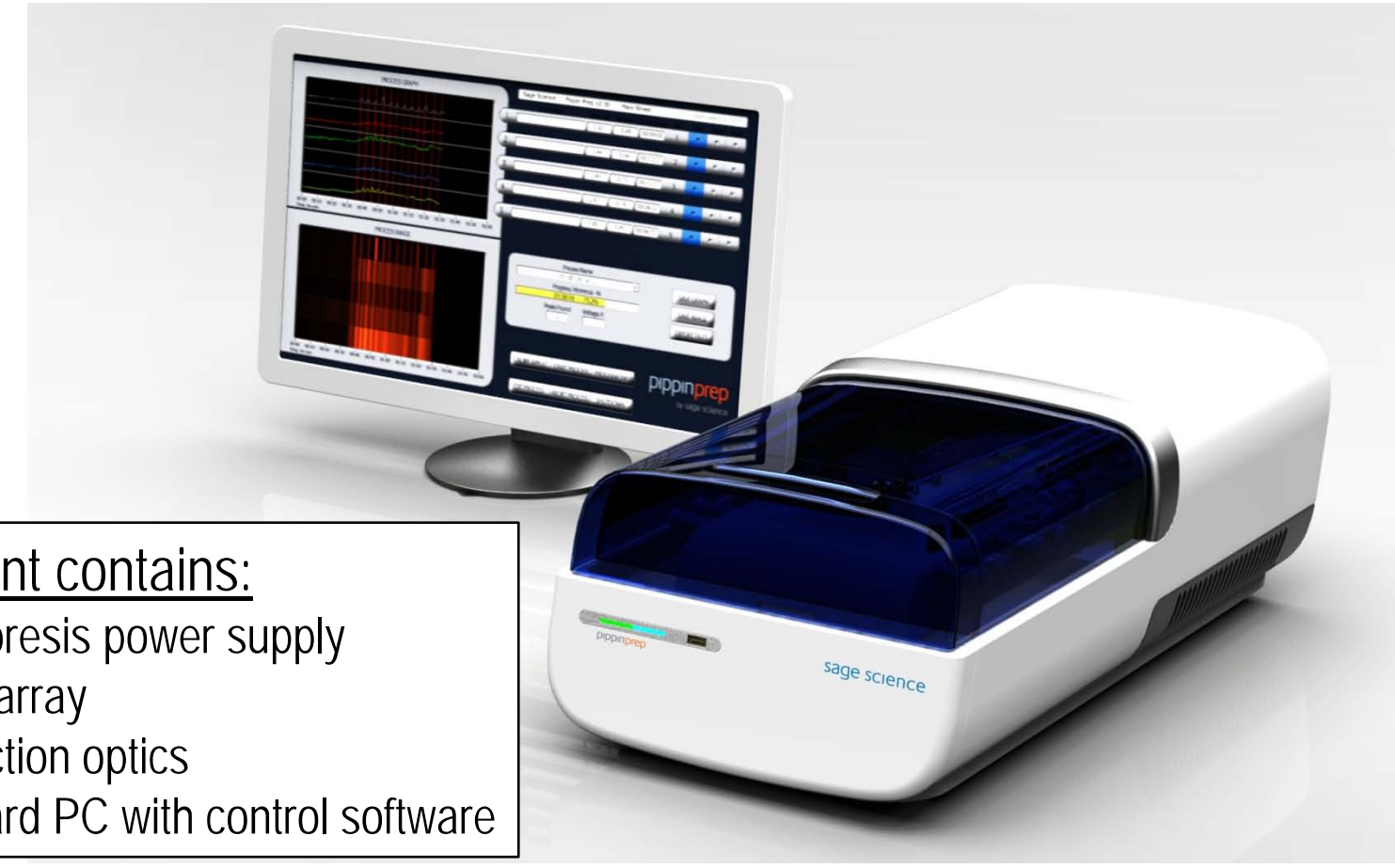# Expanded NGS Applications for the **Pippin Prep** DNA Size Selection System

