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LEUVEN

Tagging Low Temperature Responsive Promoters in Banana Using the Luciferase Reporter Gene

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Outline

- I. Introduction
- II. High-throughput screening for low temperature responsive promoters
- III. Isolation and analysis of T-DNA flanking sequences
- IV. Identification of activated insertion(s)
- V. Conclusions and perspectives

Banana

Economy: The 4th most important food crop

Diet: Major staple food in tropical countries

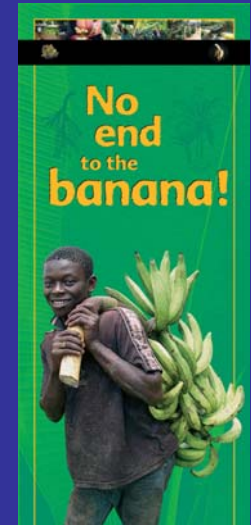
Characteristics: (Sub)tropical, vegetative propagation, parthenocarpy

Genome: 500-600 Mbp; 11 chromosomes; ploidy: 2x, 3x, 4x; genomic groups (AAA, AAB, ABB ...)

Molecular tools: Genetic transformation systems, limited sequence data (EST, BAC, SAGE libraries), molecular markers, ...

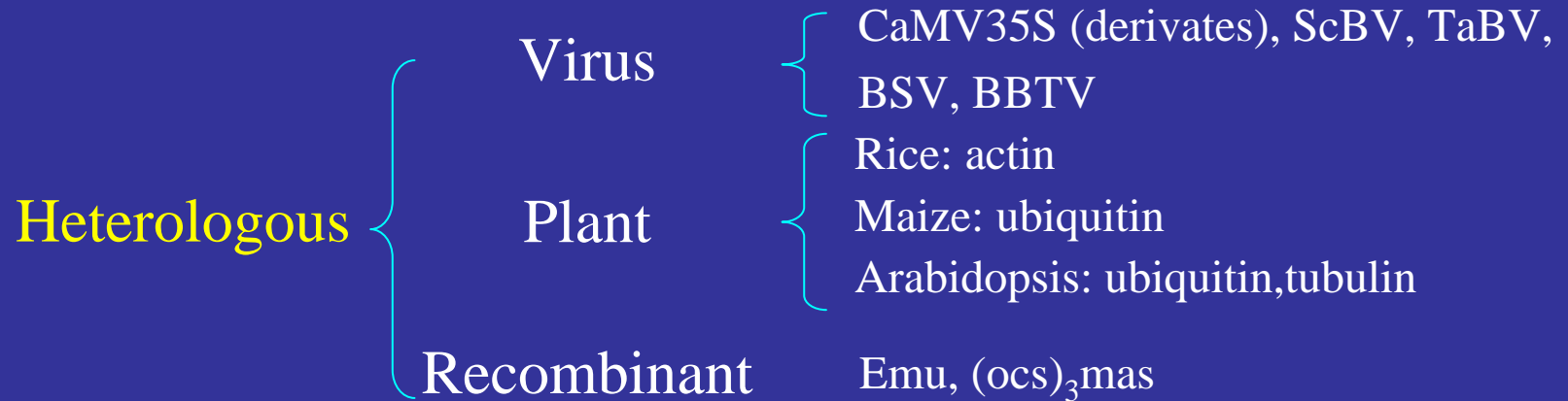
INIBAP (www.inibap.org)

Global *Musa* Genomics Consortium (www.musagenomics.org)



