Rapid DNA Sequencing for Food Authentication

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Science Group

science group Defined by science Inspired by technology Delivering innovation	sagentia	Science, technology & product development	oakland innovation	Technology consultancy for the consumer & industrial sectors
	otm	Technology advisory services to the oil & gas industry	leatherhead food research	Science & technology advisory services to the food & drinks market

TIME Quotes of the Day

“If you're paying for white tuna and you're eating tilapia, I think you'd want to know that.”

August 22, 2008

Detecting Food Adulteration

Technique	Number of ref.	Technique	Number of ref.
Sample preparation		Spectroscopic	
Microwave-assisted extraction	**384**	Mass spectrometry	**5030**
Headspace	**2571**	Fluorescence	**4807**
Solid phase extraction	**3866**	NMR	**3735**
Supercritical fluid extraction	**680**	Infrared	**2369**
Purge and trap	**151**	X-ray	**2119**
Flow injection analysis	**393**	Ultraviolet	**1429**
Pressurized liquid extraction	**436**	Atomic spectroscopy	**1046**
Microextraction	**2201**	Electron spectroscopy	**1026**
Biological		Light scattering	**891**
Biosensors	**750**	Circular dichroism	**468**
PCR	**7085**	Other	**932**
Microbiological analysis	**416**	Rheological	
Recombinant DNA	**220**	Creep	**205**
Immunological techniques	**3008**	Oscillatory shear	**203**
Others	**118**	Rheometry	**195**
Separation		Viscometry	**163**
Liquid chromatography	**8927**	Stress relaxation	**145**
Gas chromatography	**4798**	Normal stress	**32**
SDS/PAGE	**3227**	Thermal	
Capillary electrophoresis	**1155**	DSC	**551**
Supercritical fluid chromatography	**51**	Thermogravimetry	**17**
LC LC, LC-LC	**27**	The mochemical	**16**
GC GC, GC-GC	**210**	Differential thermal analysis	**9**
LC-GC	**38**	Electrochemical	
Radiochemical		Biosensors	**750**
Radioimmunoassay	**96**	Voltammetry	**532**
Isotopic	**140**	Potentiometry	**234**
Radiochemical	**31**	Amperometry	**245**
Radiometric	**18**	Polarography	**47**
Radioisotope	**8**	Conductometry	**43**
Radiotracer	**5**	Coulometry	**32**
Radiolabelling	**2**		

Number of works published in the period 2001–2011 found through a search in the database of Food Science and Technology Abstracts (FSTA)

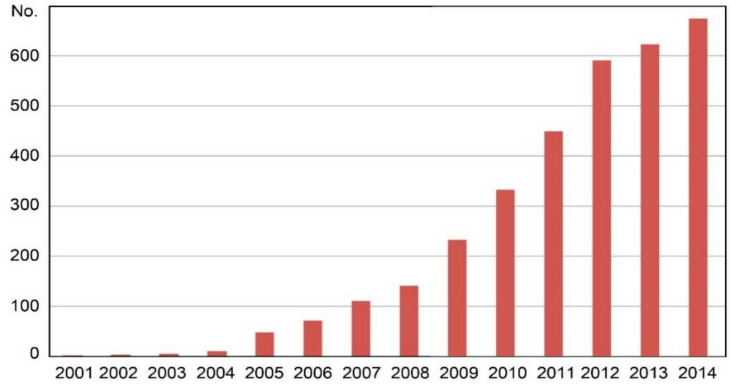
Review Article: Food Analysis: Present, Future, and Foodomics Alejandro Cifuentes 2012

DNA barcoding

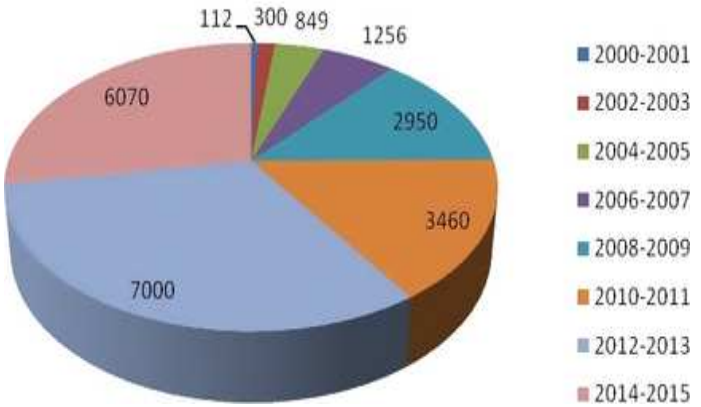
- Genetic fingerprint of a short section of DNA (<1000 base pairs)

- BOLD is a searchable high quality repository of reference sequences for all living species
- Species (breed) identification based on core assumption that DNA sequences are likely more similar to one another within species than between species
- 97% -100% species resolution

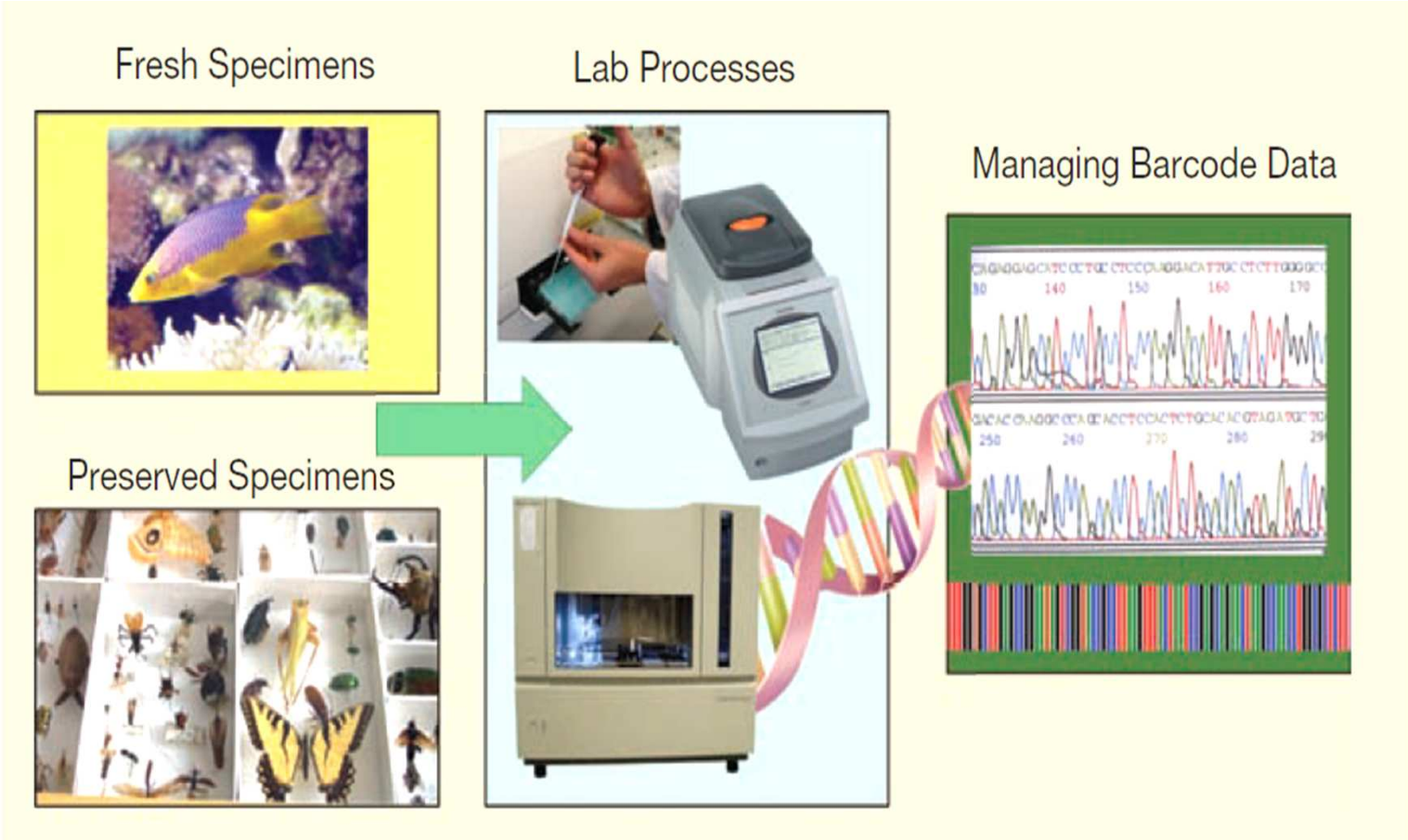
Number of scientific papers found in the database of ISI Web of Science using the keyword "DNA barcoding"
Barcaccia, G., Lucchin, M., & Cassandro, M. (2015)



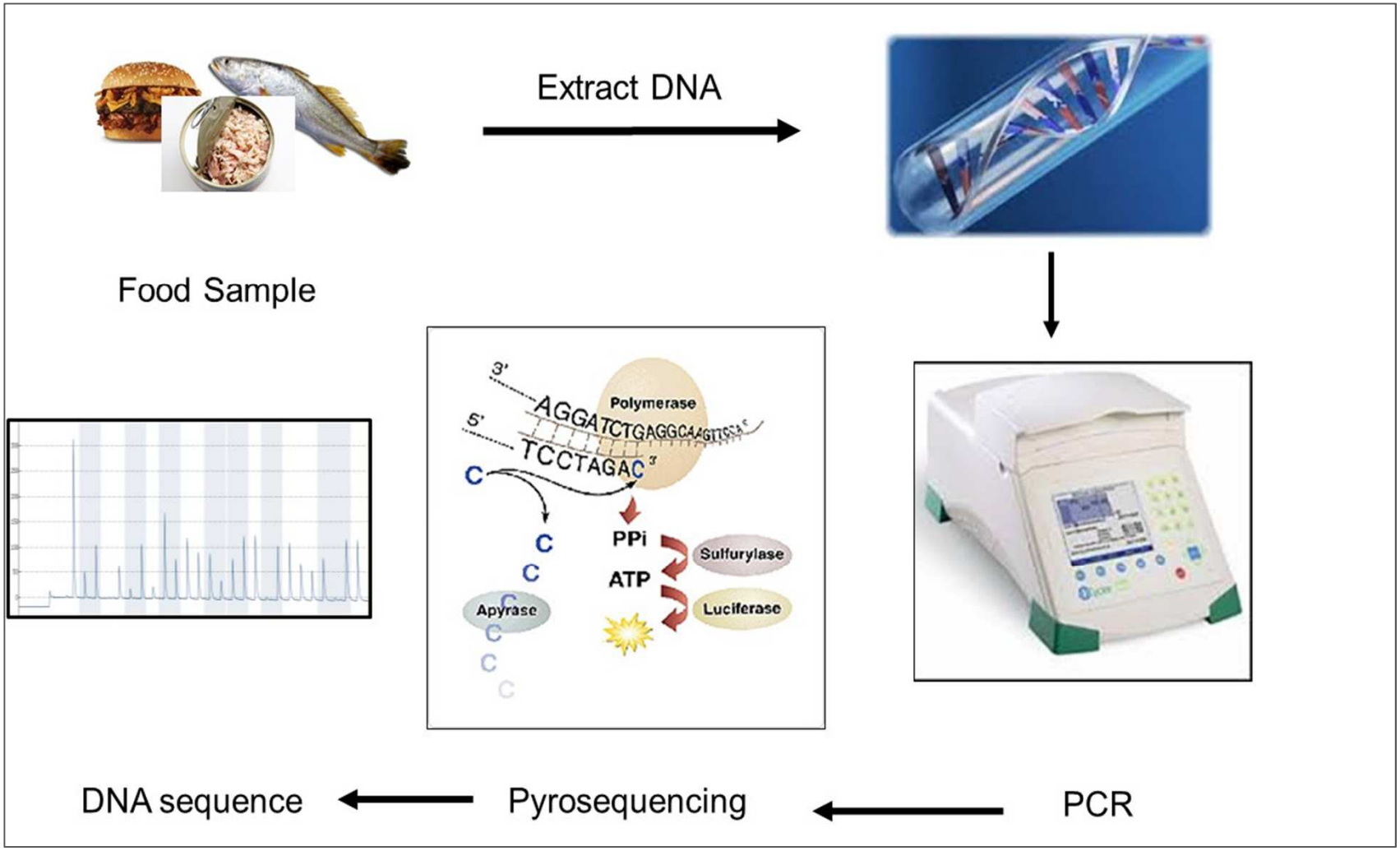
Total number (approx.) of peer reviewed manuscripts using DNA barcoding for plant identification



