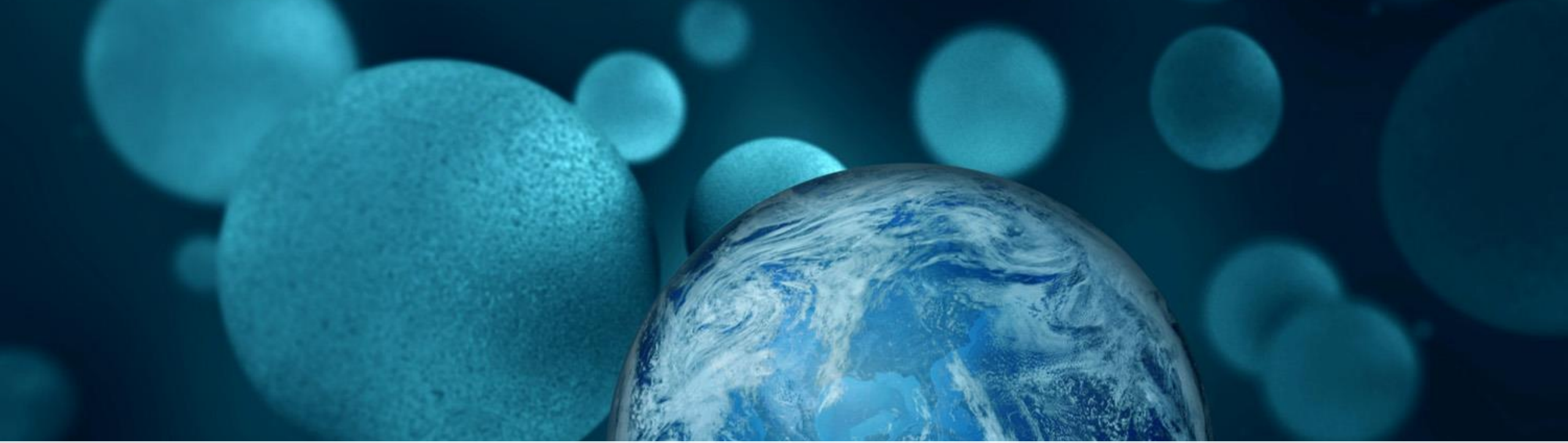

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The growing problem of food fraud: Next Generation Sequencing to detect and identify species

Dra Victoria Moleiro-San Emeterio
Molecular Key Account Manager Iberia

MRAMA, noviembre 2019

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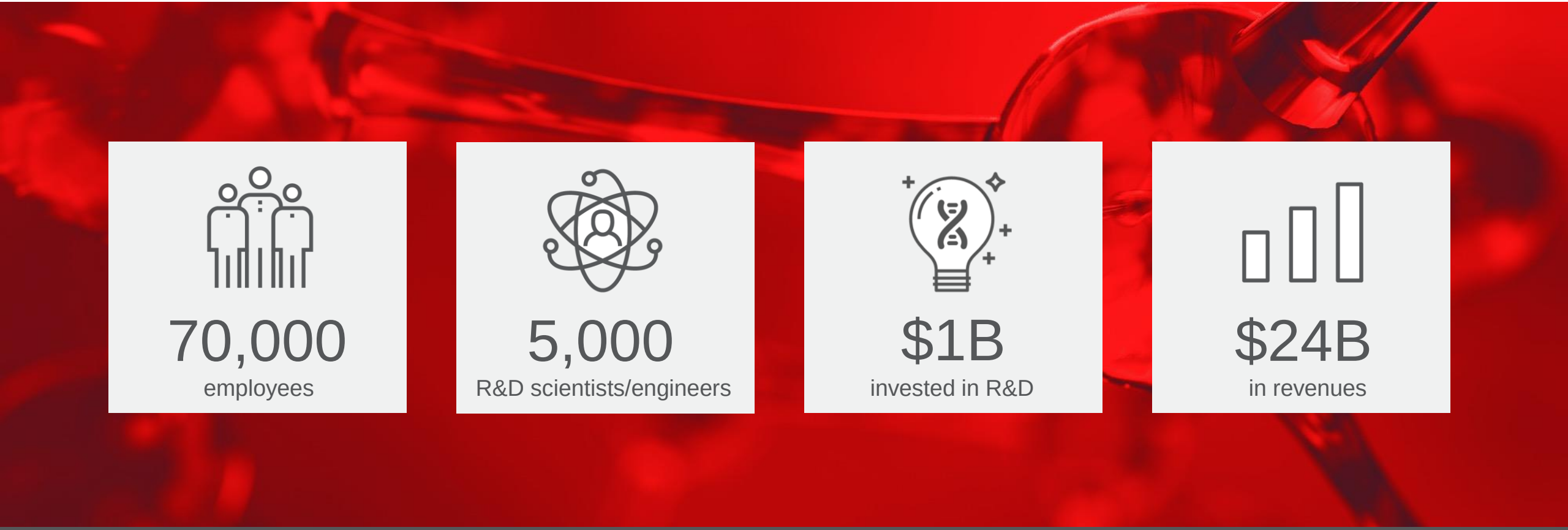
thermo scientific | fisher scientific | invitrogen | applied biosystems | unity lab services



70,000
employees

5,000
R&D scientists/engineers

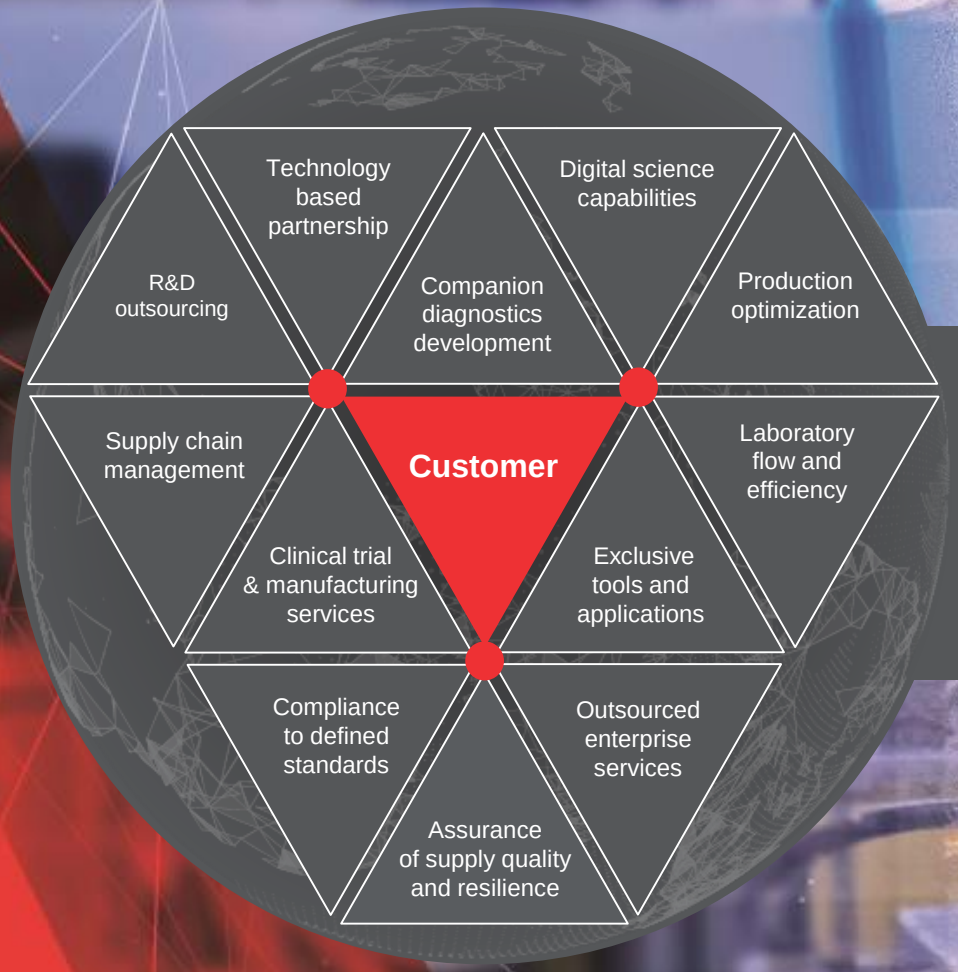
$1B
invested in R&D

$24B
in revenues

The world leader in serving science

Proprietary & Confidential

Our customer value proposition



We help our customers **accelerate innovation** and **enhance productivity**, underpinned by **quality**.

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Unique scale and depth of capabilities

Enable our customers working in…

Healthcare	Life Sciences	Applied Markets	Pharma and Biotech
• Clinical Diagnostics • Immunodiagnostics • Microbiology • Transplant Diagnostics • Healthcare Supplies Channel	• Mass Spectrometry • Electron Microscopy • Molecular & Cellular Biology • Genetic Sciences • Clinical & Next-Generation Sequencing	• Chemical Analysis • Chromatography • Electron Microscopy • Next-Generation Sequencing	• Bioproduction • API • Formulation • Clinical Trials • Manufacturing • Global Logistics, Labeling

Laboratory Equipment, Chemicals and Scientific Supplies Channel

Enterprise-Wide Services and Digital Science Solutions

Innovating Sustainable Solutions from Design to Disposal

Product Ideation Employing green chemistry and green engineering

Product Life Cycle
Taking back, refurbishing, and recycling products and packaging

Using Greener Products
Designing products with the environment in mind

Responsible Sourcing
Increasing materials efficiency and minimizing hazardous chemicals

Eco-Minded Manufacturing
Pursuing energy, water, and materials efficiencies

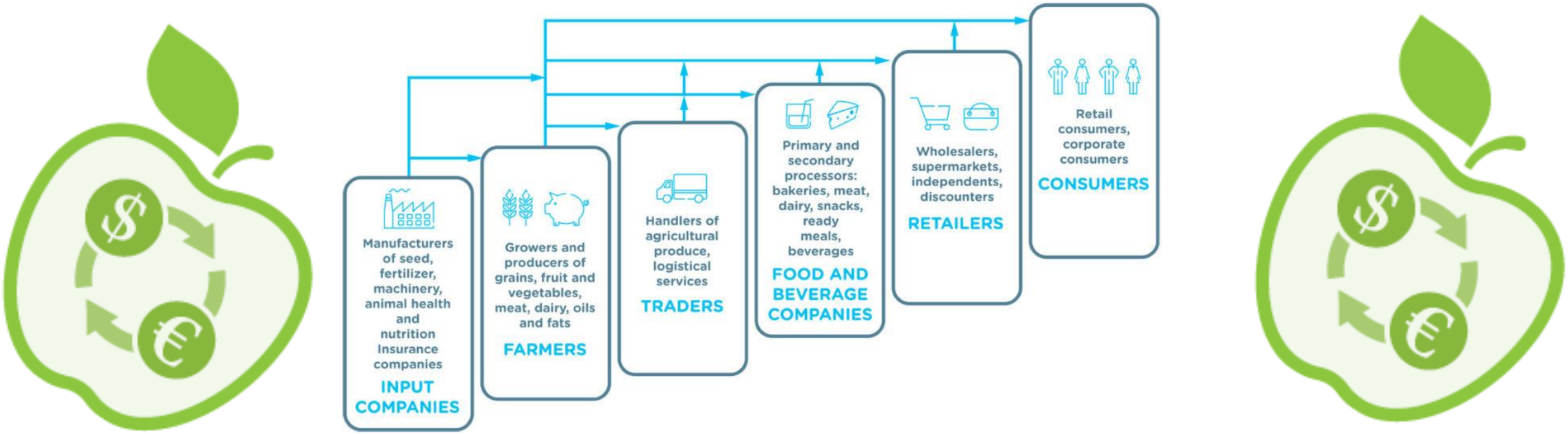
Lower-Impact Packaging and Shipping
Reducing our carbon footprint through redesigned and minimal packaging

thermofisher.com/sustainability

Environmental Sustainability: **One Continuous Pursuit**

Food Fraud

- **Food fraud** is the act of purposely altering, misrepresenting, mislabeling, substituting or tampering with any food product at any point along the farm–to–table food supply–chain.



