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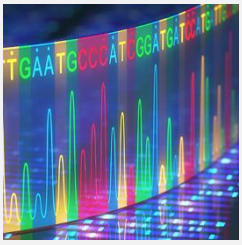
Evolve Genomix

APPLICATION NOTE

Evolve Purity: Verifying Food Composition with Next-Generation Genomic Sequencing

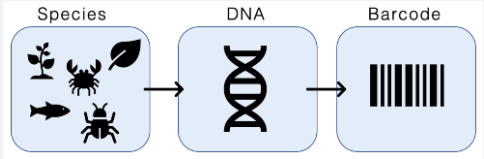
Introduction



As a team of scientists and subject matter experts in the areas of Food microbiology, Next-generation sequencing (NGS), Taxonomy, Phylogenetics and Molecular diagnostics, **Evolve Genomix** is committed to the cause of leveraging the power of NGS to underserved markets like food safety, food authenticity and protection against food fraud.

The expansion of the global food trade industry has heightened the significance of both authenticity and traceability [1]. Regarding food quality, numerous concerns arise, including chemical or microbiological adulteration and the incorporation of hazardous additives or fillers aimed at artificially inflating the prices of artisanal goods. While the issue of food adulteration and fraud is not new, it is expected to intensify in the coming years due to imminent threats to food security due to factors like global warming [1]. Foods like meat, sea food, dairy products, oils and spices are subjected to the most adulteration, fraud and mislabeling concerns [2, 3]. Consumer demand and regulatory pressure towards food manufacturers for transparency and authenticity in food labeling is all time high.

While traditional analytical methods such as spectroscopy and chromatography are good for identifying chemical contaminants or fillers, they are not adequate in properly identifying biological adulterants or verifying the composition of plant and animal products. DNA based methods are very effective in these situations as DNA is very stable against processing conditions and offers high sensitivity and specificity for the biological analytes [4].



Evolve Purity™ is a DNA metabarcoding-based NGS assay that can accurately determine the composition of both pure and complex foods, raw and processed [5, 6, 7]. It leverages the unique sequence variation of conserved gene markers across animals, plants and microbes to identify the source of the DNA in a sample. This assay does not assume anything about the composition of the food unlike other molecular assays such as PCR or immunoassays that need the knowledge of the analyte expected to be detected in a sample. For this reason, the assay workflow is practically universal for detecting any biological ingredient in any time of food or edible consumable.

In this application note, we showcase a pilot study of the Evolve Purity test performed in our lab by our trained lab personnel.

Methodology:

For the pilot study, 25 different real-world food samples, as shown in Table 1 below, along with a negative control, were tested. 2-5g of the food sample was weighed out in separate tubes. The samples were then subjected to cell lysis by incubating at 65C overnight in Lysing matrix A (from MP Biomedicals). Then DNA was extracted and purified from the respective samples using the NucleoSpin Food kit (from Machery Nagel). Universal meta barcode markers were selectively amplified from the genomic DNA of the samples using a proprietary primer mix that contained multiple markers for plant, animal and fungal ingredients. For the rice samples only, a separate primer pool was used to amplify select regions of the BADH2 gene to quantify the proportion of aromatic and wild rice variants. The amplification products were then purified separately using a bead-based purification method. Another PCR reaction was performed with amplicons of each sample to attach the sample level indices and sequencing adapters. After another round of bead-based purification, the amplicon libraries for each of the samples were quantified, normalized for concentration and then pooled together into a single library pool. This was then loaded onto a MiSeq nano flow cell after addition of the sequencing control PhiX and sequenced for 27 hours using the MiSeq v2 reagent kit (2x250 bp). The FASTQ files were then analyzed by our proprietary bioinformatic analysis pipeline to produce the final sample-level report.

Sl. No.	Type of sample	Tested for	No. of samples tested in the pilot
1	Spices	Composition, Adulterants/fillers	3
2	Rice	% of aromatic variant (for ex., Basmati)	3
3	Sea food	Composition, Adulterants, Labeling accuracy	7
4	Pet food	Composition, Adulterants, Labeling accuracy	5
5	Pre-cooked mixed meat	Composition, Labeling accuracy	2
6	Vegan meat products	Composition, Labeling accuracy, Animal contaminants	5

Table 1. Food samples tested in this pilot study using Evolve Purity™ assay

Our bioinformatic pipeline leverages standard genomic analysis tools, but combines with proprietary read thresholds, phylogenetic clustering, decision trees and a large curated database to produce a fairly comprehensive report that lists both the expected and unexpected ingredients in a food sample, with species-level resolution for most. A noise threshold based on normalized read percentage for each sample and read quality filters any noise and cross contamination from other samples in a run. The negative control that is processed and sequenced alongside all the samples provides a measure of noise in a sequencing run. The sequencing control PhiX, provided by Illumina, is included in all the sequencing runs as a benchmark for sequencing quality.

