illumina®

Nextera® Rapid Capture Enrichment Reference Guide

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

Document # 15037436 v01
January 2016

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

Revision History

Document	Date	Description of Change
Document # 15037436 v01	January 2016	• Renamed and combined some procedures as needed to improve continuity • Added reference to the Custom Protocol Selector • Corrected quantity of ET2 for 6 plex and 9 plex in Box 1 of the Exome 8 Reaction kit

15037436 Rev. J	June 2015	• Removed step to remove plate from magnetic stand before adding RSB in:
		  • Clean Up Amplified DNA
		  • Clean Up Amplified Enriched Library
		• Changed SMB mixing to invert tube instead of vortex tube
		• Updated Kit Contents:
		  • Removed individual kit configurations
		  • Removed box and tube part numbers
		• Removed Nextera from NEM and NLM descriptions
		• Corrected High Sensitivity DNA Kit part #
		• Changed title of this document to Reference Guide
		• Updated design of workflow diagram
		• Renamed and combined some procedures as needed to improve continuity
		• Simplified consumables information at the beginning of each section
		• Revised step-by-step instructions to be more succinct
		• Removed reference to obsolete Experienced User Cards and added reference to new protocol guide and checklist
		• Changed BaseSpace resource reference to helpcenter

Document	Date	Description of Change

15037436 Rev. H	September 2014	• Removed List of Tables
		• Updated Additional Resources to remove web navigation instructions and written urls
		• Removed use of plate name (eg NLT plate), except for first instance and last instance in each procedure
		• Change 'index primer' to 'index adapter' to correspond to reagent labeling
		• Added instructions to use new caps on index adapter tubes after use
		• Replaced Nextera Rapid Capture Custom Enrichment Kit (96 Samples) (catalog # FC-140-1008), Box 3 (part # 15055302) with part # 15055366
		• Added a more Streptavidin Magnetic Beads tube to the following kits to make sure that there are sufficient reagents:
		  • Nextera Rapid Capture, 8 rxn × 3 plex (Box 1 of 3), part # 15050491
		  • Nextera Rapid Capture, 8 rxn × 6 plex (Box 1 of 3), part # 15050492
		  • Nextera Rapid Capture, 8 rxn × 9 plex (Box 1 of 3), part # 15050493
		• Corrected slot number for Elute Target Buffer 2 and Stop Tagment Buffer in Nextera Rapid Capture, 8 rxn × 1 plex (Box 1 of 3), part # 15050019
		• Removed mention of Index Adapter Replacement Caps in the Nextera Rapid Capture Custom Enrichment Kit (288 Samples), catalog # FC-140-1009
		• Updated SDS link to support.illumina.com/sds.html

15037436 Rev. G	May 2014	• Replaced the E501 and E502 Index Adapters with E505 and E506 Index Adapters in the exome and expanded exome 12 plex kits, custom 48 and 96 samples kits, and updated the kit Box 3 part numbers
		• Added E502 tubes to the exome 8 rxn × 6 plex and 8 rxn × 9 plex kits
		• Replaced the E501 Index Adapter with the E517 Index Adapter in the custom 288 samples kit, updated the kit Box 3 part number, and corrected the kit configuration
		• Revised the following sections regarding the index adapter replacements:
		  • Amplify Tagmented DNA
		  • Kit Contents
		  • Index Sequences
		• Corrected the hyperlink to the Nextera Rapid Capture Enrichment Low-Plex Pooling Guidelines Tech Note in Additional Resources
		• Changed 'sample' prep to 'library' prep

Document	Date	Description of Change

15037436 Rev. F	January 2014	• Added Nextera® Rapid Capture Exome Enrichment 8 rxn × 3 plex, 8 rxn × 6 plex, and 8 rxn × 9 plex kits. Revised the following sections regarding these kits:
		  • Protocol Introduction
		  • Amplify Tagmented DNA
		  • Kit Contents
		  • Index Sequences
		  • Make DNA Stock Plate
		• Modified description of existing exome and expanded exome kits to denote the reactions and plexity of each kit
		• Changed the following plate names to clarify library vs. sample:
		  • NES (Nextera Enrichment Sample Plate) is now NEL (Nextera Enrichment Library Plate)
		  • NLS (Nextera Library Sample Plate) is now NIL (Nextera Index Library Plate)
		• Added DNA Input Recommendations for an optional cleanup step followed by requantitation of DNA samples
		• Added references to BaseSpace® for organizing samples, libraries, pools, and runs
		• Removed Low-Plex Pooling Guidelines, which are now available in a Nextera Rapid Capture Enrichment Low-Plex Pooling Guidelines Tech Note
		• Moved content from the Sequencing section to Quantify Libraries and the Nextera Rapid Capture Enrichment support pages on the Illumina website
		• Corrected the 8 Samples - Oligos box configuration

15037436 Rev. E	November 2013	• Added guidance on EDTA to DNA Input Recommendations
		• Added training and qPCR information to Additional Resources
		• Renamed the EUC to EUC and LTF
		• Added Nextera Rapid Capture Exome Enrichment (8 Samples) Kit. Revised the following sections regarding this kit:
		  • Protocol Introduction
		  • Amplify Tagmented DNA
		  • Kit Contents
		  • Index Sequences
		  • Make DNA Stock Plate
		• Corrected the 96 Samples - Box 2 configuration

15037436 Rev. D	August 2013	• Providing 8 Index 2 (i5) adapters in Box 3 of the Nextera Rapid Capture Custom Enrichment Kits (288 Samples). Revised the following sections regarding the additional indexes:
		  • 288 Samples Kit Contents
		  • Low-Plex Pooling Guidelines
		  • Index Sequences

15037436 Rev. C	June 2013	• Appended 'Enrichment' to product name

Document	Date	Description of Change
15037436 Rev. B	May 2013	• Added Nextera Rapid Capture Custom Kit information • Changed protocol for small-size capture panels • Reorganized Getting Started content and move some topics to the Appendix • Replaced Best Practices with reference to content on the Illumina website • Removed recommendation for a paired-end 76 cycle sequencing run
15037436 Rev. A	February 2013	Initial Release

Table of Contents

Overview

Chapter 1

Introduction

This protocol explains how to prepare up to 96 indexed, paired-end libraries, followed by enrichment using exome or custom probe panels and reagents provided in an Illumina® Nextera® Rapid Capture Exome, Expanded Exome, or Custom Enrichment kit. The libraries are prepared 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 Enrichment protocol offers:

- Excellent data quality with low input of 50 ng
- Fast and easy preparation of up to 96 enriched libraries in ~1.5 days, including ~5 hours of hands-on time
- High throughput, automation-friendly procedures

DNA Input Recommendations

Nextera Rapid Capture Enrichment 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. Therefore, accurate quantification of the gDNA is essential.

Use a fluorometric-based method to quantify input gDNA specific for double-stranded DNA (dsDNA), such as QuantiFluor or Qubit, and run samples in triplicate for confident measurements.

- Avoid methods that measure total nucleic acid content, such as NanoDrop or other UV absorbance methods.
- Common contaminants, such as ssDNA, RNA, and oligos, are not substrates for the assay.
- Make sure that the starting DNA does not contain more than 1 mM EDTA and is free of organic contaminants, such as phenol and ethanol. For more information, see *DNA Quantification* on page 46.
- DNA samples can contain substances that interfere with the Nextera tagmentation reaction and result in unexpected library insert sizes. To make sure that conditions are optimal before you begin library preparation, perform an optional sample cleanup, and then requantify the DNA samples.

The Nextera Rapid Capture Enrichment protocol has been optimized for 50 ng of total gDNA. A higher mass input of gDNA can result in incomplete tagmentation and larger insert sizes, which can affect enrichment performance. A lower mass input of gDNA or low quality gDNA in the tagmentation reaction can generate smaller than expected insert sizes. Smaller inserts can be lost during subsequent cleanup steps and result in lower diversity.

