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Quantitative detection of *Potato virus Y* in potato plants and aphids

Discussion of diverse applications in potato research

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Potato virus Y (PVY) – Symptomatology und Morphology



Nature Precedings : doi:10.1038/npre.2011.5734.1 : Posted 28 Feb 2011



Potato plant infected with PVY



A. Hühnlein, JKI 2009



Healthy potato plant



A. Hühnlein, JKI 2009



Electron-microscopical photograph of PVY particles, E. Zimmermann, JKI 2009

1 200 nm

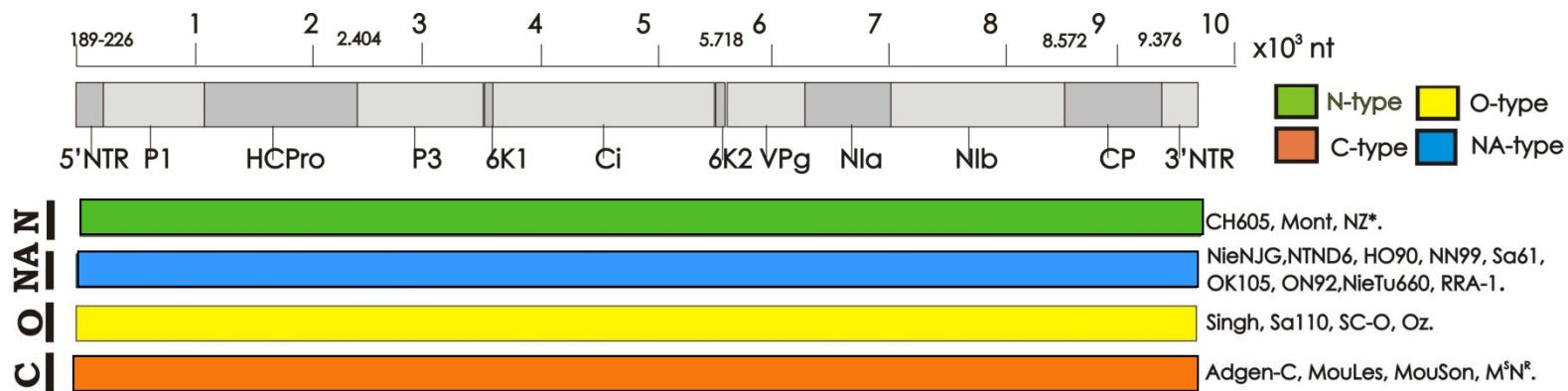


PTNRD caused by PVY^{NTN}

A. Hühnlein, JKI 2010



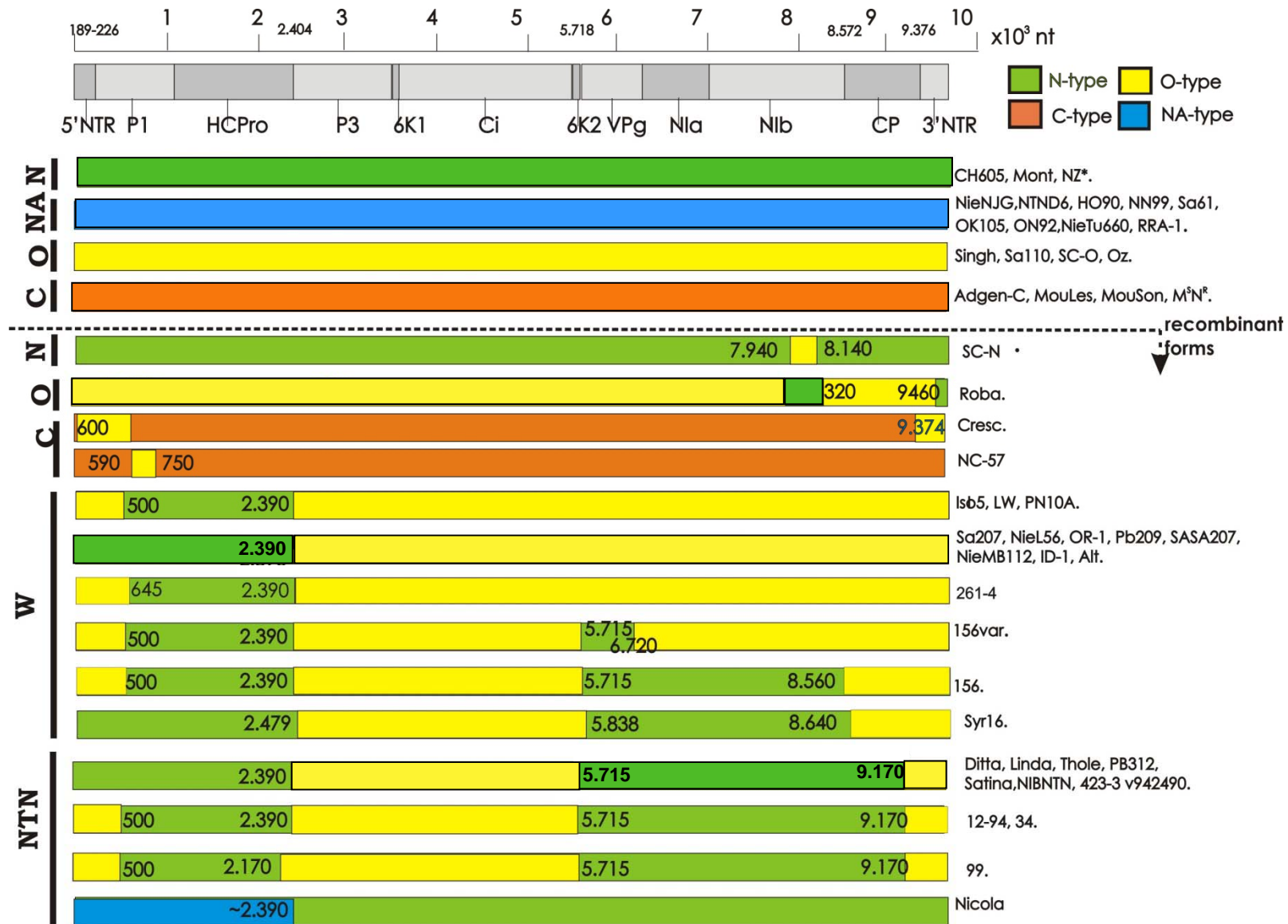
Strains of PVY



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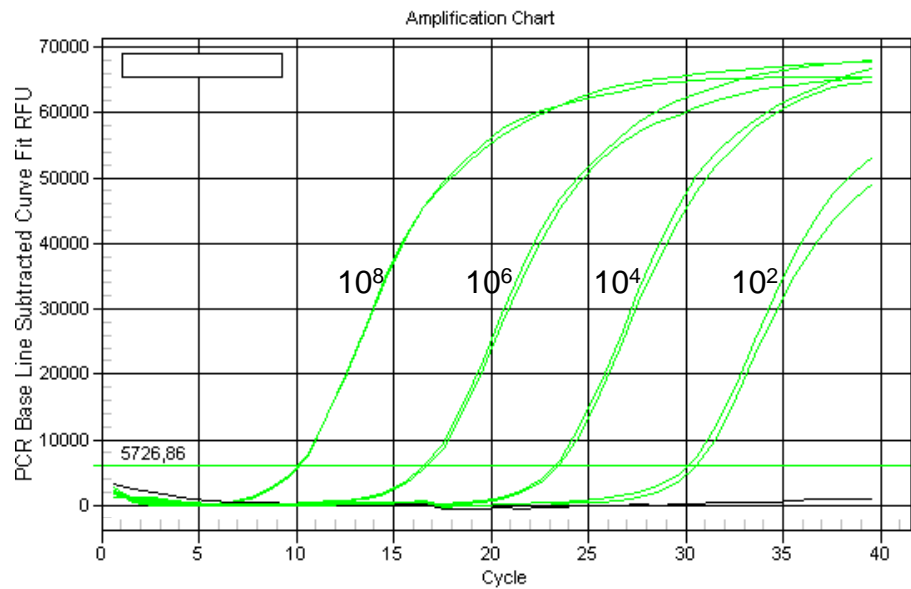
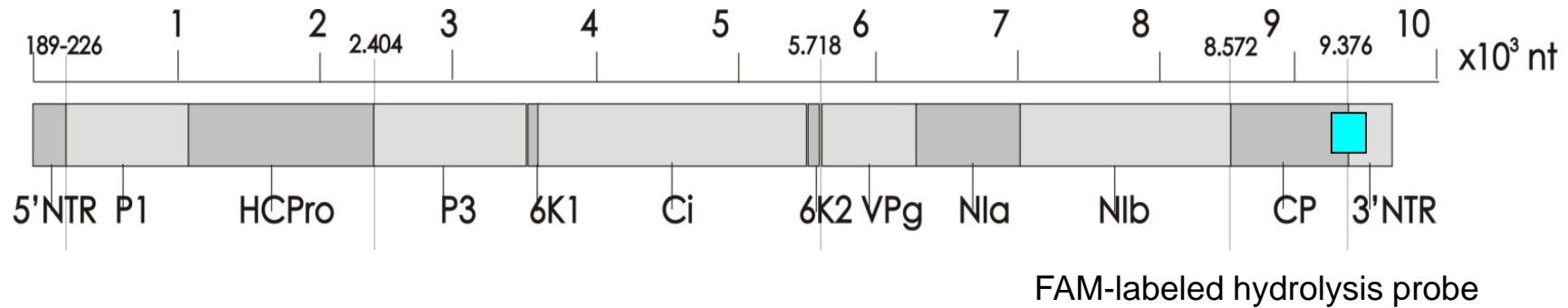