Results

The sequencing run produced more than 7500 2x250 mapped sequences on an average per sample with 86.5% reads ≥ Q30 quality. All of these were fairly acceptable metrics for the test. The final report of the test is shown in Table 2.

		Expected ingredients				Unexpected ingredients detected (Traces)	
Sample No.	Sample Label	Common Name	Scientific Name	Detected (Y/N)	Notes	Common name	Scientific name
1	Powdered Nutmeg	Nutmeg	Myristica fragrans	Y		Cumin	Cuminum cyminum
						Oregano	Origanum vulgare
2	Powdered White Pepper	White Pepper	Piper nigrum	Y		Peanut	Arachis hypogaea
						Random herb	Arachis ipaensis
						Field bindweed	Convolvulus arvensis
3	Powdered Moringa leaves	Moringa	Moringa oleifera	Y			
4	Basmati Rice 1	Basmati Rice 1		Y	98% Aromatic		
					2% Non-aromatic		
5	Basmati Rice 2	Basmati Rice 2		Y	95% Aromatic		
					5% Non-aromatic		
6	Non-Basmati Rice 1	Non-Basmati Rice		Y	100% Non-aromatic		
7	Canned Pink Salmon	Pink Salmon	Oncorhynchus gorbuscha	Y		Chum Salmon	Oncorhynchus keta
8	Canned Tuna in Veg broth	Albacore Tuna	Thunnus alalunga	Y		Pacific Bluefin Tuna	Thunnus orientalis
		Wild cabbage	Brassica oleracea	Y		Yellow fin Tuna	Thunnus albacares
		Rapeseed (oil/greens)	Brassica napus	Y			
		Wheat	Triticum aestivium	Y			
9	Cooked Shrimp	Whiteleg Shrimp	Penaeus vannamei	Y			
10	Wile caught Shrimp meat	Ocean Shrimp	Pandalus jordani	Y		Slender sole	Lyopsetta exilis
						Bigfin eelpout	Lycodes cortezianus
						Eulachon	Thaleichthys pacificus
11	Canned Sardines	Sardine	Sardina pilchardus	Y		Ocean Shrimp	Pandalus jordani
12	Herring Fillets	Atlantic Herring	Clupea harengus	Y			
13	Fish sticks	Alsaka Pollock	Gadus chalcogrammus	Y		Russian Wheatgrass	Thinopyrum junceiforme
		Wheat	Triticum aestivium	Y			
		Edible goatgrass	Aegilops speltoides	Y			
		Rye	Secale cereale	Y			
14	Dried dogfood (Duck)	Muscovy duck	Cairina moschata	Y			
		Mallard duck	Anas platyrhynchos	Y			
		Tufted duck	Aythya fuligula	Y			
		Flax seed (oil)	Linum bienne	Y			
		Sweet potato	Ipomea batatas	Y			
		Rapeseed (oil)	Brassica napus	Y			
		Yeast	Pichia fermantans	Y			
		Yeast	Pichia kluyveri	Y			
		Broccoli	Brassica oleracea	Y			
		Tomato	Solanum lycopersicum	N			
		Locust bean gum	Ceratonia siliqua	N			
		Rosemary extract	Salvia rosmarinus	N			
15	Dogfood (Red meat stew)	Beef	Bos taurus	Y		Human impurities	Home sapiens
		Lamb	Ovis aries	Y			
		Red deer	Cervus elaphus	Y			
		Curly Kale	Brassica oleracea var	Y			
		Potato starch	Solanum tuberosum	N			
		Tapioca starch	Manihot esculenta	N			
16	Dogfood (Mixed meat)	Beef	Bos taurus	Y		Pyrenean Chamois	Rupicapra pyrenaica
		Lamb	Ovis aries	Y		Human impurities	Home sapiens
		Hen	Gallus Gallus	Y			
		Blacktip Sardinella	Sardinella melanura	Y			
		Chum Salmon	Oncorhynchus keta	Y			
17	Catfood (Mixed meat)	Beef	Bos taurus	Y		Whiteleg Shrimp	Penaeus vannamei
		Wheat	Triticum aestivium	Y		Muskovy duck	Cairina moschata
		Lamb	Ovis aries	N		Banana	Musa acuminata
		Cod	Gadus morhua	N		Carrot	Daucus carota
		Turkey	Meleagris gallapavo	N		Kratom	Mitragyna speciosa
		Tomato	Solanum lycopersicum	N		Soybean	Glycine max
		Kelp	Macrocystis pyrifera	N		Onion	Allium cepa
		Pumpkin	Cucurbita pepo	N		Kale	Brassica oleracea var
		Mojave Yucca	Yucca schidigera	N		Human impurities	Home sapiens

