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Small RNAs in Plum pox virus infection and protection

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SHARCO

SEVENTH FRAMEWORK PROGRAMME

- ***WP-G2. Characterization of new and complementary genetic resistance mechanisms suitable for pyramiding durable resistance to PPV***

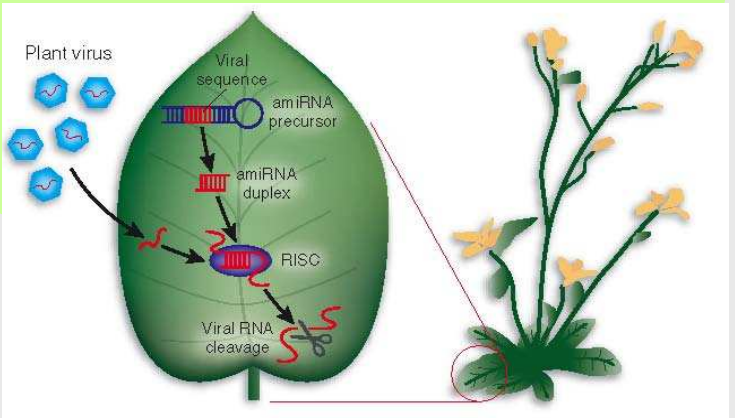
Task TG2-3b Use of pathogen-derived resistance to control PPV

- Expression of virus-derived ihRNA (INRA)

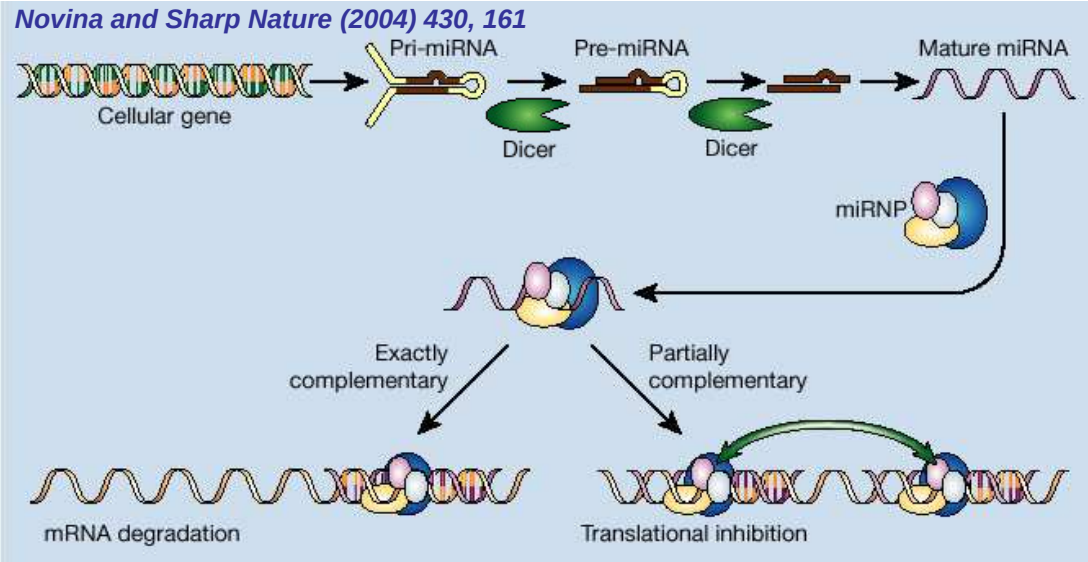
Para ver esta película, debe disponer de QuickTime™ y de un descompresor TIFF (sin comprimir).

Smith et al. (2000)

- Expression of artificial miRNAs (INRA, CSIC)



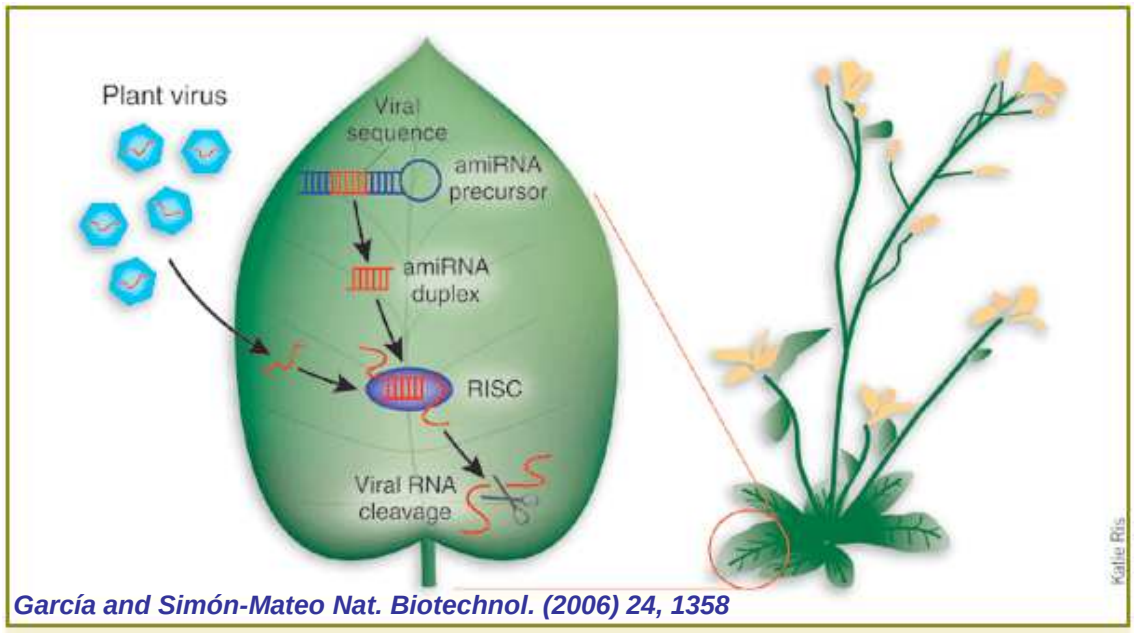
García and Simón-Mateo (2006)



Post-transcriptional gene silencing mediated by miRNAs

- *Development of multicellular organisms*
- *Adaptation to environmental changes*

