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APPLICATION NOTE

Mapping Histone post-translational modifications in the Pacific oyster using iDeal CUT&Tag and the Tissue Nuclei Extraction Module.

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Background

Mollusks represent one of the largest and most diverse phyla in the animal kingdom (Escoubas et al., 2016). Nevertheless, the different carriers of epigenetic information have only rarely been studied in these species. This lack of knowledge is particularly concerning for *Magallana gigas*, commonly known as the Pacific Oyster, which is the most widely distributed bivalve mollusk species in aquaculture worldwide (Botta et al., 2020). This gap is especially pronounced when it comes to the mapping of post-translational histone modifications. We had anecdotal evidence that chromatin immunoprecipitation (ChIP) was particularly difficult to perform with mollusk species. To address this technical challenge, we tested different ChIP-seq methods (Dellong et al., 2024) and in our hands, CUT&Tag with the Tissue Nuclei Extraction module and the iDeal CUT&Tag kit for histones gave the most convincing results.

CUT&Tag (Cleavage Under Targets & Tagmentation) is an advanced genome-wide mapping technique used to study protein-DNA interactions and chromatin modifications. It uses specific antibodies to bind chromatin-associated proteins within intact nuclei or cells. A tethered transposase is then employed to selectively cleave and insert adapter oligonucleotides to tag DNA at these sites, producing sequencing-ready fragments. This method eliminates the need for bulk chromatin fragmentation or immunoprecipitation steps, offering a more efficient, low-background alternative to traditional methods (Kaya-Okur et al., 2019).

This application note describes the successful use of the Diagenode Tissue Nuclei Extraction Module in combination with the iDeal CUT&Tag kit to map histone post-translational modifications (PTMs) in *M. gigas* tissue. We demonstrate that this approach enables efficient and reliable chromatin profiling in the Pacific oyster, a non-model organism, by successfully using tissue samples (or tissue-like materials) in CUT&Tag, provided that integrity of the nuclei is conserved. This highlights the potential for applying this method to study epigenetic regulation in mollusks and other understudied species.

Material

Nuclei Isolation

Tissue Nuclei Extraction module (Hologic Diagenode, Cat. No. C01080003)

Cleavage Under Targets and Tagmentation

iDeal CUT&Tag kit for histones (Hologic Diagenode, Cat. No. C01070020)

Antibody package for CUT&Tag (Hologic Diagenode, Cat. No. C01070022)

Primer indexes for tagmented libraries (Hologic Diagenode, Cat. No. C01011033)

Additional supplies

- Pacific oyster mantle pieces (~30 mg)
- Protease inhibitor mix 200X (Hologic Diagenode, Cat. No. C12010011)
- H3K4me3 Antibody (Abcam, Cat. No. Ab8580)
- PBS (Gibco, Cat. No. 70011-044)
- Nuclease-free water of molecular biology grade (Sigma-Aldrich, Cat. No. W4502-1L)
- 40 µm cell strainer (Sarstedt, Cat. No. 83.3945.040)
- Dounce and Pestle (Kontes Glass Company, Cat. No. 885303-0007 and Cat. No. 885301-0007)
- Plastic Pestle for 1.5 mL tubes
- DAPI (Invitrogen, Cat. No. D1306)
- SYBR (Invitrogen, Cat. No. S7563)
- Agilent High Sensitivity DNA Kit for the Bioanalyzer

Procedure

Tissue Nuclei Extraction module - Adult oyster mantle tissue lysis

- Adult oyster mantle tissues were dissected, put into 1.5mL Eppendorf tube, snap-frozen and stored at -80°C.
- To lyse the tissue, 800 µl of Tissue Lysis Buffer were added directly into the 1.5 ml Eppendorf tube and tissue was homogenized using a plastic pestle.
- Tissue suspension was transferred into a Dounce and further homogenized by 25 strokes with a size A pestle on ice.
- To remove tissue debris, cell suspension was filtered by gravity through a 40 µm cell strainer placed on top of a 25 ml tube.
- Flow-through was transferred into new 1.5 ml Eppendorf tubes and centrifuged 5 minutes at 1000 g, at room temperature.

iDeal CUT&Tag kit for histones

The procedure was executed according to the manual:

STEP 1 - ConA beads binding

- The supernatant was carefully removed, and cells are resuspended in 250 µl of Complete CT Wash Buffer 1.
- Tubes were centrifuged for 3 minutes at 600 x g at room temperature (RT) and the supernatant was removed without disturbing the cell pellet.
- The cells were resuspended in Complete CT Wash Buffer 1 using 100 µl per reaction.