Recombinant forms of PVY



Location of primers and probe for the detection of all PVY strains



Location of the amplicon (125 bp) generated by **PVY^{all}** primers



— Standards consist of 100-fold dilutions of *in vitro* RNA transcripts based on a PVY⁰ strain sequence

— NTC

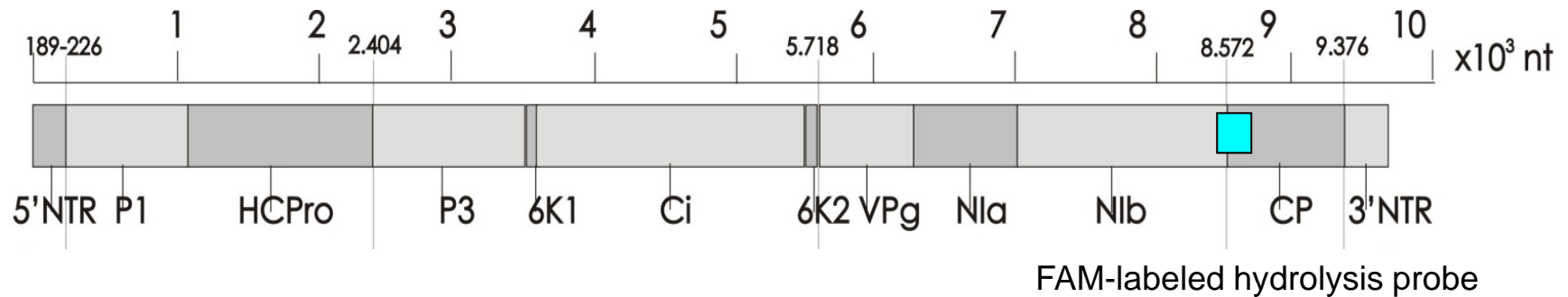
Fluoro-phore	PCR Efficiency (%)	R squared	Slope	Y-Intercept
FAM	100	1.000	-3.323	40.371



