Asia Sub Regional Training-of-Trainers Workshop on the Identification and Documentation of Living Modified Organisms

Detection and Identification of Living Modified Organisms

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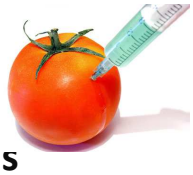
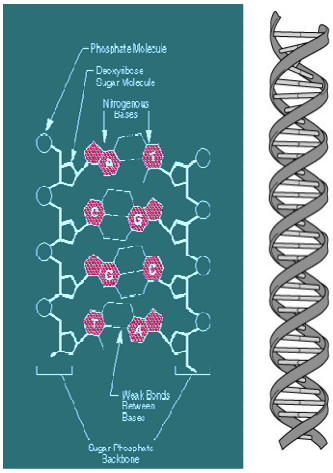
Living Modified Organisms or Genetically Modified Organisms

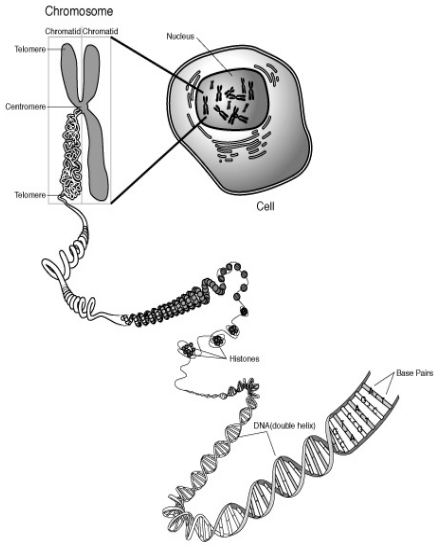


- Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology
- Living organism means biological entity capable of transferring or replicating genetic material

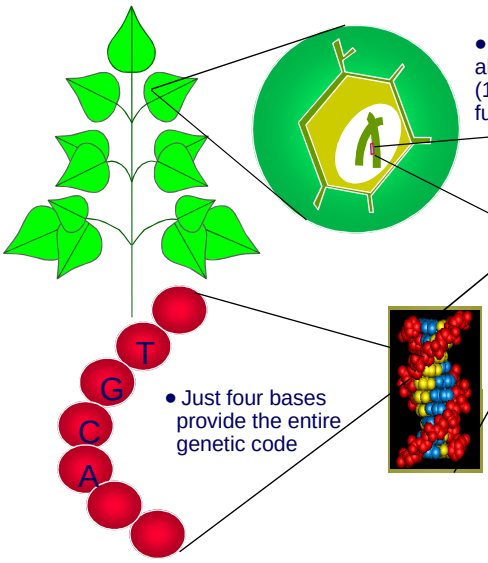
DNA (Deoxyribonucleic acid)

