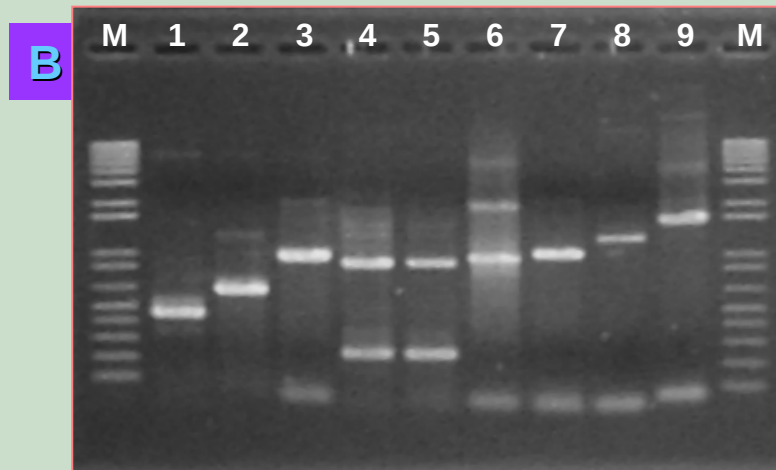
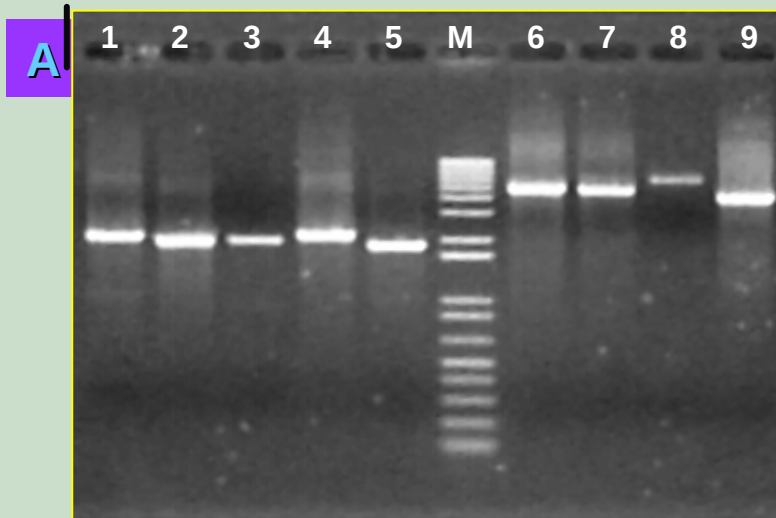


(0) LMu-primer pair.pcr: Target & Yield DNA Sizes

PCR-rx on M13 Clones:

(A) Target Inserts on clones

(B) Dual-Phase PCR Yield



No.	Virus	M13R/F	((A))	PCR	((B))
Lane	Genus	Clone	SeQ	Primers	Size
1	Poty	DsMV	1826	06 07	414
2	Poty	TuMV	1757	03 07	592
3	Poty	<u>PVA</u>	1682	03 11	<u>789</u>
4	Poty	ZaMMV	1845	02 05	<u>916</u>
5	Poty	ZaMV	1600	03 11	<u>982</u>
6	Tombus	CNV	4701	09 10	856
7	Tombus	TBSV	4776	09 10	<u>856</u>
8	<u>Carmo</u>	<u>HCRSV</u>	3910	04 08	<u>1208</u>
9	Potex	BaMV	6366	01 04	1613

Dual-Phase PCR Protocol (SOP):

