



# Pippin Prep 2011: New Applications

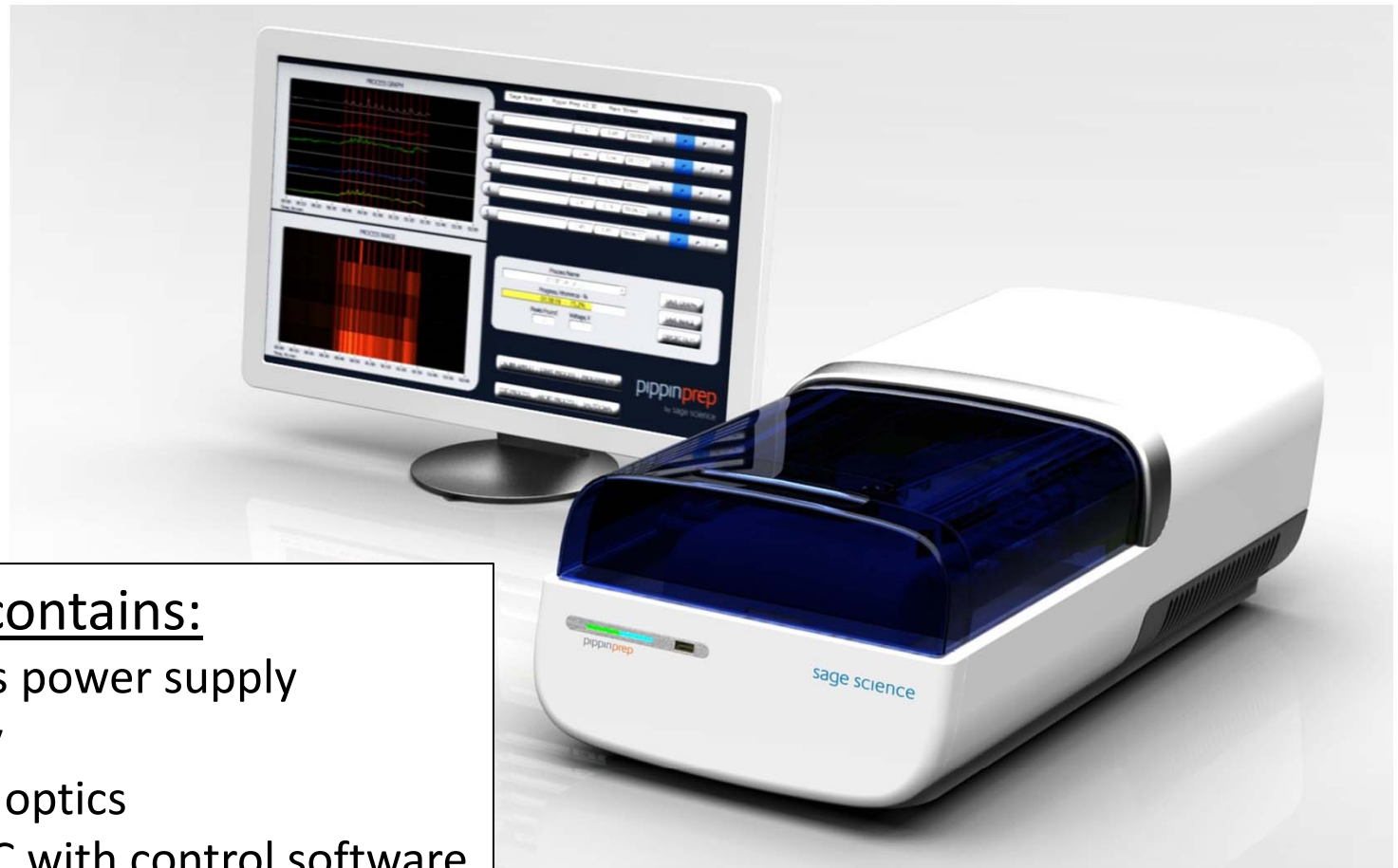
Expanded Cassette Offerings and System Improvements

