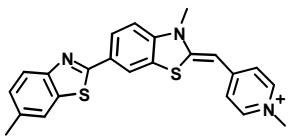
Best of Both Worlds- New Dyes for qPCR used in combination with probes

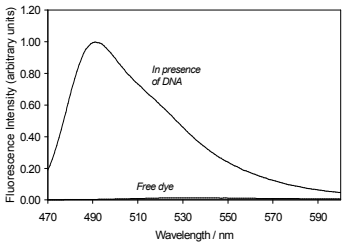
Neven Zoric

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Improving probe technology

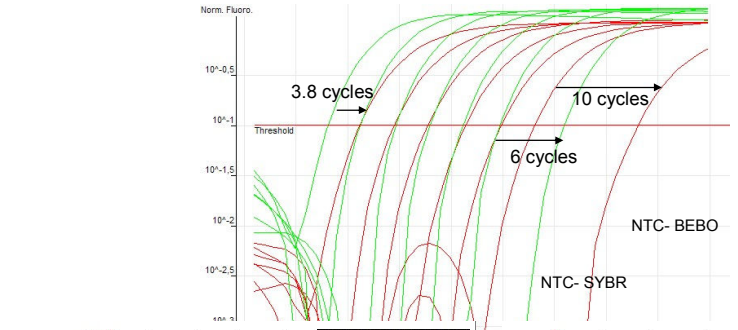
- BEBO/BOXTO – a novel series of asymmetric cyanine dyes that bind to the minor groove of DNA
- 250-fold increase in fluorescence upon binding dsDNA

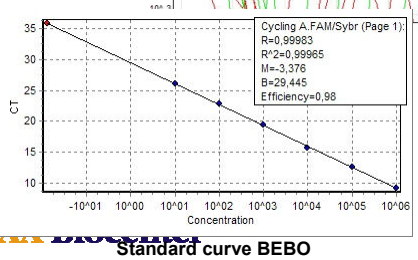




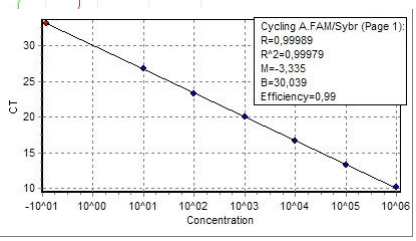
BEBO-excitation max 468nm, emission max 492nm
BOXTO-excitation max 515nm, emission max 552nm

BEBO





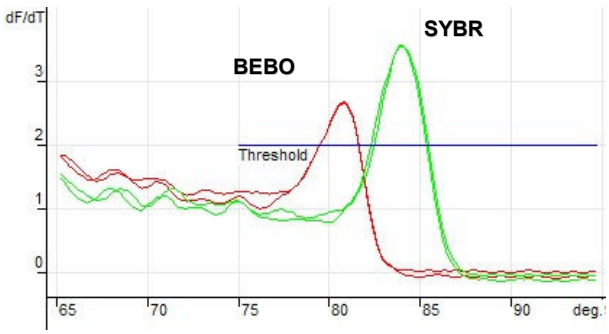
Standard curve BEBO



Standard curve SYBR

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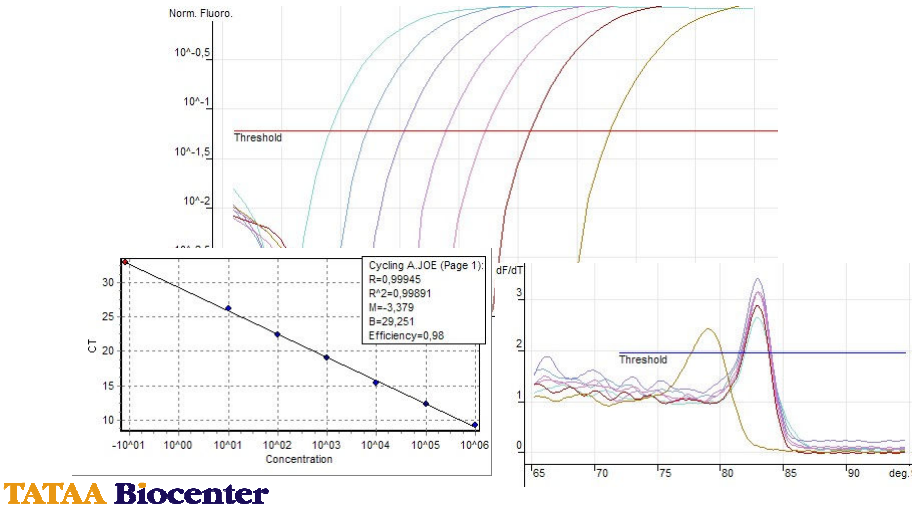
BEBO



BEBO does not stabilize dsDNA as much as SYBR.

