USER GUIDE

ion torrent
by life technologies™

Ion Xpress™ Plus and Ion Plus Library Preparation for the AB Library Builder™ System

for use with:

Ion Xpress™ Plus Fragment Library Kit for AB Library Builder™ System
Ion Plus Fragment Library Kit for AB Library Builder™ System

Catalog Number 4477597 and 4477598

Publication Number MAN0006946
Revision 5.0

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About this guide

IMPORTANT! Before using this product, read and understand the information in Appendix G, "Safety" on page 87 in this document.

Revision history

Revision	Date	Description
5.0	9 April 2013	• Added support for 400-base-read libraries.
4.0	28 January 2013	• Updated the section "(Optional) Amplify and purify the library" on page 44. • Streamlined and reorganized background information. • Added the Covaris® M220 System to Appendix A.
3.0	10 September 2012	• Added support for 300-base-read libraries.
2.0	10 August 2012	• Corrected Agencourt® AMPure® XP Reagent cartridge positions on pages 27 and 36. • Clarified input DNA cartridge position on pages 27 and 36. • Corrected DNA dilution using E1 Buffer instead of low TE in Appendix A, "Manual fragmentation of DNA" on page 57.

Other Ion library preparation kits and guides

Other library preparation kits and protocols are available. For guides and protocols, visit the Ion community at **http://ioncommunity.iontorrent.com/** and follow the links under **Protocols** > **Construct Library** > **Construct Library User Guides and Quick Reference**.

1 Product Information

Product description

Library kits

Automated preparation of high-quality Ion fragment libraries from genomic DNA (gDNA) or amplicons using the AB Library Builder™ System, requires one or more of the following kits.

- Ion Plus Library Kit for AB Library Builder™ System (Cat. no. 4477597). This kit includes:
  - Reagents for end-repair of physically fragmented gDNA or unfragmented short (<250 bp) amplicons.
  - Reagents and components for library preparation using the AB Library Builder™ System.
- Ion Xpress™ Plus Library Kit for AB Library Builder™ System (Cat. no. 4477598). This kit includes:
  - Ion Shear™ Plus Reagents, for enzymatic fragmentation of gDNA or long amplicons.
  - Reagents and components for library preparation using the AB Library Builder™ System.

Adapter kits

Use the AB Library Builder™ System library kits with the following Adapter kits (sold separately).

- Ion Plus Fragment Library Adapters (Cat. no. 4476340). This kit includes:
  - Ion-compatible adapters (P1–A).
  - Library Amplification Primer Mix.

  or
- Ion Xpress™ Barcode Adapters Kits.

  Each Barcode Adapter Kit includes the P1 adapter and barcoded A adapters that substitute for the non-barcoded adapter mix supplied by the Ion Plus Fragment Library Adapters (Cat. no. 4476340). Barcoded library preparation is otherwise identical to non-barcoded library preparation.

Procedure overview

The library preparation procedure is illustrated in Figure 1. To prepare Ion libraries on the AB Library Builder™ System, start with 50 ng–1 µg DNA. Input DNA can be short amplicons (<250 bp), long amplicons (>400 bp), or genomic DNA. Short amplicon DNA does not require shearing prior to library construction, while long amplicon and genomic DNA require fragmentation to appropriately sized fragments. Use the Ion Xpress™ Plus Library Kit for AB Library Builder™ System when automated fragmentation is desired. Use the Ion Plus Library Kit for AB Library Builder™ System with short amplicons or manually pre-fragmented genomic DNA. Perform automated size-selection using the AB Library Builder™ System, or manually size-select after library preparation is complete using gels or other methods.

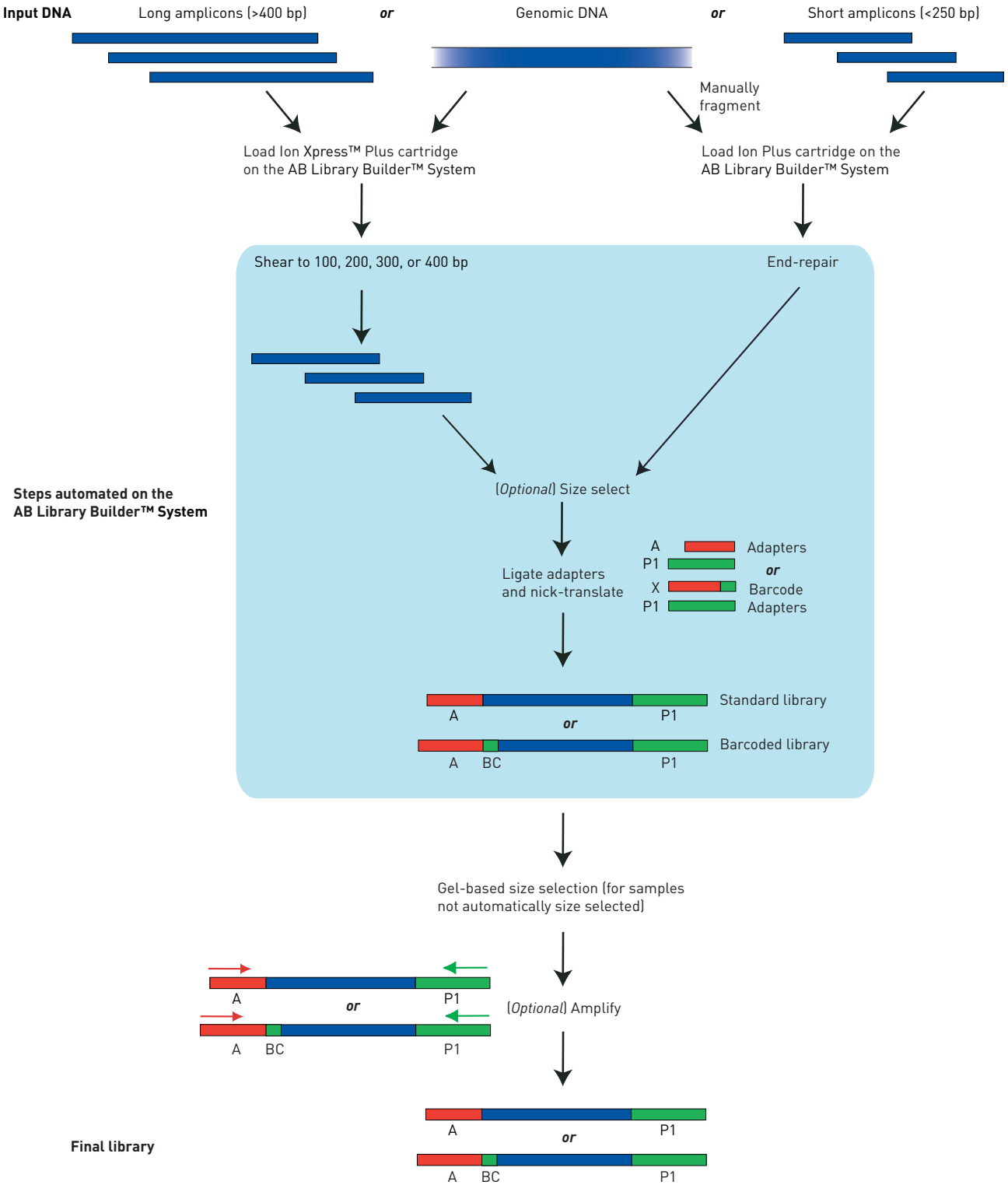
Target read length	Median insert size	Median library size
400 bases (400-base-read library)	~420 bp	~490 bp
300 bases (300-base-read library)	~320 bp	~390 bp
200 bases (200-base-read library)	~260 bp	~330 bp
100 bases (100-base-read library)	~130 bp	~200 bp

Note: The AB Library Builder™ System enables shearing of DNA to 100-, 200-, 300-, and 400-bp fragments, and can size-select 100-, 200-, or 300-bp DNA inserts.

Final amplification of the library is optional, depending on the amount of input DNA and your experimental requirements, and requires reagents not supplied in the library builder kits (see page 14).

After library construction or optional amplification, assess the size distribution and quantitate the library dilution factor of each library to prepare for downstream template preparation procedures and subsequent semiconductor-based sequencing on the Ion Personal Genome Machine® (PGM™) System.

Figure 1 **Fragment library preparation on the** AB Library Builder™ System.



Related information

For complete site preparation and operating instructions of the AB Library Builder™ System, refer to the *AB Library Builder™ System Site Preparation Guide* (Pub. no. 4465106) and the *AB Library Builder™ System User Guide* (Pub. no. 4463421), available at: **http://www.lifetechnologies.com**

Kit contents and storage

Ion Plus Library Kit for AB Library Builder™ System

The Ion Plus Library Kit for AB Library Builder™ System (Cat. no. 4477597) is shipped in three containers. Supplied reagents are sufficient for preparing up to 13 libraries. Store the kit components as directed in the following table:

Ion Plus Library Kit for AB Library Builder™ System (Cat. no. 4477597)			
Box	**Component†**	**Quantity**	**Storage**
Ion Plus Core Module for AB Library Builder™ System (Part no. 4477683)	Cartridge containing ready-to-use reagents	13 cartridges	–30°C to –10°C
	E1 Buffer	2 tubes x 700 µL	
	Ion End Repair Enzyme Mix	13 tubes x 30 µL	
LdN Buffer Module for AB Library Builder™ System (Part no. 4477681)	LdN Buffer	13 tubes x 44 µL	
AB Library Builder™ Plastics Module (Part no. 4465605)	Sample/elution tubes, tips, and tip holders	26 tips, 52 tubes	15°C to 30°C

† Do not mix any of the components provided in the kit with components from other kits.

Ion Xpress™ Plus Library Kit for AB Library Builder™ System

The Ion Xpress™ Plus Library Kit for AB Library Builder™ System (Cat. no. 4477598) is shipped in three containers. Supplied reagents are sufficient for preparing up to 13 libraries. Store the kit components as directed in the following table:

Ion Xpress™ Plus Library Kit for AB Library Builder™ System (Cat. no. 4477598)			
Box	**Component†**	**Quantity**	**Storage**
Ion Xpress™ Plus Core Module for AB Library Builder™ System (Part no. 4477682)	Cartridge containing ready-to-use reagents	13 cartridges	–30°C to –10°C
	E1 Buffer	2 tubes x 700 µL	
	LB Ion Shear™ Plus Enzyme Mix	13 tubes x 30 µL	
LdN Buffer Module for AB Library Builder™ System (Part no. 4477681)	LdN Buffer	13 tubes x 44 µL	
AB Library Builder™ Plastics Module (Part no. 4465605)	Sample/elution tubes, tips, and tip holders	26 tips, 52 tubes	15°C to 30°C

† Do not mix any of the components provided in the kit with components from other kits.

Ion Plus and Ion Xpress™ Plus Protocol Card for AB Library Builder™ System

The Ion Plus and Ion Xpress™ Plus Protocol Card for AB Library Builder™ System (Cat. no. 4477687) contains the protocol for automated library construction of Ion fragment libraries on the AB Library Builder™ System. You can obtain the protocol card from **http://www.lifetechnologies.com**.

Ion Plus Fragment Library Adapters

The Ion Plus Fragment Library Adapters (Cat. no. 4476340) is sold separately and contains enough reagents to prepare up to 10 DNA libraries.

Component	Cap color	Quantity	Volume	Storage
Adapters	Green	1 tube	100 µL	–30°C to –10°C
Library Amplification Primer Mix	White	1 tube	100 µL	

Ion Xpress™ Barcode Adapters Kits

The following Ion Xpress™ Barcode Adapters Kits are available separately:

- Ion Xpress™ Barcode Adapters 1–16 (Cat. no. 4471250)
- Ion Xpress™ Barcode Adapters 17–32 (Cat. no. 4474009)
- Ion Xpress™ Barcode Adapters 33–48 (Cat. no. 4474518)
- Ion Xpress™ Barcode Adapters 49–64 (Cat. no. 4474519)
- Ion Xpress™ Barcode Adapters 65–80 (Cat. no. 4474520)
- Ion Xpress™ Barcode Adapters 81–96 (Cat. no. 4474521)

The complete set of adapters is also available:

- Ion Xpress™ Barcode Adapters 1–96 (Cat. no. 4474517)

Each barcode kit is sufficient for preparing up to 10 libraries per barcode (10 × 16 libraries) for 50 –100 ng input, or 2 libraries per barcode for 1 µg input, and contains the following components:

Component	Cap color/ label	Quantity	Volume	Storage
Ion Xpress™ P1 Adapter	Violet/—	1 tube	320 µL	–30°C to –10°C
Ion Xpress™ Barcode X†	White/X	1 tube	20 µL each	

† X = Barcode number

Materials and equipment required but not provided

Required for library preparation

Use common molecular biology equipment, supplies, and reagents with the kits, and use common commercial kits for several purification steps where specified. Use the Agencourt® AMPure® XP Kit for DNA purification. Use the BioAnalyzer® 2100 instrument or conventional gel electrophoresis to analyze DNA fragment length distribution during library preparation.

Description†	Supplier	Cat. no.	Quantity
AB Library Builder™ System The system includes: • AB Library Builder™ Device • Tip and Tube Tray • Reagent Cartridge Rack • Barcode Reader • RS232C Cable • CommViewer Barcode Software CD-ROM • 13 empty reagent cartridges • 52 sample/elution tubes	Life Technologies	4463592	1 system
AB Library Builder™ System with Service Installation The system includes: • AB Library Builder™ Device • Tip and Tube Tray • Reagent Cartridge Rack • Barcode Reader • RS232C Cable • CommViewer Barcode Software CD-ROM • 13 empty reagent cartridges • 52 sample/elution tubes	Life Technologies	4463794	1 system
AB Library Builder™ System replacement parts: • AB Library Builder™ Tips and Tip Holders • AB Library Builder™ Tip and Tube Rack • AB Library Builder™ Cartridge Rack • AB Library Builder™ D-Ring Tool • AB Library Builder™ Barcode Reader • AB Library Builder™ Sample Tubes • AB Library Builder™ D-Rings • AB Library Builder™ Plastics Module	Life Technologies	• 4463781 • 4463776 • 4463782 • 4465603 • 4465657 • 4463779 • 4465602 • 4465605	—
Agencourt® AMPure® XP Kit	Beckman Coulter	A63880 *or* A63881	1 kit
Agilent® 2100 BioAnalyzer® instrument	Agilent	G2939AA	1 instrument

Description†	Supplier	Cat. no.	Quantity
Agilent® Bioanalyzer® High Sensitivity DNA Kit	Agilent	5067-4626	1 kit
LoBind® Tubes (1.5 mL)	Eppendorf	022431021	1 box
low-binding microcentrifuge tubes (0.65 mL)	Costar	3206	500 tubes
Microcentrifuge	Major Laboratory Supplier (MLS)‡	—	1 microcentrifuge
Vortexer	MLS	—	1 vortexer
Pipettors (1–1000 µL)	MLS	—	1 each size
Barrier pipette tips	MLS	—	1 box each
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS	—	100 mL
Nuclease-Free Water	Life Technologies	AM9932	1000 mL
(*Optional*) PicoFuge® Microcentrifuge	MLS	—	1 microcentrifuge
(*Optional*) Thermal cycler	MLS	—	1 thermal cycler
(*Optional*) Platinum® PCR SuperMix High Fidelity	MLS	12532-016	100 reactions
(*Optional*) Ion Library Quantitation Kit (required for quantitation of unamplified libraries)	Life Technologies	4468802	1 kit
(*Optional*) DynaMag™-2 magnet (magnetic rack)	Life Technologies	123-21D	1 rack
(*Optional*) RNase I	Life Technologies	AM2294 AM2295	10,000 units 25,000 units
(Optional) PureLink® Genomic DNA Kit (for cleanup after optional RNase treatment)	Life Technologies	K1820-1 K1820-2 K1821-04	50 preps 250 preps 4 × 96-well plates
(Optional) MicroAmp® Optical 8-Tube Strip (0.2 mL)	Life Technologies	4316567	1000 tubes in strips of 8
(Optional) Ethanol, absolute	Sigma-Aldrich	E7023	500 mL
(Optional) LoBind® Tubes (0.5 mL)	Eppendorf	022431005	250 tubes
(Optional) Filtered pipettor tips	MLS	—	1 box each
(Optional) CF-1 Calibration Fluid Kit	Thermo Scientific	CF-1	0.1 mL ampule
(Optional) PR-1 Conditioning Kit§	Thermo Scientific	PR-1	1 kit

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

§ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned" (refer to the Nanodrop® Conditioning Kit user's manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

Required for physical fragmentation of gDNA

Use the BioRuptor® Standard or BioRuptor® NGS Sonication System, or the Covaris® S2 or Covaris® S220 Systems when preparing DNA for the Ion Plus Library Kit for AB Library Builder™ System.

Note: The Ion Xpress™ Plus Library Kit for AB Library Builder™ System contains reagents for automated shearing and does not require pre-fragmented DNA.

Description†	Supplier	Cat. no.	Quantity
BioRuptor® Standard Sonication System with accessories (for 12 x 0.5-mL tubes)	Ion Torrent ***or*** Diagenode	4465622‡ UCD-200 TS	1 system 1 system
BioRuptor® NGS Sonication System with accessories (for 12 x 0.5-mL tubes)	Diagenode	UCD-600 TS‡	1 system
BioRuptor® Microtube Attachment and Gearplate (0.5 mL)	Ion Torrent ***or*** Diagenode	4465648 UCD-pack-05	1 1
Covaris® S2 System (110 V for U.S. customers) (220 V for international customers)	Covaris	—	1 system
Covaris® S220 System (110 V for U.S. customers) (220 V for international customers)	Covaris	—	1 system
Covaris® microTUBEs	Covaris	520045	25 snap-cap tubes

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ Also includes the BioBox (soundproof box).

(*Optional*) Required for gel-based library size-selection

Use the E-Gel® SizeSelect™ 2% Agarose Gel or the Pippin Prep™ instrument for gel-based size-selection of the libraries.

