VeriSeq NIPT Solution Sample Prep Checklist

**Process Blood Samples**

1. Centrifuge at 1600 x 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.
   - Inspect the plate for consistent volumes.
   - Make note of any inconsistencies.
   - Seal the plate and centrifuge at 5600 x 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 x 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 tubes 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.
- [ ] 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 x 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.