

Best of Both Worlds- New Dyes for qPCR used in combination with probes

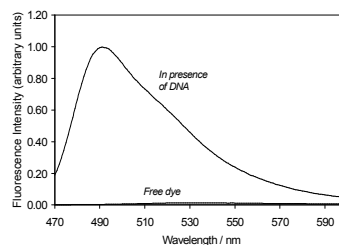
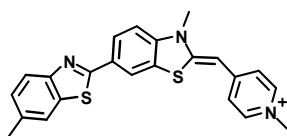
Neven Zoric

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Improving probe technology

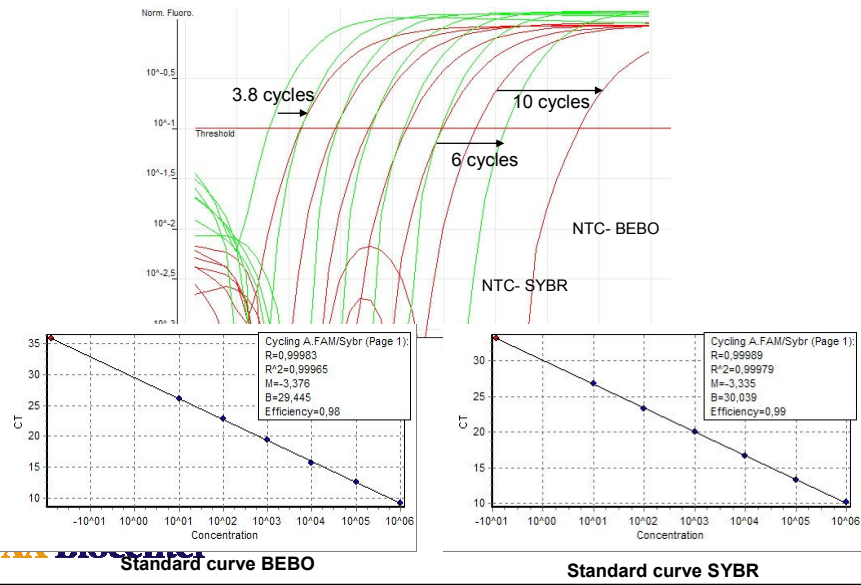
- BEBO/BOXTO – a novel series of asymmetric cyanine dyes that bind to the minor groove of DNA
- 250-fold increase in fluorescence upon binding dsDNA



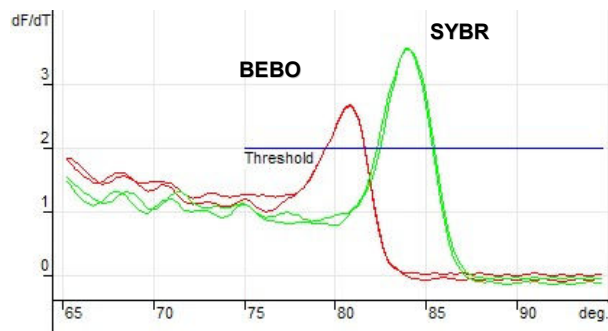
**BEBO-excitation max 468nm, emission max 492nm
BOXTO-excitation max 515nm, emission max 552nm**

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BEBO

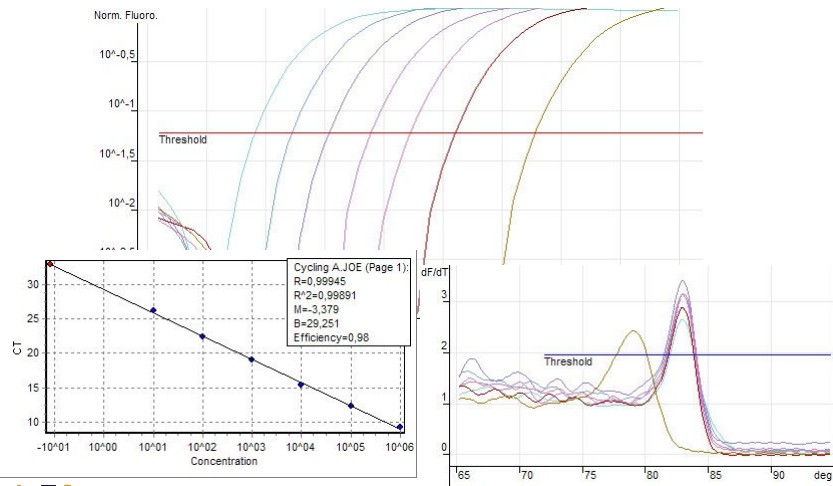


BEBO



BEBO does not stabilize dsDNA as much as SYBR.

