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ORIGINAL ARTICLE

Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelating resin Chelex 100

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Abstract A DNA extraction method using Chelex 100 is widely used for bacteria, *Chlamydomonas*, and animal cell lines, but only rarely for plant materials due to the need for additional time-consuming and tedious steps. We have modified the Chelex 100 protocol and successfully developed a rapid and simple method of DNA extraction for efficient PCR-based detection of transgenes from a variety of transgenic plant and algal species. Our protocol consists of homogenizing plant tissue with a pestle, boiling the homogenized tissue in a microfuge tube with 5% Chelex 100 for 5 min, and centrifuging the boiled mixture. The supernatant, which is used for PCR analysis, was able to successfully amplify transgenes in transgenic tobacco, tomato, potato, *Arabidopsis*, rice, strawberry, *Spirodela polyrhiza*, *Chlamydomonas*, and *Porphyra tenera*. The entire DNA extraction procedure requires <15 min and is therefore comparable to that used for bacteria, *Chlamydomonas*, and animal cell lines.

Keywords Chelex 100 · DNA extraction method · Plant material · PCR · Transgenes

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Introduction

The PCR is the most frequently used technique for amplification of specific DNA fragments in vitro. Because of its high sensitivity, PCR does not require high-quality template DNA obtained using the complicated conventional method with proteinase K, phenol, and chloroform. Numerous shortcuts have been reported for extracting DNA suitable for PCR amplification; of these, the method using the chelating resin Chelex 100 (Walsh et al. 1991) is most popular for extracting DNA from a vast array of biological materials. In the Chelex method, a boiling step alone is sufficient to extract DNA from *Escherichia coli* suitable for amplifying a gene of interest using PCR. For animal cells and *Chlamydomonas*, a rapid and simple DNA extraction protocol using Chelex 100 and/or ethylenediaminetetraacetic acid is widely used. However, a similarly uncomplicated DNA extraction protocol is not yet available for plant materials due to the difficulties in extracting DNA from limited amounts of plant tissue.

Chelex 100 has proven to be efficient in extracting DNA for use in PCR analyses in a wide range of experiments (Walsh et al. 1991; Ward 1992; Holmes et al. 1994; Cao et al. 2009). It also removes PCR inhibitors, such as heme and its metabolic products (Panaccio 1991), acidic polysaccharides (Furukawa and Bahavanandan 1983), mineral ions, and humic acids (Tsai and Olson 1992; Tebbe and Vahjen 1993). However, plant DNA extraction with Chelex 100 requires additional time-consuming and tedious steps, including ethanol treatment and autoclaving (Berthold et al. 1993) and, consequently, this method is seldom used to extract DNA from plant material.

We report here our development of a rapid and simple plant DNA extraction technique using Chelex 100 that produces DNA suitable for PCR amplification. This

method requires no ethanol treatment or autoclaving steps and can be completed in <15 min. Using this method, we were able to detect transgenes from a variety of transgenic plant and algal species.

Materials and methods

Plant materials

To evaluate our newly developed rapid DNA extraction method, we first generated transgenic plants of various species. Transgenic tobacco (*Nicotiana tabacum* cv. Xanthi), tomato (*Lycopersicon esculentum* cv. Joyful), and potato plants (*Solanum tuberosum* cv. Desiree) were produced via transforming *TBP19* (P19 protein of Tomato bushy stunt virus, GI:9790328) gene. Transgenic *Arabidopsis thaliana* plants (cv. Columbia) were grown from seeds provided by the Salk Institute. Transgenic rice (*Oryza sativa* cv. Dongjin) plantlets harboring the *nptII* (*neomycin phosphotransferase II*) gene conferring kanamycin resistance were also grown from seeds of *nptII* transgenic plants. Transgenic *Spirodela polyrhiza* plants were produced by transformation with the *nptII* gene. Transgenic *Chlamydomonas reinhardtii* lines were produced by transformation with the *aminoglycoside phosphotransferase* (*aph7*) gene for hygromycin selection. *Porphyra tenera* (red alga) samples were prepared from thallus grown in vitro. In vitro-cultured plant tissues or plants grown in a growth chamber were used for DNA extraction.

