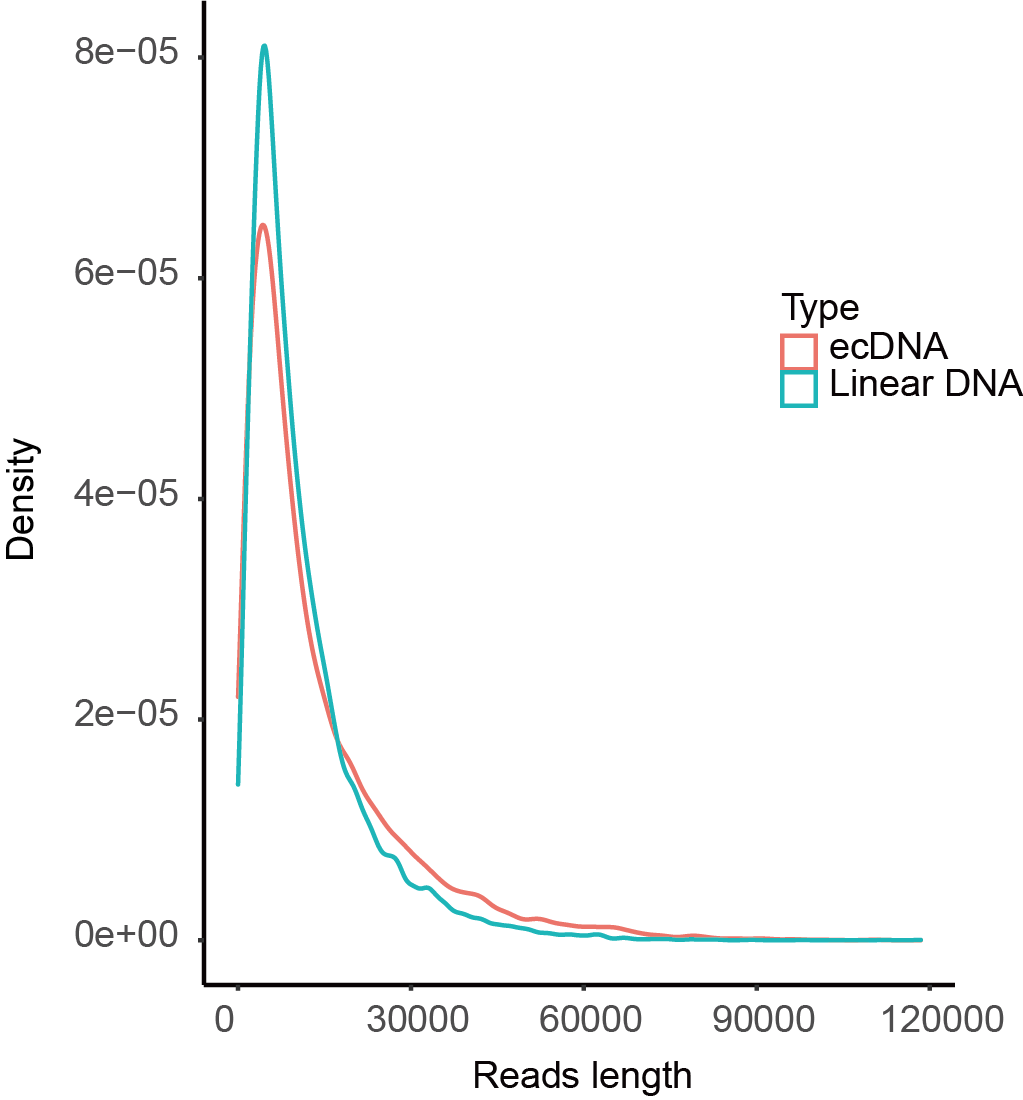
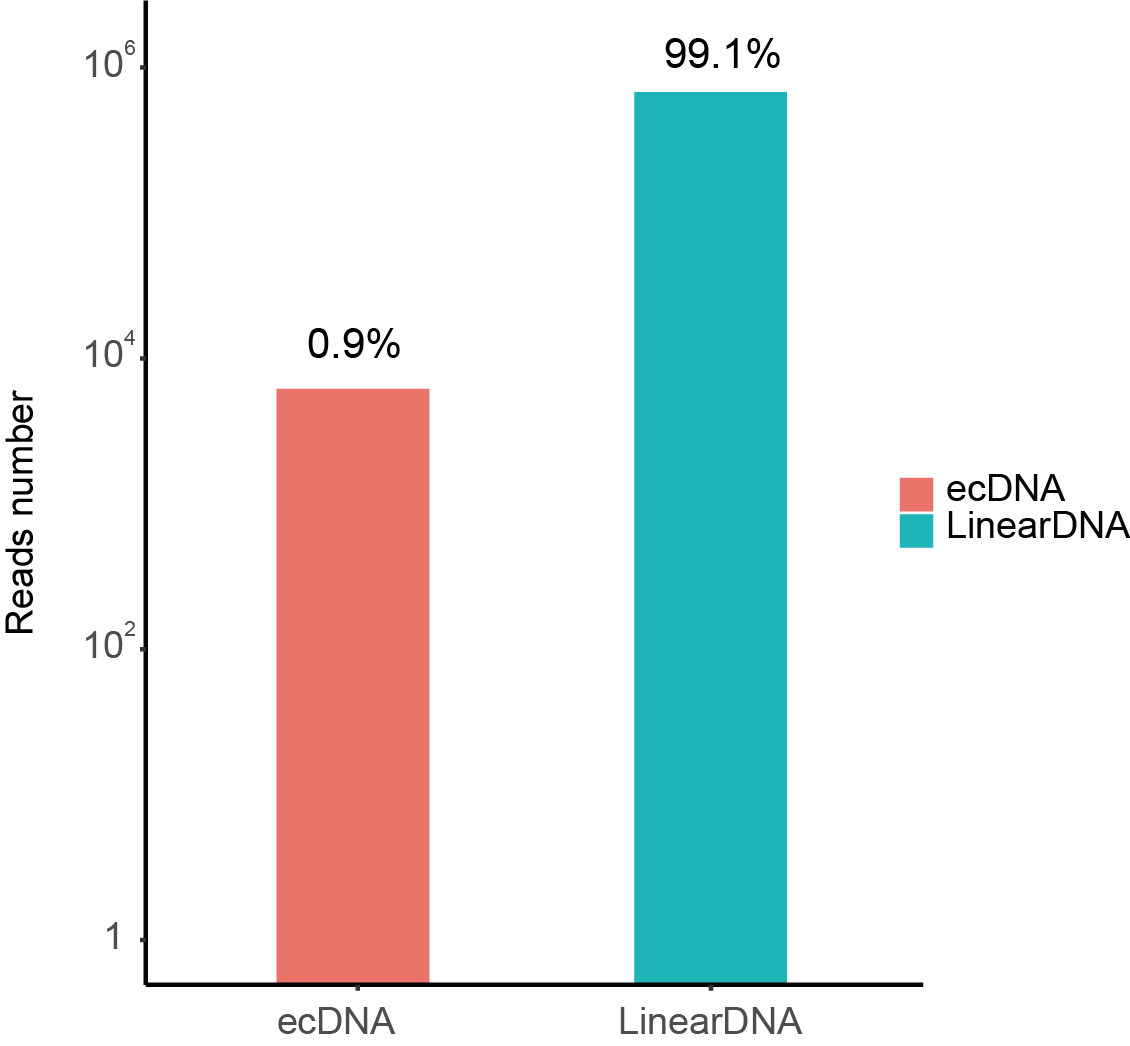
****