- Fraud can occur in the raw material, in an ingredient, in the final product or in the food's packaging

http://www.myfoodtrust.com/2018/02/12/food-fraud-whats-really-in-your-food/

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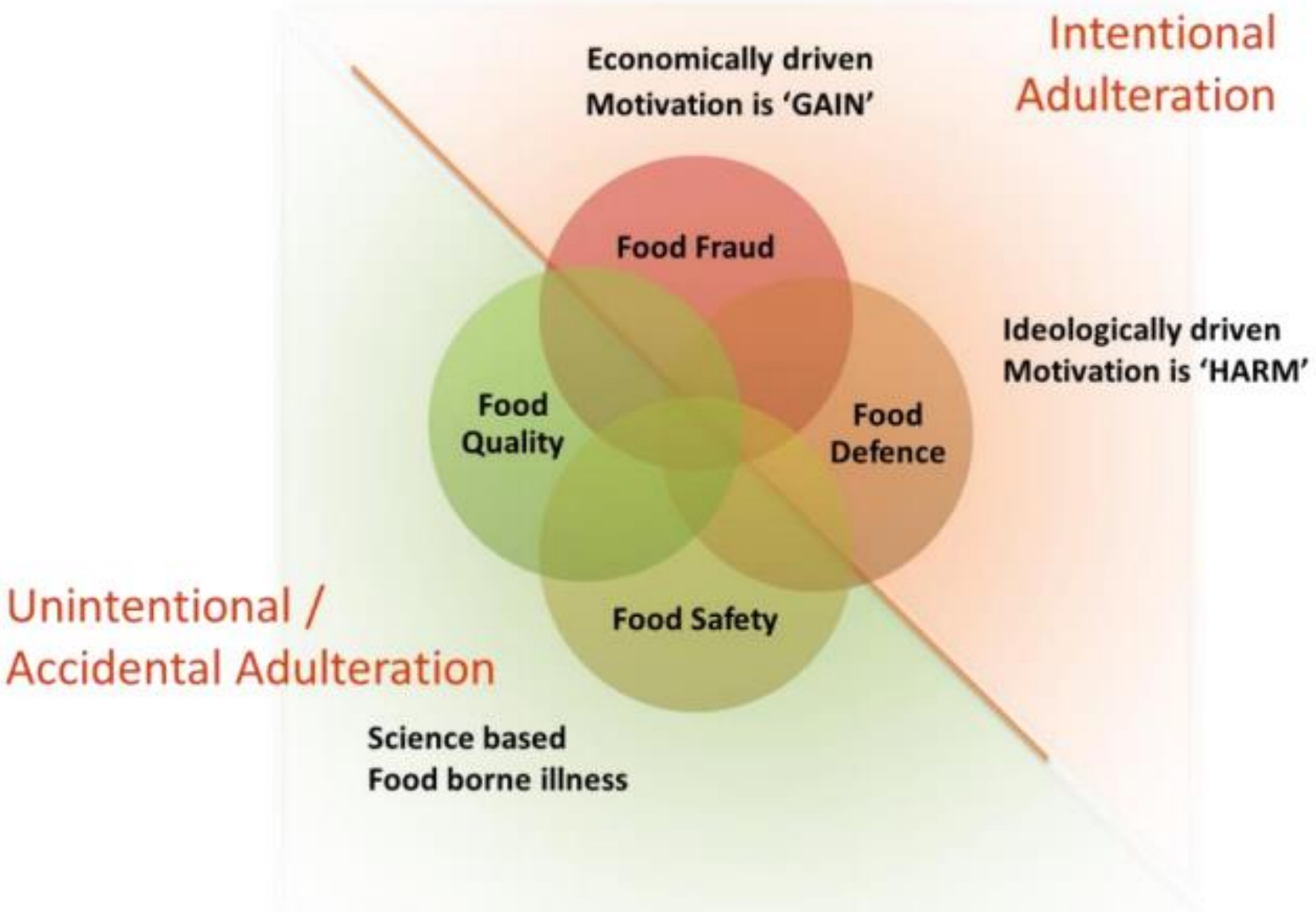
Food Risks



https://www.mygfsi.com/files/Technical_Documents/Food_Fraud_Position_Paper.pdf

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Evolution Food Fraud in Europe

https://www.focos-food.com/the-evolution-of-food-fraud-in-europe/

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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, (economic losses) 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

[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.

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Why Care About Food Authenticity and Fraud?

RTL

EU records significant jump in cross-border food fraud cases in 2018

https://www.foodnavigator.com/Article/2019/04/15/EU-records-significant-jump-in-cross-border-food-fraud-cases-in-2018

RELATED TAGS: Food fraud, Tuna, fipronil

The number of cases reported to the EU Food Fraud Network rose significantly last year. Requests from one European country to cooperate with others on food fraud cases rose to 234 in 2018, compared to 178 in 2017 and 157 in 2016, according to its annual report.

EUROPOL ABOUT EUROPOL | ACTIVITIES & SERVICES | CRIME AREAS & TRENDS | PARTNERS & AGREEMENTS | CAREERS & PROCUREMENT

HOME › NEWSROOM › FRAUD ON A PLATE: OVER 3 600 TONNES OF DANGEROUS FOOD REMOVED FROM CONSUMER MARKET

FRAUD ON A PLATE: OVER 3 600 TONNES OF

od-removed-consumer-market

nuts and almonds in hazelnut products imported to sufferers. When 1 300 kg of roasted chopped uts. In 500 kg of hazelnut paste, up to 45% of mixed monds could be determined. Due to the fact that these a potential health risk for allergy sufferers was given

-seized-in-global-opson-operation-targeting-food-fraud

Experts from Europol's Intellectual Property Crime Coordinated Coalition - IPC3 supported the Italian NAS Carabinieri and the Tribunal of Darmstadt in Germany, in the arrest of 20 individuals and the seizure of 150 000 litres of fake olive oil. The criminals, who raked in up to € 8 million every year in criminal profit, modified the colour of low quality oils to sell them on the Italian and German markets as extra virgin olive oil.

newsroom/news/150-000-litres-of-fake-extra-virgin-olive-oil-seized-%E2%80%98well-oiled%E2%80%99-gang

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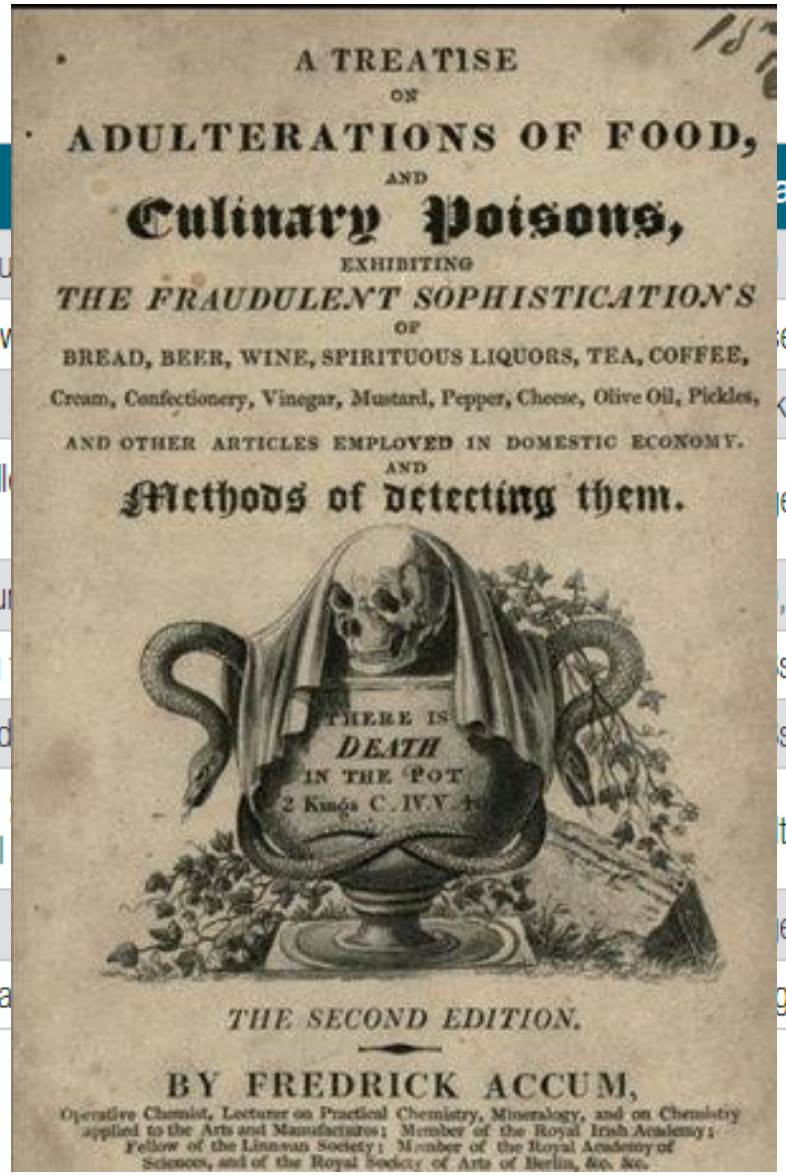
High profile food and beverage scandals

Product	Adulteration	ancial & Health Effects
Olive oil	Industrial oil denatu	deaths reported in Spain
Orange juice	Beet sugar syrup, v	ecutions of juice suppliers
Wine - Austria	Ethylene glycol	ket recall – huge damage to Austrian wine industry
Chili spices - Asia	Sudan and other ill processed foods	gest supermarket recall ever in UK costing £millions
Milk powder - China	Melamine & cyanu	,000 victims, 6 infant deaths & 54,000 babies hospitalized
Animal feed - Ireland	Adulteration of pig	s and dioxins in pork – Estimated €200 million financial losses
Animal feed - Germany	Adulteration of feed	s and dioxins in meat – restrictions on 5000 farms
Sports & tea drinks - Taiwan	Phthalates (DEHP) to replace palm oil	th effects unknown but exposure above TDI for up to 15 years
Meat & meat products - Europe	Horse meat	e scale food recalls, RASFF alerts and prosecutions of processors
Cumin spice – India, Turkey	Ground peanut & a	gerous to allergen suffers but no individuals identified



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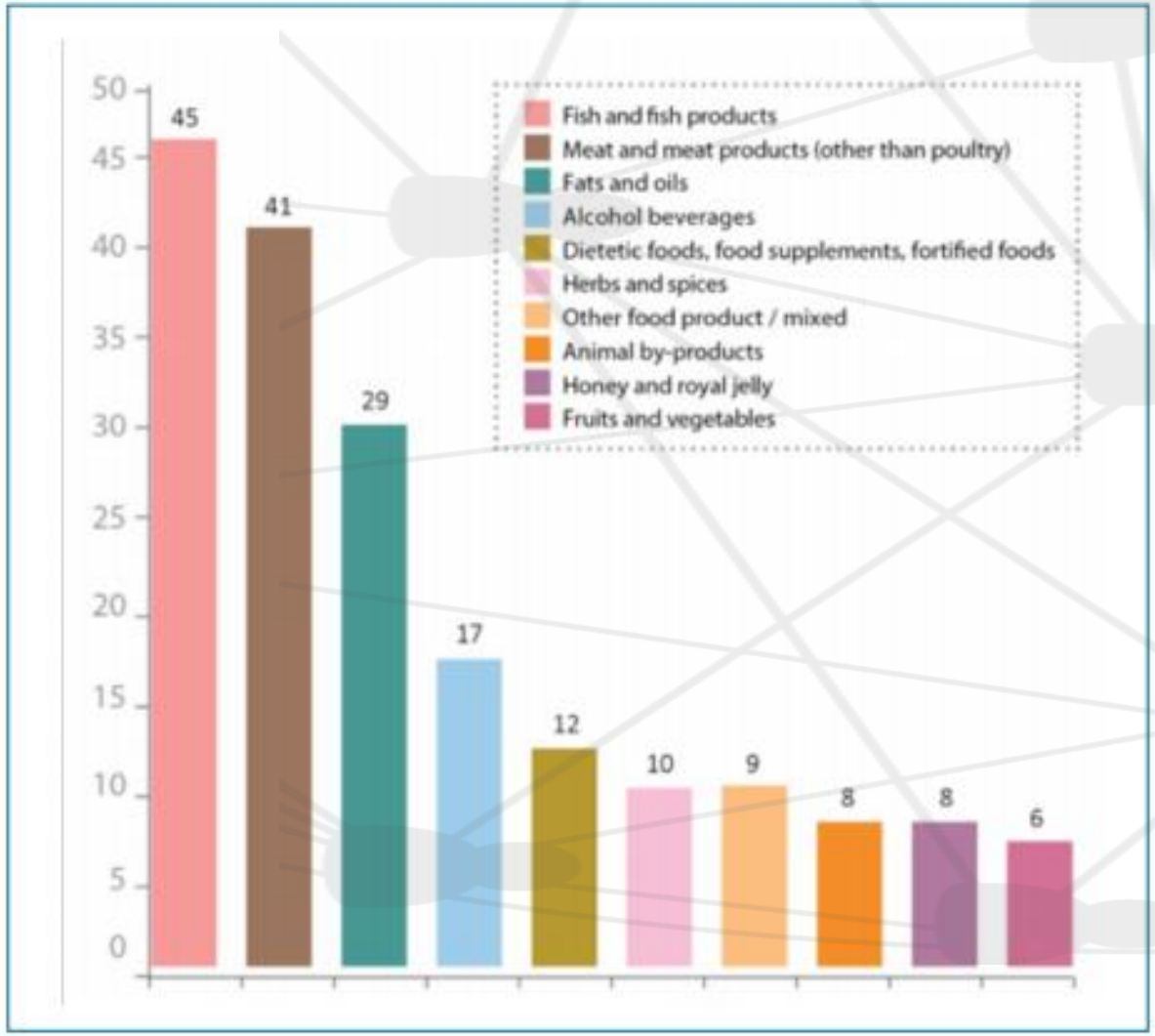
The top 10 product categories (number of requests) in the AAC-FF in 2018



https://ec.europa.eu/food/sites/food/files/safety/docs/food-fraud_network_activity_report_2018.pdf