DNA extraction procedure

The DNA extraction procedure is summarized in Fig. 1a. In a 1.5-ml microfuge tube (Eppendorf, Hamburg, Germany), shoot or leaf tissue (10–15 mg; one or two leaf discs with a diameter of 1 cm) is homogenized with a pestle for 1 min in 150–300 µl of 5% Chelex 100 (Bio-Rad, USA). The tissue is vortexed for 10 s, incubated in boiling water for 5 min, then vortexed again for 10 s, and finally centrifuged at 13,000 rpm for 1 min. The supernatant can then be used as template for PCR amplification.

PCR analysis

In our experiments, an aliquot of extracted DNA (0.5–2 µl) was used as template for the PCR. DNA, primers, *Taq* polymerase, and reaction buffers were mixed for PCR cycling (GeneAmp PCR system 9700; Applied Biosystems, Foster City, CA) and amplified under the following cycling conditions: one cycle at 94°C for 5 min, followed by 30–40 cycles of 94°C for 30 s, 52–58°C for 30 s, and 72°C for 1–2 min, and terminated by a final 5-min extension at 72°C.

The following primer pairs were used to amplify the target genes: TBP19-F (5′-CGCCATGGAACGAGCTATAC A-3′) and TBP19-R (5′-CCTCTAGAAGGTCTCAGTACC T-3′) amplified a 0.5-kb *TBP19* fragment; NPTIIF (5′-G AGGCTATTCGGCTATGACTG-3′) and NPTIIR (5′-ATC GGGAGCGGCGATACCGTA-3′) amplified a 0.7-kb *nptII* fragment; btbP-5 (5′-CCACTCGAGCTTGTGATCGCA CT-3′) and Aph7R2 (5′-TTCCGGTCGGTCGTGCCGTCC AT-3′) amplified a 0.6-kb *aph7* fragment; PtHSP70R3 (5′-A CAGGAGCCGACGCGTGCCA-3′) and PtHSP70P1KF (5′-TTGGATCCGAACCTGCCCCCGGGT-3′) amplified a 1.1-kb *heat shock protein* (*HSP 70*) fragment from *P. tenera*; PtBtub (31 5′-CTCCGASACSAGGTCRTTCA T-3′) and PtBtub51 (5′-AACAACTGGGCYAAGGGSC A-3′) amplified a 1-kb *beta tubulin* (*bTub*) fragment from *P. tenera*.

Results and discussion

Optimization of rapid DNA extraction for PCR

To develop a rapid protocol for plant DNA extraction for PCR, we modified a colony PCR protocol used for *Chlamydomonas* (Cao et al. 2009) by adding a homogenizing step and removing a cooling step (Fig. 1a). The modified protocol successfully extracted DNA from freshly harvested and liquid nitrogen-frozen tobacco leaf discs, demonstrating that DNA obtained from frozen plant samples using this method is suitable for PCR amplification. Homogenization of plant tissue is considered to be critical for successful PCR amplification after rapid DNA extraction. To test this assumption, we evaluated PCR efficiency after DNA extraction without the homogenization step and found that although the PCR did not always amplify transgenes without the homogenization step, the amplification itself appeared to be successful if the boiling time was extended or the number of PCR cycles was increased. Furthermore, at least a 5-min incubation in boiling water was required to produce rapid and consistent PCR amplification. We found that the step eliminated from the *Chlamydomonas* colony PCR protocol (chilling on ice) was not essential for PCR amplification of plant DNA: the PCR successfully amplified transgenes in all samples of DNA extracted without this step. Figure 1b, c shows the results of these experiments, illustrating the efficiency of the various modifications for extracting of tobacco plant DNA for PCR analyses.

PCR amplification of transgenes from various species of transgenic plants

Berthold et al. (1993) reported successful PCR amplification with *Chlamydomonas* and *Arabidopsis* DNA extracted

