# Sensitive and Robust Capillary Electrophoretic Analysis of Host Cell Residual DNA in Biopharmaceutical Samples

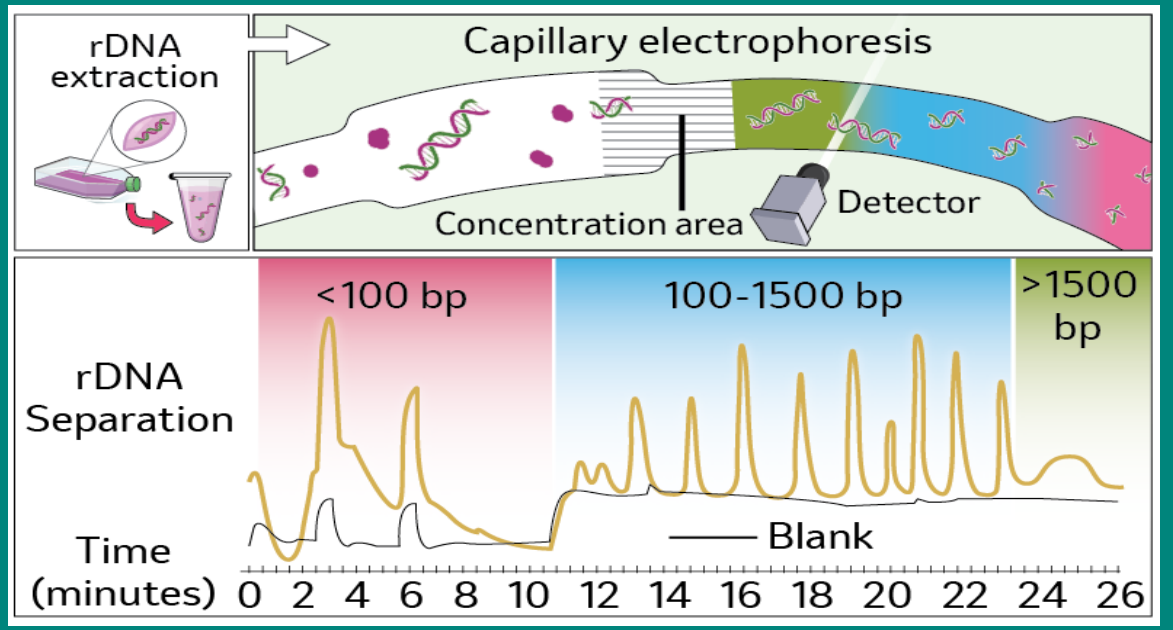
Wan Zhou, Ph.D.

Merck & Co., Inc., Rahway, NJ, USA

@ CE Pharm 2026

Session I: Application and Advancements in CE Innovations

September 28, 2026




**Sensitive and Robust Capillary Electrophoretic Analysis of Host Cell Residual DNA in Biopharmaceutical Samples**
**Laura N. Taylor, Paul F. Gillespie, Richard R. Rustandi, Matthew Schombs, Adam T. Sutton*, Wan Zhou***
***Analytical Chemistry*, 2026, Accepted**
https://doi.org/10.1021/acs.analchem.6c04185

# Acknowledgement

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- Merck Future Talent Program: Jesse Kuiper, Jeremiah James Trausch, David Foley, Kartik Narayan, Daniel J. DiStefano, and Caroline McGregor
- Prof. Lisa Holland, West Virginia University
- Adelis (BIABooster)
- Agilent (Femto Pulse)
- Dr. Cari Sänger - van de Griend

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# Agenda

1. Background
2. Innovative CE Platforms for rDNA Analysis
3. Method Performance
4. Conclusions
5. Future Directions
6. Acknowledgement

# Background

*Control residual host cell DNA size & amount during biopharmaceutical manufacturing — and the analytical challenge is harder than it looks.*

## Regulatory Recommendation

**<10 ng**
**rDNA per dose**
**WHO / FDA limit**

**<200 bp**
**Fragment size**
**requirement**

- Host cell residual DNA (rDNA) is a critical process-related impurity in vaccines and protein therapeutics
- WHO and FDA guidance: <10 ng rDNA per dose (20 ng/mL in 0.5 mL dose) and fragment sizes <200 bp

⚠ Risks: Oncogenic potential and infectivity concerns drive these strict limits

## The Analytical Challenge

**Ultra-Low Concentration Detection**
Detecting DNA at low ng/mL levels in biopharmaceutical matrices requires exceptional analytical sensitivity

**Simultaneous Sizing & Quantification**
Confirming <200 bp fragment size requires sizing capability alongside quantitation — a dual demand few methods meet

**Complex Biopharmaceutical Matrices**
Crude intermediates and formulated final products introduce matrix effects that challenge conventional analytical approaches

https://www.fda.gov/media/78428/download
WHO Expert Committee on Biological Standardization. Fifty-Sixth Report; WHO Technical Report Series, No. 941.

