Store at -20°C

Digitonin Solution

#16359

2 x 1.2 ml

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Description: The Digitonin Solution provides enough reagent to support 24 CUT&RUN or CUT&Tag assays. This product is formulated for optimal performance in the CUT&RUN and CUT&Tag assays and each lot is tested and validated using the CUT&RUN Assay Kit #86652 or CUT&Tag Assay Kit #77552. This product should be completely thawed at 90-100°C for 5 minutes before using. Please keep on ice during use and store at -20°C when finished for the day.

Background: Like the chromatin immunoprecipitation (ChIP) assay, Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and Cleavage Under Targets and Tagmentation (CUT&Tag) are powerful and versatile techniques used for probing protein-DNA interactions within the natural chromatin context of the cell (1-7). CUT&RUN provides a rapid, robust, and true low cell number assay for detection of protein-DNA interactions in the cell. Unlike the ChIP assay, CUT&RUN is free from formaldehyde cross-linking, chromatin fragmentation, and immunoprecipitation, making it a much faster and more efficient method for enriching protein-DNA interactions and identifying target genes. CUT&RUN can be performed in less than one day, from live cells to purified DNA, and has been shown to work with as few as 500-1,000 cells per assay (1,2). Instead of fragmenting all of the cellular chromatin as done in ChIP, CUT&RUN utilizes an antibody-targeted digestion of chromatin, resulting in much lower background signal than seen in the ChIP assay. As a result, CUT&RUN requires only 1/10th the sequencing depth that is required for ChIP-seq assays (1,2). Finally, the inclusion of simple spike-in control DNA allows for accurate quantification and normalization of target-protein binding that is not possible with the ChIP method. This provides for effective normalization of signals between samples and between experiments. CUT&Tag has many of the same advantages as the CUT&RUN assay in that it provides a rapid, robust, and true low cell number protocol for detection of protein-DNA interactions in the cell. In addition, the CUT&Tag assay adds an in situ adaptor DNA ligation step carried out by the pAG-Tn5 enzyme, in which an adaptor DNA is ligated directly to antibody-targeted chromatin DNA fragments in the cell. As a result, subsequent DNA library preparation is much faster and easier than library preparation following the CUT&RUN assay, free from DNA end repair, A-tailing, and adaptor ligation in vitro. CUT&Tag works very well for analyzing histone modifications, in addition to mapping some transcription factors and cofactors binding.

Storage: Digitonin Solution is supplied in nuclease-free water. Store at -20°C. *This product is stable for at least 12 months.*

Directions for Use: For the CUT&RUN and CUT&Tag assay, we recommend adding 25 µl of Digitonin Solution per 1 ml buffer. Not all cell lines exhibit the same sensitivity to digitonin, so it may be necessary to adjust the final volume of digitonin in the buffer to get optimal permeabilization of your specific cell line. Cell permeabilization can be tested by combining an equal volume of cell suspension and 0.4% Trypan Blue Stain. Optimal cell permeabilization will result in staining of >90% of the cell population.

Background References:

(1) Skene, P.J. and Henikoff, S. (2017) *Elife* 6,

(2) Skene, P.J. et al. (2018) *Nat Protoc* 13, 1006-1019.

(3) Meers, M.P. et al. (2019) *Elife* 8.