Chris Boles, Sadaf Hoda, Simran Singh, Todd Barbera, Edd Snow, Ezra Abrams

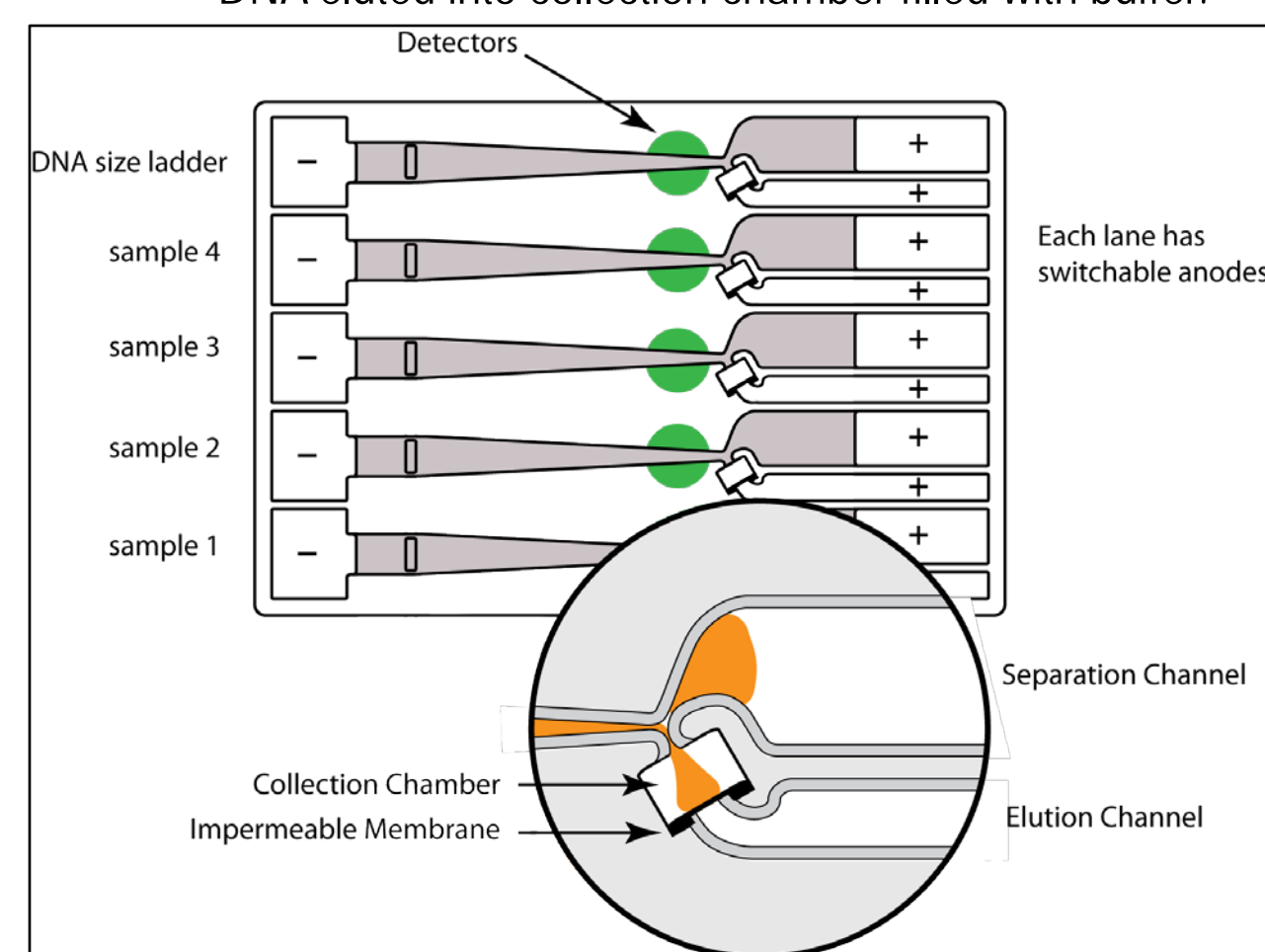Sage Science, Inc., Suite 3150, 500 Cummings Center, Beverly, MA 01915

sage science
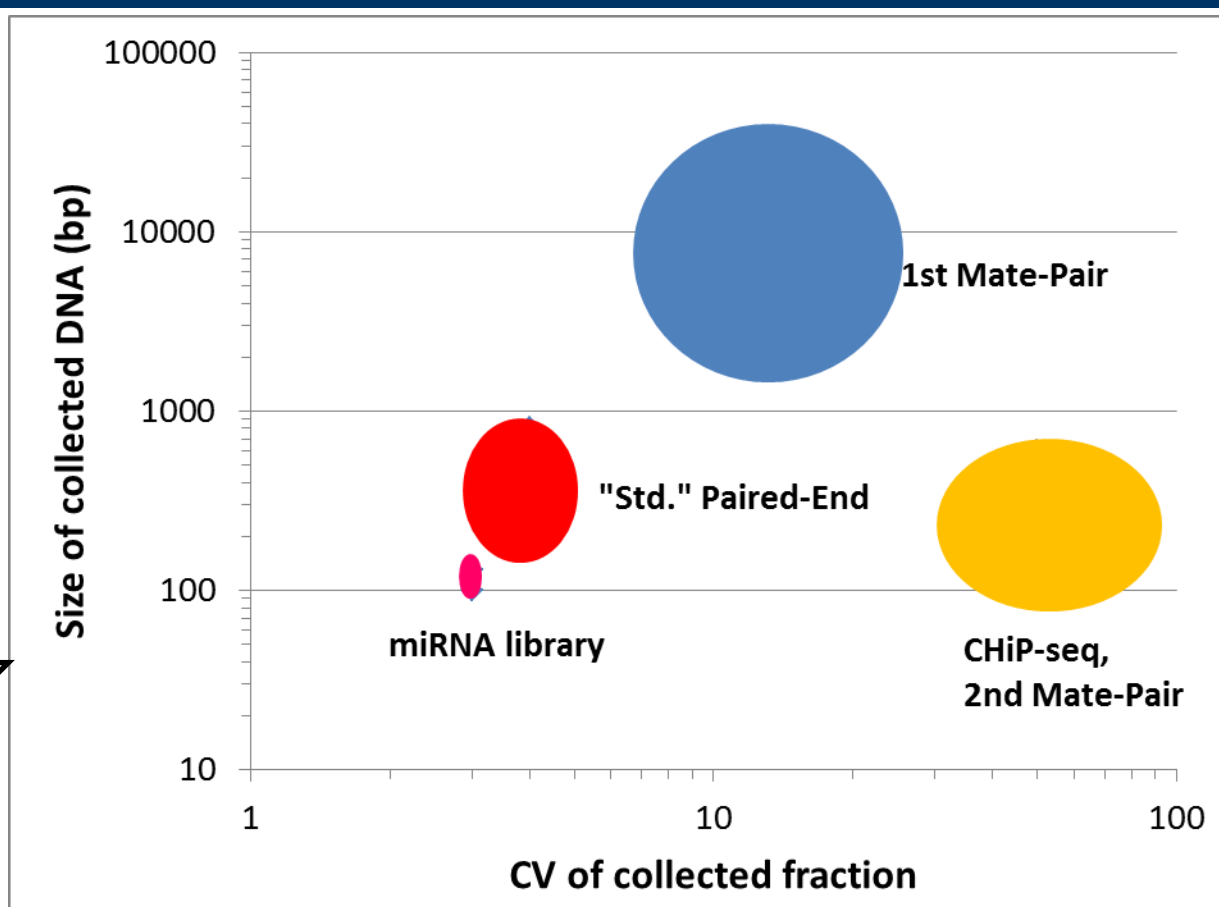
The Pippin Prep System: Overview

Instrument contains:
Electrophoresis power supply
Electrode array
DNA detection optics
Single-board PC with control software

Disposable Agarose Gel Cassettes
Five physically separate channels
DNA eluted into collection chamber filled with buffer.