# Why Existing Methods Fall Short

*Current tools force a trade-off between sensitivity, sizing capability, and practical ease — none does it all.*

| Method | High Sensitivity | Size Info | Low Complexity | High Throughput | Key Limitation |
|---|---|---|---|---|---|
| **qPCR** | ✓ | ✗ | ● | ✓ | No size information; species-specific probe design required |
| **PicoGreen** | ✓ | ✗ | ✓ | ✓ | Matrix interference in complex biopharmaceutical samples, No size info |
| **Southern Blot** | ● | ✓ | ✗ | ✗ | Labor-intensive; slow development; low throughput |
| **Agarose Gel** | ✗ | ✓ | ✓ | ✗ | Low sensitivity; poor quantification accuracy |
| **NGS** | ✓ | ✓ | ✗ | ✓ | Complex workflows; high cost; not routine QC-ready |
| **The Gap** | ✓ | ✓ | ✓ | ✓ | **Simultaneously quantify AND size rDNA — high sensitivity, low complexity, fit-for-purpose precision** |

✓ Yes ● Partial ✗ No

qPCR: quantitative polymerase chain reaction

# New CE Platforms Enter the Lab

*We evaluated two fundamentally different CE systems — one ultra-sensitive single-capillary, one high-throughput array — head to head on the same samples.*

## BIABooster

µLAS — Single Capillary System

**Mechanism** **Pressure-driven viscoelastic flow + opposing electrophoresis**

- Concentrates and separates DNA at flow/electrophoresis interface for exceptional sensitivity
- SYBR Gold intercalating dye — in-line fluorescence detection
- Sizing range: 100–1,500 bp (standard quantitative window)

## Femto Pulse

Agilent — 12-Capillary Array System

**Mechanism** **Parallel capillary electrophoresis — multiplexed array format**

- 12-capillary array enables higher-throughput parallel screening
- Independent platform — serves as orthogonal confirmation method
- Simultaneous sizing and quantification capability

**Both platforms: simultaneous DNA sizing AND quantification**

**Same biopharmaceutical samples — head-to-head evaluation**

**Key Ask** **Deliver the sensitivity and robustness required for biopharmaceutical rDNA testing**

µLAS: Micro-Laboratory for DNA analysis and separation

# BIABooster — How µLAS Technology Works

*The µLAS concentration mechanism is what gives BIABooster its exceptional sensitivity — understanding it explains all the optimization choices that follow.*

**Concentration via µLAS**
DNA concentrated at the interface of pressure-driven viscoelastic flow and counter-directional electrophoresis — overcoming the sensitivity limits of conventional CE.

**In-line SYBR Gold Detection**
SYBR Gold intercalating dye labels DNA in-line; fluorescence detection captures the signal with high sensitivity and specificity.

**Simultaneous Sizing & Quantification**
The concentration and separation step enables both fragment sizing and quantification in a single run — no trade-off between sensitivity and size information.

**Systematic Optimization Enabled**
This unique mechanism motivated systematic optimization of every parameter affecting the concentration and separation step — diluent, injection pressure, and voltage.

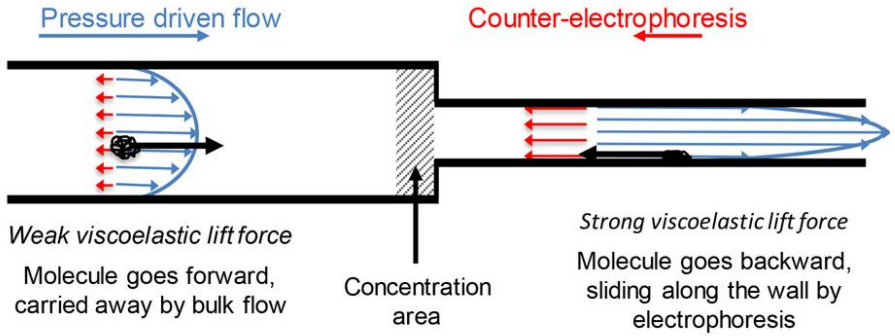
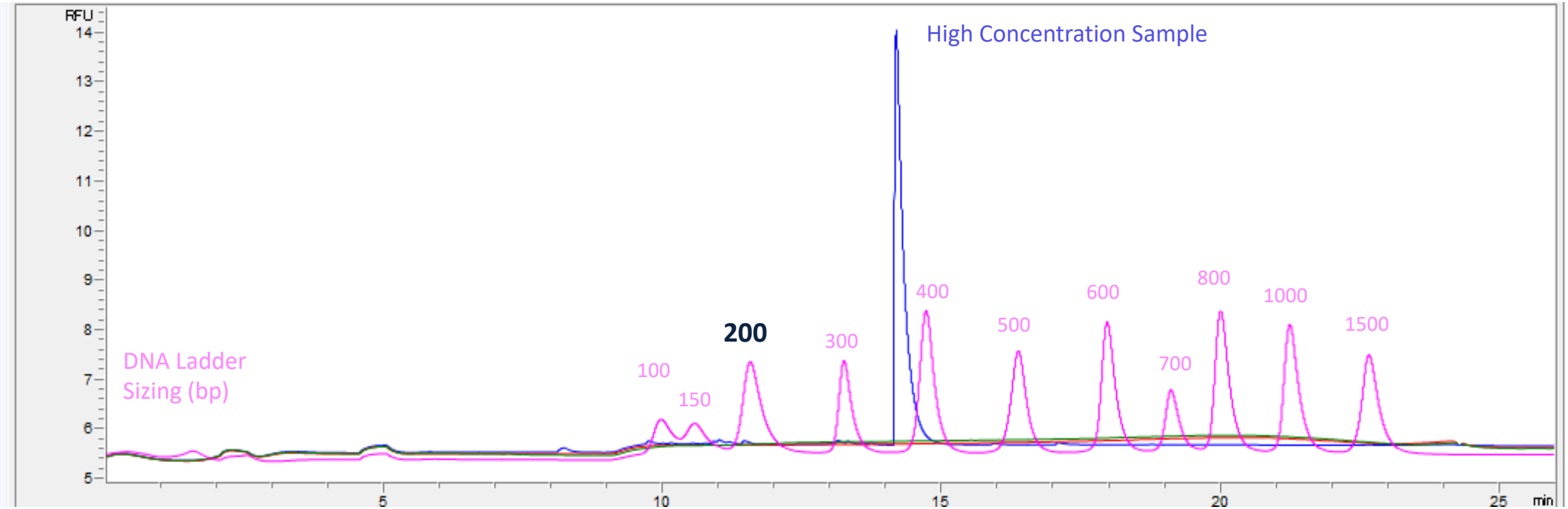



