FOOD AUTHENTICITY TESTING WITH NEXT-GENERATION SEQUENCING

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INTRODUCTION

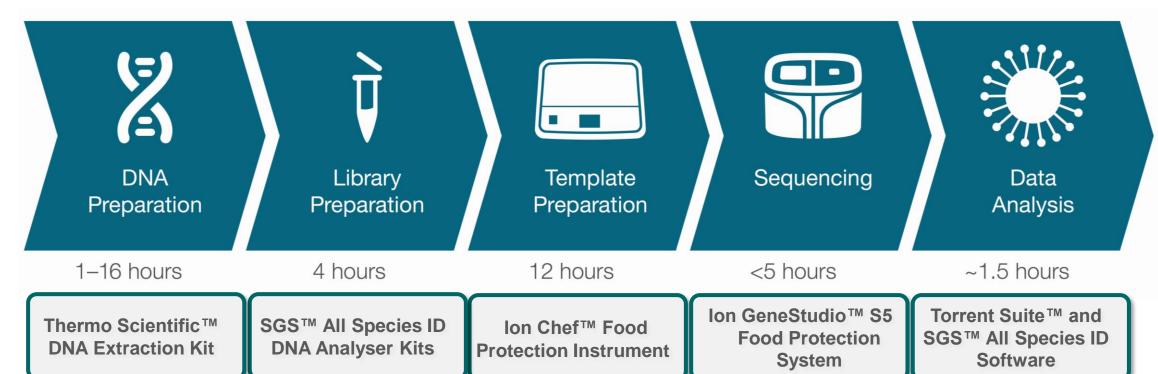
A study was executed in order to identify meat, fish and plant species in food products. The goal was to verify the compatibility of the methods included in the workflow and the identification of species from several different sample types.

- ✓ End-to-end workflow to identify all plant, fish and meat species within a food sample
- ✓ Samples from several food categories (ready-to-eat meals, fresh products, soups, ingredients, canned foods, etc)
- ✓ Comprehensive list of all species detected

MATERIALS AND METHODS

- ✓ **Homogenization** with Precellys[®] homogenization instrument (Bertin Instruments) to prepare a representative sample for DNA extraction.
- ✓ GMO Extraction kit (Thermo Fisher Scientific) with silica based spin-column technology was used to produce high-quality DNA for library preparation.
- ✓ Libraries for sequencing were prepared with SGS™ All Species Meat, Fish and Plant Analyser kits (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.
- ✓ A fully automated **templating** reaction on the lon Chef[™] Food Protection instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips.
- ✓ **Sequencing** was performed on the Ion GeneStudio[™] S5 Food Protection System (Thermo Fisher Scientific) relying on semi-conductor technology.
- ✓ Sequencing results were mapped against a database of species DNA of meat, fish and plant for data analysis. A comprehensive list of all species detected in a sample was generated by the SGS[™] All Species ID software (Thermo Fisher Scientific).

