# Whole Genome Sequencing using the Illumina MiSeq

**Module within Healthcare-Associated Infections clinical scenario**

Module Developers and Assistants

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List of learning outcomes specific for this module 

By the end of this module participants will be able to

* Appreciate the use of whole genome sequencing (WGS) in clinical microbiology
* Generate bacterial gDNA libraries and assess the library quality and quantity
* Generate short-read DNA sequences (Illumina MiSeq)

Background and summary/objectives

In this laboratory practical, we will perform whole genome sequencing of bacterial genomic DNA extracted from *Klebsiella pneumoniae* strains isolated from a healthcare outbreak setting using the Nextera XT library preparation kit (Illumina) and paired-end sequencing on a MiSeq instrument (Illumina). We will generate fastq sequencing files that will be used for subsequent genomic data analysis.

Overview of the laboratory practical

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**1.0 Principles of the procedure**

Whole genome DNA library for Illumina sequencing will be prepared using the Nextera XT Library Prep kit where using a single transposase enzymatic reaction, sample DNA is simultaneously fragmented and tagged with sequencing adapters. Short sample specific oligonucleotide barcodes are attached to the fragmented DNA. Libraries containing different indexed adapters are then constructed, quantified, pooled in equimolar amounts, and sequenced. Deconvoluting the bar codes informatically then allows multiple libraries to be sequenced on a single flow cell.

* 1. **Overview of Nextera XT library preparation**

The following diagram illustrates the Nextera XT DNA Library Prep workflow. Safe stopping points are marked between steps. Time estimates are based on processing 8 samples.

A screenshot of a phone

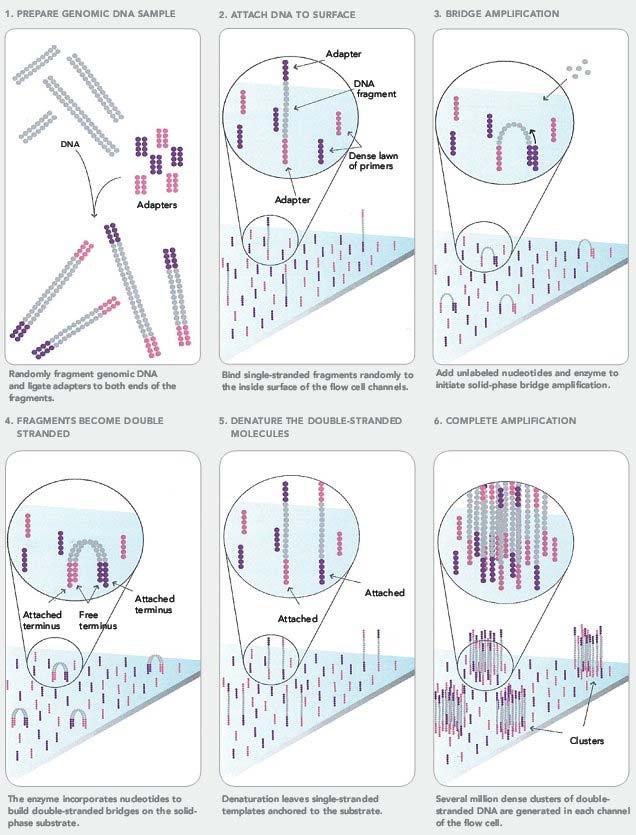
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**Fig. 1. Nextera XT library preparation workflow**

Note: Reference Guide for Nextera XT Library preparation can be found as Illumina Document: <https://support-docs.illumina.com/LP/NexteraXTRef/Content/LP/FrontPages/NexteraXT.htm> (version: 15 May 2023)

* 1. **Overview of Illumina sequencing**

(Illumina video: <https://www.youtube.com/watch?v=fCd6B5HRaZ8>)