Source: INIBAP

Promoter usage in transgenic bananas



Homologous

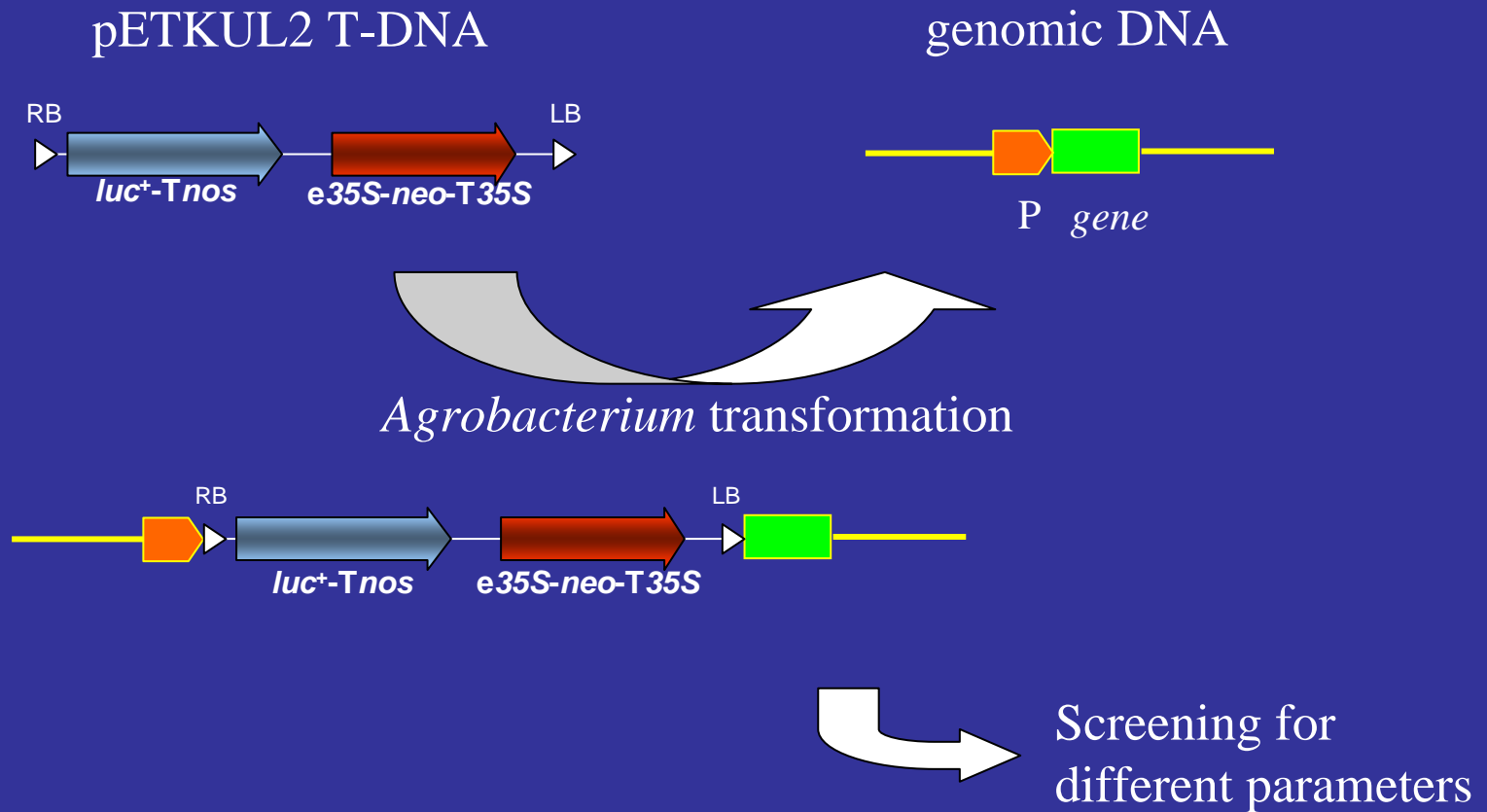
Banana: actin, EFE, ACO1, ACC



Functionality/stability, less biosafety concerns (*cis*-genic bananas)

Constitutive, tissue specificity, inducible (biotic/abiotic stress)

