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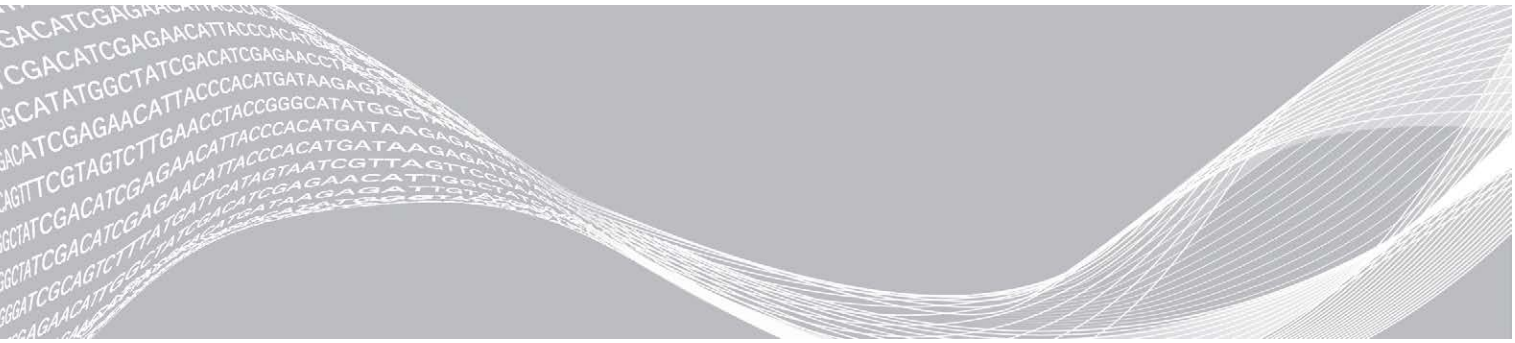
VeriSeq PGS Library Prep

Reference Guide



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Revision History

Document	Date	Description of Change
Document # 15052877 v05	August 2021	• Updated normalized library preparation information.
Document # 15052877 v04	September 2020	• Reconfigure introduction. • Added multiplexing information. • Updated Warnings and Precautions. • Updated Qubit 2.0 or greater Fluorometer in Consumables and Equipment. • Added dye aliquot best practices to Quantification Methods and Qubit Method. • Added Quant-iT™ dsDNA HS steps to Quant-iT Method. • Added cluster density information to Pooling Libraries for the MiSeq System. • Updated instructions for preparing ATM and TD in Tagment Input DNA. • Removed 1 rxn values from table in Normalize Libraries Preparation. • Removed duplicate step of adding 10 µl of each λ DNA standard to separate wells during the Quant-iT Method procedure.
Material # 20007092 Document # 15052877 v03	April 2016	• Added links to documents in Additional Resources. • Edited Quantify Unpurified SurePlex Products description—Quant-iT HS DNA Assay Kit recommendation. • Corrected step in Normalize Libraries section to vortex LNA1/LNB1 mix. • Removed SCT and SLB from Acronyms list.
Material # 20004574 Document # 15052877 v02	January 2016	• Added volume for SurePlex amplification product in Tagmentation of SurePlex WGA Product section. • Corrected index adapter instructions in PCR Amplification section. • Added volume for PCR product in PCR-Clean-Up Procedure section.
Material # 20000347 Document # 15052877 v01	September 2015	• Updated to new library prep style.
Part # 15052877 Rev. A	June 2014	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

The VeriSeq PGS Library Prep uses an engineered transposome to simultaneously fragment and tag ("tagment") SurePlex™ input DNA, adding unique adapter sequences in the process. A limited-cycle PCR reaction uses the adapter sequences to amplify the SurePlex insert DNA. The PCR reaction also adds index sequences to both ends of the DNA, enabling single-indexed sequencing of up to 12 pooled libraries and dual-indexed sequencing of up to 24 pooled libraries on the MiSeq System.

This protocol describes the procedures for preparing sequencing libraries of PGS samples.

This protocol explains how to:

- Prepare up to 12 single-index OR up to 24 dual-index, single-read libraries from SurePlex amplification products
- Prepare multiplexed library pools for cluster generation and sequencing on the MiSeq® System.

The VeriSeq™ PGS Library Preparation protocol offers:

- Fast and easy library preparation
- Low DNA input (only 1 ng unpurified SurePlex double-stranded DNA (dsDNA) required)
- Master mixed reagents to reduce reagent containers, pipetting, and hands-on time
- Innovative sample normalization that eliminates the need for library quantification before sample pooling and sequencing

Recommendations

The VeriSeq PGS solutions are manufactured in batches, called lots, in accordance with strict quality standards. Each component or reagent in each lot is tested to work with other reagents in the kit. To ensure proper performance, do not interchange, mix, or combine reagents from different kits and lots.

Library Pooling and Multiplexing

- Using alternative indexes or indexes combination can compromise your test results.
- Do not exceed the maximum multiplexing capacity of 24 pooled libraries. Exceeding the maximum of 24 pooled libraries causes an error in the MiSeq Control Software and prevents a VeriSeq PGS run from starting.
- Higher level of multiplexing might affect the data quality, resulting in poorer overall kit performance.

