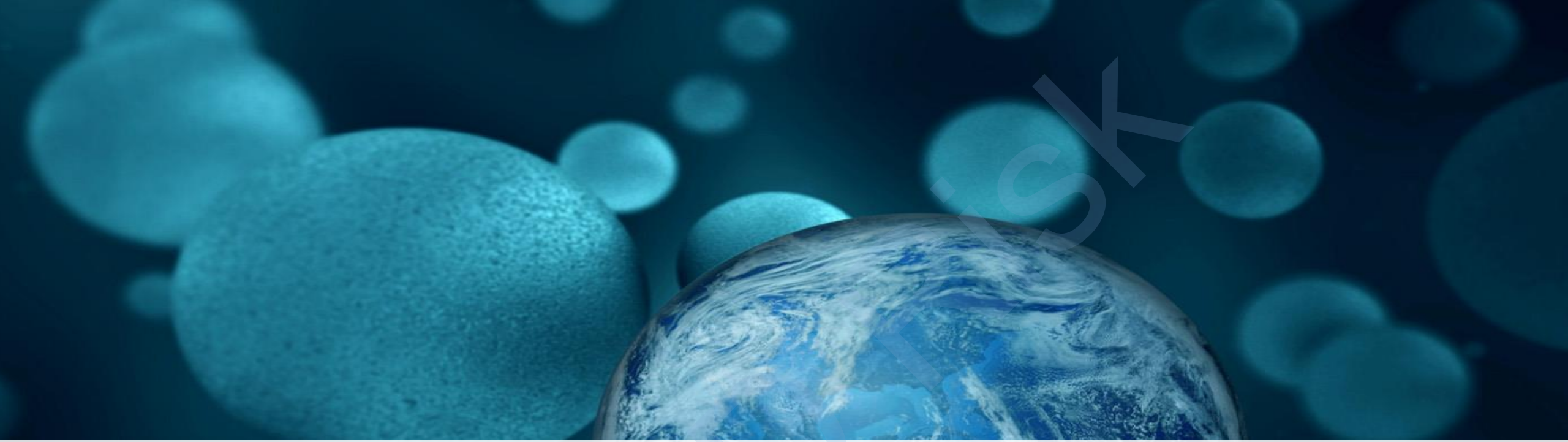

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NGS for routine food fraud analysis

Conférence Authenticité – Nouvelles technologie et solutions

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R&D Senior Staff Scientist, next gen solutions

What is Food Authenticity Testing?

Testing For:

- Purity and Authenticity
- Origin (type or country)
- Halal Food (pork and alcohol)
- Food Fraud / Adulteration
  - ✓ Addition or substitution of prohibited ingredients
  - ✓ Substitution with inferior or completely different product
  - ✓ Mislabeling

Why Care About Food Authenticity and Fraud?

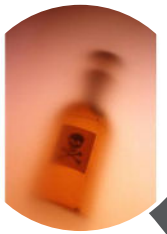
- In 2014 the Grocery Manufacturers Association (GMA) estimated that fraud cost the global food industry $10-$15 billion a year, with 10% of all commercially sold food products affected[1,2]
- Fraud resulting in a food safety or public health risk event could have significant financial or public relations consequences for the food industry or company
- Food fraud can tarnish the reputation of the food manufacturer or contract testing laboratory

Everyone involved in the food industry has a stake in ensuring that adulterated food is not being traded.

[1] GMA, Consumer Product Fraud, Deterrence and Detection, 2010, http://www.gmaonline.org/downloads/wygwam/consumerproductfraud.pdf
[2] A. Kircher, NCFPD, Tools for Protecting the Nations Food Supply, June 5, 2012.

Choosing the Right Analytical Strategy to Reduce the Food Fraud Risk

Analytical Strategy for Food Integrity



Blatantly counterfeit product ⇨ Visual and/or organoleptic verification of labels, container, content

