NicE-seq: high resolution open chromatin profiling

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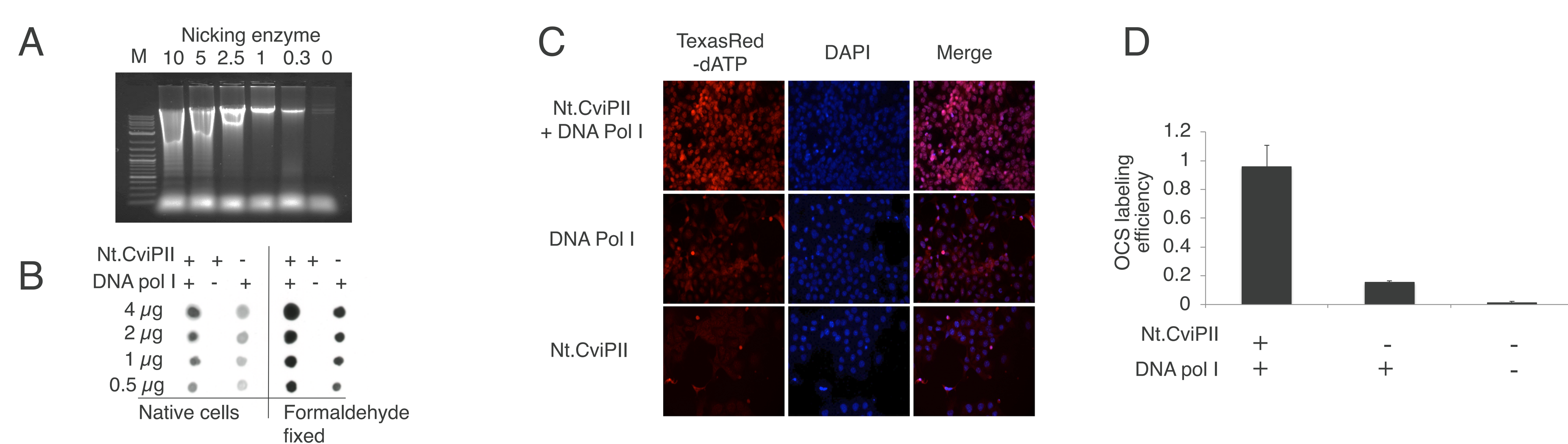
Abstract

Open chromatin profiling integrates information across diverse regulatory elements to reveal the transcriptionally active genome. Tn5 transposase and DNase I sequencing-based methods prefer native or high cell numbers. Here, we describe NicE-seq (nicking enzyme assisted sequencing) for high-resolution open chromatin profiling on both native and formaldehyde-fixed cells using 25 to 250K cells. Lower cell numbers (25 and 250 cells) require lower amounts of enzyme mix. NicE-seq captures and reveals open chromatin sites (OCSs) and transcription factor occupancy at single nucleotide resolution, coincident with DNase hypersensitive and ATAC-seq (Tn5 transposase based) sites at a low sequencing burden. OCSs correlate with RNA polymerase II occupancy and active chromatin marks, while displaying a contrasting pattern to CpG methylation. Decitabine-mediated hypomethylation of HCT116 displays higher numbers of OCSs.