Carriers of heredity



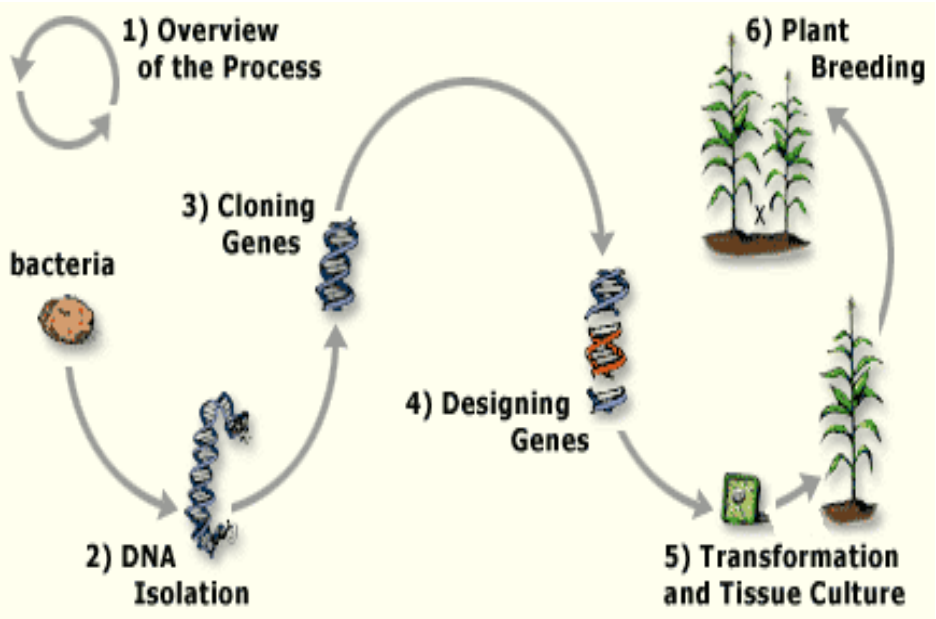


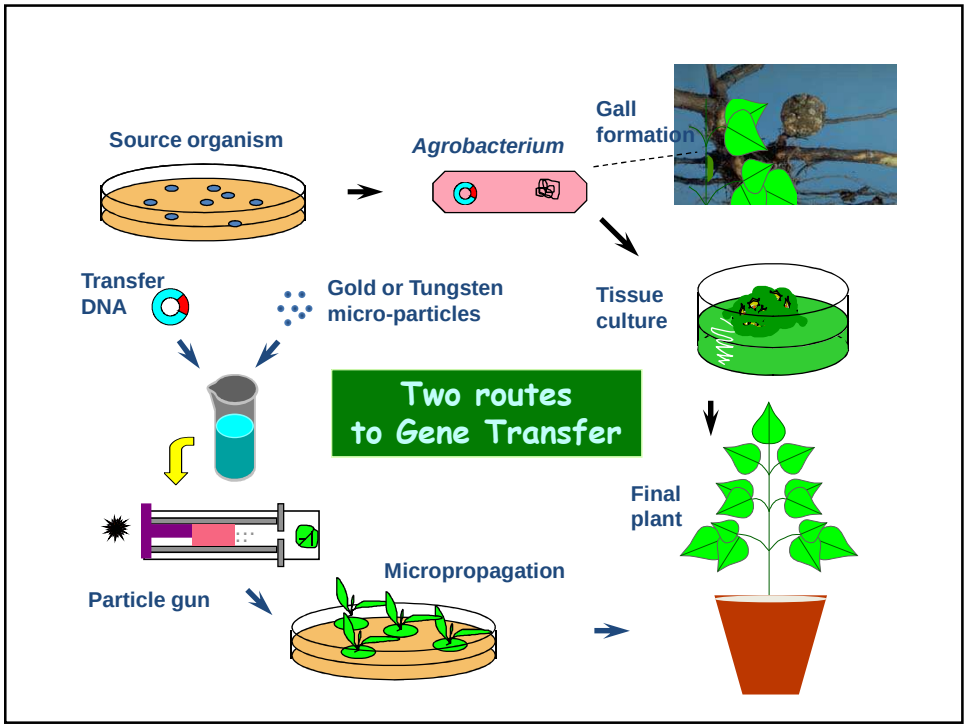
DNA The universal genetic language

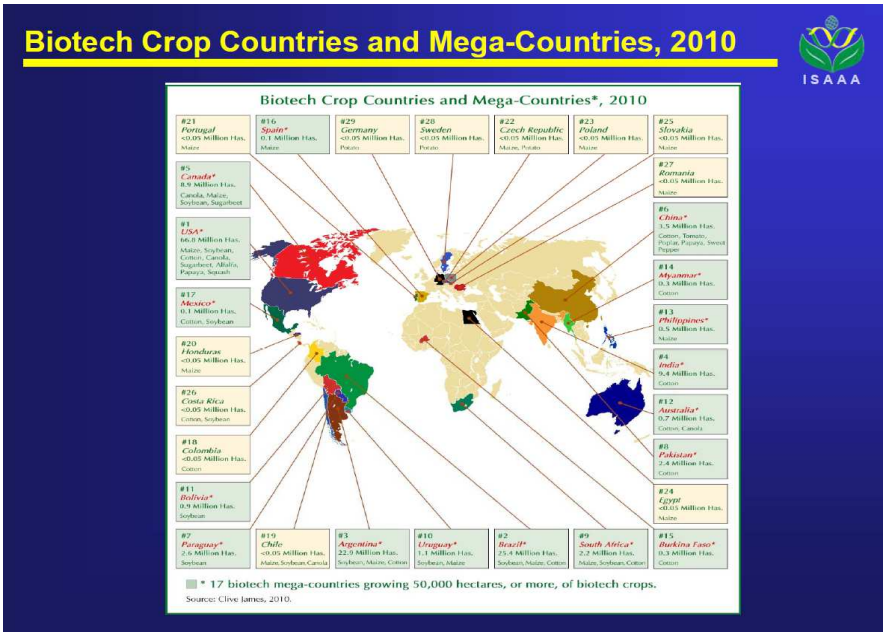


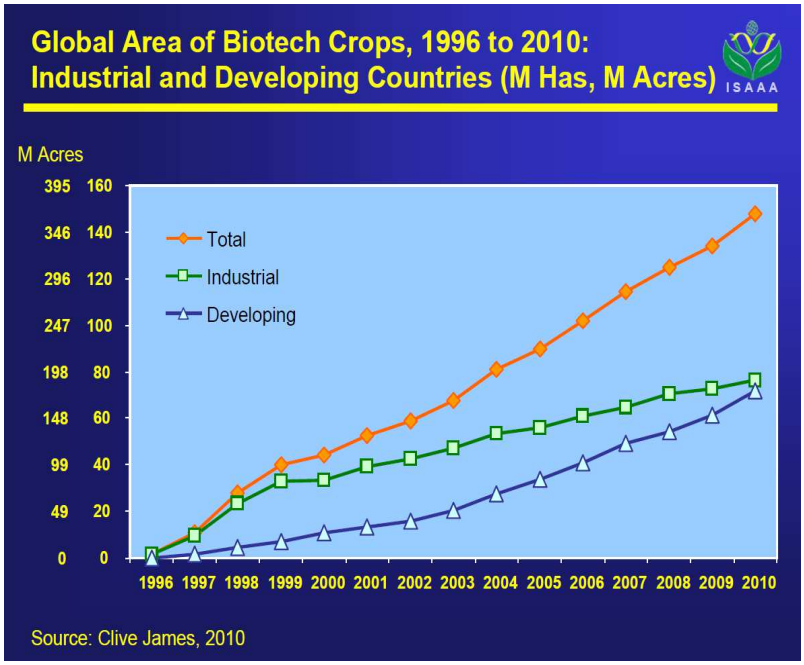
- **CELLS:**The human body has about 1, 000, 000, 000, 000 cells (1 million million), each with a full genetic 'recipe'
- A typical animal or plant cell contains 20-50,000 genes: lengths of DNA which control a single character
- Just four bases provide the entire genetic code
- A maize plant contains about 15 billion **'bases':** the sub-units in DNA which carry the genetic code

THE PROCESS ……..


1) Overview of the Process
6) Plant Breeding
3) Cloning Genes
bacteria
4) Designing Genes
2) DNA Isolation
5) Transformation and Tissue Culture


Source organism
Agrobacterium
Gall formation
Transfer DNA
Gold or Tungsten micro-particles
Tissue culture
Two routes to Gene Transfer
Final plant
Particle gun
Micropropagation


Biotech Crop Countries and Mega-Countries, 2010
ISAAA
Biotech Crop Countries and Mega-Countries*, 2010
* 17 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.
Source: Clive James, 2010.



Commercially Released Hybrids/Variety of Bt cotton of the Six events in India from 2002-till date

No.	Crop	Event	Developer	Status	Date of Approval
1	Cotton*	MON-531	Mahyco/Monsanto	Commercialized	2002
2	Cotton*	MON-15985	Mahyco/Monsanto	Commercialized	2006
3	Cotton*	Event-1	JK Agri-Genetics	Commercialized	2006
4	Cotton*	GFM Event	Nath Seeds	Commercialized	2006
5	Cotton**	BNLA-601	CICR (ICAR) & UAS, Dharwad	Commercialized	2008
6	Cotton*	MLS-9124	Metahelix Life Sciences	Commercialized	2009

*Bt cotton hybrid; ** Bt cotton variety and Bt cotton hybrid

Source: Compiled by ISAAA, 2009.

Purpose of Detection Methods

To assure purity and segregation of seeds and products thereof

To be able to trace genetic modification in breeding

To assure compliance with legislation

To be able to retrieve specific transgenic planting material in case of unauthorized material is being grown

To solve legal/IPR issues if they arise

Testing of GM Crops

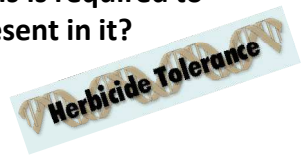
Detection

To determine whether a sample contains any transgene

OR

Identification

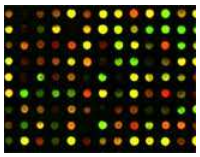
If the results are positive, further analysis is required to find which specific transgene is present in it?



Quantification

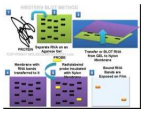
If the specific transgene has been identified, the next step is to assess compliance with threshold level (e.g.1%)

Methods of GMO detection

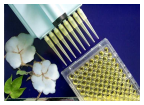


Low technological methods
Protein-based Methods
- Herbicide Bioassays
- Immunoassays