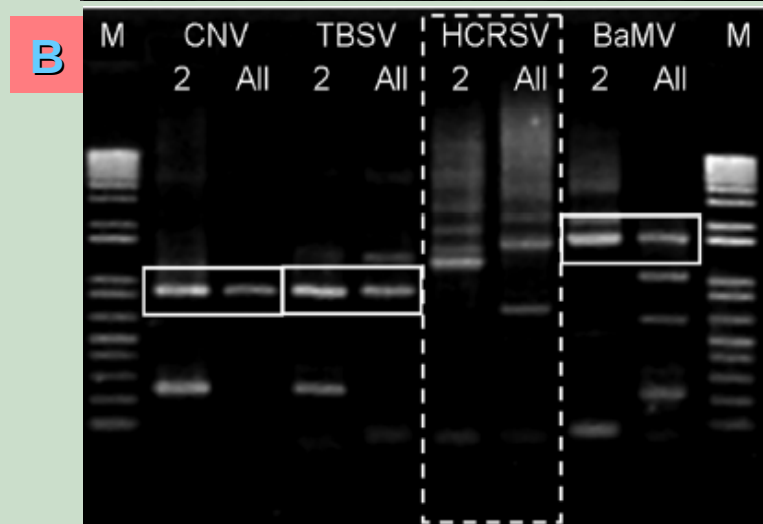
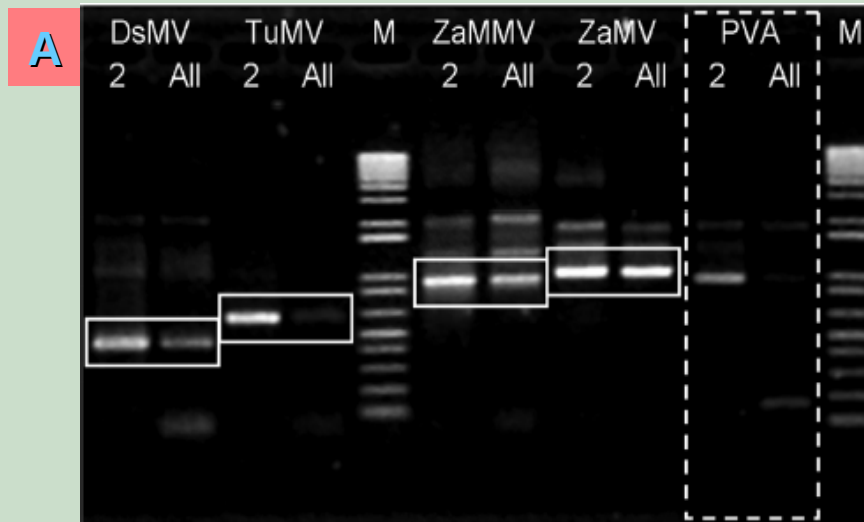
- Touch-Down Phase:** on original templates [36.0~30.0oC] x13 cycles, drop 0.5oC each
- Steady-State Phase:** on PCR yield templates [55.0oC] x23 cycles

Mu-Primer = Total 10 pmol [2/2 or 2/11] run 5/20ul on 1.5% gel
M: Size marker of 1 kb plus DNA ladder (Invitrogen Inc.).

(1) LMu-primer pair/ms.pcr: Yield DNA & Specificity

PCR-rx on Respective Target:

- 1) 2 primers pair = 10pmol
- 2) All 11 MinSet primers = 10pmol



No.	Virus	M13R/F	PCR		((A,B))
Lane	Genus	Insert	Primers		Size
1	Poty	DsMV	06	07	414
2	Poty	TuMV	03	07	592
3	Poty	ZaMMV	02	05	832
4	Poty	ZaMV	03	11	982
5	Poty	PVA	03	11	789
6	Tombus	CNV	09	10	856
7	Tombus	TBSV	09	10	856
8	Carmo	HCRSV	04	08	1208
9	Potex	BaMV	01	04	1613

Dual-Phase PCR Protocol (SOP):

- a) **Touch-Down Phase:** on original templates [36.0~30.0oC] x13 cycles, drop 0.5oC each
- b) **Steady-State Phase:** on PCR yield templates [55.0oC] x23 cycles

Mu-Primer = Total 10 pmol [2/2 or 2/11] run 5/20ul on 1.5% gel
M: Size marker of 1 kb plus DNA ladder (Invitrogen Inc.).

