

So many genes, so little time: targeting systems for gene destruction

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PARROTLAB



1785

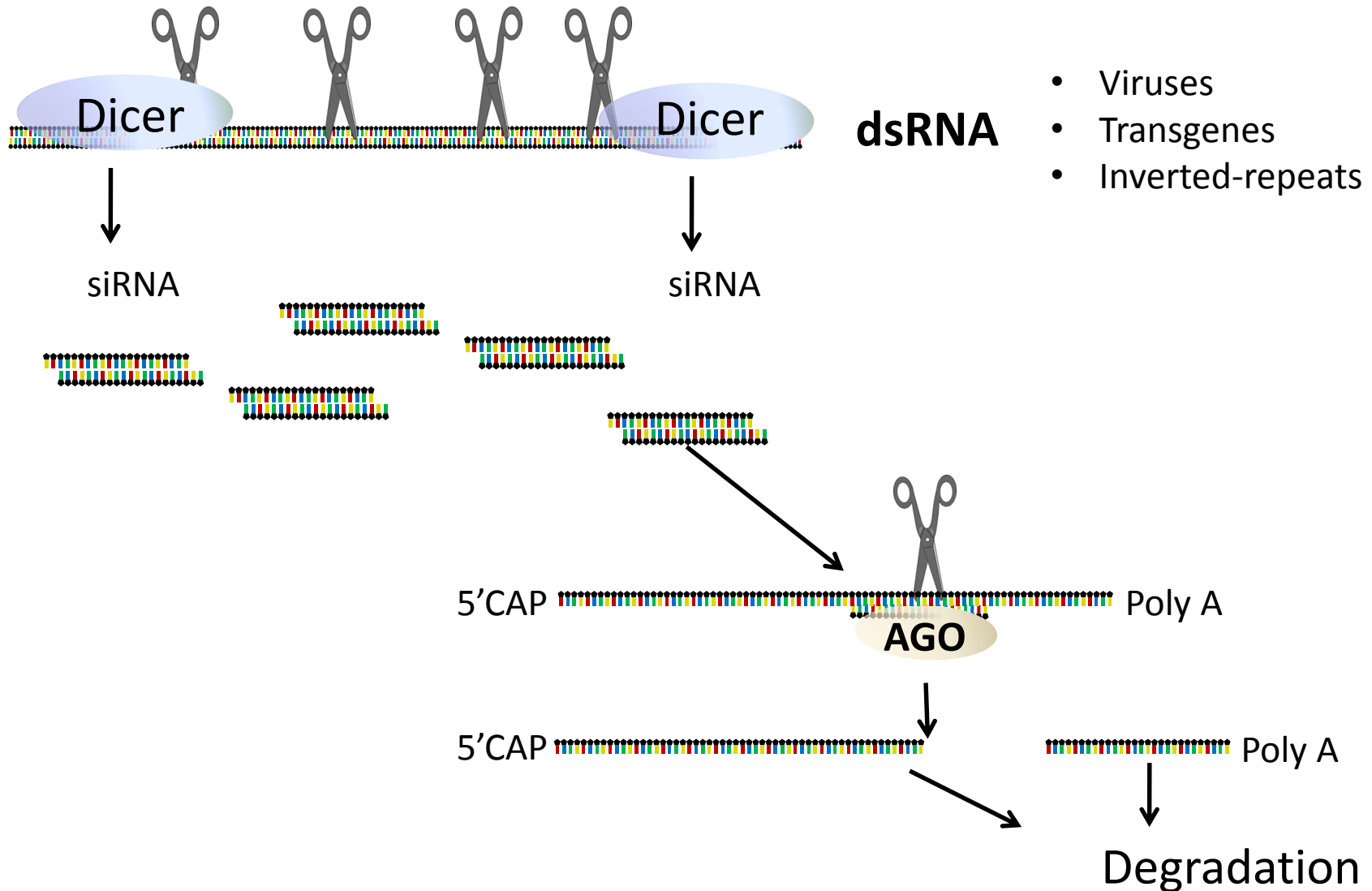
The University of Georgia

*Center for Applied Genetic Technologies
Institute for Plant Breeding, Genetics & Genomics*

