Promoter tagging in banana (*Musa* spp.) using the luciferase reporter gene - development and applications

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Objective

- Isolate promoters from banana (*Musa* spp.) with constitutive, tissue specific, and cold stress responsive expression patterns

Content

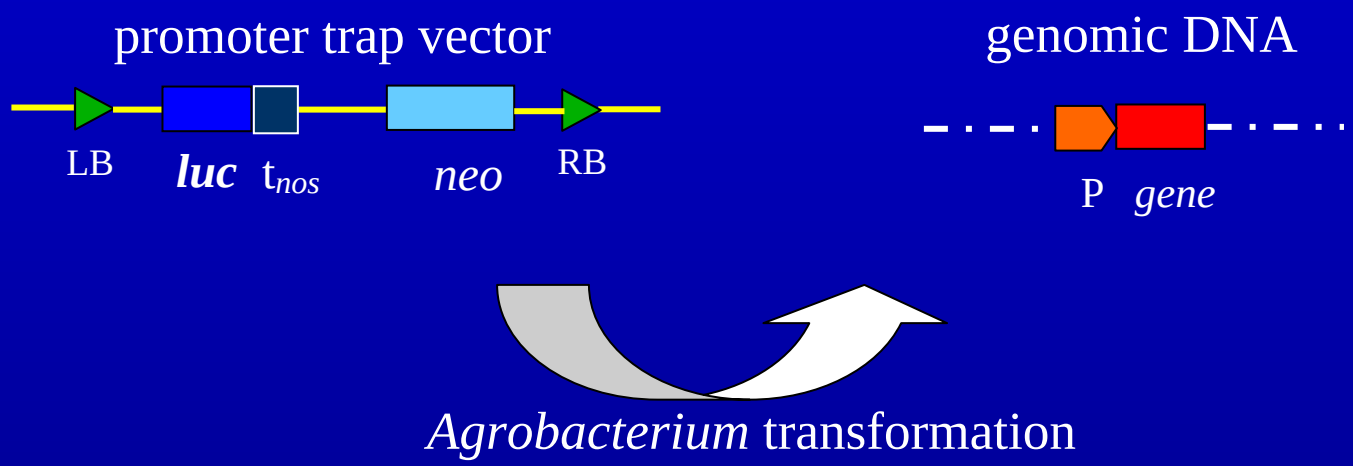
I. The promoter tagging system

II. Luciferase expression patterns

III. Tagging of cold-responsive promoters

IV. Conclusions and perspectives

I. The promoter tagging system



Luciferase reporter gene

luc

- Highly sensitive
- Non destructive screenings
- Short half-life of enzyme

luciferin + ATP + O_2 $\xrightarrow{\text{LUC}}$ oxyluciferin + AMP + PPi + CO_2 + light (562 nm)

ultrasensitive digital CCD camera system

I. The promoter tagging system

High throughput screening for LUC activation

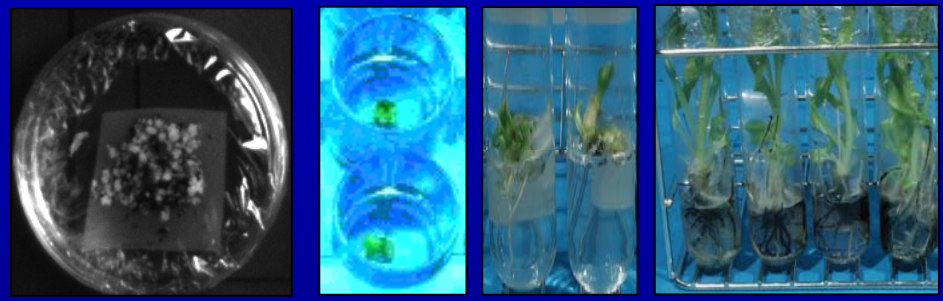
Months after *Agrobacterium* infection

1 2 3 4 5 6 ...