Chris Boles, Ph.D.  
VP & CSO, Sage Science  
AGBT 2011

sage science

# The Pippin Prep System

## Automated Preparative Gel Electrophoresis for NGS



### Instrument contains:

Electrophoresis power supply

Electrode array

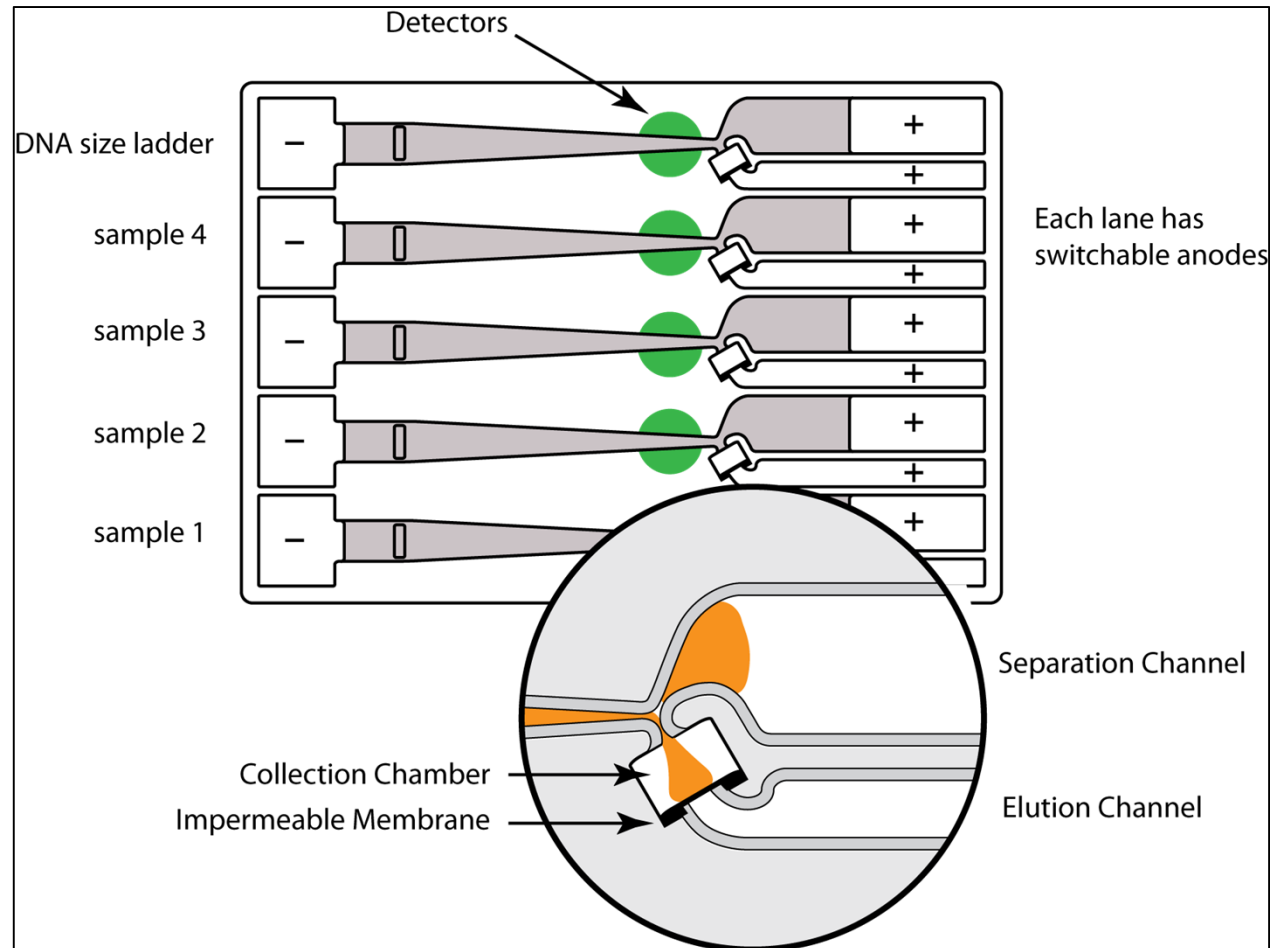
DNA detection optics

Single-board PC with control software

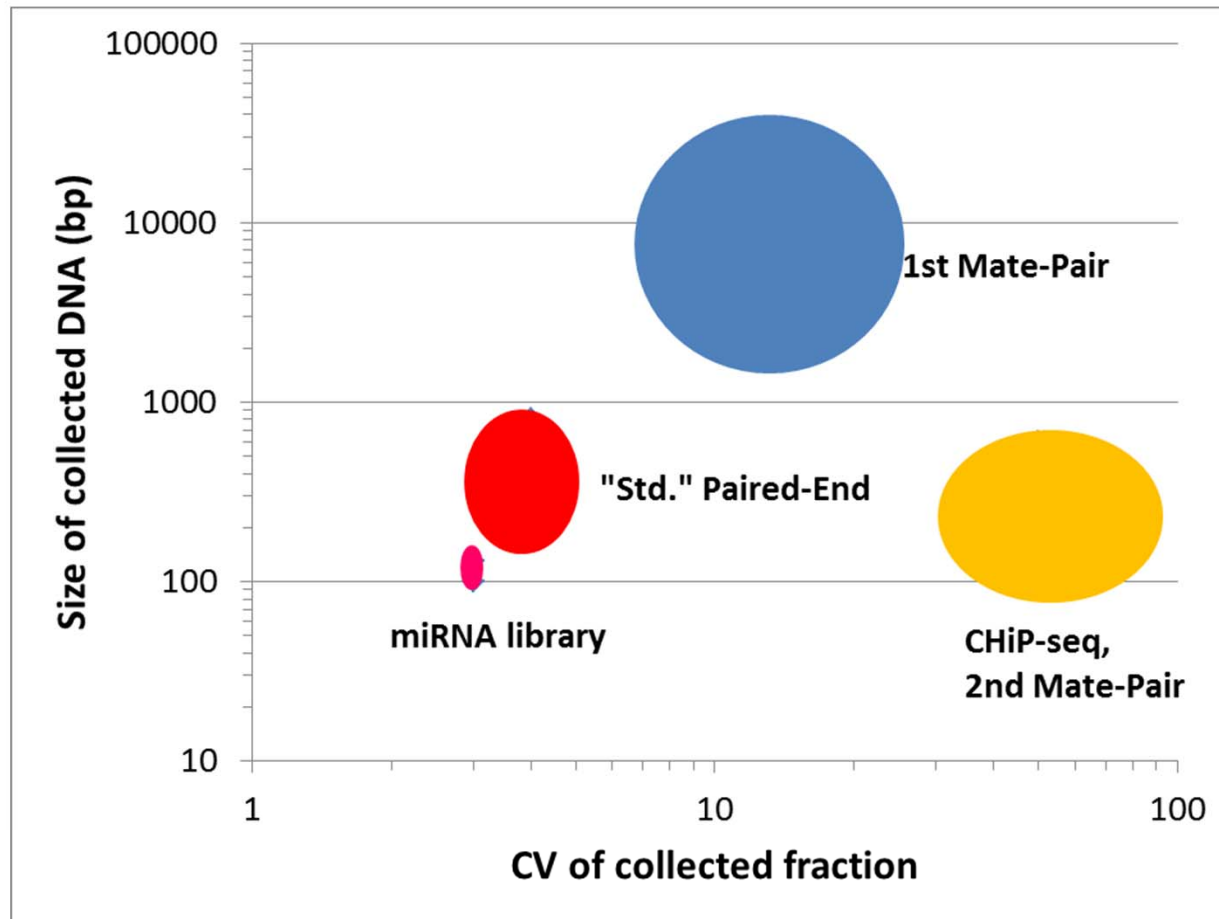


# The Pippin Prep System

## Automated Preparative Gel Electrophoresis for NGS

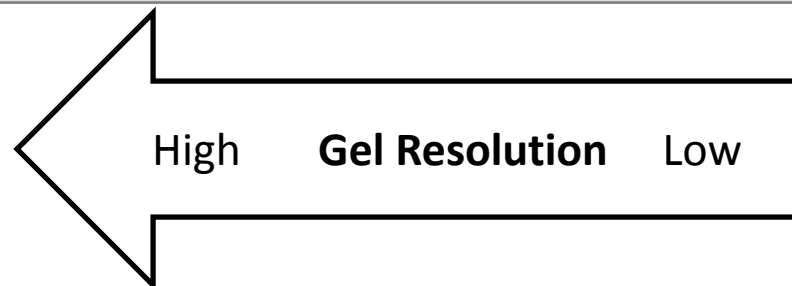
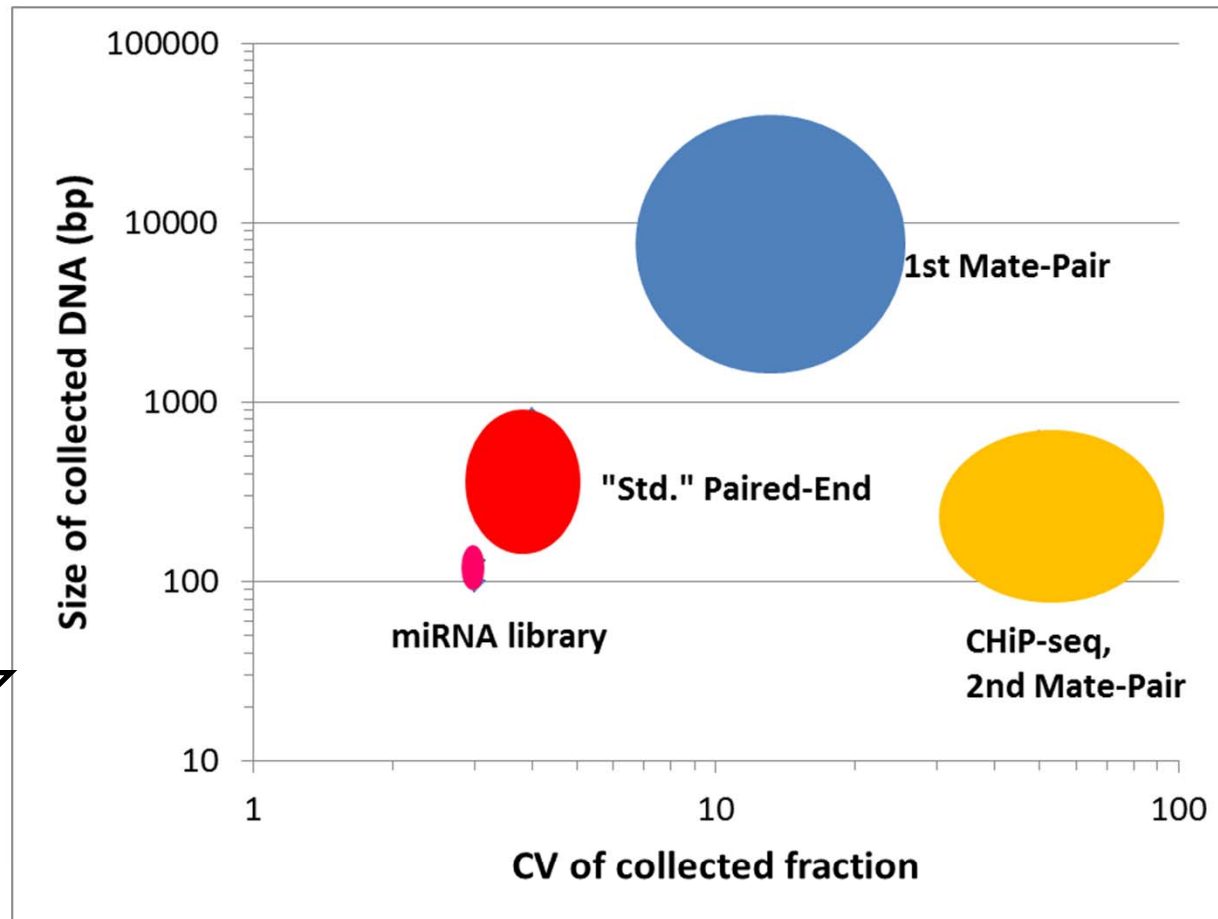
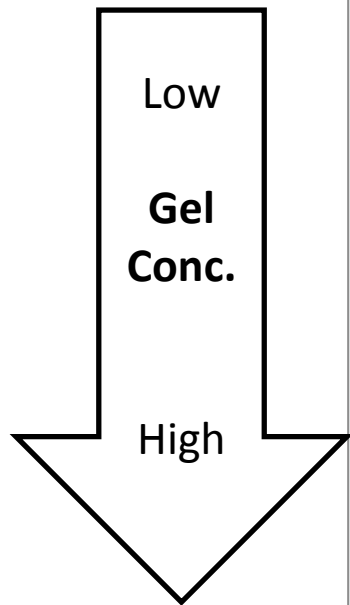