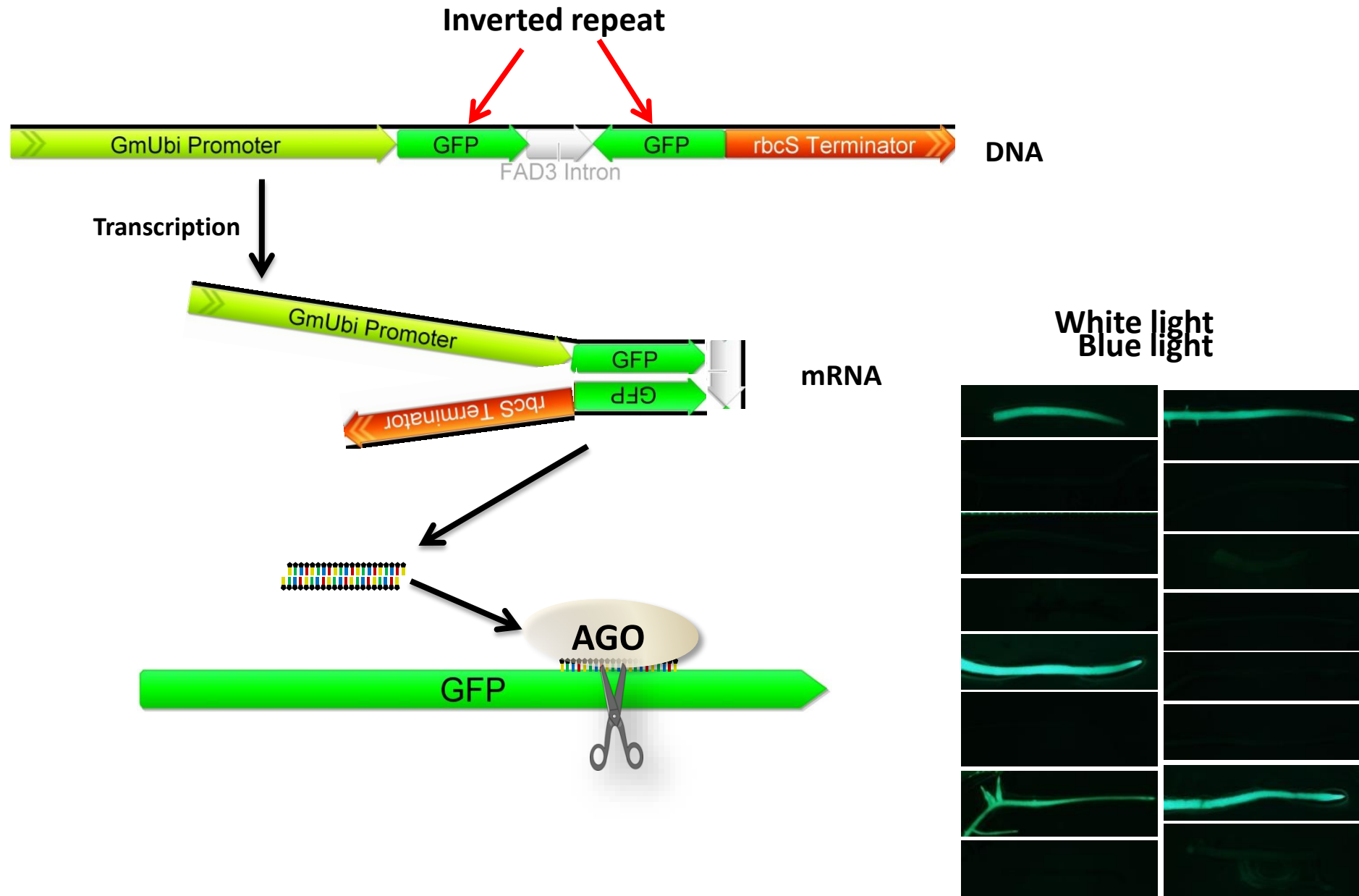


<http://www.vib.be/en/news/PublishingImages>

Double-stranded RNA induces gene-silencing



Inducing RNAi with hairpin vectors

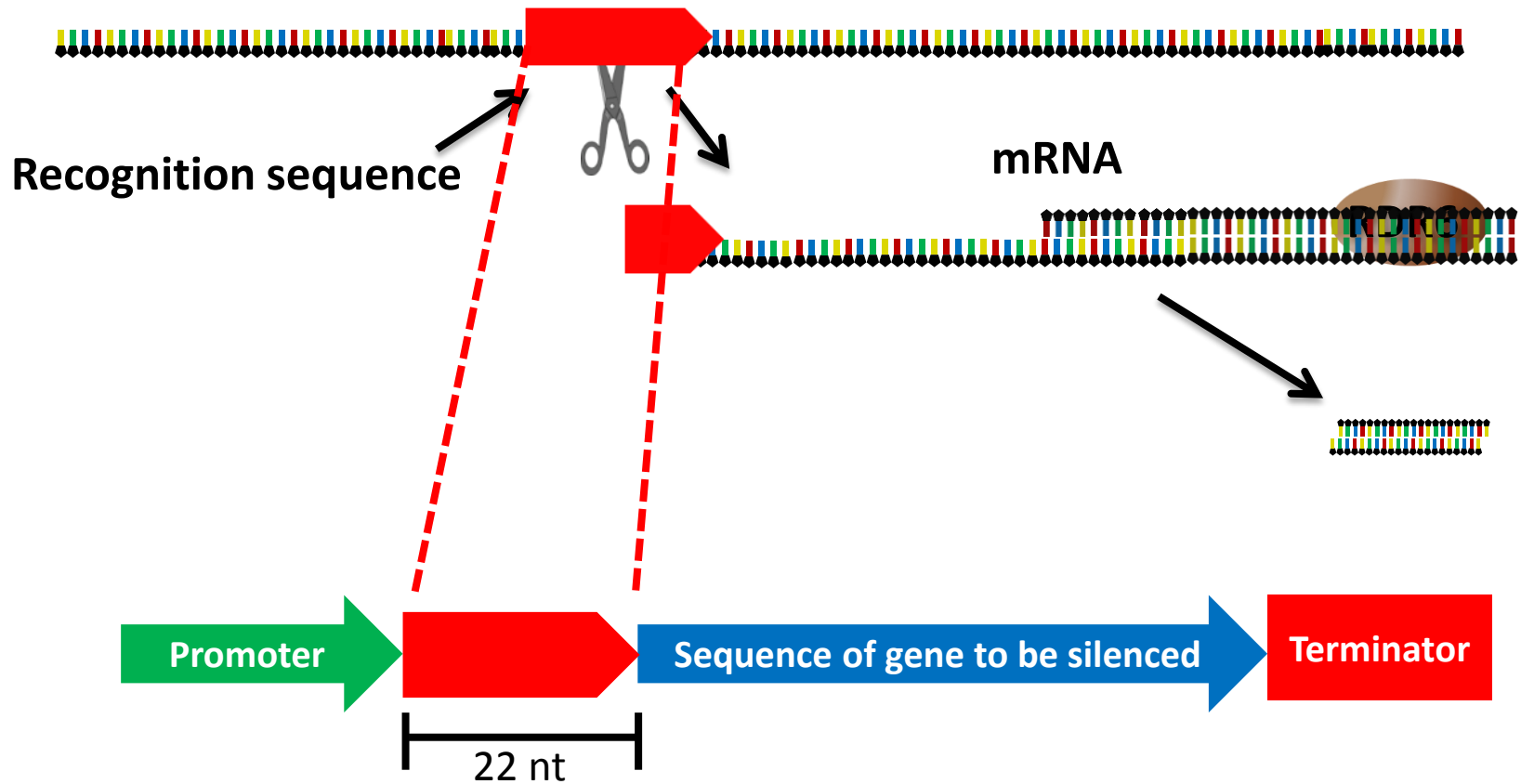


Trans-acting siRNA (tasiRNA) pathway

MIR gene



miRNA



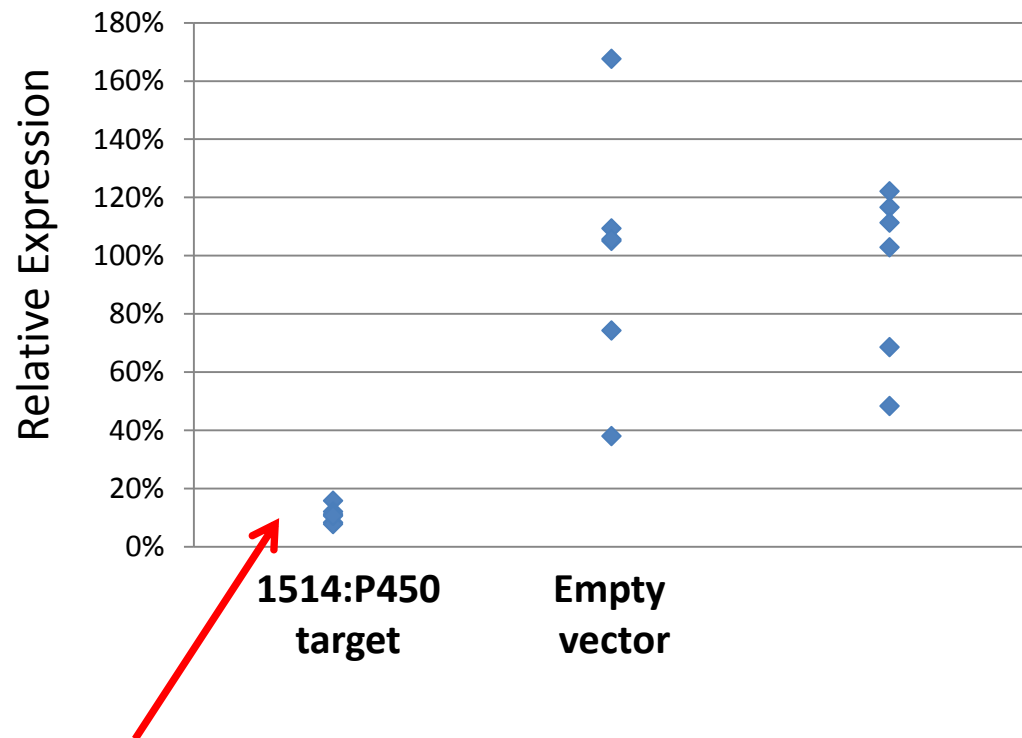
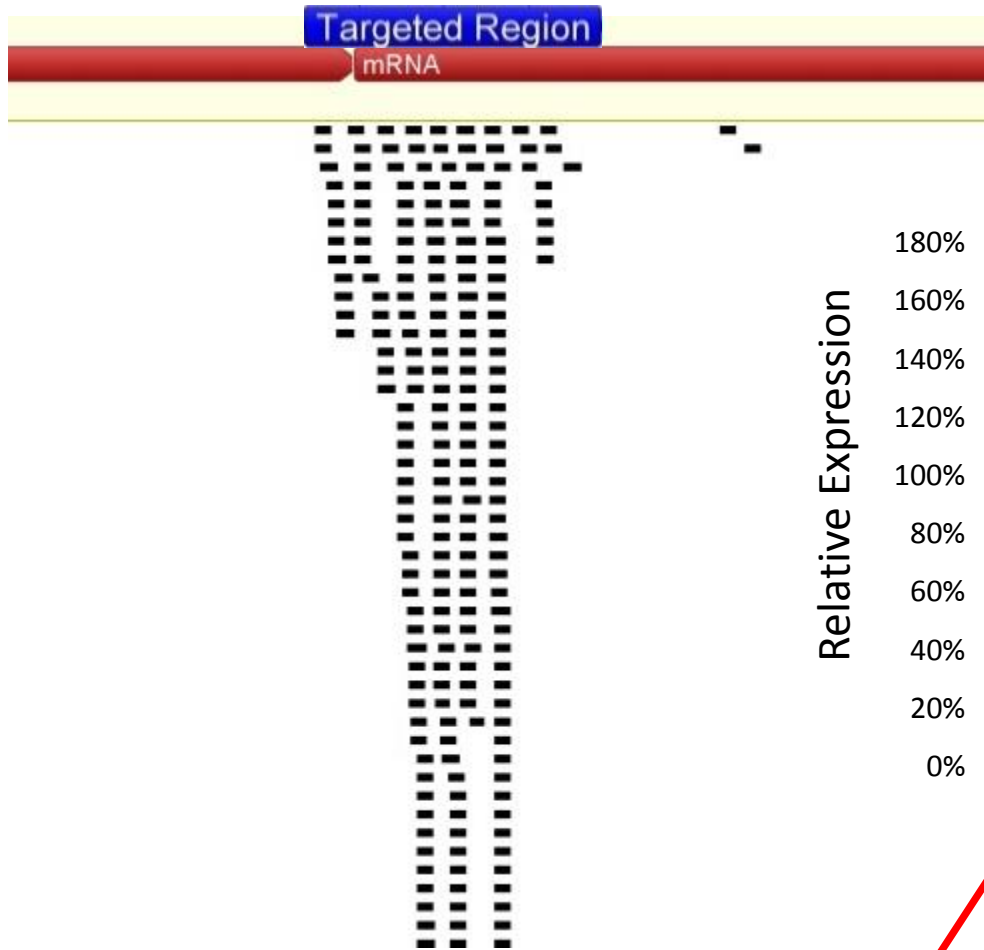
Proof-of-concept

- Identified 6 miRNA recognition sequences
 - Case study with miR1514
- P450 Endogenous Target
 - Highly expressed all tissue types
 - Nulls are embryo lethal in arabidopsis