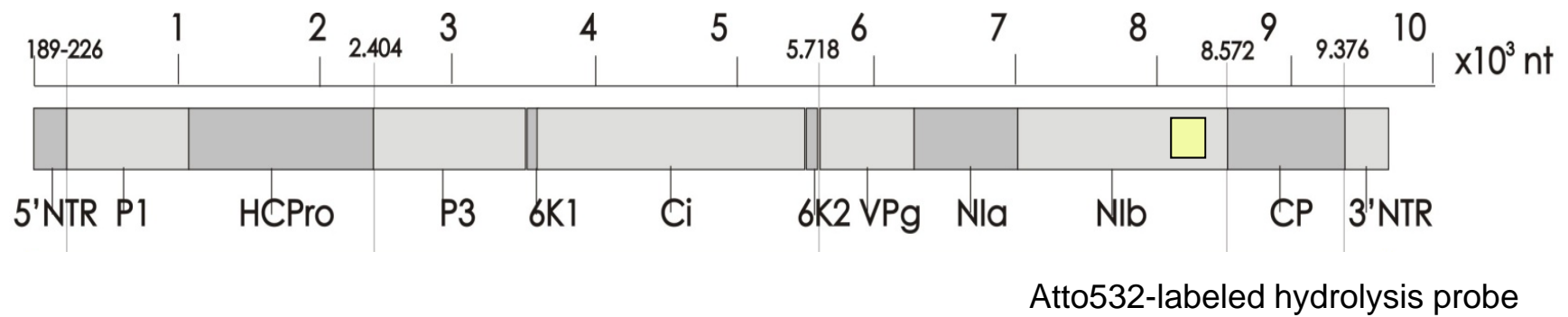
Location of primers and probes for the specific qPCR assays



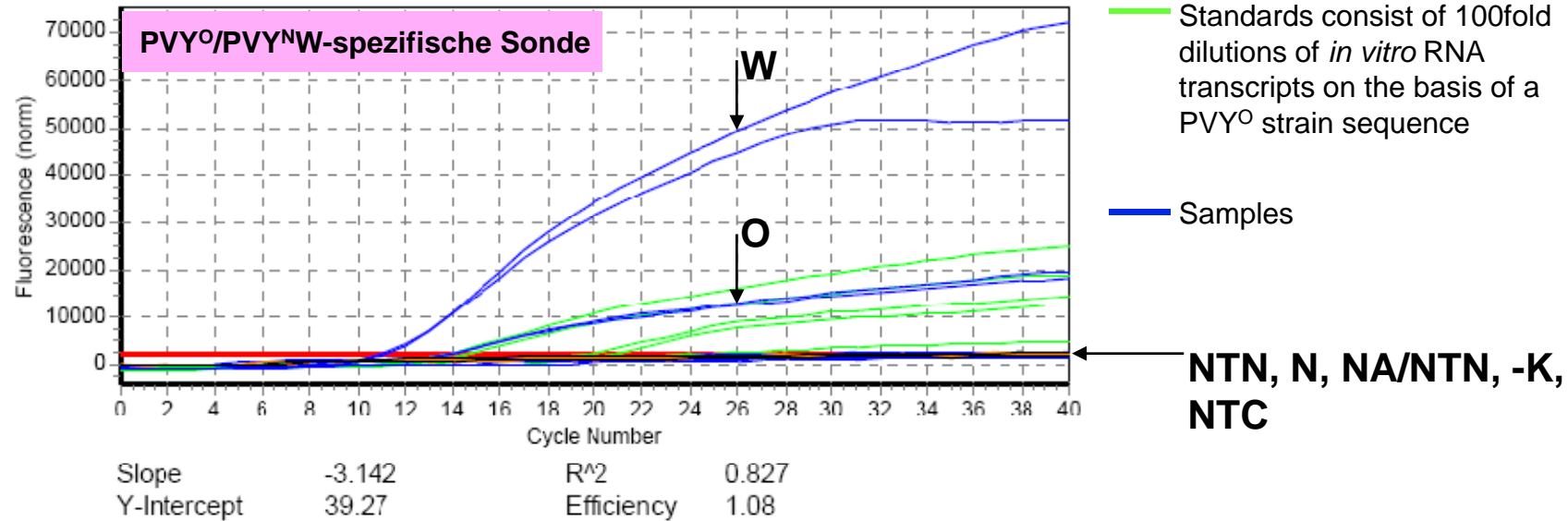
Location of the amplicon (121 bp) generated by **PVY^O/PVY^{NW}** primers