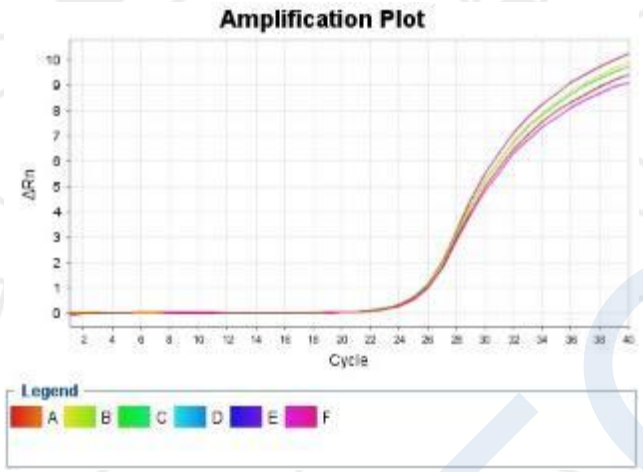
Adulteration
Enhancement
Dilution
Substitution ⇨ Chemical or biomolecular composition: chromatography, spectroscopy, DNA profile

Geographical Origin
Production method ⇨ Stable isotope analysis, trace element profile

All or some of the above! ⇨ Untargeted or targeted approach

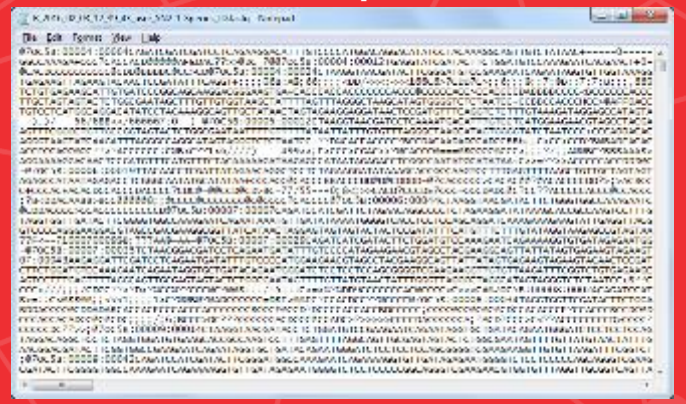
Multi-species Identification Using DNA Fingerprints

Real time PCR



Next Generation Sequencing

Fastq file



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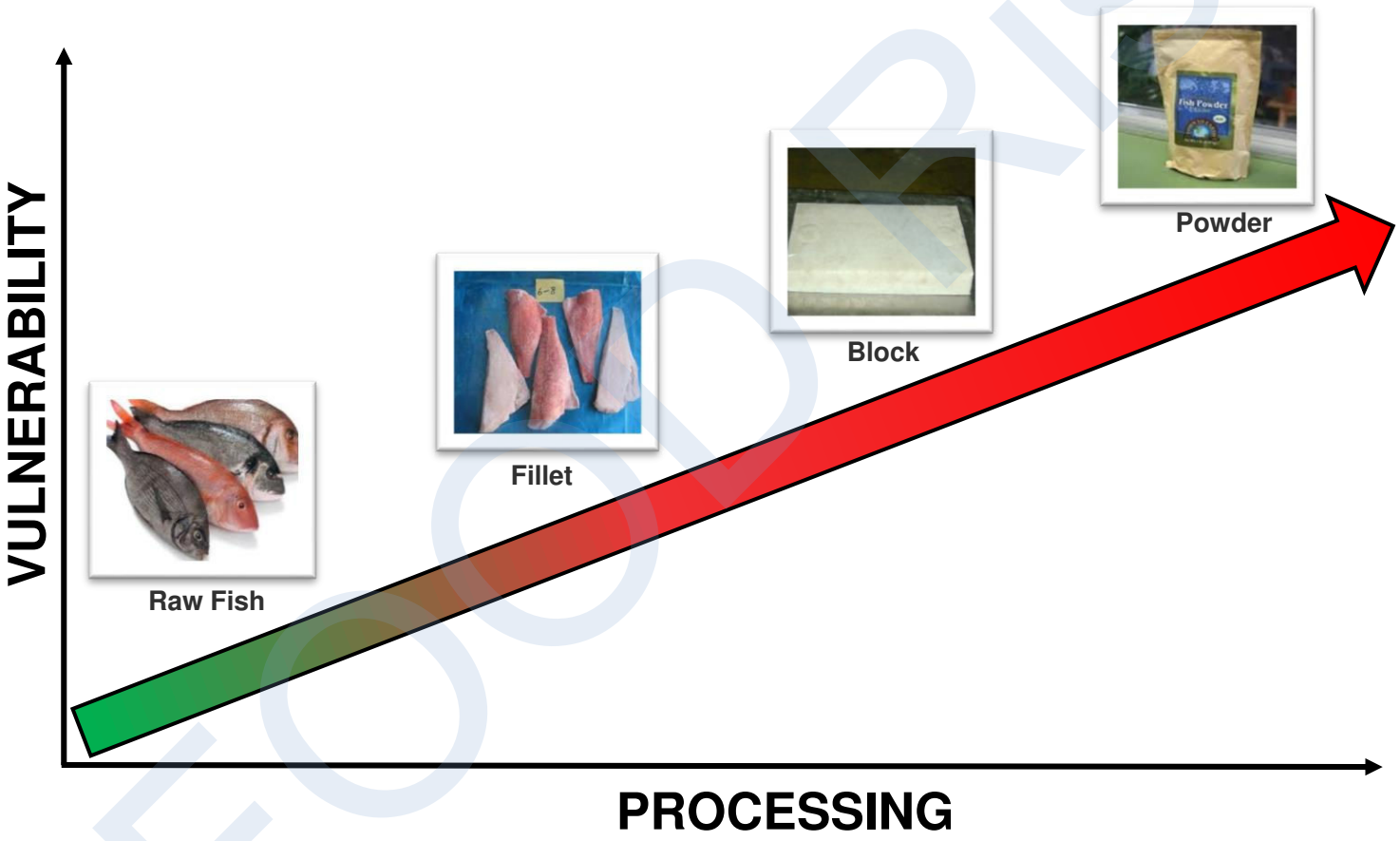
Market needs



