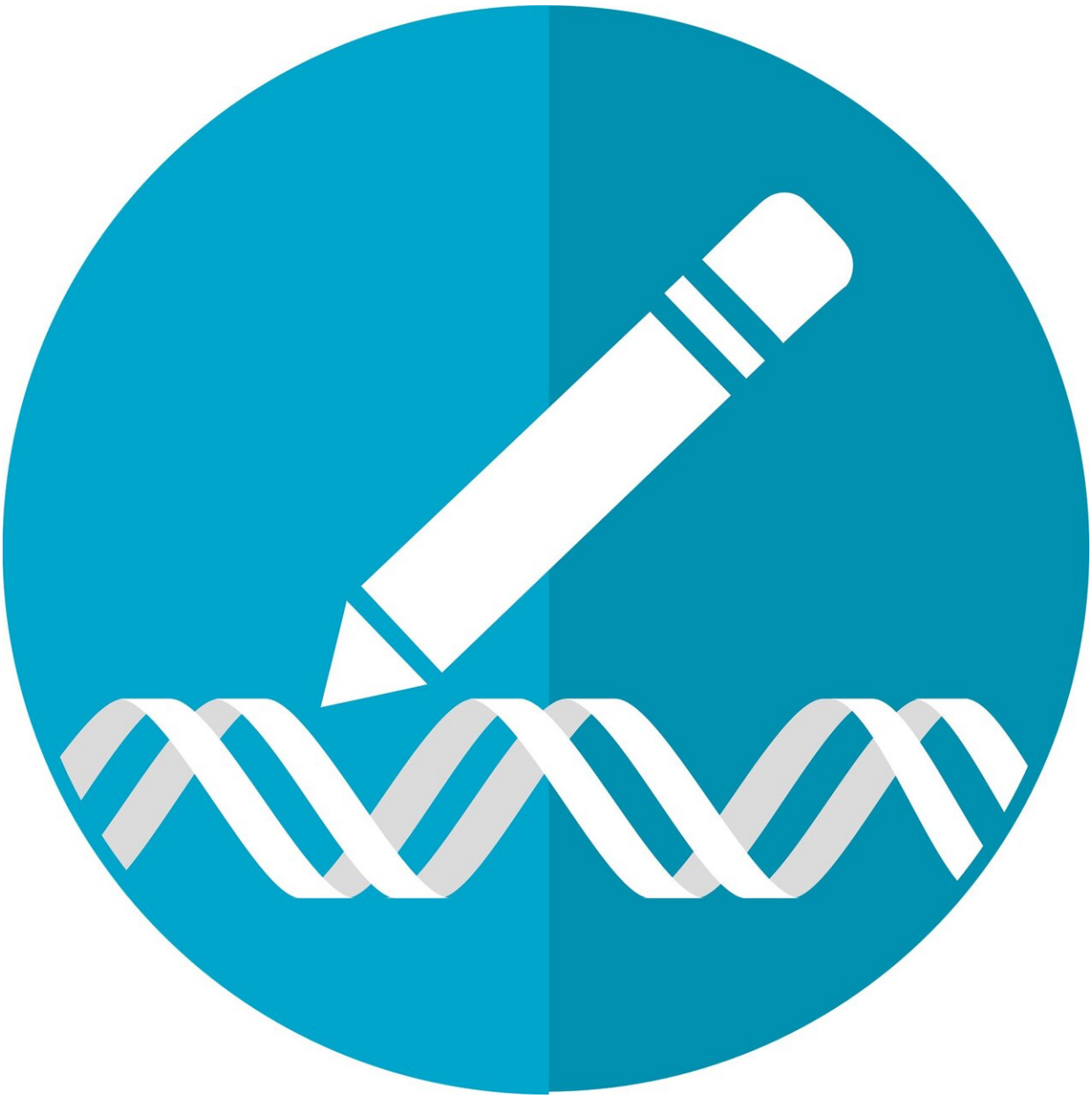
CRISPR-SNP-chip enables amplification-free electronic detection of single point mutations

April 5 2021



Credit: CC0 Public Domain

Keck Graduate Institute (KGI) Assistant Professor and University of California, Berkeley Visiting Scientist Dr. Kiana Aran first introduced the CRISPR-Chip technology in 2019. Now just two years later, she has expanded on its application to develop CRISPR-SNP-Chip, which enables detection of single point mutations without amplification in Sickle Cell Disease and Amyotrophic lateral sclerosis (ALS).

