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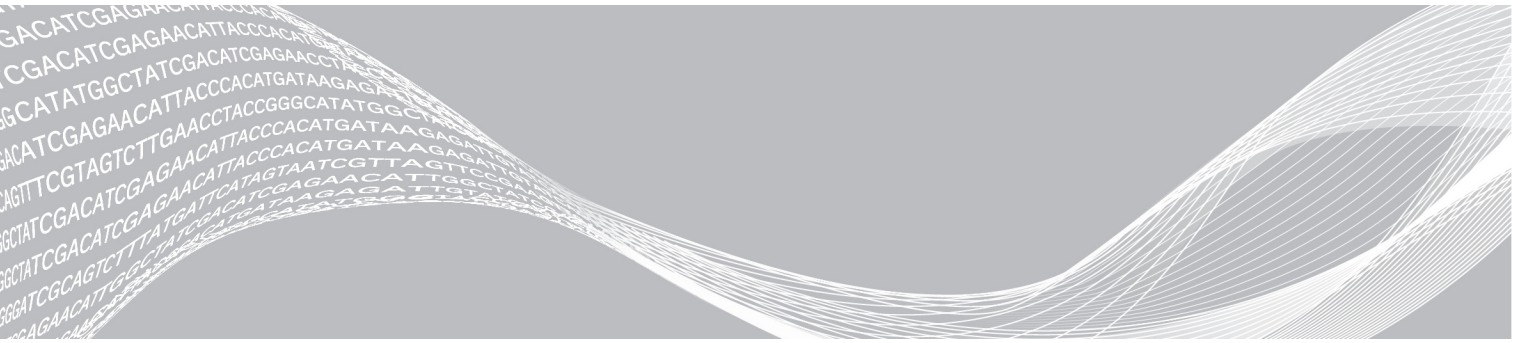
TruSeq DNA Exome

Reference Guide



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Chapter 1 Overview

Introduction

This TruSeq DNA Exome workflow protocol explains how to prepare up to 96 indexed, paired-end libraries, followed by enrichment using reagents provided in an Illumina® TruSeq® Exome 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.

This TruSeq DNA Exome protocol offers:

- Optimized shearing for exome sequencing with a 150 bp insert size
- Bead-based size selection
- Index adapters
  - The 24 sample kit contains adapter index tubes for 24 single indexes (TruSeq DNA Single Indexes, Set A and B)
  - The 96 sample kit contains an adapter index plate for 96 combinatorial dual indexes (TruSeq DNA CD Indexes)
- Compatible with single sample sequencing or lower indexing pooling levels

Illumina-IDT Exome Enrichment Workflow

TruSeq DNA Exome library prep and adapter components can be ordered separately to perform the Illumina-IDT Exome Enrichment workflow. If performing the Illumina-IDT Exome Enrichment workflow, the protocol documented in this guide can be followed through validation of libraries before hybridization and capture. Hybridization and capture continues following the IDT xGen hybridization capture protocol and reagents must be ordered separately through IDT. The libraries after IDT xGen hybridization and capture are ready for subsequent cluster generation and sequencing on Illumina platforms. For more information, see the Integrated DNA Technologies website.

DNA Input Recommendations

For best results, follow the input recommendations. Use 100 ng input gDNA. Quantify the input gDNA and assess the gDNA quality before beginning library preparation.

Quantify Input DNA

Quantify input DNA per the following recommendations:

- Successful library prep depends on accurate quantification of input DNA. Use multiple methods to verify results.
- Use fluorometric-based methods for quantification, such as Qubit or PicoGreen to provide accurate quantification for dsDNA. UV spectrophotometric based methods, such as the Nanodrop, measures any nucleotides present in the sample including RNA, dsDNA, ssDNA, and free nucleotides, which can give an inaccurate measurement of gDNA.

- Quantification methods depend on accurate pipetting methods. Do not use pipettes at the extremes of volume specifications. Make sure that pipettes are calibrated.

Assess DNA Quality

Absorbance measurements at 260 nm are commonly used to assess DNA quality:

- The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity. Values from 1.8 through 2.0 indicate relatively pure DNA.
- The presence of RNA or small nucleic acid fragments, such as nucleotides, can compromise both absorbance measurements.
- Make sure that samples are free of contaminants.

Positive Control

Illumina recommends using Coriell Institute gDNA (NA12878) as a positive control sample for this protocol.

Additional Resources

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

Resource	Description
Custom Protocol Selector	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.
TruSeq DNA Exome Checklist (document #1000000040419)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Illumina Adapter Sequences (document # 1000000002694)	Provides the nucleotide sequences that comprise Illumina oligonucleotides used in Illumina sequencing technologies.
Index Adapter Pooling Guide (document # 1000000041074)	Provides pooling guidelines for preparing libraries for Illumina sequencing systems that require balanced index combinations. Review this guide before beginning library preparation.
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.
Illumina Experiment Manager Guide (document # 15031335) and *IEM TruSeq DNA, RNA, or ChIP Quick Reference Card (document # 15037152)*	Provides information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and record parameters for your sample plate.
Local Run Manager Software Guide (document #100000002702)	Provides an overview of the Local Run Manager (LRM) software, instructions for using software features, and instructions for installing analysis modules on the instrument computer.
BaseSpace help (help.basespace.illumina.com)	Information about the BaseSpace® sequencing data analysis tool that also enables you to organize samples, libraries, pools, and sequencing runs in a single environment.
Hybridization capture of DNA Libraries using xGen Lockdown Probes and Reagents Protocol	Integrated DNA Technologies (IDT) protocol guide for use when performing the Illumina-IDT Exome Enrichment workflow. Provides information on performing hybridization through clean up of the amplified enriched library in the workflow.

Visit the TruSeq DNA Exome workflow support page on the Illumina website for access to requirements and compatibility, additional documentation, software downloads, online training, frequently asked questions, and best practices.

Chapter 2 Protocol

Introduction

This chapter describes the TruSeq DNA Exome workflow protocol.

