

October 12, 2016

Lisa Brines, Ph.D.
National List Manager
USDA/AMS/NOP, Standards Division
1400 Independence Ave. SW
Room 2648-So., Ag Stop 0268
Washington, DC 20250-0268

Dear Dr. Brines:

We respectfully submit the attached petition for consideration by the USDA NOP and the NOSB. This petition requests the addition of **short DNA tracers** to the National List of Allowed and Prohibited Substances as a non-agricultural (non-organic) substance allowed in or on processed products labeled as “organic” or “made with organic (specified ingredients)”. Specifically, the petition requests addition of short DNA tracers to §205.605. We believe you will agree that this material meets the requirements of the Organic Foods Production Act §6517, for inclusion in the National List. To wit, “...the use of such substance would not be harmful to human health or the environment; is necessary to the production or handling of the agricultural product because of the unavailability of wholly natural substitute products; and is consistent with organic farming and handling.”

Traceability is critical for confirmation of organic certification status. For small operations this is currently performed via, generally cumbersome, record-keeping, however, it now can be simplified and/or supplemented by the inclusion of short DNA tracers, especially for larger and more complex operations. Because it is added to the food, and not the packaging, a DNA tracer cannot be separated from the food, accidentally or intentionally. Traceability offers benefits in supply chain management, for example tracking problems, including detection of diversion and counterfeit products. Traceability is also critical for food safety. It simplifies and expedites trace back investigations and recall actions, which help address food safety problems at the source and limit exposure to liability. Finally, traceability is important for “transparency”, making information about the supply chain more readily available. Such transparency is increasingly demanded by customers, consumers, and government regulators, as evidenced by the Food Safety and Modernization Act. As the organic food industry continues to grow in size and complexity, improved methods for traceability are absolutely mandatory.

Organic foods are made from living things and contain large amounts of DNA. The addition of only a tiny amount of additional DNA (parts per trillion) can be very effective in providing traceability. Our tracers can identify the origin of a commodity (location, variety, date, grower, certification, etc.), intermediate

handlers (identity, location, time/date, operation, etc.), processors (identity, operation, methods and materials, time/date, etc.), and final disposition for retail (e.g., vendor authorization).

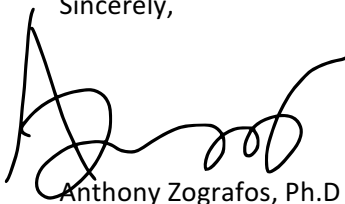
The material can be added as a powder or liquid formulation, in a wash step, or included in produce coatings. Sampling is performed with a swab or a rinse, and analysis of the short DNA sequences is a rapid and inexpensive step that can be performed at a customer site. The sequence information can then be mapped to a database with the detailed traceability information.

Short DNA tracers will be used in food production as inactive ingredients with no technical effect. They are not classified by the EPA as inert substances of toxicological concern. They are not recombinant or GMO materials. They are GRAS and already occur naturally in almost all foods. We believe there exist neither non-synthetic nor synthetic substances on the National List, nor alternative cultural methods that could be used in place of the petitioned substance.

The manufacturing of short DNA tracers starts with a naturally-occurring material, which is DNA purified from an edible plant or fungus. Since the use of full-length DNA raw material as a tracer is not economically practical, manufacturing uses naturally-occurring enzymes and nucleotides to copy a short section of the starting DNA. This process closely mimics the natural synthesis of DNA that occurs in the cells of living things. The result is millions of copies of pure short DNA.

In summary, we believe that the addition of short DNA tracers to the National List offers important advantages to the organic community. This material is naturally-derived, it is safe for the environment and the soil, and it offers traceability advantages that cannot be achieved by any other means. We hope you will agree.

Sincerely,



Anthony Zografos, Ph.D

President and CEO

ITEM A

Petition for addition of *short DNA tracers* to the National List of Allowed and Prohibited Substances as a nonagricultural (nonorganic) substance allowed in or on processed products labeled as “organic” or “made with organic (specified ingredients)”. Add to §205.605.

ITEM B

1. Substance Name

The substance’s common name is DNA (deoxyribonucleic acid). This petition is for double-stranded DNA molecules of 50 to 150 base pairs (bp) in length (“short DNA tracers”). While DNA molecules with different base sequences are all different, short DNA tracers generally have similar biological and physicochemical properties. Thus this petition is for inclusion of 50-150 bp DNA bearing any base sequence meeting the following three criteria: (1) The DNA sequence occurs naturally in a plant or fungus that is not considered pathogenic nor listed as a Select Agent¹, (2) the DNA does not code for a toxin or antibiotic resistance, (3) the DNA is manufactured via polymerase chain reaction (PCR).

Current GMO labeling legislation, as confirmed by the USDA Policy Memo dated 2016 September 19 defines bioengineered food as food “that contains genetic material that has been modified through *in vitro* recombinant deoxyribonucleic acid (DNA) techniques; and for which the modification could not otherwise be obtained through conventional breeding or found in nature.” The short DNA tracers petitioned herein are produced via PCR. PCR uses DNA, but it is **not** a recombinant technique.

The U.S. Department of Agriculture’s (USDA’s) National Organic Program (NOP) regulations prohibit the use of "excluded methods" during the production or handling of any organic product. Excluded methods are defined in the regulation as “a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes, and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology).” (7 CFR 205.1) The short DNA tracers petitioned herein are produced using none of these excluded methods.

DNA produced via recombinant techniques, genetic engineering or genetic modification, cloning, plasmids, or via chemical synthesis is specifically excluded from this petition.

2. Petitioner and Manufacturer Information

¹ Select Agents are bio-agents which since 1997 have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the “potential to pose a severe threat to public health and safety”. They are listed in 42 CFR 72.

Short DNA tracers are petitioned by SafeTraces (SafeTraces, Inc., 5627 Stoneridge Dr., Suite 315, Pleasanton, CA 94588, voice 925-326-1200). SafeTraces will also manufacture short DNA tracers.

Contact: Anthony Zografos, CEO (510) 761-6782, anthony@safetraces.com.

3. Intended or Current Use

The intended use of the substance is as an inactive ingredient with no technical effect to assist in tracing supply chains to authenticate that foods and commodities are derived from certified organic sources.

4. Intended Activities and Application Rate

The mode of action of short DNA tracers is as a trace label, which may be added to organic foods and ingredients in a dilute solution of water or ethanol, or applied externally to the product in a dilute solution of water, ethanol, or certified organic product coating or added to the product in particle form, encapsulated in a material that is certified organic or included in §205.605 or §205.606. Short DNA tracers do not affect appearance, flavor, aroma, nutritional value, or storage requirements of labeled foods and ingredients. Use of distinct short DNA tracers by different supply chain nodes allows identification of each node by its specific respective tracer, as these different short DNA tracers may be easily distinguished through testing. From subsequent points in the supply chain, up to the consumer, the short DNA tracers may be collected from the product and identified through biochemical means. Handling activities in which short DNA tracers may be especially useful for tracing the movement and authenticity of organic foods and ingredients include blending materials from multiple producers, transporters, handlers, and processors.

To help discourage counterfeiting, processors may add the short DNA tracer to the wax or other commonly used coating of high-value novel varieties for their commodities. Many fresh fruits and vegetables are commonly distributed with a wax or other coating, including apples, pears, citrus, tropical fruit, tomatoes, peppers, and cucumbers. Other coatings may be used with other fruits and vegetables. The short DNA tracers may also be added to dried goods in powder form, encapsulated in various materials that are certified organic or included in §205.605 or §205.606 such as maltodextrin, agar, etc. or added directly in liquid form to liquid commodities, such as wine, extra virgin olive oil, or honey. Especially when produced in special regions commanding a high price, these foods are valuable and commonly subject to economically-motivated adulteration. Such dilution or substitution with an inferior product is easily detected via addition of short DNA tracers.

The markets for some commodities, such as coffee and palm oil, are influenced by socio-political and socio-economic issues, and by environmental sustainability. Short DNA tracers may be added to these foods as an invisible label, guaranteeing that they are produced by certified organic, fair-trade and/or sustainable producers and handlers.

Cereal grains are commonly sorted at large facilities that handle both organic and conventional products. While organic grains are more valuable, the appearance of the two is indistinguishable to operators and consumers. Thus short DNA tracers may be added to grains, to identify the origin. This may be accomplished via a wet spray in water or alcohol, or distributed in a powder, such as maltodextrin.

Organic certification is mostly concerned with guaranteeing that unacceptable materials are not added to products, and consumers of certified organic foods mostly appreciate them for what they do not contain. The intended activity for short DNA as a tracer is a departure from this pattern, as the short DNA tracer will sometimes be added specifically to guarantee organic authenticity.

