TruSeq® DNA PCR-Free Library Prep

Reference Guide

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Revision History

Part #	Revision	Date	Description of Change
15036187	D	June 2015	Updated *Kit Contents* with reagent changes and removed box and tube part numbers Corrected CTA and CTL volumes to 2.5 µl Removed SAV for viewing in *In-Line Control DNA* Changed title of this document to Reference Guide Updated design of workflow diagram Renamed and combined some procedures as needed to improve continuity Combined HS and LS protocol options into a single workflow Simplified consumables information at the beginning of each section Revised step-by-step instructions to be more succinct Removed reference to obsolete Experienced User Cards and added reference to new protocol guide and checklist Changed BaseSpace resource reference to helpcenter
15036187	C	December 2014	Kit names changed from 'sample' prep to 'library' prep Added references to BaseSpace® for organizing samples, libraries, pools, and runs Removed use of plate name (eg, IMP plate), except for first instance and last instance in each procedure Bead cleanup procedures modified to remove EtOH before air-drying samples Added centrifuge step before each thermal cycler incubation in the LS protocol Added well volume to heat incubation procedures Updated *Prepare for Pooling* sections Updated *Additional Resources* Removed List of Tables Updated SDS link to support.illumina.com/sds.html
15036187	B	November 2013	Renamed Incubate 1 IMP to Incubate IMP HS protocol • Corrected Make DCT procedure to clarify that the DCT plate is an HSP plate • Moved centrifuge steps after incubate EUC and LTF merged into a single document per protocol Created appendix of *Supporting Information* containing *Acronyms*, *Kit Contents*, *Consumables and Equipment*, and *Indexed Adapter Sequences* Replaced *Best Practices* section with a reference to content on the Illumina website Replaced *Adapter Options* and *Pooling Guidelines* sections with a reference to the *TruSeq Sample Preparation Pooling Guide (part # 15042173)* Removed *Usage Guidelines*
15036187	A	January 2013	Initial Release

Introduction

This protocol explains how to prepare up to 96 uniquely indexed paired-end libraries of genomic DNA (gDNA) using Illumina® TruSeq® DNA PCR-Free Library Prep kits. The purpose of the protocol is to add adapter sequences onto the ends of DNA fragments to generate indexed libraries for single-read or paired-end sequencing.

The TruSeq DNA PCR-Free Library Prep protocol offers:

- Streamlined workflow
  - Master-mixed reagents to reduce reagent containers and pipetting
  - Universal adapter for preparation of single read, paired-end, and indexing
- Optimized shearing for whole-genome resequencing with 350 bp, and 550 bp insert size workflows
- Bead-based size selection reagents included in each kit
- Single workflow with options for processing low sample (LS) and high sample (HS) numbers
- Low-throughput (LT) and high-throughput (HT) kit configurations
- High throughput
  - Adapter plate allows for simultaneous preparation of 96 dual-indexed DNA samples
  - Volumes optimized for standard 96-well plate
- Advanced troubleshooting
  - Process control checks built-in for quality control
- Index adapter tags for all samples
  - Additional adapters and primers are not necessary
  - Each TruSeq DNA PCR-Free LT Library Prep Kit contains adapter index tubes recommended for preparing up to 24 samples for sequencing. Together kits A and B allow for pooling up to 24 samples
  - The TruSeq DNA PCR-Free HT Library Prep Kit contains a 96-well plate with 96 uniquely indexed adapter combinations designed for manual or automated preparation of 96 uniquely indexed samples

The protocol is compatible with no indexing or lower indexing pooling levels. The libraries generated do not require PCR amplification to enable cluster generation.

DNA Input Recommendations

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

- For a 350 bp insert size, use 1 µg input gDNA.
- For a 550 bp insert size, use 2 µg input gDNA.
- Input amounts lower than those specified results in low yield and increased duplicates.

Quantify Input DNA

Use the following recommendations to quantify input DNA:

- Successful library preparation depends on accurate quantification of input DNA. To verify results, use multiple methods.
- Use fluorometric-based methods for quantification, such as Qubit or PicoGreen.
- DNA quantification methods that rely on intercalating fluorescent dyes measure only double-stranded DNA and are less subject to the presence of excess nucleic acids.
- Do not use spectrophotometric-based methods, such as NanoDrop, which measure the presence of nucleotides and can result in 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 of 1.8–2.0 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.

In-Line Control DNA

The CTE (End Repair Control), CTA (A-Tailing Control), and CTL (Ligation Control) contain DNA fragments used as controls for the enzymatic activities of the ERP 2 (End Repair Mix 2), ATL (A-Tailing Mix), and LIG 2 (Ligation Mix 2). Each inline control contains dsDNA fragments designed to report the success or failure of a specific enzymatic activity.

The control molecules work through the design of their ends. Controls are added to the reactions before their corresponding step in the protocol. Their end structures match the end structures of a DNA molecule that has not gone through the step. If the step is successful, the control molecule is modified to participate in downstream reactions of library generation and resulting in sequencing data. If the step fails, the control molecule does not go forward in the process and no sequencing data are generated.

Table 1 In-Line Control Functions

Reagent	Function	Control	Structure of Control DNA Ends
ERP 2	End repair: Generate blunt ended fragments by 3'–> 5' exonuclease and 5'–> 3' polymerase activities	End Repair Control 1*	5' overhang at 1 end, 3' overhang at other end
ERP 2	End repair: Add 5'-phosphate groups needed for downstream ligation	End Repair Control 2*	Blunt with 5'-OH group
ATL	A-tailing: Make fragments compatible with adapters and prevent self-ligation by adding a 3'-A overhang	A-Tailing Control	Blunt with 5'-phosphate group
LIG 2	Ligation: Join 3'-T overhang adapters to 3'-A overhang inserts	Ligation Control	Single-base 3' 'A' base overhang

*End Repair Control 1 and End Repair Control 2 are separate controls included in the End Repair Control reagent

Inline controls can be used for various library insert sizes. Each is provided in ladders ranging from approximately 150–850 bp in 100 bp increments. Each control molecule has a unique DNA sequence, indicating both its function and size. The CTE1 and CTE2 controls show a narrow distribution of sizes, while the CTA and CTL controls show a broad size distribution, because the size selection step is before A-tailing.

