

Multidetetection of GMOs and plant-specific DNA in complex matrices

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Introduction

- TRACE : TRAcing food Commodities in Europe
 - Protected Designation of Origin (PDO)
 - Protected Geographical Indication (PGI)
 - Species Origin Methods (identification)



- Co-Extra : Technical challenges of GMO detection
 - Genetically Modified Organisms (GMOs)
 - Detection of (un)known GMOs
 - Sensitivity



- Application of microarrays in combination with Padlock probes

Introduction GMO detection

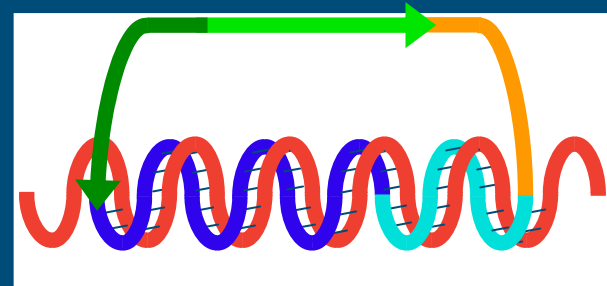
In the EU, legislation requires:

- Labelling of allowed GMOs when $> 0.9\%$ per ingredient
- Detection of unapproved and unknown GMOs
- Real Time PCR (quantitative PCR) is very sensitive, but the number of analyses increases dramatically
- Develop 'pre-screening' to reduce nr of qPCR analyses



Introduction Ligation Detection probes

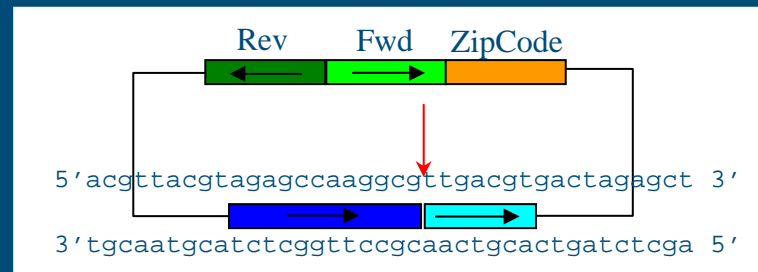
- Nice overview from Cor
- We use the Padlock probe approach
- *Bam*HI, *Eco*RI and *Hind*III-digested gDNA



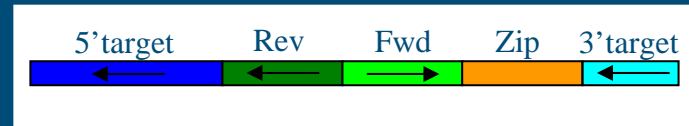
Padlock probes



- Single stranded DNA molecules (100-150 nt)
- 5' and 3' targets recognise specific sequence
- Padlock design requirements:
 - Restriction sites
 - Secondary structures
 - T_m of 5' and 3' targets and of whole molecule
 - Uniqueness of 5' and 3' targets
- After hybridisation, PLP is circularised at ↓ in ligase reaction and amplified with Fwd and Rev primer sites



Padlock probe validation



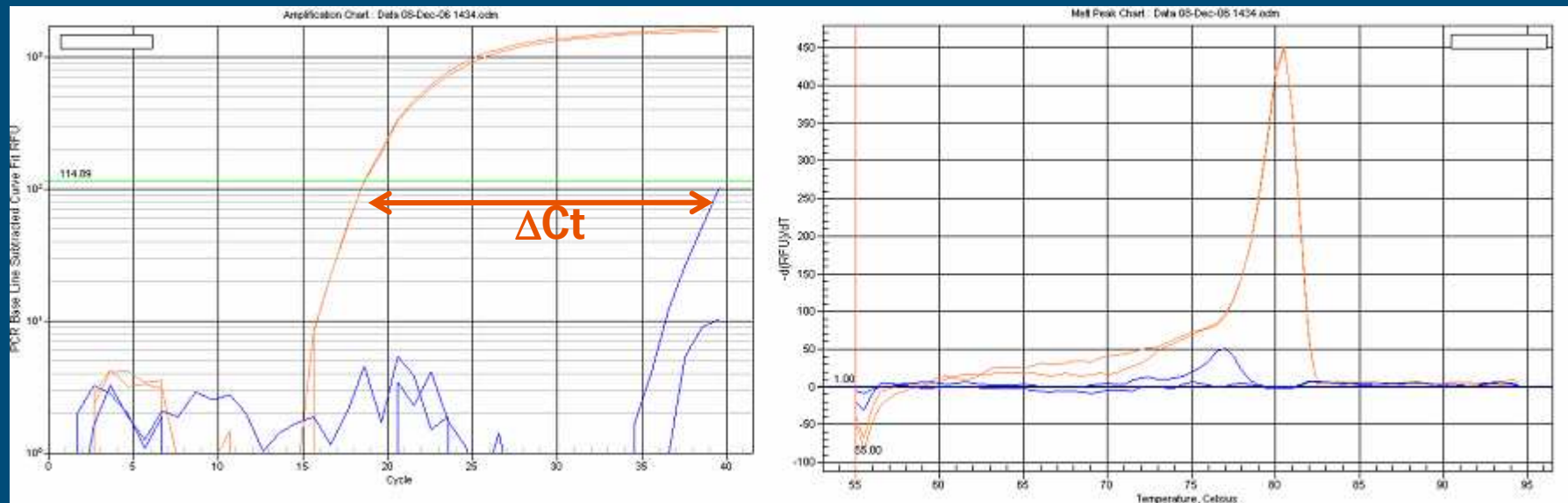
- Evaluation of newly designed probes
- Monoplex detection
- Fwd and Rev primers allow universal amplification in presence of SybrGreen
- Real Time amplification
- Analyses of amplification curves and melting curves
- Template: unique target DNA, non-target DNA and mixtures of DNA



Padlock probe validation



Amplification curve and melt curve for padlock probe Oat with oat



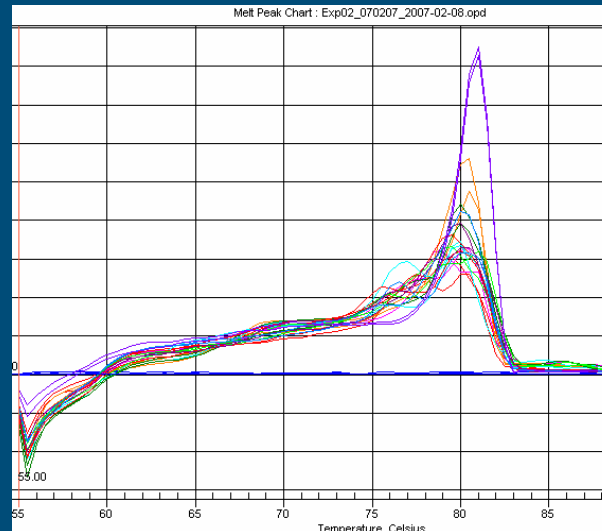
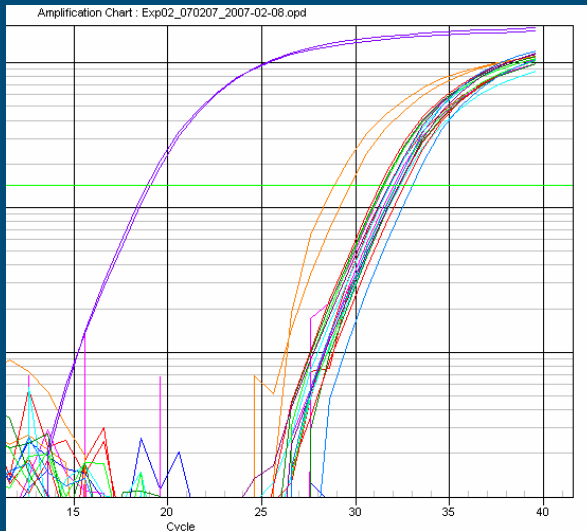
orange curve: gDNA oat
blue curve: MQ (NTC)



Padlock probe validation



Padlock probe Oat: detection of oat vs. other cereal gDNAs



	Ct value	Δ Ct
Oat	18.93	12.82
Barley	31.98	-0.23
Einkorn	31.71	0.04
Farro	29.22	2.53
Spelt	32.03	-0.28
Rye	31.80	-0.05
Wheat	31.89	-0.14
MQ	31.75	

purple curve: gDNA oat
blue curve: MQ (NTC)

Microarray hybridization

- Fwd (5'-Cy3) and Rev primers allow universal amplification of ligated Padlocks
- Unique (target-bound) cZIP code is also amplified
- cZIP is Cy3-labelled



Microarray hybridization

- Quadruplicate microarray containing 50 spots (0.5 nl 50 μ M)
- ZIP(20 nt + A₁₀C6) from Affymetrix
- Positioning spots (P) for alignment
- cZIP-Cy3 recognises spotted ZIP

P	ZIP B3	ZIP PS12	ZIP PS22	ZIP PS32	ZIP PS42	P	ZIP B3	ZIP PS12	ZIP PS22	ZIP PS32	ZIP PS42	P
	ZIP B5	ZIP PS13	ZIP PS23	ZIP PS33	ZIP PS43		ZIP B5	ZIP PS13	ZIP PS23	ZIP PS33	ZIP PS43	
	ZIP B8	ZIP PS14	ZIP PS24	ZIP PS34	ZIP PS44		ZIP B8	ZIP PS14	ZIP PS24	ZIP PS34	ZIP PS44	
	ZIP B9	ZIP PS15	ZIP PS25	ZIP PS35	ZIP PS45		ZIP B9	ZIP PS15	ZIP PS25	ZIP PS35	ZIP PS45	
	ZIP PS05	ZIP PS16	ZIP PS26	ZIP PS36	ZIP PS46		ZIP PS05	ZIP PS16	ZIP PS26	ZIP PS36	ZIP PS46	
	ZIP PS07	ZIP PS17	ZIP PS27	ZIP PS37	ZIP PS47		ZIP PS07	ZIP PS17	ZIP PS27	ZIP PS37	ZIP PS47	
	ZIP PS08	ZIP PS18	ZIP PS28	ZIP PS38	ZIP PS48		ZIP PS08	ZIP PS18	ZIP PS28	ZIP PS38	ZIP PS48	
	ZIP PS09	ZIP PS19	ZIP PS29	ZIP PS39	ZIP PS49		ZIP PS09	ZIP PS19	ZIP PS29	ZIP PS39	ZIP PS49	
	ZIP PS10	ZIP PS20	ZIP PS30	ZIP PS40	ZIP PS50		ZIP PS10	ZIP PS20	ZIP PS30	ZIP PS40	ZIP PS50	
P	ZIP PS11	ZIP PS21	ZIP PS31	ZIP PS41	ZIP PS51	P	ZIP PS11	ZIP PS21	ZIP PS31	ZIP PS41	ZIP PS51	P
P	ZIP B3	ZIP PS12	ZIP PS22	ZIP PS32	ZIP PS42	P	ZIP B3	ZIP PS12	ZIP PS22	ZIP PS32	ZIP PS42	P
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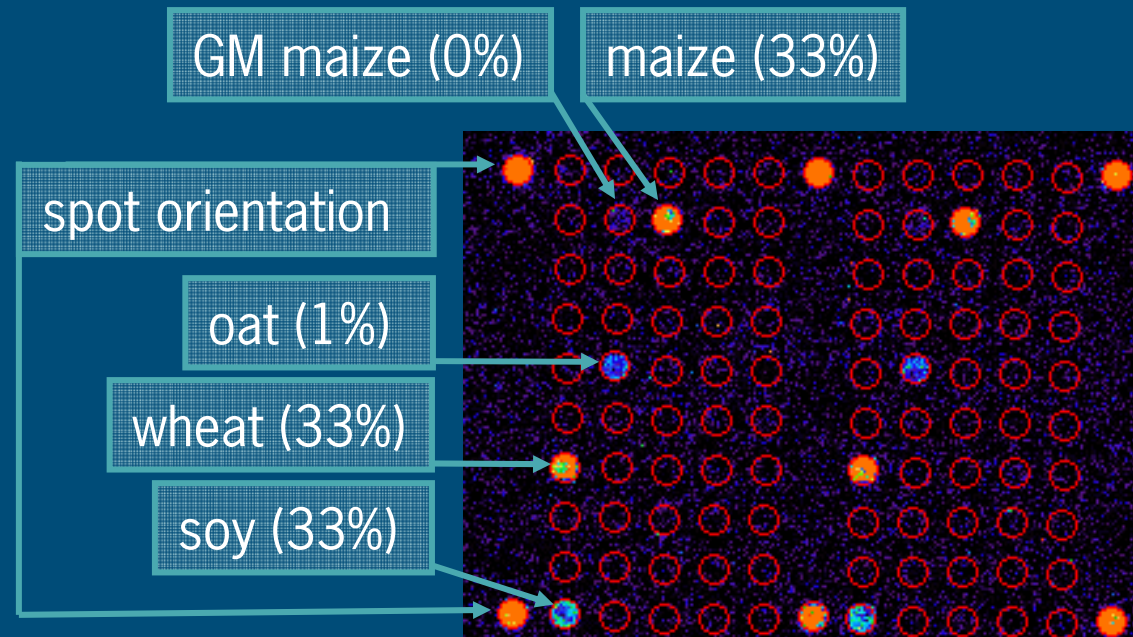


