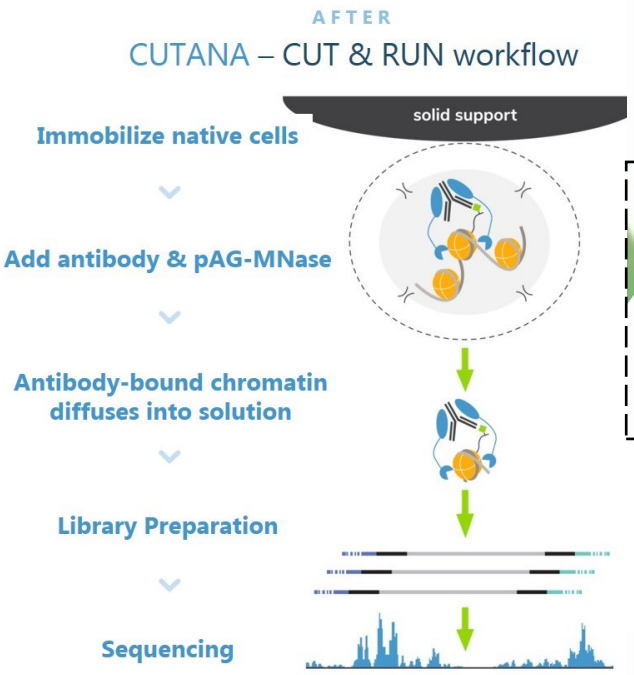
EpiCypher

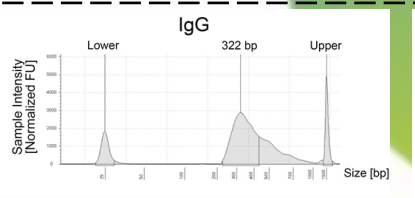
| Features | | ChIP-seq | CUTANA™ CUT&RUN | CUTANA™ CUT&Tag |
|---|---|---|---|---|
| Sample inputs | | Fragmented chromatin (*native or crosslinked*) | **Intact cells or nuclei** (*native or lightly crosslinked*) | **Nuclei** recommended* (*native or lightly crosslinked*) |
| # cells | Typical | > 1,000,000 | 500,000 | low inputs only |
| | Lowest using standard protocol | ~100,000 | **5,000** | **5,000-1,000** |
| | Protocol variations for **single cell** | No | **Hainer *et al.*, PMID 3095588** | **Kaya-Okur *et al.*, PMID 31036827** |
| Ideal for profiling | | Histone PTMs & chromatin-interacting proteins | Histone PTMs & chromatin-interacting proteins including remodelers | Histone PTMs |
| Secondary antibody recommended | | No | No | **Yes** |
| Separate library preparation steps | | Yes | Yes | **No, direct to PCR (*cells → DNA in a single tube*)** |
| Sequencing reads | | > 30 million (+ Input) | 3-8 million** | 3-8 million** |
| Signal : Noise | | Low | High | High |
| Experimental throughput | | Low | High | High |
| Normalization methods | | Drosophila spike-ins, SNAP-ChIP spike-in nucleosome panels | *E. coli* Spike-in DNA *(library prep)*, CUTANA Spike-in Nucleosome Controls *(in development)* | In development |

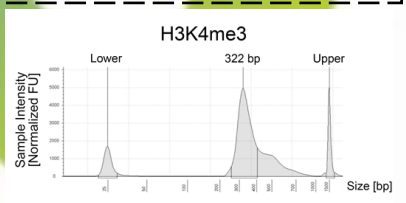



| Catalog No. | Product Name |
|---|---|
| 14-1048 | CUTANA™ CUT&RUN Kit (48 rxn) |

