Reduce environmental impact while maintaining quality and stability

Abstract

To help minimize the adverse environmental impact of packaging and shipping products on gel ice, Thermo Fisher Scientific investigated the feasibility of shipping Applied Biosystems™ AmpliTaq™ 360 and AmpliTaq Gold™ 360 DNA polymerase and master mix products at ambient temperature. This report describes stability and performance testing of these products after subjecting them to simulated summer ambient shipping conditions. Following testing, these PCR products met the same stability and performance specifications as products shipped on gel ice, throughout their stated shelf life. By shipping at ambient conditions, the need for expanded polystyrene (EPS) coolers and gel ice was eliminated, and the fuel consumption and greenhouse gas emissions from transporting the product were significantly reduced.

Introduction

The adverse environmental impact of shipping refrigerated or frozen products is tremendous. The annual carbon footprint to manufacture EPS and convert it into coolers for our AmpliTaq 360 and AmpliTaq Gold 360 products is approximately 7.7 tons. It takes nearly 17 barrels of crude oil equivalents and 47 MWh of power annually to make the EPS coolers needed to ship our genomic assay products [1]. To ship the AmpliTaq 360 and AmpliTaq Gold 360 products in a refrigerated condition, we add over 18,000 pounds of gel ice to our coolers, further increasing the mass and dimensions of each package. Factoring in the number of shipments and average distance traveled per package and the fact that most packages are shipped via air, the annual total carbon footprint for transporting AmpliTaq 360 and AmpliTaq Gold 360 products is in excess of 64 tons (CO_2-equivalents) [2].

But it's more than just energy consumption and greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often faced with the untenable decision to either burn additional fossil fuel to transport the empty cooler cross-country for reuse/recycling or to dispose of the cooler in a landfill. The best way to address the total environmental impact of cold-chain transport is to follow the hierarchy of "reduce, reuse, recycle": (1) design products for stability to ensure they can withstand the rigors of ambient shipping conditions without added refrigerant or insulation; (2) design packaging to be reusable, without increasing source material consumption; and (3) recycle locally. Thermo Fisher Scientific has opted to reduce whenever possible, reuse when it is an environmentally preferable option, and encourage our customers to recycle locally.

We have been systematically evaluating novel ways to minimize the impact of shipping products on gel ice and the CO_2 footprint generated by these products during distribution. One way to achieve this is to ship products at temperatures consistent with their demonstrated stability. By avoiding the cooler and refrigerant, a product can be shipped in a smaller box, which improves the carrier's freight density (less fuel and emissions per box) and reduces the amount of packaging materials requiring disposal or recycling. By eliminating the cooler and gel ice for these products, Thermo Fisher Scientific is helping to divert an annual total of nearly 2,000 kg (9,500 ft^3) of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and reduce the annual total carbon footprint from transport by 72 tons (CO_2-equivalents) [1,2].

For many years, AmpliTaq and AmpliTaq Gold products were shipped refrigerated on gel ice (with storage before shipping at 4°C or –20°C, depending on the product). This paper describes the results from functional, analytical, and stability testing carried out after AmpliTaq 360 and AmpliTaq Gold 360 products were exposed to established summer shipping profiles. These experiments demonstrated that by shipping our AmpliTaq 360 and AmpliTaq Gold 360 products under ambient conditions, we can supply researchers with the same superior-quality products they are used to receiving and reduce our environmental footprint in the process. This is a win for our customers (eliminating packaging waste), a win for our planet (reducing resource consumption and total carbon footprint), and a win for our company (eliminating the need to manage cold-chain transport).

Materials and methods

Components tested

This stability study was designed to measure the performance of the following products (catalog numbers provided in Table 2):

- AmpliTaq 360 DNA Polymerase with 10X AmpliTaq 360 Buffer and 25 mM $MgCl_2$
- AmpliTaq Gold 360 DNA Polymerase with 10X AmpliTaq Gold 360 Buffer and 25 mM $MgCl_2$
- AmpliTaq Gold 360 Master Mix
- 360 GC Enhancer

The recommended storage temperature for the buffers, the 25 mM $MgCl_2$ solution, and the 360 GC Enhancer solution is –20°C. The recommended storage temperature for the AmpliTaq Gold 360 Master Mix is –20°C, until thawed. Users of the master mix have the option to store it at 4°C after first use. Therefore, stability data were collected at –20°C and 4°C. At each time point, stability was measured based on set criteria for specific yield and percent specificity established at time zero of the functional testing.