DNA size ladder
sample 4
sample 3
sample 2
sample 1

Each lane has switchable anodes

Detectors

Collection Chamber
Impermeable Membrane
Separation Channel
Elution Channel

Size fractionation in NGS Applications

Low / High — **Gel Conc**

1st Mate-Pair
"Std." Paired-End
miRNA library
CHiP-seq, 2nd Mate-Pair

Size of collected DNA (bp) vs. CV of collected fraction

High — **Gel Resolution** — Low

Pippin Prep Products for NGS Applications

Application	Cassette types
PE libraries	2%
	1.5% (new, Nov. 2010)
	2% Ethidium Free (new Feb. 2011)
Small RNA libraries	3% (new, Feb. 2011)
1st Mate-pair	0.75% (new, Feb. 2011)
CHiP-seq, 2nd Mate-pair	2%, 1.5% cassettes with Closed elution modules (new, Mar. 2011)

User validation of Pippin Prep for PE Libraries

Improvement in PE Library Quality:

Tight insert size.

No LMW "shelf".

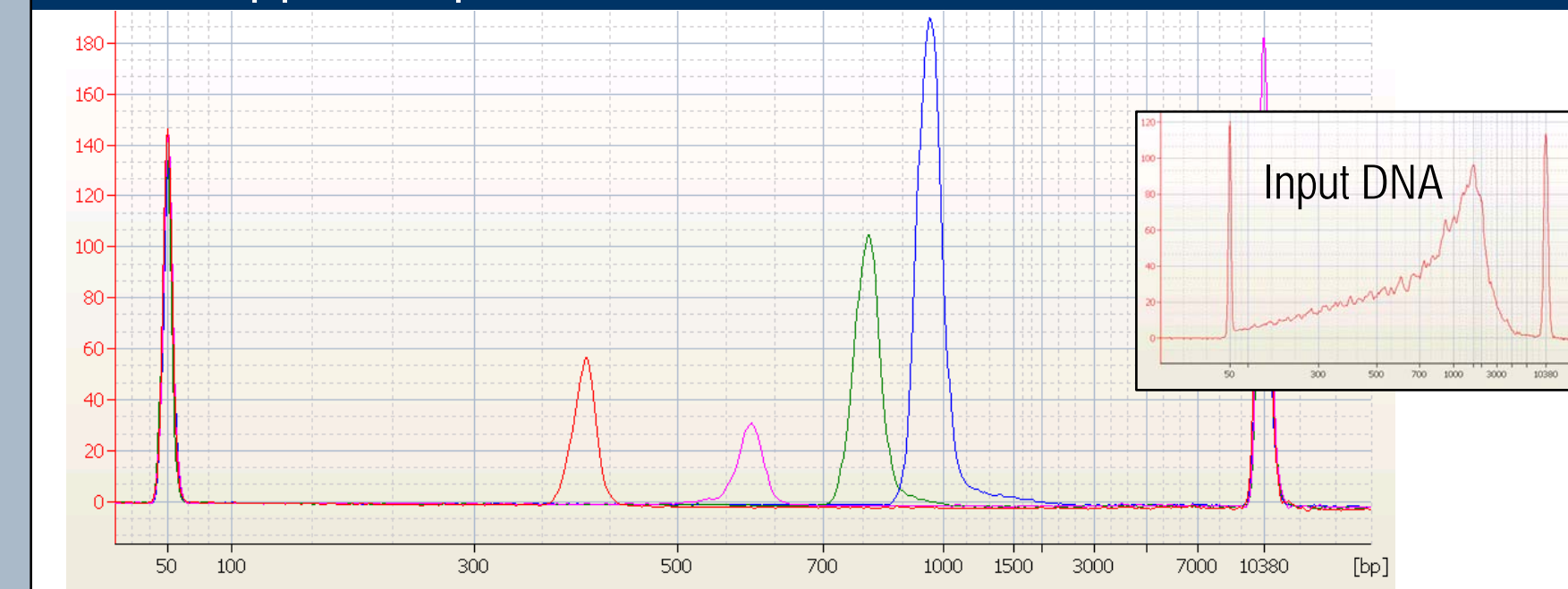Consistent, high yield.