Medium

ZZ RD1 RD2 Reg

Culture



Screening

400-600/sample
5,600-8,400/image
11,000-17,000/day

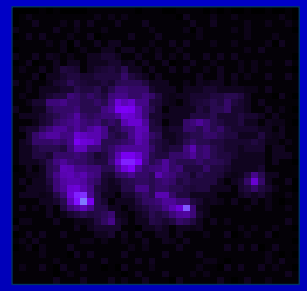
✓ ✓ ✓

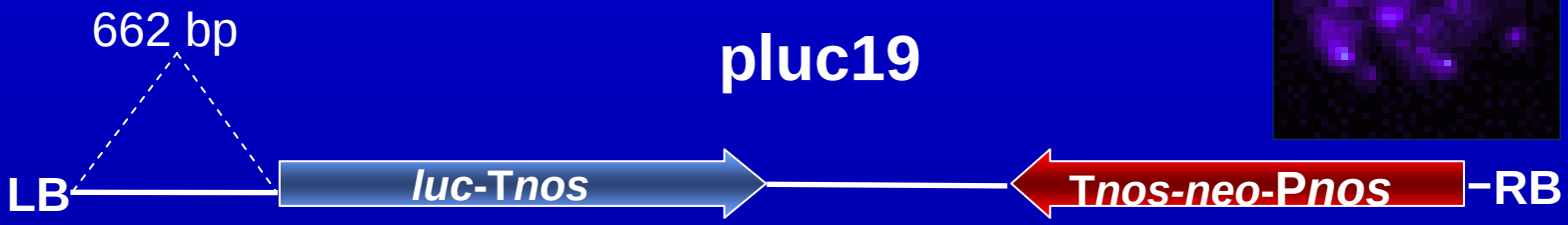
✓

I. The promoter tagging system

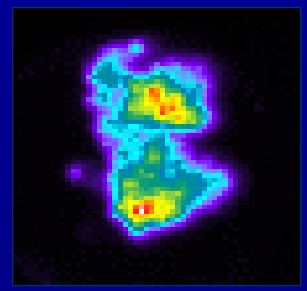
Tagging constructs

p*Ubi-luc*





pKCKUL1 T*nos-luc*$^{+INT}$



p*Ubi-luc*$^{+}$

Effect of tagging constructs on activation frequency

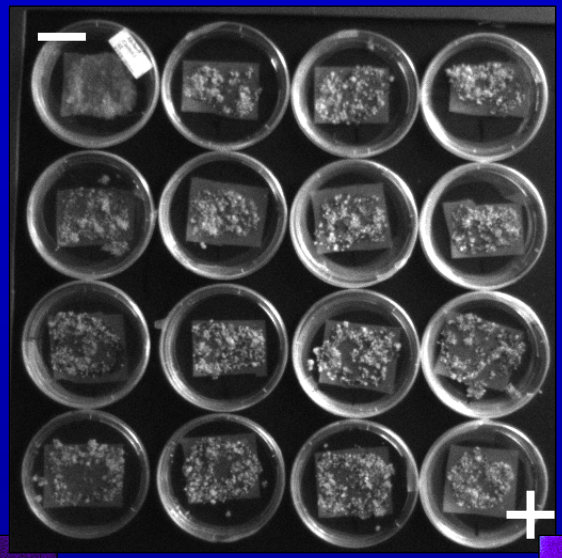
Exp.	Tagging constructs	Total # of cell colonies screened	BLA frequency* (%)
1	pluc19	1,550	0.06
	pET2	19,000	2.50
2	pET2	4,695	0.90
3	pET2	8,862	0.93
	pKC1	33,390	2.03