We did not perform routinely cell counts; instead, we preferred to use the initial weight of a piece of tissue which is easier to standardize. According to our tests, 30 mg of mantle is sufficient, even when this piece is used for a batch of three reactions. However, we routinely examined whether nuclear integrity was preserved after the lysis step by taking 4 µl of cell suspensions at this stage. We used 1 µl for visual inspection of nuclei by adding 5 µl of 1/5000 dilution of DAPI in PBS and observation under an epifluorescence microscope (at 420 nm and 400x magnification at a NIKON Eclipse TS100 microscope).

- Washed ConA beads were added.
- Tubes were placed on a rotation wheel for 8 minutes at RT.
- Tubes were briefly spun to remove the liquid from the lid. The tube was placed on a magnetic rack and left for 2 minutes until the solution was clear, and the supernatant was removed by pipetting.

STEP 2 - Cell permeabilization and primary antibody binding

- The tube was removed from the magnetic rack and the pellet resuspended in ice-cold Complete CT Antibody Buffer using 50 µl per reaction.
- 4 µg of primary antibody (Abcam Cat. No. Ab8580) was added.

Note: *the manual of the iDeal CUT&Tag kit recommends 1 µg of primary antibody. However, we routinely perform antibody testing including Western blots on target tissue and ChIP-qPCR titration experiments, to determine the ratio of chromatin to antibody in which antibody is present in excess and all modified chromatin is captured. Using the above-mentioned antibody 4 µg was established as an optimal quantity (Dellong et al., 2024).*

- The tube was placed on a rotating wheel at +4°C and incubated overnight.

STEP 3 - Secondary antibody binding

Note: *Chose the secondary antibody according to the host species of primary antibody.*

STEP 4 - Targeted tagmentation according to the protocol Version 1 (01_2024)

STEP 5 - DNA purification according to the protocol

STEP 6 - Library amplification

Before library amplification, we perform quantitative PCR to determine the number of PCR cycles required for each sample, thereby limiting the over-amplification of our libraries (and avoiding the sequencing of PCR duplicates). To do this, we use 5 µl of the final PCR mix (prepared as described in the protocol) and add 0.2 µl of SYBR (100x diluted). This mixture is used for qPCR by running the PCR program of step 6.3 (63°C annealing). Optimal cycle numbers are determined by using the number of cycles at which 1/3 of the maximum fluorescence intensity is achieved. Amplification was done with the remaining 45 µl following otherwise the protocol.

STEP 7 - Library clean-up and QC

Library clean-up was performed on the IP-Star automated system (Hologic Diagenode) using the protocol “AMPure_XP_Purification”. The beads-to-sample ratio of 1.8X was used for purification.

Results

In this application note, we present representative results from an experiment where we generated four CUT&Tag library replicates for the histone mark H3K4me3, alongside a control IgG library produced in a parallel experiment. The principal challenge in optimizing the CUT&Tag protocol for M. gigas was to achieve efficient tissue lysis, a critical step for nuclei isolation. To address this issue, we used the Tissue Nuclei Extraction module, performing only the initial steps of the protocol specifically designed for tissue lysis. Following these steps, nuclei integrity was evaluated using DAPI staining and observation by fluorescence microscopy. Across four replicates, individual cells were obtained, with the exception of the second replicate, where cell aggregates were still observed. Despite this, nuclei integrity was confirmed in all replicates (Figure 1). Following tissue lysis, the iDeal® CUT&Tag kit for histones was applied to filtered tissue lysates to map H3K4me3-enriched regions.

To assess the library quality, we used the Bioanalyzer (Agilent), revealing fragment distribution ranging from 150 bp to 2000 bp. A prominent peak at 200 bp was observed, likely corresponding to mono-nucleosomes and/or nucleosome-free regions. The remaining curve displayed a smear of poly-nucleosome fragments. Additionally, for replicates 3 and 4, a distinct peak at 350 bp was noted, indicating fragments of di-nucleosome size (Figure 2). Although the profiles obtained from the Pacific oyster were less defined into fragments expected from mono- and polysomic nucleosomes than typical profiles from model organisms or cell lines, sequencing and subsequent bioinformatic analysis yielded satisfactory results.

