# Development of a Next Generation Sequencing (NGS) workflow for food DNA analysis: How to identify meat and fish species in complex food products

Sofia Nogueira[1], Mário Gadanho[1], Sandra Chaves[1], Amanda Manolis[2], Tiina Karla[3]

[1]SGS Molecular, Lisbon, Portugal [2]Thermo Fisher Scientific, Austin, Texas, USA [3]Thermo Fisher Scientific, Vantaa, Finland

## ABSTRACT

Using next generation sequencing to detect animal and plant species in complex food samples the Thermo Scientific™ NGS Food Authenticity Workflow (Thermo Fisher Scientific) was demonstrated to provide:

✓ An untargeted approach to identify meat and fish species within a food sample
✓ Suitability for samples from several food categories (ready-to-eat meals, fresh products, soups, canned, etc)
✓ A complete end-to-end workflow
✓ Results as a comprehensive list of all identified species

## INTRODUCTION

Next Generation Sequencing (NGS) has been introduced in recent years as a very powerful DNA-based method for species identification in food products. However, the use of NGS requires the development of optimized, fit for purpose, workflow to ensure reliability of results and to maximize the advantages of this high throughput method. Taking advantage of the non-targeted and massive sequencing output obtained by NGS, an end to end workflow was developed and tested to identify meat and fish species in food products.

In the present study the NGS Food Authenticity Workflow (Figure 1) was tested for meat and fish species identification in a variety of complex food products.

Figure 1. Ion Chef™ Food Protection Instrument, Ion GeneStudio™ Food Protection NGS System and SGS™ All Species ID Analyser Kits

## MATERIALS AND METHODS

The workflow (Figure 2) was defined and optimized to meet the following criteria:

✓ Barcoding of several specific DNA regions suitable for species identification (multi-barcoding)
✓ Definition of consensus primer panels producing very small amplicons to ensure its suitability for highly processed food where DNA can be very fragmented (SGS™ All Species ID DNA Analyser Kits)
✓ Optimization with the Ion Torrent™ semi-conductor based technology with automated templating reaction on the Ion Chef™ Food Protection instrument to prepare the sample libraries for sequencing on Ion GeneStudio™ S5 Food Protection System.
✓ Development of an automated software solution for data analysis with suitable databases containing thousands of meat and fish DNA sequences for species identification (SGS™ All Species ID Software)