✓ Western Blot



✓ ELISA



✓ Lateral Flow Sticks

High technological Methods
- RNA-based methods
- DNA-based methods

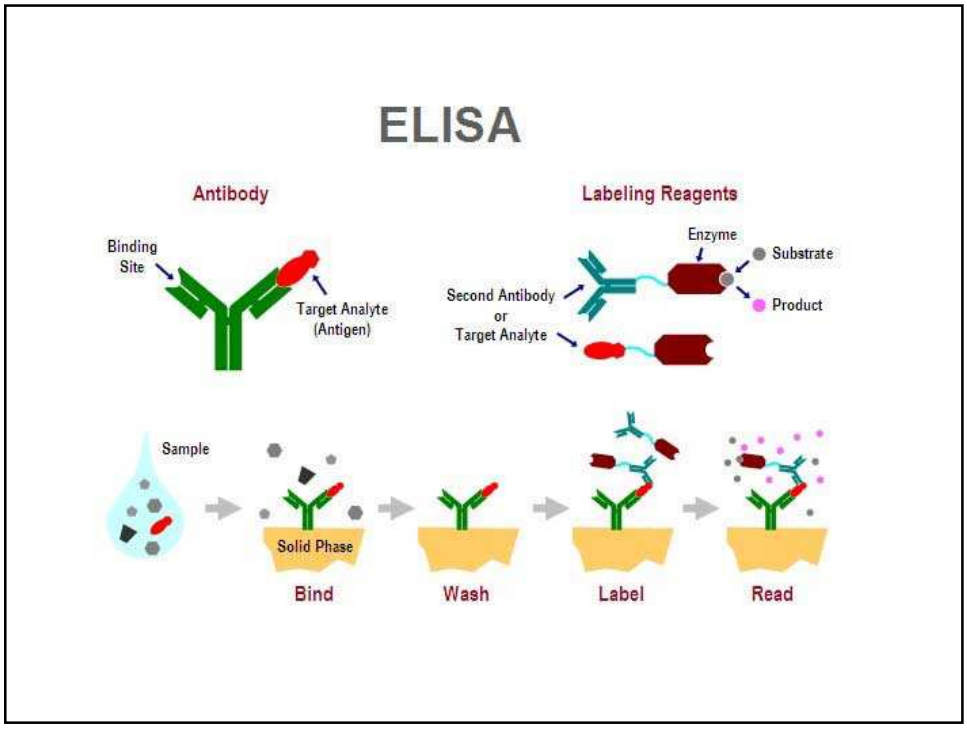
✓ Qualitative PCR

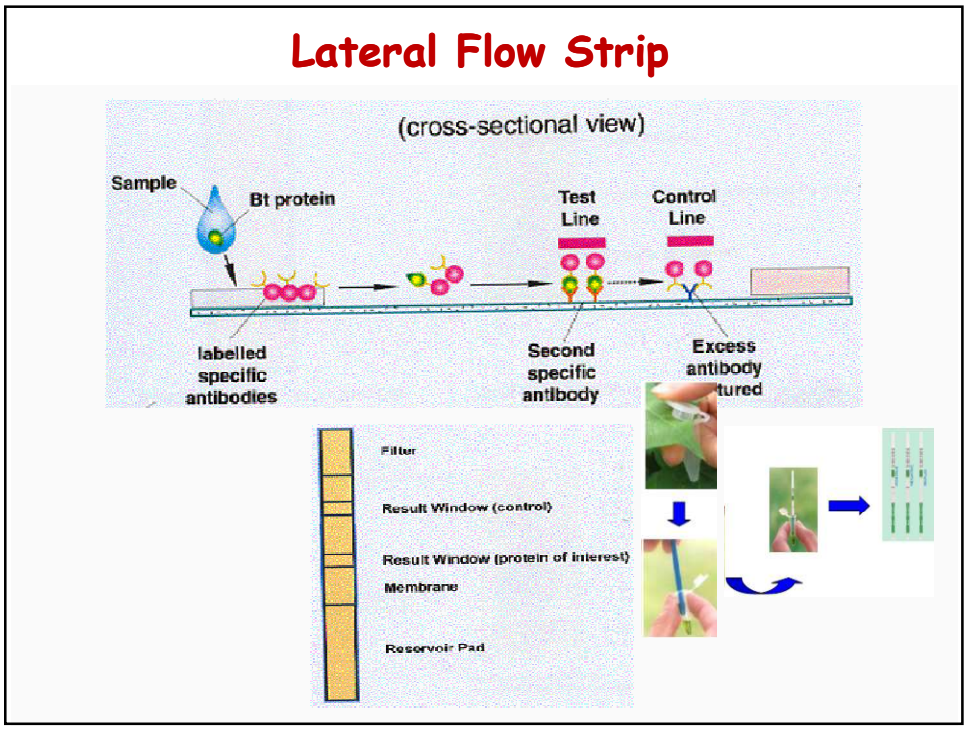
✓ Multiplex PCR
✓ Quantitative PCR: Real-time PCR

✓ Microarrays

Protein-based Methods for Detection of GMOs

1. Enzyme Linked Immunosorbent Assay (ELISA)
2. Lateral Flow Strip Methods


ELISA
Antibody
Labeling Reagents
Binding Site
Target Analyte (Antigen)
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read


Lateral Flow Strip
(cross-sectional view)
Sample
Bt protein
Test Line
Control Line
labelled specific antibodies
Second specific antibody
Excess antibody
Filter
Result Window (control)
Result Window (protein of interest)
Membrane
Reservoir Pad

Advantages of ELISA

- ✓ Reasonably sensitive
- ✓ Less susceptible to 'false positives'
- ✓ Low per sample cost
- ✓ Handles large number of samples
- ✓ Can be subjected to automation
- ✓ Detection kits available commercially

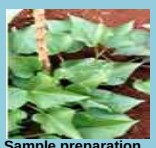
Limitations of ELISA

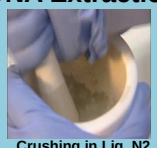
- ✓ High development costs for the assays
- ✓ GM products might be produced only during certain developmental stages or in certain plant parts and such GMs are difficult to be detected by ELISA
- ✓ In processed foods the proteins denatures easily, which makes it difficult to use ELISA for processed food fractions

DNA-based Methods for Detection of GMOs

Procedure for PCR Detection of GMOs

DNA Extraction


Sample preparation


Crushing in Liq. N2

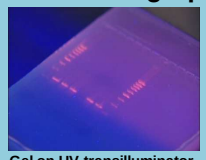

Centrifugation

PCR Analysis

Gel Electrophoresis and Photography


Electrophoresis Assemblyz


Agarose gel loading


Gel on UV-transilluminator


Polaroid Gel Photography

Advantages of PCR

- ✓ High species specificity
- ✓ High sensitivity. Detection limits ≤ 0.1% of DNA present
- ✓ Semi-quantitative PCR can provide a rough estimate of the level of contamination
- ✓ Real-Time PCR providing quite accurate quantitation in the less than 10% range
- ✓ DNA highly stable

Limitations of PCR

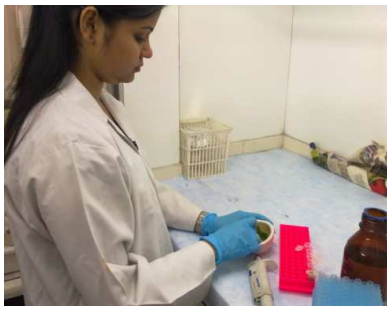
- ✓ Require relatively advanced lab. facilities and instrumentation and highly trained staff
- ✓ Cost is fairly high ($75- $300)
- ✓ Time (usually >24 hrs for results)
- ✓ Special precautions need to be taken to avoid the contamination

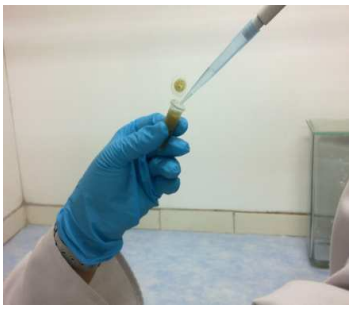
GM Planting Material grown in National Containment Facility

DNA Extraction

Isolation of Genomic DNA (containing the gene of interest) from the LMO

Using Conventional Methods (CTAB/SDS Extraction Protocol)
OR
Commercialized Kits for DNA Isolation





DNA Quantification

Extracted DNA is quantified using Nano-Drop UV-Spectrophotometer

↓

To check the quantity and quality of DNA

↓

Proceed for Polymerase Chain Reaction

Primer Designing

DNA target sequence

gggttgttgtgtatgcagtcagaaaagagagacgaatgggtgctt
ctttgctacgtttacattttcatgattgtttcgttaacggttgtgatgctt
cgattctccttgaccaaacttctaccattaatagtgaaaagacttc
tcg**asiapacificworkshoponidentificationoflmos**aacaat
tctgctagaggatttgaggtgattgataaaattaaatcagaggttg
ataaagtttgtggacgtccgggggttgttgtgtatgcagtcagaaa
agagagacgaatgggtgcttctttgctacgtttacattttcatgattg
tttcgttaacggttgtgatgcttcgattct

DNA target sequence

gggttgttgtgtatgcagtcagaaaagagagacgaatgggtgc
ttctttgctacgtttacattttcatgattgtttcgttaacggttg
tgatgcttcgattctccttgaccaaacttctaccattaatagtg
aaaagacttctcgtgctaactgaggcaatagcattgctaactt
aacaattctgctagaggatttgaggtgattgataaaattaaat
cagaggttgataaagtttgtggacgtccgggggttgttgtgtat
gcagtcagaaaagagagacgaatgggtgcttctttgctacgtt
tacattttcatgattgtttcgttaacggttgtgatgcttcgatt
ct