Inline controls are used for troubleshooting and used to identify the specific mode of failure, but are uninformative in cases where sequencing data are not generated from a library. Using the controls is optional and they can be replaced with the same volume of RSB.

Additional Resources

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

Resource	Description
TruSeq DNA PCR-Free Library Prep Protocol Guide (part # 15075699)	Provides only protocol instructions. The protocol guide is intended for experienced users.
TruSeq DNA PCR-Free Library Prep Checklist (part # 15075700)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
Dual Index Sequencing with TruSeq HT Library Prep (part # 15059916)	Provides guidelines for preparing for dual-indexing sequencing when using a TruSeq DNA PCR-Free HT Library Prep Kit.
TruSeq Library Prep Pooling Guide (part # 15042173)	Provides TruSeq pooling guidelines for preparing libraries for Illumina sequencing systems that require balanced index combinations. Review this guide before beginning library preparation.
Illumina Experiment Manager Guide (part # 15031335) and *IEM TruSeq DNA, RNA, or ChIP Quick Reference Card (part # 15037152)*	Provide information about creating and editing appropriate sample sheets for Illumina sequencing systems and analysis software and record parameters for your sample plate.
BaseSpace help (help.basespace.illumina.com)	Provides information about the BaseSpace® sequencing data analysis tool that also enables you to organize samples, libraries, pools, and sequencing runs in a single environment.

Visit the TruSeq DNA PCR-Free LT Library Prep Kit support page or TruSeq DNA PCR-Free HT Library Prep Kit support page on the Illumina website for access to requirements and compatibility, additional documentation, software downloads, online training, frequently asked questions, and best practices.

Protocol Introduction

This section describes the TruSeq DNA PCR-Free Library Prep 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 different plate types as containers.
  - Differences for each option are designated with [HS] or [LS].
  - Follow the instructions for the container that you are using.
  - You can expect equivalent results from either option. However, the [HS] option can yield more consistent results between samples.
  - The distinguishing elements of the protocol options are as follows.

Table 2 Workflow Options

Workflow Designator	HS	LS
LT Kit - Number of samples processed at the same time	> 24 with index adapter tubes*	≤ 24 with index adapter tubes*
HT Kit - Number of samples processed at the same time	> 24 with index adapter plate	≤ 24 with index adapter plate
Plate Type	96-well Hard-Shell PCR 96-well midi	96-well 0.3 ml PCR 96-well midi
Incubation Equipment	Microheating systems	96-well thermal cycler
Mixing Method	Microplate shaker	Pipetting

* Each TruSeq DNA PCR-Free LT Library Prep Kit contains enough reagents to prepare up to 24 samples. When used together, TruSeq DNA PCR-Free LT Library Prep Kits A and B allow for pooling up to 24 samples using the 12 different indexes in each kit.

- Review Best Practices before proceeding. See *Additional Resources* on page 7 for information on how to access TruSeq DNA PCR-Free Library Prep 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 28.

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

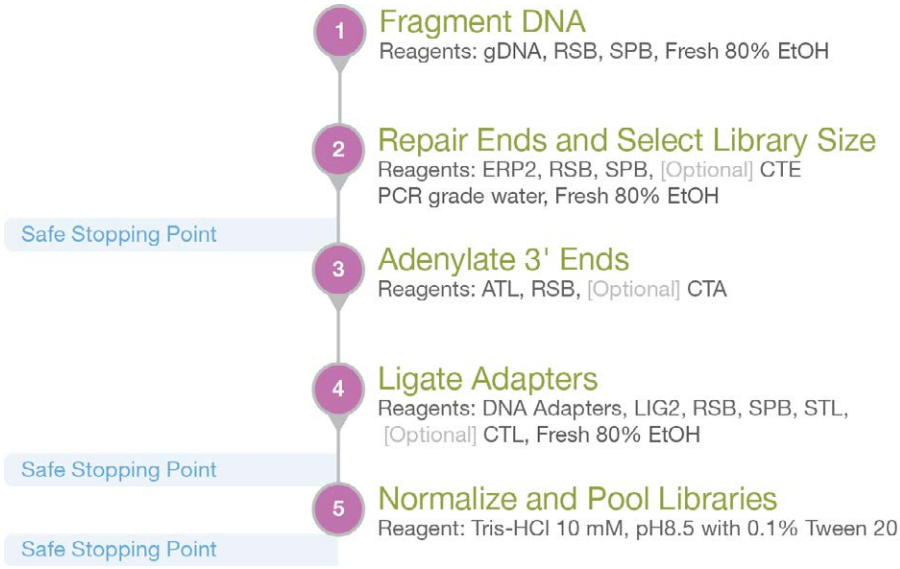
- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Centrifuge steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates.
- 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.

Library Prep Workflow

Figure 1 TruSeq DNA PCR-Free Library Prep Workflow



Prepare for Pooling

If you are pooling, use IEM or BaseSpace to record information about your samples before beginning library prep.

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

Review the planning steps in the *TruSeq Library Prep Pooling Guide (part # 15042173)* when preparing libraries for Illumina sequencing systems that require balanced index combinations.

Fragment DNA

This process describes how to optimally fragment gDNA to a 350 bp, or 550 bp insert size. Covaris shearing generates dsDNA fragments with 3' or 5' overhangs.

