Store at -20°C

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems

#47415

1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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Description: Next generation sequencing (NG-seq) is a high throughput method that can be used downstream of the Cleavage Under Targets and Tagmentation (CUT&Tag) assay to identify and quantify target DNA enrichment across the entire genome. The CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems kit is 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 CUT&Tag DNA libraries that can be combined into a single sequencing reaction. This product provides enough reagents to support up to 96 DNA sequencing libraries. This product is compatible with CUT&Tag DNA samples generated by CUT&Tag Assay Kit #77552 or CUT&Tag pAG-Tn5 (Loaded) #79561 and DNA samples from other tagmentation assays, such as ATAC-seq. This product is not compatible with SimpleChIP® Chromatin IP Kits (#9003, #9005, #56383) or the CUT&RUN Assay Kit #86652.

Background: Similar to Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag) is a powerful technique used for probing protein-DNA interactions within the natural chromatin context of the cell (1-3). CUT&Tag has many of the same advantages as the CUT&RUN assay in that it provides a rapid, robust, and true low cell number protocol for detection of protein-DNA interactions in the cell. In addition, the CUT&Tag assay adds an in situ adaptor DNA ligation step carried out by the pAG-Tn5 enzyme, in which an adaptor DNA is ligated directly to antibody-targeted chromatin DNA fragments in the cell. As a result, subsequent DNA library preparation is much faster and easier than library preparation following the CUT&RUN assay, free from DNA end repair, A-tailing, and adaptor ligation *in vitro*. CUT&Tag works very well for analyzing histone modifications, in addition to mapping some transcription factor and cofactor binding.

Specificity/Sensitivity: This kit can generate DNA libraries using CUT&Tag DNA. Note: While CUT&Tag DNA libraries generated for histone modifications typically show robust signal in Agilent Bioanalyzer or TapeStation systems analysis, libraries generated for non-histone proteins such as transcription factors and cofactors often have very weak or even no visible signal using Bioanalyzer or TapeStation systems, but still generate NG-sequencing results with high mapping rates, high numbers of identified binding peaks, and decent signal-to-noise ratios across the whole genome.

Product Includes	Item #	Kit Quantity	Storage Temp
CUT&Tag PCR Master Mix	63228	4 x 840 µL	-20°C
CUT&Tag Index 501 Primer for Illumina Systems	84876	30 µL	-20°C
CUT&Tag Index 502 Primer for Illumina Systems	27488	30 µL	-20°C
CUT&Tag Index 503 Primer for Illumina Systems	55058	30 µL	-20°C
CUT&Tag Index 504 Primer for Illumina Systems	61793	30 µL	-20°C
CUT&Tag Index 505 Primer for Illumina Systems	70447	30 µL	-20°C
CUT&Tag Index 506 Primer for Illumina Systems	87803	30 µL	-20°C
CUT&Tag Index 507 Primer for Illumina Systems	26897	30 µL	-20°C
CUT&Tag Index 508 Primer for Illumina Systems	52106	30 µL	-20°C
CUT&Tag Index 701 Primer for Illumina Systems	71100	20 µL	-20°C
CUT&Tag Index 702 Primer for Illumina Systems	88112	20 µL	-20°C
CUT&Tag Index 703 Primer for Illumina Systems	23497	20 µL	-20°C
CUT&Tag Index 704 Primer for Illumina Systems	37884	20 µL	-20°C
CUT&Tag Index 705 Primer for Illumina Systems	50105	20 µL	-20°C
CUT&Tag Index 706 Primer for Illumina Systems	65909	20 µL	-20°C
CUT&Tag Index 707 Primer for Illumina Systems	84796	20 µL	-20°C
CUT&Tag Index 708 Primer for Illumina Systems	17160	20 µL	-20°C
CUT&Tag Index 709 Primer for Illumina Systems	29209	20 µL	-20°C
CUT&Tag Index 710 Primer for Illumina Systems	41919	20 µL	-20°C
CUT&Tag Index 711 Primer for Illumina Systems	54212	20 µL	-20°C
CUT&Tag Index 712 Primer for Illumina Systems	70020	20 µL	-20°C

Storage: Store all components at -20ºC. This product is stable for 18 months if stored properly.

Background References:

(1) Kaya-Okur, H.S. et al. (2019) *Nat Commun* 10, 1930.
(2) Kaya-Okur, H.S. et al. (2020) *Nat Protoc* 15, 3264-3283.
(3) Henikoff, S. et al. (2021) *Bio Protoc* 11, e4043.

Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **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.

