NextSeq System

Denature and Dilute Libraries Guide

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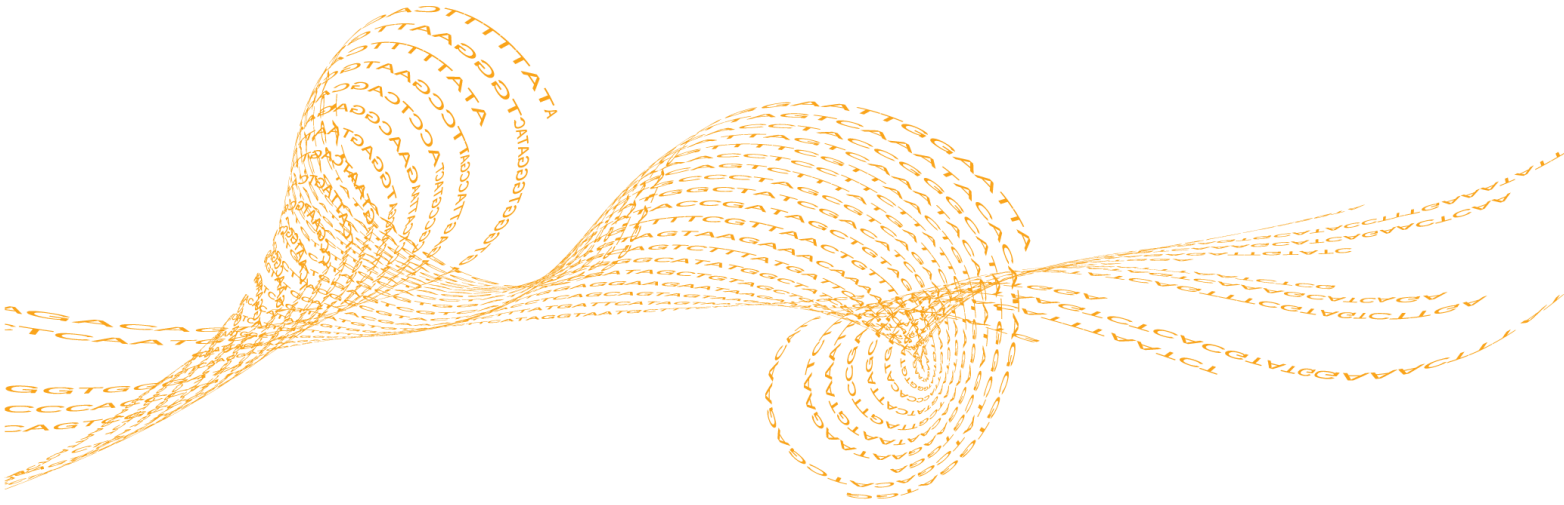

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Overview

This guide explains how to denature and dilute prepared libraries for sequencing on the Illumina® NextSeq® system.

This guide includes instructions for preparing a PhiX library for the following purposes:

- **For a control**—Prepare a PhiX library to combine with prepared libraries for use as a sequencing control. See *Denature and Dilute PhiX Control* on page 8.
- **For troubleshooting**—Prepare a PhiX library for a PhiX-only sequencing run for troubleshooting purposes. See *Prepare PhiX for a Troubleshooting Run* on page 11.

Loading Volume and Concentration

This procedure denatures and dilutes libraries to a final loading volume of 1.3 ml at a recommended concentration of 1.8 pM. In practice, loading concentration can vary depending on library preparation and quantification methods.

Protocol Variations

Follow the appropriate denature and dilute protocol depending on the normalization procedure used during library prep.

- **Standard normalization**—Libraries are normalized using standard library quantification and quality control procedures recommended in the library prep documentation. For these libraries, follow **Protocol A**. See *Protocol A: Standard Normalization Method* on page 5.
- **Bead-based normalization**—Libraries are normalized using a bead-based procedure described in the library prep documentation for methods that support bead-based normalization. For these libraries, follow **Protocol B**. See *Protocol B: Bead-Based Normalization Method* on page 7.

