SOP Reference: Sample 04

Standard Operating Procedure for:

SOP for Nucleic Acid Extraction from FF + FFPE samples and TMA construction from FFPE samples

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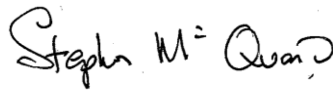
Signature of Author: 

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Introduction

For large collections of disease specific tissue blocks for extractions of DNA/RNA or for construction of control TMAs which incorporate tissue blocks from different disease states the following procedures should be followed. The choice of method for DNA/RNA extractions should be discussed with the research group and will be influenced by the downstream testing platform(s) to be used in the analyses.

For traceability and tracking purposes NIB-F-04 form (DNA-RNA tracking sheet) must be completed for each task associated with the creation of DNA/RNA aliquots and for the distribution of these samples to researchers. These procedures must be carried out by NIB or NI-MPL staff who are fully competent in all NIB protocols. Aliquots of DNA/RNA given to researchers cannot be received back into the Biobank without the approval of the NIB Scientific (Deputy) Director.

Procedure for DNA Extraction/RNA Extraction/TMA construction from FFPE samples

1. Generate a spreadsheet with the list of samples to be used in the TMA construction and DNA/RNA extractions.
2. Cut 1x3 µm and an appropriate number of tissue sections (6x5 µm or 4x10 µm) ; from each donor block as per NI-MPL-SOP-010-Microtomy
3. Dry the sections for DNA/RNA extractions at room temperature for 3 hrs and store at 4^{0}C on slide trays until required
4. Dry the 3 µm in 60˚C blower for 30mins and stain for H&E
5. The H&E is annotated for DNA extraction and TMA construction (3 cancer cores and 1 stroma core).

DNA/RNA Macrodissection

1. Take appropriate number of sections for DNA/RNA extraction to dH2O
2. Automated method: Sections to Water protocol on Tissue-Tek Prisma staining machine
3. Manual method: Dewax slides in xylene (3 x 5 minutes) and remove xylene in 99% ethanol (3 x 5 minutes)
4. Transfer to dH2O (optional)
5. Macrodissection: Using magnifying light overlay a blank tissue (5/10 µm) slide on H&E and taking a clean scalpel blade scrape the area annotated for DNA/RNA extraction into 1.5 ml eppendorf, repeat for all remaining blank sections.
6. Also see NI-MPL-HS-RA-025-Nucleic Acid Extraction.E1 and NI-MPL-HS-COSHH-00N27-DNA Extraction–E1.

DNA Extractions on FFPE samples (method 1 manual)

1. Perform DNA extraction using the Qiagen DNeasy Blood & Tissue Kit (250) (order no. 69506) as per manufacturer's instructions using the O/N incubation protocol and elution in 100 µl of AE buffer.
2. Determine DNA concentration in ng/µl and absorbance ratio of 260/280 using the Nanodrop and record data in excel spreadsheet (folder NIB Projects on S-drive). Store DNA at -20°C in NIB -20°C freezer

3. Enter the location on NIB IMS

DNA Extraction on FFPE samples (method 2 automated)

1. Perform DNA extraction on Maxwell 16 automated machine as per manufacturer's instructions using the Promega Maxwell 16 FFPE DNA Purification Kit Ref: AS1135 with O/N incubation protocol.
2. Complete NI-MPL F 045 Maxwell 16 Instrument User Log' to record the task performed.
3. Determine DNA concentration in ng/µl and absorbance ratio of 260/280 using the Nanodrop and record data in excel spreadsheet (folder NIB Projects on S-drive). Store DNA at -20°C in NIB -20°C freezer.
4. Enter the location on NIB IMS.

RNA Extraction on FFPE samples (method 1 manual)

1. Using a scalpel, remove the area of interest from the slide and transfer to a tube containing 150 µl buffer PKD (from Qiagen RNeasy FFPE kit).
2. Add 10 µl proteinase K to each sample and mix by pipetting.
3. Incubate at 56°C for 15 min, then at 80°C for 15 min.
4. Incubate on ice for 3 min, then centrifuge at full speed (20,000g) for 15 min.
5. Transfer the supernatant to a new 1.5 ml tube.
6. Add 16 µL DNase booster buffer and 10 µl DNase I solution. Mix by inverting the tube – do not vortex!
7. Incubate at room temperature for 15 min.
8. Add 320 µl Buffer RBC.
9. Add 720 µl 100% molecular grade ethanol. Vortex to mix.
10. Transfer 700 µl of the sample (including any precipitate) to an RNeasy MinElute spin column within a 2 ml collection tube. Centrifuge at full speed for 15 s. Discard the flow through.
11. Repeat step 12 until the entire sample has passed through the spin column.
12. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Centrifuge at full speed for 15 s. Discard the flow through.
13. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Centrifuge at full speed for 2 min. Discard the collection tube with the flow through.
14. Place the spin column in a new 2 ml collection tube. Open the spin column lid and centrifuge at full speed for 5 min. Discard the collection tube with the flow through.
15. Place the spin column in an RNase-free 1.5 ml tube. Add 20 µl of RNase-free water directly to the spin column membrane. Close the lid and centrifuge at full speed for 1 min. Place tubes on ice immediately.
16. Measure RNA concentration and purity using the Nanodrop.
17. Proceed to reverse transcription protocol or store samples at -80°C until required.

