diagenode

A Hologic Company

iDeal Cut&Tag Kit for Histones

Rapid Chromatin Profiling Assay

Cat. No. C01070025 (8 rxns)
Cat. No. C01070020 (24 rxns)
Cat. No. C01070021 (48 rxns)

USER GUIDE

Version 1 01_2024

Please read this manual carefully
before starting your experiment

Contents

Introduction

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.

Chromatin immunoprecipitation followed by sequencing (ChIP–seq) has been the standard technique for examining protein–DNA interactions across the whole genome.

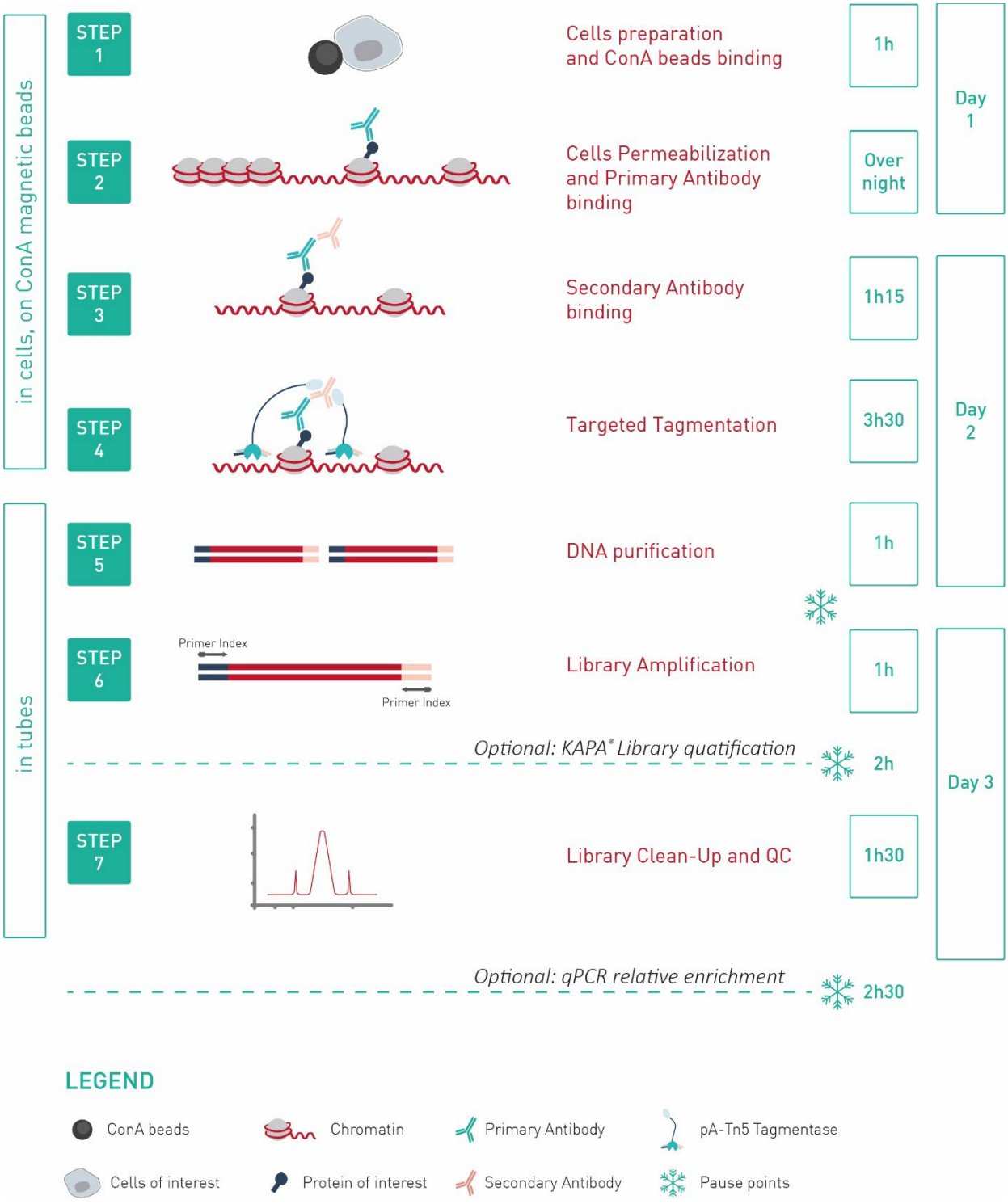
CUT&Tag-sequencing (Cleavage Under Targets and Tagmentation) is a new alternative method to ChIP-seq combining antibody-targeted controlled cleavage by a protein A-Tn5 fusion with massively parallel DNA sequencing to identify the binding sites of DNA-associated proteins.

Key features:

- Rapid and easy chromatin profiling assay for histones marks:
    - Native cells
    - No chromatin preparation (fixation and fragmentation)
    - Easy sample handling due to ConA magnetic beads
    - Integrated library prep
- High resolution and sensitivity
- Low cell number
- Lower sequencing depth

The protocol described in this manual is optimized for chromatin profiling on histone marks. The iDeal CUT&Tag protocol (see Method Overview & Timetable) involves the binding of cells on a solid phase ConA magnetic beads, allowing magnetic handling of the cells for the major steps of the protocol. Bead-bound cells are permeabilized, incubated with primary antibody against a target of interest and secondary antibody. Then, Diagenode's protein pA-Tn5 Transposase - loaded is bound to the complex. Protein A guides Tn5 transposase on chromatin to the antibody attached to its target. Tn5 transposase is activated by Mg+2 ions to insert the sequencing adaptors into genomic regions of interest. DNA is then purified and the tagmented genomic regions of interest are amplified by PCR using Diagenode's Primer Indexes for tagmented libraries. Moreover, Diagenode's iDeal CUT&Tag workflow proposes two optional protocols: intermediate quantification of unpurified library and the evaluation of relative enrichment by qPCR. The intermediate quantification allows the assessment of the library to avoid an overamplification. The qPCR analysis is an additional quality check allowing to determine the enrichment over positive region to ensure the expected performance of the protocol. Finally, the libraries are sequenced and generated reads are mapped to the appropriate reference genome for further analysis.

Kit method overview



Kit Materials

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

1. iDeal CUT&Tag kit for Histones (8 rxns - Cat. No. C01070025, 24 rxns – Cat. No. C01070020 or 48 rxns – Cat. No. C01070021)
2. Antibody Package for CUT&Tag: 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 iDeal CUT&Tag kit for Histones exists in three formats, 8, 24 and 48 rxns. The kit includes the reagents for cells preparation, antibody binding, tagmentation reactions, DNA purification and library amplification as described in Tables 1-3 below. It does not contain secondary antibodies and control antibodies that should be purchased additionally as the Antibody Package for CUT&Tag. The primary antibody of interest should be supplied by customer.

The Antibody Package for CUT&Tag (Table 4 and 5) exists in 2 versions:
- Anti-rabbit – contains species-specific controls 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 controls 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 rxns, depending on the package format. The positive and negative control antibodies are enough for 2 reactions each. Additionally, primers, specific for human DNA, for the evaluation of relative enrichment by qPCR are included.

Components supplied with the iDeal CUT&Tag Kit for Histones

NOTE: *Upon receipt, store the components at the indicated temperature*

Table 1. Components stored at +4°C.

Component	Cap color	Volume– 8 rxns Cat. No. C01070025	Volume – 24 rxns Cat. No. C01070020	Volume – 48 rxns Cat. No. C01070021	Storage
CT Binding Buffer		2 ml	6 ml	12 ml	+4°C
CT Wash Buffer 1		2.8 ml	8.4 ml	16.8 ml	+4°C
CT Antibody Buffer		400 µl	1.2 ml	2.4 ml	+4°C
5X CT Wash Buffer 2		4 ml	12 ml	24 ml	+4°C
5X CT Wash Buffer 3		4 ml	12 ml	24 ml	+4°C
ConA beads	yellow	80 µl	240 µl	480 µl	+4°C
CT Buffer E	white	80 µl	240 µl	480 µl	+4°C
CT Buffer S	red	24 µl	72 µl	144 µl	+4°C

Table 2. Components stored at -20°C.