Consumables

- gDNA samples
  - 1 µg per sample for a 350 bp insert size
  - 2 µg per sample for a 550 bp insert size
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Barcode labels
  - CFP (Covaris Fragmentation Plate)
  - CSP (Clean Up Sheared DNA Plate)
  - DNA (DNA Plate)
  - IMP (Insert Modification Plate)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [HS] 96-well midi plates (3) and 96-well Hard-Shell 0.3 ml PCR plate (1)
  - [LS] 96-well 0.3 ml PCR plates, semiskirted or skirtless (4)
- Covaris tubes (1 per sample)
- 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.
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 Turn on and set up the Covaris instrument according to manufacturer guidelines.

3 [HS] Calibrate the microplate shaker with a stroboscope and set it to 1800 rpm.

4 Apply barcodes to label plates as follows.
- DNA [midi or PCR plate]
- CFP [Hard-Shell PCR or PCR plate]
- CSP [midi or PCR plate]
- IMP [midi or PCR plate]

Procedure

Normalize gDNA

1 Quantify gDNA using a fluorometric-based method.

2 Normalize gDNA samples with RSB to a final volume of 55 µl in the DNA plate.
- 20 ng/µl for a 350 bp insert size
- 40 ng/µl for a 550 bp insert size

3 Mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

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

Fragment DNA

1 Transfer 52.5 µl DNA samples to separate Covaris tubes.
Use the wells of the CFP plate to hold Covaris tubes upright.

2 Centrifuge at 280 × g for 5 seconds.

3 Fragment the DNA using the following Covaris settings.

Table 3 350 bp Insert Settings

Covaris Setting	M220	S220	S2	E210
Duty Factor (%)	20	5	10	
Intensity	—	—	5.0	
Peak/Displayed Power (W)	50	175	23	14
Cycles/Burst	200			
Duration (seconds)	65	50	45	
Mode	—	Frequency sweeping		
Temperature (°C)	20	5.5–6		

Table 4 550 bp Insert Settings

Covaris Setting	M220	S220	S2	E210
Duty Factor (%)	20	5	10	
Intensity	—	—	2.0	
Peak/Displayed Power (W)	50	175	9	7
Cycles/Burst	200			
Duration (seconds)	45	25	45	
Mode	—	Frequency sweeping		
Temperature (°C)	20	5.5–6		

4 Centrifuge at 280 × g for 5 seconds.

5 Transfer 50 µl supernatant from each Covaris tube to the corresponding well of the CSP plate.

Clean Up Fragmented DNA

1. Vortex SPB until well-dispersed.
2. Add 80 µl SPB to each well, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.
3. Incubate at room temperature for 5 minutes.
4. [HS] Centrifuge at 280 × g for 1 minute.
5. Place on a magnetic stand and wait until the liquid is clear (~8 minutes).
6. Remove and discard all supernatant from each well.
7. Wash 2 times as follows.
   a. Add 200 µl freshly prepared 80% EtOH to each well.
   b. Incubate on the magnetic stand for 30 seconds.
   c. Remove and discard all supernatant from each well.
8. Use a 20 µl pipette to remove residual EtOH from each well.
9. Air-dry on the magnetic stand for 5 minutes.
10. Add 52.5 µl RSB to each well.
11. Remove from the magnetic stand, and then mix thoroughly as follows.
    - [HS] Shake at 1800 rpm for 2 minutes.
    - [LS] Pipette up and down.
12. Incubate at room temperature for 2 minutes.
13. [HS] Centrifuge at 280 × g for 1 minute.
14. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
15. Transfer 50 µl supernatant to the corresponding well of the IMP plate.

Repair Ends and Select Library Size

This process converts the overhangs resulting from fragmentation into blunt ends using End Repair Mix 2. 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 appropriate library size is selected using different ratios of the SPB (Sample Purification Beads).

Consumables

- ERP2 or ERP3 (End Repair Mix)
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- [Optional] CTE (End Repair Control)
- Barcode labels
  - ALP (Adapter Ligation Plate)
  - CEP (Clean Up End Repair Plate)
- 15 ml conical tube
- Freshly prepared 80% ethanol (EtOH)
- PCR grade water
- Choose from the following containers:
  - [HS] 96-well midi plates (2)
  - [LS] 96-well 0.3 ml PCR plates, semiskirted or skirtless (2)
- Microseal 'B' adhesive seals

About Reagents

- The kit contains either ERP2 or ERP3.
- Using CTE is optional. Use RSB as a substitute.
- 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
ERP2 or ERP3	-25°C to -15°C	Thaw at room temperature, and then place on ice. Return to storage after use.
CTE	-25°C to -15°C	Thaw at room temperature, and then place on ice.
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 [HS] Preheat the microheating system to 30°C.

3 [LS] 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

4 Apply barcodes to label plates as follows.
   - ALP [midi or PCR plate]
   - CEP [midi or PCR plate]

Procedure

Convert Overhangs

1 Centrifuge CTE at 600 × g for 5 seconds.

2 Add 10 µl CTE to each well.

3 Centrifuge ERP2 or ERP3 at 600 × g for 5 seconds.

4 Add 40 µl ERP2 or ERP3 to each well, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

5 [HS] Centrifuge at 280 × g for 1 minute.

6 Incubate as follows.
   - [HS] Place on the 30°C microheating system with the heated lid closed for 30 minutes, and then place on ice.
   - [LS] Place on the thermal cycler and run the ERP program. Each well contains 100 µl.

Remove Large DNA Fragments

1 Vortex SPB until well-dispersed.

2 Dilute SPB with PCR grade water to 160 µl per 100 µl of end-repaired sample.
   - When processing ≤ 6 samples, use a new 1.7 ml microcentrifuge tube.
   - When processing > 6 samples, use a new 15 ml conical tube.

   Determine the volumes using the following formulas, which include 15% excess for multiple samples.

