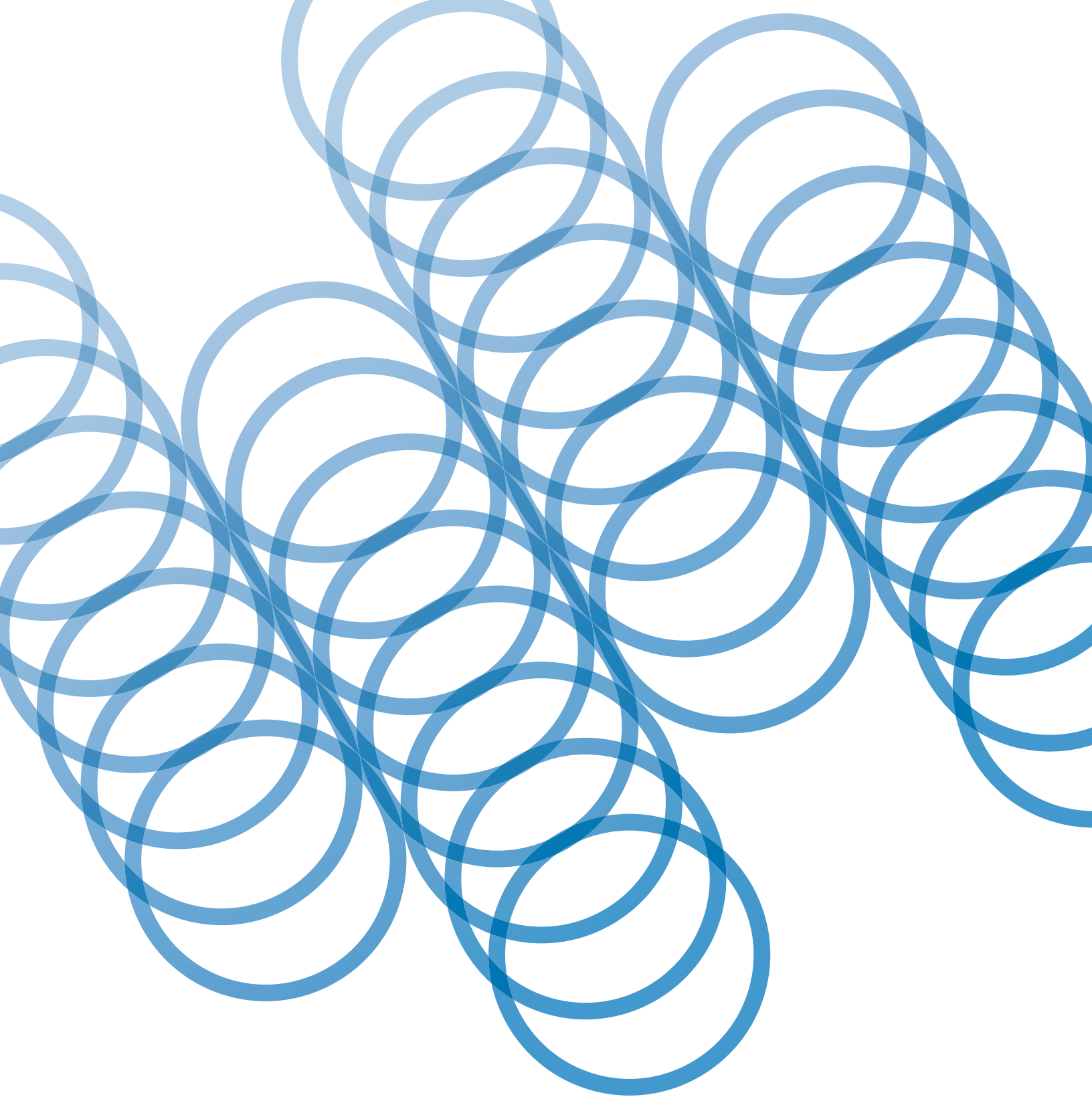

EpiCypher®
Bringing Epigenetics to Life

CUTANA™
CUT&Tag Kit Version 4
User Manual Version 4.0

Kit Version 4
User Manual Version 4.0

EpiCypher®

CUTANA™

CUT&Tag Kit

Catalog No. 14-1102-48s1: 48 Reactions (formerly No. 14-1102)
Catalog No. 14-1102-48s2: 48 Reactions (formerly No. 14-1103)
Catalog No. 14-1102-24s3: 24 Reactions

Upon receipt, store indicated components at 4°C, -20°C and room temperature (RT)

Stable for 12 months upon date of receipt.
See p. 7-8 for storage instructions.

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US & Canada: 1-800-535-5053 | International: 1-352-323-3500

Table of Contents

See EpiCypher's Tech Support Center at support.epicypher.com for sample prep variations, SNAP-CUTANA™ Spike-in Control information, assay FAQs, and troubleshooting guidance.

Background and Product Description

Cleavage **U**nder **T**argets & **Tag**mentation (CUT&Tag) is a groundbreaking epigenomic mapping strategy that builds on its predecessor immunotethering technologies CUT&RUN and ChIC[1-6]. In CUTANA™ CUT&Tag, nuclei are immobilized to a solid support and antibodies bind their chromatin targets *in situ*. A fusion of proteins A and G with prokaryotic transposase 5 (pAG-Tn5) is used to selectively cleave and tagment antibody-bound chromatin with sequencing adapters (Figure 1). Tagmented fragments are directly PCR amplified using EpiCypher's exclusive single-tube ("Direct-to-PCR") approach, yielding sequence-ready DNA[6,7].

CUT&Tag is best for mapping histone post-translational modifications (PTMs). For chromatin-associated proteins, CUT&RUN assays are strongly recommended.

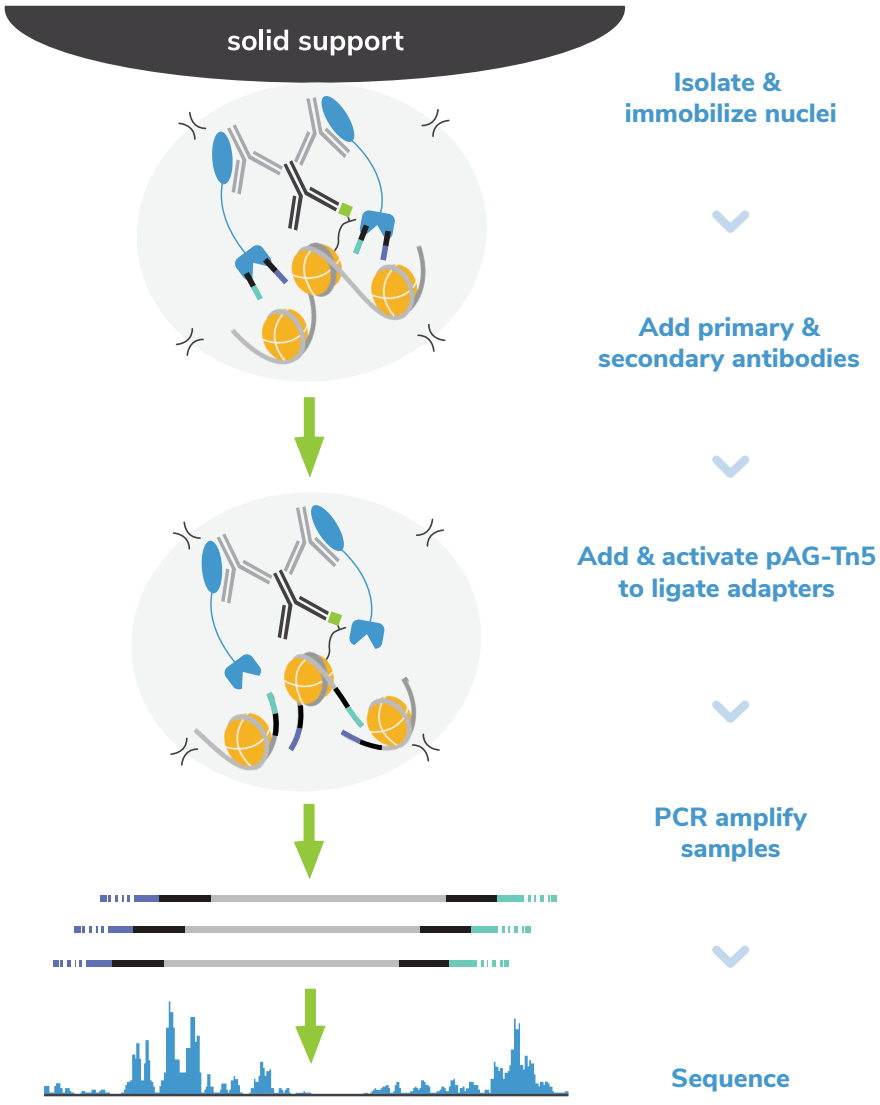


FIGURE 1
Overview of the CUTANA™ CUT&Tag workflow.

Background and Product Description

Compared to ChIP-seq, the historical go-to chromatin mapping assay, CUT&Tag provides higher quality sequencing data with improved sensitivity. By selectively targeting antibody-labeled chromatin in intact nuclei, without chromatin fragmentation or immunoprecipitation, background is dramatically reduced. Tagmentation eliminates traditional library prep, further streamlining the protocol and mitigating sample loss. These innovations enable high-resolution mapping for histone PTMs using a small number of starting cells and only 5-8 million total reads per reaction (Figure 2).

The CUTANA™ CUT&Tag Kit is ideal for genomic mapping experts that want to increase throughput and scale without sacrificing sensitivity. Our unique single-tube workflow is designed for multi-channel pipetting and can be completed in just two days [6,7]. These features, combined with reduced assay costs and exquisite signal-to-noise, position CUT&Tag to become a leading tool for chromatin research.

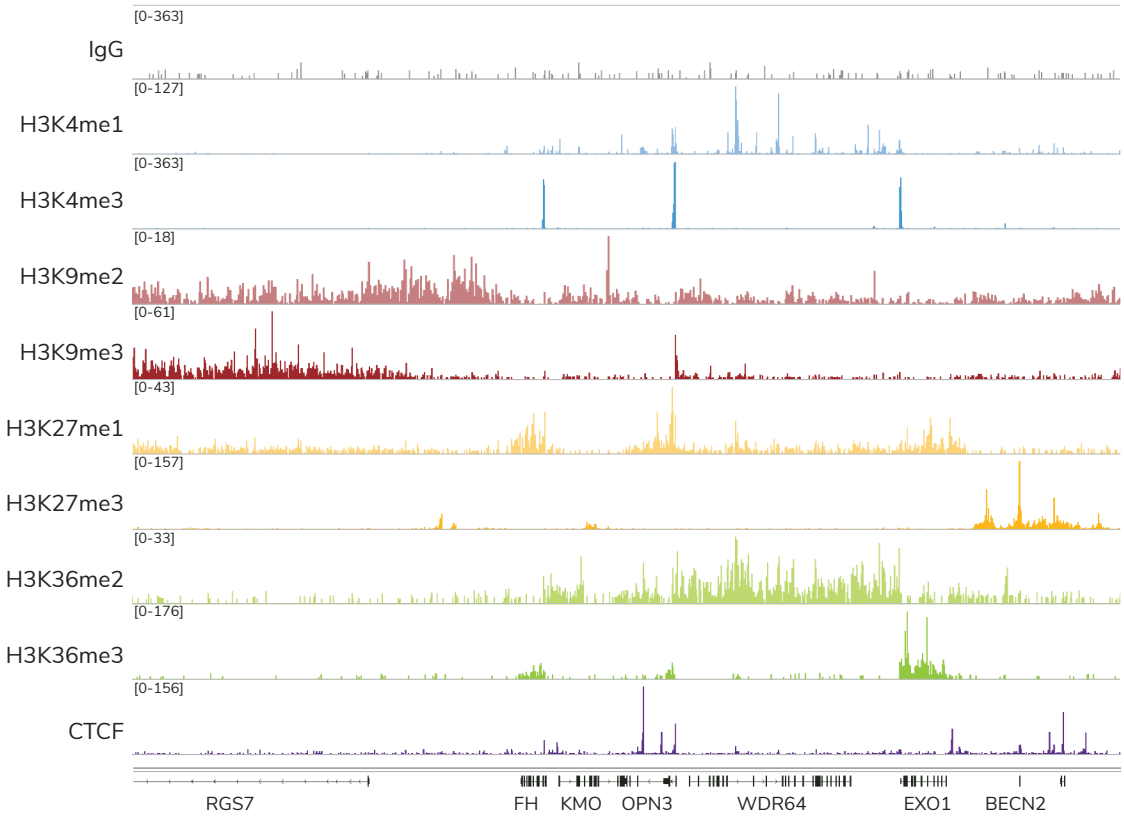


FIGURE 2

CUTANA CUT&Tag generates high-quality profiles for targets in both active and repressed chromatin regions, including active regulatory elements (H3K4me1) and promoters (H3K4me3), constitutive (H3K9me2/me3) and facultative (H3K27me1/me3) heterochromatin, active gene bodies (H3K36me2/me3), and select proteins (CTCF). Rabbit IgG is shown as a negative control. Results were generated using 100,000 K562 nuclei and 5-8 million total reads per reaction (3-5 million unique reads per reaction).

The 48 reaction kits (14-1102-48s1/s2) or 24 reaction kit (14-1102-24s3) respectively contain sufficient materials for 48 or 24 CUT&Tag reactions, including pAG-Tn5, Concanavalin A (ConA) beads, buffers, and tubes. Positive and negative control antibodies, along with our exclusive nucleosome spike-in controls, are provided to help optimize workflows, monitor experimental success, and aid troubleshooting.

