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MiSeq standard operating procedures and best practices

Please note that the MiSeq must be booked in advance at: https://cgi.sfu.ca/~mbbweb/seq/. Please contact Tim Heslip (trh2@sfu.ca) or Duncan Napier (dgnapier@sfu.ca) for access to this calendar.

Sample preparation and sample sheet

1. Obtain the newest version of the User Guide and READ it. The current version is Rev. N and can be obtained from: http://support.illumina.com/sequencing/sequencing_instruments/miseq.html.
2. Get the most recent version of guide for "Preparing Libraries for Sequencing on the MiSeq". The current version is Rev. D and can be obtained from: http://support.illumina.com/downloads/prepare_libraries_for_sequencing_miseq_15039740.html.
3. Prepare a sample sheet using the Illumina Experiment Manager software. The user guide for this software can be obtained here: http://support.illumina.com/sequencing/sequencing_software/experiment_manager.ilmn. If your library requires custom sequencing primers, be sure to specify this.
4. Make aliquots of fresh 1N or 2N NaOH and freeze for future use. Sigma supplies molecular biology grade 1N NaOH. Thaw and dilute an aliquot to 0.2N fresh for every run.
5. Keep stock libraries at 4nM
6. Always use a Bioanalyzer for determining size of library inserts and for detecting presence of primer dimers. This information is critical for calculating the appropriate dilutions prior to loading the MiSeq.
7. Store the library in EBT (not supplied with kits, can be made, 10mM Tris-Cl pH 8.5 0.1%Tween 20).
8. Always quantitate libraries with Q bit. Nanodrop quants are not sufficiently accurate.
9. When pooling libraries, it is important to quantitate the pooled library with Q bit.
10. After denaturation with fresh 0.2N NaOH, dilute denatured library with FRESH HBT. If sequencing amplicons, a separate sample preparation protocol can be found at: http://support.illumina.com/downloads/truseq_custom_amplicon_library_prep_guide_15027983.html. Please note that an additional heat step is recommended (see pages 34-37). After incubating with 0.2N NaOH, heat library to 96C for 2 minutes then snap cool on water/ice bath.
11. For V2 kits, PhiX library max is 12.5pmol
12. For V3 kits, PhiX library max is 20pmol.
13. Pooled libraries and PhiX library must be the same concentration.

14. If custom sequencing primers are needed, load these into the appropriate ports on the cartridge. If applicable, ensure that the use of custom primers is correctly indicated in the sample sheet.

Starting a run

15. After thawing in a room temperature water bath, invert thawed kit 10X and then knock on benchtop firmly several times to release bubbles. Visually ensure all chambers in the kit have completely thawed.
16. Rinse flow cell generously with ddH_2O to remove the storage buffer and dry with a Kimwipe. Gently squeeze base of flow cell to release any trapped buffer, then rinse and dry again.
17. When starting a run, the MiSeq chiller temperature should indicate 5C or less. A higher temperature may result from a recent restart of the MiSeq or could indicate a problem.
18. Make sure flow cell is seated properly by visually ensuring the two ports align with the valve on the stage

Post-run Washing

19. A post-run wash must be performed. The protocol for post-run washes can be found on pages 62-63 of the User Guide. Briefly, fresh 10% bleach is prepared by the user and added to well 17 of the wash cartridge, and fresh 0.5% Tween 20 available from the large carboy underneath the MiSeq is added to the rest of the wells. The flow cell must be left in place during **and** after the post-run wash.