Table 5 Diluted SPB for a 350 bp Insert Size

	Formula	Example Amount per 12 samples	Your Calculation
SPB	# of samples X 109.25 µl	1311 µl	
PCR grade water	# of samples X 74.75 µl	897 µl	

Table 6 Diluted SPB for a 550 bp Insert Size

	Formula	Example Amount per 12 samples	Your Calculation
SPB	# of samples X 92 µl	1104 µl	
PCR grade water	# of samples X 92 µl	1104 µl	

3 Vortex diluted SPB until well-dispersed.

4 Add 160 µl diluted SPB to each well, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

5 Incubate at room temperature for 5 minutes.

6 [HS] Centrifuge at 280 × g for 1 minute.

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

8 Transfer 250 µl supernatant to the corresponding well of the CEP plate.

9 Discard remaining diluted SPB.

Remove Small DNA Fragments

1 Vortex undiluted SPB until well-dispersed.

2 Add 30 µl undiluted SPB to each well, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

3 Incubate at room temperature for 5 minutes.

4 [HS] Centrifuge at 280 × g for 1 minute.

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

6 Remove and discard all supernatant from each well.

7 Wash 2 times as follows.
a Add 200 µl freshly prepared 80% EtOH to each well.
b Incubate on the magnetic stand for 30 seconds.
c Remove and discard all supernatant from each well.

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

9 Air-dry on the magnetic stand for 5 minutes.

10 Add 17.5 µl RSB to each well.

11 Remove from the magnetic stand, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

12 Incubate at room temperature for 2 minutes.

13 [HS] Centrifuge at 280 × g for 1 minute.

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

15 Transfer 15 µl supernatant to the corresponding well of the ALP plate.

SAFE STOPPING POINT

If you are stopping, seal the plate 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

- ATL or ATL2 (A-Tailing Mix)
- RSB (Resuspension Buffer)
- [Optional] CTA (A-Tailing Control)
- [HS] Microseal 'B' adhesive seal

About Reagents

- The kit contains either ATL or ATL2.
- Using CTA is optional. Use RSB as a substitute.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
ATL or ATL2	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
CTA	-25°C to -15°C	Thaw at room temperature, and then place on ice.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 [HS] Preheat 2 microheating systems, the first to 37°C and the second to 70°C.

3 [LS] 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

Procedure

1 Centrifuge CTA at 600 × g for 5 seconds.

2 Add 2.5 µl CTA to each well.

3 Centrifuge ATL or ATL2 at 600 × g for 5 seconds.

4 Add 12.5 µl ATL or ATL2 to each well, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

5 Incubate as follows.

[HS]

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.

[LS]

a Place on the thermal cycler and run the ATAIL70 program. Each well contains 30 µl.

Ligate Adapters

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

Consumables

- DNA Adapters (tubes or DAP)
- LIG2 (Ligation Mix 2)
- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- STL (Stop Ligation Buffer)
- [Optional] CTL (Ligation Control)
- Barcode labels
  - CAP (Clean Up ALP Plate)
  - DAP (DNA Adapter Plate)
  - TSP1 (Target Sample Plate)
- Freshly prepared 80% ethanol (EtOH)
- Choose from the following containers:
  - [HS] 96-well midi plate (1) and 96-well Hard-Shell 0.3 ml PCR plate (1)
  - [LS] 96-well 0.3 ml PCR plates, semiskirted or skirtless (2)
- [HS] Microseal 'B' adhesive seals

About Reagents

- Using CTL is optional. Use RSB as a substitute.
- Do not remove the 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
CTL	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
DNA Adapters	-25°C to -15°C	Thaw at room temperature for 10 minutes. Return to storage after use. The DAP can undergo up to 4 freeze-thaw cycles.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
STL	-25°C to -15°C	Thaw at room temperature. Return to storage after use.
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 [HS] Preheat a microheating system to 30°C.

3 [LS] 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

4 Apply barcodes to label plates as follows.
- CAP [midi or PCR]
- TSP1 [Hard-Shell PCR or PCR]

Procedure

Add Index Adapters

1 [HT kit] Remove the tape seal from the DAP.

2 Centrifuge the DNA adapters as follows.

Reagent	Speed	Duration
Adapter tubes	600 × g	5 seconds
DAP	280 × g	1 minute

3 [HT kit] Prepare the DAP as follows.
a Remove the plastic cover. Save the cover if you are not processing the entire plate at the same time.
b Apply the DAP barcode.

4 Centrifuge CTL at 600 × g for 5 seconds.

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

6 Add the following reagents in the order listed to each well, and then mix thoroughly as follows.

Reagent	Volume (µl)
CTL	2.5
LIG2	2.5
DNA adapters	2.5

- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

7 Centrifuge at 280 × g for 1 minute.

8 Incubate as follows.
- [HS] Place on the 30°C microheating system with the lid closed for 10 minutes, and then place on ice.
- [LS] Place on the thermal cycler and run the LIG program. Each well contains 37.5 µl.

9 Centrifuge STL at 600 × g for 5 seconds.

10 Add 5 µl STL to each well, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

11 [HS] Centrifuge at 280 × g for 1 minute.

Clean Up Ligated Fragments

1 Vortex SPB until well-dispersed.

2 Perform steps 2a through 2m using the **Round 1** volumes.

a Add SPB to each well, and then mix thoroughly as follows.

	Round 1	Round 2
SPB	42.5 µl	50 µl

- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

b Incubate at room temperature for 5 minutes.

c [HS] Centrifuge at 280 × g for 1 minute.

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

e Remove and discard all supernatant from each well.

f Wash 2 times as follows.

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

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

h Air-dry on the magnetic stand for 5 minutes.

i Add RSB to each well.

	Round 1	Round 2
RSB	52.5 µl	22.5 µl

j Remove from the magnetic stand, and then mix thoroughly as follows.

- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

k Incubate at room temperature for 2 minutes.

l [HS] Centrifuge at 280 × g for 1 minute.

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

3 Transfer 50 µl supernatant to the corresponding well of the CAP plate.

4 Repeat steps 2a through 2m with the new plate using the **Round 2** volumes.

5 Transfer 20 µl supernatant to the corresponding well of the TSP1 plate.

SAFE STOPPING POINT

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

Validate Libraries

Perform the following procedures to quantify libraries and check library quality.