* Baseline luciferase activity

II. Luciferase expression patterns

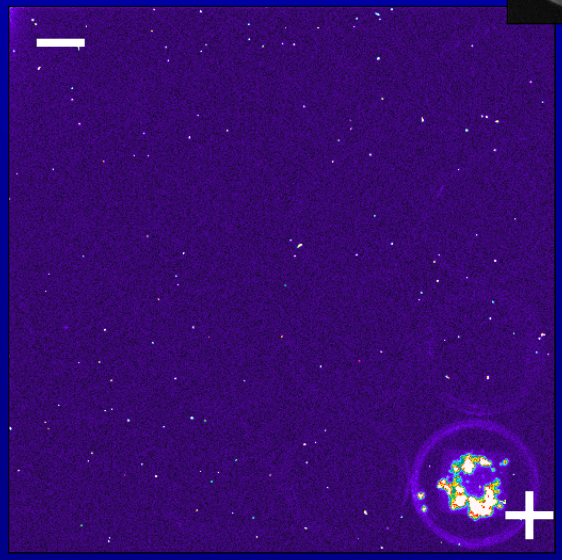
Cell colony stage

Live

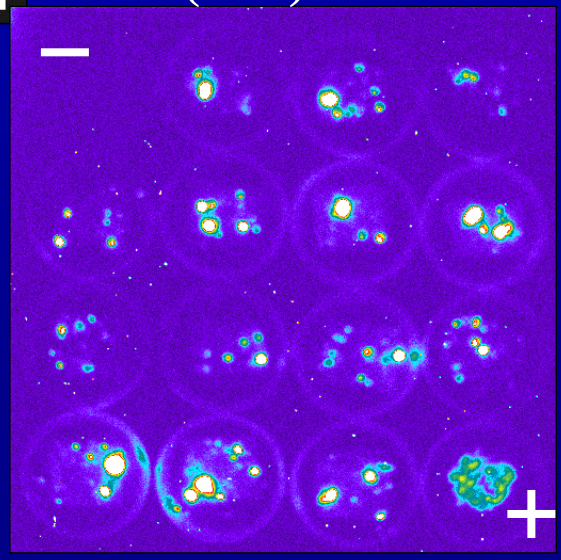


11,000 – 17,000 colonies/day

pluc19 (*luc*) 0.06%



pETKUL2 (*luc*$^{+}$) 2.5%

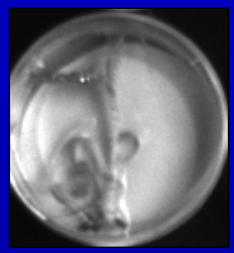


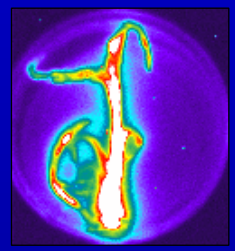
II. Luciferase expression patterns

In vitro plantlet stage

