Chapter 14

Detecting Epigenetic Effects of Transposable Elements in Plants

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Abstract

Transposable elements (TE) represent a major fraction of eukaryotic genomes and play many roles in plant epigenetics. In this chapter, we describe the use of Sequence-Specific Amplified Polymorphism (SSAP) as a reliable Transposon Display technique applicable for use in many plant species. We also discuss the interpretation of SSAP data and associated risks. This technique has potential to allow rapid screening of plant populations, especially in nonmodel or wild species.

Key words Transposable element, Epigenetics, SSAP, Transposition

1 Introduction

Transposable elements (TE) represent a major fraction of eukaryotic genomes [1] and can induce alterations in their host genome [2]. TEs indeed are highly mutagenic and silenced by overlapping epigenetic mechanisms including DNA methylation [3]. Thus, TEs represent likely candidate sequences playing a pivotal role fuelling genome structural and epigenetic reorganization [4, 5]. For a general review of their function and detection of their mobility, *see* Chapter 13. Various molecular techniques reducing genome complexity can be exploited to specifically investigate TE genome fractions [6]. Among Transposon Display strategies (i.e., high-resolution TE-anchored PCR strategy allowing the simultaneous detection of multiple insertions), Sequence-Specific Amplified Polymorphism (SSAP) is one of the most easily applicable and reliable [7, 8]. Briefly, the SSAP procedure is derived from the Amplified Fragment Length Polymorphism (AFLP) strategy, but specifically targets TEs insertions. It relies on the amplification of digested genomic DNA with primers designed at the border of TEs and generates a pool of labeled fragments containing the

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termini of inserted copies of a given TE and its flanking genomic region [9]. SSAP usually generates highly polymorphic markers that allow to reliably assessing patterns of genetic diversity within and among groups [10–12]. SSAP polymorphism may result from molecular changes at insertion sites that modify the size of the amplification product [13], but comparative SSAP banding patterns can also offer reliable insights on the genome dynamics of TE fraction among related lineages. Relying on proper TE-specific primers represents the decisive step for implementing a reliable SSAP. The reader willing to design TE-specific primers for an SSAP procedure or any of its derivatives allowing to reliably amplify genomic regions flanking insertions of a given TE would profitably consult the literature on that topic [8, 14].

In this chapter, we present a recent modification of the SSAP protocol using restriction enzymes with differential sensitivity to DNA methylation at the digestion step. This methyl-sensitive derivative of SSAP has been named Methyl-Sensitive Transposon Display (MSTD; Fig. 1a) and provides useful knowledge on the methylation environment of TE insertions [15, 16]. The isoschizomers MspI and HpaII are widely used for methyl-sensitive displays (e.g., ref. [17–19]), as both enzymes recognize the same tetranucleotide sequence (5′-CCGG-3′), but present different sensitivities to DNA methylation (ref. [20]; Fig. 1b). HpaII is sensitive to methylation of any cytosine at both strands (5′-CCGG-3′), whereas MspI cuts methylated internal cytosine (5′-C5mCGG-3′). These properties allow assessing the methylation status of internal cytosine at restriction sites (CpG methylation). However, MspI is sensitive to methylation of the external cytosine (5′-5mCCGG-3′). Hence, methylation of external cytosine on both strands (CpCpG methylation: 5′-5mCCGG-3′ and 5′-5mC5mCGG-3′) may not produce bands with this MSTD. Since HpaII cleaves when the external cytosine is methylated on one strand, whereas MspI does not, hemimethylated CpCpG sites can be detected with this MSTD.

In addition to the limits inherent to the SSAP, MSTD profiles have to be interpreted with caution because CpCpG methylation on both strands prevents the enzymes from cutting and the technique is thus blind to heavily methylated portions of the genome. The absence of selected bands in specific samples might thus reveal either restructuring of the TE insertion or increased methylation in the vicinity of the TE insertion. Accordingly, a band specific from selected samples might correspond to a transposition event or demethylation in the vicinity of a TE insertion. MSTD data are biased toward non-heavily methylated regions and chiefly assess methylation changes in this particular fraction of the genome. Furthermore, it should always be kept in mind that MSTD offers insights about the methylation status of sequences flanking a particular TE insertion (i.e., the CCGG site next to a TE insertion) and does not necessarily reflects methylation changes affecting the TE insertion itself.