Quantify Libraries

To achieve the highest quality data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of the flow cell. Optimizing cluster densities requires accurate quantification of DNA libraries. Quantify the libraries using qPCR.

- TruSeq DNA PCR-Free Library Prep library quantification has been validated using the KAPA Library Quantification Kit specified in the *Consumables and Equipment* on page 31.
- Methods other than qPCR quantify molecules that do not have adapters on both ends and do not form clusters. More of these nonclusterable molecules can be present due to the absence of PCR enrichment and quantification by methods other than qPCR can be inaccurate.

Follow qPCR instructions included in the *KAPA Library Quantification Kits for Illumina sequencing platforms Technical Data Sheet* using the KAPA standard (www.kapabiosystems.com), with the following modifications:

You can download the *KAPA Library Quantification Kits for Illumina sequencing platforms Technical Data Sheet* from the Kapa Biosystems website (www.kapabiosystems.com).

- Use at least 2 µl of the original library stock in the library dilution step to ensure accurate and reproducible quantification.
- Perform 2 additional independent (not serial) 1:10,000 and 1:20,000 dilutions using at least 2 µl of the initial diluted libraries to evaluate quantification precision.

NOTE

For guidance on handling small liquid volumes, see *Handling Liquids* in the TruSeq DNA PCR-Free Library Prep Best Practices. See *Additional Resources* on page 7 for information TruSeq DNA PCR-Free Library Prep Best Practices on the Illumina website.

The concentration of each library is calculated as indicated in Table 7–Table 8.

Table 7 350 bp Library Concentration Calculation

Dilution Factor	Calculated by qPCR instrument (pM)*		Average diluted library (pM)	Size adjusted diluted library (pM)	Undiluted library (pM) *	Undiluted library (pM)
1:10,000	A1	A2	A = (A1 + A2)/2	W1 = A x (452/470)	C1 = W1 x 10,000	(C1 + C2)/2
1:20,000	B1	B2	B = (B1 + B2)/2	W2 = B x (452/470)	C2 = W2 x 20,000	

Table 8 550 bp Library Concentration Calculation

Dilution Factor	Calculated by qPCR instrument (pM)*		Average diluted library (pM)	Size adjusted diluted library (pM)	Undiluted library (pM) *	Undiluted library (pM)
1:10,000	C1	C2	C = (C1 + C2)/2	W3 = C x (452/670)	C3 = W3 x 10,000	(C3 + C4)/2
1:20,000	D1	D2	D = (D1 + D2)/2	W4 = D x (452/670)	C4 = W4 x 20,000	

*Duplicate data points

- Obtain the calculated concentration of the 1:10,000 and 1:20,000 library dilutions, as determined by qPCR, in relation to the concentrations of the correctly annotated KAPA DNA Standards 1–6. Use the average of the replicate data points to determine the concentration of the diluted library.
- Perform a size adjustment calculation to account for the difference in size between the average fragment length of the library and the KAPA DNA Standard (452 bp).

  NOTE
  Do not use the average fragment length of the library insert size based on the Bioanalyzer results. PCR-free library fragment sizes measured on the Bioanalyzer are substantially larger than would be predicted or derived from sequencing data.

- Calculate the concentration of the undiluted library by taking account of the relevant dilution factor (eg, 1:10,000 and 1:20,000). Use the average of the replicate data points corresponding to each library DNA dilution to calculate the concentration of the undiluted library.
- If a replicate is an outlier, it can be omitted from the calculation. If multiple replicates are outliers, repeat the assay.

Quality Control

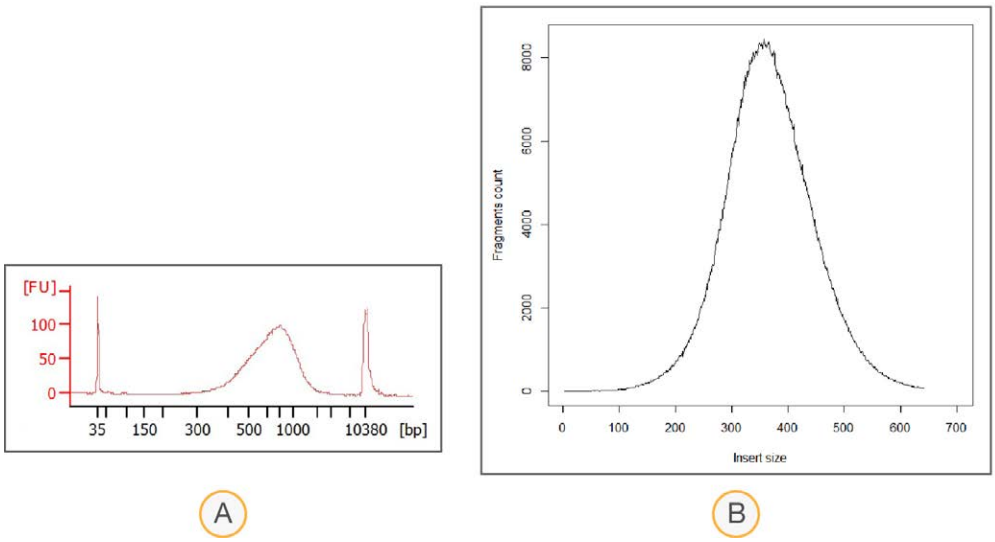
Verify fragment size by checking the library size distribution. Run samples on an Agilent Technologies 2100 Bioanalyzer for qualitative purposes only.