Pippin Prep / Manual Gel
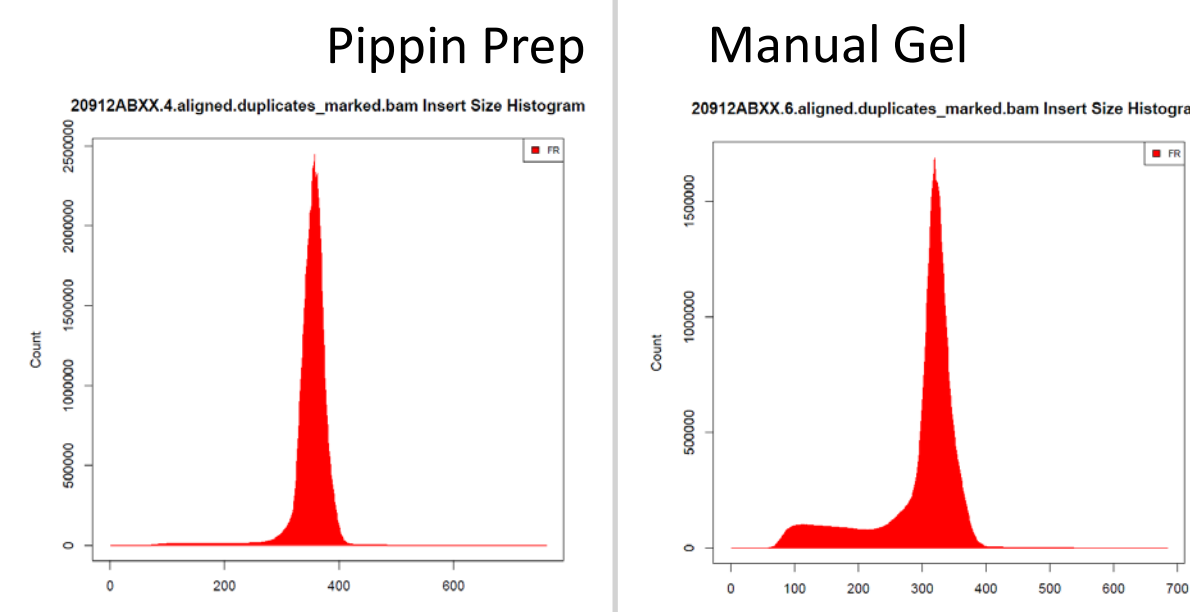
Work flow and cost efficiency:

Library production rate doubled per FTE.

10-fold decrease in PCR enrichment cost due to improved yield.

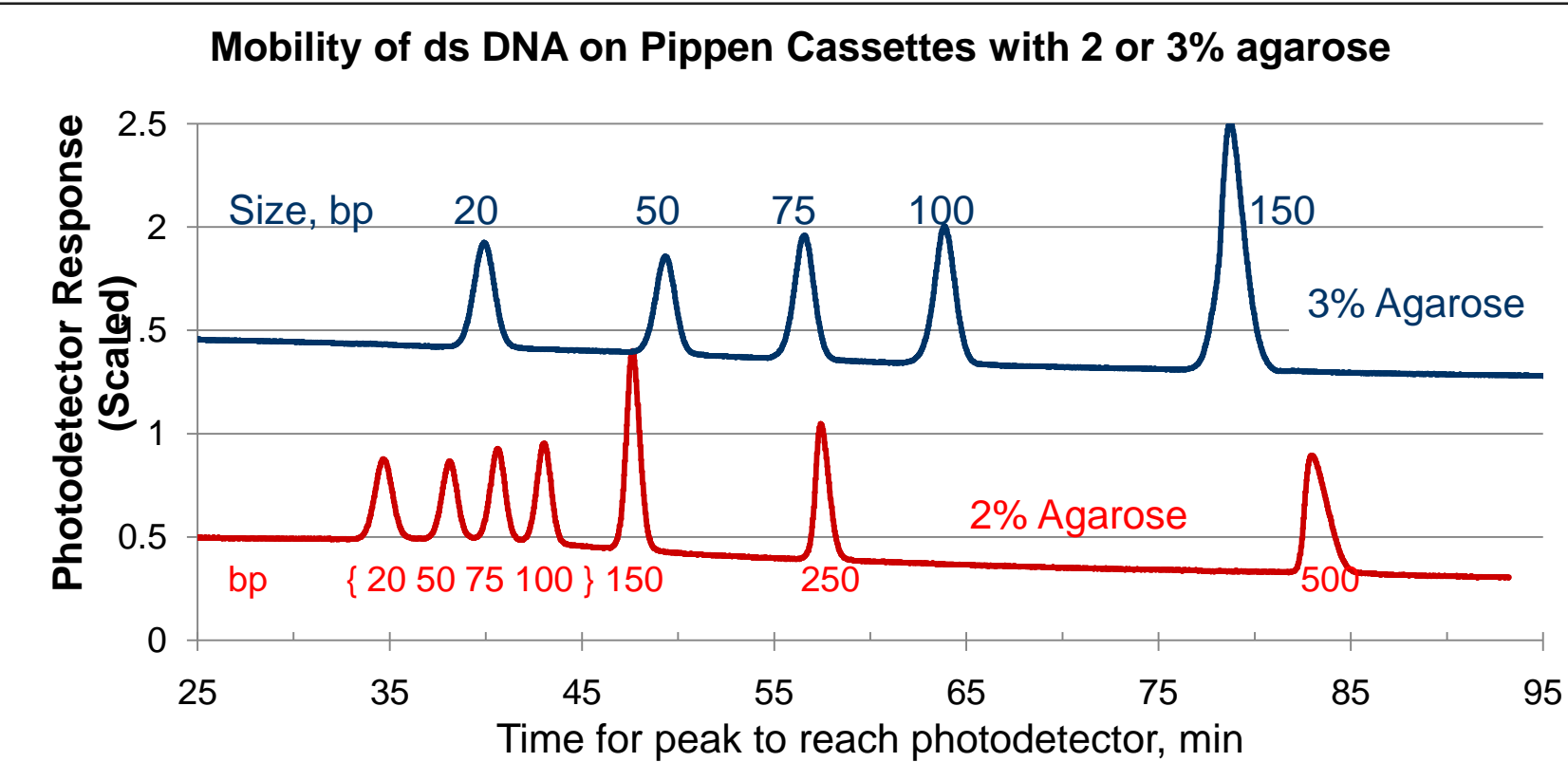Data courtesy of Broad Institute Sequencing Technology Development Group

