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Biomedical Engineering

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Casting and running of 15% PAGE gel

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Table of contents

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1 Preparation buffers

- Prepare 10% ammonium persulfate (APS) by dissolving 100 mg ammonium persulfate in 1 mL of dH2O.
- Mix the following together in 1 liter demi water to make 10x TBE buffer:
    - 108g Tris base
    - 55g Boric acid
    - 40mls 0.5M EDTA (pH 8.0)
- Autoclave the mixture

2 Casting gel

Prepare two 15% PAGE gels according to the following recipe for 15 mL:

- 5.6 mL acrylamide (19:1)
- 1500 µL 10x TBE buffer
- 7.8 mL H_2O
- 64 µL 10% ammonium persulfate
- 12.8 µL TEMED

To cast the gel:
Make sure you are using nice intact glass plates, this is especially important for the underside, as gel might run out otherwise.
Don't forget to wear gloves when handling materials that may have been in contact with acryl-amide!

- Clean the front and back glasses with soap in the sink
- Assemble the two glasses in the casting frame:
    - Place the glasses together making sure the spacer pieces on the thicker glass face the cover glass.
    - Place both glasses in the clamp with the bigger glass facing the side with the clamps
    - Place the frame and glasses on a flat surface to make sure they are properly aligned.
    - Finally place the glass plates in the clamp in the bigger casting frame, make sure there is a rubber underneath both setups.
    - Check if your construction is sealed by pouring water between the glasses, check to make sure water level stays the same.
- Next mix all the ingredients together in a falcon tube, whilst working in the fume hood also make sure you add the TEMED last since this is the catalyst that begins the polymerization reaction.
- Next mix by inverting the tube a couple of times.

- Pour the gel in between the glasses while going to the maximum might cause some spilling when inserting the comb it is useful to ensure a nice full gel.
- Insert the combs into the liquid.
- Let the gel polymerize for about 20~30 minutes, if you didn't spill any there is still some gel left in the falcon tube, this can be used to check polymerization speed.

3 Prepare samples

- Prepare 100 µM solutions in water of the unreacted oligo and the reaction product.
- Next in an Eppendorf tube mix 3 µL water with 3 µL loading dye and 700 ng of the samples (can be calculated using the MW given by manufacturer)

4 Running PAGE gel

If the gel is hardened it is time to run samples

- Take the glass plates and gels out of the fume hood and remove from casting frame
- Remove the clamps
- Wash the gels in the sink with water to remove any gel on the outside of glass
- Next with both hands remove the comb using your thumbs and pushing on either side
- One gel can be stored in the fridge for later use, wrap it in a wet paper towel and aluminum foil
- Make sure to use a running system with a straight untwisted platinum wire to ensure that the gel runs nicely
- Assemble the running system by placing the glass plate with gel against the rubber and making sure the little glass plate faces inwards and push the plates slightly up to make sure it is leak-proof
- Place a spacer plate on the side with the clamps, also pushing up.
- Place this system in the container
- Fill the entire container with 1x TBE buffer
- The lanes need to be "cleaned" by pipetting some TBE buffer into them using a normal yellow pipette tip (the flow should clear any un-polymerized gel out)
- Samples can now be loaded into the lanes, do not use any of the outer lanes as they tend to cause a so called smiling effect.
- Pipette all of the samples into the lane using the special pipette tips.
- Attach the power cables, making sure black is connected to black and red to red on both ends.
- Run the gel for 1 hour at 125 V

5 Imaging gel

- Take out the gel and rinse with water
- In a square container filled with MilliQ water remove both glass plates
- Wash the gel for 2 minutes by placing it in MilliQ and placing it on the see-saw table.
- Prepare the staining solution by adding 5 µL of SYBR safe to 50 mL of MilliQ water.
- Take the gel out of MilliQ and replace with the staining solution
- Stain the gel for 10 minutes on the see-saw table
- Pictures of the gel can be acquired by the digital image acquisition machine, make sure to place them on the correct table, focus the camera and set the correct filter.