

Gli Organismi Geneticamente Modificati in agricoltura: realità, prospettive e regole nel mercato globale

**Convegno organizzato da:
Ordine dei Dottori Agronomi e Dottori Forestali di Imperia**

OGM: lo stato dell'arte della ricerca e le possibili utilizzazioni



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CRA-FSO Sanremo (IM)**



Lavori pionieristici di trasformazione

Trasformazione mediata da *Agrobacterium*

Identificazione del plasmide Ti di *Agrobacterium* (Engler et al., 1975)

Dimostrazione della capacità di *Agrobacterium* di trasformare le cellule vegetali (Schell e Van Montagu, 1977)

Ottenute le prime piante di tabacco transgeniche da due diversi gruppi (Mary-Dell Chilton e Jeff Schell) utilizzando il plasmide Ti come vettore (Barton et al 1983; Herrera-Estrella et al., 1983)

Trasformazione diretta

Elettroporazione (Fromm et al., 1985)

Microproiettili (Klein et al., 1987)



Transformation Methods

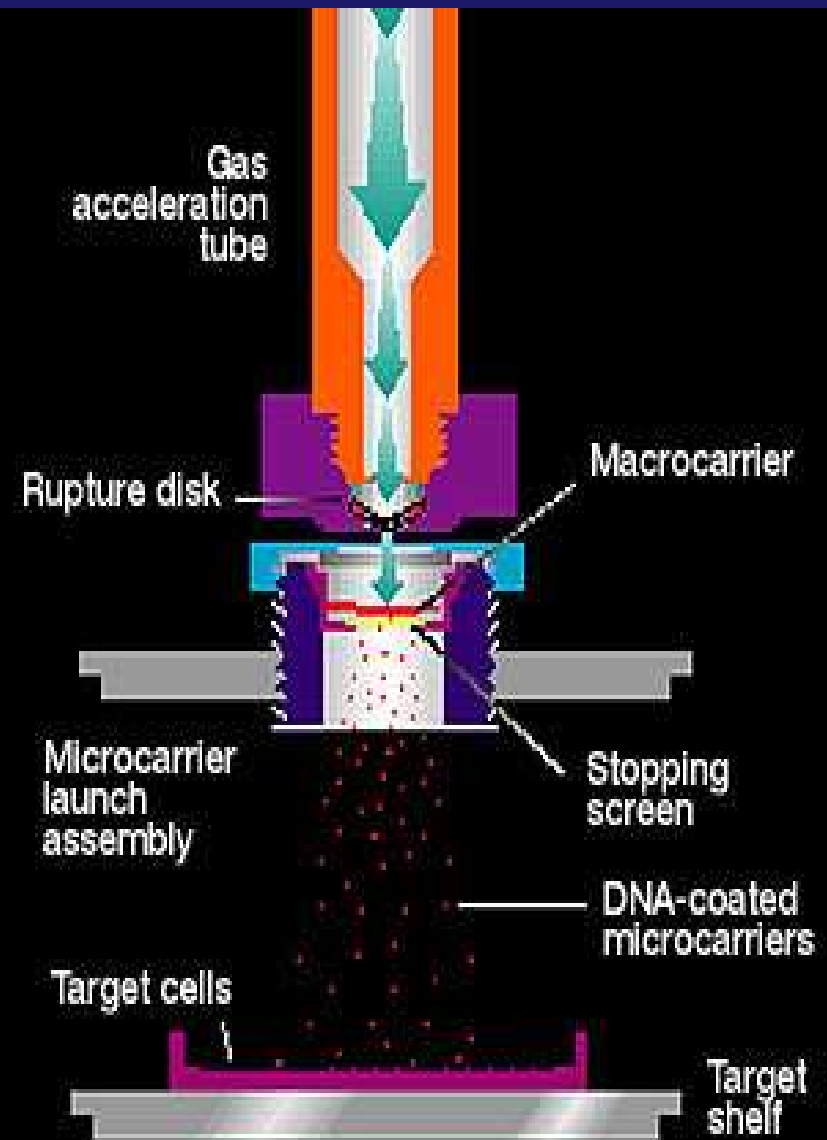
Method	Equipment/ Supply	Potential License Required
Agrobacterium	Strains	Japan Tobacco (grasses) Monsanto (canola, cotton, soybean) 4-way interference: Novartis, Monsanto, Max-Planck, U. of Leiden
Biolistics (method) for corn for wheat	Helium gun	DuPont DeKalb/Pioneer Novartis
Silicon carbide fibers	Fibers	Zeneca
Microinjection	Microscope & injector	Calgene
Pollen transformation by electroporation for corn	Electroporator	BTG DeKalb
Electroporation	Electroporator	Various depending on species (Boyce Thompson)
	MARS sequences	Mycogen
Electrophoresis	Apparatus	?



http://farm2.staticflickr.com/1300/4720070097_4082e801b6_z.jpg

Biolistic / Gene Gun

PSD-1000/He Particle Delivery System



<http://arabidopsis.info/students/anna/biolistics.gif>

Specie di *Agrobacterium* rilevanti per la trasformazione delle piante

- *A. tumefaciens*
- *A. rhizogenes*

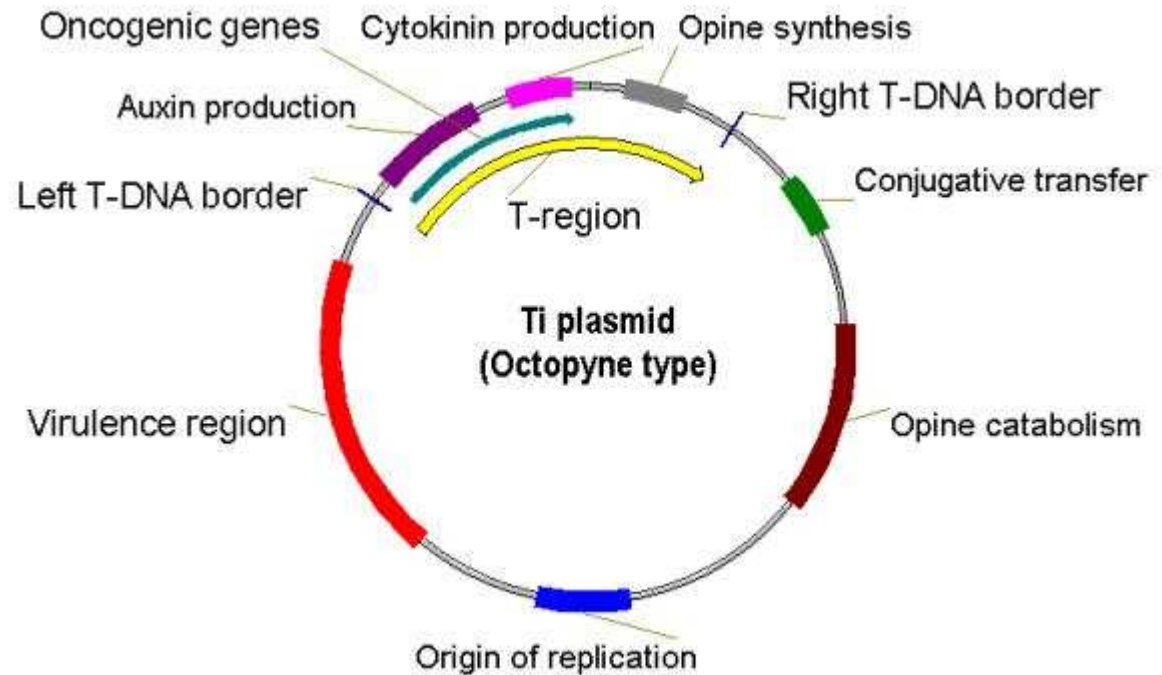
- *A. rubi*
- *A. vitis*

Plasmide generico di *A. tumefaciens* (tipo octopina)



Peter Sforza, Geanie McMeans,
Fabricio Medina-Bolivar, Dan
Tillman,
George Lacy, Mary Ann
Hansen, and John Jelesko

<http://www.patentlens.net/daisy/AgroTran/1053/version/default/part/ImageData/data>

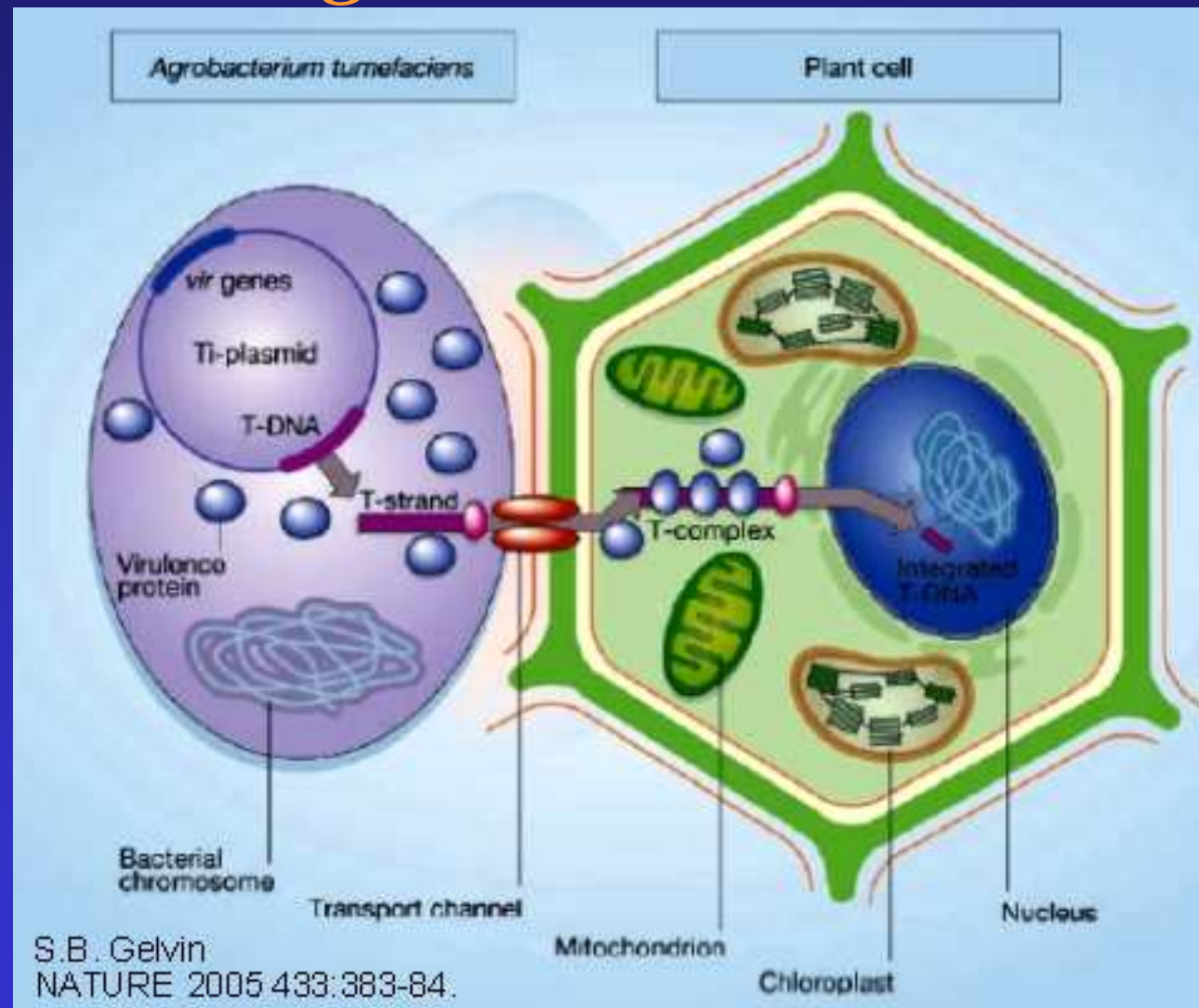


Sequenze dei bordi del T-DNA

Bordo	Sequenza
LB-TL (oct)	ggcGGCAGGATATATtcaatTGTAAC
RB TL (oct)	acTGGCAGGATATATaccgtTGTAATt
LB-TR (oct)	ggTGGCAGGATATATcgaggTGTAANA
RB TR (oct)	gaTGGCAGGATATATcgaggTGTAATt
TL (nop)	ggTGGCAGGATATATtgtggTGTAAC
TR (nop)	ttTGGCAGGATATATtggcggGTAAAC
LB-TL (Ri)	ggTGGCAGGATATATtgtgaTGTAAC
RB-TL (Ri)	acTGaCAGGATATATgttccTGTcATg
LB pGreen	ctTGGCAGGATATATtgtggTGTAAG
RB pGreen	ctTGaCAGGATATATtggcggGTAAAC
Consensus	xxTGGCAGGATATATxxxxxTGTAAN/T
P-DNA	Rommens et al., 2005, 139:1338-1349

Rommens et al(2005)*Plant Physiology* 139:1338-1349

Trasformazione mediata da *Agrobacterium*



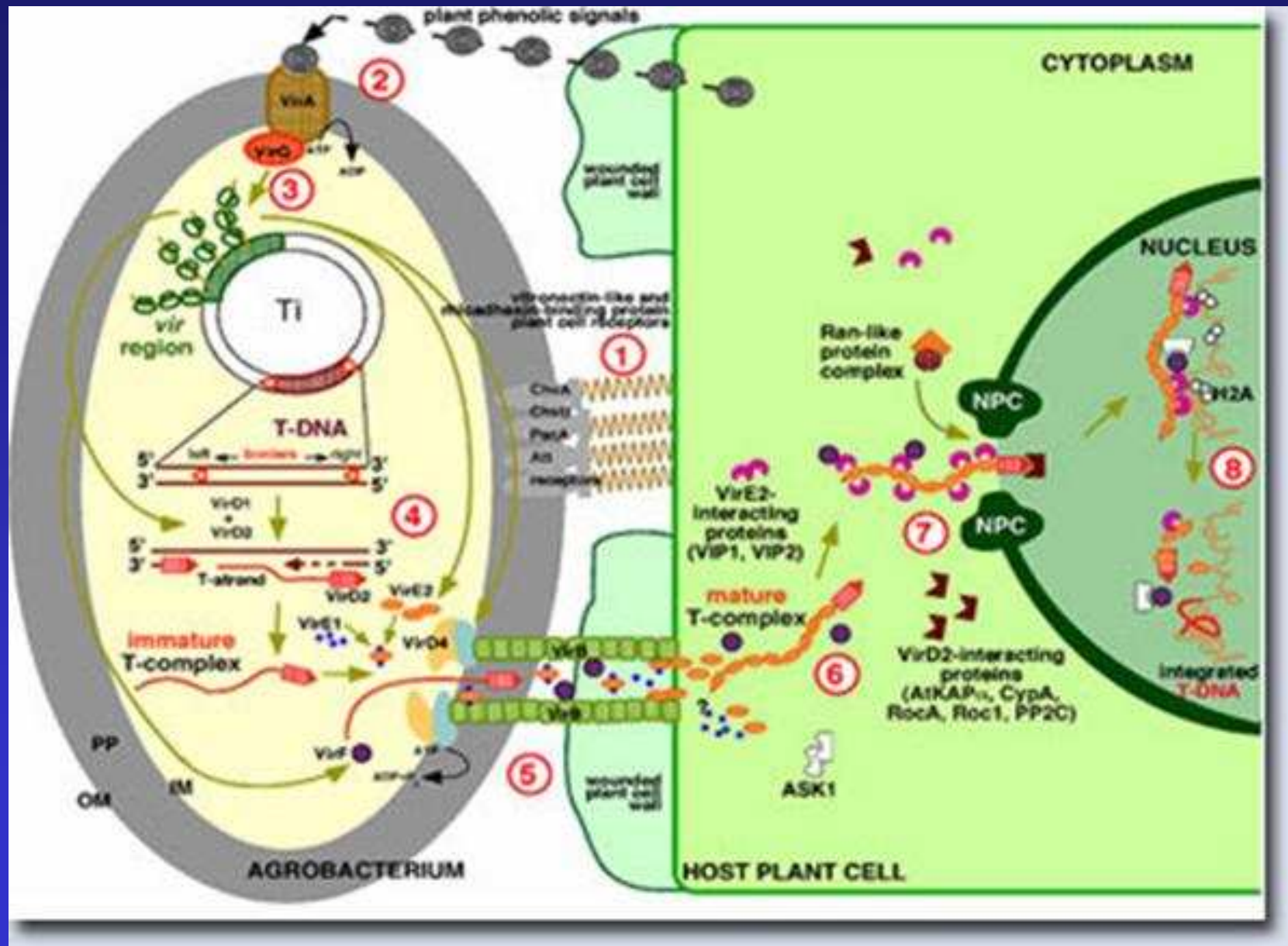
Courtesy of
[[http://biologi.uio.no/plfys/haa/gen/gmo.h](http://biologi.uio.no/plfys/haa/gen/gmo.htm)
[tm](http://biologi.uio.no/plfys/haa/gen/gmo.htm) Halvor Aarnes A gall caused by
Agrobacterium tumefaciens.