To facilitate library prep and multiplexed sequencing, the kit also includes indexing primers, a PCR master mix, and DNA purification beads. A combinatorial dual indexing primer strategy [8,9] enables the entire 48 or 24 reaction kit to be pooled in a single run. The three versions of this kit (14-1102-48s1, 14-1102-48s2, 14-1102-24s3) contain distinct primer sets, allowing up to 96 reactions to be multiplexed when kits are used together properly (see Appendix 1).

The user-friendly protocol starts with a nuclei isolation step. Although it is recommended to start with 100,000 nuclei per reaction, comparable data can be generated down to 10,000 nuclei for select targets (Figure 3), making CUT&Tag well-suited for rare cell types and low-input applications.

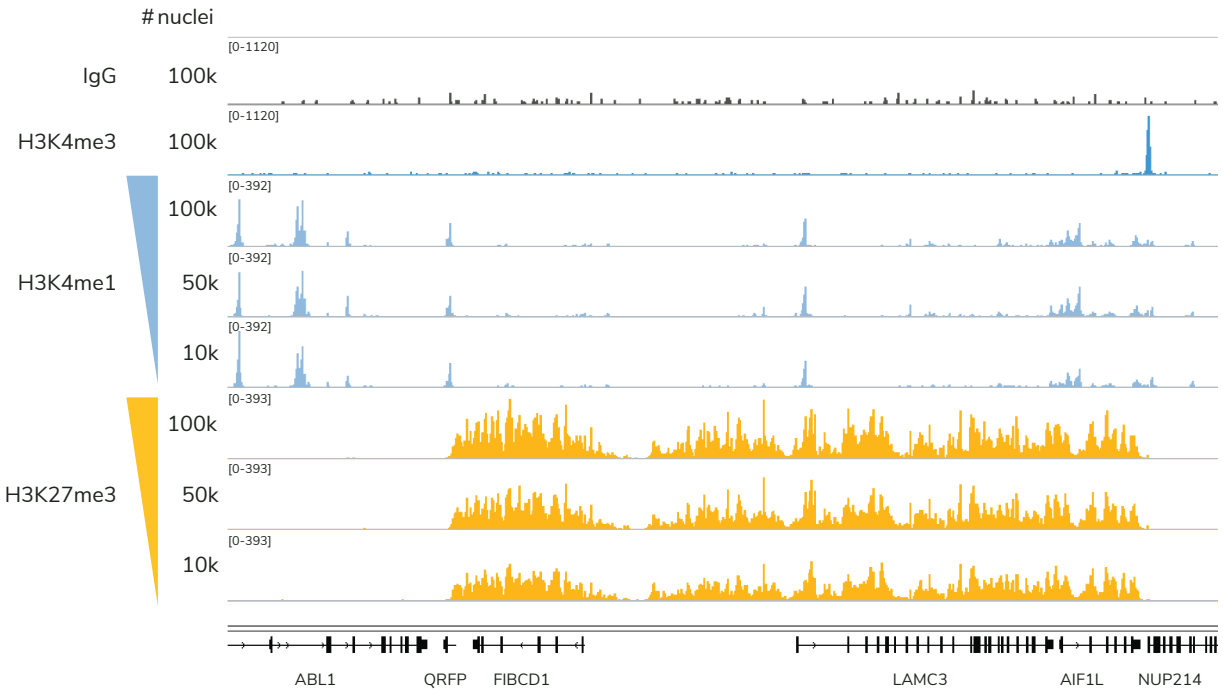


FIGURE 3

CUTANA CUT&Tag enables reliable chromatin profiling from low nuclei numbers. CUT&Tag was used to map H3K4me1 (low abundance target) and H3K27me3 (high abundance target) using decreasing numbers of K562 nuclei. Data quality at 10,000 nuclei is comparable to standard inputs of 100,000 nuclei. There are some caveats when using low nuclei numbers; see support.epicypher.com for details. H3K4me3 and IgG are shown as controls.

Included in the Kit

Kit components are stable for 12 months upon date of receipt. Store as outlined below.

Store at room temperature (RT) upon receipt:

Item	14-1102-48s1/s2	14-1102-24s3	Notes before use
8-strip Tubes	10-0009t	10-0009-07	Enables use of multi-channel pipettors.
0.5 M EDTA	21-1006t	21-1006-07	Use to prepare Antibody Buffer.
5 M NaCl	21-1013t	21-1013-07	Use to prepare Wash Buffer 2.
1 M $MgCl_2$	21-1015t	21-1015-07	Use to prepare Tagmentation Buffer.
SDS Release Buffer	21-1017t	21-1017-07	Releases tagmented fragments into solution.
SDS Quench Buffer	21-1018t	21-1018-07	Neutralizes SDS prior to indexing PCR.
DNA Purification Beads	21-1407t	21-1407-07	**DO NOT FREEZE.** Slightly viscous; thoroughly mix and pipette carefully to ensure correct volume**.**
0.1X TE Buffer	21-1025t	21-1025-07	Use to elute CUT&Tag libraries.

Store at 4°C upon receipt:

Item	14-1102-48s1/s2	14-1102-24s3	Notes before use
ConA Beads	21-1401t	21-1401-07	**DO NOT FREEZE.** Concanavalin A (ConA) beads are used for immobilizing nuclei or cells.
Bead Activation Buffer	21-1001t	21-1001-07	Use to prepare ConA beads.
Pre-Nuclei Extraction Buffer	21-1021t	21-1021-07	Use to prepare Nuclei Extraction Buffer.
Pre-Wash Buffer	21-1002t	21-1002-07	Use to prepare Wash Buffer 1 and to wash reactions after tagmentation.
Rabbit IgG Negative Control Antibody	13-0042t	13-0042-02	**SMALL VOLUME: quick spin before use.** 0.5 mg/mL stock. Add 1 µL to designated negative control reactions. Sufficient for 8 reactions.
H3K27me3 Positive Control Antibody	13-0055t	13-0055-02	**SMALL VOLUME: quick spin before use.** Rabbit monoclonal antibody, 0.5 mg/mL stock. Add 1 µL to designated positive control reactions. Sufficient for 8 reactions.
H3K4me3 Positive Control Antibody	13-0060t	13-0060-02	**SMALL VOLUME: quick spin before use.** Rabbit monoclonal antibody, 0.5 mg/mL stock. Add 1 µL to designated positive control reactions. Sufficient for 8 reactions.
Wash Buffer Enhancer 1	21-1028t	21-1028-07	Buffer additives to reduce sample clumping and improve bead handling.
Wash Buffer Enhancer 2	15-1030t	15-1030-07	

Included in the Kit

Kit components are stable for 12 months upon date of receipt. Store as outlined below.

Store at -20°C upon receipt:

Item	14-1102-48s1/s2	14-1102-24s3	Notes before use
5% Digitonin	21-1004t	21-1004-07	Thaw at RT. Use to prepare Wash Buffer 1.
1 M Spermidine	21-1005t	21-1005-07	Use to prepare Nuclei Extraction Buffer and Wash Buffer 1.
SNAP-CUTANA™ K-MetStat Panel	19-1002t	19-1002-02	**SMALL VOLUME: quick spin before use. Pipette to resuspend Panel — DO NOT VORTEX.** Panel of biotinylated nucleosomes coupled to streptavidin-coated magnetic beads. Pair with IgG, H3K4me3, and H3K27me3 control antibodies. Sufficient for 24 reactions. See support.epicypher.com for information.
Anti-Rabbit Secondary Antibody	13-0047t	13-0047-07	**SMALL VOLUME: quick spin before use.** Secondary antibody for CUT&Tag reactions using a rabbit primary antibody. Sufficient for 50 reactions in 14-1102-48s1/s2 and for 24 reactions in 14-1102-24s3.
pAG-Tn5	15-1017t	15-1017-07	**GLYCEROL CONTAINING BUFFER: quick spin before use.** 20X concentration. Proteins A and G (pAG) bind antibodies of various isotypes and host species including total IgG for rabbit, mouse, goat, donkey, rat, guinea pig, horse, and cow.
Non-Hot Start 2X PCR Master Mix	15-1018t	15-1018-07	Use for PCR amplification and indexing of CUT&Tag libraries.
i7 Primers, 10 µM (i701-i712)	18-1301 to 18-1312	18-1301 to 18-1312	**SMALL VOLUME: quick spin before use.** Included in all Kits (14-1102-48s1, 14-1102-48s2, and 14-1102-24s3).
Kit 14-1102-48s1 i5 Primers, 10 µM (i501-i504)	18-1201 to 18-1204	N/A	**SMALL VOLUME: quick spin before use.** 14-1102-48s1 and 14-1102-48s2 contain distinct i5 primers. 14-1102-24s3 contains a subset of primers in 14-1102-48s1. 14-1102-48s1 and 14-1102-48s2 each generate 48 unique pairs of barcodes; Kit 14-1102-24s3 generates 24 unique pairs of barcodes; Combined, they generate up to 96 uniquely barcoded libraries. See Appendix 1. ***NOTE:** Download i5 and i7 index sequences at epicypher.com/14-1102 under Resources.
Kit 14-1102-48s2 i5 Primers, 10 µM (i505-i508)	18-1205 to 18-1208	N/A	
Kit 14-1102-24s3 i5 Primers, 10 µM (i501-i502)	N/A	18-1201 and 18-1202	

Materials Required but Not Supplied

REAGENTS:

- Antibody to target of interest. See epicypher.com/products/antibodies/?product-filter=cuttag to see our list of CUT&Tag validated antibodies or contact techsupport@epicypher.com for recommendations.
- Secondary antibody matched to the primary antibody host species. If using rabbit primary antibodies, use the Anti-Rabbit Secondary Antibody provided with this kit. For mouse or other primary antibody species, the purchase of an additional secondary antibody is required; anti-mouse secondary antibody for CUT&Tag is available (EpiCypher 13-0048).
- CUTANA™ Protease Inhibitor Tablets (EpiCypher 21-1027)
- 0.4% Trypan blue (e.g. Invitrogen T10282)
- Molecular biology grade water, any vendor
- 100% Ethanol (200 proof), any vendor
- 1X Phosphate buffered saline (1X PBS), any vendor

EQUIPMENT:

- 1.5, 15, and 50 mL tubes
- Low-retention filter pipette tips
- Magnetic separation rack for 1.5 mL tubes (EpiCypher 10-0012)
- Magnetic separation rack for 8-strip tubes (EpiCypher 10-0008)
- 8-channel multi-pipettor (e.g. VWR 76169-250) and multi-channel reagent reservoirs (e.g. Thermo Fisher Scientific 14-387-072)
- Vortex (e.g. Vortex-Genie 2, Scientific Industries SI-0236)
- Benchtop centrifuge/mini-centrifuge with an 8-strip tube adapter (e.g. from Fisher Scientific, Benchmark Scientific)
- Tube nutator for incubation steps (e.g. VWR 82007-202)

  * **A nutator rocks tubes gently, without end-over-end rotation. Rotating tubes traps liquid in tube caps, resulting in ConA bead dry out and sample loss — do NOT rotate tubes.**

- Thermocycler with heated lid (e.g. from BioRad, Applied Biosystems, Eppendorf)
- Qubit™ 4 Fluorometer (Invitrogen Q33238 or previous version) and 1X dsDNA High Sensitivity Kit (Invitrogen Q33230)
- Capillary electrophoresis machine and required reagents, e.g. Agilent TapeStation® with D1000 ScreenTape (5067-5582) and D1000 reagents (5067-5583) or Agilent Bioanalyzer® with High Sensitivity DNA Kit (5067-4626)

Outline of Workflow

The CUTANA™ CUT&Tag Kit uses an exclusive Direct-to-PCR strategy, going from cells to PCR-amplified libraries in one tube. Advantages include:

* Improved throughput - designed for 8-strip tubes, fewer steps vs. standard CUT&Tag
* Low nuclei requirements - streamlined workflow minimizes sample loss, supports low inputs
* High reproducibility - optimized for multi-channel pipetting, reduces sample handling variation

Here, we review the main steps of the CUTANA™ CUT&Tag assay:

Step 1: Isolate nuclei and immobilize to Concanavalin A (ConA) beads

Nuclei are prepared from bulk cell populations and immobilized to activated ConA beads in 8-strip tubes. High quality sample prep is essential to CUT&Tag workflows. It is recommended to confirm cell viability, nuclear integrity, and binding to ConA beads. Avoid ConA bead clumping and dry out during the assay, which results in sample loss and reduced yields.

Step 2: Label chromatin using primary and secondary antibodies

The bead-nuclei mixture is incubated with a target-specific primary antibody overnight, followed by treatment with a species-matched secondary antibody the next day. Selection of a highly specific primary antibody is crucial to CUT&Tag assay success, as off-target binding can significantly impact data quality. At the conclusion of this step, nuclei are washed with a high-salt buffer (Wash Buffer 2) to remove unbound antibodies and prepare for tagmentation.

Step 3: Perform targeted chromatin tagmentation using pAG-Tn5

pAG-Tn5 is added to each reaction and binds antibody-labeled chromatin via the immunoglobulin binding properties of pAG. The addition of secondary antibody in prior steps increases the number of pAG binding sites, amplifying Tn5 localization and on-target signal in sequencing data.

Tn5 is activated by the addition of magnesium (Tagmentation Buffer) to cleave and append sequencing adapters to DNA proximal to antibody-bound chromatin (Figure 4). These steps are performed under high salt to minimize nonspecific Tn5 cleavage (i.e. ATAC-like signal) [5,6]. Rinsing the bead slurry with Pre-Wash Buffer effectively quenches the tagmentation reaction.

SDS Release Buffer is added to release tagmented DNA into solution. Finally, SDS Quench Buffer is added to neutralize SDS and enable PCR.

