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# **Advances in Food Authenticity Testing Using DNA Techniques**

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**Food Auditing Conference**

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## Public Analyst Scientific Services

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- Public Analyst Scientific Services (PASS) is a limited company owned by Eurofins which serves local authority food and feed safety enforcement
- Public Analysts are appointed by local authorities and must hold the Mastership in Chemical Analysis qualification awarded by the RSC
- PASS has 9 public analysts, a third of all PAs working in the UK, and works with over 200 local authorities



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## New UK Headquarters - Wolverhampton

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- Increase in laboratory capacity, 40,000 square feet, purpose-built
- Investment in state-of-the art instrumentation and expertise
- UKAS accredited to ISO:17025
- Application of 'Lean' processing





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# Eurofins Competence Centers

## Lidköping (Sweden)

- Food Chemistry
- Metals
- Pesticides

## Vejen (Denmark)

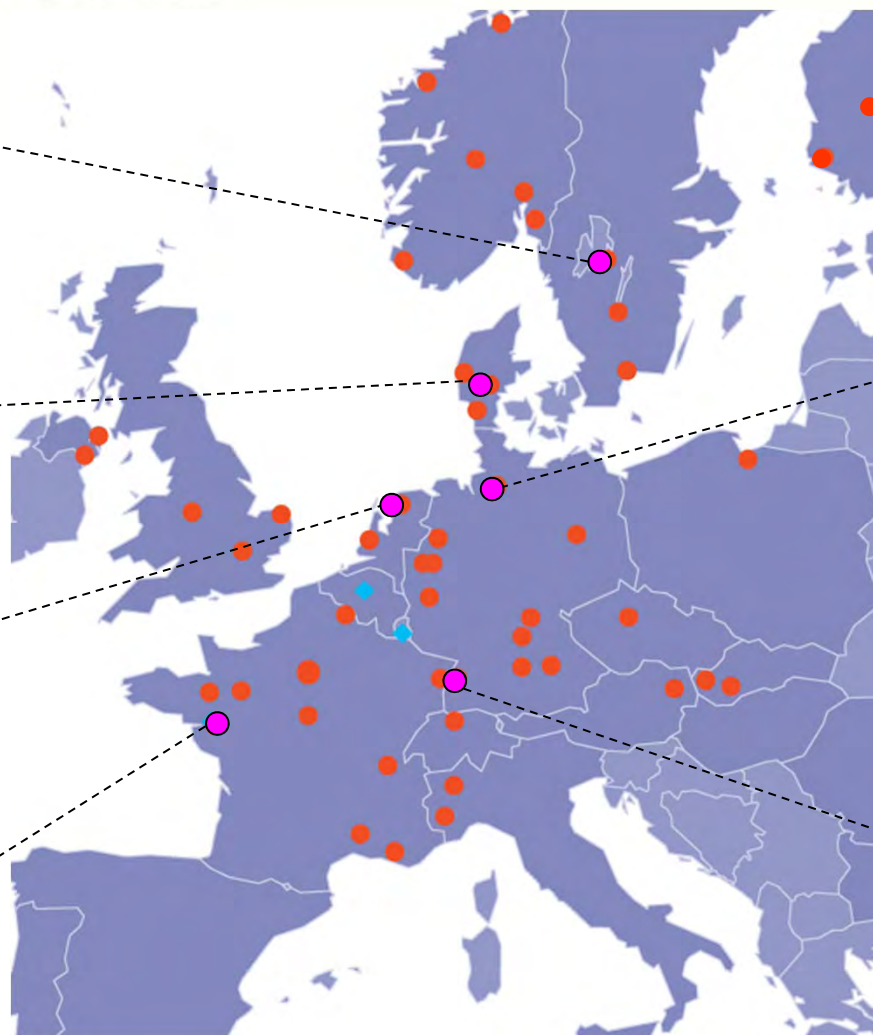
- Vitamins
- Amino Acids

## Heerenveen (The Netherlands)

- Carbohydrates &  
Dietary Fibres

## Nantes (France)

- Authenticity
- Isotopic analysis
- Allergens



## Hamburg (Germany)

- Pesticides (Dr. Specht)
- Mycotoxins (WEJ)
- Veterinary Residues (WEJ)
- Organic Contaminants (WEJ)
- Irradiation (WEJ)
- Dioxins & Persistent Organic Pollutants (GfA)
- Allergens (WEJ)

