Laboratory Operating Procedure

Isolation of DNA from formalin-fixed and paraffin-embedded tissue using the QIAamp DNA FFPE Tissue Kit

Date: 11.04.2016

Prepared by: *Vanessa Pfankuche, Florian Hansmann*

Contact: *Wolfgang.Baumgaertner@tiho-hannover.de*

Institution: *University of Veterinary Medicine Hannover (TiHo), Germany*

LOP-Version: 1

Attachments: None

Introduction

The here described method allows extraction of total DNA from formalin-fixed paraffin embedded (FFPE) tissue samples. This protocol combines a deparaffinization of FFPE tissue sections followed by a proteinase K and heat-based lysis with silica-membrane-based spin column DNA purification following. The DNA is eluted in elution buffer or water.

Sample Material

8 freshly cut sections of FFPE tissue, 5–10 µm thick
Preparation of FFPE tissue samples to limit the extent of DNA fragmentation:

- Tissue samples should be fixed in 4–10% formalin as quickly as possible after removal.
- Fixation time should be of 14–24 hours
- Samples should be thoroughly dehydrated prior to embedding

Equipment and Reagents

Successful DNA extraction demands proper microbiological aseptic technique. Therefore, only sterile, DNase-free, disposable polypropylene tubes must be used throughout the purification procedure. Latex or vinyl gloves have to be worn while handling reagents and samples and changed regularly to prevent contamination. During the entire procedure personal protection equipment consisting of lab coat, gloves and protective goggles should be used. For more information, consult the appropriate material safety data sheets (MSDSs), available from the manufacturer

Equipment

- Laminar flow
- Pipettes
- Disposable gloves
- 1.5 ml and/or 2 ml tubes
- Sterile, RNase and DNase-free pipet tips (with aerosol barriers to prevent cross contamination)
- Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C and 90°C
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Vortexer

Reagents

- QIAamp DNA FFPE Tissue Kit (Qiagen; cat. no.: 56404)
- Xylene
- Ethanol (96–100%)
- RNAse free water (DEPC-treated)

Literature

- *QIAamp DNA FFPE Tissue Handbook (6/2012)*

Preparation of reagents supplied with the QIAamp DNA FFPE Tissue Kit

Buffer ATL and AL

Before starting the procedure, check whether precipitate has formed. If necessary, dissolve by heating to 70°C with gentle agitation.

Buffer AW1

Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate.

Buffer AW2

Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate.

Before getting started

- Equilibrate all buffers to room temperature (15–25°C).
- Set a thermomixer, heated orbital incubator, heated block or water bath to 56°C and one to 90°C.

Procedure

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut up to **8** sections **5–10 µm** thick (see "Starting material").
   If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube (not supplied), and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 s.
4. Centrifuge at full speed for 2 min at room temperature (15–25°C).
5. Remove the supernatant by pipetting. Do not remove any of the pellet.
6. Add 1 ml ethanol (96–100%) to the pellet, and mix by vortexing. The ethanol extracts residual xylene from the sample.
7. Centrifuge at full speed for 2 min at room temperature.
8. Remove the supernatant by pipetting. Do not remove any of the pellet. Carefully remove any residual ethanol using a fine pipet tip.
9. Open the tube and incubate at room temperature or up to 37°C. Incubate for 10 min or until all residual ethanol has evaporated.
10. Resuspend the pellet in 180 µl Buffer ATL. Add 20 µl proteinase K, and mix by vortexing.
11. Incubate at 56°C for 1 h (or until the sample has been completely lysed).
12. Incubate at 90°C for 1 h.
    If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.
13. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
14. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.
15. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
16. Carefully transfer the entire lysate to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml

collection tube, and discard the collection tube containing the flow-through.

17. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
18. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
19. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.
20. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 30 µl RNase-free water or Buffer ATE to the center of the membrane.
21. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.