Consumables and Equipment

Consumables

The following consumables are required to denature and dilute libraries and prepare a PhiX control.

Consumables	Kit Name
HT1 (Hybridization Buffer)	Component of the NextSeq 500/550 Kit

User-Supplied Consumables	Supplier
1 N NaOH, molecular biology-grade	General lab supplier
200 mM Tris-HCl, pH 7.0	General lab supplier

The following additional consumables are required to prepare a PhiX control.

Consumables	Kit Name
PhiX, 10 nM RSB (Resuspension Buffer)	Illumina, catalog # FC-110-3002

Equipment

The following equipment is used to denature libraries that have been normalized using a bead-based method.

Equipment	Supplier
Hybex Microsample Incubator	SciGene, catalog # 1057-30-O (115 V), or equivalent SciGene, catalog # 1057-30-2 (230 V), or equivalent
Block for 1.5 ml microcentrifuge tubes	SciGene, catalog # 1057-34-0, or equivalent

Best Practices

- ***Always*** prepare freshly diluted NaOH for denaturing libraries for cluster generation. This step is essential to the denaturation process.
- To prevent small pipetting errors from affecting the final NaOH concentration, prepare at least 1 ml of freshly diluted NaOH.
- For best results, begin thawing reagents before denaturing and diluting libraries. For instructions, see the system guide for your instrument.

Protocol A: Standard Normalization Method

Use protocol A to denature and dilute libraries that have been normalized using standard library quantification and quality control procedures recommended in the library prep documentation.

NOTE
Typically, it is important that not more than 1 mM NaOH is in the final solution after diluting with HT1. However, introducing 200 mM Tris-HCl ensures that the NaOH is fully hydrolyzed in the final solution. As a result, template hybridization is not affected even if the final NaOH concentration after diluting with HT1 is greater than 1 mM.

Prepare Reagents

Prepare a Fresh Dilution of NaOH

1. Combine the following volumes in a microcentrifuge tube:
   - Laboratory-grade water (800 µl)
   - Stock 1.0 N NaOH (200 µl)
2. Invert the tube several times to mix.

NOTE
Use the fresh dilution within **12 hours**.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Store at 2°C to 8°C until you are ready to dilute denatured libraries.

Prepare RSB

NOTE
In place of RSB, you can use 10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20.

1. Remove the tube of RSB from -25°C to -15°C storage and thaw at room temperature.
2. Store thawed RSB at 2°C to 8°C until you are ready to dilute libraries.

Denature Libraries

1. Combine the following volumes of library and freshly diluted 0.2 N NaOH in a microcentrifuge tube.

Starting Library Concentration	Library	0.2 N NaOH
4 nM	5 µl	5 µl
2 nM	10 µl	10 µl
1 nM	20 µl	20 µl
0.5 nM	40 µl	40 µl

2. Vortex briefly and then centrifuge at 280 × g for 1 minute.
3. Incubate at room temperature for 5 minutes.
4. Add the following volume of 200 mM Tris-HCl, pH 7.

Starting Library Concentration	200 mM Tris-HCl, pH 7
4 nM	5 µl
2 nM	10 µl
1 nM	20 µl
0.5 nM	40 µl

5 Vortex briefly and then centrifuge at 280 × g for 1 minute.

Dilute Denatured Libraries to 20 pM

1 Add the following volume of prechilled HT1 to the tube of denatured libraries.

Starting Library Concentration	Prechilled HT1
4 nM	985 µl
2 nM	970 µl
1 nM	940 µl
0.5 nM	880 µl

The result is a 20 pM denatured library.

