



# First generation of a mini-oligo array for rapid genome wide analysis of *Plum pox virus*

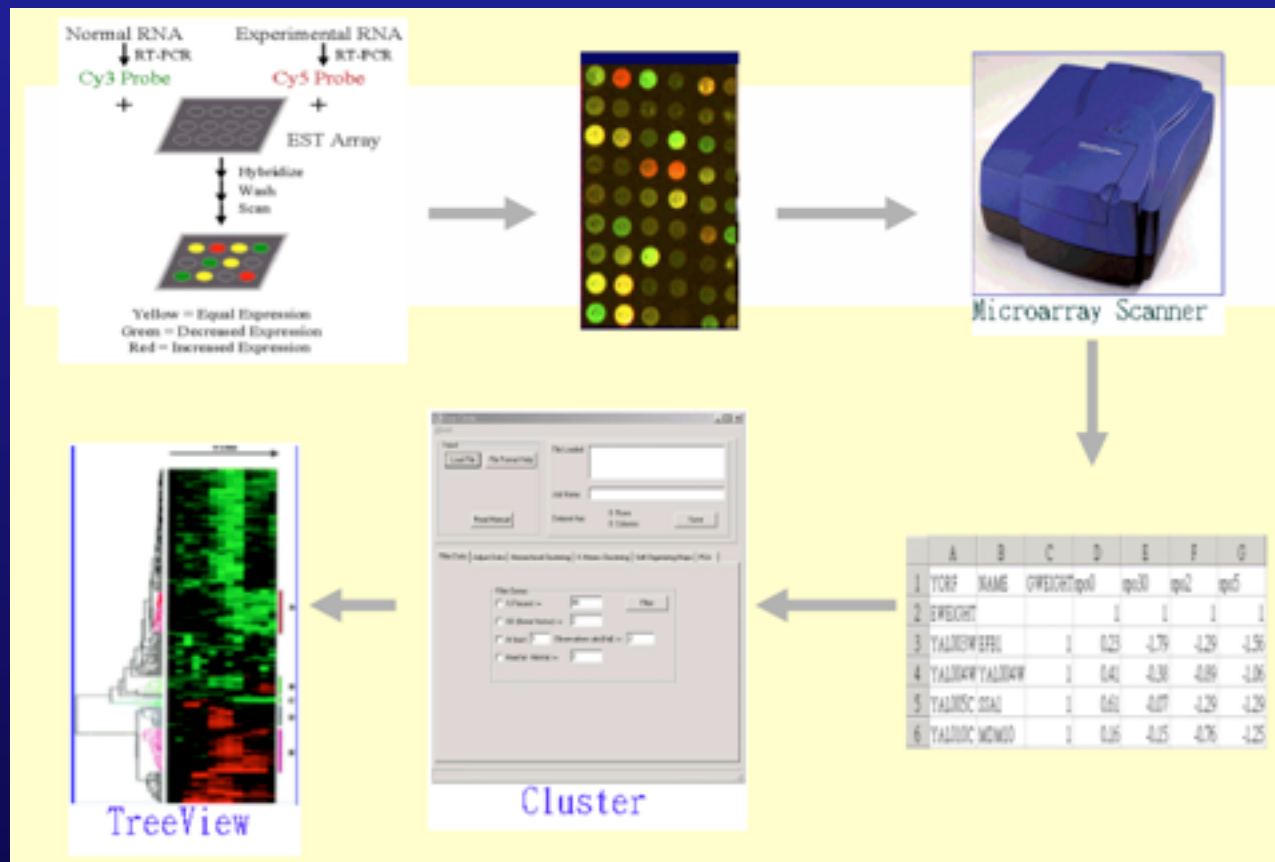
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# WPE.1.- Large scale analysis of *Plum pox virus* current diversity worldwide



## Task TE.1.1.

- Development of mini-oligos array technology for the rapid genome-wide analysis of PPV isolates



***In silico* design**

Design of probes were based on:

1.- The use of complete sequenced isolates available of 6 groups of PPV (D, M, EA, C, Rec, W)

A) to design specific probes to group

B) to design a set of PPV probes common to several groups

2.- The use of additional sequences from CP, NIb, P3-6K1 regions to design more probes and increase the number tests in such regions



