Sample evaporation: A comparison of several thermal cycler models

Introduction

Reaction evaporation is a common occurrence, and it can often be a significant factor in determining the success or failure of PCR. Evaporation can lead to a change in pH, an increase in salt concentration, and a decrease in thermal mass. Such a change in reaction chemical composition has the potential to alter the uniformity and robustness of amplification, which in many cases is critical for the correct analysis and interpretation of experimental data. This study compares evaporation levels of several thermal cyclers by measuring reaction weight loss over the course of a standard PCR run.

Materials and methods

The instruments tested in this study are shown in Table 1. The same equipment, methods, and reagents were used for each instrument.

Table 1. Instruments tested for sample evaporation.

Manufacturer	Model name	Cat. No.
Bio-Rad	C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module	185-1196
Bio-Rad	T100 Thermal Cycler	186-1096
Bioer	Life ECO Thermal Cycler	BYQ6078
Eppendorf	Mastercycler X50a	6311 000.010
Takara	Dice Touch	TP350
Thermo Fisher Scientific	ProFlex 96-well PCR System	4484075
Thermo Fisher Scientific	VeritiPro 96-well Thermal Cycler	A48141
Thermo Fisher Scientific	SimpliAmp Thermal Cycler	A24811
Biometra	TOne	846-x-070-301

Sample preparation

A single bulk mock reaction was prepared by addition of green food dye to Applied Biosystems™ TaqMan™ master mix, and the weight of the empty plate (**W1**) recorded prior to dispensing the required volume into the appropriate wells. After pipetting the mock reaction into each well, the weight of the plate with reaction mix was recorded (**W2**). The plate seal was applied and the final pre-run weight recorded (**W3**). This methodology was also followed for testing evaporation using an 8-tube strip.

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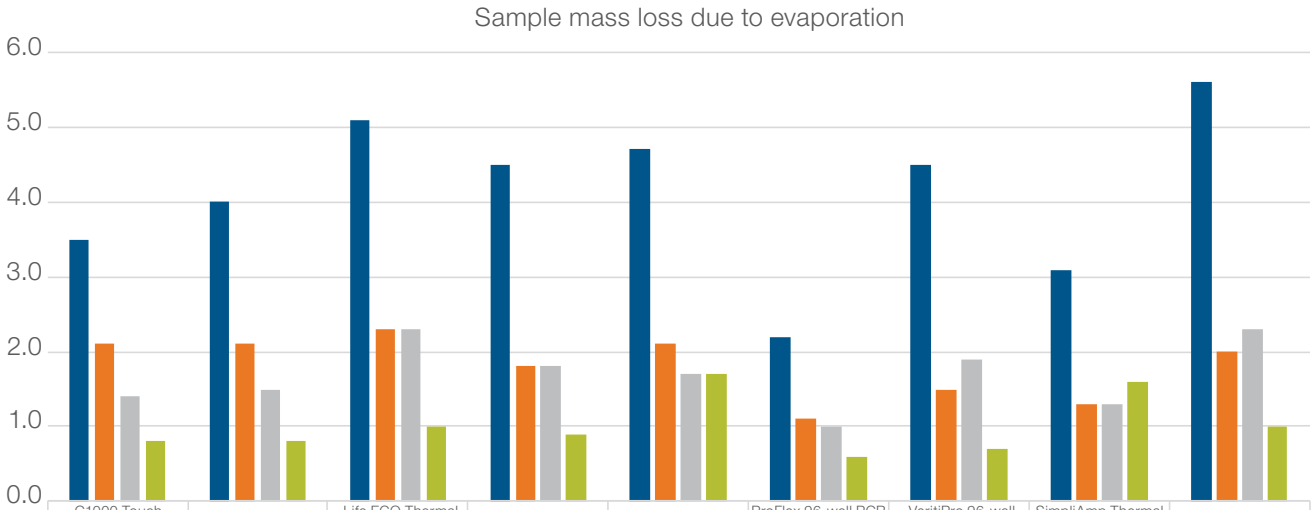
Data acquisition and analysis

The mock reactions were subjected to a standard PCR thermal profile consisting of 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. After thermal cycling, the consumable weight was recorded for a final time (**W4**) and percent sample loss was calculated using the formula below.

$$\text{Sample mass loss (\%)} = \frac{(W3 - W4)}{(W2 - W1)} \times 100$$

Results

Figure 1 shows the average percent sample mass loss due to evaporation over the course of a standard PCR thermal profile. The individual values are specified below the chart, and the group average of 1.8% is indicated as a red line.



	C1000 Touch Thermal Cycler	T100 Thermal Cycler	Life ECO Thermal Cycler	Mastercycler X50a	Dice Touch	ProFlex 96-well PCR System	VeritiPro 96-well Thermal Cycler	SimpliAmp Thermal Cycler	TOne
20 µl, 96-well plate	3.5	4.0	5.1	4.5	4.7	2.2	4.5	3.1	5.6
20 µl, 8-tube strips	2.1	2.1	2.3	1.8	2.1	1.1	1.5	1.3	2.0
50 µl, 96-well plate	1.4	1.5	2.3	1.8	1.7	1.0	1.9	1.3	2.3
50-µl, 8-tube strips	0.8	0.8	1.0	0.9	1.7	0.6	0.7	1.6	1.0

Discussion

The ability of a thermal cycler to uniformly and robustly amplify each well of the reaction plate is partially dependent on the level of evaporation that takes place. In this study, we have shown that evaporation as measured by percentage loss in weight varies between the thermal cyclers tested. This was carried out as a side-by-side comparison while utilizing the same mock reaction chemistry and methodology.

For best results, we recommend using a thermal cycler that exhibits consistently low evaporation across all plastic consumables and reaction volumes. The results presented confirm that Applied Biosystems™ thermal cyclers demonstrate consistently low evaporation, independent of the plastics and volumes examined here.

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