Detection and Identification of Living Modified Organisms

V. Siva Reddy
International Centre for Genetic Engineering and Biotechnology

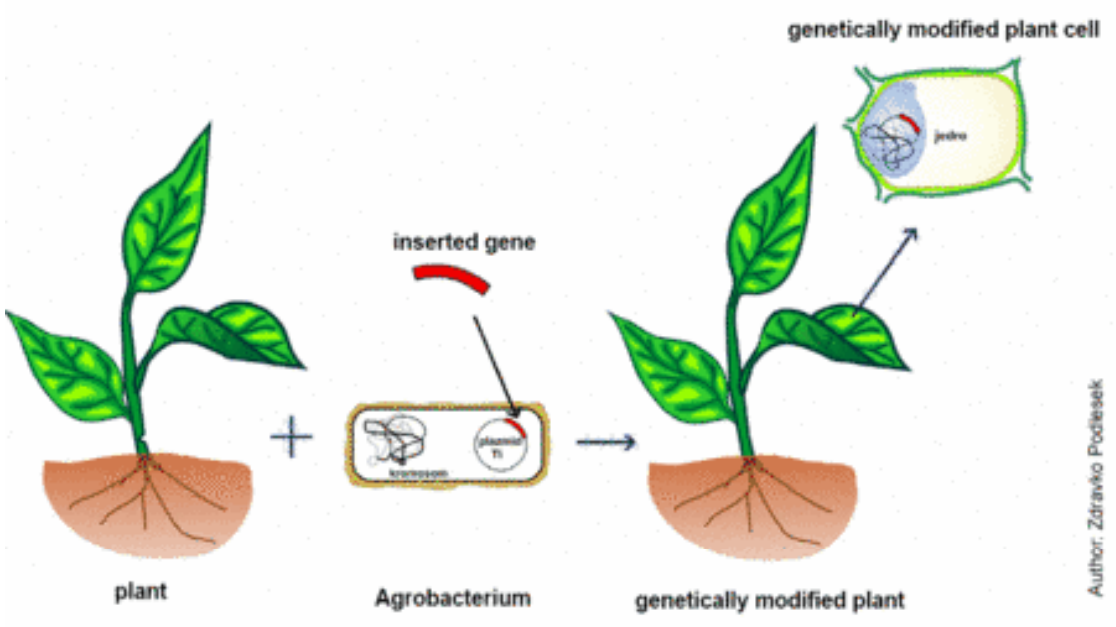
22nd November 2011 (cbd/MoEF workshop)

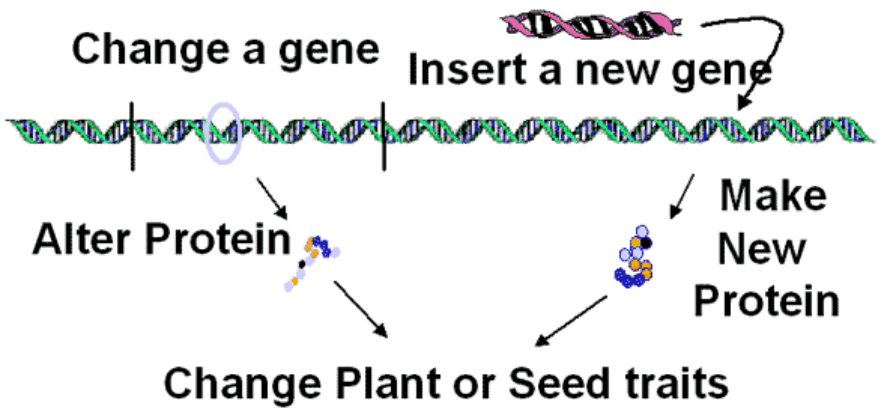
Talk will cover:

Methods of genetic transformation
Basic features of gene constructs
Current Global scenario
Current Indian Scenario
Detection strategies
- **DNA based**
- **Protein based**

LMO detection: Lab set up and equipment required

Introduction of foreign genes and creation of GMO/LMO





Methods of Plant Transformation

- *Agrobaterium*
- Gene gun
- Electroporation
- PEG up take method
- Microinjection
- Pollen tube path way
- Liposome mediated
- Viral vectors
- Apical meristem method
- Vacuum infiltration method

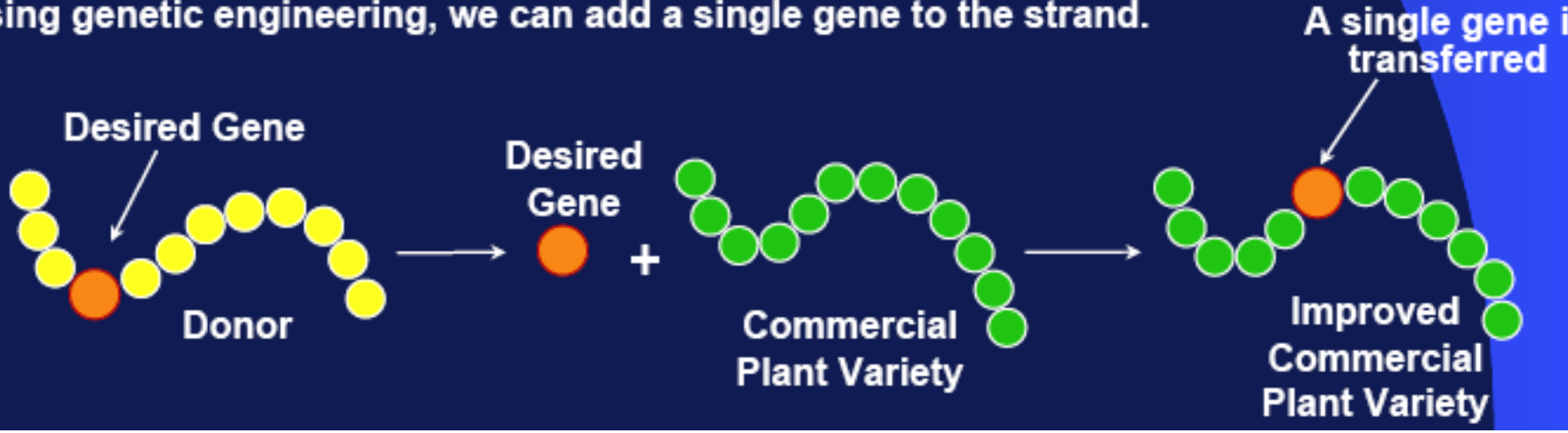
Genetic Engineering is an Extension of Traditional Plant Breeding

TRADITIONAL PLANT BREEDING

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.

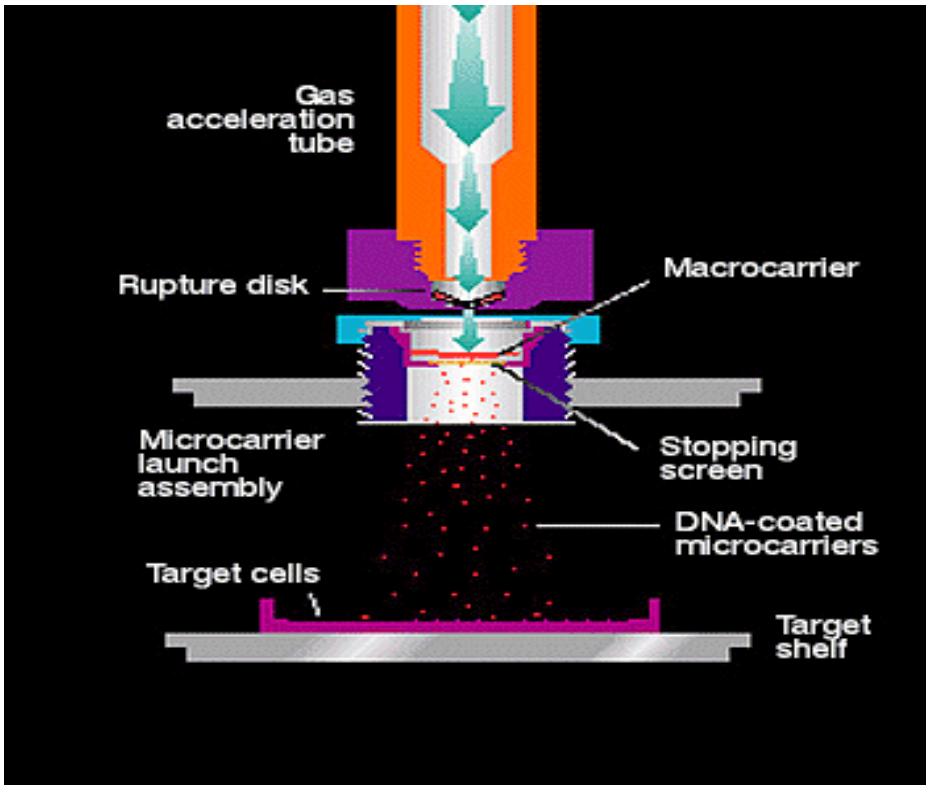
GENETIC ENGINEERING

Using genetic engineering, we can add a single gene to the strand.



