Fixed Archival Tissue

Purify DNA and Primers for Good PCR Yield!

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Abstract

The analysis of archival formalin-fixed, paraffin-embedded tissue samples becomes increasingly important for molecular biology studies. As fixation and paraffin-embedding cause alterations of proteins and nucleic acids, archival sources of DNA must be handled with care. To address the need for specificity and reproducibility, we developed an improved protocol for semi-automated DNA extraction adapted to fixed, embedded tissue samples, and a PCR approach using HPLC-purified primers.

Index Entries: Fixation; DNA-extraction; PCR-HPLC.

1. Introduction

Since the advent of the polymerase chain reaction (PCR), archival tissues have become increasingly important sources of DNA for genetic analyses. However, tissue fixation and paraffin-embedding induce DNA-alterations, and it is estimated that these conservation methods cause DNA segmentation resulting in fragments no longer than 300–400 bp (1-5). Further, additional factors as fixation time, quality of fixative, and storage conditions of fixed tissue may have negative effects on DNA and consequently may severely hamper PCR amplification. Usually details about these influences are not precisely known. We describe here useful modifications of current protocols for specific and reproducible amplification of DNA by PCR from archival tissues. These improvements are based on the extraction and purification of DNA using the QiaAmp® extraction kit with a protocol adapted to down-scaled amounts of fixed tissues, and rely on primers that were purified by high-pressure liquid chromatography (HPLC; 6).

2. Materials and Methods

Tissue Preparation

1. 8-μm thick sections are taken from each block and mounted on glass slides, then dried at 48°C overnight. The number of sections per block depends on the extension of the tissue area of interest (i.e., tumor): a total area of a minimum of 1.5 cm² and a maximum of 20 cm² of tissue is sufficient for a good DNA yield.

2. Sections are deparaffinized sequentially with xylene for 10 min twice, isopropanol for 5 min, 96% ethanol for 5 min, 80% ethanol for 5 min, 70% ethanol for 5 min, 50% ethanol for 5 min. Fresh reagents are used to prevent tissue carry-over. The extended deparaffinization including an 80% ethanol and 50% ethanol step helps to preserve tissue adherence to the glass slides.

3. Deparaffinized sections are stained in freshly prepared hematoxylin for 1 min, then washed...
twice in sterile distilled water for 2 min, and
developed in tap water four times 2 min.

4. Sections are kept in sterile distilled water until
dissection.

2.2. Tissue Microdissection
and DNA Extraction

1. Follow items 1-4, Subheading 2.1.

2. Stained sections are histologically analyzed and
the boundaries of areas of interest are marked
with a water-proof color writer on the back side
of each glass slide.

3. A sterile scalpel moistened with sterile distilled
water is used to scrape marked tissue areas off
the glass slides. For each slide use a new scal-
pel. During the following steps reagents and
materials of the QiaAmp Tissue Kit (Qiagen,
Germany) are used. The protocol is adapted to
down-scaled amounts of fixed, paraffin-embed-
ded tissues.

4. Microdissected tissue is immediately trans-
ferred to a 1.5 mL microfuge tube (Eppen-
dorf, Germany) filled with 130 μL Buffer
ATL (QiAmp).

5. For the digestion of tissue and cellular proteins
15 μL of Proteinase K (stock solution 18 mg/mL)
are added, the tube closed, and briefly vortex-
mixed. The incubation is carried out overnight
until the solution turns opaque and tissue frag-
ments are no more visible.

6. 145 μL Buffer AL (QiAmp) are added, mixed
well, and incubated at 70°C for 10 min.

7. 155 μL 96% Ethanol are added and well mixed.

8. A QiAmp spin column is placed in a 2 mL
collection tube. The mixture from step 7 is
transferred to the spin column, the cap closed,
and centrifuged at 10,000g for 1 min or
(microfuge Hettich®, Germany) until the spin
column is empty. The filtrate is discarded and
the spin column placed into a clean 2-mL col-
lection tube.

9. 400 μL Buffer AW (QiAmp) are added to the
spin column and centrifuged at 6000g. The fil-
strate is discarded. This step is repeated once.

10. To remove buffer traces, step 9 is repeated but
centrifugation is now at 12,000g for 2 min.

11. The spin column is put in a new sterile 2-mL
microfuge tube. DNA is eluted with 100 μL
warm (70°C) 10 mM Tris-HCl, pH 9.0, for 5 min.

12. DNA is washed out by centrifugation at 6000g
for 1 min.

13. Steps 11 and 12 may be repeated if the total
area of extracted tissue is more than 3 cm².
Eluates are pooled.

14. DNA concentration and purity are estimated
photometrically (OD260,280).

15. Aliquots of ready-to-use dilutions for PCR are
prepared, i.e., 10 ng/μL. Stock solutions and
dilutions are stored at −20°C.

3. Results

3.1. PCR

In our experiment DNA was extracted from
64 formalin-fixed, paraffin-embedded tissue blocks,
and from six stained slides. The DNA was used as
a template for the PCR-amplification of exon 1-3
of the von-Hippel-Lindau tumor suppressor gene
(VHL), which is a single copy gene (7). The PCR
product of exon 1 spans 240 bp, the product of
exon 2 spans 223 bp, and the product of exon 3
spans 280 bp (Fig. 1). Briefly, each VHL-exon
was amplified by a single-round PCR approach
consisting of 45 cycles. For all exons, the
annealing temperature was 65°C in a 25 μL reac-
tion volume. Nonpurified primers, as they are
commonly used for PCR, and HPLC-purified
primers were tested under several variations of
PCR conditions, which included annealing tem-
peratures, cycle numbers, pH of the reaction mix-
ture, and the amounts of template DNA. For all
exons of the VHL-gene, PCR products were more
specific and reproducible by using HPLC-puri-
fied primers compared to nonpurified primer-
based PCR products (Fig. 1). This was also
confirmed by direct sequencing. In addition,
we were able to obtain PCR-products from all
samples in all VHL-exons, which substantially
exceeded the commonly reported PCR success
rate of 70–80% using formalin-fixed tissues as a
DNA source (1–3). In contrast, DNA-templates
that stemmed from peripheral blood gave rise to
equally specific PCR-products using nonpurified
or HPLC-purified primers (Fig. 1). Although the
HPLC-purification of primers is an established
method in molecular biology (6), commonly
used to generate ultra-pure primers for sophis-