

# Removal of contaminating bacterial DNA from commercially available mastermixes

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## INTRODUCTION

Detection and typing of bacteria in clinical samples can be done by PCR using primers targeting conserved regions in the 16S RNA gene. However, the high sensitivity of the PCR reactions makes the 16S DNA detection very sensitive to DNA contamination leading to erroneous results. The origin of the contaminating DNA may vary, but it is well documented that traces of bacterial DNA, mainly from *E.coli*, exist in most of the commercially available PCR reagents and mastermixes (Ehricht, R. *et.al.*, 2007, Mühl, H. *et.al.*, 2010.). Most decontamination techniques used are hampered by incomplete removal of the contaminating DNA or result in a reduced sensitivity of the assay (Corless, C.E. *et.al.*, 2000).

In this study we tested out several different commercially available qPCR mastermixes for the presence of contaminating bacterial DNA. We also tested out the use of a new DNase (Nilsen *et.al.*, 2010) for the removal of contaminating DNA from the mastermixes to enhance the selectivity and specificity of the assay. This DNase is double-strand specific, can be easily heat inactivated and can be added directly to the mastermix before adding your template.

## Methods

Quantitative PCR was performed on a Stratagene Mx3005P machine.

16s rDNA Primer pair 1: Forward CATGAAGTCGGAA TCGCTAG. Reverse ACTCCCAT GGTGTGACGG.

Product size: 97 bp

16s rDNA primer pair 2: Forward TTAAGTGGGCGTAAA GCGCAC. Reverse ACGCATTTCACCGCTACACC

Product size: 141 bp

The presence of contaminating bacterial DNA was tested using six different commercially available qPCR mastermixes (Figure 1).

PCR : 95°C 10 min, (95°C 15 seconds, 60°C 1 min) X 45 cycles

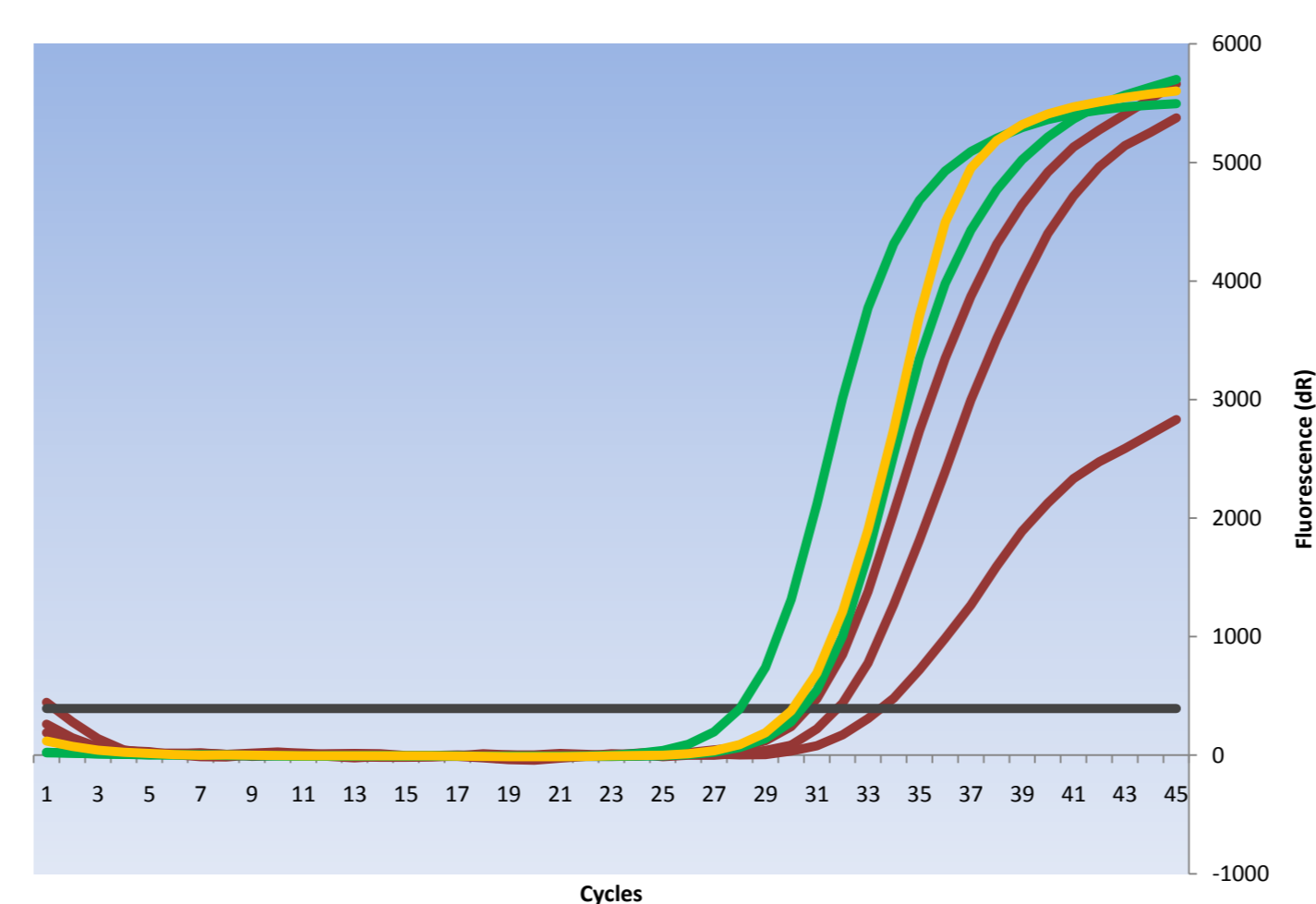
Preincubation step in contamination removal: 30 min 37°C (Figure 2 and 3)