Artificial miRNAs can provide specific antiviral resistance



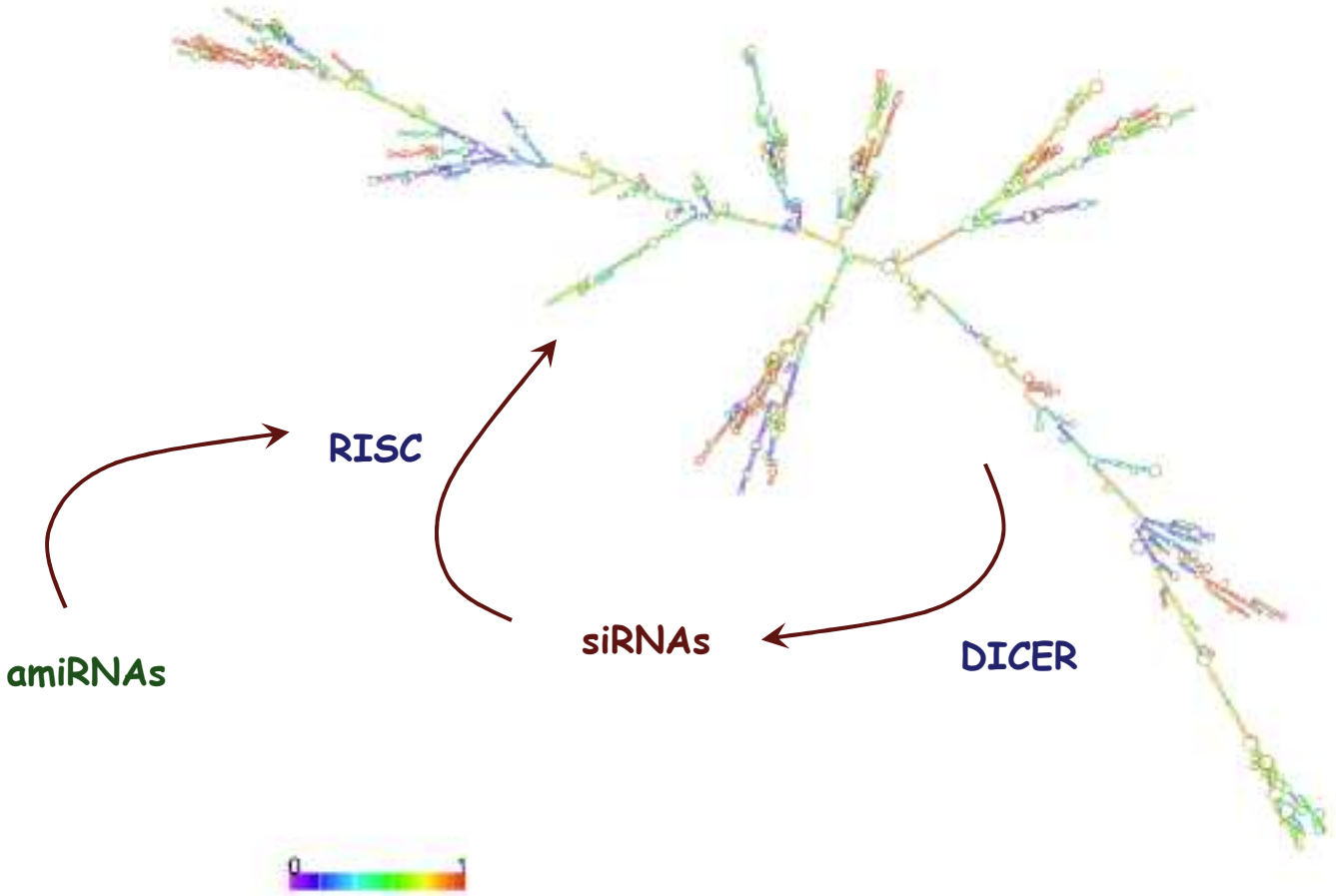
García and Simón-Mateo Nat. Biotechnol. (2006) 24, 1358

Niu et al. Nature Biotechnology (2006)
Artificial microRNAs in transgenic *Arabidopsis thaliana* confer TuMV and TYMV resistance.

Qu et al. Journal of Virology (2007)
Artificial microRNAs in transgenic tobacco plants confer efficient CMV virus resistance.

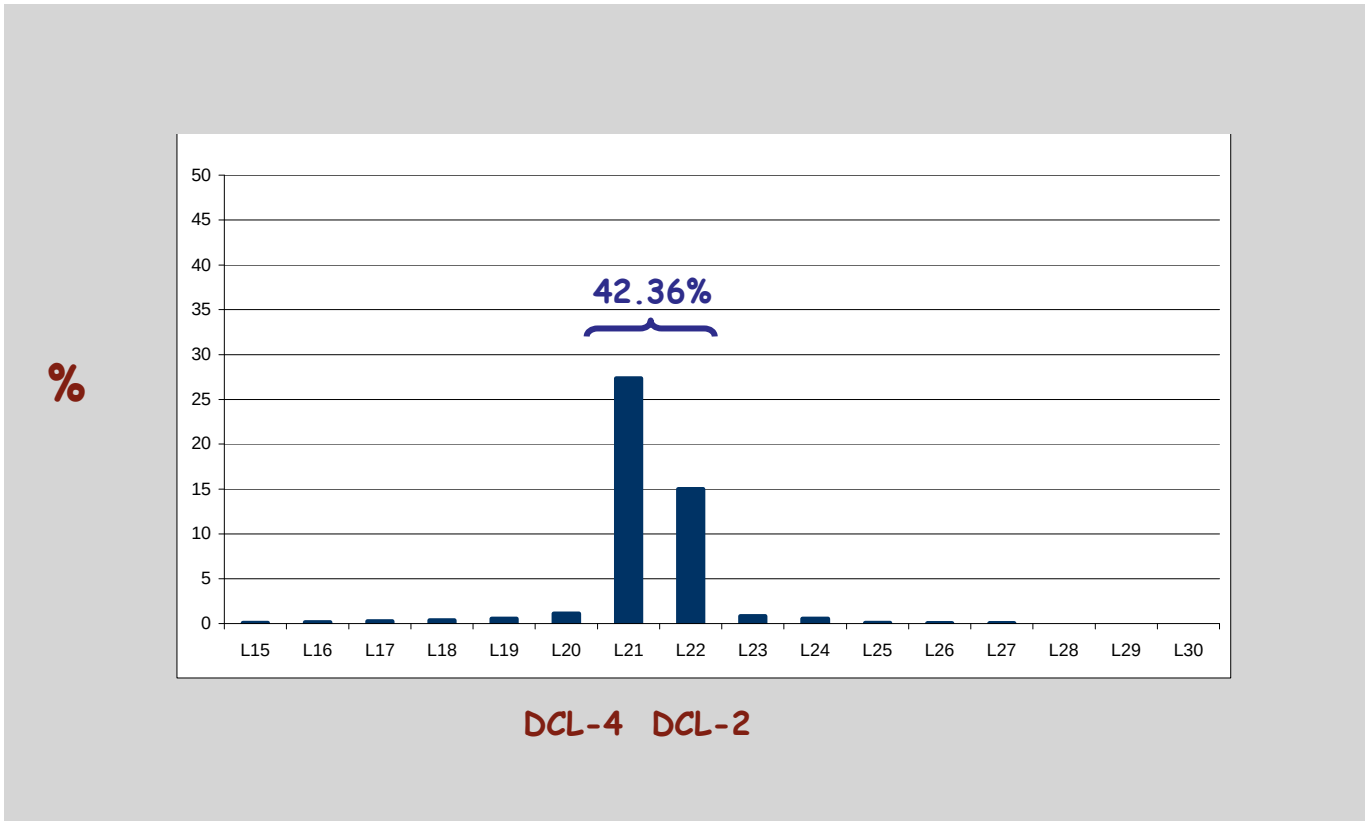
Duan et al. Journal of Virology (2008)
Artificial microRNAs in transgenic *Arabidopsis thaliana* confer efficient CMV virus resistance.