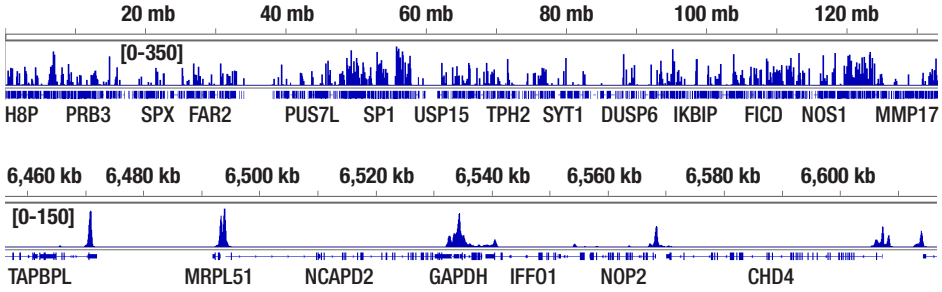

Figure 1. CUT&Tag was performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using CUT&Tag Assay Kit #77552. The DNA library was prepared using indexes i504 and i703 from CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems. The figure shows binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K4me3.

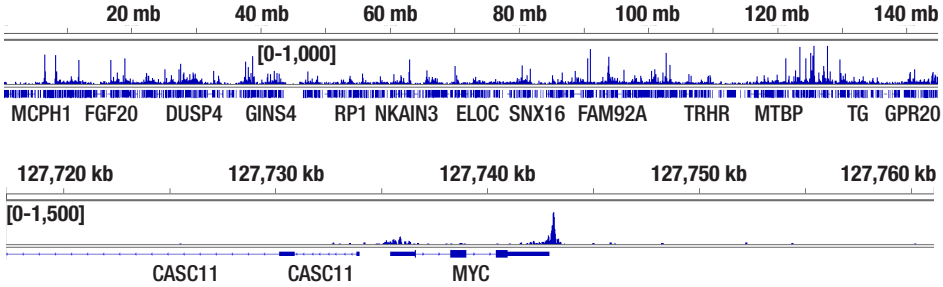

Figure 2. CUT&Tag was performed with HCT 116 cells and TCF4/TCF7L2 (C48H11) Rabbit mAb #2569, using CUT&Tag Assay Kit #77552. The DNA library was prepared using indexes i502 and i706 from CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems. The figure shows binding across chromosome 8 (upper), including MYC (lower), a known target gene of TCF4.

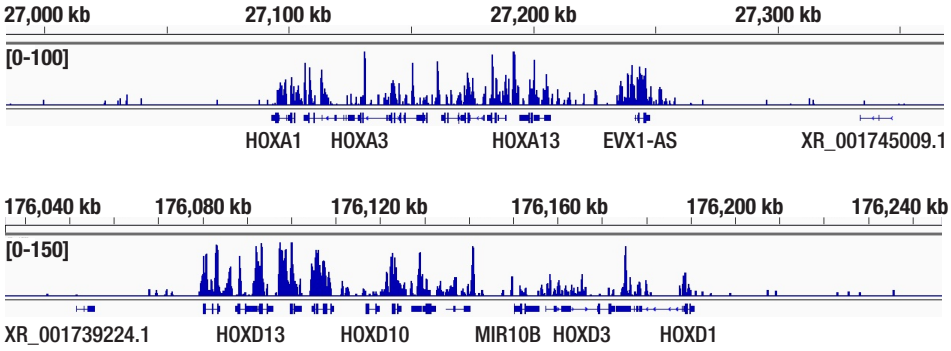

Figure 3. CUT&Tag was performed with NCCIT cells and JARID2 (D6M9X) Rabbit mAb #13594, using CUT&Tag Assay Kit #77552. The DNA library was prepared using indexes i501 and i705 from CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems. The figure shows binding across HOXA (upper) and HOXD (lower), known target genes of JARID2.

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems

Next generation sequencing (NG-seq) is a high throughput method that can be used downstream of Cleavage Under Targets and Tagmentation (CUT&Tag) assay to identify and quantify target DNA enrichment across the entire genome. The CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems kit is 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 CUT&Tag DNA libraries that can be combined into a single sequencing reaction. This product is compatible with CUT&Tag DNA sample generated by the CUT&Tag pAG-Tn5 (Loaded) #79561 and DNA samples from other tagmentation assays, such as ATAC-seq. This product is not compatible with ChIP-DNA from SimpleChIP® Chromatin IP Kits (#9003, #9005, #56383) or the CUT&RUN DNA from CUT&RUN Assay Kit #86652.

