

## NEBNext® Multiplex Oligos for MGI® (Dual Index Primer Pairs Set 1)

NEB #E9725S/L

24/96 reactions

Version 1.0\_06/24

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### The NEBNext Multiplex Oligos for MGI Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E9725S) or 96 reactions (NEB #E9725L). All reagents should be stored at  $-20^{\circ}\text{C}$ .

#### NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) NEB #E9725S

- (red) NEBNext Adaptor for MGI/Complete Genomics®

NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics

NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (1-24)

#### NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) NEB #E9725L

- (red) NEBNext Adaptor for MGI/Complete Genomics

NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics

NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (25-120)

### Overview

NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) contains adaptors and pre-plated index primer pairs that are ideally suited for multiplex sample preparation for next-generation sequencing on the MGI platform. Each kit component is required to pass rigorous quality control standards and, for each new lot the entire set of reagents, is functionally validated together by constructing and sequencing of indexed libraries on an MGI sequencing platform. Designed for use in library prep for DNA and RNA, the NEBNext Adaptor for MGI/Complete Genomics enables high-efficiency ligation and high library yields, with minimal adaptor-dimer formation. During PCR, barcodes can be incorporated via the NEBNext Dual Index Primer Pairs for MGI, thereby enabling multiplexing. The 10-base index primer pairs included in this kit are pre-mixed and are packaged in a single-use 96-well plate with a pierceable foil seal. NEBNext Multiplex Oligos for MGI can be used with NEBNext MGI products or with other standard MGI-compatible library preparation protocols.

**Please refer to the product page on NEB.com for FAQs about this product.**

Where larger volumes, customized or bulk packaging are required, we encourage consultation with the NEB Customized Solutions team. Please complete the NEB Custom Contact Form at [www.neb.com/CustomContactForm](http://www.neb.com/CustomContactForm) to learn more.

Please refer to the appropriate library prep kit-specific protocol for recommendations on using the NEBNext Multiplex Oligos for MGI.

**The following sequences are used for adaptor trimming of NEBNext adaptors for MGI/Complete Genomics:**

The adaptor sequences should be trimmed on both 5' ends and 3' ends.

Forward: AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA

Reverse: AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTG

## Section 1 Setting up the PCR Reactions

### Symbols



This caution sign signifies a step in the protocol with multiple paths leading to the same endpoint but is dependent on a user variable, like the number of samples being processed.

### 1. PCR Amplification



For < 96 samples, follow the protocol in Section 1.1. For 96 samples, follow the protocol in Section 1.2.

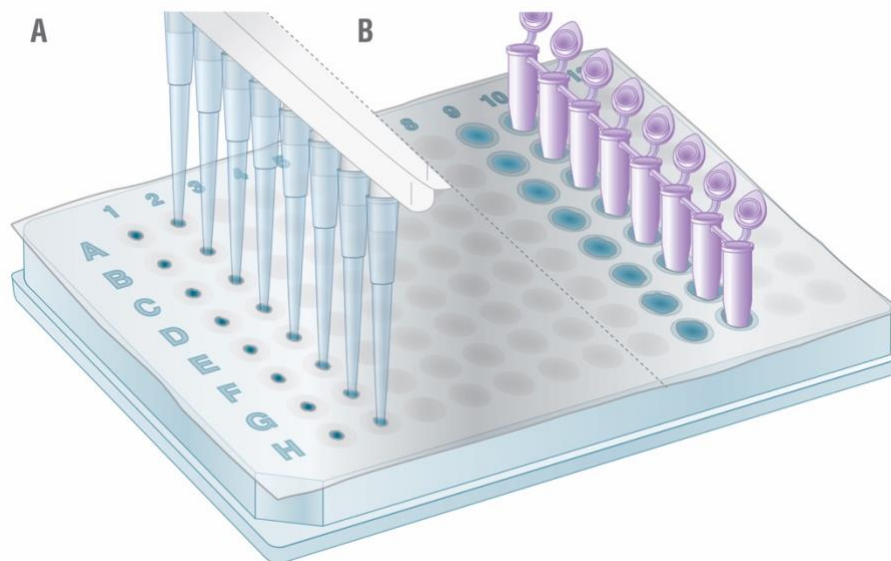
#### 1.1. Setting up the PCR reactions (< 96 samples)

