Removal of Melanin V.2

Jason E Stajich[1]
[1]UC Riverside

Version 2
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1 Works for me
dx.doi.org/10.17504/protocols.io.bj6zkrf6

stajichlab

Jason Stajich
UC Riverside

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.

EXTERNAL LINK

http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0749.2004.00155.x/abstract

DOI

dx.doi.org/10.17504/protocols.io.bj6zkrf6

PROTOCOL CITATION

Jason E Stajich 2020. Removal of Melanin. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj6zkrf6

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
NaCl	53014	Sigma Aldrich
Water		
Guanidine hydrochloride	G3272-1KG	Sigma Aldrich
70% ethanol		Fisher Scientific

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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
by Sigma Aldrich
Catalog #: 53014

3 Add **1.6 mL** mL of CTAB-Urea solution

CTAB-Urea buffer
by Jason Stajich
PREVIEW RUN

3.1 50 mM Tris-HCl, pH 7.0

3.2 1% CTAB

3.3 4M Urea

3.4 1 mM EDTA

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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 **7 Molarity (M)**

Guanidine hydrochloride
by Sigma Aldrich
Catalog #: G3272-1KG
CAS Number: 50-01-1

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
by Fisher Scientific

13 Wash again with

70% ethanol
by Fisher Scientific

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

15 Remove the supernatant.

16 Dry pellet

17 Resuspend in TE buffer

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