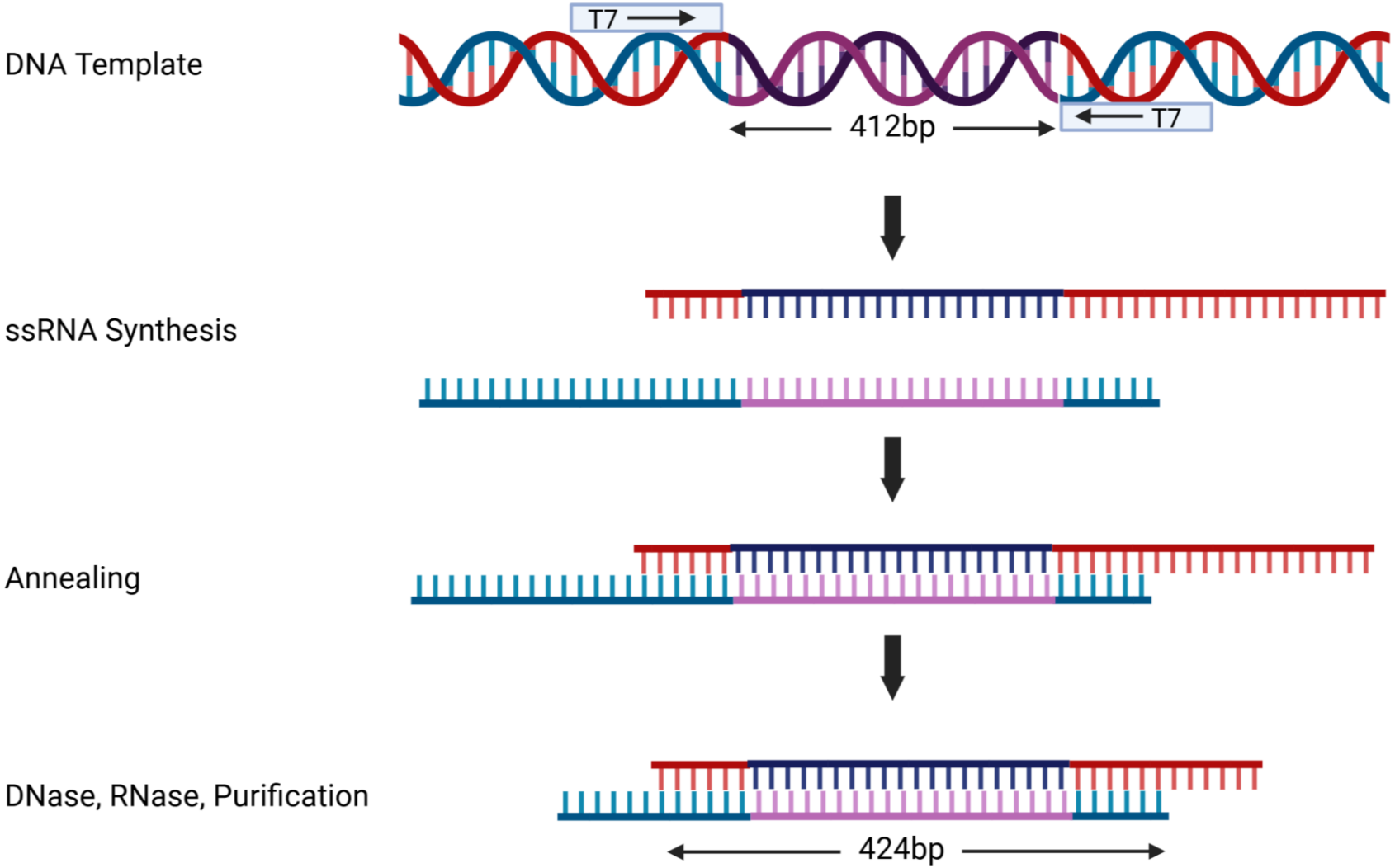
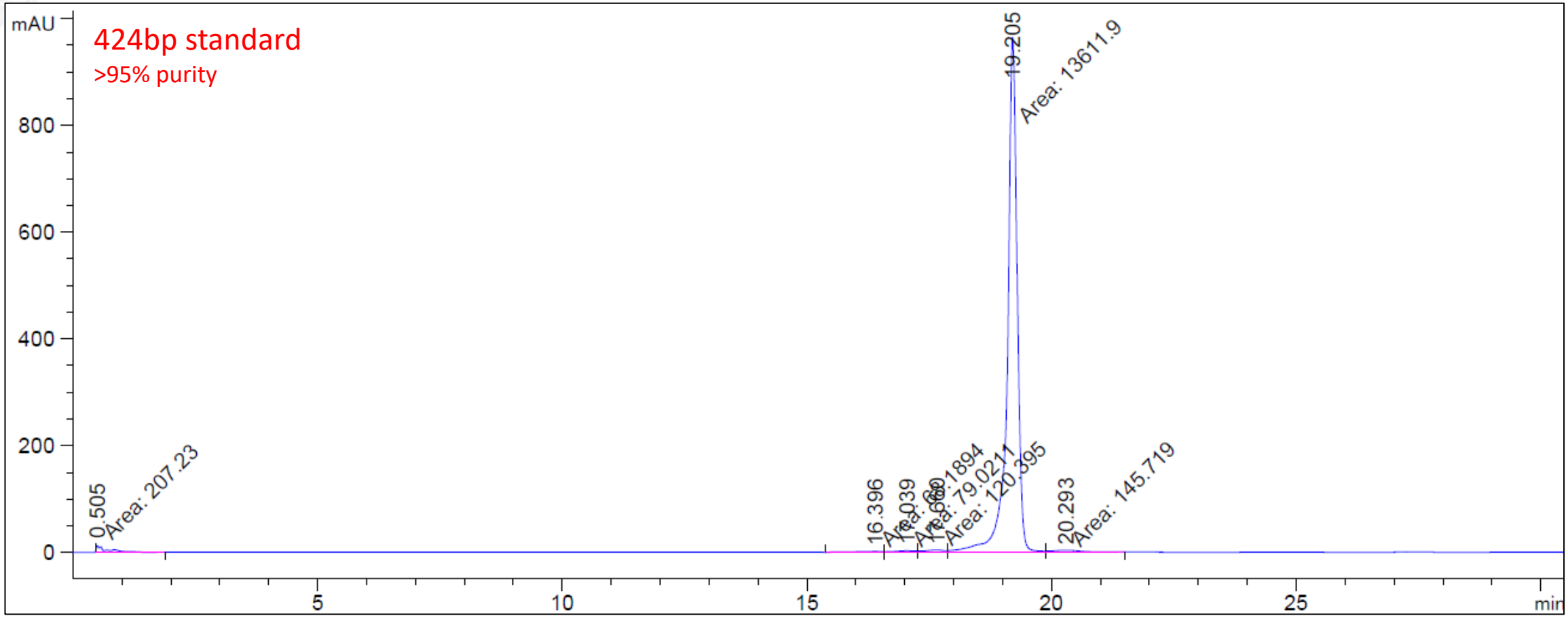
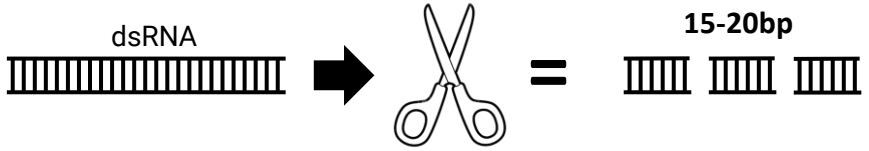
Generation of the dsRNA standard