2 Vortex briefly and then centrifuge at 280 × g for 1 minute.

3 Place the 20 pM libraries on ice until you are ready to proceed to final dilution.

Dilute Libraries to Loading Concentration

1 Dilute the denatured 20 pM library solution to 1.8 pM as follows.
- Denatured library solution (117 µl)
- Prechilled HT1 (1183 µl)

The total volume is 1.3 ml at 1.8 pM.

2 Invert to mix and then pulse centrifuge.

3 If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control* on page 8. Otherwise, see *Next Steps* on page 10.

Protocol B: Bead-Based Normalization Method

Use protocol B to denature and dilute libraries that have been normalized and pooled using a bead-based procedure described in the library prep documentation for methods that support bead-based normalization.

Bead-based normalization procedures can be variable. Depending upon library type and experience, 2–5 µl of library produces optimal results.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Store at 2°C to 8°C until you are ready to dilute denatured libraries.

Prepare Incubator

1. Preheat the incubator to 98°C.

Dilute Library to Loading Concentration

1. Combine the following volumes of pooled libraries and prechilled Hybridization Buffer in a microcentrifuge tube.

Library Pool	Prechilled Hybridization Buffer
2 µl	998 µl
3 µl	997 µl
4 µl	996 µl
5 µl	995 µl

   The total volume is 1 ml.

2. Vortex briefly and then centrifuge at 280 × g for 1 minute.
3. Transfer 750 µl diluted library to a new microcentrifuge tube.
4. Add 750 µl prechilled Hybridization Buffer.
5. Vortex briefly and then centrifuge at 280 × g for 1 minute.

Denature Diluted Library

1. Place the tube on the preheated incubator for 2 minutes.
2. Immediately cool on ice.
3. Leave on ice for 5 minutes.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control* on page 8. Otherwise, see *Next Steps* on page 10.

Denature and Dilute PhiX Control

Use the following procedure to denature and dilute a PhiX library for use as a sequencing control.

Dilute PhiX to 4 nM

1. Thaw a tube of 10 nM PhiX stock (10 µl/tube).
2. Combine the following volumes in a microcentrifuge tube.
   - 10 nM PhiX (10 µl)
   - RSB (15 µl)

   The total volume is 25 µl at 4 nM.
3. Vortex briefly and then pulse centrifuge.

NOTE
[Optional] Store the 4 nM PhiX at -25°C to -15°C for up to 3 months.

Denature PhiX

1. Combine the following volumes in a microcentrifuge tube.
   - 4 nM PhiX (5 µl)
   - 0.2 N NaOH, freshly diluted (5 µl)
2. Vortex briefly, and then pulse centrifuge.
3. Incubate at room temperature for 5 minutes.
4. Add 5 µl 200 mM Tris-HCl, pH 7.0.
5. Vortex briefly and then centrifuge at 280 × g for 1 minute.

Dilute Denatured PhiX to Loading Concentration

1. Add 985 µl of prechilled HT1 to the tube of denatured PhiX.
   The total volume is 1 ml at 20 pM.
2. Dilute the denatured 20 pM PhiX to 1.8 pM as follows.
   - Denatured PhiX (117 µl)
   - Prechilled HT1 (1183 µl)

   The total volume is 1.3 ml at 1.8 pM.
3. Invert to mix and then centrifuge at 280 × g for 1 minute.

NOTE
[Optional] Store the denatured 1.8 pM PhiX at -25°C to -15°C for up to 2 weeks. After 2 weeks, cluster numbers tend to decrease.

Combine Library and PhiX Control

For most libraries use a low-concentration PhiX control spike-in at 1% as a sequencing control.

1. Combine the following volumes of denatured PhiX control and denatured library.

Library and Concentration (Using 1.8 pM PhiX)	Volume
Denatured and diluted PhiX control at 1.8 pM	13 µl
Denatured and diluted library (from protocol A or protocol B)	1287 µl

Library and Concentration (Using 20 pM PhiX)	Volume
Denatured and diluted PhiX control at 20 pM	1.2 µl
Denatured and diluted library (from protocol A or protocol B)	1299 µl

2 Set aside on ice until you are ready to load it onto the reagent cartridge.

NOTE

The library and PhiX mixture provides a PhiX spike-in of 0.5%–2.0%. Actual PhiX percentage varies depending upon the quality and quantity of the library pool.

