Store at -20°C

# Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN)

#29580

1 Kit
(24 assays)

Cell Signaling TECHNOLOGY®

**Support:** +1-978-867-2388 (U.S.)
www.cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)
orders@cellsignal.com

**For Research Use Only. Not For Use In Diagnostic Procedures.**

| Products Included | Product # | Quantity |
|---|---|---|
| Adaptor for Illumina Systems | 42436 | 240 µl |
| USER Enzyme | 59713 | 72 µl |
| Universal PCR Primer for Illumina Systems | 12078 | 120 µl |
| Index 1 Primer for Illumina Systems | 28248 | 10 µl |
| Index 2 Primer for Illumina Systems | 41836 | 10 µl |
| Index 3 Primer for Illumina Systems | 64036 | 10 µl |
| Index 4 Primer for Illumina Systems | 83765 | 10 µl |
| Index 5 Primer for Illumina Systems | 18392 | 10 µl |
| Index 6 Primer for Illumina Systems | 27180 | 10 µl |
| Index 7 Primer for Illumina Systems | 43985 | 10 µl |
| Index 8 Primer for Illumina Systems | 68962 | 10 µl |
| Index 9 Primer for Illumina Systems | 83219 | 10 µl |
| Index 10 Primer for Illumina Systems | 90275 | 10 µl |
| Index 11 Primer for Illumina Systems | 28019 | 10 µl |
| Index 12 Primer for Illumina Systems | 39090 | 10 µl |

**Storage:** Store all components at -20ºC. This product is stable for 12 months if stored properly.

**For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com**

**Description:** Next generation sequencing (NG-seq) is a high throughput method that can be used downstream of chromatin immunoprecipitation (ChIP) and Cleavage Under Targets and Release Using Nuclease (CUT&RUN) assays to identify and quantify target DNA enrichment across the entire genome. Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) contains adaptors and primers that are ideally suited for multiplex sample preparation for NG-seq on the Illumina Systems platform. This kit can be used to generate up to 12 disctinct, barcoded ChIP-seq or CUT&RUN DNA libraries that can be combined into a single sequencing reaction. This product provides enough reagents to support up to 24 DNA sequencing libraries, and must be used in combination with the DNA Library Prep Kit for Illumina Systems (ChIP-Seq, CUT&RUN) #56795.

This product is compatible with SimpleChIP® Enzymatic ChIP Kit (Magnetic Beads) #9003, SimpleChIP® Plus Enzymatic ChIP Kit (Magnetic Beads) #9005, SimpleChIP® Plus Sonication ChIP kit #56383, and CUT&RUN Assay Kit #86652. This product is not compatible with SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002 and SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004 because agarose beads are blocked with sonicated salmon sperm DNA, which will contaminate DNA library preps and NG-seq.

**Specificity/Sensitivity:** This kit has been validated in combination with DNA Library Prep Kit for Illumina Systems (ChIP-Seq, CUT&RUN) #56795 to generate qualified DNA libraries using as little as 0.5 ng ChIP DNA or as little as 0.1 ng CUT&RUN DNA as starting materials. Libraries prepared from different starting amounts of ChIP DNA exhibit similiar Agilent Bioanalyzer System profiles, genome mapping rates, numbers of identified binding peaks, and signal-to-noise ratios across the whole genome.

NEW ENGLAND BioLabs Inc. Contains: NEBNext Oligos



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**Applications:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

# Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) Protocol

Next generation sequencing (NG-seq) is a high throughput method that can be used downstream of chromatin immunoprecipitation (ChIP) and Cleavage Under Targets and Release Using Nuclease (CUT&RUN) assays to identify and quantify target DNA enrichment across the entire genome. Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) contain adaptors and primers that are ideally suited for multiplex sample preparation for NG-seq on the Illumina Systems platform (Illumina, Inc.). This kit can be used to generate up to 12 distinct, barcoded ChIP-seq or CUT&RUN DNA libraries that can be combined into a single sequencing reaction.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of indexed libraries on the Illumina Systems sequencing platform.