PPV genome as inducer and target of viral siRNAs

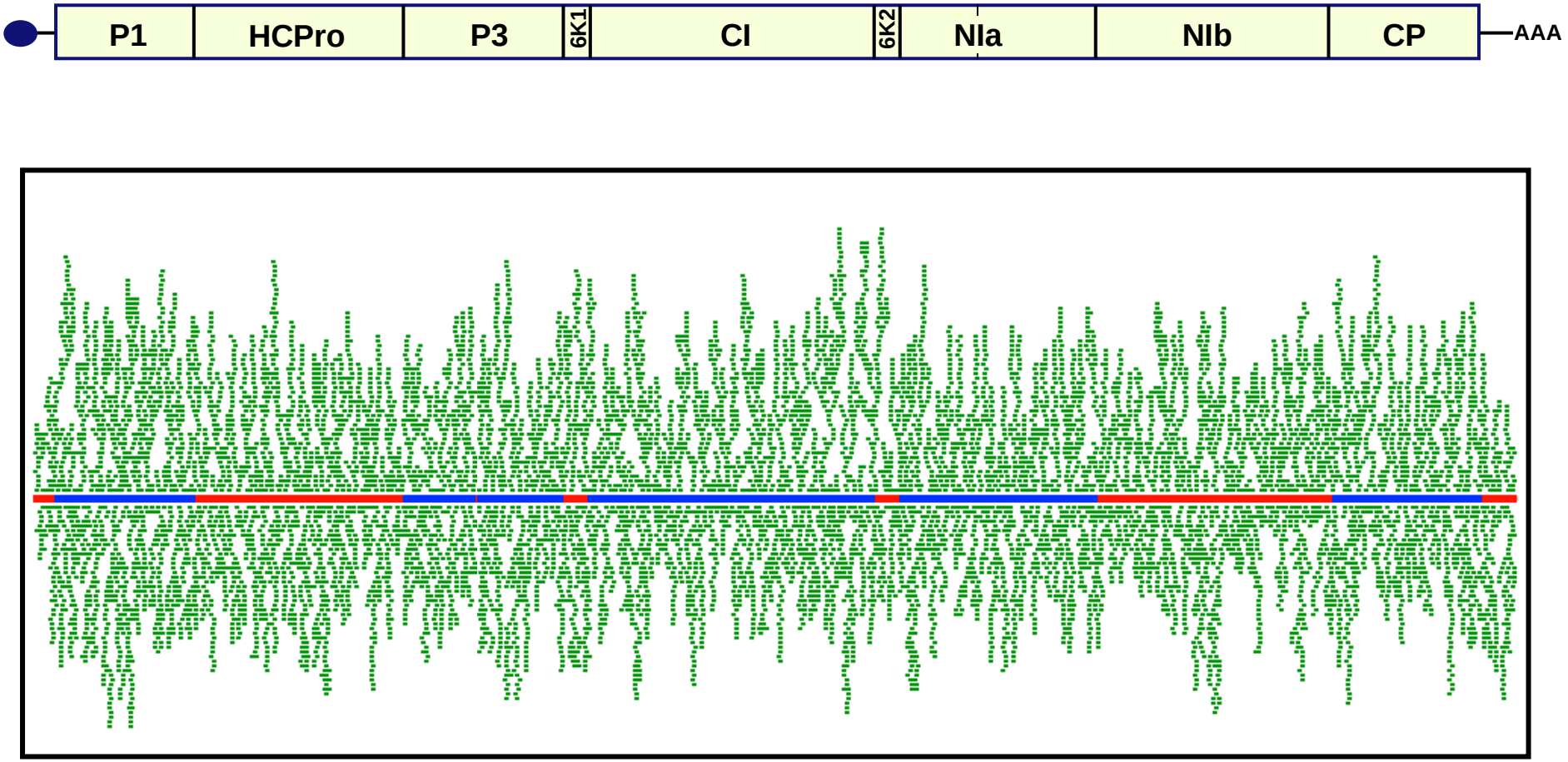


Deep-sequencing of PPV small RNAs

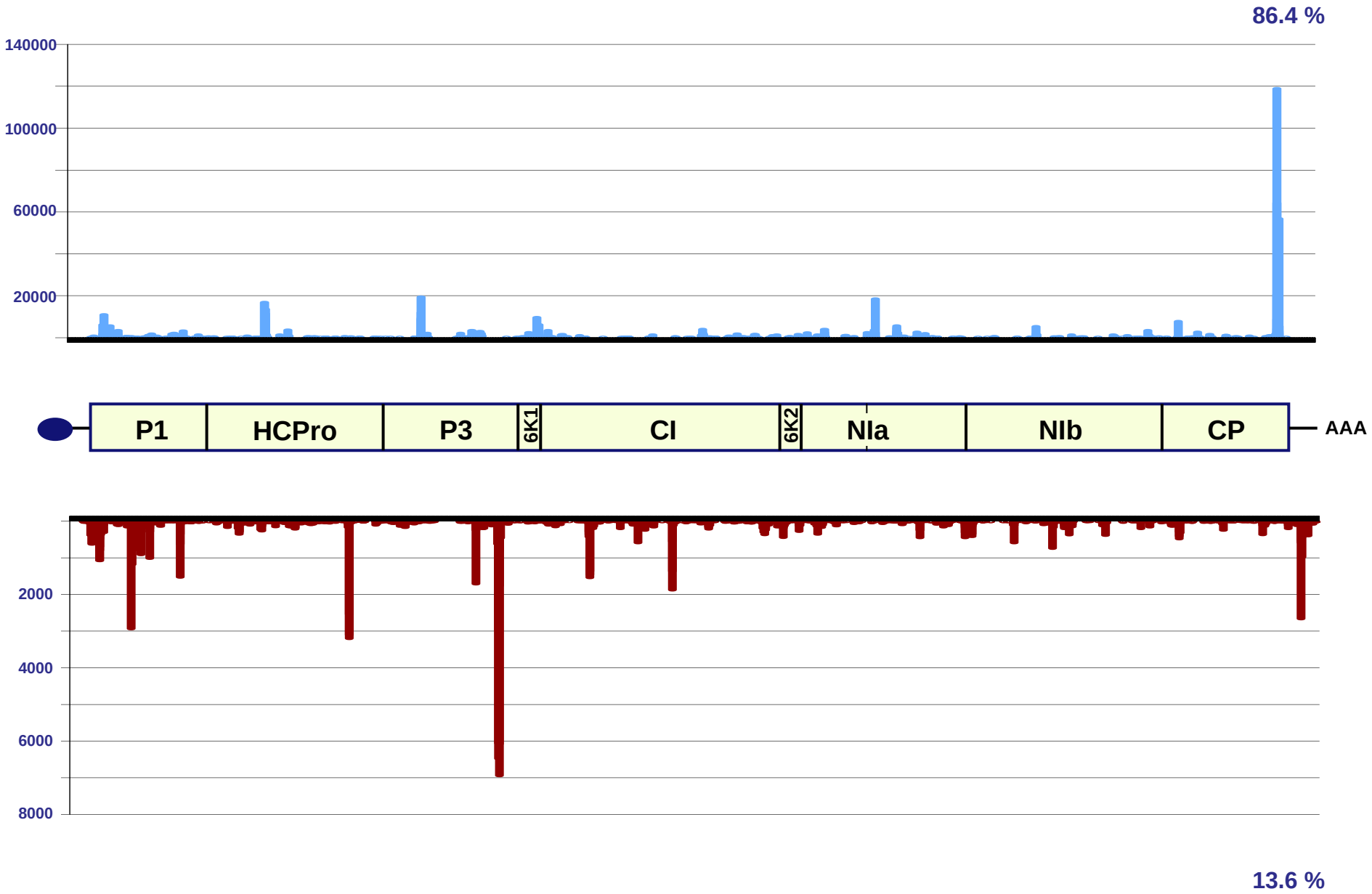
Solexa/Illumina ➲ 4.1-6.8 x 10^6 sequences



