Next Generation Sequencing for Detection of Meat, Fish and Plant Species in Pure and Mixed Species Samples

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ABSTRACT

In this study the technical experts from Thermo Fisher Scientific and SGS Molecular supported scientists at Nestle Research in the use of the Thermo Scientific™ NGS Food Authenticity Workflow (Figure 1) to test for meat, fish and spices/herbs species detection and identification at a variety of different spike levels (1% to 100%) and combinations of species (up to 5 different species combined into a sample).

INTRODUCTION

Food authenticity and fraud are topics of high interest in the food industry and highly controlled by authorities. The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients for food production. The most common method to verify species substitution and species identification is Real-Time PCR. However, PCR testing is limited by the number of targets that can be simultaneously identified and differentiated. This can be critical, especially when testing highly processed and complex food that often contain multiple different species.

The introduction of Next Generation Sequencing (NGS) into the food sector revolutionizes food authenticity testing. NGS enables accurate detection and differentiation of thousands of different species in each sample using DNA sequencing that is recognized as the most reliable method for species identification.

Figure 1. Left to right – Ion Chips and consumables, Ion Chef™ Instrument, SGS™ All Species ID Meat, Fish and Plant Analyser Kits and Ion GeneStudio™ S5 System

MATERIALS AND METHODS

All samples analyzed in this study were selected to include common species present in commercial food products. DNA was extracted as described below from different materials, including reference samples obtained from samples repositories and proficiency tests and commercial single species food products according to the label.

A total of 148 meat samples, 347 plant samples and 78 fish samples were tested.

Mixtures of species were produced by mixing DNAs to be tested with the NGS workflow proposed. Artificial DNA mixtures contained up to 5 species:
Meat DNAs up to 3 species
Fish DNAs up to 2 species
Plant DNAs up to 5 species

Additionally spiked samples where produced at different levels:
Meat spiked samples – 1%, 10% and 50%
Fish spiked samples – 1%, 2%, 5% and 10%
Plant spiked samples – 1%, 5%, 10% and 20%

MATERIALS AND METHODS

Thermo Scientific NGS Food Authenticity Workflow (See figure 1 for an overview of the NGS Food Authenticity Workflow steps and timings).
Homogenization: To prepare a representative portion of the sample homogenization using the Precellys™ homogenization instrument (Bertin Technologies) utilizing bead-beating technology was carried out.
DNA Extraction: The GMO Extraction Kit (Thermo Fisher Scientific) with silica based spin-column technology was used to produce high-quality DNA for library preparation.
DNA library preparation: DNA libraries were prepared using the SGS All Species Meat, Fish and Plant Analyser Kits (Thermo Fisher Scientific). Regions of interest were amplified using PCR with the DNA extractions of the samples sequencing adapters added. During library preparation unique barcodes (molecular tags) were added to each sample to enable sequencing and analysis of multiple samples in the same sequencing run.
Template preparation and Library pooling: After library preparation, a fully automated templating reaction on the Ion Chef™ Food Protection Instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips.
Sequencing: Performed on the Ion GeneStudio™ S5 Food Protection System (Thermo Fisher Scientific) DNA sequences were determined relying on semi-conductor based sequencing technology.
Data analysis: Results were mapped against the SGS® All Species ID Software, a database containing the DNA sequences of many thousands of meat, fish and plant species to provide an identification for all species detected in the samples.

Figure 1. Thermo Scientific NGS Food Authenticity Workflow overview

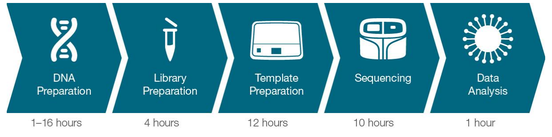

Table 1. List of meat species tested

Meat species name	Meat ommon name	Meat species name	Meat ommon name
Ovis aries	Sheep	*Tragelaphus strepsiceros*	Kudu
Capra hircus	Goat	*Felix catus*	Cat
Lepus capensis	Hare	*Rattus norvegicus*	Rat
Oryctolagus_cuniculus	Rabbit	*Vulpes vulpes*	Fox
Macropus rufus	Kangaroo	*Alces alces*	Elk
Capreolus capreolus	Roe Deer	*Coturnix japonica*	King quail
Cervus elaphus	Red Deer	*Bubalus bubalis*	Buffalo
Rangifer tarandus	Reindeer	*Camelus dromedarius*	Camel
Antidorcas marsupialis	Springbok	*Crocodylus niloticus*	Crocodile
Equus hemionus	Zebra	*Lophura inornata*	Pheasant
Lama glama	Lama	*Oryx leucoryx*	Oryx gazella
Gallus gallus	Chicken	*Alcelaphus buselaphus*	Gnu
Canis familiaris	Dog	*Bos grunniens*	Cattle Yak
Bison bison	Bison	*Equus asinus*	Donkey
Cervus dama	Fallow Deer	*Meles meles*	Badger
Equus caballus	Horse	*Tragelaphus scriptus*	Antilope
Sus scrofa	Pork	*Corvus macrorhynchos*	Daw
Bos taurus	Beef	*Mustela erminea*	Weasel
Meleagris galopavo	Turkey	*Ondatra zibethicus*	Muskrat
Cairina moscata	Duck	*Anas species*	Mallard duck
Alopochen aegptiacus	Goose	*Crocodylus siamensis*	Crocodile
Struthio camelus	Ostrich	*Phasianus colchicus*	Pheasant
Columba livia	Pigeon	*Alectoris chukar*	Partridge
Numida meleagris	Guinea fowl	*Aepyceros melampus*	Impala
Dromaius novaehollandiae	Emu		

