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Supporting Information

Enzymatic Isolation and Microfluidic Electrophoresis Analysis of Residual dsRNA Impurities in mRNA Vaccines and Therapeutics

Adriana Coll De Peña,[1] Matei Vaduva,[2] Nina S. Li,[1] Shreyas Shah,[3] Menel Ben Frej,[3] and Anubhav Tripathi[1,*]

[1]Center for Biomedical Engineering, School of Engineering, Brown University, Providence, RI, USA

[2]Department of Molecular Biology, Cell Biology, and Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI, USA

[3]Applied Genomics, Revvity, Hopkinton, MA, USA

*Correspondence should be addressed to the following author:

Anubhav Tripathi (PhD)

Center for Biomedical Engineering, School of Engineering,

182 Hope St, Providence, RI 02912, USA

Email: anubhav_tripathi@brown.edu

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Table S1. Conditions tested during the factorial analysis

Table S1: Factorial design of experiment with three parameters - mRNA, buffer, and enzyme concentrations - at three levels. The mRNA concentrations were 1/2, 1, and 2 × the initial concentration of 10 ng/µL of a 1,198 nt sample, the buffer concentrations were 1/16, 1/8, and 1/4 × the initial concentration of 9% v/v of the stock, and the enzyme concentrations were 2, 10 and 50 × the initial concentration of 0.30% v/v of the stock. For all conditions, the 700 bp dsRNA sample concentration was kept constant at 5 ng/µL.

Condition	dsRNA Concentration (ng/µL)	mRNA Concentration (× 10 ng/µL)	Buffer Concentration (× 9% v/v)	Enzyme Concentration (× 0.30% v/v)
Untreated	5	1	0	0
1	5	1/2	1/16	2
2	5	1/2	1/8	2
3	5	1/2	1/4	2
4	5	1/2	1/16	10
5	5	1/2	1/8	10
6	5	1/2	1/4	10
7	5	1/2	1/16	50
8	5	1/2	1/8	50
9	5	1/2	1/4	50
10	5	1	1/16	2
11	5	1	1/8	2
12	5	1	1/4	2
13	5	1	1/16	10
14	5	1	1/8	10
15	5	1	1/4	10
16	5	1	1/16	50
17	5	1	1/8	50
18	5	1	1/4	50
19	5	2	1/16	2
20	5	2	1/8	2
21	5	2	1/4	2
22	5	2	1/16	10
23	5	2	1/8	10
24	5	2	1/4	10
25	5	2	1/16	50
26	5	2	1/8	50
27	5	2	1/4	50

Figure S1. Comparison of the dsRNA and undesired peak areas yielded by the factorial analysis

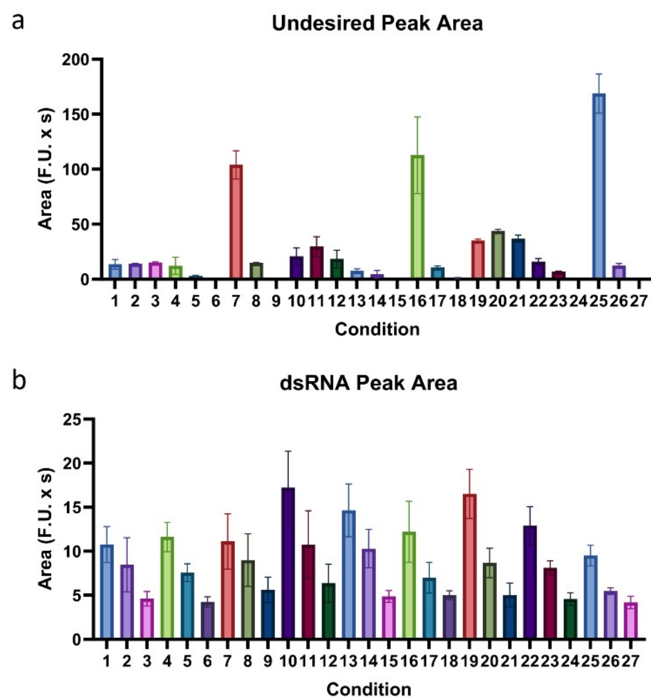

Figure S1: Comparison of the (a) undesired peak area and (b) dsRNA peak area yielded by the 27 conditions tested. From here, the best 10 conditions based on lowest undesired peak areas were further explored in the main text.

