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

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# DEVELOPMENT OF A DNA-BASED REAL-TIME PCR ASSAY TO QUANTIFY *ALLORHIZOBIUM VITIS* IN GRAPEVINE PLANTLETS

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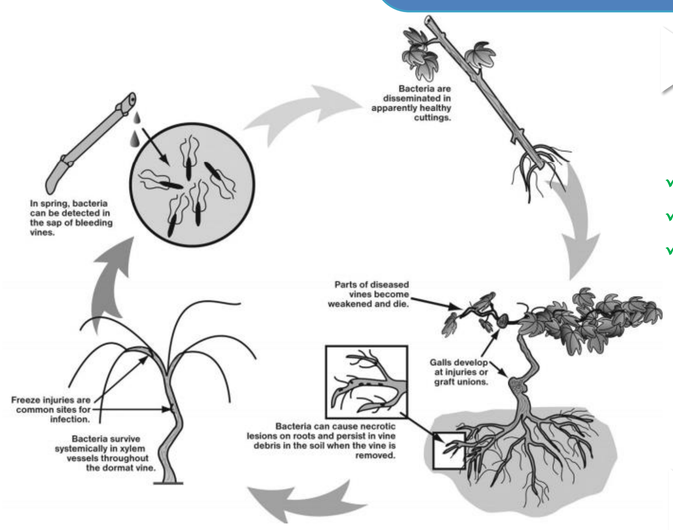
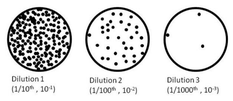
## Background



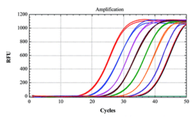

- **Crown gall**: important disease of grapevine worldwide, causing yield decrease and even plant death (Burr et al. 1998; Schroth et al. 1988)
- Causal agent: ***Allorhizobium vitis*** (previously named *Agrobacterium vitis,* Mousavi et al. 2014)
- Risk of spreading and persisting in vineyard (Kuzmanović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

**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)

## Methods

### Objectives

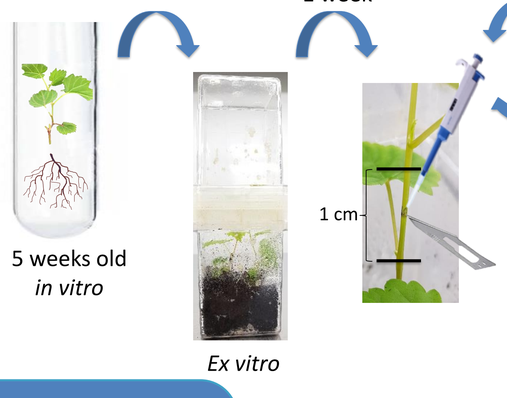
**Develop DNA-based qPCR as an alternative method for ① detection and ② quantification of *A. vitis* in grapevine plantlets by comparing and validating with plate counting assays**

**1 Primer pairs**

| | | | |
|---|---|---|---|
| Avi_1889 | 5'-TTCTGCATCGACCCGGAAAT-3' / 5'-GCGAAACATCCGCTCCAAAA-3' | 5'-ATTGGAATATCTGTCCCG-3' / 5'-GGCGAGATCGCGGATATT-3' | virD59 |
| Avi_5072 | 5'-CGTCTGGCTGATCTTGGGTT-3' / 5'-GATTGATCGCTTCCTCCGCT-3' | 5'-GGTGATGGTCCAGCATCCAA-3' / 5'-AAGCCAAACATTTTCCGCCC-3' | virD3 |
| Avi_7106 | 5'-CGGCGGAACACCCTTCATTA-3' / 5'-ACCGTTGGCATGATCTCGAA-3' | 5'-AATCCGGAGGTGATGGT-3' / 5'-GGCGTCATGTAAGCGTTG-3' | virD3cons |