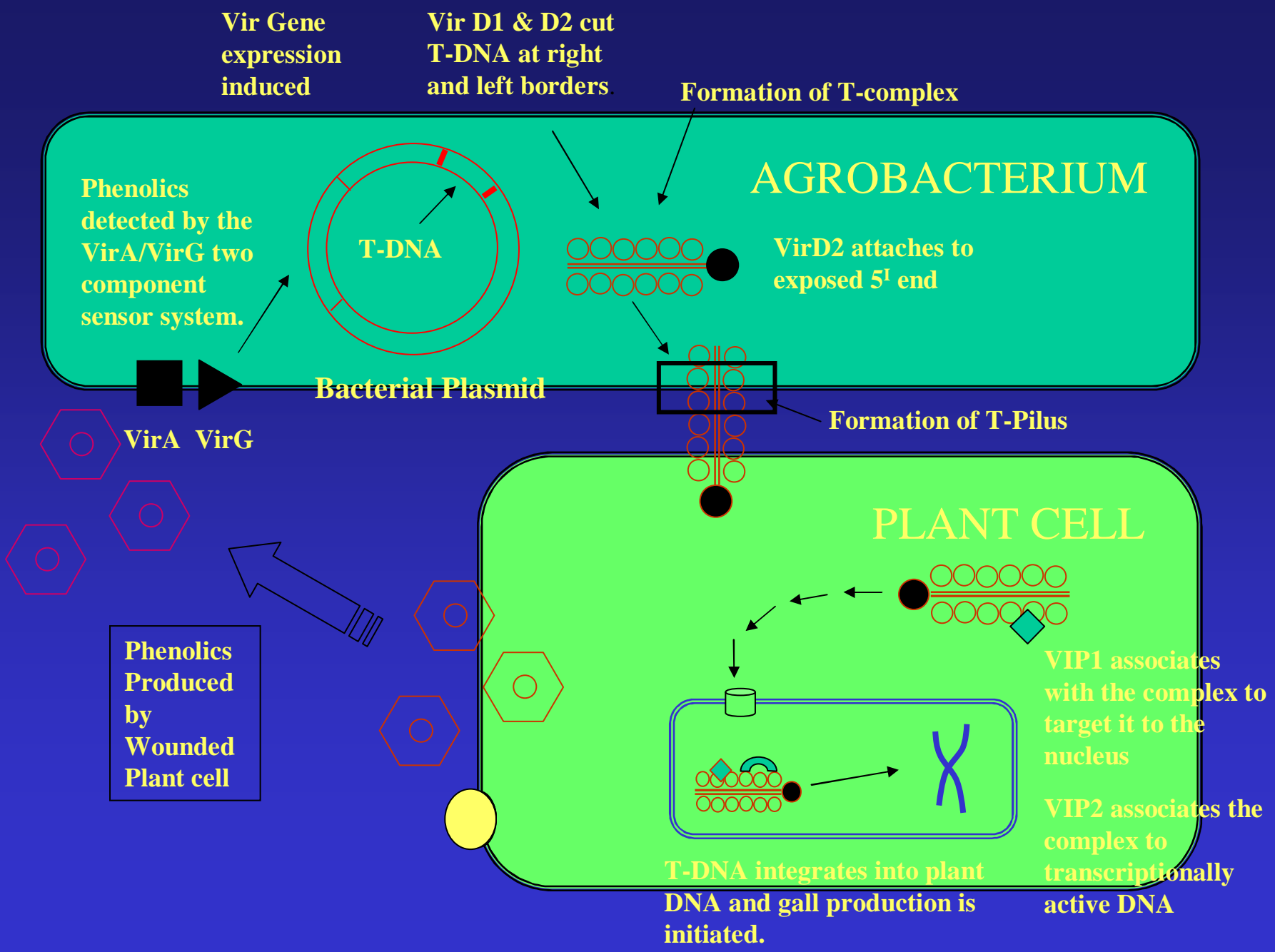


Agrobacterium rhizogenes

Catharanthus hairy roots
Kirsi-Marja Oksman-Caldentey, VTT

Modalità di formazione e di trasferimento del T-strand





Modalità di integrazione del T-DNA

Proposti numerosi modelli (elica singola o doppia?)

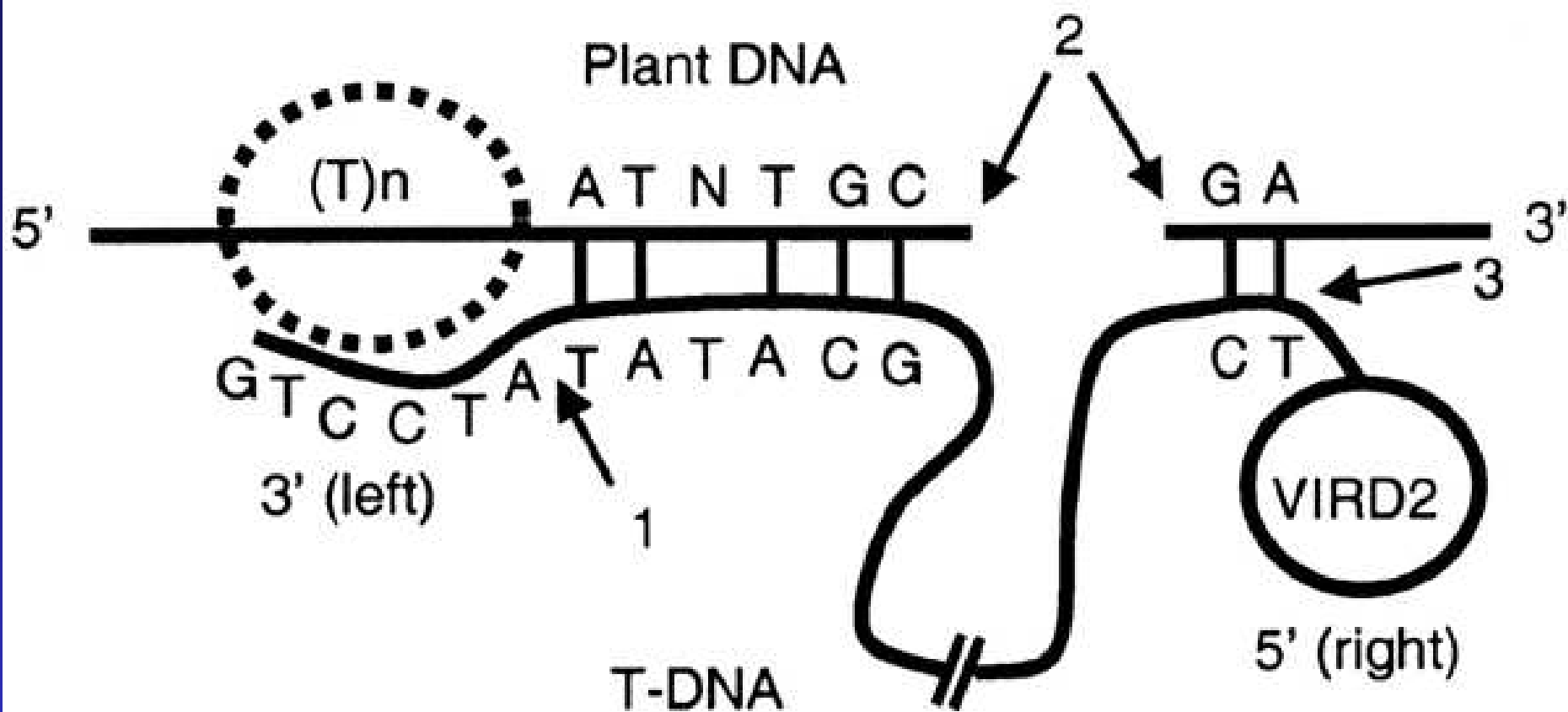
**L'integrazione è favorita da zone ricche di AT
(microomologie) con cui si appaia il LB**

**Vir D2 legata al 5' del T-DNA stimola l'attività della DNA
ligasi 1 e si lega anche ad altre proteine (Es.: DRT112)**

**L'integrazione comporterebbe una delezione del DNA
genomico**

**Nel caso classico di integrazione, al 5' del T-DNA sono
tagliate 3 basi del border**

Al 3' il numero di basi tagliate del border può variare

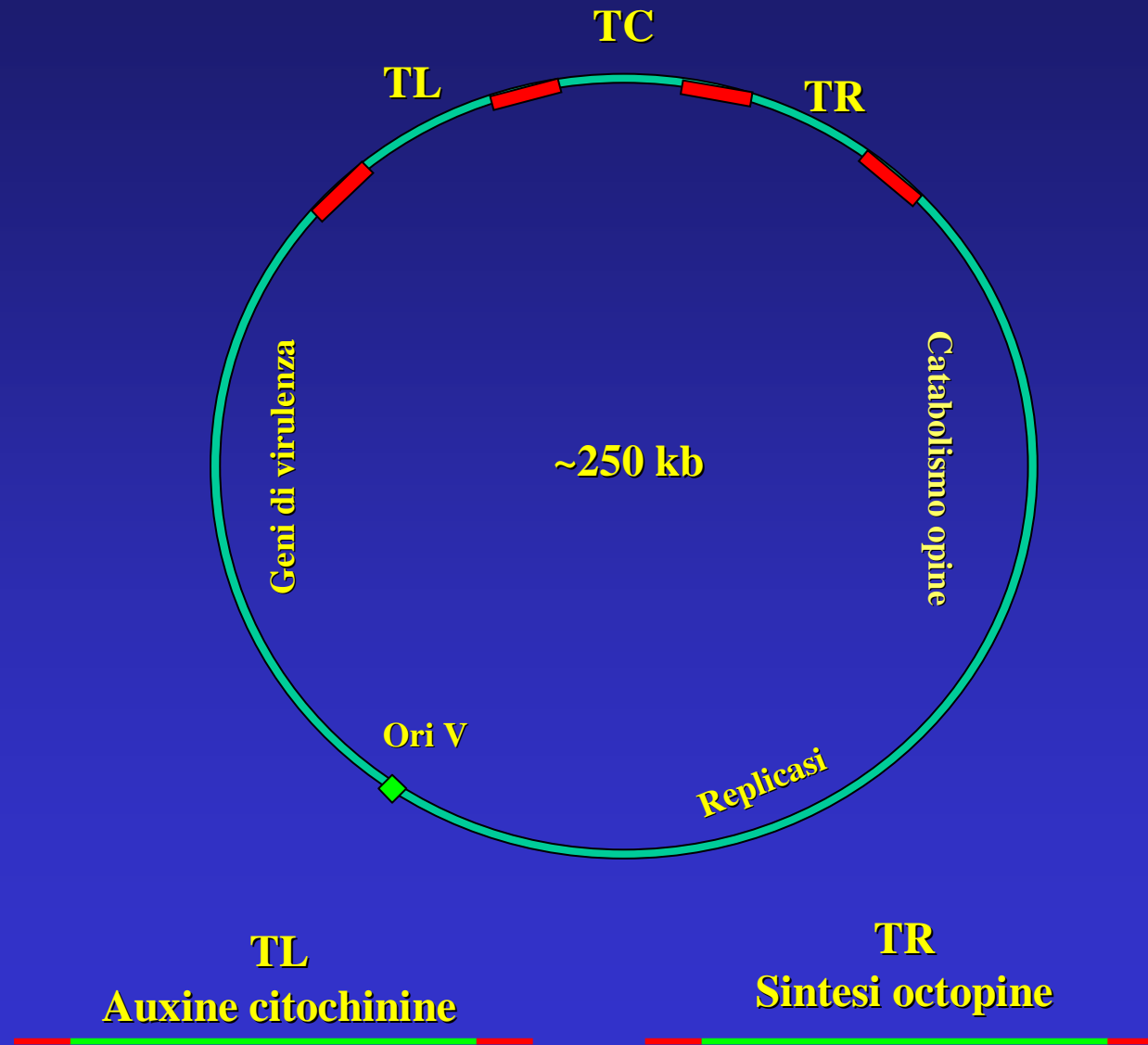


A model of the T-DNA integration process. The given example is for a putative T-DNA that, as a consequence of its integration is deleted by six nucleotides in the 3' LB [position (b) in Figure 2A] but complete in its RB. For convenience, only the upper strand of the host DNA is represented. A T-rich region [T(n)] operates as a preferential site of entry of the T-DNA LB. Starting from its 3' end, the T-DNA scans the plant DNA until it finds a microcomplementarity just downstream of the T-rich region. A nick (2) is generated in the host DNA downstream of the microcomplementarity-based duplex and used as a priming site to synthesize the complementary strand of the T-DNA until the 5' RB covalently linked to VirD2 is reached. The integration process of the newly synthesized double-stranded DNA frequently brings about a deletion in the host DNA. Recombination between the host bottom strand and the T-DNA operates at sites 1 and 3 after the action of exonucleases on both ends of the T-DNA.

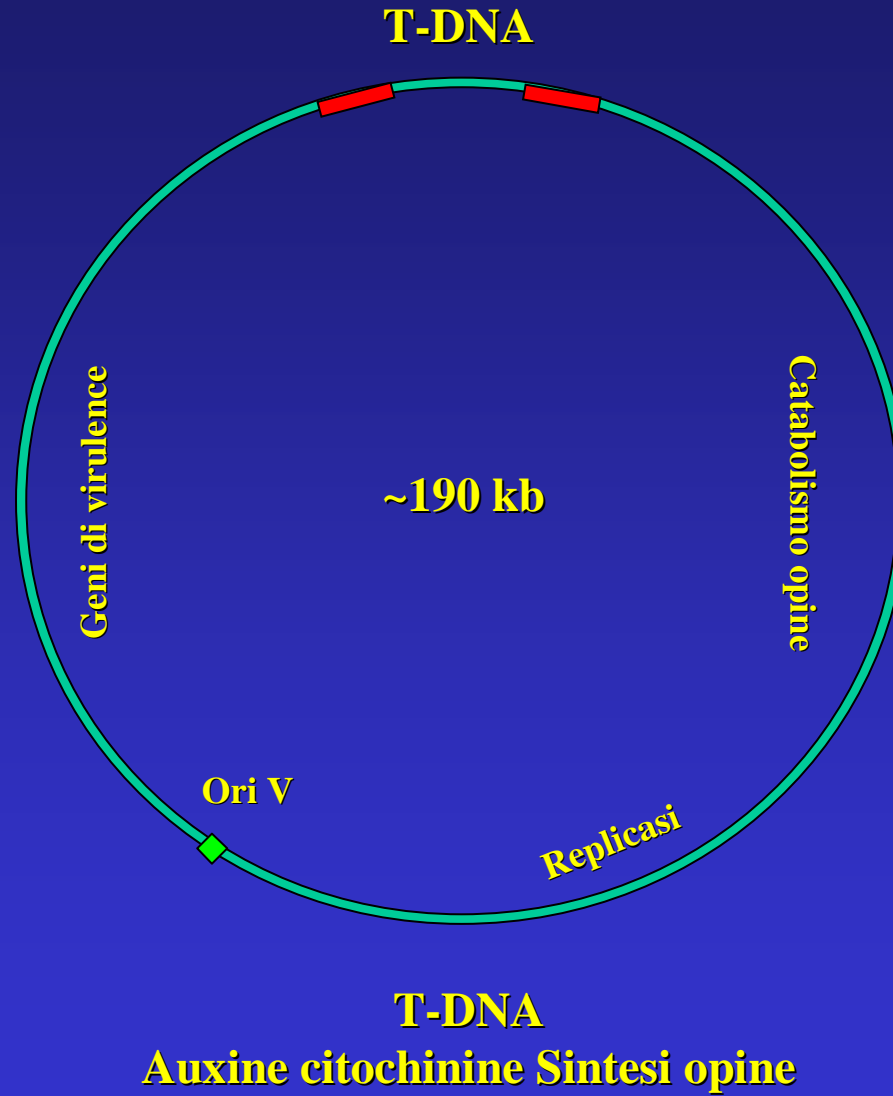
Brunaud et al., EMBO reports, 3:1152-1157

**Modifiche apportate ai plasmidi
utilizzati per la trasformazione
genetica delle piante**

Plasmide generico di *A. tumefaciens* (tipo octopina)



Plasmide generico di *A. tumefaciens* (tipo nopalina)



Ceppi di *A. tumefaciens*

Ach5 agrocinopine, octopine type

B6S3, A6 ... octopine type

Bo542 leucinopine, succinamopine,
agropine type, vir weaker than A281

C58, T37 ... nopaline types

A281 succinamopine, leucinopine,
agrocinopine

Genotypes of some useful *A. tumefaciens* strains

LBA4404 (Ach5 pTiAch5) Sm/Sp(R) in the virulence plasmid (from Tn904); all T-DNA of pTiAch5 eliminated in pAL4404 (Hoekema et al., 1983).

