Automated DNA Size Selection

NGS Library Construction

- Whole genome sequencing
- miRNA library isolation
- RNA-seq
- ChIP-seq
The Importance of NGS Sample Prep

The manipulation of DNA fragments is an essential step when preparing libraries for next-gen sequencing (NGS). Automated size selection of DNA or DNA libraries can be an invaluable tool for providing the highest-quality sample to sequence, reducing sequencing costs and improving downstream data analysis.

The Pippin Difference

In several independent studies, Pippin automated DNA size selection has been shown to provide superior libraries. Pippin has been the go-to system for optimizing samples for most of today’s NGS platforms and has been adopted by most leading labs in the sequencing community.

Pippin technology features pre-cast agarose gel cassettes. DNA fractions are collected by electro-elution using a branched channel configuration with switching electrodes. The switch timing is determined by measuring the rate of DNA migration with optical detection of labeled markers.

Users enter a target value or size range in software, and the selected DNA fragments are collected in buffer. Collections are removed with a standard pipette.

<table>
<thead>
<tr>
<th>Selection Chart</th>
<th>Pippin Prep</th>
<th>BluePippin</th>
<th>PippinHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. Sample Capacity</td>
<td>5</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Pulsed-Field</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Run Time</td>
<td>50-90 min</td>
<td>50-90 min*</td>
<td>25-45 min</td>
</tr>
<tr>
<td>Max. Size Selection</td>
<td>1.5 kb</td>
<td>50 kb</td>
<td>1.5 kb</td>
</tr>
<tr>
<td>High-Pass Size Filtering</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Max. DNA input</td>
<td>5 µg</td>
<td>5 µg</td>
<td>1.5 µg</td>
</tr>
</tbody>
</table>

* does not include pulsed-field run-times.

**Specifications**

<table>
<thead>
<tr>
<th></th>
<th>Pippin Prep</th>
<th>BluePippin</th>
<th>PippinHT</th>
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</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>&gt; 93%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproducibility</td>
<td>&gt; 92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. Size Distribution as CV</td>
<td>8%</td>
<td></td>
<td></td>
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<tr>
<td>Recovery</td>
<td>50-80%</td>
<td></td>
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</tbody>
</table>

* the specifications for the Pippin Prep, BluePippin, and PippinHT are the same for comparable size selections. Inquire for specifications for BluePippin pulsed-field collections.
Key Applications

- Whole genome sequencing
- miRNA library isolation
- RNA-seq
- ChIP-seq

Benefits of Pippin Sizing:

- Narrow-range selection provides more optimal fragment lengths for short-read NGS libraries
- High-pass size filtering provides large-length libraries for long-read sequencing
- Eliminates unwanted artifacts such as adapters, dimers, and short DNA fragments
- Provides users flexibility in setting size-selection ranges
- Provides run-to-run reproducibility and more accurate assemblies

Working with Large DNA: BluePippin and Pippin Pulse

As long-read sequencing becomes more broadly available, the ability to work with very large DNA fragments is more important than ever. With BluePippin, researchers can select targeted ranges of DNA up to 50 kb, or use the tool to remove all fragments shorter than a certain threshold size. This step significantly increases the read lengths that can be generated from long-read sequencing (see graph).

Pippin Pulse, another tool from Sage Science, can be used to quickly check the size of long DNA fragments. Using pulsed-field gel electrophoresis, the Pippin Pulse is an affordable tool that makes it easy to resolve DNA out to 800 kb or more.

* Graph excerpted from PacBio poster: Taking Advantage of Long Read Lengths with Improved Library Preparation Methods. Joan Wilson et al., 2013
In the Literature:

"Of the three sizing fractionation methods tested for target recovery efficiency, throughput, and risk of cross-sample contamination, Pippin Prep, an automated optical electrophoretic system that does not require gel extraction, was the most efficient and reproducible, with the tightest, most specific sizing."


"Bioanalyzer results suggested that [Pippin] automated size-selection libraries were substantially more consistent than gel extraction libraries. In contrast to automated size-selected samples, gel excision samples did not appear to saturate in the range of coverage observed. This is likely because size selection was imprecise or 'leaky,' with substantial representation of fragments of lengths relatively distant from the size-selection target mean. Careful practitioners can achieve roughly 50% of the precision and repeatability of automated DNA size selection."


Also from Sage Science: SageELF™ Whole Sample Fractionation

For many applications, it is useful to prepare multiple libraries of different insert sizes from the same sample. The SageELF allows for automated whole-sample fractionation based on size, resulting in 12 fractions from each sample. The tool is equipped with pulsed-field electrophoresis for resolving large DNA. Whole-sample fractionation is important for mate-pair sequencing, splice variant detection, reducing complexity in cDNA or RNA samples, and preserving precious samples.