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Prepare Ion AmpliSeq™ libraries using the Biomek™ FX^P Automated Workstation

USER BULLETIN

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Revision A.0

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Revision history: Pub. No. MAN0019590

Revision	Date	Description
A.0	17 November 2020	New user bulletin that describes Ion AmpliSeq™ library preparation on the Biomek™ FX^P Automated Workstation.

Contents

Methods

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Protocol information

Description

This user bulletin describes how to prepare the Ion AmpliSeq™ libraries using the Biomek™ FX^P Automated Workstation. The workflow for library preparation described in this bulletin is similar to the Ion AmpliSeq™ Library Kit 2.0 or Ion AmpliSeq™ Library Kit Plus workflow. For more information, see the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735) or *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003).

Additional steps for setting up and running the Biomek™ FX^P Automated Workstation and Biomek™ Software v4.1 are described. For more information, see the *Biomek FX and FXP Lab Auto Workstations User's Manual* (Pub. No. B30026AB) and *Biomek 4.1 Software User's Manual* (Pub. No. 987834.AF), available at **Beckman.com/search/techdocs**.

Procedure overview

Amplify target regions from DNA or cDNA, then treat the resulting amplicons with FuPa Reagent to partially digest the primers and phosphorylate the amplicons. Next, ligate the amplicons to barcode adapters and purify them. Normalize or quantify libraries and combine them (optional) prior to template preparation and sequencing.

Note: The automated method described here is not compatible with Ion AmpliSeq™ Exome Kits.

The following kits are used in this automation protocol.

Kit	Requirements
Ion AmpliSeq™ Library Kit 2.0 (Cat. No. 4480441 (96 reactions), or Cat. No. 4480442 (384 reactions)) *or* Ion AmpliSeq™ Library Kit Plus[1] (Cat. No. A35907 (96 reactions), or Cat. No. A38875 (384 reactions))	Each kit contains reagents for the rapid preparation of either 96 or 384 libraries containing 12–24,576 amplicons per reaction. Due to higher dead volume requirements for library preparation using the automated platform, you will need more than one kit to set up 96 or 384 reactions. Each kit uses a 96-well plate-based protocol for easy sample handling and tracking, and for compatibility with automation and high-throughput laboratories.

(continued)

Kit	Requirements
Ion Library Equalizer™ Kit (Cat. No. 4482298)	This kit provides an optional streamlined method for normalizing library concentration without the need for quantification.
One of the following barcode adapters kits:	
(Recommended) IonCode™ Barcode Adapters Kit (Cat. No. A29751)	Each kit provides 384 different barcode adapters, sufficient for ~3840 Ion AmpliSeq™ libraries.
Ion Xpress™ Barcode Adapters Kit (Cat. No. 4474517)[2]	Each kit includes reagents sufficient for preparing up to 40 Ion AmpliSeq™ libraries per barcode (40 × 16 libraries).

[1] Cat. No. 4488990 is also supported for preparing <24 samples.

[2] Other kit configurations are available (see "Ion Xpress™ Barcode Adapters Kits" on page 9).

Kits used in this protocol

Ion AmpliSeq™ Library Kits

Use Ion AmpliSeq™ Library Kit 2.0 or Ion AmpliSeq™ Library Kit Plus for library preparation. If preparing the maximum number of libraries per kit, multiple kits may be required to accommodate for higher dead volume requirements that are associated with the automated platform.

Ion AmpliSeq™ Library Kit 2.0 (96 or 384 reactions)

The Ion AmpliSeq™ Library Kit 2.0 (Cat. No. 4480441) provides reagents for preparing 96 libraries for 1- or 2-pool panels.

The Ion AmpliSeq™ Library Kit 2.0 (Cat. No. 4480442) provides reagents for preparing 384 libraries for 1- or 2-pool panels.

Contents	Amount		Storage
	Cat. No. 4480441 (96 reactions)	Cat. No. 4480442 (384 reactions)	
5X Ion AmpliSeq™ HiFi Mix (red cap)	480 µL	4 × 480 µL	–30°C to –10°C
FuPa Reagent (brown cap)	192 µL	4 × 192 µL	
Switch Solution (yellow cap)	384 µL	4 × 384 µL	
DNA Ligase (blue cap)	192 µL	4 × 192 µL	
Ion AmpliSeq™ Adapters (green cap)	192 µL	4 × 192 µL	
Platinum™ PCR SuperMix High Fidelity (black cap)	3 × 1.6 mL	12 × 1.6 mL	
Library Amplification Primer Mix (white cap)	192 µL	4 × 192 µL	
Low TE	2 × 6 mL	8 × 6 mL	15°C to 30°C[1]

[1] Can be stored at –30°C to –10°C for convenience.

Ion AmpliSeq™ Library Kit Plus (96 or 384 reactions)

The Ion AmpliSeq™ Library Kit Plus (Cat. No. A35907) provides reagents for manually preparing 96 libraries for 1- or 2-pool panels.

The Ion AmpliSeq™ Library Kit Plus (Cat. No. A38875) provides reagents for manually preparing 384 libraries for 1- or 2-pool panels.

Note: If preparing the maximum number of libraries per kit, multiple kits may be required to accommodate for higher dead volume requirements associated with the automated platform.

Component	Amount		Storage
	Cat. No. A35907 (96 reactions)	Cat. No. A38875 (384 reactions)	
5X Ion AmpliSeq™ HiFi Mix (red cap)	480 µL	4 × 480 µL	–30°C to –10°C
FuPa Reagent (brown cap)	192 µL	4 × 192 µL	
Switch Solution (yellow cap)	384 µL	4 × 384 µL	
DNA Ligase (blue cap)	192 µL	4 × 192 µL	
25X Library Amp Primers (pink cap)	192 µL	4 × 192 µL	
1X Library Amp Mix (black cap)	4 × 1.2 mL	16 × 1.2 mL	
Low TE	2 × 6 mL	8 × 6 mL	15°C to 30°C[1]

[1] Can be stored at –30°C to –10°C.

Ion Library Equalizer™ Kit

The Ion Library Equalizer™ Kit (Cat. No. 4482298) provides an optional, streamlined method for normalizing library concentration without quantification. This kit should be used when library yields are consistently above the minimum expected concentration.

The Ion Library Equalizer™ Kit contains reagents sufficient for 96 libraries.

Component	Amount	Storage
Equalizer™ Primers (pink cap)	200 µL	2°C to 8°C
Equalizer™ Capture (purple cap)	1 mL	
Equalizer™ Elution Buffer (clear cap)	10 mL	
Equalizer™ Beads (orange cap)	300 µL	
Equalizer™ Wash Buffer (clear cap)	35 mL	15–30°C[1]

[1] Can be stored at 2–8°C.

Barcode adapters kits

Ion Xpress™ Barcode Adapters, IonCode™ Barcode Adapters, or Ion Torrent™ Dual Barcode Adapters are required to run multiple libraries per sequencing chip, and are ordered separately.

IonCode™ Barcode Adapters Kit

Each IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751) provides 384 different barcode adapters, sufficient for ~3840 Ion AmpliSeq™ libraries.

Contents	Quantity	Storage
IonCode™ Barcode Adapters 1–384 (3840 reactions): • IonCode™ 0101–0196 in 96-well PCR Plate (red) • IonCode™ 0201–0296 in 96-well PCR Plate (yellow) • IonCode™ 0301–0396 in 96-well PCR Plate (green) • IonCode™ 0401–0496 in 96-well PCR Plate (blue)	1 set of 4 plates (10 reactions per barcode)	–30°C to –5°C

Ion Xpress™ Barcode Adapters Kits

Each kit provides 16 different barcode adapters, sufficient for ~640 Ion AmpliSeq™ libraries.

