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Selection of Efficient Taq DNA Polymerase to Optimize T-DNA Genotyping Method for Rapid Detection of Mutant Arabidopsis thaliana Plants

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Abstract

Plants harbor homologues of various animal genes involved in phosphorus metabolism, telomere biology and other cellular processes. Compared to experiments with many other multicellular organisms, research in the model plant *Arabidopsis thaliana* takes advantage of short generation time and an ever increasing arsenal of genetic and transgenic tools, including large collections of T-DNA knockout and activation lines. The availability of thousands of publicly available transgenic *Arabidopsis* lines provides a unique opportunity to address a number of important biological questions. However, identification of individual T-DNA mutant plants from a pool of seeds provided by a biological stock distribution center remains a laborious and time-consuming procedure. Here we compared a number of commercial Taq DNA polymerases commonly used for routine PCR genotyping to identify a single polymerase most suitable for genotyping T-DNA mutant plants. Our data indicate that Emerald Amp GT PCR Master Mix provides the most reliable, quick and simple DNA genotyping tool to determine the presence of a T-DNA insertion and to establish whether an individual *A. thaliana* plant is heterozygous or homozygous for the mutant allele.

Introduction

In the past few decades, the small weed *Arabidopsis thaliana* from the Brassicaceae family has become one of the most popular model organisms for addressing fundamental questions concerning biological functions of evolutionarily conserved genes [1]. Since the *Arabidopsis* genome harbors homologues of many important animal genes, knowledge gained from studies in *Arabidopsis* has important implication for research in many other organisms, including humans. For example, 48–60 % of *Arabidopsis* genes involved in protein synthesis have homologues in other eukaryotic genomes, further highlighting conservation of gene functions [2]. Insertional mutagenesis with transfer DNA (T-DNA) is a routine method to generate large numbers of *Arabidopsis* mutants with insertions in coding regions of known genes [3]. Tens of thousands of transgenic lines carrying random T-DNA insertions throughout the *A. thaliana* genome have been generated and deposited into public seed stock centers [4–6]. However, when a biological stock center such as ABRC distributes seeds for a particular mapped T-DNA line, the provided pool of seeds often includes plants not only homozygous for the T-DNA insertion but also heterozygous and even wild-type. Thus, a preliminary screening of potentially mutant plants will require PCR genotyping that tests for the presence of wild-type and mutant T-DNA alleles. While this analysis is a necessary first step for many follow-up genetic assays, PCR genotyping often remains a laborious and time-consuming procedure. The correct choice of a reliable DNA polymerase can save researchers many long hours at the bench and simplify routine genotyping of multiple T-DNA mutants. Here we analyze and compare a number of commercially available Taq DNA polymerases to identify one most suitable for quick and easy genotyping of multiple T-DNA mutant plant lines.

Material and Methods

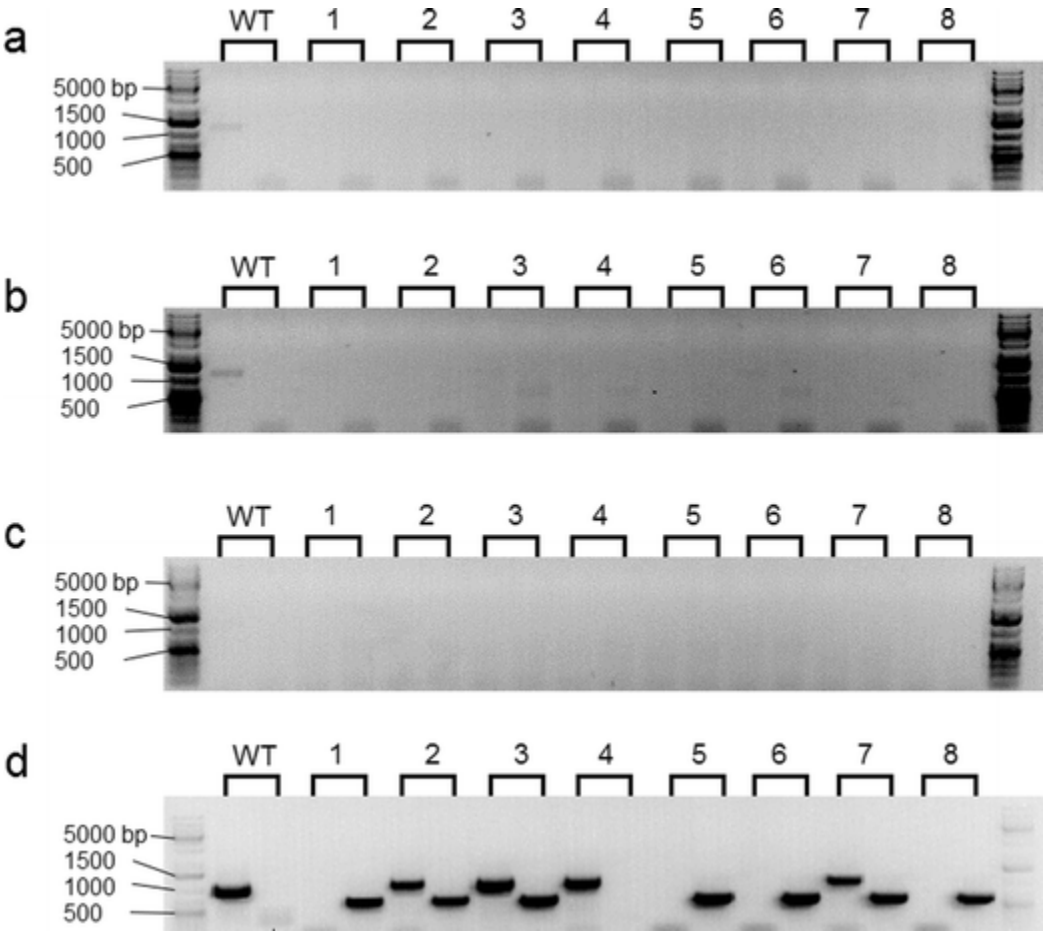
Seeds for wild-type *A. thaliana* (Columbia ecotype, #CS6673) and T-DNA mutant lines SALK_100012 (insertion in At5g56130), GABI_462A04 (insertion in At5g55550 gene), SAIL_547_C10 (insertion in At5g57050 gene) and SALK_022236 (insertion in At5g55310 gene) were purchased from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus, OH). Seeds were cold treated overnight at 4 °C and then placed in an environmental growth chamber where they were grown for 3 weeks under a 16-h light/8-h dark photoperiod at 23 °C. Genomic DNA from one rosette leaf of each individual plant was extracted as previously described [7]. PCR genotyping for SALK lines was performed with T-DNA-specific primer LBa1 5′-TGGTTCACGTAGTGGGCCATCG-3′ and gene-specific primers 56130-LP 5′-AACAGCTACATGTGTCCCGTC-3′ and 56130-RP 5′-TACCGGAATGACTAAACGCTG-3′ for SALK_100012 line or 55310-LP 5′-CTCCGTGAGATTTCGAGACAG-3′ and 55310-RP 5′-CCTGCTTTCCCATTTCTTACC-3′ for SALK_022236 line. For the SAIL line SAIL_547_C10, we used T-DNA-specific primer LB1 5′-GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC-3′ and gene-specific primers 57050-LP 5′-TTCCTTCTCCTCTTTTCTCCG-3′ and 57050-RP 5′-TTGATCCGAGATCGATGAATC-3′. For the GABI-Kat line GABI_462A04, we used T-DNA-specific primer 8474 5′-ATAATAACGCTGCGGACATCTACATTTT-3′ and gene-specific primers 55550-LP 5′-GCTTCATTGTCTTTGCAGACC-3′ and 55550-RP1 5′-AAACCATTAAATGGGGACGAG-3′. All primers were ordered from Integrated DNA Technologies, USA.

The following DNA polymerases were tested: Phusion High-Fidelity DNA polymerase (ThermoFisher Scientific, USA, cat #F530S), Ex-Taq DNA polymerase (Clontech, USA, cat #RR001A), Taq DNA polymerase (SibEnzyme, Russia, cat #E331), Emerald Amp GT PCR Master Mix (Clontech, USA, cat #RR310A). Ten nanograms of genomic DNA and 10 μM of each primer were used in all PCR reactions; all other components of PCR reactions were added according to the manufacturer's suggested protocol for each individual polymerase. All PCR reactions were performed in T100™ and C1000 Touch™ Thermal Cyclers (Bio-Rad). Expected PCR product sizes for all wild-type alleles and mutant alleles were predicted with the iSect T-DNA Primer Design Tool (http://signal.salk.edu/tdnaprimers.2.html). For all reactions, the following PCR protocol was used: 2 min at 96 °C, 35 cycles of 96 °C (10 s), 57 °C (25 s) and 72 °C (1.5 min), followed by 5 min at 72 °C. PCR products were run on a 1 % agarose gel in 1X TBA and visualized using a Bio-Rad Molecular Imager Chemidoc XRS+ scanner with Image Lab™ Software.

