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

Abstract

To help minimize the adverse environmental impact of packaging and shipping products on gel or dry ice, we investigated the feasibility of shipping our Thermo Scientific™ and Invitrogen™ 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 Thermo Scientific and Invitrogen DNA ladders is approximately 14.9 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 for shipping DNA ladders is in excess of 63 tons CO_2-e [2].

But it’s more than just greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often faced with the untenable decision of whether to 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 insulated packaging; (2) design packaging to be reusable, without increasing source material consumption; and (3) recycle locally. We have opted to reduce material consumption whenever possible, reuse when it is an environmentally preferable option, and encourage our customers to recycle locally.

We are systematically evaluating novel ways to minimize the impact of shipping our products on gel or dry ice and the CO_2 footprint generated 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 helps improve 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, we are helping to divert an annual total of nearly 4,503 kg (14,950 ft^3) of EPS from landfills and incinerators by replacing EPS with recyclable corrugated paper packaging, and to reduce the net annual carbon footprint by 67.5 tons CO_2-e [1,2,4].

For many years, Thermo Scientific and Invitrogen DNA ladders were shipped refrigerated on gel or dry ice (with storage after shipping at +4°C or –20°C, depending on the product). This paper describes results from functional and stability testing carried out after the DNA ladders were exposed to established summer shipping profiles and multiple freeze-thaw cycles. These experiments demonstrated that by shipping certain DNA ladders under ambient conditions, we can supply researchers with the same superior-quality products they are used to receiving and also reduce our environmental footprint in the process.

This is a win for our customers (reducing 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 (minimizing the need to manage cold-chain transport).

Materials and methods

Products tested

This study measured the performance of each of our DNA ladders listed in Table 1 as well as the associated sample loading buffers.

Table 1. DNA ladders evaluated for maintaining quality and functionality at simulated summer shipping conditions. Multiple catalog numbers represent multiple dispense volumes or varieties.

Description	Cat. No.
NoLimits DNA Fragments	SM1391, SM1381, SM1401, SM1761, SM1411, SM1421, SM1431, SM1441, SM1601, SM1611, SM1451, SM1621, SM1631, SM1641, SM1461, SM1651, SM1471, SM1481, SM1661, SM1491, SM1671, SM1681, SM1691, SM1701, SM1571, SM1711, SM1501, SM1721, SM1731, SM1511, SM1741, SM1521, SM1751, SM1531, SM1771, SM1541
λ DNA/EcoRI+HindIII Marker	SM0191, SM0192, SM0193
λ DNA/EcoRI Marker	SM0281
pUC19 DNA/MspI (HpaII) Marker	SM0221, SM0222, SM0223
ϕX174 DNA/BsuRI (HaeIII) Marker	SM0251, SM0252, SM0253
pBR322 DNA/BsuRI (HaeIII) Marker	SM0271
GeneRuler DNA Ladders	SM0311, SM0312, SM0313, SM0314, SM0318, SM1331, SM1332, SM1333, SM1334, SM1338, SM0321, SM0322, SM0323, SM0324, SM0328, SM0241, SM0242, SM0243, SM0244, SM0248, SM0371, SM0372, SM0373, SM0378, SM0331, SM0332, SM0333, SM0334, SM0338, SM1191, SM1192, SM1193, SM1198
GeneRuler Express DNA Ladder	SM1551, SM1552, SM1553, SM1558
GeneRuler Ultra Low Range DNA Ladder	SM1211, SM1212, SM1213, SM1218
GeneRuler High Range DNA Ladder	SM1351, SM1352, SM1353, SM1358
O'RangeRuler DNA Ladders	SM1303, SM1308, SM1313, SM1318, SM1323, SM1328, SM0613, SM0618, SM0623, SM0628, SM0633, SM0638, SM0643, SM0648, SM0653, SM0658
MassRuler DNA Ladders	SM1243, SM1253, SM1263, SM1273, SM1283, SM1288, SM1293, SM1298, SM0383, SM0393, SM0403
FastRuler DNA Ladders	SM1103, SM1108, SM1113, SM1118, SM1123, SM1128, SM1233, SM1238
ZipRuler Express DNA Ladders	SM1373, SM1378
E-Gel Sample Loading Buffer	10482055
E-Gel DNA Ladder	10488099, 12352019, 10488090, 10488091, 10488099, 12373031, 10488100, 10488096
λ DNA/HindIII Marker	SM0101, SM0102, SM0103
Low DNA Mass Ladder	10068013
High DNA Mass Ladder	10496016
1 Kb Plus DNA Ladder	10787018, 10787026
1 Kb DNA Extension Ladder	10511012*
100 bp DNA Ladder	15628019, 15628050
50 bp DNA Ladder	10416014
6X DNA Loading Dye	R0611
6X MassRuler DNA Loading Dye	R0621
6X Orange DNA Loading Dye	R0631
6X DNA Loading Dye & SDS Solution	R1151
6X TriTrack DNA Loading Dye	R1161
TrackIt Cyan/Orange Loading Buffer	10482028
TrackIt Cyan/Yellow Loading Buffer	10482035
BlueJuice Gel Loading Buffer (10X)	10816015
TrackIt DNA Ladders	10488085, 10488058, 10488043, 10488023

