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# Polymerase study: Improved detection of *Salmonella* and *Campylobacter* through the optimized use of DNA polymerases in diagnostic real-time PCR

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## Aim

To investigate the performance of different DNA polymerases and master mixes in real-time PCR and their resistance towards inhibitors in matrices relevant for food safety, using validated PCR assays for *Salmonella* and *Campylobacter*.

## Introduction

To improve food safety it is important to pursue fast, well performing and low-cost methods for detection of foodborne pathogens. Though real-time PCR offers several advantages compared with classical microbiology, the choice of a suitable DNA polymerase has been shown to optimize method performance considerably<sup>1</sup>.

## Screening:

**Table 1.** 16 DNA polymerases and 4 master mixes screened in standardized *Salmonella* and *Campylobacter* qPCR assays<sup>2,3</sup> on a 10-fold dilution series of purified DNA, ranked after performance.

DNA Polymerase or Master mix	Performance on <i>Salmonella</i>			Performance on <i>Campylobacter</i>			Con-clusion	Price [USD/U]
	Rating	LoD [ $\mu\text{g/ml}$ ]	Max dR	Rating	LoD [ $\mu\text{g/ml}$ ]	Max dR		
Tth DNA Polymerase (Roche)	+++	$1.2 \times 10^{-6}$	42602	+++	$6.6 \times 10^{-7}$	48971	+++	1.22
VeriQuest™ Probe qPCR Master Mix (Affymetrix)	+++	$1.2 \times 10^{-5}$	38063	+++	$6.6 \times 10^{-7}$	43067	+++	0.92
AmpliQ™ Gold® (Applied Biosystems)	+++	$1.2 \times 10^{-6}$	34546	++	$6.6 \times 10^{-7}$	28147	+++	0.75
HotMaster® Taq DNA Polymerase (5 Prime)	+++	$1.2 \times 10^{-5}$	39244	++	$6.6 \times 10^{-7}$	16543	+++	0.66
TaKaRa Ex Taq® Hot Start Version (TaKaRa Bio Inc)	+++	$1.2 \times 10^{-5}$	37222	++	$6.6 \times 10^{-7}$	24549	+++	1.04
AmpliQ™ DNA Polymerase (Applied Biosystems)	++	$1.2 \times 10^{-5}$	40053	++	$6.6 \times 10^{-7}$	24930	++	0.80
AmpliQ™ 360 DNA Polymerase (Applied Biosystems)	++	$1.2 \times 10^{-5}$	39484	++	$6.6 \times 10^{-7}$	35547	++	0.56
AmpliQ™ Gold® 360 DNA Polymerase (Applied Biosystems)	+++	$1.2 \times 10^{-5}$	41110	++	$6.6 \times 10^{-7}$	28090	++	1.29
TaqMan® Fast Advanced Master Mix (Applied Biosystems)	++	$1.2 \times 10^{-6}$	34546	++	$6.6 \times 10^{-6}$	28331	++	1.20
SG qPCR Master Mix (EURx)	+++	$1.2 \times 10^{-6}$	30894	+	$6.6 \times 10^{-6}$	6996	IC	0.46
HotStarTaq® Master Mix kit (Qiagen)	+++	$1.2 \times 10^{-5}$	37751	+	$6.6 \times 10^{-4}$	14648	IC	0.99
PicoMaxx High Fidelity PCR System (Agilent Technologies)	+	$1.2 \times 10^{-3}$	24797	++	$6.6 \times 10^{-6}$	19700	IC	1.00
FastStart Taq DNA Polymerase (Roche)	+++	$1.2 \times 10^{-5}$	39644	-	$6.6 \times 10^{-2}$	5091	IC	1.07
MyTaq™ HS DNA polymerase (Bioline)	+	$1.2 \times 10^{-5}$	5448	+	$6.6 \times 10^{-7}$	5338	+	0.66
MyTaq™ DNA Polymerase (Bioline)	+	$1.2 \times 10^{-5}$	5126	+	$6.6 \times 10^{-6}$	5733	+	0.33
Titanium™ Taq DNA Polymerase (Clontech)	-	$1.2 \times 10^{-5}$	688	+	$6.6 \times 10^{-6}$	8584	-	2.68
OneTaq® DNA Polymerase (New England Biolabs)	-	ND		+	$6.6 \times 10^{-5}$	12860	-	0.20
Phusion® High-Fidelity DNA Polymerase with GC buffer (New England Biolabs)	-	$1.2 \times 10^{-5}$	2676	-	ND		-	0.84
Pfu DNA Polymerase (Fermentas)	-	1.2	2358	-	ND		-	0.68
Herculase II Fusion DNA Polymerase (Agilent Technologies)	-	ND		-	ND		-	0.07

+++ Very good, ++ Good, + Intermediate, - Poor, ND not detected, IC inconclusive, \*LoD could be lower.

## Conclusions

- The performances of the tested DNA polymerases varied considerably, reinforcing the importance of careful selection of an appropriate DNA polymerase for the PCR assay and sample type in question.
- For *Salmonella* in minced meat samples, HotMaster Taq, AmpliQ Gold and VeriQuest were found to be the best performing alternative DNA polymerases.
- For *Campylobacter* in chicken feces samples, VeriQuest and ExTaq were found to be the best performing alternative DNA polymerases.

## Further evaluation of top 5:

**Table 2.** The performance of the four polymerases and the master mix, with the best results in the screening, on three different DNA extractions methods on:

- A. Meat artificially contaminated with *Salmonella* and  
B. Feces artificially contaminated with *Campylobacter*.

DNA Polymerase or master mix	Magnetic beads based DNA extraction			Lysis by boiling			Non-extracted		
	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR
Tth	++	$10^2$	59615	+	$10^3$	47634	+	$10^5$	11710
VeriQuest MM	++	$10^2$	28736	++	$10^2$	17216	++	$10^4$	7430
AmpliQ Gold	++	$10^2$	64637	++	$10^2$	147690	++	$10^4$	12874
HotMaster Taq	++	$10^2$	35691	+++	$10^2$	97583	++	$10^4$	23048
TaKaRa ExTaq HS	-	$10^3$	27058	-	$10^4$	47569	-	ND	257

DNA Polymerase or master mix	Qiagen kit extraction			Magnetic beads based DNA extraction			Lysis by boiling		
	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR	Rating	LoD [CFU/ml]	Max dR
Tth	++	$10^3$	22022	-	NA		-	$10^6$	SD
VeriQuest MM	++	$10^2$	12661	-	NA		+	$10^4$	5578
AmpliQ Gold	+	$10^3$	6643	-	NA		-	NA	
HotMaster Taq	+	$10^3$	9088	-	NA		-	$10^6$	SD
TaKaRa ExTaq HS	++	$10^2$	12661	+	$10^3$	7861	-	$10^6$	SD

+++ Very good, ++ Good, + Intermediate, - Poor, NA no amplification, SD single detection, \*LoD could be lower, but only  $10^2$  to  $10^6$  CFU/ml was tested

Decreasing purity of DNA extractions

## Materials and Methods

16 commercially available DNA polymerases and 4 master mixes were included (see Table 1) These were evaluated on a dilution series of purified *Salmonella* ser. Typhimurium and *Campylobacter jejuni* DNA, analyzed by standardized real-time PCR assays<sup>2,3</sup> using the accompanying PCR buffers for each polymerase.

The 5 best performing polymerases/kits were further evaluated using minced pork meat samples (diluted in BPW 1:10 and enriched for 18 h at 37°C followed by artificial contamination with *Salmonella* ser. Typhimurium,  $10^2$ - $10^6$  CFU/ml) and chicken feces samples (artificially contaminated with *Campylobacter jejuni*,  $10^2$ - $10^6$  CFU/ml). DNA extraction was performed on the samples by three different methods (Magnetic beads-based (KingFisher), lysis by boiling, and non-extracted for *Salmonella* and QIAamp® Fast DNA Stool Mini Kit (Qiagen), magnetic beads-based, and lysis by boiling for *Campylobacter*) followed by real-time PCR.

Polymerases were rated based on shape of amplification curves, amplification efficiency (AE), linear range and linearity of standard curve ( $R^2$ ) and max fluorescence (Max dR)

