Institut für HIV Forschung

Agarose Gel Electrophoresis

Item	Manufacturer	Order Number
LE Agarose	Biozym	840004
Ethidium Bromide (1%)	Roth	2218.2
6X Loading Dye	Thermo	R0611
1X TAE	Merck & Millipore	1.06174.1000
100bp DNA Ladder	AnalytikJena	845-st-1010100
1kbp DNA Ladder	AnalytikJena	845-st-1020100

This procedure uses Ethidium Bromide (EtBr) which is a known carcinogen (cancer causing agent). At all times glove MUST be worn. As an extra precaution, we use only white gloves when working with EtBr and agarose gels. Everything past the red line on the floor of the molecular lab is potentially contaminated, so DO NOT touch anything on that side of the room with gloves. All EtBr work must done in the fume hood and anything that comes into contact with it must be put in a specialized waste container, this includes gloves, tips, and gels.

Running a Gel

1. Fill a bucket up with ice and bring it to the molecular lab
2. Using blue gloves, determine which percentage gel you wish to run and measure the correct amount of agarose using the weigh boats near the scale and agarose
    a. Gels are typically made in concentrations from 0.7% to 2%, depending on the size of the DNA you are working with (larger DNA requires higher concentrations of agarose)
    b. To create a 2% gel, you will need 2g agarose per 100ml TAE buffer. You can adjust the amount of agarose as needed
3. Pour the agarose into the Erlenmeyer flask labelled “Agarose Gel EtBr Free” that is located next to the microwave
4. Measure 50ml (or 100ml depending on the size of the gel desired) of 1X TAE buffer using the graduated cylinder near the sink and pour it into the flask with the agarose
5. Swirl the flask a few times to dissolve the agarose a bit and microwave it (use the gel mark) ***THE FLASK WILL BE HOT AFTER THIS, USE THE ORANGE HEAT GLOVES***
    a. Optionally you can put tissue paper into the top to prevent it from boiling over
    b. Half way through, open the door and swirl it again
6. During this time, discard your blue gloves and put on white gloves
7. The mixture is ready once it is boiling
8. Open the fume hood and (while wearing the orange heat gloves) transport the agarose solution inside the hood
9. Pour the solution into the flask labelled “Agarose Gel EtBr”

10. Let it cool for about 2-5 minutes while you assemble the casting tray in the mount. Be sure to choose the size right size for your experiment and tighten the mount well as well as add your combs (you can do this after pouring the gel as well)
11. Swirl the mixture and add 3µl EtBr per 100ml solution. Swirl again to mix
12. Pour the solution slowly into the casting tray. If bubbles form, use a pipette to drag them to the edges of the gel.
13. Let cool for 15-20 minutes. The gel will become cloudy and thick during this time
14. Prepare the electrophoresis chamber (use the one that will fit your casting tray) by filling it with 1X TAE Buffer.
    a. Use blue gloves to fill the 1000ml cylinder with 1X TAE buffer. The large chamber needs ~750ml while the smaller one only needs ~300ml
    b. Buffer may already be in the chamber.
        i. If the buffer is perfectly clear and colorless, make sure there about 1cm of buffer above the raised middle section of the chamber. Add buffer if needed.
        ii. If it is not perfectly clear and colorless, dispose of it by pouring it into the waste beaker located on the work bench and adding one ComPhorSafe sheet to the solution. This can be left overnight and it will bind the EtBr and the sheet can be disposed of in the EtBr bin while the buffer can be poured down the sink.
15. Remove the combs from your gel carefully by lifting on both sides
16. Loosen the mount and take your casting tray with your gel in it to the chamber and place both the tray and gel on the raised middle section of the chamber. The buffer should completely cover your gel and it should be fully submerged, otherwise add additional buffer
    a. It is important to position your wells so that your samples will run towards the red electrode. DNA is negatively charged so will go to the positive (red) electrode.
    Remember: Runs to Red
17. Retrieve your samples, loading dye, and DNA ladder from the 4°C and place them on ice
18. There are 3 ways to prepare your sample:
    a. In the reaction tube that your sample is in
    b. In a new Eppy tube
    c. Using parafilm

    Either way, you will need at least 10µl of stained DNA. Mix 10µl of your sample with 2µl of 6X Loading Dye. Pipette mix several times.
19. Add 10µl of DNA ladder to first well (and last if you wish)
20. Add 10µl of your stained sample to the wells. Pipette slowly to avoid blowing your sample out of the well
    a. A good tip here is to place your free hand under the pipette to stabilize it
    b. The loading dye contains sucrose so your sample will quickly sink once ejected into the liquid, be sure that you are over the well
21. Place the top on the chamber and ensure that the electrodes are connected. Run the gel at 80-150V for 1-1.5hr depending on the gel concentration and size you are using
    a. For DNA fragments <1kbp use 5 V/cm, for >1kbp use 4-10 V/cm
        i. Distance measured is cm between electrodes.
    b. Run the gel until the blue marker (dye marker) is 50%-60% of the way down the gel
22. Once finished, turn off the power and remove the lid. You can now analyze your gel, once finished dispose of the gel in the EtBr waste bin under the gel workstation

Analyzing Your Gel

Agarose gels can be visualized with UV light. The gel imager next to the workstation is equipped to photograph your gel while exposed to UV light

1. Turn on the PC and the photo printer
2. Click the INTAS software symbol on the Desktop
    a. Optional – type in your name for the 'User' and name your gel
3. Open the door of the gel imager and clean the glass. Place your gel of the left side of the glass.
4. Turn on the white light and line up your gel so it is centered on the computer screen
    a. The zoom is located on the lower portion of the camera. Do not touch the camera unless it is absolutely necessary
5. Move the plastic shields so that they stand straight up and form a barrier between yourself and the gel. Close the shutter door on the imager
6. Turn off the white light and turn on the UV light
    a. WARNING, UV light degrades DNA so prolonged exposure will destroy your product and you will not be able to visualize it any more. This can happen within minutes for smaller DNA products. Take your photo as soon as possible after turning on the UV
    b. If a longer time is needed to enhance your photo, use the longer wavelength UV light. The switches to control the wavelength are located on the platform the gel is sitting on in the front side
7. Once satisfied with your picture, click 'Freeze' and then print
8. Turn off the UV light, open the shutter door of the imager and retrieve your gel. Gels are discarded in the red bin beneath the gel workstation
    a. If you wish to keep your gel overnight, it can be stored in TAE buffer, wrapped in plastic, in 4°C. This is not advisable as DNA can diffuse into the gel. Any gel extractions or pictures should be performed immediately after run