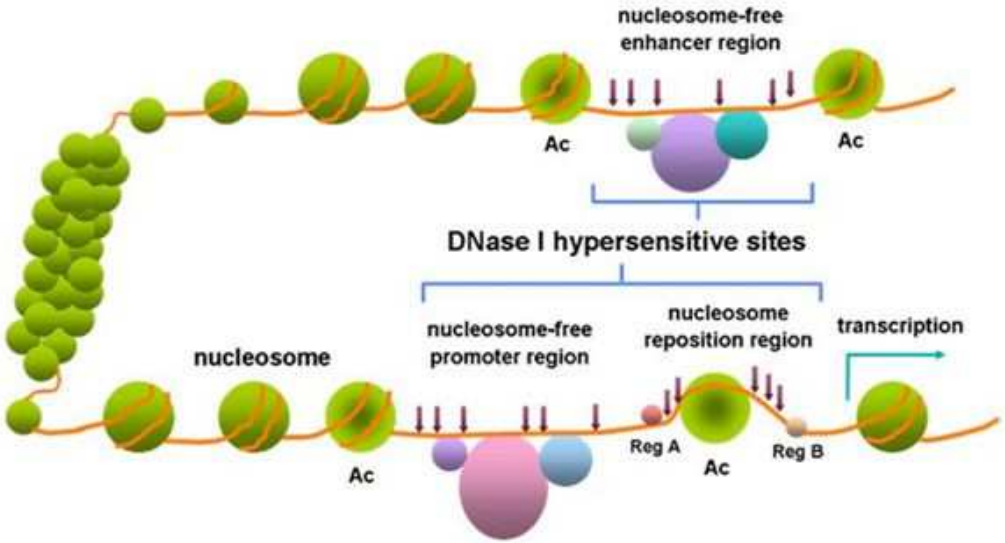
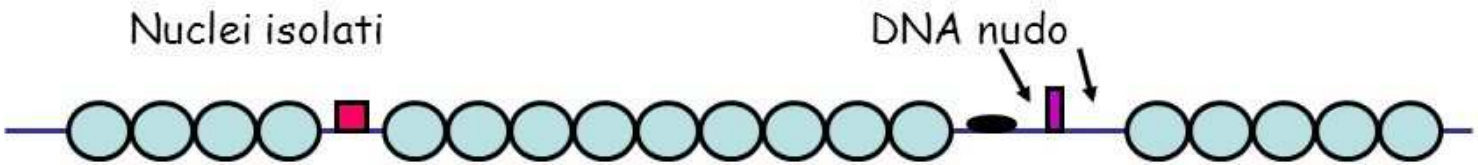
Saggio di ipersensibilita' alla DNasi I

- Regioni ipersensibili alla DNAsi I si trovano in connessione con geni attivamente trascritti:
  - nelle regioni codificanti
  - nelle regioni regolatrici
- Il saggio di ipersensibilita′ alla Dnasi I puo′ rivelare regioni regolatrici remote
- Le basi esatte per l′ipersensibilita′ non si conoscono (assenza di nucleosomi, distorsioni della doppia elica dovute a legame di fattori)
- Regioni di ipersensibilita′ alla Dnasi I sono state trovate in associazione a
  - Promotori
  - Enhancers
  - LCRs
  - silencers
  - MARs (Matrix Attachment Regions o Scaffold-Associated Regions)
  - insulators

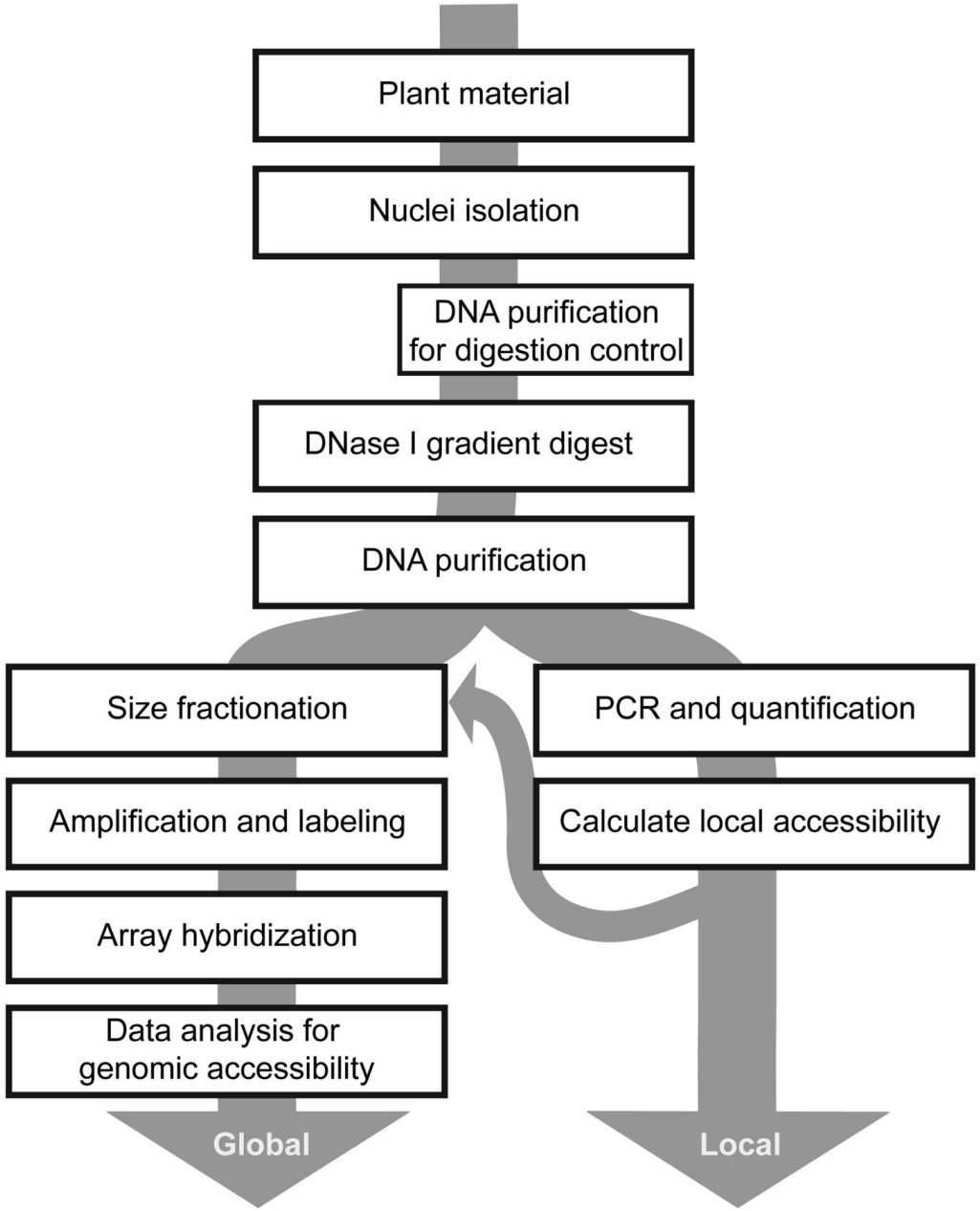


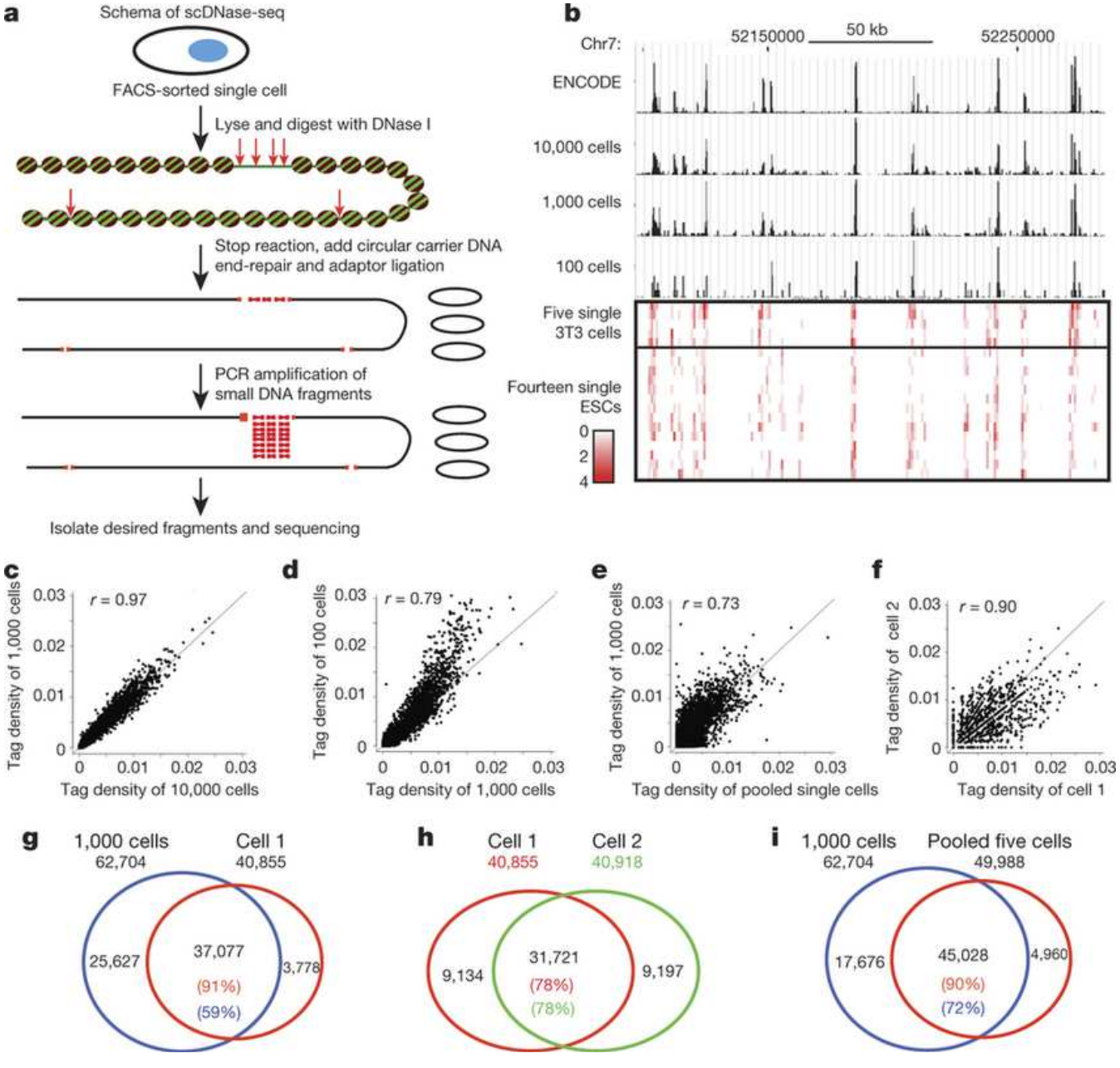
Come si dimostra che i nucleosomi sono esclusi da una regione?

Mappatura di siti ipersensibili alla DNasi I



Si usano quantità molto piccole di DNAsi I in modo da avere solo circa un taglio per molecola
Questo permette di tagliare solo il DNA nudo e non quello nucleosomale.
La cromatina viene poi deproteinizzata


Plant material
Nuclei isolation
DNA purification for digestion control
DNase I gradient digest
DNA purification
Size fractionation
Amplification and labeling
Array hybridization
Data analysis for genomic accessibility
Global
PCR and quantification
Calculate local accessibility
Local


a
Schema of scDNase-seq
FACS-sorted single cell
Lyse and digest with DNase I
Stop reaction, add circular carrier DNA
end-repair and adaptor ligation
PCR amplification of
small DNA fragments
Isolate desired fragments and sequencing
b
Chr7:
52150000
50 kb
52250000
ENCODE
10,000 cells
1,000 cells
100 cells
Five single
3T3 cells
Fourteen single
ESCs
0
2
4
c
r = 0.97
Tag density of 1,000 cells
Tag density of 10,000 cells
d
r = 0.79
Tag density of 100 cells
Tag density of 1,000 cells
e
r = 0.73
Tag density of 1,000 cells
Tag density of pooled single cells
f
r = 0.90
Tag density of cell 2
Tag density of cell 1
g
1,000 cells
62,704
Cell 1
40,855
25,627
37,077
(91%)
(59%)
3,778
h
Cell 1
40,855
Cell 2
40,918
9,134
31,721
(78%)
(78%)
9,197
i
1,000 cells
62,704
Pooled five cells
49,988
17,676
45,028
(90%)
(72%)
4,960