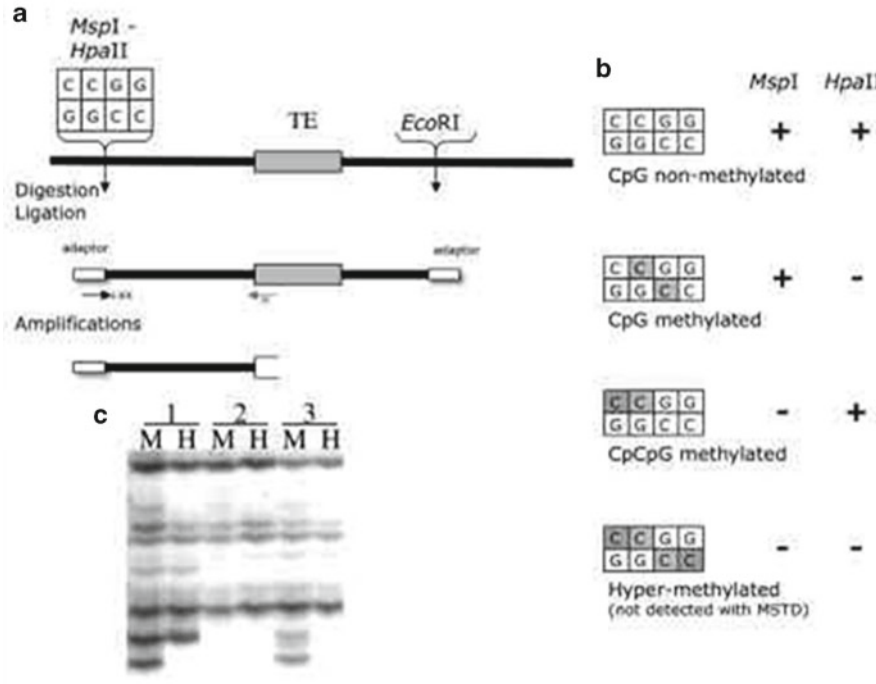

Fig. 1 Principle of the methyl-sensitive transposon display (MSTD). (**a**) Schematic representation of the high-resolution TE-anchored PCR strategy allowing the simultaneous detection of multiple insertions. After digestion of genomic DNA with rare cutter (e.g., MspI/HpaII) and frequent cutter (e.g., EcoRI) restriction enzymes, adaptors are ligated to DNA fragments. PCR amplifications are carried out using a primer complementary to the rare cutter adaptor and a labeled (*asterisk*) primer specific to the targeted transposable element (TE). (**b**) Methylation sensitivity of isoschizomer enzymes (MspI and HpaII) and interpretation of resulting banding pattern as a function of presence (+)/absence (–) of a given MSTD band. (**c**) An example of MSTD banding pattern for three samples (1–3). Comparison of band presence/absence in MspI (M) and HpaII (H) profiles reveals the methylation state of restriction sites flanking the corresponding TE insertion

MSTD was successfully employed to detect methylation changes following genomic shocks such as interspecific hybridization and/or genome duplication (15). Insights offered by this method can be valuably compared to data derived from Methyl-Sensitive Amplified Polymorphism (MSAP), combining AFLP and methylation-sensitive restriction enzymes to allow random detection of methylation changes across the genome [21]. When contrasting these two methods, Parisod et al. [15] were able to detect significantly more CpG methylation changes in regions flanking TE insertions than in random sequences following recent hybridization and genome duplication in Spartina, indicating that TEs were the most (epigenetically) targeted compartment subject to rapid evolution during allopolyploid speciation. We anticipate that the MSTD method will remain useful for rapid screening of populations, most particularly in nonmodel or wild species where genomic resources and information are limited.

2 Materials

2.1 Digestion

1. Tango Buffer [10×]: 33 mM Tris-acetate (pH 7.9); 10 mM Mg-acetate; 66 mM K-acetate; and 0.1 mg/mL BSA.
2. EcoRI [10 U/mL]: rare cutter enzyme (5′-GAATTC-3′) (*see* **Note 1**).
3. MspI/HpaII [10 U/mL]: frequent cutter enzymes recognizing the same tetranucleotide sequence (5′-CCGG-3′), but displaying differential sensitivity to DNA methylation.

2.2 Ligation

1. EcoRI-adaptors [100 mM]: 5′-CTCGTAGACTGCGTACC-3′ and 5′AATTGGTACGCAGTCTAC-3′. Preparation: mix equal volumes of the two adaptors [final concentration: 50 mM] and warm up to 95 °C for 5 min, then allow to cool down to room temperature. Then dilute at 1/10 for a final concentration of 5 mM. MspI/HpaII-adaptors [100 mM]: 5′-GACGATGAGTCTAGAA-3′ and 5′-CGTTCTAGACTCATC-3′. Preparation: mix equal volumes of the two adaptors [final concentration: 50 mM] and warm up to 95 °C for 5 min, then allow to cool down to room temperature.
2. ATP [20 mM].
3. T4 DNA ligase [5 U/mL].

2.3 PCR Preselective Amplification

1. EcoRI + Aprimer [10 mM]: 5′-GACTGCGTACCAATTCA-3′.
2. MspI/HpaII + C primer [10 mM]: 5′-GATGAGTCTAGAA CGGC-3′.
3. Rxn Buffer [10×]: 200 mM Tris pH 8.4 + 500 mM KCl.
4. Equimolar dNTPs [10 mM].
5. $MgCl_2$ [25 mM].
6. Taq polymerase [5 U/mL].