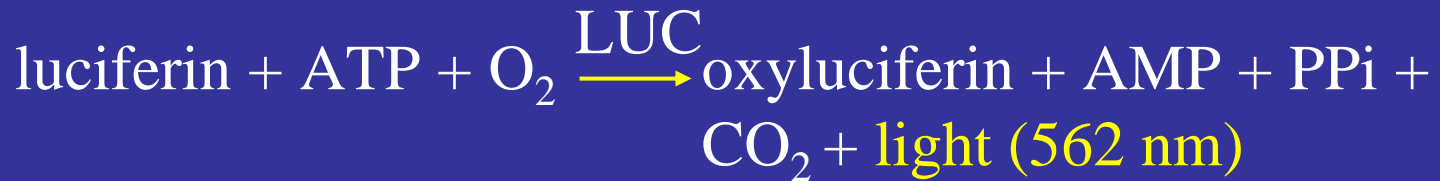
Promoter tagging via *Agrobacterium* transformation



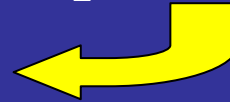
Luciferase reporter gene system



luc



ultrasensitive digital
CCD camera system



II. High-throughput screening for low temperature responsive promoters

Banana promoter tagging

Months after
Agrobacterium
infection

1 2 3 4 5 6 7 8 ...



Phase

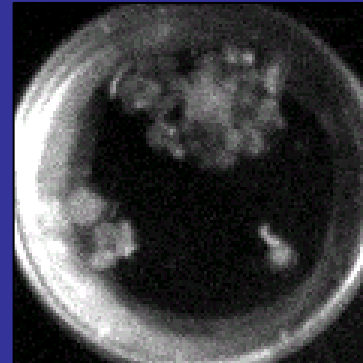
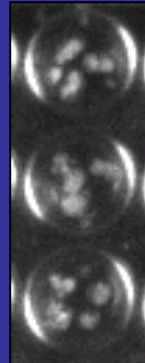
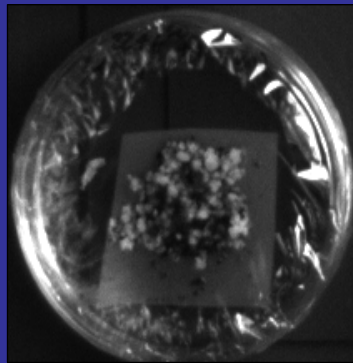
Cell colonies

Cell
cultures

Regenerating
cultures

Plantlets

Culture



400-600/Petri Dish
5,600-8,400/image

Screening



II. High-throughput screening for low temperature responsive promoters

Banana - low temperature

Low temperature stress
chilling / freezing



Source: www.texasriviera.com



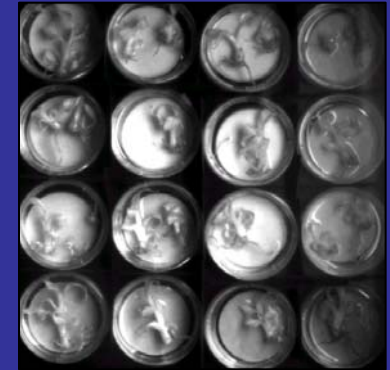
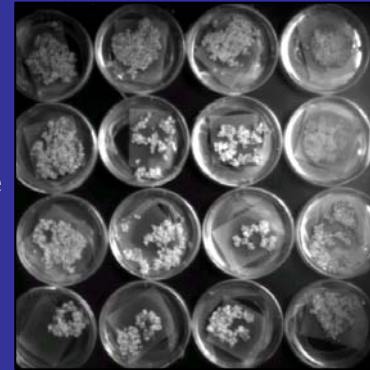
8°C

Real-time luciferase screening

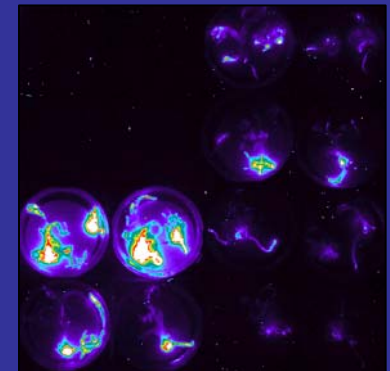
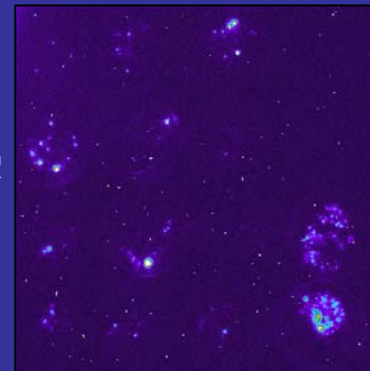
Cell colonies

