I. Workflow Overview

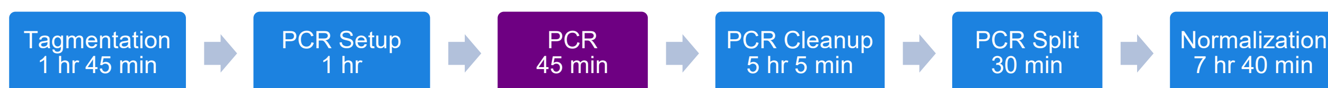


Figure 1. Workflow overview for NGS library preparation on the Apollo system with the Nextera XT library prep kit. Blue and purple boxes indicate steps performed on and off the Apollo system, respectively. Indicated times are estimates for completion of each protocol, and include reagent and equipment setup, heating/cooling/thawing of reagents and hardware, incubation of reactions, and automated liquid-handling processes.

II. Materials Required

Input cDNA

This protocol is designed for preparation of Illumina® NGS libraries on the Apollo system using purified cDNA generated with the SMART-Seq® v4 Ultra® Low Input RNA Kit for Sequencing as input material.

Nextera XT DNA Library Preparation Kit and Nextera XT Index Kit

This protocol is intended for high-throughput preparation of 96 NGS libraries in parallel, which requires the 96-reaction size of the Nextera XT DNA Library Preparation Kit (Illumina, Cat. No. FC-131-1096). Illumina offers many Nextera XT Index Kits. Please refer to the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942) for index primer selection options and criteria.

Apollo Consumables

The protocol and script described in this protocol-at-a-glance have been validated using the following consumables. **Please do not make any substitutions.**

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	768 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	72 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	48 strips
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	2 reservoirs
Apollo 1.1 mL MiniTubes	Takara Bio	640088	Box of 960 minitubes	40 minitubes
Hard-Shell Plate	Bio-Rad	HSP-9601	Box of 50 plates	9 plates

Additional Materials Required

- Single-channel pipette: 10 µl, 20 µl, 200 µl, and 1,000 µl
- Eight-channel pipette (recommended): 20 µl and 200 µl
- Filter pipette tips: 2 µl, 20 µl, 200 µl, and 1,000 µl
- Minicentrifuge for 1.5-ml tubes
- Minicentrifuge for 0.2-ml tubes or strips or low-speed benchtop centrifuge for 0.2-ml strips (recommended; e.g., Eppendorf 5804 R, Cat. No. 5805000017)
- PCR thermal cycler
- Nuclease-free low-adhesion 1.5-ml tubes (USA Scientific, Item No. 1415-2600) or LoBind tubes (Eppendorf, Cat. No. 022431021)
- Agencourt AMPure XP PCR purification kit (60 ml; Beckman Coulter, Item No. A63881)
  - Use this kit for purification of the amplified DNA.

NOTE: Agencourt AMPure XP beads need to come to room temperature before the container is opened. Therefore, we strongly recommend preparing 10-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 chances of bead contamination. Mix well to disperse the beads before adding them to your reactions. The beads are viscous, so pipette slowly.

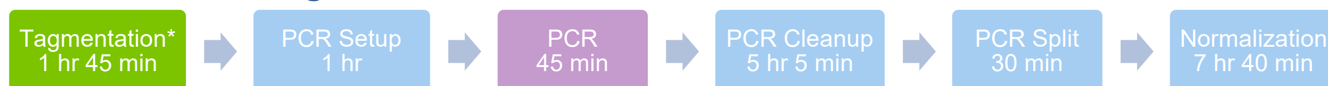
- 100% ethanol (molecular biology grade)
- Nuclease-free water (molecular biology grade)
- NaOH (1 N, pH >12.5, molecular biology grade)

III. Apollo System Best Practices

- Clean the work surfaces, including the retention plates, with 70% ethanol at least once a week.
- Restart the instrument before every run.
- 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.

IV. Protocols

A. Protocol: Tagmentation



*For each protocol, the corresponding step in the workflow diagram is indicated in green.

In this step, input cDNA is tagmented (tagged and fragmented) by the Nextera XT transposome.

Materials Required

Reagents	Storage conditions	Source
Sample (purified cDNA)	–20°C	User
ATM (Amplicon Tagment Mix)	–20°C	Nextera XT DNA Library Preparation Kit
TD (Tagment DNA Buffer)	–20°C	
NT (Neutralize Tagment Buffer)	–20°C	

Consumables	Source	Cat. No.	Quantity	Usage/run
Apollo Filter Tips	Takara Bio	640084	Box of 960	192 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125	24 strips
Hard-Shell Plate	Bio-Rad	HSP-9601	Box of 50	2 plates

1. Before you begin, check that:
   - All work surfaces have been cleaned with 70% ethanol within the past week.
   - The instrument has been restarted during the current work day.
   - Any deformed plastics are discarded.
2. Prechill Apollo blocks:
   - On the touchscreen, press **Maintenance** and then press **User Maintenance**.
   - Select the following subscript: `SetTemp10C_v1-1.scb`.
   - Proceed to Step 3 (below) while the instrument is chilling. Chilling takes about 2 minutes.

