Applications
Note 126 | March 2006

Automated isolation of plant genomic DNA using ChargeSwitch® technology

Dmitry A. Filatov, University of Birmingham, d.filatov@bham.ac.uk

Abstract

Isolation of genomic DNA from plant tissues often represents a bottleneck, as it requires substantial amount of manual work. This application note describes a way to automate this process using Invitrogen ChargeSwitch magnetic beads on the Eppendorf epMotion® 5075 LH automated pipetting system.

Introduction

Isolation of genomic DNA is required for many downstream processes, such as amplification of genes using polymerase chain reaction (PCR), genotyping of single nucleotide polymorphisms (SNPs) or other molecular genetic markers, and sequencing. DNA isolation from plant tissues can be more complicated than DNA isolation from blood, or other animal tissues, as plant tissues are often difficult to grind and may contain substantial amounts of polyphenolic compounds, which require additional purification steps. Often researchers grind plant samples in liquid nitrogen using the classical mortar and pistil, making it impossible to extract more than 20-30 samples per day. Yet, many applications (such as marker-assisted plant breeding, or population genetic studies) require extraction of genomic DNA from hundreds to thousands of samples. Here we describe a way to automate this laborious, time consuming process using Invitrogen CST magnetic beads technology on the epMotion 5075 LH from Eppendorf. The CST technology is based on pH-dependent DNA binding and it does not require any hazardous compounds. Unlike column-based methods, magnetic beads-based DNA isolation yields fairly high molecular weight DNA because it does not involve passing DNA through porous membranes, which may lead to DNA fragmentation. The resulting DNA is suitable for a wide range of downstream applications, including PCR, restriction digestion, cloning and genetic analysis, based on most commonly used genetic markers (SNPs, microsatellites, RFLPs, AFLPs). The procedure described below is based on the standard Invitrogen protocol and was adapted for the Eppendorf epMotion 5075 LH automated pipetting system equipped with a plate gripper tool.

Equipment and Consumables

Automated pipetting system

Eppendorf epMotion 5075 LH liquid handling station (#5075 000.008) with a gripper tool (#5282 000.018), 1-channel dispensing tool TS1000 (#5280 000.053) or 8-channel dispensing tool TM1000-8 (#5280 000.258), reservoir rack (#5075 754.002) with three 30 ml and three 100 ml reservoirs (#0030 126.505 and #0030 126.513, respectively) and Invitrogen 96-well magnetic separator (#CS15096). Our system was equipped with optional heating/cooling thermal module (#5075 757.001), hence we used a thermo-adapter for 96 well PCR plates (#5075 787.008) to cool extracted DNA.

Other equipment

Qiagen TissueLyser mixer-mill (#85220) with 96-well plate adapters (#69984), Eppendorf centrifuge 5810 (#5810 000.017) with a swing-bucket rotor A-4-81-MTP (#5810 725.003).

Consumables

Invitrogen ChargeSwitch gDNA Plant Kit (#CS18000 or #CS18000-10), ABgene 96-deep well plates (#AB-0932), ABgene square cap sealing mats (#AB-0662), Eppendorf twin-tech PCR 96-well plates (#0030 128.648), Eppendorf epTIPS 1000 µL tips (#0030 003.985), Qiagen stainless steel 5 mm grinding beads (#69989). DNA extraction from 96 samples requires ~4ml of paramagnetic CST beads from the Invitrogen ChargeSwitch gDNA Plant Kit, two 96 deep well plates, one 96 well PCR plate for collection of purified DNA, one sealing mat, 96 stainless steel 5 mm beads for grinding and up to 8 boxes of 1000 µL tips (depending on whether 1-channel or 8-channel tools are used, and whether the tips are changed at every wash step).