Plantlets

Live



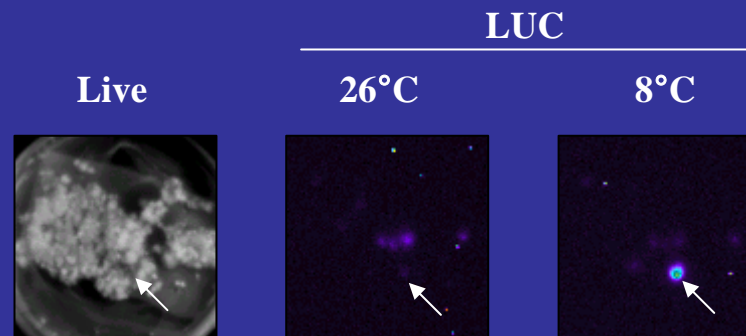
LUC



II. High-throughput screening for low temperature responsive promoters

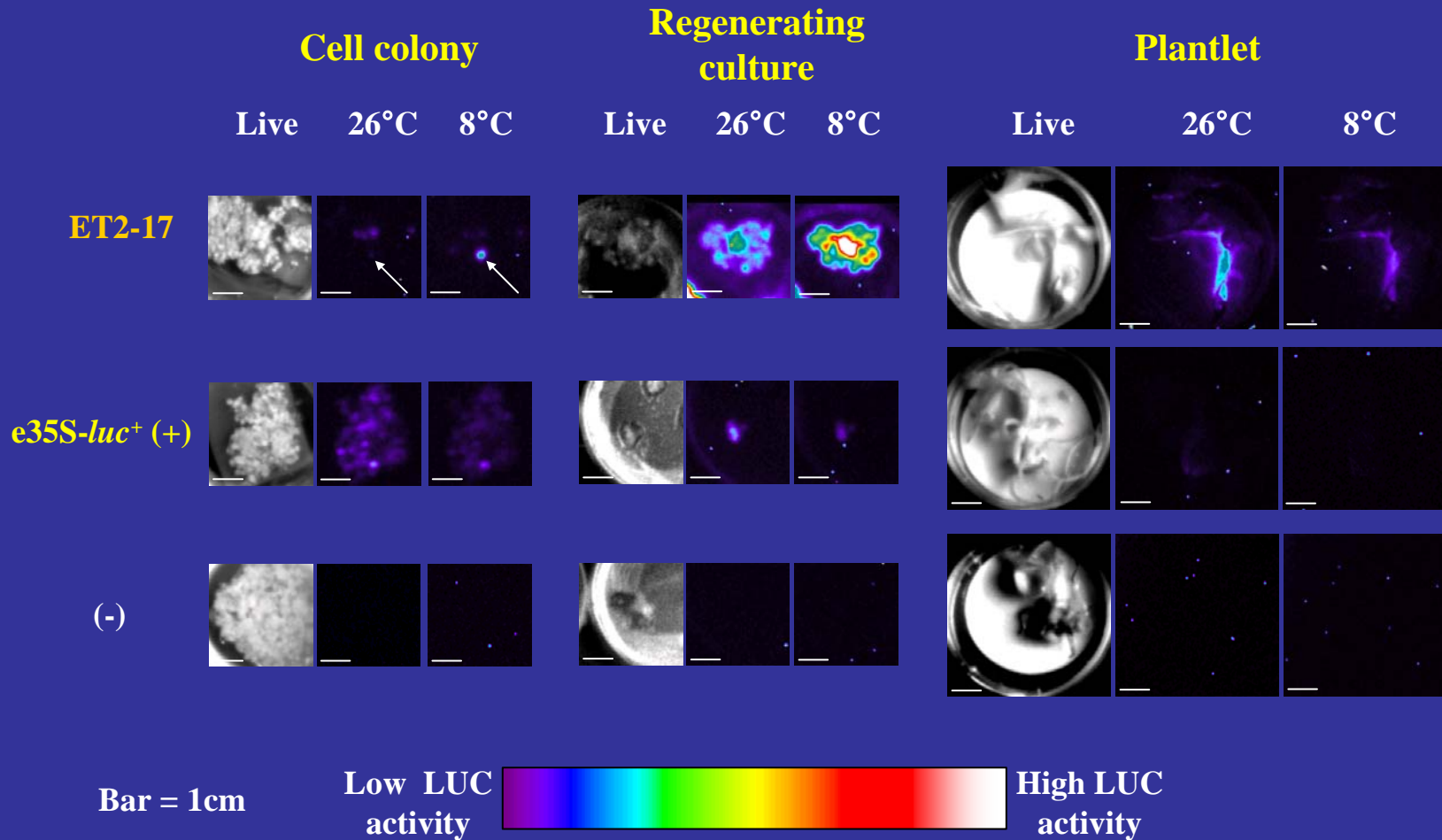
Luciferase activation in transgenic cell colonies (~16,000)