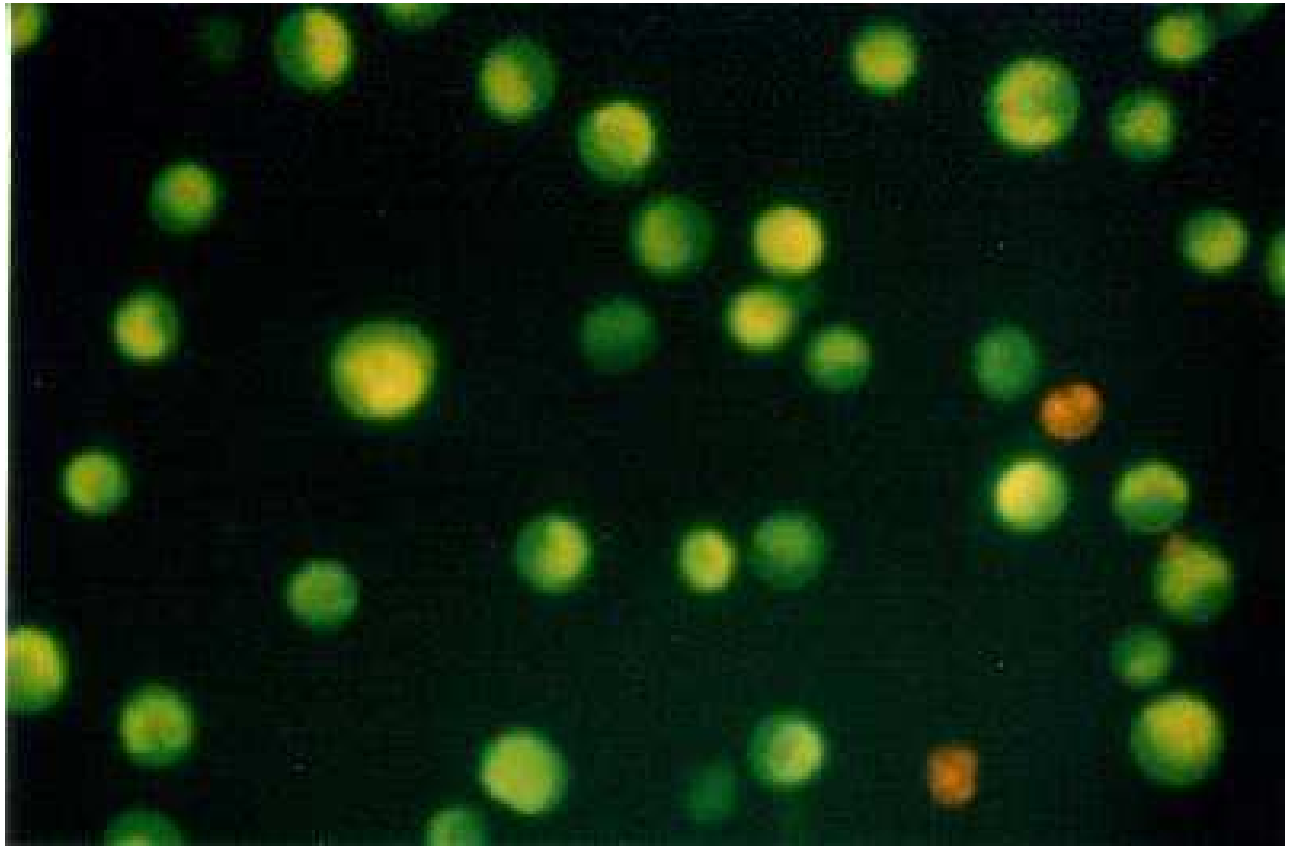
Agrobactrium mediated transformation of tobacco
Co-cultivation and selction

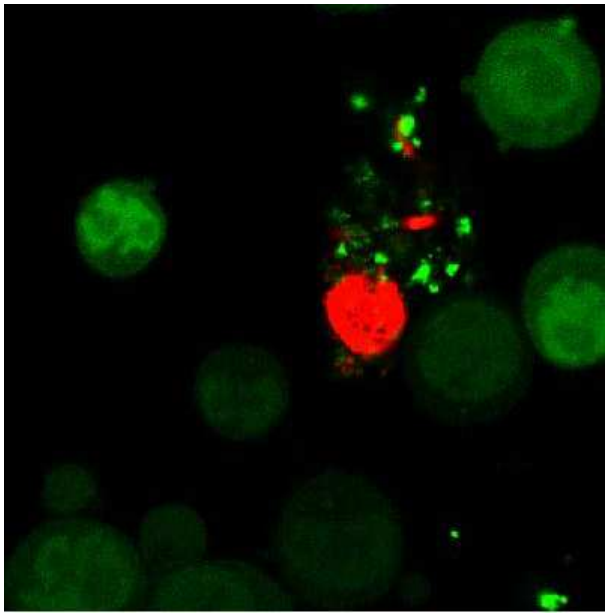
Gene gun
Particle delivery system (PDS) He 1000



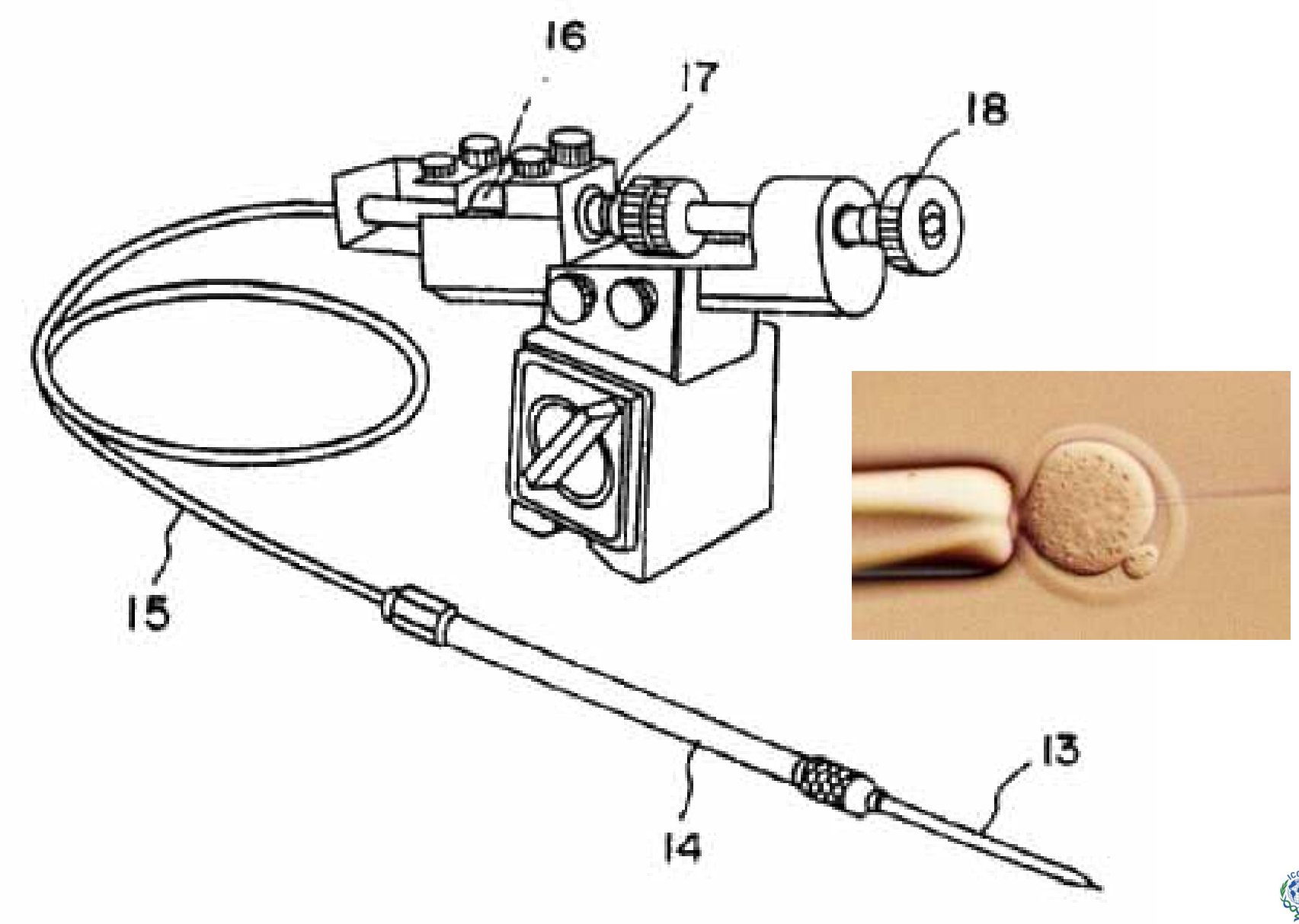
Electroporation of plant cells (protoplasts) with recombinant DNA

