

Development of a new microarray-based diagnostic tool to detect novel viral pathogens involved in potentially devastating animal diseases.

Christian Bellehumeur^{1,2}

Josée Harel^{1,2}, Yvan L'Homme³, Luke Masson⁴ et Carl A Gagnon^{1,2}

- 1- Centre de recherche en infectiologie porcine et avicole (CRIPA), Université de Montréal, St-Hyacinthe, Québec, Canada
- 2- Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Université de Montréal, St-Hyacinthe, Québec, Canada
- 3- St-Hyacinthe Laboratory, Canadian food inspection agency, St-Hyacinthe, Quebec, Canada
- 4- Biotechnology research institute (BRI), National research council Canada (NRC), Montréal, Québec, Canada

CAHLN / RCTLSA

Université
de Montréal



GREMIP



Canadian Swine
Health Board
Conseil canadien
de la santé porcine

Introduction

Identification of virus involved in a disease

Proven method!

Coronavirus implicated in SARS outbreak (2003)

Rota et al, 2003, Science 300(5624): 1394-1399

Detection of pH1N1 (2010)

Greninger et al, 2010, PLoS One 5(10): e13381

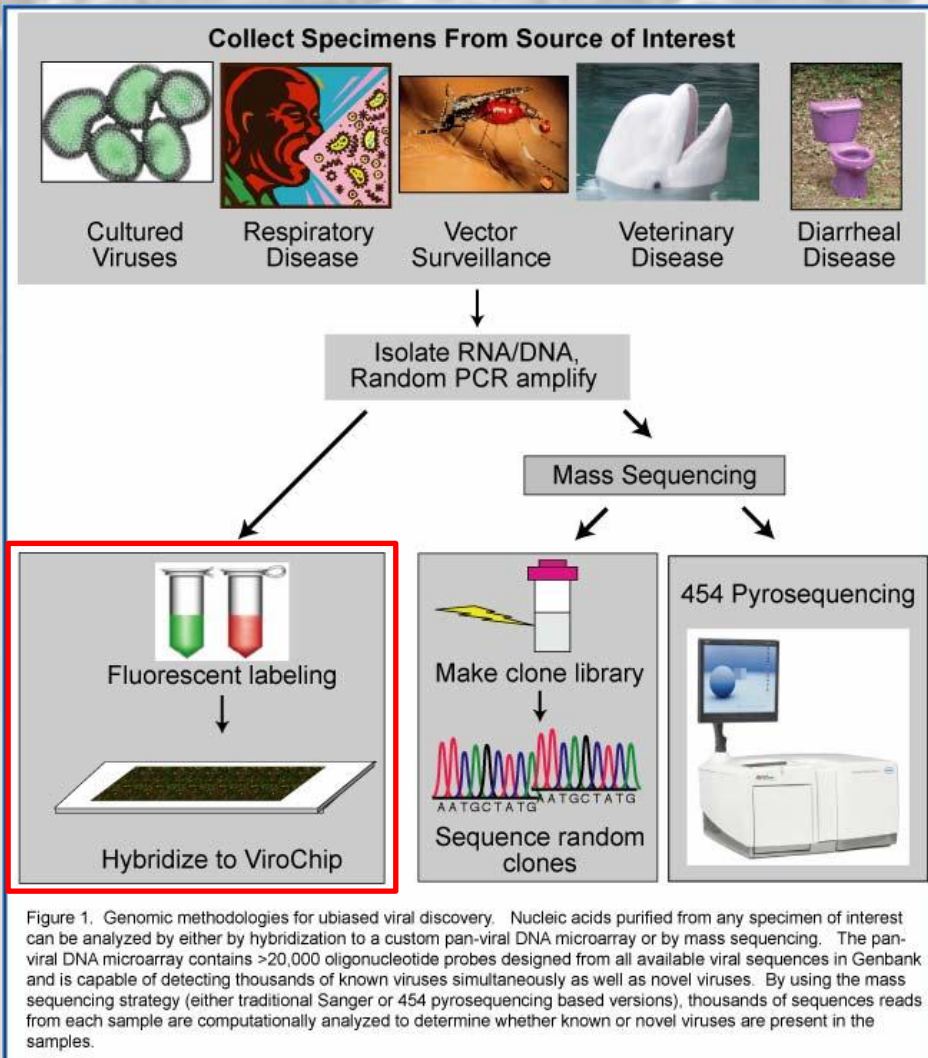
Others...

