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Transcriptional regulatory network in potato – potato virus Y interaction signaling

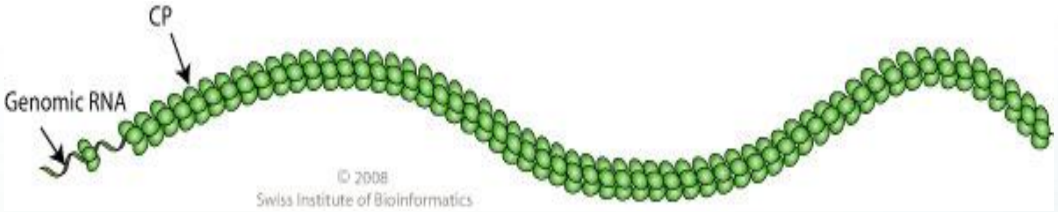
Tjaša Lukan

National Institute of Biology, Ljubljana, Slovenia
Department of Biotechnology and Systems biology

August 2016

Potato virus Y

VIRUS CLASSIFICATION	
Group	IV: (+) sense RNA viruses
Family	Potyviridae
Genus	Potyvirus
Species	Potato virus Y (PVY)



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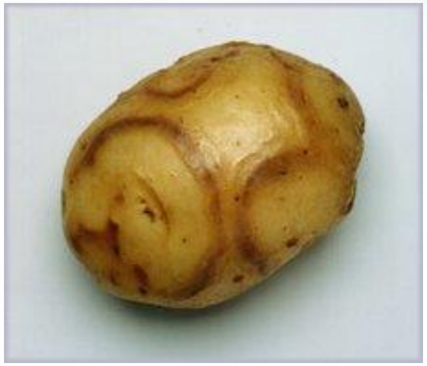
The level of crop damage is determined by:

- **the strain of PVY**
- **the host tolerance**

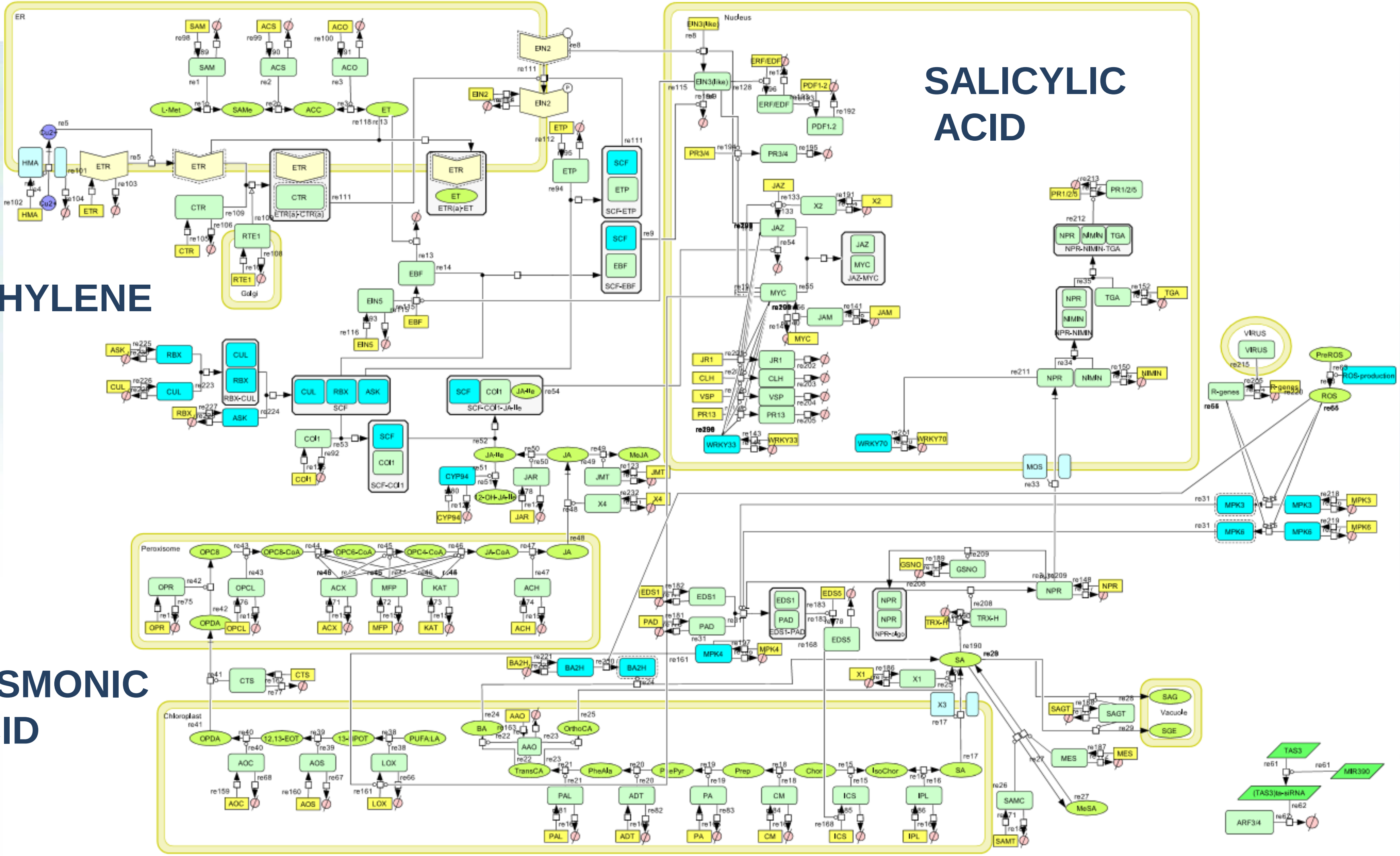
Potato tuber necrotic ringspot disease



Symptoms after PVY infection

	Host			
	Susceptible		Resistant	
	SENSITIVE	**TOLERANT**	**HYPERSENSITIVE RESPONSE**	**EXTREME RESISTANCE**
CULTIVAR	Igor	***Desiree***	***Rywal***	***PW363***
SYMPTOMS	necrosis or other symptoms	mild or no symptoms	necrotic lesions	no symptoms

Plant immune signaling



ETHYLENE

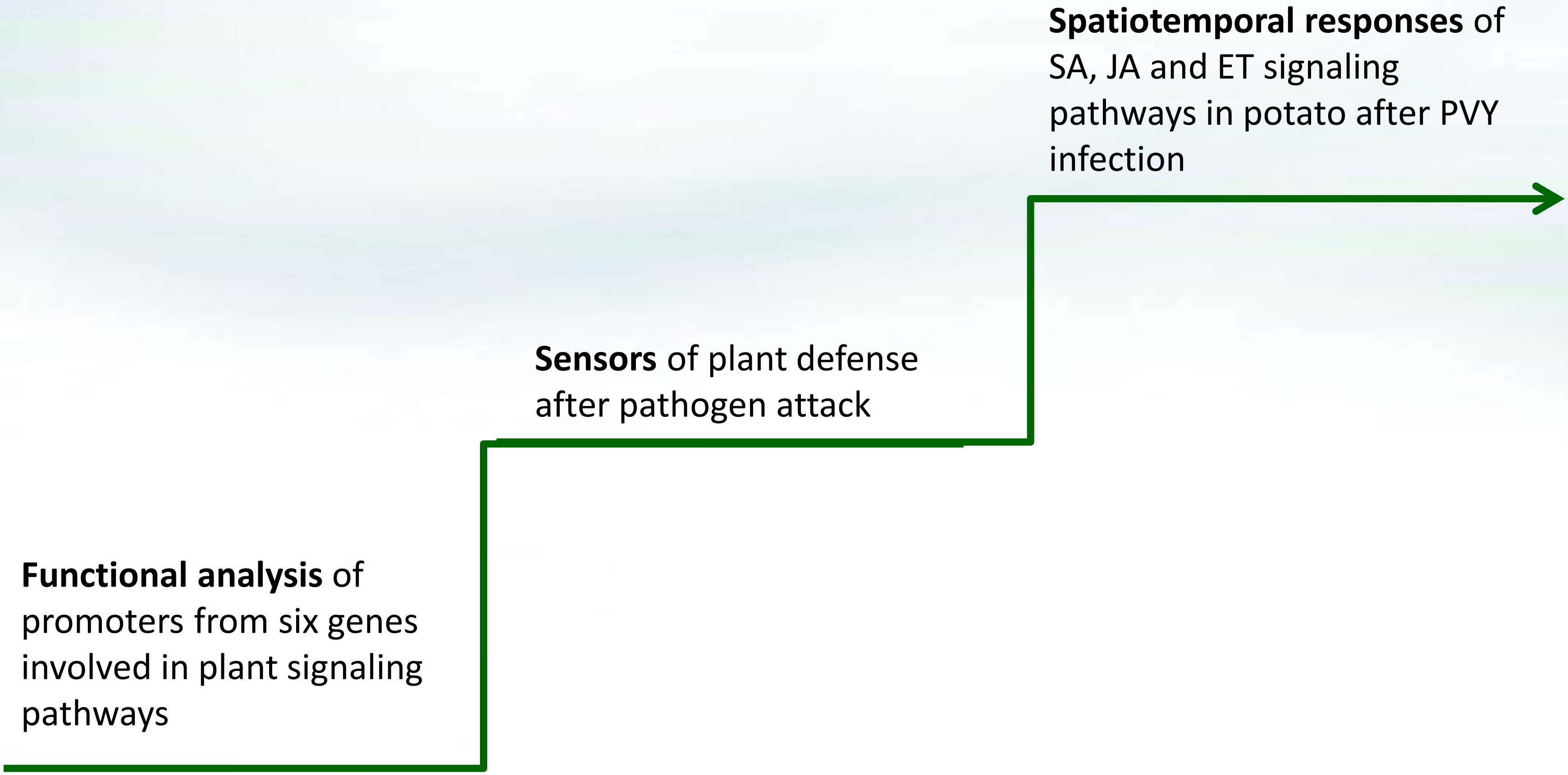
SALICYLIC ACID

JASMONIC ACID

Aim

Studies of potato-PVY interaction → different genes have different spatiotemporal gene expression behaviour