**Fig. 2. Overview of Illumina sequencing.** Reproduced from SEQanswers.com.

**2.0 Tagmentation of genomic DNA (~20 min)**

***Personal Protective Equipment required***

*Lab coat, nitrile gloves and safety specs*

***Hazardous substances  
80% Ethanol*** *– Flammable, Irritant****Sodium Hydroxide*** *– Corrosive, Irritant, Skin Sensitizer****TD buffer*** *(contains Formamide) – Toxic, Irritant, Teratogen – expectant or new mothers should avoid handling this chemical****LDR Formamide*** *(MiSeq reagent kit) – Toxic, Teratogen – expectant or new mothers should avoid handling this chemical****PR2 Incorporation Buffer*** *(MiSeq reagent kit) – Irritant, Skin Sensitizer****Library Normalisation Wash 1*** *(Nextera XT sample prep. kit) – Mutagen, Teratogen – expectant or new mothers should avoid handling this chemical****Library Normalisation Additives 1*** *(Nextera XT sample prep. kit) – Mutagen, Teratogen – expectant or new mothers should avoid handling this chemical****Dye Concentrate*** *(Agilent high sensitivity DNA kit) (contains DMSO) – Irritant, Flammable*

Table 1. Tagmentation reagents

|  |  |  |
| --- | --- | --- |
| Item | Quantity | Storage |
| Amplicon Tagment Mix (ATM) | 1 tube (7 μl) | Ice Bucket |
| Tagment DNA Buffer (TD) | 1 tube (12 μl) | Ice Bucket |
| Neutralize Tagment Buffer (NT) | 1 tube (7 μl) | Room Temperature |
| 1ng Input DNA | Provided at: 5μl @ 0.2ng/μl | -15°C to -25°C |

1. Ensure all reagents are adequately mixed by gently inverting the tubes 3–5 times, followed by a brief spin in a microcentrifuge.
2. Retrieve the tube containing your genomic DNA (in a 0.2 ml PCR tube, labelled with your group number).
3. Add 10 μl of TD Buffer to the DNA sample. Pipette to mix.
4. Add 5μl of ATM and gently pipette up and down 5 times to mix. Briefly spin down the 0.2 ml PCR tube with the tagmentation mix.
5. Place the sample in a thermocycler and run the following program (with heated lid):

|  |  |
| --- | --- |
| Thermal Cycler Setting | |
| Program Name | Nextera XT Tagmentation |
| Total Volume: | 20 μl |
| Parameters: | 55°C for 5 minutes  Hold at 10°C |

1. Once the tubes have reached 10°C immediately remove from the thermocycler.
2. Add 5μl of NT buffer and pipette mix gently 5 times to ensure that the sample is thoroughly mixed.
3. Pulse spin in a microcentrifuge.
4. Incubate the sample at room temperature for 5 minutes.

**3.0 PCR amplification (~ 15 min prep and 30 min to run PCR or omit PCR)**

Retrieve the following reagents and consumables from the ice bucket:

|  |  |  |
| --- | --- | --- |
| Item | Quantity | Storage |
| Nextera PCR Master Mix (NPM) | 1 tube (17 μl) | Ice Bucket |
| Index 1(i7) & 2 (i5) Primer Mix (index) | 1 tube (10 μl) | Ice Bucket |

1. To your tagmented DNA sample, add 15μl of NPM and pipette mix 5 times.
2. Add 10μl of the Index Primer Mix.
3. Replace the lid and pulse spin in a microcentrifuge.
4. Place the sample tube onto a thermocycler using the following parameters:

|  |  |
| --- | --- |
| Thermal Cycler Setting | |
| Program Name | Nextera XT PCR |
| Total Volume: | 50 μl |
| Parameters: | 72°C for 3 minutes  95°C for 30 seconds  12 cycles of:  95°C for 10 sec  55°C for 30 sec  72°C for 30 sec  72°C for 5 minutes  Hold at 10°C |

**4.0 Clean-up of libraries using pre-made PCR products (~ 30 min)**

|  |  |  |
| --- | --- | --- |
| Item | Quantity | Storage |
| Resuspension Buffer | 1 tube (55 μl) | Room Temperature |
| AMPure XP beads (XP) | 1 tube | Room Temperature |
| Fresh 80% ethanol | 1 tube (15 ml) | N/A |
| DNA library | 1 tube (50 μl) | 10°C (or 4°C) |

