From analytical methods for labeling towards analytical methods for control: Knowledge-technology-based approaches

Tom Ruttink, Dany Morisset
Jana Zell, Kristina Gruden, Marc De Loose

29 & 30 September 2010

Enlargement/Networking Workshop on Harmonisation of GMO Analysis

European Commission TAIEX
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Agriculture and Fisheries Policy Area

IT-based strategy supporting GMO detection

Anal Bioanal Chem
DOI 10.1007/s00216-009-3218-6

FEATURE ARTICLE

Knowledge-technology-based discovery of unauthorized genetically modified organisms

Tom Ruttink · Dany Morisset ·
Bart Van Droogenbroeck · Nada Lavrač ·
Guy L. M. Van Den Eede · Jana Žel · Marc De Loose

Anal Bioanal Chem
DOI 10.1007/s00216-009-3287-6

ORIGINAL PAPER

Molecular toolbox for the identification of unknown genetically modified organisms

Tom Ruttink · Rolinde Demeyer · Elke Van Gulck ·
Bart Van Droogenbroeck · Maddalena Querci ·
Isabel Taverniers · Marc De Loose

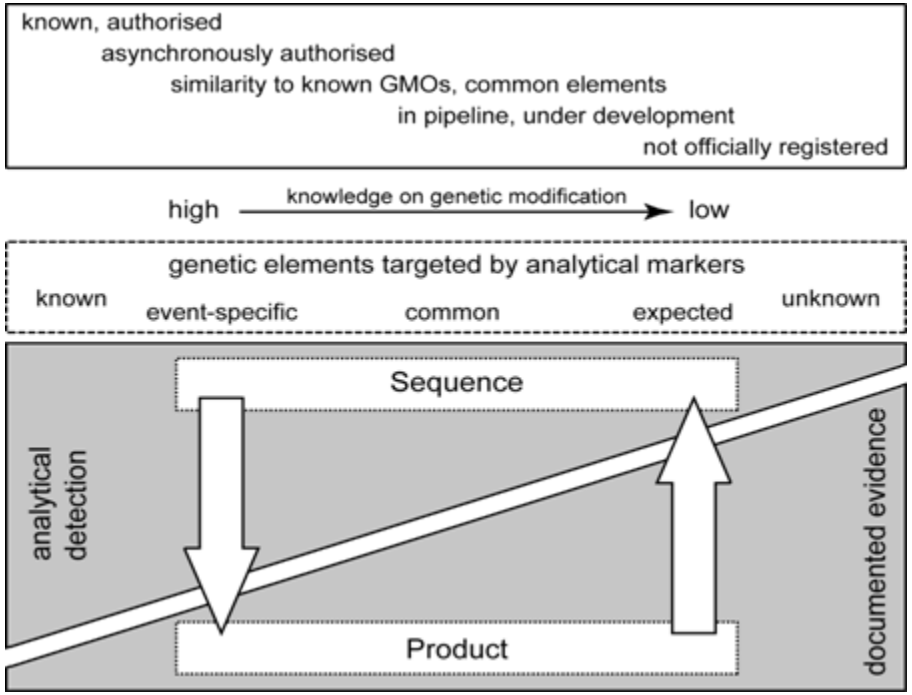
focus on unauthorized GMO discovery

current routine screening designed to detect, identify, quantify known, authorized GMOs
testing blind samples: no prior knowledge on sample composition is used
discovery of some UGM products is possible but:
very low chance, not in admixtures, indirect evidence, only UGMs with screening elements

goals:
improve the use of documented evidence
to discover rare, unexpected UGMs

optimization of product selection,
optimization of choice of analytical tests

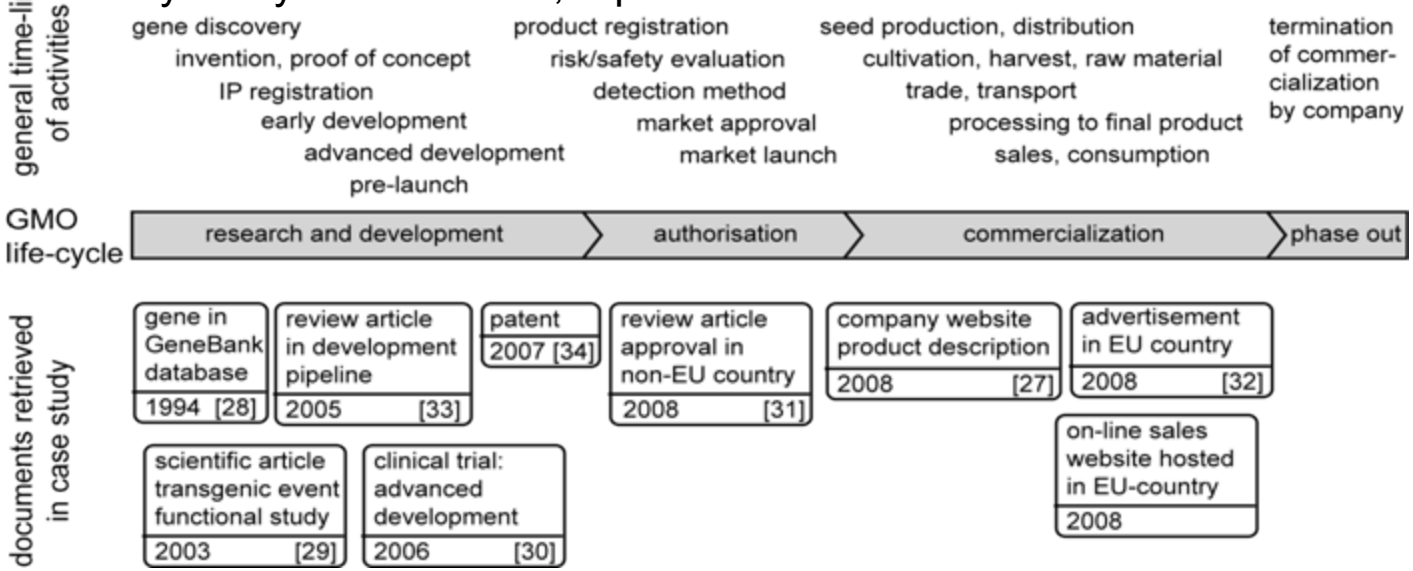
develop a novel method for event identification
by anchor-PCR fingerprinting:
provide direct evidence for UGM
discover masked UGM events



UGM discovery by Knowledge technologies: web based search

proposed conceptual framework:

1. design model to describe the R&D, authorization, commercialization phase of GMO `life cycle`
2. collect documents about novel GMO-derived products on the Web
3. match documented evidence to the model: trace the GMO `life-cycle` per product
4. identify products that do not comply with authorization
5. collect DNA sequence information on novel events
6. select suspicious products for targeted analytical confirmation
7. proof of concept: novel unauthorized GMO discovered on the EU market in 2008, identity confirmed by analytical evidence, reported in RASFF



How can documentation-based evidence complement routine screening?

