VeriSeq™ PGS Library Preparation on the Apollo™ System - Protocol-At-A-Glance

I. Introduction

When run with the included VeriSeq scripts, this protocol can process **up to 96 samples per batch** into 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 96 samples for VeriSeq PGS on the Apollo system. Total = total time 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. Run = time it takes for the program to run.

III. List of Required Components

Reagents	Storage conditions	Source	Cat. No.
Sample (amplified SurePlex products)	–20°C	User	
VeriSeq Library Preparation Kit-PGS	See manufacturer's protocol	Illumina	RH-101-1001
Nextera® Index Kit (96 Indexes, 384 Samples)	See manufacturer's protocol	Illumina	FC-121-1012
0.1 N NaOH	Room temperature	User	N/A
70% ethanol (prepared fresh)	Room temperature	User	N/A

NOTE: For abbreviation, the VeriSeq Library Preparation Kit-PGS will be referred to as 'VeriSeq Kit' for the remainder of this protocol.

Additional Materials Required

The following consumables must be purchased separately and were used to validate protocols and scripts. **Do not make any substitutions.**

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	696 tips
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	4 reservoirs
Apollo 1.1 mL MiniTubes	Takara Bio	640088	Box of 960 minitubes	40 minitubes
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	112 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	112 strips
Hard-Shell 96-Well PCR Plates	Bio-Rad	HSP9601	Box of 50 plates	9 plates

General lab equipment, reagents, and consumables

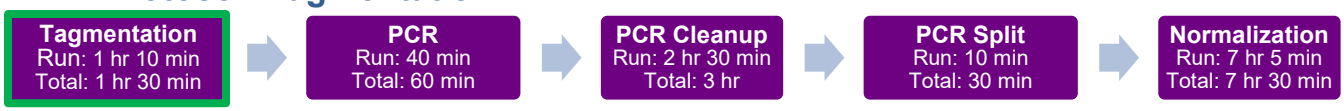
- Single-channel pipettes: 10 µl, 20 µl, 200 µl, and 1,000 µl
- Eight-channel pipettes (recommended): 20 µl and 200 µl
- Filter pipette tips: 2 µl, 20 µl, 200 µl, and 1,000 µl
- PCR thermal cycler
- 100% ethanol (EtOH; molecular biology grade)

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.** 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 and before (or after) each run.
- **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 any 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.**
- The Apollo system is specifically calibrated for the consumables indicated in this protocol. **Using alternative consumables may cause the run to fail.**

V. Protocols

A. Protocol: Tagmentation



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

Materials Required

Reagents	Storage conditions	Source
Sample (amplified SurePlex products)	–25°C to –15°C	User
ATM (Amplicon Tagment Mix)	–25°C to –15°C	VeriSeq Kit
TD (Tagment DNA Buffer)	–25°C to –15°C	VeriSeq Kit
NT (Neutralize Tagment Buffer)	2°C to 8°C	VeriSeq Kit

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	192 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	24 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	24 strips
Hard-Shell 96-Well PCR Plates	Bio-Rad	HSP9601	Box of 50 plates	2 plates

1. Prior to starting the protocol, do the following:
   a) Thaw ATM and TD on ice. Gently invert tubes 3–5 times to make sure all reagents are adequately mixed.
   b) Allow NT to warm to room temperature and visually inspect it to make sure there is no precipitate. If there is, vortex until all precipitate is resuspended.
2. On the benchtop, aliquot the reagents into the consumables as described in the table below and in Figure 2:

Component	Consumable	Volume/tube (12 rxns)
Sample (amplified SurePlex products)	Hard-Shell 96-Well PCR Plates	5 µl
ATM (Amplicon Tagment Mix)	Apollo 0.2 mL PCR 8-tube Strips, Clear	70 µl
TD (Tagment DNA Buffer)	Hard-Shell 96-Well PCR Plates	130 µl
NT (Neutralize Tagment Buffer)	Hard-Shell 96-Well PCR Plates	70 µl

3. 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.
4. Load Apollo consumables onto the Apollo work surface according to the layout in Figure 2. First, load the Apollo consumables that do not initially hold reagents (see table in step 2 above). Then, just before the run, load the Apollo consumables containing temperature-sensitive reagents and samples onto the system.

   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. To access the `VeriSeq_1_Tagmentation_v6.scb` script, press [Maintenance] > [User Maintenance]. Then, select the `VeriSeq_1_Tagmentation_v6.scb` script.