## 1.- Complete sequenced isolates

To design specific probes common to several types, specific of types and specific of isolate

## 2.- CP, NIb, P3-6K1 regions



A total of 1,440 probes were designed as candidate probes *in silico*

In order to select only the most suitable probes:  
A database was elaborated including parameters:

Position  
Accession Number/Name  
Probe Sequence  
Type  
Sequence Definition  
Sequence Length  
Quality  
Rating  
Position  
Length  
Tm  
GC%  
Hairpin dG(Internal)  
Hairpin Bond(Internal)  
Self Dimer dG(Internal)  
Self Dimer Bond(Internal)  
Run/Repeat Length

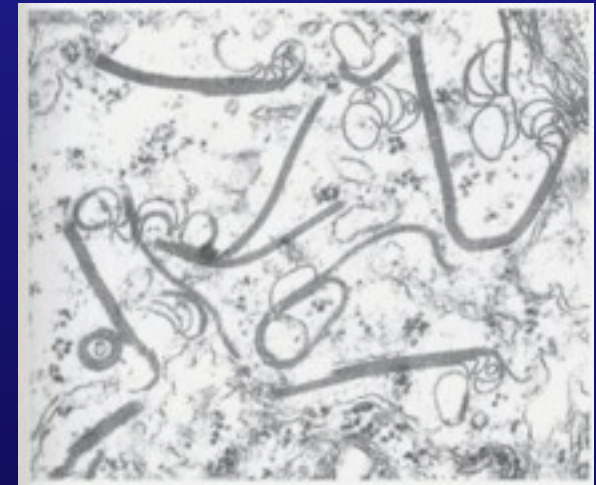
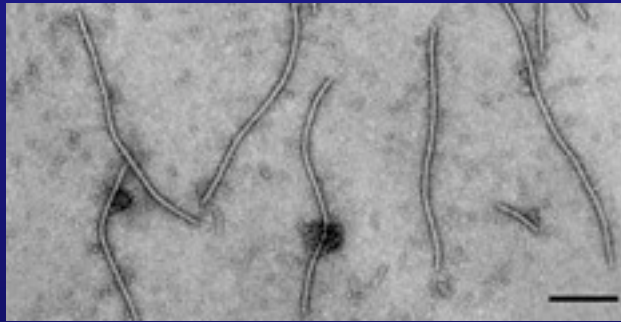


PPV probes.xls													
Nuevo Abrir Guardar Imprimir Importar Copiar Pegar Formato Deshacer Rehacer Autosuma Orden A-Z Orden Z-A Galería Herramientas Zoom Ayuda													
Verdana 10 N K S W % 125% Ayuda													
	Hojas			Gráficos		Gráficos SmartArt		WordArt					
	A	B	C	D	E	F	G	H	I	J	K	L	
	Positior	Accession Numt	Probe Sequence	Type	Sequence Definition	Sequence L	Quality	Rating	Position	Length	Tm	GC%	
1	137	X16415	GCAAGTCAAGATGTCAACCATT	D- M- Rec-W	Plum pox virus RNA genome.	9.787	Best	79,4	5.442	22	53,6	40,9	
2	185	DQ431465	TGACGCAGCAATCCACCA	EA	Plum pox virus isolate El Amar, comp	9.791	Best	86,9	8.471	18	54,6	55,6	
3	199	AY184478	ATCAGGATAATGCGGACAGAGT	C	Plum pox virus isolate SoC polyprote	9.795	Best	87,2	2.368	22	55,1	45,5	
4	238	X16415	GTCCAGAGAACCGCCAAGT	D-M-EA-REC-W	Plum pox virus RNA genome.	9.787	Best	87,5	7.143	19	55	57,9	
5	269	X16415	TCTACTGTGCCGAAGAGCG	D-M-REC-W	Plum pox virus RNA genome.	9.787	Best	83	8.285	19	54,9	57,9	
6	332	X16415	TTGGAAAGGCTGGAAGAGAA	D-W	Plum pox virus RNA genome.	9.787	Best	75	9.607	21	55	47,6	
7	333	AJ243957	TGGAAAGGGCTGGAAGGAGA	M	Plum pox virus strain M, complete ge	9.786	Best	75	8.643	19	55	57,9	
8	406	DQ431465	AGGGTCTTCGGTGTCTTCAA	EA	Plum pox virus isolate El Amar, comp	9.791	Best	75	1.968	20	55,1	50	
9	407	X16415	AGGGCTTCGGTGTCTTCAA	D-M-REC-w	Plum pox virus RNA genome.	9.787	Best	75	1.961	19	55,2	52,6	
10	421	AY184478	TCAAGGCAAGTTCGGAGCA	C	Plum pox virus isolate SoC polyprote	9.795	Best	87,5	7.703	19	54,9	52,6	
11	460	AY184478	AGCAGCAATATAAGGAGGAACG	C	Plum pox virus isolate SoC polyprote	9.795	Best	85,7	1.680	22	54,4	45,5	
12	463	X16415	AACAGTATCAAGAGGAGCGTG	D-REC-W	Plum pox virus RNA genome.	9.787	Best	81,9	238	21	53,7	47,6	
13	469	AJ243957	GTAGAGAGGAGCGTGAGAGATT	M	Plum pox virus strain M, complete ge	9.786	Best	75	7.063	22	54,9	50	
14	504	DQ431465	GGTCTGATGCCGTTGTTG	EA	Plum pox virus isolate El Amar, comp	9.791	Best	86,8	6.902	19	54,6	57,9	
15	538	AY184478	TTACTGAAGAGGCTCCAACCTG	C	Plum pox virus isolate SoC polyprote	9.795	Best	81,2	9.260	22	54,1	45,5	
16	591	DQ431465	CTCTTCAACAACCTTCAACGA	EA	Plum pox virus isolate El Amar, comp	9.791	Best	87,5	4.768	22	55	45,5	
17	639	DQ431465	AGGATGTCTGAGGCTTCTCTG	EA	Plum pox virus isolate El Amar, comp	9.791	Best	78,9	1.838	21	54,7	52,4	
18	645	X16415	TCCGAGGCATGACTACAACCT	D-M-W-rec (izq)	Plum pox virus RNA genome.	9.787	Best	87,5	7.996	21	54,9	47,6	
19	663	DQ431465	CTGTTTATGCGGAAAGTGGCT	EA	Plum pox virus isolate El Amar, comp	9.791	Best	75	4.876	21	55,1	47,6	
20	669	AY184478	ATGCGGAGAGTAGCCAACAA	C	Plum pox virus isolate SoC polyprote	9.795	Best	87,5	2.603	20	55	50	
21	692	AY184478	AAAGGCGAACGGGCAGAA	C	Plum pox virus isolate SoC polyprote	9.795	Best	75	127	18	54,9	55,6	
22	726	X16415	CGTAAGCGTGTAGTCGGTAAC	D-W	Plum pox virus RNA genome.	9.787	Best	87,5	4.453	21	54,9	52,4	
23	726	DQ431465	CGTAAGCGTTTGGTTGCCA	EA	Plum pox virus isolate El Amar, comp	9.791	Best	75	4.487	19	54,8	52,6	
24	746	AY184478	CTACATTTCGGAAGGCCAGC	C	Plum pox virus isolate SoC polyprote	9.795	Best	84,3	4.660	20	54,1	55	
25	809	AY184478	GCCACGCTACGACATTACTT	C	Plum pox virus isolate SoC polyprote	9.795	Best	84,6	6.042	20	54,1	50	
26	820	X16415	ATCTTGTGTTGGACGAGGCA	D	Plum pox virus RNA genome.	9.787	Best	87,3	2.476	20	55,2	50	
27	831	AY912058	GACGAGGCAACCAAGAGATT	W	Plum pox virus isolate 48-922, comp	9.786	Best	86,5	5.132	21	54,5	47,6	
28	876	AY028309.2	GGCTTCCACCATGTCCACA	Rec	gi 49659680 gb AY028309.2  Plum p	9.786	Best	77,8	6.834	19	55,2	57,9	
29	893	AY184478	TGGCAAGGGCGAGATAACC	C	Plum pox virus isolate SoC polyprote	9.795	Best	75	8.746	19	55,1	57,9	
30	903	AJ243957	GAGATAACCAAGGAATGAGCG	M	Plum pox virus strain M, complete ge	9.786	Best	87,5	5.236	22	55,1	50	
31	906	AY028309.2	ATAACACCAGGGATGAGCGG	Rec	gi 49659680 gb AY028309.2  Plum p	9.786	Best	75	2.245	20	55,1	55	
32	907	X16415	TAACACCAGGAAATGAGCGG	D-W	Plum pox virus RNA genome.	9.787	Best	87,5	7.441	21	55	47,6	
33	916	DQ431465	GGATGAGTGGCTTCGTTGTG	EA	Plum pox virus isolate El Amar, comp	9.791	Best	86	6.720	20	54,4	55	





