I. Introduction

This protocol is for use with the PrepX DNA Library Kit, 96 Samples (Cat. No. 640102), which accommodates rapid, walkaway automation of NGS library preparation on the Apollo™ Library Prep system. When run with the `PrepX ILMN 32i` script, this kit can be used to process **three batches of up to 32 samples per batch** into 220-, 320-, 520-, or 870-base-pair-read DNA libraries suitable for sequencing on Illumina® platforms. **Read this Protocol-At-A-Glance in its entirety before you begin with particular attention paid to the Apollo System Best Practices.**

II. Workflow Overview

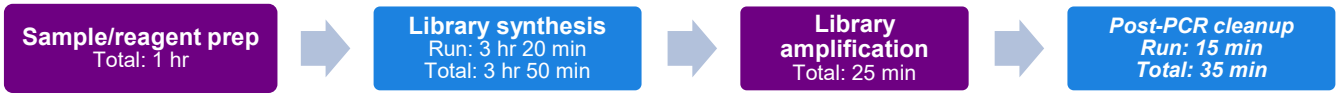


Figure 1. Library preparation workflow overview for one batch of up to 32 samples on the Apollo system with the PrepX ILMN DNA Library Kit - B. Blue and purple boxes indicate steps performed on and off the Apollo system, respectively. The italicized text indicates that the post-PCR cleanup requires a separate protocol. Run = the run time in minutes on the Apollo system, if applicable. Total = total time in minutes spent, including thawing of reagents, reagent and equipment setup, heating and cooling of thermal blocks, incubation of reactions, and automated liquid-handling processes, if applicable.

III. List of Components

PrepX DNA Library Kit, 96 Samples (Cat. No. 640102. Also sold separately.)	
Box 1. (Store at –20°C)	
PrepX DNA 32i Ligase Buffer, 96	3 x 450 µl
PrepX PCR Master Mix	4 x 700 µl
PrepX Complete 32i Ligase Enzyme	3 x 45 µl
PrepX Intermediate 32i Enzymes Strips Complete, Orange Strips	24 x 4-tube yellow strips
Box 2. (Store at 4°C)	
PrepX Molecular Grade Water	2 x 100 ml
PrepX Cleanup Beads	4 x 6.8 ml
PrepX 2.5M NaCl	10 ml

Additional Materials Required

Sequencing Indexes

- PrepX Complete ILMN Barcodes 1-96 (Cat. No. 640107)

Apollo Consumables

The following consumables must be purchased separately from Takara Bio USA, Inc. and were used to validate protocols and scripts. **Do not make any substitutions.**

Apollo consumables	Cat. No.	Quantity	Usage/32-rxn run
Apollo Piercing Tips	640085	Box of 1,000 tips	8 tips
Apollo Filter Tips	640084	Box of 960 tips	368 tips
Apollo Reservoirs	640087	Box of 100 reservoirs	2 reservoirs
Apollo Microtiter Plates	640083	Box of 25 plates	1 plate
Apollo 1.1 mL MiniTubes	640088	Box of 960 minitubes	96 minitubes
Apollo 0.2 ml PCR 8-Tube Strips, Clear	640082	Box of 125 strips	27 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	640086	Box of 125 strips	27 strips

General lab equipment, reagents, and consumables

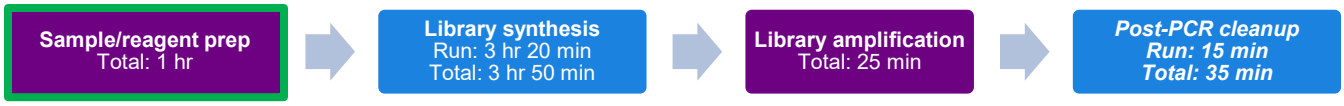
- Single-channel pipettes: 10 µl, 20 µl, 200 µl, and 1,000 µl
- Eight-channel pipettes (recommended): 20 µl and 200 µl
- Filter pipette tips: 2 µl, 20 µl, 200 µl, and 1,000 µl
- PCR thermal cycler
- Covaris instrument and related materials for DNA shearing
- DNA LoBind Tubes (Eppendorf, Cat. No. 0030108051)
- 100% ethanol (EtOH; molecular biology grade)

IV. Apollo System Best Practices

- **Read this Protocol-At-A-Glance in its entirety before you begin.**
- **Before beginning library preparation, follow the Illumina index pooling guidelines to select the right barcode adapters for your set of samples.** (Barcode adapter sequences are in Appendix B.) To avoid index read failure due to registration failure, confirm that the combination of barcode adapter sequences you intend to use will maintain color balance for each base of the index read being sequenced. For more information, visit: https://www.illumina.com/informatics/sample-experiment-management/sequencing-experiment-setup.html
- Clean the work surfaces, including the retention plates, with 70% ethanol at least once a week.
- **Restart the instrument before every run. Also, between each subprotocol, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.**
- Discard any deformed plastics.
- Separate partial tube strips with scissors and remove resulting plastic overhangs.
- Spin down reagents before placing them on the deck to avoid air bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
- Ensure plastics are properly seated on the deck surface **with caps/lids removed**. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.
- Empty the waste box before every run. **An accumulation of tips in the waste box may cause the run to fail.**

V. Protocols

A. Protocol: Sample and Reagent Prep



For each protocol, the corresponding step in the workflow diagram is indicated in with a green outline.

