TruSeq® Nano DNA Library Prep for NeoPrep™ Reference Guide

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Revision History

Document	Date	Description of Change
Material # 20000943 Document # 15049722 v01	October 2015	Changed loading order to load samples first Changed loading workflow on reagent plate guide and library card guide Added 3 minute wait to drain oil from vial into library card Renamed button Home at the conclusion of unloading libraries *Consumables* table: • Removed 20 µl and 200 µl pipettes. They are specified in *Pipettes and Tips*. • Removed 1000 µl pipettes as they are standard lab items Moved *Acronyms* to end of Supporting Information
Part # 15049722 Rev. C	June 2015	Added required pipettes to: • *Pipette Tip Requirements* • *Consumables and Equipment* Added step to vortex DMB in *Prepare Samples for Loading* Removed part numbers and package layouts from *Kit Contents* Updated reagent names in *Reagent Plate Contents*
Part # 15049722 Rev. B	April 2015	Added required pipette tip and calibration information to: • New *Pipette Tip Requirements* • *Consumables and Equipment* • *Tips and Techniques* • *Load the Library Card* Changed BaseSpace resource reference to helpcenter
Part # 15049722 Rev. A	March 2015	Initial release.

Introduction

This protocol explains how to prepare up to 16 indexed paired-end libraries of genomic DNA (gDNA) using the reagents provided in the Illumina® TruSeq® Nano DNA Library Prep Kit for NeoPrep™. The purpose of the protocol is to add adapter sequences onto the ends of DNA fragments. The result is indexed paired-end libraries for single read or paired-end sequencing. The libraries are ready for subsequent cluster generation and sequencing.

The protocol offers:

- Streamlined workflow
- All reagents required for library prep, quantification, and normalization are included
- 30 minutes hands-on time
- Optimized shearing for whole-genome resequencing with 350 bp and 550 bp insert size workflows
- A disposable library card allowing for simultaneous preparation of up to 16 DNA samples
- Use of 16 default index adapters, plus 8 alternate index adapters, allowing up to 24-plex pooling with additional NeoPrep runs
- Universal adapters for preparation of single read, paired-end, and indexed libraries

Additional Resources

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

Resource	Description
TruSeq Nano DNA Library Prep for NeoPrep Protocol Guide (document # 15059579)	Provides only protocol instructions. The protocol guide is intended for experienced users.
TruSeq Nano DNA Library Prep for NeoPrep Checklist (document # 15068125)	Provides a checklist of the protocol steps. The checklist is intended for experienced users.
NeoPrep Library Prep System Guide (document # 15049720)	Provides an overview of instrument components and software, instructions for performing library prep runs, and procedures for proper instrument maintenance and troubleshooting.
Illumina Experiment Manager Guide (document # 15031335) and *IEM NeoPrep Quick Reference Card (document # 15061111)*	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.
TruSeq Library Prep Pooling Guide (document # 15042173)	Provides TruSeq pooling guidelines for preparing libraries for Illumina sequencing systems that require balanced index combinations. Review this guide before beginning library preparation.

Visit the TruSeq Nano DNA Library Prep Kit for NeoPrep and NeoPrep System support pages on the Illumina website for requirements and compatibility, additional documentation, software downloads, online training, frequently asked questions, and best practices.

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 25 ng input gDNA. Do not use more than 50 ng gDNA.
- For a 550 bp insert size, use 75 ng input gDNA. Do not use more than 150 ng 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.

Positive Control

Illumina recommends using Coriell Institute gDNA (NA 12878) as a positive control sample for this protocol.

Pipette and Tip Requirements

Use the following required pipettes and tips. Other pipettes and tips are not supported and can result in reagents not dispensing properly and run failure.

Table 1 Required User-Supplied Pipettes and Tips

Volume	Use	Product Name	Supplier
20 µl	≤ 20 µl	Pipet-Lite XLS+ 8-channel LTS, 2 µl to 20 µl	Rainin, catalog # L8-20XLS+
		One of the following: • LTS tips 20 µl. Presterilized. Filter • ART Barrier Pipette Tips 20 µl; 20 µl SoftFit-L	• Rainin, catalog # RT-L10F • Fisher Scientific, catalog # 2749RI
200 µl	21–200 µl	Pipet-Lite XLS+ 8-channel LTS, 20 µl to 200 µl	Rainin, catalog # L8-200XLS+
		One of the following: • LTS tips 200 µl. Presterilized. Filter • ART Barrier Pipette Tips 200 µl; 200 µl SoftFit-L	• Rainin, catalog # RT-L200F • Fisher Scientific, catalog # 2769RI

Tips and Techniques

Sealing a Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking
  - Vortexing
  - Centrifuge
- 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.

Covaris Tubes and Instruments

- Make sure that pipettes are calibrated before beginning. Uncalibrated pipettes can lead to variations in insert size, and reagents not dispensing properly, resulting in run failure.
- To ensure reproducible DNA shearing, centrifuge samples before fragmenting.
- Load DNA samples into the Covaris tube slowly to avoid creating air bubbles. However, air bubbles might not be preventable.
- Use the wells of a plate or another device to hold the Covaris tubes upright.
- Remove the Intensifier from the E220 instrument before fragmenting with a microTUBE-15.
- If using a Covaris instrument other than specified, contact Covaris.

Handling the Library Card

- To avoid instrument damage, do not place the library card guide on the library card during library card verification or a run.
- Use the library card latch release to load and remove the library card from the library card stage.
  - Do not snap the library card into place.
  - Oil and reagents in a used library card can splash out of the card and onto the instrument.
- Hold the used library card level when removing it from the instrument to avoid spilling its contents.

Library Card Loading Guidelines

- Load the library card while it is on the library card stage to avoid spilling or disturbing the loaded contents.
- The NeoPrep Control Software guides you through the steps to set up a run and load the library card. Use the loading procedures in this guide as a reference.
- Do not open the compartment door during library card verification or a run.
- Change your gloves after loading the oil.
- Transfer contents from the reagent plate to the corresponding wells on the library card.
- Reference the corresponding colors and well labels on the reagent plate and library card guides.
- Use the pipettes and tips specified in *Pipette and Tip Requirements* on page 7 and *Consumables and Equipment* on page 31. Other pipettes and tips are not supported and can result in reagents not dispensing properly and run failure.
- Use a multichannel pipette to load reagents, samples, and adapters.
- To avoid instrument damage, make sure that the library card guide is removed from the library card before starting the run.

