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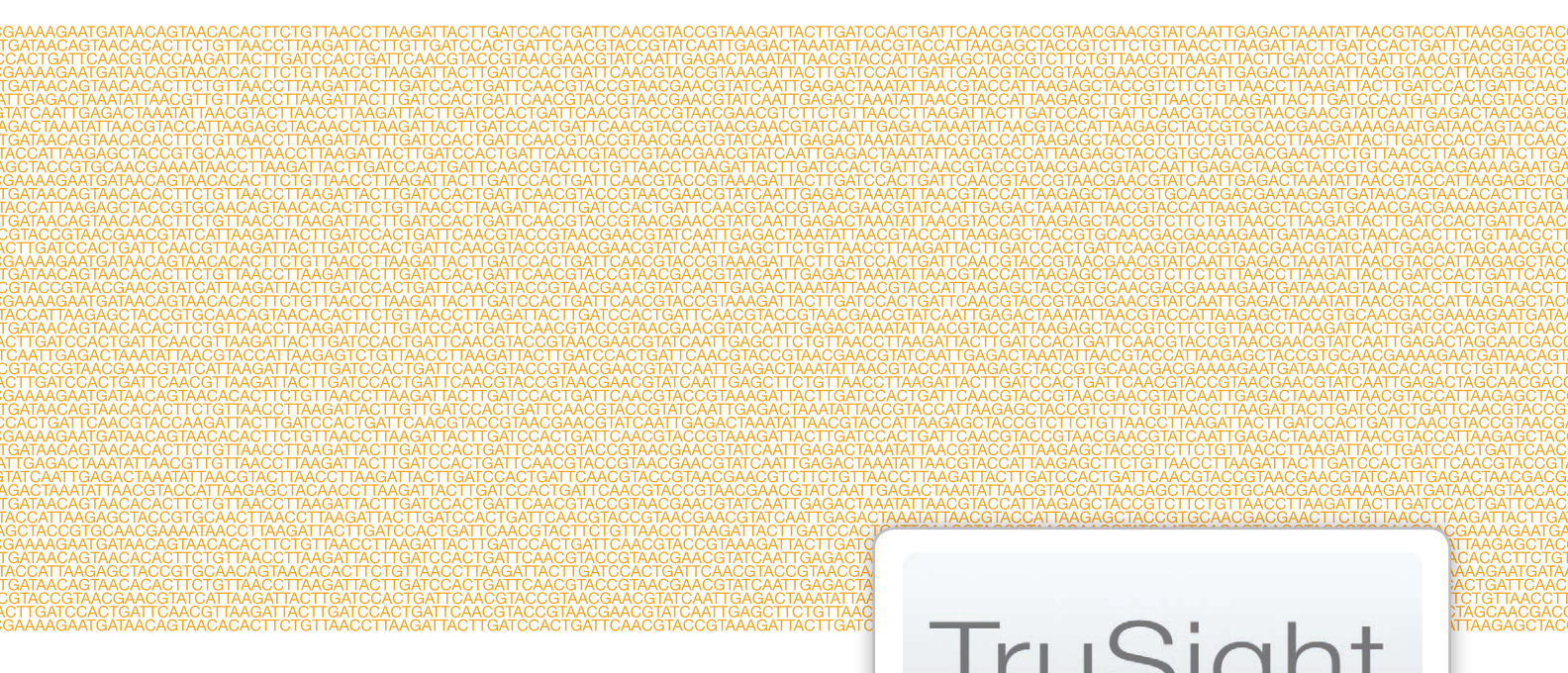
TruSight® Rapid Capture Reference Guide



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Document # 15043291 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 # 15043291 v01	January 2016	• 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 references to Custom Protocol Selector and new protocol and checklist.
Part # 15043291 Rev. A	May 2013	Initial release.