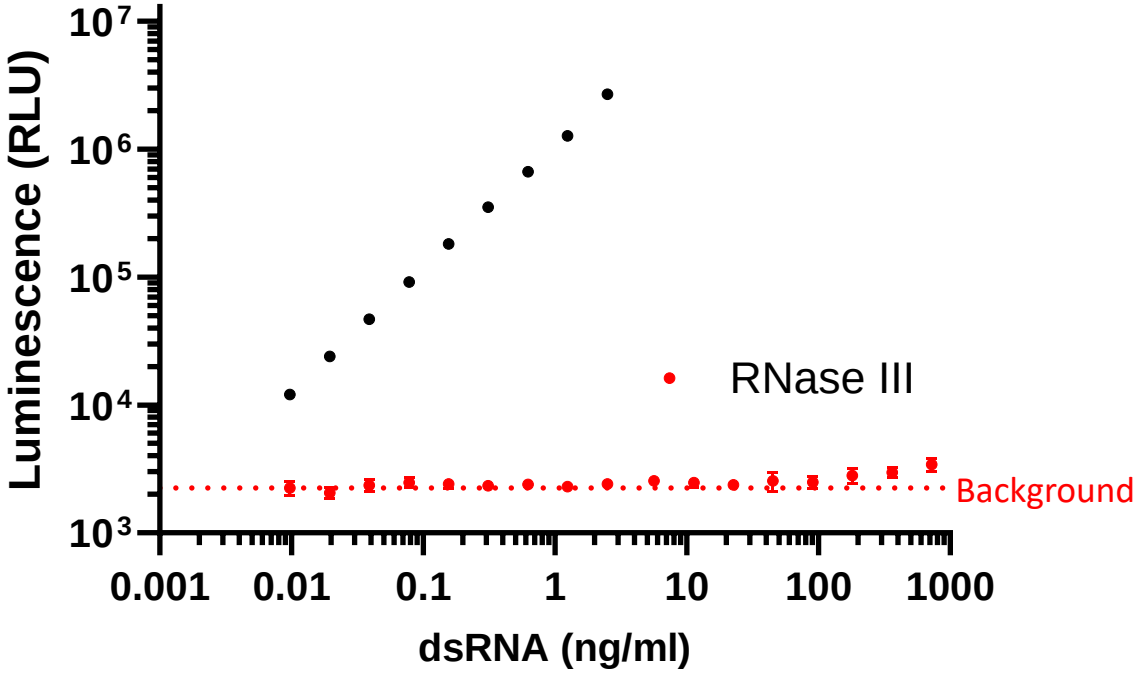
dsRNA Standard

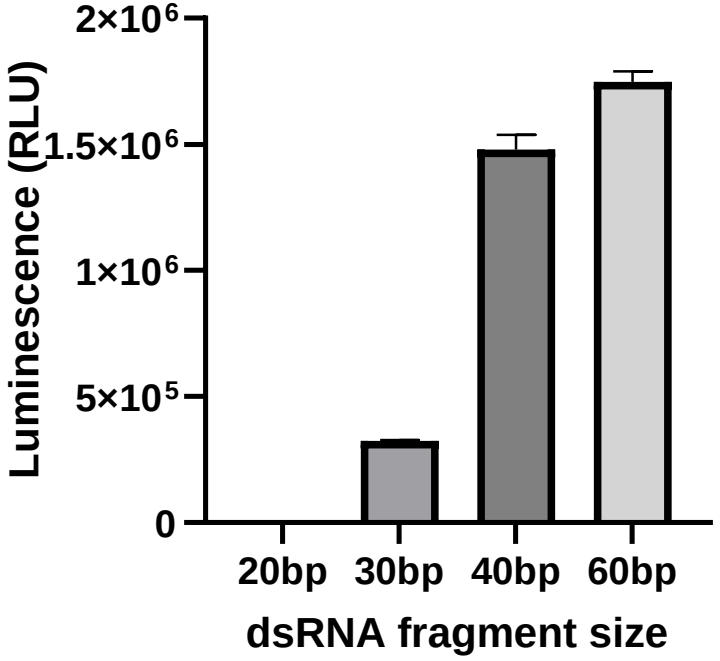


Detection of dsRNA fragments ≥30bp

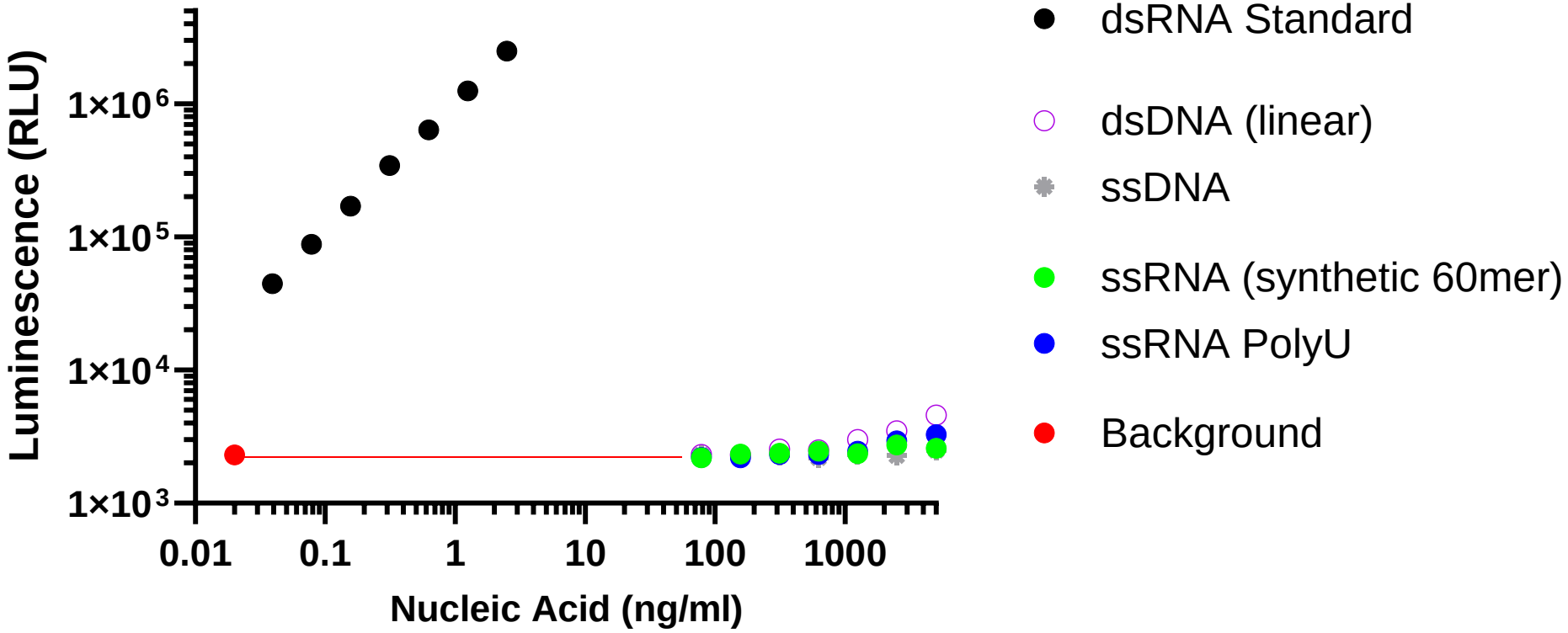


RNase III

