Micropipetting 101 Learning Lab

Gel Loading Mastery Activity

Teacher's Instructions

This activity can be performed individually or in groups. The smaller the groups, the more hands-on experience each student will gain.

Activity setup

Each group will need
- One 2-20 µl micropipette
- One Silicone Practice Gel
- Micropipette tips
- Micropipetting practice dyes
    - 500 µl for groups of 4 students
    - 250 µl for groups of 2 students
- Beaker or similar container for used tips

For use with Micropipetting 101 Learning Lab.
Cat. No. KT-1510-10

Teacher's notes

Using the gels

Silicone gels are an inexpensive, durable tool for practicing loading gels. A common problem for students using gel electrophoresis systems for the first time is losing their sample due to lack of experience pipetting into a gel. We recommend students practice with silicone gels as a way to avoid the frustration of losing samples when performing authentic electrophoresis investigations.

Bubbles

Because silicone is a hydrophobic material, air bubbles will tend to stick in the wells of a submerged silicone gel much more firmly than in an agarose gel. This can interfere with loading the gel. If using the gel while submerged in buffer or water, first hold the gel under a running faucet. This will force water into the wells, reducing the amount of air bubbles trapped in the wells.

Loading dye

Loading dyes can come in several different colors. For this activity we provide three colors to practice with. Darker loading dyes are often easier to use for new users, because they can be easier to see, but this can vary depending on the background the gel is on. For this activity, use whichever color practice dye best meets your needs.
We recommend practicing loading these gels in as realistic a scenario as possible by placing the gels in an electrophoresis system. If using blueGel™, the silicone gels fit precisely in the regular gel trays. Turning on the blue light illuminator will make certain colors of loading dye much easier to see in the wells of the gel.

Making your own practice loading dye

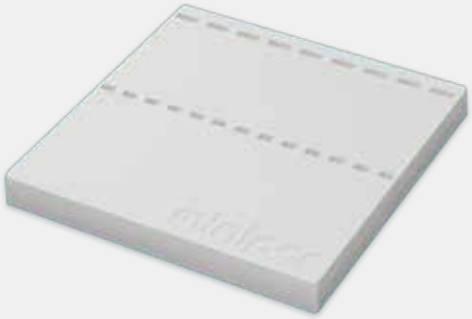
The practice dye supplied here contains glycerol. Loading dye can also be made using sucrose. To make your own 1X practice loading dye, dissolve 0.7 grams of sucrose (table sugar) in 10 ml water and add food coloring.

Reducing waste - micropipette tips

General pipetting best practices dictate that a new micropipette tip should be used every time you pipette a new volume of liquid. Unfortunately, this also creates a considerable amount of plastic waste. For this activity, to reduce unnecessary waste, we suggest reusing tips.

Gel loading practice exercises

Today you will be using a silicone practice gel. The gel you are using is the exact dimensions of a regular blueGel™ agarose gel. Because it is made of silicone, you cannot run your samples on this gel, but it is durable and reusable making it a good tool to practice with.



How to load a gel

• Using a 2-20 µl micropipette with a new tip, pick up the sample you wish to load.

• Load between 5 and 15 µl in the larger wells. Load between 5 and 10 µl in the smaller wells.

• Place the tip of your micropipette just inside the upper edge of the well. There is no need to try to get your tip near the bottom of the well.

• When dispensing liquid into a well, only press the plunger to the first stop. Pressing to the second stop will add bubbles to the well and may displace your sample.

• After dispensing your sample, remove the pipette tip from the buffer before releasing the plunger.

Tips of the trade

• Loading dye is more dense than the buffer your gel sits in. This means your sample will sink when it is added to the well and will displace the liquid that is already there. There is no need to get your tip to the bottom of the well.

• Don't stab the gel. Putting your tip too deep in a well or against the side of the well can result in puncturing the gel. In an agarose gel, this can damage the gel and create a hole that your sample may leak out of. You will know that you have stabbed the well if the sample does not leave the pipette tip when you depress the plunger.

• Two hands on the pipette! You are aiming for a small target. Use your dominant hand to operate the pipette. Use your other hand to steady your pipette by placing a finger on the pipette shaft near where it meets the pipette tip.

• Steady your arms by resting your elbows on the lab bench.

Self-assessment scoring guide

0- I didn't just miss the well; I missed the gel.
1- That was very difficult; I stabbed the well or missed the well on most of my tries.
2- That wasn't easy, but by my third try I got the hang of it.
3- Easy. All of my sample is in the well with no spillover.

Directions

1. Dry run

Normally, when you load a gel, it is submerged in buffer. As a practice, you will first try loading a dry gel. This can be a little easier because you can better see what you are doing.

- Place your silicone gel on your lab bench.
- Place a new tip on your pipette and pick up 10 µl of practice loading dye.
- Add 10 µl of practice dye to one of the wells. Don't worry if the dye sticks to the side of the well or does not go all the way to the bottom of the well.
- Now try adding loading dye to two more wells.

Self-assessment score: _____

2. Getting smaller

The larger size wells are what you will likely use when loading an agarose gel, but once you've gotten the hang of the larger wells, try the smaller wells in the second row for your next challenge.

- Add 7 µl of loading dye to one of the small wells. Don't worry if the dye sticks to the side of the well or does not go all the way to the bottom of the well.
- Now try adding loading dye to two more wells.

Self-assessment score: _____

3. Adding buffer

Typically, gels are loaded while submerged in electrophoresis buffer. Because we are only practicing, you can use water to submerge your gel today. Place your gel in a gel electrophoresis chamber or in another low walled container. Add just enough water so the entire gel is barely submerged.

- Because this gel is made of silicone, bubbles stick in the wells more firmly than in a typical agarose gel. If there are bubbles in the wells of the gel, hold your gel under a running faucet. This will force water into the wells. Then, submerge your gel in the gel chamber. This step is not needed for a regular agarose gel.
- Add 10 µl of loading dye to at least two of the larger wells.

Self-assessment score: _____

4. Final challenge

Once you feel comfortable with the larger wells, test your micropipetting skill with the smaller wells.

- Add 7 µl of loading dye to at least two of the smaller wells.

Self-assessment score: _____

Final assessment

Add up your self-assessment scores to determine your true skill at gel loading: _____

0-5	**Amateur**	I should practice a few more times before trying on a real gel.
6-8	**Intermediate**	I'm starting to get the hang of things.
9-11	**Pro**	I'm ready for the real thing!
12	**Gel Loading Master!**	I was born with a micropipette in one hand and a gel in the other!