Laboratory Operating Procedure

Running prepared sequencing libraries on the Illumina MiSeq platform

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LOP-Version: 1

Attachments:

Introduction

This LOP describes the steps required to set up a run on the Illumina MiSeq instrument

Sample Material

Libraries (pooled if required) prepared according to the APHA Illumina Library Preparation LOP

Equipment and Reagents

(general remarks and list of equipment and material needed, bullet points)

Equipment

- Pipettes
- Illumina MiSeq Instrument

Reagents

- 2N NaOH
- MiSeq v2 300 cycle Reagent Kit
- PhiX Control Kit
- 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20

General remarks

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Literature

- *(e.g. Kit handbooks, publications)*

Before getting started

- Retrieve a MiSeq V2 300 cycle kit from -20 °C storage. Place the reagent cartridge in room temperature water to thaw. Select the pool that is going to be sequenced. It will take 20-30 minutes to thaw completely.
- Prepare the samplesheet, indicating the samples to be run and their corresponding indexes. This can be done using the Illumina Experiment Manager, of generated from the library preparation worksheet. The .csv file should be named with the lot number of the reagent kit and saved in the 'samplesheets' folder on the instrument.

Procedure

1. - **Denaturation of the library**

- Dilute 5 µl of 2 N NaOH (Illumina) in 20 µl PW1 pure water in a microcentrifuge tube

- Add 2.5 µl of diluted NaOH to a fresh microcentrifuge tube. Add 7.5 µl of a library pool and incubate at room temperature for 5 minutes.

- Add 940 µl HT1 to the denatured library. To this add 50 µl of a pre-prepared PhiX control (see appendix 1).
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2. - **Loading the Instrument**

- On the MiSeq instrument, select the MiSeq Control Software. Select sequence and follow the onscreen instructions

- Remove the Flowcell from the kit stored at 2-8 °C. Rinse the flowcell with Molecular Biology Grade Water and dry with lint free tissue. Taking care not to get ethanol in the small rubber ports clean the glass portion of the flowcell with lens tissue and molecular biology grade ethanol. Insert the flowcell into the instrument and follow the onscreen instructions.

- Insert the PR2 reagent bottle into the instrument

- Retrieve the reagent cartridge and invert several times to ensure reagents are thoroughly mixed. Also check that all reagents are completely thawed. Pierce the foil cover on reagent port 17 (labelled Sample) on the reagent cartridge with a pipette tip. Add 600 µl of the phiX-spiked diluted denatured library pool (from step 1) to reagent port 17.

- Insert the reagent cartridge into the instrument and start the sequencing process. Ensure to wait until all checks have been completed before finally pressing 'Start'. If any errors are detected refer to the MiSeq Instrument Manual.

- When the run is complete (approximately 26 hours) fill each well of the MiSeq wash tray with 5 ml 0.5% Tween. Ensure the 500 ml wash bottle has at least

350 ml 0.5% Tween. Replace the reagent cartridge and PR2 buffer bottle with the wash bottle and wash tray as directed on-screen. Click ‘Wash’ to clean the instrument prior to the next run.

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Appendix 1

Preparation of PhiX control

1. Combine the following volumes to dilute the PhiX library to 4 nM:
    - 10 nM PhiX library (2 µl)
    - 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (3 µl)
2. Combine the following volumes of 4 nM PhiX library and freshly diluted 0.2 N NaOH in a microcentrifuge tube:
    - 4 nM PhiX library (5 µl)
    - 0.2 N NaOH (5 µl)
3. Vortex briefly to mix the 2 nM PhiX library solution.
4. Centrifuge the template solution to 280 xg for 1 minute.
5. Incubate for 5 minutes at room temperature to denature the PhiX library into single strands.
6. Add the following volume of pre-chilled HT1 to the tube containing denatured PhiX library to result in a 12.5 pM PhiX library.
    - Denatured PhiX library (10 µl)
    - Pre-chilled HT1 (1590 µl)

The denatured 12.5 pM PhiX library can be stored at +2° to +8 °C and used for up to 3 months.