Library Card Loading Techniques

- Use proper library card loading techniques and specified loading angles.
- Pipette to the first stop to avoid creating bubbles.
- Insert pipette tips perpendicular to the well.
- Insert the pipette tips to the bottom of the well while dispensing. Do not lift the tips until the reagents are dispensed completely.
- Dispense at an angle by pointing pipette tips under the well label and dotted well outline on the library card guide.
- The pipette loading angle depends on the item being dispensed. The angle is specified in each step of the control software loading guide and is depicted in the protocol.
- An icon represents the loading angle and the volume is specified on the control software loading guide. For example, (5 µl)

Icon	Description
	Point the pipette tip toward the well label and dotted well outline on the left.
	Point the pipette tip perpendicular in the well.
	Point the pipette tip toward the well label and dotted well outline on the right.

- Increase the pipette angle if liquid is not dispensing from the pipette tips.

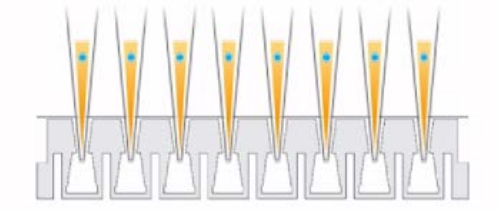
Handling Samples

- Always track the location of each sample.
- Change tips between each sample to avoid cross-contamination.
- Do not centrifuge samples before loading.
- Some space might be present in the pipette tips during transfer from the sample plate to the library card.
- Both 350 bp and 550 bp sample insert sizes can be included in a single run.

Collecting Libraries

- Unload the library card while it is on the library card stage.
- The NeoPrep Control Software guides you through the steps to unload the library card. Use the procedures in this guide as a reference.
- Do not use a 20 µl pipette. It does not fit properly into the library card well.
- Insert pipette tips perpendicularly and touch the tips to the bottom of the collection wells.
- Hold down the library card with one hand while removing the tips from the collection ports to prevent any movement of the card.
- An icon represents the required pipette angle, and the volume is specified on the control software unloading guide. For example, (10 µl)
- Inspect each pipette tip to make sure that a blue library droplet is present in the tips indicated by the control software.

Figure 1 Library Droplet in Pipette Tips



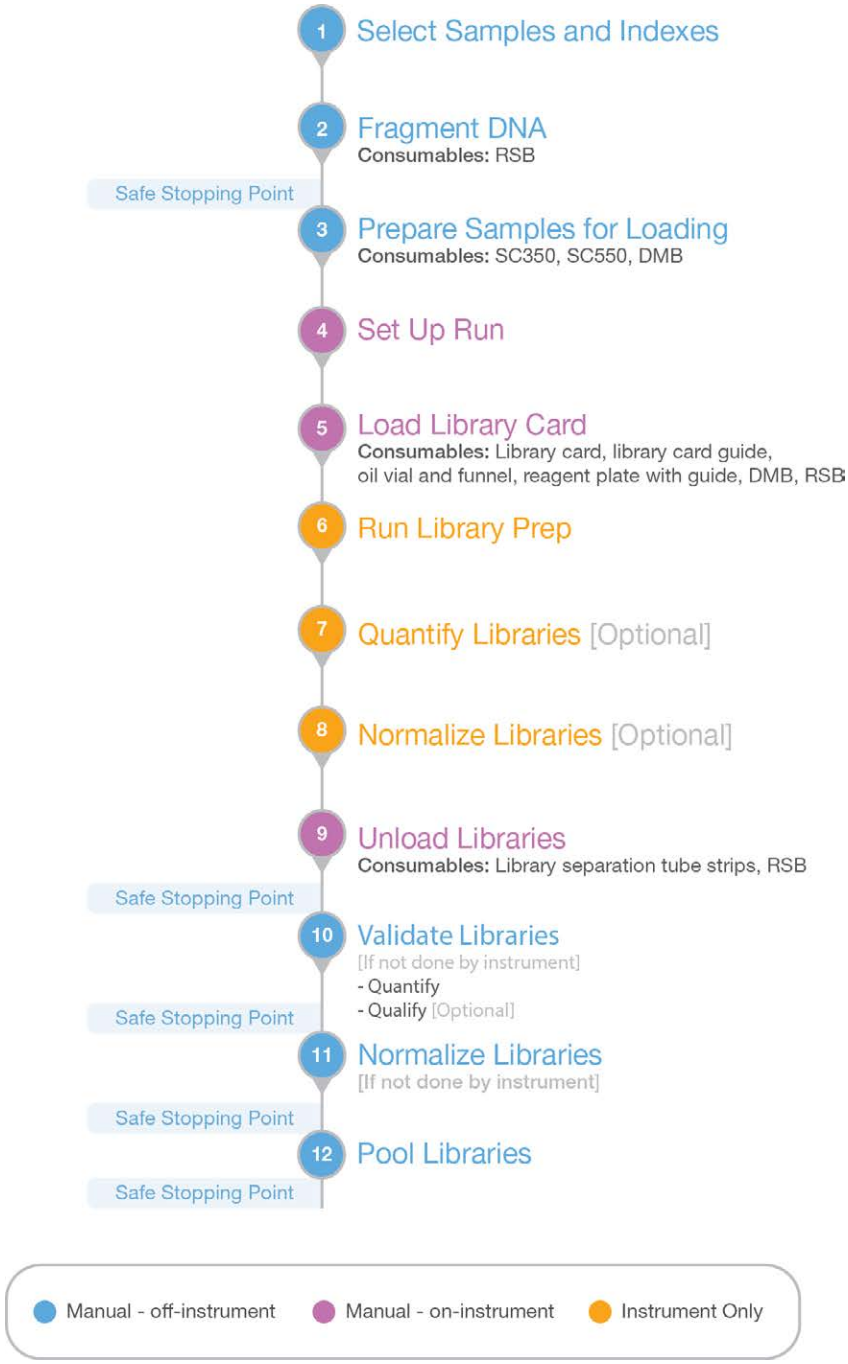
- If a blue library droplet is not visible in each expected pipette tip, do the following:
  - Transfer the extracted liquid to the corresponding plate well containing RSB.
  - Do not dispense the liquid back into the library card, which can introduce air gaps and interfere with library extraction.
  - Use a single-channel pipette to repeat the transfer 1 time for the wells that did not contain the blue droplet. Do not attempt the transfer more than 2 times.
- Vigorously pipette up and down in RSB to dislodge the blue library droplet from the pipette tip.

