Technical Note

DNA Fragment Library Size Range Selection

Apollo 324™ System

Overview

This technical note presents instructions for adjusting experimental conditions when using the Apollo 324™ System to produce DNA fragment libraries with custom molecular weight range (size in base pairs) profiles.

The binding of DNA with magnetic beads used by the Apollo 324 System depends, in part, on the concentrations of polyethylene glycol (PEG) and salt (NaCl) in the DNA solution, and the lengths of individual fragments. User modifications to the PEG and buffer concentrations used by the Apollo 324 System selects the size range of the library produced. By making relatively straightforward modifications to the PEG and salt concentrations of BeadX™ tubes used by the Apollo 324 System during fragment library cleanup and size selection, researchers can extend the laboratory utility of the system beyond standard next-generation sequencing library preparation to automate the production of fragment libraries compatible with ChIP (Chromatin Immunoprecipitation), RNA, and *de novo* sequencing applications. This document presents buffer conditions and buffer modification instructions for producing libraries compatible with each of these four applications.

Additionally, the ability to produce DNA libraries of varying size ranges can be used to assist in the characterization and optimization of new application workflows.

The Apollo 324 System

The Apollo 324 System is a benchtop workstation that uses magnetic bead technology to automate next-generation sequencing library preparation. The robotic benchtop instrument component of the system features two heating/cooling units for 96-well PCR plates, the BeadX magnetic bead capture system, control software and a dedicated touchscreen interface.

The Apollo 324 performs end-repair, A tail addition, and adapter ligation on input fragmented DNA. The system then proceeds to perform double bead-based cleanup and molecular weight range selection to produce an application-ready DNA library.

Size Range Requirements for DNA Fragment Libraries

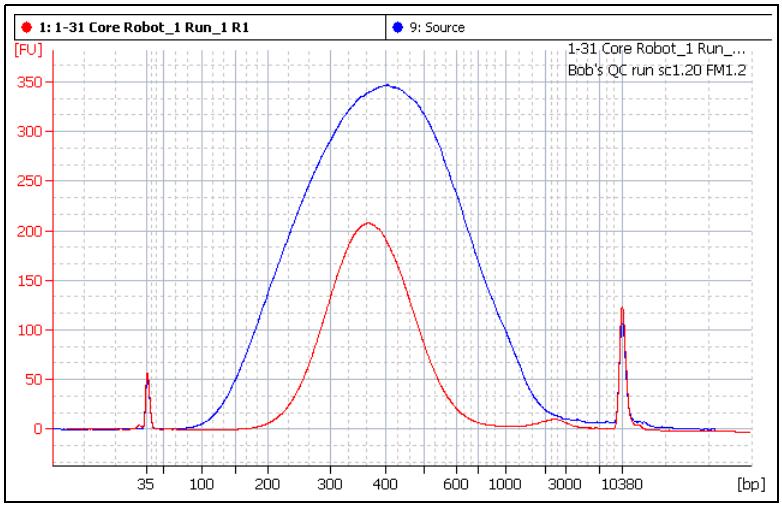
The PrepX™ DNA Library Kit developed for the Apollo 324 System produces DNA fragment libraries with a size range of 270–500 base pairs (bp). This corresponds to the optimal DNA fragment library input size range for next-generation sequencing applications using the Illumina® Genome Analyzer, HiSeq™ and MiSeq™ next-generation sequencing systems. Other genetic analysis applications, such as ChIP-Seq and various RNA-Seq applications, have different optimal DNA library size ranges.

This technical note presents experimental conditions for automatically producing DNA fragment libraries with four distinct size range profiles:

- Low range capture (150–300 bp) for RNA analysis applications
- Mid range capture (270–500 bp) for genomic sequencing applications
- High range capture (500–700 bp) for *de novo* sequencing applications
- >200 bp range capture for ChIP analysis applications

Size Range Measurement and Definition

The size range of a DNA fragment library can be measured using the Agilent® 2100 Bioanalyzer. A DNA fragment library is prepared for analysis using the Agilent High Sensitivity DNA Kit. Using size standards to characterize electrophoretic separation of DNA fragments, the Agilent Bioanalyzer can convert elapsed time of electrophoresis to DNA fragment molecular weight, where molecular weights are expressed as DNA base pairs. Data from such analyses are presented as electropherograms plotting fluorescence intensity against electrophoretic mobility.