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

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

8. Close the instrument door and press [RUN].

   NOTE: The run time is 1 hour and 10 minutes.

9. When the run is complete, remove the products from Block 4 and discard the consumables in Blocks 1, 2, and 3.
10. Proceed immediately to the VeriSeq PCR Protocol (Section V.B).

    NOTE: This is **NOT** a safe stopping point to store the tagmented DNA.

Tagmentation

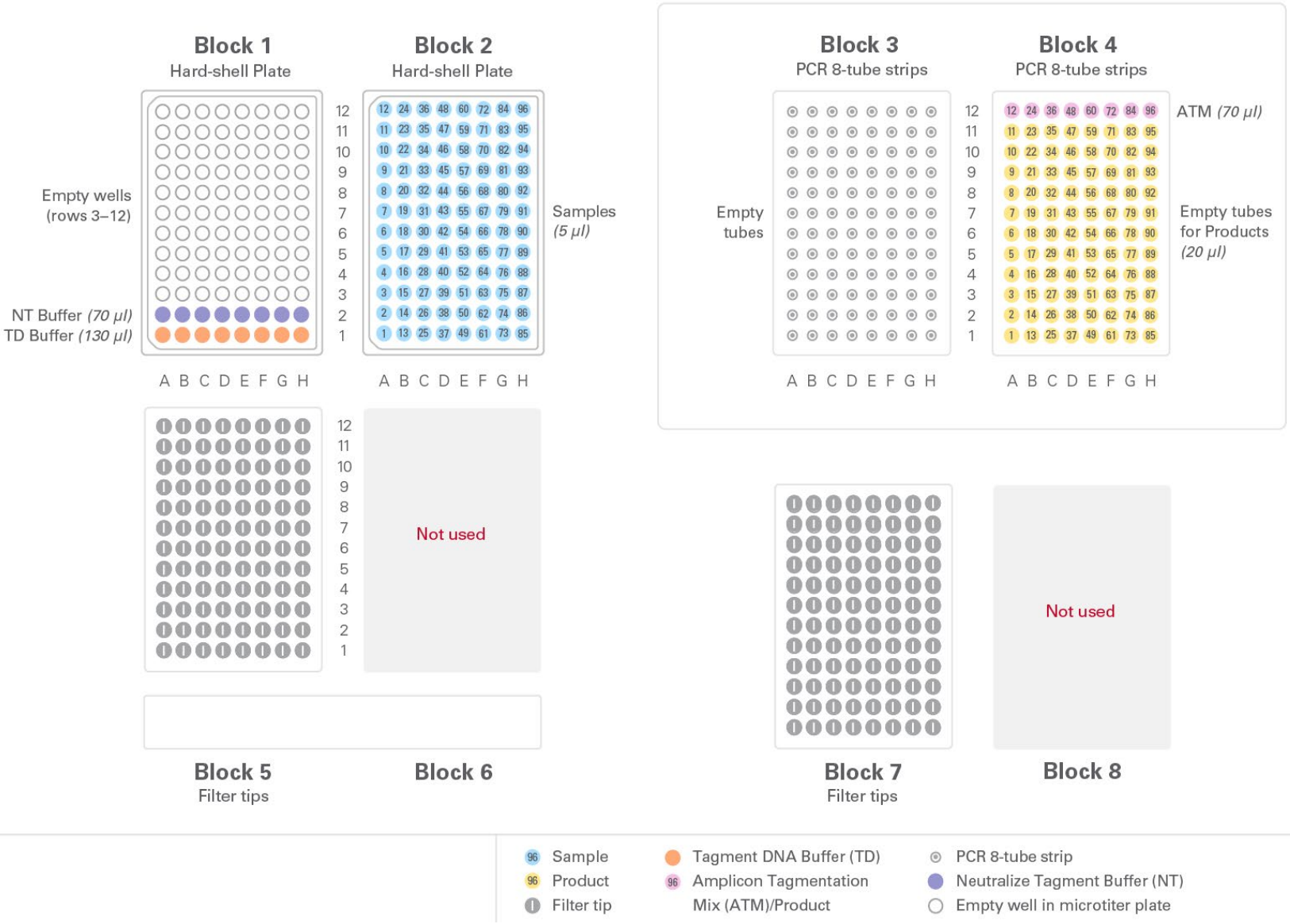
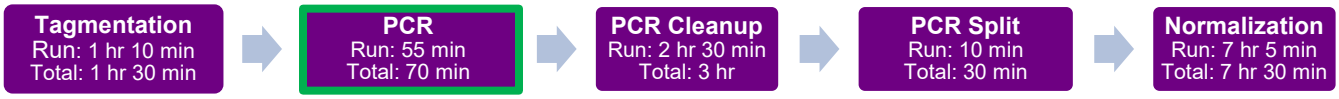


Figure 2. Deck layout for Tagmentation of 96 samples. For this protocol, use script VeriSeq_1_Tagmentation_v6.scb. Run time is 1 hour, 10 minutes.

