FOOD AUTHENTICITY TESTING WITH NEXT-GENERATION SEQUENCING

Amanda Manolis[1], Nicole Prentice[2] and Tiina Karla[3]
[1]Thermo Fisher Scientific, Austin, Texas, USA, [2]Thermo Fisher Scientific, Basingstoke, Hampshire, UK, [3]Thermo Fisher Scientific, Vantaa, Uusimaa, Finland

OVERVIEW

The Thermo Scientific™ NGS Food Authenticity Workflow (Thermo Fisher Scientific) utilizes next-generation sequencing to detect animal, fish and plant species in food samples

- ✓ Untargeted approach 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)
- ✓ End-to-end workflow
- ✓ Comprehensive list of all species detected

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.

MATERIALS AND METHODS

- ✓ **Homogenization** was performed to prepare a representative portion of the sample for DNA extraction. Homogenization was carried out in a Precellys® homogenization instrument (Bertin Instruments) utilizing bead beating technology
- ✓ **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). Regions of interest were amplified with PCR from the DNA extractions of food samples and sequencing adaptors added. During the library preparation unique barcodes (i.e. molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run.
- ✓ 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** was performed on the Ion GeneStudio™ S5 Food Protection System (Thermo Fisher Scientific) relying on semi-conductor based sequencing technology.
- ✓ Sequencing results were mapped against our database of species DNA of meat, fish and plant for **data analysis**. A comprehensive list of all species detected in a sample as well as an estimate of their abundance was generated by the SGS™ All Species ID software (Thermo Fisher Scientific).

WORKFLOW

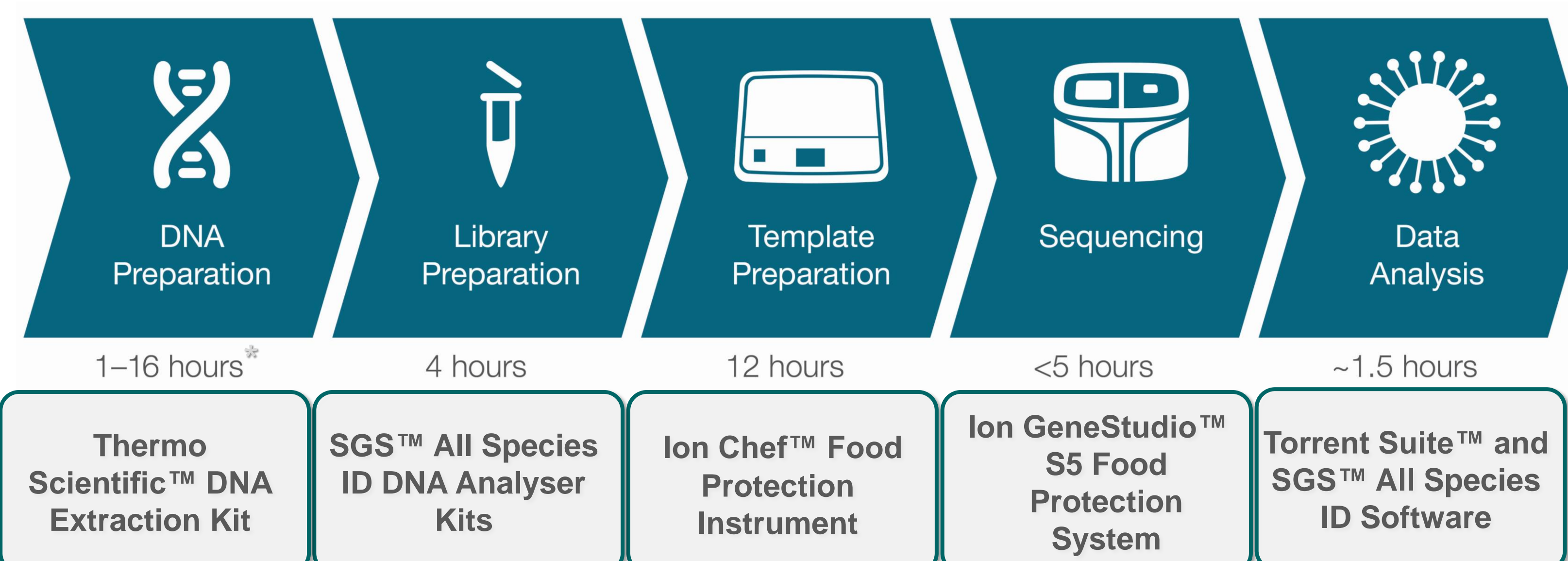| DNA Preparation	Library Preparation	Template Preparation	Sequencing	Data Analysis
1–16 hours*	4 hours	12 hours	<5 hours	~1.5 hours
Thermo Scientific™ DNA Extraction Kit	SGS™ All Species ID DNA Analyser Kits	Ion Chef™ Food Protection Instrument	Ion GeneStudio™ S5 Food Protection System	Torrent Suite™ and SGS™ All Species ID Software