Next Steps

After denaturing and diluting your libraries and preparing the optional PhiX control, you are ready to load libraries onto the reagent cartridge and set up the sequencing run. See the *NextSeq 500 System Guide (document # 15046563) or NextSeq 550 System Guide (document # 15069765)*.

Prepare PhiX for a Troubleshooting Run

Use the following procedure to denature and dilute a PhiX library for use as a PhiX-only sequencing run. Performing a PhiX-only run is helpful in confirming instrument performance or for troubleshooting purposes. A PhiX-only run requires 100% PhiX library at recommended volumes and loading concentration.

Before proceeding, prepare reagents as described in *Prepare Reagents* on page 5.

Dilute PhiX to 4 nM

1. Thaw a tube of 10 nM PhiX stock (10 µl/tube).
2. Combine the following volumes in a microcentrifuge tube.
   - 10 nM PhiX (10 µl)
   - RSB (15 µl)

   The total volume is 25 µl at 4 nM.
3. Vortex briefly and then pulse centrifuge.

NOTE
[Optional] Store the 4 nM PhiX at -25°C to -15°C for up to 3 months.

Denature PhiX

1. Combine the following volumes in a microcentrifuge tube.
   - 4 nM PhiX (5 µl)
   - 0.2 N NaOH, freshly diluted (5 µl)
2. Vortex briefly, and then pulse centrifuge.
3. Incubate at room temperature for 5 minutes.
4. Add 5 µl 200 mM Tris-HCl, pH 7.0.
5. Vortex briefly and then centrifuge at 280 × g for 1 minute.

Dilute Denatured PhiX to Loading Concentration

1. Add 985 µl of prechilled HT1 to the tube of denatured PhiX.
   The total volume is 1 ml at 20 pM.
2. Dilute the denatured 20 pM PhiX to 1.8 pM as follows.
   - Denatured PhiX (117 µl)
   - Prechilled HT1 (1183 µl)

   The total volume is 1.3 ml at 1.8 pM.
3. Invert to mix and then centrifuge at 280 × g for 1 minute.
4. Set aside on ice until you are ready to load the library onto the reagent cartridge.

Revision History

Document	Date	Description of Change
Document # 15048776 v02	January 2016	Added procedure for denaturing and diluting libraries that have been normalized using a bead-based procedure. Organized procedures as Protocol A and Protocol B. Add instructions to dilute PhiX to 1.8 pM for use as a control.
Document # 15048776 v01	October 2015	Removed extra vortex and centrifuge steps from PhiX preparation instructions. Removed instructions for using NCS v1.2 software.
Part # 15048776 Rev. E	May 2015	Changed title to the NextSeq System Denature and Dilute Libraries Guide. This guide applies to the NextSeq 500 and NextSeq 550 systems.
Part # 15048776 Rev. D	October 2014	Corrected library volume to 2995 µl when combining libraries with a PhiX spike-in and when using NCS v1.2. Added information about performing a PhiX-only run for troubleshooting purposes.
Part # 15048776 Rev. C	September 2014	Updated URL for Safety Data Sheets (SDS) to support.illumina.com/sds.html. Updated NextSeq product markings from ™ to ®.
Part # 15048776 Rev. B	August 2014	Added instructions for preparing a library loading concentration of 1.8 pM, and reduced loading volume from of 1.3 ml. This change requires NCS v1.3. Corrected volumes for denaturing and diluting a 0.5 nM library. Updated URL for Safety Data Sheets (SDS) to support.illumina.com/sds.ilmn.
Part # 15048776 Rev. A	January 2014	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

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

Table 2 Illumina Customer Support Telephone Numbers

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

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

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

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