1. Retrieve your DNA library sample from the thermal cycler. Briefly pulse spin in a microcentrifuge.
2. Label a new 1.5 ml tube with your group number.
3. Transfer 50μl of the PCR product (your DNA library) from the original PCR tube to the new clean 1.5 ml tube.
4. Vortex the AMPure XP beads for 30 seconds to ensure that the beads are evenly dispersed.
5. Add 30μl of AMPure XP beads to the tube. Pipette mix 10 times.
6. Incubate at room temperature for 5 minutes.
7. Place the tube on a magnetic stand for 2 minutes or until the supernatant has cleared.
8. Carefully remove and discard all the supernatant from each well (using a 200μl pipette tip).

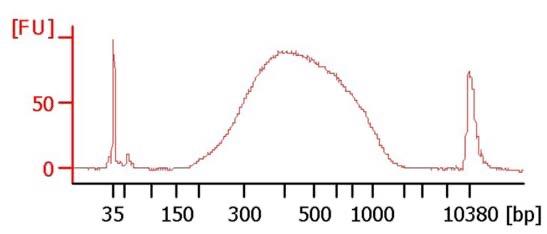
(Please note: Your DNA is now bound to the Ampure XP beads, avoid disturbing the beads. If any beads are inadvertently aspirated into the tips, dispense the beads back into the tube and let it rest on the magnet for 2 minutes and confirm that the supernatant has cleared)

1. Add 200μl of 80% ethanol to the tube whilst still on the magnetic stand. Incubate for approximately 30 s and carefully remove and discard the supernatant. Do not remove the beads.
2. Repeat the ethanol wash in step 9.
3. If required use a P10 pipette to remove excess ethanol, so as to not remove the beads.
4. With the samples still on the magnetic stand, allow the beads to air-dry for 5 minutes.
5. Remove the tube from the magnetic stand and add 52.5μl of RSB.
6. Gently pipette mix up and down 10 times.
7. Incubate at room temperature for 2 minutes.
8. Place the tube back on the magnetic plate for 2 minutes (or until the supernatant has cleared).
9. Label a new tube ‘CAN’ (Clean Amplified NTA) & your group number.
10. Carefully transfer 30 μl of the supernatant (this is your purified DNA library) to the CAN tube.

**5.0 Library Quality and Quantity Check (Bioanalyzer, Agilent) (~ 10 min)**

Note: the library quality and quantity can also be checked on a Tape Station (Agilent) instrument. Tapestation –<https://www.youtube.com/watch?v=XI74ZZghR5o> and more details at <https://www.youtube.com/watch?v=c-I4aioxMBo> . Bioanalyser - <https://www.youtube.com/watch?v=V4tvmhdBSFs>

* The size distribution of your library can be checked by running 1μl of it on an Agilent Technologies 2100 Bioanalyzer using a High Sensitivity DNA chip.
* Typical libraries show a broad size distribution from ~250-1000bp, with an average of ~400–500bp, as shown in the figure below:



**Fig. 1. Example of DNA library size distribution.**

**Setting up the Chip Priming Station**

* 1. Insert the syringe into the clip.

1. Slide it into the hole of the luer lock adapter and screw it tightly to the chip priming station.
2. Check base plate is in position C.
3. Adjust the syringe clip to the lowest position.

**Checking the Chip Priming Station for Good Seal — Seal Test**

1. Make sure the syringe is tightly connected to the Chip Priming Station.
2. Pull the plunger of the syringe to the 1.0ml position (plunger pulled back).
3. Place an empty chip in the Chip Priming Station.
4. Close the Chip Priming Station and make sure to lock it by pressing the cover, the lock of the latch will audibly click when it closes.
5. Press the plunger down until it is locked by the clip.
6. Wait for 5 seconds and press the side of the clip to release the plunger.
7. Appropriate sealing is verified if the plunger moves back up to the 0. ml mark within less than 1 second.

**Preparing the Gel-Dye Mix**

1. Allow High Sensitivity DNA dye concentrate (blue) and High Sensitivity DNA gel matrix (red) to equilibrate to room temperature for 30 min.
2. Add 15μl of High Sensitivity DNA dye concentrate (blue) to a High Sensitivity DNA gel matrix vial (red).
3. Vortex solution well and spin down. Transfer to spin filter.
4. Centrifuge at 2240 g ± 20 % for 10 min. Protect solution from light. Store at 4 °C.

**Loading the Gel-Dye Mix**

1. Allow the gel-dye mix equilibrate to room temperature for 30 min before use.
2. Put a new High Sensitivity DNA chip on the chip priming station.
3. Pipette 9.0 μl of gel-dye mix in the well, marked G.
4. Make sure that the plunger is positioned at 1ml and then close the chip priming station.
5. Press plunger until it is held by the clip.
6. Wait for exactly 60seconds then release clip.
7. Wait for 5seconds, and then slowly pull back the plunger to the 1 ml position.
8. Open the chip priming station and pipette 9.0μl of gel-dye mix in the two additional wells marked G.

**Loading the Marker**1. Pipette 5μl of marker (green) in all sample and ladder wells. Do not leave any wells empty.

**Loading the Ladder and the Samples**

1. Pipette 1μl of High Sensitivity DNA ladder (yellow) in the ladder well.
2. In each of the 11 sample wells pipette 1μl of sample (used wells) or 1μl of marker (unused wells).
3. Put the chip horizontally in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
4. Run the chip in the Agilent 2100 Bioanalyzer within 5 min.

**Starting the Run**

1. Select the High Sensitivity assay from the Assay menu.
2. Enter details in the sample name table.
3. Click the Start button in the upper right of the window to start the chip run.
4. The incoming raw signals are displayed in the Instrument context.
5. After the run is finished, remove the chip.

**Average Library Fragment Size**

1. When viewing the results of the run, navigate to the ‘Region Table Bar’.
2. Move the blue bars to either side of the curve.
3. The average length in bp will be displayed
4. Use this value to calculate the molarity using the values from the Qubit quantitation assay.

**5.1 Library quantification using Qubit**

Accurate quantification of purified double stranded DNA library can be achieved by Qubit dsDNA HS Assay and measurement on a Qubit fluorometer.

|  |  |  |
| --- | --- | --- |
| Item | Quantity | Storage |
| Qubit® dsDNA HS Reagent | 1 tube | Room Temperature (protect from light) |
| Qubit® dsDNA HS Buffer | 1 tube | Room Temperature |
| Qubit® dsDNA HS Standard #1 | 1 tube | 4°C |
| Qubit® dsDNA HS Standard #2 | 1 tube | 4°C |

* 1. Set up and label 0.5 ml Qubit assay tubes and label them with the number of your sample(s) and your group on the lid. Note: do not write on the tube walls as the fluorescence is read through the side of a tube.
  2. In a 1.5 ml Eppendorf tube prepare Qubit working solution by mixing 199 ml of dsDNA HS buffer with 1 ml dsDNA Reagent per sample. Mix thoroughly by vortexing and pulse spin.
  3. Add 198 ml of the Buffer-Reagent solution into a Qubit assay tube and add 2 ml of your DNA library sample. Mix thoroughly by vortexing and pulse spin.
  4. Measure the DNA library concentration on a Qubit fluorometer at settings for dsDNA high sensitivity kit. Note: the standards are already set and measured for you.

Note: Qubit protocol details <https://tools.thermofisher.com/content/sfs/manuals/Qubit_dsDNA_HS_Assay_UG.pdf> and a video available at <https://www.youtube.com/watch?v=6HtnVUHMX_8>

**6.0 Library Normalisation**

After the Nextera XT PCR cleanup step (4.0), the library is double stranded DNA and can be quantified using a fluorometric method, such as Qubit (step 5.1) or PicoGreen. The library fragment size distribution is checked using the Agilent’s Tape station or Bioanalyzer instrument (step 5.0). Bioanalyzer traces or qPCR only are not an acceptable method for quantifying Nextera libraries.\* Although a Bioanalyzer trace is a good method for assessing final library size, it is not accurate for quantification due to a broad sample size distribution. Quantify samples with Qubit or PicoGreen.