Component	Cap color	Quantity	Volume per tube	Storage
Ion Xpress™ P1 Adapter	Violet	1 tube	320 µL	–30°C to –10°C
Ion Xpress™ Barcode X	White	16 tubes (1 tube per barcode)	20 µL each	

The following Ion Xpress™ Barcode Adapters Kits are available:

- Ion Xpress™ Barcode Adapters 1–16 Kit (Cat. No. 4471250)
- Ion Xpress™ Barcode Adapters 17–32 Kit (Cat. No. 4474009)
- Ion Xpress™ Barcode Adapters 33–48 Kit (Cat. No. 4474518)
- Ion Xpress™ Barcode Adapters 49–64 Kit (Cat. No. 4474519)
- Ion Xpress™ Barcode Adapters 65–80 Kit (Cat. No. 4474520)
- Ion Xpress™ Barcode Adapters 81–96 Kit (Cat. No. 4474521)
- *(Complete set)* Ion Xpress™ Barcode Adapters 1–96 Kit (Cat. No. 4474517)

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Instruments and Equipment	
Biomek™ FX^P Automated Workstation	A31844 (Beckman Coulter™)
Static Peltier Automated Labware Positioner (ALP)	A93938 (Beckman Coulter™)
One of the following thermal cyclers: • GeneAmp™ PCR System 9700[1] or GeneAmp™ PCR System 9700 96-Well[1] • 2720 Thermal Cycler[1] • Veriti™ 96-Well Thermal Cycler • ProFlex™ 96-well PCR System	See web product pages
MicroAmp™ Splash-Free 96-Well Base	4312063
MicroAmp™ Optical Film Compression Pad	4312639
(Optional) ALPS 3000 Automated Microplate Heat Sealer (or equivalent)	AB3000
96-well plate centrifuge	MLS
Reagents and consumables	
Biomek™ P1000 Span-8 Conductive Sterile Filtered Tips	987925 (Beckman Coulter™)
Automation Tips for Beckman™ Liquid Handling Systems with AP96 Dispense Head (20 µL, Sterile, Filtered)	918-021
Automation Tips for Beckman™ Liquid Handling Systems with AP96 Dispense Head (130 µL, Sterile, Filtered)	919-021
Alpaqua™ 96R Ring Magnet Plate	A001219 (Alpaqua™)
Beckman Coulter™ 24-Position Tube Rack	373661 (Beckman Coulter™)
Beckman Coulter™ Tube Rack Inserts, 11mm Diameter	373696 (Beckman Coulter™)
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483354, 4483352
MicroAmp™ Clear Adhesive Film	4306311
Eppendorf™ DNA LoBind™ Tubes, 2.0 mL, PCR clean, colorless	022431048 (Eppendorf™)

(continued)

Item	Source
Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate, Sterile	3960 (Corning™)
Agencourt™ AMPure™ XP Reagent	A63880, A63881, or A63882 (Beckman Coulter™)
Nuclease-free Water	AM9932
Absolute ethanol	MLS
One or more of the following kits for nucleic acid isolation and quantification	
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
PureLink™ Genomic DNA Mini Kit	K1820-00
(RNA only) SuperScript™ VILO™ cDNA Synthesis Kit *or* Ion Torrent™ NGS Reverse Transcription Kit	11754250 A45003
(Recommended for DNA quantification) TaqMan™ RNase P Detection Reagents Kit	4316831
(Recommended for RNA quantification) Qubit™ RNA HS Assay Kit	Q32852 or Q32855
One or more of the following kits for library preparation and quantification	
Ion AmpliSeq™ Library Kit 2.0	4480441 (96 reactions) or 4480442 (384 reactions)
Ion AmpliSeq™ Library Kit Plus[2]	A35907 (96 reactions) or A38875 (384 reactions)
Ion Xpress™ Barcode Adapters Kit	Various[3]
Ion Library TaqMan™ Quantitation Kit	4468802
Ion Library Equalizer™ Kit	4482298
If you are not using the Ion Library Equalizer™ Kit for library normalization, select one of the following kits:	
Qubit™ Fluorometer[4] and Qubit™ dsDNA HS Assay Kit	Q33238, Q32851 or Q32854
Agilent™ 2100 Bioanalyzer™ and Agilent™ High Sensitivity DNA Kit	G2939BA, 5067-4626 (Agilent™)

[1] Supported but no longer available for purchase.
[2] Cat. No. 4488990 is also supported for preparing <24 samples.
[3] For Cat. Nos., see "Ion Xpress™ Barcode Adapters Kits" on page 9.
[4] Qubit™ 2.0 Fluorometer or later

Before you begin

Workflow

Workflow duration depends on the sample type and whether 1- or 2-pool primer pools are used. Approximate duration times reflect on-deck procedures only and do not include thermal cycling.

Biomek™ FX^P Automated Workstation workflow

7 min

(RNA samples only) Reverse transcribe RNA (see page 17)

If starting from RNA samples, reverse transcribe RNA, then proceed to preparing cDNA target amplification reactions.

9–14 min

Set up cDNA target amplification reactions (see page 17)

OR

Set up DNA target amplification reactions (see page 22)

If starting from RNA samples, prepare cDNA target amplification reactions using one of the following methods, depending on the number of primer pools in your panel.

- "Prepare cDNA target amplification reactions – single primer pool panels" on page 19.
- "Prepare cDNA target amplification reactions – 2 primer pool panels" on page 20.

If starting from DNA samples, prepare DNA target amplification reactions using one of the following methods, depending on the number of primer pools in your panel.

- "Prepare DNA target amplification reactions – single primer pool panels" on page 22.
- "Prepare DNA target amplification reactions – 2 primer pool panels" on page 23.

3–5 min

Partially digest amplicons (see page 26)

- For single-pool libraries, see "Set up partial digestion reactions – single primer pool libraries" on page 26.
- For 2-pool libraries, see "Set up partial digestion reactions – 2 primer pool libraries" on page 27.

7 min

Ligate adapters to amplicons (see page 31)

When sequencing multiple libraries on a single chip, ligate a different barcode adapter to each library.

38–58 min

Equalize libraries (see page 32)

OR

Purify and quantify unamplified libraries by qPCR (see page 37)

Equalize libraries or purify and elute unamplified libraries, then determine library concentration by qPCR.

Procedural guidelines

The procedure described in this user bulletin has many steps that refer to a specific configuration of the Biomek™ FX^P Automated Workstation. To ensure proper function, you need to properly map the labware to the instrument that is used to perform the protocol. We recommend for you to confirm all transfers using the **Simulator** mode and confirm all liquid transfers using mock solutions prior to running real samples. For more information on running the **Simulator** mode, see the *Biomek Software User's Manual* or *Help documentation*. For a list of suitable mock solutions, see “Recommended reagent substitutions for calibration” on page 16.

The methods presented here are capable of processing 1–4 plates in parallel for each procedure. For simplicity, only the maximum throughput is described. Each configuration has a specific instrument setup prompt that guides the deck setup. For convenience, the empty deck can be set up as shown in Figure 1. In general, all labware ends in a suffix 1–4, indicating which samples will be processed. For example, plate1 will be processed using tips1 and plate2 will be processed using tips2, etc.

Figure 1 Example empty deck setup

All reagents are stable at room temperature for up to 48 hours and can be processed on non-temperature controlled deck. In the absence of a Static Peltier ALP, the evaporation time during bead cleanup must be extended. You must first validate the required drying time in the absence of a Static Peltier ALP.

The protocol described in this user bulletin is intended for high throughput applications and the 96-channel head is utilized wherever possible. To maximize throughput, place the reagents into the appropriate labware. The following table provides fill volume recommendations however your system may vary.

Table 1 Reagent fill volume recommendation

Reagent	Labware	Dead volume	Recommended fill volume per well	Estimated uses per plate
FuPa Reagent	MicroAmp™ EnduraPlate™	5 µL	25 µL	10
DNA Ligase	MicroAmp™ EnduraPlate™	5 µL	25 µL	10
Switch Solution	MicroAmp™ EnduraPlate™	5 µL	45 µL	10
1X Library Amp Mix + Equalizer™ Primers	MicroAmp™ EnduraPlate™	20 µL	220 µL	4
Agencourt™ AMPure™ XP Reagent	Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate	50 µL	1,000 µL	Varies
Equalizer™ Capture	MicroAmp™ EnduraPlate™	5 µL	45 µL	4
Washed Equalizer™ Beads	MicroAmp™ EnduraPlate™	5 µL	30 µL	4
5X Reaction Buffer or 5X VILO™ Reaction Mix	MicroAmp™ EnduraPlate™	5 µL	25 µL	10
10X RT Enzyme Mix or 10X SuperScript™ Enzyme Mix	MicroAmp™ EnduraPlate™	5 µL	15 µL	10

Reagents should be filled as described in Table 1 and used multiple times to minimize dead volumes in each run. All reagents from AmpliSeq kits are ready to be used with the exception of 1X Library Amp Mix + Equalizer™ Primers and Washed Equalizer™ Beads.