EHA101, genotype C58 pTiBo542; T-region::aph, Km(R); A281 derivative harboring pEHA101, T-DNA replaced with nptII, elimination of T-DNA boundaries unconfirmed, super-virulent (Hood et al., 1986).

EHA105 is a Km(S) derivative of EHA101 (Hood et al., 1993).

AGL1, genotype is AGL0 (C58 pTiBo542) recA::bla, T-region deleted Mop(+) Cb(R) [AGL0 is an EHA101 with the T-region deleted, which also deletes the aph gene] (Lazo et al., 1991).

A281, reconstructed strain, derivative of A136 (cured C58) harboring pTiBo542, super-virulent (Hood et al., 1986).

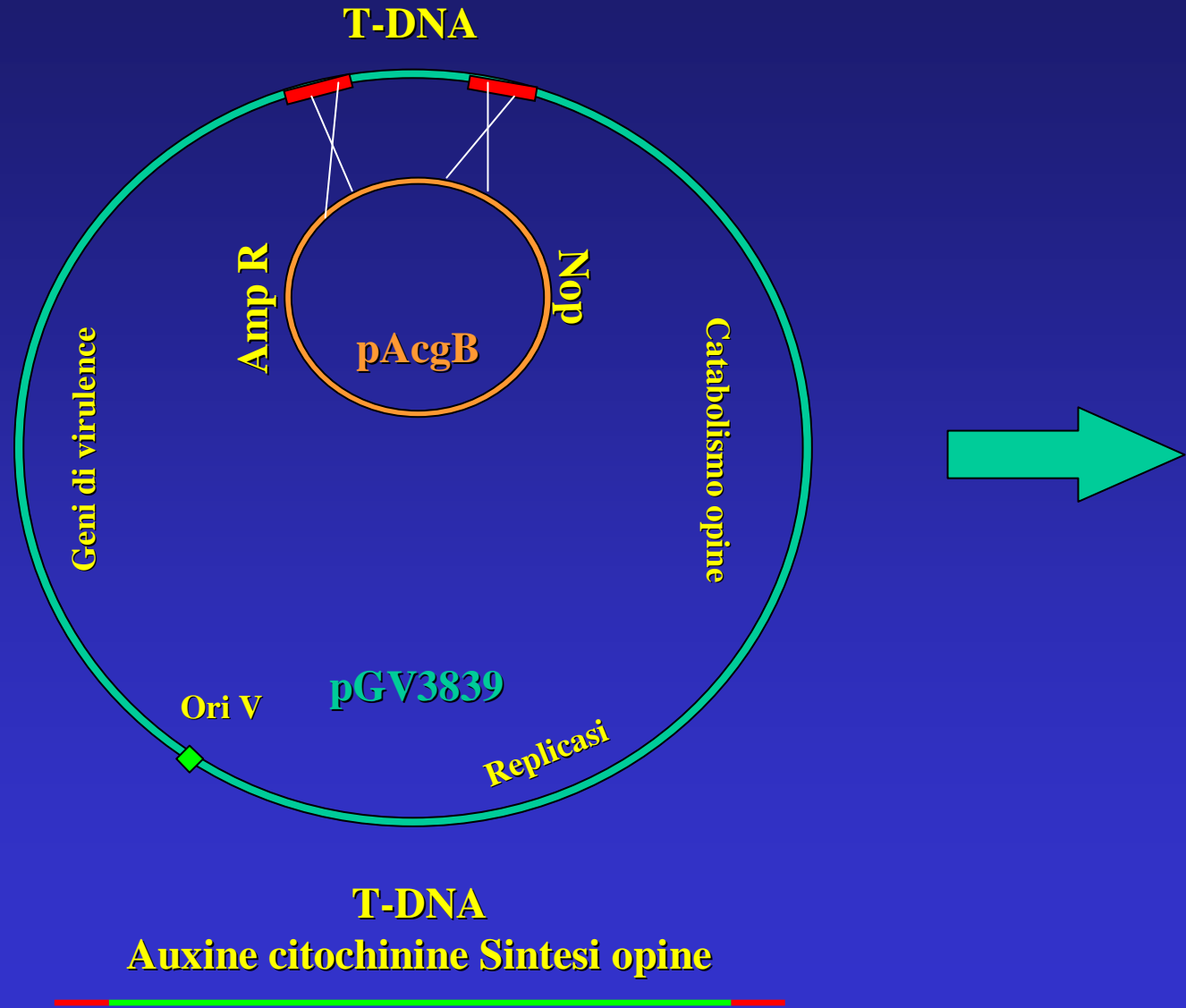
Evoluzione dei vettori di trasformazione di *A. tumefaciens*

Vettori cointegrati

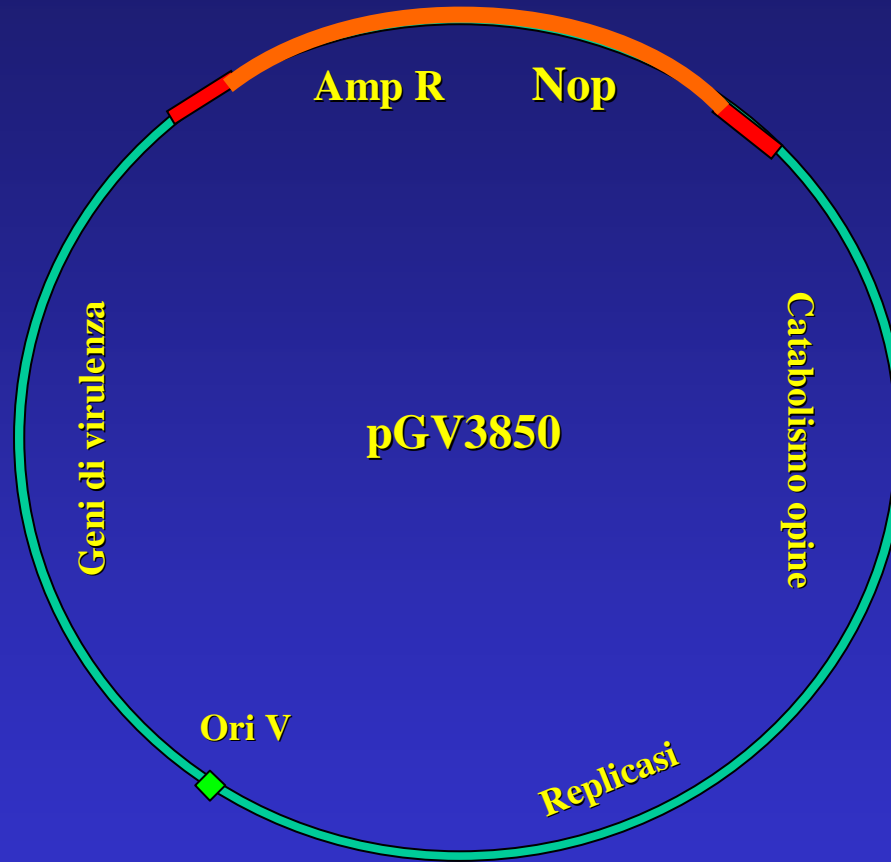
Vettori binari

Altri

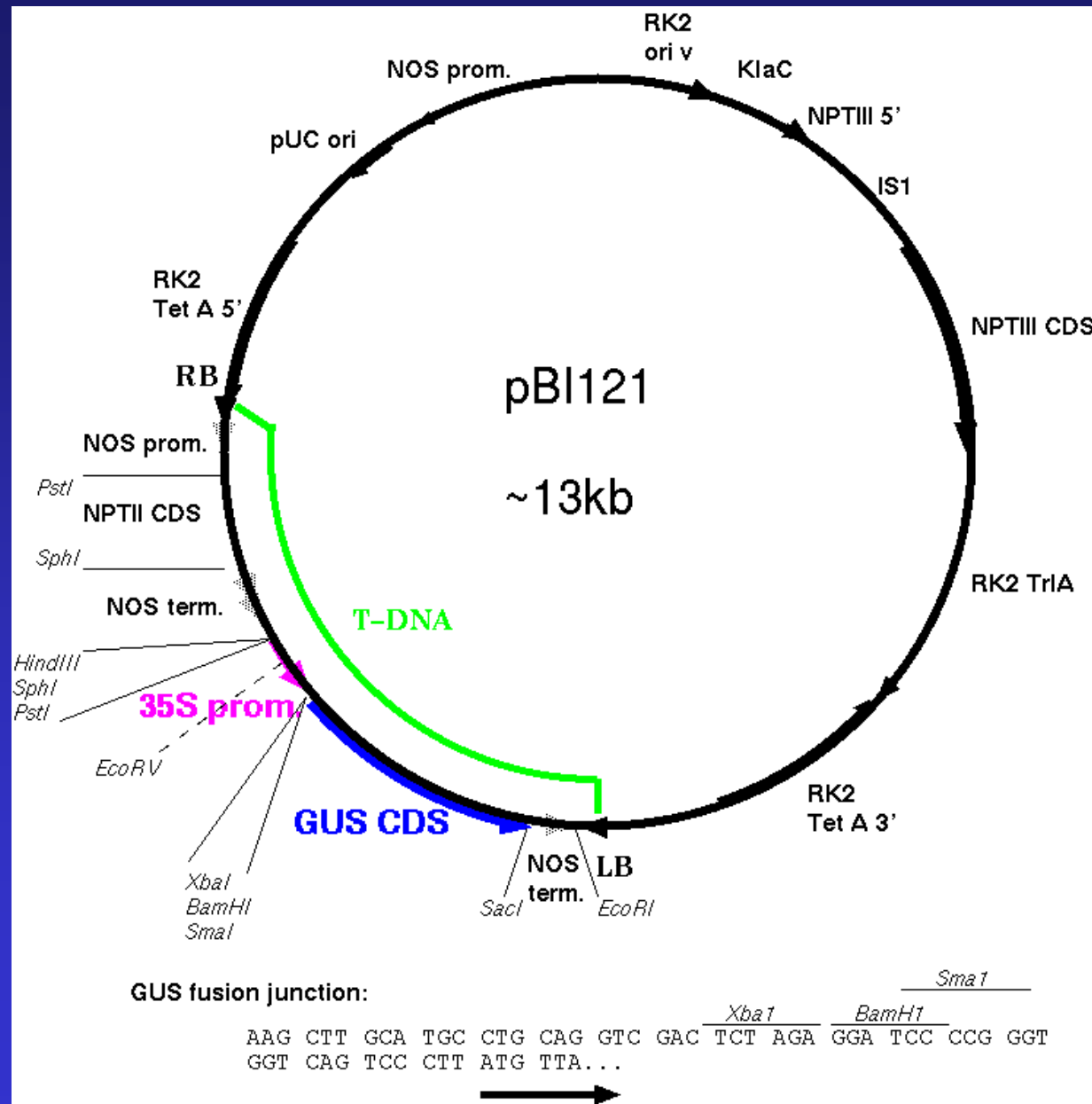
COSTRUZIONE DI pGV3850 MEDIANTE RICOMBINAZIONE OMOLOGA



Plasmide cointegrato



Vettore binario



Altri vettori

Plant Molecular Biology **42**: 819–832, 2000.

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819

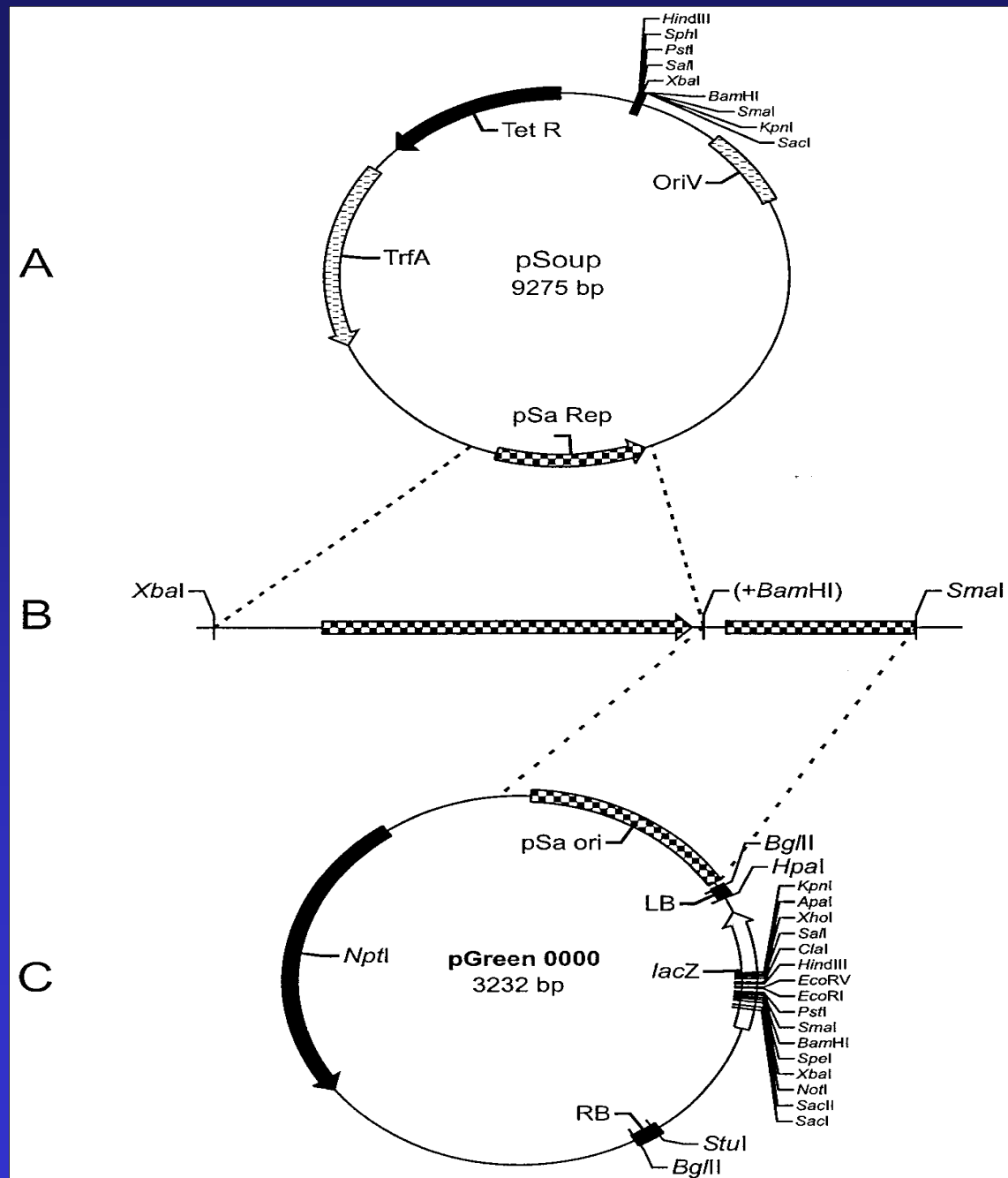
pGreen: a versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation

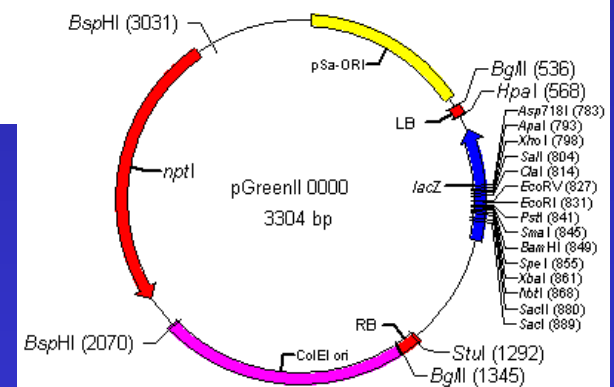
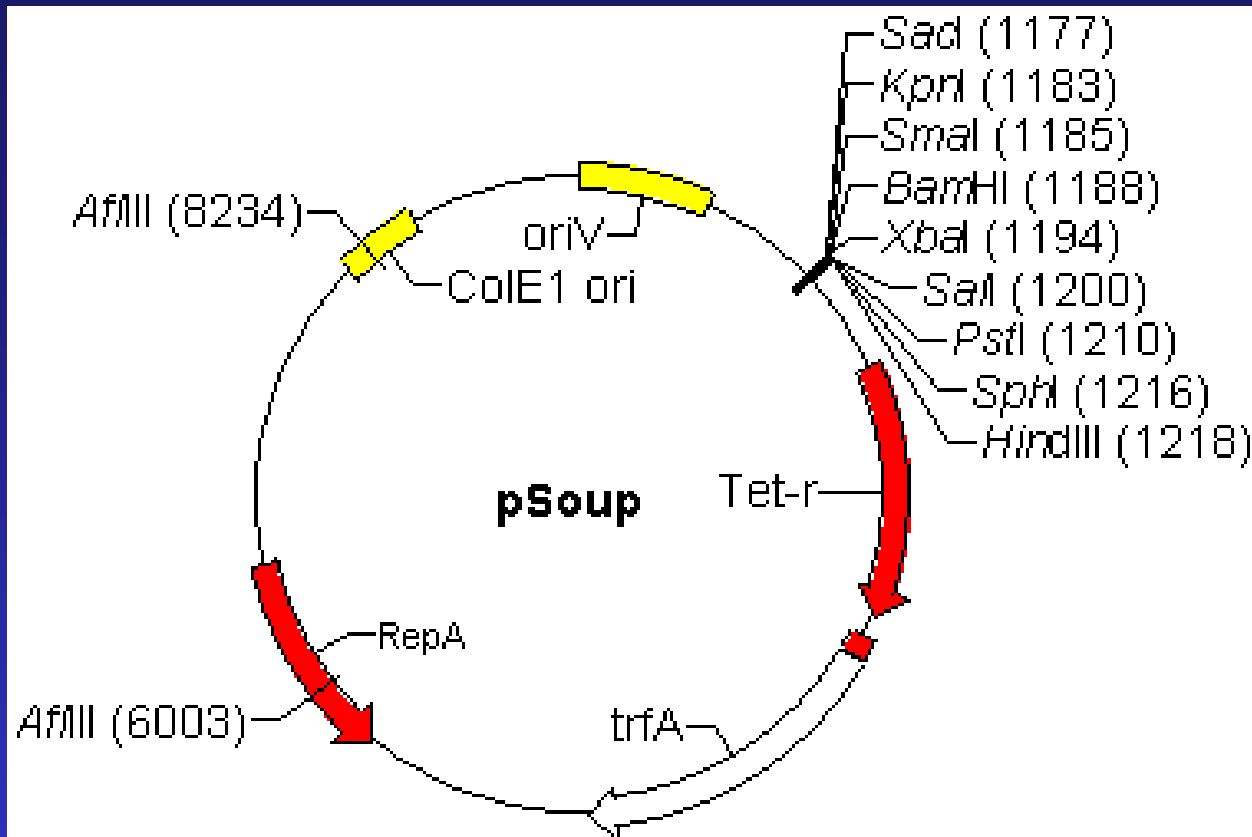
Roger P. Hellens, E. Anne Edwards, Nicola R. Leyland, Samantha Bean and Philip M. Mullineaux

John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK (author for correspondence; e-mail:

hellens@bbsrc.ac.uk; fax: C44 1603 456844)

Sistema PGreen/pSoup





pGreen con T-DNA minimo (2534 bp)

CGTTAACAAAGCTTGGGACCGCGGTGGAATTCCAGCTATGAAGG

AATTGTTTCGAACCCTGGCGCCACCTTAAGGTCGATACTTCC

Hpa I

Hind III

Eco RI

LB

RB

TTAGAGATCTTGGCAGGATATATTGTGGTGTAAA

AATCTCTAGAACCGTCCTATATAACACCACATTTGC

Bgl II

Acl I

CCTTGACAGGATATATTGGCGGGTAAACTAA

GGAACTGTCCTATATAACCGCCCATTTGATT

Stu I

Strumenti per l'analisi del numero di copie del transgene e dei siti di integrazione

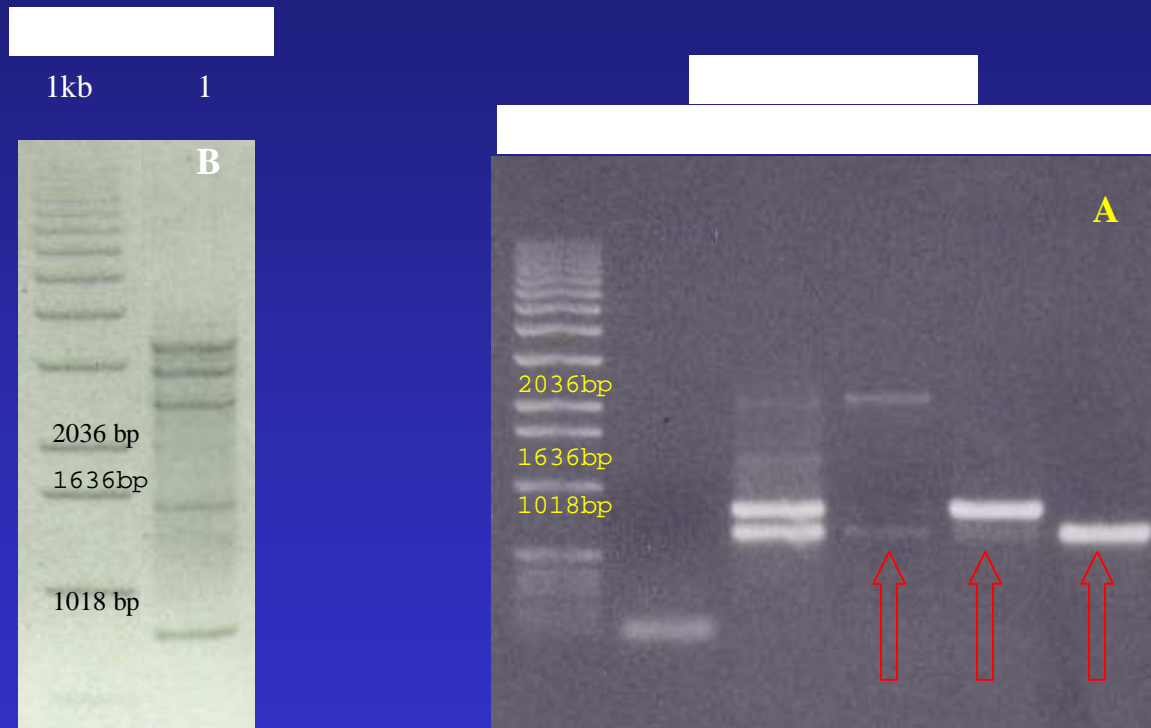
- Segregazione
- Southern blot
- Quantitative PCR
- Sequenziamento delle giunzioni
- Ibridazione in situ (FISH)

Sequenziamento delle giunzioni mediante AL-PCR

Passi fondamentali:

- Digestione
- Ligazione
- Amplificazioni nested
- Sequenziamento

Prodotti di amplificazione mediante AL-PCR



**Le bande ottenute sono sequenziate
direttamente o previo clonaggio in vettore**

Prodotti di amplificazione ottenuti mediante AL-PCR

CLONI TRANS GENICI	LIBRERIE			
	ScaI		RsaI	
	LB	RB	LB	RB
Clone 426	2800bp 2000bp 180bp	300bp	2000bp 900bp 700bp	1600bp
Clone 428	400bp	Non verificato	6500bp	3100bp 2500bp
Clone 441	Non verificato	Non verificato	1800bp 700bp	3200bp 2900bp 2300bp 1600bp

Caso tipico d'integrazione

```
1 NTTNAGTTGCCNCNTTTTGGCTGTCAAGATGCGAAAGCACGTGAGAGACAAGTAAATC
61 ACGAAAGCATTTCGGGAGATGTGGAGACTTTATTACCATAACAATCATAGCGAAGATAG
121 GCATCAAGGAATCGTTTGGAAATCCACCGCGGTCCCAAGCTTTGTTAACGTTACACCACA
181 ATATATCCAGAGCTATCCTTTGGAGCACGTTGAACTCCTAAGATGTTATCTAGACATAAG
241 GTAGAATTTTGGAAATTTCTCCAACCTCCTATTTAAGTCTACAGTTTCACTTTAACGTCT
301 GCTAGCTCCTTATTTTATCAGCTAGTTGTTGAGTTAAGTCTTGAATGTGTTTCATACAGC
361 ATGTCAATATTATTTGCTCATTTCAGATCTTTTAGTGCAGTTCTATCCTTAGCATCTG
421 ACTTAAGTTTATCAGTTAAATCATTCCTTTATATCTTTTAAGCTGACATTCTCAAGTTTTA
481 AGTTATCATTTTCAACACGCATAGGTTCACTATTCTCAACTAGCATCTTAATCCTATTAG
541 ACATATCAATATGAGACCTGCAAATATTAGTAACAACAGATAAAATCTAAACGTACCTGCC
601 CAGGCCGTCNACCACGCNNGGGCCCTATAGTANNGNNGNNGNNGNNGNNGNNGNNGNNG
```

RoI ABC

Adattatore

Left Border

Genoma

CL 441 RsaI 700bp LB

Delezione del tratto terminale del T-DNA

```
1  NNNNNNNNNNNNNNTGNCTGTCAGANGCGAAAAGCACGTGAGAGACAAGTAAATCACGAAAG
61  CATTTTTTCGGGAGATGTGGAACATGTTTCAGGAGACGGTTCAGGAGCTGATTCAGAAGGCG
121 GTCCGGGAGTCGGTTCAGGAGCCAGTTCGGGAGTTGTGGTTGGAACAGGTTGTTCTTCAA
181 TAGCAAATGGATCAAGATGTATGAGATATGACTTTGTTCATATGATGAGCACTAGCAGGAA
241 AATGGAAAAGAATGGAATGTGTTCCAAAAACATAACATGCCGTGATACATATAGTTTTTG
301 ATGGTTTTCTCTGGCGATTACCTCAGGACTTCAAGGGGACGTTGTAGGTTGCTTTTTGGT
361 GGTGTTTAGAACGTAATAATATGTTTCAAATTTGTGTTTTTCGATTGGTTAGGGTTTTGCG
421 ATGGTGTGGTGGTCGTGGGTGGATTCCGGTGGTGGCAGGAGGTGGTTGTGATGGTGTGTA
481 TCGTCTGGAGAAGGTGATGGTGTATGGTGTATGTCGGCTGTAGTGCAGAGGAAGGAGAGGGA
541 GAGGTGTAAACTGAGGCTAGGGTTTCAAGATTAAGTTTATATATGTCTAGGGTTTTCTCA
601 AAGGATGGTAATCGGACCAGGCTTGGCTAAGTCCAGCTAGTGGTAGGTCAAGAGTGGTCC
661 TGGACTGACCTTGGGTTCGTATGGCTCGATGGTGTATGGCCAGGCAATCCTCGTGTGTTGG
721 TTTTATGTACCTNNNCCGNNNNNNNNNNNACCCGTGCCCTATANTNNNNN
```

Cl 426 RsaI 900bp LB

```
1
TTTTAGTTGCCCNTTTTGGCTGTCAAGATGCGAAAAGCACGTGAGAGACAACCTGCCCG
61  GGCCGTCGACCACGCGTGCCCTATAGTANNNNNNNNNNN
```

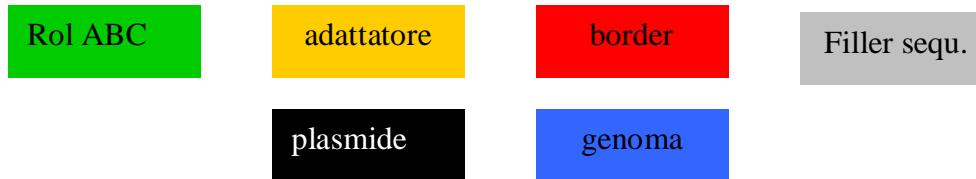
Rol ABC

Genoma

Cl 426 ScaI 200bp LB

Mancato riconoscimento del border

```
1 CAGTTGCCNNNTTTTGGCTGTCAAGATGCGAAAGCACGTGAGAGACAAGTAAATCACGA
61 AAGCATTTTTTCGGGAGATGTGGAGACTTTATTACCATAACAATCATAGCGAAGATAGGCAT
121 CAAGGAATCGTTTGGAAATCCACCCGGTCCCAAGCTTTGTTAACGTTACACCACAATAT
181 ATCCTGCCAAGATCTAATTCCGGGGATCGGAAATCCAGAAGCCCAGAGAGGTTGCCGCCTT
241 TCGGGCTTTTTCTTTTTCAAAAAAAAAAATTTNAAAANNANNTGGTGGGGCCGGCCCCCG
301 GGTTGGGGGCAAAGGNCCNAAGGGGGNAACNNTTGGGGTTGGCCNCGGGGGAACC
361 GGGGNNNTAACNNATTGGCCNNAANCCNAGGGGANCAAAAANNNGNAAAAAAATTCNA
421 AANTGNAAANNNAANTGNAAAAANCANTTGGGGGAANNANTTGNCGGAANGAGGGGGGT
481 TGNAAACTGNTGAN CN GAAAGGNNNNTAANGNTCCCCGGGNCNTTTTGNNTAANGANT
541 TCCCGGNCC
```



Cl 426 RsaI 700bp LB

Riarrangiamento del T-DNA

```
1 CGCGCAATANAATGAAAATTGCCAAAATTAGCTATCGGTCTTCTGAAAAGAAGGCCGAC
61 ATGTTTTCATAGACCATGCAAAGTCATACTACCTGAAACTGATAAATAACGACAAAGAAA
121 TTTCCACGCCGGAGATGATATCGAATATGTTCTGTTAAGTTAAAATAAGCTGCGAGCCAT
181 GCGCGATTGTCCTGTTTTATTAATATAGTACCTGCCCGGGCCGTCGACCACGCGGTGGC
241 NCTATANNANNA
```

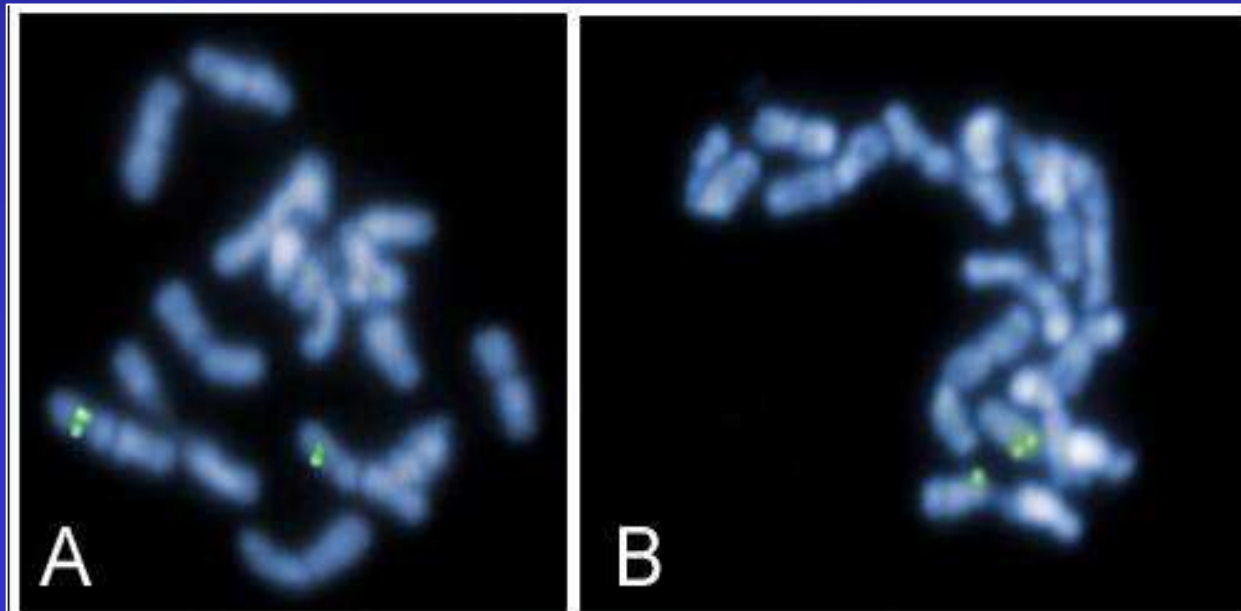
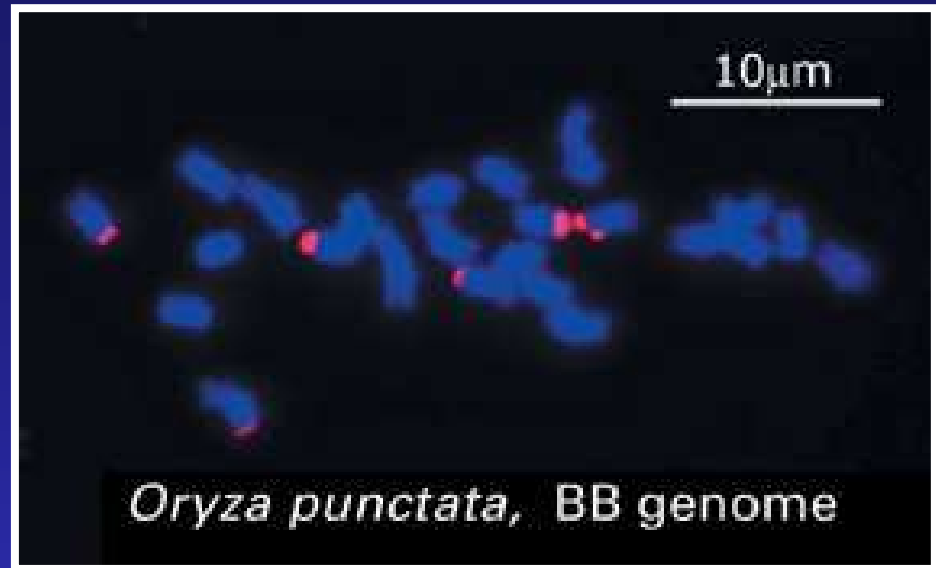
Rol ABC