Location of the amplicon (195 bp) generated by **PVY^N/PVY^{NTN}** primers



Spezifität der Hydrolyse-Sonden



PVY-Isolates used for assay optimization

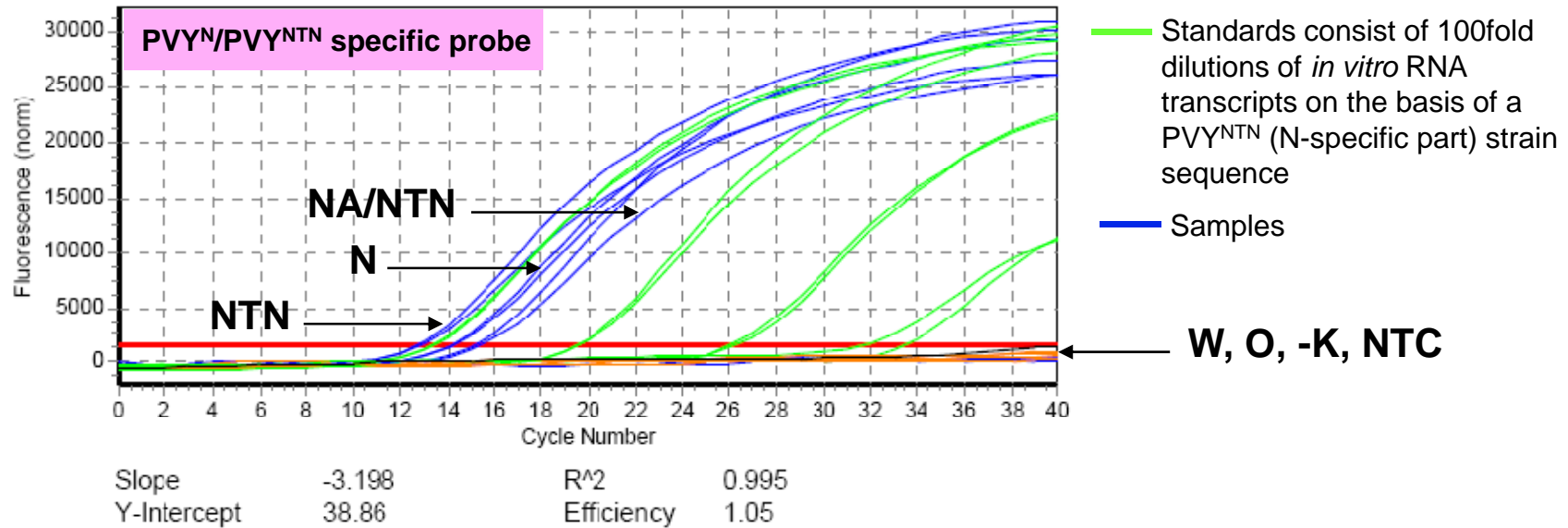
<ul style="list-style-type: none"> • PVY^N (CH605, Switzerland) N • PVY^O (12, New Zealand) O • PVY^{NTN} (Gr99, Poland) NTN 	<ul style="list-style-type: none"> • PVY^{NA/NTN} (Nicola, JKI) NA/NTN • PVY^{NW} (261/4, JKI) W
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Specificity of designed probes



Nature Precedings : doi:10.1038/npre.2011.5734.1 : Posted 28 Feb 2011



- Epidemiological Studies

- Differences in transmission of PVY strains/isolates by *Myzus persicae*
- Differences in accumulation of PVY strains/isolates in potato plants