* This product has been discontinued.

Here we describe how each of the DNA ladders was subjected to simulated summer shipping conditions and subsequently analyzed for quality and functional performance. These are 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 stability tests were conducted. At each time point in this study, the sample "stressed" with ambient shipping was evaluated side by side with a matched control that was kept at the recommended storage temperature. Representative samples were also subjected to real-time stability and freeze-thaw tests to further substantiate the change to ambient shipping conditions.

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 temperature (+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 encountered during shipping, samples were placed in a cycling environmental chamber (Envirotronics™ EnviroFSH1800) programmed to reproduce a "worst-case" 144 hr (6-day) summer temperature profile (sequentially run two times for a total of 288 hours) (Figure 1). The original 144 hr profile was developed and verified to simulate global ambient shipping conditions [3]. This profile mimics temperature extremes encountered in over 2,500 shipments during summer months between the latitudes of 59.9° north and 37.8° south. Other than consideration of freeze-thaw cycles, winter ambient shipping conditions were not tested.

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: clarity of the solution or color (if ready-to-use DNA ladder was used), uniform viscosity, and the absence of precipitate. Samples were prepared for electrophoresis by mixing the DNA ladder with specific amounts of Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water and Invitrogen™ 10X BlueJuice™ Gel Loading Buffer (or the gel loading buffer supplied with the ladder), according to the instructions in the product manuals.

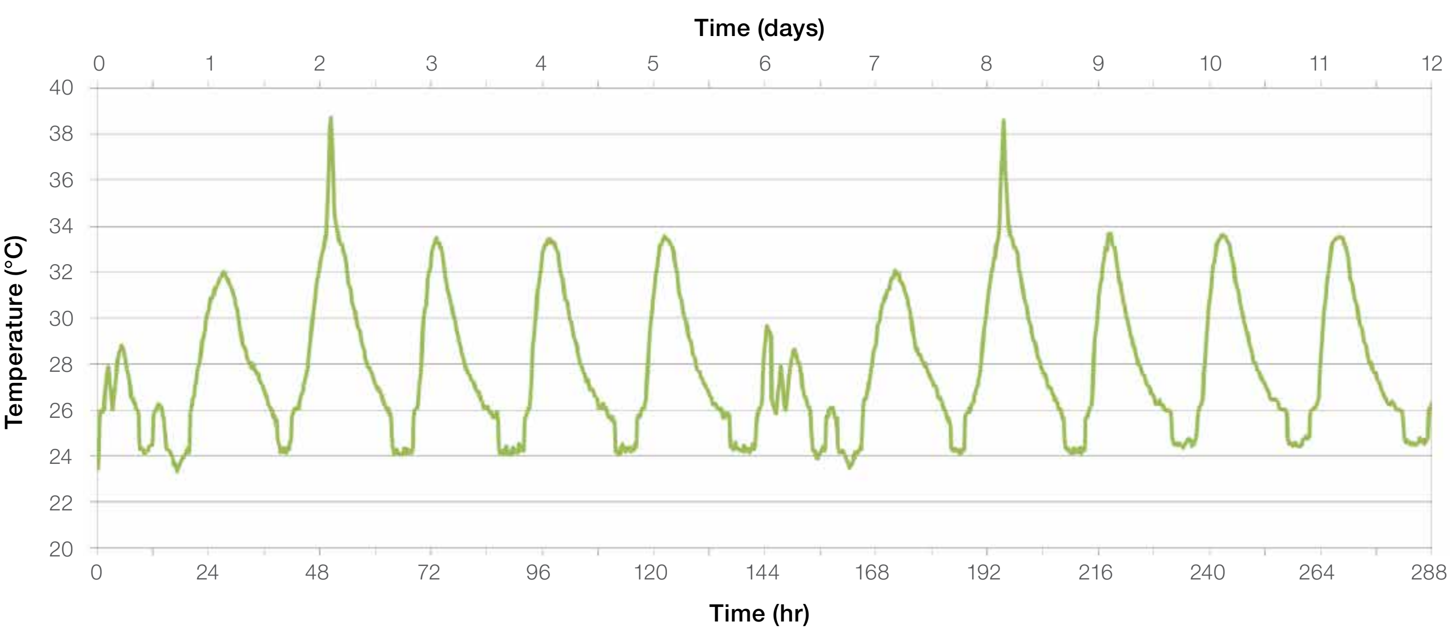

Figure 1. Summer temperature profile used to simulate shipping conditions. This summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° north and 37.8° south.

For the Invitrogen™ E-Gel™ Ultra Low Range DNA Ladder and E-Gel™ 50 bp DNA Ladder, Invitrogen™ E-Gel™ EX 4% and 2% Agarose Gels were used, respectively. Gels were run and documented using the Invitrogen™ E-Gel™ Power Snap Electrophoresis System.

For the Invitrogen™ Ultra Low Range DNA Ladder and Thermo Scientific™ Ultra Low Range Ladders, 4–5% agarose gels (made with Invitrogen™ UltraPure™ Low Melting Point Agarose) were used for electrophoresis. For all other DNA ladders, 0.8–3% agarose gels (made with Invitrogen™ UltraPure™ Agarose) were used. All gels were run in a Horizon™ 11-14 Horizontal Gel Electrophoresis Apparatus with 1X Invitrogen™ UltraPure™ TAE buffer, using a Thermo Scientific™ Owl™ EC-105 Gel Electrophoresis Power Supply. All gels were stained with ethidium bromide and photographed on a ProteinSimple™ AlphaImager™ 3400 system.

Freeze-thaw stress test

To assess whether freezing and thawing from ambient shipping would shear the DNA, representative DNA ladders with fragments larger than 10,000 bp were subjected to additional freeze-thaw cycles following the simulated shipping conditions. The Invitrogen™ 1 Kb DNA Extension Ladder (Cat. No. 10511012*), the Invitrogen™ High DNA Mass Ladder (Cat. No. 10496016), and the Invitrogen™ 1 Kb Plus DNA Ladder (Cat. No. 10787018 and 10787026) were all subjected to 20 freeze-thaw cycles by placing them in a –20°C freezer for 2 hours and then at room temperature for 30 minutes (Figure 2).

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 as shown by the equation below.

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

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 48 months for ladders. Previously aliquoted vials of the stressed and control samples were placed in incubators, 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 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. Table 2 describes the testing status for the DNA ladders in this study.

