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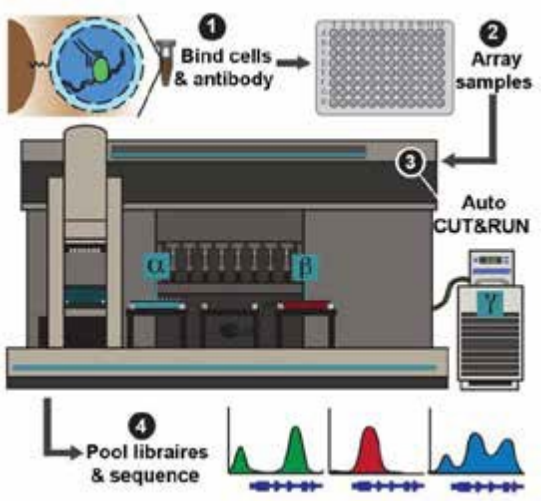
AutoCUT&RUN



Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is an antibody-targeted chromatin profiling method developed by the Henikoff Lab to examine the genomewide occupancy of transcription factors and chromatin modifying proteins as well as histone modifications and histone variants. This method is broadly applicable across different species and cell types and uses micrococcal nuclease tethered to a protein A/G fusion to bind an antibody of choice and cut the immediately adjacent DNA, thereby releasing the antibody targeted DNA for subsequent deep sequencing analysis. The procedure is carried out in situ, avoiding crosslinking and solubilization issues, and produces extremely low backgrounds as compared to ChIP, make profiling possible using low cell numbers and reduced sequencing depth without loss of quality.

CUT&RUN has been automated (AutoCUT&RUN) using a Beckman Biomek FX liquid-handling robot to facilitate high-throughput chromatin profiling in 96 well format. This platform allows sample-to-Illumina library processing of 96 samples in two days. In addition, AutoCUT&RUN has been validated for profiling histone modifications and transcription factors in frozen tissue samples taken from tumor xenografts indicating this method can be used to examine the epigenetic landscape of clinically relevant samples.

Sample processing

Users will culture cells / process tissue and bring to homogenous suspension, bind to Concanavalin A coated magnetic beads, permeabilize, and treat with desired antibodies prior to sample submission.

Submitted samples are arrayed into a 96 well PCR plate for robotic processing which includes; binding of pAG-MNase to localized antibodies, cleavage, release, and purification of chromatin targets, and adapter ligation and PCR amplification of libraries.

After completion of library amplification and cleanup, the 96 well sample plate undergoes TapeStation analysis to provide data for process QC, individual sample evaluation, and quantification for pooling. Users will be provided with this report to review prior to pooling.

A panel of 48 unique adapter pairs is used to generate libraries and each pool of 48 barcoded samples is run on 2 lanes of a flow cell for paired-end 25x25 bp NextGen Sequencing (separate charges apply).

References

Targeted in situ genome-wide profiling with high efficiency for low cell numbers.

- https://www.ncbi.nlm.nih.gov/pubmed/29651053

Automated in situ chromatin profiling efficiently resolves cell types and gene regulatory programs

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6302505/

Improved CUT&RUN chromatin profiling and analysis tools

- https://www.biorxiv.org/content/10.1101/569129v2.full

Validated antibodies

The use of a protein A-protein G-MNase fusion (pAG-MNase) makes this method compatible with most antibodies, however, in some cases pre-incubation with a rabbit or guinea-pig secondary antibody may still be required for efficient binding of certain mouse primaries etc. In addition, the small amount of E. coli DNA that is carried over from the pAG-MNase preparation can be used for internal calibration of samples without adding additional heterologous spike-in DNA.

ANTIBODY	VENDOR	DILUTION
IgG	Abcam ab46540	1 to 50
IgG	ABIN101961	1 to 50
H3K27me3	Cell Signaling 9733S	1 to 100
H3K27ac	Abcam ab4729	1 to 100
H3K27ac	Abcam ab45173	1 to 100
H3K27ac	Millipore MABE647	1 to 50
H3K4me	Abcam ab8895	1 to 100
H3K4me2	Millipore 07-030)	1 to 100
H3K4me3	Active Motif 39159	1 to 100
H3K9me3	Abcam ab8898	1 to 100
H2A.Z	Active Motif 39113	1 to 100
H3.3	Abnova H0000302-M01*	1 to 100
PolII S5P	Cell Signaling D9N5I	1 to 50
CTCF	Millipore 07-729	1 to 100

Requires Rabbit anti-mouse secondary

ANTIBODY	VENDOR	DILUTION
NPAT	Thermo Fisher PA5-66839	1 to 50
cMYC	Cell Signaling D38NF	1 to 50
Ring1B	Abcam ab101273	1 to 50
CBX7	Abcam ab21873	1 to 50
Suz12	Abcam ab12073	1 to 100
Ezh2	Diagenode C15410039	1 to 100
Sox2	Abcam ab92494	1 to 50
Nanog	Abcam ab109250	1 to 50
FoxA1	Abcam ab23738	1 to 50
FoxA2	Millipore 07-633)	1 to 50
GATA4	Santa Cruz sc-25310X	1 to 50
SOX17	R&D Systems AF1924	1 to 50
EOMES	Abcam ab23345	1 to 50