Document Number: **SASoM/METHOD/053.v5**

Title: **DNA Maxi-Prep using Qiagen Endo-free kit**

Version: **v5**

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SOP History		
Number	Date	Reason for Change
v1	29/08/2013	Original
v2	01/06/2015	Update
v3	01/06/2017	Update
V4	01/06/2019	Update
V5	01/06/2021	Update

1.0 Purpose –

This SOP describes the current procedure for DNA Maxi-Prep using Qiagen Endo-free kit for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in DNA Maxi-Prep using Qiagen Endo-free kit work.

3.0 Responsibilities –

All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

It is assumed that the bacteria are DH5α or equivalent. If you are using STBL3 bacteria for plasmid prep, then these should be cultured at 30°C instead of 37°C.

Culturing bacteria:

1. Make a starter culture: in a tube inoculate 3mL of LB plus antibiotic (typically Amp®, Ampicillin/Carbenicillin at 100ug/mL) with a small amount of frozen bacterial glycerol stock scrapped from stock cryovial using a sterile yellow tip. Culture this tube at 37°C with shaking for 8 hrs. Transfer 500uL of this to between 50mL and 2L of LB plus antibiotic (depending on plasmid) and culture overnight at 37°C with shaking.

2. Transfer culture to a 500mL screw capped bottle and centrifuge at 6,000g for 15 minutes at 4°C. Discard supernantant and invert on white paper towel to remove remaining liquid. Autoclave waste stream for supernatant and paper.

   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

Maxiprep procedure – use the Qiagen Endofree kit:

1. **Resuspend the bacterial pellet in 10 ml Buffer P1.** For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1. If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. **Add 10 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.** Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO2 in the air. If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously coloured suspension. If the suspension contains localized colourless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously coloured suspension is achieved.

   <u>During the incubation prepare the QIAfilter Cartridge: Screw the cap onto the outlet nozzle of the QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge in a convenient tube</u>.

3. **Add 10 ml chilled Buffer P3 to the lysate, and mix immediately and thoroughly by vigorously inverting 4–6 times. Proceed directly to step 4. Do not incubate the lysate on ice.** Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution. It is important to transfer the lysate into the QIAfilter Cartridge immediately in order to prevent later disruption of the precipitate layer. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colourless. A homogeneous colourless suspension indicates that the SDS has been effectively precipitated.

4. **Pour the lysate into the barrel of the QIAfilter Cartridge plus cap. Incubate at room temperature (15–25°C) for 10 min. Do not insert the plunger!** Important: This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Maxi Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging. If, after the 10 min incubation, the precipitate has not floated to the top of the solution, carefully run a sterile pipet tip around the walls of the cartridge to dislodge it

5. **Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Maxi Cartridge and filter the cell lysate into a 50 ml tube. Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. Approximately 25 ml of the lysate is generally recovered after filtration.**

6. **Add 2.5 ml Buffer ER to the filtered lysate, mix by inverting the tube approximately 10 times, and incubate on ice for 30 min.** After the addition of Buffer ER the lysate appears turbid, but will become clear again during the incubation on ice.

7. **Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.** Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

8. **Apply the filtered lysate from step 6 to the QIAGEN-tip and allow it to enter the resin by gravity flow.** The presence of Buffer ER may cause the lysate to become turbid again. However, this does not affect the performance of the procedure.

9. **Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.** Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid preparations. The second wash is particularly necessary when large culture volumes or bacterial strains containing large amounts of carbohydrates are used.

10. **Elute DNA with 15mL Buffer QN.** Collect the eluate in a 30 ml endotoxin-free or pyrogen-free tube. Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps. Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

Important: For all subsequent steps use endotoxin-free plasticware (e.g., new polypropylene centrifuge tubes) or pretreated glassware.

If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

11. **Precipitate DNA by adding 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x g for 30 min at 4°C. Carefully decant the supernatant.** All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x g for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

12. **Wash DNA pellet with 5 ml of endotoxin-free room-temperature 70% ethanol (add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the kit) and centrifuge at ≥15,000 x g for 10 min. Carefully decant the supernatant without disturbing the pellet.** Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x g for 60 min at 4°C. The 70% ethanol removes precipitated salt and

replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

13. **Air-dry the pellet for 5–10 min, and redissolve the DNA by adding a suitable volume of endotoxin-free dH_20, usually 200uL and place tube in fridge overnight.** Redissolve DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers.

14. **Quantify DNA using the nanodrop protocol.**

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with water.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1	Risk assessments –	Gen – 009 – General Laboratory Safety COSHH – 009 – DNA isolation
8.2	SOP -	Equip – 013 – Nanodrop Equip – 022 – Gilson Pipettes

9.0 Approval and sign off –

Author:

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Signature:  Date: 19/04/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 19/04/2021

QA release by:

Name: Peter Mullen

Position: QA Manager

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STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE