Performance of microarrays for diagnostics of viral infections

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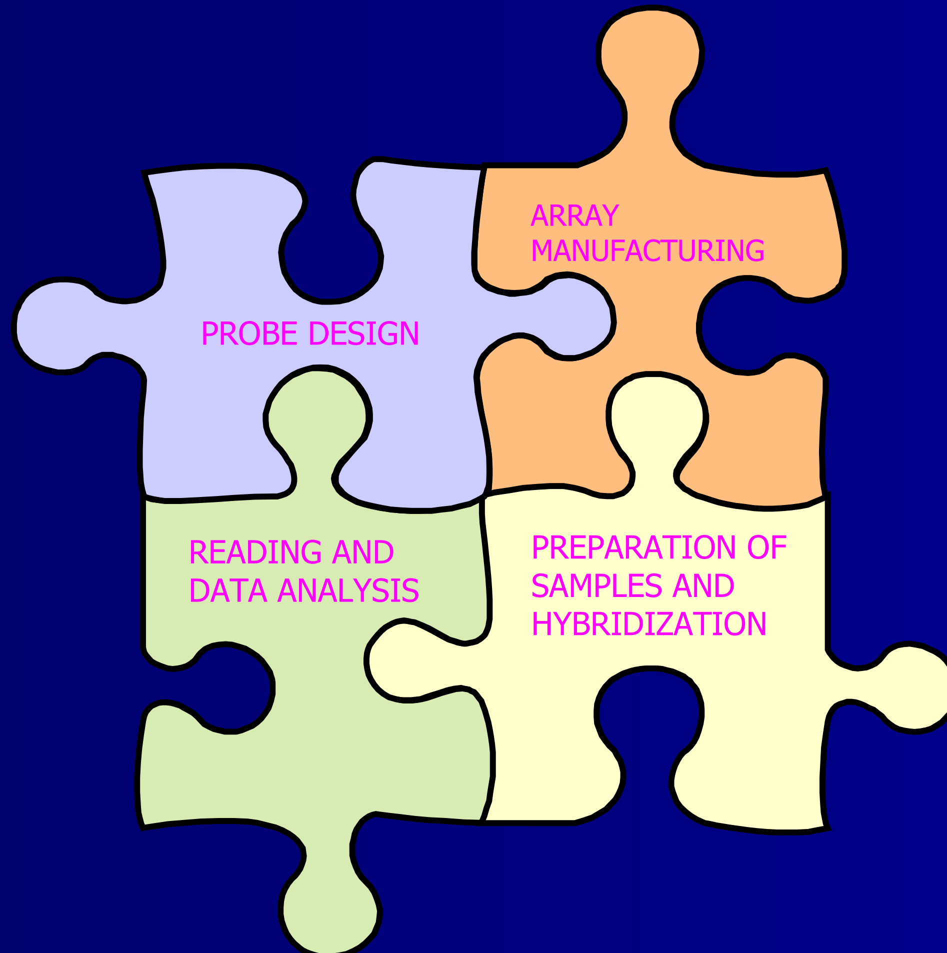
Abstract

- Plant viruses represent an important thread for crops and generally are present in mixed infections involving the presence of several viruses in one sample. Here we summarize the design and results of tests of a microarray for the detection of major viral potato pathogens alone and in mixed infections.
- Short synthetic single-stranded oligomers (40 nt) were used as capture probes. A microchip detecting potato viruses, PVA, PVS, PVM, PVX, PVY and PLRV, in both single and mixed infections was developed and tested. The chip was also designed to distinguish between the main strains of PVY and PVS.
- The design of capture probes using the concepts of hybridization in a solution without taking into account the real conditions on microarrays, in particular the presence of a charged surface requires excessive testing of the chip to validate the sequences.
- The results were compared to routine testing results by ELISA. The microarray tests confirmed in all cases the presence of viruses found previously by ELISA and simultaneously found further pathogens. The presence of these additional pathogens was then confirmed by RT-PCR and sequencing. Our results show that for samples containing several pathogens a well designed microarray may become a high performance practical tool.

THE MICROARRAY PUZZE



Statistics of probe performance

Perfect Match Probe Performance Category	Number on U133A GeneChip	Percent of U133A GeneChip
Responsive	169,154	68.2 %
Unresponsive	26,731	10.8 %
Invariant	50,914	20.5 %
AFFX Quality Control	1,166	0.5 %

> 30 % of probes with poor performance!!!

