How to make a sample sheet on BaseSpace?

Login Basespace (lmtmiseq4@gmail.com)—runs— click right corner new run—instrument run set up—instrument platfrom -- Run name--local—illumina Dragen fastq generation 3.9.34—not specified—not specified—1 index--paired end—159 8, 159 0—sample name ---i7 index in read1– fastq—gzip—under submit Run --export sample sheet.

Login—runs— click right corner new run—instrument run set up—Run name

--local—illumina Dragen Enrichment-3.8.4—not specified—not specified—1 index

--paired end—159 8, 159 0—sample name ---i7 index –index2(if run duel indexes, 10 random seq)--next—hg38 custom Alt Aware

--illumina\_exome\_targetedRegions\_V12.hg38.bed—somatic—Cram—yes fastq—gzip—export

sample sheet.

How to pool samples?

Quantify sample using Qubit and Tapestation. Measure both ng/ul and nM.

Dilute each sample to 10nM or 4nM based on Qubit results using low TE.

Pool samples with equal volume. Still 10nM in concentration.

Dilute the pool sample to 1nM using the provided buffer on magnet.

Add iphex (1pM, in reagent paper box) 0.5ul in 23 ul or 2%

Add iphex (1pM, in reagent paper box) 2.5ul in 50 ul or 5%

How to run Nextseq2000?

Take out reagent kit and put in room temp for 4-8 hrs then put 4C O/N.

If reagent kit was ON at 4C, put at room temp for 2 hr.

Take out flow cell, warm up to RT, or 37C 5 min.

Start-setup new run—select sample sheet – Run Review – output folder:external /nextseq runs(check space) –-denature on board –-reverse 10 times—slightly tap—insert flowcell –remove shell – add samples--taps—insert cartridge -- Instrument check (humidity out of range warning, ignore) and fluidics check (sounds from punching holes) take 20 mins

Start with a final concentration 1nM

24ul samples add to bottom of the well

Bed file and samples sheet in desktop sample sheet folder

Human use S31285117—bed file

disk management –Delete run to free space

if disk management can’t delete, go to computer—usr-local-illumina-runs-deleted old runs.

How to retrieves I2 fastq files containing molecular barcodes?

OverrideCycles Y154;I8;U10;Y154

Put OverrideCycles info in front of [BCLConvert\_Settings]

the UMI info will put into the fastq header line.

Illumine tech support 8008094566 case# 02412089

https://support-docs.illumina.com/SW/BCL\_Convert/Content/SW/BCLConvert/SampleSheets\_swBCL.htm

How to requeue on Nestseq2000?

Process management

Requeue

Sample sheet choose desktop—sample sheet

Output folder usr—local—illumina—images

How to check the quality of the run?

USR—LOCAL—ILLUMINA—RUNS—20210619—PrimaryAnalsis Matrix

External drive-Runs---20210619—PrimaryAnalsis Matrix

Loading concentration should be 95-99% if 90% add 10% more for next run if 80% add 10% more in next run.

Total reads >400

How to requeue on Miseq?

Data—illumina—miseq analysis—20210619—data—intensities—basecalls

Data—illumina—miseq analysis—20210619—change sample sheet

IE—local—Miseq reporter 2.6.2

Tips

XT has no UMI

XT low index 1-192 differ from XT index A01-H12

How to run sureselect libraries on Miseq?

I\_n\_ \_c\_o\_l\_u\_m\_n\_ \_7\_ \_u\_n\_d\_e\_r\_ \_**I5\_Index\_ID**,\_ \_e\_n\_t\_e\_r\_ \_*MolBC* f\_o\_r\_ \_a\_l\_l\_ \_s\_a\_m\_p\_l\_e\_s\_.\_ \_I\_n\_ \_c\_o\_l\_u\_m\_n\_ \_8\_ \_u\_n\_d\_e\_r\_ \_**index2**,\_ \_e\_n\_t\_e\_r\_ \_t\_e\_x\_t\_ \_“N\_N\_N\_N\_N\_N\_N\_N\_N\_N\_” \_f\_o\_r\_ \_a\_l\_l\_ \_s\_a\_m\_p\_l\_e\_s\_ \_t\_o\_ \_r\_e\_p\_r\_e\_s\_e\_n\_t\_ \_t\_h\_e\_ \_d\_e\_g\_e\_n\_e\_r\_a\_t\_e\_ \_1\_0\_-\_n\_u\_c\_l\_e\_o\_t\_i\_d\_e\_ \_m\_o\_l\_e\_c\_u\_l\_a\_r\_ \_b\_a\_r\_c\_o\_d\_e\_ \_t\_a\_g\_g\_i\_n\_g\_ \_e\_a\_c\_h\_ \_f\_r\_a\_g\_m\_e\_n\_t\_.\_

Adjust the MiSeq Reporter settings to generate FASTQ files for index reads. Once changed, this setting is retained for future runs. To change this setting, open the file **MiSeq Reporter.exe.config**. Under the **<appSettings>** tag, add **<add key="CreateFastqForIndexReads" value="1"/>**. You must restart the instrument for this setting change to take effect.

How to unlock a snakemake folder

# Navigate to working directory

cd /path/to/working/dir

# Load the right version of snakemake

modue load snakemake

# Unlock the working directory

snakemake --unlock

# remove a git directory

rm -rf .git

rm -r dir

# config.json need to be removed if the repo updated.