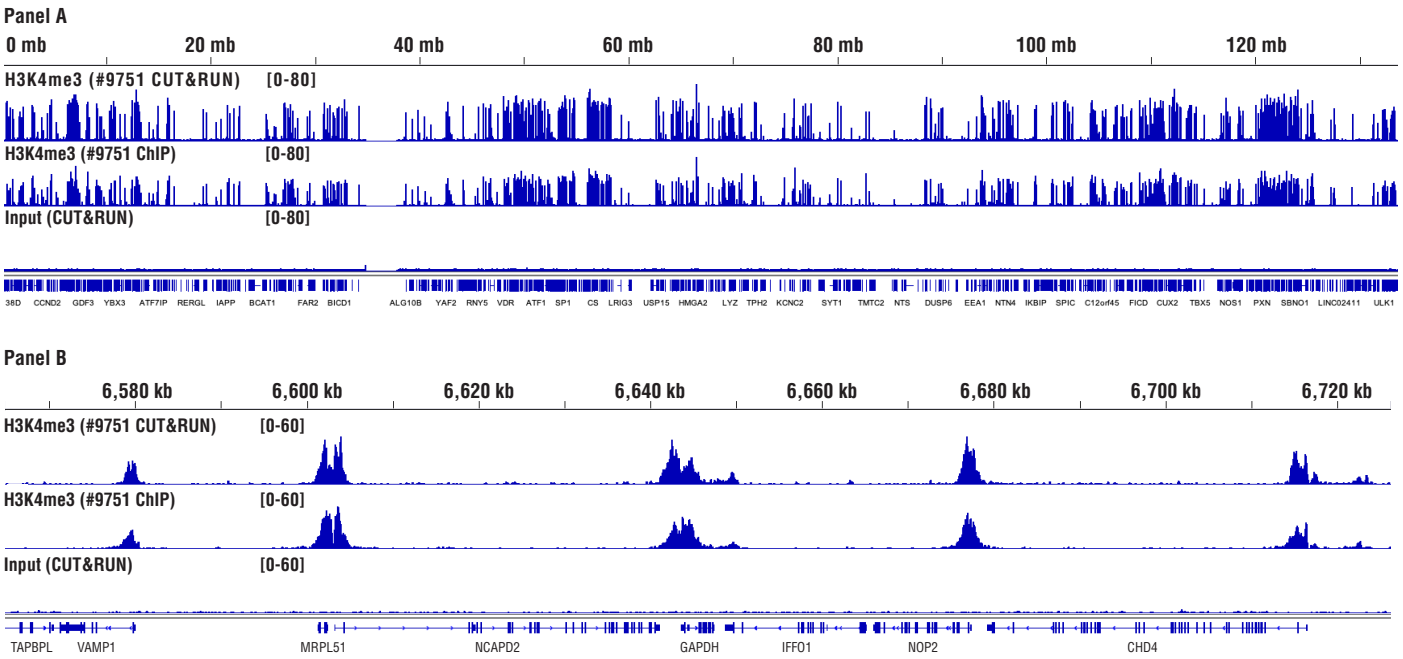
(4) Meers, M.P. et al. (2019) *Mol Cell* 75, 562-575.e5.

(5) Kaya-Okur, H.S. et al. (2019) *Nat Commun* 10, 1930.

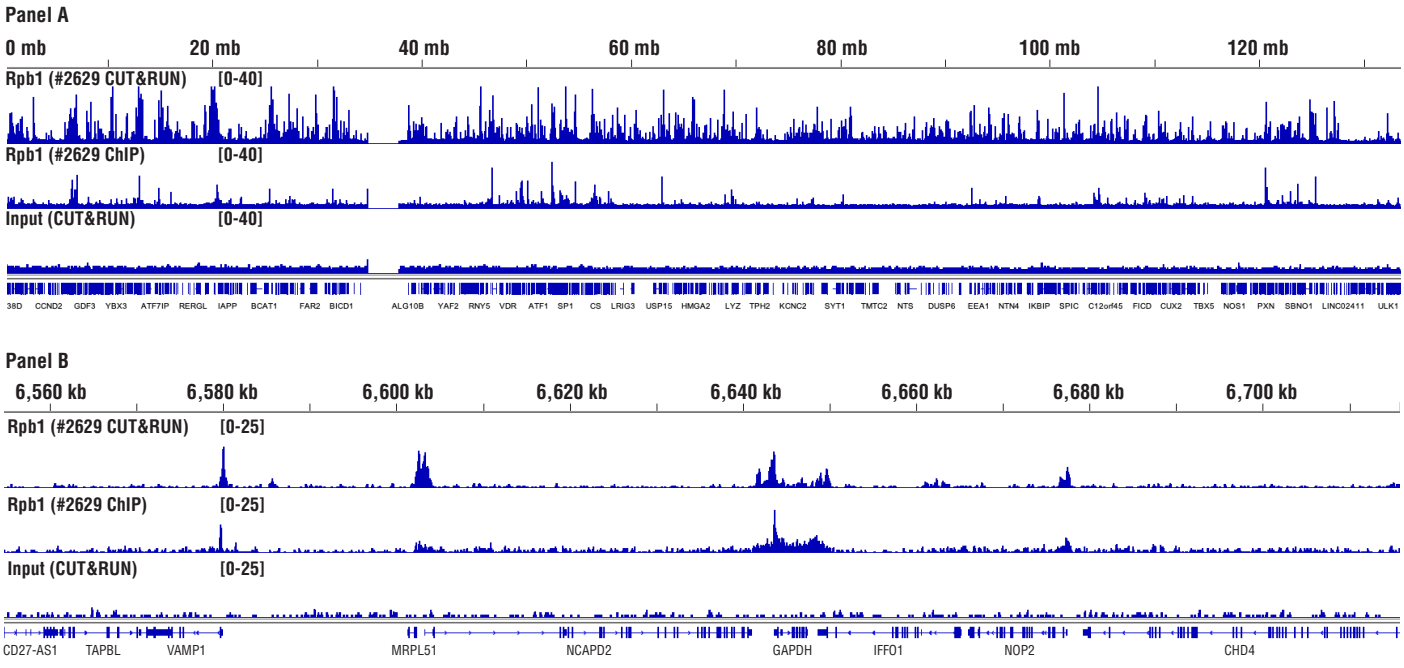
(6) Kaya-Okur, H.S. et al. (2020) *Nat Protoc* 15, 3264-3283.

