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Opinion Article

Restriction Enzymes: A Tool for DNA Detection and Manipulation

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DESCRIPTION

Restriction enzymes are a type of enzyme that can recognize and cut specific sequences of DNA. They are widely used in molecular biology for manipulating DNA fragments, such as cloning, mapping, and sequencing. However, restriction enzymes can also be used as a target for DNA-based sensing and structural rearrangement, which is a novel application of DNA nanostructures. DNA nanostructures are artificial assemblies of DNA molecules that can form various shapes and patterns based on the Watson-Crick base pairing rules. DNA nanostructures can be designed to perform different functions at the nanoscale, such as sensing, computing, and drug delivery. One of the advantages of DNA nanostructures is their modularity and programmability, which allow them to interact with various types of inputs and outputs.

One way to use restriction enzymes as a target for DNA-based sensing and structural rearrangement is to integrate them with a DNA structure that can undergo conformational changes or cleavage upon enzyme recognition. For example, reconfigured a three-arm DNA switch that can detect three different restriction enzymes and produce complex output signals based on cyclic Förster Resonance Energy Transfer (FRET) between three dyes. The three-arm DNA switch consists of three double-stranded DNA arms that are connected by single-stranded loops. Each arm contains a recognition site for a specific restriction enzyme (BamHI, EcoRI, or HindIII) and a dye (Cy3, Cy5, or Cy7) at the end. The three dyes form a FRET cycle that can be monitored by fluorescence spectroscopy.

The three-arm DNA switch can respond to the presence or absence of the three restriction enzymes in different ways. If no enzyme is present, the switch remains intact and the FRET cycle is active. If one enzyme is present, it cleaves one arm of the switch and disrupts the FRET cycle. If two enzymes are present, they cleave two arms of the switch and create a new FRET pair between the remaining arm and one of the cleaved arms. If all three enzymes are present, they cleave all three arms of the switch and abolish the FRET signal completely. Therefore, by measuring the fluorescence intensity and ratio of the three dyes, one can determine which enzymes are present in the sample.

Another way to use restriction enzymes as a target for DNA-based sensing and structural rearrangement is to harness their cleavage activity to expose an active toehold into the DNA structure, which can then initiate a strand displacement reaction with another DNA strand in solution. For example, modified one arm of the three-arm DNA switch by adding a short sequence at the end that can hybridize with a complementary strand labeled with Alexa Fluor 488 dye. This strand acts as an output signal that can be released from the switch upon enzyme cleavage.

The modified three-arm DNA switch can respond to the presence or absence of BamHI enzyme in different ways. If no enzyme is present, the switch remains intact and the output strand is bound to the switch. If BamHI enzyme is present, it cleaves the modified arm of the switch and exposes an active toehold that can be displaced by another strand in solution that has a longer complementary sequence to the modified arm. This strand acts as an input signal that can displace the output strand from the switch and release it into solution. Therefore, by measuring the fluorescence intensity of Alexa Fluor 488 dye, one can determine whether BamHI enzyme is present in the sample.

In summary, restriction enzymes can be used as a target for DNA-based sensing and structural rearrangement by integrating them with a DNA structure that can change its conformation or cleavage state upon enzyme recognition. This approach can enable complex logic operations and signal amplification based on DNA nanostructures.

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