1514:P450 target construct



P450 mRNA reduced in 1514:P450 events



Validating six tasiRNAs



Empty
vector

Wild
type

1509

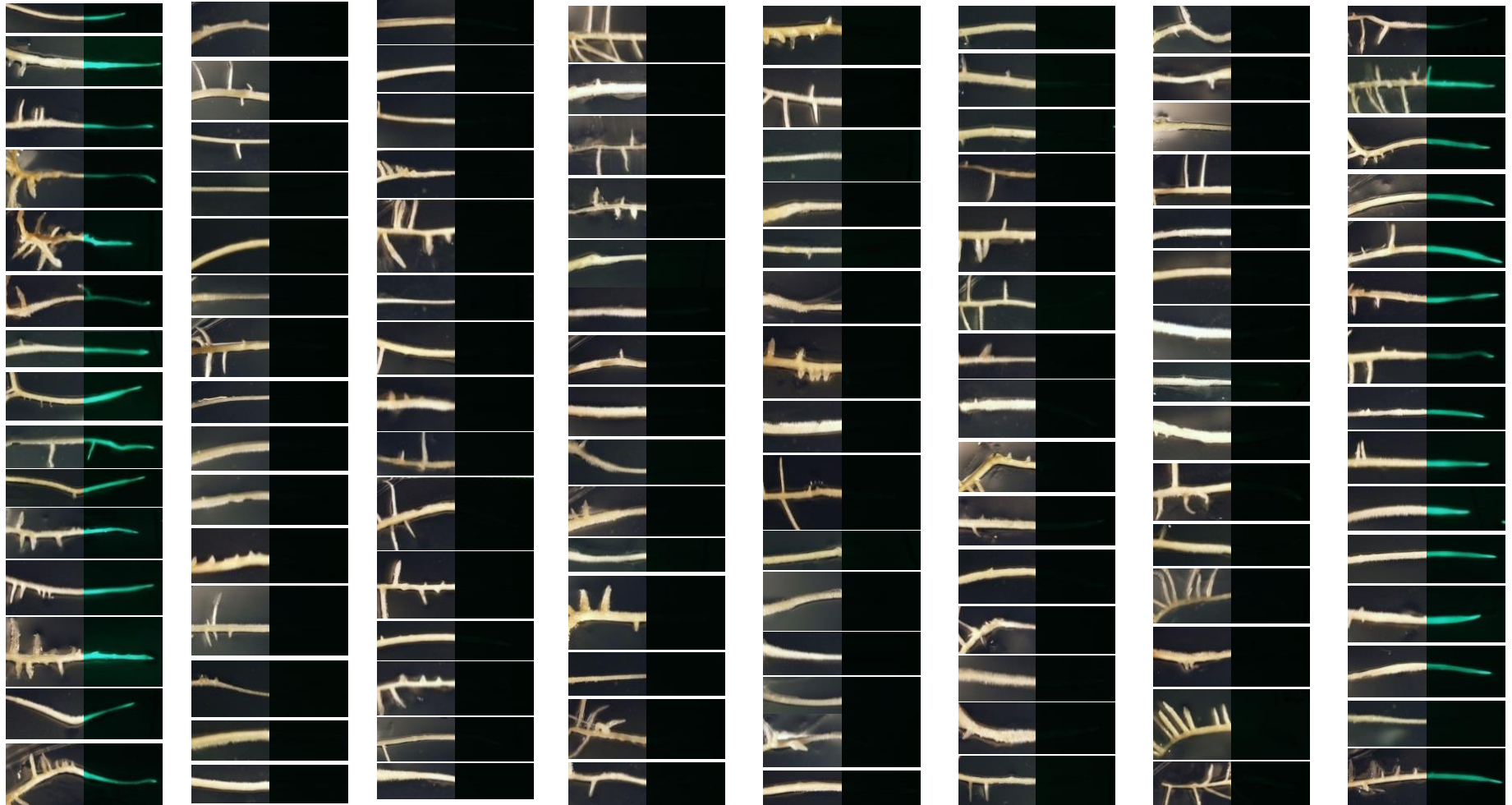
1510

1510a.2

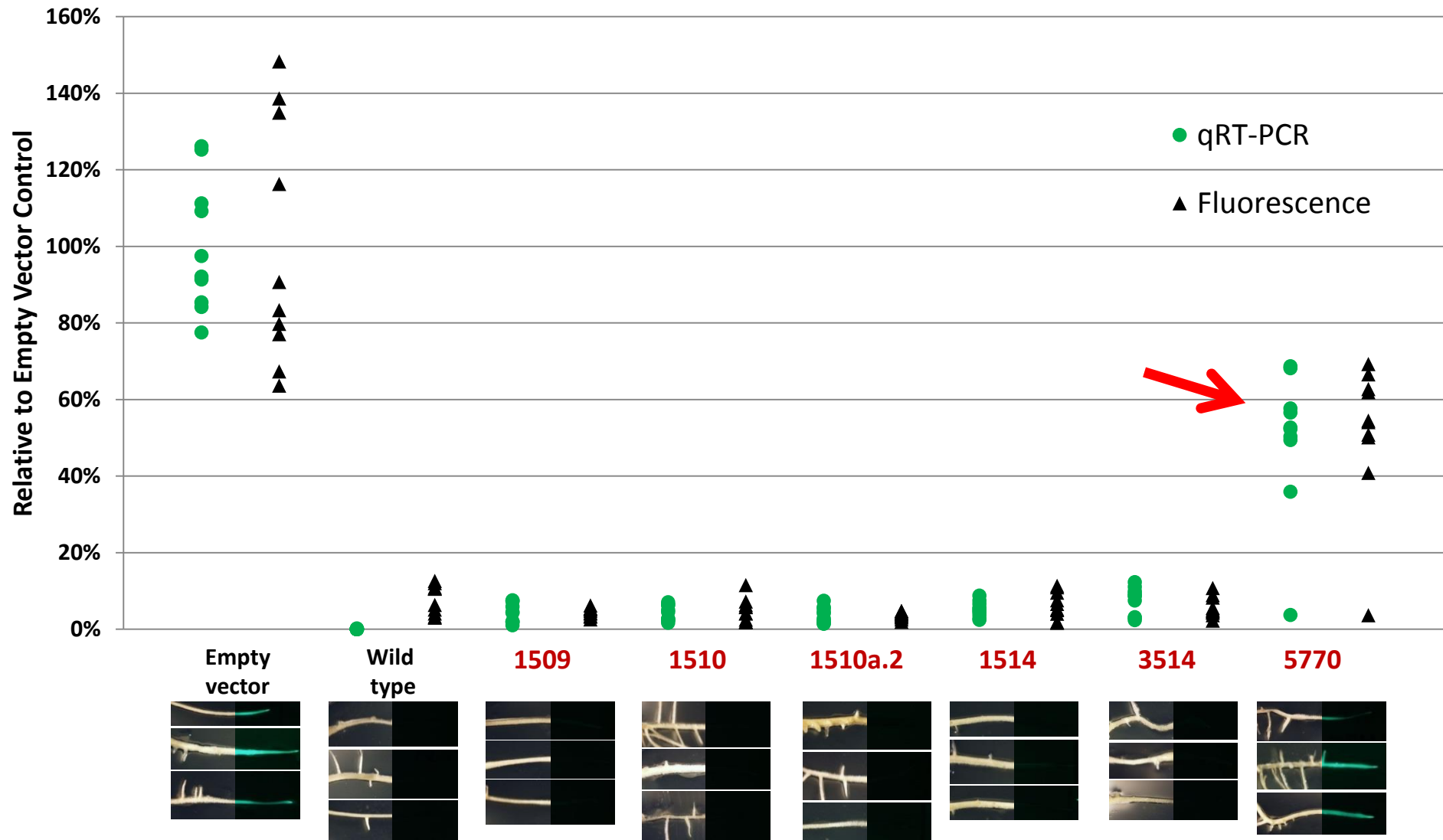
1514

3514

5770

