

Microarray for detection of fruit-tree viruses: developing and testing

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Targeted viruses

- Apple mosaic virus (ApMV) - Iiarvirus - **Bromoviridae**
- Prunus necrotic ringspot virus (PNRSV) - Iiarvirus - **Bromoviridae**
- Prune dwarf virus (PDV) - Iiarvirus - **Bromoviridae**
- Apple stem grooving virus (ASGV) - Capillovirus - **Flexiviridae**
- Apple stem pitting virus (ASPV) - Foveavirus - **Flexiviridae**
- Apple chlorotic leafspot virus (ACLSV) - Trichovirus - **Flexiviridae**
- Plum pox virus (PPV) - Potyvirus - **Potyviridae**

Outline...

- design of specific probes for each of targeted virus
- assessing the detection limit of the microarray
- detection of viruses in natural samples
 - cDNA targets
 - (RT)-PCR products
- comparing of different labeling method

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Designing of the probes

Possibility of hybridization

[Kane et al., 2000]

\geq 85% similarity

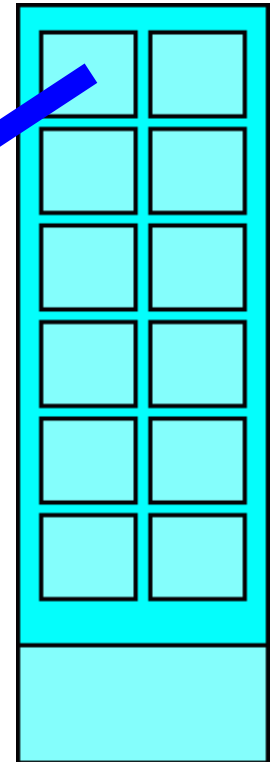
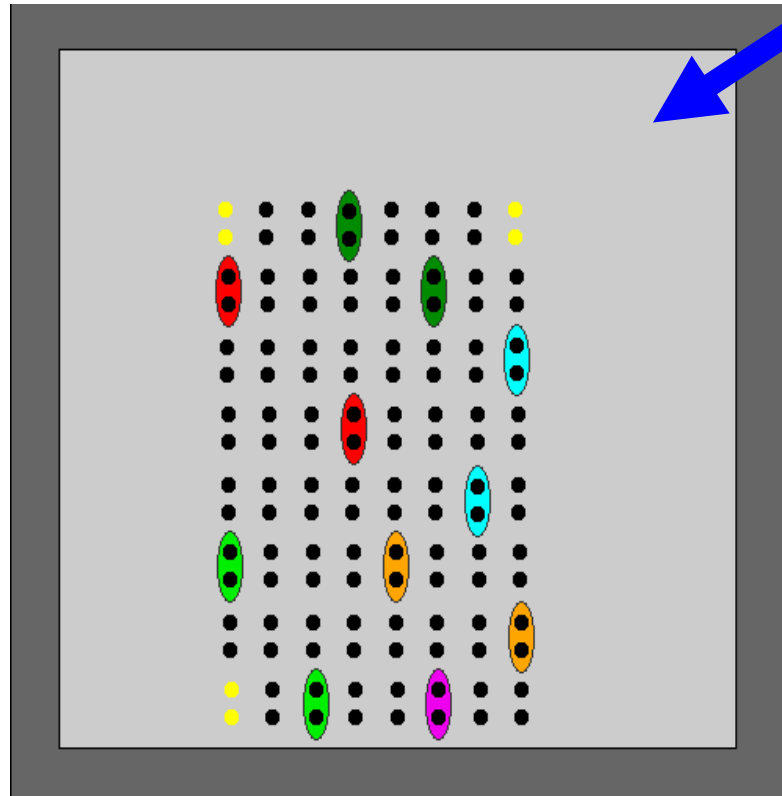
\geq 15 nt homologic region

- at least 3 different probes for each virus
- “control” oligos (dr. Neil Boonham), rRNA

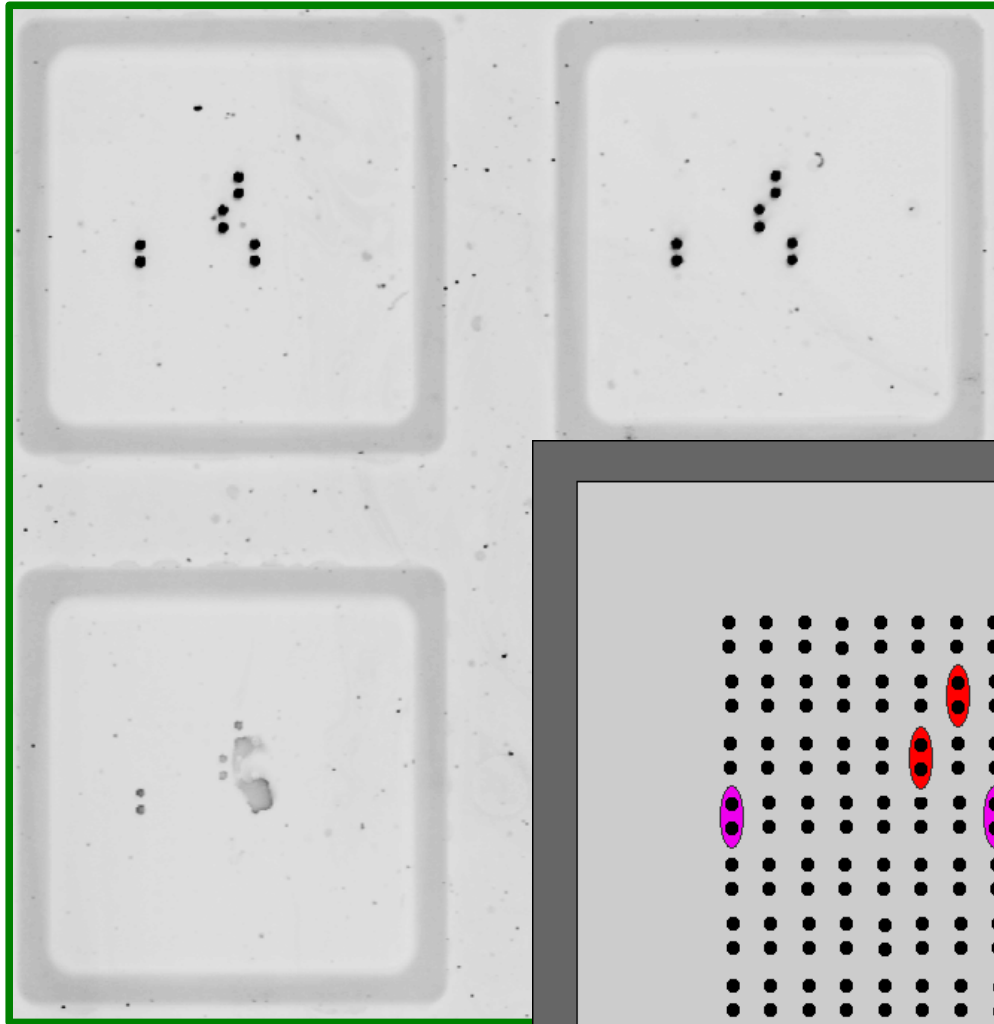
Microchip layout

- Plastic support, commercially printed (Lambda)

