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News

How Can DNA Amplification Possible at Body Temperature?

Molecular logic operation and computing are increasingly gaining attention, especially for chemical sensing, intelligent diagnostics, and molecular robotics. Among various molecules, DNA is a prominent material for computing owing to its inherent capability to bind with its complement in a sequence-specific manner according to the Watson–Crick base-pairing rule. There is thus an emerging demand for a cascadable DNA amplification reaction that efficiently senses an input DNA, generates an output DNA in a single-stranded form, and allows the generated DNA to stably hybridize with its pre-designed target in one pot. Such type of DNA amplification reaction should avoid the short amplified sequences and high-temperature conditions that melt away the hybrid formed by the generated DNA and its target.

To approach this amazing possibility Researchers from Tokyo Institute of Technology (Tokyo Tech), and the University of Electro-Communications, Japan, developed a way to get million-fold DNA amplification and targeted hybridization that works at body temperature (37°C/98.6°F). The method, named L-TEAM (Low-TEmperature AMplification), is the result of more than five years of research and offers several advantages over traditional PCR, the dominant technique used to amplify DNA segments of interest. With its easy-to-use, one-pot design, L-TEAM avoids the need for heating and cooling steps and specialized equipment usually associated with PCR. That means it is a well-organized, reasonably priced method that can importantly prevent protein denaturation, in so doing opening a new route to real-time analysis of living cells. In their study published inOrganic & Biomolecular Chemistry, the researchers introduced synthetic molecules called locked nucleic acids (LNAs) into the DNA strands, as these molecules are known to help achieve greater stability during hybridization.

"We were surprised to discover the novel effect of LNA in overcoming the common leak problem in DNA amplification reactions," said Ken Komiya, assistant professor at Tokyo Tech's School of Computing. *"We plan to investigate the mechanisms behind leak amplification in detail and further improve the sensitivity and speed of L-TEAM."* Our low-temperature amplification (L-TEAM) reaction comprises two DNA species used as templates, called the converter and amplifier; one primer DNA as an input; a DNA polymerase with strand displacement ability; and a nicking endonuclease. Upon binding of the input to the converter, the DNA polymerase extends the input and transforms a recognition site of the nicking endonuclease to a double-stranded form. The nicking endonuclease then nicks the extended input and generates a DNA strand of a fixed length as a signal. The signal DNA bound on the converter is displaced by the DNA polymerase when it again extends the nicked strand from the cut point. Though with a advantageous result, the addition of LNA led to an astonishing discovery. The researchers noticed a reduced level of "leak" amplification, a type of non-specific amplification that has long been an issue in DNA amplification studies as it can lead to a slip-up in the diagnosis of disease, that is, a false positive. In the near future, the method could be used to detect short nucleic acids such as microRNA for medical diagnostics. By enabling highly sensitive nucleic acid detection, their method could improve disease diagnostics and accelerate the development of biosensors, for example, for food and environmental applications.In particular, it could facilitate point-of-care testing and early disease detection. MicroRNAs are now increasingly recognized as promising biomarkers for cancer detection and may hold the key to uncovering many other aspects of human health and environmental science.

In addition, Komiya explained that L-TEAM paves the way to practical use of DNA computing and DNA-controlled molecular robotics. *"The original motivation behind this work was the construction of a novel amplified module that is essential to build advanced molecular systems,"* he said. *"Such systems could provide insights into the operational principle behind living things."*

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DNA Amplification, Amplified Sequences, Molecular Techniques, Polymerase Chain Reaction, L-TEAM

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