Note: Both the Ion Plus and Ion Xpress™ Plus Kits for AB Library Builder™ System enable automated size-selection of the libraries. Size-selection gels are only required for *optional* off-instrument size-selection.

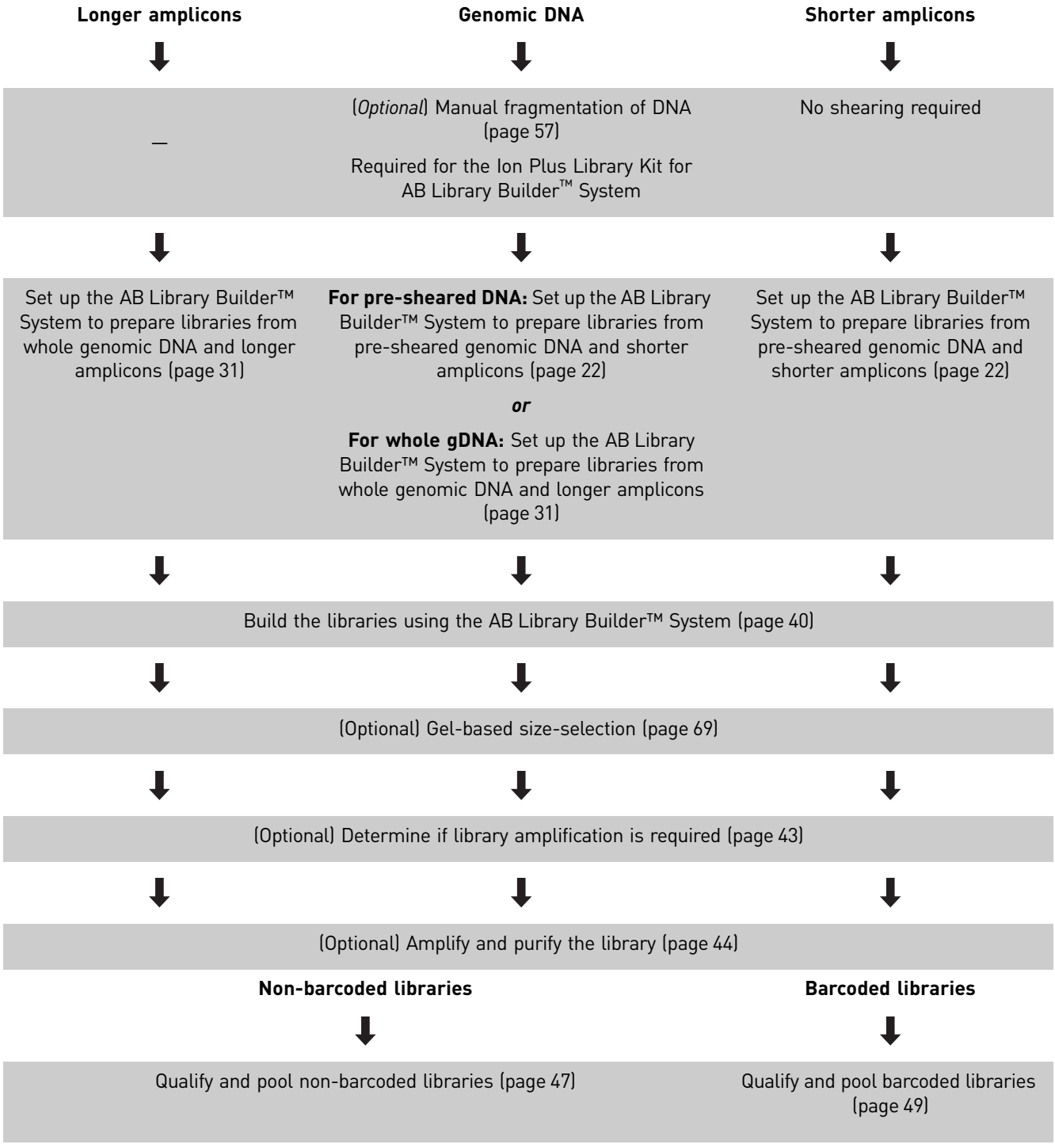
Description†	Supplier	Cat. no.	Quantity
E-Gel® iBase™ unit and E-Gel® Safe Imager™ transilluminator combo kit, for library size-selection	Life Technologies	G6465	1 kit
E-Gel® SizeSelect™ 2% Agarose, required for E-Gel® size-selection method	Life Technologies	G6610-02	10/pack
50 bp DNA ladder (1 µg/µL), required for E-Gel® size-selection method	Life Technologies	10416-014	1 ladder
Pippin Prep™ instrument, for library size-selection	Life Technologies	4471271	1 instrument
2% Agarose Gel Cassettes for the Pippin Prep™ instrument	Life Technologies	CSD2010 CSD2050	10 cassettes 50 cassettes

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

2 Methods

Workflow

Start with high-quality, RNA-free DNA. Completely thaw AB Library Builder™ System cartridges at room temperature or on ice for ≤2 hours.



Procedural guidelines

- Use good laboratory practices (GLP) to minimize cross-contamination of products. If possible, prepare libraries in a separate location from template preparation.
- When handling barcoded adapters, do not cross-contaminate. Change gloves frequently and open one tube at a time.
- Perform all steps requiring LoBind® Tubes with Eppendorf® LoBind® Tubes (0.5-mL Cat. no. 022431005 and 1.5-mL Cat. no. 022431021).
- When preparing to use reagents:
  - Store enzymes at –30°C to –10°C before use.
  - Thaw all reagents on ice or at room temperature before use.
  - Keep thawed reagents on ice when not in use.
- Mix reagents thoroughly before use, especially if reagents were frozen and thawed.

Input DNA requirements

- High-quality RNA-free DNA is required. The quality of the input DNA has a significant impact on the quality of the resulting library. A number of commercially available kits are available for isolation of high molecular weight, RNA-free genomic DNA. DNA extraction from a variety of tissue sources may be automated on the AB Library Builder™ System using iPrep™ automated nucleic acid extraction kits. See **www.lifetechnologies.com** for more details. See Appendix C, "Evaluating the quality of input DNA" on page 79 for more information about assessing the integrity and size of your input DNA material and performing an optional RNase treatment procedure.
- For guidelines on preparing amplicon DNA, refer to the *Prepare Amplicon Libraries without Fragmentation using the Ion Plus Fragment Library Kit User Bulletin* (Pub. no. MAN00006846) and the *Prepare Amplicon Libraries Requiring Fragmentation with the Ion Xpress™ Plus Fragment Library Kit User Bulletin* (Pub. no. MAN00007044).

Guidelines for using the AB Library Builder™ System

To install and set up the AB Library Builder™ System, refer to the *AB Library Builder™ System Site Preparation Guide* (Pub. no. 4465106) and the *AB Library Builder™ System User Guide* (Pub. no. 4463421).

IMPORTANT! To avoid data loss or run cancellation, always follow these practices:

- Before you insert or remove a protocol card, power off the instrument.
- Before you power on the instrument, insert the protocol card, then close the instrument door.
- To pause the instrument during a run, press **Stop** before you open the instrument door.
- When you are not performing a run or instrument test, you can open the instrument door with the power off or on.
- Do not move instrument components such as the platform, magnets, and syringes while the instrument is powered on.

Note: To ensure the best pipetting performance, use the cartridge rack and tip and tube rack shipped with the instrument; these racks are calibrated with the instrument at the factory. Before using other racks on a specific instrument, run the installation test to qualify the racks for use on that instrument. Refer to the *AB Library Builder™ System User Guide* (Pub. no. 4463421) for details.

Note: Wear gloves when you handle samples or load the cartridges, tips, and tubes in the rack.

Set up the AB Library Builder™ System to prepare libraries from pre-sheared genomic DNA and shorter amplicons

About the Ion Plus Library Kit for AB Library Builder™ System

Use the Ion Plus Library Kit for AB Library Builder™ System to end-repair, ligate, and purify fragment libraries from pre-sheared genomic DNA and shorter amplicons. Refer to Appendix A, "Manual fragmentation of DNA" on page 57 for example protocols for shearing DNA. The AB Library Builder™ System is then able to size-select 100-, 200-, or 300-bp DNA fragments using the Ion Plus Library Kit for AB Library Builder™ System.

Automated size-selection produces libraries that may have a broader size distribution, resulting in a reduced mean read length and total sequencing throughput relative to gel-based size-selected libraries. To bypass automated size-selection on the AB Library Builder™ System, select option **2: No SizeSelection** at the appropriate on-screen prompt during protocol set-up on the AB Library Builder™ System. Libraries created with option **2: No SizeSelection** must be size-selected manually by gels or other methods prior to use in downstream protocols. See Appendix B, "Gel-based size-selection" on page 69 for other size-selection methods.

Set up the AB Library Builder™ System

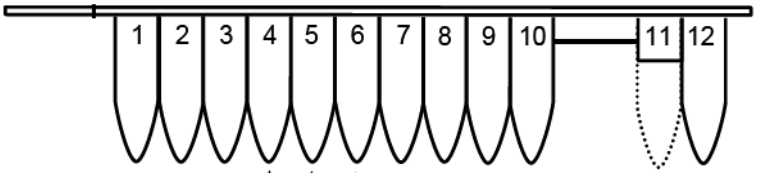
Materials Provided in the Ion Plus Library Kit for AB Library Builder™ System	Materials Provided in the Ion Plus Fragment Library Adapters and Ion Xpress™ Barcode Adapter Kits	Other Materials and Equipment
• AB Library Builder™ cartridges • E1 Buffer • LdN Buffer tubes • Ion End Repair Enzyme Mix • Sample/elution tubes, tips, and tip holders	Adapter mix: • **For non-barcoded libraries** – Use the Adapters (P1-A) from the Ion Plus Fragment Adapters Kit • **For barcoded libraries** – Use the P1 Adapter and barcoded A adapters provided in the Ion Xpress™ Barcode Adapter Kits.	• AB Library Builder™ System and accessories • Agencourt® AMPure® XP Reagent • Pipettors and pipette tips

Before you begin

- Completely thaw an appropriate number of cartridges for ≤2 hours at room temperature, 2°C to 8°C, or on ice. One cartridge is required per sample. Thawed cartridges do not require additional mixing.

IMPORTANT! Avoid leaving the cartridges at room temperature for longer than necessary to completely thaw them. Avoid repeated freeze-thawing of unused cartridges.

- Thaw one tube of LdN Buffer for each cartridge to be used. If you see a white precipitate in the LdN Buffer, warm the solution at 37°C to redissolve the precipitate.
- Thaw an appropriate amount of E1 Buffer as specified in the following table and the tables on page 26.
- Manually shear genomic DNA or prepare short amplicon DNA.
- Inspect the AB Library Builder™ fragment cartridges. Each cartridge contains 12 compartments for reagents:



Cartridge Compartment Numbers	Quantity
1	1300 µL
2–3	1200 µL
4	900 µL
5	30 µL
6–7	Empty
8	20 µL
9	20 µL
10	Empty (user to add 400–550 µL Agencourt® AMPure® XP Reagent beads)
11	Empty (user to add 50 µL diluted adapters in LdN Buffer)
12	Empty (user to add 50 µL pre-sheared input DNA diluted in E1 Buffer)

IMPORTANT! Do not switch the supplied pre-filled reagents with any other reagents. The protocols are specifically optimized with the reagents supplied with the kit.

1. Place the pre-sheared or short amplicon DNA on ice. Dilute 50 –1000 ng input DNA to 50 µL E1 Buffer.

2. Insert or change the protocol card and power on the instrument:

IMPORTANT! Do not remove the protocol card while the instrument is on. Removing the card stops the run, and it may cause instrument data file loss. To remove the card, see 2c on page 24.

If you accidentally remove the protocol card during a run, power off the instrument immediately to minimize potential for instrument data loss.

For guidelines on handling protocol cards, see the *AB Library Builder™ System User Guide* (Pub. no. 4463421).

a. Confirm that the power switch is in the off position.

   Note: If you insert the card while the instrument is on, the instrument will not recognize the card.

b. Open the card slot.

c. To remove a card that is already installed in the slot, push the button located below the card slot (see the following photo), then pull the card out of the slot. Place the card in the plastic cover in the box.

IMPORTANT! Do not remove the protocol card while the instrument is on.

d. Insert the appropriate protocol card in the slot with the arrow on the protocol card pointing toward the instrument and the label facing left.

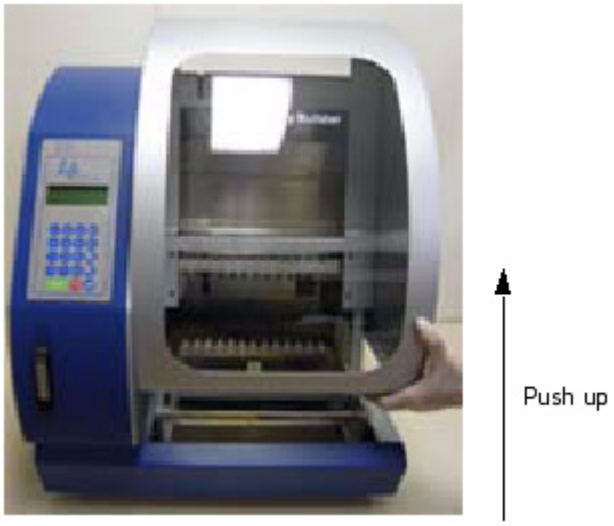
e. Push the card completely into the card slot, then close the card slot.

f. Close the door to the AB Library Builder™ Device.

g. Power on the instrument.

   When the card is fully inserted in the correct orientation and the instrument powered on, the display briefly shows information including the instrument version, the protocol card version **AB Library Builder Ion Xpress Plus Library Protocol v. 1.00**, then displays the Main menu.

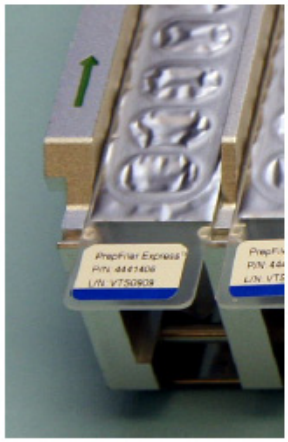
3. Open the instrument door (push up the door), then remove the tip and tube rack and the cartridge rack:

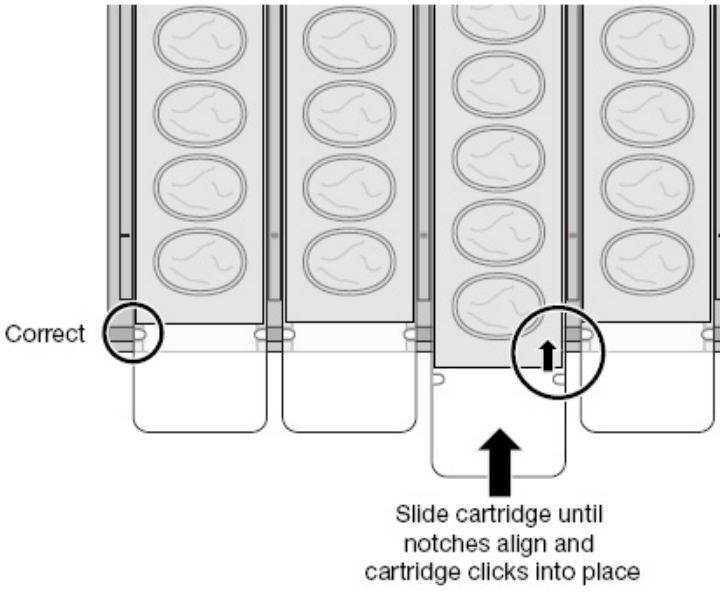


4. Gently tap each cartridge on the laboratory bench until any liquid droplets underneath the foil seal fall into the bottom of the wells.

5. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

   Note: An incorrectly loaded cartridge rack may cause the instrument to stop during a run.





6. Load adapters in the cartridges:

   a. Dilute the appropriate adapters in E1 Buffer according to the amount of DNA input, as shown in the following tables.

      - **For non-barcoded libraries**: Use the Adapters provided in the Ion Plus Fragment Library Adapters (Cat. no. 4476340).

Component	Volume									
	50–100 ng input	100–200 ng input	200–300 ng input	300–400 ng input	400–500 ng input	500–600 ng input	600–700 ng input	700–800 ng input	800–900 ng input	900–1000 ng input
Adapters	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
E1 Buffer	49 µL	48 µL	47 µL	46 µL	45 µL	44 µL	43 µL	42 µL	41 µL	40 µL
Total	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**

      - **For barcoded libraries:** Use the P1 Adapter and barcoded A adapters provided in the Ion Xpress™ Barcode Adapter Kits.

Component	Volume									
	50–100 ng input	100–200 ng input	200–300 ng input	300–400 ng input	400–500 ng input	500–600 ng input	600–700 ng input	700–800 ng input	800–900 ng input	900–1000 ng input
Ion Xpress™ P1 Adapter	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
Ion Xpress™ Barcode X†	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
E1 Buffer	48 µL	46 µL	44 µL	42 µL	40 µL	38 µL	36 µL	34 µL	32 µL	30 µL
Total	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**

† X = Barcode chosen.

   b. Mix the diluted adapters thoroughly by pipetting up and down, and transfer 50 µL of the diluted adapters to one LdN Buffer tube per library.

   c. Tap the LdN Buffer tube with the added adapters or pulse-spin, to ensure that all of the liquid is in the bottom of the tube.

   d. Unscrew and discard the cap from each LdN Buffer tube containing the added adapters. Place the tubes in the empty holes in position 11 of each cartridge in the cartridge rack.

7. Puncture the foil covering position 10 on each cartridge, then thoroughly resuspend and transfer the correct amount of Agencourt® AMPure® XP Reagent to the bottom of each position 10 according to the following table:

Library Size	Amount of Agencourt AMPure XP Reagent to Add:	
	Auto Size-Selection Protocol	No Size-Selection Protocol
100 bp	550 µL	500 µL
200 bp	450 µL	500 µL
300 bp	400 µL	500 µL
400 bp	Not supported	500 µL

Note: Do not puncture positions 1–9. These wells are automatically punctured by the AB Library Builder™ System.