# Size fractionation in NGS Applications





# Size fractionation in NGS Applications



# Pippin Prep Products for NGS Applications

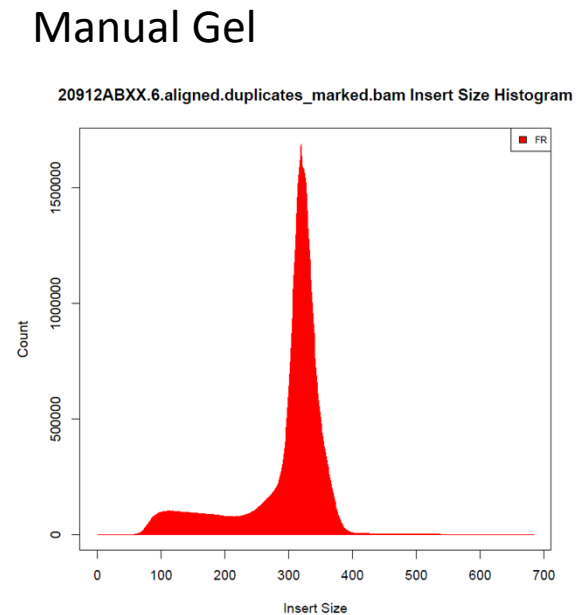
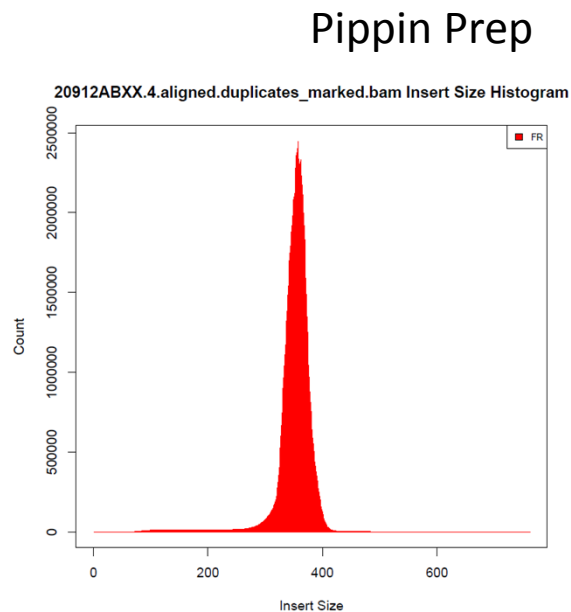


<b><u>Application</u></b>	<b><u>Cassette types</u></b>
PE libraries	2% 1.5% (new, Nov. 2010) 2% Ethidium Free (new Feb. 2011)
Small RNA libraries	3% (new, Feb. 2011)
1 <sup>st</sup> Mate-pair	0.75% (new, Feb. 2011)
CHiP-seq, 2 <sup>nd</sup> Mate-pair	2%, 1.5% cassettes with Closed elution modules (new,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

# Pippin Prep for PE Libraries: 2% Cassettes



## Improvement in PE Library Quality:



Tight insert size.  
No LMW “shelf”.  
Consistent, high yield.