1. it increases efficiency of analytical testing:
2. risk-based selection of suspicious products enriches the lab with suspicious samples (less `negative results` compared to random sampling)
3. it is used to optimize the choice of tests and reduce the number of tests per product (case-by-case; reduce operational cost)
4. the Matrix approach may be updated with new elements,to detect novel UGM events: documented evidence identifies the `most urgent` cases and guides the best choice for new screening methods (manage development cost).
5. it contains information to design a specific detection method for targeted confirmation of a suspicious product (a guided analysis is more efficient than a blind reconstruction)
6. it discovers products for which no routine analytical test exists
7. Web-based searches enhance market coverage and reduce time to discovery after UGM release
8. fast discovery crucial to limit further spreading of an UGMs in the food, feed, pharma chains

conceptual framework and real-life case study described in:

Ruttink *et al*. (2009) Knowledge-technologies-based discovery of unauthorized GMOs Anal Bioanal Chem

scientific reviews on novel GMO traits

25 APRIL 2008 VOL 320 SCIENCE www.sciencemag.org

Plant Genomes

Selected Plant-Made Pharmaceuticals

Company	Plant	Grown in	Drug or product	Disease	Status
Human drugs					
Protalix Biotherapeutics	carrot	cell culture	glucocerebrosidase	Gaucher disease	Phase III trial*
Biolex Therapeutics	duckweed	indoor chambers	alpha interferon	hepatitis C	Phase II trial*
SemBioSys Genetics	safflower	field	insulin	diabetes	Phase I/II trial †
Meristem Therapeutics	corn	field	lipase	cystic fibrosis	Phase III trial †
Other products					
Ventria Bioscience	rice	field	lactoferrin, lysozyme	diarrhea	Efficacy trial §
Cobento	Arabidopsis	greenhouse	human intrinsic factor	Vitamin B-12 deficiency	Approved ††
Planet Biotechnology	tobacco	field	secretory antibody vaccine	tooth decay	E.U. approved
Dow AgroSciences	tobacco	cell culture	poultry vaccine	Newcastle disease	USDA approved
CIGB, Cuba	tobacco	greenhouse	vaccine purification antibody	hepatitis B	On market

* Ongoing; † Projected late 2008; § Completed; †† In Ukraine.

Steps along the way. No plant-made human drug has made it through final clinical trials, but several "pharmed" proteins are close to or on the market as supplements, a vaccine reagent, and a medical device.

other references

- www.molecularfarming.com:pmps-and-pmips.html
- http://www.isaaa.org/Kc/inforesources/publications/pocketk/Pocket_K26_(English).pdf

Table 1. Plant-derived pharmaceuticals for the treatment of human diseases that are in the pipeline for commercialization.

Product	Class	Indication	Crop
Various single-chain Fv antibody fragments	Antibody	Non-Hodgkin's lymphoma	Viral vectors in tobacco
CaroRx	Antibody	Dental caries	Transgenic tobacco
E. coli heat-labile toxin	Vaccine	Diarrhea	Transgenic maize Transgenic potato
Gastric lipase	Therapeutic enzyme	Cystic fibrosis, pancreatitis	Transgenic maize
Hepatitis B virus surface antigen	Vaccine	Hepatitis B	Transgenic potato Transgenic lettuce
Human intrinsic factor	Dietary	Vitamin B12 deficiency	Transgenic *Arabidopsis*
Lactoferrin	Dietary	Gastrointestinal infection	Transgenic maize
Norwalk virus capsid protein	Vaccine	Norwalk virus infection	Transgenic potato
Rabies glycoprotein	Vaccine	Rabies	Viral vectors in spinach
Cyanoverin-N	Microbicide	HIV	Transgenic tobacco
Insulin	Hormone	Diabetes	Transgenic safflower
Lysozyme, Lactoferrin, Human serum albumin	Dietary	Diarrhea	Transgenic rice

Source: Compiled by ISAAA, 2007.

Cobento

COBENTO

Cobento A/S
Gustav Wieds Vej 10
DK-8000 Aarhus C, Denmark
Phone: (+45) 86 20 50 95
Fax: (+45) 86 20 50 99
E-mail: info@cobento.dk

Search

Vitamin B12-binding proteins

The transfer of vitamin B12 from food to tissue cells involves three vitamin B12-binding proteins:

- **Haptocorrin (HC)** is a vitamin B12-binding protein present in saliva and blood. It binds to vitamin B12 in the oral cavity. Vitamin B12 bound to HC from the oral cavity is released when gastric juices from the stomach denature HC.

- **Intrinsic factor (IF)**, is a vitamin B12-binding protein synthesized in gastric parietal cells and secreted into gastric juice. In the stomach, vitamin B12 is separated from food and transferred to IF. The IF-B12 complex binds to an intestinal receptor. Vitamin B12 then passes into the blood circulation, while IF is degraded by intestinal cells.

- **Transcobalamin (TC)** is the only vitamin B12-binding protein that delivers vitamin B12 to tissue cells. When vitamin B12 enters the blood stream from the intestinal IF-B12 complex, TC binds to vitamin B12 and delivers it to the tissue.

Cobento Coban

COBENTO

Cobento A/S
Gustav Wieds Vej 10
DK-8000 Aarhus C, Denmark
Phone: (+45) 86 20 50 95
Fax: (+45) 86 20 50 99
E-mail: info@cobento.dk

Search

Coban

Coban tablets have recently been developed by Cobento as a food supplement containing both intrinsic factor and vitamin B12.

Recombinant human intrinsic factor (rhIF) is collected from dried, powdered transgenic Arabidopsis leaves, which express this protein. Vitamin B12 is added to the protein mixture prior to tablet preparation.

Each Coban tablet contains 135 mg purified rhIF and 3.6 mcg vitamin B12.

Cobento rhIF

COBENTO

Cobento A/S
Gustav Wieds Vej 10
DK-8000 Aarhus C, Denmark
Phone: (+45) 86 20 50 95
Fax: (+45) 86 20 50 99
E-mail: info@cobento.dk

Search

Recombinant human intrinsic factor (rhIF)

Cobento produces recombinant human intrinsic factor (rhIF) using transgenic plants. This protein's amino acid sequence identically matches naturally occurring human intrinsic factor (100%). Like human intrinsic factor, recombinant human intrinsic factor is naturally glycosylated to provide the same vitamin B12 binding characteristics. Recombinant human intrinsic factor not only binds to vitamin B12 but also delivers it to the intestinal receptors exactly as human intrinsic factor. Additionally, rhIF is purified to remove any other possible proteins.

rhIF technical parameters

- Stability: 2 years when stored at 4 °C (lyophilized powder or solution)
- Solubility: Soluble in suitable aqueous buffers (e.g. 0.2M sodium phosphate at pH 7.4)
- Specificity: Binds and delivers vitamin B12 to intestinal receptors
- Activity: >80% of rhIF molecules bind to vitamin B12
- Production: According to cGMP
- Availability: 1mg glass vials

Cobento CobaSorb

COBENTO

Cobento A/S
Gustav Wieds Vej 10
DK-8000 Aarhus C, Denmark
Phone: (+45) 86 20 50 95
Fax: (+45) 86 20 50 99
E-mail: info@cobento.dk

Search

The CobaSorb test - a non-radioactive Schilling test

Cobento is patent pending a non-radioactive Schilling test, called the CobaSorb test. This new vitamin B12 absorption test has been successfully tested in a clinical trial involving 37 patients (*Hvas et al. 2006. The Hematology Journal; 91 (6), 805-808. "The effect of recombinant human intrinsic factor on the uptalke of vitamin B12 in patients with evident vitamin B12 deficiency*).

