

Guidelines to Prepare a Sample Metadata File for Demultiplexing NEBNext Direct GS Libraries using Picard Tools

NEBNext DIRECT[®] GENOTYPING SOLUTION PANELS (NEB #E9500/#E9530)

1. Download an NEBNext Direct GS sample_metadata.txt file for use with Picard tools from the product page for the NEBNext Direct GS Target Enrichment Kit (NEB #E9530).
2. Update the file with the barcodes used in your experiment. Each step to demultiplex a sequencing run with Picard tools has its own requirements for identifying metadata, so there are some columns with redundant information. The sample barcodes are defined in the order in which they are read by the sequencer, with the inline barcode defined as barcode1 and the i7 barcode defined as barcode2.
3. The sample_metadata.txt file can be edited in Excel and saved as a tab delimited text file (.txt).

Example Sample Metadata File for Picard:

library_name	OUTPUT_PREFIX	barcode_name	A		B	
			barcode_sequence_1	BARCODE_1	barcode_sequence_2	BARCODE_2
1	1	5GS001-A1-DGS01	TTCGCTCA	TTCGCTCA	ATTACTCG	ATTACTCG
2	2	5GS002-B1-DGS01	TATGGCAC	TATGGCAC	ATTACTCG	ATTACTCG
3	3	5GS003-C1-DGS01	CGTATCTC	CGTATCTC	ATTACTCG	ATTACTCG
4	4	5GS004-D1-DGS01	GTCATCGT	GTCATCGT	ATTACTCG	ATTACTCG
5	5	5GS005-E1-DGS01	TTACCGAC	TTACCGAC	ATTACTCG	ATTACTCG
6	6	5GS006-F1-DGS01	TTCCCTTT	TTCCCTTT	ATTACTCG	ATTACTCG
7	7	5GS007-G1-DGS01	CAAGGTAC	CAAGGTAC	ATTACTCG	ATTACTCG
8	8	5GS008-H1-DGS01	CCGCTTAA	CCGCTTAA	ATTACTCG	ATTACTCG
9	9	5GS009-A2-DGS01	GTCCTTGA	GTCCTTGA	ATTACTCG	ATTACTCG
10	10	5GS010-B2-DGS01	GAATCACC	GAATCACC	ATTACTCG	ATTACTCG
11	11	5GS011-C2-DGS01	TTACGTGC	TTACGTGC	ATTACTCG	ATTACTCG
12	12	5GS012-D2-DGS01	CAATGCGA	CAATGCGA	ATTACTCG	ATTACTCG
13	13	5GS013-E2-DGS01	TCGTCTGA	TCGTCTGA	ATTACTCG	ATTACTCG
14	14	5GS014-F2-DGS01	GAACGAAG	GAACGAAG	ATTACTCG	ATTACTCG
15	15	5GS015-G2-DGS01	AGACCTTG	AGACCTTG	ATTACTCG	ATTACTCG
16	16	5GS016-H2-DGS01	GACCAACT	GACCAACT	ATTACTCG	ATTACTCG

- A. The barcode_sequence_1/BARCODE_1 columns should contain the inline sample indexes that were sequenced in Read1.
- B. The barcode_sequence_2/BARCODE_2 columns should contain the pool indexes that were sequenced from the i7 site (Index1).

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