3. Prepare reagents as indicated in the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942):
   a. Thaw ATM and TD buffer on ice.
   b. In preparation for the PCR Setup protocol (Section IV.B, below), thaw NPM and Index 1 and 2 primers at room temperature. Allow approximately 20 minutes for thawing.
   c. Make sure that the NT buffer is at room temperature. Visually inspect the NT buffer to make sure that there is no precipitate. If there is a precipitate, vortex until all particulates are resuspended.
   d. After thawing, gently invert the tubes 3–5 times to make sure all reagents are adequately mixed.
4. Load consumables onto the work surface and aliquot reagents, following the layout in Figure 2 (next page):

Component	Consumable	Volume
Sample (100–150 pg of cDNA)	Hard-Shell Plate, Rows 1–12	5 µl per well
ATM (Amplicon Tagment Mix)	Apollo 0.2 ml PCR 8-Tube Strips, Clear	70 µl per tube (12 reactions per tube)
TD (Tagment DNA Buffer)	Hard-Shell Plate, Row 1	130 µl per well (12 reactions per well)
NT (Neutralize Tagment Buffer)	Hard-Shell Plate, Row 2	70 µl per well (12 reactions per well)

NOTES:
- The Apollo system is specifically calibrated for the consumables indicated in the table above. Using alternative consumables may cause the run to fail.
- Ensure that there are no air bubbles. Keep ATM and TD on ice and NT at room temperature.
- Do not place reagents on the Apollo deck until you are ready to start the run.

5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box.
   NOTE: An accumulation of tips in the waste box may cause the run to fail.
7. Set up the run.
   a. From the chill-down run in Step 2, press **OK** to return to the **User Maintenance** menu.
   b. Select the following subscript: `NXTforSSv4_1_Tagmentation_v1.scb`
8. Before you begin the run, check that:
   - No overhangs from partial strips are present.
   - Reagents were spun down to remove bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
   - Plastic consumables are properly seated on the deck surface with caps/lids removed.
9. Start the tagmentation run.
   a. Close the instrument door.
   b. Press **Run**. The run time is 1 hour, 11 minutes.
10. When the run is complete, discard the consumables in Blocks 1, 2, and 3.
11. From the touchscreen, press **OK** to return to the **User Maintenance** menu. Do not leave the **User Maintenance** menu or turn off the instrument.
12. Proceed immediately to the PCR Setup protocol (Section IV.B, below).
    NOTE: This is **NOT** a safe stopping point to store the tagmented DNA. Proceed immediately to PCR Setup.

Tagmentation

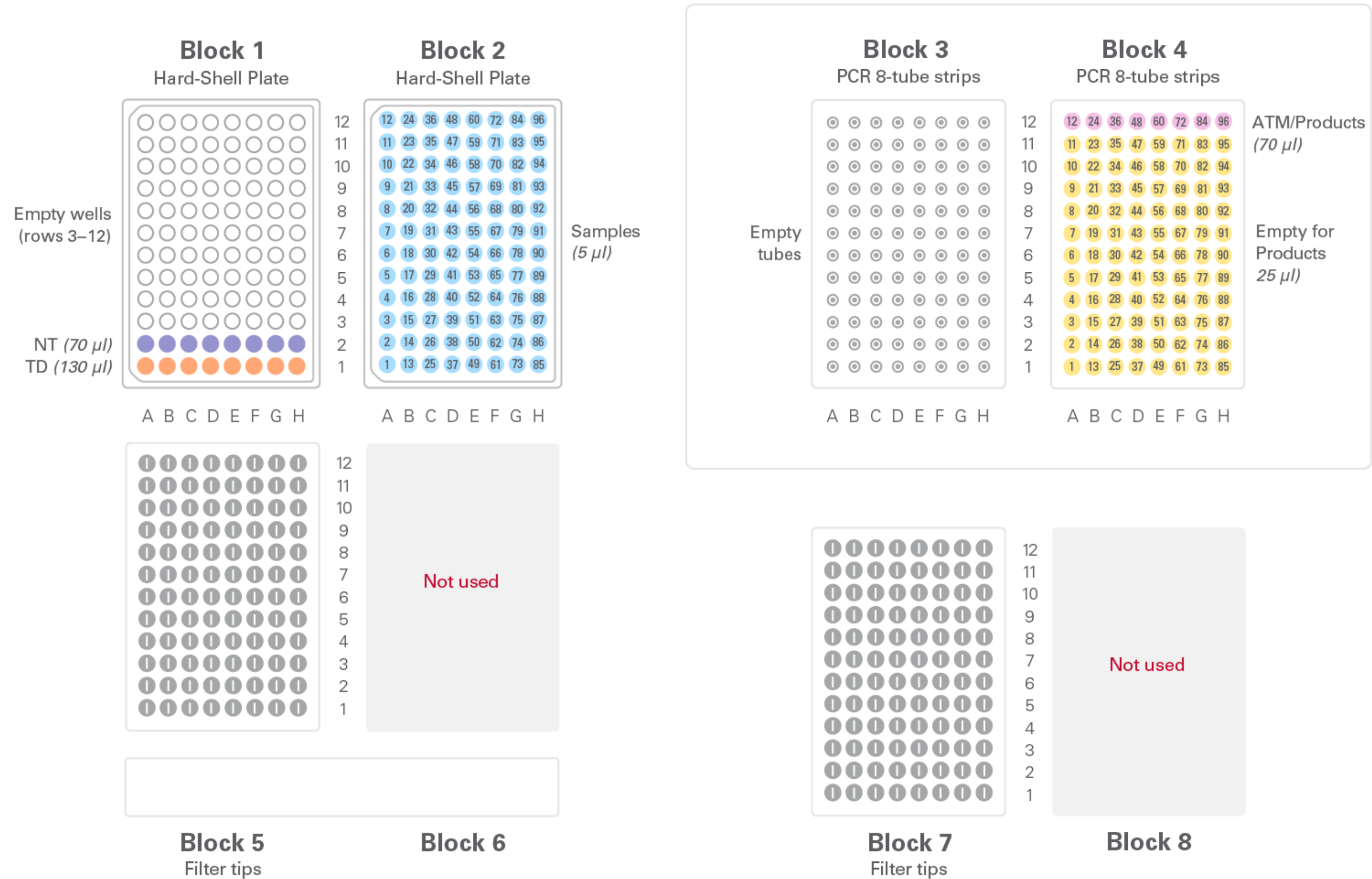
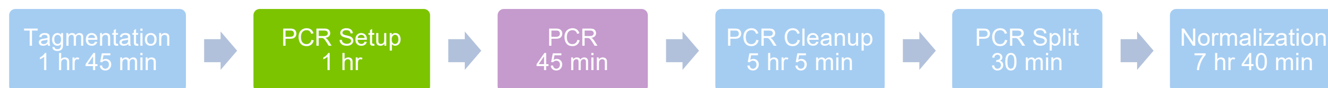