Because it is easily amplified, a modern Polymerase Chain Reaction Thermo Cycler can routinely detect even a single molecule of short DNA tracer in a 1-gram sample, which is about 100 molecules in a typical 4 ounce serving of food. This is about one trillionth of 1 part per trillion. The material is non-toxic, and the cost is low. So to obtain maximum sensitivity producers are likely to include short DNA tracers in food at levels as high as 1 milligram per ton, which is 1 ppb.

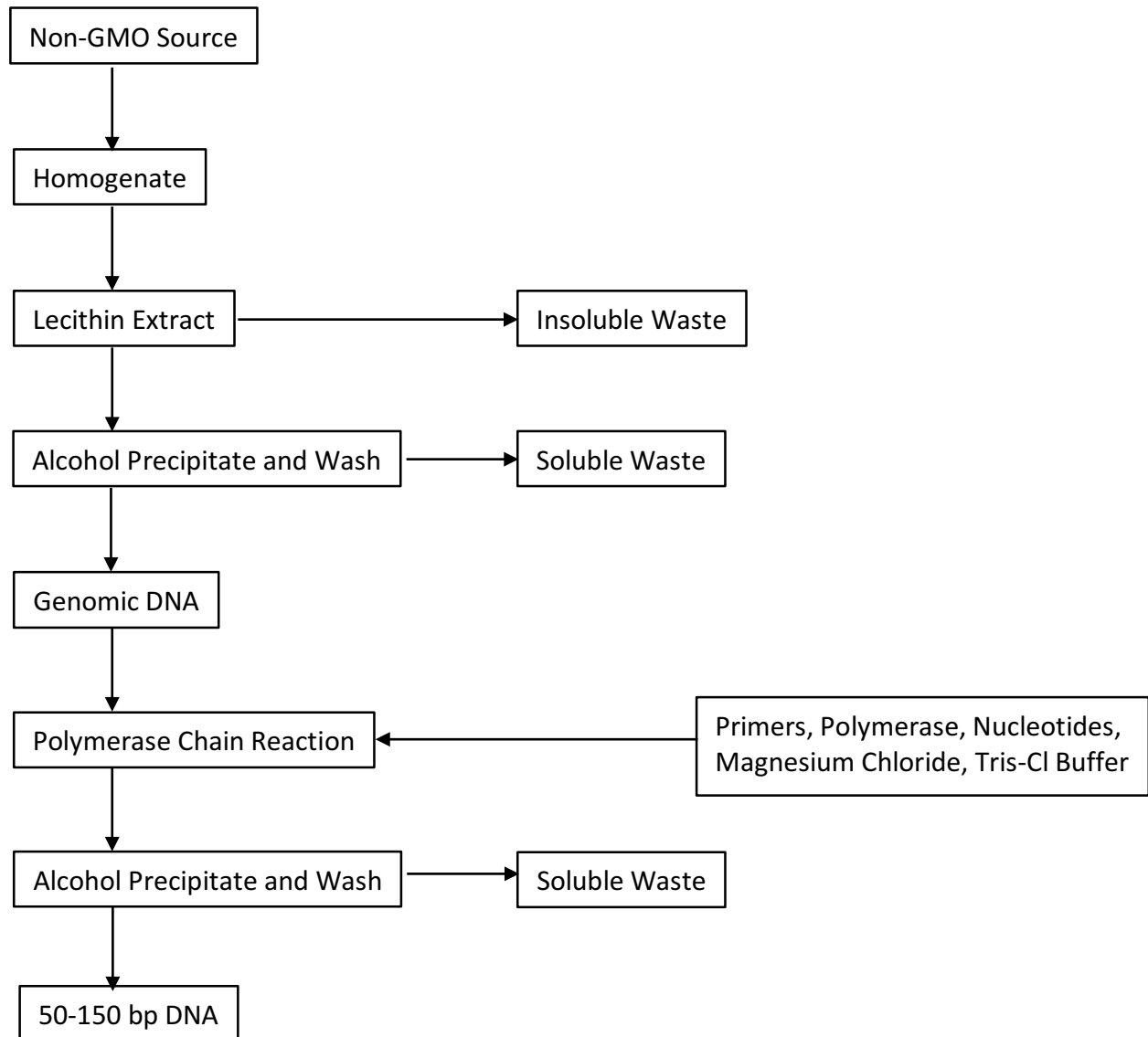
5. Manufacturing Process

Because DNA is necessary for life, all foods naturally contain large quantities of biologically-produced DNA. This naturally occurring DNA is biochemically synthesized inside the cells of all living things. The natural process requires template DNA, complementary primers, nucleotide triphosphates, and a complex DNA polymerase enzyme. The same biochemical process, using the same ingredients, is used to manufacture short DNA outside of a cell, in two steps. First full-length genomic DNA is purified from a natural source, to serve as the template. Then this template is used to biochemically copy a short targeted region of 50-150 bp in length. This process does not involve genetic modification, and no genes are produced or transferred. All living things naturally contain large quantities of DNA, which may be purified for use as a template. Sources used for the petitioned material may include living organisms not normally found in the environment where food is produced, processed and consumed. One example is the red alga *Gelidium amansii*, from which commercial agar is commonly derived.

Extraction of DNA from natural sources is commonly performed as a demonstration in science classes. In fact, the process is so simple that one can do it at home by grinding a strawberry or banana, dissolving it with the help of salt and shampoo or dish soap, and separating the DNA using cold alcohol. A video of this demonstration is at

<https://www.youtube.com/watch?v=67KXatgoNKs>, and a copy of a school lesson plan (http://www.shsu.edu/~agr_www/documents/DNALAB.pdf) is included as an Appendix.

Manufacturing Flow Chart



Step 1 – Isolation of genomic template DNA from natural source

Industrial manufacturing of short DNA tracers is slightly more sophisticated, but begins with the exact same principles of this demonstration.

First the source material is selected to ensure that it meets the restrictions listed above (paragraph 1). Enough tissue for a manufacturing lot of short DNA tracer is procured from the source material. The tissue is trimmed and washed, as necessary, to make certain that it is clean and pure of material from other species. Then it is homogenized, through mechanical grinding. This is commonly performed in batches using one or two minutes in a blender. Grinding also may be facilitated via freezing and/or drying. The grinding step breaks down cell walls, which contain the DNA within physically and biochemically tough compartments inside the cells. DNA molecules are typically very long. Thus grinding causes some shearing of the molecules. This step must be optimized to release the DNA without breaking it into pieces that are too small, on average, to be detected later as tracers.

The homogenate is then dissolved in water with addition of 1-10% w/w lecithin. The lecithin breaks up proteins and lipids, including membranes, just as the shampoo or dish soap does in the class demonstration above. Subsequent steps in purification require relatively gentle handling, as these protein and lipid components normally stabilize DNA in its natural state. Once the DNA is free in the solution it is especially susceptible to mechanical shearing. DNA that is naturally short in length, such as bacterial DNA, is somewhat less susceptible to shearing.

Undissolved solid material is then separated, via centrifugation. The clarified liquid is taken from the top, and 0.1-10% sodium chloride is added and stirred to dissolve. Sodium in the salt serves to bind and neutralize the negative charges on the DNA, so that the molecules may be forced to clump together in the next step.

Next cold alcohol is added. Alcohol acts as an anti-solvent for water, causing the DNA to be physically forced out of solution, form a solid, which is then separated from the solution via centrifugation. The solution, containing most of the sodium chloride and lecithin, is removed from the top and discarded; the crude DNA pellet is re-suspended in cold alcohol and washed. Washing includes gentle stirring to distribute impurities away from the DNA, followed by centrifugation to pellet the DNA again, leaving impurities in solution.

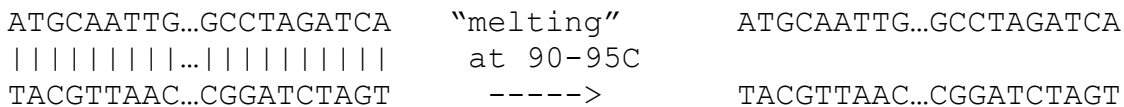
Step 2 – Biochemical copying of target region of template

After the genomic DNA has been isolated, it may be used as a template for the second step, which is biochemical copying. The laboratory biochemical process reproduces the natural DNA synthesis process, with a few differences. The main difference is that the lab method requires a pair of single stranded DNA 20-30 bases in length, called primer oligonucleotides. These DNA molecules are designed to bind to either end of the target 50-150 bp region on the template. They serve to delineate the short region of the genomic DNA that is targeted for copying.

