Universal ProbeLibrary – Evaluation on Different qPCR Instruments

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Abstract

The **Universal ProbeLibrary** (UPL) is a fast, specific and flexible format for quantitative real-time PCR experiments. Just 90 UPL probes provide transcriptome-wide coverage in a number of organisms (human, mouse, rat, arabidopsis, drosophila, primates and C. *elegans*). The probes are short but use the LNA technology to reach suitable melting temperatures (T_m); they can therefore bind effectively to several locations along the DNA. Consequently, the specificity of each assay depends on the interplay of the selected primers and the Universal Probe. The specific primers for each assay are selected with the help of ProbeFinder, a convenient, free web-based assay design software. ProbeFinder can meet a variety of design needs, including designs that detect transcript variants and gene family members.

However, important qPCR parameters like the T_m are dependent on the reagents used in the reactions and results may vary in sensitivity and specificity, depending on the choice of instrument. Here we demonstrate the performance of UPL on different instruments. In addition, we also evaluate dual color hydrolysis probe assays that use a new dye.

For all reactions, we used the **FastStart Universal Probe Master (Rox)**, a new reagent mix for qPCR.

Introduction

There are numerous qPCR instruments on the market. Those instruments can be divided into the following main categories:

- instruments that use the reference dye ROX for quantification
- instruments that have different light sources (laser, halogen, LED)
- instruments that use different types of reaction vessels (plates, capillaries)

Since we have already demonstrated the performance of UPL in the capillary format (LightCycler® 2.0 Instrument), we focused on the plate format and selected the following instruments:

- **A**: AB 7900HT Real-time PCR System (Rox, laser)
- **B**: AB 7500 Real-time PCR System (Rox, halogen)
- **C**: Bio-Rad iQ5 Real-time PCR Detection System (halogen)
- **D**: Stratagene Mx 3000P® QPCR System (Rox, halogen).

For the evaluation we decided to use the same master on all instruments in order to avoid inexplicable results caused by the chemicals included in each reaction mix.

For these tests, we selected the **FastStart Universal Probe Master (Rox)**. This master contains a Rox derivative which can be used in all halogen and laser-equipped instruments regardless of the filters they use. Further, the concentration of the Rox derivative does not need to be adjusted for different instruments.

This master reagent mix showed excellent overall performance on different targets that had:

- extreme GC content
- different lengths

and independent of the format used, e.g.:

- hydrolysis probe (Taqman® format) assays (fig.1 and 2)
- genotyping (hydrolysis probe format) (fig. 3)

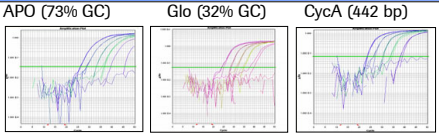

Fig.1: Amplification of different target DNAs using FastStart Universal Probe Master (Rox). APO; Glo; Cyc A; dilutions from 50 – 0.005ng human gen. DNA/well, Instrument A).

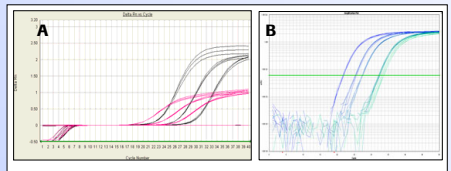

Fig. 2: Applications using FastStart Universal Probe Master (Rox): A) Duplex reaction; globin (FAM-BHQ, black lines) and actin (Vic-TAMRA, AoD ABI, primer limited, red lines), Instrument B.
B) Detection of miRNA let 7a, Instrument A.

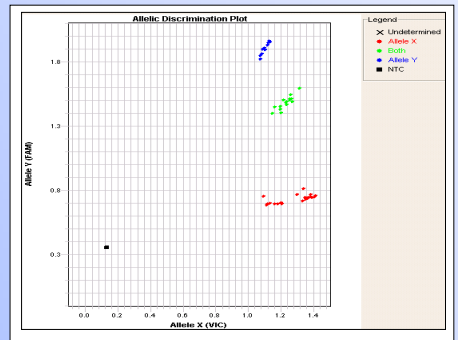

Fig. 3: SNP-Genotyping using FastStart Universal Probe Master (Rox). Gene expression assay of Applied Biosystems, ADAM 11; 10 µl, 384-block, 10ng/well; Instrument A.

