Epigenome Profiling with CUT&RUN and CUT&Tag

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CUT&RUN (cleavage under target and release using nuclease) and CUT&Tag (cleavage under target and tagmentation) are two novel methods focused on the characterization of protein-DNA interactions in situ. They are characterized by a lower background and higher signal-to-noise ratio compared to ChIP-seq. Therefore, they are also better suited for lower sample input.

In CUT&RUN chromatin is fragmented through antibody-directed tethering of a protein A and/or protein G MNase fusion protein in immobilized permeabilized cells or isolated nuclei, similarly to ChIC. Fragments are analyzed via next generation sequencing. CUT&Tag employs a hyperactive Tn5 fused to protein A and/or protein G to direct tagmentation of genomic DNA and NGS analysis. Both methods can be used on native or lightly fixed samples.

CUT&Tag is easier and quicker than CUT&RUN because of the simultaneous chromatin fragmentation and addition of sequencing adapters. In contrast, CUT&RUN is better suited for less abundant target proteins such as transcription factors or chromatin modifiers (as opposed to histone markers) because of less stringent wash steps. CUT&RUN also has a higher resolution thanks to nuclease digestion as opposed to tagmentation.

High signal-to-noise ratio
Targeted digestion of chromatin leads to greatly reduced background signal. MNase and Tn5 both have higher resolution than ChIP-seq.

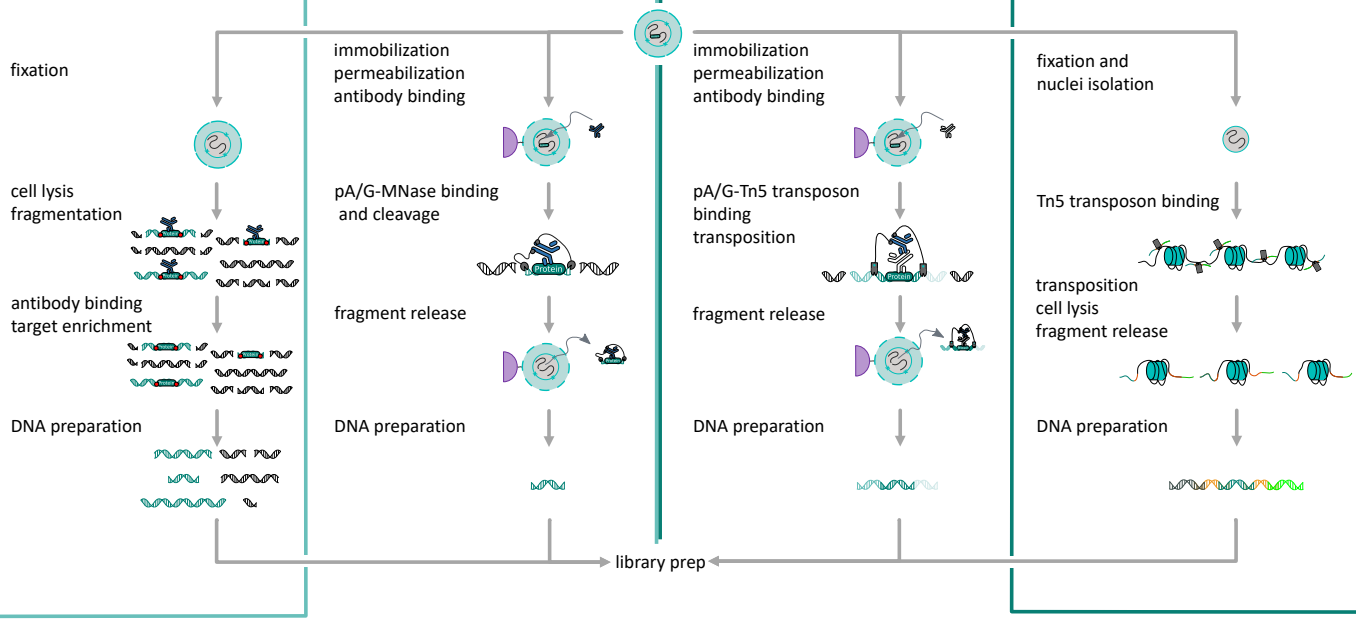
Fewer sequencing reads
Thanks to the increased signal-to-noise ratio CUT&RUN and CUT&Tag require 10% of ChIP-seq sequencing depth. Accurate quantitation using heterologous spike-in DNA.

Low sample requirements
Low starting cell numbers needed depending on the antigen; single-cell profiling is possible using combinatorial indexing.

Optimized protocol
In-situ method without mechanical chromatin fragmentation. Simple and fast protocol amenable for automation. Library prep within one day.

Method Comparison

ChIP-seq	CUT&RUN	CUT&Tag	ATAC-seq
10^7 cells	10^2 - $5x10^5$ cells	10^2 - $5x10^5$ cells	$5x10^2$ - $20x10^5$ cells
cell lysates	whole cells or nuclei	whole cells or nuclei	nuclei
heterogeneous fragment size	homogeneous fragment size	homogeneous fragment size	heterogeneous fragment size
high background	low background	low background	high background
low resolution	very high resolution	high resolution	high resolution
NGS $3x10^7$ reads	NGS $3x10^6$ reads	NGS $3x10^6$ reads	NGS $5\text{-}20x10^7$ reads
LM PCR lib. amp.	LM PCR lib. amp.	PCR lib. amp.	PCR lib. amp.
quantification difficult	easy spike-in	easy spike-in	



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