Screening	Treatment	Luciferase activity at 26°C		Response at 8°C	
		No.	Frequency (%)	Induced % (No.)	Repressed % (No.)
1	RT	155	0.98	-	-
2	26°C → 8°C	96	0.61	0.05 (10)	0.57 (84)



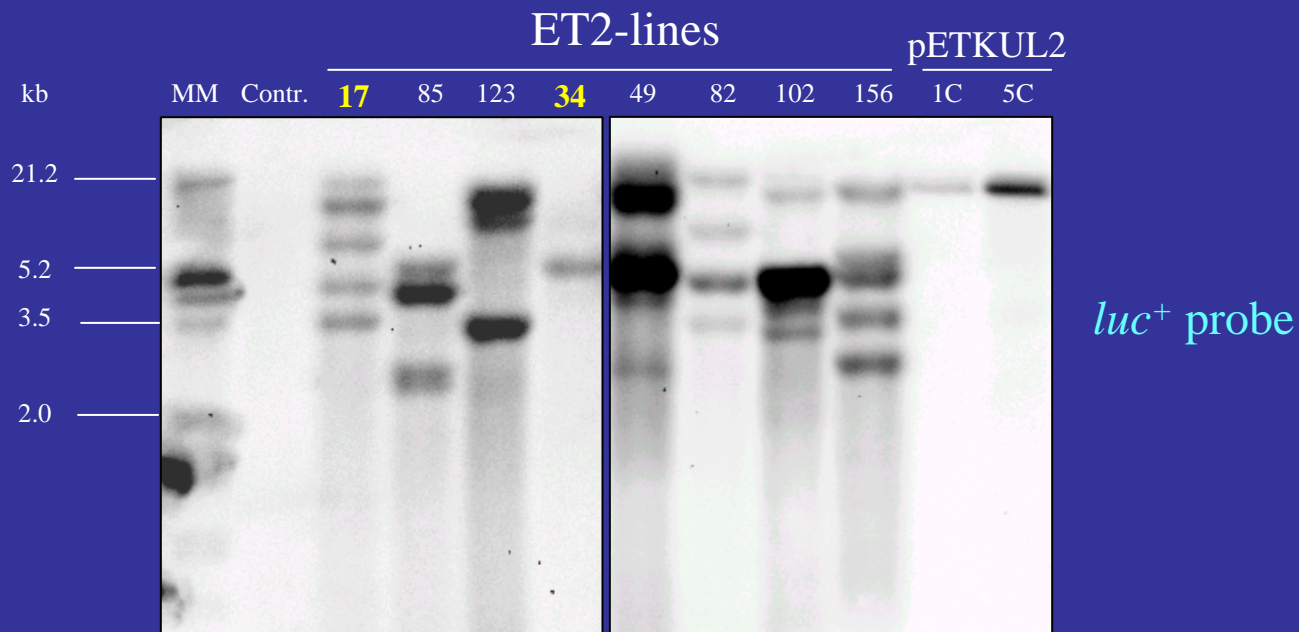
II. High-throughput screening for low temperature responsive promoters

