Store at 4°C

Concanavalin A Magnetic Beads and Activation Buffer

#93569

Cell Signaling TECHNOLOGY®

Support: +1-978-867-2388 (U.S.)
cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

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

Products Included	Product #	Quantity	Storage Temp
Concanavalin A Magnetic Beads	82307	240 µl	4°C
Concanavalin A Bead Activation Buffer	91275	5 ml	4°C

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Concanavalin A Magnetic Beads and Activation Buffer kit provides enough reagents to support 24 CUT&RUN or CUT&Tag assays. This product is tested and validated using the CUT&RUN Assay Kit #86652 or CUT&Tag Assay Kit #77552. This product should be stored at 4°C. Please do not freeze the Concanavalin A Magnetic Beads!

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: Store at 4°C. Do not freeze the Concanavalin A Magnetic Beads! *This product is stable for at least 12 months.*

Directions for Use: For the CUT&RUN and CUT&Tag assays, we recommend using 10 µl Concanavalin A Magnetic Beads per reaction. Before use, the Concanavalin A Magnetic Beads should be washed 2 times with 10X volume of Concanavalin A Bead Activation Buffer and resuspended in a volume of Concanavalin A Bead Activation Buffer equal to the initial volume of bead suspension. Activated beads should be used within one day.

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.

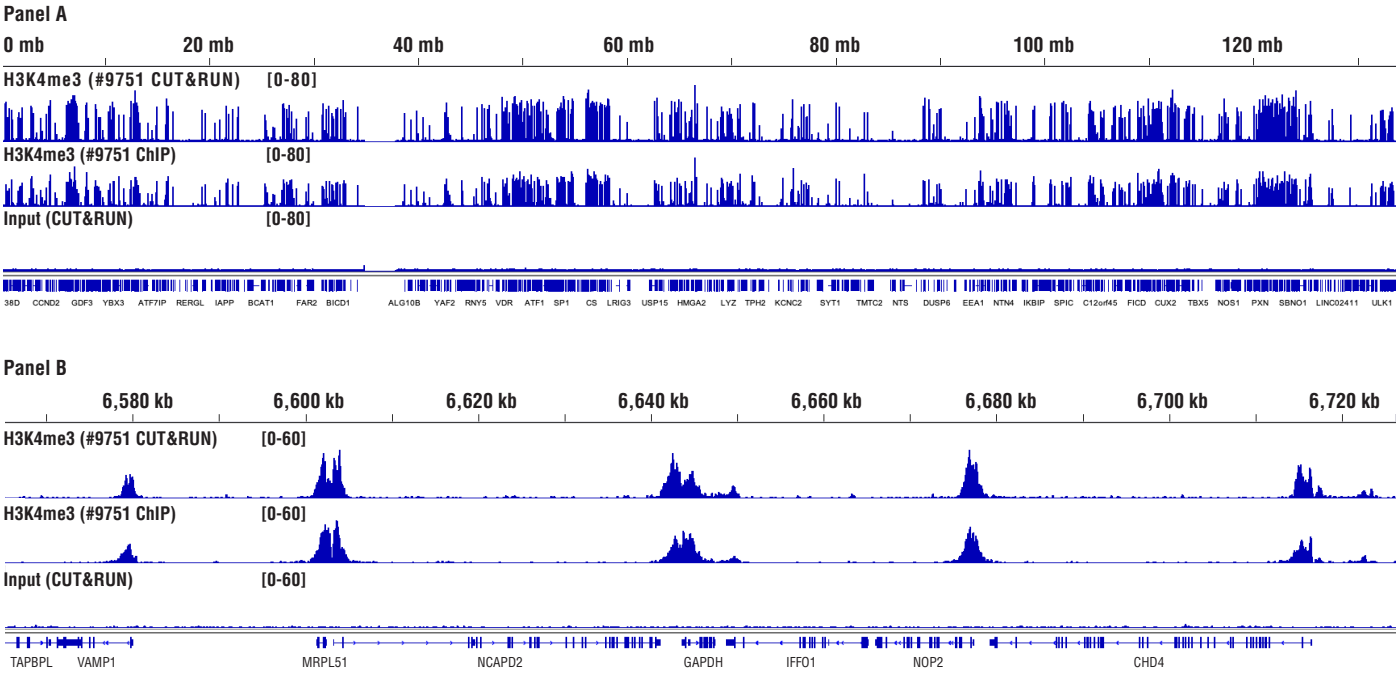
(4) Meers, M.P. et al. (2019) *Mol Cell* 75, 562-575.e5.