2.4 PCR Preselective Amplification

1. Labeled TE-specific primers (*see* **Note 2**).
2. MspI/HpaII selective primers were similar to that of preselective primer, with the addition of two variable nucleotides (=MspI/HpaII + CXX primer).
3. Primers used for this step were otherwise similar to those listed in Subheading 2.3.

3 Methods

3.1 Digestion (See Note 3)

1. Add 5 mL of Tango Buffer to 12.7 mL of sterile water.
2. Add 0.1 mL (1 U) of EcoRI.

3. Add 0.2 mL (2 U) of MspI (alternatively, HpaII) and gently mix.
4. Add 250 ng of DNA in 7 mL to this mix (final volume 25 mL) and gently mix.
5. Incubate at 37 °C for 3 h.
6. Deactivate restriction enzymes at 70 °C for 15 min.

3.2 Ligation (See Note 3)

1. Add 3 mL of Tango Buffer to 8.5 mL of sterile water.
2. Add 1 mL of ATP.
3. Add 1 mL of EcoRI-adaptors.
4. Add 1 mL of MspI/HpaII-adaptors and vortex.
5. Add 0.5 mL (5 U) of T4 DNA ligase and gently mix.
6. Add this ligation mix (15 mL) to the 25 mL of digestion mix (final volume 40 mL) and gently mix.
7. Incubate at room temperature (22 °C) overnight.
8. (Optional) 5 μL of product can be visualized by electrophoresis on a 1 % agarose gel and stained with ethidium bromide or similar in order to verify the success of digestion.
9. Dilute the digestion–ligation mix four times (e.g., 30 μL of product in 90 μL of sterile water) (*see* **Note 4**).

3.3 Performance of PCR Preselective Amplification

1. Add 2 μL of reaction (Rxn) Buffer to 12.7 μL of sterile water.
2. Add 0.5 μL of dNTP.
3. Add 1.6 μL of $MgCl_2$.
4. Add 0.5 μL of EcoRI + primer A.
5. Add 0.5 μL of MspI/HpaII + primer C and vortex.
6. Add 0.2 μL of Taq polymerase (1 U).
7. Add 18 μL of this preselective mix to 2 μL of diluted digestion–ligation mix (to a final volume 20 μL).
8. Place in a thermocycler to perform this PCR amplification: 94 °C for 180 s, followed by 28 cycles at 94 °C for 30 s, 60 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 180 s.
9. Dilute the preselective amplification products 1:10 with sterile water (e.g., 10 μL of product in 190 μL of water).

3.4 Performance of PCR Selective Amplification

1. Add 2 μL of Rxn Buffer to 11.1 μL of sterile water.
2. Add 0.5 μL of dNTP.
3. Add 1.6 μL of $MgCl_2$.
4. Add 0.8 μL of TE-specific primer.
5. Add 0.8 μL of MspI/HpaII + CXX primer and vortex.

6. Add 0.2 μL of Taq polymerase (1 U).
7. Add 17 μL of this selective mix to 3 μL of diluted preselective amplification product (final volume 20 μL).
8. Place in a thermocycler to perform this touch-down PCR amplification: 94 °C for 120 s, followed by 13 cycles at 94 °C for 30 s, 65 °C to 56 °C (decreasing by 0.7 °C per cycle) for 30 s, and 72 °C for 60 s, followed by 25 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s, and a final extension at 72 °C for 300 s.
9. Prepare the amplification products according to your electrophoresis protocol (*see* **Note 4**).

4 Notes

1. EcoRI is very widely used but can be variable in its sensitivity to CpG methylation [20]. SSAP protocols using Csp6 instead of EcoRI have been developed [8, 14] and might be profitably used for MSTD. Unfortunately, Csp6 is a four-base restriction enzyme (i.e., frequent cutter) and might provide too much SSAP bands for the analysis of complex genomes. Furthermore, with such frequent cutter, it might happen that the TE itself presents a restriction site. This would induce the amplification of a band internal to the TE instead of a band containing the flanking genomic DNA, resulting in confusing results, especially in the case of retrotransposons bordered by two identical long terminal repeats.
2. It is vital to label the TE primer in order to highlight bands containing the termini of an inserted TE and its flanking genomic region. The TE primer can be radioactively labeled with P33 or with fluorochromes. Amplification products labeled with P33 can be visualized after electrophoresis on 6 % Long Ranger denaturing gel for 5 h (75 V, limited to 2,000 W) by autoradiography. Amplification products labeled with fluorochromes can be visualized with automatic sequencers after electrophoresis.
3. MspI and HpaII have to be used on the same samples in parallel in order to provide an MSTD. Accordingly, preparing two mixes in parallel at each step (one for the MspI reactions and one for the HpaII reactions) can improve reliability and comparability of the MSTD profiles. Although SSAP and MSTD approaches generate reliable consistent patterns, it is strongly advisable to perform the protocol several times on selected samples in order to estimate the error rate (*see* refs. 22, 23 for further details).
4. Diluted digestion–ligation product can be stored at –20 °C.

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