**Supplemental Figure 1. The length distribution of reads identified as ecDNAs or linear DNAs.**

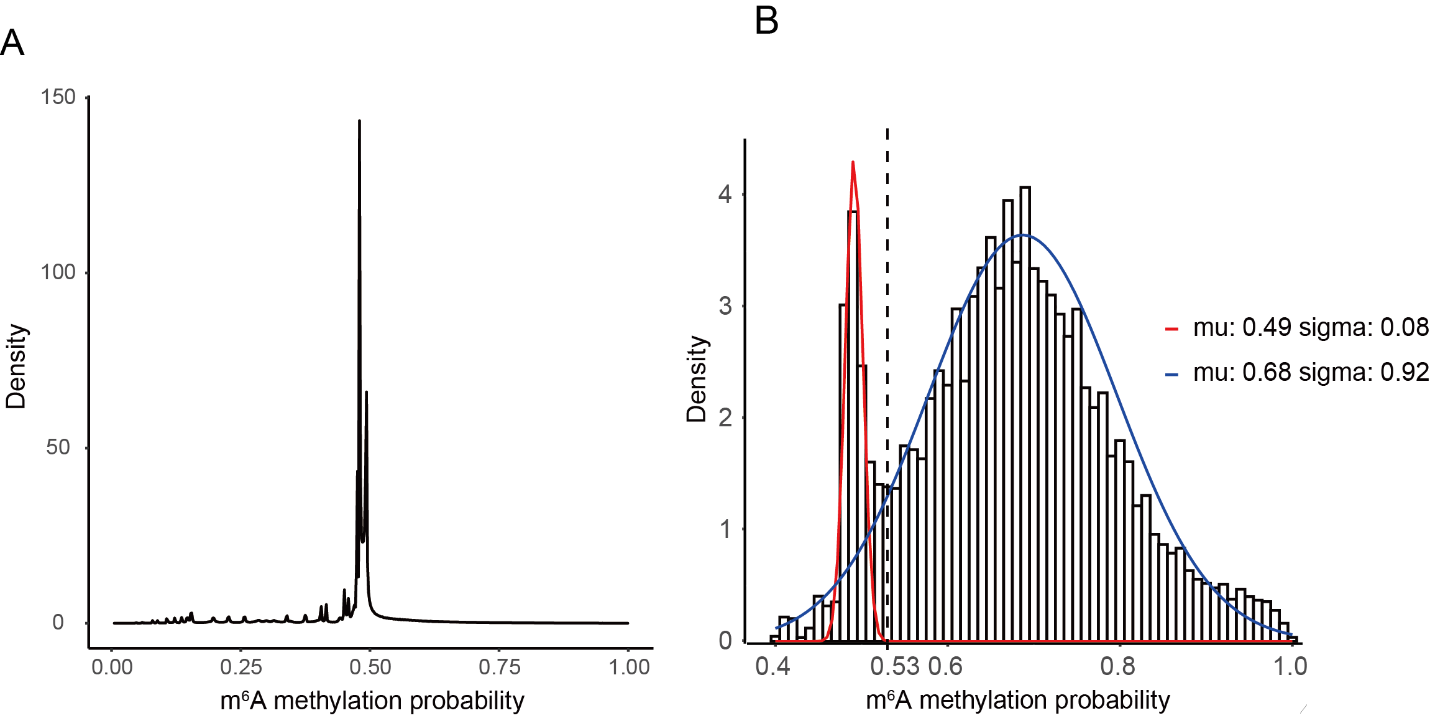
Graphical user interface

Description automatically generated with medium confidence

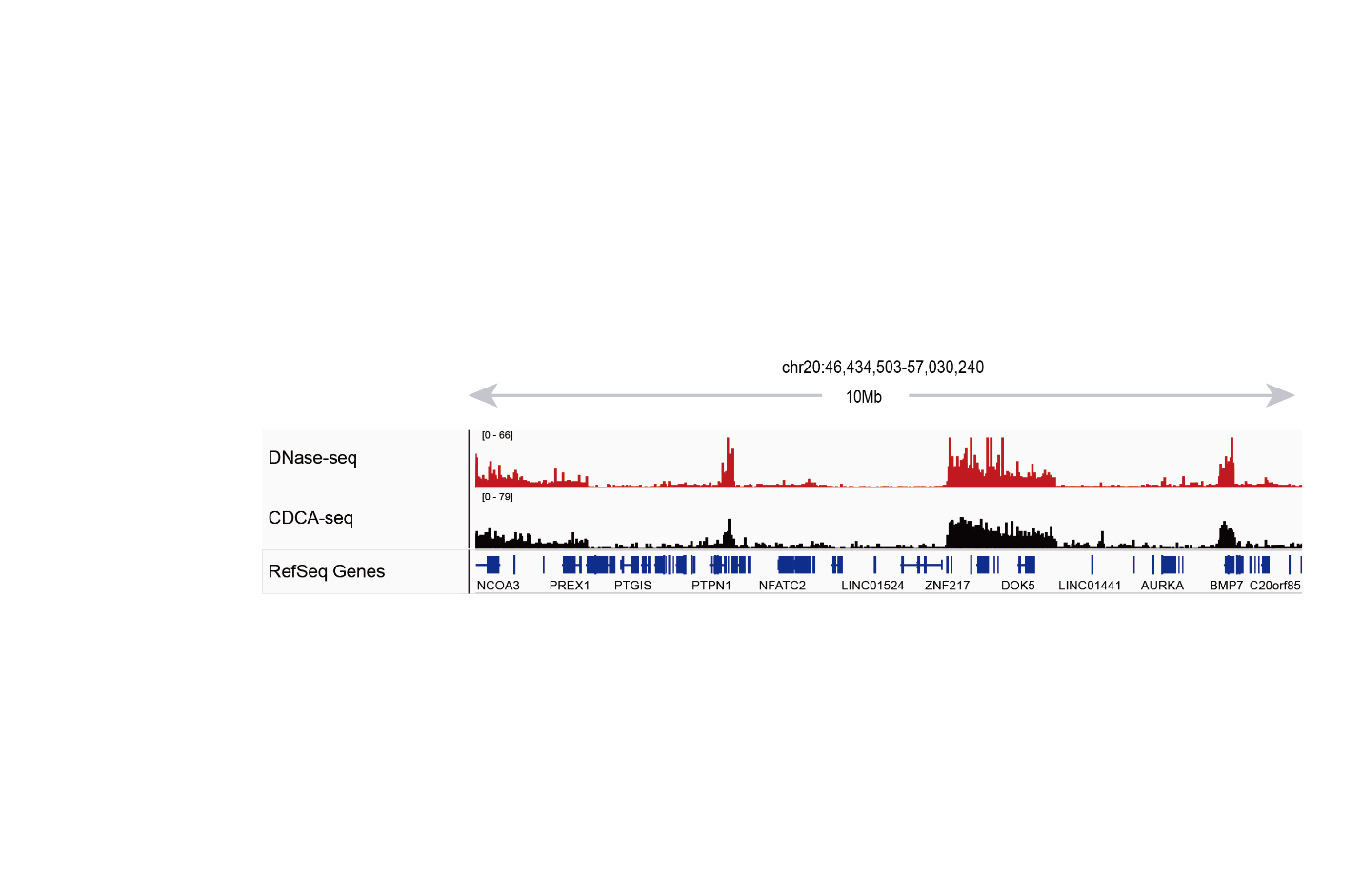
**Supplemental Figure 2. The PCR validation of the identified ecDNAs.** The red box indicates the expected DNA size. We found many other structure variations around the junction region, so the PCR targeted fragments are not unique.



