Optimizing Pulsed Field Gel Electrophoresis for Accessible Sample QC in Long Read Sequencing Workflows

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03/25/25

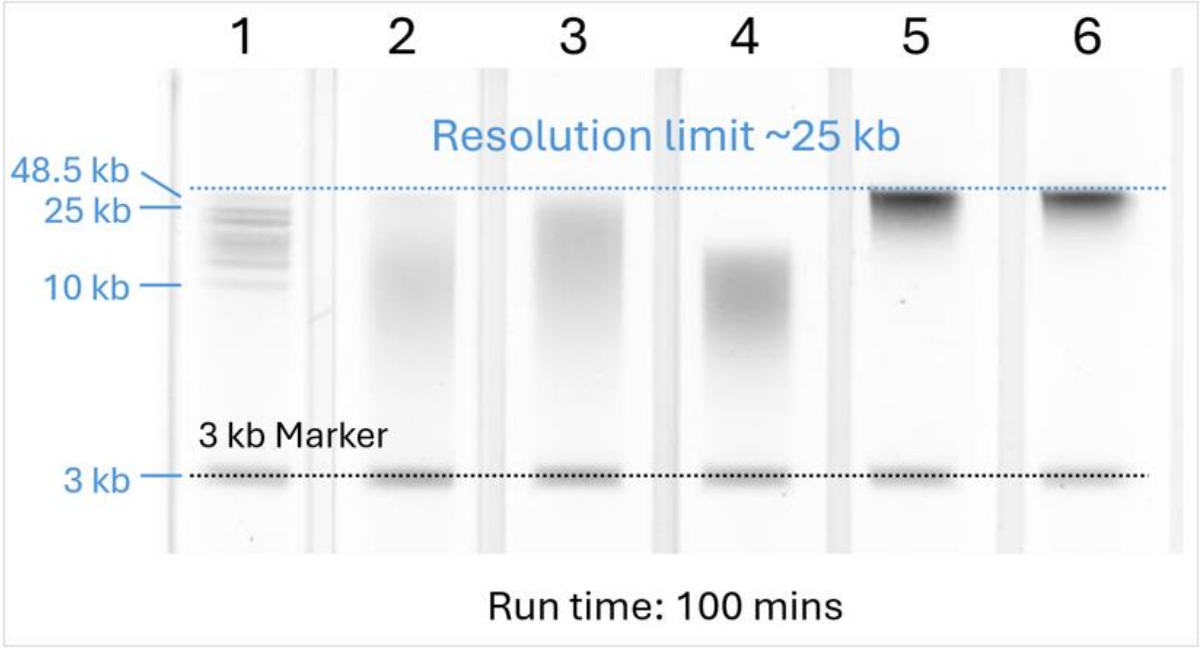
Goal: Fragment Length QC for Large DNA

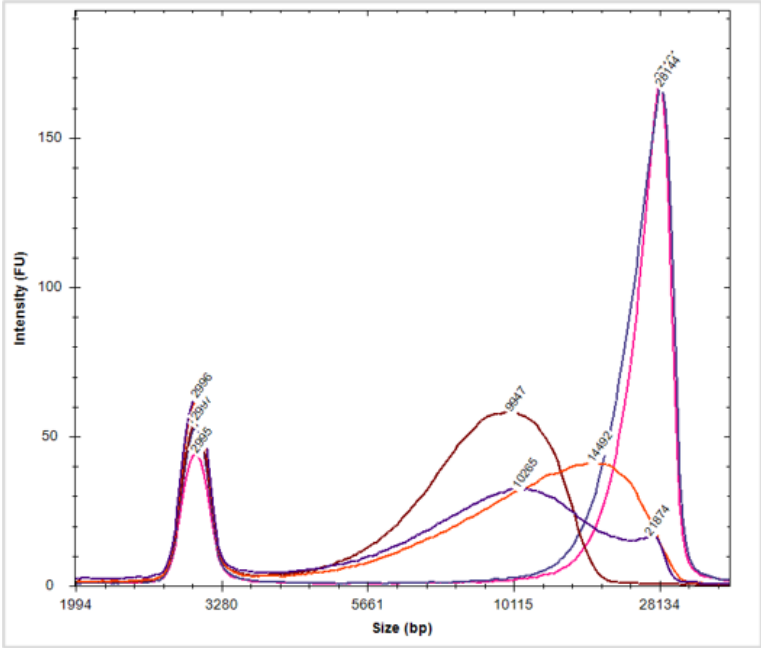
Resolution to 50 kb+ in < 2 hrs

DC electrophoresis equipment is **ubiquitous** and **accessible**, but suffers from a **resolution limit of ~25 kb**

Lane 1
GeneRuler High Range DNA Ladder

Lanes 2-6
gDNA sheared under different conditions

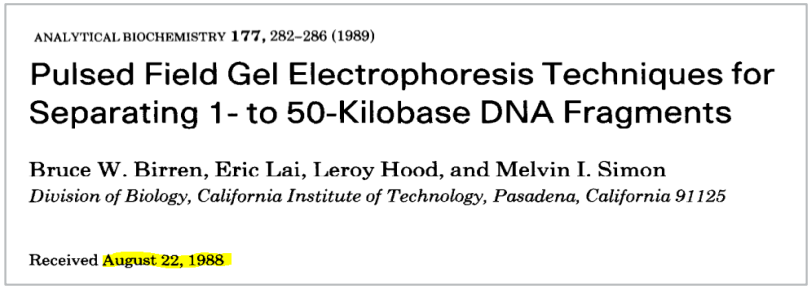




Pulsed-field electrophoresis equipment exists for this purpose, but is generally **expensive** and/or **operationally burdensome**

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Hypothesis: A Solution Exists within the Parameter Space of Established Electrophoresis Techniques

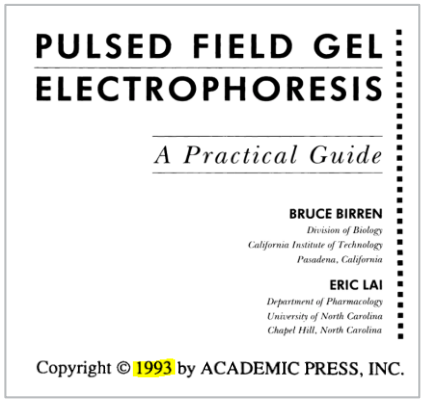