- Safety Research

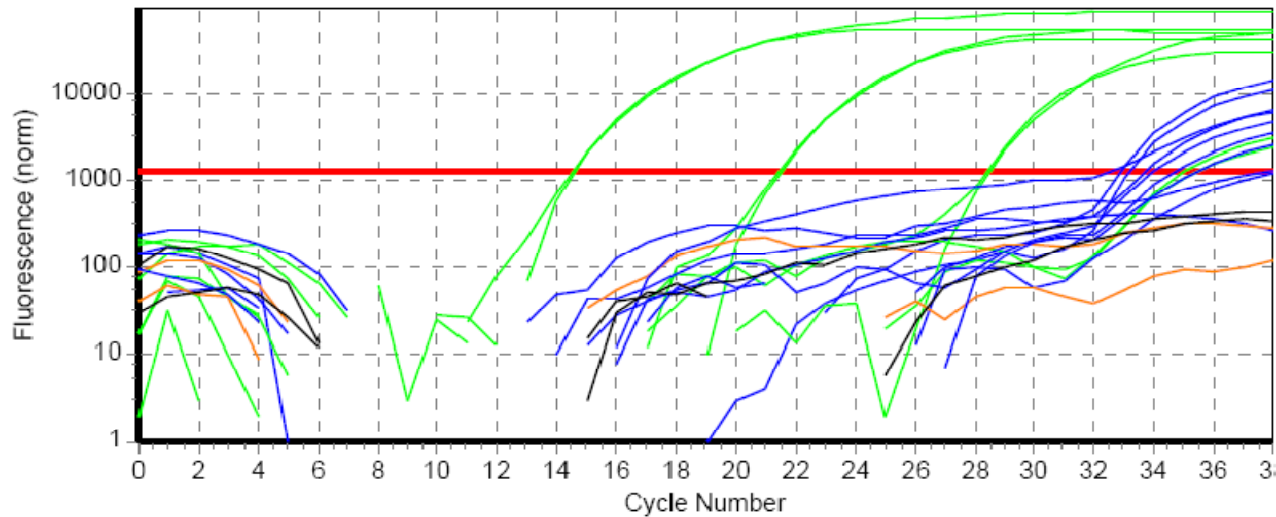
- Alterations in resistance level of genetically modified potato plants
- Changes in virus uptake of aphids (*Myzus persicae*) feeding on genetically modified potato plants

- Certification of Seed Potatoes(?)

- Sensitive detection method allows selling of virus free material with a theoretical threshold of 0.
- Determination of the resistance level in potato plants (quantitative/qualitative resistance)

