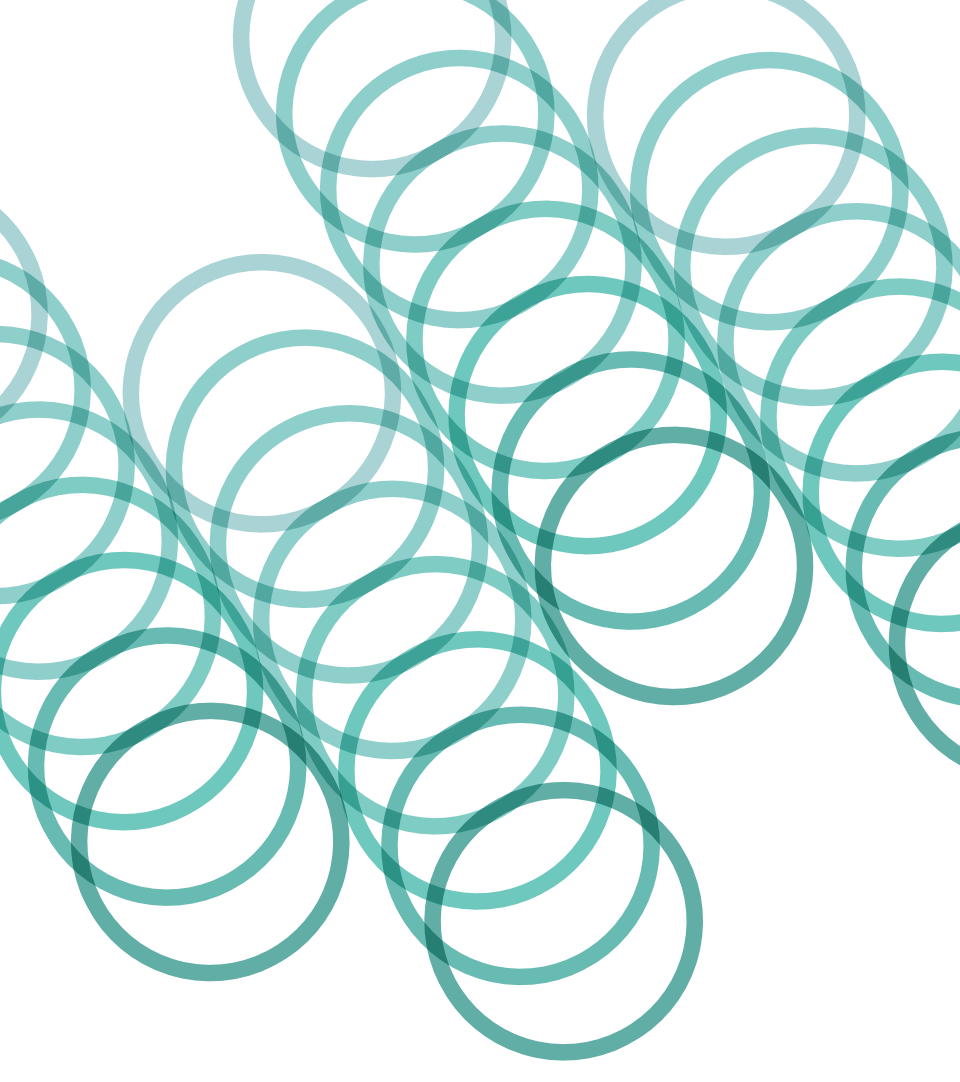

EpiCypher®
Bringing Epigenetics to Life

CUTANA™ Quick Cleanup DNA Purification Kit Version 1

User Manual Version 1.1

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EpiCypher®

CUTANA™

Quick Cleanup DNA Purification Kit

Kit Version 1
Catalog No. 14-0052

Sufficient for
48 CUT&RUN reactions
-OR-
96 CUT&Tag reactions

Store at room temperature (RT)
Stable for 1 year upon date of receipt.
See p. 7 for information.

Table of Contents

See EpiCypher's Tech Support Center at support.epicypher.com for additional FAQs and troubleshooting guidance.

Overview

EpiCypher's CUTANA™ Cleavage Under Targets & Release Using Nuclease (CUT&RUN) and Cleavage Under Targets and Tagmentation (CUT&Tag) assays enable epigenomic mapping with unprecedented sensitivity and speed[1-5].

The purification of high-quality DNA is essential for CUT&RUN and CUT&Tag assays. Unlike many kits that purify DNA using spin columns, the CUTANA™ Quick Cleanup DNA Purification Kit uses a SPRI bead-based purification strategy. In this approach, SPRI beads are added to DNA at specific ratios to enable simultaneous size selection and purification of target DNA. Higher bead:DNA ratios capture DNA fragments of varying lengths, while lower ratios preferentially recover longer fragments. Of note, these paramagnetic beads are compatible with 8-strip tubes, allowing streamlined integration in CUTANA CUT&RUN and CUT&Tag workflows.

Below we outline the protocols and applications described in this manual. In each case, bead ratios have been empirically optimized to maximize DNA yields.

CUT&RUN DNA PURIFICATION (PAGE 8)

Use to purify CUT&RUN-enriched DNA. Designed for use with the CUTANA™ CUT&RUN DIY Protocol (epicypher.com/cutana-cut-and-run-protocol) and CUTANA™ pAG-MNase (EpiCypher 15-1016 and 15-1116). Please note:

- Our **standard** CUT&RUN DNA cleanup protocol uses a 1.4X SPRI bead ratio to capture mononucleosomal fragments (~140-170 bp), which works for the majority of targets tested by EpiCypher's genomics team. Using this protocol, the Quick Cleanup DNA Purification Kit can process 48 CUT&RUN reactions.
- If you are mapping transcription factors or chromatin proteins that generate small, subnucleosomal fragments (<120 bp), a 1.8X SPRI bead ratio may help improve recovery. See this Tech Support Article for the latest details.

NOTE FOR CUTANA™ CUT&RUN KIT USERS (EPICYPHER 14-1048)

- Your CUT&RUN kit comes with sufficient SPRI beads to purify CUT&RUN DNA using the standard 1.4X bead ratio.
- If you are using a 1.8X SPRI bead ratio to help improve recovery of small fragments, the CUTANA™ Quick Cleanup DNA Purification Kit can be used to supplement your supply of SPRI beads. See this Tech Support Article for details.

CUT&TAG SEQUENCING LIBRARY PURIFICATION (PAGE 12)

Use to purify CUT&Tag sequencing libraries. Designed for use with EpiCypher's Direct-to-PCR DIY Protocol (epicypher.com/cutana-cut-and-tag-protocol) and CUTANA pAG-Tn5 (EpiCypher 15-1017 & 15-1117). Please note:

- In Direct-to-PCR CUT&Tag, DNA purification is not performed until after tagmentation and indexing PCR, resulting in DNA fragments of ~300 bp. A 1.3X SPRI bead ratio is used to capture longer DNA fragments.
- A total of 96 CUT&Tag libraries may be purified using this kit.
- **NOT** for CUTANA™ CUT&Tag Kit users (EpiCypher 14-1102 & 14-1103), which includes necessary DNA purification reagents.

SEQUENCING LIBRARY CLEANUP (PAGE 18)

Use to remove adapter dimers from CUT&RUN libraries (~150 bp) and primer dimers from CUT&Tag libraries (25-100 bp). Please note:

- This protocol is specifically optimized to remove DNA fragments <180 bp. A lower SPRI bead ratio is used to capture larger-sized library fragments, while smaller contaminating fragments are left behind in the supernatant.
- Note that cleanup is performed on a normalized pool of sequencing libraries. The protocol is **NOT** meant for cleanup of individual libraries, as this risks sample loss.

Included in the Kit

Kit components are stable for 1 year upon date of receipt.

Store at room temperature (RT) upon receipt:

Item	Catalog No.	Notes before use
8-strip Tubes	10-0009d	Enables use of multi-channel pipettors.
SPRIselect Reagent manufactured by Beckman Coulter Inc.	21-1405d	**DO NOT FREEZE.** Reagent is slightly viscous. Thoroughly mix prior to use and pipette carefully to ensure correct volume is transferred. Use to purify DNA.
0.1X TE Buffer	21-1025d	Use to elute DNA.

Materials Required but Not Supplied

MATERIALS:

- 100% Ethanol (200 proof), any vendor
- Molecular biology grade water, any vendor

EQUIPMENT:

- Low-retention filter pipette tips
- 8-channel multi-channel pipettor (e.g. VWR 76169-250)
- Magnetic separation rack for 8-strip tubes (e.g. EpiCypher 10-0008)
- Benchtop centrifuge/mini-centrifuge with an 8-strip tube adapter (e.g. from Fisher Scientific, Benchmark Scientific)
- Vortex (e.g. Vortex-Genie® 2 Mixer, Scientific Industries SI-0236)
- Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit (Invitrogen Q33230)
- Qubit™ 4 Fluorometer (Invitrogen Q33238) or comparable model
- **For CUT&Tag DNA Cleanup and Library Cleanup:** Capillary electrophoresis machine and 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)

DESCRIPTION

This method is used to purify CUT&RUN-enriched DNA as part of the CUTANA™ CUT&RUN DIY Protocol (epicypher.com/cutana-cut-and-run-protocol).

Our **standard** CUT&RUN DNA cleanup protocol described herein uses a 1.4X SPRI bead ratio to capture mononucleosomal fragments (~140-170 bp), which generates robust profiles for most targets. However, if you are mapping transcription factors or chromatin proteins that generate small, subnucleosomal fragments (<120 bp), a 1.8X SPRI bead ratio may help improve recovery. See this Tech Support Article for the latest details.