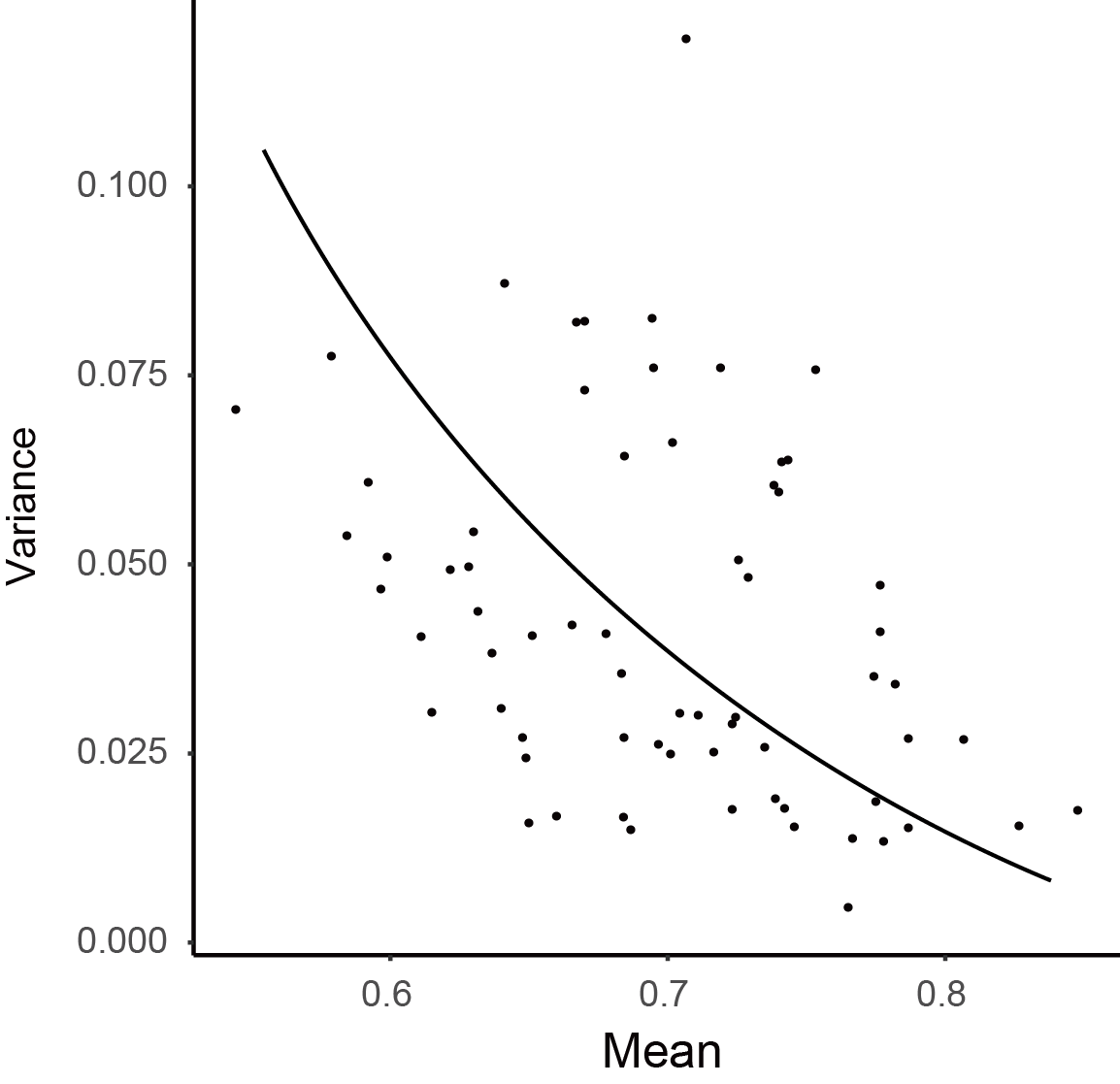
**Supplemental Figure 3. The counts of reads identified as ecDNAs or linear DNAs.**



**Supplemental Figure 4. The m6A possibility distribution of the treated and non-treated samples.** The signal data were transformed to the m6A sites with possibility on each site. The m6A possibility distribution of the non-treated negative sample, which has no detectable m6A, was below 0.52. The two peaks (0.5,0.7) could be classified as positive sites and negative sites. The cut-off value was set as 0.53, and the m6A calling specificity and sensitivity is 0.99 and 0.92.



