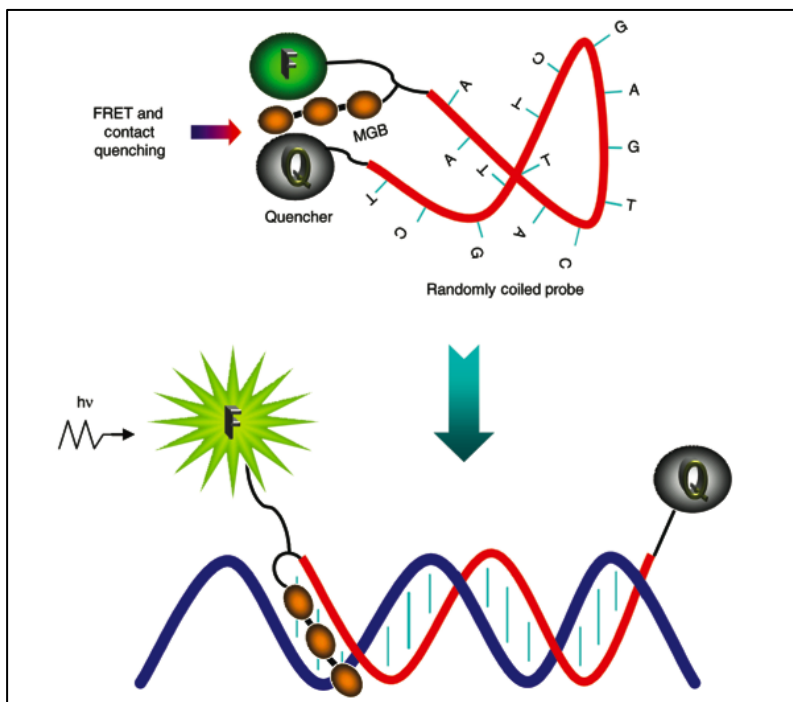


MGB Alert® Real-Time PCR – The Technology Behind the MGB Alert® Probes

MGB Alert® real-time PCR products offer performance advantages through unique probe technologies. In the following sections, we will discuss these key technologies known as the Minor Groove Binder (MGB) and Super Bases as well as melt curve analysis.

Minor Groove Binder (MGB)

MGB is a crescent shaped molecule that is bound to the probe oligonucleotide. Refer to Picture 1 for an illustration.



Picture 1: MGB Alert® probe (with Pleiades design) before and after target hybridization.

The MGB molecule serves two functions in a Pleiades probe design.

1. When the probe is not hybridized to a target, the MGB molecule acts as an additional quencher to the attached fluorophore enhancing the signal-to-background ratio for test results.
2. When the probe is hybridized to a target, the MGB molecule fits into the minor groove of the double helix structure enhancing the bond between the probe and the target.



The primary advantage of a probe with an attached MGB molecule is a significantly increased melting temperature, T_m . This advantage can minimize or eliminate the need to design probes that avoid A-T rich target regions or that require a longer length of bases to compensate for low T_m 's. Higher T_m 's and shorter probe lengths typically provide greater target specificity.

Super Bases

Nanogen's proprietary modified bases, known as Super Bases, compensate for common issues related to probe design.

Super ATM and Super TTM-

Problem: a probe designed for an A-T rich target region must have a longer length to overcome a low T_m . A and T bases form only two hydrogen bonds when hybridized to their complementary base. This bonding capacity is low compared to the three hydrogen bonds for G and C bases and can be easily broken at relatively low temperatures.

Solution: a Super A or Super T base can form three hydrogen bonds with its complementary base. Replacing one or more normal A or T bases with one or more Super A or Super T bases in a probe sequence increases the T_m and allows for improved hybridization in spite of a shorter length probe.

Super GTM -

Problem: a probe designed for a G-C Rich target region can form secondary structures from G-G self association. Secondary structures can prevent sufficient hybridization.

Solution: a Super G base can form only two hydrogen bonds when hybridized to a complementary base. This lower bonding capacity allows secondary structures from G-G self association to be easily broken at relatively low temperatures.

Super NTM -

Problem: a target region may have one or more bases that are highly variable or unknown making it difficult to design a probe that will hybridize.

Solution: a degenerative Super N base can be incorporated into the probe to hybridize to these unknown or variable bases.

