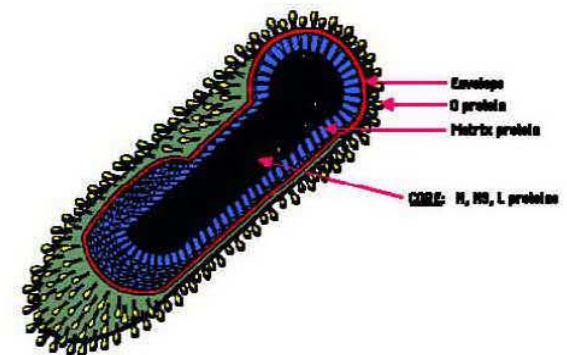
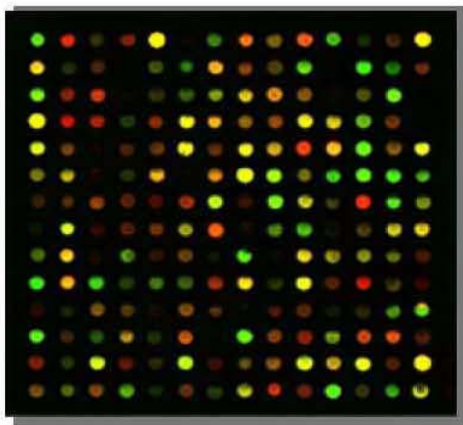


Microarray-based detection of fish viruses

Kyle Garver, Patrick Tang,
Laura Hawley & Jon Richard



Fisheries and Oceans
Canada



Virus Detection Method #1: Assay by Cell Culture

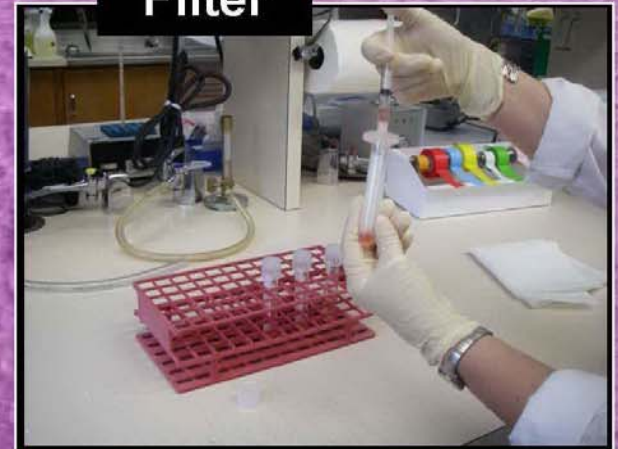
Dissection



Homogenize



Filter



Inoculate



Monitor



Virological Analysis by Cell Culture

- **Advantages**
 - Detects live infectious agents capable of producing pathogenesis in cells.
 - Obtain virus isolates that can be used in pathogenicity studies.
 - Sensitive
- **Disadvantages**
 - Laborious process
 - Slow turn-around time for results (up to 28 days)
 - Not all viruses grow in cell culture

Cell Culture Limitations



→ Electron Microscopy

= ?