Warnings and Precautions

- Check the documentation and safety data sheets (SDS) for guidelines on handling, preparing, and disposing kit components, intermediate mixtures, or wastes. For more information, see the SDS for this kit at support.illumina.com/sds.html.
- Avoid cross-contamination during the library preparation steps. Contamination of reagents can compromise your test results.
  - Discard used materials without passing them over open containers.

  - To minimize the risk of contamination, label and use separate reservoirs for each reagent.
  - Make sure that index containers do not contact each other.
  - Change caps between each index use.
  - Change gloves if they come in contact with indexes.
- Incubation times, incubation temperature, and pipetting volumes that differ from those specified in this protocol can lead to suboptimal library quality and compromise your test results.
- Shipping conditions might differ from storage conditions. Some kit components must be brought to room temperature before use.
- All components have an expiration date. Do not use kit components beyond the expiration date printed on component labels.
- Do not add sodium azide as a preservative to any of the components.
- This kit is intended for use by qualified laboratory staff.

DNA Input Recommendations

The VeriSeq PGS solution begins with DNA amplification from a single embryonic cell or multiple embryonic cells using the SurePlex DNA Amplification Kit. For more information, see the *SurePlex Summary Protocol (part # 15053626).*

The VeriSeq PGS Library Prep Kit protocol is optimized for 1 ng of input SurePlex amplified DNA. Illumina® strongly recommends quantifying the starting SurePlex amplified dsDNA. Steps for quantification are included in this protocol.

DNA Input Quantification

VeriSeq PGS Library Prep uses an enzymatic DNA fragmentation step and thus can be more sensitive to DNA input compared to mechanical fragmentation methods. The ultimate success of the assay strongly depends on using an accurately quantified amount of input DNA library. Therefore, the correct quantification of the DNA library is essential.

To obtain an accurate quantification of the DNA library, quantify the starting DNA library using a fluorometric based method specific for duplex DNA such as the Quant-iT HS DNA Assay Kit. Avoid methods that measure total nucleic acid content (eg, NanoDrop or other UV absorbance methods) because common contaminants such as ssDNA, RNA, and oligos are not substrates for the VeriSeq PGS assay. These contaminants can cause an overestimation of viable material, resulting in poorer overall performance.

Additional Resources

The following documentation is available for download from the Illumina website.

Resource	Description
VeriSeq PGS Library Prep Protocol Guide (document # 1000000000812)	Provides only protocol instructions. The protocol guide is intended for experienced users. For new or less experienced users, see the VeriSeq PGS Library Prep Reference Guide.
VeriSeq PGS Library Prep Checklist (document # 1000000000813)	Provides a checklist of the protocol steps. The checklist is intended for experienced users. For new or less experienced users, see the VeriSeq PGS Library Prep Reference Guide.

Resource	Description
BlueFuse Workflow Manager Quick Reference Guide (document # 15056206)	Provides information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and recording parameters for your sample plate.
SurePlex Summary Protocol (document # 15053626)	Describes the protocol for cell lysis and DNA amplification from an embryo biopsy using the SurePlex DNA Amplification Kit.
BlueFuse Multi Software Reference Guide (document # 15053620)	Provides information about the BlueFuse® Multi sequencing data analysis tool.
MiSeq Reagent Kit v3-PGS Reagent Prep Guide (document # 15055896)	Describes the method for the preparation of reagents from the VeriSeq PGS Kit-MiSeq.
VeriSeq PGS Guidance Technical Note (document # 1000000138837)	Provides guidance on concentration requirements of undiluted SurePlex samples for VeriSeq PGS library preparation, in addition to the quality control metrics supplied in BlueFuse Multi and Sequence Analysis Viewer (SAV).

Visit the VeriSeq PGS Library Prep support pages on the Illumina website for requirements and compatibility, additional documentation, software downloads, online training, frequently asked questions, and best practices.

For more information about updates to the MiSeq Control Software, visit the MiSeq support pages on the Illumina website.

Protocol

Introduction

This chapter describes the VeriSeq PGS Library Prep protocol.

- Follow the protocols in the order shown, using the specified volumes and incubation parameters. Incubation times, incubation temperatures, and pipetting volumes other than those specified or **missing any centrifuge or mixing steps will lead to suboptimal** DNA sequencing libraries and compromise your test results.
- Review Best Practices from the VeriSeq PGS support page on the Illumina website.
- Review your kit contents and make sure that you have all required consumables and equipment.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.
- Do not reuse index adapter tube caps. Use the index adapter replacement caps supplied in the kit.

Sealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Mixing steps
  - Centrifuge steps
  - Vortexing steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.

- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).

Handling Beads

- Pipette bead suspension slowly.
- When mixing, mix thoroughly.
- To remove the supernatant, use a multichannel pipette and 200 µl barrier pipette tips with the plunger down. Place the tip opposite the aggregated beads and aspirate the supernatant. Dispense any aspirated beads back into the plate, and then leave the plate on the magnet for 2 minutes or until the liquid is clear.
- To avoid sample loss, confirm that no beads remain in the pipette tips after resuspension and mixing steps.
- When washing beads:
  - Use the appropriate magnet for the plate. For more information, see *Consumables and Equipment* on page 22.
  - Dispense the liquid on the opposite side of the bead pellet so that the beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

The following diagram illustrates the workflow using the VeriSeq PGS Library Prep Kit. This protocol is performed in the post-PCR lab.