Rol ABC

Genoma

Cl 426 RsaI 700bp RB

Ibridazione in situ (FISH)



Eliminazione dei marcatori

Metodi disponibili per ottenere piante marker-free

- Cotrasformazione e segregazione
- Ricombinazione sito specifica:
 - sistema *cre/loxP*
 - sistema *attP*

Costituzione di genotipi T1 attraverso co-trasformazione con 2 ceppi di *Agrobacterium tumefaciens* di cui uno porta il gene marcatore selezionabile (gene *NPTII*) e l'altro il gene utile (gene *35SGUS*)



Identificazione di genotipi primari T1 che esprimono entrambi i geni



Autofecondazione



Analisi delle progenie T2

Analisi espressione dei 2 geni

Espressione gene *NPTII*



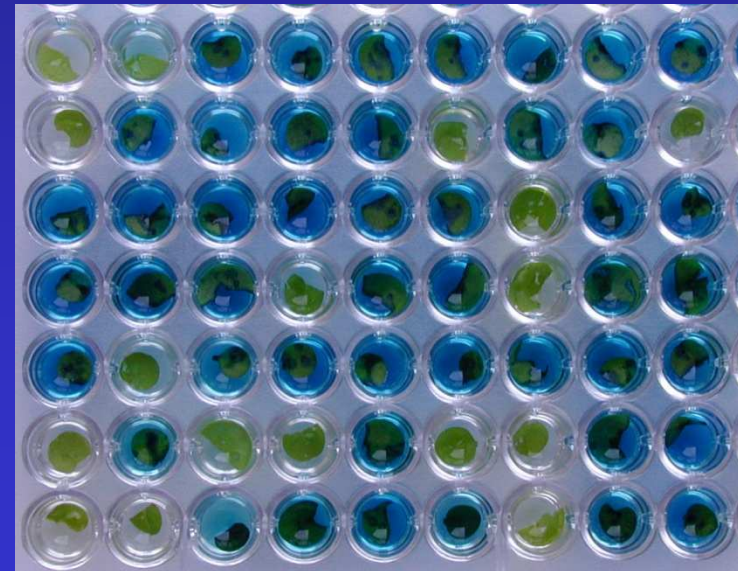
Rigenerazione su terreno selettivo



Espressione gene *35SGUS*



Saggio istochimico



Risultati espressione delle progenie

Genotipi T1	Progenie T2			
	Espressione (N° di piante per classe)			
	GUS ⁺ Km ^R	GUS ⁺ Km ^S	GUS ⁻ Km ^R	GUS ⁻ Km ^S
C2	129	0	42	0
D4	113	0	5	29
8	207	0	4	63
N2	316	0	104	1
B4	59	23	25	8
N1	138	5	6	1
N1	120	5	24	1

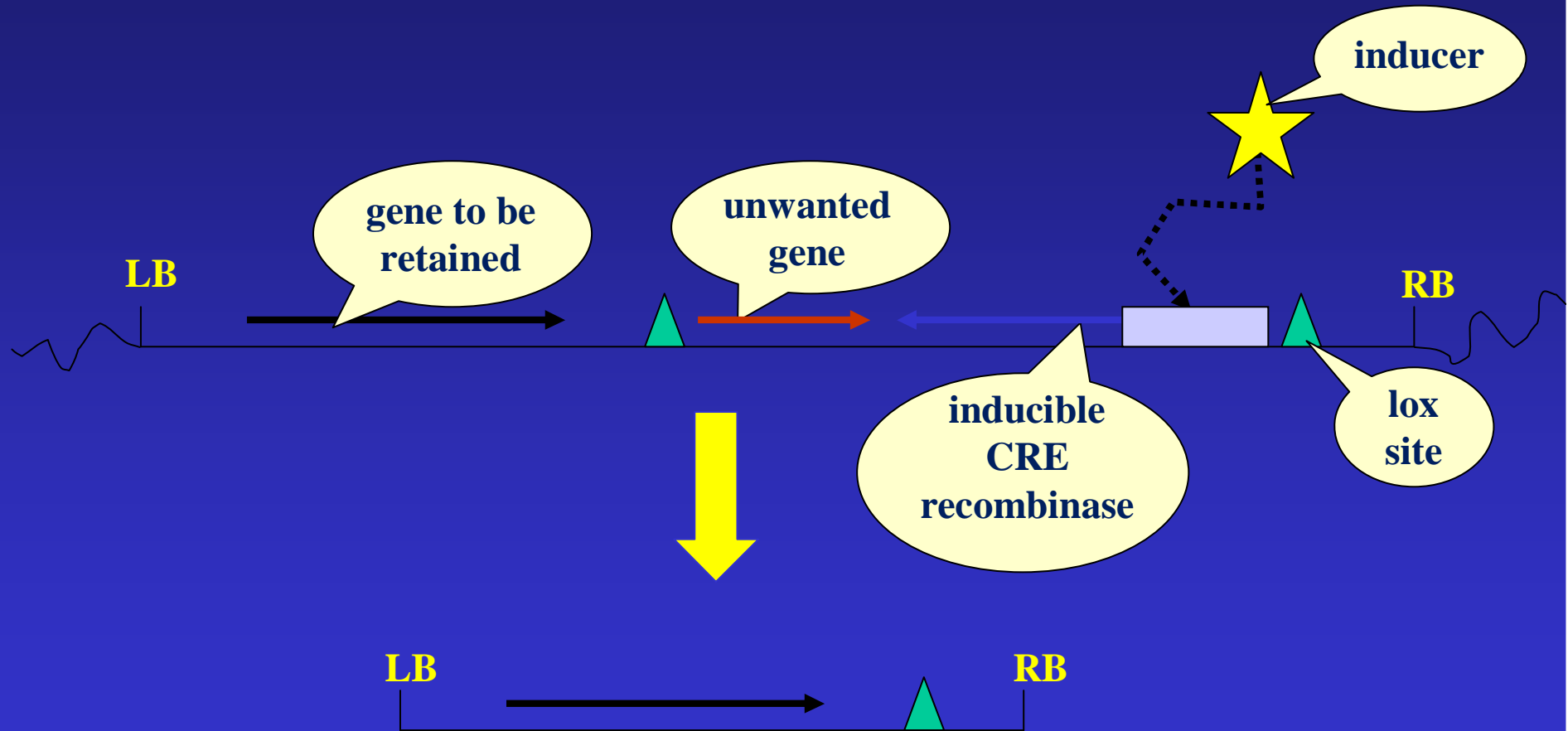
Segregazione

Genotipi T1	Progenie T2	
	Ipotesi di segregazione (test X^2)	
	GUS ^(+/-)	Km ^(R/S)
C2	3:1	>63:1
D4	3:1	3:1
8	3:1	3:1
N2	3:1	>63:1
B4	3:1	3:1
N1	15:1	15:1
N1	3:1	15:1

Site-specific recombination-mediated excision of marker genes

- eg. CRE/Lox consisting of:
- CRE recombinase
 - 34bp lox recombination sites; substrate for the recombinase

Removal of superfluous genes from a transgenic plant using inducible CRE/lox



Phil Mullineaux John Innes Centre, Colney, Norwich
(phil. mullineaux@bbsrc.ac.uk)

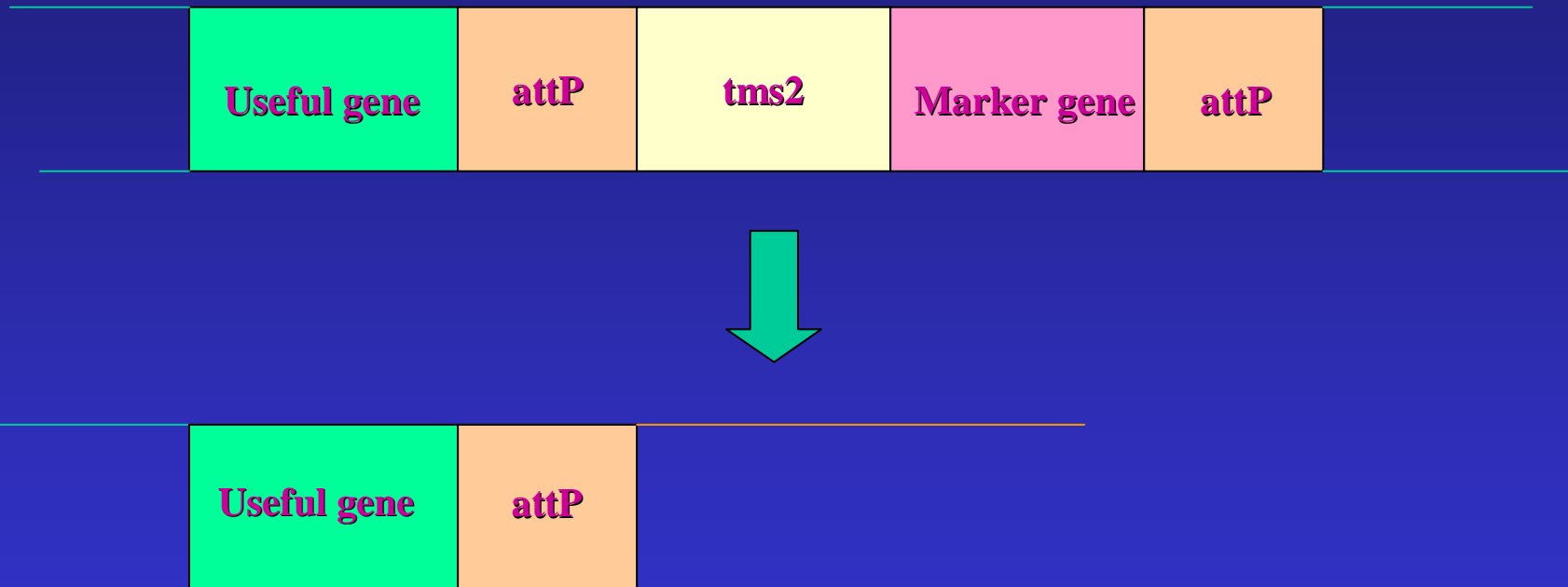
NR4 7UH, UK

Ricombinazione intracromosomica: *attP*

Sequenze di ricombinazione di 352 pb (regione di attacco del batteriofago λ nelle regioni *attB* del DNA di *E. coli*)

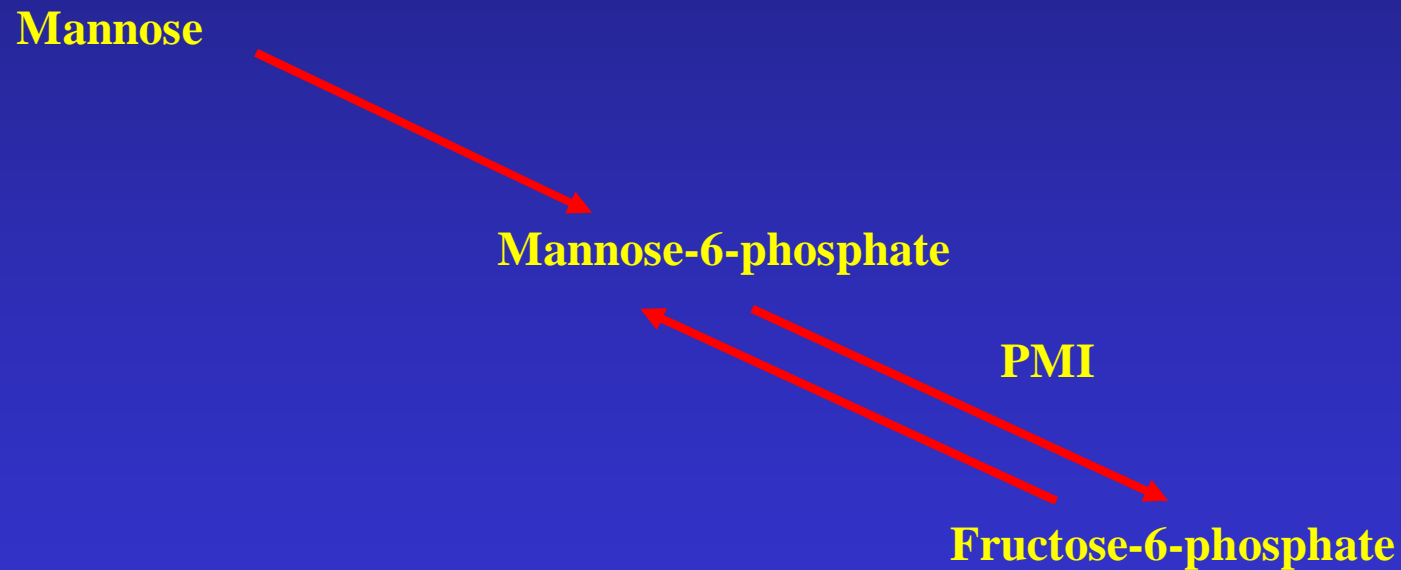
La ricombinazione è ricombinasi indipendente

Site specific recombination: the attP system



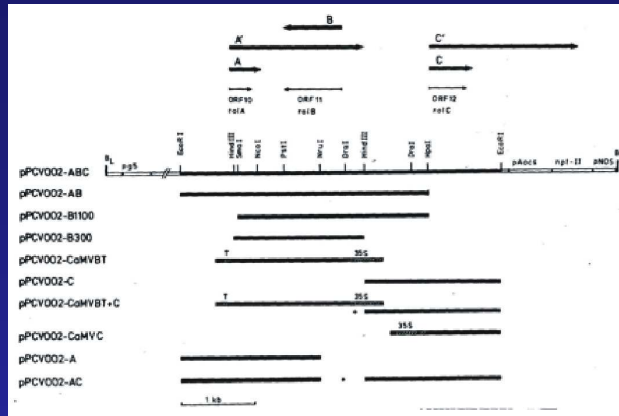
Alternatives to antibiotic resistance markers

Example : Use of Phosphomannose Isomerase (PMI)

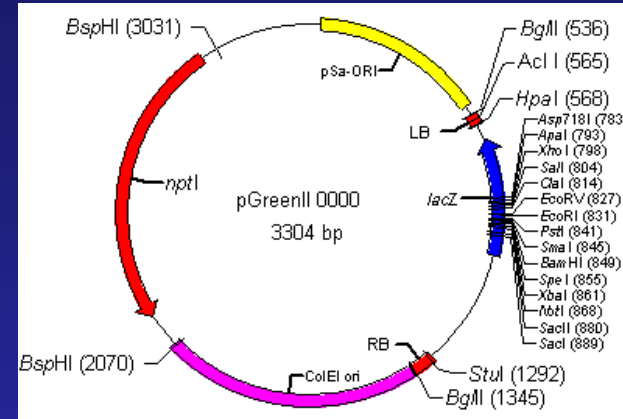


Selection on mannose as sole C source and against mannose-6-phosphate accumulation

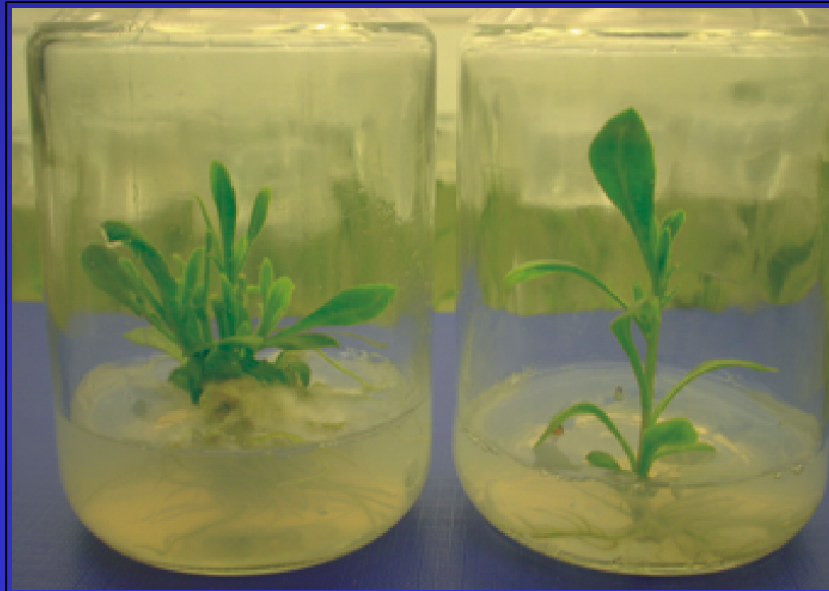
Rol C e rol ABC conferiscono vantaggio selettivo