→ Serum
Neutralization

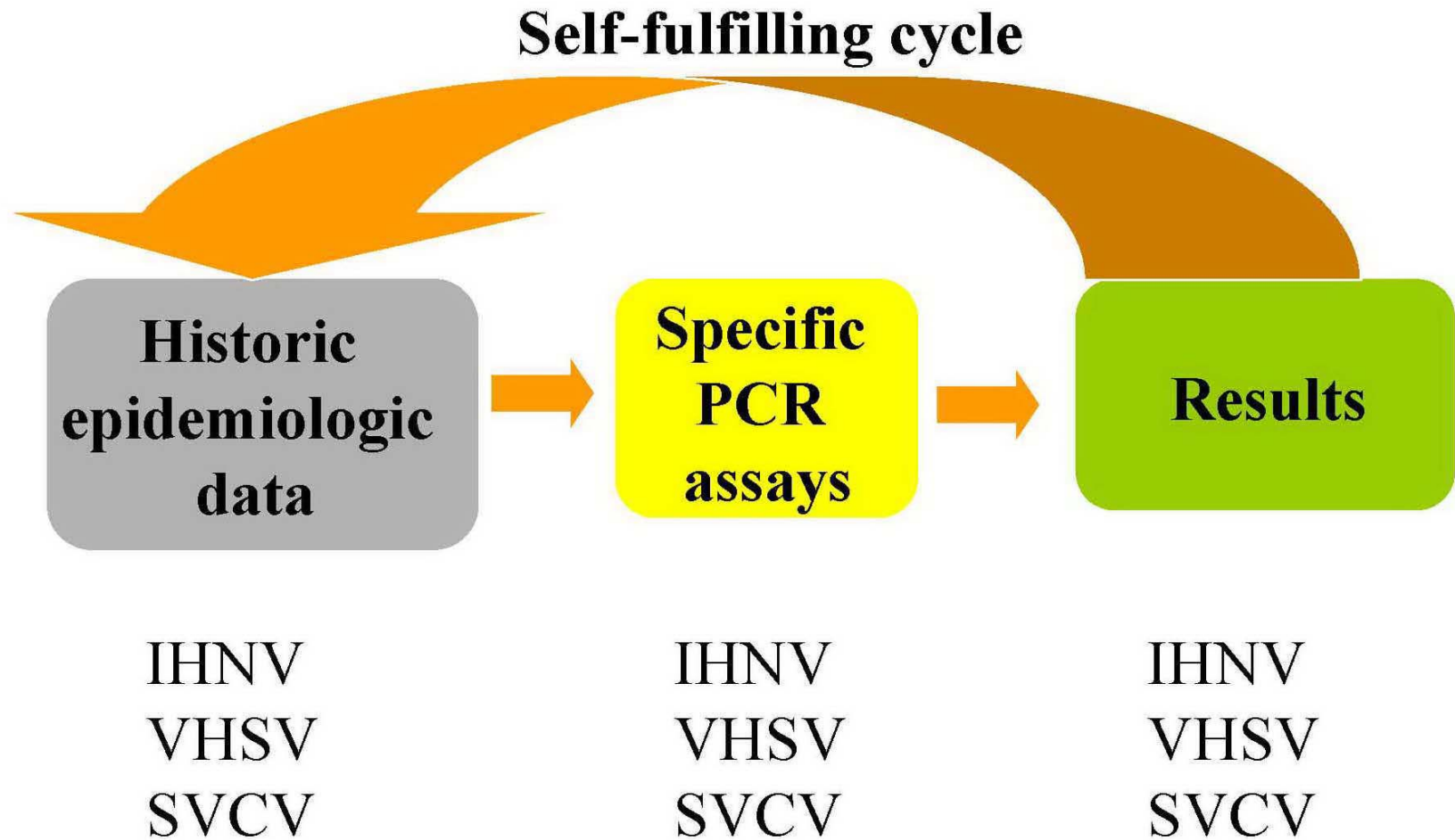
→ PCR

More tests = Longer time for results

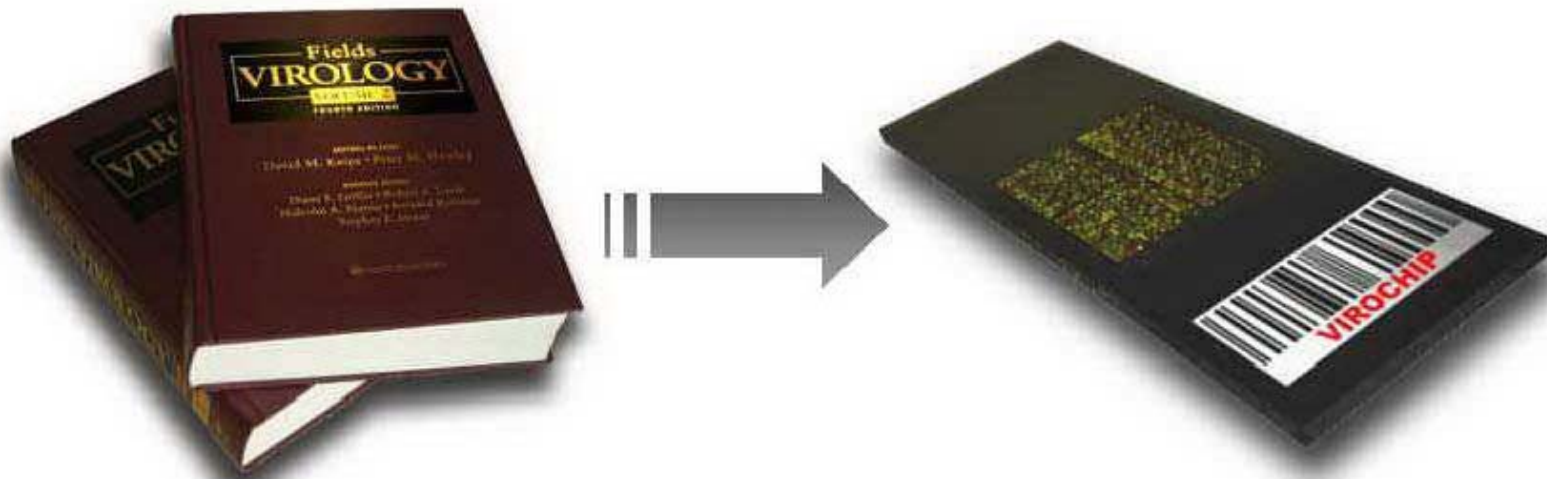
Virus Detection Method #2: PCR-based

- **Advantages**
 - Rapid turnaround time (<1 day)
 - Highly sensitive, specific & reproducible
- **Disadvantages**
 - Does not definitely determine whether there is active, viable virus in the sample.
 - High equipment cost
 - Specificity limits detection of “new” viruses

PCR-based Tests



The “Virochip” Premise



- Develop a DNA microarray for simultaneous screening of ALL viruses
- Develop a comprehensive picture of which viruses are present and how frequently
 - without preconceptions

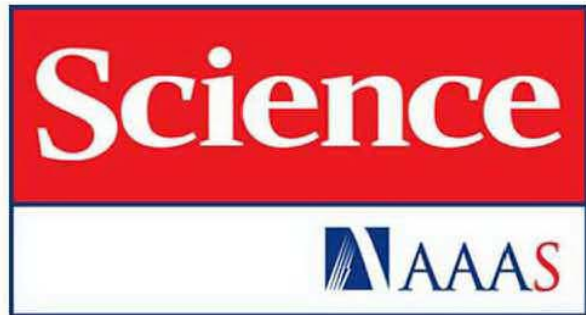
The “Virochip”

- Originally developed at University of California, San Francisco in 2001
 - Joe DeRisi, PhD
 - Don Ganem, MD
 - Dave Wang, PhD (WUSTL)

The Virochip ver. 4



- Oligonucleotides representing all known viruses at the species, genus and family levels
 - updated August 2007
- 16,000 oligonucleotides (70-mers)
 - emphasis on human and animal viruses
 - common bacterial pathogens