Figure 1 Workflow Diagram

Quantify Unpurified SurePlex Products

Library preparation uses an enzymatic DNA fragmentation step, which can be more sensitive to dsDNA input compared to mechanical fragmentation methods. The success of the assay depends on using accurately quantified input dsDNA.

Avoid methods that measure total nucleic acid content (eg, NanoDrop or UV absorbance methods). Common contaminants such as ssDNA, RNA, and oligonucleotides are not substrates for the VeriSeq PGS assay.

Reduced quantification accuracy dramatically lowers the number of clusters generated during sequencing, resulting in less data per sample. This reduction in data significantly impacts aneuploidy calls for affected samples.

Use 1 of the following kits to quantify the dsDNA content of unpurified SurePlex products.

- The Qubit dsDNA HS Assay Kit (tube format) measures dsDNA concentration in individual tubes and requires a Qubit 2.0 or later.
- The Quant-iT HS DNA Assay Kit (96-well plate format) measures dsDNA concentration in a microplate and requires a microplate reader. This is the recommended method to use to accurately and reproducibly quantify SurePlex PCR product while reducing hand-on time.

NOTE

The Quant-iT HS DNA Assay Kit leads to more consistent libraries.

Consumables and Equipment

- 96-well PCR plate
- Molecular grade water
- For tube format:
  - Qubit dsDNA HS Assay Kit
  - Qubit Assay Tubes (1 tube per sample)
  - Qubit 2.0 or greater Fluorometer
  - Adhesive PCR seal
- For plate format:
  - Quant-iT HS dsDNA Assay Kit
  - 96-well nonbinding black microplate
  - Microplate reader
  - Adhesive PCR seal

About Reagents

- Diluted Quant-iT dsDNA HS reagent and Qubit dsDNA HS reagent are stable for at least 3 hours at room temperature, kept away from light.

Preparation

1 Protect the Qubit dsDNA HS reagent from light.

2 Allow quantification reagents to reach room temperature.

Procedure

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 to the wells containing molecular-grade water.

5 Seal the plate and briefly vortex to mix.

6 Centrifuge at 280 × g for 1 minute.

7 Set aside on wet ice.

Quantification Methods

NOTE

Review the manufacturer instruction manuals and safety recommendations before quantifying the samples.

Keep the Qubit or Quant-iT dsDNA HS reagent concentrate and the working solution away from light.

Make sure that all quantification reagents are at room temperature before performing the readings.

Dye should not undergo multiple freeze and thaw cycles. Best practice is to aliquot it into single-use aliquots.

Qubit Method

1 Prepare the working solution according to the manufacturer instructions.

2 To calibrate the Qubit fluorometer, add 10 µl of each standard to 190 µl of working solution. For more information, see the Qubit dsDNA HS Assay Kit user guide.

3 Add 10 µl of the 1/10 diluted SurePlex sample and 190 µl working solution to each assay tube. Briefly vortex to mix.

NOTE

If necessary, adjust to 1–20 µl of sample and 180–199 µl of working solution for a total volume of 200 µl.

4 For optimal fluorescence, incubate the assay tubes for 2 minutes.

5 Calculate the concentration of each 1/10 diluted SurePlex sample as described by the Qubit dsDNA HS Assay Kit user guide. Convert the units to ng/µl.

Quant-iT Method (Recommended Quantification Method)

1 Dispense the Quant-iT™ dsDNA HS reagent in single-use aliquots (standard 8-well PCR strip format) to prevent freeze and thaw cycles. Protect the aliquots from light. Store the aliquots at 2-8°C upon use. Prior to use, bring one aliquot to temperature in the dark.

2 Prepare the working solution according to the manufacturer instructions by aliquoting the volume of Quant-iT™ dsDNA HS buffer required for the experiment and bringing it to room temperature (never use Quant-iT™ buffer cold). Store the Quant-iT™ buffer bulk cold.

3 Add 190 µl working solution to the microplate wells that will contain samples or controls.

4 Add 10 µl of each 1/10 diluted SurePlex sample to separate wells. Create duplicates or triplicates of the unknown samples. Pipette to mix.

NOTE

If necessary, adjust to 1–20 µl of sample and 180–199 µl of working solution for a total volume is 200 µl.

5 Add 10 µl of each λ DNA standard to separate wells. Do not introduce nuclease into the tubes of DNA standard as you remove aliquots for the assay. Create duplicates or triplicates of the standards. Pipette to mix.

6 Measure the fluorescence using a microplate reader.
Standard fluorescein wavelengths (excitation/emission at ~480/530 nm) are appropriate for this dye. The fluorescence signal is stable for 3 hours at room temperature, protected from light.

7 Calculate the concentration of each 1/10 diluted SurePlex sample using the plate reader software. Convert the units to ng/µl.
Use a standard curve to determine the DNA concentration. For λ DNA standards, plot amount vs. fluorescence and fit a straight line through the data points.

