Custom Primers and TaqMan® Probes shipped at ambient temperature reduce environmental impact and retain their quality and stability

Abstract

To minimize the adverse environmental impact of packaging and shipping products on gel or dry ice, Thermo Fisher Scientific investigated the feasibility of shipping its Custom Primers and TaqMan® Probes at ambient temperatures. This report describes stability testing of dye-labeled primers and MGB, TAMRA™, and QSY® probes after subjecting them to simulated summer shipping conditions. Analytical and stability testing demonstrated that Custom Primers and TaqMan® Probes that underwent simulated summer ambient-temperature shipping conditions maintained the same integrity and functionality as primers and probes that were kept at the recommended storage condition. By shipping at ambient temperatures, the need for expanded polystyrene (EPS) coolers and added refrigerant is eliminated and the fuel consumption and greenhouse gas emissions from transporting the product are 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 Custom Primers and TaqMan® Probes is approximately 6 tons CO_2-equivalents (CO_2e) [1]. Factoring in the number of shipments, the average distance traveled per package, and the fact that most packages are shipped via air, the annual total carbon footprint for transporting Custom Primers and TaqMan® Probes is 32 tons CO_2e [2].

There are other facts to consider beyond the greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often put in the untenable position of deciding whether to burn additional fossil fuels to transport the empty cooler across 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 the product for stability to ensure it can withstand the rigors of ambient shipping conditions without added refrigerant or insulation; 2) Design the packaging to be reusable, without increasing source material consumption; and 3) Recycle locally. We have opted to reduce whenever possible, reuse when it is an environmentally preferable option, and to encourage our customers to recycle locally.

Thermo Fisher Scientific has been systematically evaluating novel ways to minimize the impact of shipping Life Technologies™ products on gel or dry ice, and the CO_2 footprint left by these products during distribution. Here we demonstrate that selected Custom Primers and TaqMan® Probes are stable at ambient temperatures during shipping. By avoiding the cooler and refrigerant, the product can be shipped in a smaller, corrugated cardboard 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 or dry ice for these products, Thermo Fisher Scientific is helping to divert an annual total of nearly 1,826 kg (5,062 cubic feet)

life technologies

of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and to reduce the annual total carbon footprint by 38 tons CO_2e [1,2].

In 2009, we investigated the stability of five TaqMan® Assays: TaqMan® Gene Expression, Custom TaqMan® Gene Expression, TaqMan® MicroRNA, TaqMan® Drug Metabolism Genotyping, and TaqMan® SNP Genotyping Assays [3]. These assays comprise a preformulated set of unlabeled locus-specific oligonucleotide primers and minor grove binder–nonfluorescent quencher (MGB-NFQ) probes labeled with a fluorescent dye (VIC® or FAM™ dye), and are supplied in liquid form. A total of 42 different TaqMan® Assays were selected to represent the widest range of performance as well as chemical, sequence, and structural motifs. Assays were subjected to simulated summer ambient shipping conditions and subsequently analyzed for physical integrity and functional performance. Stressed samples were compared to controls in analytical HPLC and functional real-time PCR assays. In all cases, simulated ambient shipping of the assays had no effect on their quality, integrity, or functional performance. This study provided ample evidence for the stability of a wide range of structural motifs and oligonucleotide sequences under ambient shipping conditions and also demonstrated the stability of the VIC® and FAM™ dyes and the MGB moiety at the concentrations found in the assays.

For many years, Custom Primers and TaqMan® Probes have been shipped refrigerated on gel ice (with storage after shipping at +4°C or –20°C, depending on the product). Building on our 2009 study, this paper describes results from stability testing carried out after the Custom Primers and TaqMan® Probes were exposed to established summer shipping temperature profiles. These experiments demonstrate that by shipping selected Custom Primers and TaqMan® Probes under ambient conditions, not only can we supply researchers with the same superior-quality product they are used to receiving, but we can also reduce our environmental footprint in the process. This is a win for our customers (eliminating packaging waste and extra costs associated with refrigerated shipments), 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

