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Brief Communication

Profiling plant histone modification at single-cell resolution using snCUT&Tag

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Chromatin immunoprecipitation with sequencing (ChIP-seq) with population cells or tissues yields ensemble epigenomic profiles that only represent the population average, which eliminates cell-to-cell epigenetic heterogeneity. Chromatin immunocleavage with sequencing (ChIC-seq) (Ku *et al*., 2019) that based on antibody-guided chromatin cleavage under targets is a practical alternative to ChIP-seq. Many single-cell ChIC-based methods, such as scChIC-seq (Ku *et al*., 2019), CUT&Tag (Kaya-Okur *et al*., 2019), CoBATCH (Wang *et al*., 2019), ACT-seq (Carter *et al*., 2019), and 10× scCUT&Tag (Bartosovic *et al*., 2021; Wu *et al*., 2021), have been developed and applied to study cell-type profiling, developmental trajectory, epigenetic heterogeneity, and transcriptional regulation in animals. However, due to the existence of cell walls, it is difficult to obtain single cells in plants. On the other hand, the existing scChIC methods require many single-cell barcoding procedures after tagmentation, which might lead to some DNA leakage and reduced mapping efficiency. Hence, a robust single-cell ChIC-seq method for plant epigenomic research is required emergently. In our previous study, we developed a rapid ChIC-based chromatin profiling protocol, nucleus CUT&Tag (nCUT&Tag) (Ouyang *et al*., 2021). Here, we combined the nCUT&Tag assay with 10× Single Cell ATAC, developing an easy-to-use single-nucleus CUT&Tag (snCUT&Tag) method in rice.

We reformulated the bulk-cell nCUT&Tag with the droplet-based single-cell barcoding technology to develop snCUT&Tag (Figure 1a). Briefly, ~10^6 of fixed rice-seedling nuclei were collected and immuno-cleaved with protein G-Tn5 (Vazyme, Nanjing, China, cat no. TD901). Following Tn5-assisted *in situ* tagmentation, ~20 000 Tn5-indexed nuclei were loaded onto the 10× Genomics Single Cell ATAC microfluidics device, in which each nucleus is partitioned in a gel-bead-in-emulsion droplet and barcoded with unique oligonucleotides (Figure 1a). Finally, the chromatin features of every single nucleus could be captured by pooling and sequencing the barcoded DNA fragments.

We first profiled the H3K4me3 histone mark in seedlings from the Xian group rice (Minghui 63) using snCUT&Tag. We generated over 480 million read pairs, of which 98.7% were indexed with a unique barcode (Figure 1c). After mapping the reads to Minghui 63 reference genome and removal of duplicates, we obtained the H3K4me3 profiles of 3679 single nuclei, with a median of 401 fragments per nucleus (Figure 1c). For the 3679 aggregate single nuclei, we called 28 795 peaks (Figure 1b,c).

Next, we compared the aggregate snCUT&Tag data with bulk-cell nCUT&Tag (Ouyang *et al*., 2021) and eChIP-seq data (Zhao *et al*., 2020). The aggregate snCUT&Tag peaks exhibited a high degree of consistency with nCUT&Tag and eChIP-seq data (Figure 1b). Coinciding with the high consistency, global scatter-plots displayed significant correlations among the snCUT&Tag, nCUT&Tag, and eChIP-seq libraries (Figure 1d). As demonstrated by few reads falling in non-peak regions (Figure 1b), the snCUT&Tag data exhibited a significantly high fraction of reads in peaks (FRiP) value (median FRiP = 0.923) (Figure 1c,e). snCUT&Tag displayed similar peak signal profiles to nCUT&Tag and eChIP-seq, with H3K4me3 peaks mainly enriching at transcription start sites (Figure 1f). These results indicated that snCUT&Tag is a robust method that can be used to profile single-nucleus chromatin features.