NOTE

The fluorescence of the Quant-iT dsDNA HS reagent bound to dsDNA is linear from 0 ng to 100 ng. For best results at the low end of the standard curve, force the line through the background point (or through 0, if background has been subtracted).

Template Dilution to 0.2 ng/µl

NOTE

The SurePlex samples and controls must be diluted according to the BlueFuse Workflow Manager calculations. Make sure to use the 1/10 diluted SurePlex samples and controls from the previous steps. Do not use stock or undiluted stock material.

1 Using BlueFuse Workflow Manager, enter the calculated dsDNA concentration (ng/µl) of the 1/10 diluted SurePlex sample concentration into the **1/10 dsDNA (ng/ul)** column of the VeriSeq PGS–MiSeq Assay Plate.
The BlueFuse Workflow Manager calculates the molecular-grade water required to prepare 5 µl of diluted sample at the final concentration of 0.2 ng/µl.

2 According to the BlueFuse Workflow Manager calculations, add the appropriate volumes of 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.

NOTE

Do not pipette to mix. This method leads to poor index representation.

5 Set aside on wet ice.

Tagment Input DNA

In this step, the SurePlex amplification product is tagmented (tagged and fragmented) by the VeriSeq PGS transposome.

Consumables

- ATM (Amplicon Tagment Mix)
- TD (Tagment DNA Buffer)
- NT (Neutralize Tagment Buffer)
- SurePlex amplification product (diluted at 0.2 ng/µl)
- 96-well PCR plate

- Adhesive PCR seal
- PCR 8-tube strips

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
ATM	-25°C to -15°C	Thaw on ice for 20 minutes. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly. Do not overcentrifuge.
TD	-25°C to -15°C	Thaw on ice for 20 minutes. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly. Do not overcentrifuge.
NT	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Briefly vortex, and then briefly centrifuge.

Procedure

Tagmentation of SurePlex WGA Product

1 Label a new PCR plate VTA (VeriSeq Tagment Amplicon Plate).

2 Calculate the total volume of TD for all reactions. Using a multichannel pipette, divide the volume equally among the wells of a PCR 8-tube strip, or use a reservoir.

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 Make sure that each well contains a volume of 20 µl. Record any nonuniform volumes.

9 Immediately place on a thermal cycler with a heated lid and run the following program:
- 55°C for 5 minutes
- Hold at 10°C

NOTE

To prevent overtagmentation, proceed ***immediately*** to the next step.

Neutralization of the Tagmented SurePlex DNA

1 Calculate the total volume of NT buffer required for all reactions. Using a multichannel pipette, divide the volume equally among the wells of a PCR 8-tube strip.

2 Add 5 µl NT Buffer to each well.

3 Mix at 1800 rpm for 1 minute.

4 Centrifuge at 280 × g for 1 minute.

5 Make sure that each well contains a volume of 25 µl. Record any nonuniform volumes.

6 Incubate at room temperature for 5 minutes.

NOTE

Proceed ***immediately*** to the next step.

Amplify Tagmented DNA

In this step, the tagmented DNA is amplified using a limited-cycle PCR program. The PCR step adds Index 1 (i7) and Index 2 (i5) adapters. Use the full amount of input DNA to ensure high-quality sequencing results. Do not add extra PCR cycles.

Consumables and Equipment

- NPM (Nextera® PCR Master Mix)
- Index 1 primers (N701 to N712)
- Index 2 primers (S503 and S504)
- TruSeq® Index Plate Fixture
- Adhesive PCR seal
- Plate sealer

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
NPM	-25°C to -15°C	Thaw on ice for 20 minutes. Invert each tube to mix. Centrifuge briefly using a 1.7 ml Eppendorf tube.
Index adapters (i5 and i7)	-25°C to -15°C	Thaw at room temperature for 20 minutes. Invert each tube to mix. Centrifuge briefly using a 1.7 ml Eppendorf tube.

2 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

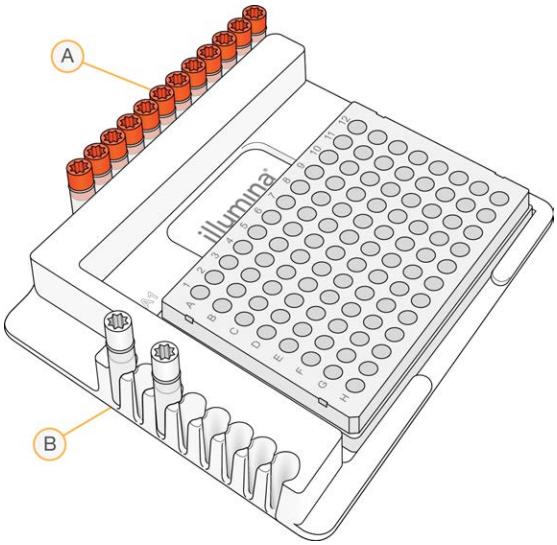
1 Print the sample assay plate layout using the BlueFuse Workflow Manager.

NOTE

If less than a full set of libraries is pooled for sequencing, use the default index layout and use the BlueFuse Workflow Manager to make sure that the correct index adapters are selected.

