

The BluePippin System: Automated Size-selection of High Molecular Weight DNA

Chris Boles, Ezra Abrams, Simran Singh, Sadaf Hoda, Todd Barbera
Sage Science, Beverly, MA



Introduction

A new version of the Pippin Prep electrophoresis system has been developed with enhancements to the optical system and electrophoresis power supply. The new optical system is designed for fluorescein-like dyes, and offers the potential to use DNA markers labeled with covalently-linked fluorescein analogues as internal standards. For band capture applications, cassettes with thiazole orange, a blue-responsive fluorescent DNA dye, will be offered.

The new power supply can operate in three modes:

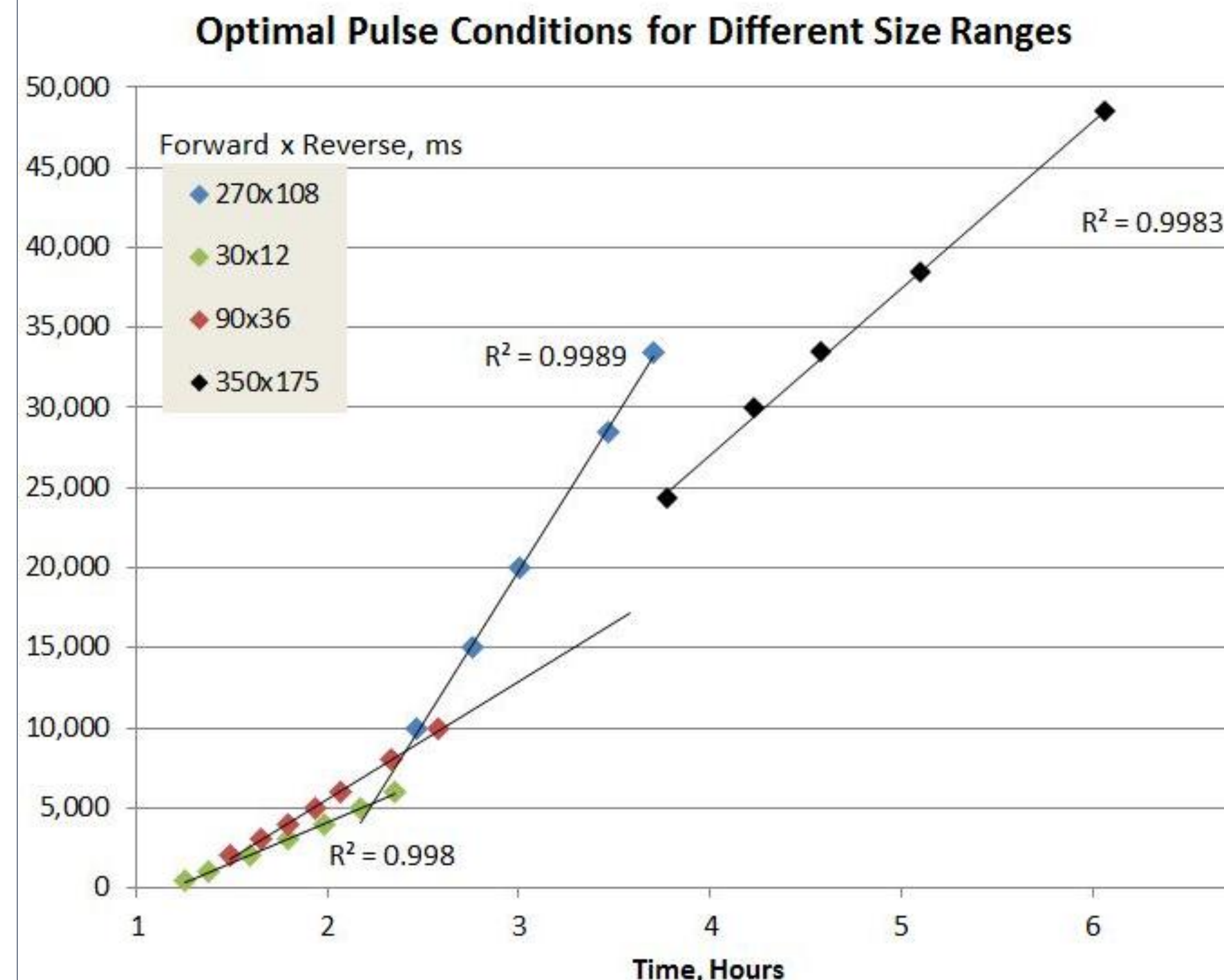
- 1) a high voltage DC mode (150V), for very fast separation of low molecular weight DNA (50-1500 bp),
- 2) a low voltage DC mode (25V) for high-resolution separation of DNA 1-6kb in size (CV's <10%), and
- 3) alternating field mode (100V, alternating electrode polarity at programmable millisecond time scales) for separation of DNA from 2-50kb in size.