Figure 1. Thermo Scientific NGS Food Authenticity Workflow overview
*only a limited number of samples require extended 16 hour incubation versus 1 hour in DNA preparation stage

RESULTS

Table 1. Samples analyzed for fish species

Product	Labelled fish species	Result
FISH PATTIES	Whitefish, Pollock	*Coregonus sp* (Whitefish), *Pollachius virens* (Pollock)
CANNED TUNA	Tuna	*Katsuwonus pelamis* (Skipjack tuna)
MACKEREL IN TOMATO SAUCE	Mackerel	*Scomber scombrus* (Atlantic mackarel)
SALMON SOUP	Rainbow trout	*Oncorhyncys mykiss* (Rainbow trout)
FISH FINGERS	Cod	*Gadus morhua* (Atlantic cod), *Melanogrammus aeglefinus* (Haddock)
FISH WITH ALMOND CRUST	Alaska pollock	*Theragra chalcogramma* (Alaska pollock)
SMOKED SALMON PIZZA	Trout	*Salmo salar/trutta* (Salmon/Trout)
FISH PATTIES	Trout, Cod	*Salmo salar/trutta* (Salmon/Trout), Gadus morhua (Atlantic cod)
SALMON ROLLS	Trout	*Salmo salar/trutta* (Salmon/Trout)
CANNED SARDINE	Sardine	*Sardina pilchardus* (Sardine)
PICKLED HERRING	Herring	*Clupea harengus/pallasii* (Pacific herring)
FISH ROLLS	Roach, Pollock	*Rutilus rutilus* (Roach), *Pollachius virens* (Pollock)
SALMON LOAF	Trout, Pollock	*Salmo salar/trutta* (Salmon/Trout), *Pollachius virens* (Pollock)
FROZEN SALMON CUBES	Trout, Pink salmon	*Oncorhynchus gorbuscha* (Pink Salmon)
FROZEN POLLOCK	Alaska Pollock	*Theragra chalcogramma* (Alaska pollock)
SMOKED SPRAT	Sprat	*Sprattus sprattus* (Sprat)
CRISPY COD FILES	Cod, Pollock	No result

