technical report

Troubleshooting Polyacrylamide Gel Electrophoresis (PAGE)

A. Introduction

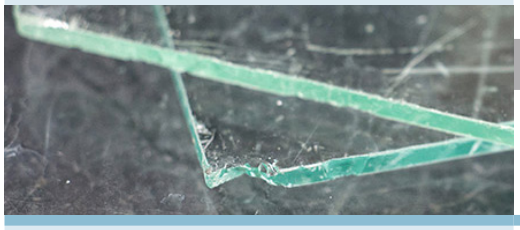
The IDT gel electrophoresis group runs preparatory polyacrylamide gels to purify certain oligonucleotides and can run up to 500 gels a day based on demand. Running that many gels means that this group has had a lot of experience with refining electrophoresis techniques and resolving gel issues. The following guide, provided by our gel electrophoresis team, contains troubleshooting tips for many of the gel issues they have come across during their work.

B. Troubleshooting

1. Pouring

The gel leaks

CAUSE: Is either glass plate chipped/cracked on the bottom?



SOLUTION: Pour a new gel using different plates.

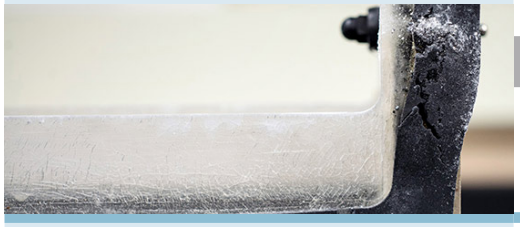
CAUSE: Are the glass plate and spacers clamped together tightly enough?

SOLUTION: Tighten the clamps—finger tight. Overtightening can lead to broken plates.

CAUSE: Are the glass plate and spacers even at bottom?

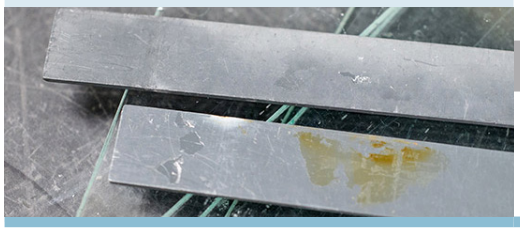
SOLUTION: Pour a new gel after standing the gel plates upright and pressing down on the spacers and plates to even out the bottom of the spacers with the bottom of the glass plate.

CAUSE: Is there a flawed casting box gasket?



SOLUTION: Clear any dried gel bits, ensure there are no scrapes/gouges/holes. If using a casting stand, place a spacer under the gasket (use like a shim).

CAUSE: Are the glass plates and spacers cleaned properly?



SOLUTION: Pour a new gel, after carefully washing the glass plates and spacers and rinsing with ethanol.

(Plates and spacers that are not clean may have bumps so that there are small gaps between the spacer and glass.)

The gel is not polymerizing or polymerizes too slowly

CAUSE: Was the correct catalyst concentration used?	SOLUTION:Adjust the concentration of the catalyst and pour a new gel.
CAUSE: Are the reagents old?	SOLUTION: Pour a new gel with freshly made gel reagents. Using fresh ammonium persulfate solution is key.
CAUSE: Is the pH is too low?	SOLUTION: Pour a new gel with freshly made gel reagents.
CAUSE: Is the acrylamide degassed?	SOLUTION: Pour a new gel with degassed acrylamide.

The gel sandwich separates

CAUSE: Is the plate glass dirty?	SOLUTION:Pour a new gel with well-cleaned plates that have been rinsed with ethanol.
CAUSE: Was the gel used before it was completely polymerized?	SOLUTION: Wait >60 minutes for polymerization before using the gel. To test, keep some residual gel solution in the pouring flask and suck up some of the solution into a glass pipet. Check the pipet for gel polymerization.

2. Set up

The buffer leaks when the gel is set up on the gel box

CAUSE: Is the area between the glass plates and spacers filled to the top?

SOLUTION: If possible, top off the gel, or pour a new gel and start over.

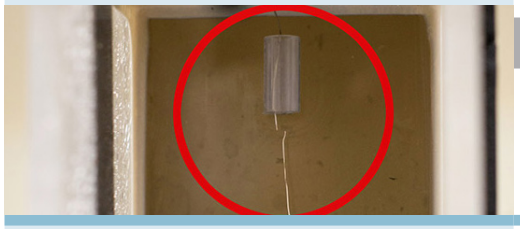
CAUSE: Is a gasket ripped/torn/ bunched?

SOLUTION: Straighten out or replace the gasket.

3. Running

There are no bubbles in buffer when running

CAUSE: Is one of the platinum wires broken or not connected to its lead?



SOLUTION: Rerun the gel in a functional gel box.

CAUSE: Are the platinum wires not covered by gel running buffer?

SOLUTION: Add more gel running buffer to the buffer chambers.

CAUSE: Is one or both leads not connected properly?

SOLUTION: Adjust the leads.

CAUSE: Is the gel running buffer concentration too low?

SOLUTION: Remake the gel running buffer.

CAUSE: Is the gel running buffer level inadequate?

SOLUTION: Add more gel running buffer to the buffer chambers.

CAUSE: Is the gel in contact with both buffer chambers?

SOLUTION: Ensure the gel is in contact with both upper and lower buffer chambers. Remove tape if used during casting.