Step 4: Indexing PCR and library cleanup

The 2X Non-Hot Start PCR Master Mix and i5 and i7 Primers are added to the entire reaction mixture. The kit uses a combinatorial dual indexing primer strategy, meaning that each CUT&Tag library will contain a distinct pair of i5 and i7 barcodes, or indexes[8,9]. This strategy enables multiplexing of up to 48 (or 24) libraries using various combinations of the four (or two) i5 Primers and twelve i7 Primers in each kit. The three versions of this kit (14-1102-48s1, 14-1102-48s2, 14-1102-24s3) contain distinct primer sets, allowing up to 96 reactions to be multiplexed when kits are used together properly. See Appendix 1.

Step 4: Indexing PCR and library cleanup *(continued)*

The kit cycling parameters include two steps prior to PCR amplification (Figure 4). The first step fills in and repairs 3' gaps caused by Tn5 tagmentation. The second step uses ligated adapter DNA as a primer to extend 3' ends.

The subsequent PCR steps are specifically optimized for CUT&Tag fragments. Briefly, i5 Primers anneal to i5 adapters on tagmented DNA and i7 Primers anneal to i7 adapters (Figure 4). Each primer incorporates a unique index and the required Illumina P5/P7 flow cell sequences during PCR. Because adapter-ligated chromatin is the only suitable template for PCR using i5 and i7 Primers, tagmented DNA is selectively amplified and barcoded — even in the presence of cell debris. This Direct-to-PCR approach enriches target DNA and greatly streamlines library prep [6,7].

Step 5: Analysis of library quality

Purified CUT&Tag libraries are examined using the Qubit Fluorometer and the Agilent Bioanalyzer or TapeStation to determine library concentration and fragment size distribution. The PCR parameters in this protocol amplify fragments compatible with Illumina paired-end sequencing, with an average fragment size of ~300 bp (including adapter DNA). See **Protocol: Section VIII** for a discussion of expected results.

Step 6: Illumina® next-generation sequencing

Once libraries are quantified, they are diluted, pooled, and sequenced on an appropriate Illumina sequencing platform; see **Protocol: Section IX** and Appendix 1. For information on expected results from control reactions, see support.epicypher.com.

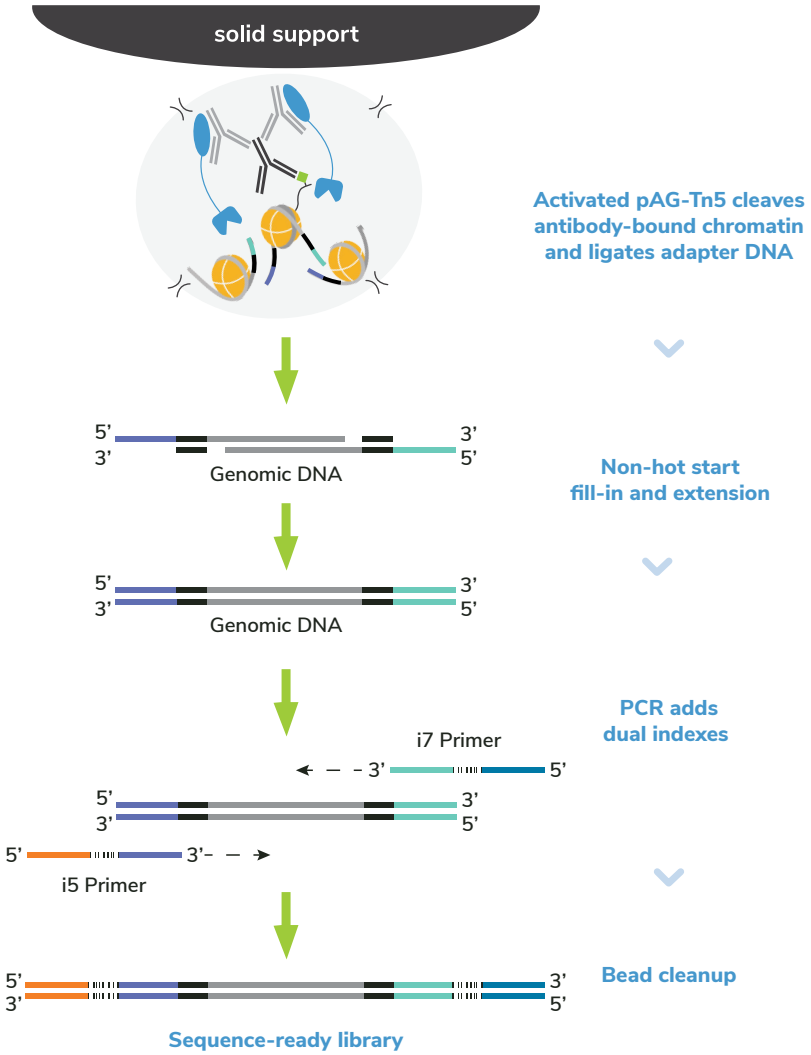


FIGURE 4

Overview of tagmentation, indexing PCR, and bead cleanup sections of the CUTANA CUT&Tag workflow. Activated pAG-Tn5 cleaves and ligates sequencing adapters to DNA proximal to antibody-labeled chromatin. Tagmented DNA is selectively repaired and amplified using i5 and i7 Primers that recognize ligated adapter DNA sequences. The i5 and i7 Primers add indexes (barcodes) to 5' and 3' ends, respectively, during PCR, generating dual-barcoded libraries for multiplexed sequencing. Libraries are bead-purified, quantified, analyzed by capillary electrophoresis (e.g. TapeStation), and used for Illumina sequencing.

Here we outline best practices for CUT&Tag success and assay optimization. For our most up-to-date recommendations, visit support.epicypher.com.

SAMPLE INPUTS FOR CUT&TAG

- Freshly isolated, unfixed (i.e. native) nuclei are the preferred input for CUT&Tag.
- 100,000 nuclei per reaction is recommended. For nuclei prep, harvest 100,000 cells per reaction plus 20% excess to account for sample loss.
- Using more than 100,000 nuclei does **NOT** improve yield and may inhibit PCR.
- Visit support.epicypher.com if using whole cells, adherent cells, tissues, cryopreserved samples, or cross-linked nuclei and cells.

IMPORTANT NOTE ON SAMPLE QUALITY

- High quality sample prep is essential for CUT&Tag success. Check cell count, viability, and morphology at initial cell harvest. Count extracted nuclei to ensure you are not losing sample during centrifugation (spin longer if needed).
- Confirm nuclei binding to ConA beads — a critical part of the CUT&Tag workflow.
- On Day 2 of CUT&Tag, it is crucial that ConA beads are resuspended. Vortex to keep beads in solution where indicated in the protocol. Excessive clumping leads to sample loss and poor yields.

COMPATIBLE TARGETS

- Histone PTMs generate the most robust and reliable profiles using CUT&Tag.
- Mapping chromatin-associated proteins with CUT&Tag is **NOT** recommended. For these targets we recommend CUTANA CUT&RUN assays, which generate robust profiles for diverse target classes (epicypher.com/technologies/cutana-cutrun-assays/).

ANTIBODY SELECTION

- Test histone PTM antibodies from multiple vendors (Figure 5). Select the antibody that gives the best balance of expected target enrichment, low background, and high yields.
- Lysine methylation PTM antibodies can be directly validated in CUT&Tag using the SNAP-CUTANA K-MetStat Panel (EpiCypher 19-1002).
- Antibodies that work well in ChIP-seq are **NOT** guaranteed success in CUT&Tag.
- Visit epicypher.com/products/antibodies/?product-filter=cuttag to shop our antibodies or email techsupport@epicypher.com for recommendations.

ASSAY OPTIMIZATION OVERVIEW

- CUT&Tag optimization, including antibody validation, is reviewed in Figure 5. For further guidance, visit support.epicypher.com.
- CUT&Tag success depends on many factors, including cell type, nuclei number, target abundance, and antibody quality. In addition, an antibody that performs reliably at 100,000 nuclei may fail at lower nuclei inputs.
- CUT&RUN may be preferrable in some cases (epicypher.com/technologies/cutana-cutrun-assays/).

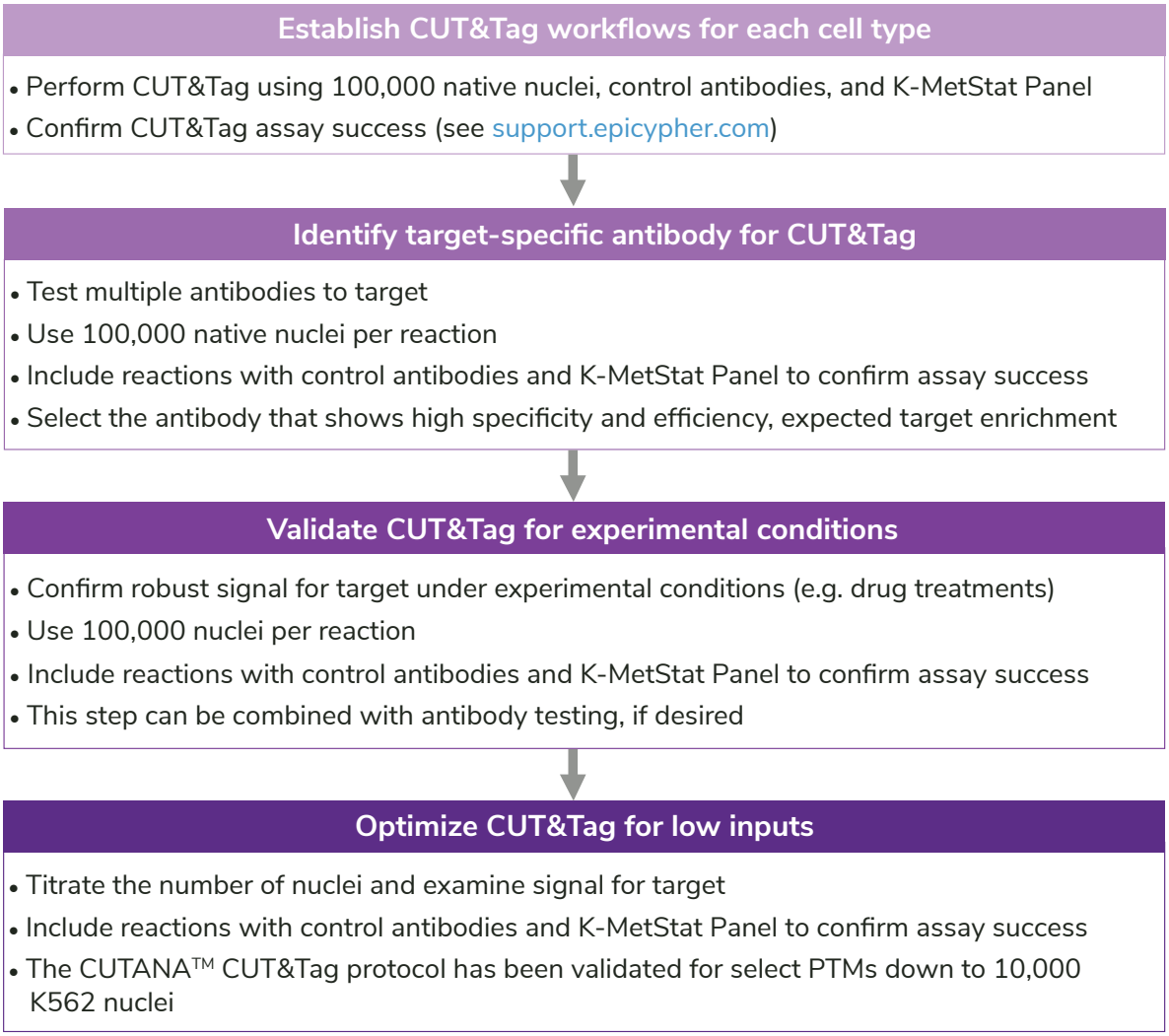


FIGURE 5

Development and optimization guidelines for successful CUT&Tag workflows.

EXPERIMENTAL CONTROLS

- This kit includes multiple quality control checks (Figure 6) to ensure reliable CUT&Tag workflows.
- Each kit comes with H3K4me3 and H3K27me3 positive control antibodies, IgG negative control antibody, and the SNAP-CUTANA™ K-MetStat Panel of spike-in controls.
- Control reactions spiked with the K-MetStat Panel should be included in **every** experiment to determine assay success and aid troubleshooting (support.epicypher.com).