Results and Discussion

To identify the most reliable DNA polymerase for rapid genotyping of T-DNA insertional *Arabidopsis* mutants, we first tested four different Taq DNA polymerases with SALK_100012 line plants harboring a T-DNA insertion in the second intron of At5g56130 gene. For each plant, two separate PCR reactions were performed: one with primers 56130-LP + 56130-RP testing for the wild-type allele and one with primers LBa1 + 56130-RP testing for the presence of T-DNA allele. Both PCR reactions for each plant were run side-by-side on agarose gel to separate PCR products (Fig. 1).

Fig. 1



Genotyping of *Arabidopsis thaliana* plants from SALK_100012 line with **a** Phusion High-Fidelity DNA polymerase; **b** Ex-Taq DNA polymerase; **c** Taq DNA polymerase; **d** Emerald Amp GT PCR Master Mix. WT—DNA from wild-type *Arabidopsis* plant (positive control for wild-type PCR band); 1–8—individual plants from SALK_100012 line. Molecular weight DNA standards are shown on each side. Loading order for each DNA sample: PCR reaction for wild-type allele, PCR reaction for T-DNA mutant allele

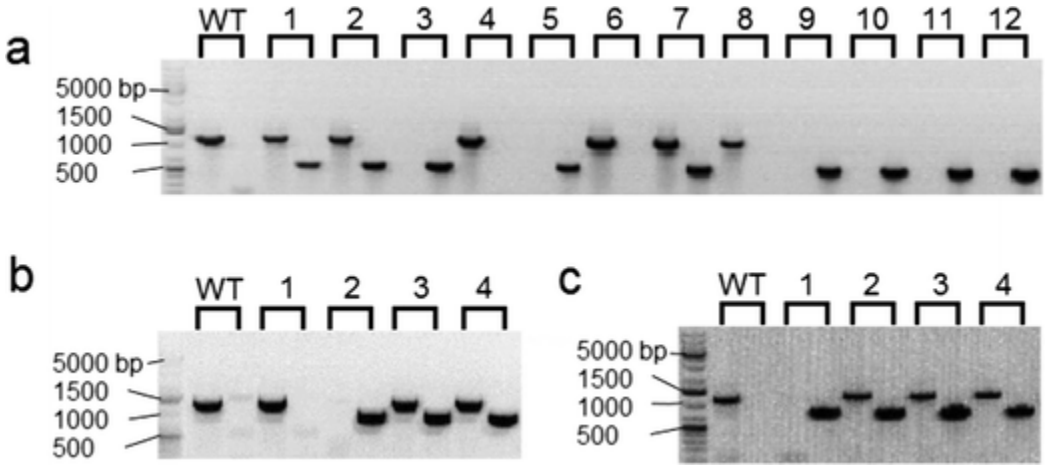
No clearly visible PCR products were observed for T-DNA mutant allele in reactions with Phusion High-Fidelity DNA polymerase (Fig. 1a), Ex-Taq DNA polymerase (Fig. 1b), and SibEnzyme's Taq DNA polymerase (Fig. 1c). Furthermore, for the wild-type allele, we detected only a faint PCR band in the positive control reaction with DNA from a wild-type (WT) *Arabidopsis* plant in case of Phusion and Ex-Taq DNA polymerases (Fig. 1a–b). In both of these cases, the PCR band for the wild type allele was not present in any reaction with plant DNA that came from the SALK_100012 seed stock. The SibEnzyme's Taq DNA polymerase produced no visible band in any PCR reaction, even with positive control wild type DNA (Fig. 1c).