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

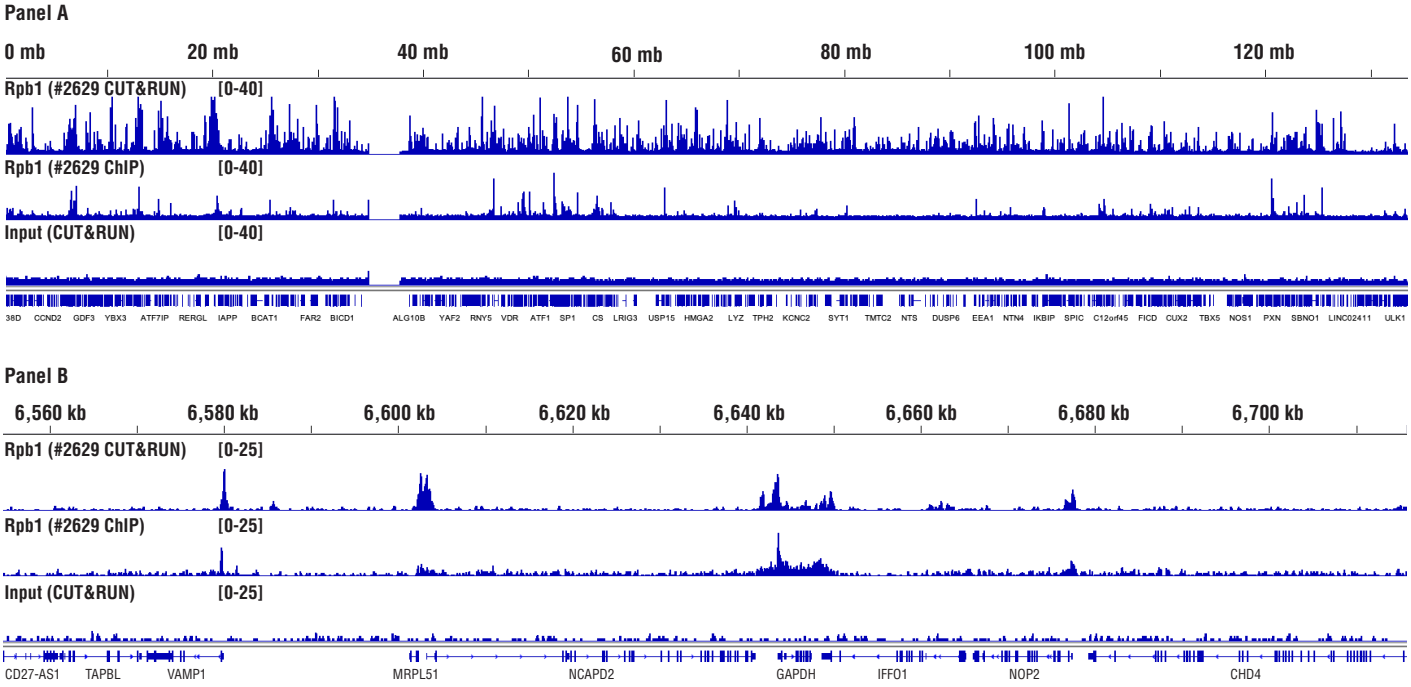
(6) Kaya-Okur, H.S. et al. (2020) *Nat Protoc* 15, 3264-3283.

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

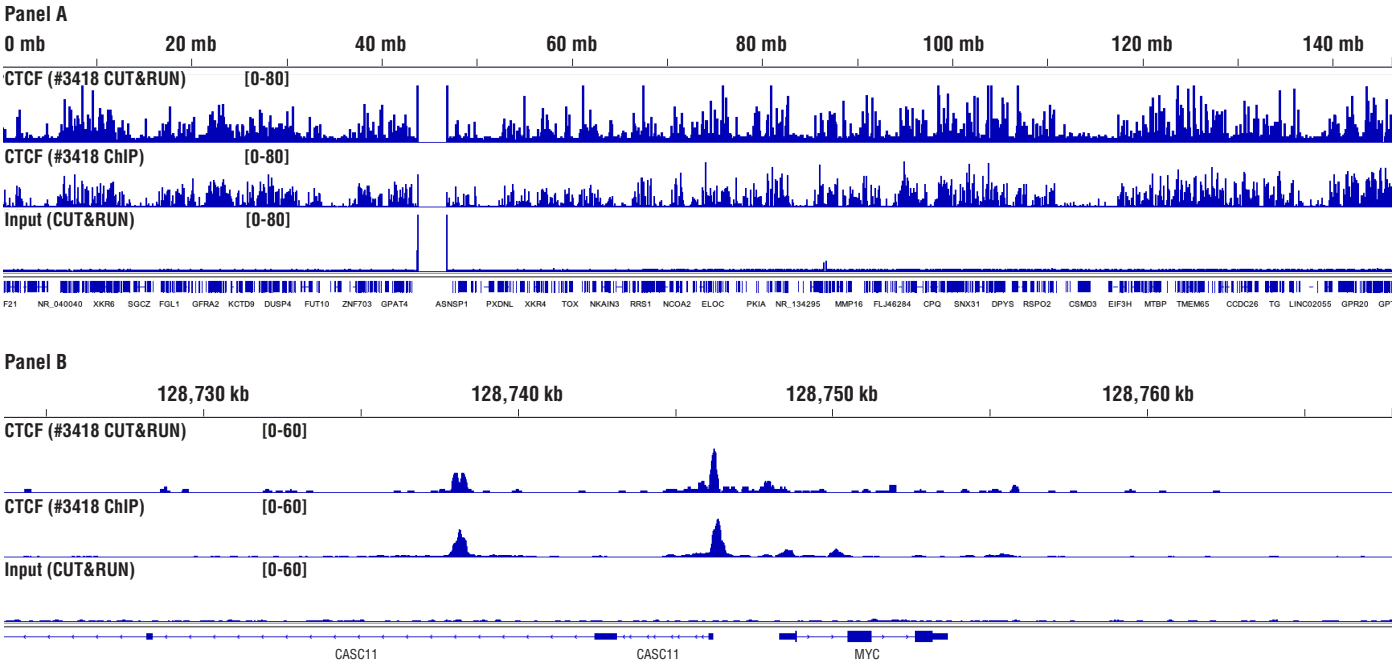
Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **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.



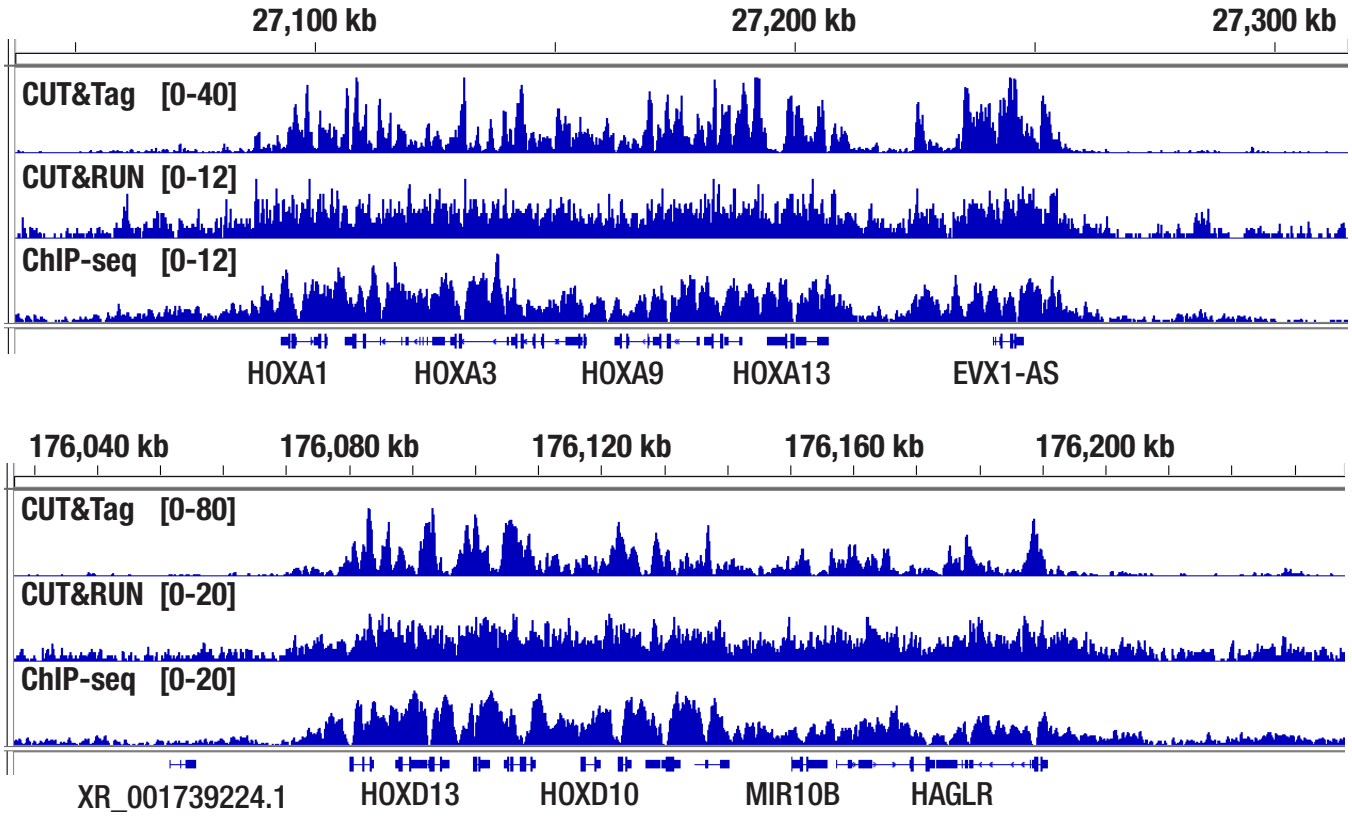
CUT&RUN and ChIP assays were performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of H3K4me3 across chromosome 12 (upper), while Panel B compares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