Photo & video source:
https://youtu.be/QgqmTJ4jJuM
https://www.adelis-tech.com

µLAS: Micro-Laboratory for DNA analysis and separation

# BIABooster — How the whole electropherograms look like?



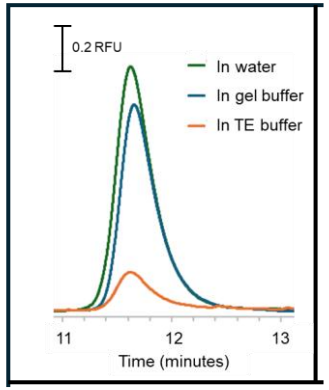
# Optimizing Conditions — Getting Every Parameter “Right”

*Systematic optimization of three key variables — **sample diluent, injection pressure/time, and separation voltage** — was essential to unlock the platform's full potential in rDNA analysis.*

## Sample Diluent

**Parameter**

**Gel buffer vs. water vs. TE buffer**



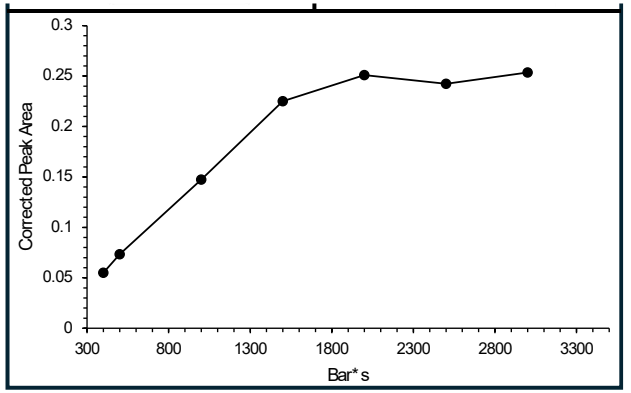

**Optimal Outcome**

Gel buffer: **RSD ≤ 3%**
Water: RSD up to 20%
TE buffer: lower signal intensity

## Injection Pressure / Time

**Parameter**

**Pressure × time product (bar·s)**



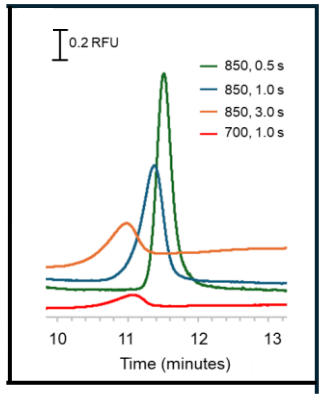

**Optimal Outcome**

Optimal at **1500 bar·s** (5 bar × 300 s)
Plateau response above threshold
No benefit beyond this point

## PM High Voltage (HV)

**Parameter**

**Voltage & rise time**




**Optimal Outcome**

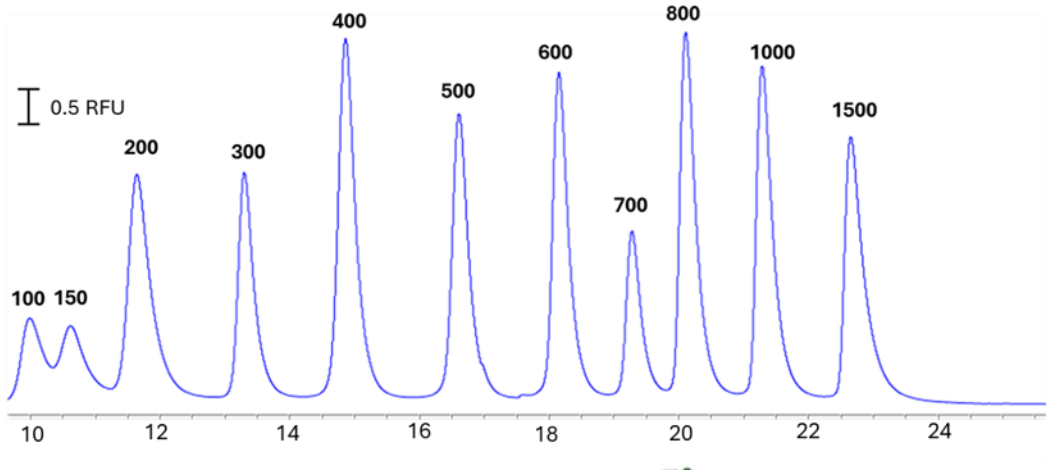
**850 V**, rise time 0.5 s
Highest peak area
Best S/N ratio: **S/N = 64**