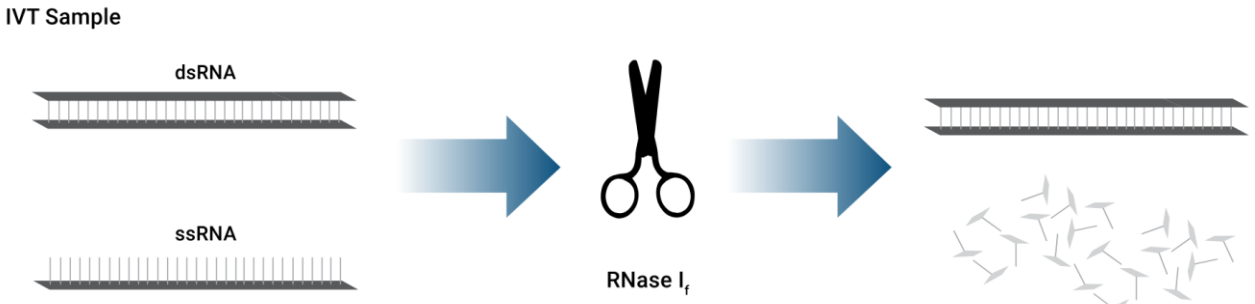


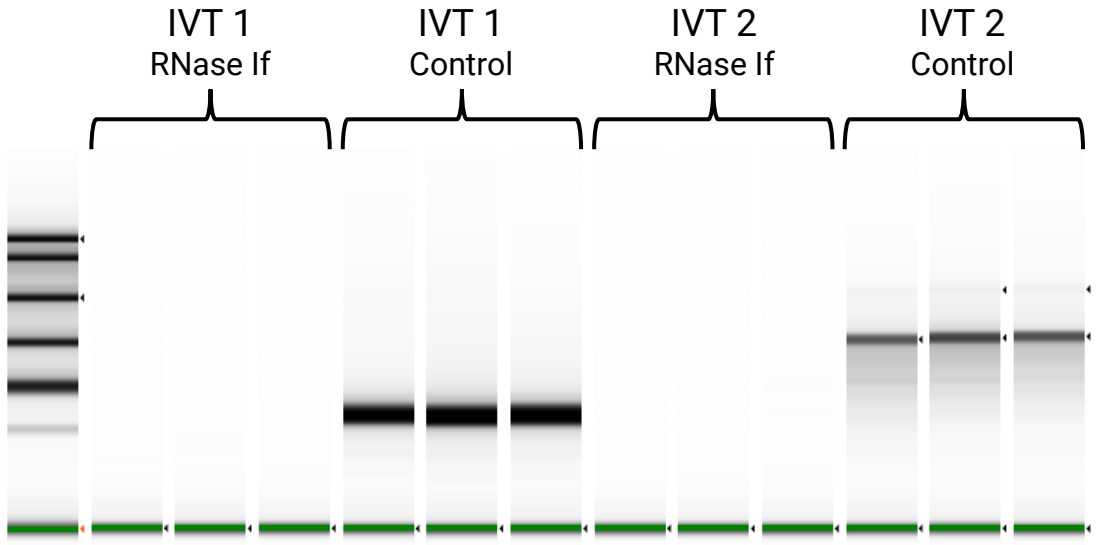


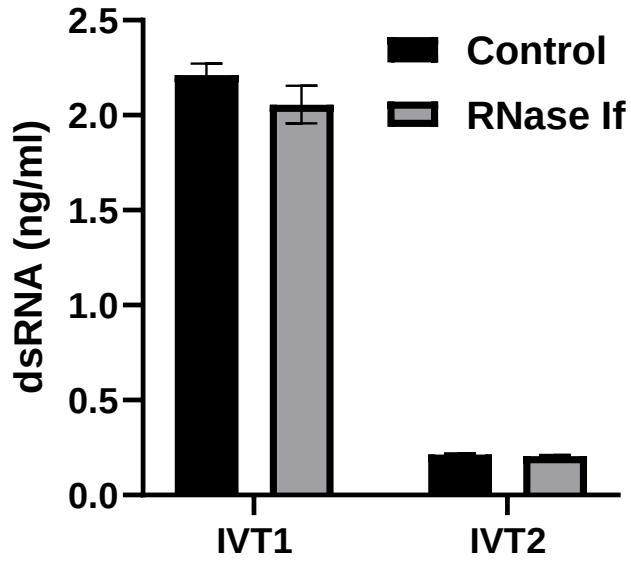
Specific detection of dsRNA



Specific detection of dsRNA in IVT products