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

Additional Resources

Visit the Nextera Rapid Capture Enrichment kit support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

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

Resource	Description
Custom Protocol Selector	http://support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
Nextera Rapid Capture Enrichment Protocol Guide (document # 15075701)	Provides only protocol instructions. The protocol guide is intended for experienced users.
Nextera Rapid Capture Enrichment Checklist (document # 15075702)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Nextera Rapid Capture Enrichment Low-Plex Pooling Guidelines Tech Note	Provides pooling guidelines and dual indexing strategies for Nextera Rapid Capture Enrichment library preparation.
Sequencing with Nextera Rapid Capture Enrichment Kits (document # 15037435)	Provides guidelines for preparing for sequencing when using a Nextera Rapid Capture Enrichment kit.
Sequencing Library qPCR Quantification Guide (document # 11322363)	Describes a qPCR method for quantifying sequencing by synthesis (SBS) libraries generated using the Illumina library prep protocols.

Protocol

Chapter 2

Introduction

This section describes the Nextera Rapid Capture Enrichment protocol.

- Follow the protocol in the order described using the specified parameters.
- Review Best Practices before proceeding. See *Additional Resources* on page 4 for information on how to access Nextera Rapid Capture Best Practices on the Illumina website.
- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. See *Consumables and Equipment* on page 42.
- Include a common index in each column. A common index facilitates pipetting operations when dispensing index adapters and pooling indexed libraries. For more information, see the *Nextera Rapid Capture Enrichment Low-Plex Pooling Guidelines Tech Note*.
- Nextera Rapid Capture kits support the following reactions and plexity. For more information on the kit configurations, see *Kit Contents* on page 34.

Samples	Enrichment Reactions	Plexity
8	8	1
24	8	3
48	8	6
72	8	9
24	2	12
48	4	12
96	8	12
288	24	12

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. Different methods are available depending on the sequencing instrument you are using. See the Nextera Rapid Capture Enrichment support page for more information.

Tips and Techniques

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

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.

Sealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Microseal 'A' adhesive film is effective for thermal cycling and easy to cut when using fewer than 96 wells.

Plate Transfers

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

Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Pipette bead suspension slowly.
- When mixing, mix thoroughly.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

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

Figure 1 Nextera Rapid Capture Enrichment Workflow

Tagment Genomic DNA

This step uses the Nextera transposome to tagment gDNA, which is a process that fragments DNA and then tags the DNA with adapter sequences in a single step.

Consumables

- SPB (Sample Purification Beads)
- ST2 (Stop Tagment Buffer 2)
- TD (Tagment DNA Buffer)
- TDE1 (Tagment DNA Enzyme)
- gDNA (50 ng per sample)
- Tris-HCl 10 mM, pH 8.5
- 96-well midi plate (1)
- Microseal 'B' adhesive seals

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
gDNA	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
TD	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly.
TDE1	-25°C to -15°C	Thaw on ice. Gently invert the thawed tubes 3–5 times, and then centrifuge briefly. Set aside on ice.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Keep at room temperature for later use in the protocol.
ST	15°C to 30°C	Check for precipitates. If present, vortex until all particulates are resuspended.

2 Preheat a microheating system with midi plate insert to 58°C.

3 Use IEM to determine what index adapters to use or BaseSpace Prep tab to organize your samples, libraries, pools, and run.

4 Label a new midi plate NLT with a marker.

Procedure

Quantify and Normalize gDNA

1 Quantify gDNA using a fluorometric method, such as QuantiFluor or Qubit.

2 Normalize gDNA in Tris-HCl 10 mM, pH 8.5 to 10 ng/µl.

3 Requantify the normalized gDNA using the same fluorometric quantification method.

4 Dilute the normalized gDNA in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl (50 ng total).

Tagment DNA

1 Add the following items in the order listed to each well of the NLT plate.

Item	Volume (µl)
Normalized gDNA	10
TD	25
TDE1	15

2 Shake at 1800 rpm for 1 minute.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the 58°C microheating system with the lid closed for 10 minutes.

5 Add 15 µl ST to each well.

6 Shake at 1800 rpm for 1 minute.

7 Centrifuge at 280 × g for 1 minute.

8 Incubate at room temperature for 4 minutes.

Clean Up Tagmented DNA

This step uses SPB (Sample Purification Beads) to purify the tagmented DNA from the Nextera transposome. The cleanup step removes the Nextera transposome that can otherwise bind to DNA ends and interfere with downstream processes.

Consumables

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- 96-well Hard-Shell 0.3 ml PCR plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex SPB before each use.
- Vortex SPB frequently to make sure that beads are evenly distributed.
- Aspirate and dispense SPB slowly due to the viscosity of the solution.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
RSB	-25°C to -15°C	Thaw at room temperature. RSB can be stored at 2°C to 8°C after the initial thaw.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Label a new Hard-Shell PCR plate NLA with a marker.

Procedure

1 Add 65 µl SPB to each well.

2 Shake at 1800 rpm for 1 minute.

3 Incubate at room temperature for 8 minutes.

4 Centrifuge at 280 × g for 1 minute.

5 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

6 Remove and discard all supernatant from each well.

7 Wash 2 times as follows.

   a Add 200 µl freshly prepared 80% EtOH to each well.
   b Incubate on the magnetic stand for 30 seconds.
   c Remove and discard all supernatant from each well.

8 Using a 20 µl pipette, remove residual 80% EtOH from each well.

9 Air-dry on the magnetic stand for 10 minutes.

10 Remove from the magnetic stand.

11 Add 22.5 µl RSB to each well.

12 Shake at 1800 rpm for 1 minute.

13 Incubate at room temperature for 2 minutes.

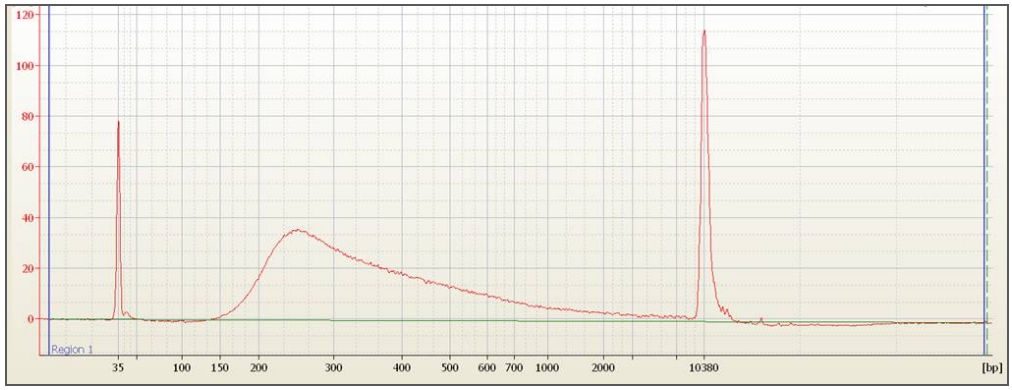
14 Centrifuge at 280 × g for 1 minute.

15 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

16 Transfer 20 µl supernatant to the corresponding well of the NLA plate.

17 [Optional] Run 1 µl of the reaction on an Agilent Technologies 2100 Bioanalyzer using a High Sensitivity DNA chip. You can expect to see a broad distribution of DNA fragments with a size range from ~150 bp to ~1 kbp.

Figure 2 Example Post-Tagmentation Library Distribution



Amplify Tagmented DNA

This step amplifies purified tagmented DNA and adds index adapters using a 10-cycle PCR program. This PCR step adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequencing adapters required for cluster amplification.

Consumables

- Index 1 (i7) adapters and orange tube caps
- Index 2 (i5) adapters and white tube caps
- NLM (Library Amp Mix)
- 1.7 ml microcentrifuge tubes (1 per index adapter tube)
- Microseal 'A' film
- Microseal 'B' adhesive seal
- [Optional] TruSeq Index Plate Fixture Kit

NOTE
Use Microseal 'A' when sealing the plate before placing on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Index adapters (i5 and i7)	-25°C to -15°C	Only remove adapters being used. Thaw at room temperature for 20 minutes. Vortex each tube to mix. Centrifuge briefly using a 1.7 ml Eppendorf tube.
NLM	-25°C to -15°C	Thaw on ice.

