**AN AWAKENING IN NEXT-GENERATION MOLECULAR DIAGNOSTICS**

CRISPR and next-generation sequencing are transforming molecular diagnostics, from infectious disease to early cancer detection. **WHERE MIGHT THE SCIENCE LEAD?**

CRISPR as a diagnostic tool

In 2016, the Zika virus outbreak in the Americas set the stage for the first approved CRISPR–Cas9 diagnostic. Since then, CRISPR diagnostics have been used to detect the Lassa and Ebola viruses, along with SARS-CoV-2. Infectious disease outbreaks have driven development of CRISPR. But any setting that requires high specificity of a known genetic target, such as the analysis of tumour samples, identification of inherited genetic variants or non-invasive prenatal testing (NIPT), is a possible candidate for CRISPR diagnostics. CRISPR is best known as a gene editing system in which a Cas9 enzyme is paired with a guide RNA engineered to complement a target DNA. When the target DNA is identified, Cas9 cleaves the sequence at a defined site, permitting gene excision or insertion.

**The mechanism behind CRISPR diagnostics is similar to gene editing, in that it uses an engineered guide RNA. But it typically employs different Cas proteins, namely Cas12, Cas13 and Cas14. Those proteins can be modified to generate fluorescent signals in the presence of a target nucleotide sequence, whether DNA or RNA, making the system suitable for assays or imaging.**

While most researchers develop their own CRISPR diagnostics, two commercial platforms have recently arisen to speed the process. SHERLOCK was developed by Feng Zhang’s group at the Broad Institute, and DETECTR was created by Jennifer Doudna, who received a Nobel Prize for the discovery of CRISPR-Cas9, along with her team at the University of California, Berkeley.

Both platforms use CRISPR to offer rapid in vitro identification of target nucleotide sequences at attomolar sensitivity. As with any molecular diagnostic, CRISPR tests need sufficient target DNA for detection. For that reason, they often rely on an amplification step before detection. That is most frequently accomplished through polymerase chain reaction (PCR), a tried-and-true method that requires thermocyclers to amplify nucleotide sequences. Unlike true PCR-based diagnostics, such as reverse transcription PCR (RT-PCR), CRISPR diagnostics can use other amplification methods, too, which presents an advantage.

“When you start thinking about doing this in a clinician’s office, or in the field, they don’t have thermocyclers,” says Matthew Poling, Product Manager for Genoma Cybernetics at Thermo Fisher Scientific. “For these diagnostics to move into patient-centric point-of-care work, LAMP is becoming more promising.” LAMP, or loop-mediated isothermal amplification, is an emerging technique that permits the amplification of nucleotide sequences at a constant temperature. Rather than separating double-stranded DNA fragments, LAMP amplifies a DNA strand, so-called ‘up’ and amplify double-stranded DNA. The technique can also be adapted to detect RNA sequences. Along with CRISPR reagents, Thermo Fisher Scientific offers various LAMP polymers and lyophilization-compatible enzymes. These lyo-ready enzymes are glycerol-free and remain stable when shipped and stored, making them better suited for use in the field or at the point-of-care.

**Using CRISPR for cancer diagnostics**

Among the clinical fields that might benefit from CRISPR diagnostics, oncology is perhaps the largest. While it’s not yet possible to walk into a doctor’s office and ask for a CRISPR test, researchers are working on it. In a proof-of-principle experimental setting, scientists used the SHERLOCK platform to detect known cancer mutations. Others are taking different approaches.

Harve Dev, a clinical leader and group leader in the Early Detection Programme at the Cancer Research UK Cambridge Centre, is currently working to identify prevalent biomarkers in prostate cancer. He is developing a CRISPR-based platform called ProCASP that can map genetic variants in individual tumours to help predict their susceptibility to certain drugs.

“The aim of it,” Dev says, “is to map the functional contribution of specific genes within an individual patient’s tumours.” Dev says that he hopes his work will eventually translate into a diagnostic that informs a patient’s treatment plan. “If there were another layer of information that we could add to that,” he says, “we think that’s going to be really important in shaping the specific treatment that individual patients receive.”

CRISPR diagnostics still face challenges. For example, current CRISPR diagnostics cannot yet be leveraged in a high-throughput setting. Also, all CRISPR-based diagnostic platforms require a single known target sequence, which can be particularly challenging in certain cancers and other multifactorial inherited disorders. Until CRISPR tools have advanced to be able to multiple genes at once, diagnostics that require this type of multiplexing may be more suited to next-generation sequencing tools.

**Next generation sequencing in diagnostics**

Like CRISPR, next-generation sequencing (NGS) technologies have quickly advanced from a standard research application to a complex diagnostic tool.

“When NGS became available, it enabled hypothesis-free experiments, where you don’t need to know what you’re looking for,” says Zana Kapustina, R&D manager at Thermo Fisher Scientific in Vilnius, Lithuania. Today, rapid parallel sequencing enables researchers to test for unknown genetic variants, as in rare diseases, or screen for a panel of possible genetic variants all at once. The method has become foundational to oncology, namely in the development of liquid biopsies or companion diagnostics for precision therapies, and it is being investigated for applications in the diagnosis of hereditary hearing loss, genetic cardiomyopathies and autosomal dominant polyolcyclic kidney disease, among others. NGS also permits single cell sequencing, which allows the discovery of mutations at the level of an individual cell.

NGS techniques are, at this point, well codified, but a few persistent challenges remain for researchers. One is library preparation. In any NGS screen, researchers start by fragmenting sample DNA into small segments and labelling them for easy identification. The fragments are then sequenced and the results are analysed. That can be a time-consuming process. “Unfortunately,” Kapustina says, “we cannot skip library prep, but we are trying to make those workflows as compact and affordable as possible.” Thermo Fisher offers a range of standardized reagents and support library preparation for both Ion Torrent and Illumina sequencers, along with expertised services for those developing specific diagnostics.

Another challenge for researchers is sample stability. “It’s very important for people using liquid handlers or robotic systems, as they [add their samples and] leave it for a period of time—up to a few days,” says Sigita Činčiūtė, product manager at Thermo Fisher in Vilnius. Thermo Fisher actively consults with researchers to help them build workflows and diagnostics that will take such considerations into account.

**Expanding the diagnostic toolkit**

The arrival of technologies suitable for molecular diagnostics is not very common. The molecular diagnostics market is still dominated by decades-old PCR and ELISA tests. The development of CRISPR and NGS diagnostics presents a rare chance to expand that portfolio.

Both techniques have proven easy to adapt and scale, allowing for the rapid development and testing of new diagnostic candidates, and they are both relatively low cost, making them more palatable for insurers and health systems. They also complement each other. NGS allows for the identification of unknown variants or the sequencing for several known biomarkers at once. Single-sequence CRISPR diagnostics are well-suited for use in the field or point-of-care. It’s unlikely that CRISPR and NGS diagnostics will supplant more established methods. Rather they will almost certainly expand access to different kinds of diagnostic information presently out of reach. Dev, for his part, is already using multiple tools to diagnose prostate cancer.

“What diagnostic tool we end up relying upon, it’s going to be multiformal,” he says. “None of these tools are likely to exist in isolation” That is good news for those pursuing CRISPR diagnostics, or for the development of NGS and CRISPR diagnostics applications.

**REFERENCES**


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