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Removal of Melanin V.2

 In 1 collection

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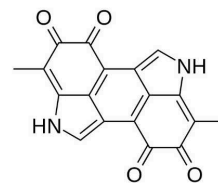
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External link: <http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0749.2004.00155.x/abstract>

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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**

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⊗ Water

⊗ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

⊗ Guanidine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G3272-1KG**

⊗ 70% ethanol **Fisher Scientific**




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1

Add


 Water

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

2

Add  130 μL  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 μ L μ L [M] 7 Molarity (M)
 Guanidine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G3272-1KG**
- 9 Add 2 2 μ L 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