Processes in DNA Barcoding



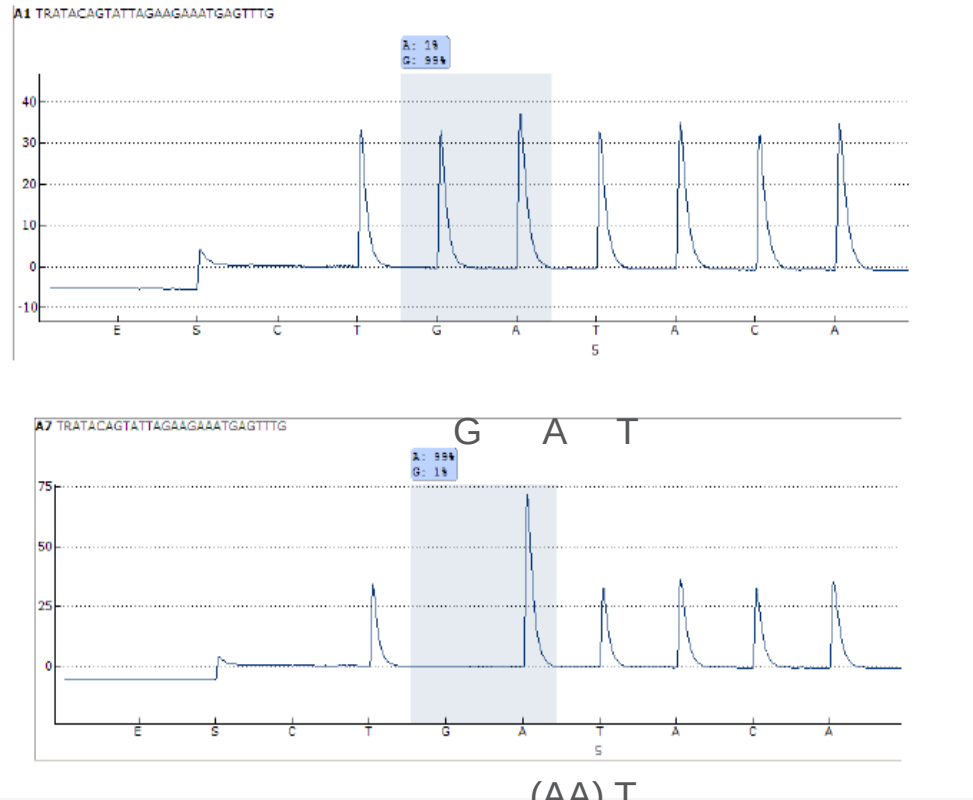
ASPECT (Authentication and Speciation Test)

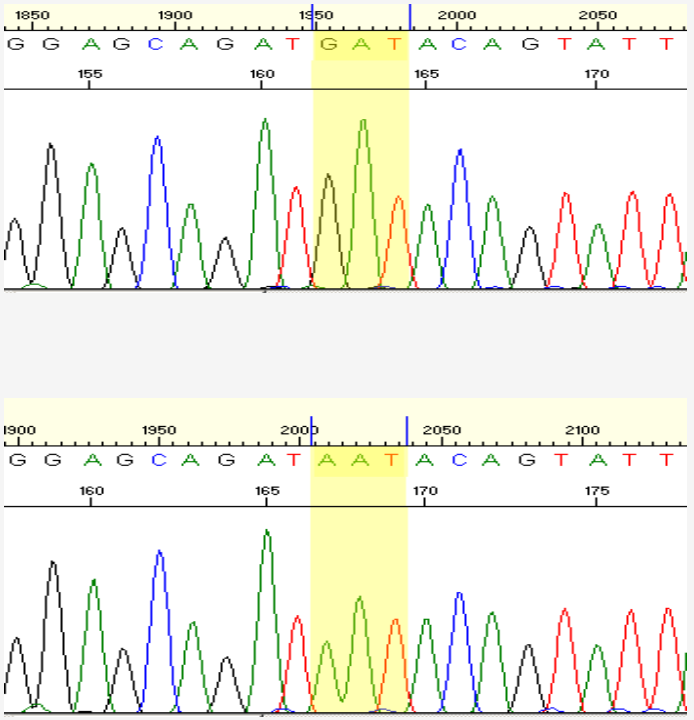


Pyrosequencing for food authentication

Why choose pyrosequencing?

- offers the highest accuracy with readouts of **99.9%** for the longest read length which is currently **700 base pairs**





Quantitative identification of plant genera in food products using PCR and Pyrosequencing((R)) technology

Article in Food Control 18(8):921-927 · August 2007

leatherhead food research

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

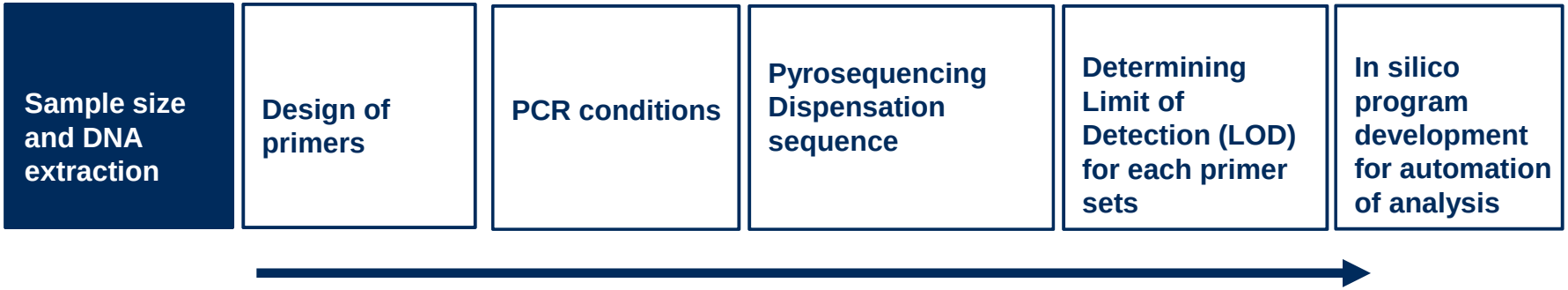
Pyrosequencing as a Tool for Rapid Fish Species Identification and Commercial Fraud Detection

Cristian De Battisti,*[,†] Sabrina Marciano,[†] Cristian Magnabosco,[‡,⊥] Sara Busato,[†] Giuseppe Arcangeli,[‡] and Giovanni Cattoli[†]

Application of Pyrosequencing® in Food Biodefense

Article in Methods in molecular biology (Clifton, N.J.) 1315:363-75 · June 2015

Development of the ASPECT method



Sampling

- *Homogenous*
- *Size*

DNA extraction efficiency

- *adulterant DNA extract at same efficiency*
- *Admixture of authenticated raw horsemeat in beef background at 0.1, 0.5, 1, 2, 5,10, 20 and 50% (w/w)*
- *Numerous commercially available test kits were assessed*