Data source: Expression Analysis
(technical note – Affymetrix microarrays)

Causes of the Poor Performance of Probes

- Sequence errors
- Unexpected behavior
  (different from probe design parameters)
  - Affinity
  - Dynamics
  - T_m

Computer assisted standard probe design

- Takes into account rules and restrictions on the sequence
  - Hairpins, self-complementarity, …
- Excludes cross-hybridization
- Optimizes probes for given experimental conditions (temperature, concentration, …)
- Quantitative predictions based on the nearest neighbor model

The nearest neighbour model

- Binding energy depends on:

2. Base composition (GC:AT)
3. Base sequence (AT/TA≠TA/AT)
4. The total binding energy is the sum of dinucleotide energies:

E(GGCT) = E(GG)+E(GC)+E(CT)

Standard probe design – the nearest neighbour model

Comparison of published NN free energy (dG) parameters at 37°C

Sequence	Parameter, kcal/mol							
	Gotoh	Vologodskii	Breslauer	Blake	Benight	SantaLucia	Sugimoto	Unified
AA/TT	-0.43	-0.89	-1.66	-0.67	-0.93	-1.02	-1.20	-1.00
AT/TA	-0.27	-0.81	-1.19	-0.62	-0.83	-0.73	-0.90	-0.88
TA/AT	-0.22	-0.76	-0.76	-0.70	-0.70	-0.60	-0.90	-0.58
CA/GT	-0.97	-1.37	-1.80	-1.19	-1.26	-1.38	-1.70	-1.45
GT/CA	-0.98	-1.35	-1.13	-1.28	-1.52	-1.43	-1.50	-1.44
CT/GA	-0.83	-1.16	-1.35	-1.17	-1.03	-1.16	-1.50	-1.28
GA/CT	-0.93	-1.25	-1.41	-1.12	-1.56	-1.46	-1.50	-1.30
CG/GC	-1.70	-1.99	-3.28	-1.87	(-1.65)	-2.09	-2.80	-2.17
GC/CG	-1.64	-1.96	-2.82	-1.85	-2.44	-2.28	-2.30	-2.24
GG/CC	-1.22	-1.64	-2.75	-1.55	-1.67	-1.77	-2.10	-1.84
Average	-0.92	-1.32	-1.82	-1.20	-1.36	-1.39	-1.64	-1.42
Init. w/term. G·C	NA	NA	+2.60	NA	NA	0.91	+1.70	0.98
Init. w/term. A·T	NA	NA	+2.60	NA	NA	1.11	+1.70	1.03
Sodium concentration, M	0.0195	0.195	1.0	0.075	0.115	1.0	1.0	1.0
Rank of stacking matrix	8	8	11	8	9	10	11	12

$$T_m(^\circ C) = \frac{dH}{dS + R\ln(c/4)} + 16.6\log_{10}\left[K^+\right] - 273.15$$