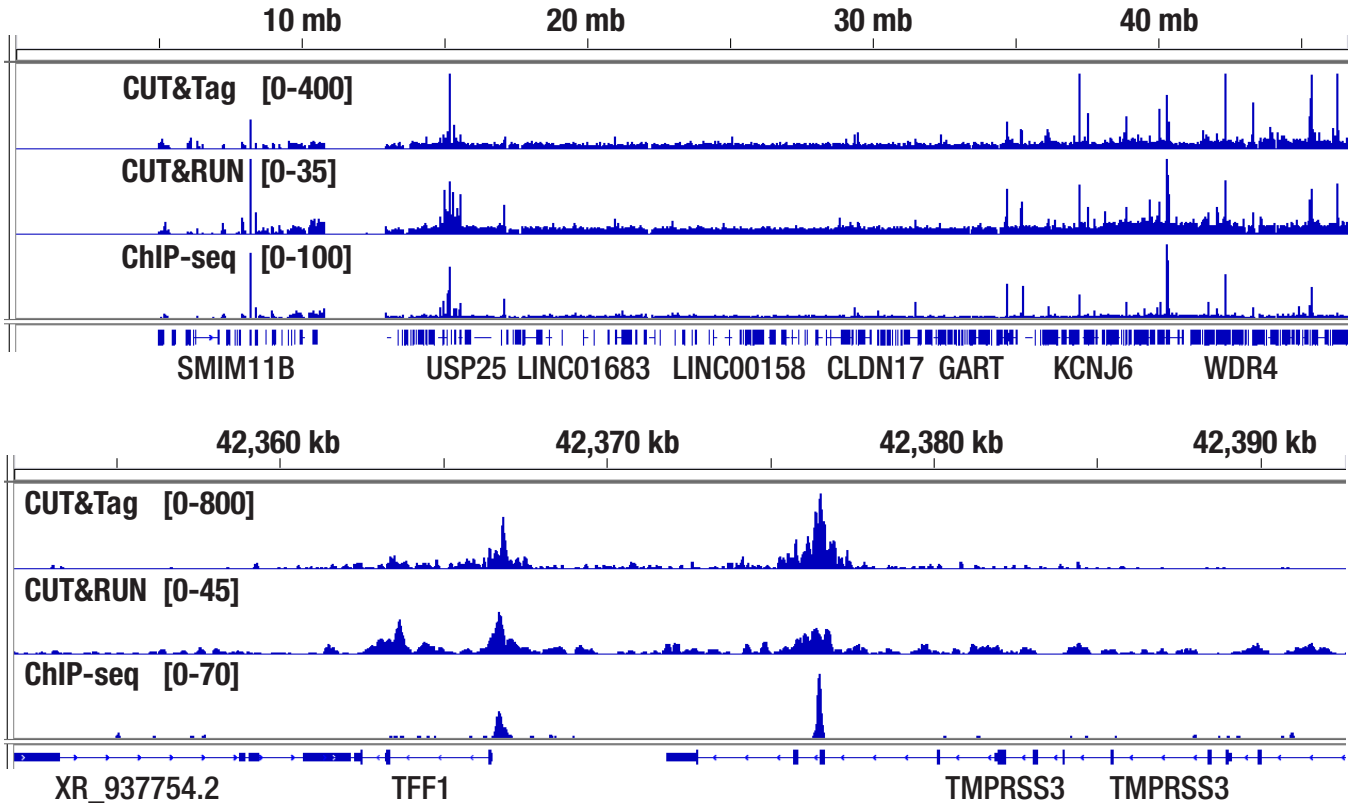
CUT&RUN and ChIP assays were performed with HeLa cells and Rpb1 CTD (4H8) Mouse mAb #2629. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of Rpb1 across chromosome 12 (upper), while Panel B compares enrichment at the GAPDH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