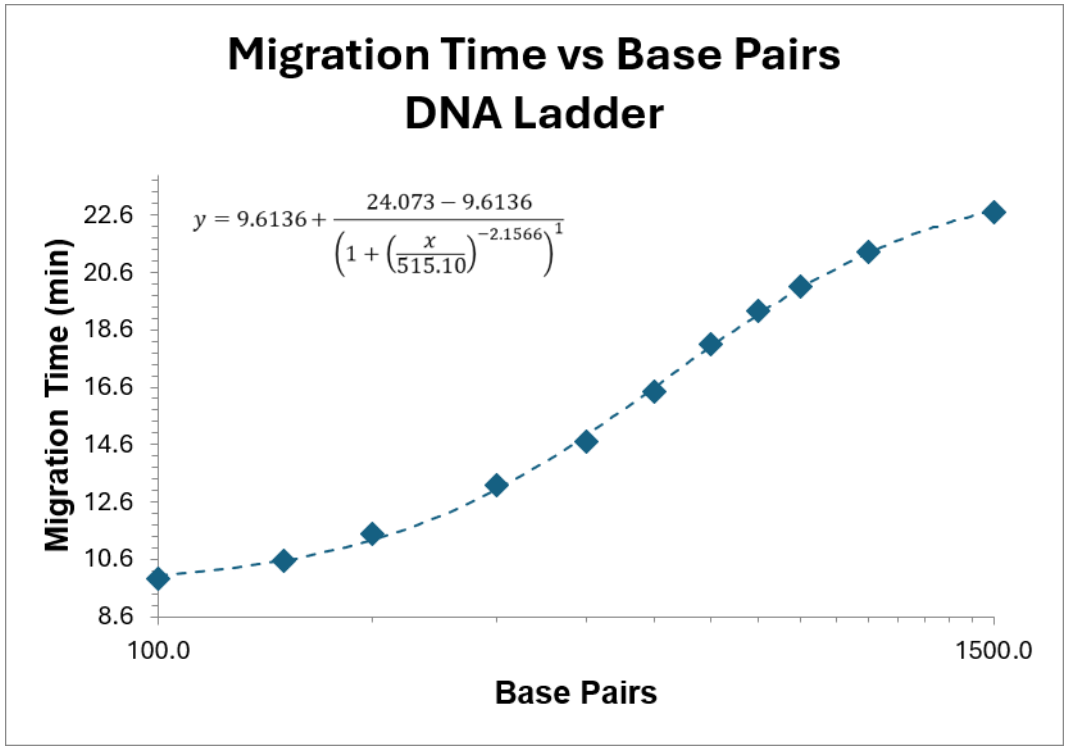
TE: Tris-EDTA

# Sizing Capability — How Broad Can We Go?

*Both platforms well at ~ 200 bp regulatory recommended detection threshold.*

**Dynamic range:** 100-1500 bp

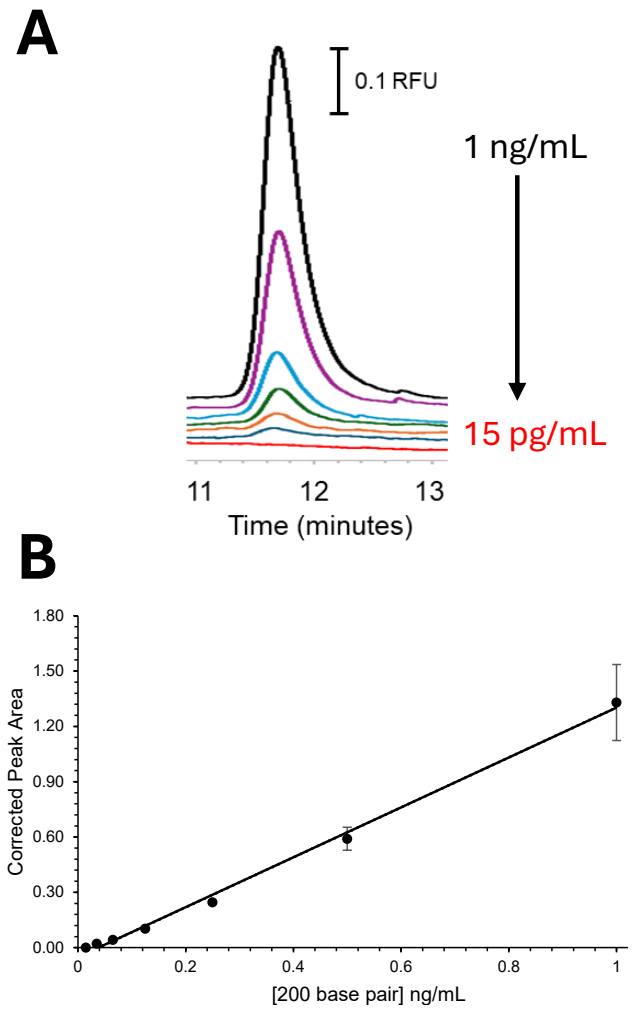



*5-Parameter logistic regression curve*

# Quantitation Sensitivity — How Low Can We Go?

*Both platforms well below the 20 ng/mL regulatory recommended detection threshold.*




**0.035**

BIABooster LOD (ng/mL)

**LOD:** 0.035 ng/mL (S/N = 3.3) at 200 bp

**LOQ:** 0.065 ng/mL (S/N = 10) at 200 bp

**Linear Range:** 0.035 – 1 ng/mL

Over 200-500X than detection threshold

# Now to the Real World — Our Biopharmaceutical Samples

*Analyzing standards is necessary but not sufficient — the true test is performance on actual manufacturing samples with complex matrices.*



### Sample 1 — In-Process Intermediate (IPI)

**Yeast-Derived**

Upstream manufacturing intermediate; high complexity matrix with significant rDNA expected.

### Sample 2 — Final Product

**CHO Cell-Derived**

Extensively purified; rDNA expected near or below detection limits.

**Testing Strategy**

Analyze both samples on BIABooster; confirm with Femto Pulse and/or E-gel orthogonal methods.