Figure 2. Loading layout for tagmentation.

B. Protocol: PCR Setup



In this step, tagmented DNA is prepared for amplification.

Materials Required

Reagents	Storage conditions	Source
Tagmented DNA	4°C	Section IV.A
NPM (Nextera PCR Master Mix)	–20°C	Nextera XT DNA Library Preparation Kit
Index 1 primers (N7XX)	–20°C	Nextera XT Index Kit
Index 2 primers (S5YY)	–20°C	

Consumables	Source	Cat. No.	Quantity	Usage/run
Apollo Filter Tips	Takara Bio	640084	Box of 960	96 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125	12 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125	12 strips
Hard-Shell Plate	Bio-Rad	HSP-9601	Box of 50	1 plate

1. Before you begin, check that:
   - All work surfaces have been cleaned with 70% ethanol within the past week.
   - The instrument has been restarted during the current work day.
   - Any deformed plastics are discarded.
2. Prechill Apollo blocks if the instrument was turned off after the last subscript. The blocks should be chilled as follows:
   a. On the touchscreen, press **Maintenance** and then press **User Maintenance**.
   b. Select the following subscript: `SetTemp10C_v1-1.scb`.
   c. Proceed to Step 3 (below) while the instrument is chilling. Chilling takes about 2 minutes.
3. Prepare reagents as indicated in the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942):
   a. Thaw NPM and Index 1 and 2 primers at room temperature (if not already thawed during the previous protocol). Allow approximately 20 minutes for thawing.
   b. After thawing, gently invert the tubes 3–5 times to make sure all reagents are adequately mixed and briefly centrifuge the tubes in a microcentrifuge.

4. Load consumables onto the work surface and aliquot reagents, following the layout in Figure 3 (next page):

Component	Consumable	Volume
Tagmented DNA	Apollo 0.2 ml PCR 8-Tube Strips, Clear	25 µl per tube
Index 1 primers (N7XX)	Apollo 0.2 ml PCR 8-Tube Strips, Clear	5 µl per tube (1 reaction per tube)
Index 2 primers (S5XX)	Hard-Shell Plate, Rows 1–2	34 µl per well (6 reactions per well)
NPM (Nextera PCR Master Mix)	Hard-Shell Plate, Rows 3–4	102 µl per well (6 reactions per well)

NOTES:

- The Apollo system is specifically calibrated for the consumables indicated in the table above. Using alternative consumables may cause the run to fail.
- Ensure that there are no air bubbles.
- Do not place reagents on the Apollo deck until you are ready to start the run.

5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box.

   NOTE: An accumulation of tips in the waste box may cause the run to fail.
7. Set up the run.
   a. From the chill-down run in Step 2, press **OK** to return to the **User Maintenance** menu.
   b. Select the following subscript `NXTforSSv4_2_PCRSetup_v1.scb`
8. Before you begin the run, check that:
   - No overhangs from partial strips are present.
   - Reagents were spun down to remove bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
   - Plastic consumables are properly seated on the deck surface with caps/lids removed.
9. Start the PCR setup run.
   a. Close the instrument door.
   b. Press **Run**. The run time is 28 minutes.
10. When the run is complete, remove the products from Block 3 and discard the consumables in Blocks 2 and 4.
11. Cap the tubes containing product and spin down briefly.
12. From the touchscreen, press **OK** to return to the **User Maintenance** menu. Do not leave the **User Maintenance** menu or turn off the instrument if you are continuing to the PCR Cleanup protocol after the PCR protocol.
13. Proceed immediately to PCR (Section IV.C, below) on a benchtop thermal cycler.

    NOTE: This is **NOT** a safe stopping point to store the DNA. Proceed immediately to PCR.