This product provides enough reagents to support up to 24 DNA sequencing libraries, and must be used in combination with the DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795.

**Compatible Assay kits:**

SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

SimpleChIP® Plus Sonication Chromatin IP Kit #56383

DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795

CUT&RUN Assay Kit #86652

**Non-Compatible SimpleChIP® kits:**

SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004

**Note:** Agarose beads are blocked with sonicated salmon sperm DNA, which will contaminate DNA library preps and NG-seq.

**Required Reagents:**

Reagents Included:

1. • (red) Adaptor for Illumina Systems #42436
2. • (red) USER Enzyme #59713
3. • (blue) Universal PCR Primer for Illumina Systems #12078
4. • (blue) Index 1 Primer for Illumina Systems #28248
5. • (blue) Index 2 Primer for Illumina Systems #41836
6. • (blue) Index 3 Primer for Illumina Systems #64036
7. • (blue) Index 4 Primer for Illumina Systems #83765
8. • (blue) Index 5 Primer for Illumina Systems #18392
9. • (blue) Index 6 Primer for Illumina Systems #27180
10. • (blue) Index 7 Primer for Illumina Systems #43985
11. • (blue) Index 8 Primer for Illumina Systems #68962
12. • (blue) Index 9 Primer for Illumina Systems #83219
13. • (blue) Index 10 Primer for Illumina Systems #90275
14. • (blue) Index 11 Primer for Illumina Systems #28019
15. • (blue) Index 12 Primer for Illumina Systems #39090

Reagents Not Included:

a. Enzymes and buffers appropriate for ChIP or CUT&RUN Illumina Systems NG-seq library preparation: provided in DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795

b. Nuclease-free Water #12931

c. AMPure XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect Reagent Kit (Beckman Coulter, Inc. #B23317)

d. Freshly prepared 80% Ethanol

e. 1X TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)

f. 10 mM Tris-HCl (pH 8.0-8.5)

g. Magnetic Separation Rack #7017/#14654

h. Agilent Bioanalyzer System and Agilent High Sensitivity DNA Kit (Agilent Technologies, Inc.)