## Freiburg (Germany)

- GMOs (GeneScan)



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# Food Safety Enforcement

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- Food businesses are responsible in law for the safety and correct labelling of their products
- In the UK the Food Standards Agency is the competent authority for the enforcement of food safety law
- It delegates this responsibility to local authorities
- The work is traditionally carried out by Environmental Health and/or Trading Standards and, e.g. premises inspection, sampling and analysis
- Samples for chemical analysis must be submitted to a Public Analyst working in an official food control laboratory

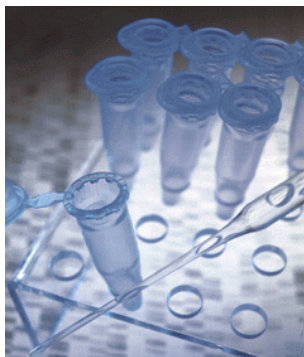


- Labs can be called upon to perform any test on any sample as required by enforcement bodies
- Public Analyst labs traditionally focussed on chemical analysis techniques
- For authenticity testing and food fraud prevention DNA analysis has become one of the methods of choice
- The profession has embraced molecular biology techniques, particularly DNA analysis





## “Authenticity”

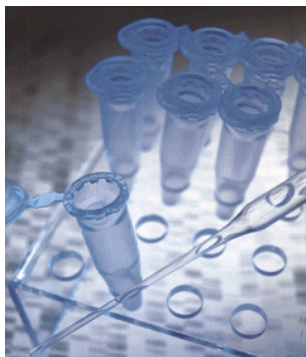


- The quality of being authentic or of established authority for truth and correctness
- Genuineness; the quality of being genuine or not corrupted from the original
- Truthfulness of origins, attributions, commitments, sincerity, and intentions; not a copy or forgery



## Molecular Biology (DNA based techniques)

- PCR – CE (Capillary Electrophoresis/Lab-on-a Chip)
- Real-time PCR (semi-quantitative)
- DNA Sequencing







- Polymerase Chain Reaction – (PCR)
- DNA Molecular Markers
  - Restriction Fragment Length Polymorphism (RFLP)
  - Insertion / Deletions events (INDELS)
  - Micro satellites - simple sequence tandem repeats such as CACACACA (SSTR)
  - Single nucleotide polymorphisms (SNP) single base pair changes in a sequence



- Extraction of good quality DNA from samples – well documented methods for most food and feed matrices and kits are commercially available
- Thermo-cycler required for the PCR stage
- Incubation facilities for restriction enzymes
- Separation and detection of DNA by Lab-on-a-Chip and Real Time PCR technology



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# Food Authenticity

DNA techniques are available for:

- Fish speciation
- Durum wheat pasta
- Basmati rice
- Thai Jasmine rice
- Fruit juice adulteration with other fruits, e.g. orange with mandarin
- Meat speciation
- Genetically modified organisms (GMOs)





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# Fish Species

FISH

Cod or Pangasius?



