VeriSeq™ PGS Library Prep Reference Guide

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Material # 20007092
Document # 15052877 v03
April 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html
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## Revision History

<table>
<thead>
<tr>
<th>Document</th>
<th>Date</th>
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<tr>
<td>Material # 20007092 Document # 15052877 v03</td>
<td>April 2016</td>
<td>• Added links to documents in Additional Resources.</td>
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<tr>
<td></td>
<td></td>
<td>• Edited Quantify Unpurified SurePlex Products description—Quant-iT HS DNA Assay Kit is recommended for ≥ 24 samples.</td>
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<td></td>
<td></td>
<td>• Corrected step in Normalize Libraries section to vortex LNA1/LNB1 mix.</td>
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<td>• Removed SCT and SLB from Acronyms list.</td>
</tr>
<tr>
<td>Material # 20004574 Document # 15052877 v02</td>
<td>January 2016</td>
<td>• Added volume for SurePlex amplification product in Tagmentation of SurePlex WGA Product section.</td>
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<tr>
<td></td>
<td></td>
<td>• Corrected index adapter instructions in PCR Amplification section.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Added volume for PCR product in PCR-Clean-Up Procedure section.</td>
</tr>
<tr>
<td>Material # 20000347 Document # 15052877 v01</td>
<td>September 2015</td>
<td>• Updated to new library prep style.</td>
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Chapter 1

Overview

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Introduction

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

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.
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.

Warnings and Precautions

- Check the documentation and safety data sheets 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.ilmn.
- Avoid cross-contamination during library preparation. Discard used materials without passing them over open containers. Labeling and using separate reservoirs for each reagent helps minimize the risk of contamination. Contamination of reagents can compromise your test results.
- Avoid index cross-contamination during library preparation. Make sure that index containers do not contact each other and change caps between index uses. If gloves come in contact with indexes, change gloves.
- Incubation times, incubation temperature, and pipetting volumes that are not 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 (e.g., 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.

<table>
<thead>
<tr>
<th>Resource</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VeriSeq PGS Library Prep Protocol Guide (document # 100000000812)</td>
<td>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.</td>
</tr>
<tr>
<td>VeriSeq PGS Library Prep Checklist (document # 100000000813)</td>
<td>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.</td>
</tr>
<tr>
<td>BlueFuse Workflow Manager Quick Reference Guide (document # 15056206)</td>
<td>Provides information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and recording parameters for your sample plate.</td>
</tr>
<tr>
<td>SurePlex Summary Protocol (document # 15053626)</td>
<td>Describes the protocol for cell lysis and DNA amplification from an embryo biopsy using the SurePlex DNA Amplification Kit.</td>
</tr>
<tr>
<td>BlueFuse Multi Software Reference Guide (document # 15053620)</td>
<td>Provides information about the BlueFuse Multi sequencing data analysis tool.</td>
</tr>
<tr>
<td>MiSeq Reagent Kit v3-PGS Reagent Prep Guide (document # 15055896)</td>
<td>Describes the method for the preparation of reagents from the VeriSeq PGS Kit-MiSeq.</td>
</tr>
<tr>
<td>VeriSeq PGS-MiSeq QC Assessment Guide</td>
<td>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).</td>
</tr>
</tbody>
</table>

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.
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 skipping any mixing steps can lead to suboptimal DNA sequencing libraries and compromise your test results.


- Review your kit contents and make sure that you have all required equipment and consumables.
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 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 magnet for 2 minutes or until the liquid is clear.
- To avoid sample loss, confirm that no beads remain in 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 31.
  - Dispense liquid on the opposite side of the bead pellet so that 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**

1. **Quantification**
   - Hands-on: 15 min
   - Total: 20 min
   - Reagents: Qubit dsDNA HS Assay Kit or Quant-IT HS dsDNA Assay Kit, Molecular grade water

2. **Tagmentation**
   - Hands-on: 10 min
   - Total: 20 min
   - Reagents: ATM, TD, NT

3. **PCR Amplification**
   - Hands-on: 10 min
   - Total: 45 min
   - Reagents: NPM, Index 1 primers, Index 2 primers

4. **PCR Clean-Up**
   - Hands-on: 15 min
   - Total: 30 min
   - Reagents: RSB, AMPure XP beads

5. **Library Normalization**
   - Hands-on: 30 min
   - Total: 1hour 20 min
   - Reagents: LNA1, LNB1, LNW1, LNS1, Fresh 0.1 N NaOH

6. **Library Pooling and Loading**
   - Hands-on: 10 min
   - Total: 10 min
   - Reagents: HT1
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 Fluorometer.
- The Quant-iT HS DNA Assay Kit (96-well plate format) measures dsDNA concentration in a microplate and requires a microplate reader. This method is recommended for ≥ 24 samples per processing batch.