Market needs – Highly processed food

Canned meat chili:

- *Bos taurus* – Beef
- *Sus scrofa* – Pork
- *Meleagris gallopavo* – Turkey
- *Gallus gallus - Chicken*

Market needs – Highly processed food

Canned Tuna:

- *Thunnus albacares* – Yellowfin Tuna
- *Thunnus obesus* – Bigeye Tuna
- *Thunnus alalunga* – Albacore Tuna
- *Katsuwonus pelamis* – Skipjack Tuna

Challenge

Validation / Recognitation / Standardization

What Reference Method ?

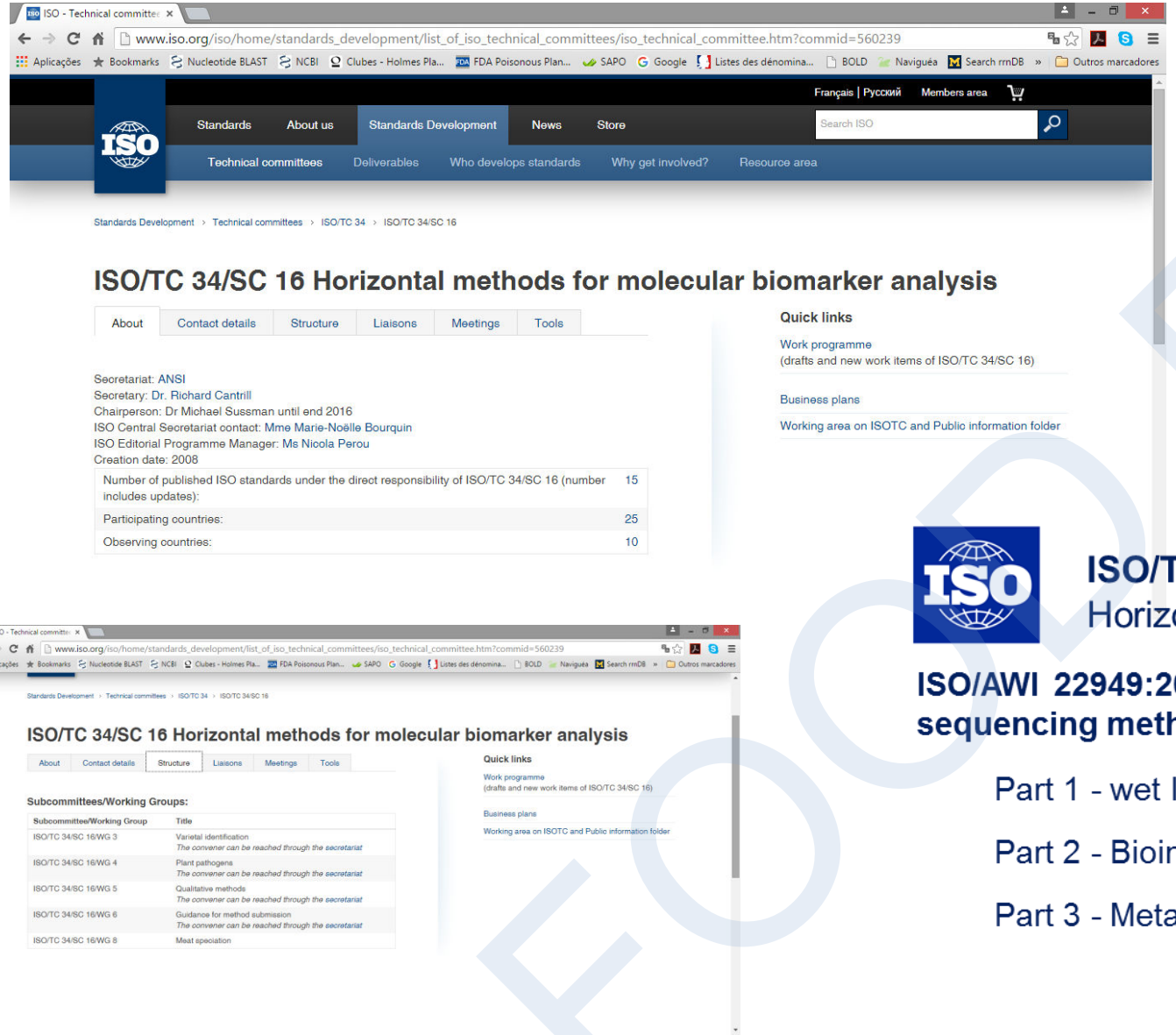
NGS standardization

- NGS has been introduced in the food sector for routine analysis in the last 3-4 years – Normalization needed

- ISO TC 34 – Food products
  - ISO TC 34/SC 9 – Microbiology
