Technische Universiteit
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Biomedical Engineering

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Agarose Gel Electrophoresis

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1 Gel preparation

Estimated bench time: 30 minutes
Estimated total time: 1 hour
Purpose: Preparing a gel on which the samples can be loaded.

1.1 Materials

- 1X TAE buffer
- Agarose gel comb
- Agarose gel tray
- Autoclave tape
- Balance
- Erlenmeyer
- Microwave
- Pipettes and tips
- Purified agarose
- SybrSafe

1.2 Setup & Protocol

- Determine how many PCR mixtures will be analysed. When you plan on analysing >8 samples, prepare a larger gel (130 ml), when you have ≤8 samples, you can make a smaller gel (65 ml). Pick a gel tray corresponding to the gel you will make.
- Close the sides of the gel tray using autoclave tape. Make sure there are no openings left. Pick the right comb for the gel, i.e. make sure that your gel contains enough trays to load the samples.
- Determine the expected size of the amplicon. The amplicon size determines the percentage of the gel you will be making. An overview of percentages corresponding to sizes in base pair is shown below:

Percent agarose gel (w/v)	DNA Size Resolution (kb=1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

- Weigh the correct amount of agarose for a gel volume of either 65 ml (small gel) or 130 ml (large gel). A 1.0% gel corresponds to 1 gram of agarose in 100 ml.
- Add TAE buffer to the Erlenmeyer with agarose up to the correct volume.
- Weigh the Erlenmeyer containing agarose-TAE.
- Heat the mixture for ~2 min in the microwave at maximal power. When the mixture starts boiling, stop the microwave (approximately every 30 seconds). Carefully shake the mixture such that the agarose is well dissolved in the TAE buffer.
- Weigh the Erlenmeyer again and add H_2O to correct for the volume loss caused by heating.

- Let the mixture cool down to 50-60°C and add SybrSafe (10.000X stock). Wear nitrile gloves to prevent contact with SybrSafe. Mix well.
- Load the mixture into the gel tray with the comb in it and let it solidify on the bench for approximately 30 minutes.

2 Sample preparation

Estimated bench time: 5 minutes start-up + 1 minute per sample
Estimated total time: 5 minutes start-up + 1 minute per sample
Purpose: Loading dye is used to enable visual tracking of DNA migration during electrophoresis. Moreover, loading dye contains glycerol which ensures that the sample forms a layer at the bottom of the well.

Wear gloves, as you are working with DNA.

2.1 Materials

- Loading dye
- PCR samples
- Pipettes and tips

2.2 Setup & Protocol

- Prepare the samples with 6X loading dye, i.e. add 1 volume of loading dye to 5 volumes of sample. Pipette up and down to mix the loading dye with the sample.

3 Loading of the ladder and samples

Estimated bench time: 5 minutes start-up + 1 minute per sample
Estimated total time: 5 minutes start-up + 1 minute per sample
Purpose: Loading the DNA samples within the wells created by the well comb.

Wear gloves, as you are working with DNA.

3.1 Materials

- 1X TAE buffer
- Agarose gel electrophoresis system
- DNA ladder
- Pipettes and tips
- Prepared samples
- Solidified agarose gel
- 1X TAE buffer

3.2 Setup & Protocol

- Remove the autoclave tape from the solidified gel. Place the gel on the gel tray within the electrophoresis system. Make sure that the comb is located at the negative electrode.
- Add TAE buffer to the gel electrophoresis system until the gel is completely submerged by the TAE buffer.
- Carefully remove the gel comb from the agarose gel.
- Load either the DNA ladder or the samples within the wells. Make sure that the sample or ladder sinks to the bottom of the well. Load approximately 300 ng of DNA, i.e. ~5 µl of ladder and ~20 µl of PCR product.

4 Running the agarose gel

Estimated bench time: 2 minutes
Estimated total time: 60 minutes
Purpose: Letting the DNA move through the gel.

- Run the gel for approximately 60 minutes at 100V.

5 Analysis of the gel using an agarose gel imager

Estimated bench time: 10 minutes
Estimated total time: 10 minutes
Purpose: Taking an image of the gel.

Note: wear gloves while carrying the gel as the TAE buffer may be irritating.

5.1 Materials

- ImageQuant system
- The gel to be analysed

5.2 Setup & Protocol

- Take out the gel tray from the agarose gel system. Carry the gel tray to the ImageQuant system.
- Turn on the ImageQuant system while it is still closed. Start-up the software while the system is still closed as it may turn on UV light as it boots.
- Turn off the UV light and turn on trans illumination. Place the gel in the ImageQuant system. Zoom in or out using the lens and make sure the image is focused. Close the door and turn off illumination with white light.
- Turn on the UV light and take a picture of the gel. Tweak the exposure time to obtain a better image.
- Turn off UV light, take out the gel and clean the system.