# Plum pox virus (PPV): structure



**Potyviridae family Potyvirus genus**  
**Flexuous rods particles (700 x 11 nm)**

**Single-stranded RNA molecule of about 10,000 nucleotides coated by up to 2,000 subunits of a single coat protein (CP)**

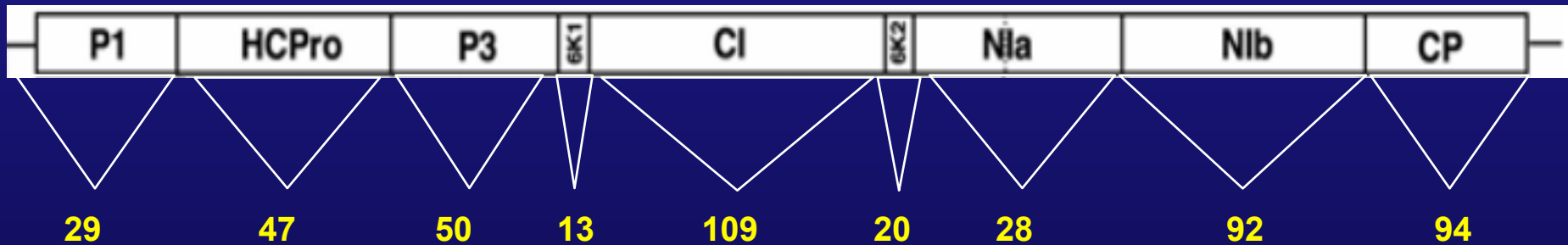
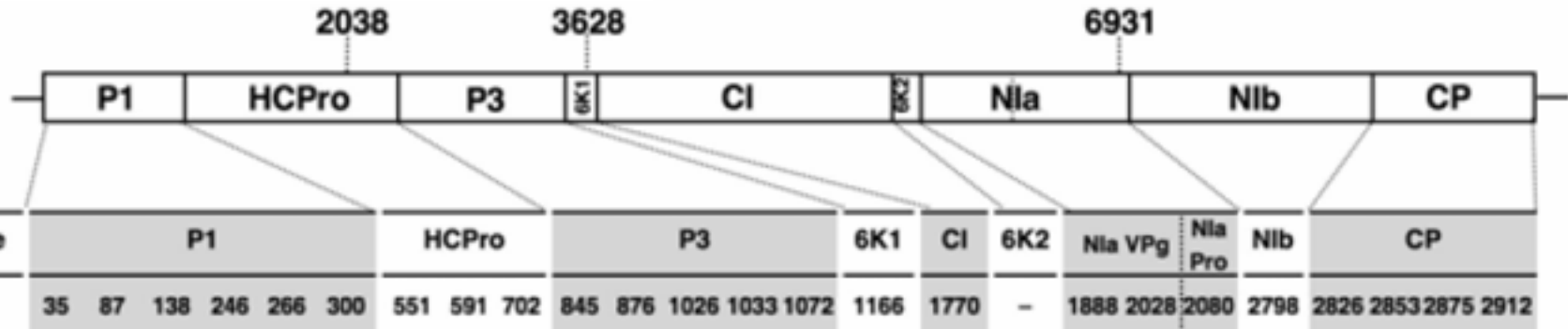


## Identification of *Plum pox virus* Pathogenicity Determinants in Herbaceous and Woody Hosts

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**A**



482 probes final candidate probes  
56 were selected to test if the approach was possible



***In vitro* assays**

## Concentration of oligo probes in spotting buffer ?

Different concentrations have been tested

3-4 micro Molar (Dawson et al., 2005) (29-mer)

