Restoration and Reconstruction of DNA Length

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Serena Bonin and Federica Tavano

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11.1 Introduction and Purpose

Among the archive tissues, autopsy tissues may represent an important resource for the study of rare diseases, neuropathology, cardiopathology, or molecular epidemiology, because of the possibility to analyze both pathological and normal tissues not available from other sources. The tissue fixation step is usually performed in a buffered formaldehyde solution, in the dark for about 24 h before paraffin wax embedding. This procedure is not usually followed for autopsy tissues, which are generally fixed for longer periods of time. Several factors, such as postmortem interval, the type of fixative, and the fixation time could affect the quality and utilization of nucleic acids from autoptic archive tissues. For example, the extensive DNA degradation that is often found in autoptic archival tissues (on average, fragments less than 100 bases long are produced) restricts PCR amplification to very short sequences. This protocol provides a method to partially restore DNA using a pre-PCR treatment, which fills single-strand breaks. This method allows the amplification of longer sequences ranging 300 bases without any modification to the usual DNA extraction procedure [1, 2]. This method could be applied even to DNA extracts from Bouin's fixed tissues [2].

11.2 Protocol

11.2.1 Reagents

- *Pre-PCR DNA restoration treatment solution*: 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 2% Triton X-100, and 200 μM of each dNTP
- *Taq DNA Polymerase* (e.g., Amersham)

S. Bonin (✉) and F. Tavano
Department of Medical, Surgical and Health Sciences, University of Trieste, Cattinara Hospital, Strada di Fiume 447, Trieste, Italy

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11.2.2 Equipment

- *Disinfected*[1] *adjustable pipettes,* range: 2–20 µl, 20–200 µl, 100–1,000 µl
- *Autoclaved PCR tubes* (0.2 ml)
- *Thermoblock*
- *Thermomixer* (e.g., Eppendorf)

11.2.3 Method

- Incubate DNA samples for 1 h at 55°C in 100 µl of Pre-PCR DNA restoration treatment solution.
- After this incubation, add 1 unit of Taq DNA Polymerase and perform DNA polymerization at 72°C for 20 min.
- Store the treated samples at –20°C until they are processed.
- Just before PCR amplification, proceed with the denaturation step: incubate 10 µl of restored DNA solution at 95°C for 5 min and then immediately chill on ice.
- Add the PCR solution to the sample and proceed with the amplification.

[1]Clean the pipettes with alcohol or another disinfectant and leave them under the UV lamp for almost 10 min. Alternatively, it is possible to autoclave the pipettes according to the provider instructions.

References

1. Bonin S, Petrera F, Niccolini B, Stanta G (2003) PCR analysis in archival postmortem tissues. Mol Pathol 56(3): 184–186
2. Bonin S, Petrera F, Rosai J, Stanta G (2005) DNA and RNA obtained from Bouin's fixed tissues. J Clin Pathol 58(3): 313–316