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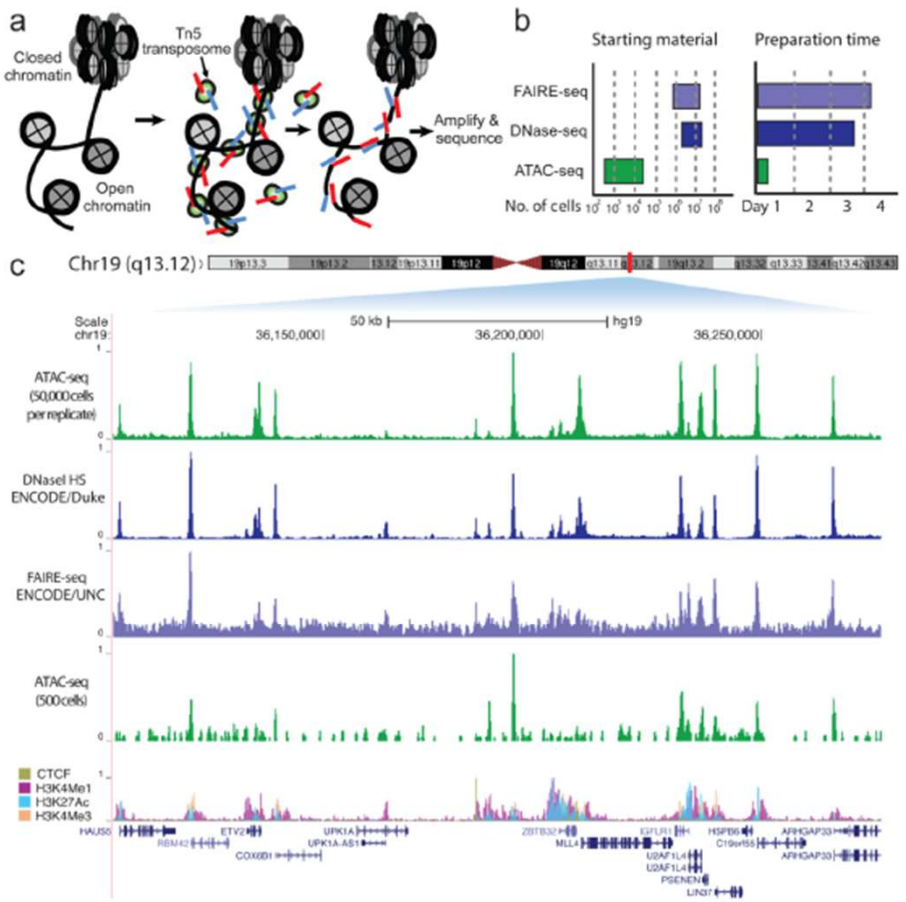

Figure 1. ATAC-seq is a sensitive, accurate probe of open chromatin state
(**a**) ATAC-seq reaction schematic. Transposase (green), loaded with sequencing adapters (red and blue), inserts only in regions of open chromatin (nucleosomes in grey) and generates sequencing library fragments that can be PCR amplified. (**b**) Approximate reported input material and sample preparation time requirements for genome-wide methods of open chromatin analysis. (**c**) A comparison of ATAC-seq to other open chromatin assays at a locus in GM12878 lymphoblastoid cells displaying high concordance. Lower ATAC-seq track was generated from 500 FACS-sorted cells.

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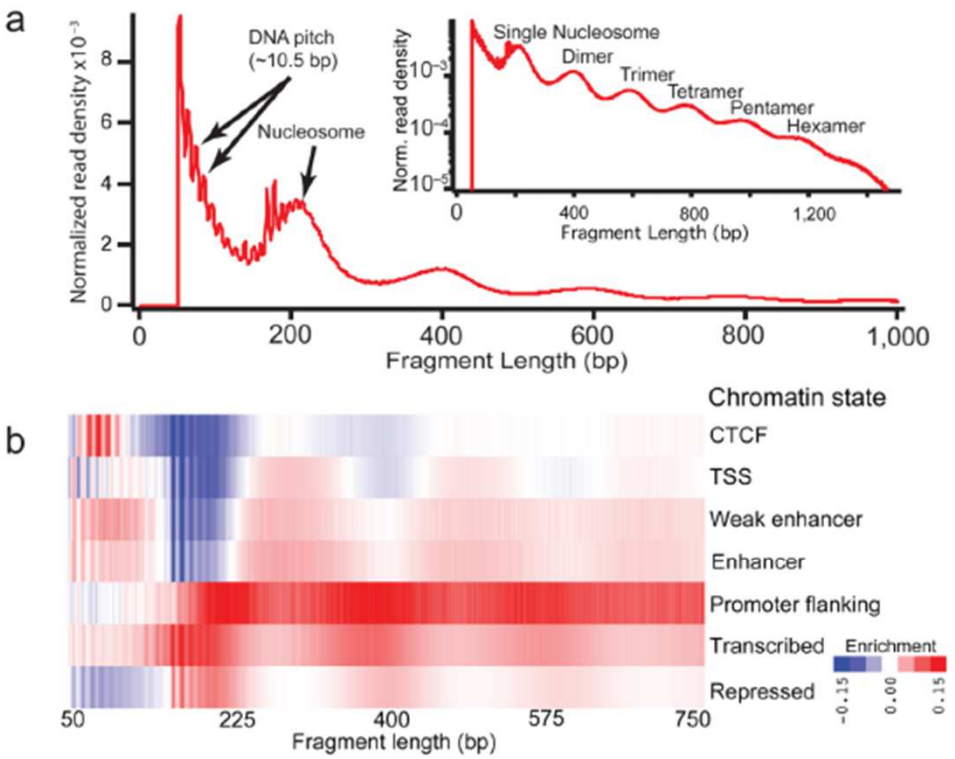