**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 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 is stable for at least 3 hours at room temperature, kept away from light.

### Preparation

1. 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

Use either the Qubit or the Quant-iT method to quantify the dsDNA.

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.

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.
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 that will contain samples or controls.
3 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.

4 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.

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).

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**

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/µl) 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>-25°C to -15°C</td>
<td>Thaw on ice for 20 minutes. Invert to mix, or pulse centrifuge. Do not overcentrifuge.</td>
</tr>
<tr>
<td>TD</td>
<td>-25°C to -15°C</td>
<td>Let stand for 30 minutes to bring to room temperature. Briefly vortex, and then briefly centrifuge.</td>
</tr>
<tr>
<td>NT</td>
<td>2°C to 8°C</td>
<td>Let stand for 30 minutes to bring to room temperature. Briefly vortex, and then briefly centrifuge.</td>
</tr>
</tbody>
</table>

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.

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

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
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.

\[\text{NOTE}\]
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 the sealed plate at -25°C to -15°C for up to 7 days.
Clean Up PCR

This step uses AMPure XP beads to purify the library DNA, and provides a size selection step that removes 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
- RSB (Resuspension Buffer)
- AMPure XP beads
- 96-well deep well plate
- 96-well PCR plate
- 50 ml conical tube
- Adhesive PCR seal
- Absolute ethyl alcohol (EtOH) (to prepare fresh 80% EtOH)
- Molecular grade water

About Reagents
- Make sure that AMPure XP beads are completely mixed before use. Homogeneous resuspension is essential for consistent cluster density on the flow cell.

Preparation

1. Prepare the following consumables.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSB</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Let stand for 30 minutes to bring to room temperature.</td>
</tr>
<tr>
<td>AMPure XP Beads</td>
<td>2°C to 8°C</td>
<td>Let stand for 30 minutes to bring to room temperature.</td>
</tr>
</tbody>
</table>

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

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.

NOTE
If the PCR product volume is insufficient (< 45 µl), adjust the volume of AMPure XP beads to 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 collect droplets. 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 Using a multichannel pipette set to 95 µl, remove and discard all 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, opposite of the aggregated beads. 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.
   c Immediately remove and discard all supernatant from each well.

   NOTE 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.

   NOTE Do not overdry the beads. Beads that are overdried 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] Perform quality control. For more information, see PCR Clean-Up Quality Control on page 28.

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

This step normalizes the quantity of each library 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

- LNA1 (Library Normalization Additives 1)
- LNB1 (Library Normalization Beads 1)
- LNW1 (Library Normalization Wash 1)
- LNS1 (Library Normalization Storage Buffer 1)
- 10 N NaOH (to prepare fresh 0.1 N NaOH)
- 96-well deep well plate
- 96-well PCR plate
- 15 ml conical tube
- Adhesive PCR seal
- Molecular grade water

About Reagents

- Do not use a P200 pipette to handle LNB1.
- Mix only the amounts of LNA1 and LNB1 required for the current experiment. To preserve stability, do not freeze or mix LNB1 with LNA1 unless used immediately.
- Make sure that LNB1 is well mixed before use. Homogeneous resuspension is essential for consistent cluster density on the flow cell.
- Store remaining LNA1 and LNB1 separately at their respective temperatures.

