



NGS for routine food fraud analysis

Conférence Authenticité – Nouvelles technologie et solutions

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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

Foods prone to fraud

USP
FOOD FRAUD
DATABASE

USP Adds 800 New Records to Food Fraud Database

40% of oregano tested was adulterated - Forbrugerrådet Tænk

By Joseph James Whitworth

- Olive oil, milk, honey and apple and orange reported to have been adulterated
- May be diluted with water or other substitutes
- Or cheap alternative ingredients used as a substitute for the luxury ingredient

(*study didn't examine breakfast cereals and other products)

Source: Global study in Journal of Food Science

BBC NEWS MAGAZINE

11 February 2013 Last updated at: 12:35

Horsemeat scandal: How often does food fraud happen?

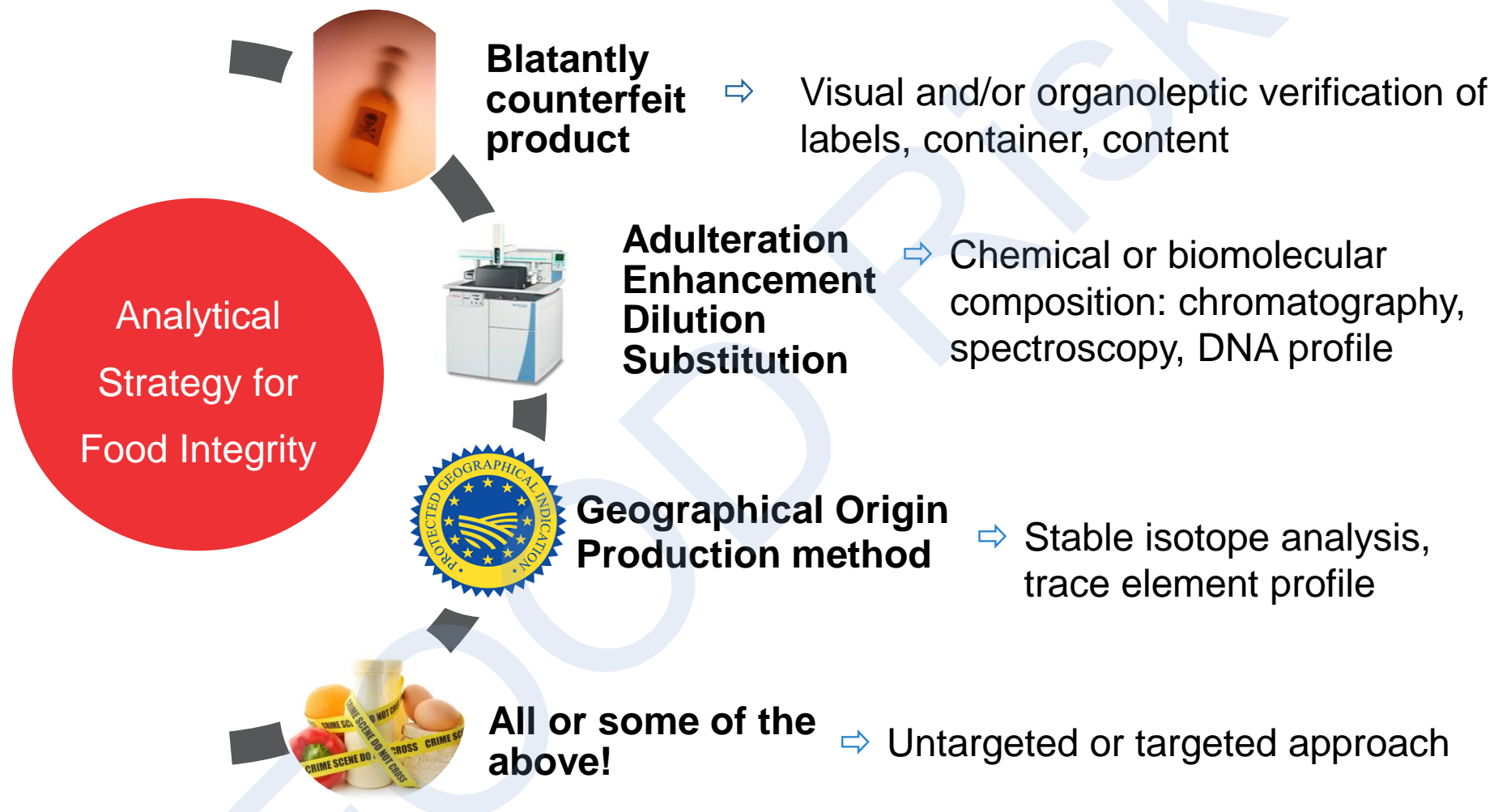
By Tom de Castella and Brian Wheeler
BBC News Magazine

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

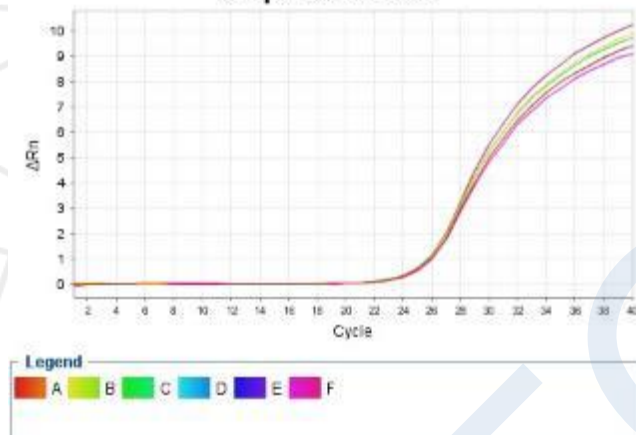


Multi-species Identification Using DNA Fingerprints

Real time PCR



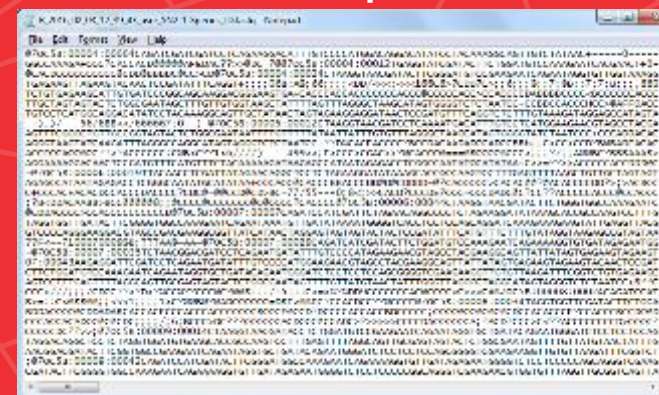
Amplification Plot

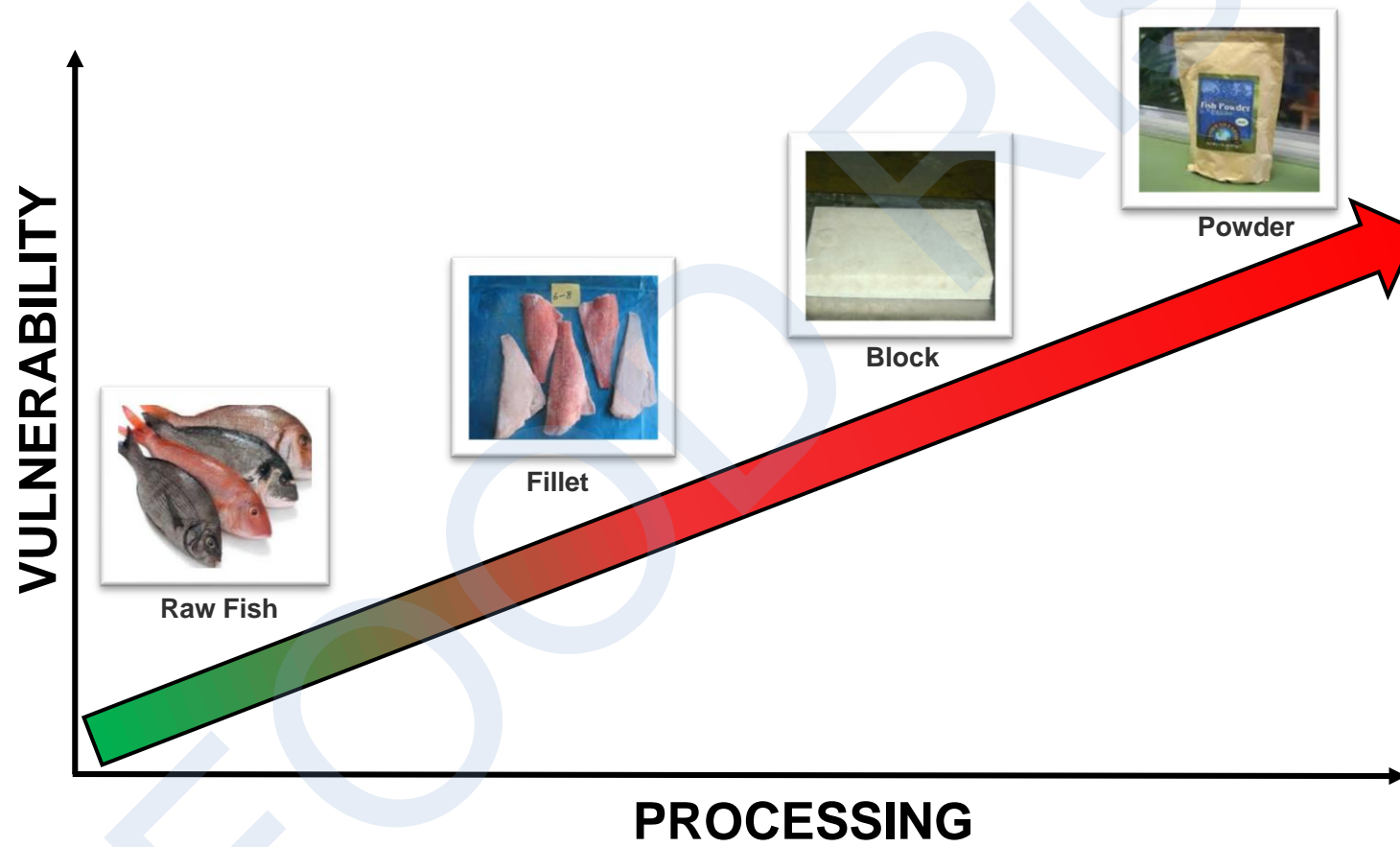


Next Generation Sequencing



Fastq file







Canned meat chili:

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



Canned Tuna:

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

ThermoFisher
S C I E N T I F I C



Validation / Recognition / Standardization



What Reference Method ?

afnor
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

www.iso.org/home/standards_development/list_of_iso_technical_committees/iso_technical_committee.htm?commid=560239

Standards Development > Technical committees > ISO/TC 34 > ISO/TC 34/SC 16

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

Quick links

- Work programme (drafts and new work items of ISO/TC 34/SC 16)
- Business plans
- Working area on ISOTC and Public information folder

Secretariat: ANSI
Secretary: Dr. Richard Cantrill
Chairperson: Dr Michael Sussman until end 2016
ISO Central Secretariat contact: Mme Marie-Noëlle Bourquin
ISO Editorial Programme Manager: Ms Nicola Perou
Creation date: 2008

Number of published ISO standards under the direct responsibility of ISO/TC 34/SC 16 (number includes updates):	15
Participating countries:	25
Observing countries:	10

www.iso.org/home/standards_development/list_of_iso_technical_committees/iso_technical_committee.htm?commid=560239

Standards Development > Technical committees > ISO/TC 34 > ISO/TC 34/SC 16

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

Subcommittees/Working Groups:

Subcommittee/Working Group	Title
ISO/TC 34/SC 16/WG 3	Varietal identification <i>The convenor can be reached through the secretariat</i>
ISO/TC 34/SC 16/WG 4	Plant pathogens <i>The convenor can be reached through the secretariat</i>
ISO/TC 34/SC 16/WG 5	Qualitative methods <i>The convenor can be reached through the secretariat</i>
ISO/TC 34/SC 16/WG 6	Guidance for method submission <i>The convenor can be reached through the secretariat</i>
ISO/TC 34/SC 16/WG 8	Meat speciation



