DEVELOPMENT AND COMMERCIAL IMPLEMENTATION OF A SOURCE ASSURANCE AND TRACEABILITY METHOD BASED ON DNA BARCODES

Clotilde L.[1)], Zografos A.[1)],Trump, M. A. [1)]Birley A.[2)],

[1)] SafeTraces, Inc. – 5627 Stoneridge Drive, #315, Pleasanton, CA 94588, USA
[2)] Borton Fruit - 2550 S Borton Rd, Yakima, WA 98903

Abstract–We present results from the development and commercial implementation of a novel method for source assurance and traceability that is based on DNA barcodes, which are externally applied onto or in foods.This development is unrelated to DNA barcoding as it applies to species detection in biology.

The method employs a set of sixty-four distinct, short (~100base pairs) DNA sequences. These sequences have already been recognized by the United States Food and Drug Administration as food safe. We employ a commercial off-the-shelf auto sampler, which we refer to as a dispenser. The dispenser is loaded with vials containing each sequence, and it is used to create unique combinations of these sequences. We refer to these combinations as "barcodes." The dispenser is connected to a main database, which ensures that each created barcode is unique.

The resulting barcodes are used to tag foods. In the first commercial implementation at Borton Fruit(Washington State, USA), the barcodes are matched to source information and are applied to apples mixed in with the standard wax coating. We demonstrate that the barcodes are stable much longer than the apples' shelf life. We demonstrate that commingled fruitsfrom multiple sources can be correctly identified with accuracy. We discuss the development of a fast assay to read the barcodes in approximately 15 minutes and present our plan to further decrease the time in the near future. Finally, we discuss planned applications in other commodities.

Keywords: DNA Barcoding, Traceability, Source Assurance, Food Fraud

1. INTRODUCTION

We live in a world of globalization, a world in which political and commercial realities are the drivers for increasing supply chain efficiencies, accountability, and security. One key to achieving gains in all three areas lies in the area of traceability. For the global food industry, supply chain traceability is, today more than ever, a high visibility issue. Traceability is the ability to trace the source of foods and their ingredients, from fork to farm.

Food traceability today entails a complex system of hand offs along the supply chain from producer to packer, distributor, retailer, and ultimately the consumer. Investigations frequently take several weeks to complete [1].Advances in bio-engineering have produced a material that enables the development of an efficient, effective, and low cost food tracing system.This material is a combination of short DNA sequences which are not normally present in the ambient environment [2].These sequences can be either synthetic or genomic DNA drawn from organisms (e.g., *Thermotoga maritima*) that are not expected to be present in the food environment [3].

We use these sequences, referred to as *tracers* or *tags*, to form unique combinations which we call*DNA barcodes.*This DNA barcoding method is different from the taxonomic method that uses a short genetic marker in an organism's **DNA** to identify it as belonging to a particular species.

In this approach we employ, for example, 64 distinct 100 base pair DNA sequences. Each sequence represents a specific bit in a 64-bit set; presence of the sequence sets the bit to 1, and absence of the sequence sets it to 0. By employing only 64 sequences we can create 2^{64} unique combinations, which we refer to as "DNA barcodes". The major advantage of this approach is that we can analyze 2^{64} unique barcodes by employing only 64 probes and sets of primers for detection via the Polymerase Chain Reaction (PCR) process. This makes our approach highly scalable and sets us apart from others which, in practicality, can only be used for authentication or identification but not traceability, because of the limited number of unique sequences they could economically support.