Tobacco leaf protoplasts expressing GFP (Transient assay)

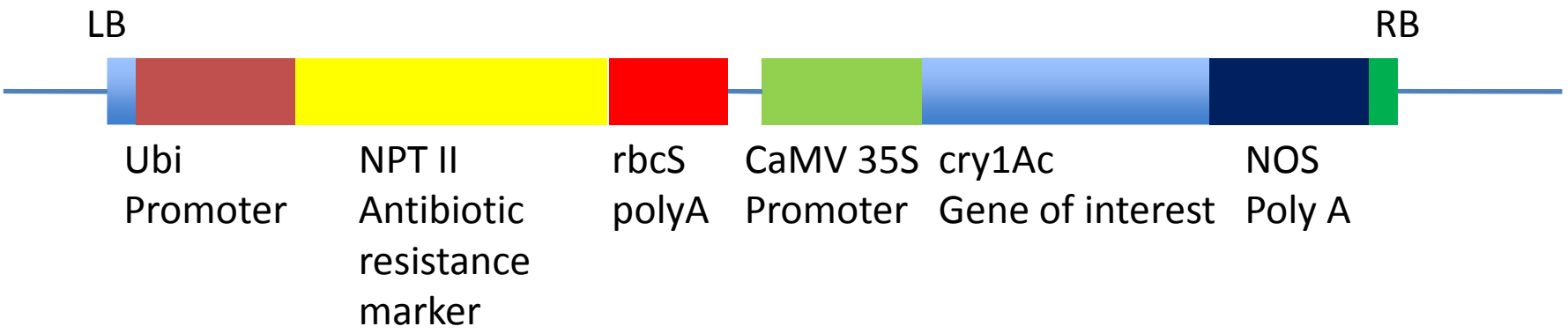




Transformation of DNA through microinjection



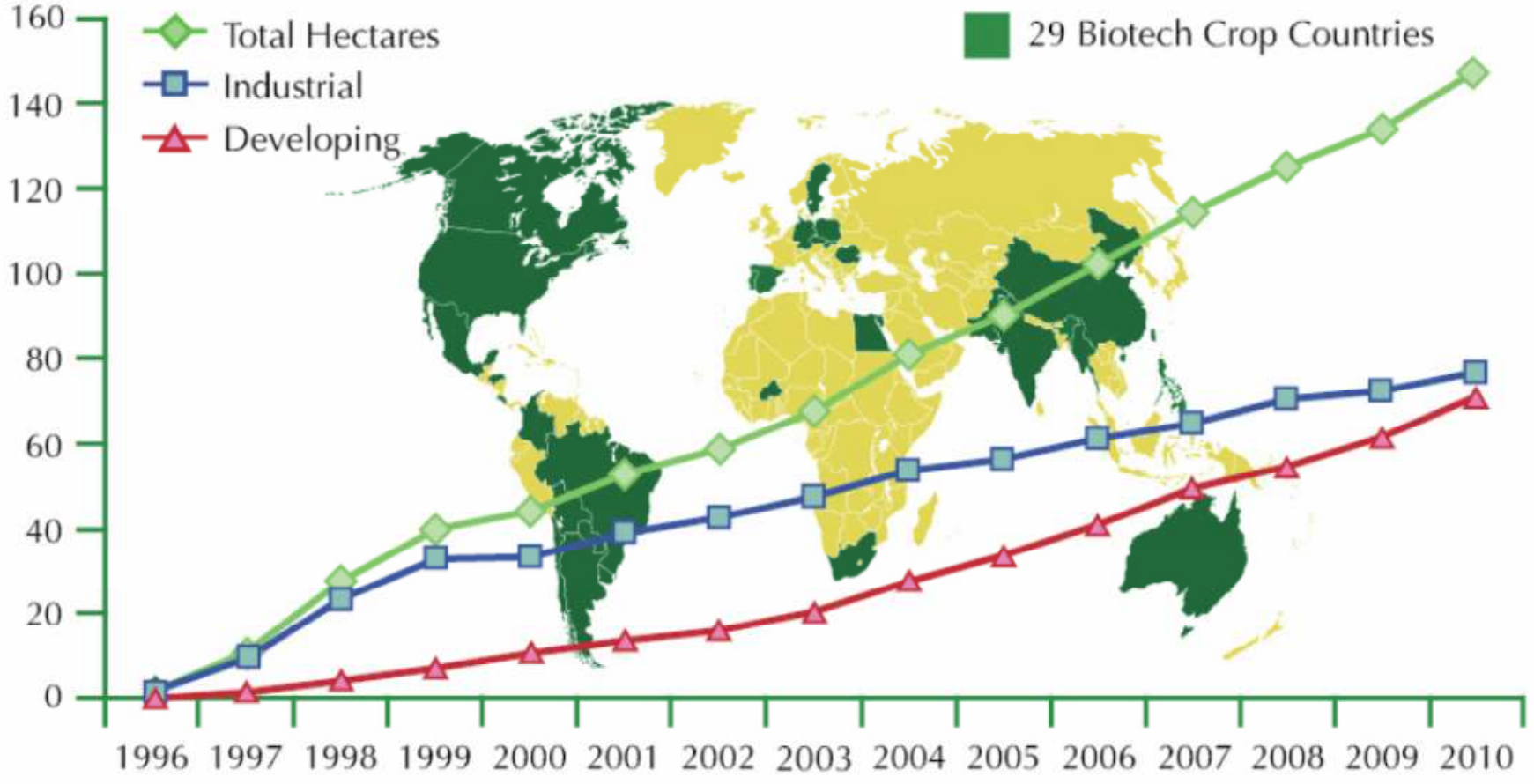
Basic transformation gene construct



Wide range of crops

16 Field Crops	14 Vegetables	16 Fruits	11 other crops
Alfalfa	Broccoli	Apple	Chicory
Barley	Cabbage	Banana	Cocoa
Canola	Carrot	Cantaloupe	Coffee
Cassava	Cauliflower	Cherry	Garlic
Clover	Cucumber	Citrus	Lupins
Cotton	Eggplant	Coconut	Mustard
Flax	Lettuce	Grape	Oil Palm
Maize	Onion	Kiwi	Oilseed Poppy
Rice	Pea/Bean	Mango	Olive
Safflower	Pepper	Melon	Peanut
Sorghum	Potato	Papaya	Tobacco
Soybean	Spinach	Pineapple	
Sugar Beet	Squash	Plum	
Sugar Cane	Tomato	Raspberry	
Sunflower		Strawberry	
Wheat		Watermelon	

