Sylphium
molecular ecology

eDNA qPCR hot start mix **qPCR detection kit**

For environmental samples

#SYL1003 validation report

Document date: 31 January 2025

For general laboratory and research use only.

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1. Validation report eDNA qPCR hot start mix

The validation of the eDNA qPCR hot start mix was performed with the IPC detection kit (SYL113) of Sylphium molecular ecology according to the protocol[1].

1.1. In silico validation

Specific component	Ref number:	Lot number:
eTaq DNA polymerase	SYL1003/2	211115
eTaq qPCR hot start mix	SYL1003/1	220114

Table 1. Specific components covered under this validation.

General components/equipment:	Manufacturer:	Reference number:
10 x Primer/probe mix	Sylphium molecular ecology	SYL113/1
Positive control 10^7/2 µl	Sylphium molecular ecology	SYL113/2
Real-Time PCR System	Applied Biosystems	7300
Rox Reference dye	Gene-on	F153
PCR plate without skirt	Sarstedt	721.978.202
SafeSeal micro tube 2 ml	Sarstedt	72.695.500
Biosphere filter tips	Sarstedt	70.760.213
Biosphere filter tips	Sarstedt	70.760.211
Biosphere filter tips	Sarstedt	703.050.255
Sealing tape	Sarstedt	951.994

Table 2. General components, consumables and equipment used for this validation.

1.1. Detection limit, fluorescence output signal and efficiency

The limit of detection, fluorescence output and efficiency were determined using a positive control standard curve. The standard curve was made as follows: 10^6, 10^5, 10^4, 10^3, 100, 10 and 1 target copies per 25 µl reaction mix. The reactions were performed in triplicate.

Results:

The limit of detection (LoDqPCR) for the eDNA qPCR hot start mix was determined to be 1 copy per reaction. (**Figure 1&2, Table 3**).

The fluorescence output signal was at least 100 (9 for a positive signal, 0,01 for the background) times stronger than the background signal (**Figure 1**) The efficiency of the eDNA qPCR hot start mix in combination with used primer/probe set was 98,4%.

