USER GUIDE

HOLOGIC®
Diagenode

Universal CUT&Tag Kit
Rapid Chromatin Profiling Assay

8 rxns

Cat. No. C01070029

24 rxns

Cat. No. C01070024

48 rxns

Cat. No. C01070028

Content

Please read this manual carefully before starting your experiment.

Introduction

The association between proteins and DNA influences various vital cellular functions, such as cellular proliferation and epigenetic regulation. It is, therefore, crucial to understand these interactions and the mechanisms by which they control and guide gene expression.

CUT&Tag sequencing (Cleavage Under Targets and Tagmentation) is an alternative to ChIP-seq. It uses a fusion of protein A and Tn5 transposase to perform antibody-guided, controlled cleavage of chromatin. Coupled with high-throughput DNA sequencing, CUT&Tag enables researchers to pinpoint the binding sites of DNA-associated proteins.

The Universal CUT&Tag Kit is engineered to unlock high-resolution chromatin profiling for non-histone targets, including transcription factors and chromatin-associated proteins—that have traditionally been more challenging to analyze with standard CUT&Tag workflows. While expanding its capabilities for these regulatory proteins, the kit maintains full compatibility with histone mapping, ensuring comprehensive coverage of chromatin components. Optimized for low input and scalable to high-throughput formats, it incorporates IgG normalization to produce consistent, high-fidelity data with minimal background noise across diverse biological contexts.

Key Features

- **Expanded Target Range:** Optimized for profiling non-histone targets such as transcription factors and chromatin-associated proteins.
- **Histone Mapping Supported:** Maintains full compatibility with histone modifications for versatile chromatin profiling.
- **Low Input Requirement:** 5-50K cells for histones, 5-100K for modifiers/remodelers, and 100–300K for transcription factors.
- **High-Throughput Ready:** Compatible with 48/96 strip-well formats.
- **Superior Data Quality:** IgG normalization enhances confidence in signal interpretation, while an optimized amplification protocol minimizes the risk of over-amplification and reduces background noise.

The Universal CUT&Tag protocol (see Method Overview & Timetable) involves the binding of cells to solid-phase ConA magnetic beads, allowing magnetic handling of the cells for the major steps of the protocol. Bead-bound cells are permeabilized and then incubated with a primary antibody specific to the target of interest, followed by a secondary antibody. Hologic Diagenode's adaptor-loaded pA-Tn5 Transposase is next introduced, binding to the antibody complex. Guided by protein A, the Tn5 transposase is directed to the chromatin, precisely targeting the antibody-bound

regions for tagmentation. Activation of the Tn5 transposase by Mg^{2+} ions triggers the insertion of sequencing adaptors into the targeted genomic regions. Finally, DNA is purified, and the adaptor-tagged fragments are selectively amplified by PCR using Hologic Diagenode's Primer Indexes, enabling the generation of high-quality libraries for sequencing. The intermediate quantification protocol applied at this step allows assessment of the library to avoid overamplification and ensure that only the optimal number of PCR cycles is used.

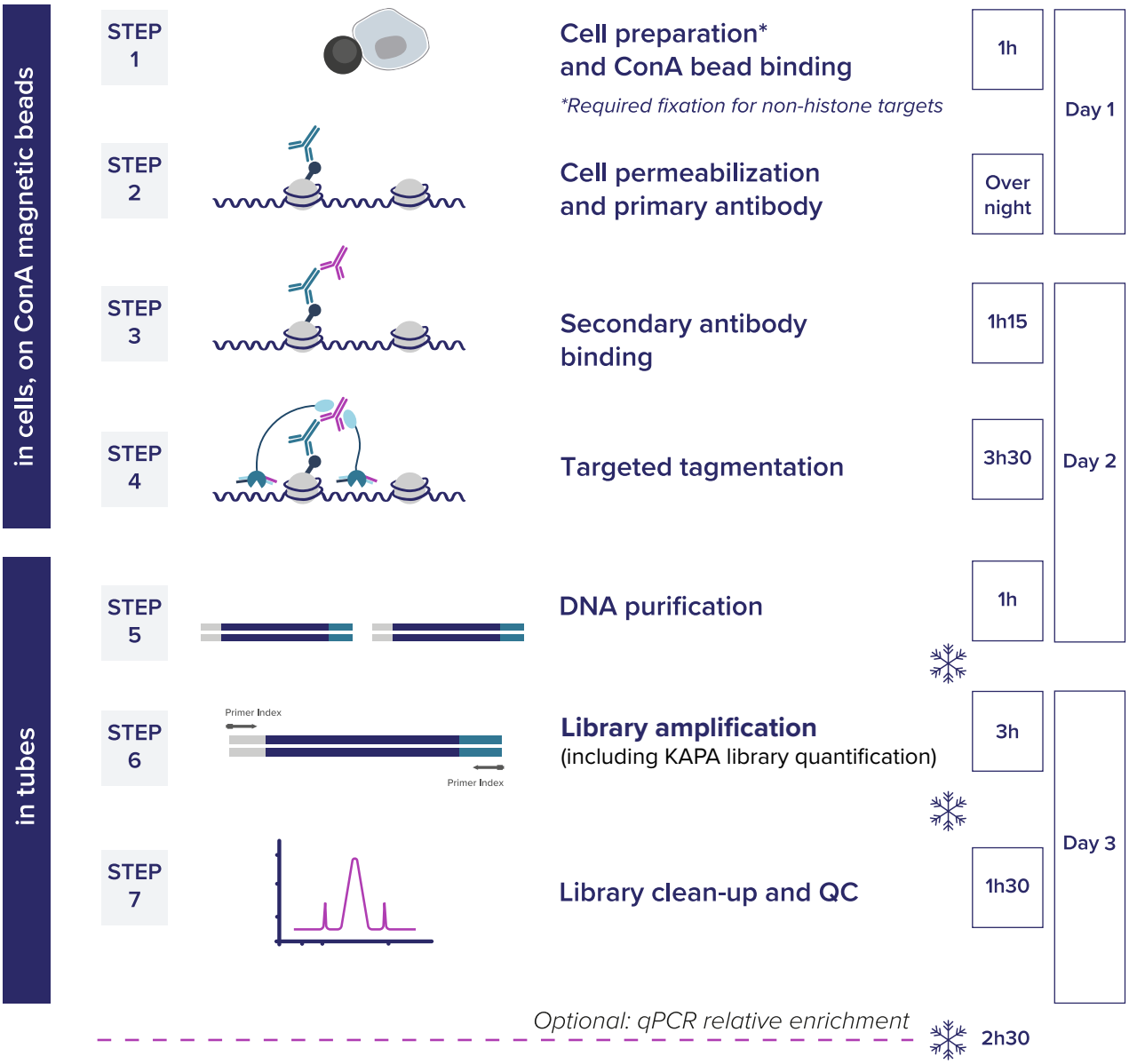
Hologic Diagenode's Universal CUT&Tag workflow offers two optional protocols:

A. Evaluation of relative enrichment by qPCR

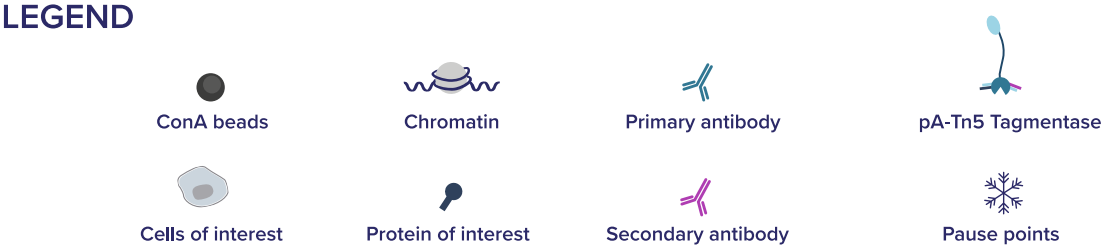
B. Genomic DNA input control

The qPCR analysis is an additional quality check that determines enrichment over a known positive region, ensuring the expected performance of the protocol. The Genomic DNA Input protocol is an optional, separate workflow used for normalization in CUT&Tag experiments.

Kit Method Overview & Timetable



LEGEND



Kit Materials

The following products should be ordered and used for the complete Universal CUT&Tag workflow:

1. **Universal CUT&Tag Kit** (8 rxns – Cat. No. C01070029, 24 rxns – Cat. No. C01070024 or 48 rxns – Cat. No. C01070028)
2. **Antibody Package**
   - Anti-rabbit (8 rxns - C01070026 or 24 rxns - C01070022)
   - Anti-mouse (8 rxns - C01070027 or 24 rxns - C01070023)
3. **Primer Indexes for Tagmented Libraries** (Cat. No. C01011032 - C01011037)

The **Universal CUT&Tag Kit** is available in three formats (8, 24 and 48 reactions). The kit includes the reagents for cell preparation, antibody binding, tagmentation reactions, DNA purification, and library amplification, as described in Tables 1–3 below. It does not include secondary or control antibodies, which are available for separate purchase as part of the Antibody Package. The primary antibody of interest should be supplied by the customer.

The **Antibody Package** (Tables 4 and 5) is available in two versions:

- **Anti-rabbit:** contains species-specific control antibodies: anti-rabbit secondary antibody, rabbit H3K27me3 positive control antibody, and rabbit negative IgG control. The anti-rabbit Antibody Package should be used if the primary antibody of interest is raised in rabbit.
- **Anti-mouse:** contains species-specific control antibodies: anti-mouse secondary antibody, mouse H3K27me3 positive control antibody, and mouse negative IgG control. The anti-mouse Antibody Package should be used if the primary antibody of interest is raised in mouse.

Each package contains enough secondary antibody to perform 8 or 24 reactions, depending on the package format. The positive and negative control antibodies are sufficient for two reactions each. Primers specific to human DNA are also included to assess the relative enrichment by qPCR.

If positive control antibodies against non-histone proteins are required, rabbit CTCF antibody (Cat. No. C15410210) or mouse RNA polymerase II antibody (Cat. No. C15200253) can be purchased separately. Negative rabbit and mouse IgG controls are also available separately (Cat. No. C15410206 and C15400001, respectively).