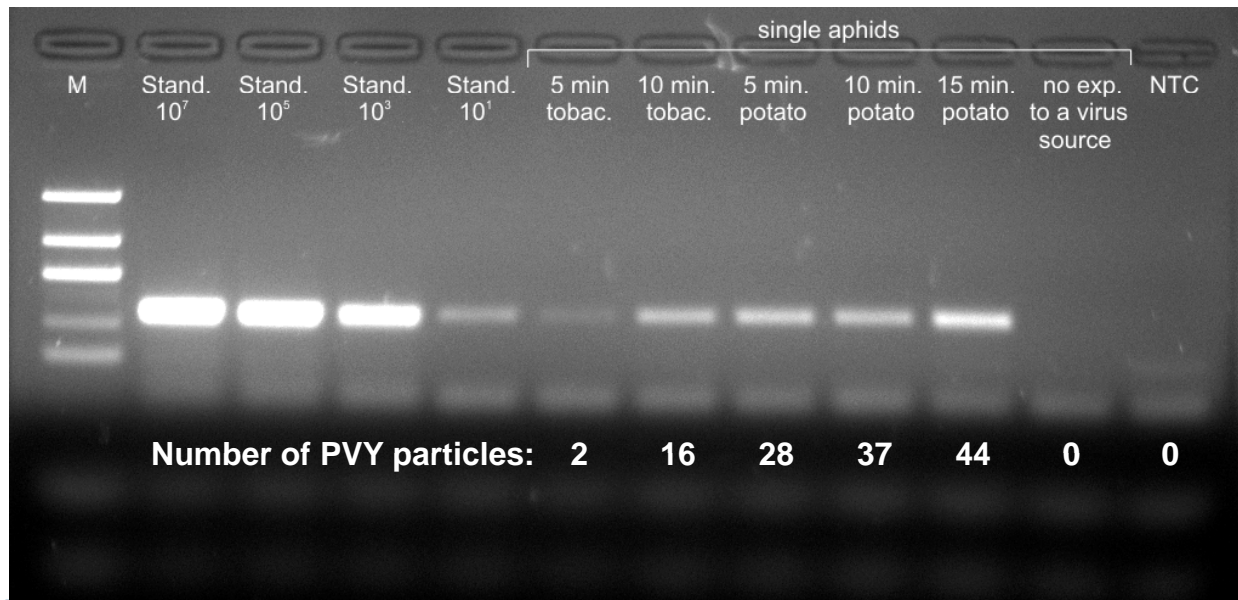


Detection of PVY in single aphids (*M. persicae*)



- NTC
- Single aphid with no exposure to a PVY source
- Single aphids fed on PVY-infected leaves of tobacco or potato for 5, 10 or 15 minutes
- Standards in 100fold dilutions consist of *in vitro* RNA transcripts on the basis of a PVY^{NTN} (N-specific part) strain sequence

Slope	-3.439	R ²	1.000
Y-Intercept	38.68	Efficiency	0.95



The number of virus particles have to be multiplied by 20, since only 2 of 40 µl cDNA reaction mix were used for qPCR.



Possible applications of a quantitative detection method of PVY



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- Safety Research
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Examination of possible alterations in the resistance level to PVY in potato plants



Cultivar	Genetically modified
a	Yes (GMO1)
a	Yes (GMO2)
a	Yes (GMO3)
a	No (WT)
b	Yes (GMO1)
b	Yes (GMO2)
b	No (WT)
c	No
d	No
e	No
f	No



Sampling



Photographic documentation of the experiment

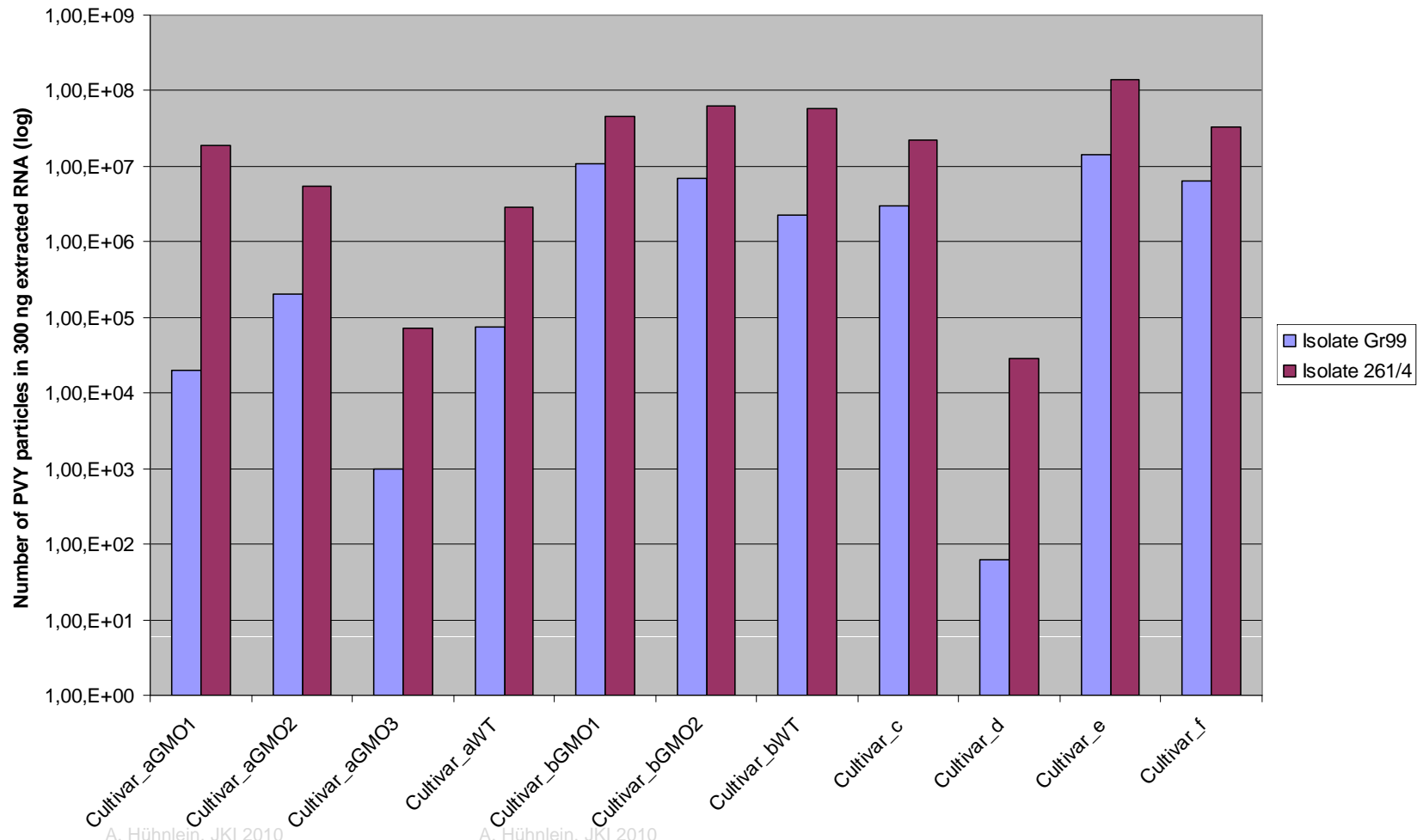
Every cultivar/line was mechanically infected with PVY^{NW} (261/4) or PVY^{NTN} (Gr99).