In brief, the blood samples are collected from patients before and after ingestion of:

- Day 1: vitamin B12 (non-radioactive)
- Day 2: vitamin B12 in complex with rhIF

The blood samples are subsequently tested for changes in serum B12 and holo-TC levels.

The CobaSorb test eliminates the problems involved with the traditional Schilling test, i.e. the risk of disease transfer induced by the use of human gastric IF, the inconvenient use of radioactive cobalamin, and the 24-hour urine collection from patients.

generation of event

Eur. J. Biochem. **270**, 3362–3367 (2003) © FEBS 2003 doi:10.1046/j.1432-1033.2003.03716.x

Human intrinsic factor expressed in the plant *Arabidopsis thaliana*

Sergey N. Fedosov[1], Niels B. Laursen[1,2], Ebba Nexø[3], Søren K. Moestrup[4], Torben E. Petersen[1], Erik Ø. Jensen[5] and Lars Berglund[1,2]

[1]*Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, Denmark;* [2]*Cobento Biotech A/S, Science Park, Aarhus C, Denmark;* [3]*Department of Clinical Biochemistry, AKH Aarhus University Hospital, Denmark;* [4]*Department of Medical Biochemistry, University of Aarhus, Denmark;* [5]*Laboratory of Gene Expression, Department of Molecular and Structural Biology, University of Aarhus, Denmark*

Intrinsic factor (IF) is the gastric protein that promotes the intestinal uptake of vitamin B_{12}. Gastric IF from animal sources is used in diagnostic tests and in vitamin pills. However, administration of animal IF to humans becomes disadvantageous because of possible pathogenic transmission and contamination by other B_{12} binders. We tested the use of recombinant plants for large-scale production of pathogen-free human recombinant IF. Human IF was successfully expressed in the recombinant plant *Arabidopsis thaliana*. Extract from fresh plants possessed high B_{12}-binding capacity corresponding to 70 mg IF per 1 kg wet weight. The dried plants still retained 60% of the IF activity. The purified IF preparation consisted of a 50-kDa glycosylated protein with the N-terminal sequence of mature IF. Approximately one-third of the protein was cleaved at the internal site …PSNP↓GPGP. The key properties of the preparation obtained were identical to those of native IF: the binding curves of vitamin B_{12} to recombinant IF and gastric IF were the same, as were those for a B_{12} analogue cobinamide, which binds to IF with low affinity. The absorbance spectra of the vitamin bound to recombinant IF and gastric IF were alike, as was the interaction of recombinant and native IF with the specific receptor cubilin. The data presented show that recombinant plants have a great potential as a large-scale source of human IF for analytical and therapeutic purposes.

Keywords: *arabidopsis*; cobalamin; intrinsic factor; recombinant.

patent application

(19) AUSTRALIAN PATENT OFFICE

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(54) Title
Transgenic plants expressing cobalamin binding proteins

(21) Application No: 2007237286

(22) Application Date: 2007.11.30

(43) Publication Date : 2007.12.20

(43) Publication Journal Date : 2007.12.20

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(71) Applicant(s)
Cobento A/S

(72) Inventor(s)
Berglund, Lars Erik; Fedosov, Sergey Nikolaevich; Petersen, Torben Ellebaek; Nexo, Ebba; Jensen, Erik Ostergaard; Laursen, Niels Bech

(74) Agent/Attorney
PIZZEYS, Level 14 324 Queen Street, Brisbane, QLD, 4000

2007237286 30 Nov 2007

ABSTRACT

The present invention relates to the use of transgenic plants for the expression of vitamin B12 (cobalamin) binding proteins. Plant cells are transformed with nucleotide sequences adapted for expression and secretion of vitamin B12 binding proteins. The present invention also relates to the use of recombinant vitamin B12 binding proteins from plants in analytical tests and treatment of vitamin B12 deficiency. Also disclosed is a method for purification of recombinant vitamin B12 binding proteins.

cloning vector, strategy and sequence of extensin signal peptide sequence (AF104327) fused to the mature human intrinsic factor encoding region (X76562) and part of the 3`UTR

patent application

http://india.bigpatents.org/view/7045/3b7d2b881e0

BigPatents India

"Transgenic Plants Expressing Cobalamin Binding Proteins."

Application 141/DELNP/2004 published 2006-02-24, filed 2004-01-20

The present invention relates to the use of transgenic plants for the expression of vitamin B12 (cobalamin) binding proteins. Plant cells are transformed with nucleotide sequences adapted for expression and secretion of vitamin B12 binding proteins. The present invention also relates to the use of recombinant vitamin B12 binding proteins from plants in analytical tests and treatment of vitamin B12 deficiency. Also disclosed is a method for purification of recombinant vitamin B12 binding proteins.

Applicant

Cobento Biotech Aps
Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark

Inventor

1. LARS ERIK BERGLUND 2. TORBEN ELLEBAEK PETERSEN 3. SERGEY NIKOLAEVICH FEDOSOV 4. EBBA NEXO 5. NIELS BECH LAURSEN 6. ERIK OSTERGAARD JENSEN

International Info

Classification: C12N 15/82
Publication Number: WO 03/006661
Application Date: 2002-07-12

Priority Information

0117171.9 UNITED KINGDOM 2001-07-13

clinical trial

Red Cell Disorders • Brief Report

The effect of recombinant human intrinsic factor on the uptake of vitamin B12 in patients with evident vitamin B12 deficiency

Anne-Mette Hvas
Thora Buhl
Niels Bech Laursen
Birger Hesse
Lars Berglund
Ebba Nexo

We report on the use of recombinant human intrinsic factor (rhIF) in a new vitamin B12 absorption test. Holotranscobalamin (holoTC) was measured before and 24 hours after intake of three 9-µg doses of vitamin B12 (B12) and again 24 hours after intake of the same dose of B12 together with rhIF (rhIF-B12). Nine patients with evident vitamin B12 deficiency had a significantly higher increase in holoTC after intake of rhIF-B12 than after intake of B12. Twenty-eight patients with suspected vitamin B12 deficiency showed no additional increase in holoTC after intake of rhIF-B12. We conclude that rhIF promotes B12 absorption among patients with evident vitamin B12 deficiency.

Key words: intrinsic factor, holotranscobalamin, vitamin B12 deficiency, vitamin B12 absorption.

Haematologica 2006; 91:805-808

From the Department of Clinical Biochemistry, Skejby Hospital and Aarhus Hospital, Aarhus University Hospital, Aarhus, Denmark (A-MH, EN); Clinic of Clinical Physiology and Nuclear Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (TB, BH); Protein

The absorption of vitamin B12 is quite complicated requiring intrinsic factor (IF) to ensure efficient uptake.[1,2] After absorption vitamin B12 is bound to transcobalamin; the fraction of transcobalamin saturated with vitamin B12 is referred to as holotranscobalamin (holoTC). Suitable methods for the measurement of holoTC

range (<200 pmol/L). We examined 37 such patients living in the County of Aarhus or the city of Copenhagen, Denmark, recruited between May 2004 and December 2004. None of the patients received vitamin B12 treatment and none was a vegetarian. Vitamin B12 absorption was evaluated by analysis of holoTC in blood samples

advertisement

TERAZ NAPRAWDĘ PRZYSWAJALNA POSTAĆ WITAMINY B12

Dziś, dzięki unikatowemu połączeniu witaminy B12 z Intrinsic Factor możemy pozbyć się problemów związanych z niedoborem witaminy B12.

