Challenge
Flexibility to use various types of plastic consumables while ensuring reliable and reproducible qPCR results.

Solution
The real-time thermal cycler qTOWERiris by Analytik Jena allows for the use of different qPCR plastic types without affecting the results obtained.

Intended audience
qPCR performing laboratories demanding flexible use of PCR plastic consumables.

Free choice of plastic ware in qTOWERiris

Introduction

The correct choice of plastic for any qPCR experiment is essential to ensure the best fit to the measurement device and thus optimal results. Many manufacturers offer a variety of types of qPCR plastic consumables which differ in material, color, well geometry, format, and volume range[1,2]. In this application note, selected properties of white qPCR plastic are introduced which consistently deliver appropriate results using the qTOWERiris real-time cycler from Analytik Jena.

First, the type of format has to be chosen which defines the sample throughput and volume range. Single tubes (0.2 mL, 0.1 mL) or eight-tube strips (0.2 mL, 0.1 mL) are more suitable for less samples while 24-, 48-, 96-well plates (0.2 mL, 0.1 mL) and 384-well plates (0.02 mL) offer throughput capabilities adapted to the required needs.

In addition, the profile type of the qPCR plastic defines the height properties and likewise the volume range (Figure 1). Low profile (LP) plastic has a total fill volume of 0.1–0.15 mL. Short reaction vessels benefit from minimized air space above the liquid, which reduces evaporation and enhances thermal conductivity in the cycler block. High profile (HP) plastics, sometimes referred to as standard profile, offer a total fill volume of 0.2–0.35 mL and an increased vessel height.

Another aspect is the geometrical structure of plates. Outer framing is available as non-skirt, half-skirt, and full-skirt (Figure 2). Half- and full-skirted plates provide more robustness and an even surface on the edges for robotic grippers. While the half-skirt reaches halfway of the total height of the plate, the full-skirt spans from the top of the plate surface to the bottom of the wells.

Sealing of the qPCR vessels can be achieved by either transparent plastic caps or optical clear foils for tubes and plates. Caps offer tight closing with minimal evaporation since they are located inside the upper edge of the reaction vessels. However, caps need to be clamped with pressure and thus are not accessible for automation. As the sealing foils sit on the surface over the whole area of all reaction vessels of a particular plastic format, evaporation can occur more easily. The huge benefit of foils is their usability for automation as they can be applied and removed from qPCR plastics by special sealing instruments.

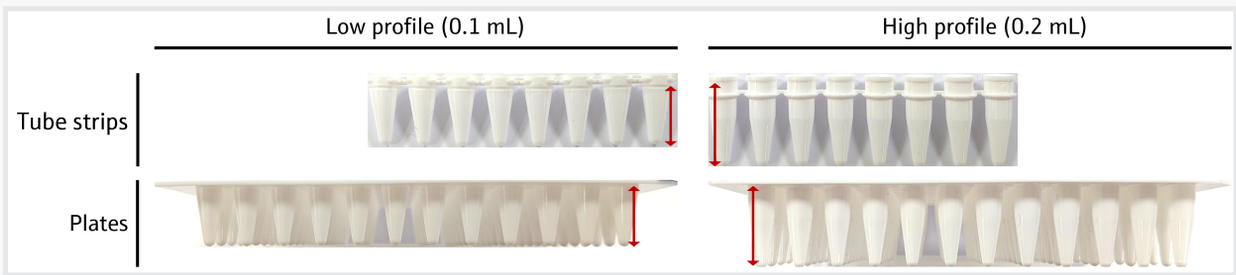

Figure 1: Geometry of PCR plastic strips and 96-well plates. Low profile exhibits shorter height with 0.1 mL (100 µL) volume range, while high profile with longer height offer volumes of up to 0.2 mL (200 µL).

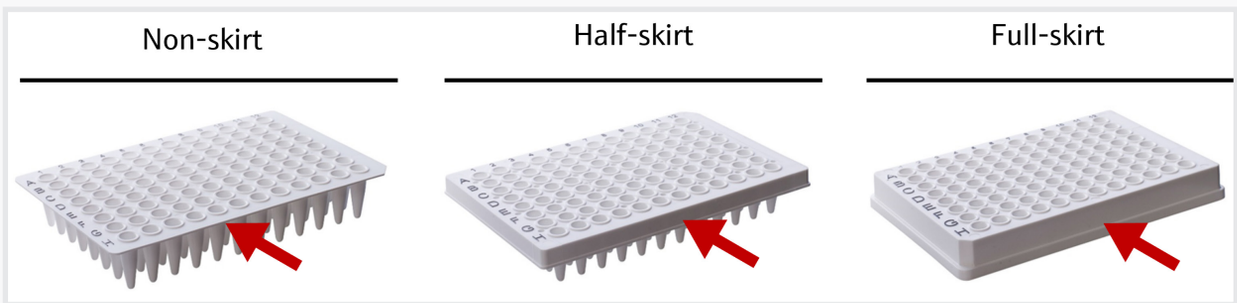

Figure 2: Structure of different edge types from 96-well PCR plates. Non-skirted plates do not exhibit any outer framing. Half-skirted plates extend a halfway outer frame. Full-skirted plates have a outer framing covering the whole height of the plates.