Diagnostic Service

Affordable

Fast Results

Expertise



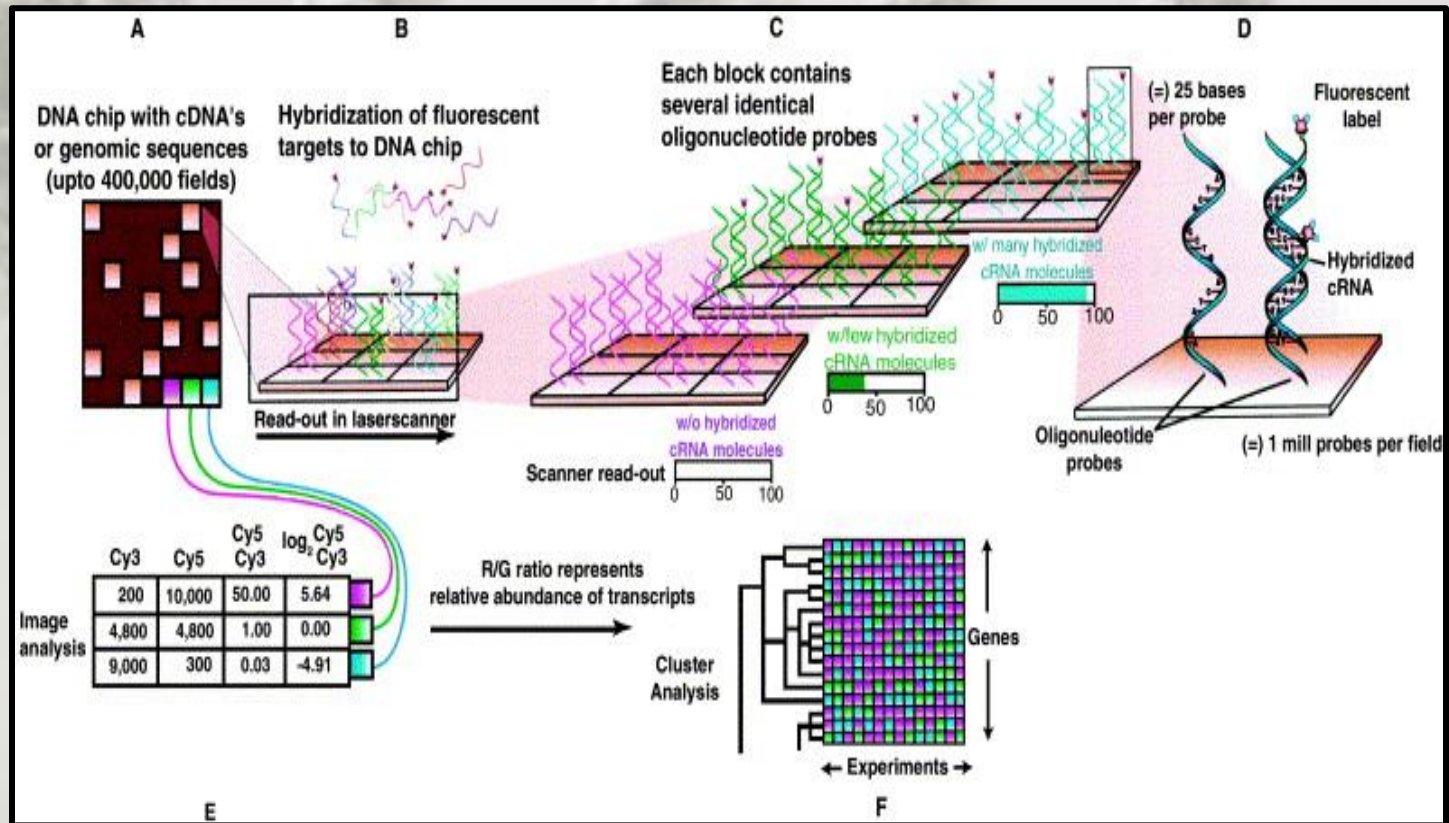
The DNA chip

How does it working?

