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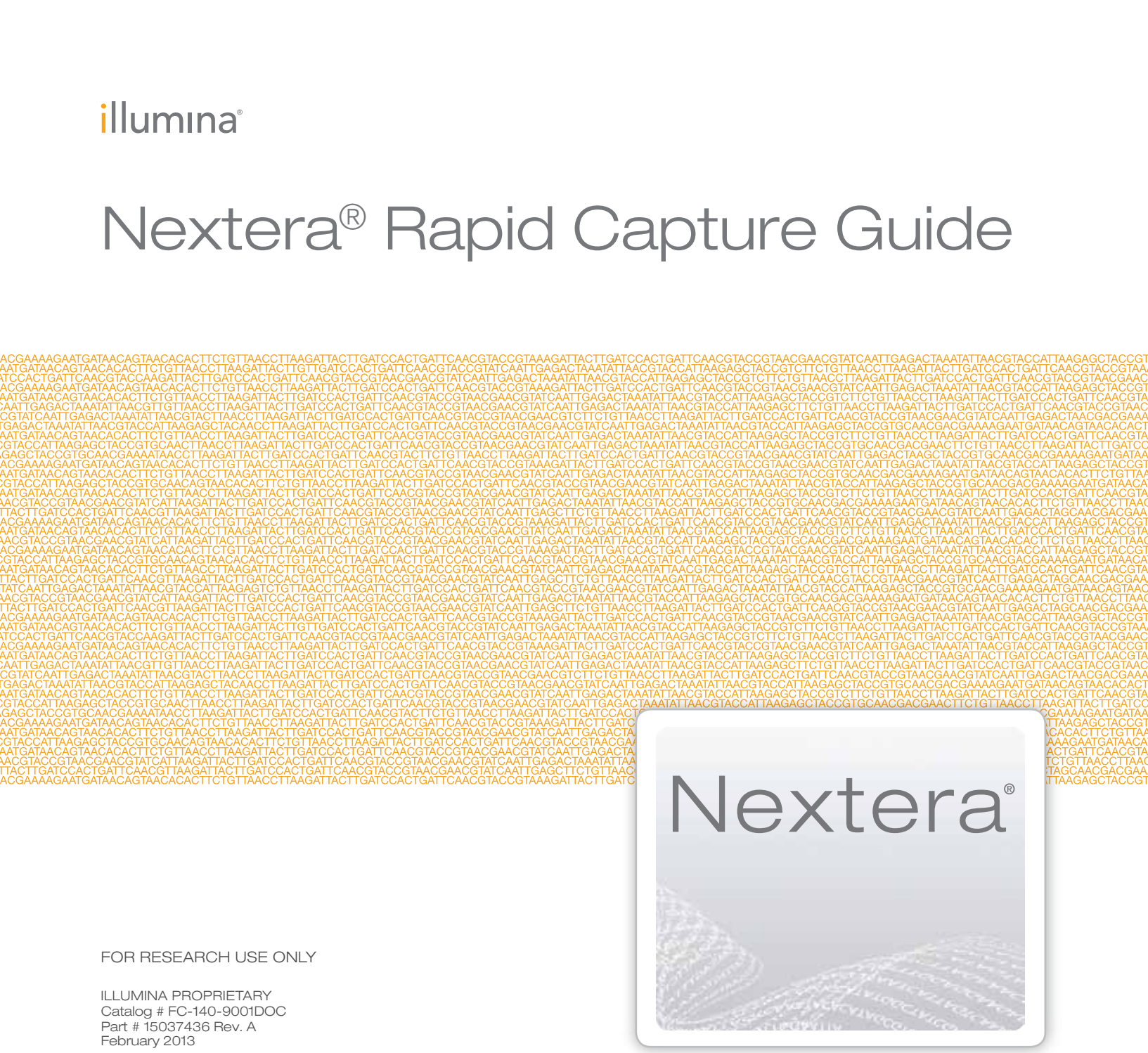
# Nextera® Rapid Capture Guide

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# Revision History

| Part # | Revision | Date | Description of Change |
|---|---|---|---|
| 15037436 | A | February 2013 | Initial Release |

Part # 15037436 Rev. A

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# Overview

Chapter 1



# Introduction

This protocol explains how to prepare up to 96 indexed paired-end libraries, followed by enrichment using exome probe panels and reagents provided in an Illumina® Nextera® Rapid Capture Exome or Expanded Exome kit, for subsequent cluster generation and DNA sequencing. The goal of this protocol is to fragment and add adapter sequences onto template DNA to generate indexed sequencing libraries that can be carried through enrichment for targeted resequencing applications.

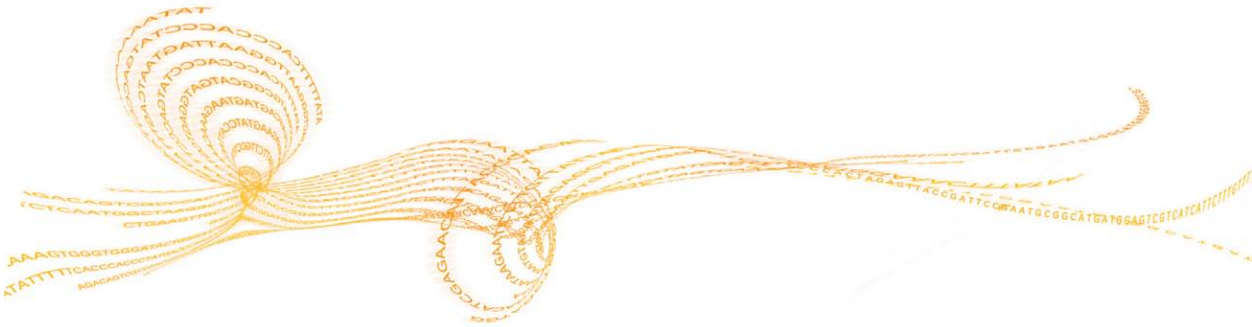
The Nextera Rapid Capture protocol offers:

- Fast and easy sample preparation
  - Prepare up to 96 enriched libraries in approximately 1.5 days, including approximately 5 hours of hands-on time
  - High manual throughput, automation-friendly procedures with no fragmentation bottlenecks
- Low DNA input and excellent data quality
  - Excellent data quality with low input of 50 ng
  - Access precious samples with no impact to performance
  - Ability to archive samples for additional analysis
- High enrichment rates, low duplicates, and exceptional coverage uniformity
  - Efficient use of sequencing and reliable variant calling
  - Reduced hands-on time with the most cost-effective, high-throughput workflow

# Getting Started

Chapter 2





# Introduction

This chapter explains standard operating procedures and precautions for performing the Nextera Rapid Capture protocol. You will also find details on the kit contents and lists of standard equipment and consumables.

The protocols described in the rest of this guide assume that you are familiar with the contents of this chapter, have implemented all the recommendations, have confirmed your kit contents, and have obtained all of the requisite equipment and consumables.

# Acronyms

Table 1 Nextera Rapid Capture Acronyms

| Acronym | Definition |
|---|---|
| CEX | Coding Exome Oligos |
| dsDNA | Double-stranded DNA |
| EE1 | Enrichment Elution Buffer 1 |
| EEX | Expanded Exome Oligos |
| EHB | Enrichment Hybridization Buffer |
| ET2 | Elute Target Buffer 2 |
| EWS | Enrichment Wash Solution |
| gDNA | Genomic DNA |
| HP3 | 2N NaOH |
| HSP | Hard Shell Plate |
| NEA | Nextera Enrichment Amplification Plate |
| NEC1 | Nextera Enriched Clean Up Plate 1 |
| NEC2 | Nextera Enriched Clean Up Plate 2 |
| NEH1 | Nextera Enrichment Hyb Plate 1 |
| NEH2 | Nextera Enrichment Hyb Plate 2 |
| NEM | Nextera Enrichment Amplification Mix |
| NES | Nextera Enrichment Sample Plate |
| NEW1 | Nextera Enrichment Wash Plate 1 |
| NEW2 | Nextera Enrichment Wash Plate 2 |

| Acronym | Definition |
|---|---|
| NLA | Nextera Library Amplification Plate |
| NLC | Nextera Library Clean Up Plate |
| NLM | Nextera Library Amplification Mix |
| NLS | Nextera Library Sample Plate |
| NLT | Nextera Library Tagment Plate |
| PCR | Polymerase Chain Reaction |
| PPC | PCR Primer Cocktail |
| RFU | Relative Fluorescence Unit |
| RSB | Resuspension Buffer |
| SMB | Streptavidin Magnetic Beads |
| SPB | Sample Purification Beads |
| ST | Stop Tagment Buffer |
| TD | Tagment DNA Buffer |
| TDE1 | Tagment DNA Enzyme 1 |

# Kit Contents

Check to make sure that you have all of the reagents identified in this section before proceeding to the sample preparation and enrichment procedures. Nextera Rapid Capture kits are available in the following configurations.

Table 2 Nextera Rapid Capture Kits

| Kit Name | Catalog # | Enrichment Reactions (12-plex) |
|---|---|---|
| Nextera Rapid Capture Exome Kit (24 Samples) | FC-140-1001 | 2 |
| Nextera Rapid Capture Exome Kit (48 Samples) | FC-140-1002 | 4 |
| Nextera Rapid Capture Exome Kit (96 Samples) | FC-140-1003 | 8 |
| Nextera Rapid Capture Expanded Exome Kit (24 Samples) | FC-140-1004 | 2 |
| Nextera Rapid Capture Expanded Exome Kit (48 Samples) | FC-140-1005 | 4 |
| Nextera Rapid Capture Expanded Exome Kit (96 Samples) | FC-140-1006 | 8 |

## Nextera Rapid Capture Exome and Expanded Exome 24 Samples Kit Contents

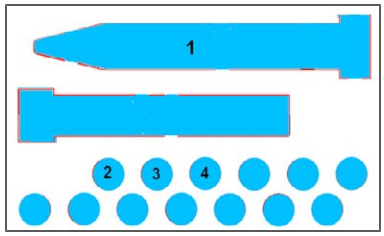
This section details the contents of both the Nextera Rapid Capture Exome and Expanded Exome 24 Samples kits. These kits contain enough reagents to support 24 samples and two 12-plex enrichment reactions. Each kit contains four boxes of reagents and an index replacement caps box. The Exome and Expanded Exome kits differ in the oligo contents of Box 4.

### 24 Samples - Box 1

#### Store as specified

This box is shipped at room temperature. As soon as you receive your kit, store the components as specified below.

Figure 1 Nextera Rapid Capture, 24 Samples - Box 1 of 4, part # 15034781



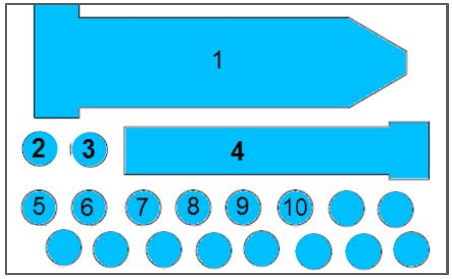

| Slot | Reagent | Part # | Description | Storage Temperature |
|---|---|---|---|---|
| 1 | SPB | 15037172 | Sample Purification Beads | 2° to 8°C |
| 2 | SMB | 15039631 | Streptavidin Magnetic Beads | 2° to 8°C |
| 3 | ET2 | 15013008 | Elute Target Buffer 2 | 2° to 8°C |
| 4 | ST | 15033016 | Stop Tagment Buffer | 15° to 30°C |

## 24 Samples - Box 2

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 2 Nextera Rapid Capture, 24 Samples - Box 2 of 4, part # 15034787




| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | RSB | 15026770 | Resuspension Buffer |
| 2 | TDE1 | 15038061 | Tagment DNA Enzyme |
| 3 | TD | 15027866 | Tagment DNA Buffer |

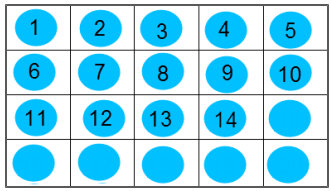
| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 4 | EWS | 15037119 | Enrichment Wash Solution |
| 5 | NLM | 15037037 | Nextera Library Amplification Mix |
| 6 | EHB | 15038763 | Enrichment Hybridization Buffer |
| 7 | HP3 | 11324596 | 2N NaOH |
| 8 | PPC | 15031752 | PCR Primer Cocktail |
| 9 | NEM | 15037047 | Nextera Enrichment Amplification Mix |
| 10 | EE1 | 15037034 | Enrichment Elution Buffer 1 |

## 24 Samples - Box 3

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 3 Nextera Rapid Capture, 24 Samples - Box 3 of 4, part # 15034783

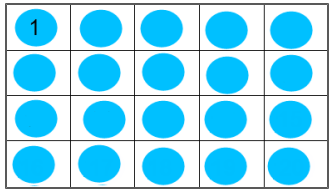



| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | E501 | 15032391 | E501 Index Adapter |
| 2 | E502 | 15032392 | E502 Index Adapter |
| 3 | N701 | 15031793 | N701 Index Adapter |
| 4 | N702 | 15031794 | N702 Index Adapter |
| 5 | N703 | 15031795 | N703 Index Adapter |
| 6 | N704 | 15031796 | N704 Index Adapter |
| 7 | N705 | 15031797 | N705 Index Adapter |
| 8 | N706 | 15031798 | N706 Index Adapter |
| 9 | N707 | 15031799 | N707 Index Adapter |
| 10 | N708 | 15031800 | N708 Index Adapter |
| 11 | N709 | 15031801 | N709 Index Adapter |
| 12 | N710 | 15031802 | N710 Index Adapter |
| 13 | N711 | 15031803 | N711 Index Adapter |
| 14 | N712 | 15031804 | N712 Index Adapter |

## 24 Samples - Box 4

### Store at at -15° to -25°C

You will receive one of the following box 4, depending on the kit ordered: Exome or Expanded Exome. This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.




Nextera Rapid Capture Exome, 24 Samples - Box 4 of 4, part # 15039642

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | CEX | 15034575 | Coding Exome Oligos |

Nextera Rapid Capture Expanded Exome, 24 Samples - Box 4 of 4, part # 15039643

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | EEX | 15034685 | Expanded Exome Oligos |

## Index Adapter Replacement Caps Box

### Store at 15° to 30°C

This box is shipped at room temperature. As soon as you receive your kit, store the components at 15° to 30°C.

Alternate Kit, Index Adapter Replacement Caps Box, part # 15026762

| Quantity | Part # | Description |
|---|---|---|
| 1 | 15026585 | i7 Index Tube Caps, Orange |
| 1 | 15026586 | i5 Index Tube Caps, White |

# Nextera Rapid Capture Exome and Expanded Exome 48 Samples Kit Contents

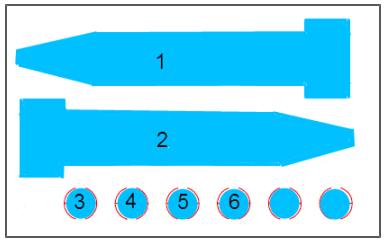
This section details the contents of both the Nextera Rapid Capture Exome and Expanded Exome 48 Samples kits. These kits contain enough reagents to support 48 samples and four 12-plex enrichment reactions. Each kit contains four boxes of reagents and an index replacement caps box. The Exome and Expanded Exome kits differ in the oligo contents of Box 4.

