Steve Lewis 00:00
Steve, Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm your host, Steve Lewis, and I want to welcome you to the first of what we're calling the Mol Bio Minutes. These are mini episodes that we'll release in between full episodes this season. Regular full episodes will continue to be released on their regular monthly schedule. These Mol Bio Minutes are shorter in length and will use a slight variation of our artwork in your streaming platforms so that you can easily spot these episodes. They will feature some of the amazing talent we have inside of Thermo Fisher Scientific and our speakers will rotate to cover various topics that we believe will be quite relevant to those working day-to-day in the lab using molecular biology methods. Today, you'll be hearing from Augustė Užuotaitė speaking about migration of different forms of DNA in agarose gel electrophoresis. We hope you learn something helpful.

Augustė Užuotaitė 01:13
Hi everybody. I'm happy to join this amazing Mol Bio podcast. My name is Augustė, and today we're going to dive into the fascinating world of gel electrophoresis, a technique that is a staple in almost every biology lab. But first things first, what is gel electrophoresis you may ask?

Well, imagine a racetrack, but instead of cars or runners, we have DNA molecules. And instead of racing for the finish line, they're racing through a gel. Just like in any race, not all racers are the same. DNA molecules have different sequences and conformations, and each has its unique speed. So this speed, or migration rate, is what allows us to separate them and analyze them.

Now, the gel here isn't like the gel in your hair. It's a porous matrix, and the DNA molecules maneuver their way through these pores. The smaller they are, the quicker they can navigate. But there's more to it than size. The sequence, right? The ATs, the GCs of the DNA that can influence their speed. And then there's shape, or conformation. Is it linear, is it circular, or is it super coiled? Each has its own influence on the migration speed, adding layers of complexity to this DNA race.

For those of you who want a visual, I'll be here for you. Picture a marathon with runners of all sizes wearing different shoes or choosing different paths. That's your DNA in gel during electrophoresis. So we'll start simple with the basics of single-stranded DNA and double-stranded DNA. Then we'll kick it up a notch, discussing other forms of DNA. And of course, we'll point you to resources where you can find more information about each of these topics.

So picture this, you've ordered a 500 base pair double stranded DNA string from a certain supplier, but it took an unexpected detour in the shipment, right. That happens a lot, experiencing some temperature fluctuations during the shipment. Now your PI, or your boss, wants you to check if the DNA is still intact before starting the experiment; wee don't want to waste any more reagents. This is where your understanding of gel electrophoresis steps in. You'll need to pick the right gel type, buffer, and electrophoresis system. Think of this as setting up the racetrack for DNA molecules. You'll need a DNA ladder. This is like your ruler, allowing you to measure your DNA molecules based on their size. Then comes the decision making. How much of the sample should you load? What voltage should you set? How long should you let the gel run? These are like setting the distance and pace of your race. Then you'll decide which DNA stain to use and how to capture the perfect image. This is your finish line, highlighting the end result of the race. Once you're done with the electrophoresis, you'll hopefully see a single band that matches your 500 base pair product. This is like your racer crossing the finish line at the expected time. But remember, just like human athletes, DNA molecules can surprise you. Their sequence composition can influence their speed with AT-rich molecules potentially trailing behind GC-rich molecules.

So as we level up, we'll delve into more complex scenarios. What if your DNA mix isn't just a single type? And remember the speed of DNA migration in a gel depends not just on its size and sequence composition, but also on how tightly it packs itself. So first, let's remember that DNA isn't always in the form we would like it to be. Let's think again about single-stranded DNA. This speedy little molecule tends to migrate faster than our regular double-stranded DNA, but it has a quirky habit of forming secondary structures, like hairpin loops. It's like a runner deciding to do summersaults mid race. These unexpected shapes can throw off our measurements, making it tricky to determine the true size of the DNA molecule. To handle this, we need to run our gel under denaturing conditions. These conditions break down the secondary structures, forcing the DNA to run straight. It's like telling our somersaulting runner, " No flips, come on just run!" Some of these common denaturing conditions are just using a certain buffer, like glyoxal or DMSO and sodium phosphate buffer, sodium hydroxide, EDTA buffer and formaldehyde or formamide in MOPS buffer, or just using polyacrylamide gels with urea.

Next up, let's talk about circular DNA, like plasmids. Now, these are fascinating. A plasmid can appear in three different formats, super coiled, linearized, and nicked. Even though they all contain the same DNA, their conformations are different, and that changes their migration speed. A super coiled plasmid is the Usain Bolt of the DNA world, zipping through the gel much faster than its linearized or nicked counterparts. So imagine you've isolated a plasmid from E. coli, and you run gel electrophoresis instead of one band, you see three. Now you know that these could be the three forms of the same plasmid. The lowest band would likely be the super cold plasmid, the middle band would be the linearized plasmid, and the upper band would likely be the nicked. Now let's say you've purified a super coiled plasmid, and you want to confirm that it's the one you need. You perform restriction digestion, an enzyme that cuts your plasmid at a specific spot, you run a gel with both super coiled and the linearized plasmid. What will you see in your gel? Even though both are the same size, the super coiled DNA will migrate quicker, as we now know, than the linear one, so you should see two bands.

All these examples highlight that gel electrophoresis is not just a simple race, but rather a complex dance of size, shape, and sequence. It challenges us not to only understand the fundamental principles of electrophoresis, but also the unique characteristics of the DNA molecules we're working with. In the end, it's all about understanding your DNA. Smaller DNA molecules will migrate quicker and appear lower on a gel. AT-rich molecules may be slower than GC-rich molecules of the same size, and that's okay. DNA molecules of the same size, but different conformations, again, will migrate differently. And always choose the right gel type for your sample analysis.

So arm yourself with knowledge, ask the right questions, and don't be afraid to dig deeper. Gel electrophoresis is a journey full of fascinating surprises, and understanding its nuances can lead to the exciting discoveries you're about to unravel. You can find additional resources in the notes and on thermofisher.com. Thanks for joining us today. We hope you learned something new, and don't forget, we have additional resources that can help you polish your nucleic acid electrophoresis work.

Steve Lewis 08:34
That was Augustė Užuotaitė, Scientist III at Thermo Fisher Scientific, with helpful information about DNA form migration in agarose gel electrophoresis. Be sure to check out the episode notes to access the helpful resources she referenced. An interesting, full-length episode is coming soon, but until then, cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti and Matthew Stock.