Coban jest produktem unikalnym i jedynym w swoim rodzaju. Nie posiada konkurencji, ani na polskim rynku, ani na rynku ogólnoświatowym.

Głównym składnikiem preparatu jest glikoproteina Intrinsic Factor, która jest substancją, występującą tylko w przewodzie pokarmowym zdrowych ludzi, gdzie odpowiedzialna jest za przyswajanie witaminy B12 z przewodu pokarmowego do krwi. Inne produkty postrzegane jako konkurencyjne, nie gwarantują absorpcji witaminy B12. Tylko Coban posiada Intrinsic Factor – białko identyczne z ludzkim, które daje GWARANCJĘ przyswojenia przez organizm witaminy B12.

Tabletki Coban zawierają witaminę B12 w najbardziej stabilnej formie, wykorzystywanej w suplementach diety oraz składniki bioaktywne wspomagające przyswajanie tej witaminy – sproszkowane liście rzodkiewnika pospolitego (Arabidopsis thaliana) i wyodrębnioną z tej rośliny glikoproteinę, odpowiedzialną za kompleksowanie i wchłanianie tej witaminy (Intrinsic Factor).

W procesie przyswajania witaminy B12 przez organizm w znacznym stopniu za etap jej wchłaniania odpowiedzialny jest białkowy czynnik wewnętrzny (Intrinsic Factor). Należy nadmienić, że część populacji nie wytwarza tej substancji lub występuje ona w niewystarczającej ilości; z tego też względu w preparatach zawierających witaminę B12 jest wprowadzana jako aktywny składnik.

Pomimo dostarczania witaminy B12 wraz z pożywieniem, stwierdza się jej niedobory. Dzieje się tak ze względu na mniejszą sprawność systemu wchłaniana. Sprawność systemu wchłaniania witaminy B12 jest mniejsza u ludzi starszych, u których pomimo wystarczającej podaży z pożywieniem, stwierdza się jej niedobory. Zwiększone zapotrzebowanie na witaminę B12 występuje również u kobiet w ciąży i karmiących oraz u wegetarian, ze względu na brak w diecie produktów pochodzenia zwierzęcego.

Victoria Pharma Sp. z o.o.
ul. Królowej Marysieńki 94A
02-954 Warszawa, Polska

Victoria Pharma

www.kompass.com/pl/DK137641

www.balticnordic.com/en/company-information/QKSMSK/Victoria-Pharma-AS.html

Victoria Pharma A/S

Contact Information for Victoria Pharma A/S
Visiting Address:
Gladsaxevej 356
DK-2860 SØBORG
Denmark

Postal Address:
Gladsaxevej 356
DK-2860 SØBORG
Denmark

Telephone:
+45 38 28 05 05
Cellphone:

Fax Number:
+45 36 45 95 17

Homepage:
http://www.victoria-pharma.com

Contact Persons for Victoria Pharma A/S
Contact Person:

NACE Classification for Victoria Pharma A/S

www.hotfrog.dk/Firmaer/Victoria-Pharma

Victoria Pharma

Lyskær 15, 2730 Herlev, Hovedstadsområdet
p: 44928688
Hjemmeside - Ikke opgivet
Email adresse - ikke opgivet

Victoria Pharma A/S

Lyskær 15
2730 Herlev
Dania
Telefon : +45 44 92 86 88
Liczba zatrudnionych (w całej firmie)
Od 1 do 10
Data założenia
2004
Numer rejestracyjny
27653855
SE-nr
DK 27653855

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COMPANY REPORT

Previous | 1 of 1 company | Next EXPORT SEND TO PRINT

VICTORIA PHARMA A/S

Private company

Number of shareholders	3
Number of subsidiaries	0

CONTACT INFORMATION

Location HERLEV, Denmark

STATUS & ACCOUNT INFORMATION

Legal form	Limited company	**Last available year**	2007
Template	Industrial company	**Account(s) published in**	DKK
Status	Active	**Size**	Very Small

INDUSTRY / ACTIVITIES

NACE Rev. 1.1 Core code :
5146 - Wholesale of pharmaceutical goods
US SIC Core code :
512 - Drugs, drug proprietaries, and druggists' sundries wholesale dealing in
NAICS 2002 Core code :
4242 - Drugs and Druggists' Sundries Merchant Wholesalers

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Efekt! PR
ul. Czerska 18 / 390
00-732 Warsaw, Poland
ph.: (22) 408 87 01 to 03

WITAMINA B12 W PRZYSWAJALNEJ FORMIE

Witamina B12 bywa nazywana czerwoną witaminą i odgrywa ważną rolę w naszym życiu.

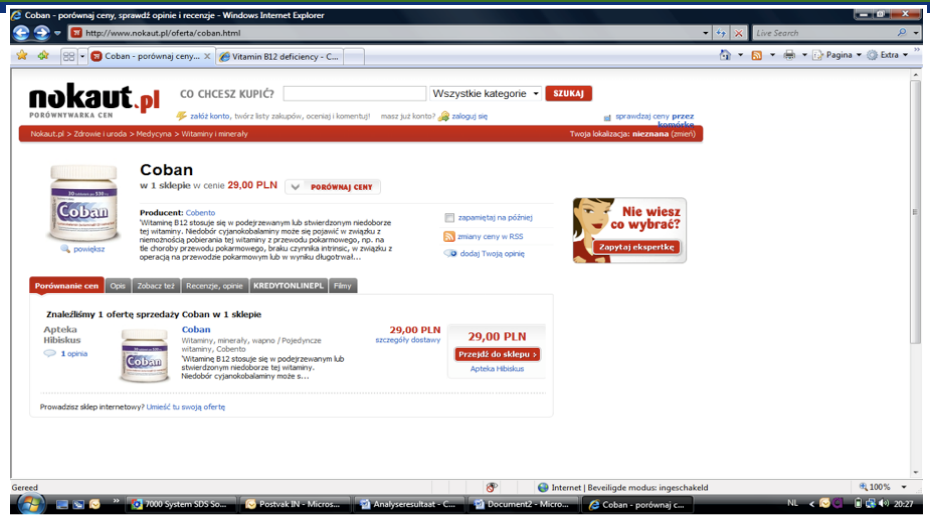
Organizm człowieka potrzebuje witaminy B12, m.in. do wytwarzania krwinek czerwonych i utrzymania układu nerwowego w prawidłowym stanie. Natomiast jej niedobór może doprowadzić do poważnych problemów zdrowotnych.Człowiek wytwarza witaminę B12 jedynie w niewielkich ilościach, dlatego musi być ona dostarczana w odpowiedniej diecie. Jednak pomimo podaży witaminy B12 wraz z pożywieniem, często stwierdza się jej niedobory.

Coban jest produktem unikalnym i jedynym w swoim rodzaju na polskim i ogólnoświatowym rynku- zawierającym witaminę B12 w najbardziej stabilnej formie cyjanokobalaminy. Głównym składnikiem preparatu jest glikoproteina Intrinsic Factor, białko identyczne z ludzkim, które daje gwarancję przyswojenia przez organizm witaminy B12.

