# Denaturing and Diluting Libraries for the NextSeq™ 500

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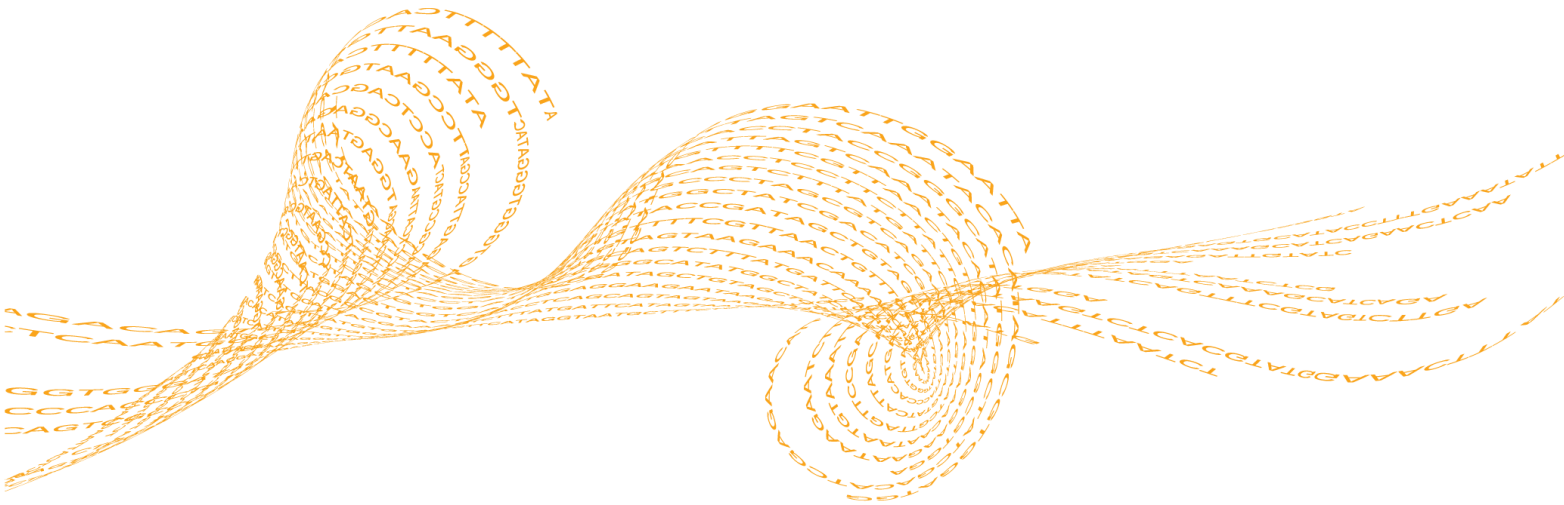


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# Introduction

This guide explains how to denature and dilute libraries after sample preparation to prepare them for sequencing on the Illumina® NextSeq™ 500.

This guide also explains how to prepare a PhiX control and combine libraries with the PhiX control before loading them onto the reagent cartridge.

NOTE

***The denaturation and dilution process is not necessary for all library types.*** Some library preparation protocols result in a ready-to-use normalized concentration of pooled libraries. For more information, see the documentation for the Illumina kit you used to prepare your libraries.

## Required Consumables

The following consumables are required to denature and dilute libraries.

| Illumina-Supplied Consumable | Kit Name |
|---|---|
| HT1 (Hybridization Buffer) | Component of the NextSeq 500 Kit |

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

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

| Illumina-Supplied Consumable | Kit Name |
|---|---|
| PhiX, 10 nM<br>RSB (Resuspension Buffer) | Illumina, catalog # FC-110-3002 |
| HT1 (Hybridization Buffer) | Component of the NextSeq 500 Kit |

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

## 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 0.2 N NaOH.

# Prepare Reagents

The following reagents are required to denature and dilute libraries for sequencing on the NextSeq.

- **0.2 N NaOH**—A fresh dilution of NaOH is required to denature libraries for cluster generation.
- **HT1**—HT1 (Hybridization Buffer) is used to dilute denatured libraries and dilute the PhiX control.
- **RSB**—RSB (Resuspension Buffer) is used to dilute the PhiX control.

## Prepare a Fresh Dilution of NaOH

1. Prepare 1 ml of 0.2 N NaOH by combining 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

A fresh dilution of 0.2 N NaOH is required for denaturing libraries and preparing a PhiX control. Set aside the remaining dilution for use within the next **12 hours**. Otherwise, discard the remaining dilution of 0.2 N NaOH.

## Prepare HT1

1. Remove the tube of HT1 from -15°C to -25°C storage and set aside at room temperature to thaw.
2. When thawed, store at 2°C to 8°C until you are ready to dilute denatured libraries.