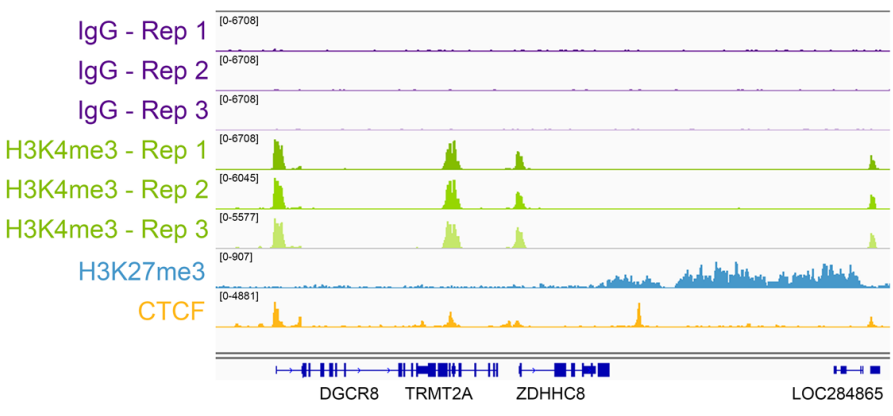
利用獨特的 **pAG-MNase 技術**將 antibody binding 的 protein-DNA complex 區段切下來，並 release 至細胞外。

=> 省略了傳統的 sonication fragmentation 或 enzyme digestion 以及 immunoprecipitation 等步驟。




**CUT&RUN DNA Fragment Size Distribution Analysis.** CUT&RUN was performed using the CUTANA ChIC/CUT&RUN Kit starting with 500,000




**Representative gene browser tracks.** A representative 150 kb window at the TRMT2A gene is shown for three replicates ("Rep") of IgG and H3K4me3 antibody controls (included in the kit). Representative tracks are also shown for H3K27me3 (EpiCypher Catalog No. 13-0030) and the transcription factor CTCF (EMD Millipore Catalog No. 07-729) antibodies. The CUT&RUN kit produced the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).

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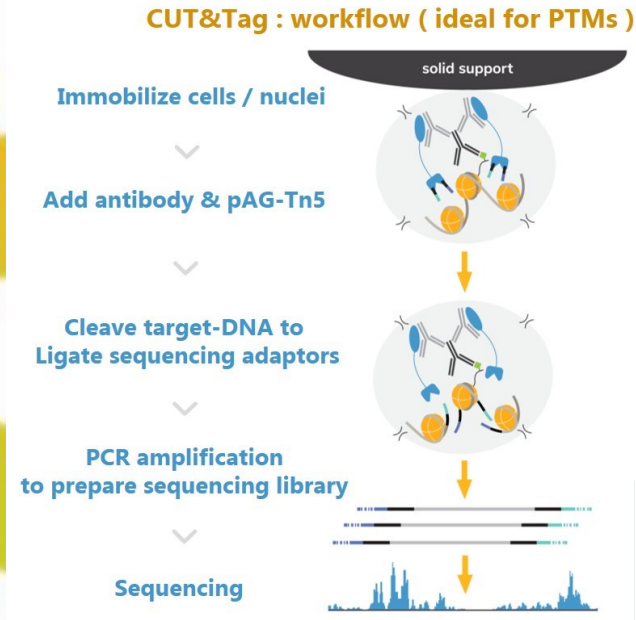
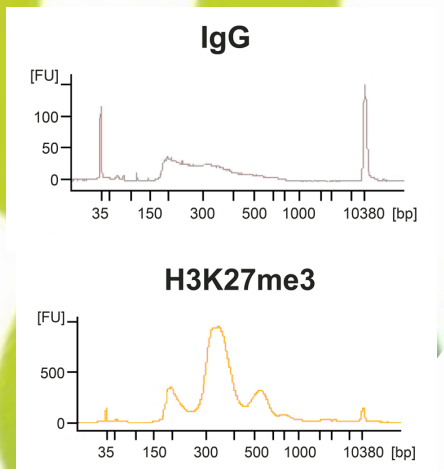


