Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series from Thermo Fisher Scientific 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, we welcome HIV researcher, Dr Ryan Jeep. Ryan offers an informative and insightful look at HIV research and the molecular biology techniques that support his area of work. We hope you enjoy.

Ryan Jeep, PhD 00:43
So my name is Ryan Jeep. I've worked in HIV drug discovery and drug resistance for a little over five years, through both my graduate training as well as my postdoc experience at Colorado State University. And I got into HIV research, really for the opportunity to contribute directly to translational research as well as to kind of explore more general questions regarding HIV biology. And through this experience, I've really directly observed how molecular biology and molecular techniques and approaches underpin HIV research and efforts toward a cure.

We'll start with a very quick outline of HIV biology and infection. HIV is a retrovirus. Retroviruses are a type of virus that are RNA viruses that actually encode a viral enzyme that reverse transcribes their RNA into DNA, and then they integrate that DNA into the infected host cells genome. So from a practical standpoint, this means that individuals infected with HIV are always infected, and this is because the viral genome, the HIV genome itself, actually integrates into the genome of the infected white blood cells. Because of this, there is no cure. So spoiler alert, we'll talk a little bit about some exciting efforts toward a cure later. But from a practical standpoint, currently, there is no cure available. This means that people infected with HIV are required to maintain and sustain lifelong treatment to suppress viral replication and reduce the chances of transmission. Current treatments aim to minimize viral replication, to preserve immune function, to reduce transmission, and overall, to let people lead mostly normal, symptom free lives. So these treatment plans are mostly successful. However, drug resistance is one of the major barriers to treatment, and we will get a bit more into drug resistance in a little bit. So in this episode, I really want to cover how molecular biology goes hand in hand with the HIV field. I've broken this into three parts. In part one, we'll go over HIV detection. In part two, we'll talk more about HIV treatment and drug resistance. And in part three, we'll go over some really exciting avenues that are currently underway to establish a cure.

Okay, so in part one, we're going to discuss HIV detection and viral load measurements. So staying true to the theme of this podcast, really getting into the depths of the molecular biology that underpins HIV, one of the most common and foundational methods for detecting HIV is quantitative reverse transcription polymerase chain reaction, or, as I'm sure most of us know it qRT-PCR. qRT-PCR is a very common method for quantifying the viral load. And viral load is a fancy way of saying the amount of virus that is circulating in a bloodstream. So to give you a brief rundown of qRT-PCR in the HIV field, you have a blood sample that is collected. You separate the plasma from red blood cells. You purify the RNA, and then you use qRT-PCR to detect the viral RNA that's present in those samples. Now, like I mentioned earlier, qRT-PCR is a way to directly detect the HIV RNA in a bloodstream, which reflects the amount of viral replication that's happening over the course of an infection. There are other ways to measure HIV infection, but these are mostly indirect responses, and you get this via measuring the

immune response or the presence of antibodies or even the presence of HIV antigens themselves. qRT-PCR is extremely reliable. It has a very high detection sensitivity. On the lower end. qRT-PCR assays are able to detect HIV RNA at quantities as low as 54 RNA copies per milliliter. And this can be even lower for HIV-2. RT-PCR strategies also exist to distinguish HIV-1 from HIV-2, in addition to their detection sensitivities, and There are also constant and ongoing improvements in detection sensitivity to detect various strains. And there's even qRT-PCR based assays to detect the actual activity of HIV viral proteins themselves in parallel to detecting viral load. So in addition to detecting viral load, these PCR methods are also highly applicable to other areas of research. These can include monitoring the efficacy of treatment over time, viral replication kinetics, you can monitor latent reservoirs of viral infection, and you can even monitor gene expression in infected cells. Okay, part one is all about HIV detection. In part two, we're really going to move into HIV treatment, into HIV drug resistance, and into a little bit of my own research in the HIV field.

So as we mentioned before, there are many treatments that are available that are successful in suppressing viral loads. And when you suppress viral loads, you lessen disease progression, and you decrease the chance of transmission. In some cases, the evolution of drug-resistant strains causes a sharp increase in viral load, which is known as viral rebound, and this really sharp increase in viral load causes treatment failure. So even people who are on antiretroviral therapy start to experience symptoms, their viral load increases, which increases disease progression and increases chances of transmission. So here's where we get into my own PhD work and my own experience in the field. So my PhD work really revolved around developing sensitive technologies to detect infectivity that can then be applied to a myriad of research and development purposes, but most notably for our purposes, really sensitive drug resistance detection. And the eventual goal of this sort of research in developing these sensitive tools is to develop personalized treatment plans based on specific mutant strains that are circulating. So highly personalized treatment plans are not currently in wide use. So in part two, we're going to talk about a typical approach that we take in a research lab setting that could potentially be applied to the real world. And all of the approaches that we currently use in a lab really heavily rely on molecular biology and molecular techniques.