## 48 Samples - Box 1

### Store as specified

This box is shipped at room temperature. As soon as you receive your kit, store the components as specified below.

Figure 4 Nextera Rapid Capture, 48 Samples - Box 1 of 4, part # 15038194



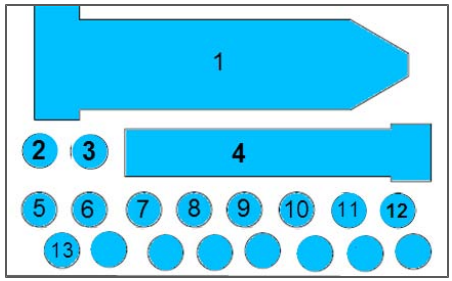

| Slot | Reagent | Part # | Description | Storage Temperature |
|---|---|---|---|---|
| 1–2 | SPB | 15037172 | Sample Purification Beads | 2° to 8°C |
| 3–4 | SMB | 15039631 | Streptavidin Magnetic Beads | 2° to 8°C |
| 5 | ET2 | 15013008 | Elute Target Buffer 2 | 2° to 8°C |
| 6 | ST | 15033016 | Stop Tagment Buffer | 15° to 30°C |

## 48 Samples - Box 2

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 5 Nextera Rapid Capture, 48 Samples - Box 2 of 4, part # 15034782



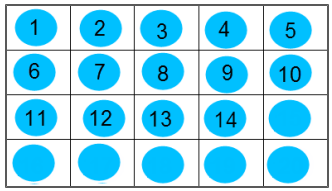

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | RSB | 15026770 | Resuspension Buffer |
| 2–3 | TDE1 | 15038061 | Tagment DNA Enzyme |
| 4 | EWS | 15037119 | Enrichment Wash Solution |
| 5 | TD | 15027866 | Tagment DNA Buffer |
| 6–7 | NLM | 15037037 | Nextera Library Amplification Mix |
| 8 | EHB | 15038763 | Enrichment Hybridization Buffer |
| 9 | HP3 | 11324596 | 2N NaOH |
| 10 | PPC | 15031752 | PCR Primer Cocktail |
| 11–12 | NEM | 15037047 | Nextera Enrichment Amplification Mix |
| 13 | EE1 | 15037034 | Enrichment Elution Buffer 1 |

## 48 Samples - Box 3

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 6 Nextera Rapid Capture, 48 Samples - Box 3 of 4, part # 15038196

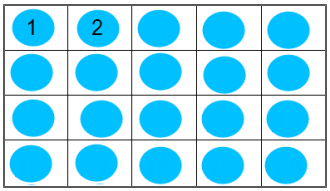


| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | E501 | 15032391 | E501 Index Adapter |
| 2 | E502 | 15032392 | E502 Index Adapter |
| 3 | N701 | 15031793 | N701 Index Adapter |
| 4 | N702 | 15031794 | N702 Index Adapter |
| 5 | N703 | 15031795 | N703 Index Adapter |
| 6 | N704 | 15031796 | N704 Index Adapter |
| 7 | N705 | 15031797 | N705 Index Adapter |
| 8 | N706 | 15031798 | N706 Index Adapter |
| 9 | N707 | 15031799 | N707 Index Adapter |
| 10 | N708 | 15031800 | N708 Index Adapter |
| 11 | N709 | 15031801 | N709 Index Adapter |
| 12 | N710 | 15031802 | N710 Index Adapter |
| 13 | N711 | 15031803 | N711 Index Adapter |
| 14 | N712 | 15031804 | N712 Index Adapter |

## 48 Samples - Box 4

### Store at at -15° to -25°C

You will receive one of the following box 4, depending on the kit ordered: Exome or Expanded Exome. This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.




Nextera Rapid Capture Exome, 48 Samples - Box 4 of 4, part # 15039644

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1–2 | CEX | 15034575 | Coding Exome Oligos |

Nextera Rapid Capture Expanded Exome, 48 Samples - Box 4 of 4, part # 15039645

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1–2 | EEX | 15034685 | Expanded Exome Oligos |

### Index Adapter Replacement Caps Box

#### Store at 15° to 30°C

This box is shipped at room temperature. As soon as you receive your kit, store the components at 15° to 30°C.

Alternate Kit, Index Adapter Replacement Caps Box, part # 15026762

| Quantity | Part # | Description |
|---|---|---|
| 1 | 15026585 | i7 Index Tube Caps, Orange |
| 1 | 15026586 | i5 Index Tube Caps, White |

## Nextera Rapid Capture Exome and Expanded Exome 96 Samples Kit Contents

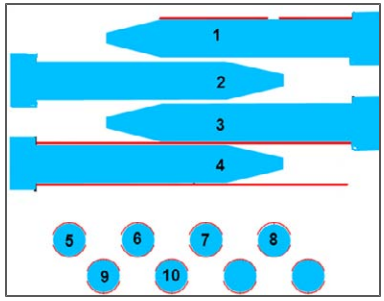
This section details the contents of both the Nextera Rapid Capture Exome and Expanded Exome 96 Samples kits. These kits contain enough reagents to support 96 samples and eight 12-plex enrichment reactions. Each kit contains four boxes of reagents and an index replacement caps box. The Exome and Expanded Exome kits differ in the oligo contents of Box 4.

### 96 Samples - Box 1

#### Store as specified

This box is shipped at room temperature. As soon as you receive your kit, store the components as specified below.

Figure 7 Nextera Rapid Capture, 96 Samples - Box 1 of 4, part # 15034784



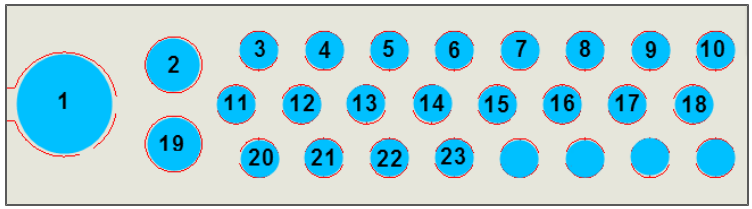

| Slot | Reagent | Part # | Description | Storage Temperature |
|---|---|---|---|---|
| 1–4 | SPB | 15037172 | Sample Purification Beads | 2° to 8°C |
| 5–8 | SMB | 15039631 | Streptavidin Magnetic Beads | 2° to 8°C |
| 9 | ET2 | 15013008 | Elute Target Buffer 2 | 2° to 8°C |
| 10 | ST | 15033016 | Stop Tagment Buffer | 15° to 30°C |

## 96 Samples - Box 2

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 8 Nextera Rapid Capture, 96 Samples - Box 2 of 4, part # 15034789




| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1 | RSB | 15026770 | Resuspension Buffer |
| 2 | EWS | 15037119 | Enrichment Wash Solution |
| 3–6 | TDE1 | 15038061 | Tagment DNA Enzyme |

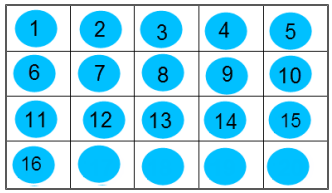
| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 7–8 | TD | 15027866 | Tagment DNA Buffer |
| 9–12 | NLM | 15037037 | Nextera Library Amplification Mix |
| 13–14 | EHB | 15038763 | Enrichment Hybridization Buffer |
| 15 | HP3 | 11324596 | 2N NaOH |
| 16 | PPC | 15031752 | PCR Primer Cocktail |
| 17–18 | NEM | 15037047 | Nextera Enrichment Amplification Mix |
| 19 | EWS | 15037119 | Enrichment Wash Solution |
| 20–21 | NEM | 15037047 | Nextera Enrichment Amplification Mix |
| 22–23 | EE1 | 15037034 | Enrichment Elution Buffer 1 |

## 96 Samples - Box 3

### Store at -15° to -25°C

This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.

Figure 9 Nextera Rapid Capture, 96 Samples - Box 3 of 4, part # 15034786



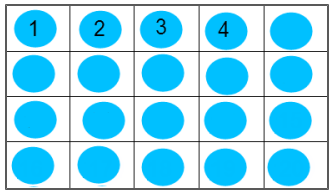

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1–2 | E501 | 15032391 | E501 Index Adapter |
| 3–4 | E502 | 15032392 | E502 Index Adapter |
| 5 | N701 | 15031793 | N701 Index Adapter |
| 6 | N702 | 15031794 | N702 Index Adapter |
| 7 | N703 | 15031795 | N703 Index Adapter |
| 8 | N704 | 15031796 | N704 Index Adapter |
| 9 | N705 | 15031797 | N705 Index Adapter |
| 10 | N706 | 15031798 | N706 Index Adapter |
| 11 | N707 | 15031799 | N707 Index Adapter |
| 12 | N708 | 15031800 | N708 Index Adapter |

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 13 | N709 | 15031801 | N709 Index Adapter |
| 14 | N710 | 15031802 | N710 Index Adapter |
| 15 | N711 | 15031803 | N711 Index Adapter |
| 16 | N712 | 15031804 | N712 Index Adapter |

## 96 Samples - Box 4

### Store at at -15° to -25°C

You will receive one of the following box 4, depending on the kit ordered: Exome or Expanded Exome. This box is shipped on dry ice. As soon as you receive your kit, store the components at -15° to -25°C.




Nextera Rapid Capture Exome, 96 Samples - Box 4 of 4, part # 15039646

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1–4 | CEX | 15034575 | Coding Exome Oligos |

Nextera Rapid Capture Expanded Exome, 96 Samples - Box 4 of 4, part # 15039647

| Slot | Reagent | Part # | Description |
|---|---|---|---|
| 1–4 | EEX | 15034685 | Expanded Exome Oligos |

## Index Adapter Replacement Caps Box

### Store at 15° to 30°C

This box is shipped at room temperature. As soon as you receive your kit, store the components at 15° to 30°C.

Alternate Kit, Index Adapter Replacement Caps Box, part # 15026762

| Quantity | Part # | Description |
|---|---|---|
| 1 | 15026585 | i7 Index Tube Caps, Orange |
| 1 | 15026586 | i5 Index Tube Caps, White |

# Consumables and Equipment

Check to ensure that you have all of the necessary user-supplied consumables and equipment before proceeding to the sample preparation and enrichment procedures.

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

Table 3 User-Supplied Consumables

| Consumable | Supplier |
|---|---|
| 1.7 ml microcentrifuge tubes | General lab supplier |
| 20 µl barrier pipette tips | General lab supplier |
| 20 µl multichannel pipettes | General lab supplier |
| 20 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 multichannel pipettes | General lab supplier |
| 1000 µl single channel pipettes | General lab supplier |
| Adhesive seal roller | General lab supplier |
| 96-well flat clear bottom black microplates<br>Note: Used when quantifying samples with a SpectraMax M5 spectrofluorometer. | Corning, part # 3904 |
| 96-well storage plates, round well,<br>0.8 ml (“MIDI” plate) | Fisher Scientific,<br>part # AB-0859 |

| Consumable | Supplier |
|---|---|
| Hard-Shell 96-well PCR Plates (“HSP” plate) | Bio-Rad, part # HSP-9601 |
| Aluminum foil | General lab supplier |
| [Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) Note: Used to concentrate a pooled library. Another option is to use a vacuum concentrator. | Millipore, part # UFC503008 |
| Conical centrifuge tubes (15 ml or 50 ml) | General lab supplier |
| Distilled water | General lab supplier |
| Ethanol 200 proof (absolute) for molecular biology (500 ml) | Sigma Aldrich, part # E7023 |
| Microseal ‘A’ film | BioRad, part # MSA-5001 |
| Microseal ‘B’ adhesive seals | BioRad, part # MSB-1001 |
| RNase/DNase-free 8-well PCR strip tubes and caps | General lab supplier |
| RNase/DNase-free multichannel reagent reservoirs, disposable | VWR, part # 89094-658 |
| Tris-Cl 10 mM, pH 8.5 | General lab supplier |
| [Optional] TruSeq Index Plate Fixture Kit Note: Recommended for setting up indexed PCR primers. This part is reusable. | Illumina, catalog # FC-130-1005 |
| Ultra pure water | General lab supplier |

Table 4 User-Supplied Equipment

| Equipment | Supplier |
|---|---|
| [Optional] 2100 Bioanalyzer Desktop System | Agilent, part # G2940CA |

| Equipment | Supplier |
|---|---|
| [Optional] DNA 1000 Chip | Agilent, part # 5067-1504 |
| [Optional] High Sensitivity DNA Chip | Agilent, part # 4067-4626 |
| DNA Engine Multi-Bay Thermal Cycler<br>See *Thermal Cyclers* on page 22. | Bio-Rad, part # PTC-0240G or PTC-0220G, with Alpha Unit, part # ALS-1296GC |
| High Speed Micro Plate Shaker | VWR, catalog #<br>• 13500-890 (110V/120V) or<br>• 14216-214 (230V) |
| Magnetic stand-96 | Life Technologies, part # AM10027 |
| Microcentrifuge | General lab supplier |
| Microplate centrifuge | General lab supplier |
| MIDI plate insert for heating system | Illumina, catalog # BD-60-601 |
| QuantiFluor dsDNA System<br>or similar fluorometrically-based DNA quantification system | Promega, catalog # E2670 |
| SpectraMax M5 spectrofluorometer<br>or similar fluorometrically-based DNA quantification system | Molecular Devices, part # 0112-0159 |
| Tru Temp Microheating System | Illumina, catalog #<br>• SC-60-503 (115V) or<br>• SC-60-504 (220V) |
| [Optional] Vacuum concentrator<br>Note: Used to concentrate a pooled library. Another option is to use Amicon Ultra-0.5 centrifugal filter units. | General lab supplier |
| Vortexer | General lab supplier |

## Thermal Cyclers

The following table lists the recommended settings for the Illumina recommended thermal cycler, as well as other comparable models. Validate any thermal cyclers not listed below if your lab has not yet performed the Nextera Rapid Capture 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 |

# Tracking Tools

Illumina provides the following tools for sample tracking and guidance in the lab:

NOTE
You can download these documents from the Illumina website at www.illumina.com. Go to the Nextera Rapid Capture Exome support page, then click **Documentation & Literature**.