The bioinformatic analysis was conducted using Bowtie2 for the alignment to the reference genome, followed by peak calling with MACS2 using default parameters. Profiles and heatmaps were generated using DeepTools (computeMatrix and plotHeatmap) on a local Galaxy instance. H3K4me3 profiles on protein-coding genes show a characteristic peak at

the transcription start site (TSS), a weaker signal along the gene body, and a dip at the transcription end site (TES). The four replicates exhibit similar profiles, except for the second replicate, which shows a slight decrease in signal intensity. This decrease may be attributed to less efficient tissue grinding during the initial steps of the protocol, as observed under fluorescence microscopy, and is consistent with the bioanalyzer results. The IgG signal in the profile and heatmap was distinctly different from the H3K4me3 samples. The weak IgG signal confirms the specificity of the H3K4me3 immunoprecipitation. While the IgG control identifies some regions with non-specific signals, it provides a narrower scope compared to an input control (Figure 3). Intermediate quantification of unpurified libraries step enabled us to determine accurate number of amplification cycles. We determined that the IgG libraries required more amplification cycles, between 20 and 25, to generate sufficient DNA for sequencing, whereas the anti-H3K4me3 antibody libraries consistently required fewer cycles, and never less than 16.

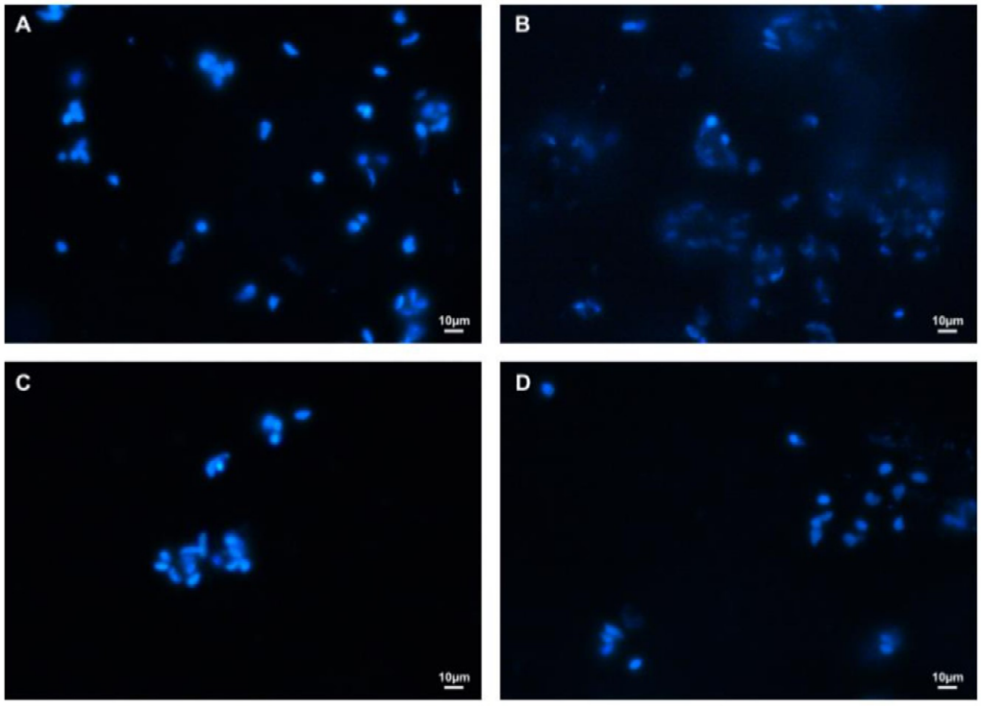

Figure 1. Nuclei from mantle tissue lysis. *Nuclei stained with DAPI observed under fluorescence microscopy. (A) replicate 1, (B) replicate 2, (C) replicate 3 and (D) replicate 4. Scale bar 10µm. Blue dots indicate intact nuclei.*