To prepare 1X Library Amp Mix + Equalizer™ Primers, combine 211.5 µL Platinum™ PCR SuperMix High Fidelity (from Ion AmpliSeq™ Library Kit 2.0) or 1X Library Amp Mix (from Ion AmpliSeq™ Library Kit Plus) with 8.5 µL Equalizer™ Primers per well.

To prepare Washed Equalizer™ Beads, wash beads as described in “Wash the Equalizer™ beads” on page 34, then add the indicated volume of washed beads to each well.

Import project and method files

Before you begin, contact Technical Support or your support representative to obtain the `AmpliSeq_1or2Pool.zip` file (see “Customer and technical support” on page 40).

The `AmpliSeq_1or2Pool.zip` file contains all the necessary IMP and BMF files that are required to carry out the procedures described in this user bulletin.

1. Download, then extract the `AmpliSeq_1or2Pool.zip` file.
2. Open the Biomek™ Software.

Import project files

3. Click **Project** ▸ **New Project**, then in the **Create project** dialog, enter the new project name. Click **OK**.
   A new project is created, to which you can now import a project file.
4. Click **Project** ▸ **Import Project**, then in the **Import Project** window select **AmpliSeq_1or2Pool.imp** from the appropriate folder. Click **Open**.
   Import Project window opens, displaying a list of all project items in the project file to import.
5. Confirm that all project items are selected, click **OK**, then close the **Import Project** window.

Import method files

6. Click **File** ▸ **Import**, then in the **Import Method** window select all the BMF files from the appropriate directory.
7. Click **Open** to import the selected method files, then click **Yes** to confirm the selections.

Starting the Biomek™ FX^P Automated Workstation

Before you begin any method creation or optimization, make sure that the Biomek™ FX^P Automated Workstation is properly installed and functioning.

Note: Homing axes can be done prior to starting each method or daily.

1. Open the Biomek™ Software.
2. Click **Instrument** ▸ **Home All Axes**.
3. Make sure that all air bubbles have cleared from the Span-8 supply lines prior to using the instrument.

Recommended reagent substitutions for calibration

Use the following reagent substitutions during initial setup of the Biomek™ FX^P Automated Workstation for calibration. You can also use the recommended reagent substitutions for troubleshooting of any performance problems and inconsistencies.

Reagent	Recommended substitution
DNA	Water
Primers	
5X Ion AmpliSeq™ HiFi Mix	40% glycerol solution in water
FuPa Reagent	50% glycerol solution in water
DNA Ligase	
Switch Solution	20% Polyethylene Glycol 8000 (PEG-8000) solution in water
Agencourt™ AMPure™ XP Reagent	

Set up cDNA target amplification reactions

Reverse transcribe RNA, then set up cDNA target amplification reactions using one of the following procedures, depending on the number of primer pools in your Ion AmpliSeq™ panel:

- For single-pool designs, see "Prepare cDNA target amplification reactions – single primer pool panels" on page 19.
- For 2-pool designs, see "Prepare cDNA target amplification reactions – 2 primer pool panels" on page 20.

If starting from DNA samples, see "Set up DNA target amplification reactions" on page 22.

Prepare reverse transcription reactions

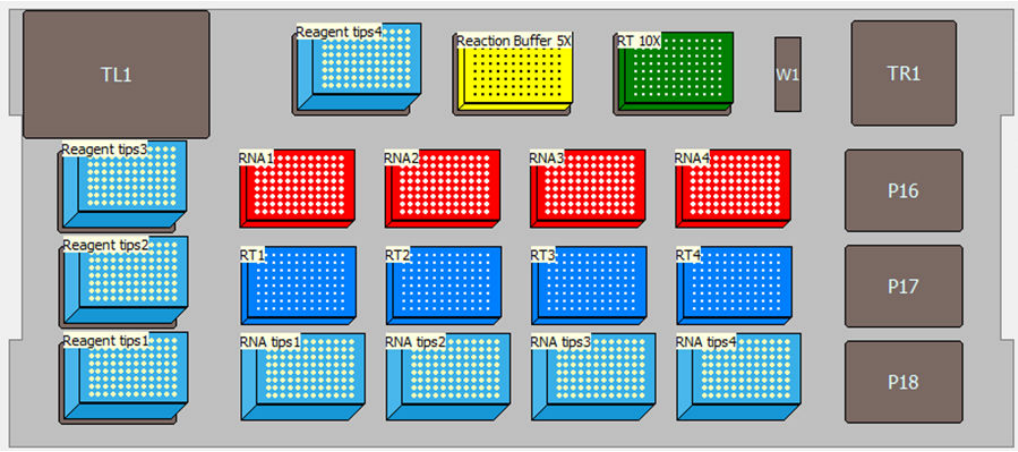
1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Reverse_Transcribe** from the appropriate folder.
2. Centrifuge the 5X Reaction Buffer and the 10X RT Enzyme Mix reagent plates at 500–1,000×g for 30–60 seconds.

   IMPORTANT! Ensure that the fill volume is sufficient for the number of plates being processed plus 5 µL dead volume per well.
3. Place the 5X Reaction Buffer plate at position **PeIt96_1**.
4. Place the 10X RT Enzyme Mix plate at position **PeIt96_2**.
5. Place extracted RNA samples (1–100 ng in 7 µL) and reaction plates on MicroAmp™ Splash-Free 96-Well Bases as described in the following table.

Sample plates	RNA position	Reaction plate position
Set 1	P4	P5
Set 2	P7	P8
Set 3	P10	P11
Set 4	P13	P14

6. Place P20 tips at positions **P1**, **P2**, **P3**, **P6**, **P9**, **P12**, **P15**, and **P19**.
7. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

8. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

9. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to "Reverse transcribe RNA".

Reverse transcribe RNA

Place a MicroAmp™ Optical Film Compression Pad on the plate, load it in the thermal cycler, then run one the following programs depending on the kit that was used to set up the reverse transcription reactions.

Table 2 SuperScript™ VILO™ cDNA Synthesis Kit conditions

Temperature	Time
42°C	30 min
85°C	5 min
10°C	Hold

Table 3 NGS Reverse Transcription Kit conditions

Temperature	Time
25°C	10 min
50°C	10 min
85°C	5 min
10°C	Hold

STOPPING POINT Samples can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

Prepare cDNA target amplification reactions – single primer pool panels

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **cDNA_Amplification** from the appropriate folder.

2. Prepare the PCR Master Mix in an appropriate container and aliquot into four 2-mL tubes as described in the following table.

# of Sample plates *(# of samples)*	5X Ion AmpliSeq™ HiFi Mix	5X Primer Mix	Water	Total master mix	Volume per 2-mL tube
1 *(96 samples)*	544 µL	544 µL	272 µL	1,360 µL	320 µL
2 *(192 samples)*	928 µL	928 µL	464 µL	2,320 µL	560 µL
3 *(288 samples)*	1,312 µL	1,312 µL	656 µL	3,280 µL	800 µL
4 *(384 samples)*	1,696 µL	1,696 µL	848 µL	4,240 µL	1,040 µL

3. Place the 4 tubes into column 1 of a Beckman Coulter™ 24-Position Tube Rack, then place the rack at position P17.

4. Place sample and reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

Sample plates	cDNA position	Reaction plate position
Set 1	P4	P10
Set 2	P5	P11
Set 3	P6	P12
Set 4	P7	P13

5. Place P20 tips at positions P1, P2, P3, and P19.

6. Place P1000 tips position P16.

7. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

8. Confirm the deck setup on the instrument matches the display and follow any prompts.

The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

9. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to "Amplify the targets" on page 25.

Prepare cDNA target amplification reactions – 2 primer pool panels

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **cDNA_Amplification** from the appropriate folder.

2. Prepare the PCR Master Mix in an appropriate container and aliquot into four 2-mL tubes as described in the following table.