**Supplemental Figure 5. Large aggregate CDCA-seq signal enrichments match closely with DNase-seq accessibility peaks.** (Chr20:46,434,503-57,030,240)

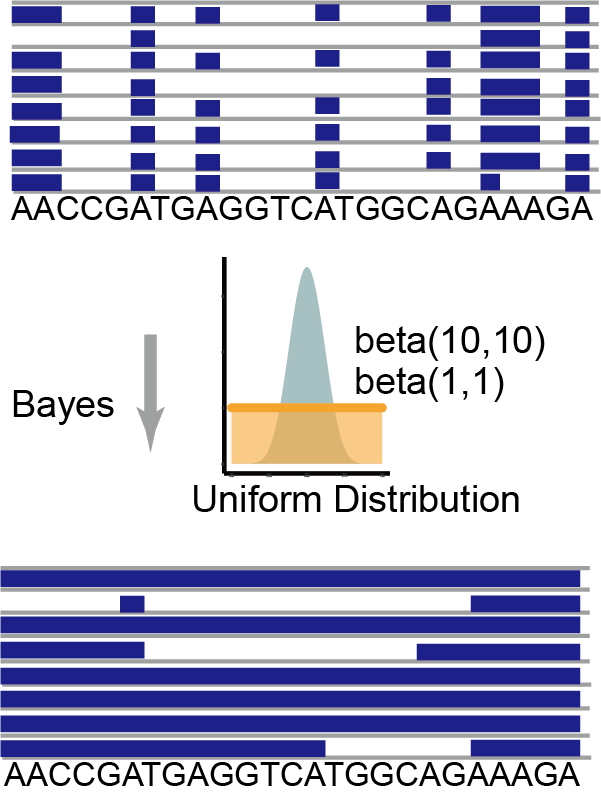
****

**Supplemental Figure 6. The m6A methylation deviation is related to the average m6A methylation.** The genome was sized into 50bp bins. The average m6A methylation was calculated as (total m6A in all covered reads)/(total adenosine in all covered reads). The deviation was represented by the bin methylation deviation, which aggregates the methylation in each bin.

Chart

Description automatically generated

**Supplemental Figure 7. The correlation between two sample replicates with and without exonuclease treatment.** The methylated bins (methylated count>2, bin=50bp) are 77.69% overlapped between non-exonuclease-digested SMAC-seq and exonuclease-digested CDCA-seq.

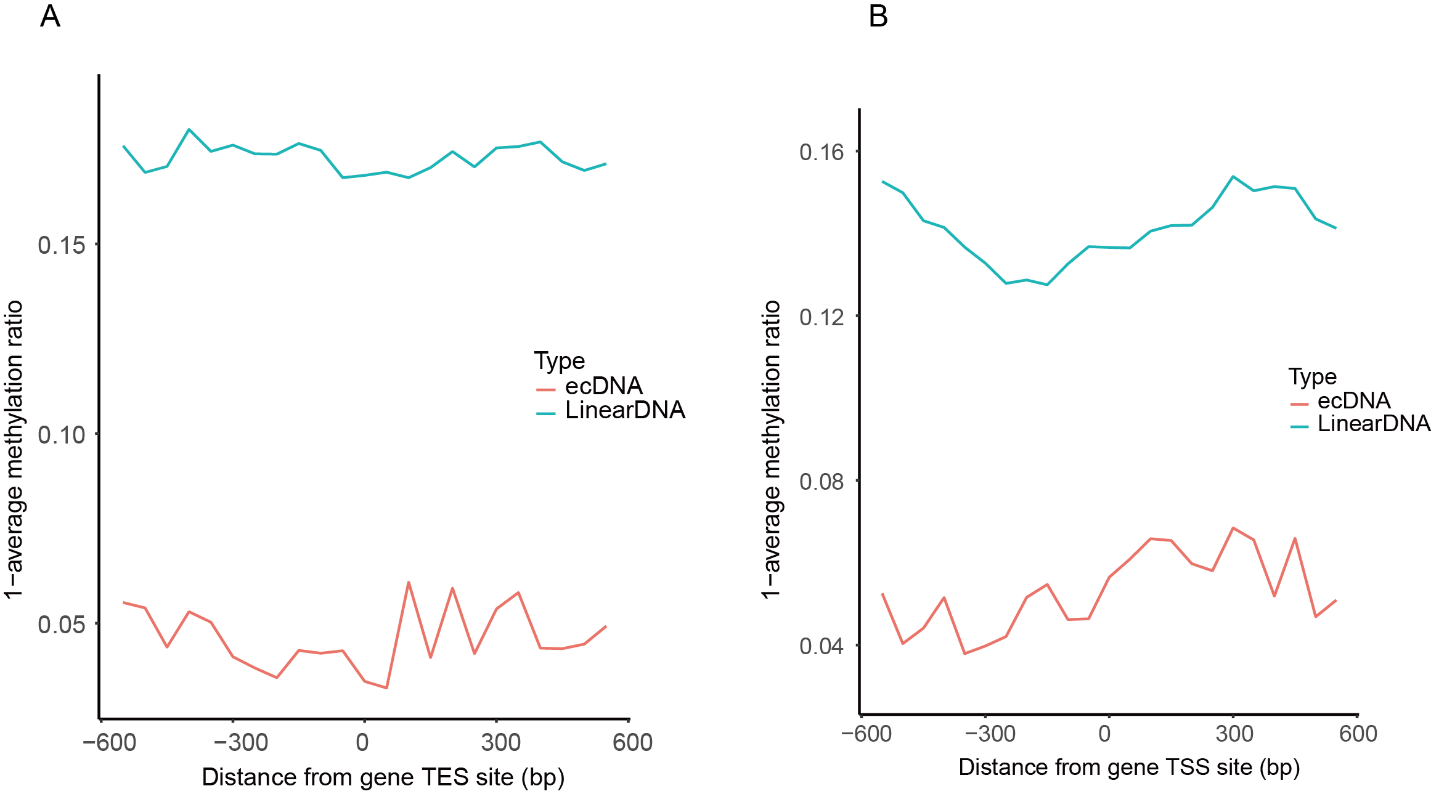


**Supplemental Figure 8. Outlines of the algorithm to configure the methylation level in genome resolution and single-molecule resolution.** In genome resolution, the methylation fraction represents the average methylation fraction of multiple reads covered in the regions. In the single molecular resolution, we adopted a Bayesian procedure to aggregate methylation probabilities and derived the accurate single-molecule accessibility calls over 50bp windows.