Screening during *in vitro* regeneration



III. Isolation and analysis of T-DNA flanking sequences

Southern analysis



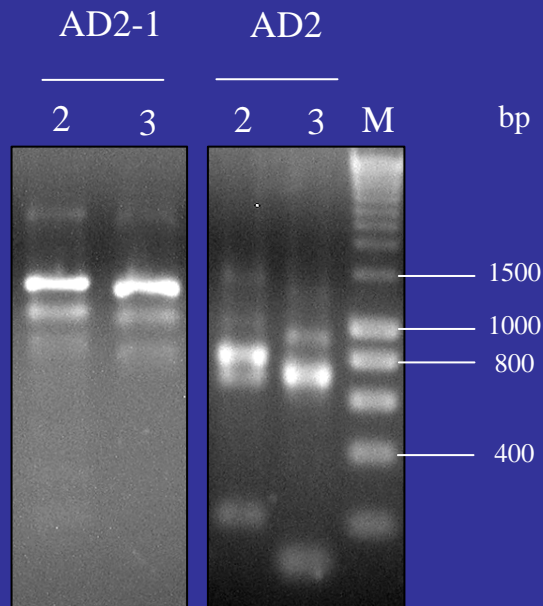
Min. No. T-DNA copies: 5 3 3 1 4 4 4 5

Average: 3.5

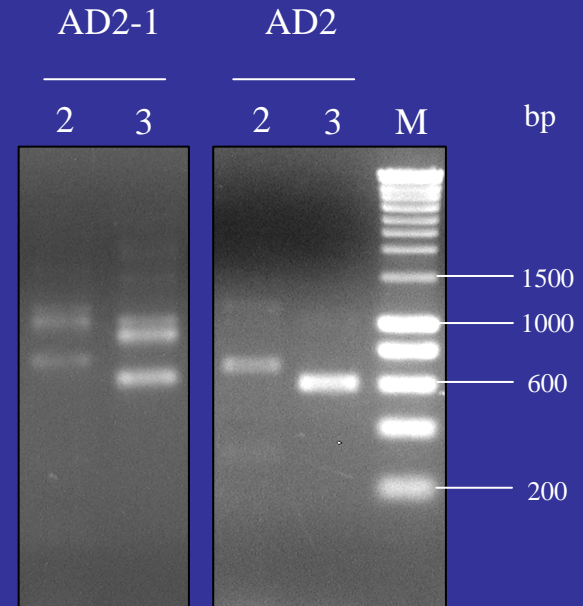
III. Isolation and analysis of T-DNA flanking sequences

TAIL-PCR

ET2-17



ET2-34



III. Isolation and analysis of T-DNA flanking sequences

Amplification of RB flanking sequences

No. of specific PCR amplicons

ET2-Line	No. T-DNA copies (Southern)	TAIL-PCR			I-PCR		Different sequences
		AD2	AD2-1	AD2-5	<i>BsrGI</i>	<i>BclII</i>	
17	5	3	5	2	2	2	5
156	5	2	1	2	2	2	3
49	4	4	5	1	NT	4	4
179	4	2	3	3	3	5	4
85	3	2	1	1	2	NT	3
111	3	2	3	1	1	3	2
34	1	1	2	1	2	2	1
Average	3.3	2.3	2.9	1.6	2.0	3.0	3.1

