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NextSeq 500 and NextSeq 550 Sequencing Systems

Denature and Dilute Libraries Guide

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Table of Contents

Overview

This guide explains how to denature and dilute prepared libraries for sequencing on the Illumina® NextSeq™ 500 or 550 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. Refer to *Denature and Dilute PhiX Control Using Protocols A, B, C, and D* on page 23, *Denature and Dilute PhiX Control Using Protocol E* on page 25, and *Denature and Dilute PhiX Control Using Protocol F and G* on page 27.
- **For troubleshooting**—Prepare a PhiX library for a PhiX-only sequencing run for troubleshooting purposes. Refer to *Prepare PhiX for a Troubleshooting Run* on page 29.

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 for high output kits and 1.5 pM for mid output kits. 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 procedure used during library prep. Use a library-specific normalization method if available, otherwise use the standard or bead-based normalization as appropriate.

- **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**. Refer to *Protocol A: Standard Normalization* on page 3.
- **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**. Refer to *Protocol B: Bead-Based Normalization* on page 5.
- **AmpliSeq™ for Illumina normalization**—For all libraries prepared using the standard AmpliSeq for Illumina workflow, follow **Protocol C**. Refer to *Protocol C: AmpliSeq for Illumina Panels Normalization Method* on page 6.
- **AmpliSeq Library Equalizer™ for Illumina normalization**—For all libraries prepared using the AmpliSeq Library Equalizer for Illumina workflow, follow **Protocol D**. Refer to *Protocol D: AmpliSeq Library Equalizer for Illumina Normalization Method* on page 9.
- **TruSight™ Tumor 170 Library Denaturation and Dilution**—For TruSight Tumor 170 libraries, follow **Protocol E**. Refer to *Protocol E: TruSight Tumor 170 Library Denaturation and Dilution Method* on page 11.

- **TruSight Oncology 500 Library Denaturation and Dilution**—For compatible TruSight Oncology 500 libraries, follow **Protocol F**. Refer to *Protocol F: TruSight Oncology 500 Library Denaturation and Dilution Method* on page 12.
- **TruSight Oncology 500 HRD Library Denaturation and Dilution**—For compatible TruSight Oncology 500 HRD libraries, follow **Protocol G**. Refer to *Protocol G: TruSight Oncology 500 HRD Library Denaturation and Dilution Method* on page 16.

Best Practices

- To prevent small pipetting errors from affecting the final NaOH concentration, prepare at least 1 ml freshly diluted NaOH.
- For best results, begin thawing reagents before denaturing and diluting libraries. For instructions, refer to the *NextSeq 500 System Guide (document # 15046563) or NextSeq 550 System Guide (document # 15069765)*.

Consumables and Equipment

Consumables

The following consumables are required to denature and dilute libraries.

Consumable	Supplier
HT1 (Hybridization Buffer)	Illumina, provided in the NextSeq 500/550 Kit
[Protocol C] Low TE	Illumina, provided in the AmpliSeq Library PLUS kit

User-Supplied Consumables	Supplier
1 N NaOH, molecular biology grade	General lab supplier
[Protocols A, B, C, and D] 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
[Protocols E, F, and G] HP3 (2 N NaOH)	Illumina, provided in library prep kit contents

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
Heat block for 1.5 ml microcentrifuge tubes	SciGene, catalog # 1057-34-0, or equivalent

Protocol A: Standard Normalization

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.

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 makes sure 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)

   The result is 1 ml 0.2 N NaOH.
2. Invert the tube several times to mix.

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

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

1. Remove 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 and 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

High Output Kits

1. Add the following volumes to dilute the denatured 20 pM library solution to 1.8 pM.
   - 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 Using Protocols A, B, C, and D* on page 23. Otherwise, refer to *Next Steps* on page 28.

Mid Output Kits

1. Add the following volumes to dilute the denatured 20 pM library solution to 1.5 pM.
   - Denatured library solution (97 µl)
   - Prechilled HT1 (1203 µl)

   The total volume is 1.3 ml at 1.5 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 Using Protocols A, B, C, and D* on page 23. Otherwise, refer to *Next Steps* on page 28.

Protocol B: Bead-Based Normalization

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 and do not have a library-specific normalization protocol.

