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Establishment of a convenient ChIP-seq protocol for identification of the histone modification regions in the medicinal plant *Andrographis paniculata*

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Abstract

Histone modification is essential for gene transcription. In medicinal plants, investigation of the regulation of the secondary metabolites biosynthesis related genes (SMBRGs) has attracted significant attention. However, how histone modifications regulate these important genes is still elusive in medicinal plants, which might be due to the limit of application of high throughput sequencing methods (e.g. ChIP-seq) targeting the histone marks in the genome. Here, we established a convenient ChIP-seq protocol which uses the Tn5 tagmentation library construction strategy to identify genome-wide H3K27me3 enrichment regions in *Andrographis paniculata*. We believe this protocol will facilitate further investigations on how histone modifications regulate the SMBRGs.

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Introduction

In medicinal plants, one of the important issues is the investigation of the transcriptional regulation of SMBRGs that are directly related to the final production of the high-value active secondary metabolites[1]. Gene (e.g. SMBRGs) transcription and regulation is complicated in plants and is usually associated with multi epigenetic regulatory factors, such as histone modifications[2]. However, currently, the role of histone modifications in regulating SMBRGs remains elusive, likely due to the lack of publicly available sequencing data (e.g. Chromatin Immunoprecipitation Sequencing, ChIP-seq) about genome-wide histone modification profiles in medicinal plants. Although histone ChIP-seq[3] technology has been widely used in the model plants and the main crops, the limited application in medicinal plants largely hinder the investigation of the roles of histone modifications in regulating SMBRGs. Therefore, to improve the application of histone ChIP-seq in medicinal plants, we adopted the Tn5 tagmentation library construction strategy[4,5] and optimized a convenient histone ChIP-seq protocol in *Andrographis paniculata* (*A. paniculata*), one of the traditional medicinal plants[6]. We believe that this protocol will facilitate the investigation of the relationship between histone modifications and SMBRGs in most herbal medicinal plants.

The overview of this histone ChIP-seq protocol is shown in Fig. 1a (for the full method see the Supplemental Protocol). In brief, the healthy leaf of *A. paniculata* was fixed by formaldehyde, frozen with liquid nitrogen and ground to a powder for the subsequent nuclei extraction. The chromatin was then sonicated into around 500−1,000 bp fragment size by a sonication equipment (e.g the Covaris series). Next, the chromatin fragments were pulled-down by the ChIP-seq grade antibodies which were recognized by the Protein A or Protein G magnetic beads. Then, the enriched chromatin/DNA on beads were tagged by the Tn5 transposases[4]. After elution and recovery (removal of protein and reverse crosslinking), the tagged DNA was amplified using the primers with specific sequencing adaptors for the final ChIP-seq library construction. This complete histone ChIP-seq experiment can be performed in two days.

Making use of this protocol, we constructed two H3K27me3 (tri-methylation of lysine 27 on histone H3) ChIP-seq libraries in *A. paniculata* (Supplemental Table S1). A high correlation was observed between two biological replicates, indicating the high reproducibility of this method (Fig. 1b; Supplemental Fig. S1). Since H3K27me3 is one of the major suppressive histone modifications in plants[7], as expected, the H3K27me3 signals were negatively correlated with the gene expressions (Fig. 1c). Furthermore, using the MACS2 software[8], we identified 27,880 H3K27me3-enriched regions (Fig. 1d; Supplemental Table S2), which were mostly located in the exon and intergenic regions (Fig. 1e). Finally, we found that some andrographolide (the main secondary metabolites in *A. paniculata* leaf) biosynthesis-related genes[6], such as 1-deoxy-D-xylulose 5-phosphate synthase (*DXS*, *CXN00001821*) and cytochrome P450 monooxygenases (*CYP*, *CXN00006065*) had different H3K27me3 modification patterns which were correlated with their expression levels, suggesting that histone modifications were potentially involved in regulating andrographolide biosynthesis. This