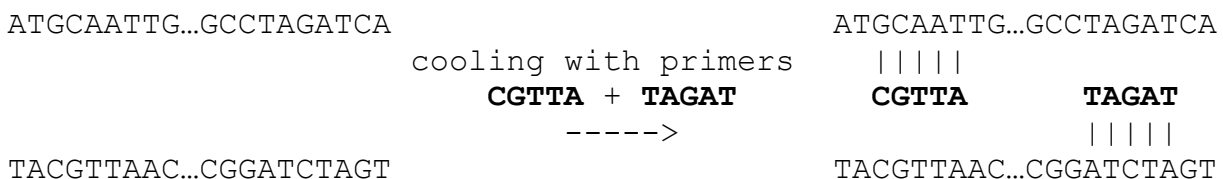
| Component | Function | Amount |
|-------------------------------|-----------------------------|--------------------------|
| Tris-HCl, pH 8.4 | pH buffer | 20 mM |
| KCl | stabilize enzyme | 50 mM |
| MgCl ₂ | bind enzyme to DNA | 1-5 mM |
| Deoxynucleoside triphosphates | building blocks for DNA | 200 μM (each) |
| template DNA (from step 1) | original for copying | 1 μg – 1 mg/L |
| <i>Taq</i> DNA polymerase | enzyme makes new DNA | 5M U*/L |
| Primers | sets template target region | 50 nM – 1 μM |
| Water | diluent | amount dictated by scale |

* One standard Unit of enzyme is a functional measurement. This amount incorporates 10 nmol dNTP into new DNA product material in 30 minutes.

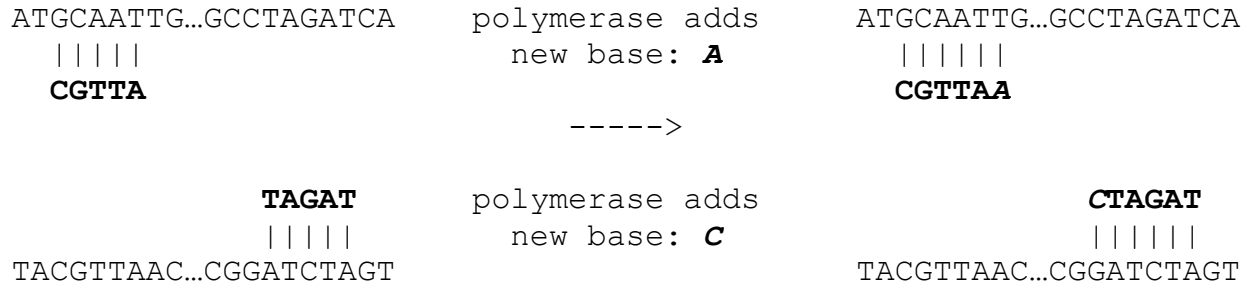
In the manufacturing process all ingredients are added together. Then the reaction vessel is heated to 90-95 °C for 2-5 minutes, to completely denature and separate the two complementary strands of the template DNA. This is shown schematically below:



Then the mixture is cooled to 50-60 °C for 15-45 seconds. Cooling allows the two complementary strands of DNA to pair up again. But because the primers are present in vast excess to the template, they out-compete it, attaching to the template DNA preferentially, displacing the original complementary strand, producing a characteristic overhang structure. In the schematic below the primers are shown in bold letters for clarity. (While actual primers used are 20-30 bases long, these are depicted schematically as only 5 bases in length.)



Next the reaction is warmed to 60-80 °C for 30-180 seconds. This allows the polymerase enzyme to bind to the primer overhang and extends the primer, making new DNA that is complementary to the template. Making new DNA requires a full set of energy-rich triphosphates corresponding to the four DNA nucleosides (i.e., adenosine triphosphate for “A”, guanine triphosphate for “G”, thymidine triphosphate for “T”, and cytidine triphosphate as a precursor for “C”). The polymerase enzyme matches complementary incoming nucleoside triphosphates to the single stranded template DNA in a process known as base pairing. For example, where the template has A, the polymerase associates it with an incoming T. (According to standard Watson-Crick base-pairing, T is complementary to A, A pairs with T, C matches G, and G matches C.) The polymerase then covalently attaches the incoming new nucleotide to the primer DNA, elongating it. Because the two strands of DNA are anti-parallel, the two new DNA strands grow out from the primers in opposite directions.



Then the DNA polymerase proceeds to the next unpaired base on the template, binds the appropriate incoming nucleobase, and attaches it to the growing primer strand. Because a pair of primers is used, two DNA polymerase enzymes can act simultaneously on the opposite strands of the template DNA. Thus both strands act as templates, so both strands are copied. The result of one round of the reaction is a new double-stranded DNA molecule of the target 50-150 bp region, for every molecule of template. The new DNA has the exact same sequence as the target region of the original template DNA. And the template DNA is preserved.

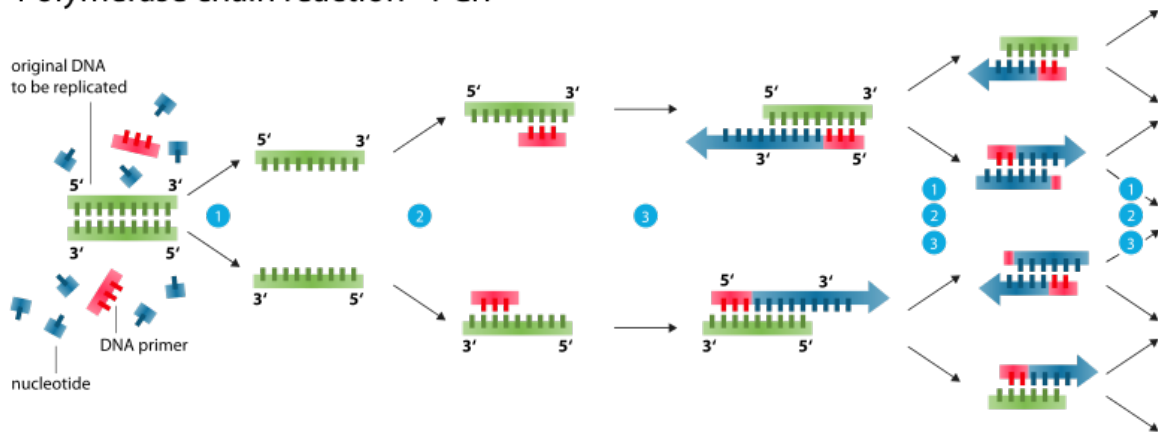


Next the reaction is heated to 90-95 °C again, for 15-60 seconds. This melts apart the DNA paired strands, again, making them available to serve as templates. But now, in addition to the original template DNA, the reaction also contains 50-150 bp DNA products from the first reaction cycle. And these new DNA molecules can also serve as templates for additional DNA production. When the temperature is cycled back to 50-60 °C again, the primers re-anneal, and then at 60-80 °C elongation is repeated. In this second cycle twice as much DNA is made, because twice as many molecules are available to function as template. (Primers and monomer nucleoside triphosphates are included in excess.)

By simply cycling the temperature, the biochemical reaction is repeated 25 to 35 times. Theoretically this would produce 2^{25} to 2^{35} molecules from a single template molecule. In fact, the yield is significantly lower, even under ideal conditions, just as most industrial chemical processes proceed at less than theoretical efficiency. Depending on the sequence of the copied DNA, typically 25-35 cycles produces around 1,000,000 copies from each template molecule.

This biochemical reaction is known as the Polymerase Chain Reaction (PCR), and it has become a standard technique for biology. PCR is the method of choice for preparation of short DNA tracers from templates. In addition to use for preparation of short DNA in the relatively large quantities required for use as tracer ingredients, PCR may be used in the laboratory for rapid and sensitive detection and identification of short DNA tracers in food. This method does not require insertion, deletion, or modification of any genetic material, and it takes place with no living organism. PCR is reviewed at https://en.wikipedia.org/wiki/Polymerase_chain_reaction. The figure below depicts the general reaction scheme.

Polymerase chain reaction - PCR



After preparation the biochemically produced short DNA tracer may be transferred into a water-based solvent for frozen storage, or it may be precipitated with alcohol and stored dry.

Short DNA tracers may also be directly manufactured from genomic DNA. This process involves treatment with restriction endonuclease enzymes, which cut the full-length DNA into specific/defined shorter pieces. Then the desired/target short DNA tracer is purified from undesired/non-target short DNA in the mixture via molecular sizing or ion exchange. This method is widely used for purification of fragments of DNA for subsequent biochemical amplification via PCR. However, it is currently prohibitively expensive for preparation of short DNA in quantities suitable for use as a tracer.

6. Ancillary Substances

For many applications the short DNA tracer may be added as a dilute solution in water, certified organic alcohol, or a mixture. These solvents serve as vehicles to ensure even distribution and/or adherence to the product. In order to protect and preserve the short DNA tracer, distilled or otherwise highly purified water or alcohol are used. The water or alcohol may be evaporated, so that it is not a part of the final product. For example, water solutions of short DNA tracer are sprayed onto leafy green produce and allowed to air dry.

As described above, for other applications short DNA tracer is added to wax coating. These are commercially available blends of allowable waxes e.g., carnauba wax, used off-the-shelf with existing processing equipment. For food such as apples and oranges, the wax serves to reduce drying, assist in flowing, and sometimes to produce an appealing clean shine.

