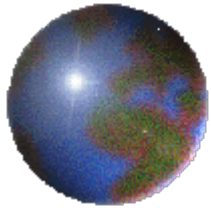


The logo consists of the letters 'U' and 'H' in a white, bold, sans-serif font on a red background.

Universität Hamburg



Detection of tobamoviruses: Proposal for general primers and specific capture oligo- nucleotides.

Günter Adam, Peter Willingmann and Cornelia Heinze
University of Hamburg
and
Plant Protection Federal State Hamburg

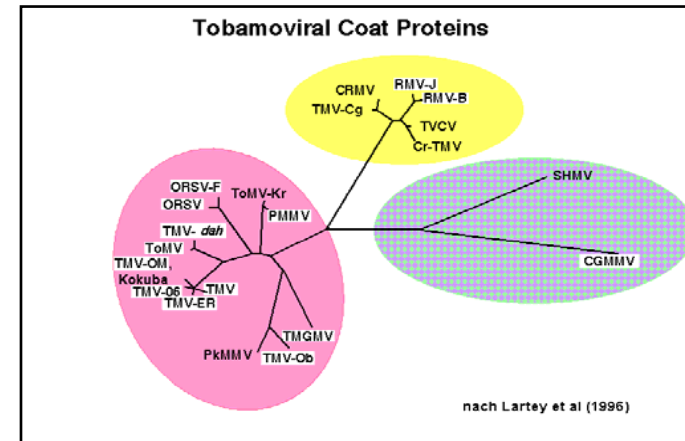
Closing meeting COST Action 853: 21.- 22. of May

Sant Feliu de Guixols



Why replace serology with NA-based detection for Tobamoviruses?

- Tobamoviruses form three clusters according to:
 - ▣ Host range
 - ▣ Sequences
 - ▣ Genome organisation
 - ▣ Serology
- If one aligns the aa-sequences of the CP in the solanaceous cluster, one detects large homologous areas, giving rise to crossreacting antibodies



```

TMV  MSYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQ 40
ToMV MSYSITSPSQFVFWSSVWADPIELLNVTSSLGNQFQTQQ 40
*****:***** ** .*****:***:*****

TMV  ARTVVQRQFSEVWK PSPQVTVRFPDSDFKVYRYNAVLDP 80
ToMV ARTTVQQQFSEVWK PFPQSTVRFPGDVYKVYRYNAVLDP 80
*** .*:***** ** * . . :*****

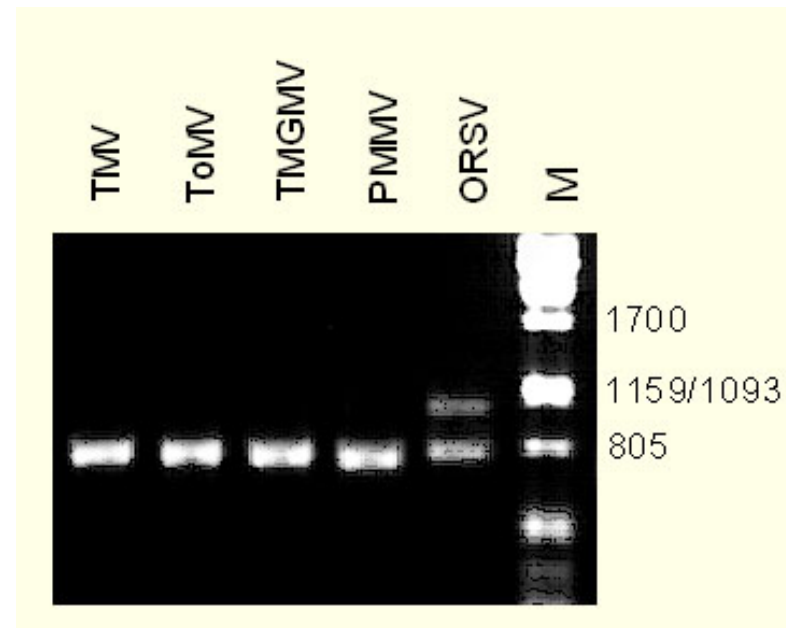
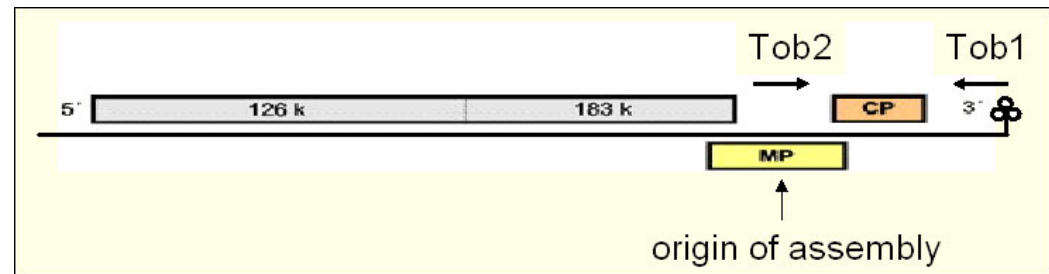
TMV  VTALLGAFDTRNRIIEVENQANPTTAE TLDATRRVDDATV 120
ToMV ITALLGTFDTRNRIIEVENQQSPTTAE TLDATRRVDDATV 120
:*****:***** .***** *****

TMV  AIRSAINNLIVELIRGTGSYNRSSFESSGLVWTSGPAT
ToMV AIRSAINNLVNELVRGTGLYNQNTFESMSGLVWTSAPAS
*****: **:* ** : .:*** ** ** . ** :
    
```