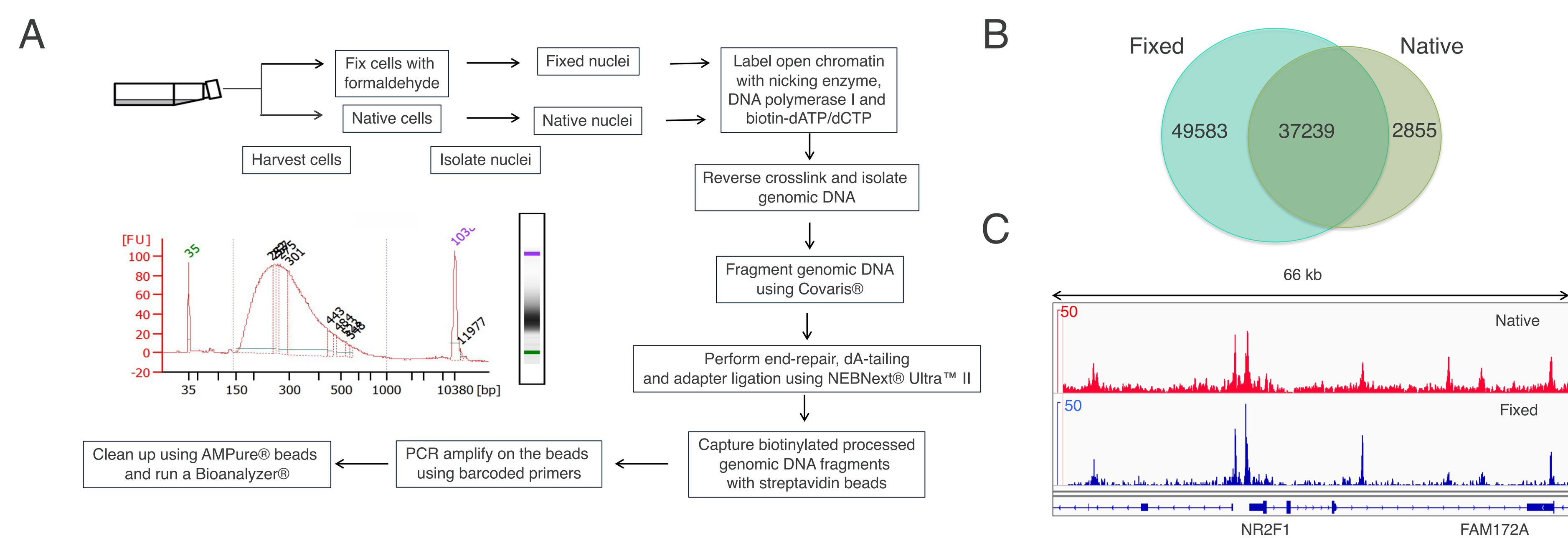
Results

Nicking enzyme mediated tagging of the open chromatin



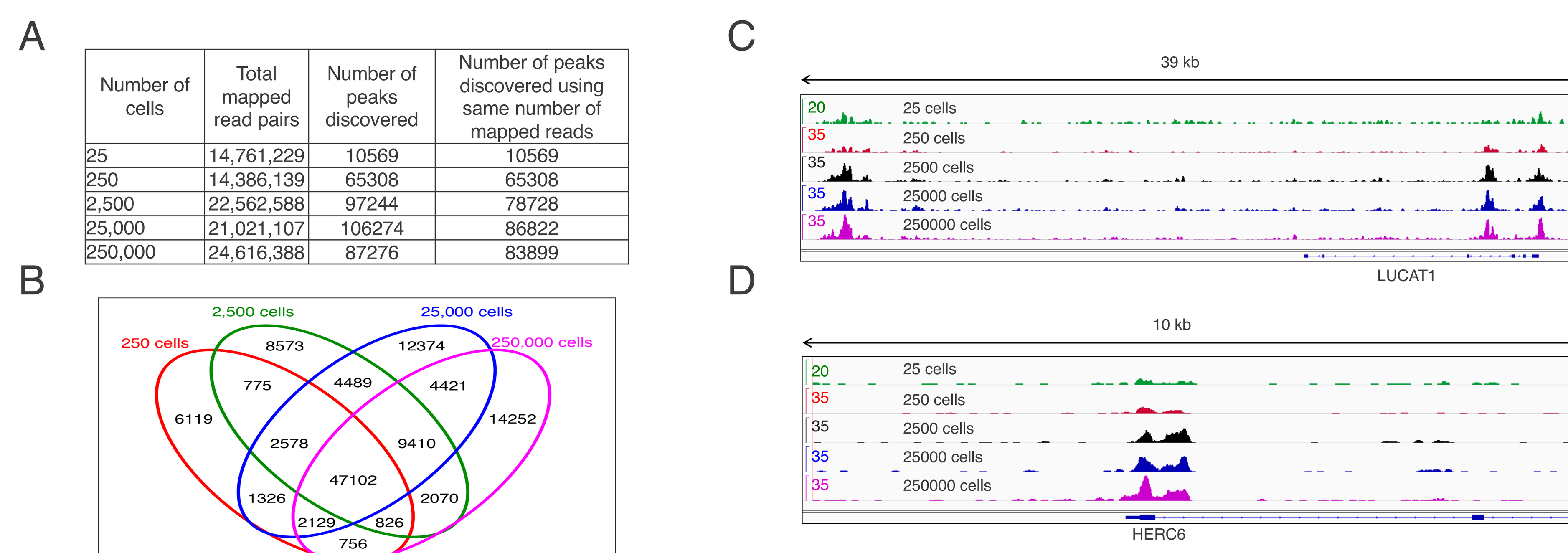
A: Nicking of crosslinked chromatin using varying amounts of Nt.CviPII showing increased nicking at higher enzyme concentration. B: Dot blot showing labeling of open chromatin by Nt.CviPII nicking enzyme in both native and formaldehyde-fixed HCT116 cells. The level of labeling was revealed using HRP-conjugated goat anti-biotin antibody. C: Open chromatin labeling in fixed HeLa cells using dNTPs supplemented with TexasRed-dATP. DNA staining was performed using DAPI (blue) and TexasRed stain (red) represents labeled OCSs. Magenta stain (Merge) represents the colocalization. D: Labeling efficiency of OCSs in all three assayed conditions. The y-axis represents the ratio of the intensity of the red pixels to the intensity of the blue pixels (OCS labeling efficiency).