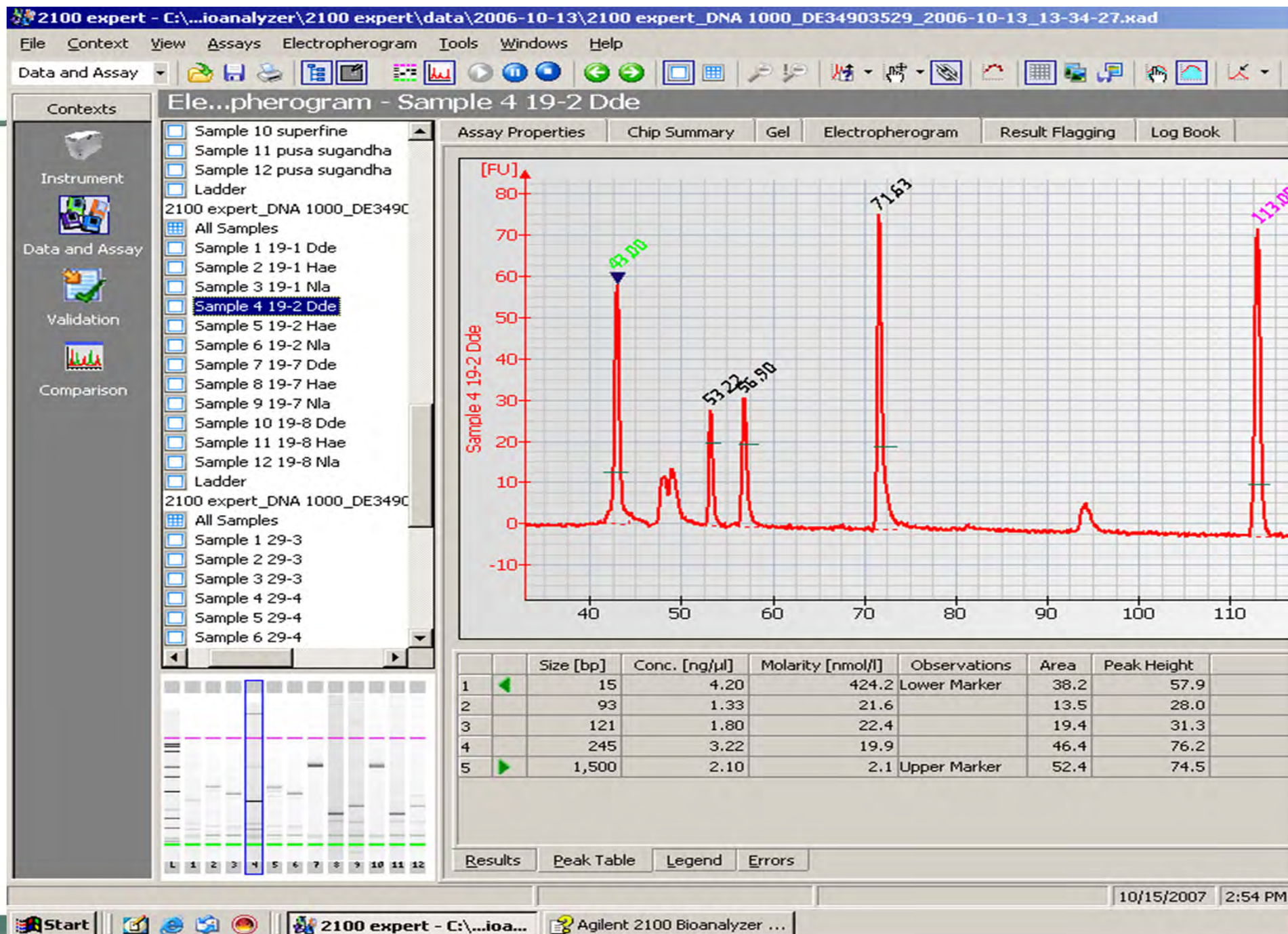


- Method uses PCR-RFLP
- A region of a specific gene is amplified by PCR using universal primers
- Produces a single PCR product of the same size from all samples
- Can be done on raw, cooked and some canned fish (not tuna)