Mono Color UPL Assays

For the evaluation of UPL-based assays on different instruments we always used the same concentration of primers and probes (200nM primers and 100nM probes). The following targets were amplified:

- GAPDH (see fig.4)
- HPRT (see fig.5)
- amylase (see fig.6).

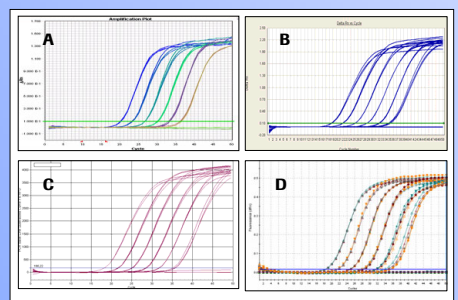

Fig. 4: Quantification of GAPDH using UPL probe no. 60 on different instruments. A: Instrument A; B: Instrument B; C: Instrument C; D: Instrument D.

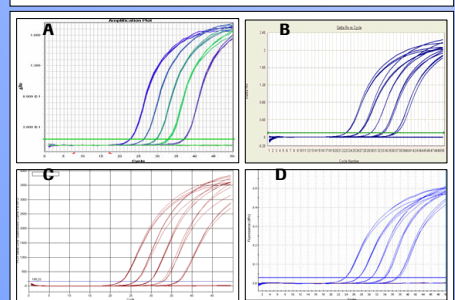

Fig. 5: Quantification of HPRT using UPL probe no. 73 on different instruments. A: Instrument A; B: Instrument B; C: Instrument C; D: Instrument D.

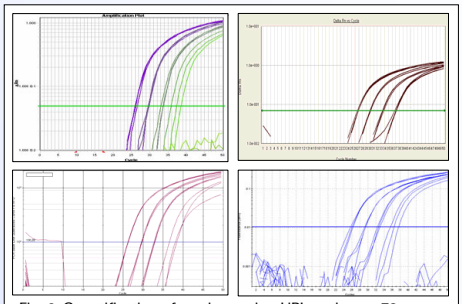

Fig. 6: Quantification of amylase using UPL probe no. 72 on different instruments. A: Instrument A; B: Instrument B; C: Instrument C; D: Instrument D.

Result: All assays worked equally well on the instruments evaluated.

Dual Color UPL Assay

Dual color assays are of general interest since they can generate more specific information (relative quantification; comparison of two parameters) while saving time and costs.

We analyzed the performance of 2 assays in two ways:

- Can both assays be performed/detected in the same reaction?
- Do single and dual color assays have different sensitivities?

The targets chosen for the assays were GAPDH (FAM-labeled) and PGK1 (Rho-labeled) (fig.7 and 8).

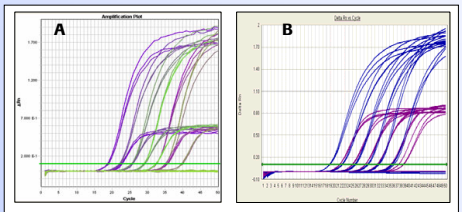

Fig. 7: Dual color UPL assay for GAPDH and PGK1 on A: Instrument A and B: Instrument B.

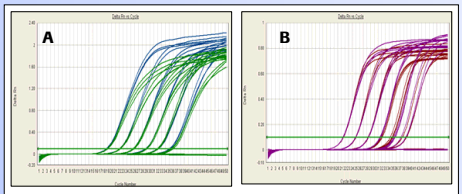

Fig. 8: Comparison of dual color UPL-assay with single color assay. GAPDH (A); PGK1 (B) on Instrument B.

Result: No significant interference (C_t-shift, cross-talk, ampitude) could be detected.

Conclusions

If the **FastStart Universal Probe Master (Rox)** is used in UPL assays, these assays work equally well on several qPCR platforms. Rox, different ramp rates, and different light sources do not significantly influence these assays. UPL worked well even in dual color assays.

Therefore, UPL assays have proved to be a more specific and robust alternative to SYBR Green assays and an inexpensive alternative to pre-designed assays.

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