Components are supplied with the Universal CUT&Tag Kit.
NOTE: *Upon receipt, store the components at the indicated temperatures.*

Table 1. Components stored at **+4°C**

Component	Cap Color	Volume – 8 rxns Cat. No. C01070029	Volume – 24 rxns Cat. No. C01070024	Volume – 48 rxns Cat. No. C01070028
UCT Binding Buffer		1.6 ml	4.8 ml	9.6 ml
UCT Wash Buffer 1		400 µl	1.2 ml	2.4 ml
UCT Antibody Buffer		4.4 ml	13.2 ml	26.4 ml
UCT Wash Buffer 2		4 ml	12 ml	24 ml
ConA beads	Yellow	80 µl	240 µl	480 µl
UCT Buffer E*	White	56 µl	180 µl	360 µl
UCT Buffer S*	Red	50 µl	100 µl	200 µl

** Include excess volume for optimizations and input protocol, if necessary.*

Table 2. Components stored at **-20°C**

Component	Cap Color	Volume – 8 rxns Cat. No. C01070029	Volume – 24 rxns Cat. No. C01070024	Volume – 48 rxns Cat. No. C01070028
UCT Tagmentation Buffer*		2x 800 µl	2x 2.6 ml	4x 2.6 ml
Spermidine 50X		200 µl	600 µl	1.2 ml
Protease inhibitor Mix 200X	Red	56 µl	170 µl	340 µl
pA-Tn5 Transposase loaded*	Red	5 µl	15 µl	30 µl
2x High-Fidelity Mastermix	Violet	160 µl	480 µl	960 µl
Proteinase K *	Clear	24 µl	72 µl	144 µl
5% BSA	Black	96 µl	290 µl	580 µl

** Include excess volume for optimizations and input protocol, if necessary.*

Table 3. Components stored at **room temperature (RT)**

Component	Cap Color	Volume – 8 rxns Cat. No. C01070029	Volume – 24 rxns Cat. No. C01070024	Volume – 48 rxns Cat. No. C01070028
ChIP DNA Binding Buffer		8 ml	24 ml	2x 24 ml
DNA Wash Buffer*		0.8 ml	2.2 ml	4.4 ml
DNA Elution Buffer	White	200 µl	600 µl	1.2 ml
Spin Columns		8	24	48
Collection Tubes (2 ml)		8	24	48

**Before first use, the DNA Wash Buffer must be prepared by adding 100% ethanol:*

- *Universal CUT&Tag Kit (8 reactions – Cat. No. C01070029): Add 3.2 mL of 100% ethanol to 0.8 mL of DNA Wash Buffer.*
- *Universal CUT&Tag Kit (24 reactions – Cat. No. C01070020): Add 8.8 mL of 100% ethanol to 2.2 mL of DNA Wash Buffer.*
- *Universal CUT&Tag Kit (48 reactions – Cat. No. C01070021): Add 17.6 mL of 100% ethanol to 4.4 mL of DNA Wash Buffer.*

Components supplied with the Antibody Package (not included in the Universal CUT&Tag Kit).

NOTE: *Upon receipt, store the components at the indicated temperatures.*

Table 4. Components included in the Antibody Package (Anti-Rabbit)

Antibody Package (Anti-Rabbit)	**Volume – 8 rxns** **Cat. No. C01070026**	**Volume – 24 rxns** **Cat. No. C01070022**	**Storage**
ChIP-seq grade rabbit antibody H3K27me3 (1 µg/µl)	2 µl	2 µl	-20°C
Rabbit IgG (1 µg/µl)	2 µl	2 µl	-20°C
Anti-rabbit Secondary antibody	8 µl	24 µl	-20°C
ChIP-seq grade TSH2B primer pair (human)*	8 µl	24 µl	-20°C
ChIP-seq grade GAPDH TSS primer pair (human)*	8 µl	24 µl	-20°C

NOTE: *The rabbit H3K27me3 antibody and rabbit IgG are available separately (Cat. No. C15410195 and C15410206, respectively).*

Table 5. Components included in the Antibody Package (Anti-Mouse)

Antibody Package (Anti-Mouse)	**Volume – 8 rxns** **Cat. No. C01070027**	**Volume – 24 rxns** **Cat. No. C01070023**	**Storage**
ChIP-seq grade mouse antibody H3K27me3 (1 µg/µl)	2 µl	2 µl	-20°C
Mouse IgG (1 µg/µl)	2 µl	2 µl	-20°C
Anti-mouse Secondary antibody	8 µl	24 µl	-20°C
ChIP-seq grade TSH2B primer pair (human)*	8 µl	24 µl	-20°C
ChIP-seq grade GAPDH TSS primer pair (human)*	8 µl	24 µl	-20°C

NOTE: *The mouse H3K27me3 antibody and mouse IgG are available separately (Cat. No. C15200181 and C15400001, respectively).*

* Equivalent primers specifically for mouse or rat DNA can be purchased separately:

- Rat GAPDH promoter +0.3 kb primer pair (Hologic Diagenode Cat. No. C17031046)
- Rat TSH2B coding region primer pair (Hologic Diagenode Cat. No. C17031043)
- Mouse GAPDH promoter primer pair (Hologic Diagenode Cat. No. C17021045)
- Mouse TSH2B coding region primer pair (Hologic Diagenode Cat. No. C17021042)

Required Materials Not Provided

Equipment

- Gloves (to be worn at all steps)
- Standard cell-culture consumables
- Cell counter system
- Ice
- 15-mL and 50-mL tubes
- RNase/DNase-free 1.5-mL and 2.0-mL tubes
- 0.2-mL low-binding PCR tube strips or PCR plates with caps
- Refrigerated centrifuge for 1.5-mL and 0.2-mL tubes
- Centrifuge for 15-mL and 50-mL tubes
- Vortexer
- Thermocycler
- Thermomixer
- Magnetic rack for 1.5-mL tubes
- Hologic Diagenode magnetic rack for 0.2-mL tubes, DiaMag02 (Cat. No. B04000001), or a 96-well-plate magnetic rack
- Rotating wheel
- Agilent Fragment Analyzer (Agilent) or equivalent
- qPCR instrument and associated tube strips/plates

Reagents

- Cell culture medium
- Sterile Trypsin-EDTA (for adherent cells)
- Phosphate-buffered saline (PBS)
- Ultra-pure, DNase-/RNase-free distilled water
- 100% ethanol, molecular grade
- 80% ethanol, molecular grade
- 10 mM Tris-HCl, pH 8.0
- 1M Tris-HCl, pH 8.0
- Tween 20
- KAPA Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems, use a reference compatible with your qPCR instrument)
- Antibody specific to an epitope of interest (CUT&Tag grade antibodies – www.diagenode.com)

- AMPure XP beads (Beckman Coulter, Inc. #A63881)
- Agilent High Sensitivity DNA Kit (Agilent Technologies) for a Fragment Analyzer
- 37% formaldehyde (Sigma F8775), required for non-histone proteins only
- 1.25 M glycine solution, required for non-histone proteins only
- Primer Indexes for Tagmented Libraries Kit (Hologic Diagenode)
  - 8 UDI for tagmented libraries (Cat. No. C01011035)
  - 24 UDI for tagmented libraries – Set I (Cat. No. C01011034)
  - 24 UDI for tagmented libraries – Set II (Cat. No. C01011036)
  - 24 UDI for tagmented libraries – Set III (Cat. No. C01011037)

Additional Supplies for Optional Protocols

- OPTIONAL PROTOCOL A. Relative enrichment evaluation by qPCR
  - qPCR SYBR® Green Mastermix
  - Primer pairs for epitope of interest (5 µM of each), designed and supplied by customers
- OPTIONAL PROTOCOL B. Genomic DNA Input for CUT&Tag
  - MicroChIP DiaPure columns (Hologic Diagenode, Cat. no. C03040001)
  - XL GenDNA Extraction Module (Hologic Diagenode, Cat. no. C03030020)

Remarks Before Starting

1. General recommendations

- Read the complete manual before first use
- Decontaminate the working area and tools with DNase AWAY™ reagent
- Wear gloves at all steps
- Add enzymes last to the reaction solutions and thoroughly incorporate them by pipetting up and down several times. Never vortex reagents.
- We strongly recommend using one positive (H3K27me3) and one negative (IgG) control.
- Unless otherwise stated, the lid of the thermocycler should be set to **105°C**.

2. Number of cells per reaction

The Universal CUT&Tag protocol has been developed to support chromatin profiling from histones, chromatin-associated proteins, and transcription factors.

- The protocol accommodates a wide range of cell numbers per reaction, from 5,000 up to 300,000 cells.
- Minimum cell inputs are required for each target type to ensure successful results, with optimal amounts recommended for best performance:

1. **Histones:**
   - Minimum: 5,000 cells per reaction
   - Optimal: 50,000 cells per reaction
2. **Non-histone chromatin proteins:**
   - Minimum: 10,000 cells per reaction
   - Optimal: 100,000 cells per reaction
3. **Transcription factors:**
   - Minimum: 100,000 cells per reaction
   - Optimal: 300,000 cells per reaction

NOTE: *For abundant transcription factors such as CTCF, starting with lower input amounts is possible if required.*

We recommend running your experimental samples at least in duplicate.

3. Sample Types

For **histone profiling**, the following sample types can be used:

- Fresh cells
- Cryopreserved cells
- Fixed cells
- Fresh or snap-frozen tissues

To ensure consistency across experimental results, we recommend using the same sample type throughout the study.

Fixation

Fixation is optional for histone profiling and does not provide additional benefit in terms of data quality or resolution.

However, fixed cells may be used when the same cell batch is also intended for non-histone protein profiling.

In such cases, fixation should follow the procedure described in the section “Cells fixation (required for non-histone protein profiling).”

Cryopreservation Guidance

- Ensure cell viability is greater than 90% before freezing.
- Freeze cells at a minimum concentration of 1 million cells/mL.
- Use standard freezing media and procedures appropriate for the cell type.

Tissue Guidance (Histone Targets)

For histone profiling from tissues, nuclei must first be extracted using the Hologic Diagenode Tissue Nuclei Extraction Module (Cat. No. C01080003) according to the manufacturer’s protocol. Extracted nuclei are then bound to ConA beads.

Non-Histone Protein Profiling

For chromatin-associated non-histone proteins and transcription factors, only fixed cells can be used.

Fixation must be performed on freshly collected cells.

Cryopreserved cells cannot be used for non-histone protein profiling because freezing can disrupt protein–protein and protein–DNA interactions required for accurate CUT&Tag targeting.

Tissue Guidance (Non-Histone Targets)

If profiling non-histone proteins or transcription factors from tissue samples, please contact us for tailored guidance, as fixation and extraction requirements differ from histone-based applications.