B. Protocol: VeriSeq PCR



Materials Required

Reagents	Current temperature	Source
Tagmented DNA sample (from Section V.A)	On ice	User
NM (Nextera PCR Master Mix)	On ice	User
Index 1 primers (N701 to N712)	On ice	Section V.A.
Index 2 primers (S503 and S504)	On ice	Section V.A.

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	104 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	16 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	16 strips
Hard-Shell 96-Well PCR Plates	Bio-Rad	HSP9601	Box of 50 plates	1 plate

1. Prior to starting the protocol, do the following:
   a) Thaw NM, Index 1 primers, and Index 2 primers at room temperature (approximately 20 min to thaw).
   b) After thawing, gently invert tubes 3–5 times to make sure all reagents are adequately mixed. Briefly centrifuge tubes in a microcentrifuge.
2. On the benchtop, aliquot the reagents into the consumables as described in the table below and in Figure 3:

Component	Consumable	Volume/tube (12 rxns)
NM (Nextera PCR Master Mix)	Apollo 0.2 mL PCR 8-tube Strips, Clear	196 µl
Index 1 primers (N701 to N712)	Hard-Shell 96-Well PCR Plates	7 µl
Index 2 primers (S503 and S504)	Apollo 0.2 mL PCR 8-tube Strips, Clear	70 µl

3. 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.
4. Load Apollo consumables onto the Apollo work surface according to the layout in Figure 3. First, load the Apollo consumables that do not initially hold reagents (see table in step 2 above). Then, just before the run, load the Apollo consumables containing temperature-sensitive reagents and samples onto the system.

   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. To access the `VeriSeq_2_PCRSetup_v3.scb` script, press [Maintenance] > [User Maintenance]. Then, select the `VeriSeq_2_PCRSetup_v3.scb` script.
6. Install the metal retention plates on Blocks 3 and 4.
7. 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.
8. Close the instrument door and press [RUN].

   NOTE: The run time is 15 minutes.

9. Turn off the instrument and proceed to PCR on a benchtop thermal cycler.

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

10. 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 the PCR Cleanup protocol (Section V.C), the plate can remain on the thermal cycler overnight. Alternately, the PCR plate may be sealed and stored at –25°C to –15°C for up to one week.