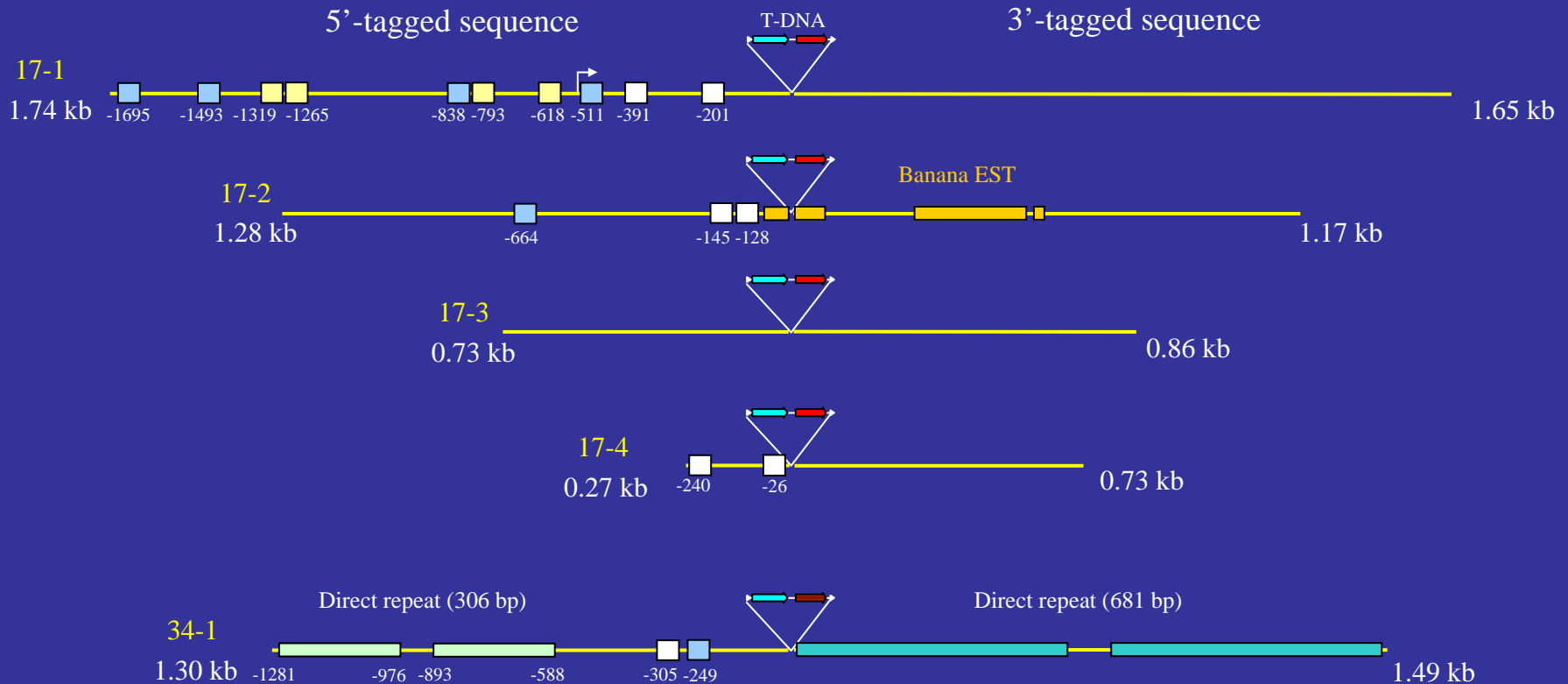
NT = Not Tested

III. Isolation and analysis of T-DNA flanking sequences

Analysis of T-DNA flanking sequences

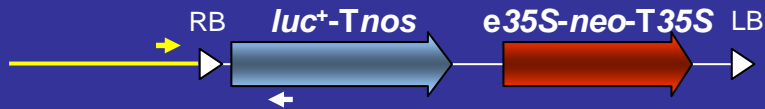
Bioinformatic tools: PlantCARE, PLACE

■ = TATA box ■ = DRE-like ■ = ABRE-like ▸ = Transcription start site (TSSP)