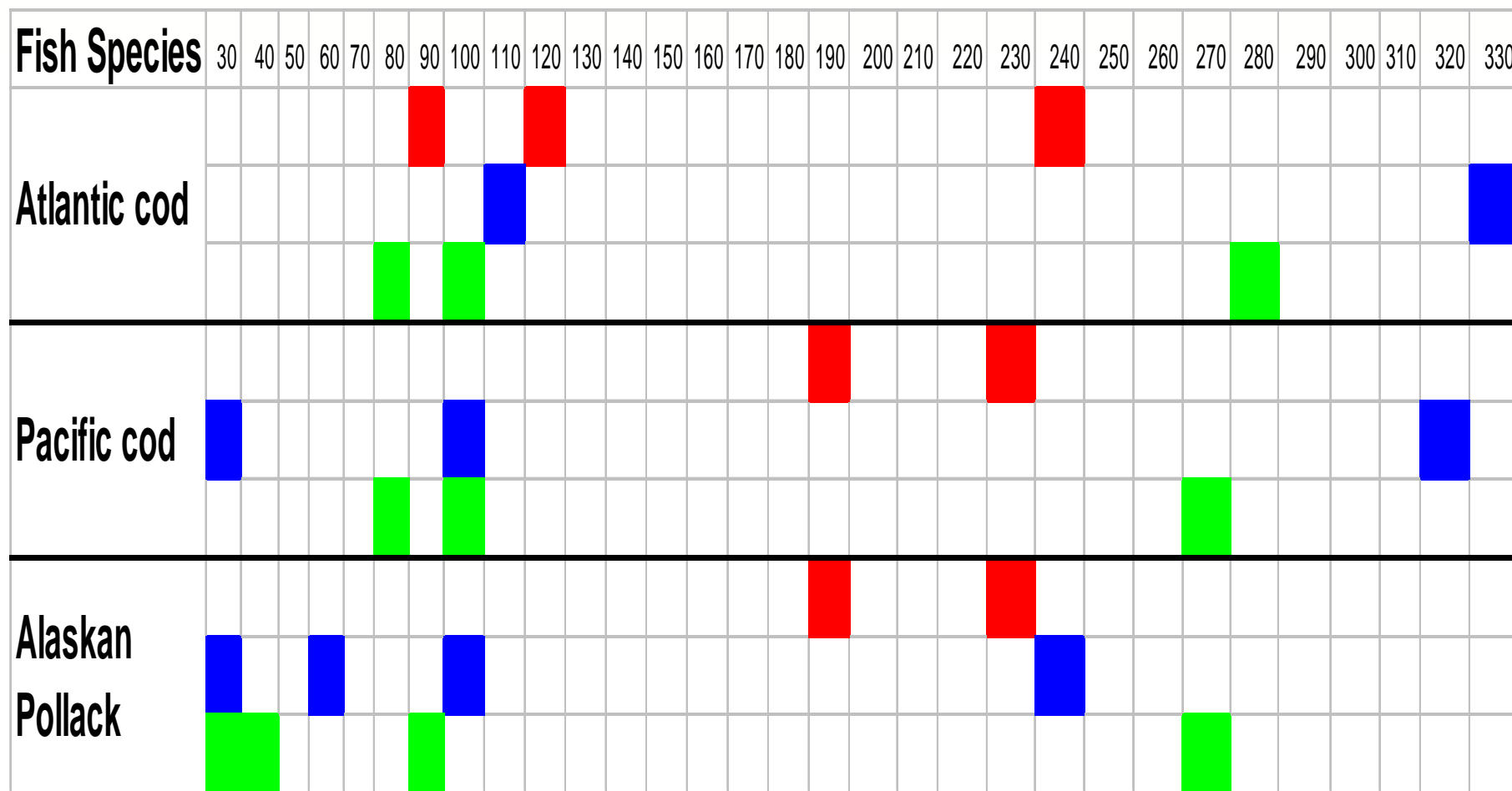
- PCR product is cut using 3 different restriction enzymes to produce a variety of DNA fragments
  - DdeI cuts C<sup>A</sup>TNAG
  - HaeIII cuts GC<sup>A</sup>CC
  - NlaIII cuts CATG<sup>A</sup>
- The fragments produced provide a simple fingerprint typical of the species
- The sample fragment profile from the 3 enzyme digests can then be matched to profiles in the reference database







Restriction Enzyme		Ddel	HaeIII	NlaIII
<b>Gadus macrocephalus Pacific cod</b>		<b>247, 209</b>	<b>335, 130, 37</b>	<b>292, 106, 98</b>
<b>Gadus morhua Atlantic cod</b>		<b>248, 123, 95</b>	<b>332, 111, 47</b>	<b>290, 107, 98</b>



