

Oct 07, 2015

DNA Extraction for college laboratory setting

DOI

dx.doi.org/10.17504/protocols.io.dwu7ev

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Genetics



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OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.dwu7ev

External link: <http://www.genetics.org/content/193/2/367.full>

Protocol Citation: James M. Burnette III, Susan R. Wessler 2015. DNA Extraction for college laboratory setting. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.dwu7ev>

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Protocol status: Working

Created: September 26, 2015

Last Modified: March 22, 2018

Protocol Integer ID: 1716



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.

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)

15 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.




- 1 Label one tube for each plant.
- 2 Harvest **2-3** seedlings and place in a mortar. Fill with about **50 ml** of liquid nitrogen. Grind tissue with pestle.
- 3 Add **1 ml** of extraction buffer to the tube.
- 4 Add **120 µl** of 10% SDS. Mix by inverting.


Note

If preparing more than one sample, prepare each sample to this step and place on ice.

- 5 Incubate tube(s) at 65 °C for 20 minutes.


 00:20:00

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

 00:05:00**Note**

Stopping point: Samples can be frozen for a future class period. Thaw samples before starting with step 7.

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

 00:05:00

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

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

Note

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

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



00:05:00

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.

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

13 Spin 5 minutes.

00:05:00

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

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

00:05:00

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.

00:05:00