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## Development of a DNA-based real-time PCR assay to quantify *Allorhizobium vitis* overtime in grapevine plantlets

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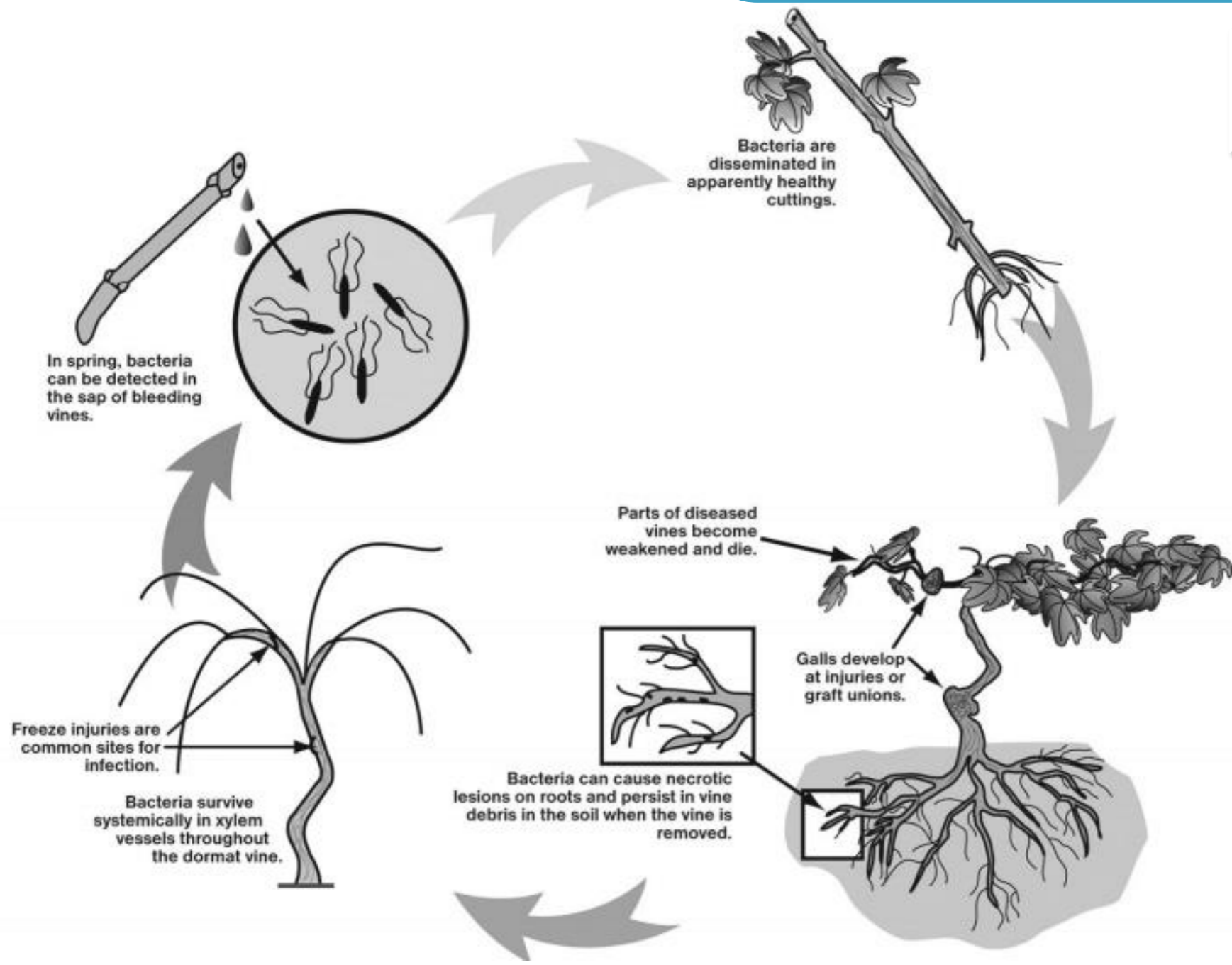
**<https://hal.univ-reims.fr/hal-03122636v1>**

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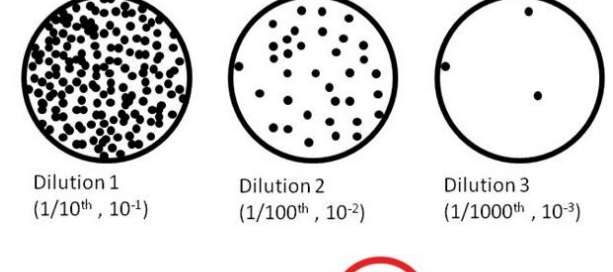
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## Background