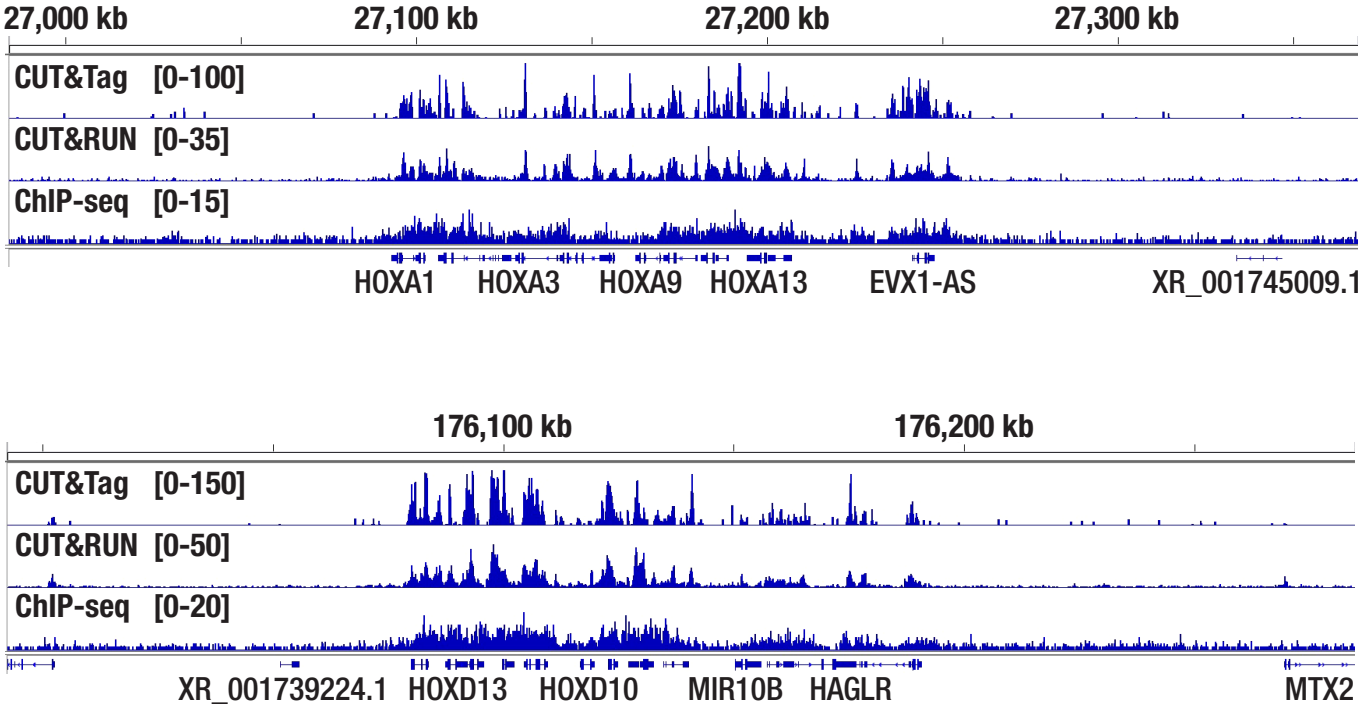
CUT&RUN and ChIP assays were performed with HCT 116 cells and CTCF (D31H2) XP® Rabbit mAb #3418. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of CTCF across chromosome 8 (upper), while Panel B compares enrichment at the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