Products tested. Custom Primers are 5'-labeled oligos that come with a choice of six dyes: 6-FAM™, TET™, VIC®, HEX™, NED™, or PET™ dye. The Custom Primer Pairs also come with an unlabeled oligo in a separate tube. Primers and Primer Pairs may be HPLC purified and can be ordered in two or three different quantities, with the largest having the highest concentration. For this study, four different labeled primers at the highest concentration were selected to represent the variety of primer types and dyes available (Table 1). The FAM™ and VIC® dyes were not tested with the primers because the 2009 study demonstrated the stability of these dyes in the TaqMan® Assays under ambient shipping conditions. Additionally, the Sequence Detection Primers, which are unlabeled, were not tested because the 2009 study established that unlabeled oligos are not affected by simulated ambient shipping conditions. All primers tested were formulated in Tris-EDTA (TE) buffer and were not HPLC purified. HPLC purification has no impact on the stability of the oligo or dye. Formulations in water were not evaluated because the pH of Tris buffers is known to vary inversely with temperature [4,5], something that does not occur in water, making TE a higher-risk formulation for ambient shipping.

TaqMan® MGB Probes incorporate a 5' reporter dye (FAM™, VIC®, TET™, or NED™ dye) and a 3' nonfluorescent quencher, with the MGB moiety attached to the quencher molecule. The TAMRA™ probes incorporate a 5' reporter dye (FAM™, TET™, or VIC® dye) and a 3' TAMRA™ quencher dye. The TaqMan® QSY® Probes can be ordered with a 5' reporter dye (FAM™, VIC®, ABY®, or JUN® dye) and the QSY® quencher. All TaqMan® Probes are HPLC purified and supplied at a single concentration in TE buffer. The MGB, TAMRA™, and QSY® probes were each tested with their respective dyes, with the exception of the MGB probe. This probe was not tested with VIC® dye because our own unpublished studies have shown that FAM™ is more labile than VIC® at elevated temperatures; therefore, FAM™ was used to represent a "worst-case" scenario. Because the 2009 study showed that variation in sequence and length did not affect oligo stability, a single sequence was chosen for all primers and probes:

5' - TGGACAGCCACCGACGAGAGCCTGG - 3'

Table 1. Custom Primers and TaqMan® Probes represented in this study.

Product Description	Reporter dye	Cat. No.
Custom Primers		
Sequence Detection Primers, 10,000 pmol, 80,000 pmol, 130,000 pmol	None	4304970, 4304971, 4304972
Custom 5'-Labeled Primer Pair Di-Repeats, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, **NED™**, PET®, 6-FAM™, VIC®, TET™	4304976, 4304977, **4304978**
Custom 5'-Labeled Primer, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, **PET®,** 6-FAM™, VIC®, TET™	450007, 450006, **450017**
Custom 5'-Labeled Primer Pair, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, PET®, 6-FAM™, VIC®, **TET™**	450056, 450059, **450062**
Custom 5'-Labeled Primer Pair Di-Repeat + Tail, 10,000 pmol, 80,000 pmol, **300,000 pmol**	**HEX™**, NED™, PET®, 6-FAM™, VIC®, TET™	4304979, 4304981, **4304982**
TaqMan® Custom Probes		
TaqMan® MGB Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™**, VIC®, **NED™, TET™**	4316034, 4316033, **4316032**
TaqMan® TAMRA™ Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**VIC®, 6-FAM™, TET™**	450025, 450024, **450003**
TaqMan® QSY® Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™, VIC®, ABY®, JUN®**	4482777, 4482778, **4482779**

Products tested are in **bold**

Creating replicates. To help eliminate manufacturing lot variability when creating the replicates, individual tubes of the primers and probes were manufactured, pooled, and aliquoted into the same packaging tube at the same fill volume as specified for the manufactured product. A total of 10 lots for each primer and probe were used to create five replicate stress tubes and five replicate control tubes. The control tubes were kept at –20°C for the duration of the study.

Simulated shipping conditions. To simulate temperatures experienced during shipping, samples were placed in a cycling environmental chamber (Thermotron® S-16) programmed to reproduce a "worst-case" 288-hour (12-day) summer temperature profile (Figure 1). This profile is adopted from one developed and validated by Amgen to simulate global ambient shipping conditions and mimics product temperature extremes encountered during transit of over 2,500 shipments during summer months between the latitudes of 59.9° N and 37.8° S [6]. Testing of winter ambient conditions was not considered, due to the low risk of exposing the Custom Primers and TaqMan® Probes to cold conditions.

