DNA Ladders shipped at ambient temperature reduce environmental impact and retain their quality and stability

Abstract

In order 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 Life Technologies™ DNA Ladders at ambient temperature. This report describes stability and performance testing of these products after subjecting them to simulated summer ambient shipping conditions and freeze-thaw cycles. Analysis by gel electrophoresis shows that products shipped under ambient conditions meet the same stability and performance specifications as products shipped on gel or dry ice. By shipping at ambient conditions, 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 Life Technologies™ DNA Ladders is approximately 5.5 tons CO_2-equivalents (CO_2-e) [1]. 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 from transporting DNA Ladders is in excess of 29 tons (CO_2-e) [2].

But it's about more than just greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often faced with the untenable decision 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 insulated packaging; 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 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. One way to achieve this is to ship a product at a temperature consistent with its demonstrated stability. By avoiding the cooler and refrigerant, the product can be shipped in a smaller corrugated box, which improves the carrier's freight density (lower 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 is helping to divert an annual total of nearly 1,670 kg (5,545 ft^3) of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and to reduce the annual total carbon footprint from transport by 34.5 tons (CO_2) [1,2].

For many years, Life Technologies™ DNA Ladders have been shipped refrigerated on dry or gel ice (with storage after shipping at +4°C or -20°C, depending on the product). This paper describes results from functional

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and stability testing carried out after the DNA Ladders were exposed to established summer shipping profiles and multiple freeze-thaw cycles. These experiments demonstrate that by shipping certain DNA Ladders 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 the 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 for managing cold-chain transport).

Materials and methods

Products tested. This study measured the performance of each of our E-Gel® and DNA Ladders listed in Table 1 as well as the E-Gel® Sample Loading Buffer.

Table 1. DNA Ladders evaluated for quality and functionality after simulated summer shipping conditions. Multiple catalog numbers reflect different product sizes.

DNA Ladder	Cat. No(s).
E-Gel® Sample Loading Buffer	10482055
E-Gel® 25 bp DNA Ladder	10488095
E-Gel® 50 bp DNA Ladder	10488099
λ DNA/*Hin*d III Fragments	15612013
ΦX174 RF DNA/*Hae* III Fragments	15611015
Low DNA Mass Ladder	10068013
High DNA Mass Ladder	10496016
1 Kb Plus DNA Ladder	10787018, 10787026
1 Kb DNA Extension Ladder	10511012
250 bp DNA Ladder	10596013
100 bp DNA Ladder	15628019, 15628050
50 bp DNA Ladder	10416014
25 bp DNA Ladder	10597011
10 bp DNA Ladder	10821015

In this paper, we describe how each of the DNA Ladders was subjected to simulated summer shipping conditions and subsequently analyzed for quality and functional performance. This is assessed via gel electrophoresis using our established Quality Assurance protocols and acceptance criteria. To ensure that ambient shipping did not affect the long-term stability of the DNA Ladders, accelerated and real-time stability tests were conducted. At each time point in this study, the ambient shipping stressed sample was evaluated side by side with a matched control that was kept at the recommended storage temperature.

Sample preparation. For each standard, several vials of a single lot were chosen from inventory. When available, multiple lots were tested. For each lot, one set of vials was subjected to an ambient shipping stress profile and a matching set of control vials was held at the recommended storage conditions (+4˚C or -20˚C). Immediately following the 12 day ambient shipping simulation, the control and stressed samples were aliquoted into several 1.5 mL tubes for use in accelerated and real-time stability studies as well as freeze-thaw tests.

Simulated shipping conditions. To simulate temperatures experienced during shipping, samples were placed in a cycling environmental chamber (Enviro FSH1800, Envirotronics, Grand Rapids, MI) programmed to reproduce a "worst-case" 144 hr (6 day) summer temperature profile (sequentially run two times for a total of 288 hr) (Figure 1). This profile was developed and validated by Amgen to simulate ambient shipping conditions globally [3]. This profile mimics product temperature extremes encountered during transit from over 2,500 shipments during summer months between the latitudes of 59.9° north and 37.8° south. Testing of winter ambient conditions was not considered due to the low risk of exposing the DNA Ladders to cold conditions.

Functional tests. All ladders were analyzed by agarose gel electrophoresis. Prior to being prepared for gel electrophoresis, the DNA Ladders were evaluated for consistent physical appearance. Gel electrophoresis samples were prepared by mixing the ladder with specific amounts of UltraPure™ DNase/RNase-Free Distilled Water and 10X BlueJuice™ Gel Loading Buffer as detailed in Table 2.

