

Index Pooling Guidelines

NEBNEXT DIRECT[®] CANCER HOTSPOT PANEL (NEB #E7000)

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/E7000 – NEBNext Direct Cancer HotSpot Panel (NEB #E7000).

For the HiSeq[®]/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[®]/NextSeq[®]/MiniSeq[®] 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 #E7000S.

| 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 #E7000L.

| 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 3: Valid index primer combinations for NEB #E7000X.

| PLEX | INDEX PRIMER (WELL POSITION) |
|------|---|
| 2 | DHT18 (B6) and DHT22 (B10) DHT37 (D1) and DHT42 (D6) DHT52 (E4) and DHT76 (G4) DHT68 (F8) and DHT95 (H11) |
| 3 | DHT4 (A4), DHT11 (A11) and DHT12 (A12) DHT15 (B3), DHT22 (B10) and DHT24 (B12) DHT25 (C1), DHT31 (C7) and DHT33 (C9) DHT37 (D1), DHT42 (D6) and DHT48 (D12) DHT49 (E1), DHT54 (E6) and DHT55 (E7) DHT64 (F4), DHT69 (F9) and DHT71 (F11) DHT76 (G4), DHT77 (G5) and DHT83 (G11) DHT87 (H3), DHT93 (H9) and DHT94 (H10) |
| 4 | DHT1 (A1), DHT2 (A2), DHT3 (A3) and DHT4 (A4) DHT5 (A5), DHT6 (A6), DHT8 (A8) and DHT10 (A10) DHT13 (B1), DHT14 (B2), DHT15 (B3) and DHT16 (B4) DHT17 (B5), DHT18 (B6), DHT19 (B7) and DHT20 (B8) DHT25 (C1), DHT26 (C2), DHT27 (C3) and DHT30 (C6) DHT28 (C4), DHT29 (C5), DHT32 (C8) and DHT35 (C11) DHT37 (D1), DHT38 (D2), DHT39 (D3) and DHT40 (D4) DHT45 (D9), DHT46 (D10), DHT47 (D11) and DHT48 (D12) DHT49 (E1), DHT50 (E2), DHT51 (E3) and DHT52 (E4) DHT56 (E8), DHT58 (E10), DHT59 (E11) and DHT60 (E12) DHT61 (F1), DHT62 (F2), DHT63 (F3) and DHT69 (F9) DHT64 (F4), DHT65 (F5), DHT66 (F6) and DHT67 (F7) DHT73 (G1), DHT74 (G2), DHT75 (G3) and DHT76 (G4) DHT80 (G8), DHT82 (G10), DHT83 (G11) and DHT84 (G12) DHT85 (H1), DHT86 (H2), DHT87 (H3) and DHT89 (H5) DHT91 (H7), DHT94 (H10), DHT95 (H11) and DHT96 (H12) |

Table 4: For combinations of valid indexes, ensure that you will have signal in both the red and green channels in each cycle. See examples below:

| GOOD | | | | BAD | | | |
|--------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|
| PRIMER | INDEX SEQUENCE | PRIMER | INDEX SEQUENCE | PRIMER | INDEX SEQUENCE | PRIMER | INDEX SEQUENCE |
| DHT1 | T T A C C G A C | DHT41 | G A C G T C A T | DHT9 | C G C A A C T A | DHT56 | T A T G G C A C |
| DHT2 | A G T G A C C T | DHT42 | C T T A C A G C | DHT10 | C G T A T C T C | DHT57 | C T C G A A C A |
| DHT3 | T C G G A T T C | DHT43 | T C C A T T G C | DHT11 | G T A C A C C T | DHT58 | C A A C T C C A |
| DHT4 | C A A G G T A C | DHT44 | A G C G A G A T | DHT12 | C G G C A T T A | DHT59 | G T C A T C G T |
| | ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ | | ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ | | ✓ X ✓ X ✓ ✓ ✓ ✓ | | ✓ ✓ ✓ ✓ ✓ X ✓ ✓ |

Tables 5, 6 and 7 lists each index sequence color coded to correspond to the red/green channel.

Table 5: Index sequences for NEB #E7000S.

| 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 |

Table 6: Index sequences for NEB #E7000L.

| 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 #E7000X.

| 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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