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Automated DNA Extraction Protocols for Next-Generation Sequencing Food Authenticity Application

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INTRODUCTION

The Thermo Scientific™ NGS Food Authenticity Workflow is based on next-generation sequencing technology to identify meat, fish and plant species. With semi-automated workflow and an extensive database thousands of species can be identified and more than a hundred food and feed products can be simultaneously analyzed. This poses a challenge for sample preparation due to the high workload and time required to manually extract DNA from multiple samples. To overcome this issue an automated extraction method was developed using the Thermo Scientific™ KingFisher™ Flex Purification system.

The KingFisher Flex is an automated magnetic particle-based system for DNA, RNA, protein and cell purification for samples of various origin. The magnetic particle technology combines the speed and efficiency of automation with high quality purification capability. The KingFisher technology uses magnetic rods to transfer particles through various purification phases of binding, mixing, washing and elution, offering a solution with minimal hands-on time.

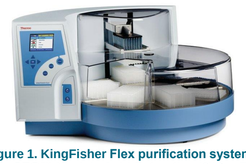

Figure 1. KingFisher Flex purification system and Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing

The KingFisher Flex Purification System was compared to the manual method using the Imegen™ GMO Extraction Kit (Thermo Fisher Scientific) spin column protocol. DNA from 48 samples of various food categories including dried, frozen, liquid and canned foods was extracted using both methods and then sequenced with Ion™ GeneStudio™ S5 Food Protection System according to the NGS Food Authenticity Workflow. The sequencing data from both methods was compared to evaluate equivalency. The sequencing results obtained using the KingFisher Flex protocol were comparable to sequencing results obtained with the manual GMO Extraction Kit . The study demonstrated that the automated KingFisher Flex workflow reduces hands-on-time and possibly also reduces the risk of cross-contamination in the DNA extraction process.

MATERIALS AND METHODS

Samples were homogenized and a DNA extraction step performed on 200 mg sample. DNA was extracted from each sample with GMO Extraction kit, and automated method with KingFisher Flex Purification system and Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing (Thermo Fisher Scientific). Extracted DNA's were quantified with Invitrogen™ Qubit™ Fluorometer (Thermo Fisher Scientific) and libraries for sequencing were prepared using SGS™ All Species ID Food DNA Analyser Kit (Thermo Fisher Scientific). Unique barcodes (i.e. molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run. Sample libraries were prepared for sequencing by Ion Chef™ Food Protection Instrument and the loaded Ion chips were sequenced on Ion GeneStudio™ S5 Food Protection System. Results were analyzed and reported with SGS All Species ID Software (Thermo Fisher Scientific).

RESULTS

Table 1. Species detected with both methods of Dry Food category

Product	Expected species*	Contaminants*
Oregano	Origanum sp. / vulgare / majorana / syriacum (oregano)	Field bindweed, Lamb's ear[GMO], Desert shrub[KF], Hyptis plants[KF]
Cinnamon	Cinnamomum zeylanicum (cinnamon)	Coriander[KF], Tomato[KF]
Black pepper	Piper nigrum (pepper)	Wheat
Cashew nuts	Anacardium occidentale (cashew), Juglans regia (walnut)	
Pistachio nuts	Pistacia vera (pistachio)	
Wheat flour	Triticum aestivum (wheat)	
Soy mince	Glycine max (soybean)	
Rye flour	Secale cereale (rye)	Wheat, Oat, Cabbage
Rosemary	Rosmarinus officinalis (rosemary)	

Table 2. Species detected with both methods of Liquid Food category

Product	Expected species*	Contaminants*
Meat soup	Sus scrofa (pork), Bos taurus (beef)	
Fish soup	Oncorhynchus mykiss (rainbow trout) Pollachius virens (saithe)	
Bolognese sauce	Bos taurus (beef), Sus scrofa (pork)	Chicken[KF]
Veggie soup (carrot)	Allium ampeloprasum (leek) Allium cepa (onion) Capsicum sp. (pepper)[GMO] Daucus carota (carrot)[GMO]	Common bead[GMO], Cabbage[GMO], Wheat[GMO], Pistachio[GMO], Oat[GMO], Rye[GMO], Cashew[GMO], Oregano[KF]
Pesto sauce	Anacardium occidentale (cashew)[GMO] Allium sativum (garlic)[GMO] Ocimum basilicum (basil)[GMO]	Coriander [GMO]
Oat milk	Avena vaviloviana (oat)	Phalaris grass
Chicken soup	Gallus (chicken)	
Fish soup frozen	Pollachius virens (saithe)	

