Removal of Melanin

Jason Stajich

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.

Citation: Jason Stajich Removal of Melanin. **protocols.io**
dx.doi.org/10.17504/protocols.io.chdt25
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Protocol

Step 1.
Add water is added to 100- 200 µL DNA/RNA solution until a volume of 400 µL is reached.

Step 2.
Add 130µL 5M NaCl.

AMOUNT
130 µl Additional info:

Step 3.
Add 1.6 mL of CTAB-Urea solution

PROTOCOL

. CTAB-Urea buffer

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Step 3.1.
50 mM Tris-HCl, pH 7.0

Step 3.2.
1% CTAB

Step 3.3.
4M Urea

Step 3.4.
1 mM EDTA

Step 4.
Mix samples (by hand).

Step 5.
Incubate overnight at 4°C.

DURATION
15:00:00

Step 6.
Centrifuge for 15 minutes at max speed at 4°C.

DURATION
00:15:00

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

ANNOTATIONS
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This is a critical step! Not all DNA will form a pellet at the bottom, but it will also adhere to the side of the tube and some will float on top of the solution. Be very careful in removing the solution! During this removal it is probably needed to perform additional centrifugation steps to reduce risk of losing DNA.

Step 8.
Resuspended in 400 µL 7M guanidine hydrochloride

AMOUNT
400 µl Additional info:

Step 9.
Add 2 Vol of EtOH (100%)

Step 10.
Incubate on ice for 1 hour

DURATION
01:00:00

Step 11.
Centrifuge for 15 min at 4 °C

DURATION
00:15:00

Step 12.
Wash with 70% EtOH

Step 13.
Wash second time with 70% EtOH

Step 14.
Centrifuge 10 min at max speed at RT.

DURATION
00:10:00

Step 15.
Remove the supernatant.

Step 16.
Dry pellet

Step 17.
Resuspend in TE