

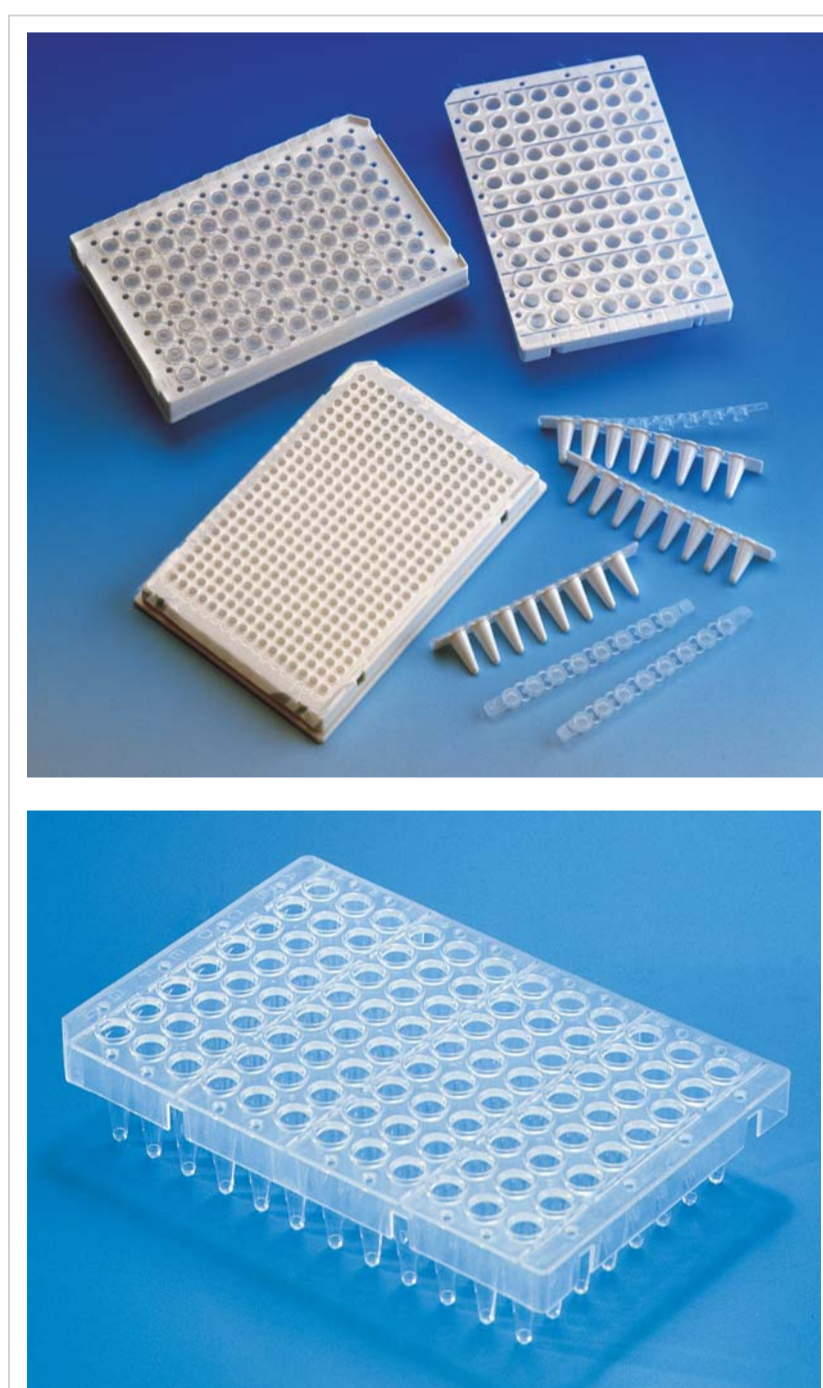


## INTRODUCTION

SYBR® Green I offers a simple and economical approach to DNA quantification whereby the fluorescent signal increases in direct proportion to the accumulation of double-stranded DNA products. Accurate quantification of low copy-number sequences and rare targets is dependent on reaction sensitivity and reproducibility. Both sensitivity and reproducibility can be increased by using opaque PCR plates in place of transparent PCR plates and by ensuring the integrity of the seal is maintained throughout the duration of the PCR process. Novel surface treatments for PCR plates can also improve reaction sensitivity and in particular reproducibility, making it possible to detect as few as 10 copy numbers of template DNA per reaction.