Bead-based normalization procedures can be variable. Depending upon library type and experience, 2–5 µl 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 and Ice Bucket

1. Preheat the incubator to 98°C.
2. Prepare ice bucket.

Dilute Library to Loading Concentration

1. Combine the following volumes of pooled libraries and prechilled HT1 in a microcentrifuge tube.

Library Pool	Prechilled HT1
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 HT1.
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 Using Protocols A, B, C, and D* on page 23. Otherwise, refer to *Next Steps* on page 28.

Protocol C: AmpliSeq for Illumina Panels Normalization Method

Use protocol C to denature and dilute libraries prepared using the standard AmpliSeq for Illumina workflow. Final loading concentration and volume vary depending on library preparation and quantification methods. For information about the number of libraries supported per sequencing run, use the Illumina support website to refer to the AmpliSeq for Illumina support page for your panel.

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)

   The result is 1 ml 0.2 N NaOH.
2. Invert the tube several times to mix.

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 Low TE

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

Denature and Dilute Libraries

Dilute Libraries

1. In a new 96-well LoBind PCR plate, dilute each library to 2 nM using Low TE.

Pool Libraries

1. Transfer equal volumes of each 2 nM library from the plate to a 1.5 ml LoBind tube.
   If applicable, make sure to use separate tubes for DNA and RNA libraries.
2. Vortex each tube to mix.
3. Centrifuge each tube briefly.
4. If DNA and RNA libraries are to be grouped in a single sequencing run, combine the DNA and RNA library pools at the following ratio of DNA to RNA:

Panel	DNA to RNA ratio
AmpliSeq for Illumina Myeloid Panel	8:1

Panel	DNA to RNA ratio
AmpliSeq for Illumina Childhood Cancer Panel	5:1
AmpliSeq for Illumina Comprehensive Panel v3	25:1

5. After combining the pools, vortex tube to mix, and then centrifuge briefly.

Denature Libraries

1. Combine the following volumes in a microcentrifuge tube.

Reagent	Volume (µl)
Pooled libraries	10
0.2 N NaOH	10

2. Vortex briefly, and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.
4. Add 10 µl 200 mM Tris-HCl, pH 7.0 to the tube containing 2 nM pooled libraries.
5. Vortex briefly, and then centrifuge briefly.

Dilute Denatured Libraries to 20 pM

1. Add 970 µl prechilled HT1 to the tube of 2 nM denatured library pool.
   The result is a 20 pM denatured library.
2. Vortex briefly, and then centrifuge briefly.
3. Place the 20 pM libraries on ice until you are ready to proceed to final dilution.

Dilute Libraries to Final Loading Concentration

1. Use prechilled HT1 to dilute the denatured 20 pM library solution to 1.1–1.9 pM at a final volume of 1.3 ml.
2. Invert to mix, and then centrifuge briefly.

SAFE STOPPING POINT

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

Protocol D: AmpliSeq Library Equalizer for Illumina Normalization Method

Use protocol D to denature and dilute libraries prepared using the AmpliSeq Library Equalizer for Illumina workflow. Libraries prepared using the AmpliSeq Library Equalizer for Illumina workflow are normalized to a starting concentration ready for sample pooling. For information about the number of libraries supported per sequencing run, use the Illumina support website to refer to the AmpliSeq for Illumina support page for your panel.

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)

   The result is 1 ml 0.2 N NaOH.
2. Invert the tube several times to mix.

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.

Denature and Dilute Libraries

Pool Libraries

1. Transfer equal volumes of each library from the plate to a 1.5 ml LoBind tube.
   If applicable, make sure to use separate tubes for DNA and RNA libraries.
2. Vortex each tube to mix.
3. Centrifuge each tube briefly.
4. If DNA and RNA libraries are to be grouped in a single sequencing run, combine the DNA and RNA library pools at the following ratio of DNA to RNA.

Panel	DNA to RNA ratio
AmpliSeq for Illumina Myeloid Panel	8:1
AmpliSeq for Illumina Childhood Cancer Panel	5:1
AmpliSeq for Illumina Focus Panel	7:3
AmpliSeq for Illumina Comprehensive Panel v3	25:1

5. After combining the pools, vortex tube to mix, and then centrifuge briefly.

Denature Libraries

1. Combine the following volumes in a microcentrifuge tube.

Reagent	Volume (µl)
Pooled libraries	10
0.2 N NaOH	10

2. Vortex briefly, and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.
4. Add 10 µl 200 mM Tris-HCl, pH 7.0 to the tube containing pooled libraries.
5. Vortex briefly, and then centrifuge briefly.