Promoters of genes from signaling pathways → sensors

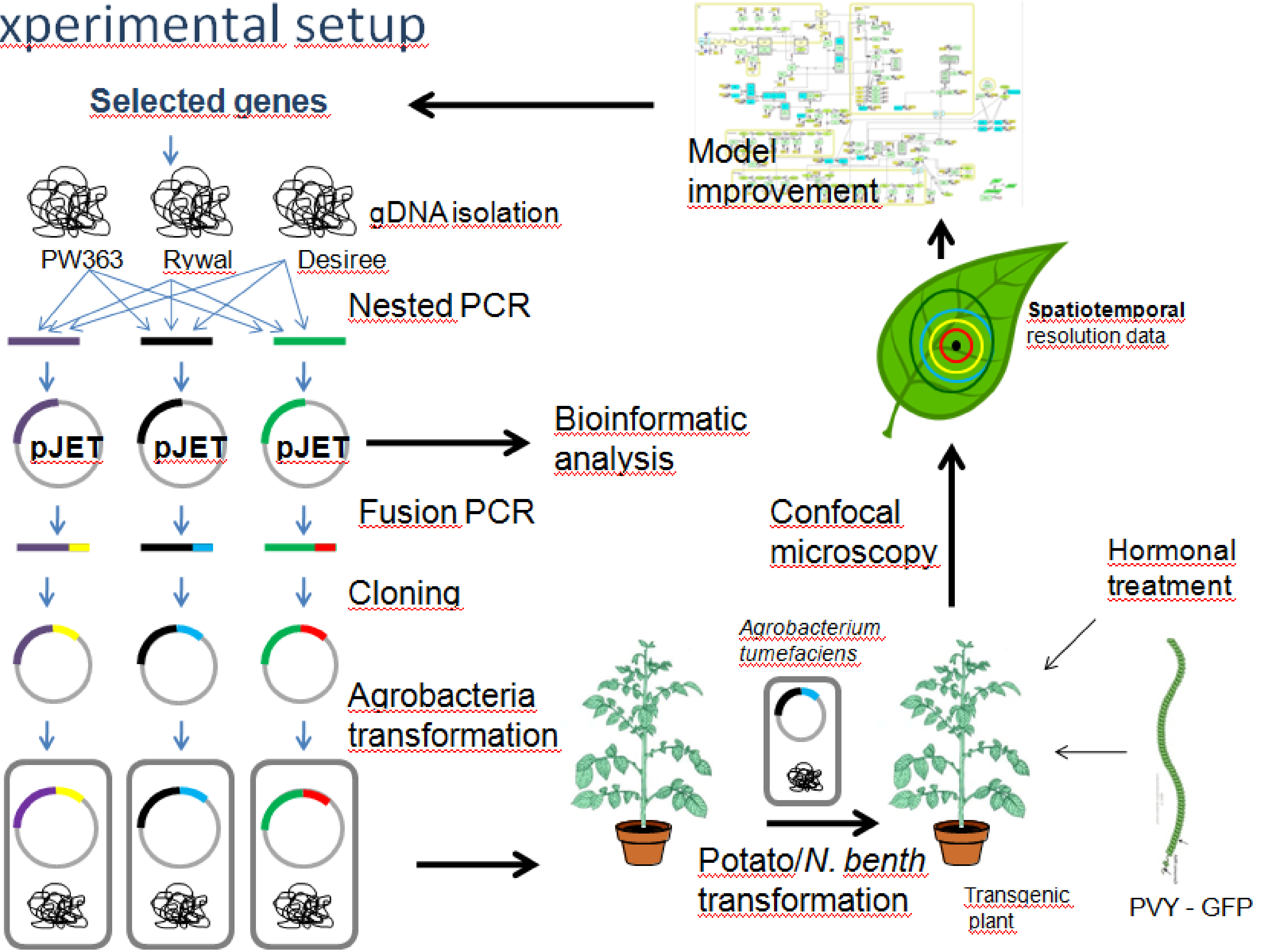


Functional analysis of promoters from six genes involved in plant signaling pathways

Sensors of plant defense after pathogen attack

Spatiotemporal responses of SA, JA and ET signaling pathways in potato after PVY infection

Experimental setup



Selected genes

gDNA isolation

PW363 Rywal Desiree

Nested PCR

pJET pJET pJET

Bioinformatic analysis

Fusion PCR

Cloning

Agrobacteria transformation

Potato/*N. benth* transformation

Agrobacterium tumefaciens

Transgenic plant

PVY - GFP

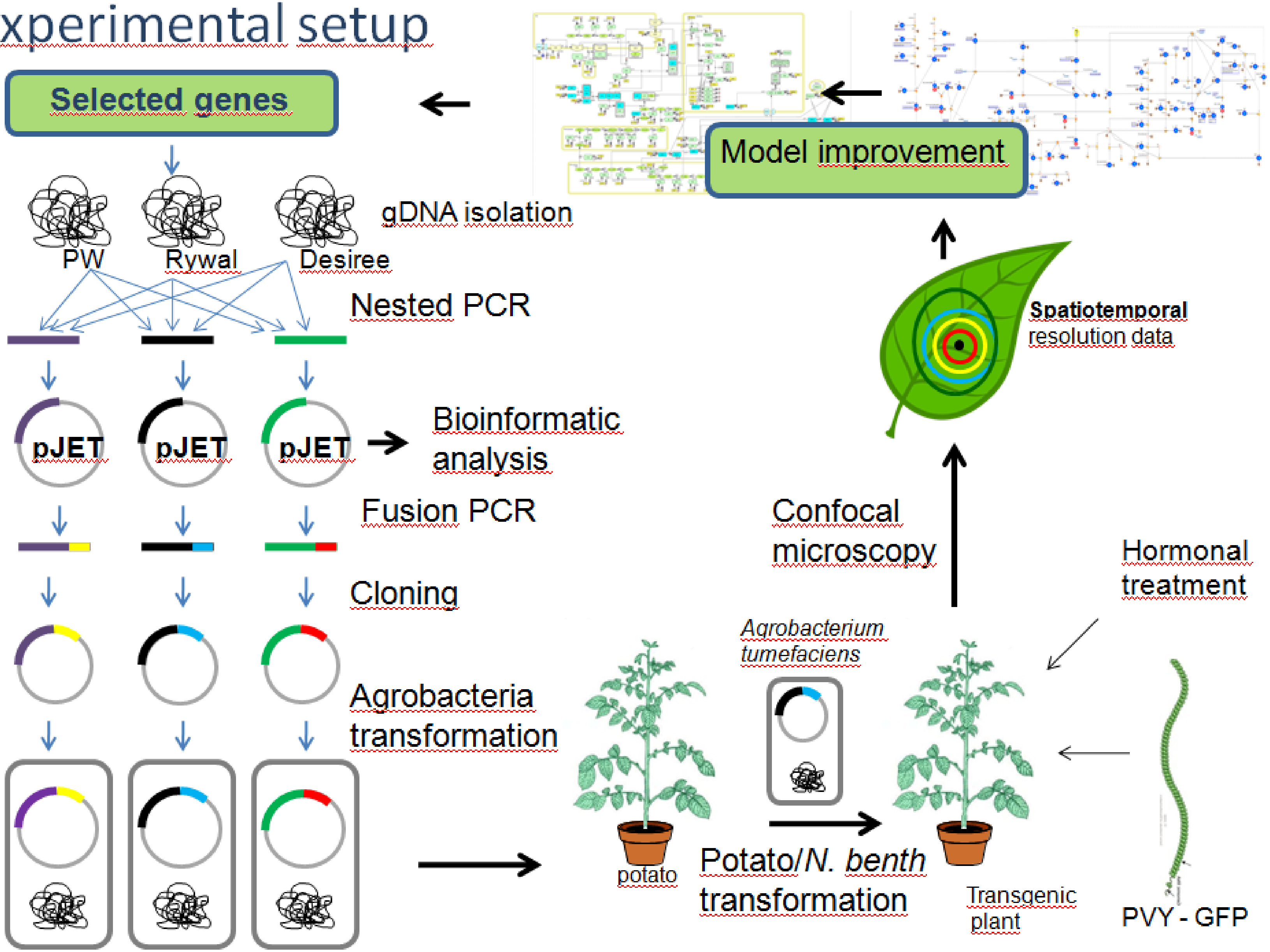
Hormonal treatment

Confocal microscopy

Spatiotemporal resolution data

Model improvement

Experimental setup



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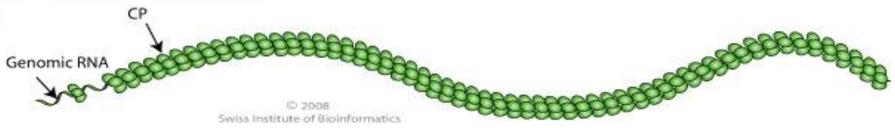
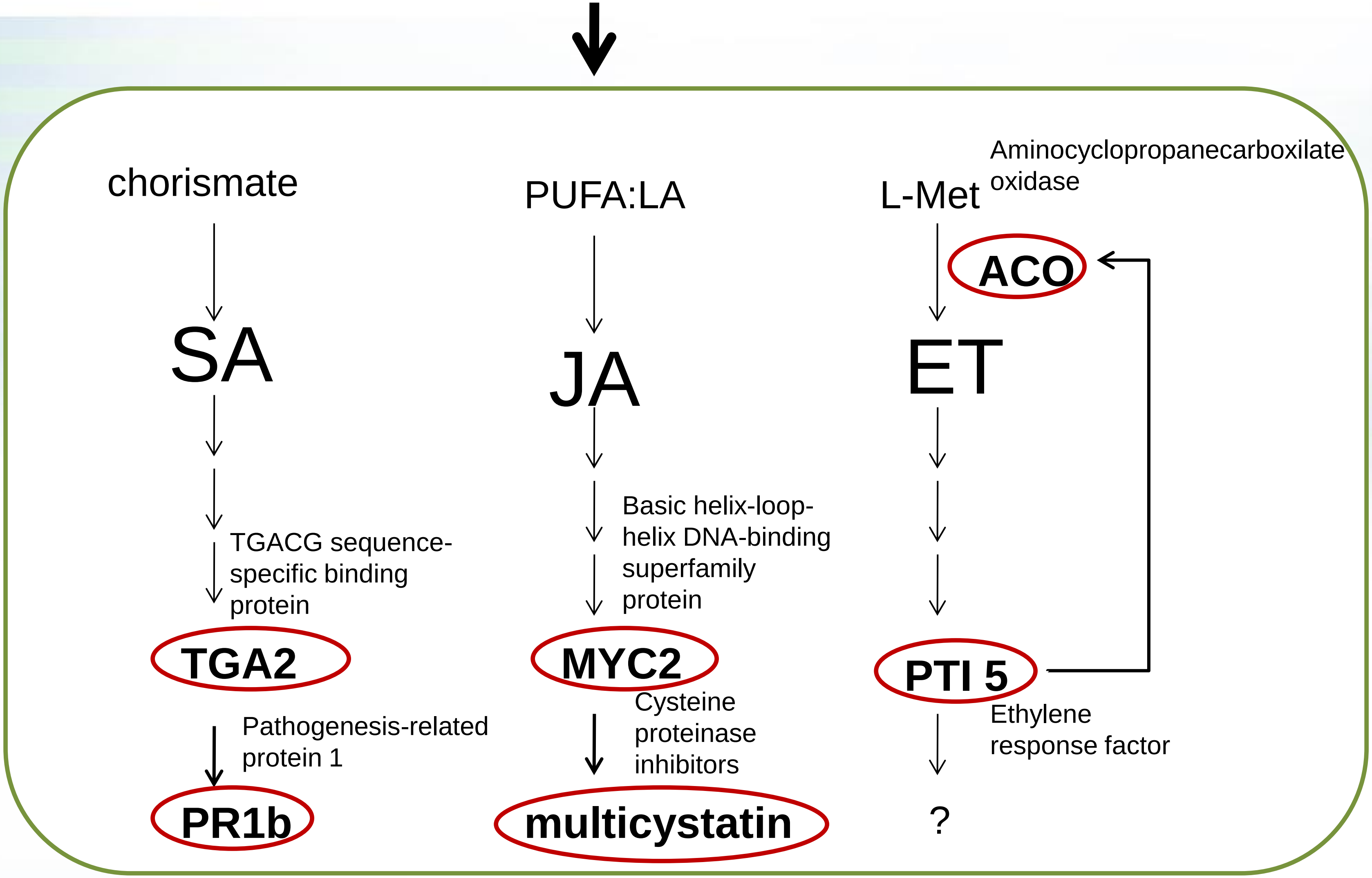