- **Experienced User Card (EUC)** to guide you through the protocol, but with less detail than provided in this user guide. **New or less experienced users are strongly advised to follow this user guide and not the EUC.**
- **Lab Tracking Form (LTF)** to record information about library preparation such as operator name, sample and index information, start and stop times, reagent lot numbers, and barcodes.
  - Create a copy of the lab tracking form for each time you perform this protocol to prepare a library for sequencing.
  - Use it online and save it electronically or print it and fill it out manually.
- **Illumina Experiment Manager (IEM)** to create your sample sheet using a wizard-based application. The sample sheet is used to record information about your samples for later use in data analysis. The IEM guides you through the steps to create your sample sheet based on the analysis workflow for your run. The IEM provides a feature for recording parameters for your sample plate, such as sample ID, dual indices, and other parameters applicable to your sample plate.
  - To create a Nextera Rapid Capture sample sheet using IEM:
    - Click **Create Sample Sheet**
    - Click the **HiSeq 2000/1000 HiScanSQ GA** instrument
    - Click the **HiSeq Enrichment** application
    - Select the **Nextera Enrichment** sample prep kit in the workflow parameters
  - When using IEM for sample sheet generation, make sure you select **Use Adapter Trimming** when you create your sample sheet. Shorter inserts can lead to sequencing into the adapter and this feature helps filter out adapter sequence from the final sequencing data.
  - The manifest specifies targeted regions for the aligner and variant caller, which results in faster analysis times and visualization of results specific for these targeted regions.

- When a run completes for HiSeq®, HiScan SQ™, or GA instruments, the product manifest file must be copied to the runfolder before running the analysis. A sample sheet must be also created using IEM or other spreadsheet application.

NOTE

- You can download IEM from the Illumina website. Go to the Nextera Rapid Capture Exome support page, then click **Downloads**. A MyIllumina account is required to download the software.
- IEM can be run on any Windows platform.
- For instructions on how to use the IEM application, see the *Illumina Experiment Manager User Guide* and quick reference card. Go to the Nextera Rapid Capture Exome support page, then click **Documentation & Literature**.

# DNA Input Recommendations

Nextera Rapid Capture library preparation uses an enzymatic DNA fragmentation step and thus can be more sensitive to DNA input compared to mechanical fragmentation methods. The ultimate success of enrichment strongly depends on using an accurately quantified amount of input DNA library. Therefore, accurate quantitation of the gDNA library is essential.

Illumina recommends quantifying the starting gDNA using a fluorometrically-based method specific for double-stranded DNA (dsDNA) such as QuantiFluor and running samples in triplicate to obtain more confident measurements. Methods that measure total nucleic acid content (e.g. nanodrop or other UV absorbance methods) should be avoided because common contaminants such as ssDNA, RNA and oligos are not substrates for the Nextera Rapid Capture assay. For more information, see *DNA Quantitation* on page 79.

**The Nextera Rapid Capture protocol has been optimized for 50 ng of total gDNA.** A higher mass input of gDNA may result in incomplete tagmentation and larger insert sizes, which can impact enrichment performance. Conversely, a low mass input of gDNA or low quality gDNA in the tagmentation reaction may generate smaller than expected insert sizes, which can be lost during subsequent clean-up steps resulting in lower diversity.

To minimize gDNA sample input variability into the tagmentation step, Illumina strongly recommends a two-step method of gDNA normalization. After the initial quantification, gDNA samples are first normalized to 10 ng/ul. Samples are then re-quantified using a similar fluorometric-based method and normalized to a final 5 ng/ul.

# Best Practices

Adhere to the following best practices when preparing libraries for sequencing using this protocol. Several components of this kit are shipped at one temperature and stored at a warmer temperature. The components are stable at either temperature, but should be used at the warmer temperature. To avoid delay during sample preparation, each component should be stored according to the recommendations in *Kit Contents* on page 7.

## Ensuring Consistency

- **Use multichannel pipettes**—To make sure there is consistency across samples, use a multichannel pipette where possible. Calibrate pipettes periodically.
- **Pre-aliquot reagents**—Minimize freeze-thaw cycles to no more than four. If you do not intend to consume the reagents in one use, dispense the reagent into aliquots after the initial thaw and refreeze the aliquots in order to avoid excessive freeze-thaw cycles. However, if you aliquot, you might not have enough reagents for the full number of reactions over multiple uses.
- **Use plate shakers**—To minimize sample loss from manual resuspension, use the recommended plates and plate shakers to mix samples.

## Handling Magnetic Beads

- **Use at room temperature**—Prior to use, allow the beads to reach room temperature. Use a 25°C water bath as necessary.
- **Vortex until well-suspended**—Immediately prior to use, vortex the beads until they are well-suspended and the color appears homogeneous.
- **Mix samples thoroughly**—After adding the beads to your samples, mix thoroughly using a plate shaker at the recommended speed and time. For some steps where complete resuspension of the beads is essential for optimal performance, after a timed shake Illumina recommends pipetting the sample up and down 10 times to ensure complete resuspension of the sample.
- **Allow maximum binding**—For best results, incubate your bead/sample mixtures at room temperature for the entire duration indicated in the protocol. After the recommended incubation time, collect the bead/sample mixture to the bottom of the plate with a brief centrifugation.

- **Slowly aspirate cleared solution**—After placing the plate on the magnetic stand, wait for the solution to clear before proceeding. Keep the plate on the magnetic stand when slowly aspirating cleared solution, taking care not to disturb the separated beads.

## Avoiding Cross-Contamination

- **Change tips between samples**—Always use fresh pipette tips between samples and between dispensing index primers.
- **Mix plates as directed**—Mix samples using the methods specified in the protocol.
- **Use aerosol-resistant tips**—Using aerosol-resistant pipette tips reduces the risk of nucleic acid carry-over and sample-to-sample cross-contamination. If aerosol-resistant tips are not available, ensure careful pipetting to avoid contamination.

## Washing During SPB Clean-Up

- **Prepare fresh 80% ethanol**—Always prepare fresh 80% ethanol for wash steps. Ethanol can absorb water from the air impacting your results.
- **Remove all ethanol from wells**—Make sure that you remove all ethanol from the bottom of the wells as it might contain residual contaminants. Use a P20 multichannel pipette to remove residual ethanol and accelerate drying.
- **Allow complete evaporation**—Allow at least ten minutes of drying time on the magnetic stand at room temperature for complete evaporation. Residual ethanol can impact the performance of subsequent reactions.
- **Avoid overdrying samples** —Do not exceed the recommended drying time as overdrying samples can negatively impact sample recovery.

## Freeze/thawing for Small Number of Samples

- Depending upon the kit that you have (24, 48, or 96 samples), each reagent supplied with your assay kit contains sufficient volume to process the specified number of samples at once, using an 8-channel pipette and either a reservoir or an 8-well strip tube. When processing smaller sample batches using a reagent reservoir, dead volume and pipetting error losses can increase. To make sure there is an accurate reagent volume for all samples, single-pipette the reagent into each well.
- To store remaining reagent, Illumina recommends freezing aliquots, rather than repeatedly freezing and thawing the supplied reagent tubes.

## Prevent PCR Product Contamination

The PCR process is commonly used in the laboratory to amplify specific DNA sequences. Unless proper laboratory hygiene is used, PCR products can contaminate reagents, instrumentation, and genomic DNA (gDNA) samples, causing inaccurate and unreliable results. PCR product contamination can shut down lab processes and significantly delay normal operations.

Make sure that the lab is set up appropriately to reduce the risk of PCR product contamination:

- **Physically Separate Pre-PCR and Post-PCR Areas**
  - Physically separate laboratory space where pre-PCR processes are performed (DNA extraction, quantification, and normalization) from the laboratory space where PCR products are made and processed (post-PCR processes).
  - Never use the same sink to wash pre-PCR and post-PCR troughs.
  - Never share the same water purification system for pre-PCR and post-PCR processes.
  - Store all supplies used in the protocols in the pre-PCR area, and transfer to the post-PCR area as needed.
- **Use Dedicated Equipment and Supplies**
  - Dedicate separate full sets of equipment and supplies (pipettes, centrifuges, oven, heat block, etc.) to pre-PCR and post-PCR lab processes, and never share between processes.
  - Dedicate separate storage areas (freezers and refrigerators) to pre-PCR and post-PCR consumables.

Because the pre- and post-amplification reagents are shipped together, it is important to unpack the reagents in the pre-PCR lab area, and then move the post-amplification reagents to the proper post-PCR storage area.

## Pre-PCR and Post-PCR Lab Procedures

To prevent PCR product contamination, it is important to establish lab procedures and follow best practices. Illumina recommends daily and weekly cleaning of lab areas using 0.5% Sodium Hypochlorite (10% Bleach).

CAUTION

To prevent sample or reagent degradation, make sure that all vapors from the cleaning solution have fully dissipated before beginning any processes.

## Daily Cleaning of Pre-PCR Area

A daily cleaning of the pre-PCR area using a 0.5% Sodium Hypochlorite (10% Bleach) solution helps to eliminate PCR product that has entered the pre-PCR area.

Identify pre-PCR areas that pose the highest risk of contamination, and clean these areas with a 0.5% Sodium Hypochlorite (10% Bleach) solution before beginning any pre-PCR processes. High-risk areas might include, but are not limited to, the following items:

- Bench tops
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards

## Daily Cleaning of Post-PCR Area

Reducing the amount of PCR product in the post-PCR area helps reduce the risk of contamination in the pre-PCR area. Daily cleaning of the post-PCR area using a 0.5% Sodium Hypochlorite (10% Bleach) solution helps achieve this.

Identify post-PCR areas that pose the highest risk of contamination, and clean these areas with a 0.5% Sodium Hypochlorite (10% Bleach) solution daily. High-risk areas might include, but are not limited to, the following items:

- Thermal cyclers
- Bench space used to process amplified DNA
- Door handles
- Refrigerator/freezer door handles
- Computer mouse
- Keyboards

## Weekly Cleaning of All Lab Areas

Once a week, perform a thorough cleaning of the pre-PCR and post-PCR areas using 0.5% Sodium Hypochlorite (10% Bleach).

- Clean all bench tops and laboratory surfaces.
- Clean all instruments that are not cleaned daily.
- Thoroughly mop lab floors.
- Make sure that personnel responsible for weekly cleaning are properly trained on prevention of PCR product contamination.

### Items Fallen to the Floor

The floor is contaminated with PCR product transferred on the shoes of individuals coming from the post-PCR area; therefore, anything falling to the floor must be treated as contaminated.

- Disposable items that have fallen to the floor, such as empty tubes, pipette tips, gloves, lab coat hangers, must be discarded.
- Non-disposable items that have fallen to the floor, such as a pipette or an important sample container, must be immediately and thoroughly cleaned with a 0.5% Sodium Hypochlorite (10% Bleach) solution to remove PCR product contamination.
- Clean any lab surface that has come in contact with the contaminated item. Individuals handling anything that has fallen to the floor, disposable or non-disposable, must discard their lab gloves and put on a new pair.

# Dual Indexing Principle

The dual indexing strategy uses two 8 base indices, Index 1 (i7) adjacent to the P7 sequence, and Index 2 (i5) adjacent to the P5 sequence. Dual indexing is enabled by adding a unique Index 1 (i7) and Index 2 (i5) to each sample from 12 different Index 1 (i7) adapters (N701–N712) and two different Index 2 (i5) adapters (E501 and E502). In the Index adapter name, the N refers to Nextera, the E refers to enrichment, 7 or 5 refers to Index 1 (i7) or Index 2 (i5), respectively, and 01–12 refers to the Index number. The following list of index sequences is provided for generating sample sheets to demultiplex the samples:

Table 5 Nextera Rapid Capture Index Adapter Sequences

| Index 1 (i7) | Sequence | Index 2 (i5) | Sequence |
|---|---|---|---|
| N701 | TAAGGCGA | E501 | TAGATCGC |
| N702 | CGTACTAG | E502 | CTCTCTAT |
| N703 | AGGCAGAA | | |
| N704 | TCCTGAGC | | |
| N705 | GGACTCCT | | |
| N706 | TAGGCATG | | |
| N707 | CTCTCTAC | | |
| N708 | CAGAGAGG | | |
| N709 | GCTACGCT | | |
| N710 | CGAGGCTG | | |
| N711 | AAGAGGCA | | |
| N712 | GTAGAGGA | | |

NOTE

While the E500 series Index 2 (i5) sequences in the Nextera Rapid Capture kits are identical to S500 series Index 2 (i5) sequences in other Nextera kits, the Index 2 (i5) adapters are not interchangeable across kits.

# Low-Plex Pooling Guidelines

Follow these guidelines when pooling less than 6 libraries. Illumina uses a green laser to sequence G/T and a red laser to sequence A/C. At each cycle at least one of two nucleotides for each color channel need to be read to ensure proper registration. It is important to maintain color balance for each base of the index read being sequenced, otherwise index read sequencing could fail due to registration failure. Follow the instructions described here to determine which libraries are pooled pre-enrichment.

The Nextera Rapid Capture kits support pre-enrichment pooling of up to 12 different indexed samples. Illumina recommends the following:

- For pooling 12 samples pre-enrichment, pool samples with Index 1 (i7) 701–712 (any Index 2 (i5)), followed by a single-index sequencing run.
- For pooling <12 samples, set up a single index workflow sequencing run using different Index 1 (i7) indices (any Index 2 (i5)). Illumina provides compatible i5 indices in this kit, but when pooling less than 6 i7 indices use the combinations in Table 6 for proper color balancing.
- See Table 7 for pooling details when using both Index 1 (i7) and Index 2 (i5), followed by a dual indexed sequencing run.

NOTE
Illumina provides enough reagents to pool 12 samples at a time. If you choose to pool less than 12 samples during the enrichment, the reagents provided in your kit may not support the number of samples as noted on the kit box.

NOTE
There is sufficient volume in each Index 1 (i7) tube for 16 reactions and in each Index 2 (i5) tube for 24 reactions. Plan your experiment accordingly so you do not to run out of index primers.

