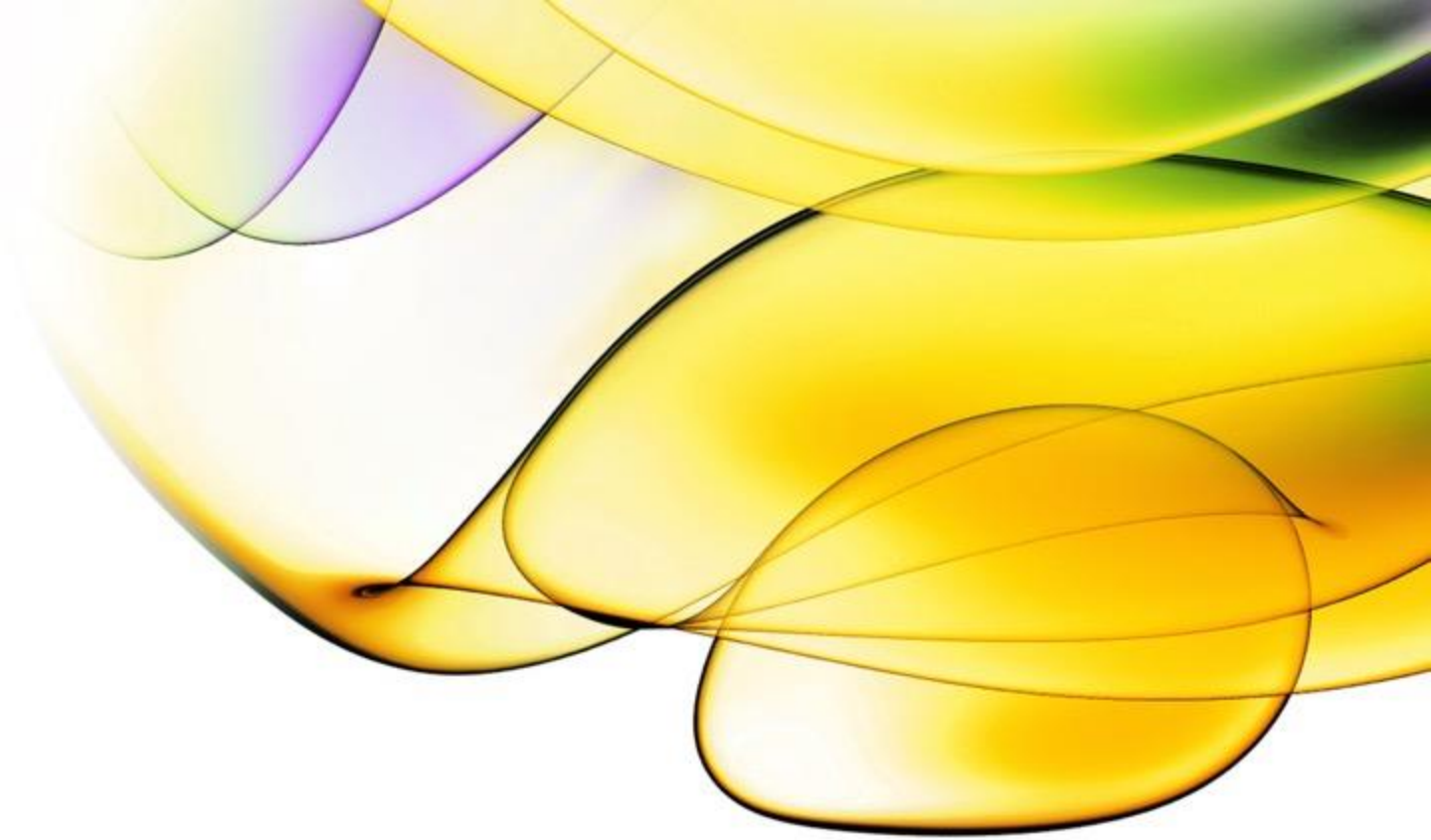


Accelerating Biologics Analysis with the LabChip™ GXII Touch™ System

CE Pharm 2024

Agenda

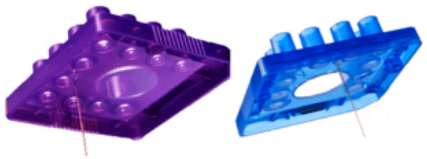
LabChip Virtual Demo

Why LabChip?

Journal Club

What’s coming for LabChip?

LabChip® GXII Touch™ Biologics Characterization System



Validated and Ready to Use Assays

- Titration (Concentration and Sizing)
- Purity/Impurity Analysis
- Stability (Degradation/Fragmentation)
- N-Glycan Profiling
- Charge Variant Profiling
- DNA/RNA Analysis

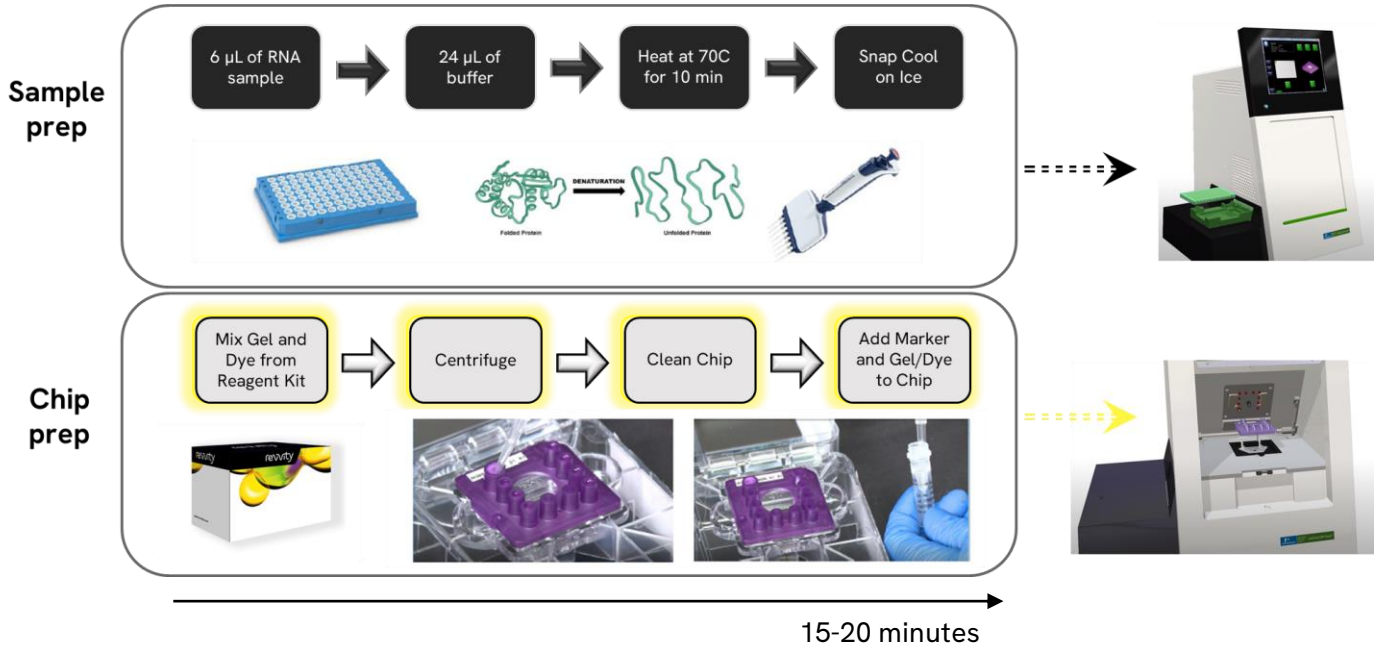
Patented LabChip® Microfluidic Technology

- Multiple-use chip: 400 samples
- Minimal Sample Consumption: 2 µL
- Fast Analysis Speed: ~60 seconds/sample
- High Throughput: 96/384 -well microplate

Modern Design

- Compact Footprint
- Touch Screen
- Fully automated workflow
- CFR 21 Part 11 Compliance

How To Run



Gel/Dye preparation may require as little as 10 minutes of hands-on time every 3 weeks.

Why LabChip®?