200 mg – 2g sample; CTAB method

Development of the ASPECT method

Sample size and DNA extraction	Design of primers	**PCR conditions**	Pyrosequencing Dispensation sequence	Determining Limit of Detection (LOD) for each primer sets	In silico program development for automation of analysis

Conserved PCR and sequencing primers

Aligning sequences to allow discrimination between species

```
cow     NNNNNNNNNNNNATG---ACTAACATTCGAAAGTCCCACCCACTAATAAAAATTGTAAACNNNNNNNNNNNN
sheep   NNNNNNNNNNNNATG---ATCAACATCCGAAAAACCCACCCACTAATAAAAATTGTAAACNNNNNNNNNNNN
goat    NNNNNNNNNNNNATG---ACCAACATCCGAAAGACCCACCCATTAATAAAAATTGTAAACNNNNNNNNNNNN
horse   NNNNNNNNNNNNATG---ACAAACATCCGGAAATCTCACCCACTAATTAAAATCATCAATNNNNNNNNNNNN
donkey  NNNNNNNNNNNNATG---ACAAACATCCGAAAATCCCACCCGCTAATTAAAATCATCAATNNNNNNNNNNNN
ostrich NNNNNNNNNNNNATGGCCCCCAACATTCGAAAATCGCACCCCCTGCTCAAAATTATCAACNNNNNNNNNNNN
emu     NNNNNNNNNNNNATGGCCCCTAACATCCGAAAATCCCACCCTCTACTCAAAATCATCAACNNNNNNNNNNNN
turkey  NNNNNNNNNNNNATGGCACCCAATATCCGAAAATCACACCCCCTATTAAAAACAATCAACNNNNNNNNNNNN
```

Optimising cycling conditions

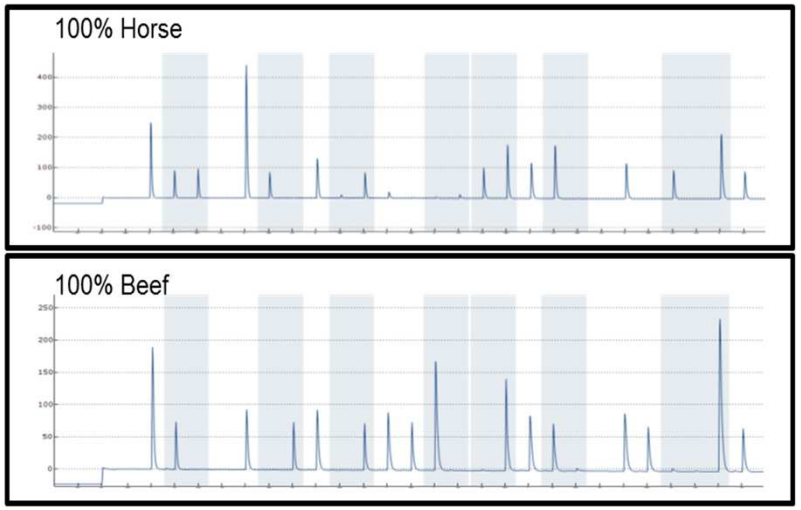
- *end point PCR conditions*
- *Amplicons tested on Lab-on-chip*

Universal primer sets for meat/fish

Development of the ASPECT method

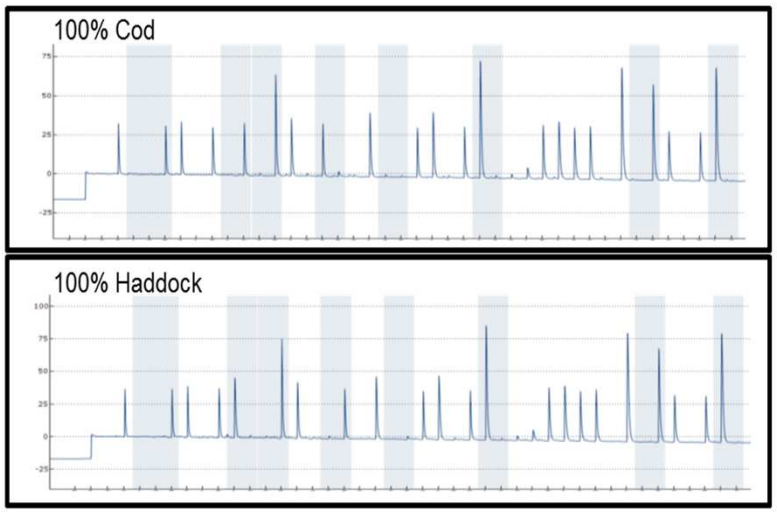
Sample size and DNA extraction	**Design of primers**	**PCR conditions**	**Pyrosequencing Dispensation sequence**	**Determining Limit of Detection (LOD) for each primer sets**	**In silico program development for automation of analysis**