11. Proceed to the PCR Cleanup protocol (Section V.C).

PCR Setup

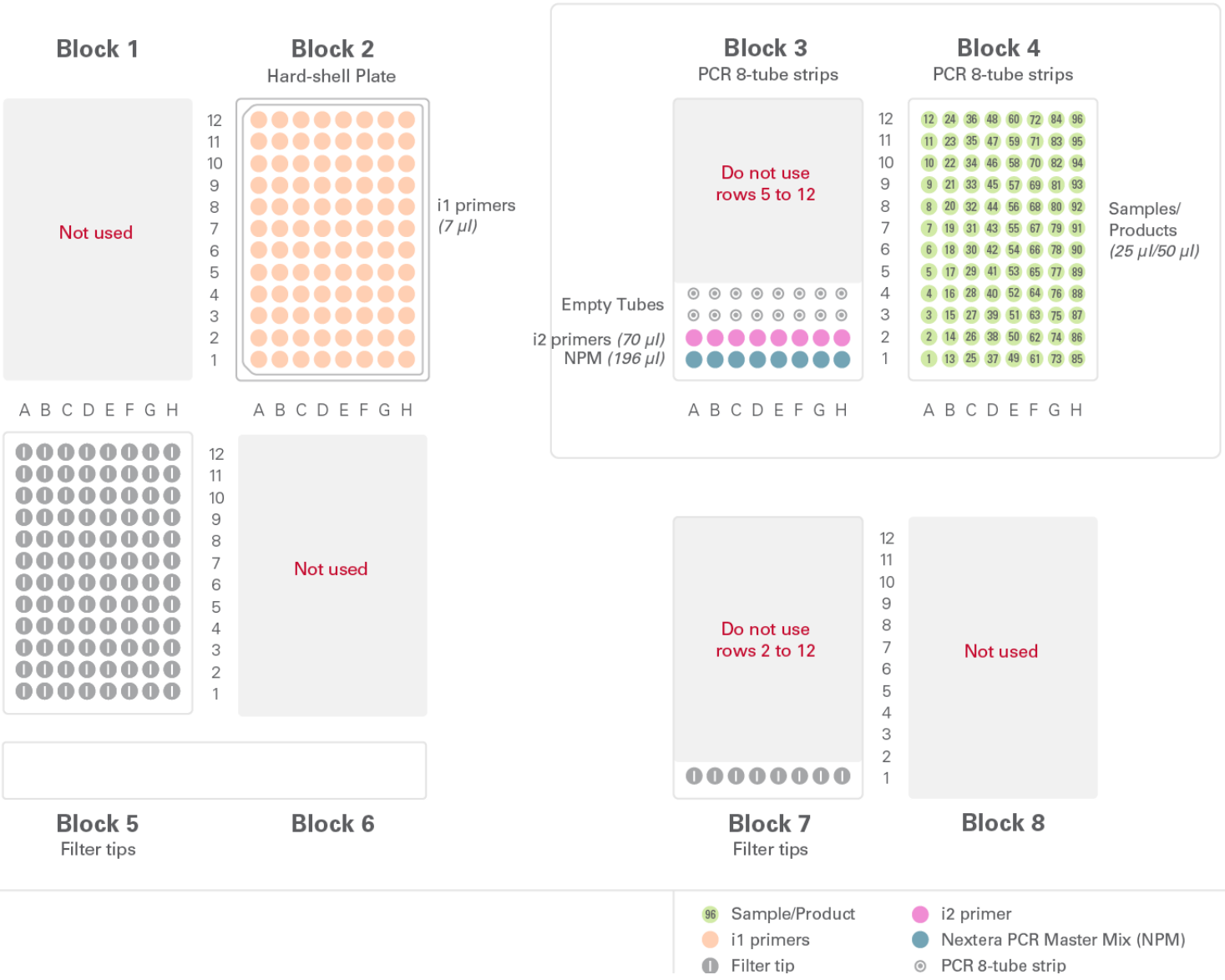
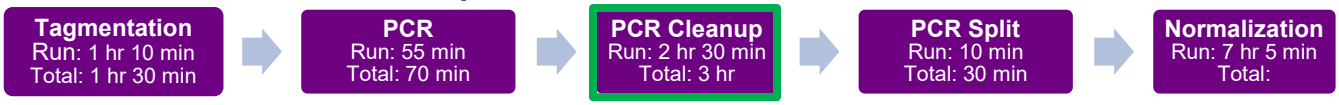


Figure 3. Deck layout for PCR Setup from 96 samples. For this protocol, use script `VeriSeq_2_PCRSetup_v3.scb`. Run time is 15 minutes.

C. Protocol: PCR Cleanup



Materials Required

Reagents	Current temperature	Source
RSB (Resuspension Buffer)	–25°C to –15°C	VeriSeq Kit
AMPure XP Beads	2°C to 8°C	Beckman Coulter
Ethanol	Room temperature	User
Nuclease-free water	Room temperature	User

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	192 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	24 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	24 strips
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	4 reservoirs
Hard-Shell 96-Well PCR Plates	Bio-Rad	HSP9601	Box of 50 plates	3 plates

NOTE: AMPure XP 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 by vortexing for 30 seconds to evenly disperse the beads before adding them to your reactions. The beads are viscous, so pipette slowly.

1. On the benchtop, aliquot the reagents into the consumables as described in the table below and in Figure 4:

Component	Consumable	Volume/tube (12 rxns)
RSB (Resuspension Buffer)	Hard-Shell 96-Well PCR Plates	220 µl
AMPure XP Beads	Hard-Shell 96-Well PCR Plates	220 µl
Ethanol	Apollo Reservoirs	12 ml
Nuclease-free water	Apollo Reservoirs	10 ml

2. 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.
3. Load Apollo consumables onto the Apollo work surface according to the layout in Figure 4. First, load the Apollo consumables that do not initially hold reagents (see table in step 1 above). Then, just before the run, load the Apollo consumables containing temperature-sensitive reagents and samples onto the system.

   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.
4. To access the `VeriSeq_3_PCRCleanup_v13.scb` script, press [Maintenance] > [User Maintenance]. Then, select the `VeriSeq_3_PCRCleanup_v13.scb` script.
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 2 hours and 30 minutes.