BOXTO



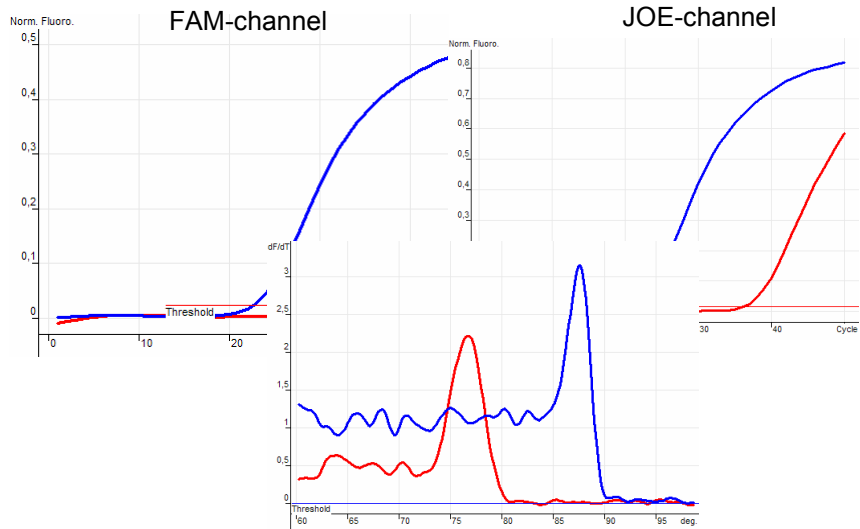
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Best of Both Worlds?

- **DNA binding dyes**
 - + non-specific products can be detected using melting curves
 - non-specific products may be included in quantification
- **Sequence specific probes**
 - + target specific
 - other products may form and disturb the amplification and never be detected

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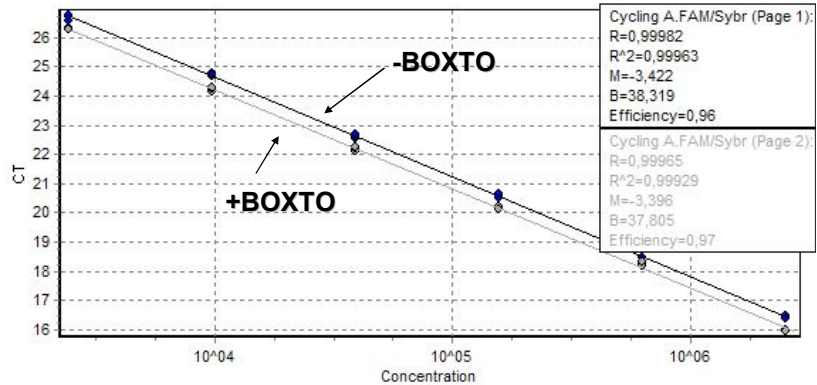
Best of Both Worlds!



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“Internal control” of all probed reactions, in every reaction.