80 micro Molar (Calebro et al., 2004) (35-mer)

10 micro Molar (Pasquini et al. 2008) (70-mer)

# To improve hybridization in oligoarrays

COST ACTION 853

“Agricultural biomarkers for array technology”

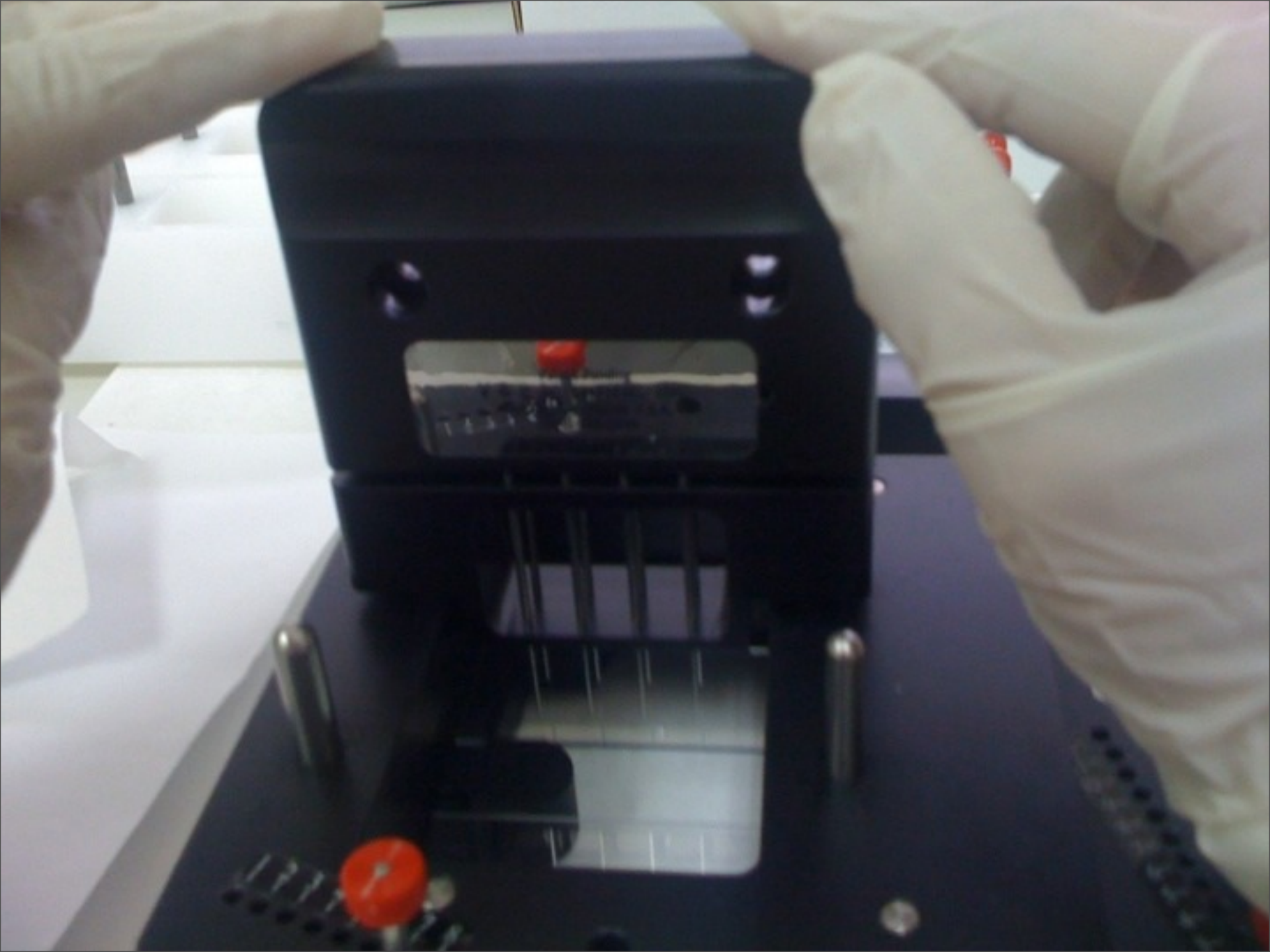
5'-NH<sub>2</sub>-C<sub>12</sub>-TTTTT-18 to 28 mer (Brodossy et al.)