ANALYTICAL BIOCHEMISTRY 177, 282–286 (1989)

Pulsed Field Gel Electrophoresis Techniques for Separating 1- to 50-Kilobase DNA Fragments

Bruce W. Birren, Eric Lai, Leroy Hood, and Melvin I. Simon
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PULSED FIELD GEL ELECTROPHORESIS

A Practical Guide

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Deoxyribonucleic Acid, Theory of Techniques for Separation

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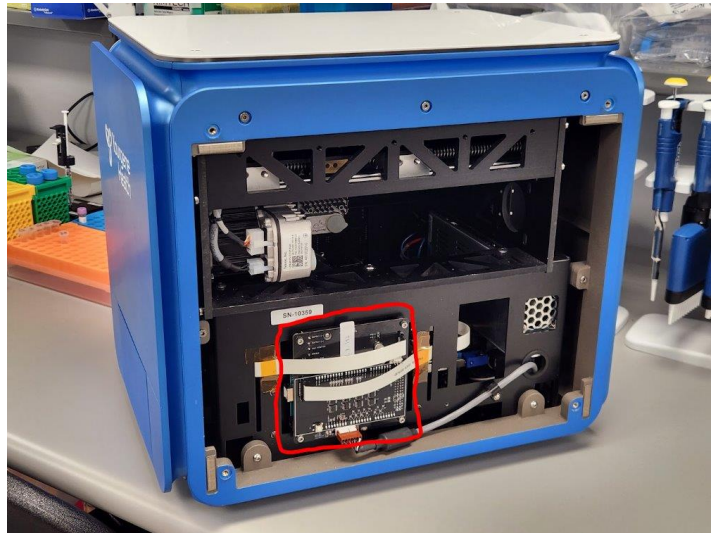
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Parameter	Range to Explore
Electric field strength	1-10 V/cm
Electrophoresis mode	Direct current (DC) Symmetric field inversion Asymmetric field inversion
Switching times	10-2000 ms
Gel percentage	0.3% 0.4% 0.5%
Buffer composition	1xTBE, 0.5xTBE
Gel dimensions	Length: 3-6 cm Thickness: 4-10 mm
Gel format	Pre-cast cassette, no circulating buffer

