Fast Protocol for DNA Extraction from Formalin-Fixed Paraffin-Embedded Tissues

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9.1 Introduction and Purpose

This protocol provides a fast method to obtain a crude extract of genomic DNA from microdissected or entire sections of FFPE tissue for PCR analysis. It is based on the method described by Higuchi [1] with modifications in the deparaffinization steps and in the procedure for recovering the tissue. For the described home-made method, approximately 1 cm^2 tissue (3–5 µm thick sections) is required. Sectioning is followed by deparaffinization, tissue microdissection when needed, and proteolytic hydrolysis with Proteinase K. There are also available commercial kits for extraction of DNA from FFPE (i.e., Qiagen, Roche, Ambion…), to this purpose visit the companies' websites for instruction.

The time required for the complete procedure is about 1.5 h and an additional overnight incubation.

9.2 Protocol

9.2.1 Reagents

Note: Reagents from specific companies are reported here, but might be substituted by reagents of comparable quality from other vendors.

- *Xylene* (ultrapure) (AppliChem, Fluka or Sigma-Aldrich)
- *Absolute ethanol* (AppliChem, Sigma-Aldrich)
- *25 mg/ml Proteinase K, stock solution* (Sigma P2308): Dissolve 25 mg of Proteinase K in 1 ml of dd H_2O

[1]Alternatively, the ready-to-use Proteinase K solution (Qiagen 19133) can be used; it has a concentration of about 20 mg/ml (store in aliquots at +4°C).

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(final concentration of Proteinase K is 3.26 mg/ml in digestion buffer). Store the solution in aliquots at −20°C. Do not refreeze after thawing.[1]
- *1× Hydrolysis buffer*: 50 mM Tris HCl pH 8.5, 1 mM EDTA pH 8.0, 0.5% (v/v) Tween 20. Store the buffer in aliquots at –20°C. Do not refreeze after thawing.

9.2.2 Equipment

- *Clean*[2*] *adjustable pipettes,* range: 1–10 µl, 10–100 µl, 100–1,000 µl
- *Nuclease-free aerosol-resistant pipette tips*
- *2 ml Safelock and 1.5 ml microreaction tubes (nuclease free)*
- *Microtome, with new blades*
- *Cooling plate for cooling down the paraffin-embedded tissue*
- *Centrifuge* suitable for centrifugation of 2 ml microreaction tubes at ~17,900 × g
- *Thermoblock*
- *Thermomixer* (e.g., Eppendorf)
- *UV-light Photometer (wavelength* [λ]*: 260 and 280 nm)*

9.2.3 Method

- Cool the paraffin blocks on a cooling plate or at −20°C in order to cut the sections.
- In order to avoid DNase cross-contaminations use a new, clean microtome blade for every paraffin block and clean the microtome with xylene. Cut 3–5 µm-thick sections together representing at least 1 cm^2 area of tissue and mount them on microscope slides. As a negative control an open microreaction tube containing Hydrolysis buffer may be put in the area of the cutting. It is closed after the cutting procedure and run in parallel with the other microreaction tubes containing tissue material.
- Deparaffinize sections twice in xylene for 10 min each and twice in absolute ethanol for 10 min each before air drying.
- Moisten the tissue with the Hydrolysis buffer and scratch off the tissue using another sterile microscope slide in a way one would prepare blood smears. Transfer the moist tissue clump into a 2 ml Safelock microreaction tube.[3] If microdissection is necessary, the tissue might be microdissected by scraping off the area of interest from the section using a sterile scalpel blade and transferring it into a new 2 ml Safelock microreaction tube. It might be helpful to moisten the tip of the blade so that the scratched-off tissue will adhere to it.
- Add 200 µl Hydrolysis buffer. The Hydrolysis buffer should cover the tissue completely. If the tissue adheres to the wall of the microreaction tubes, briefly spin it down.
- Add 30 µl Proteinase K (25 mg/ml) to a final concentration of 3.26 mg/ml.
- Incubate overnight (at least 16 h); but for microdissected tissue, incubate for a maximum of 5 h at 56°C while shaking (450 rpm).
- Inactivate Proteinase K by incubation at 95°C for 10 min.
- Centrifuge for 10 min at 14,000 rpm at room temperature.
- Transfer the supernatant into a new microreaction tube (0.5 or 1.5 ml tube) and discard the pellet.
- Determine the DNA concentration photometrically at 260 nm[4] and use the digestion buffer as blank. The A260/A280 ratio will be low because the lysate is a crude DNA extract containing cellular proteins. When working with microdissected tissue fragments it might be difficult or even impossible to obtain reliable absorbtion values at A260.
- Use the DNA extract for PCR analysis, or store it at −20°C.

[2]Clean pipettes with DNase Away™ to avoid DNase and DNA contamination. Alternatively, it is possible to clean pipettes by first using mild detergent containing aqueous solutions, followed by application of antiseptic alcohol solution (e.g., 70% (v/v) ethanol) or another disinfectant and then leaving them under UV light for at least 10 min.

[3]Depending on the Thermomixer, smaller microreaction tubes may be used.

[4]The concentration of dsDNA expressed in µg/µl is obtained as follows: $[DNA] = A_{260} \times \text{dilution factor} \times 50 \times 10^{-3}$. A clean DNA preparation should have a A_{260}/A_{280} ratio of 1.5–2. This ratio is decreased by the presence of proteins, oligo- and polysaccharides. Concentration estimation can be also affected by phenol contamination, as phenol absorbs strongly at 260 nm and therefore can mimic higher DNA yield and purity.

9.2.4 Troubleshooting

- If the yield of DNA is low or DNA is absent by UV measurement, decrease the dilution factor and measure the concentration again.
- If DNA is absent, a nuclease contamination could have occurred. In such case, clean the workspace and pipettes with DNase Away™ and repeat the extraction using freshly made reagents. These hints may not apply when working with tissue fragments isolated with the help of microdissection. In these cases, the DNA contents may not be detectable in the crude lysates or by photometry.

Reference

1. Higuchi R (1989) Simple and rapid preparation of samples for PCR. PCR technology; principles and applications for DNA amplification. Stockton, New York