Microarray results

Mix of 5 padlock probes (25 pM each) and 4 gDNAs (200 ng)

Slide contains array in quadruplicate

(Here: 1 % oat in 3 * 33 % plant DNA background)



Microarray results (DNA mixtures)

		PLP maize	PLP oat	PLP soy	PLP wheat	PLP GM maize
33% wheat	0.1% oat	++	-	++	++	-
33% soy	1% oat	++	+	++	++	-
33% maize	10% oat	++	++	++	++	-
33% wheat	0.1% maize	-	++	++	++	-
33% soy	1% maize	+	++	++	++	-
33% oat	10% maize	++	++	++	++	-



Microarray results (GMO in DNA mixtures)

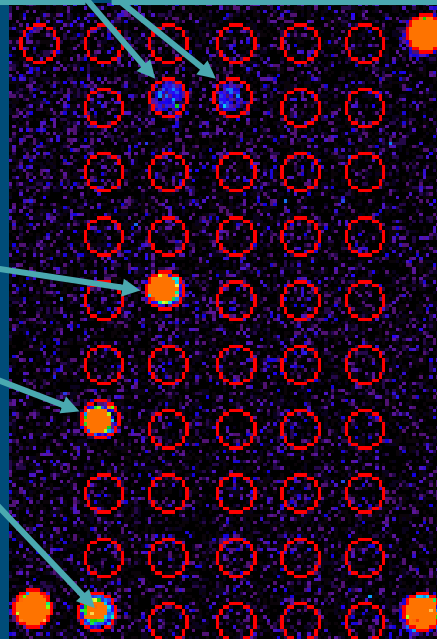
MON810 (0.5%)

maize (1%)

oat (33%)

wheat (33%)

soy (33%)



		PLP wheat	PLP oat	PLP soy	PLP maize	PLP MON810
33% wheat	0.05 % MON810 (0.1 % maize)	+++	+++	++	-	-
33% soy	0.5 % MON810 (1.0 % maize)	+++	+++	++	+	+
33% oat	5.0 % MON810 (10 % maize)	+++	+++	++	++	++



