Steve Lewis 00:09
Welcome to another Mol Bio Minute mini episode within the Speaking of Mol Bio podcast series. Our overall series is all about molecular biology and its trending applications in life sciences, and these mini episodes provide tips, tricks and resources to help you be successful in the lab when doing your molecular biology work. Today's Mol Bio Minute topic is the last of our nucleic acid electrophoresis topics, but it's a great one. We have Aistė Polikaitytė covering common challenges and troubleshooting solutions for agarose gel electrophoresis with nucleic acids. I'm pretty sure you'll learn something new, or you'll be reminded of things you may have forgotten to consider. Enjoy.

Aistė Polikaitytė 01:02
Hello. I'm Aistė, and today I will talk about the most common issues in gel electrophoresis analysis and the ways you can troubleshoot them. I think most can relate to this. It's 4pm in the evening. You have already finished with all of your experiments, and the last thing you need to do is to check your electrophoresis results. You place the gel in your imager, and all you see is a huge smear all over the gel. Yeah, you are probably not alone, because I bet that everyone who works in the lab has experienced something like that at least once in their career. Either it’s smeared, faint, poorly separated nucleic acid bands, all of them can be avoided. So listen to this episode and learn how to avoid the most common issues.

First, let's deep dive into why you are seeing faint bands in your gel. The most common cause is that you are loading too little of your DNA. Make sure to load at least 100 to 200 nanograms of nucleic acid per well. Also you should always use molecular biology grade reagents and labware to avoid contamination with nucleases that lead to sample degradation. And this is especially important when you are working with RNA samples.

These issues might not be the only reason why the bands are faded after your electrophoresis. Some issues might be related to staining of a gel. Make sure you are using the highly sensitive nucleic acid stain, properly mixing in the stain while preparing your gel. And if you are post-staining your gel, make sure you are agitating your gel to ensure even distribution of the dye throughout the gel. Do not forget to use the appropriate light source that is compatible with the DNA stain you are using. What is also important is to monitor the migration of DNA in your gel, because small-sized molecules can run off a gel. To monitor your DNA migration in the gel, you should use an appropriate loading buffer. Make sure to choose an appropriate loading dye for your application, and to know what loading buffer you should select, check out the selection guide linked in the notes.

Another common issue you can see in your electrophoresis is smeared, or poorly separated bands. Usually smeared bands occur due to improper preparation of the gel. Gel can be prepared too thick, so make sure that your gel is 3-4 millimeter thickness for the best results. Avoid forming bubbles, do not damage the wells and allow your gel sufficient time to fully solidify. Very high voltage can lead to heating of the gel that leads to smeared bands. So please make sure you are running your gel at appropriate voltage. Also use compatible buffer and do not run your gel for very long time. All of these can result in smeared bands. All of these problems related to gel preparation can easily be avoided if you use already precast E-gels. You can check them out in the link in the notes.

Other than gel preparation, smeared bands can also be caused by sample itself. If your sample has a high concentration of salts, it's best to purify your sample prior loading, or you can dilute your sample. If your sample has a high concentration of proteins, it can bind to DNA and slow the migration of it, leading to smeared bands. So for the best results, purify your samples or use loading dye with SDS in its composition. Just heat your samples prior loading to the gel. Also, if you are working with single-stranded DNA or RNA, you should use denaturing agarose or polyacrylamide gel. This avoids secondary structures to form which can lead to smeared samples. And of course, Do not overload your samples, as this can also lead to poorly separated bands.

Finally, if after electrophoresis, you see bands that migrated or separated abnormally, ensure that you selected the correct concentration of the gel and your gel is homogenous. DNA migration in the gel can also be altered by certain contaminants that are present in your sample. For example, some nucleic acid stains intercalate into the double-stranded DNA structure and this leads to slower DNA migration. This is especially important when you are analyzing DNA of different conformations. For example, super coiled, relaxed or linear DNA. If you are doing such analysis, make sure you are post staining your gels. Also, if you are performing restriction digestion sometimes the ends of DNA might be cohesive and join again together forming an additional band that is visible in your gel electrophoresis. If you are analyzing such samples, make sure you are using loading dye with SDS in it, and heat your samples prior to loading. This could avoid additional bands from forming. Sometimes your DNA might be AT-rich, have a lot of Poly(A) sequences, or loads of modifications. Such DNA migrates differently than the normal DNA so you should be aware of that when analyzing your gel electrophoresis results.

In this episode, I summarize the most common gel electrophoresis issues and how you can avoid them. For more information and more electrophoresis troubleshooting tips, you can find in the troubleshooting guide that is linked in the notes. Also, if you want to learn more about gel electrophoresis, you can check out the webinar that is also linked in the notes. I hope that thanks to this episode, you will no longer have to worry about the bad gel or smeared bands ruining your evening.

Steve Lewis 08:20
That was Aistė Polikaitytė, scientist at Thermo Fisher Scientific, with lots of helpful troubleshooting tips to use for when you run your next gel. You can find the resources that she mentioned linked in the Episode Notes. We'll have a new full episode dropping soon, so stay tuned for that, but until then, cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock.