

Get in the “groove” with new molecular technology

By George Corpus, CLSp(MB)

Polymerase chain reaction (PCR) is a process that produces many copies of the nucleic-acid sequence of interest for the purpose of detection and/or quantification (for example, a genetic mutation associated with a disease such as cystic fibrosis, or a foreign agent such as a virus or a bacteria). The process involves cycling at least two different temperatures several times to make millions of copies of the nucleic-acid sequence of interest. One cycle includes a temperature for separating double-stranded nucleic acid into single strands, and a second temperature for annealing of sequence-specific primers and for extension of the copy using the primers as a starting point.

The enzyme, called a DNA polymerase, makes the copy strand. Normally, after the PCR process, gel electrophoresis is used to detect the PCR products. The addition of fluorescent probes to the PCR process, however, allows for real-time detection, thus eliminating the need for gel electrophoresis.

Real-time PCR has rapidly become a standard method for clinical molecular biology laboratories because of its ability to provide sensitive and highly specific results. Sensitivity, automation, and turnaround time have made real-time PCR particularly useful for pathogen detection, especially for organisms difficult to grow in culture.

Strain variability of genetic sequences from pathogens, however, complicates probe and primer design, and interpretation of results. While designers typically target regions that are conserved across various strains, these regions can often be too short or problematic for traditional real-time PCR chemistries. Additionally, variants with unknown polymorphisms in these conserved regions may escape detection. The minor groove binder (MGB) probe technology overcomes some of these obstacles.

The MGB probe technology utilizes a minor groove binder attached to single-stranded DNA probes to boost the stability and raise the melting temperature (T_m) of the DNA duplex formed when probes bind to complementary targets. This

enables the use of shorter probes capable of detecting short conserved regions when developing assays to detect multiple strains. 5'-MGB probe allows post-amplification melt-curve analysis confirming real-time amplification results.

MGB — the minor groove binder

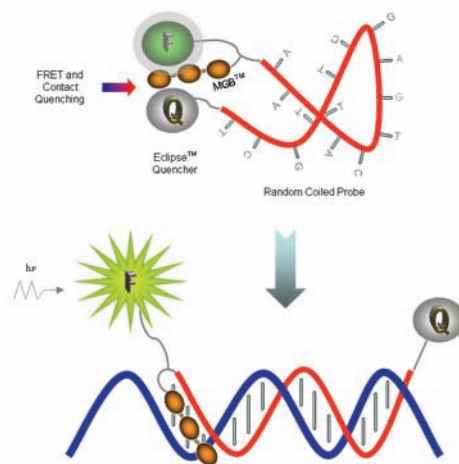
Minor groove binders are a potent class of naturally occurring antibiotics that bind to duplex DNA specifically in the minor groove. Minor groove binders are long, flat molecules composed of several similar subunits that are held together by peptide bonds that can adopt a “crescent” shape. This shape allows the MGB moiety to fit snugly into the minor groove — the deep narrow space between the two phosphate-sugar backbones in the double helix.

The MGB moiety is stabilized in the minor groove by hydrophobic interactions. By attaching the MGB moiety to the 5'-end, which is shown in the graphic here, of a DNA probe during synthesis on a commercial synthesizer or post-synthetically to an amine-modified oligo, the MGB moiety folds back into the minor groove and stabilizes the DNA probe-target duplex. The effect of this stabilization is an increase in melting temperature,

allowing the use of shorter probes, which can improve mismatch discrimination.

Prior to the introduction of MGB technology, researchers typically needed to increase the size of the probe in order to produce melting temperatures consistent with efficient PCR. Longer probes reduce design flexibility when restricted by small target regions and are less sensitive to mismatch discrimination.

Another key differentiating feature of the 5'-MGB real-time PCR technology used by Florida Hospital's Molecular Diagnostics Laboratory is the post-PCR melt-curve capability. With this cleaved probe, users do not have the added confidence of the melt-curve confirmation. The 5'-labeled MGB probes are resistant to the polymerase exonuclease activity. The probes remain intact after amplification and that allows the possibility of doing confirmatory melt curves and detecting unknown mutations.

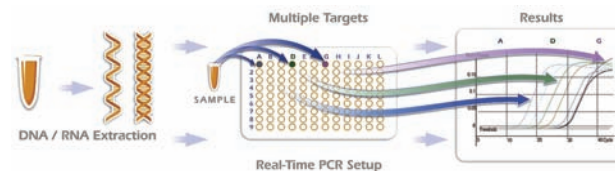


Minor groove binders are a potent class of naturally occurring antibiotics that bind to duplex DNA specifically in the minor groove.

MOLECULAR TECHNOLOGY

Universal cycling

In the Florida Hospital's Molecular Diagnostics Laboratory, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and BK virus (BK) tests were developed using the real-time PCR reagents from the company that provides the lab's MGB probe technology. With the MGB real-time PCR technology, the laboratory is able to perform all three tests (BK, CMV, and EBV), if needed, from one sample extraction since it has optimized the three tests to use the same internal control templates. All that is needed is to extract one specimen to place in all three PCR master mixes. This certainly saves a lot of time.



Another key benefit of the MGB real-time PCR technology is that all reagents run under the same cycling condition — universal cycling condition. This reduces the chance of errors in the lab because one cycling condition is used for every test.

When bringing in new tests, the set up is simple. The optimal PCR cycling condition is already known; all that needs to be done is to validate the specimen types and extraction protocol, and analyze the results for the new test.

The MGB real-time PCR technology also allows multiple tests to be run on the same thermal cycler. This enables labs to run low-volume tests without waiting for enough samples for a batch, which greatly improves lab efficiency and turnaround time.

Summary

The advantages of MGB real-time PCR technology are:

- use of short, or highly conserved, specific sequence;
- specific primers and non-cleavable probes;
- post-PCR melt-curve analysis;
- universal cycling conditions;
- open platform; compatible with most real-time PCR instruments;
- unsurpassed sensitivity;
- multiplexing with a rich proprietary dye set; and
- the ability to tailor designs to detect most mutations. □

George Corpus, CLSp(MB), is the manager of the Molecular Diagnostics Laboratory at Florida Hospital in Orlando, FL, which uses TaqMan Roche Molecular Systems and MGB Alert from Wesco's Epoch Biosciences.