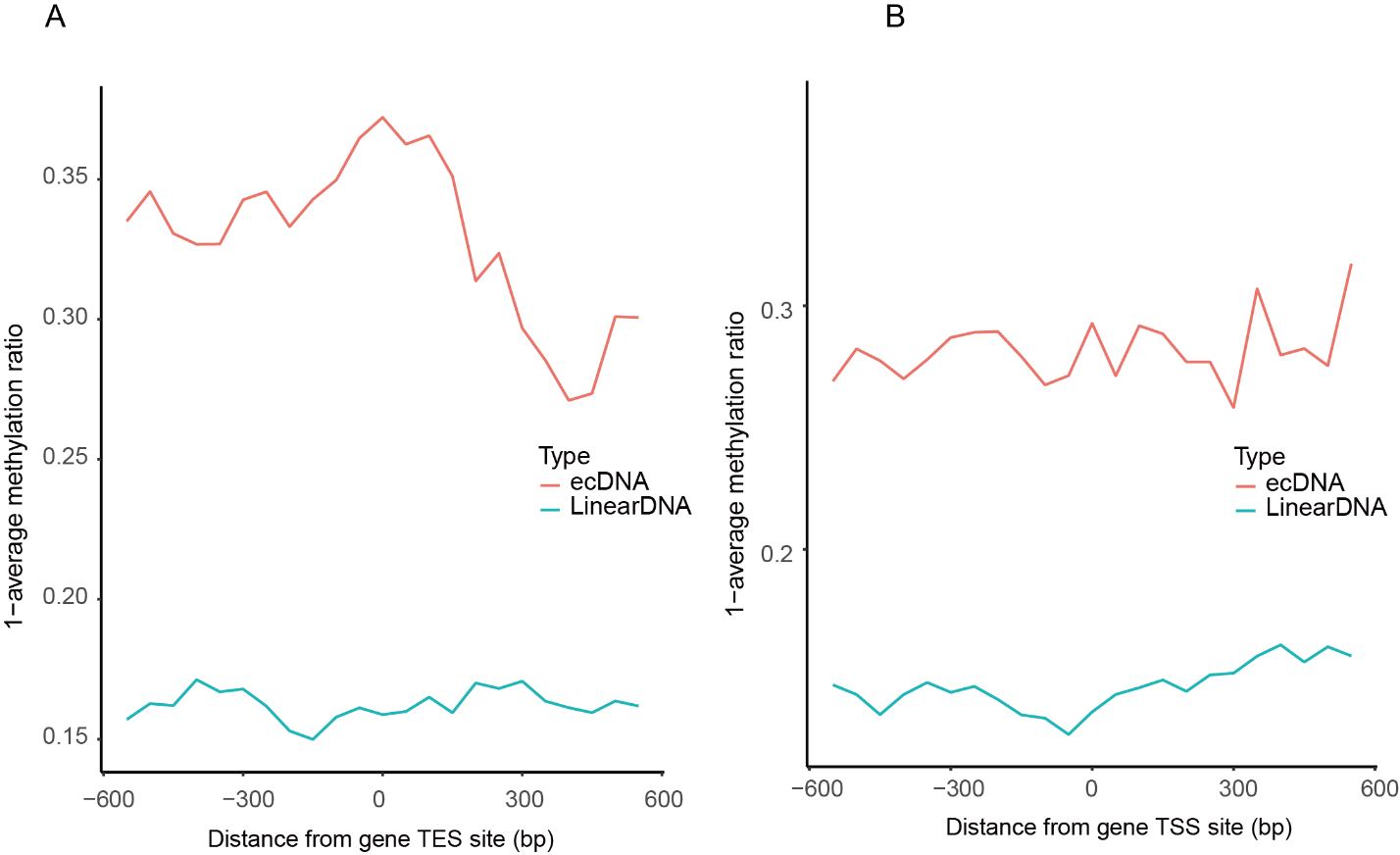
Chart

Description automatically generated with medium confidence

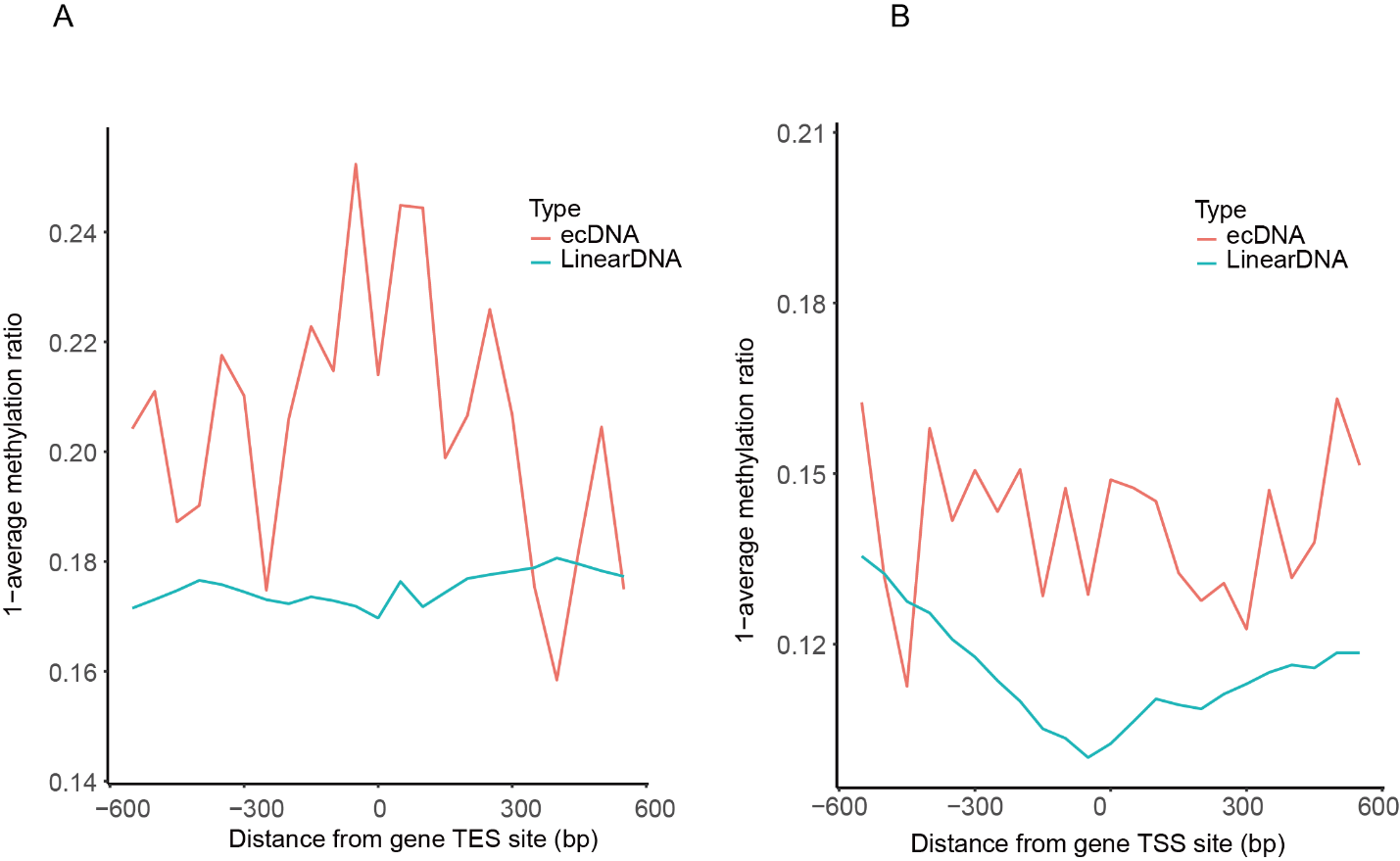
**Supplemental Figure 9. Gene ontology analysis of these ecDNA carried genes.** (<http://geneontology.org/>)