Dilute Denatured Libraries

1. Add 970 µl prechilled HT1 to the tube of denatured library pool.
2. Vortex briefly, and then centrifuge briefly.
3. Place the libraries on ice until you are ready to proceed to final dilution.

Dilute Libraries to Final Loading Concentration

1. Combine the following volumes to dilute the denatured library solution to the final loading concentration.
   - Denatured library (95 µl)
   - HT1 (1205 µl)
2. Invert to mix, and then centrifuge briefly.

SAFE STOPPING POINT

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

Protocol E: TruSight Tumor 170 Library Denaturation and Dilution Method

Use protocol E to denature and dilute TruSight Tumor 170 libraries.

Use the following procedures to achieve optimal coverage:

- Sequence 16 libraries (eight DNA and eight RNA) per run with a NextSeq High Output flow cell to achieve maximum coverage for each library.
- If you are sequencing DNA libraries only, you can sequence up to 10 libraries.
- If you are sequencing RNA libraries only, you can sequence up to 16 libraries.
- Contact Illumina Technical Support if you are sequencing other combinations of DNA and RNA libraries.

Prepare HT1

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

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Denature Libraries

1. Incubate each pooled library tube in a heat block at 96°C for 2 minutes.
2. Invert each tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 11 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

Choose one of the following dilution procedures to produce a denatured library solution. If you are sequencing the same number of DNA and RNA libraries, pool at a 4:1 ratio of DNA to RNA. If you are sequencing an unequal number of libraries (for example, seven DNA + three RNA), contact Illumina Technical Support.

Sequence DNA and RNA Libraries Simultaneously

1. Transfer 20 µl denatured DNA library to a new, screw-top microcentrifuge tube.
2. Add 5 µl denatured RNA library to the tube.
3. Add 475 µl HT1 to the tube to make a 1:20 dilution.
4. Vortex to mix, and then centrifuge briefly.

Sequence DNA Libraries

1. Transfer 10 µl denatured DNA library to a new, screw-top microcentrifuge tube.
2. Add 190 µl HT1 to the tube to make a 1:20 dilution.
3. Vortex to mix, and then centrifuge briefly.

Sequence RNA Libraries

1. Transfer 10 µl denatured RNA library to a new, screw-top microcentrifuge tube.
2. Add 190 µl HT1 to the tube to make a 1:20 dilution.
3. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured library solution into a new, snap-cap microcentrifuge tube.
2. Add 1360 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol E* on page 25. Otherwise, refer to *Next Steps* on page 28.

Protocol F: TruSight Oncology 500 Library Denaturation and Dilution Method

Use protocol F to pool, denature, and dilute libraries prepared using a compatible TruSight Oncology 500 workflow. Libraries prepared using compatible workflows are normalized to a starting concentration ready for sample pooling.

TruSight Oncology 500 libraries that are RNA only require PhiX control to ensure sequencing quality. PhiX control is optional for TruSight Oncology 500 DNA only libraries or combined DNA and RNA libraries.

Use the following procedures to achieve optimal coverage:

- If sequencing DNA libraries only, sequence up to eight libraries.
- If sequencing DNA libraries and RNA libraries simultaneously, sequence up to eight DNA and eight RNA libraries.
- If sequencing RNA libraries only, sequence up to 16 libraries.

Select one of the following options to pool, denature, and dilute the libraries.

- Sequence DNA libraries only. Refer to *Denature and Dilute DNA Libraries* on page 13.
- Sequence DNA libraries and RNA libraries simultaneously. Refer to *Denature and Dilute DNA and RNA Libraries* on page 14.
- Sequence RNA libraries only. Refer to *Denature and Dilute RNA Libraries* on page 15.

Denature and Dilute DNA Libraries

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Libraries

1. Thaw DNA Normalized Library (NL) plate to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PDL (Pooled DNA Libraries).
3. Transfer 10 µl of each normalized DNA library from the NL plate to the PDL tube.
4. Vortex the PDL tube to mix.
5. Centrifuge the PDL tube briefly.