4. Cell Preparation and ConA Bead Binding

Depending on your experimental design, two options are available for cell preparation and ConA bead binding:

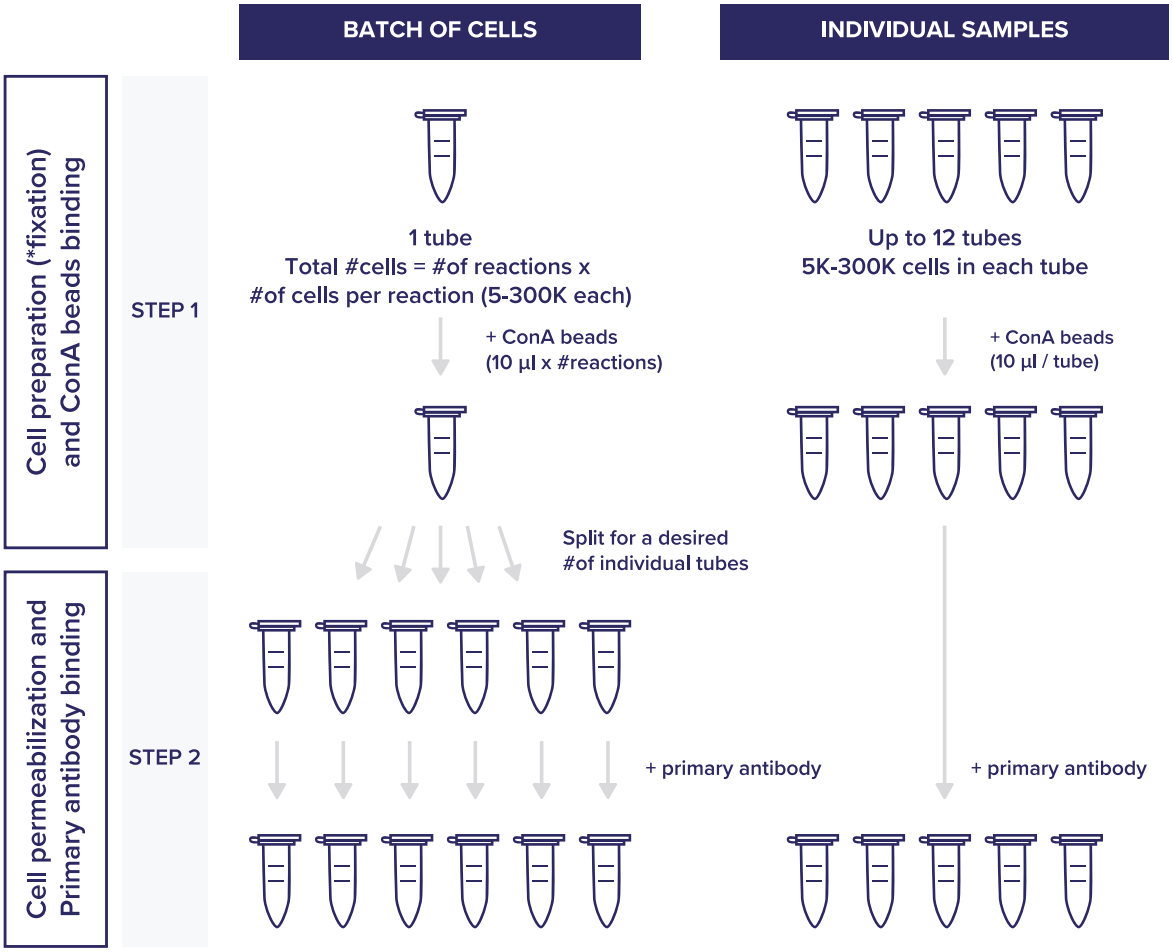


Figure 1. *Schema showing two options for cell preparation—batch of cells vs. individual samples.*

- Starting with a **batch** of cells containing enough material for each planned experiment. This batch is determined by the number of reactions to be run (including the negative and positive controls) and the number of cells used per reaction (5,000–300,000 range). Calculate the total number of cells required per batch (e.g., prepare 500,000 cells for 10 reactions of 50,000 cells each for cell preparation and ConA bead binding). The cells bound to ConA beads must be split into several reactions before primary antibody binding (step 2). When possible, this option is preferred to limit tube-to-tube variability and cell loss.
- Starting directly with **individual tubes** containing the desired number of cells per reaction (e.g., prepare 10 tubes of 50,000 cells each). Each tube (sample) will be processed separately during all steps of the protocol.

- **Important note for non-histone protein profiling:** If you are profiling non-histone chromatin proteins or transcription factors, cells should be fixed according to the protocol (either per batch or individually) prior to starting the experiment.

5. Complete UCT Buffer Preparation

UCT buffers provided in the kit must be supplemented with Protease Inhibitor Mix (200x), Spermidine (50x), and 5% BSA prior to use, according to the tables in the Buffers Preparation section. Determine the volume of Complete UCT buffers needed per experiment and prepare them accordingly. Complete UCT buffers are stable only for a short time. Keep the Complete UCT buffers at +4 °C until use. Discard any unused buffer by day 1 or 2 of the protocol.

6. ConA Beads Preparation

Determine the number of reactions to be run, including positive and negative controls, and calculate the required amount of ConA beads needed per experiment. 10 µL of ConA beads are used per reaction. Scale according to the number of reactions, wash the beads, and resuspend them in the required amount of UCT Binding Buffer, depending on your initial number of reactions.

ConA beads may show some clumping during the process, which is normal. However, it is important to handle the beads gently and keep them as a liquid suspension by careful pipetting to avoid drying, as bead drying can be harmful and may lead to sample loss or reduced performance. Variation in the volume of beads will decrease reproducibility. Do not freeze the beads.

7. Antibodies

The quality of the antibody used in Universal CUT&Tag is one of the crucial factors for assay success. It is recommended to use only validated antibodies that specifically recognize the target. Hologic Diagenode offers extensively validated and high-performance CUT&Tag-grade antibodies. Each batch is validated, and batch-specific data is available on the website www.diagenode.com.

The negative (IgG) and the positive (H3K27me3) control antibodies, which monitor the efficiency of your assay, are available in the Antibody Package. We recommend using these controls at least once per experiment, for both, histone nad non-histone targets.

For non-histone proteins, if needed, the PolII (Hologic Diagenode Cat. No. C15200004) or CTCF (Hologic Diagenode Cat. No. C15410210) antibodies can be used as well. Sequencing the IgG control sample enables peak-calling normalization and helps minimize potential false positives by ensuring consistent and reliable results. Please check the FAQs section for the specificity of each control.

Typically, 1 µg of antibody is sufficient per reaction, and additional titration is generally not required. However, it is advisable to review the technical datasheet for each antibody, as the optimal amount may vary depending on the antibody's performance. If more than 1 µg of primary antibody is used in a reaction, it may also be necessary to proportionally increase the amount of secondary antibody.

The Antibody Package is formulated to provide sufficient secondary antibody for use with up to 1 µg per reaction. If you plan to use more than 1 µg per reaction, an additional purchase will be required.

8. Tagmentation

We recommend using a 1:250 dilution of the adaptor-loaded pA-Tn5 transposase included in the kit (as described in Step 4). This dilution is effective across a wide range of cell types and targets, provided that the kit specifications regarding cell number and antibody amount are followed.

If your experiment uses an input or antibody amount outside the recommended range, further optimization of the pA-Tn5 dilution (typically between 1:100 and 1:500) may be necessary, depending on factors such as cell number, cell type, or the antibody used.

The kit includes enough pA-Tn5 for the recommended number of reactions at recommended 1:250 dilutions. However, if a higher amount of pA-Tn5 is required for optimization , additional pA-Tn5 Transposase loaded can be purchased separately (Hologic Diagenode, Cat. No. C01070001).

9. Library Amplification

The PCR amplification should yield libraries with sufficient concentration for sequencing. The requirements for a final library concentration depend on the type of sequencer used and may vary among different sequencing service providers. The usual range of a final purified library is between 5–20 nM in a final volume of 10–15 µl, but we recommend inquiring with your sequencing platform.

The number of PCR cycles affects the library complexity. It is important to avoid over-amplification during library preparation to minimize amplification biases. The number of PCR cycles should be optimized based on cell number and target abundance. **The total number of PCR cycles should not exceed 17**.

For each new project, we strongly recommend amplifying libraries for a limited number of cycles (usually 6–10 PCR cycles) and performing an intermediate quantification of unpurified libraries using the KAPA® Library Quantification Kit. This step allows you to estimate the library yield and determine if additional re-amplification is required to produce a sufficient quantity of libraries while avoiding over-amplification. For established experiments, the intermediate quantification might be skipped by amplifying samples for the required number of cycles directly.

For intermediate quantification, use a KAPA Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems) that is compatible with your qPCR instrument. Please read the complete manual before first-time use and follow the manufacturer's recommendations.

The library yield at this step should be in the range of 10–30 nM, assuming some sample loss during the final library purification to reach the final yield in the range of 5–20 nM. If this is not the case, additional PCR cycles should be added to re-amplify the libraries until the required yield is obtained.

The number of re-amplification cycles should be chosen based on the assumption that each PCR cycle doubles the copy number of the amplified DNA molecules, so that after n cycles you have 2^n copies of DNA. For example, 10 cycles produce 2 ➔ 4 ➔ 8 ➔ 16 ➔ 32 ➔ 64 ➔ 128 ➔ 256 ➔ 512 ➔ 1,024 copies (2^10). However, be aware that real PCR efficiency might be lower than 100%, so it is recommended to add one extra cycle to compensate.

Example:

KAPA quantification returns a 2 nM library concentration, which is less than required for sequencing. We need to reach approximately 10 nM final concentration, so the sample should be re-amplified by 3–4 additional cycles, with an expected yield of 8–16 nM, respectively: 2 nM ➔ 4 nM ➔ 8 nM ➔ 16 nM.

10. Primer Indexes

Hologic Diagenode's **Primer indexes for tagmented libraries**, which are compatible with the Universal CUT&Tag Kit, are available in multiple formats to suit different experimental needs. Users can choose between single indexing and unique dual-indexing (UDIs), as well as from several index sets. However, UDIs are strongly recommended, as they provide more accurate sample identification by significantly reducing the risk of index hopping during the sequencing step.

For more detailed information about the differences between the available kits and recommended pooling guidelines for the indexes, please refer to the Primer indexes for tagmented libraries manual.

11. Library Clean-Up and QC

Library purification using Agencourt AMPure® XP (Beckman Coulter) is the preferred method because sequence complexity is conserved. Do not use silica-based filters for library purification. A successful library should show a smear ranging from 150 to 2000 bp, yielding about 5–20 nM, with no presence of adapter dimers (see Example of results).

If adapter dimers (a sharp peak at around 150 bp) are present, perform an additional clean-up step with AMPure XP beads. Be aware that a second round of purification

may reduce the library yield. The presence of a sharp peak around 40–50 bp does not require additional purification. This peak corresponds to a free oligonucleotide used for pA-Tn5 loading. This oligonucleotide does not contain a full adapter sequence and will not cluster on the Illumina sequencing flow cell.

12. Quantification of Libraries

Determine the concentration of the libraries, which should fall within the range of 5–20 nM, in a final volume of 10–15 µl. There are several approaches available for library quantification, including real-time PCR, UV absorption, fluorescence detection, or sizing and quantification using the Bioanalyzer or Fragment Analyzer (Agilent). It is important to understand the benefits and limitations of each approach.

Real-time PCR-based approaches, such as the KAPA Library Quantification Kit from Kapa Biosystems, quantify the library molecules that carry the Illumina adapter sequences on both ends, and therefore reflect the quantity of the clustering-competent library molecules. This approach assumes a relatively uniform size of the sheared or fragmented starting gDNA inserts used for library construction.

The Fragment Analyzer (Agilent) provides sizing and quantification information about the analyzed library, but does not assess its clustering efficiency.

The TapeStation (Agilent) offers lower resolution than the Fragment Analyzer. If the TapeStation is the only available option, it can be used for library assessment, but please be aware that its lower resolution may limit the accuracy of CUT&Tag library profile visualization.

UV absorption/fluorescence detection-based methods (i.e., NanoDrop® (Thermo Scientific), Qubit® 2.0 Fluorometer (Life Technologies), or Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies)) simply quantify total nucleic acid concentration. These methods do not detect the presence of adapters and offer no information about the size of the library molecules. They can be used only on purified libraries. We recommend using fluorescence-based assays over spectrophotometric methods.

