

DNBSEQ Dual Barcode Library Pooling Guide

MGI Tech Co., Ltd

Announcement

This document lists the dual-adapter sequences of MGIEasy UDB Universal Library Prep Set/MGIEasy UMI Universal Library Prep Set for DNBSEQ Platforms. It also document provides recommendations for barcode balance when pooling dual barcode libraries.

This guidance only lists the information of MGIEasy UDB Universal Library Prep Set/MGIEasy UMI Universal Library Prep Set. MGI is unable to provide pooling guide for unauthorized suppliers.

Equally pooling strategy is recommended in this guidance. If unequal pooling is needed, check the base content balance rule (table 1 & 2) for special pooling strategy. Use the recommended barcode set (like Set B in UDB Primers Adapter Kit) to pool with other libraries.

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Base Content Balance

Base Content Balance Rule is the basic rule of sequencing in MGI Platform. Check the base content before pooling every time when dealing with special pooling situation (including but not limited to unequal pooling/multi-library-type pooling)

For base balance, each base content is not less than 12.5% and not more than 62.5% in single sequencing position in the same lane. (see examples in table 1 and table 2)

Table 1 perfect balanced 8 barcode Pooling strategy (8 barcode from one entire column)

Sample 1	A	G	G	A	C	G	T	A	G	A
Sample 2	C	T	G	A	A	C	C	G	A	A
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	C	C	T	G	A	A	G	G	A	T
Sample 7	T	T	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	C	T	A	C	C
Signal%	25.0%	25.0%	25.0%	25.0%	25.0%	25.0%	25.0%	25.0%	25.0%	25.0%

Table 2 unacceptable 9 barcode Pooling strategy (barcodes from different column)

Sample 1	A	G	G	A	C	G	T	A	G	T
Sample 2	A	C	G	A	A	G	G	T	C	C
Sample 3	G	A	A	C	G	T	G	T	C	G
Sample 4	T	C	C	G	T	G	A	C	T	C
Sample 5	A	A	T	T	C	A	C	T	G	T
Sample 6	G	C	T	G	A	A	G	G	A	T
Sample 7	T	G	C	C	T	T	A	C	T	G
Sample 8	G	G	A	T	G	A	T	A	C	C
Sample 9	G	A	C	G	G	T	C	G	A	G
A signal%	33.3%	33.3%	22.2%	22.2%	22.2%	33.3%	22.2%	22.2%	22.2%	0%
T signal%	22.2%	0%	22.2%	22.2%	22.2%	33.3%	22.2%	33.3%	22.2%	33.3%
C signal%	0%	33.3%	33.3%	22.2%	22.2%	0%	22.2%	22.2%	33.3%	33.3%
G signal%	44.4%	33.3%	22.2%	33.3%	33.3%	33.3%	33.3%	22.2%	22.2%	33.3%

MGIEasy UDB/UMI Library Prep Set Pooling Guide

This chapter is about the pooling strategy of MGIEasy UDB/UMI Library Prep Set.

Table 3. MGIEasy UDB/UMI Library Prep Product

Product	Lot. No.	Barcode Group	规格
MGIEasy UDB Primers Adapter Kit A	1000022801	Set A	96 RXN
MGIEasy UDB Primers Adapter Kit B	1000022802	Set B	96 RXN
MGIEasy UDB Universal Library Prep Set	1000022803	Set A	16 RXN
MGIEasy UDB Universal Library Prep Set	1000022804	Set B	96 RXN
MGIEasy UDB Universal Library Prep Set	1000022805	Set A+ Set B	192 RXN
MGIEasy Duplex UMI Universal Library Prep Set	1000018643	Set A	16 RXN
MGIEasy Duplex UMI Universal Library Prep Set	1000019376	Set A	96 RXN

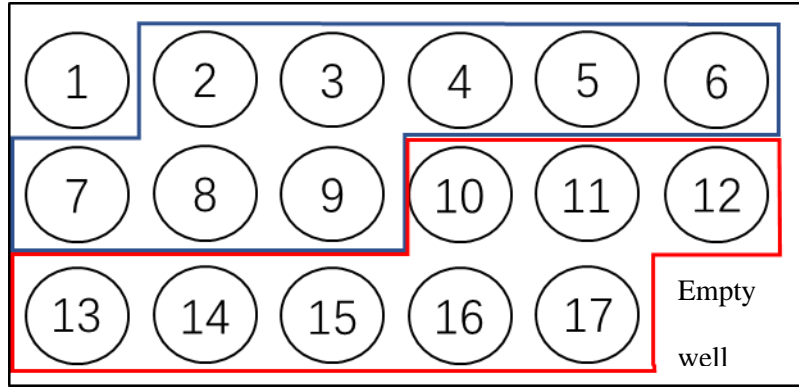


Set B is the only workable set for pooling with other libraries not listed above (like Amplicon libraries). Check the Pooling plan before purchase.

For 16 RXN Library Prep Set, 2 group of barcodes (8 barcodes for 1 group) are loaded: UDB PCR Primer Mix-57~ UDB PCR Primer Mix-64; UDB PCR Primer Mix-89~ UDB PCR Primer Mix-96. Pool at least one group (8 barcodes) in a run is recommended.

Table 4. Two Barcode groups that can be combined in 16 RXN Library Prep Set

(First group: blue frame, Second group: red frame)



For 96 RXN Library Prep Set, eight wells of each column are preset as a balanced dual barcode combination. Use table 5 to plan X-plex pooling ($X \geq 8$) strategies. Examples of each barcode combination in 8 to 13-plex pool are color-coded in plate layout of Table 6.

Table 5. Dual barcode Pooling Guide

Plexity	Combinations
8X	X entire column
8X+1	X entire column 1 random well
8X+2	X entire column 2 random well
8X+3	X entire column 3 random well
8X+4	X entire column 4 random well
8X+5	X entire column 5 random well
8X+6	X entire column 6 random well
8X+7	X entire column 7 random well

Table 6. Dual barcode 8-13 samples Pooling example

8	9	10	11	12	13	11
1	9 17	25 33	41 49	57 65	73 81	89
2	10 18	26 34	42 50	58 66	74 82	90
3	11 19	27 35	43 51	59 67	75 83	91
4	12 20	28 36	44 52	60 68	76 84	92
5	13 21	29 37	45 53	61 69	77 85	93
6	14 22	30 38	46 54	62 70	78 86	94
7	15 23	31 39	47 55	63 71	79 87	95
8	16 24	32 40	48 56	64 72	80 88	96