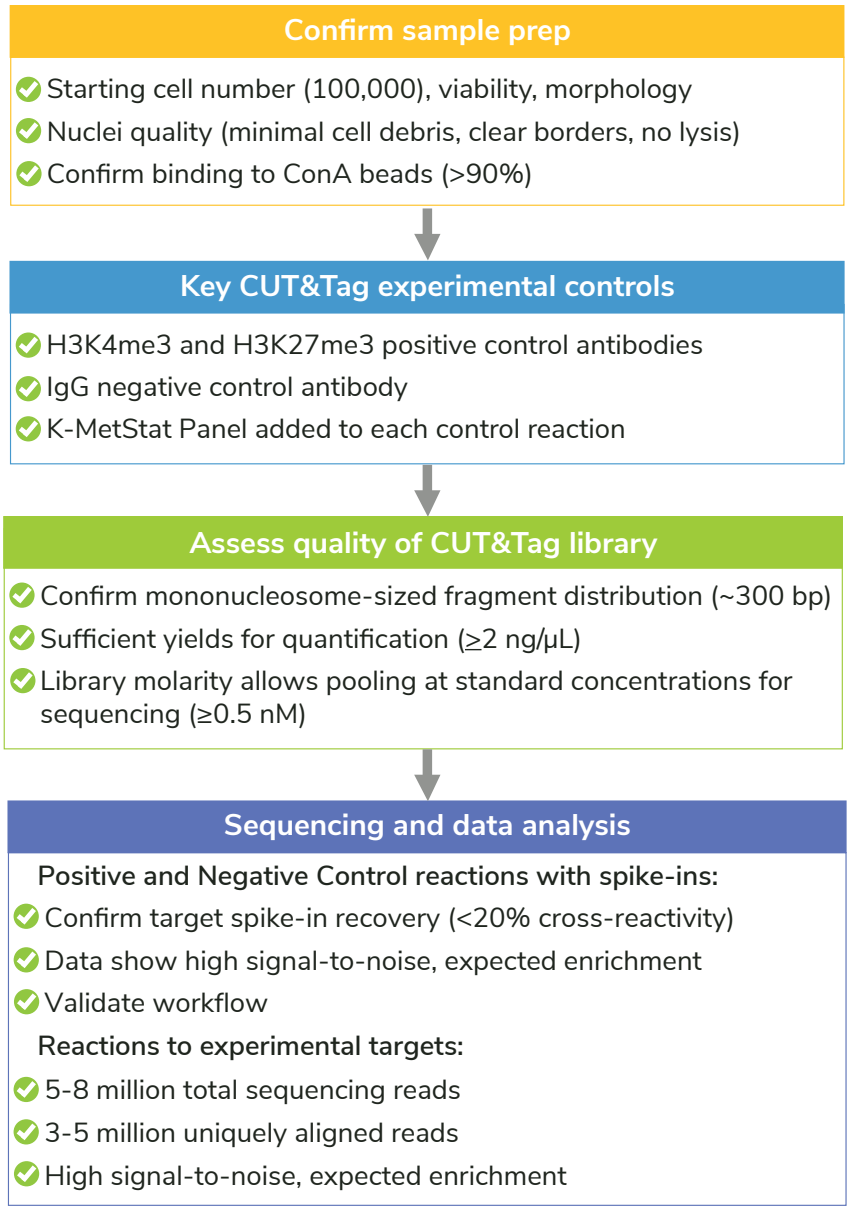


FIGURE 6

The CUTANA CUT&Tag Kit comes with multiple controls to ensure success.

SECTION I: CUT&TAG BUFFER PREP (~30 MIN)

IMPORTANT NOTES ON BUFFER PREP

* These buffers (Figure 7) are prepared **FRESH** on Day 1 of every CUT&Tag experiment.
* Volumes in Table 1 are per CUT&Tag reaction and include 20% excess to account for pipetting errors. You do NOT need to add additional volume.
* Add Wash Buffer Enhancers 1 and 2 to reduce sample clumping and improve bead handling.

1. Gather kit reagents stored at 4˚C and -20˚C needed for Day 1: **ConA Beads, Bead Activation Buffer, Pre-Nuclei Extraction Buffer, Pre-Wash Buffer, Digitonin, Spermidine, Wash Buffer Enhancer 1, Wash Buffer Enhancer 2, K-MetStat Panel, H3K4me3, H3K27me3, and IgG Control Antibodies**. Place on ice to thaw or equilibrate.
2. Reconstitute protease inhibitor tablet (EpiCypher 21-1027) as instructed in product data sheet or on product page to prepare a **25X Protease Inhibitor** stock. After buffer prep, the remaining 25X stock can be stored for 12 weeks at -20˚C.
3. In a new tube, prepare **Nuclei Extraction Buffer** by combining Pre-Nuclei Extraction Buffer, 25X Protease Inhibitor, and 1M Spermidine as outlined in Table 1. Place on ice.

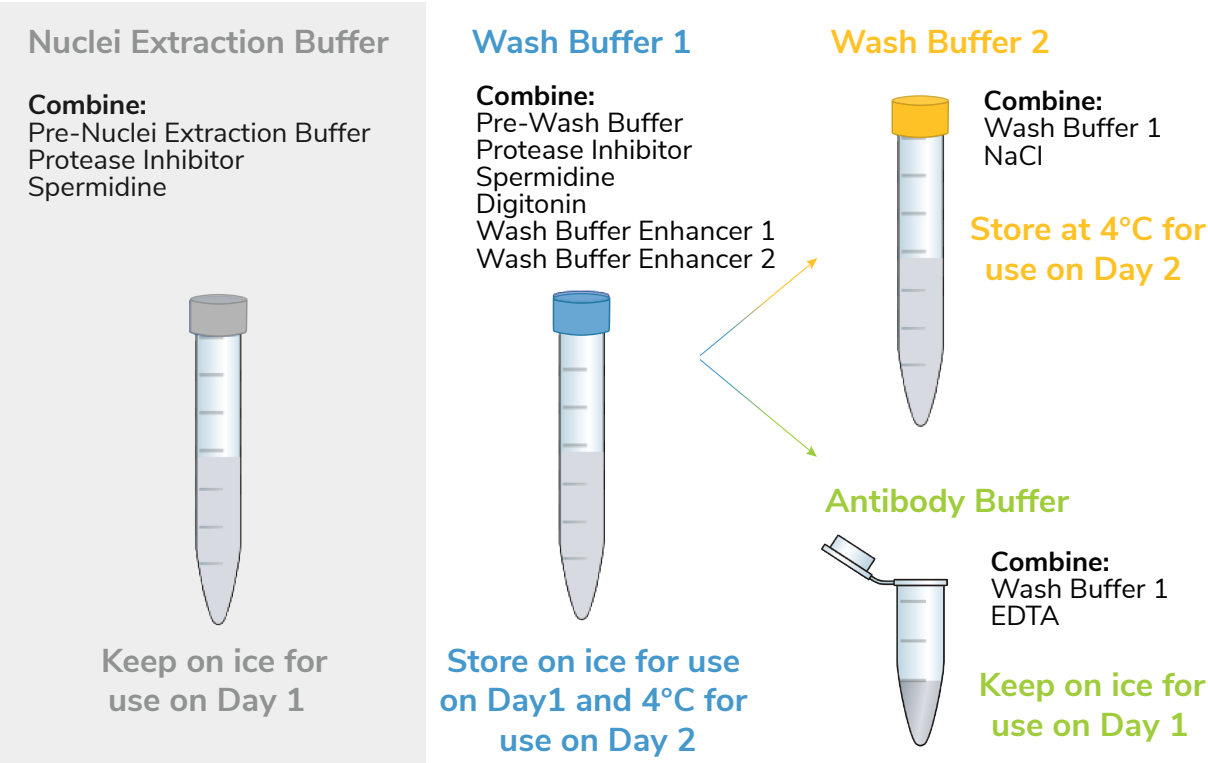


FIGURE 7

Schematic of CUT&Tag buffer preparation. See Table 1 for volume calculations.

4. To a tube labeled **Wash Buffer 1**, add Pre-Wash Buffer; 25X Protease Inhibitor; 1M Spermidine; 5% Digitonin; 1,000X Wash Buffer Enhancer 1; and 2,500X Wash Buffer Enhancer 2 as outlined in Table 1. If using whole cells, Digitonin MUST be optimized for efficient permeabilization (see support.epicypher.com). As prepared, **Wash Buffer 1** contains 150 mM NaCl. Place on ice.
5. To a tube labeled **Wash Buffer 2**, combine **Wash Buffer 1** and 5 M NaCl as outlined in Table 1. As prepared, **Wash Buffer 2** contains 300 mM NaCl. Store **Wash Buffer 2** at 4˚C for Day 2.
6. In a new 1.5 mL tube labeled **Antibody Buffer**, combine **Wash Buffer 1** and 0.5 M EDTA as described in Table 1. Place on ice.
7. Store remaining **Wash Buffer 1** at 4˚C for use on Day 2.

Buffer Sample Scaling Calculations

COMPONENT	[Final]	1rxn	8rxn	16rxn
Nuclei Extraction Buffer - store on ice for use on Day 1				
Pre-Nuclei Extraction Buffer	-	120 µL	960 µL	1.92 mL
25X Protease Inhibitor	1X	5 µL	40 µL	80 µL
1 M Spermidine	0.5 mM	0.063 µL	0.5 µL	1.0 µL
Wash Buffer 1 - store on ice for use on Day 1 and at 4°C for use on Day 2				
Pre-Wash Buffer	-	1.43 mL	11.5 mL	23 mL
25X Protease Inhibitor	1X	60 µL	480 µL	960 µL
1 M Spermidine	0.5 mM	0.75 µL	6 µL	12 µL
5% Digitonin	0.01%	3 µL	24 µL	48 µL
1,000X Wash Buffer Enhancer 1	1X	1.5 µL	12 µL	24 µL
2,500X Wash Buffer Enhancer 2	1X	0.6 µL	4.8 µL	9.6 µL
Wash Buffer 2 - store at 4°C for use on Day 2				
Wash Buffer 1	-	601 µL	4.8 mL	9.6 mL
5 M NaCl	300 mM	18.6 µL	148.8 µL	298 µL
Antibody Buffer - store on ice for use on Day 1				
Wash Buffer 1	-	60 µL	480 µL	960 µL
0.5 M EDTA	2 mM	0.25 µL	2 µL	4 µL

TABLE 1

Combine reagents as instructed in the table to prepare CUT&Tag Buffers. Calculations for 8 and 16 reactions are provided. All buffers include 20% extra volume to account for pipetting error - no additional overage is needed.

SECTION II: BEAD ACTIVATION (~30 MIN)

TIPS FOR WORKING WITH MAGNETIC CONA BEADS

* Do **NOT** use ConA beads that have been frozen and/or appear black, granular, or clumpy.
* Do **NOT** let ConA beads dry out. Avoid disturbing beads with pipette while on magnet.
* Activated ConA beads should be kept on ice and used within four hours of activation.

8. Gently resuspend **ConA Beads** and transfer 11 µL per reaction to a 1.5 mL tube.
9. Place tube on a magnet, allow slurry to clear. Pipette to remove supernatant.
10. Remove tube from magnet. Immediately add 100 µL per reaction cold **Bead Activation Buffer** and pipette to resuspend. Return tube to magnet, allow slurry to clear, and pipette to remove supernatant.
11. Repeat the previous step one time.
12. Resuspend beads in 11 µL per reaction cold **Bead Activation Buffer** (e.g. for 8 reactions, resuspend in 88 µL Bead Activation Buffer). Place on ice.

SECTION III: NUCLEI PREP AND BINDING TO BEADS (~30 MIN)

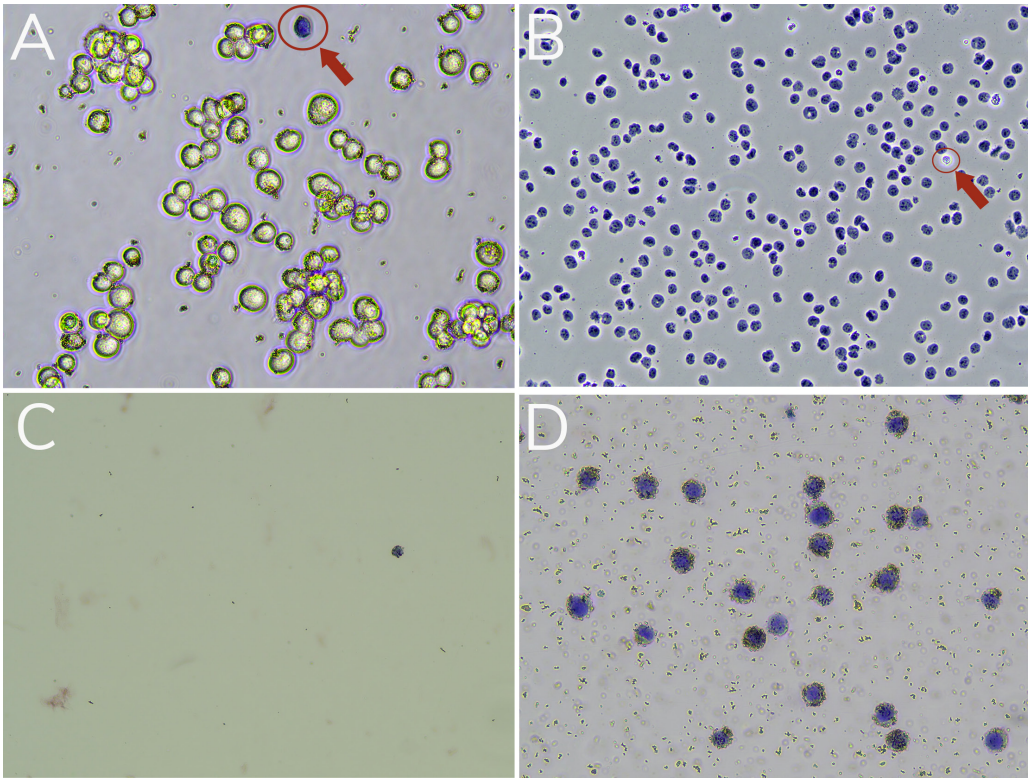
MODIFICATIONS FOR SAMPLE TYPES

* **High-quality sample prep is essential to CUT&Tag.** The quality control steps in this protocol support robust nuclei extraction for successful CUT&Tag.
* The CUTANA CUT&Tag protocol is designed for native nuclei extracted from suspension cells. If using adherent cells, cross-linked samples, frozen nuclei/cells, or other sample types, visit support.epicypher.com.

