Proteinase K DNA extraction from urine, blood serum or plasma.

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DNA extraction represents a crucial step for downstream nucleic acid based molecular methods used to characterize the genetic features of human tissue samples as well as of viral, prokaryote, fungal and parasitic agents. Proteinase K (EC 3.4.21.64, protease K, endopeptidase K, Tritirachium alkaline proteinase, Tritirachium album serine proteinase, Tritirachium album proteinase K) is a broad-spectrum serine protease commonly used in molecular biology to digest protein and remove contamination from nucleic acid preparations by inactivating nucleases that degrade DNA or RNA during purification [1]. This enzyme is active in the presence of chemicals that denature proteins, such as SDS and urea, chelating agents such as EDTA, sulfhydryl reagents, as well as trypsin or chymotrypsin inhibitors. Proteinase K excels at isolating undamaged DNA or RNA, as most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.5–1% SDS. The Proteinase K/SDS protocol was developed as an efficient but simple DNA extraction procedure applicable to various types of clinical specimens (cell free specimens). The procedure allows direct PCR amplification of nucleic acids without purification or precipitation of DNA [2]. Proteinase K DNA extraction continues to represent the most efficient and fast nucleic acid extraction procedure ever devised [3,4,13–16,5–12].

Procedure

1. Take vacutainer containing anticoagulated whole blood sample (Plasma from violet top tube) or a vacutainer containing whole blood without anticoagulant (Serum from red top tube) and spin at 3,000 G for 5 minutes.

2. Place a 200 µL aliquot of either urine, serum or plasma in a 1.5 mL microcentrifuge tube.

3. Add 500 µL of Proteinase K Cell Lysis Buffer (stored at room temperature in a Styrofoam rack next to the Thermomixer in the Main Lab).

4. Add 50 µL of 10% SDS and 40 µL of Proteinase K (10 mg/ml).

5. Vortex for 30 seconds.

6. Incubate at 55ºC in the Thermomixer at 350 RPM for 90 minutes.

7. Add 200 µL of 5 M NaCl and mix by manual inversion.

8. Spin at 16,000 G for 15 minutes.

9. Collect supernatant and transfer to a new 1.5 mLmicrocentrifuge tube.

10. Add 600 µL of 100% Isopropanol.

11. Incubate at -20ºC for at least 1 hour or overnight if an interruption in the process is warranted.

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

13. Discard supernatant by inversion and allow DNA pellet to air dry over absorbent tissue paper for 10 to 15 minutes.

14. Add 500 µL of chilled (-20ºC) 70% ethanol and mix manually.

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

16. Discard supernatant by inversion and allow DNA pellet to air dry over absorbent tissue paper for 10 to 15 minutes.

17. Resuspend DNA in 50 µL of dH_20 and use as *stock DNA solution* for immediate PCR (usually the purpose of Proteinase K extractions is for immediate use of clinical samples or relatively few PCRs only).

18. Incubate *stock DNA solution* in thermomixer or water bath for 30 minutes at 70ºC.

19. If stock DNA solution is to be used for future applications (weeks to months into the future) store at -20ºC.

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 (weeks) at 4°C in 10:1 M Tris-EDTA
    - Medium-term storage (months) at –80°C in 10:1 M Tris-EDTA
    - Long-term storage (years) at as –80°C as ethanol precipitate or FTA card immobilized.
    - Long-terms storage (decades) at –164°C in 10:1 M Tris-EDTA

2. **Proteinase K Cell Lysis Buffer** consists of 10 mM ph8.0 TRIS-HCl, 2 mM pH 8.0 EDTA and 400 mM NaCl.

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

4. Evaluate genomic DNA integrity in 1% agarose gel electrophoresis by loading 5 µL per well of a 1 µL working DNA solution + 4 µL of dH_2O + 3 µL of 6x O orange loading buffer running at 6 volts per cm og gel length for 50 minutes per inch of travel. 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 banding patterns.

5. Functional applicability of extracted DNA should be assessed depending on downstream procedures. For endpoint PCR applications, samples should be screened for the presence of housekeeping genes or suitable conserved genes such as KIR3DL2 or KIR3DL3 (see corresponding protocol "Killer-cell Immunoglobulin-like Receptor (KIR) genotyping" available from http://lgvh.hostingerapp.com/Protocolos/Hum_KIR.pdf.