13. Relative Enrichment Evaluation by qPCR (Optional Protocol A)

It is possible to analyze the relative enrichment of targeted genomic regions prior to sequencing, in order to ensure that the expected enrichment is observed. For control libraries (IgG and H3K27me3), use primer pairs included in the Antibody Package. The TSH2B promoter is an expected positive region for H3K27me3, while the GAPDH TSS represents a negative locus.

Each specific antibody will require user-designed control primers tailored to the target of interest. Keep in mind that the relative enrichment is not normalized and may not be fully accurate.

To have sufficient DNA left for sequencing, we recommend using no more than 10% of the total DNA library for qPCR. You can dilute the DNA (1/10 or more) to perform sufficient qPCR reactions. qPCR reactions should be performed at least in duplicate, although performing in triplicate is recommended to help identify potential outliers.

14. Data Normalization Using Either IgG or Input

We recommend normalization as an essential step for reliable peak calling in CUT&Tag experiments. Using IgG controls for normalization is strongly advised, as they effectively account for background signal and minimize false positives while integrating seamlessly into the standard workflow. IgG control samples can be processed in parallel with target samples, making this approach both convenient and consistent.

Genomic DNA Input normalization is available as an optional method; however, our analyses show that it provides no additional benefit over IgG controls in reducing background or improving data quality. Moreover, the Genomic DNA Input protocol requires a separate workflow and the purchase of additional reagents, making it less practical for routine use.

For these reasons, we recommend IgG-based normalization over Genomic DNA Input, as both methods yield comparable results but IgG offers greater convenience and does not require extra materials. However, for users who specifically prefer or require DNA Input normalization, a detailed protocol is provided in this manual (Optional Protocol B).

PROTOCOL

Buffer Preparation

Stock UCT buffers are prepared without Protease Inhibitor Mix (200X), Spermidine (50X), and 5% BSA. Prepare the required amount of Complete UCT buffers for each set of experiments, and discard any leftovers. All volumes of Complete UCT buffers are given for one sample; scale up according to the number of reactions, including some excess (already included in the kit). Once freshly prepared, the Complete UCT buffers must be kept at 4°C until use.

Complete UCT Wash Buffer 1: Prepare **50 µl per sample** by mixing:

Reagents	Volume for 1 sample
UCT Wash Buffer 1	50 µl
Protease Inhibitor Mix 200x	0.25 µl

Complete UCT Antibody Buffer: Prepare **550 µl per sample** by mixing:

Reagents	Volume for 1 sample
UCT Antibody Buffer	550 µl
Protease Inhibitor Mix 200x	2.75 µl
Spermidine 50X	11 µl
5% BSA	11 µl

Complete UCT Wash Buffer 2: Prepare **500 µl per sample** by mixing:

Reagents	Volume for 1 sample
UCT Wash Buffer 2	500 µl
Protease Inhibitor Mix 200x	2.5 µl
Spermidine 50X	10 µl

Complete UCT Tagmentation Buffer: Prepare **200 µl per sample** by mixing:

Reagents	Volume for 1 sample
UCT Tagmentation Buffer	200 µl
Protease Inhibitor Mix 200x	1 µl
Spermidine 50X	4 µl

STEP 1

Day 1 – 1 hour

Cell Preparation and ConA Bead Binding

Cell preparation and ConA binding can be performed as a batch or as individual samples. The batch of ConA-bound cells will be split into the desired number of individual reactions, starting from Step 2: Permeabilization and Primary Antibody Binding. Individual samples are processed separately through the protocol.

ConA Bead Preparation:

1.1 Determine the number of reactions to be run, including both the negative and positive controls. Resuspend the **ConA beads** by pipetting up and down several times, and transfer the required amount of beads into a clean 1.5 ml tube. 10 µl of beads are required per sample. Scale accordingly.

1.2 Add the **required volume of UCT Binding Buffer**. Use 90 µl of buffer for every 10 µl of ConA beads (per sample). Scale the volumes according to the number of reactions. Mix thoroughly by pipetting up and down several times.

1.3 Place the tube on a magnetic rack and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the bead pellet.

1.4 Repeat steps 1.2 and 1.3.

1.5 Remove the tube from the magnetic rack and resuspend the beads in **UCT Binding Buffer** using the original beads volume (i.e., 10 µl per reaction × number of reactions). Keep the suspension at room temperature until the cells are ready (see Step: Cells Collection and Preparation), then proceed directly to the cell binding step 1.14.

Cell Collection and Preparation (for histone and non-histone protein profiling):

Depending on your target, two approaches are possible for cell preparation.

The initial cell collection steps (up to Step 1.13) are identical for both histone and non-histone protein profiling.

The workflows diverge after cell collection:

Histone targets ➔ proceed directly to ConA Bead Cell Binding

Non-histone targets ➔ ➔ cells must be freshly collected and fixed before ConA Bead Cell Binding.

A. Histone Profiling

- **Sample types:** Fresh cells, cryopreserved cells, fixed cells, and fresh or snap-frozen tissues can be used for histone profiling.
- **Fixation guidance:** Fixation is not mandatory and provides no additional advantage for histone profiling. However, fixed cells may be used when the same cell batch will also be used to profile non-histone proteins. In that case, follow the fixation protocol described in the section "Non-Histone Protein Profiling."
- **Fresh-frozen tissues:** When working with tissue samples, nuclei should first be extracted using the Hologic Diagenode Tissue Nuclei Extraction Module (Cat. No. C01080003) in accordance with its protocol. The extracted nuclei are then bound to ConA beads.

Fresh Adherent Cells:

1.6 Pre-warm **PBS**, **culture medium**, and **sterile trypsin-EDTA** to 37°C.

1.7 Collect the required number of cells, either as a batch or as individual samples. Remove the medium and rinse the cells with **pre-warmed PBS** (10 ml for a 75 cm^2 culture flask). Gently shake the flask for 2 minutes.

1.8 Remove the PBS and add pre-warmed, **sterile trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the cells. Gently shake the culture flask for 1–2 minutes or until the cells start to detach.

NOTE: *The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary, as prolonged treatment with trypsin may damage the cell surface and the binding capacity on ConA beads. Regularly check whether the cells are beginning to detach. If necessary, use an enzyme-free dissociation method, such as scraping or a rubber policeman.*

1.9 Immediately add **pre-warmed culture medium** to the cells when they are detached. To ensure proper inactivation, the volume of medium must be twice the volume of trypsin-EDTA. Transfer the cell suspension to a 15 ml tube.

1.10 Rinse the flask by adding **10 ml of pre-warmed PBS**. Add this volume to the tube containing the cells.

1.11 Centrifuge for 10 minutes at 500 × g (room temperature) and carefully remove the supernatant without disturbing the cell pellet. Proceed directly to point 1.12 of the protocol.

Fresh Suspension Cells:

- Collect the required number of cells, either as a batch or as individual samples. Use a 15 ml tube for batch preparations or a 2 ml tube for individual samples.

- Centrifuge for 10 minutes at 500 × g at room temperature, and carefully remove the supernatant without disturbing the cell pellet. Proceed directly to step 1.12 of the protocol.

Cryopreserved Cells (for histones profiling only):

- Thaw the desired number of cells, either as a batch or individually, in a 37°C water bath for 2 minutes.
- Transfer them to a 15 ml tube for batch preparations or a 2 ml tube for individual samples, then proceed directly to step 1.13.

1.12 Resuspend the cells in **warm PBS**: use 10 ml of PBS for batch preparations or 1 ml for individual samples. Take a 10 µl aliquot, add 10 µl of Trypan blue, and count the viable cells.

1.13 Transfer the desired number of cells, either as a batch or individually, to a 1.5 ml tube. Centrifuge for 3 minutes at 600 × g at room temperature, and carefully remove the supernatant without disturbing the cell pellet. Proceed directly to cell binding (step 1.14).

B. Non-Histone Protein Profiling

- **Sample types:** Fixed cells only.

  IMPORTANT: *Fixation must be performed on freshly collected cells.*

  Cryopreserved cells cannot be used for non-histone protein profiling, as freezing can disrupt protein–protein and protein–DNA interactions required for accurate CUT&Tag targeting.
- **Fixation requirement:** Fixation is required before starting the CUT&Tag protocol and must be performed after completing the common cell collection steps.

Cells fixation (required for non-histone protein profiling):

- Prepare a 0.1% formaldehyde solution: add **1.4 µl of 37% fresh formaldehyde to 0.5 ml of PBS** at room temperature (RT). Work under the chemical hood. This amount is sufficient for fixing up to 2.5 million cells per batch or per individual sample. Scale up if more cells are needed.
- Resuspend collected cells in the formaldehyde solution and gently rotate for 5 minutes at RT.
- Quench the fixation by adding **50 µl of 1.25 M glycine**, mix by gentle pipetting, and rotate for 5 minutes at RT.
- Centrifuge at 600 × g for 5 minutes at RT and gently remove the supernatant.
- Resuspend cells in **250 µl of PBS**. Centrifuge at 600 × g* for 5 minutes at RT.

- Remove the supernatant and proceed with cell binding as described below.

NOTE: *For sensitive, fragile cells, centrifugation at 300 × g is recommended.*

PAUSE POINT: *Fixed cell pellets can be frozen at –80°C for up to 6 months. Thaw the cells for 2 minutes on ice and proceed with cell binding.*

ConA Bead Cell Binding:

1.14 Resuspend the prepared cells in **Complete UCT Wash Buffer 1**, using **50 µl** per reaction. If preparing cells in bulk, scale according to the number of reactions (e.g., 600 µl of Complete UCT Wash Buffer 1 for a batch of 12 reactions). Mix thoroughly by pipetting up and down several times.

1.15 Add the **washed ConA beads** (from step 1.5) to the cell suspension (in bulk or individually). Mix gently by pipetting up and down several times.

1.16 Place the tube on a rotating wheel for 20 minutes at +4°C.

1.17 Briefly spin the tube to collect any liquid from the cap. Place the tube on a magnetic rack and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet. Proceed directly to step 2.

Optional: Recommended for a new project, cell type, or cell amount. Check the supernatant under a microscope to ensure efficient cell binding. If the binding is efficient, the supernatant should contain no cells or only a very few. If binding is not efficient, troubleshooting is recommended, as this may be related to damaged cell surfaces (for example, due to over-trypsinization) or bead drying.

STEP 2

Day 1 – Overnight

Cell Permeabilization & Primary Antibody Binding

2.1 Remove the tube from the magnetic rack, and resuspend the pellet in **50 µl of ice-cold Complete UCT Antibody Buffer** per reaction. For batch processing, scale the volume according to the number of reactions. Mix thoroughly by pipetting up and down several times.

2.2 Distribute **50 µl** of the mix into 0.2 ml low-binding tube strips or a plate (one well corresponds to one reaction with the desired number of cells).

NOTE: *From now on, all reaction tubes will be treated individually rather than processed in batches.*

2.3 Add **1 µg** of **primary antibody** to each reaction tube and mix gently by pipetting up and down several times.

2.4 Close the strips or the plate using caps, and place it on a rotating wheel at +4°C for overnight incubation.

NOTE: *We recommend using cap strips with the PCR plates to prevent the beads from sticking to the sealing film.*

STEP 3

Day 2 – 1h15

Secondary Antibody Binding

NOTE: *Choose the secondary antibody according to the host species of the primary antibody. The secondary antibodies (anti-rabbit, anti-mouse) are included in the CUT&Tag Antibody Package, which is available separately.*

3.1 Prepare **100 µl of diluted secondary antibody** per reaction by mixing **1 µg** of secondary antibody and **Complete UCT Antibody Buffer**. Scale the volume according to the number of reactions. Mix gently by pipetting up and down several times, then hold on ice until use.