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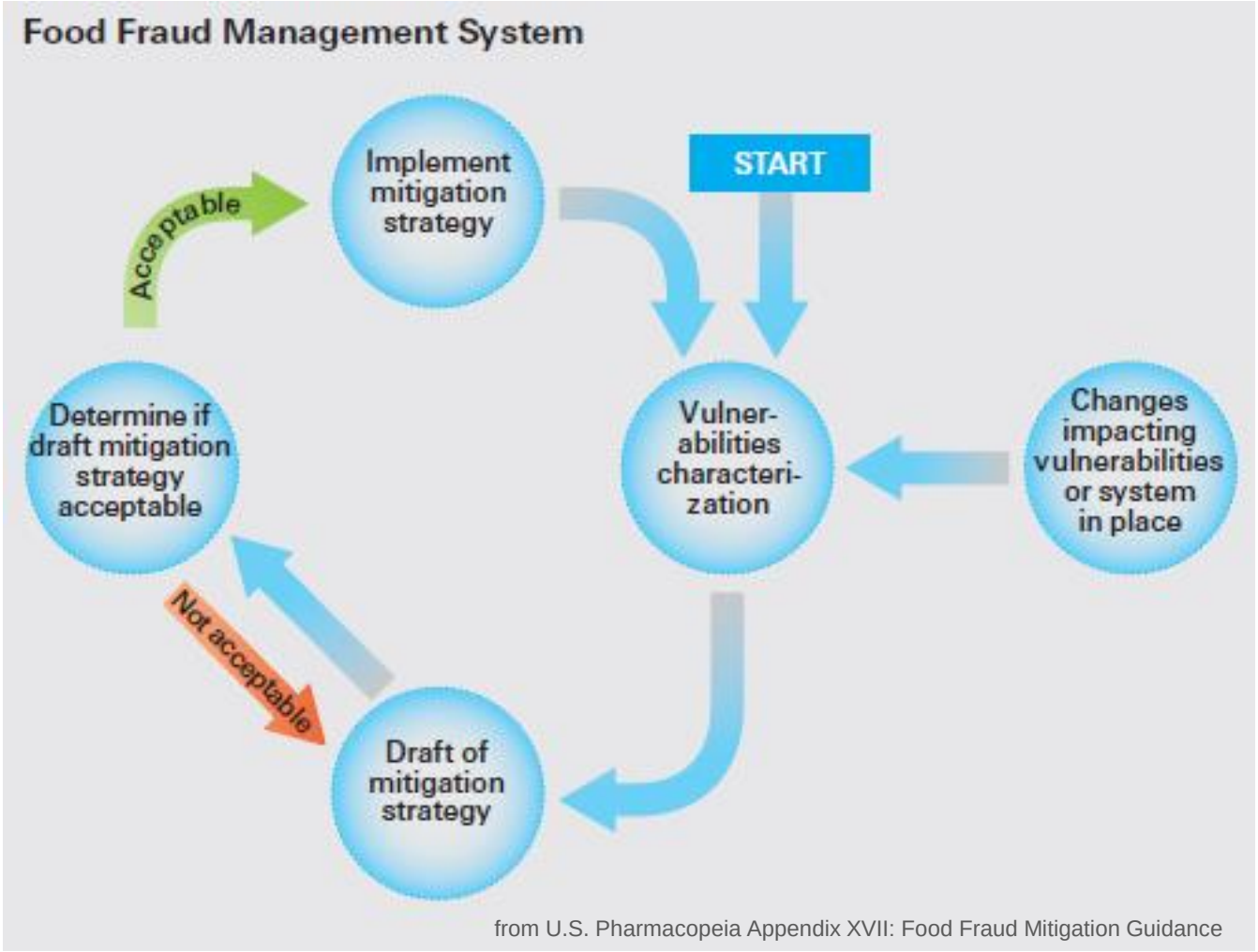
How Can a Business Protect Itself Against Food Fraud?

Continuous process:

- Evaluation to characterize food fraud vulnerabilities
- Design & review of a mitigation strategy
- Implementation & testing
- Regular review & update, particularly as changes are introduced, e.g. new supplier



from U.S. Pharmacopeia Appendix XVII: Food Fraud Mitigation Guidance

Excerpt from: https://www.nestle.com/asset-library/documents/library/documents/suppliers/food-fraud-prevention.pdf

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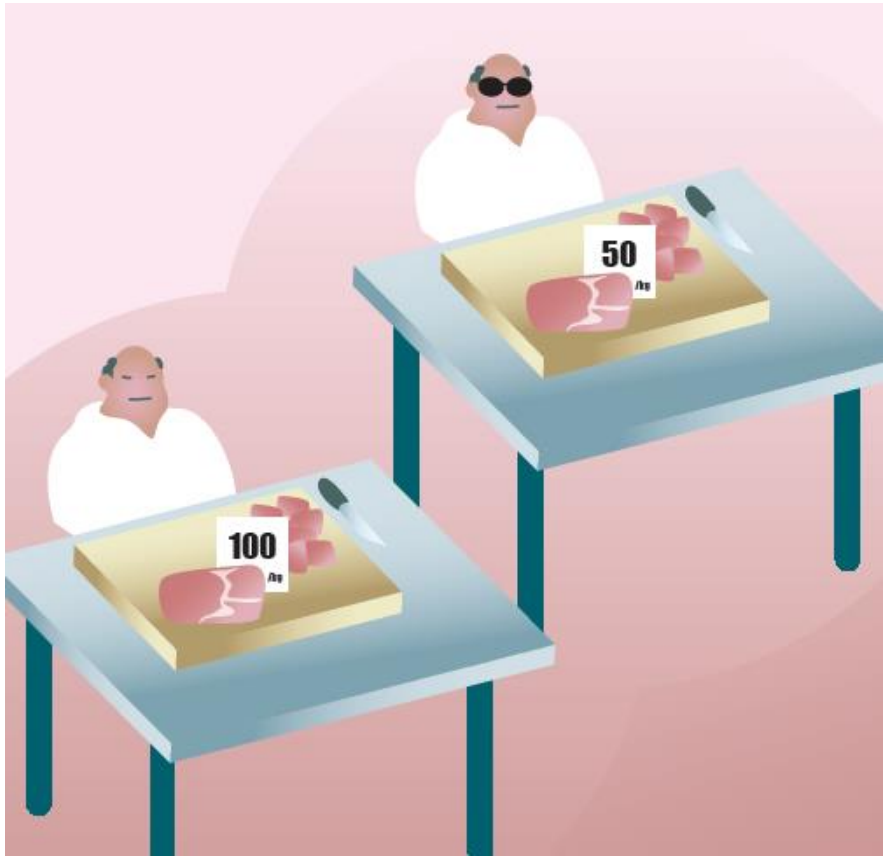
Vulnerability Assessment

- Know your materials & risks
- Know your suppliers
- Know your supply chain
- Know your existing control measures



If the price of a valuable food seems too good to be true it probably is!

Excerpt from: https://www.nestle.com/asset-library/documents/library/documents/suppliers/food-fraud-prevention.pdf

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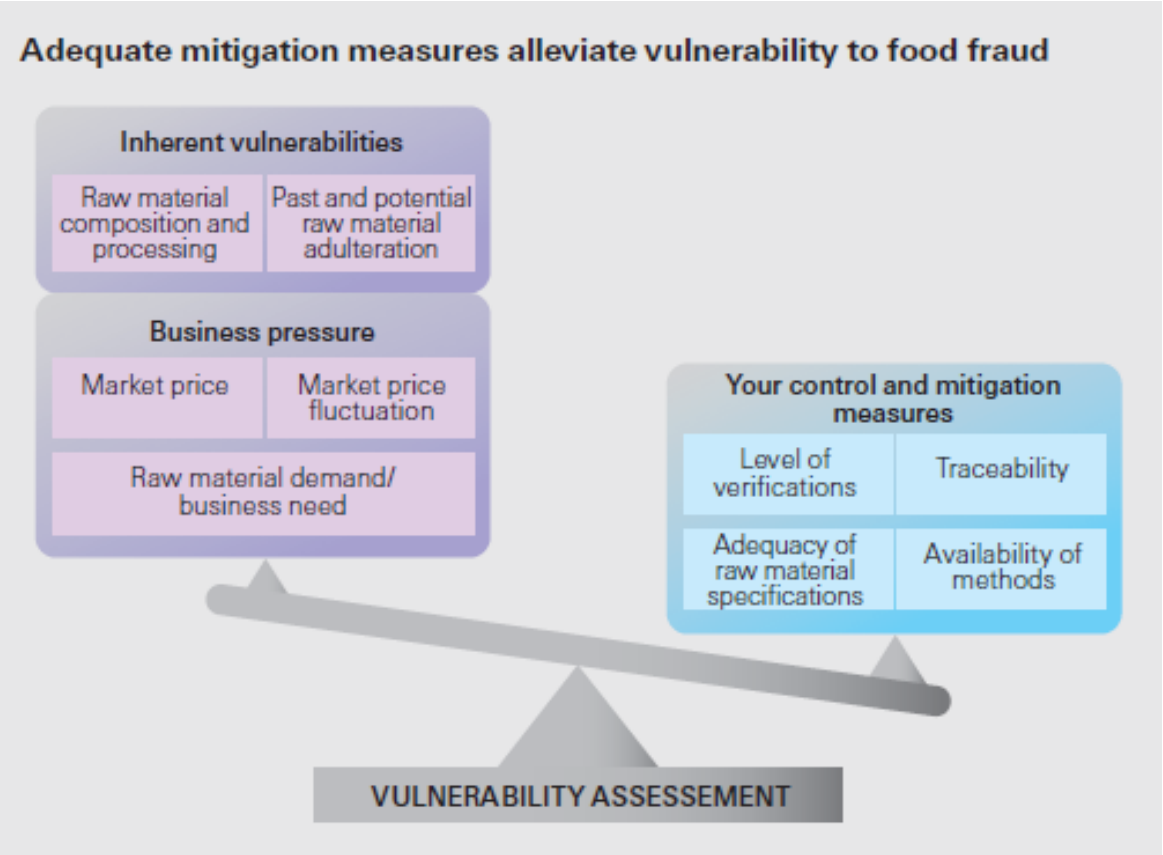
Mitigation Against Food Fraud

- Raw material specifications
- Supplier relationship
- Supplier audit
- Supply chain transparency and simplification
- Alert system
- Analytical surveillance



Excerpt from: https://www.nestle.com/asset-library/documents/library/documents/suppliers/food-fraud-prevention.pdf

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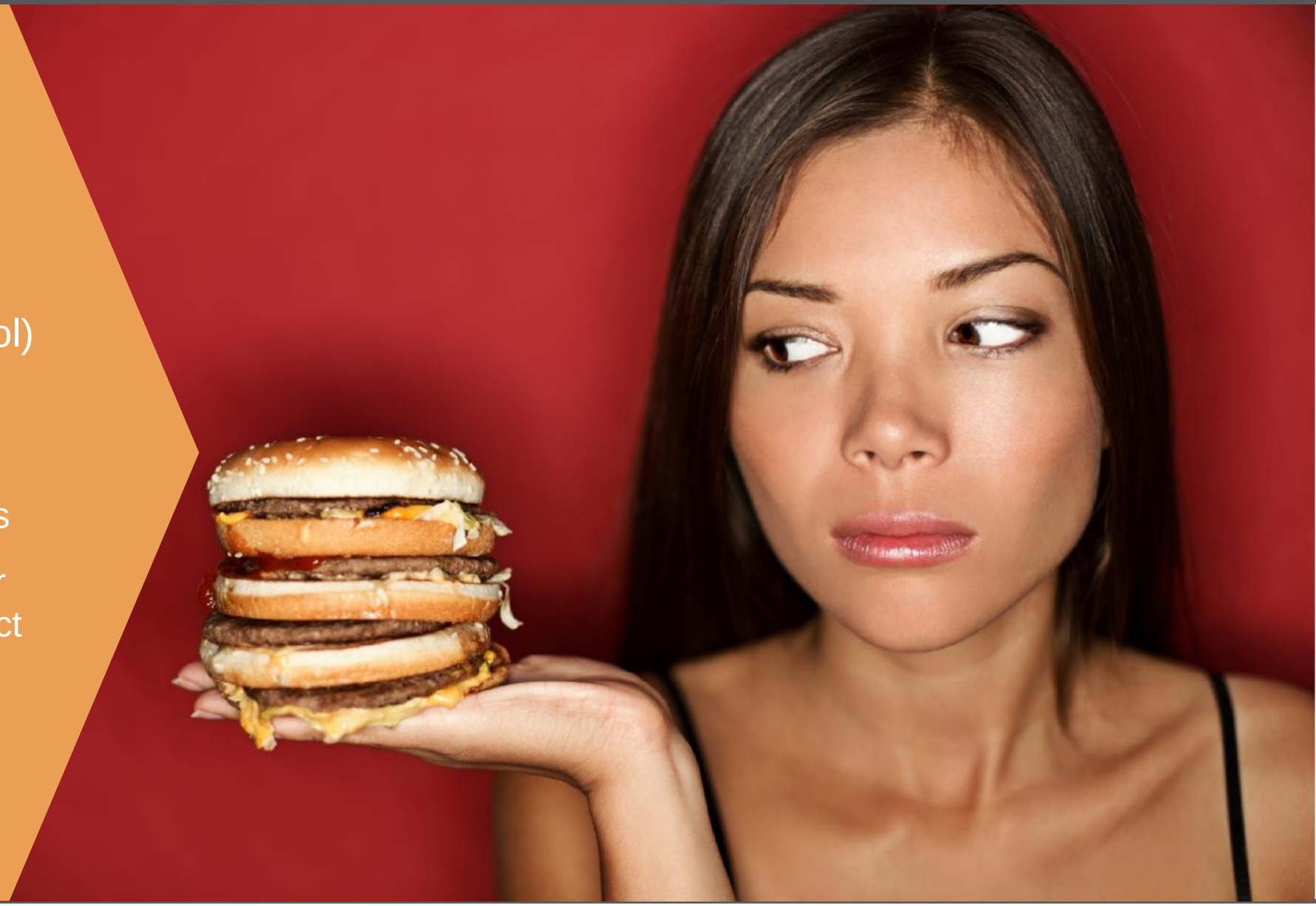
What is Food Authenticity Testing?

Testing For:

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



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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

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Food solutions: Comprehensive solutions to protect our food supply

Gas Chromatography

Ion Chromatography

Liquid Chromatography

Molecular Spectroscopy

Isotopic and Elemental Analysis

Next Generation Sequencing and RTPCR Molecular Analysis

Maintain Safety Standards

Detect Contamination

Confirm Authenticity

Determine Purity

Ensure Quality

Helping our customers to deliver healthier and safer food products

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SCENE DO NOT CROSS
CRIME SCENE DO NOT CR
SCENE DO NOT CROSS
CRIME SCENE DO NOT

Chemical fingerprints

- Materials have a fingerprint, a unique chemical signature that allows the sample to be identified
- To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) is used to measure stable isotopes and identify the isotope fingerprint of a material or product
- IRMS traces carbon, nitrogen, sulfur, oxygen, and hydrogen isotopes by detecting their natural variations, which can reveal the origin and history of samples.