**6.1 Manual Library Normalisation (15 min)**

Manual library normalization for Nextera XT bacterial genomic DNA libraries before MiSeq sequencing involves adjusting library concentrations to ensure equal representation of each sample in the sequencing run.

**1. Quantify the DNA libraries**

* **Assess fragment sizes**: Use an Agilent Bioanalyzer or TapeStation (step 5.0), Nextera XT libraries typically have fragments between 300–500 bp.
* **Measure DNA concentration**: Use the Qubit dsDNA HS kit (step 5.1) to quantify the concentration of your libraries.

**2. Calculate the DNA input**

* Determine the molar concentration of your libraries using the formula:

**3. Dilute libraries to normalise concentrations**

* Aim for a final normalised concentration of 4 nM for each DNA sample library. Use molecular grade water for library diluteion.
* Calculate the dilution of the libraries using the following equation:

Where: c1 is initial concentration of the library, V1 volume of initial library that will be diluted, c2 concentration of final library (i.e. 4nM), V2 desired volume of final library (i.e. 15 ml)

**4.Pool the normalised libraries**

* Combine equal volumes of each normalised library into a single tube to create the pooled library.
* Mix thoroughly by pipetting or vortexing gently.
* Continue with step 7.

**6.2 Bead-Based Library Normalisation (1hr 15 min, not demonstrated)**

Note 1: Reagents contain formamide: to be conducted inside fume hood

Note 2: Libraries need to be normalised manually if the final library yield is < 10 nM. (Protocol at <https://support-docs.illumina.com/LP/NexteraXTRef/Content/LP/Nextera/XT/DiluteLibraries.htm> )

|  |  |  |
| --- | --- | --- |
| Item | Quantity | Storage |
| Library Normalisation Additives1 (LNA1) | 1 tube | Ice Bucket |
| Library Normalisation Beads1 (LNB1) | 1 tube | Ice Bucket |
| Library Normalisation Wash (LNW1) | 1 tube | Ice Bucket |
| Library Normalisation Storage Buffer1 (LNS1) | 1 tube | Room Temperature |
| Fresh 0.1 N NaOH | 1 tube | Room Temperature |
| 96-well plate | 1 plate | N/A |
| 15 ml conical tube | 1 tube | N/A |

1. Remove the LNA1, LNB1 and LNW1 from the ice bucket (and LNS1 from its storage location) and bring to room temperature. Vortex for approximately 1 minute prior to use, ensuring any precipitate is fully resuspended.
2. Retrieve the ‘CAN’ tubes and, pulse-spin in a microcentrifuge. Pipette mix the sample thoroughly.
3. Label a 96-well plate ‘Normalisation’
4. Transfer 20μl of supernatant from each ‘CAN’ tube to a separate well in the ‘Normalisation’ plate.
5. Volumes of LNA1 and LNAB required:

|  |  |  |
| --- | --- | --- |
| Sample number | Reagent | Volume |
| 12 | LNA1 | 550μl |
| LNB1 | 100μl |

