The CRISPR Journal
Volume 2, Number 2, 2019
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DOI: 10.1089/crispr.2019.29053.oma

The CRISPR Journal

FIRST CUTS

Chipping in on Diagnostics

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A New Report Unveils a Promising Portable Platform That Allows for Rapid Detection of Nucleic Acid Mutations Using CRISPR and Graphene

While CRISPR has ushered in a revolution for the programmable manipulation of DNA and RNA to study biological processes or treat disease, it has more recently emerged as a powerful tool for molecular detection.[1] Point-of-care diagnostic applications, such as clinical diagnostics, genotyping, and testing in low-resource settings, require nucleic acid detection tools that are sensitive, specific, rapid, inexpensive, and straightforward to use. CRISPR systems afford the means for rapid recognition of short nucleic acid stretches, which can then be leveraged for identifying specific nucleic acids,[2–6] allowing rapid, sensitive detection with inexpensive and simple to use formats.

A new study in *Nature Biomedical Engineering* by Reza Hajian and colleagues, under the direction of Kiana Aran at the Keck Graduate Institute and University of California, Berkeley,[7] uses a catalytically inactivated CRISPR protein to recognize nucleic acid targets on a graphene-based field-effect transistor (gFET). The digital reader-based approach described by Hajian *et al*. demonstrates an encouraging pairing of gFET technologies with CRISPR-Cas9 and adds to the ever-growing toolbox of CRISPR-based detection systems. (The study also involves collaborators from two San Diego–based start-ups, Cardea Biosciences and Nanosens Innovations.)

Electronic methods for programmable detection of nucleic acids commonly rely on interactions at a material interface to generate a detectable signal. Field effect transistor (FET)–based nucleic acid detection methods rely on sensing current changes between source and drain electrodes due to binding and unbinding events.[8] FET-based biosensors employing target interactions with probes, such as oligonucleotides, morpholinos, or peptide nucleic acids, have achieved detection limits in the femtomolar range, but require at least one hour to reach this level of sensitivity.[9,10]

The approach, termed CRISPR, described by Haijan *et al*., termed CRISPR-Chip, recognizes the nucleic acid target with a catalytically inactivated (''dead'') Cas9 (dCas9) complexed with a target-specific guide RNA and immobilized on a three-terminal gFET. After an initial calibration of the chip, the binding of any target DNA sequences to the dCas9 ribonucleoprotein complex will alter the conductivity properties of the graphene and be sensitively detected as a change in current by the device, signifying target presence. As a proof-of-principle demonstration of the technology, the authors show detection of an integrated blue fluorescent protein gene in genomic DNA with sensitivity in the low femtomolar range.

As Duchenne muscular dystrophy (DMD) is a disease frequently characterized by deletions in exon 3 or exon 51 of the X-linked dystrophin gene, the authors developed a CRISPR-Chip assay for the rapid diagnosis of DMD from purified (unamplified) patient genomic DNA samples (Fig. 1). Including the calibration time, the test was capable of accurately sensing DMD deletions in 30 min or less with a limit of detection of 1.7 fM, which is within the clinically relevant range for genotyping.

Going Digital

These early results further demonstrate the feasibility of using CRISPR-based screening for rapid DNA detection in healthcare settings. Given the high specificity of Cas9,[11] testing the sensitivity of CRISPR-Chip in contexts such as cell-free DNA detection of cancer[4,5] could further showcase the precision of the technology. While the current study required purified genomic DNA, compatibility with unpurified samples will be a critical element for field-ready diagnostics.[12,13] Additionally, testing of CRISPR-Chip robustness within different sample matrices (e.g., serum or saliva), ease of use, and portability given the complexity of the chip—will be crucial for ensuring broad applicability.

Further engineering of the described methods might allow higher sensitivity and spatial[4,14] or in-sample multiplexing[5]

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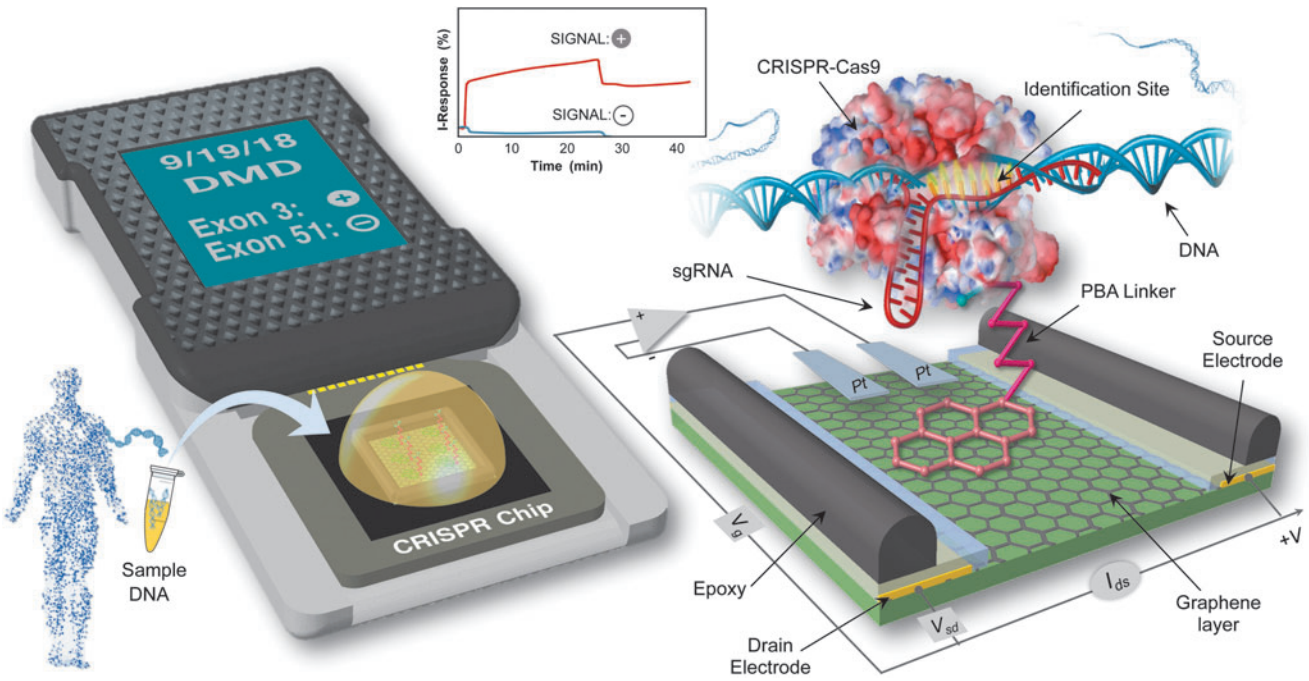