8. Carefully remove the intact circular seal covering position 12 on each cartridge and pipet 50 µL of the pre-sheared DNA into the bottom of each position 12.

9. Load the tip and tube rack in the following order:

IMPORTANT! If you are processing fewer than 13 samples, load the tips and tubes in the same positions as the reagent cartridges in the cartridge rack.

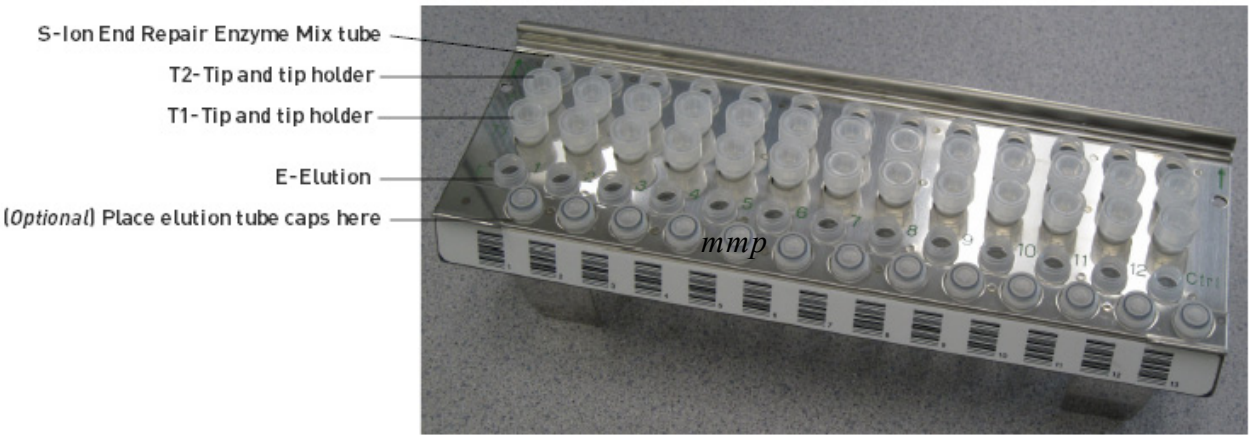
a. **Row S** (fourth row): Briefly spin the Ion End Repair Enzyme Mix tube from the Ion Plus Library Kit for AB Library Builder™ System so that all of the liquid is in the bottom of the tube. Place the tube in Row S.

IMPORTANT! Only use cartridges from the Ion Plus Core Library Module with the End Repair Enzyme Mix included in the module and pre-sheared DNA. Do not use cartridges from the Ion Plus Core Library Module for shearing whole DNA with the LB Ion Shear™ Plus Enzyme Mix provided in the Ion Xpress™ Plus Core Library Module.

b. **Rows T1 and T2** (second and third rows): Load with AB Library Builder™ Tips inserted into tip holders.

Note: Two sets of tips and tip holders are required per sample.

c. **Row E** (first row): Load with *labeled* sample/elution tubes, with the caps removed and secured:



10. Insert the racks into the AB Library Builder™ Device:

IMPORTANT!

- Insert the cartridge rack first before inserting the tip and tube rack. Loading the racks in a different order can cause the instrument to stop during a run.
- Use only AB Library Builder™ Sample Tubes (sample/elution tubes). Other tubes may be picked up by the nozzle tips due to differences in tube height and shape, stopping the run.

a. Insert the loaded cartridge rack into the instrument.

IMPORTANT! Before inserting the cartridge rack into AB Library Builder™ Device, ensure that the cartridges are completely thawed, particularly reagents in cartridge wells 2 and 3.

WARNING! Do not touch the surface of the heat block. The temperature of the heat block can reach 95°C. Touching the block can cause burns.

b. Insert the loaded tip and tube rack into the instrument with row E in the front.

11. To start the run, proceed to "Build the libraries using the AB Library Builder™ System" on page 40.

Set up the AB Library Builder™ System to prepare libraries from whole genomic DNA and longer amplicons

About the Ion Xpress™ Plus Library Kit for AB Library Builder™ System

Use the Ion Xpress™ Plus Library Kit for AB Library Builder™ System for automated enzymatic shearing of whole genomic and longer amplicon DNA, in addition to library construction. The AB Library Builder™ System using the Ion Xpress™ Plus Library Kit for AB Library Builder™ System enables shearing of DNA to 100-, 200-, 300-, and 400-bp fragments. The AB Library Builder™ System is then able to size-select 100, 200, or 300-bp DNA fragments using the Ion Xpress™ Plus Library Kit for AB Library Builder™ System.

IMPORTANT! The final concentration of EDTA in the input DNA solution must be ≤0.1 mM for accurate and precise automated fragmentation of the DNA on the AB Library Builder™ System. If necessary, reduce the EDTA concentration of the input DNA solution to ≤0.1 mM by dilution using Nuclease-Free Water or 10 mM Tris, pH 7.5—8. Alternatively, exchange the buffer using ethanol precipitation, spin-column or bead-based methods.

Automated size-selection produces libraries that may have a broader size distribution, resulting in a reduced mean read length and total sequencing throughput relative to gel-based size-selected libraries. To bypass automated size-selection on the AB Library Builder™ System, select option **2: No SizeSelection** at the appropriate on-screen prompt during protocol set-up on the AB Library Builder™ System. Libraries created with option **2: No SizeSelection** must be size-selected manually by gels or other methods prior to use in downstream protocols. See Appendix B, "Gel-based size-selection" on page 69 for other size-selection methods.

Set up the AB Library Builder™ System

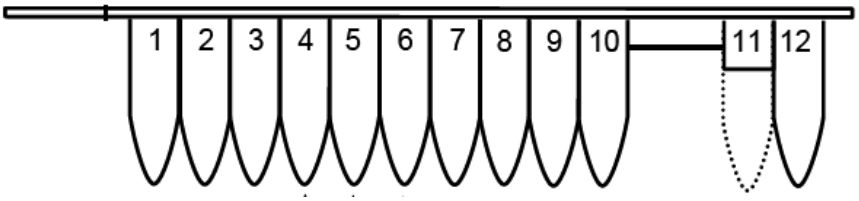
Materials Provided in the Ion Xpress™ Plus Library Kits for AB Library Builder™ System	Materials Provided in the Ion Plus Fragment Library Adapters and Ion Xpress™ Barcode Adapter Kits	Other Materials and Equipment
• AB Library Builder™ cartridges • E1 Buffer • LdN Buffer tubes • LB Ion Shear™ Plus Enzyme Mix • Sample/elution tubes, tips, and tip holders	Adapter mix: • **For non-barcoded libraries** – Use the Adapters (P1-A) from the Ion Plus Fragment Adapters Kit • **For barcoded libraries** – Use the P1 Adapter and barcoded A adapters provided in the Ion Xpress™ Barcode Adapter Kits.	• AB Library Builder™ System and accessories • Agencourt® AMPure® XP Reagent • Pipettors and pipette tips

Before you begin

- Completely thaw an appropriate number of cartridges for ≤2 hours at room temperature, 2°C to 8°C, or on ice. One cartridge is required per sample. Thawed cartridges do not require additional mixing.

IMPORTANT! Avoid leaving the cartridges at room temperature for longer than necessary to completely thaw them. Avoid repeated freeze-thawing of unused cartridges.

- Thaw one tube of LdN Buffer for each cartridge to be used. If you see a white precipitate in the LdN Buffer, warm the solution at 37°C to redissolve the precipitate.
- Thaw an appropriate amount of E1 Buffer as specified in the following table and the tables on page 36.
- Prepare genomic or long amplicon DNA.
- Inspect the AB Library Builder™ fragment cartridges. Each cartridge contains 12 compartments for reagents:



Cartridge Compartment Numbers	Quantity
1	1300 µL
2–3	1200 µL
4	900 µL
5	30 µL
6–7	Empty
8	20 µL
9	20 µL
10	Empty (user to add 400–550 µL Agencourt® AMPure® XP Reagent beads)
11	Empty (user to add 50 µL diluted adapter in LdN Buffer)
12	Empty (user to add 50 µL whole, unsheared input DNA diluted in E1 Buffer)

IMPORTANT! Do not switch the supplied pre-filled reagents with any other reagents. The protocols are specifically optimized with the reagents supplied with the kit.

1. Place the unsheared genomic or long amplicon DNA on ice. Dilute 50 –1000 ng input DNA to 50 µL E1 Buffer.

   IMPORTANT! The final concentration of EDTA in the input DNA solution must be ≤0.1 mM for accurate and precise automated fragmentation of the DNA on the AB Library Builder™ System. If necessary, reduce the EDTA concentration of the input DNA solution to ≤0.1 mM by dilution using Nuclease-Free Water or 10 mM Tris, pH 7.5—8. Alternatively, exchange the buffer using ethanol precipitation, spin-column or bead-based methods.

2. Insert or change the protocol card and power on the instrument:

   IMPORTANT! Do not remove the protocol card while the instrument is on. Removing the card stops the run, and it may cause instrument data file loss. To remove the card, see 2c on page 34.

   If you accidentally remove the protocol card during a run, power OFF the instrument immediately to minimize potential for instrument data loss.

   For guidelines on handling protocol cards, see the *AB Library Builder™ System User Guide* (Pub. no. 4463421).

   a. Confirm that the power switch is in the off position.

      Note: If you insert the card while the instrument is on, the instrument will not recognize the card.

   b. Open the card slot.

c. To remove a card that is already installed in the slot, push the button located below the card slot (see the following photo), then pull the card out of the slot. Place the card in the plastic cover in the box.

IMPORTANT! Do not remove the protocol card while the instrument is on.

d. Insert the appropriate protocol card in the slot with the arrow on the protocol card pointing toward the instrument and the label facing left.

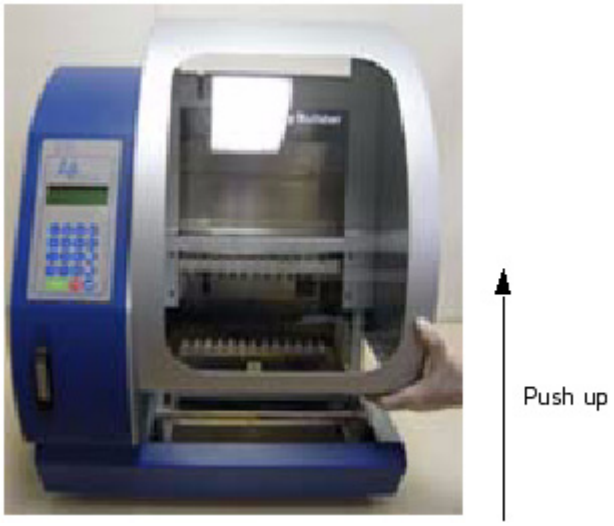
e. Push the card completely into the card slot, then close the card slot.

f. Close the door to the AB Library Builder™ Device.

g. Power on the instrument.

When the card is fully inserted in the correct orientation and the instrument powered on, the display briefly shows information including the instrument version, the protocol card version (**AB Library Builder Ion Xpress Plus Library Protocol v. 1.00**), then displays the Main menu.

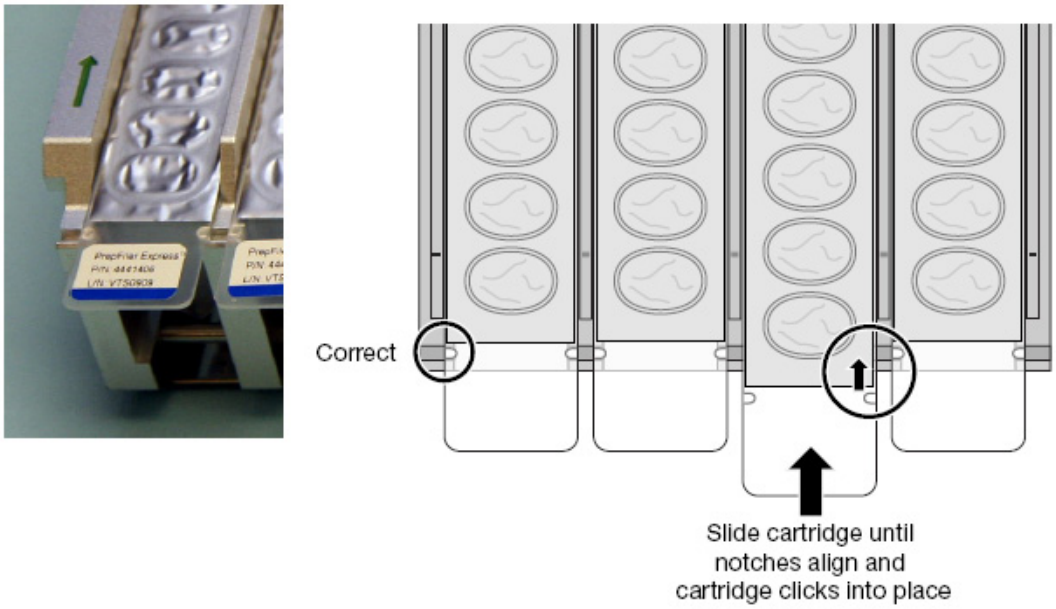
3. Open the instrument door (push up the door), then remove the tip and tube rack and the cartridge rack:



4. Gently tap each cartridge on the laboratory bench until any liquid droplets underneath the foil seal fall into the bottom of the wells.

5. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

   Note: An incorrectly loaded cartridge rack may cause the instrument to stop during a run.



6. Load adapters in the cartridges:

   a. Dilute the appropriate adapters in E1 Buffer according to the amount of DNA input, as shown in the following tables.

      - **For non-barcoded libraries**: Use the Adapters provided in the Ion Plus Fragment Library Adapters (Cat. no. 4476340).

Component	Volume									
	50–100 ng input	100–200 ng input	200–300 ng input	300–400 ng input	400–500 ng input	500–600 ng input	600–700 ng input	700–800 ng input	800–900 ng input	900–1000 ng input
Adapters	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
E1 Buffer	49 µL	48 µL	47 µL	46 µL	45 µL	44 µL	43 µL	42 µL	41 µL	40 µL
Total	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**

      - **For barcoded libraries:** Use the P1 Adapter and barcoded A adapters provided in the Ion Xpress™ Barcode Adapter Kits.

Component	Volume									
	50–100 ng input	100–200 ng input	200–300 ng input	300–400 ng input	400–500 ng input	500–600 ng input	600–700 ng input	700–800 ng input	800–900 ng input	900–1000 ng input
Ion Xpress™ P1 Adapter	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
Ion Xpress™ Barcode X†	1 µL	2 µL	3 µL	4 µL	5 µL	6 µL	7 µL	8 µL	9 µL	10 µL
E1 Buffer	48 µL	46 µL	44 µL	42 µL	40 µL	38 µL	36 µL	34 µL	32 µL	30 µL
Total	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**	**50 µL**

† X = Barcode chosen.

   b. Mix the diluted adapters thoroughly by pipetting up and down, and transfer 50 µL of the diluted adapters to one LdN Buffer tube per library.

   c. Tap the LdN Buffer tube with the added adapters on the table, or pulse-spin, to ensure that all of the liquid is in the bottom of the tube.

   d. Unscrew and discard the cap from each LdN Buffer tube containing the added adapters. Place the tubes in the empty holes in position 11 of each cartridge in the cartridge rack.

7. Puncture the foil covering position 10 on each cartridge, then thoroughly resuspend and transfer the correct amount of Agencourt® AMPure® XP Reagent to each position 10 according to the following table:

Library Size	Amount of Agencourt® AMPure® XP Reagent to Add:	
	Auto Size-Selection Protocol	No Size-Selection Protocol
100 bp	550 µL	500 µL
200 bp	450 µL	500 µL
300 bp	400 µL	500 µL
400 bp	Not supported	500 µL

Note: Do not puncture positions 1–9. These wells are automatically punctured by the AB Library Builder™ System.

8. Carefully remove the intact circular seal covering position 12 on each cartridge and pipet 50 µL of the whole, unsheared DNA into the bottom of each position 12.

9. Load the tip and tube rack in the following order:

IMPORTANT! If you are processing fewer than 13 samples, load the tips and tubes in the same positions as the reagent cartridges in the cartridge rack.

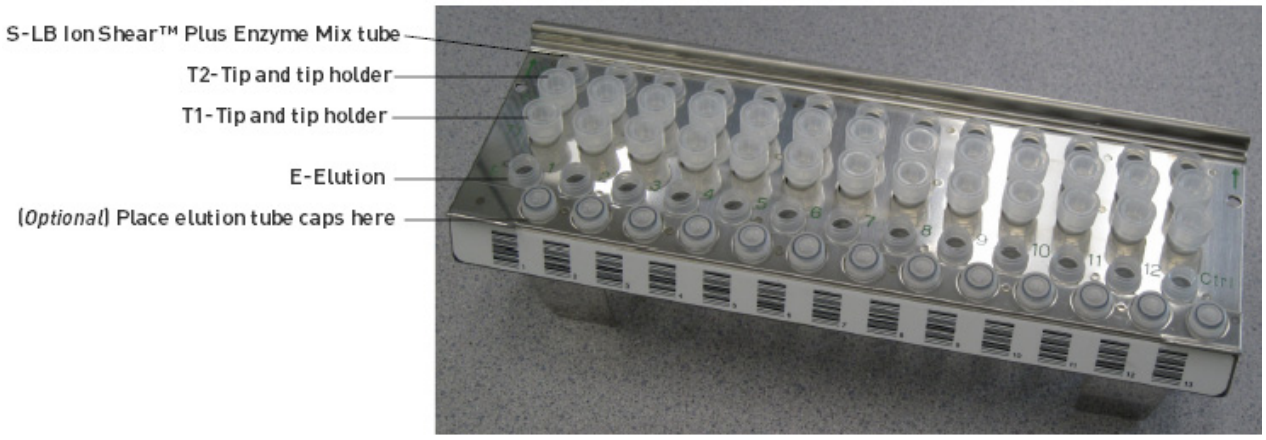
a. **Row S** (fourth row): Briefly spin the LB Ion Shear™ Plus Enzyme Mix tube supplied in the Ion Xpress™ Plus Library Kit for AB Library Builder™ System so that all of the liquid is in the bottom of the tube. Place the tube in Row S.