Materials Required

Reagents	Storage conditions	Source
Sample (DNA)	–20°C	User
Plate containing barcode adapters	–20°C	Takara Bio
PrepX DNA 32i Ligase Buffer, 96	–20°C	Takara Bio
PrepX Complete 32i Ligase Enzyme	–20°C	Takara Bio
PrepX Molecular Grade Water	4°C	Takara Bio
PrepX Cleanup Beads	4°C	Takara Bio
PrepX 2.5M NaCl	4°C	Takara Bio
70% ethanol (prepared fresh)	Room temperature	User

NOTE: PrepX Cleanup Beads need to come to room temperature before the container is opened. Therefore, **we strongly recommend preparing ~1-ml aliquots upon receipt** and then refrigerating the aliquots. Individual tubes can be removed for each experiment, allowing them to come to room temperature more quickly (≥30 minutes). This will also decrease the chance of bead contamination. Mix well to disperse the beads before adding them to your reactions. The beads are viscous, so pipette slowly.

Apollo consumables	Cat. No.	Quantity	Usage/32-rxn run
Apollo 0.2 ml PCR 8-Tube Strips, Clear	640082	Box of 125 strips	17 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	640086	Box of 125 strips	8 strips
Apollo Microtiter Plates	640083	Box of 25 plates	1 plate

1. Prepare up to 32 strips of 4-well strip tubes by cutting up to 16 8-tube strips in half and trimming the resulting plastic overhangs. The samples and reagents must be distributed in sets of four to be in the correct orientation to process up to 32 samples. **Do not use 8-tube strips instead of 4-tube strips.**
2. For each sample, prepare 1–100 ng of appropriately-sized DNA as follows:
   a) Quantify DNA using a Qubit Fluorometer or your preferred method.
   b) Assess the size using a fragment analyzer.
   c) (Optional) Further fragment the samples using a Covaris instrument according to the parameters in Appendix A and per the manufacturer's instructions.
   d) Quantify the DNA again, then prepare the samples at the desired input amount. We recommend using an input amount of 1–100 ng fragmented DNA in a volume of 15 µl (0.067–6.67 ng/µl).

3. Prepare the barcode adapters as follows:
   a) Place the barcode adapter plate in a thermal cycler set to the following annealing conditions:

	Temperature	Time
Step 1	95°C	5 min
Step 2	70°C	15 min

NOTE: This protocol is designed for the generation of up to 8 libraries at a time, so no more than 8 barcodes are used during a given run. Store the plate with the remaining barcode adapters at –20°C until the next use. Do not repeat the annealing process.Move the plate from the thermal cycler to a benchtop and allow it to cool to room temperature.

   b) Add 6 µl of PrepX Molecular Grade Water to each well containing a barcode adapter (96 wells total; use the layout shown in Appendix B, and **always change tips to avoid cross contamination**).
   c) Transfer one batch of barcode adapters into fresh 8-tube strips. Cap and place on ice until used.

4. Prepare the barcode adapter master mix ("Adapter MM") for **each** of the desired barcode adapters (up to 32) as described in the table below. Add 15 µl of Adapter MM to each tube of a new Apollo 0.2 ml PCR 8-tube strip. Mix thoroughly with a pipette. Cap and keep on ice.

Component	Volume/rxn
Barcode adapter (e.g., well A1)	2 µl
PrepX Molecular Grade Water	13 µl
Total volume	**15 µl**

5. Prepare the ligation master mix ("Ligation MM") in one PCR tube as described in the table below. Mix thoroughly with a pipette. Keep on ice until aliquoted.

Component	Volume/rxn	Volume/32 rxns + 1 rxn excess
PrepX DNA 32i Ligase Buffer, 96	12 µl	396 µl
PrepX Complete 32i Ligase Enzyme	1 µl	33 µl
PrepX Molecular Grade Water	2 µl	66 µl
Total volume	**15 µl**	**495 µl**

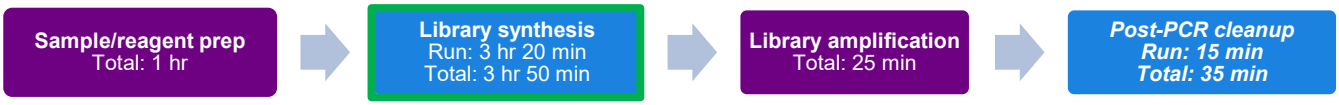
6. On the benchtop, aliquot the reagents into the consumables as described in the table below:

Component	Consumable	Volume/rxn
Sample	New Apollo 8-tube strips	15 µl
Adapter MM	Apollo 8-tube strips (from Step 4)	15 µl
Ligation MM	1 new Apollo 8-tube strip	15 µl
PrepX Molecular Grade Water	1 Apollo reservoir	15 ml
PrepX Cleanup Beads*	New Apollo 8-tube strips	200 µl
PrepX 2.5M NaCl	Apollo microtiter plate, Row 1 (Figure 2)	100 µl
70% ethanol	1 Apollo reservoir	15 ml

*The PrepX Cleanup Beads should be warmed to room temperature and mixed well prior to use.

