Steve Lewis 00:10
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 the essential elements of fast PCR. This is Laurynas Alijošius with insights and advice on the topic.

Laurynas Alijošius 00:38
Hello, everybody. Today we're going to dig deep into the world of PCR, the polymerase chain reaction. There's one thing - PCR takes a lot of time. Let's imagine one scenario. So let's say you're in the lab at 4pm and you would love to go with your friends and have a dinner at six, but you still have to do one PCR reaction that takes like four hours. Whoa, I mean, it would be pretty tough day, right? You've been working hard all day and just miss your dinner that you've been planning all week. What are we going to do here? Can we just skip the dinner or skip the PCR? Or can we do maybe both? And we actually, can. We actually can. I'm going to assure you, there's a way to do it, and that's where the fast PCR mode comes in to save your dinner with your friends.

So let's talk about a fast PCR. So what is it? Let's say standard PCR usually can take like two hours or even more hours of this process. And when we talk about the fast PCR, it can be done in less than 20 minutes even. It can be done very quick. You're just having a cup of coffee in the meantime, and wham, it's done already. This fast PCR saves time, and that can also help you to streamline your work in the lab more efficiently. The time is not the only thing we're saving, actually. Using fast PCR instead of the standard one, you can make your PCR even like more cost effective. So with fast PCR, you would need less reagents per reaction. So that means you can perform more reactions with the same amount of PCR reagent. And it's also a sustainable choice, the fast PCR, because less waste per reaction will be produced. And also using shorter PCR programs on your instruments, it means that you will consume less energy.

Okay, okay, so we know about the benefits a little bit, but how do we do the fast PCR? Let's break it down here. So first up, let's talk about your PCR instrument. So for a fast PCR, you want to have a fast ramp speed, optimally from, let's say, four to six degrees per second, and some instruments have even faster ramp speeds. But it's important to keep in mind that if you want to use very fast ramp speeds, it's good to test them out and optimize and see what kind of products are forming, is the PCR going successfully. Our portfolio, we have Applied Biosystems ProFlex, we have VeritiPro and SimpliAmp thermal cyclers that are all equipped to support fast PCR because they have ramp rates of four degrees per second and even higher.

Next, let's talk about choosing good PCR plastics for your fast PCR. You may want to think about using the plastics with thinner walls, because they allow for faster heat transfer through the plastic for the reaction to occur. And you may also want to think about using plastics with the sample wells that have lower volume, because that also helps for a quicker reaction to occur more efficiently. So for example, if you want to do fast PCR, you might want to choose like, let's say, a well that it has 0.1 milliliter volume for fast PCR, while conversely, for standard PCR, the volume of the wells is usually 0.2 milliliter. In any case, when you're selecting your plastics always make sure that you select the PCR plastic that is compatible with your PCR instrument. And if you like to know more about these PCR plastics, in the

notes you will find a link to our plastics selection tool that can help you find the right plastics for your fast PCR or just any other PCR application.

And now let's move on to the main element of the PCR, the DNA polymerase. The DNA polymerases can be wild type, for example, traditional *Taq* DNA polymerases. They can be engineered one, that means that they were changed from the wild type. For example, we have Platinum II Taq Hot-Start DNA polymerase. And the main difference between the wild type polymerases and an engineered one, in terms of PCR, is that the wild type ones usually are slower. So let's say their DNA sequence elongation time could take even like one minute for one kilobase. On the other hand, if you decide to use the engineered DNA polymerase, you can reach elongation speed up to five seconds for one kilobase. I mean, that's crazy speed. The difference feels like a race between a horse and a spaceship. You guessed it, in our lab, we love to use the engineered polymerases for a fast PCR, because they are just many, many times quicker. When we talk about the engineered DNA polymerases another nice feature is a hot start option to have available with the DNA polymerase, which is nowadays almost like a standard for the DNA polymerase to have. And what does the hot start do? It helps you ensure that your race for DNA amplification starts only when you're ready. So it's like a trigger or like some activation mechanism. And there's two types of hot start technology. It can be activated either by chemical modification, which takes up to 10 minutes and a bit slower, or it can be activated by antibody or aptamer mediated technology, which is much, much quicker and only takes seconds. So for example, our Platinum II Taq Hot-Start DNA polymerase, this uses the aptamer antibody mediated technology, which is the quicker one. Engineered DNA polymerases have multiple other beneficial features. For example, it can be the high-fidelity DNA polymerase or thermal stable, or highly processive. But what do these all terms mean? So let's say, if you're using a high-fidelity DNA polymerase, this certain polymerase would help you to have less errors introduced during DNA amplification, and that's real good. For example, if you are using PCR to later sequence those samples, so for sequencing purposes, when you need like precise, original sequences amplified. Or if you're using thermal stable polymerase, that means that this enzyme will last you long because it will be more stable, and in that case, it will bring you a more consistent performance overall, over time. If you talk about the high processivity of DNA polymerases, that kind of polymerase can synthesize long stretches of DNA without dissociating from the template strand. So it means that, in the end, it would also help you increase the speed of your DNA amplification.

