Document Number:	**SASoM/METHOD/048.v5**
Title:	**DNA Sequencing at the University of Dundee**
Version:	**v5**
Author:	**Amy Dorward**

Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	29/08/2013	Original
V2	10/08/2015	Update
V3	10/10/2016	Amend
V4	10/10/2018	Update and Amend Detail
V5	11/11/2020	Update

1.0 Purpose –
This SOP describes the current procedure for Dundee plasmid DNA sequencing 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 getting plasmid DNA sequenced at the DNA Sequencing & Services, MRC PPU, MSI / WTB / JBC Complex, College of Life Sciences, University of Dundee, Dundee, DD1 5EH.

3.0 Responsibilities –
All staff involved 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 –

Before placing an order you will need to obtain a Purchase Order number (PO) from Medical school Procurement – this PO number will be 'pasted' into a box when you go online to place your order / make payment.

Place your order at: http://www.dnaseq.co.uk/

The Reynolds lab have an existing account with Dundee sequencing –

Dundee user code is 1504.
Username: Reynolds
Password: reynlab

Alternatively, an account can be created for your own lab, in which case these details would be used to log in.

Each sequencing reaction requires a plasmid concentration of 500-600 ng in 20 μL (approximately 25-30 ng/μL concentration).
Ensure your plasmid is diluted in water or a buffer that does not contain EDTA (e.g. Tris-EDTA buffer) as this can inhibit the sequencing reaction.

For sequencing primers, the facility provides a number of standard primers free of charge – this list can be found on the website:
https://www.dnaseq.co.uk/resources/primers/standard-primers

If custom primers are required, these can be synthesised by the facility at an additional cost, mixed in with the DNA sample or sent with samples as separate aliquots.

- If adding primer in with 20 μL aliquot of DNA, this is at concentration of 12 pmol.
- If sending primers separately, a minimum of 10 μL of 3.2 μM primer stock to be sent. Per standard reaction approximately 3-4 pmol (1μL primer) is used.

Samples can be submitted in a number of ways:

- Online via the "submit online" tool.
- Submit via excel – where an excel form is downloaded and either emailed to the facility or included with your samples when handed in/sent to the facility.

Clearly label sample tubes the same as entered online or on the excel form.

Tubes can be handed in directly to Dundee University by leaving samples in the DNA sequencing box at reception desk in MSI building (note: card access required out with working hours) or posted to:

DNA Sequencing and Services
Medical Sciences Institute
School of Life Sciences
University of Dundee

Dundee
DD1 5EH
Tel – 01382 388019
Email – info@dnaseq.co.uk

An email will be sent to the address linked to the account used to submit the samples to notify when sequencing results are available to view online. Results are routinely available within 24 hours of receipt of samples.

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 100% ethanol or water.

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

8.0 Related documents –
8.1 Risk assessments –
8.2 SOPs - SASoM/METHOD/053 DNA Maxi-Preps

9.0 Approval and sign off –

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Management Approval:		
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