chorismate

SA

TGACG sequence-specific binding protein

TGA2

Pathogenesis-related protein 1

PR1b

PUFA:LA

JA

Basic helix-loop-helix DNA-binding superfamily protein

MYC2

Cysteine proteinase inhibitors

multicystatin

L-Met

Aminocyclopropanecarboxilate oxidase

ACO

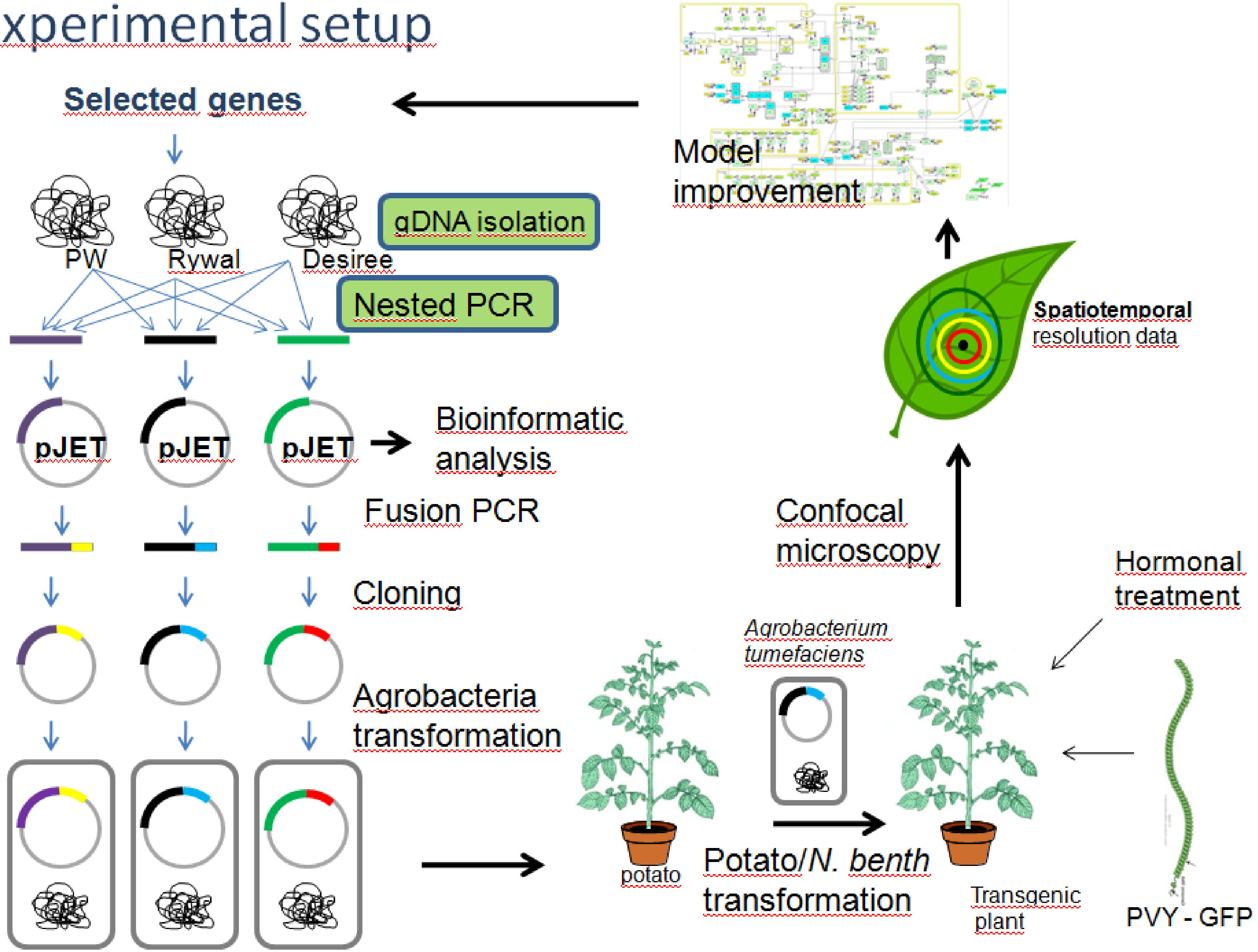
ET

PTI 5

Ethylene response factor

?

Experimental setup



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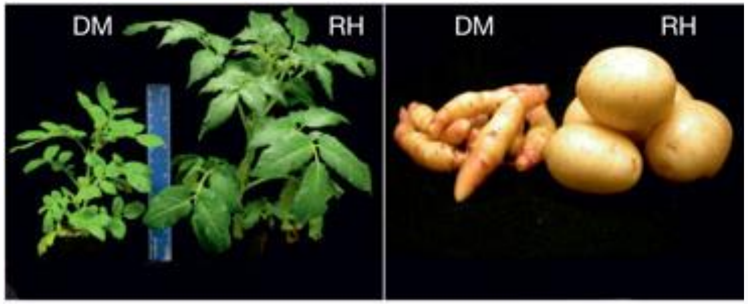
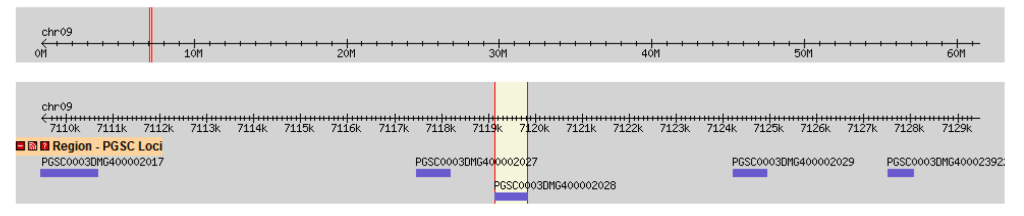
Model improvement

identification of genes
in potato genome
(Phureja - diploid monozygot
line (DM))

↓



identification of promoter sequence
(Potato genome browser)



↓

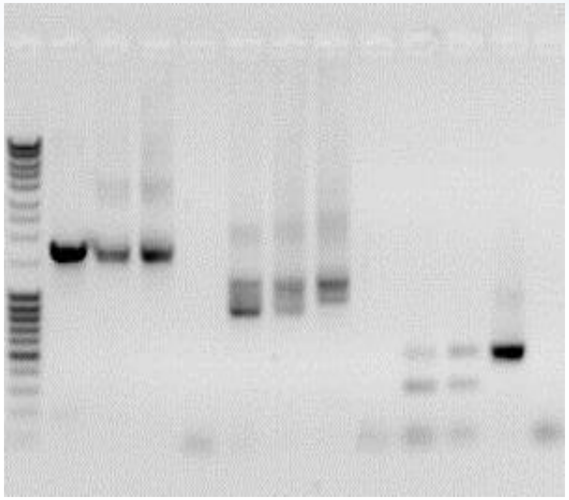
```
-->  -->          -->  -->    promoter      -->-->          ATG    <--  <--      gene
----------------------------------------------------------------------------------------
===========================
```

primer design

↓

Nested PCR
(touchdown)

← gDNA isolation
(PW, Rywal, Desiree)



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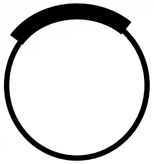
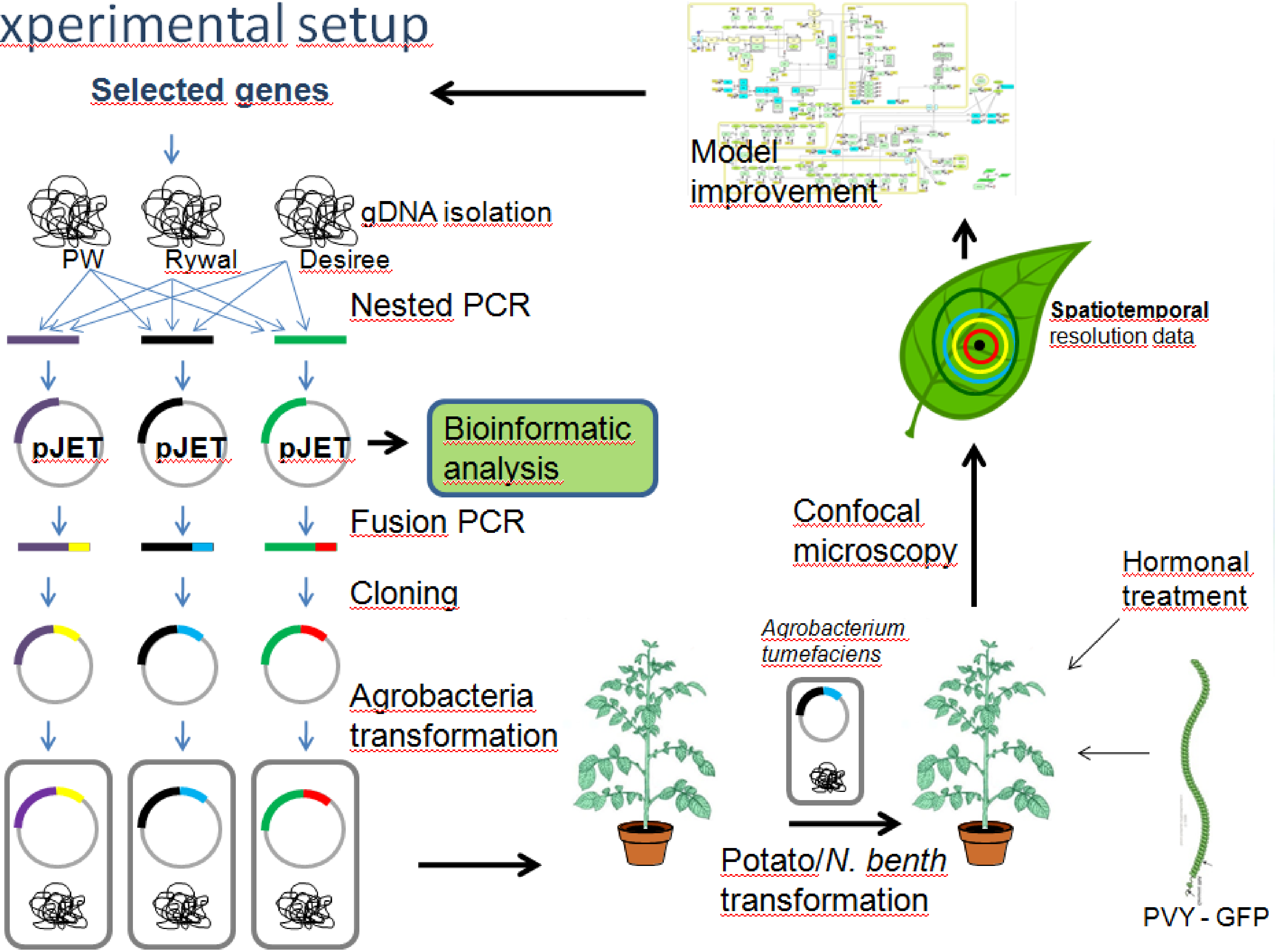
cloning into pJET