(2a) Array-hyb*CLE: ms.pcr*dig & Ab*ap|CDP-star

2.TuMV ● ● ●	1.DsMV ● ● ●	5.PVA ● ● ●
3.ZaMMV ● ● ●	4.ZaMV ● ● ●	8.HCRSV ● ● ●
6.CNV ● ● ●	7.TBSV ● ● ●	9.BaMV ● ● ●

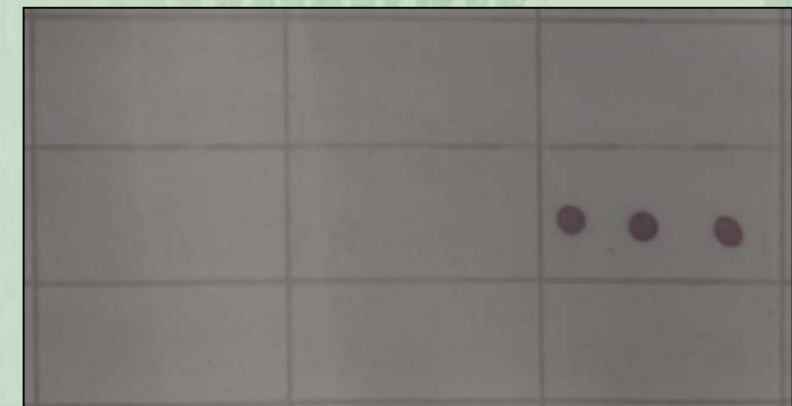
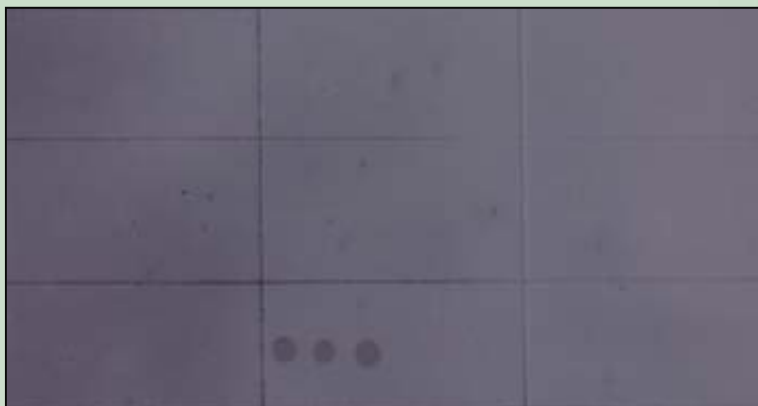
Target: MS PCR yield with DIG-labeled
 Probe-Array: Nine 50-mer Array-probe
 PreHyb/Hyb: 42/45oC 1.5/12.0hr with
 Hyb Kit, Roche
 Wash/Signal: 45/48oC & anti-DIG Ab|ap
 CDP*Kit, NEB
 Exposure: RToC 16.0hr



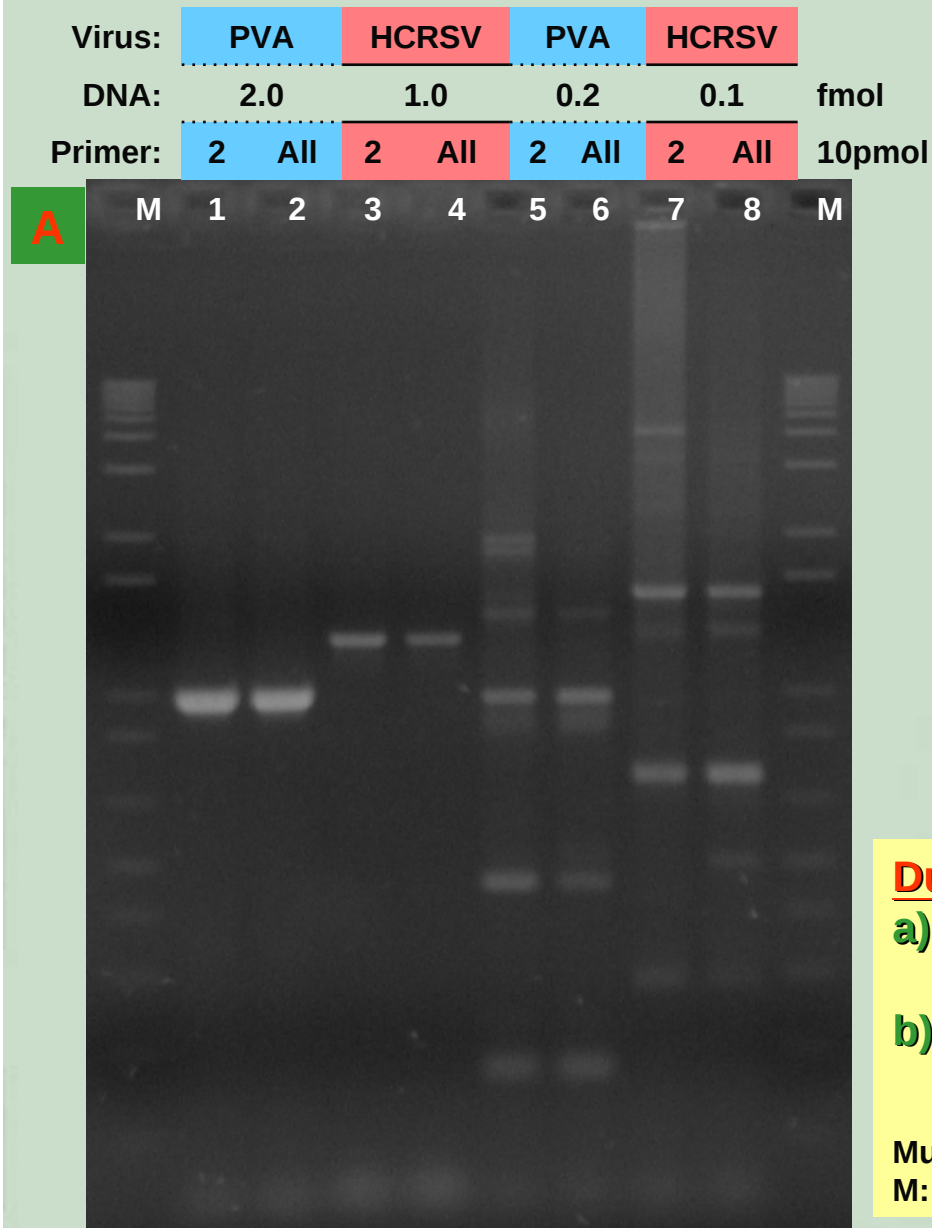
(2b) Array-hyb*Dye: ms.pcr*dig & Ab*ap|NBT-kit