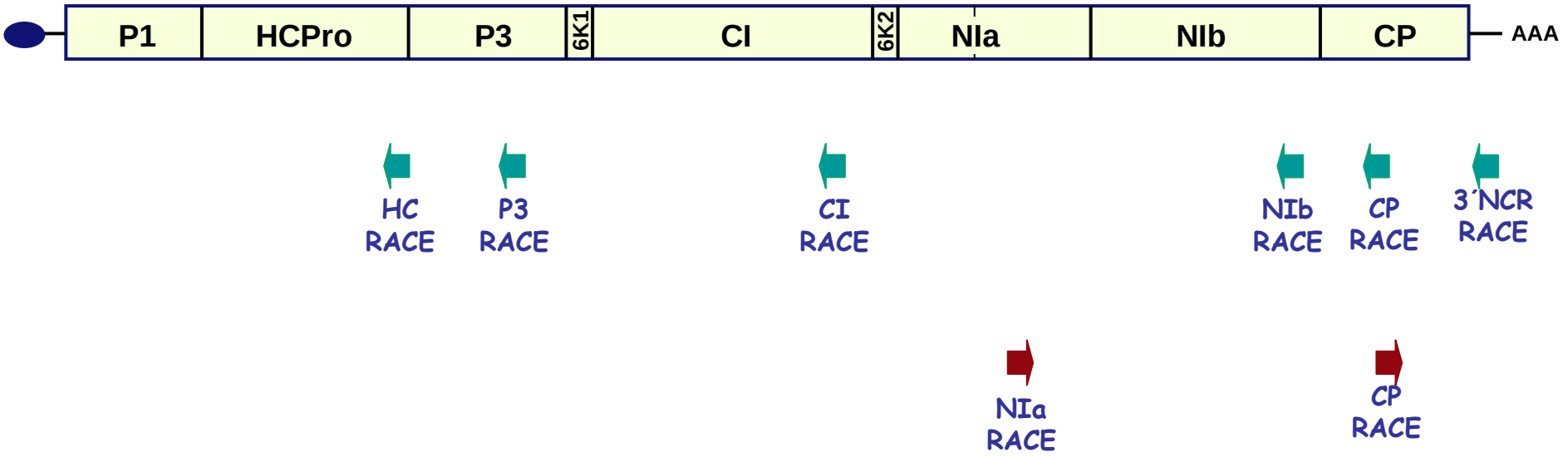
PPV small RNAs are distributed throughout the viral genome



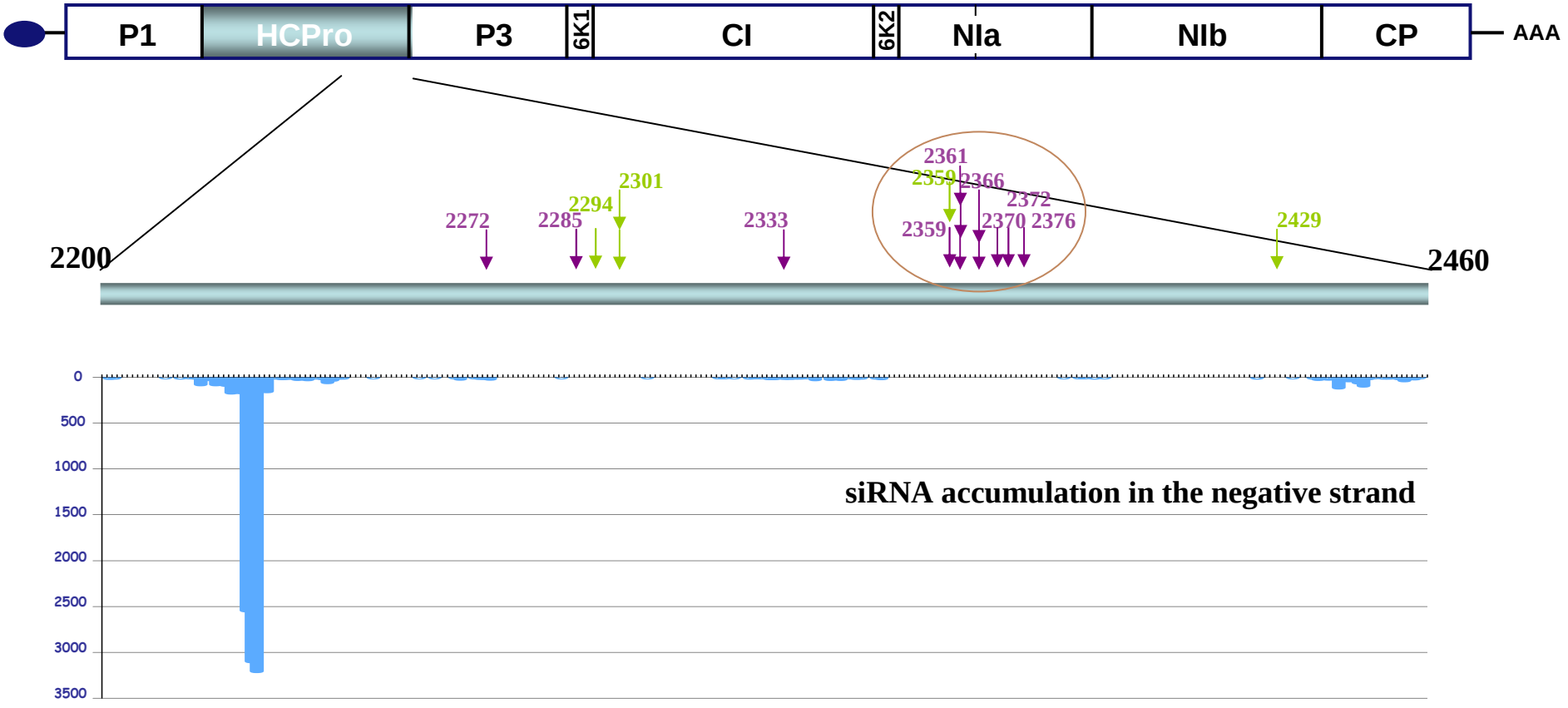
PPV small RNA are unevenly accumulated



Identification of RISC-mediated cleavages in PPV genome in natural infection by Rapid Amplification of cDNA Ends (RACE)



RACE in PPV-infected *N. benthamiana* plants



Preferred cleavage sites in PPV genome do not overlap with siRNA accumulation hot spots

