

# Index Pooling Guidelines

## NEBNEXT DIRECT<sup>®</sup> CUSTOM READY PANEL (NEB #E6631)

To download a sample sheet with the index sequences, visit the “Usage Guidelines & Tips” panel in the “Other Tools & Resources” tab on [www.neb.com/E6631](http://www.neb.com/E6631) – NEBNext Direct Custom Ready Panels (NEB #E6631).

For the HiSeq<sup>®</sup>/MiSeq, Illumina uses a red laser/LED to sequence bases A and C and a green laser/LED to sequence bases G and T. For each cycle, both the red and the green channel need to be read to ensure proper image registration (i.e. A or C must be in each cycle, and G or T must be in each cycle). If this color balance is not maintained, sequencing the index read could fail.

For the NovaSeq<sup>®</sup>/NextSeq<sup>®</sup>/MiniSeq<sup>®</sup> which utilize 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles.

Tables 1, 2 and 3 list some valid combinations (up to 4-plex) that can be sequenced together. For combinations > 4 choose any 4-plex combination and add any other index as needed.

For valid index combinations beyond what are listed in Tables 1–3, refer to Tables 4–7.

**Table 1: Valid index primer combinations for NEB #E6631S.**

PLEX	INDEX PRIMER
2	D01 and D02 D03 and D04 D05 and D06 D07 and D08
3	D01, D02 and D03 D02, D03 and D04 D03, D04 and D05 D04, D05 and D06 D05, D06 and D07 D06, D07 and D08
4	D01, D02, D03 and D04 D01, D04, D05 and D08 D02, D03, D04 and D05 D02, D04, D06 and D07 D03, D04, D05 and D06 D03, D05, D07 and D08 D04, D05, D06 and D07 D04, D05, D07 and D08 D05, D06, D07 and D08

**Table 2: Valid index primer combinations for NEB #E6631L.**

PLEX	INDEX PRIMER
2	D01 and D02 D03 and D04 D05 and D06 D07 and D08 D09 and D10 D11 and D12 D17 and D23
3	D01, D02 and D03 D02, D05 and D16 D04, D05 and D06 D07, D08 and D09 D10, D11 and D12 D11, D12 and D14 D13, D17 and D22 D17, D23 and D24
4	D01, D02, D03 and D04 D02, D04, D06 and D07 D03, D04, D14 and D17 D05, D06, D07 and D08 D07, D08, D13 and D22 D08, D14, D21 and D22 D09, D10, D11 and D12 D13, D14, D16 and D17 D15, D17, D22 and D23 D16, D17, D20 and D24



Table 6: Index sequences for NEB #E6631L.

INDEX PRIMER	INDEX SEQUENCE READ							
D01	A	T	T	A	C	T	C	G
D02	T	C	C	G	G	A	G	A
D03	C	G	C	T	C	A	T	T
D04	G	A	G	A	T	T	C	C
D05	A	T	T	C	A	G	A	A
D06	G	A	A	T	T	C	G	T
D07	C	T	G	A	A	G	C	T
D08	T	A	A	T	G	C	G	C
D09	C	G	G	C	T	A	T	G
D10	T	C	C	G	C	G	A	A
D11	T	C	T	C	G	C	G	C
D12	A	G	C	G	A	T	A	G
D13	A	G	T	C	A	A	C	A
D14	A	G	T	T	C	C	G	T
D15	A	T	G	T	C	A	G	A
D16	C	C	G	T	C	C	A	T
D17	G	T	C	C	G	C	A	C
D18	G	T	G	A	A	A	C	G
D19	G	T	G	G	C	C	T	T
D20	G	T	T	T	C	G	G	A
D21	C	G	T	A	C	G	T	A
D22	G	A	G	T	G	G	A	T
D23	A	C	T	G	A	T	A	T
D24	A	T	T	C	C	T	T	T

Table 7: Index sequences for NEB #E6631X.