2 Arrange the index primers in the TruSeq Index Plate Fixture, as follows:
- Index 1 (i7) adapters: N701–N712 in columns 1–12
- Index 2 (i5) adapters: S503 in row A, S504 in row C

Figure 2 TruSeq Index Plate Fixture (24-Plex Libraries)



A Columns 1–12: Index 1 (i7) adapters (orange caps)
B Rows A and C: Index 2 (i5) adapters (white caps)

3 Place the plate on the TruSeq Index Plate Fixture.

4 Add index adapters according to the sample assay plate layout.
- Add 5 µl of each Index 1 (i7) adapter to each column.
- Add 5 µl of each Index 2 (i5) adapter to each row.

NOTE

To avoid cross-contamination, change tips between each well. Discard the original index caps and apply the new caps provided in the kit. After use, remove all index adapter tubes from the working area.

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 Make sure that each well contains a volume of 50 µl. Record any nonuniform volumes.

9 Place on the thermal cycler and run the saved program.

SAFE STOPPING POINT

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

Clean Up PCR

This step uses AMPure XP beads to purify amplified DNA from the samples, and provides a size selection step that removes both the short library fragments and Primers from the population.

NOTE

After PCR cleanup, samples are double-stranded DNA of a few hundred base pairs, which can be quantified using a Bioanalyzer chip. Optionally, analyze samples using qPCR or library normalization. For more information, see the *Sequencing Library qPCR Quantification Guide (part # 11322363)*.

Consumables

Item	Part Number	Storage	Supplied By
Resuspension Buffer (RSB)	15027913	-25°C to -15°C	Illumina
AMPure XP beads		2°C to 8°C	User
96-well storage plates, round well, 0.8ml (deep well plate)		Room temperature	User
PCR Plates		Room temperature	User
Adhesive PCR seal		Room temperature	User
50 ml conical tube		Room temperature	User
Molecular Grade Water		Room temperature	User
Absolute ethyl alcohol (EtOH) (to prepare fresh 80% EtOH)		Room temperature	User

About Reagents

- Make sure that AMPure XP beads are mixed thoroughly and brought to room temperature before use. Homogeneous resuspension is essential for consistent cluster density on the flow cell.
- Prepare fresh ethanol for the wash steps. Ethanol can absorb water from the air impacting your results.
- Assemble reaction at room temperature, use of any other temperature **will** lead to suboptimal DNA sequencing libraries and **will** compromise your test results.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
RSB	-25°C to -15°C	Thaw at room temperature. Let stand for 30 minutes to bring to room temperature.
AMPure XP Beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Thoroughly vortex the AMPure XP beads.

3 Prepare fresh 80% EtOH from absolute ethyl alcohol.

WARNING

Ethanol is highly flammable, keep away from all sources of ignition at all times.

Procedure

1 Centrifuge the VTA plate at 280 × g for 1 minute to collect condensation.

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.

WARNING

If the PCR product volume is insufficient (< 45 µl), adjust the volume of the AMPure XP beads to a 1:1 ratio before dispensing the PCR product.

5 Mix at 1800 rpm for 1 minute.

6 Incubate at room temperature for 5 minutes. Do not shake the plate.

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

8 Place on a magnetic stand and wait until the liquid is clear (~2 minutes). Keep the plate on the stand during the following steps.

9 Discard the supernatant from each well.

10 Wash 2 times, as follows.

a Using a multichannel pipette, add 200 µl freshly prepared 80% EtOH to each row on the opposite side of the aggregated beads, being careful not to disturb the bead pellet at the bottom of the well. Do not resuspend the beads.

b Incubate on the magnetic stand for ≤ 30 seconds. Start the timer after dispensing 80% EtOH into the first well being careful to not exceed 30 seconds for each row.

c Immediately remove and discard all supernatant from each well and discard to appropriate waste container.

WARNING

Do not incubate the plate for more than 30 seconds. Longer incubation time results in reduced yield, inconsistent size selection, and tailing into the larger size range, which can lead to suboptimal DNA sequencing of libraries.

11 Using a multichannel pipette and fine pipette tips, remove residual EtOH from each well.

12 Air-dry on the magnetic stand for 15 minutes, or until beads are completely dry.

WARNING

Do not over dry the beads. Beads that are over-dried appear cracked.

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 and wait until the liquid is clear (~2 minutes).

18 Transfer 45 µl of each supernatant from each well to a new PCR plate.

19 **[Optional]** Run a Quality Control check to determine the success of the library preparation. For more information on measuring dsDNA concentration, see *PCR Clean-Up Quality Control* on page 20.

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

The quantity of each library is normalized to ensure more equal library representation in the pooled library.

NOTE

The normalized library is single-stranded DNA, which cannot be resolved well on an agarose gel or a Bioanalyzer chip. Use qPCR to quantify the normalized library. For more information, see the *Sequencing Library qPCR Quantification Guide (part # 11322363)*.