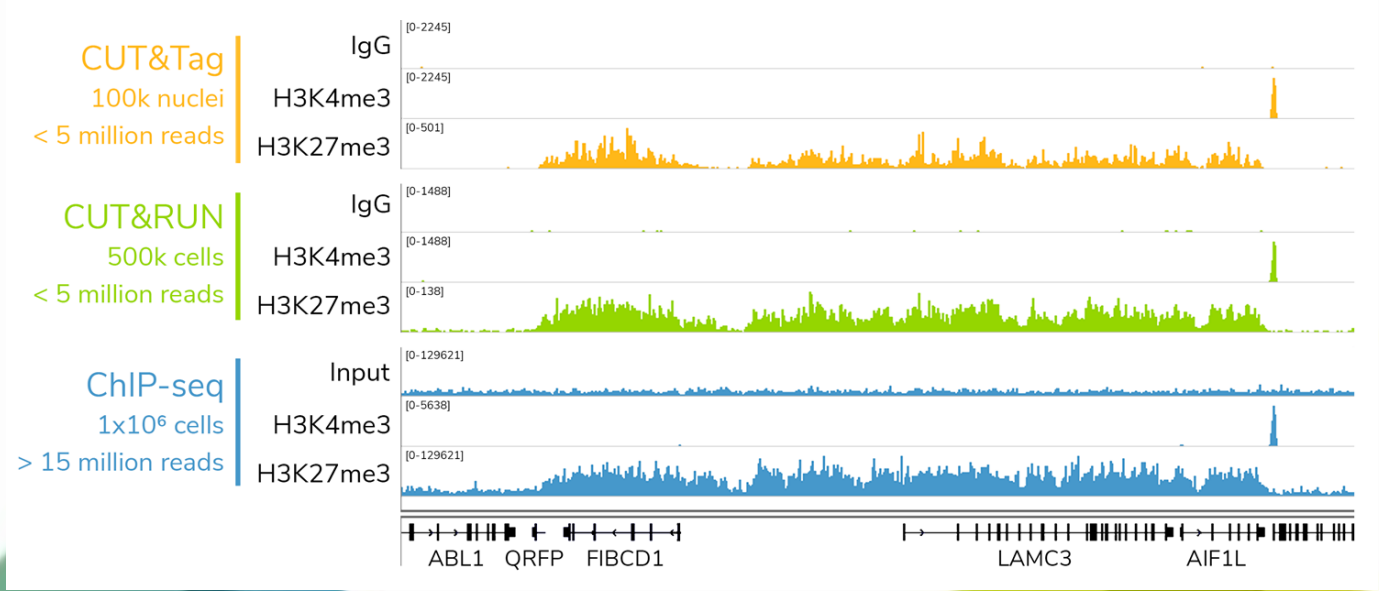

在 immobilize 的細胞/nuclei 直接加入 1st/2nd antibody, 利用獨特的 **pAG-Tn5(transposone) 技術**將 antibody labeled chromatin loci 區段切下來，並接上 adaptor，便於後續 PCR amplification 及 **NGS library** 製備。

=> 省略了傳統的 sonication fragmentation 或 enzyme digestion 以及 immunoprecipitation 和 **NGS library 製備**等步驟。

| Catalog No. | Product Name |
|---|---|
| 15-1017 (50 RXN) | CUTANA™ pAG-Tn5 for CUT&Tag |
| 15-1117 (250 RXN) | |




**CUT&Tag DNA Size Distribution Analysis:** BioAnalyzer traces of DNA purified after CUT&Tag using IgG and H3K27me3 negative/positive control antibodies confirms pAG-Tn5 primarily enriches for mononucleosome fragments (~300 bp peak).




**CUTANA™ assays generate similar results at a fraction of the cell input and sequencing depth used in ChIP-seq.** A representative 300 kb region is shown for CUTANA assays compared to ChIP-seq using H3K4me3 and H3K27me3 antibodies (EpiCypher 13-0041 and 13-0030, respectively). IgG (EpiCypher 13-0042) and ChIP Input controls are shown for comparison (controls are scaled to the track with the highest signal in each approach). The number of K562 cells and total read depth for each experiment are indicated. **A minimum of 3-5 million reads are recommended for CUTANA assays, however in this experiment even low read depth for CUT&Tag produced high quality results.**