Store at -20°C

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

#47538

1 Kit
(96 assays)

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

Orders: 877-616-2355 (U.S.)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Storage Temp
Adaptor for Illumina Systems	42436	960 µl	-20°C
USER Enzyme	59713	288 µl	-20°C
Index Primer Orange Tube Caps	54631	24 ea	room temp
Index Primer White Tube Caps	70942	16 ea	room temp
Index 501 Primer for Illumina Systems	86676	60 µl	-20°C
Index 502 Primer for Illumina Systems	92596	60 µl	-20°C
Index 503 Primer for Illumina Systems	29576	60 µl	-20°C
Index 504 Primer for Illumina Systems	46325	60 µl	-20°C
Index 505 Primer for Illumina Systems	67343	60 µl	-20°C
Index 506 Primer for Illumina Systems	89663	60 µl	-20°C
Index 507 Primer for Illumina Systems	27649	60 µl	-20°C
Index 508 Primer for Illumina Systems	40566	60 µl	-20°C
Index 701 Primer for Illumina Systems	60797	40 µl	-20°C
Index 702 Primer for Illumina Systems	79999	40 µl	-20°C
Index 703 Primer for Illumina Systems	18697	40 µl	-20°C
Index 704 Primer for Illumina Systems	26125	40 µl	-20°C
Index 705 Primer for Illumina Systems	39467	40 µl	-20°C
Index 706 Primer for Illumina Systems	51808	40 µl	-20°C
Index 707 Primer for Illumina Systems	58724	40 µl	-20°C
Index 708 Primer for Illumina Systems	65787	40 µl	-20°C
Index 709 Primer for Illumina Systems	75272	40 µl	-20°C
Index 710 Primer for Illumina Systems	99422	40 µl	-20°C
Index 711 Primer for Illumina Systems	10812	40 µl	-20°C
Index 712 Primer for Illumina Systems	38569	40 µl	-20°C

Storage: Upon receipt, #54631 and #70942 tube caps should be removed from the kit box and stored at room temperature. Store remaining components at -20ºC. This product is stable for 12 months if stored properly.

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 (Dual 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 96 distinct, 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 96 DNA sequencing libraries, and must be used in combination with 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 DNA exhibit similar Agilent Bioanalyzer System profiles, genome mapping rates, numbers of identified binding peak, 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 (Dual 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 (Dual 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 (Illumina, Inc.). This kit can be used to generate up to 96 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 96 DNA sequencing libraries, and must be used in combination with 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

ChIP-seq 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:

a. • (red) Adaptor for Illumina Systems #42436
b. • (red) USER Enzyme #59713
c. ○ (white) Index 501 Primer for Illumina Systems #86676
d. ○ (white) Index 502 Primer for Illumina Systems #92596
e. ○ (white) Index 503 Primer for Illumina Systems #29576
f. ○ (white) Index 504 Primer for Illumina Systems #46325
g. ○ (white) Index 505 Primer for Illumina Systems #67343
h. ○ (white) Index 506 Primer for Illumina Systems #89663
i. ○ (white) Index 507 Primer for Illumina Systems #27649
j. ○ (white) Index 508 Primer for Illumina Systems #40566
k. • (orange) Index 701 Primer for Illumina Systems #60797
l. • (orange) Index 702 Primer for Illumina Systems #79999
m. • (orange) Index 703 Primer for Illumina Systems #18697
n. • (orange) Index 704 Primer for Illumina Systems #26125
o. • (orange) Index 705 Primer for Illumina Systems #39467
p. • (orange) Index 706 Primer for Illumina Systems #51808
q. • (orange) Index 707 Primer for Illumina Systems #58724
r. • (orange) Index 708 Primer for Illumina Systems #65787
s. • (orange) Index 709 Primer for Illumina Systems #75272
t. • (orange) Index 710 Primer for Illumina Systems #99422
u. • (orange) Index 711 Primer for Illumina Systems #10812
v. • (orange) Index 712 Primer for Illumina Systems #38569
w. Index Primer Orange Tube Caps #54631
x. Index Primer White Tube Caps #70942

Reagents Not Included:

a. Enzymes and buffers appropriate for ChIP or CUT&RUN Illumina Systems 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.)