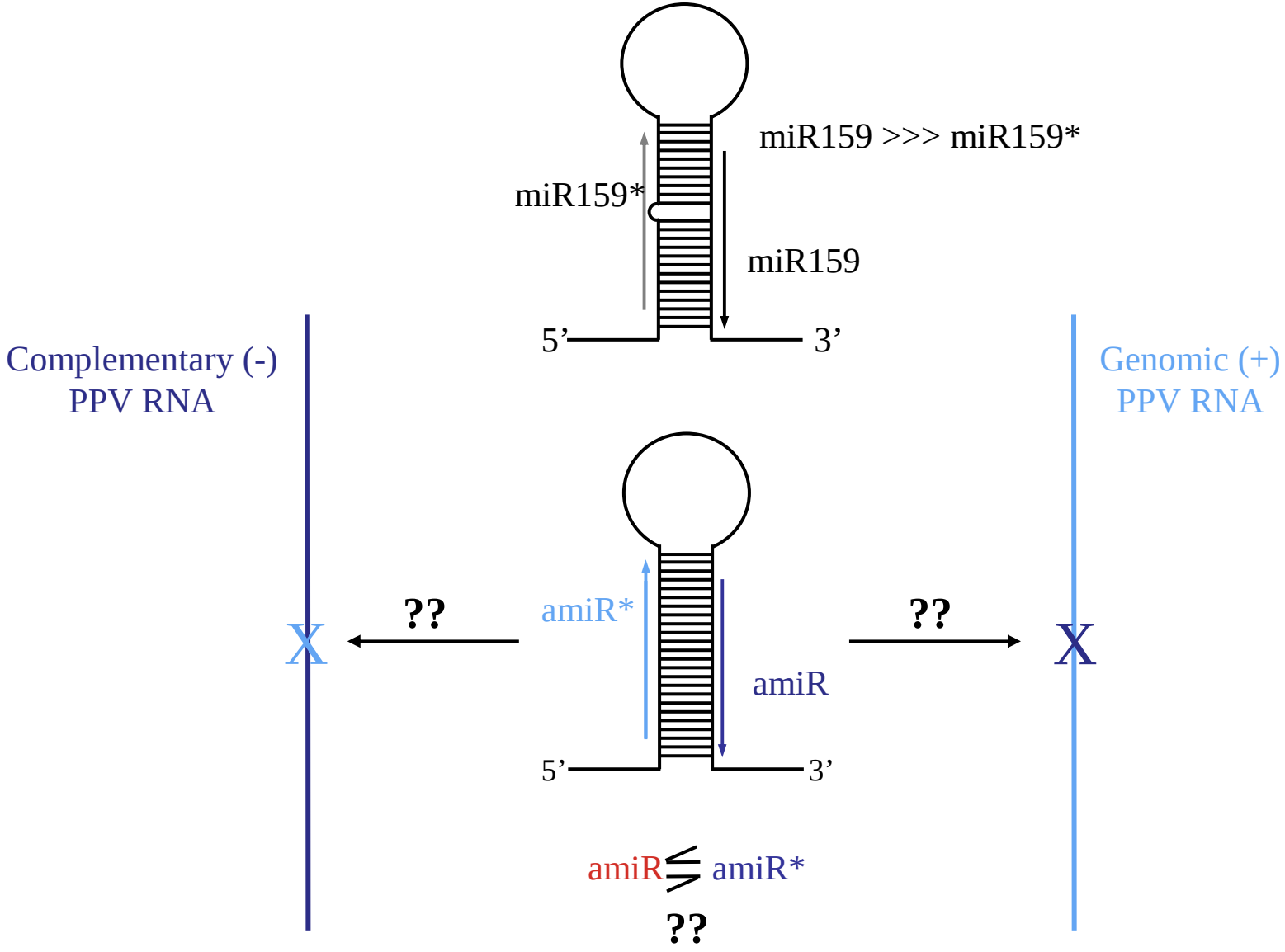
Selection of PPV sequences for amiRNA constructs

- Cleavages detected by RACE
- Accumulation of siRNAs during the natural infection
- Free energy at the ends of the target sequence
- Nucleotide at the 5´end

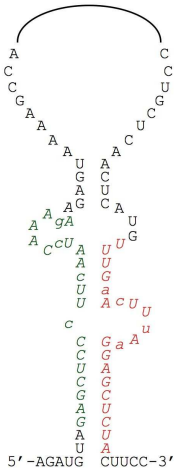
PPV sequences for amiRNA

		SEQUENCES	Energy 5' (-)/(+)	Cleavage site	vsiRNAs	
					–	+
NIb	A	5'UGCUUCAGUGUCAGUGU AAAG	9/6.8	8482 (10x)	1	142
	B	5'UUCUGUCUCAGAUGCUU CAGU	7.8/8	8494 (4x)	8	506
	C	5'UACGGGGCUUUUCCAUC UUUUU	9.3/7	8436 (6x)	13	13
CP	D	5'UUGGCAUGUAUGCUUUU UCAU	10.4/5.2	9302 (11x)	3	372
	Dm	5'UUGGCAUGUAUGCUUUU	10.4/6.8	9302		
	E	5'UUGCGCUGAAUUCCAUA GCUU	9.3/8	9321 (6x)	27	0
3´ NCR	F	5'CAGGUAGAGUUUAUGAU AGAU	9/7.1	9590 (3x)	62	17
	G	5'UAGACUCUCACCCAGGU AGAG	8.4/9.3	9602 (6x)	9	0
	H	5'AUGAUUAGACUCUCACCC AGG	7.1/11.2	9608 (9x)	57	8
	Hm	5'UUGAUUAGACUCUCACCC AGG	7.1/11.2	9608		

amiRNA structure



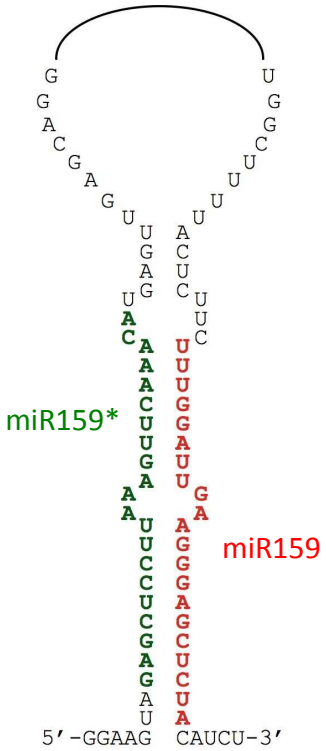
miR159 expression



5′-AGAUGUA*GAGCUCCcUUcAAUcCAAAgA*AGAGUAAAAGCCA-110-CCUGCUCAACUCATG*UUUGaAcUUuAaGGAGCUCUA*CUUCC-3′

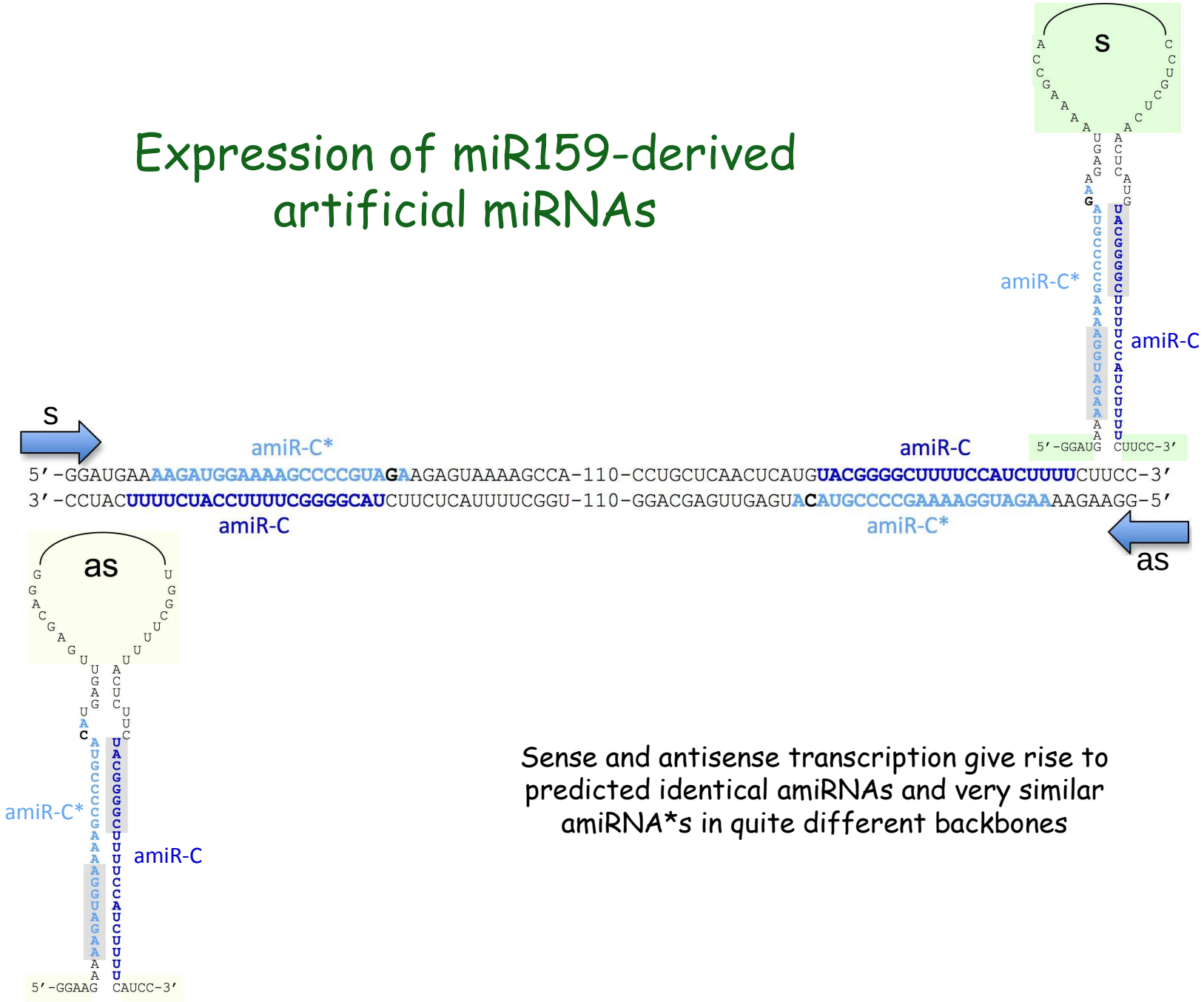
3′-UCUAC**AUCUCGAGGGAAGUUAGGUUU**CUUCUCAUUUUCGGU-110-GGACGAGUUGAGU**ACAAACUUGAAAUUCCUCGAG**AUGAAGG-5′

