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NextSeq 1000 and 2000

Denature and Dilute Libraries Guide

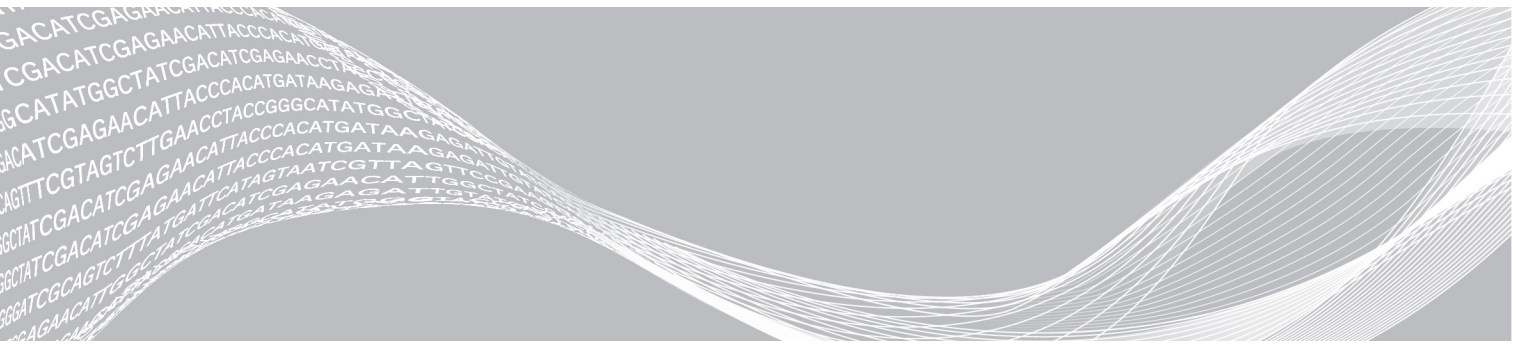

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Table of Contents

Revision History

Document #	Date	Description of Change
1000000139235 v01	November 2020	Removed 10mM Tris HCL pH 8.5.
1000000139235 v00	October 2020	Initial release.

Overview

This guide explains how to manually denature and dilute prepared libraries for sequencing on the Illumina® NextSeq™ 1000 and NextSeq™ 2000 Sequencing Systems. Manual denature and dilution is recommended for low concentration libraries that are unable to meet the recommended loading concentration and volume stated in the *NextSeq 1000 and NextSeq 2000 Sequencing System Guide (document # 1000000109376)*.

When initiating a run in the NextSeq 1000/2000 Control Software, make sure the Denature and Dilute On Board checkbox is not selected. See the *NextSeq 1000 and NextSeq 2000 Sequencing System Guide (document # 1000000109376)* for more information on run setup.

Loading Volume and Concentration

This procedure denatures and dilutes libraries to a final loading volume of 200 µl. Loading concentration can vary depending on library preparation and quantification methods.

Recommended Loading Concentrations

The optimal loading concentration depends on the library type and insert size. The following table provides the recommended final loading concentrations.

Library Type	Final Loading Concentration (pM)
AmpliSeq™ for Illumina Library PLUS	75
Illumina DNA Prep	75
Illumina DNA Prep with Enrichment	100
TruSeq DNA Nano 350	120
TruSeq DNA Nano 550	150
TruSeq Stranded mRNA	100
100 % PhiX	65
Illumina Stranded Total RNA with Ribo-Zero Plus	75
Illumina Stranded mRNA Prep	75
Illumina DNA PCR-Free	100

Consumables and Equipment

Consumables

The following consumables are required to denature and dilute libraries.

Consumables	Supplier
1 N NaOH	General lab supplier
400 mM Tris-HCl, pH 8.0	General lab supplier
Disposable gloves, powder-free	General lab supplier
Water, laboratory grade	General lab supplier
[Optional] PhiX Control v3	Illumina catalog # FC-110-3001

The following consumables for the denaturing and diluting libraries and PhiX are provided in the NextSeq 1000/2000 Reagents kits and Illumina library prep kits.

