DNA Extraction Procedure Using SDS

Jiang Hu

Abstract

Citation: Jiang Hu DNA Extraction Procedure Using SDS. **protocols.io**
dx.doi.org/10.17504/protocols.io.jfpcjmn
Published: 17 Aug 2017

Materials

- Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich
- ✓ Liquid Nitrogen by Contributed by users
- 2-Mercaptoethanol View 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

Cut tissue and grind by liquid nitrogen.

Step 1.

Transfer grinded tissue to 15 ml tube, Add 6ml TEN, 700ul 20% SDS□56℃preheat), 150ul beta mercaptoethanol and 150 ul proteinase K, rapidly mix.

Step 2.

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

Step 3.

Cool down to room temperature then add equal volume of Tris saturated phenol and mix, centrifuge at room temperature (□16 ℃) with 14000 RPM for 10 min, then save supernatant.

Step 4.

Add chloroform and isoamyl alcohol (24:1) to supernatant, then mix, centrifuge with 14000 RPM for 10 min, save supernatant.

Step 5.

Resuspend nuclei pellet with 0.8 X volume of frozen isopropyl alcohol, wash twice with cold ethanol

75%, then dry.

Step 6.

Add 200 ul TEN and 2 ul RNAse (100 mg/ml), 37 ℃ for 30 min, incubation period precipitation with Tip dolly, precipitate dissolve completely.

Step 7.

Add buffer (TEN and 200 ul 20% SDS) and 40 ul Protease K up to 2 ml, then incubate for 30 min at 56 ℃, repeat steps 5 and 6.

Step 8.

Add 300 ul EB (pH8.0) to dissolve.

Step 9.