- Follow the protocol in the order described, using the specified volumes and incubation parameters.
- The protocol provides a single workflow with options for using plates or tubes as containers.
  - Differences for each option are designated with [Plate] or [Tube].
  - Follow the instructions for the container that you are using.
  - Guidelines for using plates vs. tubes are as follows:

Table 1 Workflow Options

	Plates	Tubes
Workflow designator	[Plate]	[Tube]
Number of library prep samples processed at the same time	> 24	≤ 24
Container	• 96-well Hard-Shell PCR plates • 96-well midi plates • 1.5 ml microcentrifuge tubes • 8-tube strips	• 1.5 ml microcentrifuge tubes • 8-tube strips
Mixing method	• Microplate shaker • Pipette	• Pipette
Incubation Equipment	• Microheating systems • 96-well thermal cycler • Heat block	• Heat block • Thermal cycler

- Review best practices before proceeding. See *Additional Resources* on page 2 for information on how to access TruSeq DNA Exome best practices on the Illumina website.
- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. For more information, see *Supporting Information* on page 32.

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***.
- 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. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Microseal 'A' adhesive film is used for thermal cycling steps to prevent evaporation.

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

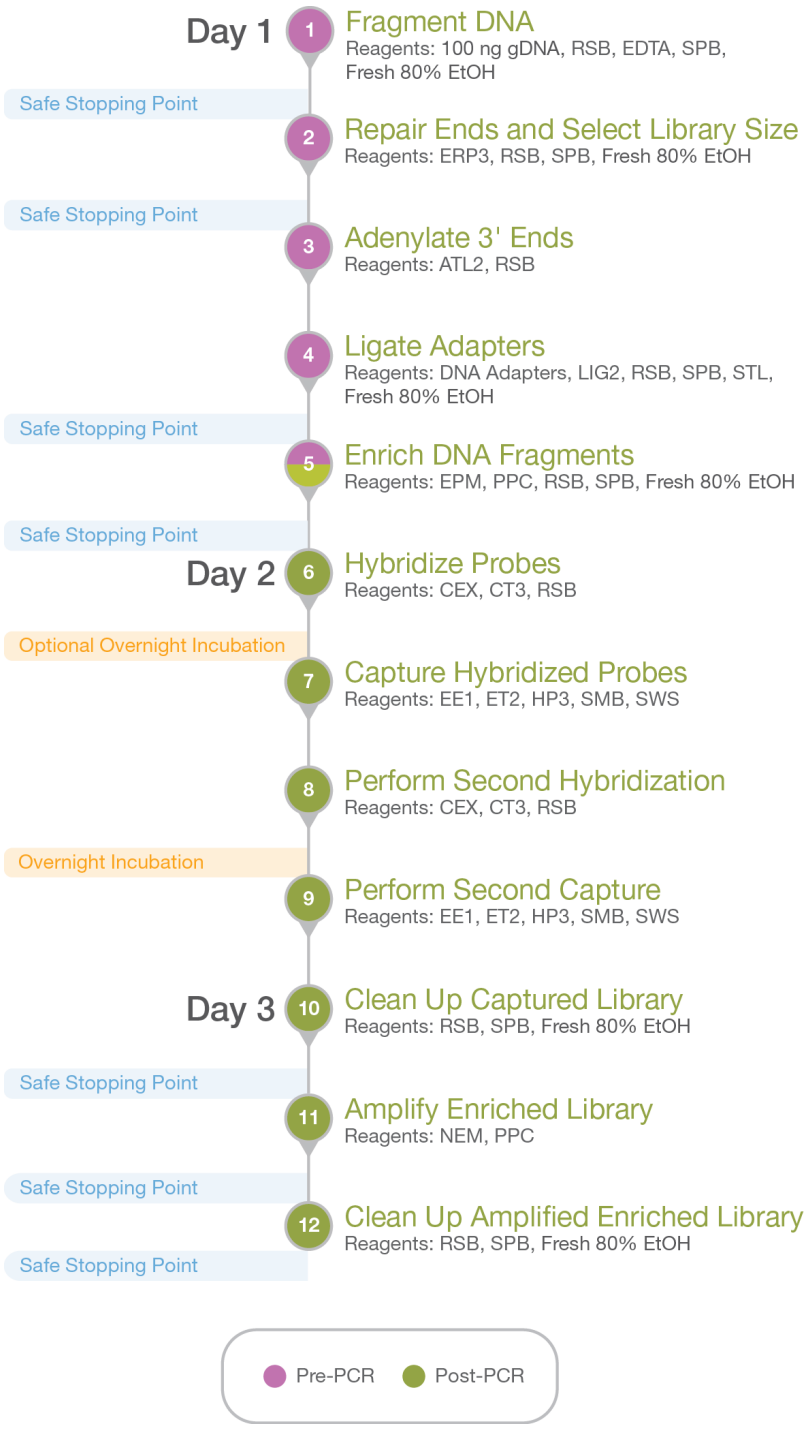
- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Do not freeze beads.
- Pipette bead suspensions slowly.
- Before use, allow the beads to come to room temperature.
- Immediately before use, vortex the beads until they are well dispersed. The color of the liquid must appear homogeneous. Vortex throughout protocol as necessary to keep homogenous.
- If beads are aspirated into 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 specified magnetic stand for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnetic stand until the instructions specify to remove it.
  - Do not agitate the plate while it is on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

This diagram represents the Illumina-only workflow. If performing the Illumina-IDT Exome Enrichment workflow, follow this protocol up to Hybridize Probes and then switch to the IDT xGen hybridization protocol. For more information, see the *Hybridization capture of DNA libraries using xGen Lockdown Probes and Reagents* protocol guide found on the Integrated DNA Technologies website.



Prepare for Pooling

When pooling samples for sequencing, use IEM, LRM, or BaseSpace Prep Tab to record information about your samples before beginning library preparation.

- Use IEM to create and edit sample sheets for Illumina sequencing systems and analysis software.
- Use LRM and BaseSpace Prep Tab to organize samples, libraries, pools, and a run for Illumina sequencing systems and analysis software.

TruSeq DNA Exome kits support the following reactions and plexity. For more information on the kit configurations, see on page 32.

Samples	Enrichment Reactions	Plexity
24	8	3
96	8	12

Fragment DNA

This process describes how to optimally fragment gDNA to a 150 bp insert size. Covaris shearing generates dsDNA fragments with 3' or 5' overhangs.

