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. Sample preparation

Manufacturer	Model name	Cat. No.
Thermo Fisher Scientific	Applied Biosystems™ ProFlex™ 96-Well PCR System	4484075
Thermo Fisher Scientific	Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
Bioer	GeneMax™ Thermal Cycler	BYQ6067
Bio-Rad	C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module	185-1196
Bio-Rad	T100™ Thermal Cycler	186-1096
Eppendorf	Mastercycler™ Nexus GX2	6336 000.015
Eppendorf	Mastercycler™ Nexus Gradient	6331 000.017
Eppendorf	Mastercycler™ Pro S	6325 000.510
SensoQuest	Labcycler Gradient	011-101, 012-103
Takara	Dice™ Touch	TP350

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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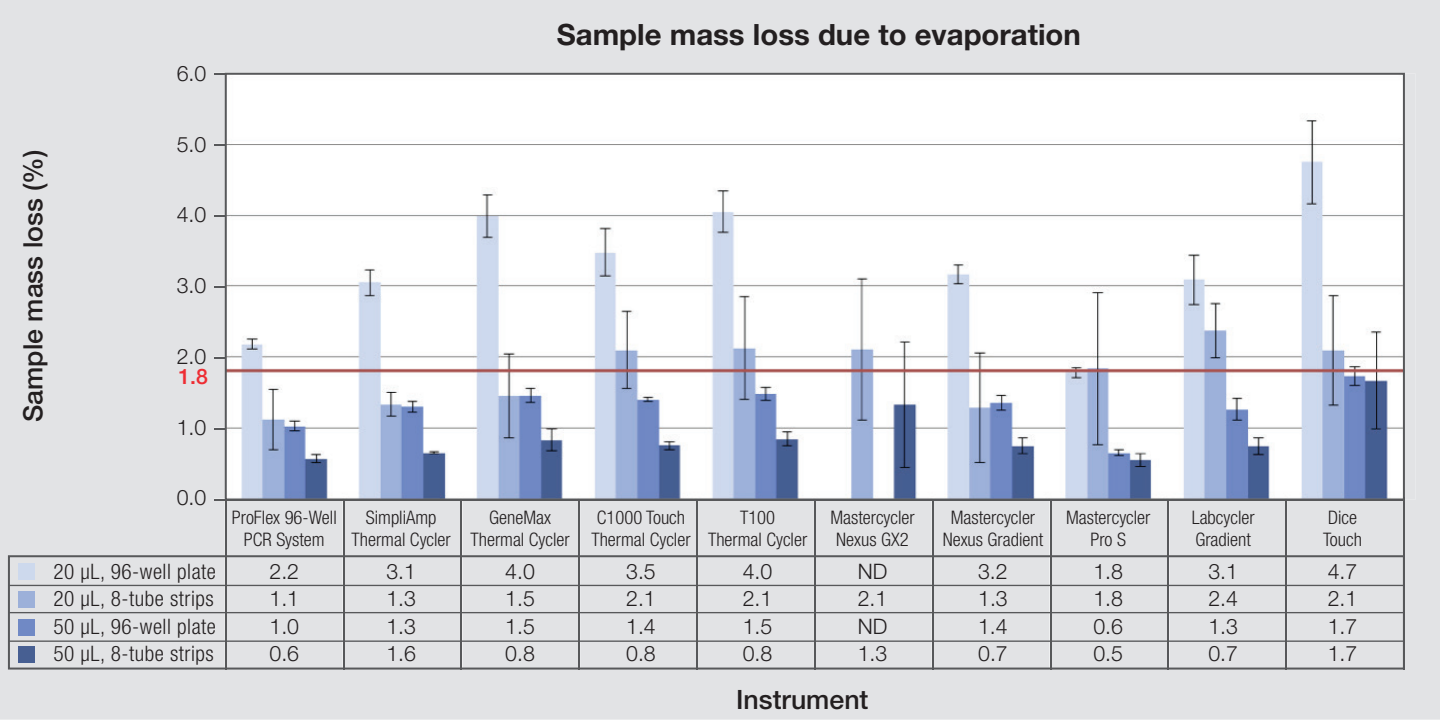
Data acquisition and analysis

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.



	ProFlex 96-Well PCR System	SimpliAmp Thermal Cycler	GeneMax Thermal Cycler	C1000 Touch Thermal Cycler	T100 Thermal Cycler	Mastercycler Nexus GX2	Mastercycler Nexus Gradient	Mastercycler Pro S	Labcycler Gradient	Dice Touch
20 µL, 96-well plate	2.2	3.1	4.0	3.5	4.0	ND	3.2	1.8	3.1	4.7
20 µL, 8-tube strips	1.1	1.3	1.5	2.1	2.1	2.1	1.3	1.8	2.4	2.1
50 µL, 96-well plate	1.0	1.3	1.5	1.4	1.5	ND	1.4	0.6	1.3	1.7
50 µL, 8-tube strips	0.6	1.6	0.8	0.8	0.8	1.3	0.7	0.5	0.7	1.7

Figure 1. Percent sample mass loss of thermal cyclers. Results from two reaction volumes and two plastic consumables are shown. ND = not determined.

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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