Meat Authentication



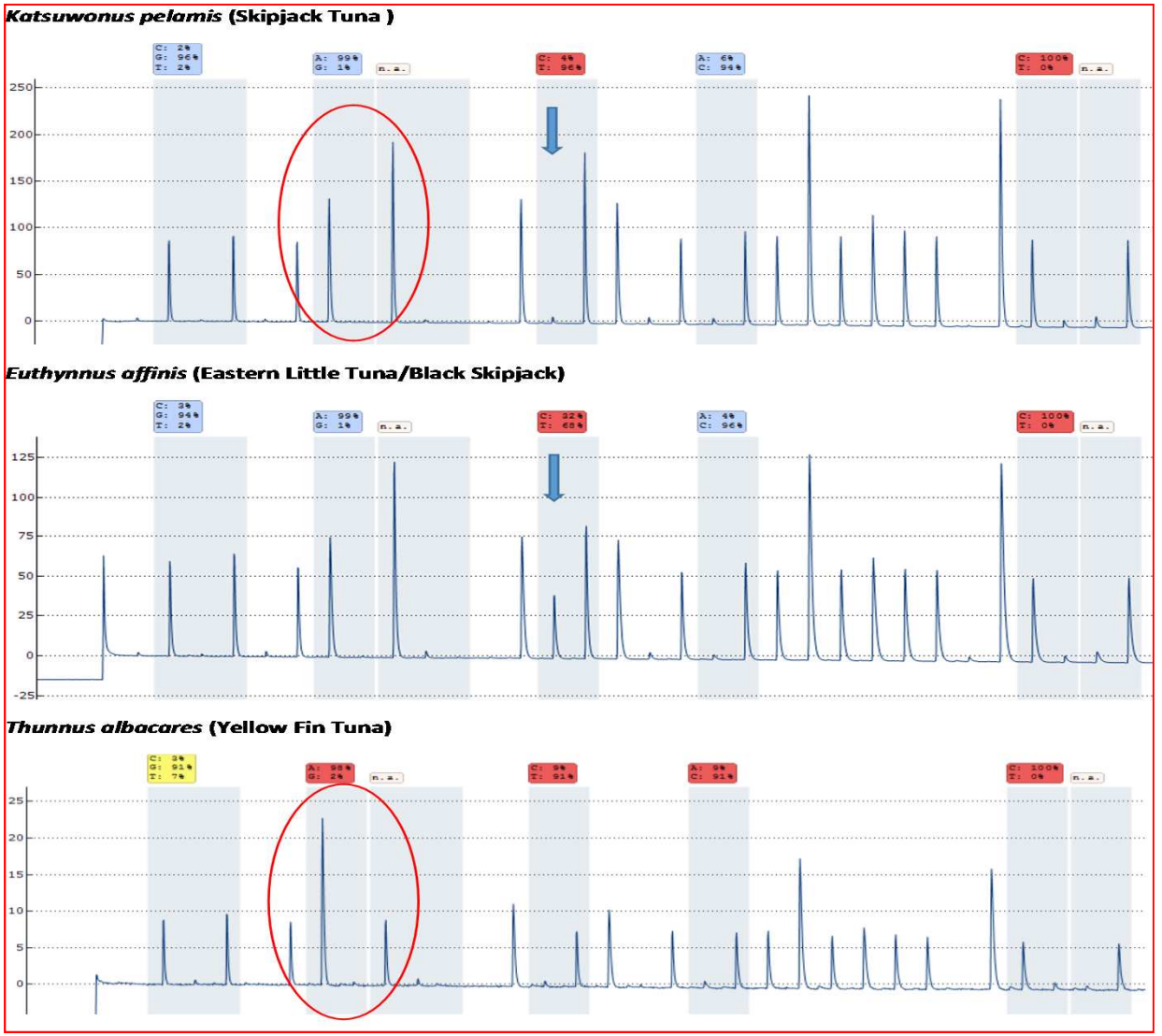
Horse vs Beef

Fish Authentication

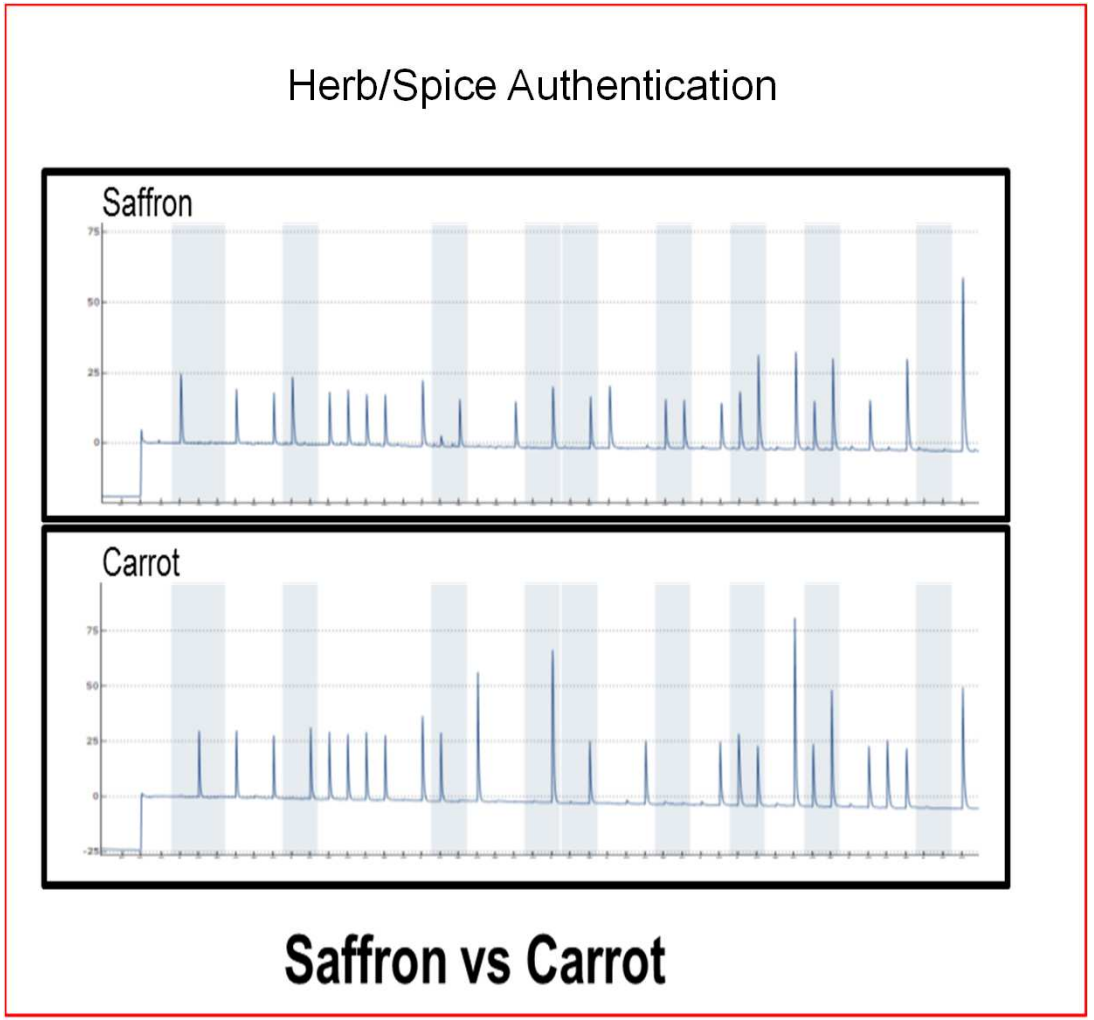


Cod vs Haddock

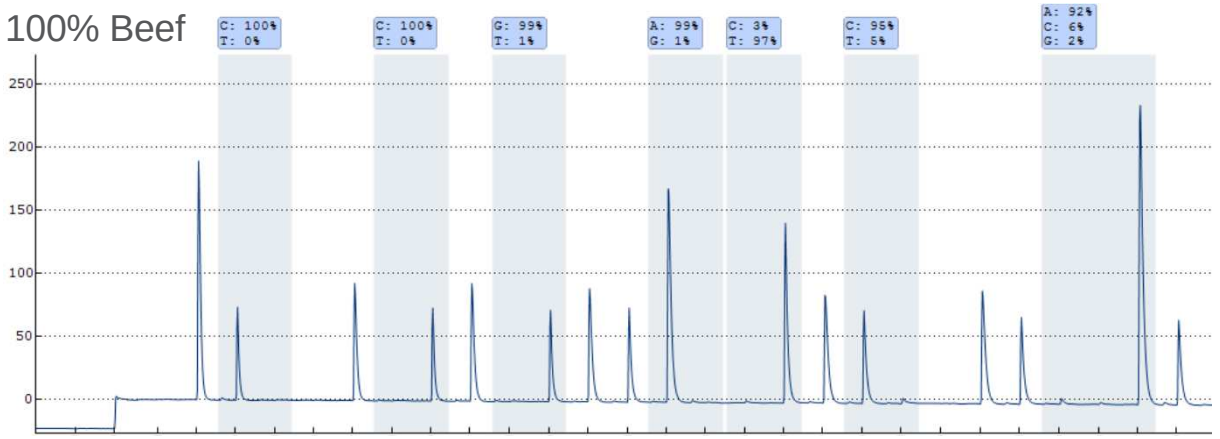
Fish Authentication



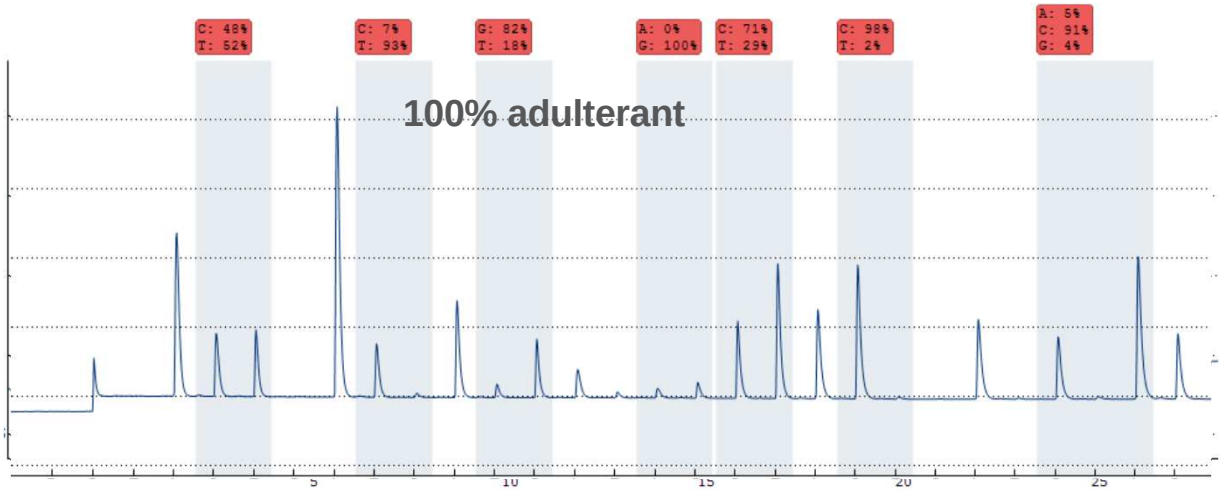