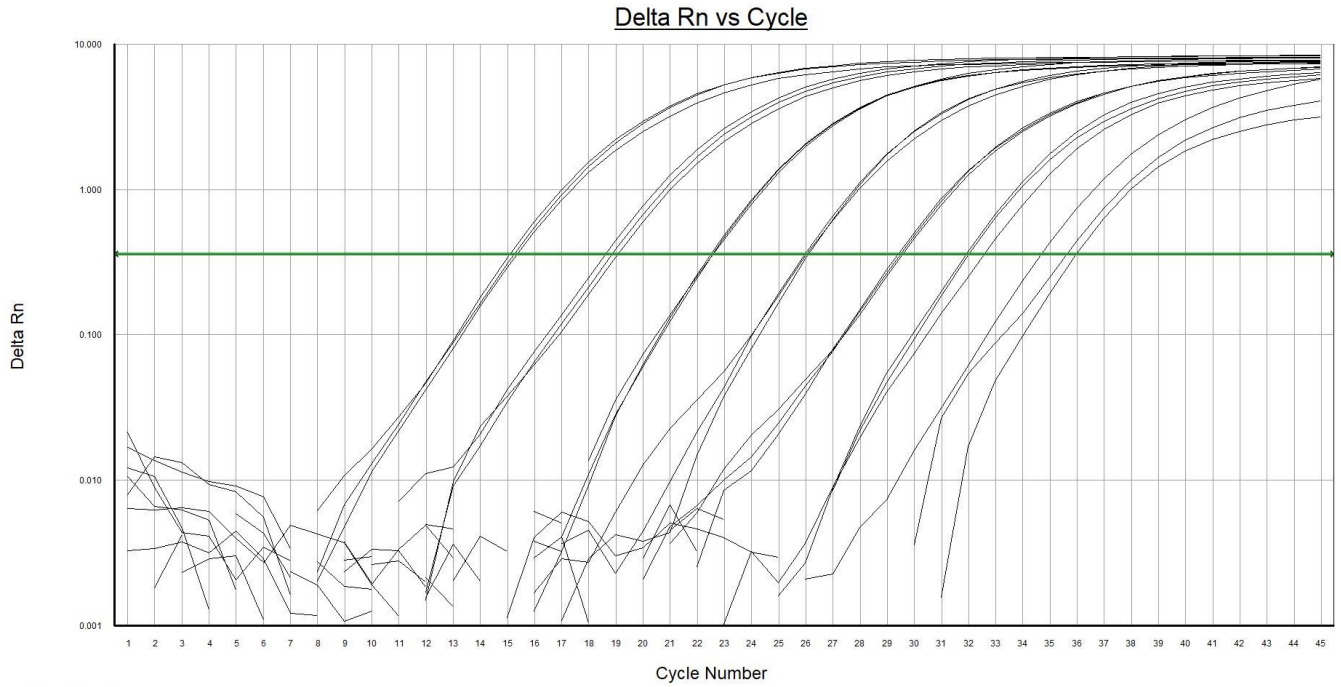

Figure 1. **Logarithmic fluorescent output curves with eDNA qPCR hot start mix** based on 10^6, 10^5, 10^4, 10^3, 100, 10 and 1 target DNA copies per 25 µl reaction mix.

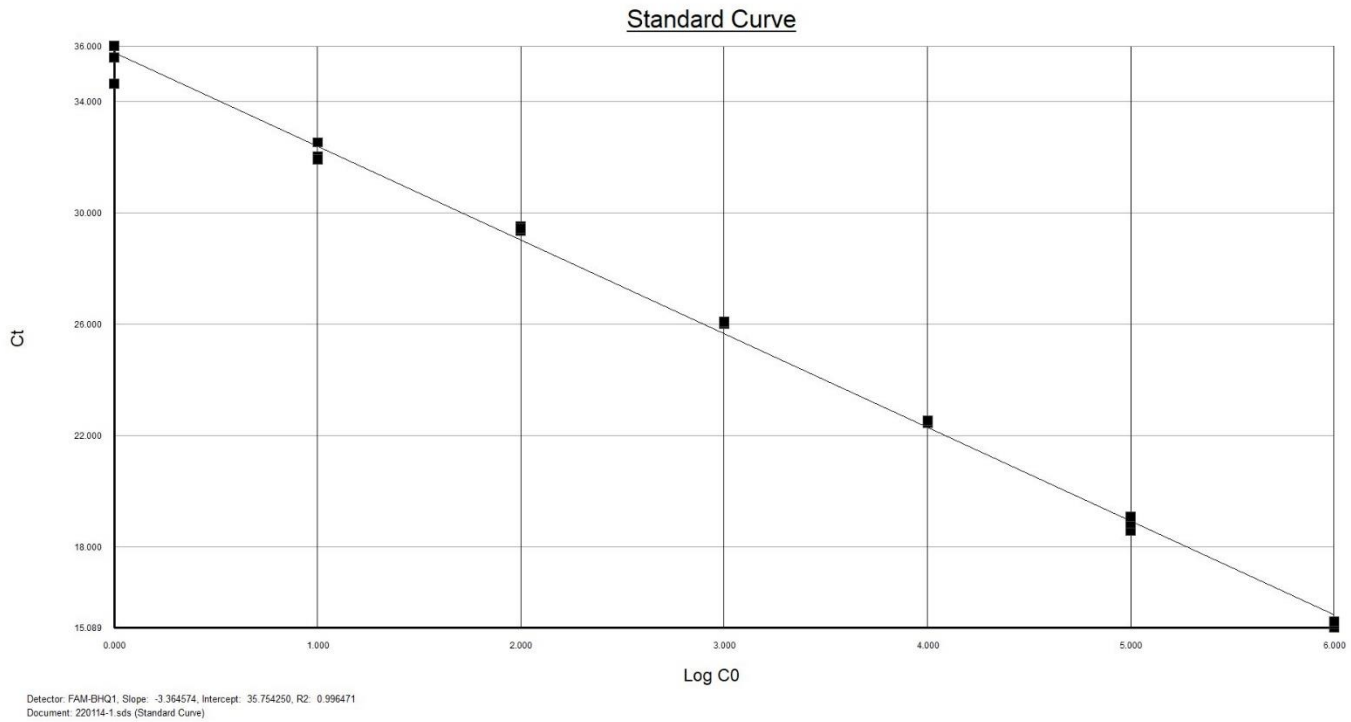

Figure 2. **Standard curve of SYL113 with eDNA qPCR hot start mix** based on 10^6, 10^5, 10^4, 10^3, 100, 10 and 1 target DNA copies per 25 µl reaction mix.

Slope	-3.36
efficiency	98.4%
R^2	0.996

Table 3. Values obtained from the standard curve.

1.2. Resistance to humic acids

The performance of the eDNA qPCR hot start mix has been tested with an increasing amount of humic acids (Sigma Aldrich). Samples were made according to Uchii et al[2]. The samples contained 0 ng, 250 ng, 500 ng and 1000ng/µl humic acids and a fixed amount of 200 target DNA copies/µl (fig 3). The qPCRs were performed in duplicate with a reaction volume of 50 µl and a sample volume of 5µl. The eDNA qPCR hot start mix was compared with best performing PCR hot start mix (EMM) from the study by Uchii et al[2]. according the manufactures protocols of eDNA qPCR hot start mix and EMM[3].

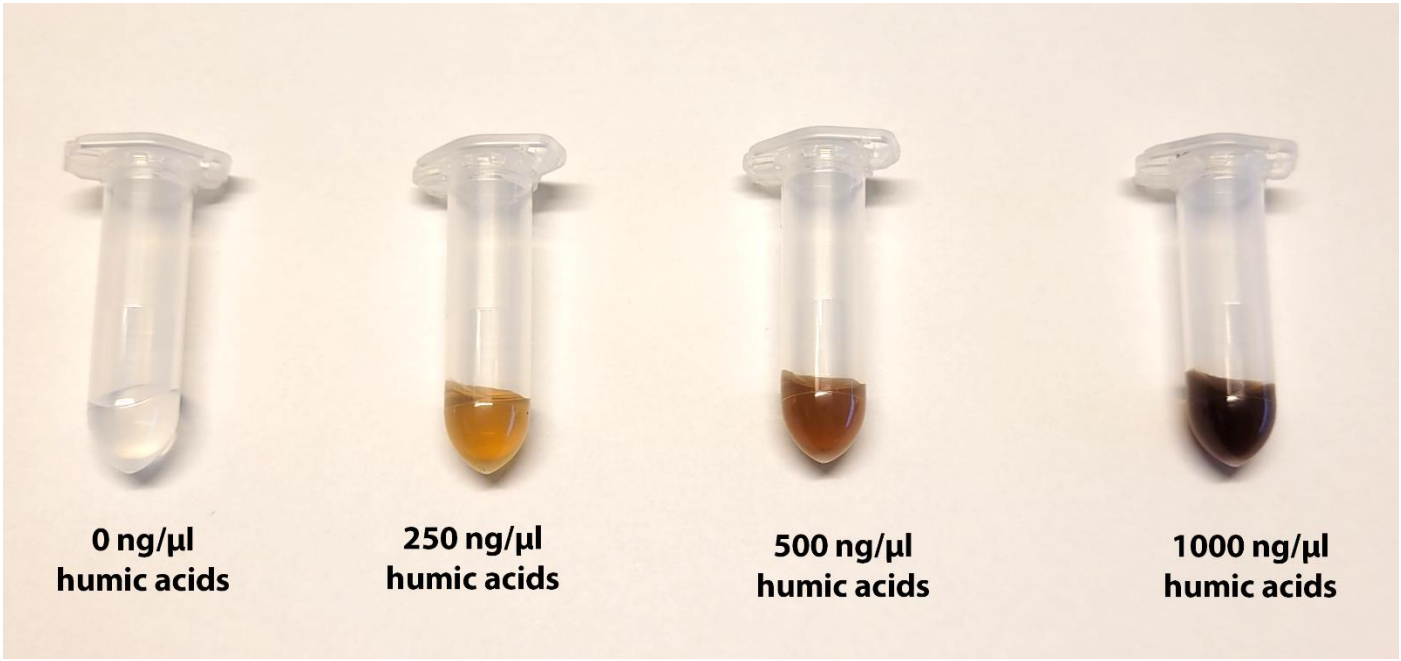

Figure 1. Prepared samples with an increasing amount of humic acids and a fixed amount of 200 target DNA copies per µl.