miR159 miR159*



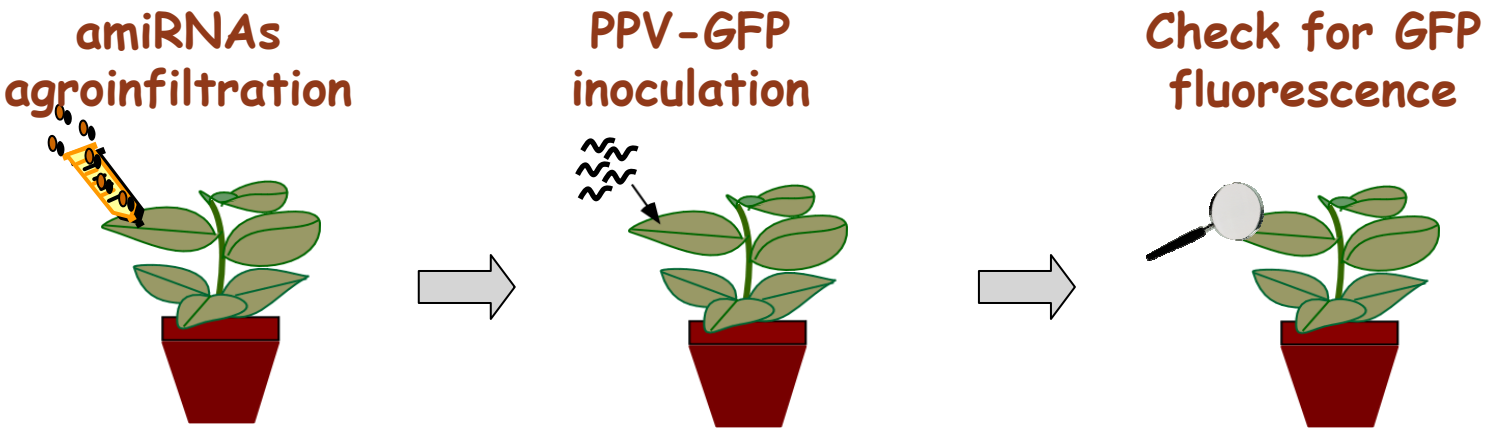
miR159 precursor is transcribed from the antisense strand of a genomic locus in Arabidopsis.

Expression of miR159-derived artificial miRNAs

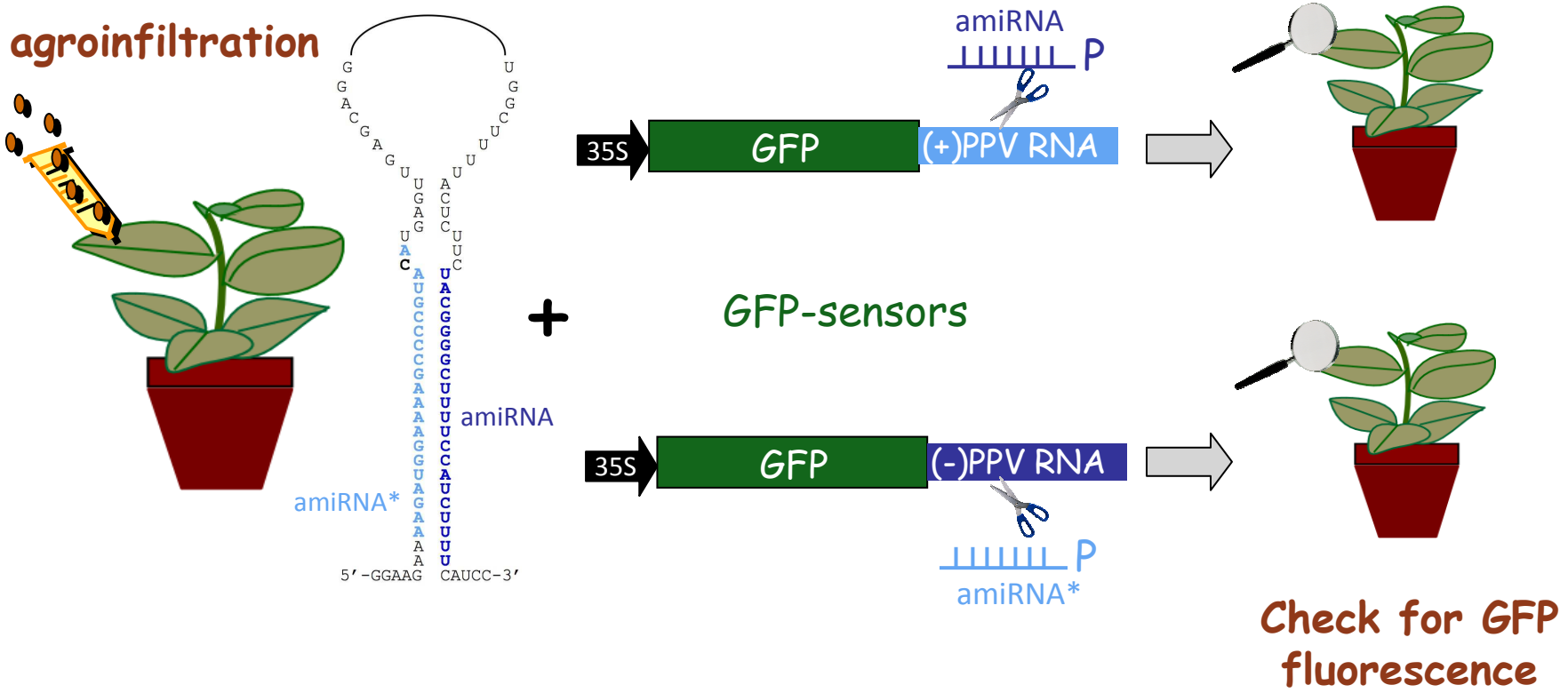


Sense and antisense transcription give rise to predicted identical amiRNAs and very similar amiRNA*s in quite different backbones