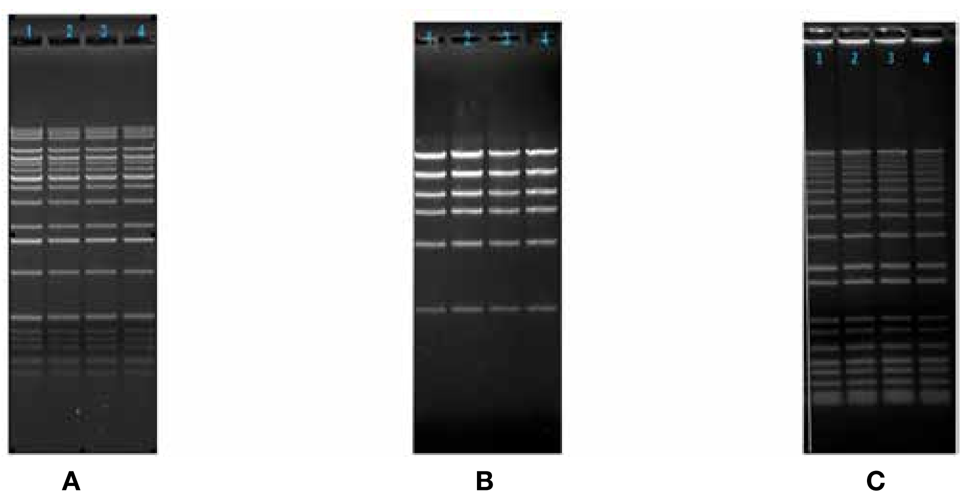

Figure 2. Representative gel images from freeze-thaw stress test. Results of the freeze-thaw stress test for **(A)** 1 Kb DNA Extension Ladder,* **(B)** High DNA Mass Ladder, and **(C)** 1 Kb Plus DNA Ladder. Lane 1: control that was held at –20°C; lane 2: sample that was subjected to the summer shipping simulation but not the freeze-thaw cycles; lane 3: sample that was subjected to the freeze-thaw cycles but not the summer shipping simulation; lane 4: sample that was subjected to both the freeze-thaw cycles and summer shipping simulation.

* This product has been discontinued.

Table 2. Stability testing plan to evaluate quality and functionality of DNA ladders after simulated summer shipping conditions. Multiple catalog numbers represent multiple dispense volumes or varieties. "X" denotes that the testing is complete. "*" denotes that while these ladders were not tested directly, results were extrapolated from other similar ladders.

Description	Cat. No.	Accelerated stability	Real-time stability
NoLimits DNA Fragments	SM1391, SM1381, SM1401, SM1761, SM1411, SM1421, SM1431, SM1441, SM1601, SM1611, SM1451, SM1621, SM1631, SM1641, SM1461, SM1651, SM1471, SM1481, SM1661, SM1491, SM1671, SM1681, SM1691, SM1701, SM1571, SM1711, SM1501, SM1721, SM1731, SM1511, SM1741, SM1521, SM1751, SM1531, SM1771, SM1541	*	*
λ DNA/EcoRI+HindIII Marker	SM0191, SM0192, SM0193	X	X
pUC19 DNA/MspI (HpaII) Marker	SM0221, SM0222, SM0223	X	X
λ DNA/EcoRI Marker	SM0281	*	*
φX174 DNA/BsuRI (HaeIII) Marker	SM0251, SM0252, SM0253	X	X
pBR322 DNA/BsuRI (HaeIII) Marker	SM0271	X	X
GeneRuler DNA Ladders	SM0311, SM0312, SM0313, SM0314, SM0318, SM1331, SM1332, SM1333, SM1334, SM1338, SM0321, SM0322, SM0323, SM0324, SM0328, SM0241, SM0242, SM0243, SM0244, SM0248, SM0371, SM0372, SM0373, SM0378, SM0331, SM0332, SM0333, SM0334, SM0338, SM1191, SM1192, SM1193	X	X
GeneRuler Express DNA Ladder	SM1551, SM1552, SM1553	X	X
GeneRuler Ultra Low Range DNA Ladder	SM1211, SM1212, SM1213	X	X
GeneRuler High Range DNA Ladder	SM1351, SM1352, SM1353	X	X
O'RangeRuler DNA Ladders	SM1303, SM1313, SM1323, SM0613, SM0623, SM0633, SM0643, SM0653	X	X
MassRuler DNA Ladders	SM1263, SM1283, SM0383, SM0393, SM0403	X	X
FastRuler DNA Ladders	SM1103, SM1108, SM1113, SM1118, SM1123, SM1128, SM1233, SM1238	X	X
ZipRuler Express DNA Ladders	SM1373	*	*
E-Gel Sample Loading Buffer	10482055	X	X
E-Gel Ultra Low Range DNA Ladder	10488096	X	X
E-Gel 50 bp DNA Ladder	10488099	X	X
λ DNA/HindIII Marker	SM0101, SM0102, SM0103	X	X
Low DNA Mass Ladder	10068013	X	X
High DNA Mass Ladder	10496016	X	X
1 Kb Plus DNA Ladder	10787018, 10787026	X	X
1 Kb DNA Extension Ladder	10511012*	X	X
100 bp DNA Ladder	15628019, 15628050	X	X
50 bp DNA Ladder	10416014	X	X