Application to Herbs and Spices



ASPECT as a rapid and cost effective screening tool



Admixtures of authentic horsemeat in beef
0.1, 0.2, 0.5, 1, 2, 10, 20 and 50% w/w



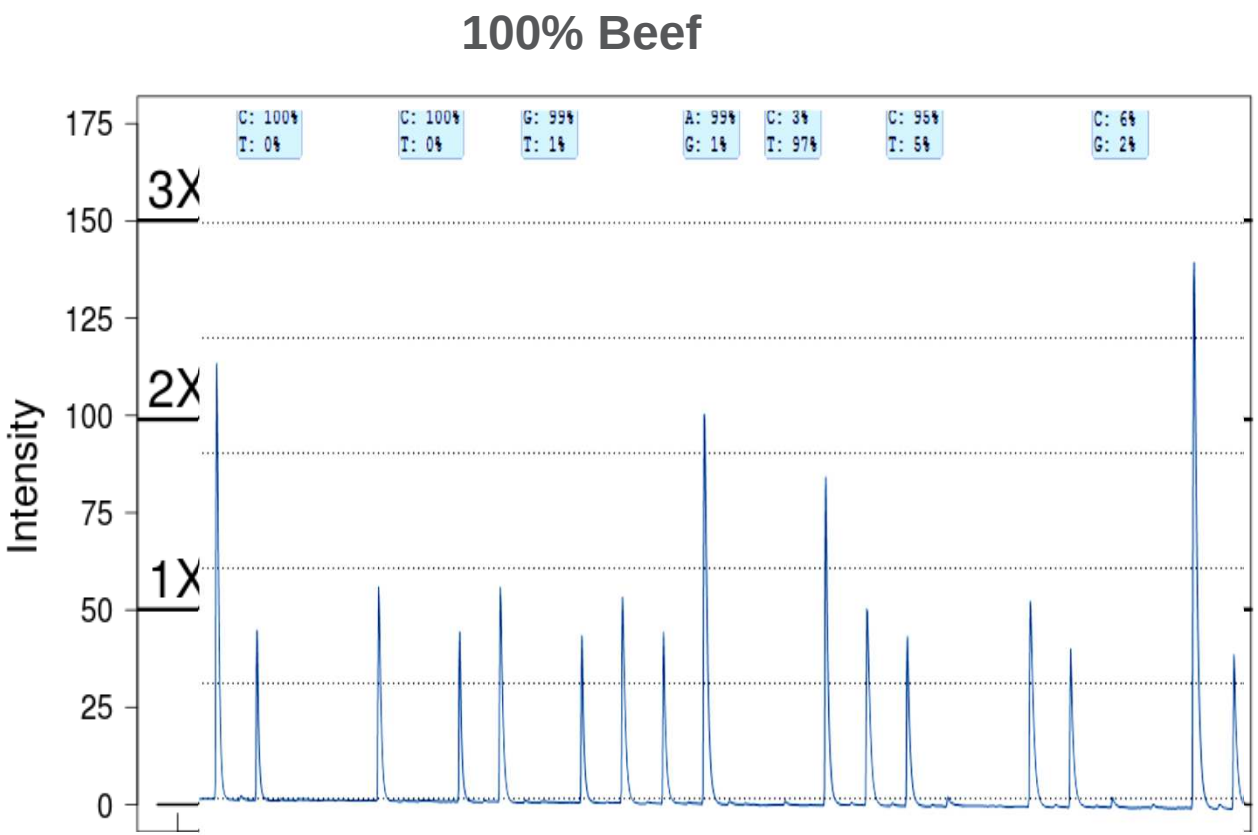
In-house validation and limit of detection (LOD) of ASPECT