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Overview

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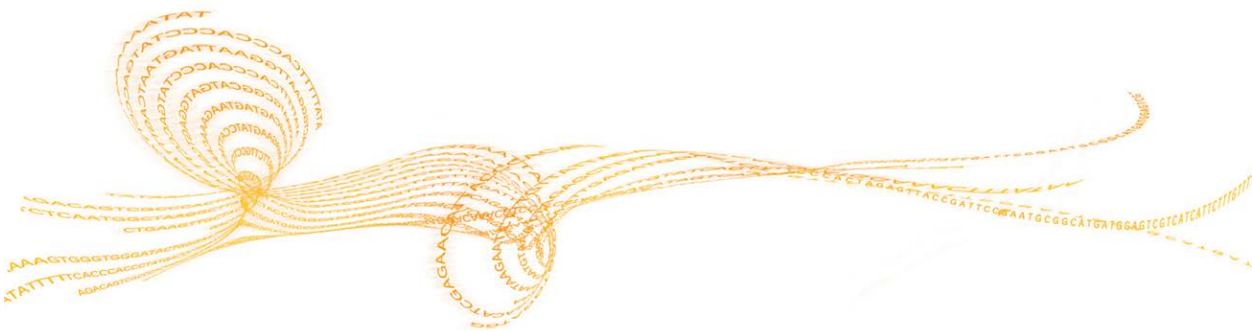


Introduction

This protocol explains how to prepare up to 96 indexed, paired-end libraries, followed by enrichment using a TruSight® Content Set and reagents provided in an Illumina® TruSight Rapid Capture kit. 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 TruSight Rapid Capture protocol offers:

- Excellent data quality with low input of 50 ng
- Fast, 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

Using an enzymatic DNA fragmentation step allows TruSight Rapid Capture library preparation to be more sensitive to DNA input than mechanical fragmentation methods. Accurate quantification of the starting gDNA is essential to enrichment success.

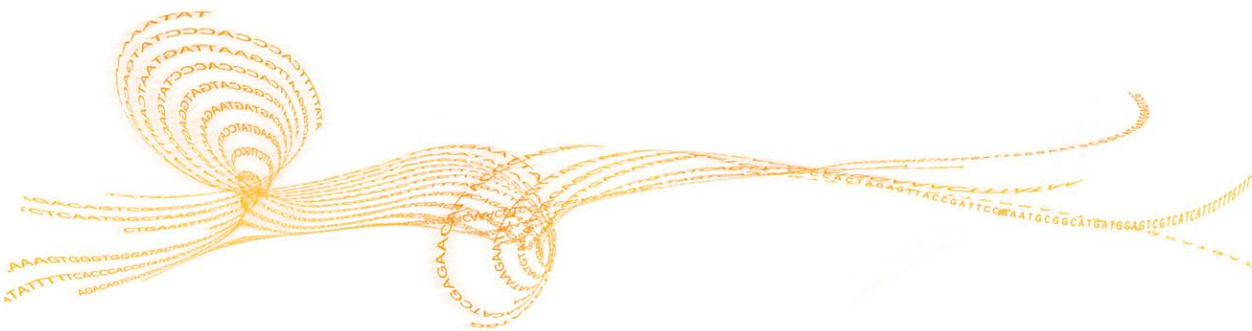
- Quantify gDNA with a fluorometric method specific for double-stranded DNA (dsDNA) and run samples in triplicate.
- Avoid methods that measure total nucleic acid content, such as NanoDrop or other UV absorbance methods.
- Perform a 2-step method of gDNA normalization to minimize gDNA sample input variability during the tagmentation step.
  - After initial quantification, gDNA samples are normalized to 10 ng/µl.
  - Using a similar fluorometric method, samples are quantified and normalized to a final 5 ng/µl.
- The TruSight Rapid Capture protocol is optimized for 50 ng of total gDNA.
  - A higher mass input of gDNA can result in incomplete tagmentation and larger insert sizes, affecting enrichment performance.
  - A lower mass input of gDNA or low quality gDNA in the tagmentation reaction can generate smaller insert sizes, which can be lost during subsequent cleanup steps and cause lower diversity.

Additional Resources

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

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.
TruSight Rapid Capture Protocol Guide (document # 1000000005002)	Provides only protocol instructions. The protocol guide is intended for experienced users. For new or less experienced users, see the TruSight Rapid Capture Reference Guide.
TruSight Rapid Capture Checklist (document # 1000000005003)	Provides a checklist of the protocol steps. The checklist is intended for experienced users. For new or less experienced users, see the TruSight Rapid Capture Reference Guide.

Protocol



Introduction

This chapter describes the TruSight Rapid Capture protocol.

- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- Review Best Practices from the TruSight Rapid Capture support page on the Illumina website.
  - Include a common index in each column. A common index facilitates pipetting operations when dispensing index adapters and pooling indexed libraries later in the protocol.