The workflow was tested on a group of 90 complex samples of meat and fish including 27 artificial DNA mixtures and 63 real food products. Real food products were selected to include different processing treatments. Dry, canned, fresh, frozen and liquid food products were selected.
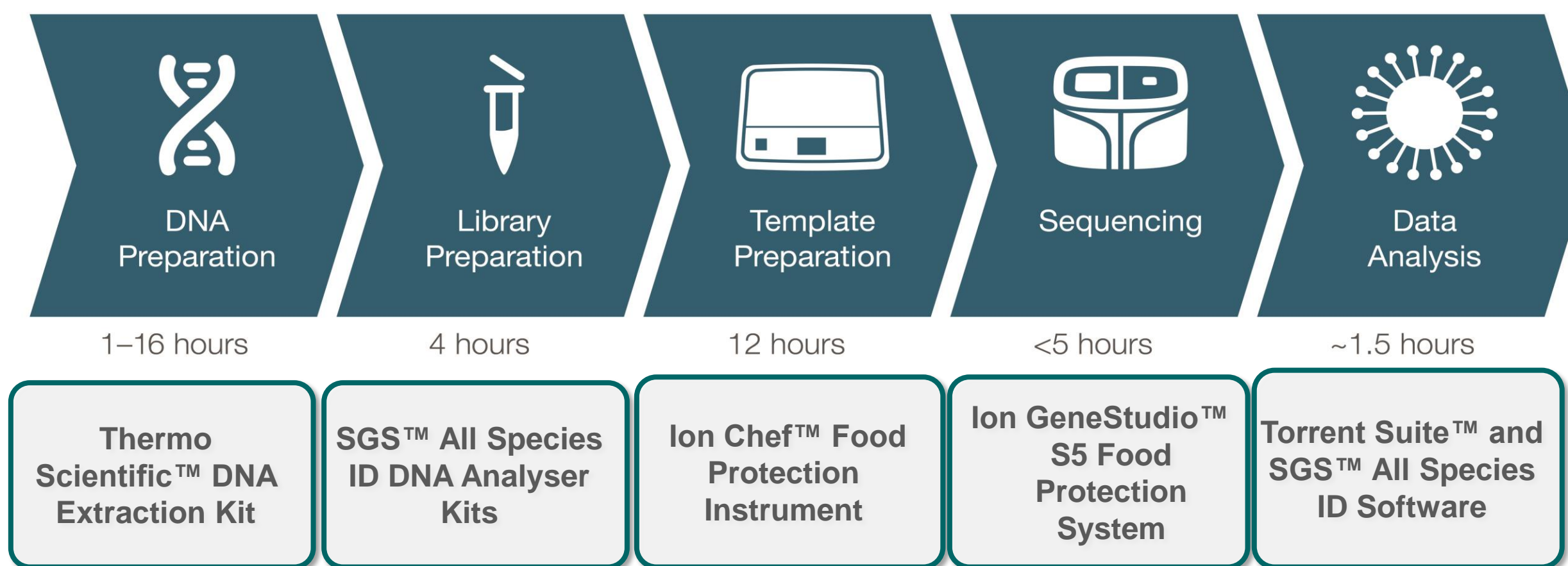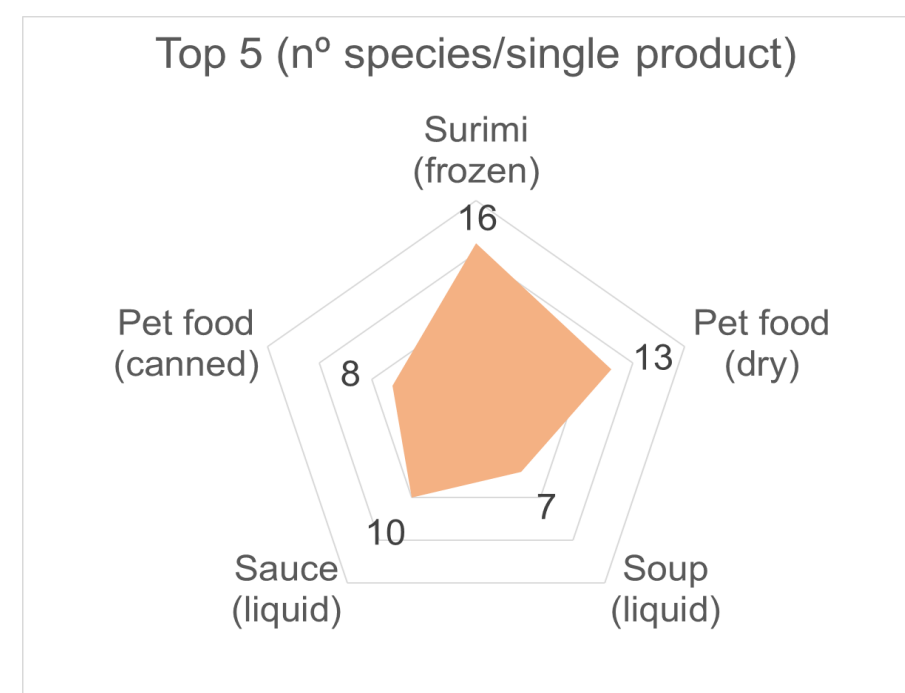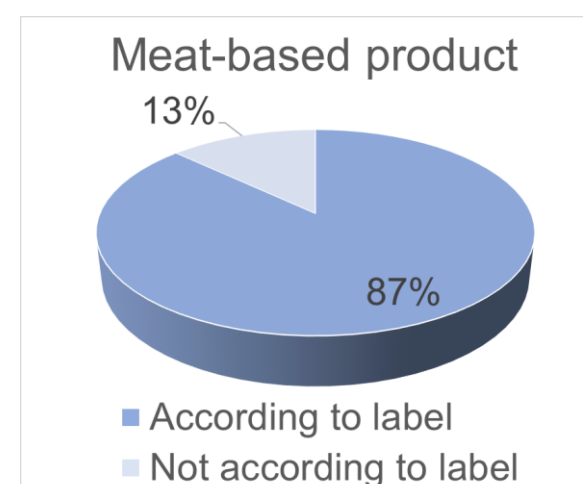
Figure 2. Thermo Scientific NGS Food Authenticity Workflow overview

