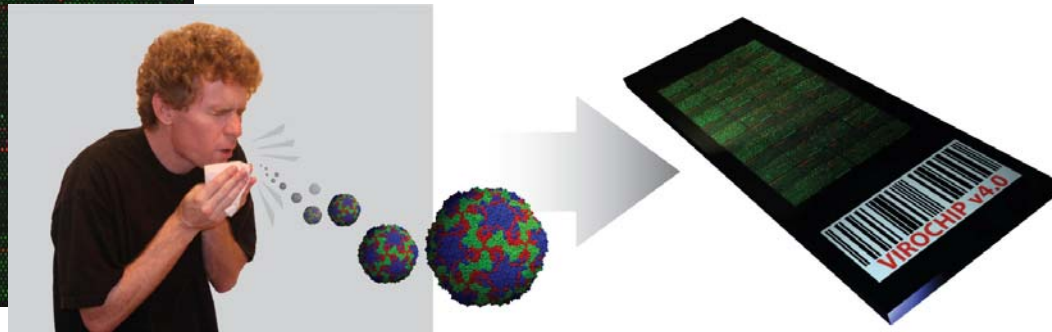
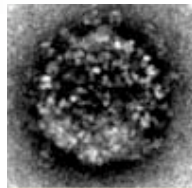
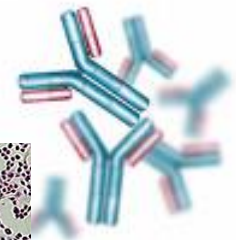
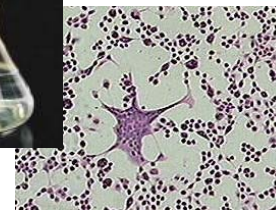


*Virochip overview:  
a microarray for viral  
detection and discovery*



Amy Kistler, Ph.D., M.P.H.  
Ganem and DeRisi labs, UCSF  
15 March 2010  
Microarray Workshop  
Teramo, Italy



# Why develop a virochip?

## 1. Significant proportion of undiagnosed acute infections



*e.g., acute respiratory tract infections--*

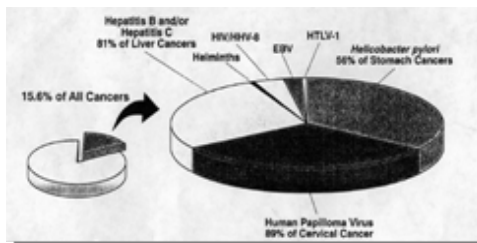
*15 – 40% unknown cause!*

- *encephalitis*
- *gastroenteritis*

## 2. Need for rapid identification of emerging viral pathogens



## 3. Mounting evidence for a viral role in chronic diseases

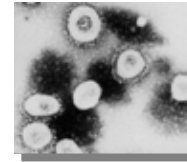


- *autoimmune disorders?*
- *inflammatory states?*

# Why not apply conventional technologies?

## Low specificity methods:

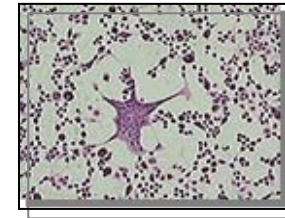
(+) no assumptions about viral agent



electron microscopy

(-) Sensitivity & scaling problems--

- not all viruses present at sufficient titers [EM]
- not all viruses are cultivable [culture]
- not easily adapted to high throughput approaches

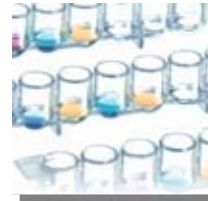


culture

---

## High specificity methods:

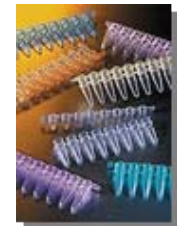
(+) exquisite sensitivity, throughput



ELISA, immunofluorescence

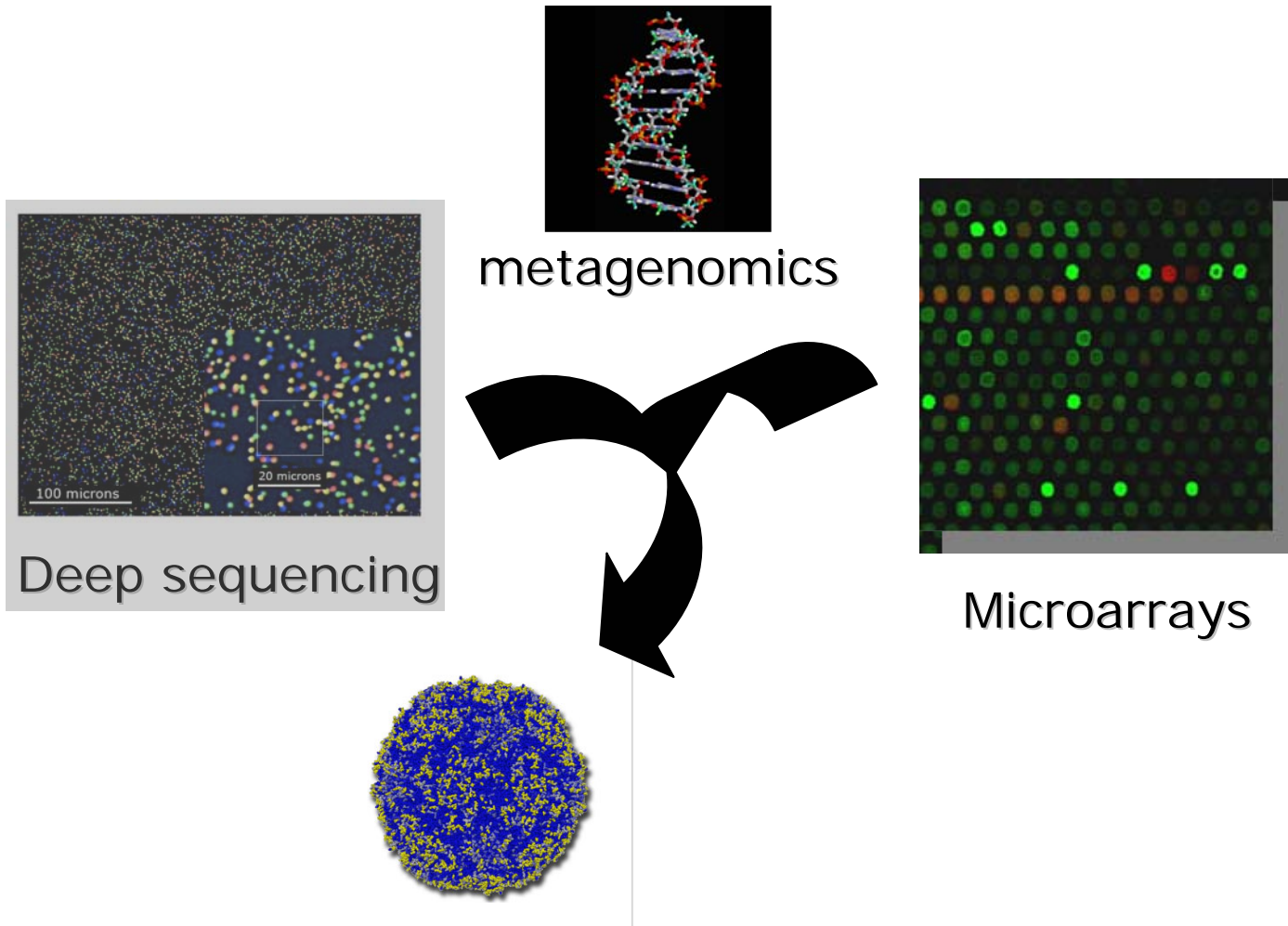
(-) *too* specific--

- Can only detect what you already know
- Rapid viral evolution requires constant revision/expansion of assays