Since the epigenomic data can be used to predict chromatin interactions (Fulco *et al*., 2019; Ouyang *et al*., 2020), we examined the H3K4me3 snCUT&Tag data and predicted cell-type specific enhancer–promoter interactions according to the activity-by-contact model (Bartosovic *et al*., 2021; Fulco *et al*., 2019). Firstly, we performed dimensionality reduction and clustering according to Bartosovic *et al*. (2021). The results showed that rice seedlings were partitioned into 17 cell clusters, with significantly high enrichment-signal specificity (Figure 1g). Furthermore, we annotated cell types for the 17 clusters by investigating the marker peak-associated functional genes. For instance, a marker peak in cluster 6 showed specific H3K4me3 enrichment around a malate dehydrogenase-coding gene, which was reported as a marker gene of mesophyll cells in maize (Marand *et al*., 2021), implying that cluster 6 should be classified as mesophyll cells. Then, we predicted enhancer–promoter interactions. Our results demonstrated that the predicted chromatin interactions were well-overlapped with H3K4me3 ChIA-PET data, and many enhancer-centred chromatin interactions showed cell-type biases that only occurred in specific cell clusters (Figure 1h). Expectedly, ~51.5% (15 881 of 30 839) of predicted enhancers overlapped with ATAC-seq peaks (Figure 1i), showing significant enrichment of ATAC-seq signals around the centre of the predicted enhancers (Figure 1j,k). Our results indicate that snCUT&Tag can be applied to dissect epigenetic heterogeneity and predict cell type-specific enhancer–promoter interactions, which might facilitate the annotation of regulatory elements and reconstruction of 3D genome structures.

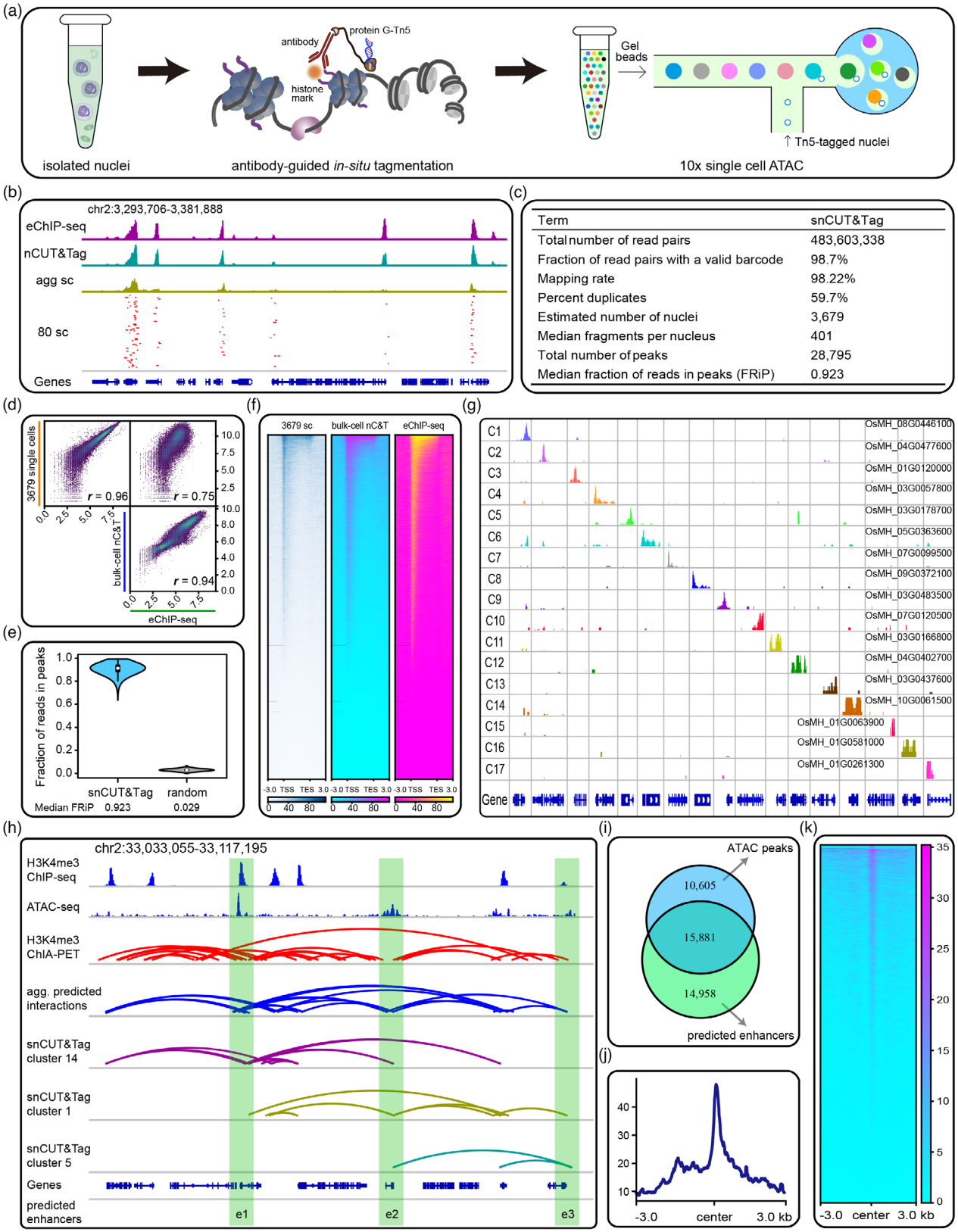
(a)
antibody
protein G-Tn5
histone mark
Gel beads
Tn5-tagged nuclei
isolated nuclei
antibody-guided *in-situ* tagmentation
10x single cell ATAC
(b)
chr2:3,293,706-3,381,888
eChIP-seq
nCUT&Tag
agg sc
80 sc
Genes
(c)
Term | snCUT&Tag
Total number of read pairs | 483,603,338
Fraction of read pairs with a valid barcode | 98.7%
Mapping rate | 98.22%
Percent duplicates | 59.7%
Estimated number of nuclei | 3,679
Median fragments per nucleus | 401
Total number of peaks | 28,795
Median fraction of reads in peaks (FRiP) | 0.923
(d)
3679 single cells
bulk-cell nC&T
eChIP-seq
r = 0.96
r = 0.75
r = 0.94
(e)
Fraction of reads in peaks
snCUT&Tag
random
Median FRiP 0.923 0.029
(f)
3679 sc
bulk-cell nC&T
eChIP-seq
-3.0 TSS TES 3.0
(g)
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
Gene
OsMH_08G0446100
OsMH_04G0477600
OsMH_01G0120000
OsMH_03G0057800
OsMH_03G0178700
OsMH_05G0363600
OsMH_07G0099500
OsMH_09G0372100
OsMH_03G0483500
OsMH_07G0120500
OsMH_03G0166800
OsMH_04G0402700
OsMH_03G0437600
OsMH_10G0061500
OsMH_01G0063900
OsMH_01G0581000
OsMH_01G0261300
(h)
chr2:33,033,055-33,117,195
H3K4me3 ChIP-seq
ATAC-seq
H3K4me3 ChIA-PET
agg. predicted interactions
snCUT&Tag cluster 14
snCUT&Tag cluster 1
snCUT&Tag cluster 5
Genes
predicted enhancers
e1
e2
e3
(i)
ATAC peaks
10,605
15,881
14,958
predicted enhancers
(j)
-3.0
center
3.0 kb
(k)
-3.0 center 3.0 kb

