OPEN ACCESS Freely available online

Biochemistry & Analytical Biochemistry

ISSN: 2161-1009

Editorial

A Brief Note on Cycling Probe Technology

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DESCRIPTION

Cycling Probe Technology (CPT) is a molecular biological method that may be used to detect certain DNA sequences. CPT operates in an isothermal environment. In some cases, CPT is a viable alternative to PCR. Unlike PCR, however, CPT does not make numerous copies of the target DNA, and the signal amplification is linear, as opposed to PCR's exponential amplification of the target DNA. CPT employs a sequence-specific chimeric probe that binds to a complementary target DNA sequence and acts as an RNase H substrate. Cleavage happens at the RNA internucleotide connections, causing the probe to separate from the target and make space for the next probe molecule. For application in CPT, integrated electrokinetic systems have been created.

Probe

Cycling probe method detects the presence of a specific DNA sequence using a chimeric nucleic acid probe. An RNA fragment is placed between two DNA segments in the chimeric probe. Four purine nucleotides are contiguous in the RNA segment. The probes should have a length of fewer than 30 nucleotides and be designed to reduce intra-probe and inter-probe interactions.

Process

Cycling probe technology is based on a cyclic, isothermal process that starts with the chimeric probe's hybridization with the target DNA. The probe becomes a suitable substrate for RNase H once it has been hybridised, and RNase H, an endonuclease, cleaves the RNA part of the probe, yielding two chimeric fragments. The freshly cleaved fragments' melting temperature (Tm) is lower than the original probe's melting temperature. The cleaved fragments detach from the target DNA because the CPT reaction is kept isothermally slightly above the melting temperature of the original probe. The target DNA is free to hybridise with a fresh probe once it has been detached, restarting the cycle. The pieces become observable after they have been split and separated. Fluorescence is a typical approach for detecting fragments. A fluorescent marker is attached to the probe's 5' end and a quencher is attached to the probe's 3' end in this procedure. The quencher and fluorescent marker separate when RNase H cleaves the probe, increasing the fluorescent marker's intensity. Alternatively, cleaved fragments can be identified through amplification (e.g., PCR) or additional modification to allow for various chemical detection methods.

The CPT process can be tweaked to maximize specificity and efficiency when working with low amounts of target DNA. Probe cleavage efficiency has been proven to improve as the time allotted is increased. The use of a probe that isn't prone to inter-probe and intra-probe interactions, as well as raising RNase H concentrations, has been shown to boost specificity.

Advantages

CPT has a lesser risk of cross contamination than PCR since cycling probe method does not include amplification of target DNA. CPT is also faster than PCR and does not necessitate the use of a specialized thermocycler. CPT does not necessitate passing CPT goods through a gel.

Disadvantages

CPT assays are more expensive than PCR because they require specific chimeric probes. Because CPT probes are so specialized, each unique experiment requires a new probe, which adds to the expense. Clinical implementation is restricted not just by cost, but also by the possibility of nonspecific RNases other than RNase H in samples.

Applications

CPT can be used to identify certain DNA sequences and, as a result, genotypes. CPT, for example, can be used to differentiate GMO from non-GMO produce. CPT can be used instead of cell culture in clinical settings to discover antibiotic resistance in pathogens.

CPT's fundamental function is to determine whether or not a specified sequence is present in a sample. However, because cleaved probes accumulate at a linear rate, the amount of target DNA can be calculated. As a result, CPT has been used to

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Received: December 08, 2021; **Accepted**: December 22, 2021; **Published**: December 29, 2021

Citation: Saki T (2021) A Brief Note on Cycling Probe Technology. Biochem Anal Biochem. 10:181

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estimate the amount of non-coding repetitions found in organisms. Other technologies, like as molecular beacons and qPCR, can be utilised in conjunction with CPT.