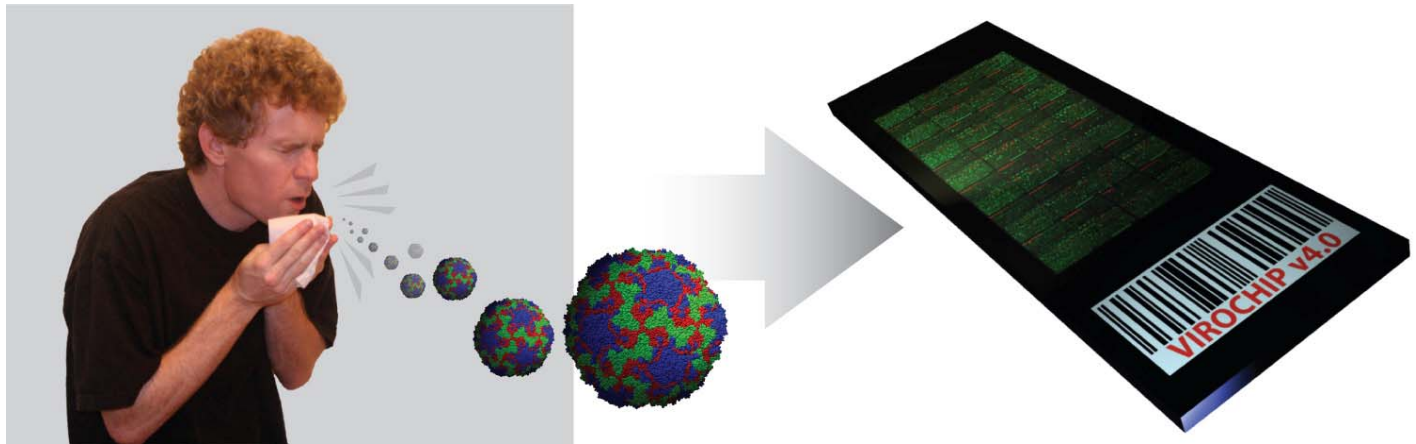
PCR

# A 21st century approach to viral detection

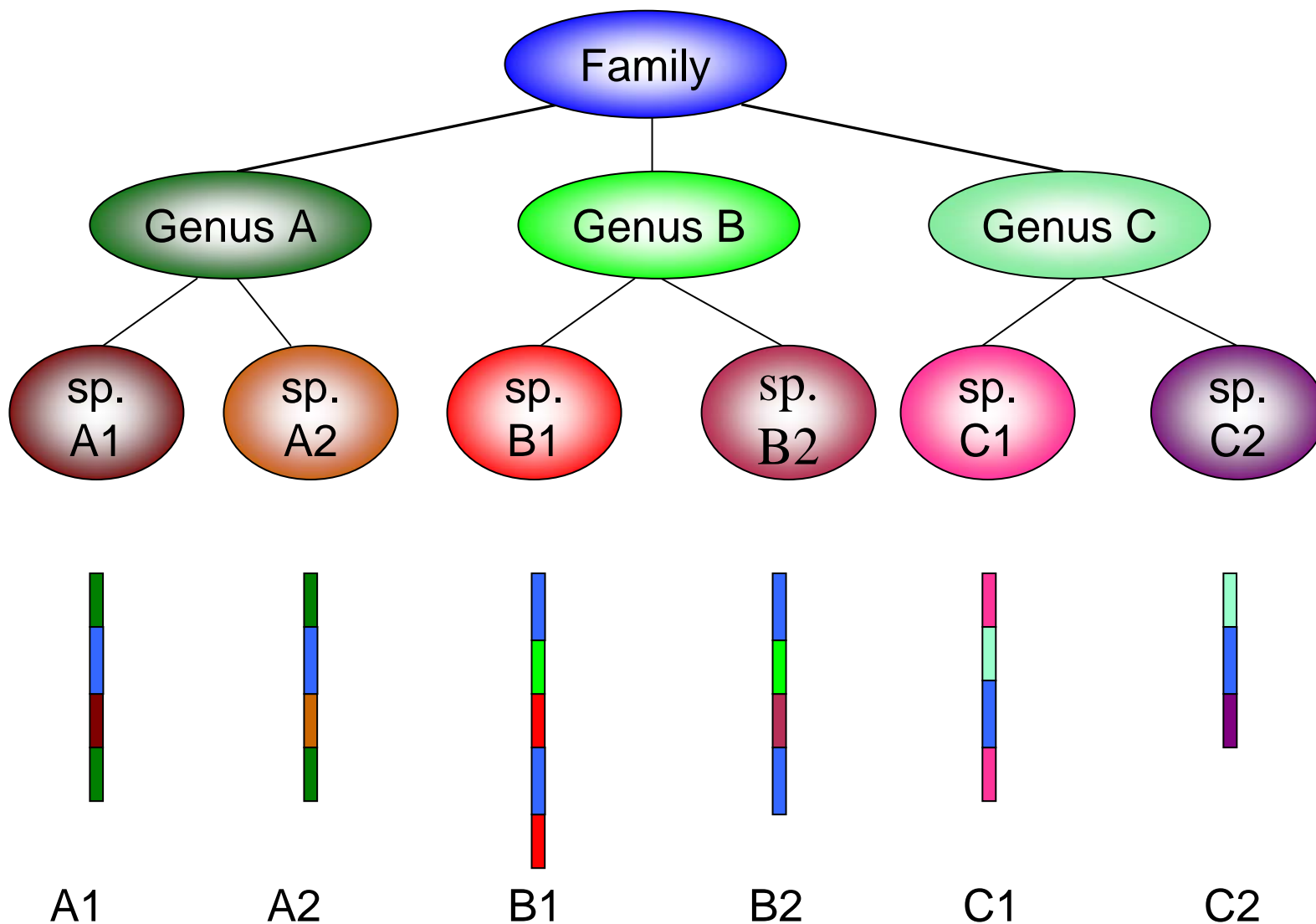


# The “Virochip” premise

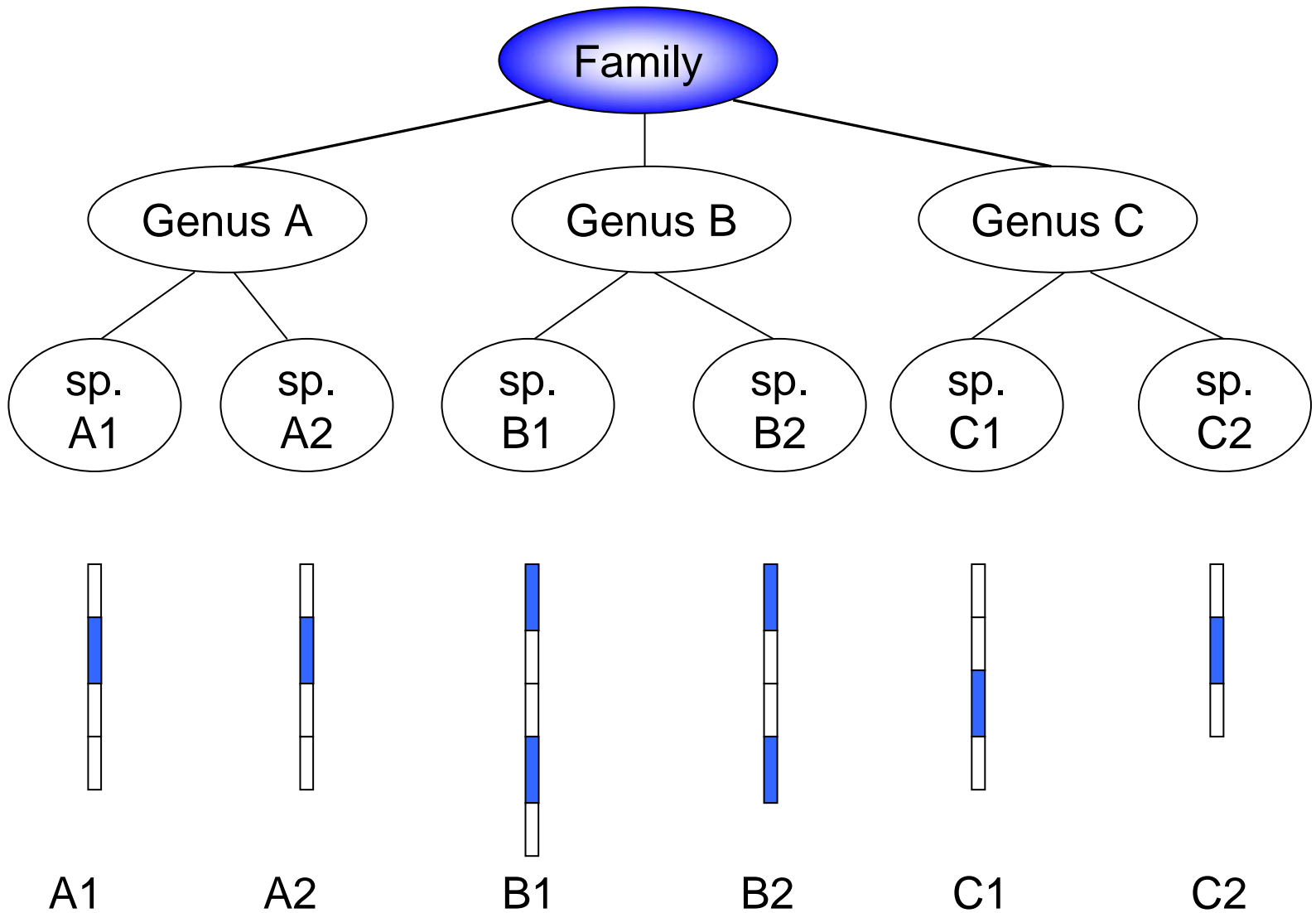
- Simultaneous screening for *all* viruses