For the E-Gel® 25 bp DNA Ladder and the E-Gel® 50 bp DNA Ladder, an E-Gel® EX 4% Agarose Gel and E-Gel® EX 2% Agarose Gel were used, respectively. Gels were run on an E-Gel® iBase™ Power System and gel images were collected using an E-Gel® Imager System with UV Light Base.

For the 10 bp DNA Ladder, 4% UltraPure™ Low Melting Point (LMP) Agarose gels were used for gel electrophoresis. For all other DNA Ladders, 0.8–3% UltraPure™ Agarose gels were used. All gels were run in

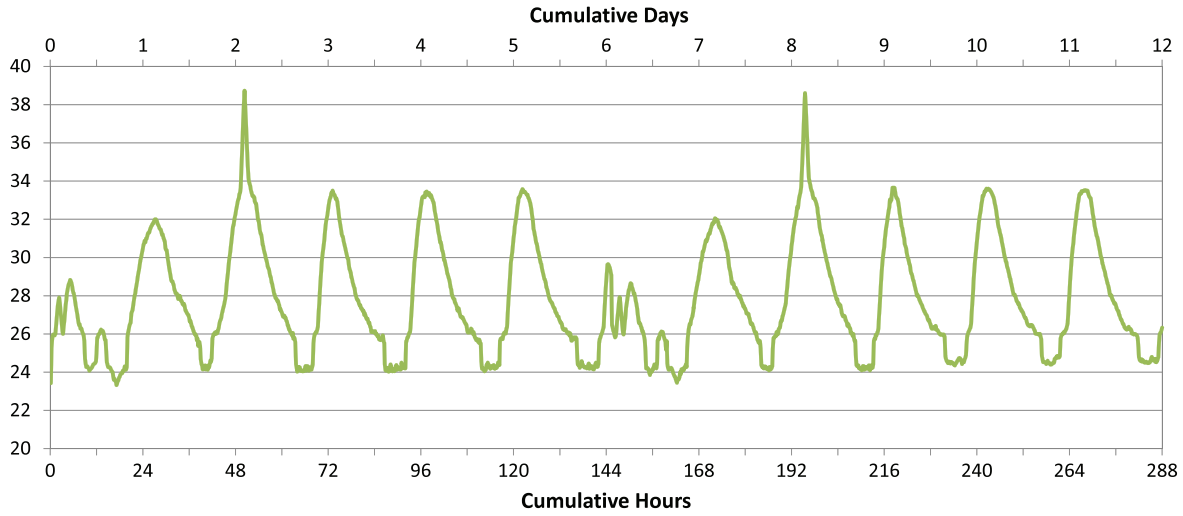

Figure 1. Summer temperature profile used to simulate shipping conditions over 288 hr. The summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° north and 37.8° south (profile derived from the Amgen protocol as described).

a Horizon 11-14 Horizontal Gel Electrophoresis Apparatus with 1X UltraPure™ TAE buffer using a Model 250 Gel Electrophoresis Power Supply. All gels were stained with ethidium bromide and photographed on an AlphaImager® 3400 system (ProteinSimple, Santa Clara, CA). Table 3 details the gel running and staining conditions.

Freeze-thaw stress test. To ensure that freezing and thawing during ambient shipping does not shear the DNA, DNA Ladders with fragments larger than 10,000 bp were subjected to additional freeze-thaw cycles following the simulated shipping conditions. The 1 Kb DNA Extension Ladder Cat. No. (10511012), the High Mass DNA Ladder (10821015), the λ DNA/*Hin*d III Fragments (15612013) and the 1 Kb Plus DNA Ladder (10787018 and 10787026) were all subjected to 20 freeze-thaw cycles simulated by holding the samples at room temperature for 30 minutes and then in a -20°C freezer for 2 hours.

Table 2. DNA Ladder sample loading preparation for gel electrophoresis.