    - WG 25 - Whole-genome sequencing for typing and genomic characterization
  - ISO TC 34/SC 16 - Horizontal methods for molecular biomarker analysis
    - WG 8 – Meat speciation

- ISO TC 276 – Biotechnology
    - WG 3 – Analytical methods

NGS standardization



ISO/TC 34/SC 16
Horizontal methods for molecular biomarker analysis

ISO/AWI 22949:2018 Detection and identification of animal species by DNA sequencing methods

Part 1 - wet lab operations

Part 2 - Bioinformatics and pipeline validation

Part 3 - Metadata and sequence repository

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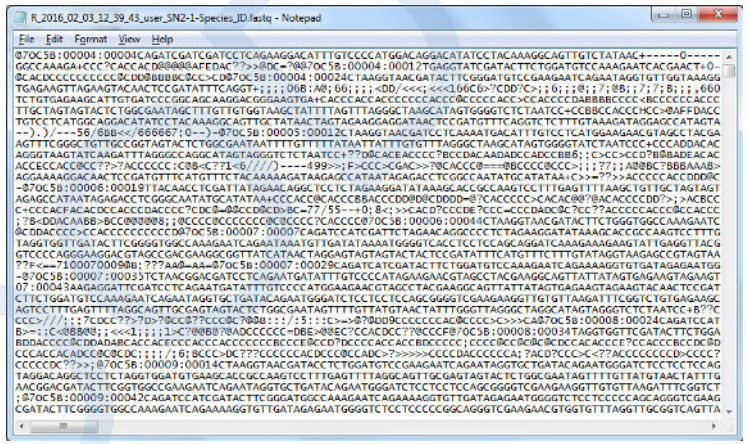
Part 1 - wet lab operations

- Follow manufacturer instructions … the easiest step !