So what is the real secret sauce to achieve the fast pace of the PCR? And there's one magic word that works wonders with PCR. And the word is optimization. So optimizing your PCR reaction can lead to some beautiful results, and more importantly, it can improve your efficiency of your PCR reaction. And in the end, that's what we want, right?

Let's jump into it. First thing that we can optimize could be the amount of the DNA polymerase that you use for your PCR reaction. But this thing depends on specific DNA polymerase. So let's say that both increasing or decreasing the amount of units can improve the efficiency of your PCR. So for example, it could be like using from half a unit or the whole unit of a fast DNA polymerase for a 25 microliter of PCR reaction volume. But that amount of units can also be different. So it can vary depending on your DNA polymerase. And I think it's also good to take note that what it says in the manual of the DNA polymerase, because it can be different for each one of them. The other important aspect is the length

of your oligonucleotide primers. So these oligos are like short DNA fragments that help you target your region of interest in your DNA template sequence. Typically, primers are usually designed to be from 18 to 25 nucleotides in length. However, you can use even longer primers and that may allow you to run a two-step PCR protocol instead of the standard three-step protocol. And how it's possible is that because long primers have higher melting temperatures and higher melting temperatures allow you to perform the annealing step of the PCR at the higher temperature, and you can even try to join the annealing and elongation step together to be performed at the same time. So this would allow you to significantly shorten the PCR run time. I think that master mixes and pre-mixed buffers are very good choice for PCR buffer for a fast PCR, but if you need to do some custom optimization of your PCR buffer or if you just curious to learn more and go deeper into this, you can access the resource link in the notes that reviews the six considerations for the PCR step optimization.

Okay, so we talked about the setup of your PCR reaction. But what about the cycling program for the fast PCR? First, you can lower the number of your PCR cycles in general, although that correlates with the amount of the starting DNA template. I think a good starting amount of the PCR cycles would be something like 30 cycles. Like, that's a good starting point to start working from there. But let's say if you have, like, a lot of DNA material, then you can lower the number of your PCR cycles significantly, like, even down to 25 or even like 20 cycles instead of like the standard, like 35 or 40 or 30 cycles. If you have lots of sample, lots of template DNA, this could be the way you can make your PCR faster. Next cool trick for your cycling program could be the shortening the steps of your PCR protocol that we just mentioned earlier. So just using a two-step protocol instead of the standard three-step that can also significantly shorten the duration of your PCR cycling program. We did that, but what else? Maybe we can shorten something else. So when you set up your number of PCR cycles and when you choose if you want to do the two-step or three-step version PCR protocol, you can start shaving the duration of your every PCR step. If you usually do the initial denaturation step for like, three or four, maybe, even minutes, you can maybe try to shorten it down to one minute or like to half a minute. In this case, that's where the new engineered DNA polymerases come in with their strong performance. Because the advanced engineered DNA polymerases they have very short times for each step of the PCR. So you can really cut down the duration of every step if you use the engineered ones.

So there you have it. You set up your fast PCR. You pick the ramp speed of your PCR instrument. You pick the right PCR consumables, and you selected the polymerase that is convenient for you, and you got your PCR reaction mix and your program optimized, and bam, look at that. Your PCR took way, way, way less time than usual. And just like that, your dinner with friends is saved too. So you can have a good time you can share with your closest people. So you can make it in time for your dinner. And we hope that you found this episode helpful, and that you found something that you can apply for your PCR experiments. So thank you for tuning in. Until the next time, have happy experiments, have happy dinners, and may your PCR is running as fast as lightning.

Steve Lewis 15:51
That was Laurynas Alijošius, Scientist III at Thermo Fisher Scientific. As always, for these Mol Bio Minutes episodes, we recommend that you check out our Episode Notes to find links to the helpful resources that Laurynas 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.