Results:

the eDNA qPCR hot start mix was able to detect the target DNA in all samples (0 - 1000 ng humic acids/µl sample). The EMM was unable to detect the target DNA above 500 ng humic acids/µl sample. At all humic acid concentrations, the eDNA qPCR hot start mix showed a higher resistance to humic acids than EMM as evidenced by a lower CT value (**Figure 4-6**).

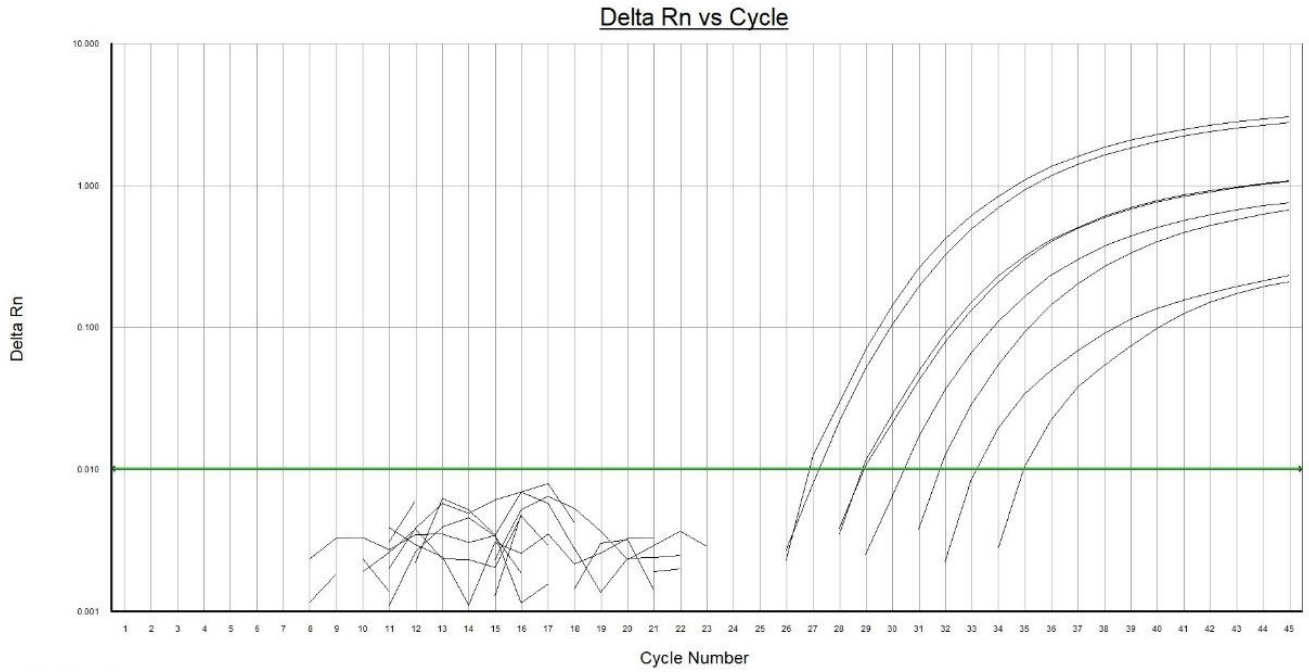

Figure 4. **Logarithmic fluorescent output curves of eDNA PCR hot start mix** at 0, 250, 500 and 1000ng humic acids /µl sample.