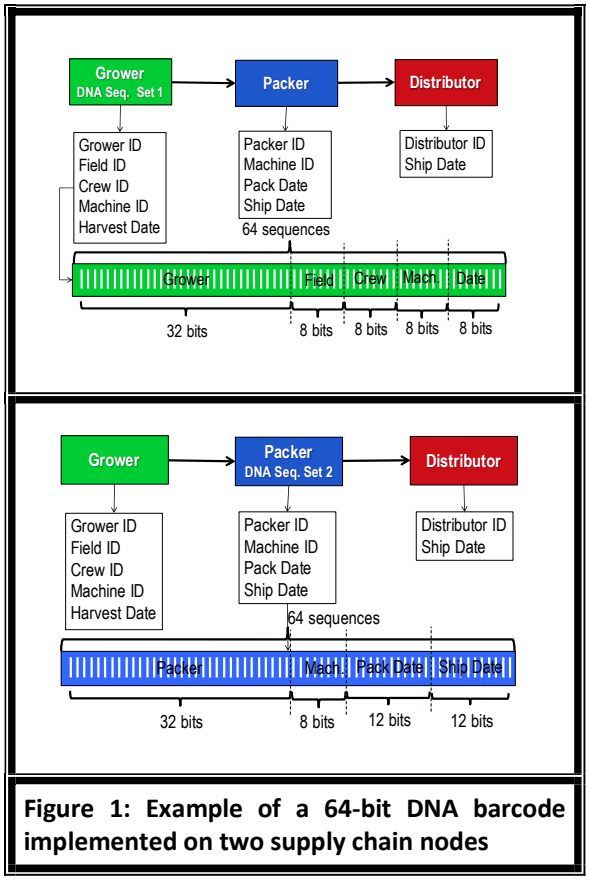

Figure 1: Example of a 64-bit DNA barcode implemented on two supply chain nodes

Different sets of sequences can be combined to generate distinct sets of barcodes assigned to growers, packers, re-packers, distributors, and processors as shown in Figure 1. A single piece of produce might bear many different barcodes, each identifying distinct steps in the supply chain.However, multiple barcodes associated with the same step in the supply chain (e.g., multiple grower barcodes) would produce an inconclusive analysis, indicating supply chain mismanagement or intentional fraud.We have previously shown this method to be effective also in the rapiddetection of fraud or adulteration of liquid goods [4].

2. EXPERIMENTAL

In a laboratory experiment, duplicates of 7 fruit varieties were coated with carnauba waxes containing a combination of up to three different tracers, effectively forming DNA barcodes. Carnauba wax is used as a coating to improve appearance and extend the shelf life of the product. The amount of wax applied was approximately equal to the rate of commercial applications of 1 L of wax per 1000kg of product. The fruitsbelonged todifferent varieties of apples and citrus. They had the following DNA barcodesrepresenting as 0 for absence or 1 for presence of Tracers 1, 2, and 3: 000 for the Red Delicious apples, 100 for Green apples, 010 for Yellow apples, 001 for Jazz apples, 110 forFuji apples, 011 for oranges, and 111 for lemons. The 14 fruits were commingled in a basket, stored at room temperature, and tested over time. At Days 0, 4, 5, 7, 11, 20, 21, and 26, each fruit was washed individually under running warm water, patted dry with a paper towel, and swabbed over a 5x5cm area, using a dry cotton-tipped swab. The swab was suspended in 500 μL of TE buffer and shaken vigorously for 10 sec. The unpurified suspensions were then tested in duplicate on the Chai Open qPCR system. An internally developed 16-min protocol was followed to test for the presence or absence of the individual tags making the DNA barcode. The purpose of this experiment was to evaluate both the stability of the barcodes over time, as well as our ability to correctly identify each piece of fruit under adverse conditions such as commingling and storage at room temperature. The results are presented in the following section.

The DNA barcodes were also applied on a commercial apple packing line. The tracers were added directly to the carnauba wax tank. The wax was collected at the site of application and tested over time prior to and post-addition of Tracers. Simultaneously, a set of 3 apples coming off the production linewas tested at the same time points.All wax samples were diluted in TE buffer, and the apples were processed as before, using dry swabbing methods. All samples were tested in triplicate by qPCR following the previously mentioned protocol.The results are presented in the following section.

3. RESULTS AND DISCUSSION

Figure 2 presents the results of the laboratory experiment evaluating the stability of the barcodes over time and the ability to correctly identify the barcodes under adverse conditions (i.e., commingling and storage at room temperature).