Component	Cap color	Volume– 8 rxns Cat. No. C01070025	Volume – 24 rxns Cat. No. C01070020	Volume – 48 rxns Cat. No. C01070021	Storage
CT Tagmentation Buffer		2.4 ml	2x 3.6 ml	4x 3.6 ml	-20°C
Spermidine 50X		912 µl	2.75 ml	5.5 ml	-20°C
Protease inhibitor Mix 200X	red	228 µl	700 µl	1.38 ml	-20°C
pA-Tn5 Transposase loaded *	red	3.2 µl	15 µl	30 µl	-20°C
2x High-Fidelity Mastermix	violet	200 µl	600 µl	1.2 ml	-20°C
Proteinase K	clear	20 µl	60 µl	120 µl	-20°C
5% BSA	black	8 µl	24 µl	48 µl	-20°C

** Including excess volume for optimizations if necessary.*

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

Component	Cap color	Volume – 8 rxns Cat. No. C01070025	Volume – 24 rxns Cat. No. C01070020	Volume – 48 rxns Cat. No. C01070021	Storage
ChIP DNA Binding Buffer		12 ml	36 ml	2x 36 ml	RT
DNA Wash Buffer *		700 µl	2.2 ml	4.4 ml	RT
DNA Elution Buffer	white	200 µl	600 µl	1.2 ml	RT
Spin columns		8	24	48	RT
Collection Tubes (2 ml)		8	24	48	RT

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

iDeal CUT&Tag kit for Histones (8 rxns – Cat. No. C01070025): add 2.8 ml of 100% ethanol to 700 µl of DNA Wash Buffer.

iDeal CUT&Tag kit for Histones (24 rxns – Cat. No. C01070020): add 8.8 ml of 100% ethanol to 2.2 ml of DNA Wash Buffer.

iDeal CUT&Tag kit for Histones (48 rxns – Cat. No. C01070021): add 17.6 ml of 100% ethanol to 4.4 ml of DNA Wash Buffer.

Components supplied with the Antibody Packages for CUT&Tag (not included in the kit iDeal CUT&Tag)

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

Table 4. Components included in the Antibody Packages for CUT&Tag – anti-rabbit

Antibody Package for CUT&Tag 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

Table 5. Components included in the Antibody Packages for CUT&Tag – anti-mouse

Antibody Package for CUT&Tag anti-mouse	**Volume – 24 rxns Cat. No. C01070020**	**Volume – 48 rxns Cat. No. C01070021**	**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

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

- Rat GAPDH promoter +0.3 kb primer pair (Diagenode Cat. No. C17031046-50)
- Rat TSH2B coding region primer pair (Diagenode Cat. No. C17031043-50)
- Mouse GAPDH promoter primer pair (Diagenode Cat. No. C17021045-50)
- Mouse TSH2B coding region primer pair (Diagenode Cat. No. C17021042-50)

Required Materials Not Provided

Equipment

- Gloves to wear at all steps
- Cell culture standard consumables
- Cell counter system
- Ice
- 15 and 50 ml tubes
- RNase/DNase-free 1.5 ml and 2.0 ml tubes
- 0.2 ml PCR Tubes, PCR-clean
- 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
- Diagenode magnetic rack for 0.2 ml tubes, DiaMag02 (Cat. No. B04000001)
- Rotating wheel
- Agilent Bioanalyzer 2100 or Fragment Analyzer (Agilent) or equivalent
- Qubit® Fluorometer (Thermo Fisher Scientific) or equivalent
- qPCR instrument and associated tube strips/plates

Reagents

- Cell line of interest
- Cell medium
- Sterile Trypsin-EDTA (for adherent cells)
- Phosphate-buffered saline (PBS) buffer
- Ultra-pure DNase/RNase-free Distilled water
- 100% Ethanol, molecular grade
- 80% Ethanol, molecular grade
- 10 mM Tris-HCl, pH 8.0
- Antibody to an epitope of interest (CUT&Tag or ChIP-seq grade antibodies – www.diagenode.com)
- AMPure XP beads (Beckman Coulter, Inc #A63881)
- Agilent High Sensitivity DNA Kit (Agilent Technologies) for Bioanalyzer or Fragment analyzer
- Primer indexes for tagmented libraries kit (Diagenode)
    - 8 SI for tagmented libraries (Cat. No. C01011033)
    - or 24 SI for tagmented libraries (Cat. No. C01011032)
    - or 8 UDI for tagmented libraries (Cat. No. C01011035)
    - or 24 UDI for tagmented libraries – Set I (Cat. No. C01011034)

- or 24 UDI for tagmented libraries – Set II (Cat. No. C01011036) or 24 UDI for tagmented libraries - Set III (Cat. No. C01011037)

Additional supplies for optional protocols

- For KAPA quantification
  - 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)
- For qPCR relative enrichment
  - qPCR SYBR® Green Mastermix
  - Primer pairs for epitope of interest (5 µM of each) designed and supplied by customer
- For fixation (required only for non-histones targets):
  - 37% formaldehyde, Molecular Grade
  - 1,25 M glycine solution

Remarks before starting

1. General recommendations

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

This protocol has been optimized for **histone profiling**. However, some **transcription factors** and **co-factors** might be successfully studied upon a mild cells fixation prior cell binding to conA beads (Step 1, Optional protocol).

2. Starting material

This protocol is compatible with **10,000-300,000 cells** per reaction with a typical input of 50,000 cells. We recommend treating a maximum of 12 reactions at one time to limit handling time. We recommend working at least in duplicates for your experimental samples.
Depending on your experimental design, two options are possible for cell preparation and conA binding:

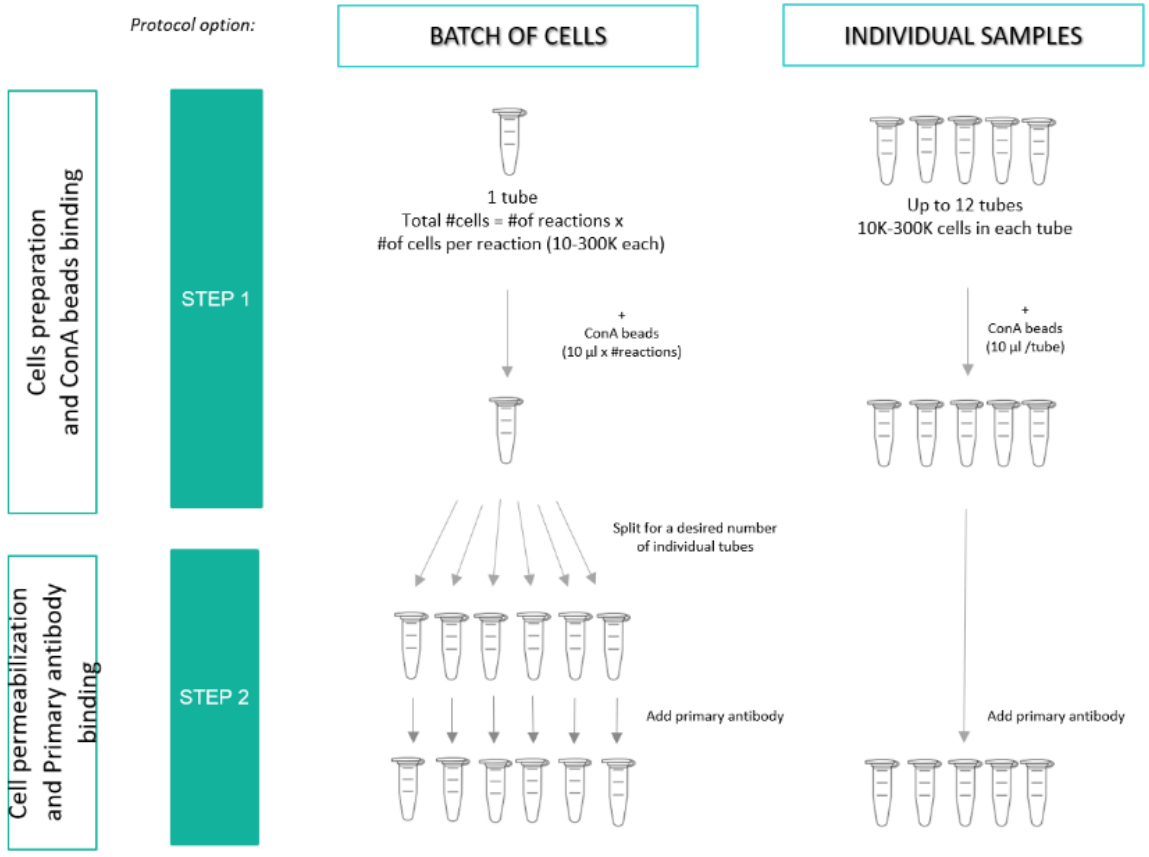


Figure 1 : Schema showing 2 options for cells preparation: batch vs individual samples.