Table 6 Libraries Pooled: 6 or Fewer; Sequencing Workflow: Single Index

| Plex | Index 1 (i7) Selection | Index 2 (i5) Selection |
|---|---|---|
| 1-plex (no pooling) | Any Index 1 adapter | Any Index 2 adapter |
| 2-plex | • [option 1] N702 and N701<br>• [option 2] N702 and N704 | |
| 3-plex | • [option 1] N701, N702, and N704<br>• [option 2] N703, N705, and N706 | |
| 4- or 5-plex | • [option 1] N701, N702, N704, and any other Index 1 adapter<br>• [option 2] N703, N705, N706, and any other Index 1 adapter | |
| 6-plex | N701, N702, N703, N704, N705, and N706 | |

Table 7 Sequencing Workflow: Single or Dual Index

| Plex | Index 1 (i7) Selection | Index 2 (i5) Selection |
|---|---|---|
| 7–12 plex, Dual Index | • [option 1] N701, N702, N704, and any other Index 1 adapter (as needed)<br>• [option 2] N703, N705, N706, and any other Index 1 adapter (as needed) | E501 and E502 |
| 7–12 plex, Single Index | • N701–N706 and any other Index 1 adapter (as needed) | Any Index 2 (i5) adapter |

The table below is an example of the acceptable combinations for four pooled samples. Alternatively, please check the real sequences of each index in Table 7 to make sure each base position will have signal in both color channels for the index read. Both i5 indices (E501 and E502) are compatible with each other

| Sample | Good Index 1 | | Bad Index 1 | |
|---|---|---|---|---|
| 1 | 701 | TAAGGCGA | 702 | CGTACTAG |
| 2 | 702 | CGTACTAG | 703 | AGGCAGAA |
| 3 | 704 | TCCTGAGC | 711 | AAGAGGCA |
| | | √ √ √ √ √ √ √ √ | | x √ x x √ x x √ |

√=signal in both color
x=signal missing in one color channel

# Protocol

Chapter 3



# Introduction

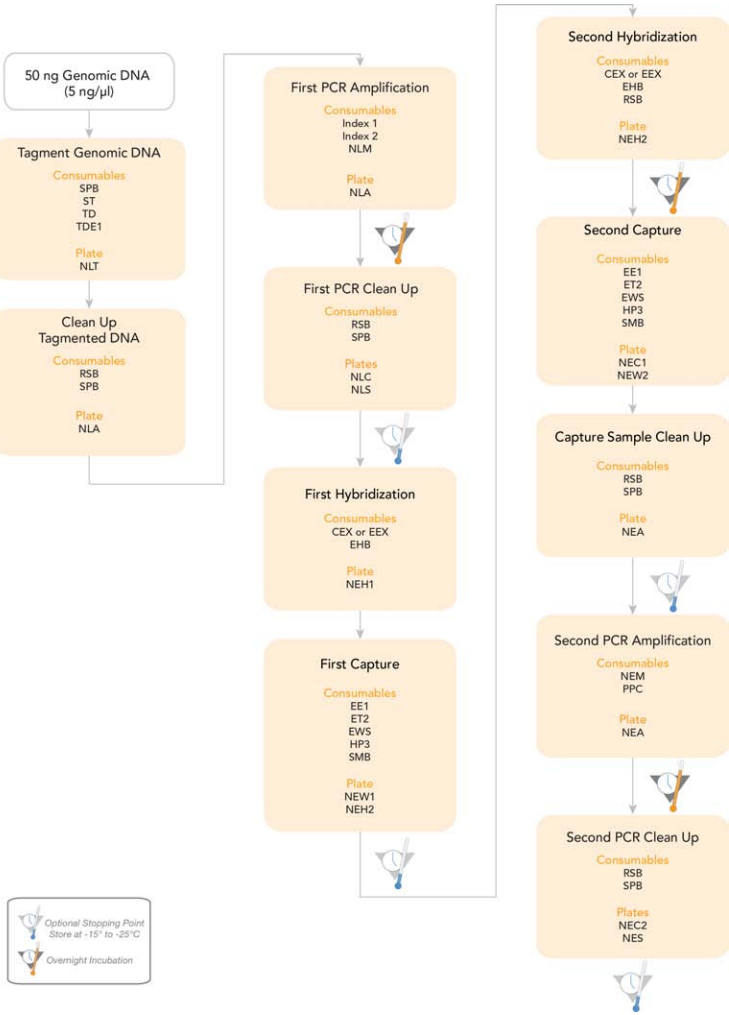
This chapter describes the Nextera Rapid Capture protocol.

- Review *Best Practices* on page 26 before proceeding.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- If you are pooling, record information about your samples before beginning library preparation for later use in data analysis.
  - For optimal sample tracking and quality control, fill out the Lab Tracking Form as you perform the sample preparation. For more information, see *Tracking Tools* on page 23.
  - Illumina recommends arranging samples that will be combined into a common pool in the same row.
  - Each column should contain a common index. This will facilitate pipetting operations when dispensing indexed adapters and pooling indexed libraries later in the protocol. For more information, see *Low-Plex Pooling Guidelines* on page 32.

# Sample PrepWorkflow

The following diagram illustrates the workflow using a Nextera Rapid Capture kit. Safe stopping points are marked between steps.

Figure 10 Nextera Rapid Capture Workflow



# Tagment Genomic DNA

This process tagments (tags and fragments) the gDNA by the Nextera transposome. The Nextera transposome simultaneously fragments the gDNA and adds adapter sequences to the ends, allowing amplification by PCR in subsequent processes.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Sample Purification Beads (SPB) | 1 tube | 2° to 8°C | Illumina |
| Stop Tagment Buffer (ST) | 1 tube | 15° to 30°C | Illumina |
| Tagment DNA Buffer (TD) | 1 tube | -15° to -25°C | Illumina |
| Tagment DNA Enzyme (TDE1) | 1 tube | -15° to -25°C | Illumina |
| 96-well MIDI plate | 1 | 15° to 30°C | User |
| gDNA (5 ng/µl) | 50 ng | -15° to -25°C | User |
| Microseal 'B' adhesive seals | 2 | 15° to 30°C | User |
| RNase/DNase-free Reagent Reservoir (for multi-sample processing) | 1 | 15° to 30°C | User |
| RNase/DNase-free Strip Tubes and Caps (for multi-sample processing) | 3 | 15° to 30°C | User |
| Tris-Cl 10 mM, pH 8.5 | as needed | 15° to 30°C | User |

## Preparation

- Remove the Tagment DNA Buffer, Tagment DNA Enzyme 1, and gDNA from -15° to -25°C storage and thaw on ice.
  - After thawing, ensure all reagents are adequately mixed by gently inverting the tubes 3–5 times, followed by a brief spin in a microcentrifuge.

- Remove the Sample Purification Beads from 2° to 8°C storage and let stand to bring to room temperature.
- Make sure the Stop Tagment Buffer has no precipitate. If there is precipitate, vortex until all particulates are resuspended.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Distribute the Stop Tagment Buffer, Tagment DNA Buffer, and Tagment DNA Enzyme 1 into separate eight-tube strips, dispensing equal volumes into each of the wells.
  - Pour the Sample Purification Beads into a multichannel reagent reservoir.
- Place a MIDI plate insert on the microheating system.
- Pre-heat the microheating system to 58°C.
- Label a new 96-well MIDI plate **NLT** (Nextera Library Tagment) with a smudge resistant pen.
- Use the Illumina Experiment Manager to determine the index primers to be used. Record the index primer positions on the Lab Tracking Form. For more information, see *Tracking Tools* on page 23.

## Procedure

NOTE
Ensure the reaction is assembled in the order described for optimal kit performance. The reaction does not need to be assembled on ice.

1. Perform the following steps to normalize your gDNA samples:
   a. Quantify your gDNA samples using a fluorometric method such as QuantiFluor or Qubit.
   b. Normalize your gDNA samples in Tris-Cl 10 mM, pH 8.5 to 10 ng/µl.
   c. Re-quantify the 10 ng/µl normalized sample using the same fluorometric quantification method.
   d. Based on this quantification, further dilute your gDNA samples in Tris-Cl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl (50 ng total) for use in the tagmentation reaction.
2. Add 10 µl of gDNA at 5 ng/µl (50 ng total) to each well of the new 96-well MIDI plate labeled NLT.
3. Add 25 µl of Tagment DNA Buffer to each well of the NLT plate.
4. Add 15 µl of Tagment DNA Enzyme 1 to each well of the NLT plate.

5 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.

6 Centrifuge the NLT plate to 280 xg for 1 minute.

7 Place the sealed NLT plate on the pre-heated microheating system. Close the lid and incubate at 58°C for 10 minutes.

8 Remove the NLT plate from the microheating system.

9 Remove the adhesive seal from the NLT plate.

10 Add 15 µl Stop Tagment Buffer to each well of the NLT plate.

11 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.

12 Centrifuge the NLT plate to 280 xg for 1 minute.

13 Incubate the NLT plate at room temperature for 4 minutes.

14 Proceed to *Clean Up Tagmented DNA* on page 41.

# Clean Up Tagmented DNA

This process purifies the tagmented DNA from the Nextera transposome and is critical because the Nextera transposome can bind tightly to DNA ends and will interfere with downstream processes if not removed.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Resuspension Buffer (RSB) | 1 tube | -15° to -25°C | Illumina |
| Sample Purification Beads (SPB) | 1 tube | 2° to 8°C | Illumina |
| 96-well hard-shell plate (HSP) | 1 | 15° to 30°C | User |
| Freshly prepared 80% ethanol (EtOH) | 400 µl per sample | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 2 | 15° to 30°C | User |
| RNase/DNase-free Reagent Reservoirs (for multi-sample processing) | 3 | 15° to 30°C | User |

## Preparation

- Remove the Resuspension Buffer from -15° to -25°C storage and thaw at room temperature.

NOTE
The Resuspension Buffer can be stored at 2° to 8°C after the initial thaw.

- Review *Handling Magnetic Beads* on page 26.
- Make sure that the Sample Purification Beads are at room temperature.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Pour the Resuspension Buffer, Sample Purification Beads, and 80% EtOH into separate multichannel reagent reservoirs.
- Label a new 96-well HSP plate **NLA** (Nextera Library Amplification) with a smudge resistant pen.

## Procedure

1. Remove the adhesive seal from the NLT plate.
2. Add 65 µl of well-resuspended Sample Purification Beads to each well of the NLT plate.
3. Mix thoroughly as follows:
   a. Seal the NLT plate with a Microseal 'B' adhesive seal.
   b. Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute.
4. Incubate the NLT plate at room temperature for 8 minutes.
5. Centrifuge the NLT plate to 280 xg for 1 minute.
6. Place the plate on the magnetic stand for 2 minutes or until the liquid appears clear.
7. Remove the adhesive seal from the NLT plate.
8. Using a 200 µl single channel or multichannel pipette set to 130 µl, remove and discard all of the supernatant from each well of the NLT plate.

NOTE
Leave the NLT plate on the magnetic stand while performing the following 80% EtOH wash steps (9–11).

9. With the NLT plate remaining on the magnetic stand, slowly add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads. Wait 30 seconds.
10. Remove and discard the 80% EtOH from each well of the NLT plate.
11. Repeat steps 9 and 10 once for a total of two 80% EtOH washes.
12. Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NLT plate without disturbing the beads.
13. With the NLT plate remaining on the magnetic stand, incubate the plate at room temperature for 10 minutes to dry.
14. Remove the NLT plate from the magnetic stand.
15. Add 22.5 µl of Resuspension Buffer to each well of the NLT plate. Do not touch the beads with the pipette tips.

16 Mix thoroughly as follows:
   a Seal the NLT plate with a Microseal 'B' adhesive seal.
   b Shake the NLT plate on a microplate shaker at 1800 rpm for 1 minute

17 Incubate the NLT plate at room temperature for 2 minutes.

18 Centrifuge the NLT plate to 280 xg for 1 minute.

19 Place the NLT plate on the magnetic stand for 2 minutes or until the liquid appears clear.

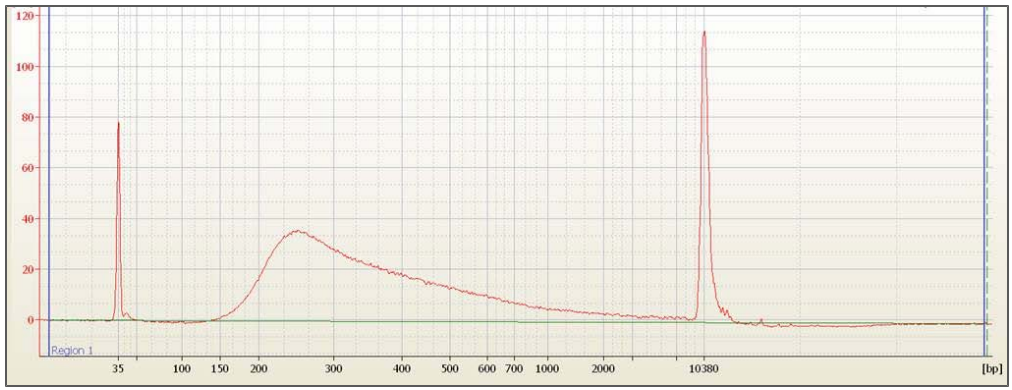
20 Remove the adhesive seal from the NLT plate.

21 Transfer 20 µl of clear supernatant from each well of the NLT plate to the corresponding well of the new HSP plate labeled NLA. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 10 µl to perform two consecutive transfers of 10 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

22 [Optional] Load 1 µl of the tagmentation reaction remaining in the NLT plate on an Agilent Technologies 2100 Bioanalyzer using a Agilent High Sensitivity DNA chip. Check the size of the sample, which should produce a broad distribution of DNA fragments with a size range from approximately 150 bp—1 kb.

Figure 11 Example Nextera Rapid Capture Post-Tagmentation Library Distribution



# First PCR Amplification

This process amplifies the purified tagmented DNA via a limited-cycle PCR program. It also adds index 1 (i7) and index 2 (i5) needed for sequencing, as well as common adapters (P5 and P7) required for cluster generation and sequencing. It is critical to use the full amount of recommended input DNA and it is imperative that no extra cycles are added to the PCR process to ensure the generation of libraries that produce high-quality sequencing results.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Index 1 primers (i7, N701–N712) | 1 tube each index | -15° to -25°C | Illumina |
| Index 1 Tube Caps, Orange | 1 per Index 1 primer tube | 15° to 30°C | Illumina |
| Index 2 primers (i5, E501–E502) | 1 tube each index | -15° to -25°C | Illumina |
| Index 2 Tube Caps, White | 1 per Index 2 primer tube | 15° to 30°C | Illumina |
| Nextera Library Amplification Mix (NLM) | 1 tube | -15° to -25°C | Illumina |
| 1.7 ml microcentrifuge tubes | 1 per index primer tube | 15° to 30°C | User |
| Microseal 'A' film | 1 | 15° to 30°C | User |
| Microseal 'B' adhesive seal | 1 | 15° to 30°C | User |
| RNase/DNase-free Strip Tube and Caps (for multi-sample processing) | 1 | 15° to 30°C | User |
| [Optional] TruSeq Index Plate Fixture Kit | 1 | 15° to 30°C | User |

### Preparation

- Remove the Nextera Library Amplification Mix from -15° to -25°C storage and thaw on ice.
- Remove the following from -15° to -25°C storage and thaw at room temperature:
  - Index 1 primers (i7, N701–N712) (only remove primers being used)
  - Index 2 primers (i5, E501–E502) (only remove primers being used)

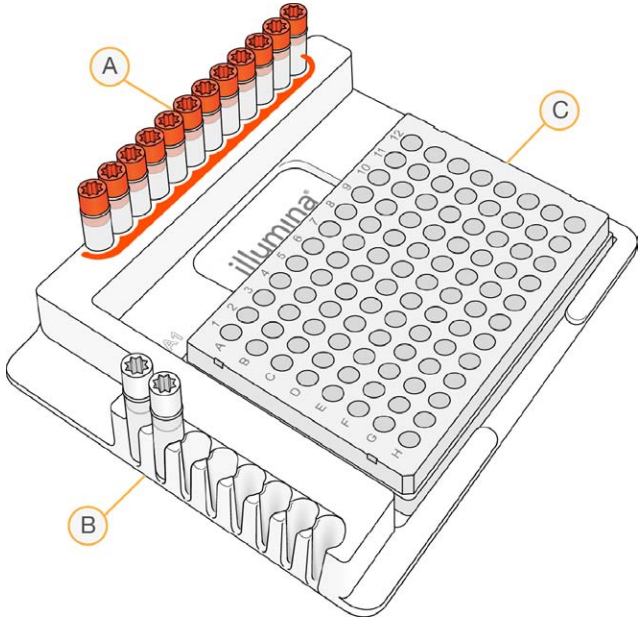
NOTE
Nextera Rapid Capture kits are designed to work only with Index 2 primers with the "E" prefix. Index 2 primers from other Nextera sample prep kits should not be used.