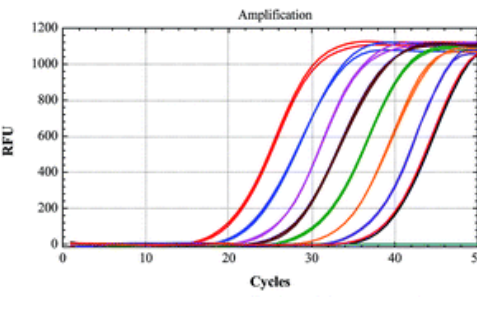


### PLATE COUNTING



- ✓ Simple
- ✓ Reliable
- ✓ Quantify living bacterial cells
- ✗ Non-storable samples
- ✗ Contamination
- ✗ Technical repetitive mistakes
- ✗ Risk of false-negative
- ✗ Time-consuming
- ✗ Intensive labor

### DNA-BASED qPCR



- ✓ Storable samples
- ✓ Sensitive
- ✓ Accurate, specific
- ✓ Rapid
- ✗ No distinction between living/dead cells
- ✗ Risk of overestimation

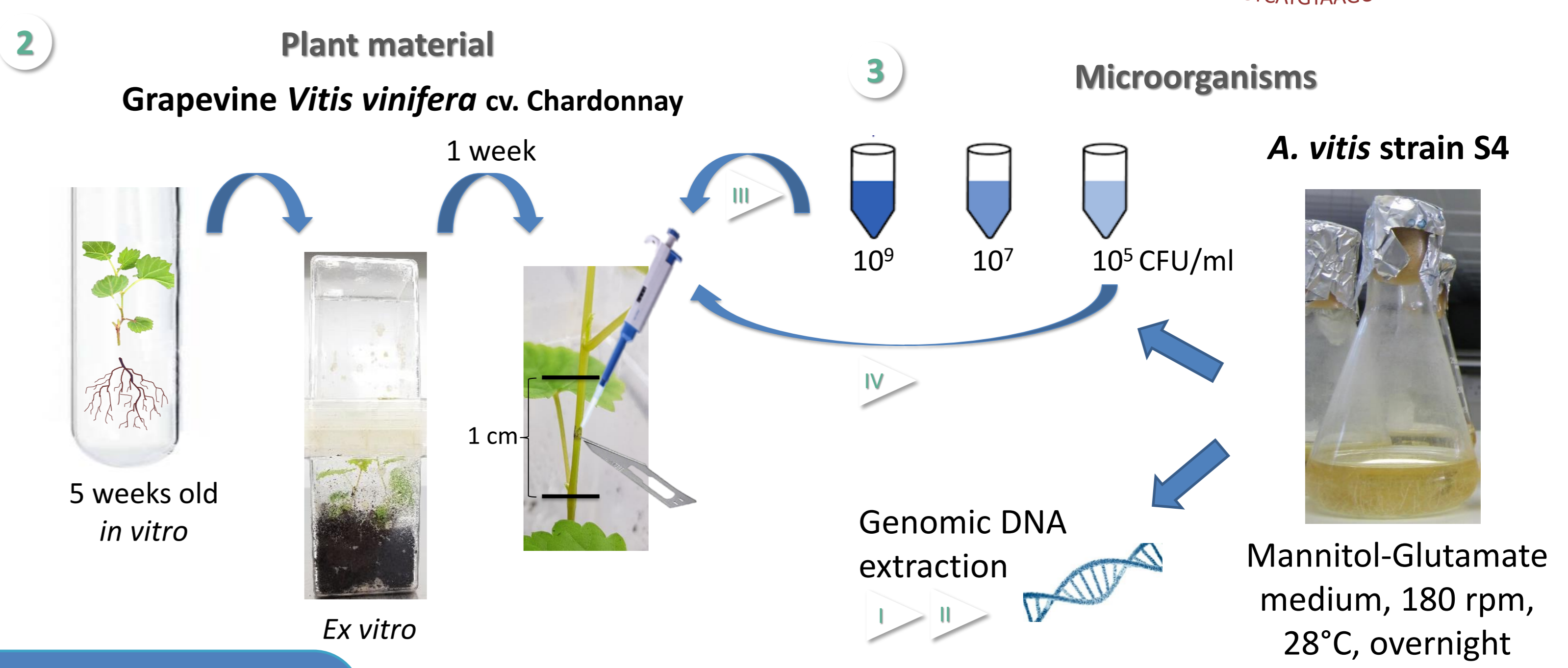
(Ross and Somssich 2016)