- Starting with a **batch** of cells containing enough material per planned experiment. This batch is determined by the number of reactions to be run (including the negative and positive controls) and the amount of cells used per one reaction (10,000-300,000 range). Calculate a total number of cells required per batch (e.g., prepare 100,000 cells for 10 reactions of 10,000 cells for cells preparation and cells binding to ConA beads). The cells bound to ConA beads can be further 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 a desired number of cells per one reaction (e.g., prepare 10 tubes of 10,000 cells each). Each tube (sample) will be processed separately during all steps of the protocol.

We recommend using **fresh cells**, but it is possible to cryopreserve cells and use them later for the assay, following these guidelines:

- Check that viability is higher than 90% before freezing
- Freeze the cells at a concentration of 1 million cells/ml
- Freeze cells with the standard reagents and procedure adapted to the cell type

3. Complete CT Buffer preparation

CT buffers provided in the kit must be supplemented with Protease Inhibitors Mix (200x), Spermidine (50x) and 5% BSA prior to use accordingly to the tables in Buffers Preparation section. Determine the volume of Complete CT Buffers required per one experiment and prepare Complete CT Buffers. Complete CT buffers are stable only for a short period of time. Keep the Complete CT buffers at +4°C until use. Discard what is not used within day 1 and 2 of the protocol.

4. 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 accordingly, wash the beads and resuspend them in the required amount of CT Binding Buffer depending on the number of reactions you want to process.
Make sure the beads do not dry out during the procedure as this will result in reduced performance. Always keep the beads homogenous in suspension when pipetting. Variation in the volume of beads will decrease reproducibility. Do not freeze the beads.

5. Antibodies

The quality of antibody used in iDeal CUT&Tag is one of the crucial factors for assay success. It is recommended using only validated antibodies that specifically recognize the target. Diagenode offers extensively validated and high-performance CUT&Tag grade antibodies. Each batch is validated, and batch-specific data are available on the website www.diagenode.com.
The negative (IgG) and a positive (H3K27me3) control antibody to monitor the efficiency of your assay are available in the Antibody Package for CUT&Tag. We recommend using these controls at least once per experiment. Please check FAQs section for specificity of each control.
The required amount of antibody of interests (provided by customer) may vary depending on its performance. For optimal results, please check the recommendations in the technical datasheet or perform a titration experiment.

6. Tagmentation

We recommend using 1/250 dilution of pA-Tn5 Transposase included in the kit (as described in the Step 4). This condition works for varieties of cell types and targets, but an additional optimization (from 1/100 down to 1/500 dilution) might be required depending on cell number, cell type or antibodies used.

7. Library amplification

The PCR amplification should yield libraries with a sufficient concentration for sequencing. The requirements for a final library concentration depend on the used sequencer 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 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 an over-amplification to reduce amplification biases. The number of PCR cycles should be optimized depending on the cell number and the target abundance. **The total number of PCR cycles should not exceed 16.**

For each new project, we strongly recommend amplifying libraries for a limited number of cycles (usually 8-10 PCR cycles) and performing an intermediate quantification of unpurified libraries using KAPA® Library Quantification Kit. This step allows estimating the library yield and determining if additional re-amplification is required to produce a sufficient amount of libraries while avoiding over-amplification. For established experiments, the intermediate quantification might be skipped by amplifying samples for required number of cycles directly.

For intermediate quantification, use a KAPA Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems) compatible with your qPCR instrument. Please read the complete manual before first time use and follow the recommendation of the manufacturer. 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 a range of 5-20 nM. If it is not the case, additional PCR cycles should be added to re-amplify the libraries until required yield.

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 examples, 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 that.
Example:
KAPA quantification returns 2 nM library concentration which is less than required per sequencing. We need to reach approximately 10 nM final concentration so the sample should be re-amplified for 3-4 additional cycles with an expected yield between 8 and 16 nM respectively: 2nM → 4nM → 8nM → 16 nM.

8. Primer indexes

Diagenode's primer indexes for tagmented libraries, compatible with the iDeal CUT&Tag Kit, are available in several formats, allowing a choice between single or unique dual-indexing and several numbers of different indexes. For more details about the differences between the kits and pooling guidelines for the indexes, please check the primer index for tagmented libraries manual: https://www.diagenode.com/en/documents/primer-indexes-for-tagmented-libraries_manual

9. 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 (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 at around 40-50 bp do not require an additional purification. This peak corresponds to a free oligonucleotide used for pA-Tn5 loading. This oligonucleotide does not contain a full adaptor sequence and will not cluster on Illumina flow cell.

10. Quantification of libraries

Determine the concentration of the libraries that should be between 5-20 nM in a final volume 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 sheared or fragmented starting gDNA inserts used for library construction.

The Bioanalyzer system or Fragment Analyzer (Agilent) provide sizing and quantification information about the library analysed, but not about the clustering competency.

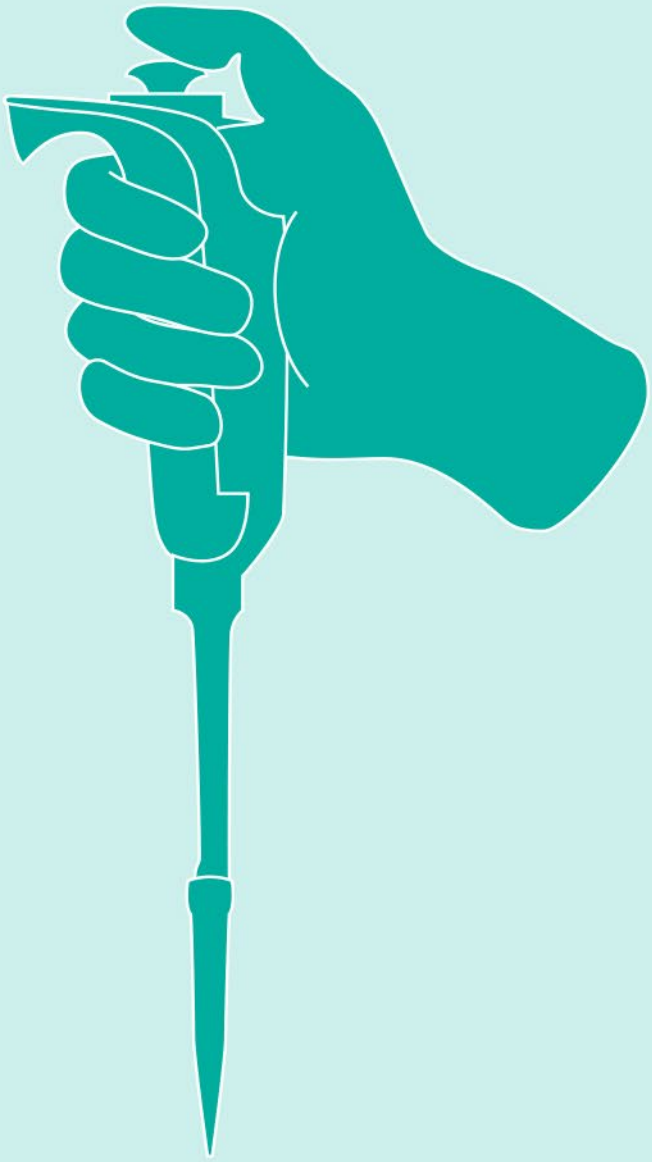
UV absorption/fluorescence detection-based methods (i.e., Nanodrop® (Thermo Scientific), Qubit®2.0 Fluorometer (Life Technologies), or Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) simply quantify total nucleic acid concentration. These methods do not discriminate adapters presence and offer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

11. Relative enrichment evaluation by qPCR (Optional Protocol B)

It is possible to analyze the relative enrichment of targeted genomic regions prior to the sequencing to ensure that an expected enrichment is observed. For control libraries (IgG and H3K27me3), use primer pairs included in the Antibody package for CUT&Tag. TSH2B promoter is an expected positive region for H3K27me3 while GAPDH TSS is negative locus. Each specific antibody will require specific control primers designed by the user. Keep in mind that the relative enrichment is not normalized and might be not fully accurate.