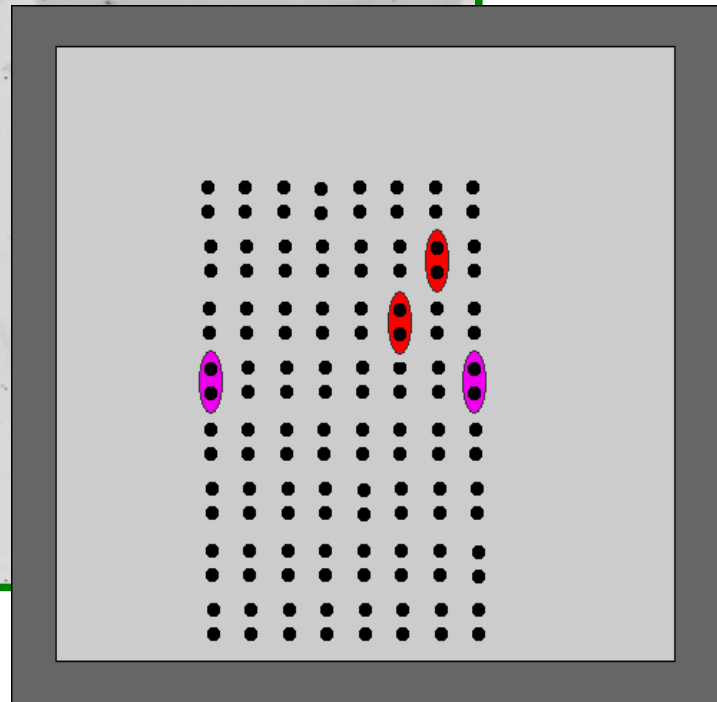
- each dot - 0,16mm
- double-dots
- controll oligos (rRNA)
- frames, different samples
- sensitive to dust



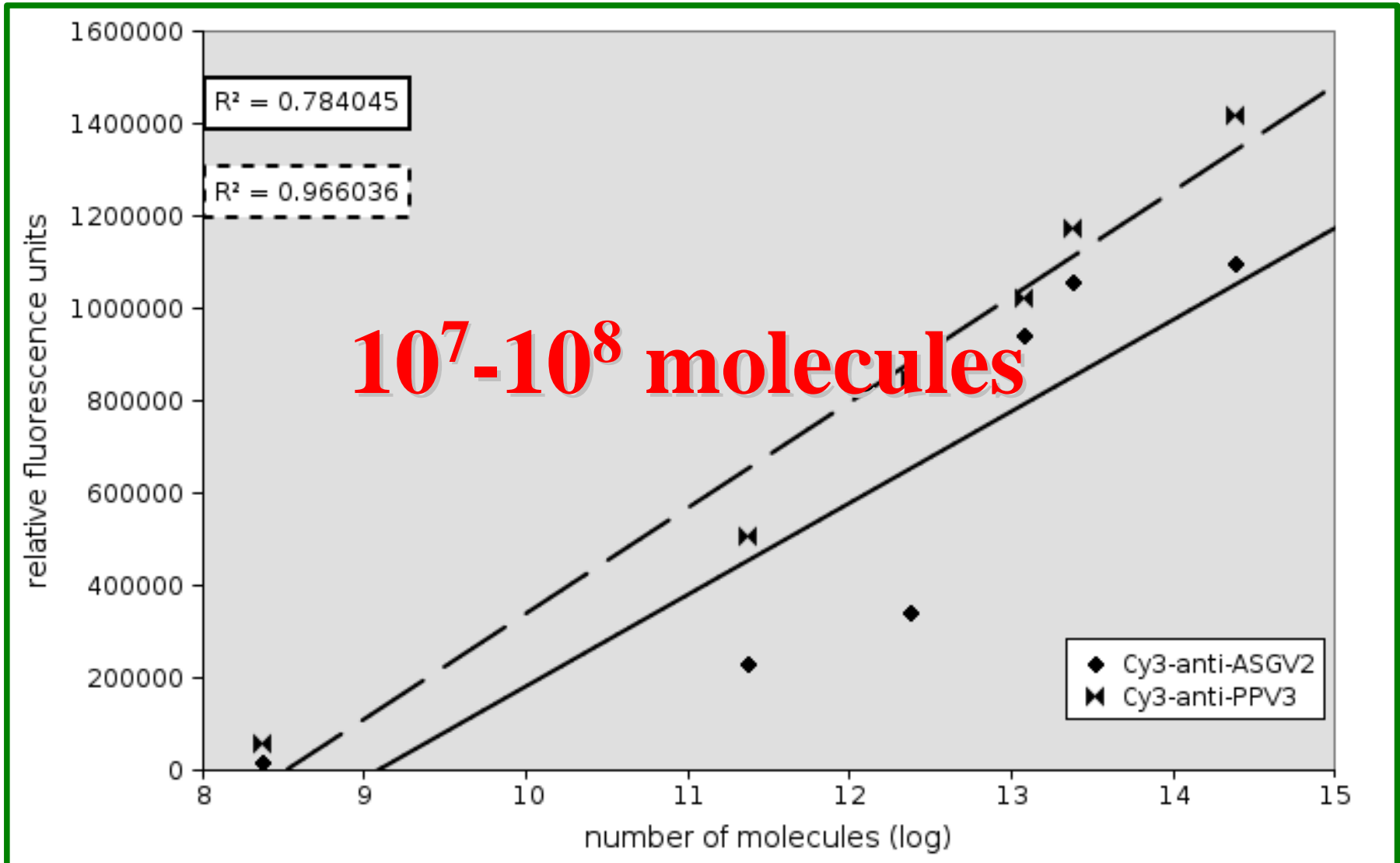
Limits of the detection



- dilution series of Cy3 directly labeled complementary oligos



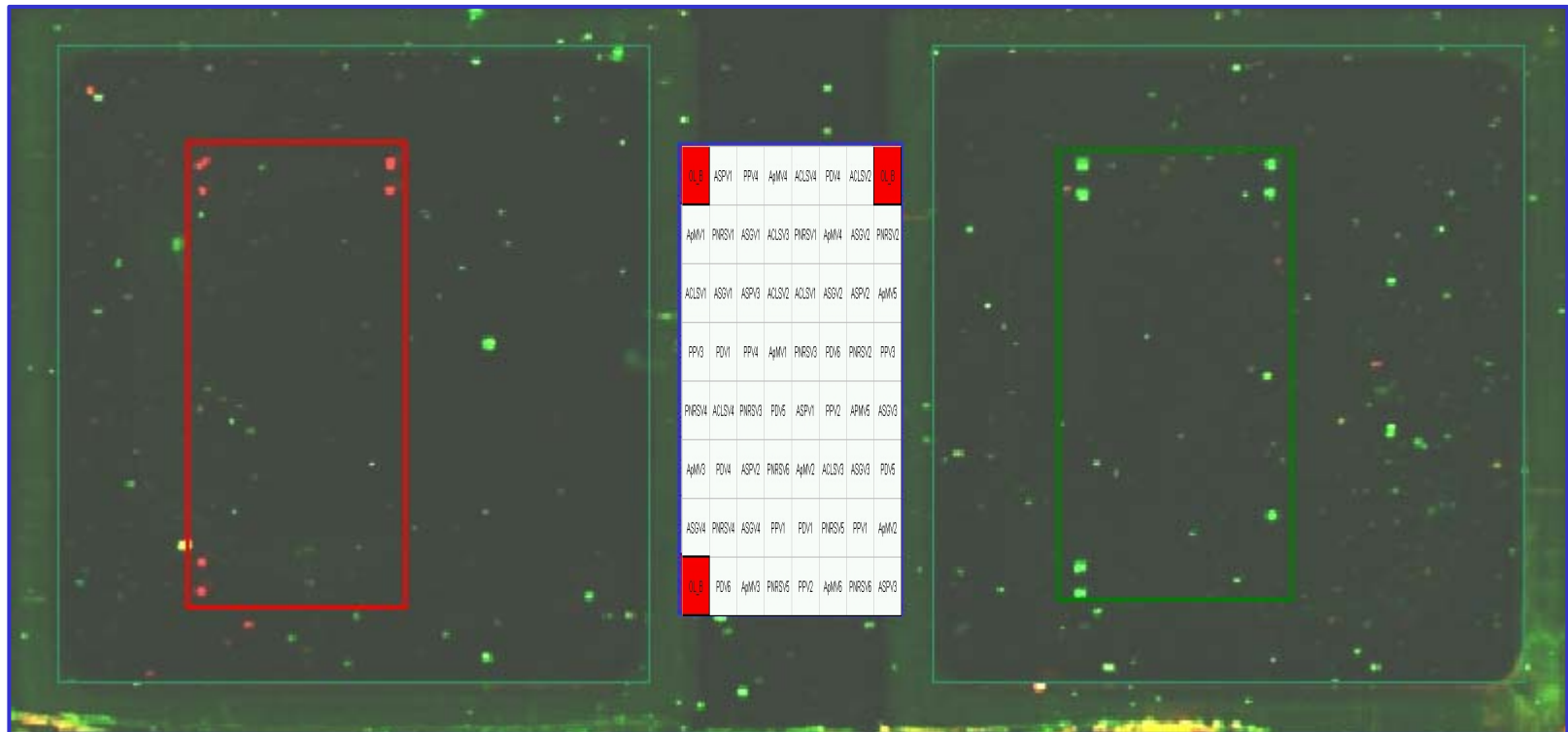
Limits of the detection



Microarray testing

- **cDNA-targets:**

- post-labeling (aa-dUTP), RP or specific priming
- no virus detected, only control oligos (rRNA)



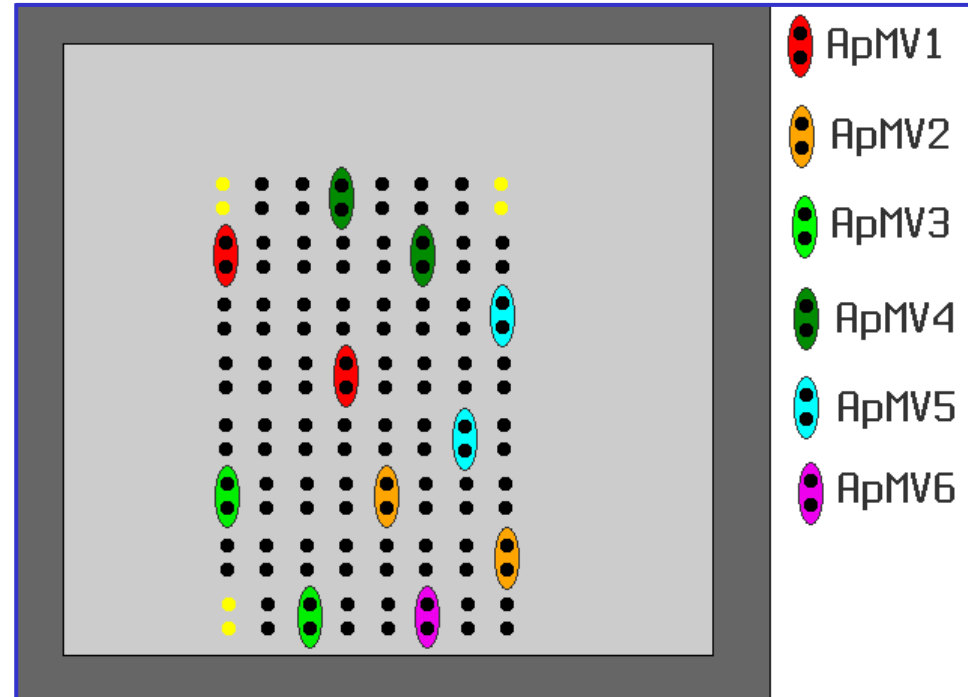
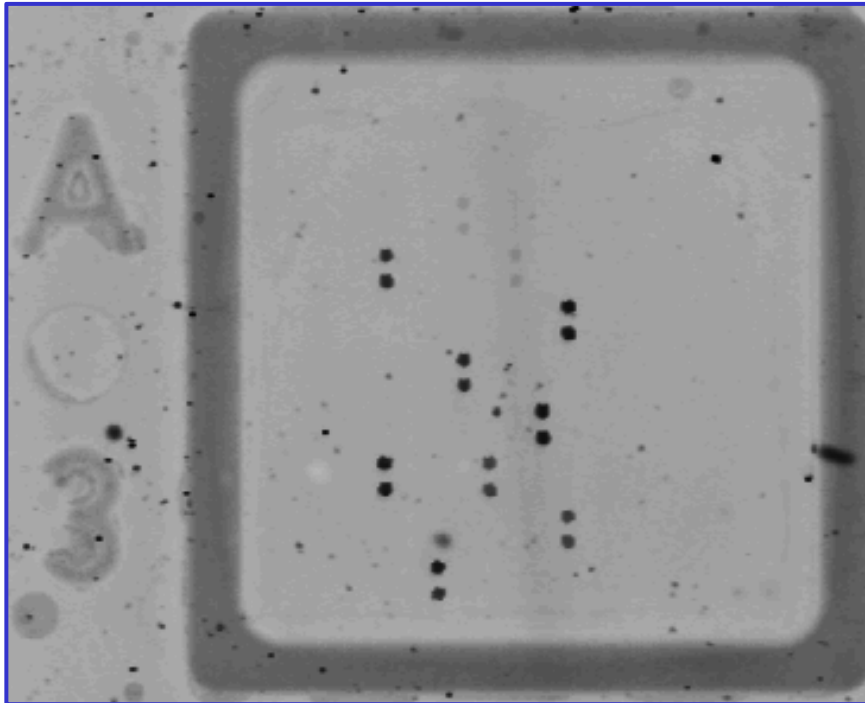
Microarray testing

- cDNA-targets:

- post-labeling (aa-dUTP), RP or specific priming
- no virus detected, only control oligos (rRNA)
- PCR: viral cDNA presented in each target
- real-time PCR: $10^4 - 10^5$ molecules of viral cDNA in each target => **NOT** detectable by microarray
- targets or signal amplification needed...

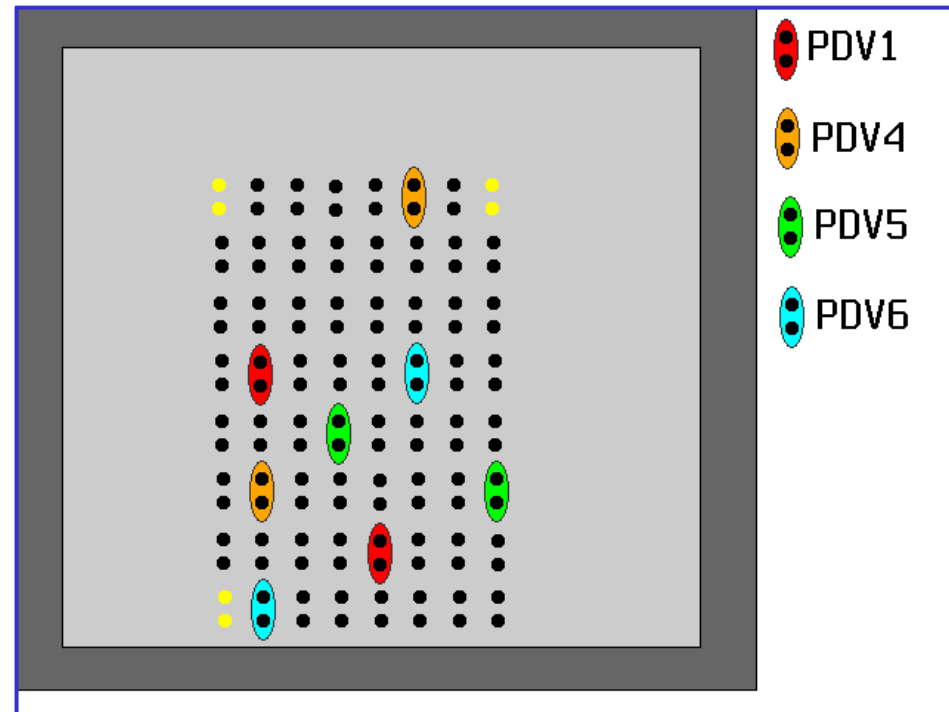
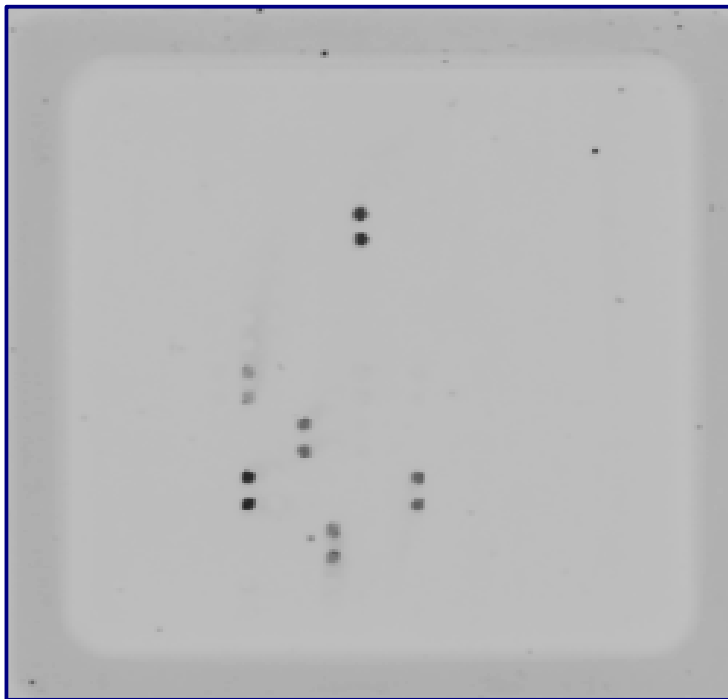
Microarray testing

- PCR-products: ApMV (post-labelling)
 - targeted probes: ApMV1, 2, 3, 4, 5 (not ApMV6)
 - no cross-hybridization



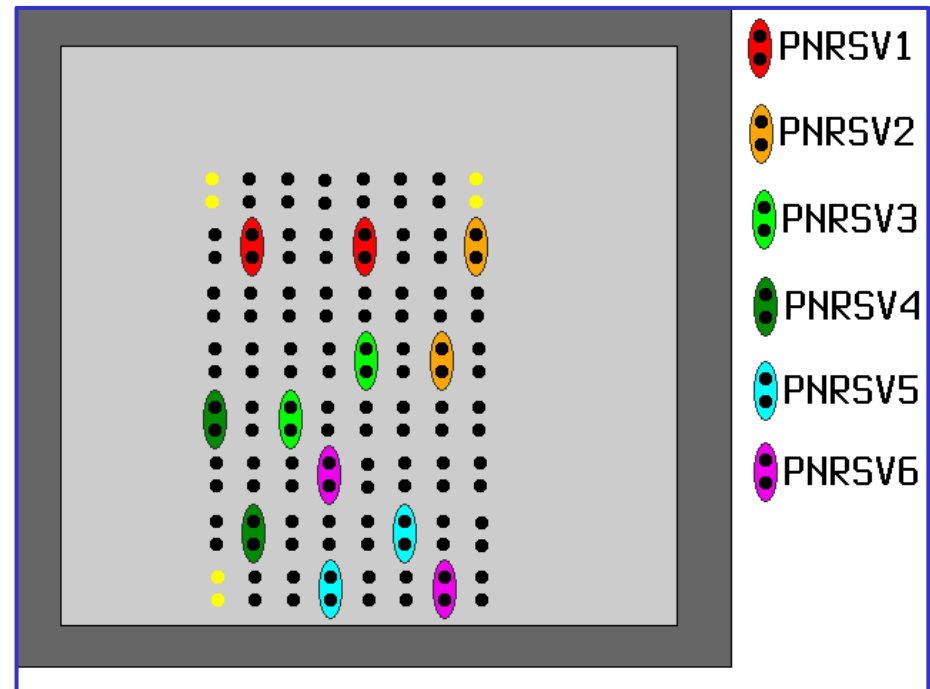
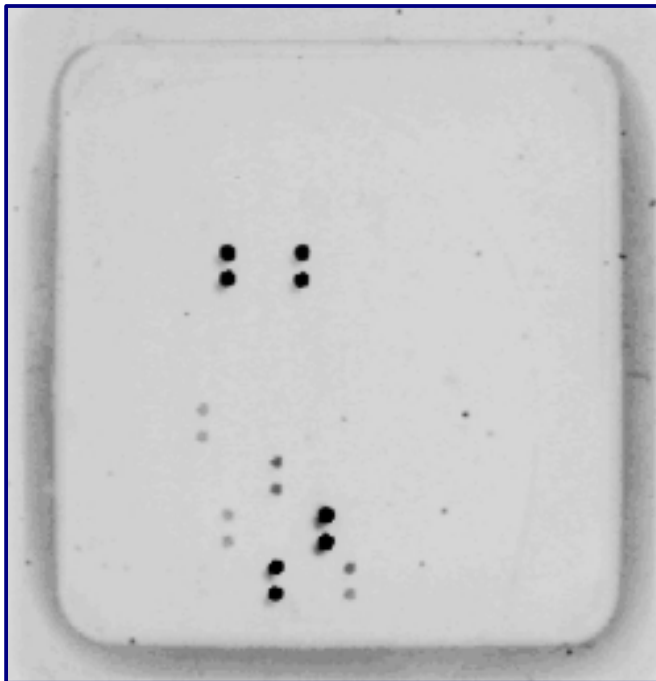
Microarray testing

- PCR-products: PDV (post-labelling)
 - targeted probes: PDV1, 4, 5 (not PDV6)
 - no cross-hybridization



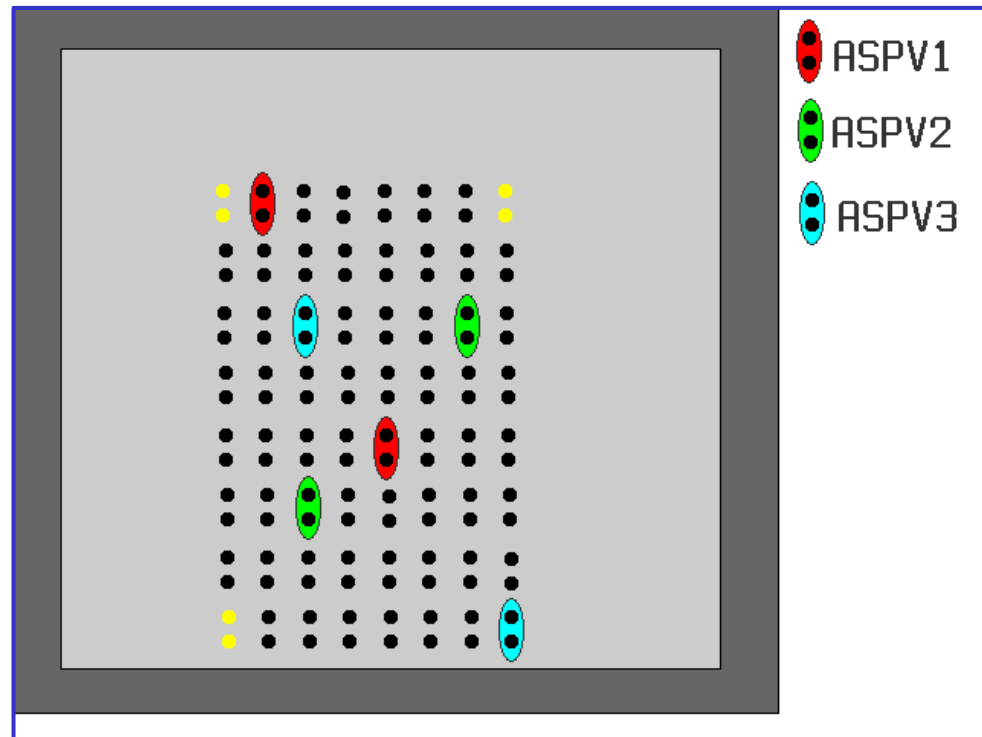
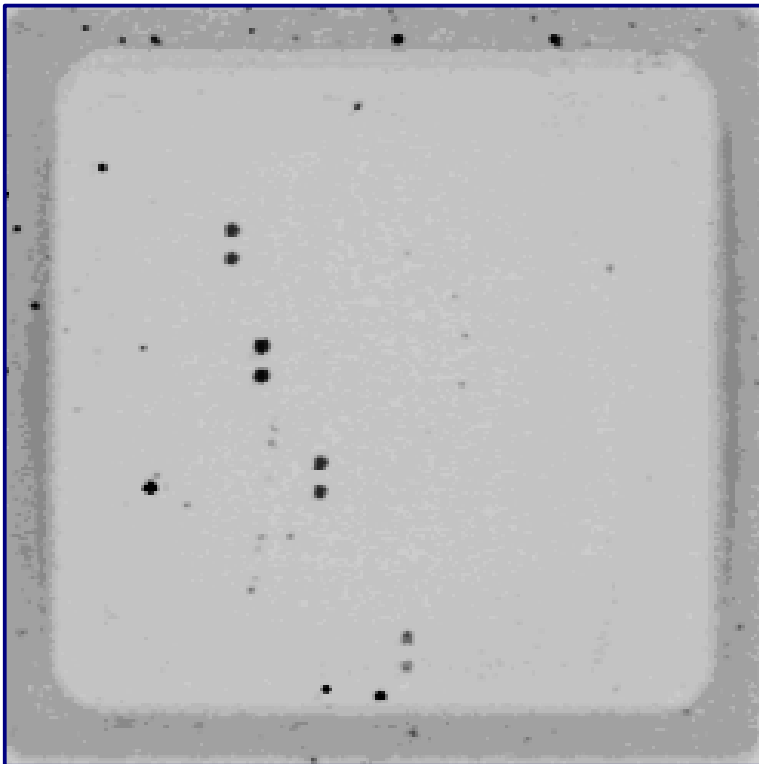
Microarray testing

- PCR-products: PNRSV (post-labelling)
 - targeted probes: PNRSV1, 4, 5, 6 (not PNRSV2, 3)
 - occasional cross-hybridization onto ASGV2



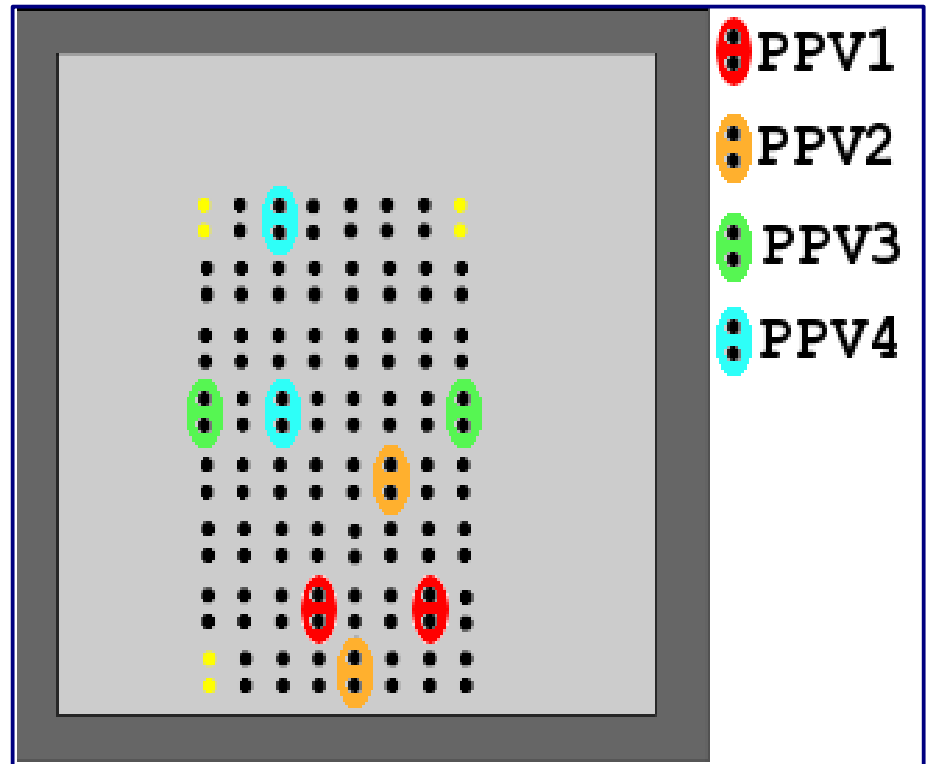
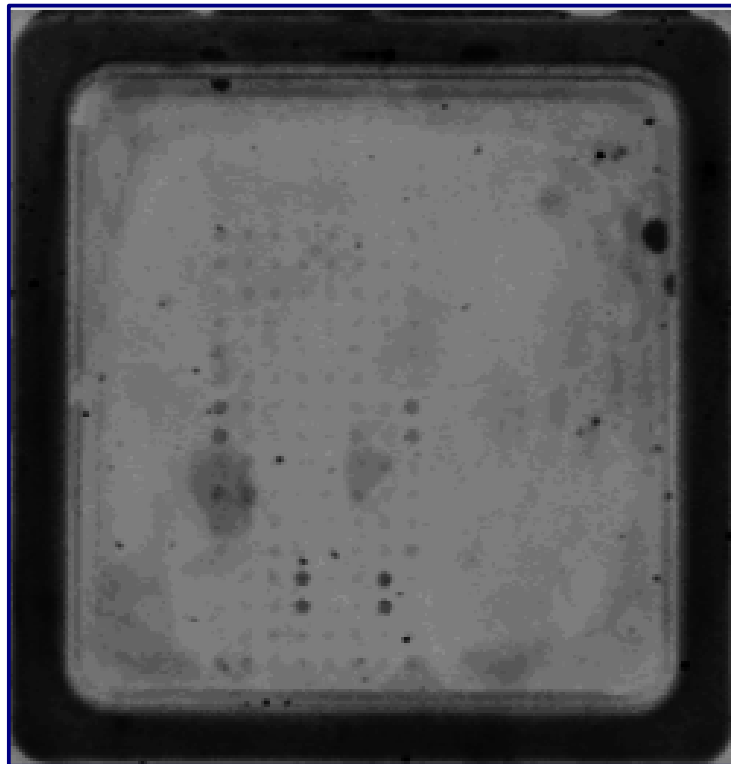
Microarray testing

- PCR-products: ASPV (post-labelling)
 - targeted probes: ASPV1, 3 (not ASPV2)
 - no cross-hybridization



Microarray testing

- PCR-products: PPV (post-labelling)
 - targeted probes: PPV1, (not PPV2, 3, 4)
 - PPV3 cross-hybridization?



Microarray testing

- **PCR-products:**
 - post-labeling
 - weak or no signals for probes with hybridization site near the 3'-end of target
 - no similar effect for 5'-end of target
 - occasional cross-hybridization onto ASGV2, control oligos (rRNA)

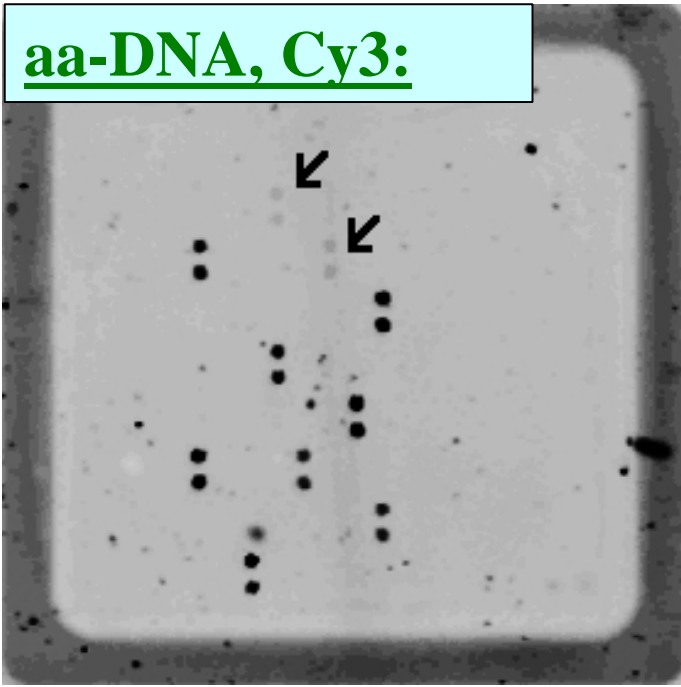
Microarray testing

- **Different labeling method:**

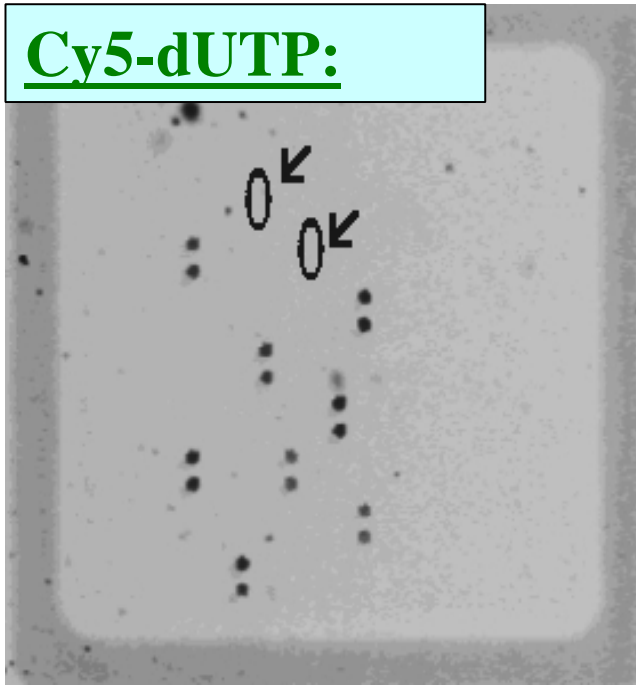
- post-labeling (aa-dUTP) by Cy3/Cy5
- direct labeling
- Cy3-primer labeling

- **Could any of the targeted method enhance sensitivity of the microarray?** (influence of labeling technique on sensitivity of detection)

aa-DNA, Cy3:



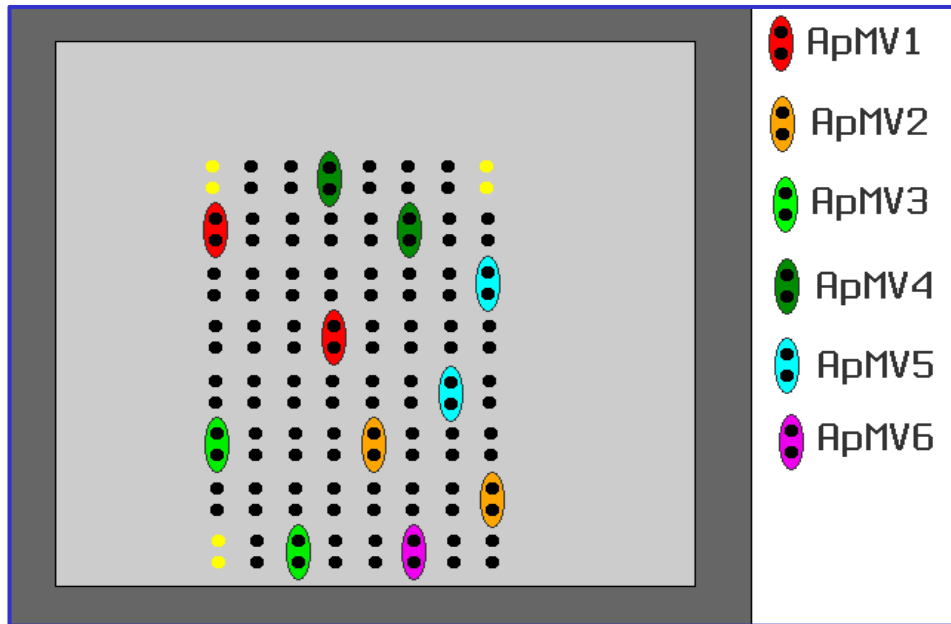
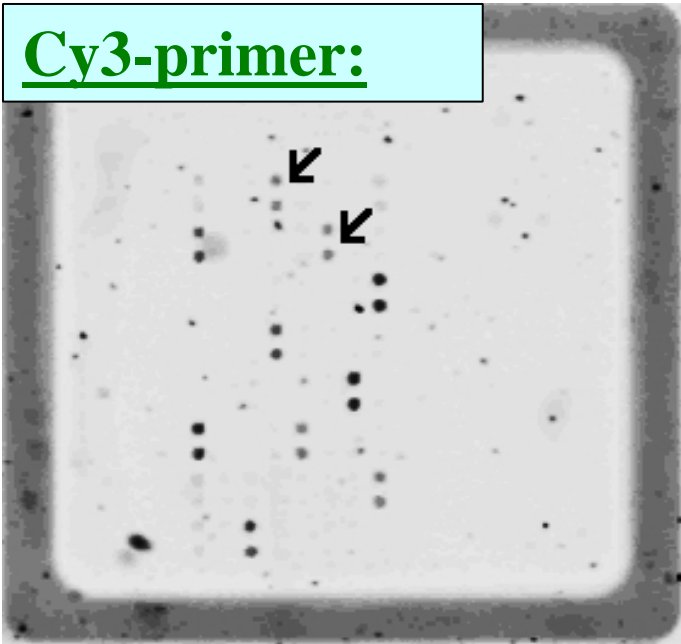
Cy5-dUTP:



ApM

• 3'-end
hybridization
arrowed

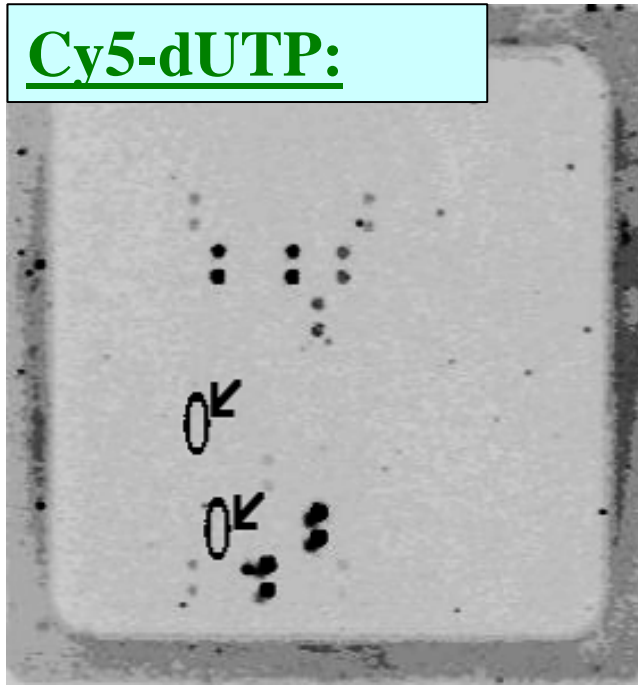
Cy3-primer:



aa-DNA, Cy3:



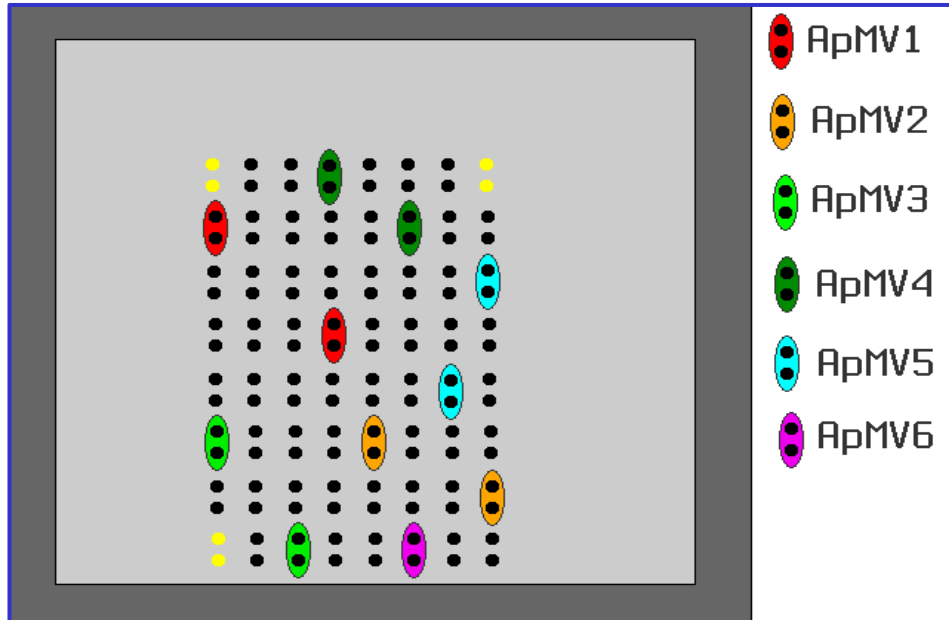
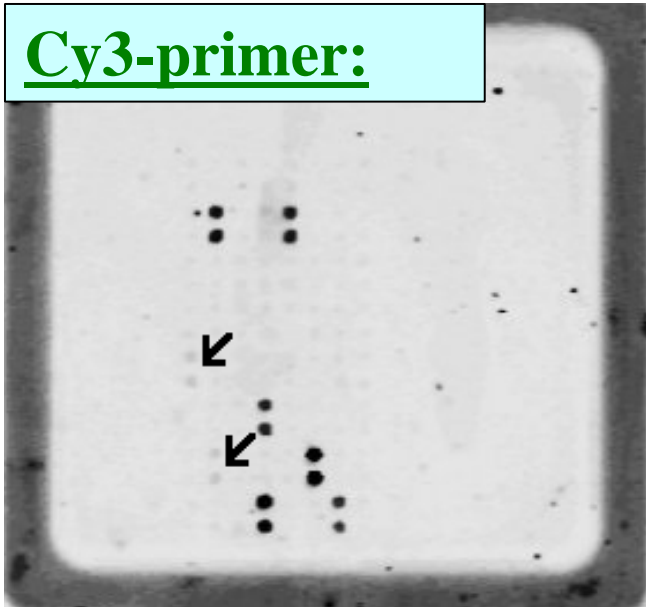
Cy5-dUTP:



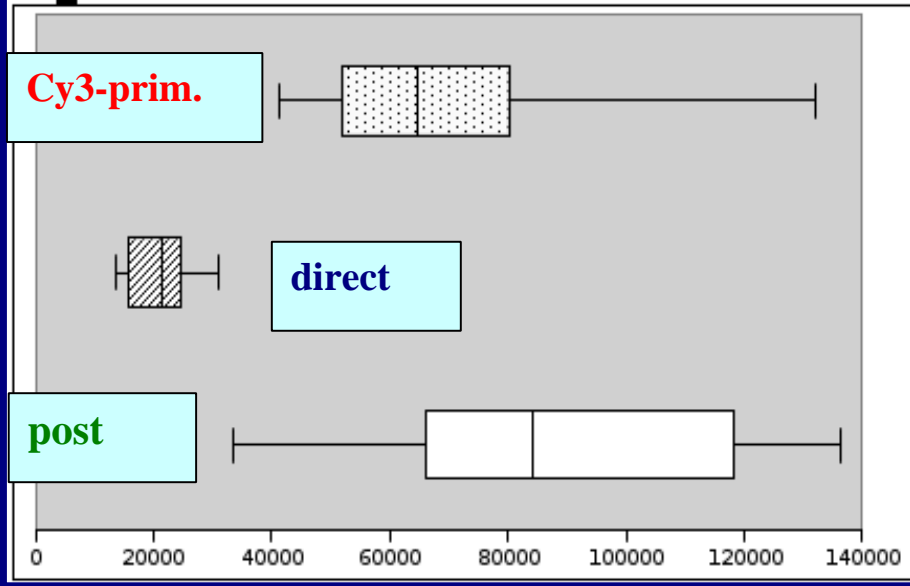
PNRSV

- 3'-end hybridization arrowed

Cy3-primer:

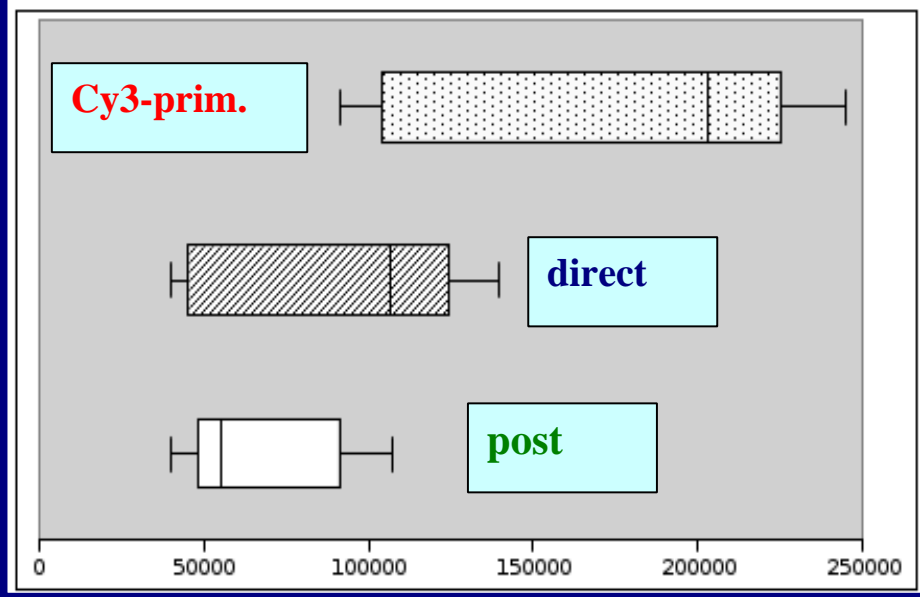


ApMV

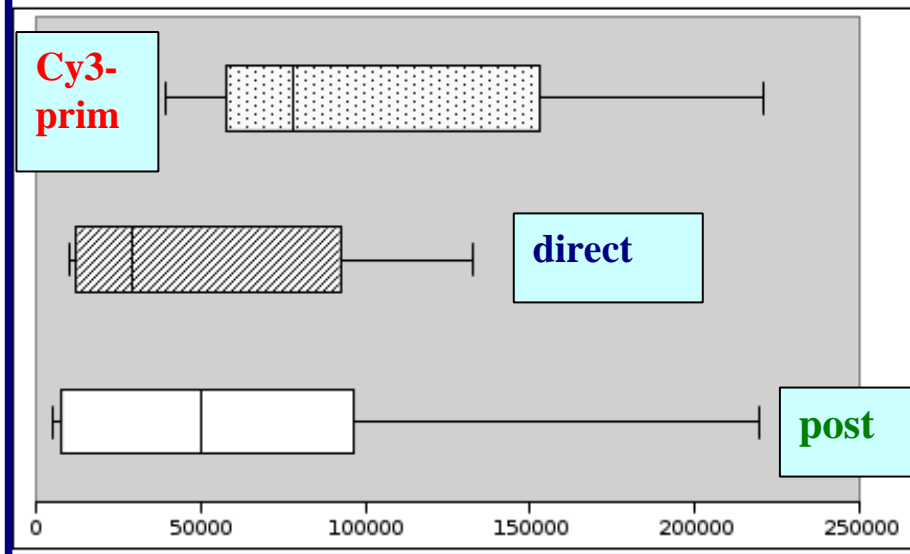


Different labeling comparison

PDV



PNRSV



Microarray testing

- **Different labelling method:**

- both post-labelling and direct incorporation of Cy5-dUTP comparable
- weakest signal by Cy3-primer method, but still sensitive
- Cy3-primer for ApMV: hybridization on control oligos => cross-hybridization or plant DNA amplified?

Microarray testing

- **SUMMARY:**

- cDNA-targets under detection limit of the microarray. Signal amplification needed.
- PCR-products successfully detected (ApMV, PNRSV, PDV, ASGV, ASPV)
- except ASGV2 probe, no cross-hybridization of PCR-products observed
- direct labeling – the best SNR values
- signal intensities: Cy3-primer labeling \approx post-labelling

Microarray testing

What is the next?

- ASGV and ACLSV hybridization
- find unsuitable probes (ASGV2?)
- multiplex RT-PCR, RT-PCR with degenerated primers
- signal amplification – using cDNA
 - ingenereed particles of CPMV [Soto et. al 2006], dendrimers, secondary hybridization, ...

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Targeted viruses

+ssRNA

ASPV, ASGV, ACLSV, PPV

RNA

6,5 - 9,8 kb

poly-(A) tail

ApMV, PNRSV, PDV

RNA1

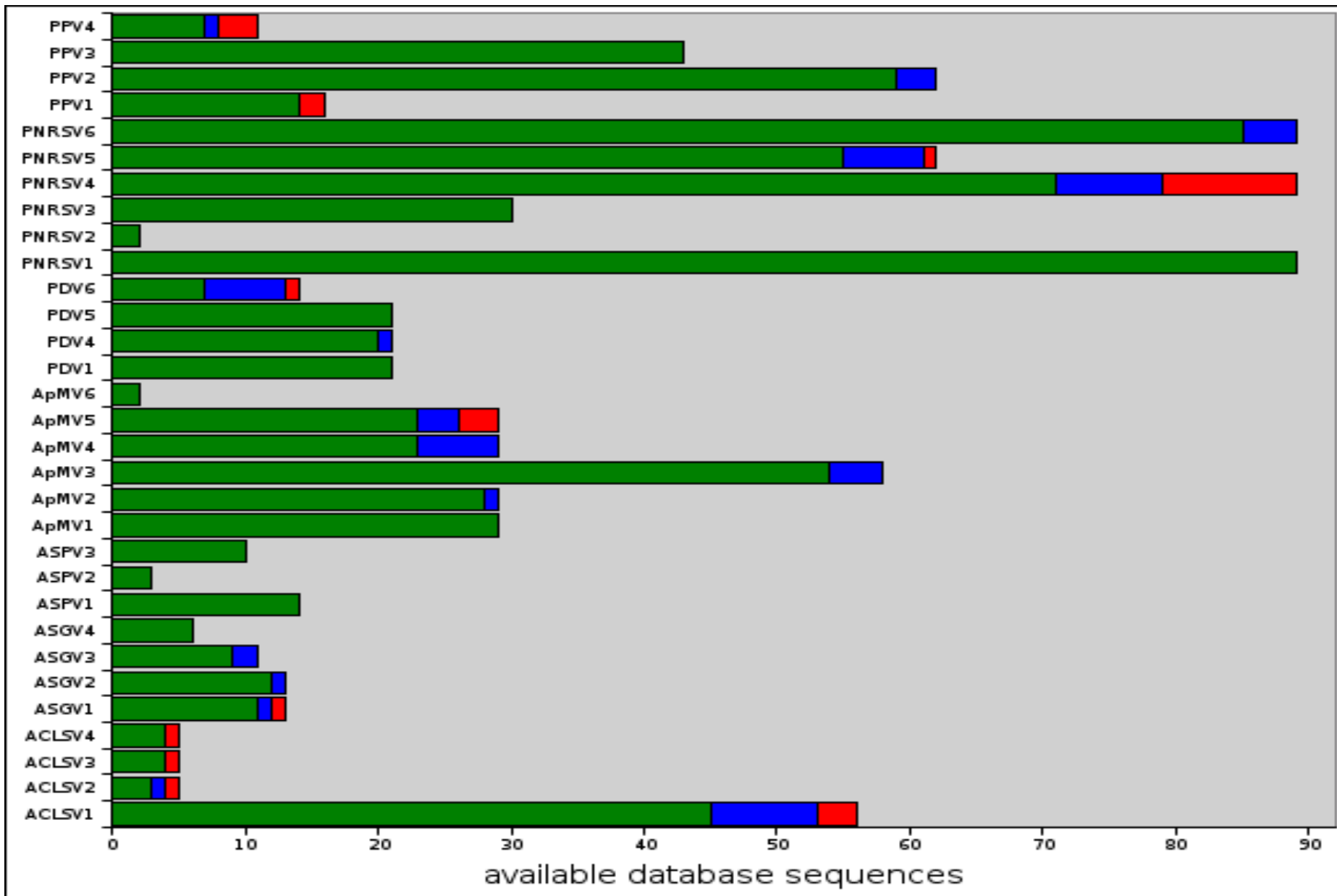
RNA2

RNA3

2 - 3,5 kb

without poly-(A) tail

Designing of the probes



Microchip development

- probe parameters (on conservative region):
 - $dG \leq -60,5$ kcal/mol
 - T_m : 60-65°C
 - minimum of dimers and hairpins ($dG \geq -1$ kcal/mol)
 - **length: 30-40 nt**
- BLAST comparison
- at least 3 different probes for each virus
- “control” oligos (Neil Boonham), 16S rRNA

Microchip testing

- PCR-products: ASGV (post-labelling)
 - targeted probes: ASGV1, 2, 3 (not ASGV4)
 - no cross-hybridization, **very low signal / only 1 of 3 oligos**