Calculation of the melting temperature

$$\textit{free energy}\quad G = H - TS$$

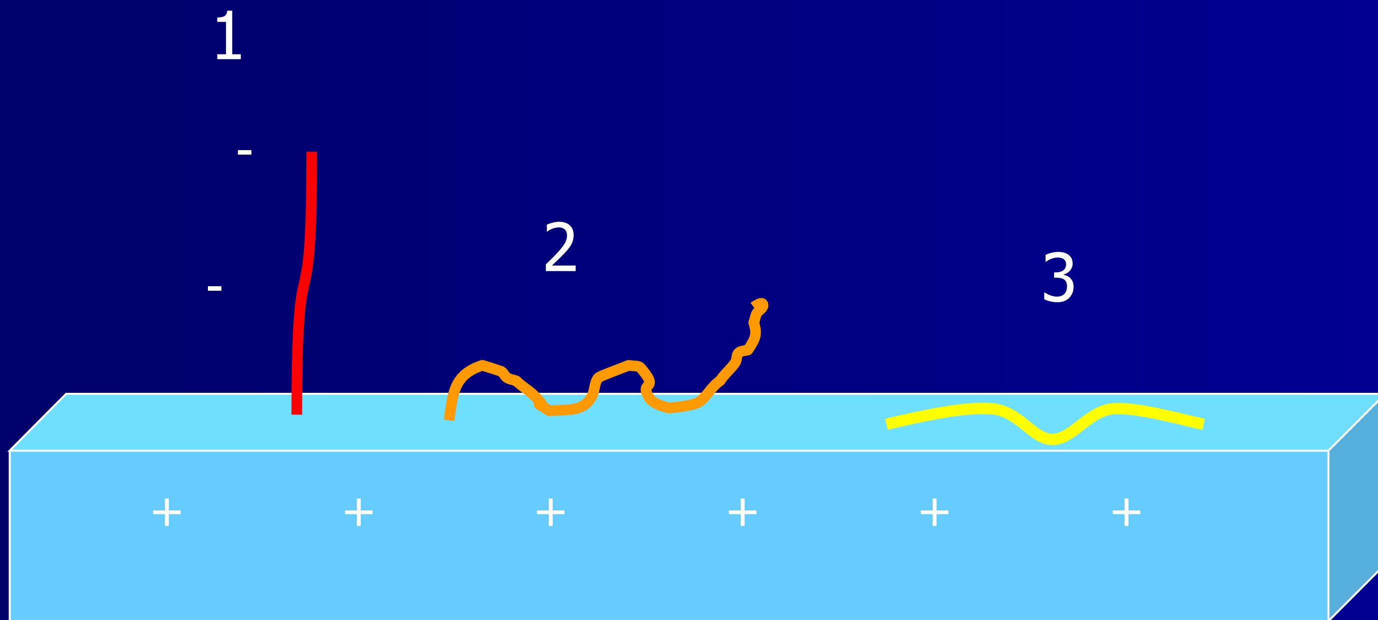
$$T_m(^\circ C) = \frac{dH}{dS + R\ln(c/4)} + 16.6\log_{10}\left[K^+\right] - 273.15$$

dH and dS from experimental data **in solution**

Hybridization on surface-bound oligonucleotides

- Oligonucleotide (probe) and the formed duplex in close contact with surface
- Bulk solution models = only first approximation

DNA-Surface Modes of Binding



Single Molecule Spectroscopy Experiments

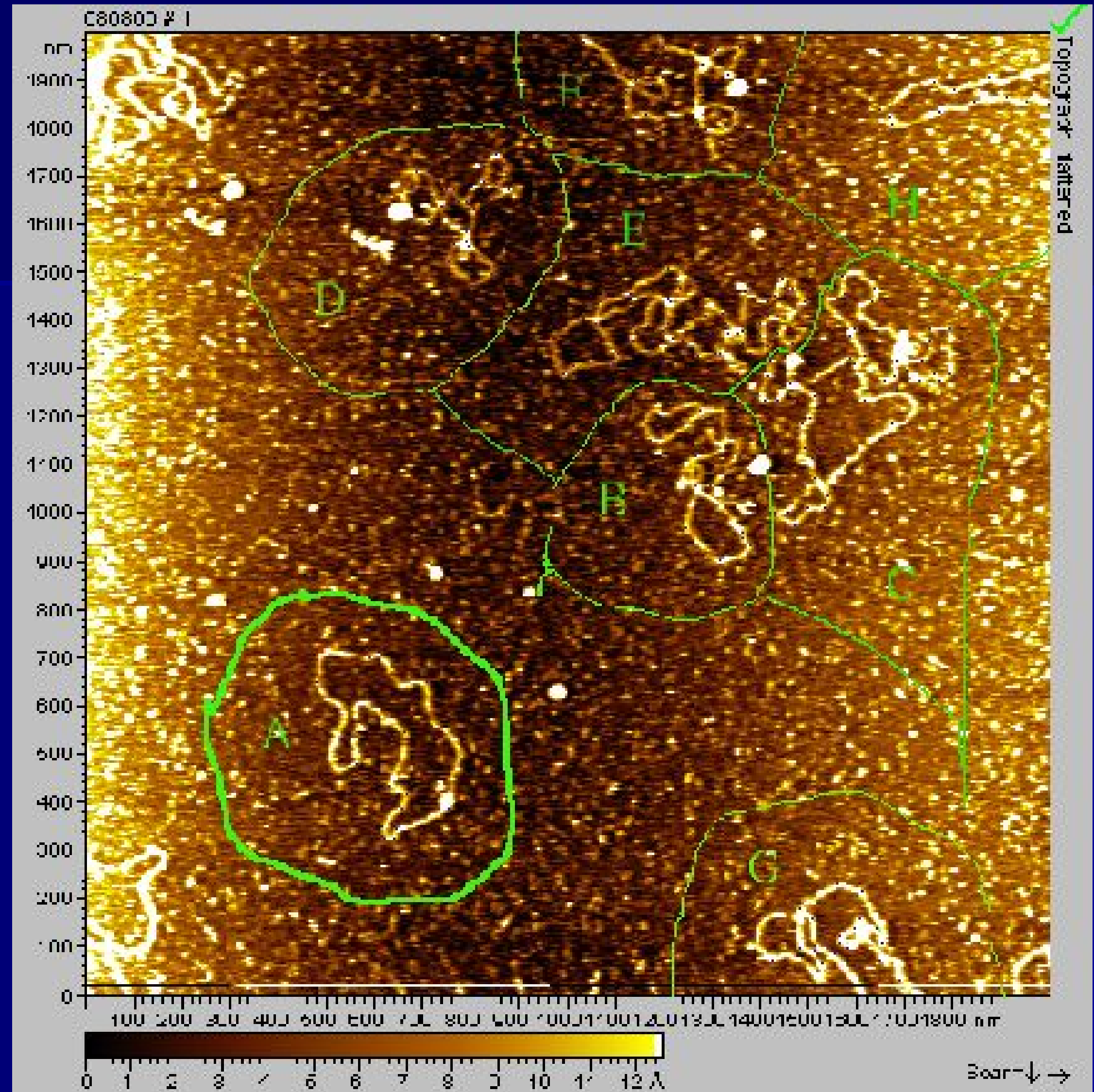
- Results:
  - significant fraction of the surface attached probes is not available for hybridization
  - surface attached probes spend significant time collapsed on the surface (binding mode 3).