Table 2. Samples analyzed for plant species

Product	Labelled plant species	Result
BELL PEPPER SPICE	Bell pepper	*Capsicum sp* (Pepper, chili, cayenne, paprika etc)
MEAT & VEGETABLE MEAL	Onion	*Allium cepa* (Onion)
BEEF SOUP	Leek, parsley	*Allium ampeloprasum* (Wild leek), *Anethum graveolens/Foeniculum vulgare* (Dill/Fennel), *Brassica napus* (Rapeseed), *Pedroselinum crispum* (Parsley)
SALMON SOUP	Onion, celery, leek, dill, black pepper	*Allium cepa* (Onion), *Anethum graveolens/Foeniculum vulgare* (Dill/Fennel), *Apium graveolens* (Celery)
SWEET & SOUR SAUCE	Onion, celery, green pepper, red pepper	*Allium cepa/Allium schoenoprasum* (Onion/Chives), *Capsicum sp.* (Pepper, chili, cayenne, paprika etc)
OREGANO	Oregano	*Origanum sp./vulgare/majorana/syriacum/onites* (Oregano), *Convolvulus arvensis* (Field bindweed)
CINNAMON	Cinnamon	*Cinnamomum zeylanicum* (Cinnamon)
CHIVES POWDER	Chives	*Allium schoenoprasum* (Chives)
CORIANDER POWDER	Coriander	*Coriandrum sativum* (Coriander)
GARLIC POWDER	Garlic	*Allium sativum* (Garlic)
GRILLING SPICE	Bell pepper, black pepper, coriander, garlic, chili	*Allium cepa* (Onion), *Capsicum sp.* (Pepper, chili, cayenne, paprika etc), *Allium sativum* (Garlic)
SMOKED TOFU	Soy	*Glycine max* (Soybean)
PELMENIS	Wheat, onion, black pepper, dill	*Triticum aestivum* (Wheat), *Allium cepa* (Onion), *Secale cereale* (Rye), *Hordeum vulgare* (Barley), *Avena sp.* (Oats)

Table 3. Samples analyzed for meat species

Product	Labelled meat species	Result
CANNED PORK & BEEF	Pork, Beef	Sus scrofa (Pig), Bos taurus (Cow)
CANNED CHICKEN	Chicken	Gallus gallus (Chicken)
CANNED HAM	Pork	Sus scrofa (Pig)
MINCED TURKEY	Turkey	Meleagris gallopavo (Turkey)
MINCED BEEF & CHICKEN	Beef, Chicken	Gallus gallus (Chicken), Bos taurus (Cow)
MINCED PORK & CARROT	Pork	Sus scrofa (Pig)
CHICKEN BACON PATTY	Chicken	Gallus gallus (Chicken)
BEEF & PORK PATTIES	Beef, Pork	Bos taurus (Cow), Sus scrofa (Pig)
SAUTÉED REINDEER	Reindeer	Rangifer tarandus (Reindeer)
SAUTÉED RED DEER	Red deer	Cervus elaphus (Red deer)
PORK WITH VEGETABLES	Pork	Sus scrofa (Pig)
BEEF SOUP	Beef	Bos taurus (Cow)
MINCED MEAT&CHEESE	Pork, Beef	No result
SLICED HAM	Pork	Sus scrofa (Pig)
LIVERWURST	Pork	Sus scrofa (Pig)
BRATWURST	Pork, Beef	Sus scrofa (Pig), Bos taurus (Cow)
SAUSAGE	Chicken, Beef, Pork, Turkey	Gallus gallus (Chicken), Sus scrofa (Pig), Bos taurus (Cow)
OX MEAT CHIPS	Beef	Bos taurus (Cow)
SMOKED MEATS	Pork, Turkey, Chicken	Sus scrofa (Pig), Meleagris gallopavo (Turkey), Gallus gallus (Chicken)
MEAT PELMENIS	Pork, Beef	Sus scrofa (Pig), Bos taurus (Cow)
BEEF KEBAB	Beef	Bos taurus (Cow)