Consumables

Item	Part Number	Storage	Supplied By
Library Normalization Additives 1 (LNA1)	15025391	-25°C to -15°C	Illumina
Library Normalization Beads 1 (LNB1)	15022566	2°C to 8°C	Illumina
Library Normalization Wash 1 (LNW1)	15022565	2°C to 8°C	Illumina
Library Normalization Storage Buffer 1 (LNS1)	15025139	15°C to 30°C	Illumina
10 N NaOH (to prepare fresh 0.1 N NaOH)		Room temperature	User
Molecular Grade Water		Room temperature	User
96-well storage plates, round well, 0.8ml (deep well plate)		Room temperature	User
96-well PCR Plates		Room temperature	User
15 ml conical tubes		Room temperature	User
Adhesive PCR seal		Room temperature	User

About Reagents

- Make sure that LNB1 is mixed well before use. Homogeneous resuspension is essential for consistent cluster density on the flow cell. Only use a P1000 pipette to resuspend LNB1.
- Mix only the amounts of LNA1 and LNB1 required for the current assay. To preserve stability, do not freeze or store premixed LNB1 with LNA1.
- Store remaining LNA1 and LNB1 separately at their respective, required temperatures.
- Assemble reaction at room temperature, use of any other temperature **will** lead to suboptimal DNA sequencing libraries and **will** compromise your test results.

Preparation

WARNING

LNA1 and LNW1 contain 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.html.

1 Prepare the following consumables. Once open, store separately at their respective recommended temperatures.

Reagent	Storage	Instructions
LNA1	-25°C to -15°C	Prepare under a fume hood. Thaw at room temperature. Let stand for 30 minutes to bring to room temperature. If needed, use a 20°C to 25°C water bath. Vortex vigorously, and then inspect in front of a light to make sure that all precipitate has dissolved.
LNW1	2°C to 8°C	If refrigerated, thaw at room temperature, and let stand for 30 minutes. If needed, use a 20°C to 25°C water bath.
LNB1	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex vigorously for at least 1 minute, and then invert to resuspend. Make sure that no pellet is present at the bottom of the tube.
LNS1	15°C to 30°C	If refrigerated, thaw at room temperature and let stand for 30 minutes. Invert to mix.

2 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

NOTE

Conduct all normalization steps at room temperature.

WARNING

Resuspend the LNB1 bead pellet completely. The use of a P1000 ensures that the beads are homogeneously resuspended and that there is no bead mass at the bottom of the tube. This step is essential for achieving consistent cluster density on the flow cell.

1 In a new 15 ml conical tube, prepare the LNA1/LNB1 mix according to the number of reactions.

Reagent	8 rxn*	12 rxn*	24 rxn*	48 rxn*	96 rxn*
LNA1	367 µl	550 µl	1100 µl	2200 µl	4400 µl
LNB1	67 µl	100 µl	200 µl	400 µl	800 µl

*Includes 20% excess.

2 Vortex thoroughly until LNA1/LNB1 mix is homogenized.

3 Label a new deep-well plate LNP (Library Normalization Plate).

4 Pour the LNA1/LNB1 mix into a reservoir.

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

6 Add 20 µl dsDNA from the Clean-Up PCR procedure to each well.

7 Mix at 1800 rpm for 30 minutes.

NOTE

Incubation of greater than or less than 30 minutes can affect library representation and cluster density.

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

9 Place on a magnetic stand and wait until the liquid is clear (~2 minutes). 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.

WARNING

Do not aspirate beads during this step as bead loss results in reduced yields. If any beads are inadvertently aspirated into the tips, dispense the beads back into the plate and let the plate rest on the magnet for 2 minutes. After incubation, confirm that the supernatant has cleared, and then resume the removal of the supernatant.

12 Wash beads as follows.

a Keep on the magnetic stand and add 45 µl LNW1 to each well.
b Seal and shake at 1800 rpm for 5 minutes.
c To prevent magnetic bead aggregation, pulse centrifuge to collect any droplets.
d Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
e Remove and discard all supernatant from each well.

13 Wash beads a **second** time.

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.

NOTE

Incubate beads with NaOH for five minutes. Only in the event of non-resuspended beads should an additional five minute incubation be performed.

NOTE

Make sure that the contents of each well is resuspended. If any wells are not resuspended, pipette to mix, and then shake for another 5 minutes.

17 Centrifuge at 280 × g for 1 minute.

18 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

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 the PCR plate containing LNS1 and supernatant at 280 × g for 1 minute.

22 **[Optional]** Perform quality control using qPCR. For more information, see the *Sequencing Library qPCR Quantification Guide (document # 11322363)*.

NOTE

The normalized libraries consist of single-stranded DNA (ssDNA), which cannot be resolved on agarose gels or Bioanalyzer chips.

SAFE STOPPING POINT

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

Pool Libraries for the MiSeq System

Equal volumes of normalized libraries are combined, diluted in HT1, and heat-denatured before being transferred to the flow cell for cluster generation and sequencing.

Consumables

Item	Part Number	Storage	Supplied By
HT1 (Hybridization Buffer)	15027041	-25°C to -15°C	Illumina
MiSeq reagent tray	15049598	-25°C to -15°C	Illumina
MiSeq flow cell	15049599	2°C to 8°C	Illumina
PR2, Incorporation Buffer	15041807	2°C to 8°C	Illumina
Eppendorf DNA LoBind 1.5 mL Microcentrifuge tube		Room temperature	User
PCR 8-tube strip or tube		Room temperature	User
Ice bucket		Room temperature	User

Preparation

1 Generate your MiSeq sample sheet with BlueFuse Workflow Manager.

2 Make sure that the MiSeq system is ready for use.

3 Prepare the following consumables.

WARNING

Certain sequencing components contain 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 content in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at support.illumina.com/sds.html.