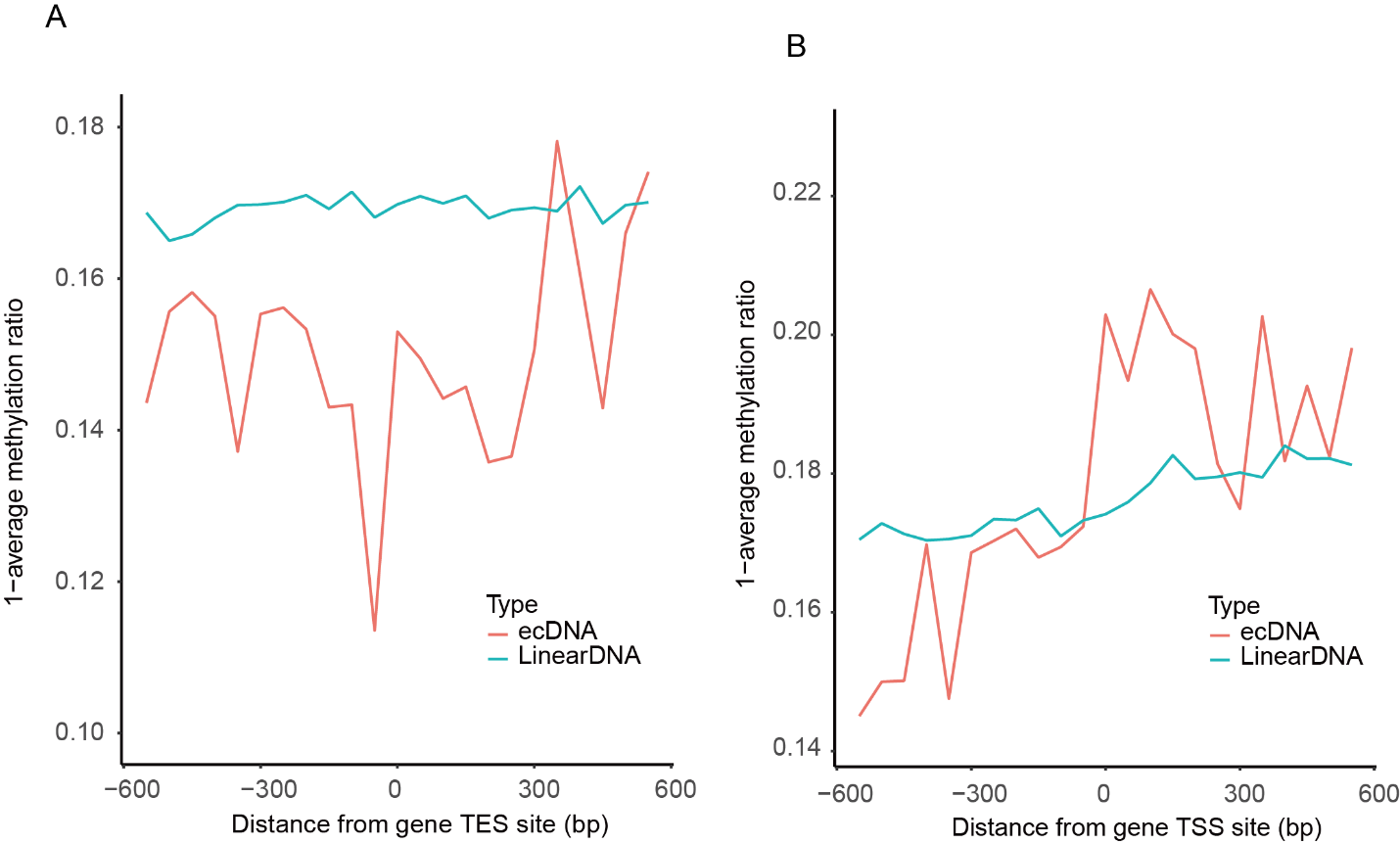
**Supplemental Figure 10. Average CDCA-seq profile around TSSs and TES of group II gene.** The group II genes have more open chromatin structure on ecDNA than linear DNA (Figure 2C).



