

AGAROSE GEL PREPARATION AND DNA QUANTIFICATION

1. Prepare lambda DNA of 2 ng/ μ L, 5 ng/ μ L and 10 ng/ μ L concentration from the stock solution available
2. E.g., Promega, comes in a concentration of 450 ng/ μ L solution, to make a 2 ng/ μ L (prepare:

$$\begin{aligned}\text{Stock lambda (450 ng/\mu L)} &= 4.45 \mu\text{L} \\ \text{3:1 buffer (TE:5X dye)} &= 995.55\mu\text{L}\end{aligned}$$

Store in 4°C until ready to use.

2. Prepare 0.8% agarose gel (0.8 g agarose powder per 100ml of 1X TBE) in a flask. Swirl to mix and microwave until the agarose is completely melted. Stir-mix using a magnetic stirrer and add 3.0 μ L of 10 mg/mL **ethidium bromide** solution (**WEAR GLOVES** when handling ethidium bromide) for every 100 ml of agarose (see also note “c” below). Continue stirring until the solution has cooled down (enough so that the flask can be held by the hand for about 10 sec or longer without getting burnt, about 50°C, but not cool enough for the gel to start solidifying). Pour carefully (to avoid air bubbles) onto the gel mold with the appropriate combs set in place depending on the number of samples you have.
3. Let the gel solidify (approx. 30 minutes), then remove combs carefully and set mold in the electrophoresis tank. Use 1X TBE as electrophoresis buffer, add just enough to cover the gel surface.
4. Load 5 μ L of each lambda (10, 25, 50ng) in the first 3 wells, which will serve as standards.
5. Take 1 μ L of total genomic DNA and dilute 200X by adding

our use of lambda DNA.

b. Take a picture if you want for your records. Try to use just 1 film by checking on the correct setting of the camera and the position of your gel before taking a picture. Place gel in the middle of the UV box and set camera at 5.6 - 8.0 for aperture and 0.5 or 1.0 sec for exposure time.

c. If you don't want to handle concentrated ethidium bromide, you may also skip adding the ethidium bromide directly to your gel by using the "used ethidium bromide solution" located in a dark bottle under the fume hood. After electrophoresis, place your gel in a tray and add the used ethidium bromide solution, and stain the gel for about 30 min with gentle mixing. Return used ethidium bromide solution to bottle. Use fresh ethidium bromide solution for gel to be used for blotting.