5'-NH<sub>2</sub>-C<sub>6</sub>- 20 mer (Grundmann et al.)

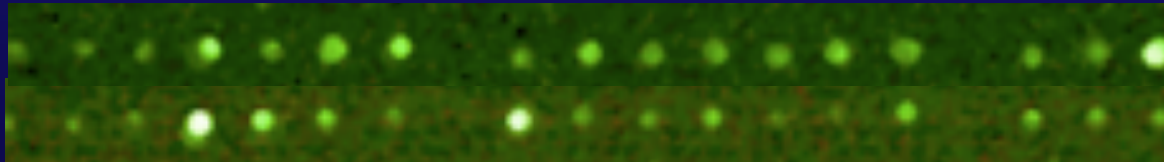
**Spacer preceding probe**



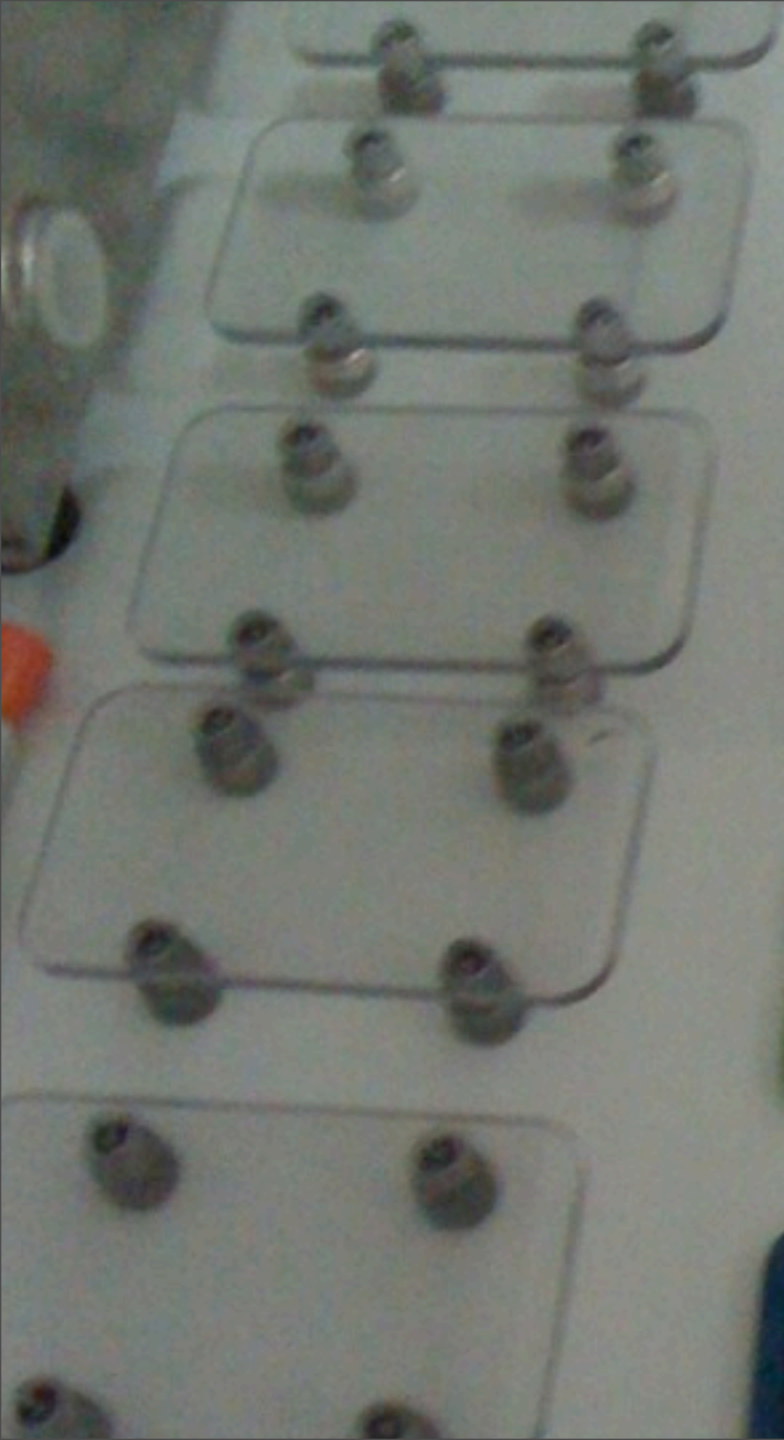




Different spotting conditions have been tested  
(Genepix 4000b)