For some applications DNA may be used in a powder form, produced by spray drying to encapsulate with a carrier material. These carriers provide a form of DNA that is stable and dry, suitable for dry foods, such as spices and grains. Suitable certified organic carriers for such formulations include sugars, starches, gums, gels, and gelatin.

7. Previous reviews

Biochemically produced DNA is GRAS. It is nominally covered under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, which were amended in 2013 to include synthetic DNA, as well as recombinant DNA (http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf). However, under these guidelines, the 50-150mer sequences for which we petition are specifically excluded from regulation, because they are presumed to be safe. Section 3 of the Guidelines describes these exclusions:

“Section III-F-1. Those synthetic nucleic acids that:

- (1) can neither replicate nor generate nucleic acids that can replicate in any living cell, and
- (2) are not designed to integrate into DNA, and
- (3) do not produce a toxin.

“Section III-F-2. Those that are not in organisms, cells, or viruses, and that have not been modified or manipulated to render them capable of penetrating cellular membranes.

“Section III-F-3. Those that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.”

Thus NIH considers short DNA tracers safe, and this material is not regulated.

The natural breakdown products of DNA are deoxyribonucleotides, often called simply nucleotides. In 2011-2012 NOSB considered a petition for Nucleotides derived via industrial scale hydrolysis of yeast RNA as an additive for baby formula. Nucleotides from RNA are only slightly different from those derived from DNA. The Technical Report commissioned by NOSB found that these Nucleotides are safe and healthy. Although the Handling Subcommittee recommended adding synthetic nucleotides to the National List, the motion failed on a 7-8 vote, primarily because the Board found inclusion of nucleotides in infant formula to be non-essential.

8. Regulatory Authority

A GRAS Notification for short DNA tracers was submitted to FDA, and a No Questions Letter was received on August 25, 2014. This letter is included as an Appendix. A part of this letter states, “based on its ubiquity in food, FDA concluded that nucleic acids themselves do not raise safety concerns. ... Moreover, the small size of the nucleic acids involved would not ordinarily be expected to remain intact after digestion or be biologically active.”

We are not aware of any EPA, FDA, or US State regulatory authority registrations for DNA. We note that, although it is not considered an essential nutrient, DNA is used legally as a dietary supplement, for which FDA registration is not required.

9. Chemical Abstracts Service (CAS) Number and Product Labels

DNA does not have a Chemical Abstract Service (CAS) number. Any such identifier would be assigned on a sequence-specific basis. This petition is for DNA polynucleotides of 50-150 bp comprising any sequence of the four naturally-occurring 2-deoxyribose-containing nucleotides: deoxyadenosine, deoxycytidine, deoxyguanosine, and (deoxy)thymidine. An example of such a material is:

CATGGGCGTTCGGCACTACCGACACGAACCTCAGTTAGCGTACATCCTACCAGAGGTCTGTGGCCCCGTGGTCAAAGTGC GGTTTCGTATTTGCTGCT. (The sequence is given for only one strand of the double-stranded DNA, in the 5' to 3' direction. The complementary opposite strand of the molecule is assumed.)

10. The substance's physical properties and chemical mode of action:

(a) Physical properties

Double-stranded short DNA tracers (50-150 bp) are unbranched macromolecules. This means that the molecules are relatively large, linear structures, 25-75 times longer than their width. At high concentrations they tangle, increasing solution viscosity. DNA molecules are also polyanions, due to their phosphate backbone, bearing 100-300 negative charges per molecule. The negative charges make the DNA very easy to dissolve in water.

Short DNA tracers are colorless, odorless, and flavorless. Although high concentrations are somewhat viscous, in the small amounts used for traceability, short DNA tracers do not affect mouthfeel.

(b) chemical interactions with other substances, especially substances used in organic production;

In nature DNA's negative charges function partly to promote the functional association of histones and other nucleoproteins. They also allow reversible binding to important structural polyamines common in plant and animal material, e.g., spermidine. In water, metals pair weakly with DNA. But when alcohol is added, displacing >64% of the water, the metal-DNA complex precipitates out of solution. This phenomenon is commonly used to assist in purification of DNA.

Most natural functionality of DNA is related to genetic information – coding for the synthesis of RNA and proteins. Transcription and translation of this information requires the action of specific proteins inside of cells. While these proteins interact with naturally occurring DNA, 50-150bp DNA is too small to participate.

(c) toxicity and environmental persistence;

When manufactured as described here, short DNA tracers are indistinguishable from the DNA that is naturally present in all living things, except that short DNA tracers are much smaller molecules, and they are present only outside of living cells. Naturally occurring DNA is moderately stable in most foods, especially in the dark at low temperatures. This stability is afforded by sequestration within the cell membrane and organelles, and by binding to nucleoproteins to form tightly folded chromatin. In living cells natural DNA is constantly monitored for damage and errors, which are either repaired, or broken down and reassembled. This quality control is provided by specialized enzyme complexes. On the other hand, the short DNA tracers petitioned herein are naked. They do not benefit from such stabilization, and they are destroyed in the environment.

Compared to other macromolecules, such as RNA and protein, DNA is a remarkably stable molecule. This is one reason that it is such an effective medium for storing information – both biological information in living organisms, and supply chain information as an inactive food ingredient with no technical effect. When used as a tracer, it is stable for a few months when dry, for example in the wax coating on an apple, or in cereal or pasta. On the other hand, in the presence of water short DNA tracers slowly degrade. They are subject to hydrolysis, which breaks the DNA into smaller and smaller pieces, destroying the information encoded. This effect is increased at temperatures above 212 °F and in acidic pH (pH < 7). DNA is also susceptible to damage from ultraviolet (UV) light, such as sunlight. In addition, raw foods contain defensive and digestive enzymes that destroy DNA. Indeed, cooking destroys DNA rapidly. In flour >99% of DNA is destroyed by baking cookies, and in ground beef >99.9% is destroyed by browning in a skillet. Thus short DNA tracers are destroyed during normal use, and they do not persist in the environment.

DNA is completely biodegradable, and almost all organisms readily use it as a food. When eaten, DNA is rapidly broken up into individual nucleotides. In the diet, nucleotides serve important biochemical functions, including raw materials for making new DNA, and sources of energy, such as the nucleotide ATP (Carver, 2003). Although as a food ingredient short DNA tracers are actually nutritious, DNA is not considered essential for the human diet. This petition does not claim any nutritional value for short DNA tracers, as the amount proposed for addition to food is biologically insignificant.

We note that nucleotides (the primary products of DNA breakdown) were formerly included in the National List under the “Nutrient Vitamins and Minerals” classification. As of January 2012, nucleotides and other additives not specified in the rule must be petitioned and reviewed on an individual basis.

Short DNA tracers are only 50-150 bp long, which is too short to code for biological information. For example, by comparison, the smallest known gene is 406 bp in length (coding for the highly conserved nucleoprotein histone H4). Naturally occurring genetic material is well-known to undergo natural recombination reactions, adding and swapping between genomes. This provides is an important means for generating mutations, especially in bacteria and viruses. But small molecules of DNA 50-150 bp in length are too short to contain the functional elements (Linear Terminal Repeats, LTR) that are required for natural recombination. There is no evidence that these molecules can be assimilated by living things, other than as food, which entails digestion and thereby destruction.

(c) environmental impacts from its use or manufacture;

The petitioned substances are found in all living cells. The manufacture of the substance is carried out in controlled environments. Any environmental contamination resulting from production and purification of DNA would be subject to regulations governing waste discharges from the laboratories. Anticipated use of the material for food traceability may be expected to result in occasional small environmental releases. Because DNA is not stable in the environment, and extremely small amounts are used as a processing aid or disposed as industrial waste, environmental impact is negligible.

(d) effects on human health;

As stated above, DNA is a nutrient that is normally abundant in human diets. The amount of short DNA added for tracing purposes is less than one trillionth the amount found in a typical meal. Normal digestive processes completely destroy short DNA tracers, breaking them down into individual nucleotides. The nucleotides are then absorbed into the body. Up to 5% may be incorporated into tissues (Carver, 2003), while the remainder is “burned” for energy.

Regarding nucleotides, on April 25, 2012, ICF International presented a Technical Evaluation to the NOSB that indicated,

“Nucleotides play important roles in many specific biological processes, including cellular signaling, and are constituents of biologically important coenzymes, molecules required for the normal functioning of cellular enzymes. Nucleotides are considered ‘conditionally essential’ nutrients. This means that they are not normally required in the diet, but must be supplied to some individuals during certain disease states because those individuals cannot synthesize the compound internally, or their need for the compound is greater than their capacity for synthesis (Fürst and Stehle, 2004). This may be especially true of nucleotides during periods of rapid growth or in the case of some diseases affecting infants (Singhal, et al., 2010).”

We also note that nucleotides were formerly included under the “Nutrient Vitamins and Minerals” classification in the National List. As of January 2012 nucleotides and other additives not specified in the rule must be petitioned and reviewed on an individual basis.