NicE-seq (Nicking Enzyme assisted sequencing) for open chromatin capture



A: Schematic showing the different steps involved in preparation of libraries for NicE-seq. B: A Venn diagram showing the overlap between the OCSs identified using fixed and native HCT116 cells. C: A screenshot of the IGV browser showing the overlap between OCSs identified in native (top panel in red) and fixed (bottom panel in blue) HCT116 cells in a 66-kb window.

NicE-seq using 25-250,000 cells

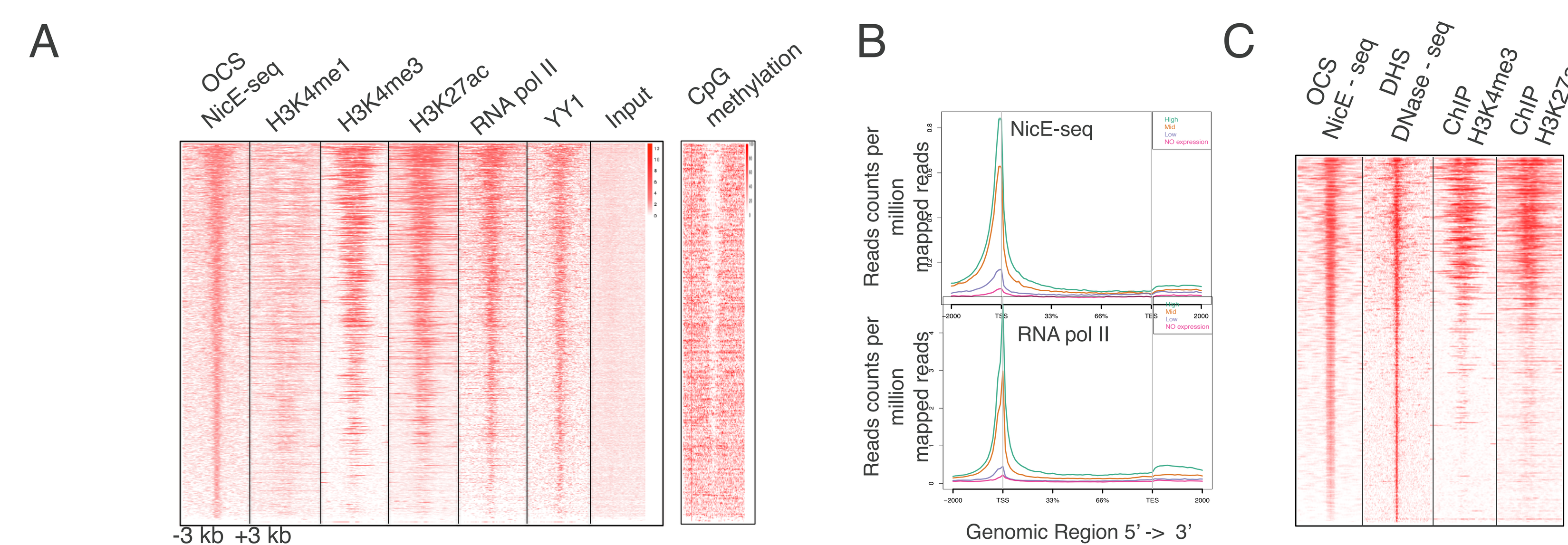


Number of cells	Total mapped read pairs	Number of peaks discovered	Number of peaks discovered using same number of mapped reads
25	14,761,229	10569	10569
250	14,386,139	65308	65308
2,500	22,562,588	97244	78728
25,000	21,021,107	106274	86822
250,000	24,616,388	87276	83899