Consumables

- gDNA samples (100 ng per sample)
- EDTA
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [Plate] 96-well midi plates (3)
  - [Tube] 1.5 ml microcentrifuge tubes and 8-tube strips
- Covaris tubes (1 per sample) or plate
- 15 ml conical tube
- [Plate] Microseal 'B' adhesive seal

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. Store at 2°C to 8°C after the initial thaw.
EDTA	-25°C to -15°C	Thaw at room temperature.
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.

2 Prepare fresh 80% ethanol.

3 Turn on and set up the Covaris instrument according to manufacturer guidelines.

4 [Plate] Calibrate the microplate shaker with a stroboscope and set it to 1200 rpm.

Procedure

Normalize gDNA

1 Quantify gDNA using a fluorometric-based method.

2 Create shearing buffer premix in a 15 ml conical tube.
- RSB (5 ml)
- EDTA (10 µl)

If the starting DNA sample concentration is below < 20 ng/µl, add more EDTA to make sure that the final concentration of EDTA is 1 mM in 50 µl of shearing buffer.

3 Normalize 100 ng gDNA samples with shearing buffer premix to a final volume of 50 µl, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

Fragment DNA

1 Transfer 50 µl DNA samples to separate Covaris tubes or plate wells.

2 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

3 Fragment the DNA using the following Covaris settings.

Covaris Setting	M220	S2	S220	E220	LE220
Duty Factor (%)	20	10	10	10	30
Intensity	—	5	—	—	—
Peak Power (W)	50	—	175	175	450
Cycles/Burst	200	200	200	200	200
Duration (seconds)	375	280	280	280	360/rack; 420/tube
Temperature (°C)	20	7	7	7	7
Water Level	—	12	12	6	6
Intensifier	—	—	—	Yes	—

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

5 Transfer 50 µl sample from each Covaris tube or plate well to a new midi plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

Clean Up Fragmented DNA

1 Vortex SPB until well-dispersed.

2 Add 100 µl SPB to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

3 Incubate at room temperature for 5 minutes.

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

5 Place on a magnetic stand and wait until the liquid is clear (~8 minutes).

6 Remove and discard all supernatant from each well or from the tube.

7 Wash 2 times as follows.

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

8 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

9 Incubate on the magnetic stand for 30 seconds.

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

11 Air-dry on the magnetic stand until dry (~5 minutes).

12 Add 62.5 µl RSB to each well or to the tube.

13 Remove from the magnetic stand, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

14 Incubate at room temperature for 2 minutes.

15 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

17 Transfer 60 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

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

Repair Ends and Select Library Size

This process converts the overhangs resulting from fragmentation into blunt ends using ERP3 (End Repair Mix). The 3' to 5' exonuclease activity of this mix removes the 3' overhangs and the 5' to 3' polymerase activity fills in the 5' overhangs. Following end repair, the library size is selected using SPB (Sample Purification Beads).

Consumables

- ERP3 (End Repair Mix)
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [Plate] 96-well midi plates (2)
  - [Tube] 1.5 ml microcentrifuge tubes and 8-tube strips
- [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
ERP3	-25°C to -15°C	Thaw at room temperature, and then place on ice. Return to storage after use.

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.

3 [Plate] Preheat the microheating system to 30°C.

4 [Tube] Save the following ERP program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 30°C for 30 minutes
- Hold at 4°C
- Each tube contains 100 µl.

Procedure

Convert Overhangs

1 Add 40 µl ERP3 to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

2 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

3 Incubate as follows.
- [Plate] Place on the 30°C microheating system with the heated lid closed for 30 minutes, and then place on ice.
- [Tube] Place on the thermal cycler and run the ERP program.

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

Optimize Fragment Length

1 Vortex SPB until well-dispersed.

2 Add 90 µl SPB to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

3 Incubate at room temperature for 5 minutes.

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

6 Transfer 185 µl supernatant to the corresponding well of a new midi plate or to a new 1.5 ml microcentrifuge tube.

7 Vortex SPB until well-dispersed.

8 Add 125 µl SPB to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

9 Incubate at room temperature for 5 minutes.

10 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

12 Remove and discard all supernatant from each well or from the tube.

13 Wash 2 times as follows.

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

14 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

15 Incubate on the magnetic stand for 30 seconds.

16 Use a 20 µl pipette to remove residual EtOH from each well or from the tube.

17 Air-dry on the magnetic stand until dry (~5 minutes).

18 Add 20 µl RSB to each well or to the tube.

19 Remove from the magnetic stand, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

20 Incubate at room temperature for 2 minutes.

21 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

23 Transfer 17.5 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

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

Adenylate 3′ Ends

A single 'A' nucleotide is added to the 3′ ends of the blunt fragments to prevent them from ligating to each other during the adapter ligation reaction. A corresponding single 'T' nucleotide on the 3′ end of the adapter provides a complementary overhang for ligating the adapter to the fragment. This strategy ensures a low rate of chimera (concatenated template) formation.

Consumables

- ATL2 (A Tailing Mix)
- RSB (Resuspension Buffer)
- [Plate] Microseal 'B' adhesive seals

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
ATL2	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 [Plate] Preheat 2 microheating systems, one to 37°C and another to 70°C.

3 [Tube] Save the following ATAIL70 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 37°C for 30 minutes
- 70°C for 5 minutes
- 4°C for 5 minutes
- Hold at 4°C
- Each tube contains 30 µl.

Procedure

1 Centrifuge ATL2 briefly.

2 Add 12.5 µl ATL2 to each well or tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

3 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

4 [Plate] Incubate as follows.

a Place on the 37°C microheating system with the lid closed for 30 minutes.
b Move to the 70°C microheating system with the lid closed for 5 minutes.
c Place on ice for 5 minutes.

5 [Tube] Place on the thermal cycler and run the ATAIL70 program.

6 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

Ligate Adapters

This process ligates multiple indexing adapters to the ends of the DNA fragments, which prepares them for hybridization onto a flow cell.

Index adapters are included in the TruSeq Exome Kit for TruSeq DNA Exome workflow. If performing the Illumina-IDT Exome Enrichment workflow, index adapters must be ordered separately. For information on compatible index adapters, see on page 32.