Handling Library Separation Tube Strips

- Label the tubes to support tracking sample location.
- Use the wells of a plate or another device to hold the library separation tube strips upright.
- Do not centrifuge library separation tube strips.

Library Prep Workflow

Figure 2 Workflow Diagram



Samples are fragmented manually using a Covaris Focused-ultrasonicator. Then, the samples are size-selected and concentrated using Digital Microfluidics Beads (DMB) and a Sample Concentration Solution buffer (SC350 or SC550). Oil, samples, reagents, and adapters are loaded into the library card for a run on the NeoPrep System. A run includes library prep, and optional quantification, and normalization. The NeoPrep Control Software guides you through the run setup and library card loading steps. After

the run is complete, the control software guides you through the process of collecting your libraries from the library card and separating them from the oil. If quantification and normalization were performed by the NeoPrep System, the libraries are ready for pooling, denaturing, and clustering.

Before proceeding:

- Review Best Practices, available on the Illumina website. See *Additional Resources* on page 5.
- Review *Supporting Information* on page 30. Confirm kit contents and make sure that you have the requisite equipment and consumables for this protocol.

Select Samples and Indexes

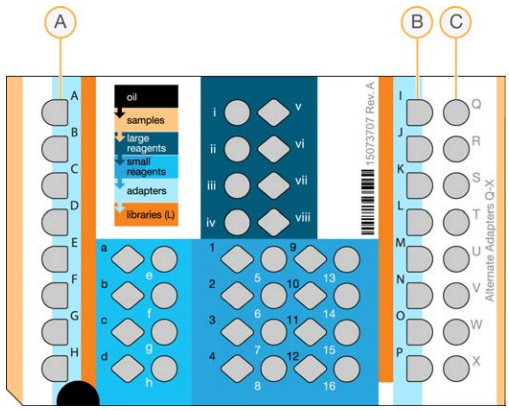
Before beginning library preparation, plan for your NeoPrep System run.

1 Select the samples to use for library prep. Each kit is single-use for 1 NeoPrep System run and each NeoPrep System run prepares up to 16 samples.

2 Plan the sample locations on the sample plate and the library card.
- Place samples 1–8 in column 1, A–H
- Place samples 9–16 in column 2, A–H

3 Use the default index adapters in the order that they are arranged in the reagent plate and arrange the samples used with those index adapters accordingly.

Figure 3 TruSeq Nano DNA Library Prep for NeoPrep Index Adapters



A Adapters A–H (default for samples 1–8)
B Adapters I–P (default for samples 9–16)
C Adapters Q–X (alternate)

- Indexes are single-use.
- Each sample requires a unique index in a library prep run.
- Each kit includes 24 single-index adapters, allowing for pooling up to 24 samples with multiple NeoPrep System runs.
- For the index adapter layout, see *Reagent Plate Contents* on page 30.
- For the index adapter sequences, see *Index Adapter Sequences* on page 34. The TruSeq Nano DNA Library Prep for NeoPrep adapters are TruSeq LT single-index adapters.
- Review the planning steps in the *TruSeq Library Prep Pooling Guide (document # 15042173)* for Illumina sequencing systems that require balanced index combinations.

4 [Optional] Use IEM or BaseSpace Prep tab to record information about your samples and indexes. The information is used during the run setup.

Fragment DNA

This process describes how to shear input gDNA into 350 bp and 550 bp dsDNA fragments required for loading into the library card.

Consumables

- RSB (Resuspension Buffer)
- 96-well 0.3 ml PCR plates (2)
- Covaris microTUBE-15 AFA Beads Screw-Cap (1 per sample)
- gDNA samples
  - 25 ng for 350 bp inserts
  - 75 ng for 550 bp inserts
- Microseal 'B' adhesive seal

Preparation

1. Remove RSB from 2°C to 8°C storage.
2. Turn on the Covaris instrument and follow manufacturer instructions to set up your instrument.
3. Make sure that pipettes are calibrated before beginning. Uncalibrated pipettes can lead to variations in insert size, and reagents not dispensing properly, resulting in run failure.

Procedure

1. Quantify gDNA samples using a fluorometric-based method that uses dsDNA binding dyes such as Qubit or QuantiFlour.
2. Normalize gDNA samples with RSB in a final volume of 15 µl in separate wells of a new PCR plate:
   - For a 350 bp insert size—1.67 ng/µl of sample
   - For a 550 bp insert size—5 ng/µl of sample
3. Transfer 15 µl of each normalized DNA sample to a separate microTUBE-15.
4. Centrifuge, using a microTUBE adapter, at 3000 × g for 1 minute.
5. Fragment the DNA on a Covaris using the appropriate settings for your instrument.

Table 2 Covaris S220 or E220 Settings

Setting	350 bp Insert	550 bp Insert
Duty factor	20%	
Peak Incident Power	18 W	
Cycles per burst	50	
Duration	45 seconds	22 seconds
Temperature	20°C	
Water Level—S220	15	
Water Level—E220	10	

Table 3 Covaris M220 Settings

Setting	350 bp Insert	550 bp Insert
Duty factor	20%	
Peak Incident Power	30 W	
Cycles per burst	50	
Duration	42 seconds	23 seconds
Temperature	20°C	

6 Centrifuge, using a microTUBE adapter, at 600 × g for 5 seconds.

7 Transfer 15 µl fragmented DNA from each microTUBE-15 to a separate well of a new PCR plate.

SAFE STOPPING POINT

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

Prepare Samples for Loading

This process describes how to perform size-selection using SC350 or SC550. Both 350 bp and 550 bp insert sizes can be included in a single run. DMB binds the DNA in preparation for loading onto the library card.

Consumables

- DMB (Digital Microfluidics Beads)
- SC350 (Sample Concentration Solution 350) for 350 bp inserts
- SC550 (Sample Concentration Solution 550) for 550 bp inserts
- 1.5 ml microcentrifuge tubes (1 per library insert size)
- 96-well 0.3 ml PCR plate
- Microseal 'B' adhesive seal
- RNase/DNase-free reagent reservoirs (2)

About Reagents

SC350 and SC550

- When running both 350 bp and 550 bp insert sizes on the same library card, use a separate, new 1.5 ml microcentrifuge tube for each solution.
- When creating separate mixtures for both 350 bp and 550 bp insert sizes, add the DMB volume specified to each tube containing SC.
- If you are creating separate mixtures for both 350 bp and 550 bp insert sizes, pour each mixture into a separate, new reagent reservoir.
- Make sure that the mixture added to the sample plate well contains the SC that corresponds to the desired library insert size.