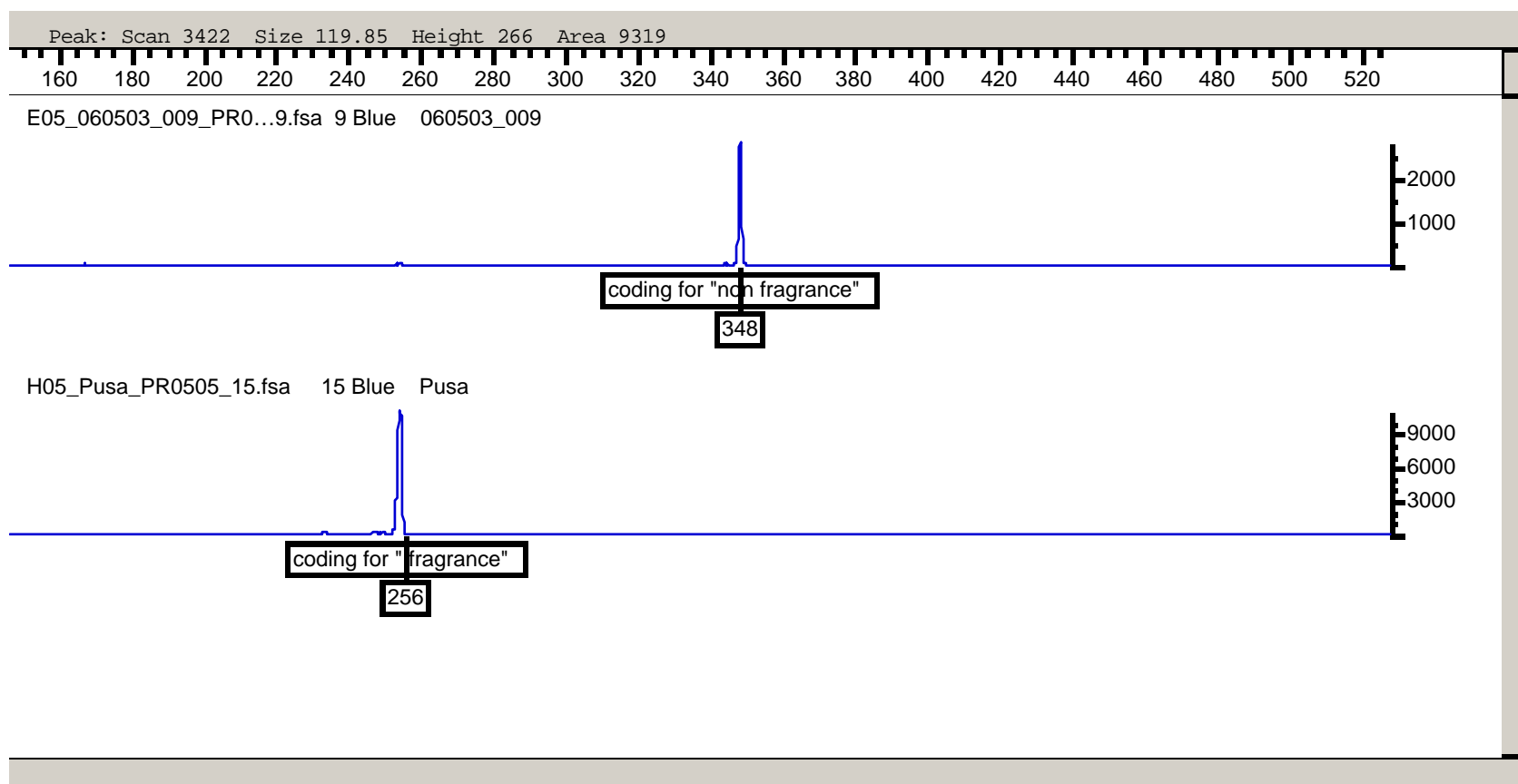


## The Problem:

- Regularly, non-fragrant rice is sprayed with the fragrance of the fragrant-rice, typically **2-acetyl-1-pyrroline** but sold as premium fragrant rice.
- Thai (Jasmine) rice and Basmati Rice have a natural flavour (also **2-acetyl-1-pyrroline**).



