Document Number:	**SASoM/METHOD/001.v5**
Title:	**DNA Electrophoresis using Agarose Gels**
Version:	**v5**
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Effective from:	01/01/2021
Valid to:	01/01/2023

SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

1.0 Purpose –
This SOP describes the current procedure for agarose DNA electrophoresis in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved with agarose DNA electrophoresis.

3.0 Responsibilities –
All staff involved in agarose DNA electrophoresis 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 –
Gel preparation
Solution volume, voltage and timing are given for the small electrophoresis tank. Update volumes etc if another tank is used.

Prepare 250 ml of 1x TBE (0.5x TBE can be used instead): 25ml of 10x TBE (e.g. Invitrogen Cat No 15581-044) and distilled or filtered water till 250ml.

Assemble electrophoresis tank.

Prepare 75ml of agarose gel in a 200 ml glass beaker:

	0.75% gel for long genomic DNA: 5-10 kb	*2% gel for short PCR products: 200bp-1kb*
Agarose	0.56 g	1.5 g
1x TBE	75 ml	75 ml
SYBR safe dye (e.g. Invitrogen S33102)	7.5 µl	7.5 µl

NB: low percentage gels are fragile

Mix, cover beaker with small glass, paper or weighting boat. ???

Boil gel in microwave (~2 min; do NOT cover beaker with foil!).

Pour gel to tank (takes ~40 ml).

Leave gel for ~ 30 min to polymerase (check by the remaining gel in the beaker).

When gel is ready add 1x TBE to the tank to cover gel +~1mm above

Mixing samples with Loading buffer

Before application onto the gel the DNA samples have to be mixed with a loading buffer. E.g. mix Invitrogen cat. No. 10482-035 loading buffer with sample in the proportion 1:2, (3µl of buffer + 6 µl of sample).

There are many other commercially available loading buffers. Typical buffer contains TRIS and 30-40% of glycerol or sucrose to make sample dense enough for easy application: heavy sample quickly sinks to the bottom of the well when applied. Typical loading buffer also includes a dye(s) to trace progress of electrophoresis. These are some commonly used dyes:

Dye	Dyes migration in 0.7% agarose
Xylene cyanol FF	Along with ~4000bp dsDNA
Bromphenol blue	Along with ~300bp dsDNA
Orange G	Along with ~50bp dsDNA

Loading DNA samples & DNA ladder

Samples: Apply 7µl DNA/Loading buffer into the well of the gel.

DNA ladder: Add 3µl of appropriate DNA size marker to a well.

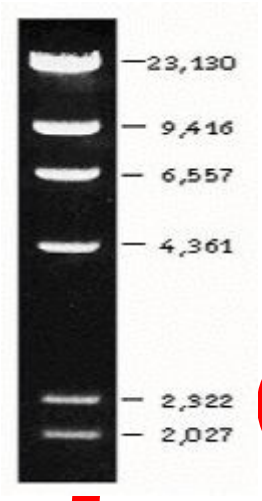
One of the commonly used markers is Lambda digested by Hind-III (e.g. Invitrogen 10488-064). It produces the following DNA fragments:

Fragment (bp)	%	ng DNA in 1 µg sample
23130	47.7	477
9416	19.4	194
6557	13.5	135
4361	9.0	90
2322	4.8	48
2027	4.2	42
564	1.1	11
125	0.3	3

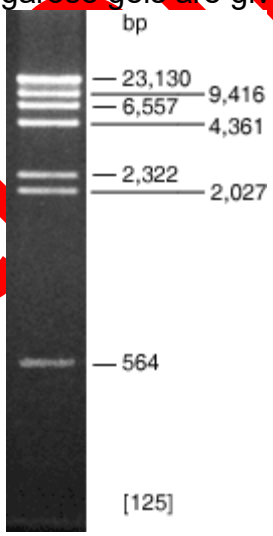
The top 6 bands are clearly visible. The bottom 2 bands may or may not be visible depending on staining conditions, amount of applied Lambda-Hind-III, transilluminator and camera performance. In good conditions SYBR-safe may detect less than 1ng per band.

23130 and 4361 fragments have “cohesive sticky ends” (cos) and can form an additional product of ~ 27k. An optional denaturing step can be used to avoid the appearance of this product on gel - heat 5µl aliquot of Lambda at 65°C for 5 min and place on ice.

Examples of Lambda Hind-III migration in agarose gels are given in the table below:



0.75% - 1% gel



1.5 - 2% gel

Running electrophoresis

Optional: start electrophoresis at low voltage until specimens enter the gel (25v for ~15 min).

Complete electrophoresis at 75v until Bromphenol blue (~300bp) or Orange G (50bp) is at the edge of the gel (~ 45 min)

Optional: Stain gel with SYBR Safe (if it has not been added to buffer earlier).

Take a picture of the gel using the Gel doc system

Preventing contamination by PCR products

If running agarose gels to analyse PCR products: never open tubes with PCR products in the area where pre-PCR steps are performed and never use pipettes that are used for the pre-PCR steps (e.g. RNA/DNA extraction and PCR set-up). Gels with PCR products can be prepared and run in the photo-room.

Always remember that electrophoresis room is contaminated by PCR products. Ideally, consider using periodic UV illumination to destroy DNA contamination in electrophoresis room as well as in your pre-PCR areas/ PCR cabinets (e.g. Sygma cat nums Z169625, W4136, Z566195, Z566225 or similar, if available) – see more references on prevention of PCR contamination in section 8.4. .

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 detergent.

7.0 Training –

All staff should under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/016
RA/GEN/005 and 037

8.2 SOP SASoM/EQUIP/026
Use and maintenance of gel tanks used for Agarose DNA Electrophoresis

8.3 SOP SASoM/EQUIP/049
Use and Maintenance of the Gel Documentation System

8.4 Some references for UV irradiation protocols preventing DNA/PCR contamination:

- Sarkar & Sommer. 1990. Shedding light on PCR contamination. Nature 1990, 343: 27
- Sarkar & Sommer. 1991. Parameters affecting the susceptibility of PCR contamination to UV inactivation. BioTechniques 10: 589-594.
- Cone et al 1993 Protocol for Ultraviolet Irradiation of Surfaces to Reduce PCR Contamination, Genome Res. 3: S15-S17
- http://www.escogb.co.uk/datasheets/SCR_PCR.pdf

9.0 Approval and sign off –

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