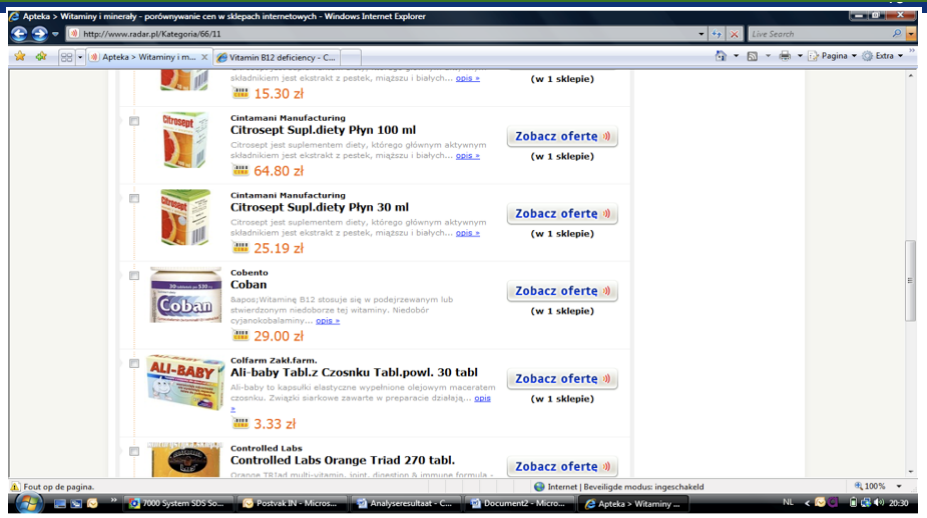
Tabletki **Coban** zawierają witaminę B12 w najbardziej stabilnej formie cyjanokobalaminy- wykorzystywanej w suplementach diety- oraz składniki bioaktywne wspomagające przyswajanie tej witaminy- sproszkowane liście rzodkiewnika pospolitego (Arabidopsis thaliana) i wyodrębnioną z tej rośliny glikoproteinę, odpowiedzialną za kompleksowanie i wchłanianie tej witaminy (Intrinsic Factor).

Celem zaplanowanych działań agencji jest kształtowanie wizerunku produktu oraz edukacja na temat niedoborów witaminy B12.

online sales



www.nokaut.pl/oferta/coban.html



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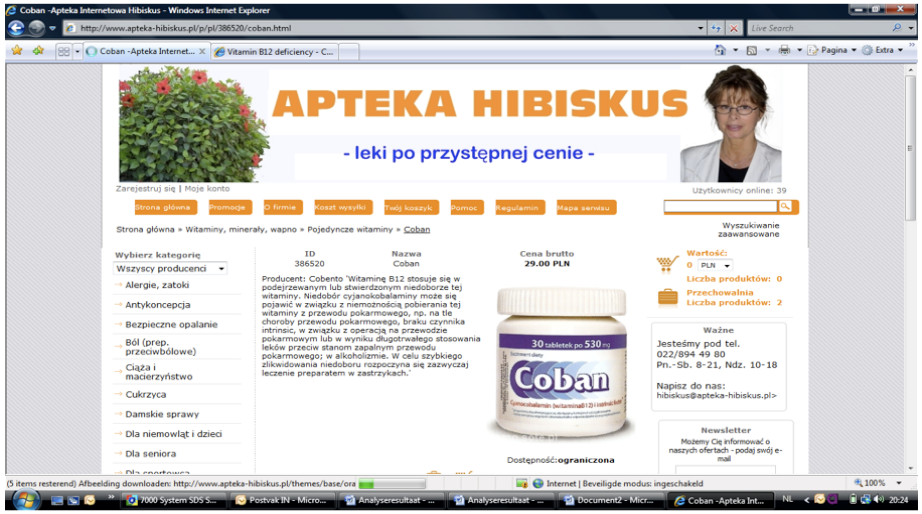

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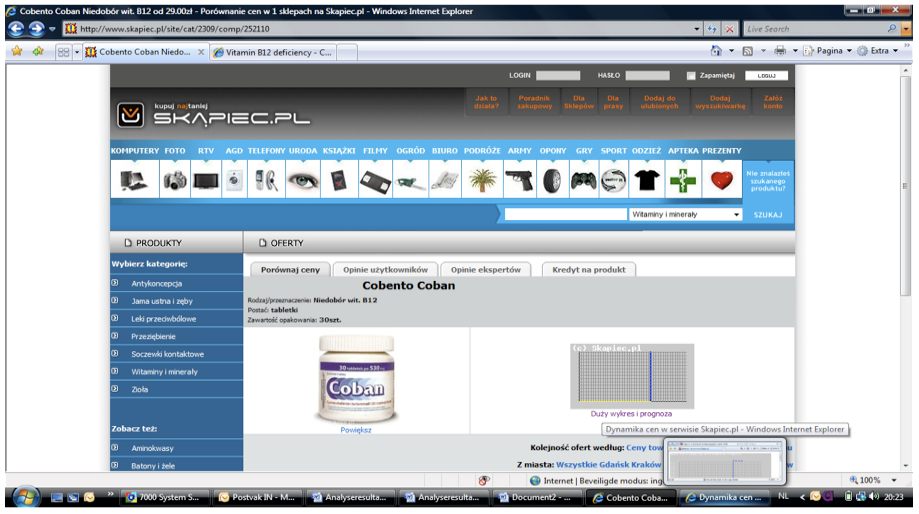
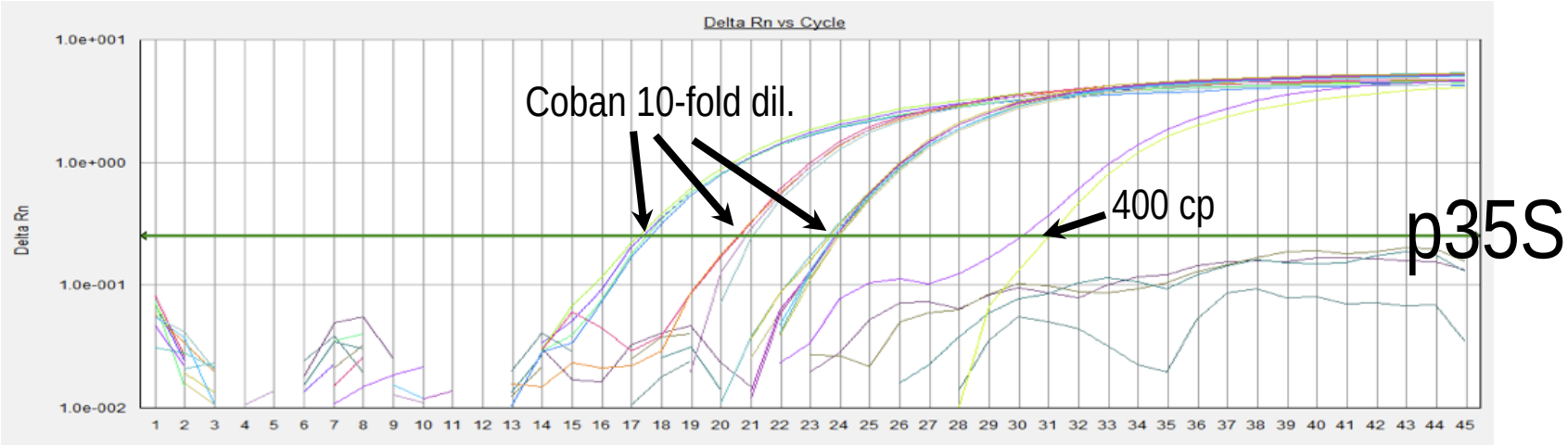


