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 your host, Steve Lewis, bringing you another Mol Bio Minutes mini episode. Today, Thermo Fisher's Aistė Serapinaitė joins us to explain 1-Step RT-PCR. She covers its advantages and applications in the labs, as well as considerations and tips to make your 1-Step RT-PCR successful.

Aistė Serapinaitė 00:44
Hello everyone. I'm Aistė, an R&D scientist, and for many years, I've been working with DNA and RNA amplification methods and their improvements. So today I want to tell you more about a widely used molecular biology method that can simplify your RNA research and save you valuable time. It's 1-Step RT-PCR , or reverse transcription polymerase chain reaction. For many years, detecting RNA used to be a long and multi-step process. It was time consuming and error prone, but now with 1-Step RT-PCR, you can work faster, with greater accuracy and with less risk of contamination. So let's take a closer look at how it all happens.

So RT-PCR is used to detect RNA by converting it into cDNA or complementary DNA and amplifying specific sequences. And there are two main approaches, one step and two step. So in this episode, we'll focus on the one step method, discussing what it is, how it works, and what are its advantages. So what exactly is 1-Step RT-PCR? In simple terms, it's an all-in-one method that allows RT and PCR to occur in a single tube using a single reaction mixture. So this means that instead of handling multiple steps separately, first transcribing RNA to cDNA and then amplifying it, we did all in a single quick reaction. And now let's walk through how this reaction actually works.

So the whole process begins with RNA extraction from a sample, and this can be anything from pathogen RNA to RNA from various tissue samples. And once you have your RNA, you add it to a reaction mix containing primers, dNTPs, the buffer system, and of course, the enzyme mix with reverse transcriptase and DNA polymerase. And the composition of the reaction mix has to be carefully optimized so that both enzymes would be happy and would work efficiently without interfering with each other. But you don't need to worry about all that optimization when using Thermo Fisher Scientific reagents, because we've done all the work for you. Later, after everything is mixed, the thermal cycler takes over, and the reaction starts with a reverse transcription step at a moderate 50 degrees temperature that is convenient for the reverse transcriptase, and this is later followed by the denaturation at much higher, for example, 98 degrees Celsius temperature, which inactivates the reverse transcriptase and prepares sample for the PCR amplification. And from there, cycles of denaturation, annealing, and extension begin producing millions of copies of target sequences that can be further analyzed by gel electrophoresis, sequencing, or cloning and to get the best results, there are some things that you should remember about 1-Step RT-PCR reaction. The first one is primer design.

So since reverse transcription and amplification are happening in the same tube, gene specific primers are typically used instead of random or oligo dT primers, which are common for the two-step method. And while designing primers, do not forget to design them for the RNA or cDNA template and not the genomic DNA, especially if you are working with a transcribed mRNA that differs from the DNA template. And for faster and more convenient primer design you can always use our Oligo Perfect Primer Design tool. Also, RNA is quite fragile and prone to degradation, so its quality is everything. And this becomes even more critical if you want to amplify and analyze long RNA targets, for example,

those longer than 10 kilobases, and sometimes it's not the reaction or the primers that are not working, but rather, there is no longer any RNA template of that necessary length left. So make sure to work in an RNase free environment and use fresh and recently extracted RNA that wasn't frozen and thawed many times or even wasn't frozen at all. And of course, do not forget to include controls. No template control, or NTC, to check for the contamination and no RT control a sample without reverse transcriptase, to confirm that your RC amplification is from the RNA and not the DNA. And in my work, to keep no RT control clean, I usually treat all of my RNA samples with an ezDNase Enzyme, which requires only two minutes for the complete genomic DNA removal and that ensures that no false positive results will occur in my experiments.

So now that we've covered how 1-Step RT-PCR works, let's talk about why it's such a popular choice. One of the biggest advantages is convenience. With fewer pipetting steps and no need to transfer cDNA between tubes, there's lower risk of contamination user error and false positive results, which are really important for any application where accuracy of your test result is critical. Since it's all happening in a single tube, 1-Step RT-PCR improves reproducibility by minimizing handling and keeping all the conditions consistent. Also, it needs fewer tubes and fewer pipette tips, which means less plastic waste. So it's a greener option too. Nowadays, 1-Step RT-PCR mixes are highly optimized and developed, and one of them is a novel SuperScript IV UniPrime One-Step RT-PCR System. It has an advanced hot start mechanism, elevated benchtop stability, and even universal annealing temperature feature that allows you to skip calculating primer annealing temperatures. And believe me, this is really convenient when you're working with several different primer pairs. And all of this together with a reduced number of reaction steps, make 1-Step RT-PCR faster, more efficient and perfect for high throughput testing. Also, 1-Step RT-PCR is very sensitive and specific, because the primers used in the reaction are designed to match the match the RNA target precisely and that allows the detection of even low levels of RNA, such as a few femtograms. And its high sensitivity and convenience makes 1-Step RT-PCR a powerful tool that can be used in various RNA research. It can be used in gene expression studies to measure the levels of specific RNA molecules in your sample. Also in cancer research, it can help you to detect RNA biomarkers associated with different types of cancer. Also, you can monitor food water or soil microbial contamination, track plant viruses and agriculture science, or you can use it for medical research. You can detect various RNA viruses that can cause common viral infections like flu or COVID-19 or even more dangerous diseases like Ebola, Zika, or Dengue fevers. And as you know, viruses are a constant threat. They spread easily; they cause outbreaks and evolve into new variants that can resist treatments. So that's why tracking all these changes and new mutations is so important, and one way to do that is by sequencing the virus entire genome in order not to miss anything. To get the RNA virus ready for the new generation sequencing, first you need to prepare DNA library from the whole RNA virus genome. And for that, scientists can use a method called targeted RT-PCR. Basically, you break the genome into smaller overlapping pieces, let's say around 400 base pair each and use RT-PCR to copy all these pieces all at once in a single tube using dozens of primer pairs. The exact number of primers depends on the size of the virus. For example, to cover the whole Zika virus, you will need around 35 amplicons, whereas for the SARS-CoV-2, which has much bigger genome, you will need more than 100 amplicons. So this helps to make sure that no part of the genome is missing.

So we've seen how convenient 1-Step RT-PCR can be. However, it isn't perfect for every situation. Since everything happens in one tube, you can't store cDNA for later use and this makes it less ideal for experiments where you want to analyze multiple genes from the same sample over time. In those

cases, a two-step approach might be better. In the end, 1-Step RT-PCR brings speed, simplicity and reliability to RNA analysis. While it has its limitations, its convenience, low contamination risk and high reproducibility make it a powerful tool in your lab. If you're working with RNA and want fast and easy results, 1-Step RT-PCR is your go-to method, just don't forget fresh RNA, specific primers, and optimized master mix. So thank you for listening to today's episode. I hope you found it interesting and useful, and that it inspires you to try this method in your experiments. And if you want to know more about 1-Step RT-PCR, its applications and tools, just check in the links in the notes. Until next time.

Steve Lewis 10:36
That was Aistė Serapinaitė from our R&D team here 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 Aistė covered today. We'll have another Mol Bio Minutes mini episode next month, but up before that is our regular full episode that I am excited to share with you. 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.