DNA ladder	Cat. No(s).	Lot number(s) tested	Sample loading preparation for electrophoresis		
			DNA ladder (µL)	10X BlueJuice™ Gel Loading Buffer (µL)	UltraPure™ DNase/RNase-Free Distilled Water (µL)
E-Gel® 25 bp DNA Ladder	10488095	1305747	5	0	15
E-Gel® 50 bp DNA Ladder	10488099	1305773	5	0	15
ΦX174 RF DNA/*Hae* III Fragments	15611015	1305782	1	1	8
λ DNA/*Hin*d III Fragments	15612013	1384821, 1384826	2	1	7
Low DNA Mass Ladder	10068013	1305767, 1372589	4	1	5
High DNA Mass Ladder	10496016	1296583	4	1	5
1 Kb Plus DNA Ladder	10787018, 10787026	1369484, 1413081	1	1	8
1 Kb DNA Extension Ladder	10511012	1305764	1	1	8
250 bp DNA Ladder	10596013	1368858	1	1	8
100 bp DNA Ladder	15628019, 15628050	1265106, 1376599	1	1	8
50 bp DNA Ladder	10416014	1204786, 1383164	1	1	8
25 bp DNA Ladder	10597011	1305781	1	1	8
10 bp DNA Ladder	10821015	1383173	2	1	7

Accelerated and real-time stability tests. To assess the impact of ambient shipping on the long-term stability of the DNA Ladders, we conducted both accelerated and real-time stability studies. The accelerated stability testing was performed with both the control and stressed samples for 4 weeks at three different temperatures, +20, +30 and +37°C for the E-Gel® Ladders and +4, +20 and +30°C for the remaining DNA Ladders. We used the Q-Rule, which states that a product's degradation rate decreases by a constant factor (Q_{10}) when the storage temperature is lowered by 10°C, to predict product stability:

$$\text{Predicted Stability} = \text{Accelerated Stability} \times (Q_{10})^{\Delta T/10}$$

We used a value of 2 for Q_{10}, which is a conservative estimate of the activation energy required for product degradation and allows for a maximum predicted shelf life of 9 months for the E-Gel® ladders and 32 months for the other ladders. Previously aliquoted vials of the stressed and control samples were placed in incubators and removed after 1, 2, 3 and 4 weeks and stored at the recommended storage temperature until the end of the stability study. All samples were analyzed side by side using gel electrophoresis in order to evaluate whether the ambient shipping stress caused any change in quality or functional performance of the DNA Ladders as compared to the matched control.

To corroborate the results of the accelerated stability study, all DNA Ladders were also monitored in a real-time stability test for 6 months, the typical product life cycle. Products were tested at 3 months (data not shown) and again at 6 months.

Results

Freeze-thaw stress test. To evaluate the impact of the freeze-thaw cycles with and without the added stress of simulated summer shipping conditions, four samples of each DNA Ladder tested were loaded onto a single gel as described in the legend of Figure 2, which shows the results of the freeze-thaw study. As can be seen, the 1 Kb Extension, the High Mass, and the 1 Kb Plus DNA Ladders appear unaffected by 20 freeze-thaw cycles and simulated summer shipping conditions. Each ladder displays the same number of bands, with the same migration and intensity, and no additional smearing is evident, which would have suggested shearing due to the freeze-thawing cycles in the tests. This indicates that the quality and functionality of these DNA Ladders are not negatively affected by either the summer shipping simulation or the 20 freeze-thaw cycles. Results for the λ DNA/*Hin*d III Fragments were not conclusive and, consequently, will not be considered for ambient shipping pending further experiments (data not shown).