## References

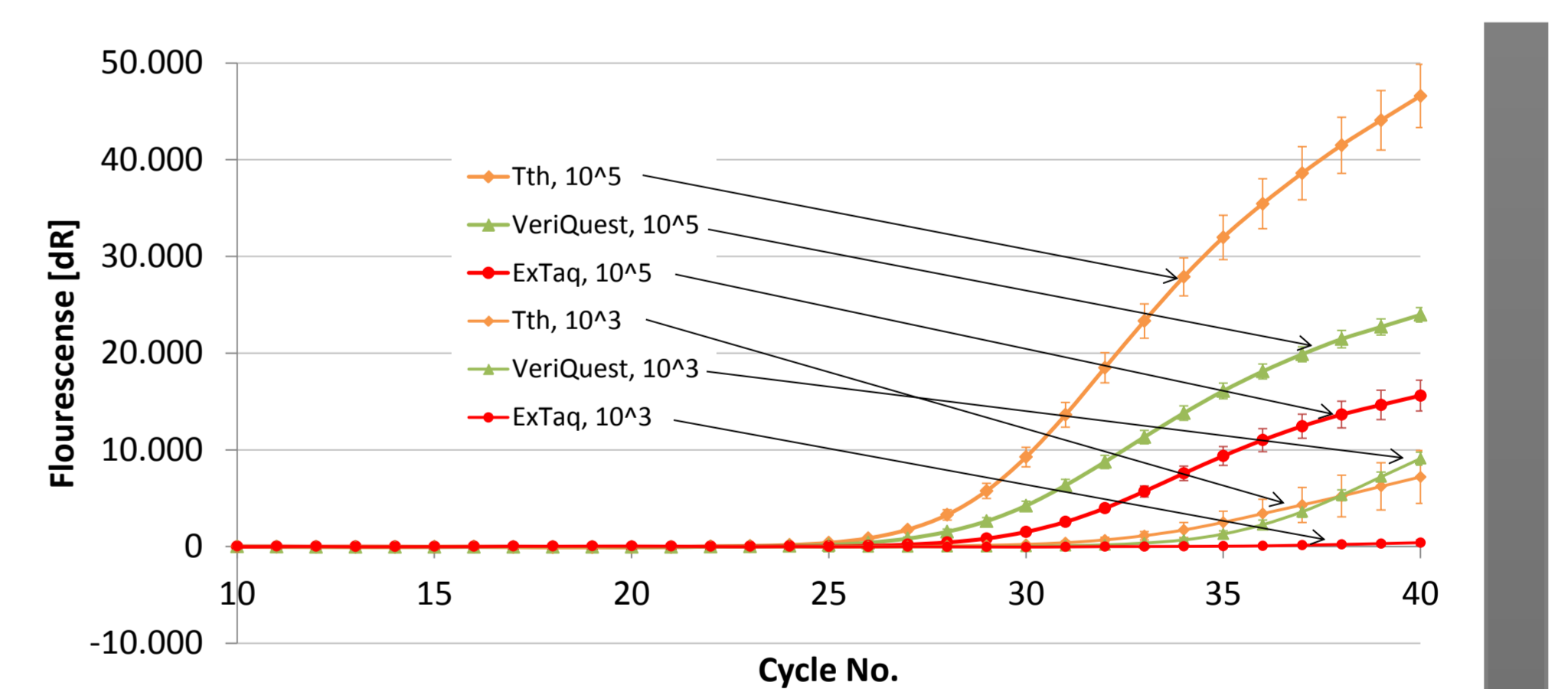
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## Acknowledgements

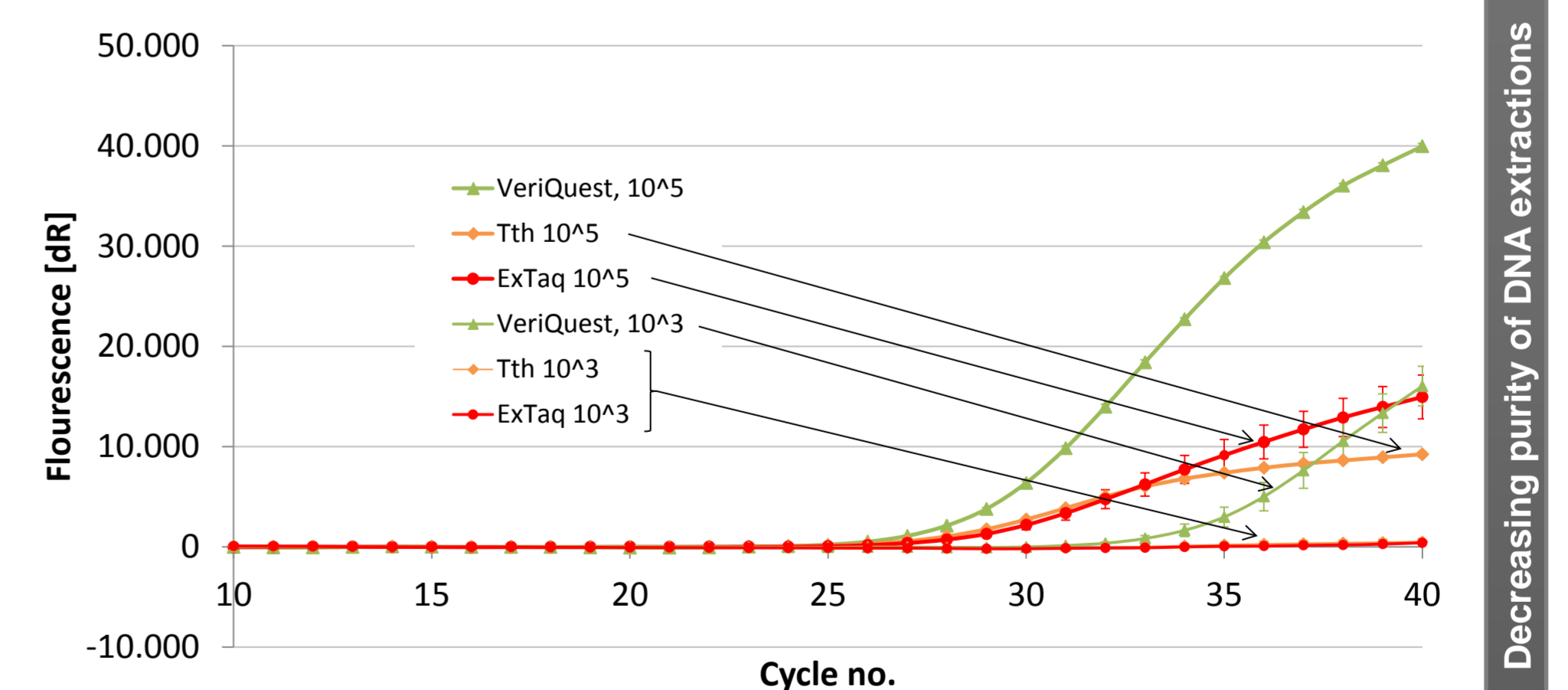
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## Examples of good, intermediate, and poor performance on high to low DNA purity:

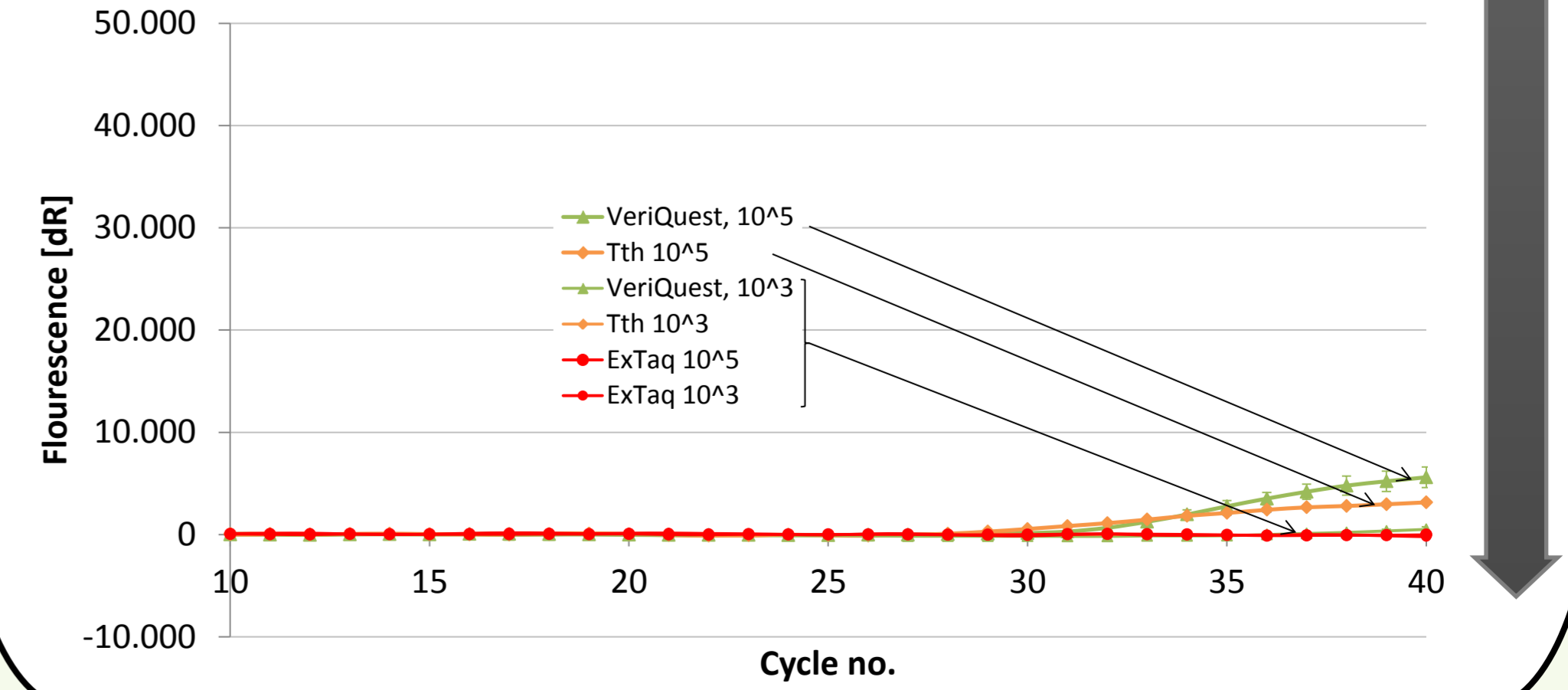
**Figure 1.** Performance on magnetic beads-based extracted meat samples



**Figure 2.** Performance on lysis by boiling extracted meat samples



**Figure 3.** Performance on non-extracted meat samples



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