(7) Henikoff, S. et al. (2021) *Bio Protoc* 11, e4043.

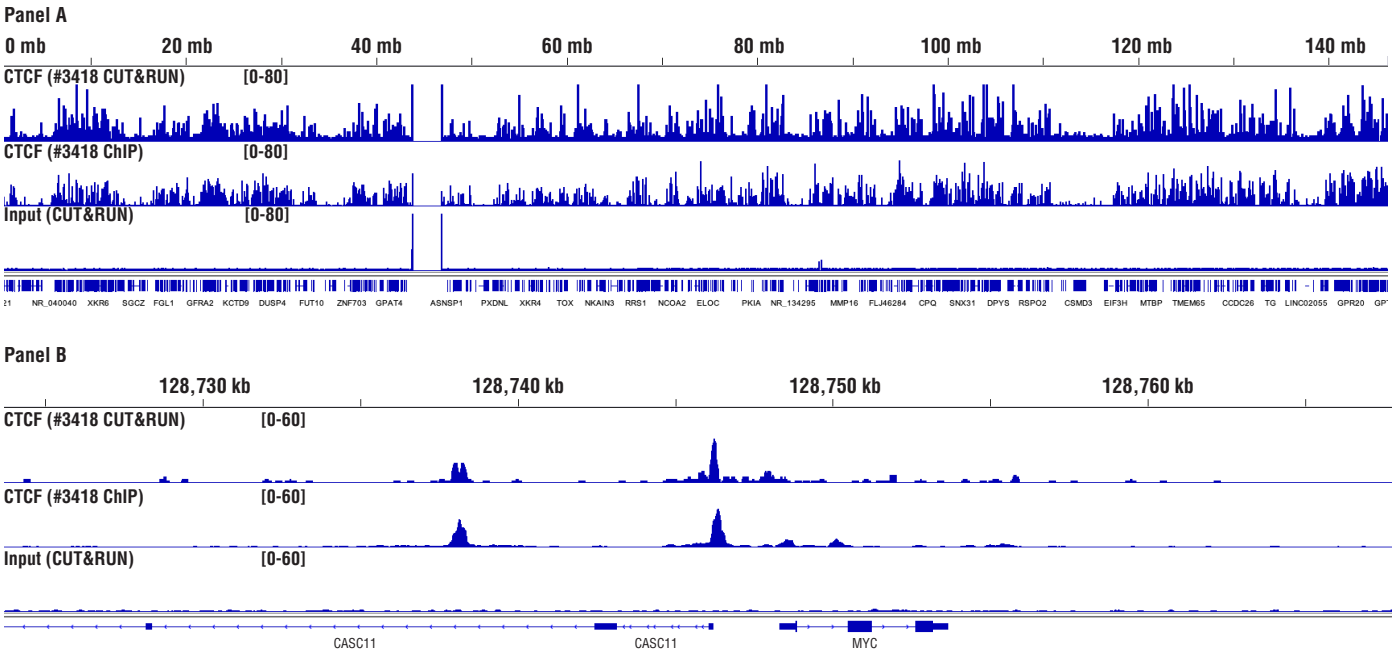
Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