M. A. Osborne *J. Phys. Chem. B,* 105 (15), 3120 -3126, 2001

Surface bound DNA

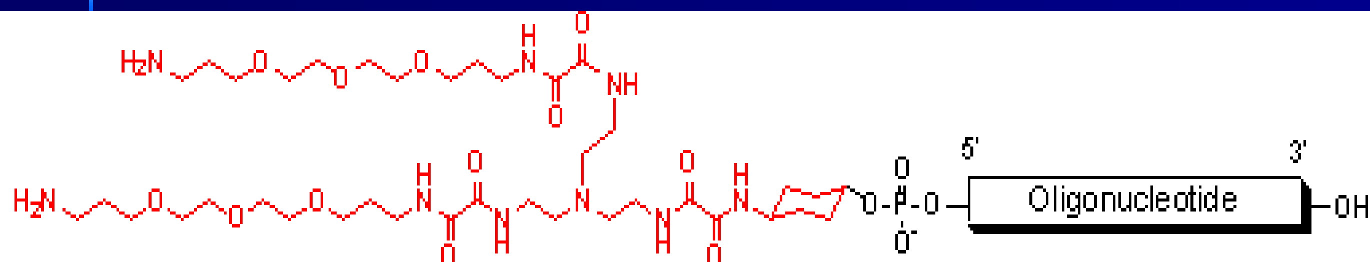
weak electrostatic interaction

AFM imaging

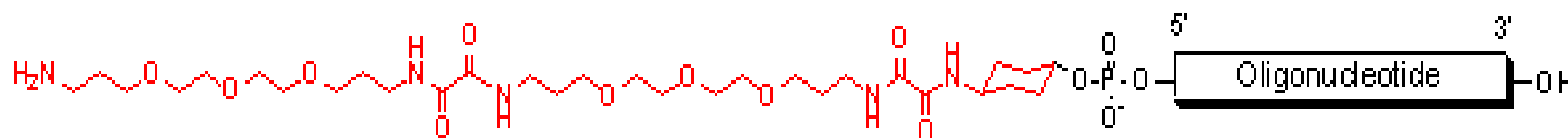


- M. Sip and P. Hinterdorfer, 2000

Amino Modified Nucleotides long tethers



2 amino groups, 31-atom long amino linker (overall) - no charge within tether



39-atom amino linker - no charge within tether

Examples of Short Oligonucleotide Probes Performance

Short oligonucleotide approach

- short synthetic single stranded oligomers (40 nt)
- length 40 nt , melting temperature 60 - 65°C, deltaG < -65 kcal/mol (Vector NTI Suite, InforMax)
- simultaneous detection of several potato viruses (PVA, PVS, PVM, PVX, PVY and PLRV)
- designed to distinguish between the main PVY and PVS strains
- at least 4 probes for each of viruses, according to results obtained from BLAST

