

Sugden Lab.

Electrophoretic Mobility Shift Assay (EMSA)

20cm Gel Preparation, Sample Separation, and Visualization:

Glass Plate Preparation:

Wash both panes of glass with windex and 70% EtOH
Dry with KimWipe
Assemble with toothed spacers on sides, winged spacer on bottom
Clamp corners, bottom and side with binder clips
Lie on a slight angle (can use microfuge tube rack)

Native PAGE Preparation:

Add in this order

63.25ml dH₂O

3.75ml 10x TBE

7.5ml 40% 37:5:1 acrylamide

0.5ml 10% APS

50ul TEMED

0.5x TBE:

50ml 10x TBE

950ml dH₂O

Gel Preparation Before Sample Separation:

Place comb between the glass panes, do not insert completely to facilitate comb removal.

Add more gel solution every 5min as needed to keep gel volume up to the top of the comb until it solidifies.

Remove winged spacer from bottom of gel and gel comb upon solidification and attach to the vertical gel apparatus, wet small square sponges in 0.5x TBE and place between the gaskets of apparatus and the gel plate. Push down on the sponges at the gasket junction with a 1000ul pipette tip to ensure a tight seal.

Bring gel apparatus to 7th floor cold room and fill lower buffer well on an angle to prevent formation of an air bubble between the glass plates. Fill the upper buffer well above the top of the back plate. Use a 3ml syringe and small bore needle to wash each well with buffer to remove unpolymerized (liquid) acrylamide.

Run gel at 10-15 V/cm (200-300V for 20cm gel) for at least 1 hr at 4C. This ensures that the entire gel is at the same temperature and that free radicals formed during the polymerization of the gel are removed before sample separation. Check after 30 minutes of running for buffer leaks.

Sample Loading and Separation:

Turn off power supply.

Load each lane with gel loading tips or P10 tips to ensure all of the sample enters the well.

Run gel at 10-15 V/cm (200-300 V for 20 cm gel) until bromophenol blue marker contained in the free probe lane has migrated at least halfway through the gel (2-4 hrs).

Just as the gel is about done running, erase the PhosphorImager Storage Screen by exposure to white light (use extended erase with the Screen Eraser in the gel camera room). Additionally, go get about 1.0-1.2lbs of dry ice and deposit it into the trap of the gel dryer and start pre-heating the gel dryer to 80C for at least 10 min.

Remove gel from apparatus and remove the smaller, back pane of glass. Be careful not to stretch the gel too badly as you do this.

Cover the gel with a piece of Whatman paper and remove the gel from the second pane of glass.

Cover the gel with a piece of Saran Wrap and place in the gel dryer underneath the plastic sheet of the gel dryer. Close gel dryer cover and dry at 80C for about 1 hr.

Remove Whatman/Gel/SaranWrap from the gel dryer. Be careful not to pull off the Saran Wrap from the gel when lifting the plastic sheet of the gel dryer off.

Trim the Whatman/Gel/SaranWrap to the size of the gel.

Tape the Whatman/Gel/SaranWrap to a PhosphorImager Exposure Cassette, place the PhosphorImager Storage Screen on top and lock into place.

Expose the screen o/n at RT (can minimally expose the screen for ~4 hrs to detect a signal if the radioisotope is fresh enough).

Visualize the screen by PhosphorImager (STORM).