		Expected ingredients				Unexpected ingredients detected (Traces)	
Sample No.	Sample Label	Common Name	Scientific Name	Detected (Y/N)	Notes	Common name	Scientific name
18	Catfood (Mixed Seafood)	Turkey	Meleagris gallapavo	Y		Ocean Shrimp	Pandalus jordani
		Wild celery	Angelica sp.	Y		Herring	Clupea harengus
		Lamb	Ovis canadensis	Y			
		Cranberries/Buckthorn fruit	Hippophae tibetana	Y			
		Salmon	Oncorhynchus sp.	N			
		Mackerel	Scomber scombrus	N			
		Cod	Gadus morhua	N			
		Tomato	Solanum lycopersicum	N			
		Kelp	Macrocystis pyrifera	N			
		Mojave Yucca	Yucca schidigera	N			
19	Vegan hotdog	Wheat	Triticum petropavloskyi	Y		Russian Wheatgrass	Thinopyrum junceiforme
		Pea	Pisum sativum	Y		Human inpurities	Home sapiens
		Edible goatgrass	Aegilops speltoides	Y		Beef	Bos taurus
		Garlic	Allium sativum	Y			
		Mustard leaves	Sinapis alba	Y			
		Onion	Allium cepa	Y			
		Violet cabbage	Moricandia moricandioides	Y			
		Rapeseed (oil/greens)	Brassica napus	Y			
		Carrot extract	Dacus carota	N			
		Radish extract	Raphanus sativus	N			
		Tomato	Solanum lycopersicum	N			
20	Vegan sausage	Soybean	Glycine max	Y		Fungal canker (from gum)	Fusarium circinatum
		Garlic	Allium sativum	Y			
		Garlic chives	Allium tuberosum	Y			
		White pepper	Piper nigrum	Y			
		Syrian Oregano	Origanum syriacum	Y			
		Majoram	Origanum majorana	Y			
21	Vegan beef pattie	Soybean	Glycine max	Y			
		Wheat	Triticum aestivum	Y			
		Coriander	Coriandrum sativum	Y			
		Wild cabbage	Brassica oleracea	Y			
22	Vegan meatballs 1	Pea	Pisum sativum	Y			
		Chickpea	Cicer arietinunm	Y			
		Corn	Zea mays	Y			
		Potato	Solanum tuberosum	Y			
		Soybean	Glycine max	Y			
		Onion	Allium cepa	Y			
		Wild cabbage	Brassica oleracea	Y			
		Rapeseed greens	Brassica napus	Y			
		Carrot	Dacus carota	Y			
		Peppers	Capsicum annum	Y			
		Lettuce	Lactuca sativa	Y			
		Honey clover	Melilotus albus	Y			
		Sage	Salvia officinalis	Y			
23	Vegan meaballs 2	Rice	Oryza sativa	Y			
		Pea	Pisum sativum	Y			
		Potato	Solanum tuberosum	Y			
		Oats	Avena fatua	Y			
		Onion	Allium cepa	Y			
		Garlic chives	Allium tuberosum	Y			
		Common hawthorn (berries)	Crataegus monogyna	Y			
		Wild tomato	Solanum pennellii	Y			
24	Meat sausage	Beef	Bos taurus	Y			
		Pork	Sus scrofa	Y			
		Peppers	Capsicum annum	Y			
		Black Pepper	Piper nigrum	Y			
		Coriander	Coriandrum sativum	Y			
		Flax seed (oil)	Linum usitatissimum	Y			
		Rapeseed (greens/oil)	Brassica napus	Y			
25	Meatballs	Beef	Bos taurus	Y			
		Pork	Sus scrofa	Y			
		Onion	Allium sp.	Y			
		Cumin	Cuminum cyminum	Y			
		Coriander	Coriandrum sativum	Y			
		Garlic	Allium sativum	Y			

Table 2. Detailed report of the Evolve Purity™ results for the real-world samples in the Pilot study

Some of the highlights of the test results were:

- The Evolve Purity™ test was able to correctly identify the biological ingredients for all the single-ingredient samples such as spices and canned fish with species-level resolution.