# of Sample plates *(# of samples)*	5X Ion AmpliSeq™ HiFi Mix	5X Primer Mix	Water	Total master mix	Volume per 2-mL tube
1 *(96 samples)*	352 µL	352 µL	176 µL	880 µL	200 µL
2 *(192 samples)*	544 µL	544 µL	272 µL	1,360 µL	320 µL
3 *(288 samples)*	736 µL	736 µL	368 µL	1,840 µL	440 µL
4 *(384 samples)*	928 µL	928 µL	464 µL	2,320 µL	560 µL

3. Place the 4 tubes of Pool 1 Master Mix into column 1 of a Beckman Coulter™ 24-Position Tube Rack.

4. Place the 4 tubes of Pool 2 Master Mix into column 2 of a Beckman Coulter™ 24-Position Tube Rack.

5. Place the Beckman Coulter™ 24-Position Tube Rack containing the master mixes at position P17.

6. Place sample and reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

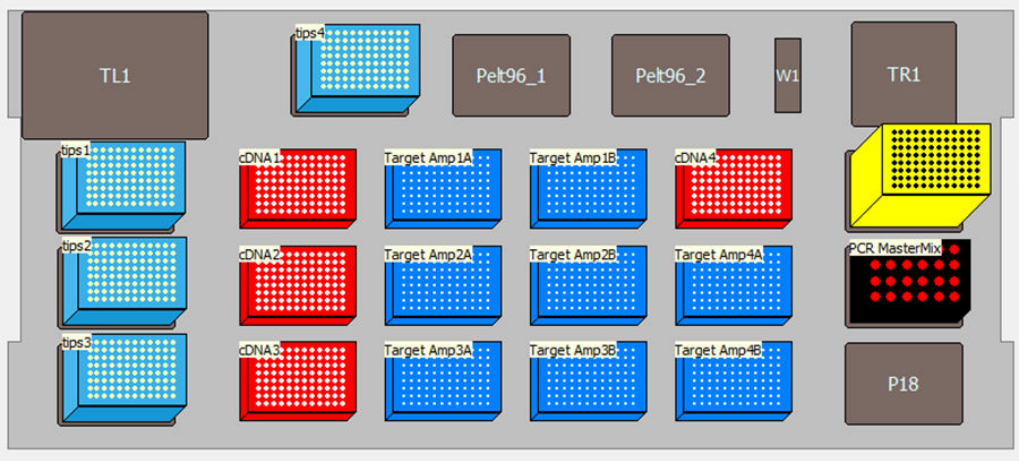
Sample plates	cDNA position	Reaction plate position	
		Pool 1	Pool 2
Set 1	P4	P7	P10
Set 2	P5	P8	P11
Set 3	P6	P9	P12
Set 4	P13	P14	P15

7. Place P20 tips at positions P1, P2, P3, and P19.

8. Place P1000 tips at positions P16.

9. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

10. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

11. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to “Amplify the targets” on page 25.

Set up DNA target amplification reactions

Set up DNA target amplification reactions using one of the following procedures, depending on the number of primer pools in your Ion AmpliSeq™ panel:

- For single-pool designs, see "Prepare DNA target amplification reactions – single primer pool panels" on page 22.
- For 2-pool designs, see "Prepare DNA target amplification reactions – 2 primer pool panels" on page 23.

If starting from RNA samples, see "Set up cDNA target amplification reactions" on page 17.

Prepare DNA target amplification reactions – single primer pool panels

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **DNA_Amplification** from the appropriate folder.

2. Prepare the PCR Master Mix in an appropriate container and aliquot into four 2-mL tubes as described in the following table.

# of Sample plates *(# of samples)*	5X Ion AmpliSeq™ HiFi Mix	2X Primer Mix	Total master mix	Volume per 2-mL tube
1 *(96 samples)*	498 µL	1,246 µL	1,744 µL	416 µL
2 *(192 samples)*	882 µL	2,206 µL	3,088 µL	752 µL
3 *(288 samples)*	1,266 µL	3,166 µL	4,432 µL	1,088 µL
4 *(384 samples)*	1,650 µL	4,126 µL	5,766 µL	1,424 µL

3. Place the 4 tubes into column 1 of a Beckman Coulter™ 24-Position Tube Rack, then place the rack at position P17.

4. Place extracted DNA samples (2–100 ng in 7 µL) and reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

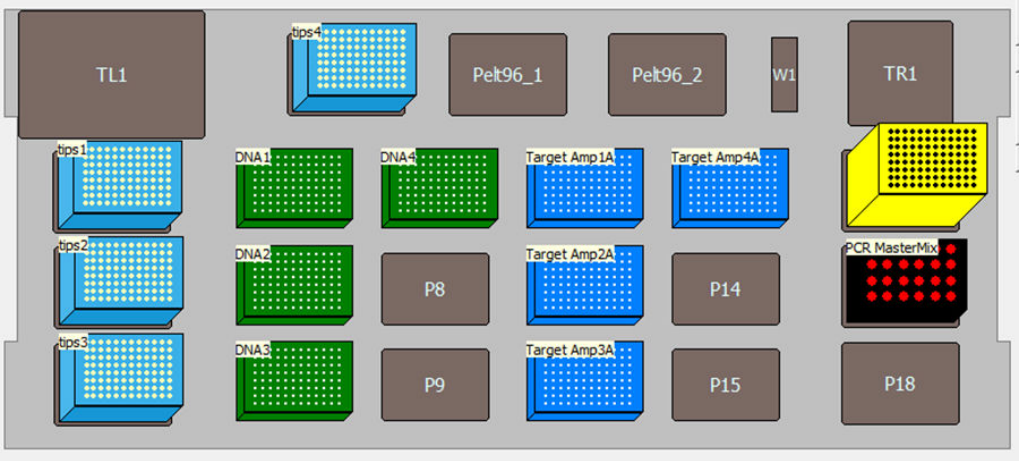
Sample plates	DNA position	Reaction plate position
Set 1	P4	P10
Set 2	P5	P11
Set 3	P6	P12
Set 4	P7	P13

5. Place P20 tips at positions P1, P2, P3, and P19.

6. Place P1000 tips position P16.

7. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

8. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

9. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to "Amplify the targets" on page 25.

Prepare DNA target amplification reactions – 2 primer pool panels

1. In the Biomek™ Software, click **File** ▸ **Open**, then in the **Open Method** window, select **DNA_Amplification** from the appropriate folder.

2. Prepare the PCR Master Mix in an appropriate container and aliquot into four 2-mL tubes as described in the following table.

# of Sample plates *(# of samples)*	5X Ion AmpliSeq™ HiFi Mix	2X Primer Mix	Total master mix	Volume per 2-mL tube
1 *(96 samples)*	306 µL	766 µL	1,702 µL	248 µL
2 *(192 samples)*	498 µL	1,246 µL	1,744 µL	416 µL
3 *(288 samples)*	690 µL	1,726 µL	2,416 µL	584 µL
4 *(384 samples)*	882 µL	2,206 µL	3,088 µL	752 µL

3. Place the 4 tubes of Pool 1 Master Mix into column 1 of a Beckman Coulter™ 24-Position Tube Rack.

4. Place the 4 tubes of Pool 2 Master Mix into column 2 of a Beckman Coulter™ 24-Position Tube Rack.

5. Place the Beckman Coulter™ 24-Position Tube Rack containing the master mixes at position P17.

6. Place extracted DNA samples (2–100 ng in 7 µL) and reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

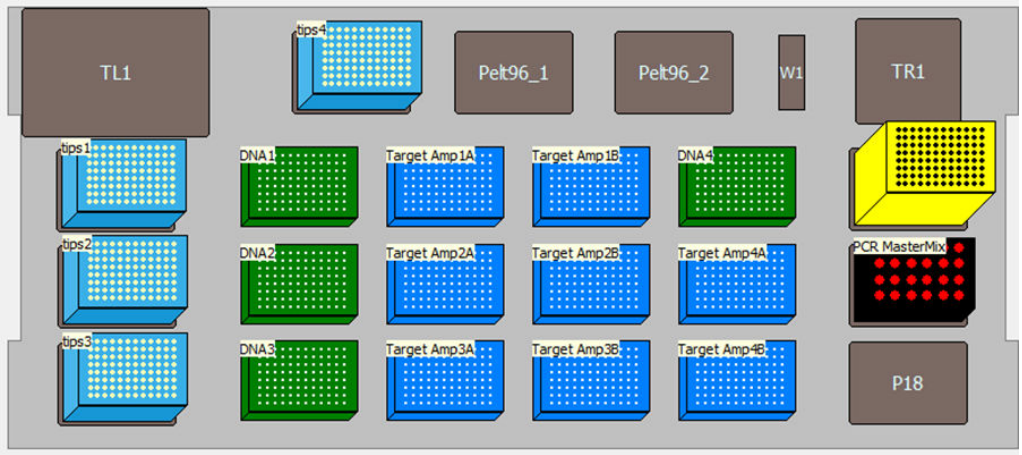
Sample plates	DNA position	Reaction plate position	
		Pool 1	Pool 2
Set 1	P4	P7	P10
Set 2	P5	P8	P11
Set 3	P6	P9	P12
Set 4	P13	P14	P15

7. Place P20 tips at positions P1, P2, P3, and P19.

8. Place P1000 tips at positions P16.

9. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution** ▸ **Run**.

10. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

11. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to “Amplify the targets” on page 25.

Amplify the targets

Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to amplify target regions.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 min
Cycle (see Table 4 for recommended number of amplification cycles)	Denature	99°C	15 sec
	Anneal and extend	60°C	4 min/8 min/16 min[1]
Hold	—	10°C	Hold

[1] 4 minutes for ≤1536 primer pairs per pool; 8 minutes for 1,537–6,144; 16 minutes for 6,145–24,576.

Table 4 Recommended number of amplification cycles

Note: Cycle numbers can be increased when input material quality or quantity is questionable. The cycle number does not need to be adjusted when using the Ion AmpliSeq™ Sample ID Panel.

Primer pairs per pool	Recommended number of amplification cycles	
	Normal DNA/RNA	FFPE DNA/RNA
gene fusion	27	30
12–24	21	24
25–48	20	23
49–96	19	22
97–192	18	21
193–384	17	20
385–768	16	19
769–1,536	15	18
1,537–3,072	14	17
3,073–6,144	13	16

Table 4 Recommended number of amplification cycles *(continued)*

Primer pairs per pool	Recommended number of amplification cycles	
	Normal DNA/RNA	FFPE DNA/RNA
6,145–12,288	12	15
12,289–24,576	11	14

Cycle number recommendations in the preceding table are based on 10-ng DNA input. Adjust cycle number from the preceding table for lower or higher DNA input:

Amount of DNA starting material	Adjustment to cycle number
1 ng (300 copies)	+3
10 ng (3,000 copies)	0
100 ng (30,000 copies)	–3

Note:

- Cycle number can be increased when input material quality or quantity is questionable.
- If two primer pools for a single panel fall into different cycling categories, use the greater number of cycles.
- When amplifying multiple samples in a single PCR plate, ensure that the input across the samples is roughly equivalent so that the selected cycle number for target amplification is optimal for all the samples in the run.

STOPPING POINT PCR products can be stored at 10°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

Partially digest amplicons

- For single-pool libraries, see “Set up partial digestion reactions – single primer pool libraries” on page 26.
- For 2-pool libraries, see “Set up partial digestion reactions – 2 primer pool libraries” on page 27.

Set up partial digestion reactions – single primer pool libraries

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Fupa_Digestion** from the appropriate folder.
2. Centrifuge the FuPa Reagent plate at 500–1,000×g for 30–60 seconds, then place the plate at Pelt96_1.

   IMPORTANT! Make sure that the fill volume is sufficient for the number of plates being processed plus 5 µL overage per well to account for dead volume.

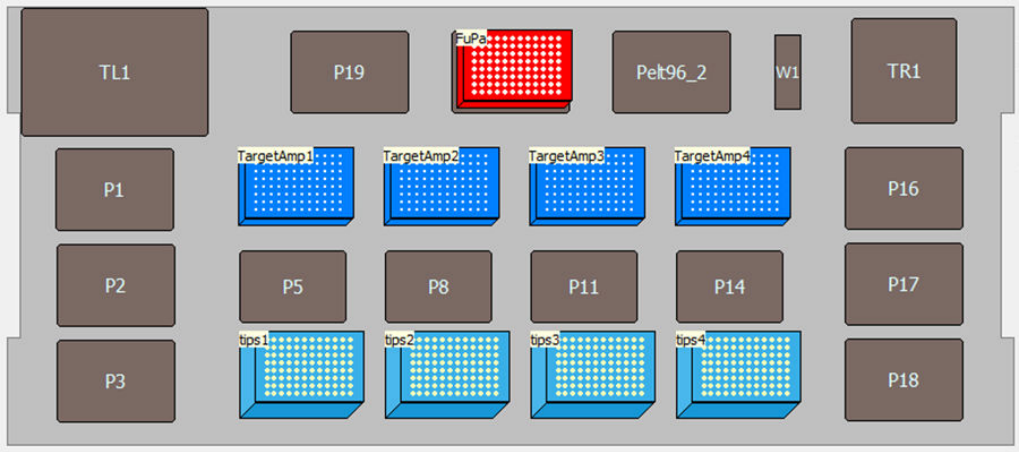
3. Place the target amplification reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

Target amplification reaction plates	Position
Set 1	P4
Set 2	P7
Set 3	P10
Set 4	P13

4. Place P20 tips at positions P6, P9, P12, and P15.

5. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

6. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

7. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to "Partially digest amplicons" on page 29.

Set up partial digestion reactions – 2 primer pool libraries

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Fupa_Digestion** from the appropriate folder.

2. Centrifuge the FuPa Reagent plate at 500–1,000×g for 30–60 seconds, then place the plate at Pelt96_1.

IMPORTANT! Make sure that the fill volume is sufficient for the number of plates being processed plus 5 µL overage per well to account for dead volume.

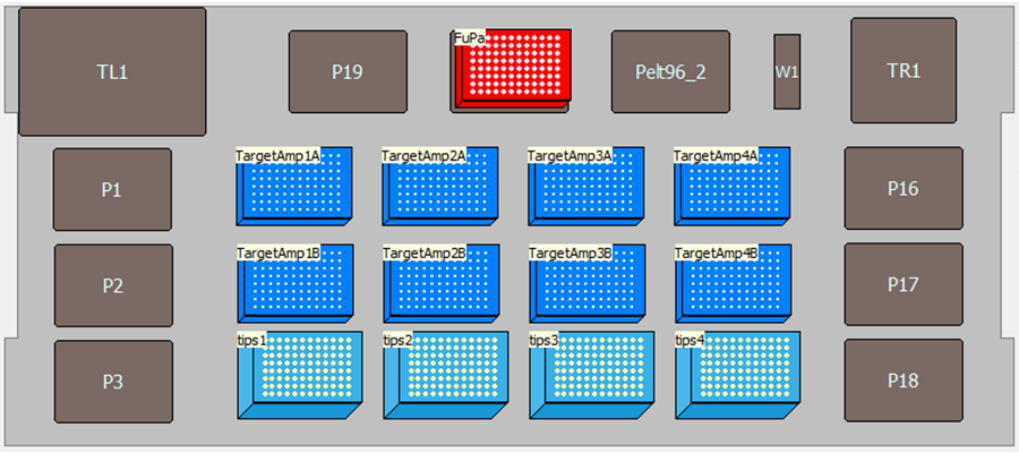
3. Place the target amplification reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

Target amplification reaction plates	Position	
	Pool 1	Pool 2
Set 1	P4	P5
Set 2	P7	P7
Set 3	P10	P11
Set 4	P13	P14

4. Place P20 tips at positions P6, P9, P12, and P15.

5. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution** ▸ **Run**.

6. Confirm the deck setup on the instrument matches the display and follow any prompts.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

7. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to “Partially digest amplicons” on page 29.

Partially digest amplicons

Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Temperature	Time
50°C	10 min
55°C	10 min
60°C	20 min
10°C	Hold (for up to 1 hour)

Ligate adapters to amplicons

When sequencing multiple libraries on a single chip, you *must* ligate a different barcode adapter to each library. DNA and RNA libraries from the same sample also require different barcodes.

Ion Torrent™ Dual Barcode Adapters are provided at the appropriate concentration. No further handling is necessary. If using Ion Torrent™ Dual Barcode Adapters, proceed to "Set up ligation reactions" on page 31.

IonCode™ Barcode Adapters are provided at the appropriate concentration and include forward and reverse adapters in a single well. No further handling is necessary. If using IonCode™ Barcode Adapters, proceed to "Set up ligation reactions" on page 31.

Ion Xpress™ Barcode Adapters require handling and dilution as described in "(Ion Xpress™ Barcode Adapters only) Combine and dilute adapters" on page 30.

(Ion Xpress™ Barcode Adapters only) Combine and dilute adapters

For each barcode X selected, prepare a mix of Ion P1 Adapter and Ion Xpress™ Barcode X at a final dilution of 1:4 for each adapter in a MicroAmp™ EnduraPlate™ 96-well plate.