3.2 Remove the strips or plate, containing the cells and primary antibody (from step 2.4), from the rotating wheel.

3.3 Briefly spin the strips or plate to collect any liquid from the cap. Place the strips or plate on a magnetic rack, and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

3.4 Remove the strips or plate from the magnetic rack and add **100 µl of diluted secondary antibody** per reaction. Mix gently by pipetting up and down several times.

3.5 Close the strips or plate with caps and incubate on a rotating wheel for 45 minutes at +4 °C.

3.6 Briefly spin the strips or plate to collect any liquid from the cap. Place the strips or plate on a magnetic rack and wait for 2 minutes, until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

3.7 Wash the pellet **twice** as follows:

- Remove the strips or plate from the magnetic rack and add **200 µl of Complete UCT Antibody Buffer** per reaction. Mix gently by pipetting up and down several times.
- Place the strips or plate back on the magnetic rack and wait for 2 minutes, until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

3.8 Proceed directly to **Step 4**.

STEP 4

Day 2 – 3h30

Targeted Tagmentation

(pA-Tn5 Binding – Activation – Inactivation)

4.1 Prepare a 1:250 dilution of **loaded pA-Tn5 transposase by mixing loaded pA-Tn5 transposase and Complete UCT Wash Buffer 2** as shown in the table below. 100 µl of diluted loaded pA-Tn5 transposase is needed per reaction; scale the volume according to the number of reactions. Mix gently by pipetting up and down several times and hold on ice until use.

pA-Tn5 preparation table:

# of reactions	Loaded pA-Tn5 Transposase	Complete UCT Wash buffer 2	Total volume
1	0.4 µl	99.6 µl	100 µl
2	0.8 µl	199.2 µl	200 µl
3	1.2 µl	298.8 µl	300 µl
4	1.6 µl	398.4 µl	400 µl
5	2 µl	498 µl	500 µl
6	2.4 µl	597.6 µl	600 µl
7	2.8 µl	697.2 µl	700 µl
8	3.2 µl	796.8 µl	800 µl

4.2 Remove the strips or plate from the magnetic rack (from step 3.8) and add **100 µl of the 1:250 dilution of loaded pA-Tn5 transposase** per reaction. Mix gently by pipetting up and down several times.

4.3 Close the strips or plate using caps and incubate on a rotating wheel for 1 hour at +4°C.

4.4 Briefly spin the strips or plate to collect any liquid from the cap. Place the strips or plate on a magnetic rack and wait for 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

4.5 Wash the pellet **twice** as follows:

- Remove the strips or plate from the magnetic rack and add **200 µl of Complete UCT Wash Buffer 2** per reaction. Mix gently by pipetting up and down several times.
- Place the strips or plate back on the magnetic rack and wait for 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

4.6 Briefly spin the strips or plate in a tabletop centrifuge. Place the strips or plate on a magnetic rack and remove the remaining liquid without disturbing the pellet.

4.7 Add **200 µl of Complete UCT Tagmentation Buffer** per reaction. Mix gently by pipetting up and down several times.

4.8 Close the strips or plate using caps and incubate for 1 hour at 37°C in a thermocycler with the lid set to **37°C or off**.

4.9 Remove the strips or plate from the thermocycler and briefly spin them to collect any liquid from the cap.

4.10 Stop the tagmentation reaction by adding **6.7 µl of UCT Buffer E, 2 µl of UCT Buffer S**, and **2.5 µl of proteinase K** per sample.

4.11 Close the strips or plate using caps, vortex to mix, and incubate for 1 hour at 55°C in a thermocycler with the lid set to 55°C or off.

4.12 Proceed directly to **Step 5**.

STEP 5

Day 2 – 1h

DNA Purification

NOTE: *Before the first use of the Universal CUT&Tag kit, 8.8 ml of absolute ethanol must be added to 2.2 ml of DNA Wash Buffer (for 24 rxns kit), and 17.6 ml of absolute ethanol must be added to 4.4 ml of DNA Wash Buffer (for 48 rxns kit). Never leave the bottle open during storage to avoid evaporation.*

5.1 Remove the strips or plate from the thermocycler (step 4.12) and briefly spin them to collect any liquid from the cap. Place the strips or plate on a magnetic rack and wait for 2 minutes until the solution is clear.

5.2 Carefully **transfer the supernatant** to a clean 2 ml tube (one tube per reaction) without disturbing the pellet.

5.3 Add **1 ml of ChIP DNA Binding Buffer** to each reaction tube. Mix gently by pipetting up and down several times.

5.4 Transfer **600 µl of the mixture** into the provided spin column placed in a 2 ml collection tube. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through. Repeat this step once more (for a total of two times).

5.5 Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through. Repeat this step once more (for a total of two times).

5.6 Centrifuge again at ≥10,000 x g for 1 minute to dry the column and remove any remaining liquid from it.

5.7 Transfer the column to a new 1.5 ml microcentrifuge tube and add **22 µl of DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the tagmented DNA.

5.8 Store at 4 °C until PCR is performed, or continue immediately with Step 6: Library Amplification.

STEP 6

Day 2 – 1h

Library Amplification

For each new project, we strongly recommend determining the optimal number of amplification cycles for each sample by following the protocol for Intermediate Quantification of Unpurified Libraries Using KAPA® Library Quantification Kit – Illumina. This step allows you to estimate the library yield and determine if additional re-amplification is required to produce a sufficient amount of library while avoiding over-amplification. For established experiments, the intermediate quantification may be skipped by amplifying samples for the required number of cycles directly.

NOTE: *Primer indexes for the tagmented libraries kit are required for this step and must be purchased separately (see "Required Materials Not Provided"). Please refer to the corresponding manuals for guidance on index pooling. Use different indexes for samples that will be sequenced in the same lane.*

6.1 Transfer **20 µl of purified DNA** from step 5.8 into a 0.2 ml tube strip or plate well, and add **1 µl of primer index pair for tagmented libraries** to each sample.

6.2 Add **20 µl of 2x High Fidelity Mastermix** to each sample and mix gently by pipetting up and down several times. Close the strips or plate with caps.

6.3 Run the following PCR program:

Step	Temperature	Time & Cycles	
Gap filling	72°C	5 minutes	
Initial denaturation	98°C	30 seconds	
Denaturation	98°C	10 seconds	6–10* Amplification Cycles
Annealing/Extension	63°C	10 seconds	
Final extension	72°C	1 minute	
Hold	4°C	∞	

** For each new project, we recommend amplifying each sample for 6-10 cycles and then determining the additional number of cycles according to the below protocol for intermediate library quantification. For known samples, this step can be omitted, and the previously determined number of cycles can be applied.*

Perform intermediate library quantification if necessary (refer to Optional STEP 6A) or proceed directly to Step 7: Library Clean-Up and QC.

PAUSE POINT: *After PCR amplification, the unpurified libraries can be stored on ice during the optional quantification using the KAPA® Library Quantification Kit. If necessary, additional PCR cycles can be performed to re-amplify the libraries before purification.*

Intermediate Quantification of Unpurified Libraries Using KAPA® Library Quantification (2h)

NOTE: *We recommend choosing a KAPA Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems) compatible with your qPCR instrument. Please read the full manual before first-time use and follow the manufacturer's recommendations. For accurate quantification, we recommend running at least duplicates of both samples and standards.*

6.4 Prepare a library dilution buffer containing 10 mM Tris-HCl, pH 8.0, and 0.05% Tween 20.

NOTE: *For 50 ml of library dilution buffer, add 500 µl of 1 M Tris-HCl, pH 8.0, and 23 µl of Tween 20, and increase the volume to 50 ml with ultrapure DNase/RNase-free water.*

6.5 In a 1.5 ml tube, prepare a 1/1000 dilution of the library DNA by mixing 1 µl of unpurified library DNA sample (from step 6.3) and 999 µl of library dilution buffer. Vortex for 10 seconds. Keep the remaining unpurified library DNA samples on ice for the duration of this step.

6.6 Prepare the KAPA Master Mix (according to the number of reactions):

Reagent	Volume for 1 reaction
KAPA mix containing Primer Premix*	12 µl
Nuclease-free water	4 µl
Total	16 µl

**Pay attention when adding the Primer Premix to the KAPA mix (follow the manufacturer's manual).*

6.7 Pipette 16 µl of the KAPA Master Mix into qPCR plate wells.

6.8 Add 4 µl of 1/1000-diluted library DNA or ready-to-use standard (1–6) into the wells.

6.9 Run the following qPCR program:

Step	Temperature	Time & Cycles	
Initial activation/denaturation	95°C	5 minutes	
Denaturation	95°C	30 seconds	35 cycles
Annealing/Extension	60°C	45 seconds + data acquisition	

NOTE: *Melting curve analysis is optional and, in certain circumstances, may provide a useful indication of possible primer- and/or adaptor-dimer contamination of libraries. Please refer to the KAPA® Library Quantification Kit manual for more information.*

6.10 Enter the standard curve values in the qPCR software:

Standard Name	dsDNA concentration (pM)
Standard 1	20
Standard 2	2
Standard 3	0.2
Standard 4	0.02
Standard 5	0.002
Standard 6	0.0002

6.11 Calculate the library DNA concentration using the standard curve. Please refer to the KAPA® Library Quantification Kit manual for more information.

NOTE: *Keep in mind that the values need to be multiplied by 1000 to take the dilution into account. The library yield at this step should be in the range of 10–30 nM, assuming some sample loss during the purification step.*

6.12 If necessary, repeat PCR on under-amplified samples for the required number of additional cycles. Use the following program for re-amplification.

Step	Temperature	Time & Cycles	
Denaturation	98°C	10 seconds	Additional cycles*
Annealing/Extension	63°C	10 seconds	
Final extension	72°C	1 minute	
Hold	4°C	∞	

**Please determine the number of re-amplification cycles before starting.*

NOTE: *For samples processed in a plate at the Library Amplification step (step 6), separating them into individual 0.2 ml low-binding tubes before re-amplification is required so that additional PCR cycles can be applied to each sample individually.*

STEP 7

Day 2 – 1h30

Library Clean-Up and QC

7.1 Take the AMPure® XP beads out of the fridge and place them on a rotating wheel at room temperature until they are in complete suspension.

7.2 Add **40 µl of AMPure® XP beads** to **40 µl of unpurified library DNA samples**. Mix thoroughly by pipetting up and down several times or by vortexing for a few seconds to resuspend the beads.

7.3 Incubate at room temperature for 10 minutes.

7.4 Place the strips or plate on a magnetic rack and wait for 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the bead pellet.

7.5 Leave the strips or plate on the magnetic rack and wash the pellet **twice** as follows:

- Add **200 µl of freshly prepared 80% ethanol** without disturbing the **bead pellet**, and wait for 30 seconds.
- Carefully aspirate the supernatant by pipette and discard it without disturbing the pellet.