Purified DNA is prepared for sequencing using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001 & 14-1002), enabling genome-wide profiling of histone PTMs and chromatin-associated proteins with exquisite sensitivity.

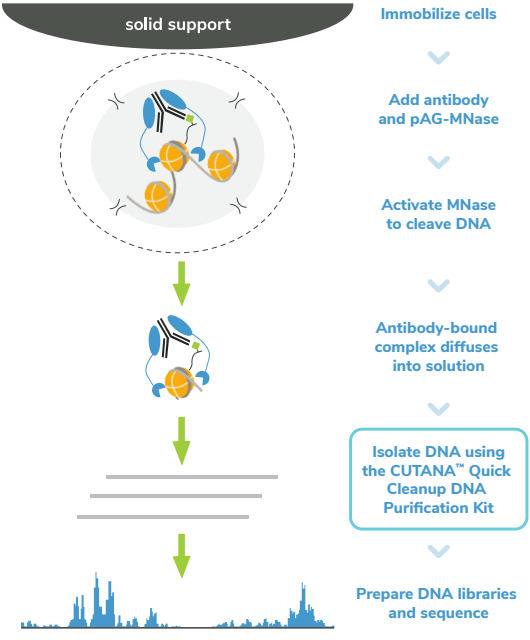


FIGURE 1

CUT&RUN releases antibody-bound chromatin into solution. DNA is extracted using the CUTANA™ Quick Cleanup DNA Purification Kit and prepared for sequencing (e.g. using the CUTANA™ CUT&RUN Library Prep Kit, EpiCypher 14-1001 & 14-1002).

CUT&RUN ASSAY TIPS

CUT&RUN is our go-to chromatin mapping assay for all users. Our protocol is validated for diverse histone PTM and protein targets across many cell types. Below we describe best practices for CUT&RUN success.

CUT&RUN assay optimization and key controls

- Validate workflows using 500,000 freshly harvested unfixed cells per reaction. We recommend using a cell line (e.g. K562) to avoid wasting precious samples.
  - View recommendations for specific sample types at support.epicypher.com.
- Validate workflows with positive control (H3K4me3, EpiCypher 13-0060; H3K27me3, EpiCypher 13-0055) and negative control (IgG, EpiCypher 13-0042) antibodies. Use with the SNAP-CUTANA™ K-MetStat Panel of spike-in controls (EpiCypher 19-1002) to confirm workflow success and guide troubleshooting. See support.epicypher.com for guidance.
- For experimental targets, test antibodies that bind distinct epitopes and/or from multiple vendors. Select the antibody that gives the best balance of expected target enrichment, low background, and high yields. Note: ChIP antibodies are **NOT** guranteed to work in CUT&RUN. Shop our CUT&RUN validated antibodies at epicypher.com/cut-and-run-antibodies or email techsupport@epicypher.com for recommendations.
- For proteins that generate small fragments (<120 bp), consider purifying CUT&RUN DNA using a higher ratio of SPRI beads (1.8X) to maximize recovery.
- After validating your workflow and identifying antibodies for experimental targets, you can further optimize for precious samples/low inputs. Our CUT&RUN protocol is validated for select PTMs down to 5,000 cells. However, success at lower inputs varies by target abundance, antibody performance, and sample quality.

Tips for CUT&RUN success

- High quality sample prep is essential for CUT&RUN success. Check cell count, viability, and morphology at initial cell harvest AND after resuspending in CUT&RUN Wash Buffer. Cells should show minimal lysis and expected morphology.
- Confirm cell binding to ConA beads - a key part of the CUT&RUN workflow.
- On Day 2 of CUT&RUN, it is critical that ConA beads are resuspended. Vortex to keep beads in solution. Excessive clumping leads to sample loss and poor yields.
- For more guidance, see our CUT&RUN support center (support.epicypher.com).

CUT&RUN DNA PURIFICATION (~30 MIN)

The starting input for this protocol is 84 µL CUT&RUN-enriched chromatin in 8-strip tubes.

