Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Illumina MiSeq Preparation and Loading of Samples

1. PURPOSE

To prepare libraries for loading onto Illumina's MiSeq Sequencer.

2. MATERIALS & EQUIPMENTS

Samples

- Dengue or Zika libraries

Reagents

- Illumina MiSeq Reagent Kit v3 600-cycle (Illumina, MS-102-3003)
- Illumina PhiX control
- 1.0 N NaOH
- Nuclease – free ultrapure water

Consumables

- 1.5ml Eppendorf DNA Lo-Bind tubes

Equipments

- Ilumina MiSeq Sequencer

3. PROCEDURE

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 NaOH.
- For best results, begin thawing reagent cartridge before denaturing and diluting libraries.

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Step 1

Prepare a fresh dilution of NaOH:

a. Combine the following volumes in a 1.5 ml tube
   - Nuclease-free water – 800 ul
   - Stock 1.0N NaOH – 200 ul
   - The result is 1 ml of 0.2N NaOH

b. Invert tube several times to mix

Step 2

Prepare HT1:

a. Remove HT1 from – 25°C to – 15°C storage and thaw at room temperature.
b. Store at 2°C to 8°C until ready to dilute denatured libraries.

Step 3

Denature 4 nM Library:

a. Combine the following volumes in a 1.5 ml tube
   - 4 nM library (5 ul)
   - 0.2 N NaOH (5 ul)

b. Vortex briefly and centrifuge at 280 x g for 1 min
c. Incubate at room temperature for 5 mins
d. Add 990 ul prechilled HT1 to tube containing denatured library.
   The result is 1 ml of a 20 pM denatured library.

Step 4

Dilute Denatured 20pM Library:

a. Dilute to desired concentration using following volumes

Concentration	6 pM	8 pM	10 pM	12 pM	15 pM	20 pM
20 pM library	180 µl	240 µl	300 µl	360 µl	450 µl	600 µl
Prechilled HT1	420 µl	360 µl	300 µl	240 µl	150 µl	0 µl

b. Invert to mix and pulse centrifuge

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Step 5

Denature and Dilute PhiX Control

a. Dilute PhiX to 4 nM by combining the following volumes in a 1.5 ml tube
   - 10 nM PhiX library (2 ul)
   - 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (3 ul)

b. Denature PhiX control by combining following volumes in a 1.5 ml tube
   - 4 nM PhiX library (5 ul)
   - 0.2 N NaOH (5 ul)

c. Vortex briefly to mix
d. Centrifuge at 280 x g for 1 min
e. Incubate at room temperature for 5 mins

f. Dilute denatured PhiX to 20 pM by adding prechilled HT1
   - Denatured PhiX library (10 ul)
   - Prechilled HT1 (990 ul)

   The results is 1 ml of a 20 pM PhiX library

g. Invert to mix

h. Dilute denatured PhiX to 12.5 pM by adding prechilled HT1
   - 20 pM denatured PhiX library (375 ul)
   - Prechilled HT1 (225 ul)

   The result is 600 ul of a 12.5 pM PhiX library.

i. Invert to mix

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Step 6

Combine Library and PhiX Control

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

	Most Libraries (1% Spike-In)	Low-Diversity Libraries (≥ 5% Spike-In)
Denatured and diluted PhiX	6 µl	30 µl
Denatured and diluted library (from protocol A, B, C, or D)	594 µl	570 µl

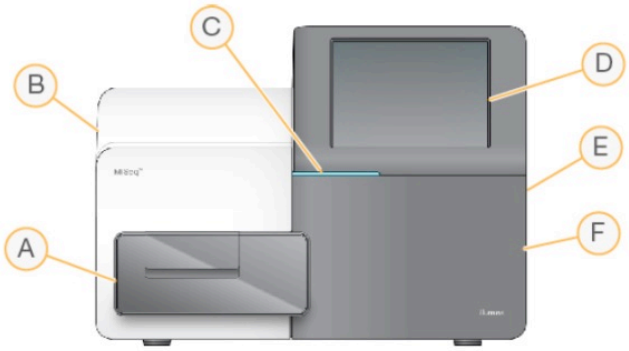
b. Set aside on ice until ready to load onto the reagent cartridge

Step 7

Loading onto MiSeq Sequencer (overview)

Components

The MiSeq comprises a touch screen monitor, a status bar, a power button with adjacent USB ports, and three compartments.