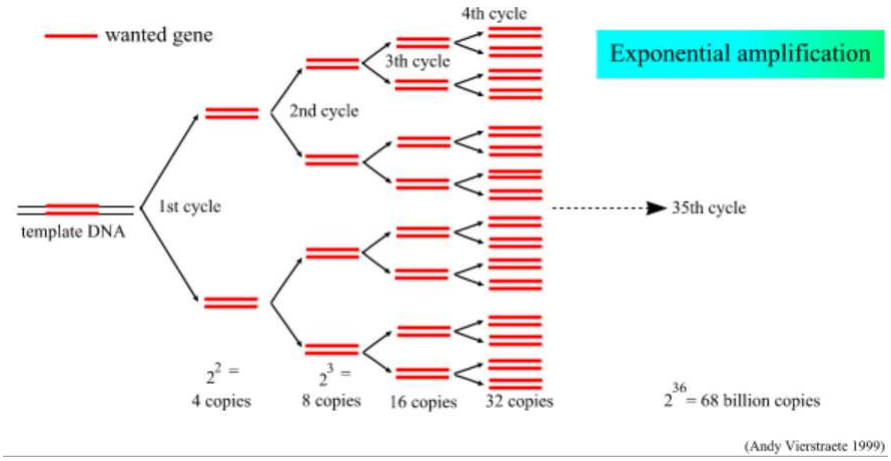
Primers

Tgatgcttcgattctccttgaccaaacttctaccattaatagtg

aaaagacttctcgtgctaactgaggcaatagcattgctaactta
acgatt attga

acaattctgctagaggatttgaggtgattgataaaattaaat

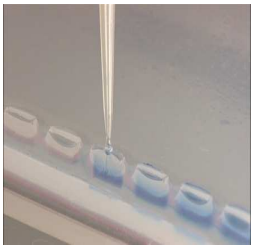
Polymerase Chain Reaction



PCR Set up

PCR Programming

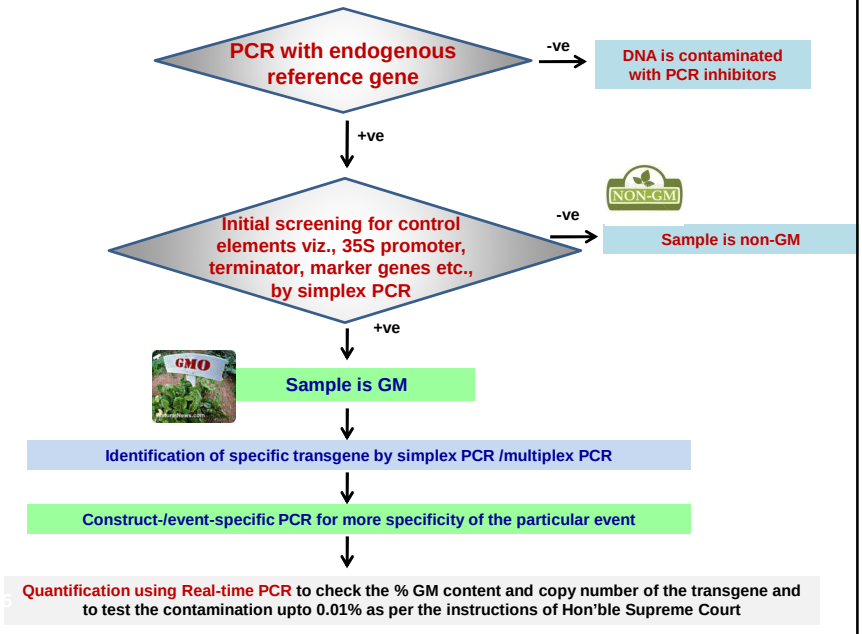
Analysis of PCR Products



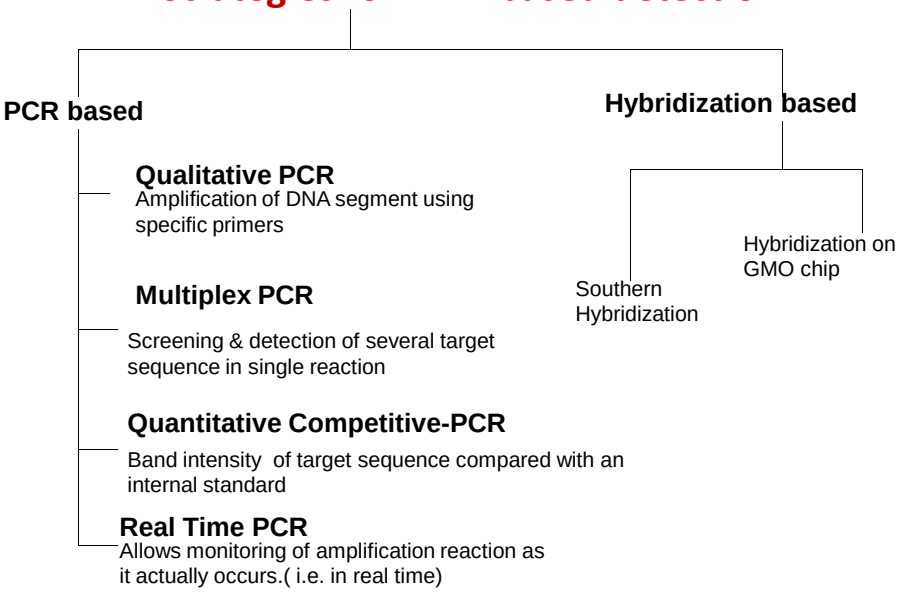
Agarose Gel Electrophoresis

Analysis of PCR amplicons on Gel Documentation System

MOLECULAR DIAGNOSIS OF GM MATERIAL



Strategies for DNA based detection



Qualitative PCR Analysis

PCR-based Methods for GM Detection

Screening methods

Specific methods

- First tool for the analysis of unknown samples
- Allowing to detect sequences that are commonly found in transgenic events, in particular, some promoters, terminators and selectable marker genes

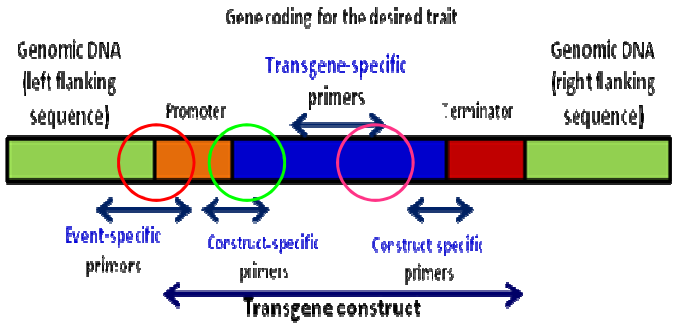
- Useful for confirming positive results obtained with screening methods
- Allow the tracking of events within the seed company during development of the variety

Gene-specific method

Construct-specific method

Event-specific method

PCR based Detection Methods



The transgenic construct showing primer's target positions (Event-specific, Construct-specific and Gene-specific; shown by double-headed arrows)

Identification, standardization and validation of endogenous reference genes in different GM crops

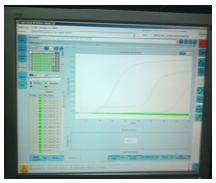
Characteristics of Endogenous Reference Gene

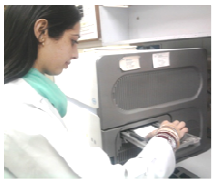
- ✓Species specificity
- ✓Low heterogeneity among cultivars
- ✓Stable and low copy number in the genome (a single copy is best)

Purpose of having Reference Gene

- ✓To confirm the origin of plant materials
- ✓To exclude the false-negative
- ✓To normalize the quantitative result

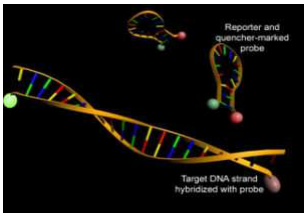
Quantitative – Real Time PCR



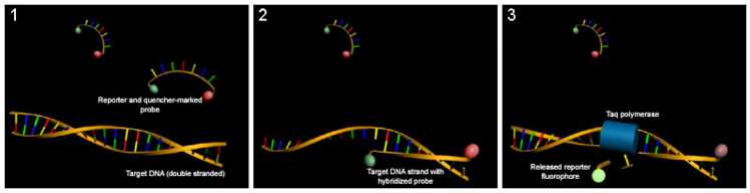


- Real-time monitoring of the amplification reaction
- To estimate the initial quantity of specific template DNA
- Advantages: Sensitivity, reproducibility, dynamic range, throughput