Preparation

1. If the sample plate was stored, thaw it at room temperature, and then centrifuge at 280 × g for 1 minute.
2. Prepare the following consumables:

Item	Storage	Instructions
DMB	2°C to 8°C	Let stand for 10 minutes to bring to room temperature.
SC350	2°C to 8°C	Let stand for 10 minutes to bring to room temperature.
SC550	2°C to 8°C	Let stand for 10 minutes to bring to room temperature.

Procedure

1. Add 1 of the following to a new 1.5 ml microcentrifuge tube:
   - For a 350 bp insert size—900 µl SC350
   - For a 550 bp insert size—700 µl SC550
2. Vortex DMB until well-dispersed. Do not centrifuge DMB.
3. Add 100 µl DMB to the microcentrifuge tube containing SC350 or SC550. Vortex for 5 seconds.
4. Pour the DMB and SC mixture into a new reagent reservoir.

5 Add 35 µl DMB and SC mixture to each well of the sample plate. Pipette to mix.

6 Shake or vortex at 1400 rpm for 12 minutes.

Set Up Run and Load Library Card

This process describes how to set up a NeoPrep System run, which includes loading oil, samples, reagents, and adapters into the library card. Load the library card and start the run within 90 minutes.

The NeoPrep Control Software guides you through the steps to set up a run and load the library card. Use the procedures in this section as a reference. For more information on the NeoPrep Control Software, see the *NeoPrep Library Prep System Guide (document # 15049720)*.

Consumables

- Library card
- Library card guide
- Oil vial
- Oil funnel
- DMB (Digital Microfluidics Beads)
- TruSeq Nano DNA - NeoPrep reagent plate
- [Optional] RSB (Resuspension Buffer)

WARNING

The reagent plate contains hazardous materials. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and a laboratory coat. Handle the used reagent plate as chemical waste. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at support.illumina.com/sds.html.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
Reagent plate	-25°C to -15°C	Let stand for 15 minutes to bring to room temperature.
RSB	2°C to 8°C	If stored, let stand for 10 minutes to bring to room temperature.

Set Up the Run

1 Vortex the reagent plate for 3 seconds.

2 Centrifuge at 600 × g for 5 seconds.
If you are not using the reagent plate immediately, set aside on ice.

3 Select **Prepare Libraries** on the NeoPrep System Welcome screen.

4 Do the following and then select **Next**.
- If running in BaseSpace mode, select a run.
- If running in standalone mode, use the following options to select a protocol:
  - Select **Select by barcode**, and then scan the reagent plate barcode or enter the reagent plate serial number.
  - Select **Select by name**, and then select **TruSeq Nano DNA**.

5 Configure the run. Select **Next**.
For more information, see *Configure the Run* in the *NeoPrep Library Prep System Guide (document # 15049720)*.
- The following processes are available:
  - **Prep Library**—Prepares libraries and must be selected.
  - [Optional] **Quantify**—Quantifies samples during the run, after library prep is complete.
  - [Optional] **Normalize**—Normalizes the final libraries to 10 nM, after quantification is complete. This option can only be selected if Quantify is also selected.
- Configuration options for TruSeq Nano DNA are as follows:

Table 4 Configuration Options

Parameter	Default	Options
Sample Count	16	1–16
PCR Cycles	6	4–15
Insert Size (bp)	No default	350, 550, Mixed

NOTE
Only the default PCR Cycles setting is supported.

  - Select the Insert Size. If the Mixed Insert Size option is selected, the insert size for each sample must be specified when confirming the run settings in step 6.

6 Review the run and sample information. Select **Next**.
For more information, see *Confirm Run* in the *NeoPrep Library Prep System Guide (document # 15049720)*.
If the Mixed Insert Size option was selected for the run configuration, the insert size must be selected for each sample in the Sample Info tab.

7 Enter the consumable tracking information. Select **Next**.
For more information, see *Track Consumables* in the *NeoPrep Library Prep System Guide (document # 15049720)*.

8 Open the library card compartment door, slide the latch release to the right, and then place the library card on the library card stage.

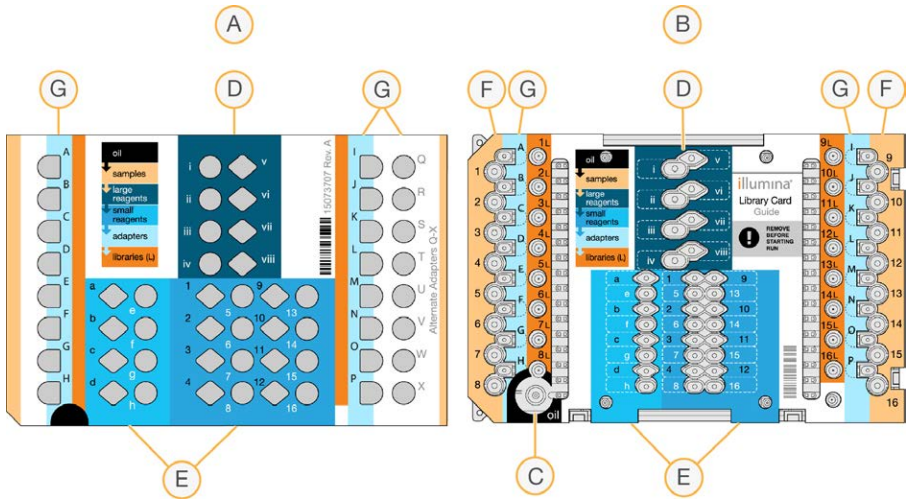
WARNING
To avoid instrument damage, make sure that the library card guide is not on the library card.

9 Close the library card compartment door. Select **Verify Library Card**. Do not open the compartment door during library card verification.

Load the Library Card

1 When library card verification is complete, open the library card compartment door and place the library card guide on the library card.