1 Dilute the DNA library 1:5 with water.

2 Run 1 µl diluted DNA library on a High Sensitivity DNA chip.

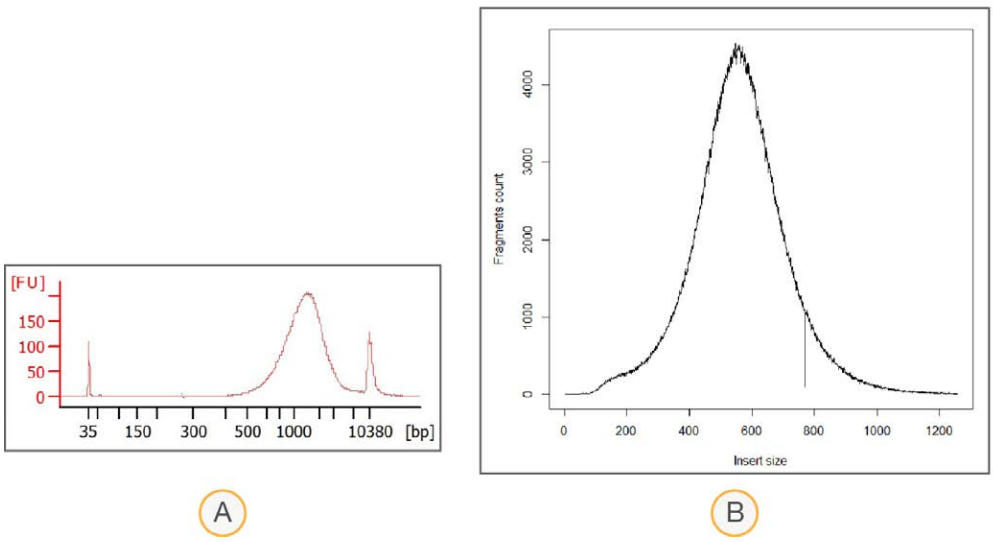
Library fragment sizes measured on the Bioanalyzer are substantially larger than would be predicted or derived from sequencing data. The larger size is because of the anomalous migration of fragments on the chip due to the presence of certain structural features, which would normally be removed if a subsequent PCR-enrichment step were performed. Figure 2–Figure 3 show a comparison between library fragment sizes derived by a Bioanalyzer and the corresponding insert sizes derived from the alignment of paired-end reads to a suitable reference sequence.

Figure 2 Example 350 bp Insert Library Distribution



A Bioanalyzer
B Paired-End Alignment

Figure 3 Example 550 bp Insert Library Distribution



A Bioanalyzer
B Paired-End Alignment

Normalize and Pool Libraries

This process describes how to prepare DNA templates for cluster generation. Indexed DNA libraries are normalized to 2 nM in the DCT plate and then pooled in equal volumes in the PDP plate. Non-indexed DNA libraries are normalized to 2 nM in the DCT plate.

Consumables

- Choose from the following containers:
  - [HS]
    - 96-well midi plate (1) (for pooling > 40 samples)
    - 96-well Hard-Shell 0.3 ml PCR plates (2) (second plate for pooling ≤ 40 samples)
  - [LS]
    - 96-well midi plate (1) (for pooling > 40 samples)
    - 96-well 0.3 ml PCR plates, semiskirted or skirtless (2) (second plate for pooling ≤ 40 samples)
- Microseal 'B' adhesive seals
- Tris-HCl 10 mM, pH8.5 with 0.1% Tween 20
- Barcode labels
  - DCT (Diluted Cluster Template)
  - PDP (Pooled DCT Plate) (for pooling only)

Preparation

1. Apply barcodes to label plates as follows.
   - DCT [PCR or Hard-Shell PCR plate]
   - [For pooling only] PDP [midi (> 40 samples) or PCR (≤ 40 samples) or Hard-Shell PCR plate]

Procedure

Make DCT

1. Transfer 5 µl library to the corresponding well of the DCT plate.
2. Normalize the library concentration with Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20 to 2 nM, and then mix thoroughly as follows.
   - [HS] Shake at 1000 rpm for 2 minutes.
   - [LS] Pipette up and down.

   NOTE
   Depending on the yield quantification data of each library, the final volume of each well can vary from 5–100 µl.
3. [HS] Centrifuge at 280 × g for 1 minute.
4. Do the following,
   - To pool libraries, proceed to the next step in the workflow.
   - For libraries that are not pooled, proceed to cluster generation. For more information, see the system guide for your Illumina platform.

Make PDP

1 If pooling 2–24 samples, transfer 5 µl of each normalized library to a single well of the PDP plate.

2 If pooling 25–96 samples, do the following.
   a Transfer 5 µl of each column of normalized library to column 1 of the PDP plate, and then mix thoroughly as follows.
      — [HS] Shake at 1800 rpm for 2 minutes.
      — [LS] Pipette up and down.
   b [HS] Centrifuge at 280 × g for 1 minute.
   c Transfer the contents of each well of column 1 to well A2.

3 Mix thoroughly as follows.
   - [HS] Shake plate at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

4 [HS] Centrifuge at 280 × g for 1 minute.

5 Proceed to cluster generation. For more information, see the system guide for your Illumina sequencing platform.

SAFE STOPPING POINT

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

Supporting Information

The protocols provided in this guide assume that you are familiar with the contents of this section and that you have the required equipment and consumables.

Acronyms

Acronym	Definition
ALP	Adapter Ligation Plate
ATL	A-Tailing Mix
CAP	Clean Up ALP Plate
CEP	Clean Up End Repair Plate
CFP	Covaris Fragmentation Plate
CSP	Clean Up Sheared DNA Plate
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
DAP	DNA Adapter Plate
DCT	Diluted Cluster Template Plate
DNA	Customer Sample DNA Plate
ERP	End Repair Mix
HS	High Sample
HT	High Throughput
IEM	Illumina Experiment Manager
IMP	Insert Modification Plate
LIG	Ligation Mix
LS	Low Sample
LT	Low Throughput
PDP	Pooled Dilution Plate
RSB	Resuspension Buffer
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
TSP1	Target Sample Plate 1

Kit Contents

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

The TruSeq DNA PCR-Free LT Library Prep Kit is available in a Set A and a Set B. Each TruSeq DNA PCR-Free LT Library Prep Kit contains enough reagents to prepare up to 24 samples. When used together, sets A and B allow for pooling up to 24 samples using the 12 different indexes in each kit.

