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

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Protocol status: Working
We use this protocol and it's working

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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.

Materials

STEP MATERIALS

- Buffer ATL **Qiagen Catalog #**19076
- Proteinase K **Thermo Fisher Scientific Catalog #**EO0491
- Buffer AL, Lysis buffer **Qiagen Catalog #**19076
- EtOH
- Buffer AW1 **Qiagen Catalog #**19081
- Buffer AW2 **Qiagen Catalog #**19072
- Buffer AE **Qiagen Catalog #**19077

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Troubleshooting

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 00:02:00

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

DNA isolation

12 Place slides in xylene 3 × 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 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 00:05:00

20 Shake and invert samples (do not vortex).

21 Add 160 µl ATL buffer
Buffer ATL **Qiagen Catalog #**19076

22 Add 40 µl prot K (20 mg/mL)
Proteinase K **Thermo Fisher Scientific Catalog #**EO0491

23 vortex sample 00:00:15
Make sure all tissue is in the liquid

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

25 Vortex samples and spin down

26 Incubate at 98°C 10 min. 98 °C 00:10:00

27 Spin down at 16600 rcf. 1 min. 00:01:00

28 Add 200 µl AL buffer and mixvery well by vortexing!
Buffer AL, Lysis buffer **Qiagen Catalog #**19076

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

30 Incubate at RT 00:05:00

31 Spin down the eppendorf cups at full speed 1 min.

32 Transfer up to 600 µl lysate to the QIAamp MinElute Column.

33 Spin down at 8.000 rpm 00:01:00

34 Place the QIA Column in a clean 2 ml collection tube, discard the flow-through make sure the column tip is clear of droplets

35 Add 500 µl AW1 buffer to the column
Buffer AW1 **Qiagen Catalog #**19081

36 Spin down for at 8.000 rpm and place the column in a new tube 00:01:00

37 Add 500 µl AW2 buffer (QIAmp micro-kit) to the column
Buffer AW2 **Qiagen Catalog #**19072

38 Spin down at 8.000 rpm and place the column in a new tube 00:01:00

39 Spin down at full speed to dry the membrane 00:03:00

40 Place the QI column in a properly marked eppendorf cup, discard collection tube

41 Add 20 to 50 µl (depending on sample size) of AE buffer to the column and incubate.
00:05:00 20 °C room temperature
Buffer AE **Qiagen Catalog #**19077

42 Spin down at full speed. 00:03:00

43 Throw away the column, close the eppendorf cup and store the DNA at 4 °C