Fastq file



NGS standardization

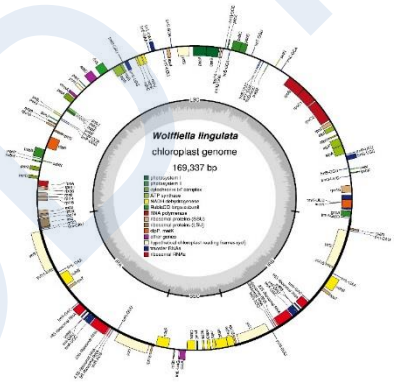
DNA sequencing

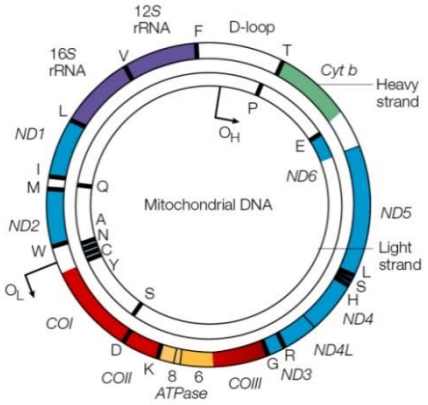
General primers

Gene(s)?
COI
cytB
16S
12S
ITS
matK
TRN
rbcl

Part 2 - Bioinformatics and pipeline validation

- Target DNA region(s) to be amplified
- Consensus primers used for PCR library construction





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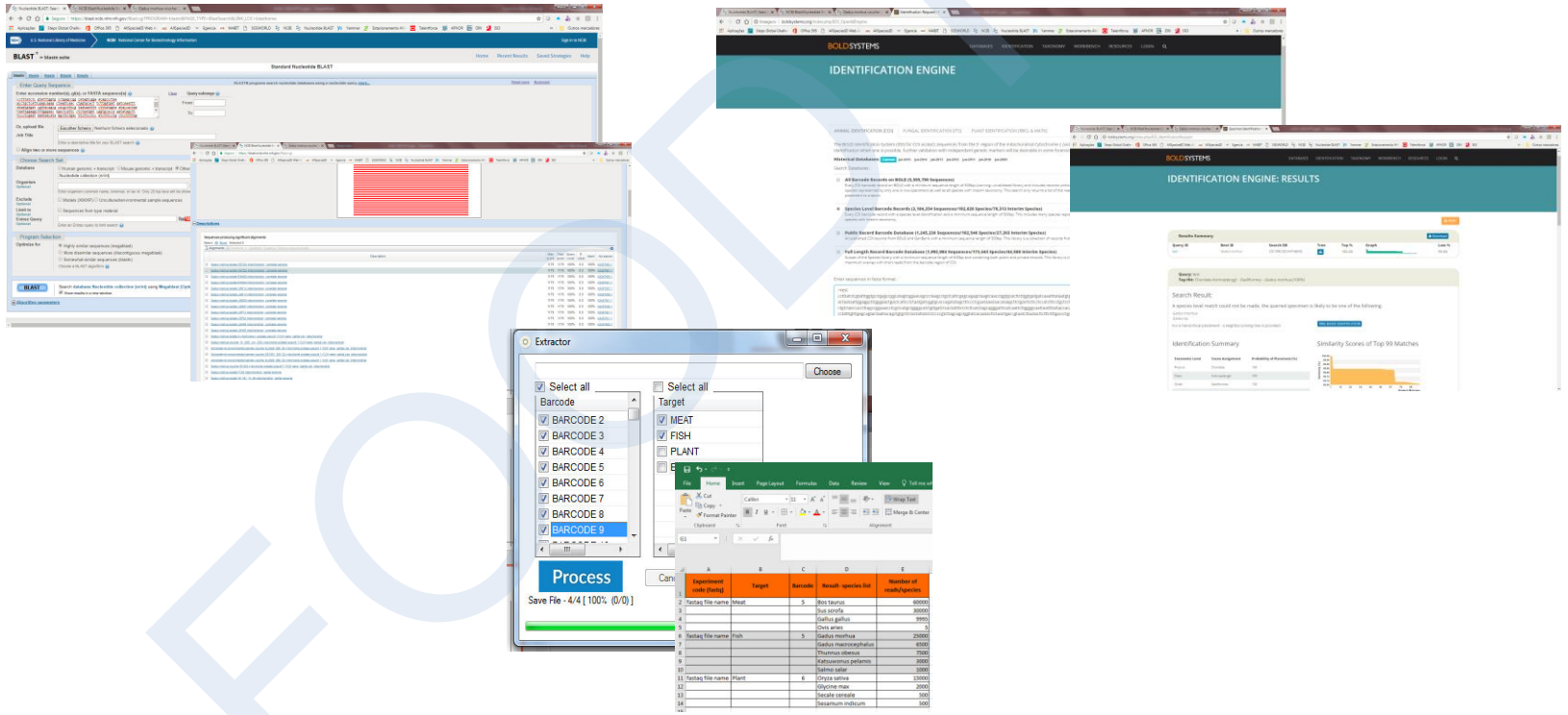
NGS standardization