FIG. 1. Getting a read on the CRISPR-Chip. The chip consists of dCas9 molecules complexed with a custom single guide RNA (the dRNP) attached to the surface of the gFET. The immobilized dRNP scans the patient genomic DNA sample until it identifies and binds to the target sequence. Binding of the target DNA to the dRNP complex triggers an electrical signal output within 30 min. dRNP, dCas9 ribonucleoprotein; gFET, field-effect transistor. (Adapted from Hajian *et al.,* 2019,[7] courtesy of K. Aran.)

to expand throughput and increase adoption of the technology. CRISPR-Chip could also be used to test *in vitro* affinity and kinetics of guide RNAs for a given target, although with genomic DNA this assay would characterize binding to both on-target and off-target sites, which would be indistinguishable.

There are many immediate applications for portable nucleic acid detection, including in-field tracking of viral epidemics in low-resource settings or deployment in clinics without sophisticated testing labs. In addition, increased portability would be compatible with at-home testing of nucleic acids for diagnosing diseases like flu or the common cold. Overall, the flexibility of easily designed CRISPR systems for new targets, and the portability of a convenient electronic test, positions the CRISPR-Chip detection system as an exciting addition to an already impressive breadth of applications for CRISPR-based detection systems.

Disclosures

The authors are co-inventors on patent applications filed by the Broad Institute for CRISPR nucleic acid detection technologies; co-founders of Sherlock Biosciences; and advisors for Beam Therapeutics. J.S.G. is a campus advisor for Benchling, Inc.

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DOI: 10.1089/crispr.2019.29054.mrw

Base Editors and Off-Targeting: The Deaminase Matters

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Of Mice and Rice: A Pair of Reports Show That Cas9-Linked Cytosine, but Not Adenine, Base Editors Yield Genome-Wide Non-sequence-Specific Off-Targeting

CRISPR-Cas-mediated gene editing has made it easier for scientists to obtain the genetic variation needed for their research and in medicine offers a bountiful array of therapeutic possibilities. Using standard Cas9 and Cpf1/Cas12a systems, researchers can induce specific double-stranded breaks that lead to desired insertions and deletions (indels) via errors in DNA repair and, at a much lower frequency, swaps, and insertions with the aid of a DNA donor.

Most genetic variation within organisms, however, is due to single-nucleotide polymorphisms—point mutations are the most common alterations associated with human diseases.[1] Although specific single-base changes can be introduced using a DNA donor, this method is inefficient because it usually relies on homologous recombination. The recent development of base editing offers a very promising approach to engineer base substitutions without creating double-stranded breaks, but as two recent papers highlight,[2,3] the specter of off-target mutations that has enveloped CRISPR genome editing methods will have to be addressed here as well.

The Development of DNA Base Editors

To create, recreate, and correct SNPs with higher efficiency, scientists have used Cas9–guide RNA (Cas9–gRNA) complexes to bring DNA-modifying enzymes to specific sites in the genome where these enzymes can perform base substitutions. As described elsewhere in this issue (see pages 81–90), Alexis C. Komor in David Liu's lab at Harvard University made the first such base editor by fusing a rat cytidine deaminase (APOBEC1) to the N-terminus of a nuclease-inactive Cas9 (dCas9) to yield C-to-T (and G-to-A) base edits.[4]

Cytidine deaminases generally act on RNA to convert a cytosine to uracil, but APOBEC1 can also modify single-stranded DNA (ssDNA).[5] During DNA replication or repair, this uracil can be converted to a thiamine, yielding a C-to-T change. DNA editing by the APOBEC1-dCas9 fusion protein occurs within a five-nucleotide window of the protospacer on the ssDNA strand not paired with the gRNA.[4] Window size and position can be altered using different linkers between APOBEC1 and dCas9, and editing efficiency can be altered depending on sequence context.

The base editing efficiency of this system (0.8–7.7% of targeted sequences *in vivo*) was further improved by making two changes aimed at reducing an edited cell's use of repair mechanisms that favor conversion of the U:G mismatch back to C:G.[4] By using a nickase form of Cas9 [nCas9 (D10A)] to generate a single-stranded cut on the strand paired with the gRNA, Komor and colleagues improved base editing efficiency, likely by stimulating DNA repair mechanisms that use the uncleaved (and edited) strand as a repair template.[4] Fusing a uracil-DNA glycosylase inhibitor (UGI, from a bacteriophage of *Bacillus subtilis*[6]) to the C terminus of nCas9[4] further increases C-to-T editing by reducing the activity of the ubiquitous uracil-DNA glycosylases that normally remove uracil residues found within DNA, thereby converting the U:G base editing intermediate back to C:G. The final form, APOBEC1-nCas9-UGI, also called BE3 (third-generation base editor), produced 15–75% C-to-T

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