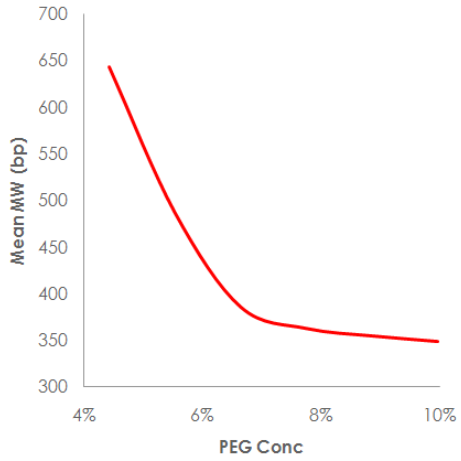


Electropherogram visualization of mid range size selection of the PrepX DNA fragment library. The blue line represents unprocessed fragmented DNA input. The red line represents the fragment library prepared and range-selected using the PrepX DNA Library Kit.

Adjusting PEG Concentrations for Size Range Selection

Magnetic carboxyl-coated beads used in the IntegenX BeadX magnetic capture system reversibly bind DNA in the presence of PEG and sodium chloride[1]. PEG is a DNA precipitant, and the capture and binding of DNA fragments of different lengths by the beads depends on the relative concentration of PEG in the bead solutions. Increasing the PEG concentration in a DNA solution favors the precipitation and capture of shorter DNA fragments. Salt concentration in the solution is a significant factor affecting the degree of affinity of DNA strands for the magnetic beads. Higher concentrations of salt favor stronger affinity of DNA for the magnetic beads; lower concentrations of salt favor dissociation. Manipulating the concentrations of these solutes during the final cleanup of processed DNA libraries enables size range selection[2].

During automated DNA fragment library preparation on the Apollo 324 System, the final cleanup steps are: the “Bead 2 capture” to remove larger, higher molecular weight material, such as poorly fragmented DNA and the “Bead 3 capture” to remove smaller, low molecular weight material such as adapters and adapter dimers. By adjusting the relative PEG and salt concentration in the bead solutions used in these steps, you can tailor the size range selection and capture of the desired DNA fragments.



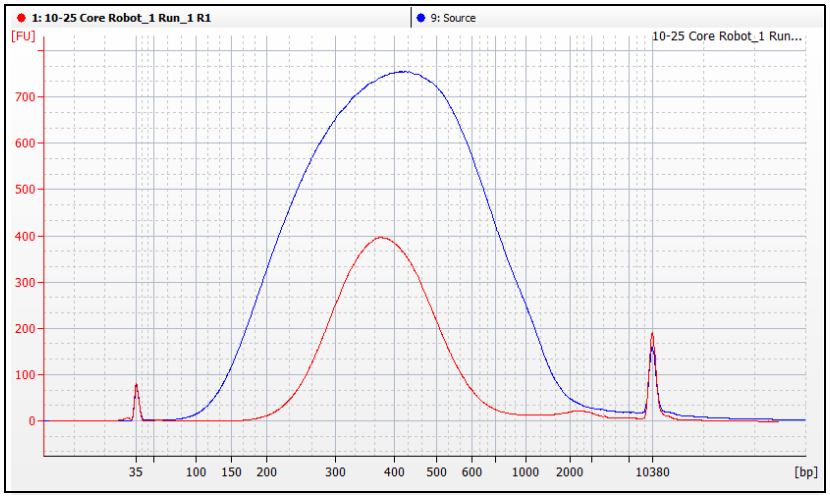
Plot of PEG concentration vs. fragment size. The plot shows higher PEG concentrations selecting for precipitation of shorter DNA fragments.