- ***Global or Pan-Viral*** approach via ***cross-hybridization***
- Develop a comprehensive picture of which viruses are present in any sample ***without preconceptions***



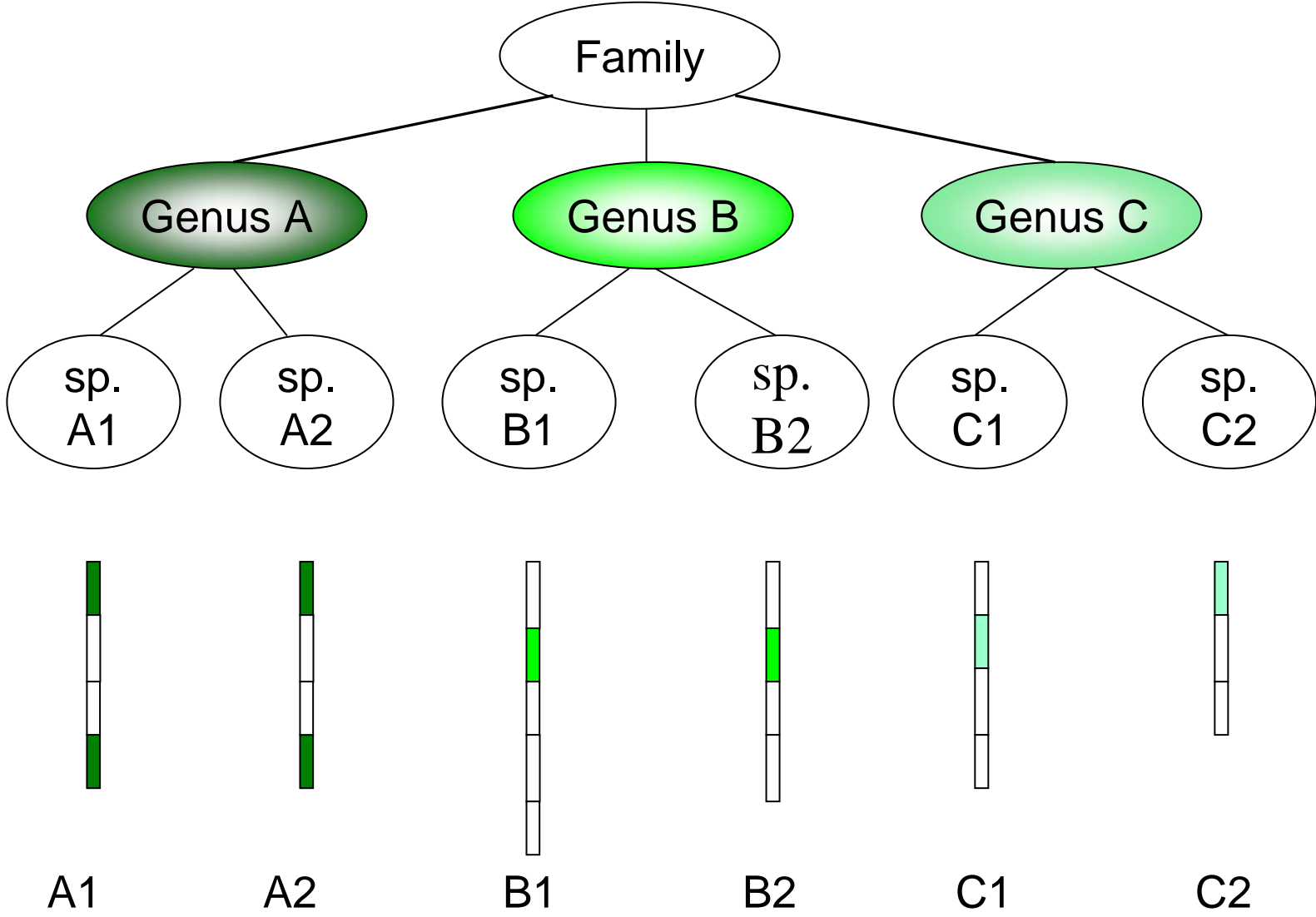
# Strategy: utilize sequence conservation