Figure S2. Factorial analysis of the buffer, enzyme, and mRNA concentration experiment

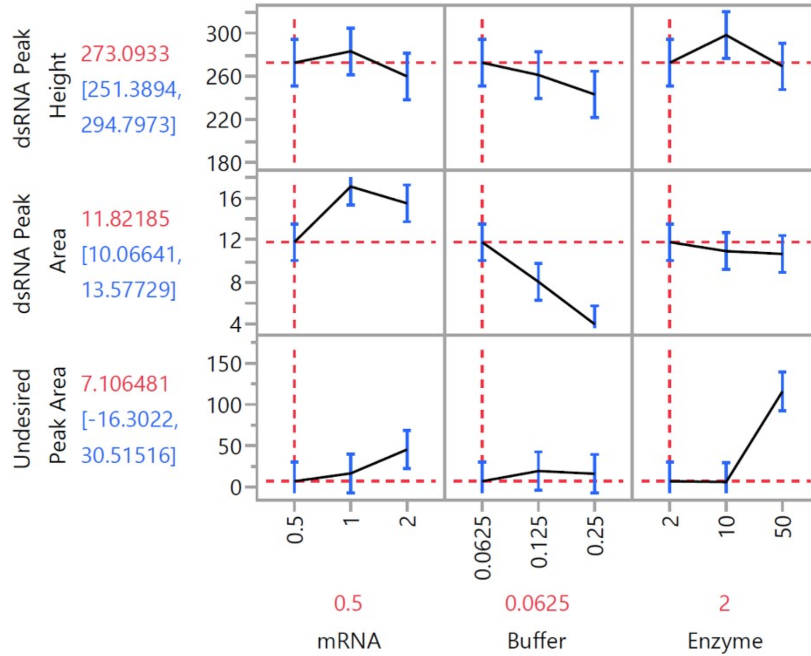

Figure S2: Factorial analysis changing three parameters - mRNA, buffer, and enzyme concentrations - at three levels. During the design of experiments, the mRNA concentrations were 1/2, 1, and 2 × the initial concentration of 10 ng/µL, the buffer concentrations were 1/16, 1/8, and 1/4 × the initial concentration of 9% v/v of the stock, and the enzyme concentrations were 2, 10 and 50 × the initial concentration of 0.30% v/v of the stock. For the statistical analysis, a factorial analysis was performed on JMP Pro 17, with the goal of maximizing the dsRNA Peak Height and the dsRNA Peak Area and minimizing the Undesired Peak Area. The figure was generated using JMP Pro 17.

Figure S3. Assessment of the dsRNA limit of detection

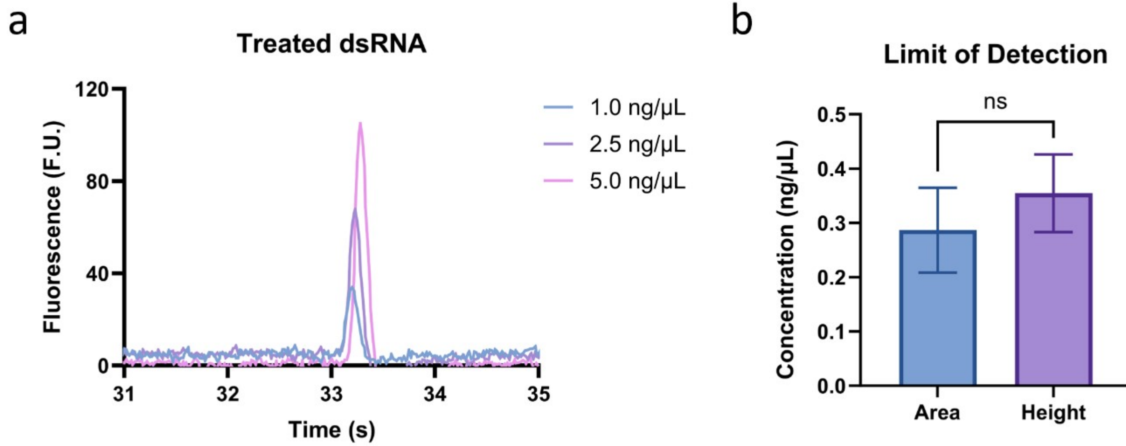

Figure S3: Assessment of the LOD of the system post enzymatic treatment, estimated by analyzing a 700 bp dsRNA sample at 1.0, 2.5, and 5 ng/µL. (a) Electropherogram of the three dsRNA concentrations. (b) Comparison of the average LOD estimated from the three samples using Equations 1 (area) and 2 (height). "*" indicates the significance level in the difference (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$), while "ns" indicates no statistical difference. This figure was created using GraphPad Prism.