Get Answers Easier

Pre-validated plug-and-play assays provide answers on day 1

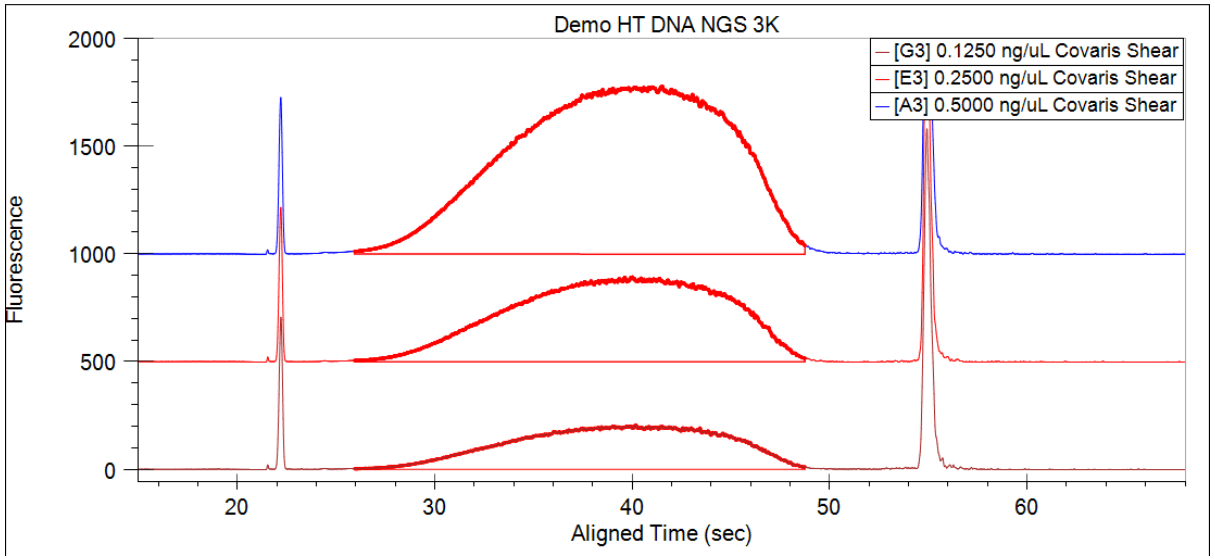
Finish Your Project Faster

Separation times up to 30x faster than other methods allow for more screening, better informed decisions, and reaching targets faster.

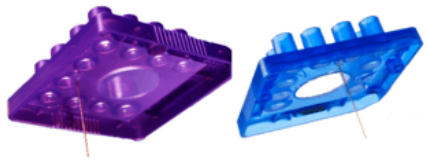
Keep Costs Low

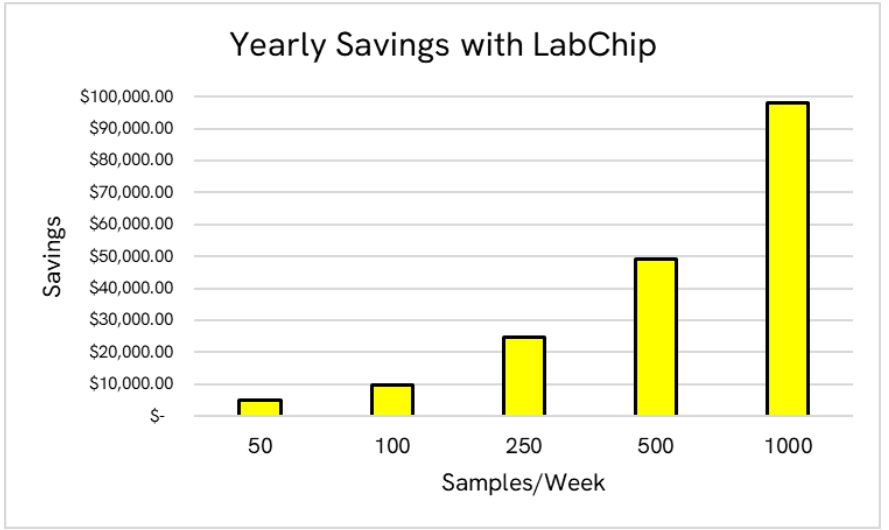
Reusable microfluidic chips keep analysis costs low – saving more than $100k/year for many of our customers.

Why LabChip®?



Samples/ Week	Cost (CE)	Cost (LabChip)	Savings with LabChip
50	$ 314.73	$ 76.63	**$ 238.10**
100	$ 629.46	$ 153.26	**$ 476.21**
200	$ 1258.93	$ 306.52	**$ 952.41**
500	$ 3147.32	$ 766.30	**$ 2381.03**
1000	$ 6294.64	$ 1532.59	**$ 4762.05**



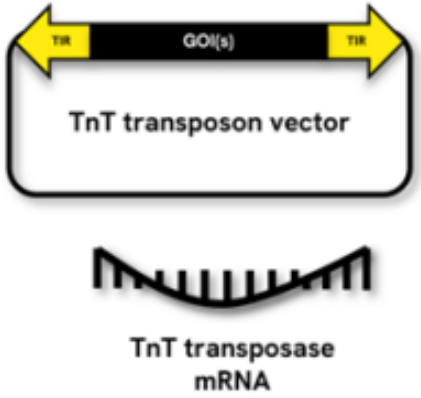


Journal Club

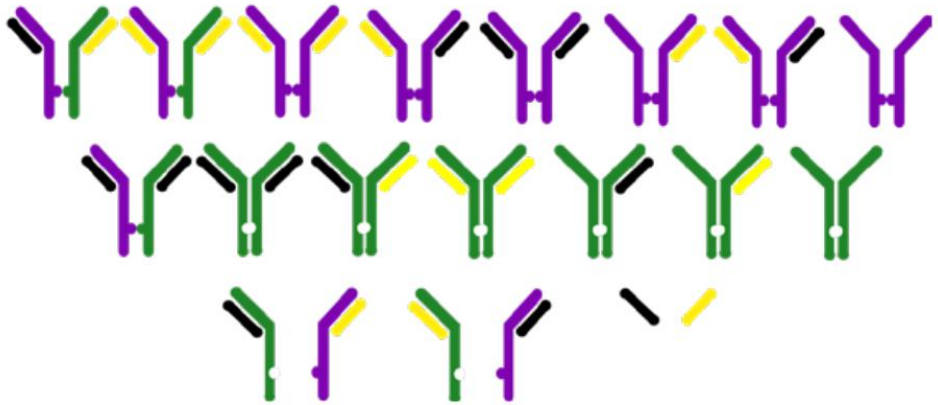
GXII Touch™ Optimizing BioProcessing

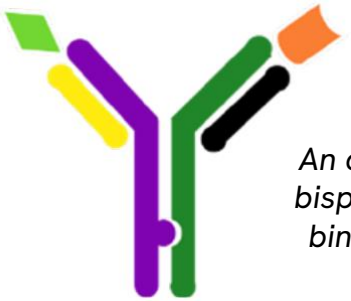
revvity APPLICATION NOTE

Streamlining the development of bispecific antibodies from expression to quality assessment with Revvity's biotherapeutic workflow solutions.



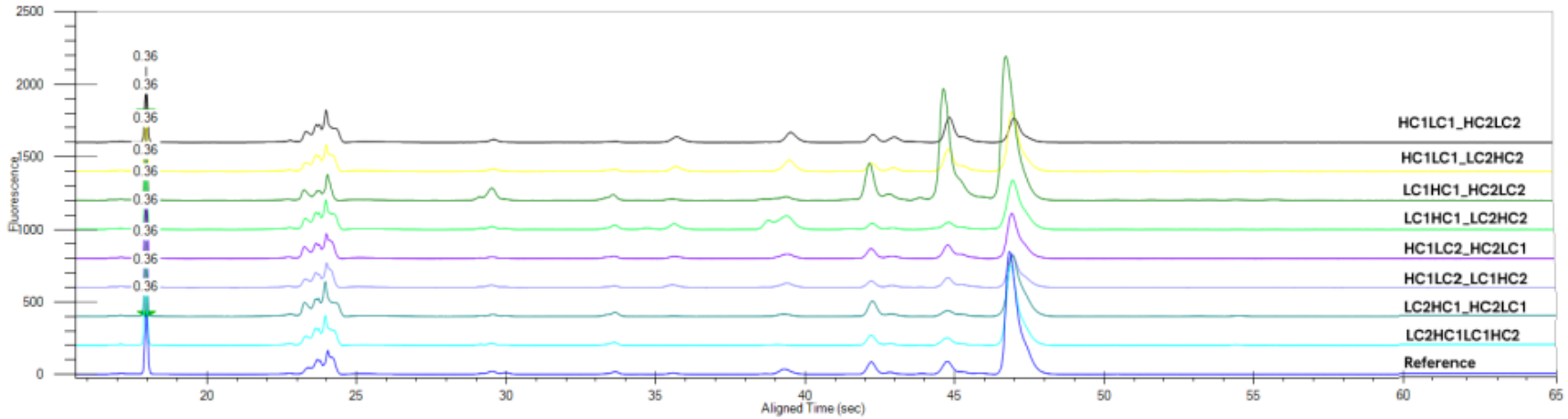
Transfection conditions	Vector 1	Vector 2
1	HC1LC1	HC2LC2
2	HC1LC1	LC2HC2
3	LC1HC1	HC2LC2
4	LC1HC1	LC2HC2
5	HC1LC2	HC2LC1
6	HC1LC2	LC1HC2
7	LC2HC1	HC2LC1
8	LC2HC1	LC1HC2