In contrast, all reactions with Emerald Amp GT PCR Master Mix produced clearly distinguishable and self-evident PCR results (Fig. 1d). Specifically, we detected a very strong PCR band in the positive

control reaction with WT DNA. As expected for a wild-type plant, primer combination for the mutant allele produced no band in this case (Fig. 1d). Moreover, in all PCR reactions with the Emerald Amp GT PCR Master Mix, we were able to assign genotype status to each individual plant from SALK_100012 mutant line. Specifically, plants #1, 5, 6, and 8 are homozygous for the T-DNA insertion; plants #2, 3, 7 are heterozygous for the T-DNA insertion; and plant #4 is wild-type as it has no T-DNA-specific PCR band (Fig. 1d). These data indicate that the Emerald Amp GT PCR Master Mix clearly outperforms all other tested DNA polymerases in successfully genotyping mutant *Arabidopsis thaliana* plants generated by the Salk Institute for Biological Studies (SALK T-DNA lines).

To test if the Emerald Amp GT PCR Master Mix can also efficiently genotype plants from other T-DNA collections, we analyzed individual plant DNA from the SAIL [5] and GABI-KAT [6] insertional mutagenesis collections. Indeed, we detected strong and specific bands for both wild-type and mutant alleles in all T-DNA lines tested (Fig. 2). Our genotyping results indicate that in the GABI_462A04 line (T-DNA insertion in At5g55550 gene) plants #3, 5, 9, 11 and 12 are homozygous for the T-DNA insertion; plants #1, 2, 7 are heterozygous for the T-DNA insertion; and plants #4, 6, 8 are wild type (Fig. 2a). Similarly, PCR genotyping reveals that in the SAIL_547_C10 line (T-DNA insertion in At5g57050 gene) plants #3 and 4 are heterozygous for the T-DNA insertion; plant #2 is homozygous for the T-DNA insertion; and plant #1 is wild type (Fig. 2b). Furthermore, since to date the SALK T-DNA collection is still the largest collection of mapped insertional T-DNA lines in *Arabidopsis*, we confirmed the reliability of PCR results generated with Emerald Amp GT PCR Master Mix by genotyping plants from another SALK T-DNA line (SALK_022236 with T-DNA insertion in At5g55310 gene). Similar to results with SALK_100012 line, we observed strong PCR bands for all analyzed plants, and identified plants #2, 3, and 4 as heterozygous for the T-DNA insertion; and plant #1 as homozygous for the T-DNA insertion (Fig. 2c). We conclude that Emerald Amp GT PCR Master Mix is equally efficient at genotyping T-DNA lines from all three major T-DNA insertional mutagenesis collections and that it produces strong and reliable PCR results from the first try, greatly decreasing time and effort required for successful genotyping of mutant *Arabidopsis* plants. Thus, the Emerald Amp GT PCR Master Mix appears to be suitable for genotyping T-DNA mutants in *Arabidopsis thaliana* though its suitability for genotyping mutants in other plants will need to be tested.

Fig. 2



Genotyping of *Arabidopsis thaliana* plants from GABI_462A04 line (**a**), SAIL_547_C10 line (**b**) and SALK_022236 line (**c**) with Emerald Amp GT PCR Master Mix. WT—DNA from wild-type *Arabidopsis* plant (positive control for wild-type PCR band); 1–12 and 1–4—individual plants from each T-DNA line. Molecular weight DNA standards are shown on the *left*. Loading order for each DNA sample: PCR reaction for wild-type allele, PCR reaction for T-DNA mutant allele

Conclusions

A. thaliana plants display an extraordinary tolerance to genome stress and are often able to survive inactivation of many genes which would be lethal in mammals [8]. The development of a convenient and rapid PCR method for quick identification of T-DNA mutants would allow fast genotyping of large amount of samples. Here we compare four commonly used DNA polymerases and identify Emerald Amp GT PCR Master Mix as the best choice for efficient genotyping of T-DNA lines from all three major T-DNA insertional mutagenesis collections (SALK, SAIL and GABI-KAT). While some degree of PCR optimization will likely be required for individual primer sets when analyzing different T-DNA mutants, our data indicate that Emerald Amp GT PCR Master Mix provides the most reliable source of Taq polymerase to initiate this optimization procedure. Moreover, the premix contains all components required for PCR reaction (DNA polymerase, reaction buffer, dNTPs, and loading dye), further simplifying set-up and genotyping procedure.

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