# VeriSeq NIPT Solution v2 Sample Prep Checklist



## Process Samples

- ☐ 1 Complete the following steps for each aliquot:
  - ☐ a Centrifuge at 1600 × g for 10 minutes at 4°C.
  - ☐ b Begin plasma isolation within 15 minutes.
- ☐ 2 Inspect to confirm that each tube contains at least 1.5 ml plasma above the buffy coat.
- ☐ 3 Uncap the tubes and load them into the tube carriers.

## Isolate Plasma

- ☐ 1 Enter the Batch ID and username.
- ☐ 2 Load a sample sheet or click **No Sample Sheet**.
- ☐ 3 Select the batch size.
- ☐ 4 Select the number of no template controls (NTCs).
- ☐ 5 Load the samples, tips, and plates (barcode facing right) onto the carrier.
- ☐ 6 Observe the automated steps.
- ☐ 7 When finished, click **Unload** to unload the deck.
- ☐ 8 Remove the Intermediate Plasma deep-well plate.
  - ☐ a Inspect the plate for consistent volumes.
  - ☐ b Note any inconsistencies.
  - ☐ c Seal the plate, load with balance, and centrifuge at 5600 × g for 10 minutes.
- ☐ 9 Click **Yes**.
- ☐ 10 Remove the plate seal and reload the plate onto the carrier.
- ☐ 11 Observe the automated steps.
- ☐ 12 When finished, click **Unload** to unload the deck.
- ☐ 13 When prompted by the Workflow Manager, empty the carriers and deck.
- ☐ 14 Remove the Final Plasma deep-well plate.
- ☐ 15 Inspect the plate for consistent volumes, visible cell pellets, and excessive hemolysis.
- ☐ 16 Invalidate samples with a visible cell pellet or excessive hemolysis.
- ☐ 17 Enter comments about affected wells.

### SAFE STOPPING POINT

If you are stopping, seal the Final Plasma plate and store at 2°C to 8°C for up to 7 days.

## Extract cfDNA

- ☐ 1 Load tips.
- ☐ 2 Enter the location of the first and last tips for each tip rack.
- ☐ 3 Scan the Extraction Box barcodes.
- ☐ 4 Enter the user name or reagent preparer initials.
- ☐ 5 Scan the Accessory Box barcodes.
- ☐ 6 Enter the user name or reagent preparer initials.
- ☐ 7 Unseal the Final Plasma deep-well plate, and load plates (barcode facing right) onto carrier.
- ☐ 8 For partial plate batches, apply a trimmed plate seal over the unused wells (columns 4–12 for 24 sample batches and columns 7–12 for 48 sample batches).
- ☐ 9 Load the DNA Binding plate onto the vacuum manifold.
- ☐ 10 Select the **Are DNA Binding Plate Columns Sealed?** checkbox, and then click **OK**.
- ☐ 11 Pour the reagents into tubs and load.
- ☐ 12 Transfer reagents to deep-well reservoirs and load.
- ☐ 13 Wait for the reagent volume check to complete.
- ☐ 14 Confirm that the vacuum waste is not more than half full (empty recommended).
- ☐ 15 Observe the automated steps.
- ☐ 16 Centrifuge the DNA Binding plate at 5600 × g for 10 minutes.
- ☐ 17 During centrifugation, clean the vacuum with 70% EtOH.
- ☐ 18 After centrifugation, unseal the wells containing samples on the DNA Binding plate and place it on top of the cfDNA Elution plate.
- ☐ 19 Observe the automated steps.

- ☐ 20 After incubation, select the **Plates are assembled as indicated** checkbox.
- ☐ 21 Centrifuge the DNA Binding plate at 5600 x g for 2 minutes.
- ☐ 22 Inspect the cfDNA Elution plate for consistent volumes
- ☐ 23 Seal and retain the cfDNA Elution plate for library preparation.
- ☐ 24 When finished, click **Unload** to unload the deck.
- ☐ 25 Unload all carriers and clean the ML STAR deck.
- ☐ 26 Enter comments about affected wells.
- ☐ 27 Perform one of the following steps:
  - To continue to Prepare Libraries, click **Yes**.
  - To stop, click **Exit**.

### SAFE STOPPING POINT

If you are stopping, seal the cfDNA Elution plate and store at -25°C to -15°C for up to 7 days.

## Prepare Libraries

- ☐ 1 Scan the Library Prep Box barcodes.
- ☐ 2 Enter the user name or reagent preparer initials.
- ☐ 3 Scan the Accessory Box barcodes.
- ☐ 4 Enter the user name or reagent preparer initials.
- ☐ 5 Load tips.
- ☐ 6 Enter the location of the first tip for each tip rack.
- ☐ 7 Load plates.
- ☐ 8 Pour reagents into the deep well reservoirs and load.
- ☐ 9 Pour reagents into tubs and load.
- ☐ 10 Wait for the reagent volume check to complete.
- ☐ 11 Observe the automated steps.
- ☐ 12 When finished, click **Unload** to unload the deck.
- ☐ 13 Inspect the Libraries plate for consistent volumes.
- ☐ 14 If storing, seal and retain the Libraries plate.
- ☐ 15 Unload the carriers and clean the deck.
- ☐ 16 Enter comments about affected wells.
- ☐ 17 Perform one of the following steps:
  - To continue to Quantify Libraries, click **Yes**.
  - To stop, click **Exit**.
- ☐ 18 Unless you are stopping, proceed immediately with quantification.

