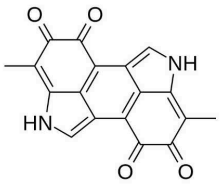
Aug 22, 2020 Version 2

Removal of Melanin V.2



In 1 collection

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OPEN ACCESS

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Protocol status: Working
This is transcribed from work published in this study http://dx.doi.org/10.1111/j.1600-0749.2004.00155.x and http://1000.fungalgenomes.org/home/protocols/removal-of-melanin/

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Protocol Integer ID: 40889

Disclaimer

Abstract

Principle is that CTAB is charging the anionic nucleotides whereby neutral polysaccharides/ melanins are remaining in supernatant. This method also uses urea with the idea that the presence of urea helps to solubilize hydrophobic compounds that would otherwise potentially interact with the hydrophobic core of the CTAB micelles.

Materials

STEP MATERIALS

- NaCl **Sigma Aldrich Catalog #**53014
- Water
- Guanidine hydrochloride **Sigma Aldrich Catalog #**G3272-1KG
- 70% ethanol **Fisher Scientific**
- 70% ethanol **Fisher Scientific**

Protocol materials

- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Water
- Guanidine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G3272-1KG
- 70% ethanol **Fisher Scientific**
- 70% ethanol **Fisher Scientific**
- Water
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Guanidine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G3272-1KG
- 70% ethanol **Fisher Scientific**
- 70% ethanol **Fisher Scientific**

1

Add
Water
to ~ 100 µL - 200 µL DNA/RNA solution until a volume of 400 µL is reached.

2 Add 130 µL [M] 5 Molarity (M)
NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014

3 Add 1.6 mL mL of CTAB-Urea solution

Protocol

NAME
CTAB-Urea buffer

CREATED BY
Jason Stajich

PREVIEW

3.1 50 mM Tris-HCl, pH 7.0

3.2 1% CTAB

3.3 4M Urea

3.4 1 mM EDTA

4 Mix samples (by hand).

5 Incubate overnight at 4 °C
Overnight

6 Centrifuge for 00:15:00 at max speed at 4 °C

7 Remove the solution. Be very careful in this step!

8 Resuspended in 400 µL µL [M] 7 Molarity (M)
Guanidine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G3272-1KG

9 Add 2 2 µL VOLUMES Vol of EtOH (100%)

10 Incubate on ice for
01:00:00

11 15300 x g, 4°C, 00:15:00

12 Wash with
70% ethanol **Fisher Scientific**

13 Wash again with
70% ethanol **Fisher Scientific**

14 20800 x g, Room temperature, 00:10:00 , (or maximum speed)

15 Remove the supernatant.

16 Dry pellet

17 Resuspend in TE buffer