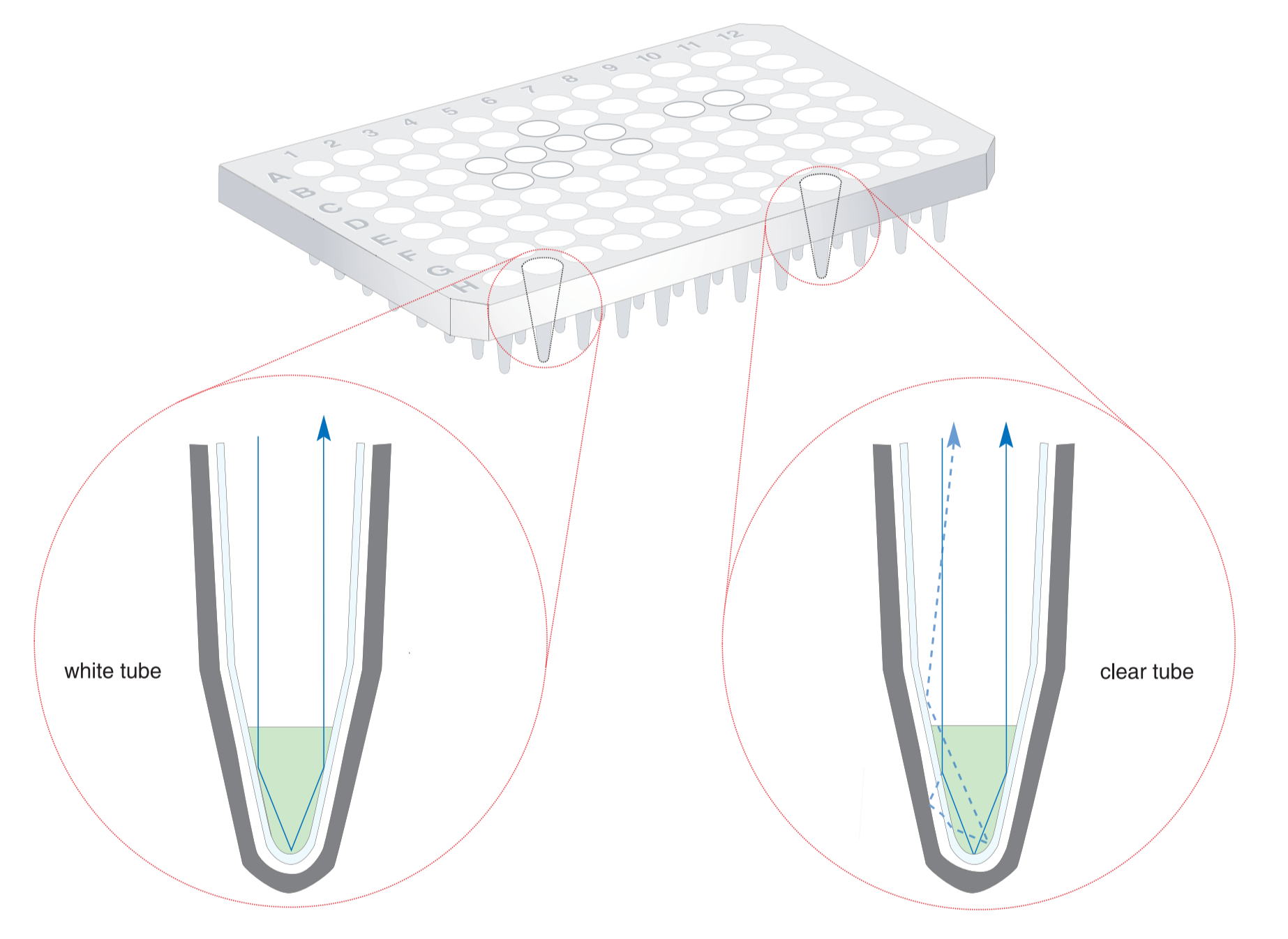
## MATERIALS AND METHODS

Quantitative PCRs were performed using ABgene® ABsolute™ SYBR® Green Fluorescein Mix (AB-1219) with human genomic template DNA and GAPDH primers. ABgene® Thermo-Fast® 96 PCR plates (AB-0900, AB-0900/w) were sealed using Clear Seal Diamond (AB-3799). A Bio-Rad® Laboratories iCycler iQ™ Real-Time PCR detection system (black anodised block) was used throughout.



A range of ABgene® Thermo-Fast® white and transparent polypropylene consumables.

Figure 1. Light is reflected from the interior of a white tube wall to the fluorescent detector ensuring uniform signal detection. In contrast, light can be scattered in a transparent tube by refraction through the tube wall to the heated block from where it can then be refracted back through the tube wall causing variability in the signal detected.



## RESULTS

### Transparent Versus White PCR Plates

White microwell plates are commonly used for fluorescent applications because the pigment used to colour the plates causes the signal to be reflected without absorption in contrast to black plates that absorb signal. Transparent plates are primarily used for PCR and QPCR applications to allow visualisation of the well contents. The disadvantage of using transparent plates for QPCR is that the signal can pass through the well wall (figure 1) where it may be absorbed by the heated block (if black), or reflected (if silver). Results show that white plates offer an advantage over transparent plates in