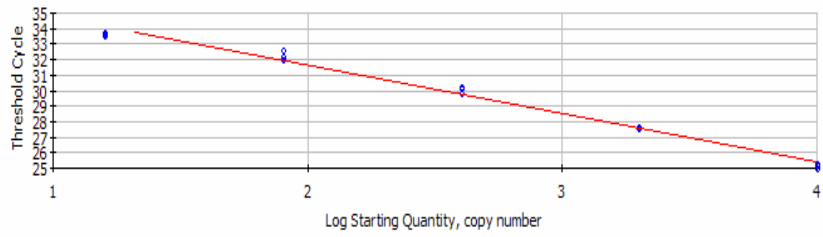
Addition of BOXTO to probed assay



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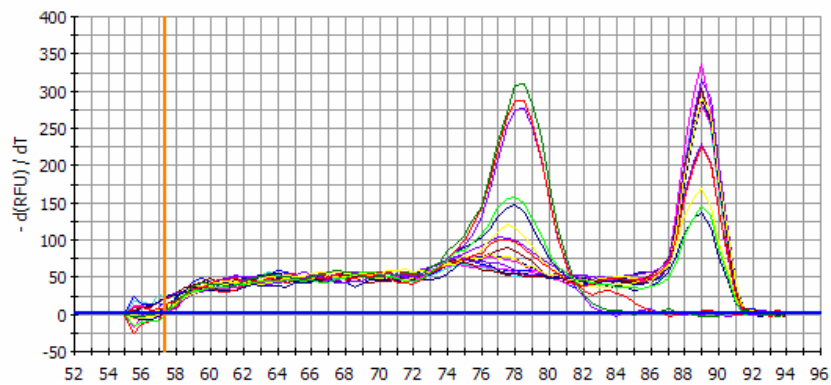
Correlation Coefficient: 0,993 Slope: -3,080 Intercept: 37,775 $Y = -3,080 X + 37,775$
PCR Efficiency: 111,2 %

□ Unknowns
● Standards



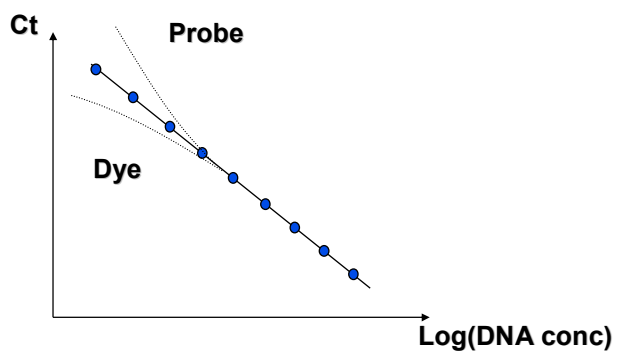
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Melting curves of primer dimers

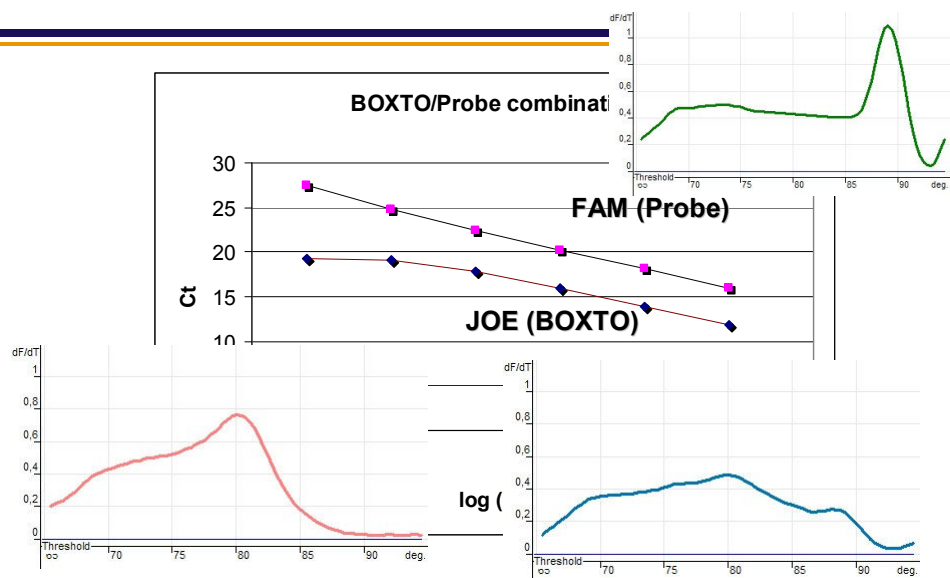


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Effect of PD's on dilution series