10 pg of genomic *E.coli* DNA was added in Figure 3 after preincubation and DNase inactivation, to test integrity of primers and probe.

In figure 3 we tested out five different concentrations of dsDNase(0 – 5 U dsDNase pr 25 µl qPCR reaction)

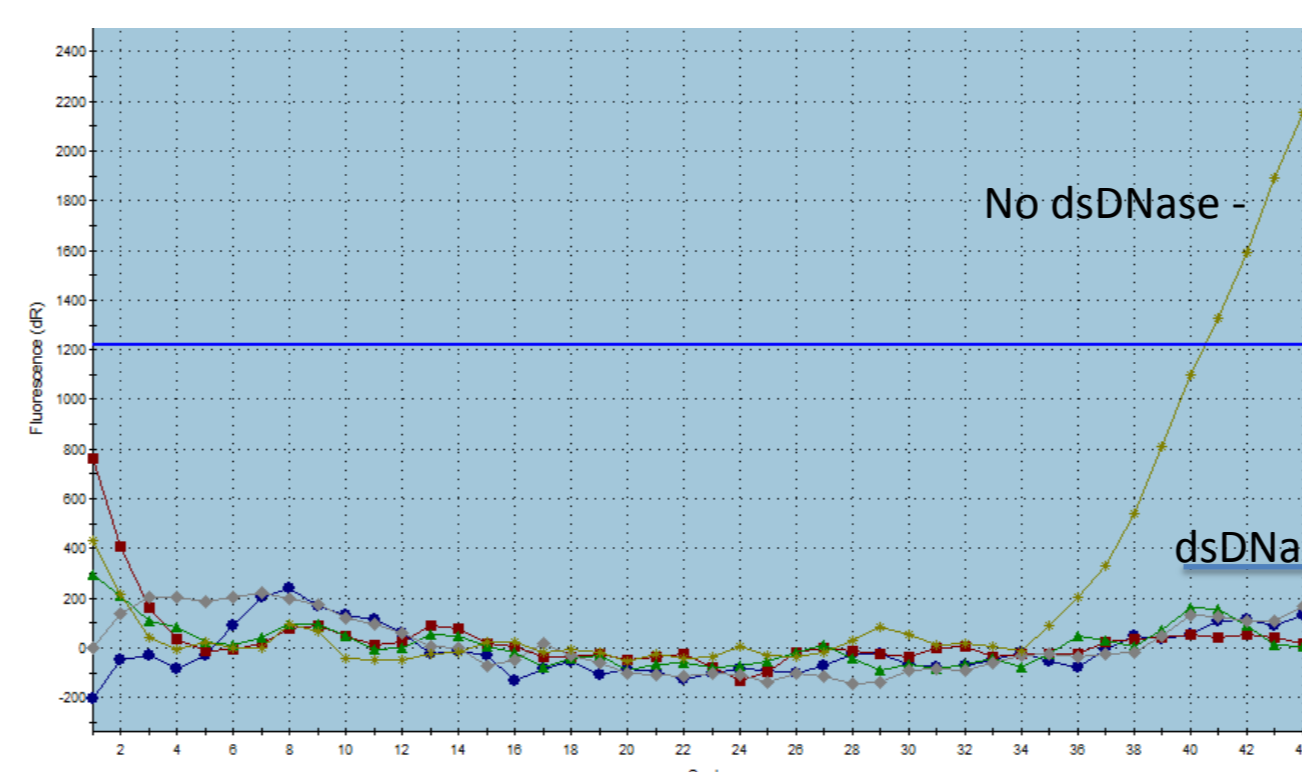
## RESULTS

Figure 1. Contaminating bacterial DNA in all qPCR mastermixes tested



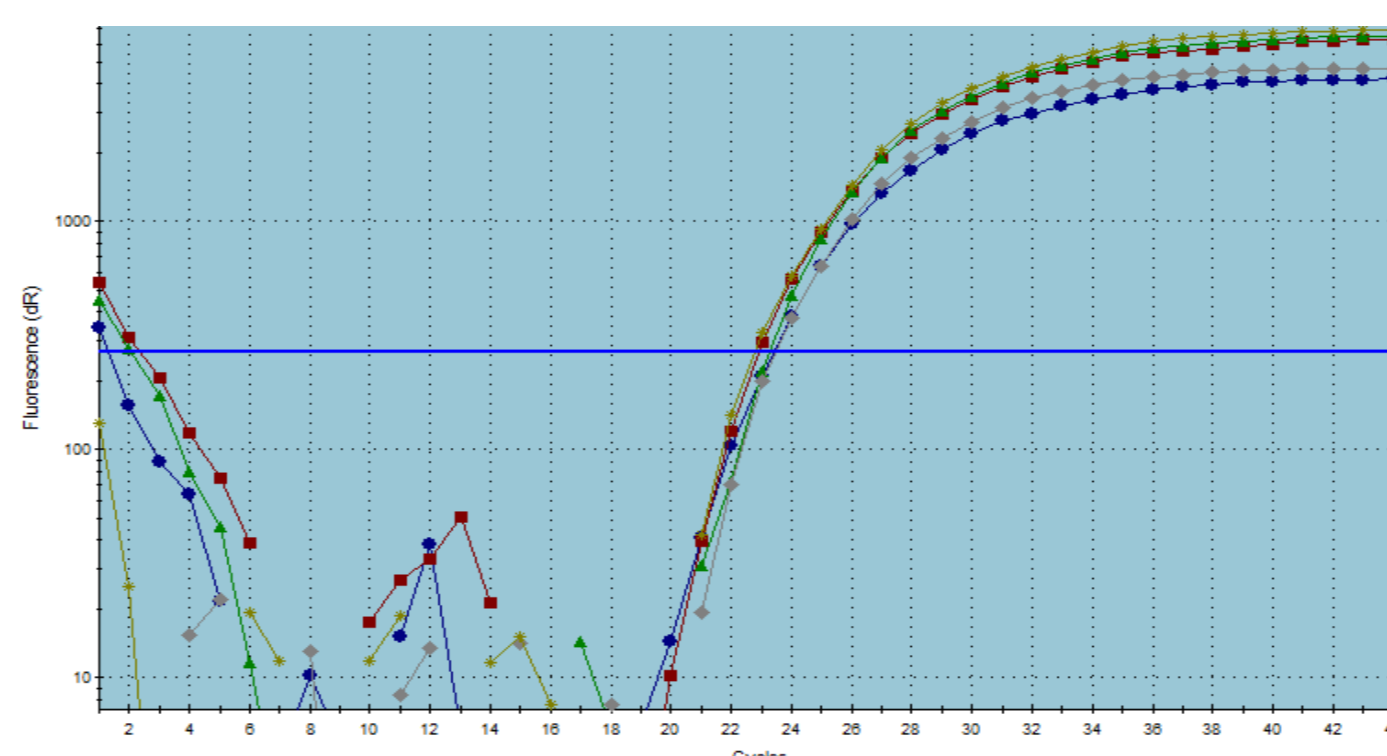
No template controls of six different commercially available qPCR mastermixes, were tested for contaminating bacterial DNA using broad-range 16S rDNA primers. All six mastermixes contained significant amounts of bacterial DNA.

Figure 2. Double-strand specific DNase removes contaminating bacterial DNA



The qPCR mixes were preincubated at 37°C for 30 min with and without dsDNase (0-5 U dsDNase) before qPCR was performed. The lack of amplification signal in the dsDNase treated samples indicates removal of contaminating bacterial DNA. qPCR was performed with bacterial 16S rDNA specific primers and probe.

Figure 3. dsDNase does not degrade primers or probes in the qPCR mastermix



0-5 Unit dsDNase was added to the qPCR mastermix and preincubated at 37°C for 30 min. After the nuclease-inactivation step at 60°C for 15 min, 10 pg genomic *E.coli* DNA was added and qPCR performed. The sensitivity of the qPCR was not affected after pre-incubation with the dsDNase

## CONCLUSION

Contaminating DNA in qPCR mastermixes is a problem when detecting bacteria

All qPCR mastermixes tested here contain bacterial DNA

A double-strand specific nuclease can be added directly into your PCR mastermix to remove contaminating bacterial DNA

The dsDNase does not affect the sensitivity of the qPCR

## REFERENCES

Corless, C.E. *et.al.*, (2000)  
J Clin Microbiol. 38(5):1747-52

Ehricht, R. *et.al.*, (2007)  
Biologicals. 35(2):145-7

Mühl, H. *et.al.*, (2010)  
Diagn Microbiol Infect Dis. 66(1)41-49

Nilsen, I.W. *et.al.* (2010) PLoS one 5(4):  
e10295