Figure 2. ATAC-seq provides genome-wide information on chromatin compaction
(**a**) ATAC-seq fragment sizes generated from GM12878 nuclei (red) indicate chromatin-dependent periodicity with a spatial frequency consistent with nucleosomes, as well as a high frequency periodicity consistent with the pitch of the DNA helix for fragments less than 200 bp. (Inset) log-transformed histogram shows clear periodicity persists to 6 nucleosomes. (**b**) Normalized read enrichments for 7 classes of chromatin state previously defined[17].

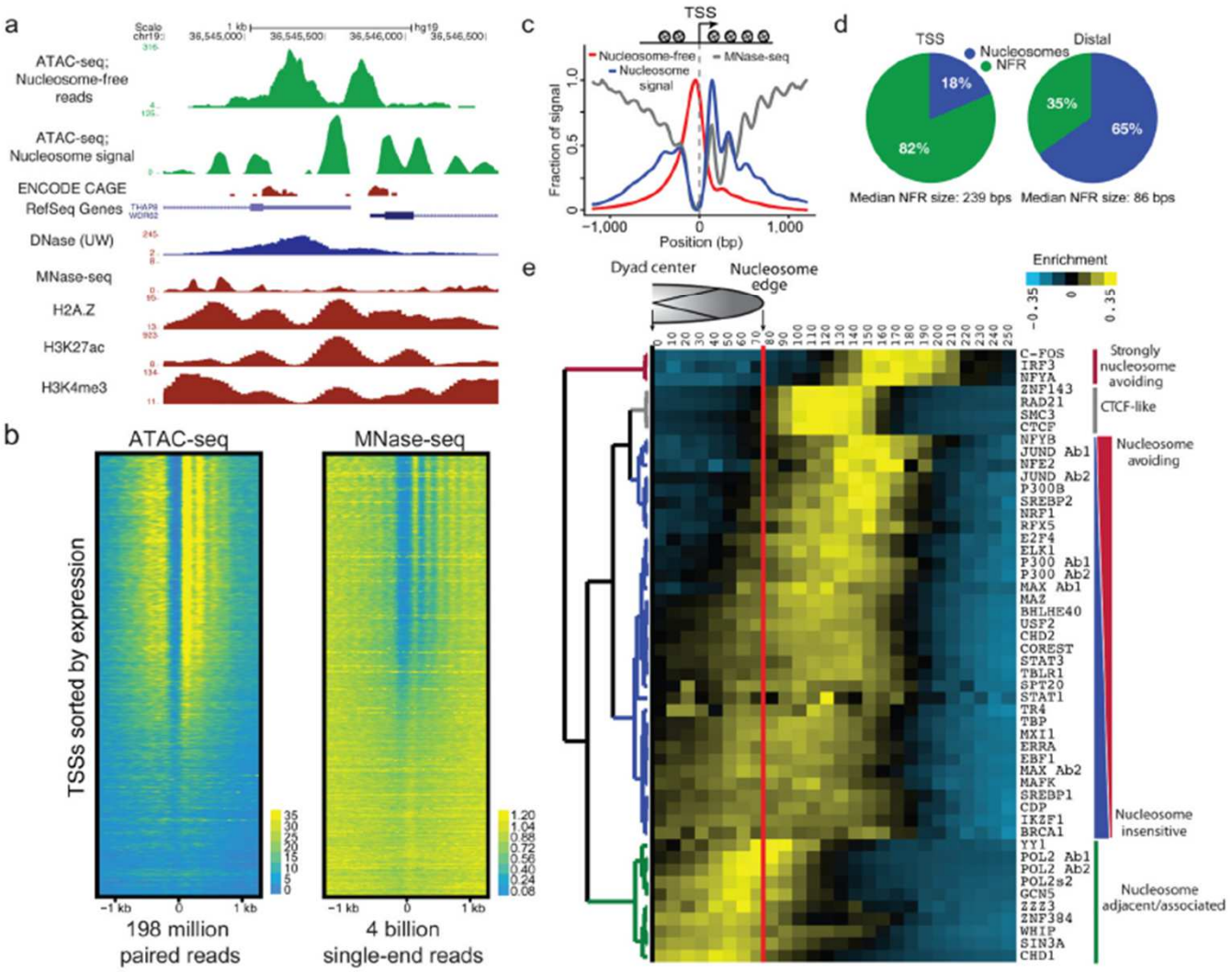

Figure 3. ATAC-seq provides genome-wide information on nucleosome positioning in regulatory regions

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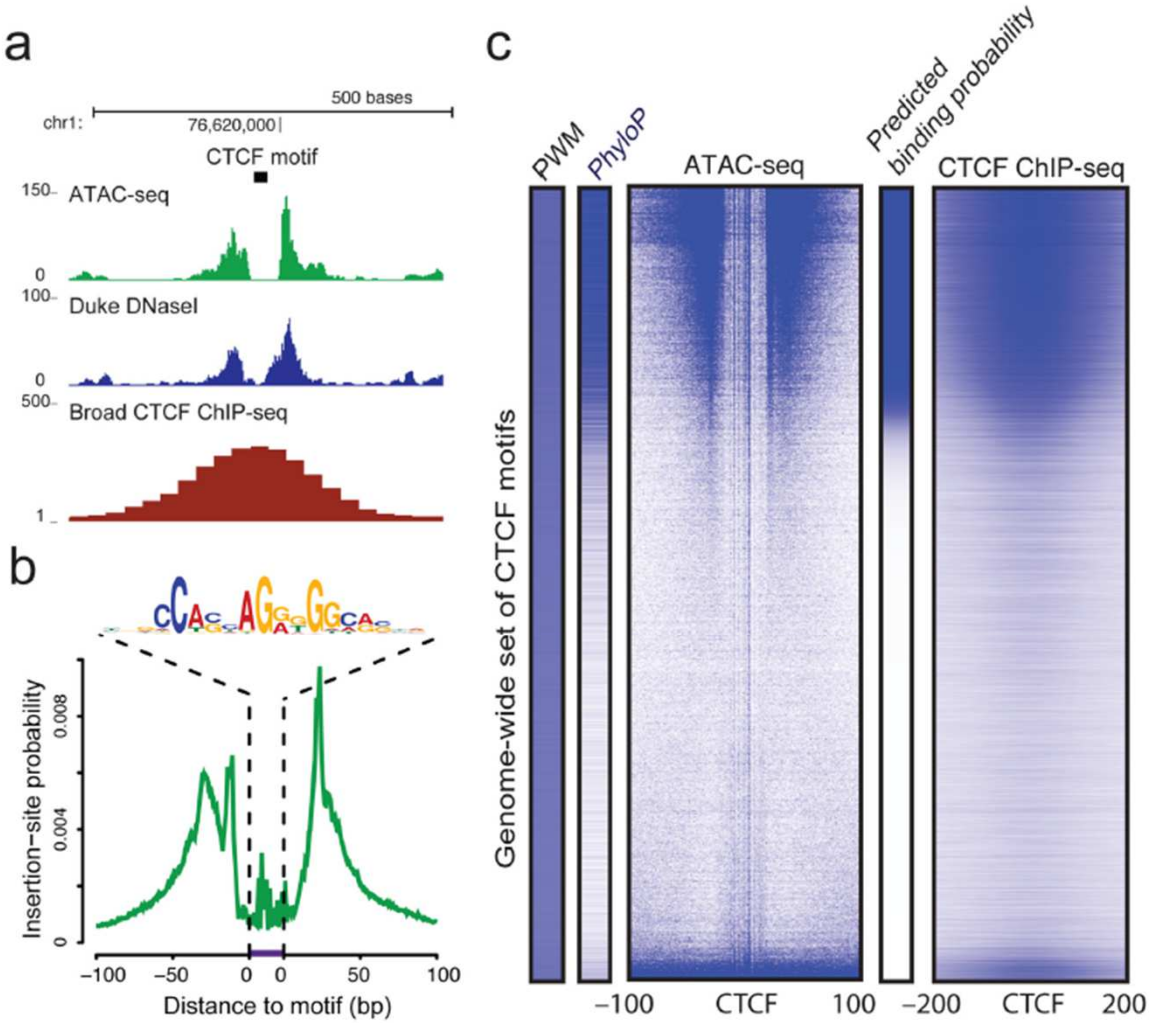