Some experts believe that dietary supplementation with DNA (and RNA) may improve health. In one recent publication dietary DNA was found to extend the lifespan of rats (Xu, 2013). This observation has not been replicated, and the idea is not widely accepted.

(e) effects on soil organisms, crops, or livestock.

Short DNA tracers are not intended for use on soil, organisms, crops, or livestock. However, disposal and spills may be expected occasionally to release small amounts into the environment. Fortunately, almost all life forms readily use foreign DNA as food source. It is theoretically possible that a significant source of extra DNA might shift populations within a biome, favoring those that can efficiently utilize DNA as an energy source. However, the amount added for tracing is not nutritionally significant, and so it will not have such an influence on ecology.

11. Safety Information

This product is considered non-hazardous. Per OSHA 29 CFR 1910.1200, Commonwealth of Australia [NOHSC: 1005,1008(1999)], and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, this product does not require a Material Safety Data Sheet (MSDS) or Safety Data Sheet (SDS). Nonetheless, we have attached SDS from two commercial suppliers of chemically synthetic DNA, which is comparable to natural DNA on the molecular level.

The US National Institute of Environmental Health Studies has not generated a health report document for DNA, and DNA is not listed by the National Toxicology Program.

12. Research Information

Committee on Recombinant DNA Molecules Assembly of Life Sciences, National Research Council. 1974. "Potential Biohazards of Recombinant DNA Molecules" *Proc. Nat. Acad. Sci. USA*, 71(7):2593-2594.

The short DNA tracers described in this petition specifically exclude recombinant DNA (rDNA), which is the topic of this white paper. Nonetheless, it set the standard for DNA safety, in general. Published by the National Academy of Sciences, this was one of the first definitive publications describing the hazards of rDNA technology. It called for a new NIH committee for regulation of rDNA, encouraged debates about safety, and recommended moratoria on rDNA experiments that might increase pathogenicity. This publication led directly to the February 1975 Asilomar International Conference on Recombinant DNA Molecule Research.

US Department of Health and Human Services. 2010. "Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA," 75 FR 62820.

This Federal Regulation pertains to commercial orders for manufacturing of DNA. Specifically, it recommends voluntary screening for "sequences of concern," defined as unique base sequences derived from or encoding Select Agents and Toxins (q.v.). This petition specifically excludes DNA containing sequences derived from Select Agents and pathogenic organisms, as well as DNA encoding any toxin.

US Centers for Disease Control and Prevention (CDC) and USDA. 2013. Select Agents and Toxins List. <http://www.selectagents.gov/SelectAgentsandToxinsList.html>.

This is the current official list of Select Agents and Toxins - organisms and toxins that are considered to pose severe threats to health, including the health of humans, livestock, and crops. Listed agents are generally considered risks for bioterror activity. Thus research and commercial activities performed with them is highly restricted under 7 CFR 331, 9 CFR 121, and 42 CFR 73. The DNA described in this petition specifically excludes DNA obtained from these sources.

It is noted that practically all living things produce toxins, which are chemicals developed specifically to kill or incapacitate other living things sharing the environment. These toxins are produced according to genetic instructions encoded by DNA. Thus it would be impossible to identify a completely non-toxic organism from which to derive short DNA tracers. For example, cinnamon contains cinnamaldehyde, a natural flavoring chemical that is well-tolerated in humans, but happens to be quite toxic to many fungi and insects. This petition allows the use of DNA from the various *Cinnamomum spp.* organisms that produce the cinnamon toxin, as long as that DNA does not include genes or fragments of genes that code for the toxin.

12. Petition Justification Statement

E. Inclusion of a Non-Synthetic, Non-Agricultural Substance in the National List, §205.605(a)

Certified organic foods justifiably command a higher price than conventionally produced foods. However, the two products are often completely indistinguishable on the material level. Labeling is commonly counterfeited. Testing for residues of materials not on the National List is expensive and unreliable. And in fact, the organic method is not based on lingering residues, but on the agriculture process. Thus there is a significant incentive for fraud. Consumers and

processors need means for tracking organic foods back to their sources, to ensure compliance with organic labeling regulations. And certifiers need tracing to support the claims of growers and processors. At this point tracking and tracing rely on paper files and electronic records. As the organic food industry grows and matures, it needs to achieve the next level of assurance through improved traceability.

Short DNA may be added at the source, pre-harvest or post-harvest, like an invisible edible barcode. It can also be added at subsequent points in supply and processing. Different short DNA tracer sequences can be used to code for various locations, handlers, activities, and even dates. If there is a question about authenticity, the short DNA tracers can be measured to recreate the supply chain record for the commodity or product.

It is imperceptible to consumers and potential fraudsters, because it is invisible, tasteless, and odorless. It is philosophically acceptable to consumers of organic products, because DNA is naturally derived and already naturally present in foods.

In a hypothetical example of the use of short DNA tracers, consider a grower/shipper of a variety of organic apple. They label their fruit by adding a unique short DNA tracer to a wax coating applied during processing. When they visit a large grocer to sell their organic apple variety, the produce manager shows them similar apples bearing counterfeit labels bearing the USDA Organic seal. The grower can swab the surface of apples from both sources, put them into a test device, and show the grocer how only the authentic product bears the short DNA tracer. Testing currently takes less than an hour, and less than \$100, and the pace of modern technology will make it even faster and less expensive in the future. The test result identifies the specific short DNA tracer molecule used in the coating wax, among millions of possible sequences. That tracer is linked to the grower's supply chain. The grower may choose to use only a single tracer, to indicate the source. Or the grower may use many different short DNA molecules. In the latter case, the various sequences detected are linked to specific locations, harvest crews, date/time stamps, carriers, etc.

As an alternative to short DNA for tracing food, we are aware of Grainfetti. Grainfetti is ¼ inch square pieces of paper bearing printed alphanumeric codes that are mixed into grain. To our knowledge, this is the only other food tracer added to the food, rather than being applied to food packaging. Grainfetti is designed to prevent theft/diversion, and it is not suitable for certified organic products, in which both the paper and ink would be prohibited as ingredients. Grainfetti is GRAS and nominally edible. In practice it is impossible to completely remove Grainfetti from grain. Furthermore, Grainfetti has very limited use as it is not suitable for foods that are larger than grains or for moist foods.

The growing demand for improved food traceability and safety is reflected in many publications, including the following recent examples:

- Private Eyes in the Grocery Aisles. *New York Times*, April 5, 2015. http://www.nytimes.com/2015/04/05/business/private-eyes-in-the-grocery-aisles.html?_r=0. DNA tracers can be used for detection of fraud, and for improved tracing of disease outbreaks, through food source tracking.

- Food Traceability Gaining Popularity as Consumers Demand Transparency. *Organic Monitor*, January 16, 2015. <http://www.organicmonitor.com/r1601.htm>. Consumers of organic foods are especially demanding of transparency and traceability.
- Quick DNA Scan Could Ensure Food is Safe to Eat. *Scientific American*, February 4, 2015. <http://www.scientificamerican.com/article/quick-dna-scans-could-ensure-food-is-safe-to-eat/>. DNA tracers can accelerate food trace-back. Technology promises to put such testing into consumers' own hands soon.
- A New Weapon in Food Safety: Tracking Everything We Eat from Seed to Stomach *Fast Company*, September 8, 2016. <https://www.fastcompany.com/3063134/food-safety-track-and-trace-data>. Sluggish tracking for Chipotle's many foodborne disease outbreaks in 2015 led to increased disease and disgust among consumers. With no clear definition for "ethical," "sustainable," or even "healthy," complete transparency is becoming the universal standard for a company's trustworthiness with consumers. Food producers save money by investing in traceability, because they discover supply chain problems and solve them easily.
- Public demands what feds suggest: Traceability via food labels. *Food Safety News*, March 7, 2016. <http://www.foodsafetynews.com/2016/03/public-demands-what-feds-suggest-traceability-via-food-labels/#.V9cmA5MrJp9>. FSMA requires food processors to acquire and use more information about their supply chain.
- Spray on traceability for contaminated food. *Food Quality News*, January 14, 2015. <http://www.foodqualitynews.com/R-D/Tracing-contaminated-food-back-to-source-thanks-to-DNA-Trax>. DNA tracers in food can be used to track sources in under 1 hour. They can also be used to detect fraud.
- DNA Spray-On Technology Could Revolutionize Food Traceability. *Food Safety News*, November 17, 2014. <http://www.foodsafetynews.com/2014/11/dna-laced-spray-technology-could-revolutionize-food-traceability/#.VGquAlegJaV>. The advantage of having a DNA barcode directly on fresh produce is that it significantly reduces the potential for traceback information to be lost.
- Spray-on DNA seen as produce traceability solution. *The Packer*, November 11, 2014. <http://www.thepacker.com/fruit-vegetable-news/Spray-on-DNA-seen-as-produce-traceability-solution-282323281.html>. Because food products can be traced back more quickly, fewer people will get sick from eating contaminated products, which will result in fewer lawsuits for food processors.
- Benefits of Food Traceability. *Food Safety Magazine*, July 14, 2015. <http://www.foodsafetymagazine.com/newsletter/benefits-of-food-traceability>. As more consumers demand local, sustainable, organic, non-genetically modified foods, traceability is the only tool that can prove to the consumers they are in fact purchasing and consuming a product that makes these claims.

