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Genome-wide quantification of TF binding at single DNA molecule resolution

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ABSTRACT

Precise control of gene expression requires the coordinated action of multiple factors at cis-regulatory elements (CREs). We recently developed Single Molecule Footprinting (SMF) to simultaneously resolve the occupancy of multiple proteins including Transcription Factors (TFs), RNA Pol II (Pol II) and nucleosomes on single DNA molecules genome-wide. The technique combines the use of cytosine methyltransferases to footprint the genome with bisulfite sequencing to resolve TF binding patterns at CREs. DNA footprinting is performed by incubating permeabilized nuclei with recombinant methyltransferases. Upon DNA extraction, whole genome or targeted bisulfite libraries are prepared and loaded on Illumina sequencers. The protocol can be completed in 4-5 days. Analysis can be performed in 2 days using a dedicated R package. The protocol can be executed in any laboratory with access to high- throughput sequencing. Our method can be used to analyse how TFs cooperate and antagonize to regulate transcription.

ATTACHMENTS

Kleinendorst_et_al_preprint.pdf

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