13. Collect starting cells (in tissue culture flask, tube, etc.). Count and confirm cell integrity as follows:
    A. Transfer 10 µL cells to a fresh tube.
    B. Add 10 µL 0.4% Trypan Blue. Pipette 3 times to mix.
    C. Transfer 10 µL of the cell-Trypan Blue mixture to a cell counting slide. Obtain cell counts, determine viability (>80% is ideal), and confirm expected cellular morphology using a brightfield/phase microscope or cell counter. See Figure 8A (p. 19).

14. Harvest 100,000 cells per reaction in a fresh tube. To account for sample loss and allow quality checks, it is recommended to collect at least 20% excess cells (e.g. for 8 reactions, harvest ~1 million cells).
15. Spin cells 600 x g, 3 min, RT. Pipette to remove supernatant. Perform a 1X PBS wash if needed.
16. Add 100 µL per reaction thoroughly mixed and cold **Nuclei Extraction Buffer** (e.g. for 8 reactions, add 800 µL Nuclei Extraction Buffer). Resuspend by gentle yet thorough pipetting. Incubate for 10 min on ice.
17. Spin 600 x g for 5 min at 4°C. Pipette to remove supernatant. The pellet should change from pale yellow (cells) to white (nuclei). If samples are not forming a pellet, increase spin time.
18. Gently resuspend nuclei in 105 µL per reaction cold **Wash Buffer I**.
19. Transfer 10 µL nuclei to a new tube. Count and evaluate nuclei integrity using Trypan Blue staining as in Step 13. Nuclei should be unclumped and free of cellular debris with clear borders. Total nuclei counts should be at ~100,000 per reaction. See Figure 8B for expected results.
20. To extracted nuclei, add 10 µL per reaction **activated ConA Beads**. Gently vortex to mix and quick spin in a mini-centrifuge to collect slurry; beads should not settle.
21. Incubate for 10 min at RT. Nuclei will adsorb to beads.
22. If using a multi-channel pipettor (recommended), place a multi-channel reagent reservoir on ice. Fill with cold **Antibody Buffer**.

    Retrieve compatible 8-strip tube magnetic rack. If using the EpiCypher 8-strip tube magnet, use the high-volume side unless otherwise noted.
23. After the 10 min incubation, place tubes on a magnet and allow slurry to clear.
24. If bead binding was successful, the supernatant should not contain nuclei. To confirm, transfer 10 µL supernatant to a new 1.5 mL tube. Perform Trypan Blue staining as in Step 13. See Figure 8C for expected results.
25. Pipette to remove and discard remaining supernatant. If using multi-channel pipettors, remove and replace buffers one strip at a time to avoid bead dry out.
26. Immediately add 55 µL per reaction cold **Antibody Buffer** to each reaction. Remove tubes from magnet and pipette to resuspend.
27. To confirm nuclei binding to ConA beads, transfer 10 µL bead slurry to a new 1.5 mL tube. Perform Trypan Blue staining as in Step 13. See Figure 8D for expected results.
28. Aliquot 50 µL bead slurry per reaction to **8-strip Tubes**. Gently vortex tubes and quick spin.



Sample	Success Metrics	Troubleshooting Tips
Starting cells Figure 8A	Cells show expected morphology, are unclumped, and >80% viable. Acceptable viability depends on cell type and experimental conditions.	EEvaluate cell culture conditions; use fresh media and troubleshoot contamination issues. Increase spin time if losing sample. See support.epicypher.com for specific recommendations for your sample type (tissues, adherent cells, etc.)
Extracted Nuclei Figure 8B	Nuclei should be >95% "dead" (Trypan Blue positive). Nuclei should have clear borders, be unclumped, and free of cell debris.	Monitor cells during extraction by Trypan Blue staining to optimize incubation time. If nuclei extraction is incomplete, a second incubation with fresh Nuclei Extraction Buffer can be added, but may result in sample loss.
Nuclei coupled to ConA beads Figure 8C, D	Supernatant **(C)** contains very few nuclei and the reaction slurry **(D)** shows that all nuclei are bound to ConA beads.	Ensure that ConA beads were never frozen, cells and nuclei were not clumped, beads did not become clumped or dried out, and all buffers were correctly prepared.

FIGURE 8

Validation of sample prep using Trypan Blue staining. **(A)** Starting cells are mostly viable (bright white and round). A dead cell (blue, Trypan positive) is circled in red. **(B)** Successful nuclei harvest shows Trypan Blue stained nuclei. An intact cell (bright white, Trypan negative) is circled in red. **(C)** Following ConA bead binding, the supernatant has minimal nuclei. **(D)** The final reaction slurry contains nuclei successfully conjugated to activated ConA beads (brown specks).

SECTION IV: PRIMARY ANTIBODY BINDING (~30 MIN + OVERNIGHT)

ANTIBODY BINDING NOTES

* Add K-MetStat Panel to control reactions **BEFORE** adding the primary antibody.
* Do **NOT** rotate or invert tubes. Rotation causes ConA beads to stick to tube lids and dry out, reducing yields. Use a nutator for incubations and elevate tube caps as suggested.

29. Quick spin the **K-MetStat Panel**. Pipette to resuspend - do **NOT** vortex stock.
30. Add the **K-MetStat Panel** to reactions designated for H3K4me3, H3K27me3, and IgG Control Antibodies. Add 2 µL if using 100,000 nuclei per reaction.

    For lower nuclei numbers, prepare a dilution of the K-MetStat Panel in **Antibody Buffer** on the day of the experiment, as outlined below. Add 2 µL diluted K-MetStat Panel to each reaction.

Number of nuclei	Panel dilution	Volume per reaction
100,000	Stock	2 µL
50,000	1:2	2 µL
20,000	1:5	2 µL
10,000	1:10	2 µL

31. Gently vortex tubes and quick spin.
    Add 0.5 µg primary antibody (or manufacturer's recommendation) to each experimental reaction.

    For control reactions, add 1 µL respectively of **H3K4me3, H3K27me3, or IgG Control Antibody**.

    For antibodies stored in viscous glycerol solutions, ensure accurate pipetting: aspirate slowly, check tip for accuracy, and pipette up and down to clear the solution from tip.
32. Gently vortex to mix and quick spin. Incubate overnight at 4˚C on a nutator, gently rocking with tube caps elevated (Figure 9). Do **NOT** invert or rotate tubes end-over-end.

FIGURE 9

8-strip tubes should be placed on a nutator at a 45 degree angle with caps elevated.

SECTION V: SECONDARY ANTIBODY BINDING (~1 HR)

IMPORTANT NOTES ON CONA BEAD MIXING

* **Resuspension of ConA beads is essential for assay success.** Mix as instructed in each step.
* Beads often become clumpy after overnight incubation. Vortex frequently to keep in solution. The end of a pipette tip can be cut off to help mix plant nuclei or preserve delicate samples.

33. Gather reagents at 4˚C and -20˚C for Day 2: **Pre-Wash Buffer, Wash Buffer 1, Wash Buffer 2, Anti-Rabbit Secondary Antibody, pAG-Tn5, Non-Hot Start 2X PCR Master Mix, i5 and i7 Primers**. Place on ice to thaw or equilibrate.
34. Prepare **Tagmentation Buffer** in a 1.5 mL tube. Per reaction, combine 59.4 µL **Wash Buffer 2** and 0.6 µL **1 M $MgCl_2$** (10 mM final concentration). Place on ice. Recipe includes 20% excess volume to account for pipetting error.
35. If using a multi-channel pipettor (recommended), place a multi-channel reagent reservoir on ice. Fill with cold **Wash Buffer 1**.
36. Remove tubes from 4˚C incubation and quick spin to collect liquid. Beads may settle overnight (Figure 10). This will not impact results.
37. Place tubes on magnet and allow slurry to clear. Pipette to remove supernatant. Remove and replace buffers one tube strip at a time to avoid ConA bead dry out.
38. Take tubes off magnet. Add 50 µL cold **Wash Buffer 1** to each reaction and resuspend bead slurry by gentle pipetting and/or vortexing. If pipetting, avoid losing beads in tips and expel all material back into tubes. Vortexing can be used to help resuspend beads and is recommended for viscous samples. Always quick spin tubes after mixing to avoid bead loss.
39. Add 0.5 µg secondary antibody to each reaction. Secondary antibody must match primary antibody host species. For kit control antibodies and rabbit primary antibodies, use 0.5 µL **Anti-Rabbit Secondary Antibody**. Anti-mouse secondary is also available (EpiCypher 13-0048). Gently vortex ~5 sec to mix.
40. Quick spin tubes and place on nutator, caps elevated, for 30 min at RT.
41. Gently vortex tubes ~5 sec and quick spin. Place tubes on magnet and allow slurry to clear. Pipette to remove supernatant.

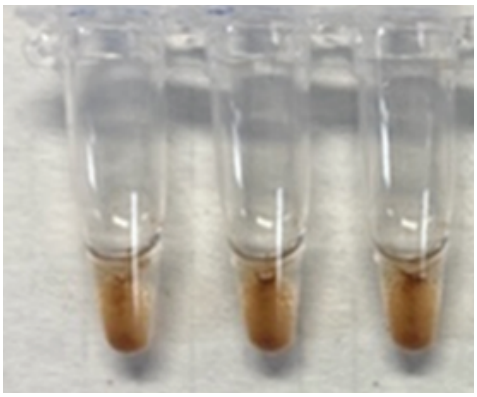


FIGURE 10

Settling of ConA beads after overnight incubation at 4˚C.

42. Keeping tubes on magnet, add 200 µL cold **Wash Buffer 1** to each reaction. Pipette to remove supernatant. Repeat the wash one time, keeping tubes on magnet.
43. Take tubes off magnet. Add 50 µL cold **Wash Buffer 2** to each reaction and resuspend by pipetting (avoid bead loss, expel all material back into tubes) and/or vortexing, followed by a quick spin.

SECTION VI: PAG-TN5 BINDING AND TARGETED TAGMENTATION (~4 HRS)

44. Add 2.5 µL **pAG-Tn5** to each reaction. Gently vortex tubes ~5 sec to mix.
45. Quick spin tubes and place on a nutator, caps elevated. Incubate 1 hour at RT.
46. Gently vortex tubes ~5 sec and quick spin. Place tubes on magnet and allow slurry to clear. Pipette to remove supernatant.
47. Take tubes off magnet. Add 200 µL cold **Wash Buffer 2** to each reaction and resuspend by pipetting (avoid bead loss, expel all material back into tubes). Quick spin tubes, place on magnet, and allow slurry to clear. Pipette to remove supernatant. Repeat one time for a total of two washes.
48. Take tubes off magnet. Add 50 µL cold **Tagmentation Buffer** to each reaction and resuspend by pipetting (avoid bead loss, expel all material back into tubes).
49. Gently vortex tubes ~5 sec and quick spin. Incubate 1 hour in a thermocycler set to 37˚C (heated lid at 47˚C). During the incubation, transfer 60 µL per reaction **Pre-Wash Buffer** to a new tube and let equilibrate to RT.
50. Gently vortex tubes ~5 sec and quick spin. Place tubes on magnet and allow slurry to clear. Pipette to remove supernatant and take tubes off magnet.
51. Add 50 µL RT **Pre-Wash Buffer** to each reaction. Gently but thoroughly pipette 3-5 times to resuspend the bead slurry. Expel all material back into tubes.
52. Quick spin and place tubes on magnet, allow slurry to clear. Pipette to remove supernatant.
53. Take tubes off magnet. Add 5 µL RT **SDS Release Buffer** to each reaction, vortex ~10 sec at max speed to mix, and quick spin. **Do NOT pipette**.
54. Incubate reactions for 1 hour in a thermocycler set to 58˚C (heated lid at 68˚C). Vortex ~10 sec at max speed to mix.
55. Quick spin tubes. Add 15 µL RT **SDS Quench Buffer** to each tube without touching sample with pipette tip. **Do NOT pipette** — sample may be very viscous from this point on, pipetting may clog tips and cause sample loss.
56. Vortex ~10 sec at max speed and quick spin. Keep tubes at RT. Sample may not fully homogenize at this point: this is normal.

SECTION VII: INDEXING PCR AND LIBRARY CLEANUP (~1 HR)

INDEXING PCR OPTIMIZATION

* Use the minimum number of PCR cycles needed to accurately quantify DNA libraries (≥2 ng/µL). EpiCypher typically uses 16 cycles to map histone PTMs from 100,000 K562 nuclei.
* For i5 and i7 index sequences, go to epicypher.com/14-1102 and download the Multiplexing Primers spreadsheet under Resources.

57. Assign a unique pair of **i5 and i7 Primers** to each CUT&Tag reaction as instructed in Appendix 1. Quick spin the i5 and i7 Primers before each use.
58. For indexing PCR, add primers and PCR Master Mix directly to reactions in 8-strip tubes at RT. To each reaction add: 2 µL **i5 Primer**, 2 µL **i7 Primer**, and 25 µL **Non-Hot Start 2X PCR Master Mix**. Gently vortex to mix and quick spin.

    Do **NOT** remove ConA beads. Do **NOT** put tubes on ice until after primers and PCR Master Mix are added. Change tips between each addition to prevent cross-contamination. **Do NOT pipette to mix**.
59. Place reaction in a thermocycler with a heated lid set to 105˚C. Perform PCR using the parameters below to amplify tagmented DNA from 100-700 bp.

    We recommend using 16 PCR cycles, which generates robust PTM profiles from 100,000 K562 nuclei. Optimize as needed for lower nuclei inputs, low abundance targets, or if troubleshooting low yields; see support.epicypher.com.

