

Preparing a PhiX Control for a Run on the MiSeq

FOR RESEARCH USE ONLY

This document describes a change in the protocol for preparing a PhiX control with a higher cluster density target that is possible with upgraded hardware and reagents provided in the MiSeq Reagent Kit v2.

Please use the following protocol for preparing a PhiX control in place of the protocol described on page 76 of the *MiSeq System User Guide*, Part # 15027617 Rev. D.

Prepare a Fresh Dilution of NaOH



CAUTION

Using freshly diluted NaOH is essential in order to completely denature samples for cluster generation on the MiSeq.

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

Denature and Dilute PhiX Control

PhiX is used as an internal control for all libraries except TruSeq Amplicon libraries (either Custom Amplicon or Cancer Panel) and Nextera XT libraries.

Use the following instructions to denature and dilute the 10 nM PhiX library to 12.5 pM. This should result in a cluster density of 1000–1200 K/mm².

- Combine the following volumes to dilute the PhiX library to 4 nM:
 - 10 nM PhiX library (2 μ l)
 - 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (3 μ l)
- Combine the following volumes of 4 nM PhiX library and 0.2 N NaOH in a microcentrifuge tube to result in a 2 nM PhiX library:
 - 4 nM PhiX library (5 μ l)
 - 0.2 N NaOH (5 μ l)
- Vortex briefly to mix the 2 nM PhiX library solution.
- Centrifuge the template solution to 280 xg for one minute.
- Incubate for five minutes at room temperature to denature the PhiX library into single strands.

- Add the following volume of pre-chilled HT1 to the tube containing denatured PhiX library to result in a 20 pM PhiX library.

- Denatured PhiX library (10 μ l)
- Pre-chilled HT1 (990 μ l)



NOTE

You can store the denatured 20 pM PhiX library up to three weeks at -15° to -25°C. After three weeks, cluster numbers tend to decrease.

- Dilute the denatured 20 pM PhiX library to 12.5 pM as follows:
 - 20 pM denatured PhiX library (375 μ l)
 - Pre-chilled HT1 (225 μ l)
- Discard the remaining dilution of 0.2 N NaOH.

Technical Assistance

For questions, visit the Illumina support page at <http://support.illumina.com> or log in to your MyIllumina account for access to support bulletins. If you do not find the information you need, contact Illumina Technical Support by email or phone.

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