Epigenomic profiling solutions at MedGenome

Elucidating the epigenetic profile of cells in development and disease can provide critical insights into understanding the mechanisms of gene expression during these processes. Chromatin immunoprecipitation-sequencing (ChIP-seq) has been a mainstay for investigating protein-chromatin interactions. However, there are several limitations to this method and accurately establishing the transcriptional or chromatin state has been a challenging endeavor that usually requires a large number of cells, lengthy protocols, and high sequencing depth with no guarantee of clear results.

One alternative strategy developed to overcome some of the limitations of ChIP assays is Cleavage Under Targets and Tagmentation[1] (CUT&Tag). CUT&Tag assays take advantage of a Tn5 transposase that is fused with protein A, which directs the enzyme to the antibody bound target on chromatin. The Tn5 transposase is pre-loaded with sequencing adapters (generating an assembled protein A-Tn5 adapter transposome) to carry out antibody-targeted tagmentation. Adding Mg^{2+} activates the Tn5 transposase and results in adapter flanked fragments of DNA cut near the protein of interest. The workflow is compatible with next-generation sequencing (NGS) for high resolution, genome-wide profiles of histone post-translational modifications (PTMs) and chromatin-associated proteins (Figure 1). CUT&Tag can be used to profile the chromatin landscape at a single cell resolution with higher efficiency and lower sequencing depths than other available methods.

At MedGenome, we have tested and optimized the laboratory protocol and bioinformatics analysis pipeline to offer an end-to-end CUT&TAG service for epigenomic profiling.

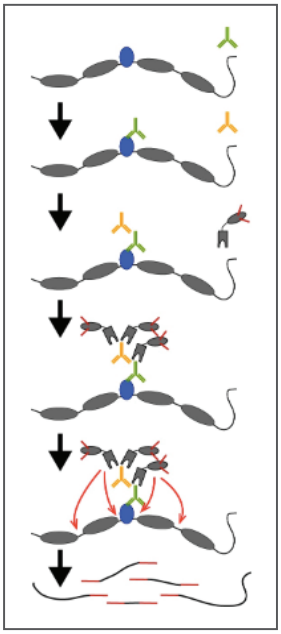

Figure 1. *Schematic of CUT&Tag technology.*

CUT&Tag technique overview adapted from Kaya-Okur et al., 2019. Briefly, following antibody incubation, the transposome with Tn5 is added, resulting in cleavage of DNA and addition of sequencing adapters (red) to DNA fragments (gray).

Sample Types & Requirements
• Fresh or frozen cells/tissue
• 50 k-500 k viable cells per reaction, including a negative control
• Primary antibody against histone mark or transcription factor
• Secondary antibody (species-specific)
• Shipping- cryopreserved cells and all antibodies should be shipped on dry ice

Experimental Workflow

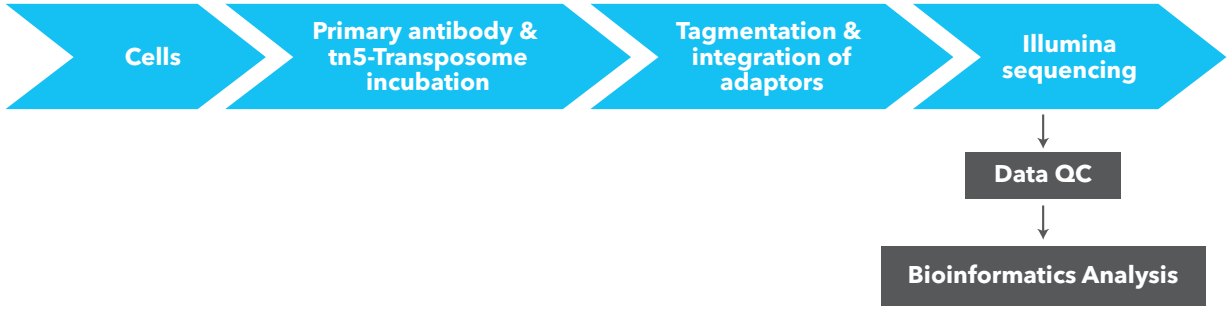

Figure 2. *CUT&Tag experimental workflow.*

Results

We performed CUT&Tag against H3K27me2, a histone residue that is often modified for epigenetic regulation. Starting with K562 cells, we used a primary antibody against H3K27me2, followed by the unique secondary antibody that produces the transposome. Thus in intact cells, we were able to specifically target the chromatin regions bound by H3K27me2. The protein bound DNA was simultaneously cut and then tagged with adapters for efficient sequencing. After clean up and QC, we then proceeded with standard library prep for Illumina sequencing and bioinformatics analysis