# Family level probes hit sequences conserved across multiple genera

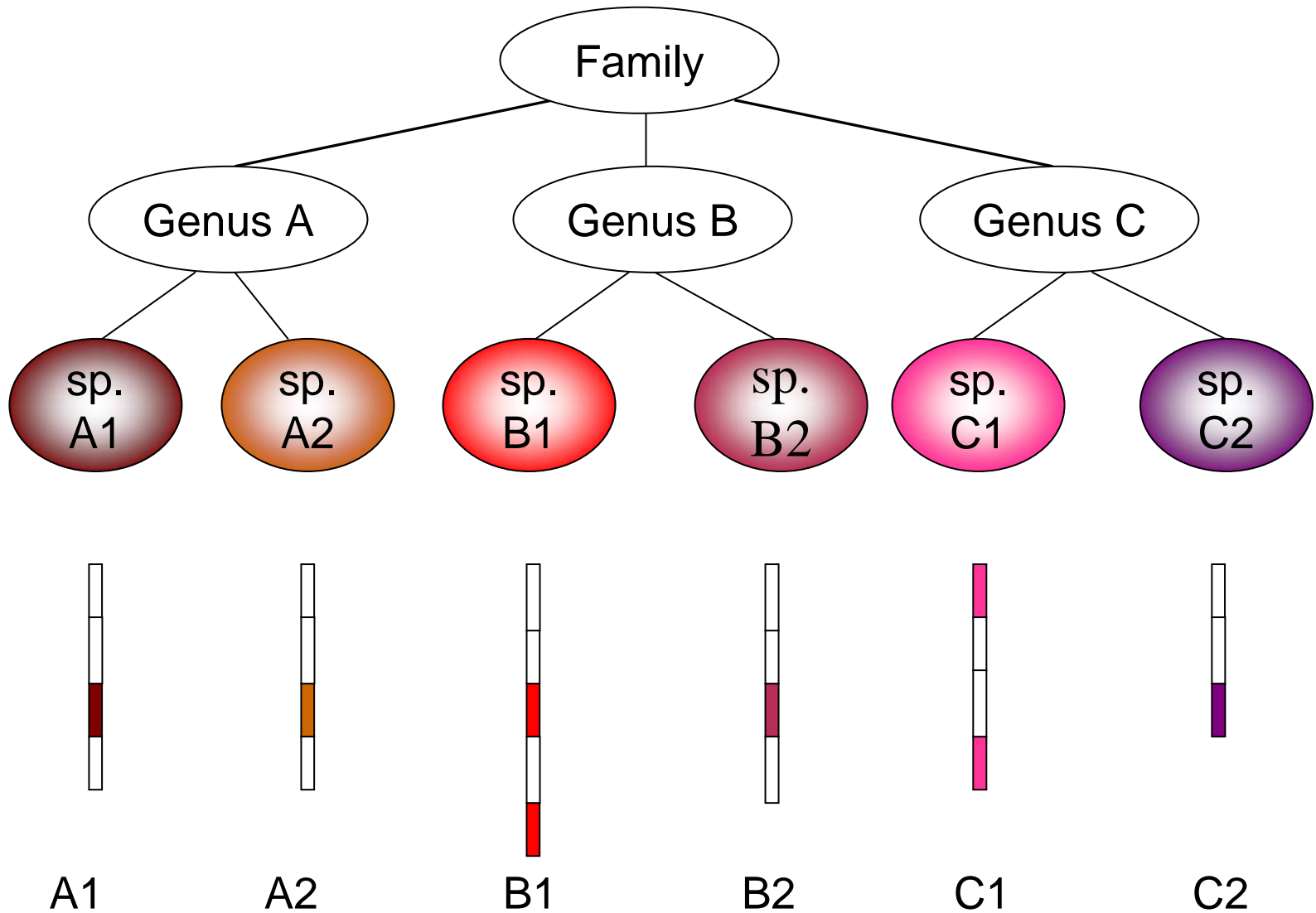


# Genus level probes hit sequences conserved across multiple species

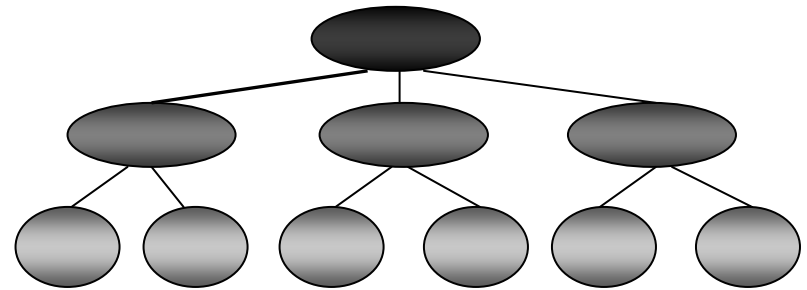




# Species probes are sequences unique to terminal nodes of viral taxonomy

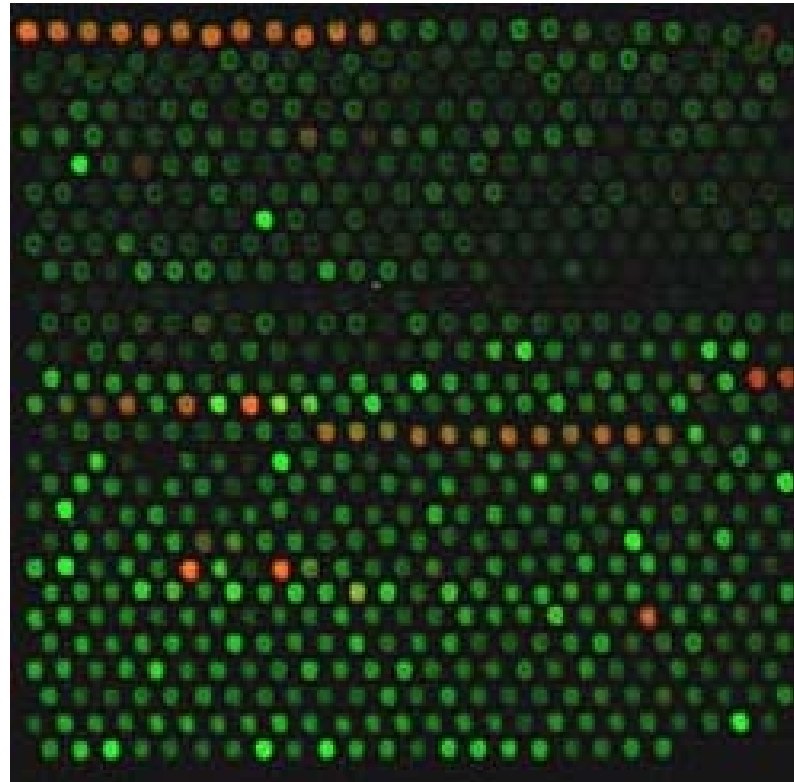
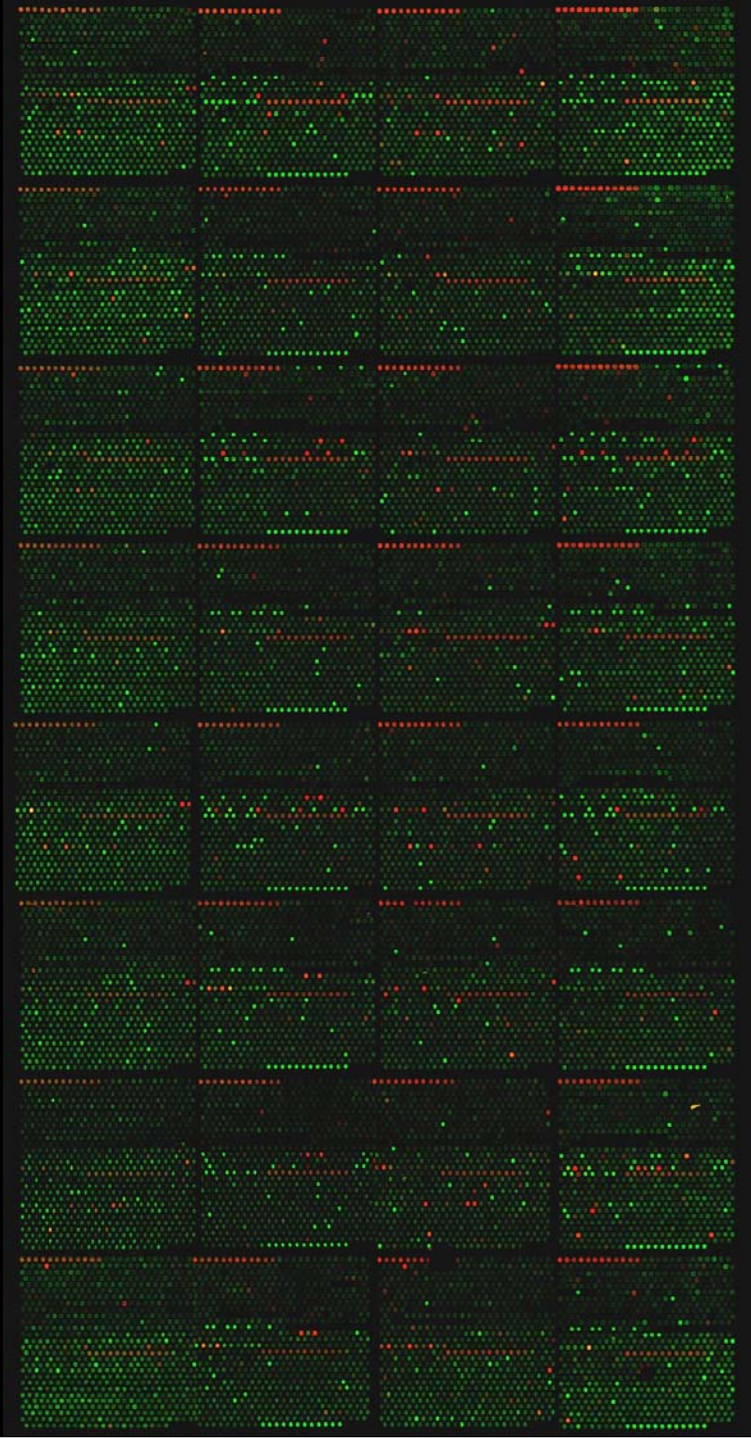


