Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis bringing you another episode of our Mol Bio Minutes mini episodes. Today's topic is staining considerations for nucleic acid separation and analysis in agarose gel electrophoresis. This is Paulius Palaima with all sorts of helpful information, reminders and resources on the topic.

Paulius Palaima 00:44
My name is Paulius Palaima, and I'm a product manager at Thermo Fisher Scientific. Imagine you need to perform nucleic acid electrophoresis. What stain should you use to best visualize your experiment? In this little segment, I will go over the diversity of the stains available, staining different forms of nucleic acids, the associated methods, as well as some safety considerations and overall recommendations for these experiments.

Nucleic acid stains are a very diverse reagent segment, and a lot of them have different aspects in which they have advantages or disadvantages. One thing to consider when you are selecting your stain for your experiment is the size of the molecule. Small-molecule stains have the particular advantage of impacting the migration of the nucleic acid that you are testing less. They have less artifacting, they generally are much easier to work with in different types of settings of your experiment. However, they tend to be classified more often that they are mutagenic, meaning that something that is, let's say, ethidium bromide, is a small-molecule state, and it can cross the cell membrane and then bind to the DNA. So, you have to have dedicated working environments, usually, for these stains. When you're working with large-molecule states, the crossing of the membrane usually is no longer an issue, because it just cannot cross the cell membrane and it's generally considered as a safe stain. However, in their case, they can impact the migration of your nucleic acid significantly more if you are using in-gel staining and can lead to some serious artifacting.

Additionally, you have to consider the wavelength that your stain is excited at. So, the most common stain, ethidium bromide, is excited at UV light, while others can be excited under blue light or even green light. And this can impact the downstream of your experiment. So, if you don't really need your sample to maintain its integrity for any downstream applications, say you're just looking at the PCR result, which you will discard afterwards, UV light might be enough. However, if you're working on an experiment you will use the product for downstream applications, blue light or green light that help to maintain your sample integrity, they don't cause DNA degradation, is probably the preferred method.

So now let's move into how do you stain an agarose gel during your electrophoresis? So, there are primarily two methods. The first one is in-gel staining, where you add your nucleic acid stain into the agarose mix, first allowing it to cool, and then adding it. And the second one is a post-run stain, meaning that you submerge your gel, which has been already run in a buffer that contains the nucleic acid stain of your choice. Both methods have their pros and cons. So, the in-gel staining is a particularly quick staining, meaning you don't need a secondary step to take after your gel has been run and it can help you visualize your progress much quicker. So meaning, because the stain is already in the agarose gel, you can stop your run at any given point and check your progress if you're not sure about

your experiment. The disadvantage of this method is that when you use large molecule stains, they can have significant artifacting. Additionally, there could be artifacting like a strong background, or you can see a shadow. The increased background illumination can be caused by the overabundance of the stain in the gel. This can manifest as a washed out look to your gel. The shadow manifests as the stain is moving in the opposite direction in the gel from your DNA sample or your nucleic acid. What this means is that smaller fragments that you're trying to visualize will be more difficult to see. And finally, it is the artifacting due to, say, large-molecule stains that you might use for your analysis, because they can cause some drag and some general linear artifacts within your gel if you're using them for your nucleic acid analysis, when in-gel staining. The post-run stains provide a much more even staining, meaning they don't really have the background that could be seen in in-gel stains. However, you cannot check your progress as you are running the experiment. They are a bit more time consuming because you are adding an additional step to your electrophoresis, and you risk some diffusion of the bands if they are left for too long.

Now, let's talk about the different types of nucleic acids and how the different stains interact with them. The majority of experiments that you will be performing with double-stranded DNA being it linear plasmid, or super coiled. In the majority of the cases, this is not going to be an issue, and you can use any stain, say SYBR Safe or SYBR Gold, to visualize them. The more interesting element is when you are analyzing single-stranded molecules, like single-stranded DNA or RNA. First of all, not all stains can stain these nucleic acids efficiently. And an example of this can be SYBR Safe, which does not show a significant affinity to single-stranded molecules, while SYBR Gold, on the other hand, has a strong affinity to single-stranded DNA, or minimally affected affinity when compared to the double-stranded molecules. Don't be surprised if you need to use more stain in these types of experiments than compared to usual double-stranded DNA analysis. This is a common challenge for single-stranded molecule analysis and performing electrophoresis.

So, let's move into the safety considerations. Ideally, the stain that you choose and the selected imaging source does not damage your sample or pose a danger to yourself. So, this is in reference to the ethidium bromide as well as UV light. So ethidium bromide is a well known carcinogen and it uses UV light for its excitation. Don't forget to choose stains that can be excited by blue light or green light, as they are much safer and they don't cause bleaching of the sample. So, meaning they don't degrade your sample as you illuminate them and you're trying to visualize them. This is also particularly important in the downstream applications as mentioned before, say, cloning. Finally, the most popular stain, as mentioned before, like ethidium bromide, requires dedicated laboratory space due to its mutagenicity. Considering safer, less mutagenic stains, like SYBR Safe, can improve your laboratory environment and help reduce the space that is allocated to nucleic acid electrophoresis. You can even have a small bench top allocated to that.

So overall thoughts on recommendations for using stains for nucleic acid analysis is that there is not one stain to cover all your needs. Consider your sample, your imaging system, and your goal. Make sure that you use the correct illumination compatible with the stain that you are using and documenting your experiments. Use the resources available to choose the right stain and optimize using something other than your very precious sample. And think about safety and environmental aspects where you can implement them and make your laboratory environment safer.

To help you better decide on what stain to use in your experiment, you can visit our website and find our DNA stain selection guide there. Thank you for joining us today, and we hope you learned something new or have a new resource to help you be successful in your nucleic acid electrophoresis workflow.

Steve Lewis 09:27
That was Paulius Palaima Product Manager at Thermo Fisher Scientific. As always, for these Mol Bio Minutes episodes, we recommend that you check out the episode notes to find the links to the helpful resources that Paulius covered today. We'll have another Mol Bio Minutes episode next month. But up before that is a great interview and discussion I had about some amazing science. Stay tuned for that to drop and until then, cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti and Matthew Stock.