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 TruSight Rapid Capture 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.

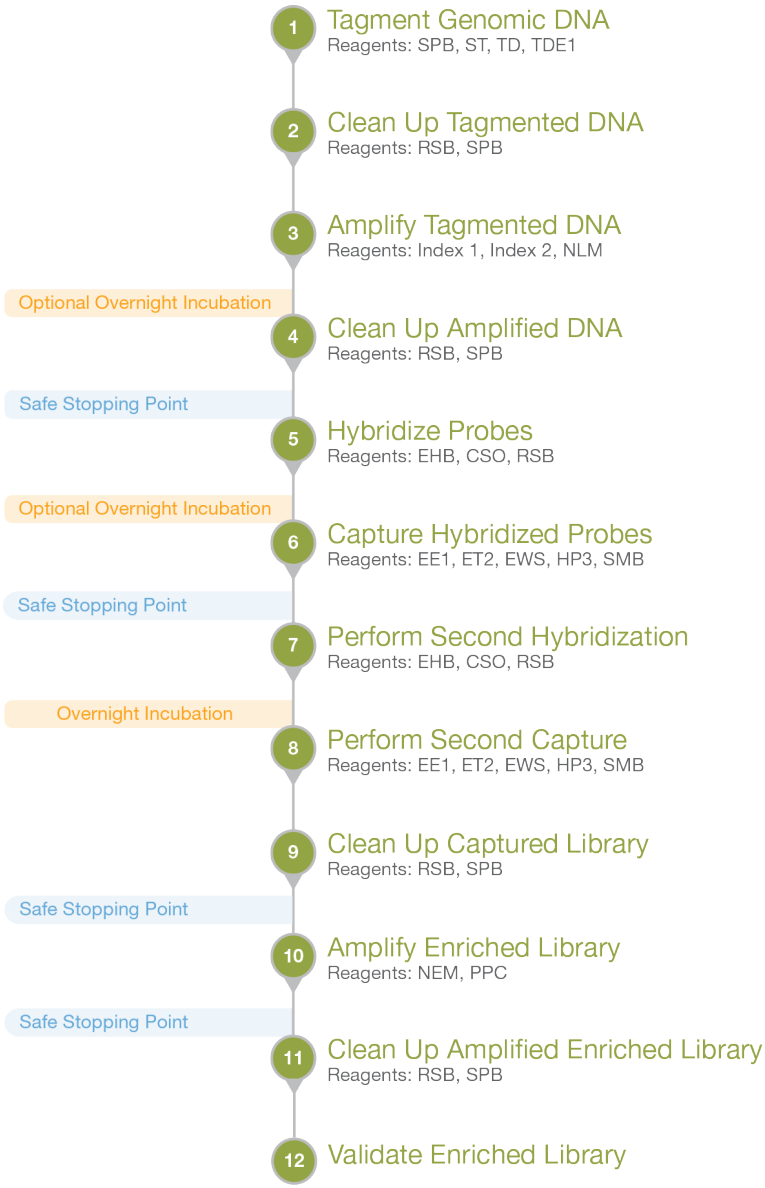
Handling Beads

- 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 TruSight Rapid Capture kit. Safe stopping points are marked between steps.

Figure 1 TruSight Rapid Capture 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)
- ST (Stop Tagment Buffer)
- 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. Invert to mix, and then centrifuge briefly.
TD	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
TDE1	-25°C to -15°C	Thaw on ice. Invert to mix, and then centrifuge briefly. Set aside on ice.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Set aside at room temperature.
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.

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 a new midi plate.
   - Normalized gDNA (10 µl)
   - TD (25 µl)
   - TDE1 (15 µl)

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	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% EtOH.

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 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 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 minutes).

