

Guidelines for Setting Up a Sample Sheet for Illumina[®] Sequencing

NEBNext DIRECT[®] GENOTYPING SOLUTION PANELS (NEB #E9500B/#E9530B)

1. Download an NEBNext Direct GS sample sheet containing all 768 barcode combinations 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.
3. Add optional, user specific information in the remaining (Data) fields.
4. Save the sample sheet as a csv file (.csv) and transfer the sample sheet to an Illumina sequencer.

Example Sample Sheet for Illumina Sequencing:

[Header]								
IEMFileVersion	4 A							
Investigator Name								
Experiment Name								
Date								
Workflow	GenerateFASTQ							
Application	FASTQ Only							
Assay	TruSeq HT B							
Description								
Chemistry								
[Reads]								
20	C							
75	D							
[Settings]								
[Data]								
Sample_ID	Sample_Nan	Sample_Plats	Sample_Wel	Inline_ID	Index	I7_Index_ID	Index2	Sample_Project
1				5G5001-A1	TTCCGCTCA	DG501	ATTACTCG	
2				5G5002-B1	TATGGCAC	DG501	ATTACTCG	
3				5G5003-C1	CGTATCTC	DG501	ATTACTCG	
4				5G5004-D1	GTTCATCGT	DG501	ATTACTCG	
5				5G5005-E1	TTACCGAC	DG501	ATTACTCG	
6				5G5006-F1	TTCCCTTT	DG501	ATTACTCG	

- A. For use with Illumina Experiment Manager v5, change this value to 5.
- B. For use with Illumina Experiment Manager v5, change the Assay field to “TruSeq Nano DNA”.
- C. 20 cycles of Read1 sequences the 12 nt unique molecular identifier (UMI) followed by the 8 nt inline sample index.
- D. 75 cycles of Read2 sequences the targeted regions containing loci of interest.
- E. The Inline_ID/Index0 fields indicate the sample barcodes that are added during 5´ Adaptor ligation. This column will not be recognized by the sequencer and is for documentation purposes only.
- F. The I7_Index_ID/index fields instruct the sequencer to read 8 cycles of Index1 to sequence the pool index. The pool index is added to each pool of samples that is processed through the NEBNext Direct GS Target Enrichment Kit as a single reaction using an indexed PCR primer mix during the Library Amplification step of the protocol.
- G. If a single target enrichment library pool is sequenced alone (only one i7 barcode is used), these fields should be left blank in order to instruct the sequencer to omit the Index 1 read.

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