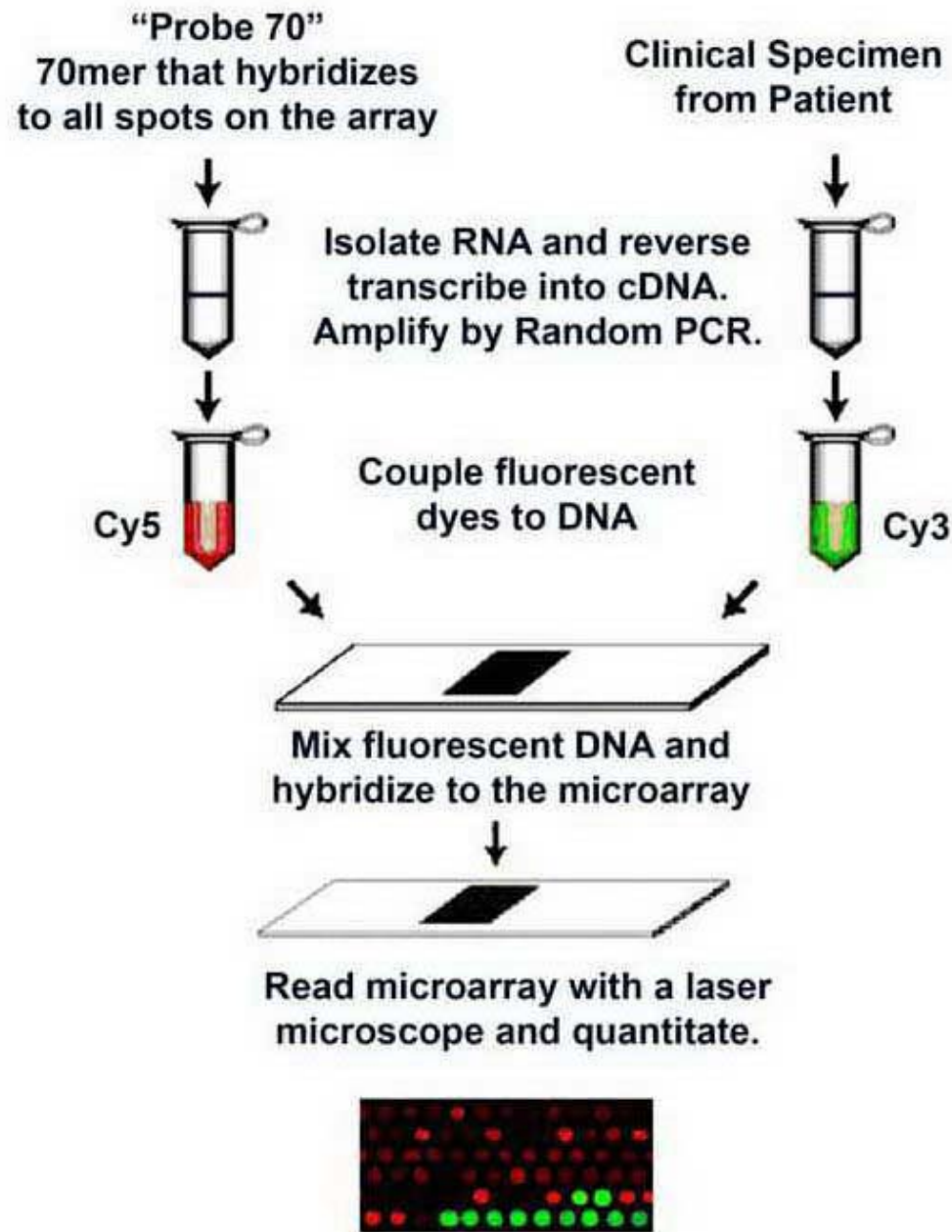


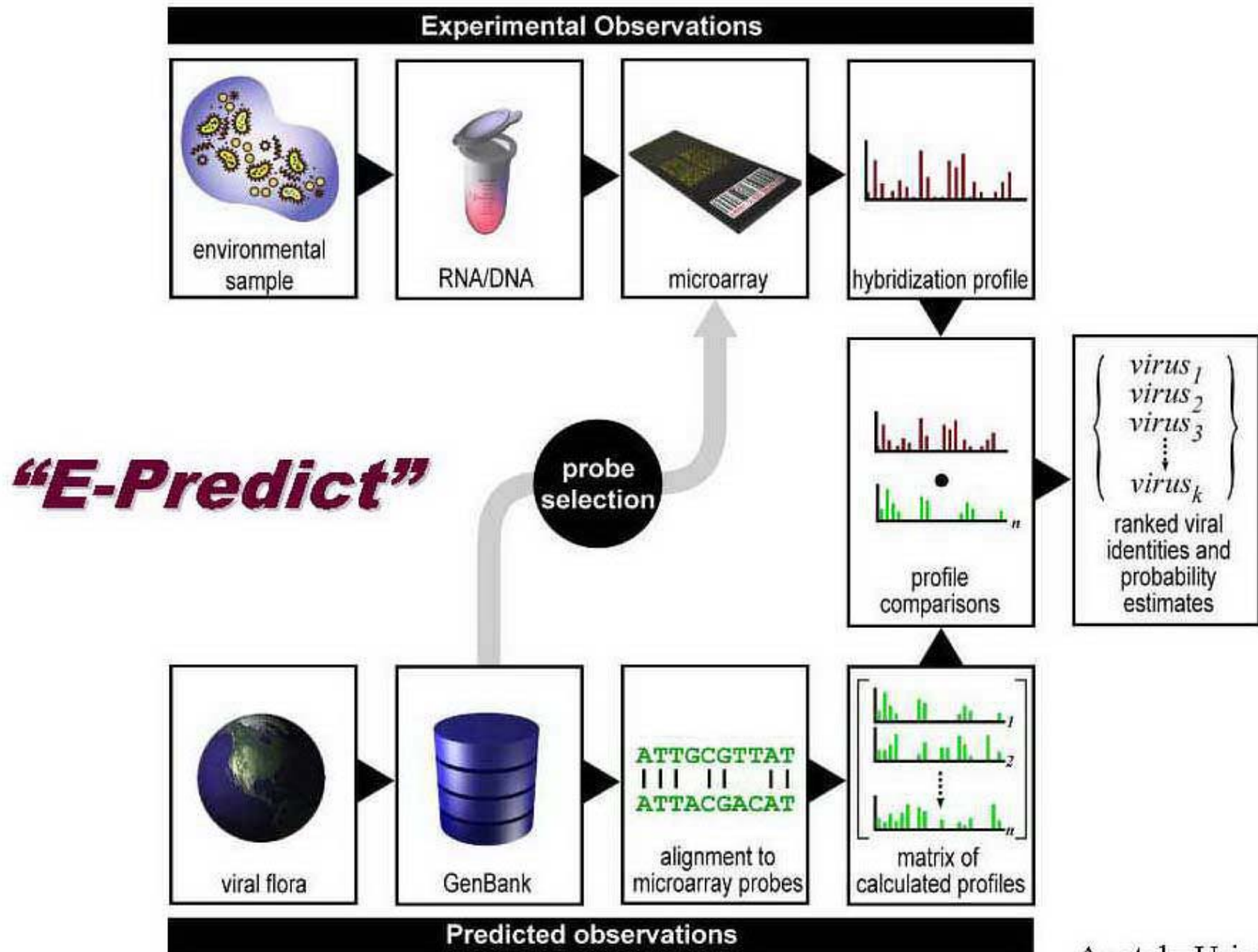
Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Paul A. Rota, *et al.*

30 MAY 2003 VOL 300 SCIENCE www.sciencemag.org

Virus Detection in Clinical Specimens





Anatoly Urisman

Detection of Fish Viruses?

- RNA virus
 - Cell culture (EPC) amplified VHSV
 - Family: Rhabdoviridae
 - Genus: Novirhabdovirus
- DNA virus
 - Cell culture (EPC) amplified stickleback iridovirus
 - Family: Iridoviridae
 - Genus: ??