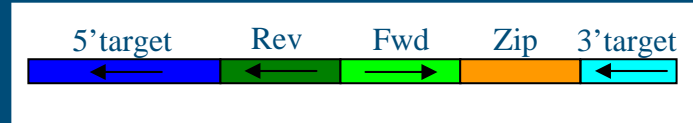
Conclusions and future plans

- Multiplex padlock ligation detection works well
- Sensitive detection down to at least 1%

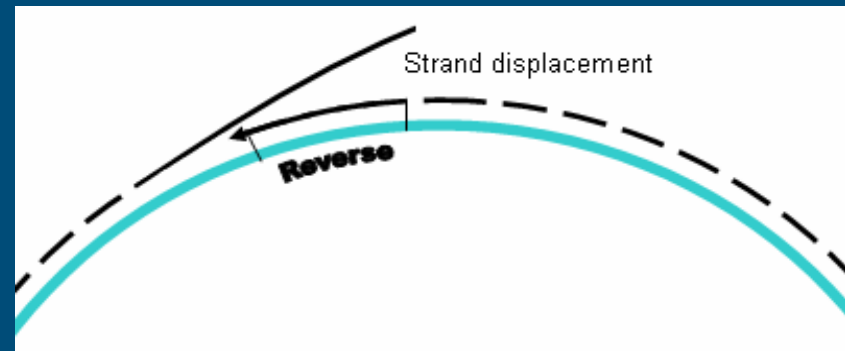
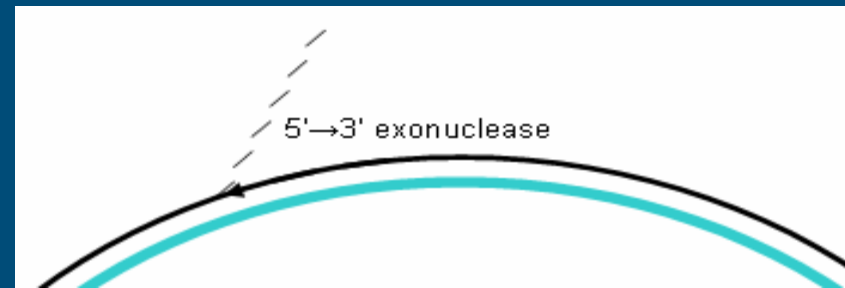
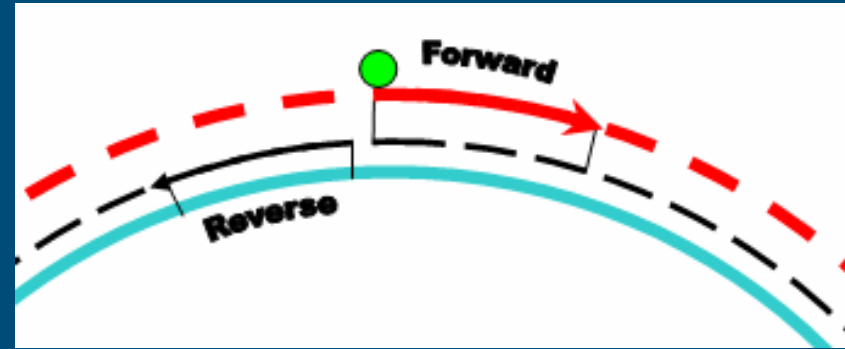
- Develop and validate more PLPs
- Optimise system (sensitivity)



Circular amplification



- Ideal start situation: 'Rolling circle Amplification'
- HotStar Taq DNA pol.
 - 5'-3' exonuclease
- Vent exo⁻ DNA polymerase
 - no 5'-3' exonuclease
 - strand displacement
 - (no hot start...)



Team members:

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Microarray preparation

5' aminomodified C₆ oligonucleotides; spacer of A₍₁₀₎

Spot diameter: 0.12 mm (0.5 nl of a 50 μM solution)

BioRobotics MicroGrid II spotter



Perkin Elmer ScanArray Express HT



ultraGAPS slides; UV cross linking