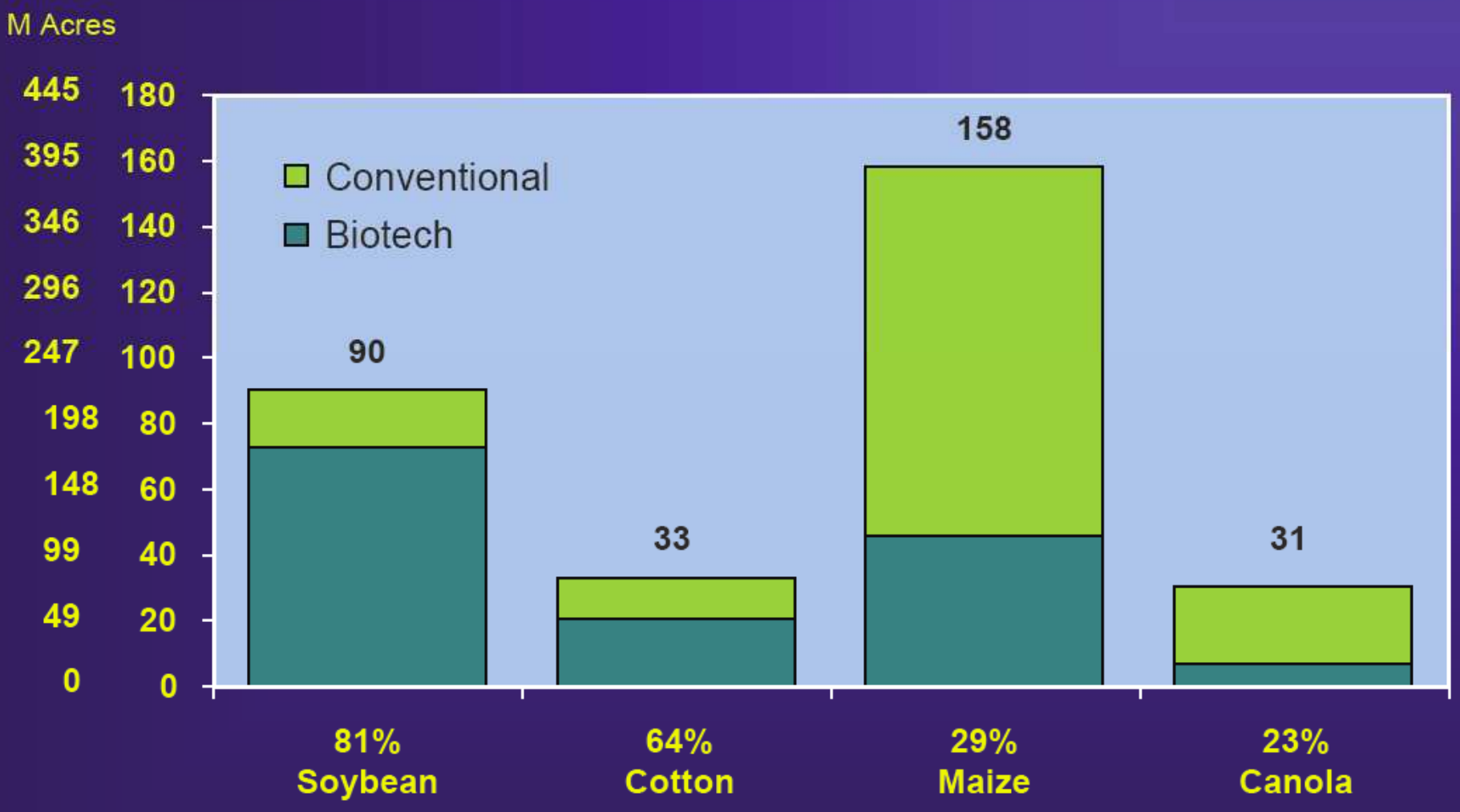
GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996-2010)



A record 15.4 million farmers, in 29 countries, planted 148 million hectares (365 million acres) in 2010, a sustained increase of 10% or 14 million hectares (35 million acres) over 2009.

Source: Clive James, 2010.

Global Adoption Rates (%) for Principal Biotech Crops (Million Hectares, Million Acres), 2010



Source: Clive James, 2010

Commercial Release of Bt Cotton Events in India, 2002 to 2010

No.	Crop	Event	Developer	Status	Year 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, Dharwa	Commercialized	2008
6	Cotton*	MLS-9124	Metahelix Life Sciences	Commercialized	2009

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

Insect resistance: Bt toxins

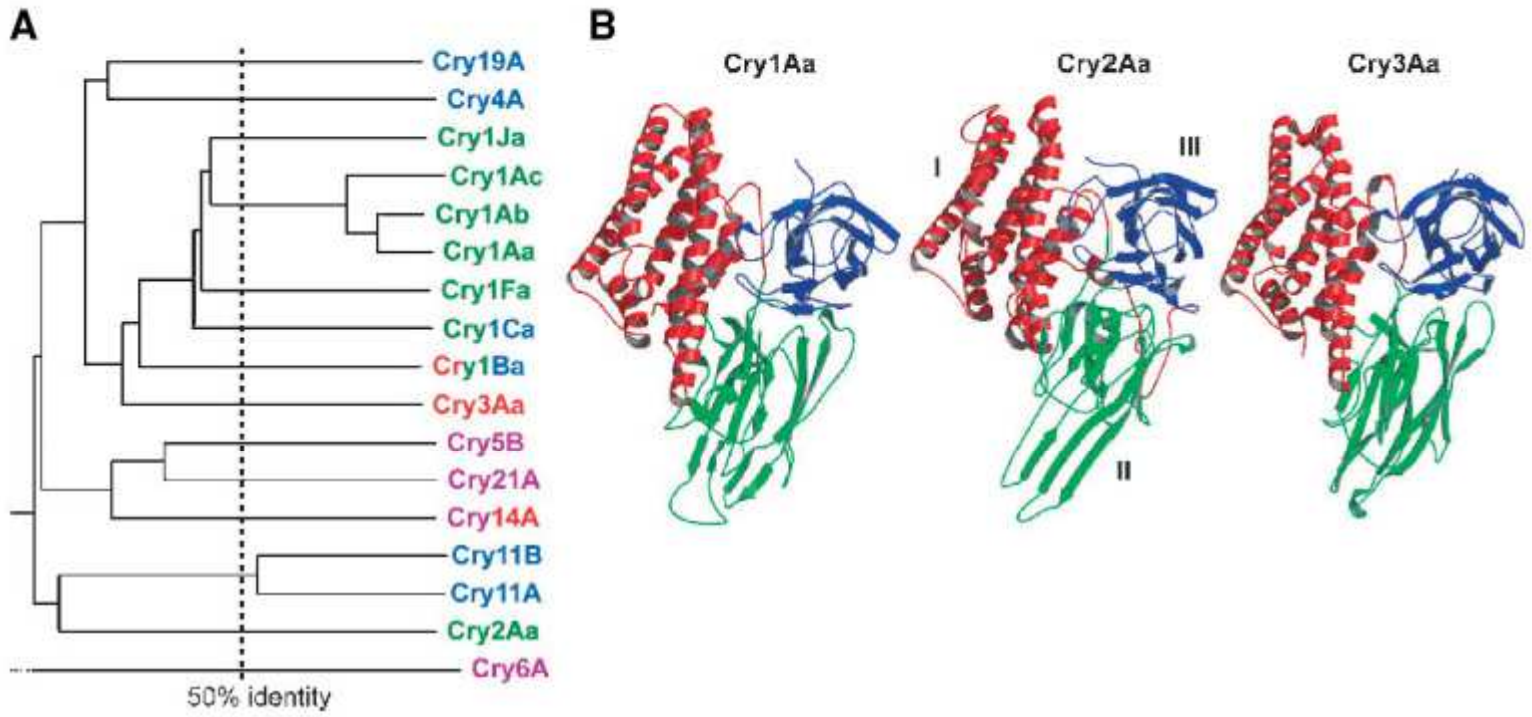


Figure 1. Cry proteins have diverse properties but highly conserved structure. **A:** A phylogenetic tree based on ClustalW alignment using Phylogeny Interference Package (version 3.5c). Only (putative) domains I-III were used in these comparisons. Colors indicate known susceptible organisms: blue, Diptera; green, Lepidoptera; red, Coleoptera; magenta, nematodes. **B:** Three-dimensional structures of Cry1Aa (left), Cry2Aa (middle) and Cry3Aa (right) drawn using MOLSCRIPT. Toxin domains I, II and III colored red, green and blue, respectively.