Experimental setup



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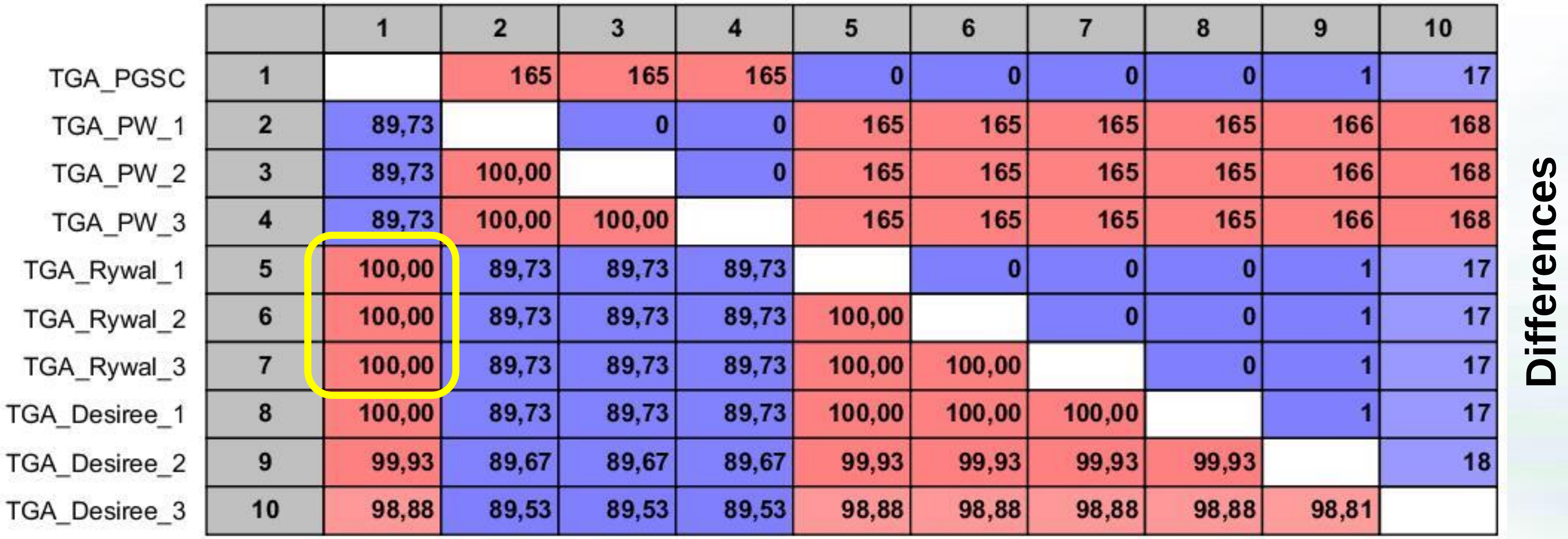
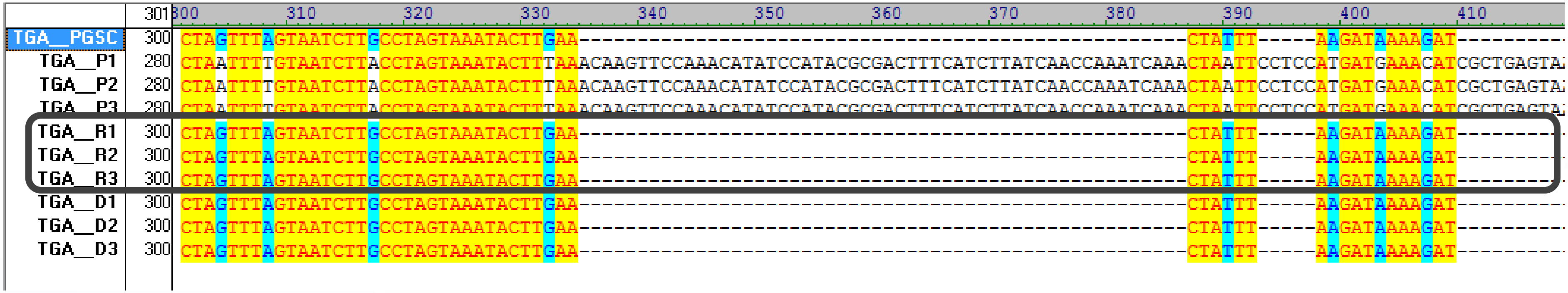
Bioinformatic analysis of TGA promoter sequences



		1	2	3	4	5	6	7	8	9	10
TGA_PGSC	1		165	165	165	0	0	0	0	1	17
TGA_PW_1	2	89,73		0	0	165	165	165	165	166	168
TGA_PW_2	3	89,73	100,00		0	165	165	165	165	166	168
TGA_PW_3	4	89,73	100,00	100,00		165	165	165	165	166	168
TGA_Rywal_1	5	100,00	89,73	89,73	89,73		0	0	0	1	17
TGA_Rywal_2	6	100,00	89,73	89,73	89,73	100,00		0	0	1	17
TGA_Rywal_3	7	100,00	89,73	89,73	89,73	100,00	100,00		0	1	17
TGA_Desiree_1	8	100,00	89,73	89,73	89,73	100,00	100,00	100,00		1	17
TGA_Desiree_2	9	99,93	89,67	89,67	89,67	99,93	99,93	99,93	99,93		18
TGA_Desiree_3	10	98,88	89,53	89,53	89,53	98,88	98,88	98,88	98,88	98,81	

Differences

Percent identity



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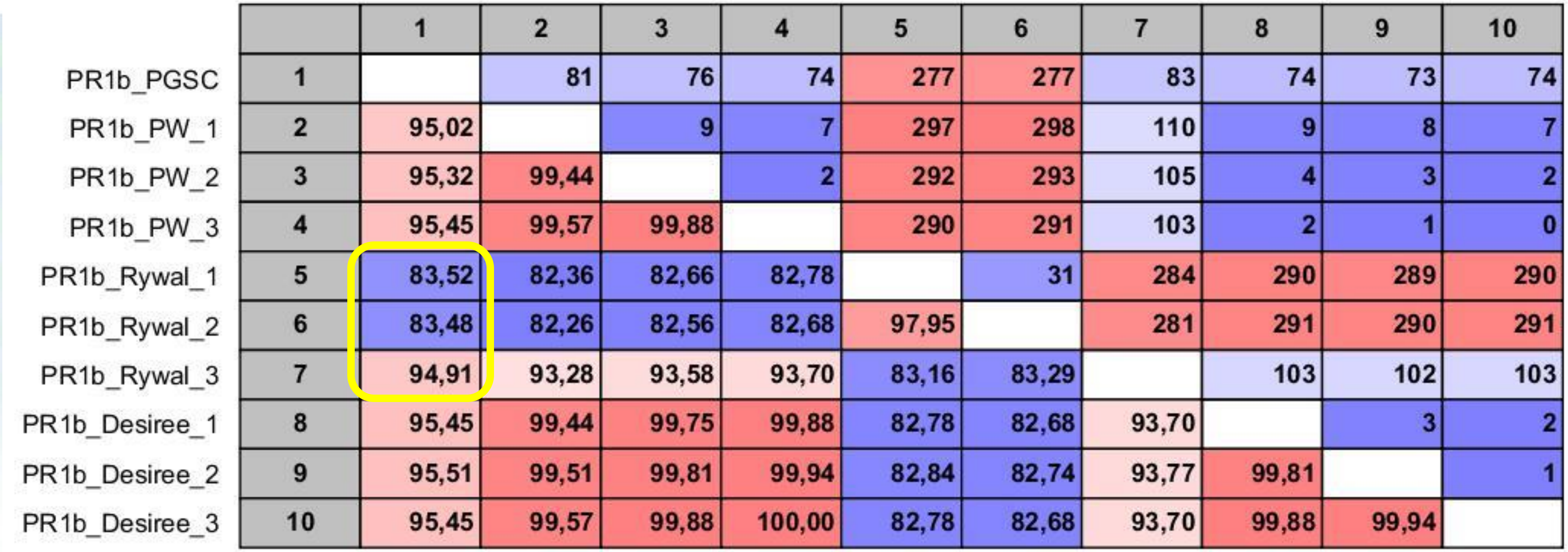
            301 300       310       320       330       340       350       360       370       380       390       400       410
TGA__PGSC   300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__P1     280 CTAATTTTGTAATCTTACCTAGTAAATACTTTAAACAAGTTCCAAACATATCCATACGCGACTTTCATCTTATCAACCAAATCAAACTAATTCCTCCATGATGAAACATCGCTGAGTA
TGA__P2     280 CTAATTTTGTAATCTTACCTAGTAAATACTTTAAACAAGTTCCAAACATATCCATACGCGACTTTCATCTTATCAACCAAATCAAACTAATTCCTCCATGATGAAACATCGCTGAGTA
TGA__P3     280 CTAATTTTGTAATCTTACCTAGTAAATACTTTAAACAAGTTCCAAACATATCCATACGCGACTTTCATCTTATCAACCAAATCAAACTAATTCCTCCATGATGAAACATCGCTGAGTA
TGA__R1     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__R2     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__R3     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__D1     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__D2     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
TGA__D3     300 CTAGTTTAGTAATCTTGCCTAGTAAATACTTGAA----------------------------------------------------CTATTT-----AAGATAAAAGAT---------
```

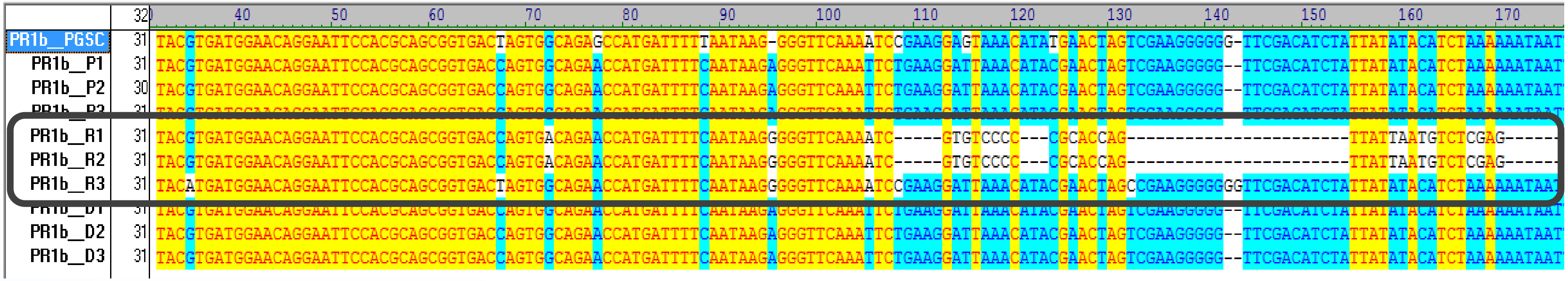
Bioinformatic analysis of PR1b promoter sequences



		1	2	3	4	5	6	7	8	9	10
PR1b_PGSC	1		81	76	74	277	277	83	74	73	74
PR1b_PW_1	2	95,02		9	7	297	298	110	9	8	7
PR1b_PW_2	3	95,32	99,44		2	292	293	105	4	3	2
PR1b_PW_3	4	95,45	99,57	99,88		290	291	103	2	1	0
PR1b_Rywal_1	5	83,52	82,36	82,66	82,78		31	284	290	289	290
PR1b_Rywal_2	6	83,48	82,26	82,56	82,68	97,95		281	291	290	291
PR1b_Rywal_3	7	94,91	93,28	93,58	93,70	83,16	83,29		103	102	103
PR1b_Desiree_1	8	95,45	99,44	99,75	99,88	82,78	82,68	93,70		3	2
PR1b_Desiree_2	9	95,51	99,51	99,81	99,94	82,84	82,74	93,77	99,81		1
PR1b_Desiree_3	10	95,45	99,57	99,88	100,00	82,78	82,68	93,70	99,88	99,94	

Differences

Percent identity