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BOXTO



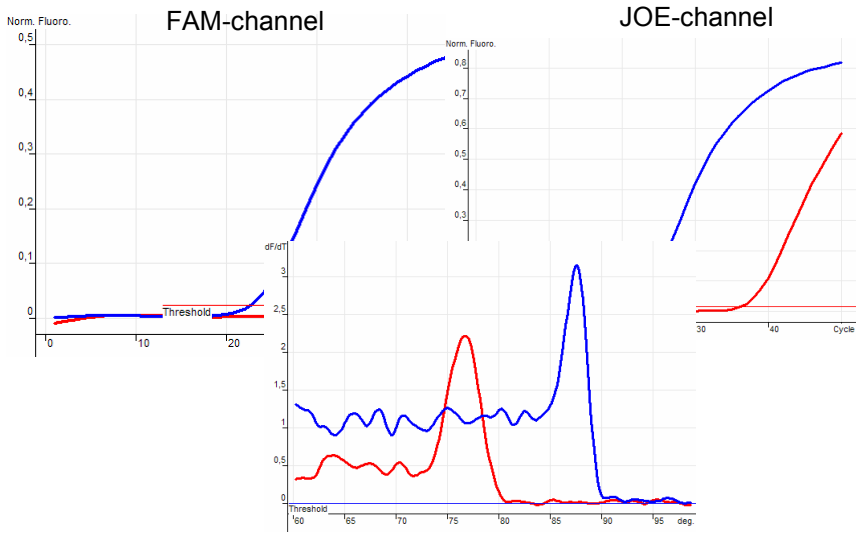
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Best of Both Worlds?

- **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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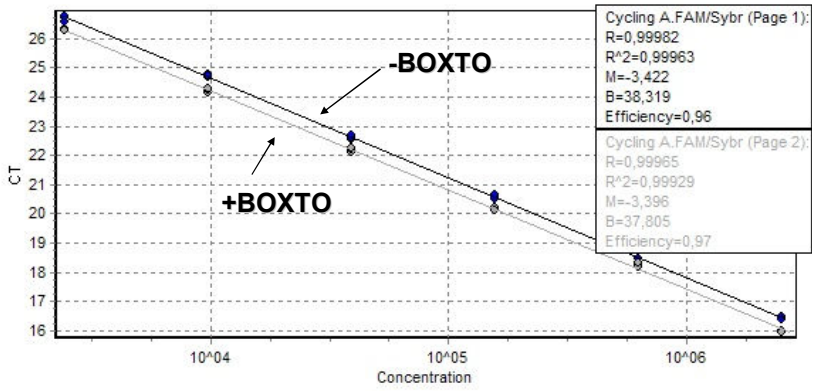
Best of Both Worlds!



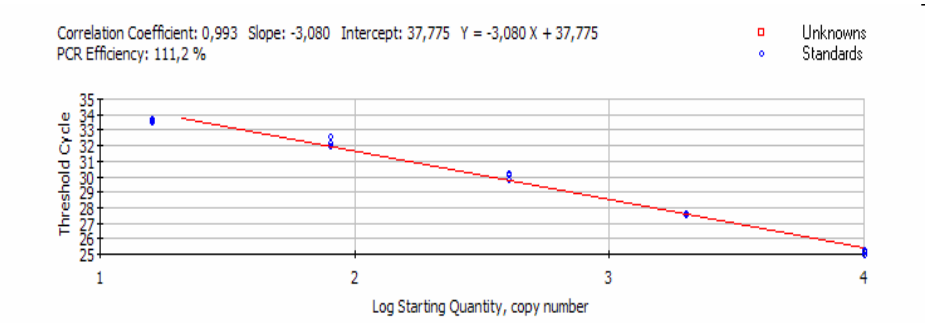
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"Internal control" of all probed reactions, in every reaction.

