

# Comparison of DNA Extraction Protocols for Down-stream Food Authenticity Next-Generation Sequencing Application

Amanda Manolis<sup>1</sup>, Nicole Prentice<sup>2</sup>, Tiina Karla<sup>3</sup>, Milja Tikkanen<sup>3</sup>

<sup>1</sup>Thermo Fisher Scientific, Austin, Texas, USA, <sup>2</sup>Thermo Fisher Scientific, Basingstoke, UK, <sup>3</sup>Thermo Fisher Scientific, Vantaa, Finland

## INTRODUCTION

DNA extraction is a crucial part of successful sequence analysis when studying the species authenticity of food products. The Thermo Scientific™ NGS Food Authenticity Workflow is able to identify meat, fish and plant species from various food, feed and ingredients. With semi-automated next generation sequencing workflow and an extensive database, thousands of species can be identified and more than a hundred samples can be simultaneously analyzed. As multiple species are analyzed from a variety of sample types the DNA extraction method needs to perform robustly regardless of the variables.

This study was conducted to compare the performance of two DNA extraction kits designed for food samples. Foods from different categories were tested to challenge the method including heavily processed foods, fresh and frozen foods, ready-to-eat meals, liquid foods and dried food products.

Imegen™ GMO Extraction Kit (Thermo Fisher Scientific) and NucleoSpin® Food Kit (Macherey-Nagel) were used to extract the DNA from a homogenized samples. Both kits employ silica-based DNA purification using spin column technology to efficiently extract meat, fish or plant DNA from food samples. DNA from 31 samples of various food categories including dried, frozen, liquid and canned foods was extracted 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 GMO extraction kit were comparable to sequencing results obtained with the NucleoSpin Food kit. The study demonstrated that the GMO extraction kit is able to extract DNA from various food types and categories for successful sequencing analysis of the species present in a sample.

## MATERIALS AND METHODS

Samples were homogenized with Precellys® homogenizer (Bertin Instruments) and a DNA extraction step performed on 200 mg sample. DNA was extracted from each sample with GMO Extraction kit and NucleoSpin Food kit. 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 GeneStudio™ S5 Food Protection System. Results were analysed and reported with SGS® All Species ID Software (Thermo Fisher Scientific).

## RESULTS

Table 1. Meat samples

Product	Detected species	
	NucleoSpin Food	GMO DNA extraction
Sauteed reindeer	Reindeer	Reindeer
Canned beef	Pork	Pork
	Beef	Beef
Ground beef patties	Beef	Beef
	Pork	Pork
Mixed vegetables and meat	Pork	Pork
Canned chicken	Chicken	Chicken
Beef soup	Beef	Beef
Canned ham	Pork	Pork
Sauteed game meat	Red deer	Red deer
Beef and pork patties	Beef	Beef
	Pork	Pork
Ground beef stick with cheese	Beef	Beef
	Pork	Pork
Liverwurst	Pork	Pork

Table 2. Fish samples

Product	Detected species	
	NucleoSpin Food	GMO DNA extraction
White fish patties	Lake whitefish	Lake whitefish Whitefish Pollock
	Whitefish	
	Pollock	
	Freshwater whitefish Northern pike	
Tuna (in water)	Skipjack tuna	Skipjack tuna
	Frigate tuna	
Mackerel in tomato sauce	Atlantic mackerel	Atlantic mackarel
Salmon soup	Rainbow trout	Rainbow trout Pollock
	Pollock	
Fish fingers	Atlantic cod	Atlantic cod Haddock
	Haddock	
White fish with almond crust	Alaska pollock	Alaska pollock
Smoked salmon pizza	-	Atlantic salmon/Brown trout
Fish and veggie patties	Atlantic salmon/Brown trout	Atlantic salmon/Brown trout Atlantic cod
	Atlantic cod	
Salmon rolls	Atlantic salmon/Brown trout	Atlantic salmon/Brown trout

Table 3. Plant samples

Product	Detected species	
	NucleoSpin Food	GMO DNA extraction
Bell pepper spice	Pepper Apiopetalum glabratum	Pepper Apiopetalum glabratum
Cinnamon	-	Cinnamon
Mackerel in tomato sauce	-	-
Mixed vegetables and meat	Onion	Onion
Beef soup	Carrot	Carrot Leek Rapeseed Parsley
	Leek	
	Rapeseed	
	Parsley	
Salmon soup	Onion	Onion Dill/Fennel Celery Parsnips
	Dill/Fennel	
	Celery	
	Parsnips	
Sweet & sour sauce	-	Onion Pepper Carrot
Lettuce & beetroot mix	Beetroot	Beetroot
Oregano	Oregano/Marjoram/Syrian oregano Field bindweed	Oregano/Marjoram/Syrian oregano Field bindweed
Tea	Tea plant Leek	Tea plant
Cereal	Oat	-
	Oregano/Marjoram/Syrian oregano	
	Common wheat	



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

Table 4. Sample result summary

Kit	Extraction protocol	Food products	Result	Missing result
Meat ID	MN kit (1 h incubation)	11	11/11	
	GMO kit (1 h incubation)	11	11/11	
Fish ID	MN kit (1 h incubation)	9	8/9	1
	GMO kit (1 h incubation)	9	9/9	
Plant ID	MN kit (1 h incubation)	11	8/11	3
	GMO kit (1 h incubation)	11	9/11	2

## CONCLUSIONS

The spin-column based method was proven to be suitable for extracting DNA from food samples for down-stream NGS analysis.

Both tested kits performed without any problems for meat products, all declared species were detected. Some more variation was observed with fish samples where the smoked salmon pizza sample with NucleoSpin extracted DNA failed to produce any result in sequence analysis. Also an additional species (Northern pike) was detected in White fish patties. However, this can also be a true result of trace amount as pikes and white fish inhabit the same waters.

More variation was observed when sequencing plant products. Some species like Field bindweed and Apiopetalum glabratum can be true contaminants of the plant sample derived from the natural habitat of the declared species. The undeclared but detected species in tea and cereal sample extracted with NucleoSpin Food kit are most likely due to a cross-contamination between samples.

Tomato species was not found from tomato sauce (Mackerel in tomato sauce) with either of the DNA extraction methods. 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. Also the sweet & sour sauce proved to be difficult for the NucleoSpin food kit to extract DNA from.

Overall, the results showed that the spin-column based GMO Extraction kit is suitable for DNA extraction of meat, fish and plant samples.

## TRADEMARKS

© 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Precellys® is a registered trademark of Bertin Instruments SGS is a registered trademark of SGS Group Management S. A. NucleoSpin® is a registered trademark of Macherey-Nagel. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others.

LT2496A  
September 2019

**ThermoFisher**  
SCIENTIFIC