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Poster Presentation

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Determination of plasmid content in eukaryotic and prokaryotic cells using Real-Time PCR

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Background

Determination of the plasmid content in prokaryotic cells during plasmid DNA (pDNA) production and in eukaryotic cells after transfection is crucial for DNA vaccine development. In *Escherichia coli*, pDNA is usually determined after plasmid extraction, either by UV absorbance

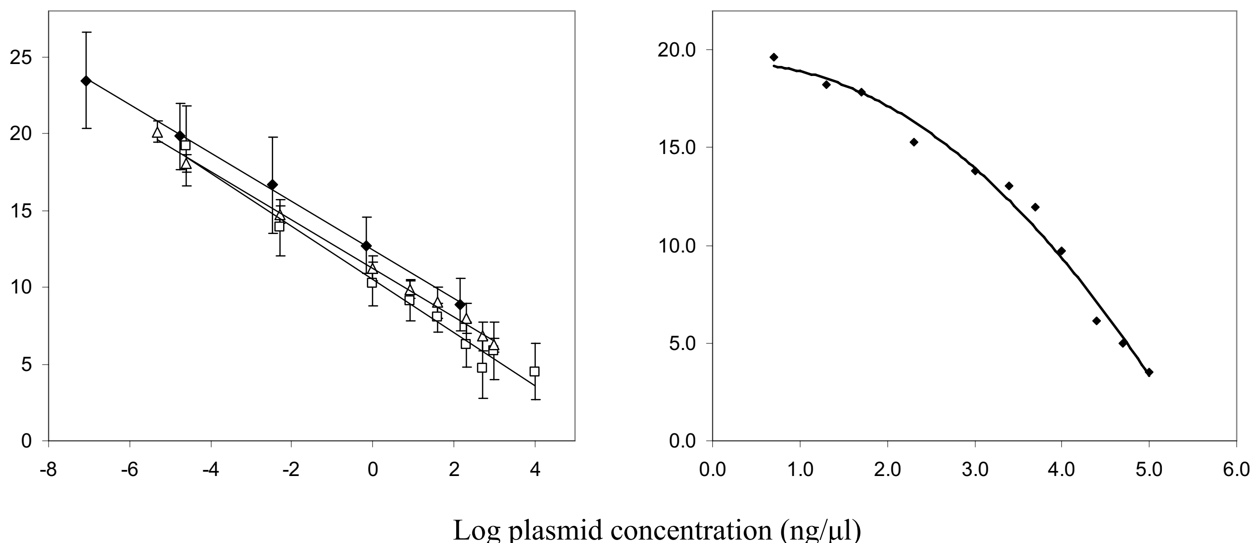

Figure 1
Variation of threshold cycle numbers using plasmid-free *E. coli* (left) and CHO cells (right) spiked with pDNA. In *E. coli*, 5×10^4 (□), 2.5×10^5 (△) and 3.5×10^6 (▲) cells were spiked with 8.5×10^{-4} to 8.5 ng of pDNA. With CHO, 1.2×10^4 cells were spiked with pDNA masses ranging from 5 pg to 100 ng. A linear working range was obtained from 5 pg to 2.5 ng and was subsequently used for quantitation.

or by densitometry of ethidium bromide-stained agarose gels. Fluorescence microscopy techniques are mainly used in eukaryotic cells. These techniques are time-consuming and labour-intensive and can not be used for process control. Thus, a Real-Time PCR method was developed to monitor the plasmid content of *E. coli* and Chinese Hamster Ovary (CHO) cells.

Results

Real-Time PCR with a 108 bp amplicon enabled the detection of quantities as low as 4 copies of pDNA per cell in *E. coli* and 100 copies/cell in CHO cells transfected with high copy-number plasmids (see figure 1). The procedure can be performed in less than 30 minutes and requires no sample pre-treatment. Analysis of pDNA number in *E. coli* harbouring a ColE1 plasmid revealed that copy number reached a maximum during exponential phase of growth and that this number decreased up to 80% upon entry into stationary phase. Additionally, the half-life of pDNA in transfected CHO cells was 20 hours and around 100 copies of plasmid were still detected 6 days after transfection.

Conclusion

Monitoring the pDNA content on producing and recipient cells is crucial for DNA vaccine development. The Real-Time PCR method developed on this work provides *quasi*-online results and is suitable for process control and optimisation. The procedure was first developed for *E. coli* and was quickly adapted to CHO cells. It is therefore likely that it can be modified for application with other prokaryotic and eukaryotic systems.

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