VeriSeq PGS Library Prep Checklist

Quantification–Qubit Method

- ☐ 1 Prepare 1/10 dilution of each SurePlex sample or control.
- ☐ 2 Prepare the working solution according to the manufacturer instructions.
- ☐ 3 Add 10 µl of each standard to 190 µl of working solution.
- ☐ 4 Add 10 µl of the 1/10 diluted SurePlex sample and 190 µl working solution to each assay tube. Briefly vortex.
- ☐ 5 Incubate the assay tubes for 2 minutes.
- ☐ 6 Calculate the concentration of each 1/10 diluted SurePlex sample. Convert the units to ng/µl.
- ☐ 7 Compare the measured concentration of the samples to the values in the VeriSeq PGS-MiSeq QC Assessment Guide.

Quantification–Quant-iT Method

- ☐ 1 Prepare 1/10 dilution of each SurePlex sample or control.
- ☐ 2 Prepare the working solution according to the manufacturer instructions.
- ☐ 3 Add 190 µl working solution to the microplate wells.
- ☐ 4 Add 10 µl of each 1/10 diluted SurePlex sample to separate wells. Create duplicates or triplicates. Pipette to mix.
- ☐ 5 Add 10 µl of each λ DNA standard to separate wells. Create duplicates or triplicates. Pipette to mix.
- ☐ 6 Measure the fluorescence.
- ☐ 7 Calculate the concentration of each 1/10 diluted SurePlex sample. Convert the units to ng/µl.
- ☐ 8 Compare the measured concentration of the samples to the values in the VeriSeq PGS-MiSeq QC Assessment Guide.

Quantification–Template Dilution to 0.2 ng/µl

- ☐ 1 Using BlueFuse Workflow Manager, enter the calculated dsDNA concentration (ng/µl) of the 1/10 diluted SurePlex sample concentration into the VeriSeq PGS–MiSeq Assay Plate.
- ☐ 2 According to the BlueFuse Workflow Manager calculations, add molecular-grade water to a new PCR plate.
- ☐ 3 Add 5 µl of the 1/10 diluted SurePlex sample to each well of the plate containing molecular-grade water.
- ☐ 4 Vortex, and then centrifuge the plate at 280 × g for 1 minute.
- ☐ 5 Set aside on wet ice.

Tagment Input DNA

- ☐ 1 Label a new PCR plate VTA.
- ☐ 2 Calculate the total volume of TD. Divide the volume equally among the wells of a PCR 8-tube strip.
- ☐ 3 Add 10 µl TD Buffer to each well.
- ☐ 4 Add 5 µl ATM to the wells containing TD Buffer.
- ☐ 5 Add 5 µl SurePlex amplification product (diluted at 0.2 ng/µl) to each sample well.
- ☐ 6 Mix at 1,800 rpm for 1 minute.
- ☐ 7 Centrifuge at 280 × g for 1 minute.
- ☐ 8 Place on a thermal cycler with a heated lid and run the program:
  - 55°C for 5 minutes
  - Hold at 10°C
- ☐ 9 Calculate the total volume of NT buffer. Divide the volume equally among the wells of a PCR 8-tube strip.
- ☐ 10 Add 5 µl NT Buffer to each well.
- ☐ 11 Mix at 1800 rpm for 1 minute.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Incubate at room temperature for 5 minutes.

Amplify Tagmented DNA

- ☐ 1 Save the following program on a thermal cycler with a heated lid:
  - 72°C for 3 minutes
  - 95°C for 30 seconds
  - 12 cycles of:
    - 95°C for 10 seconds
    - 55°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 4°C
- ☐ 2 Print the sample assay plate layout.
- ☐ 3 Arrange the index primers in the TruSeq Index Plate Fixture, as follows:
  - N701–N712 in columns 1–12
  - S503 in row A, S504 in row C
- ☐ 4 Place the plate on the TruSeq Index Plate Fixture.
- ☐ 5 Add index adapters. Change tips between each well.
  - Add 5 µl of each Index 1 (i7) adapter to each column.
  - Add 5 µl of each Index 2 (i5) adapter to each row.
- ☐ 6 Add 15 µl NPM to each well.
- ☐ 7 Mix at 1800 rpm for 1 minute.
- ☐ 8 Centrifuge at 280 × g for 1 minute.
- ☐ 9 Place on the thermal cycler and run the saved program.

SAFE STOPPING POINT

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

Clean Up PCR

- ☐ 1 Centrifuge the VTA plate at 280 × g for 1 minute.
- ☐ 2 Add an appropriate volume of beads to a trough.
- ☐ 3 Add 45 µl AMPure XP beads to each required well of a clean deep well plate.
- ☐ 4 Transfer 45 µl PCR product from the VTA plate to the plate containing beads.
- ☐ 5 Mix at 1800 rpm for 1 minute.
- ☐ 6 Incubate at room temperature for 5 minutes.
- ☐ 7 Pulse centrifuge.
- ☐ 8 Place on a magnetic stand until the liquid is clear. Keep the plate on the stand during the following steps.
- ☐ 9 Discard the supernatant from each well.
- ☐ 10 Wash 2 times with 200 µl 80% EtOH.
- ☐ 11 Remove residual EtOH.
- ☐ 12 Air-dry on the magnetic stand for 15 minutes, or until beads are completely dry.
- ☐ 13 Add 50 µl RSB to each well.
- ☐ 14 Remove the plate from the magnetic stand.
- ☐ 15 Mix at 1800 rpm for 1 minute.
- ☐ 16 Centrifuge at 280 × g for 1 minute.
- ☐ 17 Place on a magnetic stand until the liquid is clear.
- ☐ 18 Transfer 45 µl of each supernatant from each well to a new PCR plate.

SAFE STOPPING POINT

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

Normalize Libraries

- ☐ 1 Prepare 0.1 N NaOH.
- ☐ 2 Prepare the LNA1/LNB1 mix according to the number of reactions. For 24 rxn, add 200 µl of LNB1 to 1100 µl of LNA1.
- ☐ 3 Vortex thoroughly until LNA1/LNB1 mix is homogenized.
- ☐ 4 Label a new deep-well plate LNP.
- ☐ 5 Pour the LNA1/LNB1 mix into a reservoir.
- ☐ 6 Transfer 45 µl LNA1/LNB1 mix to each well.
- ☐ 7 Add 20 µl dsDNA to each well.
- ☐ 8 Mix at 1800 rpm for 30 minutes.
- ☐ 9 Pulse centrifuge .
- ☐ 10 Place on a magnetic stand until the liquid is clear. Keep the plate on the stand during the following steps.
- ☐ 11 Remove and discard all supernatant from each well.
- ☐ 12 Discard the tips in an appropriate hazardous waste container. Change tips between samples.
- ☐ 13 Wash 2 times.
  - Add 45 µl LNW.
  - Remove from magnetic stand.
  - Shake at 1800 rpm for 5 minutes.
  - Briefly centrifuge at 280 × g.
  - Place on a magnetic stand until liquid is clear.
  - Remove and discard all supernatant.
- ☐ 14 Add 30 µl 0.1 N NaOH to each well.
- ☐ 15 Remove from the magnetic stand.
- ☐ 16 Mix at 1800 rpm for 5 minutes.
- ☐ 17 Centrifuge at 280 × g for 1 minute.
- ☐ 18 Place on a magnetic stand until the liquid is clear.
- ☐ 19 Add 25 µl of LNS1 to each well of a new PCR plate.
- ☐ 20 Transfer 25 µl of supernatant from the LNP plate to the new PCR plate containing LNS1.
- ☐ 21 Vortex, and then centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

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

Pool Libraries

- ☐ 1 Centrifuge the plate at 280 × g for 1 minute.
- ☐ 2 According to the sample sheet, transfer 5 µl of each normalized library to pool into a LoBind tube.
- ☐ 3 Vortex and centrifuge the pooled library.
- ☐ 4 Transfer 15 µl library pool to a new PCR tube or PCR 8-tube strip.
- ☐ 5 Add 85 µl HT1.
- ☐ 6 Record the volumes of library pool and HT1 dispensed in the table below.

Reagent	Recommended Volume	Actual Volume
Pool library	15 µl	
HT1	85 µl	
Total	100 µl	100 µl

- ☐ 7 Gently vortex and centrifuge the pool/HT1 mixture.
- ☐ 8 Place on the preprogrammed thermal cycler and run the POOL program.
- ☐ 9 Transfer 600 µl of HT1 into a second clean LoBind tube. Set aside in an ice-water bath.
- ☐ 10 Transfer 100 µl of denatured pool/HT1 mixture to the LoBind tube with HT1. Set aside on wet ice.
- ☐ 11 Sequence your library according to the *MiSeq System Guide (document # 15027617)*.

Acronyms

Acronym	Definition
ATM	Amplicon Tagment Mix
HT1	Hybridization Buffer
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNS1	Library Normalization Storage Buffer 1
LNP	Library Normalization Plate
LNW1	Library Normalization Wash 1
NPM	Nextera PCR Master Mix
NT	Neutralize Tagment Buffer
PGS	Preimplantation Genetic Screening
RSB	Resuspension Buffer
SCT	Single-Cell Tagment
SLB	Sample Lysis Buffer
TD	Tagment DNA Buffer
VTA	VeriSeq Tagment Amplicon Plate