Denature Libraries

1. Incubate the PDL tube in a heat block at 96°C for 2 minutes.
2. Invert the PDL tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 13 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Transfer 10 µl denatured PDL tube to a new, screw-top microcentrifuge tube.
2. Add 190 µl HT1 to the tube to make a 1:20 dilution.

3. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

Denature and Dilute DNA and RNA Libraries

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Libraries

1. Thaw both DNA and RNA Normalized Library (NL) plates to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PDL (Pooled DNA Libraries).
3. Transfer 10 µl each normalized DNA library from the NL plate to the PDL tube.
4. Label a 1.5 ml screw top microcentrifuge tube PRL (Pooled RNA Libraries).
5. Transfer 10 µl each normalized RNA library from the NL plate to the PRL tube.
6. Vortex each tube to mix, and then centrifuge briefly.

Denature Libraries

1. Incubate each pooled library tube in a heat block at 96°C for 2 minutes.
2. Invert each tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 14 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Transfer 20 µl denatured DNA library (PDL) to a new, screw-top microcentrifuge tube.
2. Add 5 µl denatured RNA library (PRL) to the tube.
3. Add 475 µl HT1 to the tube to make a 1:20 dilution.
   - If you are sequencing the same number of DNA and RNA libraries, pool at a 4:1 ratio of DNA to RNA.
   - If you are sequencing an unequal number of libraries, contact Illumina Technical Support.
4. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

Denature and Dilute RNA Libraries

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Libraries

1. Thaw RNA Normalized Library (NL) plate to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PRL (Pooled RNA Libraries).
3. Transfer 10 µl of each normalized RNA library from the NL plate to the PRL tube.
4. Vortex the PRL tube to mix.
5. Centrifuge the PRL tube briefly.

Denature Libraries

1. Incubate the PRL tube in a heat block at 96°C for 2 minutes.

2. Invert the PRL tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 15 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Transfer 10 µl denatured PRL tube to a new, screw-top microcentrifuge tube.
2. Add 190 µl HT1 to the tube to make a 1:20 dilution.
3. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. Proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27.

Protocol G: TruSight Oncology 500 HRD Library Denaturation and Dilution Method

Use protocol G to pool, denature, and dilute libraries prepared using a compatible TruSight Oncology 500 HRD workflow. Libraries prepared using compatible workflows are normalized to a starting concentration ready for sample pooling.

PhiX control is optional for the following libraries:

- TruSight Oncology 500 HRD DNA only libraries
- Combined DNA and RNA libraries

Term	Description
Paired Libraries	DNA libraries from samples enriched with both TSO 500 probes (OPD2) and HRD probes (OPD3). Two enriched libraries are present for each sample.
Unpaired Libraries	DNA libraries from samples enriched with TSO 500 probes (OPD2) only. One enriched library is present for each sample.
Mixed Libraries	A pool of DNA libraries that contains both paired and unpaired libraries.

Matched Run	A sequencing run that contains all paired libraries. Matched sequencing runs do not contain unpaired libraries.
Unmatched Run	A sequencing run that contains at least one unpaired library. Unmatched sequencing runs contain paired and unpaired libraries.

Use the following procedures to achieve optimal coverage:

Matched Run

- If sequencing DNA libraries only, sequence up to eight libraries.
- If sequencing DNA libraries and RNA libraries simultaneously, sequence up to eight DNA and eight RNA libraries.
- To ensure adequate diversity, sequence a minimum of three paired libraries.

Unmatched Run

- If sequencing DNA libraries only, sequence up to eight TSO 500 enriched libraries with one to seven HRD enriched libraries per run.
- If sequencing DNA libraries and RNA libraries simultaneously, sequence the same number of RNA libraries and TSO 500 enriched libraries (up to eight RNA libraries and eight TSO 500 enriched DNA libraries) with one to seven HRD enriched libraries per run.
- To ensure adequate diversity, sequence a minimum of three TSO 500 enriched libraries.

Select one of the following options to pool, denature, and dilute the libraries.