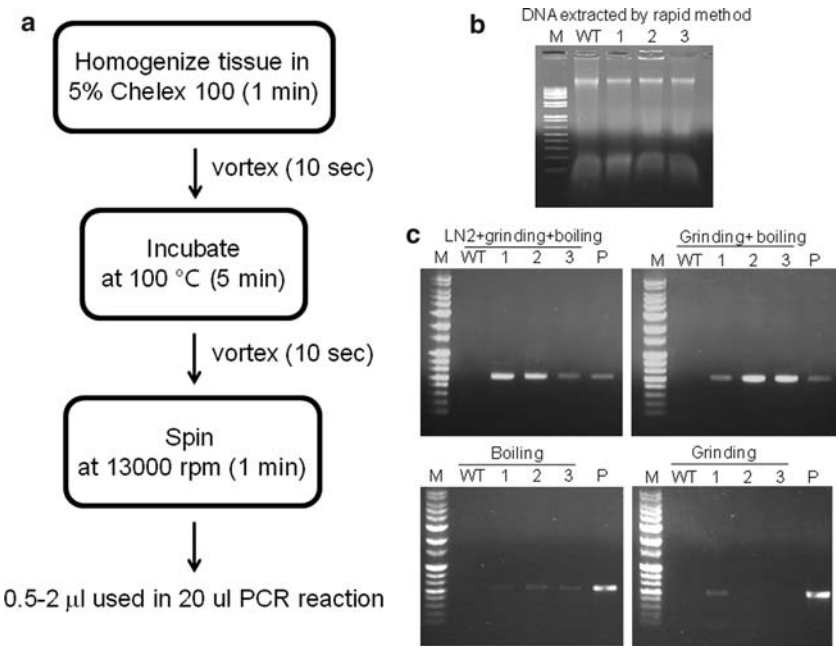

Fig. 1 Rapid extraction of DNA from tobacco plant and PCR amplification of transgene. **a** An novel rapid procedure for DNA isolation using Chelex 100. **b** Gel electrophoresis of DNA extracted from tobacco leaf with the newly developed Chelex 100 method. **c** PCR amplification of *TBP19* from transgenic tobacco plants using the method shown in **a**. *LN2+grinding+boiling* PCR amplification using the method with LN2 (liquid nitrogen) treatment, *Grinding+boiling* PCR amplification using the grinding and boiling method, *Boiling* PCR amplification using grinding and boiling method without homogenizing step, *Grinding* PCR amplification using the grinding and boiling method without incubation at 100°C. *M* 1-kb DNA size marker, *WT* wild-type tobacco, *lanes 1–3* transgenic plants, *P* positive control, isolated DNA using the Qiagen kit for transgenic plants

using Chelex 100, ethanol, and autoclaving steps, but they failed to amplify a transgene in tomato. To develop a universal plant DNA extraction protocol, we tested the applicability of our modified Chelex 100 method with DNA from various species. In this study, we used our method to extract DNA from tomato tissues cultured in vitro and grown in a growth chamber; the DNA was subsequently tested by PCR amplification. We found that the transgene from the DNA of transgenic tomato plants was successfully amplified (Fig. 2a; lanes 12, 14, 16, 61, and 64) and that the samples without a band were from non-transgenic plants. This result was confirmed by PCR amplification of DNA isolated with a Qiagen kit (DNeasy Plant Mini kit, Qiagen, Valencia, CA) (Fig. 2a; lanes 11 and 63). These findings indicate that our rapid DNA extraction method produces high-quality DNA from tomato plant samples for PCR.

This method was evaluated using a diverse range of plant species including potato, *Arabidopsis*, rice, strawberry, *S. polyrhiza* (hydrophyte), *C. reinhardtii* (green algae), and *Porphyra tenera* (red alga), and the genes of interest were successfully amplified from all species tested (Fig. 2). Although the amplification of some DNA samples produced only faint PCR bands following electrophoresis in an agarose gel, increasing the number of PCR cycles or the quantity of the DNA template produced stronger bands, whereas the same conditions still produced no signal from the DNA of wild-type plants lacking the transgene.

Conventional methods used to extract DNA from plant and algal species for PCR, such as the cetyl-trimethylammonium bromide procedure, require many tedious steps to remove the polysaccharides that act as potent PCR inhibitors (Furukawa and Bahavanandan 1983). However, PCR amplification of DNA extracted using our method successfully amplified *nptII* from a transgenic strawberry plant and *HSP70* and *bTub* from *P. tenera*, indicating that 5% Chelex 100 removed or sufficiently reduced the activity of PCR inhibitors.