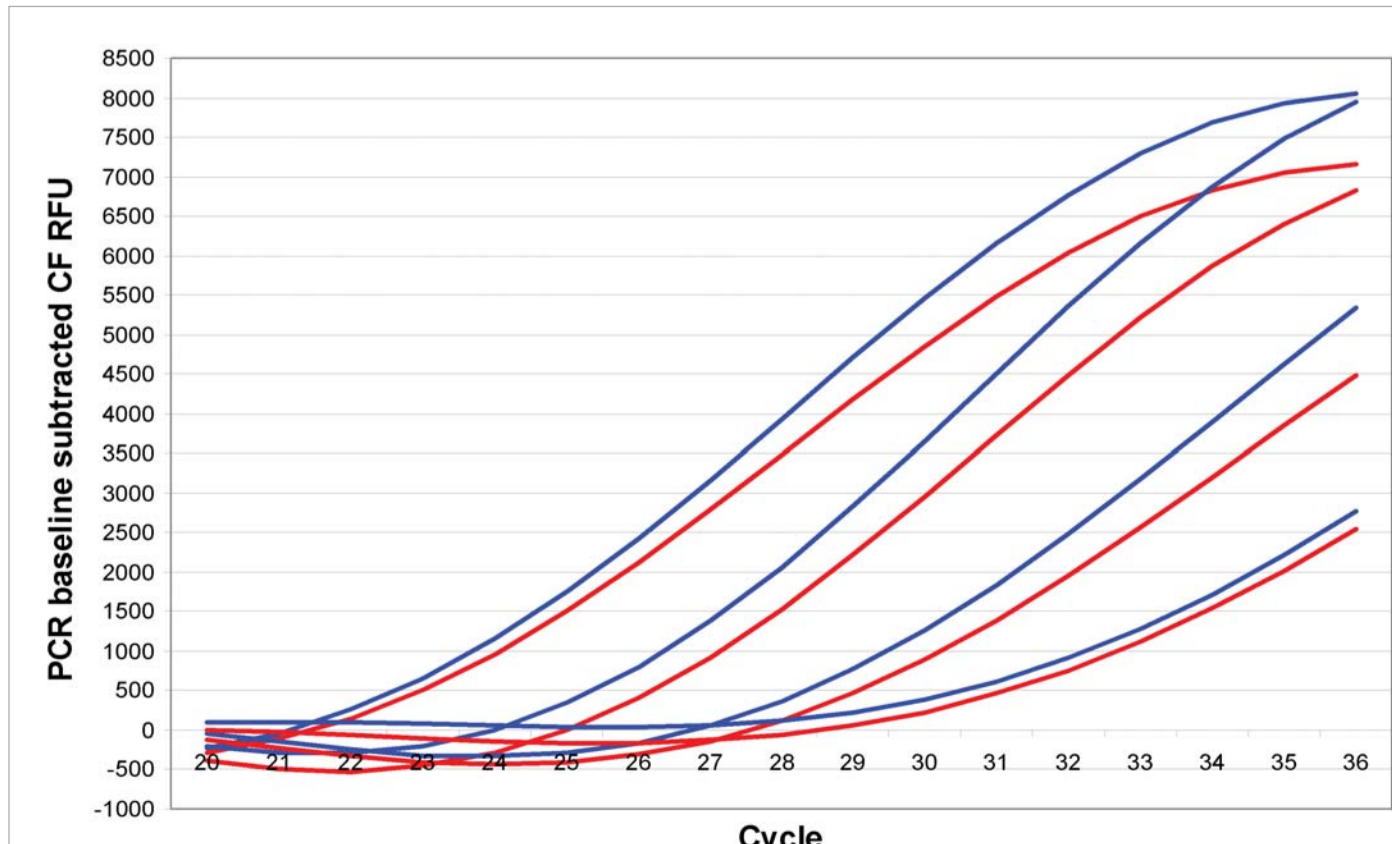