References

1. Jany KD, Lederer G, Mayer B. Amino acid sequence of proteinase K from the mold Tritirachium album Limber. Proteinase K - a subtilisin-related enzyme with disulfide bonds. *FEBS Lett*. 1986;199(2):139-144. doi:10.1016/0014-5793(86)80467-7

2. Goldenberger D, Perschil I, Ritzler M, Altwegg M. 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

3. Rohland N, Hofreiter M. Ancient dna extraction from bones and teeth. *Nat Protoc*. 2007;2(7):1756-1762. doi:10.1038/nprot.2007.247

4. Wang JH, Gouda-Vossos A, Dzamko N, Halliday G, Huang Y. DNA extraction from fresh-frozen and formalin-fixed, paraffinembedded human brain tissue. *Neurosci Bull*. 2013;29(5):649-654. doi:10.1007/s12264-013-1379-y

5. Bellemare A, John T, Marqueteau S. Fungal genomic DNA extraction methods for rapid genotyping and genome sequencing. In: *Methods in Molecular Biology*. Vol 1775. Humana Press Inc.; 2018:11-20. doi:10.1007/978-1-4939-7804-5_2

6. Olekšáková T, Žurovcová M, Klimešová V, Barták M, Šuláková H. DNA extraction and barcode identification of development stages of forensically important flies in the Czech Republic. *Mitochondrial DNA Part A DNA Mapping, Seq Anal*. 2018;29(3):427-430. doi:10.1080/24701394.2017.1298102

7. Gómez-Acata ES, Centeno CM, Falcón LI. Methods for extracting 'omes from microbialites. *J Microbiol Methods*. 2019;160:1-10. doi:10.1016/j.mimet.2019.02.014

8. Lenore Ackerman A, Anger JT, Khalique MU, et al. Optimization of DNA extraction from human urinary samples for mycobiome community profiling. *PLoS One*. 2019;14(4). doi:10.1371/journal.pone.0210306

9. Kallassy H, El Khoury LY, Eid M, Chalhoub M, Mansour I. Comparison of four DNA extraction methods to extract DNA from cigarette butts collected in Lebanon. *Sci Justice*. 2019;59(2):162-165. doi:10.1016/j.scijus.2018.09.003

10. Gryp T, Glorieux G, Joossens M, Vaneechoutte M. Comparison of five assays for DNA extraction from bacterial cells in human faecal samples. *J Appl Microbiol*. 2020;129(2):378-388. doi:10.1111/jam.14608

11. Frazer Z, Yoo C, Sroya M, et al. Effect of Different Proteinase K Digest Protocols and Deparaffinization Methods on Yield and Integrity of DNA Extracted From Formalin-fixed, Paraffin-embedded Tissue. *J Histochem Cytochem*. 2020;68(3):171-184. doi:10.1369/0022155420906234

12. Koshy L, Anju AL, Harikrishnan S, et al. Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays. *Mol Biol Rep*. 2017;44(1):97-108. doi:10.1007/s11033-016-4085-9

13. Garbieri TF, Brozoski DT, Dionísio TJ, Santos CF, Neves LT das. Human DNA extraction from whole saliva that was fresh or stored for 3, 6 or 12 months using five different protocols. *J Appl Oral Sci*. 2017;25(2):147-158. doi:10.1590/1678-77572016-0046

14. Zhou J, Bruns MA, Tiedje JM. DNA recovery from soils of diverse composition. *Appl Environ Microbiol*. 1996;62(2):316-322. doi:10.1128/aem.62.2.316-322.1996

15. Dabney J, Meyer M. Extraction of highly degraded DNA from ancient bones and teeth. In: *Methods in Molecular Biology*. Vol 1963. Humana Press Inc.; 2019:25-29. doi:10.1007/978-1-4939-9176-1_4

16. Nasiri H, Forouzandeh M, Rasaee MJ, Rahbarizadeh F. Modified salting-out method: High-yield, high-quality genomic DNA extraction from whole blood using laundry detergent. *J Clin Lab Anal*. 2005;19(6):229-232. doi:10.1002/jcla.20083

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