Simulated ambient temperature

To simulate temperatures encountered during shipping, samples were placed in an environmental chamber programmed to reproduce a "worst-case" 240-hour (10-day) summer temperature profile (Figure 1), previously developed from testing profiles established at Amgen [3]. This profile mimics temperature extremes encountered in over 2,500 shipments followed during summer shipment of products between the latitudes of 59.9° north and 37.8° south. The maximum temperature reached was 39°C. This study did not test the ambient "winter profile" model, as there was (1) known product stability at reduced temperatures (not reported) and (2) ample data indicating stability to >10 freeze/thaw cycles. After simulation of summer ambient shipping was completed, aliquots of samples were stored at both –20°C and 4°C.

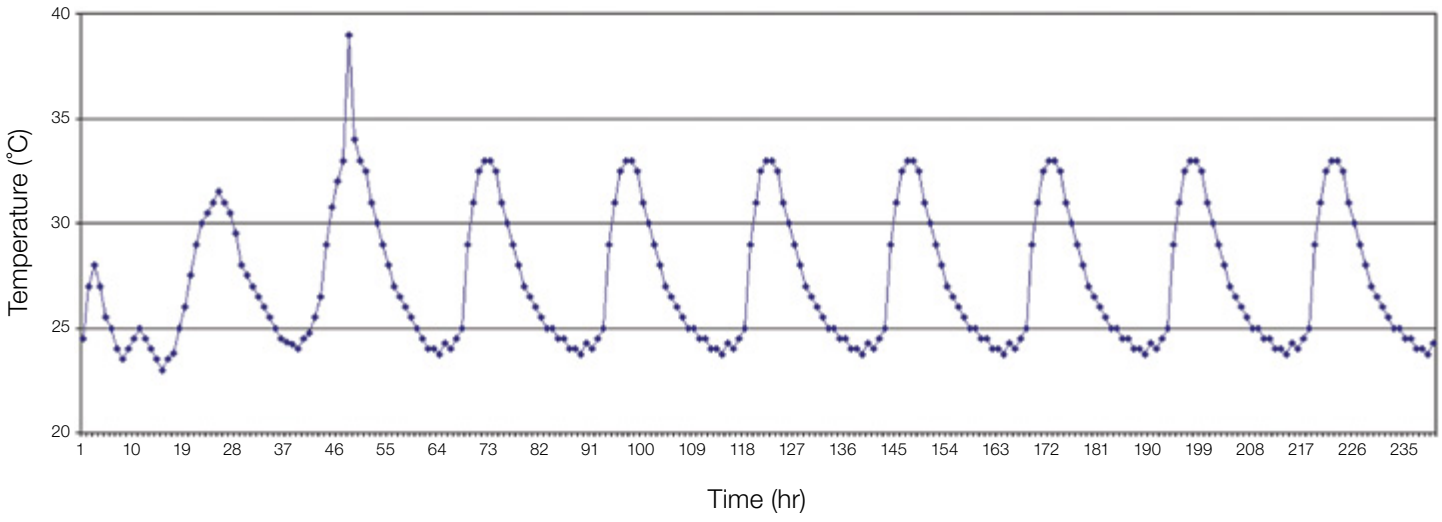

Figure 1. Summer temperature profile used to simulate shipping temperatures. This 240-hour summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° north and 37.8° south (profile derived from the Amgen protocol [3], as described).

Functional testing—specificity and specific yield

For both of the accelerated studies and the real-time study, functional testing was performed using the Applied Biosystems™ GeneAmp™ PCR System 9700 thermal cycler (standard mode; now discontinued) equipped with a 96-well silver block. PCR final reaction volume was set to 50 µL, and the reactions contained 1 ng/µL of template DNA and 200 nM of each primer. Additionally, for the AmpliTaq Gold 360 and AmpliTaq 360 10X buffers, final concentrations of 0.025 U/µL of enzyme (AmpliTaq Gold or AmpliTaq 360 DNA polymerase, respectively) and 2 mM $MgCl_2$ were included.