Table 2. List of fish species tested

Fish species name	Fish common name	Fish species name	Fish common name
Salmo salar	Atlantic Salmon	*Trisopterus luscus*	Norway pout
Thunnus albacares	Yellowfin tuna	*Cynoglossus senegalensis*	Witch flounder
Gadus morhua	Atlantic cod	*Oncorhynchus chrysogaster*	Pink salmon
Hippoglossus hippoglossus	Pacific halibut	*Lophius piscatorius*	Angler
Limanda limanda	Common dab	*Oncorhynchus nerka*	Sockeye salmon
Merluccius merluccius	European hake	*Pangasianodon hypophthalmus*	Silver carp
Melanogrammus aeglefinus	Haddock	*Scomber scombrus*	Atlantic mackerel
Katsuwonus pelamis	Skipjack tuna	*Oncorhynchus gorbuscha*	Pink salmon
Thunnus alalunga	Albacore	*Merluccius hubbsi*	Argentine hake
Pleuronectes platessa	European plaice	*Merluccius productus*	North Pacific hake
Molva molva	Ling	*Macruronus magellanicus*	Patagonian grenadier
Sander lucioperca	Pike-perch	*Merluccius gayi*	South Pacific hake
Pollachius pollachius	Pollack	*Thunnus obesus*	Bigeye tuna

Table 3. List of plant species tested

Plant species name	Plant common name	Plant species name	Plant common name
Origanum species	Origanum	*Laurus_nobilis*	Sweet bay
Allium schoenoprasum	Wild chives	*Manihot_esculenta*	Cassava
Allium sativum	Garlic	*Mentha spicata*	Spearmint
Anethum graveolens	Dill	*Myristica fragrans*	Nutmeg
Argemone species	Prickly poppy	*Ocimum basilicum*	Sweet basil
Avena sativa	Oat	*Oryza_sativa*	Rice
Brassica napus	Rape	*Panicum miliaceum*	Millet
Capsicum annuum	Cayenne pepper	*Papaver somniferum*	Opium poppy
Carum carvi	Caraway	*Petroselinum crispum*	Parsley
Ceratonia siliqua	Carob	*Pimpinella anisum*	Anis
Conium maculatum	Poison henlock	*Piper nigrum*	Black pepper
Coriandrum sativum	Coriander	*Rosmarinus_officinalis*	Rosemary
Crocus sativus	Saffron	*Sesamum indicum*	Sesame
Cuminum_cyminum	Cumin	*Sinapis_alba*	White mustard
Curcuma longa	Turmeric	*Sorghum_bicolor*	Sorghum
Elettaria cardamomum	Cardamom	*Thymus_vulgaris*	Garden Thyme
Foeniculum vulgare	Sweet fennel	*Triticum_aestivum*	Wheat
Glycine max	Soybean	*Triticum_durum*	Durum wheat
Hordeum vulgare	Barley	*Zingiber_officinale*	Garden Ginger
Juniperus_communis	Juniper		

RESULTS

All pure (100%) meat, plant and fish species were detected and correctly identified.

For the meat samples spiked at 1% two out of 81 meat species were not detected (2.5%). These samples were both cooked beef spiked with 1% pork.

For all meat samples with spike level above 1%, all the species were correctly identified.

Of the ten fish samples spiked with the most common fish species at 1%, all were detected and correctly identified.

170 plant samples were spiked at 1%, 29 of these were not detected (17.1%)

All plant species were detected for the 46 plant samples spiked at 5%.

A few samples didn't originate results since no DNA could be obtained due to high sample processing .

CONCLUSIONS

The Thermo Scientific NGS Food Authenticity Workflow was shown to detect and correctly identify 100% of meat (n=49), fish (n=26) or plant (n= 39) species at a spike level of 5% or higher).

For meat samples at a spike level of 1%, 79/81 (97.5%) of the species were detected and correctly identified.

For fish samples at a spike level of 1%, 10/10 (100%) of the species were detected and correctly identified.

For plant samples at a spike level of 1%, 143/170 (82.9%) of the species were detected and correctly identified. At a level of 5% all plant species were detected.

Combining up to five species for plant, three species for meat or two species for fish samples had no effect on the detection or correct identification of the species present.

When combined, all targets could be analyzed simultaneously in a single NGS run which reduces NGS costs compared with having to carry out separate runs.

The workflow could differentiate very closely related species with important commercial impact like for Bigeye and Yellowfin tuna that are know to be very difficult to distinguish by DNA sequencing.

The workflow is defined to work with highly processed food (including canned food) by analysing very short DNA fragments. However products originating very low or no DNA can't be analysed.

The identification success of the workflow depends on the number of different species included in the databases. Nevertheless the current databases for meat and fish ID include many thousands of species entries that makes unlikely the absence of and ID result.

For plant ID, the present database is mostly focused on spices, herbs and cereals

At spike levels 1-5% all species were detected making the workflow appropriate for food species ID analysis.

TRADEMARKS/LICENSING

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LT2495A
September 2019