Reagent	Storage	Instructions
MiSeq reagent tray	-25°C to -15°C	Thaw in a room temperature water bath for 1 hour, or overnight at 2°C to 8°C. Do not allow the water to exceed the maximum water line printed on the reagent cartridge. Invert 10 times to mix, visually inspect all positions are thawed.
MiSeq Flow Cell	2°C to 8°C	Rinse the Flow Cell with deionized water and clean with ethanol ensuring there are no smudges.
HT1	-25°C to -15°C	Thaw at room temperature and invert to mix. Set aside at 2°C to 8°C.

4 Save the following DENATURE program on a thermal cycler with a heated lid:
   - 96°C for 3 minutes
   - 4°C for 5 minutes
   - Hold at 4°C

Procedure

NOTE

Prepare a fresh library pool for each use.

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.
The recommended cluster density of the MiSeq VeriSeq PGS workflow ranges from 1,100 K/mm2 to 1,600 K/mm2. Adjust the volume of the library pool to keep the cluster density in the recommended cluster density range. Under- and over-clustering might impact the quality of the results.
[For experienced users] Reduce the volume of the library pool to < 15 µl to prevent overclustering (cluster density ≥ 1400K/mm^2). The optimal cluster density ranges from 1200–1400K/mm^2.

5 Add 85 µl HT1.
If the volume of library pool was reduced to prevent over clustering, adjust the volume of added HT1 so that the total volume in the PCR tube is 100 µl.

NOTE

If a repeat run is required, prepare aliquots of the library pool using the remaining library pool mixture. Store the pool for up to 7 days at -25°C to -15°C.

If library pools are prepared simultaneously, store the second library without HT1 for up to 7 days at -25°C to -15°C.

6 Record the volumes of library pool and HT1.

7 Gently vortex and centrifuge the pool/HT1 mixture.

8 Immediately place on the preprogrammed thermal cycler and run the DENATURE program.

9 Transfer 600 µl of HT1 into a second clean LoBind tube. Set aside in an ice-water bath.

10 When the denaturation is complete, immediately transfer 100 µl of denatured pool/HT1 mixture to the LoBind tube with HT1. Set aside on wet ice.

WARNING

HT1 diluted/denatured library pools are not stable for storage. Only undiluted libraries without HT1 are stable for up to 7 days when stored at -25°C to -15°C.

Perform heat denaturation immediately before loading the library into the MiSeq reagent cartridge.

11 Sequence your library according to the *MiSeq System Guide (document # 15027617)*.

Supporting Information

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
ATM	Amplicon Tagment Mix
HT1	Hybridization Buffer (Hyb 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
TD	Tagment DNA Buffer
VTA	VeriSeq Tagment Amplicon Plate

PCR Clean-Up Quality Control

To determine the success of the library prep, perform 1 quality control method from the following options:

- Use the Qubit dsDNA HS Assay Kit or the Quant-iT HS Assay Kit to measure the concentration of the library prep. Avoid methods that measure total nucleic acid content (eg, NanoDrop or UV absorbance methods). Before proceeding, make sure that there is at least 1.5 ng/µl of sample material.
- Quality control the PCR-clean up samples using a High Sensitivity Bioanalyzer chip. You can analyze samples with qPCR before library normalization. For more information, see the *Sequencing Library qPCR Quantification Guide (document # 11322363)*.

VeriSeq PGS Library Prep Kit Contents

Reagents for VeriSeq PGS are sold under catalog number RH-101-1001 to include enough reagents for processing 96 samples.

The SurePlex DNA Amplification System kit (PR-40-415101-00) and the MiSeq Reagent Kit v3-PGS (RH-102-1001) are also available to order separately.

Kit Name	Catalog #	# Samples	# Indexes
VeriSeq PGS Kit	RH-101-1001	96	24

VeriSeq PGS Kit Contents

Quantity	Consumable
2	SurePlex DNA Amplification System
1	VeriSeq Library Prep Kit-PGS
1	VeriSeq Index Kit PGS
4	MiSeq Reagent Kit v3-PGS
1	Index Adapter Replacement Caps

TruSeq Index Plate Fixture VeriSeq PGS Library Prep

Use the index plate fixture to arrange the index primers during the PCR Amplification steps.

Consumable	Catalog #
TruSeq Index Plate Fixture Kit (2 Fixtures)	FC-130-1005

SurePlex DNA Amplification System Kit, Store at -25°C to -15°C

Quantity	Reagent
1	Cell Extraction Buffer
1	Extraction Enzyme Dilution Buffer
1	Cell Extraction Enzyme
1	SurePlex Pre-Amp Enzyme
1	SurePlex Amplification Buffer
1	SurePlex Amplification Enzyme
2	Nuclease-free water

VeriSeq Library Prep Kit-PGS Box 1, Store at -25°C to -15°C

Quantity	Reagent	Description
1	ATM	Amplicon Tagment Mix
2	TD	Tagment DNA Buffer
1	NPM	Nextera PCR Master Mix
4	RSB	Resuspension Buffer
1	LNA1	Library Normalization Additives 1
2	LNW1	Library Normalization Wash 1
1	HT1	Hybridization Buffer (Hyb Buffer)

VeriSeq Library Prep Kit-PGS Box 2, Store at 2°C to 8°C

NOTE

This box is shipped at room temperature. Store at 2°C to 8°C upon arrival.