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

NGS standardization

DNA sequencing

Part 2 - Bioinformatics and pipeline validation

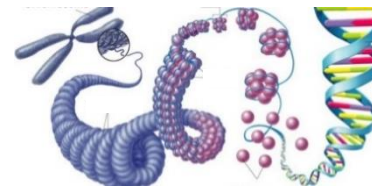
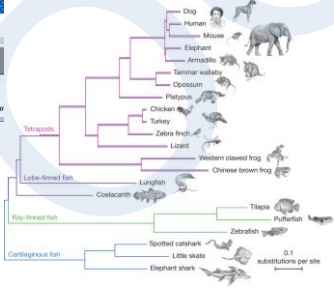
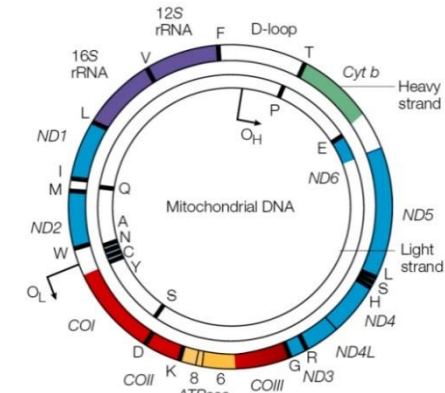
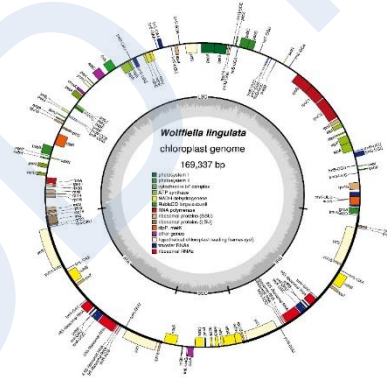
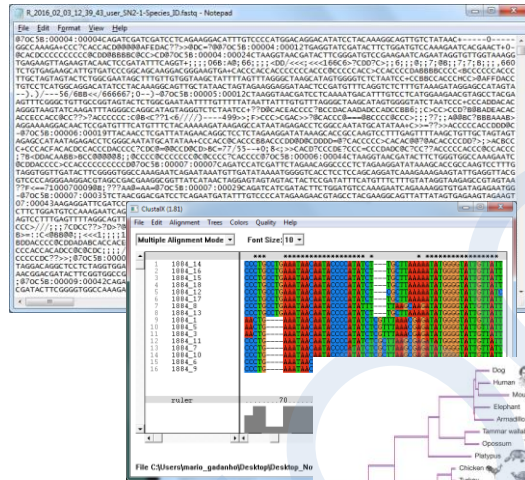


General primers



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

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



Part 3 - Metadata and sequence repository

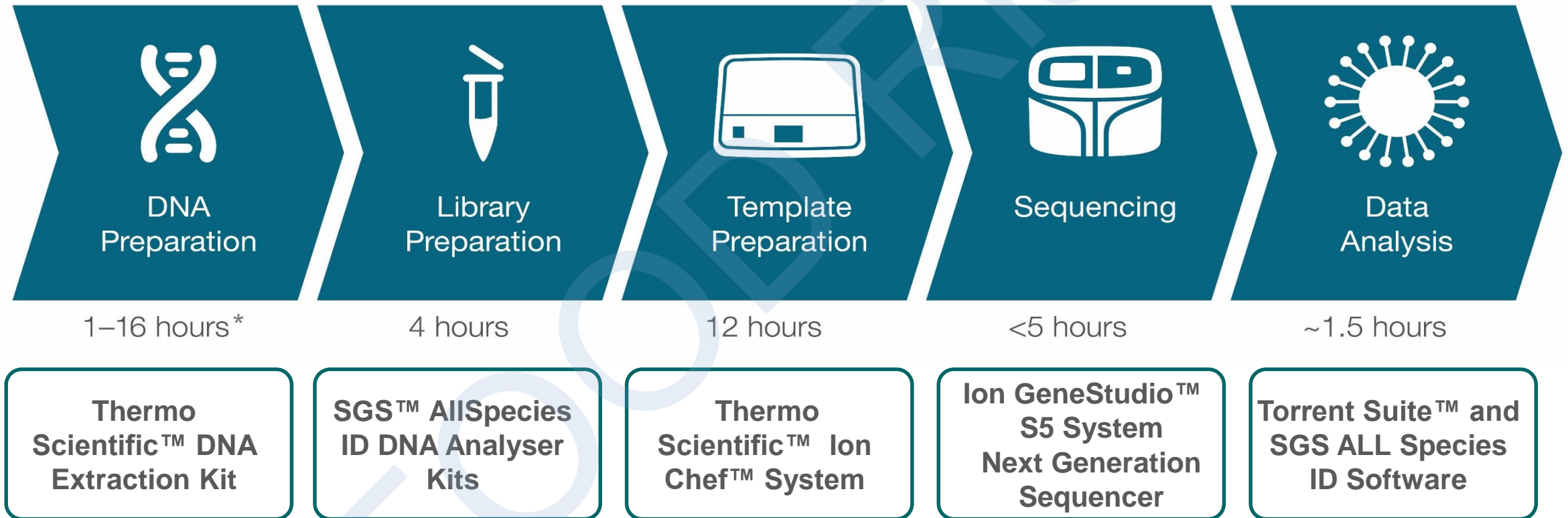
- Reference DNA sequence database used for identification
- Comparison of DNA sequences obtained with reference database