At each time point, an aliquot of each buffer or AmpliTaq Gold 360 Master Mix was removed from storage at both –20°C and 4°C. Each assay was performed in six replicates for each temperature. Three no-template controls (NTCs) per amplicon were included for each temperature and in each plate using primers for average GC content (AGC) and high GC content (HGC) amplicons (Table 1). While testing the performance of the 360 GC Enhancer with AmpliTaq Gold 360 Master Mix, the enhancer was used as a 10X solution (5 µL of 360 GC Enhancer/50 µL PCR reaction). Assessment of total yield, percent specificity, and specific yield of PCR samples was performed using a Bioanalyzer™ instrument (Agilent Technologies).

CEPH control human DNA served as the PCR template in all studies. Two different primer pairs were chosen to amplify regions of DNA with average and high GC (Table 1).

Table 1. Characteristics of AGC and HGC amplicons used in this study.

Amplicon	Size (bp)	Percent GC
AGC	553	62.2%
HGC	597	76.7%

Following simulated summer ambient shipping, aliquots of all of the samples were stored at both –20°C and 4°C, and each time point analysis was performed on samples stored at both temperatures. PCR specific yield and specificity were measured for the samples that were subjected to simulation of summer ambient shipping and gel ice shipping.

Analytical testing—volume, salt concentration, and dNTP integrity

In addition, salt concentrations (K^+ and Mg^{2+}) were measured throughout these studies for all buffers and AmpliTaq Gold 360 Master Mix. dNTP integrity was measured at time zero, and then intermittently throughout the study.

Using HPLC, aliquots of AmpliTaq Gold 360 Master Mix from both the –20°C and 4°C storage conditions were investigated for dNTP degradation. The height and retention time of each sample was evaluated. Using ion chromatography, K^+ and Mg^{2+} concentrations were evaluated and compared to a standard curve; results were compared against the –20°C temperature point and/or the initial time point.

Results

Functional testing—specificity and specific yield

The two key parameters of the functional quality control method utilized to gauge the success of the PCR reactions were yield and specificity of the amplified DNA. In this study, both a DNA region with AGC content and one with HGC content were amplified using either AmpliTaq Gold 360 DNA Polymerase in conjunction with the 10X buffer and 25 mM $MgCl_2$ solution, or AmpliTaq Gold 360 DNA Master Mix. These amplifications were performed for all samples throughout the 64-week test period.

In Figures 2–4, Tukey-Kramer analysis shows the variability in the specificity and specific yields of the PCR reactions. These results are presented across shipping treatments, time points (weeks), and storage temperatures. Specificity was retained for both the AGC amplicon and the HGC amplicon throughout the study with both shipping conditions (gel ice and simulated summer ambient shipping). As demonstrated in Figures 3 and 4, both amplicons were generated using AmpliTaq Gold 360 Master Mix, and no significant differences in specific yield were observed between the shipping conditions for any time point. While some minor assay variation occurred over the time course, no loss of specificity or yield was observed over the 12-month stability study (Figures 2–4). Importantly, exposure to the ambient shipping conditions did not shorten the products' shelf life.