# Virochip probes are conserved across multiple levels of the viral taxonomy



**End goal = maximize our capability to:**

1. detect all known viral family members
2. detect novel, uncharacterized viruses
3. distinguish distinct viral species

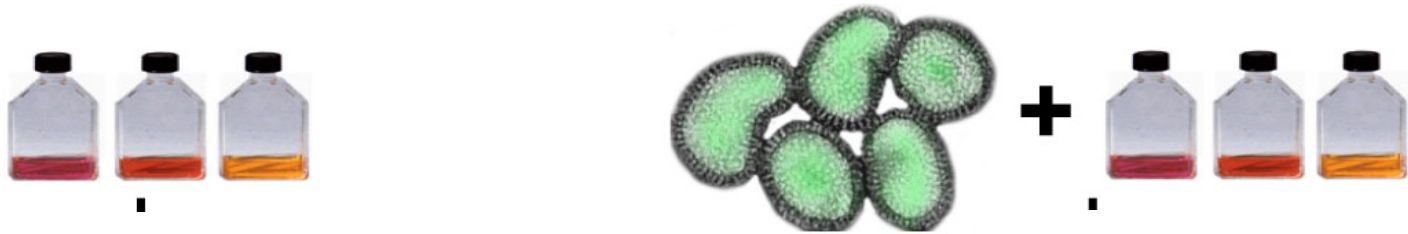


*Virochip, version 5.0*

17,000+ viral sequences  
representing *all* viral species in  
GenBank as of June 2009.

>1000 unique viral reference genome sequence records  
>200,000 partial sequence records

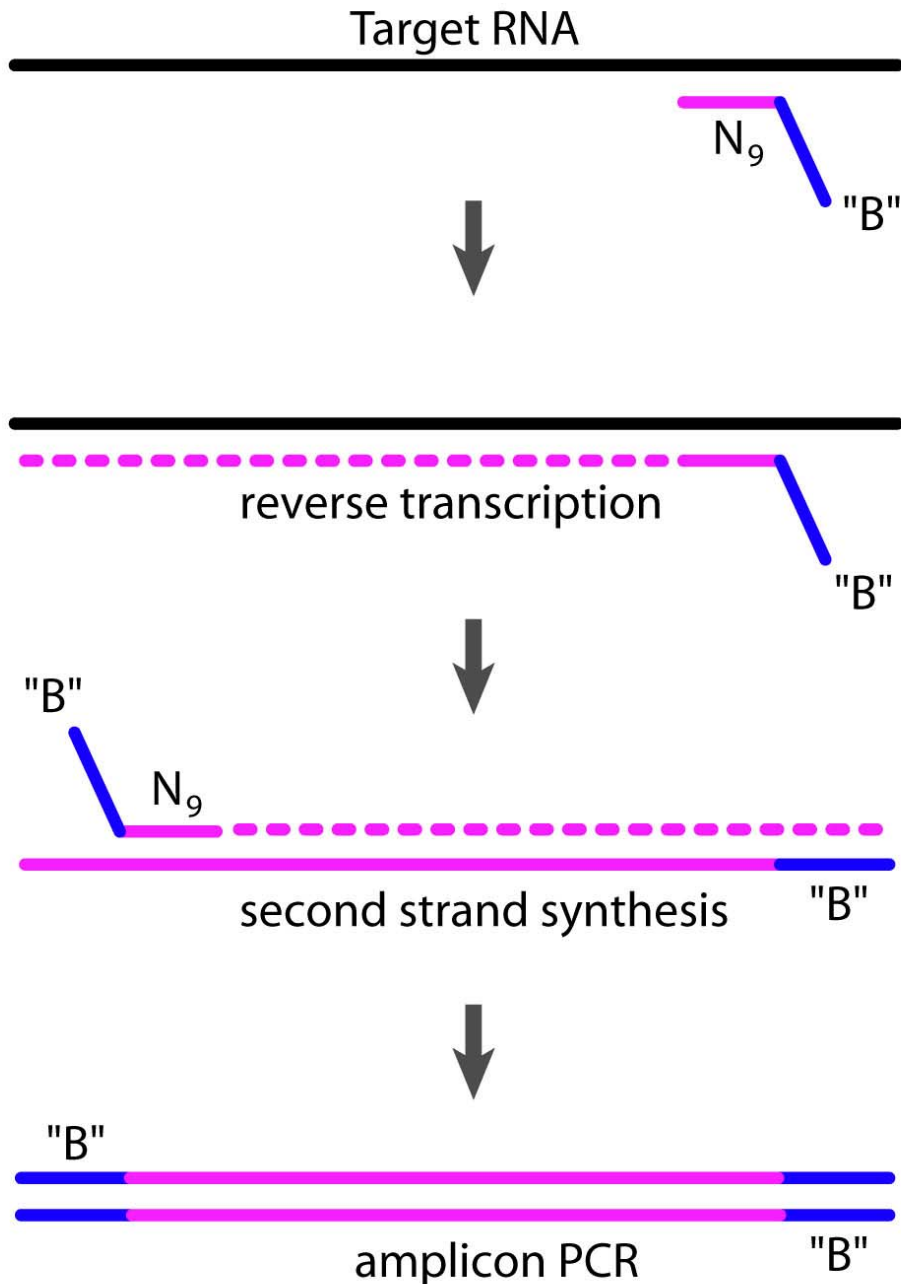
# What does empirical data look like?



