**Protocol for denaturing gels**

Based on Native PAGE protocol written by Jessica Chen and Liliya Yatsunyk

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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.

**Samples for the gel:** (No ions should be included in the any of the samples)

Samples can be prepared the same day as the gel and should consist of **3 µg DNA in 10 µL** **water**. To each 10 μL of the sample, add 3 μL of formamide then put in annealer for 5 min at 95 °C to unfold the DNA! Allow samples to cool at RT for 5 minutes before loading. Load 10 µL of sample for both 10 and 15-lane gels.

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. Assemble the following mixture:

For 1 lane: 4 µL dT mix + 6 µL water + and 3 µL formamide

For 2 lanes: 7 µL dT mix + 10 uL water + 5 uL formamide

After assembling sample, boil for 5 min at 95 °C. Load 10 µL of dT sample per lane.

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 formamide, and 8 μL of the water, and load 8 µL, usually in an outermost lane and NOT next to a ladder.

Blue/orange 6x loading dye, made by Promega in Madison, WI; ref G190A

**Making gel and running buffer**

Rinse all equipment with DI water and ethanol, let it air dry or pat dry.

Assemble the gel set up. Pack a glass sandwich with one notched glass plate, two plastic strips aligned to the sides, and a blank glass plate on top. Hold the “glass sandwich” and slide it inside the gel casting bag. Put the setup inside the gel castor, **behind the solid plastic block**. Choose the right comb – the choices are: 10 lane and 15 lane combs. Test and see if the comb fits, if not, you have to move the spacers further apart.

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.

Prepare the gel solution according to the table below (volume 40 mL for one gel)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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** |
| 5x TBE (make sure there is no precipitation; always use new TBE | 1x | **8 mL** | **8 mL** | **8 mL** |
| H2O |  | **1 mL** | **3 mL** | **7 mL** |
| Add all the components, stir bar and set the beaker on the heater (set to 50 °C) until all the urea dissolves. Be **extra** careful as to not overheat (beaker should only be slightly warm to the touch). The solution being too warm will cause APS to instantly polymerize the acrylamide. 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** |

\*larger amount of ions might slow down the gel.

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.

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.

Wait for 30 min for your gel to set. (Keep the leftover gel solution in your flask 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).

While gel solidifies prepare **700 mL of** **running buffer** that contains only1xTBE (140 mL of 5x TBE then bring up to final volume).

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. Have a **syringe** ready to flush the wells with running buffer to prevent further polymerization.

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). Rinse the wells three times under tap with DI water to get rid of the unpolymerized acrylamide. I recommend using a syringe filled with your running buffer. The syringe needle goes all the way into the well, and works better flushing out the guck, especially in a urea denature gel.

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).

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.

Pre-migrate for at least **30 min** setting the power supply to **300 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)

After pre-migrating, wash the wells once more (especially important for denaturing gels). 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. Wash until urea can no longer be seen being displaced from the well. This usually means Prewashing at least 2 times.

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 **300 V (maximum voltage)** for **120 min**.

**Staining the gel using Stains All**

Filter the Stains All solution just before the end of your gel run.

Disconnect the wires from the power supply and bring the gel tower and tray to the sink. Dump the buffer.

Unscrew and remove the gel. Rinse it under DI water.

Remove the spacer strips without pressing them further into the gel.

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!

DO NOT touch the gel with your bare hands or gloves – polynitrile stains the gel yellow

Cut the lanes off of the gel with one of the plastic spacers, leaving about ½ cm left to tell where each lane is

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

Pour just enough STAINS-ALL into the gel tray to cover the gel entirely. Cover with aluminum foil and let sit for 15 minutes

Pipette the STAINS-ALL back into the storage bottle. Rinse the gel 3x under DI water, still in the tray

Cover the gel with DI water in the tray and let it sit in direct sunlight. 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

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.