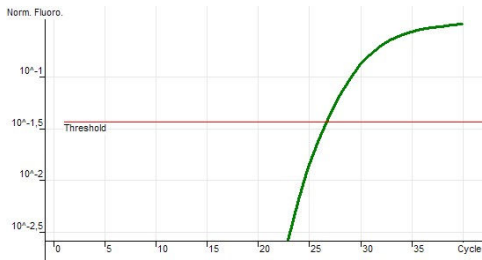
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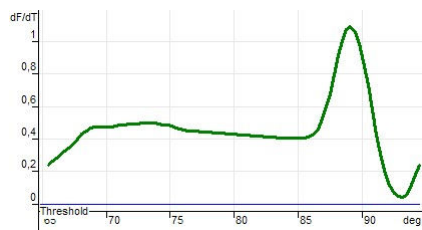
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How to use **BOXTO-FAM** combination?

Quantify using FAM labeled probe



Evaluate melting curve using BOXTO

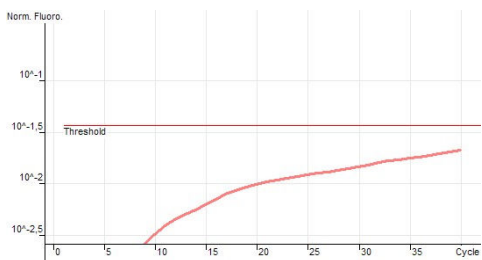


Specific product! Quantification is accurate

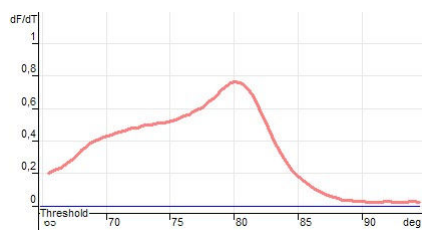
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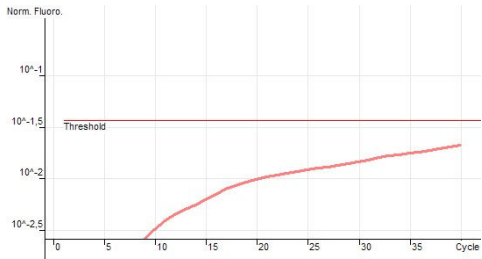


**Non-specific product! Target is non-detectable.
May be due to considerable primerdimer formation.
May be false negative.**

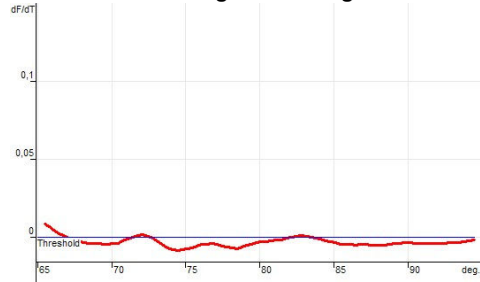
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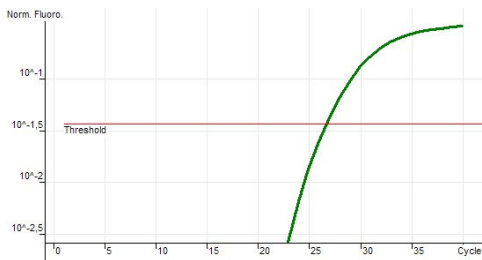


No product amplification. True negative.

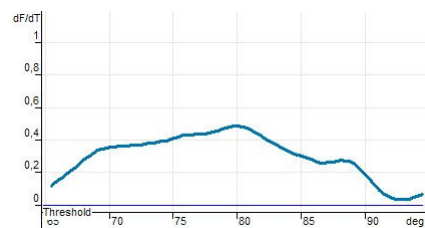
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Evaluate melting curve using BOXTO



**Both specific and non-specific products are amplified.
Quantification may be underestimated!**

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Conclusions

- BOXTO can be used with established FAM-labeled probed assays
- BOXTO works in combination various probe-technologies

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Conclusions

- BOXTO does not interfere with the PCR
- BOXTO can readily detect primer-dimer formation
- BOXTO can be used as a closed-tube quality control assay for probed assays.

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Aknowledgments

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