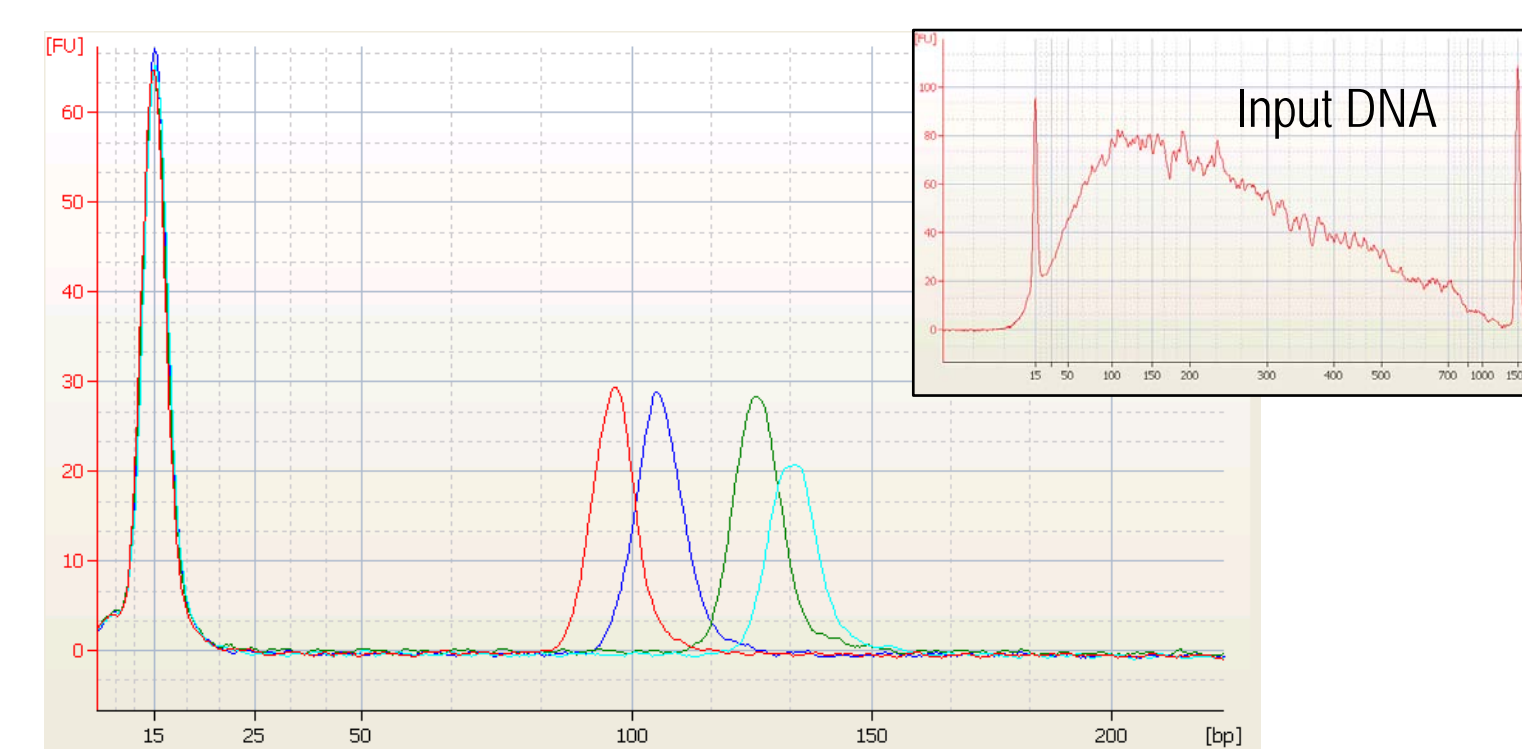## Pippin Prep 1.5% cassette: Low CVs out to 1.5kb

Input DNA

Programmed	Actual	CV (%)
400 tight	408(364-454)	3
600 tight	598 (545-674)	3
800 tight	811(690-973)	5
1000 tight	977(851-1361)	6

New Pippin Prep 3% cassette: miRNA libraries

Optimal size resolution in 50-200 bp region

Mobility of ds DNA on Pippen Cassettes with 2 or 3% agarose

Photodetector Response (Scaled) vs. Time for peak to reach photodetector, min

Size, bp: 20 50 75 100 150 — 3% Agarose

bp: { 20 50 75 100 } 150 250 500 — 2% Agarose

Example of 3% cassette performance on gDNA

Input DNA

Programmed	Actual	CV(%)
90 tight	97 (82-114)	5
100 tight	106(90-129)	5
120 tight	127 (109-144)	4
130 tight	134 (115-149)	4

0.75% Pippin Prep cassettes for Mate-Pairs

Marker size (kb): 0.5 1 2 3 4 5 6 8 10

Genomic DNA
Marker lane
Time, hh:mm

Size range: 1 - 10 kb
Run times: 0.6- 1.5 hr.
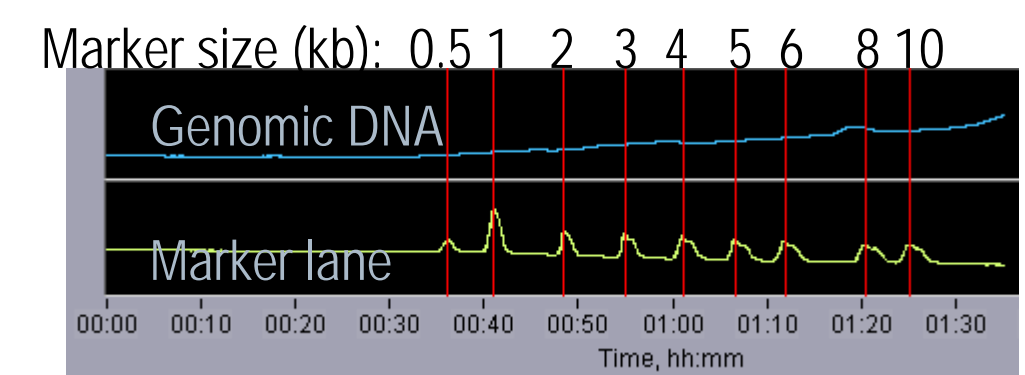
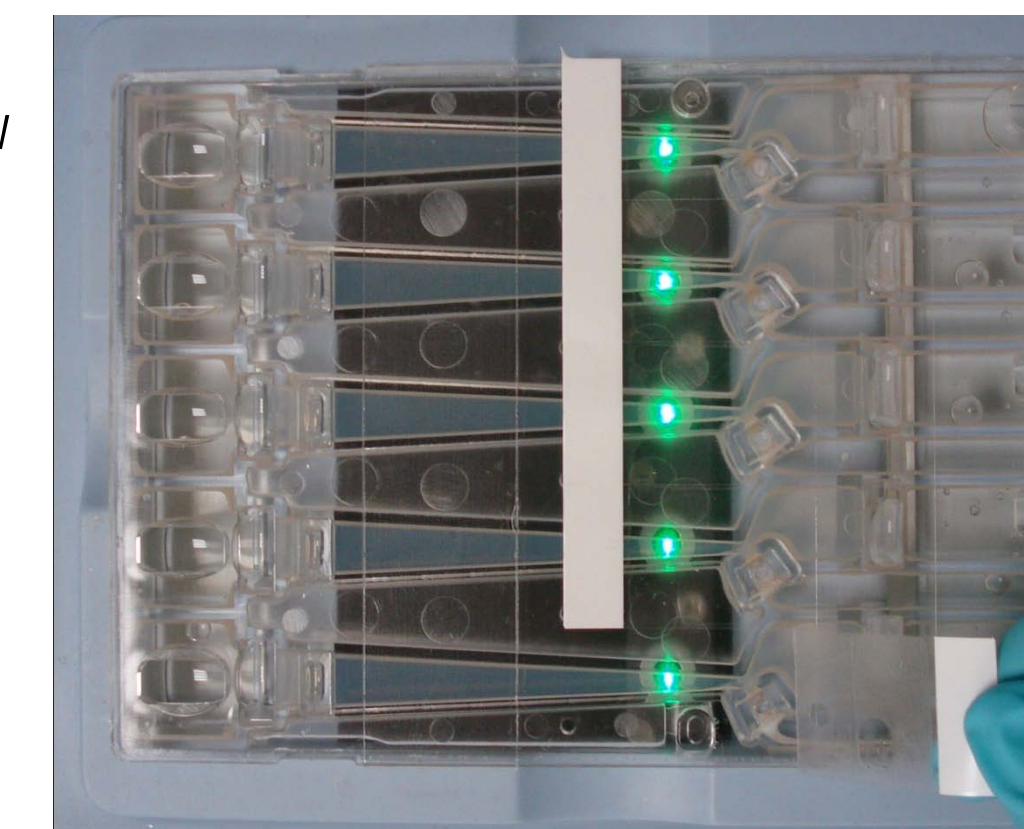| Size (kb):	8.2	7.1	7.4	9.1	9.6
CV:	12%	10%	12%	13%	16%

5 µg/lane input:
CVs ~10-16%
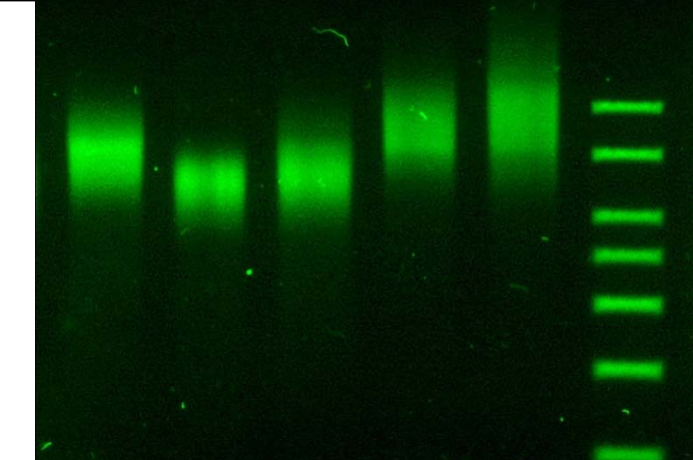
Cassette modifications for CHiP-seq