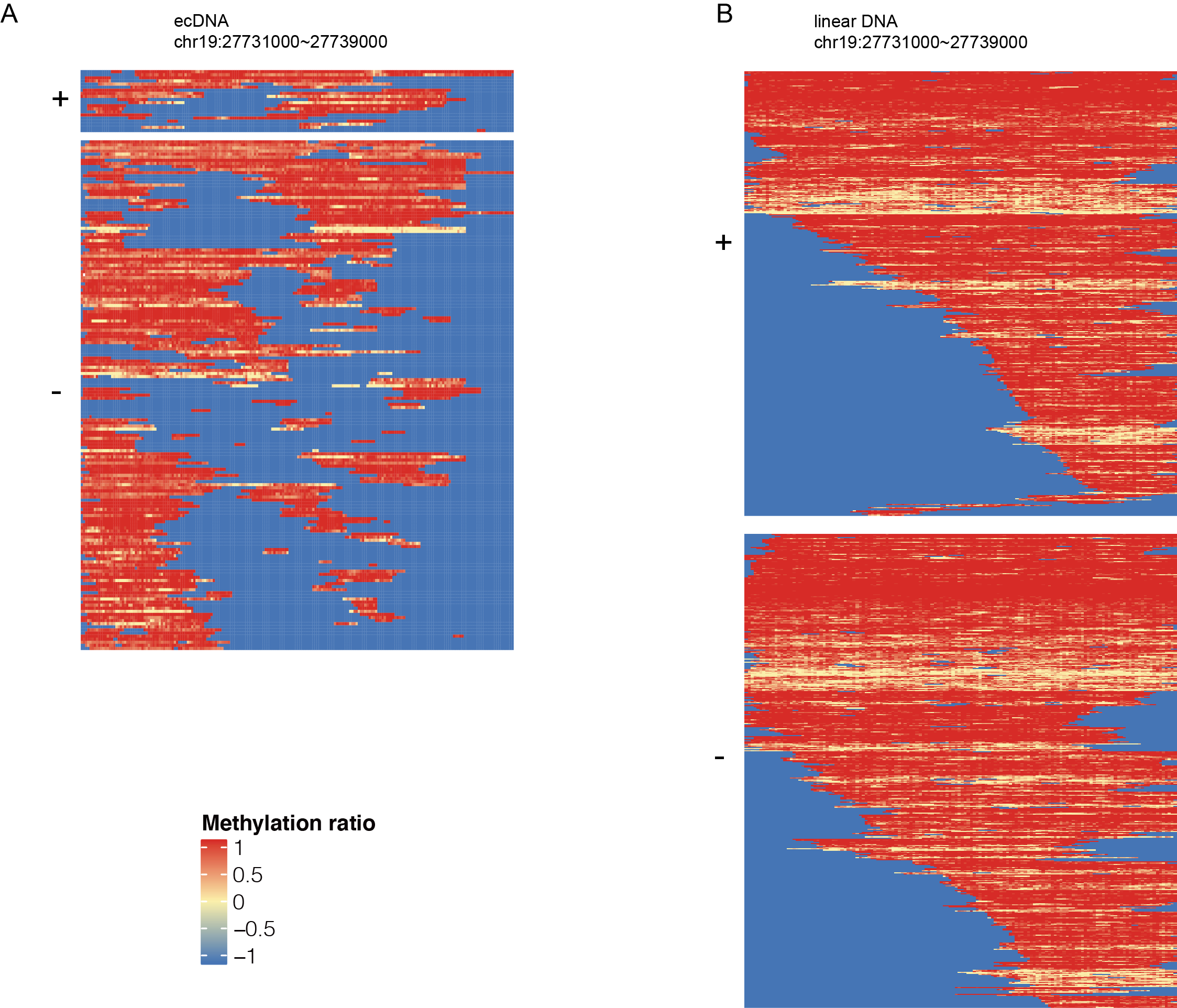
**Supplemental Figure 11. Average CDCA-seq profile around TSSs and TES of group I gene.** The group I genes have more open chromatin structure on linear DNA than ecDNA (Figure 2C).



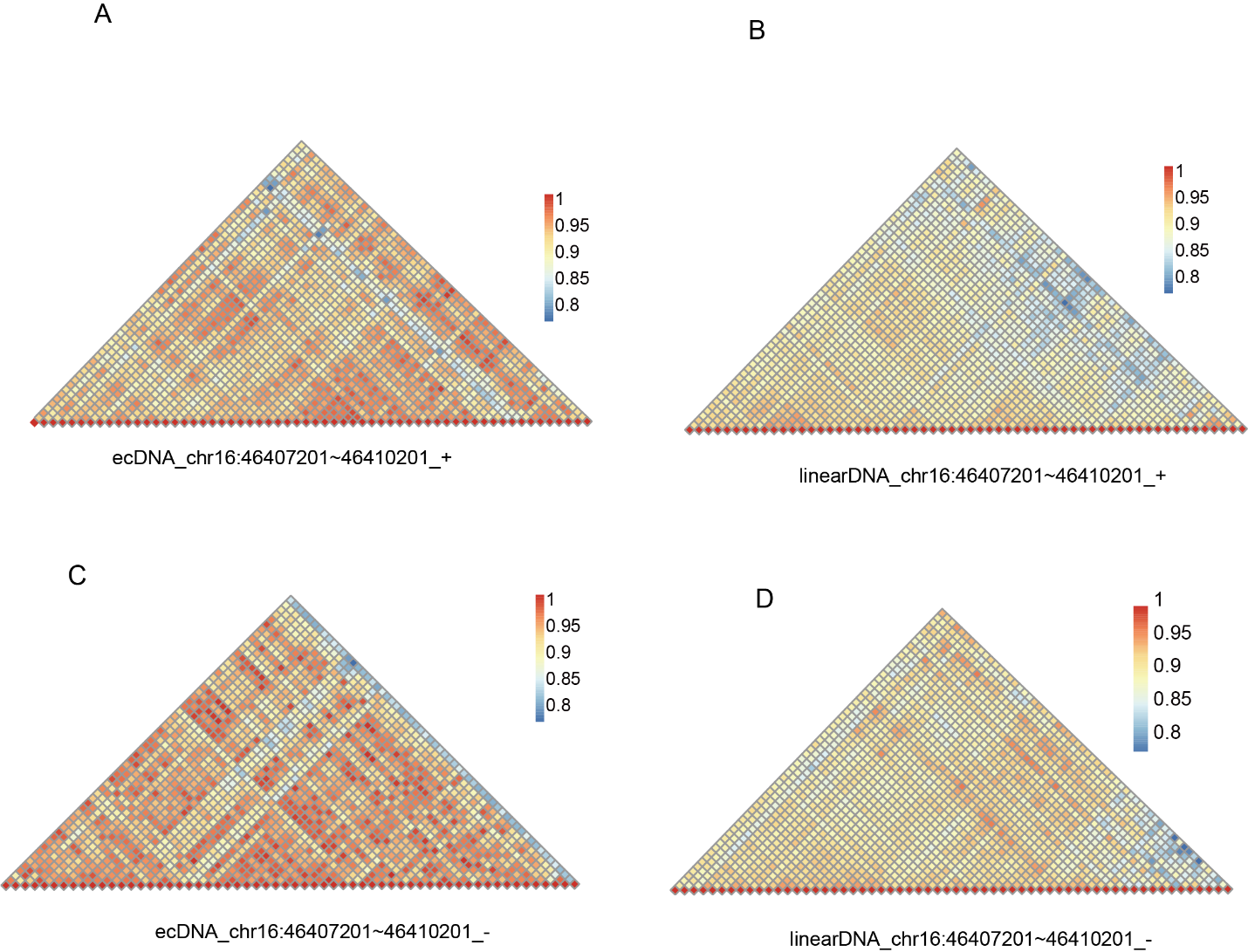
**Supplemental Figure 12. Average CDCA-seq profile around TSSs of genes with high expression level.** The top quantile defines the high expression genes in RNA-seq.