Consumables

- DNA Adapters (tubes or index adapter plate)
- LIG2 (Ligation Mix 2)
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- STL (Stop Ligation Buffer)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [Plate] 96-well midi plate and 96-well Hard-Shell 0.3 ml PCR plate
  - [Tube] 1.5 ml microcentrifuge tubes and 8-tube strips
- [Plate] Microseal 'B' adhesive seals

About Reagents

- Do not remove LIG2 from storage until instructed to do so in the procedure.
- Return LIG2 to storage immediately after use.
- 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
DNA Adapters	-25°C to -15°C	Thaw at room temperature for 10 minutes. Return to storage after use.
STL	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
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.

3 [Plate] Preheat a microheating system to 30°C.

4 [Tube] Save the following LIG program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 30°C for 10 minutes
- Hold at 4°C
- Each tube contains 37.5 µl.

Procedure

Add Index Adapters

1 Centrifuge the DNA adapters as follows.

Reagent	Speed	Duration
Adapter tubes	N/A	5 seconds
Index Adapter Plate	280 × g	1 minute

2 [Plate] Remove the plastic cover from the index adapter plate. Save the cover if you are not processing the entire plate.

3 Remove LIG2 from -25°C to -15°C storage.

4 Add the following reagents in the order listed to each well or to the tube.
- RSB (2.5 µl)
- LIG2 (2.5 µl)
- DNA adapters (2.5 µl)

5 Mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

6 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

7 Incubate as follows.
- [Plate] Place on the 30°C microheating system with the lid closed for 10 minutes, and then place on ice.
- [Tube] Place on the thermal cycler and run the LIG program.

8 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

9 Centrifuge STL briefly.

10 Add 5 µl STL to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

11 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

Clean Up Ligated Fragments

1 Vortex SPB until well-dispersed.

2 Perform steps 3 through 18 using the **Round 1** volumes.

3 Add SPB to each well or to the tube.

	Round 1	Round 2
SPB	42.5 µl	50 µl

4 Mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

5 Incubate at room temperature for 5 minutes.

6 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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 or from the tube.

9 Wash 2 times as follows.

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

10 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

11 Incubate on the magnetic stand for 30 seconds.

12 Use a 20 µl pipette to remove residual EtOH from each well or from the tube.

13 Air-dry on the magnetic stand until dry (~5 minutes).

14 Add RSB to each well or to the tube.

	Round 1	Round 2
RSB	52.5 µl	27.5 µl

15 Mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

16 Incubate at room temperature for 2 minutes.

17 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

19 Transfer 50 µl supernatant to the corresponding well of a new midi plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

20 Repeat steps 3 through 18 with the new plate or tube using the **Round 2** volumes.

21 Transfer 25 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

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

Enrich DNA Fragments

This process uses PCR to selectively enrich those DNA fragments that have adapter molecules on both ends and to amplify the amount of DNA in the library. PCR is performed with PPC (PCR Primer Cocktail) that anneals to the ends of the adapters. Minimize the number of PCR cycles to avoid skewing the representation of the library.

NOTE

Fragments with no adapters cannot hybridize to surface-bound primers in the flow cell. Fragments with an adapter on 1 end can hybridize to surface bound primers, but cannot form clusters.

Consumables

- EPM (Enhanced PCR Mix)
- PPC (PCR Primer Cocktail)
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [Plate] 96-well Hard-Shell 0.3 ml PCR plate
  - [Tube] 1.5 ml microcentrifuge tubes or 8-tube strips
- [Plate] Microseal 'A' film
- [Plate] Microseal 'B' adhesive seals

NOTE

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

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
PPC	-25°C to -15°C	Thaw at room temperature. Invert to mix, then centrifuge at 600 × g for 1 minute. Do not vortex. Return to storage after use.
EPM	-25°C to -15°C	Thaw on ice. Invert to mix, then centrifuge at 600 × g for 1 minute. Do not vortex. Return to storage after use.
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.

3 Save the following PCRNano program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 3 minutes
- Do 8 cycles (Illumina-only workflow) or 10 cycles (Illumina-IDT workflow) of:
  - 98°C for 20 seconds
  - [Plate] 60°C for 20 seconds
  - [Tube] 60°C for 15 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 4°C
- Each well or tube contains 50 µl.

Procedure

Amplify DNA Fragments

1 Place the plate or tube on ice and add 5 µl PPC to each well or to the tube.

2 Add 20 µl EPM to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 20 seconds.
- [Tube] Pipette up and down.

3 Centrifuge briefly.

4 Place on the preprogrammed thermal cycler and run the PCRNano program.

Clean Up Amplified DNA

1 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

2 Vortex SPB until well-dispersed

3 Add 35 µl SPB to each well or to the tube.

4 Mix thoroughly, as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

5 Incubate at room temperature for 5 minutes.

6 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

8 Transfer 82 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

9 Vortex SPB until well-dispersed.

10 Add 82 µl SPB to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

11 Incubate at room temperature for 5 minutes.

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

13 Remove and discard all supernatant from each well or from the tube.

14 Wash 2 times as follows.

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

15 Centrifuge briefly.

16 Incubate on the magnetic stand for 30 seconds.

17 Use a 20 µl pipette to remove residual EtOH from each well or from the tube.

18 Air-dry on the magnetic stand until dry (~5 minutes).

19 Add 17.5 µl RSB to each well or to the tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

20 Incubate at room temperature for 2 minutes.

21 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

23 Transfer 15 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

SAFE STOPPING POINT

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

Check Libraries

Quantify Libraries

1 Quantify the libraries using the Qubit dsDNA HS Assay Kit (Illumina-only workflow) or the Qubit dsDNA BR Assay Kit (Illumina-IDT workflow).

a Use 1 µl as the loading volume.
b Use the dsDNA and high sensitivity settings.
c Record STD1 and STD2 readings.
d Measure the library concentration in duplicate and use the average of the 2 measurements.

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

Check Library Quality

1 Check the library size distribution on an Agilent Technologies 2100 Bioanalyzer:
- If using a High Sensitivity DNA chip:
  - Dilute the DNA library 1:10 (Illumina-only workflow) or 1:30 (Illumina-IDT workflow) with RSB to achieve ~2.5ng/µl.
  - Run 1 µl diluted DNA library.
- If using a DNA 1000 chip, run 1 µl undiluted DNA library.