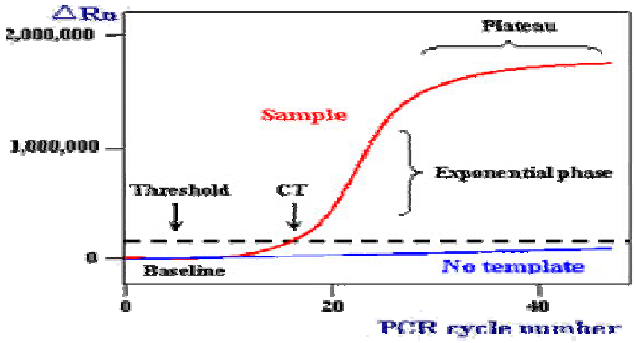
Real-Time Polymerase Chain Reaction



The method of choice to quantitatively measure amounts of transgene DNA in GM sample



Principle for Quantification by Real Time PCR



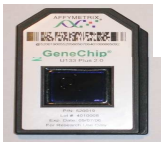
Exponential amplification $=10^{(-1/slope)}$

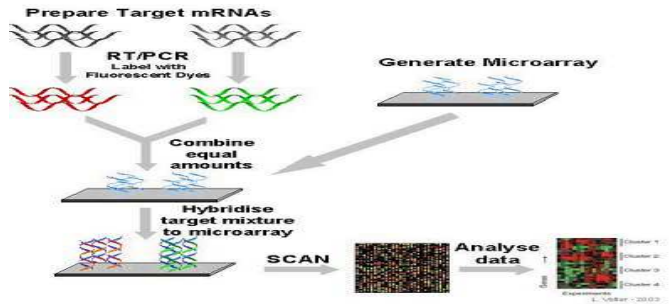
Efficiency $=[10^{(-1/slope)}]$ -1

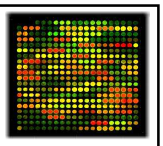
Recent Techniques Available For GM Detection

Micro-array based method

- Automated rapid screening
- Based on nucleic acid hybridisation
- Specific probes are attached to the chip







Different DNA Microarray Approaches used for GM Detection

Multiplex DNA array-based PCR

Rudi *et al*., 2003 in transgenic maize

Peptide nucleic acid array approach

Germini *et al*., 2005 for five transgenic events in two plant species

Ligation detection reaction coupled with universal array technology

Bordoni *et al*., 2004, 2005 in transgenic maize

These methods used fluorescent probes, which require costly material and are photosensitive, thus limiting the common use of microarrays for GM detection

Microarray based detection system of GM food ingredients (Leimanis *et al.* 2006)

- Developed a multiplex DNA microarray chip for simultaneous identification of nine GMOs, five plant species and three GMO screening elements, *i.e*., the 35S promoter, nos terminator and nptII gene
- The chips include several controls (*e.g*. presence of CaMV)
- The targets were biotin labeled
- Arrays were detected using a colorimetric methodology
- Sensitivity of experiments carried out in five different laboratories
- Limit of detection was lower than 0.3% GMO for all the tests

Leimanis et al. (2006) A microarray-based detection system for genetically modified (GM) food ingredients. *Plant Mol. Bio.* 61: 123-139.

Contents of GMO Chip

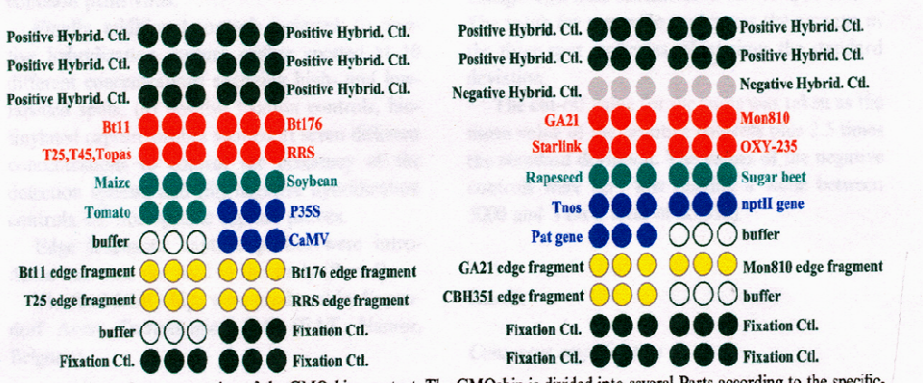


Figure 1. Schematic representation of the GMOchips content. The GMOchip is divided into several Parts according to the specificity of the capture probes: Part 0 for the various controls (●), Part 1 for the specific GMO probes (●), Part 2 for the plant probes (●), Part 3 for the small elements (●) and Part 4 for the edge fragments (●). Order of the capture probes can vary from one image to another. To avoid any misunderstanding below, each capture probe giving a positive signal is indicated by an arrow and its name.

DualChip® GMO: Principle

	Transformation event	Maize	Soybean	Rapeseed	Cotton
1	MON 15985				✓
2	MON 531				✓
3	MS8, RF3, MS8 X RF3			✓	
4	Bt176 maize	✓			
5	GA 21	✓			
6	MON 863	✓			
7	MON 1445				✓
8	MON 531 X MON 1445				✓
9	MON15985 X MON1445				✓
10	RRS // GTS 40/3/2 // MON 40-3-2		✓		
11	GT 73			✓	
12	MON603 // NK603	✓			
13	MON863 X MON603	✓			
14	MS1, RF1, MS1 X RF1			✓	
15	MS1, RF2, MS1 X RF2			✓	
16	GA21 X MON 810	✓			
17	MON 810	✓			
18	MON863 X MON810	✓			
19	NK 603 X MON 810	✓			
20	T45			✓	
21	Bt11	✓			
22	T25	✓			
23	TC 1507 // DAS1507	✓			
24	Topas 19/2 // HCN92			✓	

- 1st prototype published 2006 by Leimanis *et al*. Plant Mol. Biol.
- screening system
  one analysis → 14 results
- Sensitivity: 0.1%
- Accuracy rate: 95%
- 400 ng DNA starting material

DualChip® GMO: Principle

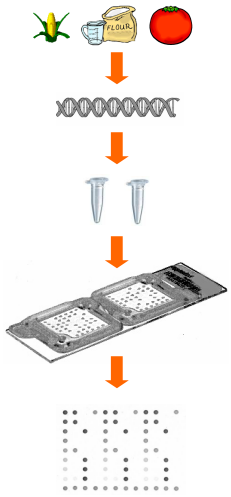
DNA extraction from GMO sample

Amplification through multiplex PCR with biotinylated primers

Hybridization of PCR products on 1 DualChip GMO

Detection of hybridization with Silverquant® detection

Data analysis and results



3 h

1h 20 min

1h 30 min

10 min

DNA based GM detection work at NBPGR

National Bureau of Plant Genetic Resources

Nodal Agency for:

Import Permit

Quarantine Processing

Issue of Phytosanitary Certificate

For Germplasm/ Transgenic Planting Materials

DBT Import Clearance

- **Para 4**
  - applicant to certify to NBPGR
    - material being imported conform to the description given in the import clearance
  - NBPGR to retain 5% of the seed in the safe custody
- **Para 5**
  - supplier to certify that the imported transgenic material contains
    - transgenes conforming to those described in the permission
    - no embryo-genesis deactivator (terminator) gene

Facilities at NBPGR for GM detection

Capacity Building in GM Detection at NBPGR

- NBPGR has been actively involved in testing of transgenes in the imported transgenic material since 2000.
- DBT project "National Containment/Quarantine facility for testing of Transgenic Material"

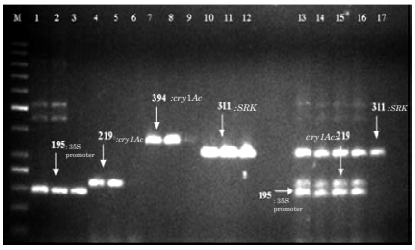
- Established a State-of- Art National Containment Facility of CL-4 level
- Well-equipped transgenic testing laboratory for PCR based testing of imported transgenic lines

Imported Transgenic Planting Material kept in Gene bank (1997-till date)
Total No. of imports: 135 constituting 2879 accessions

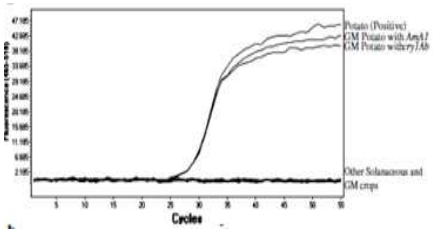
Crop & No. of Imports	Transgenes	Traits	Country of Import
Zea Mays (44)	*cry1Ab, cry1A.105, cry2Ab2, cry1F* *gat, cp4epsps, mepsps* *Gus & control elements*	Insect resistance Herbicide tolerance	USA, South Africa, Philippines
Oryza sativa (41)	*cry1Ac, cry1Ab, cry1Ca, cry1gC, GFM-cry1A, cry2A* *AmA1, ferritin, crtI, lcy* *Basta, cp4epsps, bar* *Xa-21* *HAS, ScFv, AFP-AG*	Insect resistance High nutritional quality Herbicide tolerance Bacterial pathogen resistance Nematode resistance	USA, Belgium, Philippines, UK, Switzerland, Vietnam, China
Gossypium hirsutum (26)	*cry1Ac, cry2Ab, cry1Ab-cry1Ac, cp4epsps, cry1F, vip3A, cry2Ae, cry1Ab* *Cp4epsps* *35S-rolA, B, C & Mannosyl transferase* *At ANP1, AtSOS2, At A-20, At CBF3, At SOS1*	Insect resistance Herbicide tolerance Drought tolerance Salinity and drought tolerance	China, USA
Brassica spp. (8)	*cry9C, cry1Ba, cry1Ca* *Barnase, barstar, bar* *Osmades-1*	Insect resistance Male sterility and restoration of male fertility & glufosinate ammonium herbicide resistance Reduced apical dominance	Belgium, The Netherlands, Australia
Lycopersicon esculentum (3)	*AVP1* *arg*	Increased salt and drought tolerance Insect resistance	USA
Glycine max (3)	*Cp4epsps*	Herbicide tolerance	USA
Triticum aestivum (2)	*HAS, ScFv, AFP-AG* *Cp4epsps*	Nematode resistance Herbicide tolerance	Germany, USA
Cicer arietinum (2)	*Bean-alpha amylase inhibitor*	Insect resistance	Australia, Scotland
Nicotiana tabacum (1)	*Alternate oxidase*		Canada
Solanum tuberosum (1)	*RB*	Late blight resistance	USA

Standardization of Endogenous Reference Genes

1. Molecular characterization of Bt cauliflower with multiplex PCR and validation of endogenous reference gene in Brassicaceae family



2. Validation of ST-LS as reference gene for detection of GM potato using Real Time PCR



Specificity analysis of the potato ST-LS1 gene using qualitative real time PCR
a. Amplification plot generated from 11 different crops (*Solanum tuberosum, Solanum melongena, Lycopersicon esculentum, Capsicum annum, Datura stramonium, Petunia hybrida, Gossypium, Zea mays, Oryza sativa, Brassica oleracea var. Botrytis, Abelmoschus esculentus*)

Source: 1. Randhawa G.J, R Chhabra and M Singh (2008) Molecular Characterization of Bt Cauliflower with Multiplex PCR and Validation of Endogenous Reference Gene in Brassicaceae Family. *Current Science*. 95, No.12 :1729-31

2. Randhawa G.J, M Singh & R Sharma (2009) Validation of ST-LS1 as an endogenous reference gene for detection of AmA1 and cry1Ab genes in genetically modified potatoes using multiplex and real time PCR. *Amer. J. Pot. Res.*, 86: 398–405.

Initial screening through Simplex / multiplex PCR-based amplification of marker & reporter genes for screening GM crops

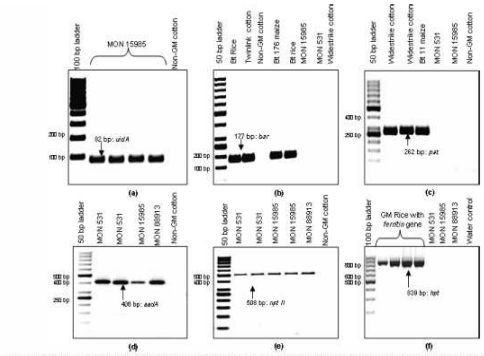


Figure 1. Simplex PCR amplicons in different transgenic seed materials for their respective selectable marker genes using the specific primer pairs for the detection of (a) *uidA*; (b) *bar*; (c) *pat*; (d) *aadA*; (e) *npt II*; and (f) *hpt* genes.

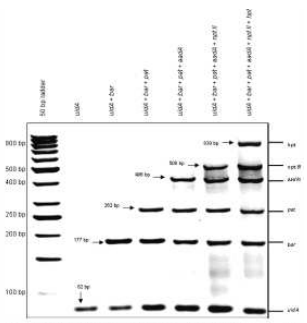


Figure 2. Multiplex PCR assay for testing of primer interference using equivalent DNA mix of six different GM events, i.e., MON 531 of cotton, MON 15985 of cotton, Widestrike cotton, Bt rice, GM rice with the *hpt/bar* gene and Bt176 of maize.

Simultaneous amplification of six commonly used marker genes *viz.*, *nptII, aadA, bar, pat, hpt* and *uidA*

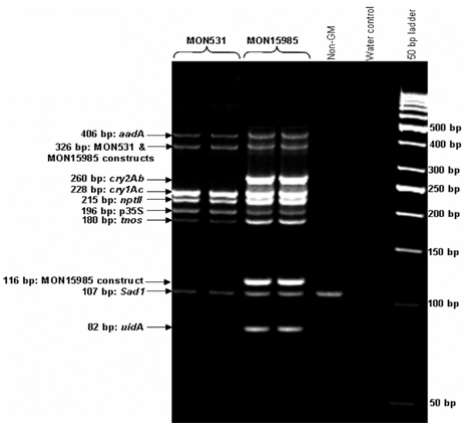
Randhawa G.J, R Chhabra and M Singh (2009) Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for screening of genetically modified crops. *J. Agri. Food Chem.* 57 (12): 5167-5172.

Identification of specific transgene by simplex PCR /multiplex PCR

Development of qualitative and quantitative PCR assays

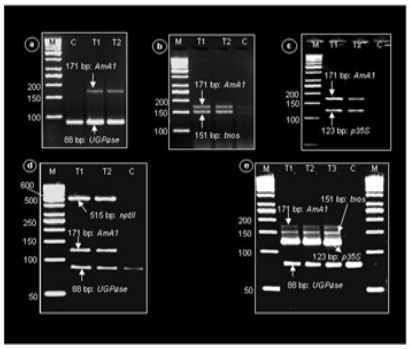
Event	Multiplex PCR	Transgenes + reference gene involved
Bt cotton MON 531	Heptaplex	fs-ACP + cry1Ac + 35S promoter + nos term. + nptII + aadA + cry1Ac construct
Bt cotton MON 15985	Decaplex	fs-ACP + cry1Ac + cry2Ab + 35S promoter + nos term. + nptII + aadA + uidA + cry1Ac construct + cry2Ab construct
Bt Rice	Triplex	cry1Ac, nptII + α-tubulin
Bt Brinjal	Quadraplex	cry1Ac, caMV 35S promoter, aadA + β- fructosidase
Bt Brinjal	Triplex	cry1Ab, 35S promoter + β- fructosidase
Bt cauliflower	Triplex	cry1Ac, 35S promoter + SRK
Bt Okra	Quadraplex	cry1Ac, nptII, 35s promoter + chloroplast t-RNAomat
GM tomato	Quadraplex	Avp1, nptII, 35S promoter + LAT52,
GM tomato	Triplex	Osmotin + 35S promoter + LAT52,
GM potato	Triplex/ Quadraplex	RB gene, CaMV 35S promoter, npt II marker + UGPase
GM potato	Triplex/ Quadraplex	Ama1 gene, CaMV 35S promoter, nos terminator, nptII + UGPase
GM potato	Triplex/ Quadraplex	cry1Ab gene, CaMV 35S promoter, nos , nptII + UGPase

Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events



Transgene- and construct-specific multiplex PCR for discrimination of two Bt cotton events, i.e., MON531 and MON15985 using primer pairs for *cry1Ac* and *cry2Ab* transgenes, *nptII*, *aadA*, and *uidA* marker genes, *CaMV* 35S promoter, *nos* terminator, endogenous *Sad1* gene, and specific gene constructs in MON531/MON15895 and MON15985.