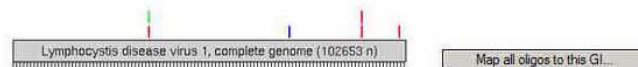
VHSV

<input type="text"/> UID ↓ ↑	<input type="text"/> SPECIES ↓ ↑	<input type="text"/> FAMILY ↓ ↑	BCViro4P3- 231:23267 *Cy3* Infected Cell cult RNA known fish isolates <input type="checkbox"/> ↓ ↑	BCViro4P3- 241:23269 *Cy3* Infected Cell Cult DNA Known Fish isolate <input type="checkbox"/> ↓ ↑	BCViro4P3- 493:23364 *Cy3* EPC Cell Cult Known Pos RNA <input type="checkbox"/> ↓ ↑
<input type="checkbox"/> 9633477_310_rc	Snakehead rhabdovirus	Rhabdoviridae .. Novirhabdovirus	52170	60207	74
<input type="checkbox"/> 5042321_38_rc	Viral hemorrhagic septicemia virus	Rhabdoviridae .. Novirhabdovirus	46856	65224	101
<input type="checkbox"/> 9633477_270_rc	Snakehead rhabdovirus	Rhabdoviridae .. Novirhabdovirus	43928	65203	68
<input type="checkbox"/> 29501369_nt3078_rc	Woolly monkey hepatitis B Virus	Hepadnaviridae .. Orthohepadnavirus	30699	776	2933
<input type="checkbox"/> 3046973_97_rc	Feline astrovirus	Astroviridae .. Mamastrovirus	25055	65178	206
<input type="checkbox"/> 5042321_110_rc	Viral hemorrhagic septicemia virus	Rhabdoviridae .. Novirhabdovirus	13009	10752	59
<input type="checkbox"/> 9632550_400_rc	Viral hemorrhagic septicemia virus	Rhabdoviridae .. Novirhabdovirus	11703	38554	100
<input type="checkbox"/> 15216207_nt4031	Phocid herpesvirus 1	Herpesviridae .. Varicellovirus	10051	3649	300
<input type="checkbox"/> 222595_111_rc	Suid herpesvirus 1 strain Kaplan	Herpesviridae .. Varicellovirus	8429	11458	152
<input type="checkbox"/> 9632550_362_rc	Viral hemorrhagic septicemia virus	Rhabdoviridae .. Novirhabdovirus	8238	34645	53
<input type="checkbox"/> 9632550_324_rc	Viral hemorrhagic septicemia virus	Rhabdoviridae .. Novirhabdovirus	7635	32097	71

- Cell culture samples containing viral hemorrhagic septicemia virus (*Rhabdoviridae*)
- Highest intensity oligos displayed

Stickleback isolate

gi|9695413|ref|NC_001824.1| Lymphocystis disease virus 1, complete genome (102653 n)



OLIGO

9695413_1412_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus
 9695413_1412_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus
 32482462_44_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus
 2582499_nt1028 | Wiseana iridescent virus | Iridoviridae .. Iridovirus | Iridoviridae
 9695413_3637 | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Iridoviridae
 9695413_3637_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Iridoviridae
 9695413_4028_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus

	START	END	ALIGN	MATCH
+	35276	35345	70	70
+	35334	35397	69	50
+	71654	71724	71	62
+	71916	71985	71	47
+	90901	90970	70	70
+	90901	90970	70	70
+	100676	100745	70	70

gi|48843719|ref|NC_005902.1| Lymphocystis disease virus - isolate China, complete genome (186250 n)



OLIGO

32482462_44_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus
 2582499_nt1028 | Wiseana iridescent virus | Iridoviridae .. Iridovirus | Iridoviridae
 335215_nt2793 | Tipula iridescent virus | Iridoviridae .. Iridovirus | Iridoviridae
 335215_nt2793_rc | Tipula iridescent virus | Iridoviridae .. Iridovirus | Iridoviridae
 9695413_1412_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus
 9695413_3637 | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Iridoviridae
 9695413_3637_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Iridoviridae
 9695413_4028_rc | Lymphocystis disease virus 1 | Iridoviridae .. Lymphocystivirus | Lymphocystis disease virus

	START	END	ALIGN	MATCH
+	31663	31733	71	70
+	31925	31993	70	49
+	117143	117196	56	40
+	117143	117196	56	40
+	128917	128989	73	50
+	130082	130151	71	60
+	130082	130151	71	60
+	131578	131647	71	60

- Cell culture samples containing iridovirus from stickleback (*Iridoviridae*)
- High intensity oligos mapped onto available viral genomes

Diagnostic Use of the Virochip

- Regular updating of oligos on array
 - Bioinformatics expertise required
 - Initial expense for prototype arrays and purchase of oligos
- Sample preparation, amplification and hybridization
 - Minimal training required
 - Basic molecular biology laboratory (PCR)
 - Microarray scanner
- Data analysis
 - Simplified with E-predict algorithm
 - Borderline virus predictions will need expert follow-up

Diagnostic Use of the Virochip

- Material cost ~ \$100 per assay
 - oligos, glass slides, PCR reagents
- Turn-Around Time
 - 24 to 72 hours
- Sensitivity
 - 10^3 to 10^6 copies per sample
 - >90% compared to specific PCR for respiratory specimens
- Specificity
 - Up to 100% depending on availability and number of negative controls



Acknowledgments

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Chelsea DeColle

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Gary Marty
John Robinson