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Nucleic acid sequence-based amplification

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Nucleic acid sequence-based amplification (NASBA) is a primer-dependent technology that can be used for the continuous amplification of nucleic acids in a single mixture at one temperature.

Introduction

Nucleic acid amplification has rapidly become one of the most important tools for molecular biologists. Technology based on the polymerase chain reaction (PCR) uses repeated thermal denaturation of double-stranded DNA and extension by a thermostable DNA polymerase of two primers, each annealed to the complementary template, to achieve DNA amplification of greater than one million-fold in about three to four hours. This method requires rapid changes in sample temperature, for which specialized thermal cycling instruments are usually required.

Nucleic acid sequence-based amplification offers a simple and rapid alternative method for nucleic acid amplification, which can yield an RNA amplification of a billion-fold in about two hours. In addition, as NASBA is a continuous, isothermal process, specialized equipment is not required.

NASBA amplification process

A standard NASBA reaction mixture contains T7 RNA polymerase, RNase H, AMV (avian myeloblastosis virus) reverse transcriptase, nucleoside triphosphates, two specific primers and appropriate buffer components. Primer 1 is about 45 bases in length, with an average of 20 bases at the 3' end that are complementary to the 3' side of the target sequence. The 5' end of this primer contains a promoter sequence that is recognized by T7 RNA polymerase. Primer 2 is about 20 bases in length and is derived from the opposite (5' direction) side of the target sequence.

One of the properties of the NASBA system is the ability to amplify directly specific sequences of single-stranded RNA. When using a natural RNA template, amplification is achieved by adding the template to the standard NASBA reaction. In the so-called 'non-cyclic' phase of NASBA, primer 1 anneals to the RNA target sequence. AMV reverse transcriptase extends the 3' end of primer 1, forming a cDNA copy of the template and resulting in an RNA:DNA hybrid. RNase H hydrolyses RNA from such RNA:DNA hybrids. Consequently, the original RNA is destroyed, leaving a single strand of DNA to which primer 2 anneals. Reverse transcriptase synthesizes the second DNA strand, rendering the promoter region double-stranded. The third enzyme, T7 RNA polymerase, transcribes RNA copies from the now transcriptionally-active promoter, generating as many as 100 copies from each template molecule. Each new RNA molecule is now available as template for reverse transcriptase in the so-called 'cyclic' phase of the NASBA process. In the cyclic phase, primer 2 binds to the template first and reverse transcriptase extends it, generating an RNA:DNA hybrid; RNase H hydrolyses the RNA strand, primer 1 binds to the resulting single-stranded DNA, and reverse transcriptase synthesizes DNA, again yielding a transcriptionally-active promoter. All these steps occur simultaneously, enabling amplification in a continuous, homogeneous and isothermal fashion.

Features of the technology

In practice, NASBA is surprisingly robust. Prepared template samples are added to NASBA reaction mixtures that contain kit components and appropriate primers. Incubation at a single temperature for 1.5 to 2 hours achieves an amplification of about 10^9-fold. In a model system, template input of 10^4 molecules results in synthesis of about 2 × 10^13 full-length RNA molecules (1.3 g of RNA in two hours).

The NASBA process requires fewer 'cycles' than PCR: with NASBA, 10 - 100 copies of RNA are generated in each transcription step, so only four to five 'cycles' are required to achieve a million-fold amplification. Consequently, both total incubation time and overall error frequency are reduced. The fidelity of NASBA has been tested by sequencing the RNA product directly; 90 per cent is readable.

The specificity of NASBA has been confirmed in model systems. Using 10^4 molecules of template, the presence of 1 g of human placental DNA does not significantly change the product yield. 'No template' control reactions remain unchanged by addition of excess heterologous DNA.

The sensitivity of NASBA: samples with as few as ten molecules of input produce positive results. Samples of HIV-infected cells or infectious HIV virus particles diluted in human plasma have been detected with the same sensitivity as model systems. False positives are not seen.

NASBA can also be used for double-stranded nucleic acids as templates, which must first be converted to single-stranded molecules compatible with the process.

Applications

As a research tool, NASBA simplifies both detection and sequencing of mRNA sequences. With the absence of thermal denaturation, contaminating genomic DNA is not amplified. As NASBA does not require specialized equipment, it is suitable for researchers with limited requirements or budgets.

NASBA also has clinical applications: screening of infectious diseases and genetic disorders, diagnosis and monitoring of cancer, and testing for disease susceptibility. As few as three HIV particles could be detected in a 100-l sample of reconstituted human plasma. NASBA can also be applied to food screening, veterinary diagnostics, aquaculture and, to some degree, forensic science.

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