Development of genus-specific primers

- ❖ We wanted the CP as target
- ❖ For the areas to chose genus-specific primers we selected functional sequence stretches
- ❖ The first selection was cluster 1 specific!





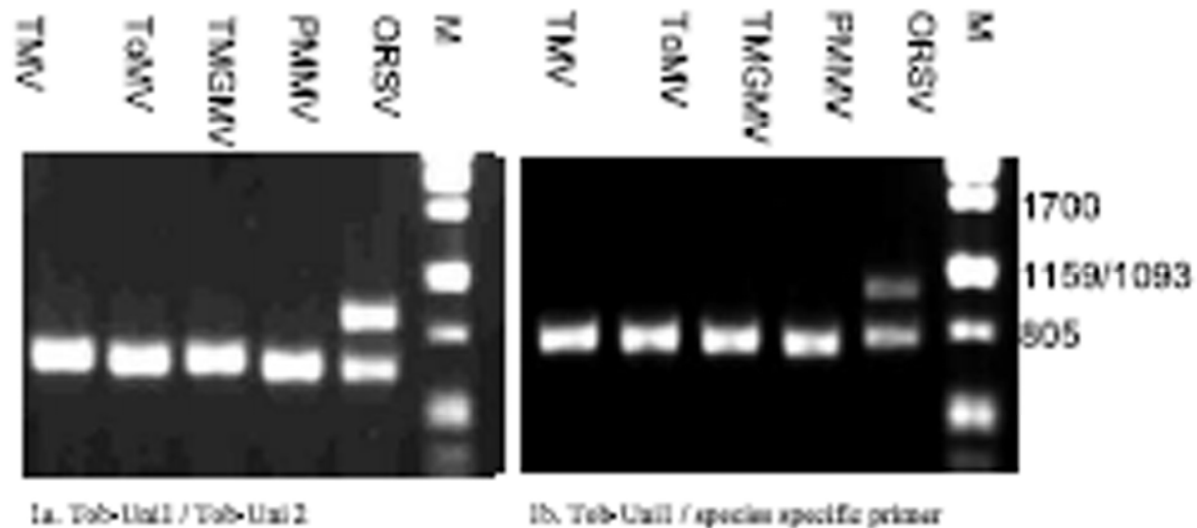
How many primers are necessary?

- As seen before we have three clusters.
- The reverse primer for all three is the same, however, specific forward primers were necessary for each cluster