To have sufficient DNA left for sequencing, we recommend not using 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 be able to identify potential outliers.

PROTOCOL



BUFFERS PREPARATION

All volumes of Complete CT buffers are given for one sample (an excess included). Scale up accordingly to the number of reactions. Once freshly prepared, the Complete CT buffers must be kept at +4°C until use. Prepare the Complete CT buffers required per one set of experiment and discard leftovers of the day.

Complete CT Wash Buffer 1: Prepare **350 µl per sample** by mixing:

Reagents	Volume for 1 sample
CT Wash Buffer 1	350 µl
Protease Inhibitor Mix 200x	1.75 µl
Spermidine 50X	7 µl

Complete CT Antibody Buffer: Prepare **50 µl per sample** by mixing:

Reagents	Volume for 1 sample
CT Antibody Buffer	50 µl
Protease Inhibitor Mix 200x	0.25 µl
Spermidine 50X	1 µl
5% BSA	1 µl

Complete CT Wash Buffer 2: Prepare **2500 µl per sample** by mixing:

Reagents	Volume for 1 sample
5X CT Wash Buffer 2	500 µl
Protease Inhibitor Mix 200x	12.5 µl
Spermidine 50X	50 µl
Water	1937.5 µl

Complete CT Wash Buffer 3: Prepare **2500 µl per sample** by mixing:

Reagents	Volume for 1 sample
5X CT Wash Buffer 3	500 µl
Protease Inhibitor Mix 200x	12.5 µl
Spermidine 50X	50 µl
Water	1937.5 µl

Complete CT Tagmentation Buffer: Prepare **300 µl per sample** by mixing:

Reagents	Volume for 1 sample
CT Tagmentation Buffer	300 µl
Protease Inhibitor Mix 200x	1.5 µl
Spermidine 50X	6 µl

STEP 1

Cell preparation and ConA beads binding

Day 1 1h

ConA beads preparation

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

1.2 Add **the required volume of CT Binding Buffer.** 90 µl of buffer is used per every 10 µl of conA beads (one sample). Scale 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 beads pellet.

1.4 Repeat the steps 1.2 and 1.3 once.

1.5 Remove the tube from the magnetic rack, resuspend the beads in **CT Binding Buffer** adding the original volume of beads (this means 10 µl per reaction x number of reactions). Hold at room temperature until the cells are ready (Step: Cells Collection) and then, proceed directly to cell binding step.

Cell collection

The cell collection and ConA-binding can be performed as a batch or as individual samples. The batch of ConA bound cells will be split into a desired number of individual reactions starting from Step 2: Permeabilization and Primary antibody binding. Individual samples are procced separately through the whole protocol.

For adherent cells:

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

1.7 Collect a required number of cells 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 cells surface and binding capacity on ConA beads. Regularly check if the cells start to detach. If necessary, use an enzyme-free dissociation method instead, such as scraping or rubber policeman.*

1.9 Immediately add **pre-warmed culture medium** to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 15 ml tube.

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

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

For suspension cells:

- Collect the required number of cells as a batch or as individual samples. Use 15 ml tube per batch preparation or 2 ml tube per individual samples.
- Centrifuge for 10 minutes at 500 x g (at room temperature) and carefully remove the supernatant without disturbing the cells pellet. Go directly to point 1.12 of the protocol.

For Cryopreserved cells:

- Thaw the desired number of cells per batch or per individual samples for 2 minutes at 37°C water bath.
- Transfer them to a 15 ml tube per batch preparation or 2 ml tube per individual samples and go directly to step 1.13.

1.12 Resuspend the cells in 1-10 ml of **warm PBS**. Use 10 ml of PBS for batch preparation or 1 ml per individual samples. Take an aliquot of 10 µl, add 10 µl of Trypan blue and count the viable cells.

1.13 Transfer the desired number of cells per batch or individual samples in a 1.5 ml tube, centrifuge for 3 minutes at 600 x g (at room temperature) and carefully remove the supernatant without disturbing the cells pellet. Proceed directly to cell binding.

Optional, required for profiling of non-histones proteins

- *Prepare 0,1 % of formaldehyde solution: add 1,4 µl of 37% fresh formaldehyde to 0,5 ml PBS at RT. Work under the chemical hood.*
- *Resuspend cells in formaldehyde solution and gently rotate for 5 min at RT.*
- *Quench the fixation by adding 50 µl of 1.25 M Glycine, mix by gentle pipetting and rotate for 5 min at RT.*
- *Centrifuge at 600 x g for 5 min at RT and gently remove the supernatant.*
- *Resuspend cells in 250 µl of PBS. Centrifuge at 600 x g for 5 min at RT, remove the supernatant and proceed with cell binding as described below.*

Cell Binding

1.14 Resuspend the cell pellet in **250 µl of Complete CT Wash Buffer 1 (batch or individual samples)** at room temperature.

NOTE: *Always use the 250 µl whatever is the cell amount.* ***Do not scale*** *up/down accordingly to the cells amount.*

1.15 Centrifuge for 3 minutes at 600 x g at room temperature and carefully remove the supernatant without disturbing the cell pellet.

1.16 Resuspend the cells in **Complete CT Wash Buffer 1** using 100 µl per reaction. For batch, scale accordingly to the number of reactions (e.g., 1200 µl of Complete CT Wash Buffer 1 for 1 batch of 12 reactions). Mix thoroughly by pipetting up and down several times.

1.17 Add washed ConA beads (from step 1.5) to the cell suspension (per batch or per individual samples). Mix gently by pipetting up and down several times.

1.18 Place on rotating wheel for 8 minutes.

1.19 Briefly spin the tube to remove the liquid from 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.

STEP 2

Cell permeabilization and primary antibody binding

1 Day 1 ⏳ overnight

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

2.2 Distribute **50 µl** of the mix into 1.5 ml **individual tubes** (one tube = one reaction of desired quantity of cells).

NOTE: *From now on, all the reaction tubes are treated individually and not anymore processed by batch.*

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

NOTE: *The required amount of antibody per reaction varies and we recommend following the recommendation of manufacturers. Use 1 µg of positive (H3K27me3) and negative (IgG) controls included in the Antibody Package for CUT&Tag.*

2.4 Place the tubes on a rotating wheel at +4°C and incubate overnight.

STEP 3

Secondary antibody binding

2 Day 2 ⌛ 1h15

NOTE: *Chose the secondary antibody accordingly to the host species of primary antibody. The secondary antibody is included in the Antibody Package for CUT&Tag (anti-rabbit and anti-mouse) available separately.*

3.1 Prepare 100 µl of diluted secondary antibody per reaction (1/100 dilution) by mixing **1 µl of the secondary antibody and 99 µl of Complete CT Wash Buffer 2.** Scale according to the number of reactions. Mix gently by pipetting up and down several times and hold on ice until use.

3.2 Remove the tubes containing the cells and primary antibody (from step 2.4) from the rotating wheel.

3.3 Briefly spin the tubes to remove the liquid from cap. Place the tubes 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 tubes 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 Place the tubes on a rotating wheel at room temperature for 45 minutes.

