Biotechnology

CRISPR tool interrogates a line-up of viral suspects

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Rapid, reliable identification of an unknown viral infection is challenging. Use of CRISPR technology can simultaneously detect nucleic acids of many viruses and pinpoint specific ones, such as the virus that causes COVID-19. **See p.277**

The current severe effects of the global pandemic of COVID-19 reveals our vulnerability to emerging infectious diseases[1,2]. It also highlights the need for tools to detect a broad range of disease-causing agents, both known and recently emerged, that could threaten public health. However, the genetic diversity of the potential perpetrators, which include viruses, bacteria, fungi and protozoa, presents a practical difficulty. Molecular methods that detect nucleic acids are uniquely suited to this task because such infectious agents contain DNA, RNA, or both, that enables their recognition and identification. Feasible surveillance methods for tracking emerging global infections must have broad detection capability, be suited to high-throughput use and have low cost per test. On page 277, Ackerman *et al.*[3] describe an attempt to meet these requirements using a diagnostic detection platform they have created, called CARMEN (combinatorial arrayed reactions for multiplexed evaluation of nucleic acids).

CARMEN is an extension of SHERLOCK, a diagnostic platform previously developed by some members of the same team[4] that was built around the biotechnology tool CRISPR, which can be used to selectively edit nucleic acids. CRISPR is based on a bacterial defence system. Its use as a laboratory tool depends on a 'guide' RNA (also termed a CRISPR RNA) present in a complex with a Cas enzyme. If the guide RNA binds to a nucleic-acid target that is complementary to it in sequence, Cas is activated and cleaves the target.

Some Cas proteins cut target nucleic acids only at a specific site related to the guide sequence. However, Cas13 is different from other Cas proteins in that it digests only RNA and not DNA[5], and exerts its RNA-cleaving activity on any nearby RNA that it encounters. This property can be used to generate a signal that indicates the presence of a sequence of interest.

This principle underlies SHERLOCK and CARMEN. A reporter RNA is cleaved in a non-sequence-specific manner by Cas13 if it is activated through recognition of a specific sequence. This cleavage generates a fluorescent signal by separating two components attached to the reporter RNA: a fluorescence quencher and a fluorescent molecule (Fig. 1). CARMEN retains the sensitive and specific detection achieved by SHERLOCK, and adds the capability for simultaneous detection of multiple nucleic-acid targets. This makes the workflow compatible with a high-throughput, miniaturized setting that enables rapid turnaround at a low cost per test.

To detect specific nucleic acids using CARMEN, the process begins with the amplification of target viral nucleic acids (if present) in a specimen by methods such as the polymerase chain reaction (PCR) or recombinase polymerase reaction (RPA). The amplified nucleic acid can be the product of a specific amplification reaction targeting a single viral sequence, or can be the product of pooled reactions used to potentially amplify a range of different viral sequences. The sample of amplified RNA is given a unique colour code by the use of four fluorescent dyes mixed in a ratio that provides one of 1,050 possible colour combinations. Oil is then added to generate emulsified one-nanolitre droplets. The authors prepared such droplets for all the different amplification reactions carried out. They also generated a series of emulsified droplets with unique colour codes containing the components needed to detect the presence of specific viral sequences. Each detection mixture comprised a quenched fluorescently labelled reporter RNA and Cas13 bound to a guide RNA needed to detect a viral target.

All the emulsified droplets are mixed in a

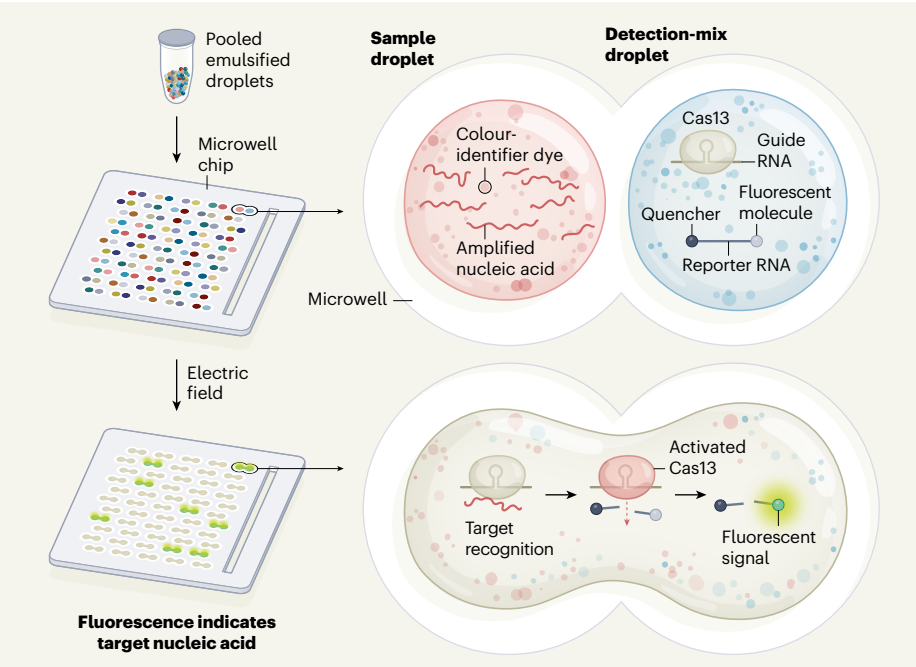

Figure 1 | A method to detect viral infection. Ackerman *et al.*[3] present a platform called CARMEN that can identify a range of infections in a given sample, including infection by the virus that causes COVID-19. Two categories of emulsified droplet are needed for this platform — sample droplets and droplets that enable the detection of a specific nucleic acid. Each different type of sample or detection droplet contains a unique dye-based colour identifier. Mixed droplets are loaded onto a chip containing microwells that hold two random droplets, and the colour codes are recorded. If a sample has viral sequences of interest, these nucleic acids are amplified when droplets are prepared. The detection-mix droplets contain the enzyme Cas13 in complex with a guide RNA that enables the recognition of a particular target RNA sequence. They also contain a reporter RNA, which is tagged by a fluorescent molecule that does not fluoresce owing to a quencher molecule attached to the RNA. Each pair of droplets in a well merges when an electric field is applied. If the Cas13 complex recognizes its target RNA in amplified nucleic acid, Cas13 is activated and cleaves the reporter RNA. This removes the quencher and generates a fluorescent signal, which reveals a particular viral infection in the person from whom the sample came.