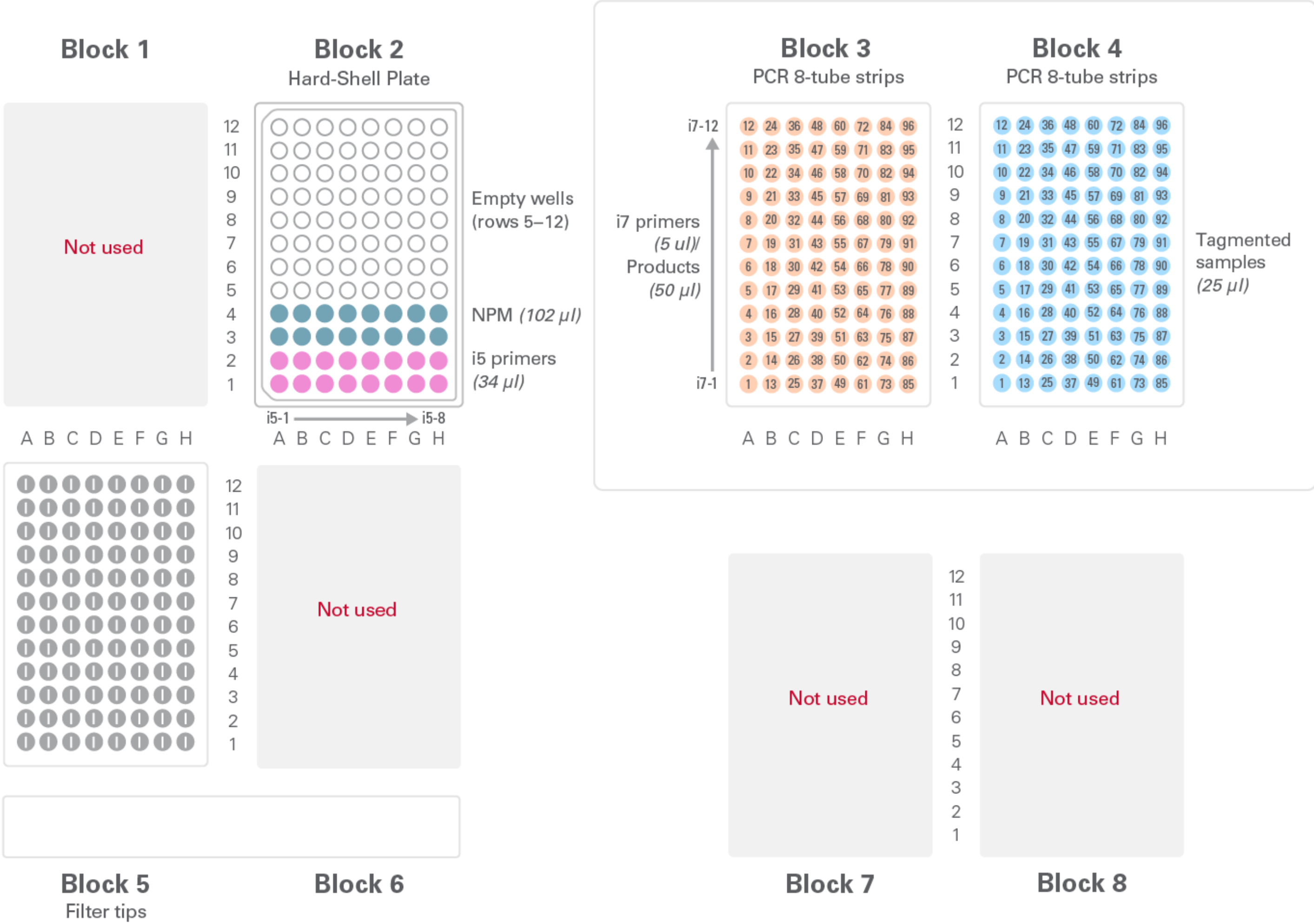


Figure 3. Loading layout for PCR setup. On Block 2, i5 primers vary by column (as indicated by gray horizontal arrow). On Block 3, i7 primers vary by row (indicated by gray vertical arrow).

C. Protocol: PCR

In this step, the tagmented DNA is amplified via a limited-cycle PCR program, resulting in incorporation of i7 and i5 index sequences and adapters required for cluster formation.

Materials Required

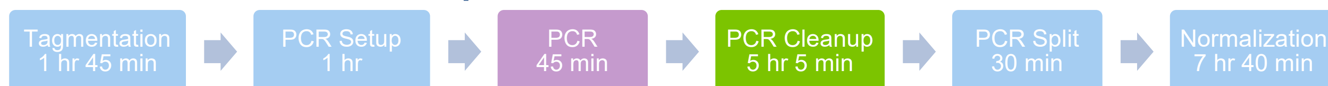
Reagents	Storage conditions	Source
Combined sample and PCR reagents	4°C	Section IV.B

1. Transfer Apollo PCR tubes containing PCR reactions prepared in the previous section (Section IV.B, above) to a preheated thermal cycler. Perform PCR using the following program:

   72°C 3 min
   95°C 30 sec
   12 cycles:
   95°C 10 sec
   55°C 30 sec
   72°C 30 sec
   72°C 5 min
   10°C forever

SAFE STOPPING POINT: If you do not plan to proceed immediately to PCR Cleanup (Section IV.D, below), samples can be left overnight in the thermal cycler or the sealed tubes can be stored at –20°C for up to one week.

D. Protocol: PCR Cleanup



In this step, DNA is purified and size-selected with AMPure XP beads, resulting in removal of short library fragments and primers.

Materials Required

Reagents	Storage conditions	Source
PCR products	10°C/–20°C	Section IV.C
RSB (Resuspension Buffer)	–20°C	Nextera XT DNA Library Preparation Kit
100% Ethanol	Room temperature	User
Nuclease-free water	Room temperature	

Consumables	Source	Cat. No.	Quantity	Usage/run
Apollo Filter Tips	Takara Bio	640084	Box of 960	192 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125	12 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125	12 strips
Apollo Reservoirs	Takara Bio	640087	Box 100	2 reservoirs
Hard-Shell Plate	Bio-Rad	HSP-9601	Box of 50	3 plates

1. Before you begin, check that:
   - All work surfaces have been cleaned with 70% ethanol within the past week.
   - The instrument has been restarted during the current work day.
   - Any deformed plastics are discarded.
2. Prepare reagents as indicated in the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942):
   a. Thaw RSB at room temperature.