The image displays a workflow for DNA sequence identification. It includes:

- BLAST Interface:** A screenshot of the NCBI BLAST web interface showing search parameters and results.
- SOLiS IDENTIFICATION ENGINE:** A screenshot of the SOLiS web interface showing search results and an identification summary.
- Excel Spreadsheet:** A screenshot of an Excel spreadsheet with columns for 'Barcode', 'Target', 'Barcode', 'Barcode', and 'Barcode'. An 'Extractor' dialog box is overlaid on the spreadsheet, showing a list of barcodes (Barcode 2 through Barcode 9) and a 'Process' button.

Barcode	Target	Barcode	Barcode	Barcode
Barcode 2	Meat	5	Bos taurus	40000
Barcode 3			Bos taurus	30000
Barcode 4			Capra hircus	30000
Barcode 5			Ovis aries	30000
Barcode 6			Capra hircus	20000
Barcode 7			Capra hircus	10000
Barcode 8			Capra hircus	10000
Barcode 9			Capra hircus	10000

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

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

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

FOOD Risk

PCR



NGS



What's in my lasagna ?



Does my lasagna contain beef meat ?



Does my lasagna contain horse meat ?



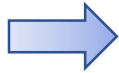
Does my lasagna contain pork meat ?

Targeted
a few (dozen) species

UNtargeted
Several thousands of species



homogenization



Total DNA extraction



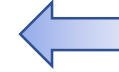
DNA quantification



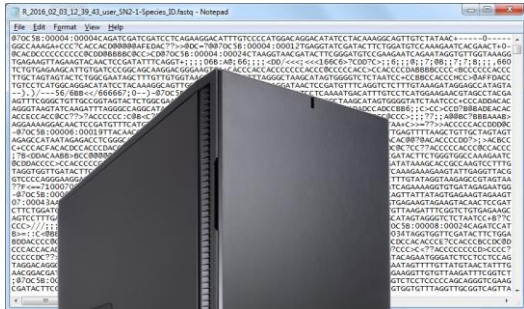
PCR amplification



Library preparation



Sequencing



Data analysis

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.

Limitations

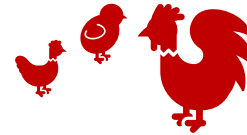
× **No geographical origin**

NGS result is a list of species.



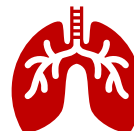
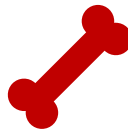
× **No age or « genre » identification**

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

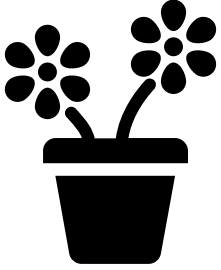


× **No organ or tissu identification**

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



Plant species identification

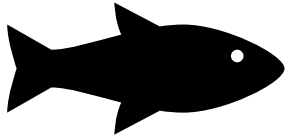


✓ **5088** plant species

- 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

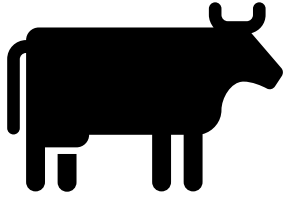


✓ **6615** fish species

- 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 » ?

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 !