DNA Extraction Procedure Using SDS Version 2

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Abstract

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Materials

Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich
✓ Liquid Nitrogen by Contributed by users
2-Mercaptoethanol by Sigma Aldrich
Buffer EB 19086 by Qiagen
Chloroform:Isoamyl alcohol 24:1 C0549 by Sigma Aldrich
2 x 0.5ml LongLife™ Proteinase K [5mg/ml] 786-038 by G-Biosciences
2 x 0.5ml LongLife™ RNase [10U/?l] 786-040 by G-Biosciences
1 Liter STE Buffer [10X] (100mM Tris.HCl (pH8.0), 10mM EDTA, 1M NaCl) 786-569 by G-Biosciences
100g SDS (Sodium dodecyl sulfate) DG092 by G-Biosciences
1kg Tris Base RC-106 by G-Biosciences
✓ isopropyl alcohol by Contributed by users

Protocol

Preparation

Step 1.

Cut tissue and grind by liquid nitrogen.

Step 2.

Transfer grinded tissue to 15 ml tube.

Step 3.

Add 6ml TEN, 700µl 20% SDS□56℃preheat), 150µl beta mercaptoethanol and 150 µl proteinase K, rapidly mix.

AMOUNT
6 ml Additional info: TEN

AMOUNT
700 µl Additional info: 20% SDS
AMOUNT
150 µl Additional info: beta mercaptoethanol
AMOUNT
150 µl Additional info: proteinase K

Incubation

Step 4.

Incubate homogenate for 2 h at 56 ℃ , gently blending for every 5 10 min.

Step 5.

Cool down to room temperature.

Step 6.

Add an equal volume of Tris saturated phenol and mix.

Step 7.

Centrifuge at room temperature (□16 ℃) with 14000 RPM for 10 min, then save supernatant.

Step 8.

Add chloroform and isoamyl alcohol (24:1) to the supernatant, then mix.

Step 9.

Centrifuge with 14000 RPM for 10 min, save supernatant.

Step 10.

Resuspend nuclei pellet with 0.8 X volume of frozen isopropyl alcohol.

Step 11.

Wash with cold ethanol 75%. (1/2)

Step 12.

Wash with cold ethanol 75%. (2/2)

Step 13.

Let it dry.

Step 14.

Add 200 µl TEN and 2 µl RNAse (100 mg/ml).

AMOUNT
200 µl Additional info: TEN
AMOUNT
2 µl Additional info: RNAse (100 mg/ml)

Step 15.

37 ℃ for 30 min, incubation period precipitation with Tip dolly, precipitate dissolve completely.

Step 16.

Add buffer (TEN and 200 µl 20% SDS) and 40 µl Protease K up to 2 ml.

AMOUNT
200 µl Additional info: 20% SDS
AMOUNT
40 µl Additional info: Protease K

Step 17.

Incubate for 30 min at 56 ℃.

Step 18.
Add chloroform and isoamyl alcohol (24:1) to supernatant, then mix.

Step 19.
Centrifuge with 14000 RPM for 10 min, save supernatant.

Step 20.
Resuspend nuclei pellet with 0.8 X volume of frozen isopropyl alcohol.

Step 21.
Wash twice with cold ethanol 75%. (1/2)

Step 22.
Wash twice with cold ethanol 75%. (2/2)

Step 23.

Let it dry.

Step 24.

Add 300 µl EB (pH 8.0) to dissolve.

AMOUNT
300 µl Additional info: EB