- For multi-sample processing:
  - Use a multichannel pipette.
  - Dispense the Nextera Library Amplification Mix in equal volumes into each of the wells of an eight-tube strip.
- Pre-program the thermal cycler with the following program and save as **NLM AMP**:
  - Choose the pre-heat lid option and set to 100°C
  - 72°C for 3 minutes
  - 98°C for 30 seconds
  - 10 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 10°C

## Setup Index Primers

1. Vortex the index primer tubes for 5 seconds.
2. Centrifuge the index primer tubes to 600 xg for 5 seconds. Use empty 1.7 ml microcentrifuge tubes as tube adapters for the microcentrifuge.
3. Arrange the index primers in a rack on ice (i.e. the TruSeq Index Plate Fixture) using the following arrangement:
   a. Arrange the Index 1 Primer tubes (orange caps) vertically, aligned with columns 1-12.
   b. Arrange the Index 2 Primer tubes (white caps) horizontally, aligned with rows A-H.

Figure 12 Index Plate Fixture




**A** Index 1 Primer tubes (orange caps)
**B** Index 2 Primer tubes (white caps)
**C** NLA plate

## Procedure

NOTE
For pooling libraries prior to enrichment, it is recommended to pool libraries so all Index 1 (i7) indices are unique. Choose Index 1 and Index 2 primers for PCR accordingly. For information, see *Low-Plex Pooling Guidelines* on page 32.

1 Add 5 µl of Index 1 (i7, N7xx) primer to each well of the NLA plate.

2 Add 5 µl of Index 2 (i5, E5xx) primer to each well of the NLA plate.

3 Add 20 µl of Nextera Library Amplification Mix to each well of the NLA plate.

4 Mix thoroughly as follows:
   a Seal the NLA plate with a Microseal 'A' film.
   b Shake the NLA plate on a microplate shaker at 1200 rpm for 1 minute

5 Centrifuge the NLA plate to 280 xg for 1 minute.

6 Place the sealed NLA plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NLM AMP** program.

SAFE STOPPING POINT

If you do not plan to immediately proceed to *First PCR Clean Up* on page 48, the NLA plate can remain on the thermocycler overnight. If you are stopping, replace the Microseal 'A' with a Microseal 'B' adhesive seal and store the NLA plate at 2° to 8°C for up to two days.

# First PCR Clean Up

This process uses Sample Purification Beads to purify the library DNA and remove unwanted products.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Resuspension Buffer (RSB) | 1 tube | 2° to 8°C | Illumina |
| Sample Purification Beads (SPB) | 1 tube | 2° to 8°C | Illumina |
| 96-well HSP plate | 1 | 15° to 30°C | User |
| 96-well MIDI plate | 1 | 15° to 30°C | User |
| Freshly prepared 80% ethanol (EtOH) | 400 µl per sample | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 3 | 15° to 30°C | User |
| RNase/DNase-free Reagent Reservoirs (for multi-sample processing) | 3 | 15° to 30°C | User |

## Preparation

- Review *Handling Magnetic Beads* on page 26.
- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- For multi-sample processing:
  - Use a multichannel pipette.
  - Pour the Resuspension Buffer, Sample Purification Beads, and 80% EtOH into separate multichannel reagent reservoirs.
- Label a new 96-well MIDI plate **NLC** (Nextera Library Clean Up) with a smudge resistant pen.
- Label a new 96-well HSP plate **NLS** (Nextera Library Sample) with a smudge resistant pen.

## Procedure

1. Remove the NLA plate from the thermocycler and centrifuge to 280 xg for 1 minute.
2. Remove the adhesive seal from the NLA plate.
3. Transfer 50 µl of clear supernatant from each well of the NLA plate to the corresponding well of the new 96-well MIDI plate labeled NLC.
4. Vortex the Sample Purification Beads until the beads are well dispersed, then add 90 µl of well-resuspended Sample Purification Beads to each well of the NLC plate.
5. Mix thoroughly as follows:
   a. Seal the NLC plate with a Microseal 'B' adhesive seal.
   b. Shake the NLC plate on a microplate shaker at 1800 rpm for 1 minute.
6. Incubate the NLC plate at room temperature for 10 minutes.
7. Centrifuge the NLC plate to 280 xg for 1 minute.
8. Remove the adhesive seal from the NLC plate.
9. Place the NLC plate on the magnetic stand for 2 minutes or until the liquid appears clear.
10. Carefully remove and discard all of the supernatant from each well of the NLC plate.

NOTE
Leave the NLC plate on the magnetic stand while performing the following 80% EtOH wash steps (11–13).

11. With the NLC plate remaining on the magnetic stand, slowly add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads. Wait 30 seconds.
12. Remove and discard the 80% EtOH from each well of the NLC plate.
13. Repeat steps 11 and 12 once for a total of two 80% EtOH washes.
14. Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NLC plate without disturbing the beads.
15. Let the NLC plate stand at room temperature for 10 minutes to dry on the magnetic stand.
16. Remove the NLC plate from the magnetic stand.

17 Add 27 µl of Resuspension Buffer to each well of the NLC plate. Do not touch the beads with the pipette tips.

18 Mix thoroughly as follows:
   a Seal the NLC plate with a Microseal 'B' adhesive seal.
   b Shake the NLC plate on a microplate shaker at 1800 rpm for 1 minute.

19 Incubate the NLC plate at room temperature for 2 minutes.

20 Centrifuge the NLC plate to 280 xg for 1 minute.

21 Remove the adhesive seal from the NLC plate.

22 Place the NLC plate on the magnetic stand for 2 minutes or until the liquid appears clear.

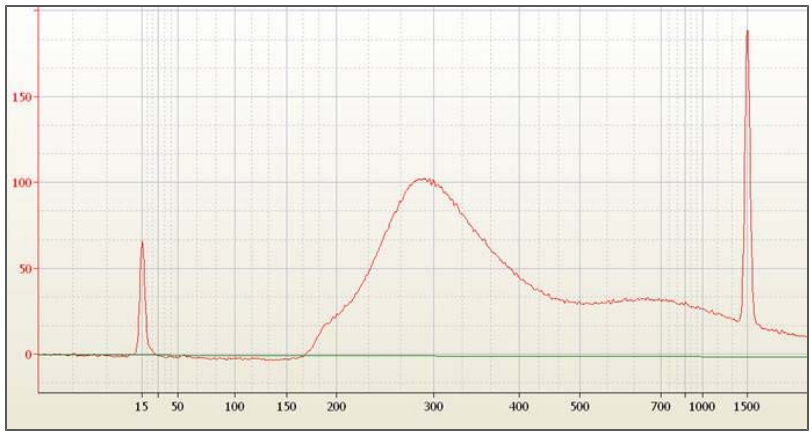
23 Transfer 25 µl of clear supernatant from each well of the NLC plate to the corresponding well of the new HSP plate labeled NLS. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 12.5 µl to perform two consecutive transfers of 12.5 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

24 Quantify the library in the NLS plate using a fluorometric method. See *DNA Quantitation* on page 79 for an example protocol using the Promega QuantiFluor method.

25 [Optional] Load 1 µl of the library on an Agilent Technologies 2100 Bioanalyzer using a Agilent DNA 1000 Chip. Check the size of the library, which should produce a distribution of DNA fragments with a size range from approximately 150 bp—1 kb.

Figure 13 Example Nextera Rapid Capture Post-PCR, Pre-Enriched Library Distribution




NOTE

The sample peak should not be significantly shifted compared to the example shown in Figure 13, although traces may differ depending on sample quality. A larger peak distribution (> 350 bp) can be indicative of > 50 ng gDNA input going into tagmentation and may result in lower on-target reads. Conversely, a smaller sample peak distribution (< 225 bp) can be indicative of < 50 ng gDNA or low quality gDNA, which may result in reduced library diversity or elevated duplicates.

SAFE STOPPING POINT

If you do not plan to proceed to *First Hybridization* on page 52 immediately, the protocol can be safely stopped here. If you are stopping, seal the NLS plate with a Microseal 'B' adhesive seal and store it at -15° to -25°C for up to 14 days.

# First Hybridization

This process mixes the DNA library with capture probes to targeted regions of interest. The recommended hybridization time makes sure that targeted regions bind to the capture probes thoroughly. This process also describes how to combine multiple libraries with different indices into a single pool prior to enrichment.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| One of the following, depending on the kit you are using:<br>• Coding Exome Oligos (CEX)<br>• Expanded Exome Oligos (EEX) | 1 tube | -15° to -25°C | Illumina |
| Enrichment Hybridization Buffer (EHB) | 1 tube | -15° to -25°C | Illumina |
| 96-well HSP plate | 1 | 15° to 30°C | User |
| [Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) | 1 per pooled sample | 15° to 30°C | User |
| Microseal 'B' adhesive seal | 1 | 15° to 30°C | User |
| RNase/DNase-free Strip Tubes and Caps (for multi-sample processing) | 2 | 15° to 30°C | User |

## Preparation

- Remove the following from -15° to -25°C storage and thaw them at room temperature:
  - Enrichment Hybridization Buffer
  - One of the following, depending on the kit you are using:
    - Coding Exome Oligos
    - Expanded Exome Oligos

- For multi-sample processing:
  - Use a multichannel pipette.
  - Distribute the Coding Exome Oligos or Expanded Exome Oligos, and Enrichment Hybridization Buffer into separate eight-tube strips, dispensing equal volumes into each of the wells.
- Remove the NLS plate from -15° to -25°C storage, if it was stored at the conclusion of *First PCR Clean Up* and thaw on ice.
  - Centrifuge the thawed NLS plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the thawed NLS plate.
- Pre-program the thermal cycler with the following program and save as **NRC HYB**:
  a. Choose the pre-heat lid option and set to 100°C
  b. 95°C for 10 minutes
  c. 18 cycles of 1 minute incubations, starting at 94°C, then decreasing 2°C per cycle
  d. 58°C for forever
- Label a new 96-well HSP plate **NEH1** (Nextera Enrichment Hyb 1) with a smudge resistant pen.

## Pool Libraries

1. Reference the table below for the amount of DNA libraries to use for enrichment. Illumina recommends using 500 ng of each DNA library, quantified by QuantiFluor. See *DNA Input Recommendations* on page 25.
   - If your are pooling libraries, combine 500 ng of each DNA library.
   - If the total volume is greater than 40 µl, concentrate the pooled sample using either a vacuum concentrator or Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) according to manufacturer's instructions.
     - If you are using a vacuum concentrator, Illumina recommends concentrating samples with a no heat and medium drying rate setting.
     - If you are using a Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa), it is not required to pre-rinse the device prior to use. The majority of volume will filter through in 5 minutes, but up to 30 minutes may be required depending on the starting volume.
   - If the pooled sample volume after concentrating is less than 40 µl, bring the volume up to 40 µl with Resuspension Buffer.
2. The recommended pre-enrichment pooling strategy is to pool libraries so that each contains a unique Index 1/i7 index. With this pooling approach samples can be

sequenced using a single index read workflow, as described in the HiSeq and GAIIx user guides.

- If Index1/i7 indexes are not unique, ensure that libraries with different Index 2/i5 indexes are included (e.g. N703/E501 and N703/E502). With this approach, sequence samples using a dual index read workflow, as described in the HiSeq and GAIIx user guides.
- See *Low-Plex Pooling Guidelines* on page 32 for further details.

Table 8 DNA Libraries for Enrichment

| Library Pool Complexity | Total DNA Library Mass (ng) |
|---|---|
| 1-plex | 500 |
| 2-plex | 1000 |
| 3-plex | 1500 |
| 4-plex | 2000 |
| 5-plex | 2500 |
| 6-plex | 3000 |
| 7-plex | 3500 |
| 8-plex | 4000 |
| 9-plex | 4500 |
| 10-plex | 5000 |
| 11-plex | 5500 |
| 12-plex | 6000 |

## Procedure

1 Thoroughly vortex the Enrichment Hybridization Buffer tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

NOTE
If crystals and cloudiness are observed, vortex the Enrichment Hybridization Buffer tube until it appears clear.

2 Add the following reagents in the order listed below to each well of the new 96-well HSP plate labeled NEH1.

| Reagent | Volume (µl) |
|---|---|
| DNA library sample or library pool from NLS plate | 40 |
| Enrichment Hybridization Buffer | 50 |
| Coding Exome Oligos or<br>Expanded Exome Oligos | 10 |
| **Total Volume per Sample** | **100** |

3 Mix thoroughly as follows:
   a Seal the NEH1 plate with a Microseal 'B' adhesive seal. Make sure that the plate is tightly sealed to prevent potential evaporation. Use an adhesive seal roller to apply force to the seal and make sure the seal is secured.
   b Shake the NEH1 plate on a microplate shaker at 1200 rpm for 1 minute.

4 Centrifuge the NEH1 plate to 280 xg for 1 minute.

5 Place the sealed NEH1 plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NRC HYB** program.
***Incubate the plate at the 58°C holding temperature for at least 90 minutes and up to a maximum of 24 hours***. Do not remove the plate from 58°C incubation until you are ready to proceed to *First Capture* on page 56.

# First Capture

This process uses streptavidin beads to capture probes hybridized to the targeted regions of interest. Two heated wash procedures remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for a second round of hybridization.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| 2N NaOH (HP3) | 1 tube | -15° to -25°C | Illumina |
| Elute Target Buffer 2 (ET2) | 1 tube | 2° to 8°C | Illumina |
| Enrichment Elution Buffer 1 (EE1) | 1 tube | -15° to -25°C | Illumina |
| Enrichment Wash Solution (EWS) | 1 tube | -15° to -25°C | Illumina |
| Streptavidin Magnetic Beads (SMB) | 1 tube | 2° to 8°C | Illumina |
| 1.7 ml microcentrifuge tube | 1 | 15° to 30°C | User |
| 96-well HSP plate | 1 | 15° to 30°C | User |
| 96-well MIDI plate | 1 | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 6 | 15° to 30°C | User |

## Preparation

- Remove the 2N NaOH, Enrichment Elution Buffer 1, and Enrichment Wash Solution from -15° to -25°C storage and thaw at room temperature.
- Remove the Elute Target Buffer 2 and Streptavidin Magnetic Beads from 2° to 8°C storage and let stand at room temperature.

