

Center for Health Genomics and Informatics

Illumina Experiment Manager

Cheat Sheet for Sample Sheets only (not sample plates)

For official Illumina IEM support, visit:

https://support.illumina.com/sequencing/sequencing_software/experiment_manager/documentation.html

For MiSeq runs:

- 1) Open IEM
- 2) Select "Create Sample Sheet"
- 3) In Instrument Selection window - select "MiSeq" and Next
- 4) MiSeq Application Selection window:
 - a) Select Category and Application and Next
 - i) Examples:
 - (1) 16S libraries: category "Targeted Resequencing", application "Metagenomics 16S rRNA"
 - (2) Standard fragment libraries (e.g. Nextera XT, NEB Ultra, etc.): category "Small Genomic Sequencing", application "Resequencing"
 - (3) Amplicon libraries: category "Other", application "FASTQ only"
 - b) Note that many applications require a Manifest to be uploaded to the sequencing instrument prior to run set up. If you do not have this, select category "other" and "FASTQ only" as application.
- 5) Workflow Parameters window:
 - a) Reagent Cartridge Barcode: enter "SampleSheet" or a Project Name. This is the name under which the generated .csv file will be saved as.
 - b) Library Prep Workflow: select your library prep kit. If you do not see your kit or are using a custom library prep kit, select the kit that the indexes you used to make your libraries are compatible with. See document: https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences-1000000002694-09.pdf
 - c) Index Adapters: select the kit that your indexes are from (this will then provide the indexes for ONLY that kit in the next window)
 - d) Index Reads:
 - i) 0 (None) - library is not indexed
 - ii) 1 (Single) - libraries have only one 6-bp index attached
 - iii) 2 (Dual) - libraries each have two 8-bp indexes attached
 - e) Enter an experiment name. Enter the investigator name and project description. These should be the same as entered on the CHGI Sample Submission excel worksheet.
 - f) Select Read Type
 - i) Paired End – run includes Read 1 and Read 2 (2 x bp read, e.g. 2x150bp)
 - ii) Single Read – run includes only Read 1 (a single direction sequencing run, e.g. 75bp in one direction only)

- g) Cycles per Read: enter one more than the number of cycles for each read. E.g. for a 300 cycle cartridge with paired end reads of 2 x 150bp, enter 151 for both reads.
- h) Leave Workflow-Specific Settings in the right hand table as default.
- 6) Sample Selection window:
 - a) If a previous sample plate has not been created, add a blank row for each of your libraries.
 - b) For each library enter information in the table (only Sample ID and index information are required), selecting the correct index for each sample, the index sequence will auto-populate and you can cross check with your kit.

For NextSeq runs:

- 1) Open IEM
- 2) Select "Create Sample Sheet"
- 3) In Instrument Selection window - select "NextSeq/MiniSeq" and Next
- 4) NextSeq Application Selection window:
 - a) "NextSeq FASTQ only" is the only option, select Next.
- 5) Workflow Parameters window:
 - a) Reagent Cartridge Barcode: enter "SampleSheet" or a Project Name.
 - b) Library Prep Workflow: select your library prep kit. If you do not see your kit or are using a custom library prep kit then select the kit that the indexes you used to make your libraries are compatible with. See document https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences-1000000002694-09.pdf
 - c) Index Adapters: select the kit that your indexes are from (this will then provide the indexes for ONLY that kit in the next window)
 - d) Index Reads:
 - i) 0 (None) - library is not indexed
 - ii) 1 (Single) - libraries have only one 6bp index attached
 - iii) 2 (Dual) - libraries each have two 8bp indexes attached
 - e) Enter Experiment name, investigator name, and project description
 - f) Select Read Type
 - i) Paired End reads – run will include Read 1 and Read 2 (2 x bp read)
 - ii) Single Read – run will include only Read 1 (a single direction sequencing run, e.g. 75bp in one direction only)
 - g) Cycles per Read: enter one more than the number of cycles for each read. E.g. for a 300 cycle cartridge with paired end reads of 2 x 150bp, enter 151 for both reads.
 - h) Leave Workflow-Specific Settings in the right hand table as default.
- 6) Sample Selection window:
 - a) If a previous sample plate has not been created, add a blank row for each of your libraries.
 - b) For each library enter information in the table (only Sample ID and index information are required), selecting the correct index for each sample, the index sequence will auto-populate and you can cross check with your kit.