Using the PrepX DNA Library Kit for Custom Fragment Size Selection

The basic PrepX DNA Library Kit for the Apollo 324 System produces DNA fragment libraries with a size range of 270–500 base pairs (bp). This corresponds to the mid bp range, which is the optimal DNA fragment library input size range for next-generation sequencing applications using the Illumina® Genome Analyzer and the Illumina HiSeq 2000 next-generation sequencing systems.

The Apollo 324 System gives you the flexibility to automate DNA library preparation for applications requiring different optimal DNA library size ranges. You can customize fragment size targets for your library DNA by modifying the bead chemistry, adjusting the PEG concentrations of the BeadX tubes used during library cleanup and size range selection.

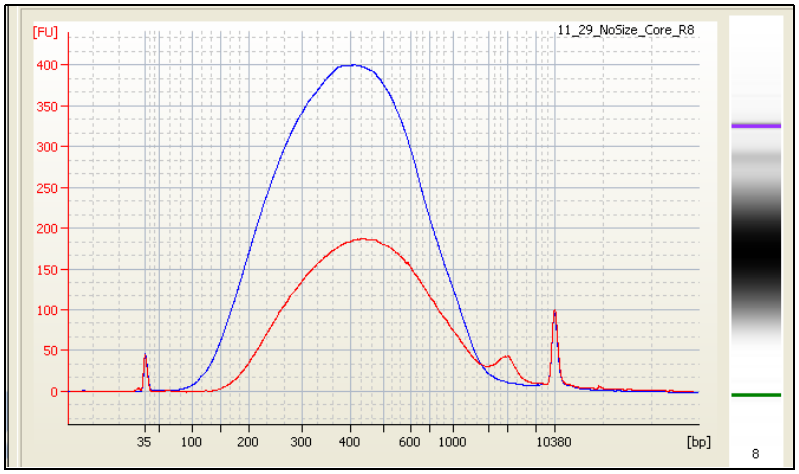
Mid Range Overlay (Standard PrepX Kit)



Tube Position	PEG Solution
Bead 3	11% PEG, 1.25M NaCl
Bead 2	8% PEG, 2.5M NaCl
Bead 1	20% PEG, 2.5M NaCl

Electropherogram of fluorescence intensity vs. fragment bp for mid range-selected fragment library. The blue line represents unprocessed fragmented DNA input. The red line represents the fragment library prepared and range-selected using mid range BeadX conditions during final cleanup and size selection.

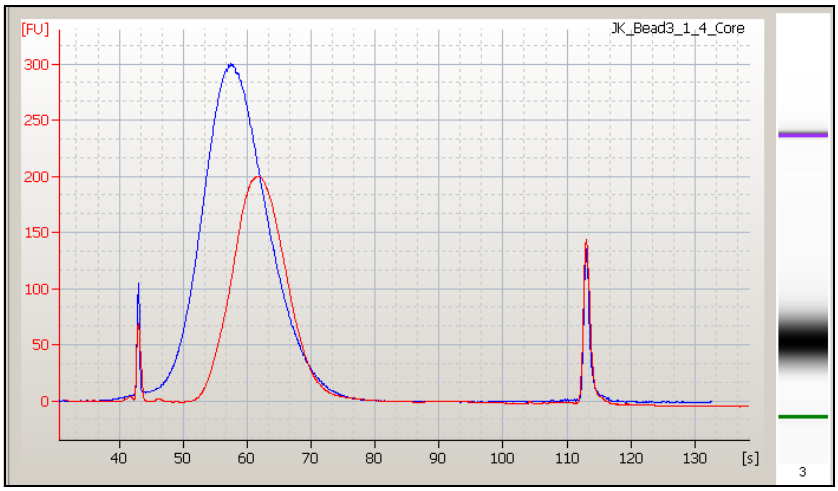
>200 BP Range Overlay (ChIP-Seq optimized)



Tube Position	PEG Solution
Bead 3	25% PEG, 1.25M NaCl
Bead 2	0% PEG, 2.5M NaCl
Bead 1	20% PEG, 2.5M NaCl

Electropherogram of fluorescence intensity vs. fragment size for the >200 bp range-selected fragment library. The blue line represents unprocessed fragmented input DNA. The red line represents the fragment library prepared and range-selected using >200 bp range BeadX conditions during final cleanup and size selection.