It is normal to see some remaining adapter dimer (~150 bp) and a secondary peak, as a concatemer of the library (~550 bp–1000 bp).

Figure 1 Example 150 bp Insert Library Distribution (pre-enrichment) on a High Sensitivity DNA Chip at 1:10 Dilution

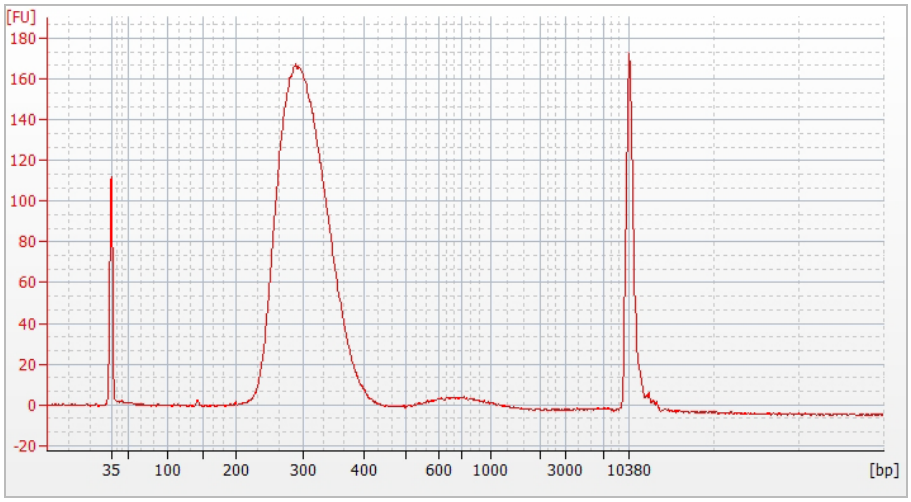
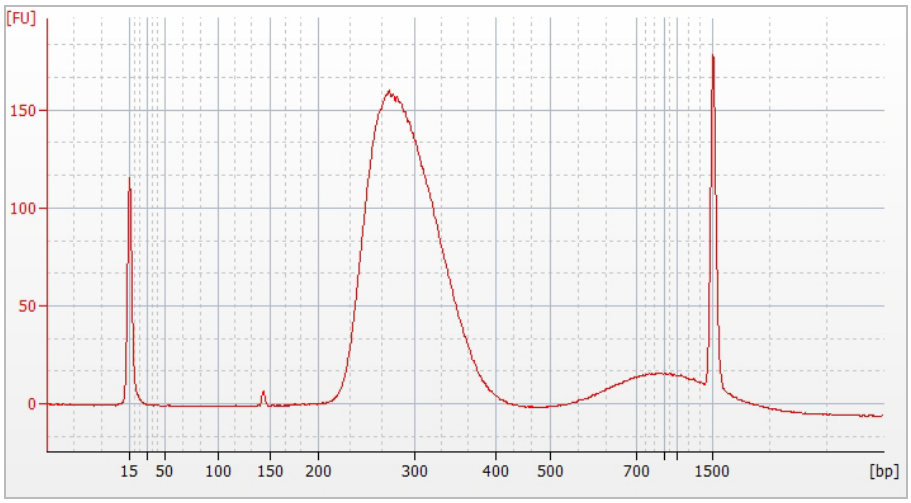


Figure 2 Example 150 bp Insert Library Distribution (pre-enrichment) on a DNA 1000 Chip Undiluted



NOTE

If performing the Illumina-IDT Exome Enrichment Workflow, do not proceed with the Illumina protocol as documented in the remainder of this guide, switch to the IDT xGen hybridization protocol. For more information, see the *Hybridization capture of DNA libraries using xGen Lockdown Probes and Reagents* protocol guide found on the Integrated DNA Technologies website.

If you are following the TruSeq DNA Exome Workflow using the TruSeq Exome Kit, continue with the sections that follow.

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

- CEX (Coding Exome Oligos)
- CT3 (Capture Target Buffer 3)
- RSB (Resuspension Buffer)
- 1.5 ml microcentrifuge tubes and 8-tube strips
- [Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) (1 per pooled sample)

About Reagents

- Before using CT3, vortex to resuspend the thawed 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
CEX	-25°C to -15°C	Thaw at room temperature.
CT3	-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 TE 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
- 58°C for 90 minutes
- Hold at 58°C
- Each tube contains 100 µl.

NOTE

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

Pool Libraries

1 Combine the following amount of each DNA library, making sure that each library has a unique index.

Plexity	Each Library	Total Pool
3-plex	250 ng	750 ng
6-plex	200 ng	1200 ng
9-plex	150 ng	1350 ng
12-plex	100 ng	1200 ng

- 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), it is not required to rinse the device before use. Most of the volume filters through in 5 minutes, but up to 30 minutes can be required 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 reagents in the order listed to a new 8-tube strip. Pipette to mix.
- DNA library pool (40 µl)
- CT3 (50 µl)
- CEX (10 µl)

2 Centrifuge briefly.

3 Place on the preprogrammed thermal cycler and run the TE HYB program.

4 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)
- HP3 (2 N NaOH)
- SMB (Streptavidin Magnetic Beads)
- SWS (Streptavidin Wash Solution)
- 1.5 ml microcentrifuge tubes or 8-tube strips

About Reagents

- SWS can be cloudy after reaching room temperature.
- Make sure that you use SMB (2 ml tube) and not SPB (15 ml tube) for this procedure.
- 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.
HP3	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
SWS	-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 heat block to 50°C.

Procedure

First Bind

1 Add 250 µl SMB to a new 1.5 ml microcentrifuge tube.

2 Immediately transfer the total sample volume (~100 µl) from the thermal cycler to the 1.5 ml microcentrifuge tube containing SMB. Pipette to mix.

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 Incubate at room temperature for 25 minutes.