A: The number of total mapped reads and the number of peaks identified before and after normalizing the total mapped reads to the level of 250 cells. B: A Venn diagram showing the overlap between the OCSs identified from 250 to 250,000 cells. C: A screenshot of the IGV browser showing the alignment of identified OCSs from 25 to 250,000 cells in a 39-kb window. D: A screenshot of the IGV browser showing the alignment of identified OCSs from 25 to 250,000 cells in a 10-kb window

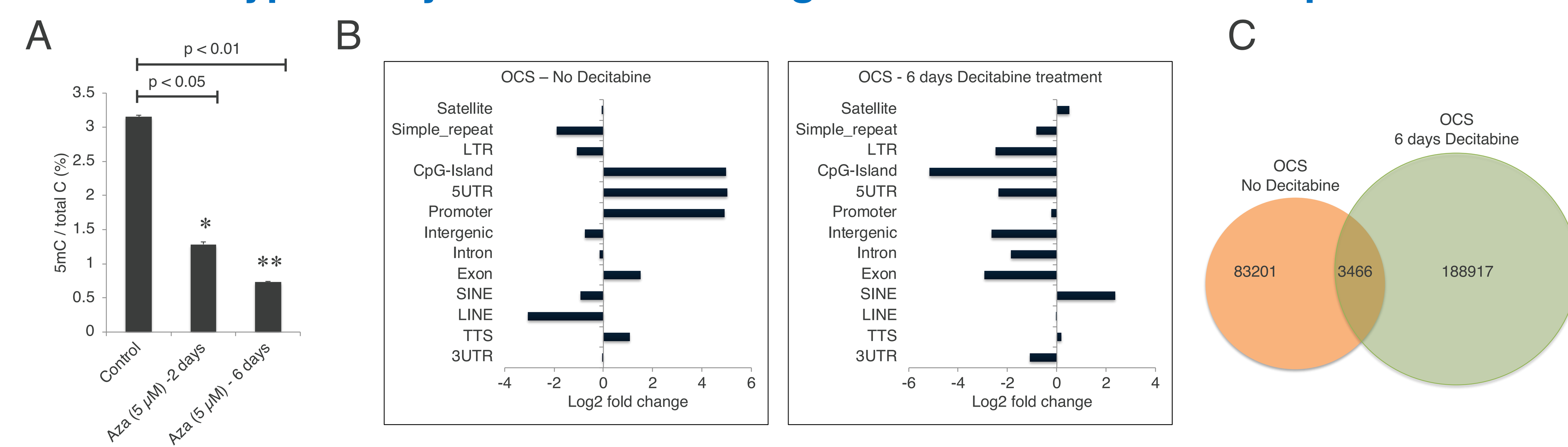
Results

NicE-seq identifies transcriptionally active open chromatin regions



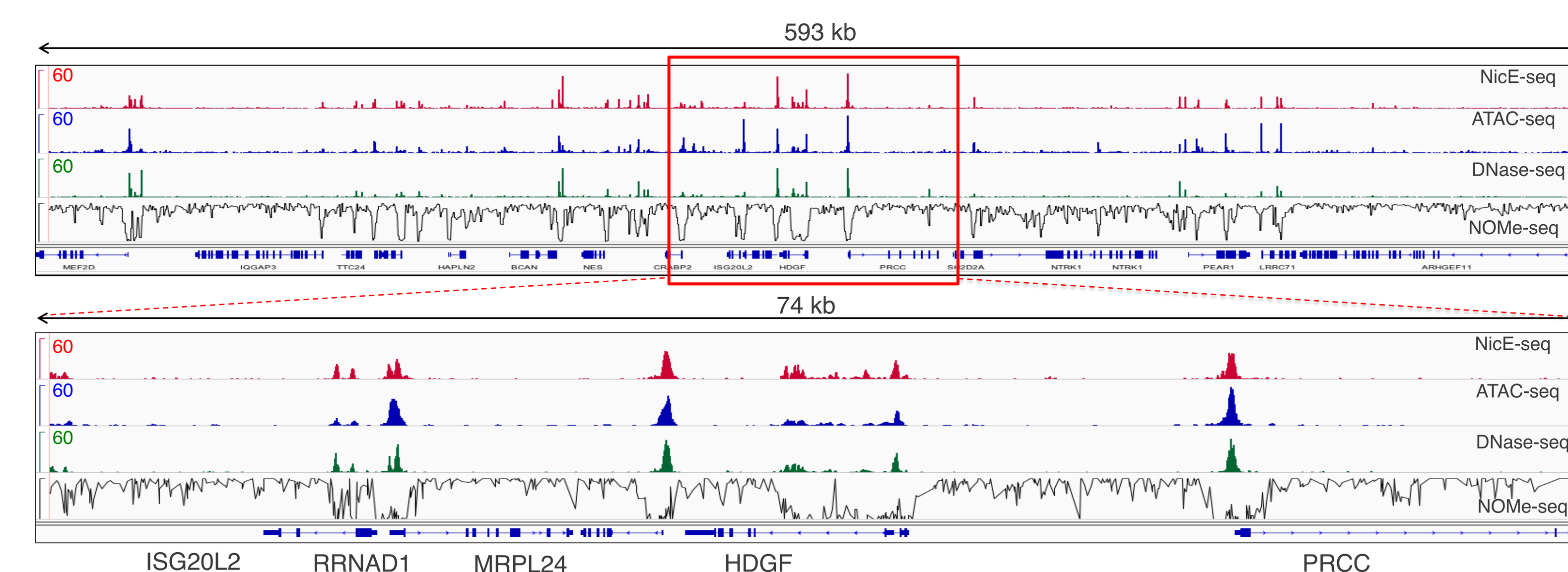
A: A heat map showing the correlation of NicE-seq peaks in a ±3-kb window with occupancy of H3K4me1, H3K4me3, H3K27ac, RNA pol II and YY1 across ChIP-seq data from ENCODE for HCT116 cells. Input was used to show lack of enrichment. Plotting a heat map using whole genome bisulfite sequencing data for HCT116 cells showed an inverse correlation between OCSs and CpG methylation. B: Metagene plot showing the distribution of sequencing tag densities for high (turquoise), medium (orange), low (purple), and no expression (pink) genes (based on the expression level in the RNA-seq data set) in the NicE-seq experiment (top panel) and ChIP RNA pol II experiment (bottom panel) in a 2-kb window upstream of the transcription start site (TSS) and downstream of the transcription termination site (TTS). C: A heat map showing the correlation of peaks common to NicE-seq and DNase-seq with ChIP H3K4me3 and ChIP H3K27ac peaks in a ±3-kb window.