IMPORTANT! Do not use cartridges from the Ion Plus Core Library Module for shearing whole DNA with the LB Ion Shear™ Plus Enzyme Mix provided in the Ion Xpress™ Plus Core Library Module. Cartridges provided in the Ion Xpress™ Plus Core Library Module should be used only with the LB Ion Shear™ Plus Enzyme Mix included in the same kit lot. Do not mix Ion Xpress™ Plus cartridges and LB Ion Shear™ Plus Enzyme Mix from separate kit lots.

b. **Rows T1 and T2** (second and third rows): Load with AB Library Builder™ Tips inserted into tip holders.

Note: Two sets of tips and tip holders are required per sample.

c. **Row E** (first row): Load with *labeled* sample/elution tubes, with the caps removed and secured:



10. Insert the racks into the AB Library Builder™ Device:

IMPORTANT!

- Insert the cartridge rack first before inserting the tip and tube rack. Loading the racks in a different order can cause the instrument to stop during a run.
- Use only AB Library Builder™ Sample Tubes (sample/elution tubes). Other tubes may be picked up by the nozzle tips due to differences in tube height and shape, stopping the run.

a. Insert the loaded cartridge rack into the instrument.

IMPORTANT! Before inserting the cartridge rack into AB Library Builder™ Device, ensure that the cartridges are completely thawed, particularly reagents in cartridge wells 2 and 3.

WARNING! Do not touch the surface of the heat block. The temperature of the heat block can reach 95°C. Touching the block can cause burns.

b. Insert the loaded tip and tube rack into the instrument with row E in the front.

11. To start the run, proceed to "Build the libraries using the AB Library Builder™ System" on page 40.

Build the libraries using the AB Library Builder™ System

Note: For additional instructions on instrument operation, see the *AB Library Builder™ System User Guide* (Pub. no. 4463421).

Start the run

1. Press **START** to select the AB Library Builder™ System Kit option.
2. Confirm that you have loaded and inserted the cartridge rack and tip and tube rack correctly.
3. Follow the on-screen prompts in the following paths:

Menu 1: Choose Size-Selection Method	Menu 2: Choose Shear Method	Menu 3: Choose Target Shear-size	Menu 4: Choose Target Size-Selection Size
1: Auto SizeSelection	1: Auto Shearing	1: 100-bp shear and size-selection	—
		2: 200-bp shear and size-selection	—
		3: 300-bp shear and size-selection	—
	2: Custom Shearing	Enter custom shear time	1: 100-bp size-selection
			2: 200-bp size-selection
			3: 300-bp size-selection
	3: Pre-Sheared	1: 100-bp size-selection	—
		2: 200-bp size-selection	—
		3: 300-bp size-selection	—
2: No SizeSelection	1: Auto Shearing	1: 100-bp shear	—
		2: 200-bp shear	—
		3: 300-bp shear	—
		4: 400-bp shear	—
	2: Custom Shearing	Enter custom shear time	—
	3: Pre-Sheared	—	—

Note: Automated size-selection produces libraries that may have a broader size distribution, resulting in a reduced mean read length and total sequencing throughput relative to gel-based size-selected libraries. To bypass automated size-selection on the AB Library Builder™ System, select option **2: No SizeSelection** at the appropriate on-screen prompt during protocol set-up on the AB Library Builder™ System. Libraries created with option **2: No SizeSelection** must be size-selected manually by gels or other methods prior to use in downstream protocols. See Appendix B, "Gel-based size-selection" on page 69 for alternative size-selection methods.

Note: The pre-programmed automated shear protocols are optimized to maximize the yields of libraries with 100-, 200-, 300-, and 400-bp insert sizes from standard DNA preparations. Some users may want to customize the automated shear conditions for specific DNA preparations or downstream applications. To

customize shearing, select protocol **2: Custom Shearing** from the second menu and follow the on-screen prompts to input a custom shear time (in minutes). The pre-optimized shear times for various insert sizes are shown in the following table.

Nominal Shear Size	Actual Shear Size	Pre-programmed Shear Time
100 bp	~130 bp	38 min
200 bp	~260 bp	19 min
300 bp	~380 bp	12 min
400 bp	~460 bp	9 min

4. (*Optional*) Scan the sample, elution tube, and sample lane barcodes [refer to the *AB Library Builder™ System User Guide* (Pub. no. 4463421)].

5. Close the door to the AB Library Builder™ Device.

6. Press **START**.

   The screen shows the current step and the approximate incubation time remaining.

IMPORTANT! Do not open the door during a protocol run. To pause or cancel the run, see *AB Library Builder™ System User Guide* (Pub. no. 4463421).

Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the digital display shows the Main menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original positions as described in *AB Library Builder™ System User Guide* (Pub. no. 4463421).

7. At the end of the run, the instrument beeps briefly and the digital display shows "Finished Protocol". To unload the instrument:

   a. Press  to return to the Main menu, then open the instrument door.

   b. Remove the elution tubes, confirm that they are properly labeled, then cap the elution tubes. The tubes will contain ~30 µL for non-size-selected and 40–50 µL for size-selected libraries.

      Note: Libraries may have a brown tint, which may affect subsequent PCR steps. If desired, place each tube in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle, then transfer the supernatant to a new tube.

   c. Remove the tip and tube rack and cartridge rack.

   d. Properly dispose of the used reagent cartridges, tips, and tubes.

   e. Close the instrument door.

   f. Clean the tip and tube rack as needed.

Note: No cooling period is required between runs.

STOPPING POINT Store the DNA in a supplied Sample Tube at 2°C to 8°C for short-term storage or at –30°C to –10°C for long-term storage. Otherwise, proceed directly to "(Optional) Determine if library amplification is required" on page 43 for automatically size-selected libraries, or Appendix B, "Gel-based size-selection" on page 69 to manually size-select the libraries.

Set up for a new run

WARNING! If the instrument is used with any iPrep™, PrepFiler® Express, PrepFiler® Express BTA, or PrepSEQ® Express cartridges, do not clean the instrument with acids, or bases (such as bleach). Acids and bases can react with the guanidine thiocyanate in the lysis buffer and generate toxic gas.

1. Follow the set-up procedures for a new run (see page 22).

   Note: To set up for a new run using the same protocol card, leave the instrument ON. To set up for a new run with a different protocol card, power OFF the instrument, then change the protocol card (see step 2 on page 24).

2. Start the run (see "Start the run" on page 40).

(*Optional*) Determine if library amplification is required

Estimate the number of template preparation reactions that can be performed with the unamplified library, to determine if the yield of the unamplified library is sufficient for your experimental needs.

Note: In general, libraries do not require amplification. However, this procedure may be helpful when preparing libraries from a new sample type for the first few times.

Quantify the unamplified library by qPCR with the Ion Library Quantitation Kit (Cat. no. 4468802). This kit directly determines the library dilution that gives a suitable concentration for template preparation (~26 pM).

1. Determine the Template Dilution Factor (TDF) for the unamplified library with the Ion Library Quantitation Kit. Follow the instructions in the *Ion Library Quantitation Kit User Guide* (Pub. no. 4468986), and dilute the unamplified library for the qPCR as follows:
   - 50 ng-input: 1:500 dilution
   - 100 ng-input: 1:1000 dilution
   - 1 µg-input: 1:2000 dilution
2. Calculate the number of template preparation reactions that can be performed with the unamplified library as follows:

   No. of reactions =

   [(library volume in µL) × TDF] ÷ [volume per template preparation reaction in µL]

   For the volume per template preparation reaction, see the specific user guide for the appropriate template preparation kit.

   If the estimated number of template preparation reactions is sufficient for your experimental requirements, no amplification is necessary.
3. Proceed to either amplify or further qualify the library, according to your experimental needs.

Library Amplification	Proceed to...
Yes	"(Optional) Amplify and purify the library" on page 44
No	• **Non-barcoded libraries:** "Qualify and pool non-barcoded libraries" on page 47 • **Barcoded libraries:** "Qualify and pool barcoded libraries" on page 49

(*Optional*) Amplify and purify the library

Amplify the library

Materials Provided in the Ion Plus Fragment Library Adapters	Other Materials and Equipment
• Library Amplification Primer Mix ***or*** Use the PCR primer mix (T_PCR_A and P1amp) prepared as instructed in Appendix E on page 83	• Platinum® PCR SuperMix High Fidelity • Thermal cycler • 0.2-mL PCR tubes • 1.5-mL LoBind® tubes

1. Adjust the volume of the unamplified library as described below:

Size-selection Method:	Automated	E-Gel® SizeSelect™ Agarose Gel		Pippin Prep™ Instrument	
DNA input amount	**50 ng–1 µg**	**50 ng to <1 µg**	**1 µg**	**50 ng to <1 µg**	**1 µg**
Volume, unamplified library	40–50 µL	~30 µL	~60 µL	40 µL	40 µL
Volume to amplification rxn (step 2)	40–50 µL	25 µL†	50 µL	25 µL	50 µL‡

† Save the remainder for trouble-shooting.

‡ Adjust volume to 50 µL with Low TE or Nuclease-Free Water.

2. Combine the following reagents in an appropriately sized tube and mix by pipetting up and down:

Component	Volume by Input DNA†	
	50 ng to <1 µg	**1 µg**
Platinum® PCR SuperMix High Fidelity	100 µL	200 µL
Library Amplification Primer Mix ***or*** PCR primer mix (T_PCR_A and P1amp)‡	5 µL	10 µL
Size-selected DNA	25 µL	50 µL
Total reaction mix volume	**130 µL**	**260 µL**

† PCR reaction volumes can be halved.

‡ Prepared according to Appendix E on page 83.

3. Split the 260-µL reaction mix into two 0.2-mL PCR tubes, each containing about 130 µL.

   If desired, you can split the 130-µL reaction into two 0.2-mL PCR tubes, each containing about 65 µL, and split the 260-µL reaction mix into three 0.2-mL PCR tubes, each containing about 86 µL.

4. Place the tubes into a thermal cycler and run the following PCR cycling program. Set the number of cycles according to the second table:

Stage	Step	Temperature	Time
Holding	Denature	95ºC	5 min
Cycling†	Denature	95ºC	15 sec
	Anneal	58ºC	15 sec
	Extend	70ºC	1 min
Holding	—	4ºC	∞

† Set the number of cycles according to the following table.

Number of Cycles by Library Input	
50 to <1 µg	**1 µg**
8	5

Note: Minimize the number of cycles to avoid over-amplification, production of concatemers, and introduction of PCR-induced errors. Reduce the number of cycles if concatemers are formed.

5. Pool the PCR samples in a new 1.5-mL Eppendorf® LoBind® Tube.

Purify the library

Required Materials and Equipment

- 1.5-mL LoBind® tubes
- Agencourt® AMPure® XP Kit
- Magnetic rack such as the DynaMag™-2 magnet
- Low TE
- Freshly prepared 70% ethanol

IMPORTANT! Use ***freshly prepared 70% ethanol***. A higher percentage of ethanol causes inefficient washing of smaller-sized molecules. A lower percentage of ethanol could cause sample loss.

1. Add the indicated volume of Agencourt® AMPure® XP Reagent (1.5X sample volume) to each sample:
   - 50 to <1 µg input: 195 µL
   - 1 µg input: 390 µL
2. Pipet up and down 5 times to thoroughly mix the bead suspension with the DNA, then pulse-spin the tube and incubate the mixture for 5 minutes at room temperature.
3. Place the sample tube in a magnetic rack such as the DynaMag™-2 magnet for 3 minutes or until the solution is clear, then carefully remove and discard the supernatant without disturbing the bead pellet.

4. Without removing the tubes from the magnet, add 500 µL of freshly prepared 70% ethanol to each sample. Incubate for 30 seconds, turning the tube around twice in the magnet to move the beads around. After the solution is clear, remove and discard the supernatant without disturbing the pellet.
5. Repeat step 4 for a second wash.
6. To remove residual ethanol, pulse-spin the tube, place it back in the magnetic rack and carefully remove any remaining supernatant with a 20-µL pipettor without disturbing the pellet.
7. Keeping the sample on the magnet, air-dry the beads at room temperature for ≤5 minutes.
8. Remove the tube from the magnet and add 50 µL of Low TE to the pellet. Pipet the mixture up and down 5 times, then vortex the sample for 10 seconds to mix thoroughly.
9. Pulse-spin and place the sample in the magnetic rack for at least 1 minute. After the solution is clear, transfer the supernatant containing the eluted DNA into a new 1.5-mL LoBind® tube without disturbing the pellet.

STOPPING POINT Store the libraries at –30°C to –10°C. Before use, thaw on ice. To reduce the number of freeze-thaw cycles, store each library in several aliquots.

Finalize the libraries workflow

Finalize the barcoded or non-barcoded libraries as follows:

Non-barcoded Libraries	Barcoded Libraries
Assess the library size distribution (page 47)	Assess the size distribution of individual barcoded libraries (see page 49)
⬇	⬇
Determine the library dilution required for template preparation (page 48)	Pool barcoded libraries using qPCR (see page 50) ***or*** Pool barcoded libraries using Bioanalyzer® instrument quantitation (see page 50)
⬇	⬇
Proceed to template preparation (page 49)	Proceed to template preparation (see page 51)

Qualify and pool non-barcoded libraries

For barcoded libraries, go to "Qualify and pool barcoded libraries" on page 49.

Assess the library size distribution

If you performed a Bioanalyzer® instrument analysis to determine the library molar concentration, you can use the size distribution information from that run. If you performed quantitation using the Ion Library Quantitation Kit (Cat. no. 4468802), perform the following procedure to assess the library size distribution.

Required Materials and Equipment

- Agilent® Bioanalyzer® instrument
- Agilent® High Sensitivity DNA Kit
- (*Optional*) Low TE

To assess the size distribution of each non-barcoded library, analyze an aliquot of a 1:20 dilution in Low TE on the Agilent® Bioanalyzer® instrument with the Agilent® High Sensitivity DNA Kit. Follow the manufacturer's instructions.

See Figure 14, Figure 15, and Figure 16 on pages 85 and 86 for examples of the expected result.

IMPORTANT! Ensure that there are no excessive primer-dimers immediately adjacent to the marker or that there are no excessive over-amplification products (concatemers). For more information, contact Life Technologies Technical Support.

Determine the library dilution required for template preparation

Quantitate a non-barcoded library to determine the dilution that results in a concentration within the optimal range for template preparation (PCR-mediated addition of library molecules onto Ion Sphere™ Particles) using an appropriate template preparation kit.

- **Unamplified libraries:** Determine the library dilution by qPCR with the Ion Library Quantitation Kit (Cat. no. 4468802).
- **Amplified libraries:** Determine the library dilution by qPCR or by Bioanalyzer® instrument analysis.

Quantitation Method	Features
Ion Library Quantitation Kit (qPCR)	• Quantitative real-time PCR (qPCR) methodology. • Directly determines library dilution. • Higher precision for quantitation. A single dilution of a library is usually sufficient for an optimized template preparation procedure. • Higher sensitivity for detection. The Ion Library Quantitation Kit is recommended for low-yield libraries. Libraries with insufficient material for detection by Bioanalyzer® instrument analysis may have material that is detectable by qPCR and sufficient for sequencing. • Unamplified and low-yield libraries also contain unadapted and improperly adapted fragments. The Ion Library Quantitation Kit accurately quantifies the properly adapted libraries with minimal impact from background material.
Bioanalyzer® instrument analysis	• Determines a molar concentration for a library, from which the library dilution is calculated. • Concentration is part of the output of the Bioanalyzer® instrument analysis to assess the library size distribution, so an additional quantitation procedure is unnecessary. • Lower precision for quantitation. Titration of a library over a 4-fold concentration range based on Bioanalyzer® instrument analysis must be performed for optimized template preparation.

Determine the library dilution with the Ion Library Quantitation Kit

Use the Ion Library Quantitation Kit (Cat. no. 4468802) to directly determine the library dilution by quantitative real-time PCR (qPCR). Follow the instructions in the *Ion Library Quantitation Kit User Guide* (Pub. no. 4468986).

The dilution factor calculated by this kit will result in a library concentration suitable for use with Ion template preparation kits.

Note: If you previously quantified an unamplified library with the Ion Library Quantitation Kit and did not amplify the library, you do not need to repeat the qPCR.

Determine the library dilution from Bioanalyzer® instrument analysis

Only amplified libraries can be quantitated using the Bioanalyzer® instrument.

1. From the Bioanalyzer® instrument analysis used to assess the library size distribution, determine the molar library concentration in pmol/L using the Bioanalyzer® software. If necessary, follow the manufacturer's instructions to perform a region analysis (smear analysis) to place the entire distribution of library molecules within a single peak.
2. Determine the dilution factor that gives a concentration of ~26 pM (~15.5 × 10^6 molecules per µL). This concentration is suitable for downstream template preparation. Use the following formula:

   Dilution factor = (Library concentration in pM)/26 pM

   Example: The library concentration is 10,000 pM.

   Template Dilution Factor = 10,000 pM/26 pM = 385

   Thus, 1 µL of library mixed with 385 µL of Low TE (1:385 dilution) yields approximately 26 pM. Use this library dilution for template preparation.

   Note: Because Bioanalyzer® instrument quantitation is not as precise as qPCR, when you perform the template preparation procedure, you will need to prepare 3 serial dilutions of the library at 0.5X library dilution (~13 pM), 1X library dilution (~26 pM), and 2X library dilution (~52 pM) to ensure that one or more dilutions are in the optimal concentration range.

Proceed to template preparation

Prior to template preparation, dilute an appropriate aliquot of each library (based on your template kit requirements) using the preceding calculations.

Note: Diluted libraries should be stored at 2°C to 8°C and used within 48 hours. Store undiluted libraries at –10°C to –30°C.

The libraries are ready for downstream template preparation using an appropriate template preparation kit.

Template preparation documentation is available on the Ion Community at **http://ioncommunity.iontorrent.com/**. Follow the links under **Protocols > Prepare Template > Prepare Template User Guides and Quick Reference**.

Qualify and pool barcoded libraries

Pooling barcoded libraries in equimolar amounts ensures equal representation of each barcoded library in the sequencing run. Barcoded libraries are individually quantitated and pooled. This section describes alternative pooling procedures according to the library quantitation method.

For non-barcoded libraries, follow "Qualify and pool non-barcoded libraries" on page 47.

Assess the size distribution of individual barcoded libraries

Required Materials and Equipment
• Agilent® Bioanalyzer® 2100 instrument • Agilent® High Sensitivity DNA Kit • Low TE

Analyze an aliquot of each barcoded library with an Agilent® High Sensitivity DNA Kit, as indicated in the following table. Follow the manufacturer's instructions.

See "Bioanalyzer® instrument analysis of DNA and libraries" on page 81 for example traces. Individual barcoded libraries display the same size distributions as nonbarcoded libraries.

Library Type	Unamplified		Amplified	
Input amount	100 ng	1 µg	10–100 ng	1 µg
Library aliquot	1 µL	1 µL, 1:5	1 µL, 1:10	1 µL, 1:10

IMPORTANT! Ensure that excessive primer-dimers (immediately adjacent to the marker) or over-amplification products (concatemers) are not present. For more information, contact Life Technologies Technical Support.

Pool barcoded libraries using qPCR

Both unamplified and amplified barcoded libraries can be quantitated for pooling using the Ion Library Quantitation Kit (Cat. no. 4468802). Use the Ion Library Quantitation Kit to directly determine the dilution factor by quantitative real-time PCR (qPCR) for each individual barcoded library. Follow the instructions in the *Ion Library Quantitation Kit User Guide* (Pub. no. 4468986).

1. Dilute each barcoded library according to its dilution factor.

   For example, if the dilution factor is 350, mix 1 µL of the final library with 349 µL of Low TE.

2. Prepare at least 20 µL of a barcoded library pool by mixing equal volumes of the diluted barcoded libraries. The library pool will be at the correct concentration for template preparation. No further dilution of the library pool is necessary.

Pool barcoded libraries using Bioanalyzer® instrument quantitation

Only amplified barcoded libraries can be quantitated for pooling using the Bioanalyzer® instrument.

1. From the Bioanalyzer® instrument analysis used to assess the individual barcoded library size distribution, determine the molar concentration in pmol/L of each barcoded library using the Bioanalyzer® software. If necessary, follow the manufacturer's instructions to perform a region analysis (smear analysis) to place the entire distribution of library molecules within a single peak.

2. Prepare an equimolar pool of barcoded libraries at the highest possible concentration.

   STOPPING POINT (*Optional*) Store the library pool at –30°C to –10°C. To reduce the number of freeze-thaw cycles, store the library pool in several aliquots. Thaw on ice.

3. Determine the molar concentration of the library pool.
   - Use the combined concentration of the library pool calculated for your library pooling algorithm.
   - Alternatively, confirm the concentration of the library pool by analyzing 1 µL of the library pool on the Bioanalyzer® instrument with an Agilent® High Sensitivity DNA Kit.

Determine the molar concentration of the library pool using the Bioanalyzer® software. If necessary, follow the manufacturer's instructions to perform a region analysis (smear analysis) to place the entire distribution of library molecules within a single peak.

4. Determine the dilution factor that gives a concentration of ~26 pM (~15.5 × 10^6 molecules per µL). This concentration is suitable for downstream template preparation. Use the following formula:

   Dilution factor = (Library pool concentration in pM)/26 pM

   Example:

   The library pool concentration is 10,000 pM.

   Dilution Factor = 10,000 pM/26 pM = 385

   Thus, 1 µL of library pool mixed with 385 µL of Low TE (1:385 dilution) yields approximately 26 pM. Use the library dilution for template preparation.

   Note: Because Bioanalyzer® instrument quantitation is not as precise as qPCR, when you perform the template preparation procedure, you will need to prepare 3 serial dilutions of the library at 0.5X library dilution (~13 pM), 1X library dilution (~26 pM), and 2X library dilution (~52 pM) to ensure that one or more dilutions are in the optimal concentration range.

Proceed to template preparation

Prior to template preparation, dilute an appropriate aliquot of each library (based on your template kit requirements) using the preceding calculations.

Note: Diluted libraries should be stored at 2°C to 8°C and used within 48 hours. Store undiluted libraries at –10°C to –30°C.

The libraries are ready for downstream template preparation using an appropriate Ion template preparation kit.

Template preparation documentation is available on the Ion Community at **http://ioncommunity.iontorrent.com/**. Follow the links under **Protocols > Prepare Template > Prepare Template User Guides and Quick Reference**.

Troubleshooting

For issues other than those listed in this section, contact Life Technologies Technical Support (see page 94).

Observation	Possible Cause	Recommended Action
Before loading the cartridges in the cartridge rack		
Precipitate in LdN Buffer tubes	LdN Buffer tubes were exposed to low temperatures during shipping or storage.	To dissolve precipitate that may have formed during shipping or storage, incubate the LdN Buffer tubes at 37°C for 5 minutes or until precipitate is no longer visible.
During the automated run		
No power (the digital display is blank and the fan does not turn on when you power ON)	AC power cord is not connected.	Check AC power cord connections at both ends. Use the correct cords.
	Fuse has blown.	Check the integrity of the fuse and replace it if necessary (refer to the *AB Library Builder™ System User Guide*, Pub. no. 4463421). If the problem persists after connecting the correct power cord and replacing the fuse, contact Life Technologies Technical Support (see page 94).
The digital display is blank, but the fan turns on when you power ON	Protocol card is not inserted correctly.	Power off the instrument and re-insert the protocol card in the proper orientation into the card slot (see page 24). Insert it completely into the slot by manually pushing the card.
	Protocol card was inserted when the instrument was powered ON.	Power off the instrument, then power on the instrument.
Error code displayed	—	See "Instrument error codes" on page 55.
Reagent cartridges, tips, or tubes are not inserted in the correct positions	—	Press **STOP** to pause the run. Open the door, add the missing items, then press **START** to resume the run. Do not open the door without pausing the run.

Observation	Possible Cause	Recommended Action
Run stops after an initial start (you may also see an error code)	• Instrument door opened during the run. • Reagent cartridges, tips, or tubes incorrectly loaded in the rack. • Racks incorrectly loaded on the instrument.	**IMPORTANT!** If you open the instrument door while the instrument is running, the run stops, and it cannot be restarted. If you need to open the instrument door during a run, first press **STOP** to pause the run, then open the door. 1. Follow the procedure in "Instrument error codes" on page 55. 2. Before starting a new run, make sure that the reagent cartridges, tips, and tubes are correctly loaded: • Slide the reagent cartridges into the cartridge rack as described on page 25. • Load the cartridge rack before the tip and tube rack for proper positioning. • Do not cap the tubes. 3. If the instrument continues to stop during the run, contact Life Technologies Technical Support.
	Reagent cartridges not completely thawed.	1. Stop the run. 2. Remove the tip and tube rack, then remove the cartridge rack. 3. Inspect cartridge wells 2 and 3 for ice. 4. If any well is frozen, close the door to the AB Library Builder™ Device, then thaw the cartridges completely. 5. Replace the tips in position T2. 6. Insert the cartridge rack then the tip and tube rack onto the AB Library Builder™ Device. 7. Restart the run.
No liquid in tip, or liquid in tip not moving	No sample added to tube, leading to wet filter barrier on the tip and blockage of nozzles.	Add samples to tubes, load new reagent cartridges, then perform the run again.
Buffer in the bottom tray	Motor movements are not smooth.	Schedule preventive maintenance annually to ensure proper motor movements.
	Reagent cartridges, tips, or tubes incorrectly loaded in the rack.	If you are processing fewer than 13 samples, make sure to load the tips and tubes in the same positions as the reagent cartridges that are loaded in the cartridge rack. See next row for recommended action when experiencing leakage from tips.
Leakage from tips or uneven liquid handling between nozzles	D-Rings are not greased regularly or they need replacement.	You can continue the run, but maintain the D-rings as scheduled. To prevent leakage, maintain or replace the D-rings (refer to the *AB Library Builder™ System User Guide*).
Blockage of tips	Too much starting material causing clumps or aggregates.	Contact Life Technologies Technical Support (see page 94). In future runs, use the sample volume recommended in the user guide for the kit you are using.

Observation	Possible Cause	Recommended Action
After the automated run		
No elution volume	Sample volume is lower than the recommended volume, leading to wet filter barrier on the tip and blockage of nozzles.	In future runs, use the recommended sample volume for the protocol you are using. Long-term operation with lower-than-recommended sample volumes can lead to issues with liquid handling performance.
Little or no library yield	Insufficient or no adapters added to the 5X Reaction Buffer tube.	Add sufficient adapter according to the adapter calculations, and insert the tube in position 11 of the cartridge (see page 26).
	Enzymes or buffer not at bottom of wells.	Tap the wells down against a hard surface to move enzymes and buffer to bottom of wells, then inspect the wells.
	EDTA concentration in the input DNA solution >0.1 mM.	Reduce the concentration of EDTA in the input DNA solution to ≤0.1 mM by dilution using Nuclease-Free Water or 10 mM Tris, pH 7.5—8. Alternatively, exchange the buffer using ethanol precipitation, spin-column or bead-based methods.
Final library is brownish	Beads in final library.	1. Place the tube with the final library in a DynaMag™-2 magnetic rack for at least 1 minute until the solution is clear of brown tint when viewed at an angle. 2. Without disturbing the pellet, carefully transfer the ***supernatant***, which contains the final library, to a new 1.5-mL LoBind® Tube.

Instrument error codes

If an extraction run is interrupted by an error, you cannot resume the interrupted run. Follow the following procedure to resolve the error before you start a new run.

If you observe an error code:

1. Make a note of the error code, including the line number. Common error codes are listed in the following table:

Code	Problem	Code	Problem
10	Failed return to origins, protocol cannot run	21	P axis time out, protocol in run
11	Limit error, protocol can not run	22	M axis time out, protocol in run
12	Failed to return to Z Axis, protocol in run	23	Y axis time out, protocol in run
13	Failed to return to P axis, protocol in run	24	Open door in motion
14	Failed to return to M axis, protocol in run	25	Abnormal input from bottom sensor in motion
15	Failed to return to Y axis, protocol in run	26	Failed to initialize heating block
16	Z axis limit error, protocol in run	27	Failed to initialize motion control board
19	Y axis end limit, protocol in run	110	System error; (Assigned greater than 10)
20	Z axis time out, protocol in run		

2. Press **ESC** to return to the Main menu.
3. If there are tips attached to the nozzles, press **1** to select the Manual screen, then press **2** to return the tips to the original position.
4. Power OFF the instrument, remove the protocol card, wait 5 minutes, insert the protocol card, then power ON the instrument.
5. Run the axis test (refer to the *AB Library Builder™ System User Guide*, Pub. no. 4463421).
6. If the axis test:
   - Is successful, start a new extraction run. Use new samples and plastics where required.
   - Is *not* successful, contact Life Technologies Technical Support (see page 94).

A Manual fragmentation of DNA

This section describes several methods to manually shear genomic DNA and large amplicons, as appropriate for input DNA amount and desired library size.

Method	Library Size	Input DNA
Covaris® S2 and S220 Systems	100-, 200-, and 300-base-read	100 ng or 1 µg
Covaris® M220 System	100-, 200-, 300-, or 400-base-read	100 ng or 1 µg
Bioruptor® Sonication System	100-, 200-, 300-, or 400-base-read	100 ng or 1 µg

Fragment DNA with the Covaris® S2 or S220 System

This section describes sonication of genomic DNA with the Covaris® S2 and S220 Systems to generate DNA fragments with a size range of 100–350 bp. The sonicated DNA is ready for end-repair with the AB Library Builder™ System. For detailed instructions on using the Covaris® System, including loading and unloading the Covaris® microTUBE™ in the microTUBE holder, see the manufacturer's instructions.

Materials and equipment needed

Description	Supplier	Cat. No.	Qty
Covaris® S2 System (110 V for U.S. customers) (220 V for international customers) The Covaris® S2 System includes: • Covaris® S2 sonicator • Latitude™ laptop from Dell™ Inc. • MultiTemp III Thermostatic Circulator • Covaris®-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris®-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris®-2 Series Machine Holder for (one) microTUBE • Covaris® microTUBE Prep Station • Covaris® Water Tank Label Kit • Covaris® microTUBEs (1 pack of 25) ***or***	Covaris	—	1 System
Covaris® S220 System (110 V for U.S. customers) (220 V for international customers)	Covaris	—	1 system
Ethylene glycol	American Bioanalytical	AB00455-01000	1 bottle
LoBind® Tubes (1.5 mL)	Eppendorf	022431021	1 box
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS	—	10 mL
(*Optional*) Shear Buffer Provided with the Ion Plus Library Kit for AB Library Builder™ System	Life Technologies	—	—

Procedure

IMPORTANT! Set the chiller temperature to between 2°C and 5°C to ensure that the temperature reading in the water bath displays 5°C. The circulated water chiller, not the water bath itself, should be supplemented with 20% ethylene glycol.

1. Fill the Covaris® water bath to level 12, then degas the water bath for 30 minutes before shearing. When you place the tube in the holder, ensure that the base of the cap is at water level and the glass portion of the tube is completely submerged.
2. Dilute DNA in an Eppendorf® LoBind® Tube:

Component	Amount
DNA	100 ng or 1 µg
E1 Buffer	Variable
(*Optional*) Shear Buffer[1]	1.2 µL
Total	**130 µL**

1 Shear Buffer reduces DNA damage from fragmentation.

3. Place a Covaris® microTUBE™ into the loading station. Keep the cap on the tube and use a tapered pipette tip to slowly transfer the diluted DNA sample through the pre-split septa. Be careful not to introduce a bubble into the bottom of the tube.
4. Shear the DNA using the following shearing conditions:

Covaris® S2 Settings

Condition	100–250-bp Fragments	350-bp Fragments
Number of cycles	6	2
Bath temperature	5°C	5°C
Bath temperature limit	12°C	10°C
Mode	Frequency sweeping	Frequency sweeping
Water quality testing function	Off	Off
Duty cycle	10%	10%
Intensity	5	5
Cycles/burst	100	100
Time	60 sec	45 sec

Covaris® S220 Settings

Condition	100–250-bp Fragments	350-bp Fragments
Bath temperature	5°C	5°C
Duty factor	10%	10%
Peak Incident Power (PIP)	175 W	175 W
Cycles/burst	100	100
Time	60 sec	90 sec

5. Place the Covaris® microTUBE™ into the loading station. Keeping the snap-cap on, insert a pipette tip through the pre-split septa, slowly remove the sheared DNA, and transfer the DNA into a new 1.5-mL Eppendorf® LoBind® Tube.
6. Either dilute an aliquot 1:50 in Low TE and analyze using a Bioanalyzer® High Sensitivity DNA LabChip® Kit, ***or*** analyze an undiluted aliquot on an agarose gel. Bioanalyzer® instrument peaks should be around 200 bp for 100–250 bp fragments, and around 350 bp for 350-bp fragments.
7. Transfer each sheared DNA sample to a separate empty 1.5-mL sample/elution tube provided in the Ion Plus Library Kit for AB Library Builder™ System or the Ion Xpress™ Plus Library Kit for AB Library Builder™ System.
8. Bring each sheared DNA sample up to a total volume of 110 µL using E1 Buffer, if necessary.

STOPPING POINT Store the sheared DNA in 1.5-mL sample/elution tubes on ice for use that day in "Set up the AB Library Builder™ System to prepare libraries from pre-sheared genomic DNA and shorter amplicons" on page 22, or store at –30°C to –10°C for long-term storage.

Fragment DNA with the Covaris® M220 System

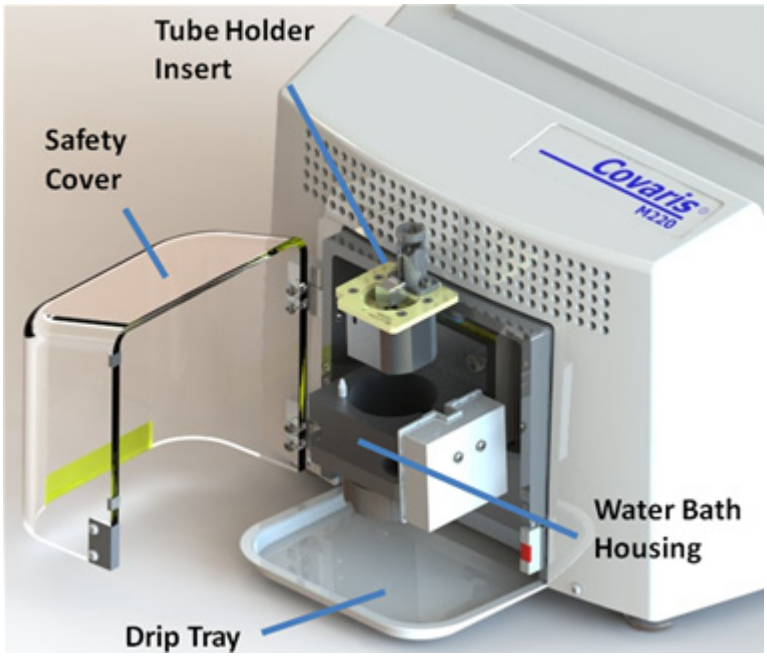
This section describes sonication of genomic DNA with the Covaris® M220 System to generate DNA fragments suitable for 100–400-base-read libraries. The sonicated DNA is ready for end-repair with the AB Library Builder™ System. For detailed instructions on using the Covaris® M220 System, including loading and unloading the Covaris® microTUBE in the microTUBE™ holder, see the manufacturer's instructions.