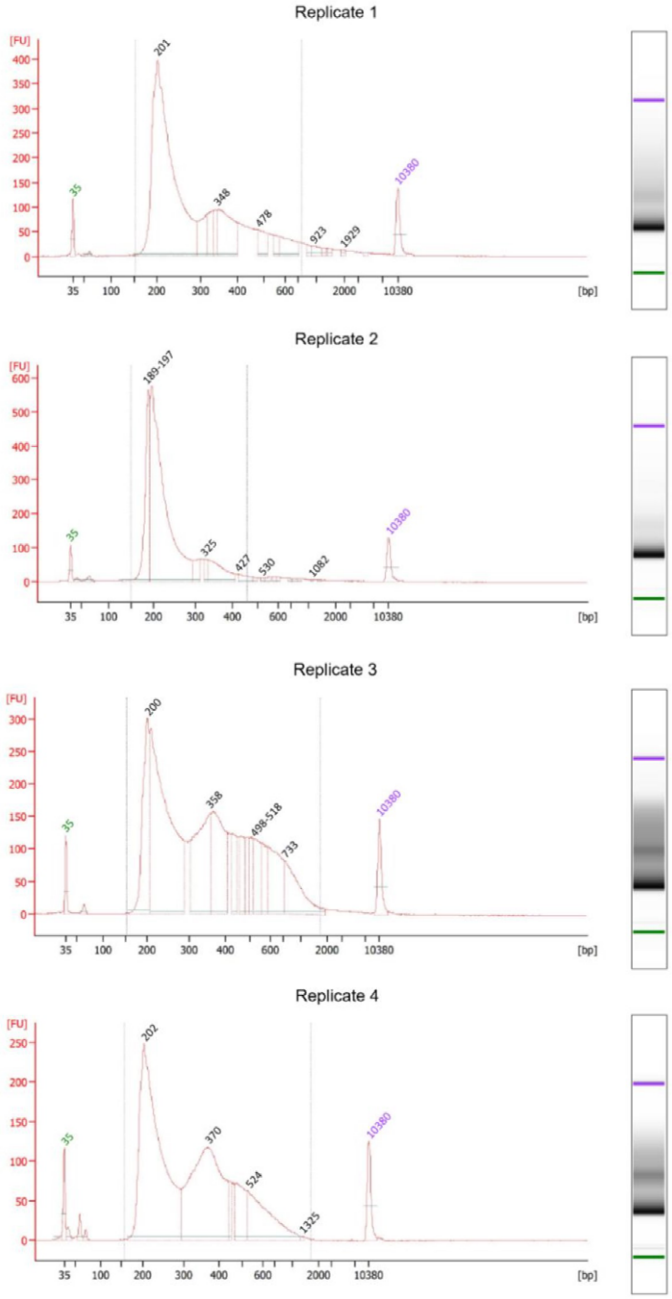

Figure 2. Library size distributions for the four H3K4me3 CUT&Tag replicates. *For each replicate: The electropherogram shows the fluorescence intensity as a function of the size of the DNA fragments (in bp) accompanied by the simulated virtual gel, illustrating the distribution of the DNA fragments. The spike-in guide peaks are visible at 35 bp and 10380 bp.*