1. For each CUT&RUN reaction, make 500 µL 85% Ethanol (EtOH) by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT). Note that these calculations include extra volume to account for pipetting error.
2. Vortex **SPRIselect** reagent (beads) thoroughly to resuspend. Ensure pipette tip is free of extra bead droplets. For a 1.4X ratio (most targets), slowly add 118 µL SPRI beads to 84 µL CUT&RUN-enriched chromatin. For a 1.8X ratio (transcription factors or targets that generate small fragments), slowly add 151 µL SPRI beads to 84 µL CUT&RUN-enriched chromatin.
3. Mix well by pipetting and/or vortexing to ensure an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.
4. Place tubes on a compatible magnetic rack for 2-5 min at RT, until the solution clears. Pipette to remove supernatant without disturbing beads.
5. Keep tubes on magnet. Add 180 µL **85% EtOH** directly onto beads. Carefully pipette to remove supernatant. Repeat one time for a total of two washes.
6. Remove tubes from magnet and quick spin with caps facing in to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
7. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 2). If beads are crackly and/or light brown, they are too dry.
8. Add 17 µL **0.1X TE Buffer** to elute DNA. Pipette and/or vortex to fully resuspend beads and quick spin. Incubate 2 min at RT.
9. Place tubes on magnet for 2 min. Transfer 15 µL CUT&RUN DNA to new **8-strip tubes**.
10. Use 1 µL to quantify DNA using the Qubit fluorometer and 1X dsDNA HS Assay Kit.

    Proceed to library prep or store DNA at -20°C.

FIGURE 2

Elute DNA when beads are "ideal."

FAQS: CUT&RUN YIELDS

What DNA yield should I expect?

There is no typical yield for CUT&RUN, as yields vary by cell type, number of cells, target abundance, and antibody quality. **For all reactions, aim for ~5 ng DNA to enable robust library prep**. For guidance, visit support.epicypher.com.

How can I determine if my CUT&RUN experiment worked before library prep?

- Check yields from the suggested control reactions on page 9. Yields from H3K4me3 positive controls should be similar to or slightly greater than IgG. H3K27me3 is a high abundance target and should have higher yields vs. IgG.
- Do **NOT** use yields from experimental targets to define workflow success.
- Do **NOT** examine fragment distribution of raw CUT&RUN DNA on the TapeStation or Bioanalyzer, as yields are too low - you won't see any peaks.

I ran my CUT&RUN DNA on the TapeStation/Bioanalyzer and don't see anything! What went wrong?

Due to the low inputs and high resolution of CUT&RUN, DNA yields are too low for detection on Bioanalyzer/TapeStation and will not provide useful information. **We only recommend fragment distribution analysis AFTER library prep**.

My yields are below 5 ng and/or same as my IgG negative control. Did my assay fail?

Yields simlar to the IgG controls do **NOT** imply CUT&RUN failure. Many low-abundance targets, such as transcription factors, generate low yields, even when using the recommended 500,000 cells. In these cases, use the total amount of CUT&RUN-enriched DNA for library prep. In EpiCypher's experience, good sequencing data with high signal-to-noise can be obtained from low yields.

Where can I get help with CUT&RUN library prep and/or sequencing?

The CUTANA CUT&RUN Library Prep Kit Manual has extensive guidance for optimizing CUT&RUN library prep and sequencing. Learn more and purchase at epicypher.com/14-1001.

The BEST indicator of CUT&RUN success is after library prep. Purified CUT&RUN libraries should show enrichment of mononucleosome-sized fragments (~300 bp) on the TapeStation or Bioanalyzer.

DESCRIPTION

This method was developed for use with the CUTANA™ CUT&Tag DIY Protocol, found at epicypher.com/cutana-cut-and-tag-protocol. Briefly, pAG-Tn5 inserts sequencing adapters at antibody-bound chromatin in intact nuclei (Figure 3). This "tagmented" DNA is selectively amplified and barcoded using indexing primers that recognize adapter DNA – even in the presence of cell debris. EpiCypher's exclusive Direct-to-PCR approach enriches target DNA and greatly streamlines library prep.

The CUT&Tag sequencing libraries are purified with the CUTANA™ Quick Cleanup DNA Purification Kit using a lower SPRIselect ratio that captures ~300 bp library fragments (~170 bp mononucleosome + sequencing adapters), while excluding smaller DNA fragments.

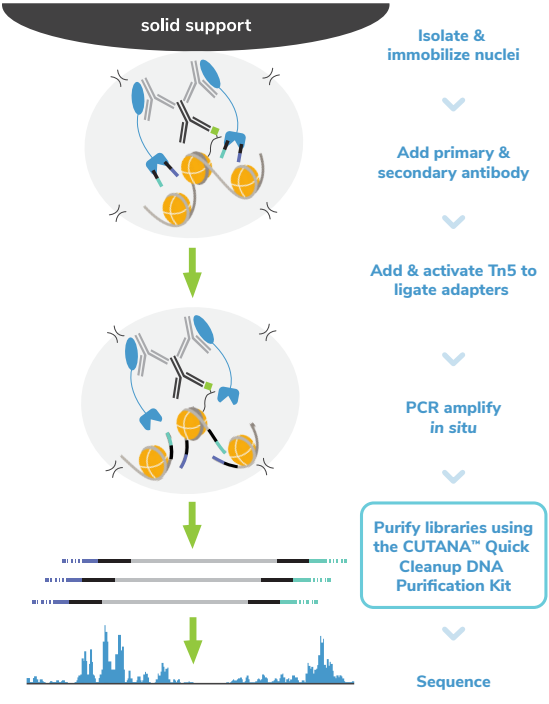


FIGURE 3

In CUT&Tag, pAG-Tn5 is used to ligate sequencing adapters at antibody-labeled chromatin. Following indexing PCR, CUT&Tag sequencing libraries are purified using the CUTANA™ Quick Cleanup DNA Purification Kit.