Materials and Methods

A standard SYBR®Green assay including melting curve was performed to compare the performance of different qPCR consumables from different manufacturers using the real-time qPCR thermal cycler qTOWERiris. For each plastic type we employed 24 replications across the whole 96-well PCR block using the thermal protocol described in Table 1. In the analysis, homogeneity of fluorescence signals from the qPCR and melting curve were considered as well as the calculated Ct values and melting temperatures (T_m) between the different plastic types.

Samples and reagents

- Low profile 8 x 0.1 mL strips (white) – manufacturer A
- High profile 8 x 0.1 mL strips (white) – manufacturer A
- Low profile 96 x 0.1 mL non-skirted plate (white) – manufacturer B
- High profile 96 x 0.1 mL non-skirted plate (white) – manufacturer C
- Low profile 96 x 0.1 mL half-skirted plate (white) – manufacturer A
- Low profile 96 x 0.1 mL full-skirted plate (white) – manufacturer A
- Optical sealing foil
- A&A qPCR-HS Mix SYBR® master mix, cat: 2008HS-100
- *Escherichia coli* K12 genomic DNA and specific primer

Instrumentation

The real-time PCR thermocycler qTOWERiris 96 and qTOWERiris 384 (Analytik Jena GmbH+Co. KG) including:
Color module 1, blue (455 nm / 515 nm) was used for FAM™ dye.

Table 1: Overview of a 3-step PCR program for all plastic consumables. Fluorescence measurement was captured at the elongation step across 30 cycles repetitions. Subsequent melting curve was performed using an increment of 1 °C per cycle.

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	2 min	8 °C/sec
2	30x	Denaturation	95 °C	5 sec	8 °C/sec
		Annealing	58 °C	5 sec	5.5 °C/sec
		Elongation	72 °C	15 sec	6 °C/sec
3		Melting curve	60 °C–95 °C	15 sec	

Results and Discussion

Fluorescent signals at the endpoint cycle ranged on average from 39,457 ± 2,060 to 60,579 ± 3,039. The amplification and melting curve results are exemplarily shown in Figure 4 between low and high profile strips. Endpoint fluorescence differed by 7.5% on average. This variation was more apparent between low and high profile non-skirted 96-well plates which differed on average for 21,000 ± 900 fluorescent units or 45%. The variance of endpoint fluorescence between strips, half-skirted and full-skirted plates was around 5% to 11%.
The differences of the captured fluorescence between plastic types, even from the same manufacturer, have to be considered when endpoint signals need to be evaluated for data analysis. Vessel material and thickness can impact fluorescence transmission in the qPCR cycler.

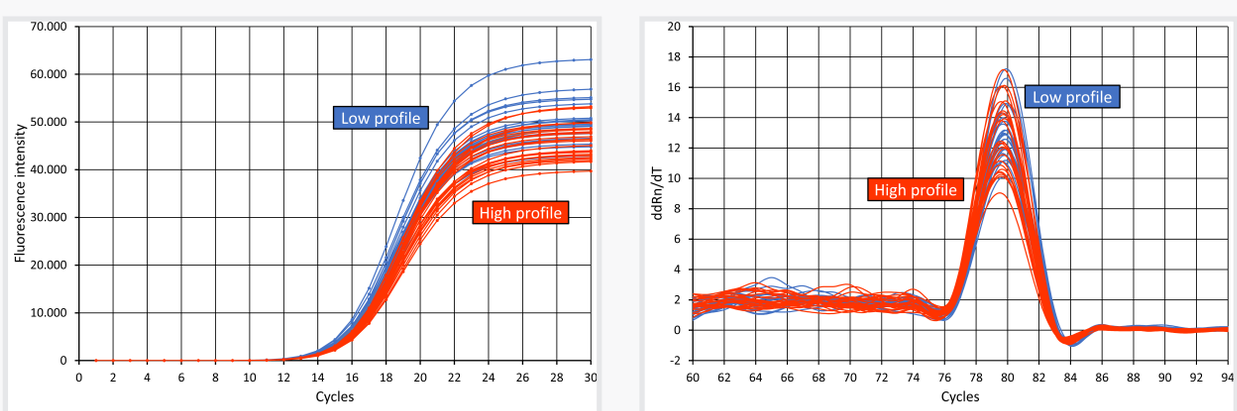

Figure 3: Comparison between low and high profile tube geometry showing highly similar amplification (left) and melting curve (right) patterns. 24 replicates were measured for each profile type.