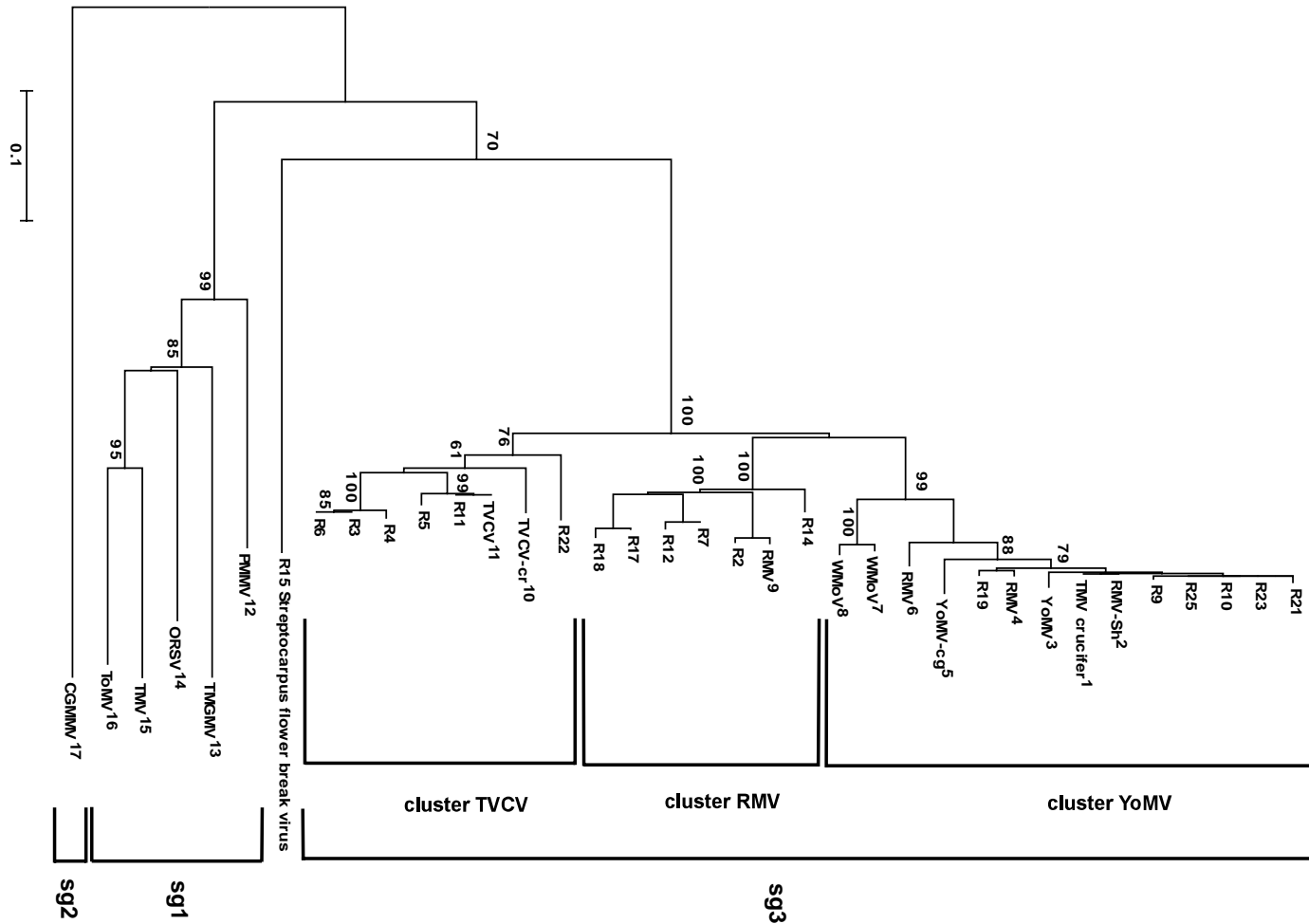
Development of a species-specific nested PCR on basis of genus specific amplicons

- For the solanaceous cluster it was tested if species-specific forward primers could be developed





The Clustering of Tobamoviruses according to CP Sequences



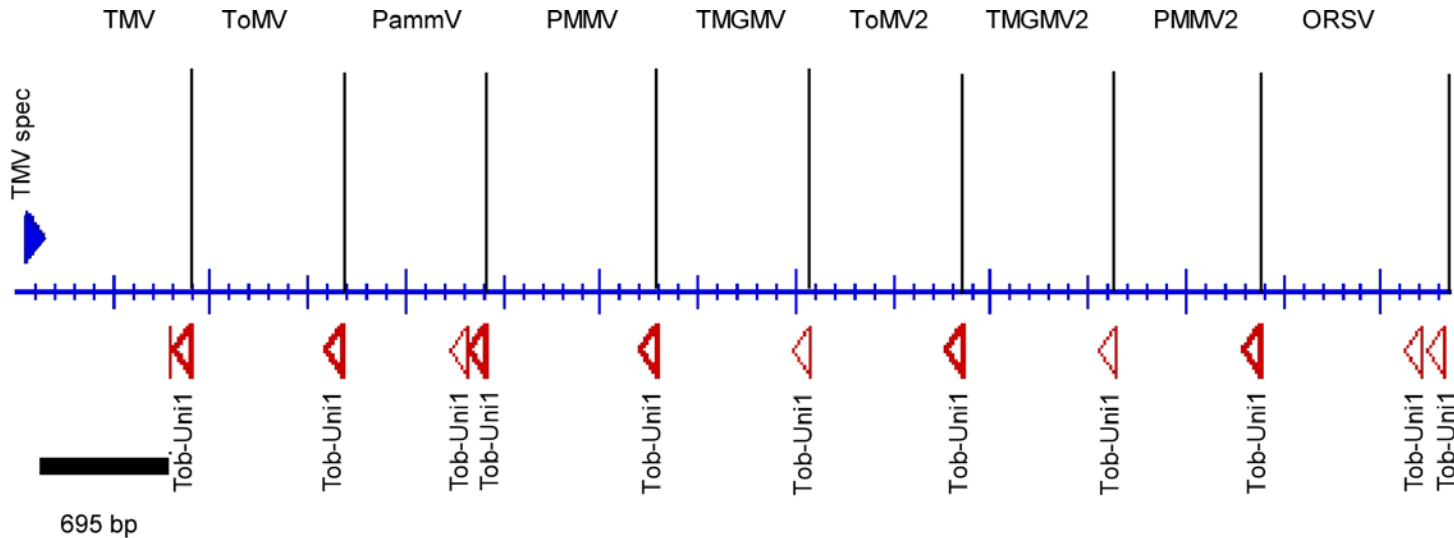


Testing of the genus-specific amplicons and species-specific primers for applicability in array applications

- To determine if the genus-specific primers and the species-specific forward primers can be combined for an array solution, we tested in silico with the program Amplify for Mac all possible combinations.




Results TMV Spec-Primer




TMV X68110
 ToMV X02144
 PammV X72586
 PMMV AB00079
 TMGMV AB078435
 ToMV2 X2144
 TMGMV2 M34077
 PMMV2 M81413
 ORSV X82130


