

# Guidelines to Prepare a Sample Sheet for Demultiplexing NEBNext Direct GS Libraries using *bcl2fastq*

## NEBNext DIRECT<sup>®</sup> GENOTYPING SOLUTION PANELS (NEB #E9500/#E9530)

1. Download an NEBNext Direct GS sample sheet for use with *bcl2fastq* from the product page for the NEBNext Direct GS Target Enrichment Kit (NEB #E9530).
2. Update the (Data) fields with the barcodes used in your experiment. The *bcl2fastq* software uses the sample barcodes to demultiplex in the order in which they are read by the sequencer. Therefore, the “index” column is required to contain the sample barcodes from the Read1 site, while the “index2” column is required to contain the sample barcodes from the i7 site.
3. Save the sample sheet as a csv file (.csv) for use with the *bcl2fastq* software. Do not use this sample sheet to run NEBNext Direct GS libraries on an Illumina sequencer.

### Example *bcl2fastq* Sample Sheet:

[Header]								
ITEMVersion	4							
Investigator Name								
Experiment Name								
Date								
Workflow	GenerateFASTQ							
Application	FASTQ Only							
Assay	TruSeq HT							
Description								
Chemistry								
[Reads]								
	20							
	75							
[Settings]								
[Data]								
Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Inline_ID	index	I7_Index_ID	index2	Sample_Project
1				5GS001-A1	TTCCGCTCA	DGS01	ATTACTCG	
2				5GS002-B1	TATGGCAC	DGS01	ATTACTCG	
3				5GS003-C1	CGTATCTC	DGS01	ATTACTCG	
4				5GS004-D1	GTCATCGT	DGS01	ATTACTCG	
5				5GS005-E1	TTACCGAC	DGS01	ATTACTCG	
6				5GS006-F1	TTCCCTTT	DGS01	ATTACTCG	
7				5GS007-G1	CAAGGTAC	DGS01	ATTACTCG	
8				5GS008-H1	CCGCTTAA	DGS01	ATTACTCG	
9				5GS009-A2	GTCCTTGA	DGS01	ATTACTCG	
10				5GS010-B2	GAATCACC	DGS01	ATTACTCG	
11				5GS011-C2	TTACGTGC	DGS01	ATTACTCG	
12				5GS012-D2	CAATGCGA	DGS01	ATTACTCG	
...				...	...	...	...	...



- A. The Inline\_ID/index columns should contain the inline sample IDs/indexes that were sequenced in Read1.
- B. The I7\_Index\_ID/index2 columns should contain the pool IDs/indexes that were sequenced from the i7 site (Index1).

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