- 1.1.1. Determine the number of libraries that will be amplified and pooled for subsequent sequencing.
- 1.1.2. Choose a valid combination of barcode primers based on the color balance guidelines in Section 2.
- 1.1.3. Thaw the NEBNext Dual Index Primer Pairs for MGI/Complete Genomics plate for 10–15 minutes at room temperature.
- 1.1.4. Remove the hard plastic plate cover. Mix briefly by vortexing and then centrifuge the plate ( $280 \times g$  for ~1 min) to collect all of the primer at the bottom of each well.
- 1.1.5. Orient the primer plate as indicated in Figure 1.1 (well A1 on the top left). With a pipette tip, pierce the desired well(s) (Figure 1.1A) and transfer the volume of primer mix required for the PCR reaction to the PCR plate/tubes (see specific library construction manual for protocol). It is important to change pipette tips before piercing a new well to avoid cross contamination of indexed primers. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

#### Note:

- a. Barcodes from each column (e.g. A1–H1; A2–H2; A3–H3) are color balanced for sequencing on the MGI DNBSEQ® platforms.
  - b. Each well contains a unique pair of index primers. There is enough primer in each well for one PCR reaction. Do not reuse primer if the seal has been previously pierced to avoid contamination with other index primers.
- 1.1.6. Proceed with the PCR reaction according to the specific library construction manual.
  - 1.1.7. Carefully seal the plate with remaining primer wells using the hard plastic cover and store it at  $-20^{\circ}\text{C}$  until its next use.

Figure 1.1. NEBNext Dual Index Primer Pairs for MGI



## 1.2. Setting up the PCR reactions (96 samples)

- 1.2.1. Thaw the NEBNext Dual Index Primer Pairs for MGI/Complete Genomics plate for 10–15 minutes at room temperature.
- 1.2.2. Remove the hard plastic plate cover. Mix briefly by vortexing and then centrifuge the plate ( $280 \times g$  for ~1 min) to collect all of the primer at the bottom of each well.
- 1.2.3. Orient the primer plate as indicated in Figure 1.1 (well A1 on the top left). With a pipette tip, pierce the wells (Figure 1.1A) and transfer the volume of primer mix required for the PCR reaction to the PCR plate (see specific library construction manual for protocol). It is important to change pipette tips before piercing a new well to avoid cross contamination of index primers. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

**Note: Each well contains a unique pair of index primers. There is enough primer in each well for one PCR reaction. Do not reuse primer if the seal has been previously pierced to avoid contamination with other index primers.**

- 1.2.4. Proceed with the PCR reaction according to the specific library construction manual.

## Section 2

### Index Pooling Guidelines



Barcodes from each column (e.g. A1–H1; A2–H2; A3–H3) are color balanced for sequencing on the MGI DNBSEQ platforms.

If multiplexing samples for circularization reaction, pool 8 libraries from each column to perform a circularization reaction. Multiple columns can be pooled for a circularization reaction.

Note:

1. To ensure proper demultiplexing, dual barcode sequencing on the DNBSEQ platforms with 10 cycles for both barcodes is required, as combinatorial dual indices are used in this product.
2. Use the barcode sequences in Table 2.1 and Table 2.2 to prepare your sample sheets if the “split barcode” option is used on the DNBSEQ instrument for demultiplexing. If demultiplexing is performed off the instrument (i.e., the “split barcode” option is unchecked when setting up the run; therefore, no sample sheets are required), use the reverse complement of column BC1 seq and BC2 seq for demultiplexing.
3. Each primer pair combination is unique (combinatorial indexing) in #E9725S and #E9725L, so the libraries generated from #E9725S and #E9725L can be pooled to achieve a total of 120 barcode pairs.