- Crown gall: important disease of grapevine worldwide, causing yield decrease and even plant death (Burr et al. 1998; Schroth et al. 1988)
- Causal agent: *Allorhizobium vitis* (previously named *Agrobacterium vitis*, Mousavi et al. 2014)
- Risk of spreading and persisting in vineyard (Kuzmanović et al. 2018) → need of detection of pathogen
- Study the interaction host-pathogen → need of quantification of bacterial population

## Methods

### Objectives

Develop DNA-based qPCR as an alternative method for ① detection and ② quantification of *A. vitis* in grapevine plantlets by comparing and validating with plate counting assays



### Primer pairs

**Avi\_1889**: 5'-TTCTGCATCGACCCGAAAT-3' / 5'-GCGAAACATCGCTCCAAA-3'  
**Avi\_5072**: 5'-CGTCTGGCTGATCTGGTT-3' / 5'-GATTGATGCTTCTCCGCT-3'  
**Avi\_7106**: 5'-CGCGGAACACCCCTTATTA-3' / 5'-ACCGTTGGCATGATCTCGAA-3'  
**virD59**: 5'-ATTGGAATATCTGTCCG-3' / 5'-GGCGAGATCGCGATATT-3'  
**virD3**: 5'-GGTGTGTCAGCATCAA-3' / 5'-AAGCAAACATTTCCGCC-3'  
**virD3cons**: 5'-AATCCGGAGGTGATGGT-3' / 5'-GGCTCATGTAAGCGTTG-3'

