This page describes work in the T. Kartzinel lab current as of: August 9, 2022

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Gel Protocols

Many people are handed a gel running protocol without the opportunity to think about how suitable parameters were identified. This can lead to trial and error when developing a new protocol, transferring to new equipment, or joining a new lab. Here are a few useful resources for planning new gel running protocols, identifying common problems, and resolving the issues efficiently.

- https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-electrophoresis-workflow.html#preparing
- https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-electrophoresis-troubleshooting.html
- https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-electrophoresis-workflow.html#voltage

Below, we list protocols that work for our current gel system. If you have trouble, review the information in the links above and check with the PI.

Glove changes and care for materials

Although post-PCR standards differ from pre-PCR, glove changes remain important to care for our experiments and materials. Here are some tips:

- Amplicon cross-contamination remains a concern. When opening strip tubes with PCR products to run a gel, change gloves frequently (when beginning, when changing tasks, when you notice dirt or droplets on your fingers).
- You wash a lot of dishes; wear gloves when you do, but change gloves when you are done to avoid getting soap / water on equipment or supplies.
- The freezers are frosty; change gloves after working in them because you will have condensation on your gloves.

Storing and reusing gel materials

- Please do not pile gel rigs and flasks in the drain rack; before beginning work each day, please put away dry materials so they don't pick up gross material from splashing and soap residue in or around the sink.
- Gel stain: we only use GelGreen in the lab. This dye is light-sensitive and must be kept in a freezer box and wrapped in foil. It can be stored at room temperature. It must be vortexed and spun before each use.
- Reusing TBE buffer: You can use the Nalgene kept on the bottom shelf of the metal rack labeled "Used TBE" to fill the gel rig a few times. The number of times

depends on how it is used and how long it is stored, but usually at least 3 times; write initials/dates on jar to keep track. (Gel quality will decline with repeated reuse, which you will see in faded / fuzzy ladders and samples.)

- Agarose gels: Use the carboy labeled “Fresh TBE” to cast your gel.
- Loading dye: we use NEB Gel Loading Dye Purple (6X). This is a mix of glycerol and a visible stain to allow you to track the progress of your gel run. Stocks and working stocks can be stored at room temperature.
- Amplicons are relatively stable at room temperature, so these can be handled at room temperature for the ~15 min it may take you to load a gel. But they should be stored in the post-PCR -20°C freezer between uses.
- Note on how to use carboys: loosen the lid on top to allow airflow when dispensing; tighten it back up to prevent evaporation when you are done!

Gel density

Pore density in gels is important. Different densities help visualize DNA of different lengths. These densities are determined based on the % of agarose in a gel (usually in the range 0.5-3.0%). For example, to prepare a 1.5% gel, weigh 0.75 g of agarose and dissolve it in 50 mL of fresh TBE buffer. Because our most commonly used gel rig is small, only 50 mL of TBE needs to be prepared. Note: when concentrations are reported in %, it is a sign that the measured quantities do not need to be ultra precise (use good technique, but do not agonize about a few decimal places on the scale.)

To prepare a gel

1. Place appropriate gel combs in the gel form (think about whether you want large or small wells, and ensure the gel form is on a flat surface).
2. Zero the balance with a weighing paper/boat on top. Weigh the appropriate amount of agarose in grams, and put it into a flask (note that 0.75g of agarose is required for a 1.5% gel, however the post-PCR scales only display 1 decimal place, so 0.7g is fine).
3. Pour 50 mL of 1x TBE buffer from the “Fresh TBE” carboy into a graduated cylinder and then into the flask. If you pour the liquid at a moderate pace while swirling the flask, it will mix evenly and you will avoid creating clumps of agarose that take time to melt in the microwave.
4. Place the flask in the microwave. Press the “Start/30 seconds” button. Remove the flask and swirl the contents. Heat the flask for another 30 seconds, but be prepared to stop the microwave as the mixture starts to boil to avoid boiling over or over-cooking the agarose. Swirl again and make sure that all of the agarose is dissolved. Once the mixture is totally clear, heat it again in bursts: allow it to begin to boil up and then stop it so it does not boil over.
5. Quickly add 1:10000 (5 uL when using 50 mL TBE) of GelGreen to the flask and

swirl. Place the flask gently on the bench to allow it to begin cooling.

6. After a few minutes, the flask should cool enough to lift without an oven mitt but uncomfortable to hold in your hands for more than a few seconds at a time. The ideal target temperature is usually 65ºC, and this is simply a rule of thumb.
7. Pour the contents of the flask into the gel cast at a moderate pace. Aim to pour the whole contents in a steady stream; don't allow a broken stream or drops to fall out onto the gel by trying to empty the flask entirely. If there are bad bubbles in the gel, pop them with a clean pipette tip, especially if they are near a comb.
8. Wash the flask quickly before residual agarose dries and hardens (see instructions under "**Cleaning of gel equipment"** later in this document)
9. Let the gel set for ~30 minutes until visibly solid.
10. Once the gel is set, put the gel in the chamber with the wells facing away from you (near the black electrode: remember, "run to red").
11. Carefully fill the chamber with TBE buffer (this buffer can be used several times -- see section on storing and reusing supplies -- but should be replaced every 2 weeks or more often if conducting many long runs). The buffer should rise to the "fill line" and be <1 cm above the top of the gel. If the gel is very thin, it is OK to top off the buffer a bit below the fill line (a few mm above the surface of the gel).
12. Then remove the combs. Wiggle them out gently by applying pressure near the edges, rather than yanking up from the middle of the comb.
13. Select a ladder based on the expected size(s) of DNA in your samples. We generally use ThermoFisher Scientific FastRuler Low Range DNA (50-1500 bp).
14. On some gel rigs, the alternate first and last well should be reserved for the ladder (these wells tend to be smaller than the "sample" wells). Otherwise, try to be consistent about where you place the ladder so you don't get confused.