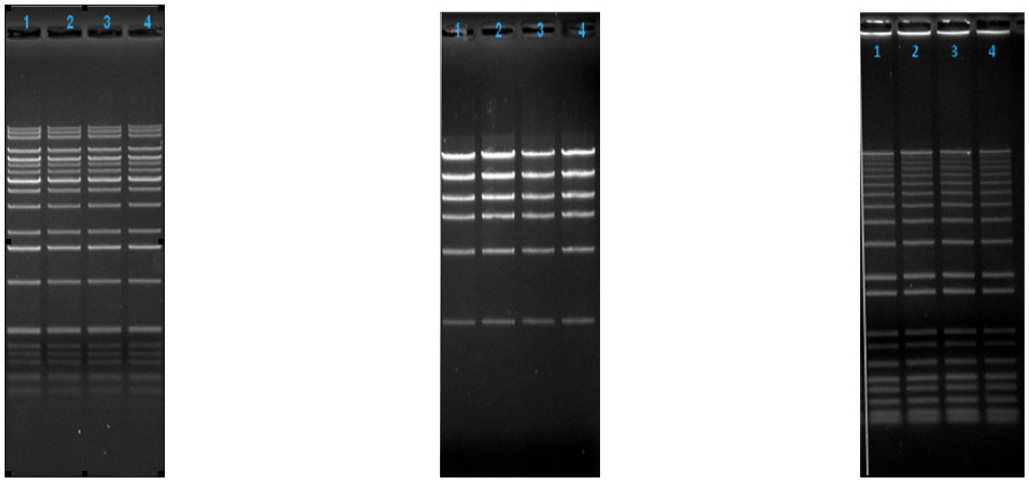

Figure 2. Representative gel images from freeze-thaw stress test. Results of the freeze-thaw stress test for (a) 1 Kb DNA Extension Ladder, (b) the High Mass DNA Ladder, and (c) the 1 Kb Plus DNA Ladder. Gel lanes were loaded as follows: 1) a control that was held at -20°C; 2) a sample that was subjected to the summer shipping profile but not the freeze-thaw cycles; 3) a sample that was subjected to the freeze-thaw cycles but not the shipping simulation; 4) a sample that was subjected to both the freeze-thaw cycles and shipping simulation.

Real-time stability tests. The results of the accelerated stability tests indicate that all of the DNA Ladders tested maintain stability for several months after simulated summer shipping conditions (data not shown). To verify this, a real-time stability study was initiated where a matched control and stressed (shipped at ambient temperature) sample were analyzed side by side by gel electrophoresis. The primary concern was for degradation of the DNA, leading to weaker bands, smearing, or different migration patterns. Figure 3 shows results after 6 months of real-time stability testing. As can be seen, in all cases, the control (lane 1) and stressed (lane 2) samples show the same number of bands, with the same migration patterns and intensity. This indicates that the long-term stability of these DNA Ladders is not negatively affected by simulated ambient shipping conditions.

With the results of the accelerated stability study and the 6-month real-time stability data, we are confident that DNA Ladders shipped under ambient conditions will maintain the same quality and functionality throughout their shelf life.

The E-Gel® Ladders are formulated with Xylene Cyanol FF and tartrazine. To address concerns that ambient shipping would affect absolute band intensity of these ladders, stressed samples were compared to controls. As can be seen in Figure 3 (a) and (b), bands in both the control and stressed samples maintain the same intensity, indicating that the dyes remain unchanged under ambient shipping temperatures. The formulation of the buffer in these ladders is identical to the E-Gel® Sample Loading Buffer, with the same constituents and dye concentrations. Because no degradation of