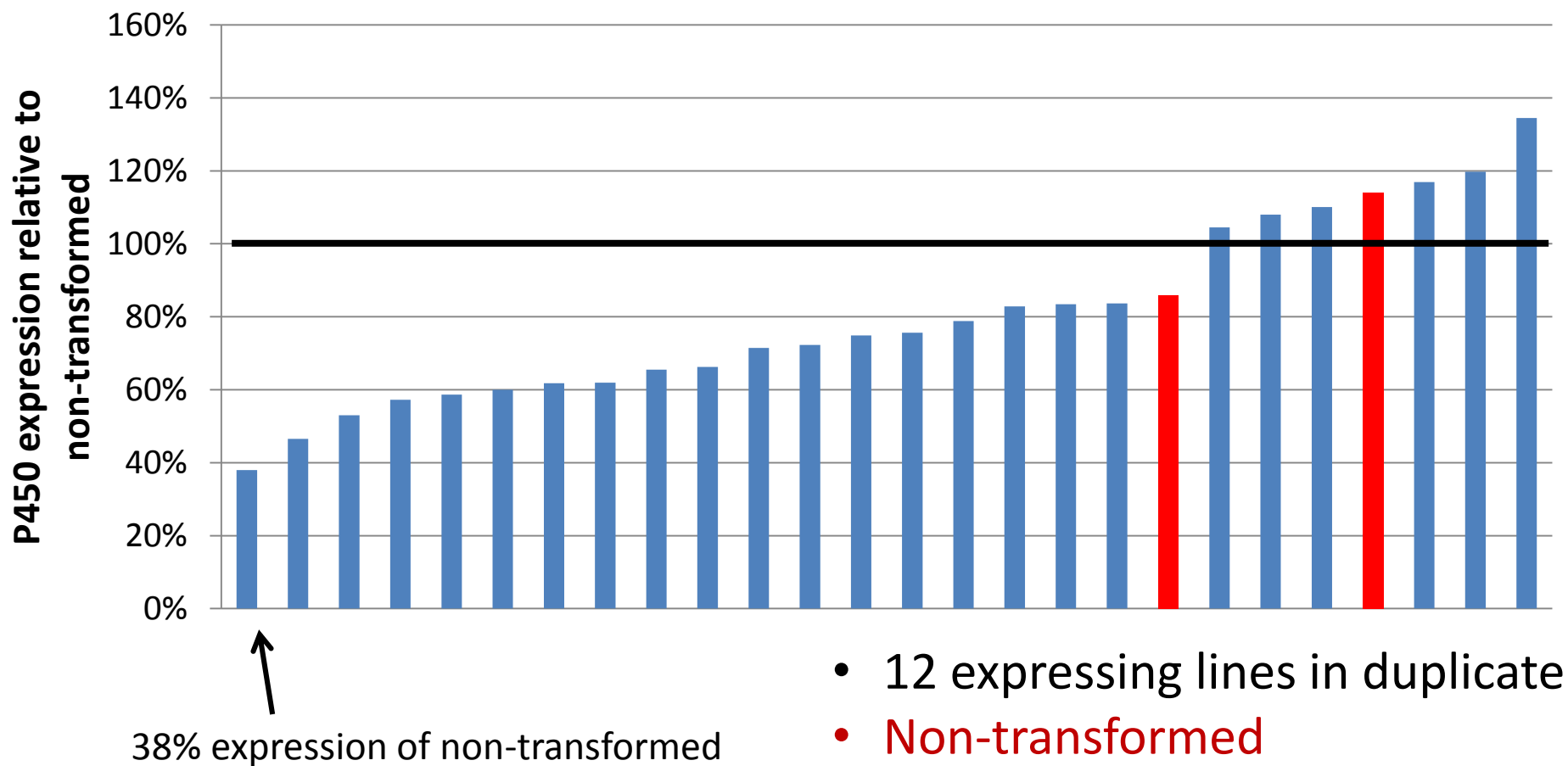


qRT-PCR and protein abundance



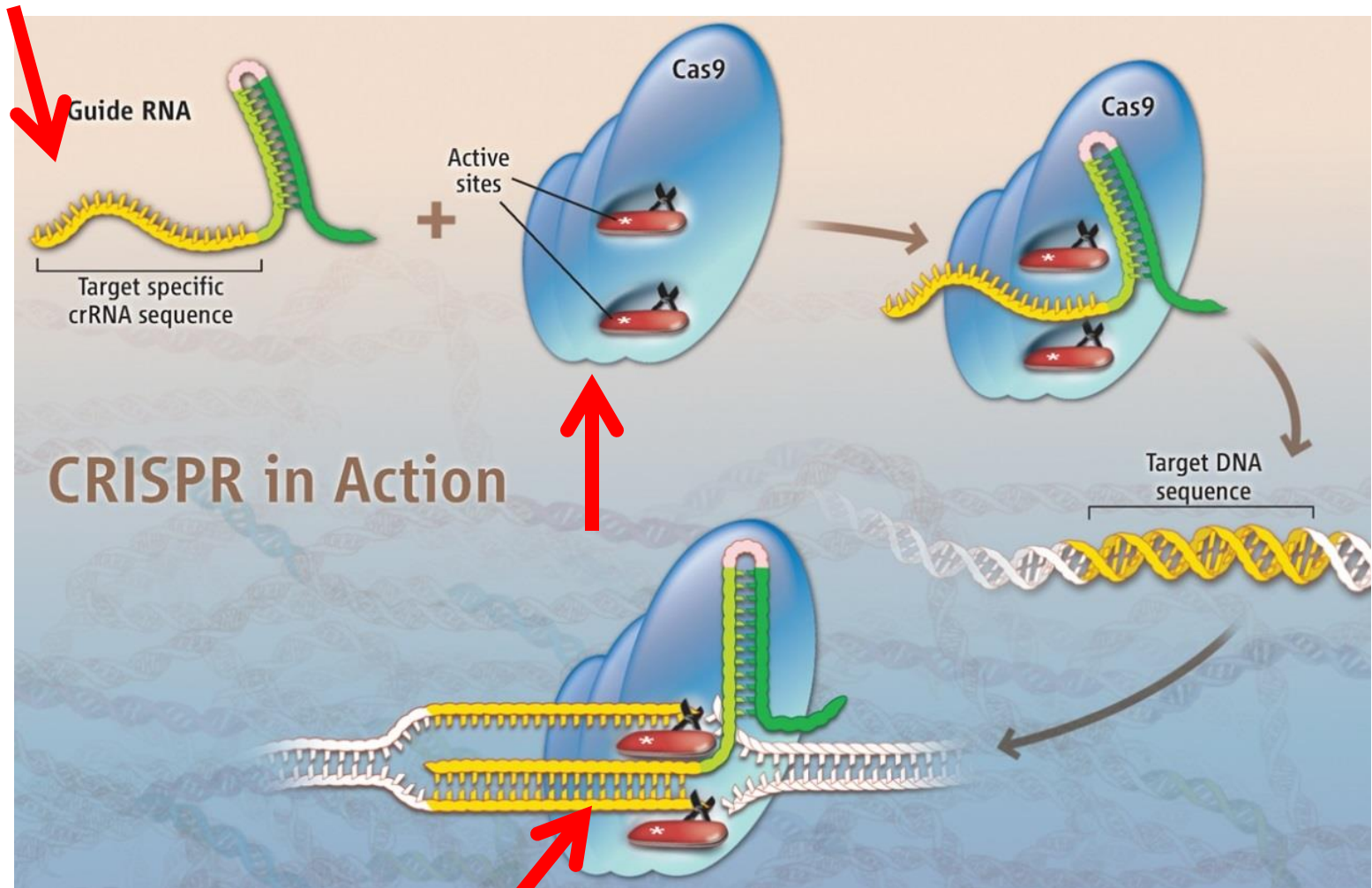
Silencing P450 in stable events

- Roots are nice, but really need transgenic plants



- 12 expressing lines in duplicate
- Non-transformed
- Lethal gene effect?

