

Photocleavage Agarose Gel Procedure

A. Purify pUC 18 plasmid DNA

1. Let the DNA thaw in its ampule in an epindorph tray. The purification procedure is posted on the self of the bio bench.
2. Use the Qiagen kits for the spin columns and buffers.
3. Do as many spin columns as you need, but do not load more than 20 μL of unpurified DNA onto any one spin column.
4. When DNA is purified, take to the UV-vis to obtain the concentration.
 - Blank 2 ml of DI H_2O and add 10 μL of purified DNA to the cuvette and take a spectrum.
 - Monitor the absorbance at 260 nm and calculate the concentration based on $\epsilon_{260\text{nm}} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$
 - Sample Calculation: $a_{260\text{nm}} = 0.0088346$ $\epsilon_{260\text{nm}} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$ and $b = 1 \text{ cm}$
 - $a = \epsilon bc$ and $c = a/\epsilon b$ so concentration in the cuvette equals, $0.0088346 / (6600 * 1) = 1.339 * 10^{-6} \text{ M}$
 - And back calculating for the stock DNA concentration equals, $(1.339 * 10^{-6} \text{ M} * 2010 \mu\text{L}) / 10 \mu\text{L} = 2.69 * 10^{-4} \text{ M} = 269 \mu\text{M}$
 - (DNA stock concentrations should be close to 200 μM to be used for photocleavage gels)

B. Prepare Photocleavage Samples and Run Agarose Gel

1. Turn on the 150 W lamp in the basement to warm up and ensure that the correct filter is placed in front of the lamp sample holder.
2. Prepare a grid laying out the appropriate components for each lanes samples:

Example:

Lane	Sample	DNA	NaCl	Tris	[complex]	H ₂ O	Total V
1	DNA only	7.4 μL	2 μL	2 μL	X	8.6 μL	20 μL
2	DNA + Complex Dark	7.4 μL	2 μL	2 μL	2.5 μL	6.1 μL	20 μL
3	DNA + Complex Irradiated	7.4 μL	2 μL	2 μL	2.5 μL	6.1 μL	20 μL
4	DNA + Complex F-P-T	22.2 μL	6 μL	6 μL	7.5 μL	18.3 μL	60 μL

You will need to calculate what volume of DNA and complex to use by $M_1V_1 = M_2V_2$ calculations. For $\text{Ru}(\text{bpy})_3^{2+}$:

- DNA in the sample should be 100 μM
- Complex in the sample should be 20 μM
- NaCl buffer in the sample should be 50 mM
- Tris buffer in the sample should be 5 mM
- Water is added to fill to a final volume of 20 μL (for 8 lane gel) or 10 μL (for a 12 lane gel)
- In the above example, stock concentrations were as follows:

$$\begin{aligned} \text{DNA} &= 269 \mu\text{M} & (20 \mu\text{L} * 100 \mu\text{M}) &= (X \mu\text{L} * 269 \mu\text{M}) \\ \text{Complex} &= 160 \mu\text{M} & (20 \mu\text{L} * 20 \mu\text{M}) &= (X \mu\text{L} * 160 \mu\text{M}) \\ \text{NaCl} &= 500 \text{mM} & (20 \mu\text{L} * 50 \text{mM}) &= (X \mu\text{L} * 500 \text{mM}) \\ \text{Tris} &= 50 \text{mM} & (20 \mu\text{L} * 5 \text{mM}) &= (X \mu\text{L} * 50\text{mM}) \end{aligned}$$

All X's are the calculated numbers put into the table.

- For lane 4, when F-P-T, all volumes including total volume are multiplied by 3 to allow for sample loss in transfer.*

3. Prepare the samples in small epindorpha and centrifuge once all components have been added.

4. Keep sample tray in the dark (in a drawer) until the gel is ready to load, meanwhile irradiate samples that require it.

For $\text{Ru}(\text{bpy})_3^{2+}$ 15 min irradiation is sufficient with a 385 nm filter

5. Prepare the agarose gel. For a 1% gel you will need to weigh out ~0.40-0.43 g of agarose into the beaker labeled "DNA".

6. To this add 40-43 ml of TAE or TBE buffer (if the nalgene bottles on the bio bench are empty, extra, concentrated buffers are stored on the shelf against the wall above the centrifuge).

7. Next add 18 μL of 1250 $\mu\text{g}/\text{mL}$ stock ethidium bromide (EtBr). Total amount of EtBr needed is 0.5 $\mu\text{g}/\text{ml}$ in gel mixture. Sample calculation:

$(18 \mu\text{L} * 1250 \mu\text{g}/\text{mL}) = (5 \mu\text{g}/\text{mL} * 45,000 \mu\text{L})$ where 45,000 μL is the total volume of the gel solution.