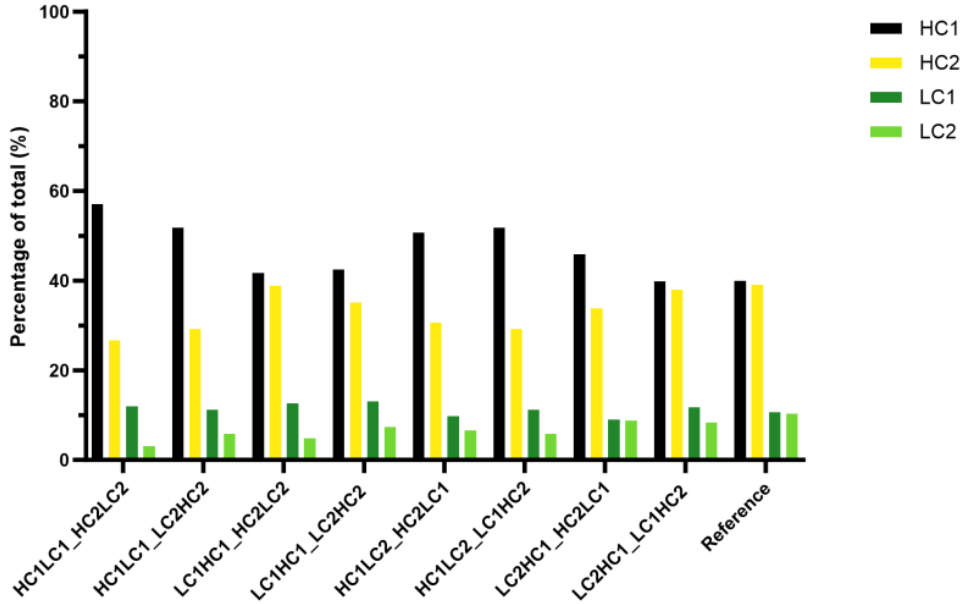




An asymmetric 4-chain bispecific antibody that binds to two different antigens.

GXII Touch™ Optimizing BioProcessing





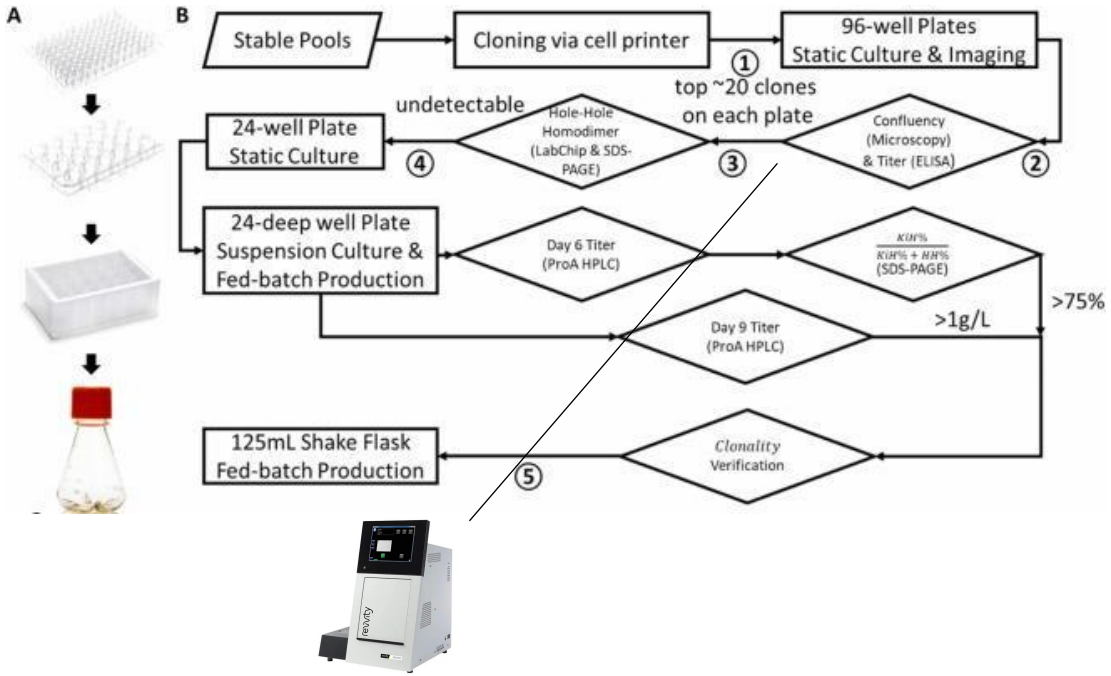
The partnership between Revvity's CHOSOURCE expression platform and the ProteinEXact assay on GXII Touch system allows acceleration of cell line development processes, contributing to the advancement of development and manufacturing of biotherapeutics

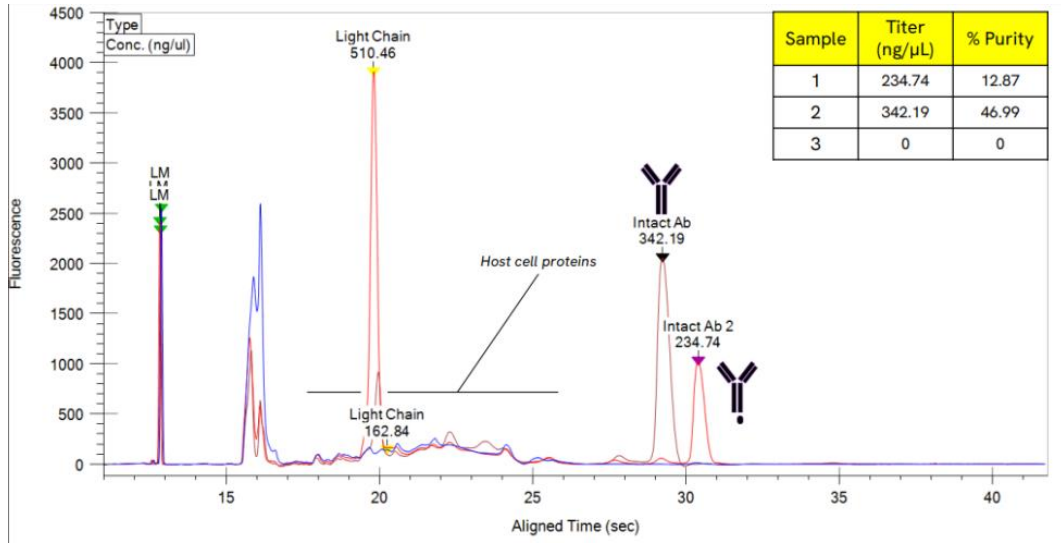
GXII Touch™ Optimizing BioProcessing

An innovative platform to improve asymmetric bispecific antibody assembly, purity, and expression level in stable pool and cell line development☆☆,☆

Yanling Wang [a,*,1], Haoran Qiu [a,1], Jeremy Minshull [b], Wilburt Tam [a], Xichan Hu [a], Carl Mieczkowski [a], Weibin Zheng [a], Chun Chu [a], Wenqiang Liu [a], Ferenc Boldog [b], Claes Gustafsson [b], Jean-Michel Gries [a], Wenfeng Xu [a]

[a] Hengenix Biotech Inc., 430 N McCarthy Blvd, Milpitas, CA, USA
[b] ATUM, 37950 Central Court, Newark, CA, USA





Sample	Titer (ng/μL)	% Purity
1	234.74	12.87
2	342.19	46.99
3	0	0

GXII Touch™ Optimizing BioProcessing

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Analytical Biochemistry 351 (2006) 122–127

ANALYTICAL BIOCHEMISTRY

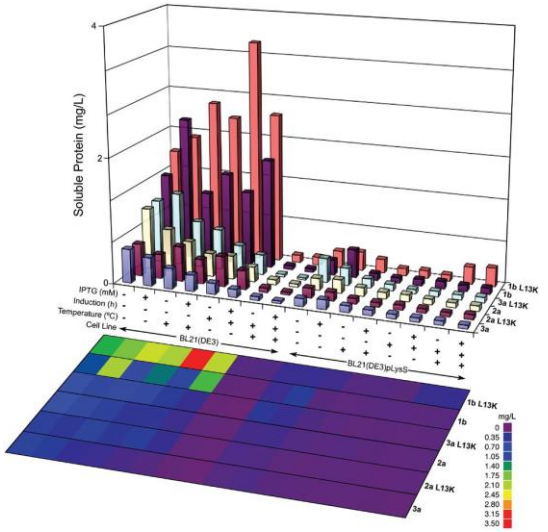
www.elsevier.com/locate/yabio

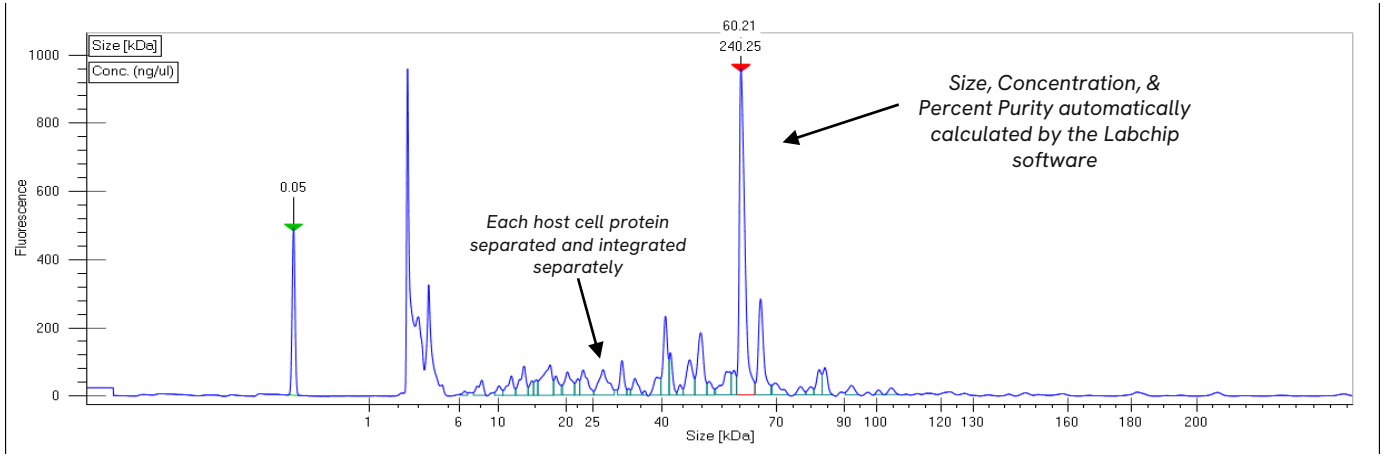
Screening factors effecting a response in soluble protein expression: Formalized approach using design of experiments

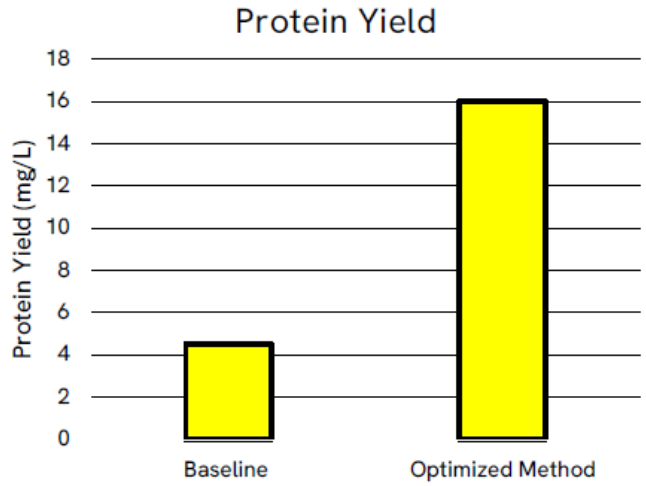
Susanne E. Swalley [1], John R. Fulghum [1], Stephen P. Chambers *

Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139-4242, USA

Received 19 October 2005
Available online 20 December 2005







mRNA Integrity Analysis

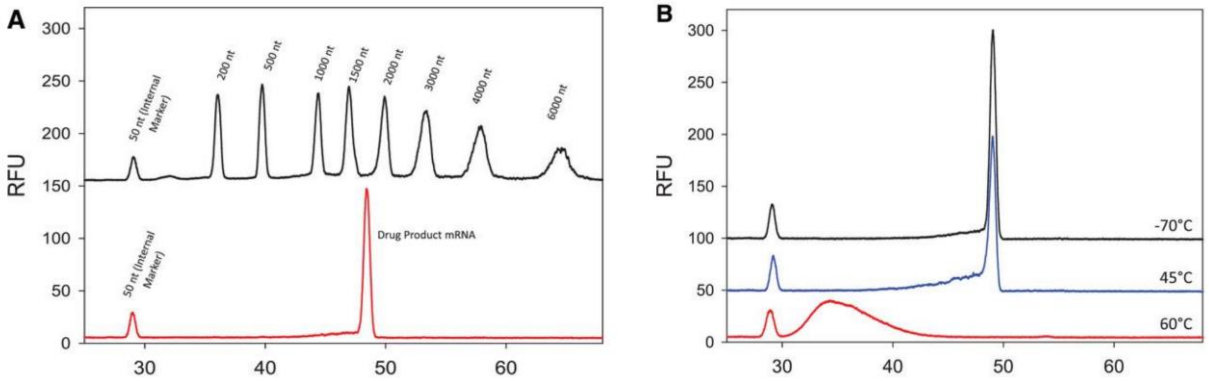
ELECTROPHORESIS Electrokinetics Fluidics Proteomics

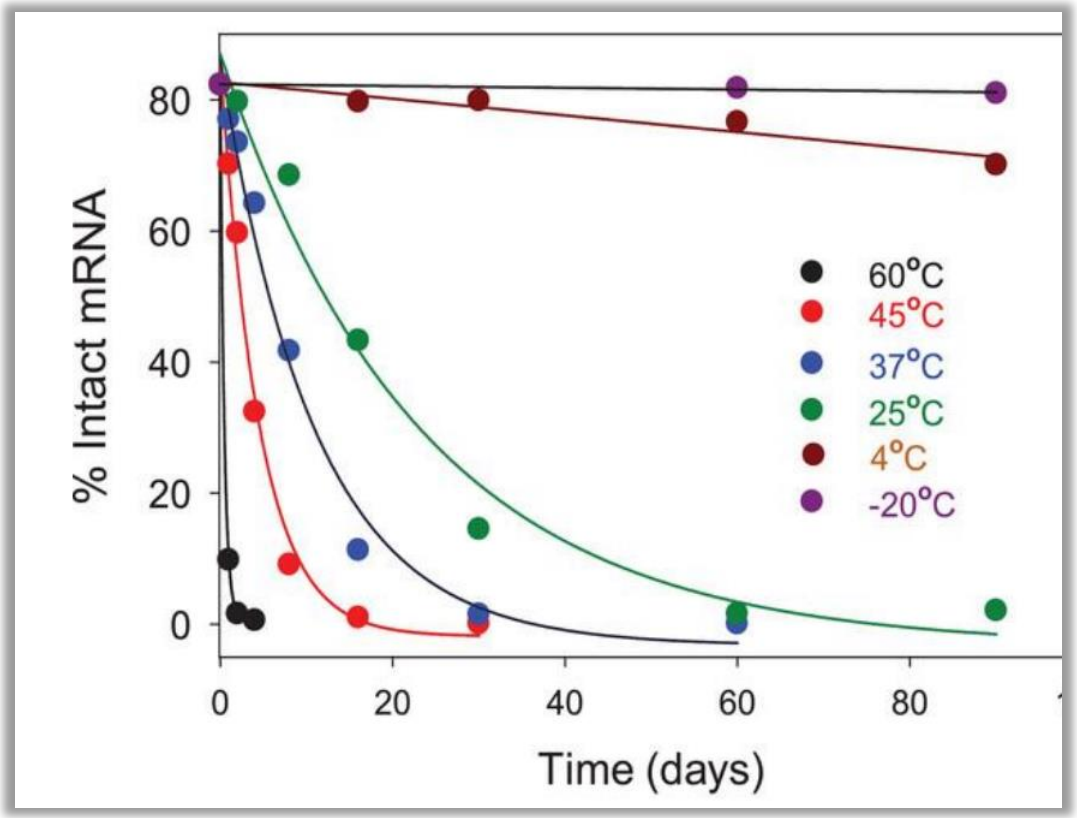
Research Article | Open Access

Development of a microchip capillary electrophoresis method for determination of the purity and integrity of mRNA in lipid nanoparticle vaccines