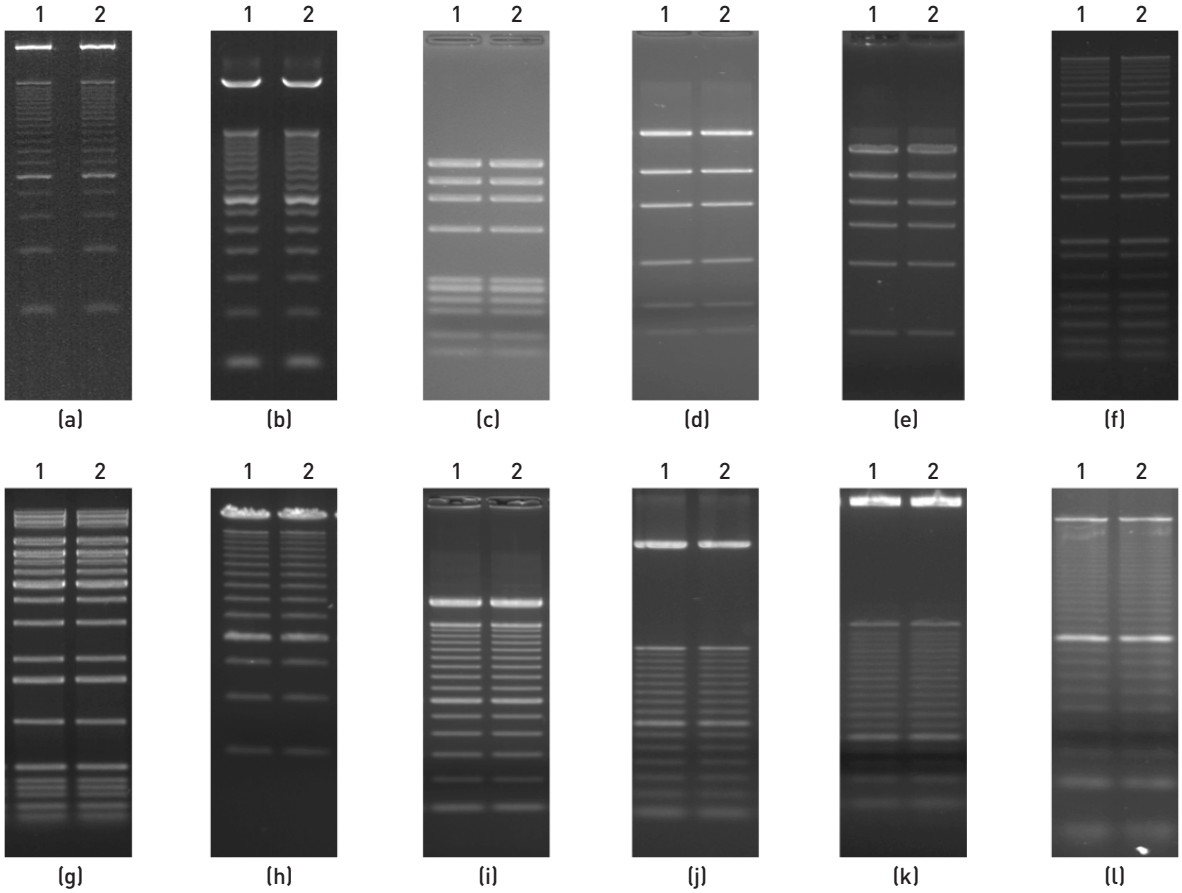

Figure 3. Representative gel images from real-time stability test at 6 months. Representative results from the real time stability test after 6 months for each DNA Ladder covered in this study. Lane 1 is a control that was kept at the recommended storage temperature throughout the study and Lane 2 is stressed sample that exposed to simulated summer shipping conditions and then kept at the recommended storage temperature until testing (6 months). (a) E-Gel® 25 bp DNA Ladder; (b) E-Gel® 50 bp DNA Ladder; (c) ΦX174 RF DNA/*Hae* III Fragments; (d) Low Mass DNA Ladder; (e) High Mass DNA Ladder; (f) 1 Kb Plus DNA Ladder; (g) 1 Kb DNA Extension Ladder; (h) 250 bp DNA Ladder; (i) 100 bp DNA Ladder; (j) 50 bp DNA Ladder; (k) 25 bp DNA Ladder; (l) 10 bp DNA Ladder.