**Isolate RNA and reverse transcribe into cDNA**

**Couple fluorescent dyes to cDNA**

**Mix fluorescent cDNA and hybridize to the microarray**

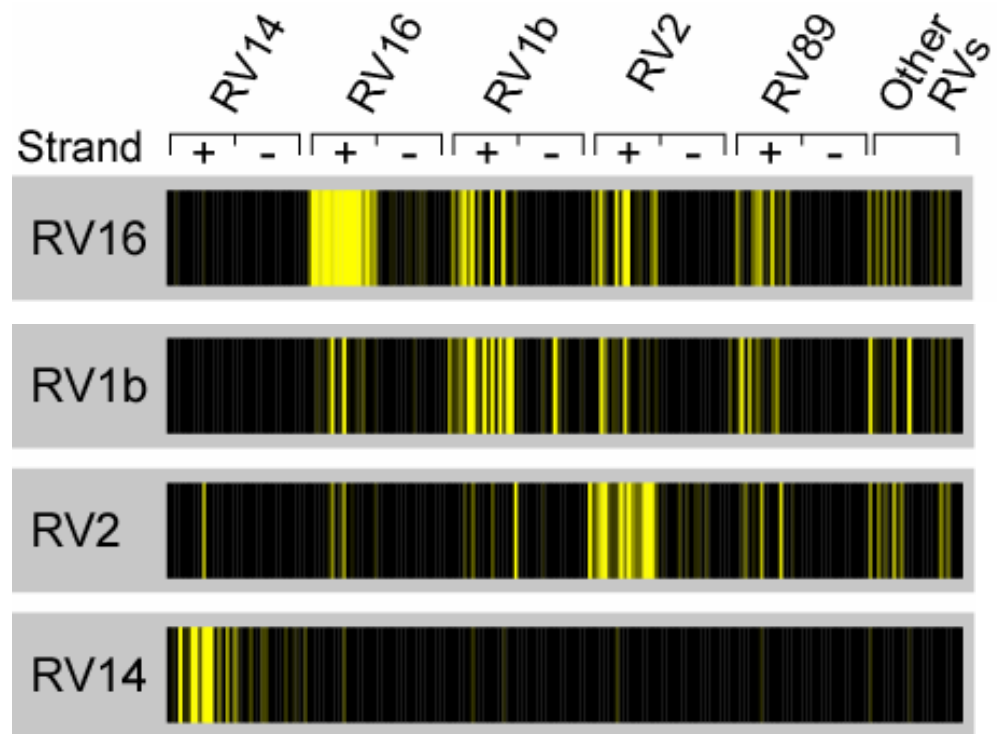


**Randomly primed  
RT-PCR  
amplification  
allows capture of  
ALL RNA  
present in  
specimens**

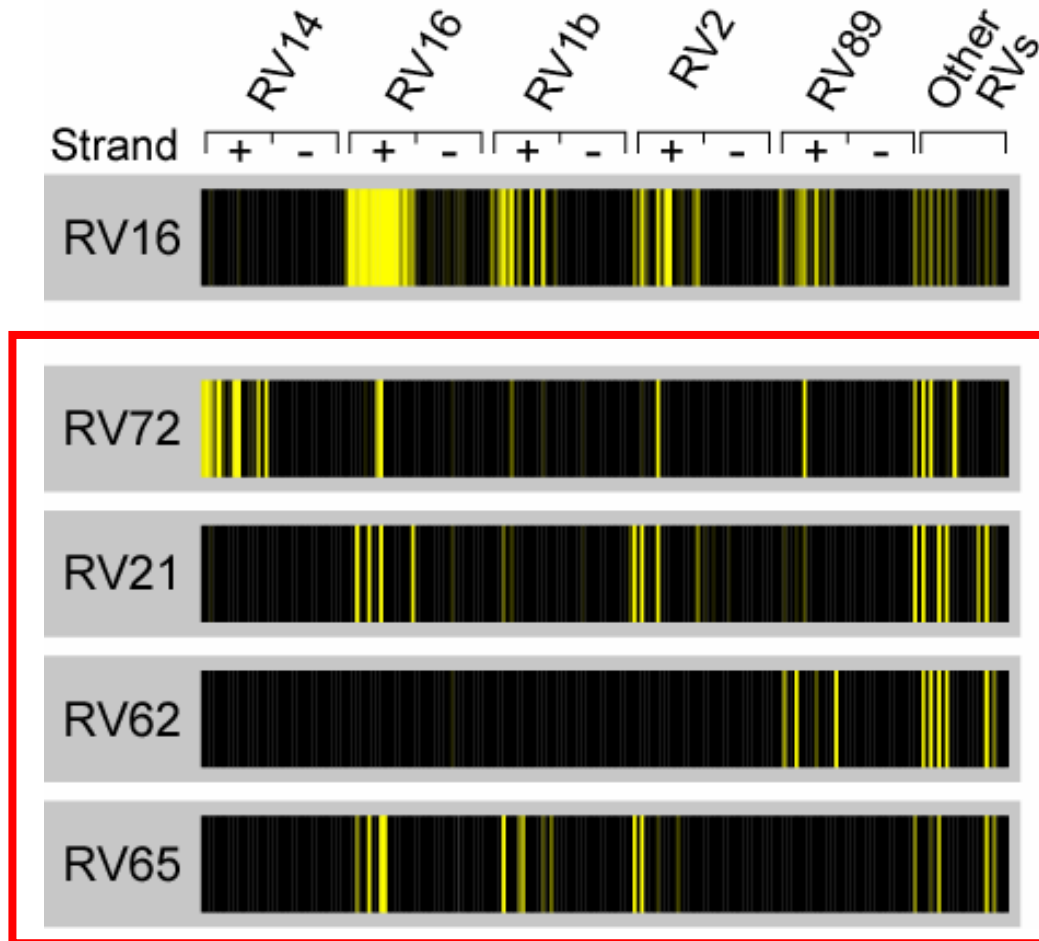
# Result: distinct hybridization profiles for diverse viruses



.... *and* for closely related viruses, too

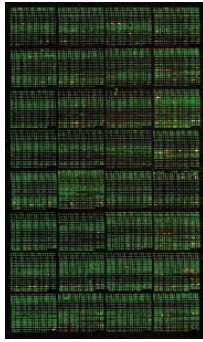


# Unrepresented viruses are also detected

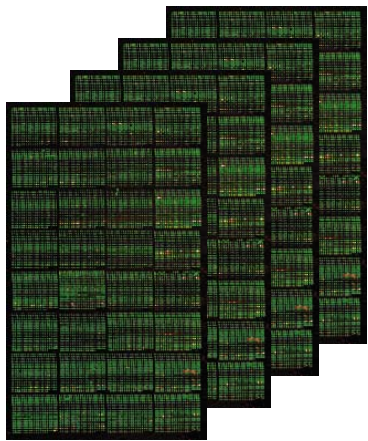




# How do we analyze the Virochip results?



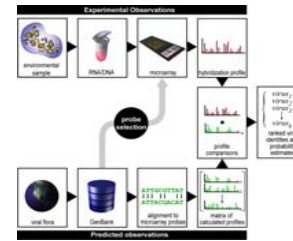
Visual inspection of each array



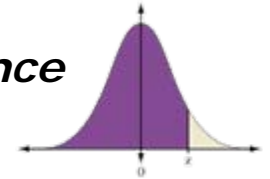
Hierarchical cluster analysis

Computational analyses

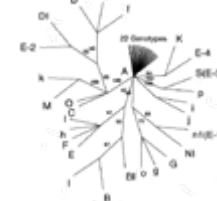
*E-predict*



*Single oligo performance (Z-score)*



*Phylogenetic (vTaxI)*



# **E-Predict: automated Virus Chip result interpretation**

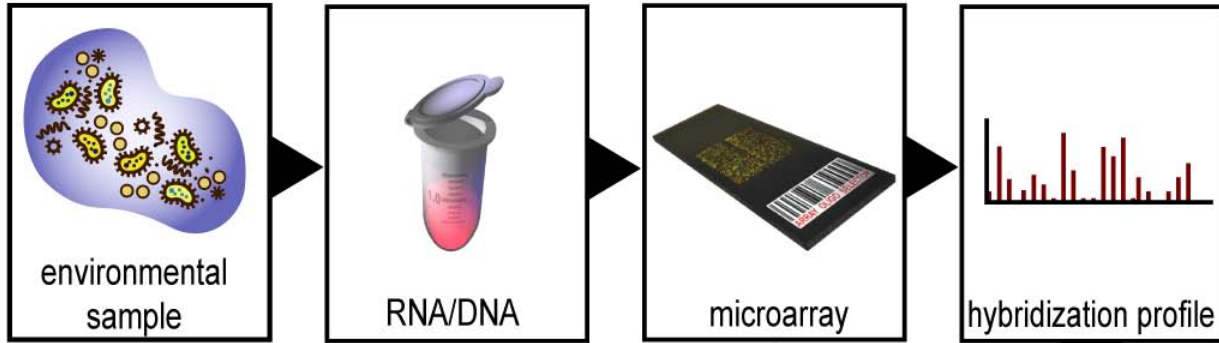
## ***Goal:***

Which virus best accounts for the observed pattern of hybridization?

