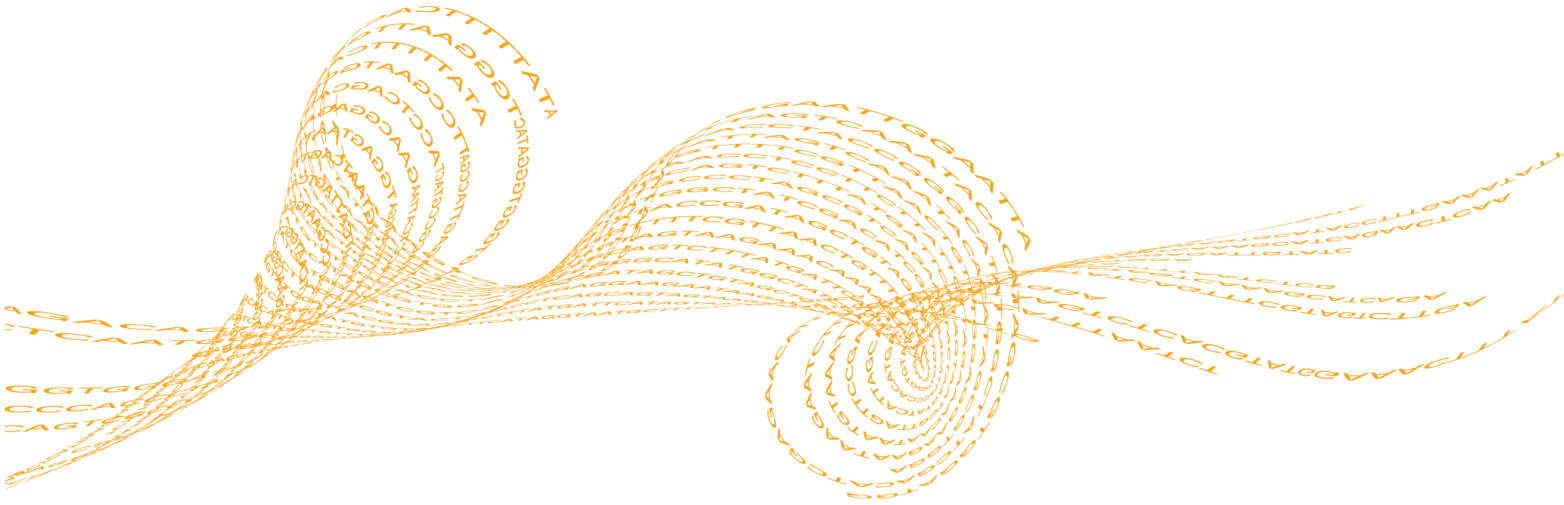
VeriSeq PGS Library Prep

Protocol Guide

For Research Use Only. Not for use in diagnostic procedures.



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Quantify Unpurified SurePlex Products

Prepare 1/10 Dilutions of SurePlex Sample and Controls

1. Vortex each sample and control.
2. Centrifuge at 280 × g for 1 minute.
3. In a new PCR plate, add 45 µl molecular-grade water to the required wells.
4. Add 5 µl sample or control.
5. Vortex to mix.
6. Centrifuge at 280 × g for 1 minute.
7. Set aside on wet ice.

Qubit Method

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

Quant-iT Method

1. Prepare the working solution according to the manufacturer instructions.
2. Add 190 µl working solution to the microplate wells.
3. Add 10 µl of each 1/10 diluted SurePlex sample to separate wells. Create duplicates or triplicates. Pipette to mix.
4. Add 10 µl of each λ DNA standard 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.

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

Procedure

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

Preparation

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

Procedure

1. Print the sample assay plate layout using the BlueFuse Workflow Manager.
2. 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
3. Place the plate on the TruSeq Index Plate Fixture.
4. 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.
5. Add 15 µl NPM to each well.
6. Mix at 1800 rpm for 1 minute.
7. Centrifuge at 280 × g for 1 minute.
8. 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

Preparation

1. Thoroughly vortex the AMPure XP beads.
2. Prepare fresh 80% EtOH from absolute ethyl alcohol.

Procedure

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. *To prevent magnetic bead aggregation, do not centrifuge longer than a pulse*.
8. Place on a magnetic stand until the liquid is clear. Keep the plate on the stand during the following steps.
9. Discard all 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

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit, at support.illumina.com/sds.ilmn.

Preparation

1 Prepare 0.1 N NaOH, as follows.

   a Add 9.9 ml molecular-grade water to a 15 ml conical tube.
   b Add 0.1 ml of 10 N NaOH (1/100 dilution).
   c Vortex to mix.

Procedure

1 Prepare the LNA1/LNB1 mix according to the number of reactions

Reagent	1 rxn	12 rxn*	24 rxn*	48 rxn*	96 rxn*
LNA1	36.8 µl	550 µl	1100 µl	2200 µl	4400 µl
LNB1	8.2 µl	100 µl	200 µl	400 µl	800 µl

*Includes 20% excess.

2 Thoroughly vortex until LNA1/LNB1 mix is homogenized.

3 Label a new deep well plate LNP.

4 Pour the LNA1/LNB1 mix into a trough.

5 Transfer 45 µl LNA1/LNB1 mix to each well.

6 Add 20 µl dsDNA to each well.

7 Mix at 1800 rpm for 30 minutes.

8 Pulse centrifuge. *To prevent magnetic bead aggregation, do not centrifuge longer than a pulse.*

9 Place on a magnetic stand until the liquid is clear. Keep the plate on the stand during the following steps.

10 Remove and discard all supernatant from each well.

11 Discard the tips in an appropriate hazardous waste container. Change tips between samples.

12 Wash 2 times, as follows.

   a Add 45 µl LNW1.
   b Remove from the magnetic stand.
   c Shake at 1800 rpm for 5 minutes.
   d Briefly centrifuge at 280 × g.
   e Place on a magnetic stand until the liquid is clear.
   f Remove and discard all supernatant.

13 Add 30 µl 0.1 N NaOH to each well.

14 Remove from the magnetic stand.

15 Mix at 1800 rpm for 5 minutes.

16 Make sure that the contents of each well is resuspended.

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 for the MiSeq System

Preparation

1. Make sure that the MiSeq System is ready for use.
2. If needed, thaw the plate at room temperature, and then vortex.
3. Save the following POOL program on a thermal cycler with a heated lid:
   - 96°C for 3 minutes
   - 4°C for 5 minutes
   - Hold at 4°C

Procedure

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	10 µl	
HT1	90 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	Pre-Implantation Genetic Screening
RSB	Resuspension Buffer
SCT	Single Cell Tagment
SLB	Single Lysis Buffer
TD	Tagment DNA Buffer
VTA	VeriSeq Tagment Amplicon Plate

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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