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Working

DNA isolation from Formalin-Fixed, Paraffin-Embedded (FFPE) material

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ABSTRACT

This protocol describes the isolation of DNA from paraffin embedded tissue. The resulting DNA can be used for CGH array hybridizations.

After removal of the paraffin, the tissue is lysed and the DNA is released from the cells. Then the DNA is isolated by using a silica-based column that binds the DNA.

STEPS MATERIALS

NAME V	CATALOG #	VENDOR ~
Buffer ATL	19076	Qiagen
Proteinase K	E00491	Thermo Fisher Scientific
Buffer AL, Lysis buffer	19076	Qiagen
EtOH		
Buffer AW1	19081	Qiagen
Buffer AW2	19072	Qiagen
Buffer AE	19077	Qiagen

H&E staining from paraffin blocks

- 1 Cut 3 μm (1x), 10 μm (2-3x, or more if needed depending on tumour area)
- 2 Mount all the section on slides with BSA 0.1%, and dry them o/n at 37°C (to a maximum of one week)
- 3 Place slides in xylene © 00:07:00 3 times
- 4 Hydrate by passing sequentially through 100%, 96%, 70% EtOH, and distilled-water
- 5 Stain slides with haematoxylin **© 00:02:00** (if solution is not fresh staining time can be extended, max 4 min.)
- 6 Wash with running tap water (00:05:00

7	Stain slides with eosin
8	Wash quickly in water
9	Dehydrate by passing sequentially through 70%, 96%, 100% EtOH, xylene
10	Remove slides from xylene and use DePex to mount a covering glass.
11	Let the 3 µm H&E slide be judged by the pathologist for dissection. Mark the tumor area and tumor percentage
DI	NA isolation
12	Place slides in xylene 3 x 7 min. © 00:07:00 3 times
13	Hydrate by passing sequentially through 100%, 96%, 70% EtOH, and water
14	Stain slides with haematoxylin 1-2 min. © 00:02:00 (if solution is not fresh staining time can be extended, max 3 min.)
15	Wash with running tap water $\textcircled{00:05:00}$
16	Place slides in distilled water
17	Dissect the tissue from the slides (when they are still slightly wet) with a scalpel or a needle
18	Place the material in a clean safelock eppendorf cup
19	Spin down in a centrifuge at full speed and get rid of the water layer \bigcirc 00:05:00
20	Shake and invert samples (do not vortex).
21	Add 160 μl ATL buffer



22 Add 40 μl prot K (20 mg/mL)



vortex sample © 00:00:15

Make sure all tissue is in the liquid

Vortex samples and spin down

25

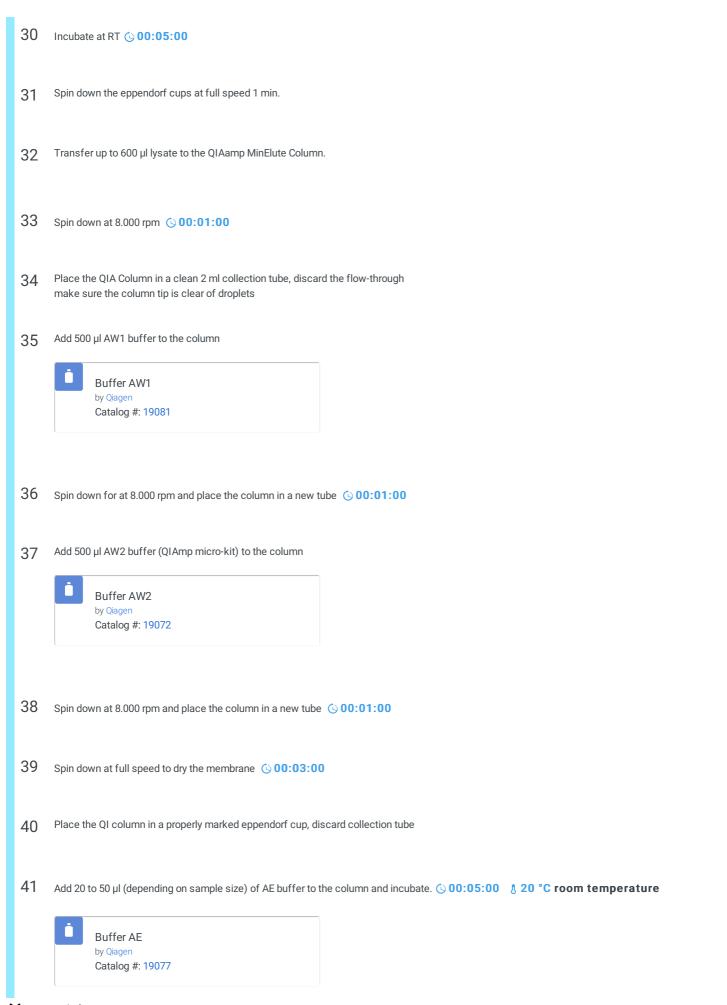
- 24 Incubate o/n at 56°C in heat-block or waterbath and vortex regularly! © 08:00:00 § 56 °C

 Check if all tissue is digested
- 27 Spin down at 16600 rcf. 1 min. (§ 00:01:00
- 28~ Add 200 μl AL buffer and mixvery well by vortexing!



29~ Add 200 μl Ethanol 100% and mix very well by vortexing!





- 42 Spin down at full speed. © 00:03:00
- Throw away the column, close the eppendorf cup and store the DNA at 8.4 °C

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