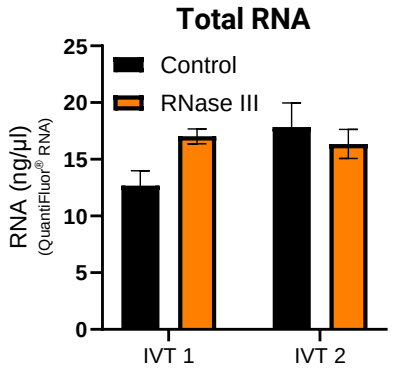


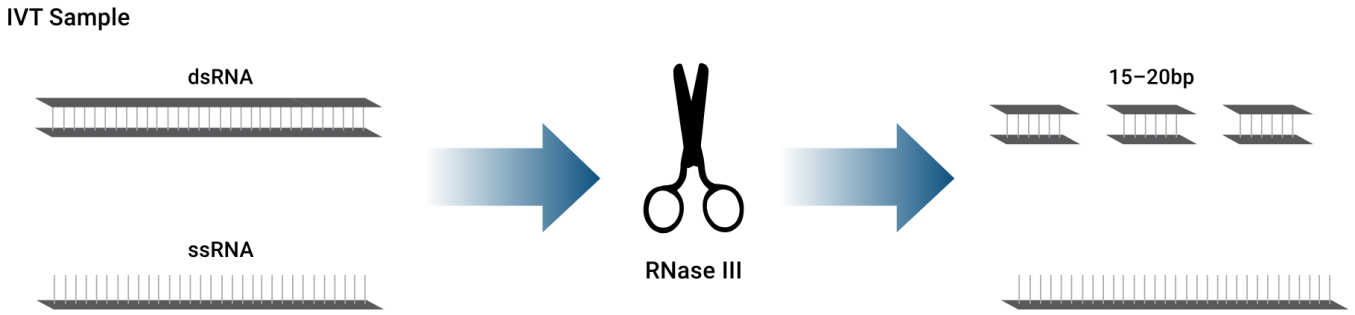


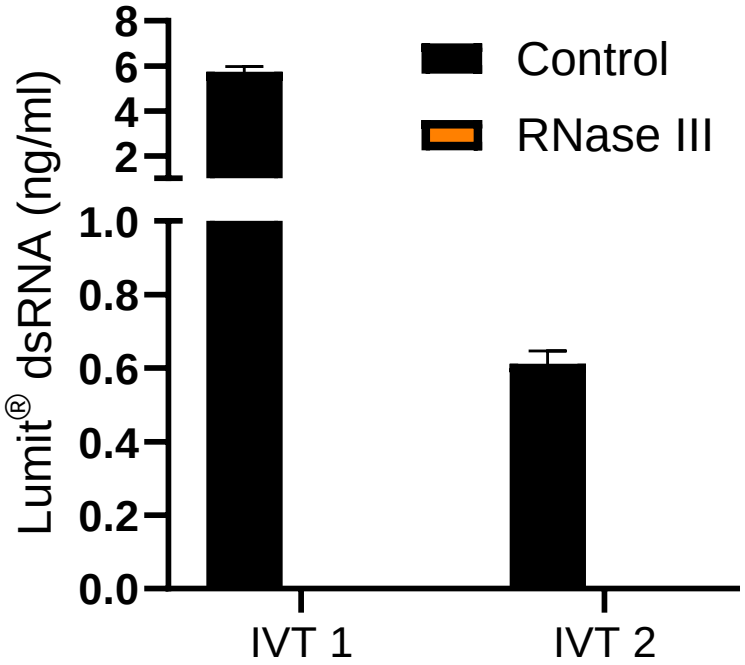
500ng/ml total RNA (or equivalent volume of digested)