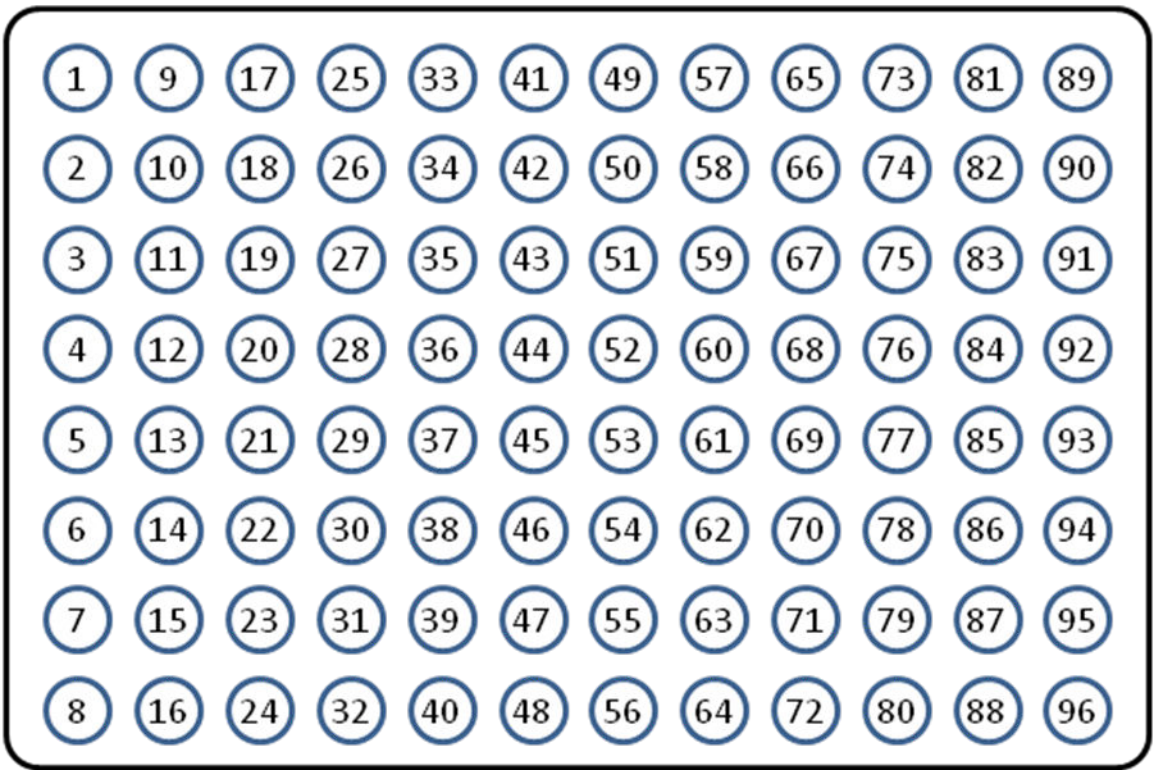
Table 5 Example barcode adapter mix for up to 40 reactions

Component	Volume
Ion P1 Adapter	20 µL
Ion Xpress™ Barcode X[1]	20 µL
Nuclease-Free Water	40 µL
Total	**80 µL**

[1] X = Barcode chosen

IMPORTANT!

- When handling barcoded adapters, be careful to avoid cross-contamination by changing gloves frequently and opening one tube at a time.
- Barcodes *must* be arrayed in the following pattern, with barcode number matching position number on the plate:



Set up ligation reactions

1. In the Biomek™ Software, click **File** ▸ **Open**, then in the **Open Method** window, select **Ligation** from the appropriate folder.

2. Centrifuge the DNA Ligase and Switch Solution at 500–1,000×g for 30–60 seconds, then place the plate at Pelt96_1.

   IMPORTANT! Make sure that the fill volume is sufficient for the number of plates being processed plus 5 µL overage per well to account for dead volume.

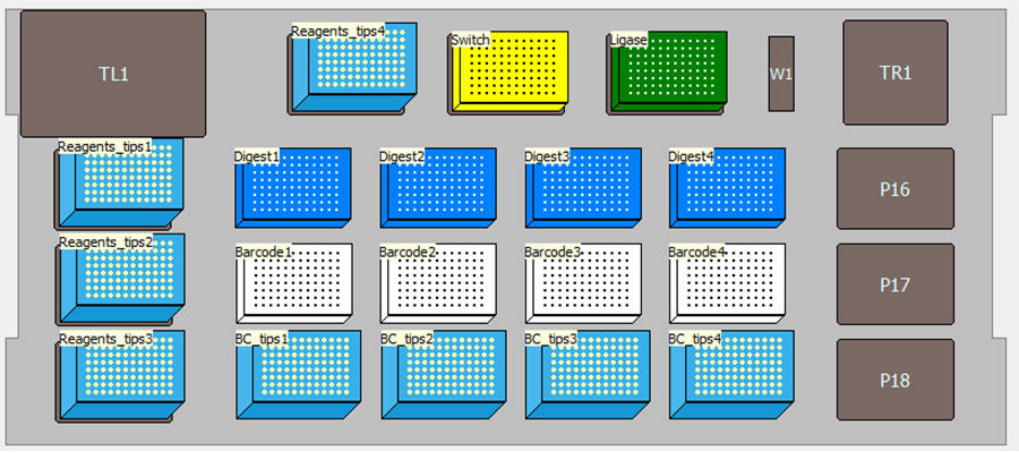
3. Place digested amplicon reaction plates on MicroAmp™ Splash-Free 96-Well Bases, at positions listed in the following table.

Digested amplicon plate	Barcode plate
P4	P5
P7	P8
P10	P11
P13	P14

4. Place P20 tips at positions P1, P2, P3, P6, P9, P12, P15, and P19.

5. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution** ▸ **Run**.

6. Confirm the deck setup on the instrument matches the display and follow any prompts.



   The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

7. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to "Ligate adapters" on page 32.

Ligate adapters

Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Temperature	Time
22°C	30 min[1]
68°C	5 min
72°C	5 min
10°C	Hold (for up to 1 hour)

[1] For libraries from FFPE samples, 60 min may increase yield.

STOPPING POINT Samples can be stored at –20°C.

Proceed to one of the following methods.

- "Option 1: Equalize libraries" on page 32
- "Option 2: Purify and quantify the unamplified library by qPCR" on page 37

If you are not planning to use the prepared libraries immediately, proceed to "Store libraries" on page 39.

Option 1: Equalize libraries

IMPORTANT! We recommend using the Ion Library Equalizer™ Kit when library yield is consistently above the minimum concentration outlined in this user bulletin. In cases where sample input is highly variable or unknown (such as RNA libraries), we recommend using the qPCR method (see "Option 2: Purify and quantify the unamplified library by qPCR" on page 37).

Before you begin

Warm all the reagents in the Ion Library Equalizer™ Kit to room temperature. Vortex and centrifuge all reagents before use.

Purify libraries

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Purify_and_Amplify** from the appropriate folder.
2. Mix the Agencourt™ AMPure™ XP Reagent Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate by inversion, then briefly centrifuge to pull down any droplets.
3. Ensure that the Agencourt™ AMPure™ XP Reagent Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate contains at least 175 µL per well, then place at position P1.
4. Place a clean tip box lid at position P2, then add at least 150 mL of 70% Ethanol.

5. Place an empty Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate at position P19.

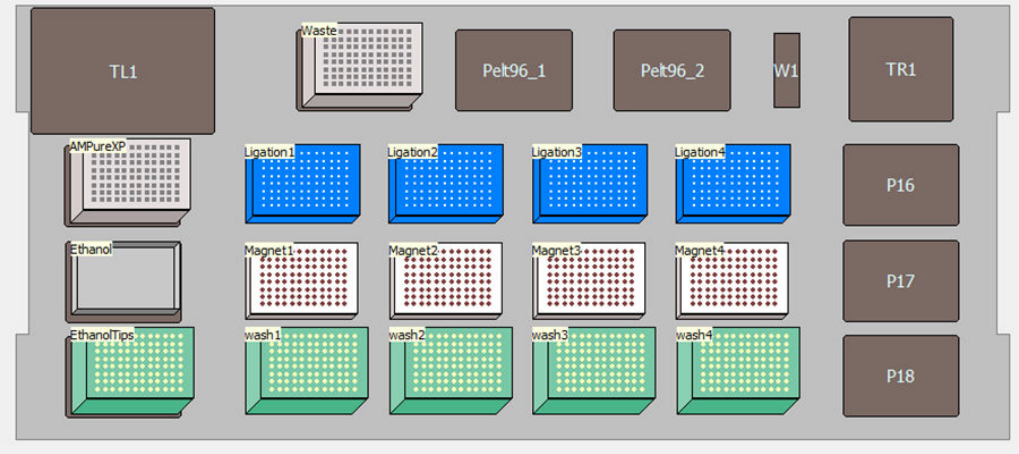
6. Place ligated library plates on a MicroAmp™ Splash-Free 96-Well Bases at positions P4, P7, P10, and P13.