| 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 |

## RESULTS

| Meat species identified |
|---|
| Alectoris chukar |
| Anas sp. |
| Bos taurus |
| Capra hircus |
| Cervus elaphus |
| Coturnix sp. |
| Equus caballus |
| Gallus gallus |
| Meleagris gallopavo |
| Oryctolagus cuniculus |
| Ovis aries |
| Phasianus colchicus |
| Struthio camelus |
| Sus scrofa |

**Meat-based product**
13%
87%
■ According to label
■ Not according to label

**Top 5 (nº species/single product)**
Surimi (frozen) 16
Pet food (dry) 13
Soup (liquid) 7
Sauce (liquid) 10
Pet food (canned) 8

**Fish-based product**
19%
81%
■ According to label
■ Not according to label

| Fish species identified | |
|---|---|
| Ammodytes marinus | Oncorhynchus mykiss |
| Atheresthes stomias | Oncorhynchus nerka |
| Auxis rochei | Oreochromis niloticus |
| Brosme brosme | Pagrus caeruleostictus |
| Chelidonichthys lucernus | Planiliza parmata |
| Clupea pallasii / harengus | Pleuronectes platessa |
| Conger conger | Pollachius virens |
| Conger japonicus | Pomadasys maculatus |
| Dactyloptena peterseni | Raja sp. |
| Decapterus maruadsi | Rastrelliger brachysoma |
| Decapterus sp. | Rastrelliger kanagurta |
| Dussumieria elopsoides | Salmo salar |
| Encrasicholina sp. | Sander lucioperca |
| Engraulis encrasicolus | Sander volgensis |
| Gadus morhua | Sarda sarda/chiliensis |
| Helicolenus dactylopterus | Sardina pilchardus |
| Helicolenus sp. | Sardinella gibbosa |
| Ilisha melastoma | Sardinella jussieu |
| Katsuwonus pelamis | Scomber scombrus |
| Lepturacanthus savala | Scyliorhinus canicula |
| Lophius Litulon | Sebastes sp. |
| Megalaspis cordyla | Selar crumenophthalmus |
| Melanogrammus aeglefinus | Selaroides leptolepis |
| Merlangius merlangus | Sprattus sprattus |
| Merluccius capensis | Stolephorus waitei |
| Merluccius merluccius | Theragra chalcogramma |
| Merluccius paradoxus | Thunnus albacares |
| Merluccius productus | Thunnus obesus |
| Micromesistius poutassou | Trichiurus lepturus |
| Nemipterus japonicus | Upeneus guttatus |
| Oncorhynchus gorbuscha | Xiphias gladius |
| Oncorhynchus keta | |