"The field of CRISPR-based diagnostics is rapidly evolving due to CRISPR programmability and ease of use," Aran says. "However, the majority of CRISPR-based diagnostics platforms are still relying on target amplifications or optical detections. The reprogrammability of CRISPR combined with optics-free highly scalable graphene transistors will allow us to bring the diagnostics power of the CRISPR to its full potential.

"The ability to detect single nucleotide polymorphisms (SNPs) is at the core of human health genetics but detection of SNPs is also very important in pharmacology, and agriculture, and is a driving force in evolutionary change such as mutations conferring resistance to antibiotics. Eliminating the need for amplification and optics will make SNP genotyping readily accessible."

Aran led the research team responsible for the work described in the paper "CRISPR-based Transistors for Amplification-free Electronic Detection of Single Point Mutations," to be published in the journal *Nature Biomedical Engineering* on April 5, 2021. It was a collaborative effort between Cardea Bio, KGI, UC Berkeley, UC Irvine, Vilnius

University, and CasZyme.

The SNP-Chip technology is an extension of previously reported CRISPR-ChipTM, a technology that is capable of detecting large insertion and deletions. It earned a spot on the cover of *Nature Biomedical Engineering* in June 2019.

With graphene transistors, the authors now utilized a few versions of CAS enzymes and gRNA designs and monitored various different electrical signals obtained from graphene transistors to construct a new version of CRISPR-ChipTM, which ultimately enabled SNP detection without amplification. The newly developed CRISPR-Chip set, called SNP-Chip, is another major milestone in reshaping nucleic-acid-based detection methods.

"Merging a diversity of CRISPR-Cas biology with electronics via Cardean Transistors opens up a whole new range of possibilities for diagnostic applications, "said Dr. Virginjus Siksnys, founder and chairman of the CasZyme management board, professor at Vilnius University, Lithuania, and co-author on the paper. "Using the Cas9 orthologue for SNP detection is just the tip of the iceberg."

In this article, the utility of SNP-Chip was validated for testing SNP mutation in samples obtained from patients with Sickle Cell Disease and ALS. In both of these clinical models, the platform was able to discriminate healthy from mutated gene within the whole human genome without amplification and by simple swapping of gRNA to target desired DNA sequences indicating the ease of platform reconfiguration for different DNA targets.

SNP-Chip has the potential to greatly impact medical diagnostics and basic research as it can dramatically reduce the time and cost of SNP geotyping, monitor the efficiency of gRNA designs, and facilitate the

quality control process involved in CRISPR-based gene editing.

"SNP-Chip's digital, direct, rapid, and accurate SNP analysis will revolutionize the screening for genetic mutations," said Irina Conboy, Ph.D., Professor of Bioengineering at UC Berkeley and co-author on the paper. "This new technology will inform the discovery of processes underlying disease and aging and will enable faster, more effective clinical translation."

Amplification-free detection of a target gene with single nucleotide mismatch specificity has the potential to streamline genetic research and diagnostics. Furthermore, it would provide more flexibility for biosensing applications previously confined to a laboratory setting.

More information: Discrimination of single-point mutations in unamplified genomic DNA via Cas9 immobilized on a graphene field-effect transistor, *Nature Biomedical Engineering* (2021). DOI: 10.1038/s41551-021-00706-z

Provided by Keck Graduate Institute