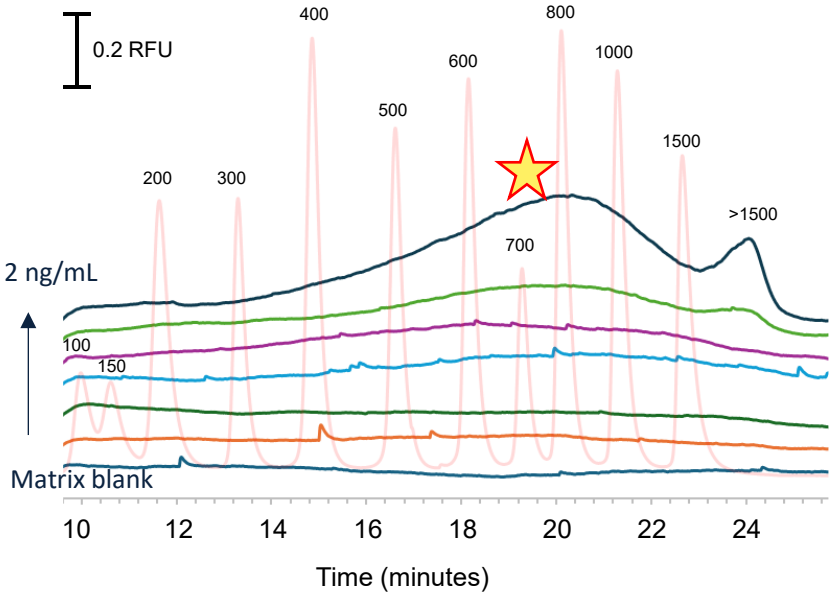
# IPI Sample — What Three Methods Agree On

*Orthogonal confirmation across BIABooster, Femto Pulse, and E-gel builds confidence that the CE result reflects true sample biology, not analytical artifact.*

**A**

**BIABooster**

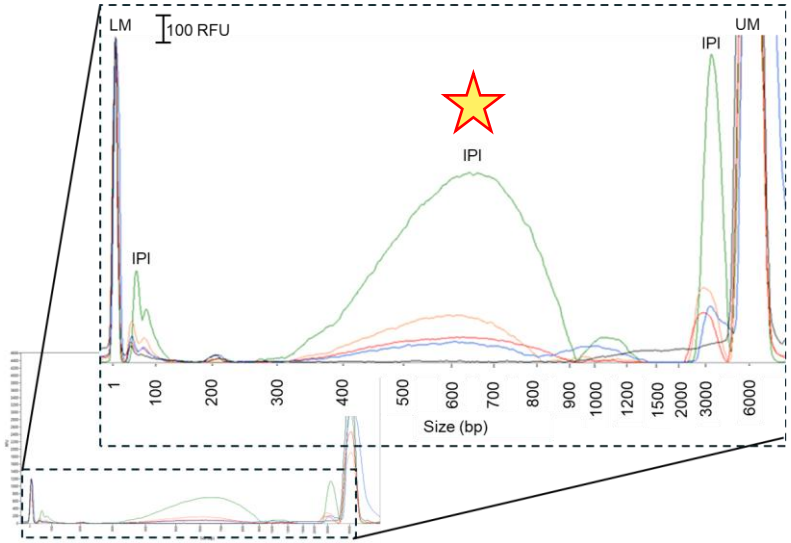
*µLAS single-capillary CE — primary method*




**B**

**Femto Pulse**

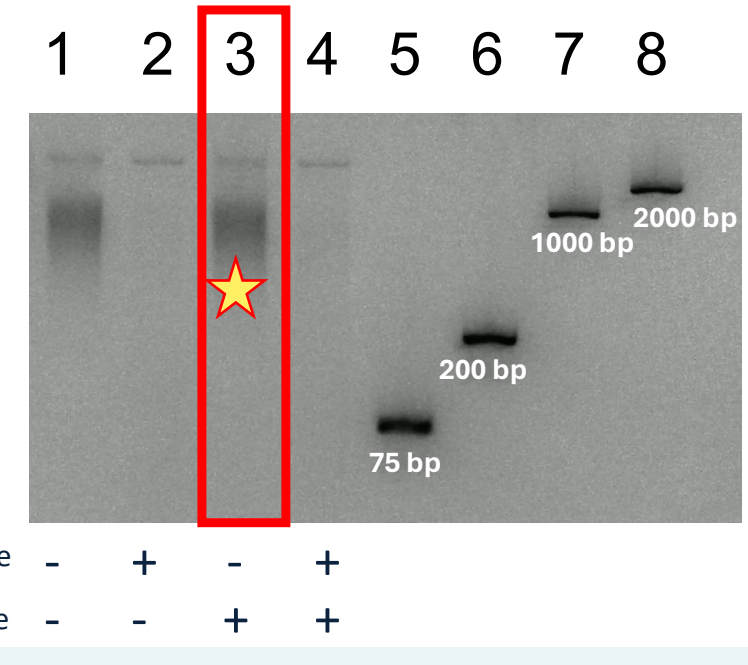
*12-capillary CE array — independent CE platform*




