Sep 12, 2022

DNA Extraction

klaus.stark[1], Kira Julia Stanzick[1]

[1]Department of Genetic Epidemiology, University of Regensburg

dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1

Kira Julia Stanzick

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.

DOI

dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1

PROTOCOL CITATION

klaus.stark, Kira Julia Stanzick 2022. DNA Extraction. **protocols.io** https://protocols.io/view/dna-extraction-cfiutkew

CREATED

Aug 19, 2022

LAST MODIFIED

Sep 12, 2022

PROTOCOL INTEGER ID

68916

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 10m **55 °C** for **00:10:00** and thereafter immediately placed **On ice**

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

Citation: klaus.stark, Kira Julia Stanzick DNA Extraction https://dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1

11 Adding of **5 mL** Cell Lysis Solution **Qiagen** and **25 µL** of Proteinase K **Qiagen** **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 **Contributed by users**

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 17 includes a Step case.
big red to brown pellet
white pellet

DNA washing 4h 23m 20s

step case

big red to brown pellet

Additional washing step to remove remaining erythrocytes

Citation: klaus.stark, Kira Julia Stanzick DNA Extraction https://dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1

18 4h

2 mL of Cell Lysis Solution **Qiagen** and **10 µL** of Proteinase K (20 mg/ml) **Contributed by users** 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)**

19 5m 20s

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**

20 12m

After centrifugation **2000 x g, 00:10:00** , the samples were incubated for **00:02:00** **On ice**

21 3m

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

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 **70 % (v/v)** ethanol **Contributed by users** were added immediately and the tubes were inverted until the pellet was detached

24 3m

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

DNA hydration 1h 2m

25 1h 2m

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

Citation: klaus.stark, Kira Julia Stanzick DNA Extraction https://dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1

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

Citation: klaus.stark, Kira Julia Stanzick DNA Extraction https://dx.doi.org/10.17504/protocols.io.5qpvobjw7l4o/v1