Supply chain tracing is already valued in conventional agriculture. There it is used to prevent fraud and manage logistics, as well as to improve food safety, by shortening the time required to execute a recall in the case of adulteration. Organic foods suffer recall rates similar to those of conventional foods, for most of the same reasons, including pathogens and undeclared allergens. Thus improved traceability will also improve safety of organic foods.

References

Carver, J. D. 2003. Advances in nutritional modifications of infant formulas. *The American journal of clinical nutrition* 77:1550S.

Fürst, P; Stehle, P. (2004). What are the essential elements needed for the determination of amino acid requirements in humans? *Journal of Nutrition* 134 (6 Suppl): 1558S-1565S.

Rizzi A, Raddadi N, Sorlini C, Nordgrd L, Nielsen KM, Daffonchio D. 2012. “The stability and degradation of dietary DNA in the gastrointestinal tract of mammals: implications for horizontal gene transfer and the biosafety of GMOs.” *Crit Rev Food Sci Nutr.* 52(2):142-161.

Singhal, A., K. Kennedy, J. Lanigan, H. Clough, W. Jenkins, A. Elias-Jones, T. Stephenson, P. Dudek, and A. Lucas. 2010. Dietary Nucleotides and Early Growth in Formula-Fed Infants: A Randomized Controlled Trial. *Pediatrics* 126:e946.

Xu M, Liang R, Guo Q, Wang S, Zhao M, Zhang Z, Wang J, Li Y. 2013. “Dietary nucleotides extend the life span in Sprague-Dawley rats,” *J. Nutr Health Aging.* 17(3):223-229. doi: 10.1007/s12603-012-0399-z.

Appendix 1



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740-3835

August 25, 2014

Anthony Zografos
DNA Trek
3997 Lyman Road
Oakland, CA 94602

via email: azografos@dnatrek.com

Re: Your Inquiry on DNATrax, ID system using small DNA sequences

Dear Mr. Zografos:

In response to your inquiry, dated July 14, 2014, about the regulatory status of DNATrax, our thinking on the status of DNATrax may be somewhat different from yours; however, the overall conclusion would be that the use of DNA in this fashion would meet GRAS (generally recognized as safe) criteria. Our understanding is that DNATrax utilizes small defined DNA segments of around 100 bases (you specifically mention the thermophilic bacterium *Thermatoga maritima* as one source of DNA in your submission) as identifiers that could be applied to fruit and vegetables to encode information that would identify the companies involved in the processing and distribution of the commodities.

In its Statement of Policy: Food Derived From New Plant Varieties (57 FR 22984, May 29, 1992), the agency stated the following:

With respect to transferred genetic material (nucleic acids), generally FDA does not anticipate that transferred genetic material would itself be subject to food additive regulation. Nucleic acids are present in the cells of every living organism, including every plant and animal used for food by humans or animals, and do not raise a safety concern as a component of food. In regulatory terms, such material is presumed to be GRAS. Although the guidance provided in section VII. calls for a good understanding of the identity of the genetic material being transferred through genetic modification techniques, FDA does not expect that there will be any serious question about the GRAS status of transferred genetic material.

Consequently, based on its ubiquity in food, FDA concluded that nucleic acids themselves do not raise safety concerns. While this use of nucleic acids would be distinct from the use of genetic material in plant cells or other food cells, the inherent rationale would still apply. Moreover, the small size of the nucleic acids involved would not ordinarily be expected to remain intact after digestion or be biologically active.

Further, the carriers you describe would ordinarily also be considered GRAS, in fact, some have been affirmed as GRAS by the agency in years past (In Title 21 of the Code of Federal Regulations, maltodextrin is affirmed GRAS in section 184.1444, salt is mentioned in section 182.1(a) as a substance the Commissioner regards as safe for its intended use). Starches, while not specified in the regulations as direct food ingredients have a long history of use in foods and would meet history of common use in food criteria as discussed in the report of the Select Committee on GRAS Substances on uses of starches in packaging materials in 1979.

Page 2 -Mr. Zografos

Based on these criteria, we conclude that the DNA and carriers and other substances used in DNATrax would be safely used as a tracer/identifier as described in the description dated June 12, 2014.


Sincerely yours,

**Robert I.
Merker -S**

Digitally signed by Robert I. Merker -S
DN: c=US, o=U.S. Government, ou=HHS,
ou=FDA, ou=People,
0.9.2342.19200300.100.1.1=1300070407,
cn=Robert I. Merker -S
Date: 2014.08.25 08:16:00 -0400

Robert I. Merker, Ph.D.
Supervisory Consumer Safety Officer
Division of Biotechnology
and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Appendix 2 – Here are two different Safety Data Sheets for short synthetic DNA, which is indistinguishable from natural DNA.

| | | | |
|--|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 1 / 7 |

1. Product and Company Identification

1.1 Product identifier

Chemical Name / Trade Name: Synthetic Nucleic Acid (Oligonucleotide)
 Index-No.: ---
 EG-No.: ---
 CAS-No.: ---
 REACH-Registration No.: ---
 Other Names: ---

1.2 Application of the substance

For Research and Development, In-Vitro-Diagnostic, Laboratory chemicals

1.3 Manufacturer's details

| | |
|---------------------------------|-------------------------------------|
| Manufacturer: | Eurofins MWG Synthesis GmbH |
| Street / No.: | Anzinger Straße 7a |
| Nat. / Post code / Town: | D-85560 Ebersberg |
| Contact: | Eurofins Genomics, Customer Support |
| Phone-No.: | (+49)-8092-8289-77 |
| Fax-No.: | (+49)-8092-8289-21084 |
| E-Mail: | support-eu@eurofins.com |

1.4 Information in case of an emergency:

Poison Information Center Mainz: (+49)-6131-19240 (24h in German and English)

2. Hazard Identification

2.1 Classification of the substance


Classification according to Regulation (EC) 1272/2008, annex VII:
 Not classified
 Classification according to Directive 67/548/EEC or Directive 1999/45/EC:
 Not applicable

2.2 Label elements

Labelling according to Regulation (EC) 1272/2008 / Directive 1999/45/EC:
 Not applicable

2.3 Other hazards

Warning: substance not fully tested

| | | | |
|--|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 2 / 7 |

3. Composition / Information on Ingredients

3.1 Compound

Oligonucleotide

4. First-Aid Measures

4.1 Description of the First-Aid Measures

General advices

If health disorders occur, get medical attention

Inhalation

Get fresh air supply immediately. If irritations present, get medical attention

Skin Contact

Wash with soap and water. If irritations present, get medical attention

Eye Contact

Flush open eye with water for several minutes. If necessary, remove contact lenses. If irritations present, get medical attention.

Ingestion

Flush mouth area with water and get medical attention

Injection

Get medical attention

4.2 Most important symptoms and effects, both acute and delayed

Not known

4.3 Recommendations to physicians

For coordination of the treatment ask the Poison Information Center Mainz (see 1.4)
No specific antidote known. Treat symptomatically and supportively

5. Fire-Fighting Measures

5.1 Fire extinguishing agents


CO₂- or Powder Spray, alcohol resistant Foam

5.2 Special hazards in case of fire

Do not inhale explosion or combustion gases, aerosols or powder

5.3 Advice for firefighting

Avoidance of contamination of the surfaced or ground water, sewage system or soil by firefighting water

| | | | |
|--|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 3 / 7 |

6. Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures

Ensure adequate ventilation, avoid dust formation, use personal protective equipment

6.2 Environmental precautions

Should not be released into the environment or sewage system

6.3 Methods for containment and cleaning up

Larger amounts should be absorb with liquid-binding material (Sand, diatomite, universal binders, acid binders, sawdust)
Disposals of the waste should be in closed bins

7. Handling and Storage

7.1 Handling

Precautions for safety handling

Use personal protective equipment (Lab coat, gloves and safety glasses)
Eating, drinking and smoking should be forbidden in relevant areas

Measures for fire- and explosion protection

Standard measures for fire protection

Measures for avoidance of dust formation or aerosols

Ensure adequate ventilation
Use fume hood while handling with large amounts

Measures for protection of the environment

Should not be released into the environment or sewage system

General hygiene measures

Use personal protective equipment

7.2 Conditions for safe storage, including any incompatibilities storage

Requirements for storage conditions

Keep container tightly closed, in a cool, dry place ($\leq 4^{\circ}\text{C}$)