The alternating field mode allows rapid separation of high molecular weight DNA in 0.75% agarose cassettes: linear phage lambda DNA (48,500 bp) can be collected in as little as 6 hours. These features make the BluePippin useful for the size fractionation steps of mate-pair (jumping) library construction.

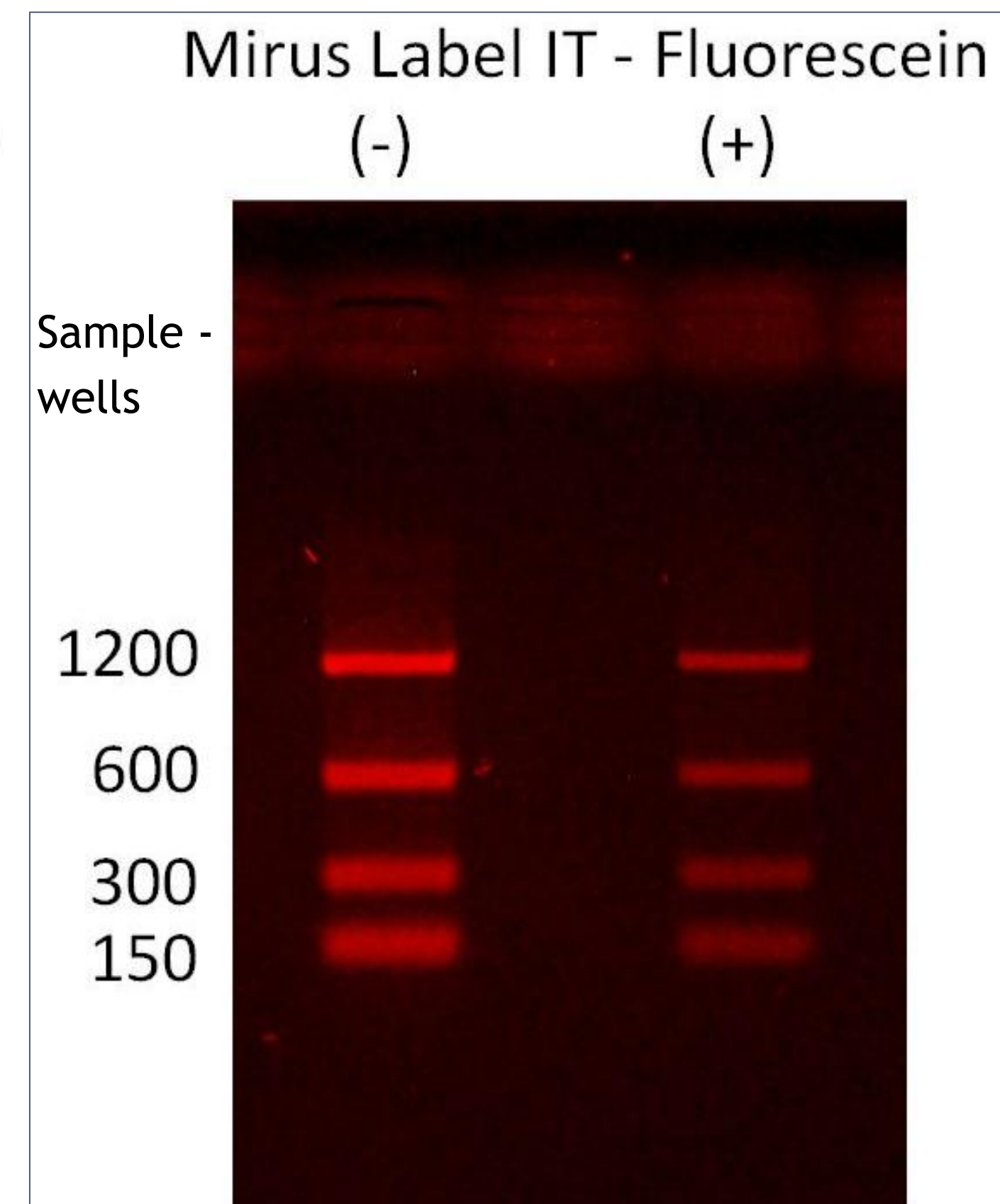
Pulse protocols for preparative pulsed field electrophoresis