Cotton hybrid seeds – Non GM

Cotton hybrid seeds – GM Bt. Cotton

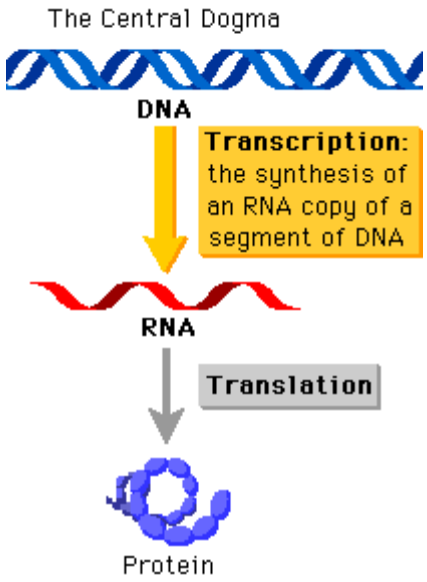
Cotton hybrid seeds mixed up with GM Bt. Cotton

Central Dogma: GMO detection

DNA

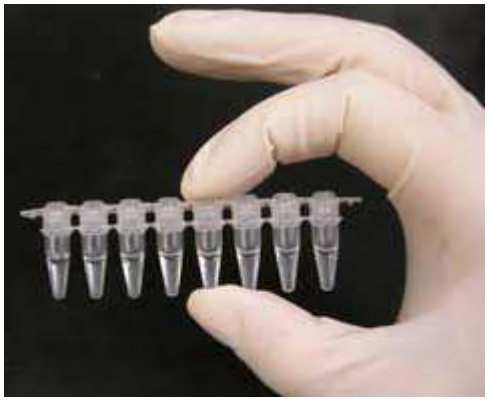
RNA

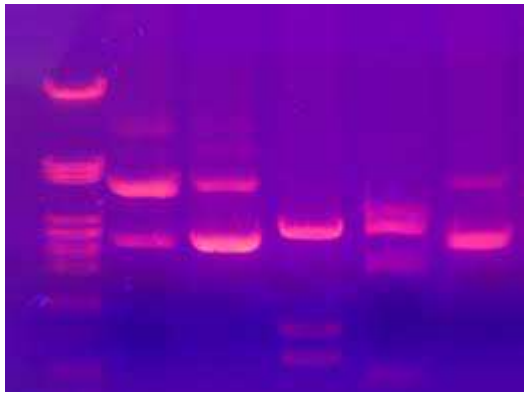
PROTEINS



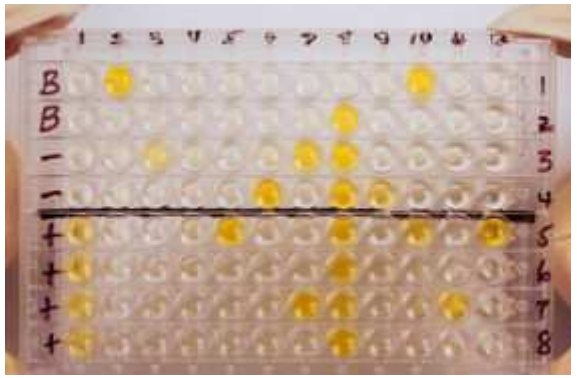
LMO detection: Lab set up and equipment required