Despite the differences in endpoint fluorescence, there is less variation between Ct and T_m values, which are crucial for the analysis of qPCR experiments. The measurements between six different qPCR plastics showed high consistency for Ct and T_m values (Figure 4). Obtained Ct values were on average 14.09 with a standard deviation of ± 0.16 and T_m values were on average 79.66 with a deviation of ±0.13. In conclusion, all tested strips and plates were adequate consumables to be used in the qTOWERiris for qPCR. It is important to note that consumables from different suppliers can affect intensity and performance of signal capture due to variations in material, vessel thickness and quality.

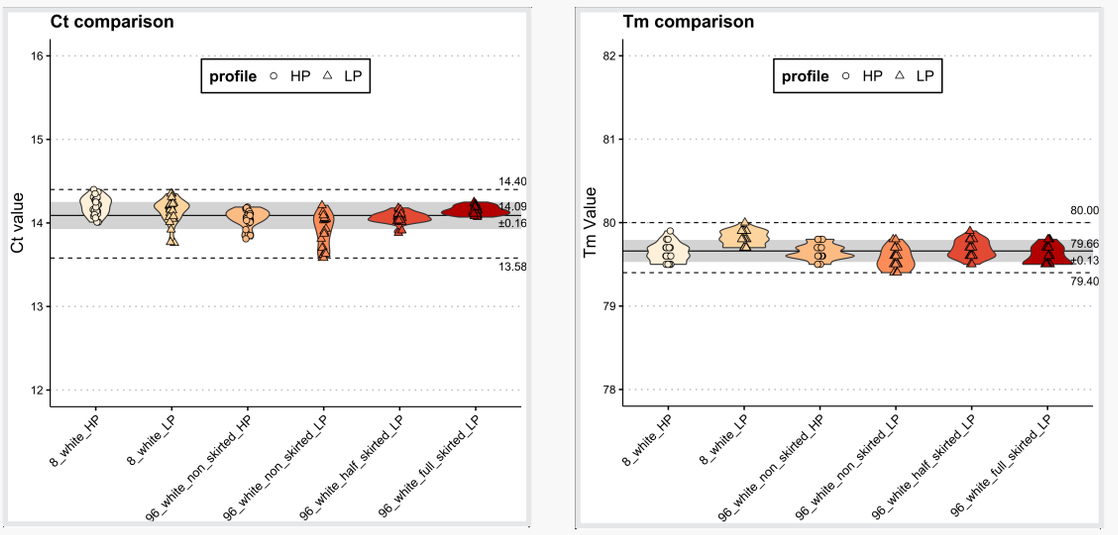

Figure 4: Comparison between low and high profile tube geometry showing highly similar amplification (left) and melting curve (right) patterns. 24 replicates were measured for each profile type. 8: 8-well strip; 96: 96-well plate; HP: high profile; LP: low profile.

Summary

Here, we introduced key features of qPCR plastic consumables and their effect on measurements using the real-time thermal cycler qTOWERiris. It could be shown, that the qTOWERiris with its open system concept allows the use of a broad range of plastic types without affecting result quality. While different qPCR plastic features (profile, outer framing) can have an impact on fluorescent signals they do not compromise Ct and T_m values when used with the qTOWERiris.

Figure 5: qTOWERiris

Recommended device configuration

Table 2: Overview of devices, accessories, and consumables

Article	Article number	Description
qTOWERiris incl. color module 1	844-00853-x*	Real-time PCR system designed in the 96-well format, operable via PC, customizable with up to 6 color modules. Available in 100 V, 115 V, and 230 V version, incl. color module 1 for excitation and detection of fluorescent dyes – FAM™, SYBR®Green, ATTO425 and Cyan500

*x=2 for 230 V, x=4 for 115 V and x=5 for 100 V

References

[1] Placek, J. Five Things to Consider Before Buying Your Next PCR Plate. (2023).

[2] PCR/qPCR Plastics Considerations - DE. https://www.thermofisher.com/de/de/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/pcr-education/pcr-qpcr-plastics/pcr-qpcr-plastics-considerations.html.

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