i. PCR tubes and PCR

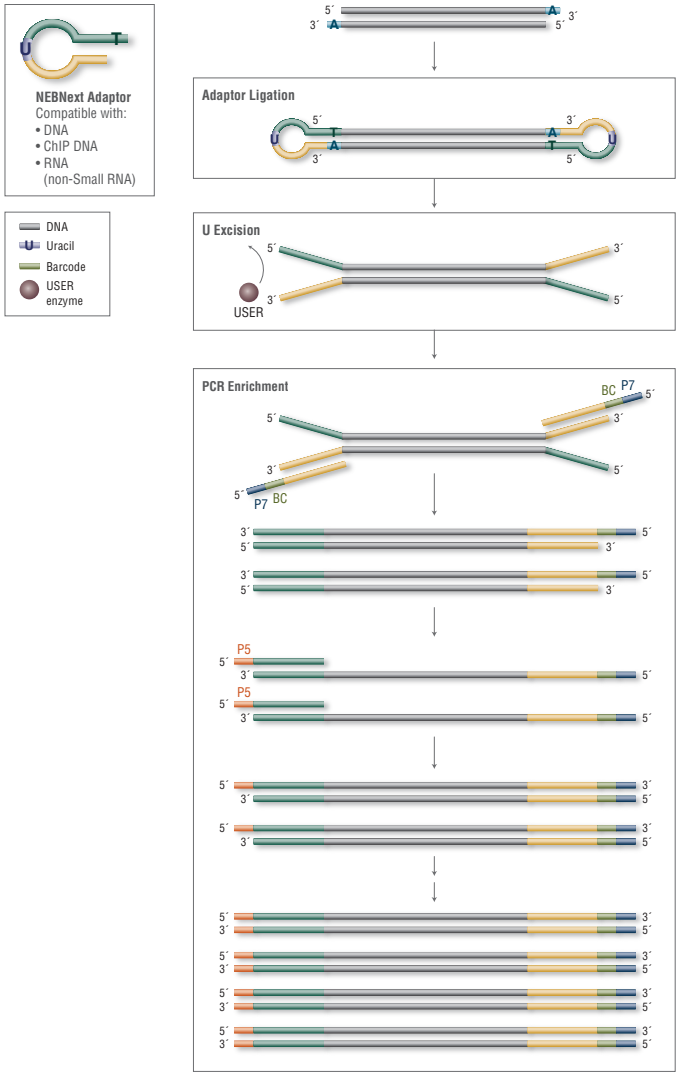


***Figure 1.*** *Workflow demonstrating the use of Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) with the (ChIP-seq, CUT&RUN) DNA Library Prep Kit for Illumina Systems #56795.*

# Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) Protocol

## I. Low Plexity Pooling Guidelines:

Illumina Systems NG-seq platforms use a red laser/LED to sequence A/C and a green laser/LED to sequence G/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 present in each cycle, and G or T must be present in each cycle). If this color balance is not maintained, sequencing the index read could fail. Please check the sequences of each index to be used to ensure that you will have signal in both the red and green channels for every cycle. See example below:

| | | | |
|---|---|---|---|
| *Index 1 Primer for Illumina Systems* | ATCACG | *Index 1 Primer for Illumina Systems* | ATCACG |
| *Index 2 Primer for Illumina Systems* | CGATGT | *Index 2 Primer for Illumina Systems* | CGATGT |
| *Index 4 Primer for Illumina Systems* | TGACCA | *Index 3 Primer for Illumina Systems* | TTAGGC |
| *Index 7 Primer for Illumina Systems* | CAGATC | *Index 4 Primer for Illumina Systems* | TGACCA |
| | ✓✓✓✓✓✓ | | ✓✗✗✓✓✓ |

The following table lists some (but not all) valid index combinations that can be sequenced together:

| | |
|---|---|
| *Pool of 2 samples* | *Index 6 and 12 Primers* |
| *Pool of 3 samples* | *Index 4, 6, and 12 Primers* |
| *Pool of 6 samples* | *Index 2, 4, 5, 6, 7, and 12 Primers* |

Multiplex Oligos for Illumina Systems (Single Index Primers) (ChIP-seq, CUT&RUN) can generate 12 different, barcoded samples if each Index primer is used only once with Universal PCR Primer for Illumina Systems. Each Index Primer is supplied in sufficient amounts to generate two libraries, but these two libraries cannot be pooled together in one sequencing lane.

For 1-plex (no pooling), use any index primer with the universal PCR primer.

## II. Index 1-12 Primers for Illumina Systems:

Each Index Primer for Illumina Systems is provided in volume of 10 µl.

| Product | Index Primer Sequence | Expected Index Primer Sequence Read |
|---|---|---|
| *Index 1 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | ATCACG |
| *Index 2 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | CGATGT |
| *Index 3 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGA GTTCAGACGTGTGCTCTTCCGATC-s-T-3´* | TTAGGC |
| *Index 4 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGA GTTCAGACGTGTGCTCTTCCGATC-s-T-3´* | TGACCA |
| *Index 5 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGA GTTCAGACGTGTGCTCTTCCGATC-s-T-3´* | ACAGTG |
| *Index 6 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | GCCAAT |
| *Index 7 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | CAGATC |
| *Index 8 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | ACTTGA |
| *Index 9 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | GATCAG |
| *Index 10 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | TAGCTT |
| *Index 11 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGA GTTCAGACGTGTGCTCTTCCGATC-s-T-3´* | GGCTAC |
| *Index 12 Primer for Illumina Systems* | *5´-CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAG TTCAGACGTGTGCTCTTCCGATC-s-T-3´* | CTTGTA |

Where -s- indicates phosphorothioate bond.

