AllGlo miniProbe: Highly Specific Homo-Labeled Probe Technology for Real-Time qPCR

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Summary

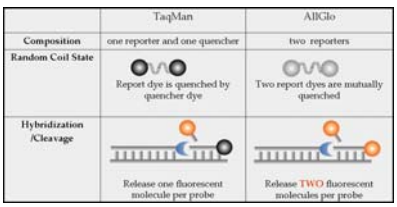
The miniProbes are AllGlo probes that are significantly shorter than the conventional dual labeled probes. The nucleic acid portion has a calculated Tm of 55-60^{0}C, and the length can be as short as 15-16 nucleotides.

Although the calculated Tm is lower than the annealing/extension temperature used in the universal protocol, the miniProbe performs better than the regular AllGlo probe that has a calculated Tm of 65-70^{0}C in TaqMan assays.

The shortened oligo length in miniProbes makes quenching more efficient and boast low fluorescence baselines. The ΔRn is at least equal or better than TaqMan BHQ probes or TaqMan MGB probes. We discovered that a miniProbe offers higher specificity than TaqMan probe or TaqMan MGB probe in detecting single point mutations. Unlike TaqMan MGB probes, we have not found a single case that a miniProbe inhibits PCR reactions.

Introduction

AllGlo probes are novel homo-labeled fluorogenic probes. Unlike conventional dual labeled probes such as TaqMan probes, an AllGlo probe is comprised of two identical reporter dyes that are capable of quenching each other and become de-quenched once the probe is cleaved. AllGlo probes do not employ a quencher and have two signal-generating reporter dyes per oligo.

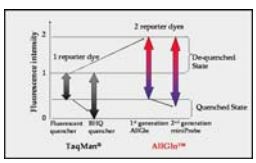


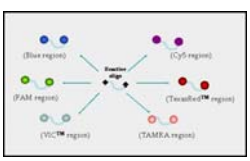
Advantages in Probe Design

- For every AllGlo probe, two reporter molecules can be released to generate the fluorescence signal, fully maximizes the ΔRn, make it one of the most sensitive probes.
- To make an AllGlo probe, it only requires one step homogenous reaction. By reacting a common intermediate with different dye species, it is easy to make probes of multiple colors.

Sensitive

Probe Sensitivity = end-point fluorescence – background fluorescence





Simple

One common intermediate makes probes of different colors

miniProbe: 2nd generation of AllGlo probe with shorter sequence and higher specificity

- Short AllGlo probe (called mini probe) has Tm = 55-60 ^{o}C, or a length as short as 15-16 nucleotide long (using universal protocol, cycling between 95 ^{o}C and 60 ^{o}C).
- Baseline fluorescence becomes very low and Δ Rn is at least equal or better than TaqMan BHQ probe or TaqMan MGB probe.
- Higher specificity than TaqMan Probes:
  - -Specificity can be as high as to detect a single point mutation.
  - -Offer tunable stringency of specificity up to one mismatch mutation.
  - -Capable of detecting degenerate sequences with equal efficiency.
- Unlike TaqMan MGB probes, AllGlo probes DO NOT inhibit PCR.
- Synthesis yield increased.

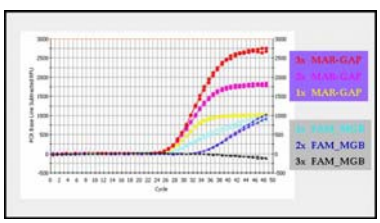
miniProbe vs TaqMan

Material and Methods:

- huGAPDH miniProbe that has Tm= 58^{o}C was labeled with MAR AllGlo dye: 5'-(MAR) CATGAC CACAGTCCATGCC (MAR)- 3'.
- huGAPDH Taqman FAM-MGB probe (Applied Biosystems Cat 4333764) is supposed to have Tm close to 70^{o}C, its sequence is unknown and might probe a different region of GAPDH cDNA.
- Real time PCR reaction was carried out in 20ul of volume using male heart cDNA (BD Clontech) as the template by the universal cycling condition (95^{o}C-15s , 60^{o}C-60s)
- A total of three concentrations of probe/primer mix were used. 1x, 2x, and 3x are based on the manufacturer's recommended concentration. 1x Taqman MGB probe/primer mix contains 250nM probe and 900nM for each unlabeled primer. 1x miniProbe/primer mix contains 500nM probe and 500nM of each unlabeled primer.
- Both MAR miniProbe (Spectra Ex 500nm/ Em525nm) and FAM-MGB probe are detected in the FAM channel of BioRad iCycler IQ.

Results:

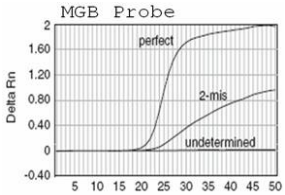
- huGAPDH miniProbe has increased signals up to 3x concentration without any inhibition.
- huGAPDH Taqman FAM-MGB probe has inhibition even at 1x concentration, 3x yielded no signal at all.



Detecting single point mutation

Background information:

- According to Yao, et al., Mol. Cell Probes 20(5), 311-6(2006), MGB probes can at best discern two mismatches as shown on the bottom.



- Four BETAM miniProbes were made with identical sequences except one unique nucleotide at the center.
- Four different plasmid templates of BETAM gene were made, correlating to the sequences of the four miniProbes.
- A total of 16 real time PCR reactions with each having a unique probe/template combination were carried out in 20ul of volume using the universal cycling condition (95^{o}C-15s , 60^{o}C-60s).

```
A Probe:     GTAAGCAGAATCATGGAGG
T Probe:     GTAAGCAGTATCATGGAGG
C Probe:     GTAAGCAGCATCATGGAGG
G Probe:     GTAAGCAGGATCATGGAGG
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A template:  --------A----------
T template:  --------T----------
C template:  --------C----------
G template:  --------G----------
```
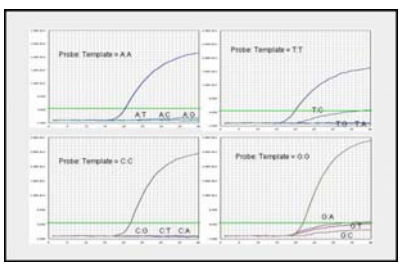

Results:

- All perfect matches generated good signals.
- Imperfect matches, even for a single mismatch generated no or weak signals.



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