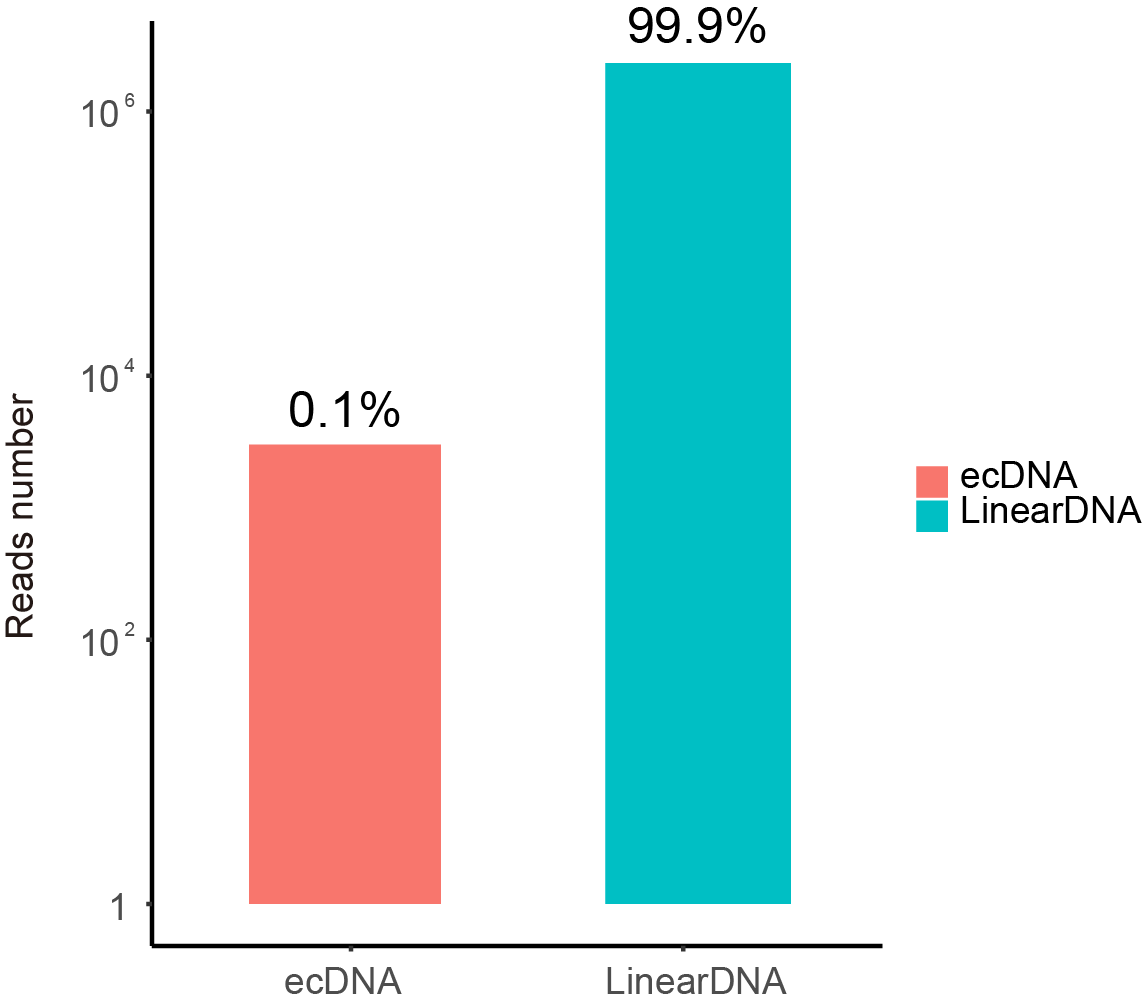
**Supplemental Figure 13. Average CDCA-seq profile around TSSs of genes with low expression level.** The bottom quantile defines the low expression genes in RNA-seq.



**Supplemental Figure 14. CDCA-seq reveal the distribution of alternative chromatin state in single molecular resolution.** A. Shown are all reads (+/-) covering linear DNA regions. B. Shown are all reads (+/-) covering the ecDNA areas.



**Supplemental Figure 15. Chromatin co-accessibility profiles for the chr16:46407201~46410201 show correlation and anticorrelation in ecDNA and linear DNA.** (A) Chromatin co-accessibility profiles for the chr16:46407201~46410201 show correlation and anticorrelation on ecDNA positive strand. (B) Chromatin co-accessibility profiles for the chr16:46407201~46410201 show correlation and anticorrelation on linear DNA positive strand. (C) Chromatin co-accessibility profiles for the chr16:46407201~46410201 show correlation and anticorrelation on ecDNA negative strand. (D) Chromatin co-accessibility profiles for the chr16:46407201~46410201 show correlation and anticorrelation on linear DNA negative strand.



**Supplemental Figure 16. The counts of reads identified as ecDNAs or linear DNAs.** The sample is directly subjected to nanopore DNA sequencing without the exonuclease digestion to remove the linear DNA.

Chart, bar chart

Description automatically generated

**Supplemental Figure 17. Comparing the megalodon and Tombo in ecDNA methylation calling.** All\_ecDNAs indicate the ecDNAs identified by minimap2. All the detected ecDNAs are processed to call methylation by Megalodon or Tombo.

**Chart

Description automatically generated**

**Supplemental Figure 18. The density distribution of the methylation ratio on ecDNAs and linear DNAs in non-exonuclease digested sample**

**Chart, line chart

Description automatically generated**

**Supplemental Figure 19. Average CDCA-seq profile (no linear DNA digestion) around transcription start site and transcription end site on ecDNAs and linear DNAs.** The sample, not digested by exonuclease, showed the lower average methylation on both eccDNAs and linear DNAs. The light red indicated the nucleosome occupancy in the CDCA-seq with exonuclease digestion. Comparison showed the approximately similar trend between the non-digested sample and digested sample. The difference may be caused by the distinct ecDNA coverage in two samples. The exonuclease treatment should not bias our analysis. (aggregated over 50-bp windows sliding every 5 bp; the sequencing depth is normalized for ecDNA and linear DNA;see Methods for details)

**Chart, scatter chart

Description automatically generated**

**Supplemental Figure 20. Pairwise scatter plot of the average bin methylation between ecDNAs and linear DNAs.** The genome is sized into 50bp bins. The methylation in each bin is the methylation ratio average of covering reads. The bins were classified into four groups: Type I- the regions are highly accessible in both linear DNA and ecDNAs; Type II- the linear DNA regions are less accessible than the ecDNA regions; Type III- the regions are not accessible in both linear DNA and ecDNAs; Type IV- the ecDNA regions have more open chromatin than the linear areas.

**Chart, bar chart

Description automatically generated**

**Supplemental Figure 21. The bins distribution among the gene elements.** The four types of regions are defined in supplemental figure 18.