## III. Set up the PCR Reaction

1. Ensure that a valid combination of index primers is used. See Section I and II to verify that correct primer combinations have been selected.
2. Add only one index primer (•) (5 µl) and 5 µl universal PCR primer (•) to each PCR tube. It is critical to change tips between tubes to avoid cross-contamination.
3. Record the index primers added to each PCR tube.
4. Add 25 µl Q5 PCR Master Mix (•) to each tube that contains primers.
5. Add 15 µl of adaptor ligated ChIP DNA for a final volume of 50 µl to the corresponding tube. Gently pipette up and down 5–10 times to mix. It is critical to change tips between samples to avoid cross-contamination.
6. Record the adaptor ligated DNA sample added to each PCR tube.
7. Quickly centrifuge and perform PCR according to recommended cycling conditions (refer to the respective protocols in DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-DNA or CUT&RUN DNA starting samples).

# APPENDIX: Quality Control of the Kit Components

The components in the SimpleChIP® ChIP-seq Multiplex Oligos for Illumina Systems (Single Index Primers) #29580 are individually validated by the functional testing listed below and must pass rigorous quality control standards. Furthermore, each set of components is functionally validated together by construction and sequencing of indexed libraries on the Illumina Systems sequencing platform.

## I. Adaptor for Illumina Systems (15 µM) (•)

5´-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT C/ideoxyU/A CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C-s-T-3´

**Quality Control Assays**

1. 16-Hour Incubation: 50 µl reactions containing this adaptor and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.
2. Endonuclease Activity: Incubation of a minimum of 5 µl of this adaptor with 1 µg of ϕX174 RF 1 DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.
3. Phosphatase Activity: Incubation of a minimum of 10 µl of this adaptor in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.
4. RNase Activity: Incubation of this adaptor with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

## II. USER Enyzme (•)

Supplied in: 50 mM KCl, 5 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 175 µg/ml BSA and 50% Glycerol

**Quality Control Assays**

1. Non-Specific DNase Activity (16 Hour): A 50 µl reaction in NEBuffer 1 containing 1 µg of Lambda DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of Lambda-HindIII DNA and a minimum of 25 units of Endonuclease VIII incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
2. Exonuclease Activity (Radioactivity Release): A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [$^3$H] E. coli DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity. A 50 µl reaction in Endonuclease VIII Reaction Buffer containing 1 µg of a mixture of single and double-stranded [$^3$H] E. coli DNA and a minimum of 10 units of Endonuclease VIII incubated for 4 hours at 37°C releases < 0.5% of the total radioactivity.
3. Endonuclease Activity (Nicking): A 50 µl reaction in UDG Reaction Buffer containing 1 µg of supercoiled ϕX174 DNA and a minimum of 50 units of Uracil DNA Glycosylase incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.
4. Phosphatase Activity: Incubation of a minimum of 10 µl of USER Enzyme at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

## III. Universal PCR Primer for Illumina Systems (10 µM) (•)

5´-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC*T-3´

**Quality Control Assays**

1. 16-Hour Incubation: 50 µl reactions containing this primer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1 µl primer and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable nonspecific nuclease degradation as determined by agarose gel electrophoresis.
2. Endonuclease Activity: Incubation of a minimum of 5 µl of primer with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in < 10% conversion to RF II as determined by agarose gel electrophoresis.
3. RNase Activity: Incubation of 1 µl of primer with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase Activity as determined by polyacrylamide gel electrophoresis.
4. Phosphatase Activity: Incubation of a minimum of 10 µl of this primer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

## IV. Index 1-12 Primers for Illumina Systems (10 µM) (•)

**Quality Control Assays**

1. 16-Hour Incubation: 50 µl reactions containing 1 µl Index [X] Primer for Illumina Systems and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing Index [X] Primer for Illumina Systems and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.
2. Endonuclease Activity: Incubation of a 50 µl reaction containing 1 µl Index [X] Primer for Illumina Systems with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in less than 10% conversion to RF II (nicked molecules) as determined by agarose gel electrophoresis.
3. RNase Activity: Incubation of a 10 µl reaction containing 1 µl Index [X] Primer for Illumina Systems with 40 ng of RNA transcript for 16 hours at 37°C resulted in no detectable degradation of RNA as determined by gel electrophoresis.
4. Phosphatase Activity: Incubation of Index [X] Primer for Illumina Systems in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM pnitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.