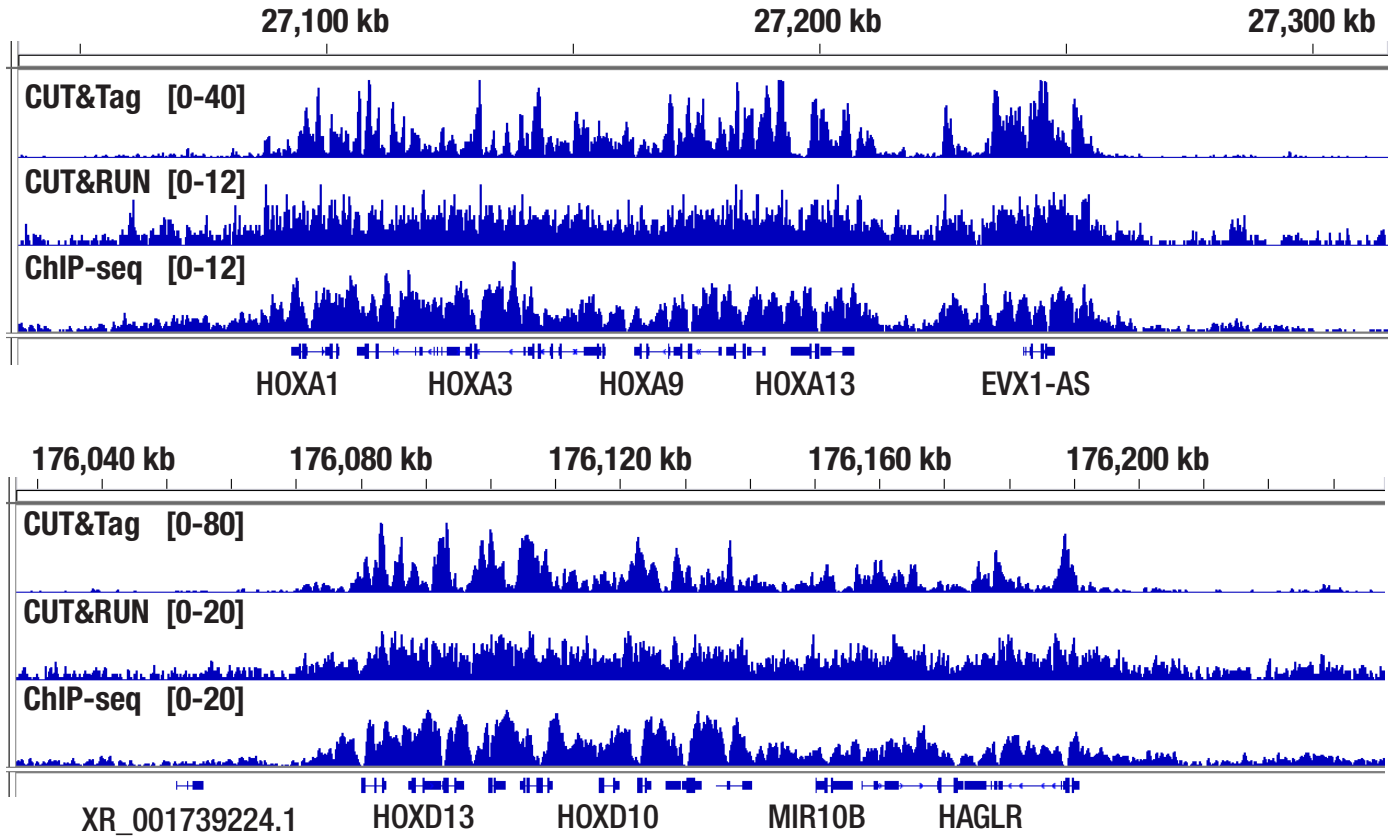
CUT&RUN and ChIP assays were performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of H3K4me3 across chromosome 12 (upper), while Panel B compares enrichment at the GAPDH gene (lower), a known target of H3K4me3. The input tracks are from the CUT&RUN input sample.