Duplex, triplex and quadruplex PCR for molecular characterization of GM Potato with improved protein quality



PCR in duplex,triplex and Quadraplex format for Detection of GM Potato with *Ama1* gene
Lane M: 50 bp DNA ladder; Lanes T1, T2, T3,: GM potato with *AmA1* gene; Lane C: Non-GM potato

Source: Randhawa G.J., R Chhabra and M Singh (2010) Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events. *Journal of Agriculture and Food Chemistry* (2010) *58* (18), pp 9875–9881.

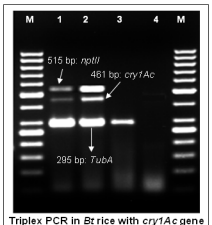
Randhawa G.J, Monika Singh & Ruchi Sharma (2009) Duplex, triplex and quadruplex PCR for molecular characterization of genetically modified potato with better protein quality. *Current Science*, 97 (1): 21-23.

Multiplex PCR assays for detection of *Bt* crops with *cry1Ac* gene

Bt Brinjal

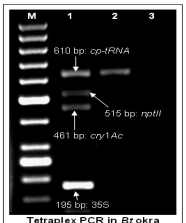
Triplex PCR in *Bt* brinjal with *cry1Ac* gene
1: Water control
2: Non-*Bt* brinjal
3-4: *Bt* brinjal
M: 50bp DNA ladder

Bt Rice



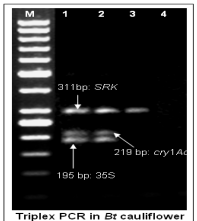
Triplex PCR in *Bt* rice with *cry1Ac* gene
1-2: *Bt* rice
3: Non-*Bt* rice
4: Water control
M: 50bp DNA ladder

Bt Okra



Tetraplex PCR in *Bt* okra with *cry1Ac* gene
1: *Bt* okra
2: Non-*Bt* okra
3: Water control
M: 50bp DNA ladder

Bt cauliflower

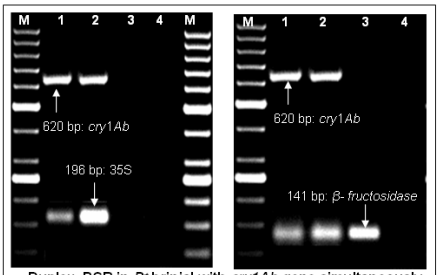


Triplex PCR in *Bt* cauliflower with *cry1Ac* gene
1-2: *Bt* cauliflower
3: Non-*Bt* cauliflower
4: Water control
M: 50bp DNA ladder

Source: **Randhawa G.J., M Singh, R Chhabra and R Sharma (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India.** ***Food Analytical Methods*** **3 (4), 295-303**

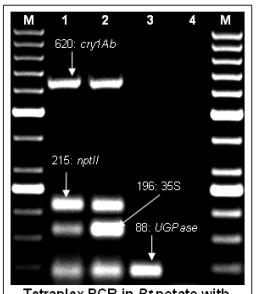
Multiplex PCR assays for detection of *Bt* crops with *cry1Ab* gene

Bt Brinjal



Duplex PCR in *Bt* brinjal with *cry1Ab* gene simultaneously detecting (i) *cry1Ab* and *CaMV* 35S *promoter; and (ii) cry1Ab and endogenous β- fructosidase* gene
1-2: *Bt* brinjal
3: Non-*Bt* brinjal
4: Water control
M: 50bp DNA ladder

Bt Potato



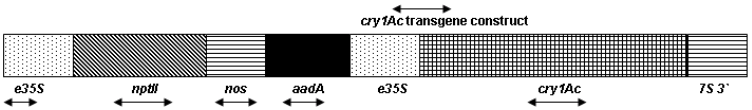
Tetraplex PCR in *Bt* potato with *cry1Ab* gene
1-2: *Bt* potato
3: Non-*Bt* potato
4: Water control
M: 50bp DNA ladder

Source: **Randhawa G.J., M Singh, R Chhabra and R Sharma (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India.** ***Food Analytical Methods*** **3 (4), 295-303**

Construct-specific PCR

I.Bt-brinjal and Bt-rice *:* Juncture of 35S promoter and *cry1Ac* gene

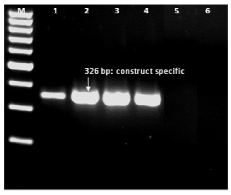
GM Cotton: Juncture of 35S promoter and *cry1Ac* gene in Bt-cotton events *viz.* BG I, BG II, Event1 and GFM-cry1A



Linear Transgene Construct of MON 531

Construct-specific PCR for detection of a part of inserted gene construct in GM Tomato (*avp1* gene)

Lane1-2: GM tomato, Lane 3: Non-GM tomato, Lane 4: Water control, M: 1kb Ladder



Construct-specific PCR for detection of a part of inserted gene construct in GM cotton events *viz.* BGI, BGII, Event1 and GFM- cry1A

Lane 1: BGI, Lane 2: BGII, Lane 3: Event1, Lane 4: GFM-cry1A
Lane 5: Non-GM cotton, Lane 6: Water control, M: 100 bp ladder

Construct-specific PCR for detection of a part of inserted gene construct in GM rice (*cry1Ac* gene)

Lane 1: Water control, Lane 2: Non-GM rice, Lane 3-6: GM rice, M: 1kb ladder

Quantitative detection

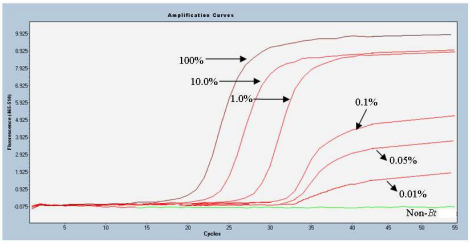
Real-time PCR assays on Light cycler®480 system

I.GM Tomato:

II. GM Cotton:

III. GM Potato:

Sensitivity of Real-Time PCR assay for *cry1Ac* gene: up to 0.01%



The experimental mean values for GM content, *i.e.*, 100, 10, 1.0, 0.1, 0.05 and 0.01 ng/µl were found similar to the theoretical values indicating that the developed assays can detect as low as 0.01 ng of genomic DNA with *cry1Ac* gene.

Test samples with 100, 10, 1.0, 0.1, 0.05 and 0.01% transgene content showed the amplification signals whereas no signal was detected in non-*Bt* sample

Oral presentation in *Fourth International conference on co-existence between GM and non-GM based agricultural supply chains* at Melbourne

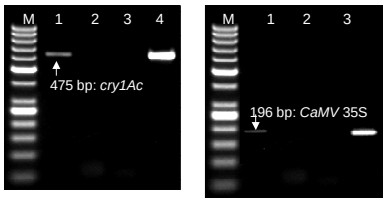
Sampling strategies for PCR-based transgene detection

- An appropriate seed sampling strategy developed to screen or/and quantify GM content in laboratory and analytical samples to be prepared as the guidelines of International Seed Testing Association (ISTA) 1999.