Compatible Reagent:

CUT&Tag pAG-Tn5 (Loaded) #79561

Non-Compatible Assay kits:

SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002
SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005
SimpleChIP® Plus Sonication Chromatin IP Kit #56383
CUT&RUN Assay Kit #86652
DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795

Required Reagents

Reagents Included:

- **a.** CUT&Tag PCR Master Mix #63228
- **b.** CUT&Tag Index 501 Primer for Illumina Systems #84876
- **c.** CUT&Tag Index 502 Primer for Illumina Systems #27488
- **d.** CUT&Tag Index 503 Primer for Illumina Systems #55058
- **e.** CUT&Tag Index 504 Primer for Illumina Systems #61793
- **f.** CUT&Tag Index 505 Primer for Illumina Systems #70447
- **g.** CUT&Tag Index 506 Primer for Illumina Systems #87803
- **h.** CUT&Tag Index 507 Primer for Illumina Systems #26897
- **i.** CUT&Tag Index 508 Primer for Illumina Systems #52106
- **j.** CUT&Tag Index 701 Primer for Illumina Systems #71100
- **k.** CUT&Tag Index 702 Primer for Illumina Systems #88112
- **l.** CUT&Tag Index 703 Primer for Illumina Systems #23497
- **m.** CUT&Tag Index 704 Primer for Illumina Systems #37884
- **n.** CUT&Tag Index 705 Primer for Illumina Systems #50105
- **o.** CUT&Tag Index 706 Primer for Illumina Systems #65909
- **p.** CUT&Tag Index 707 Primer for Illumina Systems #84796
- **q.** CUT&Tag Index 708 Primer for Illumina Systems #17160
- **r.** CUT&Tag Index 709 Primer for Illumina Systems #29209
- **s.** CUT&Tag Index 710 Primer for Illumina Systems #41919
- **t.** CUT&Tag Index 711 Primer for Illumina Systems #54212
- **u.** CUT&Tag Index 712 Primer for Illumina Systems #70020

Reagents Not Included:

- **a.** AMPure XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect Reagent Kit (Beckman Coulter, Inc. #B23317)
- **b.** 80% Ethanol (freshly prepared)
- **c.** 10 mM Tris-HCl (pH 8.0-8.5)
- **d.** Magnetic rack/stand
- **e.** Agilent Bioanalyzer system and Agilent High Sensitivity DNA Kit (5067-4626)
- **f.** PCR tubes or plate and PCR Machine

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems Protocol

SAFE STOP This is a safe stopping point in the protocol, if stopping is necessary.

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 NG-seq systems 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:

GOOD

CUT&Tag Index 7 Primers for Illumina Systems		CUT&Tag Index 5 Primers for Illumina Systems	
Index 701	ATTACTCG	Index 503	CCTATCCT
Index 702	TCCGGAGA	Index 504	GGCTCTGA
Index 703	CGCTCATT	Index 505	AGGCGAAG
Index 704	GAGATTCC	Index 506	TAATCTTA
	✔✔✔✔✔✔✔✔		✔✔✔✔✔✔✔✔

BAD

CUT&Tag Index 7 Primers for Illumina Systems		CUT&Tag Index 5 Primers for Illumina Systems	
Index 701	ATTACTCG	Index 502	ATAGAGGC
Index 702	TCCGGAGA	Index 504	GGCTCTGA
Index 703	CGCTCATT	Index 506	TAATCTTA
Index 704	GAGATTCC	Index 508	GTACTGAC
	✔✔✔✔✔✔✔✔		✔✔✘✔✔✘✔✘

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems (continued)

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

Plex	CUT&Tag Index 7 primers for Illumina Systems	CUT&Tag 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 711Index 701, Index 702, Index 703 and Index 704 Index 703, Index 704, Index 705 and Index 706	Any Index 5 Primer
4	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 primer (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 CUT&Tag Index 7 primers and a valid set of CUT&Tag Index 5 primers. Use each CUT&Tag Index 7 primer with each CUT&Tag Index 5 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
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 two 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

II. CUT&Tag Index 5 Primers for Illumina Systems:

Each CUT&Tag Index 5 Primer for Illumina systems is provided in a volume of 30 µl.