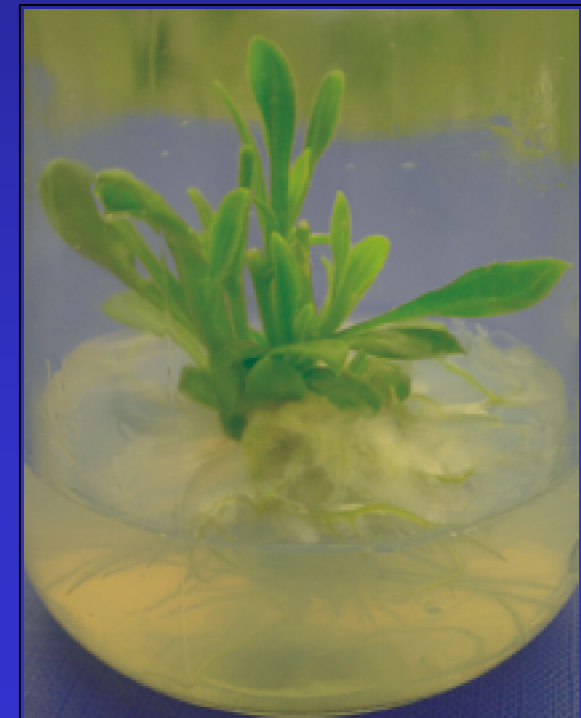
rol C gene (Spena et al.1987)



pGreen II 0000 plasmid (www.pgreen.ac.uk)



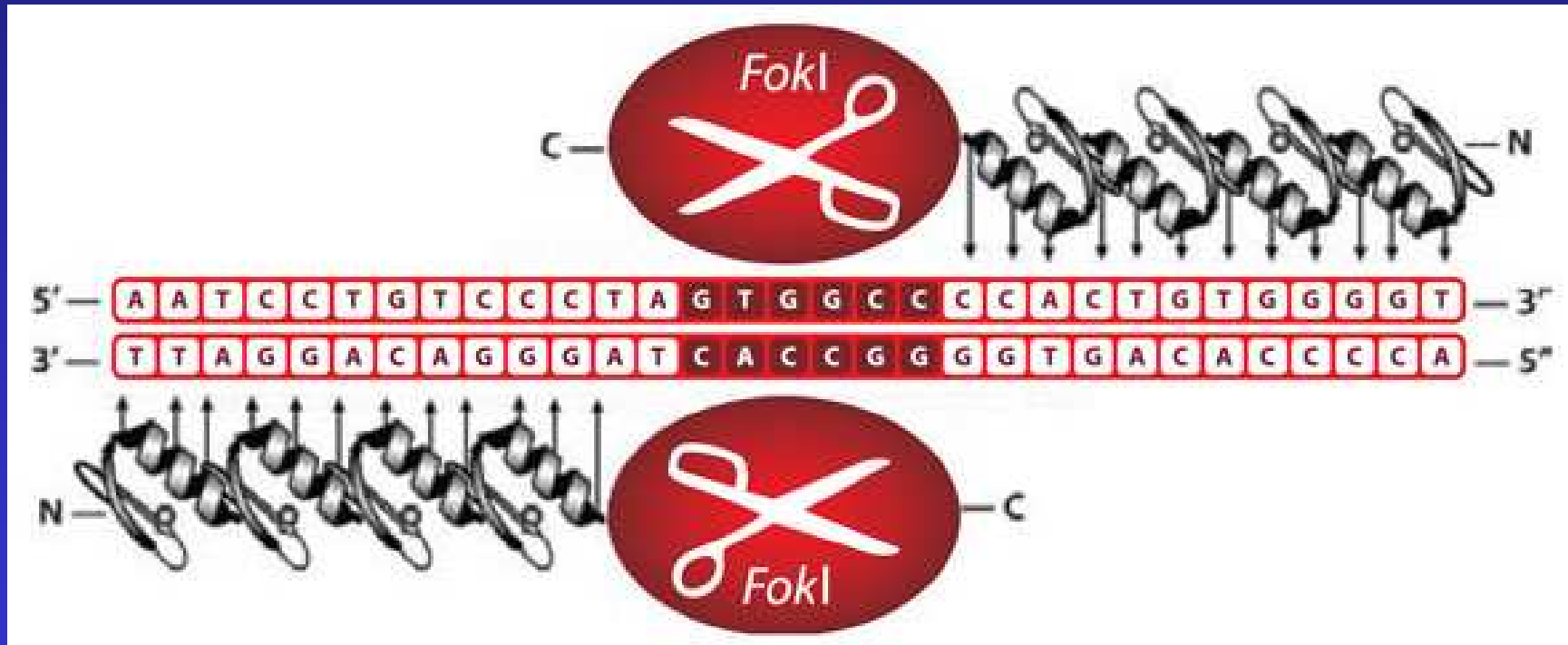
Osteospermum ecklonis L.

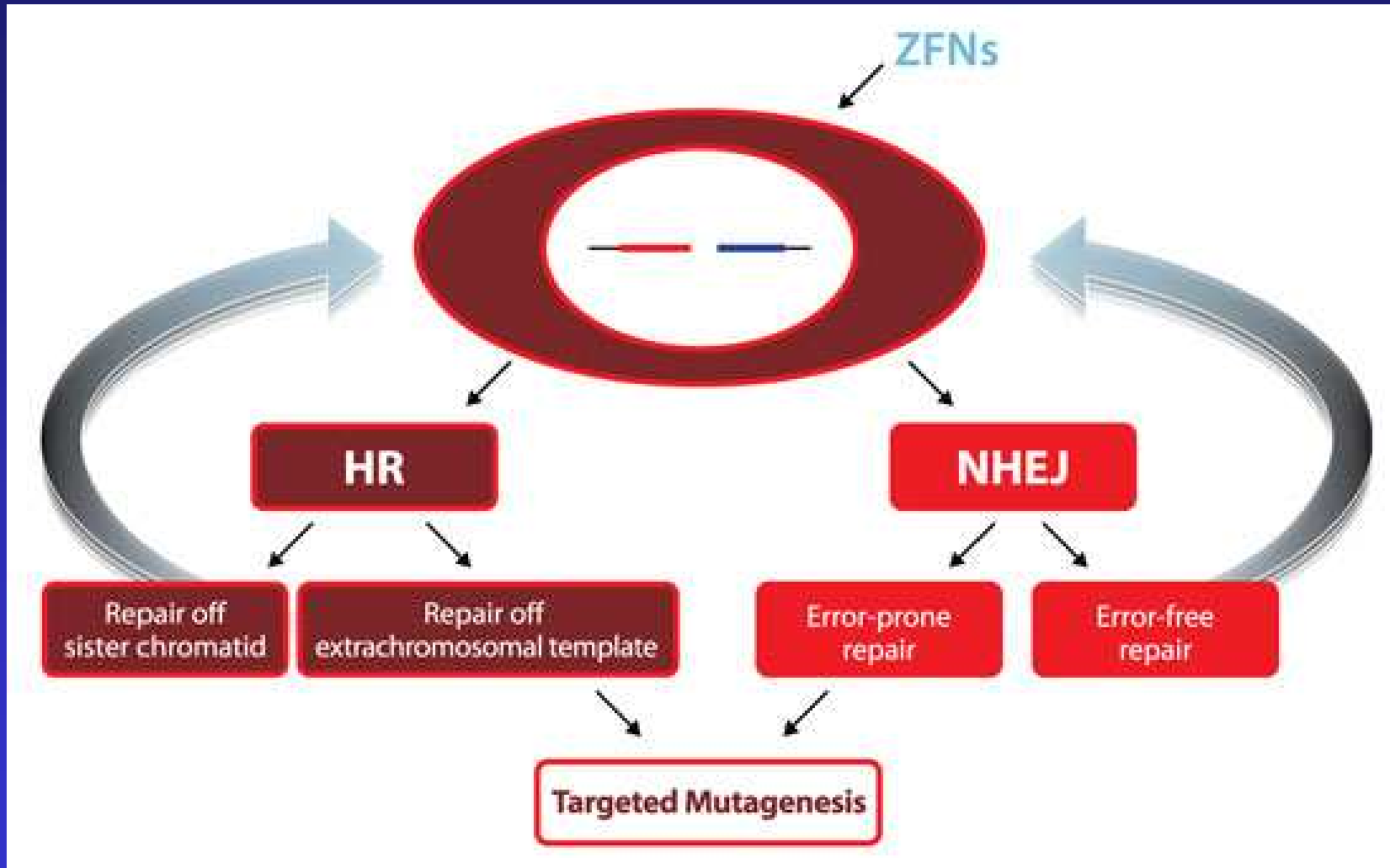


Tecniche di ingegnerizzazione di precisione:

Zink finger nucleases

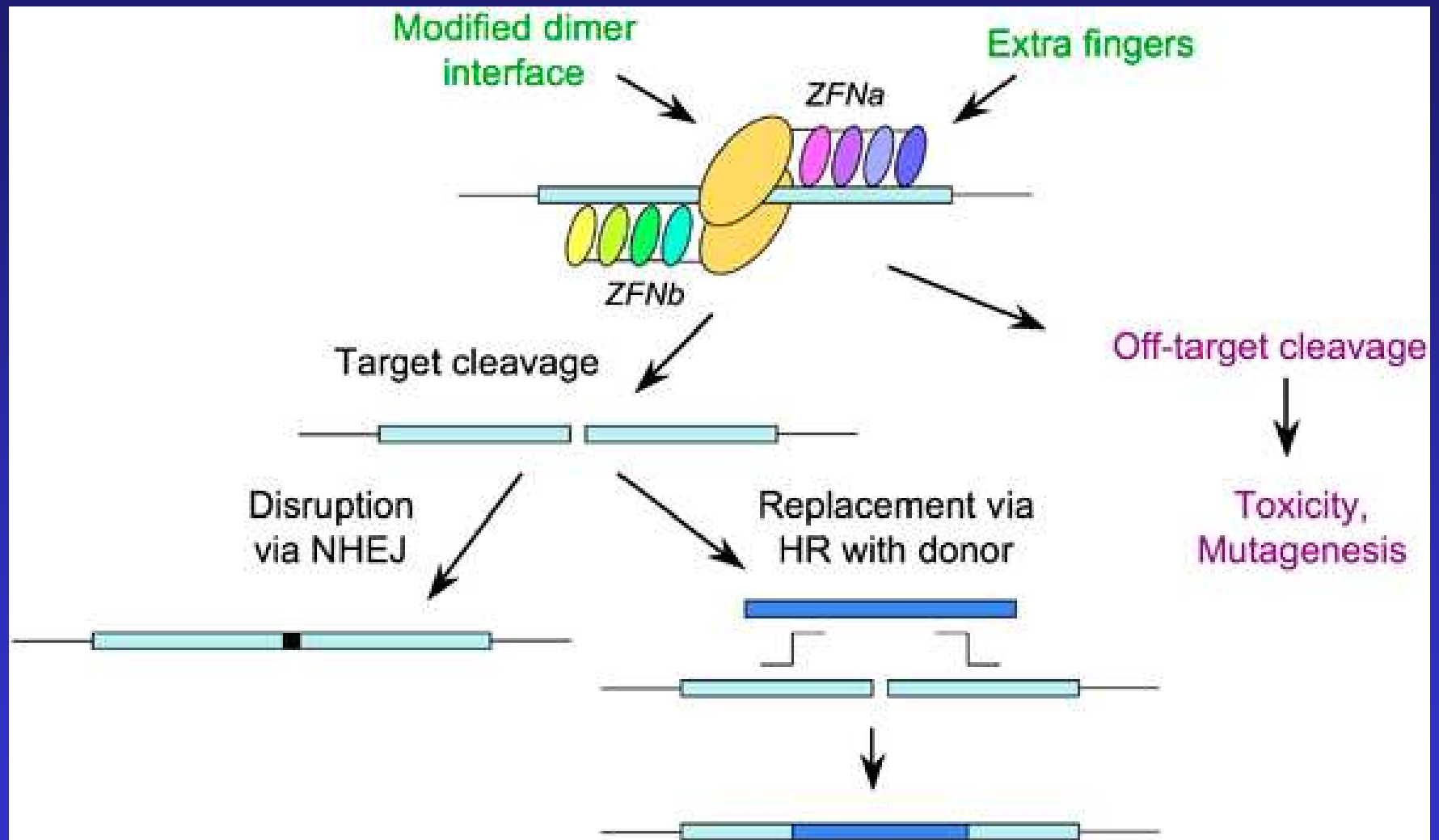
Mutagenesi sito specifica mediante taglio con Zink Finger Nucleases e successiva riparazione dei frammenti via ricombinazione non omologa





Opportunità per mutare geni di specifico interesse

	<u>DdeI</u>	GUS
ATGTTCTTCCCCTCCTGAGGGGGAAGAATTA		-
M F F P S • G E E L		
ATGTTCTTCCCCTCCCGAGGGGGAAGAATTA		+
ATGTTCTTCCCCTCCAGAGGGGGAAGAATTA		+
ATGTTCTTCCCCTCCTGGGGGGAAGAATTA		+
ATGTTCTTCCCCTCCTGGAGGGGGAAGAATTA		-
ATGTTCTTCCCCTCCTG—GGGAAGAATTA		-
ATGTTCTTCCC-----GAGGGGGAAGAATTA		-
ACGAAC -82 bp TGTAGA		-



Danziger
innovations



האוניברסיטה העברית בירושלים
The Hebrew University of Jerusalem



Alexander
Vainstain

MemoGene™ Technology: bypass current barriers to breeding.

Cross breeding



Limitation:
Expensive and time-consuming
Restricted to a given gene pool

Mutation breeding



Limitation:
Random and highly
unpredictable

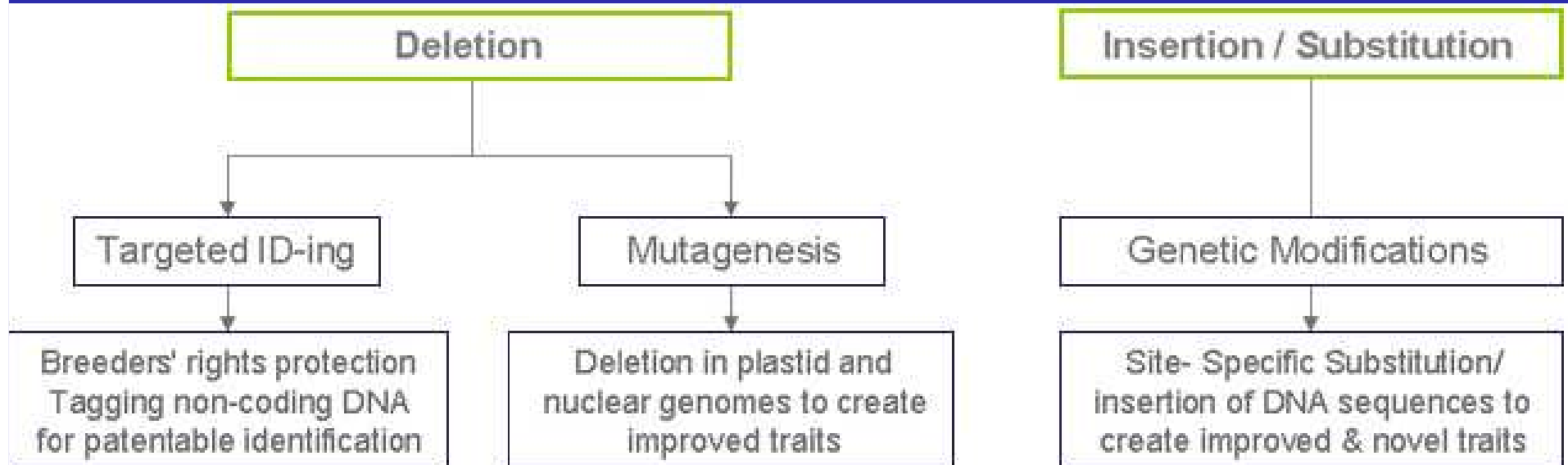
Genetic engineering



Limitation:
Highly crop/variety
specific



MemoGene™ Technology: a unique patented technology for precise plant genomic modifications toward new and better traits in crops.



