VALIDATION DATA

CUT&Tag Methods

6xHis-pAG-Tn5 was charged with EpiCypher's standard adapters (see "Technical Information" for EpiCypher 15-1017) using the loading protocol described in Application Notes above. CUT&Tag was performed on 100k native K562 nuclei with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0041), or H3K27me3 (EpiCypher 13-0055) antibodies using CUTANA™ Uncharged 6xHis-pAG-Tn5 and the CUTANA™ CUT&Tag Kit v1 (EpiCypher 14-1102/14-1103). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 4.8 million reads (IgG), 9.0 million reads (H3K4me3), and 5.4 million reads (H3K27me3). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

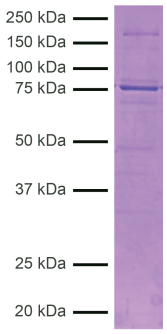

FIGURE 1 Protein gel data. CUTANA Uncharged 6xHis-pAG-Tn5 (1 µg) was resolved via SDS-PAGE and stained with Coomassie blue. The migration and molecular weight of the protein standards are indicated.

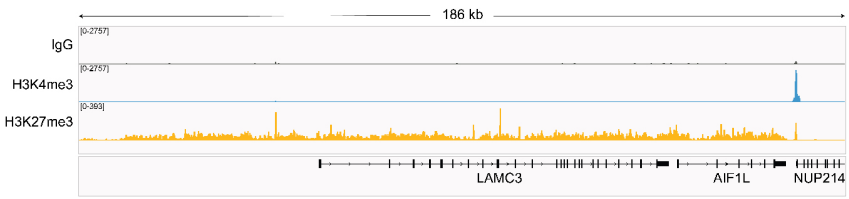

FIGURE 2 Functional validation in CUT&Tag. CUT&Tag was performed as described above. Representative sequencing tracks obtained using CUTANA Uncharged 6xHis-pAG-Tn5 show a 186 kb close up view of the LAMC3 gene. CUTANA Uncharged 6xHis-pAG-Tn5 produced clear peaks with genomic distribution profiles consistent with the known biological functions of H3K4me3 and H3K27me3 as well as minimal background in the IgG negative control.

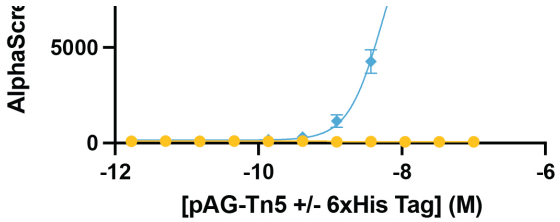

FIGURE 3 Functional validation of 6xHis binding. Various concentrations of CUTANA Uncharged 6xHis-pAG-Tn5 were incubated with biotinylated recombinant nucleosomes (rNuc; EpiCypher 16-0006). Uncharged pAG-Tn5 lacking a 6xHis tag (EpiCypher 15-1025) was used as a negative control. AlphaScreen technology (PerkinElmer/Revvity) was used to confirm functional 6xHis binding by using Nickel Chelate Donor Beads (Revvity AS101) to bind the 6xHis tag and Streptavidin Acceptor Beads (Revvity AL125) to bind biotin-rNuc. Signal (AlphaScreen counts) indicates 6xHis-pAG-Tn5 complexed with biotin-rNuc. No signal was observed in pAG-Tn5 lacking a 6xHis tag.