Table 9 TruSeq DNA PCR-Free Library Prep Kits

Kit Name	Catalog #	Number of Samples Supported	Number of Indexes
TruSeq DNA PCR-Free LT Library Prep Kit - Set A	FC-121-3001	24	12
TruSeq DNA PCR-Free LT Library Prep Kit - Set B	FC-121-3002	24	12
TruSeq DNA PCR-Free HT Library Prep Kit	FC-121-3003	96	96

TruSeq DNA PCR-Free LT Library Prep Kit

The TruSeq DNA PCR-Free LT Library Prep Kit contains 2 boxes: a Set A or Set B box and an SP Beads box.

24 Samples - Set A or Set B Box, Store at -25°C to -15°C

You receive either box A or B with the kit depending on the set you ordered. These boxes also contain plate barcode labels.

NOTE
The kit contains either ERP2 or ERP3 and either ATL or ATL2.

Set A

Quantity	Reagent	Description
1	RSB	Resuspension Buffer
1	ERP2 or ERP3	End Repair Mix
1	ATL or ATL2	A-Tailing Mix
1	LIG2	Ligation Mix 2
1	CTE	End Repair Control
1	CTA	A-Tailing Control
1	CTL	Ligation Control
1	STL	Stop Ligation Buffer
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	AD012	DNA Adapter Index 12
1	AD013	DNA Adapter Index 13
1	AD014	DNA Adapter Index 14
1	AD015	DNA Adapter Index 15
1	AD016	DNA Adapter Index 16
1	AD018	DNA Adapter Index 18
1	AD019	DNA Adapter Index 19

Set B

Quantity	Reagent	Description
1	RSB	Resuspension Buffer
1	ERP2 or ERP3	End Repair Mix
1	ATL or ATL2	A-Tailing Mix
1	LIG2	Ligation Mix 2
1	CTE	End Repair Control
1	CTA	A-Tailing Control
1	CTL	Ligation Control
1	STL	Stop Ligation Buffer
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	AD010	DNA Adapter Index 10
1	AD011	DNA Adapter Index 11
1	AD020	DNA Adapter Index 20
1	AD021	DNA Adapter Index 21
1	AD022	DNA Adapter Index 22
1	AD023	DNA Adapter Index 23
1	AD025	DNA Adapter Index 25
1	AD027	DNA Adapter Index 27

24 Samples - SP Beads Box, Store at 2°C to 8°C

Quantity	Reagent	Description
1	SPB	Sample Purification Beads

TruSeq DNA PCR-Free HT Library Prep Kit

The TruSeq DNA PCR-Free HT Library Prep Kit contains 3 boxes: a core reagent box, an Adapter Plate box, and an SP Beads box.

96 Samples - (Box 1 of 2), Store at -25°C to -15°C

This box also contains plate barcode labels.

NOTE
The kit contains either ERP2 or ERP3 and either ATL or ATL2.

Table 10 TruSeq DNA PCR-Free HT Library Prep Kit, 96 Samples (Box 1 of 2), part # 15037059

Quantity	Reagent	Description
1	RSB	Resuspension Buffer
2	ERP2 or ERP3	End Repair Mix
2	ATL or ATL2	A-Tailing Mix
2	LIG2	Ligation Mix 2
2	CTE	End Repair Control
2	CTA	A-Tailing Control
2	CTL	Ligation Control
2	STL	Stop Ligation Buffer

96 Samples - Adapter Plate Box, Store at -25°C to -15°C

Quantity	Reagent	Description
1	DAP	DNA Adapter Plate, 96plex

96 Samples - SP Beads Box, Store at 2°C to 8°C

Quantity	Reagent	Description
5	SPB	Sample Purification Beads

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol. Some items required depend on the workflow performed (HS or LS) 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.

Table 11 User-Supplied Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier
15 ml conical tubes	General lab supplier
10 µl barrier pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier

Consumable	Supplier
10 µl single channel pipettes	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
96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
High Sensitivity DNA Kit	Agilent Technologies, part # 5067-4626
Ice bucket	General lab supplier
KAPA Library Quantification Kit - Illumina/Universal	KAPA Biosystems, part # KK4824
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
microTUBE AFA Fiber 6x16mm with • Crimp-Cap or • Pre-Slit Snap-Cap (for use with Covaris M220)	Covaris, part # • 520052 or • 520045
PCR grade water	General lab supplier
Qubit assay tubes or Axygen PCR-05-C tubes	Life Technologies, catalog # Q32856 or VWR, part # 10011-830
Qubit dsDNA BR Assay Kit	Life Technologies, catalog # • 100 assays, Q32850 • 500 assays, Q32853
RNaseZap (to decontaminate surfaces)	General lab supplier
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
Tween 20	Sigma-Aldrich, part # P7949
Ultra pure water	General lab supplier

Table 12 User-Supplied Consumables - Additional Items for HS Workflow

Consumable	Supplier
96-well Hard-Shell 0.3 ml PCR plate	Bio-Rad, part # HSP-9601
96-well 0.3 ml skirtless PCR plates or Twin.tec 96-well PCR plates	E&K Scientific, part # 480096 or Eppendorf, part # 951020303

Table 13 User-Supplied Equipment

Equipment	Supplier
2100 Bioanalyzer Desktop System	Agilent Technologies, part # G2940CA
One of the following Covaris systems: • S2 • S220 • E210 • M220	Covaris M220, part # 500295 For all other models, contact Covaris
Magnetic stand-96	Life Technologies, catalog # AM10027
Microplate centrifuge	General lab supplier
Qubit 2.0 Fluorometer	Life Technologies, catalog # Q32866
Vortexer	General lab supplier
qPCR system See *qPCR Systems* on page 34.	General lab supplier

Table 14 User-Supplied Equipment - Additional Items for HS Workflow

Equipment	Supplier
High-Speed Microplate Shaker	VWR, catalog # • 13500-890 (110 V/120 V) or • 14216-214 (230 V)
SciGene TruTemp Heating System Note: Two systems are recommended to support successive heating procedures.	Illumina, catalog # • SC-60-503 (110 V) or • SC-60-504 (220 V)
Midi plate insert for heating system Note: Two inserts are recommended to support successive heating procedures.	Illumina, catalog # BD-60-601
Stroboscope	General lab supplier

Table 15 User-Supplied Equipment - Additional Items for LS Workflow

Equipment	Supplier
96-well thermal cycler (with heated lid)	General lab supplier

qPCR Systems

The following table lists the validated qPCR systems for the TruSeq DNA PCR-Free Library Prep protocol.