```
           32|   40        50        60        70        80        90        100       110       120       130       140       150       160       170
PR1b__PGSC 31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACTAGTGGCAGAGCCATGATTTTTAATAAG-GGGTTCAAAATCCGAAGGAGTAAACATATGAACTAGTCGAAGGGGG-TTCGACATCTATTATATACATCTAAAAAATAAT
PR1b__P1   31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGGCAGAACCATGATTTTCAATAAGAGGGTTCAAATTCTGAAGGATTAAACATACGAACTAGTCGAAGGGGG--TTCGACATCTATTATATACATCTAAAAAATAAT
PR1b__P2   30 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGGCAGAACCATGATTTTCAATAAGAGGGTTCAAATTCTGAAGGATTAAACATACGAACTAGTCGAAGGGGG--TTCGACATCTATTATATACATCTAAAAAATAAT
PR1b__P3   31 [illegible]
PR1b__R1   31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGACAGAACCATGATTTTCAATAAGGGGGTTCAAAATC-----GTGTCCCC---CGCACCAG----------------------TTATTAATGTCTCGAG------
PR1b__R2   31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGACAGAACCATGATTTTCAATAAGGGGGTTCAAAATC-----GTGTCCCC---CGCACCAG----------------------TTATTAATGTCTCGAG------
PR1b__R3   31 TACATGATGGAACAGGAATTCCACGCAGCGGTGACTAGTGGCAGAACCATGATTTTCAATAAGGGGGTTCAAAATCCGAAGGATTAAACATACGAACTAGCCGAAGGGGGGTTCGACATCTATTATATACATCTAAAAAATAA
PR1b__D1   31 [illegible]
PR1b__D2   31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGGCAGAACCATGATTTTCAATAAGAGGGTTCAAATTCTGAAGGATTAAACATACGAACTAGTCGAAGGGGG--TTCGACATCTATTATATACATCTAAAAAATAAT
PR1b__D3   31 TACGTGATGGAACAGGAATTCCACGCAGCGGTGACCAGTGGCAGAACCATGATTTTCAATAAGAGGGTTCAAATTCTGAAGGATTAAACATACGAACTAGTCGAAGGGGG--TTCGACATCTATTATATACATCTAAAAAATAAT
```

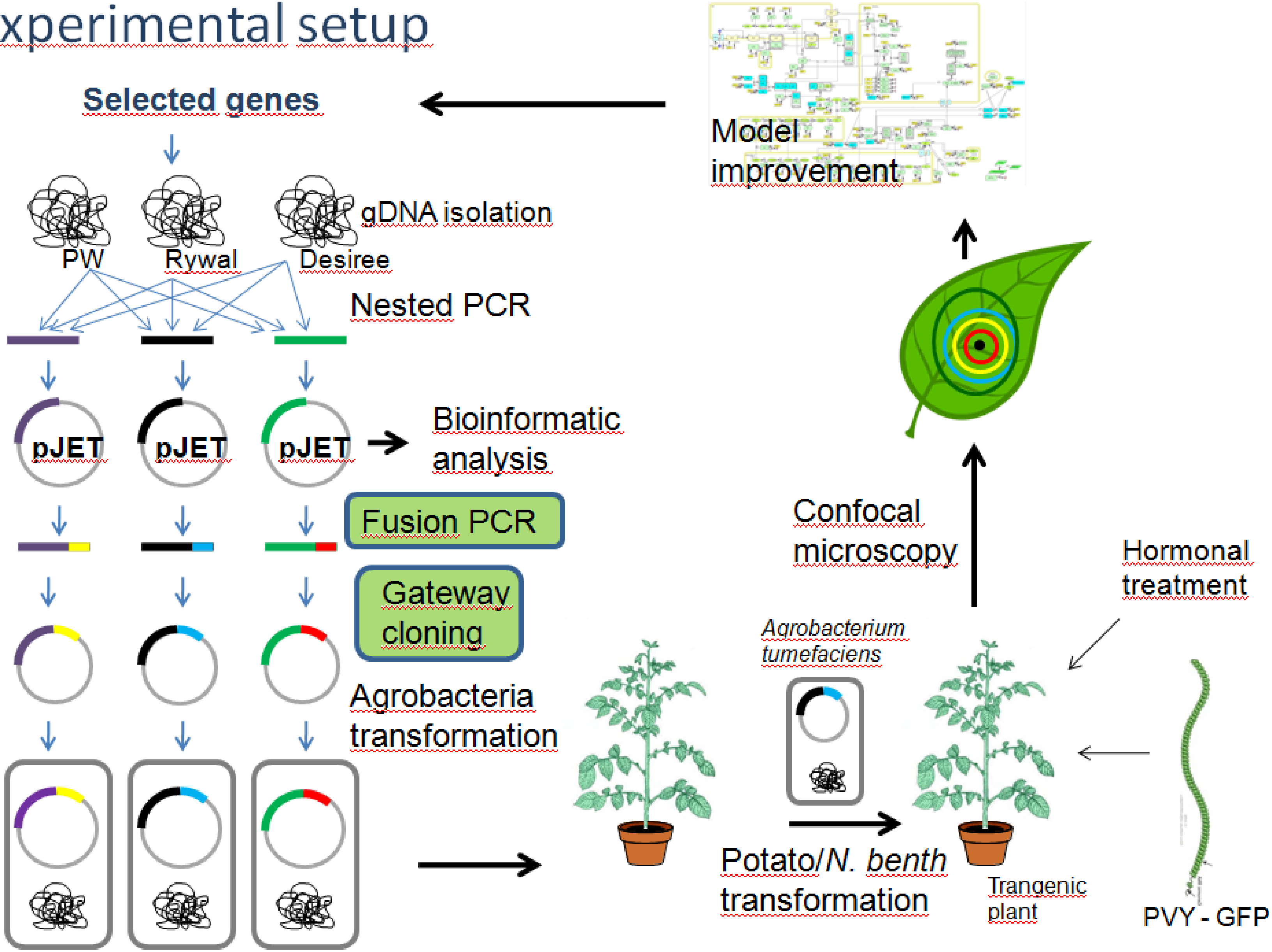
PR1b promoter specificities: ***TGA1a*** from SA-signaling pathway

Transcription factor	Regulatory element	PR1b_P1	PR1b_P2	PR1b_P3	PR1b_R1	PR1b_R2	PR1b_R3	PR1b_D1	PR1b_D2	PR1b_D3
BPC1	**AGAAAg**	6 (+)								
NAC6	**TTACGt**	30 (-)	29 (-)	30 (-)	30 (-)	30 (-)		30 (-)	30 (-)	30 (-)
OCSBF-1	**ACGTG**	32 (-)	31 (-)	32 (-)	32 (-)	32 (-)		32 (-)	32 (-)	32 (-)
C1	**cgAACTAgccg**						123 (+)			
ARR10	**AGATCcg**				137 (+)	137 (+)				
