

European Research Projects on Food & Molecular Biology Methods

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Genescan AgroFood

GMO Testing Service Worldwide

Detection of Animal Species

Analysis of Processed Food Products

Analytic Kits (GMOQuant, BosQuant, ...)

Identity Preservation

Consulting and Certifications

Genescan R&D

Research Projects of the European Commission
(Framework Program 5; www.cordis.lu)



Development of Quantitative and Qualitative Molecular Biological Methods to Identify **Plant** and **Animal** Species in Foods

**MOLSPEC-
ID**

Partner

AgroFood - R&D (o.degen@genescan.com)

Development of molecular genetic methods for the identification and quantification of **fish and seafood**

DNA-IQ

Coordination

Objectives: Monitoring of product safety and traceability

Focus : DNA-analytical methods for qualitative and quantitative identification of plant and animal species.

Comparison of nucleic acid-based methods with protein-based ones.

First steps towards “HTS”: multiplex-PCR, PCR-ELISA and chip technology.

Interlaboratory studies for the validation of methods for several species.

MOLSPEC- ID

Development of Quantitative and Qualitative Molecular Biological Methods to Identify Plant and Animal Species in Foods

- 14 Partners out of 11 European countries are involved
- 4 companies, 4 authorities, 6 research institutions (Coord.: Federal Institute for Risk Assessment)

- *Methods for*
 - DNA extraction*
 - Species Identification*
 - Quantification*



Plants : Almond, hazelnut, pea, soybean, wheat...

Animal : Beef, chicken, goat, horse, pork, ostrich...

Sequences

Searching for marker regions
Generating of a project database

DNA-extraction

Comparison of extraction methods
Estimation of DNA per kg/g tissue

Influences on diagnostic systems based on DNA-amplification (PCR):

Inhibitory substances depending on extraction method

Inhibitory structural elements of the DNA

Different DNA content of tissues has to be considered

Degradation of DNA in tissues is possible

Tissue specific rearrangements of DNA regions

Identification of wheat, rye, barley

A qualitative detection system (RFLP) for wheat, rye, barley was developed.

International interlaboratory study for the validation of the method finished.

Further 12 qualitative detection systems (RFLP or specific PCR) for almond, cattle, goat, chicken, duck, hazelnut, kangaroo, ostrich, sheep, pig, soy, turkey are available.

Next steps: New 'state-of-the-art' Real-time PCR systems

- Less time (no restriction analysis+gel).
- Less contamination problems
- Species specific probes possible
- Multiplex systems in one tube

Systems of Genescan & Eurofins for meat identification

Development of high-specific Real-time PCR systems for the identification of beef, pork, horse, kangaroo, ostrich.

New target regions: Single-copy genes for further use in quantification.

Pork-Identification based on Real-time DNA amplification.

PCR-system SUS: ABI Prism[®] 7900 HT

2 Primers & 1 probe (FAM-DQ MGB) Amplikon:79 bp. By GS.



Beef-Identification based on Real-time DNA amplification.

PCR-system BOS: ABI Prism[®] 7900 HT

2 Primers & 1 probe (VIC-DQ MGB) Amplikon:82 bp. By GS.



Horse-Identification based on Real-time DNA amplification.

PCR-system HSP: Roche Light Cycler[®]

By Eurofins Nantes.

Validation of Real-time PCR systems for identification:

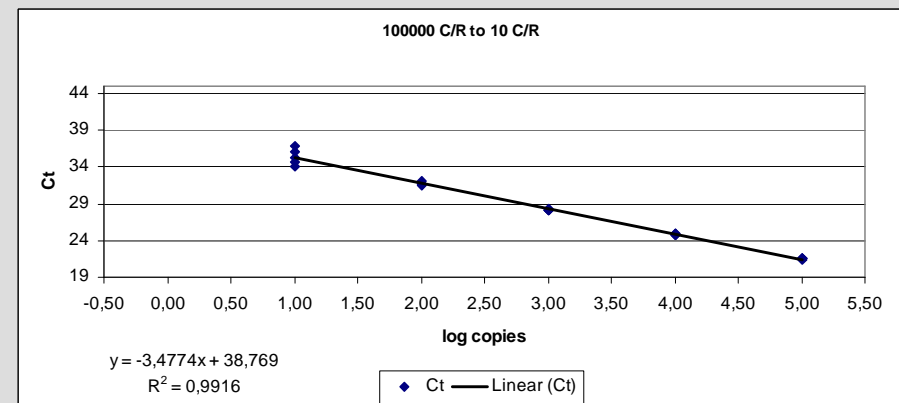
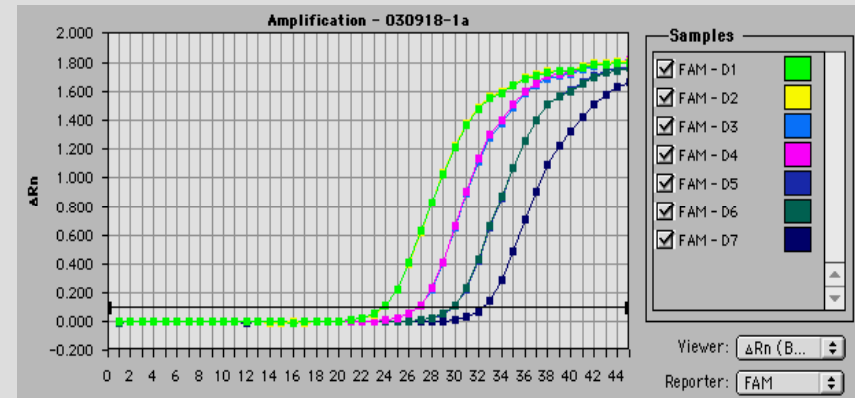
Selectivity: high-specific (SUS for pork; BOS for beef).

Applicability: Tested on model mixtures of processed food provided by BAFF, identification possible in sausages, home canned cans (Fc-value=0,8), normal cans (Fc 3,4), tropical conditions (Fc 12,2).

Linearity: 5 Replikates of different dilutions 100% meat gDNA tested.

Copies	Replicates	Cts
100000	5x	21,5
10000	5x	24,80
1000	5x	28,18
100	5x	31,74
10	5x	35,42

SUS



Systems for quantification (incl. multiplex)

Pork-system SUS: FAM-DQ MGB

Chorda-system REFERENCE: BoTMR-BHQ2

Calculation of % = copy numbers **Pork** / **Chorda**

Duplex Pork-Beef system SUS-BOS:

FAM-DQ MGB and VIC- DQ MGB in one tube

Chorda-system REFERENCE: BoTMR-BHQ2

Applicability: Tested on model mixtures of processed food provided by BAFF, identification possible in sausages, home canned cans (Fc-value=0,8), normal cans (Fc 3,4), tropical conditions (Fc 12,2).

→in meat mixtures even 0,1 % cattle or pork was reliably detected

DNA-IQ

Seafood and consumer protection (coordinated by Genescan)

Background:

- rise of consumption and trade of fish and seafood
- significant increase of variety of fish products
- decrease of the natural stock of high-quality fishes (fraud!)
- demand for fast labelling methods esp. for import/export products
- 6 Partners out of 4 European countries are involved

Methods for DNA extraction

Species Identification (PCR, Real time PCR, RFLP, SSCP)+

Quantification (Real time)



DNA-IQ

outcome

- DNA-extraction protocols for seafood.
- DNA contents of whole fish homogenates and fish eggs.
- Sequences on regions of interest.
- New fish reference PCR system (by GS).
- DNA detection systems for >20 commercial important and high-quality species: anchovy, arctic charr, cod, dab, flounder, hake, halibut, limanda, mackerel, plaice, pollack, rainbow trout, salmon, sardine, scallop, sevruga, solea, sturgeon, tuna, trout, turbot.
- International validation ring trials for
 - Identification of salmonids (SSCP)
 - Fish detection with Real time PCR
 - Quantification of European plaice

DNA-IQ

outcome

Publications of the results

Sotelo C.G., Pérez-Martín R.I. 2002. Species identification in processed seafoods. In Safety and quality in fish processing. Bremmer A. (Ed.), Woodhead Publishing, London (UK)/CRC Press.