CUT&TAG ASSAY TIPS

CUT&Tag is best for experienced genomics researchers and only recommended for histone PTM targets. Below we describe best practices for CUT&Tag success.

CUT&Tag assay optimization and key controls

- Validate workflows using 100,000 freshly harvested, unfixed nuclei per reaction. We recommend using a cell line (e.g. K562) to avoid wasting precious samples.
    - Cross-linking may be useful for some labile targets, such as lysine acetylation PTMs. Always test cross-linking in parallel with native nuclei. Do **NOT** use ChIP conditions; use our protocol at epicypher.com/cutana-cut-and-tag-protocol.
- Validate workflows with positive control (H3K4me3, EpiCypher 13-0060; H3K27me3, EpiCypher 13-0055) and negative control (IgG, EpiCypher 13-0042) antibodies. Use with the SNAP-CUTANA™ K-MetStat Panel of spike-in controls (EpiCypher 19-1002) to confirm workflow success and guide troubleshooting. See the SNAP-CUTANA User Guide at epicypher.com/protocols for guidance.
- For histone PTM experimental targets, test antibodies from multiple vendors. Validate lysine methylation PTM antibodies directly in CUT&Tag using the SNAP-CUTANA K-MetStat Panel (EpiCypher 19-1002). For other PTMs, select the antibody that gives the best balance of expected target enrichment, low background, and high yields. Note: ChIP antibodies are **NOT** guranteed to work in CUT&Tag. Visit epicypher.com/cut-and-tag-antibodies to shop our antibodies or email techsupport@epicypher.com for recommendations.
- After validating your workflow and identifying antibodies for experimental targets, you can further optimize for precious samples/low inputs. Our CUT&Tag protocol is validated for select PTMs down to 10,000 nuclei. However, success at lower inputs varies by target abundance, antibody performance, and sample quality.

Tips for CUT&Tag success

- 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 aren't losing sample during 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. Excessive clumping leads to sample loss and poor yields.
- See the CUTANA™ CUT&Tag DIY Protocol for additional information (epicypher.com/cutana-cut-and-tag-protocol) .

CUT&TAG SEQUENCING LIBRARY PURIFICATION (~30 MIN)

The starting input for this protocol is 50 µL post-indexing PCR product in 8-strip tubes.

1. For each CUT&Tag reaction, make 500 µL 85% Ethanol (EtOH) by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT). Note that these calculations include extra volume to account for pipetting error.
2. Vortex **SPRIselect** reagent (beads) thoroughly to resuspend. Slowly add 65 µL **SPRIselect** reagent (1.3X ratio) to 50 µL post-indexing PCR product in 8-strip tubes. Ensure pipette tip is free of extra bead droplets before dispensing to reactions.
3. Mix well by pipetting and/or vortexing to ensure an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.
4. Place tubes on a compatible magnetic rack for 2-5 min at RT, until the solution clears. Pipette to remove supernatant without disturbing beads.
5. Keep tubes on magnet. Add 180 µL **85% EtOH** directly onto beads. Carefully pipette to remove supernatant. Repeat one time for a total of two washes.
6. Remove tubes from magnet and quick spin with caps facing in to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
7. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 4). If beads are crackly and/or light brown, they are too dry.
8. Add 17 µL **0.1X TE Buffer** to elute DNA. Pipette and/or vortex to resuspend beads and incubate 2 min at RT.
9. Quick spin tubes and place on magnet for 2 min. Transfer 15 µL CUT&Tag libraries to new **8-strip tubes**.
10. Use 1 µL to quantify DNA using the Qubit fluorometer and 1X dsDNA HS Assay Kit.

FIGURE 4

Elute DNA when beads are "ideal."

Examine CUT&Tag sequencing libraries by TapeStation/Bioanalyzer (see Figure 5 and discussion, page 16). Store libraries at -20°C for future processing.

FAQS: CUT&TAG YIELDS

What library yield should I expect?

There is no typical yield for CUT&Tag, as yields vary by cell type, number of cells, 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. For more guidance, see the CUTANA™ CUT&Tag Kit Manual at epicypher.com/protocols.

How do I know if my CUT&Tag experiment worked?

Examine the fragment distribution of CUT&Tag libraries on the TapeStation/Bioanalyzer and confirm enrichment of mononucleosome-sized library fragments (see discussion, next page). The H3K4me3 and H3K27me3 positive control reactions and IgG negative control reactions, suggested on page 13, can also be used to confirm CUT&Tag success. If control reactions show proper enrichment in TapeStation/Bioanalyzer data (Figure 5, next page) and yields ≥2 ng/µL, you can be confident in your workflow.