Matched Run

- Sequence paired DNA libraries only. Refer to *Denature and Dilute Paired DNA Libraries for Matched Sequencing Runs* on page 17.
- Sequence paired DNA libraries and RNA libraries simultaneously. Refer to *Denature and Dilute Paired DNA and RNA Libraries for Matched Sequencing Runs* on page 19.

Unmatched Run

- Sequence paired and unpaired DNA libraries only. Refer to *Denature and Dilute Mixed DNA Libraries for Unmatched Sequencing Runs* on page 20.
- Sequence paired and unpaired DNA libraries and RNA libraries simultaneously. Refer to *Denature and Dilute Mixed DNA and RNA Libraries for Unmatched Sequencing Runs* on page 22.

Denature and Dilute Paired DNA Libraries for Matched Sequencing Runs

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Libraries

1. Thaw DNA Normalized Library (NL) plate to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PDL (Pooled DNA Libraries).
3. Transfer 10 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PDL tube.
4. Label a 1.5 ml screw top microcentrifuge tube PHL (Pooled HRD Libraries).
5. Transfer 10 µl of each normalized HRD-enriched DNA library from the NL plate to the PHL tube.
6. Vortex the PDL and the PHL tubes to mix.
7. Centrifuge the PDL and the PHL tubes briefly.

Denature Libraries

1. Incubate the PDL and PHL tubes in a heat block at 96°C for 2 minutes.
2. Invert the PDL and PHL tubes two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 18 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Label a 1.5 ml screw top microcentrifuge tube DIL1 (Dilution 1).
2. Transfer 20 µl denatured TSO 500-enriched DNA library from the PDL tube to the DIL1 tube.
3. Add 5 µl denatured HRD-enriched DNA library from the PHL tube to the DIL1 tube.
4. Add 475 µl HT1 to the tube to make a 1:20 dilution.
5. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl DIL1 denatured diluted library solution into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.

4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

To achieve optimal cluster density, the ratio of DIL1 to HT1 can be modified to adjust loading concentration. For more information, refer to the *Cluster Optimization Overview Guide (Document # 1000000071511)*.

Denature and Dilute Paired DNA and RNA Libraries for Matched Sequencing Runs

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Libraries

1. Thaw both DNA and RNA Normalized Library (NL) plates to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PDL (Pooled DNA Libraries).
3. Transfer 10 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PDL tube.
4. Label a 1.5 ml screw top microcentrifuge tube PHL (Pooled HRD Libraries).
5. Transfer 10 µl of each normalized HRD-enriched DNA library from the NL plate to the PHL tube.
6. Label a 1.5 ml screw top microcentrifuge tube PRL (Pooled RNA Libraries).
7. Transfer 10 µl of each normalized RNA library from the NL plate to the PRL tube.
8. Vortex each tube to mix, and then centrifuge briefly.

Denature Libraries

1. Incubate each pooled library tube in a heat block at 96°C for 2 minutes.
2. Invert each tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 19 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Transfer 16.25 µl denatured TSO 500-enriched DNA library from PDL to a new, screw-top microcentrifuge tube.
2. Add 3.75 µl denatured HRD-enriched DNA library from PHL to the tube.
3. Add 5 µl denatured RNA library from PRL to the tube.
4. Add 475 µl HT1 to the tube to make a 1:20 dilution.
5. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

To achieve optimal cluster density, the ratio of DIL1 to HT1 can be modified to adjust loading concentration. For more information, refer to the *Cluster Optimization Overview Guide (Document # 1000000071511)*.

Denature and Dilute Mixed DNA Libraries for Unmatched Sequencing Runs

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Mixed Libraries

1. Thaw DNA Normalized Library (NL) plate to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PML (Pooled Mixed Libraries). This tube will contain a mixture of TSO 500-enriched and HRD-enriched DNA libraries.
3. Pool DNA libraries into PML tube as follows.
   Paired libraries (enriched with both TSO 500 and HRD probes):

a. Transfer 9.75 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PML tube.

b. Transfer 2.25 µl of each normalized HRD-enriched DNA library from the NL plate to the PML tube.

Unpaired libraries (enriched with TSO 500 probes only):

a. Transfer 12 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PML tube.