Step #	Temperature	Time	Cycles	Notes
1	58˚C	5 min	1	Fill-in step
2	72˚C	5 min	1	Extension
3	98˚C	45 sec	1	DNA melting
4	98˚C	15 sec	14-21	DNA melting
5	60˚C	10 sec		Hybrid annealing/extension
6	72˚C	1 min	1	Final extension
7	4-12˚C	∞	1	Hold temperature

60. After PCR, remove reactions from thermocycler and quick spin to collect liquid.
61. Prepare 85% Ethanol (EtOH) fresh using 100% EtOH and molecular biology grade water. Make 500 µL per reaction: 425 µL 100% EtOH + 75 µL water. Note that these calculations include extra volume to account for pipetting error.
62. Vortex **DNA Purification Beads** to fully resuspend. Slowly add 65 µL **DNA Purification Beads** (1.3X reaction volume) to each reaction. **DNA Purification Beads** are added to the **entire** reaction, including ConA beads. Ensure pipette tip is free of extra droplets when dispensing to reactions.
63. Mix well by pipetting and/or vortexing to an even resuspension (critical for DNA Purification Bead binding). Quick spin tubes and incubate 5 min at RT.
64. Place tubes on a magnet for 2-5 min at RT, until solution clears. Pipette to remove supernatant without disturbing beads.
65. Keeping tubes on the magnet, add 180 µL **85% EtOH** directly onto **DNA purification beads**. Pipette to remove supernatant.
66. Repeat the previous step one time.
67. Remove tubes from magnet. Quick spin to collect liquid, with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
68. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 11). If beads are crackly and/or light brown, they are too dry.
69. Add 17 µL **0.1X TE Buffer** to each reaction to elute CUT&Tag libraries. Pipette and/or vortex to fully resuspend beads and quick spin. Incubate for 2 min at RT.
70. Place tubes on magnet for 2 min at RT.
71. Transfer 15 µL CUT&Tag libraries to new **8-strip Tubes**.

FIGURE 11

Elute DNA before beads dry out.

Safe pause point. Libraries can be stored at -20˚C.

SECTION VIII: ANALYSIS OF LIBRARY FRAGMENT SIZE (~1 HR)

72. Use 1 µL to quantify CUT&Tag libraries using the Qubit fluorometer and 1X dsDNA HS Assay Kit. See Expected Results, below.

73. Use 1 µL CUT&Tag library for analysis on the Agilent Bioanalyzer (High Sensitivity DNA Kit) or TapeStation (D1000 ScreenTape System). See Expected Results and Figure 12 on the next page.

EXPECTED RESULTS AND FAQS: CUT&TAG SEQUENCING LIBRARIES

What library yield should I expect?

There is no typical yield for CUT&Tag, as yields vary by cell type, number of nuclei, target abundance, and antibody quality. In general, aim for 2 ng/µL or ~30 ng total DNA, which will allow accurate library quantification and minimize PCR duplicates. Library molarity ≥0.5 nM for the 200-700 bp region will allow pooling at standard concentrations for sequencing.

Yields from positive and negative controls can also be used to gauge assay success. H3K27me3 should have higher yields compared to IgG. H3K4me3, a low abundance target, should have yields similar to or just slightly greater than IgG.

What is the expected fragment distribution of CUT&Tag libraries?

Traces should show predominant enrichment of mononucleosome-sized fragments, as in Figure 12 (~300 bp: ~170 bp mononucleosomes + sequencing adapters). This is the **BEST** indicator of CUT&Tag success prior to sequencing.

Why are there short and/or long fragments in my CUT&Tag library?

Peaks at ~200 bp, ~300 bp, and ~500 bp represent the "trident" patten often observed in CUT&Tag traces; see the H3K27me3 trace in Figure 12B. The trident peak pattern is not cause for concern, as it typically reflects on-target enrichment. These peaks do not impact sequencing and we do **NOT** recommend size selection, as it risks loss of the mononucleosome peak (~300 bp).

Peaks at ~25-100 bp in CUT&Tag libraries indicate primer dimers. The H3K4me3 library in Figure 12C has a primer dimer peak at ~75 bp (red text). If primer dimers comprise >5% of the library, additional cleanup should be performed on pooled libraries; see support.epicypher.com for more information.

Why are my yields low and/or fragment distribution traces showing nothing?

Visit support.epicypher.com for help troubleshooting low yields and fragment distribution traces.

EXAMPLE FRAGMENT DISTRIBUTION ANALYSIS OF CUT&TAG SEQUENCING LIBRARIES

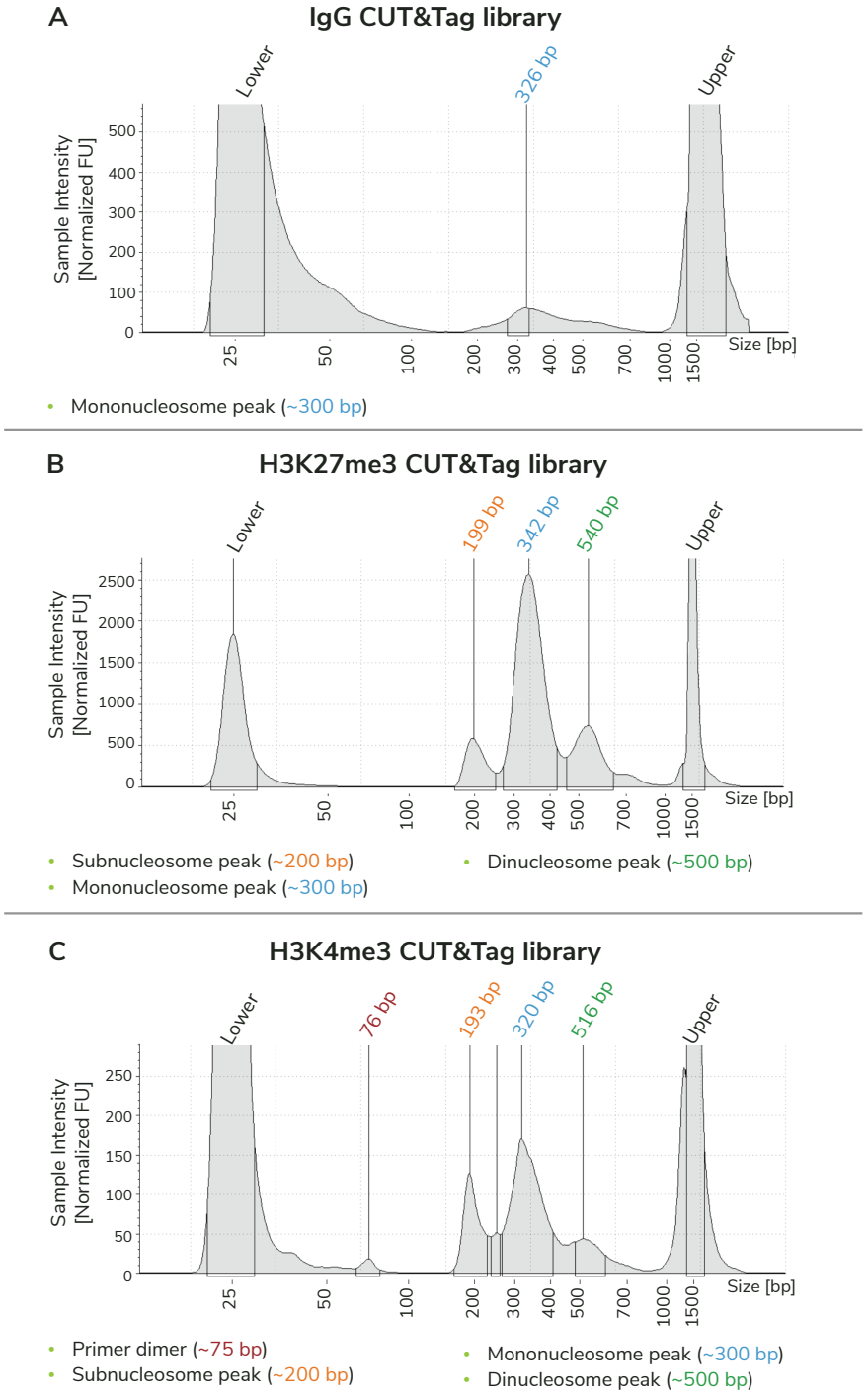


FIGURE 12

Typical TapeStation traces from CUTANA CUT&Tag libraries prepared using negative control (**A**) and positive control (**B, C**) antibodies. Subnucleosome (~200 bp; orange text) and dinucleosome (~500 bp; green text) peaks are present, forming the discussed trident patten. Minimal primer dimers are observed in the H3K4me3 library (~75 bp; red text).

SECTION IX: ILLUMINA® SEQUENCING AND DATA ANALYSIS

74. Select the appropriate Illumina sequencing platform (see **Appendix 1**) based on the number of CUT&Tag libraries and desired sequencing depth. Paired-end sequencing is recommended (2 x 50 bp cycles minimum). Libraries should be sequenced to a depth of 5-8 million **total** reads. For sufficient coverage, each library should generate 3-5 million **unique** reads (after removing multimapping, duplicate, and DAC exclusion list reads).

75. Dilute and pool libraries using molarity calculations from **Protocol: Section VIII** (200-700 bp region) and load onto the Illumina sequencer. General steps:

    a. Dilute each library to the same concentration as recommended by Illumina (support.illumina.com).

    b. Pool equimolar libraries into one tube.

    c. Dilute pooled libraries following Illumina guidelines (support.illumina.com).

    d. When setting up a multiplexed sequencing run, ensure that each library contains a unique i5 and i7 Primer pair and that dual indexes are correctly assigned. For a full list of index sequences, download the CUTANA CUT&Tag Kit Multiplexing Primers spreadsheet at epicypher.com/14-1102 under Resources.

76. EpiCypher now supports bioinformatic analysis of CUT&RUN and CUT&Tag data with **CUTANA™ Cloud** (cloud.epicypher.com), a fast, secure, user-friendly cloud computing platform to help researchers rapidly unlock insights from their data (see **Appendix 3**). CUTANA Cloud automates genomic alignment, SNAP-CUTANA Spike-in analysis, and compilation of all necessary QC statistics to determine experimental success.

77. K-MetStat Spike-in analysis of H3K4me3, H3K27me3, and IgG control reactions can validate your workflow, identify failed reactions, and troubleshoot problematic experiments, as outlined at support.epicypher.com. Genomic distribution of these controls should show expected enrichment and high reproducibility across replicates (Figures 13-14). Visit support.epicypher.com for further help with bioinformatic analysis of CUT&Tag sequencing data.

EXAMPLE DATA FROM POSITIVE AND NEGATIVE CONTROL REACTIONS

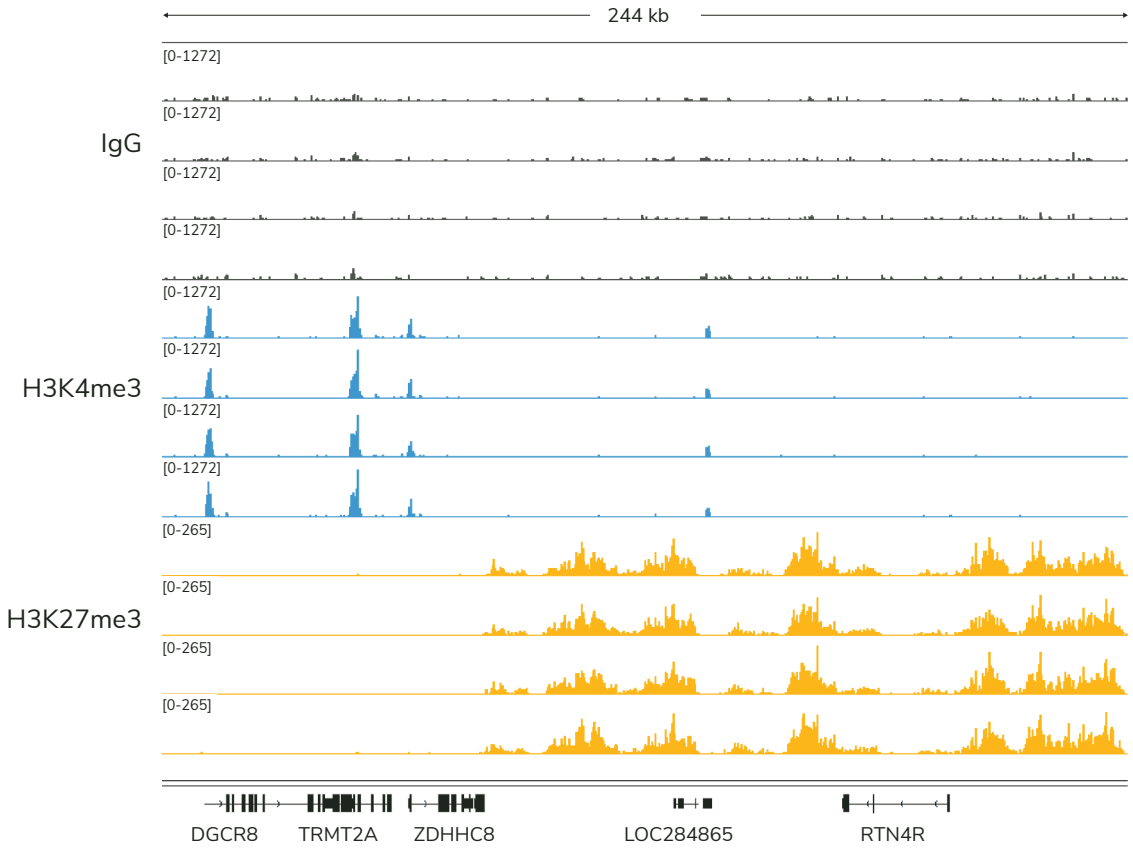
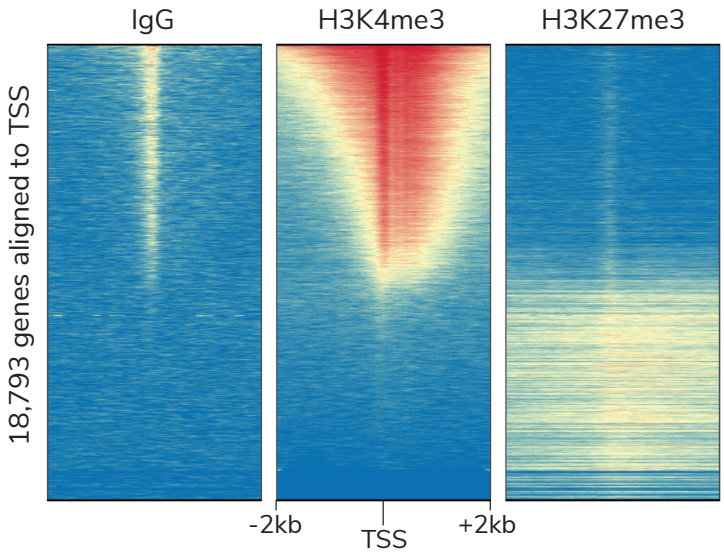


FIGURE 13

Data across four technical replicates in a CUT&Tag experiment demonstrate the reproducibility of the CUTANA™ CUT&Tag Kit. Genome browser tracks are shown for CUT&Tag replicates using 100,000 K562 nuclei with IgG, H3K4me3, and H3K27me3 control antibodies. H3K4me3 tracks show sharp peaks localized to transcription start sites (TSSs), while H3K27me3 tracks show broad peaks spread over repressed regions. IgG shows typical background enrichment.