Lumit® dsRNA signal remains when ssRNA is digested

Specific detection of dsRNA in IVT products

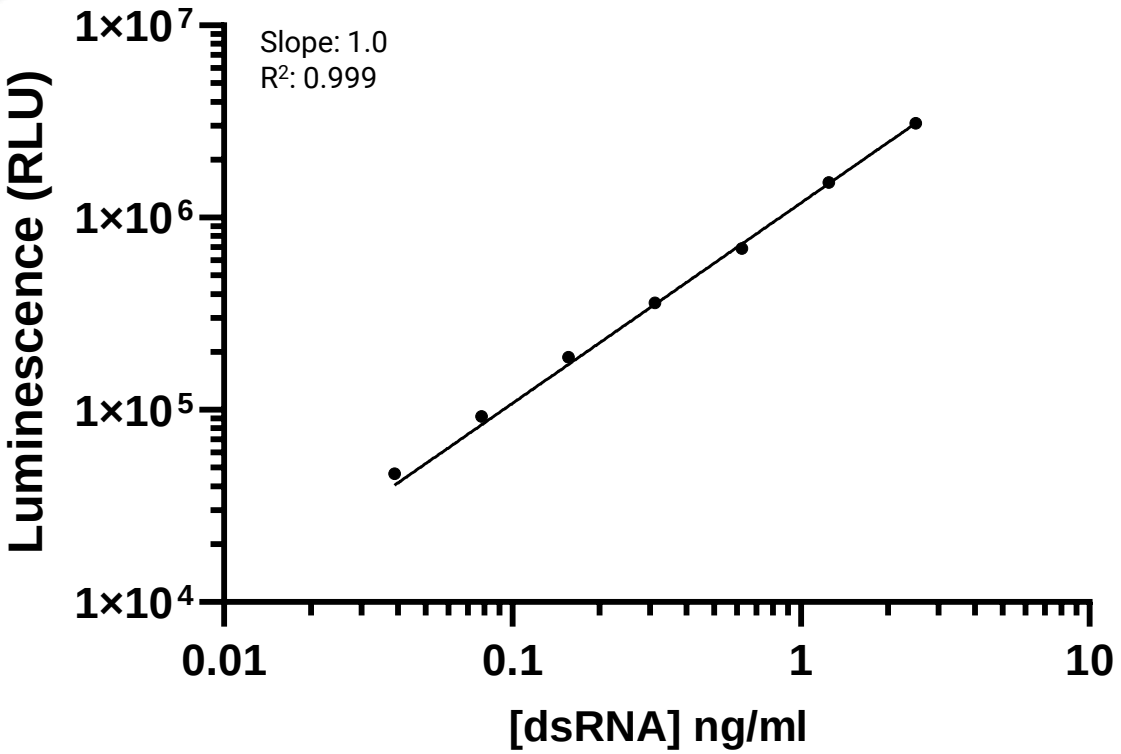






Lumit® dsRNA signal disappears when dsRNA is digested to short fragments

Assay performance characteristics



- Standard curve range: 40 – 2,500 pg/ml
- Limit of Detection (LoD): 0.4 pg/ml
- Limit of Quantitation (LoQ): 1.1 pg/ml
- Intra-assay Precision (16 replicates):

	0.13 ng/ml	0.50 ng/ml	2.0 ng/ml
Recovery	100%	102%	105%
%CV	3.2	4.4	3.6

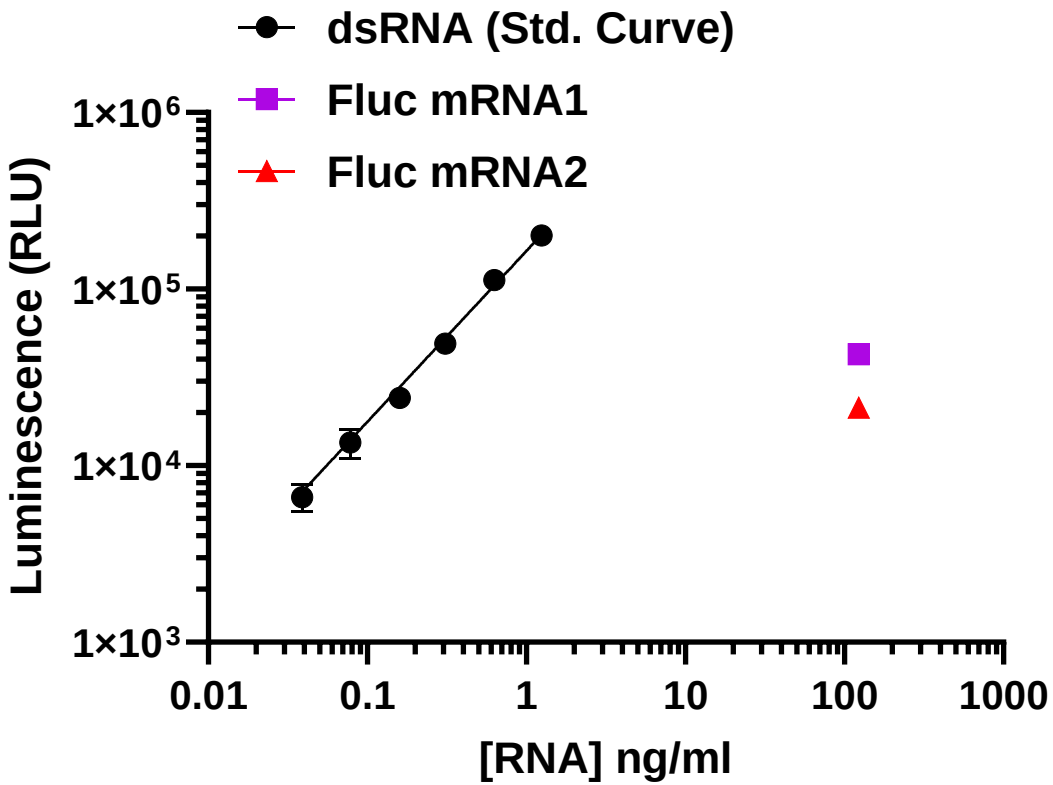
- Inter-assay Precision:

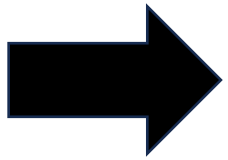
	0.13 ng/ml	0.50 ng/ml	2.0 ng/ml
Recovery	100%	98%	105%
%CV	6.2	5.2	3.6

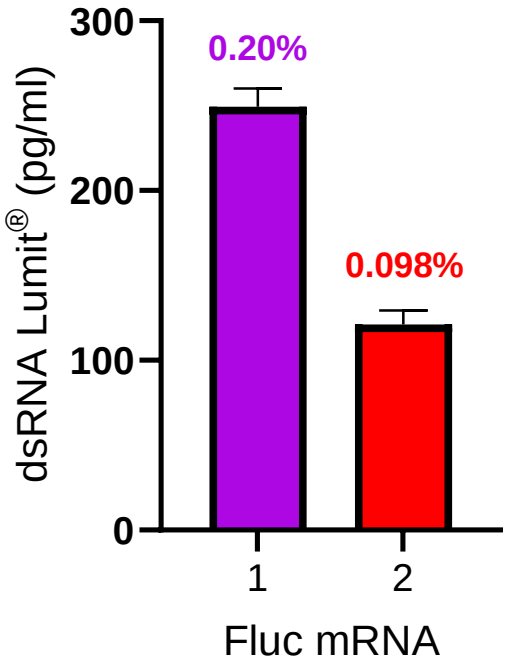
- Assay time: 75min

Application: measure dsRNA content in purified mRNA substance

Two commercially available Fluc mRNA products