## Work flow and cost efficiency:

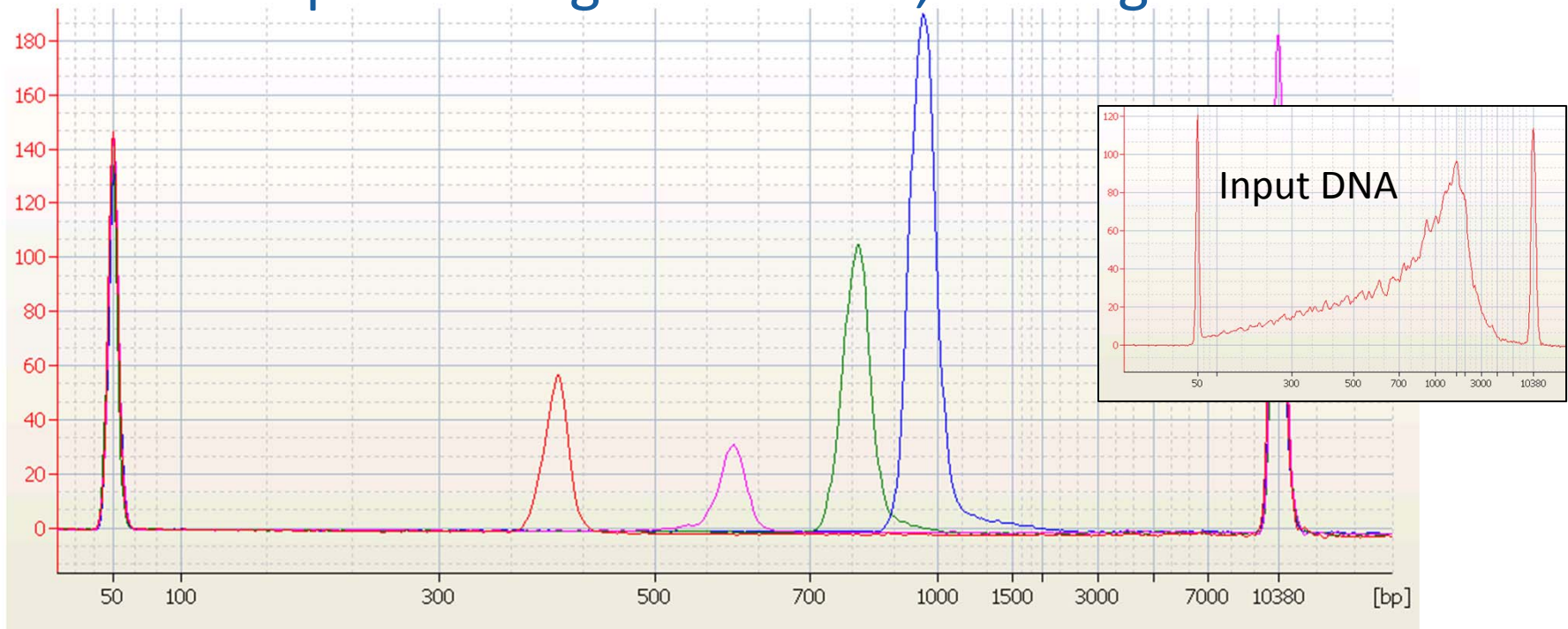
Library production rate doubled per FTE.

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

Data courtesy of Broad Institute Sequencing Technology Development Group

# Pippin Prep for PE Libraries: 1.5% cassette

Expands range to ~1.5 kb, with tight CVs.



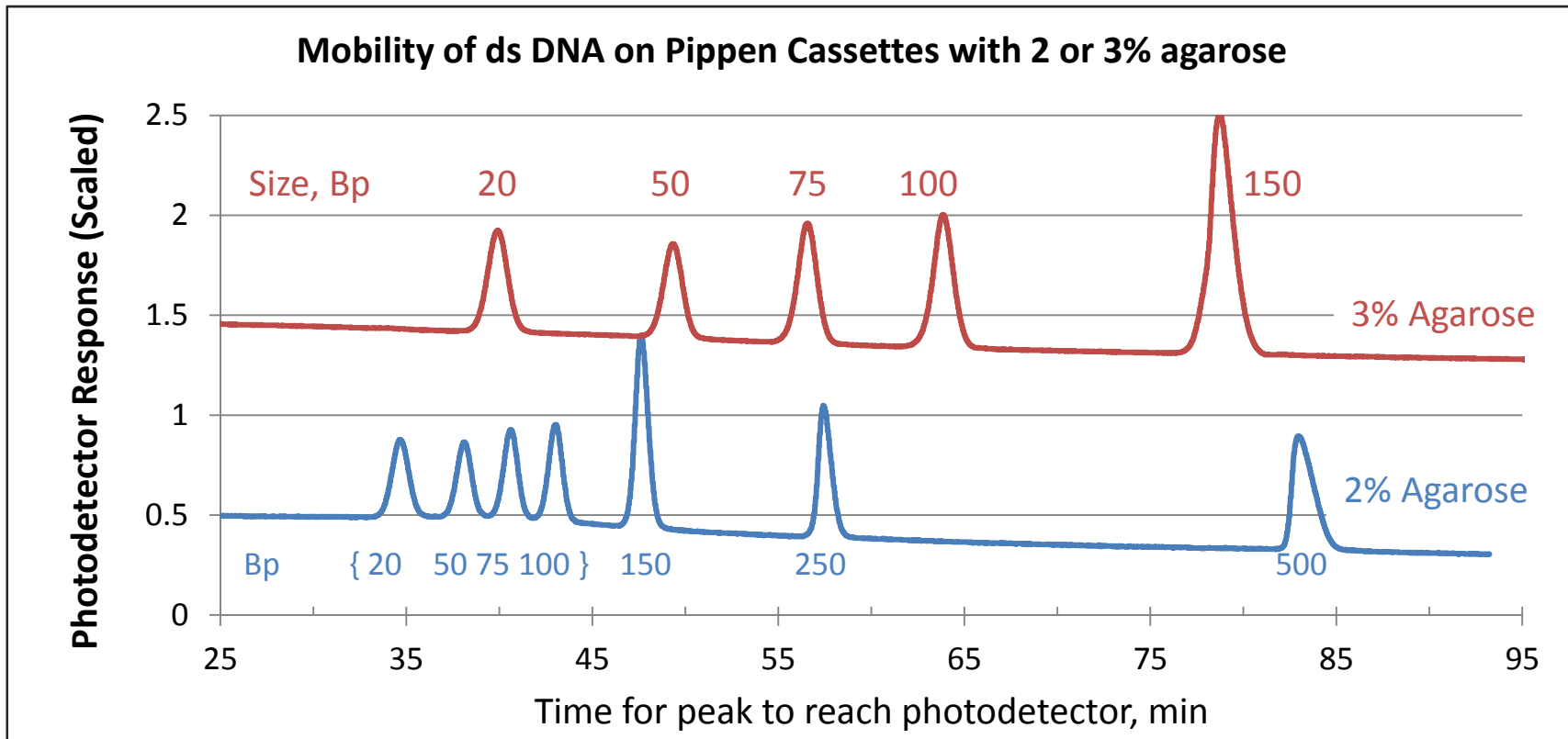
<b><u>Programmed</u></b>	<b><u>Actual</u></b>	<b><u>CV (%)</u></b>
400 tight	408(364-454)	<b>3</b>
600 tight	598 (545-674)	<b>3</b>
800 tight	811(690-973)	<b>5</b>
1000 tight	977(851-1361)	<b>6</b>





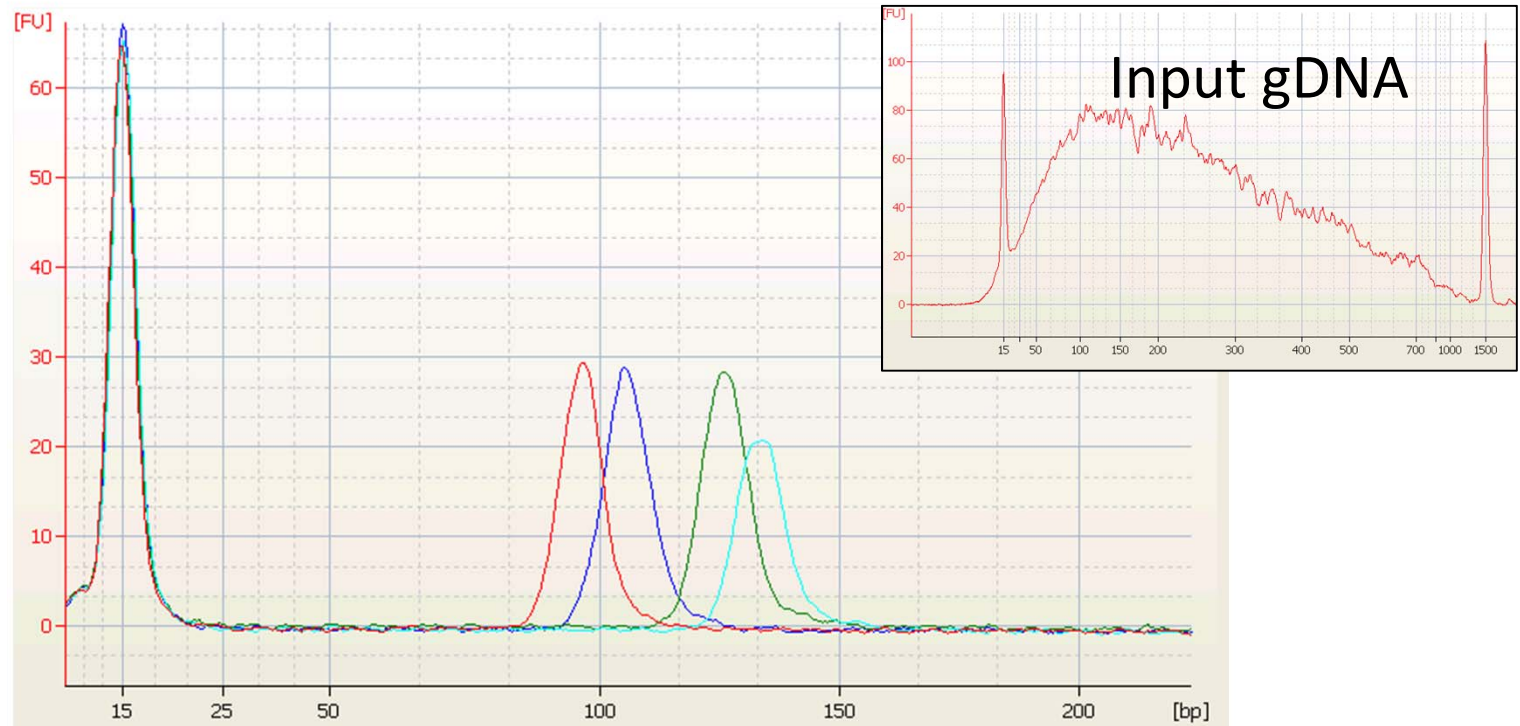
# Pippin Prep of miRNA libraries: 3% cassette

Optimal resolution in 50 – 200 bp range.



# Pippin Prep of miRNA libraries: 3% cassette

## Performance on gDNA samples

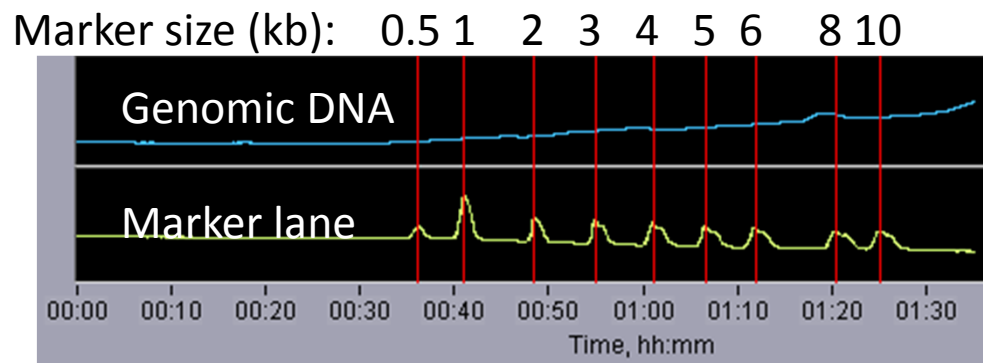


<b>Programmed</b>	<b>Actual</b>	<b>CV(%)</b>
90 tight	97 (82-114)	5
100 tight	106(90-129)	5
120 tight	127 (109-144)	4
130 tight	134 (115-149)	4

# Pippin Prep 0.75% Cassettes for Mate-Pair Libraries

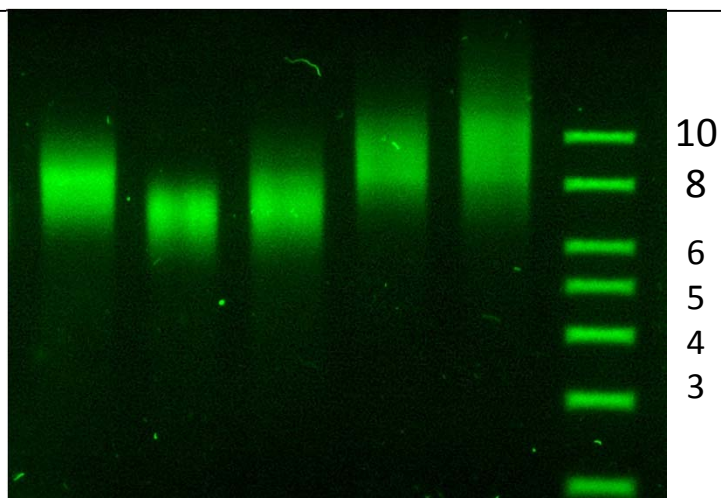


Size range: 1 - 10 kb  
Run times: 0.6- 1.5 hr.



