VeriSeq NIPT Solution Sample Prep Checklist

Process Blood Samples

- ☐ 1 Centrifuge at 1600 × g for 10 minutes.
- ☐ 2 Inspect to confirm that each tube contains at least 1.5 ml plasma above the buffy coat.
- ☐ 3 Uncap tubes and load 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 number of no template controls (NTCs) and batch size.
- ☐ 4 Load the samples, tips, and plates onto the carrier.
- ☐ 5 Observe the automated steps.
- ☐ 6 When finished, click **Unload** to unload the deck.
- ☐ 7 Remove the Intermediate Plasma deep-well plate.
  - ☐ a Inspect the plate for consistent volumes.
  - ☐ b Make note of any inconsistencies.
  - ☐ c Seal the plate and centrifuge at 5600 × g for 10 minutes.
- ☐ 8 Remove the plate seal and reload the plate onto the carrier.
- ☐ 9 Observe the automated steps.
- ☐ 10 When finished, click **Unload** to unload the deck.
- ☐ 11 When prompted by the Workflow Manager, clean the carriers and deck.
- ☐ 12 Remove the Final Plasma deep-well plate.
- ☐ 13 Inspect the plate for consistent volumes, visible cell pellets, and excessive hemolysis.
- ☐ 14 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 tip for each tip rack.
- ☐ 3 Scan Extraction Box barcodes.
- ☐ 4 Enter the user name or reagent preparer initials.
- ☐ 5 Scan Accessory Box barcodes.
- ☐ 6 Enter the user name or reagent preparer initials.
- ☐ 7 Unseal Final Plasma deep-well plate, and load plates onto carrier.
- ☐ 8 For 48-sample batch, apply a seal over unused columns 7-12.
- ☐ 9 Load the DNA Binding plate onto the vacuum manifold.
- ☐ 10 Pour the reagents into tubs and load.
- ☐ 11 Pour reagents into deep-well reservoirs and load.
- ☐ 12 Wait for the reagent volume check to complete.
- ☐ 13 Confirm that vacuum waste is empty.
- ☐ 14 Observe the automated steps.
- ☐ 15 Centrifuge the DNA Binding plate at 5600 × g for 10 minutes.
- ☐ 16 Remove vacuum manifold.
- ☐ 17 During centrifugation, clean vacuum with 70% EtOH.
- ☐ 18 After centrifugation, unseal the wells containing samples on the DNA Binding plate and place it on the cfDNA Elution plate.
- ☐ 19 Observe the automated steps.
- ☐ 20 After incubation, select the **Plates are assembled as indicated** checkbox.
- ☐ 21 Inspect the cfDNA Elution plate for consistent volumes.
- ☐ 22 Seal and retain the cfDNA Elution plate for library preparation.
- ☐ 23 When finished, click **Unload** to unload the deck.
- ☐ 24 Unload all carriers and clean the ML STAR deck.
- ☐ 25 Enter comments about affected wells.

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 Library Prep Box barcodes.
- ☐ 2 Confirm that the kit is not expired.
- ☐ 3 Enter the user name or reagent preparer initials.
- ☐ 4 Scan Accessory Box barcodes.
- ☐ 5 Enter the user name or reagent preparer initials.
- ☐ 6 Load tips.
- ☐ 7 Enter the location of the first tip for each tip rack.
- ☐ 8 Load plates.
- ☐ 9 Pour reagents into deep well reservoirs and load.
- ☐ 10 Pour reagents into tubs and load.
- ☐ 11 Wait for the reagent volume check to complete.
- ☐ 12 Observe the automated steps.
- ☐ 13 When finished, click **Unload** to unload the deck.
- ☐ 14 Inspect Libraries plate for consistent volumes.
- ☐ 15 Seal and retain the Libraries plate.
- ☐ 16 Unload the carriers and clean the deck.
- ☐ 17 Enter comments about affected wells.

SAFE STOPPING POINT

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

Quantify Libraries

- ☐ 1 Scan 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 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 384-well plate and check for liquid in the appropriate wells.
  - ☐ a Seal the plate with a foil seal.
  - ☐ b Centrifuge at 1000 × g for 20 seconds.
  - ☐ c Incubate at room temperature for 10 minutes.
- ☐ 13 Unload all carriers and clean the ML STAR deck.
- ☐ 14 After incubation, remove the foil seal and load the 384-well plate onto the microplate reader.
- ☐ 15 Save the data as an .XML file.
- ☐ 16 On the ML STAR, enter the fluorometer ID, enter comments for the run, and upload the .XML file.
- ☐ 17 Review the analysis results.
- ☐ 18 Enter comments about affected wells.
- ☐ 19 Assess the results.

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 Select the pool concentration.
- ☐ 3 Load a sample sheet or use the default.
- ☐ 4 Load tips.
- ☐ 5 Load the Denatured Library plate.
- ☐ 6 Load pooling tubes.
- ☐ 7 Pour the reagents into reagent tubs and load.
- ☐ 8 Load tips.
- ☐ 9 Enter the location of the first tip for each tip rack.
- ☐ 10 Observe the automated steps.
- ☐ 11 When finished, click **Unload** to unload the deck.
- ☐ 12 Unload the tube carrier. Cap each pooling tube, vortex, and then centrifuge briefly.
- ☐ 13 Sequence libraries as soon as possible after pooling. Store the Libraries plate at -25°C to -15°C for up to 7 days cumulative storage to enable repooling, if necessary.
- ☐ 14 Enter comments about affected wells.

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 Pool for Sequencing

- ☐ 1 Add buffer and library pool directly to the sequencer sample cartridge.
  - ▶ 900 µl Hybridization Buffer
  - ▶ 450 µl Pool A
  - ▶ Pipette to mix
- ☐ 2 Proceed with sequencing.
- ☐ 3 Confirm correct run configuration when prompted.
- ☐ 4 Repeat procedure for Pool B, if necessary.