### SAFE STOPPING POINT

If you are stopping, seal the Libraries plate prior to storage. The Libraries plate is stable for up to 7 days from date of preparation at -25°C to -15°C.

## Quantify Libraries

- ☐ 1 Scan the Accessory Box barcodes.
- ☐ 2 Enter the user name or reagent preparer initials.
- ☐ 3 Load tips onto the tip carrier.
- ☐ 4 Unseal the Libraries plate, and then load plates.
- ☐ 5 Load reagent tubes without caps.
- ☐ 6 Pour the reagents into reagent tubs and load.
- ☐ 7 Wait for the reagent volume check to complete.
- ☐ 8 Observe the automated steps.
- ☐ 9 When finished, click **Unload** to unload the deck.
- ☐ 10 Unload the Libraries plate, check for consistent volumes, seal, and store at room temperature.
- ☐ 11 Unload 96-well plates and check for consistent volumes
- ☐ 12 Unload the 384-well plate and check for liquid in the appropriate wells.
- ☐ 13 Seal the plate with a foil seal.
- ☐ 14 Centrifuge at 1000 × g for 20 seconds.
- ☐ 15 Incubate at room temperature for 10 minutes, protected from light.
- ☐ 16 Unload all carriers and clean the ML STAR deck.
- ☐ 17 After incubation, remove the foil seal and load the 384-well plate onto the microplate reader.
- ☐ 18 Double click the VeriSeq NIPT template to open it in SoftMax Pro.
- ☐ 19 Select **New Experiment** in the Home tab.
- ☐ 20 Select **Read**.
- ☐ 21 Export the data as XML as follows.
  - ☐ a Right click **Plate**, and then select **Rename**.

- ☐ b Scan the barcode of the Quantification plate, and then click **OK**.
- ☐ c In the upper-left corner of the screen, click the plate icon, and then select **Export** from the menu.
- ☐ d Select the **Expt name** checkbox, set the plate date option to raw, set the output format to XML, and then click **OK**.
- ☐ e Set the output file path and name, and then click **Save**.

☐ 22 On the ML STAR, enter the fluorometer ID, enter comments for the run, and upload the XML file.

☐ 23 Review the analysis results.

☐ 24 Enter comments about affected wells.

☐ 25 Assess the results.

- If the results pass specification, proceed to Pool Libraries. For specifications, see the quantitation QC metrics and boundaries table in the VeriSeq NIPT Solution v2 Software Guide (document # 1000000067940).
- If the results fail specification, the system aborts the method. Repeat the quantification procedures beginning with *Preparation* on page 1.

☐ 26 Perform one of the following steps:

- To continue to Pool Libraries, click **Yes**.
- To stop, click **Exit**.

### SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

## Pool Libraries

☐ 1 Place the Libraries plate on the thermal cycler and run the denature program.

☐ 2 Centrifuge the Libraries plate at 1000 × g for 20 seconds.

☐ 3 Select the pool concentration.

☐ 4 Load a sample sheet or use the default.

☐ 5 Select **Start**.

☐ 6 Load tips.

☐ 7 Load the Denatured Library plate.

☐ 8 Load pooling tubes.

☐ 9 Pour the reagents into reagent tubs and load.

☐ 10 Load tips.

☐ 11 Enter the location of the first and last tips for each tip rack.

☐ 12 Observe the automated steps.

☐ 13 Enter comments about affected wells.

☐ 14 When finished, select **Unload** to unload the deck.

☐ 15 Unload the tube carrier.

☐ 16 Cap each pooling tube, vortex, and then centrifuge briefly.

☐ 17 Click **OK**.

☐ 18 Sequence libraries as soon as possible after pooling. If necessary, seal the Libraries plate and store at -25°C to -15°C for up to 7 days cumulative storage to allow repooling.

### SAFE STOPPING POINT

If you are stopping, cap the pooling tubes and store at -25°C to -15°C for up to 7 days.

## Prepare Pooled Libraries for Sequencing

☐ 1 Add the following consumables to the reagent cartridge, and then pipette to mix.

- 900 µl Hybridization Buffer
- 450 µl Pool A

☐ 2 Proceed with sequencing on a next-generation sequencing system.

☐ 3 If necessary, repeat this procedure for Pool B.

- To achieve target cluster density range, the library plate can be repooled using a different pooling concentration on the Hamilton. Repooling invalidates the original pool.
- Alternatively, the ratio of pool to HT1 (450+900ul) can be modified to achieve target cluster density range.