HSFA1E	**cCGTCG**	202 (+)	201 (+)	202 (+)			204 (+)	202 (+)	202 (+)	202 (+)
HSFA2	**tTTTGG**	214 (-)	213 (-)	214 (-)				214 (-)	214 (-)	214 (-)
At2g41690	**gTTCGG**						216 (-)			
TGA1a	**gcgGTGACctatttgaaaactt**						255 (+)			
AZF3	**aaattATACTt**	282 (-)	281 (-)	282 (-)			285 (-)	282 (-)	282 (-)	282 (-)
TGA1A	**tTCGTCa**	320 (+)	319 (+)	320 (+)			323 (+)	320 (+)	320 (+)	320 (+)
HSFA4A	**aCTATT**	406 (+)	405 (+)	406 (+)	296 (+)	296 (+)	409 (+)	406 (+)	406 (+)	406 (+)
RIN	**ccTTAAAaacg**	434 (-)	433 (-)	434 (-)	324 (-)	324 (-)	437 (-)	434 (-)	434 (-)	434 (-)
GT-1	**aGTTAAtatcta**					381 (-)	494 (-)			
BPC1	**AGAAAg**	504 (+)	503 (+)	504 (+)	394 (+)	394 (+)	507 (+)	504 (+)	504 (+)	504 (+)
WRKY	**TGACTaactaa**				477 (-)					
At3g51080	**ttTTTCC**					409 (-)	522 (-)			
CBNAC	**tTGCTT**	577 (+)	576 (+)	577 (+)		462 (+)	575 (+)	577 (+)	577 (+)	577 (+)
Myb-15	**TAACAa**	591 (+)	590 (+)	591 (+)		476 (+)	589 (+)	591 (+)	591 (+)	591 (+)
MADS-A	**aatcctAAAATgaaaa**				636 (+)	631 (+)	744 (+)			
ERF13	**aggGCGGCtc**	643 (-)	642 (-)	643 (-)		528 (-)	641 (-)	643 (-)	643 (-)	643 (-)
DRF1.3	**aggGCGGCtc**	643 (-)	642 (-)	643 (-)		528 (-)	641 (-)	643 (-)	643 (-)	643 (-)
ESR1	**agggCGGCTc**	643 (-)	642 (-)	643 (-)		528 (-)	641 (-)	643 (-)	643 (-)	643 (-)
At3g63350	**gGGCGG**	644 (-)	643 (-)	644 (-)		529 (-)	642 (-)	644 (-)	644 (-)	644 (-)
ERF1	**GGCGGctc**	645 (+)	644 (+)	645 (+)		530 (+)	643 (+)	645 (+)	645 (+)	645 (+)
At3g51080	**ttTTTCC**	679 (-)	678 (-)	679 (-)		562 (-)	675 (-)	679 (-)	679 (-)	679 (-)
ASR-1	**ACCCA**	840 (+)	839 (+)	840 (+)	728 (+)	723 (+)		840 (+)	840 (+)	840 (+)
PEND	**tatAAGAAtt**	853 (-)	852 (-)	853 (-)				853 (-)	853 (-)	853 (-)
HSFA2	**tTTTGG**	887 (-)	886 (-)	887 (-)	776 (-)	771 (-)	887 (-)	887 (-)	887 (-)	887 (-)
CBNAC	**tTGCTT**						914 (+)			
OCSBF-1	**CACGT**	931 (+)	930 (+)	931 (+)	820 (+)	815 (+)		931 (+)	931 (+)	931 (+)
At3g51080	**GGAAAaa**	980 (+)	979 (+)	980 (+)	870 (+)	865 (+)		980 (+)	980 (+)	980 (+)
TGA1A	**tACATCa**	1052 (+)	1051 (+)	1052 (+)				1052 (+)	1052 (+)	1052 (+)
ASR-1	**ACCCA**	1104 (+)	1103 (+)	1104 (+)	994 (+)	990 (+)	1106 (+)	1104 (+)	1104 (+)	1104 (+)