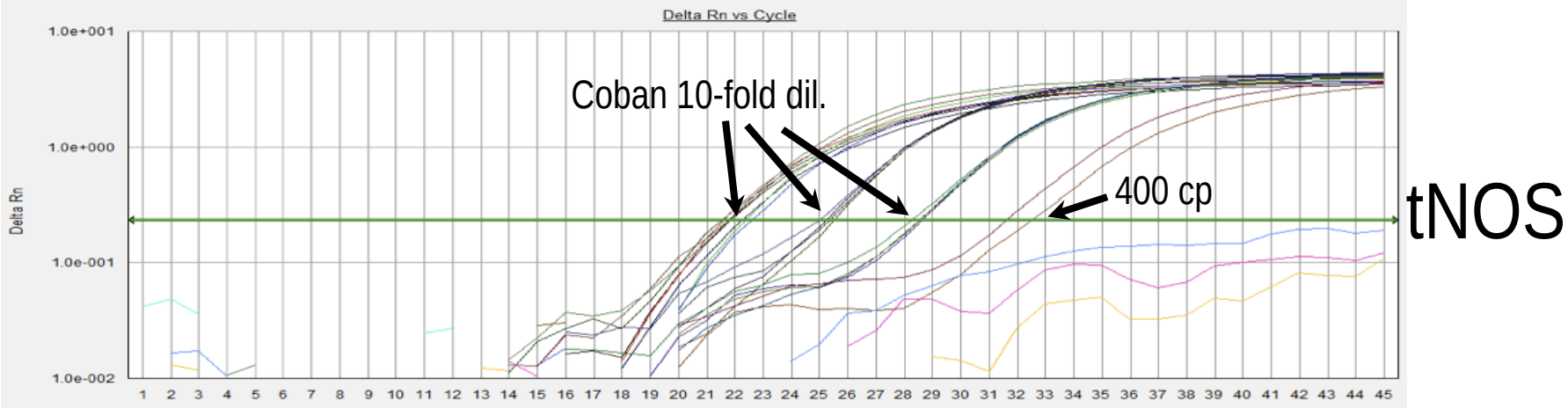
http://www.skapiec.pl/site/cat/2309/comp/252110

the product


30 tabletek 530 mg
30 tabletek 530 mg
Suplement diety
Coban
Cyanocobalamin (Witamina B12) i intrinsic factor *
* Substancja identyczna z produkowaną przez błonę śluzową żołądka i jelito cienkie, odpowiedzialna za absorbcję witaminy B12.
Producent: Cobento A/S Gustav Wieds Vej 10C DK-8000 Aarhus C, Dania
Dystrybutor: Victoria Pharma Sp. z o.o. ul. Krótka 1, 05-520 Konstancin
Składniki:
Suche liście Arabidopsis thaliana, witamina B12, stearynian magnezu, glukoza.
1 tabletka zawiera:
Cyanocobalamin 3,6 mcg
Intrinsic factor 135 mcg
Arabidopsis thaliana 120 mg
Dzienna dawka: 1 tabletka (zawiera ok. 90% zalecanej dawki dziennej witaminy B12)
Preparat nie może być stosowany jako substytut zróżnicowanej diety.
Przechowywać z daleka od dzieci.
6484
0708

p35S, tNOS in routine screening





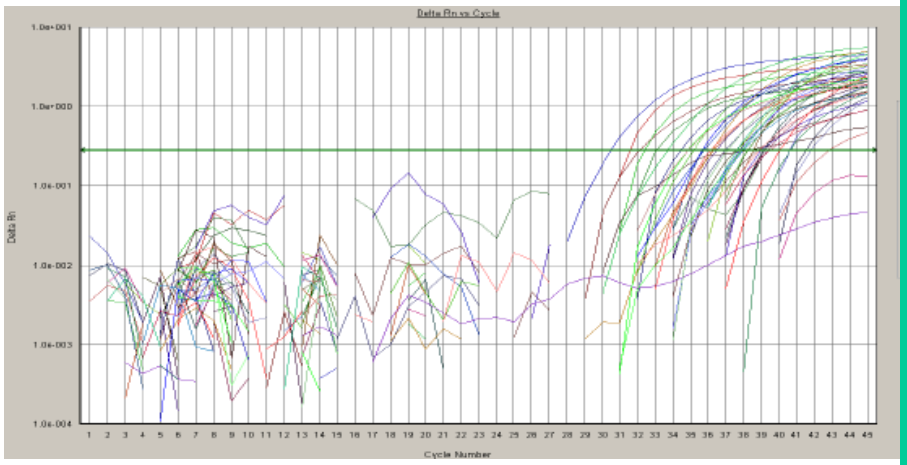
The product was reported in the RASFF:

unauthorised genetically modified (P-35S, T-NOS) Arabidopsis thaliana food supplement containing recombinant human intrinsic factor from Denmark, via Poland

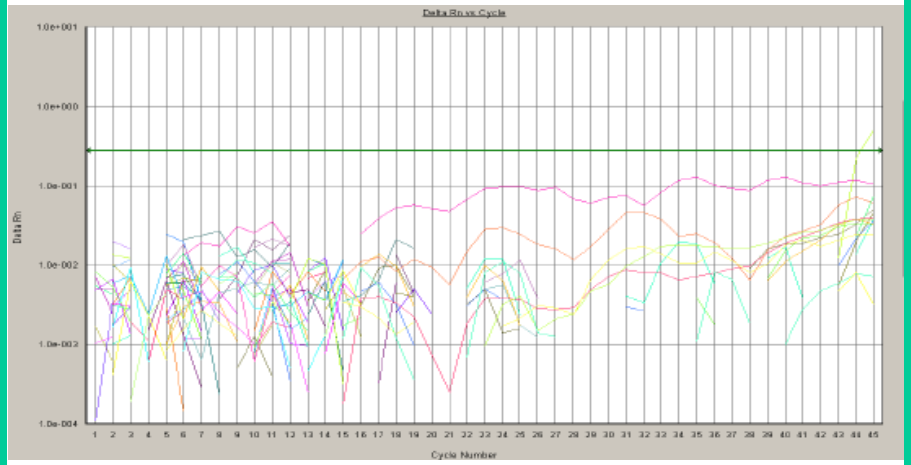
http://ec.europa.eu/food/food/rapidalert/reports/week12-2008_en.pdf

JRC pre-spotted plates

top half: positive controls



lower half: Coban tablets



none of the 39 event-specific methods is positive

(Bt11, NK603, GA21m, MON863, 1507, T25, 59122, H7-1, MON810, 281-24-236, 3006-210-23, LL62, T45, EH92-527-1, Ms8, Rf3, GT73, LL25, MON531, A2704, MIR604, Rf1, Rf2, Ms1, Topas19/2, MON1445, Bt176, MON15985, 40-3-2, GA21s, MON88017, LY038, 3272, MON89788, MON89034, DP-356043, MON88913, rice p35S::Bar, LL601, Bt63, Bt10)

