iGEM-Group – Microbiology – BMB – SDU

Title: Agarose gel DNA	**Date issued:** 2012.10.30
SOP number: SOP0020_v01	**Review date:** 2013.12.01
Version number: 01	**Written by:** Michelle Madelung

1. Purpose

To visualise or purify DNA run on an agarose gel

2. Area of application

This procedure is valid for all DNA samples

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Heating Cupboard	V18-403b-2	• Preheated	58 °C
Microwave oven	V18-403b-2	• Max strength	Never use metal in the microwave
Fume hood	V18-403b-2		
Power supply	V18-403b-2		
UV chamber (UVIDOC/TEC)	V18-403b-2		Use only when DNA is not to be used further
Transilluminator (UV light table)	V18-403b-2		Use for DNA samples to be purified
Erlenmeyer flask (500 ml) - glass			
Measuring cylinder (500 ml) - plastic			
Gel casting tray and comb	V18-403b-2		
Electrophoresis chamber	V18-403b-2		
Bull's eye spirit level	V18-403b-2		Centre the bobble in the bull's eye

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Tris-acetate-EDTA (TEA buffer)	0.4 M Trizma base 0.01 M EDTA / Titriplex III 0.2 M Sodium acetate trihydrate Acetic acid ≥ 99.8 %	Sigma / T1503-1kg MERCK / 1.08418.1000 Millipore Sigma-Aldrich / 33209-1L	10x Anne Mette	cas # 77-86-1 cas # 64-19-7
Demineralized water				
Agarose	SeaKem LE Agarose	Lonza	V18-403b-2	
Ethidium bromide	0.07 %	AppliChem	V18-403b-2	Can be carcinogenic – use glows at all times
Photo paper		Mitsubishi electric	V18-403b-2	
Weighing paper		Contact lab-manager	BMB-storage	
Tesa Universal Tape		Tesa – 19 mm	BMB-storage	

5. QC – Quality Control

% agarose gel	Range of separation (bp)
0.3	5000 – 60000
0.5	1000 – 20000
0.8	800 – 10000
1.0	400 – 8000
1.2	300 – 7000
1.5	200 – 4000
2.0	100 – 3000

Size of comb	Amount of sample (µl)
Small thin	10
Small thick	20
Big thin	25-30
Big thick	50

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

Ethidium Bromide can be carcinogenic and should therefore be handled with care. Always were glows in the agarose gel room and if something gets on the glows change into a new pair.

8. Procedure

8.1 Look in table in paragraph 5 to identify percentage of gel to be used
8.2 Add appropriate amount of agarose to 500 ml Erlenmeyer flask
8.3 Add 300 ml buffer, mark water line on flask
8.4 Place at piece of weighing paper over the opening (NEVER METAL)
8.5 Place the flask in the microwave oven for 4-5 min
8.6 Check that all agarose have been dissolved or heat extra
8.7 Add demineralized water to the line marked before to replace the water that have evaporated
8.8 Mix by swirling the liquid in the bottle
8.9 Let the agarose cool to 60 °C
8.10 Add Ethidium bromide (1 drop pr 60 ml) while swirling in fume hood
8.11 On the flask write your initials, percentage gel, the date and +/- Ethidium bromide
8.12 Place the flask in a heating cupboard (58 °C) until use
8.13 Attach tape at the open ends of the plastic agarose forms and insert a comb to form the wells in the agarose gel
8.14 Place the form on an even surface, check by using a bull's eye spirit level
8.15 Pour agarose into the form and let it set
8.16 Remove comb and tape and place the form in an agarose tub
8.17 Add buffer until the gel is submerged (this buffer can be used for three runs)
8.18 To DNA samples add loading buffer 1 µL pr. 5 µL sample, mix
8.19 When all samples are ready, load samples onto gel and add appropriate ladder to the first and last well if possible (paragraph 12)
8.20 Run the gel for 30 minutes at 75 V and unlimited amps
8.21 Take a photo of the gel in UV chamber (UVIDOC/TEC) or if the DNA is to be used further look at the gel on Transilluminator (UV light table) – run longer if necessary

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Used buffer		EtBr waste	
Agarose gel		GMO yellow waste	
Once use plastic		GMO yellow waste	

10. Time consumption

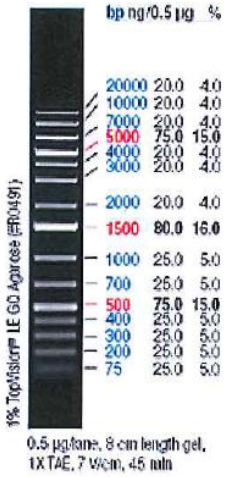
- Total-time 2 hours.
- Hands-on-time 1 hour.

11. Scheme of development

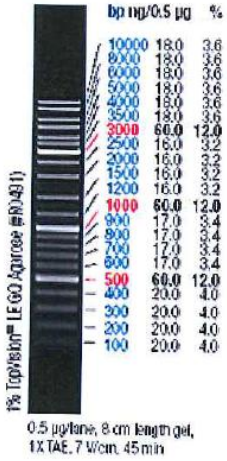
Date / Initials	Version No.	Description of changes
12.10.30 / MM	01	The SOP has been written
13.01.17 / MM & TK	01	The SOP has been approved

12. Appendixes

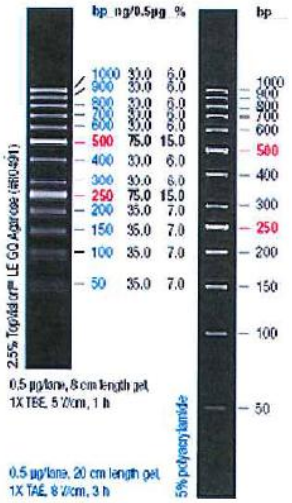
Fermentas GeneRuler™ 1 kb DNA Ladder Plus



Fermentas GeneRuler™ DNA Ladder Mix



Fermentas GeneRuler™ 50bp DNA Ladder



Fermentas GeneRuler™ 100bp DNA Ladder