Stability and integrity testing. Structural integrity changes in stressed samples compared to controls were measured by reverse-phase HPLC (RP-HPLC) and MALDI mass spectrometry. RP-HPLC samples were analyzed using an Agilent® 1200 HPLC. The HPLC column used was a Phenomenex® Clarity® 3 µm Oligo-RP, 2.0 mm ID x 50 mm. Mobile phases used were 0.1 M TEAA (triethylamine acetate) in water and 0.1 M TEAA in

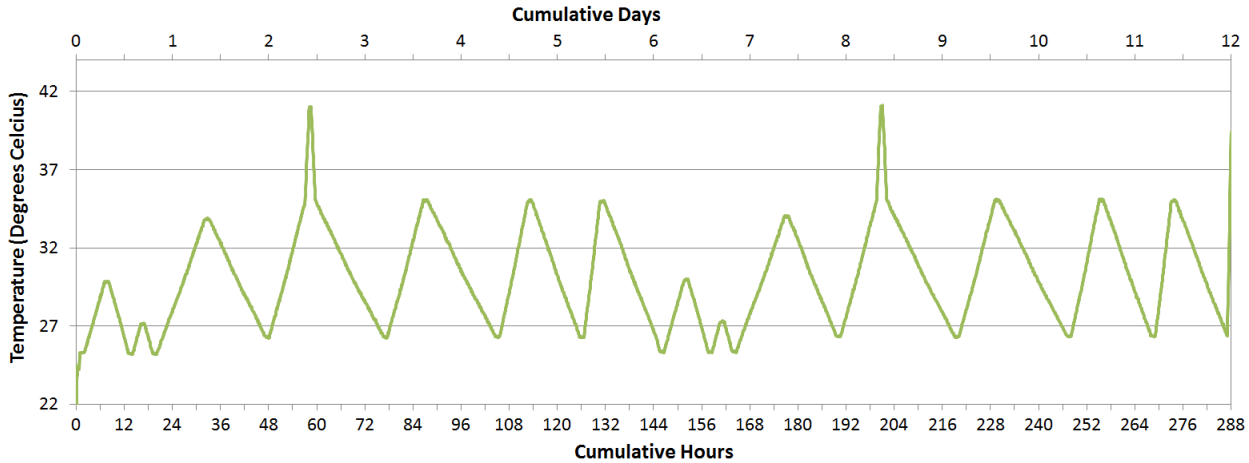

Figure 1. 288 hr summer temperature profile used to simulate shipping conditions. The summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° N and 37.8° S.

50% water/40% acetonitrile/10% methanol for the primers, MGB, and TAMRA™ probes, and 0.1 M TEAA in water and 2.0 M TEAA in 5% water/95% methanol for the QSY® probes. Absorbance was monitored at 260 nm for the oligonucleotide and at the maximum absorbance wavelength of the dye. Samples for MALDI mass spectrometry were analyzed on an AB Sciex® 4800 Plus MALDI TOF/TOF™ Analyzer.

Results

RP-HPLC. RP-HPLC was used to create peak profiles of the dye-labeled primers using UV/Vis absorbance detection. Matched test and control tubes from each assay were analyzed. An example of the data is shown in Figure 2. Test and control peak profiles were compared, and the purity (peak areas) were calculated (data not shown). For all samples analyzed, test samples were judged as identical to matched controls (no degradation), confirming that the simulated shipping stress did not affect product integrity.

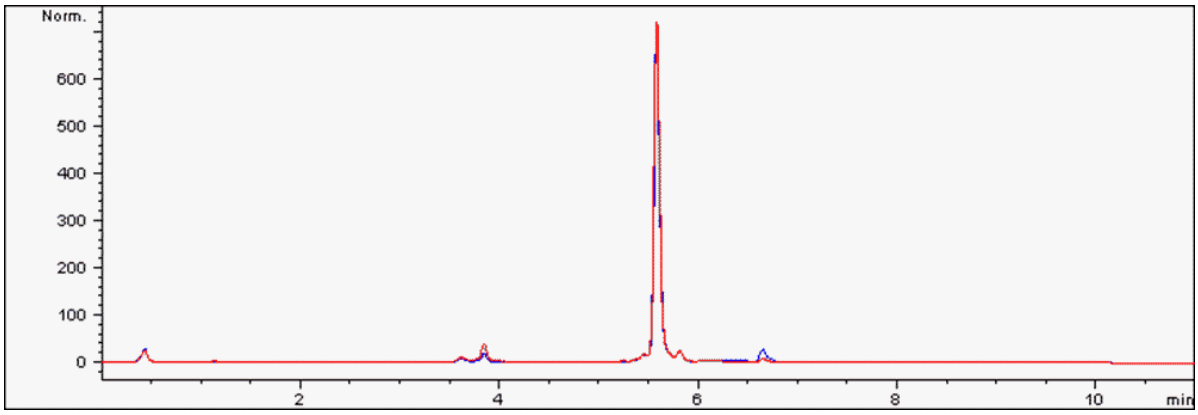

Figure 2. Simulated summer ambient shipping does not affect oligonucleotide stability—representative HPLC data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing RP-HPLC profiles of matched test and control samples. The HPLC chromatogram profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped 5'-Labeled Primer Pair Di-Repeats with the NED™ dye (red) compared to the matched control (blue).