- How to distinguish?
- Test for the chemical? Identical in natural and sprayed rice
- But: The fragrant rice has a specific gene that is absent in non-fragrant rice, the 'fragrance gene'.
- This can be detected by PCR

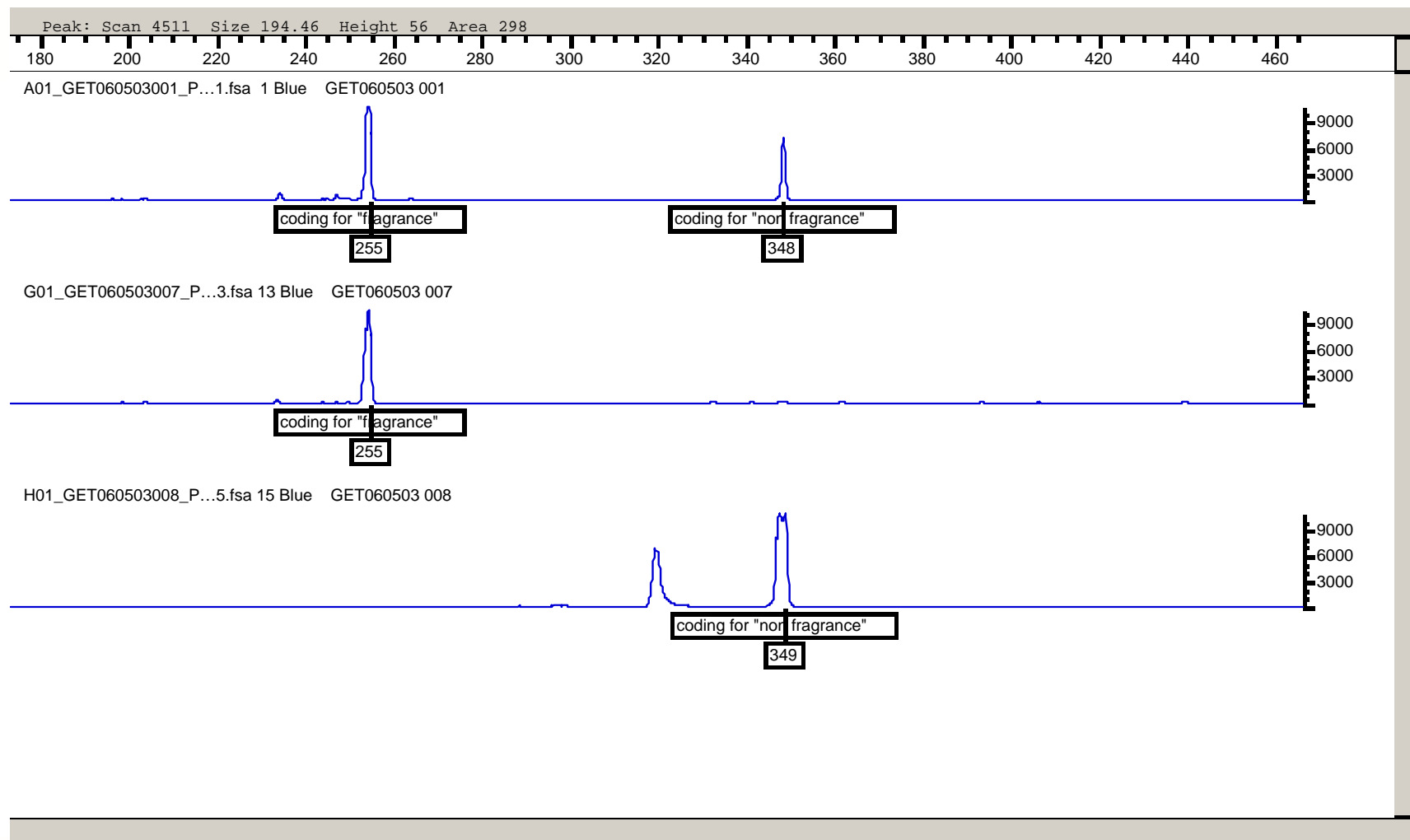






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# Fragrant Rice - Adulteration