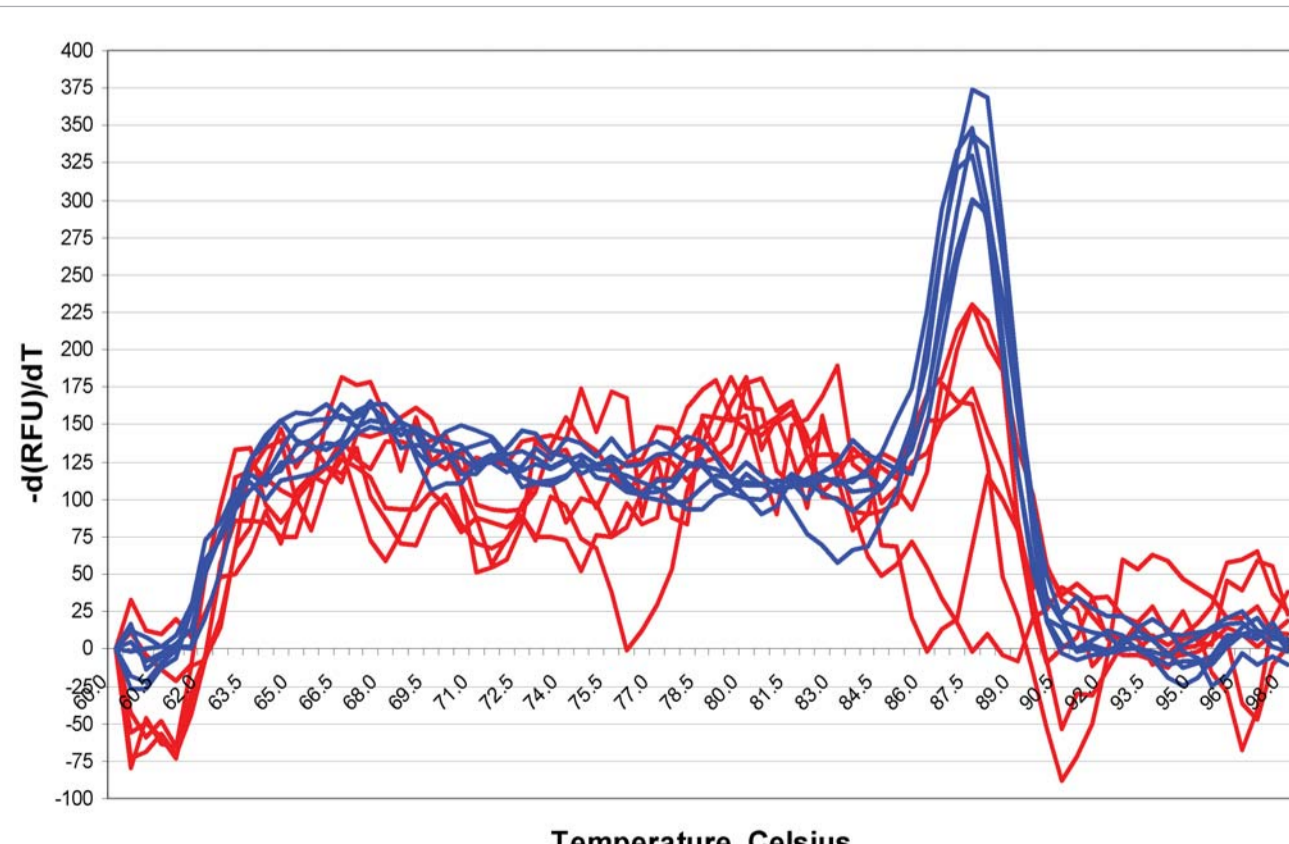