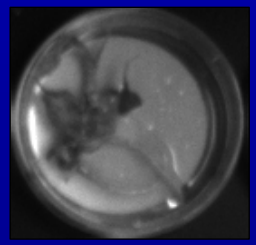
Live

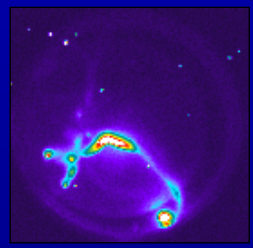
LUC

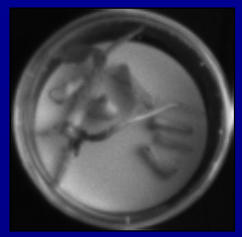


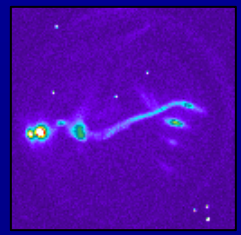


Constitutive expression



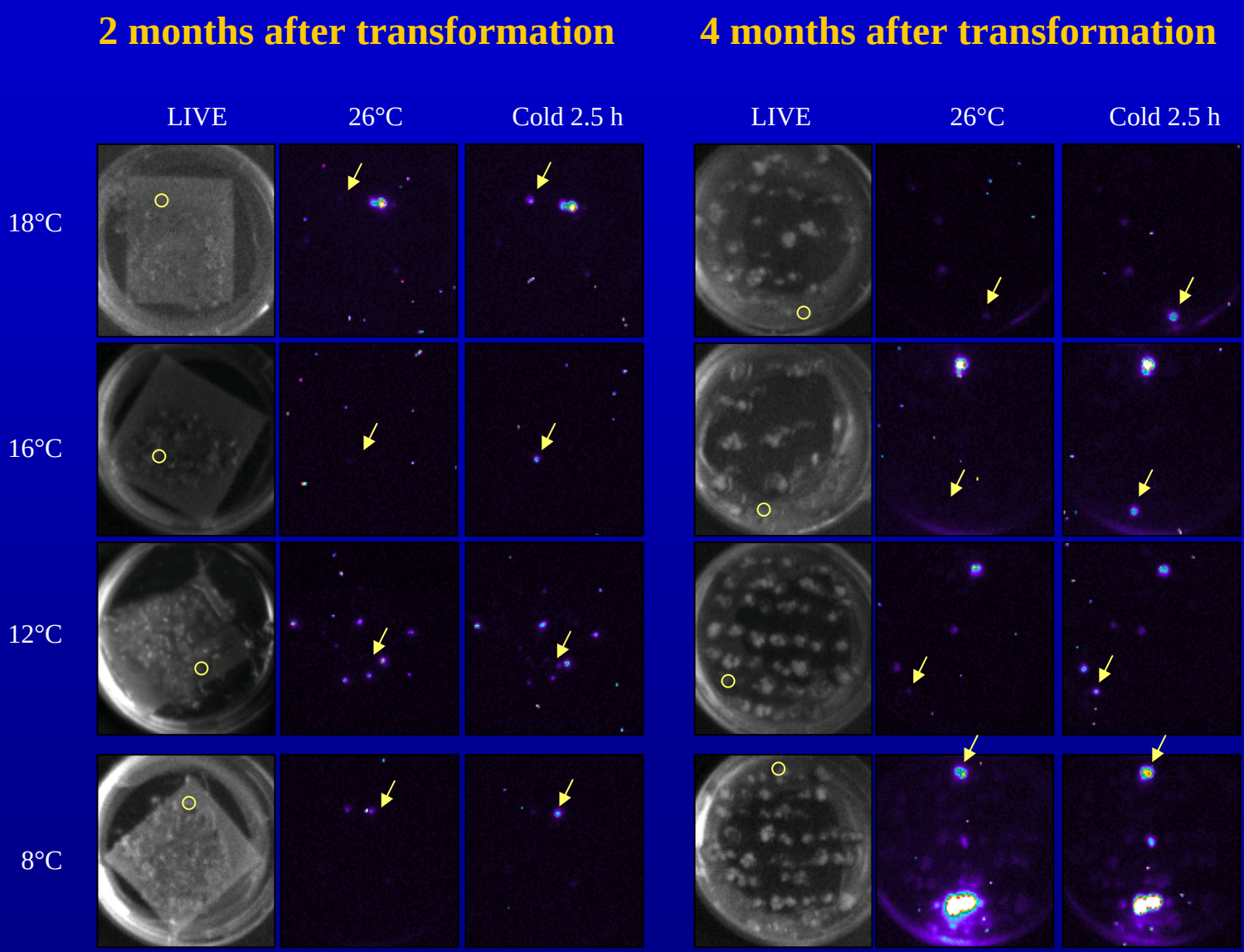






Root specific expression

III. Tagging of cold responsive promoters



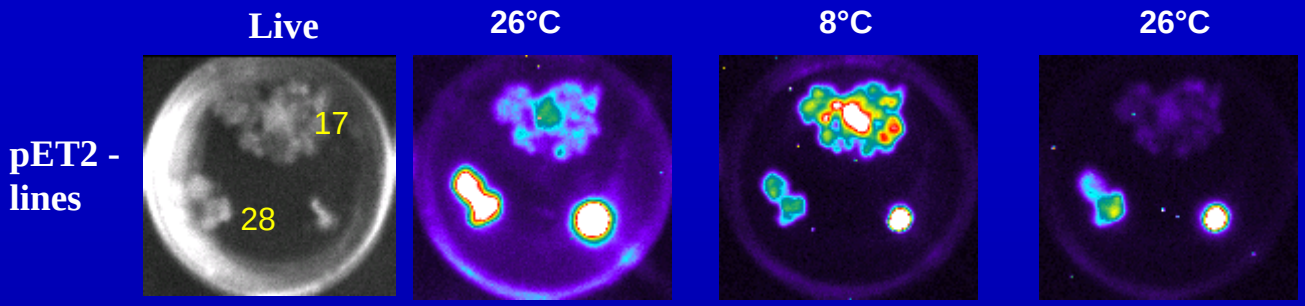
III. Tagging of cold responsive promoters

