Evaluation of Taqman® DNA Probes: Can High Quality Syntheses be used in Quantitative Real-Time PCR Assays without Gel or HPLC Purification?

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Abstract

Real-Time or quantitative (q)PCR technology is of increasing importance in genomic research. The high cost of (q)PCR DNA probes for experiments has long impeded the full utilization of qPCR. The commercial cost of dual-labeled probes for qPCR reactions is high because of the post-synthesis HPLC and/or gel purification steps required by limitations on the traditional synthetic chemistry. The recent availability of CPG quencher reagents to core DNA synthesis facilities has opened up the possibility that probes, when carefully prepared, may be used without extensive post-synthesis purification. This would substantially reduce the cost, making the synthesis of qPCR probes feasible and more affordable for any DNA synthesis laboratory. The NARG tested the hypothesis that all DNA synthesis labs are able to make quality dual-labeled probes suitable for qPCR reactions without gel and/or HPLC purification, by inviting members of the DNA synthesis community to synthesize 3-FAM, 5-FAM, 3-BHQII or -TAMRA quencher labeled 25-mers probes and submit them for quality analysis. The NARG members performed quality analyses on the probes using CE, DHPLC, and PAGE. Effectiveness in Real-Time PCR experiments was determined over a five log range of standard template concentration to assess the effect on assay efficiency and sensitivity compared to highly purified probes.

Quality Control of FRET Probes

Real-Time PCR of 3-FAM, 3-BHQII, or 3-TAMRA quencher labeled (30-mer) probes and submit them for quality analysis.
- The probes were analyzed for quality by three analytical methods: PAGE, CE, and DHPLC.
- The probes were assayed for functionality by a qPCR test that utilized a human β-Actin assay (below) and measured performance against a synthetic template to generate standard curves covering a 5-log range, either 2x10^3 to 2x10^8 or 2x10^3 to 2x10^8 molecules. 50 ng of genomic DNA was used as an unknown sample. All assays were run in duplicate on an ABI 7900 using a BioRad robot and a Tecan robot to setup the assays.
- PAGE analyses: DNA stained
- DHPLC (WAVE) Profiles

Conclusions

- Both FAM/BHQII and FAM/TAMRA quenched FRET probes are easy and inexpensive to make. 100% of the respondents said that they found the syntheses easy.
- qPCR is a very robust technique. Even a probe containing only 17.9% full-length probes (r1) allowed detection down to 200 copies when used with an optimized primer pair on a degenerate template. (Table 1, Figure 1).
- Pure probes gave larger delta Rs resulting in a larger dynamic range. (Table 1, Figure 3).

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Announcement

NAGC Research Group Presentation

Tuesday, January 01, 4:30-5:00 pm
Plaza Ballroom D