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The Journey: Exploring Electrophoresis Parameter Space

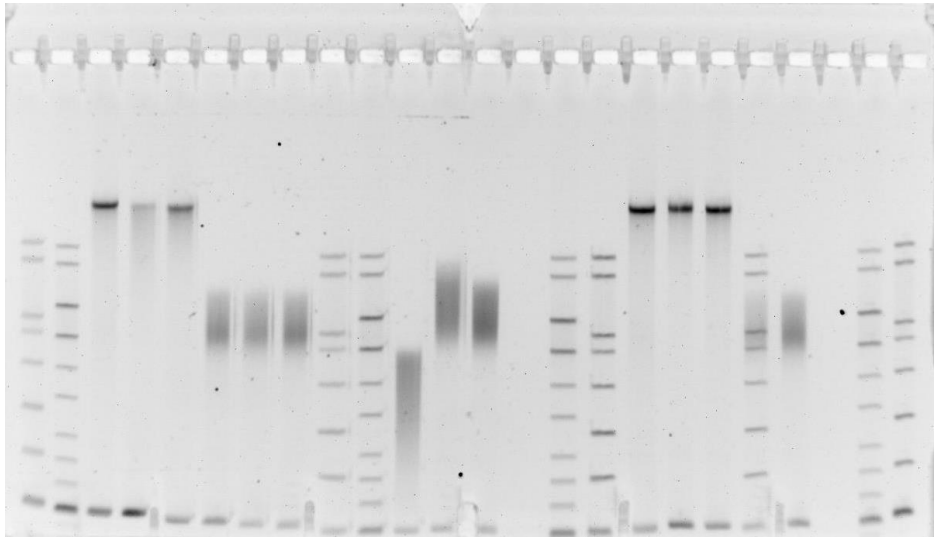
Custom Programmable Pulsed-Field Electrophoresis Power Supply