Authenticated horsemeat and beef:

- Admixtures of meat in 0.1, 0.2, 0.5, 1, 2, 10, 20 and 50% w/w
- Mixtures of horse DNA: beef DNA (following DNA extraction)

	SNV#1	SNV#2	SNV#3	SNV#4	SNV#5	SNV#7
Horsemeat in Beef (% w/w)	Mean ± standard deviation for N	Mean ± standard deviation for N	Mean ± standard deviation for N	Mean ± standard deviation for N	Mean ± standard deviation for N	Mean ± standard deviation for N
0 % (100 % Beef)	99.4 ± 0.5	98.8 ± 2.3	98.4 ± 1.8	96.9 ± 3.7	93.5 ± 0.5	90.8 ± 2.2
0.1 %	99.0 ± 0.3	98.7 ± 0.6	98.7 ± 0.1	97.8 ± 0.5	94.0 ± 0.3	93.6 ± 0.1
0.2 %	98.4 ± 1.6	98.2 ± 0.6	98.3 ± 0.5	95.1 ± 2.7	89.8 ± 6.0	88.6 ± 2.4
0.5 %	98.7 ± 1.3	98.7 ± 0.8	98.9 ± 0.1	98.1 ± 0.5	94.0 ± 1.5	93.5 ± 0.2
1 %	96.5 ± 0.1	96.6 ± 0.6	98.1 ± 0.5	96.4 ± 0.3	88.1 ± 4.3	86.2 ± 2.3
1/10 dilution of 1 %	96.5 ± 0.1	96.2 ± 0.3	98.2 ± 0.4	95.8 ± 1.0	88.7 ± 3.5	87.5 ± 3.2
1/100 dilution of 1 %	96.3 ± 0.2	96.5 ± 0.3	98.5 ± 0.4	96.5 ± 0.4	86.3 ± 7.1	84.0 ± 1.6
2 %	96.0 ± 0.4	96.1 ± 0.4	98.1 ± 0.4	96.0 ± 1.5	85.5 ± 4.2	85.2 ± 3.5
10 %	84.7 ± 0.8	82.8 ± 0.8	97.4 ± 1.7	95.0 ± 3.4	74.5 ± 6.1	71.5 ± 7.6
20 %	76.1 ± 0.4	72.4 ± 2.3	95.8 ± 2.7	90.9 ± 8.4	58.6 ± 1.3	60.3 ± 5.6
50 %	74.5 ± 0.4	67.9 ± 2.8	96.0 ± 3.3	91.9 ± 6.7	65.4 ± 1.9	54.7 ± 2.2
100 % (100% Horsemeat)	49.4 ± 1.9	7.9 ± 8.0	92.8 ± 6.3	99.9 ± 6.5	32.7 ± 1.7	6.3 ± 6. 6

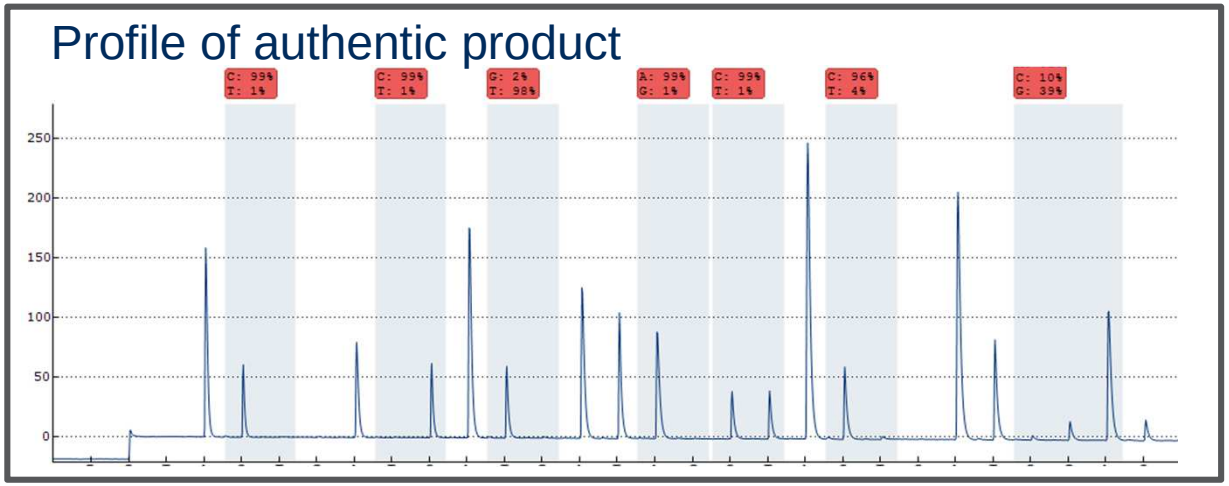
In silico



Adulteration in Highly Processed Canned Soup

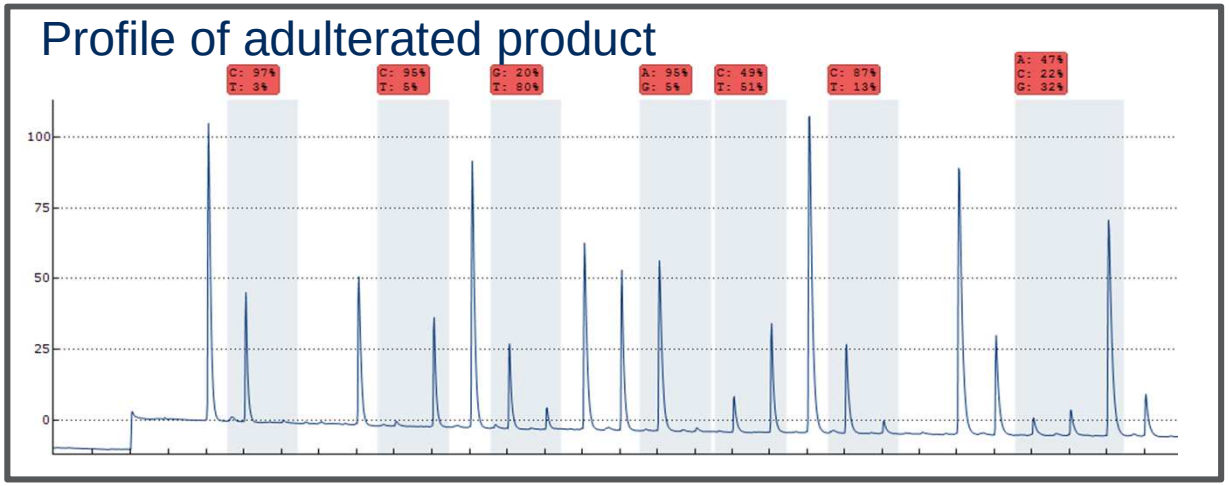
ASPECT for resolving complex mixtures & identifying unknown adulterants

