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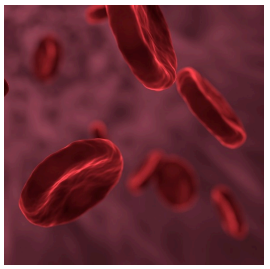
DNA extraction from whole blood using simple salting out procedure



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Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

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OPEN ACCESS

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Protocol status: Working
We use this protocol in our group and it is working.

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Keywords: DNA, DNA extraction, blood, whole blood, human blood, salting out, nuclei lysis, molecular weight dna extraction from whole blood, dna extraction, molecular weight dna extraction, extraction, dna, genetic analysis, range sequencing, procedure the purpose, procedure,

Abstract

The purpose of this protocol is high-molecular weight DNA extraction from whole blood for genetic analyses, including no amplification long-range sequencing.

Citation

S. A. Miller, D. D. Dykes, H. F. Polesky (1988)
. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research.

10.1093/nar/16.3.1215 LINK

Attachments

PDF

DNA extraction from ...

212KB

Guidelines

Storage Vial labeling:

3 lines, 6pt bold font; 1.5" label
a) Subject ID#: four digit SUSL ID (e.g. SUSL-2345), Sample type: *WBC*
b) Date of blood draw: MM/DD/YY
c) Study: [Name Study], Initials

Materials

MATERIALS

- Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S3014
- Ammonium chloride (≥ 99.5 %) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9434
- Potassium bicarbonate (≥ 99.5 %) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**90339
- EDTA 500 mM Solution pH 8.0 ULTROL® Grade **Merck Millipore (EMD Millipore) Catalog #**324504
- Trizma® hydrochloride / Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T5941
- Ethyl Alcohol 200 Proof (GR ACS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**EX0276
- 2-Propanol (99.5 %) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**278475
- Proteinase K from Tritirachium album **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P2308

Note

If using different vendors, choose molecular biology grade reagents.

Buffers and Solution Recipes

10X Red blood cell (RBC) lysis buffer (1l)

	NH4 Cl	82.91 g
	KHC O3	10.01 g
	EDTA 0.5 M	20 ml
	complete to 1000 ml with autoclaved dH2O	

* dilute to 1x before use with autoclaved dH_2O

1 M Tris-HCl, pH 8 (used to make nucleolysis buffer and TE)

	Tris - HCl	15.7 g
	Bring volume to ~ 95ml with autoclaved ddH2O	
	measure & adjust pH to	

	8
	Bring final volume up to 100 ml with autoclaved ddH2O

10X Nucleus lysis buffer (100ml)

	NaCl	23.38 g
	Tris-HCl (1 M, pH = 8)	10 ml
	EDTA 0.5 M	4 ml
	complete to 100 ml with autoclaved ddH2O	

* dilute to 1x before use with autoclaved ddH_2O

20 % SDS (used end of Day 1 for DNA extraction to poke holes in cell membrane)

	SDS	20 g
	autoclaved ddH2O	80 ml

5 M NaCl

	NaCl	146.1 g
	Bring volume to 500 ml with autoclaved dH2O	

70 % EtOH (used Day 2 of DNA extraction to “clean” DNA)

	100 % EtOH	35 ml
	ddH2O	15 ml

TE (used at end of DNA extraction to re-suspend DNA and prepare for storage)

	1 M Tris-HCl	1 ml
	0.5 M EDTA	0.2 ml
	Bring volume to 100 ml with autoclaved ddH2O	

Proteinase K reconstitution:

Solutions can be prepared in [M] 25 millimolar (mM) Tris-HCl buffer , pH 8.0 , containing [M] 1 millimolar (mM) Calcium chloride . Amounts for typical usage is [M] 50 µg/ml – [M] 200 µg/ml . Solutions are stable at pH 8 at 4 °C . It is stable in a broad range of environments: pH, buffer salts, detergents (SDS), and temperature. In the presence of 0.1 – 0.5 % SDS, proteinase K retains activity and will digest a variety of proteins and nucleases in DNA preparations without compromising the integrity of the isolated DNA.