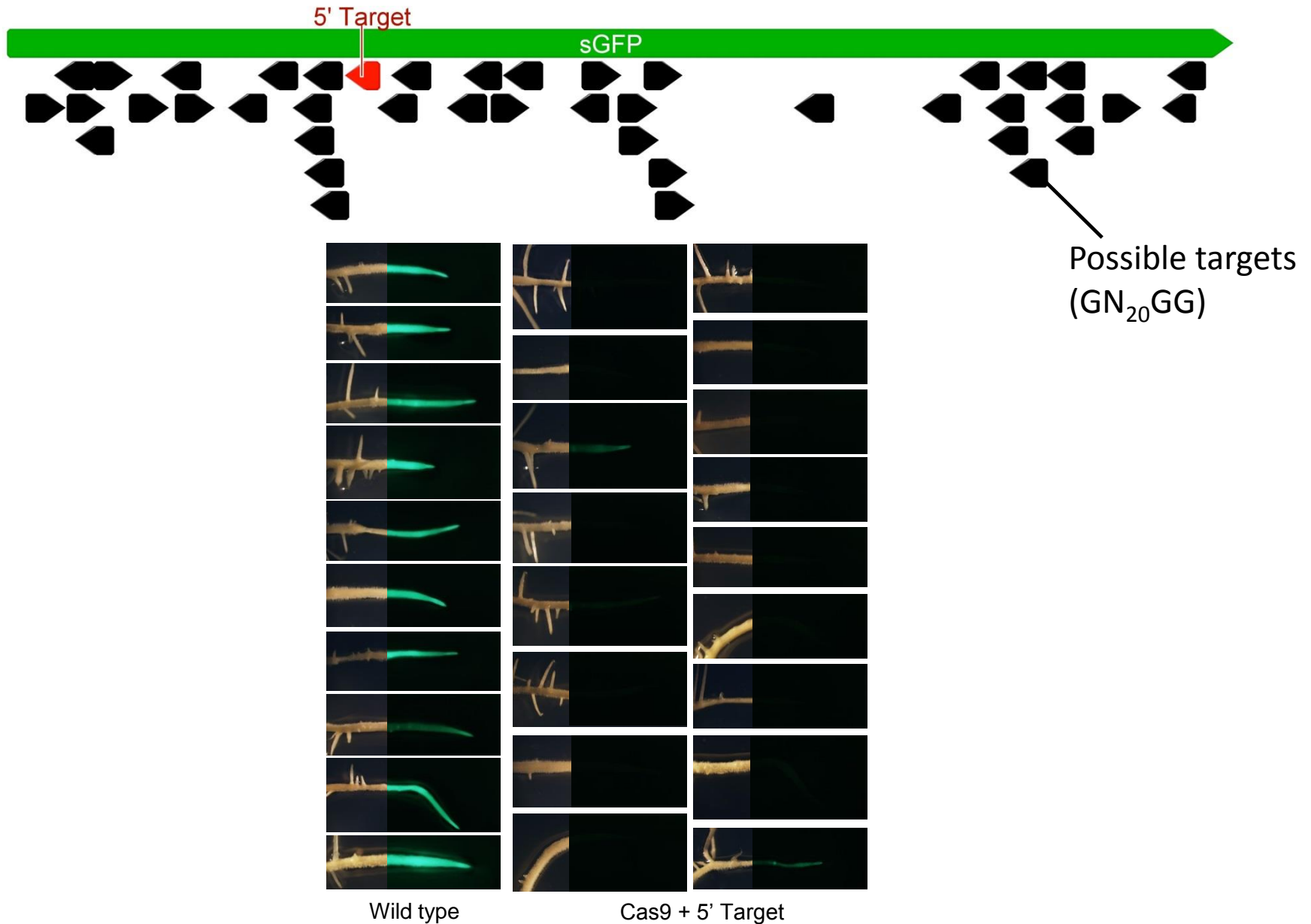
CRISPR/Cas as genome editing tool



Science Pennisi 2013


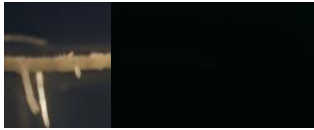
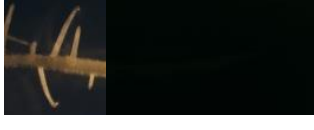
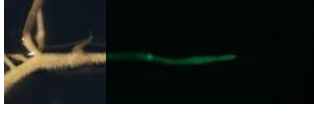
- NHEJ results in indels

