Quantification of PSTVd specific siRNAs by RT qPCR

Results presented here were obtained with the aid of infrastructure acquired through MOBITAG project (FP7-REGPOT-2008-1, GA 229518), that helped us to collaborate with:

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- specialist on bioinformatics and analysis of viroids
- visited laboratory of RNDr. J. Matoušek CSc. (Institute of Plant Molecular Biology, BC ASCR, České Budějovice, CZ) and presented his work on April 23, 2009 within project **MOBITAG**
- selected 7 abundant viroid specific siRNAs and suggested amplicons for following quantitations

Quantification of PSTVd specific siRNAs by RT qPCR

Putative mechanism of pathogenic reactions
- viroid interacts with host post-transcriptional gene-silencing (PTGS) system via **viroid-specific siRNA**

PSTVd strains (potato spindle tuber viroid) **tested:** AS1, C3

Model plants tested: *Lycopersicum esculentum* (cv. Rutgers)
Nicothiana benthamiana
Matricaria chamomilla (cv. Bona)

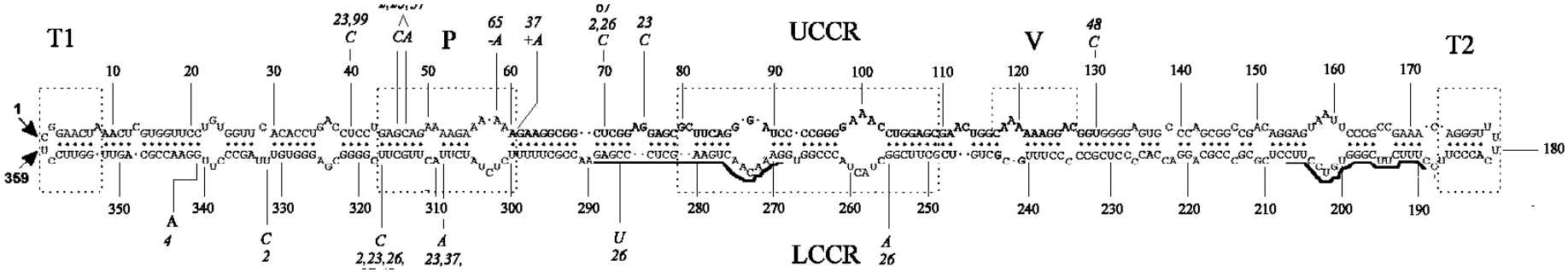
Observations:
- AS1 x C3 differs in P domain, but have similar pathogenic reactions
- same viroid strain induces different pathogenic reactions in different plant species

Hypothesis: reason are different abundances of viroid-specific siRNA compatible to different parts/domains of viroid

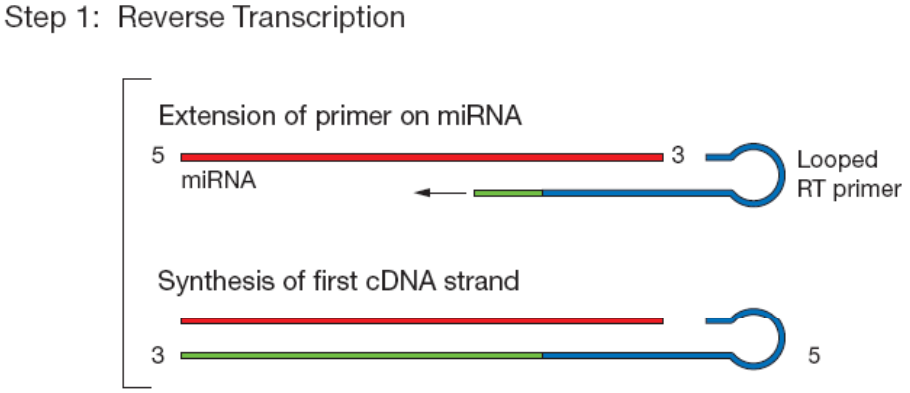
Selection of 7 viroid-specific siRNA species that accumulate during AS1 propagation in the host *Lycopersicum esculentum*

	siRNA name	compatible to
1.	5-25-193	T1 (terminal) domain
2.	17-36-73	region between T1 and P domain
3.	188-209-57	close to T2 domain
4.	258-278-65	CCR (central conserved) domain
5.	292-311-AS1/C3	P (pathogenicity) domain
6.	293-312-140	P (pathogenicity) domain
7.	301-321-288	P (pathogenicity) domain

PSTVd domains:



Detection system - Real-Time RT PCR, TaqMan probes



RT primer

- 1st part is specific to certain siRNA
- 2nd part is common (= loop)