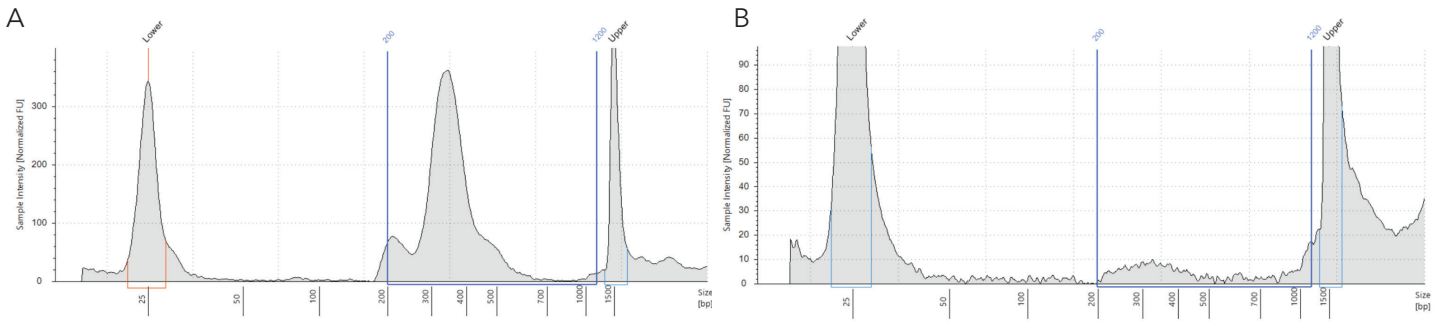

Figure 3. *Representative library QC for H3K27me2 (A) and IgG negative control (B)*

Sequencing Specs :

- Paired-end 25 bp reads
- 4 million reads per sample

Analysis

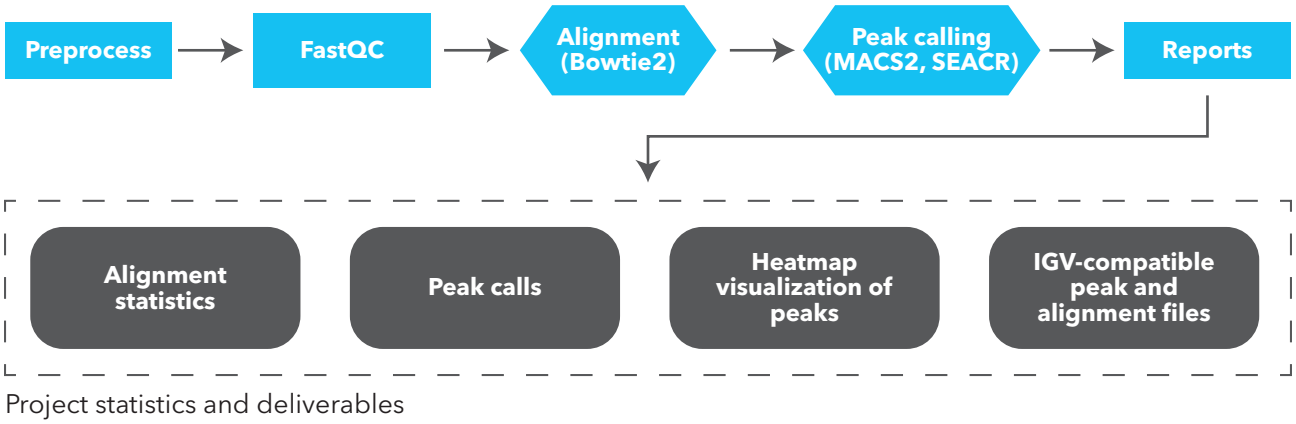

Figure 4. *CUT&Tag bioinformatics analysis workflow*

Using well-established pipelines for alignment (Bowtie2[2]) and peak calling (MACS2[3] & SEACR[4]), we can generate various data outputs to visualize protein bound regions of DNA.