A **Flow cell compartment**—Contains the flow cell stage that houses the flow cell throughout the run. Flow cell stage motors move the stage out of the enclosed optical module for flow cell loading and returns the stage when the run begins.

B **Enclosed optics compartment**—Contains optical components that enable imaging of the flow cell.

C **Status bar**—Indicates flow cell status as ready to sequence (green), processing (blue), or needs attention (orange).

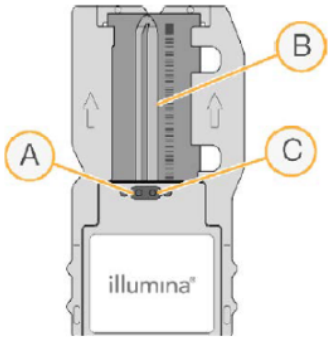
D **Touch screen monitor**—Displays the control software interface for system configuration and run setup.

E **External USB ports**—Facilitates the transfer of files and data to the instrument computer from the touch screen monitor.

F **Reagent compartment**—Contains reagents at proper temperatures, wash solutions, and a bottle for used reagents. A magnetic latch secures the reagent compartment door.

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Figure 7 MiSeq Flow Cell



A Outlet Port
B Imaging Area
C Inlet Port

Flow Cell Cap Color

The cap color of the flow cell container indicates the flow cell type:

Flow Cell	Flow Cell Cap Color
Standard Flow Cell PGS Flow Cell	Clear
Micro Flow Cell	Green
Nano Flow Cell	Yellow

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

Figure 8 Reagent Cartridge with Numbered Reservoirs

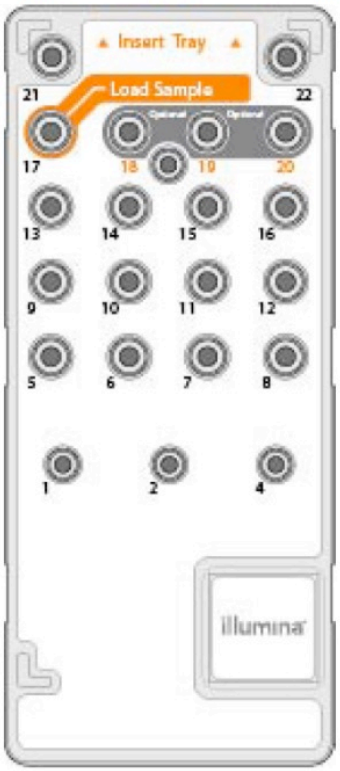


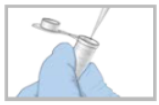
Table 1 Reagent Cartridge Reservoirs

Position	Reagent Name	Description
8	LDR	Denaturation Reagent (contains formamide)
17	Reserved	**Load Sample** (Reserved for sample libraries)
18	Reserved	Reserved for custom Read 1 primer **[Optional]**
19	Reserved	Reserved for custom Index Read primer **[Optional]**
20	Reserved	Reserved for custom Read 2 primer **[Optional]**

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu

MiSeq Workflow

Prepare the prefilled reagent cartridge for use.



Denature and dilute libraries (does not apply to all library types). See *Preparing Libraries for Sequencing on the MiSeq (document # 15039740)*.

Load the library mix onto the reagent cartridge in the designated reservoir.

From the software interface, select **Sequence** to start the run setup steps.

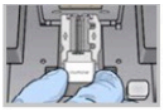
From the Sequence Mode Selection screen, select a run setup option: **Local Run Manager**, **Sample Sheet**, or **Manual**.

[Optional] From Local Run Manager selection screen select a run.
[Optional] Connect to BaseSpace or BaseSpace Onsite.

[Optional] From the Sample Sheet selection screen, browse to select a sample sheet for the run.
[Optional] Connect to BaseSpace or BaseSpace Onsite.

[Optional] Form the Manual mode screen, manually enter the run parameters. No secondary analysis is available in this mode.

Title: Illumina MiSeq Preparation and Loading of Samples		
Date last updated: 7th July 2024	Total Pages: 8	Written by: Jeanie Wu



Wash and thoroughly dry the flow cell.
Load the flow cell.

Load the PR2 bottle and make sure that the waste bottle is empty.
Load the reagent cartridge.

Review run parameters and pre-run check results.
Select **Start Run**.

Monitor your run from the MCS interface or from another computer using Local Run Manager or Sequencing Analysis Viewer (SAV).

Perform a post-run wash.