2 Save the following NLM AMP program on the thermal cycler:
- Choose the preheat 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

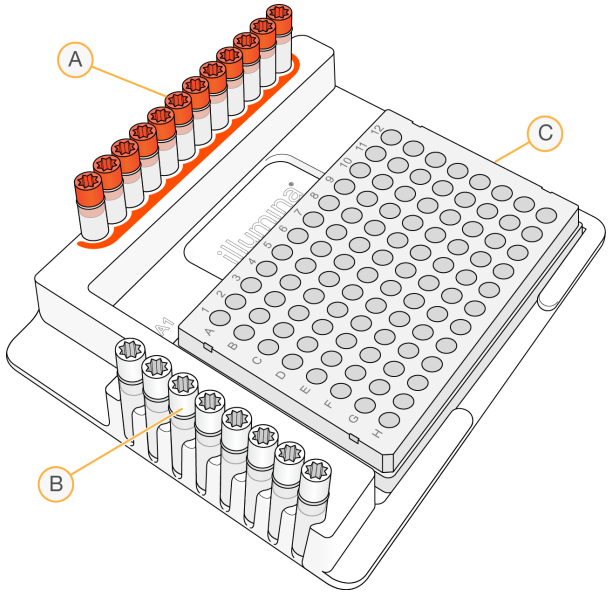
Procedure

1 Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.

2 Arrange Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.

3 Place the plate on the TruSeq Index Plate Fixture.

Figure 3 TruSeq Index Plate Fixture (96 libraries)



A Columns 1–12: Index 1 (i7) adapters (orange caps)
B Rows A–H: Index 2 (i5) adapters (white caps)
C 96-well plate

4 Using a multichannel pipette, add 5 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.

5 Using a multichannel pipette, add 5 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.

6 Add 20 µl NLM to each well.

7 Shake at 1200 rpm for 1 minute.

8 Centrifuge at 280 × g for 1 minute.

9 Place on the preprogrammed thermal cycler and run the NLM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Amplified DNA

This step uses SPB (Sample Purification Beads) to purify the DNA library and remove unwanted products.

Consumables

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- 96-well Hard-Shell 0.3 ml PCR plate
- 96-well midi plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex SPB before each use.
- Vortex SPB frequently to make sure that beads are evenly distributed.
- Aspirate and dispense SPB slowly due to the viscosity of the solution.

Preparation

1. Prepare the following consumables.

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2. Prepare fresh 80% ethanol from absolute ethanol.
3. Label plates with a marker as follows.
   - NIL - Hard-Shell PCR
   - NLC - midi

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer 50 µl supernatant to the corresponding well of the NLC plate.
3. Add 90 µl SPB to each well.
4. Shake at 1800 rpm for 1 minute.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge at 280 × g for 1 minute.
7. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8. Remove and discard all supernatant from each well.
9. Wash 2 times as follows.
   a. Add 200 µl freshly prepared 80% EtOH to each well.
   b. Incubate on the magnetic stand for 30 seconds.
   c. Remove and discard all supernatant from each well.

10 Using a 20 µl pipette, remove residual 80% EtOH from each well.

11 Air-dry on the magnetic stand for 10 minutes.

12 Add 27 µl RSB to each well.

13 Shake at 1800 rpm for 1 minute.

14 Incubate at room temperature for 2 minutes.

15 Centrifuge at 280 × g for 1 minute.

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

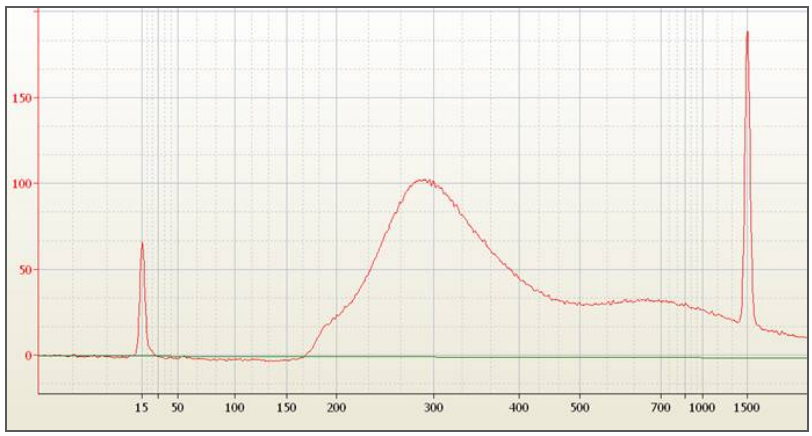
17 Transfer 25 µl supernatant to the corresponding well of the NIL plate.

18 Quantify the library using a fluorometric method, such as QuantiFluor or Qubit. For an example protocol using the Promega QuantiFluor method, see *DNA Quantification* on page 46.

NOTE
Inaccurate quantification and pooling can result in a higher representation of some samples compared to others in the same pool.

19 [Optional] Run 1 µl of the library on an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip. You can expect to see a distribution of DNA fragments with a size range from ~150 bp to ~1 kbp.

Figure 4 Example of Post-PCR, Pre-Enriched Library Distribution



NOTE
The sample peak must not be significantly shifted compared to the example shown in Figure 4, although traces can differ depending on sample quality. A larger peak distribution (> 350 bp) can indicate > 50 ng gDNA input going into tagmentation, which can result in lower on-target reads. Conversely, a smaller sample peak distribution (< 225 bp) can indicate < 50 ng gDNA or low quality gDNA, which can result in reduced library diversity or elevated duplicates.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 14 days.

Hybridize Probes

This step combines DNA libraries containing unique indexes into a single pool, and then binds targeted regions of the DNA with capture probes.

Consumables

- EHB (Enrichment Hybridization Buffer)
- One of the following, depending on the kit you are using:
  - CEX (Coding Exome Oligos)
  - EEX (Expanded Exome Oligos)
  - RCO (Rapid Capture Oligos)
- RSB (Resuspension Buffer)
- 96-well Hard-Shell 0.3 ml PCR plate (1)
- Microseal 'B' adhesive seal
- [Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) (1 per pooled sample)

About Reagents

- Before using EHB, vortex to resuspend the solution. Make sure that no crystal structures are present. If crystals and cloudiness are observed, vortex until the solution is clear.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
One of the following, depending on the kit you are using: • CEX • EEX • RCO	-25°C to -15°C	Thaw at room temperature.
EHB	-25°C to -15°C	Thaw at room temperature.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Save the NRC HYB program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 10 minutes
- 18 cycles of 1 minute each, starting at 94°C, then decreasing 2°C per cycle
- Hold at 58°C

NOTE
Incubate the plate at the 58°C holding temperature for at least 90 minutes and up to a maximum of 24 hours.

3 Label a new Hard-Shell PCR plate NEH1 with a marker.

Pool Libraries

NOTE
Pooling is not performed with the Nextera Rapid Capture Enrichment Kit (8 rxn × 1 plex).
For custom enrichment projects, avoid low levels of sample multiplexing (< 6-plex) for a small capture target size (< 2 Mb). The final enriched sample yield can be insufficient for clustering and subsequent sequencing. For more information, see the *Nextera Rapid Capture Low-Plex Pooling Guidelines Tech Note*.

1 Combine 500 ng of each DNA library. Make sure that each library has a unique index.

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

- If the total volume is > 40 µl, use a vacuum concentrator or Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) to concentrate the pooled sample to 40 µl.
- If you are using a vacuum concentrator, use a no heat setting and a medium drying rate.
- If you are using an Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa), you do not need to rinse the device before use. Most of the volume filters through in 5 minutes. Larger starting volumes can take up to 30 minutes to filter.
- If the total volume is < 40 µl, increase the volume to 40 µl with RSB.

Procedure

1 Add the following items in the order listed to each well of the NEH1 plate.

Item	Volume (µl)
DNA library sample or pool	40
EHB	50
CEX, EEX, or RCO	10

2 Shake at 1200 rpm for 1 minute.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the preprogrammed thermal cycler and run the NRC HYB program. Each well contains 100 µl.

5 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

This step uses SMB (Streptavidin Magnetic Beads) to capture probes hybridized to the targeted regions of interest. Two heated washes remove nonspecific binding from the beads. The enriched library is then eluted from the beads and prepared for a second round of hybridization.