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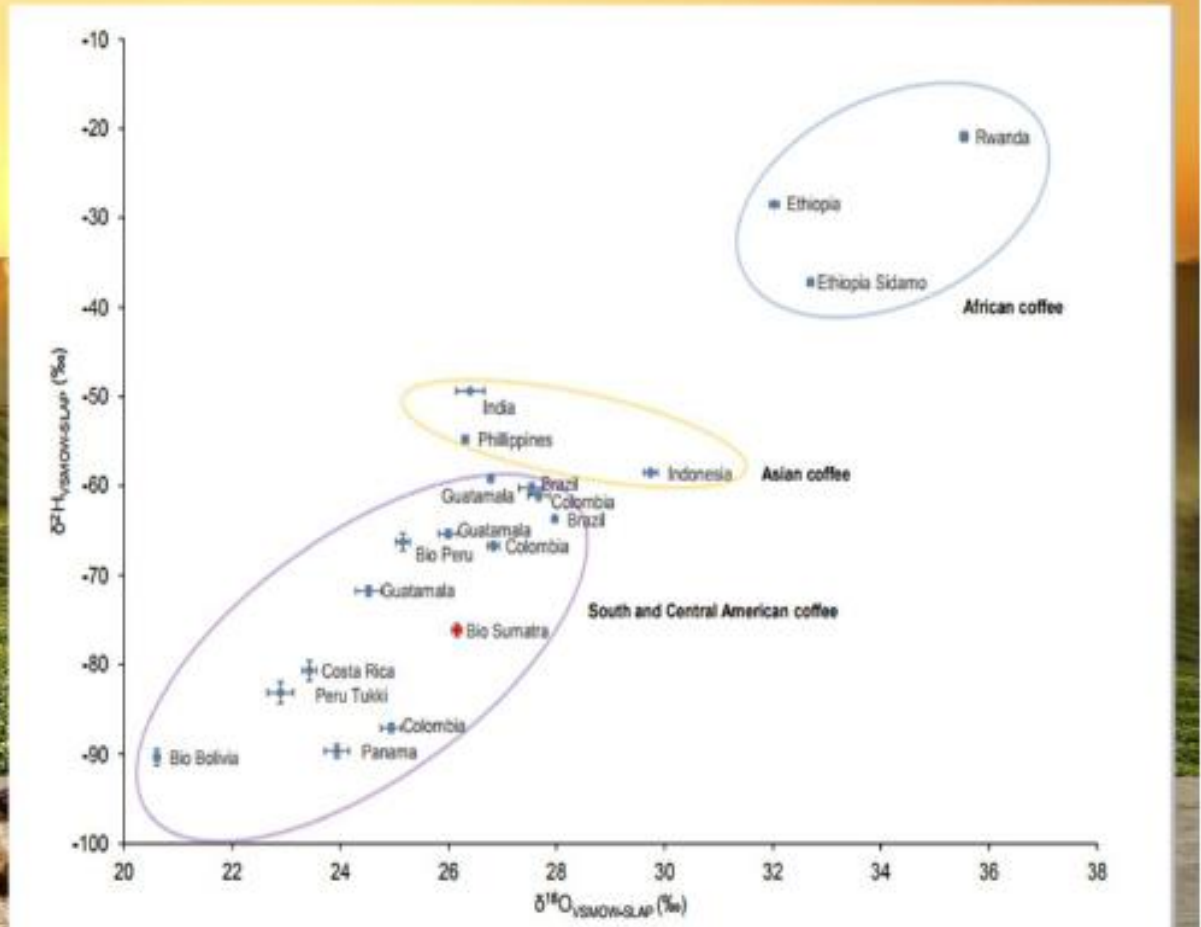
Tracing the geographical origin of coffee by IRMS

- Hydrogen and oxygen isotope fingerprints
- The *Coffea* species plants, cultivated as the source of the coffee beans, carry an isotopic fingerprint associated with local-regional rainfall
- Differentiation of American, Asian and African coffee beans (green and roasted)
- Identification of mislabeled coffee



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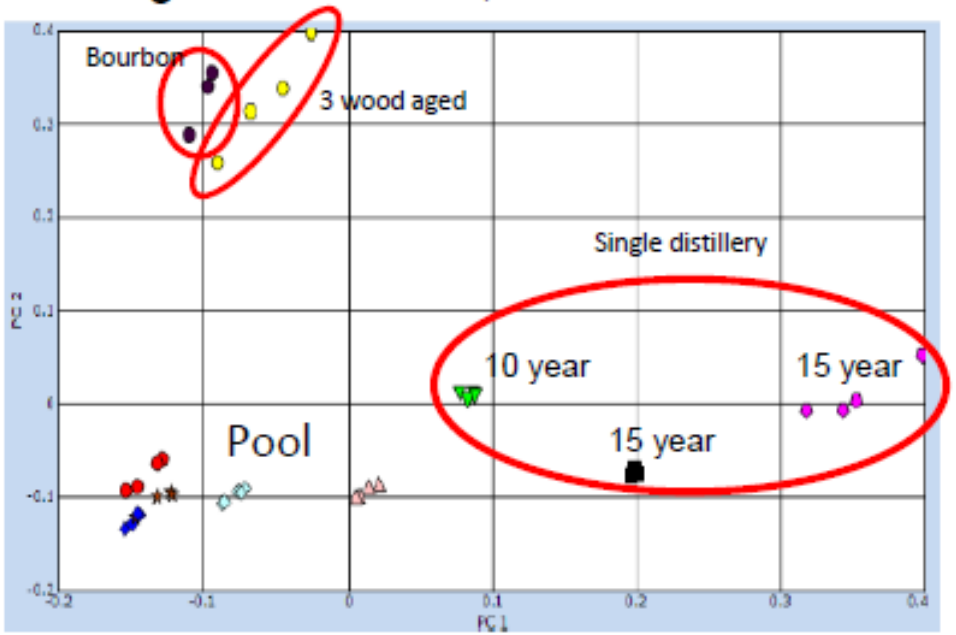
Whisky authenticity by GC-HRAM

- Study to determine the chemical differences between whisky samples?
  - Type of Whisky: Bourbon or Scotch Whisky
  - Geographical Origin: USA or Scotland, Highland or Lowland
  - Age of Whisky: 10 or 15 year aging

- Approach: A non-targeted (screening) analysis and statistical software tools (including NIST libraries, deconvolution software, elemental composition and fragment matching software)



- Bourbon and 3 wood aged clearly different from other whiskies
- Single distillery whiskies also show clear differences

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DNA fingerprints

- To visualize DNA fingerprints, molecular detection can be used, e.g. real-time PCR (qPCR) and Next Generation Sequencing (NGS)
- DNA fingerprints can provide a unique insight into food integrity investigations around applications of authenticity, adulteration and mislabeling for brand and consumer protection
- The most common method to verify species substitution and animal species identification and quantitation is real-time PCR
- NGS is a high-throughput methodology that enables rapid sequencing of the base pairs in DNA samples. Supporting a broad range of applications, including microbial profiling, food authenticity and traceability, detection of epigenetic changes, and molecular analysis.

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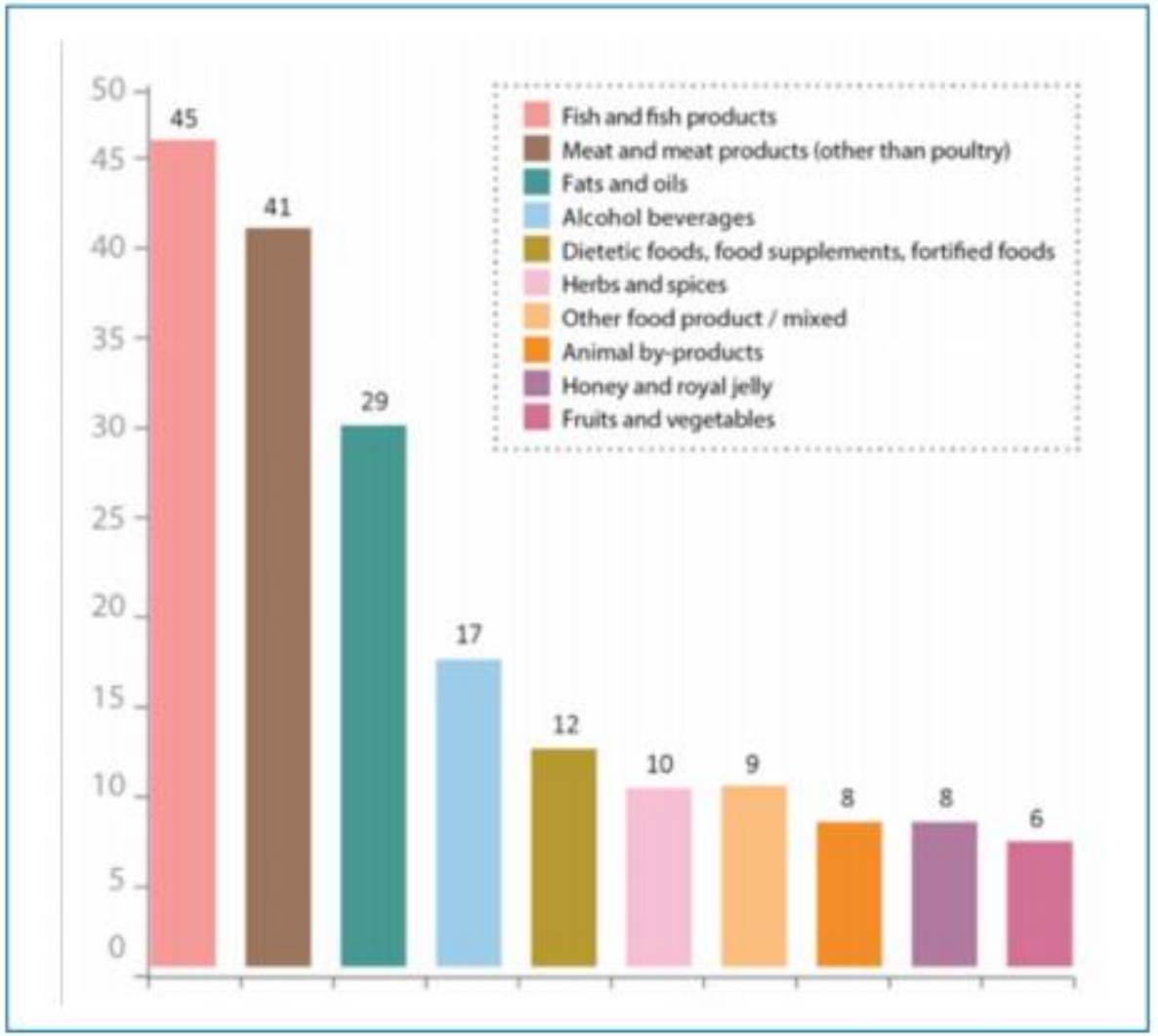
Examples of regulations related to authenticity and adulteration

Area regulated	Scope	Regulations
Adulteration - general	Prevention of adulteration	Regulation 178/2002
Authenticity - general	Fraudulent & deceptive practices	
Intentional adulteration	Prevent terrorists acts of food adulteration & requires mitigation	FSMA 2011 (US)
Melamine adulteration	Limits of 2.5 ppm in food & 1.0 ppm in infant formula	Regulation 594/2002
Authorized food colors	Prevention of use of illegal dyes	Directive 94/36/EC
Emergency measures for illegal dyes in imported foods	Provision for import controls of chilli, chilli products, curcuma & palm oil	Decision 2005/402/EC
Food Labeling	Prevention of misleading information on food labels	Regulation 1169/2011
Olive oil – vertical regulation	Compositional and labeling standards for olive oils	Regulation 1019/2002
Wine – vertical regulation	Organization of agricultural markets including wine	Regulation 1308/2013
Wine vinegar – vertical regulation	Compositional and labeling standards for wine vinegar	Regulation 479/2008
Agricultural products	Protected designations of origin Establishes PDO Establishes TSG	Regulation 607/2009, Regulation 510/2006, Regulation 509/2006
GM foods	Labelled as GM if more than 0.9%	Regulation 1829/2003
Organic foods	Labeling of organic foods	Regulation 834/2007
Traceability	Traceability must be established at all stages of food production	Regulation 178/2002

The Food Fraud Iceberg

Horse dressed like a beef…



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Top Foods & Ingredients for Fraud

1 in 5 Seafood Samples Mislabeled Worldwide, Finds New Oceana Report

Oceana Calls on President Obama to Track All Seafood from Boat to Plate as Ocean Leaders Prepare to Gather in DC for Our Ocean Conference

Press Release Date
Wednesday, September 7, 2016
Location: Washington

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Seafood Fraud was Investigated in 55 Countries

file:///C:/Users/epscshs/Downloads/https___usa.oceana.org_sites_default_files_global_fraud_report_final_low-res.pdf

