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, Thermo Fisher scientist Monika Jazdauskaitė shares her expertise with recombinase polymerase amplification reactions for target detection, as well as information on ready to use kits available for your lab. We hope you enjoy.

Monika Jazdauskaitė 00:44
Hello everyone. Thanks so much for tuning in. I am Monica, an R&D Scientist at Thermo Fisher Scientific Baltics. My focus is protein purification, DNA RNA amplification methods and their improvements. Now in this episode, I would like to share one of my personal stories. I remember when I got sick and had to go to the hospital, that doctors immediately tested me for three different viruses. What really stood out was how quickly they identified COVID-19 and started the right treatment. And this got me thinking that we are living in a time where amplification and detection methods have seen the major improvements, largely driven by the urgency of the pandemic. What is even more remarkable is that the progress didn't stop at PCR. Alternative methods were explored and adapted to deliver faster, more affordable and more accessible pathogen identification across various settings. As an example, isothermal amplification. You've already heard one of its examples in the last episode, which is LAMP. And in this episode, I will be talking about another isothermal amplification method, recombinase polymerase amplification in short way, RPA. I will cover what it is, why it matters, how it works, and why it's gaining attention, especially in the next generation sequencing library prep.

So, what is RPA? It's, as I mentioned before, isothermal amplification method working at low constant temperature, for example, 37 or 42 degrees Celsius. RPA doesn't require thermal cycling, which is the biggest difference from PCR. Think of PCR like baking a bread, you need to heat an oven to a very high temperature, then lower the heat, then bring it up again. It requires precise temperature control and time management. In contrast, RPA is more like making a stew. You set the pot on a steady, moderate heat and let it simmer. There is no need for precise temperature changes, just consistent warmth to cook the ingredients. Therefore, RPA can be greatly adapted to resource limited settings and decentralized applications.

Now, how does RPA work? Remember how I said that RPA is like making a stew? Let's stick with that. In this case, the ingredients you put into the pot are four key proteins, each with a specific role. First, you've got the recombinase protein, UvsX and UvsY. They help the primers to find the right place to bind to the DNA. If we are talking about the primers, like in PCR you only need one primer pair. However, there is a slight difference in the designing them as they are a little bit longer. Their length varies from 30 to 35 nucleotides. Also, amplicon length should be around 150 or 450 base pairs. The second protein that comes to their action is gp34. Then the third protein that comes to the mix is gp32, a single stranded binary protein keeping everything together from clumping by stabilizing the open DNA strands. And finally, you add the strand displacing DNA polymerase, which does the heavy lifting, copying DNA by building new strands without needing the heat cycles.

However, just like making the real stew, you need more than just the main ingredients. For RPA to work you also need a buffer, magnesium chloride, and a mix of nucleotides. Mix it all together, incubate at 42

degrees Celsius for 20 minutes, and you've got the rapid DNA amplification that can be analyzed by agarose gel, fluorescence detection, or other assays. Luckily, you don't have to shop around for each ingredient. There are ready -to-use RPA kits like Invitrogen Lyo-ready RPA kit that comes with everything included. I will drop the link in the notes if you are curious to check it out. But if you do want to shop around and to spice things up to your preference, I also know a link where you can request a quote for the custom RPA reaction. And the link, I will also drop it. Our RPA reagents are in a format compatible with lyophilization, which means if you need a long-term stability storage or room temperature stability, they can easily be freeze dried for that purpose.

Now, we talked how RPA works, let's go for RPA for target detection. What really stands out to me about RPA when I am doing a reaction is not just its speed or simplicity, or the fact that it works at low temperatures, but its specificity and sensitivity. For example, if you are dealing with respiratory infectious pathogens, labs using RPA can quickly and accurately tell if it's caused by *Staphylococcus aureus, Pseudomonas aeruginosa,* or *Mycoplasma pneumonia*. I think we would all agree that research methods that can quickly identify pathogens are desirable and advantageous. Now here's something cool. Not only can RPA amplify DNA, but it can also be used to detect RNA targets, even from a single copy. All you need to do is to add a few components to the reaction mix, which are reverse transcriptase, ribonuclease inhibitor and RNase H. If that's something you're curious about, I've got it all covered, as I will drop the link to those products. However, the good part is that our Invitrogen Lyo-ready RPA kit is designed to tolerate common inhibitors like ethanol, heparin, urea, humic acid, and even transport media components, making it reliable even with less than perfect samples.

Now, let's go to the sequencing part. In recent years, amplifying next generation sequencing libraries has become a really important step. Not just to get enough DNA for the sequencing, but to make sure we give the integrity and diversity of the original sample. Unlike PCR, which relies on cycling through high temperatures, RPA works at constant lower temperature. This helps to reduce thermal damage and preserves the more delicate structures in the complex libraries. Because of this, RPA has emerged as a solid alternative to traditional PCR, especially when you are working with fragile or precious samples. And here's the best part, experimental data shows that RPA delivers almost identical results to PCR in terms of coverage, alignment, GC bias, even across the wide GC range. Now if you are working with a high GC content genome, you can enhance GC coverage uniformity by adding up to 5% DMSO.

So, let's take a closer look at the workflow itself. You start with your DNA sample, fragment it, and ligate adapters. Then comes the interesting part. Instead of traditional PCR, you use an isothermal amplification step with the Invitrogen Lyo-ready RPA Kit to pre-amplify your libraries. It is mostly done following the manufacturer protocol, but there are a couple of tips worth mentioning. First of all, RPA seems to work best at a slightly lower temperature, around 37 degrees of Celsius. And as I mentioned before, if your sample has a high GC content, adding a little bit of DMSO can really improve reaction efficiency. Once your library is pre-amplified, you move to quantification and then it's ready for the sequencing. As you can see, RPA is simple, fast, and adaptable to different DNA inputs, even with those with trick GC content.

As we are coming to the end of this episode, I would like to recap the key points we have covered today. First of all, RPA is a fast isothermal amplification method, highly suited for pathogen detection. Although not lyophilized by default, our reagents are lyophilization compatible, offering potential for field deployable or long-term storage formats. In next generation library prep, RPA can replace traditional PCR working effectively at lower temperatures and tolerating high GC content with additives like DMSO. Thanks everybody for listening, and I hope this episode gave you useful insight and maybe even the inspiration to try RPA in your own lab.

Steve Lewis 11:30
That was Monika Jazdauskaitė, an R&D Scientist at Thermo Fisher Scientific. As always, for these Mol Bio Minutes mini episodes, we recommend that you check out the Episode Notes to find links to the helpful resources that she 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.