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Illumina denature and dilute

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the preparation of a library for Illumina NGS sequencing.

Materials

Consumables:

The following consumables are required to prepare DNA libraries for sequencing on the MiSeq.

Consumable	Supplier
HT1 (Hybridization Buffer), thawed and prechilled	Illumina, Provided in the MiSeq Reagent Kit
[Optional] Illumina PhiX Control	Illumina, catalog # FC-110-3001
1.0 N NaOH, molecular biology grade	General lab supplier
Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20	General lab supplier
Tris-HCl, pH 7.0	General lab supplier
[Protocol C] Low TE	Illumina, Provided in the AmpliSeq Library PLUS kit

Troubleshooting

Standard Normalization Method

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

Follow the steps most appropriate for your library and the version of Illumina Reagent Kit you are using.
Loading concentration can also vary depending on library type and quantification methods.

Chemistry	Compatible Denature and Dilute Steps
MiSeq Reagent Kit v3	**4 nM library**—Results in a 6–20 pM loading concentration.
MiSeq Reagent Kit v2	**4 nM library**—Results in a 6–20 pM loading concentration. **2 nM library**—Results in a 6–10 pM loading concentration.

The denaturation steps described in this guide make sure that the concentration of NaOH is not more than 0.001 (1 mM) in the final solution after diluting with HT1. Higher concentrations of NaOH in the library inhibit library hybridization to the flow cell and decrease cluster density.

Prepare Reagents

2 **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 of 0.2 N NaOH.

2: Invert the tube several times to mix.

NOTE
Use the fresh dilution within 12 hours.

3 **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 a 4 nM Library

4 1 Combine the following volumes in a microcentrifuge tube.
- 4 nM library (5 µl)
- 0.2 N NaOH (5 µl)

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

6 Incubate at room temperature for 5 minutes.

7 Add 990 µl prechilled HT1 to the tube containing denatured library.
The result is 1 ml of a 20 pM denatured library.

Note that the denatured library concentration may vary by Illumina platform.