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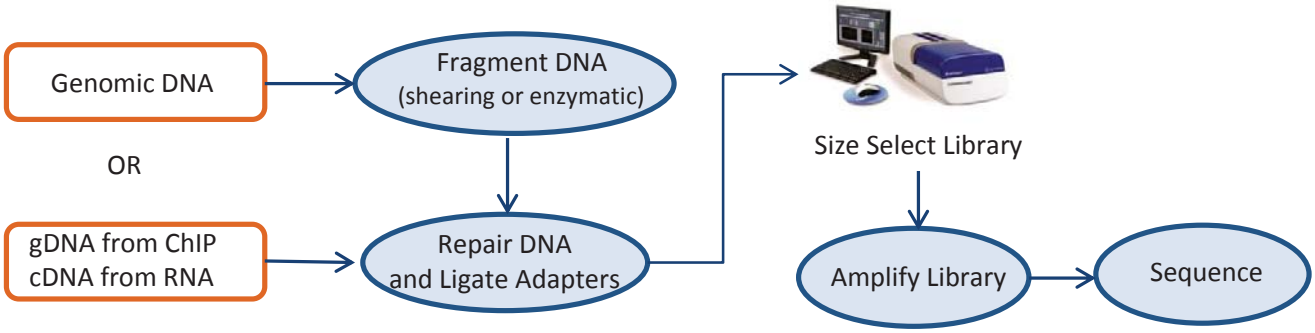
Pippin™ DNA Size-Selection Core Recommendation Guide

About this guide:
This document provides recommendations to core facility customers on techniques that can benefit from Pippin DNA size selection, and how sequencing results may be improved with size selected libraries. Depending on the sequencing platform, benefits may include higher sequencing throughput, higher quality data, increased read lengths, and improved sample-to-sample reproducibility.

About Pippin size selection:
Pippin Prep (90bp – 1.5 kb) and Blue Pippin (90bp - 50 kb) are automated DNA sizing tools from Sage Science that offer better reproducibility, accuracy, and yield than manual gels[1,2,3,4] while preventing sample cross-contamination. They use a preparative electrophoresis instrument with pre-cast, disposable gel cassettes to separate and extract DNA fragments based on user-defined size ranges using a proprietary electro-elution process.

Automated DNA sizing can be used to remove low molecular weight content such as adapter-dimers, and to collect narrow size distributions or broad fragment ranges, according to the needs of specific sequencing workflows.

Typical NGS Workflow:



Applications:

Research Area	Sequencing Workflow	Platform
Genome structure and function	Whole genome sequencing	all platforms
Structural variation/ Genome Rearrangement	Mate-pair sequencing Single molecule sequencing	Illumina Pac-Bio
Allelic variation	Paired-end sequencing	Ilumina
Genetic variation	Paired-end sequencing, Exome sequencing	Illumina Ion Torrent
Population genetics	ddRAD-seq	Illumina
Chromatin structure and regulation	ChIP-seq	Illumina Ion Torrent
Regulation of transcription/gene expression	RNA-seq, miRNA library isolation, isoform discovery	all platforms

Benefits:

Illumina (miSeq and HiSeq)	Downstream Benefit
Paired-End Library Construction[a]	Narrow and cleanly sized libraries (without LMW fragments) facilitates "stitching." Variants such as indels are more easily identified in downstream analysis.
Mate-Pair Library Construction[b]	Benefits from precise sizing of inserts for identification of structural variants and rearrangement.
RNA-seq Library Construction[c]	Improves library diversity in sequencing results (number of genes, transcription start sites, isoforms, splice sites, and promoters).
miRNA library Isolation	miRNA libraries can be isolated from lower and higher MW artifacts and provide a more efficient and unambiguous sequencing result.
ddRAD-seq	Precise size selection allows for better tuning of loci targeted for analysis and improves accuracy of results.
ChIP-seq	Narrow or broad DNA size selection provides precise size selections reproducibly, with higher yield.
Ion Torrent (PGM and Proton)	**Downstream Benefit**
Bead Template Prep[d]	Narrow fragment distribution and accurate sizing provide optimal conditions prior to emulsion PCR. This results in higher throughput per chip and better sequencing efficiency.
ChIP-Seq	Narrow or broad DNA size selection provides precise size selections reproducibly, with higher yield.
Pacific Biosciences (PacBio RS and RSII)	**Downstream Benefit**
Library Prep (20kb) with BluePippin High-Pass protocol[e]	Average read length can improve by 50% or more by removing smaller fragments from the library prior to sequencing. Overall instrument production improves significantly as Pippin sizing optimizes each ZMW to sequence longer fragments and generate more data.

a Validated by Illumina
b Recommended in Illumina's "Nextera® Mate Pair Sample Preparation Guide"
c Featured in Epicentre's" ScriptSeq™ v2 RNA-Seq Library Preparation Kit, Pippin Prep™ Compatible"
d Recommended in the "Ion Xpress™ Plus gDNA Fragment Library Preparation"
e Featured in PacBio's "Procedure & Checklist - 20 kb Template Preparation Using BluePippin™ Size-Selection System"

References

1. *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.

2. *Park et al. "An improved approach to mate-paired library preparation for Illumina sequencing,"* Methods in Next Generation Sequencing, (2013) Vol.1: 10-20

3. *Peterson et al. "Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species,"* PLoS ONE (2012) 7(5): e37135. doi:10.1371/journal.pone.0037135

4. *Quail et al. "*Evaluation and optimisation of preparative semi-automated electrophoresis systems for Illumina library preparation,**"** Electrophoresis (2012) 33(23): 3571-3528
DOI: 10.1002/elps.201200128

"Bioanalyzer results suggested that [Pippin] automated size-selection libraries were substantially more consistent than gel extraction libraries. … Careful practitioners can achieve roughly 50% of the precision and repeatability of automated DNA size selection."
—From *Peterson et al.*

"Of the three sizing fractionation methods tested for target recovery efficiency, throughput, and risk of cross-sample contamination, Pippin Prep … was the most efficient and reproducible, with the tightest, most specific sizing."
— From Duhaime et al.

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