Figure 4 Reagent Plate to Library Card Transfer Layout



- **A** Reagent plate
- **B** Library card
- **C** Oil
- **D** Large reagents
- **E** Small reagents
- **F** Samples
- **G** Adapters

2 Load the entire contents of the oil vial into the library card using the oil funnel. Wait 3 minutes for the oil to drain. Change your gloves after loading the oil.

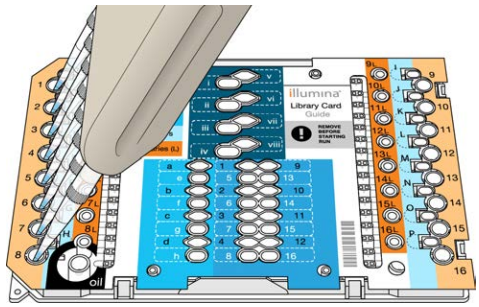
WARNING

Use the pipette tips specified in *Pipette and Tip Requirements* on page 7 and *Consumables and Equipment* on page 31. Other tips are not supported and can result in reagents not dispensing properly and run failure.

The loading angle of the pipette depends on the item being dispensed. The angle is specified in each step of the control software loading guide and is depicted in these procedures.

3 Transfer 45 µl of prepared samples 1–8.

Figure 5 Loading Samples 1–8



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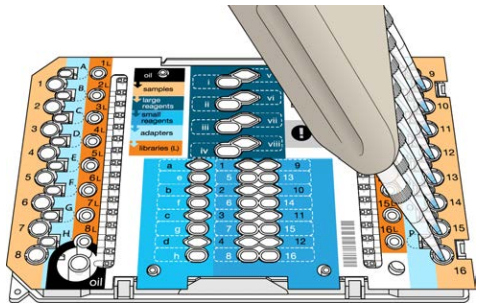
4 Transfer 45 µl of prepared samples 9–16.

Figure 6 Loading Samples 9–16

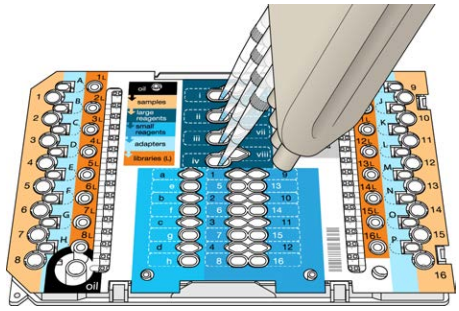


5 If you are preparing < 16 samples, add 45 µl RSB to empty sample wells.

NOTE
If you are preparing < 9 samples, the NeoPrep Control Software does not provide the RSB loading instructions for wells 9–16. Add 45 µl RSB to each empty sample well 9–16.

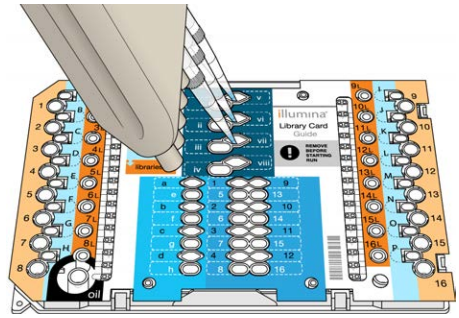
6 Transfer 125 µl of the large reagents i–iv.

Figure 7 Loading Large Reagents i–iv



7 Transfer 125 µl of the large reagents v–vii.

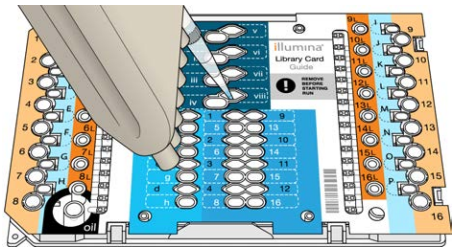
Figure 8 Loading Large Reagents v–vii



8 Vortex DMB until well-dispersed. Do not centrifuge DMB.

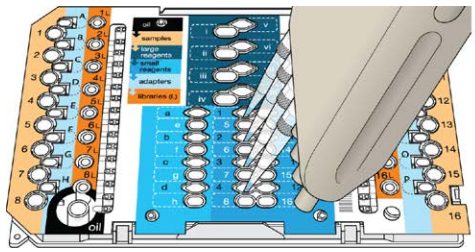
9 Add 60 µl DMB to the large reagent well viii.

Figure 9 Loading Large Reagent viii



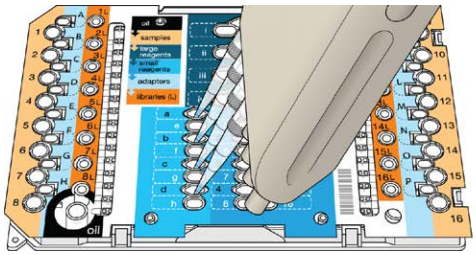
10 Transfer 15 µl of small reagents 1–4, and then 5–8.

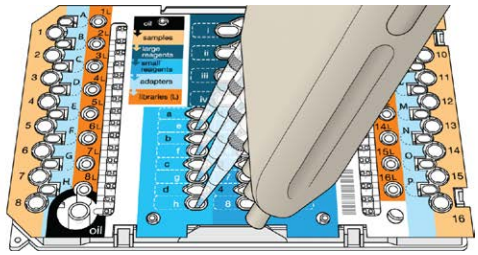
Figure 10 Loading Small Reagents 1–4, 5–8



11 Transfer 5 µl of small reagents a–d, and then e–h.

Figure 11 Loading Small Reagents a–d, e–h



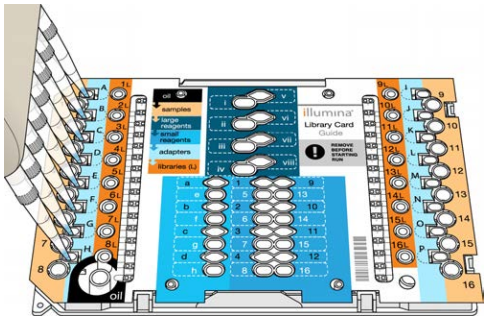


NOTE

For steps 12 and 13, if you are not using the default index adapter layout, the control software specifies which adapter to transfer to each library card well.