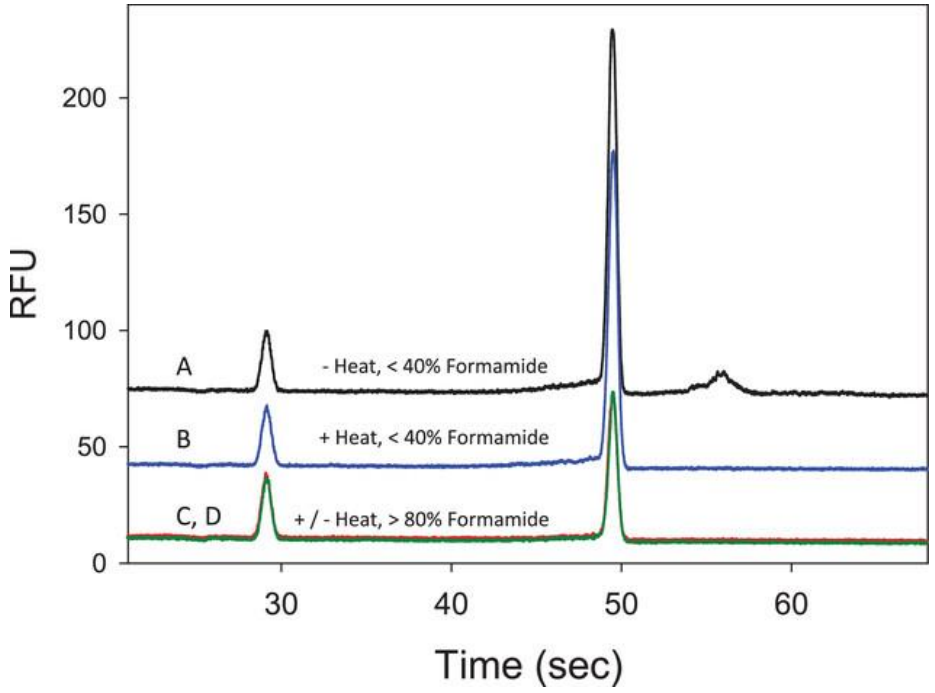
Jessica Raffaele, John W. Loughney, Richard R. Rustandi

First published: 22 November 2021 | https://doi.org/10.1002/elps.202100272 | Citations: 4

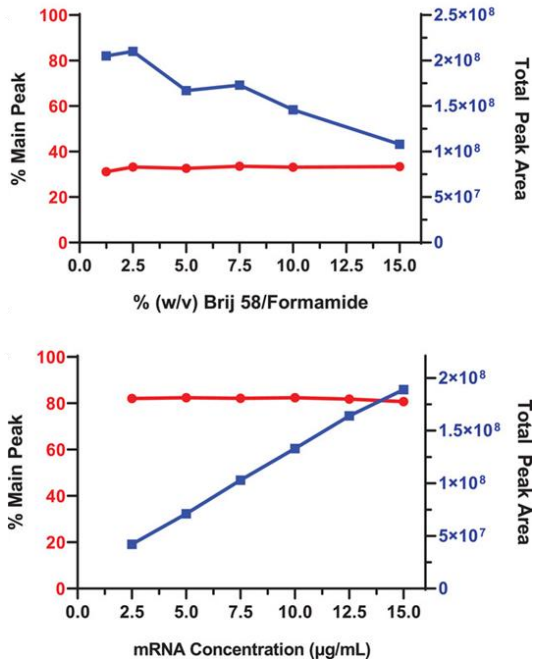




mRNA Integrity Analysis



Optimized protocols developed to ensure best signal, stability, and precision for mRNA characterization



ELECTROPHORESIS Electrokinetics Fluidics Proteomics

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Development of a microchip capillary electrophoresis method for determination of the purity and integrity of mRNA in lipid nanoparticle vaccines

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2 Materials and methods

2.1 Reagents

RNA reagent kits (Catalog# CLS960010) and RNA labchips (Catalog# 760435) were obtained from Perkin Elmer (Waltham, MA). Brij® 58 was obtained from Acros Organics (Pittsburgh, PA). Formamide was obtained from Sigma-Aldrich (St. Louis, MO). High Range RiboRuler RNA Ladder was obtained from Thermo Fisher Scientific (Norristown, PA).

2.2 mRNA-LNP preparations

LNPs containing mRNA were prepared by our Vaccine Process Development colleagues as previously described [19, 20]. mRNA was encapsulated in LNPs using a self-assembly process in which mRNA is mixed with a solution of lipids dissolved in ethanol [9]. mRNA-LNP samples contained mRNA, a cationic lipid, cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine, and poly(ethylene glycol)2000-dimyristoylglycerol. Empty LNPs were also prepared using the same process but without mRNA.

2.3 Optimized MCE sample preparation

The mRNA-LNP samples were first diluted to 100 µg/mL mRNA in a solution of 10% (w/v) Brij® 58 in formamide, then further diluted in formamide and 5 µL of 10× sample buffer from the RNA reagent kit for a final total sample volume of 50 µL (10 µg/mL mRNA final concentration). The final formamide concentration in the sample was always >80%. All final sample solutions were heated in a 70°C heating block for 10 min, then cooled on ice for at least 5 min. Samples were transferred to a 96-well plate. The RNA labchip was prepared as described in the RNA Assay Quick Guide provided by Perkin Elmer without any modifications.

2.4 Instrument and software

LabChip GXII Touch is an instrument from Perkin Elmer and was used for all experiments. This automated system performs electrophoresis using a "lab on a chip" technology. For the LabChip, gel-sieving matrix containing a blue fluorescent dye is applied to the separation channel, then sample is electrokinetically injected and mRNA binds to the fluorescent dye. Voltage is applied for separation to occur and mRNA migrates through the sieving gel matrix and separates by size. The mRNA signal is observed by fluorescent detection. Separation time is 70 s for each sample to cover the range of 50–6000 nt of RNA size. The electropherogram for each injection was transferred to Waters Empower 3 chromatography software for analysis. An example electropherogram of mRNA from an

mRNA Purity Analysis

analytical chemistry

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Article

Comprehensive Impurity Profiling of mRNA: Evaluating Current Technologies and Advanced Analytical Techniques

Julien Camperi,* Steffen Lippold, Luladey Ayalew, Brian Roper, Stephanie Shao, Emily Freund, Ariane Nissenbaum, Carolina Galan, Qinjingwen Cao, Feng Yang, Christopher Yu, and Axel Guilbaud*

Cite This: https://doi.org/10.1021/acs.analchem.3c05539

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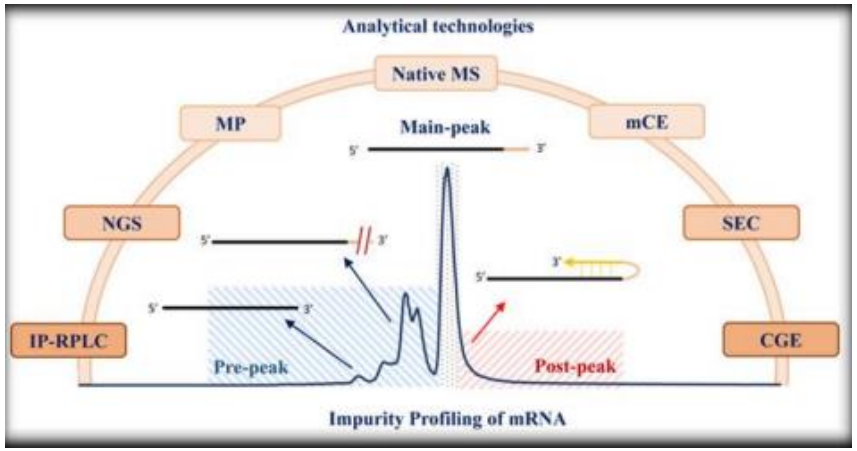
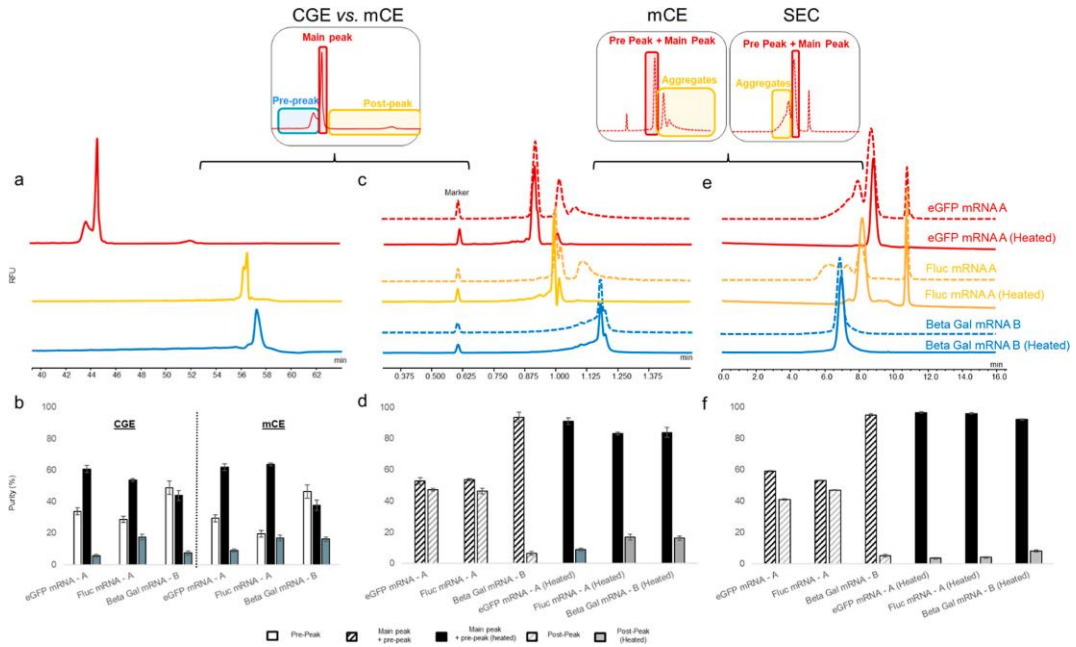