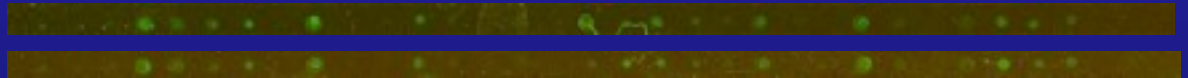




# INTRA-LAB REPRODUCIBILITY

Set of probes 1 (group specific)

SAMPLE 1 D+M (Cy3)  
SAMPLE 2 (HEALTHY CONTROL) (Cy5)



SAMPLE 1 M (Cy3) Ms79  
SAMPLE 2 D (Cy5) 4.3



Set of probes 2

SAMPLE 1 D+M (Cy3)  
SAMPLE 2 (HEALTHY CONTROL) (Cy5)



SAMPLE 1 M (Cy3) Ms79  
SAMPLE 2 D (Cy5) 4.3

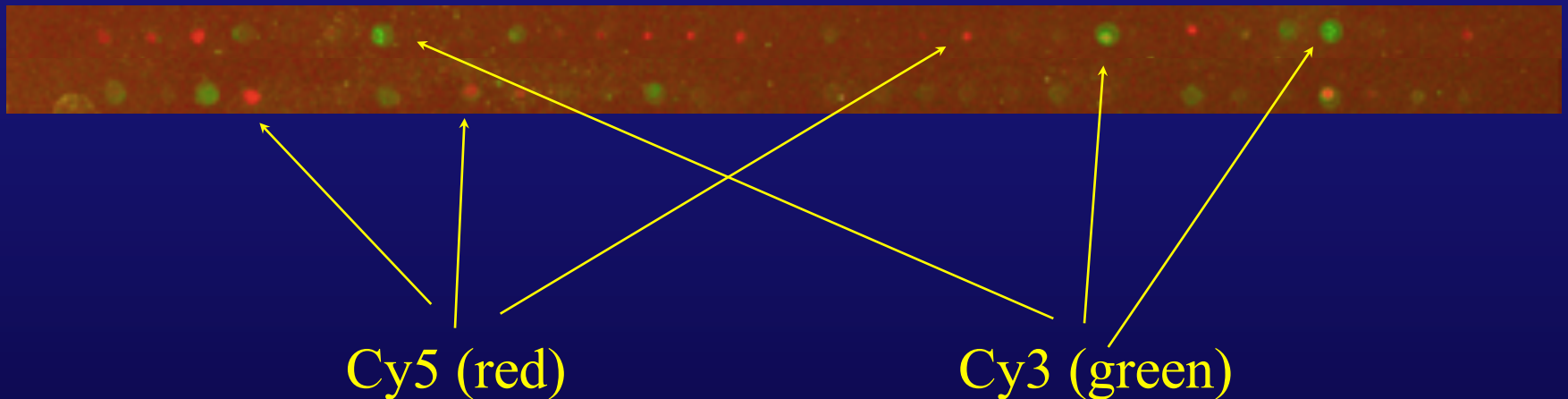


M+D

M+D



A first generation of oligo-microarrays using 4.3. isolate (type D) labelled with Cy5 (red) and Ms79 isolate (type M) labelled with Cy3 (green) are showed below. Positive hybridizations are visualized as dots (those D specific hybridizations in red and those M specific hybridizations in green)



# First generation of oligoarray

Mini-oligo arrays have been produced including 56 selected probes from the whole PPV genome present in different PPV-types.

Spotting and pos-spotting procedures have been evaluated using GenPix 4000B and suitability of probes for typing is being performed using well-characterized isolates of PPV-D,-M, -EA, in collection in Partner 6's greenhouse

# THANK YOU FOR YOUR ATTENTION



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