- A couple of spice samples had traces of other spices, which could possibly be contaminants coming from powdering machinery. But the test also found moderate to high amounts of peanut in white pepper powder, which could be a cheap filler or adulterant.

- The test was able to successfully differentiate & identify Basmati vs Non-Basmati rice varieties and even quantify the % of aromatic cultivar in the rice sample.

- The test successfully identified the multitude of ingredients, both of animal- and plant-origin, listed on the label for complex, processed or pre-cooked food such as fish sticks, hotdogs, meatballs, pet food stew and vegan meat products.

- For some of the canned seafood products, the test also identified the presence of other fish (same genus, different species or totally different genera) in trace amounts, not listed in the label. This could be due to the method and location of the fishing (open ocean) or cross contamination from the usage of facilities that were used in processing many different fish varieties.
    - For example, we found traces of Chum Salmon in canned Pink Salmon. They both are very similar and are caught in the Pacific ocean. Similarly, we found traces of Pacific bluefin tuna and yellowfin tuna in the canned Albacore tuna.
    - We also found traces of traces of different wild fishes like Bigfin eelpout, Eulachon and Slender sole in Wild caught Shrimp meat.

- We found a lot of interesting observations with the pet food, some of them possibly suggesting mislabeling.
    - The two dog food samples we tested (Red meat stew and mixed meat meal), both had traces of human DNA, perhaps stemming from contamination from operators in the food manufacturing plant. One of the dog foods also had traces of a goat-antelope (Pyrenean Chamois) DNA, which possibly came from a source of lamb meat used in the product.
    - We discovered that the two cat food samples we tested had a lot of listed ingredients missing and instead, we found a lot of extraneous DNA of both animal and plant origin that were not listed. One of the cat food samples was supposed to have lamb, turkey, cod, yucca and pumpkin, which we did not find. Instead we found ingredients that would have been possible substitutes - duck, shrimp meat, banana, carrot and soybean. The other cat food sample, which was called ‘Salmon & Mackerel recipe’ did not have either and instead had Ocean Shrimp and Herring, along with Turkey and Lamb. It is quite likely that the manufacturer substituted similar meats or the labeling was incorrect for this product.
    - In some of these complex samples minor ingredients such as food colorants, thickening agents and spices were possibly missed by our test, due to heavy processing or inadequate sampling.

- We tested different Vegan products (hot dogs, sausage, mock meat patties and balls) from different vendors. We interestingly found different plant ingredients used in each of them. While the vegan hot dog sample mostly had wheat and pea, the sausage didn't contain both and instead had Soy, as listed. Also, the meat balls from two different vendors had totally different ingredients and recipes. Our test was able to identify almost all the ingredients listed in all of these vegan products including the cereals, vegetables and spices.

- We also tested one sample each of pre-cooked mixed meat sausage and meatballs. We found all the meat ingredients (beef and pork), vegetables and spices as listed on both of them.

Discussion

The Evolve Purity™ assay has been primarily designed to provide a qualitative test for the composition of the food products. Future improvements for making it a semi-quantitative test to provide compositional ratio of ingredients are in progress. While the assay itself is very sensitive to very small amounts of DNA as evidenced in this study where it was able to detect spice ingredients in 2-5 g of pre-cooked vegan products or the trace amounts of brewer's yeast present in dried dog food sample, this study was not designed to quantify the analytical detection limits for different genomic regions from plants and animals.

This pilot study focused on sample types where adulteration and mislabeling concerns are high. The study was able to demonstrate the capabilities of the Evolve Purity™ test in detecting most, if not all, of the ingredients in both raw and processed food as well as simple and complex foods. Such a tool to ensure compositional accuracy, product purity and authenticity would benefit a diverse set of stakeholders in the entire food supply chain.

- It would help the ***food manufacturers*** to ensure compliance with labeling regulations, building brand trust, and optimizing their production processes.

- It would provide the ***large wholesalers & importers*** to compare different sourcers and also establish standards for premium quality products. It would also facilitate faster food inspection and streamline customs clearance by demonstrating product compliance and thereby limiting unnecessary delays.

- It would assist the ***retailers*** to provide the consumers with accurate product information and reduce the risk of fraud.

- It would help the ***regulatory agencies*** in enhancing food safety and promoting transparency in the food supply chain.

We invite all the above stakeholders to engage with us to explore how Evolve Purity™ and other related tests offered by Evolve Genomix (www.evolvegenomix.com) can benefit you. We would also be interested in collaboration opportunities to co-develop custom assays for your special needs.

References

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