NOTES:
- The Apollo system is specifically calibrated for the consumables indicated in the table above. Using alternative consumables may cause the run to fail.
- We recommend moving to the Library Synthesis protocol (Section V.B) immediately.

B. Protocol: Library Synthesis



Materials Required

Reagents	Current temperature	Source
Sample	On ice	User
Adapter MM	On ice	Section V.A.
Ligation MM	On ice	Section V.A.
PrepX Molecular Grade Water	Room temperature	Takara Bio
PrepX Cleanup Beads	Room temperature	Takara Bio
PrepX 2.5M NaCl	Room temperature	Takara Bio
70% ethanol	Room temperature	User
PrepX Intermediate 32i Enzymes Strips Complete, Orange Strips	On ice	Takara Bio

Apollo consumables	Cat. No.	Quantity	Usage/32-rxn run
Apollo Piercing Tips	640085	Box of 1,000 tips	8 tips
Apollo Filter Tips	640084	Box of 960 tips	240 tips
Apollo Reservoirs	640087	Box of 100 reservoirs	2 reservoirs
Apollo Microtiter Plates	640083	Box of 25 plates	1 plate
Apollo 1.1 mL MiniTubes	640088	Box of 960 minitubes	96 minitubes
Apollo 0.2 ml PCR 8-Tube Strips, Clear	640082	Box of 125 strips	4 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	640086	Box of 125 strips	4 strips

1. Turn on the instrument or, if the instrument is already on, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.
2. Load Apollo consumables onto the Apollo system work surface according to the layout in Figure 2. First, load the Apollo consumables that do not initially hold reagents (table above). Just before the run, load the Apollo consumables containing reagents, but not samples, onto the system. For information on the deck layout when processing fewer than 32 samples, please see Appendix C.
3. To access the `PrepX ILMN 32i` script, press **Library Prep** > **DNA** > **ILM**. Then, under the PrepX ILM 32i heading, select the desired library insert size (**220 bp**, **320 bp**, **520 bp**, or **870 bp**). The **Cooling** indicator will appear.
4. When the **Cooling** indicator has disappeared, and the **Run** button has appeared, load the samples, reagents, and remaining Apollo consumables onto the Apollo deck following the layout shown in Figure 2 and the instructions shown on the touch screen.

NOTE: Ensure plastics are properly seated on the deck surface with caps carefully removed. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.

5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box and remove any used consumables from the system.

   NOTE: An accumulation of tips in the waste box may cause the run to fail.

7. Close the instrument door and press **Run.**

   NOTE: The run time is 3 hours and 20 minutes.

8. When the run is complete, remove the libraries from Block 3, Row 5 (Products), cap the tubes, and place them on ice. The final DNA library product volume should be ~15 µl per tube.
9. Turn off the instrument.

   SAFE STOPPING POINT: If you do not plan to proceed immediately to the Library Amplification protocol, the products in Block 3 can be capped and stored at –20°C for up to one week.