INDEX PRIMER	WELL POSITION	INDEX SEQUENCE READ							
DHT1	A1	T	T	A	C	C	G	A	C
DHT2	A2	A	G	T	G	A	C	C	T
DHT3	A3	T	C	G	G	A	T	T	C
DHT4	A4	C	A	A	G	G	T	A	C
DHT5	A5	T	C	C	T	C	A	T	G
DHT6	A6	G	T	C	A	G	T	C	A
DHT7	A7	C	G	A	A	T	A	C	G
DHT8	A8	T	C	T	A	G	G	A	G
DHT9	A9	C	G	C	A	A	C	T	A
DHT10	A10	C	G	T	A	T	C	T	C
DHT11	A11	G	T	A	C	A	C	C	T
DHT12	A12	C	G	G	C	A	T	T	A
DHT13	B1	T	C	G	T	C	T	G	A
DHT14	B2	A	G	C	C	T	A	T	C
DHT15	B3	C	T	G	T	A	C	C	A
DHT16	B4	A	G	A	C	C	T	T	G
DHT17	B5	A	G	G	A	T	A	G	C
DHT18	B6	C	C	T	T	C	C	A	T
DHT19	B7	G	T	C	C	T	T	G	A
DHT20	B8	T	G	C	G	T	A	A	C
DHT21	B9	C	A	C	A	G	A	C	T
DHT22	B10	T	T	A	C	G	T	G	C
DHT23	B11	C	C	A	A	G	G	T	T
DHT24	B12	C	A	C	G	C	A	A	T
DHT25	C1	T	T	C	C	A	G	G	T
DHT26	C2	T	C	A	T	C	T	C	C
DHT27	C3	G	A	G	A	G	T	A	C
DHT28	C4	G	T	C	G	T	T	A	C
DHT29	C5	G	G	A	G	G	A	A	T
DHT30	C6	A	G	G	A	A	C	A	C
DHT31	C7	C	A	G	T	G	C	T	T
DHT32	C8	C	T	T	G	C	T	A	G
DHT33	C9	T	G	G	A	A	G	C	A
DHT34	C10	A	G	C	T	A	A	G	C
DHT35	C11	G	A	A	C	G	G	T	T
DHT36	C12	G	G	A	A	T	G	T	C
DHT37	D1	T	A	C	G	G	T	C	T
DHT38	D2	C	C	A	G	T	A	T	C
DHT39	D3	T	C	T	A	C	G	C	A
DHT40	D4	G	T	A	A	C	C	G	A
DHT41	D5	G	A	C	G	T	C	A	T
DHT42	D6	C	T	T	A	C	A	G	C
DHT43	D7	T	C	C	A	T	T	G	C
DHT44	D8	A	G	C	G	A	G	A	T
DHT45	D9	C	A	A	T	A	G	C	C
DHT46	D10	A	A	G	A	C	A	C	C
DHT47	D11	C	C	A	G	T	T	G	A
DHT48	D12	T	G	G	T	G	A	A	G

INDEX PRIMER	WELL POSITION	INDEX SEQUENCE READ							
DHT49	E1	A	A	G	A	C	C	G	T
DHT50	E2	T	T	G	C	G	A	G	A
DHT51	E3	G	C	A	A	T	T	C	C
DHT52	E4	G	A	A	T	C	C	G	T
DHT53	E5	C	C	G	C	T	T	A	A
DHT54	E6	T	A	C	C	T	G	C	A
DHT55	E7	G	T	C	G	A	T	T	G
DHT56	E8	T	A	T	G	G	C	A	C
DHT57	E9	C	T	C	G	A	A	C	A
DHT58	E10	C	A	A	C	T	C	C	A
DHT59	E11	G	T	C	A	T	C	G	T
DHT60	E12	G	G	A	C	A	T	C	A
DHT61	F1	C	A	G	G	T	T	C	A
DHT62	F2	G	A	A	C	G	A	A	G
DHT63	F3	C	T	C	A	G	A	A	G
DHT64	F4	C	A	T	G	A	G	C	A
DHT65	F5	G	A	C	G	A	A	C	T
DHT66	F6	A	G	A	C	G	C	T	A
DHT67	F7	A	T	A	A	C	G	C	C
DHT68	F8	G	A	A	T	C	A	C	C
DHT69	F9	G	G	C	A	A	G	T	T
DHT70	F10	G	A	T	C	T	T	G	C
DHT71	F11	C	A	A	T	G	C	G	A
DHT72	F12	G	G	T	G	T	A	C	A
DHT73	G1	T	A	G	G	A	G	C	T
DHT74	G2	C	G	A	A	T	T	G	C
DHT75	G3	G	T	C	C	T	A	A	G
DHT76	G4	C	T	T	A	G	G	A	C
DHT77	G5	T	C	C	A	C	G	T	T
DHT78	G6	C	A	A	C	A	C	A	G
DHT79	G7	G	C	C	T	T	A	A	C
DHT80	G8	G	T	A	A	G	G	T	G
DHT81	G9	A	G	C	T	A	C	C	A
DHT82	G10	C	T	T	C	A	C	T	G
DHT83	G11	G	G	T	T	G	A	A	C
DHT84	G12	G	A	T	A	G	C	C	A
DHT85	H1	T	A	C	T	C	C	A	G
DHT86	H2	G	G	A	A	G	A	G	A
DHT87	H3	G	C	G	T	T	A	G	A
DHT88	H4	A	T	C	T	G	A	C	C
DHT89	H5	A	A	C	C	A	G	A	G
DHT90	H6	G	T	A	C	C	A	C	A
DHT91	H7	G	G	T	A	T	A	G	G
DHT92	H8	C	G	A	G	A	G	A	A
DHT93	H9	C	A	G	C	A	T	A	C
DHT94	H10	C	T	C	G	A	C	T	T
DHT95	H11	C	T	T	C	G	G	T	T
DHT96	H12	C	C	A	C	A	A	C	A

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