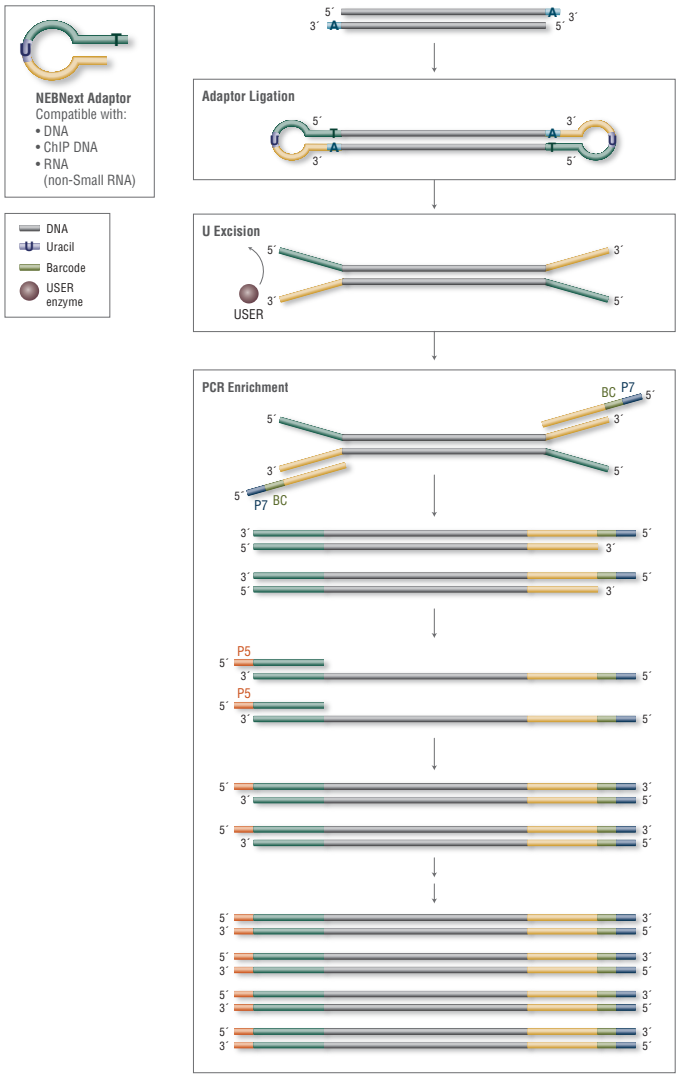

Figure 1. *Workflow demonstrating the use of Multiplex Oligos for Illumina Systems (Dual Index Primers) (ChIP-seq, CUT&RUN) with DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795.*

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

I. Low Plexity Pooling Guidelines:

The dual index primer strategy utilizes two 8 base indices within each primer. Index 7 primers contain indices that are adjacent to the P7 sequence while index 5 primers contain indices that are adjacent to the P5 sequence. Dual indexing is enabled by adding a unique index to both ends of a sample to be sequenced. Up to 96 different samples can be uniquely indexed by combining each of the 12 index 7 primers with each of the 8 index 5 primers.

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 7 Primers		Index 5 Primers	
Index 701 Primer for Illumina Systems	ATTACTCG	*Index 503 Primer for Illumina Systems*	CCTATCCT
Index 702 Primer for Illumina Systems	TCCGGAGA	*Index 504 Primer for Illumina Systems*	GGCTCTGA
Index 703 Primer for Illumina Systems	CGCTCATT	*Index 505 Primer for Illumina Systems*	AGGCGAAG
Index 704 Primer for Illumina Systems	GAGATTCC	*Index 506 Primer for Illumina Systems*	TAATCTTA
	✓✓✓✓✓✓✓✓		✓✓✓✓✓✓✓✓

Index 7 Primers		Index 5 Primers	
Index 701 Primer for Illumina Systems	ATTACTCG	*Index 502 Primer for Illumina Systems*	ATAGAGGC
Index 702 Primer for Illumina Systems	TCCGGAGA	*Index 504 Primer for Illumina Systems*	GGCTCTGA
Index 703 Primer for Illumina Systems	CGCTCATT	*Index 506 Primer for Illumina Systems*	TAATCTTA
Index 704 Primer for Illumina Systems	GAGATTCC	*Index 508 Primer for Illumina Systems*	GTACTGAC
	✓✓✓✓✓✓✓✓		✓✓✘✓✓✘✓✘