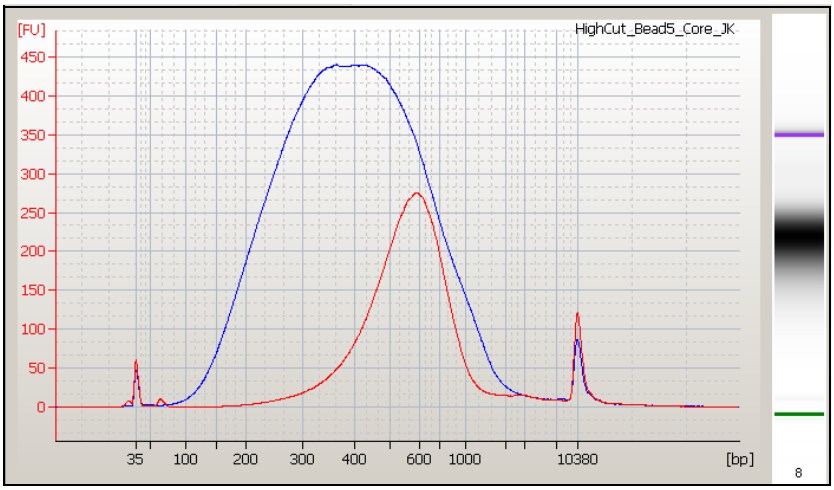
Low Range Overlay (for RNA applications)



Tube Position	PEG Solution
Bead 3	16% PEG, 1.25M NaCl
Bead 2	10% PEG, 2.5M NaCl
Bead 1	20% PEG, 2.5M NaCl

Electropherogram of fluorescence intensity vs. elapsed time of electrophoresis (seconds) for low range-selected fragment library. The blue line represents unprocessed fragmented DNA input. The red line represents the fragment library prepared and range-selected using low range BeadX conditions during final cleanup and size selection. The average size is 219 bp and the range at half-height peak width is 165–267 bp.

High Range Overlay (for *de novo* sequencing applications)



Tube Position	PEG Solution
Bead 3	8% PEG, 1.25M NaCl
Bead 2	6% PEG, 2.5M NaCl
Bead 1	20% PEG, 2.5M NaCl

Electropherogram of fluorescence intensity vs. fragment bp for high range-selected fragment library. The blue line represents unprocessed fragmented DNA input. The red line represents the fragment library prepared and range-selected using high range BeadX conditions during final cleanup and size selection. The average size is 579 bp and the range at half-height peak width is 435–816 bp.

Implementation on the Apollo 324 System

Adjusting the PEG Concentration

To create custom size targets, the bead solution in the PrepX DNA Library Kit is replaced with user-defined chemistries that select fragments in the desired size range. These customized tubes replace the four tube strips of standard bead solution in rows 9-12 of Block 3 on the work surface.

Note: No modification is required for mid range size selection. Use the PrepX DNA Library Kit (Catalog number 400007) as provided.

In the Apollo 324 automated DNA fragment library preparation, the Bead 1 solution is used for cleanup of the A-Tailing and End Repair reactions. As shown in the following table, a lower concentration of PEG is used in the Bead 2 solution to capture only the largest fragments; these beads are pelleted and discarded. The supernatant is transferred into a higher concentration PEG solution, Bead 3, which removes smaller fragments, including adapters, dimers, reaction material, enzymes, and buffers.