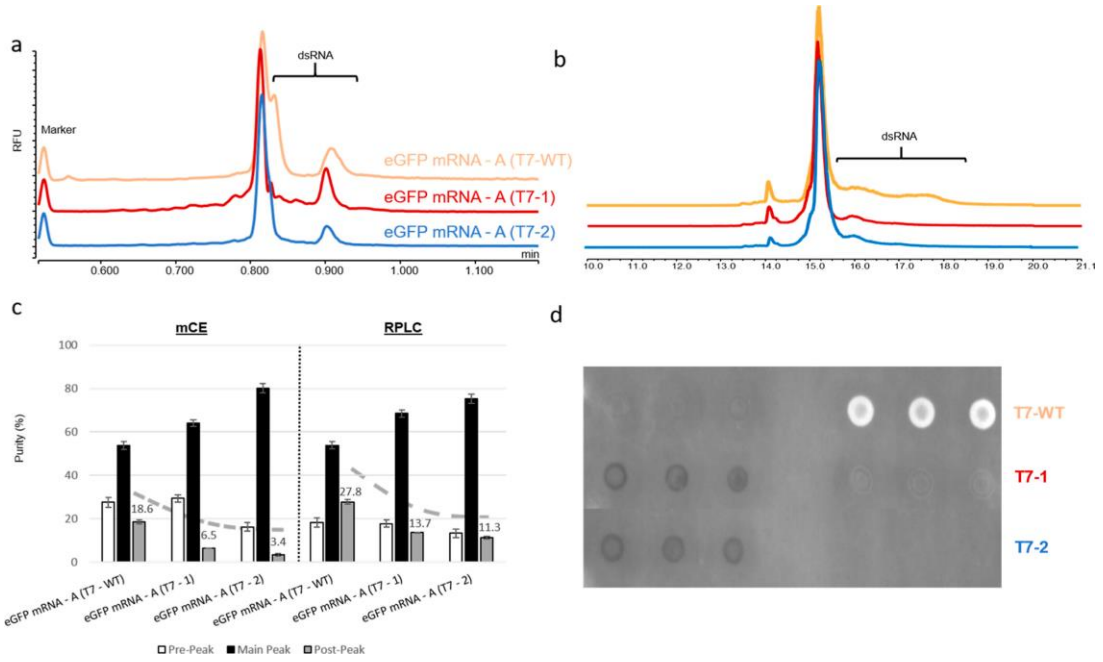
"This comparative analysis suggests that mCE represents a promising advancement in mRNA purity assessment, offering similar separation efficiency, data quality, and shorter run times (1.5 vs 60.0 min) when compared to the conventional CGE method in denaturing conditions."

mRNA Purity Analysis

Overall, it is noteworthy that mCE provided **the same informative results** as conventional CGE and SEC but with a notably smaller amount of material and in a significantly shorter time (<1.5 min).



dsRNA Analysis



Speed Doesn't Necessarily Compromise Data Quality

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

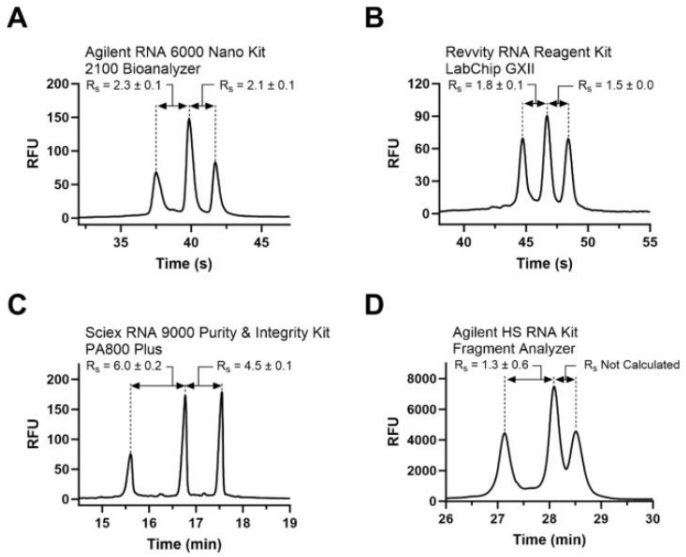
journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Comparison of capillary electrophoresis-based methods for the analytical characterization of purity and stability of *in vitro* transcribed mRNA

Prerana Mantri, Bindiya Juneja, Steven Henderson, Evan Koufos, Youmi Moon, Daniel M. Dayeh, Deanna Di Grandi, Yue Fu *, Kathir Muthusamy *, Peter M. Ihnat, Nisha Palackal, Erica A. Pyles

Protein Biochemistry, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591, United States

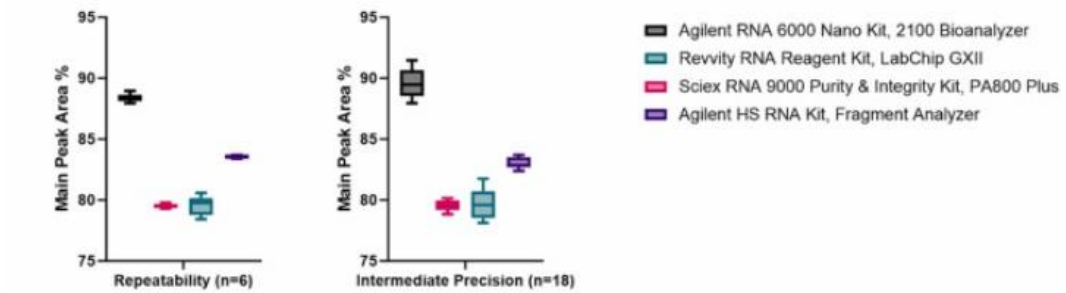
Four Metrics for mRNA Analysis
- Integrity
- Stability
- Resolution
- Precision



Half-Life of Cas 9 mRNA

Revvity RNA Reagent Kit	Sciex RNA 9000 Purity & Integrity Kit
10.5 (10.0–11.1)	11.8 (11.1–12.6)
6.0 (5.5–6.5)	6.5 (6.1–7.0)
5.0 (4.7–5.3)	4.6 (4.1–5.2)
1.6 (1.5–1.8)	1.6 (1.5–1.7)

Precision of Measurements

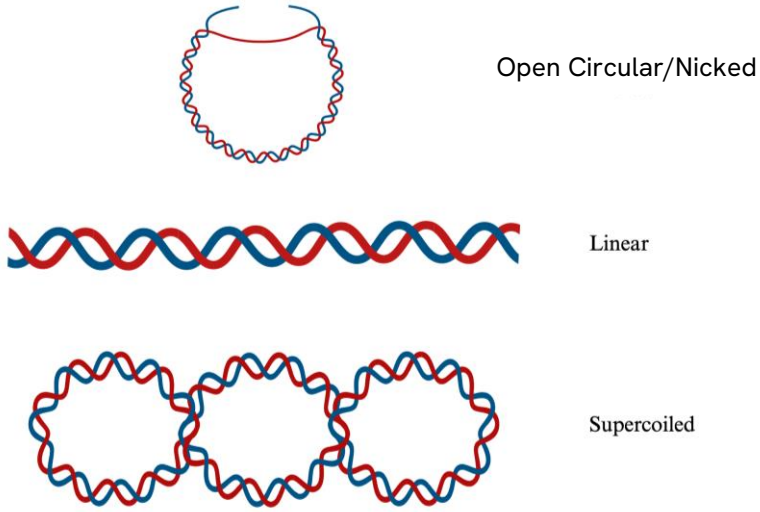


	Repeatability (n=6)			Intermediate Precision (n=18)		
CE-Based Method	Average Peak % Area	Standard Deviation	%RSD	Average Peak % Area	Standard Deviation	%RSD
Agilent RNA 6000 Nano Kit	88.40	0.35	0.40	89.63	1.10	1.23
Revvity RNA Reagent Kit	79.57	0.79	0.99	79.66	1.15	1.44
Sciex RNA 9000 Purity & Integrity Kit	79.51	0.18	0.23	79.54	0.42	0.53

What’s new?