L'ingegneria genetica nelle specie ornamentali

La trasformazione genetica
integra e non sostituisce le
tecniche classiche di
miglioramento genetico

Modificazioni del colore dei fiori

Specie	Tecnica di modificazione		Colore del fiore	
	Antisenso	Senso	Originale	Transgenico
Garofano	<i>Fht</i>	<i>Chs</i>	Rosa Arancio	Bianco Bianco
Crisantemo	<i>Chs</i>	<i>Chs</i>	Rosa Rosa	Bianco Bianco
Gerbera	<i>Chs</i> <i>Dfr</i>		Rosso Rosso	Rosa/Crema Rosa
Petunia		<i>Lc</i> <i>F3'h</i>	Bianco Lilla	Rosso Rosa

Durata dei fiori post-raccolta

Controllo biosintesi dell'etilene

Methionine



S-adenosyl methionine



(ACS)

1-Aminocyclopropane-1-carboxylic acid (ACC)



(ACD)



(ACO)

Ethylene

Durata dei fiori post-raccolta

Recettori dell'etilene

ETR1: Recettore di segnale dell'etilene.

- Il gene mutante *etr-1* induce insensibilità all'etilene
- Possibilità di utilizzare promotori tessuto specifici

Senescenza delle piante in vaso

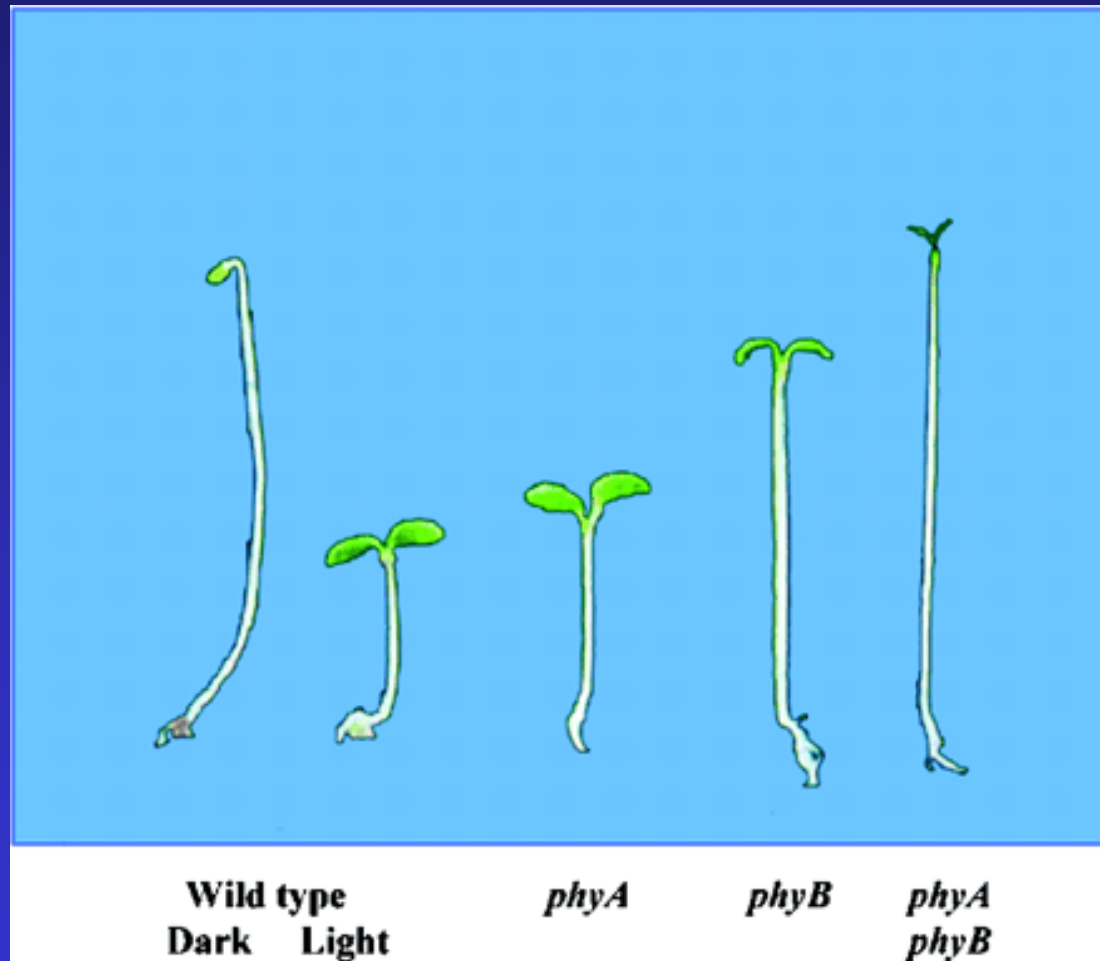
- **Durante l'allevamento in serra in condizioni di elevato investimento**
- **Durante il trasporto**
- **In appartamento**

Senescenza delle piante in vaso

Interventi

- geni del fitocromo
- geni del criptocromo
- sintesi e recettori etilene
- geni ormonali

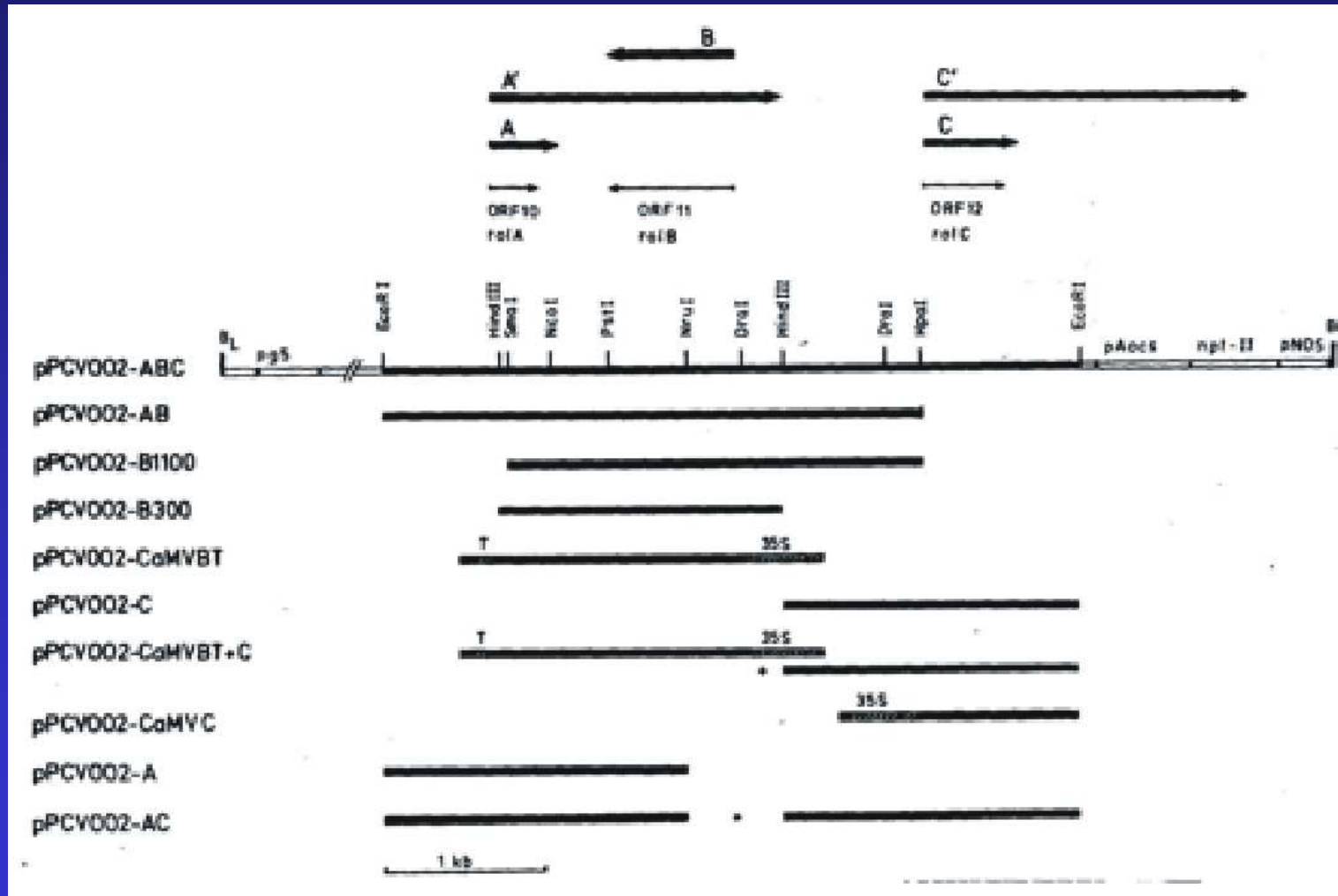
Controllo della ricerca della luce



**Esempi di trasformazione di
specie ornamentali
con Agrobacterium**

Trasformazione con i geni *rol*

TL- DNA di *A. rhizogenes*



Osteospermum ecklonis: controllo e pianta transgenica T1 (35SrolC)



Ornamental traits modification by *Rol* genes in *Osteospermum ecklonis* transformed with *Agrobacterium tumefaciens*

Authors: Giovannini, Annalisa; Zottini, Michela; Morreale, Giacomo; Spina, Angelo; Allavena, Andrea

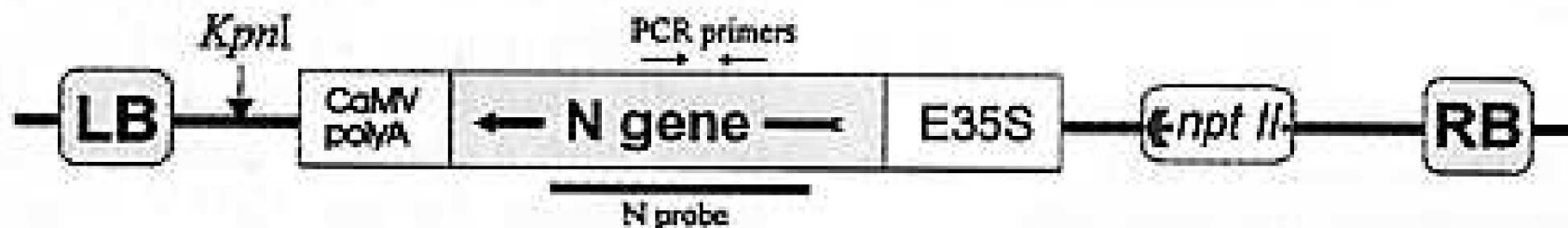
Source: In Vitro Cellular and Development Biology - Plant, Volume 35, Number 1, January 1999 , pp. 70-75(6)



Osteospermum ecklonis:
campo incroci (T_1 x varietà e
dettaglio di progenie
selezionata)



Trasformazione con il gene *N* di TSWV



GENETIC TRANSFORMATION AND HYBRIDIZATION

Evaluation of resistance in *Osteospermum ecklonis* (DC.) Norl. plants transgenic for the N protein gene of tomato spotted wilt virus

A.M. Vaira, T. Berio, G. P. Accotto, M. Vecchiati and A. Allavena

Plant Cell Reports

Volume 19, Number 10, 983-988, DOI: 10.1007/s002990000226

Table 1 Correlation of molecular properties of *O. ecklonis* clones with results from resistance assays

	Clones	Molecular properties			Resistance assays ^a	
		Transgene expression ^b	mRNA	Estimated copy number	Mechanical inoculation ^c	Vector inoculation
DM005-derived clones	Cl 301	++	nt ^e	nt	2/7	0/5
	Cl 302	+	nt	nt	0/7	0/5
	Cl 303	++	+	nt	1/7	0/5
	Cl 304	++	nt	nt	0/7	0/5
	Cl 308	++	+	nt	2/7	0/5
	Cl 309	+++	+	2	2/7	0/5
	Cl 310	++	nt	nt	0/7	0/5
	Cl 312	-	-	nt	2/7	0/5
	DM005 ^d	-	-	0	4/7	0/5
DM002-derived clones	Cl 307	+++	+	1	6/7	1/5
	Cl 313	++	+	1	4/7	0/5
	Cl 315	-	-	4	7/7	3/5
	Cl 317	++	+	3	4/7	0/5
	Cl 318	+++	+	1	5/7	0/5
	Cl 319	+++	+	1	5/7	0/5
	Cl 320	+++	+	1	6/7	0/5
	Cl 321	++	+	1	4/7	0/5
	DM002 ^d	-	-	0	4/7	0/5

^a Number of virus-free plants/no. of plants tested at the end of experiments

^b ELISA data based on Fig. 4; +, O.D. from 0.1 to 0.6; ++, O.D. from 0.6 to 1.2; + + +, O.D. higher than 1.2

^c 25°C test

^d Non-transgenic

^e Not tested

**Introduzione di
resistenze a
TSWV mediante
trasformazione
con il gene *N*
(nucleoproteina
virale)**



**Resistenza al freezing
in *Osteospermum*
indotta da espressione
di *Osmyb4*.**

**Piante trattate per una
notte a -5°C senza (A)
o con acclimatazione
per 10 giorni a 4°C (B)**

**Metabolic response to cold and
freezing of *Osteospermum*
ecklonis overexpressing *Osmyb4***

**Marina L., R. Consonni, F.
Locatelli, E. Fumagalli, A.
Allavena, I. Coraggio, M.
Mattana Plant Physiology and
Biochemistry Volume 48, Issue
9, September 2010, Pages 764–
771**

A



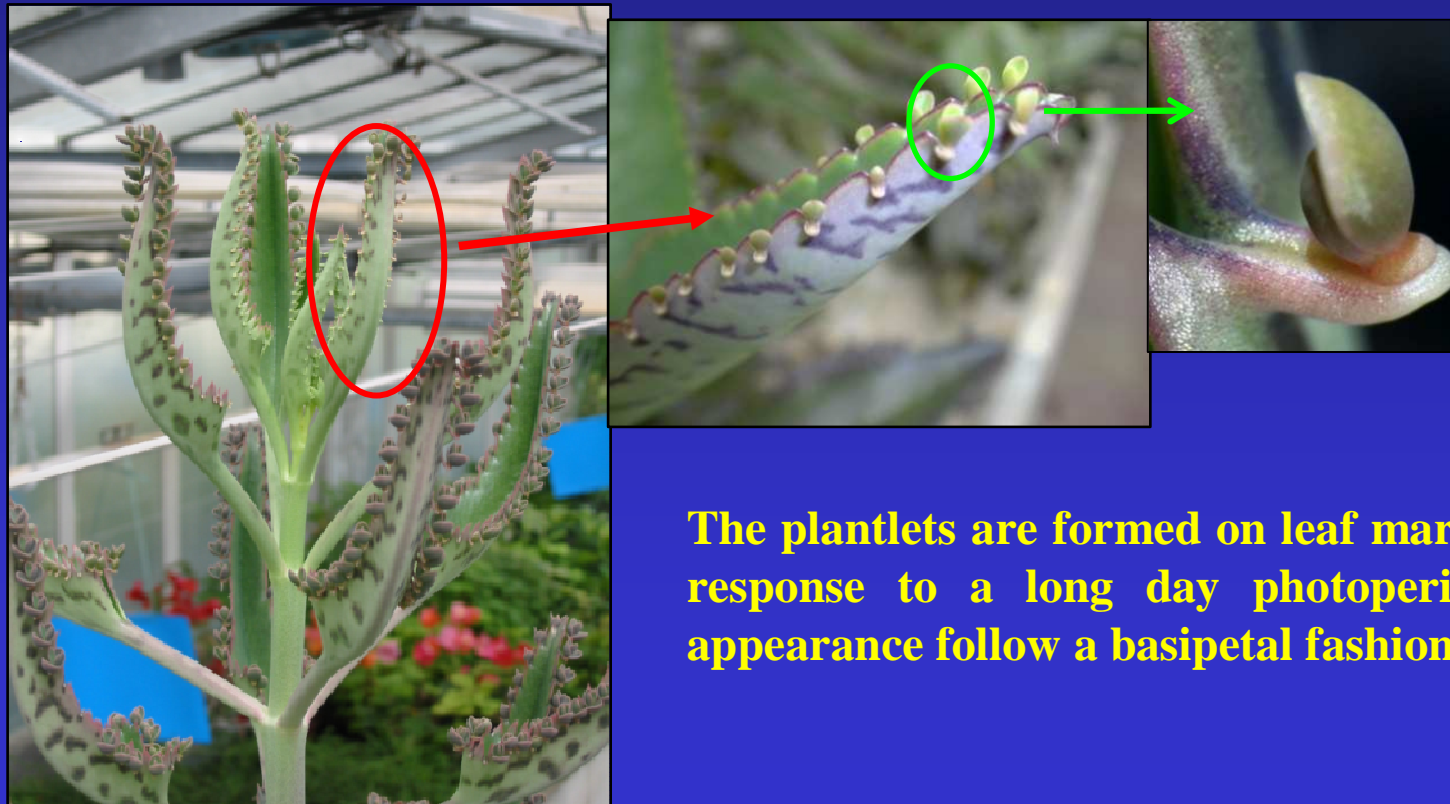
B



Studio dell'espressione genica

EPIPHILLY (VEGETATIVE VIVIPARY) in *Kalanchoe x houghtonii*

- peculiar type of asexual reproduction, leads to the formation of novel complete plantlets on mature organs (leaves, stems, roots, flower stalks)
- process is widespread to several families (Liliaceae, Graminaceae, Crassulaceae)
- may be switched on by environmental stimuli (short vegetative seasons, low light intensity, stresses)