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 the following consumables.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNA1</td>
<td>-25°C to -15°C</td>
<td>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.</td>
</tr>
<tr>
<td>LNW1</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Let stand for 30 minutes to bring to room temperature.</td>
</tr>
</tbody>
</table>
### Normalize Libraries

**Table of Contents**

- Reagent Storage Instructions
- 2° C to 8° C Let stand for 30 minutes to bring to room temperature.
- Vigorously vortex at least 1 minute, and then invert to resuspend. Make sure that no pellet is present at the bottom of the tube.
- 2° C to 8° C Let stand for 30 minutes to bring to room temperature.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNB1</td>
<td>2° C to 8° C</td>
<td>Let stand for 30 minutes to bring to room temperature. Vigorously vortex at least 1 minute, and then invert to resuspend. Make sure that no pellet is present at the bottom of the tube.</td>
</tr>
<tr>
<td>LNSI</td>
<td>2° C to 8° C</td>
<td>Let stand for 30 minutes to bring to room temperature.</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>1 rxn</th>
<th>12 rxn*</th>
<th>24 rxn*</th>
<th>48 rxn*</th>
<th>96 rxn*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNA1</td>
<td>36.8 µl</td>
<td>550 µl</td>
<td>1100 µl</td>
<td>2200 µl</td>
<td>4400 µl</td>
</tr>
<tr>
<td>LNB1</td>
<td>8.2 µl</td>
<td>100 µl</td>
<td>200 µl</td>
<td>400 µl</td>
<td>800 µl</td>
</tr>
</tbody>
</table>

*Includes 20% excess.

2 Thoroughly vortex 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 trough.

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

6 Add 20 µl dsDNA from the Clean-Up PCR step 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 Using a multichannel pipette, 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 to each well.
   
   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 and wait until the liquid is clear (~2 minutes).
   
   f Remove and discard all supernatant from each well.

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. 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 library consisted of single-stranded DNA (ssDNA), which cannot be resolved on agarose gels or Bioanalyzer chips.

**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

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

Consumables
- HT1 (Hybridization Buffer)
- 2.5 L Ice bucket
- Adhesive PCR seal
- Eppendorf LoBind Microcentrifuge Tubes
- PCR 8-tube strip

Preparation
1. Make sure that the MiSeq System is ready for use.
2. If the normalized library plate was stored frozen, thaw the plate at room temperature, and then vortex.
3. Prepare the following consumables.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reagent</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiSeq reagent cartridge</td>
<td>-25°C to -15°C</td>
<td>Thaw in a room temperature water bath for 1 hour, or overnight at 2°C to 8°C.</td>
</tr>
<tr>
<td>HT1</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Set aside at 2°C to 8°C.</td>
</tr>
</tbody>
</table>

4. 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

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.
   [For experienced users] Reduce the volume of the library pool to < 15 µl to prevent overclustering (cluster density ≥ 1400K/mm²). The optimal cluster density ranges from 1200–1400K/mm².
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 POOL 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.

NOTE
HT1 diluted/denatured library pools are not stable for storage. Only undiluted library pools 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

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Acronyms</td>
<td>27</td>
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<tr>
<td>PCR Clean-Up Quality Control</td>
<td>28</td>
</tr>
<tr>
<td>VeriSeq PGS Library Prep Kit Contents</td>
<td>29</td>
</tr>
<tr>
<td>Consumables and Equipment</td>
<td>31</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>Amplicon Tagment Mix</td>
</tr>
<tr>
<td>HT1</td>
<td>Hybridization Buffer</td>
</tr>
<tr>
<td>LNA1</td>
<td>Library Normalization Additives 1</td>
</tr>
<tr>
<td>LNB1</td>
<td>Library Normalization Beads 1</td>
</tr>
<tr>
<td>LNS1</td>
<td>Library Normalization Storage Buffer 1</td>
</tr>
<tr>
<td>LNP</td>
<td>Library Normalization Plate</td>
</tr>
<tr>
<td>LNW1</td>
<td>Library Normalization Wash 1</td>
</tr>
<tr>
<td>NPM</td>
<td>Nextera PCR Master Mix</td>
</tr>
<tr>
<td>NT</td>
<td>Neutralize Tagment Buffer</td>
</tr>
<tr>
<td>PGS</td>
<td>Pre-Implantation Genetic Screening</td>
</tr>
<tr>
<td>RSB</td>
<td>Resuspension Buffer</td>
</tr>
<tr>
<td>TD</td>
<td>Tagment DNA Buffer</td>
</tr>
<tr>
<td>VTA</td>
<td>VeriSeq Tagment Amplicon Plate</td>
</tr>
</tbody>
</table>
PCR Clean-Up Quality Control

To determine the success of the library prep, perform a 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 (e.g., NanoDrop or UV absorbance methods). Before proceeding, make sure that there is at least 1.5 ng/µl of sample material.