16 Transfer 20 µl supernatant to the corresponding well of a new Hard-Shell PCR plate.

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

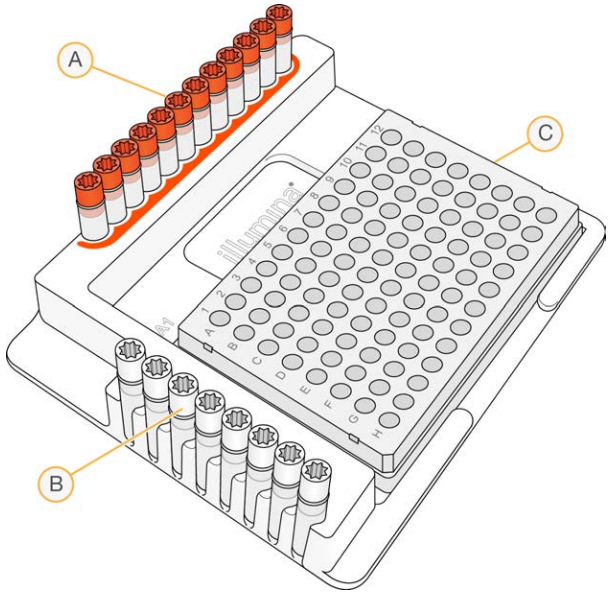
Procedure

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 2 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% EtOH.

Procedure

1 Centrifuge at 280 × g for 1 minute.

2 Transfer 50 µl supernatant to the corresponding well of a new midi 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 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 Add 27.5 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 a new Hard-Shell PCR plate.

18 Quantify the library using a fluorometric method, such as QuantiFluor or Qubit.

19 [Optional] Run 1 µl of the library on an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip.
Expect a distribution of DNA fragments with a size range from ~300 bp to ~1 kbp.

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)
- CSO (Custom Selected Oligos) from the TruSight Content Set
- RSB (Resuspension Buffer)
- 96-well Hard-Shell 0.3 ml PCR plate
- 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
CSO	-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

Pool Libraries

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), rinsing the device before use is not required. Most volume filters through in 5 minutes. Up to 30 minutes might be needed, depending on the starting volume.

- 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 a new Hard-Shell PCR plate.
   - DNA library sample or pool (40 µl)
   - EHB (50 µl)
   - CSO (10 µl)
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.

First Bind

1. Centrifuge at 280 × g for 1 minute.
2. Transfer all volumes to the corresponding well of a new midi plate.
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 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 by combining the following volumes per sample in a 1.7 ml microcentrifuge tube, and then vortex.
- EE1 (28.5 µl)
- 2 N NaOH (1.5 µl)

2 Add 23.5 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 a new Hard-Shell PCR 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)
- CSO (Custom Selected Oligos) from the TruSight Content Set
- 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
CSO	-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 sample well.
   - RSB (15 µl)
   - EHB (50 µl)
   - CSO (10 µl)

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.
2 N NaOH	-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.

Procedure

Second Bind

1 Centrifuge at 280 × g for 1 minute.

2 Transfer supernatant to the corresponding well of a new midi plate.

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 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 by combining the following volumes per sample in a 1.7 ml microcentrifuge tube, and then vortex:
   - EE1 (28.5 µl)
   - 2 N NaOH (1.5 µl)

2 Add 23.5 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 a new midi 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% EtOH.

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 Add 27.5 µl RSB to each well.

11 Shake at 1800 rpm for 1 minute.

12 Incubate at room temperature for 2 minutes.

13 Centrifuge at 280 × g for 1 minute.

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

15 Transfer 25 µl supernatant to the corresponding well of a new Hard-Shell PCR plate.

SAFE STOPPING POINT

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

Amplify Enriched Library

This step uses a 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
NEM	-25°C to -15°C	Thaw on ice.
PPC	-25°C to -15°C	Thaw on ice.

2 Save the following NEM AMP12 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 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 NEM 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% EtOH.

Procedure

1 Centrifuge at 280 × g for 1 minute.

2 Transfer 50 µl to the corresponding well of a new midi 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 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 Add 32.5 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 30 µl supernatant to the corresponding well of a new Hard-Shell PCR plate.

SAFE STOPPING POINT

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

Check Enriched Libraries

Perform the following procedures to check enriched library quality.

Quantify Libraries

Use the following procedure to quantify libraries using a fluorometric method. Alternatively, you can use qPCR. For instructions, see the *Sequencing Library qPCR Quantification Guide (document # 11322363)*.