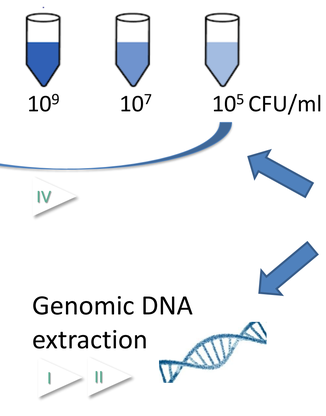
**2 Plant material**

**Grapevine *Vitis vinifera* cv. Chardonnay**



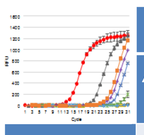

**3 Microorganisms**

***A. vitis* strain S4**




## Results

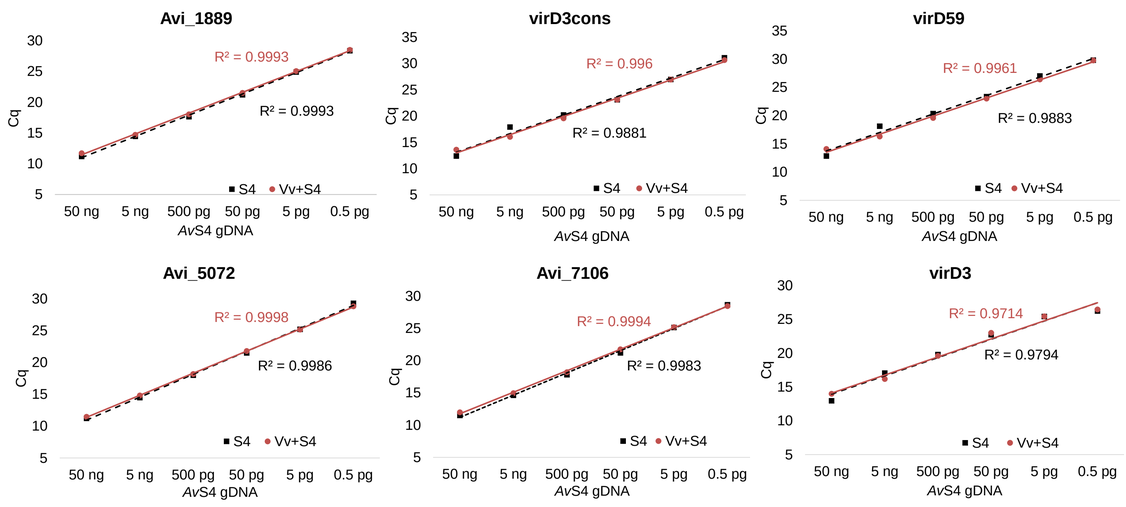
### I Primer specificity



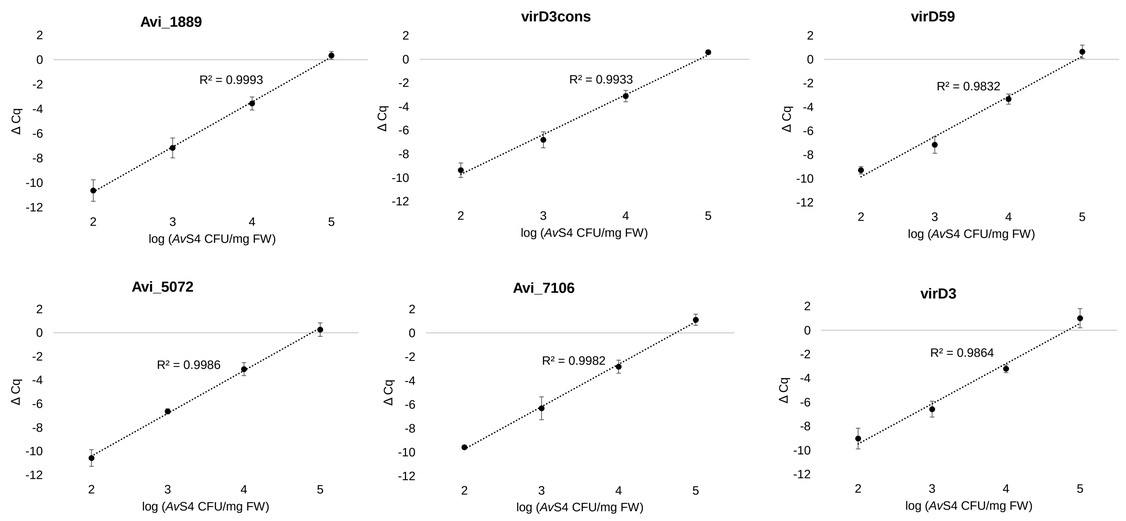
| | *A. vitis* strains | | | | | Non-*A. vitis spp.* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. vitis* S4 | *A. vitis* BT2-2 | *A. vitis* BT3-1 | *A. vitis* ET2-10 | *A. vitis* KT1-1 | *A. fabrum* C58 | *A. rhizogenes* K84 | *B. thailandensis* | *E. coli* | *P. brassicacearum* NFM 421 | *S. meliloti* 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