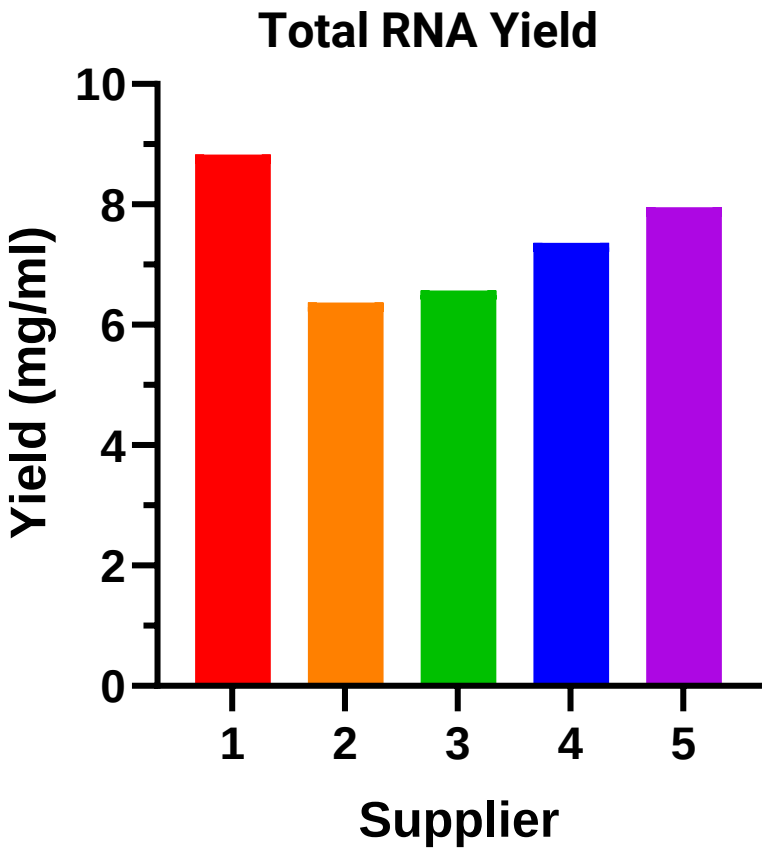


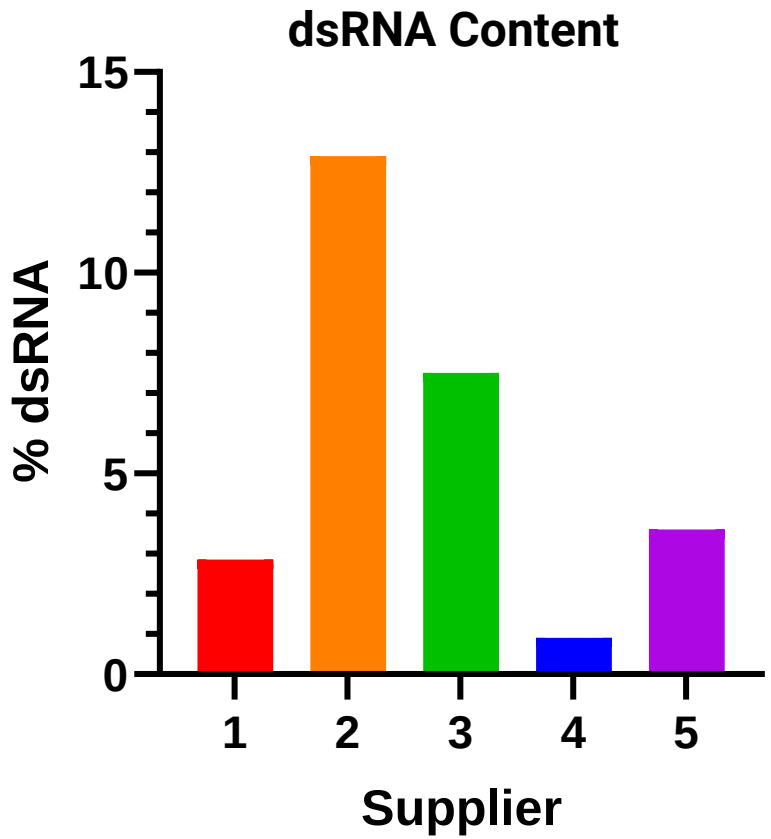


Percentage is dsRNA/Total RNA

Application: IVT process development

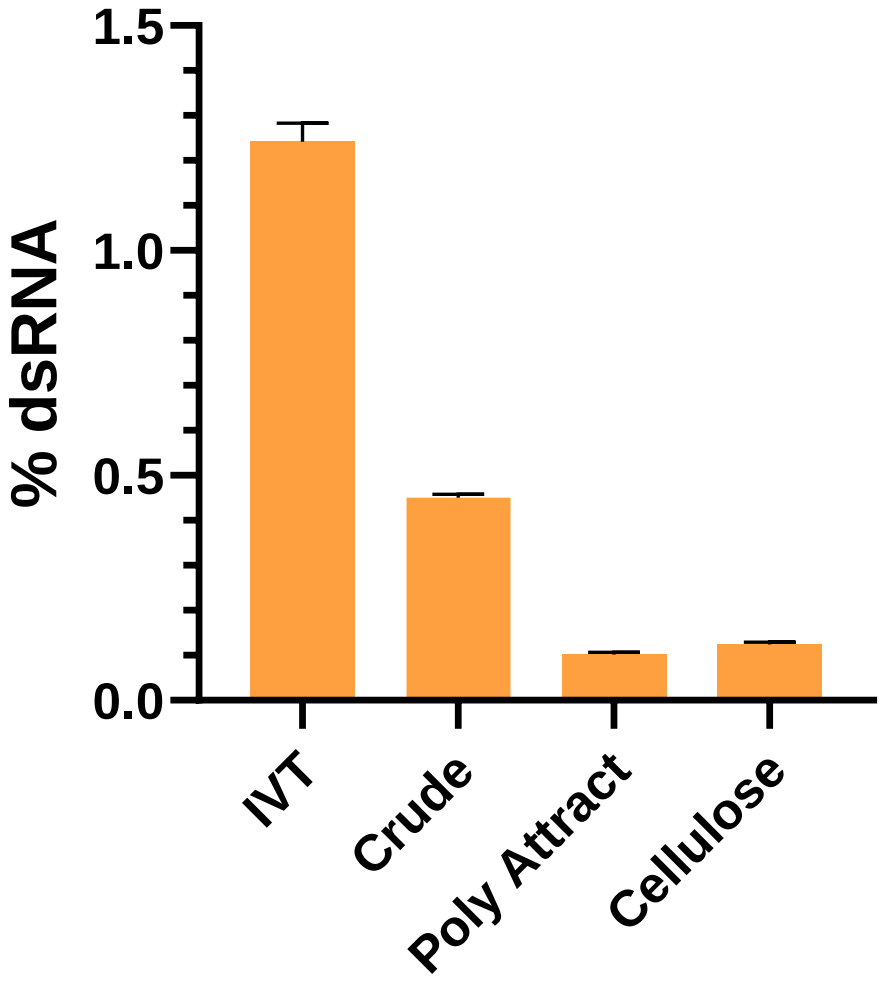
Unpurified IVT products from five commercial IVT kits were analyzed for RNA yield and dsRNA content