single tube, and its contents loaded onto a chip containing microwells that each accommodate only two droplets. The droplets distribute randomly into the microwells, to constitute what the authors refer to as a self-assembling array, such that each amplified nucleic-acid target is expected to be exposed to each detection mix, in multiple replicates in different locations on the chip. Exactly where this happens is revealed by recording the two colour codes present in each well.

The detection reactions are then initiated simultaneously in each well by merging the droplet pairs by exposure to an electric field. If an amplified viral sequence is in a well that contains Cas13 in complex with a guide RNA that can recognize this sequence, Cas13 is activated and its nonspecific RNA-cleavage activity generates a fluorescent signal from the reporter RNA. This platform is admirably innovative, marrying the desirable characteristics of the ability of the guide RNA–Cas13 complex to recognize a specific sequence with a labour-saving platform that is inherently flexible because the user can select the PCR reactions and the guide-RNA sequences used.

To illustrate the potential application of CARMEN for broad testing of virus samples, the authors show that the technique could simultaneously detect all 169 human viruses for which at least 10 genome sequences were available at the time. The authors also demonstrate that CARMEN enables comprehensive identification of different influenza strains from samples obtained from infected people. This is important, because it could allow detection of a newly emerging type of influenza. CARMEN can be adapted to detect a viral variant resulting from a mutation after the variant sequence is determined. Ackerman and colleagues report that, when CARMEN was used on samples from people infected with HIV, it could detect six known viral mutations associated with drug resistance.

Finally, the authors illustrate CARMEN's flexibility by rapidly adapting the system to detect SARS-CoV-2, the coronavirus that causes COVID-19. The authors report that CARMEN distinguishes SARS-CoV-2 from the other human coronaviruses, including four seasonal coronaviruses and the coronaviruses responsible, respectively, for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Rapid, sensitive and specific detection of SARS-CoV-2 by a method using CRISPR and Cas12 has recently been reported[6], and that technique has similarities to CARMEN but only one type of virus can be detected.

How does CARMEN compare with other emerging diagnostic platforms? In the breadth of its detection, CARMEN is most similar to the microarray methods used for the simultaneous detection of amplified nucleic acid from multiple viruses[7]. But CARMEN has the advantage of avoiding the need to manufacture in advance microarrays that contain the specific nucleic-acid sequences needed.

Commercially available multiplex PCR panels, now widely used in diagnostics in clinical laboratories, provide another possible platform[8]. These kits, described as 'sample-in, answer-out' systems, are admirably simple to use and can detect 20 or more targets in just over an hour. However, they are not modifiable by users in the way that CARMEN is – the kits come preloaded with the components needed to amplify nucleic acids and have been optimized for a specific combination of targets.

Another option is metagenomic sequencing[9], which is a next-generation sequencing approach that directly determines the sequences of any nucleic acids present without needing a PCR-based amplification step or a specifically tailored reporter probe to detect particular sequences. However, compared with CARMEN, this method requires more-complex equipment and data processing, and takes longer to generate results.

Although CARMEN incorporates numerous desirable features for the surveillance of emerging infectious disease or the identification of a viral infection, there are some concerns. First is that the CARMEN workflow includes the manipulation of amplified nucleic acid, and so there is the risk of contamination. Perhaps appropriate automated instrumentation could reduce this key vulnerability. Second, will off-target effects of Cas13, possibly resulting from binding of guide RNAs to incorrect targets, lead to nonspecific detection reactions? Third, will the generation and image analysis of the nanodrops in these chips be sufficiently robust under 'field conditions' in a range of different types of laboratory, considering the need for sophisticated fluorescent-microscopy analysis, and given that users will have different levels of experience and expertise?

Finally, the sequences used to amplify RNA and the guide RNA sequences used might need to be changed to achieve optimal sensitivity and specificity and to account for virus variation over time. These issues need to be taken into account, but they do not lessen the authors' achievement in developing a new diagnostic platform designed around the need for surveillance of global emerging infectious diseases.

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Earth science

Force takes control in mountain-height debate

Kelin Wang

What controls the height of mountain ranges? An analysis of the forces acting on mountains near tectonic-plate boundaries suggests that tectonic forces are the main controller, rather than climate-driven erosion. **See p.225**

It is common knowledge that mountain ranges were created by tectonic forces, but how their height is maintained today is a matter of debate. A widely held view is that climate-controlled erosion limits their height[1,2]. On page 225, Dielforder *et al.*[3] take a different stance. They show that, at least for mountain ranges that are near convergent tectonic-plate boundaries, tectonic force has a dominant role in controlling height.

The mountain height discussed by the authors is that of a smoothed version of the actual mountain topography, in which high peaks and deep valleys are omitted. The natural processes that maintain this mountain height can be simplified into three types (Fig. 1). The first is lateral support of mountains from tectonic force, which either prevents mountains from falling apart under their own weight or pushes them farther up against gravity. The