Appendix 2

Molecular Biology Masterclass: One day practical

Materials required (per group)

3μg Lambda DNA (e.g. 12 μL of 0.3μg/μL)

4 Units *Eco*R1 (1μL)
10X *Eco*R1 buffer (5μL)

4 Units *Hind*III (1μL)
10X *Hind*III buffer (5μL)

20μL Pre-digested λ/*Eco*R1 (diluted from stock so 20μL contains 1μg DNA)
20μL Pre-digested λ/*Hind*III (diluted from stock so 20μL contains 1μg DNA)

100μL 6X gel loading buffer

Tubes labelled (but containing only SDW)
5μL pTZ19u
5μL Control

500μL Competent cells – HB101 in LB overnight 2 x 50mL @ 37°C

Ice bucket

Finn pipettes 0.1–10μL
5–40μL
40-200μL
100-1000μL

Box of sterile yellow and blue tips
10mL Sterile water
10mL Luria Broth
2x Agar plates
10 Sterile Eppendorf tubes
marker pen
2 foam Eppendorf racks
Bunsen burner

Pot of Ethanol
Spreader

Demonstration transformation plates – 1 transformed plate and 1 control plate (see methods)

Materials required (per class)

Water bath set at 37°C
Water bath set at 42°C
Chloros pots
Mini gel rig and cast gel (see methods)
Power pack
Transilluminator
S M L Gloves

Molecular Biology Masterclass — Recipes

TE Buffer

10mM Tris (pH 7.5)	1.21g/L
1mM EDTA	0.373g/L

TBE Buffer

Working solution
0.089M Tris base; 0.089M boric acid; 0.002M EDTA

Concentrated stock solution (5X) – use at 1X		**1X**	
Tris base	54g/L	Tris base	10.8g/L
Boric acid	27.5g/L	Boric acid	5.5g/L
EDTA (pH 8.0)	3.7g	EDTA	.74g/L

6X gel loading buffer

0.25% xylene-cyanol FF
0.25% bromophenol blue
40% (w/v) sucrose in distilled water

LB Medium (Luria broth)

Tryptone	10g/l
Yeast extract	5g/l
NaCl	10g/l

Adjust to pH 7.5 with NaOH; for agar add 1.5% agar No. 2

Agar plates for blue colonies

For agar use LB medium containing 1.5% agar No.2.

Per 300ml of agar (i.e. 1x 500mL medical flat) add:

(1) 3mL of 10mg/mL ampicillin in distilled water and filter sterilise via addition with a syringe.
(2) 0.5mL of 25mg/mL X-Gal in DMF. (Check compatibility of any plastic used.) This is self-sterile.
(3) 1mL of 8mg/mL IPTG dissolved in distilled water and filter sterilise via addition with a syringe.

DNA Electrophoresis

Casting a gel:

(1) Cover the open ends of the gel tray with two layers of autoclave tape.
(2) Insert the well-forming comb.
(3) Add 0.7% agarose to 1xTBE buffer. 300mL TBE for a large rig and 50ml for a mini rig.)
(4) Melt the agarose in the microwave on full power.
(5) When the agarose is hand hot add 0.5μg/mL ethidium bromide.
(6) Pour the agarose into the gel tray to approximately halfway up the comb.
(7) When the gel is fully set remove the comb and autoclave tape.
(8) Place the tray in the gel rig and cover with 1xTBE buffer. Make sure the wells are at the negative end of the rig as the DNA runs to the postive side.

Loading the gel:

(1) Carefully load 20μL of sample into the wells.
(2) Place the lid on the rig and switch power pack on.
(3) Run the gel rig until the samples are at least ¾ along the gel.
[Mini rig @ 75volts **or** Large rig @ 200volts]