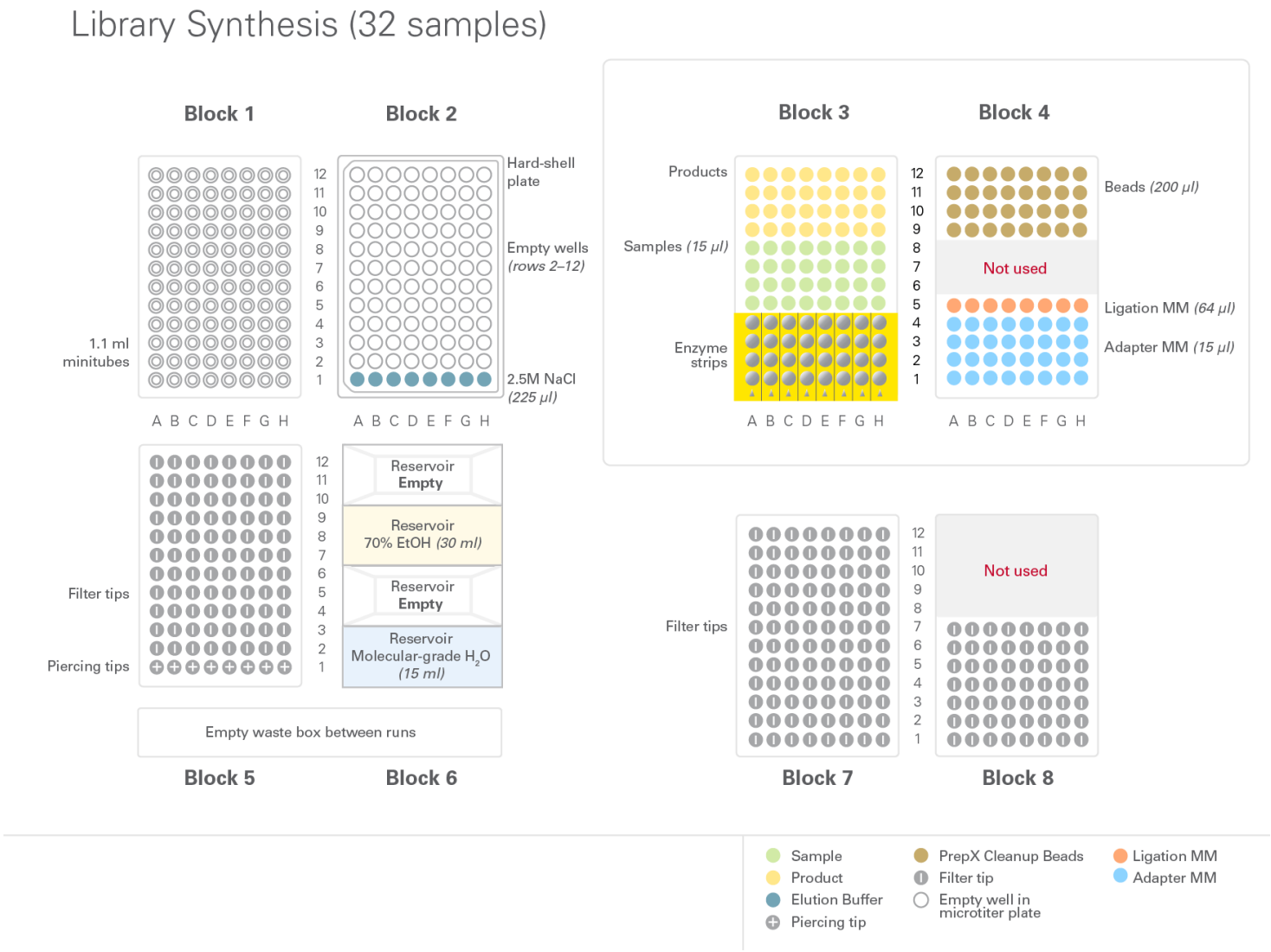
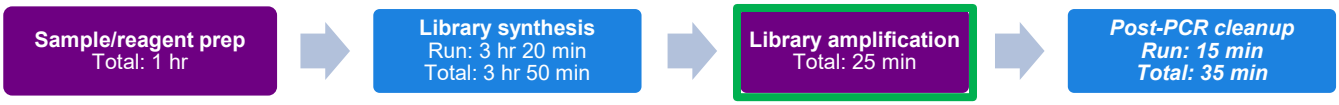


Figure 2. Deck layout for library synthesis from 32 samples. For this protocol, use script `PrepX ILMN 32i`. Run time is 3 hours and 20 minutes.

C. Protocol: Library Amplification



Materials Required

Reagents	Current temperature	Source
Products (from Block 3, Row 5)	On ice	Section V.B.
PrepX PCR Master Mix	On ice	Takara Bio
PrepX PCR Primers	On ice	Takara Bio
PrepX Molecular Grade Water	On ice	Takara Bio

Apollo consumables	Cat. No.	Quantity	Usage/32-rxn run
Apollo 0.2 ml PCR 8-Tube Strips, Clear	640082	Box of 125 strips	4 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	640086	Box of 125 strips	4 strips

1. On ice, prepare the Amplification MM in a 1.5-ml tube, per the table below. Mix the Amplification MM by vortexing gently, and then spin down. Aliquot 27 µl of this master mix into the wells indicated in Figure 2.

Component	Volume/rxn	Volume/32 rxns + 1 rxn excess
PrepX PCR Master Mix	25 µl	825 µl
PrepX PCR Primers	2 µl	66 µl
Total volume	27 µl	891 µl

NOTE: Do not change the volumes of the PrepX PCR Master Mix or the PrepX PCR primers.

2. Use the Amplification MM from Step 1 to prepare the PCR mixture as follows:

Component	Volume/rxn
Amplification Master Mix (from Step 1)	27 µl
Products (from Block 3, Row 5)	5 ng
PrepX Molecular Grade Water	Varies
Total volume	50 µl

3. Perform PCR using the following program:

98°C	30 sec
5–10 cycles:	
98°C	10 sec
60°C	30 sec
72°C	30 sec
72°C	300 sec
4°C	forever

NOTE: Depending on the sample type, the PCR conditions may need to be optimized.