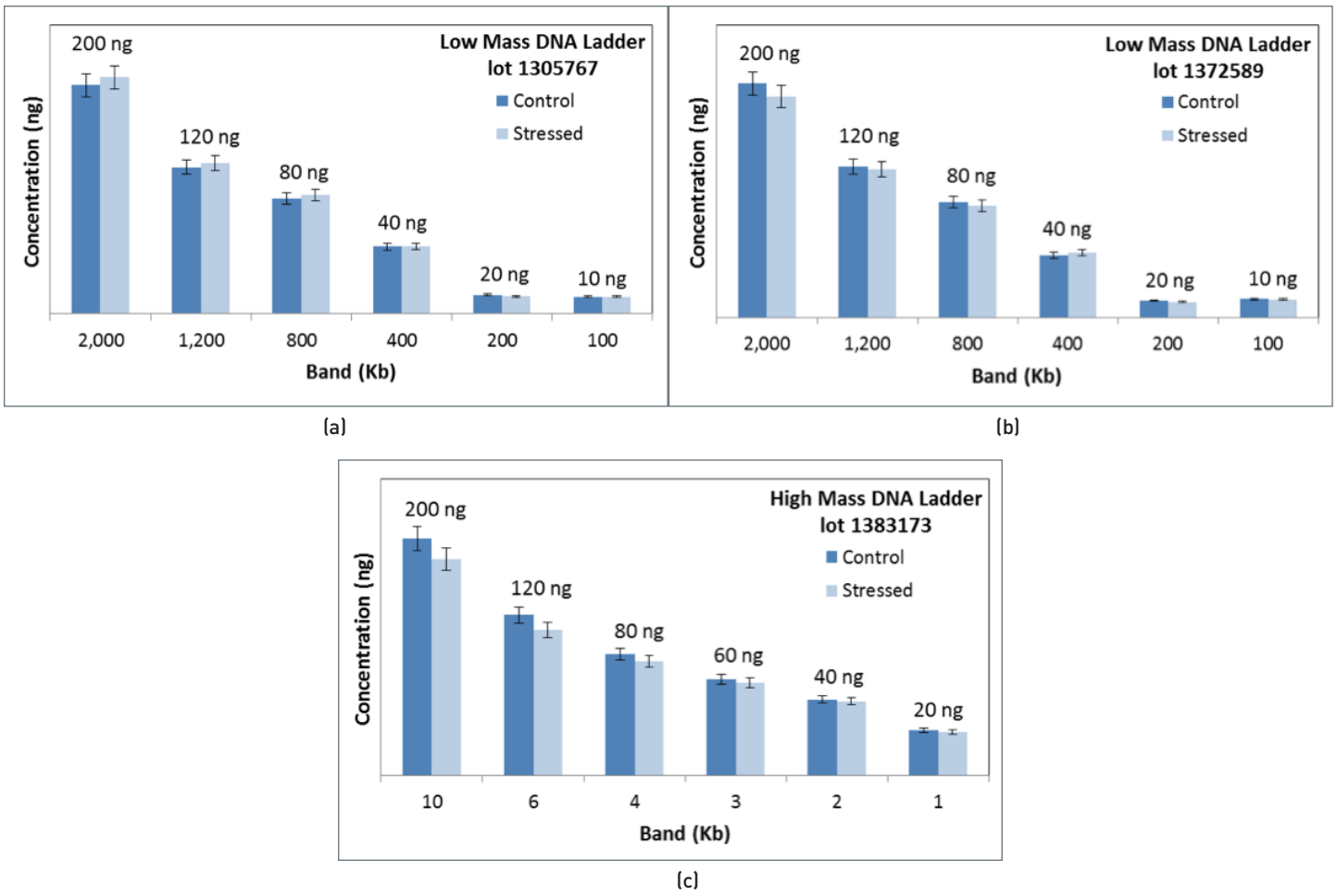

Figure 4. Quantitative analysis of high and low mass DNA ladders. Average band concentration measured for two lots (a) and (b) of the Low Mass DNA Ladder and (c) one lot of the High Mass DNA Ladder. Band concentrations for stressed samples that were exposed to simulated summer shipping conditions are shown in light blue, and band concentrations for controls that were not exposed to shipping simulations are shown in dark blue. Standard error bars are shown for each band.

the dyes in the E-Gel® Ladders was observed, we can also conclude that the E-Gel® Sample Loading Buffer will maintain the same quality and functionality under ambient shipping conditions.

Since the High and Low Mass DNA Ladders are used quantitatively, we also assessed whether ambient shipping has any impact on the amount of DNA in each band. Band concentrations were measured in each gel for the High and Low Mass DNA Ladders using GelQuant™ Express Analysis Software. Figure 4 shows the average amount of DNA measured in each band for the gels run for the real-time stability study at 6 months. No functional differences were observed between the stressed and control samples, indicating that ambient shipping conditions do not impact the quantity of DNA in each band.

Conclusions

The data described in this paper demonstrate that ambient shipping conditions have no effect on the quality, stability, or functional performance of the following products:

- E-Gel® Sample Loading Buffer, 1X
- E-Gel® 25 bp DNA Ladder
- E-Gel® 50 bp DNA Ladder
- ΦX174 RF DNA/Hae III Fragments
- Low DNA Mass Ladder
- High DNA Mass Ladder
- 1 Kb Plus DNA Ladder
- 1 Kb DNA Extension Ladder
- 250 bp DNA Ladder
- 100 bp DNA Ladder
- 50 bp DNA Ladder
- 25 bp DNA Ladder
- 10 bp DNA Ladder

For each of these standards, we were able to clearly demonstrate that ambient shipping does not affect the product quality.

These results substantiate the change to ambient shipping conditions, and provide the researcher confidence that when shipped under ambient conditions, their DNA gel electrophoresis products will exhibit no difference in functionality or stability compared to gel or dry ice–shipped products. While continuing to provide the highest quality product, we are able to reduce the annual carbon footprint of our DNA ladders by 34.5 tons and divert over 1,500 kg of EPS from landfills and incinerators.

References

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2. Data derived from U.S. EPA, climate leaders, greenhouse gas inventory protocol core module guidance (optional emissions from commuting, business travel and product transport).
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).

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