   NOTE: RSB can be stored at 4°C after initial thawing.

   b. Bring the AMPure XP beads to room temperature. Vortex the AMPure beads for 30 seconds to make sure that the beads are evenly dispersed prior to use.

   NOTE: Before each use, bring AMPure XP bead aliquots to room temperature for at least 30 minutes.
3. Load consumables onto the work surface and aliquot reagents, following the layout in Figure 4 (below):

Component	Consumable	Volume
PCR products	Apollo 0.2 ml PCR 8-Tube Strips, Clear	50 µl per tube
RSB (Resuspension Buffer)	Hard-Shell Plate, Rows 4–7	180 µl per well (3 reactions per well)
AMPure XP beads	Hard-Shell Plate, Rows 1–3	135 µl per well (4 reactions per well)
Ethanol	Apollo Reservoir	15 ml
Nuclease-free water	Apollo Reservoir	12 ml

NOTES:
- The Apollo system is specifically calibrated for the consumables indicated in the table above. Using alternative consumables may cause the run to fail.
- Ensure that there are no air bubbles.
- Do not place reagents on the Apollo deck until you are ready to start the run.

4. Install the metal retention plates on Blocks 3 and 4.
5. Empty the waste box.

   NOTE: An accumulation of tips in the waste box may cause the run to fail.
6. Set up the run.
   a. On the touchscreen, press **Maintenance** and then press **User Maintenance**.
   b. Select the following subscript: `NXTforSSv4_3_PCRCleanup_v1.scb`
7. Before you begin the run, check that:
   - No overhangs from partial strips are present.
   - Reagents were spun down to remove bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
   - Plastic consumables are properly seated on the deck surface with caps/lids removed.
8. Start the PCR cleanup run.
   a. Close the instrument door.
   b. Press **Run**. The run time is 4 hours, 25 minutes.
9. When the run is complete, remove the products on Block 4 and discard the consumables in Blocks 2, 3, 6, and 7. Visually inspect the products; cap and spin down, as necessary.

10. From the touchscreen, press **OK** to return to the **User Maintenance** menu. Do not leave the **User Maintenance** menu or turn off the instrument if you are continuing to the PCR Split protocol.
11. Proceed to the PCR Split protocol (Section IV.E, below) or store samples at –20°C for up to one week.
    SAFE STOPPING POINT: If you do not plan to proceed immediately to the PCR Split protocol (Section IV.E, below), cap the tubes and store them at –20°C for up to one week.

PCR Cleanup

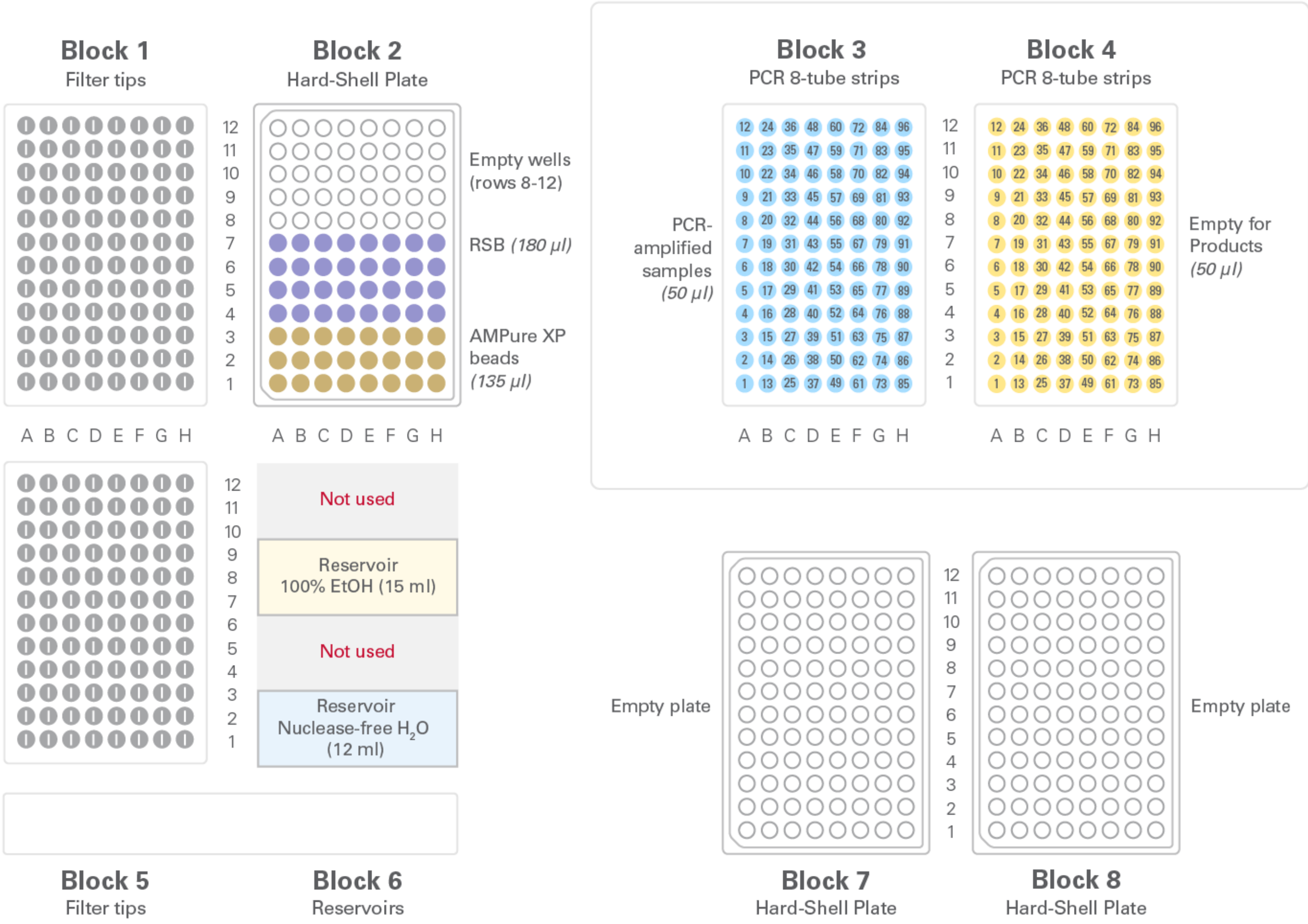
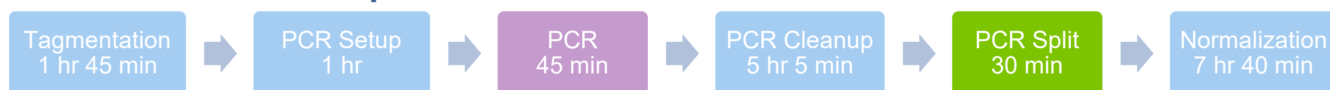