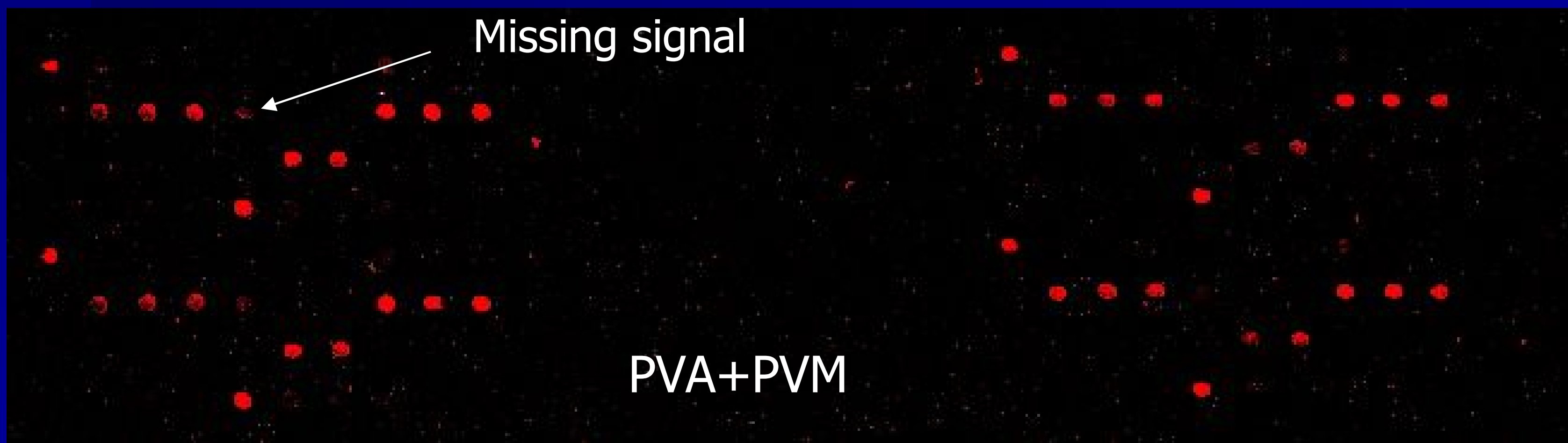
DNA-chip printmap

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cy5_RP	blank	Cy3_T3	blank	PVY5	PVY7	PVX2	PVX3	PVX4	PLRV1	PLRV2	PLRV3
B	PLRV4	PVM1	PVM2	PVM3	PVM4	PVM5	PVM6	PVA2	PVA3	PVA4	PMTV1	PMTV2
C	PMTV3	PMTV4	PMTV5	PVX4_ amino	PVS2_ amino	PVA3_ amino	PVM3_ amino	PVS2	PVS3	PVS4	PVY2	PVY3
D	PVY4	PVY6	PVS1	PVY1	PVA1	PVX1	ARA_ PPA	ARA_GCD H	blank	blank	STUB1	STUB2
E	Cy5_RP	blank	Cy3_T3	Blank	PVY5	PVY7	PVX2	PVX3	PVX4	PLRV1	PLRV2	PLRV3
F	PLRV4	PVM1	PVM2	PVM3	PVM4	PVM5	PVM6	PVA2	PVA3	PVA4	PMTV1	PMTV2
G	PMTV3	PMTV4	PMTV5	PVX4_ Amino	PVS2_ Amino	PVA3_ amino	PVM3_ amino	PVS2	PVS3	PVS4	PVY2	PVY3
H	PVY4	PVY6	PVS1	PVY1	PVA1	PVX1	ARA_ PPA	ARA_GCD H	blank	blank	STUB1	STUB2

PVM

Nonmodified p.

Amino-modified probe



Conclusion for mixed infection samples

- Several probes are necessary for a reliable detection in complex samples.
- How many?