Materials and equipment needed

Description	Supplier	Cat. No.	Qty
Covaris® M220 System (110 V for U.S. customers) (220 V for international customers) The Covaris® M220 System includes: • Covaris® M220 sonicator • Latitude™ laptop from Dell™ Inc. • SonoLab 7™ Software • Covaris® Holder microTUBE™ M220 • Covaris® microTUBE™ Screw-Cap • Covaris® microTUBE™ Prep Station	Covaris	—	1 System
Covaris® AFA-grade Water[1]	Covaris	—	1 holder
LoBind® Tubes (1.5 mL)	Eppendorf	022431021	1 box
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS	—	10 mL

1 ***Or*** highly purified water (≥ASTM Type III or ISO Grade 3).

Procedure



1. Open the Safety Cover and place the microTUBE™ Holder insert into the water bath housing.
2. Fill the Covaris® water bath housing with Covaris® AFA-grade Water using the provided wash bottle. Continue adding water until the water reaches the top surface of the microTUBE™ Holder (~15 mL) and the water level indicator in the SonoLab software turns to green.

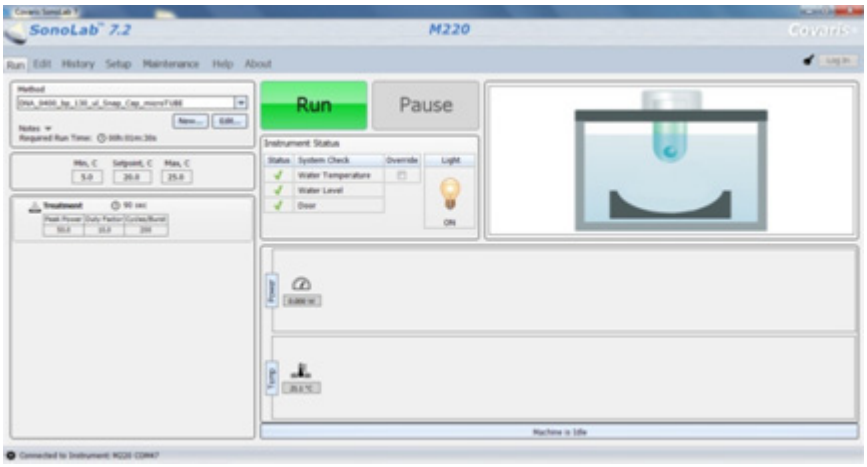
3. Dilute DNA in an Eppendorf® LoBind® Tube:

Component	Amount
DNA	100 ng or 1 µg
E1 Buffer	Variable
Total	**50 µL**

4. Place Covaris® microTUBE™ Screw-Cap into the Prep Station. Unscrew the cap and use a tapered pipette tip to slowly transfer the diluted DNA sample into the microTUBE™. Be careful not to introduce a bubble into the bottom of the tube. Close the microTUBE™.

5. Select the preloaded protocol in the SonoLab software corresponding to the desired library size. Temperature is preprogrammed for each protocol and will be automatically regulated by the M220 once the protocol is selected:

Library Size	Protocol
400-base-read	Ion_Torrent_400bp_50ul_ScrewCap_microTUBE
300-base-read	Ion_Torrent_300bp_50ul_ScrewCap_microTUBE
200-base-read	Ion_Torrent_200bp_50ul_ScrewCap_microTUBE
100-base-read	Ion_Torrent_100bp_50ul_ScrewCap_microTUBE



6. You may also enter the settings manually, as shown in the following table:

Library Size	Mean Fragment Size	Peak Incident Power	Duty Factor	Cycles per Burst	Treatment Time	Temp	Sample Volume
400-base-read	410 bp	50 W	20%	200	60 sec	20°C	50 µL
300-base-read	320 bp	50 W	20%	200	100 sec	20°C	50 µL
200-base-read	260 bp	50 W	20%	200	130 sec	20°C	50 µL
100-base-read	150 bp	50 W	20%	200	375 sec	20°C	50 µL

7. Place the Covaris® microTUBE™ in the holder, close the Safety Cover and click on "Run" in the SonoLab software.

8. Once the treatment is finished, place the Covaris® microTUBE™ into the Prep Station. Unscrew the cap, slowly remove the sheared DNA, and transfer it into a new 1.5-mL LoBind® Tube.

Assess the fragmentation profile

1. Analyze an aliquot of the fragmented DNA as described below to confirm a fragment size with a peak around 150 bp for 100-base-read libraries, 260 bp for 200-base-read libraries, 320 bp for 300-base-read libraries, and 410 bp for 400-base-read libraries.

Input DNA	Bioanalyzer® Instrument Agilent® High Sensitivity DNA Kit	Agarose Gel
100 ng	1 µL	—
1 µg	1 µL of a 1:10 dilution	5 µL

2. Bring each sheared DNA sample up to a total volume of 110 µL using E1 Buffer, if necessary.

STOPPING POINT Store the sheared DNA in 1.5-mL sample/elution tubes on ice for use that day in "Set up the AB Library Builder™ System to prepare libraries from pre-sheared genomic DNA and shorter amplicons" on page 22, or store at –30°C to –10°C for long-term storage.

Fragment DNA with the BioRuptor® Sonication System

This section describes conditions for use of the BioRuptor® UCD-200 or the BioRuptor® NGS UCD-600 Sonication System (equipped with an adapter for 0.65-mL tubes) to shear genomic DNA by ultrasonic fragmentation. The fragmentation profile is suitable for preparing 100–400-base-read libraries. Prepare the libraries by adjusting the downstream size-selection of the library molecules. Sonicate in a refrigerated cold room or on a lab bench. If you are sonicating the DNA on the lab bench, we suggest operating the BioRuptor® Sonication System in a soundproof box to reduce high-frequency noise.

Materials and equipment needed

Description	Supplier	Cat. No.	Qty
BioRuptor® Standard Sonication System with accessories (for 12 x 0.5-mL tubes) ***or***	Ion Torrent ***or*** Diagenode	4465622 UCD-200 TS	1 system 1 system
BioRuptor® NGS Sonication System with accessories (for 12 x 0.5-mL tubes)	Diagenode	UCD-600 TS	1 system
BioRuptor® Microtube Attachment and Gearplate (0.5 mL)	Ion Torrent ***or*** Diagenode	4465648 UCD-pack-05	1 1
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS	—	10 mL
E1 Buffer Provided with the Ion Plus Library Kit for AB Library Builder™ System	Life Technologies	—	—

Prepare the samples

1. In a 0.65-mL microcentrifuge tube for the BioRuptor® Sonication System, prepare 100 ng or 1 µg of your genomic DNA preparation in 50 µL of E1 Buffer. Close the cap carefully to avoid damage. Ensure that the lid is tightly sealed on the tube. Keep the samples on ice.

   IMPORTANT! The material and shape of the tube used for fragmentation of the DNA may have a profound effect on the fragmentation efficiency. This procedure is optimized for 0.65-mL tubes as specified in "Materials and equipment required but not provided" on page 14.

2. Process up to 12 samples at one time with the 12 x 0.65 mL BioRuptor® Sonication System rotor. If there are <12 samples, load tubes with 50 µL of Low TE to fill all empty slots.
3. Unscrew the removable metal ring from the rotor, insert the 12 tubes, and replace the metal ring finger tight. Do not over-tighten the metal ring.
4. Proceed to "Option 1: Sonicate the DNA with the BioRuptor® UCD-200 TS Sonication System" or to "Option 2: Sonicate the DNA with the BioRuptor® UCD-600 NGS Sonication System" on page 66.

Option 1: Sonicate the DNA with the BioRuptor® UCD-200 TS Sonication System

For 100–300-base-read libraries

1. Set the sonication parameters on the BioRuptor® UCD-200 TS Sonication System. Follow the manufacturer's instructions.

Interval ON/OFF	• **ON** (sonication time, red dial): **0.5** minutes • **OFF** (cool-down time; green dial): **0.5** minutes
Power level	**L** (low)

2. Fill the BioRuptor® Sonication System UCD-200 to 1 cm below the Fill Line with cold (<10°C) water. Add an even 1-cm layer (250 mL) of crushed ice, ensuring that the water is just at the fill line.
3. Set the timer to 15 minutes and sonicate according to the following table:

Library Size	Number of 15-min Cycles	Total Sonication Time
300-base-read	1	15 minutes
200-base-read	3	45 minutes
100-base-read	5	75 minutes

Between each cycle, remove 1 cm (150 mL) of the water from the BioRuptor® tank and add 250 mL of crushed ice to the Fill Line.

4. Remove the tubes from the rotor and store on ice.
5. Proceed to "Assess the fragmentation profile" on page 67.

For 400-base-read libraries

1. Set the sonication parameters on the BioRuptor® UCD-200 TS Sonication System. Follow the manufacturer's instructions.

Interval ON/OFF	• **ON** (sonication time, red dial): **0.5** minutes • **OFF** (cool-down time; green dial): **1.5** minutes
Power level	**L** (low)

2. Fill the BioRuptor® Sonication System UCD-200 to 1 cm below the Fill Line with cold (<10°C) water. Add an even 1-cm layer (250 mL) of crushed ice, ensuring that the water is just at the fill line.
3. Set the timer to 15 minutes and sonicate.
4. Remove 1 cm (150 mL) of the water from the BioRuptor® tank and add 250 mL of crushed ice to the Fill Line.
5. Set the timer to 9 minutes and sonicate.
6. Proceed to "Assess the fragmentation profile" on page 67.

Option 2: Sonicate the DNA with the BioRuptor® UCD-600 NGS Sonication System

1. Set the sonication parameters on the BioRuptor® UCD-600 NGS Sonication System. Refer to the instrument manual for detailed instructions:
   a. Press + or – to select the desired parameter, and press **OK**.
   b. Press + or – to change the value, and press **OK**.

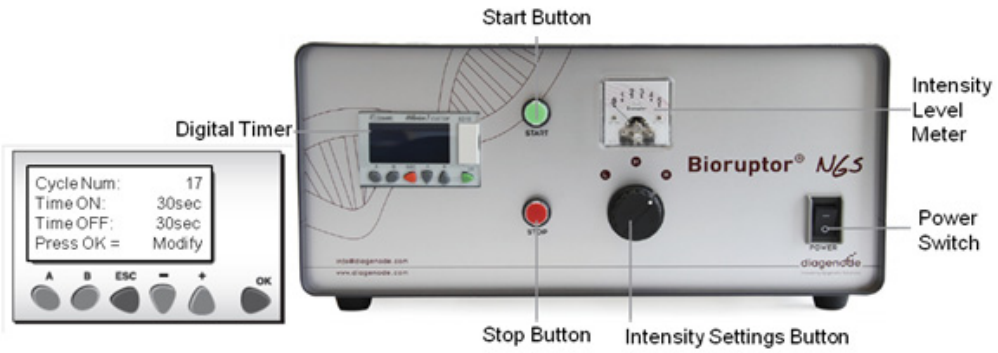
For 100- and 200-base-read libraries

Interval ON/OFF	• **ON** (sonication time, red dial): **0.5** minutes • **OFF** (cool-down time; green dial): **0.5** minutes
Power level	**17**
Intensity settings button	**H** (high)

For 300- and 400-base-read libraries

Interval ON/OFF	• **ON** (sonication time, red dial): **0.5** minutes • **OFF** (cool-down time; green dial): **1.5** minutes
Power level	**10**[1] (300-base-read) and **9**[1] (400-base-read)
Intensity settings button	**H** (high)

1 A short centrifugation step after half of the cycle numbers may improve the results.



2. Fill the BioRuptor® Sonication System to just above the Fill Line with water.
3. Switch on the Bioruptor® water cooler and set the temperature to 4°C.
4. After the set temperature reaches 4°C, insert the rotor containing tubes into the sonicator and press Start. Bioruptor® Running will display on the screen.

   Note: Ensure the temperature of the cooler stays below 10°C during the run.
5. Remove the tubes from the rotor and store on ice.
6. Proceed to "Assess the fragmentation profile" in the following section.

Assess the fragmentation profile

1. Analyze an aliquot of the fragmented DNA as described in the following table to confirm a fragment size range between 50–500 bp, with a peak around 200 bp for 100- and 200-base-read libraries, around 300 bp for 300-base-read libraries, and around 400 bp for 400-base-read libraries. See Figure 12 and Figure 13 on page 84 for example traces.

Input	Bioanalyzer® Instrument Agilent® High Sensitivity DNA Kit	Agarose Gel
100 ng	1 µL	—
1 µg	1 µL of a 1:10 dilution	5 µL

2. Proceed immediately to "Set up the AB Library Builder™ System to prepare libraries from pre-sheared genomic DNA and shorter amplicons" on page 22.

B Gel-based size-selection

Note: Size-selection is available using the AB Library Builder™ System. The AB Library Builder™ System can size-select 100-, 200-, or 300-bp libraries. For more tightly size-selected libraries or to size-select 400-base-read libraries, skip automated size-selection and perform manual, gel-based size-selection after library construction on the AB Library Builder™ System is complete.

This section describes two options for manual, gel-based size-selection:

- E-Gel® SizeSelect™ Agarose Gel, starting on page 70.
- Pippin Prep™ instrument, starting on page 75.

For each method, target the peak length of the size-selected library according to the desired read length:

Library Size	Target Peak Size
400-base-read	~470 bp
300-base-read	~390 bp
200-base-read	~330 bp
100-base-read	~200 bp

Visit the Ion Community at **http://ioncommunity.iontorrent.com/** for other library size-selection methods.

Size-select the library with the E-Gel® SizeSelect™ Agarose Gel

Start with unamplified library, non-barcoded or barcoded, prepared using the AB Library Builder™ System.

Materials Provided in the Ion Plus Fragment Kit	Other Materials and Equipment
• Low TE	• E-Gel® iBase™ unit and E-Gel® Safe Imager™ transilluminator combo kit • E-Gel® SizeSelect™ 2% Agarose Gel • **100-, 200-, or 400-base-read libraries:** 50-bp DNA Ladder (Cat. no. 10416-014; do not substitute other 50-bp ladders such as the TrackIt™ 50 bp Ladder) • **300-base-read libraries:** 100-bp DNA Ladder (Cat. no. 15628-019; do not substitute other 100-bp ladders such as the TrackIt™ 100-bp Ladder) • Nuclease-Free Water

IMPORTANT! We recommend that first-time users of the E-Gel® SizeSelect™ 2% Agarose Gel refer to the *E-Gel® Technical Guide* and *E-Gel® SizeSelect™ Agarose Gels Quick Reference*, available at **www.lifetechnologies.com**.

Prepare the E-Gel® SizeSelect Agarose Gel and iBase™ unit

1. Place the iBase™ unit on top of the Safe Imager™ transilluminator, and plug the short cord from the Safe Imager™ into the power inlet of the iBase™ unit.
2. Plug the connector of the power cord with the transformer into the Safe Imager™ transilluminator and connect the other end of the power cord to an electrical outlet.
3. Verify that the iBase™ has the "SizeSelect™ 2%" program. If not, refer to "Downloading upgrade" from the *E-Gel® Technical Guide*.
4. Remove the gel from the package and gently remove the combs from the SizeSelect™ cassette.
5. Insert the gel cassette into the E-Gel® iBase™ unit right edge first.
6. Press firmly at the left edge of the cassette to seat the gel in the base. A steady light illuminates on the iBase™ unit when the cassette is properly inserted.

Load the gel

Load the gel *without* pre-running, using the following guidelines for the most accurate size cuts:

- Load no more than 250 ng of the appropriate DNA Ladder (50-bp DNA Ladder for 100-, 200-, or 400-base-read libraries, 100-bp DNA Ladder for 300-base-read libraries).
- For size-selection of both 1 µg-input and 50–100 ng-input samples, we recommend running the 1 µg-input sample on one gel and the 50–100 ng-input on a separate gel.
- If you must run both 1 µg- and 50–100 ng-input samples on the same gel, follow the guidelines for collection described in step 8 as closely as possible. If both 1 µg- and 50–100 ng-input samples are collected at exactly the same time, the actual size of collected fragment from the 50–100 ng-input sample is always smaller than that of 1 µg-input sample.

IMPORTANT! Do not pierce the agarose at the bottom of the wells of the gel.

IMPORTANT! Do not use wells #1 and #8 at either edge of the gel (the edge effect slows the sample migration, resulting in shorter fragments). Do not use the wells right next to the ladder well in the center (to avoid potential cross contamination with the ladders).

IMPORTANT! Do not load different libraries in adjacent wells, to avoid potential cross contamination.

1. **For 1 µg-input samples**, add 20 µL of Low TE to the DNA to bring the total volume to 40 µL.
2. Add 20 µL of DNA to the loading well (top row). Use one well for 50–100 ng-input samples.

   Note: Use two adjacent wells for 1 µg-input samples, on one side of the gel. For example, use well positions 2 and 3, or well positions 6 and 7.
3. Dilute the 1 µg/µL 50-bp DNA Ladder in Low TE buffer to 25 ng/µL (1:40 dilution). Add 10 µL of diluted DNA ladder into the middle well, lane M. Load no more than 250 ng (10 µL of 1:40 dilution) of the 50-bp DNA Ladder.
4. Add 25 µL of Nuclease-Free Water to all empty wells in the top row.
5. Add 25 µL of Nuclease-Free Water to all the large wells in bottom row (collection wells), and add 10 µL to the center well (lane M) of the bottom row.