8. When the run is complete, leave the products on Block 4 and discard the consumables in Blocks 1, 2, 3, 6, and 7. Visually inspect the final products and spin down, as necessary.

   SAFE STOPPING POINT: If you do not plan to proceed immediately to the PCR Split protocol (Section V.D), seal the plate and store at –25°C to –15°C for up to one week.

9. Proceed to the PCR Split protocol.

PCR Cleanup

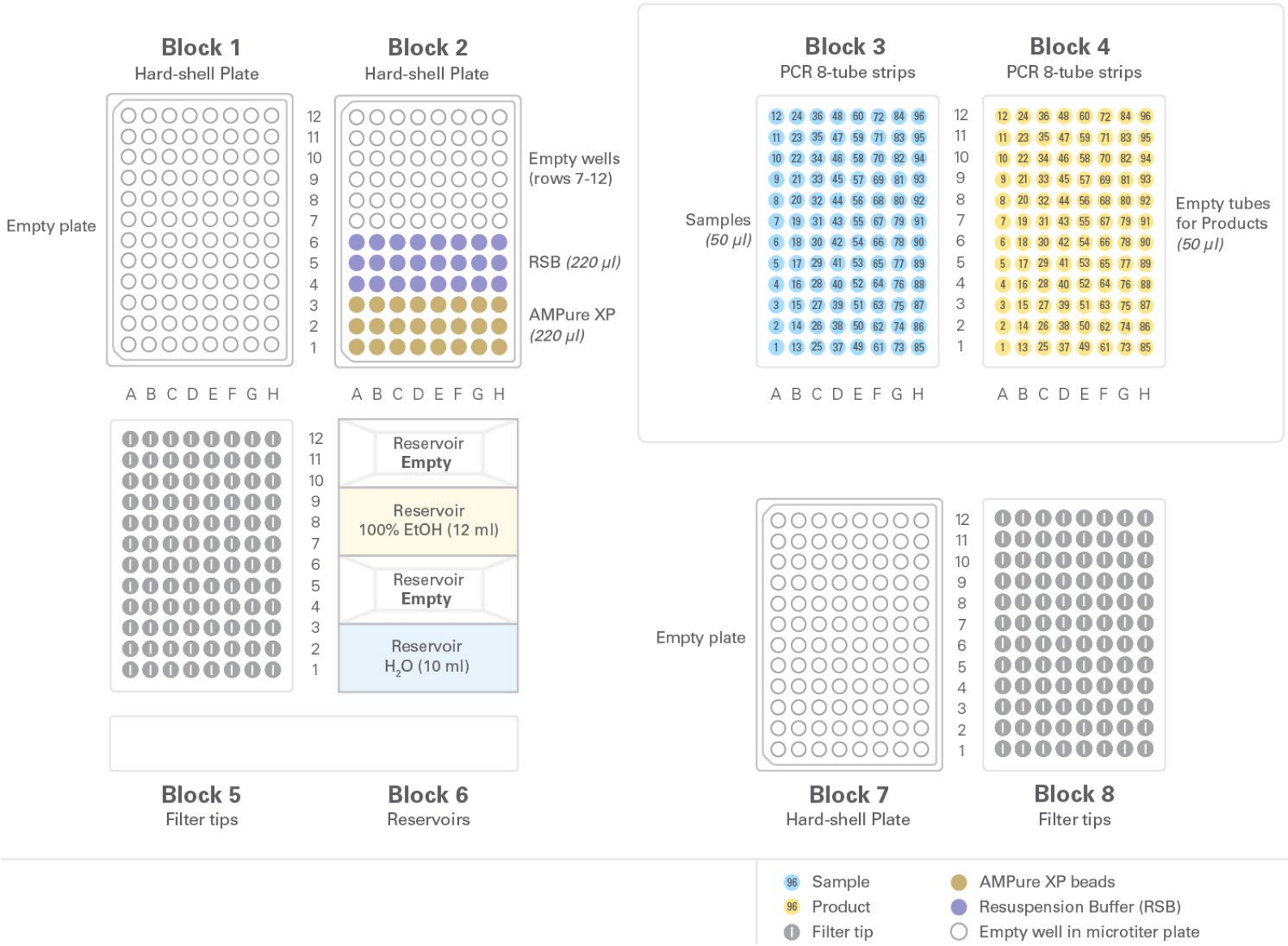
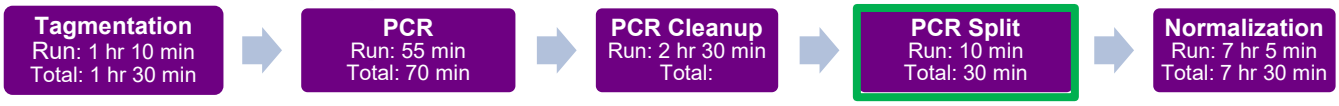


Figure 4. Deck layout for PCR Cleanup from 96 samples. For this protocol, use script `VeriSeq_3_PCRCleanup_v13.scb`. Run time is 2 hours and 30 minutes.