Part 3 - Metadata and sequence repository

- Reference DNA sequence database used for identification
- Comparison of DNA sequences obtained with reference database



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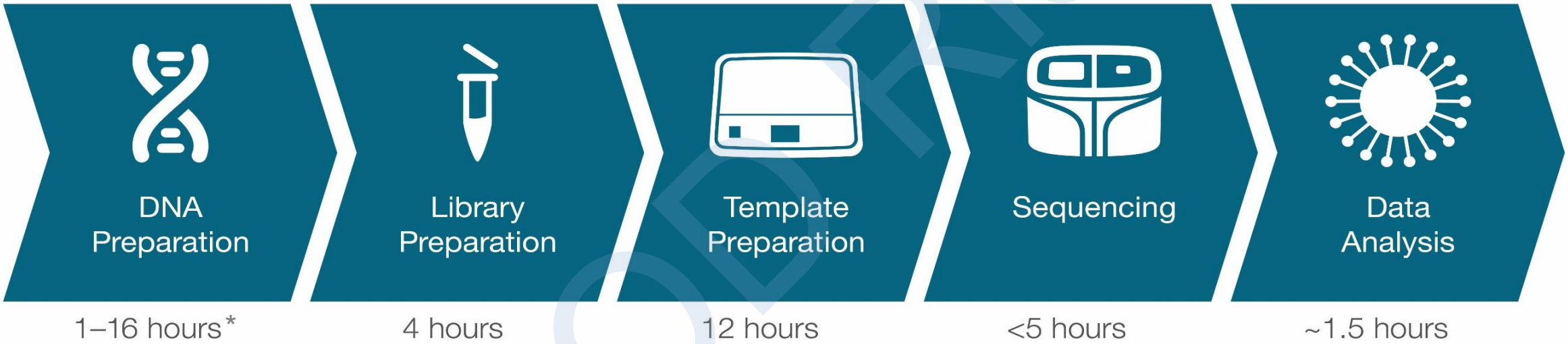
Inspect. Detect. Protect

The new **Thermo Scientific™ NGS Food Authenticity Workflow** is a complete, automated, next generation sequencing workflow and software database for multi-species ID screening



*DNA preparation time range includes overnight incubation for select few sample types

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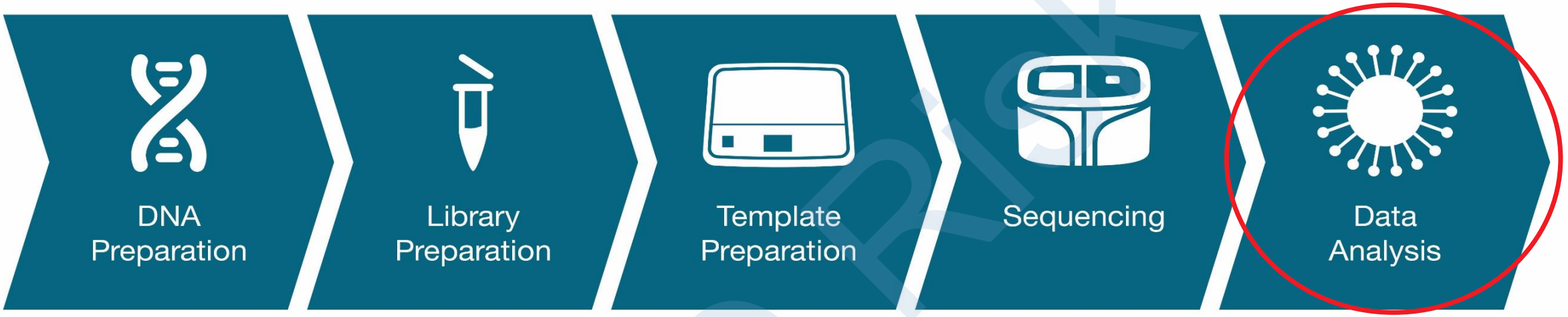
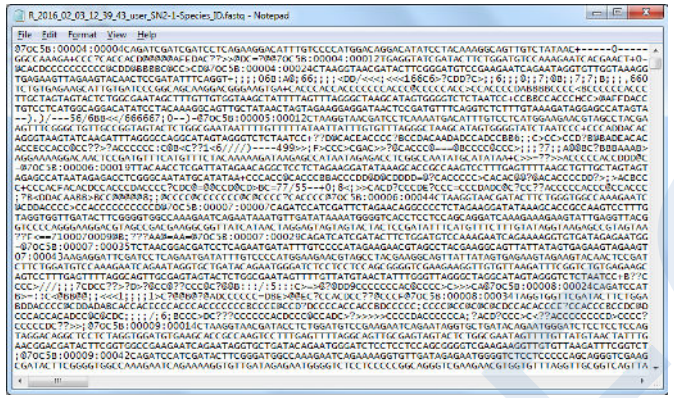
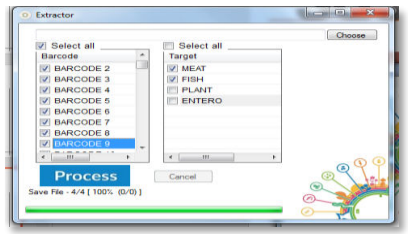
Data Analysis Made Simple with SGS All Species ID Software



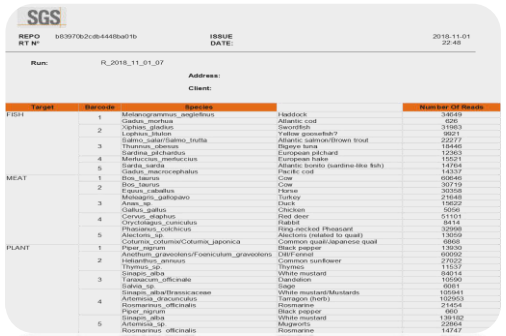
Fastq file



SGS™ All Species ID software



User-friendly output



Data analysis and results

- Validated database
- All targets detected

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Case Study: *Laurel raw material*

SITUATION

- Retailer requested species ID on spices in curry mixture (containing >10 spices according to label)

RESPONSE

- Laurel (Laurus nobilis) was not detected
- Analysis from NGS workflow indicated the sample contained large amounts of a similar plant of the laurel family but not Laurus nobilis (laurel) - potentially a toxic plant
- Supplier was substituting a similar species to Laurel but not true Laurel

VALUE DELIVERED

Rapid Response
Hours to days to sequence sample, and provide accurate analysis back

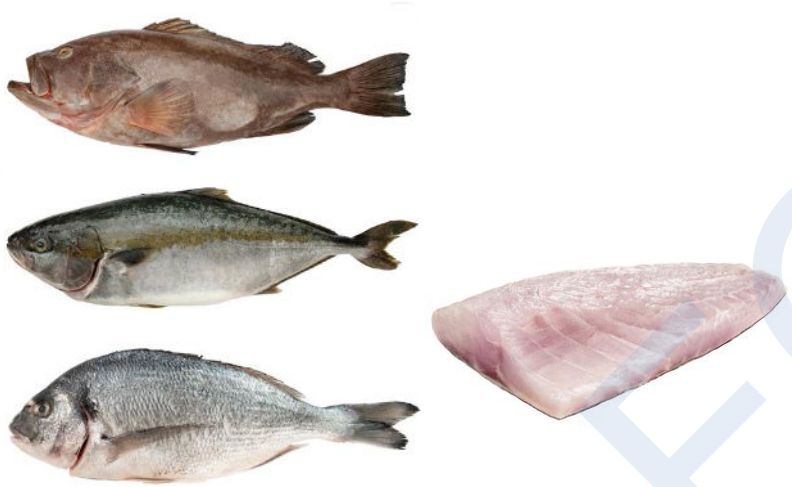
Customer Solution
Specific for *Plant Species ID* testing in food

Increased profitability
Fast and accurate analysis for prevention of intentional adulteration of food supply

Case study: Grouper fillet

SITUATION

- Retailer requested species ID for fish. Confirm grouper species authentication for a fillet
- Sanger sequencing ID that is appropriate for single species products originated a mixture of DNA sequences and no identification results



RESPONSE

- NGS Mutli-Species ID was performed, revealed 4 different fish species
- Few species identified were not commercially authorized fish species – one of the species toxic
- Supplier after knowing the NGS results confirmed that, in spite of the fillet format of the product, it was not a true fillet but processed fish sample with a fillet format

VALUE DELIVERED

Rapid Response
Hours to days to sequence sample, and provide accurate analysis back

Customer Solution
Specific for fish species ID testing in food

Scientific Expertise
Highly skilled and supportive scientists dedicated to food safety and integrity

Thank You

Phytocontrol
Laboratoire d'analyses

PCR

NGS

What's in my lasagna ?

Does my lasagna contain beef meat ? ☑ ☒

Does my lasagna contain horse meat ? ☑ ☒

Does my lasagna contain pork meat ? ☑ ☒

Targeted
a few (dozen) species

UNtargeted
Several thousands of species

Phytocontrol
Laboratoire d'analyses

homogenization

Total DNA extraction

DNA quantification

PCR amplification

Library preparation

Sequencing

Data analysis

Phytocontrol
Laboratoire d'analyses

Limitations

Must be characterized by DNA

Impossible to know if :

- A fruit juice was altered with water
- Honey was altered with glucose syrup

DNA must be extractable

DNA extraction is very difficult from fatty matrices

- Oils, butter…

DNA must be in good condition

DNA fragment size must be longer than 350bp.

Food processing (cooking, sterilization, purification, …) or food properties (acidity) fragments DNA into small pieces.

Only species in the database will be found.

If a particular species is expected or suspected, one must insure it is present in the database.

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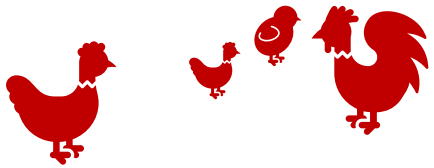
Limitations

× **No geographical origin**

NGS result is a list of species.

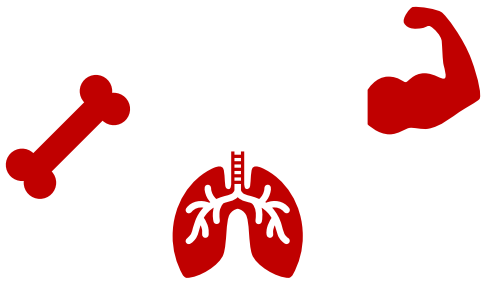
× **No age or « gendre » identification**

Gallus gallus => Chicken, rooster, egg ?
Bos taurus => Cow, beef, bull, veal ?

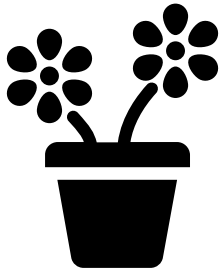


× **No organ or tissu identification**

Muscle, bone, hair, brain, … ?
Flower, root, stem, fruit, leaves, … ?



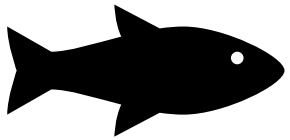
Plant species identification



✓**5088** plant species
- spices & aromatic herbs (origano, basil, cumin, saffron, pepper, …)
- veggies (carrot, leek, garlic, onions, potato, tomato, …)
- Cereals

- Which spices are present in this powder mix ?
- I found a weird looking seed in my cereal bowl. What is it ?
- Which vegetables are present in this soup ?

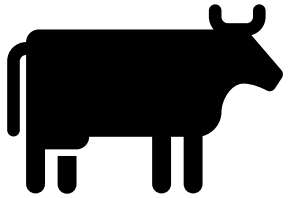
Fish species Identification



- ✓**6615** fish species
  - Tuna
  - Salmon (Atlantic, Pacific, …)
  - Trout, cod panga, …

- What kind of tuna is this fillet ?
- Which fish species was used in this prepared meal ?
- Is this smoked pacific salmon (*Onchorynchus keta*) or smoked atlantic salmon (*Salmo salar*)

Meat species identification



✓**6122** species including mammals, birds, reptiles, ….
- Beef, buffalo, pork, mutton, chicken, turkey, crocodile, …

- Is this « 100% beef minced meat » really 100% beef ?
- I suspect there might be crocodile meat in this imported fish shipment. Is it really so ?
- Has this expensive donkey milk been altered with cheaper cow milk ?
- Is this really mozzarella « di buffala » ?

Phytocontrol
Laboratoire d'analyses

Summary

- End-to-end Workflow
- NGS => untargeted search
- Very large number of species in one test :
  - 5000 plant species
  - 6100 meat species
  - 6600 fish species
- Under constant development

Thank you !