pDNA & Upstream BioProcessing

Sample Conformations



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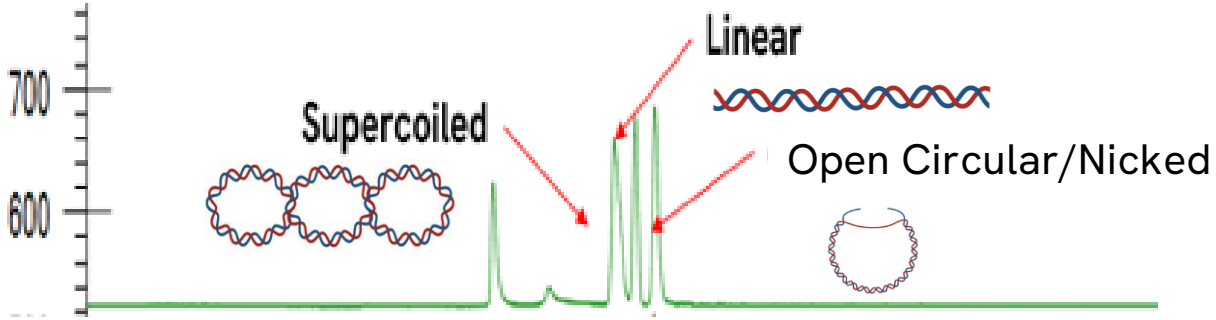
Based upon figure from Ref: DOI:10.1039/C5RA28102D

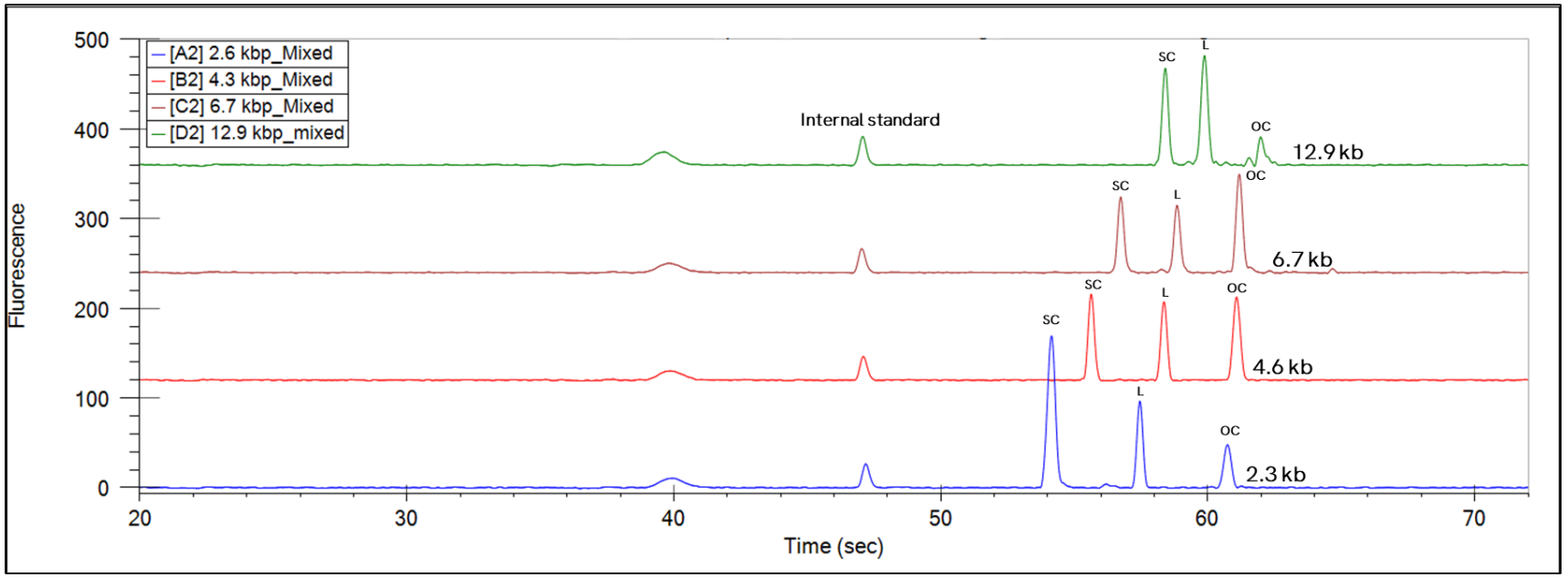
LabChip Assay Results



Created with BioRender.com 2024

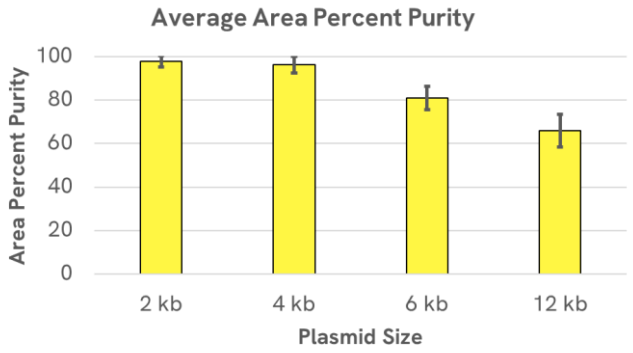
- Regulation and Safety: New Regulatory requirements from US FDA, WHO, European EMA and US Pharmacopeia requiring content of OC <20% in final GMP plasmid production
- Production efficiency: Plasmid manufacturing customers want <5% of OC/N contamination in final product
- Good storage conditions: monitor final product stability during storage to reduce cost and avoid degradation

Plasmid DNA separation and purity



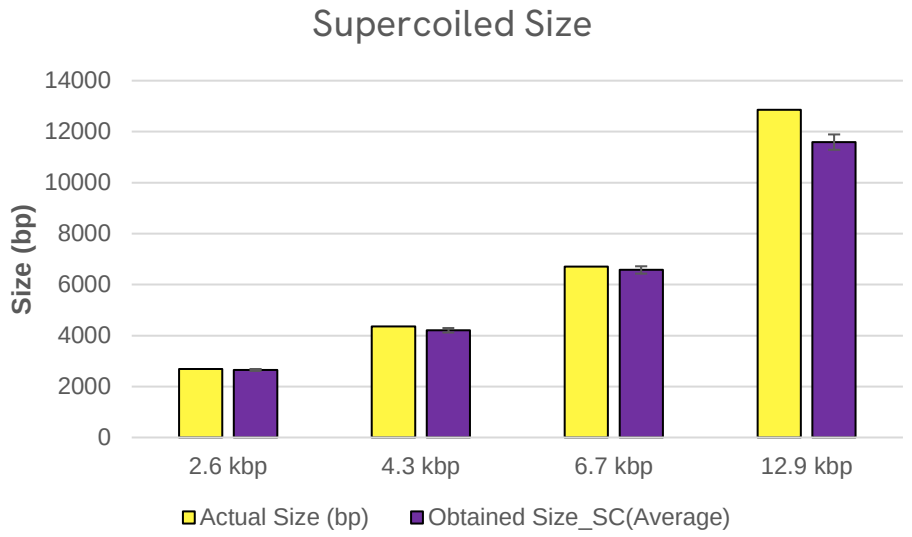
3 isoforms are resolved:
SC, L and OC/N
Sizing SC and L
Purity SC and L
Sensitivity: LOD & No carryover

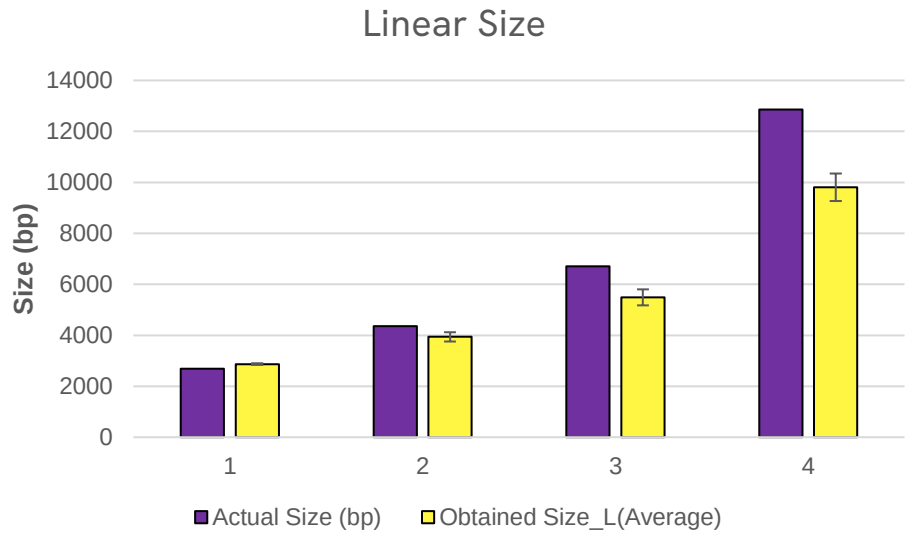
Inter-run CV of SC Percent Purity, 500 pg/uL				
Sip #	2 kb	4 kb	6 kb	12 kb
1	100	94	86.1	73.5
2	100	100	73.7	55.6
3	95.75	100	74.97	69.6
4	90.87	100	81.49	53.97
5	100	100	79.8	67.83
6	100	100	73.02	59.37
7	97.07	91.05	85.15	68.92
8	98.55	98.15	85.7	71.01
9	98.56	93.62	76.01	72.51
10	96.36	93.06	87.84	
11	98.24	96.81	83.27	
12	97.47	89.95	84.58	
AVG	**97.74**	**96.39**	**80.97**	**65.81**
ST DEV	**2.62**	**3.85**	**5.31**	**7.46**
CV	**2.68**	**3.99**	**6.56**	**11.33**