FIGURE 14

Expected results from CUTANA CUT&Tag assays using 100,000 K562 nuclei with control antibodies. Data are presented as a heatmap of signal intensity aligned to the TSS of 18,793 genes (+/- 2kb). Genes are aligned across conditions and ranked by H3K4me3 intensity from top (high signal, red) to bottom (low signal, blue).



Appendix 1: Illumina® Sequencing and Primer Selection Guide

ILLUMINA SEQUENCING PLATFORMS

* The CUTANA CUT&Tag Kit is compatible with Illumina high-throughput sequencing platforms (e.g. NextSeq 1000/2000).
* Paired-end sequencing (2 x 50 bp minimum) is recommended for CUT&Tag.
* Libraries should be sequenced to a depth of 5-8 million total reads, with a goal of generating 3-5 million uniquely aligned reads.

The table below outlines Illumina next-generation sequencing platforms and kits compatible with the multiplexing capabilities of the CUTANA CUT&Tag Kit. The number of reactions per run assumes ~5 million total reads per CUT&Tag library. Additional user optimization may be required.

* i5 & i7 primer selection is NOT optimized for color balancing on MiSeq systems. We suggest pooling with other labs on a higher throughput sequencing platform.

Platform	Cartridge	Illumina Cat No.	Read Depth	# Reactions
MiniSeq	High Output Kit (150 cycles)	FC-420-1002	25-40M	~8
HiSeq 3000/4000	SBS Kit (150 cycles) & PE Cluster Kit	FC-410-1002 PE-410-1001	350M per lane	~70 per lane
NextSeq 500/550	Mid Output v2.5 (150 cycles)	20024904	130M	~26
	High Output v2.5 (150 cycles)	20024907	400M	~80
NextSeq 1000/2000	P2 (100 cycles) v3	20046811	400M	~80
	P2 (200 cycles) v3	20046812		
NovaSeq 6000	SP v1.5 (100 cycles)	20028401	650-800M	~130
NextSeq 2000	P3 100 cycles	20040559	1B	~200
	P3 200 cycles	20040560		

PRIMER SELECTION GUIDE

* Index sequences are available in an easy-to-copy spreadsheet at epicypher.com/14-1102 under Resources.

This kit uses a combinatorial dual indexing primer strategy. Each CUT&Tag library is prepared with a distinct combination of two 8 bp barcodes, or indexes — one at the 5' end (i5 index), and the second at the 3' end (i7 index). The 48 rxn kits (14-1102-48s1 and 14-1102-48s2) come with four i5 Primers and twelve i7 Primers, which can be used to generate 48 uniquely indexed libraries for multiplexed sequencing; The 24 rxn kit (14-1102-24s3) contains two i5 Primers and twelve i7 Primers (as a subset of Primer Set 1 used in 14-1102-48s1) to generate 24 uniquely indexed libraries [8,9].

Note that multiple CUTANA CUT&Tag kits containing distinct i5 and i7 Primers can be used in combination to multiplex more than 48 reactions (Figure 15). Combining the two 48 rxn kits increases the number of unique dual index pairs to 96; Combining the 48 rxn kit 14-1102-48s2 (Primer Set 2) with the 24 rxn kit generates up to 72 unique libraries; Do **NOT** combine the 48 rxn kit 14-1102-48s1 (Primer Set 1) with the 24 rxn kit as the two kits have overlapping primer sets.

Figure 15 illustrates proper primer organization to facilitate successful i5 and i7 Primer pair selection and pipetting. Do **NOT** repeat pairs of i5 and i7 Primers in a sequencing run. If an experiment will be combined with others on a single lane or flow cell, ensure that there is no overlap of primer pairs.

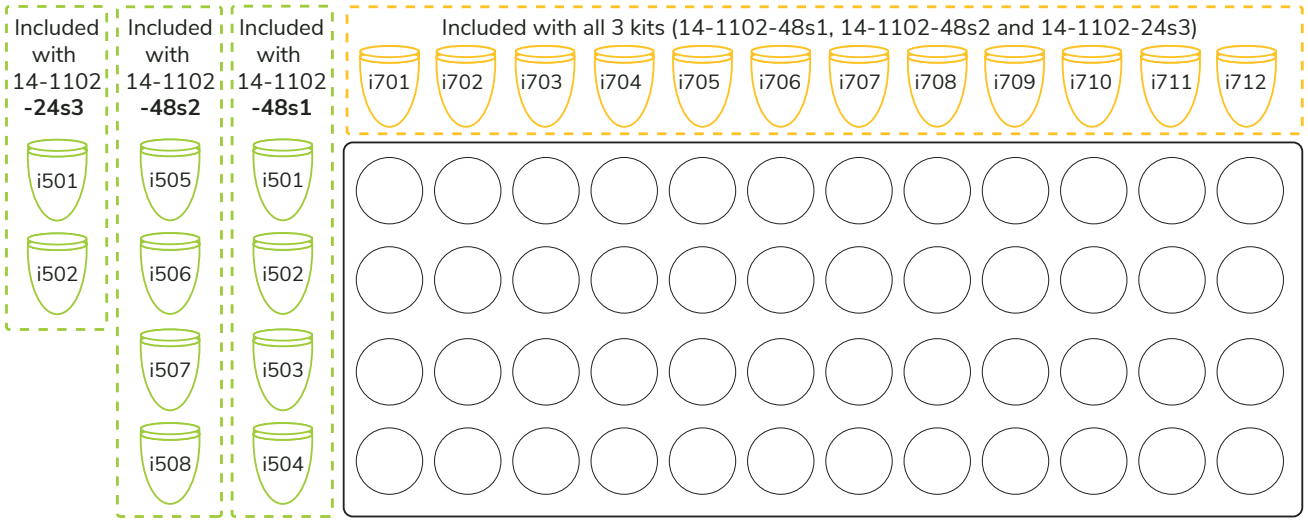


FIGURE 15

i5 and i7 Primers are organized to guide primer pair selection for successful multiplexed sequencing. Dashed orange lines indicate i7 Primers i701-i712 included with all three CUT&Tag kits. Dashed green lines indicate i5 Primers. The three versions of this kit come with distinct i5 Primers, with 14-1102-48s1 containing i501-i504, 14-1102-48s2 containing i505-i508, and 14-1102-24s3 containing i501-i502.

SELECTION OF I5 AND I7 PRIMER PAIRS FOR MULTIPLEXING (EXAMPLE)

Figure 16 provides an example of how i5 and i7 Primers can be selected across three different sequencing runs. Although the i5 and i7 Primers shown are from 14-1102-48s1, the same principles apply for 14-1102-48s2 and 14-1102-24s3.

- For Sequencing Run 1 in Figure 16 (dark blue), begin by pairing the first i5 Primer (i501) with the first i7 Primer (i701).
- Then work across the row from left to right, pairing the i501 Primer with each i7 Primer to generate 12 distinct dual indexes.
- For the six additional libraries in Sequencing Run 1, use the next i5 Primer (i502) and pair with i701. Move across the row until the desired number of dual indexes is achieved.
- In subsequent experiments, begin primer selection where the last run stopped. In Figure 16, Sequencing Run 1 ends with i502 and i706 (dark blue). When selecting primers for the next CUT&Tag experiment, begin with i502 and i707 (Sequencing Run 2; light blue).

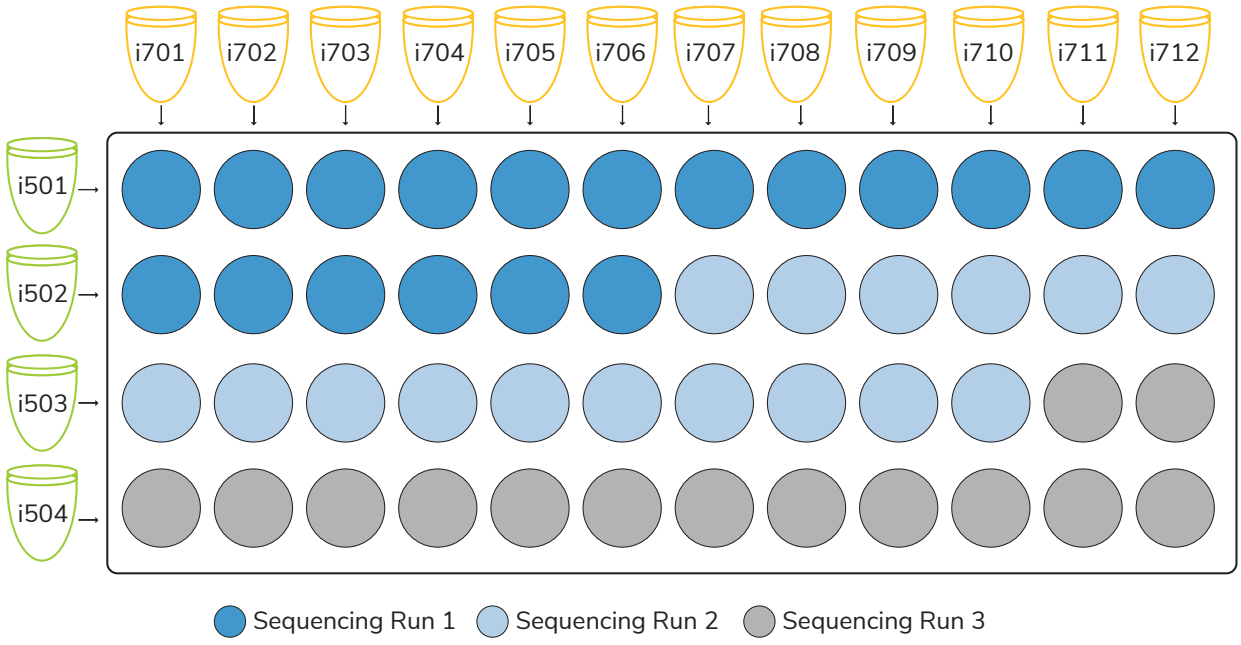


FIGURE 16

Three sequencing runs illustrate appropriate i5 and i7 Primer pair selection for dual-indexing of CUT&Tag libraries. In all sequencing runs, each library is generated using a unique pair of i5 and i7 Primers. Although primers for 14-1102-48s1 are shown, the same rules apply for i5 and i7 Primers included with 14-1102-48s2 and 14-1102-24s3.

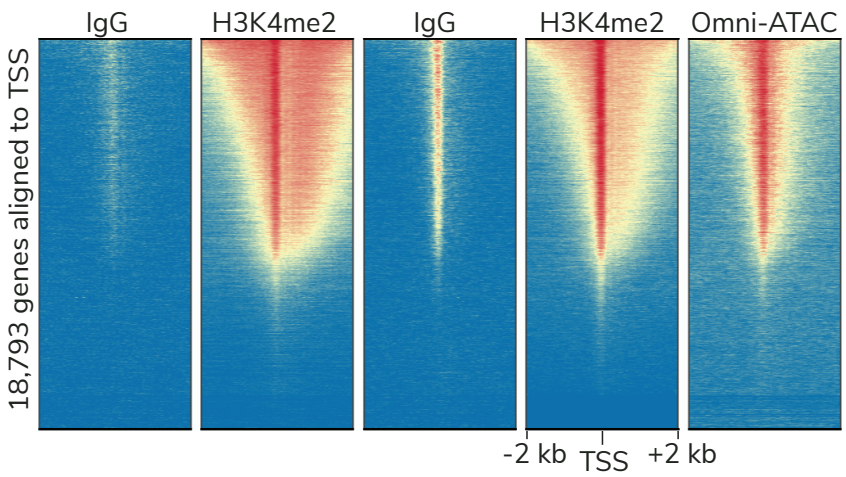
Appendix 2: CUTAC Protocol for Chromatin Accessibility Mapping

In their 2020 paper, the Henikoff group described CUTAC (Cleavage Under Target Accessible Chromatin), which enables chromatin accessibility profiling using a modified CUT&Tag protocol [7]. Key changes include:

* An H3K4me2 antibody (EpiCypher 13-0027) is used to label open chromatin.
* A No-Salt Tagmentation Buffer is used to support Tn5 activity in open chromatin.

