

Automation Tip Comparison

Performance comparison of automated tips on an automated TaqMan assay

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The challenges of performing real time PCR are greatly reduced when using the most suitable equipment. Automating TaqMan® assays can greatly help reduce pipetting errors and improve precision and accuracy. In order to ensure the best tools are used for pipetting TaqMan assays, we plated several TaqMan assays and compared the results from using Thermo Scientific™ Automation Tips and a competitor's automation tips on the Tecan™ Freedom EVO™. Thermo Scientific™ Automation Tips showed less standard deviation between threshold cycle (Ct) values in the replicates, lower coefficient of variation (%CV), and demonstrated a higher precision than the competitor's automation tips. When the copy number variation (CVN) was compared between two different DNA sources, both sets of tips had the same copy number for the TaqMan assay, validating the accuracy of the Thermo Scientific Automation Tips.

Introduction

Real Time PCR (qPCR) is an extremely valuable and widespread tool in molecular biology, having applications in research, diagnostics, and forensics. It can be used for gene expression analysis, microRNA and noncoding RNA analysis, SNP genotyping, and to determine copy number variation.

TaqMan probes are an extremely accurate method to employ when performing qPCR. They offer increased specificity when compared to primers alone. TaqMan probes are labeled with a fluorophore on the 5' end and a quencher on the 3' end. The polymerase extends from

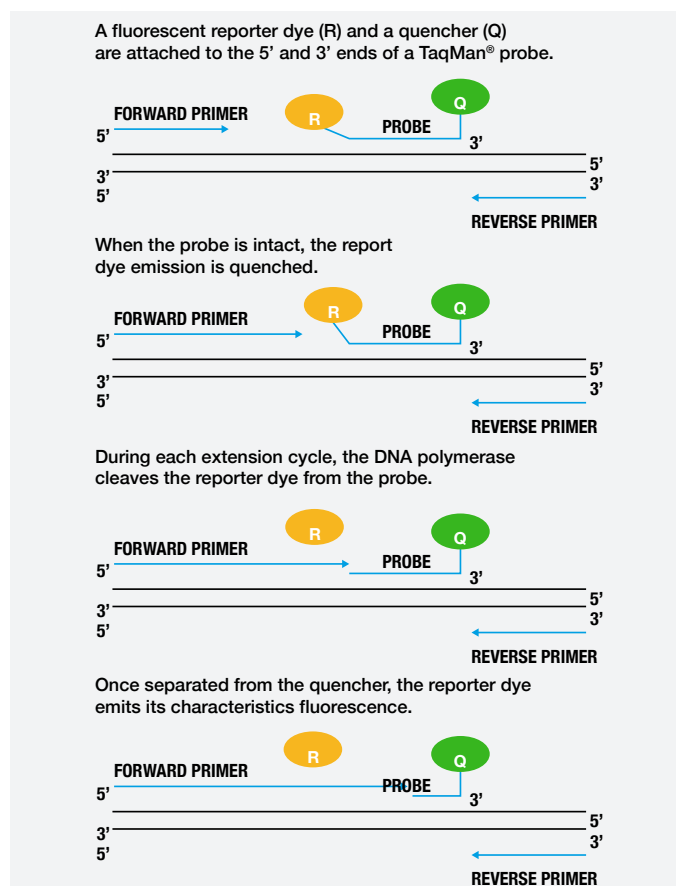


Figure 1. Overview of the TaqMan chemistry

the primer and when it reaches the TaqMan probe, the 5' to 3' exonuclease activity cleaves the fluorophore releasing it from the quencher and allowing its detection by the thermal cycler (Figure 1).

The need for precision and accuracy when performing qPCR is paramount. It is an extremely sensitive assay and slight differences due to pipetting errors can be disastrous. When combined with the large number of reactions that are often needed, this can become extremely time consuming and cause repetitive motion injuries. Automating qPCR has a huge advantage in preventing these issues and by using Thermo Scientific™ Automation Tips you can help ensure excellent repeatability, reproducibility, and accuracy, helping prevent pipetting errors that lead to incorrect data and costly reruns.

The purpose of this study is to highlight the precision and accuracy of Thermo Scientific Automation Tips for qPCR. To illustrate this we used the Tecan™ Freedom EVO™ system to automate the Applied Biosystems™ TaqPath™ ProAmp™ on two samples for CVN analysis (Figure 2). The sample preparation was automated using the Thermo Scientific™ 50 µL and 10 µL automation tips for Tecan™ LiHa and compared with equivalent competitor automation tips. The comparison demonstrates the precision of the Thermo Scientific Automation Tips and how effectively they can work on the Tecan™ Freedom EVO™ system in this and any application.

