DNA Extraction for college laboratory setting

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Abstract

This protocol is based on one described by Li et al. (2010) and has been modified to work in a college laboratory setting. The protocol is from:
James M. Burnette III and Susan R. Wessler (2013) Transposing from the Laboratory to the Classroom to Generate Authentic Research Experiences for Undergraduates *Genetics* 193:367-375; doi:10.1534/genetics.112.147355

Students need approximately 1.5 hours to extract DNA from up to five samples and the protocol can be carried out over several class periods by stopping at steps 6 and 9. Please see the full manuscript for additional details.

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Guidelines

This protocol is based on one described by LI et al. (2010) and has been modified to work in a college laboratory setting. Students need approximately 1.5 hours to extract DNA from up to five samples and the protocol can be carried out over several class periods by stopping at steps 6 and 9.

Materials list:
Extraction Buffer (100 mM Tris, pH 8.0, 50 mM EDTA and 500 mM NaCl)
10% SDS (sodium dodecyl sulfate)
5M KOAc (Potassium Acetate)
1 5 cm by 5 cm piece of Miracloth (Calbiochem, La Jolla, CA)
100% Isopropanol
70% Ethanol
Sterile water
Ice
Liquid nitrogen
65˚C heating block
Sterile 1.5 ml tubes (2 for each prep)
Mortar and pestle

All chemicals were purchased from Fisher Scientific.

Protocol

Step 1.
Label one tube for each plant.

Step 2.
Harvest **2-3** seedlings and place in a mortar. Fill with about **50 ml** of liquid nitrogen. Grind tissue with pestle.

Step 3.
Add **1 ml** of extraction buffer to the tube.

Step 4.
Add **120 µl** of 10% SDS. Mix by inverting.

NOTES
Tracey DePellegrin 26 Sep 2015
If preparing more than one sample, prepare each sample to this step and place on ice.

Step 5.
Incubate tube(s) at 65 ˚C for 20 minutes.

DURATION
00:20:00

Step 6.
Add **300 µl** 5M KOAc. Mix well by inverting several times (**important**!), then place on ice 5 minutes.

DURATION
00:05:00

NOTES
Tracey DePellegrin 26 Sep 2015
Stopping point: Samples can be frozen for a future class period. Thaw samples before starting with step 7.

Step 7.
Centrifuge for 5 minutes at >12,000 rpm. Label a second tube.

DURATION
00:05:00

Step 8.
Pass **700 µl** of the supernatant through a miracloth funnel into the second tube.

Step 9.
Add **600 µl** of isopropanol. Mix the contents thoroughly by inverting.

NOTES
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Stopping point: Samples can be frozen for a future class period.

Step 10.
Spin for 5 minutes at 14,000 rpm.

DURATION
00:05:00

Step 11.
Carefully pour off and discard the supernatant. Use a P20 set to **20 µl** to remove the remaining drops of liquid without disturbing the DNA pellet.

Step 12.
Add **500 µl** of 70% ethanol and flick the tube until the pellet comes off the bottom.

Step 13.
Spin 5 minutes.

DURATION
00:05:00

Step 14.
Pour off the ethanol. Use a P20 set to **20 µl** to remove the remaining drops without disturbing the pellet.

Step 15.
Leave the tube open on the bench to air dry for 5-10 minutes.

DURATION
00:05:00

Step 16.
Resuspend the DNA in **50 µl** TE and incubate at room temperature for 5 minutes for complete resuspension. Samples should be frozen for storage.

DURATION
00:05:00