* NOTE: EtBr is mutagen and should be handled with great care*

8. Cover the beaker with foil and place on stir plate with stir and heat both set to 6. Allow to heat and stir until it comes to a vigorous boil. Let boil a minute and remove from the heat. Gel mixture at this point should have turned from cloudy to clear.

9. While the gel mixture is cooling, take a gel tray and use masking tape to seal off both ends. Place the appropriate lane number comb in the last groove of the gel tray.

10. When gel beaker is cool enough to hold, pour mixture into the tray slowly to ensure no bubbles are produced. If there are bubbles in the poured gel, remove them immediately before the gel has time to cool and solidify.

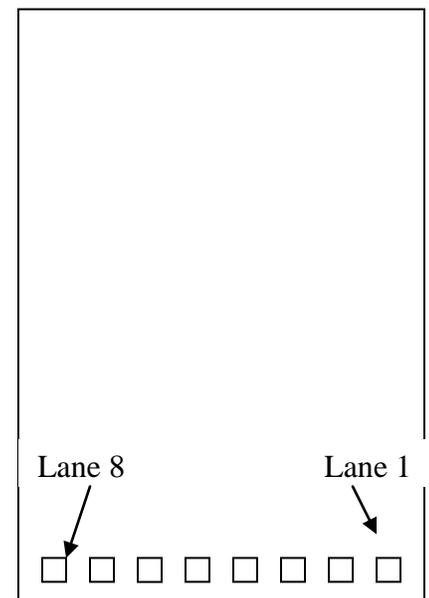
11. When gel is cool, carefully remove the lane comb and the masking tape from each end.

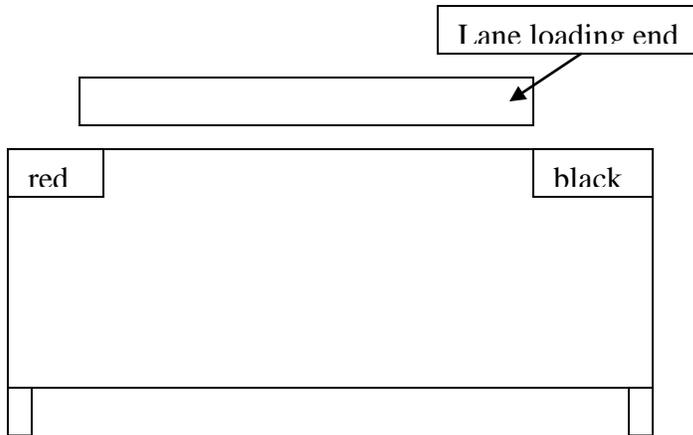
12. Retrieve the sample tray and add 4 μL of gel loading buffer (blue buffer located in the fridge in EL 1025) to each sample epindorph. Centrifuge sample to incorporate the buffer.

13. Load each sample into subsequent gel lanes, starting in this order:

If you have less than 8 lanes fill the middle-most lanes (example: if you have 4 samples begin filling in lane 3 and ending in lane 6).

14. Placed loaded gel (in its tray) into the gel tray holder with the loaded lanes on the right hand side as shown:





15. Fill the gel tray holder with used buffer first, followed by new buffer to fill completely over the gel. Do this very slowly so as to not disrupt the samples filled in the gel lanes as they are covered in buffer.

16. Slide the lid over the gel tray holder and turn on the power source. The voltage should read ~59 V. You should see bubbles flowing along the side of the apparatus.

17. Let the gel run for ~90 min or until the lanes have traveled sufficiently far.

C. Imaging the Gel

1. Spray a little methanol over the gel doc interior and wipe clean with a paper towel.
2. Turn off the gel and remove the lid. Pull gel tray out and let buffer drain back into the holder.
3. Carefully slide the gel off of the tray and onto your hand.
4. Take the gel to the gel doc station and place it centered inside.
5. Close the door and open the software on the computer (Quantity one icon on the desktop)
6. Select File → gel doc eq. This will open up a window used to display the image. Open the gel doc door and turn on the epi-light button on the gel doc station. This should give an image on the computer. You may have to press “Live Focus”
7. Center your gel in the viewing region on the computer screen with the loaded end at the top of the screen (lane 1 should now be furthest to the left).

8. Close the door and press the transilluminator button on the gel doc to see the image reappear on the computer.
9. Adjust the three knobs on top of the gel doc station to get the best size and focus.
10. Adjust the brightness on the computer, then click freeze and save.
11. Go to File → export to TIFF image and save as a TIFF
12. Email yourself with the TIFF as an attachment and it can be opened elsewhere, such as adobe photoshop, for editing.
13. In adobe photoshop invert the image for the best picture.
14. Rinse the tray and its holder in DI H₂O and then lay out to dry.