Closed elution modules:
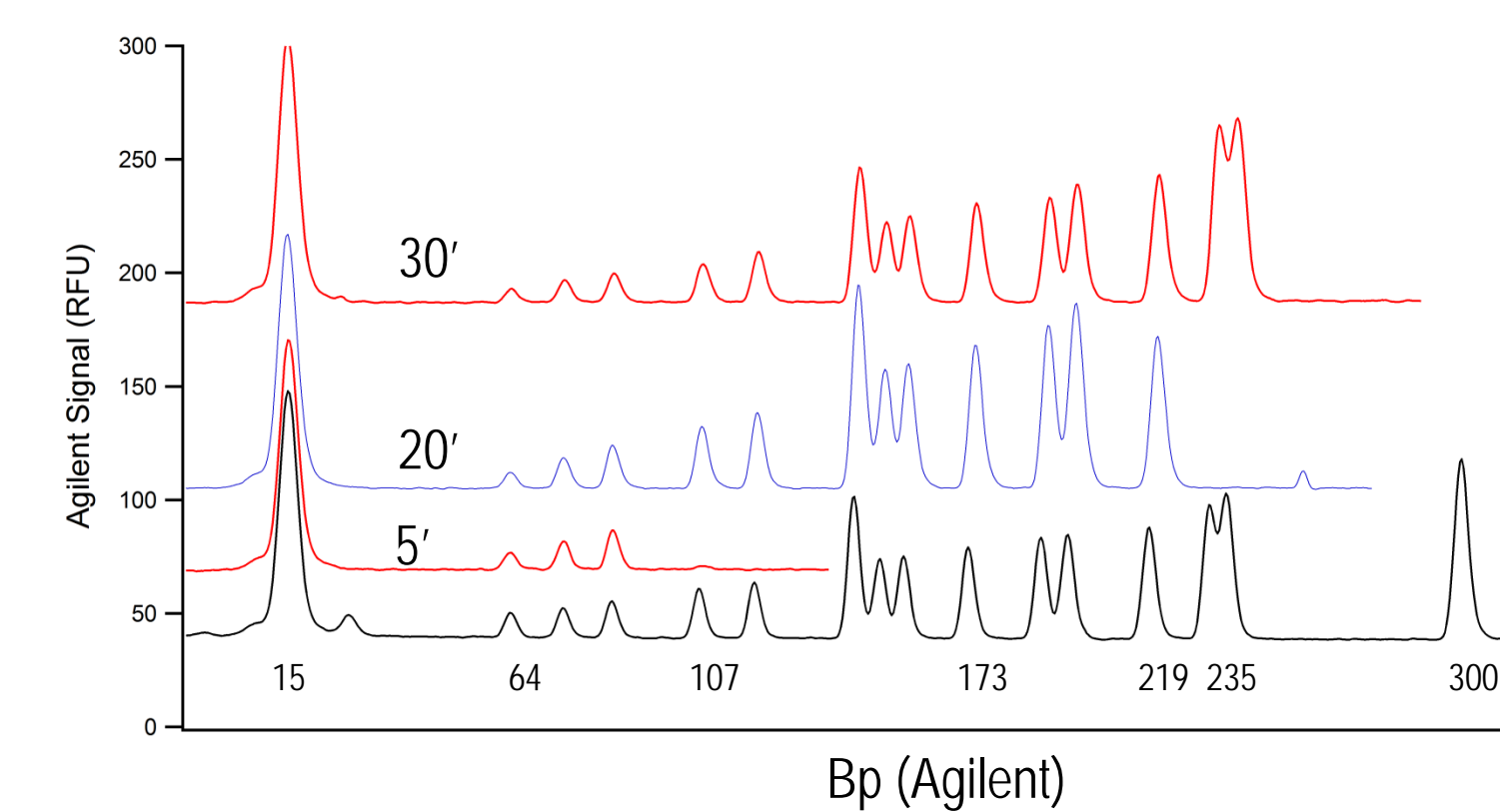Prevent electroosmotic flow into elution module.

Elution volume remains fixed at 50 µl.

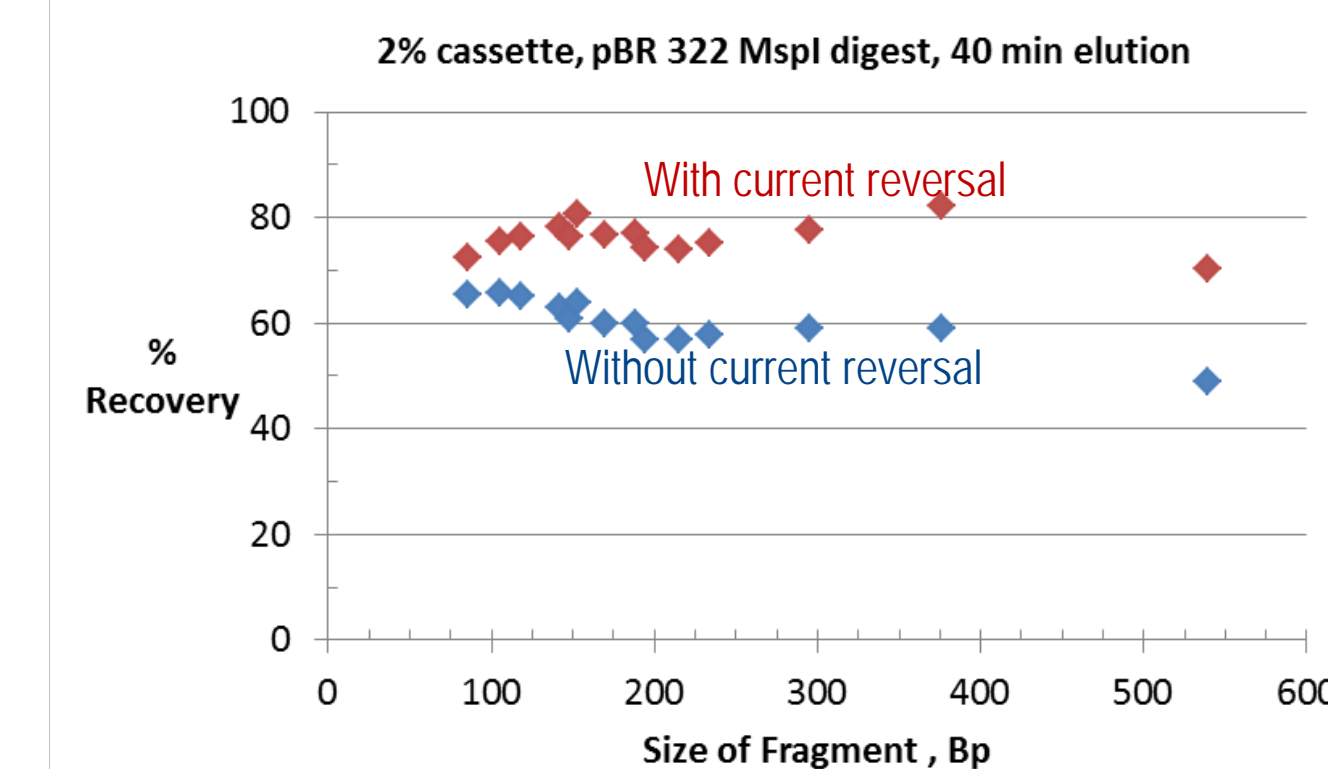Permits long elution times without overflow or manual intervention.