My yields are low - How do I optimize my workflow?

See the section on page 13 outlining key controls and guidelines for success. Start by validating the workflow in your lab using 100,000 nuclei per reaction with positive and negative control antibodies. Review additional troubleshooting steps at support.epicypher.com.

You may also consider performing CUT&RUN for your experiment, which generates high-resolution data for many targets and cell types.

I have questions about my Bioanalyzer/TapeStation results!

See the next two pages of this manual for a comprehensive discussion of CUT&Tag library fragment distribution.

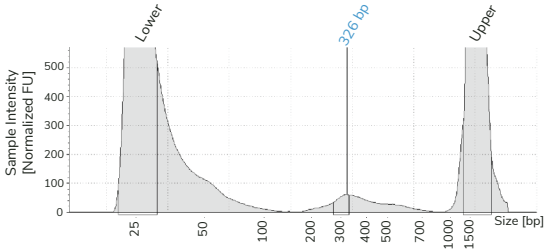
Where can I get help with CUT&Tag sequencing?

The CUTANA™ CUT&Tag Kit Manual (epicypher.com/protocols) contains information on sequencing, data analysis, and more.

The BEST indicator of CUT&Tag success is enrichment of mononucleosome-sized fragments (~300 bp = ~170 bp nucleosome + sequencing adapters) in TapeStation or Bioanalyzer results. See Figure 5 (next page) for examples.

A

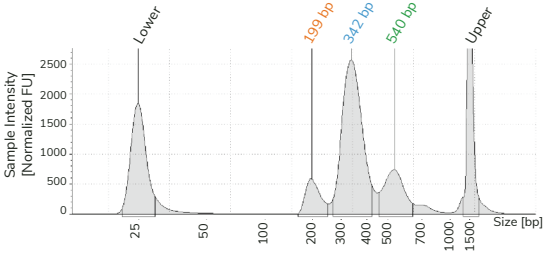
IgG CUT&Tag library



- Mononucleosome peak (~300 bp)

B

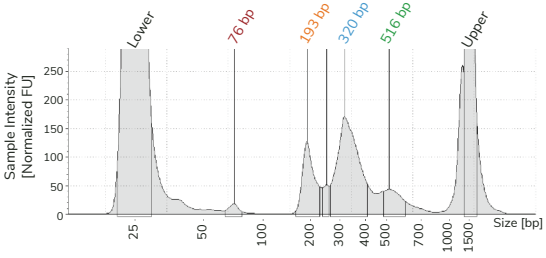
H3K27me3 CUT&Tag library



- Subnucleosome peak (~200 bp)
- Dinucleosome peak (~500 bp)
- Mononucleosome peak (~300 bp)

C

H3K4me3 CUT&Tag library



- Primer dimer (~75 bp)
- Subnucleosome peak (~200 bp)
- Mononucleosome peak (~300 bp)
- Dinucleosome peak (~500 bp)

FIGURE 5

Typical TapeStation traces from CUTANA CUT&Tag libraries targeting IgG (negative control), H3K27me3 (EpiCypher 13-0055), and H3K4me3 (EpiCypher 13-0041 shown; replaced by 13-0060). All libraries are predominantly enriched for mononucleosome-sized library fragments, indicated by the peak at ~300 bp (blue text). Subnucleosome (~200 bp; orange text) and dinucleosome (~500 bp; green text) peaks are present, forming the trident patten discussed above. Minimal primer dimers are observed in the H3K4me3 library (~75 bp; red text).

NOTES: CUT&TAG TAPESTATION/BIOANALYZER TRACES

Peaks at ~200 bp, ~300 bp, and ~500 bp ("trident pattern")

The "trident pattern" is observed for many histone PTM CUT&Tag libraries, including the H3K27me3 and H3K4me3 libraries in Figure 5B&C. These fragments are generated during tagmentation and subsequent indexing PCR. Peaks represent:

- Subnucleosome fragments: ~200 bp, orange text in Figure 5. Note that these fragments are **NOT** adapter dimer/primer dimer.
- Mononucleosome fragments: ~300 bp, blue text in Figure 5. Predominant enrichment at ~300 bp is the best metric for CUT&Tag success.
- Dinucleosome fragments: ~500 bp, green text in Figure 5.

The trident peak pattern is not cause for concern, as it typically reflects on-target enrichment. They do not impact sequencing and we do **NOT** recommend removing them, as it risks loss of the mononucleosome peak (~300 bp) .

Very small peaks (~25-100 bp): Primer dimers

Primer dimers result from indexing PCR and appear at ~25-100 bp in CUT&Tag libraries. The H3K4me3 library in Figure 5C has a primer dimer peak at ~75 bp (red text). If primer dimers comprise >5% of the library, additional cleanup should be performed as outlined in the **Sequencing Library Cleanup** portion of this manual. Removal of primer dimers is performed on the entire multiplexed sequencing pool, **NOT** individual libraries, to minimize sample loss.

Enrichment of primer dimers indicates problems with tagmentation efficiency and/or PCR amplification, and are most common when performing CUT&Tag with low starting cell numbers. Include positive and negative control reactions to validate workflows as suggested on page 13 of this manual and review troubleshooting steps in the CUTANA™ CUT&Tag Kit Manual (epicypher.com/protocols).

DESCRIPTION

This protocol is designed for removal of small fragments (180 bp or less) from CUT&RUN and CUT&Tag sequencing libraries. Cleanup is performed using pooled sequencing libraries to minimize loss - this cleanup is **NOT** for individual libraries.

Applications in CUT&RUN - Removal of Adapter Dimers

Adapter dimers result from self-ligation of sequencing adapters and are preferentially amplified due to their small size (~150 bp; see Figure 6). High levels of adapter dimers occupy valuable sequencing bandwidth and should be avoided. If >5% of the library is adapter dimers, perform cleanup as described on the next page. Note that risk of adapter dimer contamination increases when less than 5 ng of CUT&RUN-enriched DNA is used for library prep.

Applications in CUT&Tag - Removal of Primer Dimers

Primer dimers in CUT&Tag libraries are observed at ~25-100 bp on TapeStation/Bioanalyzer traces and typically comprise a small portion of the library (Figure 5, page 16). If >5% of the library is primer dimers, perform cleanup as described on the next page. Note that enrichment of primer dimers in CUT&Tag libraries often indicates problems with the workflow: low nuclei numbers, poor tagmentation efficiency, and/or indexing PCR setup.

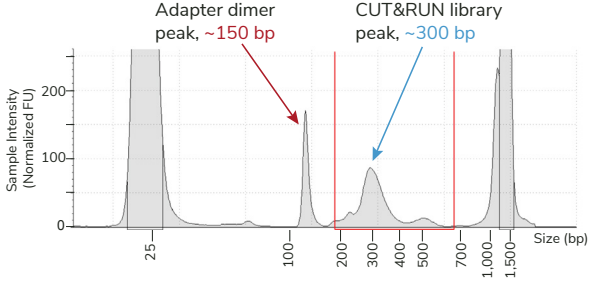


FIGURE 6

Adapter dimers observed in an H3K27me3 CUT&RUN sequencing library. The TapeStation trace shows an adapter dimer peak at ~150 bp (red arrow) and a mononucleosome-sized library peak at ~300 bp (blue arrow). 8 picograms of CUT&RUN-enriched DNA was used for library prep, highlighting the risk of adapter dimers with low library prep inputs.

SEQUENCING LIBRARY CLEANUP (~30 MIN)

The starting input for this protocol is a normalized pool of 8 (or more) sequencing libraries prepared in 8-strip tubes.

1. Prepare a normalized library pool in 8-strip tubes. Use your preferred sequencing buffer and molarity calculations from TapeStation/Bioanalyzer data (200-700 bp region):

   A. Dilute individual libraries to the same concentration, depending on final yields. 1-4 nM is ideal for NextSeq 2000 and NextSeq 500/550.

   B. Combine equimolar libraries into one tube. This is your normalized library pool for cleanup.

   C. Transfer 2 µL library pool to a new tube and set aside. Use to confirm small fragment removal after cleanup (Figure 8, page 20-21).

2. Make 1 mL 85% Ethanol (EtOH) by combining 850 µL 100% EtOH and 150 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT). Note that these calculations include extra volume to account for pipetting error.

3. Vortex **SPRIselect** reagent (beads) thoroughly to resuspend. Slowly add a 1.0X ratio **SPRIselect** reagent to library pool (e.g. add 40 µL beads to 40 µL library pool). Ensure pipette tip is free of extra bead droplets before dispensing to reactions.

4. Mix well by pipetting and/or vortexing to ensure an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.

5. Place tubes on a compatible magnetic rack for 2-5 min at RT, until the solution clears. Pipette to remove supernatant without disturbing beads.

   Optional - Purify short DNA fragments from removed fraction.
   To ensure library fragments are not lost, transfer the supernatant in Step 5 to a new tube. Note the volume of supernatant and add a 0.5X ratio **SPRIselect** reagent to the tube (e.g. add 19 µL beads to 38 µL supernatant). Mix well and purify DNA, starting at the 5 min incubation in Step 4. This material can be recombined with the library pool if loss is observed.

6. Keep tubes on magnet. Add 180 µL **85% EtOH** directly onto beads. Carefully pipette to remove supernatant. Repeat one time for a total of two washes.

