Miniaturizing qPCR reactions.

Application Note

PCR SET-UP WITH THE TECAN D300E.

TECAN.

INTRODUCTION

Since its development in 1983, PCR (polymerase chain reaction) has become an indispensable tool for a wide variety of applications in medical and life science research labs. In combination with fluorophores, it can even serve as a direct, quantitative reporting mechanism for the abundance of a target transcript in a sample (quantitative real-time PCR, qPCR). This information can be used to diagnose infectious diseases, cancers and genetic abnormalities, as well as to probe food samples for contamination with bacteria or genetically-modified organisms. As the technique has been widely available for over 30 years, there is now a plethora of different reagent sets available, but they all have one thing in common – they are a main cost of PCR experiments. Assay miniaturization therefore offers significant potential for cost savings. However, miniaturization is limited by minimum volume which can be reliably dispensed to obtain reproducible results. This application note describes the use of the Tecan D300e for setting up qPCR reactions using a range of different master mixes and reaction volumes, as well as comparing it to manual reaction set-up.

EXPERIMENTAL

Scaling down an assay is always a compromise between reagent savings and reliability of the results. The efficiency and precision of the qPCR reactions was therefore compared at different reaction volumes. The primers were pre-dispensed into a 384-well plate, then Precision Melt Supermix (Bio-Rad) was dispensed at various volumes (1, 1.5 and 2.5 µl). 1 µl of cDNA (1 ng/µl) containing 0.1 % Triton™ X-100 was dispensed into each reaction well. An additional 5 µl of 1:1 cDNA (0.4 ng/µl + 0.1 % Triton X-100) and master mix was dispensed into each well to achieve a total of 1 ng of DNA per reaction. Three replicates were performed for each point, and the results demonstrate that similar cycle thresholds (Cts) are achieved for reaction volumes down to 2 µl. (Figure 1)

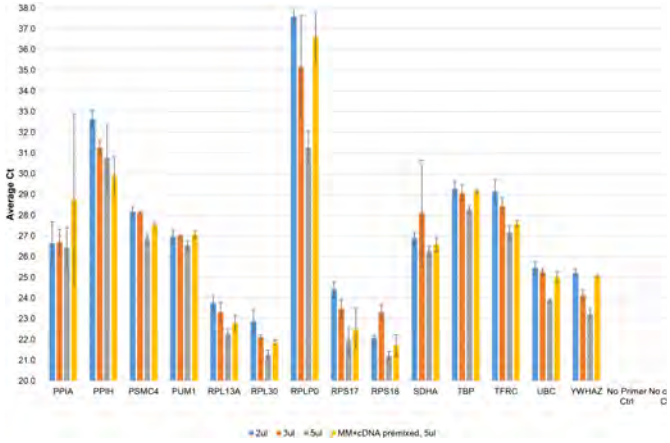

Figure 1: Comparison of Cts for different reaction volumes showing similar results for volumes down to 2 µl. Triplicates were performed for each sample and error bars represent standard deviations.

Reactions set up with the D300e were also compared with those prepared manually. Fast SYBR® Green (Thermo Fisher Scientific), master mix was dispensed into a 384-well plate which was pre-plated with primers, and then samples were added by hand. Triton X-100 or Tween® 20 was added to achieve final concentrations of 0.1 and 0.3 % respectively in total reaction volumes of 5 µl, and three replicates were performed for each point. The results show a good correlation between the manually pipetted and automated set-ups, and also indicate that no detergent is required for the reliable dispensing of the master mix (Figure 2).

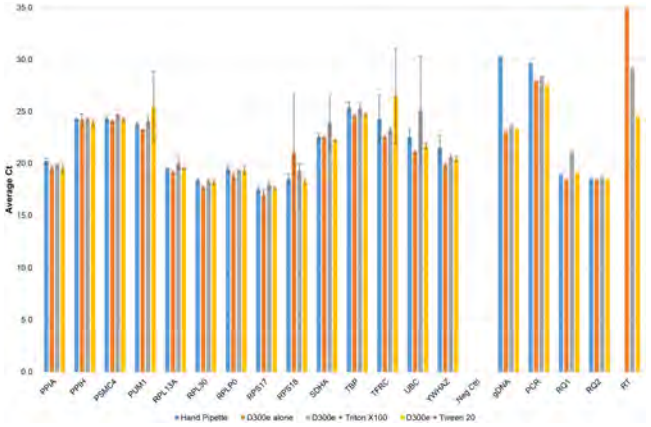

Figure 2: Comparison of Cts for reaction set-up by hand or by D300e with or without the addition of detergents in total reaction volumes of 5 µl. Fast SYBR® Green (Thermo Fisher Scientific) master mix was used and triplicates were performed for each sample and error bars represent standard deviations.

