DNA Gels

Tuesday, November 08, 2011
11:50 AM

Sean Weise

Materials:
1X TAE Buffer
Agarose (ICN 800668)
EZ-Vision DNA dye and Loading Buffer (Amresco N313-Q-1ML) 4°C
1 kb DNA Ladder (NEB N0468S) -20°C

Procedure:
1. Set up gel mold with comb in gel casting tray, tighten down to seal ends of gel mold
2. For small gels, place 50 ml of 1X TAE in a 250 ml Erlenmeyer flask, Large gel = 200 ml
3. Add 0.5 g (1%) Agarose, for large gel add 2 g, swirl flask and place smaller 50 ml flask upside down in mouth of 250 ml flask stopper
3. Microwave for ≈ 1 min
4. Pour hot Agarose solution into gel mold with comb
5. Allow gel to solidify, (takes about 0.5 hr)
6. Once gel has solidified gently place in gel tank, make sure gel is covered by TAE liquid
7. On a small piece of parafilm mix 5-10 µl DNA ladder with 1 µl DNA dye and loading buffer
8. In a 0.5 ml microfuge tube mix DNA dye with DNA of interest (1 µl of dye per 5 µl of sample)
Note: DNA dye has red visible dye that runs at about 20 bp
9. Carefully load ladder and sample with dye/loading buffer into the wells. Use a Gilson pipette for this.
10. Run get at 70V – 85V for 45 min or more to separate DNA fragments

DNA Gel Reagents:

10X TAE, 1 L
+ 48.4 g Tris
+ 7.4 g $Na_2EDTA*2H_2O$
11.5 ml glacial acetic acid
H_2O to 1 L
pH to 8.5 using NaOH or HCl

1X TAE, 1 L
In 900 ml dH_2O
+ 100 ml 10X TAE

Note: Large Fisher Gel System
Large wells hold at least 50 µl
Small wells hold at least 25 µl