

Video Article

DNA Extraction from Paraffin Embedded Material for Genetic and Epigenetic Analyses

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Abstract

Disease development and progression are characterized by frequent genetic and epigenetic aberrations including chromosomal rearrangements, copy number gains and losses and DNA methylation. Advances in high-throughput, genome-wide profiling technologies, such as microarrays, have significantly improved our ability to identify and detect these specific alterations. However as technology continues to improve, a limiting factor remains sample quality and availability. Furthermore, follow-up clinical information and disease outcome are often collected years after the initial specimen collection. Specimens, typically formalin-fixed and paraffin embedded (FFPE), are stored in hospital archives for years to decades. DNA can be efficiently and effectively recovered from paraffin-embedded specimens if the appropriate method of extraction is applied. High quality DNA extracted from properly preserved and stored specimens can support quantitative assays for comparisons of normal and diseased tissues and generation of genetic and epigenetic signatures¹. To extract DNA from paraffin-embedded samples, tissue cores or microdissected tissue are subjected to xylene treatment, which dissolves the paraffin from the tissue, and then rehydrated using a series of ethanol washes. Proteins and harmful enzymes such as nucleases are subsequently digested by proteinase K. The addition of lysis buffer, which contains denaturing agents such as sodium dodecyl sulfate (SDS), facilitates digestion². Nucleic acids are purified from the tissue lysate using buffer-saturated phenol and high speed centrifugation which generates a biphasic solution. DNA and RNA remain in the upper aqueous phase, while proteins, lipids and polysaccharides are sequestered in the inter- and organic-phases respectively. Retention of the aqueous phase and repeated phenol extractions generates a clean sample. Following phenol extractions, RNase A is added to eliminate contaminating RNA. Additional phenol extractions following incubation with RNase A are used to remove any remaining enzyme. The addition of sodium acetate and isopropanol precipitates DNA, and high speed centrifugation is used to pellet the DNA and facilitate isopropanol removal. Excess salts carried over from precipitation can interfere with subsequent enzymatic assays, but can be removed from the DNA by washing with 70% ethanol, followed by centrifugation to re-pellet the DNA³. DNA is re-suspended in distilled water or the buffer of choice, quantified and stored at -20°C. Purified DNA can subsequently be used in downstream applications which include, but are not limited to, PCR, array comparative genomic hybridization⁴ (array CGH), methylated DNA Immunoprecipitation (MeDIP) and sequencing, allowing for an integrative analysis of tissue/tumor samples.

Video Link

The video component of this article can be found at <http://www.jove.com/video/2763/>

Protocol

1. Procedural notes

- Xylene and phenol are toxic chemicals and should be handled in a fumehood. Refer to the material safety data sheet (MSDS) before use.
- Xylene dissolves certain plastics; polypropylene tubes should be used as they tolerate these compounds.
- Special tips may be purchased that contain an internal charcoal stopper. This prevents the xylene from dissolving any rubber components in the pipette. Alternatively, filter tips can be used.

2. Paraffin removal

1. In a fumehood, add 800 µl of xylene (VWR, CABDH6216-4) to the labeled tube containing your specimen and place on a rocker with gentle shaking for 5 to 15 minutes to dissolve the paraffin.

2. Pellet the sample by spinning at max speed (14,000 rpm or 16 000 x g) in a microcentrifuge for 3 minutes. Carefully withdraw the xylene supernatant and dispose in a polypropylene tube for discard in the xylene waste. Be careful not to disturb the pellet.
3. Repeat xylene wash steps 1 and 2 until paraffin is fully dissolved. This usually requires two to three washes, depending on the size of the tissue sample. A fully dissolved sample will appear soft, and will sometimes lose its structural integrity. The integrity of the sample can be gently assessed with a pipette tip.

3. Ethanol rehydration

1. Add 800 µl of 100% ethanol (v/v) molecular biology grade ethanol, vortex, then spin for 3 minutes at 14,000 rpm in microcentrifuge. Remove the ethanol supernatant, being careful not to disturb the pellet.
2. Add 800 µl of 70% ethanol (100% (v/v) ethanol diluted in distilled water (dH₂O)), vortex, and spin for 3 minutes at 14,000 rpm. Carefully remove the ethanol supernatant.
3. Add 800 µl of 50% ethanol (100% (v/v) ethanol diluted in distilled water (dH₂O)), vortex, and spin for 5 minutes at 14,000 rpm. Carefully remove as much ethanol as possible by pipetting. Be extremely careful of disturbing the pellet at this point as fully rehydrated tissue will not pellet as well as dehydrated tissue.
4. After removing the ethanol supernatant, air-dry the pellet for 5 minutes, being careful not to over dry.