1. Ensure the LNA1 and LNB1 is thoroughly mixed just prior to use.
2. Combine the required volumes of LNA1 and LNB1 in a 15ml conical tube. Vortex thoroughly for 30 seconds and invert 15-20 times.
3. Add 45μl of LNA1/LNB1 mix to each sample in the ‘Normalisation’ plate.
4. Seal the plate and shake at 1800rpm for 30 minutes.
5. Place the plate on the magnetic stand and remove the plate seal. Incubate on the magnet for 2 mins and carefully remove all of the supernatant (with multichannel). (Please note: Avoid disturbing the beads during this step).
6. Remove the plate from the magnetic stand and add 45μl of LNW1 to each sample well.
7. Seal the plate and place onto a plate shaker at 1800rpm for 5mins.
8. Place the plate on the magnetic stand and remove the plate seal. Incubate on the magnet for 2mins and carefully remove the supernatant (with multichannel).
9. Repeat wash steps 11 – 13. (Please note: Ensure excess LNW1 is removed, if required use a P10 pipette or multi-channel to remove residues).
10. Remove the plate from the magnetic stand and add 30μl of 0.1 N NaOH to each sample well to elute sample. (Please note: Only use freshly prepare 0.1N NaOH and do not store).
11. Seal the plate with and shake at 1800rpm for 5 minutes.
12. Label a new 96-well plate ‘FINAL’.
13. Pipette 30μl of LNS1 to the appropriate wells on the ‘FINAL’ plate.
14. Remove the ‘Normalisation’ plate from the shaker and check to ensure all samples are completely resuspended, if not, pipette mix and place back onto the shaker for a further 5minutes @1800rpm.
15. Place the ‘Normalisation’ plate on a magnetic stand and remove the plate seal. Incubate for 2 minutes.
16. Transfer 30μl the supernatant from the ‘Normalisation’ plate to the ‘FINAL’ plate.
17. Seal the ‘FINAL’ plate and centrifuge at 1000xg for 1 minute.

Note: For manual library normalisation (if the final library yield is < 10 nM), calculate the library molarity from the library concentration (ng/μl, as measured by HS dsDNA kit on Qubit) and from the average library size (bp, fragments as measured on Tape Station or Bioanalyzer).

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**7.0 Loading the MiSeq (40 min) demonstrated**

Preparing the 20pM PhiX (control) Library

* Gently vortex and pulse the 10nM PhiX Library.
* Combine 2μL 10nM PhiX Library and 3μL EBT buffer to make a 4nM PhiX library.
* Add 5μL 0.2M NaOH to the above Eppendorf to make 2nM PhiX library.
* Vortex the Eppendorf and pulse spin.
* Incubate for 5 minutes at room temp to denature the PhiX library.
* Add 990μL HT1 to the Eppendorf to make 20pM denatured PhiX library.

Store the 20pM denatured PhiX library between -15°C to -25°C. Dispose after 3 weeks.

**Preparing the ‘POOLED AMPLICON LIBRARY’ (PAL) tube**

* Retrieve the ‘FINAL’ plate and place plate shaker and mix for 1 minute at 1500rpm and pulse-spin in a plate centrifuge
* Place onto a magnetic stand (to collect any remaining beads)  
  Pool 5μL of sample from the ‘FINAL’ plate into an Eppendorf labelled ‘Pool’.

**Preparing the ‘DILUTED AMPLICON LIBRARY’ (DAL) tube**

* Set a heat block to 96°C and prepare an ice water bath
* Gently vortex and pulse spin the PAL tube.
* Vortex HT1 to remove all trace of precipitate.
* Label a clean 1.5mL LoBind Eppendorf with ‘DAL’.
* Add 22μL of ‘PAL’ and 588μL HT1 to the ‘DAL’ tube.
* Gently vortex and pulse-spin.
* Incubate the ‘DAL’ tube at 96°C for 2 minutes.
* Invert the ‘DAL’ tube twice and immediately place into the ice water bath.
* Incubate the ‘DAL’ tube for 5 minutes.
* Add 18μL denatured 20pM PhiX library to the ‘DAL’ tube.
* Gently vortex and pulse spin – keep on ice
* The ‘DAL’ tube is now ready for loading onto the MiSeq

**Preparing MiSeq Reagent Cartridge  
Allow approximately one hour for thawing the cartridge**

1. Place the reagent cartridge in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge up to the water line printed on the reagent cartridge. Do not allow the water to exceed the maximum water line.
2. Allow the reagent cartridge to thaw in the room temperature water bath for approximately one hour or until completely thawed.
3. Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge. Make sure that no water has splashed on the top of the reagent cartridge.
4. Invert the reagent cartridge to mix the thawed reagents, and then visually inspect that all positions are thawed.
5. Visually inspect the reagent marked IMF (Position 1) to make sure that it is fully mixed and free of precipitates.
6. NOTE: The MiSeq sipper tubes go to the bottom of each reservoir to aspirate the reagents, so it is important that the reservoirs are free of air bubbles.
7. Place the reagent cartridge on ice or set aside at 2° to 8°C until you are ready to set up your run.