Our method was successfully applied to higher plants, hydrophytes, and algae. This method differs from existing protocols for plant DNA extraction in that we were able to omit the phenol/chloroform extraction and ethanol precipitation steps. The supernatant containing the DNA template for PCR amplification, which presumably contains carbohydrates and denatured protein, had no apparent inhibiting effect on the PCR reaction. A 0.5- to 2-µl aliquot out of the total 150–300 µl supernatant was sufficient for PCR amplification. This method requires little manipulation and can easily be applied to hundreds of samples. The entire extraction procedure requires <15 min to prepare the DNA for PCR, and culture or growing time can be saved because only a small tissue sample is needed. In addition,

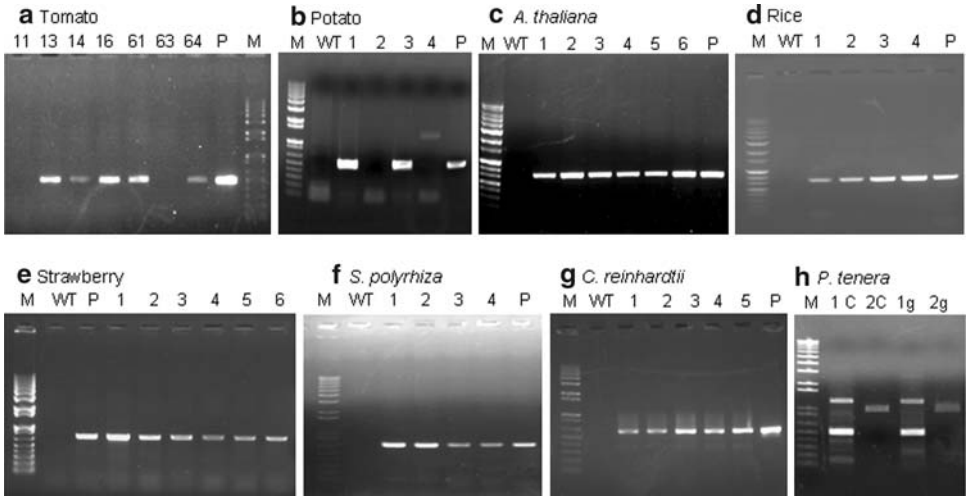

Fig. 2 PCR amplification of transgenes from tomato, potato, *Arabidopsis thaliana*, rice, strawberry, *Spirodela polyrhiza*, *Chlamydomonas reinhardtii*, and *Porphyra tenera*. Plant DNA was extracted using the method shown in Fig. 1a. **a** PCR screening of tomato plants transformed with the *TBP19* gene (0.5 kb). **b** PCR screening of potato plants transformed with the *TBP19* gene (0.5 kb). **c** PCR amplification of the *nptII* gene (0.7 kb) from T-DNA mutant lines of *A. thaliana* (*lanes*: *1* CHI-OX-4, *2* CHS-OX21, *3* F3′H-OX-24, *4* LDOX-OX-15, *5* mylo56-4BC-7, *6* Salk-112104c). **d** PCR amplification of the *nptII* gene (0.7 kb) from transgenic rice. **e** PCR amplification of the *nptII* gene (0.7 kb) from transformed strawberry plants. **f** PCR amplification of the *nptII* gene (0.7 kb) from transformed *S. polyrhiza*. **g** PCR screening of *Chlamydomonas* lines transformed with the *aph7* gene (0.6 kb). **h** PCR amplification of *HSP70* (1.1 kb) and *bTub* (1 kb) genes from *P. tenera* (*1C HSP70* amplified from DNA obtained by rapid extraction, *2C bTub* amplified from DNA was obtained by rapid extraction, *1g HSP70* amplification from DNA by the Qiagen kit and cetyl trimethylammonium bromide extraction, *2g bTub* amplification from DNA by Qiagen kit and CTAB extraction). *Lanes 1–6* independent transgenic plants, *WT* wild type, *P* positive control, isolated DNA using Qiagen kit from transgenic plant

the supernatant contains degraded RNA (Fig. 1a), suggesting the possibility that the protocol can be modified to improve RNA quality and then used for reverse transcription (RT)-PCR.

In conclusion, we have developed a rapid and simple method for DNA extraction using the chelating resin Chelex 100. Our technique prepares DNA from various higher plants and algal species that is suitable for use in PCR analyses. It differs from the conventional DNA extraction method using Chelex 100 by omitting a number of time-consuming and tedious steps normally required for DNA extraction from plant material. As shown here, the method produces reliable results and can be used routinely in the laboratory for a variety of plant and algal species.

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