DNA: complementary double strand →

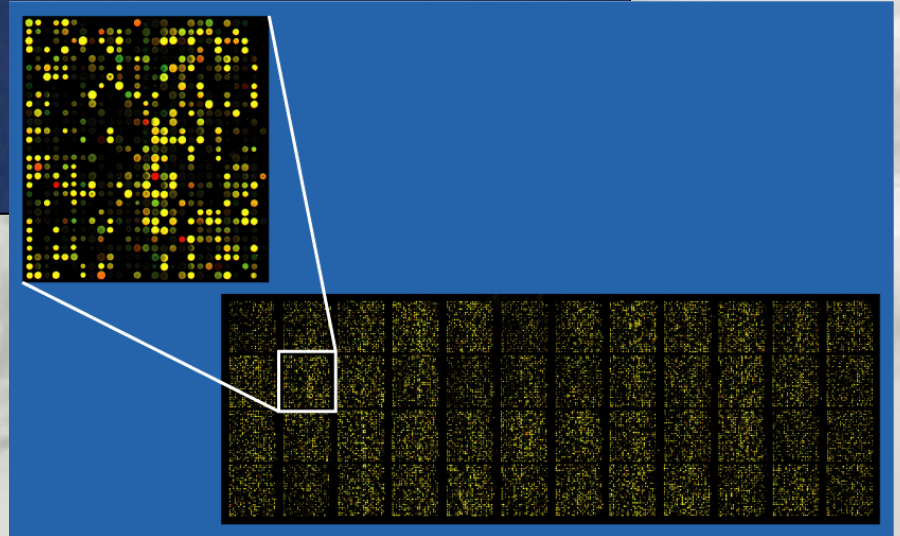
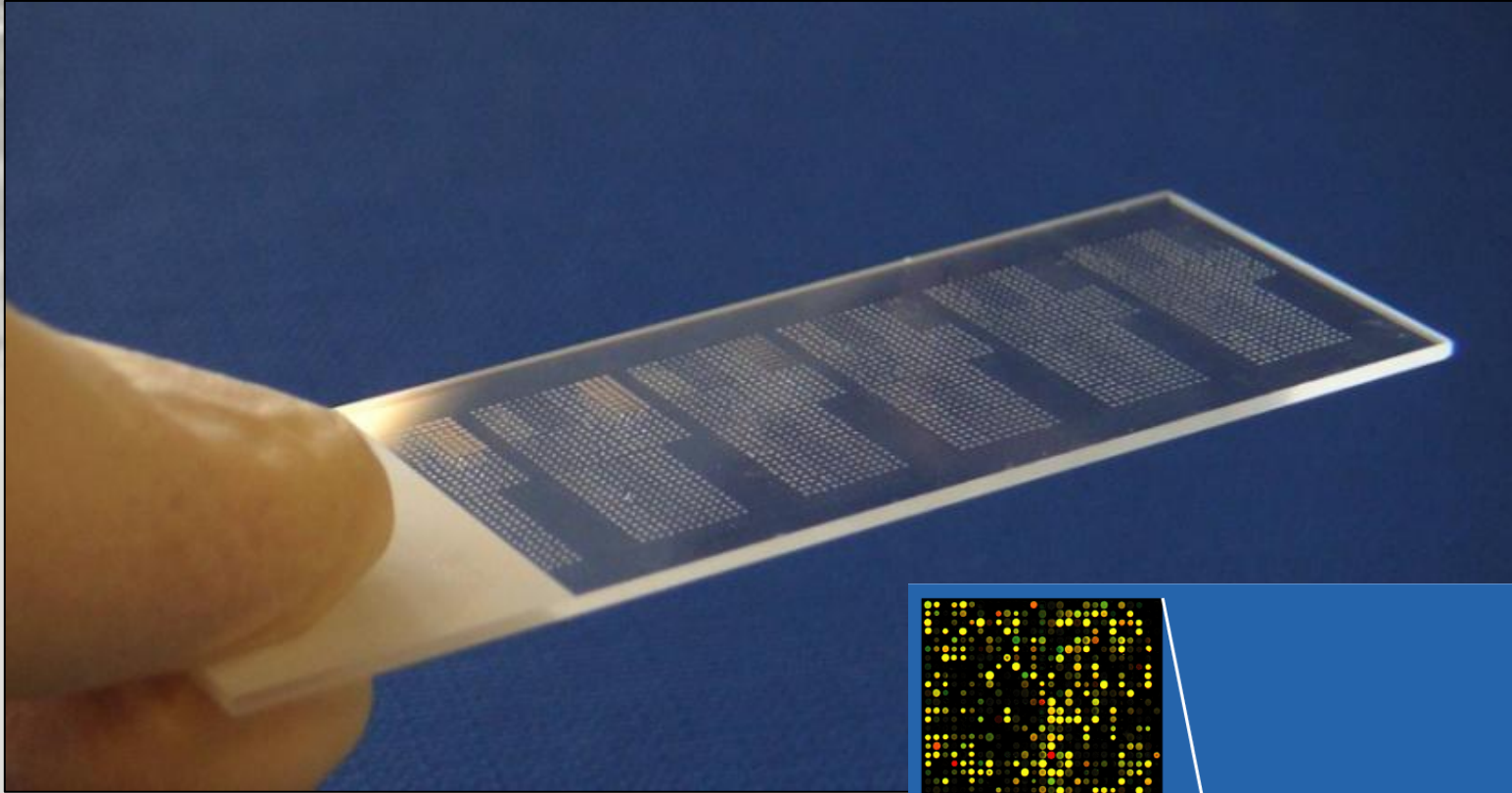
By knowing one of the two strands:

We are able to identify any DNA strand that will interact to it!



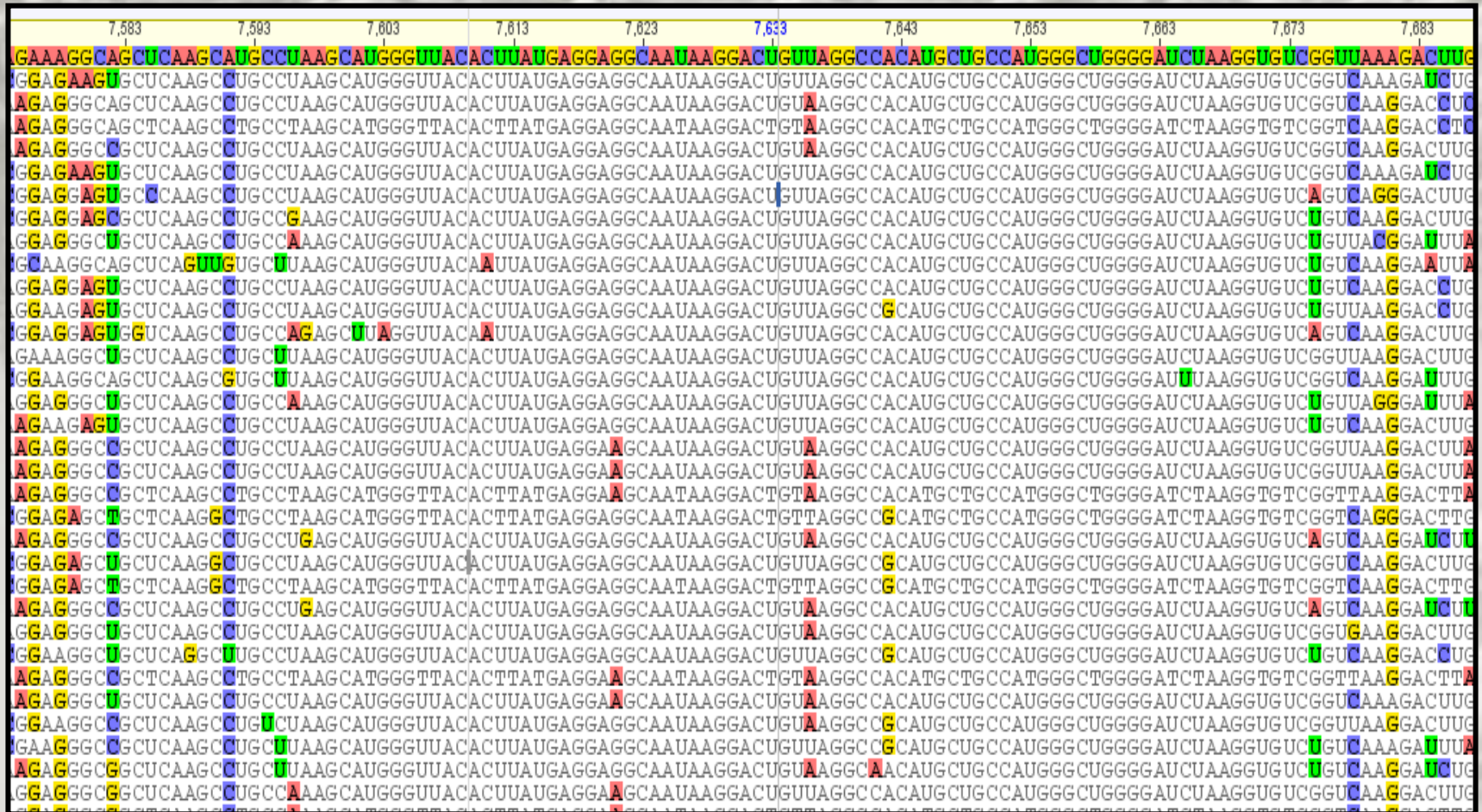
The DNA chip

How does it working?



The DNA chip

The development – Selection of conserved region



Highly conserved region

GB virus C

The DNA chip

The development – Validation

1- Validate the probes selection with the GenBank database (“blast”)

2- Supervise the DNA Chip preparation

Probes synthesis

Probes impression on the glass slide support

3- Genetic material amplification and labeling method

Select the appropriate method

Optimize the selected method

4- Validate the Microarray

The DNA chip first version

pUC	pUC	pUC	pUC	PPV	PPV	PPV	PPV	PPV	PPV	NP	NP
NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	M	M
M	M	M	M	M	M	M	M	M	M	M	M
M	M	M	M	H1	H1	H1	H1	H1	H1	H1	H1
H1	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1
H1	H1	H3	H3	H3	H3	H3	H3	H3	H3	H3	H3
H3	H3	H3	H3	H3	H3	H3	H3	H3	H3	H3	H3
H3	H3	H3	H3	N1	N1	N1	N1	N1	N1	N1	N1
N1	N1										

pUC	pUC	pUC	pUC	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV
TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV
TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV
TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV	TGEV
TGEV	TGEV	TGEV	TGEV	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS									

pUC	pUC	pUC	pUC	N1	N1	N1	N1	N1	N1	N2	N2
N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
N2	N2	N2	N2	N2	N2	neg	neg	pUC	pUC	pUC	pUC
bAct	bAct	bAct	bAct	bAct	bAct	bAct	bAct	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv	Yv
Yv	Yv	Yv	Yv								

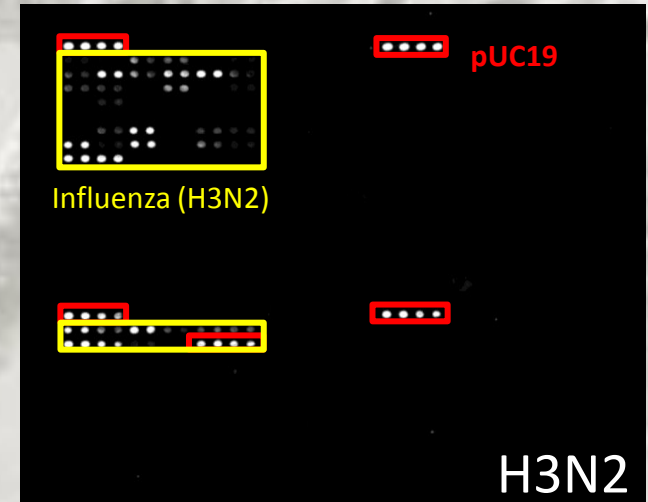
pUC	pUC	pUC	pUC	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS
PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PRRS	PCV1	PCV1	PCV1	PCV1	PCV1
PCV1	PCV1	PCV1	PCV1	PCV2	PCV2	PCV2	PCV2	PCV	PCV	PCV	PCV	PCV
PCV	PCV	PCV	PCV	PCV	PCV	PCV	PCV	PCV1	PCV1	PCV1	PCV1	PCV1
PCV	PCV	PCV	PCV	PCV1	PCV1	PCV1	PCV1	PCV2	PCV2	PCV2	PCV2	PCV2
PCV2a	PCV2a	PCV12a	PCV12a	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV
PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV
PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV	PPV
PPV	PPV	PPV	PPV									