WORKFLOW



I	FISH PRODUCTS	MEAT PRODUCTS			PLANT PRODUCTS			
Product	Detected species		Product	Detected species		Product	Detected species	
Tuna (in water)	Skipjack tuna		Canned pork & beef	Pork, Beef	\checkmark	Bell pepper spice	Pepper	
Tuna (in oil)			Canned ham	Pork	\checkmark	Potato, onion and meat	Onion	*
Mackerel in tomato sauce	Atlantic mackerel		Canned chicken	Chicken	\checkmark	Beef soup (Potato, carrot, parsley, swede, leek, rapeseed)	Wild carrot, Wild leek, Rapeseed, Parsley, Carrot, Carrot oil	*
Canned Sardine	European pilchard		Sautéed reindeer	Reindeer	✓	Colman cours (Dotate, opion, colony, look, correct, dill, black, popper)		*
Smoked salmon pizza	Atlantic salmon/Brown trout		Sautéed red deer	Red deer	V F	Salmon soup (Potato, onion, celery, leek, carrot, dill, black pepper)	Onion, Dill/Fennel, Celery, Parsnips	
Fish rolls (roach, pollock)	Common roach, Pollock		Vegetables and pork	Pork	✓	Sweet & sour sauce (Tomato, onion, carrot, celery, green/red pepper, bamboo)	Onion, Pepper	*
Crispy Cod files	-	×	Ground pork & beef stick	Pork, Beef		Cinnamon	Cinnamon	\checkmark
Pollock with almond crust	Alaska pollock		- with cheese		<u> </u>	Chives powder	Onion/chives	\checkmark
	Atlantic salmon/Brown trout, Atlantic	.[]	Pork & beef dumplings	Pork, Beef	<u> </u>	Coriander powder	Coriander	\checkmark
Fish and veggie patties	cod	1.	Smoked and sliced pork,	Pork, Turkey, Chicken		Garlic powder	Garlic	 ✓
Salmon soup	Rainbow trout, Pollock		- turkey and chicken		<u> </u>	Grilling spice (Bell & black pepper, coriander, garlic, chili)	Onion, Garlic, Chili/Pepper	*
Jumon Joap	Lake whitefish, Whitefish	۴,	- Beef soup	Beef	<u> </u>	Pasta sauce (Onion, basil, garlic, parsley, oregano)	Onion/chives, Oat, Wild leek	*
White fish patties	Pollock		Pork liverwurst	Pork	<u>√</u>	Pesto sauce (Basil, cashew, garlic)	Basil, Cashew	*
		\vdash	- Sliced ham	Pork	✓	Oregano	Oregano/Marjoram/Syrian oregano	\checkmark
	Common bream, Common dace, Ide, Ray-finned fish sp., Common roach,	1	Bratwurst (pork, beef)	Pork, Beef	✓	Теа	Tea plant	\checkmark
Lake fish patties	Bream, European perch	✓	Sausage (Chicken, pork, beef, turkey)	Chicken, Pork, Beef	*	Veggie dumplings (Wheat, potato, onion, dill, black pepper)	Rapeseed, Potato, Rye, Oat, Onion, Barley, Rapeseed, Dill/fennel	*
Fish fingers	Atlantic cod, Haddock		Ox meat chips	Beef	\checkmark	Lentil and pea sprouts	Peas	*
Smoked sprat	European sprat	\checkmark	Kebab meat	Beef	\checkmark	Smoked tofu	Soybean	\checkmark
Salmon rolls	Atlantic salmon/Brown trout	\checkmark	Beef and pork patties	Beef, Pork	\checkmark	Wok veggies (Peas, beans, corn, soy bean, sunflower seeds, kidney	Pea, Soybean, Common bean,	
Rainbow trout strips	Rainbow trout	\checkmark	Ground beef & pork	· · · · · · · · · · · · · · · · · · ·		beans, linseed)	sunflower, garlic, Corn, Parsley	*
Pickled herring	Atlantic herring	\checkmark	patties	Beef, Pork	\checkmark	Frozen peas, corn, bell pepper	Pea, Pepper, Corn	✓
Salmon loaf	Atlantic salmon/Brown trout, Pollock	\checkmark	Minced chicken	Chicken	\checkmark	French fries	Potato	\checkmark
Frozen salmon cubes	Pink salmon	\checkmark	Low fat minced beef with	Chickers Doof		TRADEMARK © 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	Thermo Fisher	
Frozen Alaska Pollock	Alaska pollock	\checkmark	chicken	Chicken, Beef	\checkmark		SCIENTIFIC	

CONCLUSIONS

90% of the fish species and 95% of the meat species that were declared as an ingredient were detected. Some plant species were not detected with samples where multiple species were listed in the ingredients, but all declared spice species were detected in spice-type samples. Also, since the products tested are real food products there is no information about the concentration of the species labelled in each product and therefore this can impact on the successful detection of species that can be present at a very low level.

The system is able to analyze several sample types and targets (meat, fish and simple plant samples) within a single sequencing run, enabling shorter processing times with lower cost.