Figure 4. Loading layout for PCR cleanup.

E. Protocol: PCR Split



In this step, the purified PCR product and RSB combined in the previous protocol (Section IV.D) are mixed together and 20 µl of purified PCR product (i.e., sequencing library) is removed for library normalization. The remaining 30 µl of library can be stored.

Materials Required

Reagents	Storage conditions	Source
Purified PCR product (sequencing library)	–20°C	Section IV.D

Consumables	Source	Cat. No.	Quantity	Usage/run
Apollo Filter Tips	Takara Bio	640084	Box of 960	96 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125	12 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125	12 strips

1. Before you begin, check that:
   - All work surfaces have been cleaned with 70% ethanol within the past week.
   - The instrument has been restarted during the current work day.
   - Any deformed plastics are discarded.
2. Prechill Apollo blocks:
   a. On the touchscreen, press **Maintenance** and then press **User Maintenance**.
   b. Select the following subscript: `SetTemp10C_v1-1.scb`.
   c. Proceed to Step 3 (below) while the instrument is chilling. Chilling takes about 2 minutes.
3. Load consumables and tubes containing purified PCR products onto the work surface following the layout in Figure 5 (next page), and uncap the PCR tubes.

   NOTES:
   - If you are proceeding directly from the previous protocol (PCR Cleanup), tubes containing purified PCR products may already be properly situated.
   - The Apollo system is calibrated for Apollo PCR tubes only. Using other tubes may cause the run to fail. Ensure that there are no air bubbles.
4. Install the metal retention plates on Blocks 3 and 4.
5. Empty the waste box.

   NOTE: An accumulation of tips in the waste box may cause the run to fail.
6. Set up the run.
   a. From the chill-down run in Step 2, press **OK** to return to the **User Maintenance** menu.
   b. Select the following subscript: `NXTforSSv4_4_PCRSplit_v1.scb`

7. Before you begin the run, check that:
   - No overhangs from partial strips are present.
   - Reagents were spun down to remove bubbles. **Bubbles at the bottoms of the tubes must be removed to ensure accurate volume delivery.**
   - Plastic consumables are properly seated on the deck surface with caps/lids removed.
8. Start the PCR split run.
   a. Close the instrument door.
   b. Press **Run**. The run time is 16 minutes.
9. Cap and remove the tubes in Block 4 and store them at –20°C (they can be stored in this manner for up to one week). These tubes contain 30 µl of library that can be used to verify the success of the library construction workflow up to this point or to repeat the normalization procedure.
10. From the touchscreen, press **OK** to return to the **User Maintenance** menu. Do not leave the **User Maintenance** menu or turn off the instrument if you are continuing to the Normalization protocol.
11. Proceed to the Normalization protocol (Section IV.F, below) with the samples in Block 3, or cap the tubes in Block 3 and store the samples at –20°C for up to one week.