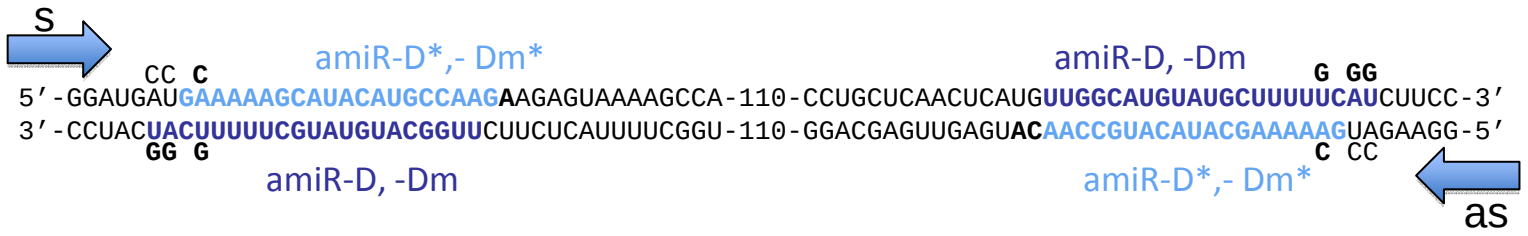
Bioassay to assess amiRNA antiviral activity

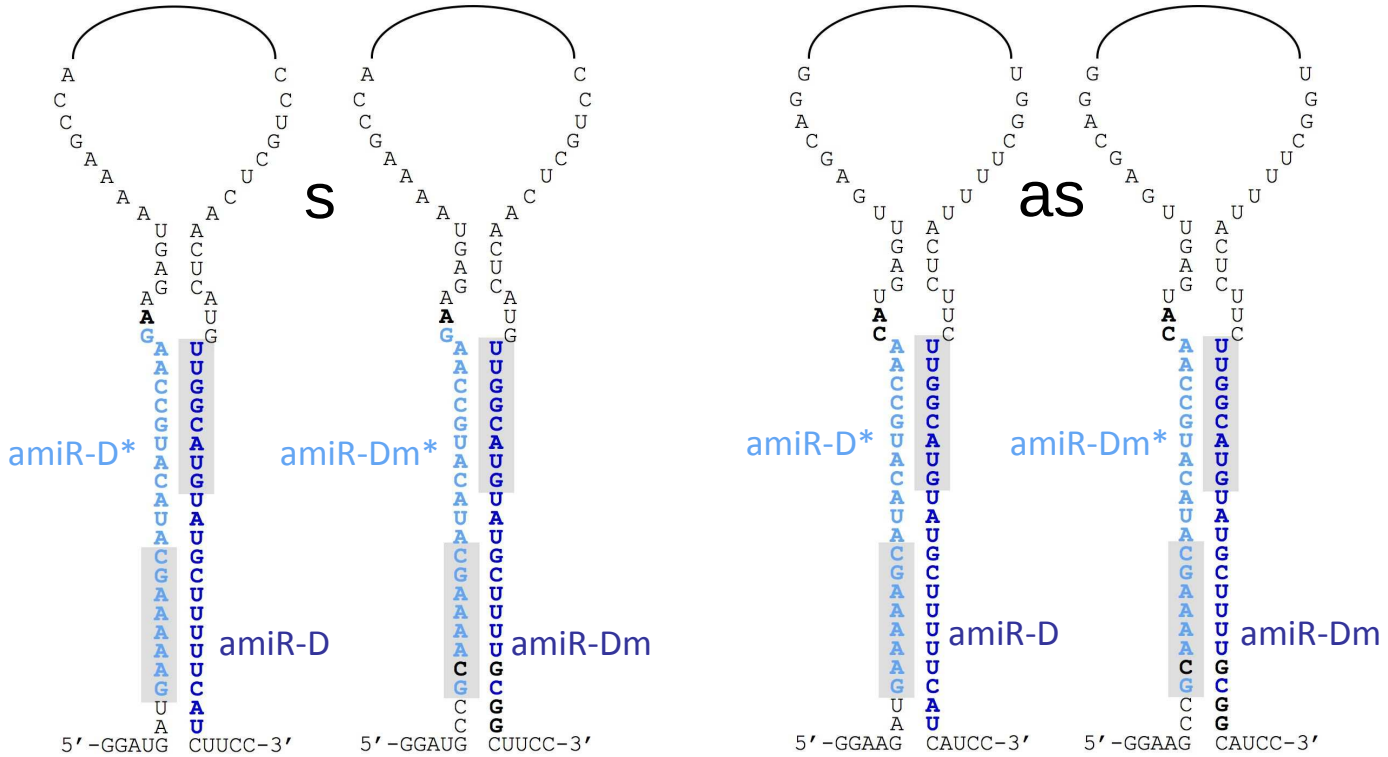


Bioassay to assess strand-specific cleavage of amiRNAs and amiRNA*s



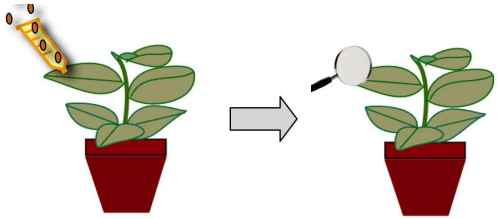
Structures of the amiR-D and amiR-Dm precursors