D. Protocol: PCR Split



Materials Required

Reagents	Current temperature	Source
Sample from PCR Cleanup protocol (Section V.C)	–25°C to –15°C	User

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	96 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	24 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	24 strips

1. On the benchtop, ensure that no air bubbles are trapped underneath the samples below. Cap and spin samples as needed to remove bubbles:

Component	Consumable	Volume/tube
Sample from PCR Cleanup protocol	Apollo 0.2 ml PCR 8-Tube Strips, Clear	50 µl

2. 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.
3. Load Apollo consumables onto the Apollo work surface according to the layout in Figure 5. First, load the Apollo consumables that do not initially hold reagents (see table in step 1 above). Then, just before the run, load the Apollo consumables containing temperature-sensitive reagents and samples onto the system.

   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.
4. To access the `VeriSeq_4_PCRSplit_v2.scb` script, press [Maintenance] > [User Maintenance]. Then, select the `VeriSeq_4_PCRSplit_v2.scb` script.
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 10 minutes.
8. When the run is complete, leave the products on Block 3 and discard the consumables in Blocks 1, 2, 5, 6, 7, and 8. Remove the tubes in Block 4 and store at –25°C to –15°C for up to 1 week. These tubes contain ~30 µl of post-PCR product that is not used for the Normalization protocol (Section V.E).

   SAFE STOPPING POINT: If you do not plan to proceed immediately to the Normalization protocol (Section V.E), seal the plate and store at –25°C to –15°C for up to one week.
9. Proceed immediately to the Normalization protocol (Section V.E)

PCR Split

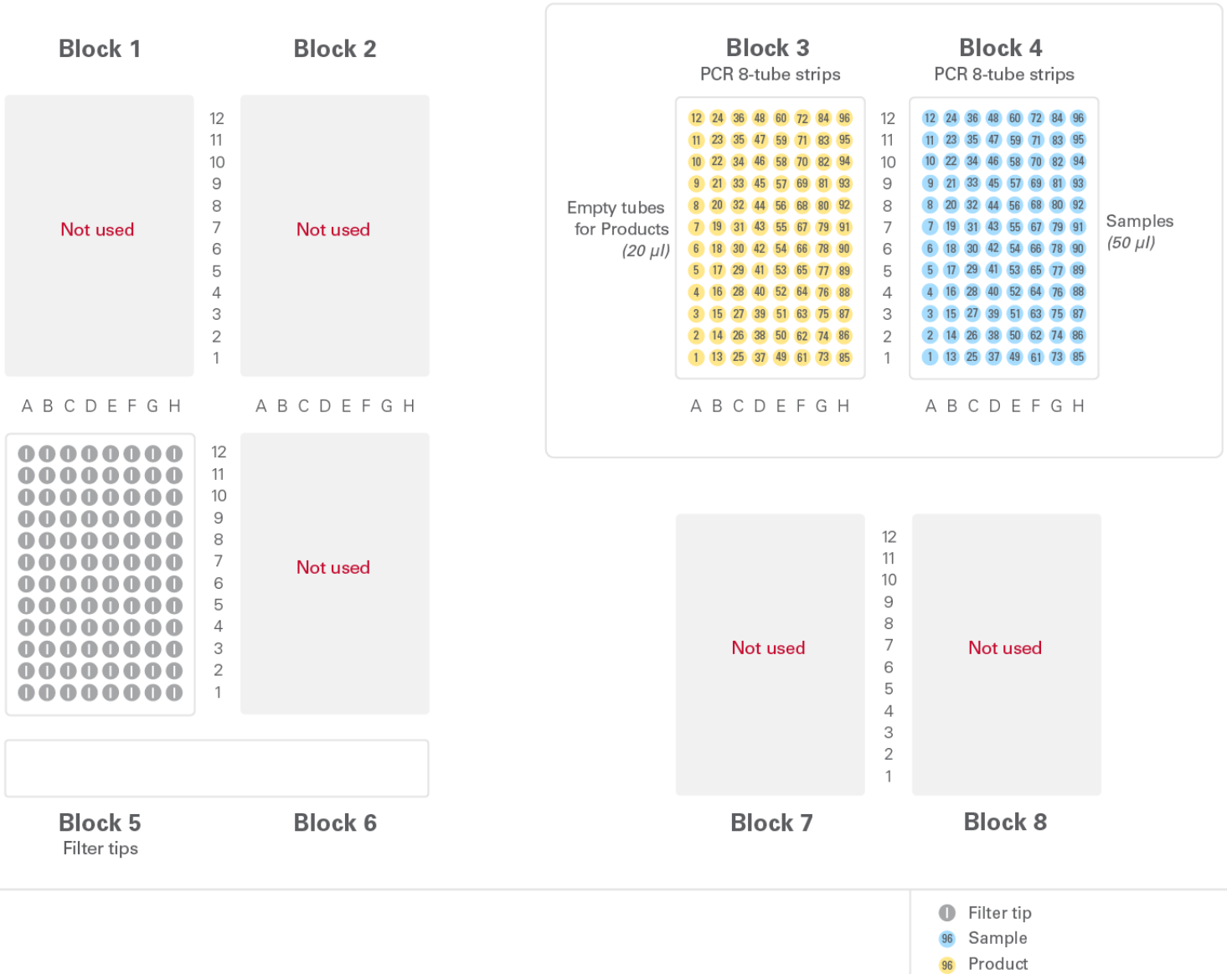
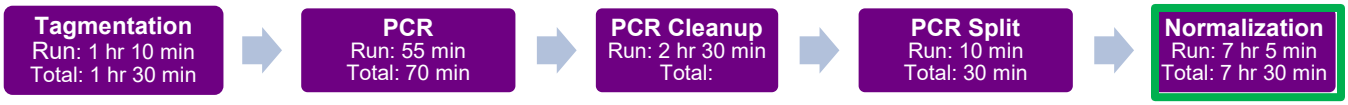