Product	Index Primer Sequence	Expected Index Primer Sequence Read
CUT&Tag Index 501 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACTATAGCCTTCGTCG-GCAGCGTCAGATGTG-s-T-3´	TATAGCCT
CUT&Tag Index 502 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACATAGAGGCTCGTCG-GCAGCGTCAGATGTG-s-T-3´	ATAGAGGC
CUT&Tag Index 503 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACCCTATCCTTCGTCG-GCAGCGTCAGATGTG-s-T-3´	CCTATCCT
CUT&Tag Index 504 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACGGCTCTGATCGTCG-GCAGCGTCAGATGTG-s-T-3´	GGCTCTGA
CUT&Tag Index 505 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACAGGCGAAGTCGTCG-GCAGCGTCAGATGTG-s-T-3´	AGGCGAAG
CUT&Tag Index 506 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACTAATCTTATCGTCG-GCAGCGTCAGATGTG-s-T-3´	TAATCTTA
CUT&Tag Index 507 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACCAGGACGTTCGTCG-GCAGCGTCAGATGTG-s-T-3´	CAGGACGT
CUT&Tag Index 508 Primer for Illumina Systems	5´-AATGATACGGCGACCACCGA-GATCTACACGTACTGACTCGTCG-GCAGCGTCAGATGTG-s-T-3´	GTACTGAC

Where -s- indicates phosphorothioate bond.

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems (continued)

III. CUT&Tag Index 7 Primers for Illumina Systems:

Each CUT&Tag Index 7 Primer for Illumina systems is provided in a volume of 20 µl.

Product	Index Primer Sequence	Expected Index Primer Sequence Read
CUT&Tag Index 701 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATCGAGTAAT-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	ATTACTCG
CUT&Tag Index 702 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATTCTCCG-GAGTCTCGTGGGCTCGGAGATGTG-s-T-3´	TCCGGAGA
CUT&Tag Index 703 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATAATGAGCG-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	CGCTCATT
CUT&Tag Index 704 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATGGAATCTC-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	GAGATTCC
CUT&Tag Index 705 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATTTCTGAAT-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	ATTCAGAA
CUT&Tag Index 706 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATACGAATTC-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	GAATTCGT
CUT&Tag Index 707 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATAGCTTCAG-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	CTGAAGCT
CUT&Tag Index 708 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATGCG-CATTAGTCTCGTGGGCTCGGAGATGTG-s-T-3´	TAATGCGC
CUT&Tag Index 709 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATCATAGCCG-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	CGGCTATG
CUT&Tag Index 710 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATTTCGCG-GAGTCTCGTGGGCTCGGAGATGTG-s-T-3´	TCCGCGAA
CUT&Tag Index 711 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATGCGC-GAGAGTCTCGTGGGCTCGGAGATGTG-s-T-3´	TCTCGCGC
CUT&Tag Index 712 Primer for Illumina Systems	5´-CAAGCAGAAGACGGCATACGAGATCTATCGCT-GTCTCGTGGGCTCGGAGATGTG-s-T-3´	AGCGATAG

Where -s- indicates phosphorothioate bond.

IV. Set up the PCR Reaction

Before starting:

- Thaw CUT&Tag Index Primers for Illumina systems and CUT&Tag DNA (or any tagmentated DNA) at room temperature. Quick spin to collect all liquid from the sides of the tube.
- 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.

1. Add the following components to a sterile PCR tube or single well of a PCR plate. Record the CUT&Tag Index 5 and CUT&Tag Index 7 primers added to each PCR tube or well.

Reagents	Volume for 1 PCR Reaction (70 µl)
CUT&Tag DNA (or any tagmentated DNA)	30 µl
CUT&Tag PCR Master Mix	35 µl
CUT&Tag Index 7 Primer for Illumina Systems (10 µM)	2.5 µl
CUT&Tag Index 5 Primer for Illumina Systems (10 µM)	2.5 µl

NOTE: It is critical to change tips between tubes to avoid cross-contamination. If starting with less than 30 µl of CUT&Tag DNA, add DNAse-free water to bring the volume up to 30 µl.

2. Thoroughly mix the reaction by pipetting up and down and perform a quick spin to collect all liquid from the sides of the tube or plate well.

   NOTE: It is critical to change tips between samples to avoid cross-contamination.

3. Place the tube on a thermocycler with a heated lid and perform PCR amplification using the following PCR cycling conditions:

   a. Gap Filling 58°C for 5 min
   b. Gap Filling Extension 72°C for 5 min
   c. Initial Denaturation 98°C for 30 sec
   d. Denaturation 98°C for 10 sec
   e. Anneal and Extension 60°C for 11 sec
   - For between 20,000 and 100,000 cells per CUT&Tag reaction, repeat steps d and e for a total of 13 cycles.
   - For 20,000 and less cells per CUT&Tag reaction, repeat steps d and e for a total of 14-16 cycles.