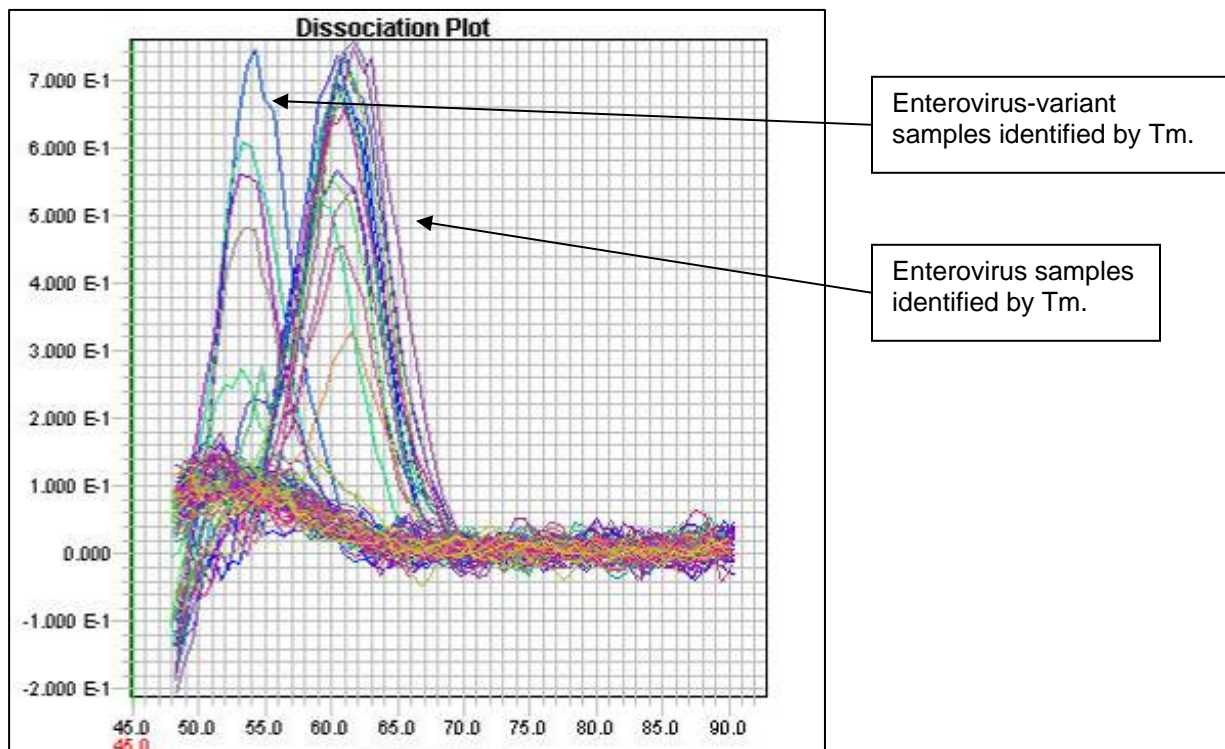
Through the selective use of Super Bases in MGB Alert probes, our products can attain higher T_m 's and shorter probe lengths to improve greater target specificity.

Melt (Dissociation) Curve Analysis

Unlike TaqMan probes, the fluorophore is not cleaved in the extension step of PCR. With an intact probe, a melt curve stage can be performed after the completion of PCR. In this stage, the real-time PCR instrument will melt all double-stranded amplicon at 95°C followed by a quick drop in temperature (which should be sufficiently below the T_m of the probe). The temperature gradually increases while the optics capture and the software interprets the change in light emitted from probe-target hybrids over this range. A plot displays the change in fluorescence (as a negative derivative) at each temperature in the range.

Key benefits of melt (dissociation) curve analysis includes:

- A specific melting temperature, T_m , for the probe-target hybrid is indicated by a peak. A unique T_m is observed when the probe hybridizes to a perfectly matching target region. By comparison, a significantly lower T_m is observed when the probe hybridizes to a target with a single-base mismatch. This allows for identification of variants of the target by T_m within the same test run. Refer to Picture 2 for an example.



Picture 2: Identification of Enterovirus and variant by T_m in melt curve analysis (courtesy of ARUP)

- Negative reactions and those that generate non-specific amplicon in PCR do not generate T_m results in melt curve analysis. This provides further confirmation of a sample's negative status within the same test run.



- Low level positive reactions or those positives that show amplification in later cycles can generate T_m results in melt curve analysis. This provides further confirmation of a sample's positive status within the same test run.

MGB Alert probes can be used in melt curve analysis to provide additional confidence in the identification of a positive sample, a positive sample that is a variant of the target, a negative sample, a negative sample that can generate non-specific amplicon in PCR, and a positive sample with a high C_t value or low concentrations of the target.