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 for Figure 2, which shows the results of the freeze-thaw study. As can be seen, the 1 Kb DNA Extension Ladder,* the High DNA Mass Ladder, and the 1 Kb Plus DNA Ladder 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 evident, which would have suggested shearing due to the freeze-thaw 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/HindIII Marker were not conclusive; consequently, this product will not be considered for ambient shipping at this time, pending further experiments (data not shown).

Real-time stability tests

The results of the accelerated stability tests indicated 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 (simulated shipping at ambient temperatures) sample of selected ladders were analyzed side by side by gel electrophoresis. The primary concern was whether degradation of the DNA led 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.

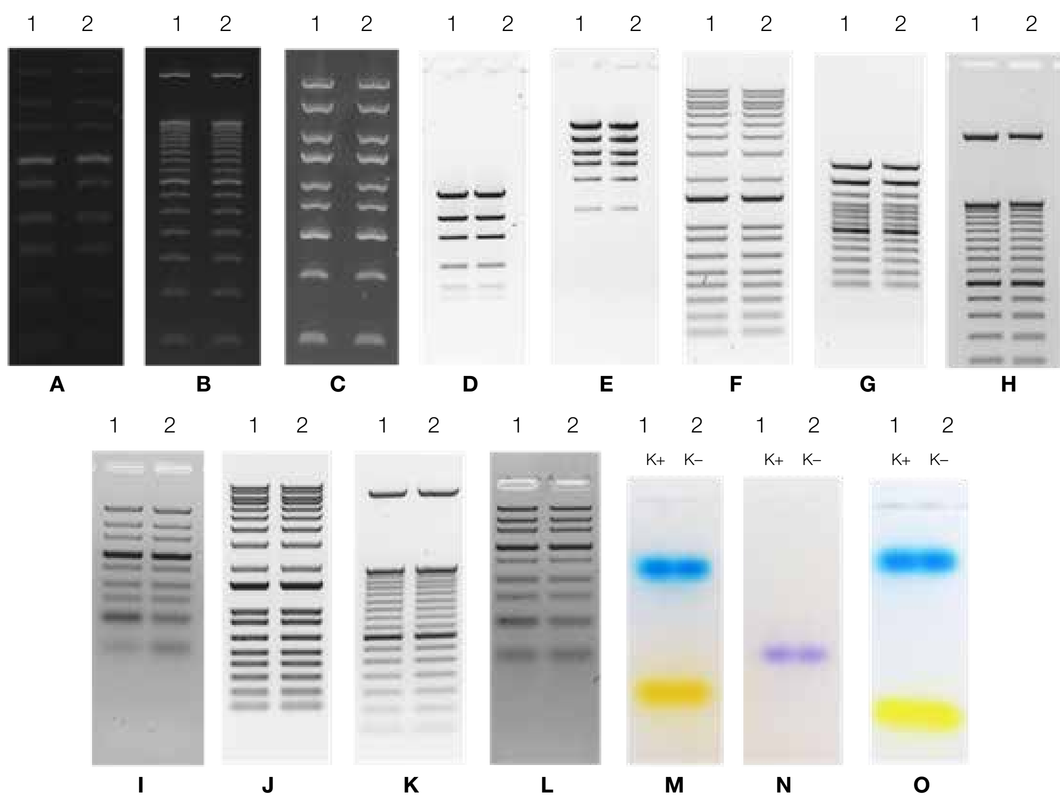

Figure 3. Representative gel images from real-time stability test at 6 months. Representative results from the real-time stability test after 6 months for each DNA ladder covered in this study. For **A–L**, lane 1: control that was kept at the recommended storage temperature throughout the study; lane 2: stressed sample that was exposed to simulated summer shipping conditions and then kept at the recommended storage temperature until testing (6 months). **(A)** E-Gel Ultra Low Range DNA Ladder; **(B)** E-Gel 50 bp DNA Ladder; **(C)** E-Gel 1 Kb Plus Express DNA Ladder; **(D)** Low DNA Mass Ladder; **(E)** High DNA Mass Ladder; **(F)** 1 Kb Plus DNA Ladder; **(G)** 100 bp DNA Ladder; **(H)** 50 bp DNA Ladder; **(I)** Ultra Low Range DNA Ladder; **(J)** Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder; **(K)** TrackIt 50 bp DNA Ladder; **(L)** TrackIt Ultra Low Range DNA Ladder. For **M–O**, K+ is control sample; K– is stressed sample. **(M)** TrackIt Cyan/Orange Loading Buffer; **(N)** BlueJuice Gel Loading Buffer; **(O)** TrackIt Cyan/Yellow Loading Buffer.

* This product has been discontinued.

Invitrogen™ DNA Ladders are formulated with xylene cyanol FF and orange G, bromophenol blue, or tartrazine. To address concerns that ambient shipping would affect dye intensity of these ladders, stressed samples were compared to controls. As can be seen in Figure 3 (M, N, and O), loading dye intensity remains the same in control and stressed samples, indicating that the dyes remain unchanged under ambient shipping conditions. The buffer compositions of our ready-to-use ladders are identical to that of TrackIt Cyan/Orange, BlueJuice Gel, and TrackIt Cyan/Yellow Loading Buffers, with the same constituents and dye concentrations. Because no degradation of the dyes in the loading buffers was observed, we can also conclude that the dyes in the DNA ladders will maintain the same quality and functionality under ambient shipping conditions.

Since some DNA ladders are used quantitatively, we also assessed whether ambient shipping has any impact on the amount of DNA in each band. Band intensities of selected ladders (High and Low DNA Mass Ladders) were measured 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.