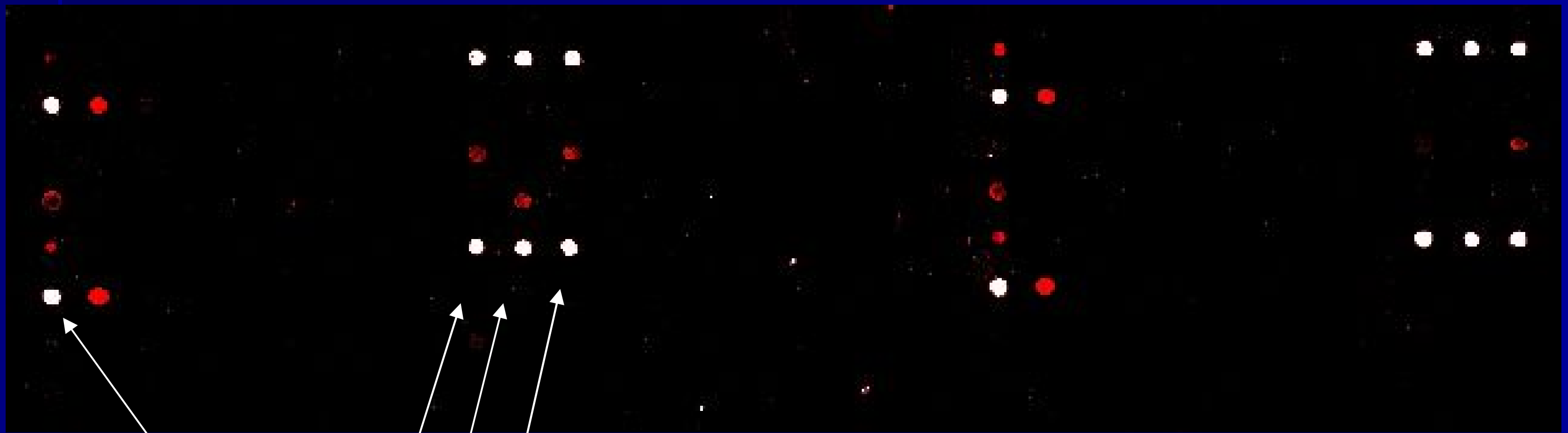
PVS

One single PVS-O probe

PVX

5 different probes for PVX

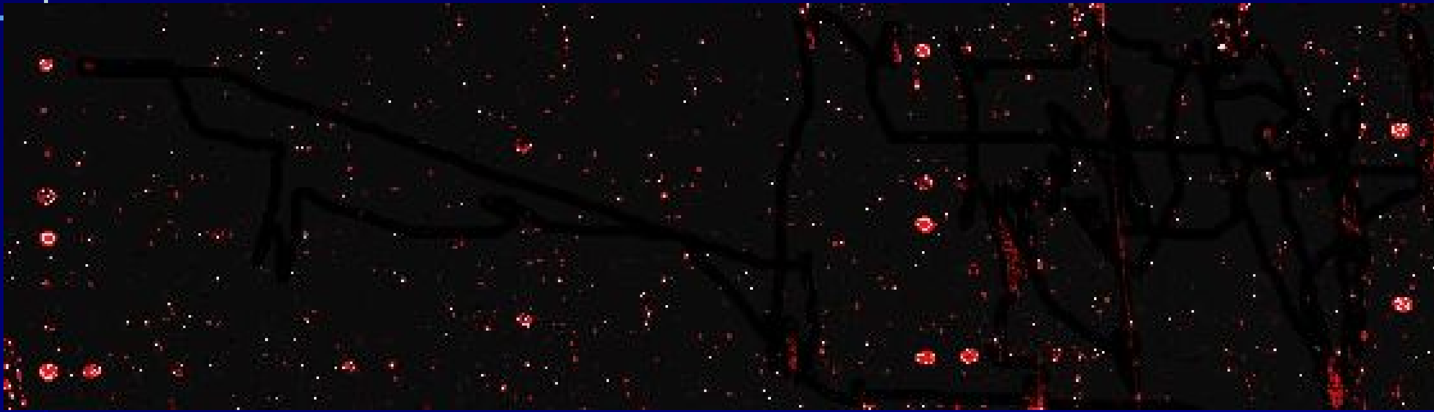
PLRV



4 different PLRV probes

PVY-O and PVY-NTN

PVY-O



PVY-NTN

Conclusions and perspectives

- Practical application possible
  - Rapid diagnostics of samples with many potential microbes
- Limitations
  - sample preparation
    - should prepare in one tube all potential targets for hybridization
  - high cost
  - theoretical background for hybridization on microarrays to be improved.
- Perspectives = overcoming the limitations

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- Bystricka D., Lenz O., Mraz I., Piherova L. Kmoch S. and Sip M.:

  Oligonucleotide-based microarray:

  A new improvement in microarray detection of plant viruses

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Collaborations

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