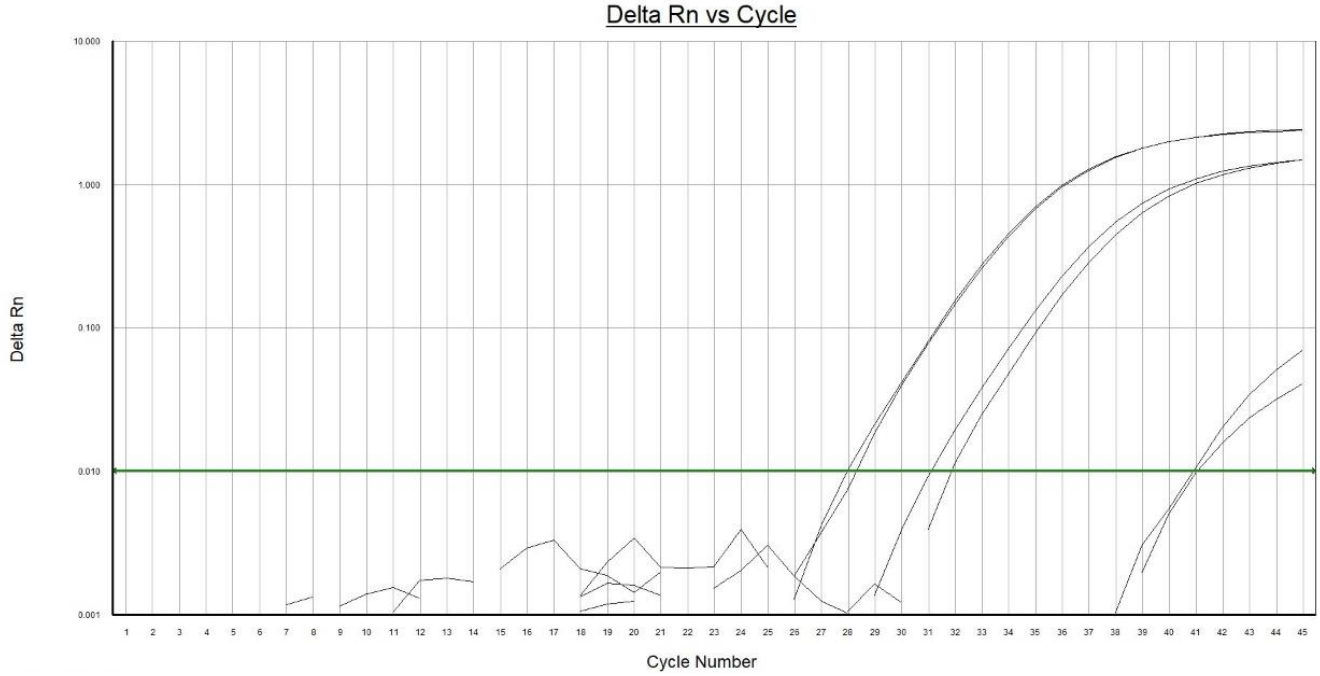

Figure 5. **Logarithmic fluorescent output curves of EMM** at 0, 250, 500 and 1000ng humic acids /µl sample.