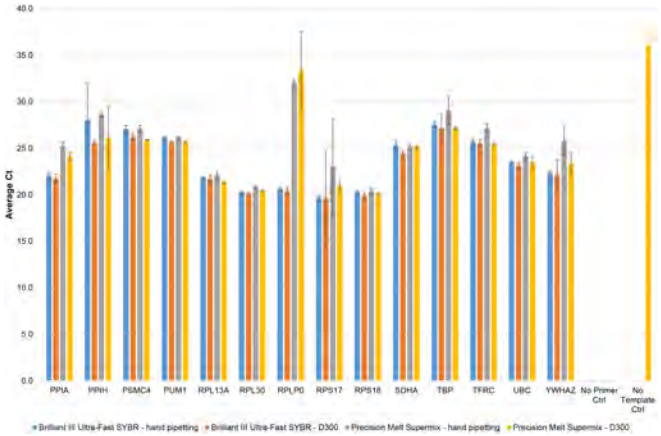

Figure 3: Comparison of Cts for reaction set-up by hand or by D300e in total reaction volumes of 5 µl. Brilliant III Ultra-Fast SYBR® (Agilent Technologies) and Precision Melt Supermix (BioRad). were used, triplicates were performed for each sample and error bars represent standard deviations.

To compare different master mixes, the experimental set-up was performed in 5 µl reaction volumes (as described previously) using Brilliant III Ultra-Fast SYBR® (Agilent Technologies) and Precision Melt Supermix (BioRad). Even though specific transcripts appear to be more easily detected by one master mix than the other, the results achieved by manual and automated set-ups are comparable, with no significant differences (Figure 3).

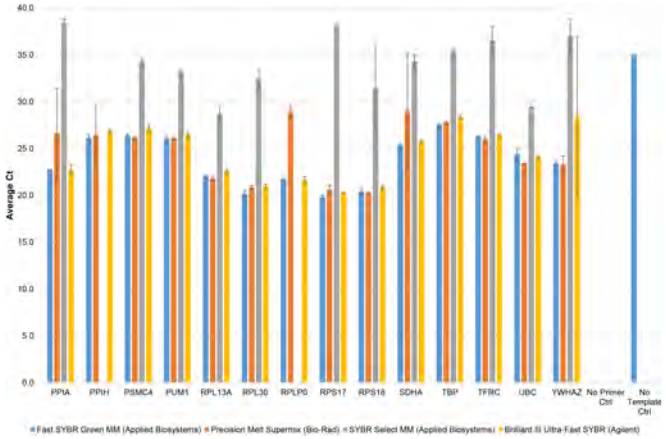

Figure 4: Comparison of Cts for reaction set-up using the D300e in total reaction volumes of 5 µl. The master mixes are indicated in the figure. Triplicates were performed for each sample and error bars represent standard deviations.

The performance of the different master mixes was further investigated using reagents from four different suppliers. The primers were once again pre-plated, but this time the samples were dispensed with surfactant (0.1 % Triton X-100), and the master mixes were dispensed without the addition of a surfactant. Again, triplicates were performed for each point. It becomes apparent from the results that the master mixes show largely comparable performance in detecting the test transcripts (Figure 4). Whether the increased Ct values for the SYBR select master mix is due to this reagent being more sensitive to detergents or lower general performance cannot be determined from these experiments, but the susceptibility of the reagent of choice to detergents must be considered for all reaction set-ups.

CONCLUSIONS

The data presented in this application note demonstrates that the D300e can be used to miniaturize qPCR reactions to volumes as low as 2 µl, which could not be achieved by manual pipetting. In addition to the reagent savings achieved through miniaturization, the greater process security and speed of the automated set-up offers further benefits for genomics workflows.

ACKNOWLEDGEMENTS

Data courtesy of Robbie Allen and Dylan Nelson from Oregon Translational Research and Development Institute, Portland, USA.

About the author

Dr. Manuel Bauer joined Tecan Switzerland as a product manager in 2013 and is responsible for the Tecan D300e and the options portfolio. He studied Biology at the University of Würzburg and Free University of Berlin. During his PhD at the ETH Zürich he focused on systems biology and has also applied various proteomics techniques during his post doc at the Biozentrum Basel.

For research use only. Not for use in diagnostic procedures.

Australia +61 3 9647 4100 **Austria** +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 28 98 63 33 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80
Germany +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Singapore** +65 644 41 886 **Spain** +34 93 490 01 74
Sweden +46 31 75 44 000 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 8125

www.tecan.com

399175 V1.0 06-2016