Detection: DNA based

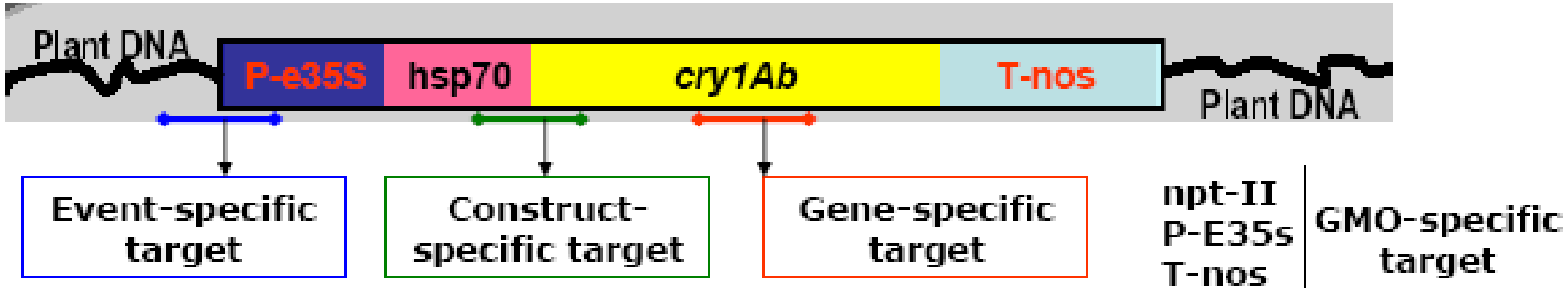




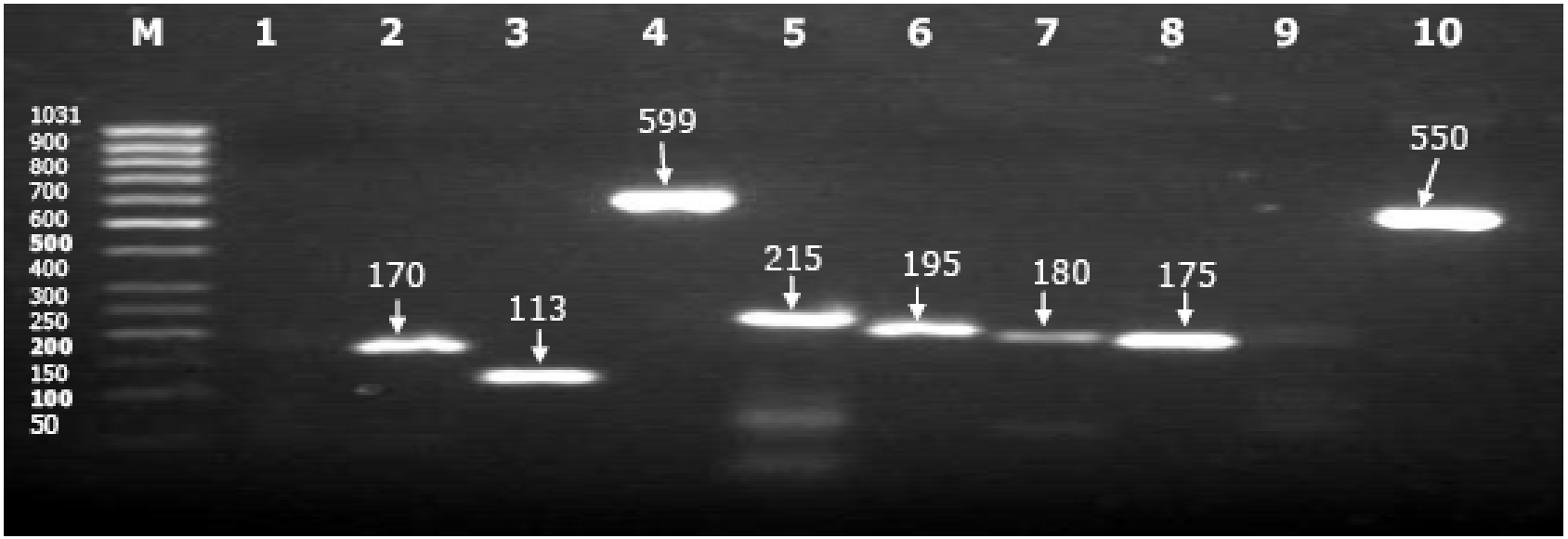
Detection: Protein based



A



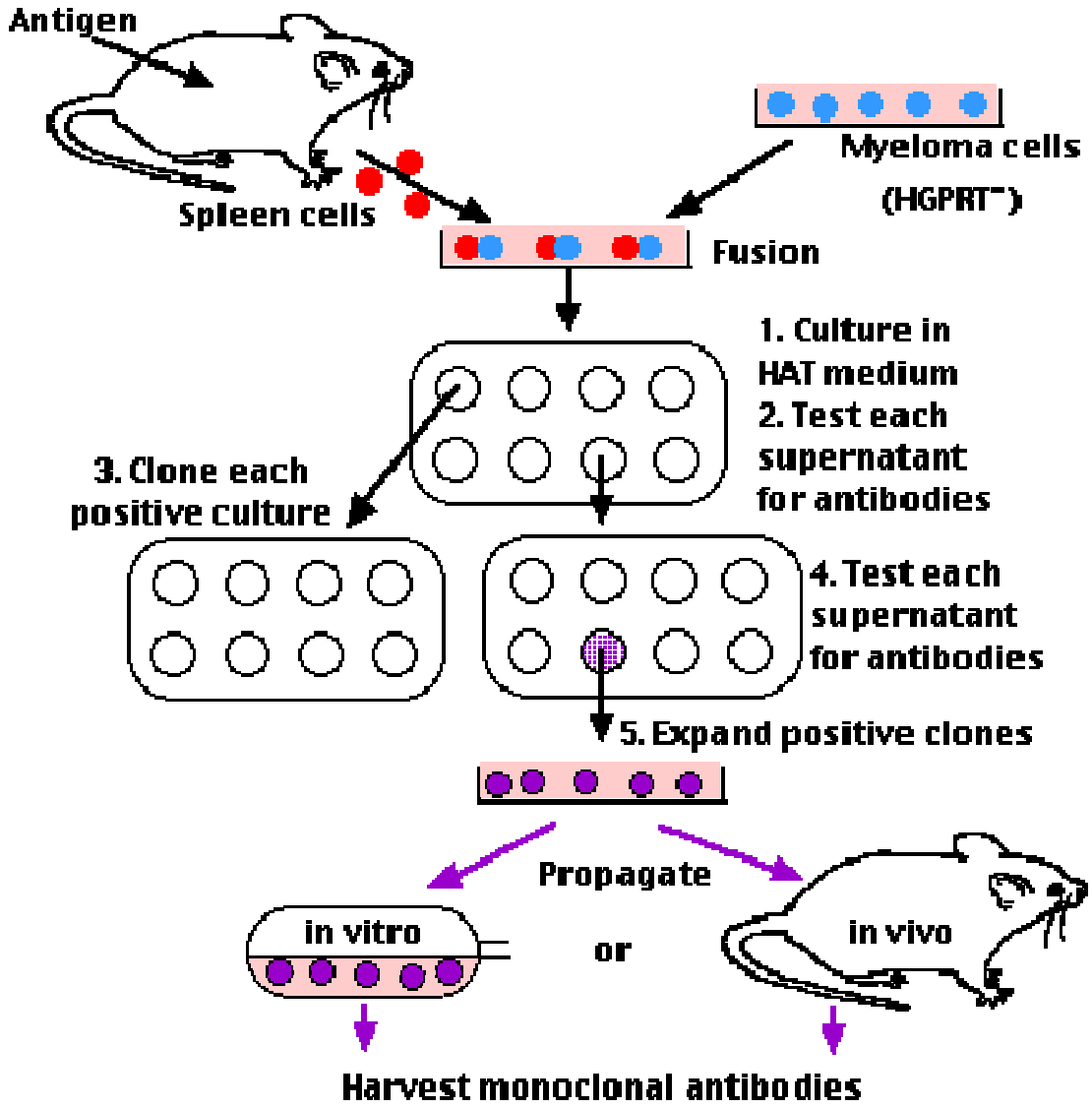
B



doi:10.1038/nprot.2007.440 Published online 23 October 2007

ELISA/Strip based detection require specific antibodies that recognize the foreign protein

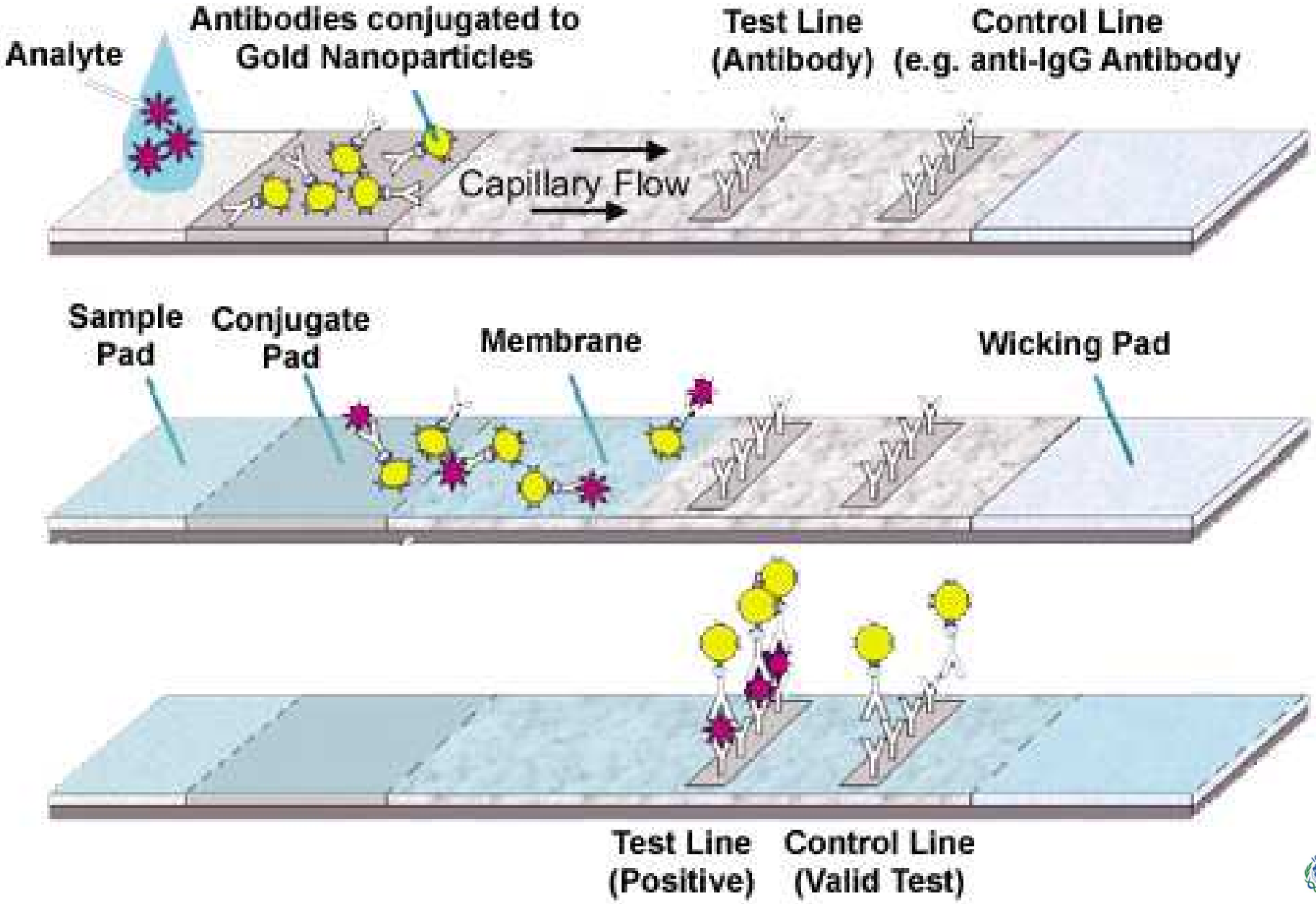
Antibodies: Monoclonal antibody production scheme