Target Size Range	Bead 2 Capture (Beads are discarded after Bead 2 capture)	Bead 3 Capture (Supernatant is discarded after Bead 3 capture)
Mid range (standard kit): 270–500 bp	8% PEG, 2.5M NaCl	11% PEG, 1.25M NaCl
>200 bp range	0% PEG, 2.5M NaCl	25% PEG, 1.25M NaCl
Low range: 150–300 bp	10% PEG, 2.5M NaCl	16% PEG, 1.25M NaCl
High range: 500–700 bp	6% PEG, 2.5M NaCl	8% PEG, 1.25M NaCl

Positions and Contents of Standard Kit and Custom Tubes for Modified PEG Solutions

Tube Position	Standard Kit (Mid Range) Tube Contents	>200 BP Range Tube Contents	Low Range Tube Contents	High Range Tube Contents
Position 4	Empty	Empty	Empty	Empty
Bead 3	30 µL of 11% PEG, 1.25M NaCl	30 µL of 25% PEG, 1.25M NaCl	30 µL of 16% PEG, 1.25M NaCl	30 µL of 8% PEG, 1.25M NaCl
Bead 2	40 µL of 8% PEG, 2.5 M NaCl	40 µL of 0% PEG, 2.5 M NaCl	40 µL of 10% PEG, 2.5 M NaCl	40 µL of 6% PEG, 2.5 M NaCl
Bead 1	80 µL of 20% PEG, 2.5M NaCl	80 µL of 20% PEG, 2.5M NaCl	80 µL of 20% PEG, 2.5M NaCl	80 µL of 20% PEG, 2.5M NaCl

Materials for Adjusting PEG Solutions

Item	Part Number	Vendor
Polyethylene Glycol (avg. wt. 8000)	P4463	Sigma-Aldrich®
5M NaCl	AM9760G	Ambion®
Reagent Grade Water	10977	Gibco®
8-tube strip (to be cut in half)	PCR-0208-C	Axygen®

Using the Standard PrepX Kit for Preparing Mid Range (270–500 bp) Libraries

Use the standard IntegenX PrepX kit in the Apollo 324 System in order to create libraries of 270–500 bp.

PEG Solution Concentrations

The PEG solution concentrations in the standard PrepX kit are as follows:

Mid Range Recipe	Bead 1 20% PEG, 2.5M NaCl	Bead 2 8% PEG 2.5M NaCl	Bead 3 11% PEG 1.25M NaCl
PEG (8000)	2.0 g	0.8 g	1.1 g
5M NaCl	5.0 mL	5.0 mL	2.5 mL
Reagent Grade Water	3.0 mL	4.2 mL	6.4 mL
Total Volume	**10.0 mL**	**10.0 mL**	**10.0 mL**

Placing the Tubes

Each PrepX tube (4-tube strip with blue seal) contains the following:

Tube Position	PEG Solution	Volume (µL)
Position 4	Empty	Empty
Bead 3	11% PEG, 1.25M NaCl	30
Bead 2	8% PEG, 2.5M NaCl	40
Bead 1	20% PEG, 2.5M NaCl	80

Place the desired number of 4-tube strips in Rows 9–12 of Block 3. Ensure that the empty tube of each strip is in Row 12 of Block 3. This is the furthest row from the front of the instrument.

Instructions for Preparing > 200 BP Range Libraries

These instructions explain how to prepare PEG solutions for use in the Apollo 324 System in order to create libraries of >200 bp.

Note: The BeadX-High kit (Catalog number 400024) is available for performing the >200 bp range method recommended for ChIP-Seq samples.

Recipe

1. Prepare the PEG solutions, using the following recipe:

> 200 BP Recipe	Bead 1 20% PEG 2.5M NaCl	Bead 2 0% PEG 2.5M NaCl	Bead 3 25% PEG 1.25M NaCl
PEG (8000)	2.0 g	0.0 g	2.5 g
5M NaCl	5.0 mL	5.0 mL	2.5 mL
Reagent Grade Water	3.0 mL	5.0 mL	5.0 mL
Total Volume	**10.0 mL**	**10.0 mL**	**10.0 mL**

2. Incubate the solutions in a water bath (approximately 60 °C) to dissolve the PEG.

Placing the Tubes

1. Pipette the following PEG solutions into each Axygen 4-tube strip being used. Use these custom tubes instead of the blue bead tubes provided by IntegenX.