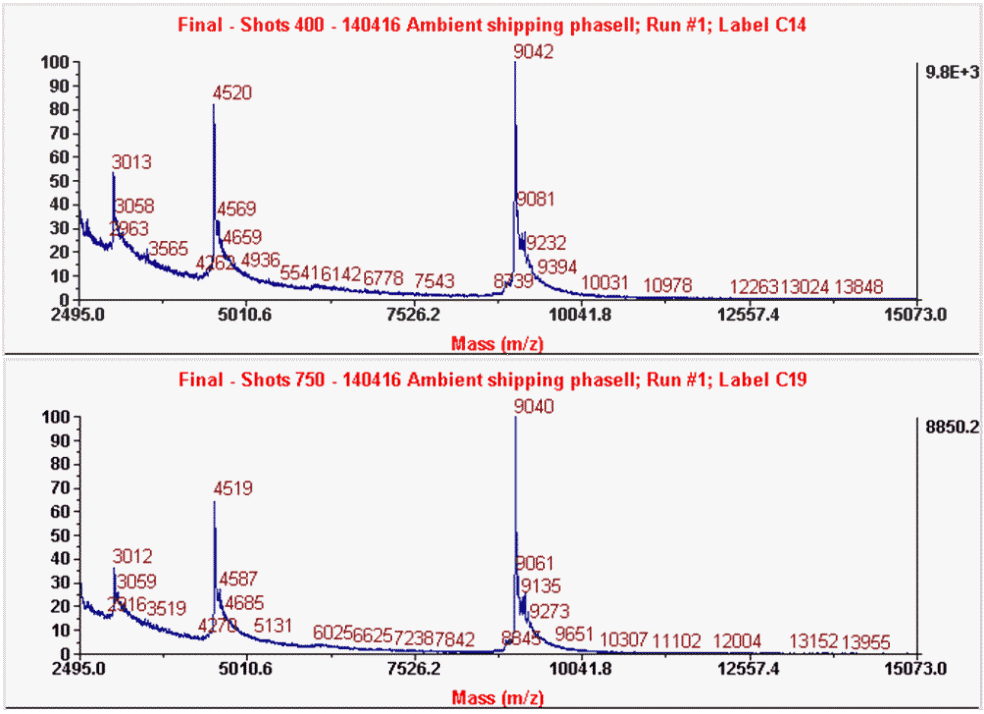

Figure 3. Simulated summer ambient shipping does not affect oligonucleotide stability—representative MALDI mass spectrometry data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing mass spectrum profiles of matched test and control samples. The profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped TaqMan® TAMRA™ Probe with the VIC™ dye (bottom) compared to the matched control (top).

MALDI mass spectrometry. MALDI mass spectrometry was used to generate mass profiles of the dye-labeled primers and probes. Again, matched test and control assays were analyzed and compared to each other. An example mass spectrum is shown in Figure 3. Test and control samples showed the same mass profiles, indicating that no degradation of the oligo, dye, or quencher occurred during the shipping simulation, further confirming that the simulated shipping stress did not affect product integrity.

Conclusions

The data described in this paper demonstrate that ambient shipping conditions have no effect on the quality and stability of Custom Primers and TaqMan® Probes. For each dye-labeled primer and probe tested, we were able to clearly demonstrate that ambient-temperature shipping conditions do not affect the product quality or integrity.

These results substantiate the change to ambient shipping conditions, and provide the researcher with confidence that when shipped under ambient conditions, their Custom Primers and TaqMan® Probes will exhibit no difference in function or stability compared to dry or gel ice–shipped products. In addition to ensuring our customers will continue to receive the highest quality possible, this study enables us to reduce the impact of transport of these products by 32 tons CO_2e. Our customers will see a reduction of 1,826 kg of EPS waste. Our planet will collectively see CO_2 emissions reduced by 38 tons every year.

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