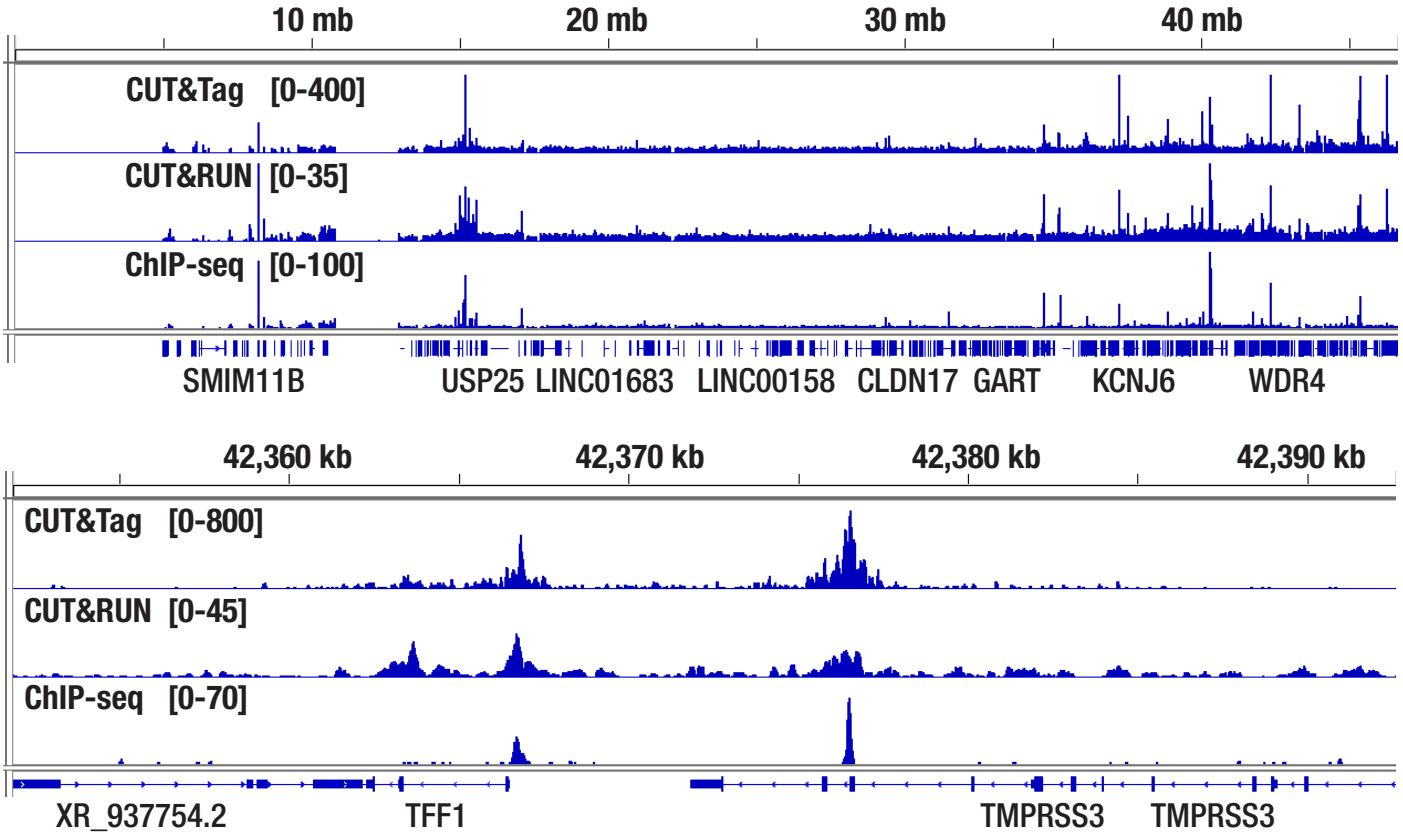
CUT&RUN and ChIP assays were performed with HeLa cells and Rpb1 CTD (4H8) Mouse mAb #2629. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of Rpb1 across chromosome 12 (upper), while Panel B compares enrichment at the GAPDH gene (lower), a known target of Rpb1. The input tracks are from the CUT&RUN input sample.