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Food Fraud: Fish

ETIQUETADO

CARACTERÍSTICAS GENERALES

SIGNOS DE FRESCURA

http://www.comunidad.madrid/servicios/salud/fraude-alimentario

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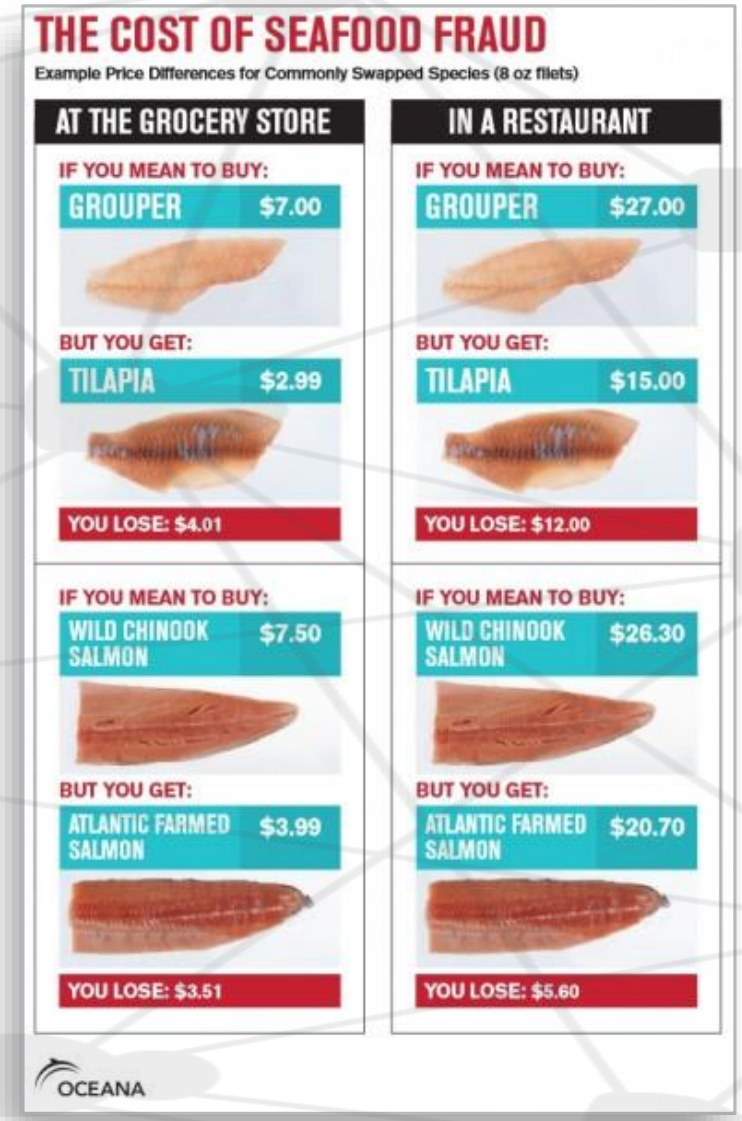
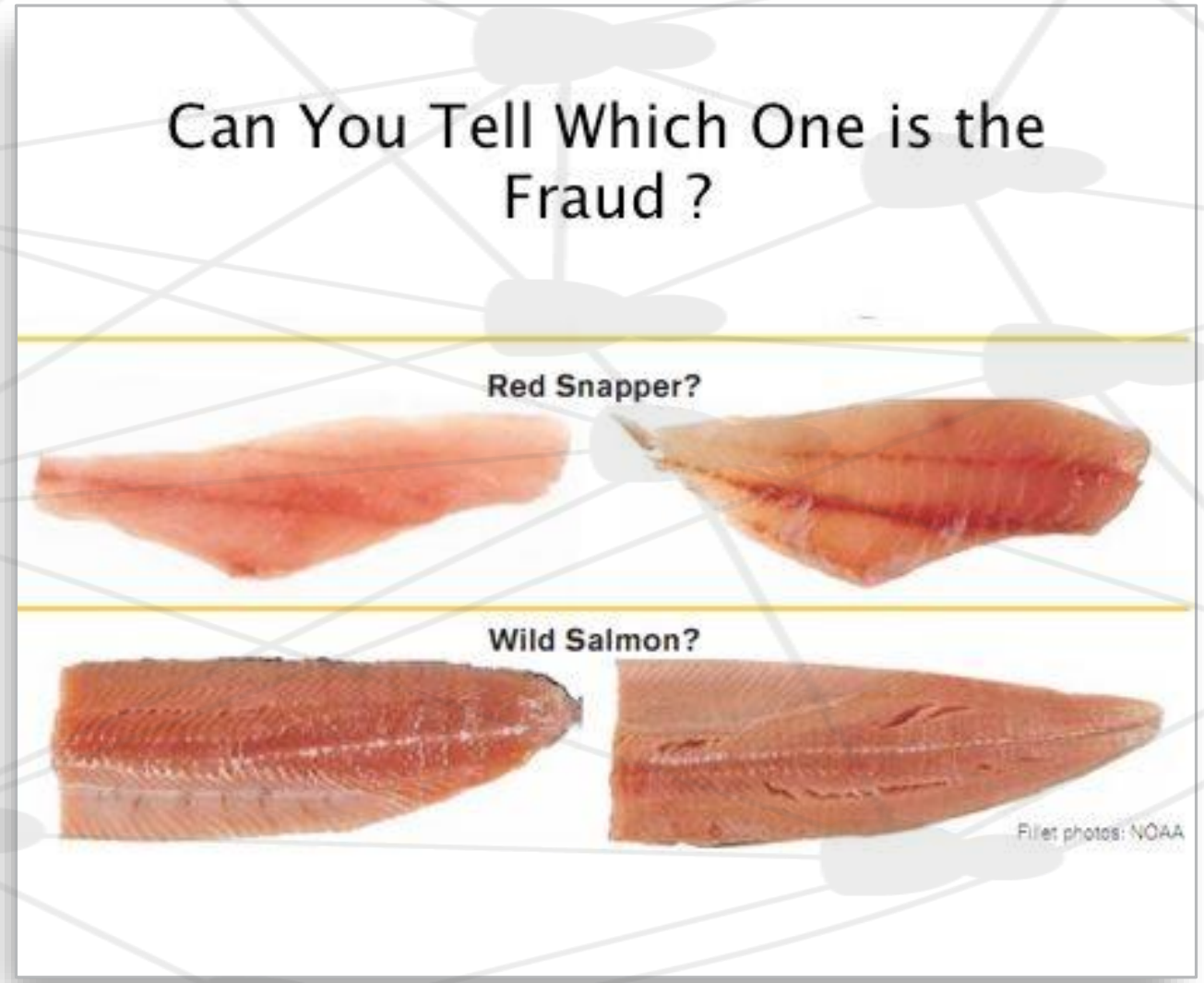
Food Fraud: Fish



https://www.foodsafetynews.com/2013/08/americans-are-getting-ripped-off-by-fish-fraud-oceana-study-finds/



https://www.treehugger.com/green-food/embait-and-switchem-report-reveals-rampant-seafood-fraud.html

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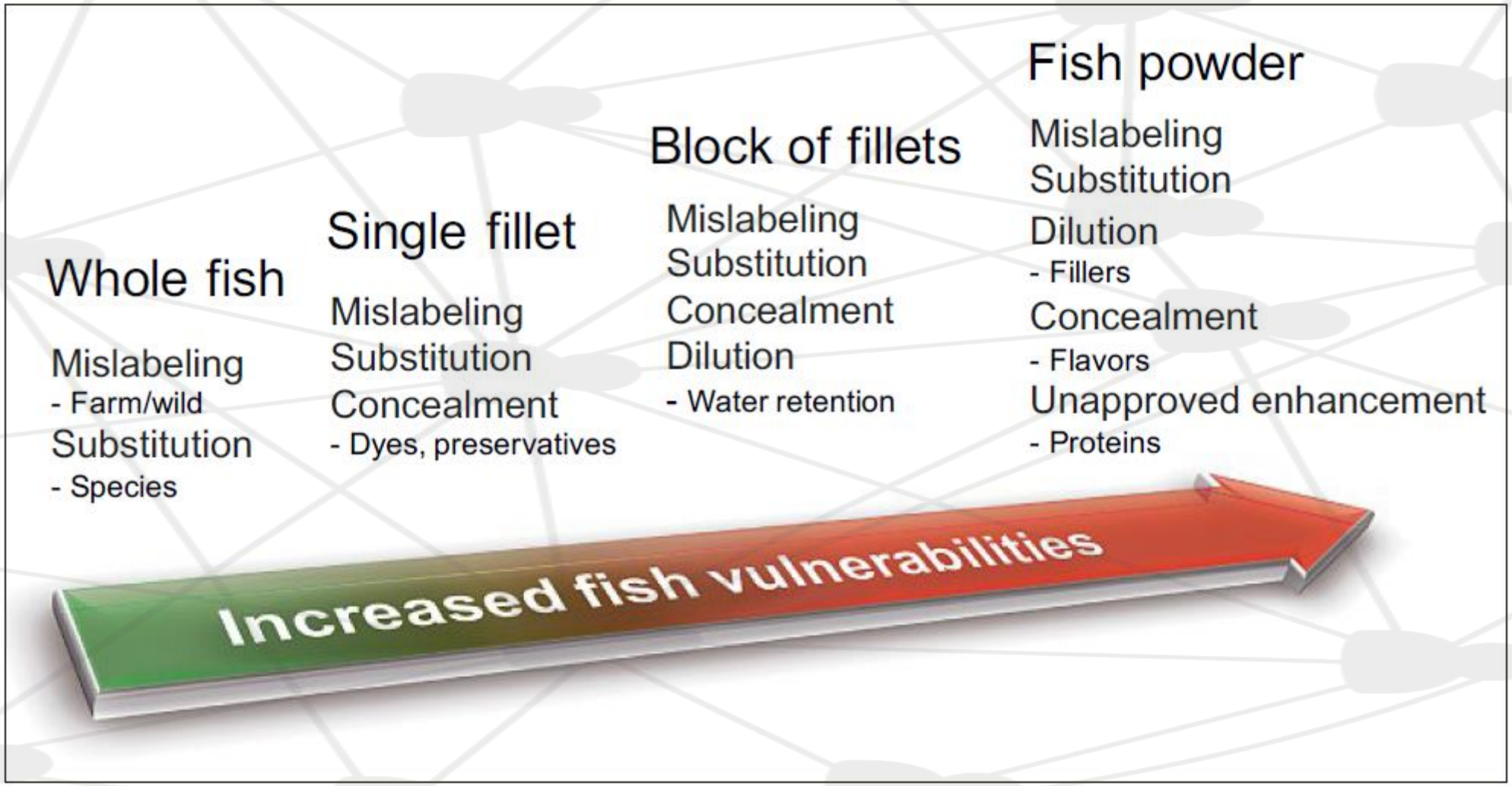
Analysis of the inherent fish vulnerabilities to adulteration



Food Adulteration: from Vulnerability Assesment to New Analytical Solutions. C Cavin et al. CHIMIA 2016, 70, No. 5. pag 330-332

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Food Fraud: Meat

French burger fraud is ‘chapter 2’ of the horsemeat scandal, campaigners warn

By Katy Askew

10-Jun-2019 - Last updated on 10-Jun-2019 at 12:23 GMT

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you think twice b

-https://www.foodnavigator.com/Article/2019/06/10/French-burger-fraud-is-chapter-2-of-the-horsemeat-scandal-campaigners-warn

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Food Fraud: Spices

Food fraud tests reveal 25% of dried oregano is adulterated

23 Jul 2015 | Andrew Don

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Share 1

RELATED ARTICLES

Scotland to tackle food fraud with dedicated crime unit

Authentic peppercorns

Adulterated peppercorns
25% Papaya Seeds

https://www.fdli.org/wp-content/uploads/2017/09/food-ad-food-fraud.pdf

BUSINESS CONSUMER AFFAIRS

Food fraud: Popular oregano brands selling adulterated products

By Esther Han

Updated 7 April 2016 –

- Latest consumer news

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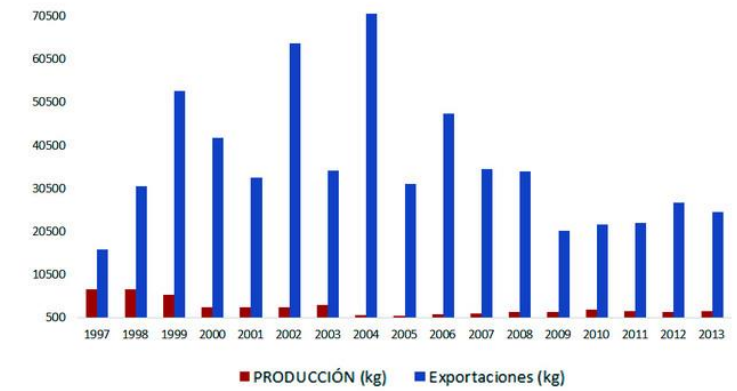
Food Fraud: Saffron

- **Don’t overpay for dyed onion or marigold powder masquerading as super - expensive saffron**



https://www.agenciasinc.es/Noticias/La-huella-quimica-confirma-el-fraude-del-azafran

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Food Fraud: Cinnamon

- Unless it says “Ceylon” on the label, your cinnamon is probably Cassia; most of the cinnamon sold in the United States is. Worse still, your “cinnamon” may actaully be ground coffee husks.

https://www.thedailymeal.com/cook/7-ingredients-arent-what-you-think-slideshow/slide-6

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PCR

PCR testing is targeted or relies on a subset of targets

PCR testing is conducted one target at a time

PCR testing has a higher limit of detection & less data density

NGS is the Next Evolution in Food Authenticity Testing

NGS

Molecular methods: RT - PCR

imegenagro

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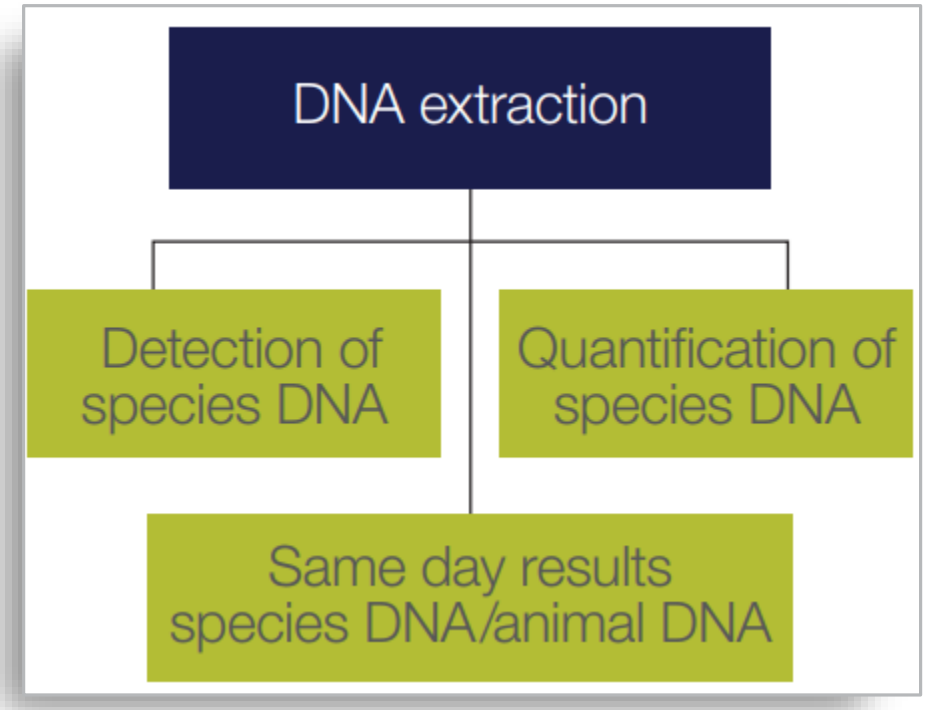
Molecular methods: RT - PCR