Consumables

- EE1 (Enrichment Elution Buffer 1)
- ET2 (Elute Target Buffer 2)
- EWS (Enrichment Wash Solution)
- HP3 (2 N NaOH)
- SMB (Streptavidin Magnetic Beads)
- 96-well Hard-Shell 0.3 ml PCR plate
- 96-well midi plate
- 1.7 ml microcentrifuge tube
- Microseal 'B' adhesive seals

About Reagents

- EWS can be cloudy after reaching room temperature.
- Vortex EWS before use.
- Make sure that you use SMB (2 ml tube) and not SPB (15 ml tube) for this procedure.
- Invert and vortex SMB to mix before use.
- Discard elution premix after use.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
EE1	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
EWS	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
HP3	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
ET2	2°C to 8°C	Let stand at room temperature. Return to storage after use.
SMB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Return to storage after use.

2 Preheat a microheating system with midi plate insert to 50°C.

3 Label plates with a marker as follows.
   - NEW1 - midi
   - NEH2 - Hard-Shell PCR

Procedure

First Bind

1 Centrifuge at 280 × g for 1 minute.

2 Transfer all (~100 µl) to the corresponding well of the NEW1 plate.

> NOTE
> If you see a greater than 15% sample loss, do not proceed with the protocol. Poor sealing or insufficient heating of the lid can cause sample loss.

3 Add 250 µl SMB to each well.

4 Shake at 1200 rpm for 5 minutes.

5 Incubate at room temperature for 25 minutes.

6 Centrifuge at 280 × g for 1 minute.

7 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

8 Remove and discard all supernatant from each well.

9 Remove from the magnetic stand.

First Wash

1 Wash 2 times as follows.

a Add 200 µl EWS to each well.
b Shake at 1800 rpm for 4 minutes.
c Pipette to resuspend the bead pellet further.
d Place on the 50°C microheating system with the lid closed for 30 minutes.
e Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
f Remove and discard all supernatant from each well.
g Remove from the magnetic stand.

First Elution

1 Create elution premix in a 1.7 ml microcentrifuge tube, and then vortex to mix.
- EE1 (28.5 µl)
- HP3 (1.5 µl)

2 Add 23 µl elution premix to each well.

3 Shake at 1800 rpm for 2 minutes.

4 Incubate at room temperature for 2 minutes.

5 Centrifuge at 280 × g for 1 minute.

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

7 Transfer 21 µl supernatant to the corresponding well of the NEH2 plate.

8 Add 4 µl ET2 to each well.

9 Shake at 1200 rpm for 1 minute.

10 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

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

Perform Second Hybridization

This step binds targeted regions of the enriched DNA with capture probes a second time. This second hybridization ensures high specificity of the captured regions.

Consumables

- EHB (Enrichment Hybridization Buffer)
- One of the following, depending on the kit you are using:
  - CEX (Coding Exome Oligos)
  - EEX (Expanded Exome Oligos)
  - RCO (Rapid Capture Oligos)
- RSB (Resuspension Buffer)
- Microseal 'B' adhesive seals

About Reagents

- Before using EHB, vortex to resuspend the solution. Make sure that no crystal structures are present. If crystals and cloudiness are observed, vortex until the solution is clear.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
One of the following, depending on the kit you are using: • CEX • EEX • RCO	-25°C to -15°C	Thaw at room temperature.
EHB	-25°C to -15°C	Thaw at room temperature.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

Procedure

1 Add the following reagents in the order listed to each well that contains a sample.

Reagent	Volume (µl)
RSB	15
EHB	50
CEX, EEX, or RCO	10

2 Shake at 1200 rpm for 1 minute.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the preprogrammed thermal cycler and run the NRC HYB program. Each well contains 100 µl.

5 Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

This step uses SMB (Streptavidin Magnetic Beads) to capture probes hybridized to the targeted regions of interest. Two heated washes remove nonspecific binding from the beads. The enriched library is then eluted from the beads and prepared for sequencing.

Consumables

- EE1 (Enrichment Elution Buffer 1)
- ET2 (Elute Target Buffer 2)
- EWS (Enrichment Wash Solution)
- HP3 (2 N NaOH)
- SMB (Streptavidin Magnetic Beads)
- 96-well midi plates (2)
- 1.7 ml microcentrifuge tube
- Microseal 'B' adhesive seals

About Reagents

- EWS can be cloudy after reaching room temperature.
- Vortex EWS before use.
- Invert SMB to mix before use.
- Discard elution premix after use.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
EE1	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
EWS	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
HP3	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
ET2	2°C to 8°C	Let stand at room temperature. Return to storage after use.
SMB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Return to storage after use.

2 Preheat a microheating system with midi plate insert to 50°C.

3 Label plates with a marker as follows.
- NEW2 - midi
- NEC1 - midi

Procedure

Second Bind

1 Centrifuge at 280 × g for 1 minute.

2 Transfer all (~100 µl) supernatant to the corresponding well of the NEW2 plate.

NOTE
If you see a greater than 15% sample loss, do not proceed with the protocol. Poor sealing or insufficient heating of the lid can cause sample loss.

3 Add 250 µl SMB to each well.

4 Shake at 1200 rpm for 5 minutes.

5 Incubate at room temperature for 25 minutes.

6 Centrifuge at 280 × g for 1 minute.

7 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

8 Remove and discard all supernatant from each well.

9 Remove from the magnetic stand.

Second Wash

1 Wash 2 times as follows.

a Add 200 µl EWS to each well.
b Shake at 1800 rpm for 4 minutes.
c Pipette to resuspend the bead pellet further.
d Place on the 50°C microheating system with the lid closed for 30 minutes.
e Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
f Remove and discard all supernatant from each well.
g Remove from the magnetic stand.

Second Elution

1 Create elution premix in a 1.7 ml microcentrifuge tube, and then vortex to mix.
- EE1 (28.5 µl)
- HP3 (1.5 µl)

2 Add 23 µl elution premix to each well.

3 Shake at 1800 rpm for 2 minutes.

4 Incubate at room temperature for 2 minutes.

5 Centrifuge at 280 × g for 1 minute.

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

7 Transfer 21 µl supernatant to the corresponding well of the NEC1 plate.

8 Add 4 µl ET2 to each well.

9 Shake at 1800 rpm for 1 minute.

10 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

This step uses SPB (Sample Purification Beads) to purify the captured library before PCR amplification.

Consumables

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- 96-well Hard-Shell 0.3 ml PCR plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex SPB before each use.
- Vortex SPB frequently to make sure that beads are evenly distributed.
- Aspirate and dispense SPB slowly due to the viscosity of the solution.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Label a new Hard-Shell PCR plate NEA with a marker.

Procedure

1 Add 45 µl SPB to each well.

2 Shake at 1800 rpm for 1 minute.

3 Incubate at room temperature for 10 minutes.

4 Centrifuge at 280 × g for 1 minute.

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

6 Remove and discard all supernatant from each well.

7 Wash 2 times as follows.

   a Add 200 µl fresh 80% EtOH to each well.
   b Incubate on the magnetic stand for 30 seconds.
   c Remove and discard all supernatant from each well.

8 Use a 20 µl pipette to remove residual EtOH from each well.

9 Air-dry on the magnetic stand for 10 minutes.

10 Remove from the magnetic stand.

11 Add 27.5 µl RSB to each well.

12 Shake at 1800 rpm for 1 minute.

13 Incubate at room temperature for 2 minutes.

14 Centrifuge at 280 × g for 1 minute.

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

16 Transfer 25 µl supernatant to the corresponding well of the NEA plate.

SAFE STOPPING POINT

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

Amplify Enriched Library

This step uses a 10-cycle or 12-cycle PCR program to amplify the enriched library.

Consumables

- NEM (Enrichment Amp Mix)
- PPC (PCR Primer Cocktail)
- Microseal 'A' film
- Microseal 'B' adhesive seal

NOTE
Use Microseal 'A' when sealing the plate before placing on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
EPM	-25°C to -15°C	Thaw on ice.
PPC	-25°C to -15°C	Thaw at room temperature.