4. Vortex the PML tube to mix.
5. Centrifuge the PML tube briefly.

Denature Libraries

1. Incubate the PML tube in a heat block at 96°C for 2 minutes.
2. Invert the PML tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 21 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Label a 1.5 ml screw top microcentrifuge tube DIL1 (Dilution 1).
2. Transfer 25 µl denatured Mixed DNA library from the PML tube to the DIL1 tube.
3. Add 475 µl HT1 to the tube to make a 1:20 dilution.
4. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution (DIL1) into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

To achieve optimal cluster density, the ratio of DIL1 to HT1 can be modified to adjust loading concentration. For more information, refer to the *Cluster Optimization Overview Guide (Document # 1000000071511)*.

Denature and Dilute Mixed DNA and RNA Libraries for Unmatched Sequencing Runs

Prepare Heat Block and Ice Bucket

1. Preheat the heat block to 96°C.
2. Prepare ice bucket.

Prepare HT1

1. Remove HT1 from -25°C to -15°C storage and thaw at room temperature.
2. Vortex to resuspend.
3. Store at 2°C to 8°C or prechill on ice until you are ready to dilute denatured libraries.

Pool Mixed Libraries

1. Thaw DNA and RNA Normalized Library (NL) plates to room temperature. Pipette to mix and centrifuge.
2. Label a 1.5 ml screw top microcentrifuge tube PML (Pooled Mixed Libraries). This tube will contain a mixture of TSO 500-enriched and HRD-enriched libraries.
3. Pool Mixed DNA libraries into PML tube as follows.
   Paired libraries (enriched with both TSO 500 and HRD probes):
   a. Transfer 9.75 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PML tube.
   b. Transfer 2.25 µl of each normalized HRD-enriched DNA library from the NL plate to the PML tube.

   Unpaired libraries (enriched with TSO 500 probes only):
   a. Transfer 12 µl of each normalized TSO 500-enriched DNA library from the NL plate to the PML tube.
4. Label a 1.5 ml screw top microcentrifuge tube PRL (Pooled RNA Libraries).
5. Transfer 10 µl of each normalized RNA library from the NL plate to the PRL tube.
6. Vortex the PML and the PRL tubes to mix.
7. Centrifuge the PML and the PRL tubes briefly.

Denature Libraries

1. Incubate each pooled library tube in a heat block at 96°C for 2 minutes.
2. Invert each tube two times to mix.
3. Centrifuge briefly, and then place on ice for 5 minutes.

SAFE STOPPING POINT

If you are stopping, store denatured libraries at -25°C to -15°C for up to 30 days. To use frozen library pools, repeat *Denature Libraries* on page 22 to redenature, mix, and cool tubes before proceeding to the next step.

Dilute Libraries

1. Label a 1.5 ml screw top microcentrifuge tube DIL1 (Dilution 1).
2. Transfer 20 µl denatured Mixed DNA library from the PML tube to the DIL1 tube.
3. Add 5 µl denatured RNA library from the PRL tube to the DIL1 tube.
4. Add 475 µl HT1 to the tube to make a 1:20 dilution.
5. Vortex to mix, and then centrifuge briefly.

Dilute Denatured Libraries to Final Loading Concentration

1. Transfer 40 µl denatured diluted library solution (DIL1) into a new, 2 ml screw top tube.
2. Add 1660 µl HT1 to the tube.
3. Vortex to mix, and then centrifuge briefly.
4. If you plan to add a PhiX control, proceed to *Denature and Dilute PhiX Control Using Protocol F and G* on page 27. Otherwise, refer to *Next Steps* on page 28.

To achieve optimal cluster density, the ratio of DIL1 to HT1 can be modified to adjust loading concentration. For more information, refer to the *Cluster Optimization Overview Guide (Document # 1000000071511)*.

Denature and Dilute PhiX Control Using Protocols A, B, C, and D

Use the following procedure to denature and dilute a PhiX library for use as a sequencing control for protocols A, B, C, and D.

Prepare PhiX

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 4 nM PhiX.

3. Vortex briefly and then pulse centrifuge.

SAFE STOPPING POINT

If you are stopping, 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

High Output Kits

1. Add 985 µl 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.

SAFE STOPPING POINT

If you are stopping, 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.