**C**

**E-gel**

*Gel electrophoresis — orthogonal reference*



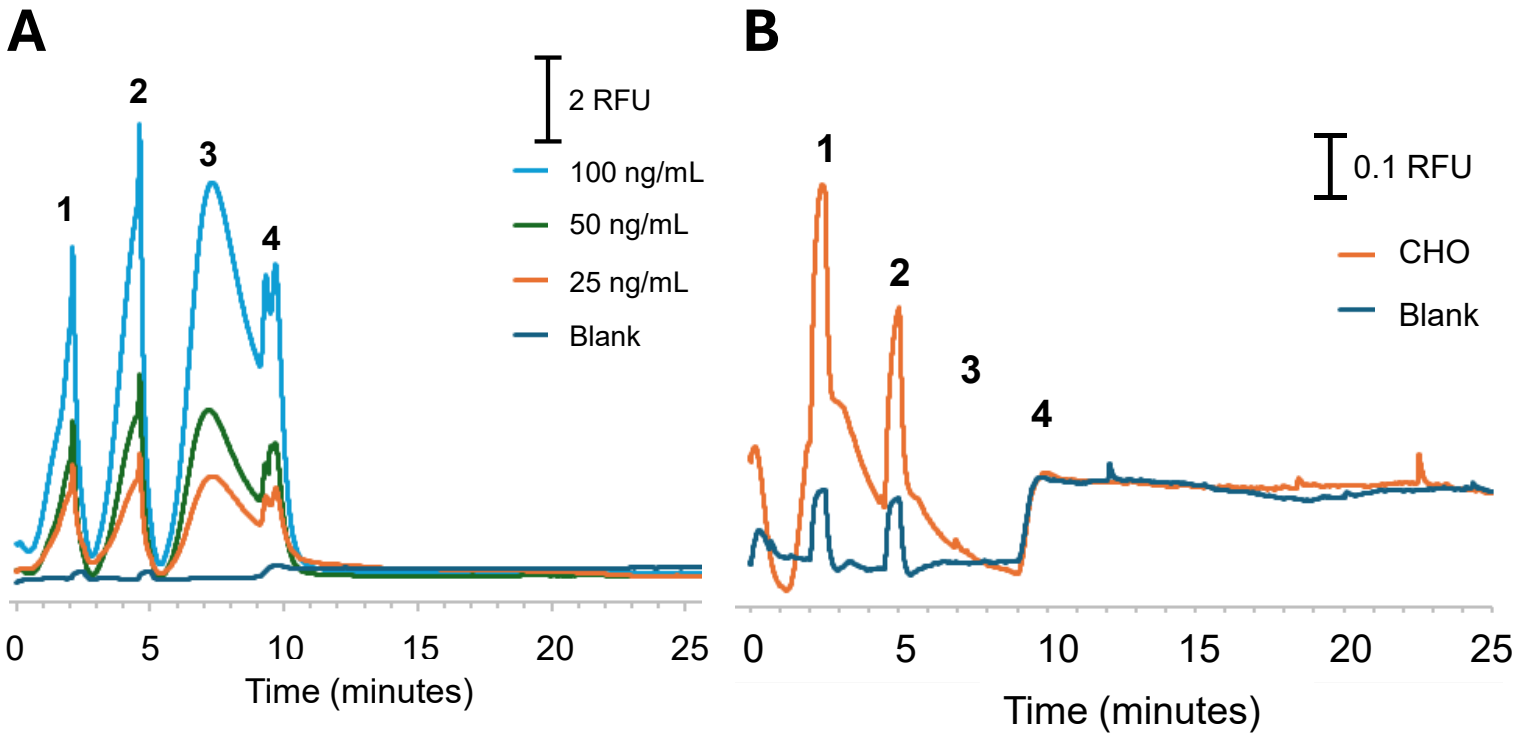

**All three orthogonal methods in agreement** — *cross-validates BIABooster result as true sample biology*

# Exploration Beyond the Standard Range

Qualitative detection extends below 100 bp and above 1500 bp — capturing fragments that matter in real biopharmaceutical samples but fall outside the quantitative window.

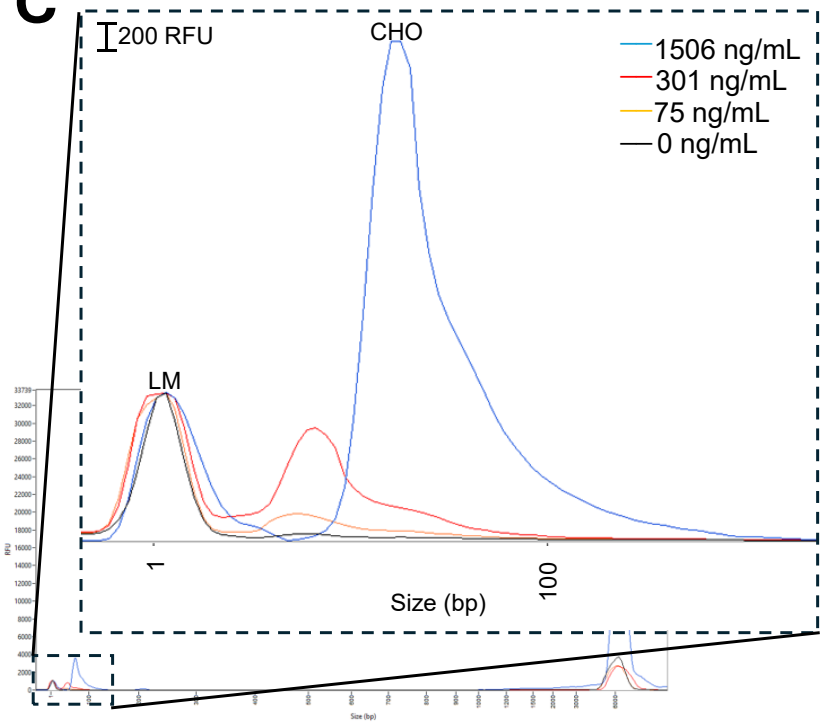
75 bp fragment DNA standard

CHO Final Product — Extensively Purified




**Femto Pulse**

*12-capillary CE array — independent CE platform*

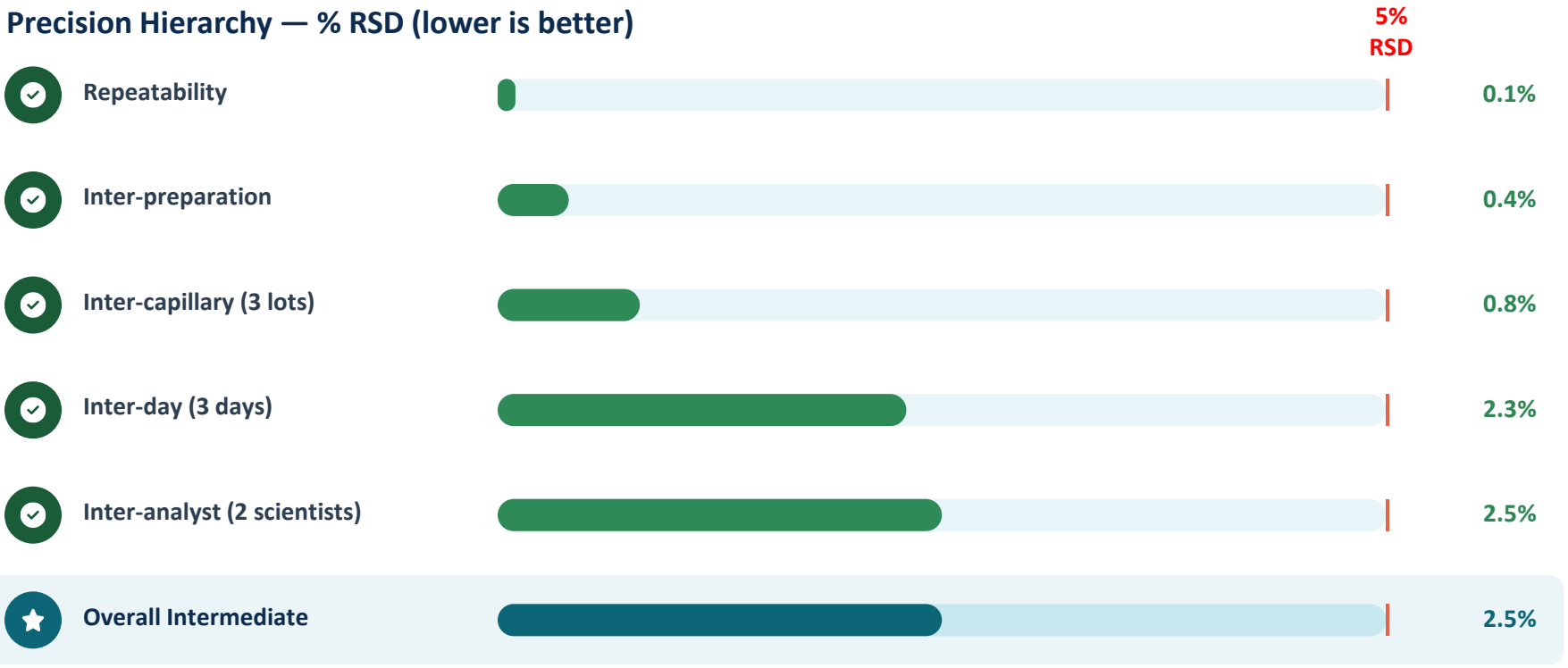



Qualitative analysis beyond 100-1500 bp

# Method Performance — Can We Trust the Numbers?

*Accurate sizing and robust performance in rDNA analysis; spiking 200, 400, and 1000 bp in biopharmaceutical samples*

**Precision Hierarchy — % RSD (lower is better)**




**<5%**

Intermediate Precision

**Analytical Method Performance**

Intermediate precision of **2.5% RSD** achieved across analysts, days, and capillary lots — supporting sensitive & robust rDNA analysis.

# Head-to-Head — BIABooster vs. the Field

When compared directly against established rDNA methods, BIABooster occupies a unique position: high sensitivity with simultaneous sizing and low operational complexity.

**BIABooster LOD: 0.035 ng/mL — high sensitivity; high sizing accuracy (99.8–104.2%), robust performance (2.5% intermediate precision) on real biopharmaceutical matrices**

| Method | Sensitivity | Direct Sizing | Probe-Free | Operational Complexity | Development Time |
|---|---|---|---|---|---|
| **BIABooster** | Very High (0.035 ng/mL) | 100–1500 bp (Qualitative sizing beyond this range) | Yes | Low | Short |
| **qPCR** | Very High | None | No | Moderate | Long |
| **PicoGreen** | High | None | Yes | Low | Short |
| **Southern Blot** | Moderate | Yes (probe) | No | High | Long |
| **NGS** | Very High | Yes | Yes | Very High | Very Long |

**Single platform covers both process stages**

**Reduced method development vs. qPCR & Southern blot**

**Robust performance, suitable for method training & transfer**

# Conclusions

*Delivers the sensitivity, sizing accuracy, and robustness that biopharmaceutical rDNA analysis demands — real sample results prove it works where it counts.*

## Sensitivity

BIABooster LOD of **0.035 ng/mL** at 200 bp — over 500X exceeds regulatory sensitivity recommendation.

## In-Process Intermediate

rDNA profile fully characterized: predominant peak at **820 bp** with qualitative detection of shoulder fragments **>1500 bp** . Profile confirmed by Femto Pulse and E-gel.

## Intermediate Precision

**<5% RSD** across all variability sources — repeatability, inter-capillary, inter-day, and inter-analyst

## Purified Product

Correctly reports **no detectable DNA** in the 100–1500 bp quantitative range; sub-100 bp fragments detected qualitatively — confirming purification efficacy.

## Sizing Accuracy

**99.8–104.2%** accuracy confirmed on real yeast IPI and CHO final product samples across 200, 400, and 1000 bp size markers.

**Single-platform solution** — BIABooster simultaneously quantifies AND sizes rDNA across the full biopharmaceutical purification spectrum, filling the gap left by qPCR, PicoGreen, and conventional gel methods.

# Future Directions

**Near-Term**
## Complex Matrices

- Evaluate simultaneous sizing & quantification in highly complex matrices: crude cell lysates, harvest material, unprocessed bulk
- Assess performance across broader expression systems beyond yeast and CHO

**Long-Term**
## Integrated Micro-CE & High Throughput

- Explore integration with micro-CE platforms: combined on-chip sample lysis, extraction, and electrophoresis in a single system
- Transform routine rDNA testing via fully integrated, miniaturized workflows
- High throughput sample preparation & analysis

# Acknowledgement

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Meet the Team:



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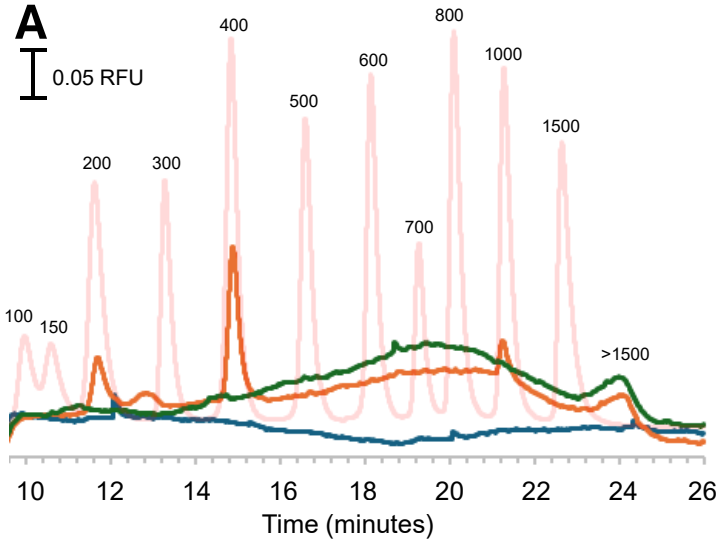
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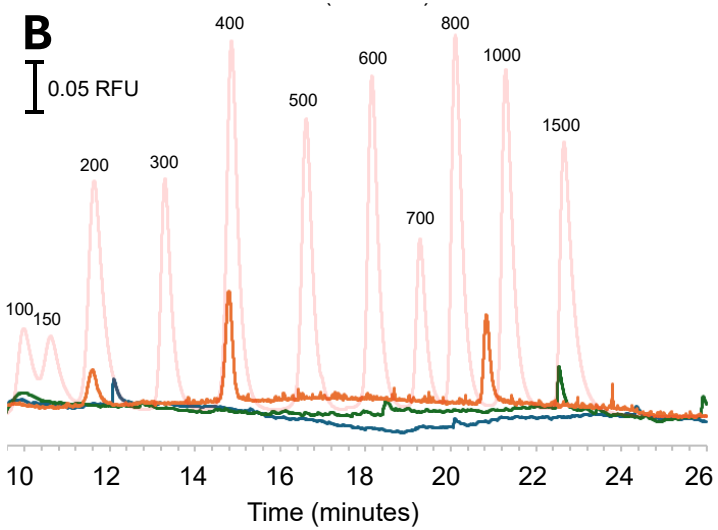
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# Analyze rDNA in Biopharmaceutical Samples

IPI (crude) → CHO (purified)

Bioprocess Purification




**Testing Strategy**

Analyze both samples on BIABooster; confirm with Femto Pulse and E-gel orthogonal methods.

# Comparison of Methods in this work and from Literature for DNA Sizing and Detection

*Current tools force a trade-off between sensitivity, sizing capability, and practical ease — none does it all.*

**Table S6: Comparison of Methods in this work and from Literature for DNA Sizing and Detection**

| Parameter | BIABooster | Femto Pulse | Other BIABooster CE | qPCR | PicoGreen Assay | Southern Blot | Agarose Gel Electrophoresis | Conventional CE |
|---|---|---|---|---|---|---|---|---|
| LOD | 0.035 ng/mL (without dilution of matrix) | 0.211 ng/mL (prior to marker addition) | 0.01 ng/mL [a] | ~3 copies/ reaction | 0.025 – 0.05 ng/mL | 0.0001 – 0.001 ng [e] | 0.5 - 5 ng/band [e] | 0.05 ng/mL |
| LOQ | 0.065 ng/mL (without dilution of marix) | 0.641 ng/mL (prior to marker addition) | ~0.033 ng/mL [b] | 10–50 copies/ reaction | 0.050-5 ng/mL [d] | 0.001 – 0.005 ng [e] | 5–10 ng/band [e] | 0.05 - 10 ng/µL |
| Dynamic Range | $10^3$ | $10^3$ | $10^3$ | $10^3$–$10^8$ | $10^4$–$10^5$ | $10^2$–$10^3$ | $10^2$ | $10^2$–$10^4$ |
| Fragment Size Range | 0.1 – 1.5 k bp | 0.1 – 6 k bp | 0.1 - 10 kbp [c] | Target-specific only | No sizing capability | 0.5–13 kbp | 100 bp - 25 kbp [c] | 35 bp-20 kbp [c] |
| Throughput | Moderate-High | High | Moderate-High | Very High | Very High | Very Low | Moderate | Moderate-High |
| Cost | Moderate - High | Moderate - High | Moderate - High | Moderate | Low | Moderate | low | Moderate-High |
| Operational Complexity | Low | Moderate | Low | High | Low | high | Low | Low |
| Sizing Accuracy | 99.8-104.2% | N/A | <3% error | N/A | N/A | 2.5 - 5% error | 2 - 10% error | 5–10% error |
| Hands-On Time | Low | Moderate - High | Low | Moderate | Very Low | high | Moderate | Moderate |
| Quantification Precision | 2.5% | N/A | ~20% | <10% | < 5% | 2.5 - 15% | Semi-quantitative | <3% |
| Reference | In this work | | 1,2 | 3,4 | 5–7 | 8–10 | 11–13 | 14,15 |

[a] For a 1000 bp standard

[b] Estimated based on LOD calculation: LOQ = 3.3 * LOD

[c] Depending on sieving matrix

[d] Depending on plate reader

[e] Loaded mass depending on detection method and probe

# BIABooster Capillary Diagram

3 different inner diameter capillaries fused together