Size (kb):	8.2	7.1	7.4	9.1	9.6
CV:	12%	10%	12%	13%	16%

5  $\mu$ g/lane input:  
CVs ~10-16%





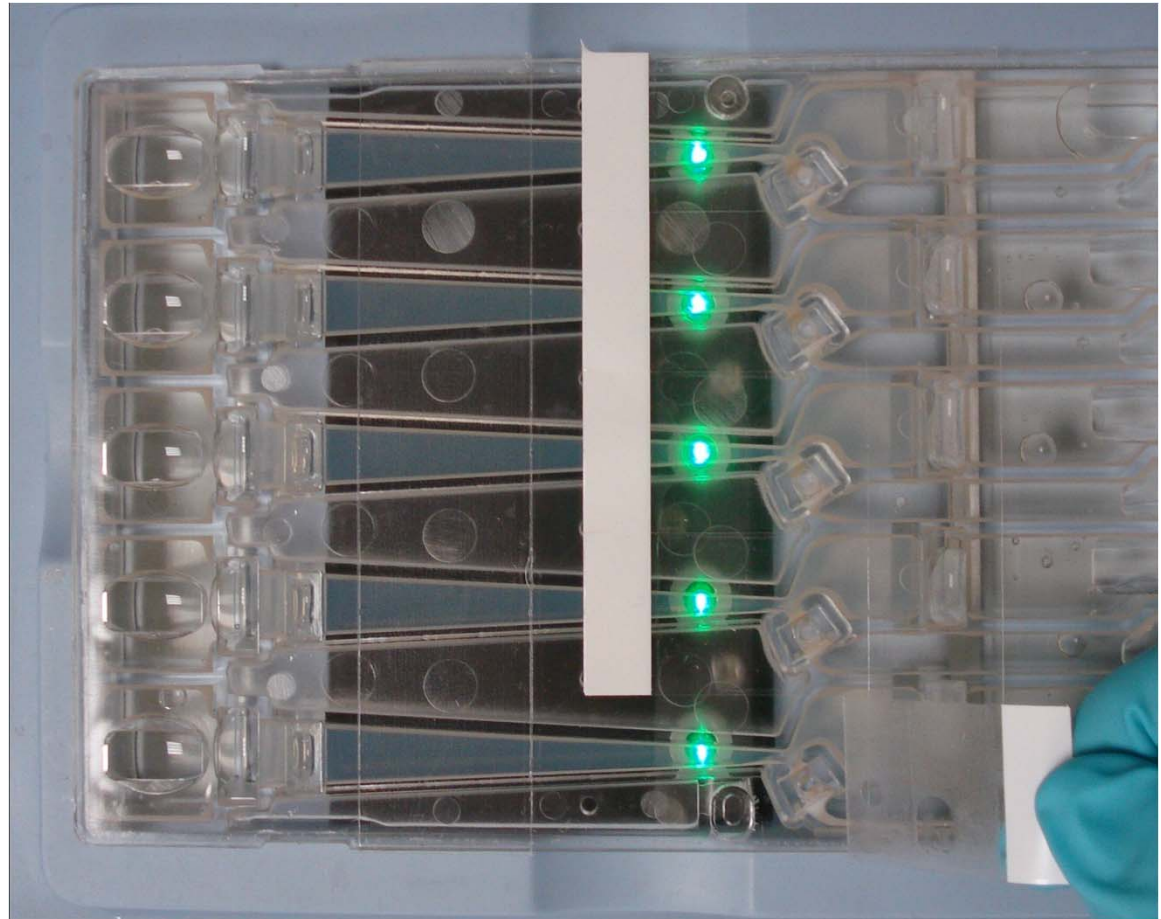
# Pippin Prep for ChIP-seq (and other broad size range applications)

## **Closed elution modules:**

Prevent electroosmotic flow into elution module.

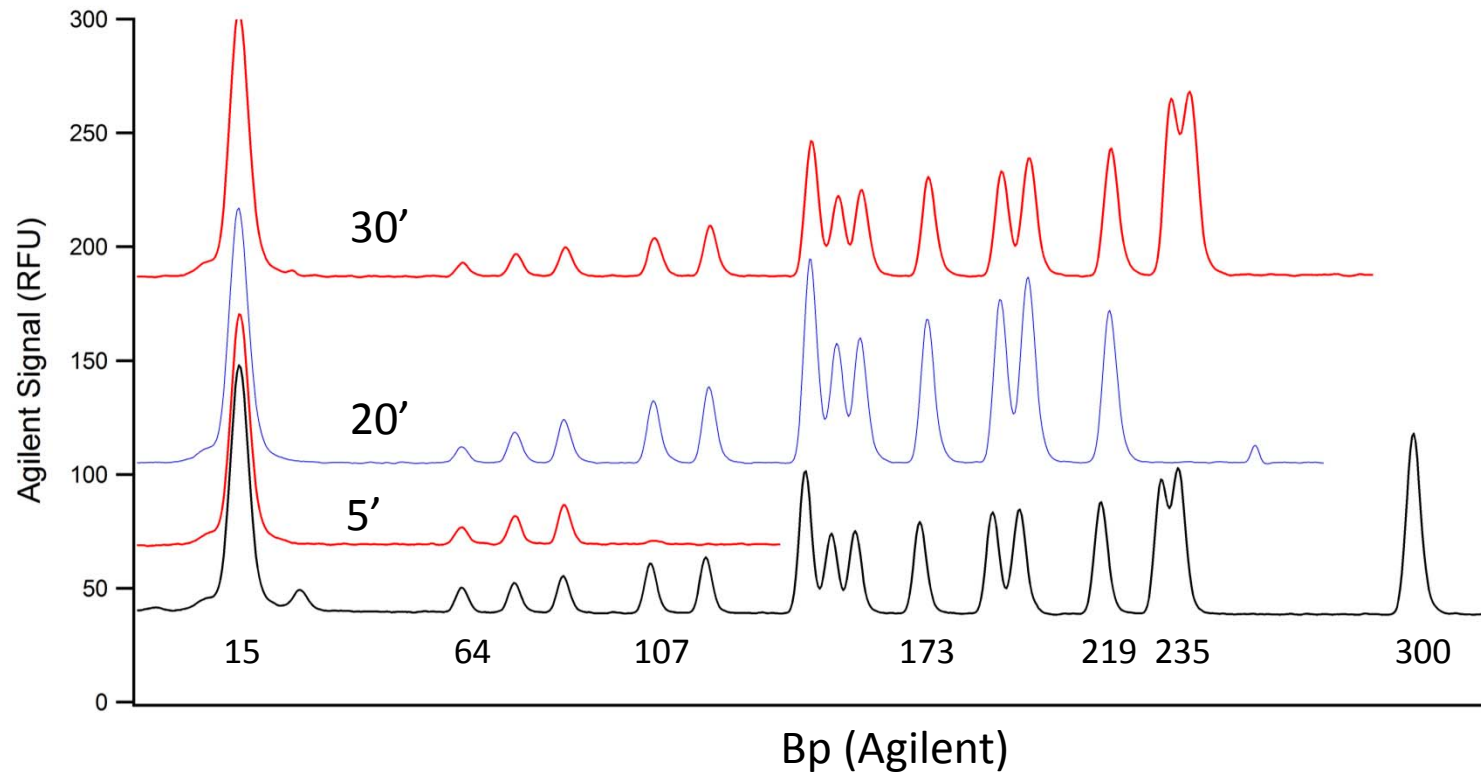
Elution volume remains fixed at 50  $\mu$ l.

