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© Pilot Exercise: Generating the Illumina SampleSheet and sharing data via BaseSpace V.3

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This protocol describes the required steps to generate Illumina SampleSheet and provides guidelines for sharing data via BaseSpace with FDA for the 2021 Pilot Exercise

V2:

- added more details regarding data transfer through BaseSpace, including steps for sharing a project with FDA-CESAN
- replaced samplesheet example.

V3:

- replaced samplesheet example

Ruth Timme, Maria Balkey, Tunc Kayikcioglu, Candace Bias, Cameron Boerner, James Pettengill 2022. Pilot Exercise: Generating the Illumina SampleSheet and sharing data via BaseSpace. **protocols.io** https://protocols.io/view/pilot-exercise-generating-the-illumina-samplesheet-b3gqqjvw Maria Balkey

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Generating Sequencing SampleSheet

- Create your SampleSheet using Excel or a text editor. Name your sample sheet according your internal protocols, and use a *.csv extension. The sample sheet is organized in sections titled Header, Reads, Settings and Data.

 Section headings are case-sensitive and shown in brackets [].

 SampleSheet.csv Details for each section are described below.
 - 1 1 Header Section.

Α	В
Parameter	Description
Investigator Name	First Name and Last Name for laboratory scientist
Experiment Name	Pilot Exercise SARS-Cov-2
Date	YYYY-MM-DD
Workflow	GenerateFASTQ
Application	FASTQ Only
Instrument Type	MiSeq
Library Prep Kit	Swift Normalase Amplicon NGS Panels (SNAP)
Index Kit	Swift Normalase Amplicon NGS Panels (SNAP)
Description	FDA Wastewater Protocol Pilot Exercise
Chemistry	Amplicon

Table 1: Header Section in Sequencing SampleSheet

Reads Section



1.2

**Enter the length of reads you are generating (could be different from 151)

Α	В		
Parameter	Description		
Number of cylces in Read 1	151		
Number of cycles in Read 2	151		

Table 2: Reader Section in Sequencing SampleSheet

1.3 Settings Section

Α	В		
ReverseComplement	0		

Table 3: Settings Section in Sequencing SampleSheet

1.4 Data Section: Include the machine ID in the Sample_Name column. e.g. sample_B01_M01234 where M01234 corresponds to the machine ID. This is the ID that will get populated to the fastq file names.

Α	В	С	D	E	F	G	Н	I	J
Sample_ID	Sample_Name	Sample_Plate	Sample_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
WPP_sample_B.01	WPP_sample_B.01_M01234	1	A1	SU001	TTGTTCCTTG	15_1	CCGAACAACA	LabAbreviation- WPP	
WPP_sample_C.01	WPP_sample_C.01_M01234	1	B1	SU002	AGGTTGTGTT	15_2	AAGAATCGGC	LabAbreviation- WPP	
WPP_sample_SA- 1.01	WPP_sample_SA- 1.01_M01234	1	C1	SU003	TATTGGTTGG	15_3	CACTTCGCTT	LabAbreviation- WPP	
WPP_sample_SA- 2.01	WPP_sample_SA- 2.01M01234	1	D1	SU004	CACTTCGCTT	15_4	TATTGGTTGG	LabAbreviation- WPP	

Table 4: Data Section in Sequencing SampleSheet (dashes converted to underscores for Illumina requirements)

1.5 If a new library or sequencing protocol is utilized for processing the pilot exercise samples, make the changes in the corresponding parameters for the sequencing sample sheet.

2 Starting your MiSeq run and connecting to BaseSpace:

When you set up the run on the MiSeq, select the option to log in to BaseSpace.

Transfer sequencing RUN through BaseSpace

- 3 Click the Runs tab in the Illumina BaseSpace website.
 - 3.1 Select the **run** that you would like to share with the FDA LFFM wastewater project team.
 - 3.2 Go to the summary tab and click at the share button.
 - 3.3 Enter the email address for the FDA team (covidtrakr@fda.hhs.gov), and then click Add
 - 3.4 Click Save Settings. Your run will be automatically shared with the FDA LFFM wastewater project team.

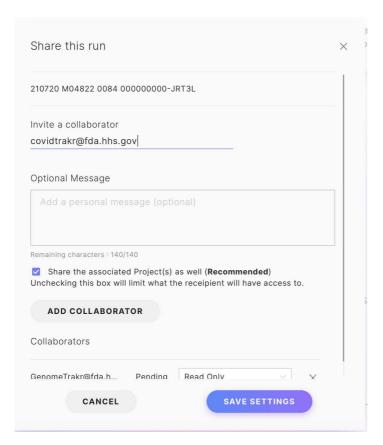


Figure 1: Sharing data via BaseSpace by Email Invitation

Transfer sequencing PROJECT through BaseSpace

- 4 Click the **Projects** tab in the Illumina BaseSpace website.
 - 4.1 Select the **project** that you would like to share with the FDA LFFM wastewater project team.
 - 4.2 Click at the share project button.
 - 4.3 Enter the email address for the FDA team (covidtrakr@fda.hhs.gov), and then click Add Collaborator.
 - **4.4** Click Save Settings. Your project will be automatically shared with the FDA LFFM wastewater project team.