**­Procedure for running DNA gels (these gels look very similar to protein gels).**

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**Attention**: unpolymerized acrylamide is a neurotoxin. For any drips and spills, wipe it off with paper towel, change gloves and spray the area with ethanol. If the spill is large, add TMED to get the acrylamide to polymerize.

**Before you start:** *1) Fetch out 10% APS, dT mix and sucrose from -80 C; 2) if running a gel at cold temp (not RT), turn on the “power” and “chiller” switches on the water bath underneath the gel setup.*

**PROCEDURE**

1. **Samples for the gel:**
2. Your samples. Samples should be annealed the day before and equilibrated in the fridge overnight. Samples should consist of **3 µg DNA in 10 µL** **buffer**. The equation below can be used to find the volume of DNA needed for this calculation; add to this volume 5 µL 2x buffer and then the requisite remaining amount of water. To each 10 μL of the sample add 3 μL of 30 % sucrose (final concentration 6.9 %). Sucrose is used to weigh the samples down (just in the same way as glycerol is). Load either 8 µL or 10 µL of sample for both 10 and 15-lane gels (I usually load 10 µL, if the bands were too thick, I try 8 µL). In my recent gels, which were 15%, 8 µL was the better choice.

$$V\_{DNA} \left(μL\right)=\frac{3 μg}{\left(MW, ^{g}/\_{mol}\right)\*(Stock Conc., μM)}\*10^{6}$$

If the volume you calculate is below 0.5 $μ$L, you can either prepare 4.5 $μg$, 9 $μg$, or simply double the volume that you make (make 20 µL instead if 10 µL**)**.

1. dT controls. We have two premixed types of dT controls/ladders: dT, and dT+90. Both consist of polythymine oligos, with 15, 24, 30, and 60 repeat sequences present at 60 µM, 30 µM, 30 µM, and 30 µM, respectively. dT+90 adds an additional T90 oligo (15 µM in the mix), and as such is expensive – use only when your samples exceed 60 nucleotides. **Dilute 5 µL of dT mix from the freezer with 5 µL of buffer and let it sit in the fridge for one hour before adding 3 µL 30% sucrose and loading either 8 or 10 µL onto the gel**.
2. Tracking dye. This is only to check the gel progress. Using dyes in UV-shadowing will interfere with the experiment. Use the stock that has three components (bromophenol blue, xylone, and yellow), usually stored on the top shelf of the refrigerator. Assemble the following mixture: **2 μL of dye stock, 3 μL of 30 % sucrose, 8 μL of buffer. Load 8 or 10 µL, usually in an outermost lane and NOT next to a ladder** (if possible skip one lane).
	1. Blue/orange 6x loading dye, made by Promega in Madison, WI; ref G190A
3. **Making gel and running buffer**
4. Rinse all equipment with DI water and ethanol, wipe thoroughly with paper towels and let it air dry.
5. Assemble the gel set up. Prepare a glass sandwich one notch and one blank glass plates, separated by two plastic strips aligned to the sides. Hold the “glass sandwich” and slide it inside the gel casting bag. Put the setup inside the gel castor, **behind the solid plastic block**. Tighten the screws. Choose the right comb – the choices are: 10 lane and 15 lane combs. Make sure it is clean. Test and see if the comb fits without resistance, if not, you have to move the spacers further apart.
6. Adjust the two knobs on the base of gel castor, so the bubble floats to the center of the white circle, indicating the castor is balanced.
7. Prepare the gel solution according to the table below (volume 40 mL for one gel)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Stock solution | Final concentration | **12%** **10 mM KCl,** **(mL)** | **15%** **10 mM KCl,** **(mL)** | **20 %** **5 mM KCl,** **(mL)** | **CAG Project****(mL)** |
| Acrylamide, 40 %, 19:1 acrylamide:bisacrylamid | 12 % for longer 20 % for shorter oligos | **12** | **15** | **20** | **12** |
| 5x TBE (make sure there is no precipitation) | 1x | **8**  | **8**  | **8** | **8** |
| 1M KCl2M MgCl2 | 10 mM | **0.4****-** | **0.4****-** | **0.2****-** | **0.06 MgCl2** |
| H2O | Bring volume to 40 mL | **19.6** | **16.6** | **11.8** | **19.94** |
| Add all the components and mix well, then add |
| APS, 10% | Dilutions 1→10 | **0.4** | **0.4** | **0.4** | **0.4** |
| TEMED | Dilutions 1→100 | **40 μL** | **40 μL** | **40 μL** | **40 μL** |

 \*larger amount of ions might slow down the gel as well as make it hot.