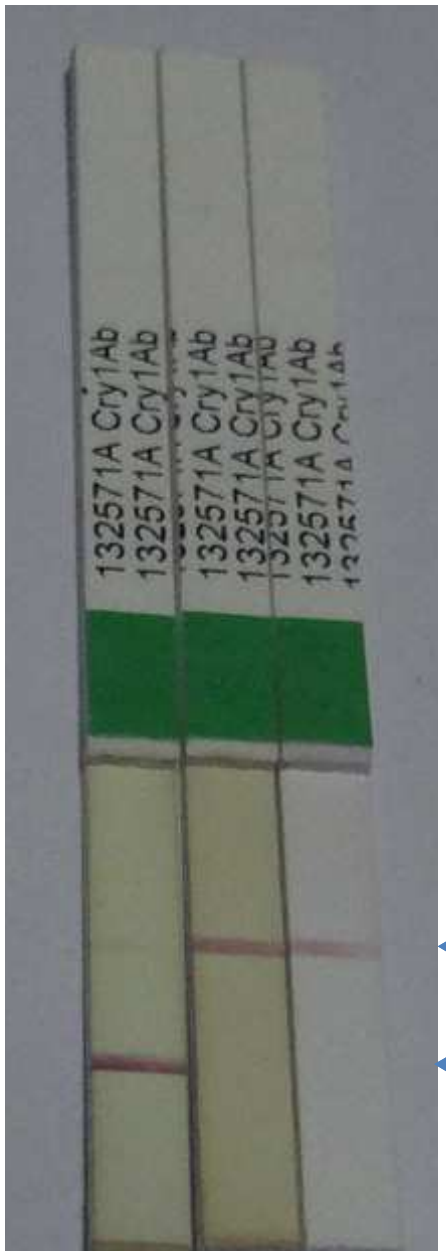
Strip based detection: Basic principles involved

- Several detection strips are available in the market to detect the same LMO
- Single strip can detect multiple events