(Expected in production March, 2011. **See website for related Tech Note.**)

Pippin Prep for CHiP-seq: Long elution times

30'
20'
5'

Agilent Signal (RFU) vs. Bp (Agilent)
15 64 107 173 219 235 300

Agilent 7500 data of DNA eluted from Pippin Cassette
pBR322 MspI digest run on 2% cassette, with elution times
From 5 – 30 minutes.
DNA analyzed on Agilent 7500, with digest as std

Pippin Prep for CHiP-seq: Consistent High Yields

2% cassette, pBR 322 MspI digest, 40 min elution

With current reversal
Without current reversal

% Recovery vs. Size of Fragment, Bp

Short current reversal in elution module before sample removal boosts recovery to >60%

Pippin Prep for CHiP-seq: Broad collections from gDNA

Input DNA
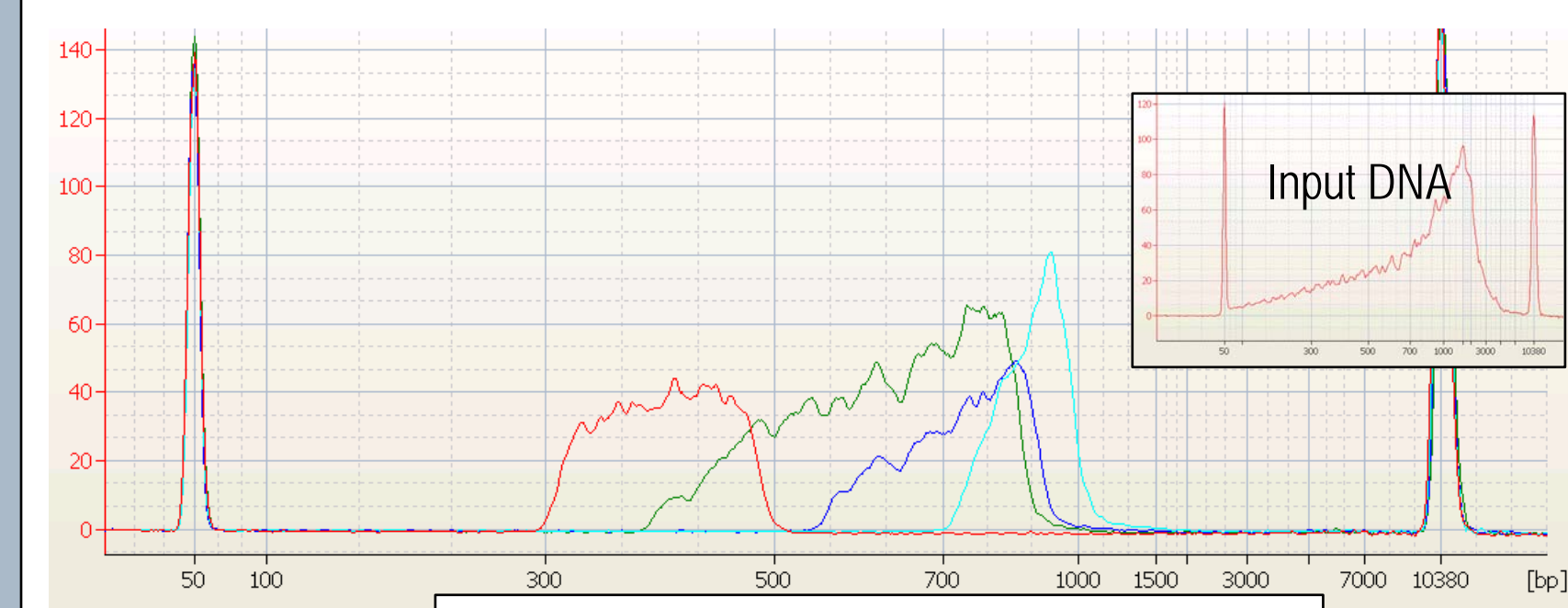
Programmed	Actual
300-500	291-523
400-900	376-925
550-950	535-976
700-1100	683-1182

Pippin Prep System Software: New Collection Modes

Selection Mode						bp Selection Criteria				Time Criteria		Band
Tight	Range	Time	Peak	Ref	Off	BP Target	BP Start	BP End	BP Pause	T Start	T End / T Pause	BP Thresh
							376	324	0			
						300						
						425	250	600	0			
												100

Tight = Capture tightest band centered on BPtarget value
Range = Collect between BPstart and BPend value
Time = Collect between Tstart and Tend
Peak = Collect first band encountered in sample after BP threshold

Pippin Prep 2011: Summary

User-validated performance in PE library construction
Benefits in throughput, process cost, and library quality

New offerings for Q'1 expand system size range: **75 - 10,000 bp**

A cassette (or two) for almost every NGS application:
Library prep for **PE reads, miRNA, mate-pair, CHiP-seq**

Come and see the Pippin Prep in our **Lanai suite: #292**.