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DNA Extraction

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We use this protocol and it's working

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Abstract

DNA extraction from frozen blood clots is challenging. Here we applied QIAGEN Clotspin Baskets and the Gentra Puregene Blood Kit for DNA extraction from 5.5 ml whole blood without anticoagulating additives.

Protocol materials

- RBC Lysis Solution **Qiagen**
- Cell Lysis Solution **Qiagen**
- Proteinase K **Qiagen**
- Protein Precipitation Solution **Qiagen**
- Isopropanol
- Proteinase K (20 mg/ml)
- Isopropanol
- ethanol
- DNA Hydration Solution **Qiagen**

Blood clot preparation and red blood cell lysis

40m 19s

1 Frozen blood clots in 15 ml tubes were transferred from -20°C to a warming cabinet 55 °C for 00:10:00 and thereafter immediately placed On ice

10m

2 The tube was inverted to loosen the clot. The blood clot was completely poured with 5 mL RBC Lysis Solution **Qiagen** into the Clotspin Basket placed on a 50 ml tube

3 To disperse the clot, centrifugate 2000 x g, 00:05:00

5m

4 The remaining clot material from the Clotspin basket was transferred through the basket to the filtrate with 00:10:00 RBC Lysis Solution **Qiagen** and the basket was discarded.

10m

5 To completely disperse the clotted material, the filtrate was **vortexed** vigorously for 00:00:03 and placed for 00:05:00 at Room temperature on a circulating **shaker (250 1/min)**.

5m 3s

6 The tubes were again **vortexed** vigorously for 00:00:03 and centrifugated 2000 x g, 00:05:00 at 2000 x g for 5 min

5m 3s

7 The supernatant was carefully discarded, taking care that the pellet remains in the tube. If no pellet was visible, about 0.5 mL of the supernatant was kept in the tube

8 The tube was **vortexed** rigorously for 00:00:10 and additional 5 mL RBC Lysis Solution **Qiagen** was added to the pellet, followed by **vortexing** for 00:00:03 and incubation on a circulating shaker **(250 1/min)** for 00:05:00 at Room temperature

5m 13s

White blood cell lysis

1d 0h 5m 30s

9 Centrifugation 2000 x g, 00:05:00 to pellet the DNA-containing white blood cells

5m

10 Discard supernatant carefully, leaving about 0.2 ml of residual liquid. Vortex rigorously for 00:00:10 — 10s

11 Adding of 5 mL Cell Lysis Solution **Qiagen** and 25 µL of Proteinase K **Qiagen** [M] 20 mg/mL as followed by rigorously **vortexing** for 00:00:10 — 10s

12 For complete lysis of DNA containing cells, incubate Overnight at 55 °C — 10s

Protein precipitation — 17m 20s

13 samples were cooled down On ice for 00:05:00 and 1.7 mL Protein Precipitation Solution **Qiagen** was added, followed by rigorous **vortexing** for 00:00:20 — 5m 20s

14 After centrifugation 2000 x g, 00:10:00 , incubate the samples for 00:02:00 On ice . — 12m

DNA precipitation — 3m

15 For precipitation of DNA, the supernatant was **carefully** transferred in a 50 ml tube, containing 5 mL Isopropanol

16 The samples were mixed by gently **inverting the tube 50 times** and centrifugated 2000 x g, 00:03:00 — 3m

17 The supernatant was **carefully** discarded, and the tube was drained on a clean piece of absorbent paper without losing the pellet. The DNA pellet was inspected visually and with two following options

STEP CASE

big red to brown pellet 9 steps

Additional washing step to remove remaining erythrocytes

DNA washing

4h 23m 20s

18 2 mL of Cell Lysis Solution **Qiagen** and 10 µL of Proteinase K (20 mg/ml) were added, followed by incubation at 55 °C for 02:00:00 in a warming cabinet **or** at Room temperature Overnight on a circulating shaker **(250 1/min)** — 4h

19 samples were cooled down On ice for 00:05:00 and 1 mL Protein Precipitation Solution **Qiagen** was added, followed by rigorous **vortexing** for 00:00:20 — 5m 20s

20 After centrifugation 2000 x g, 00:10:00 , the samples were incubated for 00:02:00 On ice — 12m

21 DNA precipitation was done with 3 mL Isopropanol . The samples were mixed by gently **inverting the tube 50 times** and centrifugated 2000 x g, 00:03:00 — 3m

22 The supernatant was carefully discarded. The tube was drained on a clean piece of absorbent paper, taking care that the pellet remained in the tube

23 5 mL of [M] 70 % (v/v) ethanol were added immediately and the tubes were inverted until the pellet was detached

24 After centrifugation 2000 x g, 00:03:00 at 2000 x g for 3 min, the supernatant was carefully discarded, and the DNA pellet was air dried at room temperature for 10 min or until the pellet got glassy — 3m

DNA hydration

1h 2m

25 Addition of 0.5 mL DNA Hydration Solution **Qiagen** for a **large** pellet **or** 0.3 mL for a **smaller** pellet was followed by incubation at 65 °C in a warming — 1h 2m

cabinet for 01:00:00 . To fully dissolve the DNA, the sample were put on a **shaker** Overnight at Room temperature

26 Samples were centrifugated briefly and the solved DNA was transferred to a 2 ml cup and **stored** at -20 °C .