Tube Position	PEG Solution	Volume (µL)
Position 4	Empty	Empty
Bead 3	25% PEG, 1.25M NaCl	30
Bead 2	0% PEG, 2.5M NaCl	40
Bead 1	20% PEG, 2.5M NaCl	80

2. Place the desired number of 4-tube strips in Rows 9–12 of Block 3. Ensure that the empty tube of each strip is in Row 12 of Block 3. This is the furthest row from the front of the instrument.

Instructions for Preparing Low Range (150–300 bp) Libraries

These instructions explain how to prepare PEG solutions for use in the Apollo 324 System in order to create libraries of 150–300 bp.

Recipe

1. Prepare the PEG solutions, using the following recipe:

Low Range Recipe	Bead 1 20% PEG 2.5M NaCl	Bead 2 10% PEG 2.5M NaCl	Bead 3 16% PEG 1.25M NaCl
PEG (8000)	2.0 g	1.0 g	1.6 g
5M NaCl	5.0 mL	5.0 mL	2.5 mL
Reagent Grade Water	3.0 mL	4.0 mL	5.9 mL
Total Volume	**10.0 mL**	**10.0 mL**	**10.0 mL**

2. Incubate the solutions in a water bath (approximately 60 °C) to dissolve the PEG.

Loading the Tubes

1. Pipette the following PEG solutions into each Axygen 4-tube strip being used. Use these custom tubes instead of the blue bead tubes provided by IntegenX.

Tube Position	PEG Solution	Volume (µL)
Position 4	Empty	Empty
Bead 3	16% PEG, 1.25M NaCl	30
Bead 2	10% PEG, 2.5M NaCl	40
Bead 1	20% PEG, 2.5M NaCl	80

2. Place the desired number of 4-tube strips in Rows 9–12 of Block 3. Ensure that the empty tube of each strip is in Row 12 of Block 3. This is the furthest row from the front of the instrument.

Instructions for Preparing High Range (500–700 bp) Libraries

These instructions explain how to prepare PEG solutions for use in the Apollo 324 System in order to create libraries of 500–700 bp.

Recipe

1. Prepare the PEG solutions, using the following recipe:

High Range Recipe	Bead 1 20% PEG 2.5M NaCl	Bead 2 6% PEG 2.5M NaCl	Bead 3 8% PEG 1.25M NaCl
PEG (8000)	2.0 g	0.6 g	0.8 g
5M NaCl	5.0 mL	5.0 mL	2.5 mL
Reagent Grade Water	3.0 mL	4.4 mL	6.7 mL
Total Volume	**10.0 mL**	**10.0 mL**	**10.0 mL**

2. Incubate the solutions in a water bath (approximately 60 °C) to dissolve the PEG.

Loading the Tubes

1. Pipette the following PEG solutions into each Axygen 4-tube strip being used. Use these custom tubes instead of the blue bead tubes provided by IntegenX.

Tube Position	PEG Solution	Volume (µL)
Position 4	Empty	Empty
Bead 3	6% PEG, 1.25M NaCl	30
Bead 2	8% PEG, 2.5M NaCl	40
Bead 1	20% PEG, 2.5M NaCl	80

2. Place the desired number 4-tube strips in Rows 9-12 of Block 3. Ensure that the empty tube of each strip is in Row 12 of Block 3. This is the furthest row from the front of the instrument.

References

1. DeAngelis MM, Wang DG, Hawkins TL (2005) Solid-phase reversible immobilization for the isolation of PCR products. *Nucleic Acids Res*. Nov 25; 23(22):4742-3.
2. Lennon NJ, Lintner RE, Anderson S, Alvarez P, Barry A, Brockman W, Daza R, Erlich RL, Giannoukos G, Green L, Hollinger A, Hoover CA, Jaffe DB, Juhn F, McCarthy D, Perrin D, Ponchner K, Powers TL, Rizzolo K, Robbins D, Ryan E, Russ C, Sparrow T, Stalker J, Steelman S, Weiand M, Zimmer A, Henn MR, Nusbaum C, Nicol R (2010) A scalable, fully automated process for construction of sequence-ready barcoded libraries for 454. *Genome Biol*. 11(2):R15. Epub 2010 Feb 5.

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