So a typical workflow starts with sequencing. So sequencing is used to determine the presence and abundance of resistance associated mutations that are circulating in blood. So just like in part one, the workflow is similar. We isolate blood samples, we extract RNA, we then generate cDNA from the RNA that we've purified via RT-PCR, and then we can either do Sanger or next generation sequencing to identify the overall mutational landscape of the genome that's circulating. And we need this really deep sequencing technology for a really complete and accurate detection of the mutational landscape. Mutations can occur anywhere, and we need to know exactly where they are and how common they are. And I really want to highlight here that at this point we really need the full sequence information. We need a complete and accurate picture of what mutations are present and where they're present. Once we know the sequence, then we can potentially develop PCR based assays to detect HIV, to treat HIV, but the sequence needs to come first. Once we have the sequences, we can then clone these sequences into a test vector or a reporter virus, and we can measure the infectivity in vitro. Now, depending on the abundance of mutations, these recombinant reporter viruses can be modified via site directed mutagenesis. We can also cut and paste small sequences of the genome using simple restriction cloning, or to clone larger sequences of the genome into our test vectors we can even use

the cDNA that we've generated from our viral RNA. We can amplify it using PCR, and then we can use cut, paste cloning. We can use Gibson cloning or even approaches like Golden Gate to construct our test vectors. Once we have these test vectors, we can then measure the infectivity against a variety of drugs. And this is what we commonly do in a lab setting. When it comes to a real-world setting, we could then use this information to construct the best collection of drugs that would work best for the specific viral strains circulating in the blood. And again, you can see in part two that the combination of many molecular techniques makes all of this feasible. We have sequencing, we have PCR, RT- PCR, restriction cloning and even more sophisticated cloning techniques like Gibson and Golden Gate. All of them are heavily utilized in HIV research in the lab, and all of them will be heavily utilized when customized, personalized treatment programs become widely available.

Okay, so moving into part three. In part three, we're going to cover some really exciting, cutting-edge avenues in the research and development field towards the development of a cure. So there are a lot of strategies for potential cures, and all of them really involve eliminating or modifying all of the infected cells such that viral replication becomes impossible. And the one I want to focus on today is the CRISPR Cas-9 system. So CRISPR Cas-9 is really widely used in this sort of research, and we use it in two main approaches. So the CRISPR Cas system at large, heavily relies on molecular biology and molecular technology. The CRISPR-Cas system relies on a nuclease and guide RNA pair in which the nuclease selectively cleaves double stranded DNA that's complementary to the guide RNA molecule. Now typically, both the nuclease and the guide RNA are expressed from plasmids. And as we all are familiar with, plasmid construction requires some really fundamental molecular techniques. We're talking PCR restriction, cloning and even higher order, more sophisticated cloning techniques to incorporate these specific DNA sequences and paste them all together to correctly encode our nuclease and our guide RNAs. There are even CRISPR Cas approaches that rely on RT- PCR to generate cDNA from the viral DNA to correctly encode our guide RNA. We can then also use PCR and sequencing to verify our plasmid products. There are two main approaches that I want to cover here.

The first approach involves targeting and deleting sequences in the viral genome that are required for transcription. So without getting too into the weeds here, all of the transcription machinery, all of the sequences that drive transcription in the virus are present at the five-prime end. If we can selectively target and delete some or all of these sequences required for transcription, we can effectively kill the virus, because we're killing viral transcription. This approach is really attractive due to the really high selectivity, and in a perfect world, we can minimize off target effects.

The second approach actually relies on generating white blood cells that are resistant to HIV infection completely. We can then deplete the white cell blood count and replace with genetically modified white blood cells that are resistant to new infection. And interestingly enough, this has actually been done in a few cases, indirectly, but I will say that this approach is less feasible for widespread application due to the really invasive procedures, very harsh treatments that are associated and the numerous risks that are associated with transplants, which are required for replenishing the white blood cell count. So despite the fact that both of these approaches still have a ways to go before widespread adoption, neither of these approaches would be imaginable without genetic and molecular technology. We have cloning, PCR, RT-PCR, and sequencing that are all really heavily relied upon to construct our plasmids,

to amplify fragments for gRNA synthesis, to verify nuclease activity and to verify the products that are created from employing this CRISPR Cas-9 system.

So in closing, I just want to remind you that the HIV pandemic really is still a huge worldwide issue. However, access to treatment and sufficient care have dramatically reduced mortality and improved the quality of life for those infected. So I hope you've learned throughout the course of this podcast that molecular techniques and molecular technology really underpin each of these triumphs in HIV research. Thanks to qRT-PCR we can very sensitively and reliably detect HIV infection, and we can also very closely monitor treatment success, despite the fact that drug resistance remains a barrier to widespread treatment success, we're constantly developing new tools and doing novel research to close that gap. And all of these approaches rely on PCR, RT-PCR, all varieties of cloning and sequencing. And the combination of all of these techniques provide the foundation for really cool, cutting-edge research involving CRISPR Cas-9 in efforts towards a cure. So I want to leave you with this just in my early career in the HIV field, I've personally witnessed and used many of these techniques, and I've seen how they've grown over the years. And what really excites me is imagining where the HIV field will end up as we constantly evolve and constantly produce newer, better technologies that all revolve around basic molecular biology.

Steve Lewis 13:43
That was Dr. Ryan Jeep, postdoctoral researcher at Colorado State University. If you'd like to learn more about the topics that Ryan covered today, check out the Episode Notes for links and additional resources. We'll have another Mol Bio Minutes episode next month, but up before that will be a full episode interview that I think you'll really enjoy. 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.