What is on your plate?

Authenticity Testing

The 2100 Bioanalyzer Instrument as Food Identification System

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Authenticity – What Do I Eat and Where is it From?

What species/variety does my food contain?
⇒ Consumer protection
⇒ Supply chain management
⇒ Protection of endangered species
⇒ Protection of economic interests
  (sustainability, customs duties, etc.)

Where does my product or its ingredients come from?
⇒ Consumer protection and supply chain management
⇒ Monitor and enforce regional protection schemes
⇒ Protection of economic interests
  (fishing rights, sustainability, protected designation of origin, etc.)
Food Fraud – A Fast Growing Problem

Estimated cost of food fraud to the industry: 10-15 bill. US$ (Food Quality & Safety 2010)

Majority of countries have strict food labeling requirements

**EU:**
All ingredients of a food product have to be stated on the label

IUU fishing regulation: Species and catch origin have to be stated for fish and fish products
(2008/1005/EC and 2009/1010/EC)

**US:**
Food Drug and Cosmetics Act, Fair Packaging and Labeling Act, Federal Meat Inspection Act
Mislabeling, Substitution and Adulteration

Factors facilitating and leading to food fraud

- Limitations of the resource (low availability of raw materials)
- Complex multinational supply chains
- Tax/customs duty evasion
- Illegal import of endangered species (CITES)
- Price pressure for finished food products on the market
Substitution and Adulteration – Not Only an Economic Problem

The Economics:

- Damage through tax or customs duties evasion
- If uncovered high costs for product recalls for manufacturers and retailers
- Loss of future revenue due to loss of brand credibility with consumer

The Health Risks:

- Adulteration of food products with harmful/toxic ingredients (eg. melamine in milk)
- Mislabeling of food products known to cause allergic symptoms/negative effects on human health (salmon, pangasius, escolar, crustaceans/shrimps)

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Common Species Authenticity Methods

Protein analysis

- Species specific antibody detection
- Isoelectric focussion (IEF) or capillary gel electrophoresis (CGE)

Issues:
- Proteins highly susceptible to processing
- Cross reactivity of antibodies
- IEF or CGE protein patterns have limited differences between species
- Protein patterns difficult to interpret, experienced user required
- Doesn’t work with mixtures

DNA analysis

- DNA more resistant to sample processing
- Methods adaptable for species specific identification or a wide range screen
- High sensitivity due to PCR amplification
Authenticity – Methods using the 2100 Bioanalyzer System

A large number of authenticity applications have been published using the 2100 Bioanalyzer

Protein Electrophoresis on the 2100 Bioanalyzer instrument:
Differentiation by varying patterns of abundant proteins

DNA Electrophoresis on the 2100 Bioanalyzer instrument:
Analysis of length differences of DNA generated by PCR either directly or in combination with enzymatic fragmentation
Mislabelling means rare fish sold: Marketplace - cbcnews

Legislation against 'food fraud' is weak, says University of Guelph biologist

DNA testing of a variety of fish bought at several Canadian grocery stores reveals products are sometimes mislabelled and some species being sold are endangered, an investigation by CBC-TV's Marketplace has found.

Marketplace purchased 103 pieces of fish — everything from halibut to pickerel, bay bass and shark — at big and small stores across Canada. While many stores had no labeling issues, 34 fish samples, or about 22 per cent, were mislabelled.

2010 CBC news, Canada

"DNA testing of a variety of fish bought at several Canadian grocery stores...some species being sold are endangered...22% were mislabelled"

Mislabeling and substitution affect 25-33% of seafood market

2014 Public

Mislabeling and substitution affect 25-33% of seafood market

2007 Chicago Sun-Times, USA

"DNA tests done on sushi described as red snapper...from 14 restaurants...not one was really red snapper"

2010 CBC news, Canada

"DNA tests done on sushi described as red snapper...from 14 restaurants...not one was really red snapper"

2010 New Brunswick, Canada

"25% of fish products labeled and sold as cod and haddock are from different species, research shows"

2010 Univ. College Dublin, Ireland

"25% of fish products labeled and sold as cod and haddock [in Ireland] are from different species"

2010 NJ.com, USA

"Sterling Seafood founder admits $60M tariff fraud by mislabeling catfish imports"

2010 NJ.com, USA

"Sterling Seafood founder admits $60M tariff fraud by mislabeling catfish imports"
RFLP Method for Fish Identification – The FishID Kit

mitochondrial target sequence

Amplification of target sequence by PCR:

RFLP Decoder Software V2

Restriction enzyme

CYTB PCR product

Dde I

Hae III

Nla III

5’ CTA I G 3’

3’ GTAGC 5’

3’ C GAG 5’