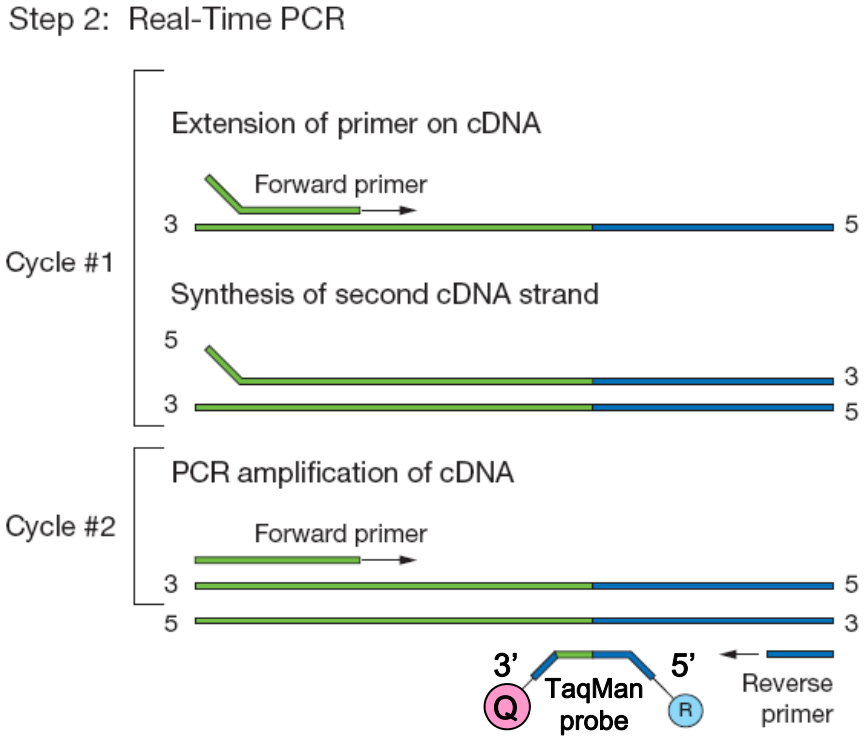
PCR oligos

- **forward** - specific to certain siRNA
- **reverse** - common (comp. to loop)
- **TaqMan probe:**

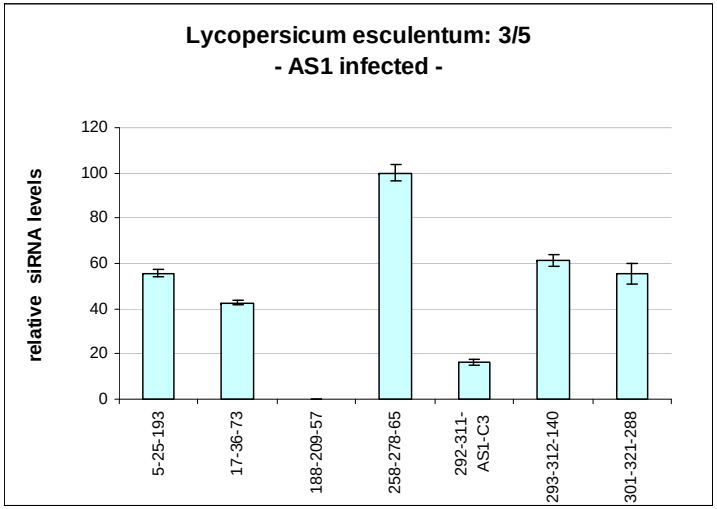
R (reporter) – FAM fluorescent dye

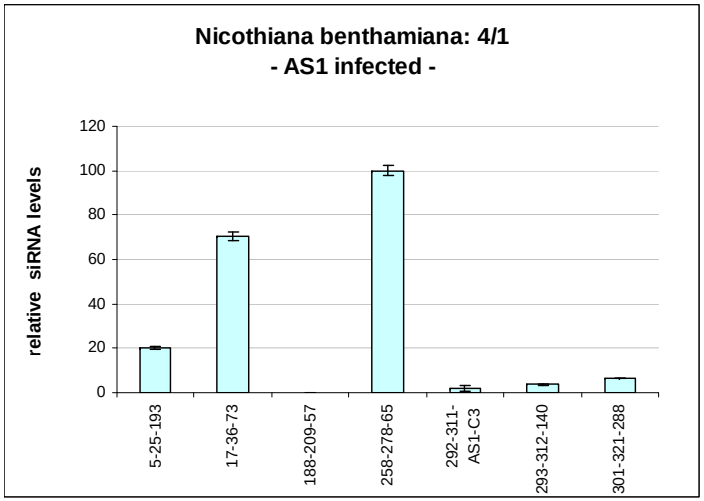
Q (quencher) - suppress reporter fluorescence

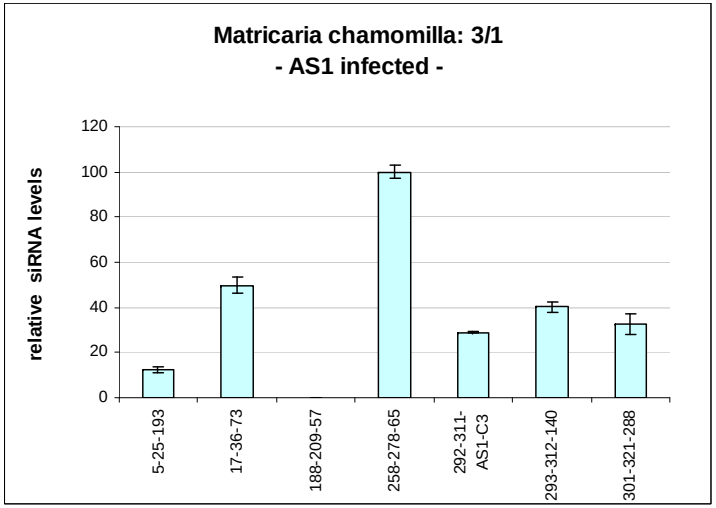
PCR product = about 50bp



Comparison of 7 siRNAs profiles in AS1 infected plants







Results – relative abundances:

- **<u>258-278-65</u> (CCR)** dominates in all 3 plants (=100%)
- **<u>188-209-57</u>** (close to T2) was not detected (=0%)

- **<u>5-25-193</u>** (T1) - 15-20% in MC & NB, 55% in LE
- **<u>17-36-73</u>** (btw T1/P) - 40-50% in LE & MC, 70% in NB

- **<u>293-311-AS1/C3</u>** & **<u>293-312-140</u>** & **<u>301-321-288</u>** (P)
- similar levels in LE and MC, lowered in NB