Quantity	Reagent	Description
1	NT	Neutralize Tagment Buffer
1	LNB1	Library Normalization Beads 1
1	LNS1	Library Normalization Storage Buffer 1

VeriSeq Index Kit-PGS, Store at -25°C to -15°C

Quantity	Component
4	Index Primers, S503, S504
12	Index Primers, N701 to N712

Index Adapter Replacement Caps, Store at Room Temperature

Quantity	Component
48 orange, 32 white	Index Adapter Replacement Caps

MiSeq Reagent Kit v3-PGS Box 1, Store at -25°C to -15°C

Quantity	Component
1	Reagent Tray
1	HT1 (Hyb Buffer)

MiSeq Reagent Kit v3-PGS Box 2, Store at 2°C to 8°C

Quantity	Component
1	PR2 Incorporation Buffer
1	MiSeq Flow Cell

Consumables and Equipment

Make sure that you have the required consumables and equipment before starting the protocol.

Some items are required only for specific workflows. These items are specified in separate tables.

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Consumables

Consumable	Supplier
10 µl barrier pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipette	General lab supplier
50 µl barrier pipette tips	General lab supplier
50 µl multichannel pipette	General lab supplier

Consumable	Supplier
50 µl single channel pipettes	General lab supplier
200 µl barrier pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
1000 µl barrier pipette tips	General lab supplier
1000 µl single channel pipettes	General lab supplier
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859
96-well nonbinding black microplates	Greiner, part # 655900
96-well PCR plates or Hard-Shell 96-well PCR plates (HSP plate)	Fisher Scientific, part # AB0600 or Bio-Rad, part # HSP-9601
Adhesive PCR seal	Fisher Scientific, part # AB0558
Adhesive seal roller	General lab supplier
Agencourt AMPure XP (60 ml kit)	Beckman Coulter, part # A63881
BioUltra, MBG, 10 N NaOH	Sigma-Aldrich, part # T72068
Eppendorf DNA LoBind Microcentrifuge Tubes, 1.5 ml	Fisher Scientific, part # 10051232
Ethanol 200 proof (absolute) for molecular biology	Sigma-Aldrich, part # E7023
Lens tissue, grade no. 105 paper	Fisher Scientific, part # 10644371
Molecular grade water or ultrapure water	Sigma-Aldrich, part # W4502 or Life Technologies, part # 10977035
PCR 8-tube strips	General lab supplier
PCR-grade water	General lab supplier
Pipetting reservoirs, multichannel, disposable	VWR, part # 89094-658
Qubit dsDNA HS Assay Kit	Life Technologies, part # Q32851
Qubit assay tubes	Life Technologies, part # Q32856
Quant-iT HS dsDNA Assay Kit, high-throughput	Life Technologies, part # Q33120
Sodium hypochlorite solution	Sigma-Aldrich, part # 239305
Sodium hydroxide solution, 10 N	Sigma-Aldrich, part # T72068
Tween 20	Sigma-Aldrich, part # P7949

Equipment

Equipment	Supplier
[Optional] Gel Electrophoresis System	N/A
High-Speed Microplate Shaker	VWR, catalog # 13500-890 (110 V/120 V) or 14216-214 (230 V) or BioShake IQ, part # 1808-0506
Magnetic stand-96	Fisher Scientific, part # AM10027
Microcentrifuge	General lab supplier
Microplate centrifuge	General lab supplier
Microplate reader (96-well plate fluorometer)	FLUOstar Omega microplate reader, BMG LabTech
Qubit 2.0 (or later) Fluorometer	Life Technologies, part # Q32866
Vortexer	General lab supplier

Thermal Cyclers

The following table lists the recommended settings for the Illumina recommended thermal cycler, and other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the VeriSeq PGS Library Prep protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate
Veriti® Thermal Cycler	Calculated	Heated	Nonskirted plate and tubes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Technical Support Telephone Numbers

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Australia	+61 1800 775 688	
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Canada	+1 800 809 4566	
China		+86 400 066 5835
Denmark	+45 80 82 01 83	+45 89 87 11 56
Finland	+358 800 918 363	+358 9 7479 0110
France	+33 8 05 10 21 93	+33 1 70 77 04 46
Germany	+49 800 101 4940	+49 89 3803 5677
Hong Kong, China	+852 800 960 230	
India	+91 8006500375	
Indonesia		0078036510048
Ireland	+353 1800 936608	+353 1 695 0506
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New Zealand	+64 800 451 650	
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Switzerland	+41 800 200 442	+41 56 580 00 00
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Thailand	+66 1800 011 304	
United Kingdom	+44 800 012 6019	+44 20 7305 7197
United States	+1 800 809 4566	+1 858 202 4566
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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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