lane

1 ladder
2 ladder
3 1K Ein
4 1K Vin
5 TH Ein
6 TH Vin

Gel Electrophoresis – Agarose Gels

(updated 1.4.07)

Gel Concentration (%)	DNA Size (Kb)
0.5	1 – 30
0.75	0.8 – 12
1.0	0.5 – 10
1.25	0.4 – 7
1.5	0.2 – 3
2 – 5	0.01 – 0.5

1. Weigh out agarose and add the appropriate volume of 1x TAE (electrophoresis buffer) in an Erlenmeyer flask. Example: 1% gel is 1g agarose in 100 ml TAE.

2. Swirl gently to suspend the powder. Microwave for ~ 5 min with stopping every ~ 30 sec to swirl the solution. Continue until boiling and ensure all agarose is dissolved.

3. Let cool to 60 C. Add 5 ul of ethidium bromide per 100 ml of agarose solution (Stock solution of ethidium bromide is 10 mg/ml and final concentration is 0.5 ug/ml). Swirl gently to ensure ethidium bromide is well mixed throughout the gel. CAUTION: *Ethidium bromide is a mutagen and potential carcinogen. Gloves should be worn and care should be taken when handling ethidium bromide solutions*

4. Cast gels in trays using casting stand and appropriate combs. Let solidify for 20 – 40 min at room temperature.

5. Submerge gel in 1x TAE in electrophoresis unit.

6. Dilute samples 1:5 with Blue Juice loading buffer. Example 50ul sample + 10 ul Blue Juice. Load 10 – 25 ul per well. For running a DNA plasmid load 100-500ng. Load 10 ul of 100bp or 1kb DNA ladder as a molecular weight marker.

DNA Ladder at 1 ug/ 10 ul:

100 ul Blue Juice
50 ul DNA ladder stock
350 ul H2O

7. Run at 75 – 100 V. In 0.1 – 1.5% agarose gels, Xylene Cyanol runs at 4-5 kb and Bromophenol Blue runs at 400-500 bp. Bromophenol Blue migrates at 4.5 cm/hr at 75 V.

Note: Samples runs from Black---→ Red

8. Visualize using Chemidoc in suzanna's lab

ChemiDot Protocol Agarose Gel

updated 3.14.07

Make sure the power pack is on. If it is not, turn on and wait for at least 10 seconds before opening up the software.

Open drawer and put gel down and center it

On computer
→Quantity One

If Quick guide is not open
→Help
→Volume Quick Guide

On Quick Guide Menu
→Select Scanner
→ChemiDot

Proceed to Step 1
→Select
→UV Transillumination

→Live Focus
→Turn on the Epi White (on machine)

Get up and change filter (lever on the top of the ChemiDot machine) to Zero (0)

- Adjust picture using zoom and focus keys (on computer or on machine)

→Freeze

Turn off the Epi White and Turn on the Trans UV light
Get up and change filter (lever on the top of the ChemiDot machine) to One

→Auto Exposure

To see all exposures in one screen
→Window
→Tile

Save best exposures of Frasor folder