- Food Standards Agency Authenticity Project - 2 Methods developed and adapted for Lab-on-a-Chip by Bangor University Food DNA Services
- Qualitative detection of common adulterant varieties using markers based on INDELS
- Quantitative detection of adulterant using 2 separate procedures – micro-satellite marker RM201 and INDEL B8



- Authenticity of cereal crops claiming to be Identity Preserved (IP), i.e. not genetically modified (GM)
- On the world market GM crops are much cheaper so potential for mislabelling and substitution is high
- Most authorised GM varieties can be identified by DNA analysis and semi-quantification is possible
- Common GMO crops – soya and maize (animal feed)
- Rice, sugar beet, potato, cotton, salmon, soft fruits



## 2 Main Techniques:

1. Protein-based - ELISA (species specific antibody / antigen reaction (cooked or raw meat). Qualitative, high limit of detection 1-2%
2. DNA-based (any terrestrial or marine animal)
  - PCR (semi-quantitative), very sensitive
  - DNA sequencing, can identify unknowns





- Illegally Traded Species of Wild Origin
- FSA Project – method developed and adapted for Bioanalyser by Bangor University Food DNA Services
- 11 mammalian species – raw, cooked or skin
- Uses PCR-RFLP, with 2 enzymes to give fragments that can be used as a fingerprint for identification



# Samples of Bush Meat



Figure 2a. Raw water buffalo meat.



Figure 2b. Cooked pig meat.

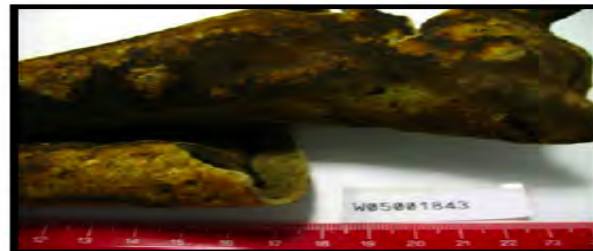


Figure 2c. Dried bovine meat.



Figure 2d. Smoked antelope meat.



Figure 2e. Raw whole pangolin.



Figure 2f. Smoked can rat.





Two restriction enzymes to yield species-specific profiles:

Fragment	Chimpanzee	Gorilla	Duiker	Bushbuck	Cane rat	Pouched rat	Bush pig	Porcupine	Zebu	African sheep
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
No. frags	5	4	3	6	3	4	3	4	6	3



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# Recent Food Scares

- 2013:**     ■ Horse DNA detected in burgers
- 2012:**     ■ Over 70 product recalls in the UK due to undeclared allergens
- 2011:**     ■ E. coli outbreak in Germany (bean sprouts)
- 2010:**     ■ Dioxins in egg products



**Expensive  
Consequences**



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## Case Study - Horse Incident

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The Food Safety Authority of Ireland tested 21 products for horse and pig.

Results were verified by Eurofins Medigenomix in Germany.

Tesco Economy burgers found to contain 29% horse

- Beef burgers, economy burgers
- Ready meals, e.g. meatballs, lasagne, shepherd's pie
- Institutional meals from prisons, hospitals and schools

Found to contain undeclared horse

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## Case Study - Horse Incident

- Real-time PCR used to identify horse and other species, e.g. pork, beef, lamb, goat, chicken, turkey
- This method is very sensitive ( $<0.01\%$ ) and semi-quantitative
- Positive results confirmed by DNA sequencing
- Provenance of horse meat?
- Hygiene?
- Veterinary medicines?





## Case Study - Horse Incident

- Pressure to produce food as cheaply as possible
- Long and complex ingredient supply chains
- Over-reliance on paperwork for traceability
- Lack of any due diligence species testing by manufacturers
- Lack of public resources to check meat for foreign species







Food companies now introducing DNA species testing regimes – a case of shutting the stable door after the horse has bolted? How can enforcement be improved?







- Collaborative working between Public Analysts and the Food Standards Agency and DEFRA on method validation and data gathering projects for food authenticity – through the Authenticity Steering Group
- The future of the UK Public Analyst laboratory network is uncertain due to funding cuts and lack of central coordination and the resources available are likely to be determined by market forces in the next few years



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Thank You