3’ GTAC 5’
PCR-RFLP – Fish Species ID on Tissue Mixtures

Fish DNA or tissue admixtures were tested successfully:
Limit of detection in admixtures: 5%
Interpretation of Results – RFLP Decoder Software

Key features of the RFLP Decoder Software:

- Ease of use, straightforward data analysis and clear results

- Agilent supplied fish species databases with 92 experimentally derived profiles from authenticated samples and 189 sequence derived, theoretical profiles

- Users can create and modify their own database

- Software indicates possible mixtures and provides mixture analysis

- Sample reports can be generated in print and as PDF
RFLP Decoder Analysis Workflow
RFLP Decoder Mixture Analysis
RFLP Decoder V2.0.0.0 – Added Flexibility

The new RFLP Decoder software adds more flexibility for new potential customer applications:

- Supports RFLP applications using 2, 3 or 4 user-definable enzymes
- Supports single well pattern matching for protein or DNA based applications
- Enables analysis of published authenticity applications for the 2100 Bioanalyzer system with user created databases
- Supports database storage locations anywhere on the hard drive or a networked drive
The Building Blocks of the Agilent Fish Species ID Solution

RFLP Decoder Software
PN G5301A

Agilent 2100 Bioanalyzer & DNA 1000 Assay
300 wells/100 samples, PN 5067-1504

Custom Primers

Agilent PCR-RFLP Kit
(PCR Mastermix, Primers & Restrictionenzymes)
100 reactions, PN 5500-0001

Custom Restrictionenzyme

Agilent DNA Isolation Kit
50 samples, PN 5500-0051

Customer's Own Method

Agilent Technologies Genomics

Home Products & Services Applications Resources & Support

Home > PCR & RT-PCR > PCR-RFLP > DNA FISH ID Ensemble
Authenticity – 2100 Bioanalyzer System Applications

Meat Species Identification

- Multiplexed PCR and 2100 Bioanalyzer instrument detection
- Analysis of bush meats or Spanish game meats
- It is known that primers used in the Agilent Fish ID solution may also produce results from certain meats but analysis of mixtures is not recommended
- horse, pork, wild boar, beef, and lamb meat was tested

Adaptation of DNA analysis techniques for the identification of illegally imported bushmeat for use on the Agilent 2100 bioanalyzer

Final Technical Report
For Food Standards Agency United Kingdom
Project Q01109

February 2008

Rob Ogden and Ross McEwing
Food DNA Services Limited

FSA Report on Project Q01109

Development of meat speciation assays using the Agilent 2100 bioanalyzer

Application
Agilent Application Note 5988-4069EN
Authenticity – DNA Patterns Using the Bioanalyzer

Basmati Testing

→ Majority of premium rice market is shared by India and Pakistan with the Basmati varieties cultured there

→ Basmati rice is mainly exported to Saudi Arabia, Kuwait and UAE, 10% are exported into the EU

→ Consumer protection (fraud) and customs tariffs (duty exemptions for certain Basmati varieties in the EU) are a driver for testing

Two SOPs have been developed by The Food Standards Agency (UK)
They will be publicly available in the future

→ SOP1 Screening
→ SOP2 Quantification of non-Basmati in Basmati
Protein analysis on the 2100 Bioanalyzer

Analysis of Milk Proteins:
Protein patterns of $\alpha$-, $\beta$-, $\kappa$-caseins, $\alpha$-lactalbumin and $\beta$-lactoglobulin are specific to a certain species.

Adulteration of milk of a certain species with a lower quality milk or milk of a different species can thus be detected.

Analysis of Wheat Proteins:
Protein patterns of gliadins and glutenins can be used to identify the wheat variety

Adulteration of durum wheat with other varieties or use of non-durum wheat in pasta can be identified.
Summary of Authenticity Testing

Importance of Authenticity Testing in the Food Market
⇒ Consumer protection, supply chain management important drivers
⇒ Large parts of food market affected by fraud for various product categories
⇒ Seafood market most severely affected by fraud
⇒ Wide array of methods available to test different aspects of authenticity

Authenticity Testing on the 2100 Bioanalyzer system
⇒ Small footprint, broad range of robust and high performing assays
⇒ Ability to resolve and analyze complex pattern profiles
⇒ Analysis of reactions with large number of markers → AFLP/MLVA or PCR-RFLP
⇒ Protein as well as DNA or RNA analysis
⇒ Published methods on milk, juices, fruits, rice, grain, wine, meat and fish

The Agilent Fish Species Identification Solution
⇒ Based on a validated method developed by a UK customer
⇒ Straightforward protocol using kit standardized reagents
⇒ Works on a wide range of samples – raw, cooked, fried, salted, dried, smoked
⇒ Analysis software identifies most likely species against a database of 92 fish profiles
⇒ Mixture analysis possible
Thank you!