4 Centrifuge briefly.

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

6 Remove and discard all supernatant from the tube.

7 Remove from the magnetic stand.

First Wash

1 Add 200 µl SWS to the tube. Pipette to mix.

2 Place on the 50°C heat block for 30 minutes.

3 Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

4 Remove and discard all supernatant from the tube.

5 Remove from the magnetic stand.

6 Repeat steps 1–5 for a total of 2 washes.

First Elution

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

2 Add 23 µl elution premix to the tube that contains the beads. Pipette to mix.

3 Incubate at room temperature for 2 minutes.

4 Centrifuge briefly.

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

6 Transfer 21 µl supernatant to a new 8-tube strip.

7 Add 4 µl ET2 to the tube. Pipette to mix.

8 Centrifuge briefly.

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

- CEX (Coding Exome Oligos)
- CT3 (Capture Target Buffer 3)
- RSB (Resuspension Buffer)

About Reagents

Before using CT3, vortex to resuspend the thawed 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
CEX	-25°C to -15°C	Thaw at room temperature.
CT3	-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 the 8-tube strip. Pipette to mix.
- DNA library pool (25 µl)
- RSB (15 µl)
- CT3 (50 µl)
- CEX (10 µl)

2 Centrifuge briefly.

3 Place on the preprogrammed thermal cycler and run the TE HYB program.

4 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)
- HP3 (2 N NaOH)
- SMB (Streptavidin Magnetic Beads)
- SWS (Streptavidin Wash Solution)
- 1.5 ml microcentrifuge tubes
- [Plate] 96-well Hard-Shell 0.3 ml PCR plate
- [Plate] Microseal 'B' adhesive seal

About Reagents

- SWS can be cloudy after reaching room temperature.
- Make sure that you use SMB (2 ml tube) and not SPB (15 ml tube) for this procedure.
- 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.
HP3	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
SWS	-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 heat block to 50°C.

Procedure

Second Bind

1 Add 250 µl SMB to a new 1.5 ml microcentrifuge tube.

2 Immediately transfer the total sample volume (~100 µl) from the thermal cycler to the 1.5 ml microcentrifuge tube containing SMB. Pipette to mix.

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 Incubate at room temperature for 25 minutes.

4 Centrifuge briefly.

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

6 Remove and discard all supernatant from the tube.

7 Remove from the magnetic stand.

Second Wash

1 Add 200 µl SWS to the tube. Pipette to mix.

2 Place on the 50°C heat block for 30 minutes.

3 Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

4 Remove and discard all supernatant from the tube.

5 Remove from the magnetic stand.

6 Repeat steps 1–5 for a total of 2 washes.

Second Elution

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

2 Add 23 µl elution premix to the tube that contains the beads. Pipette to mix.

3 Incubate at room temperature for 2 minutes.

4 Centrifuge briefly.

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

6 Transfer 21 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

7 Add 4 µl ET2 to each well or tube, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 1 minute.
- [Tube] Pipette up and down.

8 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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)
- Choose from the following containers:
  - [Plate] 96-well Hard-Shell 0.3 ml PCR plate
  - [Tube] 1.5 ml microcentrifuge tubes and 8-tube strips
- [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 Vortex SPB until well-dispersed.

2 Add 45 µl SPB to each well or tube. Pipette to mix.

3 Incubate at room temperature for 5 minutes.

4 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

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 or from the tube.

7 Wash 2 times as follows.

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

8 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

9 Incubate on the magnetic stand for 30 seconds.

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

11 Air-dry on the magnetic stand until dry (~5 minutes).

12 Remove from the magnetic stand.

13 Add 27.5 µl RSB to each well or tube. Pipette to mix.

14 Incubate at room temperature for 2 minutes.

15 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

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

17 Transfer 25 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

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

Amplify Enriched Library

This step uses an 8-cycle PCR program to amplify the enriched library.

Consumables

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

NOTE

Use Microseal 'A' when sealing the plate before placing it 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 AMP8 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 8 cycles of:
  - 98°C for 10 seconds
  - [Plate] 60°C for 35 seconds
  - [Tube] 60°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 4°C
- Each well or tube contains 50 µl.

Procedure

1 Add 5 µl PPC to each well or to the tube.

2 Add 20 µl NEM to each well or tube. Pipette to mix.

3 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

4 Place on the preprogrammed thermal cycler and run the AMP8 program.

SAFE STOPPING POINT

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

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)
- Choose from the following containers:
  - [Plate] 96-well Hard-Shell 0.3 ml PCR plate
  - [Tube] 1.5 ml microcentrifuge tubes or 8-tube strips
- [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 as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

2 Vortex SPB until well-dispersed.

3 Add 45 µl SPB to each well or tube. Pipette to mix.

4 Incubate at room temperature for 5 minutes.

5 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

7 Remove and discard all supernatant from each well or from the tube.

8 Wash 2 times as follows.

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

9 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

10 Incubate on the magnetic stand for 30 seconds.

11 Use a 20 µl pipette to remove residual EtOH from each well or from the tube.

12 Air-dry on the magnetic stand until dry (~5 minutes).

13 Remove from the magnetic stand.

14 Add 22 µl RSB to each well or tube. Pipette to mix.

15 Incubate at room temperature for 2 minutes.

16 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

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

18 Transfer 20 µl supernatant to the corresponding well of a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

NOTE

For best practice, perform normalization and pooling directly prior to sequencing. To minimize index hopping, do not store libraries in the pooled form. For more information, see *Minimize index hopping in multiplexed runs* on the Illumina website.

SAFE STOPPING POINT

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

Check Enriched Libraries

Perform the following procedures to check the quality of the enriched library.