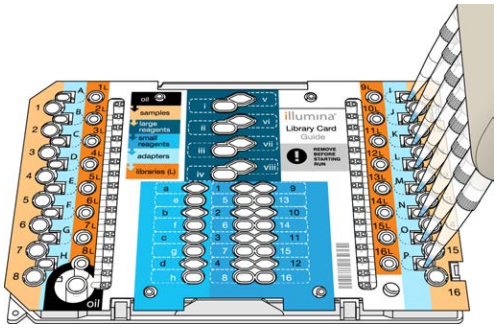
12 Transfer 3 µl of adapters A–H.

Figure 12 Loading Adapters A–H



13 Transfer 3 µl of adapters I–P.

Figure 13 Loading Adapters I–P



NOTE
If you are preparing < 9 samples, the NeoPrep Control Software does not provide the adapter loading instructions for adapters I–P. Transfer 3 µl of adapters I–P.

14 Remove the library card guide. Keep it for later use during the unloading process.

WARNING
To avoid instrument damage, make sure that the library card guide is removed from the library card.

15 Close the library card compartment door. Select **Start Run**. Do not open the compartment door until the run is complete.

16 When the run is complete, select **Next**. Libraries can remain at room temperature on a library card for up to 3 days after a run is complete.

Unload Libraries

This process describes how to collect libraries from the library card, separate libraries from the oil, and unload the library card from the instrument.

The NeoPrep Control Software guides you through the steps to unload the library card. Use the procedures in this section as a reference. For more information on the NeoPrep Control Software, see the *NeoPrep Library Prep System Guide (document # 15049720)*.

Consumables

- RSB (Resuspension Buffer)
- Library card guide
- Library separation tube strips (2)
- 96-well 0.3 ml PCR plates (2)
- Microseal 'B' adhesive seals

WARNING

The used library card contains hazardous materials. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and a laboratory coat. Handle the used library card as chemical waste. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at support.illumina.com/sds.html.

Preparation

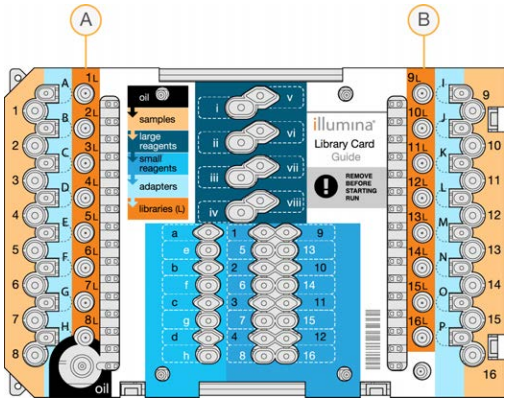
1. Remove RSB from 2°C to 8°C storage and bring to room temperature.
2. Label wells of 2 new PCR plates 1–16.
3. Label tubes of a library separation tube strip 1–8 and another library separation tube strip 9–16.

Procedure

1. Add 10 µl RSB to each well of a new PCR plate labeled 1–16.
2. Open the library card compartment door and place the library card guide on the library card.

3 Use a 200 µl pipette to transfer 20 µl from library card collection wells 1L–8L, and then 9L–16L to corresponding wells 1–16 of the plate. Pipette to mix.

Figure 14 Library Card Collection Wells



A Collection wells 1L–8L
B Collection wells 9L–16L

4 Centrifuge briefly.

5 Transfer the entire volume from plate wells 1–8, and then 9–16 to the center indent in the membrane of the corresponding library separation tubes 1–16.

6 Let stand for 10 seconds while the oil is absorbed in the tubes.

7 Transfer the entire volume from library separation tubes 1–8, and then 9–16 to the corresponding wells 1–16 of a new PCR plate.

8 Remove the library card and library card guide from the library card stage.

9 Discard the library card in accordance with applicable standards.

10 Close the library card compartment door, and then select **Home**.

11 Select from the following options:

Table 5 Post Run Options

NeoPrep System Quantification	NeoPrep System Normalization	Pooling Required	Then...
Yes	Yes	No	The protocol stops here. The final library is normalized to 10 nM. Proceed to cluster generation. For more information, see the system guide for your Illumina sequencing platform.
Yes	Yes	Yes	Proceed to *Pool Libraries*.
Yes	No	Yes or No	Proceed to *[Optional] Normalize Libraries Manually*.
No	No	Yes or No	Proceed to *[Optional] Validate Libraries Manually*.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

[Optional] Validate Libraries Manually

If quantification was not performed by the NeoPrep System, perform quantification of your DNA libraries and quality control analysis.

Consumables

- One of the following for quantification:
  - KAPA Library Quantification Kit
  - Fluorometric quantification with dsDNA binding dye reagents
- One of the following for quality check:
  - Agilent DNA 7500 Kit
  - Agilent High Sensitivity DNA Kit

Quantify Libraries

If quantification was performed by the NeoPrep System, you do not need to perform this process.

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 a fluorometric quantification method that uses dsDNA binding dyes or qPCR.

TruSeq Nano DNA Library Prep for NeoPrep library quantification has been validated using the KAPA Library Quantification Kit specified in the *Consumables and Equipment* on page 31. Follow the KAPA instructions with the KAPA standard. To calculate the library concentration in nM, perform the following insert size adjustment:

- For 350 bp libraries, use 470 bp for the average fragment length
- For 550 bp libraries, use 670 bp for the average fragment length

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

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 with water.
     - Run 1 µl diluted DNA library.
   - If using a DNA 7500 chip, run 1 µl undiluted DNA library.