Examination of possible alterations in the resistance level to PVY in potato plants



Number of PVY particles two weeks after PVY Infection



A. Hühnlein, JKI 2010

A. Hühnlein, JKI 2010

The data have to be validated by statistical analysis.



Possible applications of a quantitative detection method of PVY



- Epidemiological Studies

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- Differences in accumulation of PVY strains/isolates in potato plants

- Safety Research

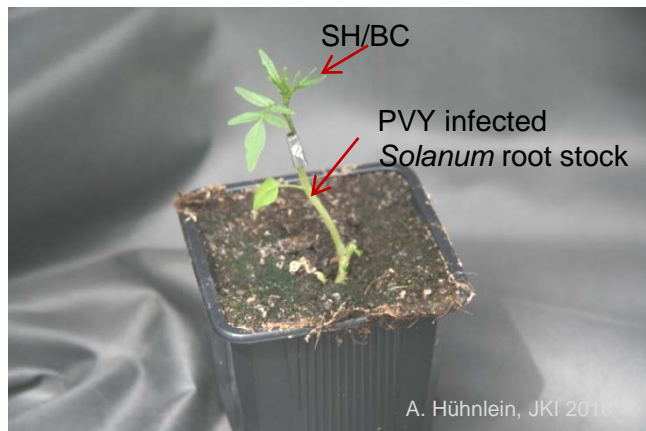
- Alterations in resistance level of genetically modified potato plants
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Determination of the resistance level in potato plants



Grafts of somatic hybrids (SH) of *Solanum tuberosum* and a resistant *Solanum* species and backcrosses (BC) with *S. tuberosum*, respectively, on PVY infected *Solanum* root stocks

here: *Solanum tarnii* (extreme resistance?) on PVY infected *S. tuberosum*

Species	Cultivar/backcross/somatic hybrid	Number of detected PVY-particles in 15 mg of leaf	Extreme Resistance
<i>S. stoloniferum</i> (Kontrolle)	Cultivar 'Ute'	0	Yes
<i>S. cardiophyllum</i>	Somatic hybrid	0	Yes
<i>S. pinnatisectum</i>	Backcross (BC2)	0	Yes
<i>S. tarnii</i>	Backcross (BC3)	19.600	?
<i>S. etuberosum</i>	Somatic hybrid	29.300	?
Average of a susceptible cultivar	Cultivar 'Princess'	~50.000.000	



Thank you!



- Thanks to all technical (and animal) assistants of the working group!