SEQUENCING LIBRARY CLEANUP (~30 MIN), *continued*

7. Remove tubes from magnet and quick spin with caps facing in to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
8. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 7). If beads are crackly and/or light brown, they are too dry.
9. Add 25 µL preferred sequencing buffer from Step 1 to elute the library pool. Larger elution volumes may be used, with the caveat that DNA concentration will be lower.
10. Pipette and/or vortex to resuspend beads and incubate 2 min at RT.
12. Quick spin tubes and place on magnet for 2 min. Transfer 24 µL eluted library pool to a new tube (8-strip tubes or a 1.5 mL tube can be used).
13. Use 1 µL to quantify the library pool using the Qubit fluorometer and 1X dsDNA HS Assay Kit.

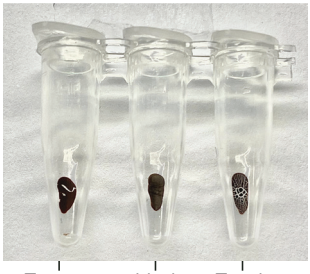


FIGURE 7

Elute DNA when beads are "ideal."

Confirm small fragment removal on the TapeStation or Bioanalyzer (Figure 8). Record final library concentration. Proceed to sequencing or store -20˚C.

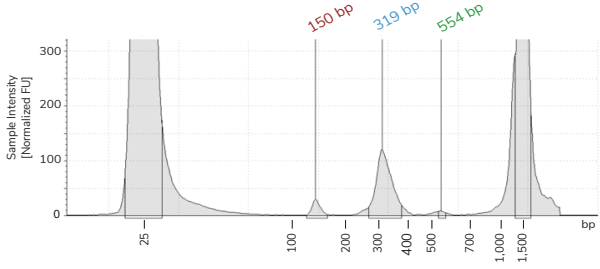
EXAMPLE: LIBRARY CLEANUP

FIGURE 8 TapeStation traces from a CUT&RUN library pool before and after removal of adapter dimers using the CUTANA™ Quick Cleanup DNA Purification Kit. Experimental workflow and observations as follow:

- Eight CUT&RUN libraries were normalized to 4 nM and pooled in one tube.
- TapeStation analysis of the library pool revealed adapter dimers at 150 bp (**A**).
- The library cleanup protocol (above) was used to remove adapter dimers and concentrate the library pool (**B**).
- The removed fraction was also purified to ensure that library fragments were not accidentally removed. As shown in (**C**), the removed fraction was enriched for adapter dimer fragments, and contains little to no library.

A

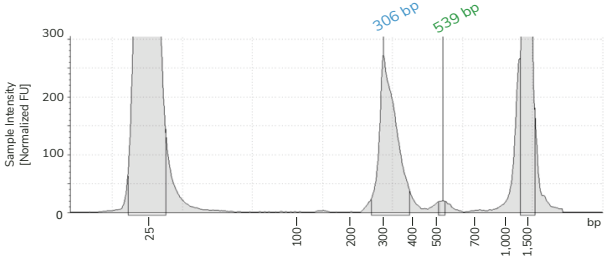
Before cleanup: 4 nM CUT&RUN library pool



- Adapter dimer peak at ~150 bp
- Note dinucleosome peak ~500 bp
- Mononucleosome library peak at ~300 bp

B

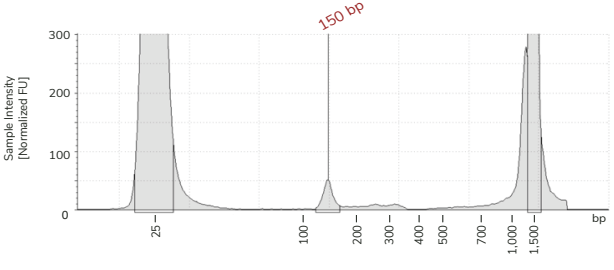
After cleanup: 6.3 nM CUT&RUN library pool



- Adapter dimer peak removed
- Enriched CUT&RUN libraries at ~300 bp ready to sequence

C

Optional removed fraction



- Adapter dimer ~150 bp removed
- Minimal library loss

References

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3. Meers MP et *al.* Improved CUT&RUN chromatin profiling tools. ***eLife*** 8, (2019).
4. Kaya-Okur HS et *al.* CUT&Tag for efficient epigenomic profiling of small samples and single cells. **Nat *Commun*** 10, 1930 (2019).
5. Kaya-Okur HS et *al.* Efficient low-cost chromatin profiling with CUT&Tag. **Nat *Protoc*** 15, 3264-3283 (2020).

Revision History

Kit Manual Version #	Date	Notes
1.1	3.12.2025	• Updated recommendations for CUT&RUN DNA purification when mapping targets that enrich small subnucleosomal fragments (<120 bp). See p. 5, 8, 9, 10. • Detailed support available within this Tech Support Center article.

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