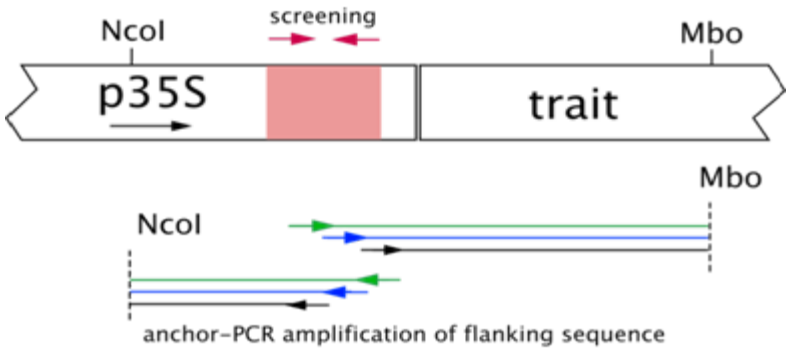
no reference genes for species that can contain GMOs are positive

(rice, cotton, soybean, maize, potato, sugar beet, oil seed)

novel method for UGM identification

fluorescent anchor-PCR fingerprinting

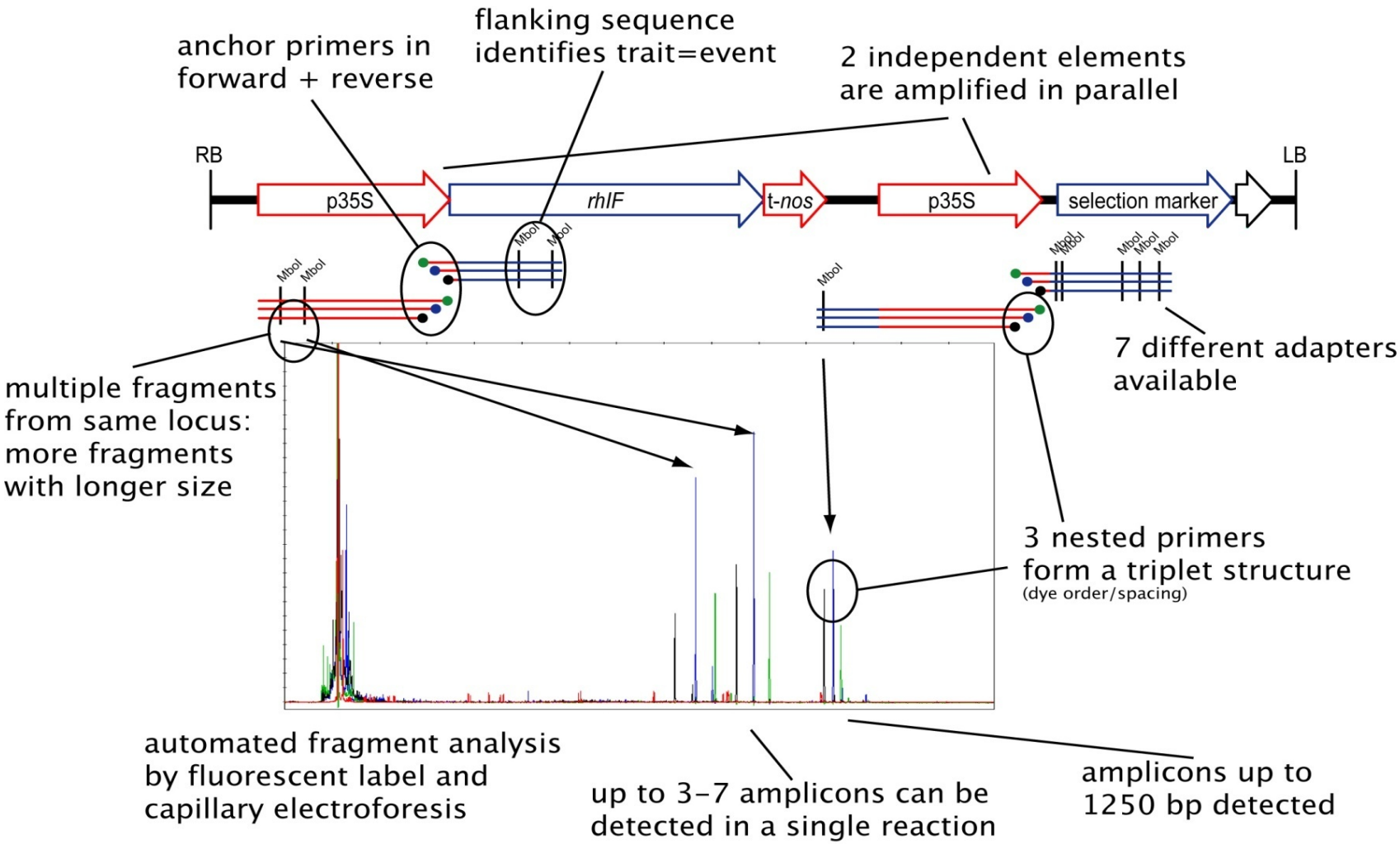
Matrix approach identifies common genetic element
Validated methods to confirm/quantify known events
`Unexplained` genetic elements:
presence of UGM inferred, but not proven
genome walking to find sequence context:



1. improved anchor-PCR method to detect flanking sequences
2. link to Matrix-approach: p35S and t-*nos* and 7 different adapters
3. fluorescent labeling and automated fragment analysis by CE
4. nested-PCR and specific 3-color triplet structure suppress false positive amplicons
5. high resolution profiling detects unique signals for each event in parallel
6. amplicons can be compared to `reference collection` of GM fingerprints of known GMOs
7. sequencing of amplicons directly identifies each event present in a sample
8. GMO fingerprinting may resolve masked UGM events in products with mixed ingredients
9. direct evidence for UGM may be helpfull to avoid false alerts in RASFF

