Sample Preparation and performing a Run on MiSeq:

- ***Prepare a Fresh Dilution 0.2 N NaOH***

  Using fresh diluted NaOH is essential in order to completely denature samples for cluster generation on MiSeq. Thus, diluted NaOH should be prepared within 12 hours of MiSeq run.

  To denature your samples, prepare 1 ml of 0.2 N NaoH. Preparing a volume of 1ml preventing small pipetting errors.

  - Laboratory-grade water (800ul)
  - Stock 1 N NaOH (200ul)

  Invert the tube several time to mix.

- ***Prepare PhiX Control***

The following instructions to prepare the 10nM PhiX library to 20pM (if you are using the V2 KIT, the 20pM sample is further diluted to 12.5pM).

1. Combine the following volume to dilute the PhiX library to 4nM:
   - 10nM PhiX library (2ul)
   - 10mM Tris-Cl, pH 8.5 with Tween 20 (3ul)

- ***Denature PhiX Control***

1. Combine the following volume of 4nM PhiX library and freshly diluted 0.2 N NaOH in a micro centrifuge tube:

   - 4nM PhiX library (2.5ul)
   - 0.2N NaOH (2.5ul)

2. Vortex briefly to mix the 2nM PhiX library solution.
3. Centrifuge to 280 xg for 1 min.
4. Incubate for 5 min at room temp to denature the PhiX library into single strands.
5. 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 (5ul)
   - Pre-Chilled HT1 (495ul)

   You can store the denatured 20pM PhiX library up to 3 Week at -15°C to -25°C. After 3 weeks, cluster numbers tend to decrease. DATE TUBE

- ***Denature sample for 4nM Library***

1. Combine the following volume of sample library and freshly diluted 0.2 N NaOH in a microcentrifuge tube:

   - 4nM sample library(calculated and prepared before) (2.5ul)
   - 0.2 N NaOH (2.5ul)

2. Vortex to mix the samples, and then centrifuge the sample solution to 280 xg for 1 min.
3. Incubate for 5 minutes at room temp to denature the DNA into single strand.

4. Add 495µl of HT1 to the tube containing the denatured sample. If you have more than 4 samples, add the following volumes of sample and pre-chilled HT1 to fresh tubes to avoid running out of HT1:

    - Denatured DNA (1ul)
    - Pre-chilled HT1 (99ul)

    This results in a **20pM** denatured library in 1mM NaOH.

5. Place the denatured DNA on ice until you are ready for final dilution

- ***Make final dilution and prep to load in the cartridge***

Use the following instructions to dilute your 20 pM DNA library further for optimal cluster density on the flowcell. The following uses volumes assuming one library will be loaded. Adjust volumes so that you have a total of a little over 600µl total for all libraries to be loaded.

1. Dilute the denatured DNA to the desired concentration using the following example (scale down in more than 4 samples to avoid running out of HT1:

Final Concentration	6 pM	8 pM	9pM	10 pM	12 pM	15 pM	20 pM
20 pM denatured DNA	180 µl	240 µl	270µl	300 µl	360 µl	450 µl	600 µl
Pre-chilled HT1	420 µl	360 µl	330µl	300 µl	240 µl	150 µl	0 µl

*Note- desired concentration depends on range and average size of fragments. As of 3/9/15, 8pM is desired for libraries of small size range in the 200s, 9pM for small size range in the 300s, 10pM if large size range.

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

3. Heat library(ies) to 96°C for 2min, followed by immediately cooling on ice-water bath (combine 3 parts ice and 1 part water) prior to loading.

    *This will assist in denaturing each molecule and separating indices.

- ***Load Library(ies) into cartridge***

    You will load 600µl into the reagent cartridge. Adjust volumes accordingly. Below are the volumes one would use if they had 5 libraries to load. Always want around 1% PhiX as a control (except in the case of low divergence like a 16S library whereby you would want 25% PhiX).

1. For 5 samples pool 125ul each sample into a fresh tube for a total volume of 625µl.
2. Add 6.25ul of prepared PhiX. Mix tube by inverting. Quick spin.
3. Load 600ul total of your denatured and pooled DNA/PhiX library sample into your cartridge.