  NOTE
  Make sure that you use the Streptavidin Magnetic Beads (2 ml tube) and not the Sample Purification Beads (15 ml tube) for this procedure.

- Pre-heat the microheating system to 50°C.

- Label a new 96-well MIDI plate **NEW1** (Nextera Enrichment Wash 1) with a smudge resistant pen.
- Label a new 96-well HSP plate **NEH2** (Nextera Enrichment Hyb 2) with a smudge resistant pen.

## First Bind

1. Remove the NEH1 plate from the thermal cycler.
2. Centrifuge the NEH1 plate to 280 xg for 1 minute.
3. Remove the adhesive seal from the NEH1 plate. Take care when removing the seal to avoid spilling the contents of the wells.
4. Transfer the entire contents (~100 µl) from each well of the NEH1 plate to the corresponding well of the new 96-well MIDI plate labeled NEW1.

   NOTE

   If an overnight First Hybridization was performed, it is normal to see a small degree of sample loss. However, if the sample loss is greater than 15%, Illumina does not recommended proceeding with the sample preparation. This amount of loss can be caused by poor sealing or not heating the lid.

5. Vortex the Streptavidin Magnetic Beads tube until the beads are well dispersed, then add 250 µl of well-mixed Streptavidin Magnetic Beads to the wells of the NEW1 plate.
6. Mix thoroughly as follows:
   a. Seal the NEW1 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEW1 plate on a microplate shaker at 1200 rpm for 5 minutes.
7. Let the NEW1 plate stand at room temperature for 25 minutes.
8. Centrifuge the NEW1 plate to 280 xg for 1 minute.
9. Remove the adhesive seal from the NEW1 plate.
10. Place the NEW1 plate on the magnetic stand for 2 minutes at room temperature or until the liquid appears clear.
11. Carefully remove and discard all of the supernatant from each well of the NEW1 plate without disturbing the beads.
12. Remove the NEW1 plate from the magnetic stand.

## First Wash

1 Make sure that the Enrichment Wash Solution tube is at room temperature, then thoroughly vortex the tube.

NOTE
It is normal that the Enrichment Wash Solution may be cloudy after vortexing.

2 Add 200 µl of Enrichment Wash Solution to each well of the NEW1 plate.

3 Mix thoroughly as follows:
   a Seal the NEW1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW1 plate on a microplate shaker at 1800 rpm for 4 minutes.
   c Remove the adhesive seal from the NEW1 plate.
   d Gently pipette the entire volume of each well up and down to ensure complete resuspension of the sample.

4 Seal the NEW1 plate with a Microseal 'B' adhesive seal.

5 Place the sealed NEW1 plate on the **pre-heated** microheating system. Close the lid and incubate at 50°C for 30 minutes.

6 Place the magnetic stand next to the microheating system for immediate access.

7 Remove the NEW1 plate from the microheating system and ***immediately*** place it on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove the adhesive seal from the NEW1 plate.

9 Immediately remove and discard all of the supernatant from each well of the NEW1 plate.

10 Remove the NEW1 plate from the magnetic stand.

11 Repeat steps 2–10 once for a total of two Enrichment Wash Solution washes.

## First Elution

1 Add the following reagents in the order listed in a new 1.7 ml microcentrifuge tube to create the elution pre-mix. Multiply each volume by the number of pooled samples being prepared. The volumes below include an excess amount for processing multiple samples.

| Reagent | Volume (µl ) |
|---|---|
| Enrichment Elution Buffer 1 | 28.5 |
| 2N NaOH | 1.5 |
| **Total Volume per Sample** | **30** |

2 Vortex the elution pre-mix tube, then add **23 µl** of the mix to each well of the NEW1 plate.

3 Mix thoroughly as follows:
   a Seal the NEW1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW1 plate on a microplate shaker at 1800 rpm for 2 minutes.

4 Let the NEW1 plate stand at room temperature for 2 minutes.

5 Centrifuge the NEW1 plate to 280 xg for 1 minute.

6 Carefully remove the adhesive seal from the NEW1 plate to avoid spilling the contents of the wells.

7 Place the NEW1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Transfer 21 µl of clear supernatant from each well of the NEW1 plate to the corresponding well of the new HSP plate labeled NEH2. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 10.5 µl to perform two consecutive transfers of 10.5 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

9 Add 4 µl of Elute Target Buffer 2 to each well of the NEH2 plate containing samples to neutralize the elution.

10 Mix thoroughly as follows:
   a Seal the NEH2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEH2 plate on a microplate shaker at 1200 rpm for 1 minute.

11 Centrifuge the NEH2 plate to 280 xg for 1 minute.

12 Store the remaining reagents as follows:
   a Place the Elute Target Buffer 2 and Streptavidin Magnetic Beads tubes in 2° to 8°C storage.
   b Place the 2N NaOH, Enrichment Elution Buffer 1, and Enrichment Wash Solution tubes in -15° to -25°C storage.
   c Discard any remaining elution pre-mix.

SAFE STOPPING POINT

If you do not plan to proceed to *Second Hybridization* on page 61 immediately, the protocol can be safely stopped here. If you are stopping, seal the NEH2 plate with a Microseal 'B' adhesive seal and store it at -15° to -25°C for up to seven days.

# Second Hybridization

This process combines the eluted DNA library from the first enrichment round with additional capture probes to targeted regions of interest. This second hybridization is required to ensure high specificity of the captured regions.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| One of the following, depending on the kit you are using:<br>• Coding Exome Oligos (CEX)<br>• Expanded Exome Oligos (EEX) | 1 tube | -15° to -25°C | Illumina |
| Enrichment Hybridization Buffer (EHB) | 1 tube | -15° to -25°C | Illumina |
| Resuspension Buffer (RSB) | 1 tube | 2° to 8°C | Illumina |
| Microseal 'B' adhesive seal | 1 | 15° to 30°C | User |

## Preparation

- Remove the following from -15° to -25°C storage and thaw them at room temperature:
  - Enrichment Hybridization Buffer
  - One of the following, depending on the kit you are using:
    - Coding Exome Oligos
    - Expanded Exome Oligos
- Make sure that the Resuspension Buffer is at room temperature.
- Remove the NEH2 plate from -15° to -25°C storage, if it was stored at the conclusion of *First Capture* and thaw on ice.
  - Centrifuge the thawed NEH2 plate to 280 xg for 1 minute.

## Procedure

1 Thoroughly vortex the Enrichment Hybridization Buffer tube until the solution is completely resuspended. Visually make sure that no crystal structures are present.

NOTE
If crystals and cloudiness are observed, vortex the Enrichment Hybridization Buffer tube until it appears clear.

2 Remove the adhesive seal from the NEH2 plate.

3 Add 15 µl of Resuspension Buffer to each well of the NEH2 plate.

4 Add 50 µl of Enrichment Hybridization Buffer to each well of the NEH2 plate.

5 Add 10 µl of one of the following reagents to each well of the NEH2 plate, depending on the kit you are using:
- Coding Exome Oligos
- Expanded Exome Oligos

6 Mix thoroughly as follows:
   a Seal the NEH2 plate with a Microseal 'B' adhesive seal. Make sure that the plate is tightly sealed to prevent potential evaporation. Use an adhesive seal roller to apply force to the seal and make sure the seal is secured.
   b Shake the NEH2 plate on a microplate shaker at 1200 rpm for 1 minute

7 Centrifuge the NEH2 plate to 280 xg for 1 minute.

8 Place the sealed NEH2 plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NRC HYB** program.
***Incubate the plate at the 58°C holding temperature overnight for at least 14.5 hours and up to a maximum of 24 hours.*** Do not remove the plate from 58°C incubation until you are ready to proceed to *Second Capture* on page 63.

# Second Capture

This process uses streptavidin beads to capture probes hybridized to the targeted regions of interest. Two heated wash procedures remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for sequencing.

NOTE
These procedures are similar to the *First Capture* on page 56.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| 2N NaOH (HP3) | 1 tube | -15° to -25°C | Illumina |
| Elute Target Buffer 2 (ET2) | 1 tube | 2° to 8°C | Illumina |
| Enrichment Elution Buffer 1 (EE1) | 1 tube | -15° to -25°C | Illumina |
| Enrichment Wash Solution (EWS) | 1 tube | -15° to -25°C | Illumina |
| Streptavidin Magnetic Beads (SMB) | 1 tube | 2° to 8°C | Illumina |
| 1.7 ml microcentrifuge tube | 1 | 15° to 30°C | User |
| 96-well MIDI plates | 2 | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 6 | 15° to 30°C | User |

## Preparation

- Remove the 2N NaOH, Enrichment Elution Buffer 1, and Enrichment Wash Solution from -15° to -25°C storage and thaw at room temperature.
- Remove the Elute Target Buffer 2 and Streptavidin Magnetic Beads from 2° to 8°C storage and let stand at room temperature.

NOTE
Make sure that you use the Streptavidin Magnetic Beads (2 ml tube) and not the Sample Purification Beads (15 ml tube) for this procedure.

- Pre-heat the microheating system to 50°C.

- Label a new 96-well MIDI plate **NEW2** (Nextera Enrichment Wash 2) with a smudge resistant pen.
- Label a new 96-well MIDI plate **NEC1** (Nextera Enriched Clean Up 1) with a smudge resistant pen.

## Second Bind

1. Remove the NEH2 plate from the thermal cycler.
2. Centrifuge the room temperature NEH2 plate to 280 xg for 1 minute.
3. Remove the adhesive seal from the NEH2 plate. Take care when removing the seal to avoid spilling the contents of the wells.
4. Transfer the entire contents (~100 µl) from each well of the NEH2 plate to the corresponding well of the new 96-well MIDI plate labeled NEW2.

   NOTE
   It is normal to see a small degree of sample loss after overnight hybridization. However, if the sample loss is greater than 15%, Illumina does not recommended proceeding with the sample preparation. This amount of loss can be caused by poor sealing or not heating the lid.

5. Vortex the Streptavidin Magnetic Beads tube until the beads are well dispersed, then add 250 µl of well-mixed Streptavidin Magnetic Beads to the wells of the NEW2 plate.
6. Mix thoroughly as follows:
   a. Seal the NEW2 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEW2 plate on a microplate shaker at 1200 rpm for 5 minutes
7. Let the NEW2 plate stand at room temperature for 25 minutes.
8. Centrifuge the NEW2 plate to 280 xg for 1 minute.
9. Remove the adhesive seal from the NEW2 plate.
10. Place the NEW2 plate on the magnetic stand for 2 minutes at room temperature or until the liquid appears clear.
11. Carefully remove and discard all of the supernatant from each well of the NEW2 plate without disturbing the beads.
12. Remove the NEW2 plate from the magnetic stand.

## Second Wash

1 Make sure that the Enrichment Wash Solution tube is at room temperature, then thoroughly vortex the tube.

NOTE
It is normal that the Enrichment Wash Solution may be cloudy after vortexing.

2 Add 200 µl of Enrichment Wash Solution to each well of the NEW2 plate.

3 Mix thoroughly as follows:
   a Seal the NEW2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW2 plate on a microplate shaker at 1800 rpm for 4 minutes
   c Remove the adhesive seal from the NEW2 plate.
   d Gently pipette the entire volume of each well up and down to ensure complete resuspension of the sample.

4 Seal the NEW2 plate with a Microseal 'B' adhesive seal.

5 Incubate the NEW2 plate on the **pre-heated** microheating system, with the lid closed, at 50°C for 30 minutes.

6 Place the magnetic stand next to the microheating system for immediate access.

7 Remove the NEW2 plate from the microheating system and ***immediately*** place it on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove the adhesive seal from the NEW2 plate.

9 Immediately remove and discard all of the supernatant from each well of the NEW2 plate.

10 Remove the NEW2 plate from the magnetic stand.

11 Repeat steps 2–10 once for a total of two Enrichment Wash Solution washes.

## Second Elution

1 Add the following reagents in the order listed in a new 1.7 ml microcentrifuge tube to create the elution pre-mix. Multiply each volume by the number of pooled samples being prepared. The volumes below include an excess amount for processing multiple samples.

| Reagent | Volume (µl ) |
|---|---|
| Enrichment Elution Buffer 1 | 28.5 |
| 2N NaOH | 1.5 |
| **Total Volume per Sample** | **30** |

2 Vortex the elution pre-mix tube, then add **23 µl** of the mix to each well of the NEW2 plate.

3 Mix thoroughly as follows:
   a Seal the NEW2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEW2 plate on a microplate shaker at 1800 rpm for 2 minutes.

4 Let the NEW2 plate stand at room temperature for 2 minutes.

5 Centrifuge the NEW2 plate to 280 xg for 1 minute.

6 Carefully remove the adhesive seal from the NEW2 plate to avoid spilling the contents of the wells.

7 Place the NEW2 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Transfer 21 µl of clear supernatant from each well of the NEW2 plate to the corresponding well of the new MIDI plate labeled NEC1. Take care not to disturb the beads.

NOTE
Illumina recommends using a 20 µl single channel or multichannel pipette set to 10.5 µl to perform two consecutive transfers of 10.5 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

9 Add 4 µl Elute Target Buffer 2 to each well of the NEC1 plate containing samples to neutralize the elution.

10 Mix thoroughly as follows:
   a Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute.

11 Centrifuge the NEC1 plate to 280 xg for 1 minute.

12 Store the remaining reagents as follows:
   a Place the Elute Target Buffer 2 and Streptavidin Magnetic Beads tubes in 2° to 8°C storage.
   b Place the 2N NaOH, Enrichment Elution Buffer 1, and Enrichment Wash Solution tubes in -15° to -25°C storage.
   c Discard any remaining elution pre-mix.

# Capture Sample Clean Up

This process uses Sample Purification Beads to purify the captured library DNA prior to PCR amplification.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Resuspension Buffer (RSB) | 1 tube | 2° to 8°C | Illumina |
| Sample Purification Beads (SPB) | 1 tube | 2° to 8°C | Illumina |
| 96-well HSP plate | 1 | 15° to 30°C | User |
| Freshly prepared 80% ethanol (EtOH) | 400 µl per sample | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 3 | 15° to 30°C | User |

## Preparation

- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- Label a new 96-well HSP plate **NEA** (Nextera Enrichment Amplification) with a smudge resistant pen.