**MiSeq Instrument Preparation Clean the Flow Cell**

1. Wash the flow cell with Millipore water.
2. Dry any excess water with a lint-free lens cleaning tissue, and visually inspect to make sure that the flow cell ports are free of obstructions and that the gasket is well seated around the flow cell ports.
3. If the gasket appear to be dislodged, gently press it back into place until it sits securely around the flow cell ports.

**Loading the Flow Cell**

1. Raise the flow cell compartment door, and then press the release button to the right of the flow cell latch. The flow cell latch opens.
2. Visually inspect the flow cell stage to make sure it is free of lint. If lint or other debris is present, clean the flow cell stage using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol. Carefully wipe the surface of the flow cell stage until it is clean and dry.
3. Hold the flow cell by the edges of the flow cell cartridge near the Illumina label.
4. Make sure the label is facing upward and place the flow cell on the flow cell stage.
5. Gently press down on the flow cell latch to close it over the flow cell. You will hear a click when the flow cell latch is secure.
6. As you close the flow cell latch, two alignment pins near the hinge of the flow cell latch properly align and position the flow cell.
7. Check the lower-left corner of the screen to confirm that the flow cell RFID was successfully read.
8. Close the flow cell compartment door.
9. Select Next on the Load Flow Cell screen. The Load Reagents screen opens.

**Loading Reagents**

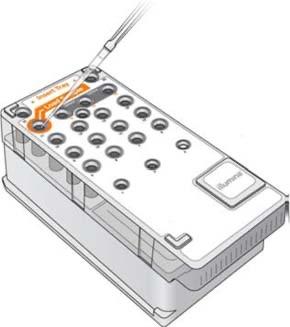
1. Remove the bottle of PR2 from fridge. Gently invert the bottle to mix the PR2 bottle, and then remove the lid.
2. Open the reagent compartment door.
3. Raise the sipper handle until it locks into place.
4. Place the PR2 bottle in the indentation to the right of the reagent chiller.
5. Make sure that the waste bottle is empty. If required, empty the contents into the appropriate waste container.
6. Slowly lower the sipper handle. Make sure that the sippers lower into the PR2 and waste bottles.
7. Check the lower-left corner of the screen to confirm that the RFID of the PR2 bottle was read successfully.
8. Select Next on the Load Reagents screen.

**Load Sample Libraries onto Cartridge**

1. Use a clean 1 ml pipette tip to pierce the foil seal over the reservoir labelled Load Samples.

NOTE: Do not pierce any other reagent positions. Other reagent positions are pierced automatically during the run.

1. Pipette 600μl of your sample libraries into the Load Samples reservoir. Take care to avoid touching the foil seal as you dispense your sample.



3. Proceed directly to the run setup steps using the MiSeq Control Software (MCS) interface.

**Load the Reagent Cartridge**

NOTE Do not leave the reagent chiller door open for extended periods of time.

1. Open the reagent chiller door.
2. Hold the reagent cartridge on the end with the Illumina label, and slide the reagent cartridge into the reagent chiller until the cartridge stops.
3. Close the reagent chiller door.
4. Check the lower-left corner of the screen to confirm that the RFID of the reagent cartridge was read successfully.
5. Close the reagent compartment door.  
   Select Next on the Load Reagents screen. The Review screen opens.

**Sample Sheet Set-up (Illumina Experiment Manager)**

The Illumina Experiment Manager is a wizard-based application that guides you through the steps to create your sample sheet.

**Starting the Run**

* After you have loaded the flow cell and reagents, the MCS interface prompts you to review run parameters and perform a pre-run check before beginning the run.