Quantify Libraries

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

1 Quantify the postenriched library using the Qubit dsDNA HS Assay Kit.

2 Use the following formula to convert from ng/µl to nM. Assume a 300 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:

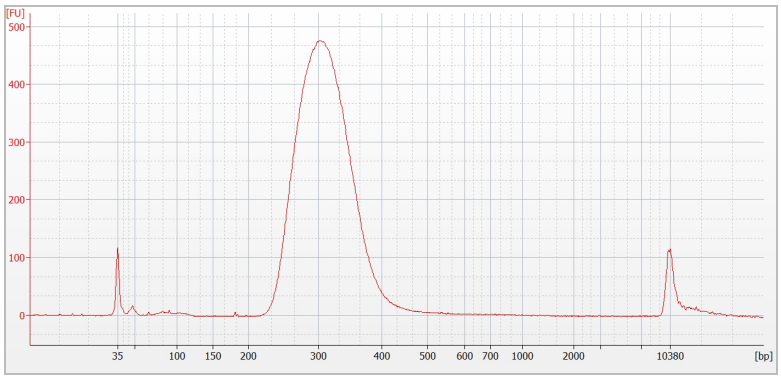
$$\frac{\text{(4 ng/µl)}}{\text{(660 g/mol * 300)}} \times 10^6 = 20 \text{ nM}$$

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

Assess Quality

1 Run 1 µl of either the pooled library, or the individual libraries, on an Advanced Analytical Fragment Analyzer with the HS-NGS High Sensitivity 474 kit or an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit.
Expect a distribution of DNA fragments with a size range from ~200 bp to ~400 bp. 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 (12-plex Enrichment) Library Distribution



Supporting Information

Introduction

The protocol described in this guide assumes that you have reviewed the contents of this section, confirmed workflow contents, and obtained all required consumables and equipment.

Product Contents

Make sure that you have all the reagents identified in this section before starting the protocol.

TruSeq DNA Exome

The following kits are available for performing the TruSeq DNA Exome workflow using Illumina reagents for library preparation and enrichment. These kits include index adapters and do not need to be ordered separately.

Kit Name	Catalog #
TruSeq Exome Kit (24 samples)	20020614
TruSeq Exome Kit (96 samples)	20020615

Illumina-IDT Exome Enrichment

The following library prep and index adapter components are available to order through Illumina to support the Illumina-IDT Exome Enrichment workflow.

From Illumina, order one catalog number for the library prep component and one catalog number for the index adapter component depending on the number of samples for your experiment.

Additional components provided by IDT are required for the IDT portion of the workflow. For more information, see the Integrated DNA Technologies website.

Library Prep Component	Catalog #
TruSeq DNA Library Prep for Enrichment (24 samples)	20020181
TruSeq DNA Library Prep for Enrichment (96 samples)	20020182

Index Adapter Component	Catalog #
IDT for Illumina-TruSeq DNA UD Indexes (24 indexes, 96 samples)	20020590
IDT for Illumina-TruSeq DNA UD Indexes (96 indexes, 96 samples)	20022370
TruSeq DNA Single Indexes (12 indexes, 24 samples) Set A	20015960
TruSeq DNA Single Indexes (12 indexes, 24 samples) Set B	20015961
TruSeq DNA Combinatorial Dual Indexes (96 indexes, 96 samples)	20015949

TruSeq Exome Kit (24 Samples)

The TruSeq Exome kit is made up of four components of reagents, library preparation, enrichment reagents, indexed adapters, and coding exome oligos. Sufficient reagents are provided to support 24 samples in 8 × 3-plex enrichment reactions.

TruSeq DNA Library Prep for Enrichment

This component contains two boxes, requiring different storage conditions.

-20°C Box, Store at -25°C to -15°C

Quantity	Reagent	Description
1	RSB	Resuspension Buffer
1	ATL2	A Tailing Mix
1	LIG2	Ligation Mix 2
1	STL	Stop Ligation Buffer
1	EPM	Enhanced PCR Mix
1	ERP3	End Repair Mix
1	EDTA	EDTA 0.5 M
1	PPC	PCR Primer Cocktail

4°C Box, Store at 2°C to 8°C

Quantity	Reagent	Description
1	SPB	Sample Purification Beads

TruSeq DNA for Enrichment

This component contains two boxes, requiring different storage conditions.

Box 1, Store at -25°C to -15°C

Quantity	Reagent	Description
1	CT3	Capture Target Buffer 3
3	NEM	Enrichment Amplification Mix
1	EE1	Enrichment Elution Buffer 1
1	HP3	2N NaOH or HP3
1	SWS	Streptavidin Wash Solution

Box 2, Store at 2°C to 8°C

Quantity	Reagent	Description
1	ET2	Elute Target Buffer 2
2	SMB	Strepravidin Magnetic Beads

TruSeq DNA Single Indexes Set A, B, Store at -25°C to -15°C

The 24 sample kit includes two sets of index adapters, Set A and Set B.

Set A

Quantity	Part Number	Description
1	AD002	DNA Adapter Index 2
1	AD004	DNA Adapter Index 4
1	AD005	DNA Adapter Index 5
1	AD006	DNA Adapter Index 6
1	AD007	DNA Adapter Index 7
1	AD0012	DNA Adapter Index 12
1	AD0013	DNA Adapter Index 13
1	AD0014	DNA Adapter Index 14
1	AD0015	DNA Adapter Index 15
1	AD0016	DNA Adapter Index 16
1	AD0018	DNA Adapter Index 18
1	AD0019	DNA Adapter Index 19

Set B

Quantity	Part Number	Description
1	AD001	DNA Adapter Index 1
1	AD003	DNA Adapter Index 3
1	AD008	DNA Adapter Index 8
1	AD009	DNA Adapter Index 9
1	AD0010	DNA Adapter Index 10
1	AD0011	DNA Adapter Index 11
1	AD0020	DNA Adapter Index 20
1	AD0021	DNA Adapter Index 21
1	AD0022	DNA Adapter Index 22
1	AD0023	DNA Adapter Index 23
1	AD0025	DNA Adapter Index 25
1	AD0027	DNA Adapter Index 27

Exome Panel (45mb), Store at -25°C to -15°C

Quantity	Reagent	Description
4	CEX	Coding Exome Oligos

TruSeq Exome Kit (96 Samples)

The TruSeq Exome kit is made up of four components of reagents, library preparation, enrichment reagents, indexed adapters, and coding exome oligos. Sufficient reagents are provided to support 96 samples in 8 × 12-plex enrichment reactions.

TruSeq DNA Library Prep for Enrichment

This component contains two boxes, requiring different storage conditions.

-20°C Box, Store at -25°C to -15°C

Quantity	Reagent	Description
3	RSB	Resuspension Buffer
2	ATL2	A Tailing Mix
1	LIG2	Ligation Mix 2
1	STL	Stop Ligation Buffer
2	EPM	Enhanced PCR Mix
2	ERP3	End Repair Mix
1	EDTA	EDTA 0.5 M
2	PPC	PCR Primer Cocktail

4°C Box, Store at 2°C to 8°C

Quantity	Reagent	Description
4	SPB	Sample Purification Beads

TruSeq DNA for Enrichment

This component contains two boxes, requiring different storage conditions.