   Note: excessive PCR cycles lead to lower library diversity and/or higher duplication rate of NGS reads.

   f. Final Extension 72°C for 1 min
   g. Hold 4°C

4. Proceed to Cleanup of PCR Amplification (Section V). (Safe Stop) Alternatively, samples can be stored at -20°C.

CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems (continued)

V. Cleanup of PCR Amplification

Before starting:

- If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use.
- Resuspend AMPure XP Beads or SPRIselect beads by tube inversion or pipetting up and down.
- Prepare 400 µl of 80% ethanol for each sample.
- Prepare approximately 20 µl of 10 mM Tris-HCl (pH 8.0-8.5) for each sample.

1. Add 70 µl (1.0X) resuspended AMPure XP beads or SPRIselect beads to 70 µl PCR reaction from Step 3 in Section IV. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix.
2. Incubate samples on bench top for at least 5 minutes at room temperature.
3. Place the tube/plate on an appropriate magnetic stand for 5 minutes to separate the beads from the supernatant.
4. Carefully remove and discard the supernatant. Be careful to remove all liquid residues but not to disturb the beads that contain DNA targets.
5. Add 200 µl freshly prepared 80% ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
6. Repeat Step 5 once for a total of two washes. Be sure to remove all visible liquid after the second wash.
7. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

   NOTE: Do not over-dry the beads. This may result in lower recovery of DNA targets. Elute the samples when the beads are still glossy looking, but when all visible liquid has evaporated. If the beads start to crack, they are too dry.
8. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 µl of 10 mM Tris-HCl (pH 8.0-8.5) per sample. Mix well by pipetting up and down 10 times. Incubate for at least 2 minutes at room temperature.
9. Place the tube/plate on the magnetic stand and wait for 5 minutes. Carefully transfer 15 µl of supernatant containing the DNA targets to a new tube. (Safe Stop) DNA libraries can be stored at -20°C until further use.
10. Measure the concentration of library DNA.

    NOTE: The yield of the amplified CUT&Tag DNA library can vary based on the DNA quantification method used. If using the Nanodrop or QIAxpert Systems, the expected reading is 10-20 ng/µL for histone targets and 5-12 ng/µL for non-histone targets. If the library concentration is lower than 3 ng/µL with the Nanodrop or QIAxpert Systems, please refer to the troubleshooting guide before sequencing your samples. If using the Qubit Fluorometric Quantification system or the Picogreen assay, the expected reading is 3-10 ng/µL for histone targets and could be lower than 1 ng/µL for non-histone targets.
11. Determine the size distribution of CUT&Tag DNA libraries using Agilent Bioanalyzer or TapeSatation systems, according to the manufacturer's instructions.

    NOTE: While CUT&Tag DNA libraries generated for histone modifications typically show robust signal in Bioanalyzer or TapeStation systems analysis, libraries generated for non-histone proteins such as transcription factors and cofactors often have very weak or even no visible signal using Bioanalyzer or TapeStation systems, but still generate NG-sequencing results with high mapping rates, high numbers of identified binding peaks, and acceptable signal-to-noise ratios across the whole genome. Therefore, we recommend sequencing DNA library preps from transcription factor and cofactor CUT&Tag reactions that do not show a signal in Bioanalyzer or TapeStation systems analysis.
12. Adjust the concentration of final purified library samples with 10mM Tris-HCl (pH 8.0-8.5) for high throughput sequencing. Refer to Illumina sequencing manual for optimal concentration and volume of library DNA required for NG-seq.

    NOTE: Usually the CUT&Tag DNA libraries from histone targets have a higher concentration than those from non-histone targets. We use the following formula to convert a library concentration from ng/µL to nM before diluting each library sample to the same concentration (nM) for pooling purposes: Concentration (nM) = 1,000,000 X Concentration (ng/µL) / library average size (bp) / 660. For CUT&Tag libraries where the Bioanalyzer or TapeStation system is unable to identify the average size of the library, we suggest using a size of 900 bp to intentionally pool more low-yield libraries than normal-yield libraries. In addition, we would also suggest pooling the libraries that have a flat signal of 5-10 fold more than the libraries that show normal sized peaks on the Bioanalyzer or TapeStation systems. This ensures an even distribution of the number of reads among all samples. Usually, a library pool concentration of 2 nM DNA is enough for NGS purposes, although a higher concentration is always welcome.