ΔG 5’	-5.2/-10.4	**-6.8**/-10.4	-5.2/-10.4	**-6.8**/-10.4

Cleavage activity of amiR-D and amiR-Dm on GFP sensors



Sense transcript

Anti sense transcript

vector

amiRNA-D

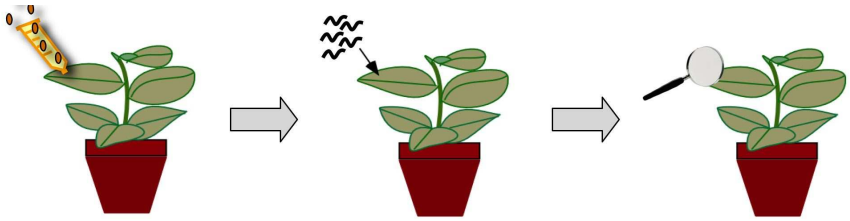
amiRNA-Dm

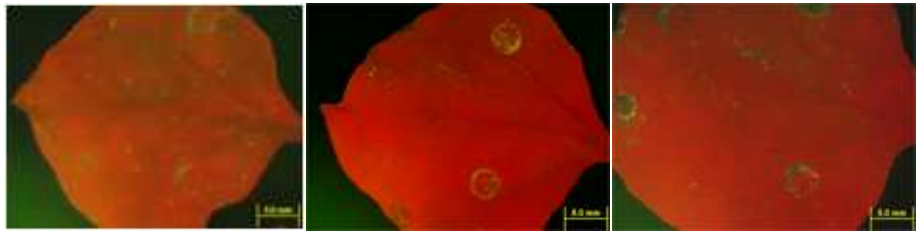
vector

amiRNA-D

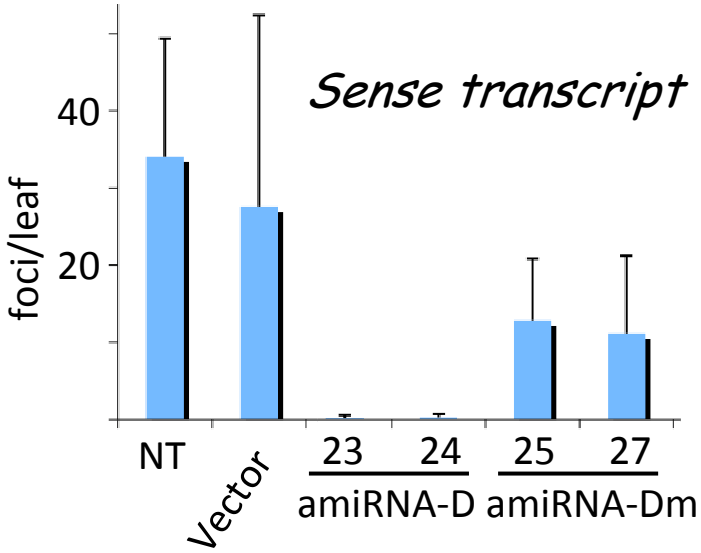
amiRNA-Dm

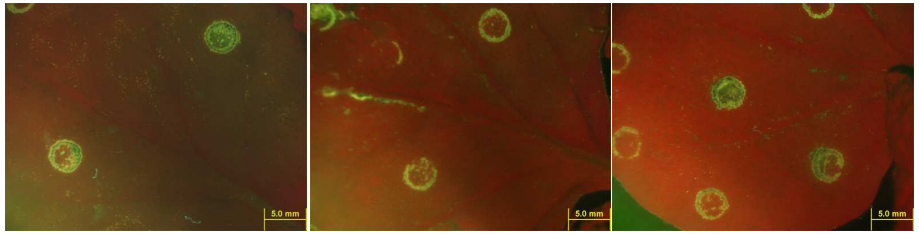
amiRNAs differs in their capacity to interfere with PPV infection



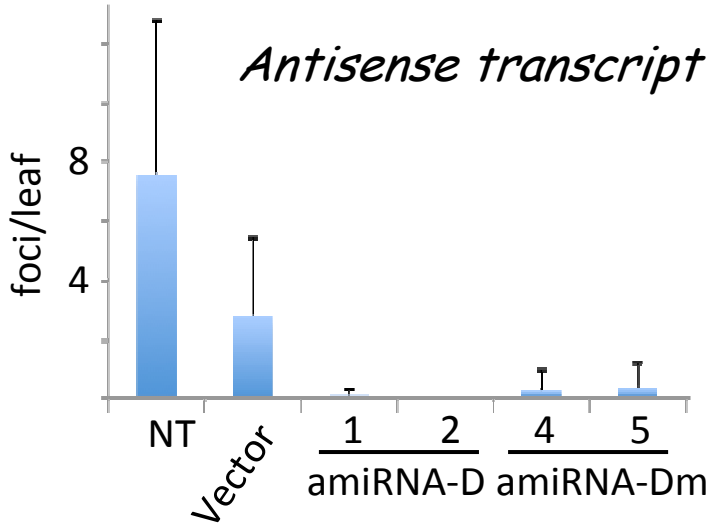


vector amiRNA-D amiRNA-Dm





vector amiRNA-D amiRNA-Dm



Features and activity of anti-PPV amiRNA

		SEQUENCES	Δ 5' (-)/(+)	Cleavage site	vsiRNAs		Cleavage on sensors (s/as)		Protection (s/as)
					–	+		*	
NIb	A	5'UGCUUCAGUGUCAGUGU AAAG	9/6.8	8482 (10x)	1	142	–/+	+/+	+/+++
	B	5'UUCUGUCUCAGAUGCUU CAGU	7.8/8	8494 (4x)	8	506	+/+	–/+	+++/+++
	C	5'UACGGGGCUUUUCCAUC UUUU	9.3/7	8436 (6x)	13	13	–/+	+/±	+++/+++
CP	D	5'UUGGCAUGUAUGCUUUU UCAU	10.4/5.2	9302 (11x)	3	372	+/+	+/–	+++/ +++
	Dm	5'UUGGCAUGUAUGCUUUU	10.4/6.8	9302			± / ±	– /–	±/+
	E	5'UUGCGCUGAAUUCCAUA CCUU	9.3/8	9321 (6x)	27	0	+/+	± / –	+++/+++
3' NCR	F	5'CAGGUAGAGUUUAUGAU AGAU	9/7.1	9590 (3x)	62	17	–/±	–/+	+/+++
	G	5'UAGACUCUCACCCAGGU	8.4/9.3	9602 (6x)	9	0	–/–	–/+	–/–
	H	5'AUGAUUAGACUCUCACCC AGAG	7.1/11.2	9608 (9x)	57	8	–/+	–/–	–/+++
	Hm	5'UUGAUUAGACUCUCACCC AGG	7.1/11.2	9608			–/+	–/+	–/ +++

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	C	5'UACGGGGCUUUUCCAUC UUUU	9.3/7	8436 (6x)	13	13	–/+	+/±	+++/+++
CP	D	5'UUGGCAUGUAUGCUUUU UCAU	10.4/5.2	9302 (11x)	3	372	+/+	+/–	+++/ +++
	Dm	5'UUGGCAUGUAUGCUUUU	10.4/6.8	9302			± / ±	– /–	±/+
	E	5'UUGCGCUGAAUUCCAUA CCUU	9.3/8	9321 (6x)	27	0	+/+	± / –	+++/+++
3' NCR	F	5'CAGGUAGAGUUUAUGAU AGAU	9/7.1	9590 (3x)	62	17	–/±	–/+	+/+++
	G	5'UAGACUCUCACCCAGGU AGAG	8.4/9.3	9602 (6x)	9	0	–/–	–/+	–/–
	H	5'AUGAUUAGACUCUCACCC AGG	7.1/11.2	9608 (9x)	57	8	–/+	–/–	–/+++
	Hm	5'UUGAUUAGACUCUCACCC AGG	7.1/11.2	9608			–/+	–/+	–/ +++

Features and activity of anti-PPV amiRNA

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	C	5'UACGGGGCUUUUCCAUC UUUU	9.3/7	8436 (6x)	13	13	–/+	+/±	+++/+++
CP	D	5'UUGGCAUGUAUGCUUUU UCAU	10.4/5.2	9302 (11x)	3	372	+/+	+/–	+++/ +++
	Dm	5'UUGGCAUGUAUGCUUUU	10.4/6.8	9302			± / ±	– /–	±/+
	E	5'UUGCGCUGAAUUCCAUA CCUU	9.3/8	9321 (6x)	27	0	+/+	± / –	+++/+++
3' NCR	F	5'CAGGUAGAGUUUAUGAU ACAU	9/7.1	9590 (3x)	62	17	–/±	–/+	+/+++
	G	5'UAGACUCUCACCCAGGU ACAC	8.4/9.3	9602 (6x)	9	0	–/–	–/+	–/–
	H	5'AUGAUUAGACUCUCACCC ACC	7.1/11.2	9608 (9x)	57	8	–/+	–/–	–/+++
	Hm	5'UUGAUUAGACUCUCACCC AGG	7.1/11.2	9608			–/+	–/+	–/ +++

Next steps…

- Assessing the efectiveness of the amiRNAs against different PPV isolates

- Preparing new amiRNA constructs to challenge hypothesis derived from the current analyses

- Transgenic expression of the most promising anti-PPV amiRNAs

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