1. Dilute the postenriched library before quantification:
   - **3-plex to 12-plex enrichments**—Add 1 µl library to 29 µl RSB in a new tube or well. Use this dilution for quantification, quality assessment, and sequencing.
   - **1-plex or 2-plex enrichments**—Use a 1:15 dilution.
2. Convert from ng/µl to nM using the following formula. 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:

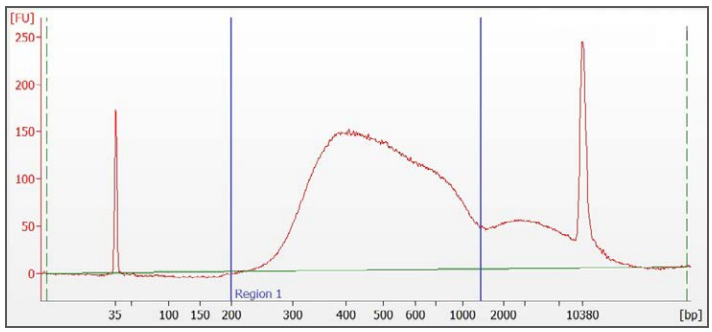
$$\frac{\text{(15 ng/µl)}}{\text{(660 g/mol * 400)}} \times 10^6 = 57 \text{ nM}$$

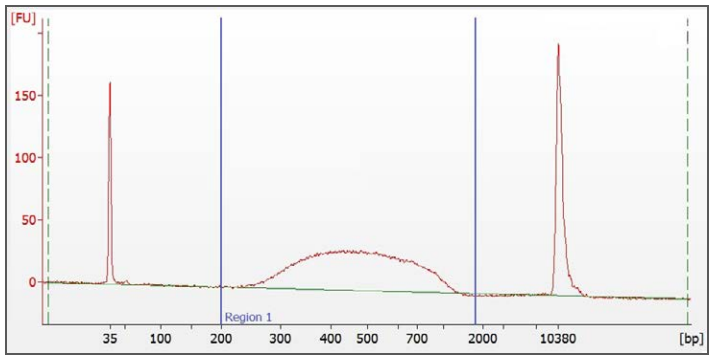
3. Choose whether to assess library quality:
   - Proceed to *Assess Quality [Optional]* on page 29.
   - Skip the assessment and proceed to cluster generation. For instructions, see the system guide for your instrument.

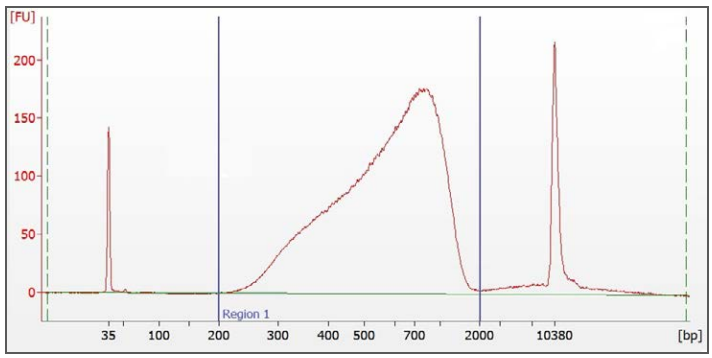
Assess Quality [Optional]

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 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 3 Example Post Enrichment Library Distributions





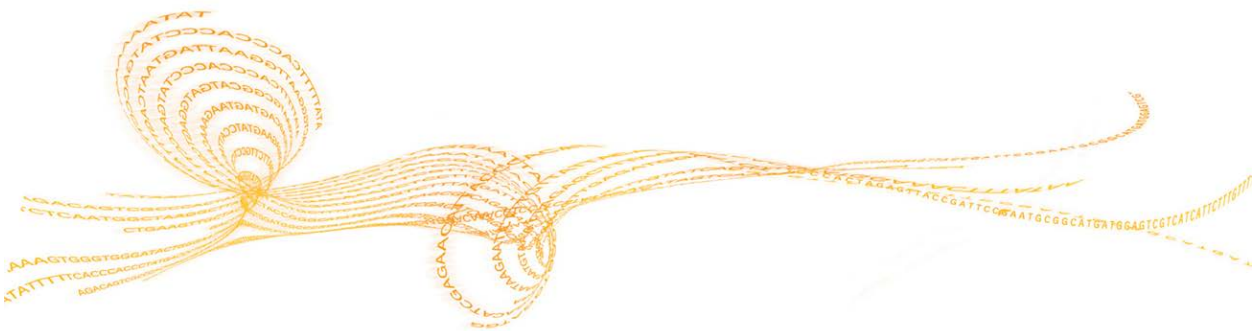


NOTE

The blue lines indicate the boundaries that were manually created to determine average library size. In the first example, a second minor peak at ~2000 bp is visible. Do not include minor peaks in the determination of average library size. The presence of these larger fragments does not affect downstream clustering and sequencing of your enriched library.