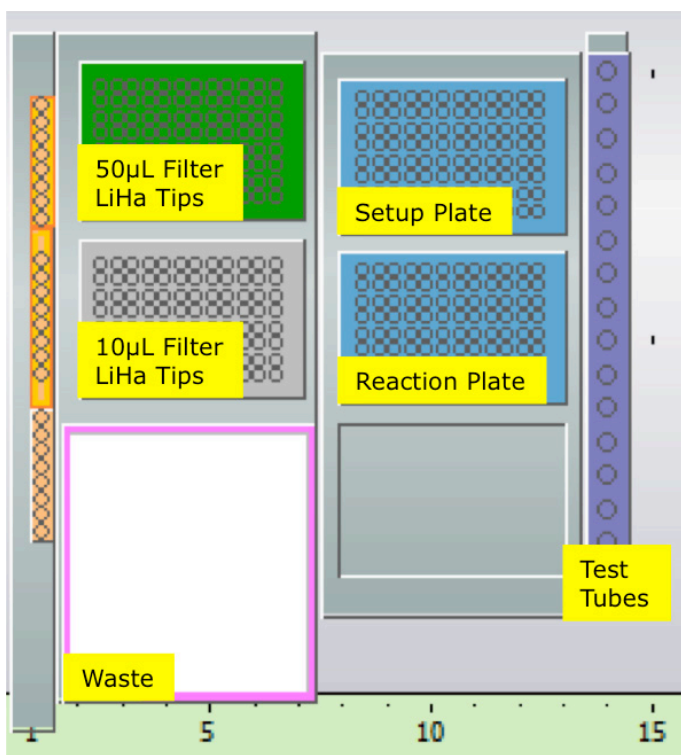


Figure 2. Deck layout using Thermo Scientific Automation Tips for TaqPath™ ProAmp™.

Materials and Methods

- Thermo Scientific Automation Tips
 - 10 µL conductive, sterile, ART barrier tips for Tecan™ LiHa - P/N 901-011
 - 50 µL conductive, sterile, ART barrier tips for Tecan™ LiHa - P/N 902-011
- Competitor's automation tips
 - 10 µl tips for Tecan™ LiHa – conductive, sterile, filtered
 - 50 µl tips for Tecan™ LiHa – conductive, sterile, filtered
- Tecan™ Freedom EVO™ Series with LiHa head was utilized for pipetting operations.
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1 mL (A28138)
- Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL (4346907)
- Applied Biosystems™ MicroAmp™ Optical Adhesive Film (4360954)
- Thermo Scientific™ Jurkat Genomic DNA (SD1111)
- Applied Biosystems™ TaqMan® Control Genomic DNA (4312660)
- Invitrogen™ RT-PCR Grade Water (AM9935)
- Applied Biosystems™ TaqMan® Copy Number Reference Assay, human, RNase P (4403328)
- Applied Biosystems™ TaqMan® Copy Number Assay, CYP2D6
- Applied Biosystems™ TaqPath® ProAmp™

Protocol

1. Make master mixes for samples according to user guide¹. Combine the TaqPath™ ProAmp™ Master Mix, the RNase P TaqMan™ Copy Number Reference Assay, the CYP2D6 TaqMan™ Copy Number Assay, the RT-PCR Grade Water, and the gDNA for 5 replicas. Repeat for each gDNA sample and a negative for each of the assays.
2. Mix the samples on an orbital shaker and spin down in a centrifuge so there are no bubbles.
3. For each sample and negative, transfer four replicas to an empty MicroAmp™ Fast Optical 96-Well Reaction Plate.
4. Seal plate with the MicroAmp™ Optical Adhesive Film.
5. Spin down in a centrifuge so there are no bubbles.
6. Using the same conditions, repeat all steps for the competitor's automation tips.
7. Run on Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System².

Real Time PCR Protocol

The samples were amplified using the following protocol:

1. Heated at 95°C for 5 min.
2. Performed 40 cycles of 95°C for 5 sec and 60°C for 30 sec.
3. Analyzed the data using the Design and Analysis Application and the Relative Quantification on the Thermo Fisher™ Connect™ Cloud.

Results

The performance of Thermo Scientific Automation Tips on a Tecan™ Freedom EVO™ system using a LiHa head was evaluated as an ideal way to perform qPCR using Applied Biosystems™ TaqPath™ ProAmp™ Master Mix. All data points were averaged from quadruplicate replicas. The Thermo Scientific Automation Tips were compared with the competitor's automation tips and displayed highly consistent threshold cycle (Ct) values within the replicas of TaqMan assays compared to TaqMan assays pipetted with the competitor's automation tips (Figure 3).