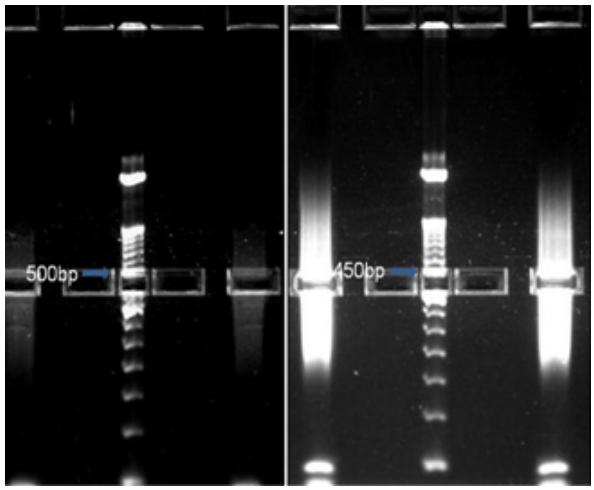
Run the gel

1. Place the amber filter over the E-Gel® iBase™ unit.
2. Select **Run SizeSelect 2%** program, and set the time to the value under **Run Time to Reference Line** in the Run Time Estimation Table in the *E-Gel® SizeSelect™ Agarose Gels Quick Reference* for the appropriate band size, as described in the following table. If you are a new user, select the shorter run time.

Library Size	Target Library Length	Run Time to Reference Line
400-base-read	470 bp	16–20 minutes
300-base-read	390 bp	15–17 minutes
200-base-read	330 bp	12–14 minutes
100-base-read	200 bp	11–12.5 minutes

3. Press **Go** on the iBase™ unit to start electrophoresis. The red light turns to green.
4. Monitor the appropriately sized ladder band to the reference line with periodic monitoring of the run. If needed, extend the run time by repeating steps 2 to 4 with very short run time settings.
5. Press **Go** again to stop the run when the band reaches the reference line.
6. Refill the collection wells to 25 µL with ~10 µL of Nuclease-Free Water. The water in the wells should form a concave surface. *Do not overfill*.
7. Repeat steps 2 to 4 with the run time set to 0.5–2.5 minutes, the value under **Run Time from Reference Line to Collection Well** in the Run Time Estimation Table in the *E-Gel® SizeSelect™ Agarose Gels Quick Reference*.
8. Monitor the middle marker well (M) frequently for the fragment length, and stop the run when the desired fragment size range is in the collection well as shown in the following figures.
    a. For 400-base-read libraries (470-bp target peak):
        - **For 1 µg-input samples**, stop the run when the 450-bp ladder band migrates into the collection well.
        - **For 50–100 ng-input samples**, stop the run when the 500-bp ladder band is at the top edge of the collection well.

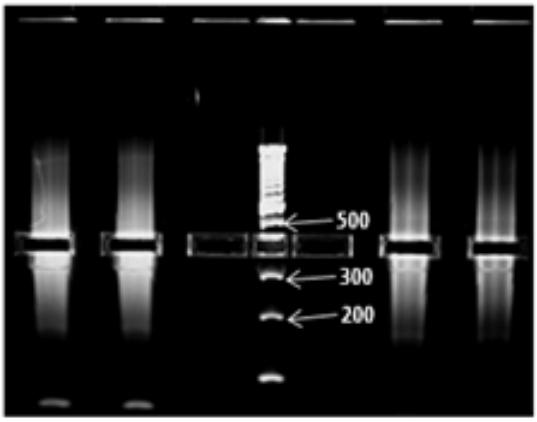
Figure 2 400-base-read library gel



50-ng input 1-µg input

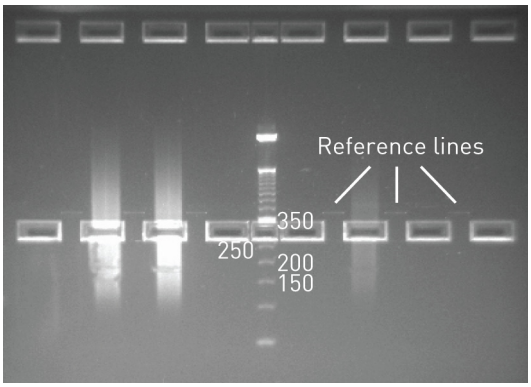
b. For 300-base-read libraries (390-bp target peak):
   - **For 1 µg-input samples**, stop the run right before the 400-bp band is about to touch the top edge of the collection well.
   - **For 50–100 ng-input samples**, stop the run when the 400-bp ladder band is in the middle of the collection well (or 500-bp ladder band is aligned with the reference lines).

Figure 3 300-base-read library gel



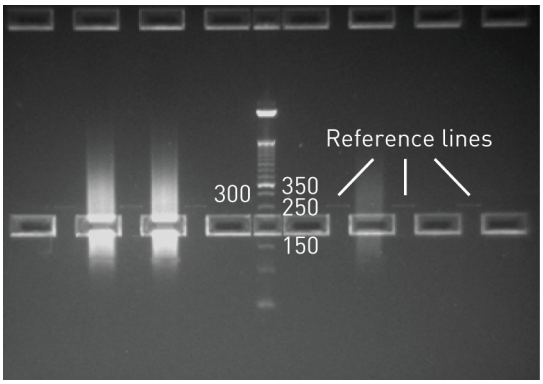
c. For 200-base-read libraries (330-bp target peak):
   - **For 1 µg-input samples**, stop the run right before the 350-bp band is about to touch the top edge of the collection well.
   - **For 50–100 ng-input samples**, stop the run when the 350-bp ladder band has just completely entered the top edge of the collection well.

Figure 4 200-base-read library gel



d. For 100-base-read libraries (200-bp target peak):
   - Stop the run when the 200-bp ladder band is in the middle of the collection well.

Figure 5 100-base-read library gel



Collect the sample

1. Pipet the solution from the collection wells, without piercing the bottom.
2. Refill the well with 10 µL Nuclease-Free Water to wash the collection well. Collect and pool the solutions. The total recovered volume is ~30 µL from each well.
3. **For 1 µg-input samples**, combine the recovered DNA from the two appropriate wells. The total volume is ~60 µL.
4. Dispose of the used gels as hazardous waste.
5. Proceed to "(Optional) Determine if library amplification is required" on page 43.

Size-select the library with the Pippin Prep™ instrument

Start with unamplified library, non-barcoded or barcoded, prepared using the AB Library Builder™ System.

Materials Provided in the Kit	Other Materials and Equipment
• Low TE	• Pippin Prep™ instrument (Cat. no. 4471271) • **100–300-base-read libraries:** 2% Agarose Gel Cassettes for the Pippin Prep™ instrument; includes Loading Solution, Marker B, and Electrophoresis Buffer (Cat. no. 4472170) • **400-base-read libraries:** 2% Dye Free (DF) Marker L Agarose Gel Cassettes for the Pippin Prep™ instrument; includes Loading Solution/Marker L mix, and Electrophoresis Buffer (Sage Science, Cat no. CDF2010) • Nuclease-Free Water • Agencourt® AMPure® XP Kit • Freshly prepared 70% ethanol • Magnetic rack

IMPORTANT! The protocol below closely follows the Pippin Prep™ instrument manual. Novice users may want to review training videos at **www.sagescience.com/resources** before using the instrument for the first time. Software version 5.8 or higher versions are required to run 2% gels with Marker L.

Define plate layout and separation parameters on the Protocol Editor screen

IMPORTANT! For consistent results, before each run calibrate the optics with the calibration fixture. Place the calibration fixture onto the optical nest. Close the lid and press "CALIBRATE" to launch the calibration window. Enter **0.80** in the "Target I ph, mA" field. Press the "CALIBRATE" button in the window, and when complete press "EXIT".

For 100–300-base-read libraries

1. From the cassette type drop-down menu, choose **2% Marker B No Overflow Detection**.
2. Select the "Tight" collection mode for each lane and then define the **BP Target** setting for each of sample 1–4 lanes used, according to the following table:

Library Size	BP Target Setting
300-base-read	390 bp
200-base-read	315 bp
100-base-read	180 bp

3. Define lanes 1–4 as sample lanes and 5 as the ladder lane by entering "5" in the reference lane box and selecting "Apply Reference to all Lanes" button. Ensure that the "Ref Lane" value for each lane is 5.

4. Set the run time for 1.5 hours.

For 400-base-read libraries

1. From the cassette type drop-down menu, choose **2% DF Marker L**.
2. Select the "Tight" collection mode for each lane and then define the **BP Target** setting for each lane as 475.
3. Define lanes 1–5 as sample lanes and press the "Use Internal Standards" button to match the lane numbers and ensure that the "Ref Lane" values match the lane numbers.
4. Set the run time for 1.5 hours.

Prepare the 2% Agarose Gel cassette for the Pippin Prep™ instrument

1. Unwrap the 2% Agarose Gel cassette, and then tip it toward the loading wells end to dislodge any air bubbles present around the elution wells and then insert the cassette into the instrument.
2. Remove the two adhesive strips covering the loading wells and elution wells.
3. Fill the loading wells with Electrophoresis Buffer to the top so that a concave meniscus forms.
4. Remove all liquid from the elution wells, and then add 40 µL of Electrophoresis Buffer.
5. Seal the elution wells with the adhesive tape strips supplied with the cassette packaging.
6. Following the Pippin Prep™ instrument user guide, confirm that the current across both the separation ports and the elution ports is within specifications.

Load the sample

IMPORTANT! Do not pierce the agarose at the bottom of the wells of the gel.

For 100–300-base-read libraries

1. Add 10 µL of Loading Solution to 30 µL of the ligated DNA. The total volume is 40 µL for each sample. If the DNA prepared using the AB Library Builder™ System is <30 µL, add enough Low TE to bring the volume to 30 µL prior to adding the Loading Solution.
2. Go to the Main screen, then choose the newly generated separation file (or a previously saved file) from the Protocol Name pull-down menu.
3. Remove 40 µL of Electrophoresis Buffer from the loading well of the designated Ref Lane, then load 40 µL of 2% DNA Marker B.
4. Remove 40 µL of Electrophoresis Buffer from one sample loading well at a time, then immediately load the entire 40-µL sample into the well.

IMPORTANT! Load the sample immediately to minimize buffer re-entering the well. Buffer in the well prevents loading the entire sample.

For 400-base-read libraries

IMPORTANT! Do not pierce the agarose at the bottom of the wells of the gel.

1. Add 10 µL of Loading Solution/marker mix (labeled Marker L) to 30 µL of the ligated DNA. The total volume is 40 µL for each sample. If the DNA prepared using the AB Library Builder™ System is <30 µL, add enough Low TE to bring the volume to 30 µL prior to adding the Loading Solution.
2. Go to the Main screen, then choose the newly generated separation file (or a previously saved file) from the Protocol Name pull-down menu.
3. Remove 40 µL of Electrophoresis Buffer from one sample loading well at a time, then immediately load the entire 40-µL sample into the well.

   IMPORTANT! Load the sample immediately to minimize buffer re-entering the well. Buffer in the well prevents loading the entire sample.

Run the instrument

1. When the ladder and all samples are loaded, close the lid of the Pippin Prep™ instrument.
2. On the Main screen, press **Start** to initiate the run.
3. When the separation is complete, transfer the DNA from the elution wells (typically 40–60 µL) with a pipette to new 1.5-mL LoBind® Tubes.
4. Add Nuclease-Free Water to the DNA to bring the volume to 60 µL.

Purify the size-selected DNA

IMPORTANT! Use *freshly prepared 70% ethanol* (1 mL plus overage per sample) for the next steps.

1. Add 108 µL of Agencourt® AMPure® beads (1.8X sample volume) to the sample, pipet up and down 5 times to thoroughly mix the bead suspension with the DNA, pulse-spin the tube, and incubate the mixture for 5 minutes at room temperature.
2. Pulse-spin and place the tube in a magnetic rack such as the DynaMag™-2 magnet for 3 minutes or until the solution is clear. Carefully remove and discard the supernatant without disturbing the pellet.
3. Without removing the tube from the magnet, add 500 µL of freshly prepared 70% ethanol to the sample. Incubate for 30 seconds, turning the tube twice in the magnet to move the beads. After the solution clears, remove and discard the supernatant without disturbing the pellet.
4. Repeat step 3 for a second wash.
5. To remove residual ethanol, pulse-spin the tube, place it back in the magnetic rack, and carefully remove any remaining supernatant with a 20-µL pipettor without disturbing the pellet.
6. Keeping the tube on the magnet, air-dry the beads at room temperature for ≤5 minutes.

7. Remove the tube from the magnetic rack, and add the indicated volume of Low TE directly to the pellet to disperse the beads.
   - 1 µg input: 50 µL
   - 50–100 ng input: 25 µL
8. Pipet the mixture up and down 5 times, then vortex the sample for 10 seconds, to mix thoroughly.
9. Pulse spin and place the tube in the magnetic rack for at least 1 minute until the solution clears. Transfer the supernatant containing the eluted DNA to a new 1.5-mL LoBind® Tube without disturbing the pellet.

   IMPORTANT! The supernatant contains the eluted DNA. Do not discard!

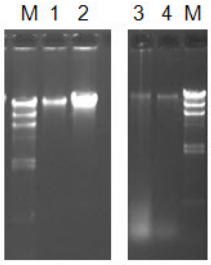
10. Proceed to "(Optional) Determine if library amplification is required" on page 43.

C Evaluating the quality of input DNA

Assess the integrity and size by gel electrophoresis

We recommend checking integrity and size of your DNA preparation by gel electrophoresis. Use of a spectrophotometer to assess DNA quality can be misleading, because many types of molecules absorb ultraviolet light.

Below are examples of high-quality DNA with no contaminating RNA (lanes 1 and 2), compared to lower quality samples containing RNA contamination (lanes 3 and 4). The RNA runs as a diffuse smear at the bottom of the gel. M is a lambda HindIII molecular weight marker:



If your DNA preparation shows RNA contamination, treat it with RNase, as described in the following section.

(*Optional*) Treat the DNA with RNase I

Treat your purified DNA with RNase I only if RNA contamination is evident.

Note: RNase I is recommended. We do not recommend RNase A, which is a site-specific endonuclease and therefore does not degrade the RNA sufficiently to remove it.

Required materials			
Description	**Supplier**	**Cat. no.**	**Quantity**
RNase I	Life Technologies	AM2294 AM2295	10,000 units 25,000 units
PureLink® columns from the PureLink® Genomic DNA Kit, or another purification technology compatible with high molecular-weight DNA	Life Technologies	K1820-1 K1820-2 K1821-04	50 preps 250 preps 4 x 96-well plates

1. Treat the DNA with RNase I according the manufacturer's instructions.
2. Remove the buffer used for RNase I treatment. For example, use a PureLink® spin column included with the PureLink® Genomic DNA Kits (follow the "Purification Procedure Using Spin Columns" protocol in the *PureLink® Genomic DNA Kits User Guide*).

IMPORTANT! The buffer used for RNase treatment interferes with library construction.

D Bioanalyzer® instrument analysis of DNA and libraries

Manually fragmented DNA

This section shows example bioanalyzer traces from manual fragmentation of DNA.

Figure 6 DNA fragmented with the Covaris® S2 System for up to 250 bp. Agilent® High Sensitivity DNA Kit analysis of fragmented *E. coli* genomic DNA diluted 1:50. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

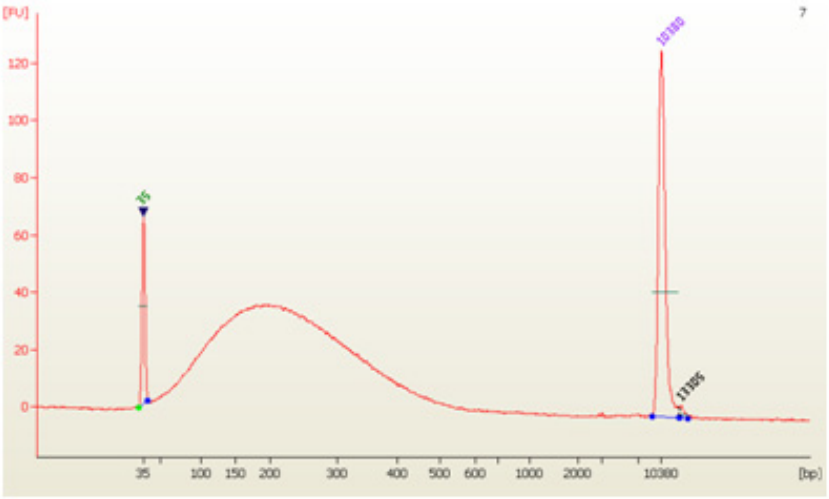


Figure 7 DNA fragmented with the Covaris® M220 System for 60 seconds for 400-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented lambda DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

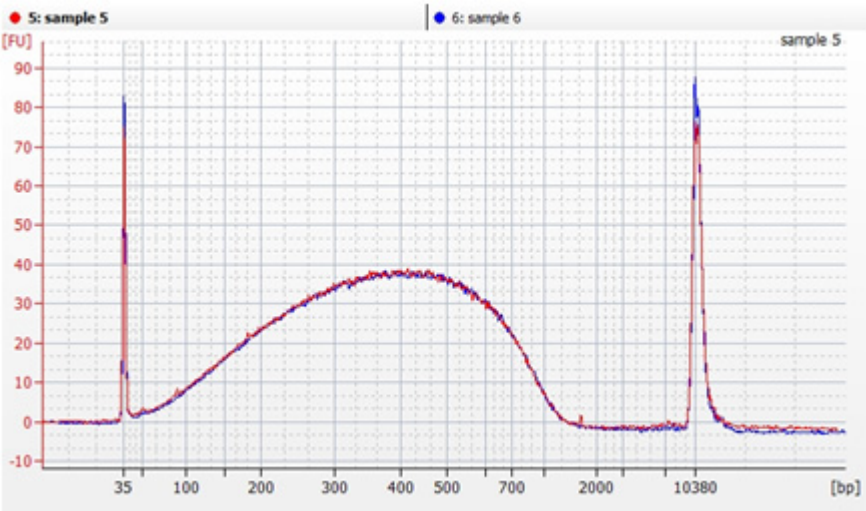


Figure 8 DNA fragmented with the Covaris® M220 System for 100 seconds for 300-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented lambda DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