Full workflow solution	• DNA extraction options for low and high sample input
High sensitivity	• Detection of mitochondrial DNA
Quantitative	• Quantification based on DNA standards • Species DNA/total animal DNA result (%)

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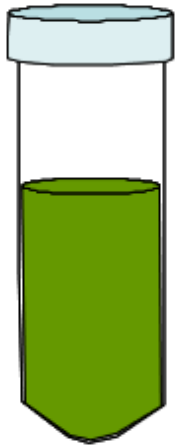
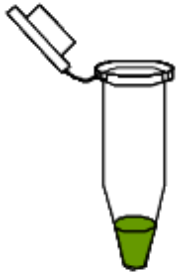
Workflow DNA Extraction



5-20 g muesta
10-20 ml Lysis buffer 1
20 µl RNAsa

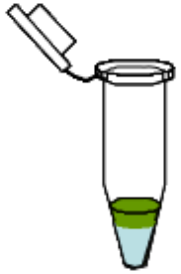
Incubar 30 min a 65ºC

Centrifugar 5 min a 3500 x g



385 µl (Muestra + Lysis buffer 1)
Añadir 25 µl Proteinase K

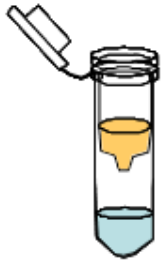
Incubar 60 min a 56ºC



Añadir 400 µl Lysis buffer 2

Incubar 10 min a 70ºC

Añadir 420 µl etanol



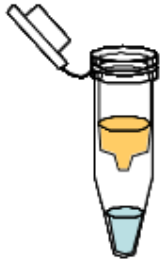
Centrifugar 1 min a 11000 x g (x2)

Añadir 500 µl Wash buffer 1

Centrifugar 1 min a 11000 x g

Añadir 600 µl Wash buffer 2

Centrifugar 1 min a 11000 x g (x2)



Añadir 100 µl H_2O a 70ºC

Incubar 3 min

Centrifugar 1 min a 11000 x g

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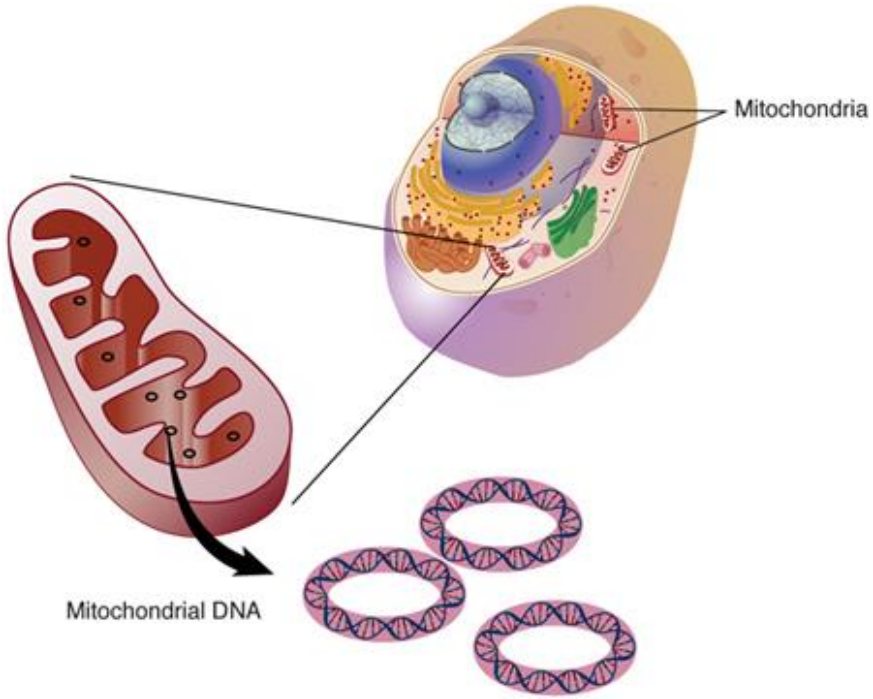
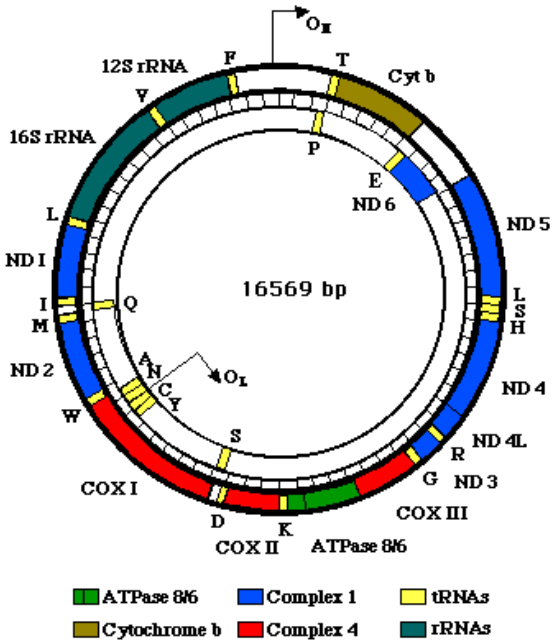
PCR *Mitochondrial DNA*

Sensitivity: It is 100 times greater using mitochondrial DNA targets than using nuclear DNA targets





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RapidFinder™ Animal Detection Kits

Product name	Part no.	Volume	Species name
GMO Extraction kit	A15570	48 rxn	Generic for Speciation & GMO
RapidFinder™ Beef ID kit	A24391	48 rxn	Bos taurus
RapidFinder™ Equine ID kit	A15570	48 rxn	Equus caballus
RapidFinder™ Pork ID kit	A24392	48 rxn	Sus scrofa
RapidFinder™ Chicken ID kit	A24393	48 rxn	Gallus gallus
RapidFinder™ Turkey ID kit	A24394	48 rxn	Meleagris gallopavo
RapidFinder™ Sheep ID kit	A24395	48 rxn	Ovis aries
RapidFinder™ Poultry ID kit	A24397	48 rxn	Several avian species, including Gallus gallus (chicken), Meleagris gallopavo (turkey), Anas platyrhynchos (duck), Struthio camelus (ostrich), and Anser (goose)
RapidFinder™ Ruminant ID kit	A24396	48 rxn	Bos taurus (beef), Ovis aries (sheep), Capra hircus (goat), Cervus elaphus (red deer), and Capreolus capreolus (roe deer)
RapidFinder™ Fish ID kit	A24398	48 rxn	Atlantic Salmon, Angler, Cod, Hake, Halibut, Seabass, Anchovy, Sole, Dab, Trout, Tiger Shark, European Eel, Red Bandfish
RapidFinder™ Quant Multi Meat Set	A24399	48 rxn	

Optimized for Applied Biosystems® 7500 FAST Real-Time PCR Instrument

More predesigned assays available (Goat, Fallow deer, Milk, Cat, Monkey, Rat…) and also Custom Assays are possible!

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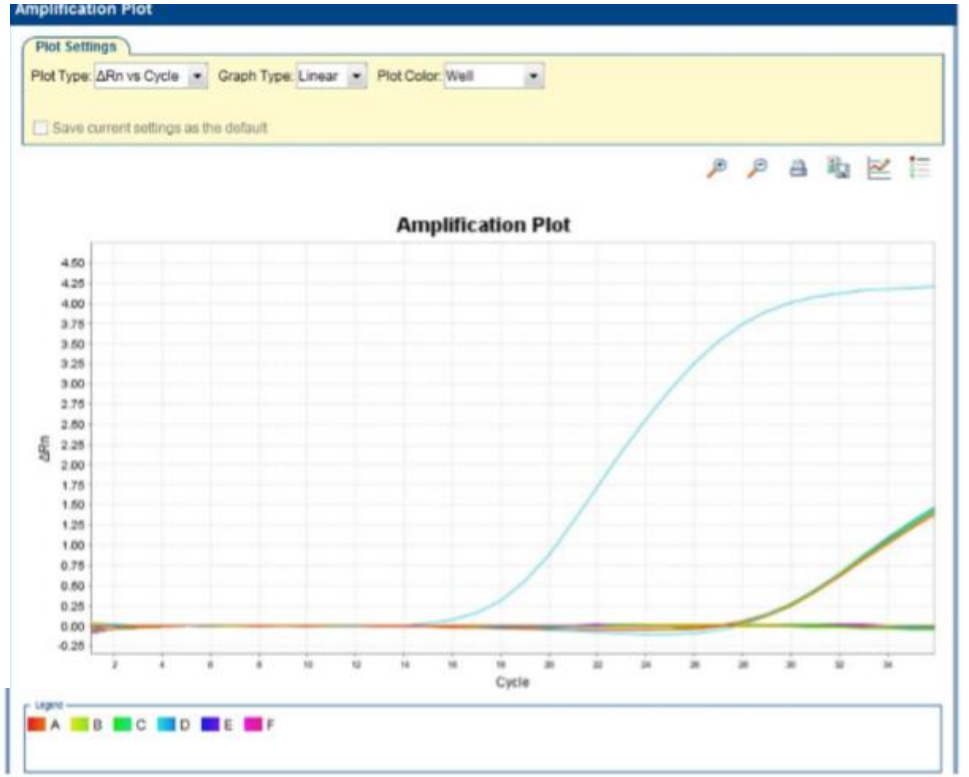
Specificity

Animal Species used during the specificity assay for the RapidFinder ™ Chicken ID Kit .

Meat Species	Results
Horse	Not detected
Mule	Not detected
Donkey	Not detected
Cow	Not detected
Swine	Not detected
Buffalo	Not detected
Fish	Not detected
Sheep	Not detected
Goat	Not detected
Deer	Not detected
Chicken	Detected
Turkey	Not detected
Duck	Not detected
Ostrich	Not detected
Goose	Not detected
Human	Not detected
Soy	Not detected
Wheat	Not detected



Chicken

IPC

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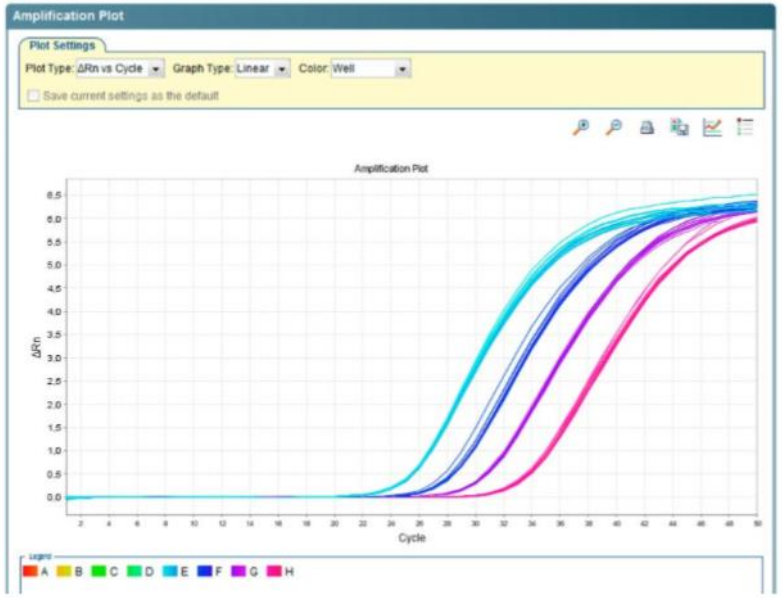
Limit of Detection



Results corresponding to the PCR amplification of samples, which contain different percentages of chicken DNA

Results for PCR limit				
	1%	**0.1%**	**0.01%**	**0.001%**
10 ng/µl of Chicken/Turkey	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
10 ng/µl of Chicken/Swine	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)

Results for PCR limit in processed samples			
	1%	**0.1%**	**0.01%**
ADN 10 ng/µl	100% (11/11)	100% (11/11)	55% (6/11)
ADN 100 ng/µl	100% (11/11)	100% (11/11)	91% (10/11)

- Adding a greater amount of DNA (200ng/µl is recommended)
- Increase the PCR cycles at 38-40 and increase the Ct cut-off.

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Legislation: e.g. *Rumiant DNA detection in feed*

COMMISSION REGULATION (EU) No 51/2013

of 16 January 2013

amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed

(Text with EEA relevance)

'*ANNEX VI*

METHODS OF ANALYSIS FOR THE DETERMINATION OF CONSTITUENTS OF ANIMAL ORIGIN FOR THE OFFICIAL CONTROL OF FEED

1. PURPOSE AND SCOPE

The determination of constituents of animal origin in feed shall be performed by light microscopy or polymerase chain reaction (PCR) in accordance with the provisions laid down in this Annex.

These two methods make it possible to detect the presence of constituents of animal origin in feed materials and compound feed. However, they do not make it possible to calculate the amount of such constituents in feed materials and compound feed. Both methods have a limit of detection below 0,1 % (w/w).

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RapidFinder™ Ruminant ID Kit

Determining the presence of DNA of ruminant (*Bos taurus*, *Ovis aries*, *Capra hircus*, *Cervus elaphus* and *Capreolus capreolus*) in any feed and food.

Master Mix Ruminant		INTERPRETATION
Ruminant	IPC	
-	+	No ruminant DNA is detected
+	+	Ruminant DNA is detected
-	-	PCR inhibitors presence in the sample*
+	-	Sample with big amount of ruminant DNA

This kit can also detect the presence of DNA in Processed Ruminant Animal Protein (PAP).

The most important innovation included in the design of this kits are the **IPC** system, needed to detect false negative result.

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Legislation/Recommendation: Fraudulent practices in food

RECOMMENDATIONS

COMMISSION RECOMMENDATION

of 27 March 2014

on a second coordinated control plan with a view to establishing the prevalence of fraudulent practices in the marketing of certain foods

(Text with EEA relevance)

(2014/180/EU)

E. Method

The following protocol should be used:

1. All samples should be submitted to an initial screening test aimed at detecting the presence of horsemeat in meat (as a ratio of mass fraction w/w) at the level of 0,5 % or above. The choice of screening method is up to the Member State.

2. Only samples positive to the screening test under para 1 should be subject to a confirmatory test using RT-PCR and targeting mitochondrial DNA aimed at detecting the presence of horsemeat in meat (as a ratio of mass fraction w/w) at the level of 1 % or above. The method used for confirmation must be calibrated to a standardised control sample of fresh meat delivered from the European Union Reference Laboratory for Animal Proteins in Feeding-stuffs.

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Quantification of meat species DNA in food and feed samples

RapidFinder™ Quant Multi-Meat

- PCR detection of a highly conserved mitochondrial genomic region from animal species
- Multi-Meat Standard, a plasmid
- Relative quantification of 0.05% of specific animal species DNA with respect to total animal DNA in a sample

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PCR

PCR testing is targeted or relies on a subset of targets

PCR testing is conducted one target at a time

PCR testing has a higher limit of detection & less data density

NGS is the Next Evolution in Food Authenticity Testing

NGS

Molecular methods: NGS – multispecies identification

Proprietary & Confidential
ThermoFisher SCIENTIFIC

Ion Torrent Applications: Food Integrity

For multiplex samples, multiple targets

Food Fraud, Authenticity, Labeling

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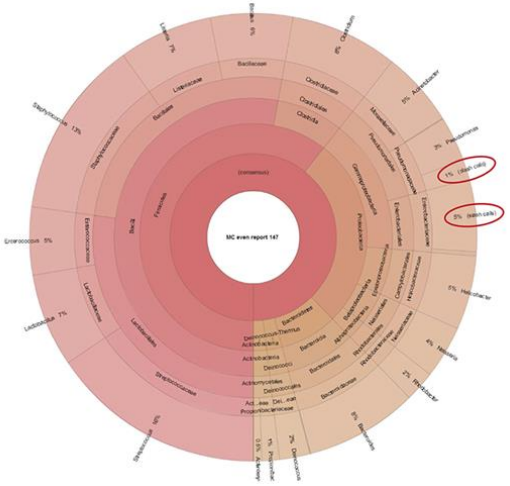
Ion Torrent Applications: Food

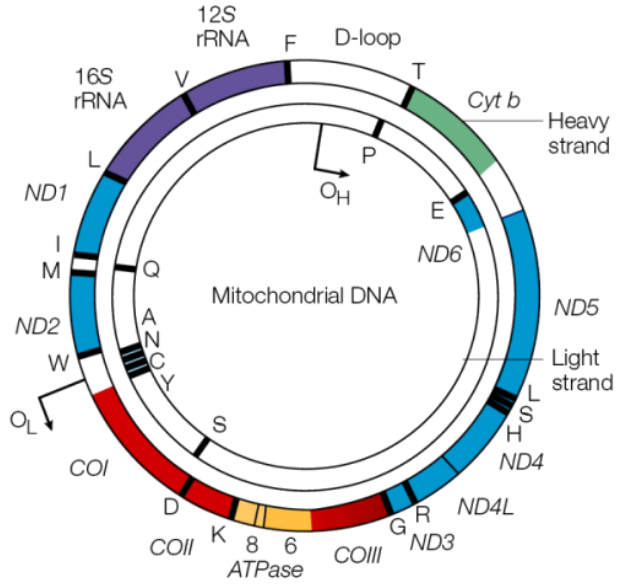
- Multi Species Identification
  - Accurate species ID by sequencing of *cytochrome* subunits and other genes (“barcodes”) even in mixed samples
  - Sequencing results show the percentage composition of different species in a sample



- Geographic Origin
  - Determination of SNP frequencies by GBS of high diversity genes

- Pathogen Detection
  - 16S gene sequencing for identification of bacterial organisms
  - 18S/ITS gene sequencing for identification of fungal organisms

- Metagenomics
  - Broad-range research analyses of mixed microbial populations.



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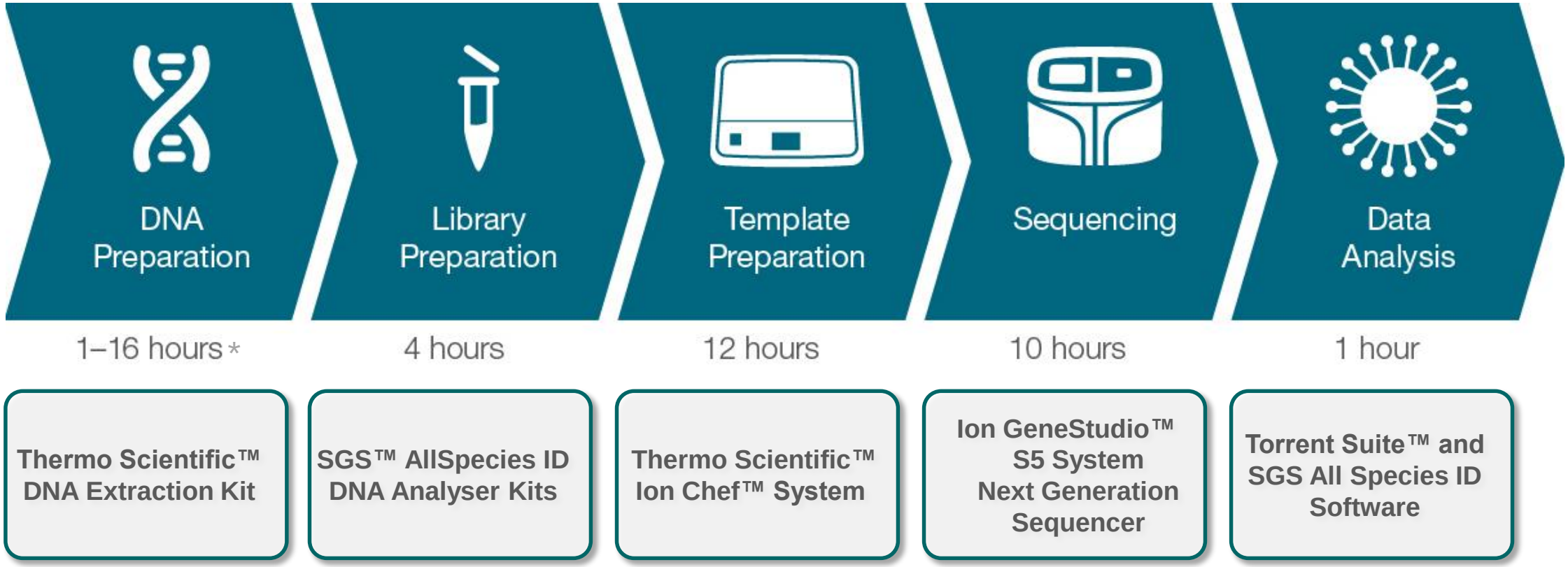
Multi-Species Screening and Identification

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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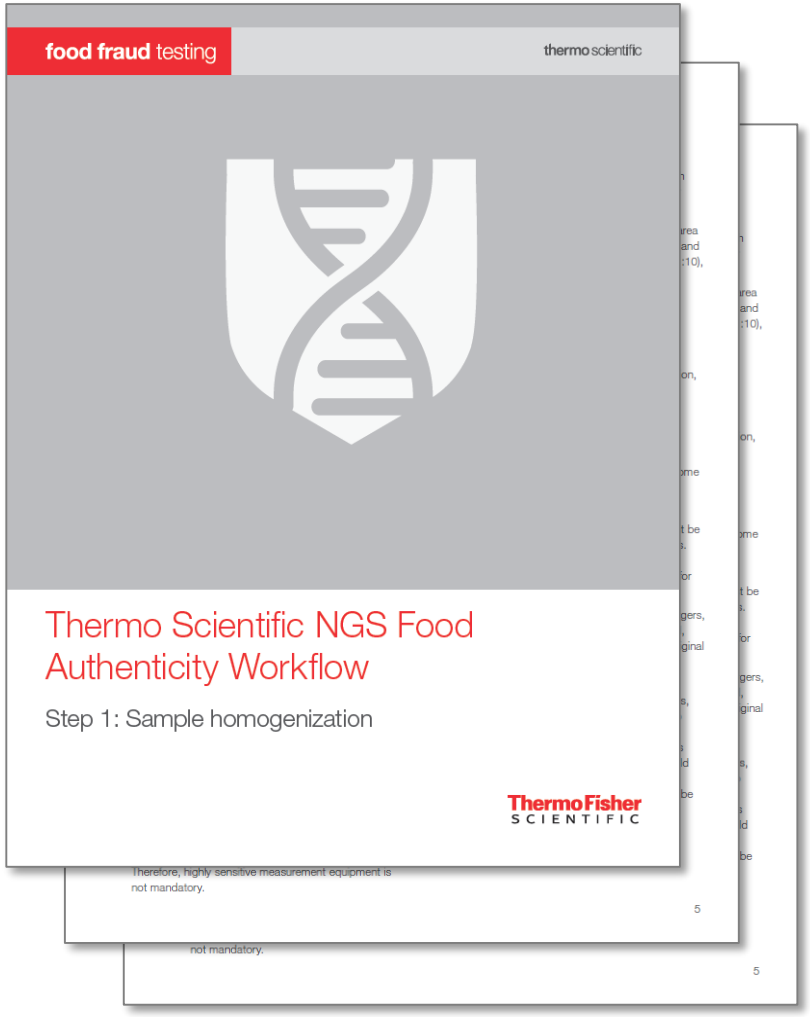
Thermo Scientific NGS Food Authenticity Workflow Guides



Sample homogenization	1.0	
DNA extraction	2a	2b
DNA quantification and dilution	3.0	
DNA amplification	4.0	
Libraries preparation	5.0	
Chip preparation DNA sequencing	6.0	
Data analysis	7.0	

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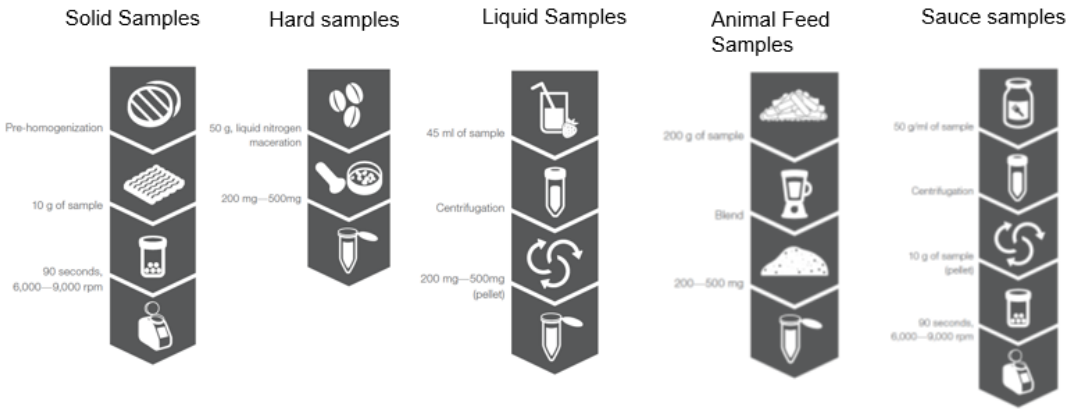
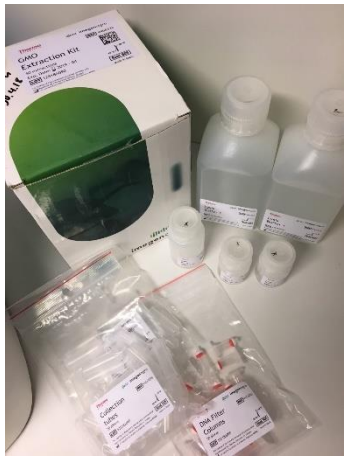
Steps 1 - 4

DNA Preparation

1–16 hours

Step 1 Sample homogenisation
Step 2a/b DNA extraction
Step 3 DNA quantification
Step 4 DNA amplification





Thermo Scientific™ DNA Extraction Kit

ThermoFisher SCIENTIFIC

SGS All Species ID Analyser Kits

- SGA All Species ID **Meat** Analyser Kits
  - SGS All Species ID Meat Analyser Kit (Barcodes **1**:1-24)
  - SGS All Species ID Meat Analyser Kit (Barcodes **1**:25-48)
- SGA All Species ID **Fish** Analyser Kits
  - SGS All Species ID Fish Analyser Kit (Barcodes **2**:1-24 & **3**:1-24)
  - SGS All Species ID Fish Analyser Kit (Barcodes **2**:25-48 & **3**:25-48)
- SGA All Species ID **Plant** Analyser Kits
  - SGS All Species ID Plant Analyser Kit (Barcodes **4**:1-24 & **5**:1-24)
  - SGS All Species ID Plant Analyser Kit (Barcodes **4**:25-48 & **5**:25-48)