Figure 15 Example 350 bp Insert Library Distribution

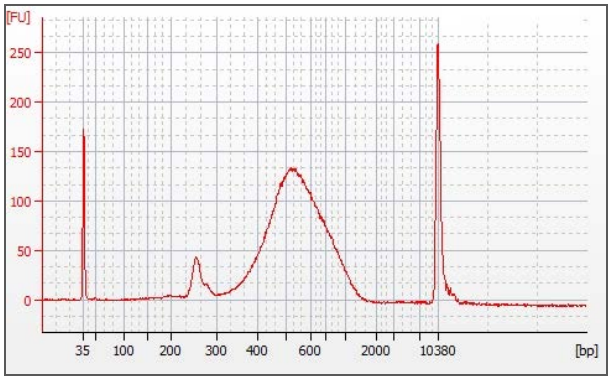
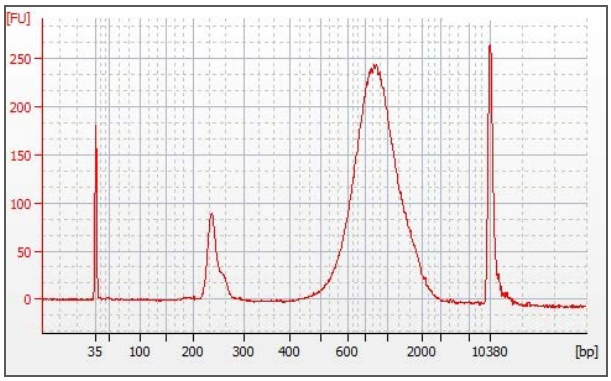
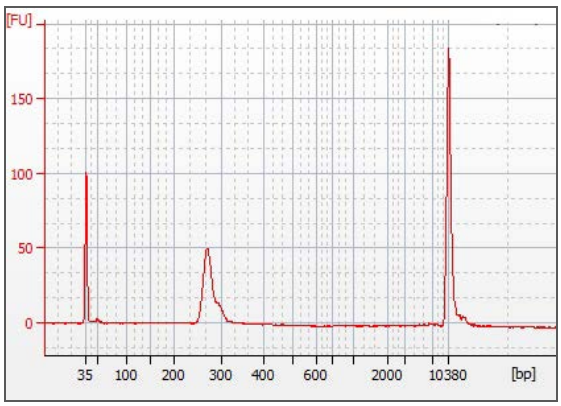


Figure 16 Example 550 bp Insert Library Distribution



A blue dye is used in the TruSeq Nano DNA Library Prep for NeoPrep reagents to aid in loading and collection. The dye appears as a characteristic peak at 200–250 bp and is not indicative of issues with the final library. Figure 17 shows the distribution of only the blue dye.

Figure 17 Reagent Blue Dye Distribution



SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

[Optional] Normalize Libraries Manually

This process describes how to prepare DNA libraries for cluster generation when normalization was not performed by the NeoPrep System. DNA libraries are normalized to 10 nM.

Do not perform this process if normalization was performed by the NeoPrep System.

Consumables

- 96-well midi plate
- Microseal 'B' adhesive seal
- Tris-HCl 10 mM, pH8.5 with 0.1% Tween 20

Preparation

1. If the DNA library plate was stored, thaw it at room temperature, and then centrifuge at 280 × g for 1 minute.
2. Label wells of a new 96-well midi plate 1–16.

Procedure

1. Transfer 5 µl from each well of the library plate to the corresponding wells of a midi plate.
2. Normalize each library to 10 nM with Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Pipette to mix.
   Depending on the quantification yield data of each sample library, the final volume in the plate can vary from 5–250 µl.
3. Select from the following options:
   - For libraries that do not require pooling, the protocol stops here. Proceed to cluster generation.
   - For libraries that require pooling, proceed to *Pool Libraries*.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Pool Libraries

This process describes how to pool normalized DNA libraries in equal volumes. Do not perform this process if you are not pooling libraries.

Consumables

- 96-well 0.3 ml PCR plate
- Microseal 'B' adhesive seal

Procedure

1. Determine the number of samples to combine for each pool. Do not pool samples with the same index.
2. Transfer 5 µl of each library to be pooled from the library plate to a single well of a new PCR plate. Pipette to mix.
   The total volume in each well is 5 times the number of combined sample libraries. For example, the volume for 2 samples is 10 µl, the volume for 12 samples is 60 µl, or the volume for 16 samples is 80 µl.
3. 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 for up to 2 months.

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.

TruSeq Nano DNA Library Prep Kit for NeoPrep Kit

Make sure that you have the items identified in this section in your TruSeq Nano DNA Library Prep Kit for NeoPrep (catalog # NP-101-1001) before starting the protocol. The kit contains 3 boxes.

Box 1, Store at 15°C to 30°C

Quantity	Description
2	Library separation tube strips
1	NeoPrep Oil vial
1	Oil funnel
1	Library Card Guide
1	NeoPrep Library Card, 16-samples

Box 2, Store at -25°C to -15°C

This box contains a TruSeq Nano DNA Library Prep for NeoPrep reagent plate covered by a reagent plate guide.

Reagent Plate Contents

The TruSeq Nano DNA Library Prep for NeoPrep reagent plate is a single-use consumable. It consists of a 96-well foil-sealed plate prefilled with library prep adapters and reagents for a single TruSeq Nano DNA Library Prep for NeoPrep run.

Figure 18 Reagent Plate

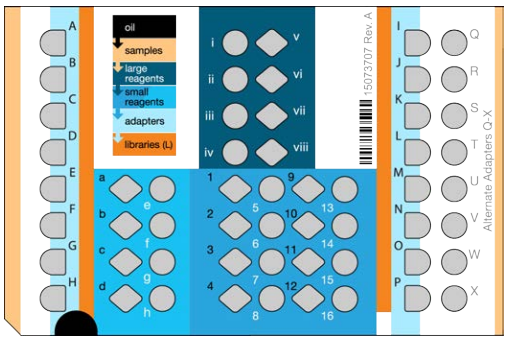


Table 6 Default Adapters

Well	Reagent	Description	Well	Reagent	Description
A	ND006	Adapter Index 6	I	ND001	Adapter Index 1
B	ND013	Adapter Index 13	J	ND010	Adapter Index 10
C	ND012	Adapter Index 12	K	ND020	Adapter Index 20
D	ND014	Adapter Index 14	L	ND008	Adapter Index 8
E	ND005	Adapter Index 5	M	ND025	Adapter Index 25
F	ND015	Adapter Index 15	N	ND011	Adapter Index 11
G	ND019	Adapter Index 19	O	ND018	Adapter Index 18
H	ND021	Adapter Index 21	P	ND023	Adapter Index 23

Table 7 Alternate Adapters

Well	Reagent	Description	Well	Reagent	Description
Q	ND002	Adapter Index 2	U	ND003	Adapter Index 3
R	ND004	Adapter Index 4	V	ND009	Adapter Index 9
S	ND007	Adapter Index 7	W	ND022	Adapter Index 22
T	ND016	Adapter Index 16	X	ND027	Adapter Index 27

NOTE

The large and small reagents and adapters are not the same as manual TruSeq library prep reagents and adapters. Do not use these reagents for manual TruSeq library prep and do not use the reagents and adapters in the manual TruSeq library prep kits for NeoPrep library prep.