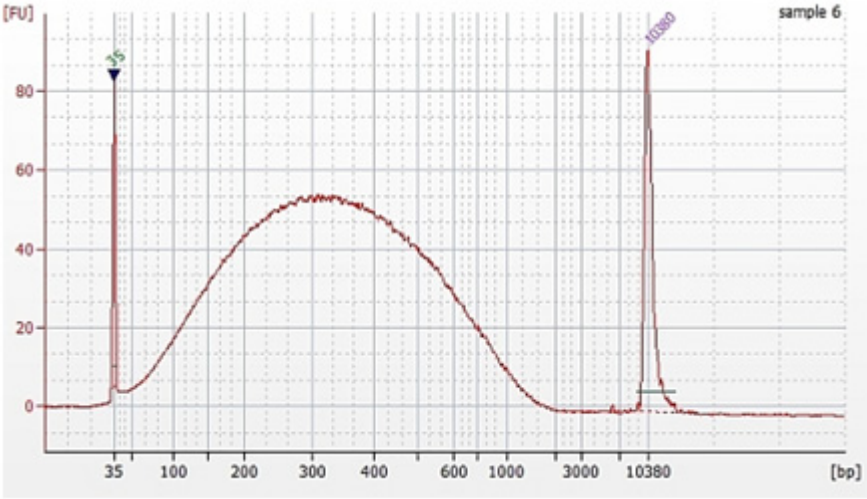


Figure 9 DNA fragmented with the Covaris® M220 System for 130 seconds for 200-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented lambda DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

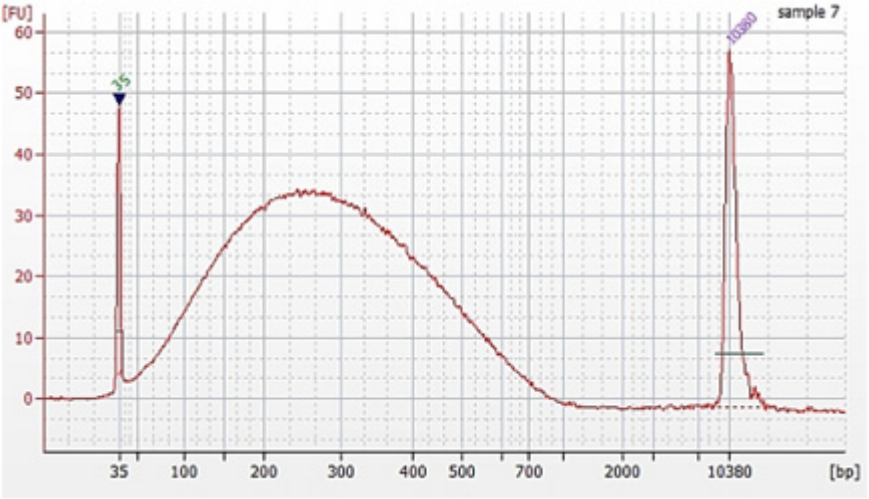


Figure 10 DNA fragmented with the Covaris® M220 System for 375 seconds for 100-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented lambda DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

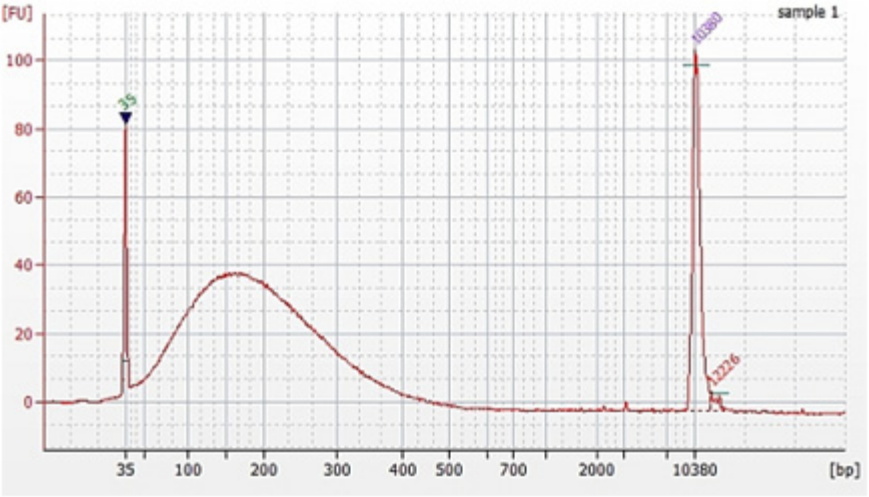


Figure 11 DNA fragmented with the Bioruptor® NGS System for 18 minutes for 400-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented *E. coli* genomic DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

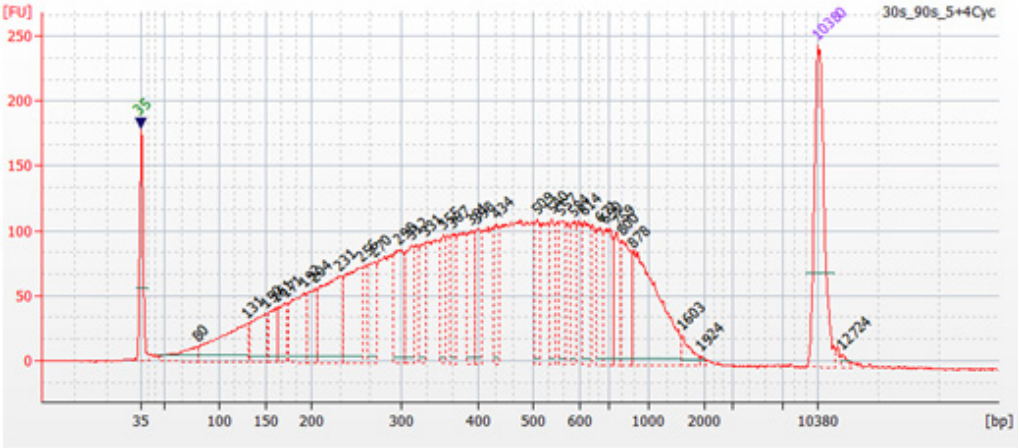


Figure 12 DNA fragmented with the Bioruptor® NGS System for 20 minutes for 300-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented *E. coli* genomic DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

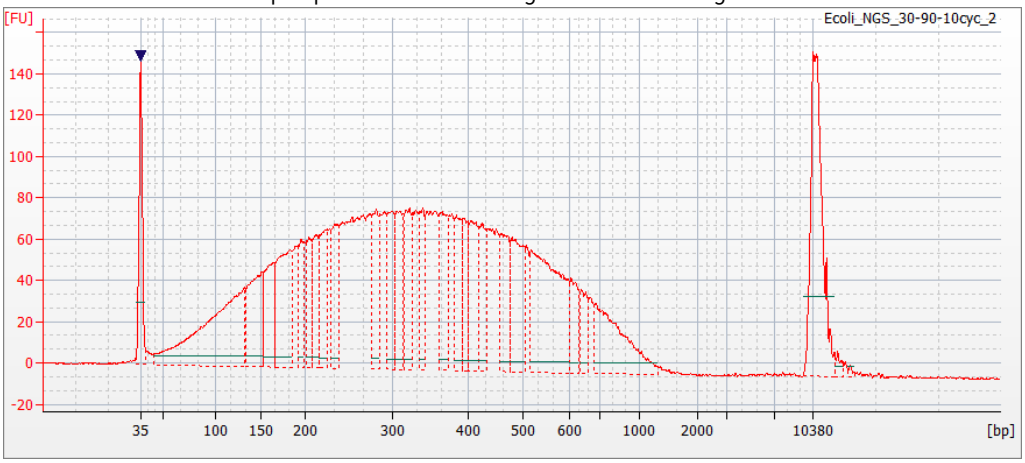
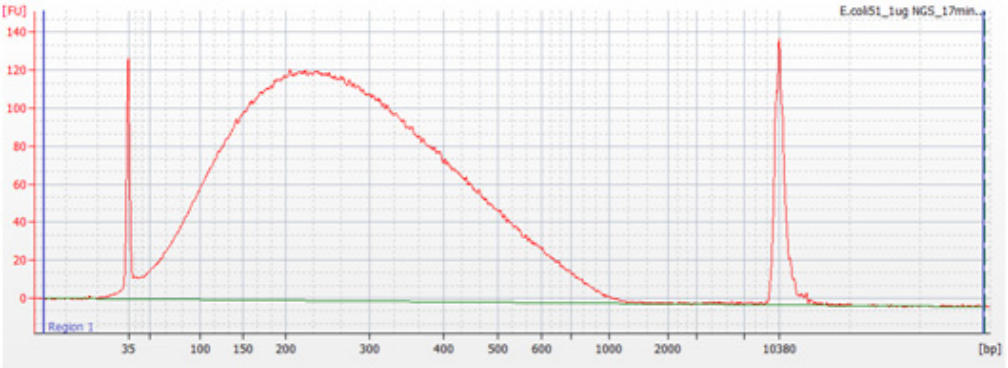


Figure 13 DNA fragmented with the Bioruptor® NGS System for 17 minutes for 100- and 200-base-read libraries. Agilent® High Sensitivity DNA Kit analysis of 1 µg fragmented *E. coli* genomic DNA. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.



Automatically sheared and size-selected DNA

This section shows example bioanalyzer traces from DNA prepared using the AB Library Builder™ System.

Figure 14 ~130-bp insert Ion Xpress™ Plus DNA fragment library. High Sensitivity DNA LabChip® Kit analysis of the unamplified library created with 100-bp automated shearing and automated size-selection on the AB Library Builder™ System. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

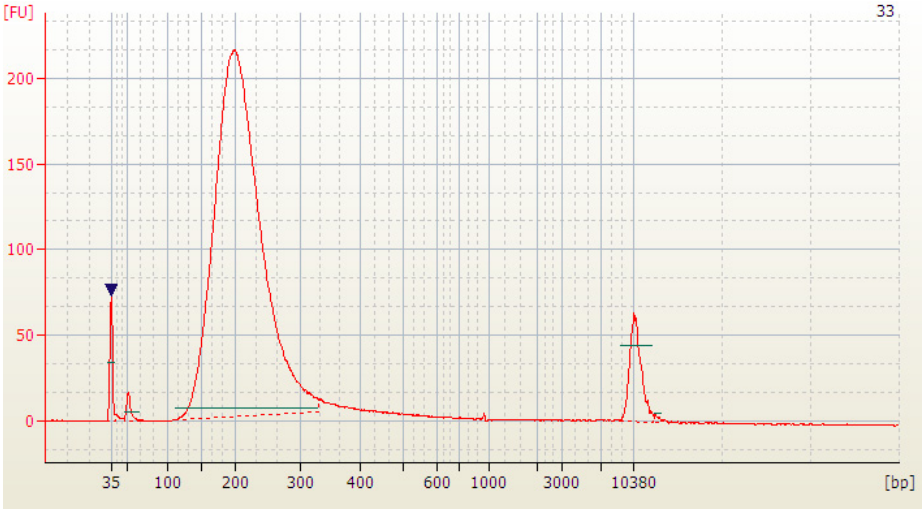


Figure 15 ~260-bp insert Ion Xpress™ Plus DNA fragment library. High Sensitivity DNA LabChip® Kit analysis of the unamplified library created with 200-bp automated shearing and automated size-selection on the AB Library Builder™ System. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.

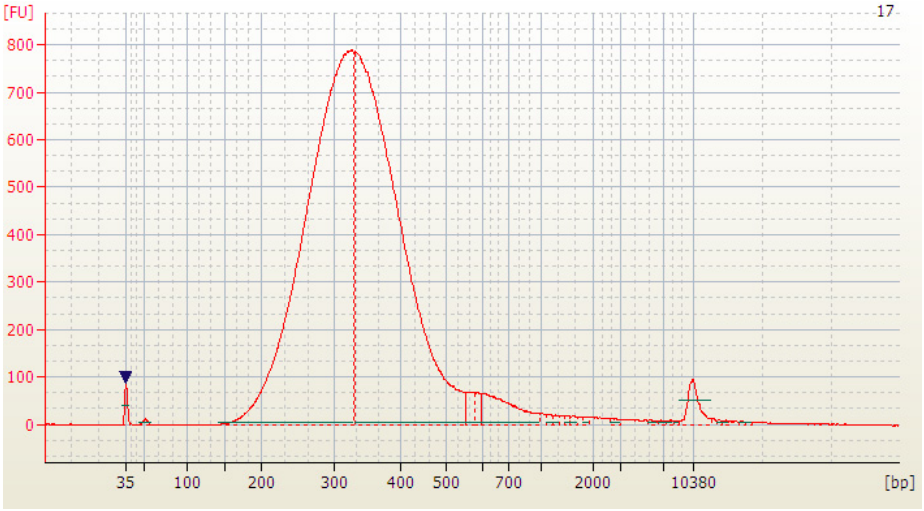
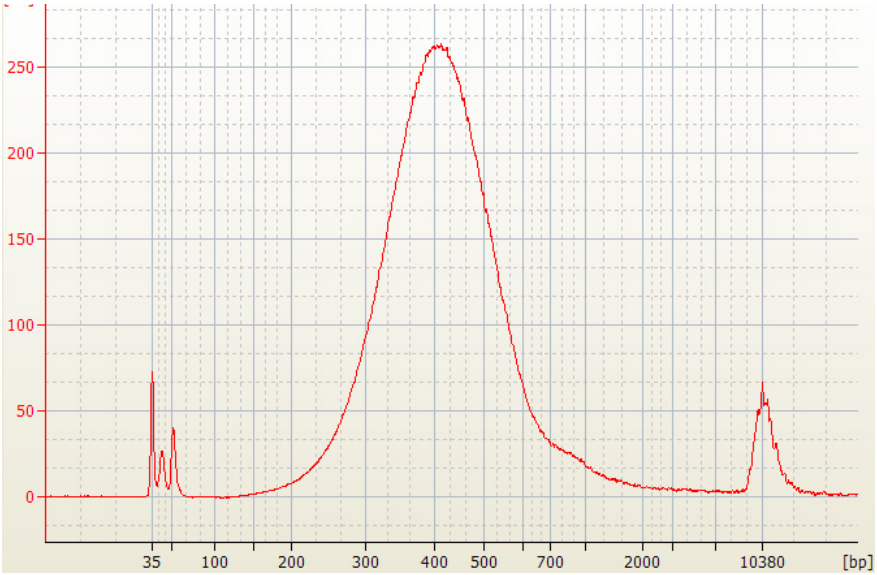


Figure 16 ~390-bp insert Ion Xpress™ Plus DNA fragment library. High Sensitivity DNA LabChip® Kit analysis of the unamplified library created with 300-bp automated shearing and automated size-selection on the AB Library Builder™ System. Peaks at 35 and 10380 bp represent low- and high-molecular weight markers.



IMPORTANT! Ensure that there are neither excessive primer-dimers immediately adjacent to the marker nor that there are no excessive over-amplification products (concatemers). For more information, contact Life Technologies Technical Support.

E Ion adapter sequences and PCR primer mix

Non-barcoded A adapter and P1 adapter sequences

In each sequence, a <*> indicates a phosphorothioate bond, for protection from nucleases and to preserve the directionality of adapter ligation.

Ion A Adapter (non-barcoded)
5'–CCATCTCATCCCTGCGTGTCTCCGACTCAG–3'
3'–T*T*GGTAGAGTAGGGACGCACAGAGGCTGAGTC–5'
Ion P1 Adapter
5'–CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT–3'
3'–T*T*GGTGATGCGGAGGCGAAAGGAGAGATACCCGTCAGCCACTA–5'

Barcode (A) adapter sequences

Barcode (A) adapter sequences are available on the Ion Community. Visit the Ion community at **http://ioncommunity.iontorrent.com/** and perform a search for "Ion 96 Barcode Set."

Prepare PCR primer mix

When amplifying the libraries (see page 44) use the Library Amplification Primer Mix supplied in the Ion Plus Fragment Library Adapters (Cat. no. 4476340), or the PCR primers prepared according to the following procedure.

Required materials			
Description†	**Supplier**	**Cat. No.**	**Quantity**
Oligonucleotides, HPLC, purified	Major Laboratory Supplier (MLS)‡	—	—
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS	—	—
Microcentrifuge tubes	MLS	—	—

† Life Technologies has demonstrated this protocol using this specific material. Substitution may adversely affect system performance.

‡ For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

1. Order oligonucleotides with HPLC purification:

Adapter	Oligo Name	Oligo Sequence (*=phosphorothioate linkage)	Length
PCR	T_PCR_A	5'-CCA TCT CAT CCC TGC GTG TC-3'	20
PCR	P1amp	5'-CCA CTA CGC CTC CGC TTT CCT CTC TAT G-3'	28

2. Thoroughly resuspend each tube of oligonucleotide to 100 µM in Low TE buffer.
3. Dilute each oligonucleotide to 10 µM in Low TE, then mix equal volumes of each oligonucleotide to make the PCR primer mix.

F

Barcode discrimination

Torrent Suite Software v3.0 or higher is recommended for sequence data analysis. The software includes tools for analysis of barcoded libraries prepared with the Ion Xpress™ Barcode Adapters 1–96.

The Ion Xpress™ Barcode Adapters 1–96 were designed for clear separation in flowspace. Barcodes are correctly assigned with high confidence in reads with up to 2 flowspace errors in the barcode region. In the rare situation of reads with 3 or more errors in the barcode region, barcodes may be misassigned. The number of allowable errors can be reduced from 2 to 1 or 0 in the Torrent Suite Software to reduce the risk of barcode misassignment. However, the number of reads assigned to a barcode will be reduced concomitantly.

The chance of barcode misassignment is much less than that of adapter, library, or templated Ion Sphere™ Particle cross-contamination. For experiments in which even a low degree of cross-contamination (<1%) will be detrimental, take measures to avoid exposure of library reagents to amplified products, particularly after the template preparation procedure.

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Safety

Note: For instrument safety and biohazard guidelines, refer to the "Safety" section in the *AB Library Builder™ System User Guide* (Pub. no. 4463421).

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies Corporation at **www.lifetechnologies.com/support**.

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5 April 2013