Profile of authentic product



100 % Lamb DNA

Profile of adulterated product



60% Lamb DNA
26% Beef DNA
14% Chicken DNA

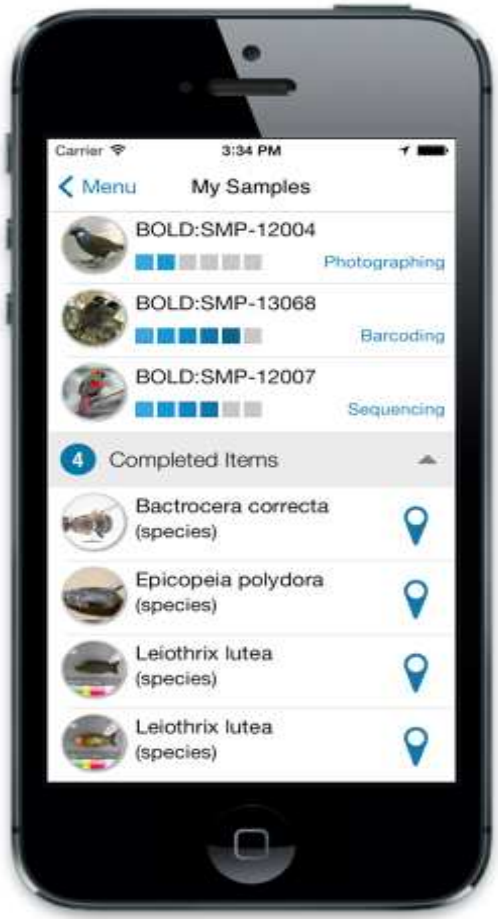
ASPECT process is applicable to:

- Meat, fish, crustaceans
- Plants, herbs and spices
- Raw and cooked foods
- Ready meals and highly processed foods (e.g. stock cubes, canned foods, animal foods, sweets and snacks)

Why choose ASPECT

- a unique non targeted DNA based authenticity test
- ***ASPECT*** process uses pyrosequencing to unambiguously identify any unsuspected adulterants in a single test
- Rapid turnover
- Low cost
- Potential point-of-need device

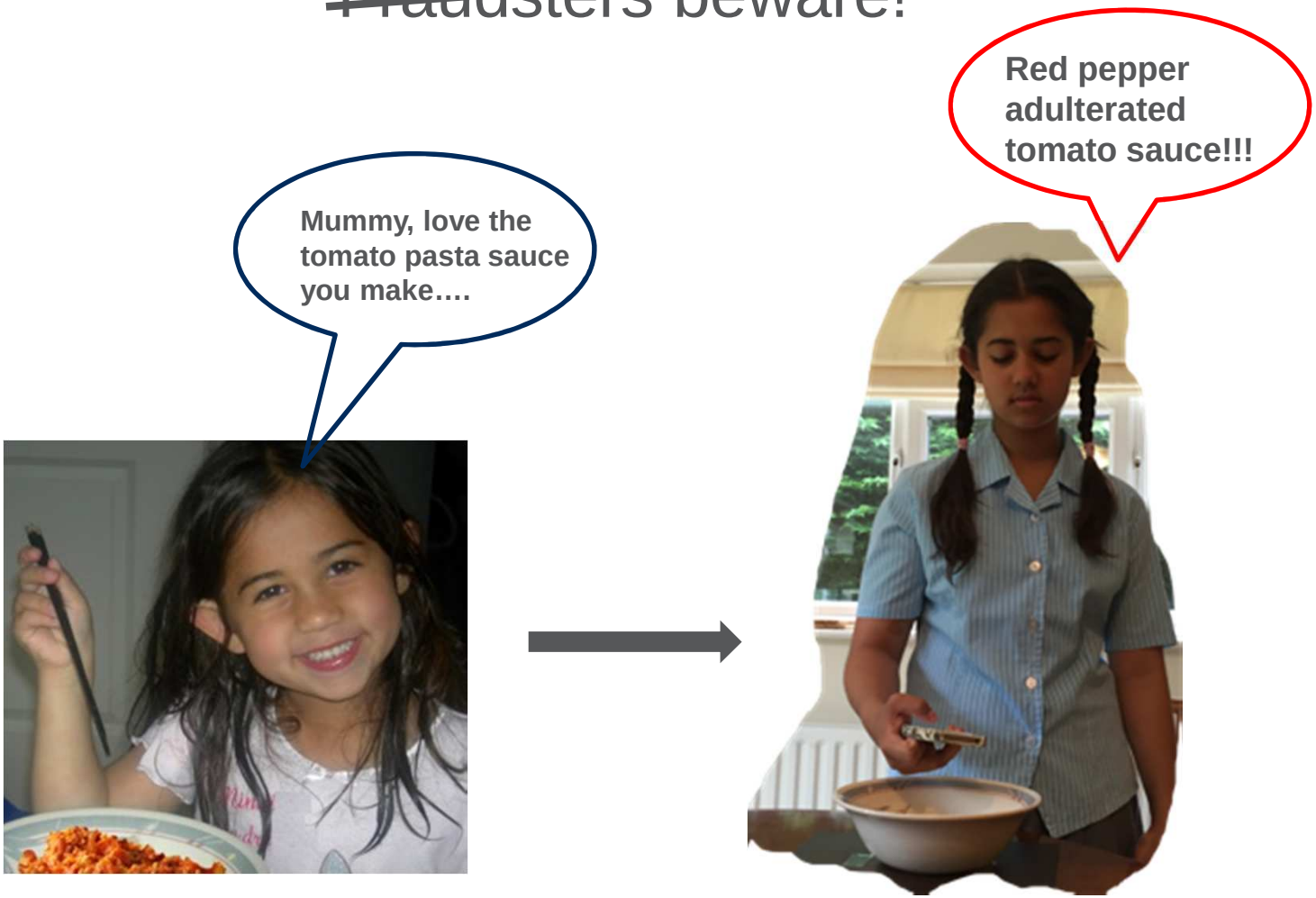
The Future: Point-of-Need
Product integrity, instantly



- Data in seconds to minutes
- Link to reference database
- A taxonomic GPS
- Usable by non-specialists

Parents

~~Fraudster~~s beware!



Thank you for listening

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