Cold responsive luciferase activity (CRLA) at 8°C of transgenic cultures (~16,000) during regeneration

	Screening for CRLA						
	1st (Colony)	2nd (Cult)	3rd (Cult)	4th (Cult)	5th (Cult)	6th (Cult)	7th (Plantlet)
Number of lines showing CRLA	106	98	42	42	42	28	26
Percentage of lines showing CRLA	0.67%	0.62%	0.26%	0.26%	0.26%	0.18%	0.16%

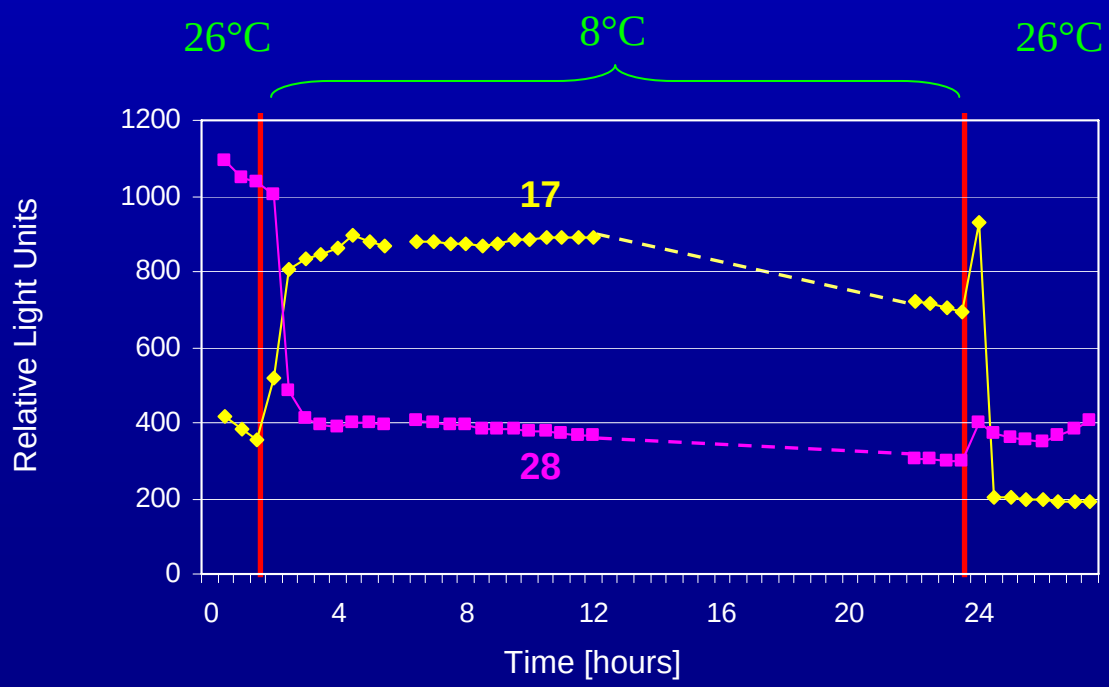
III. Tagging of cold responsive promoters