Permits long elution times without overflow or manual intervention.



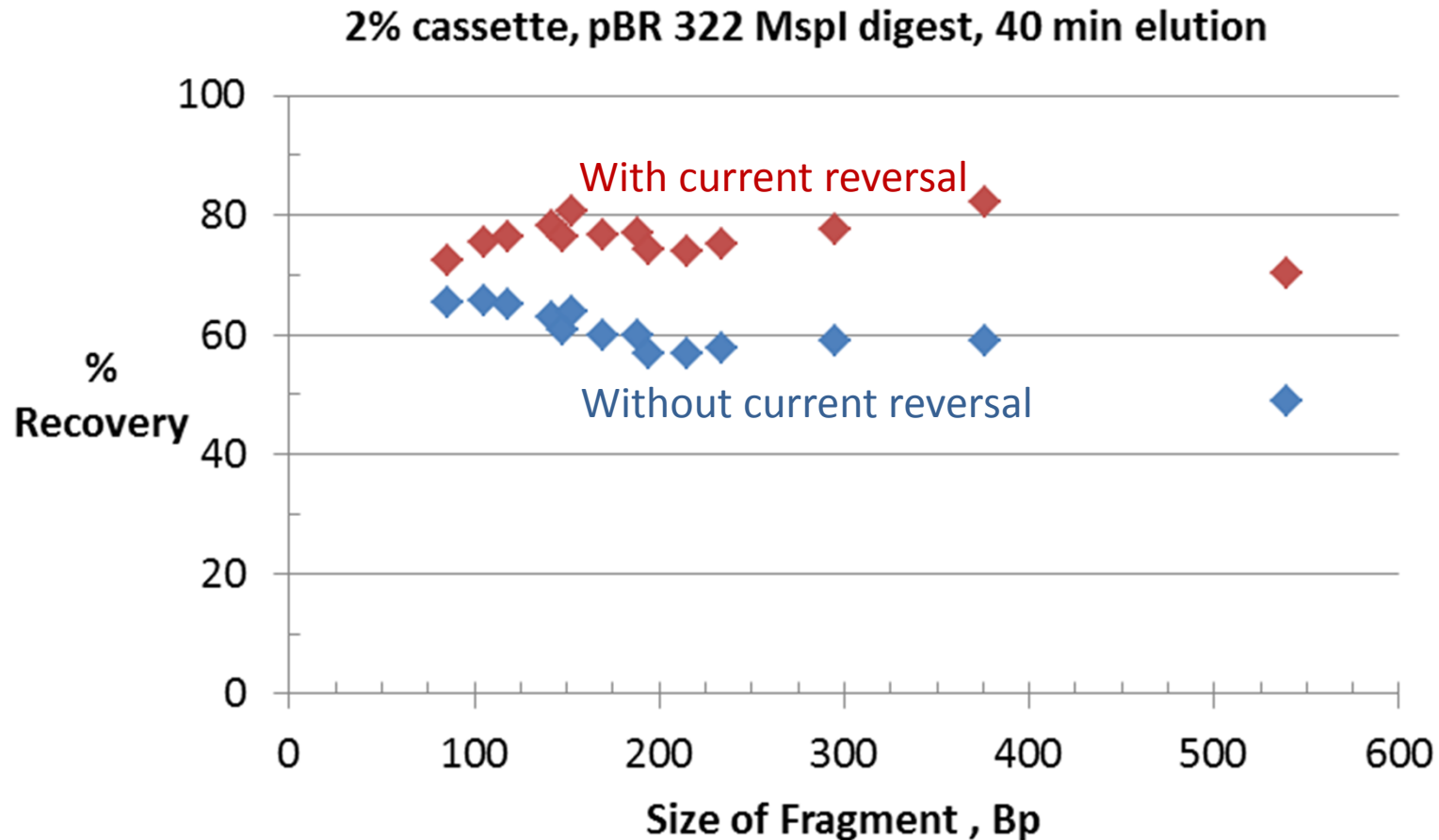


# Pippin Prep for ChIP-seq: Long Elutions



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 recoveries

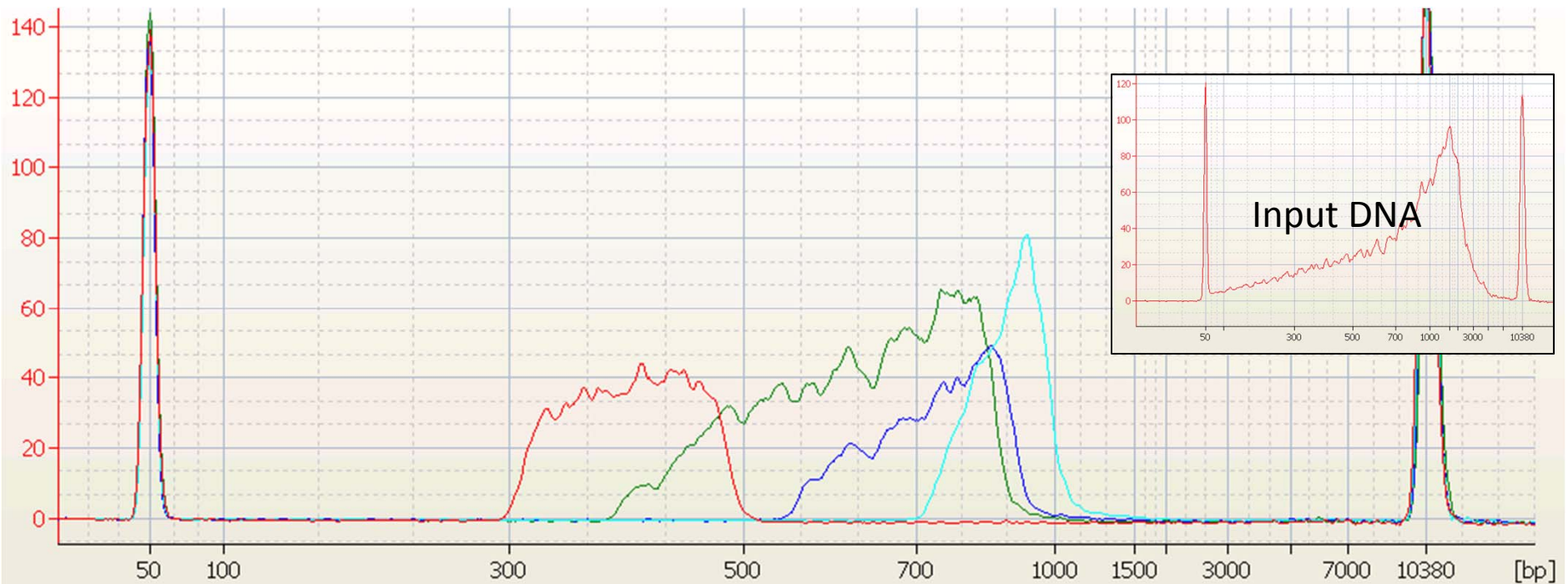


Short current reversal in elution module before sample removal  
boosts recovery to >60%



# Pippin Prep for ChIP-seq

Broad collections in 1.5% cassette (sealed elution modules)



<u>Programmed</u>	<u>Actual</u>
300-500	291-523
400-900	376-925
550-950	535-976
700-1100	683-1182

# New Pippin Prep System Software: Tabbed Browser Format



With run in progress, user can edit other protocols, review old runs, manage log files.

The screenshot shows the Pippin Prep software interface with three tabs: 'Protocol Editor', 'Log Review', and 'File Manager'. The 'Protocol Editor' tab is active and displays a chromatogram on the left and a protocol control panel on the right.

The chromatogram shows five traces (1-5) over a time range from 00:00 to 02:00. The x-axis is labeled 'Time, hh:mm'. The traces show peaks at approximately 00:40, 00:50, 01:00, 01:10, 01:30, and 01:40.

The protocol control panel shows the following data:

Sample ID	Current	Elution Timer	Reference	Idle	Separate	Elute
5 Marker B	1.94	00:00:00	5	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
4 sample 4 sequence	1.91	00:04:12	4	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
3 sample 3 sequence	1.96	00:43:09	3	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
2 sample 2 sequence	1.90	00:05:00	2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
1 sample 1 PCR	1.49	01:05:44	1	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

The protocol control panel also shows a 'Protocol Name' dropdown set to 'demonstration protocol', a progress bar at 100.0% (01:59:59), and various status indicators:

BP at LED	Voltage, V	Clock
798	99.5	26-Jan-2011 01:22:16
BP at Bridge	Remote Access	Estimated Completion Time
672	<input checked="" type="checkbox"/>	21-Feb-2118 06:30:59

At the bottom of the interface, there are buttons for 'TEST', 'START', 'PAUSE', 'STOP', 'RESERVED', 'SNAPSHOT', 'INFO', and 'SHUTDOWN'. The Pippin Prep logo and Sage Science logo are also visible.

Home screen shows **time to completion**, **bp position at LED** and **branch point**.



# New Pippin Prep System Software: New Collection Modes and Protocol Editor



Selection Mode						bp Selection criteria				Time criteria			Band
	Tight	Range	Time	Peak	Ref	BP Target	BP Start	BP End	BP Pause	T Start	T End	T Pause	BP Thresh
5						0	0	0	0	00:00:00	00:00:00	00:00:00	0
4						300	276	324	0	00:00:00	00:00:00	00:00:00	0
3						425	250	600	0	00:00:00	00:00:00	00:00:00	0