Figure 2. Amplification curve comparing the performance of transparent and white PCR plates. Multiple reactions of a template dilution series were analysed simultaneously using a clear PCR plate (red line) and a white PCR plate (blue line) and the mean values plotted.

Figure 3. Melt curves comparing the performance of transparent and white PCR plates. Multiple reactions containing 10 copies of DNA were analysed simultaneously using a clear PCR plate (red lines) and a white PCR plate (blue lines).



QPCR, with Ct values appearing up to 1 cycle earlier and endpoints values increased by up to 14% (figure 2). Although white polypropylene is known to exhibit a slightly higher degree of autofluorescence compared to clear polypropylene, we have shown that the signal-to-noise ratio is in fact higher in the white PCR plate than in an equivalent transparent PCR plate. When analysing the melt curves from low copy numbers of template DNA (10 copies per reaction), the target PCR products can be detected using a white PCR plate but cannot be detected easily using a transparent plate because the signal generated is obscured by background signal (figure 3). Studies show however, that variations in pigment type and concentration used by different plate manufacturers can affect the changes observed (data not shown).

### Sample Evaporation

One factor affecting accurate quantitation of DNA is the ability to maintain a constant reaction volume throughout. Reaction volumes of 25µl are commonly used in QPCR and a loss in reaction volume due to evaporation can have a marked effect on reaction sensitivity. Simulation studies show that even a 4% loss in reaction volume can cause delayed Ct values by up to 1 cycle, and a reduction in endpoint values of up to 20% (figure 4). This ultimately affects reproducibility and can result in an under-estimation of template DNA concentration, highlighting the importance of seal integrity during QPCR.