Figure 5. Deck layout for PCR Split from 96 samples. For this protocol, use script `VeriSeq_4_PCRSplit_v2.scb`. Run time is 10 minutes.

E. Protocol: Normalization



Materials Required

Reagents	Current temperature	Source
Sample from PCR Split protocol (Section V.D)	–25°C to –15°C	User
LNA1 (Library Normalization Additives 1)	2°C to 8°C	VeriSeq Kit
LNB1 (Library Normalization Beads 1)	2°C to 8°C	VeriSeq Kit
LNW1 (Library Normalization Wash 1)	2°C to 8°C	VeriSeq Kit
LNS1 (Library Normalization Storage Buffer 1)	Room temperature	VeriSeq Kit

Consumables	Source	Cat. No.	Quantity	Usage/96-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	112 tips
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	24 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	24 strips
Apollo 1.1 mL MiniTubes	Takara Bio	640088	Box of 960 minitubes	40 minitubes
Hard-Shell 96-Well PCR Plates	Bio-Rad	HSP9601	Box of 50 plates	3 plates

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

Component	Consumable	Volume/tube (12 rxns)
LNB1 (Library Normalization Beads 1)	Hard-Shell 96-Well PCR Plates	120 µl
LNA1 (Library Normalization Additives 1)	Apollo 1.1 mL MiniTubes	570 µl
LNW1 (Library Normalization Wash 1)	Apollo 1.1 mL MiniTubes	1.2 ml
LNS1 (Library Normalization Storage Buffer 1)	Apollo 1.1 mL MiniTubes	430 µl
0.1 N NaOH	Apollo 1.1 mL MiniTubes	700 µl

2. 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.
3. Load Apollo consumables onto the Apollo work surface according to the layout in Figure 6. First, load the Apollo consumables that do not initially hold reagents (see table in step 2 above). Then, just before the run, load the Apollo consumables containing temperature-sensitive reagents and samples onto the system.

   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.
4. To access the `VeriSeq_5_Normalization_v34.scb` script, press [Maintenance] > [User Maintenance]. Then, select the `VeriSeq_5_Normalization_v34.scb` script.
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 7 hours and 5 minutes.