Figure 1 snCUT&Tag profiles histone modification at single-cell resolution in rice. (a) Schematics of single-nucleus CUT&Tag workflow. (b) Representative H3K4me3 landscapes generated by indicated methods. Profiles of 3679 aggregate single nuclei (agg sc) and 80 selected single nuclei (80 sc) are presented. The genomic region contains 16 genes with gene IDs from OsMH_02G0056600 to OsMH_02G0058100. (c) Summary of the snCUT&Tag data. (d) Scatterplots showing Spearman correlations among snCUT&Tag, nCUT&Tag (nC&T), and eChIP-seq. (e) Violin plot showing FRiP scores of the snCUT&Tag data. Simulated random profiles are presented as control. (f) Heatmaps showing H3K4me3 peak signals across gene bodies. (g) Genomic screenshots showing aggregate marker peaks for 17 clusters (C1−C17). The genes IDs that annotated by marker peaks are presented in every row of the cell clusters. (h) Genomic screenshot showing the predicted enhancer–promoter interactions in indicated cell clusters and aggregate single cells (agg. predicted interactions). The green boxes indicate predicted enhancers (e1−e3). (i) Venn diagram showing overlapping of the predicted enhancers and the ATAC-seq peaks. (j–k) Density profiles and heatmaps showing enrichment of ATAC-seq signals around the predicted enhancers.

In conclusion, we developed a simple and practical single-nucleus ChIC-seq method (snCUT&Tag) for single-cell plant epigenomic studies. We demonstrated that snCUT&Tag could be used to dissect the cell lineage and epigenetic heterogeneity and reconstruct chromatin topology of each single nucleus. Since nCUT&Tag is applicable for studying bulk-cell chromatin features with nuclei isolated from various plant species and tissues (Ouyang *et al*., 2021), we believe that snCUT&Tag may have a broad spectrum of application in profiling single-nucleus histone marks for other types of plant materials as well. Expectedly, snCUT&Tag provides an avenue for plant single-cell epigenomic studies and may help understand the 3D genomic and epigenetic basis of transcription regulation in a single cell.

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Conflict of interest

No conflict of interest declared.

Author contributions

X.L. and G.L. supervised the research. W.O. performed the experiments with assistance from X.X., M.G., and Y.Z. S.L. and W.O. performed bioinformatic analysis. W.O. and X.L. wrote the manuscript.

Data Availability Statement

The snCUT&Tag data have been deposited in the National Genomics Data Center (https://ngdc.cncb.ac.cn/) with accession number CRA004386.

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