Applications

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Pine Needle gDNA Extraction with Invitrogen ChargeSwitch® gDNA Plant Kit on the Eppendorf ep*Motion*® 5075

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Abstract

Isolation of genomic DNA from plant tissues often represents a lot of manual working steps. In this Application Note, a way to automate this process using the Invitrogen ChargeSwitch® plant genomic DNA kit on the Eppendorf ep*Motion*® 5075 VAC or LH automated pipetting system is described.
With the help of this protocol, gDNA from 96 samples of pine needles can reliably be isolated in 60 minutes. The DNA yield is consistent across samples and the quality of DNA is sufficient for a wide range of downstream applications.

Introduction

DNA isolation from plant tissues can be more complicated than DNA isolation from blood, or other animal tissues, as plant tissues often are difficult to grind and may contain substantial amounts of polyphenolic compounds, which require additional purification steps.
To address the first issue, a bead mill is adopted for processing large number of samples. Tissue can be ground fresh in lysis buffer or ground desiccated prior to lysing. Since it is rather difficult to achieve complete lysing, it is always recommended to start with more than enough samples to compensate for incomplete lysing (i.e. 30 mg for desiccated samples and 60 mg for fresh samples). Room temperature incubation with vigorous shaking may be necessary if the samples were lysed after grinding.
To address the second issue, polyvinylpyrolidone is added to remove the polyphenols to ensure high quality downstream assays (i.e. PCR and enzymatic digestions). An additional benefit includes “neutralizing” the negatively charged polyphenols so that it will not compete with genomic DNA for the magnetic beads carrying positive charges. Thus, higher yields can be achieved.
The Invitrogen ChargeSwitch gDNA Plant kit is developed based on Invitrogen’s proprietary magnetic beads that bind DNA in low pH buffer and release DNA in high pH (> 8.5) buffer. This kit has already been validated with normal plant tissues, like leaf, on the ep*Motion* [1]. However, so far no application alike was available for pine needles. Moreover, because of the rigidity of pine needle tissue and high content of polyphenols, gDNA isolation from these samples represents a challenge.
Here, we demonstrate how to automate the purification process on the ep*Motion* 5075.

Materials and Methods

Materials

ep*Motion* 5075 VAC
Reservoir rack
Reservoirs 30 ml and 100 ml
Invitrogen ChargeSwitch gDNA Plant Kit
Invitrogen 96-well magnetic separator
Bead mill or pestles for sample grinding
Polyvinylpyrolidone (10,000 average MW)
$CaCl_2$

Sample preparation

Preparation of lysis buffer: Prepare lysis buffer containing fresh Reagent A (refer to page 6 of the kit manual for recipe).
Put the pine needles with lysis buffer in each well of a deepwell plate.
Edit the definition file of the deepwell plate on B2 so that its bottom tolerance is minimal (200 µm). Test to ensure there is no tip collision.

Table 1: Contents of the Reagent Reservoirs in the Reservoir Rack

Reservoir rack position	Size	Reservoir contents
1	30 ml	Detergent D1
2	30 ml	Magnetic beads
3	30 ml	Elution buffer
4	100 ml	Wash buffer W12
5	100 ml	Waste
6	100 ml	Waste
7	100 ml	Waste

Sample processing

Grind pine needles in 600 µl lysis buffer containing Reagent A and RNase A in a deepwell plate (2 ml DWP is preferred).
If a bead mill is used, remove the beads after lysing.
Add 60 µl 10 % SDS to pine needle lysate.
Incubate at room temperature for 5 minutes.
Add 240 µl buffer N5. Seal the plate with a film. Mix the content by vortexing or inversion for 10 seconds.
Centrifuge the plate at maximum speed for 10 minutes at room temperature.
Carefully remove the film, place the plate on position C3 of ep*Motion*.