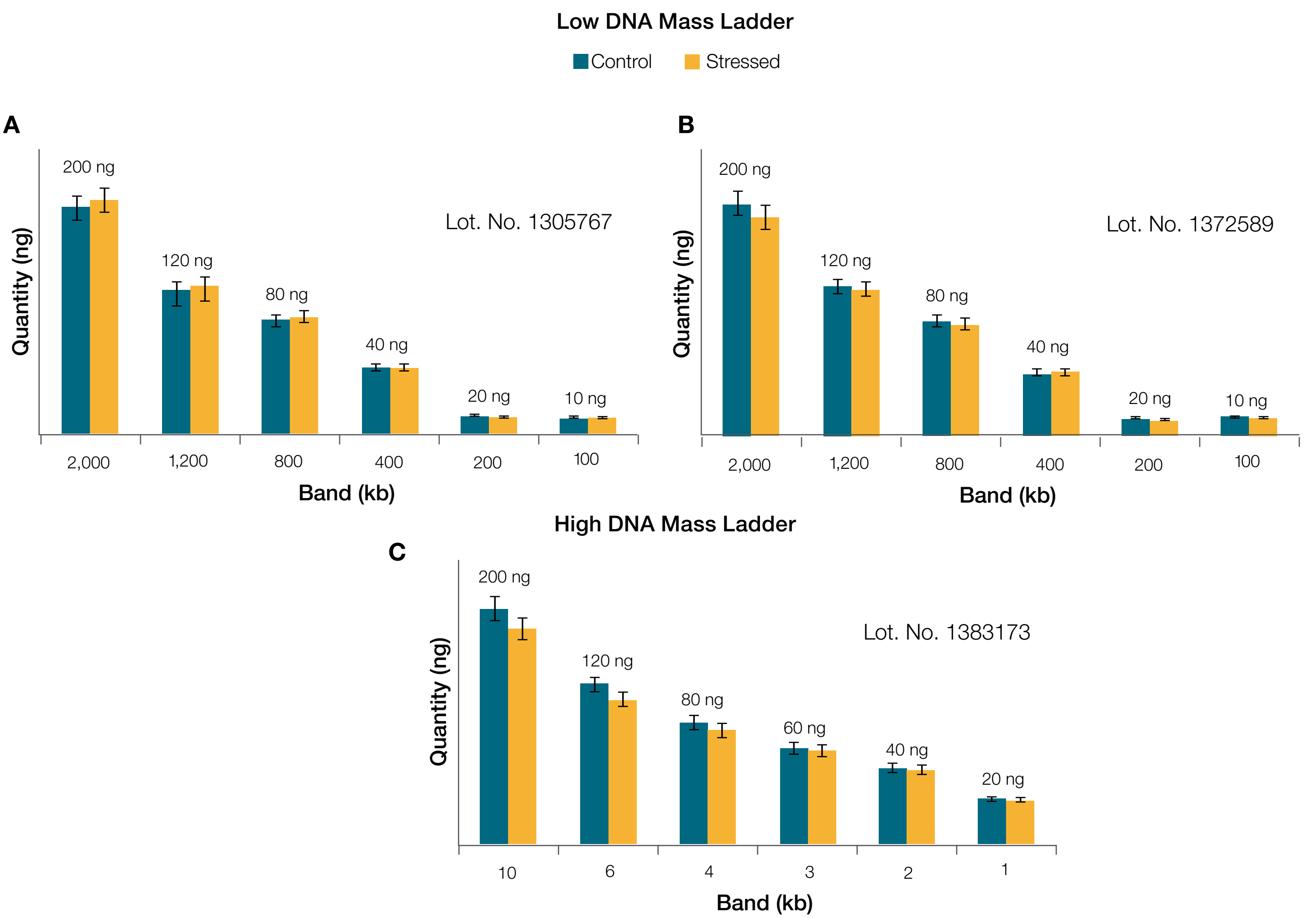

Figure 4. Quantitative analysis of Low DNA Mass and High DNA Mass Ladders. Average band quantities were measured for **(A, B)** two lots of the Low DNA Mass Ladder and **(C)** one lot of the High DNA Mass Ladder, after a 6-month real-time stability study. Band volumes for stressed samples that were exposed to simulated summer shipping conditions are shown in yellow, and band volumes for controls that were not exposed to shipping simulations are shown in teal. Standard error bars are shown for each band.

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Conclusions

The data described in this paper demonstrate that simulated ambient shipping conditions have no effect on the quality, stability, and functional performance of the following Thermo Scientific™ and Invitrogen™ products:

- NoLimits™ DNA Fragments
- λ DNA/EcoRI+HindIII Marker
- λ DNA/EcoRI Marker
- pUC19 DNA/MspI (HpaII) Marker
- ϕX174 DNA/BsuRI (HaeIII) Marker
- pBR322 DNA/BsuRI (HaeIII) Marker
- GeneRuler™ DNA Ladders
- GeneRuler™ Express DNA Ladder
- GeneRuler™ Ultra Low Range DNA Ladder
- GeneRuler™ High Range DNA Ladder
- O'RangeRuler™ DNA Ladders
- MassRuler™ DNA Ladders
- FastRuler™ DNA Ladders
- ZipRuler™ Express DNA Ladders
- E-Gel™ Sample Loading Buffer, 1X
- E-Gel™ DNA Ladder
- Low DNA Mass Ladder
- High DNA Mass Ladder
- 1 Kb Plus DNA Ladder
- 1 Kb DNA Extension Ladder*
- 100 bp DNA Ladder
- 50 bp DNA Ladder
- Ultra Low Range DNA Ladder
- TrackIt DNA Ladders
- BlueJuice Gel Loading Buffer (10X)
- TrackIt Cyan/Orange Loading Buffer
- TrackIt Cyan/Yellow Loading Buffer
- 6X DNA Loading Dye
- 6X MassRuler™ DNA Loading Dye
- 6X Orange DNA Loading Dye
- 6X TriTrack™ DNA Loading Dye
- 6X DNA Loading Dye & SDS Solution

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

These results substantiate the change from cold-chain shipping 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 function 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 US EPA, climate leaders, greenhouse gas inventory protocol core module guidance (optional emissions from commuting, business travel, and product transport).
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* This product has been discontinued.

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