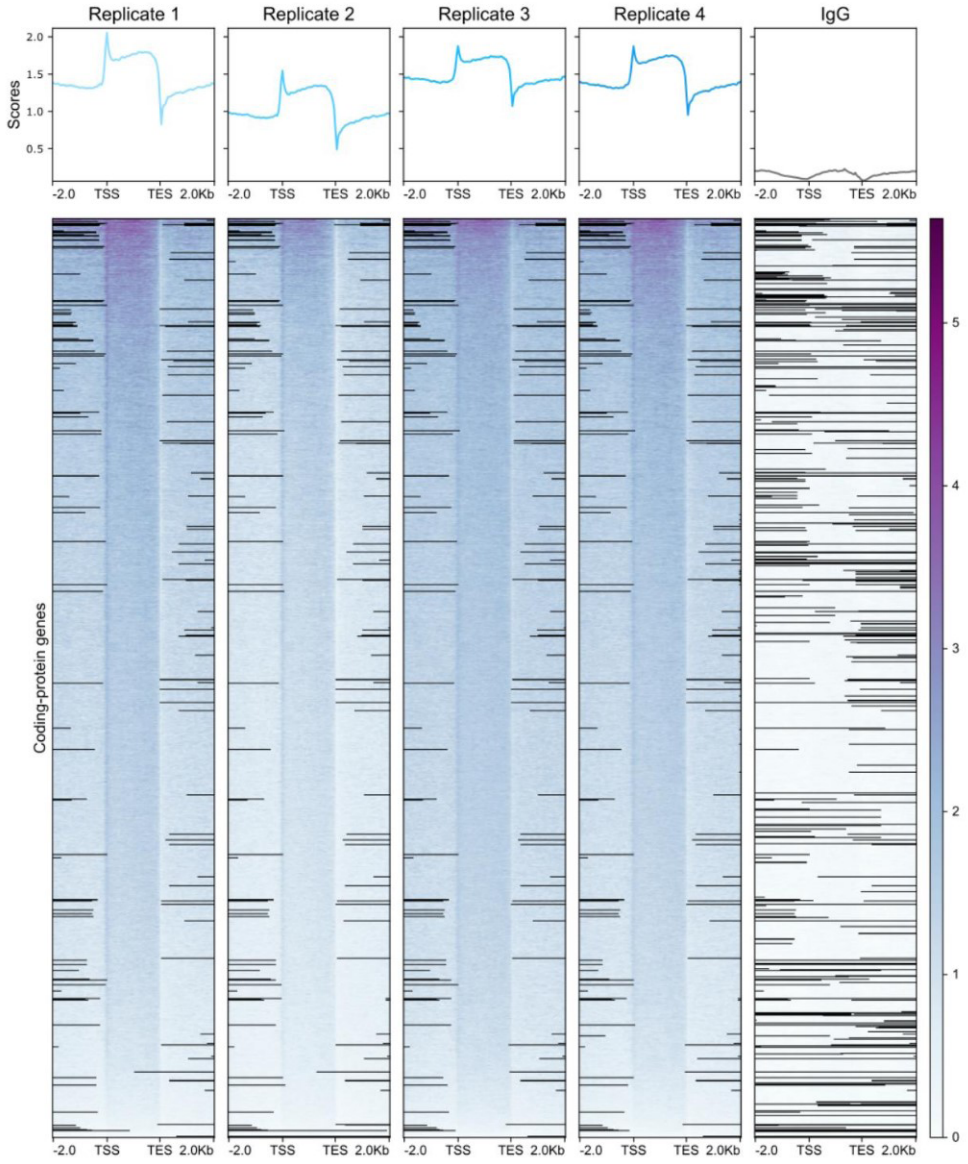

Figure 3. H3K4me3 profiles and heatmap on coding-protein genes for the four replicates. *Both profiles and heatmaps show the H3K4me3 signal on coding-protein genes (put on the same scale) with 2 kb downstream of TSS and 2 kb upstream of TES. Profiles are used to plot signal intensity (mean of scores attributed by MACS2) and on heatmaps coding-protein gene are sorted vertically in descending order in function of H3K4me3 signal intensity. Black lines represent absence of data. Replicates 1-4 profiles are very similar and show strong enrichment along the coding regions with a peak at the TSS. IgG profile is clearly different and very low indicating specific enrichment by the anti-H3K4me3 antibody.*

Conclusions

In this application note, we describe the adaptation of the CUT&Tag protocol to map histone marks in the Pacific oyster, *Magallana gigas*. Our results for H3K4me3 using the tissue lysis step of the Tissue Nuclei Extraction Module in combination with the iDeal CUT&Tag kit demonstrate that tissue samples (or tissue-like materials) can be efficiently used in CUT&Tag when cells and/or nuclei are properly individualized and when nuclei integrity is maintained, conditions that can easily be monitored by fluorescence microscopy.

The protocol described here is currently the method of choice for routine chromatin profiling in oysters. We believe that this method holds promise for advancing epigenomic studies in other mollusk species as well. However, we noted that a potential limitation of the method lies in the lack of scientific consensus on generating sequencing libraries for the "input" that is used in the bioinformatics analysis as a reference. Since fragmentation is inherently linked to antibody binding due to the nature of the technique, one would need an antibody that binds uniformly to the entire chromatin regardless of modifications.

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References

Botta, R., Asche, F., Borsum, J.S., Camp, E.V., 2020. A review of global oyster aquaculture production and consumption. Mar. Policy 117, 103952. https://doi.org/10.1016/j.marpol.2020.103952

Dellong, A., Fallet, M., Stenger, P.-L., Chaparro, C., Vidal-Dupiol, J., Clément, J., Cosseau, C., Grunau, C., 2024. Benchmarking of Chromatin Immunoprecipitation (ChIP-Seq) methods in the Pacific oyster Magallana gigas. https://doi.org/10.1101/2024.12.28.630603

Escoubas, J.-M., Gourbal, B., Duval, D., Green, T.J., Charrière, G.M., Destoumieux-Garzón, D., Montagnani, C., 2016. Immunity in Molluscs, in: Ratcliffe, M.J.H. (Ed.), Encyclopedia of Immunobiology. Academic Press, Oxford, pp. 417–436. https://doi.org/10.1016/B978-0-12-374279-7.12004-1

Kaya-Okur, H.S., Wu, S.J., Codomo, C.A., Pledger, E.S., Bryson, T.D., Henikoff, J.G., Ahmad, K., Henikoff, S., 2019. CUT&Tag for efficient epigenomic profiling of small samples and single cells. Nat. Commun. 10, 1930. https://doi.org/10.1038/s41467-019-09982-5