DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control
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Preparation of DNA Libraries and Loading on the MiSeq i100

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1.0 Purpose

1.1 This protocol describes how the Influenza DNA library is prepared before loading on the MiSeq i100 and the procedures for use of the Illumina MiSeq i100 sequencing platform.

2.0 Definitions

2.1 KLD – Illumina library denaturation buffer

2.2 RSB – Illumina resuspension buffer

2.3 PhiX – a quality control for cluster generation, sequencing, and alignment; and a calibration control for crosstalk matrix generation, phasing, and pre-phasing.

3.0 Critical Equipment

3.1 Illumina MiSeq i100

3.2 Microcentrifuge

3.3 Vortex

3.4 Adjustable pipettes – P10, P100, and P1000

4.0 Materials

4.1

MiSeq i100 Series Reagent Kit 300 cycles		
Flow Cell	Product Number	Information
5M	20126565	
25M	20126568	
50M	20141596	Only for MiSeq i100 PLUS
100M	20141599	Only for MiSeq i100 PLUS

4.2 PhiX Control v3 Illumina, FC-110-3001

5.0 Controls

5.1 PhiX – a quality control for cluster generation, sequencing, and alignment; and a calibration control for cross-talk matrix generation, phasing, and pre-phasing.

6.0 Safety Precautions

6.1 Adhere to the safety guidelines provided in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) and follow all established site-specific safety procedures, including wearing proper personal protective equipment (PPE).

7.0 MiSeq Procedures

7.1 Preparation of 360 pM PhiX v3 Control Stock

7.1.1 At room temperature, combine the following volumes of reagents to dilute the 10nM PhiX Control to 360 pM (0.360nM):

Reagent	Volume (µl)
10 nM PhiX Control	1.0 µl
RSB Solution	26.7 µl
Total	**27.7 µl (360pM PhiX Stock)**

7.1.2 Vortex briefly to mix the 360 pM PhiX solution.

7.1.3 Briefly spin the template solution.

7.1.4 Make 5 µl aliquots of the PhiX control and store for up to 4 weeks at -15°C to -25°C.

7.2 Normalization of the NDA Libraries

7.2.1 Normalize the pooled DNA library to 2nM using the concentration from the Qubit assay and genome size determination from a fragment analyzer.

NOTE: If needed, pooled libraries can be diluted to ~1.0 ng/µl or loading concentrations can be adjusted for optimal cluster density.

7.2.2 Dilute DNA libraries to 35 pM:

Reagent	Volume (µl)
Pooled **2 nM** DNA library	5.25 µl
RSB	24.75 µl
Total	**30.0 µl**

7.2.3 Vortex briefly to mix the solution.

7.2.4 Prepare diluted library solution for loading on a MiSeq i100 cartridge.

7.2.5 Store mixture on ice until ready to use.

Reagent	Volume (µl)
Pooled **35 pM** DNA library	27.5 µl
360 pM PhiX (5% of total)	2.5 µl
KLD	270.0 µl
Total	**300.0 µl**

7.3 Load Library

7.3.1 Remove the dry cartridge from the foil package (use within 4 hours of opening).

7.3.2 Avoid touching the flow cell.

7.3.3 Using a clean pipette tip, pierce the foil seal covering the reagent well labeled **Library**.

7.3.4 Pipette 250 ul of the diluted library solution into the reagent well labeled **Library**.

7.4 Begin Sequencing Run and Load Consumables

7.4.1 Select **Clear storage space** to navigate to the Completed tab on the runs screen.

7.4.2 Delete all runs except for the previous run.

7.4.3 Check that previous sequencing runs have been deleted from the instrument hard drive; only keep the previous run on the instrument.

7.4.4 Select **Start**.

7.4.5 Sign in with local account.

7.4.6 Using the slider bar, select **Manual**.

7.4.7 Select **Generate BCL files**.

7.4.8 Enter experiment run name.

7.4.9 Select paired-end and adjust cycle conditions.

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7.4.10 Select your sample sheet generated by Clarity or manually created.

7.4.11 Select **Review**.

7.4.12 On the review run screen, select **Load Consumables**.

- Reagent door opens. Wait until the dry cartridge is full extended before proceeding.

7.4.13 Place the dry cartridge in the dry cartridge tray. Gently push until it touches the back of the tray and is firmly secured.

7.4.14 Select **Next**.

7.4.15 Remove the wet cartridge from the foil pack.

7.4.16 Remove the plastic cap and load the wet cartridge.

7.4.17 Select **Close**.

7.4.18 Select **Verify Run**.

7.4.19 Confirm the run and consumables are correct, and then select **Start Run**.

7.4.20 Pre-run checks take about 15 minutes.

7.4.21 After pre-runs check is completed, the instrument will start automatically.

7.5 Monitoring the Run

7.5.1 Monitor the following metrics on the Sequencing or Runs screen:

- Run metrics are not available until cycle 26 of Read 1.
- **% ≥ Q30** – The expected number of base calls with a Q-score ≥ 30
- **Projected yield** – the expected number of bases called for the run
- **Total reads PF** – the number of paired-end reads passing filter (in millions)
- **Total % demux** – the percentage of PF reads demultiplexed for the run

7.6 **Completed Run**

7.6.1 When the run is complete, the **Next** button appears. Review the results on the Sequencing screen before proceeding.

7.6.2 To eject consumables after the run completes, select **Eject consumables**.

7.6.3 Remove, disassemble, and discard the dry cartridge in the autoclave bin.

7.6.4 Select **Next**.

7.6.5 Remove, disassemble, and discard the wet cartridge in the autoclave bin.

7.6.6 Select **Close**.

7.6.7 Select **Settings**, and then select **Open used reagent door**.

7.6.8 Remove the waste bottle and discard the contents into the hazardous waste bottle.

7.6.9 Return the empty waste bottle to the instrument.

7.6.10 Close the door and select **Continue**.

8.0 Related Procedures

8.1 LP-516 DNA Quantification with Qubit ds DNA High Sensitivity Kit

8.2 LP-519 Manual Procedure Illumina DNA Prep Library SOP

8.3 LP-520 QIAxcel Sample Preparation and Quality Control Procedure

9.0 References

9.1 Illumina MiSeq i100® System User Guide (ILLUMINA Part # 200055785 v01) https://support-docs.illumina.com/IN/MiSeqi100Series/Content/IN/FrontPages/MiSeqi100.htm

9.2 Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition

10.0 Attachments - None