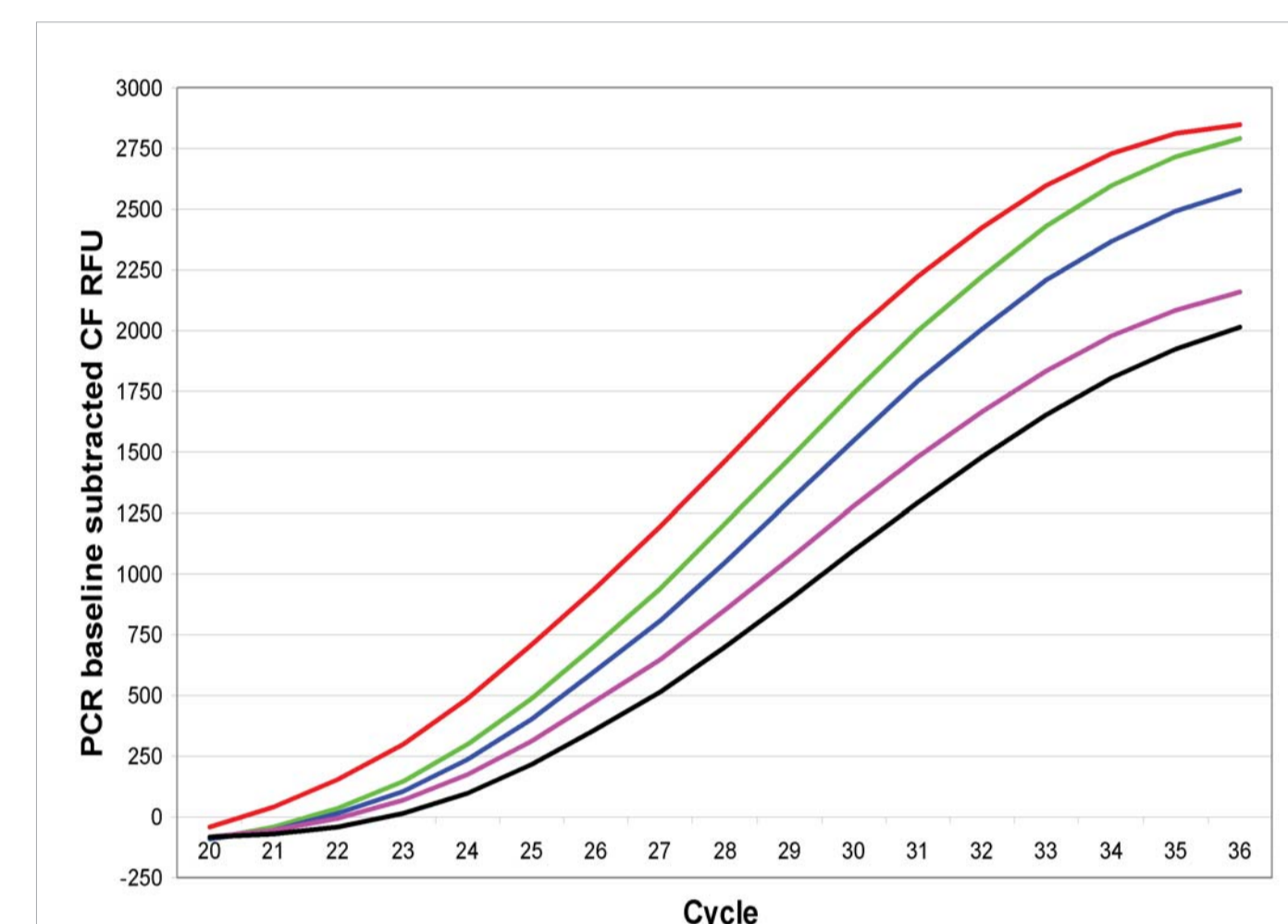


Figure 4. Amplification curve demonstrating the effect of simulated evaporation on DNA quantification. Multiple reactions with the same quantity of DNA were analysed and the mean values plotted. The reaction volume was reduced in 1µl increments.

### Surface Treatment

Current studies suggest that treating the surface of PCR plates with a novel surface treatment can also increase reaction sensitivity. Endpoint values can be increased by up to 14% and Ct values can be reduced by up to 0.5 cycles (figure 5). Further studies will reveal whether a similar advantage is observed when applied to assays utilising dual-labelled oligonucleotides.