Lateral Flow Assay Architecture



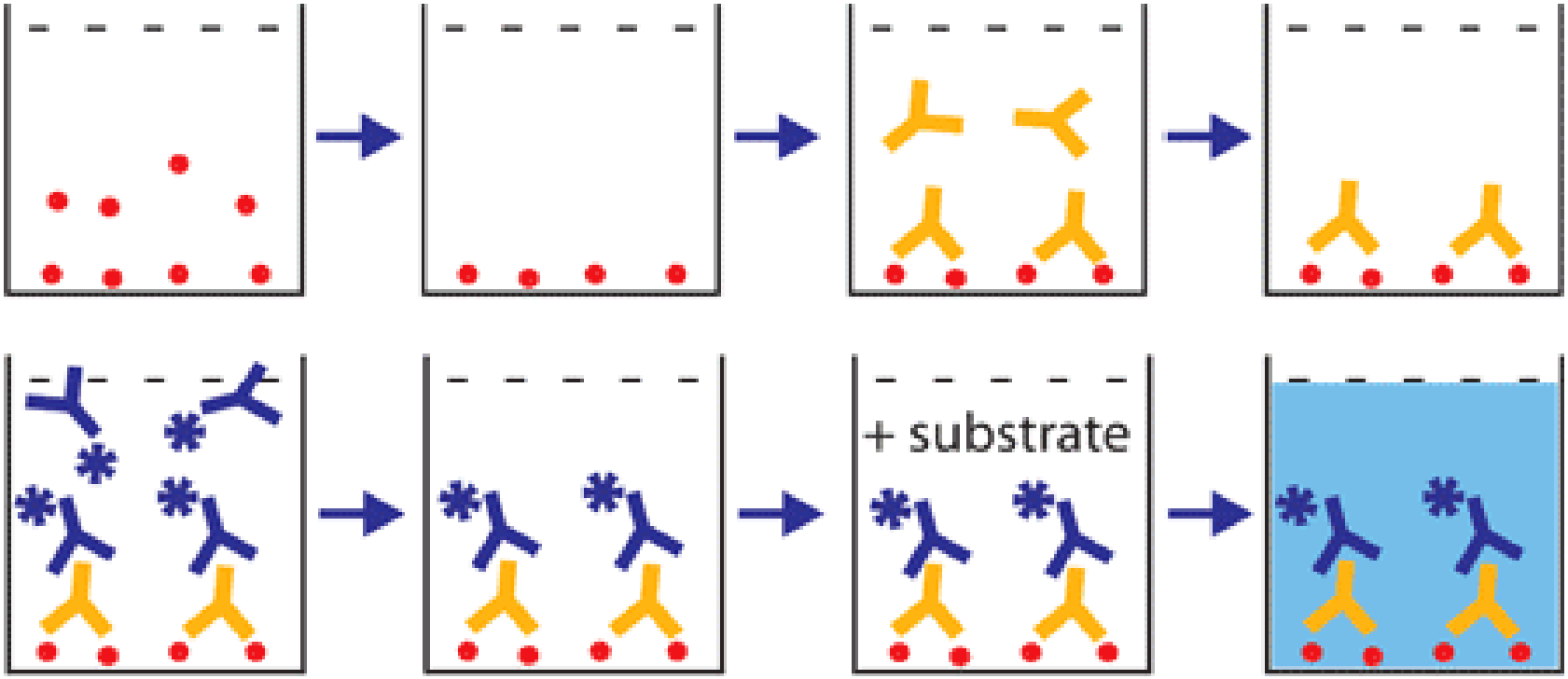
Strip test or Later flow test



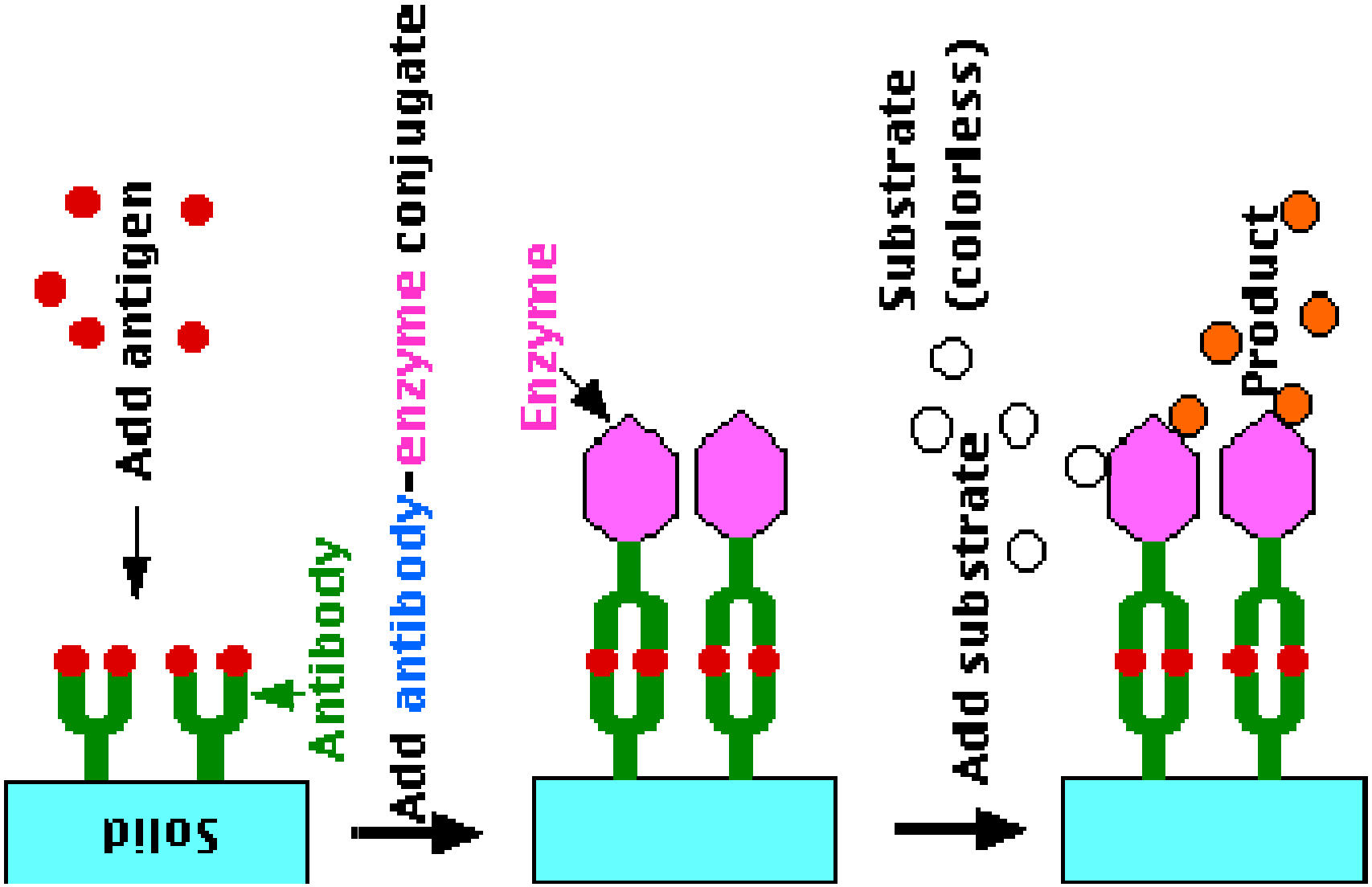
← Control line

← Test line

Direct ELISA based detection

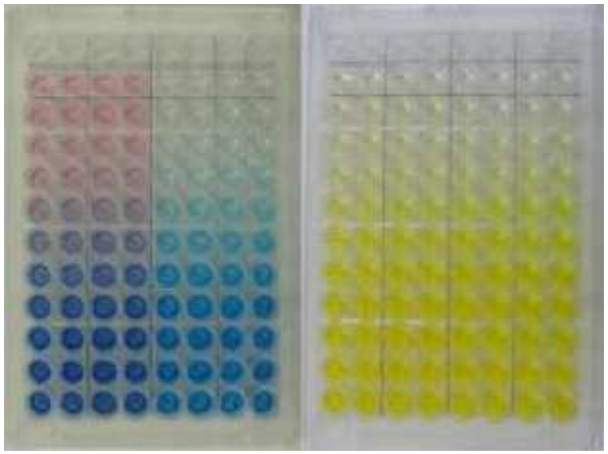


Sandwich based ELISA



HRP Substrate TMB Solutions for ELISA

TMB solutions are chromogenic reagents for peroxidase, designed for ELISA techniques, manual or automatic systems. They contain 3,3',5,5'-tetramethylbenzidine (TMB), hydrogene peroxide (H2O2), and proprietary catalyzing and stabilizing agents. Reaction with peroxidase develops an intense blue colour that can be read directly (at 650nm), or a deap yellow colour (read at 450 nm) after stop with an acid solution. Sensitivity is greater than classic substrates like OPD and ABTS, with very low background..



Detection thresholds of three transgenic maize events

CP4-EPSPS protein can be detected with high sensitivity in mixtures with low percentage of transgenic maize.

% maíz NK603	0.05%	0.10%	0.25%	0.50%	1.0%
A_{620}	0.122	0.186	0.683	0.958	1.18

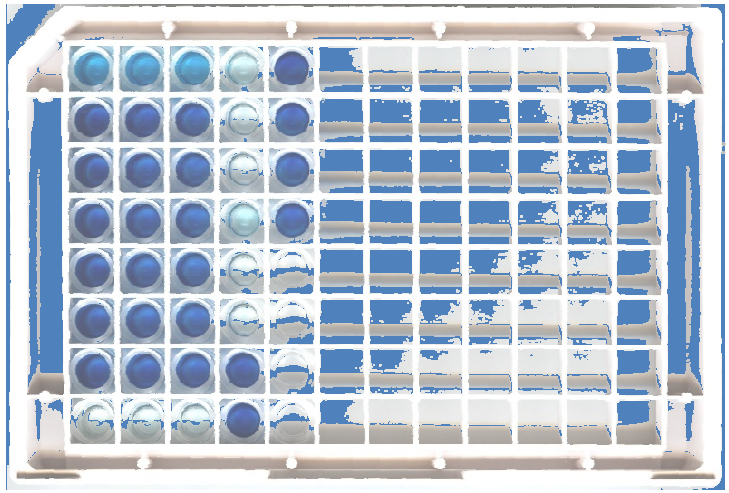
Sensitivity for detection of CRY proteins is much lower.

% de Maíz	0.05%	0.10%	0.25%	0.50%	1.0%
MON810 (A_{620})	0.06	0.058	0.064	0.069	0.086
Bt11 (A_{620})	0.064	0.073	0.104	0.153	0.229

Immunological methods commercially available for heterologous protein detection

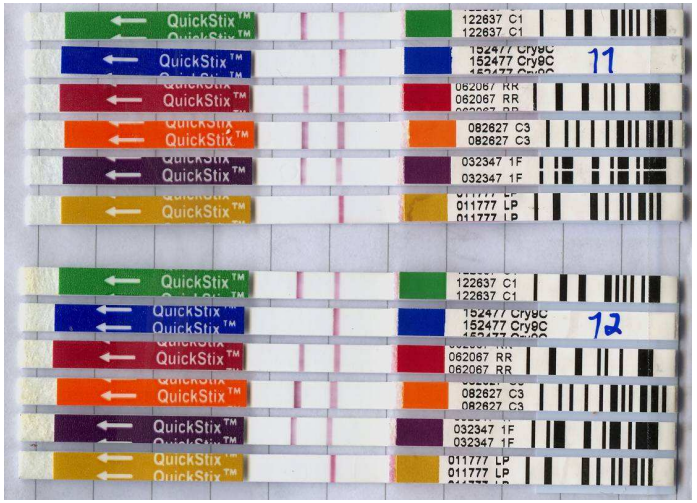
ELISA

- CP4-EPSPS (RR)
- Cry3Bb1
- Cry1Ab/1Ac
- Cry1F



Immunostrips

- CP4-EPSPS (RR)
- Cry3Bb1
- Cry1Ab/1Ac
- Cry1F
- Cry34Ab1
- Cry9C
- PAT



New GMOs with more genes and traits: A challenge for detection

Monsanto's NewLeaf Plus® potato variety, which contains a total of eleven different foreign genetic elements- Resistant to Colorado Potato Beetle And Leaf Roll Virus

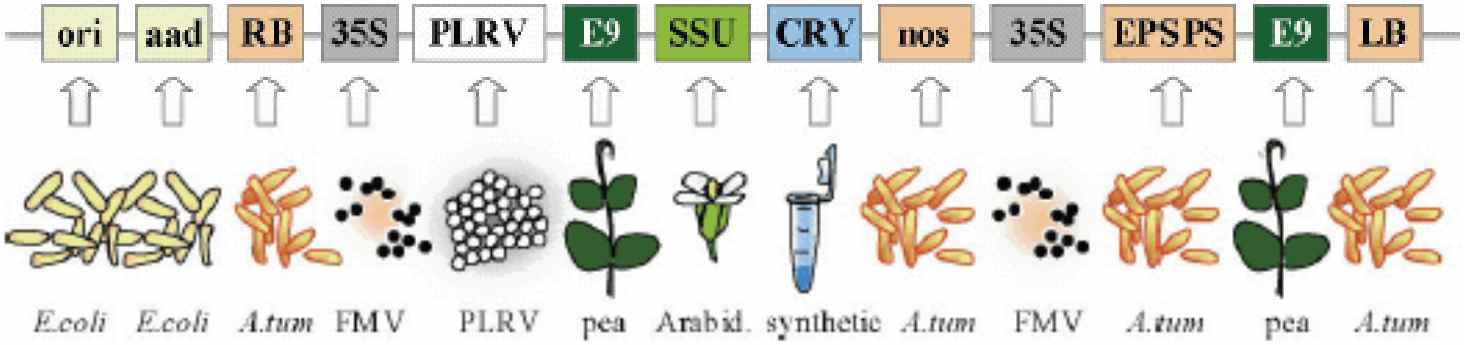


Figure 1. Schematic structure of an insertion site of NewLeaf Plus®. ori = origin of replication of *E. coli* plasmid pBR322, aad = spectinomycin resistance gene from *E. coli*; RB = right border region of *Agrobacterium tumefaciens* (*A. tum*); 35S = promoter of figwort mosaic virus; PLRV = replicase gene of potato leaf roll virus; E9 = terminator of pea E9 gene; SSU = promoter of Arabidopsis rubisco small subunit gene; CRY = synthetic gene encoding a protein identical to the cry3Aa protein of *Bacillus thuringiensis*; nos = terminator of *Agrobacterium* nopaline synthase gene; EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium*; LB = left border region of *Agrobacterium*.

http://www.isb.vt.edu/articles/dec0405.htm

Conclusions

To detect an un-approved GMO/LMO is a major task and often very difficult

RNAi based GMOs/LMOs have no protein to detect

While DNA based tests can be designed easily, ELISA based test is possible only if the specific antibodies are available

There is a need to constantly up grade the detection methods for new and staked events