3.6 Briefly spin the tubes to remove the liquid from cap. Place the tubes on a magnetic rack and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

3.7 Wash the pellet three times as follows:

- Remove the tubes from the magnetic rack and add **800 µl of Complete CT Wash Buffer 2** per reaction. Mix gently by pipetting up and down several times.
- Place the tubes on a magnetic rack and wait 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

Targeted tagmentation
(pA-Tn5 binding – activation – inactivation)

 Day 2  3h30

4.1 Prepare a 1:250 dilution of pA-Tn5 transposase loaded by mixing **1 µl of pA-Tn5 Transposase loaded** and **249 µl of Complete CT Wash Buffer 3**. 100 µl of diluted pA-Tn5 Transposase loaded is needed per reaction, scale according to the number of reactions. Mix gently by pipetting up and down several times and hold on ice until use.

4.2 Remove the tubes from the magnetic rack step 3.8 and add **100 µl of 1/250 dilution of pA-Tn5 Transposase loaded** per reaction. Mix gently by pipetting up and down several times.

4.3 Place the tubes on a rotating wheel at room temperature for 1 hour.

4.4 Briefly spin the tubes to remove the liquid from cap. Place the tubes on a magnetic rack and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

4.5 Wash the pellet three times as follows:

- Remove the tube from the magnetic rack and add **800 µl of Complete CT Wash Buffer 3** per reaction. Mix gently by pipetting up and down several times.
- Place the tubes on a magnetic rack and wait 2 minutes until the solution is clear. Carefully aspirate and discard the supernatant without disturbing the pellet.

4.6 Briefly spin the tubes on a table top centrifuge. Place the tubes on a magnetic rack and remove the remaining liquid without disturbing the pellet.

4.7 Add **300 µl of Complete CT Tagmentation Buffer** per reaction. Mix gently by pipetting up and down several times.

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

4.9 Remove the tubes from the thermomixer and briefly spin the tubes to remove the liquid from cap.

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

4.11 Vortex the tubes and incubate in a thermomixer at 55°C for 1 hour, set at 800 rpm.

4.12 Proceed directly to step 5.

STEP 5

DNA purification

Day 2 1h

*NOTE: Before the first use of the iDeal 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 Briefly spin the tubes from the step 4.12 to remove the liquid from cap. Place the tubes on a magnetic rack and wait 2 minutes until the solution is clear.

5.2 Carefully **transfer the supernatant** to a clean 2 ml tube (1 tube per reaction) without disturbing the pellet.

5.3 Add **1.5 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 mixture to a provided Spin column in a 2 ml Collection tube. Centrifuge at 10,000 x g for 30 seconds and discard the flow-through. Repeat this step for a total of three times to load the entire reaction volume to the column.

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 for a total of two times.

5.6 Transfer the column to a new 1.5 ml microcentrifuge tube and add **25 µl of DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

5.7 Store at 4°C until PCR or directly proceed with the next step: Library amplification.

NOTE: We recommend performing an intermediate quantification of your library for every new project to determine the optimal number of PCR cycles to avoid an over-amplification.

STEP 6

Library Amplification

Day 3 1h

NOTE: *The Primer indexes for tagmented libraries kit (single indexes or UDIs) are required to be used in this section. The Primer indexes must be purchased separately (see Required Materials Not Provided). Please refer to corresponding manuals for a guidance on index pooling. Use different indexes for samples that you want to sequence in the same lane.*

6.1 Transfer **21 µl of purified DNA** from step 5.7 in a 0.2 ml PCR tube and add **4 µl of Primer indexes pair for tagmented libraries** to each sample.

NOTE: *For low cells number (10,000) per reaction, use 1 µl of Primer indexes pair for tagmented libraries to each sample and 3 µl of nuclease-free water.*

6.2 Add **25 µl of 2x High Fidelity Mastermix** to each sample and mix gently by pipetting up and down several times.

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	8-16 * Amplification Cycles
Annealing/Extension	63°C	10 seconds	
Final extension	72°C	1 minute	
Hold	4°C	∞	

* *Check the before starting notes to determine the required number of cycles.*

6.4 Perform intermediate library quantification if needed (follow the protocol in Additional protocols section) or go 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 KAPA® Library Quantification Kit (see Optional Protocol A) and if necessary, additional PCR cycles can be added to re-amplify the libraries before purification*.

STEP 7

Library Clean-up and QC

Day 3 1h30

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

7.2 Add **45 µl of AMPure XP beads** to the **50 µl of unpurified library DNA samples**. Mix thoroughly by pipetting up and down several times or by vortexing a few seconds to resuspend the beads.

7.3 Incubate at room temperature for 10 minutes.

7.4 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 beads pellet.

7.5 Leave the tube on the magnetic rack and wash the pellet two times as follows:

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

7.6 Spin down the tubes in a table top centrifuge, place the tube back on the magnetic rack and remove remaining ethanol.

7.7 Leave the tube open for 2-3 minutes to let the beads 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 it from the magnetic rack.

7.8 Add **20-25 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 tube on a magnetic rack and wait 2 minutes until the solution is clear.

7.11 Carefully transfer the supernatant in a fresh tube, without disturbing the beads pellet.

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

Library Quality Check

NOTE: *After library preparation, determine the concentration of the libraries (see "Remarks before starting" section). The libraries yield will depend on different factors such as cell type, quality of the antibody used and antibody target. If necessary, dilute the libraries before performing the quality check according to manufacturer's instruction*

7.12 Run **1 µl** of each purified library on a High Sensitivity DNA chip for BioAnalyzer (Agilent) **or 2 µl on Fragment Analyzer (Agilent) 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 yielding about 5-20 nM with no presence of adapter dimers. If adapter dimers (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.*

7.14 Your libraries are now ready for pooling and sequencing. Prepare equimolar pool of each library. The library fragment sizes should be similar.

NOTE: *Individual libraries, quantified and purified according to the above protocol, can be pooled at desired molar ratios to allow multiplex sequencing. Libraries that are being pooled, must have been prepared with different indexes (for multiplexing and index pooling guidelines refer to the manual: Primer indexes for tagmented libraries). The minimal molar concentration needed to sequence the pool depends on the requirements of the sequencing platform. The total molarity is the sum of all individual libraries 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.*

$$\textit{Volume of individual library in the pool} = V(f) \times C(f) / \# \times C(i)$$

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 one pool depends on the sequencer capability.

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

Optional protocol A

Intermediate Quantification of unpurified library using KAPA® Library Quantification Kit – Illumina

⏳ 2h

NOTE: *We recommend choosing a KAPA Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems) compatible with your qPCR instrument. Please, read the complete manual before first time use and follow the recommendation* of the manufacturer. *We recommend using at least duplicates for samples and for standards quantification.*

1.1 Prepare **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 1M Tris-HCl, pH 8.0 and 23 µl of Tween 20 and increase the volume to 50 ml with Ultra-pure DNase/RNase-free water.*

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

1.3 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**

1.4 Pipet **16 µl of the KAPA Master Mix** into qPCR plate wells.

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

1.6 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 KAPA® Library Quantification Kit for more information.*

1.7 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

1.8 Calculate the library DNA concentration using the standard curve. Please refer to KAPA® Library Quantification Kit 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.*

1.9 If necessary, repeat PCR cycles to under-amplified samples for the required number of 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*

Optional protocol B

Relative enrichment evaluation by qPCR

⌛ 2h30

NOTE: *For each sample (samples of interest, negative IgG or positive H3K27me3 samples) run a positive locus primer pair (positively enriched) alongside a negative locus primer pair (not enriched). Pay attention that ChIP-seq grade TSH2B and GAPDH TSS primer pairs included in the Antibody Package for CUT&Tag are positive and negative primers for H3K27me3 control antibody and are only compatible with human samples. For samples of interest, the primers should be designed by customer.*

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

1.1 Take an aliquot of purified library DNA sample (from step 10.11) and **dilute it 1/10** using Ultra-pure DNase/RNase-free water. Do the same for negative and positive control samples.

1.2 Prepare the qPCR mix as follow:

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 qPCR instrument manufacturer recommendations		

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

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

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

$$\boldsymbol{delta\ Ct = Ct_{negative\ locus} - Ct_{positive\ locus}}$$

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

Table 5: Example of Ct values and relative enrichment of duplicate samples for positive (H3K27me), 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 negative locus**	**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 used control locus regions, so the relative enrichment can be quite different when switching to other primer pairs. Moreover, it is also dependent cells number and types but also on dilution of samples before the qPCR and abundance of the target. The relative enrichment value < 3 should be considered as background.