Plasmid DNA sizing

Calculated SC and L sizes in the same run



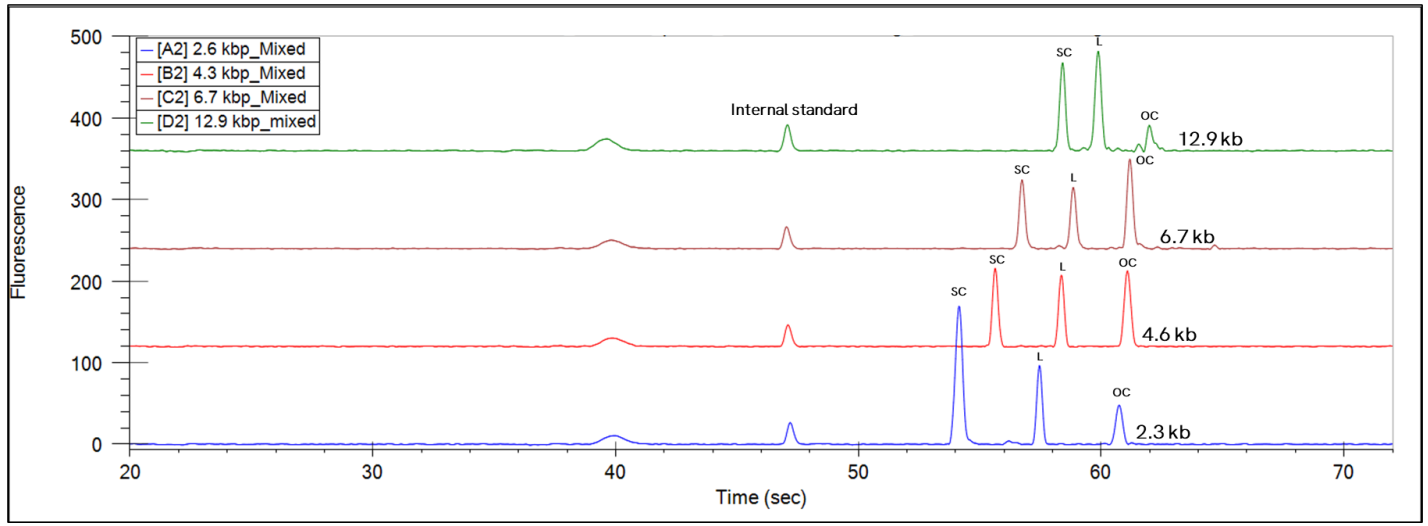


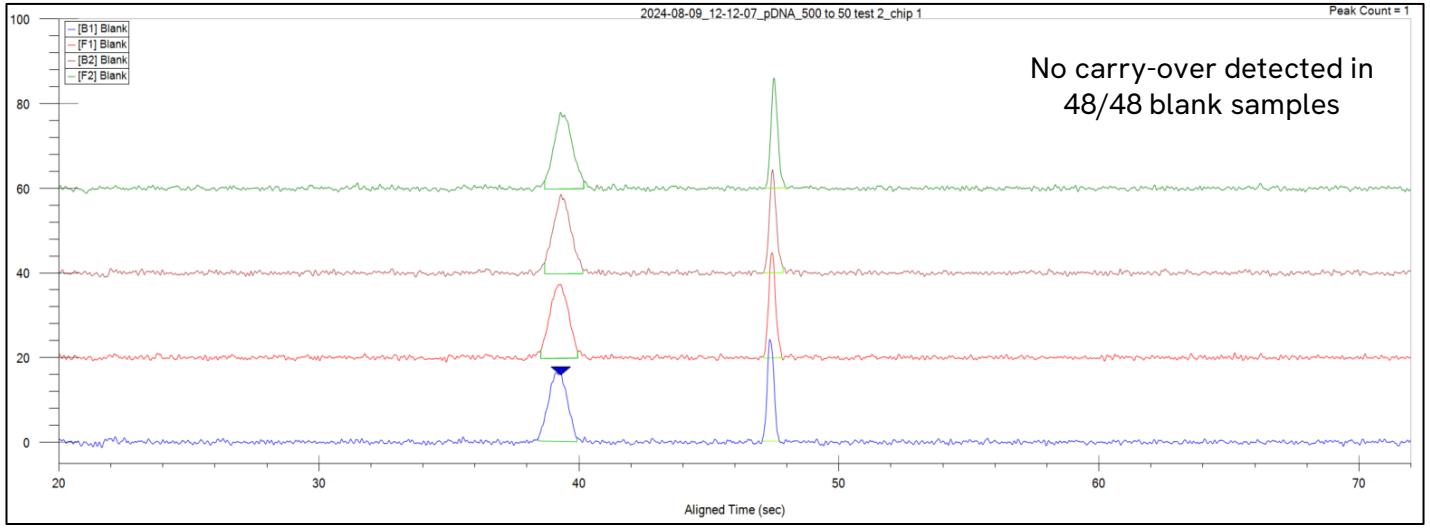
Actual Size (bp)	Obtained Size_SC(Average)	% CV	% Error	Std Dev
2686	2650	1.41	1.35	37
4361	4210	2.16	3.47	91
6706	6579	2.22	1.90	146
12856	11588	2.58	9.86	299

Actual Size (bp)	Obtained Size_L(Average)	% CV	% Error	Std Dev
2686	2870	1.05	6.85	30
4361	3943	4.67	9.58	184
6706	5490	5.64	18.13	309
12856	9809	5.51	23.70	540

Result: SC sizing is accuracy < 10%
Linear sizing accuracy is <20% for <7kbp, and <25% for > 7kbp

No Carryover measured in plasmid DNA assay

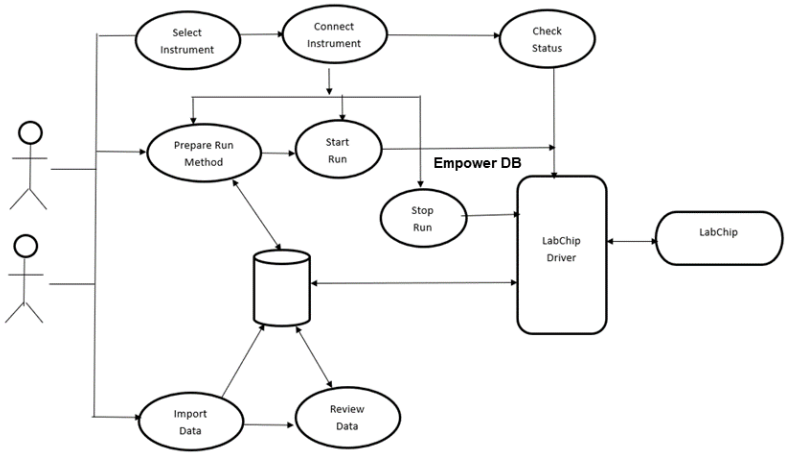


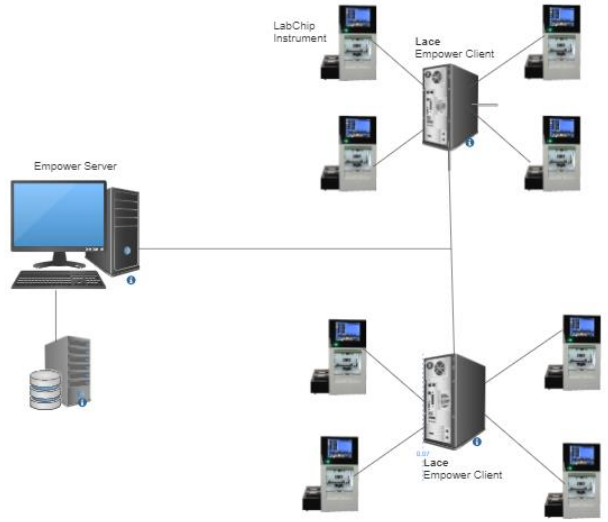


We are looking for labs to further beta test this assay in the field.
If you are interested in this analysis and would like to help ensure it meets your needs, please come by the Revvity booth, or reach out me at James.Geiger@revvity.com

LabChip for QC: Empower Drivers

No.	Requirement
1	User can import the Raw Data to Empower. User can configure the auto import of raw data
2	User can connect multiple GX Touch instruments in Empower
3	User can prepare, start and stop an assay run from Empower
4	User can check instrument and Assay run status from Empower






THANK YOU