## Procedure

1. Remove the adhesive seal from the NEC1 plate.
2. Vortex the Sample Purification Beads tube until the beads are well dispersed, then add 45 µl of the well-mixed Sample Purification Beads to each well of the NEC1 plate.
3. Mix thoroughly as follows:
   a. Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b. Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute.
4. Incubate the NEC1 plate at room temperature for 10 minutes.
5. Centrifuge the NEC1 plate to 280 xg for 1 minute.

6 Remove the adhesive seal from the NEC1 plate.

7 Place the NEC1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

8 Remove and discard all of the supernatant from each well of the NEC1 plate.

NOTE
Leave the NEC1 plate on the magnetic stand while performing the following 80% EtOH wash steps (9–11).

9 With the NEC1 plate remaining on the magnetic stand, slowly add 200 µl of freshly made 80% EtOH to each well without disturbing the beads. Wait 30 seconds.

10 Remove and discard the 80% EtOH from each well of the NEC1 plate.

11 Repeat steps 9 and 10 once for a total of two 80% EtOH washes.

12 Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NEC1 plate without disturbing the beads.

13 Let the NEC1 plate stand at room temperature for 10 minutes to dry on the magnetic stand.

14 Remove the NEC1 plate from the magnetic stand.

15 Add 27.5 µl of Resuspension Buffer to each well of the NEC1 plate. Do not touch the beads with the pipette tips.

16 Mix thoroughly as follows:
   a Seal the NEC1 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC1 plate on a microplate shaker at 1800 rpm for 1 minute

17 Incubate the NEC1 plate at room temperature for 2 minutes.

18 Centrifuge the NEC1 plate to 280 xg for 1 minute.

19 Remove the adhesive seal from the NEC1 plate.

20 Place the NEC1 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

21 Transfer 25 µl of clear supernatant from each well of the NEC1 plate to the corresponding well of the new HSP plate labeled NEA. Take care not to disturb the beads.

NOTE

Illumina recommends using a 20 µl single channel or multichannel pipette set to 12.5 µl to perform two consecutive transfers of 12.5 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

SAFE STOPPING POINT

If you do not plan to proceed to *Second PCR Amplification* on page 71 immediately, the protocol can be safely stopped here. If you are stopping, seal the NEA plate with a Microseal 'B' adhesive seal and store it at -15° to -25°C for up to seven days.

# Second PCR Amplification

This process amplifies the captured library via a limited-cycle PCR program. It is critical to use the full amount of recommended input DNA and not add extra PCR cycles to ensure libraries produce high-quality sequencing results.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Nextera Enrichment Amplification Mix (NEM) | 1 tube | -15° to -25°C | Illumina |
| PCR Primer Cocktail (PPC) | 1 tube | -15° to -25°C | Illumina |
| Microseal 'A' film | 1 | 15° to 30°C | User |
| Microseal 'B' adhesive seal | 1 | 15° to 30°C | User |

## Preparation

- Remove the Nextera Enrichment Amplification Mix and PCR Primer Cocktail from -15° to -25°C storage and thaw on ice.
  - Briefly centrifuge the thawed Nextera Enrichment Amplification Mix and PCR Primer Cocktail tubes for 5 seconds.

NOTE
If you do not intend to consume the Nextera Enrichment Amplification Mix and PCR Primer Cocktail in one use, dispense the reagents into single use aliquots and freeze in order to avoid repeated freeze thaw cycles.

- Remove the NEA plate from -15° to -25°C storage, if it was stored at the conclusion of *Second Capture* and thaw on ice.
  - Centrifuge the thawed NEA plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the thawed NEA plate.

- Pre-program the thermal cycler with the following program and save as **NEM AMP**:
  - Choose the pre-heat lid option and set to 100°C
  - 98°C for 30 seconds
  - 10 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 10°C

## Procedure

1. Add 5 µl of PCR Primer Cocktail to each well of the NEA plate.
2. Add 20 µl of Nextera Enrichment Amplification Mix to each well of the NEA plate.
3. Mix thoroughly as follows:
   a. Seal the NEA plate with a Microseal 'A' film. Use an adhesive seal roller to apply force to the film and make sure the film is secured.
   b. Shake the NEA plate on a microplate shaker at 1200 rpm for 1 minute
4. Centrifuge the NEA plate to 280 xg for 1 minute.
5. Place the sealed NEA plate on the pre-programmed thermal cycler. Close the lid, then select and run the **NEM AMP** program.

SAFE STOPPING POINT

If you do not plan to immediately proceed to *Second PCR Clean Up* on page 73, the NEA plate can remain on the thermocycler overnight. If you are stopping, replace the Microseal 'A' with a Microseal 'B' adhesive seal and store the NEA plate at 2° to 8°C for up to two days.

# Second PCR Clean Up

This process uses Sample Purification Beads to purify the enriched library DNA and remove unwanted products.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| Resuspension Buffer (RSB) | 1 tube | 2° to 8°C | Illumina |
| Sample Purification Beads (SPB) | 1 tube | 2° to 8°C | Illumina |
| 96-well HSP plate | 1 | 15° to 30°C | User |
| 96-well MIDI plate | 1 | 15° to 30°C | User |
| Freshly prepared 80% ethanol (EtOH) | 400 µl per sample | 15° to 30°C | User |
| Microseal 'B' adhesive seals | 3 | 15° to 30°C | User |

## Preparation

- Review *Handling Magnetic Beads* on page 26.
- Make sure that the Resuspension Buffer and Sample Purification Beads are at room temperature.
- Remove the NEA plate from 2° to 8°C storage, if it was stored at the conclusion of *Second PCR Amplification* and let stand to bring to room temperature.
- Label a new 96-well MIDI plate **NEC2** (Nextera Enriched Clean Up 2) with a smudge resistant pen.
- Label a new 96-well HSP plate **NES** (Nextera Enrichment Sample) with a smudge resistant pen.

# Procedure

1. Centrifuge the NEA plate to 280 xg for 1 minute.
2. Remove the adhesive seal from the NEA plate.

3 Transfer the entire contents from each well of the NEA plate to the corresponding well of the new 96-well MIDI plate labeled NEC2.

4 Vortex the Sample Purification Beads until the beads are well dispersed, then add 90 µl of the well-mixed Sample Purification Beads to each well of the NEC2 plate containing 50 µl of the PCR amplified library.

5 Mix thoroughly as follows:
   a Seal the NEC2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC2 plate on a microplate shaker at 1800 rpm for 1 minute.

6 Incubate the NEC2 plate at room temperature for 10 minutes.

7 Centrifuge the NEC2 plate to 280 xg for 1 minute.

8 Remove the adhesive seal from the NEC2 plate.

9 Place the NEC2 plate on the magnetic stand at room temperature for 2 minutes or until the liquid appears clear.

10 Carefully remove and discard all of the supernatant from each well of the NEC2 plate.

NOTE
Leave the NEC2 plate on the magnetic stand while performing the following 80% EtOH wash steps (11–13).

11 With the NEC2 plate remaining on the magnetic stand, slowly add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads. Wait 30 seconds.

12 Remove and discard the 80% EtOH from each well of the NEC2 plate.

13 Repeat steps 11–12 once for a total of two 80% EtOH washes.

14 Using a 20 µl single channel or multichannel pipette, remove any remaining 80% EtOH from each well of the NEC2 plate without disturbing the beads.

15 Let the NEC2 plate stand at room temperature for 10 minutes to dry on the magnetic stand.

16 Remove the NEC2 plate from the magnetic stand.

17 Add 32 µl of Resuspension Buffer to each well of the NEC2 plate. Do not touch the beads with the pipette tips.

18 Mix thoroughly as follows:
   a Seal the NEC2 plate with a Microseal 'B' adhesive seal.
   b Shake the NEC2 plate on a microplate shaker at 1800 rpm for 1 minute.

19 Incubate the NEC2 plate at room temperature for 2 minutes.

20 Centrifuge the NEC2 plate to 280 xg for 1 minute.

21 Remove the adhesive seal from the NEC2 plate.

22 Place the NEC2 plate on the magnetic stand for 2 minutes or until the liquid appears clear.

23 Transfer 30 µl of clear supernatant from each well of the NEC2 plate to the corresponding well of the new HSP plate labeled NES. Take care not to disturb the beads.

NOTE

Illumina recommends using a 20 µl single channel or multichannel pipette set to 15 µl to perform two consecutive transfers of 15 µl to reduce sample loss by ensuring that all of the liquid is transferred without disturbing the beads.

24 Seal the NES plate with a Microseal 'B' adhesive seal.

SAFE STOPPING POINT

If you do not plan to immediately proceed to *Validate Library* on page 76, store the sealed NES plate at -15° to -25°C for up to seven days. If the plate is stored for more than seven days, re-quantify your library to guarantee the accuracy of your enrichment results.

# Validate Library

Illumina recommends performing the following procedures for quality control analysis on your post-enriched library and quantification of the DNA library templates.

## Quantify Libraries

In order to achieve the highest quality of data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantitation of DNA library templates.

Quantify the post-enriched library in the NES plate using a fluorometric method. See *DNA Quantitation* on page 79 for an example protocol using the Promega QuantiFluor method.

Alternatively, you can quantitate libraries using qPCR according to the Illumina *Sequencing Library qPCR Quantification Guide*.

NOTE

You can download the *Sequencing Library qPCR Quantification Guide* from the Illumina website at www.illumina.com. Go to the Nextera Rapid Capture Exome support page, then click **Documentation & Literature**.

NOTE

Use the following formula to convert from ng/µl to nM. Assume a 400 bp library size or calculate based on the average size of the enriched library:

$$\frac{\text{(concentration in ng/µl)}}{\text{(660 g/mol *average library size)}} \times 10^6 = \text{concentration in nM}$$

For example:

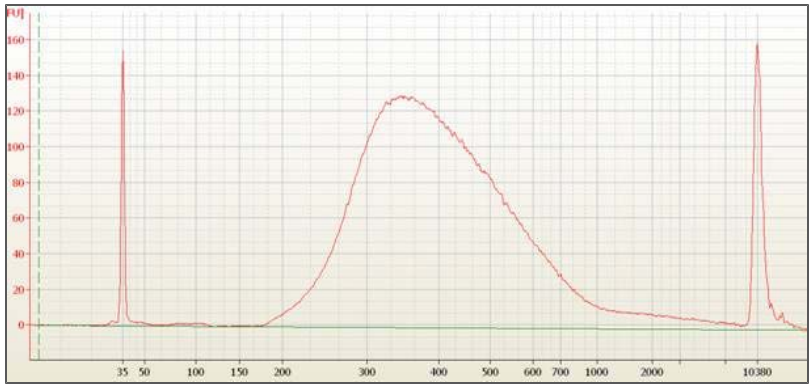
$$\frac{\text{15 ng/µl)}}{\text{(660 g/mol *400)}} \times 10^6 = 57 \text{ nM}$$

## Assess Quality [Optional]

To assess library quality, load 1 µl of the post-enriched library on an Agilent Technologies 2100 Bioanalyzer using a Agilent DNA High Sensitivity Chip. Depending on the level of indexing, an initial dilution of your sample may be necessary. For a 12-plex pool, Illumina recommends a 1:10 dilution of your sample prior to loading.

Check the size of the library, which should produce a distribution of DNA fragments with a size range from approximately 200 bp—1 kb. Depending on the level of indexing, insert size distribution may vary slightly, however the sample peak should not be significantly shifted compared to the example in Figure 14.

Figure 14 Example Nextera Rapid Capture Post-Enrichment (12-plex Enrichment) Library Distribution




NOTE

A second minor peak at ~100 bp may be present and and likely corresponds to residual single-stranded probes in the sample. The presence of these residual probes will not impact downstream clustering and sequencing of your enriched sample.

# Sequence Library

Proceed to cluster generation. For more information, see the cluster generation section of the user guide for your Illumina platform.

- When quantifying a Nextera Rapid Capture post-enriched library using a fluorometric method, clustering at 8–12 pM generates cluster densities in the range of 750,000—950, 000 clusters/mm2. However, results may vary based on your method of quantification. Illumina recommends that you determine the library concentration to cluster density relationship based on your lab instrumentation.
- A short sequencing run on a MiSeq can also be performed to optimize cluster density prior to performing a high density sequencing run.
- Nextera Rapid Capture prepared libraries contain dual 8 bp indexes. Depending on the combination of indexes used in your library pool, configure the sequencing run for either single or dual 8 bp index reads. See the *Low-Plex Pooling Guidelines* on page 32 for additional information.
- When clustering Nextera Rapid Capture libraries on the cBot™ and sequencing on the HiSeq 1000/2000, GAIIx, or in High Output mode on the HiSeq 1500/2500, new primers are required whether performing a non-indexed, single-indexed, or dual-indexed run. Use the TruSeq Dual Index Sequencing Primer Kit for Paired End runs (catalog # PE-121-1003), which is good for a single run and contains the required primers for sequencing (HP10, HP11, HP12). These primers are included with MiSeq® and HiSeq 1500/2500 rapid run reagent kits.
- For sequencing Nextera Rapid Capture libraries, Illumina recommends a paired-end 76-cycle sequencing run. Due to the smaller library sizes generated in Nextera Rapid Capture, sequencing at longer read lengths can lead to an increase in the likelihood of sequencing into the flanking adapter sequence.

# DNA Quantitation





# Procedure

Illumina recommends the QuantiFluor dsDNA assay to quantitate dsDNA samples, because it can quantitate small DNA volumes and measures DNA directly. Other techniques may pick up contamination such as RNA and proteins. Illumina recommends using a spectrofluorometer, because fluorometry provides DNA-specific quantification. Spectrophotometry might also measure RNA and yield values that are too high.

## Consumables

| Item | Quantity | Storage | Supplied By |
|---|---|---|---|
| 1X TE | as needed | 15° to 30°C | User |
| 96-well flat clear bottom black microplates | 2 per 96 samples | 15° to 30°C | User |
| 96-well MIDI plates | 2 per 96 samples | 15° to 30°C | User |
| Aluminum foil | as needed | 15° to 30°C | User |
| Conical centrifuge tube (15 ml or 50 ml) | 1 | 15° to 30°C | User |
| Lambda DNA | as needed | 2° to 8°C | User |
| Microseal 'B' adhesive seals | 1 per microplate + 1 per MIDI plate | 15° to 30°C | User |
| QuantiFluor dsDNA dye | as needed | 2° to 8°C | User |
| RNase/DNase-free Reagent Reservoir | 1 | 15° to 30°C | User |

## Preparation

- Remove the QuantiFluor dsDNA dye from to 2° to 8°C and let stand at room temperature for 60 minutes in a light-impermeable container.
- Label a new 96-well MIDI plate **Lambda DNA Stock** with a smudge resistant pen.
- Label a new 96-well MIDI plate **DNA Stock** with a smudge resistant pen.
- Label a new 96-well microplate **Lambda DNA Quant** with a smudge resistant pen.

