Protocol: Gel Electrophoresis for DNA Analysis

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Note: Protocol was taken from last year's iGEM Team. Additional modifications have been made, for easier use in the lab.

Part A: 50x TAE Buffer Preparation - 50 times concentrated

Option 1: Using EDTA disodium salt dihydrate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ *(MW: 372.24 g/mol)*

— 1L final volume

1. Prepare EDTA solution:
    - Dissolve 18.61 g EDTA in ~100 mL MilliQ H_2O.
    - Use a magnetic stirrer to dissolve.
2. Prepare Tris-acetate solution:
    - Dissolve 242.2 g Tris base in ~500 mL MilliQ H_2O.
    - Add 57.1 mL acetic acid to the Tris solution.
3. Combine solutions:
    - Add the EDTA solution to the Tris-acetate mix.
    - Adjust final volume to 1000 mL with MilliQ.

Note:

- Add acetic acid before EDTA is fully dissolved. EDTA dissolves better in low pH.
- The division has a communal TAE buffer which we are allowed to use as long as we refill it when it is low.
- If you are planning to use *EDTA* without disodium salt, you will have to dissolve it for a long time (overnight) so try to avoid this.
- Table 1 also describes the volumes that should be used for a final volume of 500 mL and 2 L.

Table 1: Recipes for 50x TAE Buffer preparation using EDTA disodium salt dihydrate for a final volume of 500 mL, 1 L and 2 L.

Final Volume	Tris base (g)	Acetic acid (mL)	EDTA disodium salt (g)	MilliQ H_2O (to final vol.)
500 mL	121.1	28.6	9.31	500 mL

1 L	242.2	57.1	18.61	1,000 mL
2 L	484.4	114.2	37.22	2,000 mL

(*Option 2: Using EDTA acid form* $C_{10}H_{14}N_2Na_2O_8$ *(MW: 292.24 g/mol) — 1 L final volume*

1. Prepare EDTA solution:
    - Dissolve 14.61 g EDTA in ~100 mL H_2O.
    - Titrate to pH ~8.0 by adding NaOH solution, using a magnetic stirrer and pH meter.
2. Prepare Tris-acetate solution:
    - Dissolve 242.2 g Tris base in ~500 mL H_2O.
    - Add 57.1 mL acetic acid to the solution.
3. Combine solutions:
    - Add EDTA solution to Tris-acetate mix.
    - Adjust volume to 1 L with MilliQ.

Note:

- For the EDTA to be dissolved, NaOH needs to be added.
- Table 2 also describes the volumes that should be used for a final volume of 500 mL and 2 L.

Table 2: Recipes for 50x TAE Buffer preparation using EDTA acid form for a final volume of 500 mL, 1 L and 2 L.

Final Volume	**Tris base (g)**	**Acetic acid (mL)**	**EDTA acid form (g)**	**MilliQ H_2O (to final vol.)**
500 mL	121.1	28.6	7.31	500 mL
1 L	242.2	57.1	14.61	1,000 mL
2 L	484.4	114.2	29.22	2,000 mL

Part B: 1x TAE Buffer Preparation (2 L total)

- Mix 40 mL of 50x TAE stock with 1.96 L of distilled H_2O in the large flask next to the gel apparatus.
- Mix thoroughly by shaking.
- Final pH of the 1x solution should be around 8.3.

- Note: Final concentrations are
    - Tris: 40 mM
    - HAc: 20 mM
    - EDTA: 1 mM

Part C: Agarose Gel Preparation

Note:

- This recipe corresponds to a concentration of 1% agarose.
- For the smaller gel electrophoresis chamber:
    - For small casting tray: 50 mL of gel solution
- For the bigger gel electrophoresis chamber:
    - For big casting tray: 150 mL of gel solution
- It is possible to store the gel in the fridge overnight (not recommended).

NOTE: Think through what you are running. Especially when preparing a gel for Gel Extraction. You can lower agarose to 0.7-0.8% for better separation in the gel.

Instructions:

1. Weigh agarose in an Erlenmeyer flask:

2. Mix it with 1X TAE Buffer.

Table 3: Recipes for 1% agarose gel depending on the tray.

1% Agarose Gel Volume	Amount of agarose	1X TAE Buffer Volume
30 mL	0.3 g	30 mL
50 mL	0.5 g	50 mL
150 mL	1.5 g	150 mL

3. Dissolve agarose:
    - Microwave in Erlenmeyer flask until the solution is clear (look carefully for any grains floating around). Use the microwave above the electrophoresis chambers.
    - Avoid overboiling. Stop and stir often.