7. Place an Alpaqua™ 96R Ring Magnet Plate at positions P5, P8, P11, and P14.

8. Place P250 tips at positions P3, P6, P9, P12, and P15.

9. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

10. Confirm the deck setup on the instrument matches the display and follow any prompts.



After ~35 minutes, a dialog appears, prompting the exchange of tips.

IMPORTANT! Do **NOT** remove or move reaction plates or magnets.

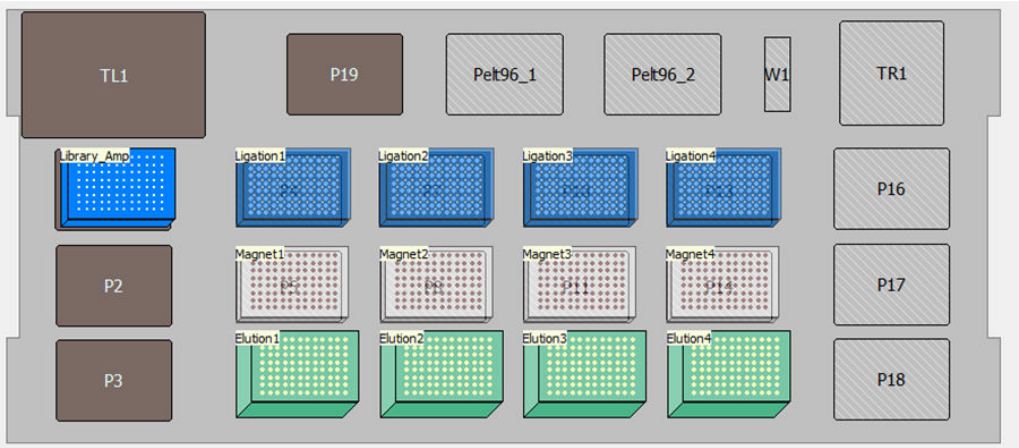
11. Remove and discard all tip boxes, waste plate (at position P19), and Ethanol (at position P2).

12. Remove the AMPure™ plate, seal it, then store at 4°C.

13. Ensure that Library Amp master plate, which contains the 1X Ion AmpliSeq™ HiFi Mix and Equalizer™ Primers, has at least 220 µL per well, then place the plate on a MicroAmp™ Splash-Free 96-Well Base at position P1.

14. Place P250 tips at positions P6, P9, P12, and P15.

15. Confirm the deck setup on the instrument to complete the script.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

16. Follow the prompts. When the script is complete, seal the plate with a MicroAmp™ Clear Adhesive Film, then centrifuge to collect the droplets.

Proceed immediately to “Amplify libraries”.

Amplify libraries

Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Stage	Temperature	Time
Hold	98°C	2 min
7 cycles	98°C	15 sec
	64°C	1 min
Hold	10°C	Hold (for up to 1 hour)

If necessary, wash the Equalizer™ Beads while cycling (see “Wash the Equalizer™ beads”).

Wash the Equalizer™ beads

If you have not previously done so, wash the Equalizer™ beads.

1. Bring the Equalizer™ Beads to room temperature and mix thoroughly.

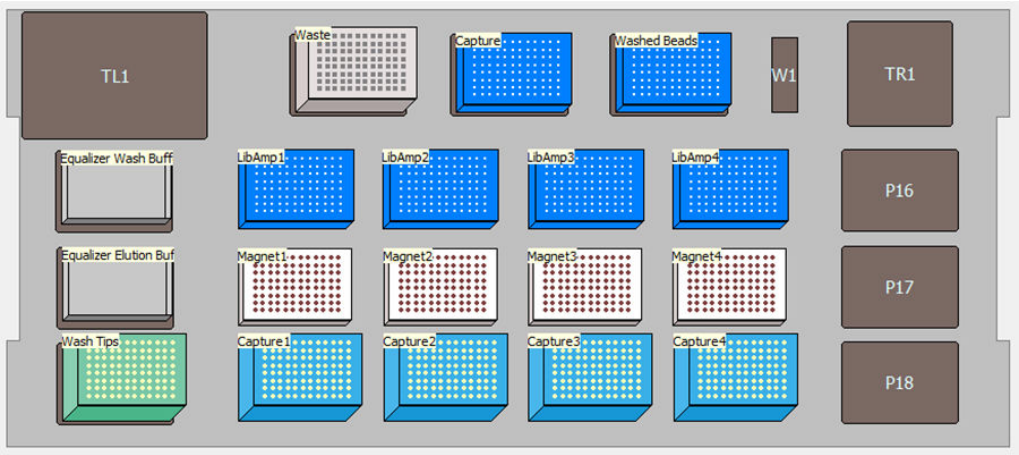
Note: Beads for multiple reactions can be prepared in bulk and can be stored in the Equalizer™ Wash Buffer at 4°C for up to 6 months until use. After 6 months, re-washed the beads with an equal volume of Equalizer™ Wash Buffer.

2. For each reaction, pipet 3 µL of beads into a clean 1.5–mL tube, then add 6 µL of Equalizer™ Wash Buffer to the beads.

3. Place the tube in a magnetic rack for 3 minutes or until the solution clears completely.
4. Carefully remove and discard the supernatant without disturbing the pellet.
5. Remove the tube or plate from the magnet, add 6 µL of Equalizer™ Wash Buffer to each reaction, then pipet up and down to resuspend.

Equalize libraries

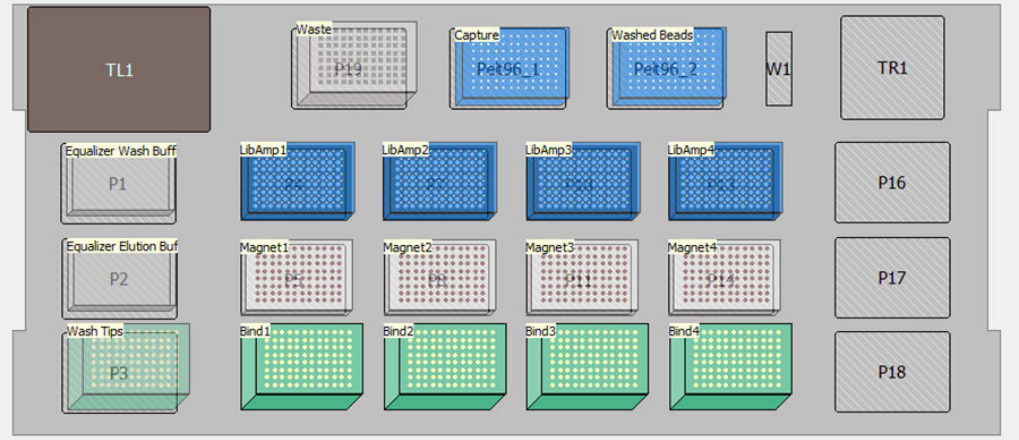
1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Equalizer** from the appropriate folder.
2. Ensure that the Equalizer™ Capture reagent MicroAmp™ EnduraPlate™ contains at least 45 µL per well, then place the plate at position Pelt96_1.
3. Ensure that the Washed Equalizer™ Beads MicroAmp™ EnduraPlate™ contains at least 30 µL per well, then place the plate at position Pelt96_2.
4. Place a clean tip box lid at position P1, then add at least 170 mL of Equalizer™ Wash Buffer.
5. Place a clean tip box lid at position P2, then add at least 80 mL of Equalizer™ Elution Buffer.
6. Place plates that contain the amplified sample (from "Purify libraries" on page 32) on a MicroAmp™ Splash-Free 96-Well Base at position P4, P7, P10, and P13.
7. Place an empty Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate plate at position P19.
8. Place an Alpaqua™ 96R Ring Magnet Plate at positions P5, P8, P11, and P14.
9. Place P20 tips at positions P6, P9, P12, and P15.
10. Place P250 tips at position P3.
11. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.
12. Confirm the deck setup on the instrument matches the display and follow any prompts.



After ~13 minutes, a dialog appears, prompting the exchange of tips.

IMPORTANT! Do **NOT** remove or move reaction plates or magnets.