4. Spin the tubes to collect the contents, and then bring each reaction volume up to 50 µl with PrepX Molecular Grade Water. Proceed to the **PrepX PCR Cleanup 32** protocol immediately **OR** PCR products may be stored at 4°C overnight.

Appendix A. Fragmentation Guidelines

Fragment the intact DNA according to the recommended settings below (for a Covaris S220 instrument). Depending on the instrument and sample type used, these settings may need to be optimized. Follow the manufacturer's instructions if using a different instrument.

Table I. Recommended Covaris S220 Instrument Settings for Desired Library Insert Sizes

Parameter	Recommended settings for library insert size		
	220 bp & 320 bp	520 bp	870 bp
Duty cycle	10%	5%	5%
Intensity	4	3	3
Cycles/burst	200	200	200
Time/cycle	9 sec	10 sec	10 sec
Number of cycles	8	5	4
Total process time	72 sec	50 sec	40 sec

Appendix B. PrepX Complete ILMN Barcodes 1-96

PrepX Complete ILMN Barcodes 1-96 (Cat. No. 640107) allows the preparation of up to 96 Illumina-ready DNA libraries using the PrepX ILMN DNA Library Kit - B. Before beginning library preparation, follow the Illumina index pooling guidelines to select the right barcode adapters for your set of samples. To avoid index read failure due to registration failure, confirm that the combination of barcode adapter sequences you intend to use will maintain color balance for each base of the index read being sequenced. Barcode adapter sequences are listed in Table II. For more information, visit: https://www.illumina.com/informatics/sample-experiment-management/sequencing-experiment-setup.html

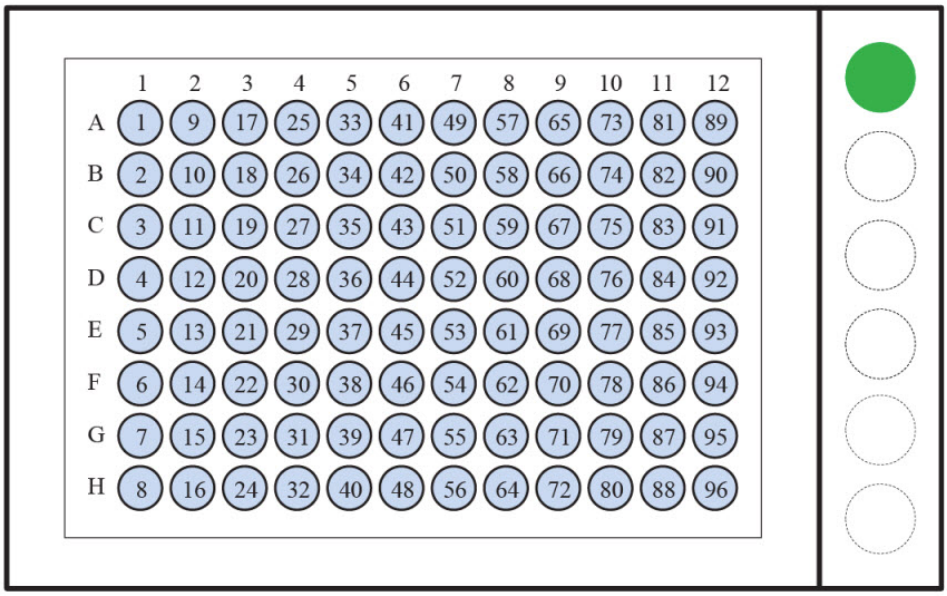


Figure 3. Contents of PrepX Complete ILMN Barcodes 1-96. The green circle in the diagram represents the PrepX PCR Primers supplied in a tube with a green cap.

Table II. Barcode adapter sequences for the PrepX Complete ILMN Barcodes 1-96. Barcode sequences are highlighted in red.

Index	Barcode adapter sequence (5´–3´)
1	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTCACG**ATCTCGTATGCCGTCTTCTGCTTG
2	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CAGCTT**ATCTCGTATGCCGTCTTCTGCTTG
3	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGCTAC**ATCTCGTATGCCGTCTTCTGCTTG
4	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATTGTA**ATCTCGTATGCCGTCTTCTGCTTG
5	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCCTAG**ATCTCGTATGCCGTCTTCTGCTTG
6	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGATCC**ATCTCGTATGCCGTCTTCTGCTTG
7	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTGTCA**ATCTCGTATGCCGTCTTCTGCTTG
8	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGCTTT**ATCTCGTATGCCGTCTTCTGCTTG
9	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATCCGC**ATCTCGTATGCCGTCTTCTGCTTG
10	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTGAAA**ATCTCGTATGCCGTCTTCTGCTTG
11	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTGGCC**ATCTCGTATGCCGTCTTCTGCTTG
12	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TAATGT**ATCTCGTATGCCGTCTTCTGCTTG
13	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATTTCG**ATCTCGTATGCCGTCTTCTGCTTG
14	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TGTACG**ATCTCGTATGCCGTCTTCTGCTTG
15	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CAGTGT**ATCTCGTATGCCGTCTTCTGCTTG
16	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GACTCA**ATCTCGTATGCCGTCTTCTGCTTG
17	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATGACT**ATCTCGTATGCCGTCTTCTGCTTG
18	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTAGGC**ATCTCGTATGCCGTCTTCTGCTTG
19	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGACCA**ATCTCGTATGCCGTCTTCTGCTTG
20	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCAGCC**ATCTCGTATGCCGTCTTCTGCTTG
21	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ACGGTC**ATCTCGTATGCCGTCTTCTGCTTG
22	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTCGAA**ATCTCGTATGCCGTCTTCTGCTTG
23	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGGTAC**ATCTCGTATGCCGTCTTCTGCTTG
24	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TATCAG**ATCTCGTATGCCGTCTTCTGCTTG
25	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CCATGT**ATCTCGTATGCCGTCTTCTGCTTG
26	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATGCGC**ATCTCGTATGCCGTCTTCTGCTTG
27	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTAGCT**ATCTCGTATGCCGTCTTCTGCTTG
28	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCCATA**ATCTCGTATGCCGTCTTCTGCTTG
29	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGTGCC**ATCTCGTATGCCGTCTTCTGCTTG
30	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTTGAC**ATCTCGTATGCCGTCTTCTGCTTG
31	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CATTAG**ATCTCGTATGCCGTCTTCTGCTTG
32	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCGGAT**ATCTCGTATGCCGTCTTCTGCTTG
33	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGATGT**ATCTCGTATGCCGTCTTCTGCTTG
34	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TGACCA**ATCTCGTATGCCGTCTTCTGCTTG
35	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCCAAT**ATCTCGTATGCCGTCTTCTGCTTG
36	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTTGTA**ATCTCGTATGCCGTCTTCTGCTTG

37	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATCACG**ATCTCGTATGCCGTCTTCTGCTTG
38	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTAGGC**ATCTCGTATGCCGTCTTCTGCTTG
39	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ACTTGA**ATCTCGTATGCCGTCTTCTGCTTG
40	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GATCAG**ATCTCGTATGCCGTCTTCTGCTTG
41	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TAGCTT**ATCTCGTATGCCGTCTTCTGCTTG
42	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GGCTAC**ATCTCGTATGCCGTCTTCTGCTTG
43	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGTCAA**ATCTCGTATGCCGTCTTCTGCTTG
44	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGTTCC**ATCTCGTATGCCGTCTTCTGCTTG
45	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATGTCA**ATCTCGTATGCCGTCTTCTGCTTG
46	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CCGTCC**ATCTCGTATGCCGTCTTCTGCTTG
47	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTAGAG**ATCTCGTATGCCGTCTTCTGCTTG
48	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTCCGC**ATCTCGTATGCCGTCTTCTGCTTG
49	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTGAAA**ATCTCGTATGCCGTCTTCTGCTTG
50	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTGGCC**ATCTCGTATGCCGTCTTCTGCTTG
51	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GTTTCG**ATCTCGTATGCCGTCTTCTGCTTG
52	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGTACG**ATCTCGTATGCCGTCTTCTGCTTG
53	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GAGTGG**ATCTCGTATGCCGTCTTCTGCTTG
54	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GGTAGC**ATCTCGTATGCCGTCTTCTGCTTG
55	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ACTGAT**ATCTCGTATGCCGTCTTCTGCTTG
56	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATGAGC**ATCTCGTATGCCGTCTTCTGCTTG
57	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATTCCT**ATCTCGTATGCCGTCTTCTGCTTG
58	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CAAAAG**ATCTCGTATGCCGTCTTCTGCTTG
59	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CAACTA**ATCTCGTATGCCGTCTTCTGCTTG
60	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CACCGG**ATCTCGTATGCCGTCTTCTGCTTG
61	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CACGAT**ATCTCGTATGCCGTCTTCTGCTTG
62	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CACTCA**ATCTCGTATGCCGTCTTCTGCTTG
63	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CAGGCG**ATCTCGTATGCCGTCTTCTGCTTG
64	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CATGGC**ATCTCGTATGCCGTCTTCTGCTTG
65	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CATTTT**ATCTCGTATGCCGTCTTCTGCTTG
66	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CCAACA**ATCTCGTATGCCGTCTTCTGCTTG
67	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGGAAT**ATCTCGTATGCCGTCTTCTGCTTG
68	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTATAC**ATCTCGTATGCCGTCTTCTGCTTG
69	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTCAGA**ATCTCGTATGCCGTCTTCTGCTTG
70	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GACGAC**ATCTCGTATGCCGTCTTCTGCTTG
71	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TAATCG**ATCTCGTATGCCGTCTTCTGCTTG
72	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TACAGC**ATCTCGTATGCCGTCTTCTGCTTG
73	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TATAAT**ATCTCGTATGCCGTCTTCTGCTTG
74	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCATTC**ATCTCGTATGCCGTCTTCTGCTTG
75	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCCCGA**ATCTCGTATGCCGTCTTCTGCTTG
76	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TCGAAG**ATCTCGTATGCCGTCTTCTGCTTG