2 Determine the appropriate number of PCR cycles:
- For capture target sizes > 2 Mb, perform 10 cycle
- For capture target sizes < 2 Mb, perform 12 cycles

3 Save the following AMP10 or AMP12 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 10 or 12 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 PPC to each well.

2 Add 20 µl NEM to each well.

3 Shake at 1200 rpm for 1 minute.

4 Centrifuge at 280 × g for 1 minute.

5 Place on the preprogrammed thermal cycler and run the AMP10 or AMP12 program. Each well contains 50 µl.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days.

Clean Up Amplified Enriched Library

This step uses SPB (Sample Purification Beads) to purify the enriched library and remove unwanted products.

Consumables

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- 96-well Hard-Shell 0.3 ml PCR plate
- 96-well midi plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex SPB before each use.
- Vortex SPB frequently to make sure that beads are evenly distributed.
- Aspirate and dispense SPB slowly due to the viscosity of the solution.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Label plates with a marker as follows.
- NEC2 - midi
- NEL - Hard-Shell PCR

Procedure

1 Centrifuge at 280 × g for 1 minute.

2 Transfer 50 µl to the corresponding well of the NEC2 plate.

3 Add 90 µl SPB to each well.

4 Shake at 1800 rpm for 1 minute.

5 Incubate at room temperature for 10 minutes.

6 Centrifuge at 280 × g for 1 minute.

7 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

8 Remove and discard all supernatant from each well.

9 Wash 2 times as follows.

a Add 200 µl fresh 80% EtOH to each well.
b Incubate on the magnetic stand for 30 seconds.
c Remove and discard all supernatant from each well.

10 Use a 20 µl pipette to remove residual EtOH from each well.

11 Air-dry on the magnetic stand for 10 minutes.

12 Remove from the magnetic stand.

13 Add 32 µl RSB to each well.

14 Shake at 1800 rpm for 1 minute.

15 Incubate at room temperature for 2 minutes.

16 Centrifuge at 280 × g for 1 minute.

17 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

18 Transfer 30 µl supernatant to the corresponding well of the NEL plate.

SAFE STOPPING POINT

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

Check Enriched Libraries

Perform the following procedures to check enriched library quality.

Quantify Libraries

Accurately quantify DNA libraries to ensure optimum cluster densities on the flow cell.

1 Quantify the enriched library using a fluorometric method.
For an example protocol using the Promega QuantiFluor method, see *DNA Quantification* on page 46.

2 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:

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

For example:

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

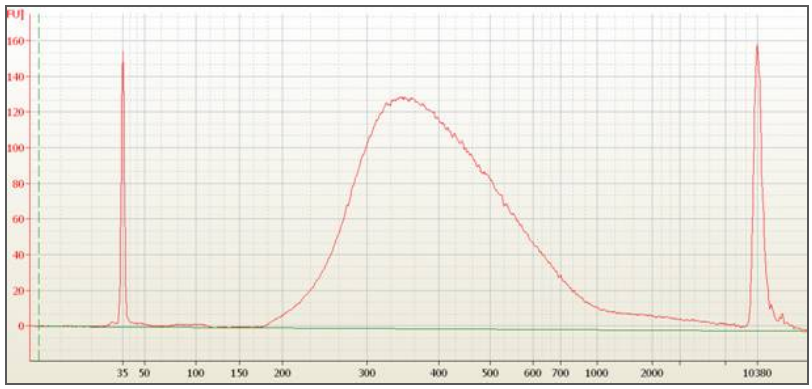
Alternatively, you can quantify libraries using qPCR according to the *Sequencing Library qPCR Quantification Guide (part # 11322363)*.

Assess Quality [Optional]

1 If the library concentration is higher than the supported quantitative range for the High Sensitivity DNA chip, dilute the library 1:10 with RSB.

2 Run 1 µl of post enriched library on an Agilent Technologies 2100 Bioanalyzer using a High Sensitivity DNA chip.
Expect a distribution of DNA fragments with a size range from ~200 bp to ~1 kbp. Depending on the level of indexing, insert size distribution can vary slightly. However, the sample peak must not be significantly shifted compared to the following example.

Figure 5 Example Post Enrichment (12-plex Enrichment) Library Distribution



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

Supporting Information

Appendix A

Introduction

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

Acronyms

Acronym	Definition
CEX	Coding Exome Oligos
EE1	Enrichment Elution Buffer 1
EEX	Expanded Exome Oligos
EHB	Enrichment Hybridization Buffer
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
HP3	2N NaOH
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
NEL	Nextera Enrichment Library Plate
NEM	Enrichment Amp Mix
NEW1	Nextera Enrichment Wash Plate 1
NEW2	Nextera Enrichment Wash Plate 2
NIL	Nextera Index Library Plate
NLA	Nextera Library Amplification Plate
NLC	Nextera Library Clean Up Plate
NLM	Library Amp Mix
NLT	Nextera Library Tagment Plate
PPC	PCR Primer Cocktail
RCO	Rapid Capture Oligos
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST	Stop Tagment Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme TDE

Kit Contents

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

Table 1 Nextera Rapid Capture Exome Enrichment Kits

Kit Name	Catalog #
Nextera Rapid Capture Exome Enrichment Kit (8 rxn × 1 plex)	FC-140-1000
Nextera Rapid Capture Exome Enrichment Kit (8 rxn × 3 plex)	FC-140-1083
Nextera Rapid Capture Exome Enrichment Kit (8 rxn × 6 plex)	FC-140-1086
Nextera Rapid Capture Exome Enrichment Kit (8 rxn × 9 plex)	FC-140-1089
Nextera Rapid Capture Exome Enrichment Kit (2 rxn × 12 plex)	FC-140-1001
Nextera Rapid Capture Exome Enrichment Kit (4 rxn × 12 plex)	FC-140-1002
Nextera Rapid Capture Exome Enrichment Kit (8 rxn × 12 plex)	FC-140-1003

Table 2 Nextera Rapid Capture Expanded Exome Enrichment Kits

Kit Name	Catalog #
Nextera Rapid Capture Expanded Exome Enrichment Kit (2 rxn × 12 plex)	FC-140-1004
Nextera Rapid Capture Expanded Exome Enrichment Kit (4 rxn × 12 plex)	FC-140-1005
Nextera Rapid Capture Expanded Exome Enrichment Kit (8 rxn × 12 plex)	FC-140-1006

Table 3 Nextera Rapid Capture Custom Enrichment Kits

Kit Name	Enrichment Reactions	Catalog #
Nextera Rapid Capture Custom Enrichment Kit (48 Samples)	4	FC-140-1007
Nextera Rapid Capture Custom Enrichment Kit (96 Samples)	8	FC-140-1008
Nextera Rapid Capture Custom Enrichment Kit (288 Samples)	24	FC-140-1009

Box Configurations

This section details the contents of each Nextera Rapid Capture Enrichment box.

Nextera Rapid Capture Exome Enrichment Kits (8 rxn)

Each kit contains 3 boxes of reagents, 1 box of oligos, and 1 box of index replacement caps.

Box 1, Store as specified

Quantity per 8 rxn kit					
1 plex	3 plex	6 plex	9 plex	Reagent	Description
1	1	2	2	SPB	Sample Purification Beads
4	4	4	4	SMB	Streptavidin Magnetic Beads
1	1	1	1	ET2	Elute Target Buffer 2
1	1	1	1	ST	Stop Tagment Buffer

Box 2, Store at -25°C to -15°C

Quantity per 8 rxn kit					
1 plex	3 plex	6 plex	9 plex	Reagent	Description
1	1	1	1	RSB	Resuspension Buffer
2	1	1	1	EWS	Enrichment Wash Solution
1	1	2	3	TDE1	Tagment DNA Enzyme
1	1	1	1	EE1	Enrichment Elution Buffer 1
1	1	1	2	TD	Tagment DNA Buffer
1	1	2	3	NLM	Library Amp Mix
2	2	2	2	EHB	Enrichment Hybridization Buffer
1	1	1	1	HP3	2N NaOH
1	1	1	1	PPC	PCR Primer Cocktail
4	3	3	3	NEM	Enrichment Amp Mix

Box 3, Store at -25°C to -15°C

Quantity per 8 rxn kit					
1 plex	3 plex	6 plex	9 plex	Reagent	Description
1	1	2	3	E502	E502 Index Adapter
1	–	1	1	N701	N701 Index Adapter
–	1	1	1	N702	N702 Index Adapter
–	–	1	1	N703	N703 Index Adapter
–	1	1	1	N704	N704 Index Adapter
–	1	1	1	N705	N705 Index Adapter
–	–	1	1	N707	N707 Index Adapter
–	–	–	1	N710	N710 Index Adapter
–	–	–	1	N711	N711 Index Adapter
–	–	–	1	N712	N712 Index Adapter

8 Reaction - Oligos, Store at -25°C to -15°C

Quantity	Reagent	Description
4	CEX	Coding Exome Oligos

Index Adapter Replacement Caps, Store at 15°C to 30°C

Description
i7 Index Tube Caps, Orange
i5 Index Tube Caps, White

Nextera Rapid Capture Exome and Expanded Exome Enrichment Kits (2 rxn x 12 plex)

These kits contain enough reagents to support 24 samples in 2 x 12-plex enrichment reactions. Each kit contains 4 boxes of reagents and 1 box of index replacement caps. The Exome and Expanded Exome Enrichment kits differ in the oligo contents of Box 4.

Box 1, Store as specified

Reagent	Description	Storage Temperature
SPB	Sample Purification Beads	2°C to 8°C
SMB	Streptavidin Magnetic Beads	2°C to 8°C
ET2	Elute Target Buffer 2	2°C to 8°C
ST	Stop Tagment Buffer	15°C to 30°C

Box 2, Store at -25°C to -15°C

Reagent	Description
RSB	Resuspension Buffer
TDE1	Tagment DNA Enzyme
TD	Tagment DNA Buffer
EWS	Enrichment Wash Solution
NLM	Library Amp Mix
EHB	Enrichment Hybridization Buffer
HP3	2N NaOH
PPC	PCR Primer Cocktail
NEM	Enrichment Amp Mix
EE1	Enrichment Elution Buffer 1

Box 3, Store at -25°C to -15°C

Reagent	Description
E505	E505 Index Adapter
E506	E506 Index Adapter
N701	N701 Index Adapter
N702	N702 Index Adapter
N703	N703 Index Adapter
N704	N704 Index Adapter
N705	N705 Index Adapter
N706	N706 Index Adapter
N707	N707 Index Adapter
N708	N708 Index Adapter
N709	N709 Index Adapter
N710	N710 Index Adapter
N711	N711 Index Adapter
N712	N712 Index Adapter

Box 4, Store at -25°C to -15°C

You receive 1 of the following, depending on the kit: Exome or Expanded Exome.

Reagent	Description
CEX	Coding Exome Oligos

Reagent	Description
EEX	Expanded Exome Oligos

Index Adapter Replacement Caps, Store at 15°C to 30°C

Description
i7 Index Tube Caps, Orange
i5 Index Tube Caps, White

Nextera Rapid Capture - Exome, Expanded Exome, and Custom Enrichment (4 rxn × 12 plex/48 Samples)

These kits contain enough reagents to support 48 samples in 4 x 12-plex enrichment reactions. Each kit contains 4 boxes of reagents and 1 box of index replacement caps. The kits differ in the oligo contents of Box 4.

Box 1, Store as specified

Quantity	Reagent	Description	Storage Temperature
2	SPB	Sample Purification Beads	2°C to 8°C
2	SMB	Streptavidin Magnetic Beads	2°C to 8°C
1	ET2	Elute Target Buffer 2	2°C to 8°C
1	ST	Stop Tagment Buffer	15°C to 30°C

Box 2, Store at -25°C to -15°C

Reagent	Description
RSB	Resuspension Buffer
TDE1	Tagment DNA Enzyme
EWS	Enrichment Wash Solution
TD	Tagment DNA Buffer
NLM	Library Amp Mix
EHB	Enrichment Hybridization Buffer
HP3	2N NaOH
PPC	PCR Primer Cocktail
NEM	Enrichment Amp Mix
EE1	Enrichment Elution Buffer 1

Box 3, Store at -25°C to -15°C

Reagent	Description
E505	E505 Index Adapter
E506	E506 Index Adapter
N701	N701 Index Adapter
N702	N702 Index Adapter
N703	N703 Index Adapter
N704	N704 Index Adapter
N705	N705 Index Adapter
N706	N706 Index Adapter
N707	N707 Index Adapter
N708	N708 Index Adapter
N709	N709 Index Adapter
N710	N710 Index Adapter
N711	N711 Index Adapter
N712	N712 Index Adapter

Box 4, Store at -25°C to -15°C

You receive 1 of the following, depending on the kit: Exome, Expanded Exome, or Custom.

Quantity	Reagent	Description
2	CEX	Coding Exome Oligos

Quantity	Reagent	Description
2	EEX	Expanded Exome Oligos

Quantity	Reagent	Description
1	RCO	Rapid Capture Oligos

The lot number for the custom kit box is specific to each customer order. The box contains 20, 120, or 240 slots, depending on the pulldowns that were ordered with your custom kit. The number of occupied slots depends on the number of pulldowns. Each tube is labeled with the Project ID.

Index Adapter Replacement Caps, Store at 15°C to 30°C

Description
i7 Index Tube Caps, Orange
i5 Index Tube Caps, White

Nextera Rapid Capture - Exome, Expanded Exome, and Custom Enrichment (8 rxn x 12 plex/96 Samples)

These kits contain enough reagents to support 96 samples in 8 x 12-plex enrichment reactions. Each kit contains 4 boxes of reagents and 1 box of index replacement caps. The kits differ in the oligo contents of Box 4.

Box 1, Store as specified

Quantity	Reagent	Description	Storage Temperature
4	SPB	Sample Purification Beads	2°C to 8°C
4	SMB	Streptavidin Magnetic Beads	2°C to 8°C
1	ET2	Elute Target Buffer 2	2°C to 8°C
1	ST	Stop Tagment Buffer	15°C to 30°C

Box 2, Store at -25°C to -15°C

Quantity	Reagent	Description
1	RSB	Resuspension Buffer
1	EWS	Enrichment Wash Solution
4	TDE1	Tagment DNA Enzyme
2	TD	Tagment DNA Buffer
4	NLM	Library Amp Mix
2	EHB	Enrichment Hybridization Buffer
1	HP3	2N NaOH
1	PPC	PCR Primer Cocktail
2	NEM	Enrichment Amp Mix
1	EWS	Enrichment Wash Solution
2	NEM	Enrichment Amp Mix
1	EE1	Enrichment Elution Buffer 1

Box 3, Store at -25°C to -15°C

Quantity	Reagent	Description
2	E505	E505 Index Adapter
2	E506	E506 Index Adapter
1	N701	N701 Index Adapter
1	N702	N702 Index Adapter
1	N703	N703 Index Adapter
1	N704	N704 Index Adapter
1	N705	N705 Index Adapter
1	N706	N706 Index Adapter
1	N707	N707 Index Adapter
1	N708	N708 Index Adapter
1	N709	N709 Index Adapter
1	N710	N710 Index Adapter
1	N711	N711 Index Adapter
1	N712	N712 Index Adapter

Box 4, Store at -25°C to -15°C

You receive 1 of the following, depending on the kit: Exome, Expanded Exome, or Custom.

Quantity	Reagent	Description
4	CEX	Coding Exome Oligos

Quantity	Reagent	Description
4	EEX	Expanded Exome Oligos

Quantity	Reagent	Description
2	RCO	Rapid Capture Oligos

The lot number for the custom kit box is specific to each customer order. The box contains 20, 120, or 240 slots, depending on the pulldowns that were ordered with your custom kit. The number of occupied slots depends on the number of pulldowns. Each tube is labeled with the Project ID.

Index Adapter Replacement Caps, Store at 15°C to 30°C

Description
i7 Index Tube Caps, Orange
i5 Index Tube Caps, White

Nextera Rapid Capture Custom Enrichment Kits (288 Samples)

The Nextera Rapid Capture Custom Enrichment (288 Samples) Kit contains enough reagents to support 288 samples in 24 x 12-plex enrichment reactions. Each kit contains 9 boxes of reagents and an index replacement caps box.