Figure 17 shows H3K4me2 CUT&Tag profiles generated using the standard tagmentation conditions vs. the modified, no-salt CUTAC tagmentation buffer. When compared to high quality Omni-ATAC-seq profiles [10], data from CUTAC assays display improved signal over background (Figure 17 A, B). Another important feature of CUTAC is the low rate of mitochondrial reads, which enables ~10-fold reduced sequencing depths compared to traditional ATAC-seq [7].

A



B

Assay	FRiP (%)
H3K4me2 CUT&Tag	63.5%
CUTAC	64.1%
Omni-ATAC	11.3%

FRiP = Fraction of reads in peaks, measure of signal-to-noise

C

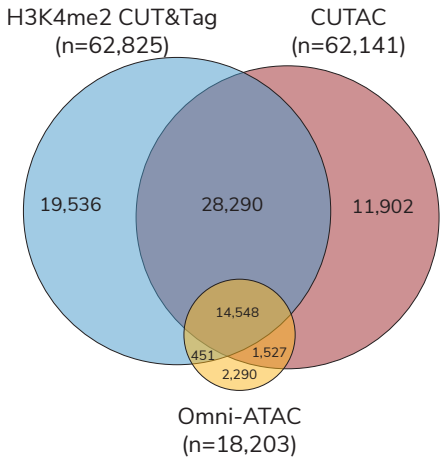


FIGURE 17

Modification of the CUTANA CUT&Tag Kit for chromatin accessibility mapping. Profiles were generated using 100,000 K562 nuclei with EpiCypher H3K4me2 or IgG negative control antibody. Tagmentation was performed under conditions for standard CUT&Tag or CUTAC. Omni-ATAC-seq data from [10] are shown for comparison. **(A)** Heatmaps show signal relative to TSS in each assay. Gene rows are aligned across conditions. **(B)** FRiP scores show that CUT&Tag and CUTAC data have higher signal-to-noise vs. Omni-ATAC-seq. **(C)** Venn diagram showing peak overlap between H3K4me2 CUT&Tag, CUTAC, and Omni-ATAC-seq.

Appendix 2: CUTAC Protocol for Chromatin Accessibility Mapping

For CUTAC assays, use the CUTANA CUT&Tag Experimental Protocol with a few modifications:

SECTION IV: PRIMARY ANTIBODY BINDING

- **Use an H3K4me2 primary antibody** (EpiCypher 13-0027).
  Add 0.5 µg to each reaction. Gently vortex to mix and incubate overnight on a nutator at 4˚C.

SECTION V: SECONDARY ANTIBODY BINDING

- At the start of Day 2, prepare the **No-Salt Tagmentation Buffer** as outlined in the table below. Store on ice.
- Note that 50 µL is needed per CUT&Tag reaction. The recipe provides enough for 10 reactions with 20% overflow to account for pipetting error.

Components	Source	[Final]	10rxn
1M TAPS, pH 8.5	Boston Bioproducts BB-2375	10 mM	6 µL
1 M $MgCl_2$	Included with CUTANA CUT&Tag Kit	5 mM	3 µL
Molecular biology grade water	Any Vendor	-	591 µL

SECTION V: SECONDARY ANTIBODY BINDING

- Use a species-matched secondary antibody. EpiCypher's H3K4me2 antibody can be used with the **Anti-Rabbit Secondary Antibody** provided in the kit.

SECTION VI: PAG-TN5 BINDING AND TAGMENTATION

- At **Step 48**, Use 50 µL cold **No-Salt Tagmentation Buffer** in place of the standard Tagmentation Buffer. Thoroughly pipette to resuspend (avoid bead loss, expel all material back into tubes).
- At **Step 49**, incubate reactions for **20 min** in a thermocycler set to 37˚C (lid to 47˚C).
  During the incubation, transfer 60 µL per reaction **Pre-Wash Buffer** to a new tube and let equilibrate to RT.
- Proceed with kit protocol at **Step 50**. No other protocol modifications are required.

For guidance on sequencing analysis, refer to [7]. Note that the IgG negative control may show slightly more background (Figure 17A). This is expected, because nonspecific tagmentation in accessible chromatin is increased by low salt.

Appendix 3: CUT&Tag data analysis with CUTANA™ Cloud

CUTANA Cloud (cloud.epicypher.com) is a web-based platform for streamlined analysis of CUT&Tag and CUT&RUN data with EpiCypher's thoroughly tested pipelines. It takes you from raw sequencing files through alignment and peak calling without the need for command line expertise (Figure 18).

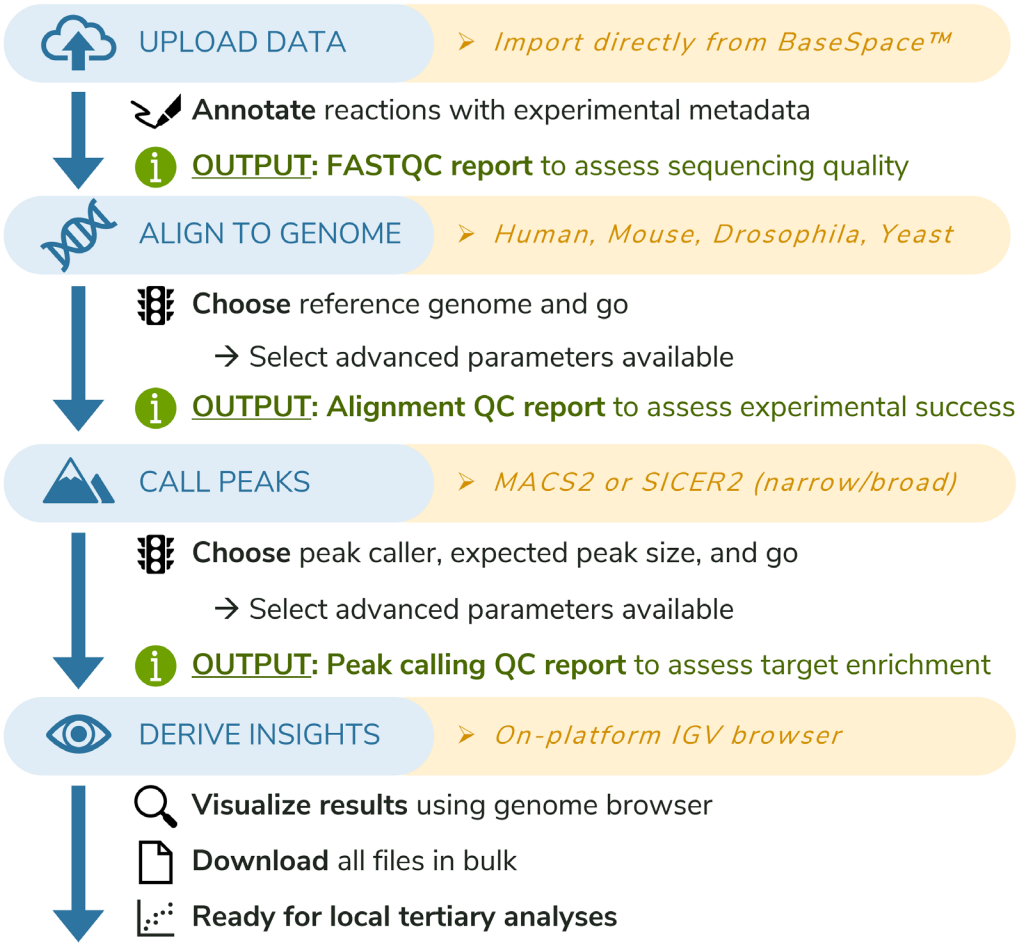


Figure 18 CUTANA Cloud Analysis Pipeline.

To access the fast and streamlined bioinformatic analysis of CUT&Tag data with CUTANA Cloud, follow the steps below:

1. Create an account
   - Sign up at cloud.epicypher.com
   - Browse the gold standard reference data
   - Set up a billing account to purchase credits
2. Prepare your experiment
   - Create a new project & experiment
   - Upload FASTQ files directly from BaseSpace, AWS, or local files
   - Enter reaction metadata to set pipeline instructions (e.g., targets, spike-ins)

Appendix 3: CUT&Tag data analysis with CUTANA™ Cloud

3. Configure and Launch Analysis

   - Select desired reactions
   - Select reference genome (hg19, hg38, T2T, mm10, dm6, sacCer3)
   - Confirm or adjust default parameters (e.g., duplicate handling, exclusion lists)
   - Select peak calling algorithms (e.g., MACS2, SICER)

4. Collect and evaluate results from QC reports (Figure 19)

   - Sequencing statistics and peak calling metrics
   - Analysis of SNAP-CUTANA Spike-in controls
   - In browser IGV tracks
   - Heatmaps from individual targets

5. Export & Explore

   - Dynamic run methods ready for publication
   - Download processed data files (BAM, BED, bigWig) for further visualization & tertiary analysis
   - Projects remain accessible in your Cloud workspace for comparison or re-analysis

A

Short Name	Total Read Pairs	Aligned Read Pairs	Uniquely Aligned Read Pairs	Unique Alignment Rate(%)	Duplication Rate(%)	chrM Bandwidth(%)	CUTANA Spike in(%)
CnT_IgG_Rep1	6270967	5612168	637185	10.16	88.5	12.97	1.06
CnT_IgG_Rep2	4721684	4352179	850766	18.02	80.24	9.92	0.85
CnT_H3K4me3_Rep1	4996191	4023260	1784727	35.72	55.29	1.86	6.12
CnT_H3K4me3_Rep2	4474170	3665496	1631192	36.46	55.13	1.85	6.34
CnT_H3K27me3_Rep1	4111130	3804082	3021754	73.5	20.17	0.45	2.6
CnT_H3K27me3_Rep2	4608423	4251426	3464451	75.18	18.11	0.39	2.04

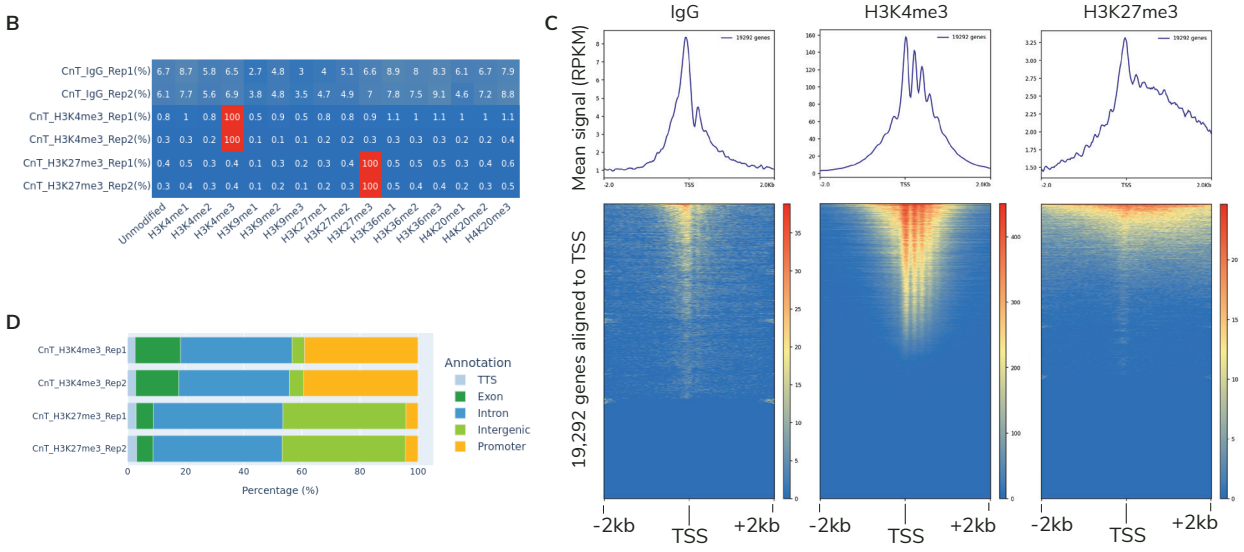


Figure 19 Select QC report figures exported from CUTANA Cloud using example CUT&Tag reactions with IgG, anti-H3K4me3, and anti-H3K27me3 antibodies in duplication. **(A)** Sequencing stats table. **(B)** Relative barcode recovery from each antibody compared to the on-target panel member defined in the reaction details. For IgG negative control, recovery is evenly spreaded across all panel members. **(C)** Heatmaps for read enrichment centered around Transcription Start Sites (TSS) of protein coding genes, each individually sorted by signal strengths. **(D)** Peaks called against IgG control sample (H3K4me3 through MACS2, and H3K27me3 through SICER2) categorized by overlapping known genomic features.

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Revision History

Kit Manual Version #	Date	Notes
4.0	10.2025	• Updated Catalog Numbers for CUTANA™ CUT&Tag kits. • Added **Wash Buffer Enhancers 1 & 2** to reduce sample clumping and improve bead handling. See p. 15 for buffer prep instructions. • New **CUTANA™ DNA Purification Beads** as a drop-in replacement to SPRI-based DNA purification beads. • Updated stock concentration of **NaCl** to 5M. • Updated pack sizes for **Pre-Nuclei Extraction Buffer**, **Pre-Wash Buffer**, **Control Antibodies** and **SNAP-CUTANA™ K-MetStat Panel**. • Updated recommendation for storage temperature of **Rabbit IgG Negative Control Antibody** to 4˚C. • Updated nuclei resuspension recommendations to use **Wash Buffer 1** instead of **Nuclei Extraction Buffer** for post extraction wash for better sample preservation. • Modified post-tagmentation handling with vortex mixing to reduce bead loss and increase DNA yield for difficult targets.