Consumables	Kit Name
HT1 (Hybridization Buffer)	Illumina provided in the NextSeq 1000/2000 Reagents kits
NextSeq 1000/2000 RSB with Tween 20	Illumina provided in the NextSeq 1000/2000 P2 (v3) Reagents kit and the NextSeq 2000 P3 Reagents kit.
Microcentrifuge tube, 1.5 ml	VWR, catalog # 20170-038, or equivalent

Equipment

The following equipment is used to denature libraries that have been normalized.

Equipment	Supplier
Centrifuge	General lab supplier
Vortexer	General lab supplier

Protocol

Prepare Reagents

Prepare a Fresh Dilution of NaOH

Prepare a fresh dilution of 0.2 N NaOH to denature libraries for sequencing. Extra volume is prepared to prevent small pipetting errors from affecting the final NaOH concentration.

1. Combine the following volumes in a microcentrifuge tube:
    - Laboratory-grade water (80 µl)
    - Stock 1.0 N NaOH (20 µl)

These volumes produce 100 µl 0.2 N NaOH.

2. Invert the tube several times to mix.

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, or on ice, until you are ready to dilute denatured libraries.

Dilute Libraries

1. Determine the required library concentration based on the desired final loading concentration.

Final Loading Concentration (pM)	Library Concentration (pM)
55	275
65	325
75	375
100	500
110	550
120	600
130	650
140	700
150	750

2. Dilute libraries to the desired library concentration using RSB with Tween 20 to a final volume of 40 µl.
3. [Optional] Add a PhiX Control.
   a. Combine the following volumes in a microcentrifuge tube to prepare PhiX to match the library concentration.
      - 10 nM PhiX (2 µl)
      - 10 mM RSB with Tween 20

   Use the following table to determine the volume (µl) of RSB with Tween 20 to use.

Initial PhiX Concentration (nM)	PhiX Volume (µl)	Library Concentration (pM)	RSB With Tween 20 Volume (µl)
10	2	275	70.7
10	2	325	59.5
10	2	375	51.3
10	2	500	38.0

Initial PhiX Concentration (nM)	PhiX Volume (µl)	Library Concentration (pM)	RSB With Tween 20 Volume (µl)
10	2	550	34.4
10	2	600	31.3
10	2	650	28.8
10	2	700	26.6
10	2	750	24.7
10	2	1000	18.0

b. Vortex briefly to mix, and then centrifuge at 280 x g for 1 minute.

c. Add 1 µl PhiX to 40 µl nondenatured library diluted to the desired library concentration.

These volumes produce a ~2% PhiX spike-in. Actual percentage varies depending on library quality and quantity.

Denature Libraries

1. Add 10 µl 0.2 N NaOH to the tube of 40 µl nondenatured library and optional PhiX.
   These volumes produce 50 µl denatured library, or 51 µl with PhiX.
2. Vortex briefly to mix, and then centrifuge at 280 × g for 1 minute.
3. Incubate at room temperature for 8 minutes.

Dilute Denatured Libraries

1. Add 10 µl 400 mM Tris-HCl, pH 8.5 to the tube of denatured library.
   These volumes produce 60 µl denatured library, or 61 µl with PhiX.
2. Vortex briefly to mix, and then centrifuge at 280 × g for 1 minute.

Dilute Denatured Libraries to Final Loading Concentration

1. Add 140 µl prechilled HT1.
   These volumes produce 200 µl final loading concentration, or 201 µl with PhiX.
2. Vortex to mix, and then centrifuge briefly.
3. Pierce the foil on the library reservoir.
4. Transfer the full volume of denatured and diluted library to the bottom of the cartridge reservoir provided with the NextSeq 1000/2000 P2 and P3 Reagents kits.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Product documentation—Available for download from support.illumina.com.

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