Requirements for storage rooms and containers


No special requirements
Do not store with foods, medicine or feeding stuff

Storage classification

Not applicable

7.3 Specific end use

No further relevant information available

| | | | |
|--|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 4 / 7 |

8. Exposure Controls / Personal Protection

8.1 Controlled parameters

Not applicable

8.2 Exposure controls

General protective and hygienic measures

Eye / face protection

Wear appropriate protective eye glasses (proofed by EN 166)

Skin / body protection

Wear appropriate protective gloves (proofed by EN 374 and EN 455, Kat. 1) and clothing (Lab coat)

Respiratory protection

In case of high dust formation wear dust mask

Protection against heat / cold

Not applicable

9. Physical and Chemical Properties

9.1 Information on basic physical and chemical properties

| | |
|-----------------------|--|
| Physical State: | Solid, dissolved in water or water based buffers |
| Appearance: | Diverse |
| Odour: | odourless |
| Odour Threshold | Not determined |
| pH-value: | Not determined |
| Melting point: | Diverse |
| Boiling point: | Not determined |
| Flash Point: | Not applicable |
| Self-igniting: | No self-igniting |
| Explosion limits: | Not applicable |
| Relative density: | Not determined |
| Solubility: | Fully soluble in water |
| Explosive properties: | None |
| Oxidizing properties: | None |


10. Stability and Reactivity

10.1 Reactivity

various

10.2 Chemical stability

No additional risks for decomposition

| | | | |
|---|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 5 / 7 |

10.3 Hazardous Reactions

Not known

10.4 Conditions to avoid

No dangerous reactions known if used according to specifications

10.5 Incompatible materials

Not known

10.6 Hazardous decomposition products

Not known

11. Toxicological Information

11.1 Information on toxicological effects

Acute toxicity
Not determined

Irritation on skin
Irritations possible

Irritation on eyes
Irritating effects

Sensitization
Not determined

Mutagenic effects
Not determined

Carcinogenicity
Not determined

Reproductive effects
Not determined

Specific organ toxicity after single or repeated exposure
Not determined


12. Ecological Information

12.1 Toxicity
Not determined

12.2 Persistence and degradability
Not available

12.3 Bioaccumulative potential
Not determined

12.4 Mobility in soil
Not determined

| | | | |
|---|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 6 / 7 |

12.5 Results of PBT and vPvB assessment

Not applicable

12.6 Other adverse effects

Water hazard class 1 (self-assessment): slightly hazardous for water. Do not allow undiluted product or large quantities of it to reach ground water, water course, sewage system or soil.

13. Disposal considerations

Waste treatment methods

Disposal must be made according to regional and national regulations
 Must not be disposed together with household garbage
 Do not allow product to reach sewage system

Contaminated packaging

Disposal must be made according to official regulations
 Recommended cleansing agents: water, if necessary together with cleansing agents

14. Transport Information

14.1 UN-Number

None

14.2 UN proper shipping name

ADR / RID

No hazardous good

IMDG-Code / ICAO-TI / IATA-DGR

None

14.3 Transport hazard class

None

14.4 Packing group

None

14.5 Environmental hazards


ADR / RID / IMDG-Code / ICAO-TI / IATA-DGR: no
 Marine Pollutant: no

14.6 Special precautions for user

Not applicable

14.7 Transport in bulk according to Annex II of MARPOL 73/78 and the IBC code

Not applicable

| | | | |
|--|--------------------------|--|----------|
| Campus Ebersberg | F_HSE_6.3-06_MSDS_en_4.0 |  eurofins | Genomics |
| Material Safety Data Sheet Oligonucleotide (unmodified and modified) English | | | 7 / 7 |

15. Regulatory Information

15.1 Safety, health and environmental regulations / specific legislation for the substance or mixture

EU-Regulations:

Not applicable

National regulations:

Water hazard class
WGK I slightly hazardous for water (self-assessment)

Other relevant regulations

Not applicable

15.2 Chemical safety assessment

Has not been carried out
Warning: substance not yet fully tested

16. Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. Eurofins MWG Synthesis GmbH assumes no legal responsibility for use or reliance upon this data.



Safety Data Sheet

SDS Number: IDT-001
Effective Date: February 12, 2003
Revision Date: February 10, 2015

1. PRODUCT AND COMPANY IDENTIFICATION

A. PRODUCT IDENTIFIERS

PRODUCT NAME : **Oligodeoxyribonucleic Acid, Unmodified**
SYNONYMS : **Synthetic DNA, Oligo**
PRODCUT NUMBER/CODES : **Proprietary Information**
INDEX # : **N/A**
CAS # : **N/A**

B. DETAILS OF SUPPLIER OF THE SAFETY DATA SHEET

COMPANY : **Integrated DNA Technologies**
: **1710 Commercial Park**
: **Coralville, IA 52241**

TELEPHONE : **1-800-328-2661**
EMERGENCY PHONE # : **24 Hour Emergency Telephone:**
: **CHEMTREC: 1-800-424-9300**

Note: CHEMTREC's emergency number is to be use only in the event of chemical emergencies involving a spill, leak, fire, exposure or accident involving chemicals.

C. RELEVANT IDENTIFIED USES OF THE SUBSTANCE OR MIXTURE:

For research and development purposes only.

2. HAZARD IDENTIFICATION

A. GHS LABEL ELEMENTS, INCLUDING PRECAUTIONARY STATEMENTS

THIS PRODUCT IS CONSIDERED NON-HAZARDOUS. Per OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)], and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, this product does not require a Material Safety Data Sheet (MSDS) or Safety Data Sheet (SDS). This product does not contain more than 1% of a component classified as hazardous and does not contain more than 0.1% of a component classified as carcinogenic. Despite the classification of certain products/components as non-hazardous, we strongly recommend using prudent laboratory practices: avoiding unnecessary contact and use of personal protective equipment, which may include gloves, eye protection, and lab coats, during the use of any laboratory reagent. IDT shall not be held liable for any damages resulting from handling or from contact with the above product.

I. PICTOGRAM: :NOT APPLICABLE
II. SIGNAL WORD :NOT APPLICABLE
III. HAZARD STATEMENTS :NOT APPLICABLE
IV. PRECAUTIONARY STATEMENTS: :NOT APPLICABLE

3. COMPOSITION/INFORMATION ON INGREDIENTS

| INGREDIENTS | CAS # | PERCENT | HAZARDOUS CLASSIFICATION |
|--|-------|---------|--------------------------|
| Oligodeoxyribonucleic acid, unmodified | N/A | 100% | NON-HAZARDOUS |

4. FIRST AID MEASURES

IDT-001-MSDS / REV 06

Oligodeoxyribonucleic Acid, Unmodified
Reviewed/Issue: 2/10/15

Page 1 of 3

- SKIN CONTACT: Remove contaminated clothing. Flush affected area with water and then wash with soap or mild detergent and water. Observe for signs of irritation. If irritation is present, get medical attention.
- EYE CONTACT: Wash eyes with large amounts of water or normal saline for at least 15 minutes. Observe for signs of irritation. If irritation is present, get medical attention.
- INGESTION: Get medical attention.
- INHALATION: Remove from exposure area to fresh air immediately. Get medical attention immediately.
- INJECTION: If accidentally injected, get medical attention.
- NOTE TO PHYSICIAN: There is no specific antidote. Treat symptomatically and supportively.
5. **FIRE FIGHTING MEASURES**
- Fire and Explosion Hazard: None known to exist.
- Fire Extinguishing Media: Dry chemical, foam, carbon dioxide or water. Care should be taken as to not aerosolize the powder, where it could become an inhalation risk.
6. **ACCIDENTAL RELEASE MEASURES**
- OCCUPATIONAL SPILL: Wear appropriate protective clothing and chemically compatible gloves. Place spillage in appropriate container for waste disposal. Wash contaminated clothing before reuse.
- REPORTABLE QUANTITY (RQ): Not Applicable
7. **HANDLING AND STORAGE**
- Observe all federal, state and local regulations. Do not breathe dust. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Keep tightly closed. Store in a cool dry place (prolonged storage at 4° C). Use prudent laboratory practices for handling and storage of chemical substances of unknown toxicity.
8. **EXPOSURE CONTROLS/PERSONAL PROTECTION**
- EXPOSURE CONTROLS: Use in a laboratory hood or other ventilated device. OSHA, ACGIH, or NIOSH has not established occupational exposure limits for this substance. Use prudent laboratory practices for handling chemical substances of unknown toxicity.
- EYE PROTECTION: Employees should wear splash proof or dust resistant safety goggles to prevent eye contact with this substance.
- CLOTHING: Employees should wear appropriate protective clothing (laboratory coat with long sleeves) and equipment to prevent skin contact with this material.
- GLOVES: Employee must wear appropriate protective gloves to prevent contact with this material.
9. **PHYSICAL AND CHEMICAL PROPERTIES**
- DESCRIPTION: Translucent or opaque flake, film or powder like substance
- PH: Not Available
- MOLECULAR WEIGHT: Varies
10. **STABILITY AND REACTIVITY**
- REACTIVITY: No adverse reactions have been reported.
- CONDITIONS TO AVOID: Prolonged Storage. Keep at 4° C.
- INCOMPATIBILITIES: Not Available
- POLYMERIZATION: Not Available
11. **TOXICOLOGICAL INFORMATION**

| | |
|---------------------------------------|--|
| TOXICITY: | The toxicological properties of this material have not been investigated. For research and development purposes only. |
| CARCINOGEN STATUS: | No information available. |
| LOCAL EFFECTS: | No information available. |
| TARGET EFFECTS: | No information available. |
| AT INCREASED RISK FROM EXPOSURE: | No information available. |
| CARCINOGEN/REPRODUCTIVE TOXIN STATUS: | |
| IARC | No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC |
| ACGIH | No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by ACGIH |
| NTP | No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by NTP |
| OSHA | No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by OSHA |