4. Cool gel:
    - Let it cool and if you can touch the glass longer it means you can pour it or/and add GelRed to the flask **(TEST ON THE FOREARM).**
    - Add a volume of GelRed 20 000 times less than volume in flask (for 150 mL it is 7,5 µL).
      Note: The GelRed should be handled with care. Use gloves and dispose of waste in the chemical waste bin directly below the casting station. It is also light sensitive.
    - Stir gently to avoid bubbles.
5. Choose an appropriate gel electrophoresis machine
    - There are two sizes; choose the smallest one if that is sufficient for the job.
    - There are different combs (for different numbers of samples) for both the big and small machine. The combs can be found in the drawer below the gel electrophoresis machines.
6. Cast the gel:
    - See the beginning of the following video for more guidance: gel electrophoresis.
    - Pour into the casting tray.
    - Insert combs (choose based on sample number).
    - Let solidify at room temp for ~20 minutes.
    - Test polymerization by poking the corner with a pipette tip if unsure.

Part D: Gel Electrophoresis

Link to video tutorial: gel electrophoresis

1. Set up the gel:
    - Remove comb.
    - Place the gel in the electrophoresis chamber. **IMPORTANT: the wells should be placed at the negative pole as DNA is negative!!!**
    - Fill the chamber with 1X TAE buffer until the gel is completely covered. However, there is a **maximum level** indicated on the chamber, so do not put too much buffer in it as it may pour out from the machine and pose a safety risk (electricity and liquid is not a good combination).
    - Note: it is possible to reuse the buffer up to 5 times**. IMPORTANT: remember to not add to the first lane and last lane, and REMEMBER TO MAKE MARKER LINES FOR BETTER VISUALIZATION!!!**
2. Load DNA ladder:
    - Load 1 µL of Ampliqon High Range DNA ladder into one well, or adjust volume depending on the manufacturer instructions if using other ladders.
3. Prepare and load samples:
    - If DNA is NOT stained (e.g., plasmid prep):
        - Mix sample with 6x loading dye (e.g., 1 µL dye + 5 µL sample).

        - Load ~10 µL per big well or less for the small well (~5 µL is ok).
        - If you want to isolate the DNA from the gel afterwards (following this protocol *Gel Extraction from Agarose Gels Using Kit*), use the full volume of your samples.
    - If DNA is already stained (e.g., PCR mix includes stain):
        - Load sample directly without additional dye.
    - Write down the loading order.
4. Put on the lid. Make sure that the colors of the cables on the lid **matches** the colors of the cable connections on the chamber.
5. Connect the cables to a power source (only the top “box” is working). NEVER put the cables in the power source before you have put the lid on.
    - On the top power source you can set the amperage, voltage and time.
    - You can run two gels with the same power source at the same time, but then the gels will run at the same voltage and amperage, and for the same amount of time.
6. Run the gel:
    - Press set to shift between the different settings: voltage, amperage and time.
    - Run at 80–100 V for 45–55 minutes (until the tracing dye reaches the bottom of the gel). Ideally, start at 60 V and then increase to 80 V (for the small gel) or 90-100 V (for the big gel).
    - Set the current to the maximum value (300–400 mA).
    - Set the time to more than one hour to prevent auto-shutdown.
    - Press run.
7. When the run is finished – and the electricity is turned off – turn off the machine, take out the cables. Then take off the lid. NEVER take off the lid before taking out the cables from the power source.

8. Visualise the gel.

Cutting the gel (this is adapted for one person only):

1. Open the box on the bottom of the gel dock.
2. Put a plastic cutting board on the UV-table. You are not allowed to cut the gel directly on the UV-table.
3. Attach the plastic protector screen on the edge of the box. The plastic screen will protect you from the UV-light.
4. Put on the face shield, which will also protect you from the UV-light.
5. Make sure that you do not expose any skin to the UV-light. Use tape if needed.
6. If there are more than one person in the room at the same time, the people who will not cut the gel must stay behind the plastic protector screen and the person cutting the gel.
7. Close the door.
8. Turn off the light.

9. Start **Trans UV**.
10. Start **Prep UV** (makes sure that the UV-light is not as strong as when the box is closed). The DNA degrades in UV-light, and therefore you want to use a lower power.
11. Excise DNA fragments from agarose gel using a clean scalpel on the plastic cutting board placed on the UV transilluminator. Remove as much excess agarose as possible.
12. Place the gel slices into separate pre-weighed eppendorf tubes (1.5 ml or 2 ml). Weigh again and subtract the tube weight to calculate gel slice weight.

Cleanup

- Dispose of gel in designated gel waste bin.
- Wipe the gel imaging station with a wet paper towel.
- Close all programs and clean up your bench.