TMV-spec : CGG TCA GTG CCG AAC AAG AA
 Tob-Uni1 : ATT TAA GTG GAS GGA AAA VCA CT

 strongest Bindung

```
CGGTCAGTGCCGAACAAGAA
|||||
CGGTCAGTGCCGAACAAGAA
```

 strong Bindung

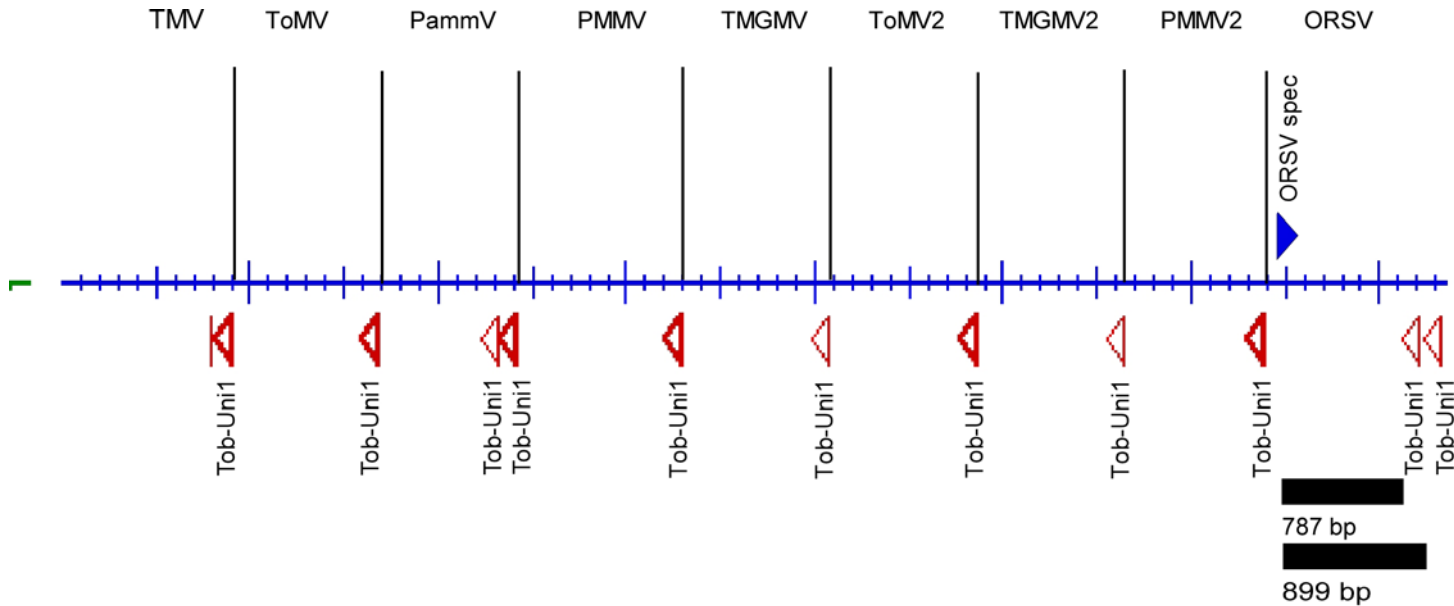
```
AGTGTTTTTCCCTCCACTTAAAT
|||||
AGTGNTTTTCCNTCCACTTAAAT
```

 weak Bindung

```
AGTGGTTGTTTCGTCCACTTAAAT
|||||
AGTGNTTTTCCNTCCACTTAAAT
```



ORSV spec. Primer



TMV X68110
 ToMV X02144
 PammV X72586
 PMMV AB00079
 TMGMV AB078435
 ToMV2 X2144
 TMGMV2 M34077
 PMMV2 M81413
 ORSV X82130

ORSV spec : AGG TGA TAG TGA TGT TGG TAT T
 Tob-Uni1 : ATT TAA GTG GAS GGA AAA VCA CT

strongest Bindung

CGGTCAGTGCCGAACAAGAA
 |||||
 CGGTCAGTGCCGAACAAGAA

strong Bindung

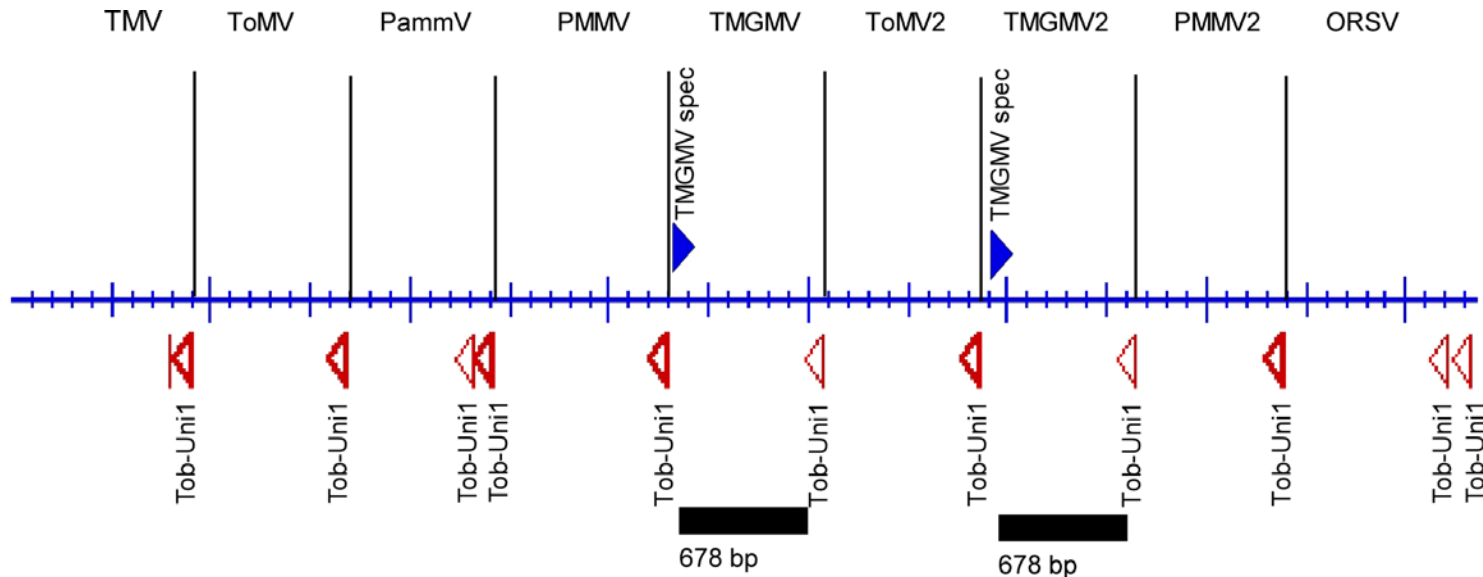
AGTGT TTTTCCCTCCACTTAAAT
 ||||| ||||| ||||| ||||| |||||
 AGTGNTTTTCCNTCCACTTAAAT

weak Bindung

AGTGGTTGTTTCGTCCACTTAAAT
 ||||| ||||| ||||| ||||| |||||
 AGTGNTTTTCCNTCCACTTAAAT





TMGMV spec. Primer




TMV X68110
 ToMV X02144
 PammV X72586
 PMMV AB00079
 TMGMV AB078435
 ToMV2 X2144
 TMGMV2 M34077
 PMMV2 M81413
 ORSV X82130

TMGMV spec : AAR TAA ATA AYA GTG GTA AGA AGG G
 Tob-Uni1 : ATT TAA GTG GAS GGA AAA VCA CT

 strongest Bindung

 strong Bindung

 weak Bindung

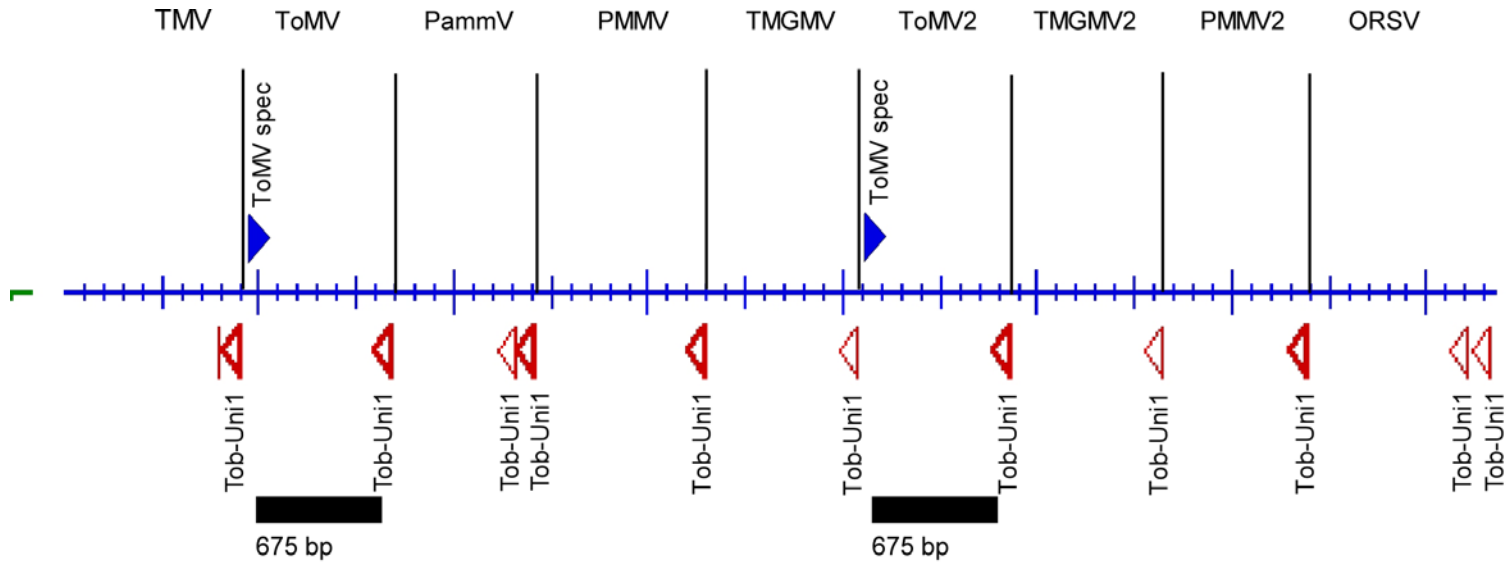
CGGTCAGTGCCGAACAAGAA
 |||||
 CGGTCAGTGCCGAACAAGAA

AGTGTTTTTCCCTCCACTTAAAT
 |||||
 AGTGNTTTTCCNTCCACTTAAAT

AGTGGTTGTTTCGTCCACTTAAAT
 |||||
 AGTGNTTTTCCNTCCACTTAAAT




ToMV spec. Primer




- TMV X68110
- ToMV X02144
- PamMV X72586
- PMMV AB00079
- TMGMV AB078435
- ToMV2 X2144
- TMGMV2 M34077
- PMMV2 M81413
- ORSV X82130

ToMV-spec : CGG AAG GCC TAA GGA AGG GAA GC
 TobUni1 : ATT TAA GTG GAS GGA AAA VCA CT

 strongest Bindung


```

CGGTCAGTGCCGAACAAGAA
|||||
CGGTCAGTGCCGAACAAGAA
  
```

 strong Bindung

```

AGTGT TTTTCCCTCCACTTAAAT
|||| |
AGTGN TTTTCCNTCCACTTAAAT
  
```

 weak Bindung

```

AGTGGTTGTTTCGTCCACTTAAAT
|||| |
AGTGN TTTTCCNTCCACTTAAAT
  
```