7.6 Spin down the strips or plate in a tabletop centrifuge, place them back on the magnetic rack, and remove the remaining ethanol.

7.7 Leave the strips or plate wells open for 2–3 minutes to let the bead pellet dry (do not over-dry the beads, as it may result in low recovery; over-dried beads become light brown with cracks), and then remove them from the magnetic rack.

7.8 Add **15 µl of 10 mM Tris-HCl**, **pH 8**, and mix thoroughly by pipetting up and down several times to resuspend the beads.

7.9 Incubate at room temperature for 5 minutes.

7.10 Place the strips or plate on a magnetic rack and wait for 2 minutes until the solution is clear.

7.11 Carefully transfer the supernatant into a fresh tube, without disturbing the bead pellet.

PAUSE POINT: *The purified libraries can be stored at - 20°C for at least one month.*

NOTE: *After library preparation, determine the concentration of the libraries (**see the "Remarks Before Starting" section**). Library yield may vary depending on factors such as cell type, antibody quality, and target specificity. If necessary, dilute the libraries before performing the quality check according to the manufacturer's instructions.*

7.12 Run **2µl** on Fragment Analyzer (Agilent) or equivalent according to the manufacturer's instructions.

7.13 Assess the library size and concentration.
NOTE: *A successful library should show a smear ranging from 150 to 2000 bp with a yield of approximately 5-20 nM with no visible adapter dimers. If adapter dimers (sharp peak around 150 bp) are present, perform an additional clean-up step with AMPure XP beads. Be aware that a second round of purification may reduce the library yield.*

7.14 The libraries are now ready for pooling and sequencing. Prepare equimolar pools of libraries. Libraries pooled together should have similar fragment sizes.

NOTE: *Individual libraries, quantified and purified according to the above protocol, can be pooled at desired molar ratios to allow multiplex sequencing. Libraries pooled together must contain different indexes (for multiplexing and index-pooling guidelines, refer to the manual "Primer Indexes for Tagmented Libraries"). The minimal molar concentration for sequencing the pool depends on the requirements of the sequencing platform. The total molarity is the sum of all individual library molarities in the final volume; e.g., if you add 5 µl of a 10 nM library to 5 µl of a 20 nM library, you have 10 µl of a 15 nM pool. If libraries are prepared from similar input amounts, they can be pooled by combining equal-volume aliquots of each library.*

Volume of individual library in the pool = V(f) x C(f) / # x C(i)

- *V(f) is the final desired volume of the pool*
- *C(f) is the final desired concentration of the pool*
- *# is the number of individual libraries in the pool*
- *C(i) is the concentration of each individual library*

The number of samples in a pool depends on the sequencer's capabilities.

OPTIONAL: Relative enrichment evaluation by qPCR. If you want to evaluate the relative enrichment after library preparation, please refer to Optional Protocol B.

Relative Enrichment Evaluation by qPCR (2h30)

NOTE: *For each sample (including samples of interest, negative controls using IgG, or positive controls using H3K27me3), run both a positive locus primer pair (targeting an enriched region) and a negative locus primer pair (targeting a non-enriched region). Please note that the TSH2B (positive control) and GAPDH TSS (negative control) primer pairs included in the Antibody Package are designed specifically for the H3K27me3 control antibody and are only compatible with human samples. For other species or targets of interest, users must design custom primers.*

qPCR reactions should be performed at least in duplicate, although performing them in triplicate is recommended to help identify potential outliers.

1.1 Take an aliquot of purified library DNA sample (from step 10.11) and dilute it 1/10 using UltraPure DNase/RNase-free water. Repeat this process for both negative and positive control samples.

1.2 *Prepare the qPCR mix as follows:*

Reagent	**Volume for 1 reaction per primer pair/locus**
2x SYBR®Green qPCR master mix	10 µl
Primer pair (5 µM of each)	1 µl
Nuclease-free Water	4 µl
Diluted purified library DNA	5 µl
Total	20 µl

1.3 Use the following PCR program:

NOTE: *These conditions may require optimization depending on the type of master mix, qPCR system used, and user-provided primer pair.*

Step	**Temperature**	**Time & Cycles**	
Denaturation	95°C	3–10 minutes*	
Amplification	95°C	30 seconds	40 Amplification Cycles
	60°C**	30 seconds	
	72°C	30 seconds + data acquisition	
Melting Curve***	Follow the qPCR instrument manufacturer's recommendations.		

**Carefully check the supplier's recommendations about Taq polymerase activation time.*

***The annealing temperature might need adjustment depending on the primer pair. The given temperature is for the control primer included in the kit.*

****Include and inspect the melting curves, based on the protocols recommended by the qPCR instrument manufacturer, to ensure that primer pairs amplify only a single specific product.*

1.4 Record the threshold cycles (Ct values) from the exponential phase of the qPCR for the purified library DNA samples, and the positive and negative controls for each primer.

1.5 Calculate the relative amount of purified library DNA for each sample by subtracting the Ct value at the positive locus from the Ct value at the negative locus for each primer pair (relative enrichment) using the following formula:

$$\textbf{delta Ct} = \textbf{Ct}_{\textbf{negative locus}} - \textbf{Ct}_{\textbf{positive locus}}$$

$$\textbf{relative enrichment} = 2^{(\textbf{delta Ct})}$$

Table 5. Example of Ct values and relative enrichment of duplicate samples for positive (H3K27me3), negative (IgG), and samples of interest (mean of qPCR replicates).

Control Samples	**Ct value TSH2B**	**Ct value GAPDH**	**delta Ct**	**Relative enrichment**
H3K27me3 #1	19.53	24.77	5.24	37.8
H3K27me3 #2	20.00	24.36	4.36	20.5
IgG #1	31.29	24.86	-6.4	0.01
IgG #2	30.99	23.73	-7.3	0.01
Experiment Samples	**Ct value positive locus**	**Ct value GAPDH**	**delta Ct**	**Relative enrichment**
Sample #1	22.13	28.23	6.10	68.59
Sample #2	24.25	29.57	5.32	39.95
IgG #1	29.30	29.10	-0.20	0.90
IgG #2	28.40	28.00	-0.39	0.80

NOTE: *The relative enrichment value is highly dependent on the control loci used and may vary significantly when switching to different primer pairs. It is also influenced by factors such as cell number and type, sample dilution prior to qPCR, and the*

abundance of the target. The relative enrichment value below 3 should be considered as background.

1.6 If the expected relative enrichment is observed, proceed to sequencing.

IMPORTANT NOTE: *To ensure accurate normalization during peak calling, it is recommended to sequence the IgG or input control alongside your target of interest. For further guidance, refer to the 'Data Analysis Recommendations' section.*

Genomic DNA Input Protocol (2h30)

NOTE: *We recommend using the same number of cells as your initial sample to better normalize the dataset. The protocol below is described for 50,000 cells per sample, in duplicate.*

Cell Lysis

1.1 Collect the required number of cells per sample (100K cells, in duplicate).

1.2 Centrifuge at 500 x g for 10 minutes at room temperature (RT). Carefully aspirate and discard the supernatant without disturbing the cell pellet.

1.3 Resuspend each sample in **100 µl of DPBS 1X** at RT (in this example, 200 µl).

1.4 Distribute 100 µl of the cell suspension into 1.5 ml tubes (50K cells in each, i.e., 100 µl).

1.5 Centrifuge at 600 x g for 3 minutes at RT. Carefully aspirate and discard the supernatant without disturbing the cell pellet.

1.6 Resuspend in **100 µl of XL GenDNA Digestion Buffer** supplemented with **0.5 µl GenDNA Proteinase K** per sample.

1.7 Incubate at 55°C for 30 minutes in a thermomixer set at 600 rpm.

Genomic DNA Purification Using DiaPure Columns

1.8 Carefully **transfer the supernatant** to a clean 2 ml tube (one tube per reaction) without disturbing the pellet.

1.9 Add **500 µl** of **ChIP DNA Binding Buffer** to each reaction tube. Mix gently by pipetting up and down several times.

1.10 Transfer the entire volume of the mixture into the provided spin column placed in a 2 ml collection tube. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through.

1.11 Add **200 µl** of **DNA Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through.

1.12 Carefully add **1 µl** of **GenDNA RNAse** directly onto the column filter and incubate for 15 minutes at room temperature (RT).

1.13 Add **200 µl** of **DNA Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through.

1.14 Centrifuge again at ≥10,000 x g for 1 minute to dry the column and remove any remaining liquid from it.

1.15 Transfer the column to a new 1.5 ml microcentrifuge tube and add **30 µl** of **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

PAUSE POINT: *Store at –20°C or continue immediately with pA-Tn5 tagmentation.*

Genomic DNA Tagmentation

1.16 Add **267 µl** of **Complete UCT Tagmentation Buffer** and **3 µl** of **pA-Tn5** per reaction. Mix gently by pipetting up and down several times.

1.17 Incubate for 1 hour at 37°C in a thermomixer set at 800 rpm.

1.18 Remove the tube from the thermomixer and briefly spin it to collect any liquid from the cap.

1.19 Stop the tagmentation reaction by adding **10 µl** of **UCT Buffer E**, **3 µl** of **UCT Buffer S**, and **2.5 µl** of **proteinase K** per sample.

1.20 Close the tube, vortex to mix, and incubate for 1 hour at 55°C in a thermomixer set at 800 rpm.

1.21 After this step, samples and input are treated the same way, so proceed directly to step 5.

Sequencing Recommendations

We recommend paired-end sequencing with a minimum read length of 50 bp, targeting 15–20 million read pairs per sample for highly abundant histone marks and 20–30 million read pairs per sample for low-abundant histone marks, chromatin-associated proteins, and transcription factors. Insufficient sequencing depth may result in reduced sensitivity and failure to detect regions of enrichment (peaks). The PhiX percentage should be applied as recommended by the sequencer (typically 1–5%).

Following the use of single or unique dual indexes, the construct will bear one or two separate indexes. The i5 index can be read in a reverse-complement workflow or in a forward-strand workflow, depending on the sequencer. For more details about the adapter sequences of the indexes, please check the Primer Index for Tagmented Libraries manual.