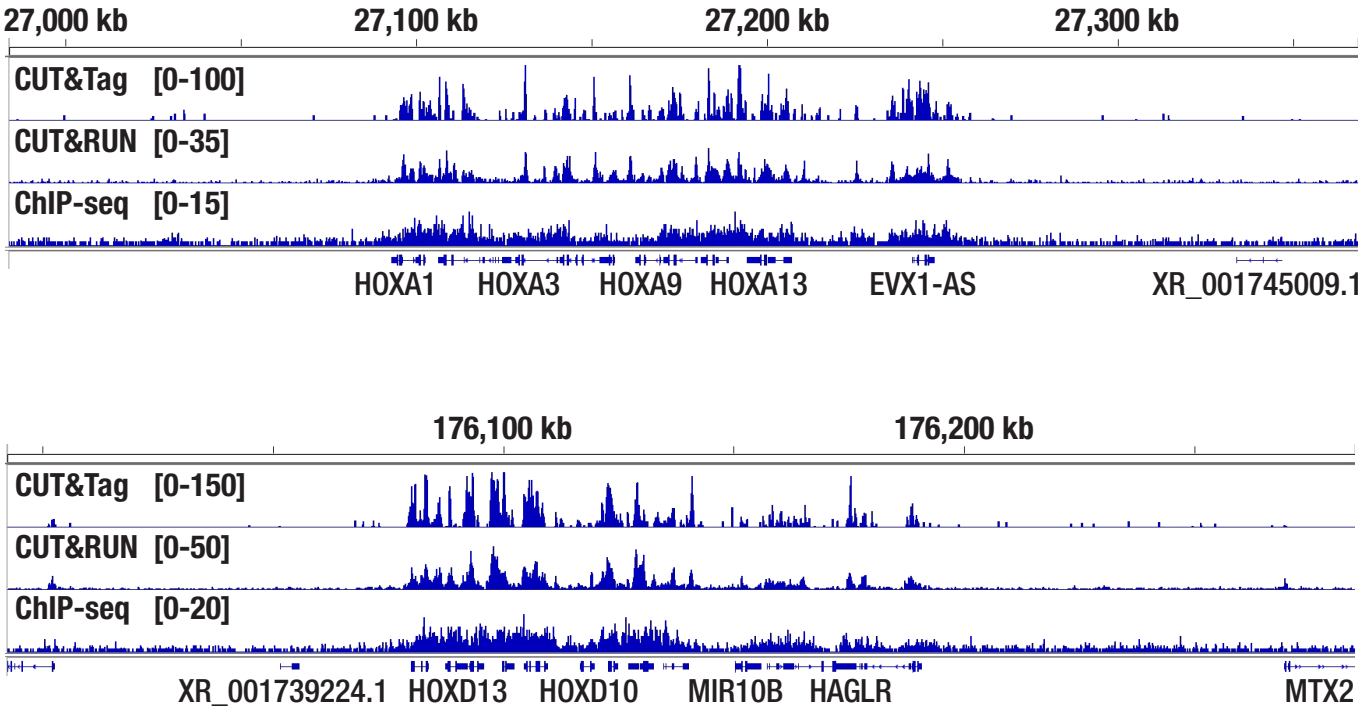
CUT&RUN and ChIP assays were performed with HCT 116 cells and CTCF (D31H2) XP® Rabbit mAb #3418. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of CTCF across chromosome 8 (upper), while Panel B compares enrichment at the MYC gene (lower), a known target of CTCF. The input tracks are from the CUT&RUN input sample.



CUT&Tag, CUT&RUN, and ChIP-seq assays were performed with NCCIT cells and Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, using the CUT&Tag Assay Kit #77552, the CUT&RUN Assay Kit #86652, or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415 for CUT&Tag samples and DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-seq and CUT&RUN samples. The upper panel compares enrichment around HoxA genes, while the lower panel compares enrichment around HoxD genes, both are known target genes of H3K27me3.



CUT&Tag, CUT&RUN, and ChIP-seq assays were performed with MCF7 cells grown in phenol red free medium and 5% charcoal stripped FBS for 4 d, then treated with β-estradiol (10 nM) for 45 min and Estrogen Receptor α (D8H8) Rabbit mAb #8644, using the CUT&Tag Assay Kit #77552, the CUT&RUN Assay Kit #86652, or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415 for CUT&Tag samples and DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-seq and CUT&RUN samples. The upper panel compares enrichment of Estrogen Receptor α across chromosome 21, while the lower panel compares enrichment around TFF1, a known target gene of Estrogen Receptor α.



CUT&Tag, CUT&RUN, and ChIP-seq assays were performed with NCCIT cells and JARID2 (D6M9X) Rabbit mAb #13594, using the CUT&Tag Assay Kit #77552, the CUT&RUN Assay Kit #86652, or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415 for CUT&Tag samples and DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795 for ChIP-seq and CUT&RUN samples. The upper panel compares enrichment around HoxA genes, while the lower panel compares enrichment around HoxD genes, both are known target genes of JARID2.