

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

NEB #E9640 S/L

24/96 reactions

Version 1.0_06/24

Table of Contents

Overview.....	1
Section 1	
Setting up the PCR Reactions.....	2
Section 2	
Index Pooling Guidelines.....	4
Kit Components.....	7
Revision History.....	7

The NEBNext Multiplex Oligos for MGI Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E9640S) or 96 reactions (NEB #E9640L). All reagents should be stored at –20°C.

NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) NEB #E9640S

- (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 #E9640L

- (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 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.

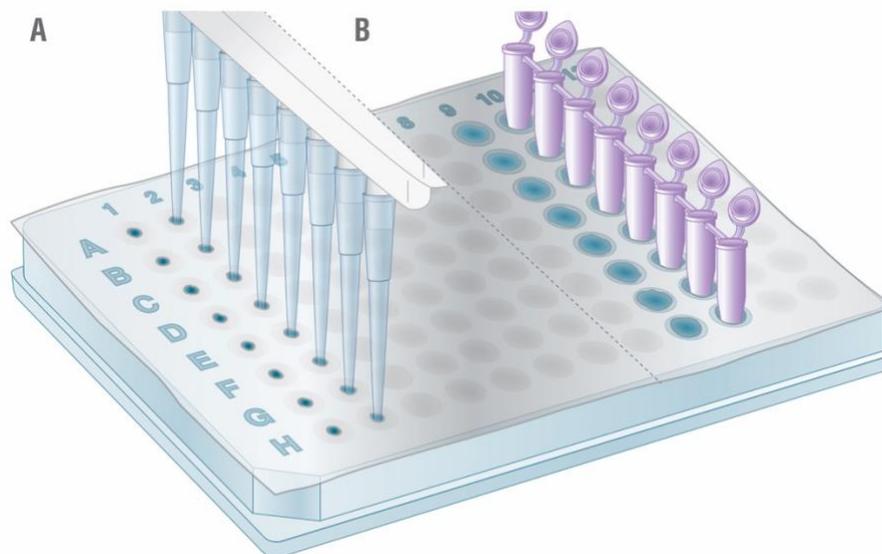
1.1. Setting up the PCR reactions (< 96 samples)

- 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°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 #E9640S and #E9640L, so the libraries generated from #E9640S and #E9640L 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	AATTCGGAGT	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	AATTCACTGT	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	AATCACTGT	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	AATCACTGT	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 #E9640S 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 #E9640L 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

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products and content are covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc (NEB). The use of trademark symbols does not necessarily indicate that the name is trademarked in the country where it is being read; it indicates where the content was originally developed. See www.neb.com/trademarks. The use of these products may require you to obtain additional third-party intellectual property rights for certain applications. For more information, please email busdev@neb.com.

B CORPORATION® is a registered trademark of B Lab IP, LLC, Inc.

MGI® and DNBSEQ® are registered trademarks of MGI Tech Co., Ltd.

COMPLETE GENOMICS® is a trademark of Complete Genomics, Inc.

© Copyright 2024, New England Biolabs, Inc.; all rights reserved