Prepping amplicons and loading dye

You can use parafilm, strip tubes, or a plate to mix amplicons with loading dye.

- Parafilm; for fewer samples
    a. Cut a strip of parafilm and pipette 2 µL of loading dye corresponding to the number of your samples. This can be done using a multi-channel pipette if preferred. "Pipette slowly" as the loading dye contains glycerol and is prone to cavitation, and prone to damaging pipettes through clogging (or getting on your gloves).
    b. Add 2 µL of PCR product to the corresponding drop of loading dye, mix (slowly) by pipetting, and then using the same tip you can inject 2 uL into the corresponding lane of the gel. This can be done with a multichannel if the dye is applied to the parafilm using a multichannel to ensure the spacing is the same.
    c. Change tips between samples.

- Strip tubes and plates; for more samples
    a. Add 2 uL of loading dye corresponding to the number of samples you are going to run in the gel. This can be done using a multi-channel pipette if preferred. When pipetting out the loading dye DON'T go to the second stop with the plunger because this will create an air bubble and makes it difficult to mix with the sample.
    b. Add 2 uL of PCR product to the corresponding well filled with loading dye. Again, DON'T go to the second stop with the plunger.
    c. Pipette 2 uL of the loading dye/PCR product mix to each well in the gel.
    d. Change tips between samples.

Running a gel

Select voltage based on the size of bands you aim to separate and the dimensions of the gel rig itself (distance between electrodes). The inter-electrode distance for our gel rig is 19 cm, and we use TBE buffer. For Sanger and metabarcoding amplicons (<1000 bp), the rule of thumb is 5 V/cm = 19 cm x 5-V/cm = 95. However, for most uses, we recommend 120 V for 30 minutes.

A template gel map can be found here.

Imaging a gel

1. When your gel is done running, remove the entire cast with the gel inside it. Place it on a paper towel to dry the bottom of the cast and place it in the imager. Turn on the imager with the power switch in the back.
2. Press "Live" to line up the gel on the screen under white light. Press "white light" and change the light type to UV. Turn on the green UV switch on the imager and close the door. Use the + button to increase the exposure to 3.2 or a value that allows you to visualize the ladder and/or bands.
3. Tap "capture image" and insert the USB stick. Save the image to the USB stick and make sure that the white dialogue text appears in the top left corner saying that the image has been saved as *name* on the USB. Tap the button to safely eject the USB.
4. Press "Home" to return to the visualizer and change the light back to "White," turn off the green UV switch, and reduce the exposure if you need to capture another gel image. Or press "Shut down" if you no longer need to use the gel visualizer and make sure you've switched off the green UV switch. After 20 seconds you can switch off the entire machine with the switch in the back near the power cord.
5. Use a kimwipe (NOT a paper towel) to clean the transilluminator surface with 70% ethanol.

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Disposing of gels and buffers

- Agarose gels can go in the red lined box in the post-PCR lab. They simply desiccate.
- Spent buffers can go into liquid hazardous waste (empty containers for waste disposal are usually saved next to the red hazmat box in the pre-PCR lab, and these containers can be brought over for use as needed).
- If a new container is required, fill out the orange chemical hygiene form and affix it to the container. Since the only liquid waste we will produce in this room is the TBE buffer, you can label it “99% EDTA buffer, 1% agarose”. Contact EHS for disposal when full.

Cleaning of gel equipment

- To wash post-PCR flasks used to pour gels, scrub them out with warm water while still warm (before agarose cools and solidifies). Check the interior for residual agarose (could be hard to see). Use the pipe cleaner to scrub out the agarose
- Wash everything with soap and water except for gel cast/combs, rig, and lid.
- The gel cast/combs, rig, and lid should be cleaned with tap water and rinsed with Milli-Q water.
- Glassware such as flasks and graduated cylinders should be kept in the tupperware with the lid closed. Glassware that is too tall for this tupperware should be kept on the benchtop with aluminum foil covers.

How to make 1x TBE buffer

- 10x TBE buffer is stored on the top shelf of the metal rack in post-PCR. Fill a 100mL graduated cylinder up to the 100 mL line with 10x TBE and pour it into the 1000mL graduated cylinder.
- Fill the 1000mL graduated cylinder near to the 1000mL mark with Milli-Q water directly from the carboy, then slowly and carefully top it up to exactly 1000mL by pouring the rest of the water from a smaller 100mL graduated cylinder (to have more control and avoid overshooting).
- Pour 1x TBE from the beaker into the “FRESH TBE BUFFER” carboy and update the “top up” date on the tape.
- Periodically, it is necessary to completely empty and rinse the carboy. As a kindness to others, if the carboy is mostly empty already, simply drain and rinse it with tap water followed by Milli-Q water before refilling. Update the lab tape labels accordingly.