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
EE1	Enrichment Elution Buffer 1
EHB	Enrichment Hybridization Buffer
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
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

Make sure that you have all reagents identified in this section before proceeding to the library preparation procedures. TruSight Rapid Capture kits are available in the following configurations.

Table 1 TruSight Rapid Capture Kits

Kit Name	Catalog #	TG Catalog #*
TruSight Rapid Capture Kit (1 Index, 8 Samples)	FC-140-1101	TG-140-1101
TruSight Rapid Capture Kit (2 Indices, 8 Samples)	FC-140-1102	TG-140-1102
TruSight Rapid Capture Kit (4 Indices, 16 Samples)	FC-140-1103	TG-140-1103
TruSight Rapid Capture Kit (24 Indices, 48 Samples)	FC-140-1104	TG-140-1104
TruSight Rapid Capture Kit (24 Indices, 96 Samples)	FC-140-1105	TG-140-1105
TruSight Rapid Capture Kit (96 Indices, 288 Samples)	FC-140-1106	TG-140-1106

* Consumables labeled TG include features to help reduce the frequency of revalidation. These consumables are available with a supply agreement and require providing a binding forecast. For more information, contact your Illumina representative.

Note regarding biomarker patents and other patents unique to specific uses of products.

Some genomic variants, including some nucleic acid sequences, and their use in specific applications might be protected by patents. Customers are advised to determine whether they are required to obtain licenses from the party that owns or controls such patents to use the product for their specific application.

TruSight Rapid Capture Kit Contents (1 Index, 8 Samples) (FC-140-1101, TG-140-1101)

Box 1

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

Box 3, Store at -25°C to -15°C

Quantity	Reagent
1 tube	Index Primer, E501
1 tube	Index Primer, N701

TruSight Rapid Capture Kit Contents (2 Indexes, 8 Samples) (FC-140-1102, TG-140-1102)

Box 1

Quantity	Reagent	Description	Storage Temperature
1	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

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

Box 3, Store at -25°C to -15°C

Quantity	Reagent
1 tube	Index Primer, E501
2 tubes	Index Primer, N701 to N702

TruSight Rapid Capture Kit Contents (4 Indexes, 16 Samples) (FC-140-1103, TG-140-1103)

Box 1

Quantity	Reagent	Description	Storage Temperature
1	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

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

Box 3, Store at -25°C to -15°C

Quantity	Reagent
1 tube	Index Primer, E501
4 tubes	Index Primer, N701 to N704

TruSight Rapid Capture Kit Contents (24 Indexes, 48 Samples) (FC-140-1104, TG-140-1104)

Box 1

Quantity	Acronym	Reagent Name	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

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

Box 3, Store at -25°C to -15°C

Quantity	Reagent Name
2 tubes	Index Primer, E501 to E502
12 tubes	Index Primers, N701 to N712

TruSight Rapid Capture Kit Contents (24 Indexes, 96 Samples) (FC-140-1105, TG-140-1105)

Box 1

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

Box 3, Store at -25°C to -15°C

Quantity	Reagent
2 tubes	Index Primer, E501 to E502
12 tubes	Index Primers, N701 to N712

TruSight Rapid Capture Kit Contents (96 Indexes, 288 Samples) (FC-140-1106, TG-140-1106)

Box 1

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

Box 2, Store at -25°C to -15°C

Quantity	Reagent	Description
12	TDE1	Tagment DNA Enzyme
3	EE1	Enrichment Elution Buffer 1

Quantity	Reagent	Description
6	TD	Tagment DNA Buffer
1	RSB	Resuspension Buffer
12	NLM	Nextera Library Amplification Mix
6	EHB	Enrichment Hybridization Buffer
6	EWS	Enrichment Wash Solution
3	HP3	2 N NaOH
3	PPC	PCR Primer Cocktail
12	NEM	Nextera Enrichment Amplification Mix

Box 3, Store at -25°C to -15°C

Quantity	Reagent
8 tubes	Index Primer, E501 to E508
12 tubes	Index Primers, N701 to N712

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 storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859
Hard-Shell 96-well PCR Plates	Bio-Rad, part # HSP-9601
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Microseal 'A' film	Bio-Rad, part # 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, part # 89094-658
Tris-HCl 10 mM, pH 8.5	General lab supplier
PCR-grade water	General lab supplier
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa)*	Millipore, part # UFC503008
[Optional] DNA 1000 Kit	Agilent Technologies, part # 5067-1504
[Optional] High Sensitivity DNA Kit	Agilent Technologies, part # 5067-4626

* Use to concentrate a pooled library. Otherwise, use a vacuum concentrator.