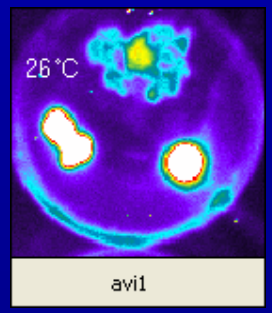
Cell culture stage



High *luc* expression

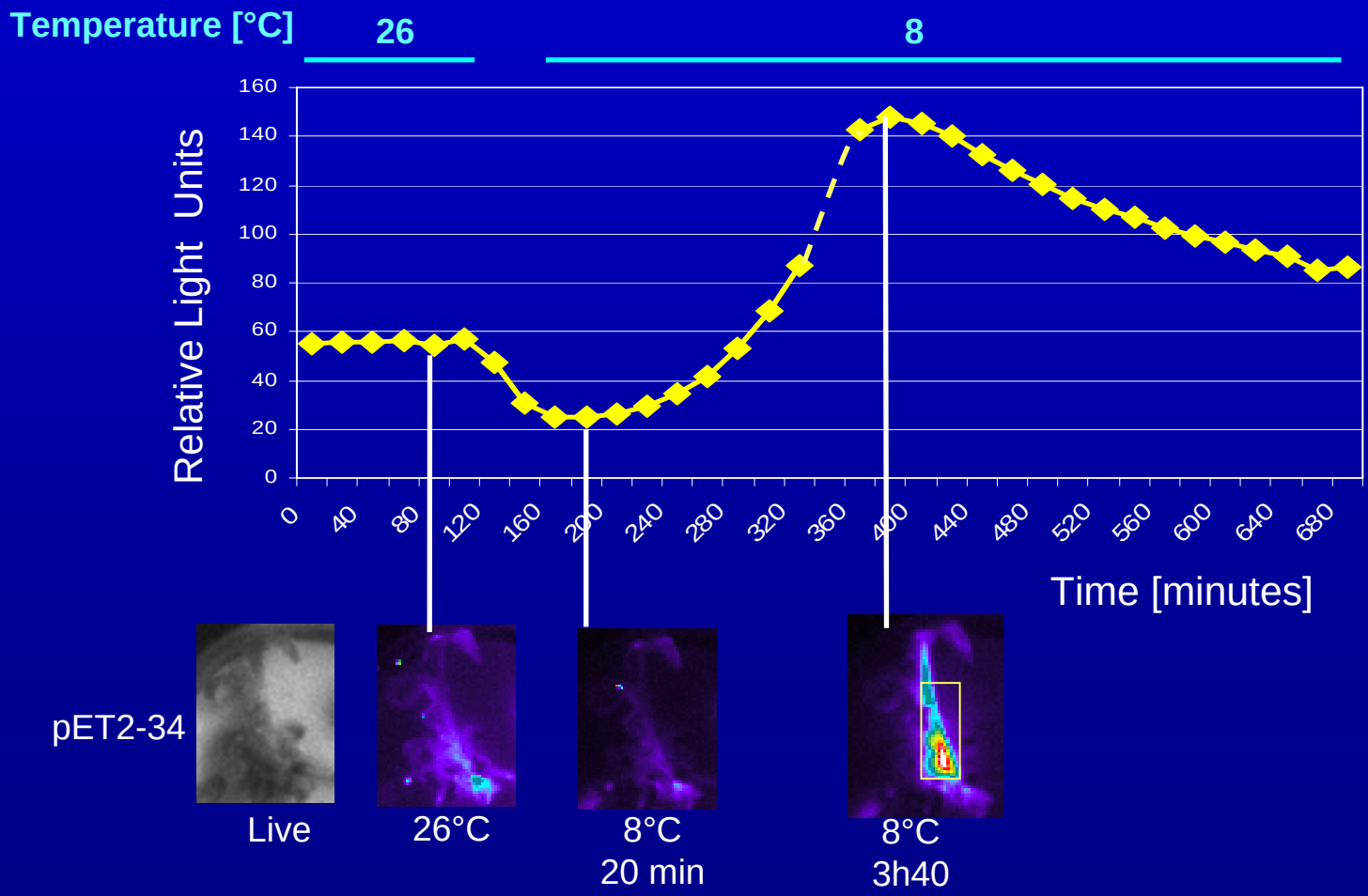
Low *luc* expression





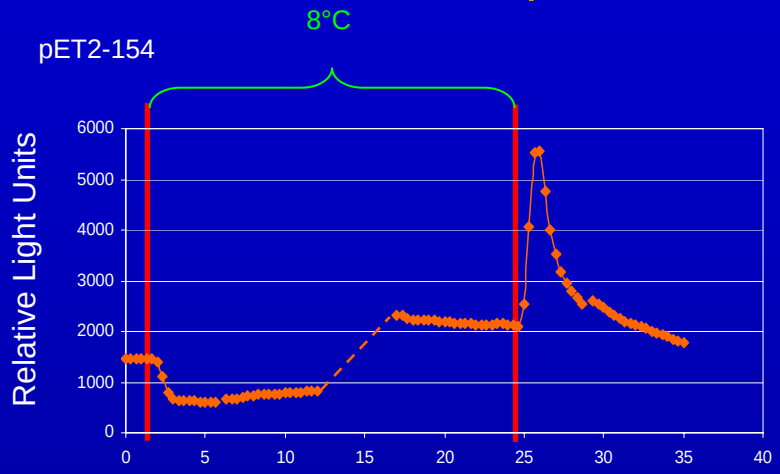
III. Tagging of cold responsive promoters