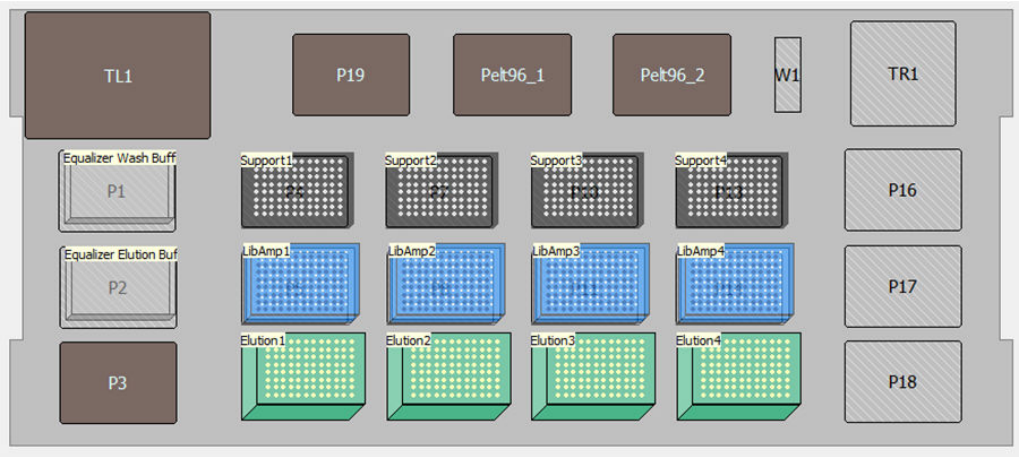
13. Remove and discard all P20 tip boxes.
14. Place P250 tips at positions P6, P9, P12, and P15.
15. Confirm the deck setup on the instrument to continue the script.



After ~26 minutes, a dialog appears, prompting the exchange of tips.

IMPORTANT! Do **NOT** remove or move reaction plates or magnets.

16. Remove and discard all tip boxes, waste plate (at position P19).
17. Remove the Equalizer™ Capture MicroAmp™ EnduraPlate™ and Washed Equalizer™ Beads MicroAmp™ EnduraPlate™, seal the plates, then store at 4°C.
18. Place P250 tips at positions P6, P9, P12, and P15.
19. Confirm the deck setup on the instrument to complete the script.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

Follow the prompts, then proceed to template preparation, or combine and store libraries (see "(Optional) Combine amplicon libraries" on page 39 and "Store libraries" on page 39).

Option 2: Purify and quantify the unamplified library by qPCR

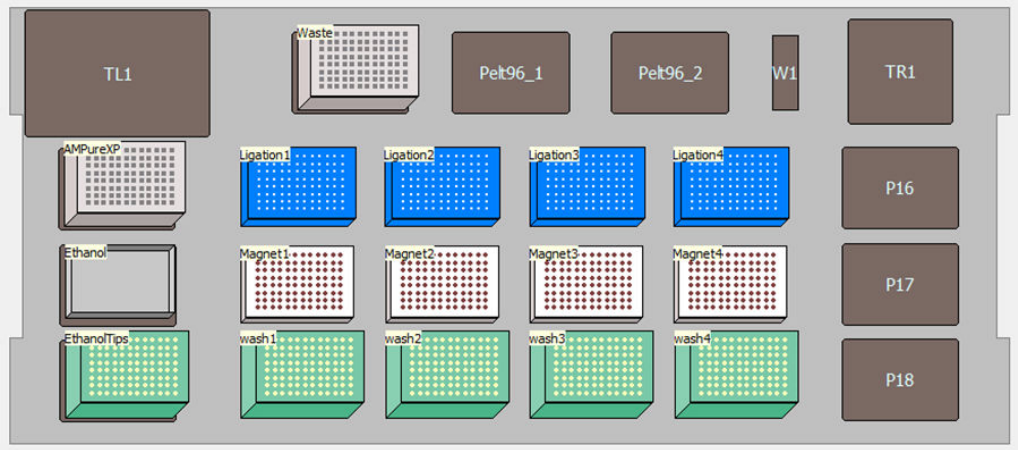
Purify and elute the unamplified Ion AmpliSeq™ library, then determine the concentration by qPCR using the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is suitable for template preparation using an Ion Torrent™ template preparation kit.

Note: The Ion Library TaqMan™ Quantitation Kit can also be used to quantify libraries that have been amplified using the procedure that is described in "Option 1: Equalize libraries" on page 32.

Purify libraries for qPCR quantification

1. In the Biomek™ Software, click **File ▸ Open**, then in the **Open Method** window, select **Purify_and_Elute** from the appropriate folder.
2. Mix the Agencourt™ AMPure™ XP Reagent Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate by inversion, then briefly centrifuge to pull down any droplets.
3. Place a clean tip box lid at position P2, then add at least 175 mL of 70% Ethanol.
4. Place an empty Corning™ 96-well Clear V-Bottom 2 mL Polypropylene Deep Well Plate at position P19.
5. Place ligated library plates on a MicroAmp™ Splash-Free 96-Well Bases at positions P4, P7, P10, and P13.
6. Place an Alpaqua™ 96R Ring Magnet Plate at positions P5, P8, P11, and P14.
7. Place P250 tips at positions P3, P6, P9, P12, and P15.
8. In the Biomek™ Software, click ▶ **(Run)** to start the method run, or click **Execution ▸ Run**.

9. Confirm the deck setup on the instrument matches the display and follow any prompts.



After ~35 minutes, a dialog appears, prompting the exchange of tips.

IMPORTANT! Do **NOT** remove or move reaction plates or magnets.

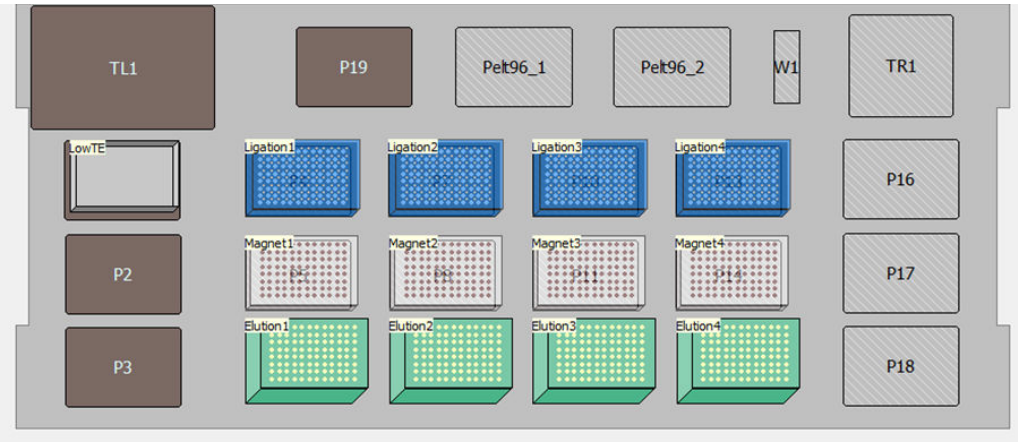
10. Remove and discard all tip boxes, waste plate (at position P19), and Ethanol (at position P2).

11. Remove the AMPure™ plate, seal it, then store at 4°C.

12. Place a clean tip box lid at position P1, then add at least 70 mL of Low TE.

13. Place P250 tips at positions P6, P9, P12, and P15.

14. Confirm the deck setup on the instrument to complete the script.



The method runs as soon as the deck setup is confirmed. The method run can be visually followed in the **Method View**; steps are highlighted in green as they are executed.

15. Follow the prompts to complete the method.

Prepare a 100-fold dilution of the library for quantification by mixing 2 µL of supernatant with 198 µL of Nuclease-Free Water, then proceed to "*Quantify library by qPCR and calculate the dilution factor*" in the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735) or *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003).

(Optional) Combine amplicon libraries

Multiple strategies for combining Ion AmpliSeq™ libraries are available. See the *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735) and *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003).

Store libraries

Libraries may be stored at 4–8°C for up to 1 month. For longer term storage, store at -20°C.

Documentation and support

Related documentation

Document	Publication number
Ion AmpliSeq™ Library Kit 2.0 User Guide	MAN0006735
Ion AmpliSeq™ Library Kit Plus User Guide	MAN0017003
Biomek FX and FXP Lab Auto Workstations User's Manual	B30026AB (**Beckman.com/search/techdocs**)
Biomek 4.1 Software User's Manual	987834.AF (**Beckman.com/search/techdocs**)

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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