Table 3. Species detected with both methods of Fresh Food category

Product	Expected species*	Contaminants*
Dill	Anethum / Foeniculum graveolens (dill)	
Coriander	Coriandrum sativum (coriander)	Tomato[GMO]
Basil	Ocimum basilicum (basil)	
Tomato	Solanum lycopersicum (tomato)	Salvia grass
Minced beef	Bos taurus (beef)	
Minced pork	Bos taurus (beef), Sus scrofa (pork)	
Minced chicken	Gallus gallus (chicken)	
Minced turkey	Meleagris gallopavo (turkey)	Pork
Flounder fillet	Platichthys flesus (European flounder)	
Perch fillet	Perca fluviatilis (European perch)	

***Species detected from both extractions unless indicated otherwise (KF = Automated, GMO = Manual)**

Table 4. Species detected with both methods of Deep Frozen Food category

Product	Expected species*	Contaminants*
French fries	Solanum tuberosum (potato), Oryza sativa (rice)	Wild grass/rice cutgrass[GMO]
Wok mix	Brassica sp. (cabbage), Capsicum sp. (paprika) Phaseolus vulgaris (common bean) Daucus carota (carrot), Allium cepa (onion)[GMO]	
Diced onion	Allium cepa (onion)	
Salami pizza	Sus scrofa (pork)	
Chicken nuggets	Gallus gallus (chicken)	
Meat dumplings	Sus scrofa (pork), Bos Taurus (beef)	
Thai cube	Gallus gallus (chicken)	
Kebab	Bos taurus (beef)	Pork
Fish fingers	Gadus chalcogrammus (pollock)	
Diced rainbow trout	Oncorhynchus mykiss (rainbow trout)	

Table 5. Species detected with both methods of Canned Food category

Product	Expected species*	Contaminants*
Tuna in sunflower oil	Katsuwonus pelamis (tuna)	Rainbow trout [GMO]
Tuna in water	Katsuwonus pelamis (tuna)	Rainbow trout [GMO]
Ham + beef	Bos taurus (beef), Sus scrofa (pork)	
Beef + ham	Bos taurus (beef), Sus scrofa (pork)	
Chicken in water	Gallus (chicken)	
Kidney beans	Phaseolus vulgaris (common bean)	Cocoa, Dill, Walnut[KF], Wheat[KF]
Tomatoes	-	Wheat, Dill[GMO], Basil[GMO], Coriander[KF] Cabbage[KF], Paprika[KF]
Reindeer	Rangifer tarandus (reindeer), Sus scrofa (pork)	Mule / White tailed deer
Mackarel	Scomber japonicas (Pacific mackerel), Scomber colias (Atlantic mackerel)	
Sardines	Sardina pilchardus (sardine)	

***Species detected from both extractions unless indicated otherwise (KF = Automated, GMO = Manual)**

Figure 2. 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

CONCLUSIONS

The automated KingFisher DNA extraction method's performance was comparable to the manual DNA method when handling fish and meat products. The only variations were the possible cross-contamination of rainbow trout in tuna samples extracted with the manual GMO kit and chicken in Bolognese sauce extracted with the automated KingFisher method.

Undeclared pork meat was detected in deep-frozen kebab product with both DNA extraction protocols which can be considered as actual undeclared species or cross contamination in the product. Mule/white-tailed deer was detected from the canned reindeer with small number of reads after sequencing and this is caused most likely due to similarity in the sequences. Additionally, possible cross contamination of pork was detected from the minced turkey extracted with both protocols.

More variation was observed when sequencing plant products. Based on the sequencing results more declared species was detected from samples extracted with the manual method, e.g. from pesto sauce where it is known that the amount of oil is interfering with DNA extraction.

Cacao was detected with both extraction protocols from canned kidney beans despite no chocolate products being handled in this study. This might be due to close sequence similarity with common beans?

Tomato species was not found from canned tomato with either DNA extraction method however was detected from fresh raw tomato. This phenomenon is most likely caused by the texture of canned tomato, high amounts of sugar and water which is not beneficial for DNA extraction. Similar result was observed with vegetable soup.

Cross contamination of flours were detected in five plant products which is likely to occur when powder-like samples are handled during the same workday within the same laboratory area. Altogether more possible cross-contaminations was observed in samples which were handled with manual GMO Extraction Kit indicating that the automated method with KingFisher Flex Purification system and PrepSeq Nucleic Acid Extraction Kit for Food and Environmental Testing protocol has a lower risk of cross-contamination.

Overall, the results showed that automated KingFisher™ Flex and PrepSeq Nucleic Acid method is a valid alternative to manual GMO™ Extraction Kit method in the NGS Food Authenticity workflow for multi-species identification.

TRADEMARKS

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