Equipment	Supplier
CFX96 Touch Real-Time PCR Detection System*	Bio-Rad, part # 185-5195
Mx3000P qPCR System	Agilent, part # 401511

* Use CFX Manager software version 3.0 with Cq Determination mode: Single Threshold; Baseline Setting:Baseline Subtracted Curve Fit and Apply Fluorescent Drift Correction for data analysis. This setting can correct for abnormalities in fluorescence intensity of the standard curve caused by the instrument. For software installation, contact Bio-Rad.

Indexed Adapter Sequences

This section describes the indexed adapter sequences.

Indexed Adapter Tube Sequences

The TruSeq DNA PCR-Free LT Library Prep Kit contains the following indexed adapter sequences.

- The index numbering is not contiguous. There is no Index 17, 24, or 26.
- The sequence contains 7 bases. The seventh base, shown in parenthesis (), is not included in the Index Read. Record only the first 6 bases in a sample sheet. For indexes 13 and above, the seventh base (in parentheses) might not be A, which is seen in the cycle 7 of the Index Read.
- For more information on the number of cycles used to sequence the Index Read, see the system guide for your Illumina sequencing platform.

Table 16 TruSeq DNA PCR-Free LT Library Prep Kit Set A Indexed Adapter Sequences

Adapter	Sequence	Adapter	Sequence
AD002	CGATGT(A)	AD013	AGTCAA(C)
AD004	TGACCA(A)	AD014	AGTTCC(G)
AD005	ACAGTG(A)	AD015	ATGTCA(G)
AD006	GCCAAT(A)	AD016	CCGTCC(C)
AD007	CAGATC(A)	AD018	GTCCGC(A)
AD012	CTTGTA(A)	AD019	GTGAAA(C)

Table 17 TruSeq DNA PCR-Free LT Library Prep Kit Set B Indexed Adapter Sequences

Adapter	Sequence	Adapter	Sequence
AD001	ATCACG(A)	AD020	GTGGCC(T)
AD003	TTAGGC(A)	AD021	GTTTCG(G)
AD008	ACTTGA(A)	AD022	CGTACG(T)
AD009	GATCAG(A)	AD023	GAGTGG(A)
AD010	TAGCTT(A)	AD025	ACTGAT(A)
AD011	GGCTAC(A)	AD027	ATTCCT(T)

Indexed Adapter Plate Sequences

The DAP in the TruSeq DNA PCR-Free HT Library Prep Kit contains the following indexed adapter sequences.

The indexed adapter sequence recorded in the sample sheet contains 8 bases, and all 8 bases are sequenced during the Index Read.

Table 18 Indexed Adapter 1 Sequences

Adapter	Sequence		Adapter	Sequence
D701	ATTACTCG		D707	CTGAAGCT
D702	TCCGGAGA		D708	TAATGCGC
D703	CGCTCATT		D709	CGGCTATG
D704	GAGATTCC		D710	TCCGCGAA
D705	ATTCAGAA		D711	TCTCGCGC
D706	GAATTCGT		D712	AGCGATAG

Table 19 Indexed Adapter 2 Sequences

Adapter	Sequence		Adapter	Sequence
D501	TATAGCCT		D505	AGGCGAAG
D502	ATAGAGGC		D506	TAATCTTA
D503	CCTATCCT		D507	CAGGACGT
D504	GGCTCTGA		D508	GTACTGAC

Figure 4 DAP Dual-Indexed Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	D701-D501	D702-D501	D703-D501	D704-D501	D705-D501	D706-D501	D707-D501	D708-D501	D709-D501	D710-D501	D711-D501	D712-D501
B	D701-D502	D702-D502	D703-D502	D704-D502	D705-D502	D706-D502	D707-D502	D708-D502	D709-D502	D710-D502	D711-D502	D712-D502
C	D701-D503	D702-D503	D703-D503	D704-D503	D705-D503	D706-D503	D707-D503	D708-D503	D709-D503	D710-D503	D711-D503	D712-D503
D	D701-D504	D702-D504	D703-D504	D704-D504	D705-D504	D706-D504	D707-D504	D708-D504	D709-D504	D710-D504	D711-D504	D712-D504
E	D701-D505	D702-D505	D703-D505	D704-D505	D705-D505	D706-D505	D707-D505	D708-D505	D709-D505	D710-D505	D711-D505	D712-D505
F	D701-D506	D702-D506	D703-D506	D704-D506	D705-D506	D706-D506	D707-D506	D708-D506	D709-D506	D710-D506	D711-D506	D712-D506
G	D701-D507	D702-D507	D703-D507	D704-D507	D705-D507	D706-D507	D707-D507	D708-D507	D709-D507	D710-D507	D711-D507	D712-D507
H	D701-D508	D702-D508	D703-D508	D704-D508	D705-D508	D706-D508	D707-D508	D708-D508	D709-D508	D710-D508	D711-D508	D712-D508

Notes

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 20 Illumina General Contact Information

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

Table 21 Illumina Customer Support Telephone Numbers

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

Safety Data Sheets

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

Product Documentation

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

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