- The detection limit up to 0.1% (by mixing 1 GM seed + 2999 non-GM seeds) at 95% probability level in GM Brinjal with *cry1Ac* gene and GM Tomato with *AVP1* gene has been achieved.

Bt Brinjal case study

1 Bt brinjal seed is mixed with a seed lot of 2999 non-GM brinjal seeds to get detection limit of 0.1% at 95% probability level



PCR amplification of *cry1Ac* gene and *CaMV* 35S promoter gene in Bt-brinjal test sample

Lane 1: Bt-brinjal test sample (1 GM brinjal seed + 2999 non-GM brinjal seeds) Lane 2: Non-GM brinjal Lane 3: Water control

Lane 4: Positive Bt-brinjal (100% GM) (with *cry1Ac* gene)

Recent Publications

1. Randhawa, GJ et. al (2010) Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events. *J Agric Food Chem 58* (18), 9875–9881
2. Randhawa G.J., Singh M and Grover M (2010) Bioinformatic analysis for allergenicity assessment of *Bacillus thuringiensis* Cry proteins expressed in insect-resistant GM food crops. *Food Chem Toxicol* doi:10.1016/j.fct.2010.11.008
3. Randhawa, GJ et. al (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India. *Food Anal Methods* 3 (4), 295-303
4. Randhawa, GJ et. al (2010) Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for screening of Genetically modified crops. *J Agric Food Chem* 57, 5167-5172
5. Tiwari S P and Randhawa G.J. (2010) Strategies to Monitor the Adventitious Presence of Transgenes in Ex Situ Collection *In J Agric Sci* 81 (5): 351-6.
6. Randhawa, GJ et. al (2010) PCR-based detection of genetically modified tomato overexpressing a mutant of *Arabidopsis* vacuolar H^+-pyrophosphatase gene employing seed sampling strategy. *Seed Sci Technol* (in press)
7. Randhawa, GJ et. al (2009) Validation of *ST-LS1* as an endogenous reference gene for detection of *AmA1* and *cry1Ab* genes in genetically modified potatoes using multiplex and real time PCR. *Am J Pot Res* 86:398-405
8. Randhawa, GJ et. al (2009) Import and Commercialization of Transgenic Crops: An Indian Perspective. *Asian Biotech Develop Review* 11(2) 115-130
9. Randhawa, GJ et. al (2009) Duplex, triplex and quadraplex PCR for molecular characterization of Genetically Modified Potato with improved protein quality *Curr Sci.* 97 (1) 21-23
10. Randhawa, GJ et. al (2009) Multiplex Polymerase Chain Reaction for detection of genetically modified potato with *cry1Ab* gene. *In J Agric Sci* 79 (5):368-71

10. Randhawa, GJ et. al (2009) Molecular Characterization of Bt Cauliflower with Multiplex PCR and Validation of Endogenous Reference Gene in Brassicaceae Family. Curr Sci. 95, No.12 :1729-31

11. Randhawa, GJ et. al (2010) Molecular diagnosis of transgenic Tomato with osmotin gene using Multiplex Polymerase Chain Reaction. Curr Sci. 96, No. 5: 689-694

Technical Article/Brochure

1. Regulation with Confidence: DNA-based Diagnostics of Genetically Modified Crops, (2010) Biotech News 5 (5), 192-195

2. DNA-based Diagnostics of Genetically Modified Crops, 2010, pp 6.

GMO Detections kits

- Available in India at a competitive price
- Excellent specificity and Sensitivity
- Robust performance with long term stability
- Only one manufacturer in Asia

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Hyderabad, AP 500033 India
Tel: 91-4023552953 Fax: 91-4023552954
e mail: amar.immunodiagnostics@gmail.com

By: Dr. Jayant K Bhanushali
Director-R & D
Amar Immunodiagnostics Pvt Ltd

LIST of GMO ELISA kits

1. Cry1Ac ELISA kit (Screening Assay)
2. Cry2A ELISA kit
3. Cry1Ac ELISA kit (WideStrike specific gene)
4. Cry1Ac ELISA kit (Chinese specific gene)
5. Cry1F ELISA kit (WideStrike specific)
6. RoundUp Ready ELISA kit
7. Cry1C ELISA kit
8. Cry1EC ELISA kit (Fusion of Cry1Ac and Cry1C)

Transfer and Commercialization of Technologies

AMAR
IMMUNODIAGNOSTICS
PCR kits for GMO detection

Amar Immunodiagnostics offers three types of qualitative kits for PCR based GMO testing.

- GMO screening kits
- Trait/event specific gene Identification kits
- Endogenous gene

1. GMO screening kits: Most transgenic plants have 35S as promoter and NOS as terminator gene. The presence of either/both of these genes in plant indicates that the plant being tested has been genetically modified and hence GMO.
2. Trait/event specific Gene Identification kit: The plant which tests positive in GMO screening kit can be further tested to establish the identity of trait/event specific gene by Trait/event specific PCR testing kit to identify transgene present in the Plant.
3. Endogenous gene: In order to ensure that the DNA purified from the plant tissue is of acceptable quality and does not contain PCR inhibitors, we provide PCR kits to detect plant specific gene.

Kit size: PCR kits are provided in 50 reactions format.
Kit contents
1.Master Mix
2.Taq polymerase
3.H_2O

Prototype PCR Amplification Program

Step	Temprature/Time
Initial Denaturation	94°C/ 30 sec
Cyclic Amplification	59°C/ 60 sec
Denaturation	72°C/ 60 sec
Primer Annealing	
Extension	94°C/5 min
Number of cycles	39 Cycles
Final Elongation	72°C/ 8 Min

The Presence or absence of amplicons generated by PCR reaction is verified by Agarose gel electrophoresis. Positive and negetive calibrators are separately available. Store at -20C

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Tel: 040-23552053, 23547202 Fax: 040-23559854
E mail:amar.immunodiagnostics@gmail.com
gurinder.randhawa@rediffmail.com

PCR kits for GMO detection

Transgenic crop/Event	Transgenes	Amplicon size (bp)	Catalog no:
Cotton			
Bollgard (Mon 531)	Cry1Ac	228	AID 101
Bollgard II (MON 15985)	Cry1Ac, Cry2A	228/240	AID 102
WideStrike	Cry1F, Cry1Ac	300/228	AID 103
MON 1445 (RoundUp Ready)	CP4EPSPS	506	AID 105
Brinjal			
For insect resistance	Cry1Ac	475	AID 107
For insect resistance	Cry1Ab	620	AID 108
Soybean			
RoundUp Ready Soybean	CP4EPSP	441	AID 100
Maize			
MON 810	Cry1Ab	170	AID 111
RoundUp Ready Maize	CP4EPSPS	441	AID 113
Tomato			
For drought and salt tolerance	Osmotin	418	AID 113
For drought and salt tolerance	Avp1-F/R	451	AID 114
Cauliflower			
For insect tolerance	Cry1Ac 1	219/354	AID 115
Mustard			
For male sterility	Barnase, barstar	300/352	AID 116
Rice			
For insect resistance	Cry1Ac	192	AID 117
Okra			
For insect resistance	Cry1Ac	391	AID 118
Potato			
For insect resistance	Cry1Ab	620	AID 119
For better nutritional quality	AmA1-171 F/R	171	AID 120
For late blight resistance	Rib	375	AID 121
Promoter and Marker Genes			
Promoter	35S	196	AID 122
Terminator	NOS	180	AID 123
Antibiotic marker	NPTII	508	AID 124
Endogenous genes			
Cotton	Rad1	107	AID 125
Brinjal	Pomtom F/R	141	AID 126
Soybean	Lectin	178	AID 127
Maize	Zein	329	AID 128
Tomato	Lat 52	92	AID 129
Tomato	PomTom	143	AID 130
Cauliflower	SRK	311	AID 131
Mustard	HMG I/Y	99	AID 132
Rice	α- tubulin	295	AID 133
Potato	UGP	88	AID 134
Okra	Chloroplast-tRNA gene	595	AID 135


THANK
YOU