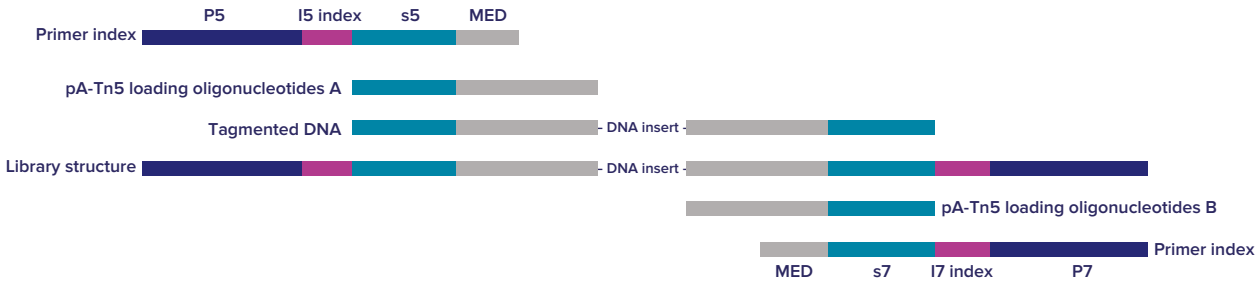


Figure 2: *Schematic representation of the Universal CUT&Tag tagmented library structure. The diagram illustrates the arrangement of adapters, indexes, and primer binding sites in a typical Illumina library prepared using tagmentation.*

- **Primer Index Row:** shows the positions of the P5 and P7 flow cell adapters (dark blue), i5 and i7 index sequences (purple), s5 and s7 sequencing primer binding sites (teal), and partial MED regions (gray).
- **pA-Tn5 Loading Oligonucleotides A and B Rows:** represent the transposase-loaded adapters that introduce adapter sequences during tagmentation.
- **Tagmented DNA:** depicts genomic DNA fragments flanked by adapter sequences after tagmentation.
- **Library Structure:** shows the final configuration of the sequencing library, including both ends with P5/P7 adapters, indexes, and sequencing primer sites.

This structure enables cluster generation on the Illumina flow cell and supports paired-end sequencing with standard sequencing primers targeting the s5/s7 regions and indexes.

Data Analysis Recommendations

In the following section, we will introduce the fundamentals of CUT&Tag data analysis, guiding you through the key steps and considerations. We will also recommend some software tools suitable for each step of the workflow.

Bioinformatics analysis is also available as a service, with both Standard and Advanced options. For more information, please contact us (www.diagenode.com).

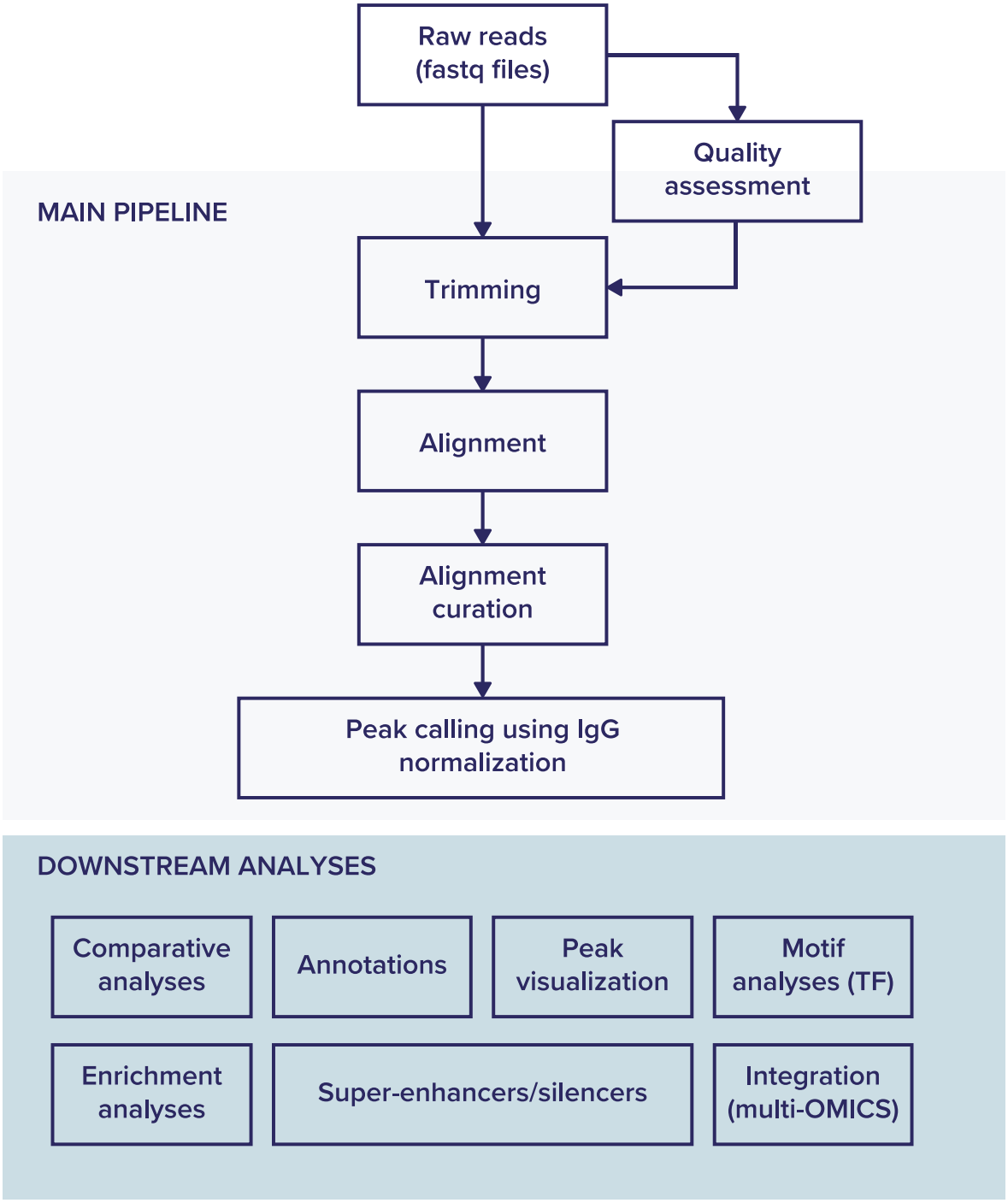


Figure 3. *Universal CUT&Tag data analysis workflow. Overview of the key steps included in CUT&Tag data analysis.*

Main Pipeline:

1. **Quality:** The quality of the raw reads in FASTQ-compressed format can optionally be checked before any analysis using FastQC [1].
2. **Trimming:** Trimming of potential Nextera adapter contamination and of low-quality bases is performed with Cutadapt [2].
3. **Alignment:** The trimmed reads are subsequently aligned to an indexed reference genome using Burrows–Wheeler Aligner (BWA) [3]. Reference genomes can be obtained through UCSC (https://genome.ucsc.edu/) or NCBI (https://www.ncbi.nlm.nih.gov/genome/).
4. **Alignment curation:** Reads mapping to regions classified as blacklisted by ENCODE [4], as well as multi-mapping reads — those mapping to multiple genomic locations with equal alignment scores — are removed using Samtools [5].
5. **Peak calling:** Peak calling is performed to detect sites of enrichment (read accumulation) along the genome. In CUT&Tag experiments, peak calling on target samples can be performed in three ways: without normalization, with IgG normalization, or with input normalization. However, normalization using either IgG or input controls is strongly recommended to ensure data reliability and to eliminate false positives by correcting for background signals. IgG and input normalization yield comparable results in terms of peak characteristics. These control conditions must be included in the sequencing workflow, and the resulting reads should be trimmed, aligned, and used to normalize peak calling on the alignment files of the target protein samples.

Downstream Analysis

After this general analysis, the peaks can be analyzed further to answer your biological questions. There are countless ways and tools for further analyses (comparative analysis, annotation, peak visualization); the project's goals determine which ones to pick. Just as in the case of the basic analysis, we recommend thoroughly going through the manual of the chosen software tool to understand its purpose and function. Recommended software and R packages include deepTools [6], the HOMER suite [7], DiffBind [8], and annotatr [9].

References

1. Simon Andrews. FastQC: A quality control tool for high throughput sequence data. 2010. URL: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

2. Martin. 'Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads'. https://doi.org/10.14806/ej.17.1.200

3. Heng Li and Richard Durbin. 'Fast and accurate short read alignment with Burrows-Wheeler transform.' In: Bioinformatics (Oxford, England) 25.14 (July 2009), pp. 1754–60. ISSN: 1367-4811. DOI:10.1093/bioinformatics/btp324. URL: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2705234%7B%5C&%7Dtool=pmcentrez%7B%5C&%7Drendertype=abstract.

4. Michael M Hoffman et al. 'Integrative annotation of chromatin elements from ENCODE data.' In:Nucleic acids research 41.2 (Jan. 2013), pp. 827–41. ISSN: 1362-4962. DOI: 0.1093/nar/gks1284.URL:http://www.ncbi.nlm.nih.gov/pubmed/23221638%20http://www.pubmedcentral.nih.gov/arti clerender.fcgi?artid=PMC3553955.

5. HengLietal.'TheSequenceAlignment/MapformatandSAMtools.'In:Bioinformatics(Oxford,England)25.16(Aug. 2009), pp. 2078–9. ISSN: 1367-4811. DOI:10.1093/bioinformatics/btp352.URL:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2723002%7B%5C &%7Dtool=pmcentrez%7B%5C&%7Drendertype=abstract.

6. Ramírez, Fidel, Devon P. Ryan, Björn Grüning, Vivek Bhardwaj, Fabian Kilpert, Andreas S. Richter, Steffen Heyne, Friederike Dündar, and Thomas Manke. deepTools2: A next Generation Web Server for Deep-Sequencing Data Analysis. Nucleic Acids Research (2016). DIO:10.1093/nar/gkw257.

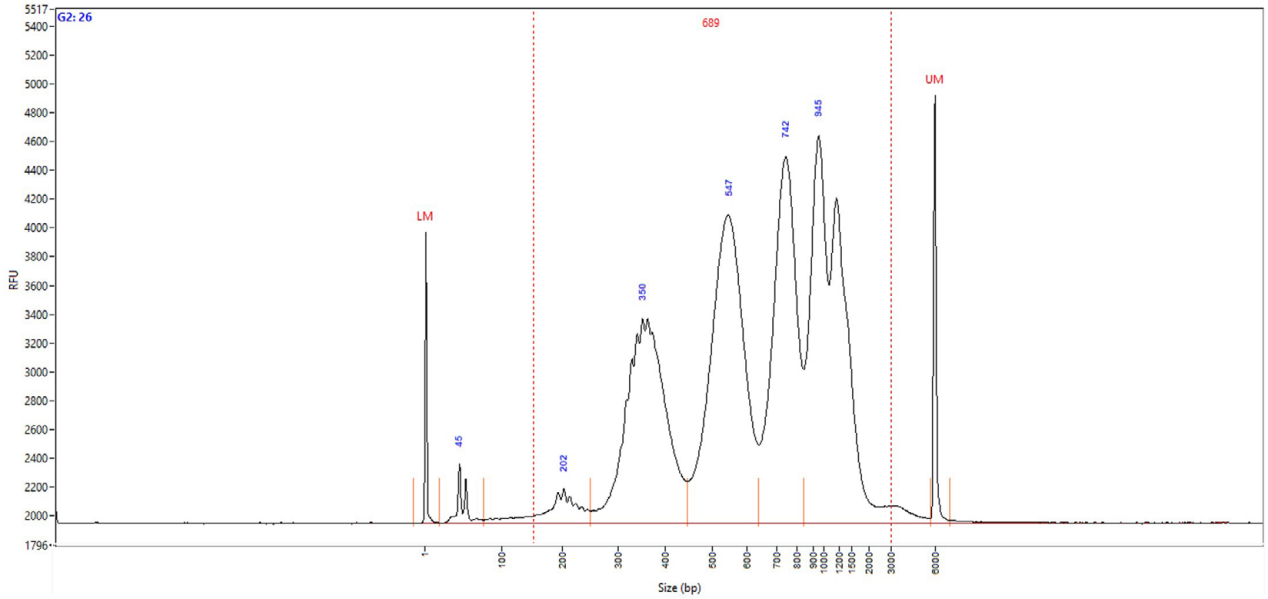
7. Heinz S, Benner C, Spann N, Bertolino E et al. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol Cell 2010 May 28;38(4):576-589.PMID: 20513432