Preparing sample lysates

Sample grinding
Leaf samples were ground in ABgene 96-deep well plates using Qiagen TissueLyser mixer-mill with 96-well plate adapters. 100 mg of leaf tissue were placed into each well of a 96 deep well plate and 1 ml of Lysis Buffer (L18), as well as 2 µl of RNase A from the Invitrogen ChargeSwitch gDNA Plant Kit (#CS18000) were added to each sample. One stainless steel 5 mm bead was used for every sample. The plates were sealed with ABgene square cap sealing mats and ground using Qiagen TissueLyser mixer-mill for 2-3 min at maximum speed. It is convenient to process two 96 well plates at the same time because TissueLyser mill requires two plates of the same weight for balance. Having two plates is also convenient at the centrifuging step (see below).

Clearing lysates
After leaf grinding 100 µl of 10% SDS was added to every sample and lysates were incubated for 5 min at room temperature. Following the 5 min incubation, 400 µl of chilled precipitation buffer (N5) was added. Samples were mixed vigorously and centrifuged for 10 min at maximum speed (4000 rpm, ~2900 g). This should result in precipitation of all the cell debris (and the steel beads) and formation of clear lysates ready for automated DNA extraction

Isolation of DNA from lysates

Labware positioning on epMotion 5075 LH
The workspace of the epMotion 5075 (figure 1) was equipped with the Invitrogen 96-well magnetic separator at position B1, the empty 96 deep well plate used for extraction at position B2, the 96 deep well plate with clear lysates at position C3, reservoir holder with two 30 ml and five 100 ml reservoirs for reagents and liquid waste at position C1, and a 96 well PCR plate for collection of purified DNA at position C2.

To better preserve extracted DNA, we placed the 96 well PCR plate into a thermoadapter. On our system, position C2 was equipped with optional heating/cooling thermal module, which was programmed to keep DNA at +4° C following the extraction. All the other positions are used for boxes of tips. The reservoir holder contained three 30 ml reservoirs for detergent D1, Charge Switch magnetic beads and elution buffer (at holder positions 1, 2 and 3, respectively), two 100 ml reservoirs, each with 100 ml of Wash Buffer (W12) at holder positions 5 and 6, and one 100 ml reservoir for liquid waste at holder position 7. The 100 ml reservoirs at positions 5 and 6 are also used for liquid waste once the Wash Buffer in these reservoirs is exhausted (see below).

Automation method
1. The program started with two reagent transfer steps to dispense 100 µl of D1 detergent and 40 µl of ChargeSwitch magnetic beads into each well of the (originally empty) 96 deep well plate at position B2. It is essential to resuspend Magnetic Beads no earlier than 30 min prior to the dispensing.
2. The second step of the protocol included sample transfer of 850 µl of clear lysate into the deep well plate containing magnetic beads. To ensure good mixing of magnetic beads and the lysate the sample transfer included the “mix after dispensing” option (5 times, 500 µl). To avoid sample cross-contamination, tips have to be changed before aspiration from every next well. DNA binding to magnetic beads takes about one minute. As processing of the 96 samples and changing from the pipetting tool to the gripper takes approximately one minute so it is not necessary to include an additional waiting step and the plate can be moved to the magnetic separator right after the completion of sample transfer step. However, the waiting step of two minutes is essential after the plate was moved to the magnetic separator to make sure the beads have formed a tight pellet.