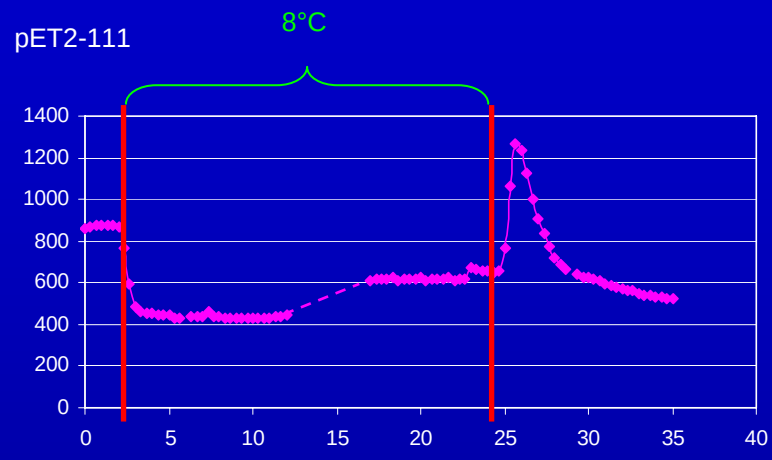
In vitro plantlet stage

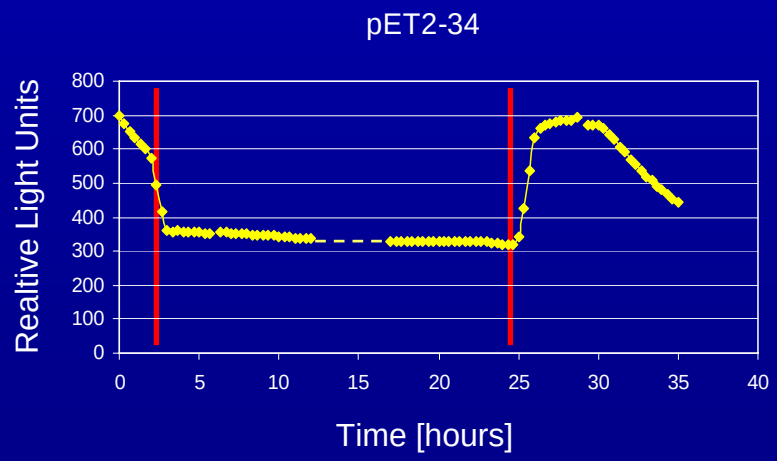


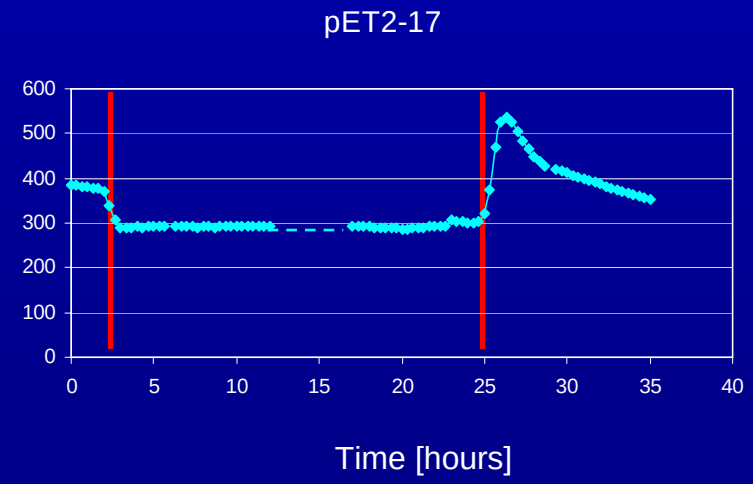
III. Tagging of cold responsive promoters

Quantification of localized LUC expression in pseudostems of *in vitro* plantlets under cold stress



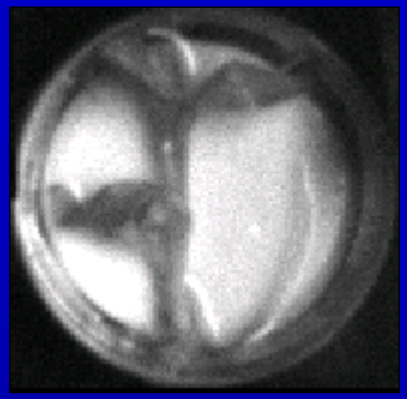


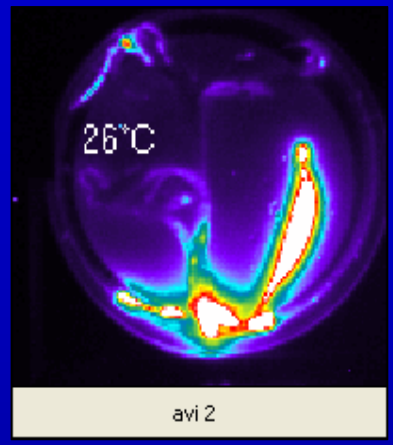




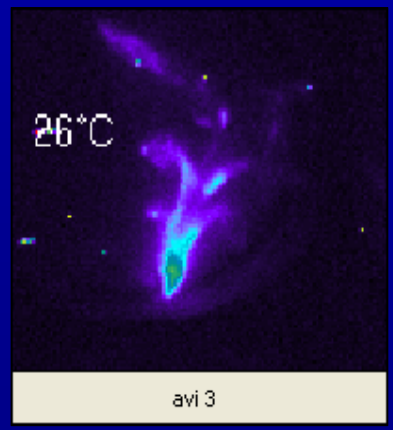
III. Tagging of cold responsive promoters

pET2-111





pET2-17



Conclusions

- Tagging constructs optimized
- Cold screening system developed
- Candidate lines obtained with enhanced and repressed CRLA patterns

Perspectives

- Confirmation of CRLA patterns in greenhouse plants
- Isolation of putative promoter sequences
- Bioinformatic analysis of promoter sequences