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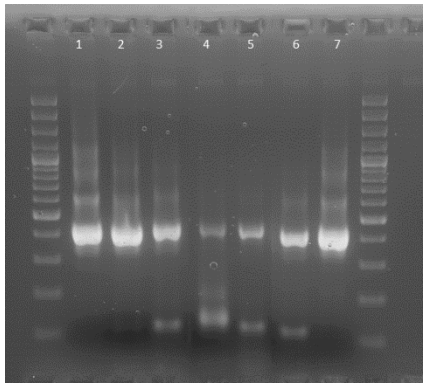
Step 5: Library preparation

The concentration of PCR products from all samples is assessed using Gel Electrophoresis to ensure that they are present in the same concentration in the final library pool



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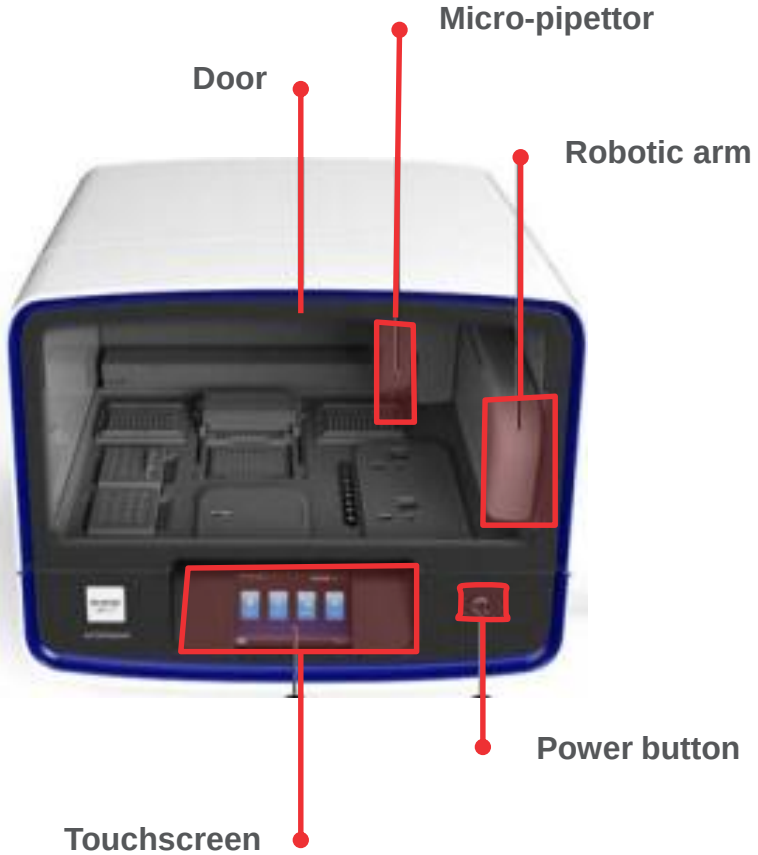
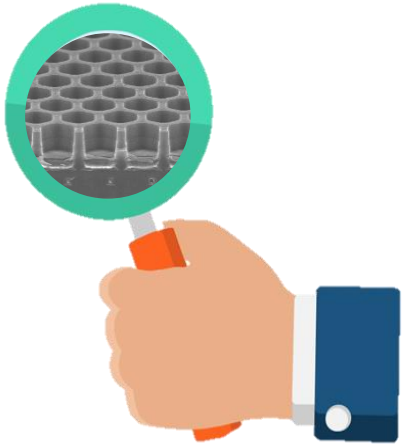
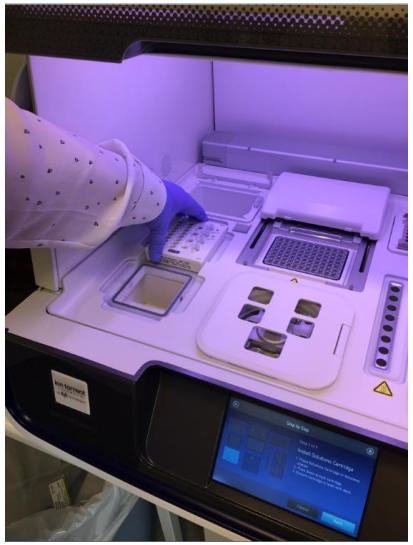
Step 6: Template and Chip preparation



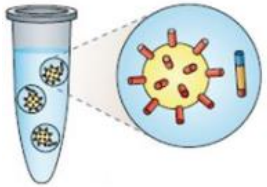


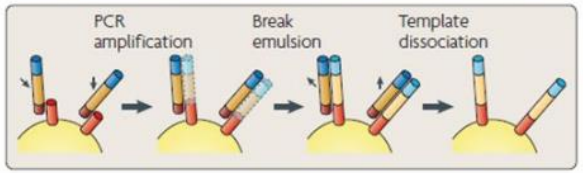
Ion Torrent Sequencing Chip

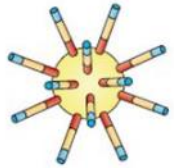


Run:

1) Library, Reagents, Oil and Ion Spheres are mixed and amplified using the on-board thermal cycler
2) Templated Ion Sphere Particles (ISPs) produced
3) Templated ISPs loaded onto chips







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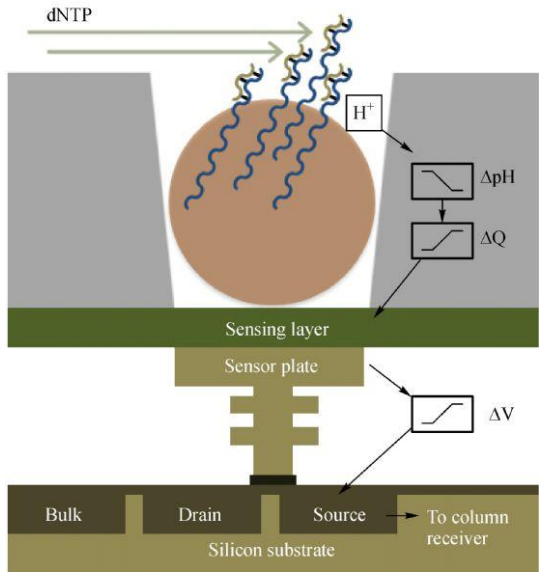
Step 6. Part 2: DNA Sequencing – Ion GeneStudio S5 Food Protection System

- Install waste container, wash solution, cleaning solution, and reagent cartridge.
- Install chip.
- Select run plan, Click **Go.**



1	2	3	4	5
Barcoded S5 Chip (1 per run)	**Reagent cartridge** (1 per init 1–2 runs)	**Wash solution** (1 per init 1–2 runs)	**Waste container** (Reusable)	**Cleaning solution** (4x use)

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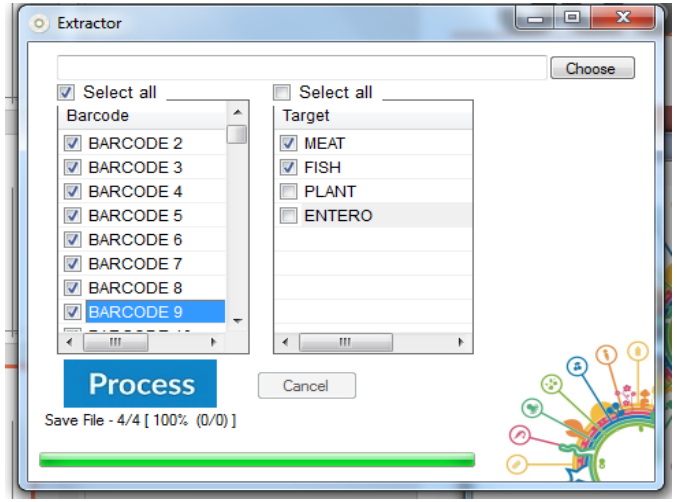
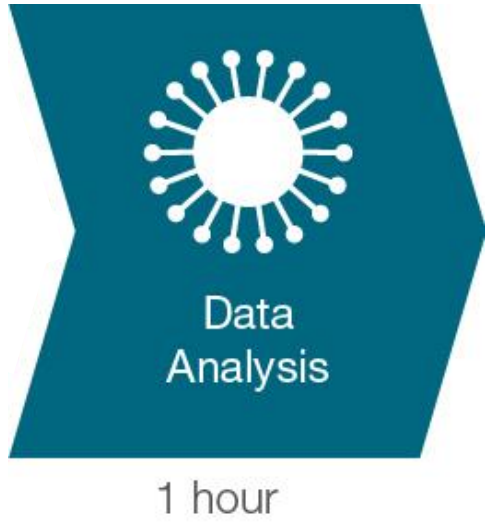
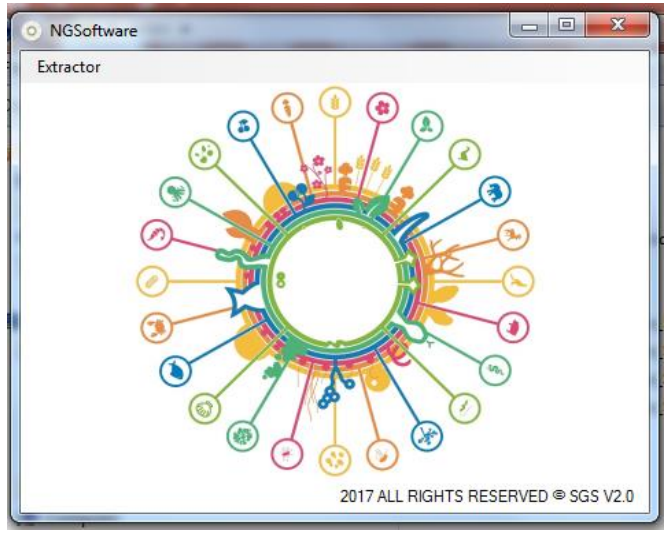
Step 7. Data Analysis – SGS All Species ID Software

Fast Q file from the S5 loaded into the SGS All Species ID Software

- Samples are identified according to barcode
- Sequences for each sample are screened against the database containing over 15,000 species
- A list of all the species contained in each sample is displayed
- The number of reads per sequence is provided, this can be indicative of the amount of a particular species in the sample but is NOT quantitative
- If a sequence is identified but not matched to the database, an ‘Unknown’ result is returned







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Example of NGS multispecies report

Target	Barcode	Species	Number Of Reads
FISH	1	Pangasianodon_hypophthalmus	3680
		Sander_lucioperca	1737
		Pleuronectes_platessa	1158
		Anguilla_japonica	1124
		Lophius_piscatorius	615
		Chanos_chanos	364
		Melanogrammus_aeglefinus	64
	2	Oncorhynchus_mykiss	630
	3	Abramis_brama	2086
		Leuciscus_leuciscus	756
		Leuciscus_idus	550
		Rutilus_rutilus	534
		Vimba_vimba	407
		Alburnus_istanbulensis	284
	4	Rutilus_rutilus	5417
		Pollachius_virens	2807
MEAT	22	Bos_taurus	19306
	23	Gallus_gallus	16534
		Bos_taurus	4217
	24	Sus_scrofa	10105
		Bos_taurus	158
PLANT	3	Origanum_sp./Origanum_onites	86660
		Origanum_sp./Origanum_vulgare	43666
		Origanum_sp./Origanum_majorana/Orig	42978
		Thymus_sp.	5159
		Convolvulus_arvensis	2772
		Lactuca_sp./Lactuca_sativa	1369
	4	Origanum_sp./Origanum_onites	71582
		Origanum_sp./Origanum_vulgare	36150
		Origanum_sp./Origanum_majorana/Orig	35415
		Laurus_nobilis	15076

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Informative and Reliable Results

- All targets are detected in a single run (meat, fish & plant)
- Reliable results in complex (multi-ingredient) and processed samples
  - DNA sequencing – most reliable method for species confirmation
  - DNA can be highly fragmented as the regions targeted are very short, e.g. 100 nucleotides
- NGS is starting to be introduced into international standardization:
  - NWIP (new work item proposal) ISO project: ISO TC 34/SC 16 ISO 22949-1
  - Molecular biomarker analysis – Detection and identification of animal species by DNA sequencing methods (Including NGS)

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Case study: Grouper fillet

SITUATION

- Retailer requested species ID for seafood. Confirm grouper species authentication
- 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 where 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

ThermoFisher SCIENTIFIC

Real time Response: Case study: Laurel raw material

SITUATION

- Retailer asked for NGS spices in Curry mixture (containing more than 10 spices according to label)

RESPONSE

- All the spices except laurel (scientifica name Laurus nobilis) could be detected
- NGS spices was performed and the majority of the sample (> 90%) was composed by a very similar plant of the laurel family but not Laurus nobilis (laurel) - potentially a toxic plant
- The supplier was mixing in the curry preparation not Laurel but something else similar to Laurel but with less than 10% of 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

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Find Out More About Multi-Species ID for Your Laboratory Today

- New video SGS just released on the Multispecies ID NGS workflow we are partnering with them on: https://www.youtube.com/watch?v=ymqMgcT0LqQ
- For more specific on the Ion sequencer is the quick demo video to get an idea of the plug and play, ease of use of the instruments:

  https://www.youtube.com/watch?v=l8Lkis9qFIo

  https://www.youtube.com/watch?v=aNA5aK5qYJc
- How Ion Torrent sequencing works works: https://www.youtube.com/watch?v=WYBzbxIfuKs
- Educational videos on our websites Learning Center (scroll to the bottom of the page for ‘Sequencing’):

  https://www.thermofisher.com/us/en/home/technical-resources/technical-reference-library/how-to-and-educational-videos

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¡Gracias!
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