## Make Lambda DNA Stock Plate

1 Add stock Lambda DNA to well A1 in the MIDI plate labeled Lambda DNA Stock and dilute it to 1 ng/µl in a final volume of 300 µl. Pipette up and down several times.

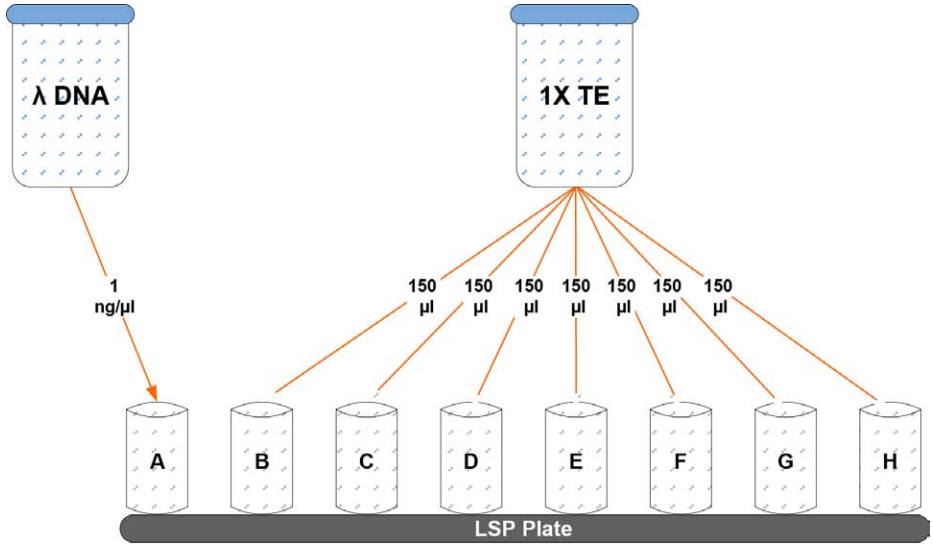
a Use the following formula to calculate the amount of stock Lambda DNA to add to A1:

$$\frac{(300\ \mu l)\ X\ (1\ ng/\mu l)}{\text{(stock Lambda DNA concentration)}} = \mu l \text{ of stock Lambda DNA to add to A1}$$

b Dilute the stock DNA in well A1 using the following formula:
(300 µl) - (µl of stock Lambda DNA in well A1) = µl of 1X TE to add to A1

2 Add 150 µl 1X TE to well B, C, D, E, F, G, and H of column 1 of the Lambda DNA Stock plate.

Figure 15 Dilution of Stock Lambda DNA Standard




3 Transfer 150 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times to mix.

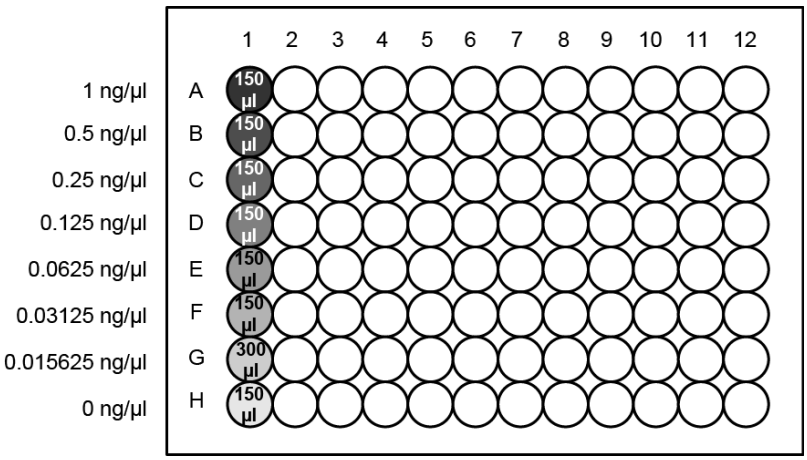
4 Transfer 150 µl of liquid from well B1 into well C1. Pipette up and down several times to mix.

5 Repeat the transfer for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.**Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 9 Concentrations of Lambda DNA

| Row-Column | Concentration (ng/µl) | Final Volume in Well (µl) |
|---|---|---|
| A1 | 1 | 150 |
| B1 | 0.5 | 150 |
| C1 | 0.25 | 150 |
| D1 | 0.125 | 150 |
| E1 | 0.0625 | 150 |
| F1 | 0.03125 | 150 |
| G1 | 0.015625 | 300 |
| H1 | 0 | 150 |

Figure 16 Serial Dilutions of Lambda DNA



6 Seal the Lambda DNA Stock plate with a Microseal 'B' adhesive seal.

## Make DNA Stock Plate

1 In the MIDI plate labeled DNA Stock, prepare the appropriate dilutions of your DNA samples using 1X TE. For accuracy, Illumina recommends measuring each sample in triplicate. Make sure at least 50 µl of diluted sample is prepared for quantification with the QuantiFluor dsDNA dye. If performing replicate measurements, scale appropriately. Illumina recommends the following dilution, depending on the DNA sample. This dilution may need to be adjusted depending on sample quality. Do one of the following:

- For high quality gDNA, dilute your samples 1:1000. For example: 2 µl of gDNA + 1998 µl of 1X TE.
- For pre-enriched Nextera libraries, dilute your libraries 1:200. For example: 2 µl of library sample + 398 µl of 1X TE.
- For post-enriched Nextera libraries, dilute your libraries at least 1:50. For example: 2 µl of post-enriched library + 198 µl of 1X TE. However, depending on the level of indexing used during the enrichment, an alternate dilution may be required. Illumina recommends a 1:100 dilution for a 12-plex enriched library.

2 Mix thoroughly as follows:
   a Cover the DNA Stock plate with a lid.
   b Shake the DNA Stock plate on a microplate shaker at 1200 rpm for 1 minute.

3 Centrifuge the DNA Stock plate to 280 xg for 1 minute

## Dilute QuantiFluor dsDNA Dye

1 Prepare a 1:200 dilution of QuantiFluor dsDNA dye into 1X TE using a conical centrifuge tube wrapped in aluminum foil.
For accuracy, Illumina recommends running each sample and standard in triplicate. For each measurement 40 µl of diluted QuantiFluor dye is required. Scale as appropriate.

NOTE
Quantifluor dsDNA dye is often still crystallized at room temperature. Before use, visually verify that the dye is fully thawed and liquid.

2 Cap the foil-wrapped tube and vortex to mix.

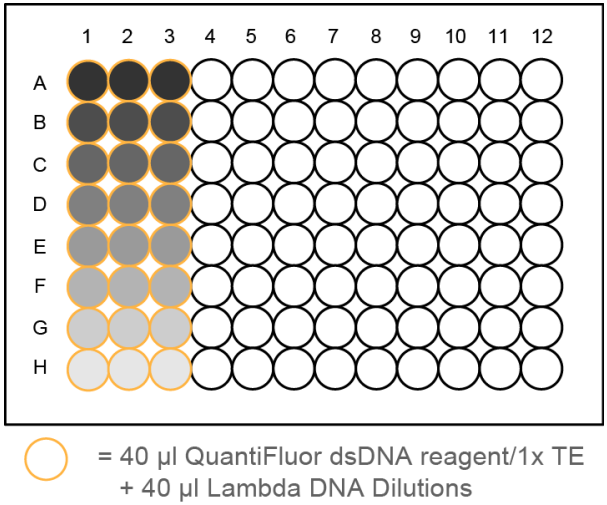
## Make Lambda DNA Quant Plate

1 Pour the diluted QuantiFluor dsDNA dye/1X TE into a clean reagent reservoir.

2 Using a multi-channel pipette, transfer 40 µl of diluted QuantiFluor dsDNA dye/1X TE into each well of columns 1–3 of the microplate labeled Lambda DNA Quant.

3 Transfer 40 µl of each stock Lambda DNA dilution from the Lambda DNA Stock plate to columns 1–3 of the Lambda DNA Quant plate.

Figure 17 Lambda DNA Quant Plate with QuantiFluor dsDNA Dye/1X TE




4 Mix thoroughly as follows:
   a Cover the Lambda DNA Quant plate with a lid.
   b Shake the Lambda DNA Quant plate on a microplate shaker at 1200 rpm for 1 minute.

5 Centrifuge the Lambda DNA Quant plate to 280 xg for 1 minute

6 Protect the Lambda DNA Quant plate from light until it is read by the spectrofluorometer.

## Make DNA Quant Plate

1. Using a multichannel pipette, transfer 40 µl QuantiFluor dsDNA reagent/1X TE dilution to each well of the microplate labeled DNA Quant that will contain a sample.
2. Remove the lid from the DNA Stock plate
3. Transfer 40 µl of each DNA sample in the DNA Stock plate to the corresponding well of the DNA Quant plate containing QuantiFluor dsDNA reagent/1X TE.
4. Mix thoroughly as follows:
   a. Cover the DNA Quant plate with a lid.
   b. Shake the DNA Quant plate on a microplate shaker at 1200 rpm for 1 minute.
5. Centrifuge the DNA Quant plate to 280 xg for 1 minute
6. Protect the DNA Quant plate from light until it is read by the spectrofluorometer.

## Read Quantitation Plate

1. Turn on the spectrofluorometer.
2. At the PC, open the SoftMax Pro program.
3. Measure fluorescence (485 nm Ex / 538 nm Em) of both the Lambda DNA Quant and DNA Quant plates according to the spectrofluorometer/software recommendations.
4. Calculate the DNA concentration of your unknown samples using the fluorescence values determined from step  as follows:
   a. Calculate the average relative fluorescence units (RFU) of the Lambda DNA standards run in triplicate on the Lambda DNA Quant plate.
   b. Calculate an Adjusted RFU by subtracting the RFU of the blank Lambda DNA standard (0 ng/µl) Row H from all unknown and standard samples.
   c. Create a scatter plot of the Lambda DNA standard curve values with the Adjusted RFU on the Y axis and DNA concentration (ng/µl) on the X axis.
   d. Determine the equation of the line for the Lambda DNA standard curve values which will be in the format of y = mx + b is equivalent to RFU = (slope*concentration) + y_int.
   e. Calculate the concentration for each unknown sample by using the RFU for each sample for y in the equation and determining the value for x in ng/µl.

f Multiply the resulting concentration by the appropriate dilution factor.

g Use the following formula to convert from ng/µl to nM. Assume a 400 bp library size or calculate based on the average size (insert + adapters) of the enriched library as determined by a Agilent Technologies 2100 Bioanalyzer trace:

$$\frac{\text{(concentration in ng/µl)}}{\text{(660 g/mol *average library size)}} \times 10^6 = \text{concentration in nM}$$

For example:

$$\frac{\text{15 ng/µl)}}{\text{(660 g/mol *400)}} \times 10^6 = 57 \text{ nM}$$

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 10 Illumina General Contact Information

| | |
|---|---|
| **Illumina Website** | www.illumina.com |
| **Email** | techsupport@illumina.com |

Table 11 Illumina Customer Support Telephone Numbers

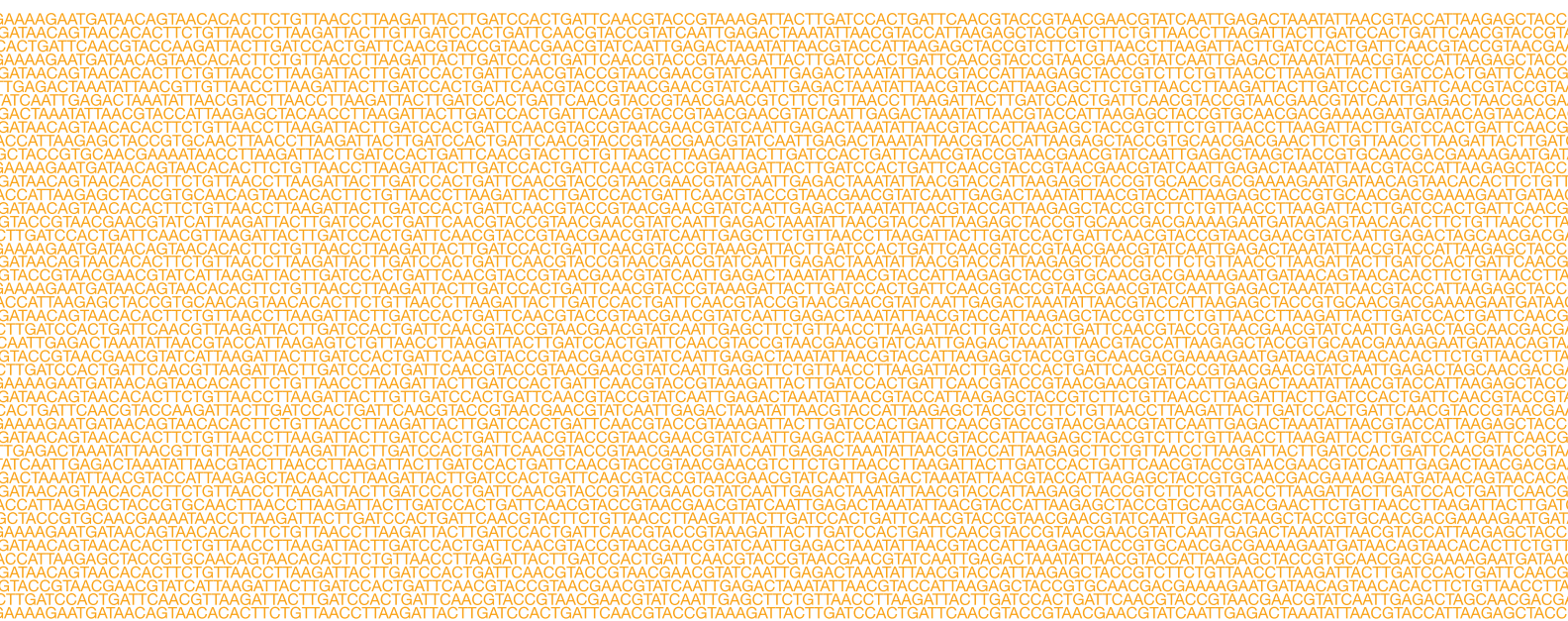
| Region | Contact Number | Region | Contact Number |
|---|---|---|---|
| North America | 1.800.809.4566 | Italy | 800.874909 |
| Austria | 0800.296575 | Netherlands | 0800.0223859 |
| Belgium | 0800.81102 | Norway | 800.16836 |
| Denmark | 80882346 | Spain | 900.812168 |
| Finland | 0800.918363 | Sweden | 020790181 |
| France | 0800.911850 | Switzerland | 0800.563118 |
| Germany | 0800.180.8994 | United Kingdom | 0800.917.0041 |
| Ireland | 1.800.812949 | Other countries | +44.1799.534000 |

## MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

## Product Documentation

Additional product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support, select a product, then click **Documentation & Literature**.



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