Methylation data from *Pseudotaxus chienii* obtained using methylation-dependent restriction-site associated DNA sequencing

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**Abstract**

*Pseudotaxus chienii* is an endangered coniferous plant that is endemic to China. Because *P. chienii* is sessile and has a long life cycle, its options for responding to drastic or rapid changes in climate are limited. To survive locally, *P. chienii* must be able to adapt, and the species shows variations in leaf size along an environmental gradient from east to west. It is important to determine whether this phenotypic variation is driven by DNA methylation. Therefore, we performed a preliminarily survey using methylation-dependent restriction-site associated DNA sequencing (MethylRAD) to investigate the methylation status of three *P. chienii* individuals from heterogeneous ecological niches. In total, 372,611 CCGG tags and 726,332 CCHGG tags were obtained. The rate of high quality methylation tags for a specific site in the genome varied from 42.31% (Gxdms3-4) to 50.01% (Jxbj3-4) and 50.18% (Zjdxg3-6). The level of CCHGG methylation (16.63%) was higher than that of CCGG (13.60%), which may be why *P. chienii* has low levels of phenotypic variation. The methylation data can be accessed using the Sequence Read Archive (SRA) database (SRP128155).

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Specifications Table

| Subject area          | Biology                |
|-----------------------|------------------------|
| More specific subject area | *Pseudotaxus chienii, Methylation* |
| Type of data          | Tables                 |
| How data was acquired | MethylRAD              |
| Data format           | Clean                  |
| Experimental factors  | Three individuals from heterogeneous ecological niches |
| Experimental features | Genomic DNA was extracted from *Pseudotaxus chienii* and digested with FspEI. Sample barcodes were introduced. Single-end sequencing was performed using an Illumina HiSeq X Ten sequencer. |
| Data source location  | Gxdms3-4 (23°29’54” N; 108°26’12” E) |
|                      | Jxbjs3-4 (26°30’35” N; 114°09’41” E) |
|                      | Zjdxg3-6 (27°52’49” N; 119°10’24” E) |
|                      | All the three individuals were kept at School of Life Sciences, Sun Yat-sen University. |
| Data accessibility    | The Methylation data have been deposited and made accessible via Bio-Project ID: PRJNA419098; BioSample accessions: SAMN08048873, SAMN08048874, and SAMN08048875; and the SRA database (SRP128155). |

Value of the data

- This dataset provides valuable information that could help predict epigenetic adaptations to future changes in climate.
- These data enhance our understanding of the mechanisms of natural phenotypic variation and may be used to provide guidance for the management of genetic resources and species conservation.

1. Data

This article provides methylation data for three *Pseudotaxus chienii* individuals from heterogeneous ecological niches. The clean data were deposited in the National Center for Biotechnology Information SRA database (SRP128155).

2. Experimental design, materials and methods

2.1. DNA samples and digestion

In this study, we selected one *P. chienii* individual from Daxiagu in Zhejiang Province (Zjdxg3-6), one from Bijiashan in Jiangxi Province (Jxbj3-4), and one from Damingshan in Guangxi Zhuang Autonomous Region (Gxdms3-4). *P. chienii* individuals across these regions possess high genetic diversity and show strong local adaptations to rapid environmental changes, which are suitable for methylation analysis [1]. Young and healthy leaves at the same developmental stage and from the same climate conditions were sampled. We extracted genomic DNA using a modified cetyltrimethylammonium bromide method [2]. Each genomic sample (200 ng) was digested separately with FspEI (New England BioLabs, cat. no. R0662L) at 37 °C for 45 min together with control DNA [3].

2.2. Adaptor ligation, amplification, and purification

A 20 µL ligation mix that included ligation master mix, 5 µM adaptor A, 5 µM adaptor B, and digested DNA was incubated at 16 °C for 1 h. The sequences of the two adaptors were as follows:
slx-ada1-BsaXI: 5’–CAAGCAGAAGACGGCATACGACCGCGCGAGTNNN–3’
3’–GCGCGCTCA–5’.
slx-ada2-BsaXI: 5’–CGACAGGTTCAGAGTTCTACAGTCCGACGATCNNN–3’
3’–TGTCAGGCTGCTAG–5’.

Ligation products were amplified in 30.4 μL of reaction mix containing 8 μM of each primer (p1: 5’–ACACTCTTTCCCTACACGACGCT–3’; and p2: 5’–GTGACTGGAGTTCAGACGTGTGCT–3’), 1.6 × High-Fidelity DNA Polymerase Buffer, 0.39 mM dNTPs, 0.4 U of Phusion High-Fidelity DNA polymerase (New England Biolabs, cat. no. M0530, Ipswich, MA, USA), and 18 μL of ligated DNA. PCR amplification was performed for 1–16 cycles using the following conditions: 98 °C for 5 s, 60 °C for 20 s, 72 °C for 10 s, and a final extension step of 5 min at 72 °C. The PCR products were separated using 8% polyacrylamide gel electrophoresis along with a 100-bp DNA ladder (New England BioLabs, cat. no. N3231). The target bands were excised, the DNA was allowed to diffuse out of the gel in nuclease-free water for 30 min at 37 °C and then amplified using the PCR conditions described above. PCR products derived from the three individuals were mixed, further purified, eluted, and quantified.

2.3. Barcoding and library pooling

Sample barcodes were introduced using PCR. Each 85-μL PCR reaction mix contained 5 × High-Fidelity DNA Polymerase Buffer, 10 mM dNTPs, 10 μM Primer3, 10 μM Index Primer, 1.6 U Phusion High-Fidelity DNA polymerase (New England Biolabs, cat. no. M0530, Ipswich, MA, USA), and 50 ng of gel-extracted PCR product. A total of 1–16 cycles of PCR were performed as described above, and following purification, the PCR products were subjected to single-end sequencing (100–150 bp) using an Illumina Hiseq X Ten sequencer (Illumina Inc., San Diego, California, USA) (Tables 1 and 2).

| Samples     | Clean data | MethylRAD tags | Quantity of data | Ratio   |
|-------------|------------|----------------|------------------|---------|
| Gxdms3-4    | 161,720,241| 25,378,549      | 793,164,733      | 10,738,505 (42.31%) |
| Jxbj3-4     | 161,720,241| 24,823,170      | 776,428,065      | 12,414,623 (50.01%) |
| Zjdxg3-6    | 161,720,241| 23,267,447      | 727,701,890      | 11,676,360 (50.18%) |
| Average     | 161,720,241| 24,489,722      | 765,764,896      | 11,609,829 (47.50%) |

Table 2
Summary of methylation site coverage.

| Sample       | CCGG               | CCWGG              |
|--------------|--------------------|--------------------|
|              | Number of sites    | Average depth      | Number of sites    | Average depth      |
| Gxdms3-4     | 242,013            | 13.34              | 462,085            | 15.53              |
| Jxbj3-4      | 256,668            | 13.31              | 521,409            | 16.69              |
| Zjdxg3-6     | 224,294            | 14.16              | 461,613            | 17.66              |
| Average      | 240,992            | 13.60              | 481,702            | 16.63              |
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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.06.045.

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