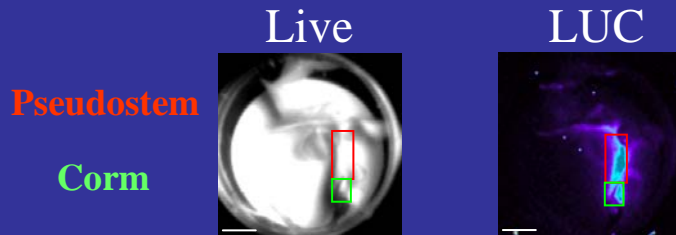


IV. Identification of activated insertion(s)

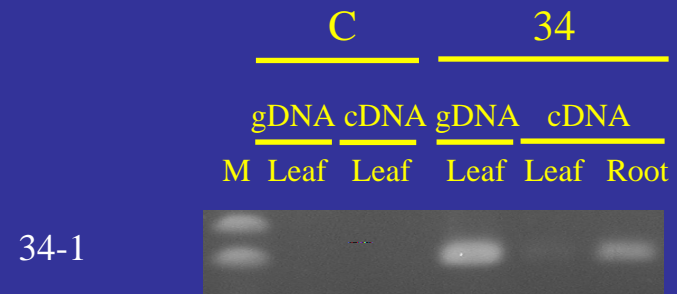
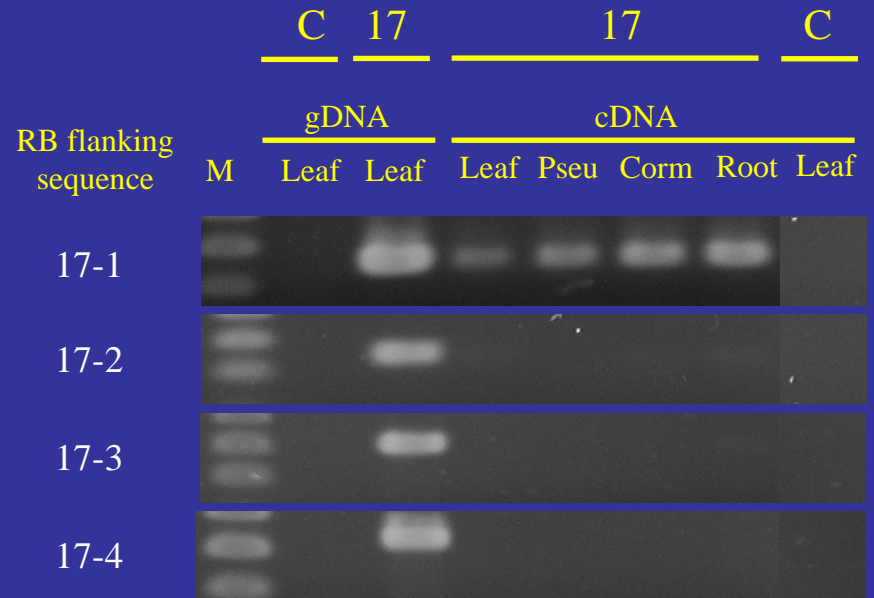
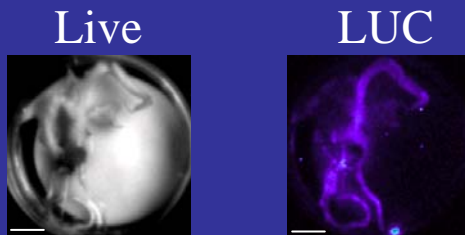
RT-PCR



ET2-17



ET2-34

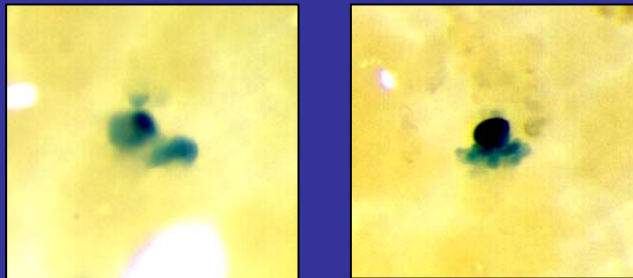


Conclusions

- Different low temperature responses identified
- Relation low temperature and *in vitro* regeneration
- Tagged lines with one to five T-DNA copies
- Isolation of T-DNA flanking sequences
- RT-PCR revealed promoter activity

Perspectives

- Back-transformation of selected putative promoter sequences



- Confirmation of low temperature and developmental regulation



Acknowledgement

VLIR-IUS program cooperation VLIR-ESPOL, Belgium-Ecuador