1. Work fast now as the polymerization happens quite rapidly. Add the solution in between the glass plates using a 25 ml pipet, up to just below the middle of the top edge of the notched plate. Keep in mind that 25 mL may not be enough. Be prepared to add more.
2. Slide in the comb. Push down one side of the comb to make contact with the gel before the other side. This way, you are less likely to trap air bubbles under the comb. *Change your gloves immediately and wash your hands.*
3. (*Make sure to put TMED and Acrylamide back at 4C*).Wait for 30 min for your gel to set. (Keep the leftover gel solution in your flask in the vent hood to help you decide whether the gel has settled. After 30 min, there will likely be a small amount of liquid on top of a thick gel in the Erlenmeyer – this is correct).
4. If running a gel at cold temperature, turn on the “power” and “chiller” switches on the water bath underneath the gel setup. Connect the tubes to the gel apparatus. It should be set to 6°C and cooling (this may take a while).
5. While gel solidifies prepare **700 mL of** **running buffer** that contains1xTBE and an appropriate amount of ions (same composition and concentration as the gel). To make 1xTBE 10 mM KCl use **140 mL of 5xTBE** and **7 mL** of 1M KCl; for 1xTBE 5 mM KCl use **140 mL of 5xTBE** and **3.5 mL** of 1M KCl; for 1xTBE 3 mM MgCl2 use **140 mL of 5xTBE** and **1.05 mL** of 2M MgCl2 solution). Prepare this in the 1000 mL graduated cylinder.
6. When gel solidified, take the “glass sandwich” out of the bag. Clean any solidified gel on the top of glass plates. Gently lift the comb up and out, watching for signs of well collapse. Wash the gel with water immediately. Have a **syringe** ready to flush the wells (at least twice) with running buffer to prevent further polymerization. Use a 25-50 mL syringe.
7. Number the wells with your sample numbers or names. This will make your sample loading easier. Now put gel into the gel running apparatus (connect to water bath hoses if running gel below RT).
8. Press the black holder panels all the way down to the glass plates and screw the knobs gently. Repeat on the other side (or use the blocking plate if you are running one gel only, in which case screw the knobs down tightly).
9. Pour in your running buffer into upper buffer chamber only, if no leak happens, keep pouring until **all wires are submerged** and all wells are refilled with the running buffer.
10. Pre-migrate for at least **30 min** setting the power supply to **150 V**. (Once you press the “run” button, look for bubbles that are released off the wires. This is the sign that your gel is running)
11. After pre-migrating, wash the wells once more (especially important for denaturing gels)
12. Load your samples (Preferably with P10) and run the gel. (Try to minimize the time from beginning loading to running, so your samples won’t diffuse as much) Typical gel run parameters are **150 V**, **150 min**, which should give a current **30-50 mA**.

*When you have ions in your running buffer the gel might overheat. If you need lower temperature you have two options: 1) cold room or 2) water bath.*