anchor-PCR fingerprinting

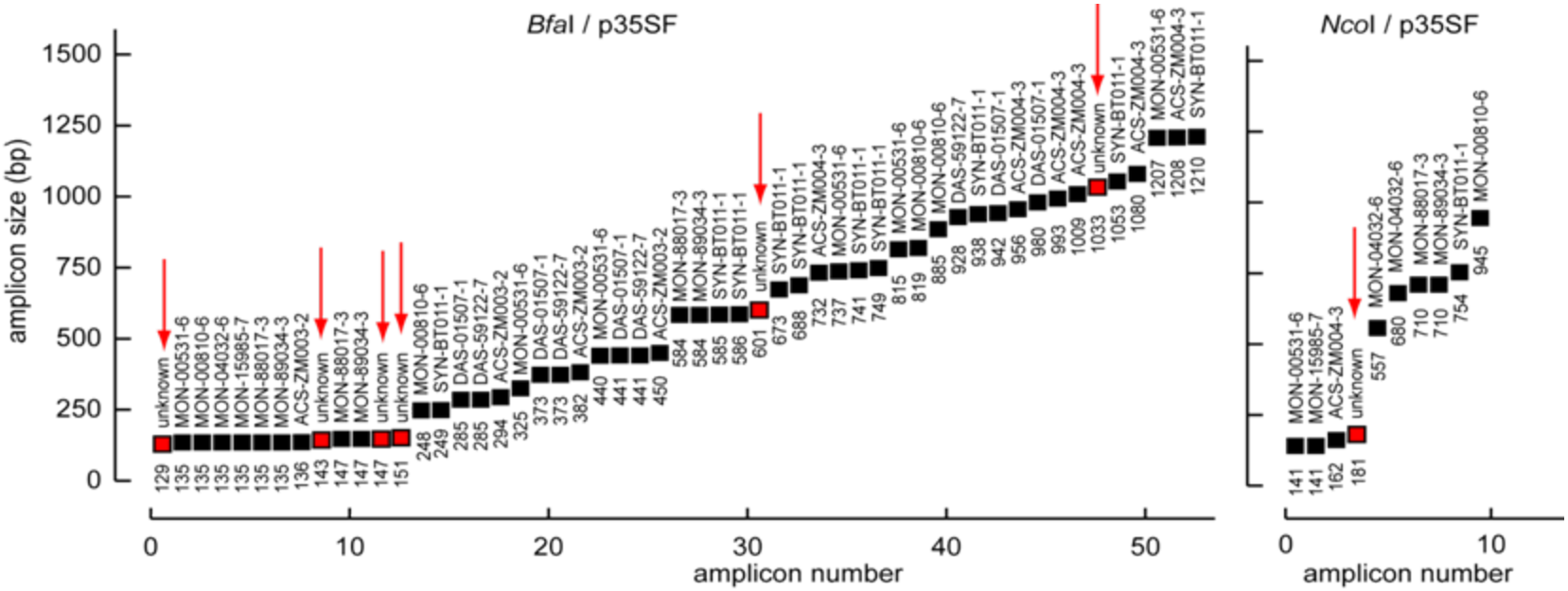
real-life example: identification of novel UGM



unique flanking amplicons for each GM event

database of fingerprints

in-silico calculated `reference` collection of GM fingerprints of authorized GMOs:
easy recognition of UGM-derived signals and detection of `masked` events
unique fragment (anchor/adapter/length) is direct evidence of UGM
selection of fragments for UGM event identification by direct sequencing



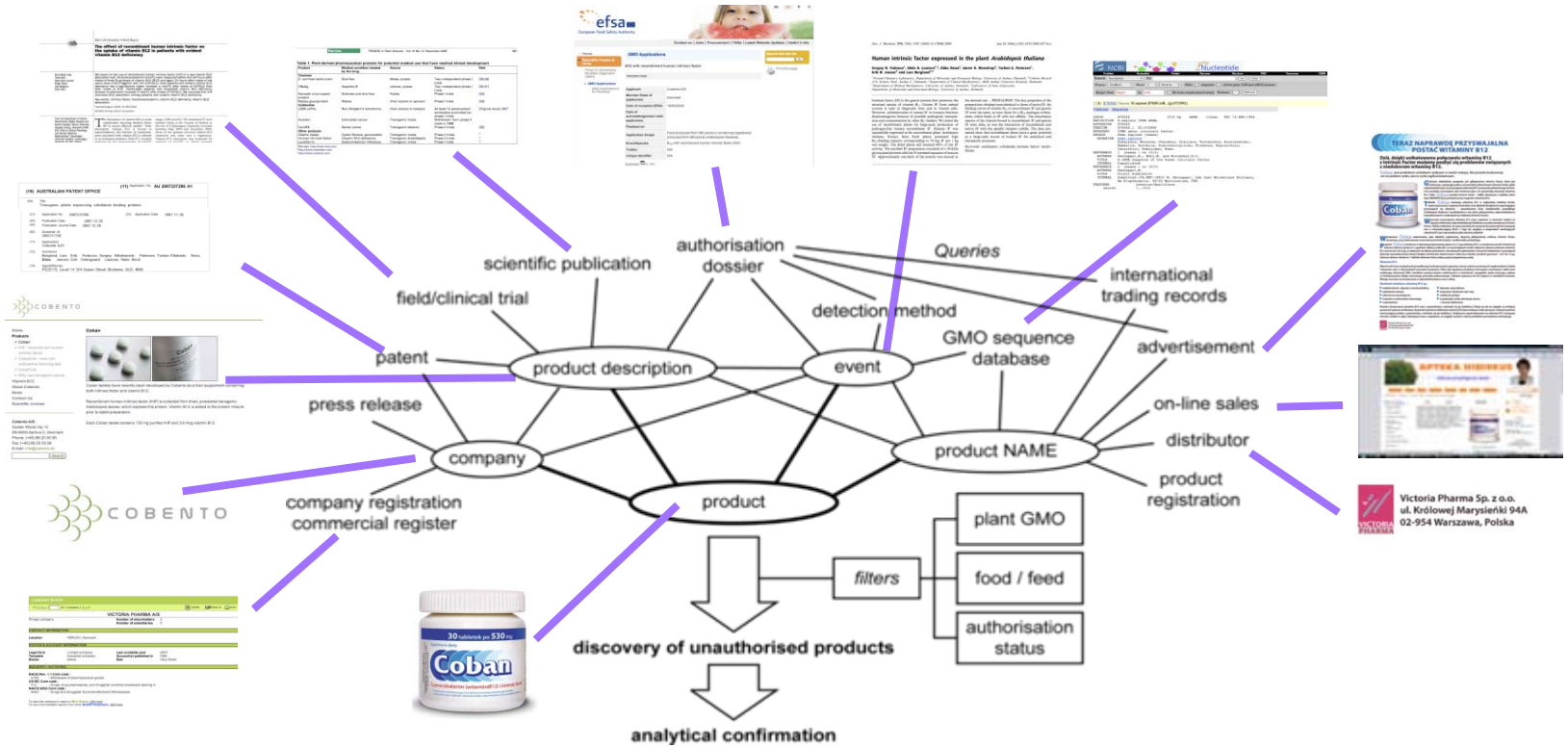
method development and proof of concept using real-life novel UGM:
Ruttink *et al.* (2009) Molecular toolbox for the identification of unknown GMOs
Anal Bioanal Chem

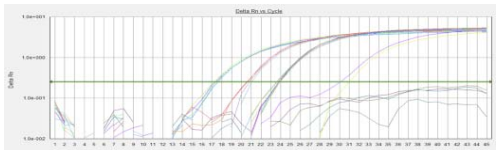
IT-based strategy supporting GMO detection

- Combining strategies from different domains

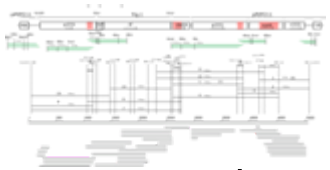
IT-based strategy supporting GMO detection

- an approach for UGM (class4) discovery





screening



sequencing

AN APPROACH FOR A SPECIFIC TYPE OF UGM DISCOVERY

Thank you for your attention

Tom Ruttink
Rolinde Demeyer
Elke Van Gulck
Bart Van Droogenbroeck
Isabel Taverniers

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Marco Mazzara
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Jana Žel
Kristina Gruden