- On a 1.5% agarose 1x TBE gel, load 5 µl of each PCR clean-up sample and 5 µl of gel loading buffer. For more information, see the Agarose Gel Electrophoresis section in the 24sure Microarray Dual Channel Reference Guide (part # 15056062).

- 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 (part # 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.

<table>
<thead>
<tr>
<th>Kit Name</th>
<th>Catalog #</th>
<th># Samples</th>
<th># Indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VeriSeq PGS Kit</td>
<td>RH-101-1001</td>
<td>96</td>
<td>24</td>
</tr>
</tbody>
</table>

VeriSeq PGS Kit Contents

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Consumable</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>SurePlex DNA Amplification System</td>
</tr>
<tr>
<td>1</td>
<td>VeriSeq Library Prep Kit-PGS</td>
</tr>
<tr>
<td>1</td>
<td>VeriSeq Index Kit PGS</td>
</tr>
<tr>
<td>4</td>
<td>MiSeq Reagent Kit v3-PGS</td>
</tr>
</tbody>
</table>

TruSeq Index Plate Fixture VeriSeq PGS Library Prep

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

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruSeq Index Plate Fixture Kit (2 Fixtures)</td>
<td>FC-130-1005</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cell Extraction Buffer</td>
</tr>
<tr>
<td>1</td>
<td>Extraction Enzyme Dilution Buffer</td>
</tr>
<tr>
<td>1</td>
<td>Cell Extraction Enzyme</td>
</tr>
<tr>
<td>1</td>
<td>SurePlex Pre-Amp Enzyme</td>
</tr>
<tr>
<td>1</td>
<td>SurePlex Amplification Buffer</td>
</tr>
<tr>
<td>1</td>
<td>SurePlex Amplification Enzyme</td>
</tr>
<tr>
<td>1</td>
<td>Nuclease-free water</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ATM</td>
<td>Amplicon Tagment Mix</td>
</tr>
<tr>
<td>2</td>
<td>TD</td>
<td>Tagment DNA Buffer</td>
</tr>
<tr>
<td>1</td>
<td>NPM</td>
<td>Nextera PCR Master Mix</td>
</tr>
<tr>
<td>4</td>
<td>RSB</td>
<td>Resuspension Buffer</td>
</tr>
<tr>
<td>1</td>
<td>LNA1</td>
<td>Library Normalization Additives 1</td>
</tr>
<tr>
<td>2</td>
<td>LNW1</td>
<td>Library Normalization Wash 1</td>
</tr>
<tr>
<td>1</td>
<td>HT1</td>
<td>Hybridization Buffer</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NT</td>
<td>Neutralize Tagment Buffer</td>
</tr>
<tr>
<td>1</td>
<td>LNB1</td>
<td>Library Normalization Beads 1</td>
</tr>
<tr>
<td>1</td>
<td>LNS1</td>
<td>Library Normalization Storage Buffer 1</td>
</tr>
</tbody>
</table>

VeriSeq Index Kit-PGS

Box 1, Store at -25°C to -15°C

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Index Primers, S503, S504</td>
</tr>
<tr>
<td>12</td>
<td>Index Primers, N701 to N712</td>
</tr>
</tbody>
</table>

Box 2, Store at Room Temperature

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 orange, 32 white</td>
<td>Index Adaptor Replacement Caps</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reagent Cartridge</td>
</tr>
<tr>
<td>1</td>
<td>HT1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PR2 Bottle</td>
</tr>
<tr>
<td>1</td>
<td>MiSeq Flow Cell</td>
</tr>
</tbody>
</table>
Consumables and Equipment