Addition of BOXTO to probed assay

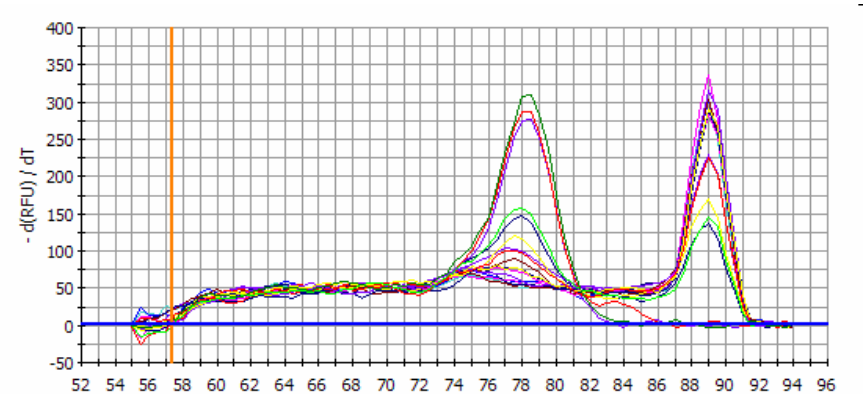


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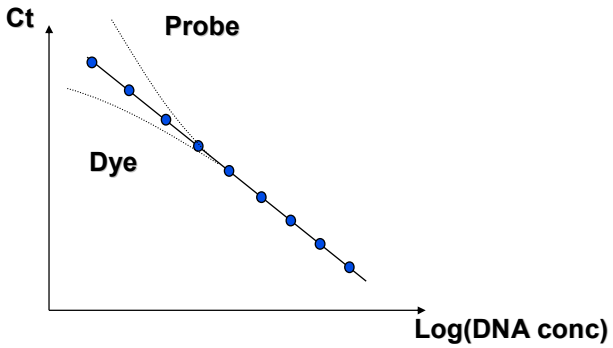
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Melting curves of primer dimers

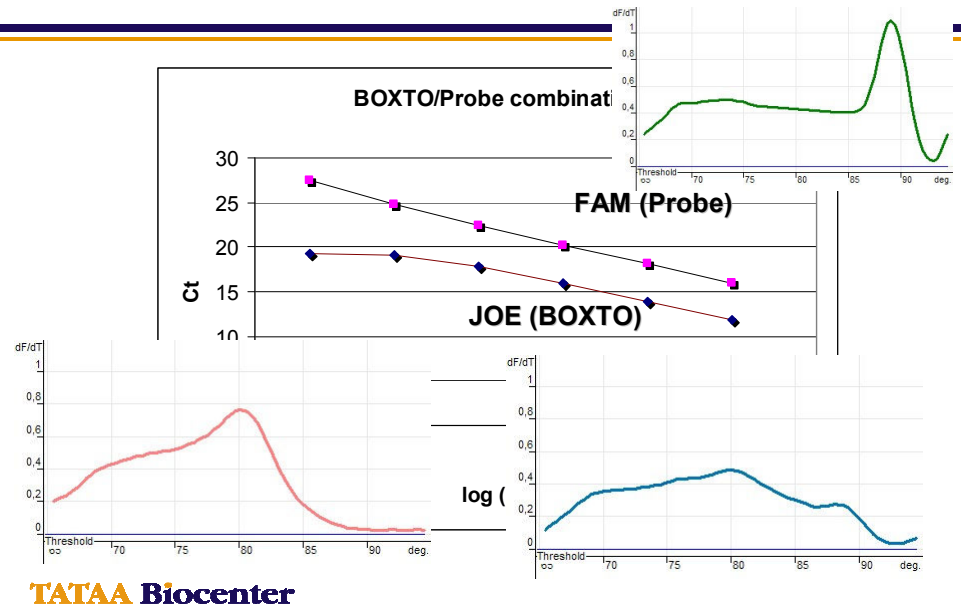


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Effect of PD´s on dilution series