The BluePippin uses a point detector that detects DNA at a single position near the branch point of the cassette channels. To determine when to start DNA collection, the system software must perform extrapolations based on the measured rate of movement of the DNA reference markers. For accurate extrapolation, the marker behavior should be linear over the size range of interest. For ordinary electrophoresis using DC power supplies, the time of appearance of a marker at the detector shows a polynomial dependence on DNA size, making extrapolation highly error prone. However, by using pulsed field power supplies, pulse timing protocols can be found that show a linear relationship between DNA size and migration time, for specific DNA size ranges. These pulse protocols are included in cassette definitions for specific DNA size ranges.



Fluorescently tagged marker DNA

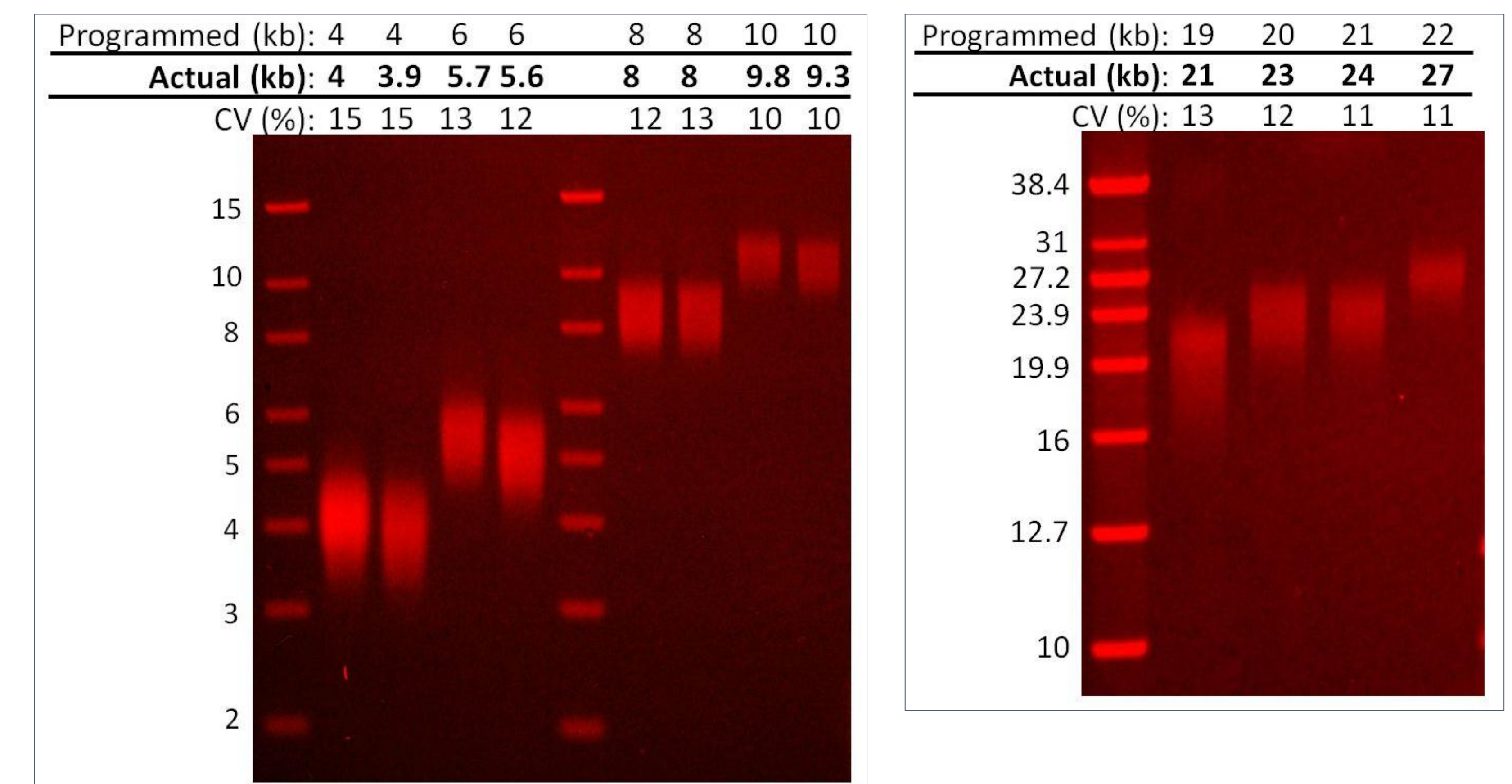
Due to high demand for dye-free cassettes, we have developed marker DNA sets that are labeled with covalently attached fluorescein groups (Label IT Fluorescein Reagent, Mirus Bio). As shown in the figure, the Mirus Bio reagent causes little, if any, detectable change in the electrophoretic mobility of the markers.



(Labeled and unlabeled marker DNA were electrophoresed in an ordinary analytical slab gel without EtBr. The gel was photographed under UV light (image not shown). The gel was then stained with EtBr and rephotographed (image shown). Comparison of the images demonstrates that the fluorescein-modified DNA comigrates with the EtBr-stainable DNA in the Mirus(+) lane.)