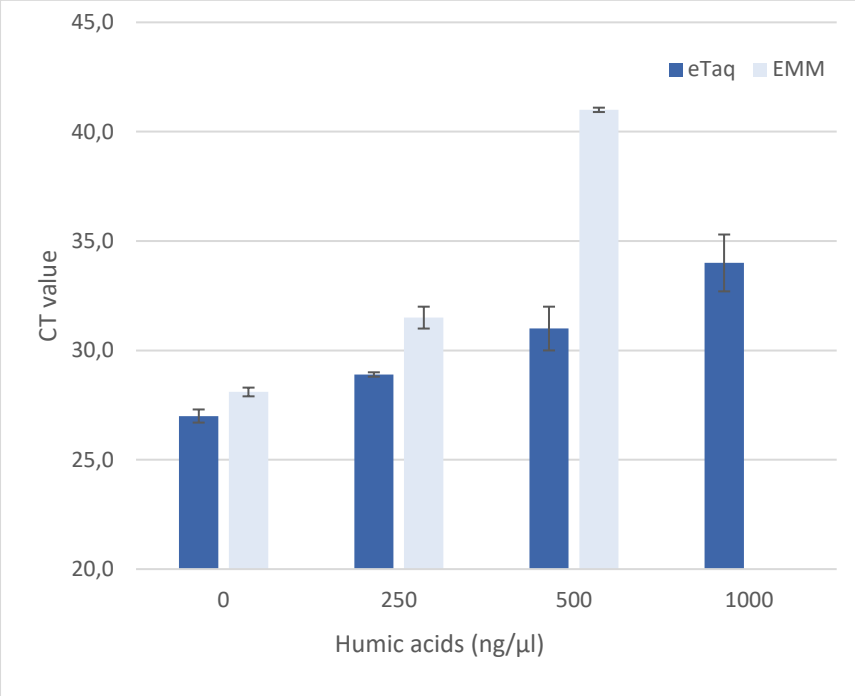

Figure 6. CT values of eDNA qPCR hot start mix and EMM at different humic acid concentrations with a fixed amount of target copies. Lower CT values are better. EMM gave no fluorescent signal at 1000 ng/µl humic acids.

1.3. Hot start properties

The eTaq enzyme is genetically modified (proprietary technology) to have an inactive form below 30°C. The hot start properties of eTaq is compared with EMM, which has an antibody mediated hot start enzym. The qPCRs were performed in duplicate with a reaction volume of 25 µl with 1000, 100, 10 and 1 target DNA copies. The mixtures were made at room temperature and after adding the target copies kept 10 minutes at room temperature before running the PCR machine.

Results:

eTaq has equal hot start properties as EMM as shown in **Figure 7&8**.

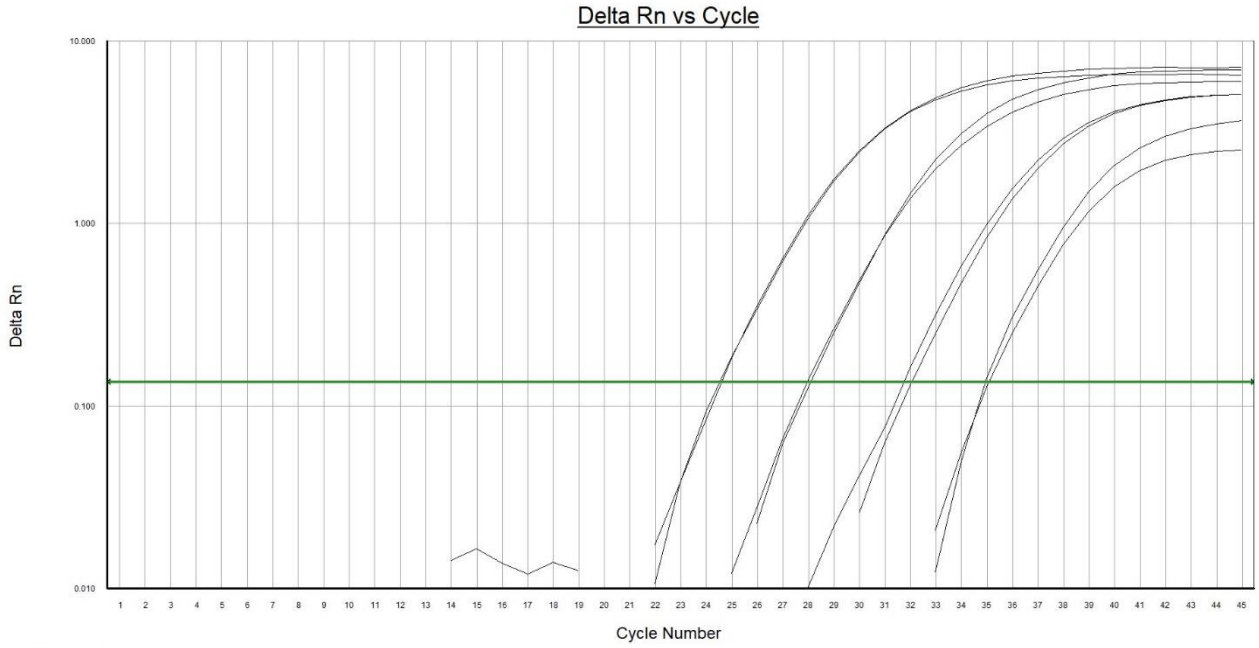

Figure 7. **Logarithmic fluorescent output curves of eTaq** with 1000, 100, 10 and 1 target copy per reaction. Reaction prepared and kept 10 minutes at room temperature before running the analysis.