Box 1, 2, and Index Adapter Replacement Caps

The Nextera Rapid Capture Custom Enrichment 288 Samples Kit contains 3 each of the Nextera Rapid Capture Custom Enrichment 96 Samples Kit boxes 1 and 2 and an Index Adapter Replacement Caps Box. For the contents of these boxes, see *Nextera Rapid Capture - Exome, Expanded Exome, and Custom Enrichment (8 rxn x 12 plex/96 Samples)* on page 38.

Box 3, Store at -25°C to -15°C

The Nextera Rapid Capture Custom Enrichment 288 Samples Kit contains 2 of these boxes.

Reagent	Description
E517	E517 Index Adapter
E502	E502 Index Adapter
E503	E503 Index Adapter
E504	E504 Index Adapter
E505	E505 Index Adapter
E506	E506 Index Adapter
E507	E507 Index Adapter
E508	E508 Index Adapter
N701	N701 Index Adapter
N702	N702 Index Adapter
N703	N703 Index Adapter
N704	N704 Index Adapter
N705	N705 Index Adapter
N706	N706 Index Adapter
N707	N707 Index Adapter
N708	N708 Index Adapter
N709	N709 Index Adapter
N710	N710 Index Adapter
N711	N711 Index Adapter
N712	N712 Index Adapter

Box 4, Store at -25°C to -15°C

Quantity	Reagent	Description
6	RCO	Rapid Capture Oligos

The lot number for the custom kit box is specific to each customer order. The box contains 20, 120, or 240 slots, depending on the pulldowns that were ordered with your custom kit. The number of occupied slots depends on the number of pulldowns. Each tube is labeled with the Project ID.

Consumables and Equipment

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

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

Consumables

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 micro plates Note: Used when quantifying samples with a SpectraMax M5 Micro Plate Reader.	Corning, catalog # 3904
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, catalog # AB-0859
Hard-Shell 96-well PCR Plates	Bio-Rad, catalog # HSP-9601
Aluminum foil	General lab supplier
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, product # E7023
Microseal 'A' film	Bio-Rad, catalog # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
RNase/DNase-free 8-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, catalog # 89094-658

Consumable	Supplier
Tris-HCl 10 mM, pH 8.5	General lab supplier
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) Note: Use to concentrate a pooled library. Otherwise, use a vacuum concentrator.	Millipore, catalog # UFC503008
[Optional] DNA 1000 Kit	Agilent Technologies, catalog # 5067-1504
[Optional] High Sensitivity DNA Kit	Agilent Technologies, catalog # 5067-4626

Equipment

Equipment	Supplier
DNA Engine Multi-Bay Thermal Cycler See *Thermal Cyclers* on page 44.	• Bio-Rad, catalog # PTC-0240G or • PTC-0220G, with Alpha Unit, catalog # ALS-1296GC
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
Magnetic stand-96	Life Technologies, catalog # AM10027
Microcentrifuge	General lab supplier
Microheating System-SciGene TruTemp Heating System	Illumina, catalog # • SC-60-503 (115 V) or • SC-60-504 (220 V)
Microplate centrifuge	General lab supplier
Midi plate insert for microheating system	Illumina, catalog # BD-60-601
QuantiFluor dsDNA System or similar fluorometric-based DNA quantification system	Promega, catalog # E2670
SpectraMax M5 Multi-Mode Micro Plate Reader or similar fluorometric-based DNA quantification system	Molecular Devices, part # M5
Vortexer	General lab supplier
[Optional] 2100 Bioanalyzer Desktop System	Agilent Technologies, catalog # G2940CA
[Optional] TruSeq Index Plate Fixture Kit Note: Recommended for setting up indexed adapters. This part is reusable.	Illumina, catalog # FC-130-1005
[Optional] Vacuum concentrator Note: Use to concentrate a pooled library. Otherwise, use Amicon Ultra-0.5 centrifugal filter units.	General lab supplier

Thermal Cyclers

Use the following recommended settings for selected thermal cycler models. Before performing library prep, validate any thermal cyclers not listed.

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

Index Sequences

The Illumina dual-index strategy adds 2 8-base indexes, Index 1 (i7) and Index 2 (i5), to each sample.

There are 12 different Index 1 (i7) adapters (eg, N705) and up to 8 different Index 2 (i5) adapters (eg, E505), depending on the kit you are using. In the Index adapter name:

- N refers to Nextera
- E refers to enrichment
- 7 refers to Index 1 (i7)
- 5 refers to Index 2 (i5)
- 01–12 refers to the index number

NOTE

See *Kit Contents* on page 34 to determine which indexes are provided in your Nextera Rapid Capture Enrichment kit.

Use the following bases for entry on your sample sheet.

Table 4 Index Adapter Sequences

Index 1 (i7)	Sequence	Index 2 (i5)	Sequence
N701	TAAGGCGA	E502	CTCTCTAT
N702	CGTACTAG	E503	TATCCTCT
N703	AGGCAGAA	E504	AGAGTAGA
N704	TCCTGAGC	E505	GTAAGGAG
N705	GGACTCCT	E506	ACTGCATA
N706	TAGGCATG	E507	AAGGAGTA
N707	CTCTCTAC	E508	CTAAGCCT
N708	CAGAGAGG	E517	GCGTAAGA
N709	GCTACGCT		
N710	CGAGGCTG		
N711	AAGAGGCA		
N712	GTAGAGGA		

DNA Quantification

Perform the QuantiFluor dsDNA assay to quantify dsDNA samples. The assay can quantify small DNA volumes and measure DNA directly. Other techniques can pick up contaminates, such as RNA and proteins. Use a spectrofluorometer for DNA-specific quantification. Spectrophotometry can also measure RNA and yield values that are too high.

Consumables

- 1X TE
- 96-well flat clear bottom black microplates (2)
- 96-well midi plates (2)
- Aluminum foil
- Conical centrifuge tube (15 ml or 50 ml)
- Lambda DNA
- Microseal 'B' adhesive seals
- QuantiFluor dsDNA dye
- RNase/DNase-free Reagent Reservoir

About Reagents

- QuantiFluor dsDNA dye often crystallizes at room temperature. Make sure that the dye is thawed and liquid.

Preparation

1 Remove the QuantiFluor dsDNA dye from to 2°C to 8°C and let stand at room temperature for 60 minutes in a light-impermeable container.

Procedure

Make Lambda DNA Stock Plate

1 Dilute lambda DNA in well A1 of a new midi plate to 1 ng/µl in a final volume of 300 µl. Pipette to mix.

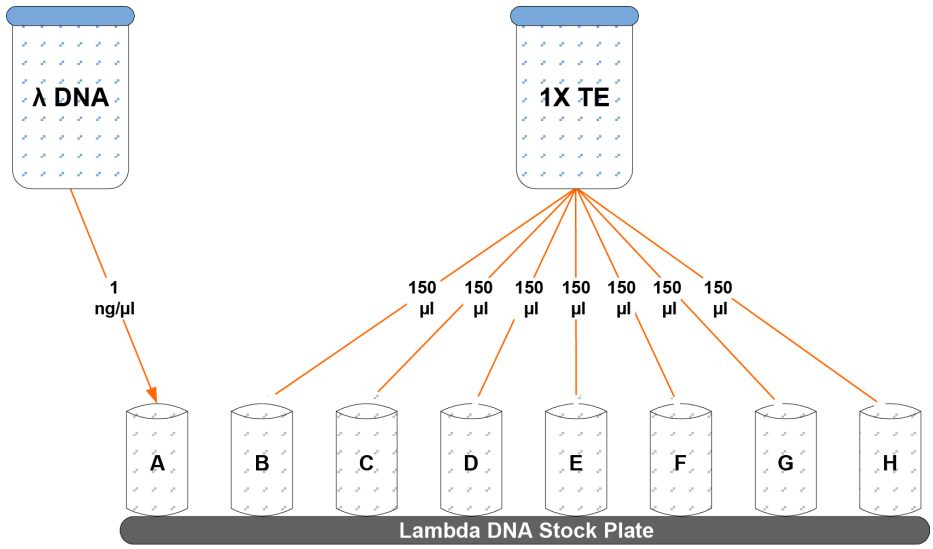
- Use the following formula to calculate the amount of lambda DNA to add to A1:

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

- Dilute 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 wells B, C, D, E, F, G, and H of column 1.