2.TuMV ● ● ●	1.DsMV ● ● ●	5.PVA ● ● ●
3.ZaMMV ● ● ●	4.ZaMV ● ● ●	8.HCRSV ● ● ●
6.CNV ● ● ●	7.TBSV ● ● ●	9.BaMV ● ● ●

Target: MS PCR yield with DIG-labeled
 Probe-Array: Nine 50-mer Array-probe
 PreHyb/Hyb: 42/45oC 1.5/12.0hr with
 Hyb Kit, Roche
 Wash/Signal: 45/48oC & anti-DIG Ab|ap
 NBT*Kit, NEB
 Exposure: RToC 16.0hr



(3) LMu-primer pair/ms.pcr: Target Dose Sensitivity



No.	Virus	M13R/F	PCR		(A,B)
Lane	Genus	Insert	Primers		Size
	Poty	DsMV	06	07	414
	Poty	TuMV	03	07	592
	Poty	ZaMMV	02	05	<u>832</u>
	Poty	ZaMV	03	11	<u>982</u>
1,5	Poty	<u>PVA</u>	03	11	<u>789</u>
	Tombus	CNV	09	10	856
	Tombus	TBSV	09	10	<u>856</u>
3,7	<u>Carmo</u>	<u>HCRSV</u>	04	08	1208
	Potex	BaMV	01	04	1613

Dual-Phase PCR Protocol (SOP):

- Touch-Down Phase: on original templates [36.0~30.0oC] x13 cycles, drop 0.5oC each
- Steady-State Phase: on PCR yield templates [55.0oC] x23 cycles

Mu-Primer = Total 10 pmol [2/2 or 2/11] run 5/20ul on 1.5% gel
M: Size marker of 1 kb plus DNA ladder (Invitrogen Inc.).

(3) LMu-primer pair/ms.pcr: Sensitivity & Specificity

Virus:	PVA		HCRSV		PVA		HCRSV		
DNA:	2.0		1.0		0.2		0.1		fmol
Primer:	2	All	2	All	2	All	2	All	10pmol

Probe: HCRSV 3'-DIG-labeled 50-mer Array-probe
 PreHyb/Hyb: 42/45oC 1.5/12.0hr with Hyb Kit, Roche
 Wash/Signal: 45/48oC & anti-DIG Ab/ap CDP*Kit, NEB
 Exposure: RToC 16.0hr