CRISPR mutagenesis in soybean



CRISPR mutagenesis in soybean



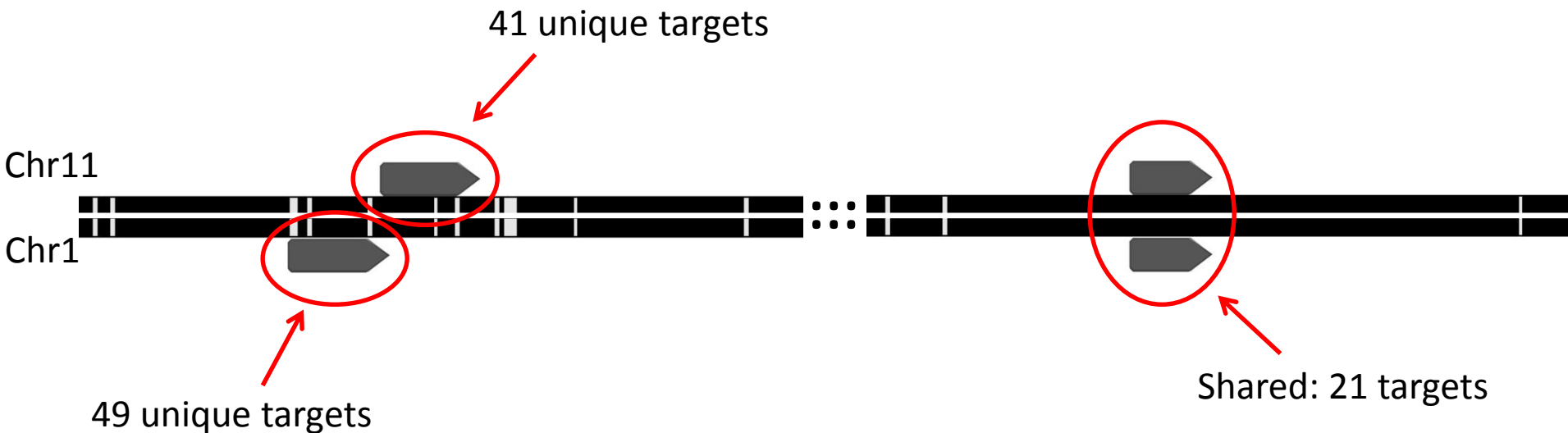
	GFP 5' Target	% reads	Δ
	CACCTTCACCTACGGCGTGCAGTGCTTCAGCCGC		
	CACCTTCACCTACGG--TGCAGTGCTTCAGCCGC	47.7%	-2
	CACCTTCACCTACG---TGCAGTGCTTCAGCCGC	47.2%	-3
	CACCTTCACCTACGGC---AGTGCTTCAGCCGC	48.0%	-4
	CACCTTCACCTACG-CGTGCAGTGCTTCAGCCGC	45.6%	-1
	CACCTTCACCTACG---TGCAGTGCTTCAGCCGC	57.9%	-3
	CACCTTCACCTACGG--TGCAGTGCTTCAGCCGC	29.3%	-2
	CACCTTCACCTACG-----TGCTTCAGCCGC	49.4%	-8
	CACCTTCACCTACGG-----GTGCTTCAGCCGC	48.4%	-6
	CACCTTCACCTACG-CGTGCAGTGCTTCAGCCGC	68.4%	-1
	CACCTTCACCTACGGCGTGCAGTGCTTCAGCCGC	16.1%	wt

- Showing two most common sequences per event

Targeting orthologous genes

- Individually?
- Both at same time?

An example with soybean DDM1



DDM1 example

Line **AAGAGGAGGTACAGTGTGAGGAGGTACC**

1	AAGAGGAGGTA---TGTGAGGAGGTACC	48.4%
	AAGAGGA-----TGTGAGGAGGTACC	44.5%
2	AAGAGGAGGTA---TGTGAGGAGGTACC	50.3%
	AAGAGGAGGTA---GTGAGGAGGTACC	47.2%

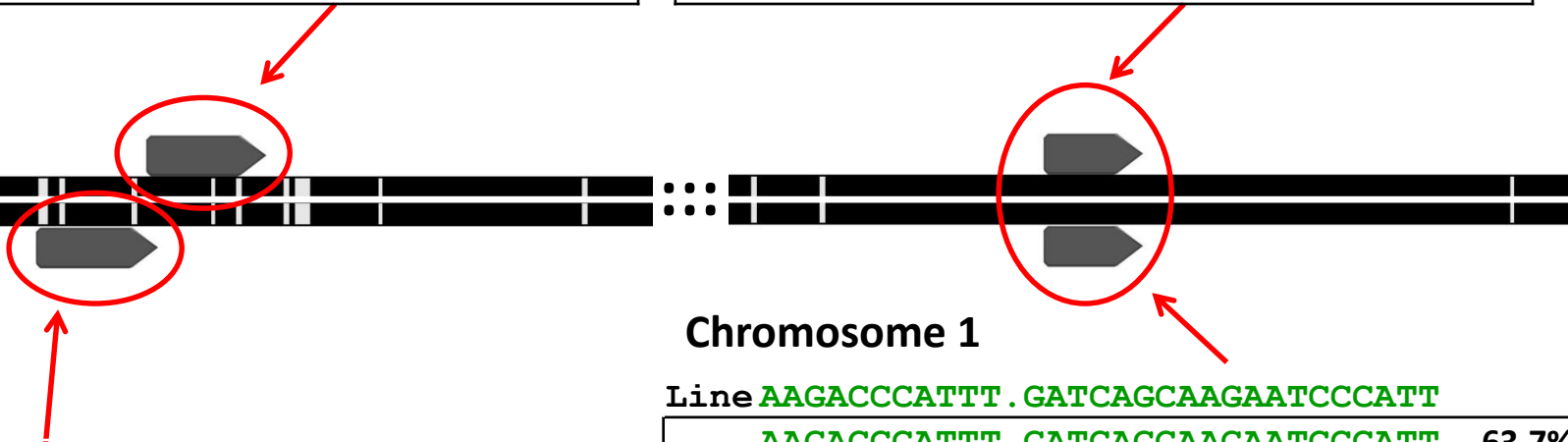
Chromosome 11

Line **AAGACCCATTT . GATCAGCAAGAATCCC**

5	AAGACCCATTT . GATCAGCAAGAATCCC	68.4%
	AAGACCCATTT . --TCAGCAAGAATCCC	13.2%
6	AAGACCCATTT . GATCAGCAAGAATCCC	92.6%
	AAGACCCATTT T GATCAGCAAGAATCCC	2.8%