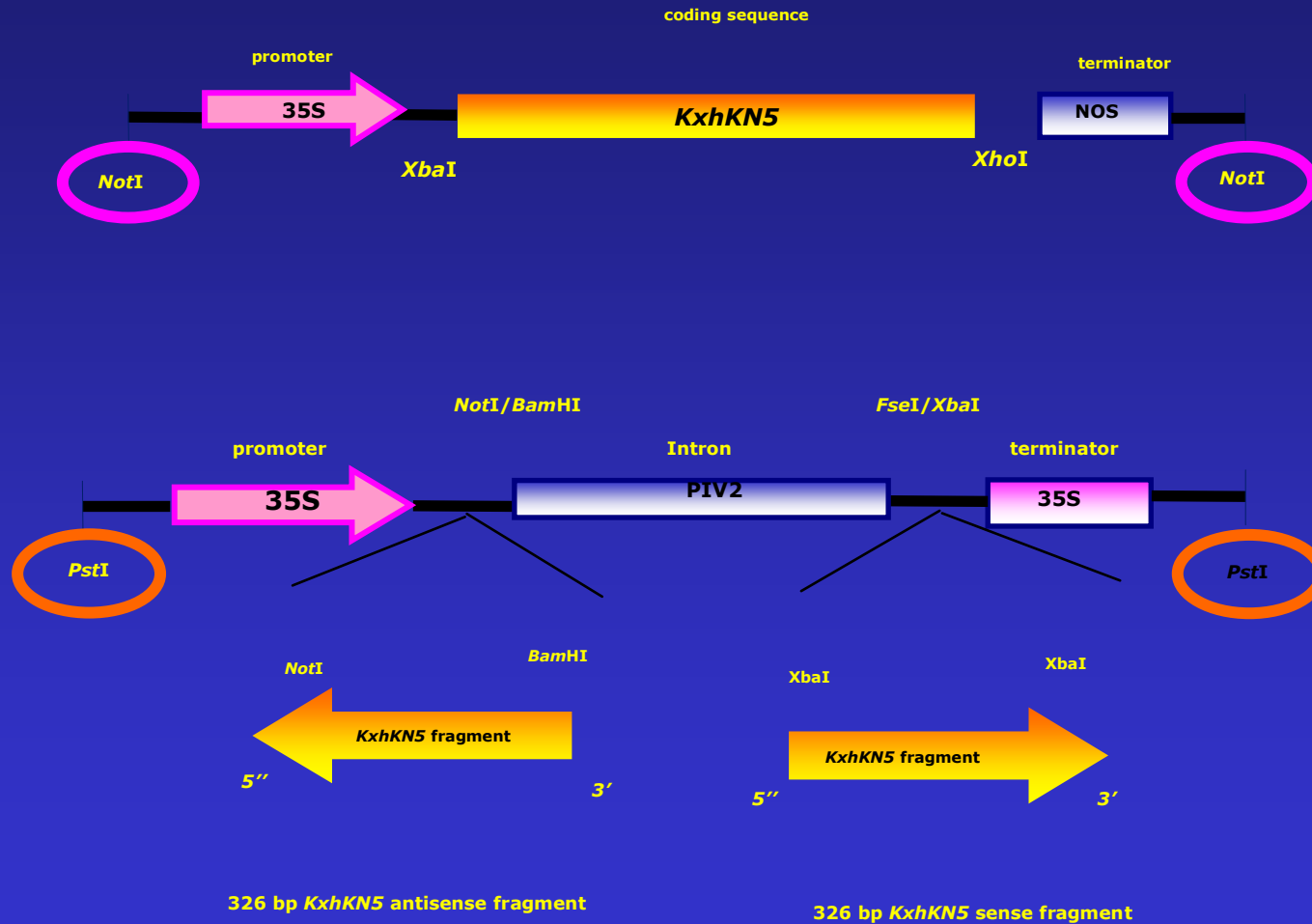


The plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion.

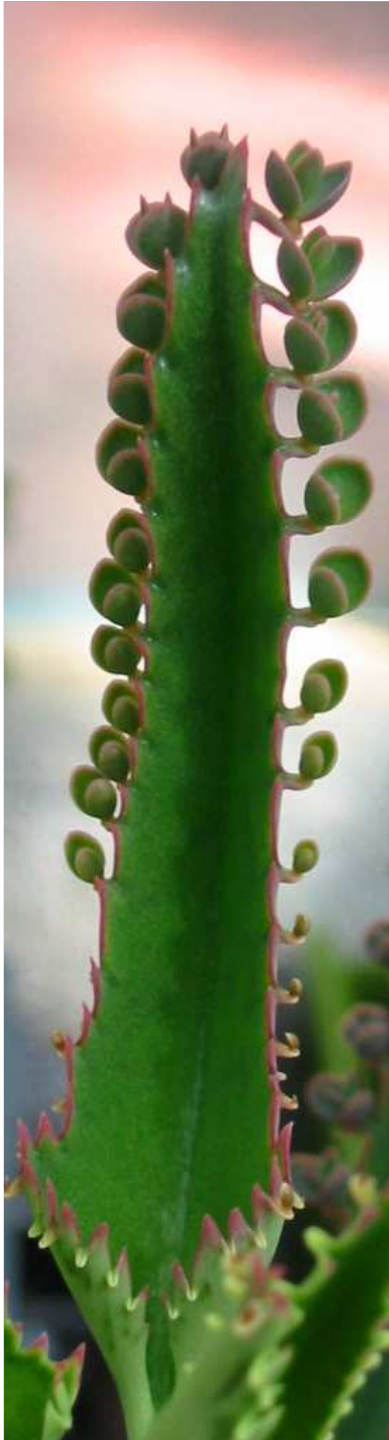
**Sequenziati 6 geni *KNOX* di cui
2 di classe 1 omologhi di *STM* e
BP di *Arabidopsis***

Sovraespressione e silenziamento di *KxhKN5*

Over-expression and silencing cassette



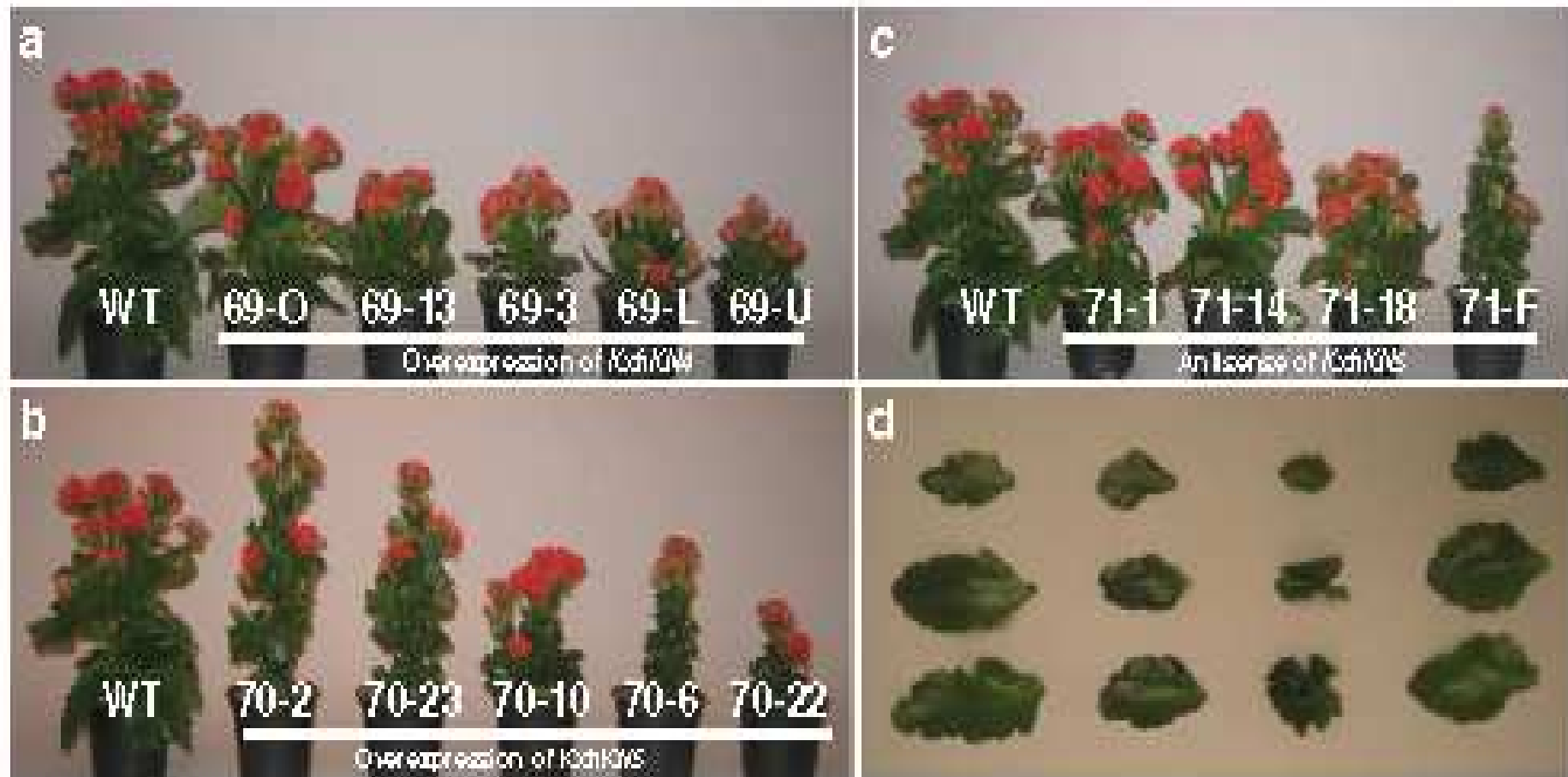
Modifica della forma delle foglie



Expression of KxhKN4 and KxhKN5 genes in Kalanchoë blossfeldiana 'Molly' results in novel compact plant phenotypes: towards a cisgenesis alternative to growth retardants.

Lütken H, Laura M, Borghi C, Ørgaard M, Allavena A, Rasmussen SK.

Plant Cell Rep. 2011 Dec;30(12):2267-79. Epub 2011 Aug 18.

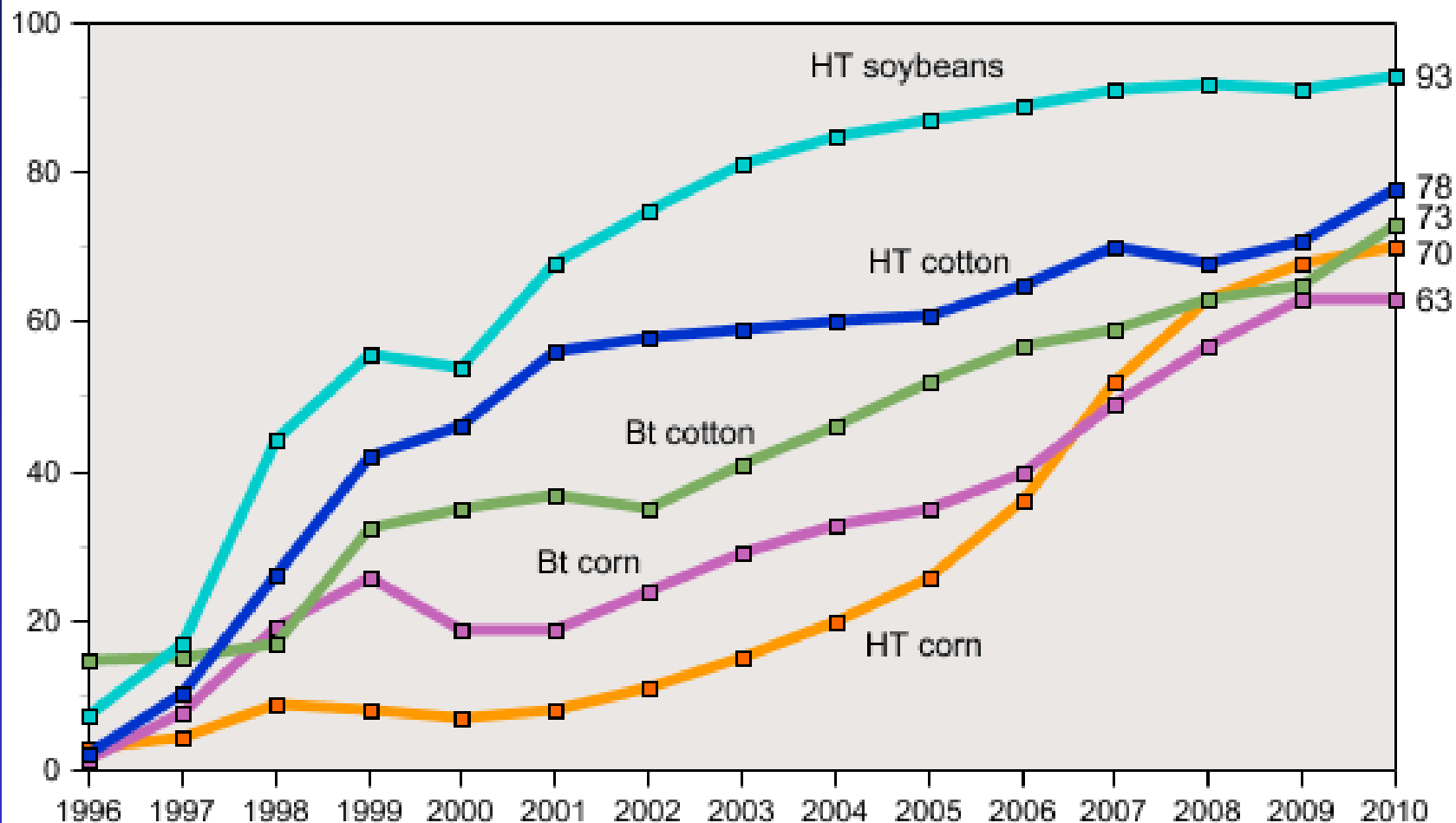


Food	Percent Modified in US	Percent Modified in world
Soybeans	93%	77%
Corn, field (Maize)	86%	26%
Cotton (cottonseed oil)	93%	49%
Hawaiian papaya	80%	
Tomatoes	Taken off the market due to commercial failure.	Small quantities grown in China
Canola	93%	21%
Sugar cane		
Sugar beet	95% (2010); planting in 2011 under controlled conditions	9%
Rice	Forecast to be on the market in 2013^[23]	
Squash (Zucchini)	13%	
Sweet Peppers		Small quantities grown in China

- [^] [a](#) [b](#) **Acreage NASS National Agricultural Statistics Board annual report, June 30, 2010. Retrieved July 23, 2010.**
- [^] [a](#) [b](#) **USA:Cultivation of GM Plants in 2009, Maize, soybean, cotton: 88 percent genetically modified GMO Compass. Retrieved July 25, 2010.**
- [^] [a](#) [b](#) **Field areas 2009, Genetically modified plants: Global cultivation on 134 million hectares GMO Compass. Retrieved July 25, 2010.**
- [^] [Ronald, Pamela and McWilliams, James](#) [Genetically Engineered Distortions](#) **The New York Times, May 14, 2010, Mentions that today 80% of Hawaiian papaya is genetically engineered. Retrieved July 26, 2010.**
- [^] [Wright, Brierley](#) [How Healthy Is Canola Oil Really? "Eating Well"](#), **March/April 2010 edition, Mentions 93% of rapeseed in the US is GM. Retrieved July 26, 2010.**
- [^] [Johnson, Stanley R. et al](#) [Quantification of the Impacts on US Agriculture of Biotechnology-Derived Crops Planted in 2006](#) **National Center for Food and Agricultural Policy, Washington DC, February 2008. Retrieved August 12, 2010.**

Rapid growth in adoption of genetically engineered crops continues in the U.S.

Percent of acres



Data for each crop category include varieties with both HT and Bt (stacked) traits.

Sources: 1996-1999 data are from Fernandez-Cornejo and McBride (2002). Data for 2000-10 are available in the ERS data product, Adoption of Genetically Engineered Crops in the U.S., tables 1-3.

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