Figure 6 Dilution of Stock Lambda DNA Standard



3 Transfer 150 µl lambda DNA from well A1 to well B1. Pipette to mix.

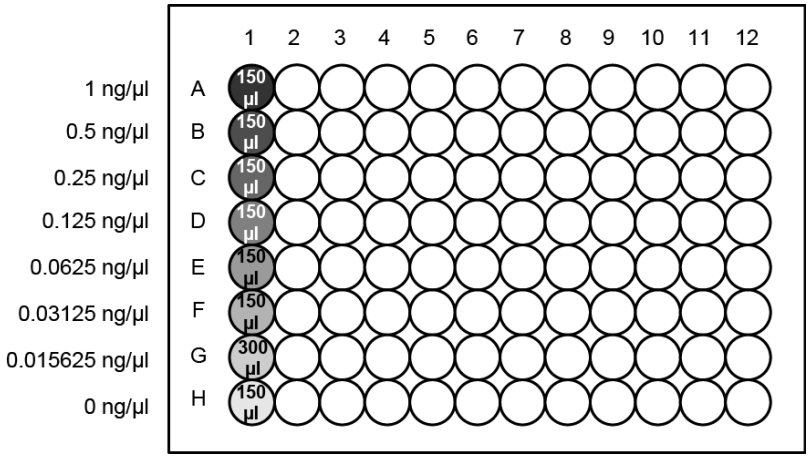
4 Transfer 150 µl from well B1 to well C1. Pipette to mix.

5 Repeat the transfer for wells D1, E1, F1, and G1, changing tips each time. Well H1 serves as the blank 0 ng/µl Lambda DNA.

Table 5 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 7 Serial Dilutions of Lambda DNA



Make DNA Stock Plate

In a new midi plate, prepare the appropriate dilutions of your DNA samples using 1X TE. Measure each sample in triplicate. Make sure that at least 50 µl of diluted sample is prepared for quantification with the QuantiFluor dsDNA dye. Scale for replicate measurements.

1. Dilute using 1 of the following options, depending on the sample quality or library type:
   - **High-quality gDNA**—Dilute 1:1000. For example: 2 µl of gDNA + 1998 µl of 1X TE.
   - **Pre-enriched Nextera Rapid Capture libraries**—Dilute 1:200. For example: 2 µl of library sample + 398 µl of 1X TE.
   - Post-enriched Nextera Rapid Capture library dilution:
     - **1-plex, 3-plex, 6-plex, and 9-plex (8 reaction kits)**—Dilute 1:50. For example: 2 µl of postenriched library + 98 µl of 1X TE.
     - **12-plex**—Dilute 1:100. For example: 2 µl of postenriched library + 198 µl of 1X TE.
2. Shake at 1200 rpm for 1 minute.
3. Centrifuge at 280 × g for 1 minute

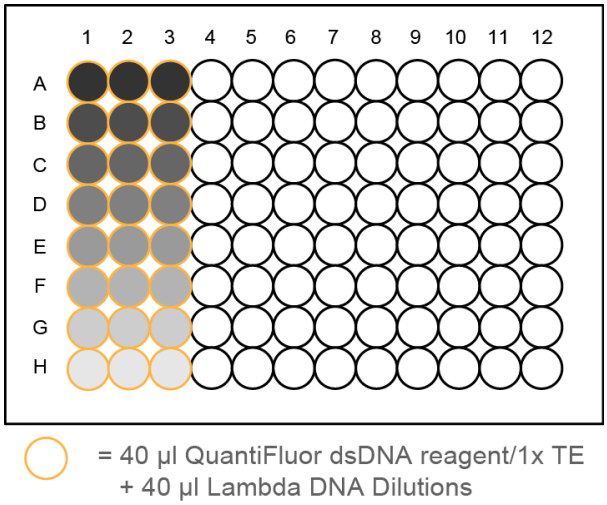
Dilute QuantiFluor dsDNA Dye

1. Prepare a 1:200 dilution of QuantiFluor dsDNA dye in 1X TE in a conical centrifuge tube wrapped in aluminum foil.
   Run each sample and standard in triplicate. For each measurement, 40 µl of diluted QuantiFluor dye is required. Scale as appropriate.
2. Vortex to mix.

Make Lambda DNA Quant Plate

1. Pour the diluted QuantiFluor dsDNA dye/1X TE into a new reagent reservoir.
2. Transfer 40 µl diluted QuantiFluor dsDNA dye/1X TE into each well of columns 1–3 of a new microplate.
3. Transfer 40 µl from each well of the lambda DNA stock plate to columns 1–3.

Figure 8 Lambda DNA Quant Plate with QuantiFluor dsDNA Dye/1X TE



4 Shake at 1200 rpm for 1 minute.

5 Centrifuge at 280 × g for 1 minute

6 Protect from light until read by the spectrofluorometer.

Make DNA Quant Plate

1 Transfer 40 µl QuantiFluor dsDNA reagent/1X TE dilution to each well of the microplate.

2 Transfer 40 µl DNA sample in the DNA stock plate to the microplate.

3 Shake at 1200 rpm for 1 minute.

4 Centrifuge at 280 × g for 1 minute

5 Protect from light until read by the spectrofluorometer.

Read Quant Plate

1 Measure fluorescence (485 nm Ex / 538 nm Em) of both the Lambda DNA quant and DNA quant plates according to the spectrofluorometer/software recommendations.

2 Calculate the DNA concentration of your unknown samples using the fluorescence values determined from step 1 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 is 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.

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

For example:

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

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 6 Illumina General Contact Information

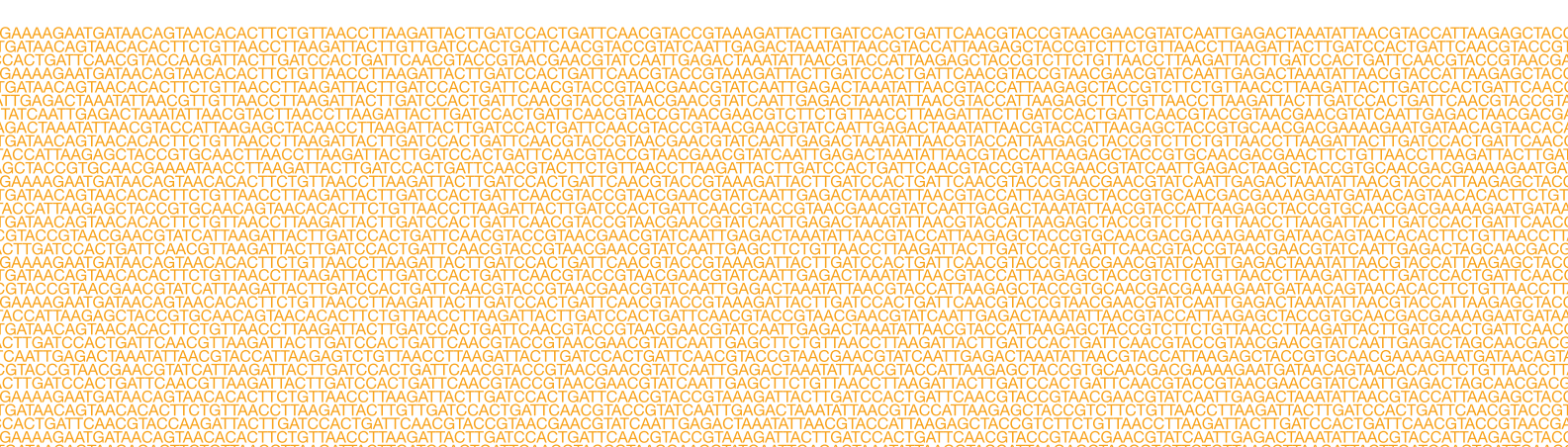
Website	www.illumina.com
Email	techsupport@illumina.com

Table 7 Illumina Customer Support Telephone Numbers

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

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

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



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com