Pippin™ Automated DNA Size Selection

NGS Library Construction

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

sage science

www.sagescience.com

Pippin Prep™

BluePippin™

PippinHT™

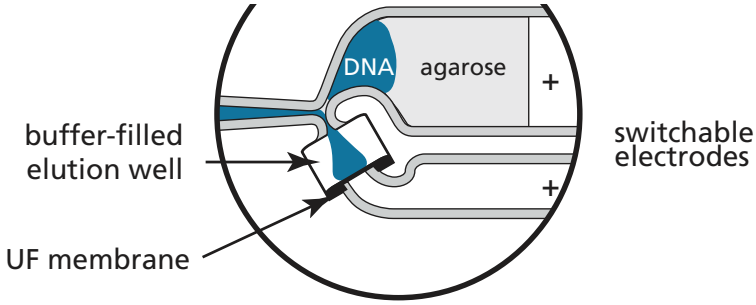
The Importance of NGS Sample Prep

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.

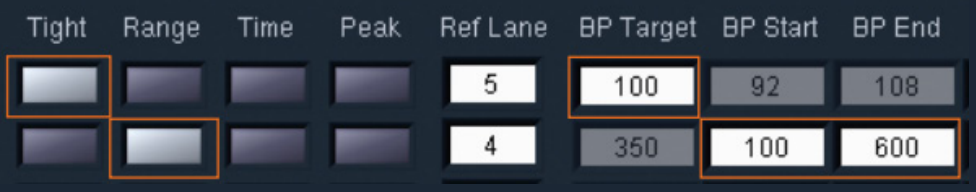
The Pippin Difference

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.



Selection Chart	Pippin Prep	BluePippin	PippinHT
Max. Sample Capacity	5	5	24
Pulsed-Field	No	Yes	Yes
Run Time	50-90 min	50-90 min*	25-45 min
Max. Size Selection	8 kb	50 kb	1.5 kb
High-Pass Size Filtering	No	Yes	Yes
Max. DNA input	5 ug	5 ug	1.5 ug

* does not include pulsed-field run-times.

Specifications*	
Accuracy	> 93%
Reproducibility	> 92%
Min. Size Distribution as CV	8%
Recovery	50-80%

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

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Key Applications

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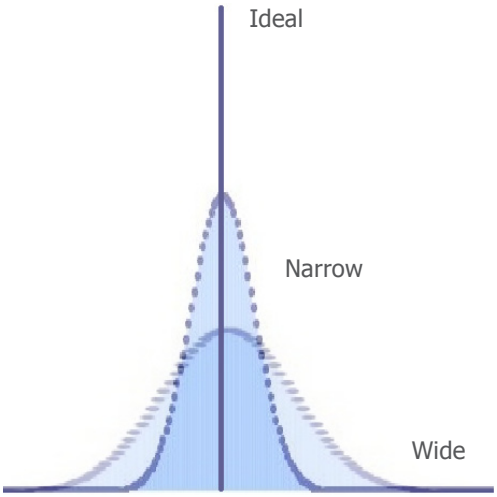
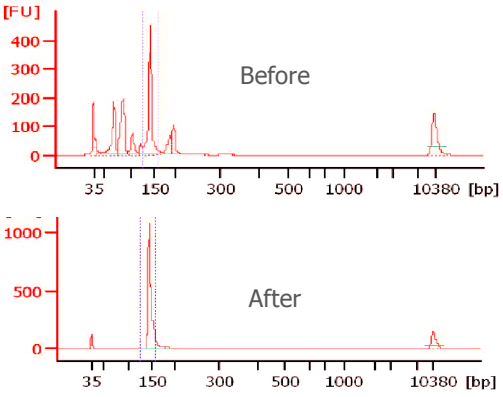
- miRNA library isolation
- RNA-seq
- ChIP-seq

Benefits of Pippin Sizing:

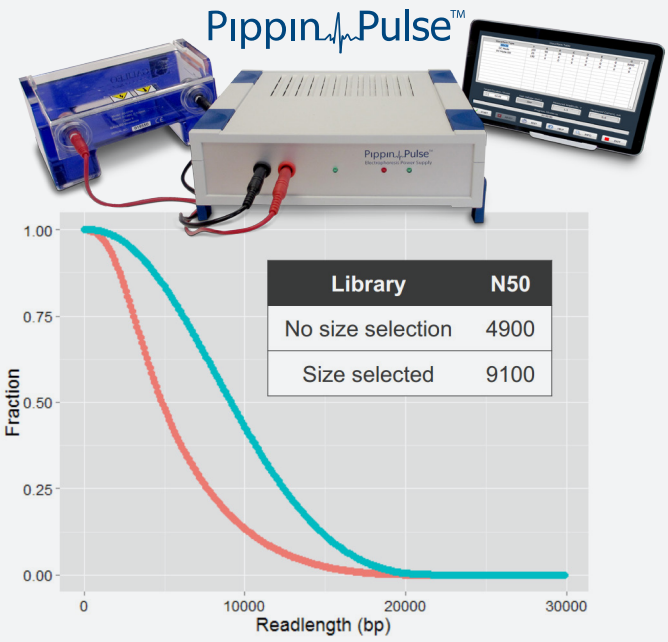
- Narrow-range selection provides more optimal fragment lengths for short-read NGS libraries
- High-pass size filtering provides large-length libraries for long-read sequencing
- Eliminates unwanted artifacts such as adapters, dimers, and short DNA fragments
- Provides users flexibility in setting size-selection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



collect narrow fragment ranges



remove unwanted artifacts



Library	N50
No size selection	4900
Size selected	9100

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

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.

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."

— *Extracted from Duhaime et al. "Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method," Environmental Microbiology (2012) 14(9), 2526–2537.*

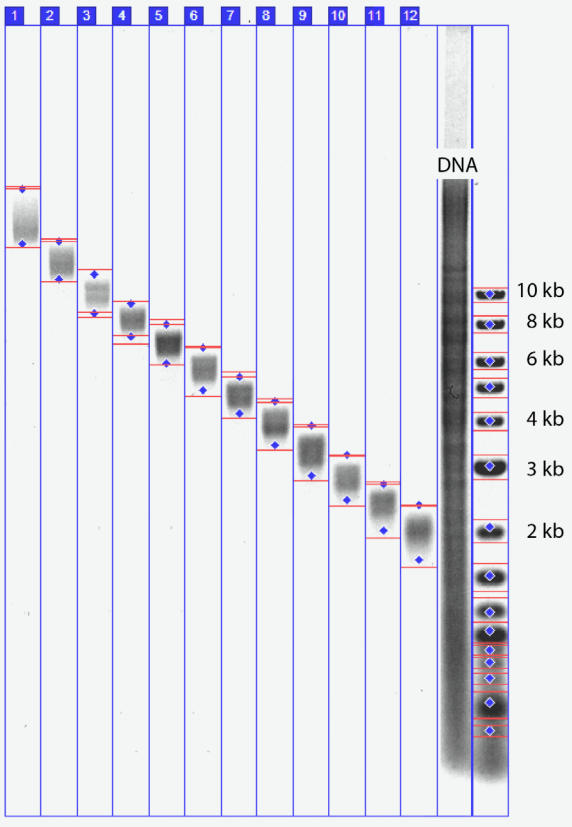
"Bioanalyzer results suggested that [Pippin] automated size-selection libraries were substantially more consistent than gel extraction libraries. In contrast to automated size-selected samples, gel excision samples did not appear to saturate in the range of coverage observed. This is likely because size 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."

— *Extracted from Peterson et al. "Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species," PLoS ONE 7(5): e37135. doi:10.1371/journal.pone.0037135*

SageELF™

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.



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