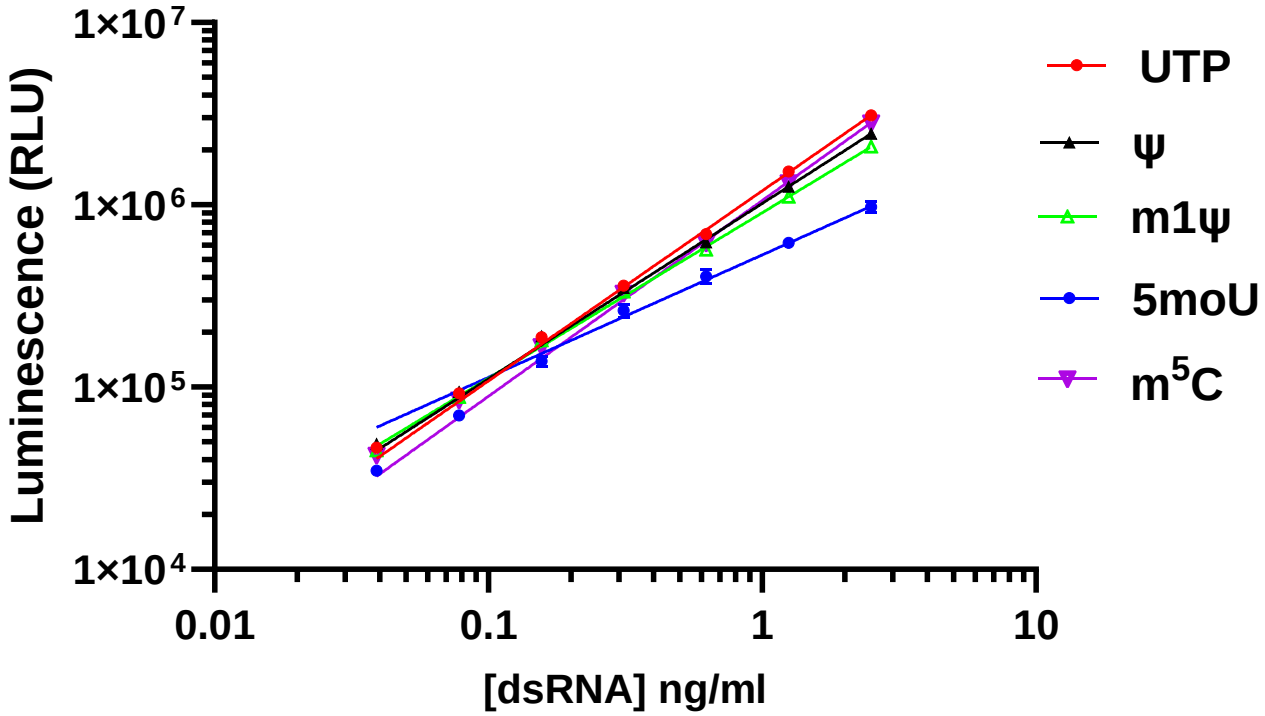


Application: purification process development

IVT was sampled throughout the purification process for dsRNA content



Performance with modified nucleotides

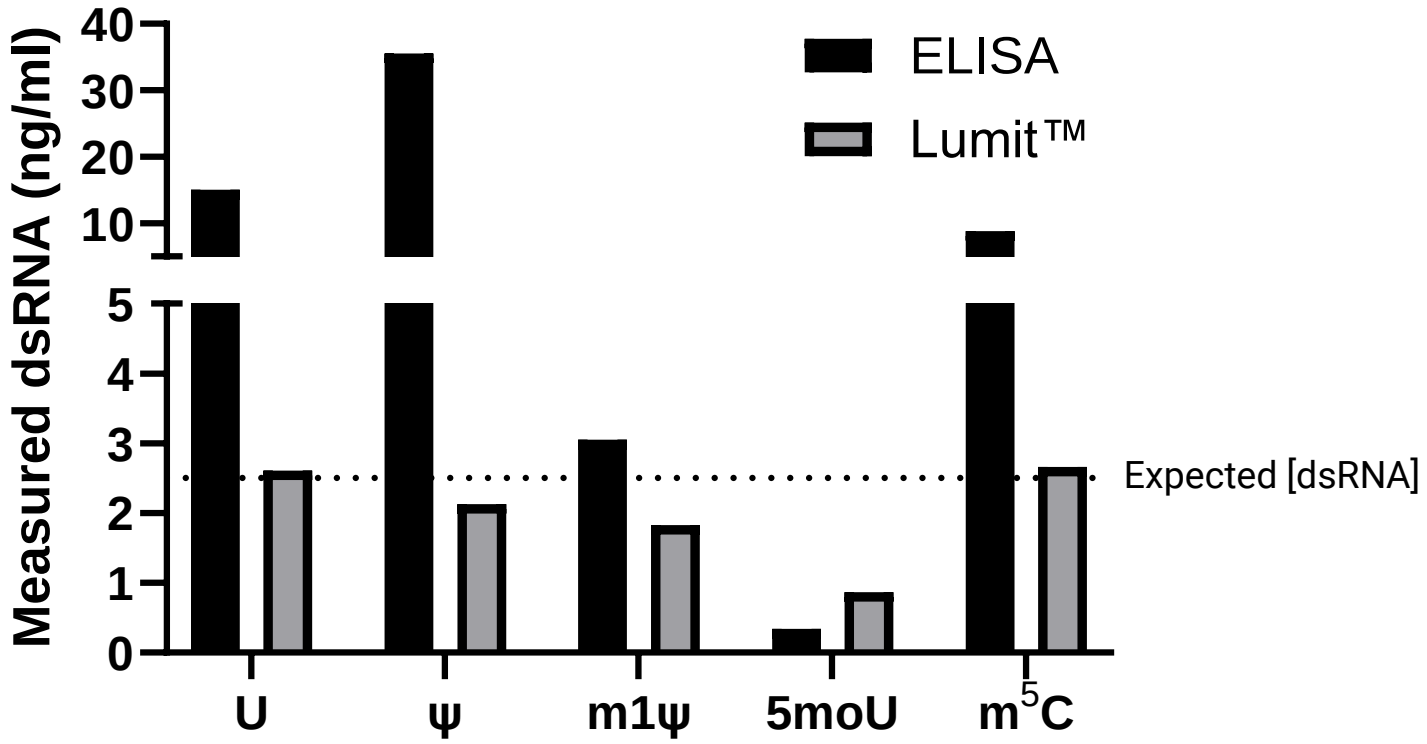


RNA modification	Average normalized recovery
Unmodified UTP	100%
Ψ	77%
m1Ψ	66%
5moU	31%
m^5C	103%

For the most accurate quantitation of samples containing modified nucleotides, we recommend using a dsRNA standard containing that same modification

Benchmarking to a commercial ELISA kit

- Samples with modified nucleotides were tested in both kits (same size and sequence)
- ELISA is a J2-based commercial kit using m1Ψ standard



	ELISA	Lumit™
LoQ (pg/ml)	123	1.1
Precision Intra	<10%	4%
Precision Inter	<15%	5%
Assay incubation time	3hrs	75min

Lumit™ dsRNA Detection Assay

A new assay technology for dsRNA detection and quantitation

- Does not use antibodies
- **Sensitive**: LoQ is 1 pg/ml
- **Specific**: no cross-reactivity with other nucleic acids
- **Quantitative**: not dependent on fragment sequence or size (≥30bp)

Easy-to-implement

- Small sample volume (nanograms of total RNA per well)
- Rapid and convenient workflow
- No immobilization or wash steps

Acknowledgements

Promega R&D

- Rich Moravec
- Anne Strouse
- Robin Hurst
- Jim Hartnett
- Mei Cong

Collaborators and alpha testers