Influenza TGEV PCV SRRP EU ctrl positif autres PPV neg ctrl SRRP US

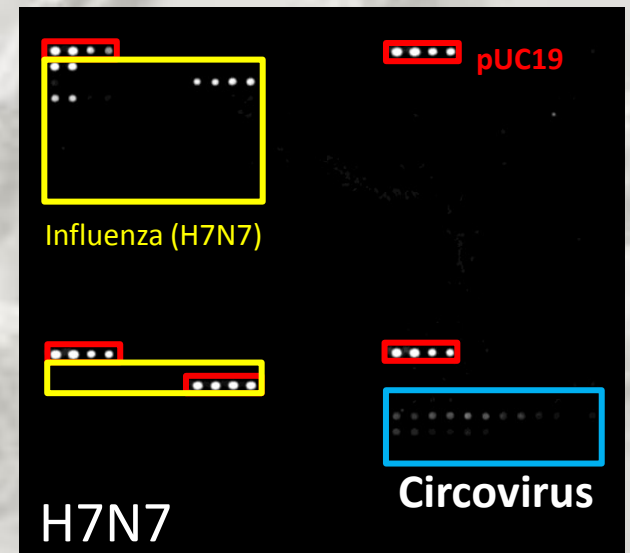
6 swine virus targeted (PRRSV, PCV, Swine Influenza, TGEV / PRCV, PPV)
And controls...

The DNA chip first version

Influenza detection (H1N1, H3N2 et H7N7)



- Capacity to detect a virus that is not circulating anymore (H7N7)
- Capacity to detect a virus not related to swine disease (H7N7)
- Possibility to detect more than one virus in a sample (H7N7 – circovirus)
- Possibility to detect an unknown virus in a sample (circovirus)



The DNA chip second version

19 viral families targeted...