1. **Staining the gel using Stains All**
2. Filter the Stains All solution just before the end of your gel run.
3. Disconnect the wires from the power supply and bring the gel tower and tray to the sink. Dump the buffer.
4. Unscrew and remove the gel. Rinse it under DI water.
5. Remove the spacer strips without pressing them further into the gel.
6. Pry the glass plates apart (this will be very hard to get going, but as soon as it starts will become much easier – do NOT rip the gel apart!
7. DO NOT touch the gel with your bare hands or gloves – polynitrile stains the gel yellow
8. Cut the lanes off of the gel with one of the plastic spacers, leaving about ½ cm left to tell where each lane is
9. Rinse out the gel tray that previously held the gel running tower and rinse the gel off the glass plate into the tray using DI. Rinse the gel 3x under DI water, dumping the water out each time
10. Pour just enough STAINS-ALL into the gel tray to cover the gel entirely. Cover with aluminum foil (use the big box on the shelf above and to the right of the gel supplies drawer) and let sit for 7-15 minutes.
11. Pipette the STAINS-ALL back into the storage bottle (turn the lights off when pipetting STAINS-ALL). Rinse the gel 3x under DI water, still in the tray
12. Cover the gel with DI water in the tray and let it sit in direct sunlight or under a light source. Sunlight is the most efficient way to destain the gel, and may produce a clear gel in 30 seconds. If your light source is less intense, this process can take up to 1 hour to produce a satisfactory destaining. As soon as all of the stain on the gel body (not staining the DNA bands themselves) has been decolored, take the gel out of the sun – do NOT overdestain
13. Obtain a transparency from the gel supplies drawer (do not wrinkle these ever). Rinse it with DI, then smoothly and gently transfer the gel from the tray to the transparency (try to only touch the corners). Dry the transparency’s outside and obtain a scanned image from the copier machine (300 dpi, JPEG, LETTER size scan, full color) or using your phone.
14. **Making STAINS-ALL**
15. IF YOU ARE MAKING STOCK FOR LATER USE:
	1. Prepare 30 mL of 0.5% Stains All in formamide: add **0.17 g** of **Stains All** and bring up the volumeto **30mL** with **formamide** in a small beaker. Mix thoroughly. You can make a larger volume of stock by adding 0.34 g Stains All in 60 mL formamide or 0.68 g in 120 mL formamide. Store this stock at 4 °C in the dark.
	2. When you are ready to use the stock, use 90 mL formamide to transfer the 30 mL solution into the bottle for Stains-All. Mix continuously. **Add** 80 mL water and **mix** thoroughly.
16. IF YOU ARE MAKING STAINS ALL DIRECTLY INTO THE STAINS ALL BOTTLE:
	1. Add 0.17 g of Stains All to a beaker and bring up the volume to 50 mL formamide. Mix thoroughly then pour this solution into the Stains All bottle. Wash the beaker with 50 mL formamide and add to the Stains All bottle. Wash the beaker again with 20 mL formamide and add to the Stains All bottle. Add 80 mL of water to the Stains All bottle and mix thoroughly. Label the bottle with the date. Store at 4°C.
17. The stain should be good for 15-20 gels. If the stain starts to have orange flakes/purple chunks when pouring out to stain a gel, filter the stain into a new bottle using the disposable filter in the gel supplies drawer.

**Denaturing gel**

**Composition of the gel: Make it in a glass beaker for later heating**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Stock solution | Final concentration | **12 % gel** | **10 % gel** | **6 % gel** |
| Urea, 60.06 g/mol | 7M | **16.8g** | **16.8g** | **16.8g** |
| Acrylamide, 40 % (19:1 acrylamide:bisacrylamide) | 12 % for longer 20 % for shorter oligos | **12 mL** | **10 mL** | **6 mL** |
| 10x TBE (make sure there is no precipitation; always use new TBE | 1x | **4 mL** | **4 mL** | **4 mL** |
| H2O |  | **5 mL** | **9 mL** | **13 mL** |
| Add all the components, stir bar and set the beaker on the heater until all the urea dissolves. Be careful as not to overheat. Then add: |
| APS, 10% | Dilutions 1→10 | **0.4 ml** | **0.4 ml** | 0.4 mL |
| TMED | Dilutions 1→100 | **40 μL** | **40 μL** | 40 μL |

**Running buffer** – 1X TBE only. No ions!

**Premigration**: set W to 15 W. Because of so much power the gel would heat up. The desired T is around 50 °C.

**Samples: A**dd 3 μL of formamide to 10 μL of your sample

**Controlls:** Add 3 μL of formamide to 10 μL of your dT mix

**Blue dye:** This is only to check the gel progress. Use the stock that has three components (bromophenol blue, xylone, and yellow). Assemble the following mixture: 3 μL of dye stock, 3 μL of formamide, and 20 μL of water.

**All samples:** Remember to add formamid right before loading. Heat all samples together at 90°C for 3 min, cool down and load

**Prerun and running the gel:**

Make sure to **prewash** the wells after prerun and immediately before loading your samples as they will be full of urea and the sample would not be able to settle properly. I usually prewash at least 2 times.