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

Sequencing Recommendations

We recommend paired-end sequencing in 50 bp or 100 bp mode targeting 15-20 M reads pairs per sample for high abundant histone marks and between 20-30M reads pairs for low abundant histone marks. Less deep sequencing leads to the loss of peaks. The PhiX % should be applied as recommended by the sequencer (usually 5%).

Following the use of single or unique dual indexes, the construct will bear one or two separated indexes. The "i5" index can be read in a reverse complement workflow or in a forward strand way depending on the sequencer. For more details about the adapter sequences of the indexes, please check the Primer index for tagmented libraries manual:
https://www.diagenode.com/en/documents/primer-indexes-for-tagmented-libraries_manual

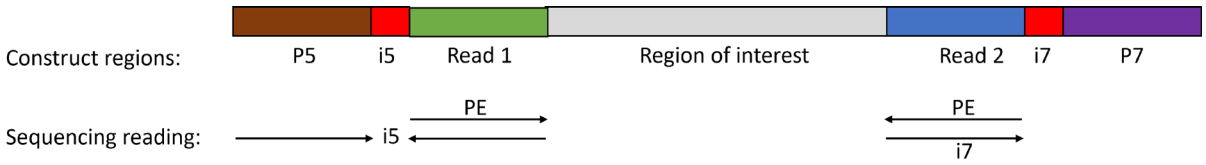


Figure 2. iDeal CUT&Tag library construct. PE (paired-end sequencing), i5/i7 (SI or UDI indexes).

Data Analysis Recommendations

In the following section we will guide you through the basics of iDeal CUT&Tag data analysis. We will also recommend some software tools suitable for each step.

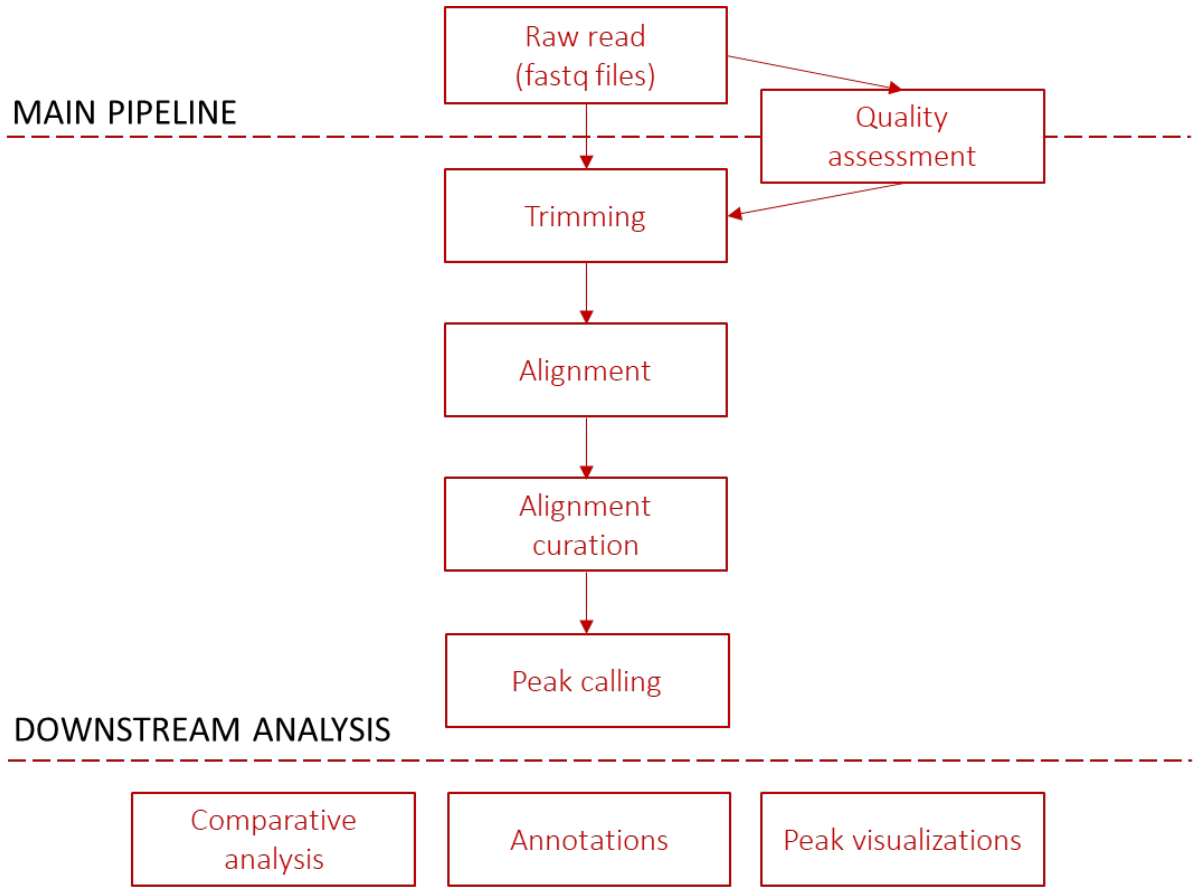


Figure 3. iDeal CUT&Tag data analysis workflow. General view of the data analysis recommendations.

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 adaptor contamination and of low-quality bases is performed with cutadapt [2]

3. **Alignment**: The trimmed reads are subsequently aligned on an indexed reference genome using Burrows-Wheeler Aligner (BWA) [3]. References genomes can be obtained through UCSC (https://genome.ucsc.edu/) or NCBI (https://www.ncbi.nlm.nih.gov/genome/).

4. **Alignment Curation**: Alignment curation involves removing reads mapped in regions classified as blacklisted by ENCODE [4] and removing multi-mapping reads, ie reads mapped to multiple

locations with the same mapping score on the reference genome, using samtools [5]. Note that due to the iDeal CUT&Tag technology and the use of Tn5, PCR duplicates are not removed from the alignment files as many fragments will have the same mapping genomic coordinates without necessarily being PCR duplicates.

5. **Peak calling**: Peak calling is performed to detect sites of enrichment (read accumulation) along the genome. Special care should be taken to ensure using proper parameters to call the peaks. (In iDeal CUT&Tag, peak calling is performed in absence of inputs, and care should be taken to choose an appropriate peak caller.)

DOWNSTREAM ANALYSIS

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

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

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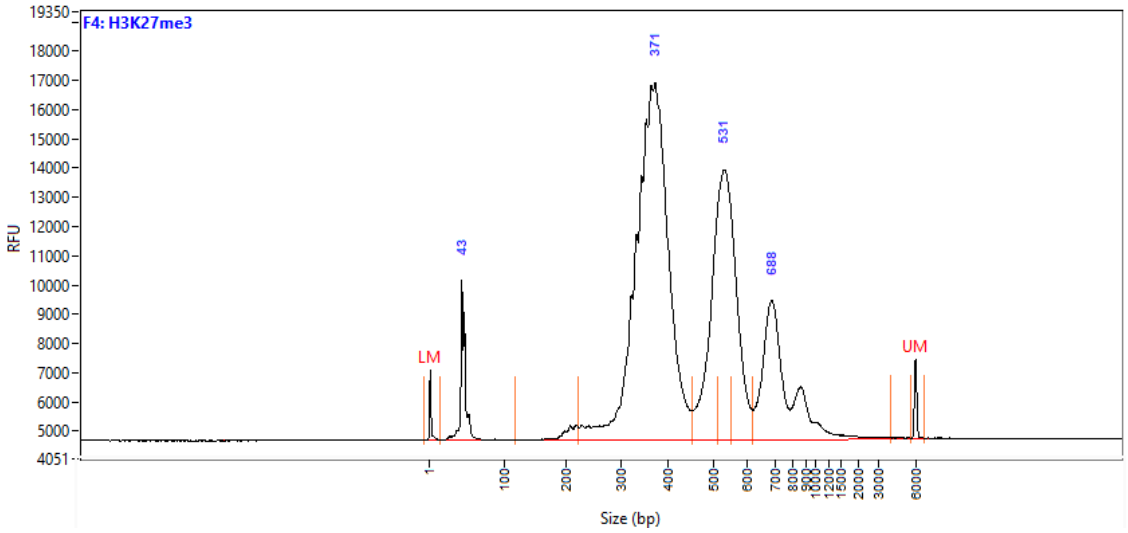
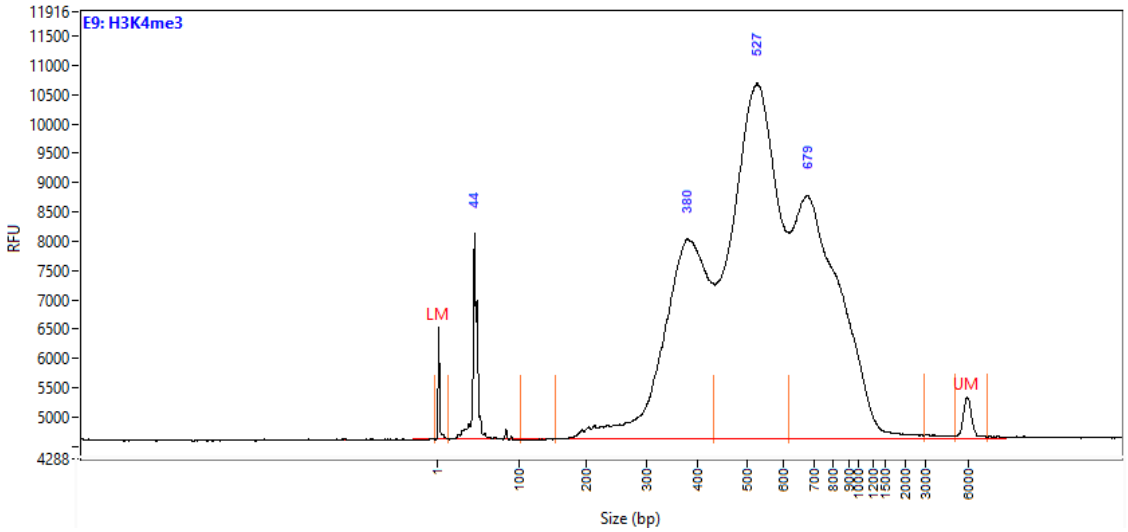
[8] Stark R, Brown G (2011). DiffBind: differential binding analysis of ChIP-Seq peak data. http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf.

[9] Cavalcante RG, Sartor MA (2017). "annotatr: genomic regions in context." Bioinformatics. R package version 1.20.0.

Example of Results

Successful CUT&Tag results showing a low background with high region-specific enrichment are presented below. Chromatin profiling has been performed on 50,000 K562 cells, using the Diagenode's iDeal CUT&Tag kit for Histones (Cat. No. C01070020), the Antibody Package for CUT&Tag anti rabbit (Cat. No. C01070022), the 24 UDI for Tagmented Libraries (Cat.No. C01011034) and H3K4me3 (Cat. No. C15410003), H3K27me3 (Cat. No. C15410069) or H3H9me3 antibodies (Cat. No., C15410193) as indicated. The libraries were sequenced on Illumina's NovaSeq6000 in 2x50 bp mode, and the data analyzed as described in the section: Data analysis recommendations.





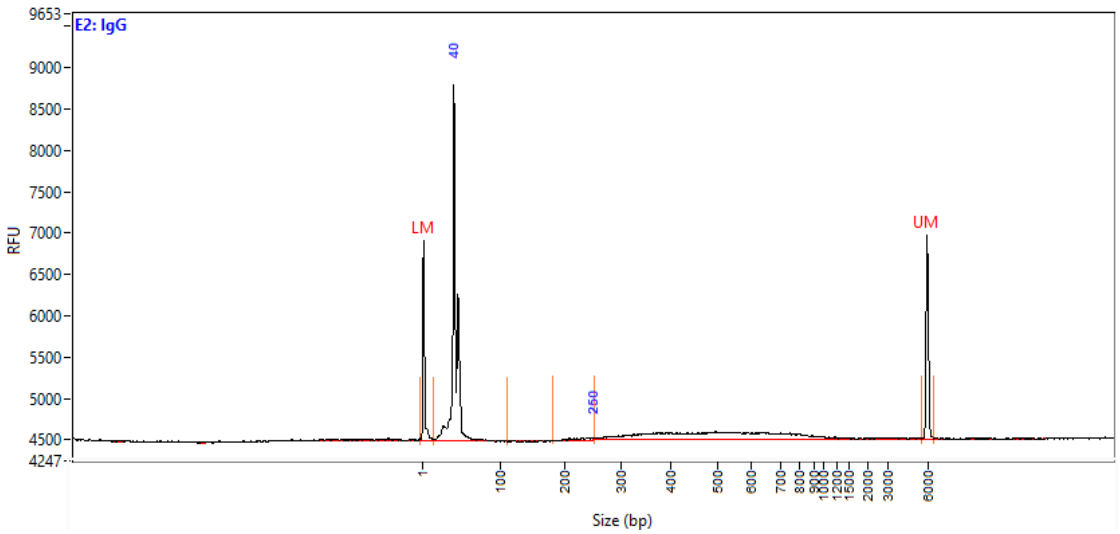


Figure 4. Typical library profiles (Agilent Fragment traces) generated by the iDeal CUT&Tag protocol using 50,000 K562 cells and H3K27me3 (top), H3K4me4 (middle) primary antibodies and IgG control (bottom). Sharp peak at around 40 bp is an excess of free oligonucleotide used for pA-Tn5 loading.

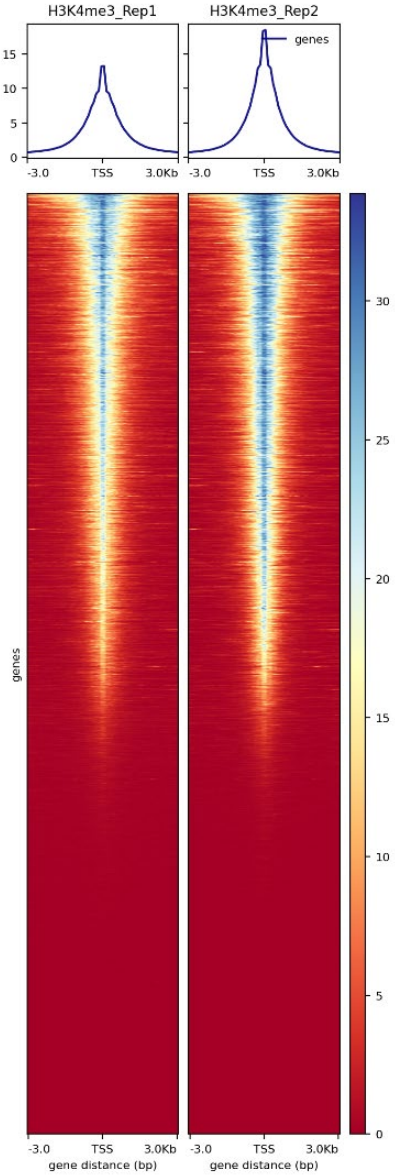


Figure 5. Enrichments at TSS of the CUT&Tag libraries. The heatmap shows the enrichment around 3kb upstream and downstream of the TSS for H3K4me3. H3K4me3 being an active chromatin mark associated with active promoters shows a narrow enrichment pattern.