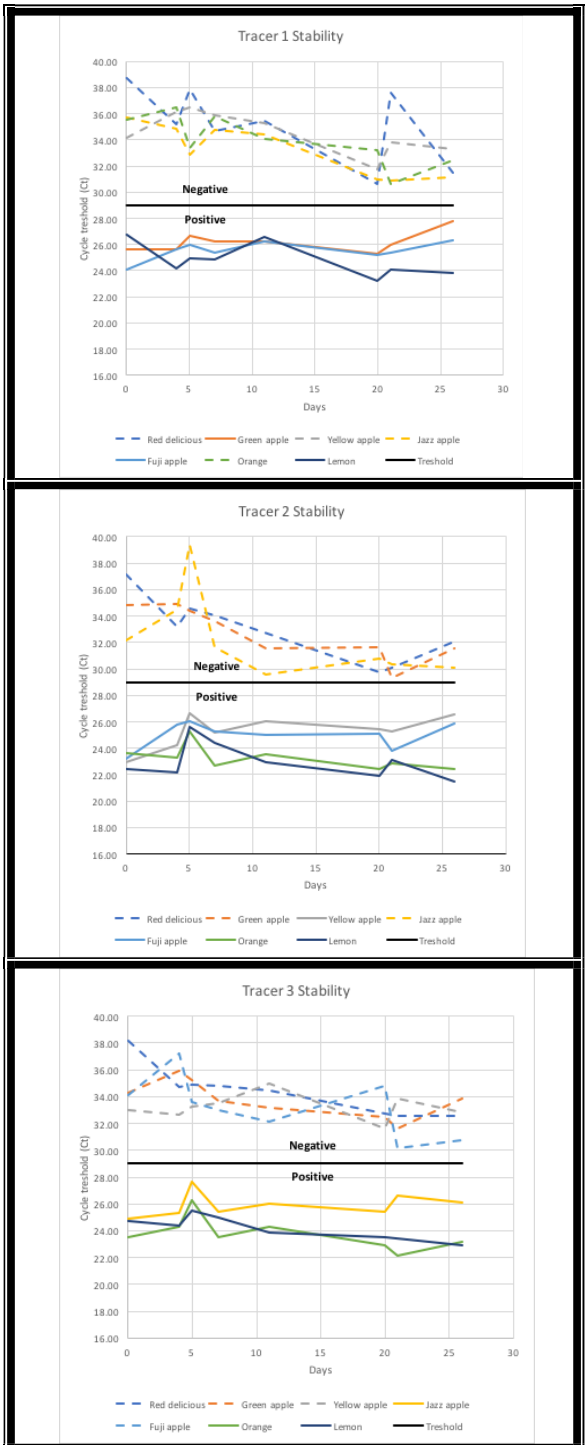

Figure 2: Stability of DNA Barcodes over time

The results are presented as Cycle Threshold (Ct; unit of measure of qPCR technology) with the lower values representing higher levels of the tracers [5].We have established a threshold of Ct_0=29 to differentiate positive from negative fruits.The results demonstrate that fruits that had been originally tagged with the DNA barcodes remain positive for the entire duration of the study (i.e., 26 days). This is generally considered longer than the shelf life of both apples and citrus when stored in room conditions.Although there is some inconsistency in the measurements on a day to day basis, that inconsistency is not sufficient to sway the results.The inconsistency is likely due to normal assay variations,including the swabbing of the produce (i.e., difference in force, duration, etc.). Each fruit was washed before each test, and the results suggest that washing does not significantly accelerate the degradation of the barcodes. In fact, the level of tracers on the fruits remained fairly constant for the duration of the experiment.

A trend is more apparent with the negative fruits, i.e. those that had been coated with untagged wax at the beginning of the experiment and had been mixed with the positive fruits. We observe that over time there is increasing transfer of the DNA barcodes from the positive to the negative fruits, even though both negative and positive fruits were washed prior to each measurement.However, all negative fruits remained negative for the duration of the experiment.