Equipment

Equipment	Supplier
DNA Engine Multi-Bay Thermal Cycler See *Thermal Cyclers* on page 40.	Bio-Rad, part # PTC-0240G or PTC-0220G, with Alpha Unit, part # ALS-1296GC
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
Magnetic stand-96	Life Technologies, part # 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
Fluorometric quantification with dsDNA binding dye reagents	General lab supplier
Vortexer	General lab supplier
[Optional] 2100 Bioanalyzer Desktop System	Agilent Technologies, part # G2940CA
[Optional] TruSeq Index Plate Fixture Kit[1]	Illumina, catalog # FC-130-1005
[Optional] Vacuum concentrator[2]	General lab supplier

[1] Reusable and recommended for setting up indexed adapters.

[2] Use to concentrate a pooled library. Alternatively, use Amicon Ultra-0.5 centrifugal filter units.

Thermal Cyclers

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

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 (N701–N712) and 2 different Index 2 (i5) adapters (E501 AND E502), 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

Use the following bases for entry on your sample sheet.

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			

* Although E500 series Index 2 sequences are identical to S500 series sequences used in other kits, the Index 2 adapters are not interchangeable.

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.

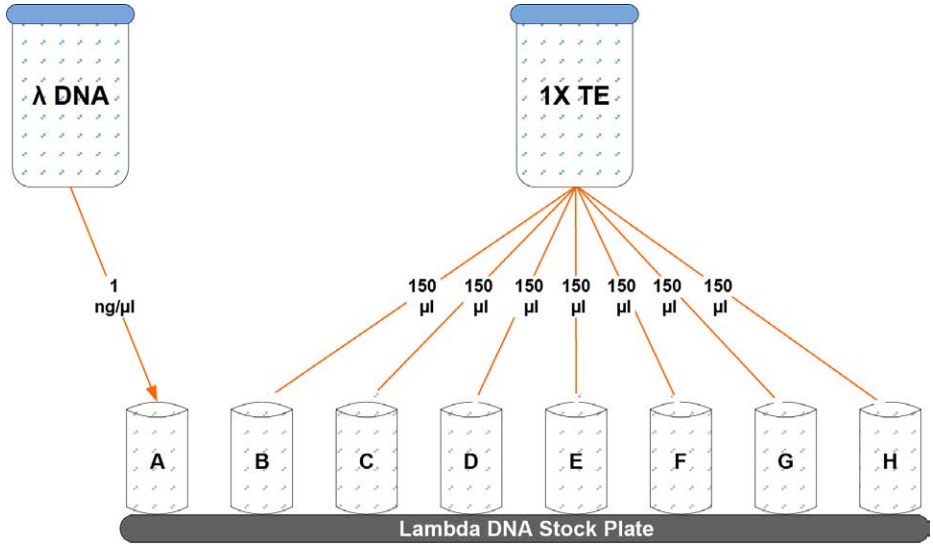
- Use the following formula to calculate the amount of lambda DNA to add to A1:

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

- 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 4 Dilution of Stock Lambda DNA Standard



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

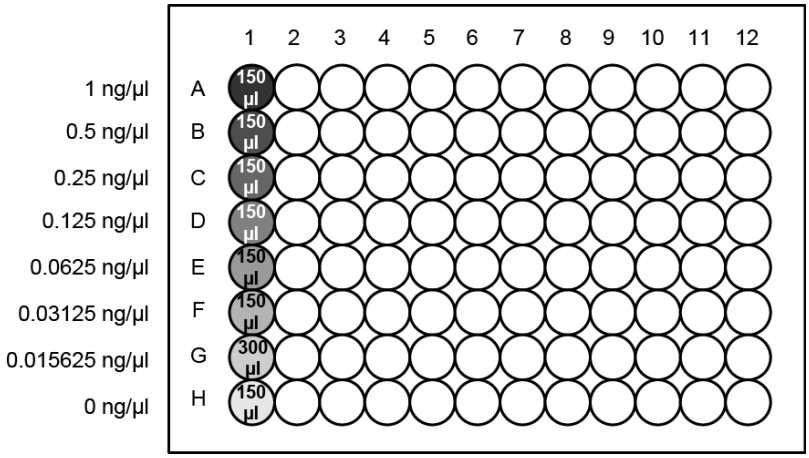
4 Transfer 150 µl from well B1 to well C1. Pipette to mix.

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 2 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 5 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 TruSight Rapid Capture libraries**—Dilute 1:200. For example: 2 µl of library sample + 398 µl of 1X TE.
   - Post-enriched TruSight 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

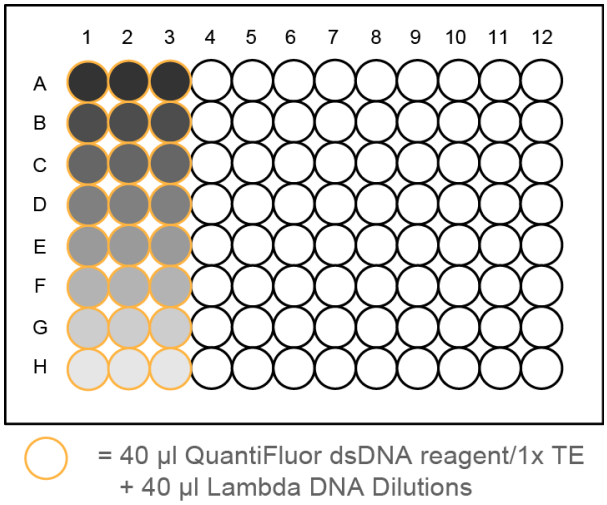
Dilute QuantiFluor dsDNA Dye

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 6 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:

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

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 3 Illumina General Contact Information

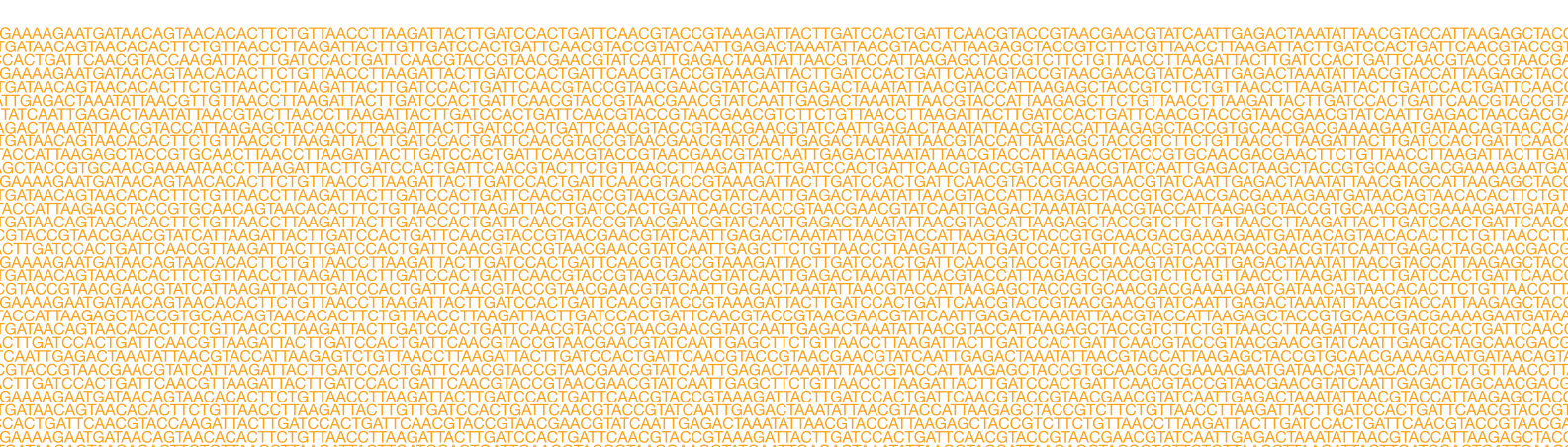
Website	www.illumina.com
Email	techsupport@illumina.com

Table 4 Illumina Customer Support Telephone Numbers

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

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

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



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