Mid Output Kits

1. Add 985 µl 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 1.5 pM as follows.
   - Denatured PhiX (97 µl)
   - Prechilled HT1 (1203 µl)

   The total volume is 1.3 ml at 1.5 pM.

3. Invert to mix and then centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, store the denatured 1.5 pM PhiX at -25°C to -15°C for up to 2 weeks. After 2 weeks, cluster numbers tend to decrease.

Prepare Library and PhiX Mixture

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 1.5 pM PhiX for Mid Output Kits)	Volume
Denatured and diluted PhiX control at 1.5 pM	13 µl
Denatured and diluted library (from protocol A, B, C, or D)	1287 µl

Library and Concentration (Using 20 pM PhiX for Mid Output Kits)	Volume
Denatured and diluted PhiX control at 20 pM	1 µl
Denatured and diluted library (from protocol A, B, C, or D)	1299 µl

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

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.

Denature and Dilute PhiX Control Using Protocol E

Use the following procedure to denature and dilute a PhiX library for use as a sequencing control for TruSight Tumor 170 libraries.

Prepare Reagents

Prepare HP3

1. Remove HP3 from 2°C to 8°C storage and bring to room temperature.

Prepare a Fresh Dilution of NaOH

1. Combine the following volumes in a microcentrifuge tube.

- RNase/DNase-free water (950 µl)
- HP3 (50 µl)

The result is 1 ml 0.1 N NaOH.

2. Invert the tube several times to mix.

Use the fresh dilution within **1 hour**.

Prepare PhiX Control

Dilute PhiX to 2 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 (2 µl)
   - RSB (8 µl)

   The total volume is 10 µl 2 nM PhiX.
3. Pipette up and down five times to mix.

Denature PhiX

1. Combine the following volumes in a microcentrifuge tube.
   - 2 nM PhiX (10 µl)
   - 0.1 N NaOH, freshly diluted (10 µl)
2. Vortex to mix, and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.

Dilute Denatured PhiX to Loading Concentration

1. Add 980 µl prechilled HT1 to the tube of denatured PhiX.
   The total volume is 1 ml at 20 pM.
2. Invert to mix, and then centrifuge briefly.

SAFE STOPPING POINT

If you are stopping, store the denatured 20 pM PhiX at -25°C to -15°C for up to 3 weeks as single-use 50 µl aliquots.

Combine Library and PhiX Control

1. Combine the following volumes of denatured PhiX control and denatured library.
   - Denatured 20 pM PhiX control (2.5 µl)

- Denatured library (1300 µl)

2. Vortex to mix, and then centrifuge briefly.
3. Set aside on ice until you are ready to load into the reagent cartridge.

Denature and Dilute PhiX Control Using Protocol F and G

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

Prepare Reagents

Prepare HP3

1. Remove HP3 from 2°C to 8°C storage and bring to room temperature.

Prepare a Fresh Dilution of NaOH

1. Combine the following volumes in a microcentrifuge tube.
   - RNase/DNase-free water (190 µl)
   - HP3 (10 µl)

   The result is 200 µl 0.1 N NaOH.
2. Invert the tube several times to mix.

   Use the fresh dilution within **1 hour**.

Prepare PhiX Control

Dilute PhiX to 2 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 (2 µl)
   - RSB (8 µl)

   The total volume is 10 µl 2 nM PhiX.
3. Pipette up and down five times to mix.

Denature PhiX

1. Combine the following volumes in a microcentrifuge tube.

- 2 nM PhiX (10 µl)
- 0.1 N NaOH, freshly diluted (10 µl)

2. Vortex to mix, and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.

Dilute Denatured PhiX to Loading Concentration

1. Add 980 µl prechilled HT1 to the tube of denatured PhiX.
   The total volume is 1 ml at 20 pM.
2. Invert to mix, and then centrifuge briefly.

SAFE STOPPING POINT

If you are stopping, store the denatured 20 pM PhiX at -25°C to -15°C for up to 3 weeks as single-use 50 µl aliquots.

Combine Library and PhiX Control (DNA Only or DNA and RNA)

1. Combine the following volumes in a microcentrifuge tube.
   - Denatured 20 pM PhiX control (2.5 µl)
   - Denatured TruSight Oncology 500 library (1700 µl)
2. Vortex to mix, and then centrifuge briefly.
3. Set aside on ice until you are ready to load into the reagent cartridge.