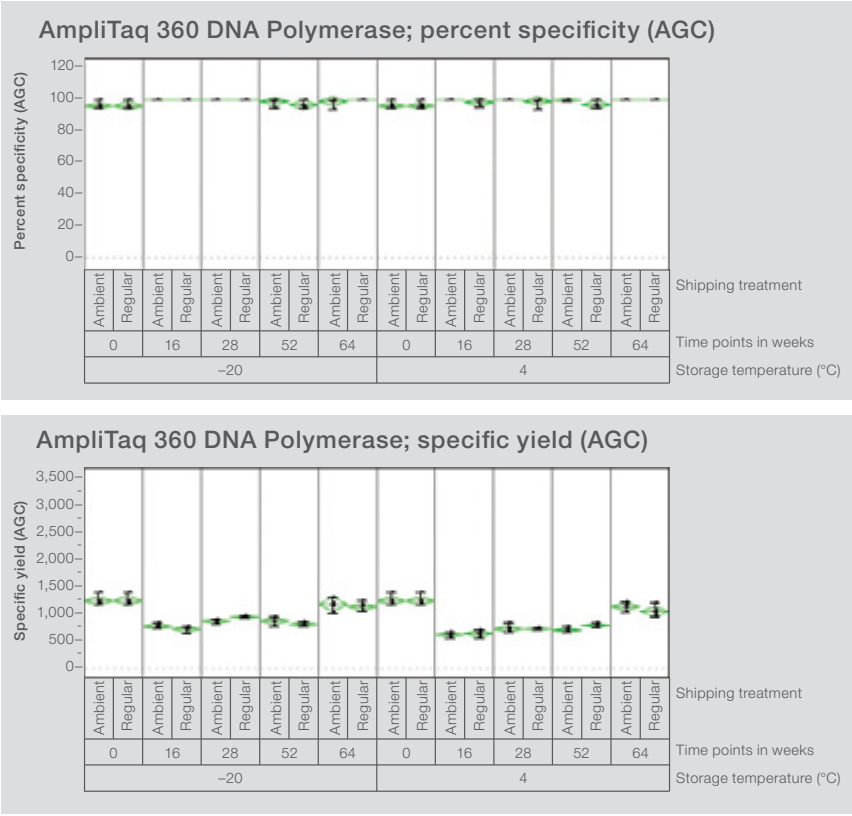

Figure 2. Specificity and specific yield of the AGC amplicon, for AmpliTaq 360 DNA Polymerase in combination with AmpliTaq 360 Buffer and 25 mM $MgCl_2$. Tukey-Kramer analysis of amplification of the AGC amplicon is shown through 64 weeks of storage.

Analytical testing—volume, salt concentration, and dNTP integrity

Ion chromatography readings were taken for 10X AmpliTaq 360 Buffer, 10X AmpliTaq Gold 360 Buffer, AmpliTaq Gold 360 Master Mix, and 25 mM $MgCl_2$ at weeks 0, 4, 12, 20, and 64. Cation analysis for K^+ and Mg^{2+} was within the specification limit set for the buffers (data not shown). pH analysis confirmed no significant difference between the gel ice and simulated summer ambient shipping samples for the same time points (data not shown).

Figure 3. Specificity and specific yield of the AGC amplicon, for AmpliTaq Gold 360 Master Mix. Tukey-Kramer analysis of amplification of the AGC amplicon is shown through 64 weeks of storage. Similar data were observed for specificity and specific yield of the AGC amplicon with AmpliTaq Gold 360 DNA Polymerase through 64 weeks of the stability study (data not shown).

Conclusion

The data described here demonstrate that ambient shipping has no detectable effect on the quality, integrity, and functional performance of the following products (catalog numbers provided in Table 2):

- AmpliTaq 360 DNA Polymerase with 10X AmpliTaq 360 Buffer and 25 mM $MgCl_2$
- AmpliTaq Gold 360 DNA Polymerase with 10X AmpliTaq Gold 360 Buffer and 25 mM $MgCl_2$
- AmpliTaq Gold 360 Master Mix
- 360 GC Enhancer

For the AmpliTaq 360 and AmpliTaq Gold 360 products, PCR functional performance for total yield, percent specificity, and specific yield showed that all of the test samples fell within specifications for these parameters.

Likewise, volume, salt concentration, and dNTP integrity were found to be unaffected over the same test period. A list of the products that were determined to be stable following ambient shipping can be found in Table 2.

These results support the change to ambient shipping, and provide the researcher confidence that when shipped under ambient conditions, these Applied Biosystems PCR products will exhibit no significant difference in function or stability compared to products shipped with gel ice. In addition to helping ensure that our customers will continue to receive the highest quality possible, this study enables us to significantly reduce the impact of transport of these products. Our consumption of nonrenewable raw materials will decrease by over 17 barrel equivalents of oil every year and reduce our water utilization by over 5,500 liters. Our customers will see a reduction of 2,000 kg of EPS waste. Our planet will see a reduction of CO_2 emissions by 72 tons every year.