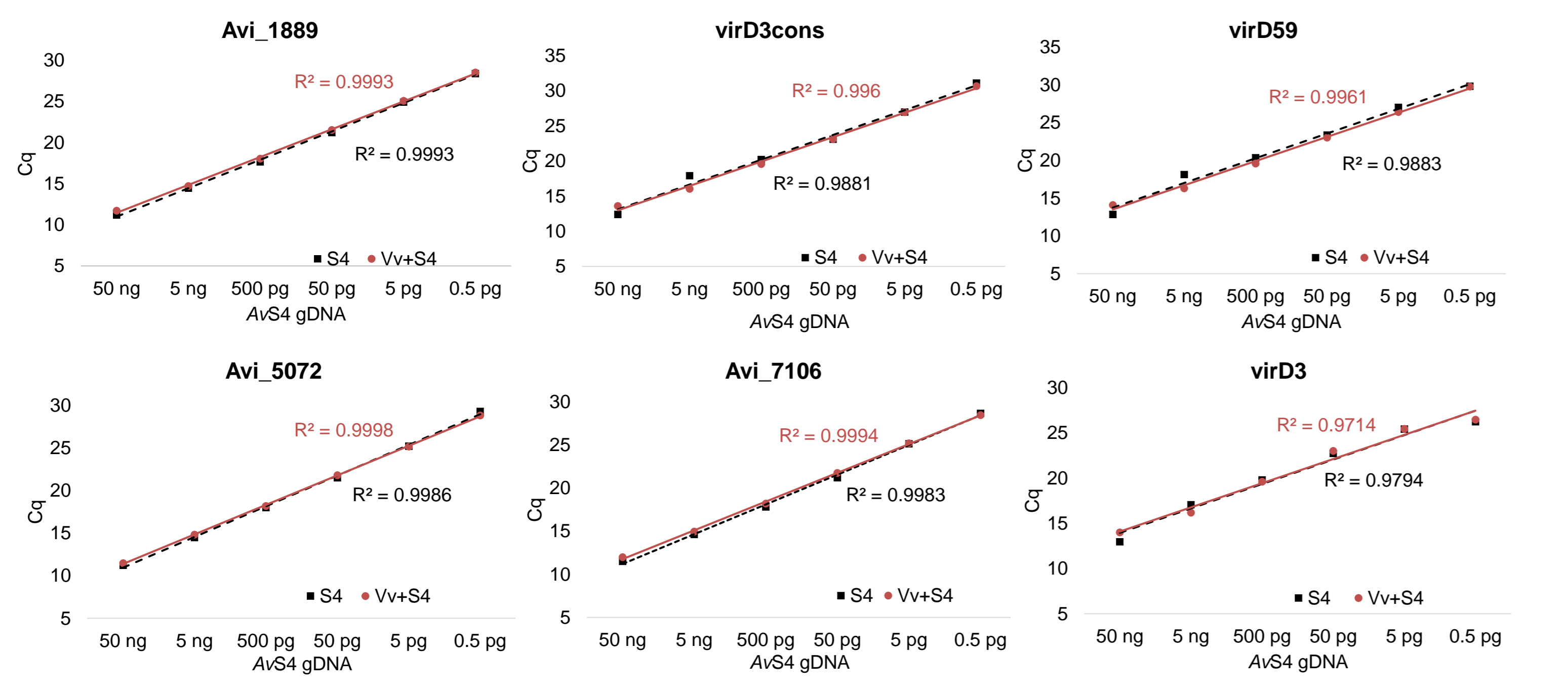
## Results

### I Primer specificity

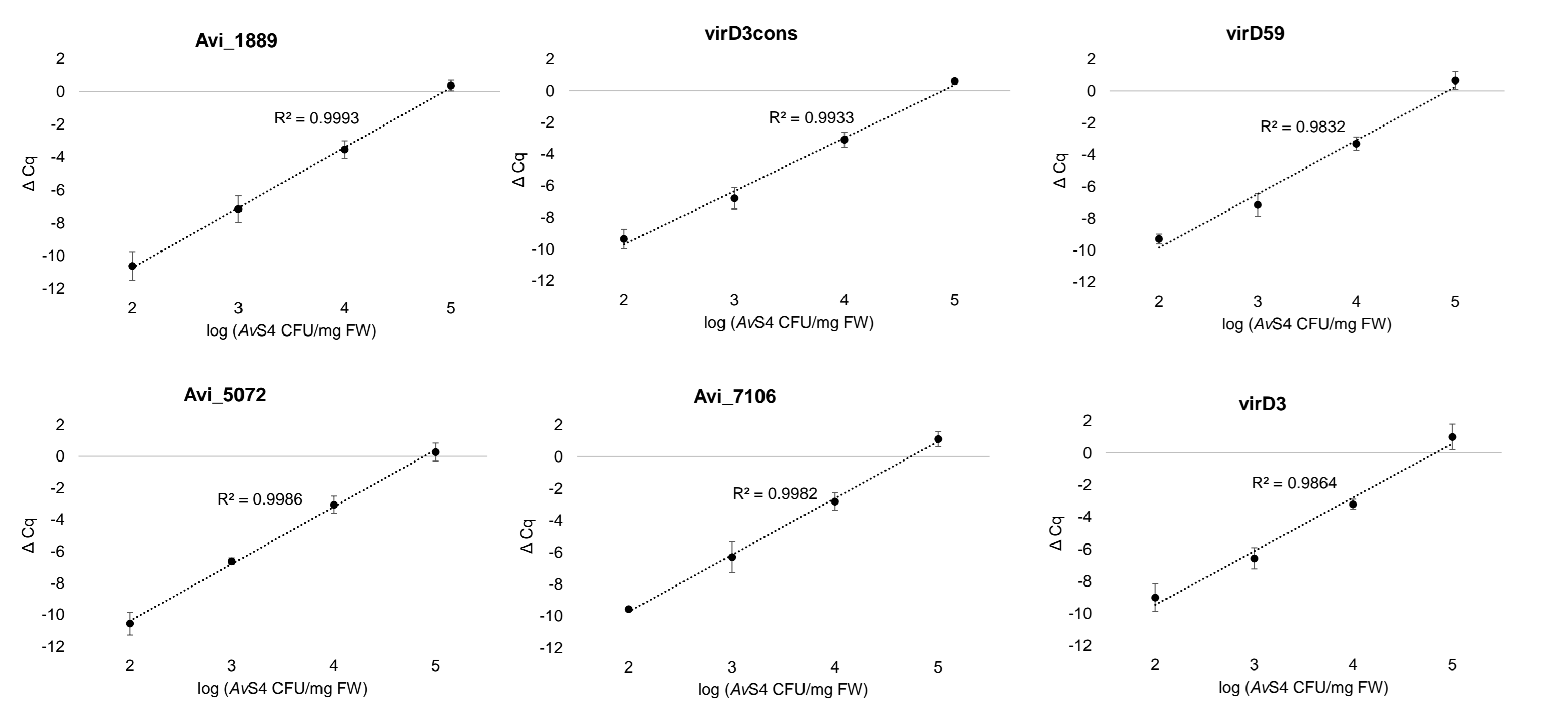
	<i>A. vitis</i> strains					Non- <i>A. vitis</i> spp.					
	<i>A. vitis</i> S4	<i>A. vitis</i> BT2-2	<i>A. vitis</i> BT3-1	<i>A. vitis</i> ET2-10	<i>A. vitis</i> KT1-1	<i>A. fabrum</i> C58	<i>A. rhizogenes</i> K84	<i>B. thailandensis</i>	<i>E. coli</i>	<i>P. brassicacearum</i> NFM 421	<i>S. meliloti</i> 1021
Avi_1889	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
Avi_5072	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
Avi_7106	✓	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗
virD3	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
virD59	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
virD3cons	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗

➤ Primers are specific for *A. vitis* S4 and some other strains of *A. vitis*.

### II Primer sensitivity

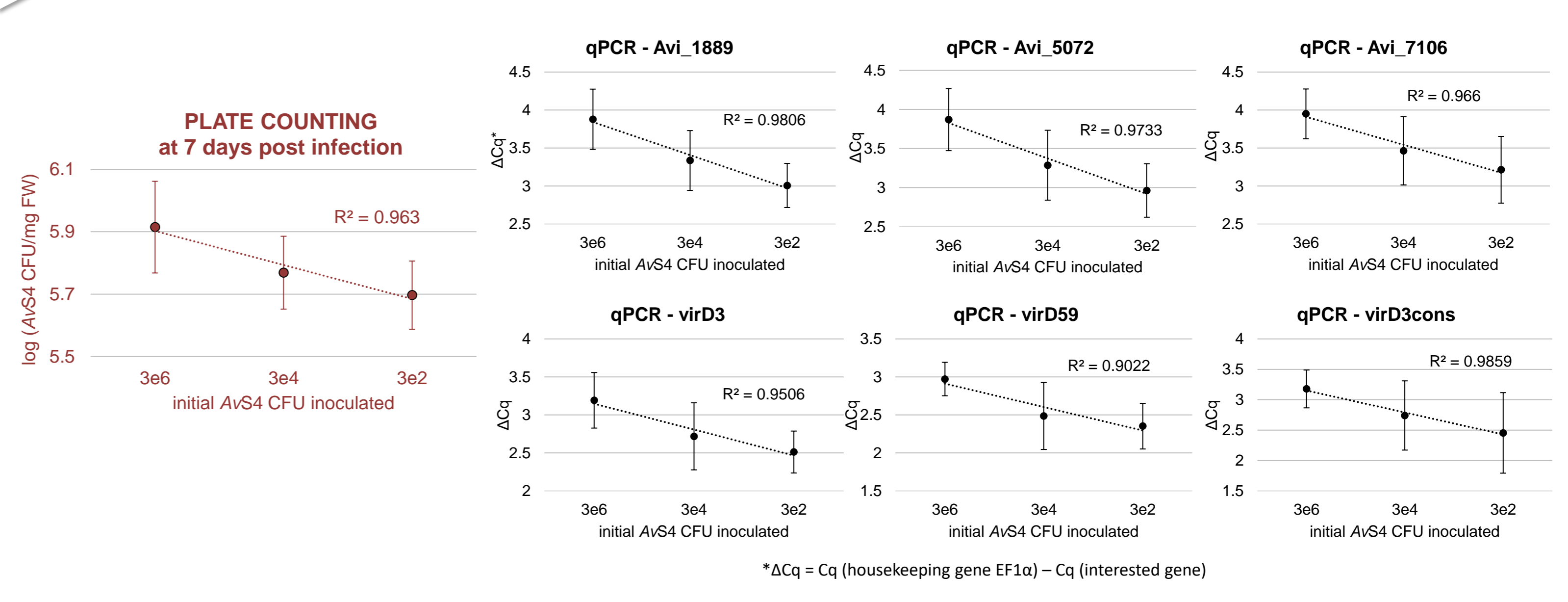


➤ Primers can quantify different levels of biomass of *A. vitis* S4.  
 ➤ Their activity is not influenced by plant gDNA matrix.

