Pippin Prep 2011: New Applications

Expanded Cassette Offerings and System Improvements

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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

- 1st Mate-Pair
- "Std." Paired-End
- miRNA library
- CHiP-seq, 2nd Mate-Pair
Size fractionation in NGS Applications

![Diagram showing size fractionation in NGS Applications](image)

- Low Gel Conc.
- High Gel Conc.

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

High Gel Resolution - Low CV of collected fraction

- miRNA library

- Size of collected DNA (bp)
## Pippin Prep Products for NGS Applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Cassette types</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE libraries</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>1.5% <em>(new, Nov. 2010)</em></td>
</tr>
<tr>
<td></td>
<td>2% Ethidium Free <em>(new Feb. 2011)</em></td>
</tr>
<tr>
<td>Small RNA libraries</td>
<td>3% <em>(new, Feb. 2011)</em></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Mate-pair</td>
<td>0.75% <em>(new, Feb. 2011)</em></td>
</tr>
<tr>
<td>CHiP-seq, 2&lt;sup&gt;nd&lt;/sup&gt; Mate-pair</td>
<td>2%, 1.5% cassettes with Closed elution modules <em>(new, Mar. 2011)</em></td>
</tr>
</tbody>
</table>
Pippin Prep for PE Libraries: 2% Cassettes

Improvement in PE Library Quality:

- 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

- Tight insert size.
- No LMW “shelf”.
- Consistent, high yield.
Pippin Prep for PE Libraries: 1.5% cassette
Expands range to ~1.5 kb, with tight CVs.

<table>
<thead>
<tr>
<th>Programmed</th>
<th>Actual</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 tight</td>
<td>408 (364-454)</td>
<td>3</td>
</tr>
<tr>
<td>600 tight</td>
<td>598 (545-674)</td>
<td>3</td>
</tr>
<tr>
<td>800 tight</td>
<td>811 (690-973)</td>
<td>5</td>
</tr>
<tr>
<td>1000 tight</td>
<td>977 (851-1361)</td>
<td>6</td>
</tr>
</tbody>
</table>
Pippin Prep of miRNA libraries: 3% cassette
Optimal resolution in 50 – 200 bp range.

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

- 3% Agarose
- 2% Agarose

- Size, Bp: 20, 50, 75, 100, 150, 250, 350, 450, 550, 650, 750, 850, 950

Graph shows the time for peak to reach photodetector in minutes and the mobility of DNA fragments in Bp on different agarose concentrations.
Pippin Prep of miRNA libraries: 3% cassette Performance on gDNA samples

<table>
<thead>
<tr>
<th>Programmed</th>
<th>Actual</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 tight</td>
<td>97 (82-114)</td>
<td>5</td>
</tr>
<tr>
<td>100 tight</td>
<td>106 (90-129)</td>
<td>5</td>
</tr>
<tr>
<td>120 tight</td>
<td>127 (109-144)</td>
<td>4</td>
</tr>
<tr>
<td>130 tight</td>
<td>134 (115-149)</td>
<td>4</td>
</tr>
</tbody>
</table>
Pippin Prep 0.75% Cassettes for Mate-Pair Libraries

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

5 µg/lane input: CVs ~10-16%

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

Marker lane

<table>
<thead>
<tr>
<th>Size (kb)</th>
<th>8.2</th>
<th>7.1</th>
<th>7.4</th>
<th>9.1</th>
<th>9.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV</td>
<td>12%</td>
<td>10%</td>
<td>12%</td>
<td>13%</td>
<td>16%</td>
</tr>
</tbody>
</table>

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

Permits long elution times without overflow or manual intervention.
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)

<table>
<thead>
<tr>
<th>Programmed</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>300-500</td>
<td>291-523</td>
</tr>
<tr>
<td>400-900</td>
<td>376-925</td>
</tr>
<tr>
<td>550-950</td>
<td>535-976</td>
</tr>
<tr>
<td>700-1100</td>
<td>683-1182</td>
</tr>
</tbody>
</table>
New Pippin Prep System Software: Tabbed Browser Format

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

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

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**.

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