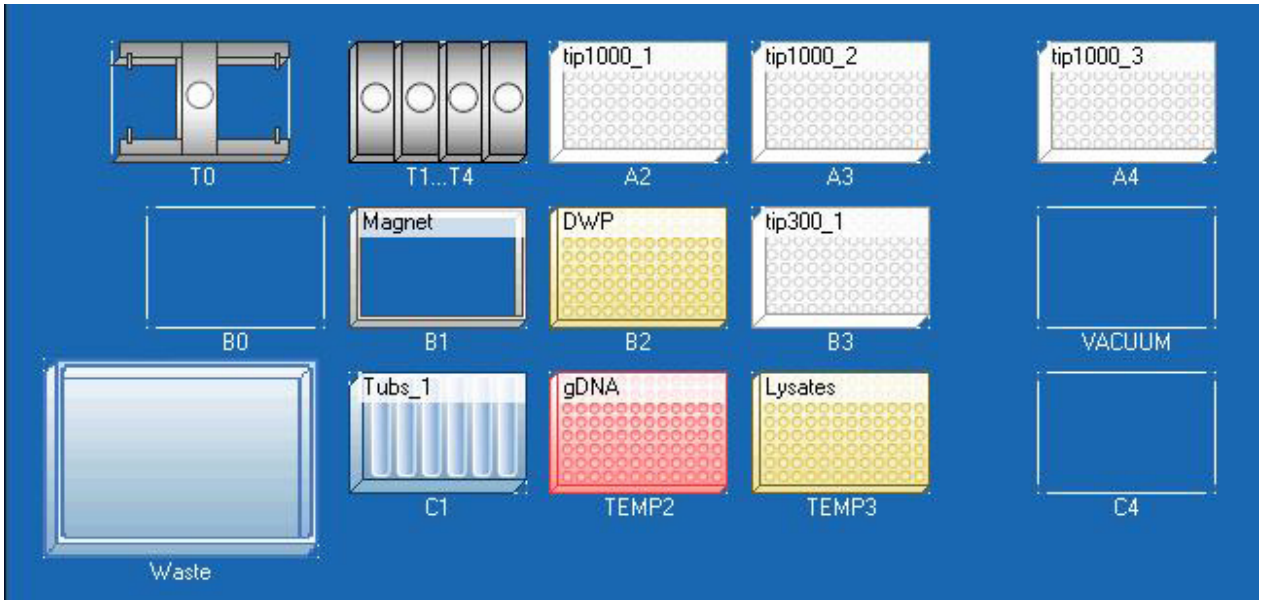

Figure 1: Screenshot taken from an ep*Motion* 5075 VAC

Results

A test run was performed with the following two types of sample:

- Desiccated samples ground with a bead mill (30 mg per well)
- Fresh samples minced then smashed with micro pestle (60 mg per well).

Eight samples each were processed on the ep*Motion* 5075 and three of each were chosen for a test on an electrophoresis gel. As shown in Figure 2, both sample pre-processing methods are viable.

In another experiment, eight samples each were processed and evaluated for yield and purity by spectrophotometric analysis. As shown in Table 2, yields and purity were comparable with both pre-processing methods.
The average purity values were lower than with other typical samples types like blood or tissue, consistent with the complex biological nature of the pine needles.

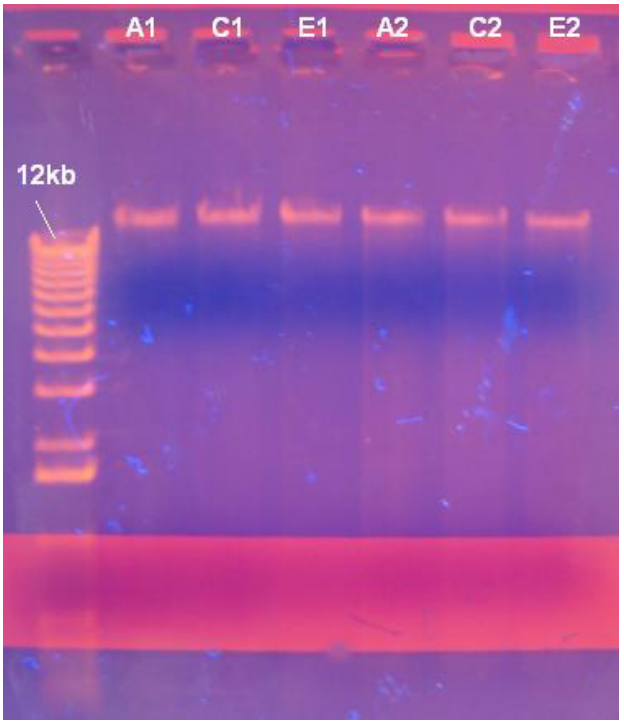

Figure 2: : Analysis of gDNA by gel electrophoresis.
Samples A1, C1, E1: desiccated pine needles
Samples A2, C2, E2: fresh pine needles.
Please note that slight degradation did occur in fresh samples.

Table 2: Spectrophotometric validation

Sample	Concentration µg/ml		OD260/OD280	
No.	Desiccated	Fresh	Desiccated	Fresh
1	43,5	55,0	1.36	1.39
2	36,6	50,7	1,34	1,49
3	40,6	64,1	1,37	1,31
4	88,4	52,9	1,14	1,38
5	43,5	50,3	1,35	1,44
6	44,9	76,2	1,36	1,31
7	89,7	59,4	1,19	1,43
8	83,6	73,6	1,18	1,43
Mean	58,9	60,3	1,29	1,40

References

[1] Eppendorf Application Note 126. www.eppendorf.com

Ordering Information Eppendorf

Product	Description	Order no. international	Order no. North America
ep*Motion* 5075 VAC		5075 000.016	960020014
Dispensing tool TM 1000-8		5280 000.258	960001061
Reservoir rack		5075 754.002	960002148
ep*Motion* Reservoir 30 ml		0030 126.505	960051009

Trademarks

ChargeSwitch is a registered trademark of Invitrogen Inc.

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