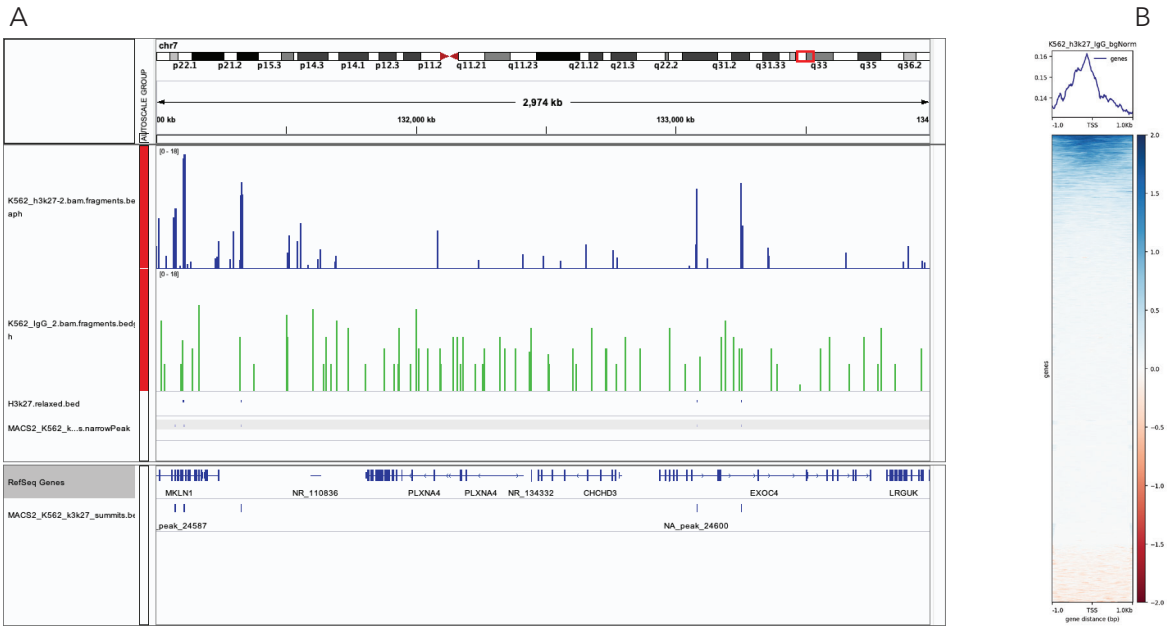

Figure 5: *Representative outputs of H3K27me2 bioinformatics analysis (A) Peak calling visualization showing H3K27me2 bound regions of the genome (blue) and the background IgG noise (green). (B) Heatmap of H3K27me2 occupancy near transcriptional start sites (TSS).*

In our experience, CUT&Tag can be performed in a single tube, in a single day with low numbers of cells and still provide sufficient captured chromatin for sequencing. In fact, with the high quality signal from CUT&Tag, we can confidently call protein-bound DNA regions with as little as 4 million reads per sample. Using this technique, we have established an efficient, validated protocol for studying epigenetic marks important for gene regulation.

Services for CUT&Tag Projects

End to End Sequencing	Basic Analysis Service	Advanced Single Cell Analysis deliverables
• Project Design • Antibody incubation and tagmentation procedures • Library Prep and Library QC • Illumina Sequencing and Data QC	• Initial output files including FastQ files, bam files, bedgraph and bed files • Peaks called by MACS2[3] and SEACR[4] • Heatmap visualization of peaks around transcription start sites (TSS) for each sample.	• Comparative analysis with other epigenomic data, such as ChIP seq, ATAC seq, FAIRE seq, as well as RNA seq data.

References

1. Kaya-Okur et al. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat Commun.* 2019 Apr 29;10(1):1930. doi: 10.1038/s41467-019-09982-5.
2. Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. *Nature Methods.* 2012, 9:357-359.
3. Zhang et al. Model-based Analysis of ChIP-Seq (MACS). *Genome Biol* (2008) vol. 9 (9) pp. R137
4. Meers, M.P. et al. Peak calling by Sparse Enrichment Analysis for CUT&RUN chromatin profiling. *Epigenetics & Chromatin* 12, 42 (2019). https://doi.org/10.1186/s13072-019-0287-4
5. Ramírez et al. "deepTools2: a next generation web server for deep-sequencing data analysis." *Nucleic Acids Research* (2016): gkw257