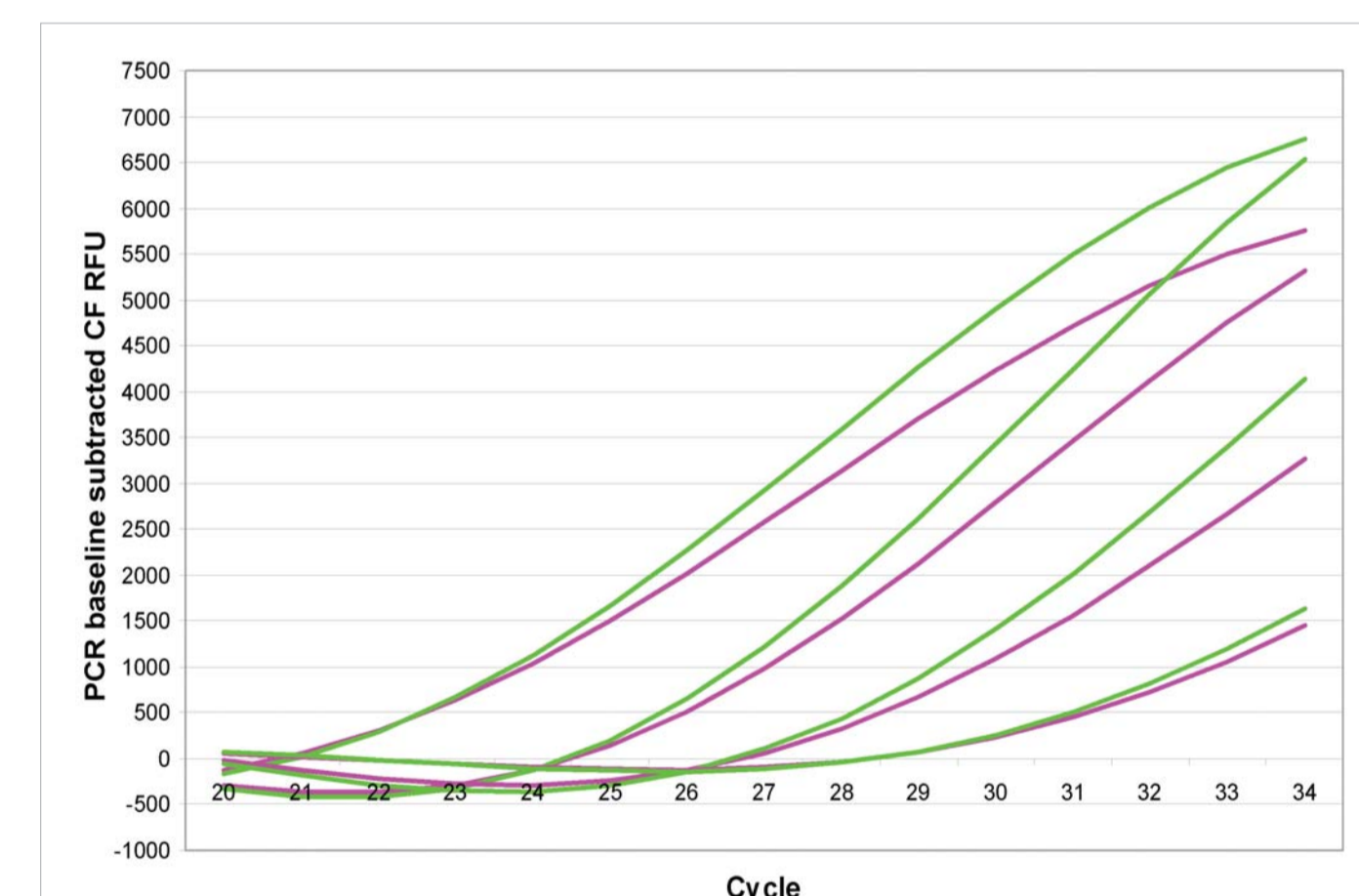


Figure 5. Amplification curve demonstrating the effect of using a PCR plate treated with a proprietary surface treatment. Multiple reactions of a template dilution series were analysed simultaneously using an untreated PCR plate (magenta lines) and a treated PCR plate (green lines), and the mean values plotted.

## CONCLUSION

- Sensitivity can be increased by using white PCR plates, enabling detection of low template DNA concentrations with SYBR® Green I.
- Sensitivity and reproducibility are compromised by losses in reaction volume due to evaporation. This can easily be prevented by using a robust sealing method.
- Sensitivity of SYBR® Green I assays can also be improved following treatment of the surface of PCR plates with a proprietary surface technology.

## ACKNOWLEDGEMENTS

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