Preparing agarose gels

WARNING: Ethidium is highly mutagenic! SYBR-Safe dye is supposed to be less mutagenic and genotoxic compared to ethidium, but any small molecule that displays high affinity for DNA should be used with caution.

(1) **Set-up the casting trays.** First, clean the casting trays and combs. These are usually found in one of the drawers by the gel running area. Make sure to remove any bits of left-over agarose, especially in the comb. Place the casting tray in the chamber, making sure the seals are water-tight. Place the comb on the slots.

(2) **Dissolve the agarose in TAE buffer.** Weigh the appropriate amount of agarose and resuspend in TAE buffer. We use 1% gels for general applications. When resolving plasmids (>5 kb), use 0.7-0.8%. For small fragments (<0.5 kb), you may need to go as high as 2.5%. Dissolve the agarose by boiling in the microwave. It is best to heat the solution in 30-second increments, mixing well in between heatings. Note: Agarose will not melt unless the solution boils.

For B2 gels (large rack), you will need around 50 mL of gel solution. For the B2A gels (small rack), you will need around 15 mL.

(3) **Cool the agarose solution and add the dye.** Allow the gel to cool to around 50 °C before adding the dye. We have ethidium bromide and SYBR-Safe dyes at 10,000x stock concentration. That means you add 1 μL for every 10 mL of gel. Thoroughly mix by swirling gently.

(4) **Pour the gel.** Pour the gel onto the casting tray, taking care not to introduce bubbles. If you do introduce bubbles, you can pop them with a pipette tip. It is best to make the gels as thin as possible. Gels 0.75 cm thick or less are appropriate for general applications (≤25 μL sample volume per well). Thicker gels may be required if you have a large sample volume, e.g., when purifying DNA fragments. Allow the gel to solidify at room temp, or at 4 °C if you're in a hurry.

Note: SYBR-Safe is very light sensitive. Do not leave the gel exposed for more than 3 hours.

(5) **Gel storage.** Place the casting tray with the solidified gel on top of wet paper towels. Wrap in plastic or place it inside a Tupperware. Ethidium gels can be stored at 4 °C for several weeks as long as the gel does not dry out. Gels containing SYBR-Safe must be stored in the dark — the dye has a shelf life of only a few days.

Note: Extended gel storage is highly discouraged because others might need to use the casting tray!

Things to look up / think about:

What is agarose?
What is the purpose of ethidium or SYBR-Safe?

Why is it important to pour thin gels?

What is the difference between agarose gels and polyacrylamide gels? Why do we use agarose gels for DNA and polyacrylamide gels for proteins?

A 0.8% agarose gel can resolve DNA fragments in the 0.5-20 kb range. Is it possible to resolve larger DNA fragments? What about Mb-size DNA (e.g., chromosomes)?