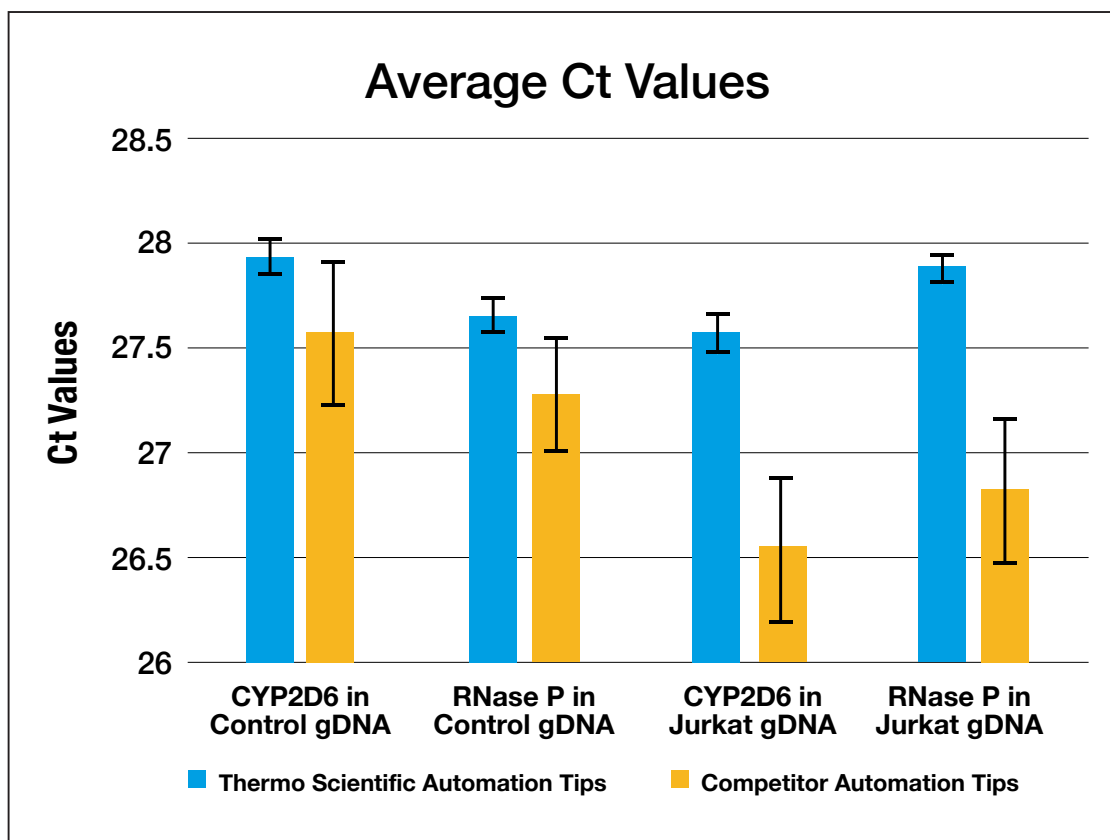


Figure 3. Quadruplicate average Ct and standard deviation for Thermo Scientific Automation Tips vs the competitor's automation tips.

Thermo Fisher™ Scientific™ Automation Tips				Competitor Automation Tips		
Sample DNA	Average Ct	Standard Deviation	%CV	Average Ct	Standard Deviation	%CV
CYP2D6 in Control gDNA	27.937	0.085	0.304	27.574	0.354	1.283
RNase P in Control gDNA	27.662	0.087	0.316	27.287	0.271	0.995
CYP2D6 in Jurkat gDNA	27.558	0.100	0.361	26.557	0.327	1.231
RNase P in Jurkat gDNA	27.888	0.062	0.221	26.845	0.330	1.228

Table 1. Quadruplicate average Ct, standard deviation, and %CV for Thermo Scientific Automation Tips vs the competitor’s automation tips. All of the Thermo Scientific Automation Tips showed a standard deviation of less than 0.1, while the competitor’s automation tips had a standard deviation closer to 0.3. In addition, %CV for the Thermo Scientific Automation Tips was almost 1% lower than the %CV for the competitor’s automation tips.

The low coefficient of variation (%CV) of Thermo Scientific™ Automation Tips (between 0.221 and 0.361) showed a higher precision than the competitor’s automation tips (between 0.995 and 1.283, Table 1).