77	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTGACT**ATCTCGTATGCCGTCTTCTGCTTG
78	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GGAACT**ATCTCGTATGCCGTCTTCTGCTTG
79	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TGACAT**ATCTCGTATGCCGTCTTCTGCTTG
80	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GGACGG**ATCTCGTATGCCGTCTTCTGCTTG
81	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTCTAC**ATCTCGTATGCCGTCTTCTGCTTG
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83	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TTTCAC**ATCTCGTATGCCGTCTTCTGCTTG
84	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GGCCAC**ATCTCGTATGCCGTCTTCTGCTTG
85	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGAAAC**ATCTCGTATGCCGTCTTCTGCTTG
86	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ACACTC**ATCTCGTATGCCGTCTTCTGCTTG
87	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCTACC**ATCTCGTATGCCGTCTTCTGCTTG
88	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATCAGT**ATCTCGTATGCCGTCTTCTGCTTG
89	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCTCAT**ATCTCGTATGCCGTCTTCTGCTTG
90	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**AGGAAT**ATCTCGTATGCCGTCTTCTGCTTG
91	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTTTTG**ATCTCGTATGCCGTCTTCTGCTTG
92	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TAGTTG**ATCTCGTATGCCGTCTTCTGCTTG
93	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CCGGTG**ATCTCGTATGCCGTCTTCTGCTTG
94	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ATCGTG**ATCTCGTATGCCGTCTTCTGCTTG
95	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TGAGTG**ATCTCGTATGCCGTCTTCTGCTTG
96	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGCCTG**ATCTCGTATGCCGTCTTCTGCTTG

Appendix C. Deck Setup for Additional Reaction Sizes

Using the `PrepX ILMN 8` and `PrepX Cleanup 8` scripts, the Apollo system can run up to eight samples per batch. If you are performing fewer than eight reactions, please use a specific deck layout based on the number of samples (Table VI).

Table III. Deck Layout Options for Various Sample Numbers

Setting up less than 8 samples	
# of samples	Columns to load
1	D or E*
2	D, E
3	C, D, E or D, E, F
4	C, D, E, F
5	B, C, D, E, F or C, D, E, F, G
6	B, C, D, E, F, G
7	A, B, C, D, E, F, G or B, C, D, E, F, G, H

*The user can choose between a deck setup centered around column D or E. The scripts are designed to accommodate either layout.

Appendix D. Library Validation

Figures 4 and 5 show example traces from pre-PCR and post-PCR library outputs using the 220-, 320-, 520-, or 870-bp size selection options.