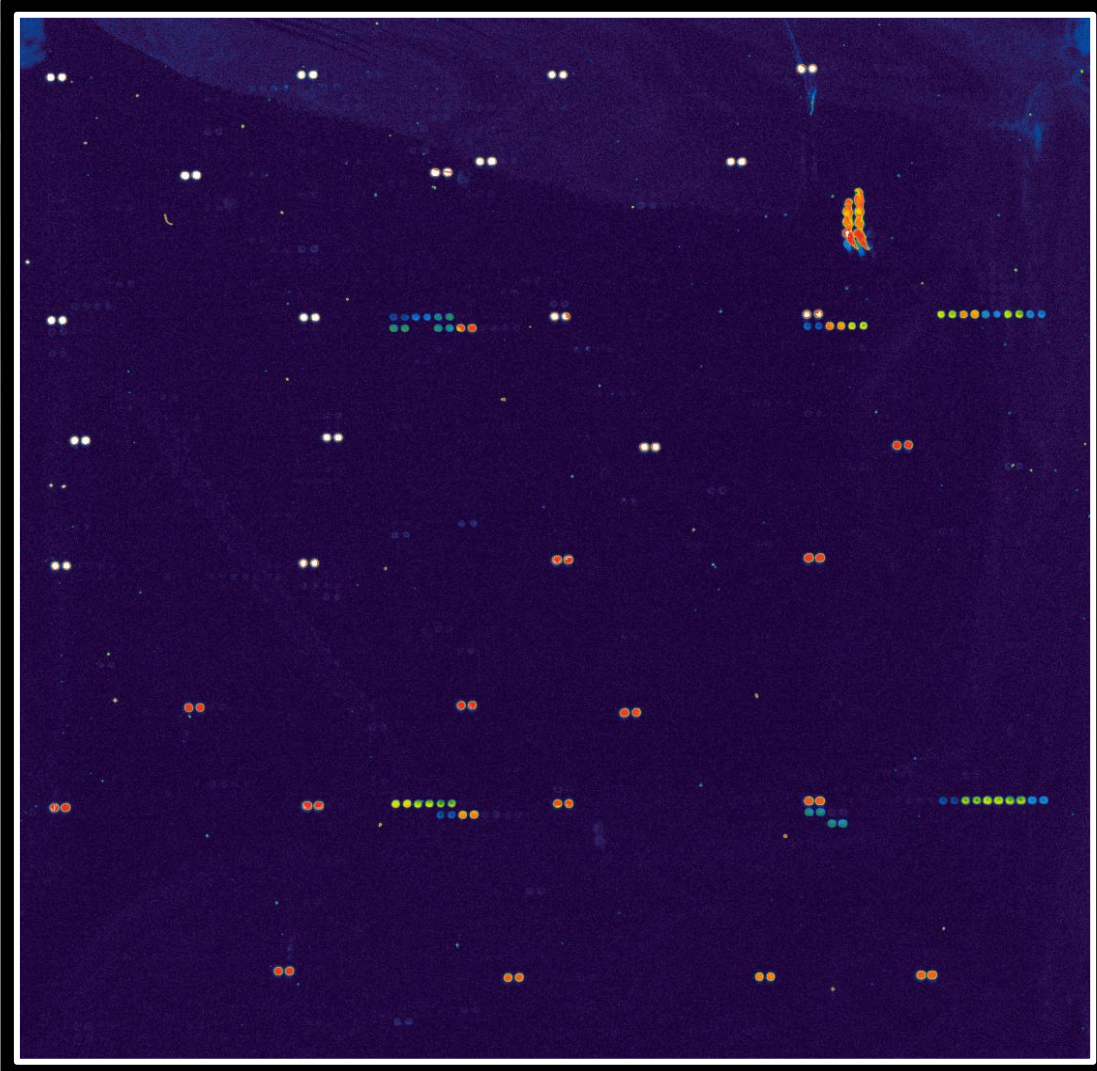
More than 360 different viruses targeted...

Virus Family	Viral genome type	Number of probes
<i>Adenoviridae</i>	DNA	476
<i>Arteriviridae</i>	RNA	71
<i>Asfarviridae</i>	DNA	10
<i>Astroviridae</i>	RNA	22
<i>Caliciviridae</i>	RNA	164
<i>Circoviridae</i>	DNA	114
<i>Coronaviridae</i>	RNA	300
<i>Filoviridae</i>	RNA	46
<i>Flaviviridae</i>	RNA	548
<i>Hepeviridae</i>	RNA	34

Virus Family	Viral genome type	Number of probes
<i>Herpesviridae</i>	DNA	564
<i>Orthomyxoviridae</i>	RNA	147
<i>Paramyxoviridae</i>	RNA	12
<i>Parvoviridae</i>	DNA	320
<i>Picornaviridae</i>	RNA	286
<i>Poxviridae</i>	DNA	156
<i>Reoviridae</i>	RNA	46
<i>Retroviridae</i>	RNA	314
<i>Rhabdoviridae</i>	RNA	160
Total:		3790

The DNA chip second version

Influenza detection (H1N1)



What is left to do...

Validate the DNA Chip with other known viruses

Test the DNA Chip with diagnostic samples where the implicated virus is unknown

Evaluate the DNA Chip sensitivity

Conclusion

Encouraging results!

- **Allow the detection of viral strains that are no longer in circulation! (H7N7)**
- **Allow the detection of multiple viruses in the same sample (H7N7 – Circovirus)**
- **Tolerance to mutations found in emerging viruses**
- **Allow the detection of an unknown virus in a clinical sample (Circovirus)**

Affordable cost, once fully developed

Available soon!...

Acknowledgement

Philippe Garneau

Miria Elias

Isabel Mandeville

Chantale Provost

Christian Savard



Canadian Swine
Health Board

Conseil canadien
de la santé porcine

Université 
de Montréal



Thank you!