DNA hypomethylation of HCT116 genome correlates with open chromatin



A: Liquid chromatography–mass spectrometry analysis showing loss of DNA methylation after treatment of HCT116 cells with 5 μM 5-aza-2′-deoxycytidine (Decitabine, an anti-cancer chemotherapeutic drug used for myelodysplastic syndromes like Acute myeloid leukemia) for 2 and 6 days. B: The log2 fold-change of OCS peaks in different genomic regions for samples treated with and without 6 days of 5 μM 5-aza-2′-deoxycytidine treatment. C: A Venn diagram showing the overlap between the OCSs identified with and without 6 days of 5 μM 5-aza-2′-deoxycytidine treatment.

Comparison of NicE-seq with existing chromatin profiling techniques



A screenshot of the IGV browser (top panel: window of 593 kb, bottom panel: window of 74 kb) showing the distribution of open chromatin peaks identified by NicE-seq, ATAC-seq, DNase-seq and NOMe-seq. NicE-seq demonstrated robust sensitivity even at 20 million reads as apposed to 100 million reads for ATAC-seq and 40 million reads for DNase-seq.

Conclusions

- NicE-seq is a novel technique that can capture open chromatin in the cellular context
  - Compatible with native and formaldehyde fixed cells
  - Identifies unique and divergent peaks on native or fixed chromatin
  - Has a dynamic range of 25-250,000 cells
  - Captures transcriptionally active open chromatin
- Accessible chromatin in cells can be visualized by NicE-see (Nicking Enzyme assisted seeing)

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