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

PCR Split

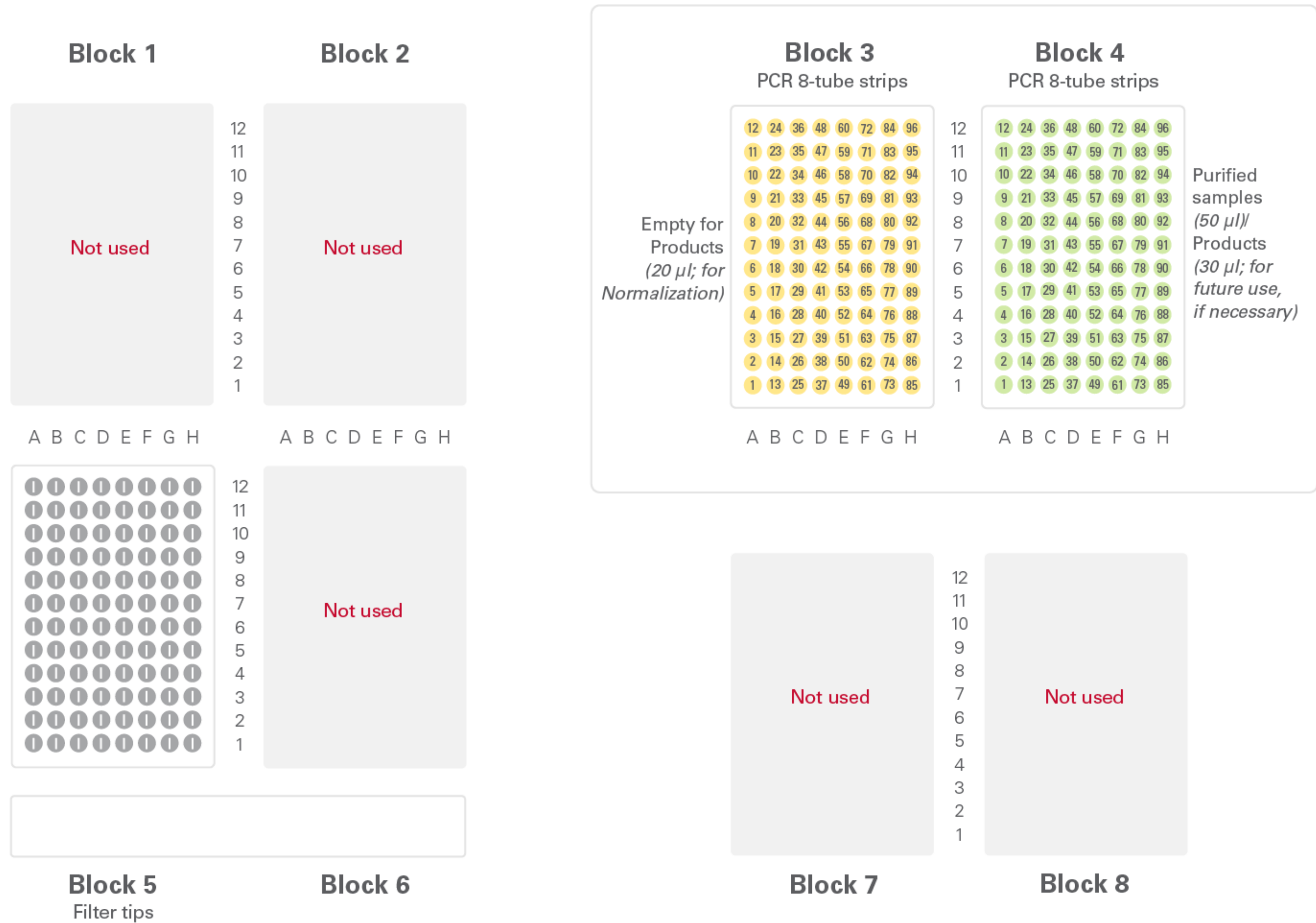
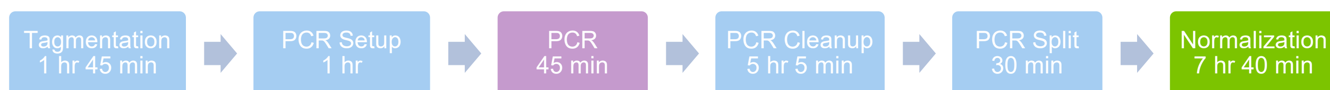


Figure 5. Loading layout for PCR split.

Filter tip
Sample/Product
Product

F. Protocol: Normalization



In this step, the quantity of each library is normalized to ensure more equal library representation in the pooled sample.

Materials Required

Reagents	Storage conditions	Source
Purified, split library	–20°C	Section IV.E
NaOH (0.1 N)	Room temperature	User (prepared from 1 N stock within the past 7 days; 7 ml is required for processing 96 samples)
LNA1 (Library Normalization Additives 1)	–20°C	Nextera XT DNA Library Preparation Kit
LNB1 (Library Normalization Beads 1)	4°C	
LNW1 (Library Normalization Wash 1)	4°C	
LNS1 (Library Normalization Storage Buffer 1)	Room temperature	

Consumables	Source	Cat. No.	Quantity	Usage/run
Apollo Filter Tips	Takara Bio	640084	Box of 960	192 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125	12 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125	12 strips
Apollo 1.1 ml MiniTubes	Takara Bio	640088	Box of 960	40 minitubes
Hard-Shell Plate	Bio-Rad	HSP-9601	Box of 50	3 plates

1. Before you begin, check that:
   - All work surfaces have been cleaned with 70% ethanol within the past week.
   - The instrument has been restarted during the current work day.
   - Any deformed plastics are discarded.
2. Prechill the Apollo blocks if the instrument was turned off after the last subscript. The blocks should be chilled as follows:
   a. On the touchscreen, press **Maintenance** and then press **User Maintenance**.
   b. Select the following subscript: `SetTemp10C_v1-1.scb`.
   c. Proceed to Step 3 (below) while the instrument is chilling. Chilling takes about 2 minutes.
3. Prepare reagents as indicated in the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942).

   NOTE: Make sure that LNS1 is at room temperature before use.

