



University of Melbourne iGEM 2014 Lab Procedure

|               |              |   |       |          |
|---------------|--------------|---|-------|----------|
| Procedure     | Name:        | DNA Gel Electrophoresis   |       |          |
|               | Description: | Used to separate DNA strands of different lengths.<br>See also <a href="#">Sub-cloning protocol</a> and <a href="#">training week video</a> .   |       |          |
|               | Trigger:     | Digesting the plasmid vector  |       |          |
| Last updated  | Name:        | Sheryl/NickYee  | Date: | 22.06.14 |
| You will need | Time:        | ~4 hours  |       |          |
|               | PPE:         | Nitrile gloves<br>UV protective gear (if using UV light)  |       |          |
|               | Equipment:   | Large gel cassette<br>Plastic gel tray<br>Electrophoresis tray<br>Well comb (15 or 20 wells)<br>800 mL buffer bottles<br>Pipettes and pipette tips<br>Sample tubes<br>Gel imaging machine   |       |          |
|               | Materials:   | <u>Agarose Gel</u> <ul style="list-style-type: none"> <li>○ 120 mL 0.5x TBE buffer           <ul style="list-style-type: none"> <li>- May be diluted with milliQ water from 5x TBE if available. Otherwise, make the buffer from scratch.</li> <li>- Some labs use TAE instead, but both serve the same purpose.</li> </ul> </li> <li>○ 1.2 g agarose (or adjust according to % desired)</li> <li>○ 10 µL ethidium bromide</li> </ul><br><u>Electrophoresis process</u> <ul style="list-style-type: none"> <li>○ Agarose gel (see above)</li> <li>○ 1 L 0.5x TBE buffer</li> <li>○ Loading dye</li> <li>○ DNA Ladder</li> </ul> |       |          |

**Casting the 1% agarose gel:**

Note:

Take note of the sizes of relevant DNA fragments being looked at

Higher % allows separation of DNA < 500 bp

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| Step 1 | Loosen each end of the gel tray, place the cassette inside with the open ends framed by the gel tray. The setup should look like a container closed on all four sides.  |
| Step 2 | Into a clean bottle, add: <ul style="list-style-type: none"> <li>- 1.2 g (or other desired amount) agarose</li> <li>- 120 mL 0.5x TBE</li> </ul>  |
| Step 3 | Microwave the bottle for approximately 2:30 minutes or until clear, and swirl to ensure mixing. Take care as the bottle will be hot!<br><br>It is vital that the gel is not solid in any way or it will fail spectacularly. |
| Step 4 | Once the bottle is just cool enough to touch, pour the solution into the cast (from Step 1) with the well comb inserted at one end. Do NOT leave to cool to room temperature as the gel will solidify in the bottle.        |

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| Step 5                                  | Ensure you are wearing nitrile gloves. Add 10 $\mu\text{L}$ ethidium bromide to the solution in the cast and mix in well with the pipette.  |
| Step 6                                  | Allow the gel to set for approximately 30 minutes. Test the gel by prodding a corner with a clean pipette tip.  |
| <b>Running the gel electrophoresis:</b> |   |
| Step 1                                  | Fill the electrophoresis tray with 0.5x TBE. The tray should contain enough TBE to cover the gel once it is placed.   |
| Step 2                                  | Place the gel into the electrophoresis apparatus, with the wells at the negative (black) electrode. Add more TBE if necessary.  |
| Step 3                                  | <p>Add 6x loading dye into each DNA sample to be used. Adjust the amount of 6x loading dye as required depending on the amount of DNA sample, so that it is diluted to 1x (e.g. for 50 <math>\mu\text{L}</math> of sample, add 10 <math>\mu\text{L}</math> 6x loading dye = 60 <math>\mu\text{L}</math> in total). Make sure you mix the samples and loading dye well before placing in the well.</p> <p>Note – To be on the safe side, make the loading dye slightly more concentrated than 1x when diluting. This can also help the DNA travel better.</p>  |
| Step 4                                  | Spin the DNA samples to ensure they are well mixed with the loading dye.  |
| Step 5                                  | <p>Pipette the following into the wells:</p> <ul style="list-style-type: none"> <li>- Well 1 = 8 <math>\mu\text{L}</math> molecular ladder</li> <li>- Well 2 = none</li> <li>- Well 3, 4 = 30 <math>\mu\text{L}</math> DNA sample 1</li> <li>- Well 5 = none</li> <li>- Well 6, 7 = 30 <math>\mu\text{L}</math> DNA sample 2</li> <li>- Well 8 = none</li> <li>- Well 9, 10 = 30 <math>\mu\text{L}</math> DNA sample 3</li> <li>- Well 11 = none</li> <li>- Well 12 = 8 <math>\mu\text{L}</math> molecular ladder</li> </ul> <p>Ensure you record the contents of the wells.</p>  |
| Step 6                                  | Connect the wires to the electrodes on the electrophoresis tray, cover the gel with the plastic lid, and use constant voltage of 150 V, 90 mA for 30-60 min.  |
| Step 7                                  | Wait for the DNA to travel down the gel. You can track its progress by checking the distance the sample buffer has moved.   |
| <b>Imaging the gel:</b>                 |   |
| Step 1                                  | Place the gel (still contained in the cassette) on a plastic transparent sheet and then onto the white tray that can be found beside the machine. Place the tray into the gel box and make sure that the circles on the tray are facing towards you. Ensure that the green filters are also in the machine.   |
| Step 2                                  | Open ImageReader LAS-3000 and wait for the sidebar on the computer screen to indicate the machine is ready to proceed.  |
| Step 3                                  | <p>Check the machine settings:</p> <ul style="list-style-type: none"> <li>- Select 'Pro' instead of 'Lite' on the homescreen.</li> <li>- In 'Darkbox Option Settings' select: <ul style="list-style-type: none"> <li>o Dark box</li> <li>o Green light</li> <li>o 575DF filter</li> <li>o 2.8 or 2.85 iris</li> <li>o Tray position 3 (or use Tray 2 or Tray 1 to 'zoom in' on smaller gels)</li> </ul> </li> <li>- Click on focusing to adjust the focusing and brightness, using the wells as a guide.</li> </ul> <p><i>Note: If the options do not appear, it may be because the wrong filters are in the machine. Check that the Green filters are in the machine and if not, ask for help in</i></p> |

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|                 | <i>changing them.</i>  |
| Step 4          | Click <Yes> and <OK> to image with the gel once finished with settings.  |
| Step 5          | Click <Focusing>, set exposure to 1 and the sensitivity/resolution to super, and adjust settings until satisfied with gel image.   |
| Step 6          | Save the image as both an 8 bit colour TIFF and an IMG file in C:\Las3000Image\Igem, recording the sample number, a description and date. Upload the image to the Dropbox.                                 |
| Version history | <i>V3 Added a few advisory notes – Sean L 2014-08-11<br/>V2 Updated voltage to match Gayle’s Adv techniques lab book. –Sean L 2014-07-29<br/>V4 Added more detail to instructions for imaging the gel.</i> |