Rapid Prototyping to Test Different Gel Cassette Dimensions



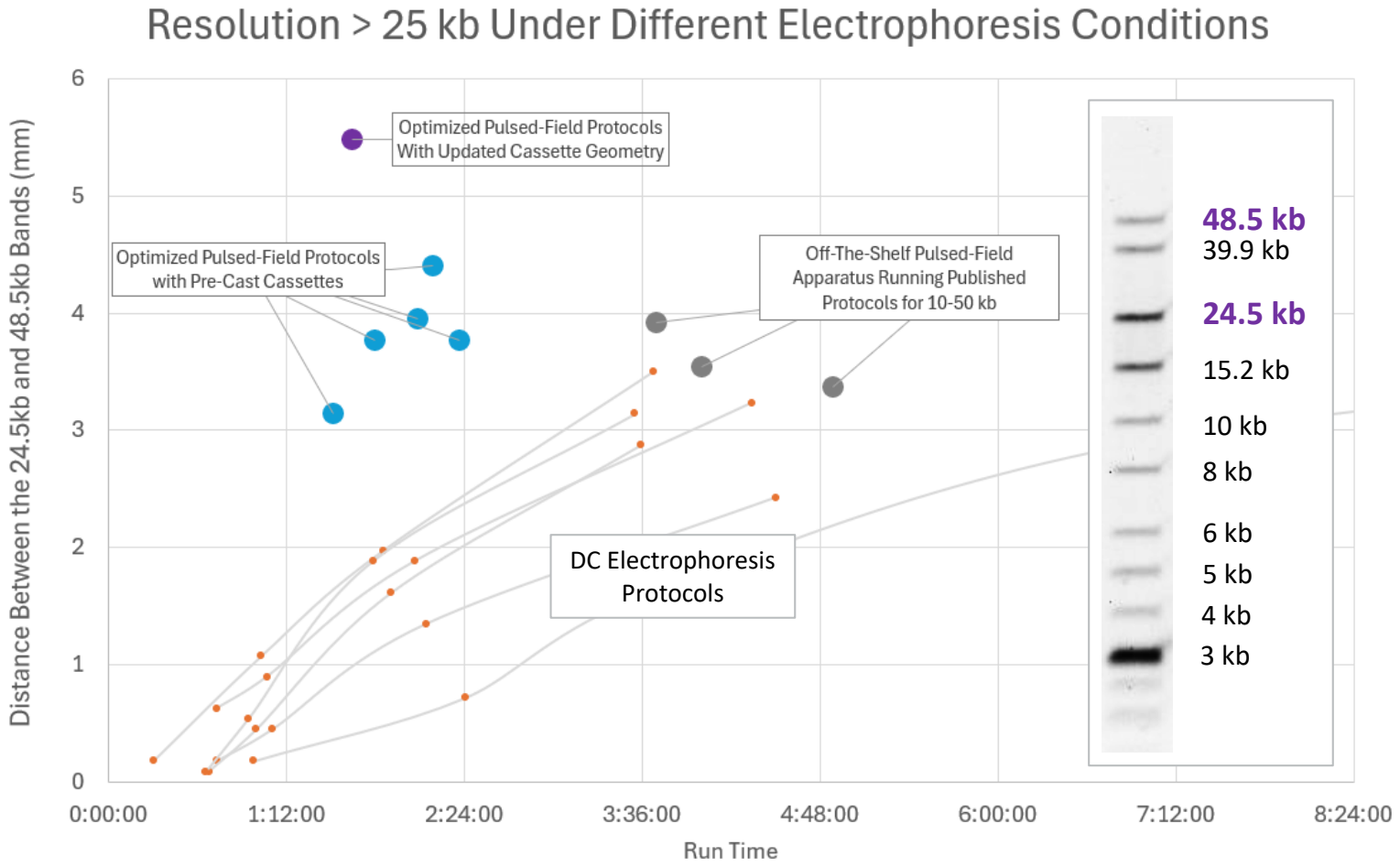
Real-Time Fluorescent Image Capture



- In-house engineering expertise was used for design, testing, and fabrication of custom hardware
- Rapid prototyping performed with the Form3 3D printer (cassette base) and laser cut acrylic (cassette top plates)
- Electrophoresis and image capture performed with LightBench® automated electrophoresis instrument
- Image analysis performed using ImageJ, MS Excel, and custom software tools written in C#

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Results: What did we find?



Parameter	Optimized Value
Electric field strength	8 V/cm (forward) 3 V/cm (reverse)
Electrophoresis mode	Asymmetric field inversion
Switching times	150-800 ms
Gel percentage	0.5% Agarose
Buffer composition	1xTBE
Gel dimensions	Length: 6 cm Thickness: 4 mm
Gel format	Pre-cast cassette, no circulating buffer

Metric	Performance
Functional Range	3-100+ kb
Run time	100 minutes
Sizing accuracy	<10% bias
Sizing precision	<10% CV