HSFA2	**CCAAAa**	1106 (+)	1105 (+)	1106 (+)	996 (+)	992 (+)	1108 (+)	1106 (+)	1106 (+)	1106 (+)
HSFA4A	**aCTATT**				1085 (+)	1076 (+)	1199 (+)			
HBP-1a	**cacaCGACAt**	1225 (+)	1224 (+)	1225 (+)	1118 (+)	1109 (+)	1232 (+)	1225 (+)	1225 (+)	1225 (+)
AGL20	**tttaaATTTAac**	1294 (-)	1293 (-)	1294 (-)	1184 (-)	1175 (-)	1298 (-)	1294 (-)	1294 (-)	1294 (-)
Myb-15	**TAACAa**	1302 (+)	1301 (+)	1302 (+)	1192 (+)	1183 (+)	1306 (+)	1302 (+)	1302 (+)	1302 (+)
HSFA2	**CCAAAa**				1294 (+)	1285 (+)	1376 (+)			
HSFA2	**CCAAAa**						1408 (+)			
SQUA	**acAAAAAtgg**	1401 (-)	1400 (-)	1401 (-)				1401 (-)	1401 (-)	1401 (-)
HSFA4A	**aCTATT**	1431 (+)	1430 (+)	1431 (+)	1323 (+)	1314 (+)	1437 (+)	1431 (+)	1431 (+)	1431 (+)
CBNAC	**AAGCAa**	1613 (-)	1612 (-)	1613 (-)	1505 (-)	1496 (-)	1616 (-)	1613 (-)	1613 (-)	1613 (-)

TCA-element involved in SA-signaling pathway present in PR1b promoter sequences from Rywal

Regulatory elements	PR1b_P1	PR1b_P2	PR1b_P3	PR1b_R1	PR1b_R2	PR1b_R3	PR1b_D1	PR1b_D2	PR1b_D3
5UTR Py-rich stretch	1	1	1				1	1	1
3-AF1 binding site						1			
AAGAA-motif	2	2	2	1	1	1	2	2	2
ABRE	1	1	1	1	1		1	1	1
ACE	1	1	1	1	1	1	1	1	1
ARE	1	1	1	1			1	1	1
AT-rich element			1	1	1	1	1	1	1
ATCT-motif	1	1	1	1	1	1	1	1	1
AT1_motif	1	1	1	1	1		1	1	1
Box 4	5	5	5	4	5	3	5	5	5
Box I	2	2	2	3	3	3	2	2	2
CAAT-box	28	28	28	26	28	27	28	28	28
CAT-box	1	1	1			1	1	1	1
CGTCA-motif	1	1	1			1	1	1	1
CTAG-motif						1			
G-Box	1	1	1	1	1		1	1	1
G-box	3	3	3	3	3	1	3	3	3
GCC box	1	1	1		1	1	1	1	1
GC-motif				1	1				
GT1-motif	3	3	3	1	2		4	3	3
HSE	3	3	3	3	3	3	4	3	3
I-box	1	1	1	1	2	1	1	1	1
LTR	1	1	1	1	1		1	1	1
MBS	1	1	1		2	1	1	1	1
MRE						1			
Skn-1_motif	1	1	1	1	1	2	1	1	1
Sp1	3	3	3	2	3	5	3	3	3
TATA-box	79	79	79	66	61	77	78	76	79
TC-rich repeats	3	3	3	3	3	3	3	3	3
TCA-element				1	1	1			
TGACG-motif	1	1	1			1	1	1	1
Unnamed__8	1	1	1				1	1	1
circadian				1	1	1			

Experimental setup



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Fusion PCR

Gateway cloning

Agrobacteria transformation

Potato/*N. benth* transformation

Agrobacterium tumefaciens

Trangenic plant