8. When the run is complete, leave the products on Block 4 and discard the consumables in Blocks 1, 2, 3, 6, 7, and 8.

   SAFE STOPPING POINT: If you do not plan to proceed immediately to the sample pooling and MiSeq sample loading, samples may be stored at –25°C to –15°C for up to one week.

9. Proceed to pooling and Illumina MiSeq® sample loading as described in the Illumina VeriSeq Library Preparation Kit-PGS user manual (available on the manufacturer's site).

Normalization

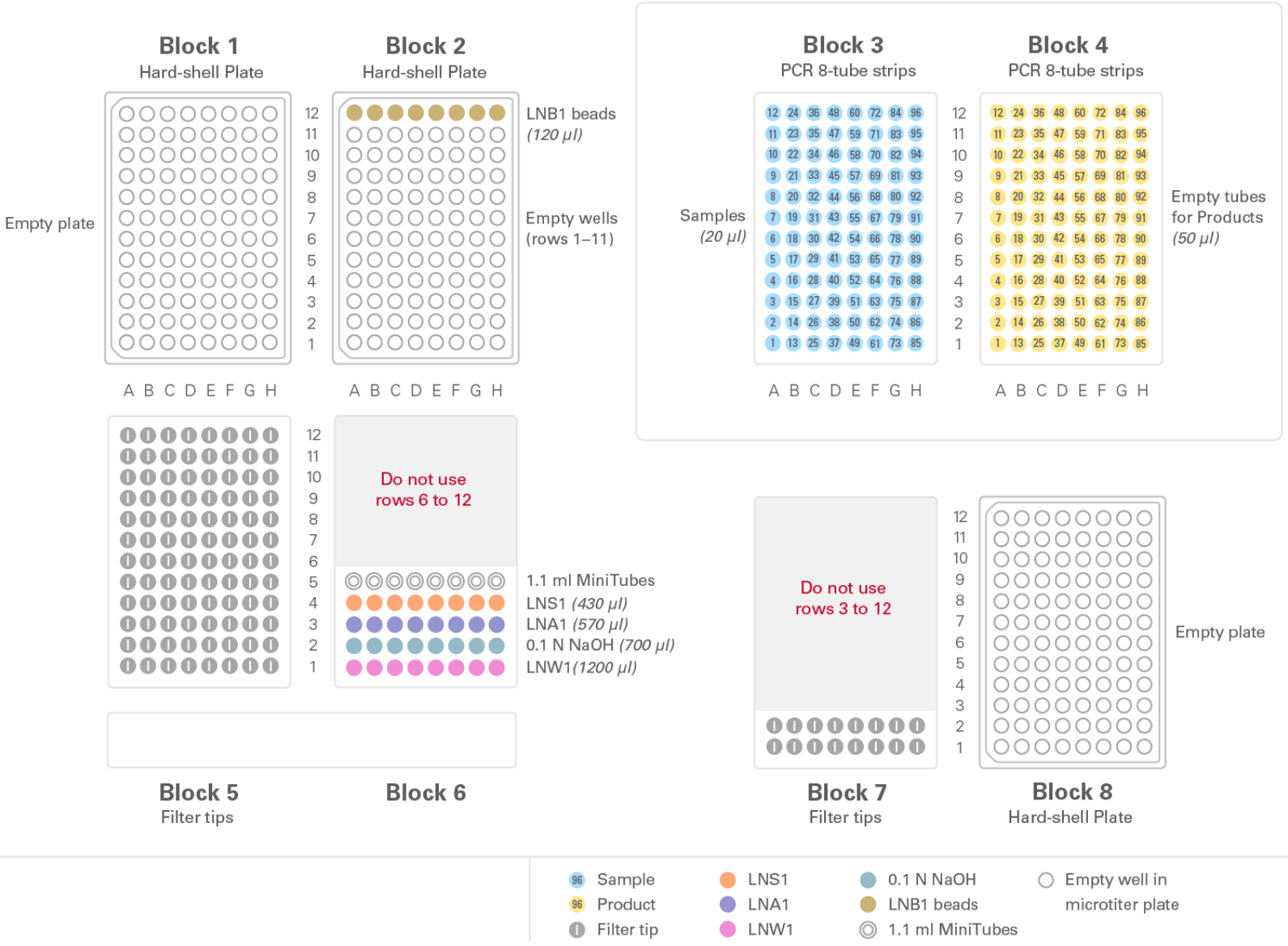


Figure 6. Deck layout for Normalization from 96 samples. For this protocol, use script `VeriSeq_5_Normalization_v34.scb`. Run time is 7 hours and 5 minutes.

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