Species not detected / Extra species detected / No result

RESULT SUMMARY

Fish samples
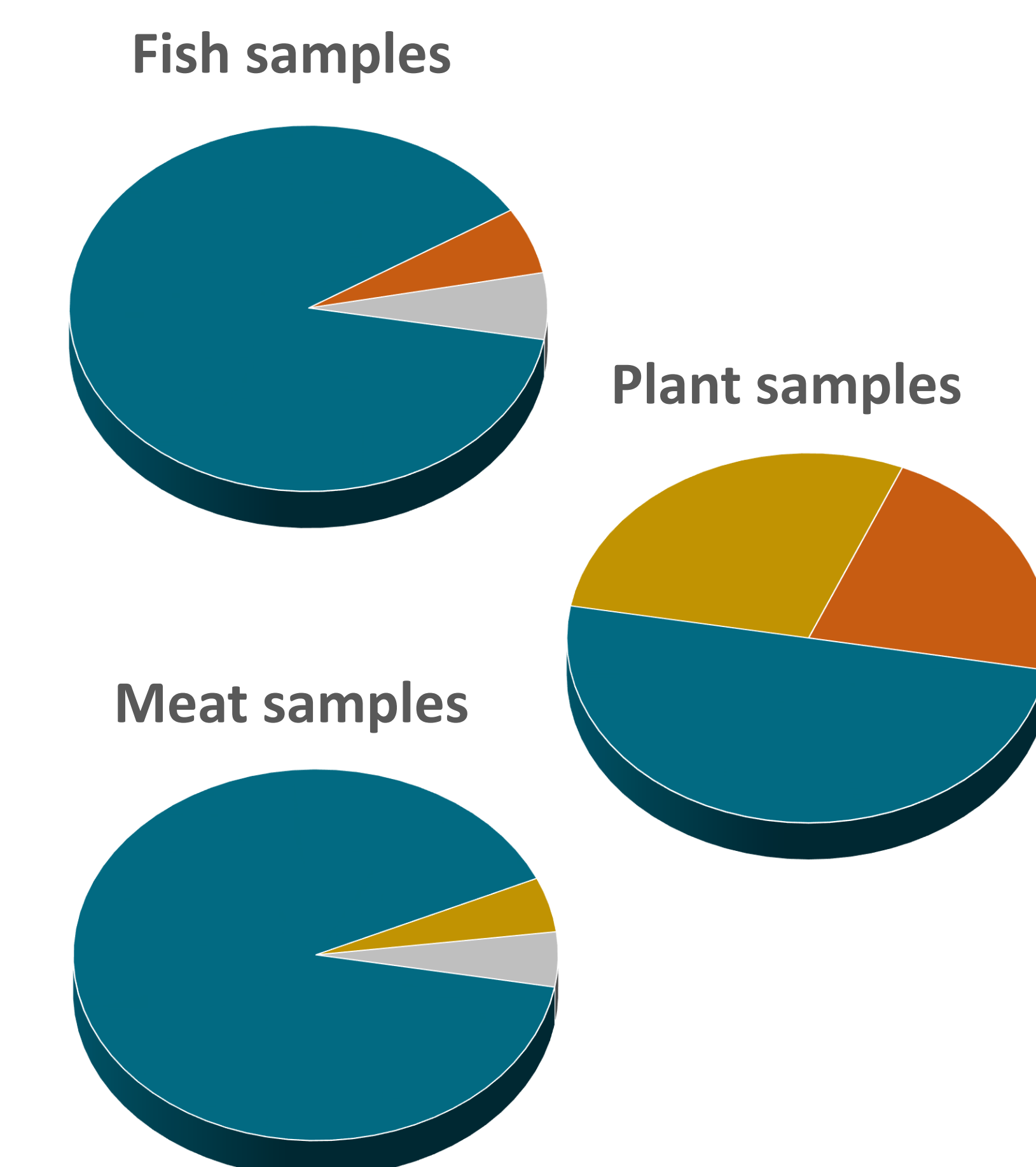
Plant samples

Meat samples

Figure 2. Species result

Number of samples where all species declared as ingredient were detected (blue)
Number of samples where not all species present in the sample were detected (yellow)
Number of samples where species not in the ingredients list were detected (orange)
Number of samples with no results from the All Species SW (grey)

CONCLUSIONS

88% of the fish species and 90% 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. However, all declared spice species were detected in spice-type samples. Based on the results further database optimization and validation of the analytics pipeline is needed to detect all species in complex plant mixes, but the system works well for fish, meat and simple plant samples.

Species not listed as ingredients were detected with fish and plant kits, however there is a possibility that the identified species are actually present in the sample even though they are not listed as ingredients.

The system is capable of analysing several sample types and targets (meat, fish and plant) within a single sequencing run, enabling shorter processing times with lower cost.

TRADEMARKS/LICENSING

ThermoFisher SCIENTIFIC