B. Horstkotte, H. Rehbein (2003) J. Food Science, 68, 2658-2666.

R.I. Pérez Martín, Carmen G. Soletto (Editors) (2003) : Authenticity of species in meat and seafood products.

O. Degen (2004): Seafood and Consumer Protection. An Official Publication of the European Community, EUR 21031.

Research Projects of the
European Commission

What do we learn?

Transferring the GMO
example to animals.

One of the main tasks of the European Union is to strengthen consumer confidence in the safety of food products.

Principles for consumer protection stating that

“you should know what you are eating” and

“consumer should not be misled”

Strong demand for systems to control food (stop fraud!).

The breakthrough of DNA based methods was the validation and standardization of DNA-based detection and quantification systems for plant GM material.

Research Projects of the European Commission

What do we learn?

- Real-time PCR is applicable for species identification on three PCR platforms: ABI Prism[®] , Biorad iCycler[®] & Roche LightCycler[®]
- >40 new species-specific PCR systems available
- 5 systems validated in international ring trails
- Real-time PCR useful for unprocessed & processed food
- Processed food display higher but tolerable SD values
- Demand for standard reference material (artificial material ? Performance?)
- Demand for standard methods, add-on protein-based methods
- Basis systems for future GM-animal/plants: salmon, cod, beef, vegetable...

Genescan R&D

New Research Projects of the European Commission
(Framework Program 6; www.cordis.lu/fp6)

- back to the GMOs –

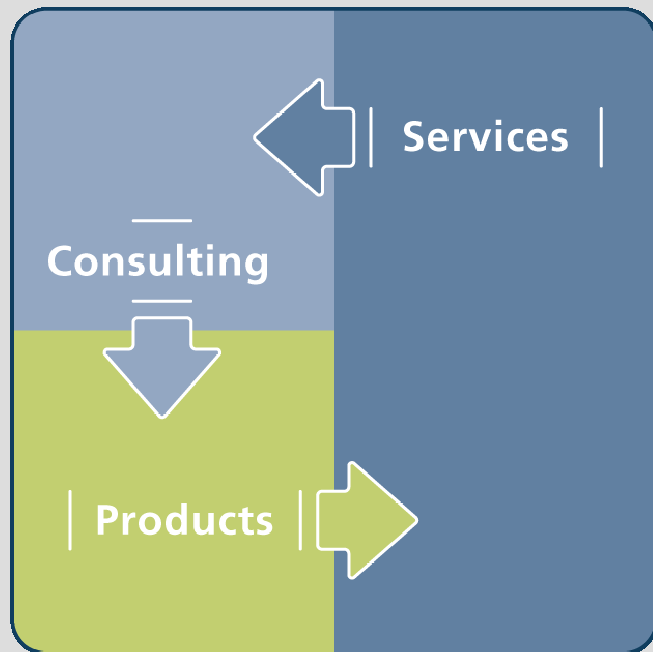
A new 'Integrated Project' on GMOs.

Focus on co-existence and traceability.

Some objectives are...

- * provide new analytical methods
- * detection of unknown GMOs
- * detection of stacked genes...

Genescan – Leader in GMO detection



THANKS FOR YOUR ATTENTION

Services

- Qualitative screening analysis to determine content for GMO
- Qualitative Identification of animals, plants and GMOs
- Quantitative analysis of GMO content

Products

- DNA based kits
- Qualitative screenings
- Quantitative analysis
- Protein based kits
- DNA extraction kits

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