Box 1, Store at -25°C to -15°C

Quantity	Reagent	Description
1	CT3	Capture Target Buffer 3
3	NEM	Enrichment Amplification Mix
1	EE1	Enrichment Elution Buffer 1
1	HP3	2N NaOH or HP3
1	SWS	Streptavidin Wash Solution

Box 2, Store at 2°C to 8°C

Quantity	Reagent	Description
1	ET2	Elute Target Buffer 2
2	SMB	Strepravidin Magnetic Beads

TruSeq DNA CD Indexes (96 indexes, 96 sample), Store at -25°C to -15°C

Quantity	Reagent	Description
1	DAP	DNA Adapter Plate, 96plex

Coding Exome Oligos, Store at -25°C to -15°C

Quantity	Reagent	Description
4	CEX	Coding Exome Oligos

Consumables and Equipment

Some items required depend on the workflow performed (Plate or Tube) and these items are specified in separate tables.

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

If performing the Illumina-IDT Exome Enrichment workflow, all user-supplied consumables and equipment for library prep listed in this section are required. Additional user-supplied consumables and equipment to complete the Illumina-IDT Exome Enrichment workflow are listed in the IDT xGen hybridization capture protocol. Make sure that you have all the necessary user-supplied consumables and equipment from both the Illumina and IDT protocols before starting the workflow. For more information, see the Integrated DNA Technologies website.

Consumables

Consumable	Supplier
1.5 ml microcentrifuge tubes	General lab supplier
15 ml conical tubes	General lab supplier
10 µl barrier pipette tips	General lab supplier
20 µl barrier pipette tips	General lab supplier
200 µl barrier pipette tips	General lab supplier
1000 µl barrier pipette tips	General lab supplier
One of the following: • High Sensitivity DNA Kit • DNA 1000 Kit	Agilent Technologies, part #: • 5067-4626 • 5067-1504
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
One of the following types, depending on your Covaris system and sample number: • microTUBE AFA Fiber Snap-Cap • 8 microTUBE strip • 96 microTUBE plate • microTUBE AFA Fiber Screw-Cap • microTUBE AFA Fiber Crimp-Cap	Covaris, part # • 520045 • 520053 • 520078 • 520096 • 520052
PCR grade water	General lab supplier

Consumable	Supplier
Qubit dsDNA HS Assay Kit (Illumina-IDT workflow)	Life Technologies, catalog # Q32850
Qubit dsDNA HS Assay Kit (Illumina-only workflow)	Life Technologies, catalog # Q32851
RNase/DNase-free 8-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) Note: Used to concentrate a pooled library. Another option is to use a vacuum concentrator.	Millipore, part # UFC503008
[Optional] Fluorometric quantification with dsDNA binding dye reagents	General lab supplier

Consumables for Plate Workflow

Consumable	Supplier
96-well storage plates, round well, 0.8 ml ('midi' plate)	Fisher Scientific, part # AB-0859
96-well Hard-Shell 0.3 ml PCR plate	Bio-Rad, part # HSP-9601
Adhesive seal roller	General lab supplier
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001

Equipment

Equipment	Supplier/Description
2100 Bioanalyzer Desktop System	Agilent Technologies, part # G2940CA
One of the following Covaris systems: • S2 • S220 • E210 • E220 • M220 • LE220	Covaris M220, part # 500295 For all other models, contact Covaris
DynaMag-2 Magnet	Life Technologies, catalog # 12321D
Minicentrifuge	General lab supplier
Qubit 2.0 Fluorometer	Life Technologies, catalog # Q32866
SpectraMax M5 spectrofluorometer or similar fluorometric-based DNA quantification system	Molecular Devices, part # 0112-0159
Thermal cycler (with heated lid) See *Thermal Cyclers* on page 38.	General lab supplier
Vortexer	General lab supplier
[Optional] Fluorometer for quantification with dsDNA binding dyes	General lab supplier
[Optional] Vacuum concentrator Note: Used to concentrate a pooled library. Another option is to use Amicon Ultra-0.5 centrifugal filter units.	General lab supplier

Equipment for Plate Workflow

Equipment	Supplier
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
Magnetic stand-96	Thermo Fisher Scientific, catalog # AM10027
Microplate centrifuge	General lab supplier
One of the following: • SciGene TruTemp Heating System Note: Two systems are recommended to support successive heating procedures. • Heat block	• Illumina, catalog # • SC-60-503 (110 V) or • SC-60-504 (220 V) • General lab supplier
Stroboscope	General lab supplier
SpectraMax M5 spectrofluorometer or similar fluorometric-based DNA quantification system	Molecular Devices, part # 0112-0159

Equipment for Tube Workflow

Equipment	Supplier
Heat Block	General lab supplier

Thermal Cyclers

The following table lists the recommended specifications for the thermal cycler. If your lab has a thermal cycler that is not listed, validate it before starting the protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, constant at 100°C	Plate
MJ Research PTC-225 DNA Engine Tetrad	Calculated	Heated, constant at 100°C	Plate
Bio-Rad C1000	N/A	Heated, constant at 100°C	Plate
Bio-Rad S1000	N/A	Heated, constant at 100°C	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Index Adapter Sequences

For information on index adapter sequences, see *Illumina Adapter Sequences (document # 1000000002694)* which provides information regarding the nucleotide sequences that comprise Illumina oligonucleotides used in Illumina sequencing technologies.

Acronyms

Acronym	Definition
ATL2	A Tailing Mix
CEX	Coding Exome Oligos
CT3	Capture Target Buffer 3

Acronym	Definition
DAP	DNA Adapter Plate
EE1	Enrichment Elution Buffer 1
EPM	Enhanced PCR Mix
ERP3	End Repair Mix
ET2	Elute Target Buffer 2
HP3	2N NaOH
IEM	Illumina Experiment Manager
LIG	Ligation Mix
LRM	Local Run Manager
NEM	Enrichment Amplification Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
SWS	Streptavidin Wash Solution

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.635.9898	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
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Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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**.