4. Load consumables onto the work surface and aliquot reagents, following the layout in Figure 6 (next page):

Component	Consumable	Volume
Purified, split library	Apollo 0.2 ml PCR 8-Tube Strips	20 µl per tube
LNB1 (Library Normalization Beads 1)	Hard-Shell Plate, Row 12	120 µl per well (12 reactions per well)
LNA1 (Library Normalization Additives 1)	Apollo 1.1 ml MiniTubes	570 µl per tube (12 reactions per tube)
LNW1 (Library Normalization Wash 1)	Apollo 1.1 ml MiniTubes	1.2 ml per tube (12 reactions per tube)
LNS1 (Library Normalization Storage Buffer 1)	Apollo 1.1 ml MiniTubes	430 µl per tube (12 reactions per tube)
NaOH (0.1 N)	Apollo 1.1 ml MiniTubes	560 µl per tube (12 reactions per tube)

NOTES:

- The Apollo system is specifically calibrated for the consumables indicated in the table above. Using alternative consumables may cause the run to fail.
- Ensure that there are no air bubbles. Keep LNB1, LNA1, LNW1, and LNS1 at room temperature during use.
- Do not place reagents on the Apollo deck until you are ready to start the run.

5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box.

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

7. Set up the run.
   a. From the chill-down run in Step 2, press **OK** to return to the **User Maintenance** menu.
   b. Select the following subscript: `NXTforSSv4_5_Normalization_v1.scb`
8. Before you begin the run, check that:
   - No overhangs from partial strips are present
   - Reagents were spun down to remove bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
   - Plastic consumables are properly seated on the deck surface with caps/lids removed.
9. Start the normalization run.
   a. Close the instrument door.
   b. Press **Run**. The run time is 7 hours, 5 minutes.
10. When the run is complete, leave the products on Block 4 and discard the consumables in Blocks 1, 2, 3, 6, and 8.
11. Proceed to pooling and sample loading as indicated in the Nextera XT DNA Library Prep Kit Reference Guide (Illumina, Part No. 15031942) and the corresponding user manual for the Illumina instrument used for sequencing.

Normalization

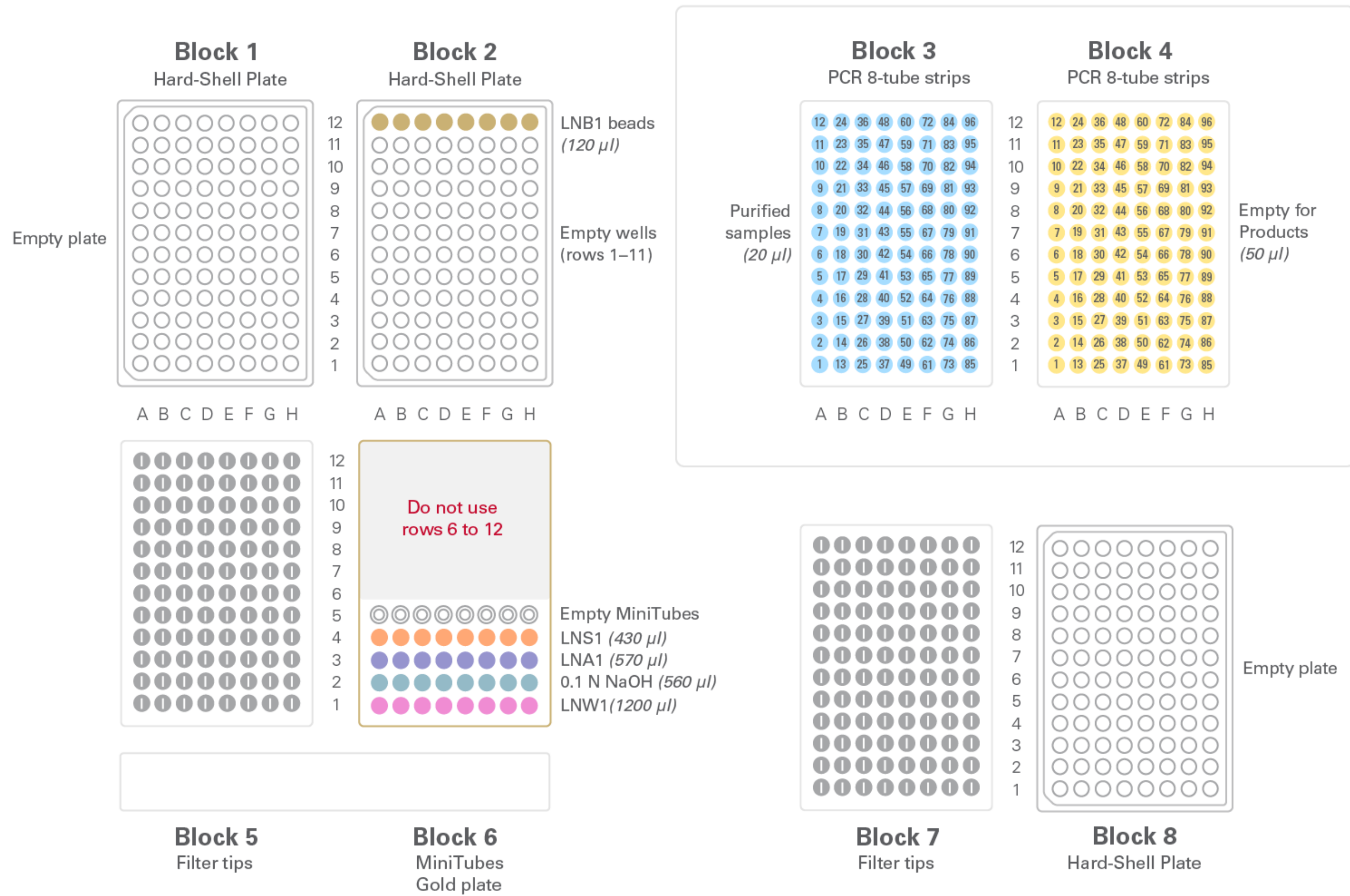


Figure 6. Loading layout for normalization.

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This document has been reviewed and approved by the Quality Department.