Table 8 Large Reagents

Well	Reagent	Description
i	BWS2	Bead Wash Solution
ii	ESL	Elution Solution
iii	BBS	Bead Binding Solution
iv	QDR	Quant Dye Reagent
v	BWS2	Bead Wash Solution
vi	ESL	Elution Solution
vii	BBS	Bead Binding Solution

Table 9 Small Reagents

Well	Reagent	Description	Well	Reagent	Description
a	FAM	FAM Dye	5	EPM2	Enhanced PCR Mix
b	QSD5	Quant Standard 5	6	EPM2	Enhanced PCR Mix
c	QSD3	Quant Standard 3	7	PPC2	PCR Primer Cocktail
d	QSD1	Quant Standard 1	8	ESL	Elution Solution
e	QSD6	Quant Standard 6	9	–	Empty
f	QSD4	Quant Standard 4	10	–	Empty
g	QSD2	Quant Standard 2	11	–	Empty
h	FAM	FAM Dye	12	–	Empty
1	ESL	Elution Solution	13	–	Empty
2	LIG4	Ligation Mix	14	–	Empty
3	ATL3	A-Tailing Mix	15	–	Empty
4	ERP4	End Repair Mix	16	–	Empty

Box 3, Store at 2°C to 8°C

Quantity	Reagent	Description
1	DMB	Digital Microfluidics Beads
1	RSB	Resuspension Buffer
1	SC350	Sample Concentration Solution 350
1	SC550	Sample Concentration Solution 550

Consumables and Equipment

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

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

Pipettes and Tips

Use the pipettes and tips specified. Other pipettes and tips are not supported and can result in reagents not dispensing properly and run failure.

Pipettes and Tips	Supplier
Pipet-Lite XLS+ 8-channel LTS, 2 µl to 200 µl	Rainin, catalog # L8-20XLS+
One of the following 20 µl pipette tips: • LTS tips 20 µl. Presterilized. Filter • ART Barrier Pipette Tips 20 µl; 20 µl SoftFit-L	• Rainin, catalog # RT-L10F • Fisher Scientific, catalog # 2749RI
Pipet-Lite XLS+ 8-channel LTS, 20 µl to 200 µl	Rainin, catalog # L8-200XLS+
One of the following 200 µl pipette tips: • LTS tips 200 µl. Presterilized. Filter • ART Barrier Pipette Tips 200 µl; 200 µl SoftFit-L	• Rainin, catalog # RT-L200F • Fisher Scientific, catalog # 2769RI

Consumables

Consumable	Supplier
1.5 ml microcentrifuge tube	General lab supplier
1000 µl barrier pipette tips	General lab supplier
96-well 0.3 ml skirtless PCR plates, or Twin.tec 96-well PCR plates	E&K Scientific, part # 480096, or Eppendorf, part # 951020303
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
microTUBE-15 AFA Beads Screw-Cap (25)	Covaris, part # 520145
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
[Optional] Positive control DNA	Coriell Institute, part # NA 12878
[Optional - for starting material quality assessment] Fluorometric quantification with dsDNA binding dye reagents	General lab supplier
[Optional - for manual library quantification] One of the following: • Fluorometric quantification with dsDNA binding dye reagents • KAPA Library Quantification Kit - Illumina/Universal	• General lab supplier • KAPA Biosystems, part # KK4824

Consumable	Supplier
[Optional - for library quality control] One of the following: • Agilent DNA 7500 Kit • Agilent High Sensitivity DNA Kit	Agilent, part # • 5067-1506 • 5067-4626
[Optional - for manual normalization] 96-well storage plates, round well, 0.8 ml (midi plate)	Fisher Scientific, part # AB-0859
[Optional - for manual normalization] Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20	General lab supplier

Equipment

Equipment	Supplier
One of the following Covaris Focused-ultrasonicators: • S220 • E220 • M220	Covaris M220, part # 500295 For all other models, contact Covaris
One of the following, depending on your Covaris Focused-ultrasonicator: • M220 NGS microTUBE-15 Starter Kit • S220 NGS microTUBE-15 Starter Kit • E220 NGS microTUBE-15 Starter Kit	Covaris, part #: • 500417 • 500418 • 500419
NeoPrep Library Prep System	Illumina, catalog # SE-601-1001
Microplate centrifuge	General lab supplier
Vortexer or microplate shaker	General lab supplier
[Optional - for starting material quality assessment and manual library quantification] Fluorometer for quantification with dsDNA binding dyes	General lab supplier
[Optional - for manual library quantification] qPCR system	General lab supplier
[Optional - for library quality control] 2100 Bioanalyzer Desktop System	Agilent, part # G2940CA

Index Adapter Sequences

The TruSeq Nano DNA Library Prep Kit for NeoPrep contains the following index adapter sequences.

- The indexes are TruSeq LT single-index adapters.
- 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 10 Indexed Adapter Sequences

Adapter	Sequence		Adapter	Sequence
ND0001	ATCACG(A)		ND0013	AGTCAA(C)
ND0002	CGATGT(A)		ND0014	AGTTCC(G)
ND0003	TTAGGC(A)		ND0015	ATGTCA(G)
ND0004	TGACCA(A)		ND0016	CCGTCC(C)
ND0005	ACAGTG(A)		ND0018	GTCCGC(A)
ND0006	GCCAAT(A)		ND0019	GTGAAA(C)
ND0007	CAGATC(A)		ND0020	GTGGCC(T)
ND0008	ACTTGA(A)		ND0021	GTTTCG(G)
ND0009	GATCAG(A)		ND0022	CGTACG(T)
ND0010	TAGCTT(A)		ND0023	GAGTGG(A)
ND0011	GGCTAC(A)		ND0025	ACTGAT(A)
ND0012	CTTGTA(A)		ND0027	ATTCCT(T)

Acronyms

Acronym	Definition
ATL	A-Tailing Mix
BBS	Bead Binding Solution
BWS	Bead Wash Solution
DMB	Digital Microfluidics Beads
EPM	Enhanced PCR Mix

Acronym	Definition
ERP	End Repair Mix
ESL	Elution Solution
FAM	FAM Dye
LIG	Ligation Mix
PPC	PCR Primer Cocktail
QDR	Quant Dye Reagent
QSD	Quant Standard
RSB	Resuspension Buffer
SC350	Sample Concentration Solution 350
SC550	Sample Concentration Solution 550

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 11 Illumina General Contact Information

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

Table 12 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 (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**.

Material # 20000943

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