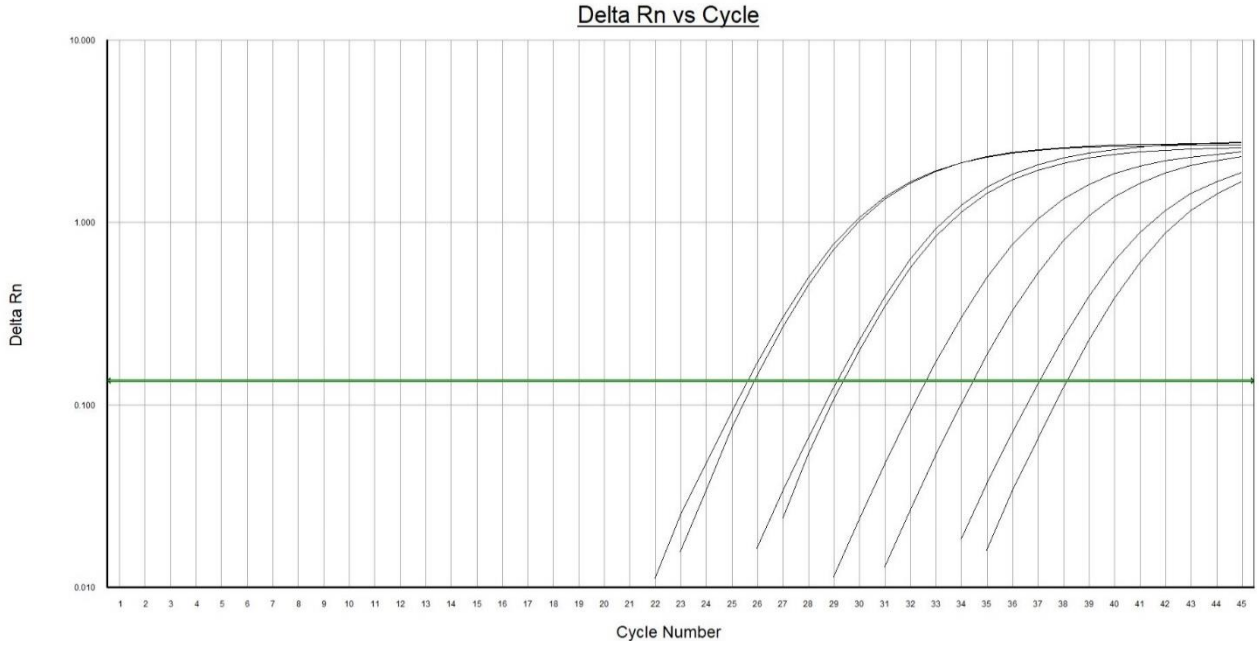

Figure 8. **Logarithmic fluorescent output curves of EMM** with 1000, 100, 10 and 1 target copy per reaction. Reaction prepared and kept 10 minutes at room temperature before running the analysis.

2. References

1. https://sylphium.com/webshop/product/syl002-environmental-dna-isolation-kit2

2. Uchii, K., Doi, H., Okahashi, T., Katano, I., Yamanaka, H., Sakata, M. K., et al. (2019). Comparison of inhibition resistance among PCR reagents for detection and quantification of environmental DNA. *Environ. DNA* 1, 359–367.

3. https://www.thermofisher.com/order/catalog/product/4396838

Sylphium

The eDNA company

Sylphium molecular ecology

P.O. box 11107
9700 CC Groningen
Netherlands

Tel.: +31 6 82 42 64 44
E-mail: info@sylphium.com

www.sylphium.com