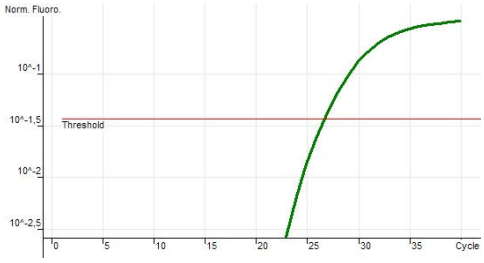
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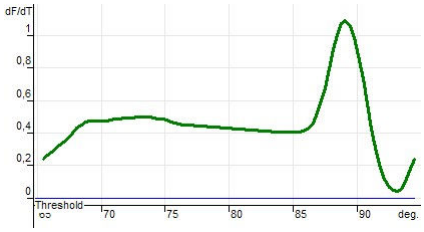
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How to use BOXTO-FAM combination?

Quantify using FAM labeled probe



Evaluate melting curve using BOXTO

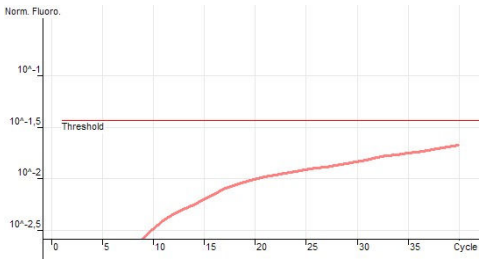


Specific product! Quantification is accurate

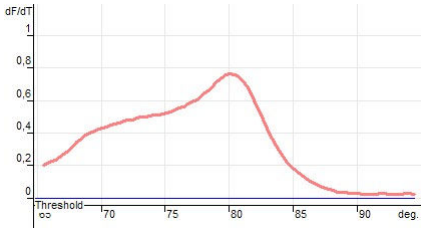
TATAA Biocenter

How to use BOXTO-FAM combination?

Quantify using FAM labeled probe



Evaluate melting curve using BOXTO

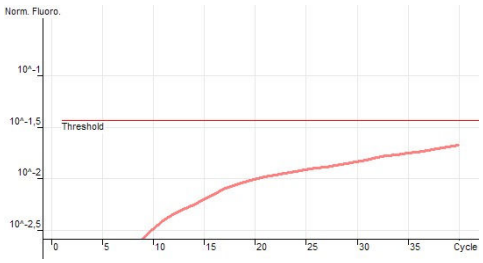


Non-specific product! Target is non-detectable.
May be due to considerable primerdimer formation.
May be false negative.

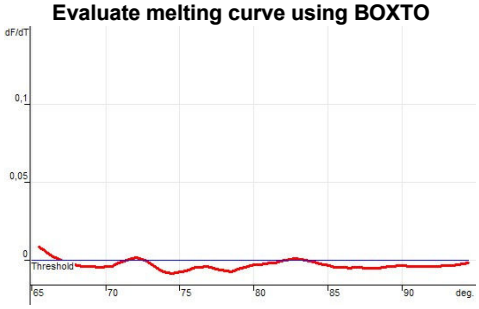
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How to use BOXTO-FAM combination?

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Evaluate melting curve using BOXTO

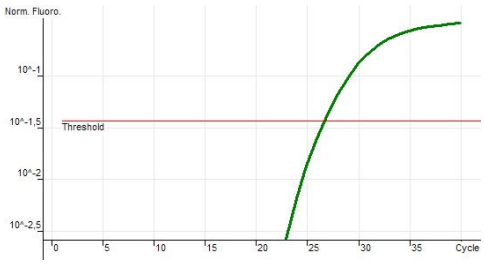


No product amplification. True negative.

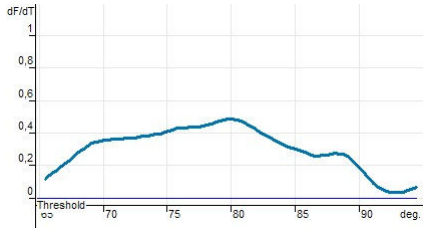
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How to use BOXTO-FAM combination?

Quantify using FAM labeled probe



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

- Kristina Lind
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