In both cases (negative or positive), there does not appear to be any dependency on the type of product (apple or citrus) or the variety.

Obviously, the level of DNA barcodes in the carnauba wax can be adjusted to create a wider separation between the initially positive and the initially negative fruits, but that may result in higher transfer rates from the positive to the negative fruits over time.More detailed optimizations depending on the type of the commodity are in progress.

Figure 3 presents the commercial implementation of the DNA barcoding system in an apple packing facility.

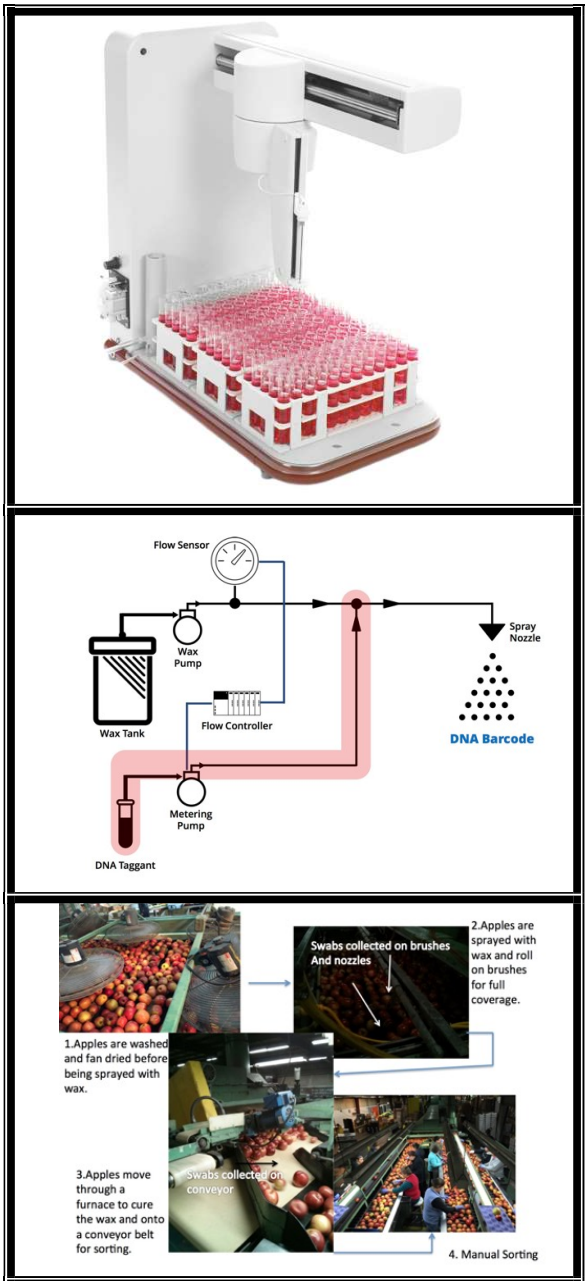

Figure 3: Commercial implementation on an apple packing line

An off-the shelf auto sampler serves to create unique DNA barcodes that are used to identify product lots.The dispenser is loaded with vials containing each sequence, and it is used to create unique combinations (mixtures) of these sequences (DNA barcodes). The dispenser is connected to a main database, which ensures that each created barcode is unique.The mixtures are then injected in the carnauba wax stream at a rate proportional to the wax flow rate. For the injection, a simple dosing system is used, as shown in Figure 3.

Figure 4 presents the results from a simpler implementation in a commercial apple packing facility. In this particular case, the DNA barcode was introduced in the entire wax tank. The purpose of the test was to evaluate the transition time from the introduction of the DNA barcodes in the wax tank to their actual detections in both the wax and on the fruit. Under the standard commercial implementation, because of the proximity of the injection system to the nozzle system, this transition time is expected to be much shorter and in the order of seconds as opposed to minutes.