**Table 2.1 Barcode sequences for NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (1-24) #E9648 (24 reactions)**

| Well ID | BC1 Id  | BC1 seq     | BC2 Id  | BC2 seq    |
|---------|---------|-------------|---------|------------|
| A1      | BC1_345 | TGATTCCGAG  | BC2_184 | CCGTACCTGA |
| B1      | BC1_344 | CCGAGAGACC  | BC2_183 | TAATCTACTT |
| C1      | BC1_346 | G TTCATACGA | BC2_164 | GTTAGGTAGG |
| D1      | BC1_343 | AACGCATTCC  | BC2_179 | AGAGTACGAC |
| E1      | BC1_339 | TAATGGCCTT  | BC2_179 | AGAGTACGAC |
| F1      | BC1_346 | G TTCATACGA | BC2_186 | TGTCGCTCAC |
| G1      | BC1_323 | AGGACGTAGA  | BC2_165 | ACACATGTCA |
| H1      | BC1_328 | CCTGAAGGAT  | BC2_175 | AACGCAACTA |
| A2      | BC1_342 | CGTCTGATAT  | BC2_179 | AGAGTACGAC |
| B2      | BC1_335 | AATTCAGAGT  | BC2_182 | GCTCGTAACT |
| C2      | BC1_343 | AACGCATTCC  | BC2_171 | GTGATCTAGA |
| D2      | BC1_324 | CTGAACCGAA  | BC2_175 | AACGCAACTA |
| E2      | BC1_340 | GTGACCGGTA  | BC2_180 | ATCGAAGGTA |
| F2      | BC1_326 | TCCGTGACTC  | BC2_181 | CAGACGTTGG |
| G2      | BC1_330 | GGATGCTACC  | BC2_177 | TTGAGTCGAC |
| H2      | BC1_329 | TTCCTTACTG  | BC2_174 | CGATAGGTCG |
| A3      | BC1_332 | GACGGTCGAG  | BC2_185 | GTCATGGACG |
| B3      | BC1_327 | AATTCAGTGT  | BC2_167 | AAGCTCTGTC |
| C3      | BC1_344 | CCGAGAGACC  | BC2_166 | CGCTCACGAT |
| D3      | BC1_337 | TTGCTTCGTT  | BC2_171 | GTGATCTAGA |
| E3      | BC1_331 | CGACGATATG  | BC2_170 | TGCTCACAGA |
| F3      | BC1_334 | TTAACGACGA  | BC2_165 | ACACATGTCA |
| G3      | BC1_338 | GGCTACTTAC  | BC2_163 | TATGGCACTG |
| H3      | BC1_336 | CCTGTAACCA  | BC2_176 | CCATACATAT |

**Table 2.2 Barcode sequences for NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (25-120)  
#E9649 (96 reactions)**

| Well ID | BC1 Id  | BC1 seq     | BC2 Id  | BC2 seq    |
|---------|---------|-------------|---------|------------|
| A1      | BC1_329 | TTCCTTACTG  | BC2_168 | CCAGAGATCT |
| B1      | BC1_343 | AACGCATTCC  | BC2_167 | AAGCTCTGTC |
| C1      | BC1_324 | CTGAACCGAA  | BC2_170 | TGCTCACAGA |
| D1      | BC1_335 | AATCCGAGT   | BC2_169 | GTGATTGCAC |
| E1      | BC1_330 | GGATGCTACC  | BC2_164 | GTTAGGTAGG |
| F1      | BC1_326 | TCCGTGACTC  | BC2_175 | AACGCAACTA |
| G1      | BC1_328 | CCTGAAGGAT  | BC2_174 | CGATAGGTCG |
| H1      | BC1_325 | GAACGTGTCG  | BC2_177 | TTGAGTCGAC |
| A2      | BC1_335 | AATCCGAGT   | BC2_181 | CAGACGTTGG |
| B2      | BC1_337 | TTGCTTCGTT  | BC2_166 | CGCTCACGAT |
| C2      | BC1_331 | CGACGATATG  | BC2_169 | GTGATTGCAC |
| D2      | BC1_332 | GACGGTCGAG  | BC2_170 | TGCTCACAGA |
| E2      | BC1_336 | CCTGTAACCA  | BC2_167 | AAGCTCTGTC |
| F2      | BC1_333 | ACGAAGGTCC  | BC2_165 | ACACATGTCA |
| G2      | BC1_338 | GGCTACTTAC  | BC2_182 | GCTCGTAACT |
| H2      | BC1_334 | TTAACGACGA  | BC2_163 | TATGGCACTG |
| A3      | BC1_324 | CTGAACCGAA  | BC2_167 | AAGCTCTGTC |
| B3      | BC1_331 | CGACGATATG  | BC2_185 | GTCATGGACG |
| C3      | BC1_326 | TCCGTGACTC  | BC2_183 | TAATCTACTT |
| D3      | BC1_327 | AATTCAGTGT  | BC2_186 | TGTCGCTCAC |
| E3      | BC1_340 | GTGACCGGTA  | BC2_168 | CCAGAGATCT |
| F3      | BC1_335 | AATCCGAGT   | BC2_184 | CCGTACCTGA |
| G3      | BC1_325 | GAACGTGTCG  | BC2_169 | GTGATTGCAC |
| H3      | BC1_326 | TCCGTGACTC  | BC2_170 | TGCTCACAGA |
| A4      | BC1_344 | CCGAGAGACC  | BC2_175 | AACGCAACTA |
| B4      | BC1_323 | AGGACGTAGA  | BC2_184 | CCGTACCTGA |
| C4      | BC1_346 | G TTCATACGA | BC2_176 | CCATACATAT |
| D4      | BC1_346 | G TTCATACGA | BC2_185 | GTCATGGACG |
| E4      | BC1_343 | AACGCATTCC  | BC2_177 | TTGAGTCGAC |
| F4      | BC1_328 | CCTGAAGGAT  | BC2_179 | AGAGTACGAC |
| G4      | BC1_339 | TAATGGCCTT  | BC2_164 | GTTAGGTAGG |
| H4      | BC1_345 | TGATTCCGAG  | BC2_178 | GGTCCGGCCG |
| A5      | BC1_343 | AACGCATTCC  | BC2_170 | TGCTCACAGA |
| B5      | BC1_346 | G TTCATACGA | BC2_169 | GTGATTGCAC |
| C5      | BC1_339 | TAATGGCCTT  | BC2_178 | GGTCCGGCCG |
| D5      | BC1_345 | TGATTCCGAG  | BC2_176 | CCATACATAT |
| E5      | BC1_341 | ACCGATTAGG  | BC2_180 | ATCGAAGGTA |
| F5      | BC1_334 | TTAACGACGA  | BC2_172 | TCTGTATAGT |
| G5      | BC1_344 | CCGAGAGACC  | BC2_173 | AACCGTCGTC |
| H5      | BC1_328 | CCTGAAGGAT  | BC2_181 | CAGACGTTGG |
| A6      | BC1_338 | GGCTACTTAC  | BC2_171 | GTGATCTAGA |
| B6      | BC1_328 | CCTGAAGGAT  | BC2_177 | TTGAGTCGAC |
| C6      | BC1_327 | AATTCAGTGT  | BC2_173 | AACCGTCGTC |
| D6      | BC1_344 | CCGAGAGACC  | BC2_176 | CCATACATAT |
| E6      | BC1_339 | TAATGGCCTT  | BC2_185 | GTCATGGACG |
| F6      | BC1_329 | TTCCTTACTG  | BC2_178 | GGTCCGGCCG |
| G6      | BC1_334 | TTAACGACGA  | BC2_179 | AGAGTACGAC |
| H6      | BC1_323 | AGGACGTAGA  | BC2_174 | CGATAGGTCG |