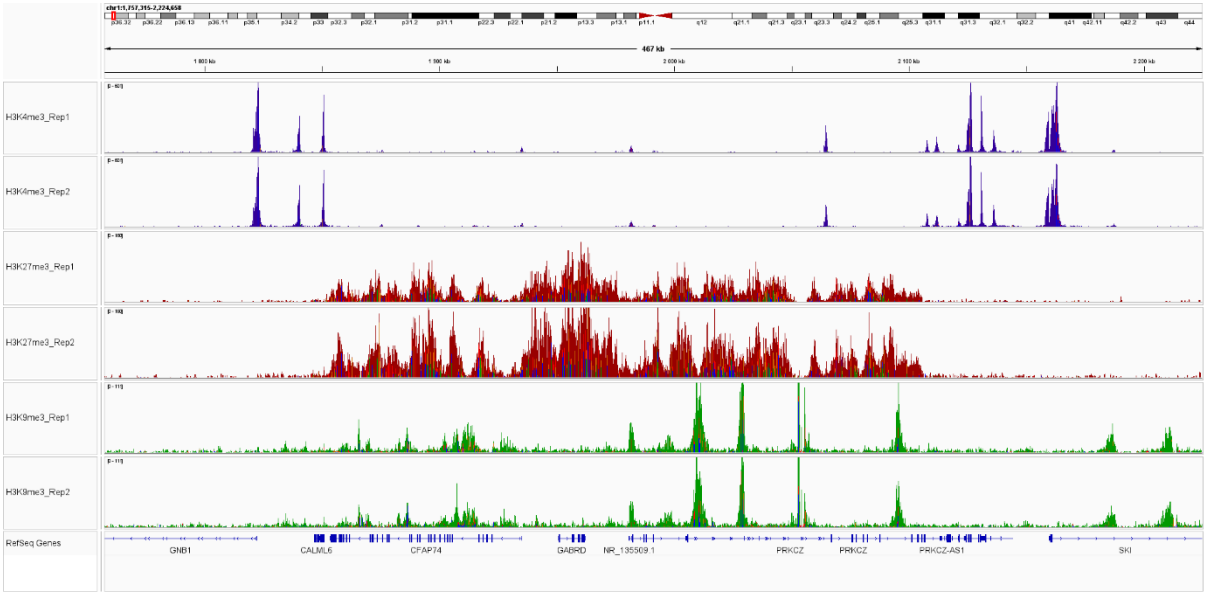


Figure 6. Sequencing profiles of the CUT&Tag libraries. Integrative genomics viewer (IGV) visualization of CUT&Tag experiments using 50.000 of K562 cells and H3K4me3 (blue), H3K27me3 (red) or H3K9me3 (green).

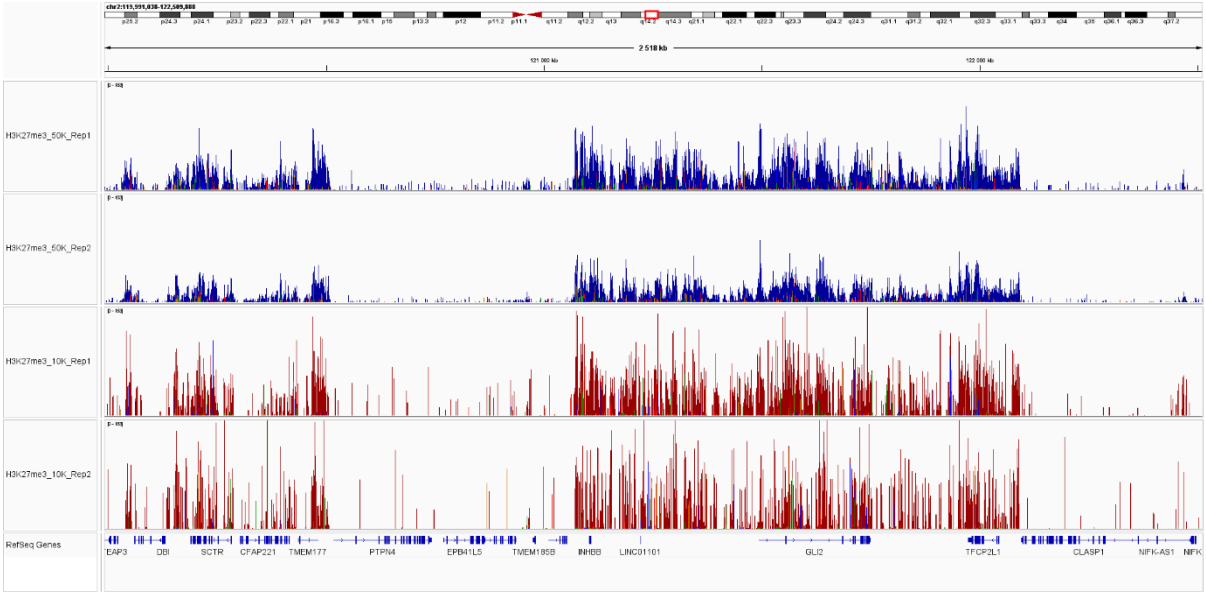


Figure 7. Sequencing profiles of the CUT&Tag libraries. Integrative genomics viewer (IGV) visualization of CUT&Tag experiments using 50.000 (blue) or 10.000 (red)) of K562 cells and H3K27me3 antibody.

FAQs section

Can I study Transcription Factors with this kit?

This protocol has been optimized for histone profiling. However, some transcription factors and co-factors might be successfully studied upon a mild cells fixation prior cell binding to conA beads (Step 1, Optional protocol).

How much antibody is needed per reaction?

The amount of antibody needed depends on different factors of which the antibody itself is the most important, generally 1 µg of antibody is enough to perform an iDeal CUT&Tag reaction. Most of Diagenode ChIP-seq grade antibodies have been tested at different concentrations to determine the optimal amount and the suggested amount of antibody is given in the data sheet. Please note, however, that this can be assay-dependent and might need to be optimized for each experimental setting. If the antibodies come from other companies, please refer to the corresponding technical data sheet. If no required amount of antibody is given, we suggest performing a titration experiment.

What is the binding capacity of ConA magnetic beads?

We recommend using 10 µl of beads per reaction whatever the range of cells (recommended 10,000 to 300,000 cells per reaction).

Can I use a monoclonal primary antibody in an iDeal CUT&Tag experiment?

iDeal CUT&Tag Kit has been validated using both monoclonal or polyclonal primary antibodies. In general, polyclonal antibody populations will recognize several epitopes, in contrast to monoclonal antibodies, which recognize a single epitope. Diagenode offers monoclonal and polyclonal antibodies validated for CUT&Tag experiment and batch-specific data are available on the website https://www.diagenode.com/en/applications/cut-and-tag

How many reads should I target when sequencing iDeal CUT&Tag libraries?

Although iDeal CUT&Tag results in high signal-to-noise ratio, we recommend between 15-20 M reads pairs per sample for high abundant histone marks and between 20-30 M reads pairs for low abundant histone marks. Less deep sequencing leads to the loss of peaks.

What is the difference between single and unique dual indexing? What is the best for iDeal CUT&Tag libraries?

With single indexing during the PCR amplification, only one primer will contain a barcode, whereas the second primer is universal. This means that only one side of the library will bear a barcode. This is enough to demultiplex the pool of samples after sequencing. Nevertheless, with some sequencers, (e.g., the NovaSeq6000), a phenomenon called index hopping has been observed. This index hopping introduces mistakes in the reading of the index and can lead to a misattribution of some reads to the wrong sample. To identify those mistakes, you can use Unique Dual-Indexing. In this case, the two PCR primers add unique barcodes to each sample, on each side of the insert. This way, if one index is wrongly associated to one sample, the second index will allow to see it. Therefore, when sequencing on NovaSeq, Unique Dual-Indexing is recommended, but not mandatory.

What is the composition of buffers included in the kit?

The composition of the buffers is proprietary.

Related products

Product	Cat. No.
iDeal CUT&Tag kit (8, 24 & 48 rxns)	C01070025 C01070020 C01070021
Antibody Package for CUT&Tag (anti-rabbit – 8 & 24 rxns)	C01070026, C01070022
Antibody Package for CUT&Tag (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
8 SI for tagmented libraries	C01011033
24 SI for tagmented libraries	C01011032
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
IPure kit v2	C03010014
Protease Inhibitor Mix 200X	C12010011
5% BSA	C03020005
Proteinase K	C06050001

Revision history

Version	Date of modification	Description of modifications
Version 1 01_2024	January 2024	Addition of the information related to the new kit format: 8 rxns
Version 1 08_2023	August 2023	Correction of small errors (point 4.6, 4.7)
Version 1 06_2023	June 2023	Addition of the protocol for non-histone proteins (Step 1)
Version 1 09_2022	Septembre 2022	Formatting corrections
Version 1 06_2022	June 2022	Manual creation

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