The results show that one minute after the DNA barcode was introduced in the wax tank, both wax and apples appear positive for the barcodes. Over the course of the ensuing 24 hours, the concentration in the wax decreased slightly. This decrease may be attributed to assay variations or degradation of the DNA in the liquid wax environment. Laboratory tests (to be published in the future) have shown that some degradation does occur through long term (several weeks) exposure of the DNA to the liquid wax. However, in a commercial implementation this is not a significant issue, since the DNA barcodes are maintained in food grade ethanol and are delivered by the injection system to the wax line just before the spray nozzle.The laboratory tests cited above have demonstrated that the DNA barcodes are stable in ethanol practically indefinitely (no degradation observed after several months of exposure).As a result, in a commercial implementation the exposure of the DNA barcodes to liquid carnauba wax is reduced to a few seconds.

Similarly, a small decrease in the presence of tracers on the waxed apples is observed over the course of the ensuing 24 hours.This decrease may be due to the decrease in the DNA concentration in the wax or normal assay variation. However, the apples remained below the positive threshold Ct_0=29. It should be also noted that a full tank of liquid wax is normally used over approximately 3 days and during such a relatively short time period, the DNA concentration in the wax remains well within the range required to produce apples below the Ct_0 threshold.

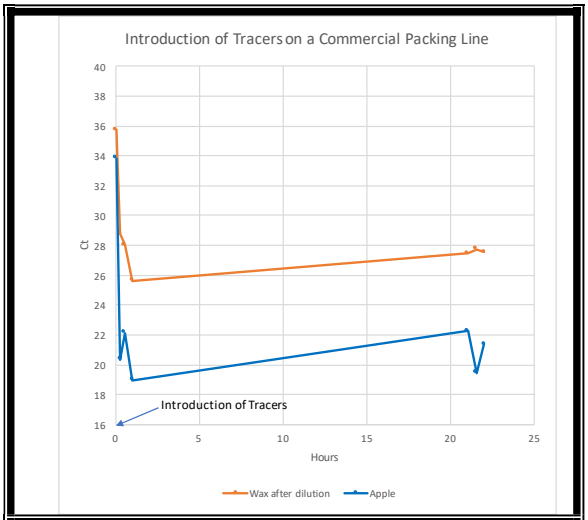

Figure 4: Simple DNA barcoding on a commercial packing line.

- We demonstrated the feasibility of using DNA barcodes for traceability in the food supply chain.
- We demonstrated stability of the DNA barcodes in excess of the normal shelf life of the produce.
- We demonstrated that cross-contamination between commingled produce is not sufficient to cause errors in the identification of the DNA barcodes. We were able to identify DNA barcodes correctly in all examined cases.
- We demonstrated simplicity of integration into the apple packing process (which is very similar to those of most pomme fruit, stone fruit, citrus, etc.).
- We developed an assay that is capable of reading the DNA barcode in approximately 15 minutes.

ACKNOWLEDGMENTS

We thank the Borton Fruit teams for their contribution to the success of this commercial implementation.

REFERENCES

[1] Hoffmann S, Maculloch B, Batz M. 2015. *Economic Burden of Major Foodborne Illnesses Acquired in the United States*. United States Department of Agriculture. Economic Information Bulletin 140.

[2] Jackson S , Rounsley S, and Purugganan M. *Comparative sequencing of plant genomes: choices to make.* Plant Cell.2006. 18(5): p. 1100-4.

[3] Ye N, Zhang X, Miao M, et al. *Saccharina genomes provide novel insight into kelp biology.* Nat Commun.201 2015. 6: p. 6986.

[4] Zografos A, Beck N, and Wang S. *Development of a DNA Barcode Based Method for Food Traceability and Detection of Adulteration or Fraud for Liquid Goods*. 2nd IMEKOFOODS. Promoting Objective and Measurable Food Quality and Safety. Italy, October 2016.

[5] Pohl, G; Shih, leM (2004). "Principle and applications of digital PCR". Expert Review of Molecular Diagnostics 4 (1): 41–7.