RNA Extraction on FFPE samples (method 1 automated)

1. Perform RNA extraction on Maxwell 16 automated machine as per manufacturer's instructions using the Promega Maxwell 16 LEV RNA FFPE Kit Ref: AS1260.

2. Complete NI-MPL F 045 Maxwell 16 Instrument User Log' to record the task performed.
3. Transfer elution tubes containing extracted RNA samples onto ice immediately after the extraction run is complete.
4. Determine RNA concentration in ng/µl and absorbance ratio of 260/280 using the Nanodrop and record data in excel spreadsheet (folder NIB Projects on S-drive). Store RNA at -80°C in NIB -80°C freezer.
5. Enter the location on NIB IMS.

DNA extraction on fresh frozen tissue

1. Thaw fresh frozen tissue and sample area for extraction. DNA can be purified from tissue samples of up to 50mg.
2. Perform DNA extraction on Maxwell 16 automated machine as per manufacturer's instructions using Promega Maxwell 16 DNA Purification Kit Ref: AS1030.
3. Complete NI-MPL F 045 Maxwell 16 Instrument User Log' to record the task performed.
4. Determine DNA concentration in ng/µl and absorbance ratio of 260/280 using the Nanodrop and record data in excel spreadsheet (folder NIB Projects on S-drive). Store DNA at -20°C in NIB -20°C freezer.
5. Enter the location on NIB IMS.

Nanodrop

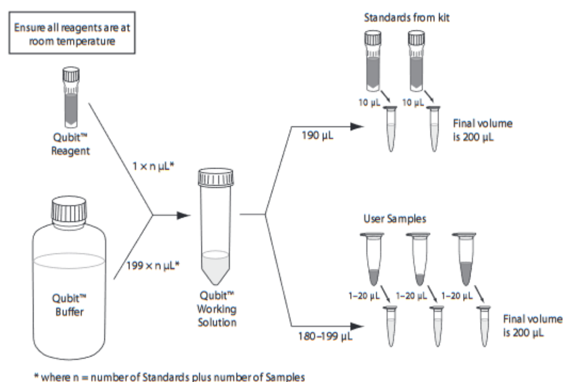
1. Open Nanodrop 2000 software, allow "changes" to computer.
2. Select 'Nucleic acid', and select 'no' when prompted to append new data.
3. Lift the arm of the nanodrop by the edge of the sample arm. Wipe the pedestal with a lint free wipe.
4. A prompt will be received to allow initialization of wavelength. Ensure the pedestal arm is down.
5. Select OK to initialize wavelength.
6. A prompt will be received to allow measurement of your blank solution. Load with 2.0µL of suitable blank, i.e. in the suspension/elution reagent as the samples, e.g. water or TE/EB buffers.
7. Select 'Blank'.
8. Enter sample ID.
9. Carefully wipe blank from the measurement pedestal and load with 2.0µL sample.
10. Select 'Measure'. Save data in folder named 'NIB FOLDER' under study number.
11. Repeat for each sample – remember to wipe off sample before next measurement (being careful not to spread sample over nanodrop).
12. At the end wipe clean.
13. Apply 2.0µl water as a sample to check that the area is completely clean. Measure water as a sample, with 'water' the sample ID.
14. Continue cleaning until the water measures zero or below value of the blank solution.
15. Copy values (concentration and purity (260/280)) onto excel spreadsheet (folder NIB Projects on S-drive).
16. Leave the area clean and tidy with the arm of the Nanodrop down.

QuBit (High Sensitivity or Broad Range)

1. Set up two Assay Tubes for the standards and one tube for each user sample.
2. Prepare the Qubit™ Working Solution by diluting the Qubit™ reagent 1:200 in Qubit™ buffer. Prepare 200 µL of Working Solution for each standard and sample.
3. Prepare the Assay Tubes according to the table below.

	Standard Assay Tubes	**User Sample** Assay Tubes
Volume of **Working Solution** (from step 2) to add	190 µL	180–199 µL
Volume of **Standard** (from kit) to add	10 µL	—
Volume of **User Sample** to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

4. Vortex all tubes for 2–3 seconds.
5. Incubate the tubes for 2 minutes at room temperature.
6. Insert the tubes in the Qubit® 2.0 Fluorometer and take readings. For detailed instructions, refer to the Qubit® 2.0 Fluorometer manual.
7. Optional: Using the Dilution Calculator feature of the Qubit® 2.0 Fluorometer, determine the stock concentration of your original sample.



TMA Construction

1. Construct TMA maps in excel, 3 for cancer cores and 1 for stroma where available, ensure asymmetry to enable identification. Constructed TMA maps are stored on QUB Sdrive/TMA maps
2. Construct TMAs as per NI-MPL-SOP-016 and store in NIB store room
3. Cut 3 µm section for H&E staining and serial sections for baseline markers (variable number depending on tumour type)
4. Carry out baseline immunos on Bond automated immunostainers in NI-MPL or NHS Tissue Pathology laboratory.

Sections from TMAs for immunohistochemistry or aliquots of DNA are requested by researchers according to *SOP Admin1 - Process of application by a researcher for the request of samples from Northern Ireland Biobank.*