12. ECOLOGICAL INFORMATION

| | |
|------------------------------------|--------------------------|
| ENVIRONMENTAL IMPACT RATING (0-4): | No data available |
| ACUTE AQUATIC TOXICITY: | No data available |
| DEGRADABILITY: | No data available |
| LOG BIOCONCENTRATION FACTOR (BCF): | No data available |

13. DISPOSAL CONSIDERATIONS

Observe all Federal, State and Local Regulations.

14. TRANSPORTATION INFORMATION

Ship as a non-regulated material. No transportation restrictions apply. The acute toxicity profile does not warrant shipment as a hazardous material (DOT) or a dangerous good (IATA).

15. REGULATORY INFORMATION

The toxicological properties of this material have not been investigated. For research and development purposes only.

16. OTHER INFORMATION

POTENTIAL HEALTH EFFECTS:

| | | |
|---------------|----------------------|---------------------------------|
| SKIN CONTACT: | Short Term Exposure: | Possible Irritant |
| | Long Term Exposure | No information available |
| EYE CONTACT: | Short Term Exposure: | Possible Irritant |
| | Long Term Exposure | No information available |
| INGESTION: | Short Term Exposure: | Possible Irritant |
| | Long Term Exposure | No information available |
| INHALATION: | Short Term Exposure: | Possible Irritant |
| | Long Term Exposure | No information available |
| INJECTION: | Short Term Exposure: | No information available |
| | Long Term Exposure | No information available |

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Strawberry DNA Extraction Lesson Plan

This lesson plan is for the extraction of DNA from strawberries. Strawberries are an exceptional fruit to use for this lesson because each individual student is able to complete the process by themselves and strawberries yield more DNA than any other fruit (i.e. banana, kiwi, etc.). Strawberries are octoploid, meaning that they have eight copies of each type of chromosome.

Primary Learning Outcomes

Students will observe first hand that DNA is in the food that they eat. Students will learn the simple method to extract DNA and why each step is necessary due to the complex organization of DNA in cells. Students will learn why it is important for scientist to extract DNA from organisms.

Assessed Georgia Performance Standards

SCSh2. Students will use standard safety practices for all classroom laboratory and field investigations.

SCSh4. Students use tools and instruments for observing, measuring, and manipulating scientific equipment and materials.

SB1. Students will analyze the nature of the relationships between structures and functions in living cells.

SB2. Students will analyze how biological traits are passed on to successive generations.

Background:

Strawberries are soft and easy to pulverize. Strawberries have large genomes; they are octoploid, which means they have eight of each type of chromosome in each cell. Thus, strawberries are an exceptional fruit to use in DNA extraction labs.

The soap helps to dissolve the phospholipid bilayers of the cell membrane and organelles. The salt is used to break up protein chains that bind around the nucleic acids. DNA is not soluble in ethanol. The colder the ethanol, the less soluble the DNA will be in it. Thus make sure to keep the ethanol in the freezer or on ice.

Procedures/Activities

Step: 1 Duration: 10 minutes

Teacher may choose prior to class to prepare the DNA extraction buffer. In a container add 900mL water, then 50mL dishwashing detergent (or 100mL shampoo), and finally 2 teaspoons salt. Slowly invert the bottle to mix the extraction buffer.



Step: 2 Duration: 40 minutes to 60 minutes depending on class cooperation

Lab procedures should be conducted as stated in the *DNA Extraction: Strawberry* lab at the end of this document. Modifications can be made based on the needs of the students. Some classes may decide for each student to add individual components of the extraction buffer to the Ziploc bag (roughly 2 tsp water, 1 tsp soap, 1 pinch salt), while other classes may choose to use the teacher prepared extraction buffer (from Step 1).

When the students add ethanol to their strawberry extract, they will see the fine white strands of DNA precipitate. The DNA will form cotton like fibers that will spool onto the stirring rod/inoculating loop/popsicle stick.

Materials and Equipment

For each student: heavy duty ziploc bag (freezer or storage bag); 1 strawberry; DNA extraction buffer (900mL water, 50mL dishwashing detergent, 2 teaspoons salt); small plastic cup to hold extraction buffer; cheesecloth to fit in small funnel (4" X 4" should be appropriate); small funnel; 50mL vial / test tube; glass rod, inoculating loop, or popsicle stick; cold ethanol, ice

Total Duration

10 minutes teacher prep before class
40-60 minutes in class

Assessment

Lab report and/or discussion questions. Discuss questions as a class to assess the students understanding and ability to communicate scientific concepts. Discuss why each step was needed and how this relates to the organization of genetic material.

Extension

The yield of DNA in this lab may be compared to that of the DNA Banana Extraction lab. Compare ploidy levels and how it may relate to the amount of DNA recovered. Use varying concentrations of ethanol (70-100%) to determine how ethanol concentration qualitatively affects the yield of DNA.



Name _____

Date _____

DNA Extraction: Strawberry

Background: The long, thick fibers of DNA store the information for the functioning of the chemistry of life. DNA is present in every cell of plants and animals. The DNA found in strawberry cells can be extracted using common, everyday materials. We will use an extraction buffer containing salt, to break up protein chains that bind around the nucleic acids, and dish soap to dissolve the lipid (fat) part of the strawberry cell wall and nuclear membrane. This extraction buffer will help provide us access to the DNA inside the cells.

Pre-lab questions:

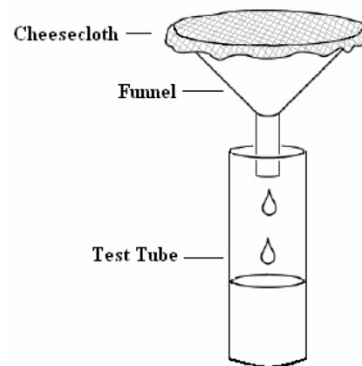
1. What do you think the DNA will look like?
2. Where is DNA found?

Materials:

heavy duty ziploc bag
1 strawberry
10 mL DNA extraction buffer (soapy, salty water)
cheesecloth
funnel
50mL vial / test tube
glass rod, inoculating loop, or popsicle stick
20 mL ethanol

Procedure:

1. Place one strawberry in a Ziploc bag.
2. Smash/grind up the strawberry using your fist and fingers for 2 minutes. *Careful not to break the bag!!*
3. Add the provided 10mL of extraction buffer (salt and soap solution) to the bag.
4. Knead/mush the strawberry in the bag again for 1 minute.
5. Assemble your filtration apparatus as shown to the right.
6. Pour the strawberry slurry into the filtration apparatus and let it drip directly into your test tube.
7. Slowly pour cold ethanol into the tube. OBSERVE ☺
8. Dip the loop or glass rod into the tube where the strawberry extract and ethanol layers come into contact with each other. OBSERVE ☺





Conclusions and Analysis

1. It is important that you understand the steps in the extraction procedure and why each step was necessary. Each step in the procedure aided in isolating the DNA from other cellular materials. Match the procedure with its function:

| PROCEDURE | FUNCTION |
|---|---|
| A. Filter strawberry slurry through cheesecloth | ___ To precipitate DNA from solution |
| B. Mash strawberry with salty/soapy solution | ___ Separate components of the cell |
| C. Initial smashing and grinding of strawberry | ___ Break open the cells |
| D. Addition of ethanol to filtered extract | ___ Break up proteins and dissolve cell membranes |

2. What did the DNA look like? Relate what you know about the chemical structure of DNA to what you observed today.

3. Explain what happened in the final step when you added ethanol to your strawberry extract. (*Hint: DNA is soluble in water, but not in ethanol*)

4. A person cannot see a single cotton thread 100 feet away, but if you wound thousands of threads together into a rope, it would be visible much further away. Is this statement analogous to our DNA extraction? Explain.

5. Why is it important for scientists to be able to remove DNA from an organism? List two reasons.

6. Is there DNA in your food? _____ How do you know?

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