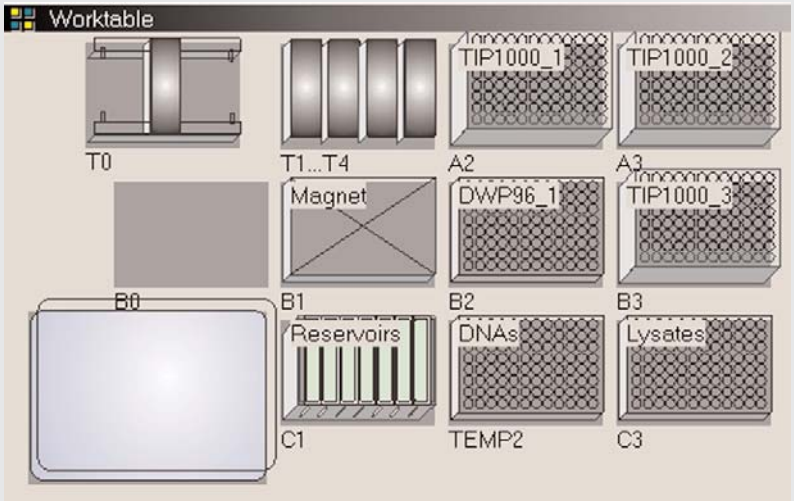

Fig. 1: Section of the epMotion 5075 LH worktable with labware

3. Once the beads with bound DNA have formed a pellet, 1 ml of the supernatant was aspirated and moved to the liquid waste reservoir at holder position 7. To ensure the pellet is not disturbed, the aspiration was done slowly, at an aspiration speed of 3 mm/sec. After supernatant aspiration, the plate was moved from the magnetic separator back to the B2 position.
4. To wash the pellet, 1 ml of the Wash Buffer was dispensed from reservoir 6 to the samples. During the washing steps it is important to ensure resuspension of the pellet; thus the Wash Buffer dispensing included "mix after dispensing" option (5 times, 800 µl). After completion of the washing step the plate was moved to the magnetic separator and the supernatant was slowly aspirated and moved back to the reservoir 6.
5. The washing step was repeated one more time as described in the previous paragraph. At this step the wash buffer from reservoir 5 was used and after the wash the aspirated supernatant was moved back to the reservoir 5. At this step the beads resuspend much better than during the first wash, hence the "mix after dispensing" option is not necessary. As the tips do not touch the pellet during dispensing step, the change of tips is not necessary.
6. To elute the DNA from the pelleted beads the plate was moved from the magnetic separator back to the position B2 and 100 µl of the elution buffer was added. To ensure efficient elution of DNA this step included "mix after dispensing" option with 20 mixing steps of 100 µl and incubation time of 10 minutes. After incubation the plate was moved to the magnetic separator and the supernatant containing eluted DNA was transferred to the 96 PCR plate at position C2. Just before the transfer of eluted DNA the thermal module at position C2 was switched on to +4° C to cool the extracted DNA.

DNA quality and quantity assessment

The concentration and quality of isolated DNA was evaluated on a 0.65% agarose gel (figure 2). The concentration of plant genomic DNA extracted with Invitrogen Charge Switch kit (CS) was approximately 5 ng/µl. The comparison with DNA extracted with a standard CTAB plant miniprep method (4 rightmost lanes) showed that Charge Switch kit yields less fragmented DNA, as there is very little smearing in the CS-extracted samples. The CTAB extraction method was performed as described (Filatov and Charlesworth 1999).

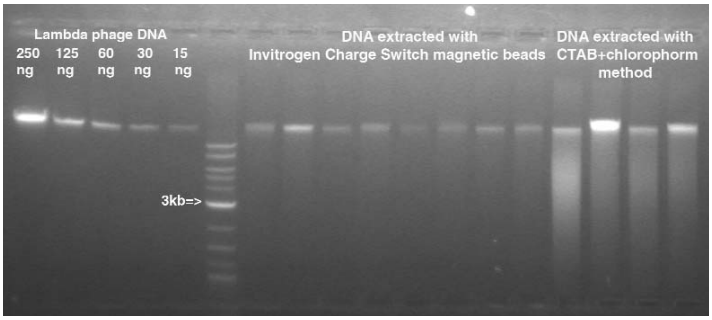

Fig. 2: Agarose gel with plant genomic DNA extracted with Invitrogen Charge Switch and CTAB+chlorophorm methods (3 ul of eluted genomic DNA were loaded into each lane).