2						0	0	0	0	00:50:00	00:55:00	00:00:00	0
1						0	0	0	0	00:00:00	00:00:00	00:00:00	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 value

# New Pippin Prep System Software: Log File Review Tab



Main Protocol Editor **Log Review** File Manager

Log File: /home/pippin/PippinPrep/Logs/2011-01-25\_23-22-14\_PPLog.bt

Protocol Name: demonstration protocol      Cassette Name: 2% Marker B No Overflow Detection      Run Time, hh:mm: 02:00

	Tight	Range	Time	Peak	Ref	Off	BP Target	BP Start	BP End	BP Pause	T Start	T End	T Pause	BP Thresh	Sample ID Template	Sig Mon	BP Range Flag	Pause Enabled
5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0	0	0	0	00:00:00	00:00:00	00:00:00	0	Marker B	<input type="checkbox"/>	none	<input type="checkbox"/>
4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	300	276	324	0	00:00:00	00:00:00	00:00:00	0	sample 4 sequence	<input type="checkbox"/>	tight	<input type="checkbox"/>
3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	425	250	600	0	00:00:00	00:00:00	00:00:00	0	sample 3 sequence	<input type="checkbox"/>	broad	<input type="checkbox"/>
2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0	0	0	0	00:50:00	00:55:00	00:00:00	0	sample 2 sequence	<input type="checkbox"/>	none	<input type="checkbox"/>
1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0	0	0	0	00:00:00	00:00:00	00:00:00	120	sample 1 PCR	<input type="checkbox"/>	none	<input type="checkbox"/>

**SIGNAL**

Photocurrent, mA

Time, hh:mm:ss

**CURRENT**

Lane Current, mA

Time, hh:mm:ss

Lane	Peak	Ladder BP
Lane 1 lph	2706	75
Lane 2 lph	3161	150
Lane 3 lph	4079	300
Lane 4 lph	5968	600
Filtered Ref	0	1200
Threshold	0	50000
SW Version	4.20	0
FW Version	2.95	0
Peaks Found	5	0

ANALYZE PEAKS      SNAPSHOT

Shows protocol details (top), interactive plots of optical and electrophoresis current data, while new run is in progress.

# Pippin Prep 2011: Summary



## **User-validated performance** in PE library construction

Benefits in throughput, process cost, and library quality

New offerings for Q'1 2011 expand system size range: **75 - 10,000 bp**

A cassette (or two) for almost every NGS application:

Libraries for **PE reads, miRNA, mate-pair, CHiP-seq**

Please visit us at poster **#27** for more details and questions. Come and see the Pippin Prep in our **Lanai suite: #292**.

Sales inquiries: [paul.ventura@sagescience.com](mailto:paul.ventura@sagescience.com)

Technical inquiries: [chris.boles@sagescience.com](mailto:chris.boles@sagescience.com), [sadaf.hoda@sagescience.com](mailto:sadaf.hoda@sagescience.com)

Company inquiries: [gary.magnant@sagescience.com](mailto:gary.magnant@sagescience.com)

Other info and contact information on our website: [www.sagescience.com](http://www.sagescience.com)