## ***Approach:***

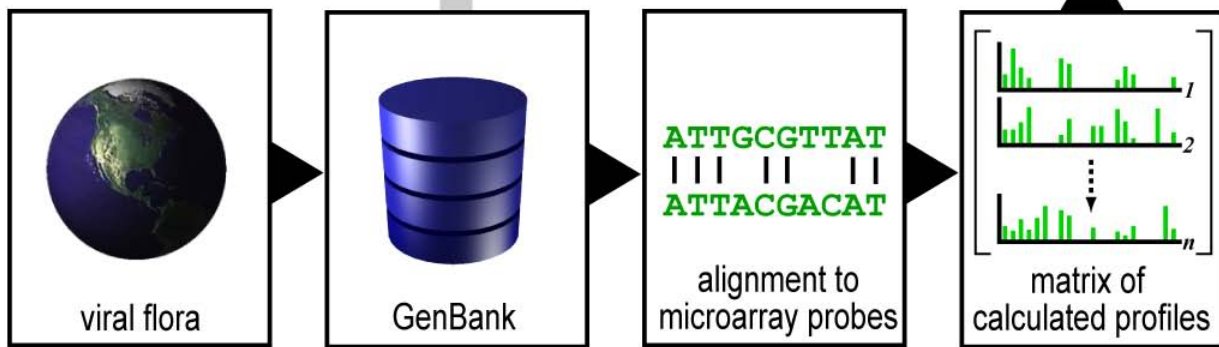
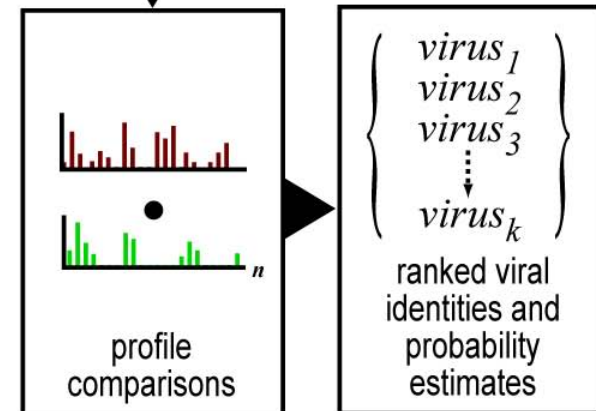
Create a library of “virtual” hybridization signatures for all known viruses. Compare observed result to these expected results.

# Experimental Observations



**"E-Predict"**

probe selection



# Predicted observations

# E-Predict: HeLa Cell RNA sample hybridization

Family: Papillomaviridae

10582	Human papillomavirus type 18	0.368195	
→ 9626069_80	Human papillomavirus type 18	Papillomaviridae	65439 ←
→ 9626069_269	Human papillomavirus type 18	Papillomaviridae	65440 ←
→ 9626069_81	Human papillomavirus type 18	Papillomaviridae	53528 ←
→ 9626069_263	Human papillomavirus type 18	Papillomaviridae	35566 ←
→ 9627356_269	Human papillomavirus type 45	Papillomaviridae	53605 ←
10593	Human papillomavirus type 45	0.214276	
→ 9627356_269	Human papillomavirus type 45	Papillomaviridae	53605 ←
→ 9626069_269	Human papillomavirus type 18	Papillomaviridae	65440 ←
→ 9626069_80	Human papillomavirus type 18	Papillomaviridae	65439 ←
→ 9626069_81	Human papillomavirus type 18	Papillomaviridae	53528 ←
→ 9626069_263	Human papillomavirus type 18	Papillomaviridae	35566 ←
37112	Human papillomavirus type 29	0.094531	
→ 9628502_277	Human papillomavirus type 29	Papillomaviridae	34210 ←
→ 9626069_269	Human papillomavirus type 18	Papillomaviridae	65440 ←
→ 9627356_269	Human papillomavirus type 45	Papillomaviridae	53605 ←
→ 13128941_271	Human papillomavirus type 84	Papillomaviridae	12112 ←
→ 9627257_277	Human papillomavirus type 10	Papillomaviridae	8039 ←

**Candidate viral family match**

**Matching viral genomes (with similarity score)**

**Array probes contributing to call (with raw signal intensity)**

# E-Predict can pick up double infections

```
Family: Orthomyxoviridae
11320   Influenza A virus                                0.537529
8486129_2_rc      Influenza A virus                                Orthomyxoviridae  65356
8486129_20_rc     Influenza A virus                                Orthomyxoviridae  52380
8486129_56        Influenza A virus                                Orthomyxoviridae  48277
8486125_53_rc     Influenza A virus                                Orthomyxoviridae  65374
8486129_29_rc     Influenza A virus                                Orthomyxoviridae  47241
Family: Paramyxoviridae
11250   Human respiratory syncytial virus                0.160976
9629198_133_rc    Human respiratory syncytial virus                Paramyxoviridae  28456
9629198_111      Human respiratory syncytial virus                Paramyxoviridae  29425
9629198_131      Human respiratory syncytial virus                Paramyxoviridae  16336
9629367_130_rc   Respiratory syncytial virus                     Paramyxoviridae  18527
9629198_133      Human respiratory syncytial virus                Paramyxoviridae  13365
```

**Here, 2 distinct viral families called**

- Each genome called has high intensity cognate oligo hybridization signal
- Call confirmed by independent serology assays and follow-up RT-PCRs

# Virochip performance on real samples

Study 1: NATURI, Naturally acquired upper respiratory infections  
82 cold specimens from asthmatic and non-asthmatic adults

Virochip vs. culture, pathogen-specific RT-PCR

AL Kistler et al., 2007.

Study 2: Acute pediatric respiratory infections  
278 specimens from hospitalized children

Test of Virochip vs. DFA, pathogen-specific RT-PCR

CY Chiu et al., 2008.



- Superior to DFA, culture
- Comparable sensitivity (85% - 90%)
- High specificity (>99%)

## **Plus....**

- Detects double infections,
- Detects viruses not routinely tested and viruses missed by DFA, culture

# Virochip exceeds standard tests in 2 cases of acute respiratory distress



- 28 yr old female presents w/ fevers, bloody sputum
- within 1 week, progresses to lung failure, intubated
- tests negative for infection by ALL standard assays
- endotracheal aspirate sent for Virochip analysis...

## **Human Parainfluenza virus 4 (HPIV4)**

- not included in standard viral pathogen test panels
- conventional wisdom: HPIV4 confers mild illness
- another false negative!

CY Chiu et al., 2006.



- 63 yr old male, CML patient, presenting w/ pneumonia
- tested negative by all standard viral panels
- Prophylactic antifungals, antibiotics
- Sample sent for Virochip analysis....

## **Human metapneumovirus**

- Part of standard viral RT-PCR panel, why missed?
- Sequence recovery: FALSE NEGATIVE!
- RT-PCR target sites are mutated in this isolate

CY Chiu et al., 2007.