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

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

### Consumables

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µl barrier pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>10 µl multichannel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>10 µl single channel pipette</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>50 µl barrier pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>50 µl multichannel pipette</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>50 µl single channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl barrier pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl multichannel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl single channel pipette</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>1000 µl barrier pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>1000 µl single channel pipette</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>96-well storage plates, round well, 0.8 ml (“deep well” plate)</td>
<td>Fisher Scientific, part # AB-0859</td>
</tr>
<tr>
<td>96-well nonbinding black microplates</td>
<td>Greiner, part # 655900</td>
</tr>
<tr>
<td>96-well PCR plates or Hard-Shell 96-well PCR plates (“HSP” plate)</td>
<td>Fisher Scientific, part # AB0600 or Bio-Rad, part # HSP-9601</td>
</tr>
<tr>
<td>Adhesive PCR seal</td>
<td>Fisher Scientific, part # AB0558</td>
</tr>
<tr>
<td>Adhesive seal roller</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Agencourt AMPure XP (60 ml kit)</td>
<td>Beckman Coulter, part # A63881</td>
</tr>
<tr>
<td>Conical centrifuge tubes, 15 ml</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Eppendorf LoBind Microcentrifuge Tubes</td>
<td>Fisher Scientific, part # 10051232</td>
</tr>
<tr>
<td>Ethanol 200 proof (absolute ethyl alcohol) for molecular biology (500 ml)</td>
<td>Sigma-Aldrich, part # E7023</td>
</tr>
<tr>
<td>Lens tissue, grade no. 105 paper</td>
<td>Fisher Scientific, part # 10644371</td>
</tr>
<tr>
<td>Molecular grade water or ultrapure water</td>
<td>Sigma-Aldrich, part # W4502 or Life Technologies, part # 10977035</td>
</tr>
</tbody>
</table>
### Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR 8-tube strips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>PCR-grade water</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Pipetting reservoirs, multichannel, disposable</td>
<td>VWR, part # 89094-658</td>
</tr>
<tr>
<td>Qubit dsDNA HS Assay Kit</td>
<td>Life Technologies, part # Q32851</td>
</tr>
<tr>
<td>Qubit assay tubes</td>
<td>Life Technologies, part # Q32856</td>
</tr>
<tr>
<td>Quant-iT HS dsDNA Assay Kit, high-throughput</td>
<td>Life Technologies, part # Q33120</td>
</tr>
<tr>
<td>Sodium hypochlorite solution</td>
<td>Sigma-Aldrich, part # 239305</td>
</tr>
<tr>
<td>Sodium hydroxide solution, 10 N</td>
<td>Sigma-Aldrich, part # T72068</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Sigma-Aldrich, part # P7949</td>
</tr>
</tbody>
</table>

### Equipment

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-Speed Microplate Shaker</td>
<td>BioShake IQ, part # 1808-0506</td>
</tr>
<tr>
<td></td>
<td>VWR, catalog #</td>
</tr>
<tr>
<td></td>
<td>• 13500-890 (110 V/120 V)</td>
</tr>
<tr>
<td></td>
<td>or</td>
</tr>
<tr>
<td></td>
<td>• 14216-214 (230 V)</td>
</tr>
<tr>
<td>Magnetic stand-96</td>
<td>Life Technologies, part # AM10027</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Microplate centrifuge</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Microplate reader (96-well plate fluorometer)</td>
<td>FLUOstar Omega microplate reader, BMG LabTech</td>
</tr>
<tr>
<td>Qubit 2.0 Fluorometer</td>
<td>Life Technologies, part # Q32866</td>
</tr>
<tr>
<td>Vortexer</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>[Optional] 2100 Bioanalyzer Desktop System</td>
<td>Agilent, part # G2940CA</td>
</tr>
<tr>
<td>[Optional] DNA 1000 Chip</td>
<td>Agilent, part # 5067-1504</td>
</tr>
<tr>
<td>[Optional] High Sensitivity DNA Chip</td>
<td>Agilent, part # 4067-4626</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Thermal Cycler</th>
<th>Temp Mode</th>
<th>Lid Temp</th>
<th>Vessel Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad DNA Engine Tetrad 2</td>
<td>Calculated</td>
<td>Heated, Constant at 100°C</td>
<td>Polypropylene plates and tubes</td>
</tr>
<tr>
<td>MJ Research DNA Engine Tetrad</td>
<td>Calculated</td>
<td>Heated</td>
<td>Plate</td>
</tr>
<tr>
<td>Eppendorf Mastercycler Pro S</td>
<td>Gradient S, Simulated Tube</td>
<td>Heated</td>
<td>Plate</td>
</tr>
<tr>
<td>Veriti® Thermal Cycler</td>
<td>Calculated</td>
<td>Heated</td>
<td>Nonskirted plate and tubes</td>
</tr>
</tbody>
</table>
Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1  Illumina General Contact Information

<table>
<thead>
<tr>
<th>Website</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="http://www.illumina.com">www.illumina.com</a></td>
<td><a href="mailto:techsupport@illumina.com">techsupport@illumina.com</a></td>
</tr>
</tbody>
</table>

Table 2  Illumina Customer Support Telephone Numbers

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