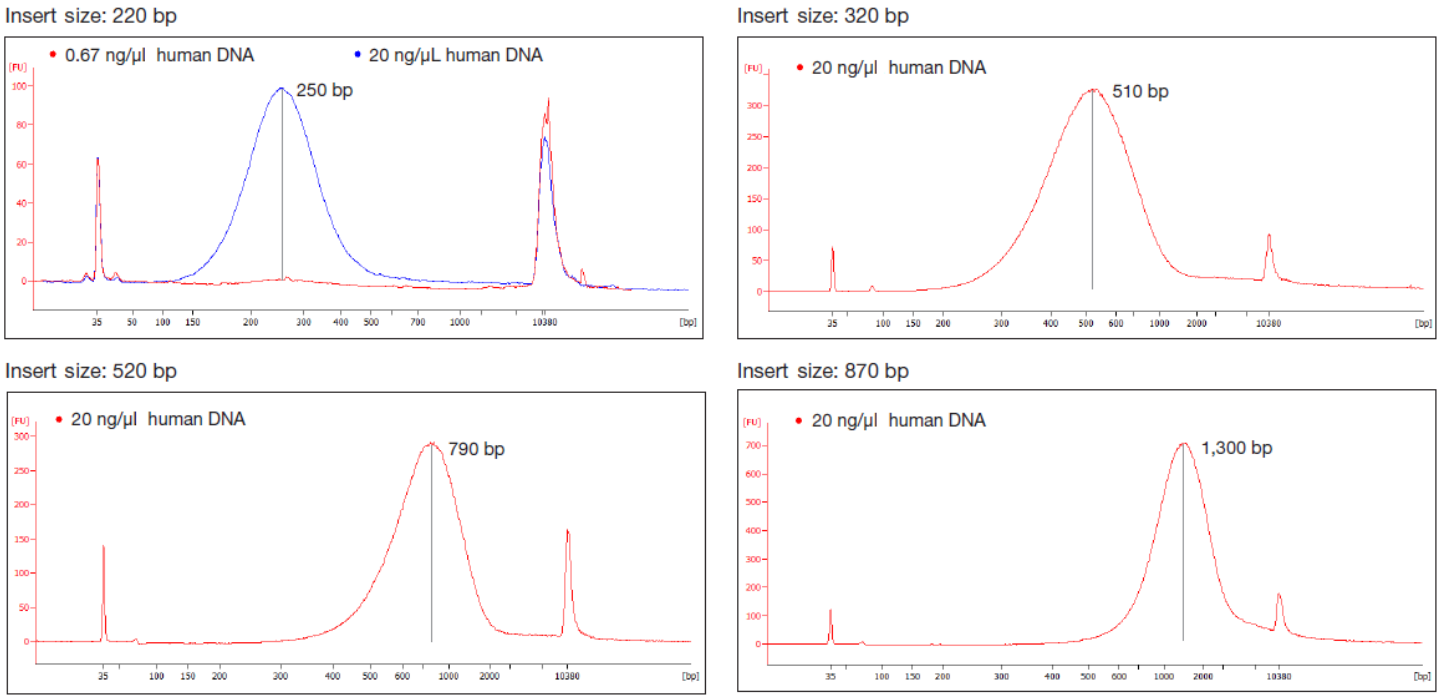

Figure 4. Bioanalyzer traces of pre-PCR library outputs. Covaris-fragmented human DNA was fragmented via sonication, processed through the Protocol: Library Amplification (Section V.C) and validated using an Agilent 2100 Bioanalyzer and Agilent's High Sensitivity DNA Kit. A no-template control is shown in blue.

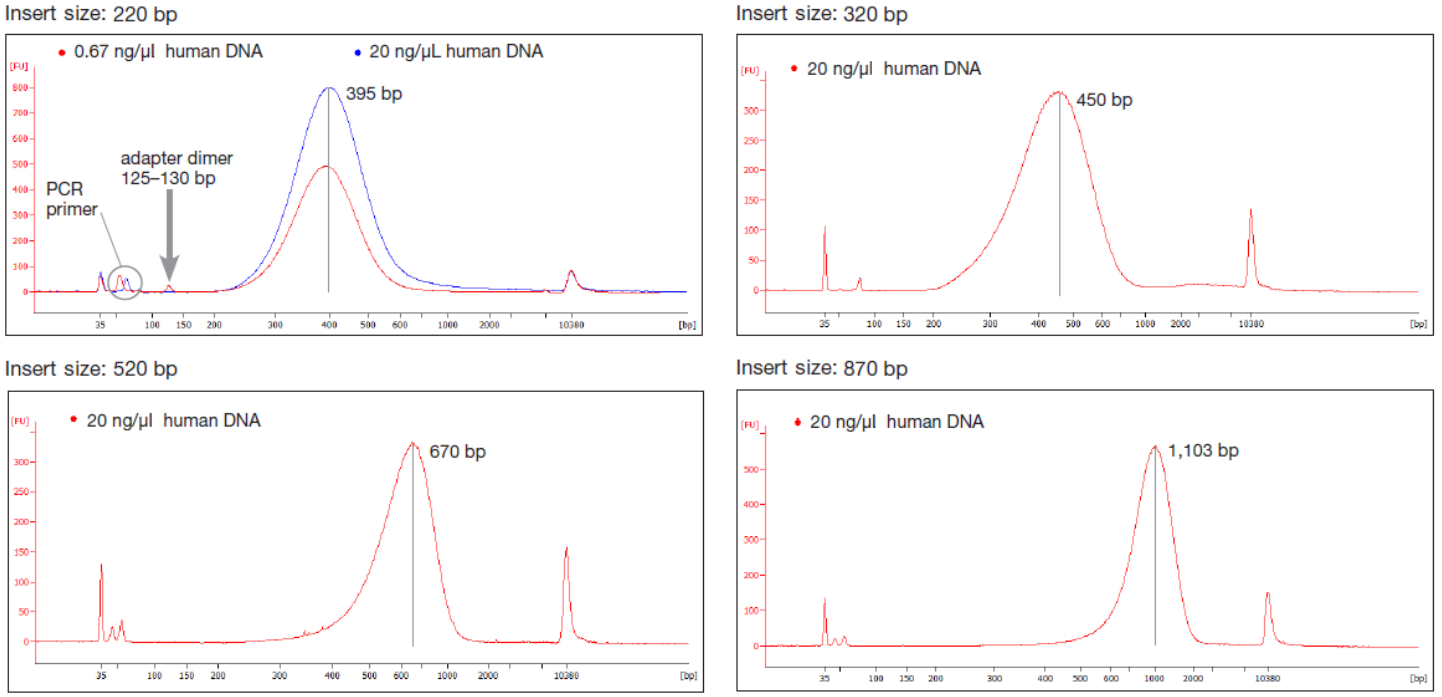

Figure 5. Bioanalyzer traces of post-PCR library outputs. The pre-PCR library outputs shown in Figure 5 were amplified in a commercial thermal cycler for a total of five cycles, then cleaned up using the **PrepX PCR Cleanup 32 Protocol-At-A-Glance**. No-template controls are shown in blue.

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