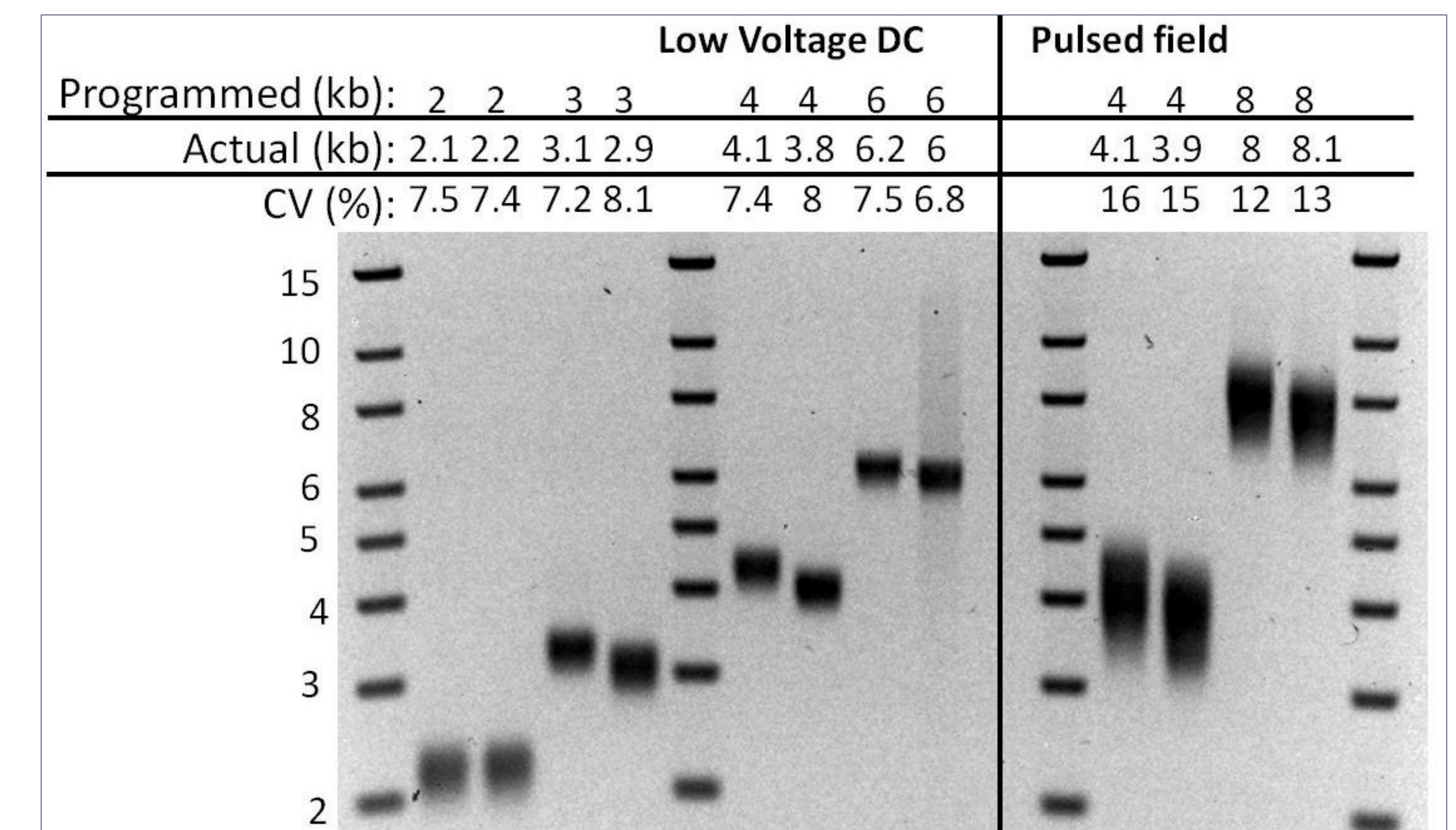
BluePippin fractionations using pulsed fields

E. coli genomic DNA was digested with restriction enzymes, and 5ug samples were fractionated in the BluePippin using dye-free 0.75% cassettes. Programmed size targets are indicated on the top line of the figure legends. The fractionated DNA was analyzed on pulsed-field slab gels, and post-stained with EtBr. Measured actual size and distribution (CV) are shown on second and third lines of the legends.