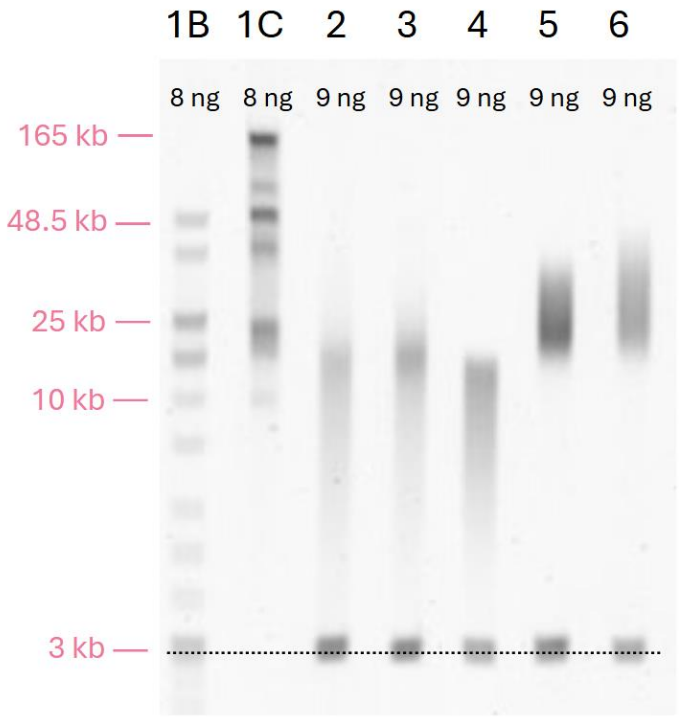
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Ongoing: External Collaboration & Validation



This protocol is now being tested at external collaborator sites:

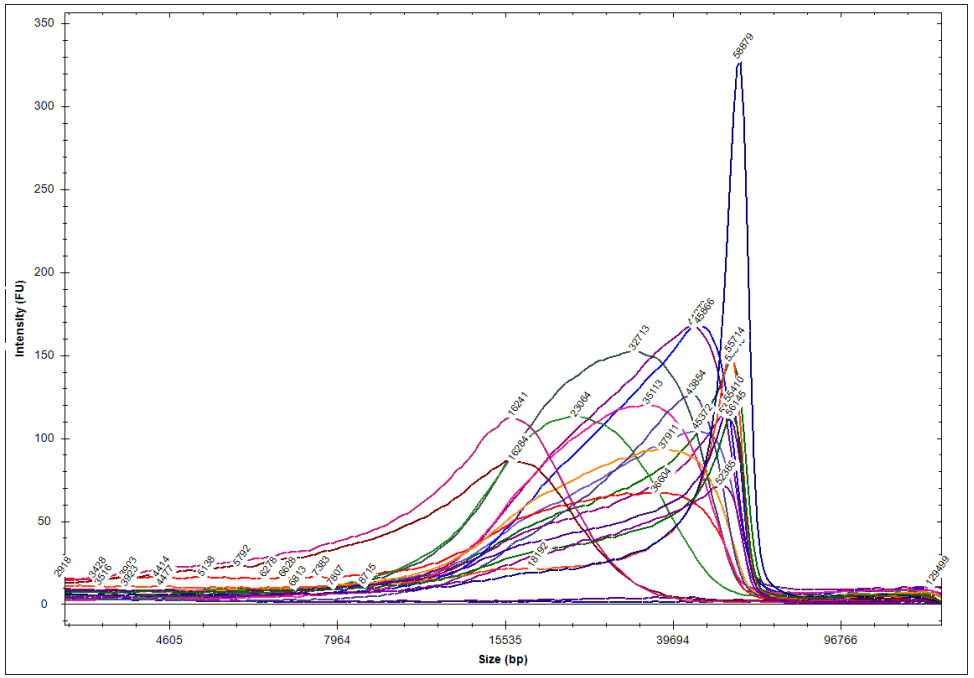
- **PacBio** (Menlo Park, CA)
- **HudsonAlpha Institute for Biotechnology** (Huntsville, AL)
- **Michael Smith Genome Science Centre** (Vancouver, Canada)



Run time: 100 mins

Lane 1B: GeneOn 50 kb DNA Ladder
Lane 1C: 165 kb Femto Pulse DNA Ladder
Lanes 2-6: gDNA sheared under different conditions

Sample provided by PacBio, Menlo Park, CA.



The Harkess Lab at the HudsonAlpha Institute for Biotechnology now relies on this protocol for fragment length QC for PacBio library construction:

- **Check integrity** of extracted DNA
- Confirm **shearing efficacy**
- **Inform molar dilution** prior to sequencing.

The traces above are taken from one of their QC runs.

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- *Camille Connor*

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- *Pawan Pandoh*
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Thanks for listening!

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