*How does E-Predict handle a novel virus for which sequence is not yet available?*

## ***Highest scoring candidate for SARS samples***

**Family: Coronaviridae**

**220713 Avian infectious bronchitis virus**

9626535_1099	Avian infectious bronchitis virus
9635576_275	Turkey astrovirus
9635572_255	Ovine astrovirus
9626535_568	Avian infectious bronchitis virus
9626535_1099_rc	Avian infectious bronchitis virus
15081544_766	Bovine coronavirus
9630726_269	Human astrovirus
15081544_766_rc	Bovine coronavirus
12175745_728	Human coronavirus 229E
9626535_727	Avian infectious bronchitis virus

Unique features:

1. Coronaviridae call for human specimen, yet few mammalian CoV oligos
2. Many avian astrovirus oligos--sequences from a distinct viral family



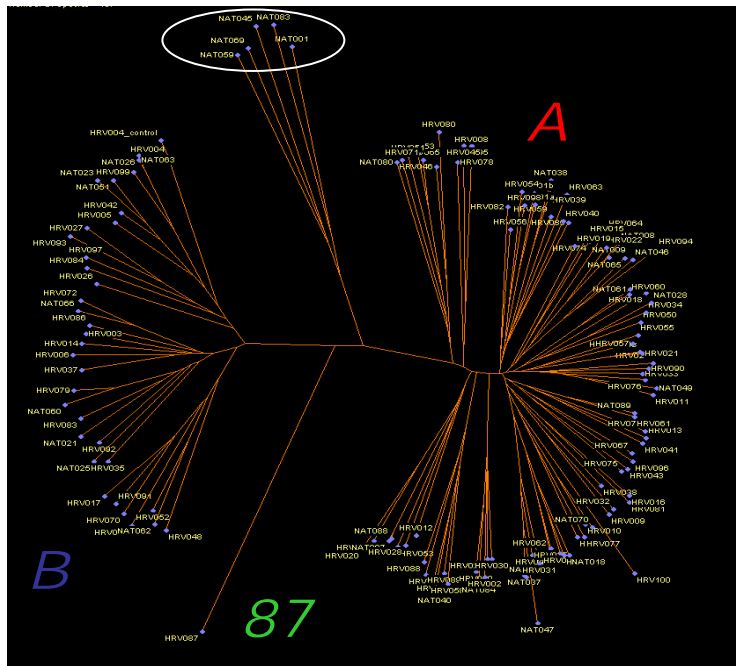
# Re-running E-predict *after SARS CoV sequence is released...*

Family: Coronaviridae				
227859	SARS coronavirus		0.371530	
9626535_1099	Avian infectious bronchitis virus	Coronaviridae		65374
9635572_255	Ovine astrovirus	Astroviridae		65344
15081544_766	Bovine coronavirus	Coronaviridae		7846
9626535_1099_rc	Avian infectious bronchitis virus	Coronaviridae		5662
15081544_766_rc	Bovine coronavirus	Coronaviridae		3845

**Same hybridization profile... Different call!**  
**By simply updating virtual hybridization profiles as new sequences become available, we can now call SARS CoV: 'software updates' increase diagnostic power of the Virochip**

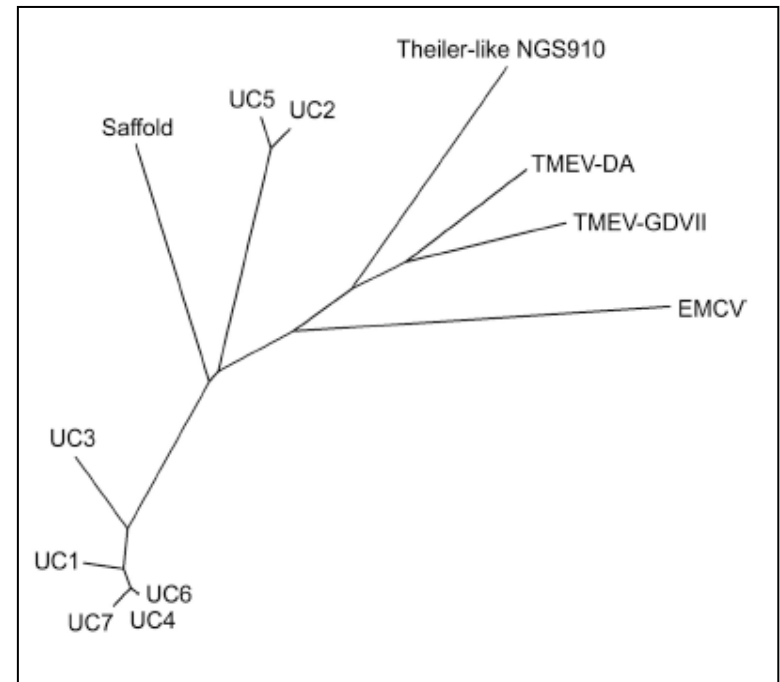
# Virochip identification of novel viral species in acute infectious disease states

a clade of human rhinoviruses detected in NATURI



AL Kistler et al., 2007

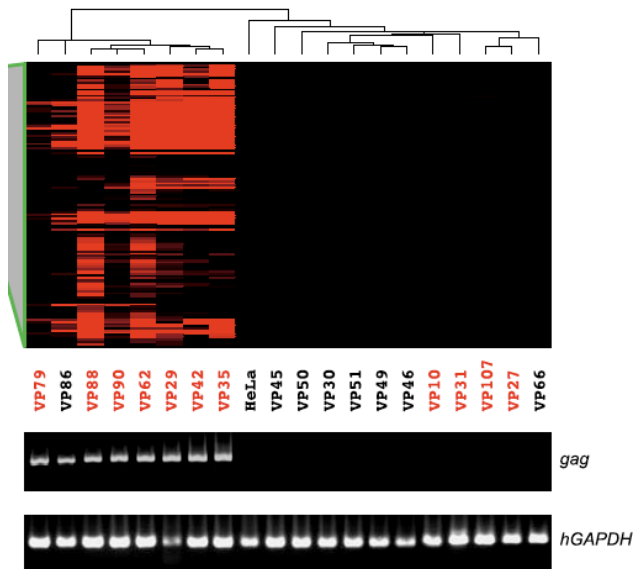
2 clades of human coronaviruses detected in ILI and stool



CY Chiu et al., 2008.

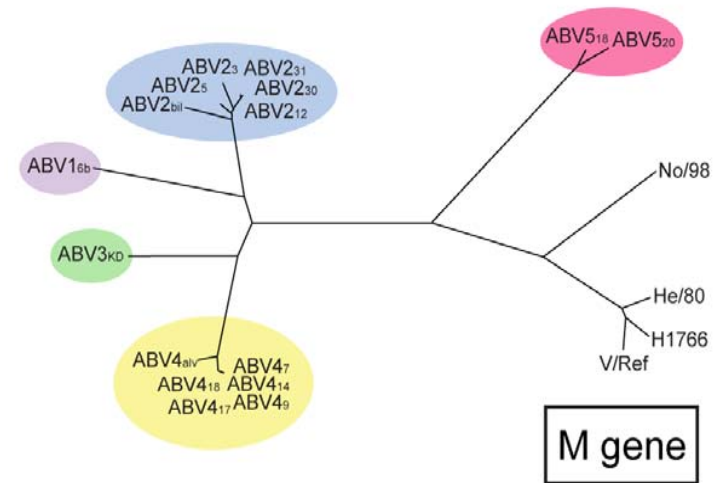
# Virochip identification of novel viral species in specimens from chronic disease states

a novel gamma retrovirus in **RNAseL**-deficient prostate cancer tumors



A. Urisman et al., 2006

a clade of divergent avian bornaviruses proventricular dilatation disease tissues



AL Kistler et al., 2008.

*Joe DeRisi  
Don Ganem  
Charles Chiu  
Alexander Greninger  
Peter Skewes-Cox  
Sharon Chao  
Nicole Fischer  
Anatoly Urisman  
Silvi Reich  
Charles Runckel  
Sarah Aarons*

*Dave Wang, Washington Univ.  
Homer Boushey, UCSF  
Bruce Patterson, Stanford Univ.  
David Schnurr, Calif. DHS  
Robert Silverman, Lerner Inst.  
Ady Gancz, Susan Clubb*



*Doris Duke Foundation  
Howard Hughes Medical Institute*

# An example: family level oligos

