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ht-HiTE: High-Throughput DNA extraction from FFPE tissue

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We use this protocol and it's working

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Abstract

This protocols outlines a high-throughput method for DNA extraction from FFPE material. The workflow has been automated on a Biomek i5 platform. 1 to 2 tissue scrolls (10 µm) or tissue macrodissected from glass slides is used as input and eluted in 60 µl after nucleic acid purification. Mean and median yield is 86.6 and 72.0 ng/µl (equivalent to 4.3 to 5.3 µg of total DNA).

Materials

- DNA lo-bind 96-well plates with 1 ml reservoirs (Eppendorf, 0030503244)
- DNA lo-bind 96-well plates with 0.5 ml reservoirs (Eppendorf, 0030503104)
- Nuclease-free water (Promega, P1199)
- Mineral oil, molecular biology grade (for example Sigma Alrich, M5904-500ML)
- Sterile Scalpel blades (for example Ruck, 2009010)
- Glass slides (for example TOMO, TOM-14)
- Tris-HCl, pH 8.0 (Merck Millipore, 648314-100ML)
- SDS (Sigma Aldrich, 71736-100ML)
- Proteinase K (NEB, P8107S)
- Ampure XP beads (Beckman Coulter, A63882)
- DNAeasy blood & tissue kit (Qiagen, 69581)

Preparation

1 Each well of a DNA lo-bind 96 deep-well plates is pre-loaded with 500 µl of mineral oil

2 From each FFPE block, 1 thin section (2 µM) and 2 to 4 thicker sections (10 µM) are placed on glass slides. Thin sections are stained with hematoxylin and eosin (H&E) and used to select and mark regions of interest. Corresponding areas of 10 µM unstained sections are macroscopically dissected using a sterile scalpel blade. The tissue fragments are placed into wells of a 96-well plate preloaded with mineral oil.

Alternatively, whole tissue scrolls (10 µM) can be placed into a well without macrodissection.

Note

Use an antistatic gun to remove charges from the plate before adding tissue. This prevents tissue cross-contamination through small displaced fragments.

Deparaffinization, Tissue Lysis and Decrosslinking

3 The plate is sealed, placed into an pre-heated oven and incubated for 15 min at 56°C. Paraffin should be completely molten and dissolved.

4 To each well, add 100 µl of the following reaction mix:

	Reagent	Concentration	Final	Volume
	Tris-HCl (pH 8.0)	1 M	0.8 M	80 µl
	SDS	10% (w/v)	1%	10 µl
	Proteinase K	20 mg/ml	0.5 mg/ml	5 µl
	H2O			5 µl
	Total			100 µl

Seal the plate and incubate:

- 1 hour at 56°C
- incubate overnight (16 to 24 hours) at 80°C

5 Optional: RNA Digestion

If downstream applications benefit from removing RNA, perform the following:

- add 4 µl of RNAse A to each well
- seal the plate, mix by vortexing
- centrifuge quickly to collect liquid at the bottom of the plate
- incubate 2 mins at room temperature

Transfer

5.1 **Option 1: Column-based purification**

- transfer 100 µl of sample per well to a fresh deep-well plate (part of Qiagen DNA Blood & Tissue 96 kit)
- adjust sample volumes to 200 µl using nuclease-free water

Opton 2: Bead-based purification

- transfer 100 µl of sample per well to a fresh shallow-well DNA lo-bind plate

> **Note**
>
> Position the pipette tip as close to the bottom of the well as possible and try to transfer as little mineral oil as possible.

DNA Purification

5.2 Option 1: Column-based purification

- prepare a 1:1 mix of Buffer AL and absolute Ethanol (400 µl per well + 10% extra)
- add 400 µl of pre-mixed Buffer AL and ethanol to each sample
- transfer 600 µl of sample with Buffer AL and ethanol onto a 96-well DNeasy column plate
- centrifuge plate for 15 min at ≥ 6,000 g and discard the flowthrough
- add 500 µl Buffer AW1
- centrifuge plate for 5 min at ≥ 6,000 g and discard the flowthrough
- add 500 µl Buffer AW2
- centrifuge plate for 15 min at ≥ 6,000 g and discard the flowthrough
- centrifuge plate for 2 min at full speed to dry sample
- place on new collection plate and add 60 µl of Buffer AE
- centrifuge plate for 4 min at ≥ 6,000 g

Note

Some of the centrifuges which are able to handle the larger plates used in this protocol do not reach speeds of 6,000 g. We have succesfully isolated DNA using speeds as low as 3,000 g.

5.3 Option 2: Bead-based purification

- add 80 µl of Ampure XP SPRI beads to each sample (0.8x ratio)
- seal the plate and mix by vortexing
- centrifuge for 30 s at 3000 g to collect liquid at the bottom of the well
- place plate on magnet and pellet beads on wall of the well
- remove supernatant
- add 200 µl of Ethanol (70%)
- wait for 30 s, remove ethanol
- repeat ethanol wash once
- air-dry beads for 10 minutes or until dry at room temperature
- add 60 µl of nuclease-free H2O, resuspend beads by pipetting and remove plate from magnet
- incubate 10 min at room temperature
- move plate to magnet, pellet beads
- transfer the supernatant containing the DNA to a fresh DNA lo-bind plate

Quality control

6 **DNA quantification**

- Option 1: measure concentrations using a dye-based method such as the Invitrogen Qubit platform
- Option 2: use a spectrometry-based method (i.e. Nanodrop). Overestimation by the Nanodrop can be corrected, refer to the manuscript for further information.

Size distribution

If downstream applications rely on high molecular weight DNA, running selected samples on a Bioanalyzer or TapeStation can be utilized for further quality control.

Protocol references

- **Oba et al.: An efficient procedure for the recovery of DNA from formalin-fixed paraffin-embedded tissue sections (PMID 35937639)**