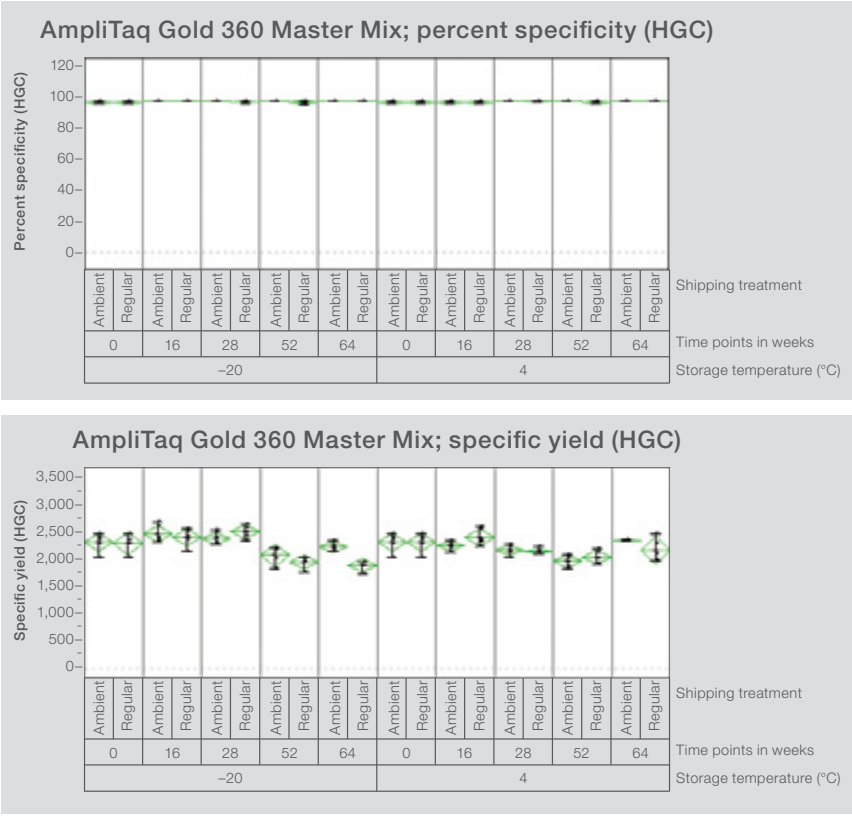

Figure 4. Specificity and specific yield of the HGC amplicon, for AmpliTaq Gold 360 Master Mix. Tukey-Kramer analysis of amplification of the HGC amplicon is shown through 64 weeks of storage. Similar data were observed for specificity and specific yield of the HGC amplicon with AmpliTaq Gold 360 DNA Polymerase through 64 weeks of the stability study (data not shown).

Table 2. AmpliTaq 360 and AmpliTaq Gold 360 products whose performance is unaltered by simulated summer ambient shipping.

Product	Size	Cat. No.
AmpliTaq 360 DNA Polymerase Includes AmpliTaq 360 DNA Polymerase, AmpliTaq 360 Buffer (10X), 25 mM $MgCl_2$, and 360 GC Enhancer	250 U	4398818
	1,000 U	4398828
	5,000 U (5 x 1,000 U)	4398895
	25 x 1,000 U	4398897
AmpliTaq 360 Buffer Kit Includes AmpliTaq 360 Buffer (10X), 25 mM $MgCl_2$, and 360 GC Enhancer	1.5 mL	4398848
AmpliTaq Gold 360 DNA Polymerase Includes AmpliTaq Gold 360 DNA Polymerase, AmpliTaq Gold 360 Buffer (10X), 25 mM $MgCl_2$, and 360 GC Enhancer	250 U	4398823
	1,000 U	4398833
	1,500 U	4398892
	3,000 U (2 x 1,500 U)	4398894
	5,000 U (5 x 1,000 U)	4398896
	25 x 1,000 U	4398898
AmpliTaq Gold 360 Buffer Kit Includes AmpliTaq Gold 360 Buffer (10X), 25 mM $MgCl_2$, and 360 GC Enhancer	1.5 mL	4398853
AmpliTaq Gold 360 Master Mix Includes AmpliTaq Gold 360 Master Mix and 360 GC Enhancer	1 mL	4398876
	5 mL	4398881
	10 x 5 mL	4398901
	50 mL	4398886

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References

1. Data produced using Compass™ Comparative Packaging Assessment online software tool (v. 1.1) (**https://trayak.com/compass/**).
2. Reference data derived from U.S. EPA, Climate Leaders, Greenhouse Gas Inventory Protocol Core Module Guidance (Optional Emissions From Commuting, Business Travel and Product Transport) (**https://nepis.epa.gov/Exe/ZyPDF.cgi/P1001177.PDF?Dockey=P1001177.PDF**).
3. Cowland R (2007) Developing ISTA Cold Chain Environmental Standards. Paper presented at the Dimensions.07 Conference, Orlando, Florida (**www.ista.org/forms/COWLAND_RAY_Dimensions07.pdf**).
4. ISTA 3A-2008 Test Protocol: **http://www.ista.org/forms/3Aoverview.pdf**.

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