Combine Library and PhiX Control (RNA Only)

1. Combine the following volumes in a microcentrifuge tube.
   - Denatured 20 pM PhiX control (16.7 µl)
   - Denatured TruSight Oncology 500 library (1646 µl)
2. Vortex to mix, and then centrifuge briefly.
3. Set aside on ice until you are ready to load into the reagent cartridge.

Next Steps

After denaturing and diluting the libraries and preparing the optional PhiX control, the next steps are to load libraries onto the reagent cartridge and set up the sequencing run. Refer to 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 27.

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 4 nM PhiX.
3. Vortex briefly and then pulse centrifuge.

SAFE STOPPING POINT

If you are stopping, 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 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.

Resources and References

The Illumina NextSeq 500 or 550 system support pages on the Illumina support site provide additional resources. These resources include training, compatible products, and other considerations. Always check support pages for the latest versions.

Revision History

Document	Date	Description of Change
Document # 15048776 v18	December 2022	Clarified list of protocol variations. Updated the protocol for denaturing and diluting TruSight Oncology 500 HRD libraries as follows: • Added list of terms and corresponding descriptions. • Clarified procedures to be used with matched sequencing runs. • Added procedures to be used with unmatched sequencing runs.
Document # 15048776 v17	June 2022	Added a new protocol for denaturing and diluting TruSight Oncology 500 HRD libraries.
Document # 15048776 v16	July 2020	Updated for internal purposes, no change to content.
Document # 15048776 v15	April 2020	Specified TruSight Oncology 500 protocols apply to all compatible TSO 500 products.
Document # 15048776 v14	March 2020	Specified NextSeq versions (NextSeq 500 and NextSeq 550) in document title.
Document # 15048776 v13	November 2019	Added the following information for TruSight Oncology 500: • Guidelines on the number of libraries and possible DNA/RNA combinations per sequencing run. • Clarification to select one of three options to pool, denature, and dilute libraries.

Document	Date	Description of Change
Document # 15048776 v12	October 2019	Added a new RNA only protocol for denaturing and diluting TruSight Oncology 500 libraries. Combined common topics in denaturation and dilution method for Protocol F. For TruSight Oncology 500, removed guidelines on the number of libraries and possible DNA/RNA combinations per sequencing run and moved the information to the TruSight Oncology 500 support pages.
Document # 15048776 v11	April 2019	Added a new protocol for denaturing and diluting TruSight Oncology 500 DNA and RNA libraries. Replaced instances of incubator with heat block in Protocols E and F. Added heat block and thermal cycler to Equipment list. Updated Denature and Dilute PhiX Control procedures to more clearly identify the compatible protocol.
Document # 15048776 v10	February 2019	Replaced Suggested Final Loading Concentration table in Protocol C with a single suggested concentration range.
Document # 15048776 v09	December 2018	Added a new protocol for denaturing and diluting TruSight Tumor 170 libraries. Added a new procedure for denaturing and diluting PhiX for TruSight Tumor 170. Added a new protocol for pooling, denaturing, and diluting TruSight Oncology 500 libraries. Added a new procedure for denaturing and diluting PhiX for TruSight Oncology 500.
Document # 15048776 v08	November 2018	Fixed AmpliSeq for Illumina Myeloid Panel pooling ratio in Protocol D.

Document	Date	Description of Change
Document # 15048776 v07	November 2018	Fixed AmpliSeq for Illumina Myeloid Panel pooling ratio in Protocol C. Added AmpliSeq for Illumina Childhood Cancer Research Assay Panel pooling ratio.
Document # 15048776 v06	October 2018	Added Protocol D for denaturing and diluting libraries prepared using the AmpliSeq Library Equalizer for Illumina workflow.
Document # 15048776 v05	July 2018	Added pooling ratio for AmpliSeq Myeloid Panel for Illumina.
Document # 15048776 v04	May 2018	Added note regarding mid output kit loading concentration. Added information on high and mid output kits. Removed caution against using PhiX with Protocol C.
Document # 15048776 v03	April 2018	Added Protocol C for denaturing and diluting AmpliSeq for Illumina Panels.
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.

Document	Date	Description of Change
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.

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

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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