Materials

- 10 ml EDTA collection tube (K_2EDTA, 5.4mg; 16 × 100mm; 10mL; Pink)
- 50 ml conical tube (Fisher catalog **# 12565268**)
- 1.5 ml clear Eppendorf tube (Fisher catalog **# 5408129**)
- 1000 µl filter pipette tips
- 5.0 ml serological pipette (Fisher catalog **# 13-678-11D**)
- 50 ml serological pipette (Fisher catalog **# 13-678-11F**)
- Gloves
- Bleach (e.g. Clorox, as 20 % fresh solution)

Equipment

- Centrifuge: Sorvall Legend XTR, or SorvallT 6000B, H10000B
- Centrifuge: Eppendorf 5417R
- Rotator

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

Prepare buffers and solutions (for recipes see '**Materials**').

1 If you use FROZEN blood, start with step-case 'FROZEN blood'. If FRESH blood is used, select step-case 'FRESH blood'.

STEP CASE

FROZEN blood 37 steps

2 In the morning take out tubes of blood from the -80 °C freezer. Record ID numbers and put in fridge (4 °C) to thaw until later in the afternoon.

3 Once blood has thawed, invert several times or place on rotator for a couple of minutes.

4 Decant the blood sample into 50 ml conical tube.

5 Rinse blood tube with 1 mL of 1x RBC lysis buffer and add to 50ml conincal tube.

6 Add *1x RBC lysis buffer* up to 50 mL .

7 Place on shaker at Room temperature for 00:30:00 .

Note

Note: Blood solution becomes transparent.

8 Centrifuge 00:15:00 at 2000 rpm at Room temperature .

9 Discard supernatant in container with [M] 20 % bleach .

Note

Note: The pellet contains the nuclei.

Note

If direct processing with Nuclei lysis, day 1, then continue **after this step** with Nuclei lysis **(step 12)**.

10 Add 700 µL 1x RBC lysis buffer and close the tube tightly. Pipette up and down to resuspend the pellet (cleans up the nuclei pellet from additional RBCs and allows for storage in small tubes).

11 Transfer to 1.5 ml **pre-labeled** clear Eppendorf tube.

12 Centrifuge 00:05:00 at 2000 rpm at Room temperature .

13 Using a 1000 µl pipette tip, discard the supernatant in container with [M] 20 % bleach .

14 Pellets can be frozen at this point at -80 °C .

Nuclei lysis (Day 1, afternoon)

15 Add 3 mL Nucleus Lysis Buffer and close tube tightly.

16 Pipette up and down to resuspend pellet.

17 Add 300 µL of [M] 10 % SDS and 70 µL Proteinase K ([M] 10 mg/ml).

18 Mix by gently swirling tube.

19 Incubate Overnight in water bath at 55 °C .

DNA extraction (Day 2, morning)

20 Add 1 mL of [M] 5 Molarity (M) NaCl to the tubes.

21 Close caps tightly and **shake vigorously**.

Note

Note: Very important to **shake thoroughly** at this step to precipitate proteins.

22 Centrifuge for 00:20:00 at 3000 rpm at Room temperature .

23 While centrifuging, label 2× 15ml conical tubes per sample.

24 Transfer supernatant to a 15 ml conical tube and then repeat centrifugation for 00:20:00 at 3000 rpm

Note

Note: The supernatant contains the DNA.

25 Transfer supernatant to next 15 ml conical tube.

26 Add equal volume of **ice-cold** *isopropanol* (usually about 4 mL).

27 Invert tubes several times and DNA will precipitate.

Note

Note: You should be able to see **white strings** (this is the DNA!).

28 Centrifuge for 00:05:00 at 3000 rpm .

29 Discard supernatant.

30 Add 1 mL [M] 70 % ethanol to each tube.

31 Centrifuge for 00:05:00 at 3000 rpm .

32 Carefully pipette off *ethanol* to not dislodge the DNA pellet.

33 Leave DNA to dry in **uncapped tubes** overnight.

Dissolve DNA

34 Add 250 µL TE and allow DNA to dissolve in 15 ml conical tube stored in fridge (4 °C).

35 Once **completely dissolved**, transfer to labeled sterile Eppendorf tube.

36 Label top with **subject ID**. Label side with **date of extraction** and **concentration**.

37 Spec all samples using **Nanodrop**. Dilute samples to stock at [M] 250 ng/µl – [M] 350 ng/µl and to working dilution at [M] 10 ng/µl .

38 Transfer samples into barcode tubes and log into DNA bank.

Citations

S. A. Miller, D. D. Dykes, H. F. Polesky. A simple salting out procedure for extracting DNA from human nucleated cells
10.1093/nar/16.3.1215