Downstream applications

DNA extracted with this method is suitable for a wide range of downstream applications. We used it for PCR amplification of a single copy nuclear gene, followed by gel-purification and sequencing of PCR products. One microliter of extracted genomic DNA was used for PCR amplification of a region of single-copy nuclear gene. PCR amplification was conducted using Expand High Fidelity PCR kit (Roche #1732650) with the following PCR conditions:
17.5 mM MgCl2 ("buffer 1")
0.5 pmol/ul each of the primers
0.4 mM each of dNTPs
and the following cycling program was used for 36 cycles:
94°C 30 sec, 57°C 30 sec, 68°C 4 min.

This resulted in strong clear bands of ~3.8 kb (figure 3). The bands were excised from the gel using razorblade and amplified PCR fragments were extracted from the gel using Qiagen gel extraction kit (#28706) and sequenced directly using ABI BigDye v3.1 sequencing kit (ABI #4336917) on the ABI3700 automatic sequencer. Sequence traces were edited and contigs assembled using ProSeq software (figure 4, also see Filatov 2002).

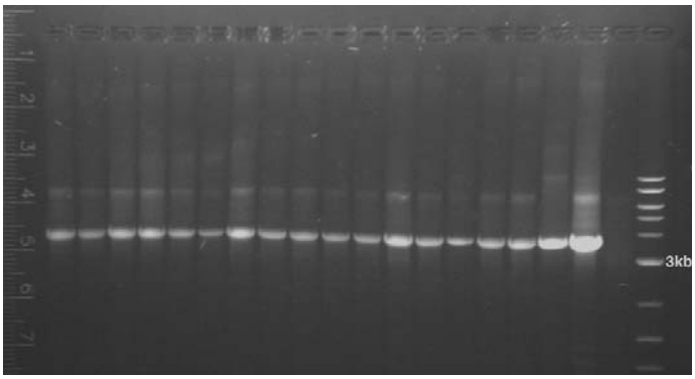

Fig. 3: Photograph of an agarose gel with PCR products. The rightmost lane is the molecular weight marker and the second rightmost lane is the negative control (no DNA added).

Fig. 4: Sequencing of a PCR fragment extracted from the gel. The sequences were edited and assembled with ProSeq software (http://helios.bto.ed.ac.uk/evolgen/filatov/proseq.html).

Conclusions

Here we described an implementation of the Invitrogen ChargeSwitch plant genomic DNA protocol on Eppendorf epMotion 5075 LH automated pipetting system. The method allows reliable isolation of 96 samples in 60 minutes (when 8-channel dispensing tool is used). The DNA yield is consistent across samples and the quality of DNA is sufficient for a wide range of downstream applications.

References

[1] Filatov D.A. and Charlesworth D., 1999, DNA polymorphism, haplotype structure and balancing selection in the Leavenworthia PgiC locus. Genetics, 153: 1423-1434.
[2] Filatov D.A., 2002, ProSeq: A software for preparation and evolutionary analysis of DNA sequence data sets. Molecular Ecology Notes 2: 621-624.

eppendorf
In touch with life

Your local distributor: www.eppendorf.com/worldwide
Eppendorf AG · 22331 Hamburg · Germany · Tel. +49 40 53801-0 · Fax +49 40 538 01-556 · E-Mail: eppendorf@eppendorf.com
Eppendorf North America Inc. · One Cantiague Road, P.O. Box 1019 · Westbury, N.Y. 11590-0207 USA
Tel. 516 334 7500 · Toll free phone 800-645-3050 · Fax +1 516 334 7506 · E-Mail:info@eppendorf.com

Application Support
Europe, International: Tel. +49 1803 666 789 · E-Mail: support@eppendorf.com
North America: Tel. 800 645 3050 ext. 2258 · E-Mail: support_NA@eppendorf.com
Asia, Pacific: Tel. +603 8023 2769 · E-Mail: support_Asia@eppendorf.com

Order-No. AA1 16PW 020/GB1/0306/2T/DigiCon · Chlorine free bleached paper