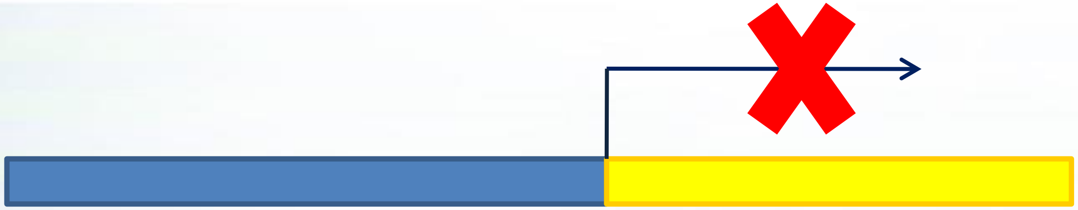
PVY - GFP

Hormonal treatment

Confocal microscopy

Model improvement

No expression or low expression



NATIVE PROMOTER

FLUORESCENT PROTEIN

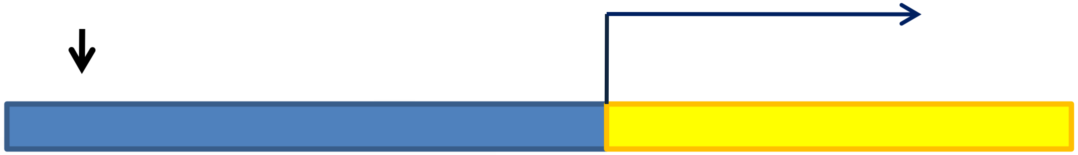
PVY infection

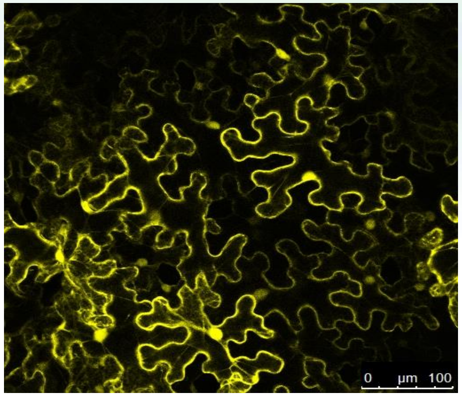
↓

signaling pathways activation

↓

Gene expression

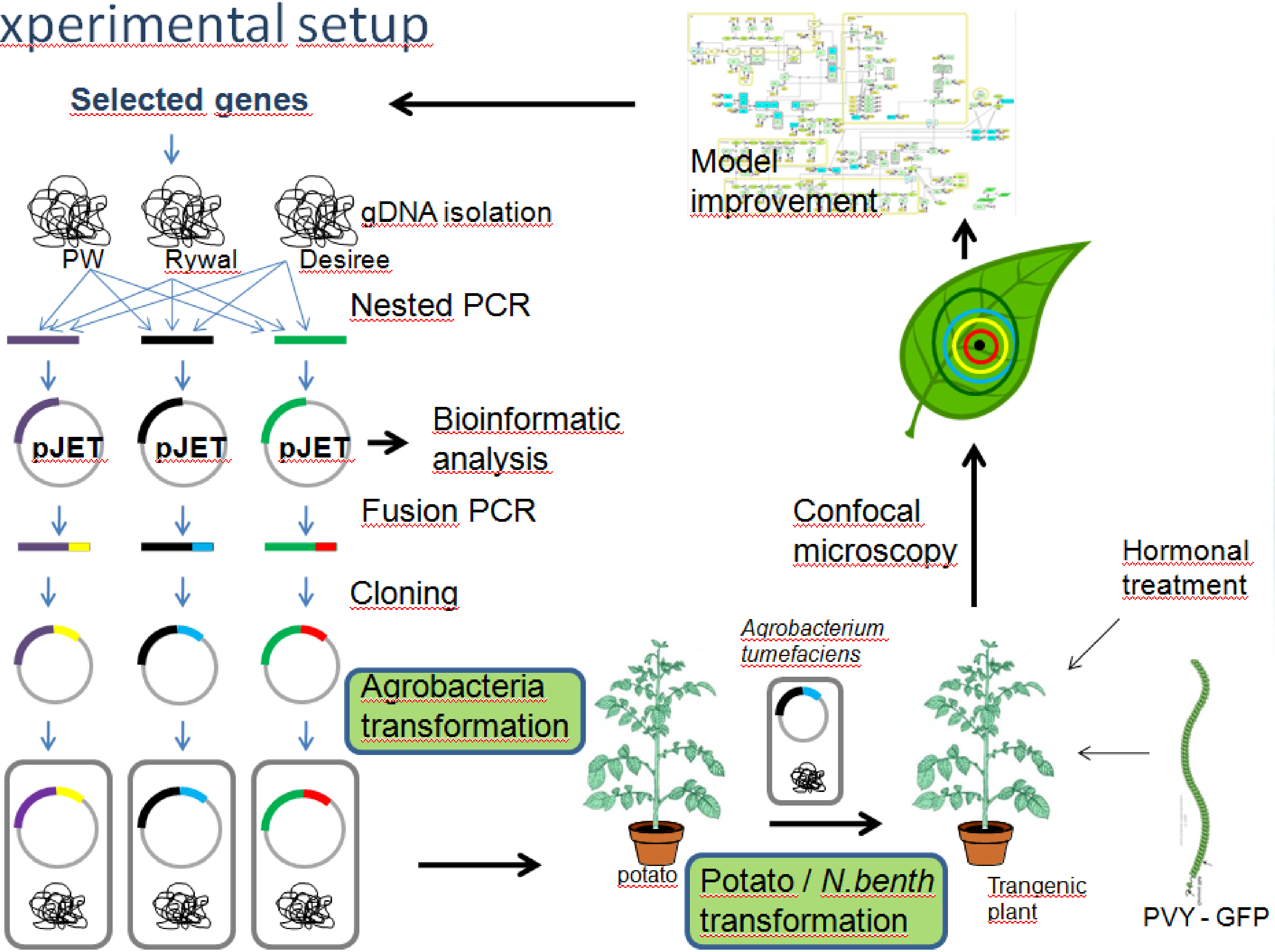




NATIVE PROMOTER

FLUORESCENT PROTEIN

Experimental setup



Selected genes

gDNA isolation

PW

Rywal

Desiree

Nested PCR

pJET

pJET

pJET

Bioinformatic analysis

Fusion PCR

Cloning

Agrobacteria transformation

potato

Agrobacterium tumefaciens

Potato / *N.benth* transformation

Trangenic plant

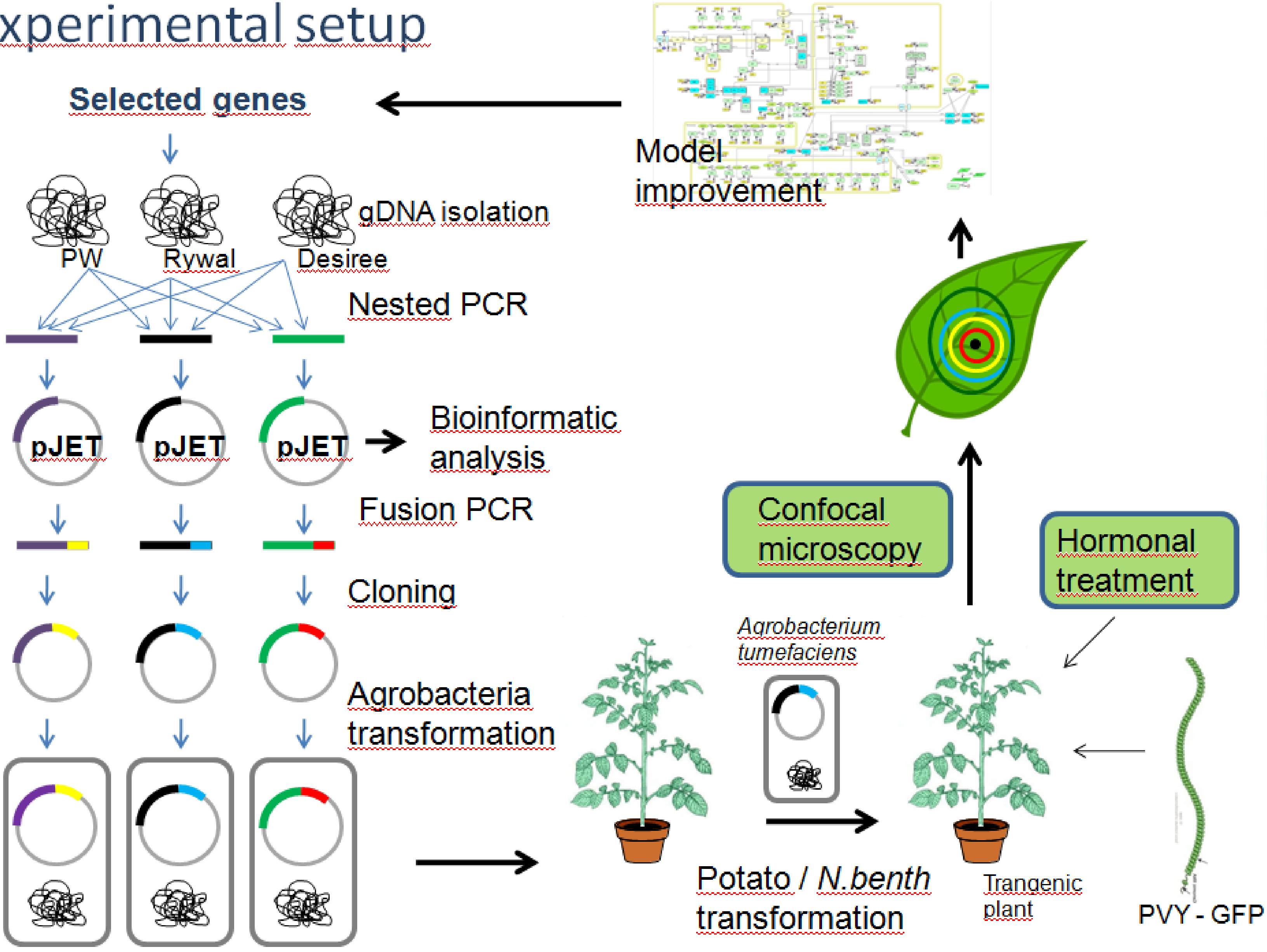
Hormonal treatment

PVY - GFP

Confocal microscopy

Model improvement

Experimental setup



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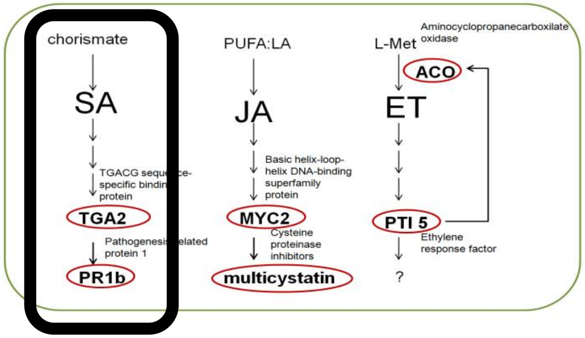
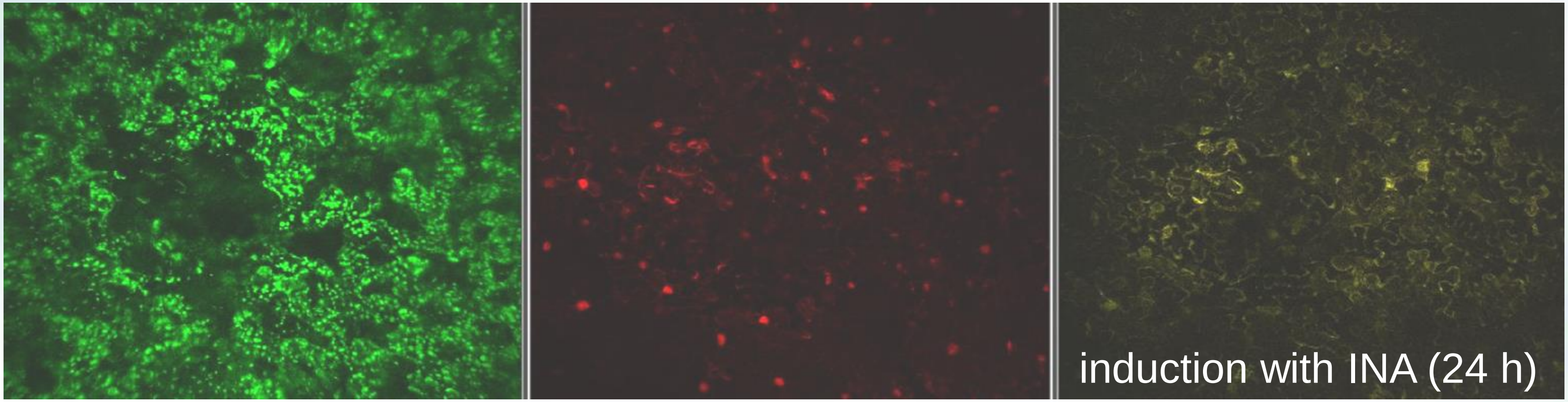
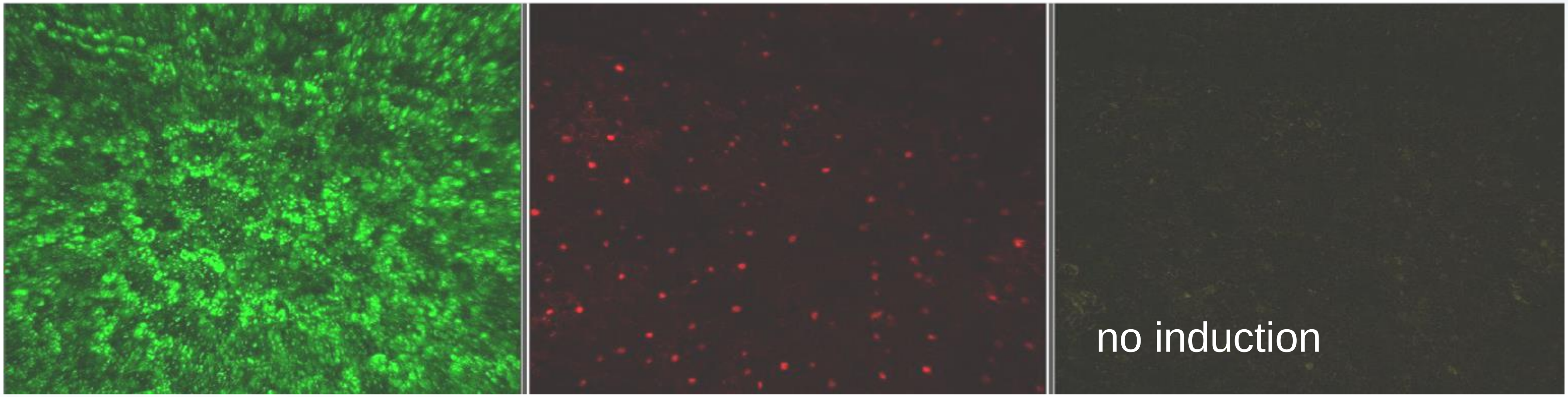
TGA2 promoter is induced by salicylic acid

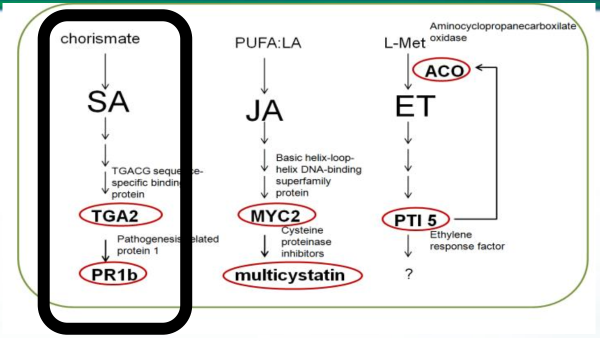


TGA2::YFP

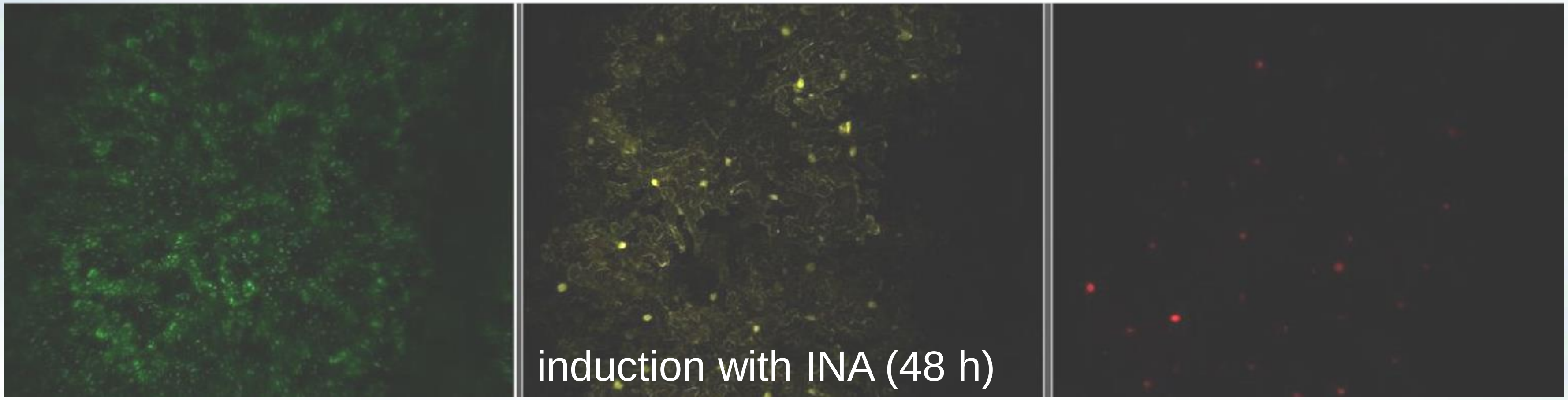


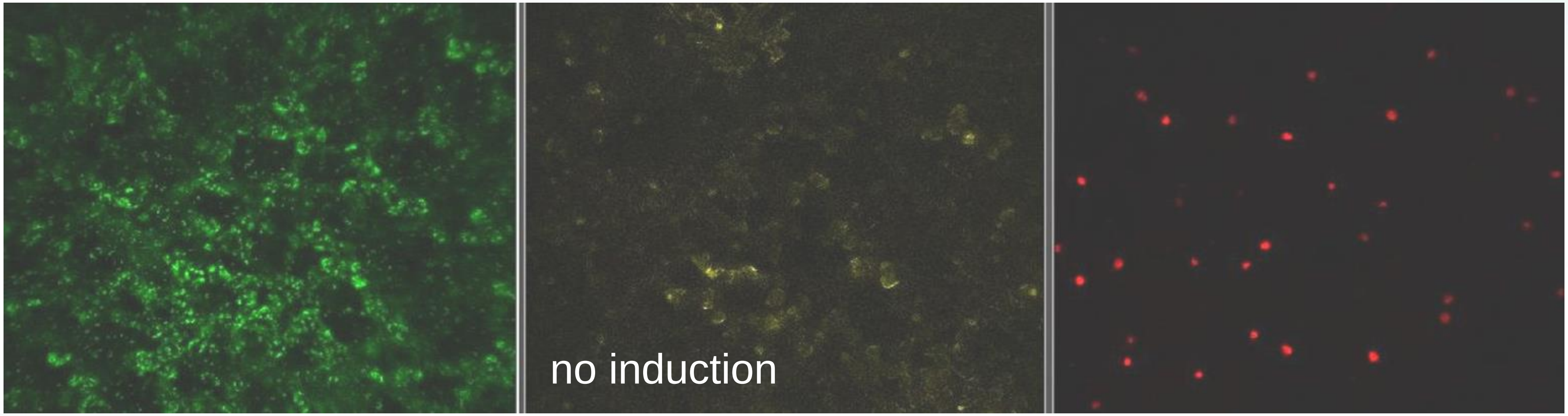


PR1b promoter is induced by salicylic acid

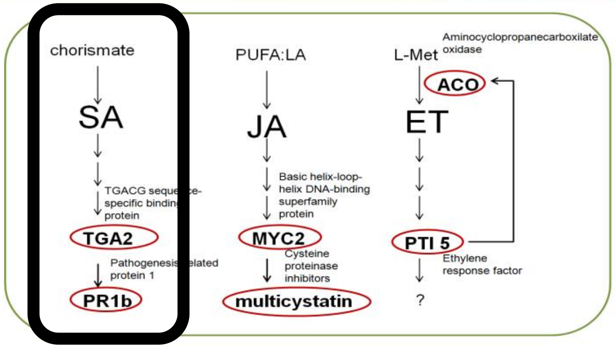


PR1b::YFP

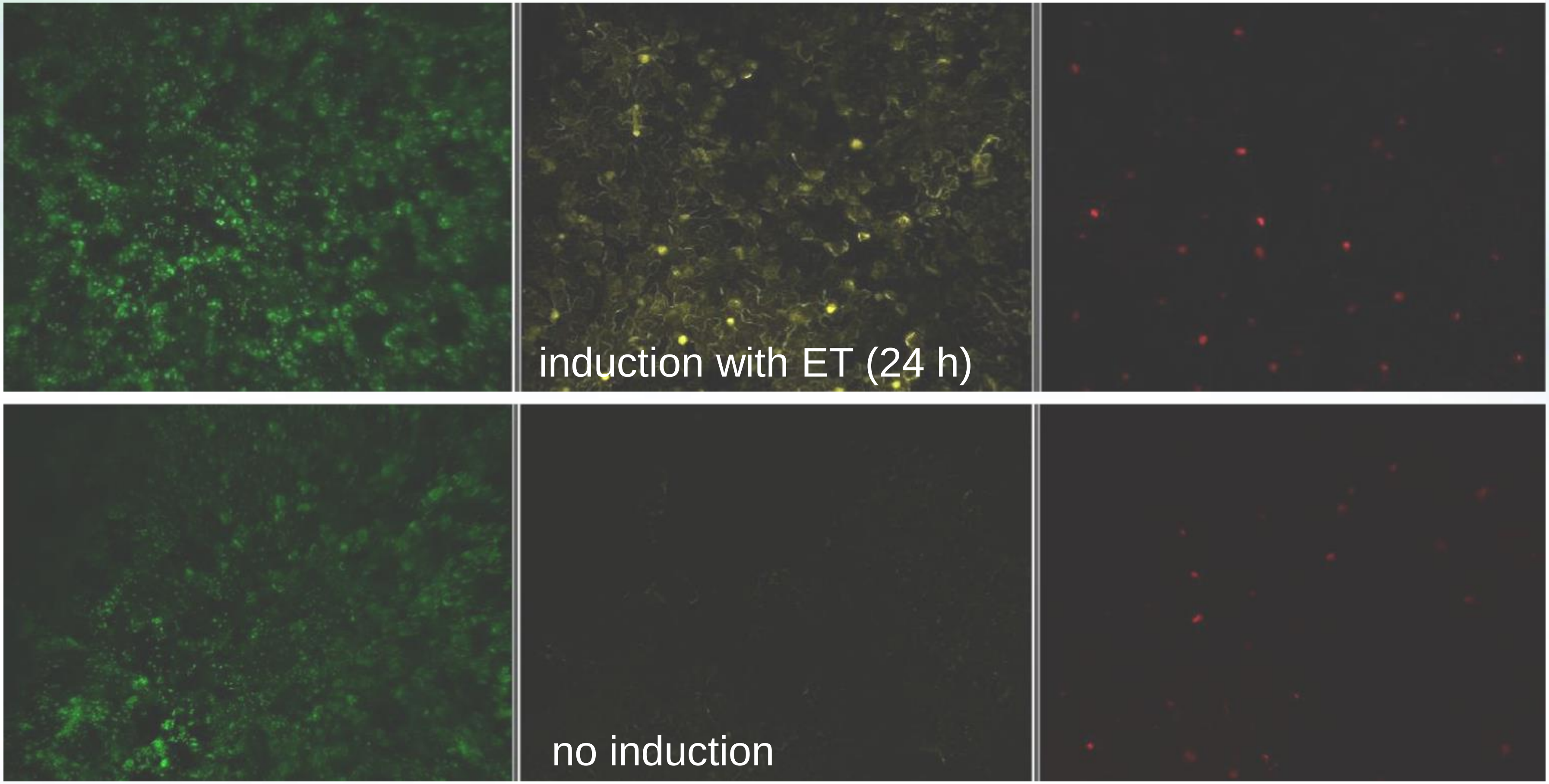

induction with INA (48 h)


no induction

PR1b promoter is induced by ethylene



PR1b::YFP



induction with ET (24 h)

no induction

Conclusions

- Promoter sequences of the same gene differ between cultivars

- Some genes can have different promoter sequences within the same cultivar

  promoter sequences of the same gene from different cultivars contain different regulatory elements

- Genes from SA signaling pathway: induction by INA

  new crosstalk identified

- Future plans: nuclear localisation signal → spatiotemporal analysis

Department of Biotechnology and Systems Biology

prof. dr. Kristina Gruden
dr. Špela Baebler
dr. Anna Coll

University of Potsdam
Dr. Katrin Messerschmidt

The James Hutton Institute
Dr. Ingo Hein

Funding:

Slovenian Research Agency