Using the Thermo Fisher™ Connect™ Cloud, the copy number of CYP2D6 was determined in each of the gDNA samples. Using both the Thermo Scientific™ Automation Tips and the competitor’s automation tips, the copy number of CYP2D6 was established to be 2 in the control gDNA and 3 in the Jurkat gDNA (Figure 4).

To ensure the quality of the data, negatives for each assay were performed. Negative samples are an extremely important part of qPCR to ensure no contamination happened during the pipetting process.

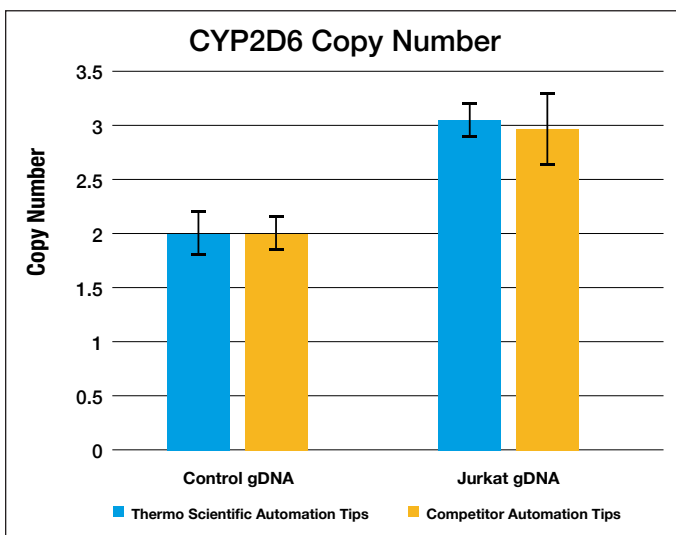


Figure 4. Copy Number difference of target gene between samples. The copy number of CYP2D6 in the control gDNA was 2, while it increased to 3 in the Jurkat gDNA. These results were proven with both Thermo Scientific Automation Tips and the competitor’s automation tips, indicating a high level of accuracy.

Thermo Scientific Automation Tips have ART barriers that maintain the integrity of the samples and provide security against aerosols and liquid contamination. The negative samples had no amplification, highlighting the effectiveness of the ART barrier used in Thermo Scientific Automation Tips and the quality in the tips (Figure 5).

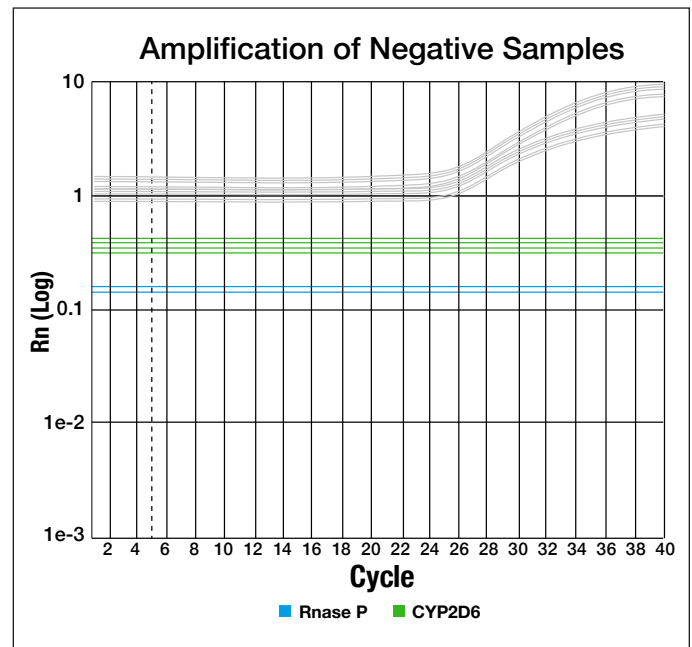


Figure 5. Negative samples for each assay using Thermo Scientific Automation Tips. The graph shows the Rn, or the magnitude of the fluorescent signal at each cycle, for the Thermo Scientific Automation Tips. The grey lines are the samples with gDNA and the highlighted green or blue lines are the negatives for each assay. The negative samples show that no leaking or spraying occurred during the pipetting process.