The gel runs too fast/slow

CAUSE: Is the gel running buffer concentration correct?	**SOLUTION:** Remake the gel running buffer.
CAUSE: Are the power supply settings correct?	**SOLUTION:** Check and readjust the power supply settings.
CAUSE: Are the power supply settings too high?	**SOLUTION:** Reduce the power settings, and dissipate heat with an aluminum plate or by recirculating the gel running buffer in the buffer chamber. Alternatively, run the gel in a cold room.

The glass plates break during the gel run

CAUSE: Are the power supply settings too high?	**SOLUTION:** Reduce the power settings, and dissipate heat with an aluminum plate or by recirculating the gel running buffer in the buffer chamber. Alternatively, run the gel in a cold room.
CAUSE: Is the gel running buffer leaking during the run, allowing heat to increase?	**SOLUTION:** Remake the gel and when setting it up in the gel box, ensure a tight contact between the plates and the box; use grease on all gaskets.

4. Gel results

Gel bands do not migrate as expected

CAUSE: Were the power supply settings set correctly?	**SOLUTION:** Check and readjust the power supply settings.
CAUSE: Is the gel concentration correct for the length of the nucleic acid samples?	**SOLUTION:** Remake the gel at the correct concentration.

CAUSE: Is the gel running buffer concentration the correct concentration?	SOLUTION: Remake the gel running buffers to the correct concentration.
CAUSE: Were the samples overloaded?	SOLUTION: Prepare a new set of samples, decreasing their quantity.
CAUSE: Does the nucleic acid sample contain secondary structure?	SOLUTION: Adjust the gel run time to account for secondary structure. Increase the run temperature while recirculating the gel running buffer to keep the glass plates cool enough not to break.
CAUSE: Does the nucleic acid sample contain poly-G motifs?	SOLUTION: Increase the run temperature while recirculating the gel running buffer to keep the glass plates cool enough not to break.
CAUSE: Does the nucleic acid sample contain modifications?	SOLUTION: Adjust the gel run time to account for the modification(s).

There is poor band resolution

CAUSE: Are the wells poorly formed and/or uneven? 	SOLUTION: Pour a new gel, adjusting the formula of the gel and/or the amount of polymerization catalyst.
CAUSE: Were the wells flushed out prior to loading the samples?	SOLUTION: Pour a new gel and flush out the wells before loading the samples.

CAUSE: Do bubbles appear in the gel?	SOLUTION: Pour a new gel using well-cleaned plates that have been rinsed with ethanol. Pour the gel slowly and steadily. If using a pipet to transfer the gel solution from flask to gel plates, keep the pipet tip submerged in the gel solution when drawing it up. If bubbles form: • Before inserting the comb, stand the plates at a tall angle and lightly tap on them with a metal object to dislodge the bubbles, sending them up out of the gel. • Or insert the comb vertically and use it to pull out the bubbles.
CAUSE: Is there uneven heat buildup during the gel run?	SOLUTION: Dissipate excess heat using an aluminum plate mounted across the gel and/or recirculating the gel running buffer in the buffer chambers
CAUSE: Is the gel concentration correct?	SOLUTION: Pour a new gel using the correct gel percentage for the length of the nucleic acids in the sample.

"Smiling bands" are produced

CAUSE: Is the gel is running too hot?	SOLUTION: Reduce the voltage. SOLUTION: Remake the gel, reducing the amount of catalyst in the gel recipe. SOLUTION: Dissipate excess heat using an aluminum plate mounted across the gel and/or recirculating the gel running buffer in the buffer chambers. SOLUTION: Run the gel with chilled gel running buffer or run in a cold room.

The gel bands are streaked

CAUSE: Are the power supply settings too high?	SOLUTION: Reduce the power settings, and dissipate heat with an aluminum plate or by recirculating the gel running buffer in the buffer chamber.
CAUSE: Were the samples overloaded?	SOLUTION: Prepare a new set of samples, decreasing their quantity.

There is no band

(Tip: Always include a size marker as a positive control for the run.)

CAUSE: Are the leads are hooked up backwards such that the gel runs in the opposite direction?	SOLUTION: Check the lead orientation.
CAUSE: Was an incorrect loading buffer used?	SOLUTION: Remake the gel loading buffer.
CAUSE: Was enough sample loaded into the well?	SOLUTION: Prepare a new set of samples, increasing the quantity.

C. Summary

Performing PAGE is an art. It takes time and practice to familiarize yourself with the various gel box accessories, reagents, gel casting stand, and the electrophoresis unit. In addition, polyacrylamide gels are much thinner than agarose gels (typically <1 mm), making them prone to bubbles, and harder to handle. Pouring these gels definitely requires a certain finesse.

We hope this troubleshooting guide has addressed any PAGE issues experienced by your lab. Please contact our Technical Support group at applicationsupport@idtdna.com if you have further questions.

D. Additional resources

Running agarose and polyacrylamide gels—Electrophoresis with agarose and polyacrylamide gels is one of the most widely used tools in molecular biology. Gels provide a simple, low-cost way to separate nucleic acids based on size for quantification and purification.

Which type of purification should I choose?—Recommendations for oligonucleotide purification based on oligo length, application, yield required, and presence of modifications.

Designing PCR primers and probes—General guidelines for designing primers and probes and for choosing target locations for PCR amplification.

Authors: Kris Enequist and Kevin York are Manager and Assistant Manager, respectively, of the IDT gel electrophoresis group.