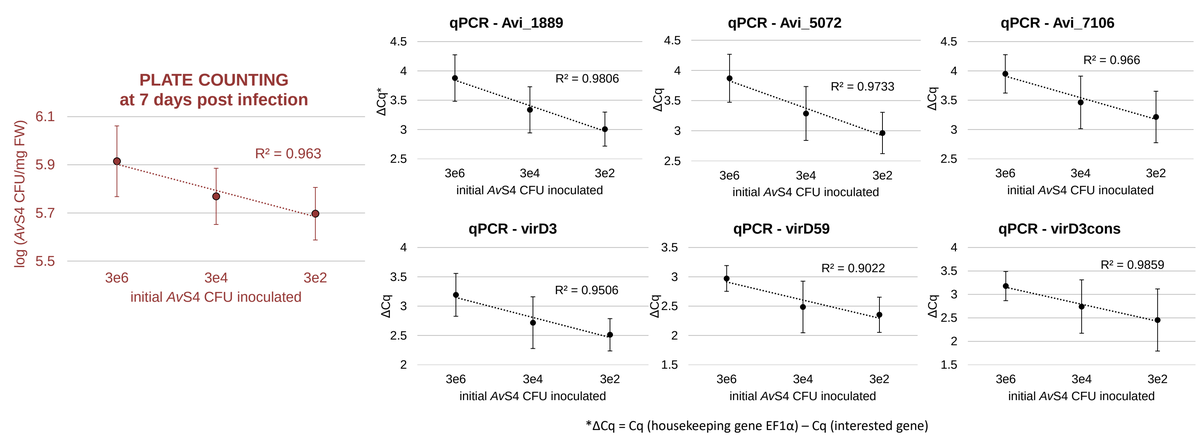



➢ Primers can quantify different levels of biomass of *A. vitis* S4.
➢ Their activity is not influenced by plant gDNA matrix.



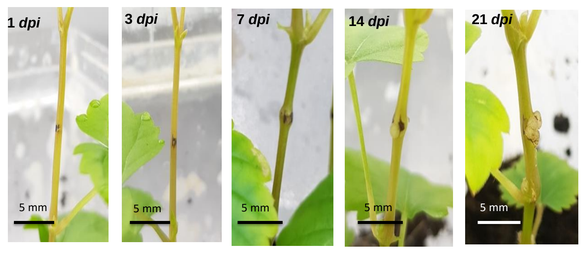

➢ Primers can detect until 300 *A. vitis* S4 cells per mg grapevine stem fresh weight.

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

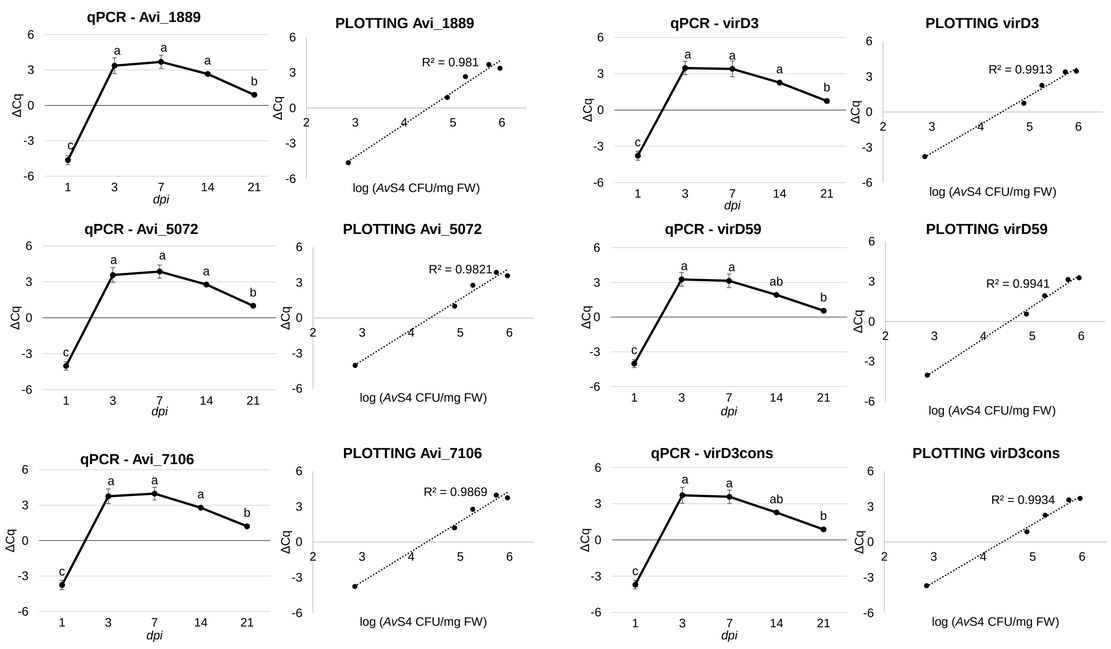



*ΔCq = Cq (housekeeping gene EF1α) – Cq (interested gene)

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








*ΔCq = Cq (housekeeping gene EF1α) – Cq (interested gene). Same letters indicate no significant difference (Tukey's test, $p < 0.05$)

➢ 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.**

### Acknowledgement

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