Animal and viral DNA extraction from FTA card-preserved dried blood spots (DBS) and tissue-smears using proteinase K.

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The "Proteinase K DNA Extraction from FTA Card Preserved Dried Blood Spots (DBS)" protocol outlines an efficient and reliable method for isolating high-quality DNA from dried blood spots preserved on FTA cards. This technique leverages the stability and long-term preservation properties of FTA cards, which safeguard nucleic acids against environmental degradation while inactivating pathogens. By incorporating Proteinase K digestion, the protocol ensures effective lysis of cellular material and optimal recovery of DNA suitable for downstream applications such as PCR, sequencing, and genotyping. This approach is particularly valuable for studies requiring minimal sample volumes, field-collected specimens, or archived materials, offering a robust solution for genetic and epidemiological research. This protocol has been successfully used to extract PCR-quality genomic and viral DNA from both fresh and frozen whole blood/serum samples obtained from humans, bats and rodents, as well as to extract mosquito DNA from mosquito smears.

Procedure

1. Aliquot 200 µl of sterile PBS into a previously labelled 1.5 mL microcentrifuge tube for each sample to be processed

2. Take three 6 mm FTA card punches from each blood/serum DBS, or a single 6 mm card punch if mosquito smears are being processed, and place them together in the previously mentioned 1.5 mL microtube.

3. Add 500 µL of Proteinase K Cell Lysis Buffer (see note 2 below), 50 µL of 10% SDS and 20 µL of 20mg/ml Proteinase K.

4. Vortex for 30 seconds.

5. Incubate in Eppendorf® Thermomixer at 55°C and 300 rpm for 1.5 hours.

6. Add 200 µL of 5M NaCl and mix by inversion (inverting 10 times).

7. Centrifuge at 16,000 G for 10 minutes.

8. Collect supernatant and transfer to new, previously labelled 1.5 mL microcentrifuge tube.

9. Centrifuge at 16,000 G for 10 minutes, again.

10. Collect supernatant and transfer to new, previously labelled 1.5 mL microcentrifuge tube.

11. Add 760 µL of ice-cold isopropanol (-20°C) to the microcentrifuge tube.

12. Centrifuge at 16,000 G for 10 minutes.

13. Discard supernatant by inverting and blotting microtube over absorbent tissue paper (do not introduce paper into microtube) and allow to air dry by placing the open microtubes in the Eppendorf® Thermomixer for 10 minutes at 70°C.

14. Add 500 µL of ice cold 70% Ethanol (-20°C) and mix by inversion.

15. Centrifuge at 9,000 G for 10 minutes.

16. Discard supernatant by inverting and blotting microtube over absorbent tissue paper (do not introduce paper into microtube) and allow to air dry by placing the open microtubes in the Eppendorf® Thermomixer for 10 minutes at 70°C.

17. Add 50 µL of either molecular-grade dH_2O (for samples intended for short-term use in a few assays) or 50 µL of 10:1 TE buffer to prepare a stock DNA solution for long-term storage..

18. Store stock DNA solution in refrigerator if intended use during first 24 hours, frozen between 0°C and -4°C if use programmed for first week or at -20°C to -30°C for long term storage (months to years).

19. Certain qPCR and end-point PCR amplification protocols may require further dilution of the stock DNA solution at a 1:3 ratio (1 volume DNA + 2 volumes dH_2O) or a 1:5 ratio (1 volume DNA + 4 volumes dH_2O) to reduce residual PCR inhibitors, such as salts and alcohols, carried over from DNA extraction.

20. The presence of PCR inhibitors in the extracted DNA should ideally be assessed by amplifying a housekeeping gene, such as 18S rRNA, as described in the following protocol: https://www.genomica.uaslp.mx/Protocolos/Mol_18S_rRNA_ENG.pdf.

Typical yield and quality

Source	DNA yield	$A^{260/280}$ ratio	$A^{260/230}$ ratio
Human dried blood spot	5 µg at 100 ± 23 ng/µL	0.5 ± 0.1	0.1 ± 0
Human serum spot	30 ng at 1.5 ± 5 ng/µL	0.7 ± 0.5	0.4 ± 2
Rat serum spot	720 ng at 14.4 ± 9.2 ng/µL	0.4 ± 0.2	0.3 ± 0.2
Rat dried blood spot	1.2 µg at 24.4 ± 8.5 ng/µL	0.6 ± 0.1	0.3 ± 0
Mosquito smear	432 ng at 86.4 ± 6.8 ng/µL	1.9 ± 0.2	1.1 ± 0.3

Notes

1. Multiple freeze-thaw cycles can degrade DNA and compromise genetic data. Make multiple aliquots to minimize impact of freeze-thaw cycles. DNA material used in a short time frame may be stored at -20 ºC. Long term storage of DNA should use ultra-low freezers, typically at or below -70C to prevent the degradation of nucleic acids. To ensure highest DNA quality, the following DNA storage strategies are recommended:

    - Short-term storage (1 week) between 0°C to -4°C in 10:1 M Tris-EDTA
    - Mid-term storage (> 1 month to years) between –20°C to -30°C in 10:1 TE buffer.
    - Long-term storage (decades) at –80°C as dry ethanol precipitate or super-diluted solution (1:10).

2. **Proteinase K Cell Lysis Buffer** consists of 10 mM 8.0 pH TRIS-HCl, 2 mM 8.0 pH EDTA and 400 mM NaCl.

3. Assess working DNA solution quality and yield as recommended in “Spectrophotometric evaluation of DNA using a Nanodrop ND-1000” multimedia protocol available in https://www.youtube.com/watch?v=k2tZTDDyxaw&feature=youtu.be).

4. Genomic DNA integrity can be assessed in 1% agarose gel electrophoresis by loading 5 µL of stock DNA + 10 µL of 6x orange loading buffer and running at 6 volts/cm of gel length for 50 minutes. Good integrity DNA should exhibit a single high molecular smear above the 10 kb marker, degraded DNA will show lower weight molecular smears whose size will vary depending on degree of degradation. DNA obtained from old blood samples or those not stored in refrigeration will normally exhibit 200 bp apoptotic ladders.

References

1. Bezerra CS, *et al*. Comparison of four extraction methods for the detection of hepatitis B virus DNA in dried blood spot samples. Microbiologyopen. 2021 Mar;10(2):e1161.

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3. Choi EH, *et al*. Rapid DNA extraction from dried blood spots on filter paper: potential applications in biobanking. *Osong Public Health Res Perspect*. 2014.

4. Goldenberger D, *et al*. A simple “universal” DNA extraction procedure using SDS and proteinase K is compatible with direct PCR amplification. *Genome Res*. 1995;4(6):368-370. doi:10.1101/gr.4.6.368.

5. Struniawski R, *et al*. Rapid DNA extraction protocol for detection of alpha-1 antitrypsin deficiency from dried blood spots by real-time PCR. *Adv Exp Med Biol*. 2013.

Revision history

1.0 Original document for use with blood, urine and saliva.

2.0 Protocol optimized for use in DBS DNA extractions.

3.0 Abbreviated protocol as optimized by MA Nieto-Villegas

4.0 Incorporates further optimizations introduced by MA Nieto-Villegas and new document format.

5.0 Additional spin/microtube transfer cycle added after step 8 as optimized by Se Guerra-Palomares.

6.0 Universal protocol for use with human and rodent DBS as well as mosquito smears.