Section: Clinical **Procedure No.** [#/v1/MM/YYYY]
PROCEDURE TITLE: DNA extraction from mucosal tissue

Review Officer: Gastroenterology and Hepatology Consultant

Review Summary: v1

Applicable To: All Gastroenterology and Hepatology Department Laboratory Staff

Date Introduced or Last Review Date: 11/2019

Next Review Date: 11/2022

Authority: Head of Clinical Function Testing, Gastroenterology and Hepatology

Replaces: New procedure

Key Words: DNA, mucosa

PURPOSE
To ensure that the procedure is conducted safely and accurate results are obtained

OUTCOME
The DNA extraction is carried out in a standardised and reproducible manner

AUTHORISED TO UNDERTAKE THE PROCEDURE
Gastroenterology and Hepatology Laboratory Staff

RISKS AND PRECAUTIONS
Appropriate PPE must be worn by laboratory technicians

STEPS OF THE PROCEDURE
Modified Morrison protocol (Repeated Bead Beating) for DNA extraction from mucosal tissue:

Reagent and sample prep
1. Thaw sample on ice for 15 minutes (any longer makes it sticky/slimy)
2. Make lysis buffer (can be stored for 1 month)
3. Prefill fresh 2 mL screw cap tubes with 0.4 g sterile zirconia beads (0.2 g of 0.1 mm, 0.2 g of 1 mm)

Cell lysis
1. Add tissue sample to screw-cap tube (use a P20 pipette tip), prefilled with zirconia beads. *Include a “mock” sample
2. Add 300 mL lysis buffer (N.B.: if precipitated, need to heat at 70°C for 15 minutes to fully dissolve).
   Lysis buffer = 500 mM NaCl,
   50 mM Tris-HCl pH 8.0,
   50 mM EDTA
   4% sodium dodecyl sulphate (SDS).
3. Homogenise in Precelleys – 3 min at 5000 rpm (setting 4)
4. Incubate at 70 °C for 10 minutes
5. Centrifuge for 5 minutes at 16 000 x g (at 4 °C). Transfer supernatant to fresh Eppendorf tube.
6. Add 200 mL lysis buffer then repeat step 3–5. Pool the supernatant

Automated purification
1. Follow instructions on Maxwell/Promega Tissue DNA Purification kit (takes 45 minutes)
2. End product is about 150 – 200 mL of DNA in elution buffer. Transfer to 1.5 mL Eppendorf tube.
3. Centrifuge (1 min @ 5000 x g) DNA in elution buffer to pellet magnetic beads; or place tubes on 1.5 mL magnetic rack and allow beads to collect at side of tube.
4. Aliquot 50 mL of the supernatant into 3 Eppendorfs, label and store in freezer as “gDNA”

Quantification of gDNA^:
1. Quantify by nanodrop and record the amount for each sample
2. Visualise on agarose gel (0.8%, 30 minutes, hyperladder 1). Load 2–3 µL.

*reagents but no tissue, to see if any background bacteria in the DNA extraction/Maxwell procedure (we will sequence and identify this)

^gDNA will be a mixture of 99% human and <1% bacteria. Size is just over 10,000 bp on agarose gel.

Use of Maxwell for gDNA Extraction from Tissue

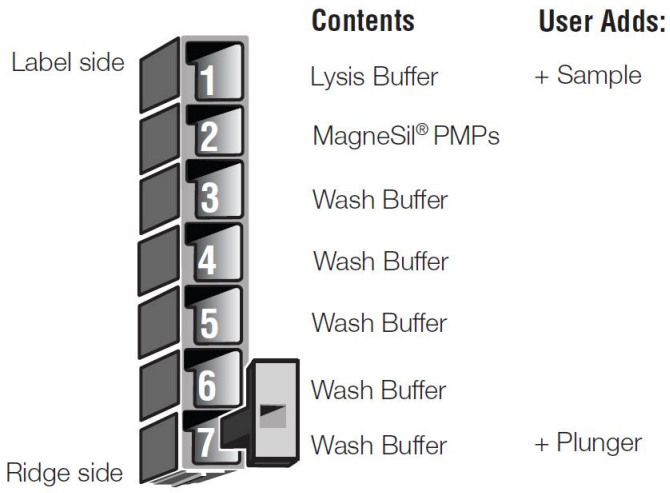
- Using samples that have been lysed through bead beating (as per Morrison RBB gDNA extraction protocol above), in approximately 400 µL volume
- Requires Promega Maxwell 16 Tissue DNA Purification kit
- Maxwell SEV mode

Set up of Maxwell machine:

- Must be in SEV mode (see appendix for how to change to SEV).
- Turn on Maxwell. Ensure SEV is shown on home screen.
- Select DNA, then scroll through and select the Tissue DNA protocol.
- Verify that the correct protocol has been chosen, then open door when prompted on the screen.

Preparing Samples:

- Take out required number of cartridges, plungers, elution tubes and elution buffer.
- Label cartridges.
- Place cartridges in preparation rack and peel off seal.
- Pipette your sample (up to 400 µL) into well 1 of the cartridge.
- Place the plunger in well 7 (ridge side) of the cartridge.



- Place the prepared cartridges into the holder in the Maxwell machine (ensure ridged side is clicked in at appropriate end – will only go in one way).
- If you have less than 16 samples, ensure the cartridges are evenly spread across holder starting from the centre.
- Label blue elution tubes corresponding to each of your samples. Place in front of the appropriate cartridge in the elution tube holder.
- Add 300 µL elution buffer to each elution tube.

Running samples:

- Once samples are prepared, press the run button to retract holder. Close the door.
- The run will start automatically. If it is interrupted at any point – your samples will be lost.
- The procedure should take approximately 45 minutes. The time remaining will be displayed on screen.
- Once the run has finished, follow instructions on screen to open door.
- Transfer the contents of the elution tubes into labelled 1.5 mL Eppendorfs.
- Discard the used cartridges and elution tubes.
- Close up machine and set UV to run.

Sample Storage:

- If there is any bead carry over in samples, place tubes on magnetic Eppendorf rack and transfer out supernatant to fresh tubes.
- RNAse treat samples if desired.
- Aliquot samples and store at -30 °C.

EVALUATION METHOD

This procedure will be kept updated by the Head of Function Testing, Department of Gastroenterology and Hepatology

SUPPORTING DOCUMENTS

Measuring bacterial density on biopsy samples using qPCR

REFERENCES