4. Tissue digestion

1. Add 200-500 µl of Lysis Buffer (formula below). Re-suspend the pellet using a pipette tip or a vortex. Extra effort at this point to fully re-suspend the tissue will result in a more complete digestion of the sample and a better yield of DNA.
2. Incubate samples at 56°C in a water bath or heating block.
3. For tissue cores, spike 20 µl of proteinase K (20 mg/ml stock solution, Invitrogen, AM2548) morning and evening.
4. Repeat above digestion steps for 2 to 5 days until the tissue is fully dissolved.

Lysis Buffer

- 10 mM Tris-HCl pH 8.0
- 100 mM EDTA pH 8.0
- 50 mM NaCl
- 0.5% SDS
- 200 µg/ml proteinase K (add just before use)

5. DNA clean up

1. Add an equal volume of buffer saturated phenol (Fisher, BP1750I-400) and mix by inversion. Spin for at least 5 minutes at 14,000 rpm in microcentrifuge.
2. Transfer the aqueous layer to a new tube. Note the interphase: is there a lot of white material?
3. Repeat steps 1 and 2 on the aqueous fraction until the interphase is clear (typically 3 or more times). Perform back extractions* when the interphase is fuzzy to increase final yield.
4. Once the interphase is clear, add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) (Fisher, BP1752I-400) to the extracted aqueous fraction of your sample. Mix for 5 minutes and then spin for 5 minutes at 14,000 rpm. This reduces residual phenol and further sharpens the interphase, facilitating extraction of the aqueous layer
5. Remove the aqueous layer to a new tube and treat with RNase A at 100 µg/ml for 1 hour at 37°C.
6. Repeat steps 1 to 4 to remove any remaining RNase A and collect the aqueous fraction. You should only need 1 or 2 buffer saturated phenol steps as there should be much less protein to remove than in the initial lysate.

*To perform back extractions add 50-100 µl of dH₂O to the sample tube containing the interphase and organic portion. Invert the tube to mix, and spin the sample at 14,000 rpm in a microcentrifuge for 5 minutes. Collect the aqueous phase and add it to the previously acquired aqueous extraction. Continue back extractions until the interphase is clear.

6. DNA precipitation

1. Estimate the volume of your collected aqueous layer.
2. Add 1/10 the volume of 3 M sodium acetate pH 5.2 and 1 volume of 100% isopropanol (v/v) molecular biology grade (or 2.5 volumes of 100% ethanol).
3. Mix well and put on ice or in a -20°C freezer for 30 minutes or overnight.
4. Spin at maximum speed (14,000 rpm) at 4°C in microcentrifuge for 10 minutes
5. Discard the supernatant.
6. Wash the pellet with 70% ice cold ethanol to remove unwanted salts.
7. Re-suspend the pellet in the buffer of choice (usually dH₂O).

7. DNA quantification

1. Quantify the concentration of DNA. The A260:A280 ratio should be ~1.8. For small quantities of DNA, measurement using a NanoDrop spectrophotometer is preferred.

2. Following quantification, gel electrophoresis should be performed to test the quality of your sample DNA and determine whether contaminating RNA has been completely degraded.
3. High quality extracted DNA is now suitable for downstream applications, or can be stored at -20°C for later use. If RNA is still present in your sample, repeat the DNA clean up and precipitation steps, and re quantify and qualify the quality of your samples.

Discussion

Biopsied or surgically excised tissues for histopathologic analysis and diagnosis are often formalin fixed and paraffin embedded (FFPE) for long term storage. With the growing interest in understanding the genetic basis of disease, the ability to extract DNA from these samples represents an invaluable source of diagnostic material that can be used for genomic analysis and translational studies. Historically FFPE samples were not considered a viable source for molecular analysis as nucleic acids may be heavily modified by protein-nucleic acid and protein-protein cross linking⁶. However, the discovery that protease digestion releases fragmented nucleic acids which are suitable for downstream analyses including PCR, array CGH, sequencing and methylation profiling, enables the use of these valuable specimens for genetic analysis⁶.

DNA extraction from paraffin-embedded tissues is a robust procedure that relies on differential solubility to purify DNA. Extracted DNA quality and quantity and the success of subsequent DNA amplification is dependent on a number of parameters before, during and after extraction. These include, but are not limited to: type and amount of tissue, the type of fixative used for tissue preservation, the duration of fixation, age of the paraffin block and storage conditions, as well as the length of the desired DNA segment to be amplified^{1,7}. Removal of paraffin from the tissue is the most critical step for successful extraction as undissolved paraffin leads to poor sample quality and inhibition of PCR amplification.

Disclosures

No conflicts of interest declared.

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