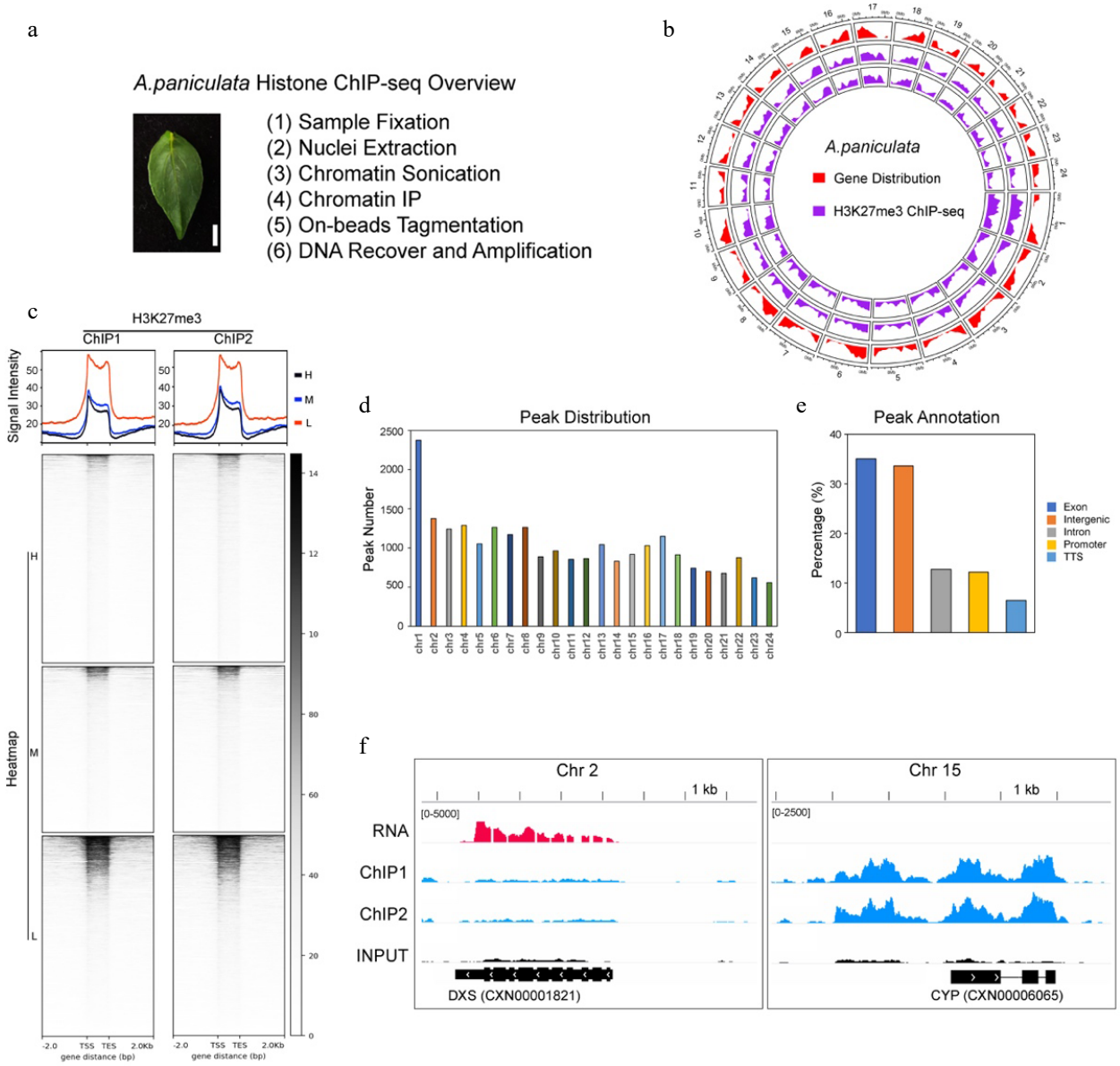

Fig. 1 Overview of the H3K27me3 ChIP-seq in *A. paniculata*. (a) A brief introduction of the histone ChIP-seq method in this study. The leaf of *A. paniculata* used in the ChIP-seq experiments. Bar = 0.5 cm. (b) A circos diagram showing the genome-wide distribution of the H3K27me3 ChIP-seq data. The red color indicates the gene location. The two violet circles indicate the two biological replicates of H3K27me3 ChIP-seq. (c) The H3K27me3 ChIP-seq profile and heatmap showing a negative correlation between H3K27me3 modifications and gene expressions. H, M and L indicate the high (FPKM > 10), middle (1 < FPKM ≤ 10) and low (FPKM ≤ 1) expression of genes, respectively. TSS, Transcript start sites; TES, Transcript end sites. ChIP1 and ChIP2 indicate two biological replicates. (d) The number of enriched peaks in the 24 chromosomes in the *A. paniculata* genome. (e) The genomic annotation of H3K27me3-enriched peaks. TTS, Transcript terminal sites. (f) The andrographolide biosynthesis-related genes, *DXS* and *CYP* genes were used as examples to show the H3K27me3 modification state in leaves.

provides evidence of the regulatory role of histone modifications in SMBRGs expression.

Materials and methods

Plant material and growth condition

A. paniculata was grown in the greenhouse (25–28 °C) for the development of healthy leaves. Leaves from about 2-month-old *A. paniculata* were used for the experiment.

ChIP-seq and data analysis

The complete optimized protocol for the preparation of histone ChIP-seq library is provided in the Supplemental Protocol. The ChIPed DNA libraries were sent for Illumina sequencing using the paired-end mode. The paired-end raw reads of ChIP-seq were then trimmed for adaptors, and low-quality reads were removed using TrimGalore (default parameters, https://github.com/FelixKrueger/TrimGalore) before they were aligned to the reference genome of *A. paniculata*[6] using Bowtie2 (https://bowtie-bio.sourceforge.net/bowtie2). The mapped reads containing >MAPQ30 were extracted by the samtools (https://github.com/samtools) and were used for the peak calling. The enriched peaks were identified by MACS2[8] under the broad mode. ChIP-seq data profiles and heatmaps were performed using Deeptools (https://deeptools.readthedocs.io/en/develop/).

Data availability

The leaf RNA-seq data of *A. paniculata* was from a previous study[6]. The H3K27me3 ChIP-seq data was submitted to the Genome Sequence Archive (https://ngdc.cncb.ac.cn/gsa/) under the accession number: PRJCA014911.

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Conflict of interest

The authors declare that they have no conflict of interest. Wei Sun is the Editorial Board member of *Medicinal Plant Biology* who was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and his research groups.

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