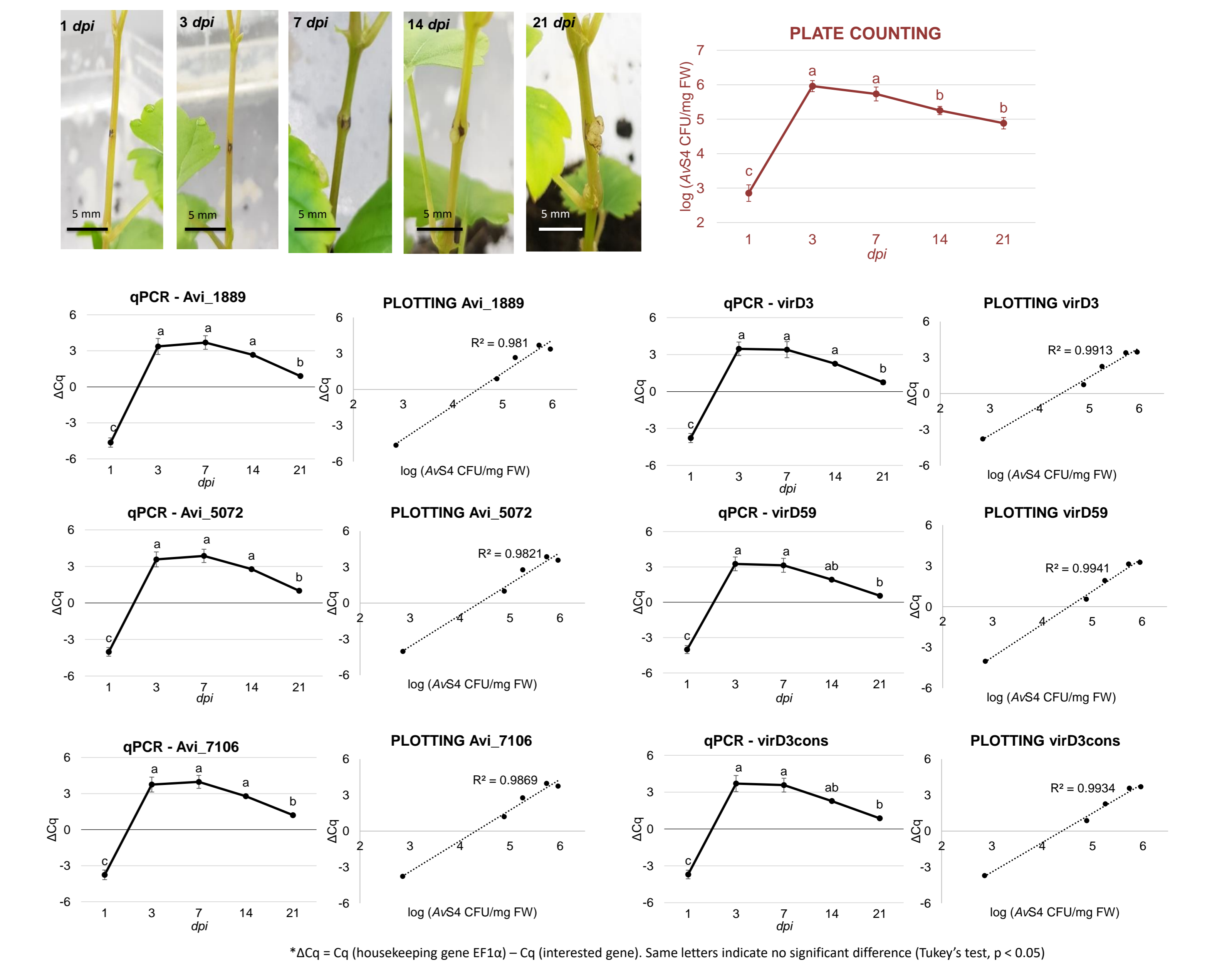


➤ Primers can detect until 300 *A. vitis* S4 cells per mg grapevine stem fresh weight.

### III Quantification of *A. vitis* S4 populations infected from different quantities



### IV Monitoring *A. vitis* S4 populations in grapevine plantlets



➤ qPCR method gave similar results as plate counting assays in quantifying *A. vitis* S4 populations in grapevine plantlets over time.

### Conclusion

qPCR as the alternative method for quantifying *A. vitis* S4 in grapevine plantlets thanks to its accuracy, rapidity, flexibility, sensitivity and specificity over plate counting assay.