Chr11

Chr1



Chromosome 1

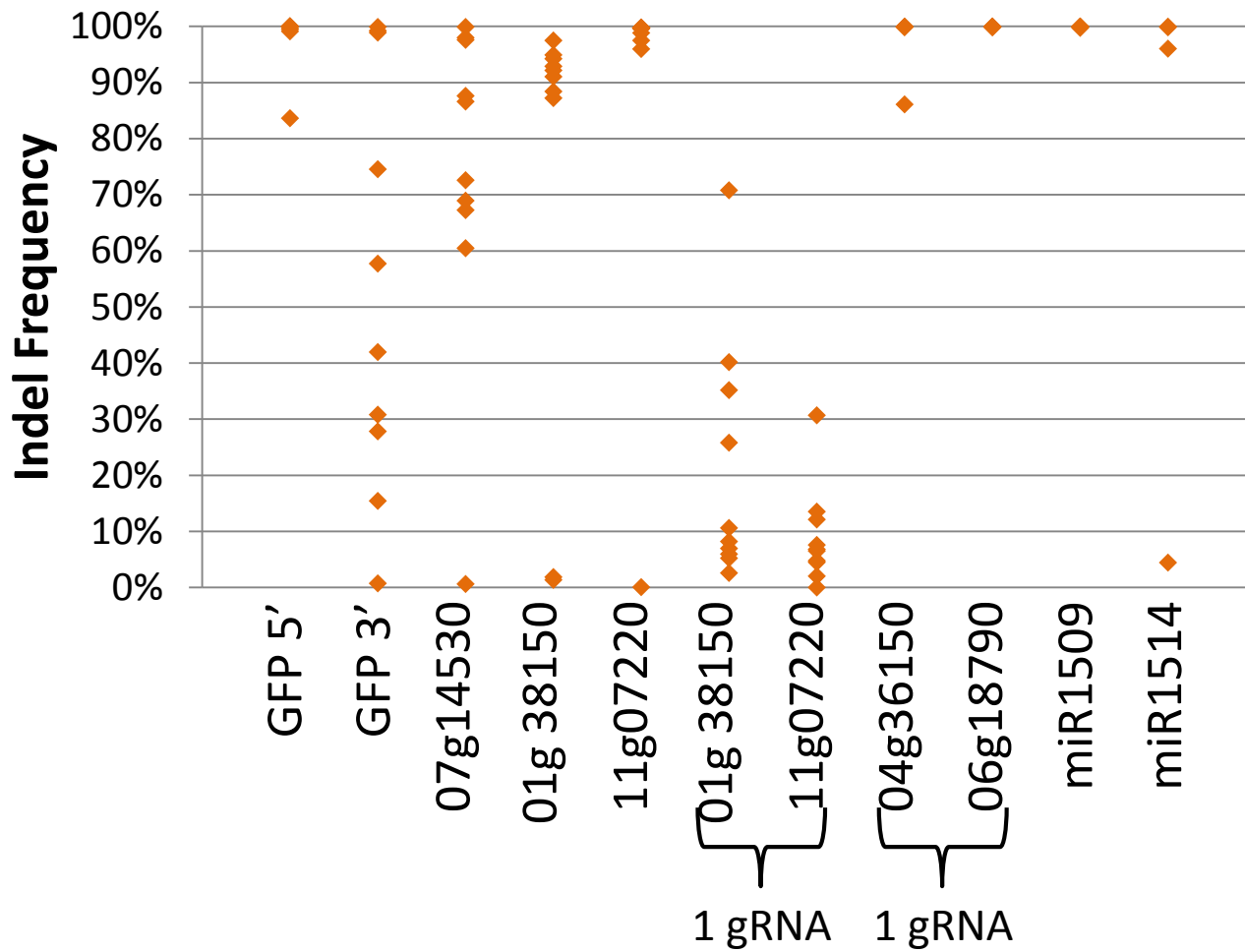
Line **AAGACCCATTT . GATCAGCAAGAATCCCATT**

5	AAGACCCATTT . GATCAGCAAGAATCCCATT	63.7%
	AAGACCCATTT T GATCAGCAAGAATCCCATT	25.5%
6	AAGACC----- . -----GCAAGAATCCCATT	34.2%
	AAGACCCATTT . G----GCAAGAATCCCATT	33.6%

Line **AAGCTACTTGAAGCTAGGATAAAGGAAGAG**

3	AAGCTACTT-----AAAGGAAGAG	46.0%
	AAGCTACTTGAAG-----TAAAGGAAGAG	43.2%
4	AAGCTACTTGAAG-----TAAAGGAAGAG	86.0%
	AAGCTACTTGAAGCTAGGATAAAGGAAGAG	8.3%

Each target works, at different efficiencies

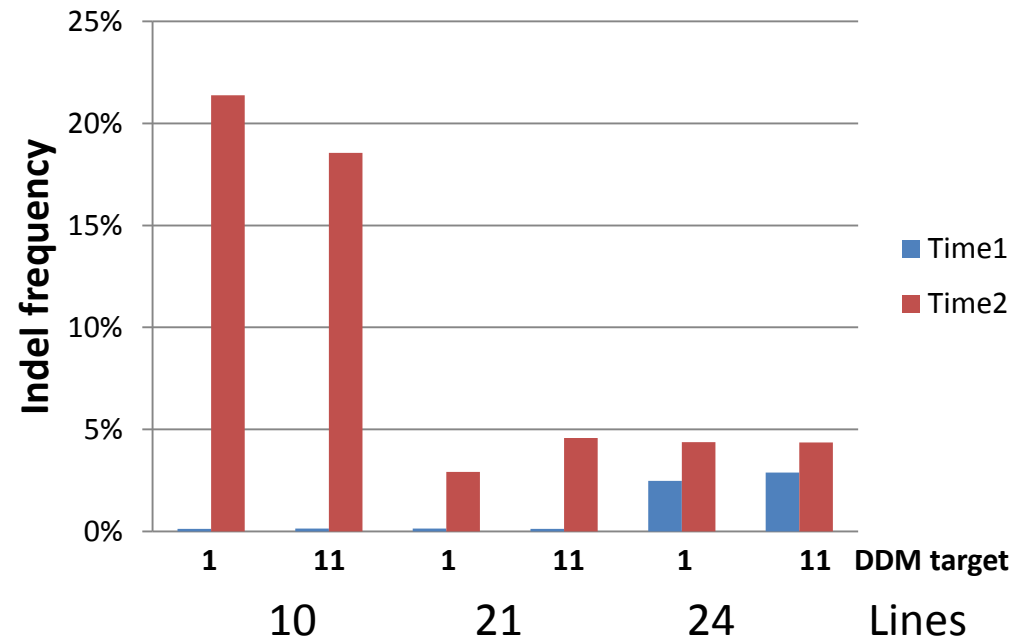


Whole plant modifications



ATTCTTGCTGATCAAA.TGGGTCTTGGGAA	
ATTCTTGCTGATCAAA.TGGGTCTTGGGAA	80.31%
ATTCTTGCTGA--AAA.TGGGTCTTGGGAA	10.13%
ATTCTTGCTGA---AA.TGGGTCTTGGGAA	4.75%
ATTCTTGCTGATCAAAATGGGTCTTGGGAA	1.13%
ATTCTTGC-----AAA.TGGGTCTTGGGAA	0.32%
ATTCTTGCTGATCAA-.TGGGTCTTGGGAA	0.21%
ATTCTTGCT---CAAA.TGGGTCTTGGGAA	0.14%
ATTCTTGCTGAT-AAA.TGGGTCTTGGGAA	0.13%

- 3/24 lines have complete Cas9
- *Agrobacterium* transformation may be necessary



Cot-node transformation

- CRISPR target to phytoene synthase
 - Sent vectors to Bob Stupar's lab



Photo: Jean-Michel Michno

CRISPR take-home

- CRISPRs are working in soybean
- Vectors available from



- More information available at
<http://parrottlab.uga.edu/parrottlab/Vectors/index.htm>

Acknowledgements



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Dena Mehalakes
Lauren Lail

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Aaron Umansky
Andrew Disharoon
Jade Newsome

Off-targeting results of 5 gRNAs