## Prepare RSB

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

# Denature and Dilute Libraries

The loading concentration for sequencing on the NextSeq 500 is approximately 3 pM, depending on library preparation and quantification methods.

This procedure includes three variations for different starting library concentrations, either 1 nM, 2 nM, or 4 nM.

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 more than 1 mM NaOH is in the final solution after diluting with HT1.

## Denature Libraries

1 Combine the following volumes of libraries 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 | 120 µl | 120 µl |

2 Discard the remaining dilution of 0.2 N NaOH or set aside to prepare a PhiX control within the next 12 hours.

3 Vortex briefly to mix the library solution, and then centrifuge at 280 × g for 1 minute.

4 Incubate for 5 minutes at room temperature to denature libraries into single strands.

5 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 |

6 Vortex briefly to mix the library solution, and then centrifuge at 280 × g for 1 minute.

## Dilute Denatured Libraries to 20 pM

1 Add the following volume of pre-chilled HT1 to the tube of denatured libraries.

| Starting Library Concentration | Pre-chilled HT1 |
|---|---|
| 4 nM | 985 µl |
| 2 nM | 970 µl |
| 1 nM | 940 µl |
| 0.5 nM | 2640 µl |

2 Vortex briefly to mix the library solution, and then centrifuge at 280 × g for 1 minute. The result is a 20 pM denatured library.

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

## Dilute Denatured Libraries to 3 pM

The following steps are an example of diluting to a loading concentration, which in the following example is 3 pM. However, the appropriate loading concentration can vary depending on library preparation and quantification methods.

1 Dilute the denatured 20 pM library solution to a 3 pM loading concentration, as follows:
   - 450 µl Denatured library solution (450 µl)
   - 2550 µl Pre-chilled HT1 (2550 µl)

   The total volume is 3 ml at 3 pM.

2 Invert several times to mix and then pulse centrifuge the solution.

3 Place the solution on ice until you are ready to load libraries onto the reagent cartridge.

# Denature and Dilute PhiX Control

Use the following steps to denature and dilute a PhiX control library.

NOTE
For storage longer than two weeks, store the PhiX library at a concentration of 4–10 nM at -15° to -25°C. When libraries are stored at 20 pM, cluster numbers tend to decrease after two weeks of storage.

## Dilute PhiX Library from 10 nM to 4 nM

1. Thaw one tube of 10 nM PhiX stock (10 µl/tube).
2. In a 2 ml Eppendorf tube, combine the following volumes:
   - 10 nM PhiX (10 µl)
   - Resuspension Buffer (15 µl)

   The total volume is 25 µl at 4 nM.
3. Vortex briefly to mix the PhiX solution, and then pulse centrifuge.

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

## Denature PhiX Library and Dilute to 20 pM

1. In a 2 ml Eppendorf tube, combine the following volumes:
   - 4 nM PhiX library (5 µl)
   - 0.2 N NaOH, freshly diluted (5 µl)
2. Vortex briefly to mix the PhiX library solution, and then pulse centrifuge.
3. Incubate for 5 minutes at room temperature to denature the PhiX library into single strands.
4. After incubation, vortex briefly to mix the PhiX library solution.
5. Centrifuge at 280 × g for 1 minute.
6. Add 5 µl 200 mM Tris-HCl, pH7.
7. Vortex briefly and then centrifuge at 280 × g for 1 minute.
8. Discard the remaining dilution of 0.2 N NaOH.
9. Add 985 µl pre-chilled HT1. The total volume is 1 ml at 20 pM.
10. Vortex briefly to mix the solution.

NOTE
[Optional] Store the denatured 20 pM PhiX library up to two weeks at -15° to -25°C. After two weeks, cluster numbers tend to decrease.

# Combine Library and PhiX Control

Illumina recommends a low-concentration PhiX control spike-in at 1% for most libraries.

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

| | Most Libraries (1%) |
|---|---|
| Denatured and diluted PhiX control, 20 pM | 5 µl |
| Denatured and diluted sample library, ~ 3 pM | 2995 µl |

2 Set the combined library and PhiX control aside on ice until you are ready to load it onto the reagent cartridge.

# Next Steps



After denaturing and diluting your libraries and preparing the optional PhiX control, you are ready to load libraries onto the thawed reagent cartridge and set up the sequencing run. For complete instructions, see the *NextSeq 500 System User Guide (part # 15046563)*.

Visit the NextSeq 500 support pages on the Illumina support website for access to documentation, software downloads, frequently asked questions, and online training.

Notes

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

| | |
|---|---|
| **Illumina 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 | Italy | 800.874909 |
| Austria | 0800.296575 | Netherlands | 0800.0223859 |
| 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 www.illumina.com/msds.

## Product Documentation

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

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