| Well ID | BC1 Id  | BC1 seq    | BC2 Id  | BC2 seq    |
|---------|---------|------------|---------|------------|
| A7      | BC1_344 | CCGAGAGACC | BC2_186 | TGTCGCTCAC |
| B7      | BC1_340 | GTGACCGGTA | BC2_183 | TAATCTACTT |
| C7      | BC1_336 | CCTGTAACCA | BC2_164 | GTTAGGTAGG |
| D7      | BC1_337 | TTGCTTCGTT | BC2_173 | AACCGTCGTC |
| E7      | BC1_325 | GAACGTGTCG | BC2_175 | AACGCAACTA |
| F7      | BC1_342 | CGTCTGATAT | BC2_185 | GTCATGGACG |
| G7      | BC1_341 | ACCGATTAGG | BC2_184 | CCGTACCTGA |
| H7      | BC1_338 | GGCTACTTAC | BC2_165 | ACACATGTCA |
| A8      | BC1_339 | TAATGGCCTT | BC2_174 | CGATAGGTCG |
| B8      | BC1_345 | TGATTCCGAG | BC2_166 | CGCTCACGAT |
| C8      | BC1_344 | CCGAGAGACC | BC2_182 | GCTCGTAACT |
| D8      | BC1_323 | AGGACGTAGA | BC2_180 | ATCGAAGGTA |
| E8      | BC1_328 | CCTGAAGGAT | BC2_167 | AAGCTCTGTC |
| F8      | BC1_343 | AACGCATTCC | BC2_163 | TATGGCACTG |
| G8      | BC1_329 | TTCCTTACTG | BC2_177 | TTGAGTCGAC |
| H8      | BC1_346 | GTCATACGA  | BC2_171 | GTGATCTAGA |
| A9      | BC1_339 | TAATGGCCTT | BC2_182 | GCTCGTAACT |
| B9      | BC1_343 | AACGCATTCC | BC2_186 | TGTCGCTCAC |
| C9      | BC1_341 | ACCGATTAGG | BC2_179 | AGAGTACGAC |
| D9      | BC1_344 | CCGAGAGACC | BC2_185 | GTCATGGACG |
| E9      | BC1_346 | GTCATACGA  | BC2_184 | CCGTACCTGA |
| F9      | BC1_340 | GTGACCGGTA | BC2_176 | CCATACATAT |
| G9      | BC1_342 | CGTCTGATAT | BC2_181 | CAGACGTTGG |
| H9      | BC1_345 | TGATTCCGAG | BC2_183 | TAATCTACTT |
| A10     | BC1_343 | AACGCATTCC | BC2_182 | GCTCGTAACT |
| B10     | BC1_335 | AATTCGAGT  | BC2_179 | AGAGTACGAC |
| C10     | BC1_328 | CCTGAAGGAT | BC2_176 | CCATACATAT |
| D10     | BC1_324 | CTGAACCGAA | BC2_181 | CAGACGTTGG |
| E10     | BC1_334 | TTAACGACGA | BC2_170 | TGCTCACAGA |
| F10     | BC1_337 | TTGCTTCGTT | BC2_185 | GTCATGGACG |
| G10     | BC1_329 | TTCCTTACTG | BC2_163 | TATGGCACTG |
| H10     | BC1_330 | GGATGCTACC | BC2_167 | AAGCTCTGTC |
| A11     | BC1_340 | GTGACCGGTA | BC2_165 | ACACATGTCA |
| B11     | BC1_336 | CCTGTAACCA | BC2_168 | CCAGAGATCT |
| C11     | BC1_338 | GGCTACTTAC | BC2_169 | GTGATTGCAC |
| D11     | BC1_329 | TTCCTTACTG | BC2_164 | GTTAGGTAGG |
| E11     | BC1_324 | CTGAACCGAA | BC2_166 | CGCTCACGAT |
| F11     | BC1_339 | TAATGGCCTT | BC2_170 | TGCTCACAGA |
| G11     | BC1_325 | GAACGTGTCG | BC2_163 | TATGGCACTG |
| H11     | BC1_323 | AGGACGTAGA | BC2_167 | AAGCTCTGTC |
| A12     | BC1_326 | TCCGTGACTC | BC2_177 | TTGAGTCGAC |
| B12     | BC1_329 | TTCCTTACTG | BC2_171 | GTGATCTAGA |
| C12     | BC1_325 | GAACGTGTCG | BC2_172 | TCTGTATAGT |
| D12     | BC1_328 | CCTGAAGGAT | BC2_182 | GCTCGTAACT |
| E12     | BC1_330 | GGATGCTACC | BC2_174 | CGATAGGTCG |
| F12     | BC1_327 | AATTCACTGT | BC2_176 | CCATACATAT |
| G12     | BC1_324 | CTGAACCGAA | BC2_178 | GGTCCGGCCG |
| H12     | BC1_323 | AGGACGTAGA | BC2_175 | AACGCAACTA |

## Kit Components

### NEB #E9725S Table of Components

| NEB #  | PRODUCT  | VOLUME              |
|--------|--|---------------------|
| E9650A | NEBNext Adaptor for MGI/Complete Genomics                        | 0.12 ml             |
| E9651A | NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics        | 0.25 ml             |
| E9648A | NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (1-24) | 1 plate (6 µl/well) |

### NEB #E9725L Table of Components

| NEB #   | PRODUCT  | VOLUME              |
|---------|--|---------------------|
| E9650AA | NEBNext Adaptor for MGI/Complete Genomics                          | 0.48 ml             |
| E9651AA | NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics          | 1 ml                |
| E9649A  | NEBNext Dual Index Primer Pairs for MGI/Complete Genomics (25-120) | 1 plate (6 µl/well) |

## Revision History

| REVISION # | DESCRIPTION | DATE |
|------------|-------------|------|
| 1.0        | N/A         | 6/24 |

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