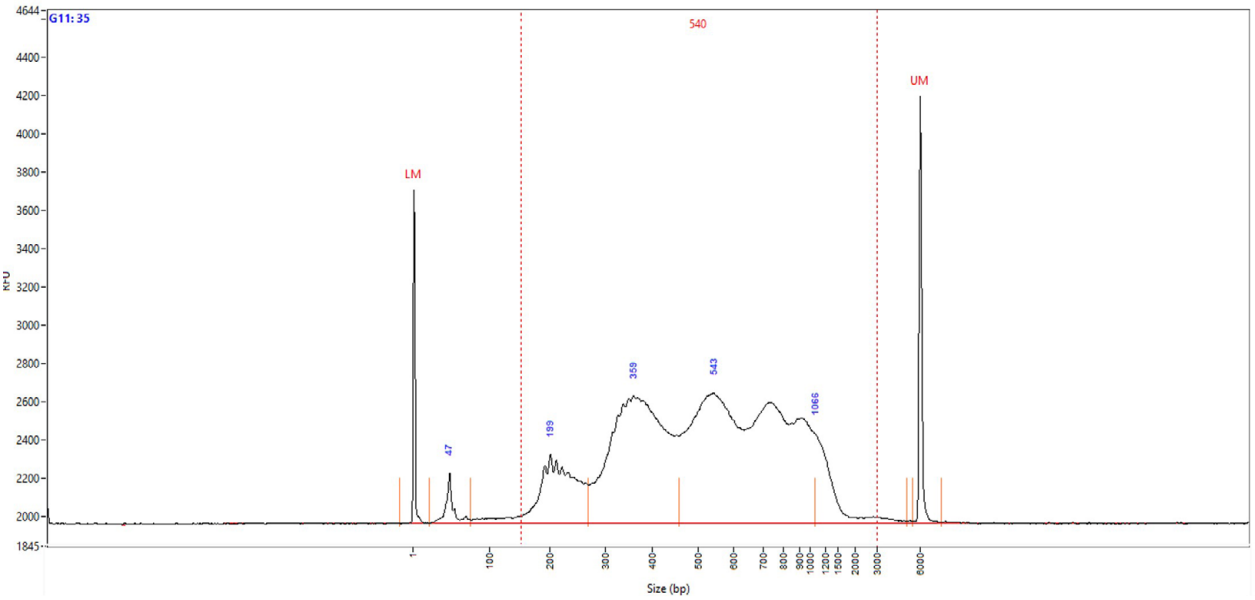
8. Stark R, Brown G (2011). DiffBind: differential binding analysis of ChIP-Seq peak data. http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf.

9. Cavalcante RG, Sartor MA (2017). "annotatr: genomic regions in context." Bioinformatics. R package version 1.20.

Example of Results

Successful Universal CUT&Tag results showing a low background with high region-specific enrichment are presented below. Chromatin profiling was performed on 50,000 or 300,000 K562 cells using Hologic Diagenode's Universal CUT&Tag kit (Cat. No. C01070024), the Antibody Packages (Cat. No. C01070022 and C01070023), the 24 UDI for Tagmented Libraries (Cat. No. C01011034), and H3K27me3 (Cat. No. C15410069), NRF1 (Cat. No. C15200013), and RNA Polymerase II (Pol II) (Cat. No. C15200253) as indicated. The libraries were sequenced on Illumina's NovaSeq X in 2 × 150 bp mode, and the data were analyzed as described in the section "Data Analysis Recommendations."





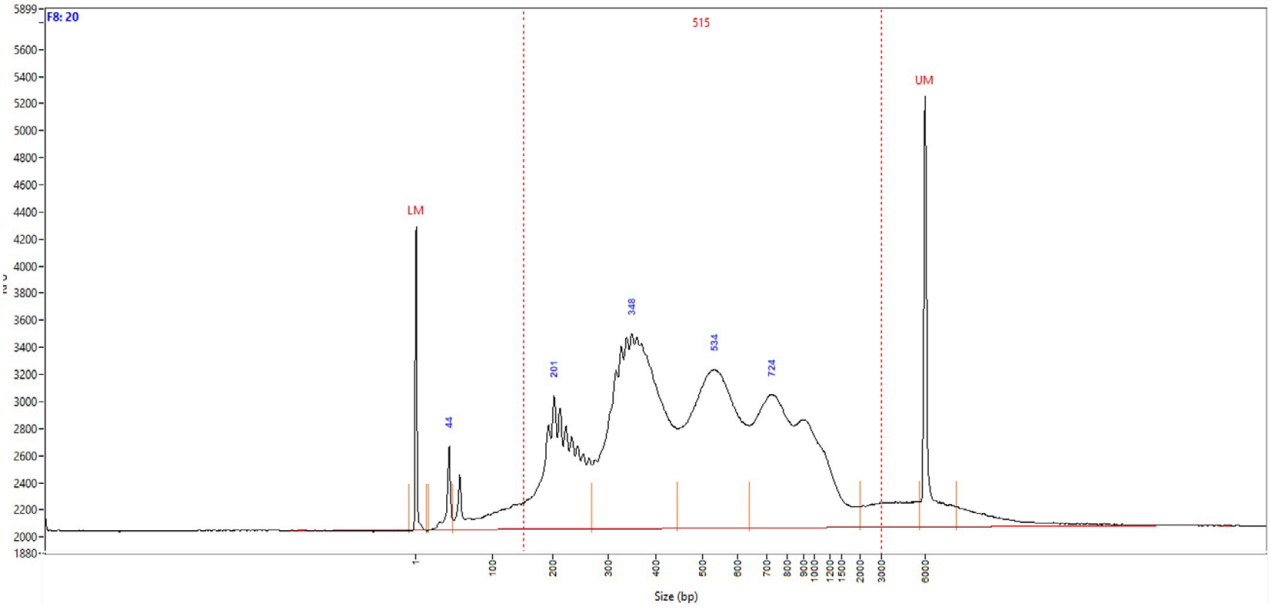


Figure 4: *Typical library profiles (Agilent Fragment Analyzer traces) generated by the Universal CUT&Tag protocol using 50,000 K562 cells: H3K27me3 (top), or 300,000 K562 cells: NRF1 (middle), and RNA Polymerase II (Pol II) (bottom).*

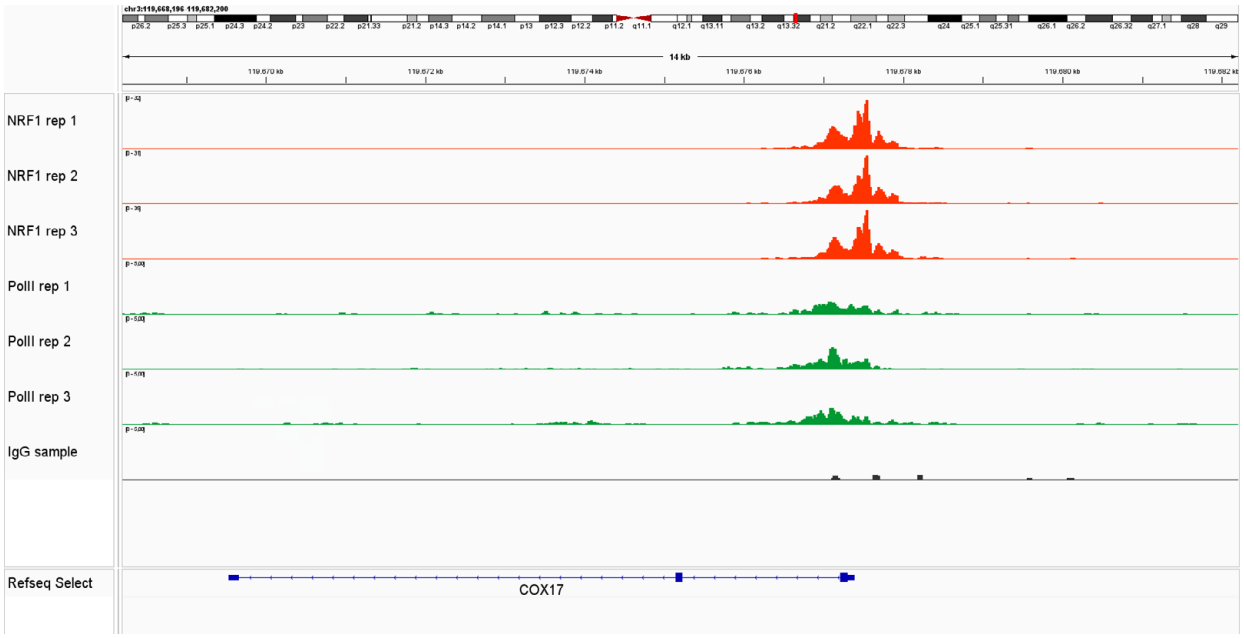


Figure 5: *IGV visualization of CUT&Tag alignment tracks generated from 300,000. K562 cells, showing regions of enrichment (peaks) for NRF1 and RNA Polymerase II (Pol II). Each target is shown with replicates and an IgG control track, which demonstrates low background signal. All target tracks exhibit clear, reproducible peak patterns and strong target specific enrichment over established positive control loci and known target regions, confirming on target specificity and assay sensitivity.*

Related Products

Product	Cat. No.
Universal CUT&Tag kit (8, 24 & 48 rxns)	C01070029 C01070024 C01070028
Antibody Package (anti-rabbit – 8 & 24 rxns)	C01070026 C01070022
Antibody Package (anti-mouse – 8 & 24 rxns)	C01070027 C01070023
pA-Tn5 Transposase - loaded	C01070001
pA-Tn5 Transposase - unloaded	C01070002
8 UDI for tagmented libraries	C01011035
24 UDI for tagmented libraries – Set I	C01011034
24 UDI for tagmented libraries – Set II	C01011036
24 UDI for tagmented libraries – Set III	C01011037
Negative control Mouse IgG	C15400001
Negative control Rabbit IgG	C15410206
Positive control (Rabbit H3K27me3 antibody)	C15410195
Positive control (Mouse H3K27me3 antibody)	C15200181
Antibody to an epitope of interest (CUT&Tag grade antibodies)	www.diagenode.com
ChIP-seq grade primer pairs	www.diagenode.com
DiaMag 0.2ml magnetic rack compatible with 0.2 ml tubes	B04000001
MicroChIP DiaPure columns	C03040001
Protease Inhibitor Mix	C12010011
5% BSA	C03020005
Proteinase K	C06050001
CTCF antibody	C15410210
PolII antibody	C15200004
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Revision history

Version	Date of modification	Description of modifications
Version 1 06_2026	June 2026	Clarification of the content concerning the sample type, the choice of the normalization method, addition of new kit format (8 rxns).
Version 1 01_2026	January 2026	Manual creation

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