Conclusions

This study evaluated the suitability of using Thermo Scientific Automation Tips on a Tecan™ Freedom EVO™ with a LiHa head to perform qPCR with TaqMan probes. Both Thermo Scientific Automation Tips and the competitor's automation tips demonstrated the control gDNA had 2 copies of the CYP2D6 gene and the Jurkat gDNA had 3 copies of the CYP2D6 gene indicating a high level of accuracy. Furthermore, the Thermo Scientific Automation Tips had lower standard deviation and lower %CV between replicas than the competitor's automation tips, proving they provide better reproducibility. The ART barriers also help ensure no contamination between samples or in the negatives, enabling the quality of the results. For over three decades Thermo Fisher Scientific has been manufacturing tips for automated liquid handling instruments. We adhere to a rigorous QC process, which includes lot-testing during production on the workstation from which the tips are created and helps ensure a low coefficient of variation. Thermo Fisher Scientific has tips available as sterile, non-sterile, filtered, non-filtered, liquid sensing, and non-liquid sensing. The sterile tips are certified free of RNase, DNase, DNA, endotoxin, bioburden, and pyrogen. Thermo Fisher Scientific has a wide range of pipet tips suitable for use in a 96 well or 384 well format, covering over 50 different liquid handlers. Our tips have equivalent performance to the robotic automation manufacturer's and other aftermarket manufacturer's tips with no need to reprogram the instrument. All of this allows for a seamless fit with identical formats to other manufacturers.

References:

1. TaqPath ProAmp User Manual https://tools.thermofisher.com/content/sfs/manuals/MAN0015758_TaqPathProAmpMMix_UG.pdf
2. QuantStudio 3/5 User Manual https://tools.thermofisher.com/content/sfs/manuals/MAN0010407_QuantStudio3_5_InstallUseMaint_UG.pdf

Ordering Information

Automation pipette tips made to fit the Tecan™ LiHa Liquid Handling System		
Product Code	Description	Packaging
901-011	10µL Conductive, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
901-021	10µL Clear, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
901-251	20µL Conductive, Sterile	96 Tips/Tray, 10 Trays/Pack
901-252	20µL Conductive	96 Tips/Tray, 10 Trays/Pack
901-253	20µL Conductive, Econopak	96 Tips/Tray, 50 Trays/Pack
901-261	20µL Clear, Sterile	96 Tips/Tray, 10 Trays/Pack
901-262	20µL Clear	96 Tips/Tray, 10 Trays/Pack
902-011	50µL Conductive, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
902-021	50µL Clear, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
902-251	50µL Conductive, Sterile	96 Tips/Tray, 10 Trays/Pack
902-252	50µL Conductive	96 Tips/Tray, 10 Trays/Pack
902-253	50µL Conductive, Econopak	96 Tips/Tray, 50 Trays/Pack
902-261	50µL Clear, Sterile	96 Tips/Tray, 10 Trays/Pack
902-262	50µL Clear	96 Tips/Tray, 10 Trays/Pack
903-011	175µL Conductive, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
903-011G	175µL Conductive, Sterile, ART Barrier, Wide Bore	96 Tips/Tray, 10 Trays/Pack
903-021	175µL Clear, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
903-033	175µL Conductive, ART Barrier, Econopak	96 Tips/Tray, 10 Trays/Pack
903-251	200µL Conductive, Sterile	96 Tips/Tray, 10 Trays/Pack
903-251G	200µL Conductive, Sterile, Wide Bore	96 Tips/Tray, 10 Trays/Pack
903-252	200µL Conductive	96 Tips/Tray, 10 Trays/Pack
903-252G	200µL Conductive, Wide Bore	96 Tips/Tray, 10 Trays/Pack
903-253	200µL Conductive, Econopak	96 Tips/Tray, 50 Trays/Pack
903-261	200µL Clear, Sterile	96 Tips/Tray, 10 Trays/Pack
903-262	200µL Clear	96 Tips/Tray, 10 Trays/Pack
904-011	1000µL Conductive, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
904-011G	1000µL Conductive, Sterile, ART Barrier, Wide Bore	96 Tips/Tray, 10 Trays/Pack
904-013	1000µL Conductive, ART Barrier, Econopak	96 Tips/Tray, 10 Trays/Pack
904-021	1000µL Clear, Sterile, ART Barrier	96 Tips/Tray, 10 Trays/Pack
904-251	1000µL Conductive, Sterile	96 Tips/Tray, 10 Trays/Pack
904-251G	1000µL Conductive, Sterile, Wide Bore	96 Tips/Tray, 10 Trays/Pack
904-252	1000µL Conductive	96 Tips/Tray, 10 Trays/Pack
904-252G	1000µL Conductive, Wide Bore	96 Tips/Tray, 10 Trays/Pack
904-253	1000µL Conductive, Econopak	96 Tips/Tray, 50 Trays/Pack
904-261	1000µL Clear, Sterile	96 Tips/Tray, 10 Trays/Pack
904-262	1000µL Clear	96 Tips/Tray, 10 Trays/Pack

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