Comparison of size resolution between low voltage and pulsed field modes

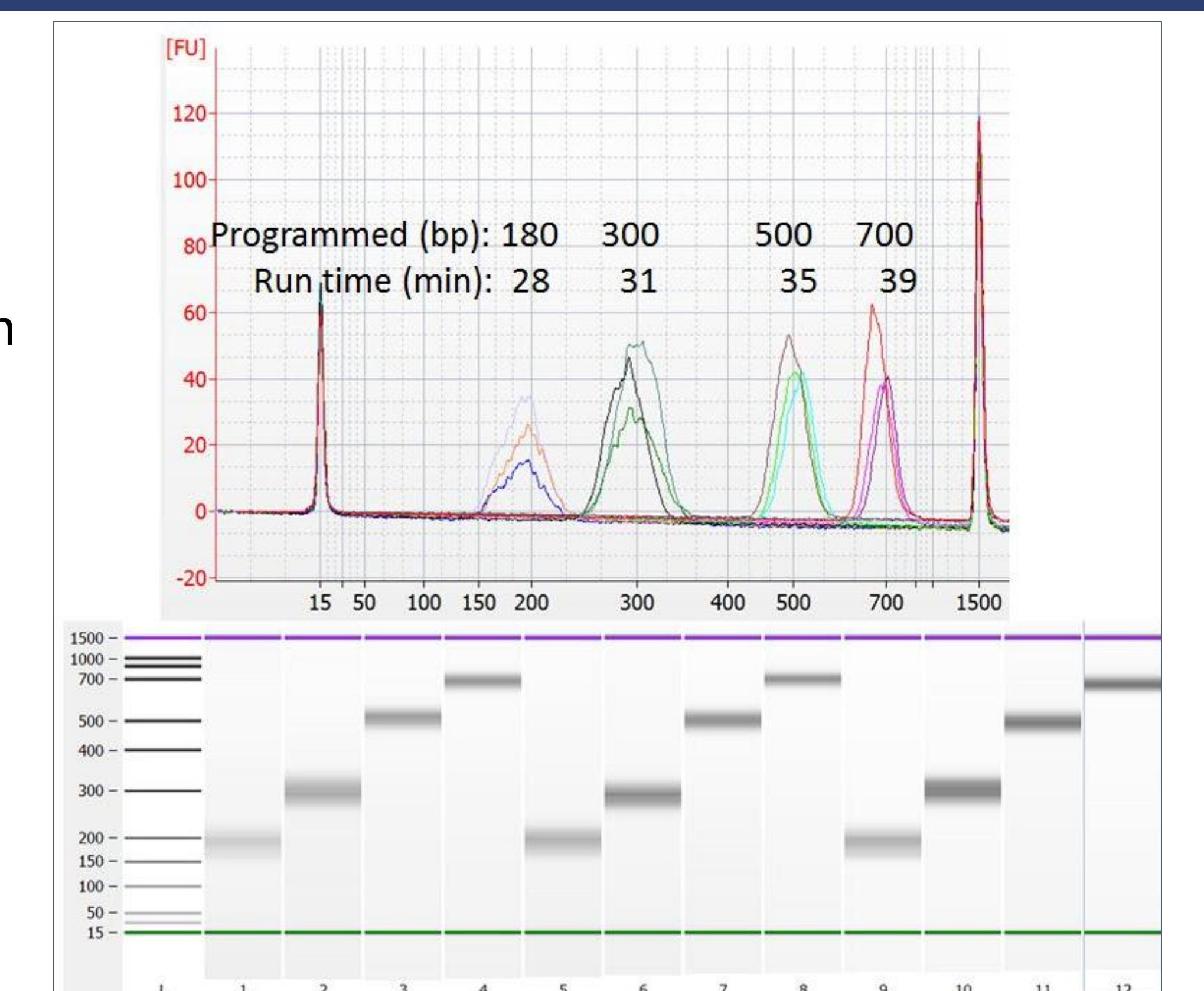
The same genomic DNA samples were fractionated using 25V DC mode (left) or pulsed field mode (right). The CVs of DNA samples fractionated using LV mode are substantially smaller than the CVs of the pulsed field samples.



The price of increased resolution is run time. 6kb in LV mode takes 6 hrs, whereas in pulsed field mode, it takes ~2 hours.

Fast LMW fractionations using high voltage mode – 3 cassettes, 4 cuts each cassette

For standard NGS PE library applications, the BluePippin can use a faster 150V power mode with 1.5% and 3% agarose cassettes. On the dye-free 1.5% cassette, tight cuts at 500 bp can be obtained at 35 minutes. This compares with 1hr 40 minutes on the original Pippin Prep operating at 100V.



Contact Information

Sales: Paul Ventura (paul@sagescience.com), Dave Cook (dave@sagescience.com)

Technical: Chris Boles (chris@sagescience.com), Sadaf Hoda (sadaf@sagescience.com)

See our other posters: # 28 and #29.