Figure 4. ATAC-seq assays genome-wide factor occupancy
(a) CTCF footprints observed in ATAC-seq and DNase-seq data, at a specific locus on chr1. (b) Aggregate ATAC-seq footprint for CTCF (motif shown) generated over binding sites within the genome (c) CTCF predicted binding probability inferred from ATAC-seq data, position weight matrix (PWM) scores for the CTCF motif, and evolutionary conservation (PhyloP). Right-most column is the CTCF ChIP-seq data (ENCODE) for this GM12878 cell line, demonstrating high concordance with predicted binding probability.

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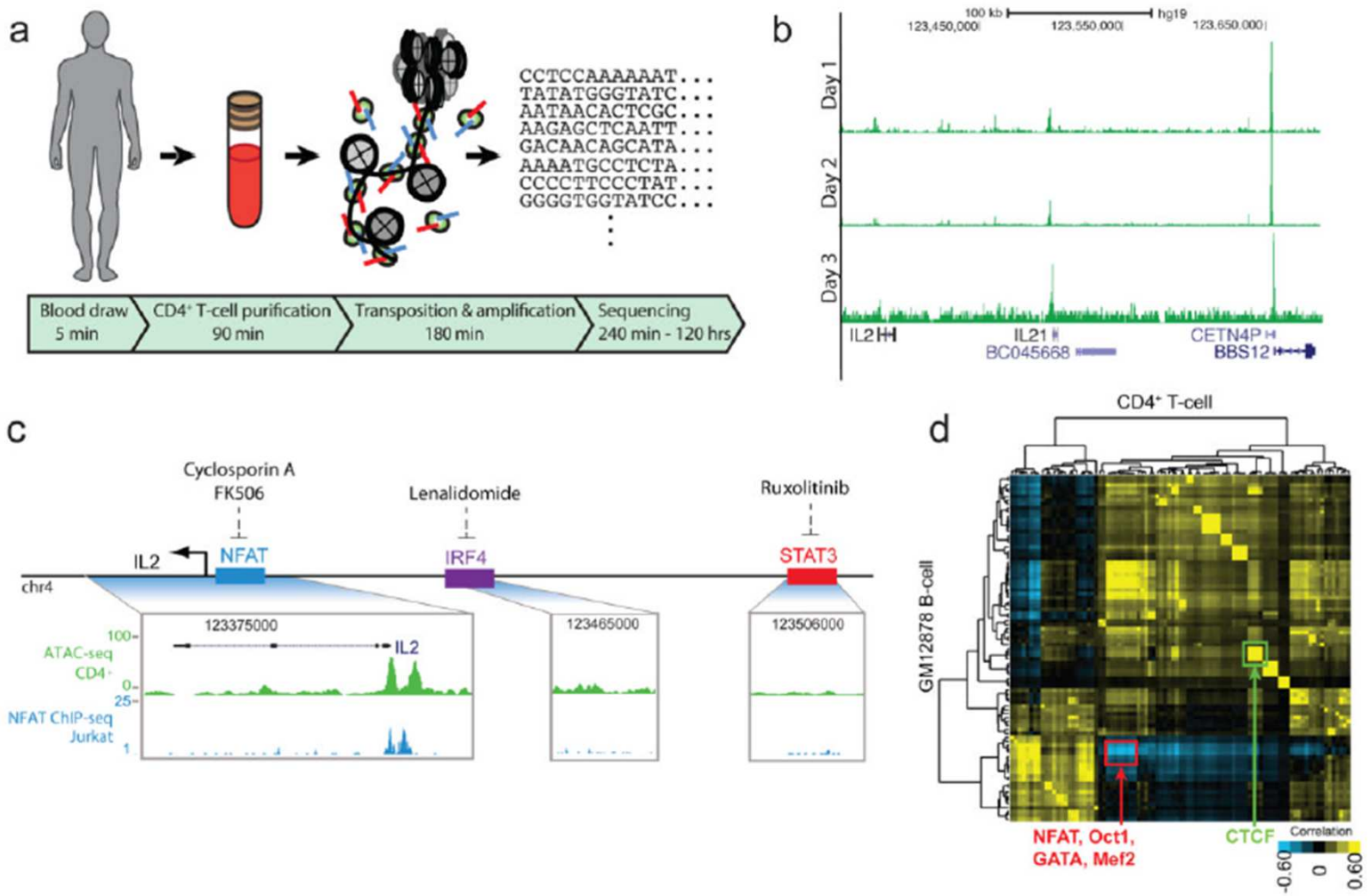
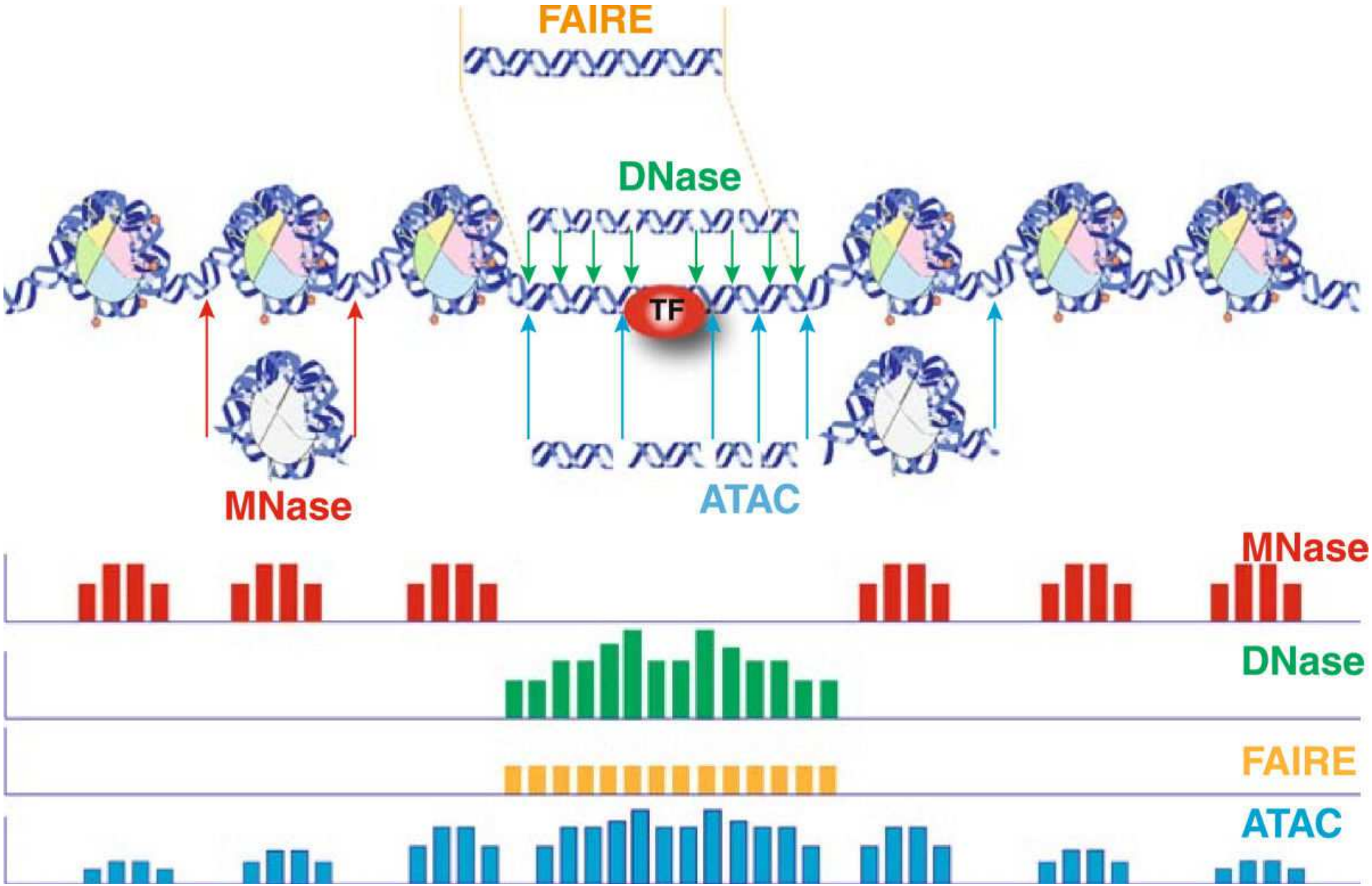


ATAC-seq enables real-time personal epigenomics


FAIRE
DNase
TF
MNase
ATAC
MNase
DNase
FAIRE
ATAC