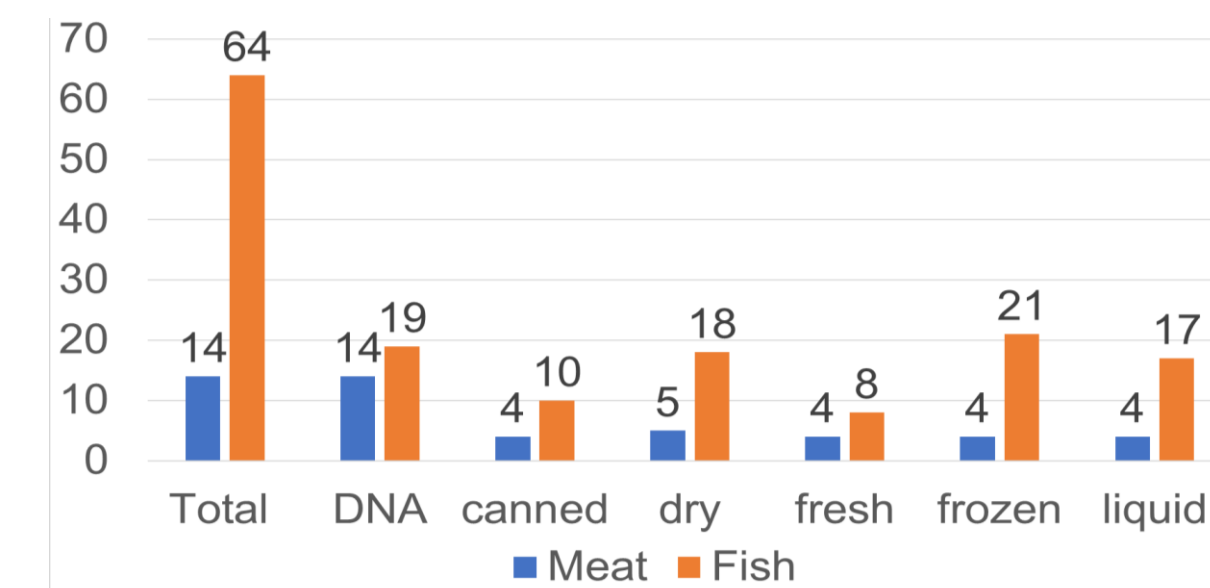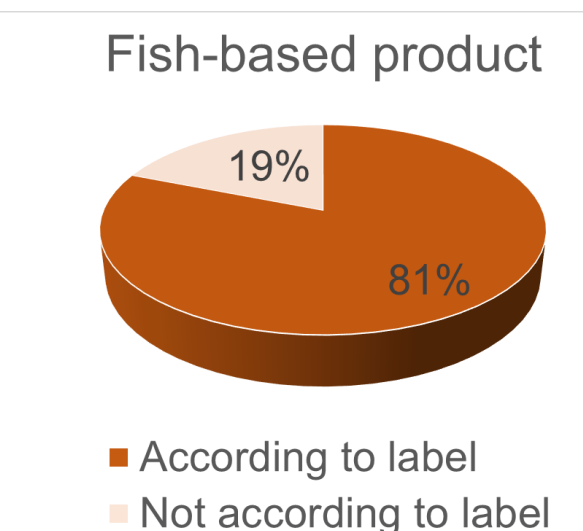| | Total | DNA | canned | dry | fresh | frozen | liquid |
|---|---|---|---|---|---|---|---|
| Meat | 14 | 14 | 4 | 5 | 4 | 4 | 4 |
| Fish | 64 | 19 | 10 | 18 | 8 | 21 | 17 |

Figure 3. Number of identified species per meat and fish category

## CONCLUSIONS

NGS workflow was effective in identifying the species present in all food samples regardless of their processing level (up to 16 species in a single product). The workflow was successfully used simultaneously for meat and fish based products analysis in a single NGS run (Figure 3).

The whole workflow from DNA extraction to species identification can take under 24 hours and requires short handling due to process automation making this a powerful and innovative solution for routine in-house screening of food samples for species identification.

SGS have partnered with Thermo Fisher Scientific who have commercialized the end-to-end NGS Food Authenticity Workflow (Thermo Fisher Scientific)

## TRADEMARKS

SGS    ThermoFisher SCIENTIFIC