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

Plex	Index 7 primers for Illumina Systems	Index 5 Primers for Illumina Systems
2	*Index 701 and Index 702* *Index 703 and Index 704* *Index 705 and Index 706* *Index 707 and Index 708* *Index 709 and Index 710* *Index 711 and Index 712*	*Any Index 5 Primer*
3	*Index 701, Index 702, and Index 703* *Index 703, Index 704, and Index 705* *Index 705, Index 706, and Index 707* *Index 707, Index 708, and Index 709* *Index 709, Index 710, and Index 711*	*Any Index 5 Primer*
4	*Index 701, Index 702, Index 703, and Index 704* *Index 703, Index 704, Index 705, and Index 706* *Index 705, Index 706, Index 707, and Index 708* *Index 707, Index 708, Index 709, and Index 710* *Index 709, Index 710, Index 711, and Index 712*	*Any Index 5 Primer*
5-12	*Any valid Index 7 4-plex combination with any other i7 Primers (as needed)*	*Any Index 5 Primer*
>12	*Any valid Index 7 4-plex combination with any other i7 Primers (as needed)*	*Index 501, Index 502, and any other Index 5 primer (as needed)* *Index 503, Index 504, and any other Index 5 primer (as needed)* *Index 505, Index 506, and any other Index 5 primer (as needed)* *Index 507, Index 508, and any other Index 5 primer (as needed)*

Some other valid combinations are listed below. Choose a **valid** set of Index 7 primers and a **valid** set of Index 5 primers. Use each Index 7 primer with each i5 primer to form desired number of primer pairs for PCR amplification of desired number of libraries.

Pool of 12 samples	*(1) A set of 4 Index 7 primers * A set of 3 Index 5 primers* *(2) A set of 3 Index 7 primers * A set of 4 Index 5 primers* *(3) A set of 6 Index 7 primers * A set of 2 Index 5 primers* *(4) A set of 12 Index 7 primers * A set of 1 Index 5 primers*
Pool of 26 samples	*(1) A set of 6 Index 7 primers * A set of 4 Index 5 primers Plus any of the Index 7 primers with any other 2 Index 5 primers (besides the set of 4)* *(2) A set of 6 Index 7 primers * A set of 5 Index 5 primers* *Use 26 of the 30 primer pairs to amplify 26 libraries*

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

II. Index 5 Primers for Illumina Systems:

Each Index 5 Primer for Illumina Systems is provided in volume of 60 µl.

Index 501 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACTATAGCCTA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	TATAGCCT
Index 502 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACATAGAGGCA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	ATAGAGGC
Index 503 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACCCTATCCTA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	CCTATCCT
Index 504 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACGGCTCTGAA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	GGCTCTGA
Index 505 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACAGGCGAAGA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	AGGCGAAG
Index 506 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACTAATCTTAA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	TAATCTTA
Index 507 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACCAGGACGTA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	CAGGACGT
Index 508 Primer for Illumina Systems	*5´-AATGATACGGCGACCACCGAGATCTACACGTACTGACA-CACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3´*	GTACTGAC

Where -s- indicates phosphorothioate bond.

III. Index 7 Primers for Illumina Systems:

Each Index 7 Primer for Illumina Systems is provided in a volume of 40 µl.

Index 701 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	ATTACTCG
Index 702 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	TCCGGAGA
Index 703 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	CGCTCATT
Index 704 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	GAGATTCC
Index 705 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	ATTCAGAA
Index 706 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	GAATTCGT
Index 707 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATAGCTTCAGGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	CTGAAGCT
Index 708 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATGCGCATTAGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	TAATGCGC
Index 709 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATCATAGCCGGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	CGGCTATG
Index 710 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATTTCGCGGAGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	TCCGCGAA
Index 711 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATGCGCGAGAGT-GACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	TCTCGCGC
Index 712 Primer for Illumina Systems	*5´-CAAGCAGAAGACGGCATACGAGATCTATCGCTGTGACTG-GAGTTCAGACGTGTGCTCTTCCGATC-s-T-3´*	AGCGATAG

Where -s- indicates phosphorothioate bond.

IV. Set up the PCR Reaction

1. Ensure that a valid combination of index 7 and index 5 primers is used. See Section I and II to verify that correct primer combinations have been selected.
2. Add only one Index 5 primer (º) (5 µl) and only one Index 7 primer (•) (5 µl) to each PCR tube. It is critical to change tips between tubes to avoid cross-contamination. If necessary, discard the original Index 5 white caps or Index 7 orange caps and apply new caps to avoid index cross-contamination.
3. Record the Index 5 and Index 7 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 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 Multiplex Oligos for Illumina Systems (Dual Index Primers) (ChIP-seq, CUT&RUN) #47538 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 Enzyme (•)

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 ηX174 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 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. Index 5 and Index 7 Primers for Illumina Systems (10 µM) (^{0}•)

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 p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

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