Case It Update: An Effective Tool for Case-based Learning and Undergraduate Research in Molecular Biology

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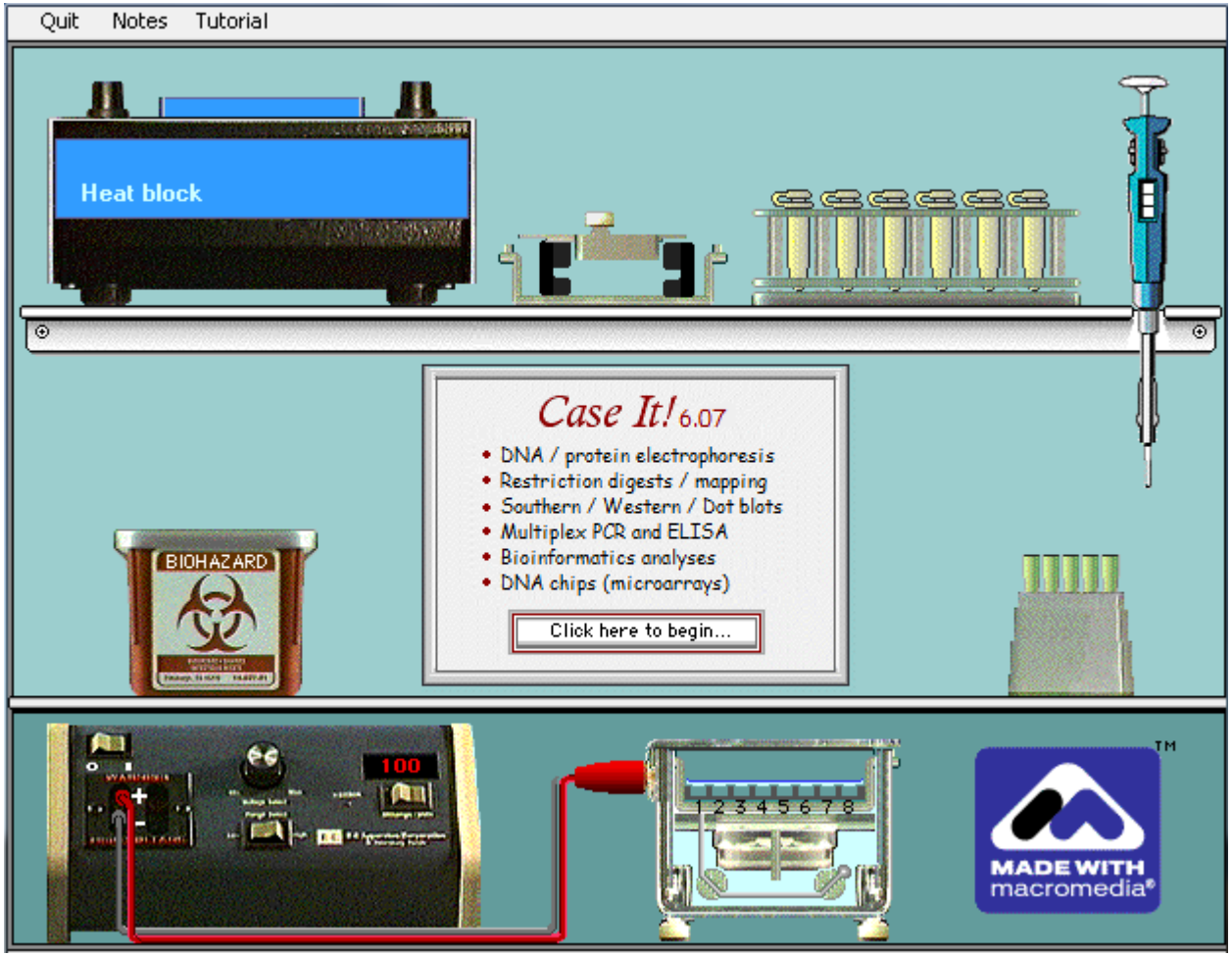

QUBES workshop, North Carolina State University, 2016

Session overview

- Introduction to the Case It! project and ScienceCaseNet
- Freshmen research applications
  - HHMI SEA-PHAGES (restriction digest of phage DNA)
  - Honey bee health
- New cases on honey bee biology
  - Virus detection by PCR (new module on qPCR)
  - Bee virus bioinformatics
- Case It! Mobile as an alternative to the Case It! simulation
- Future of the Case It! Project

History of Case It Project

- 1993 AMCBT meeting – John Jungck
- 1994 BioQUEST workshop - Peter Woodruff
- 1995 BioQUEST workshop - Joyce Cadwallader, Virginia Carson, and Bill Coleman
- 1996 BioQUEST workshop – Margaret Waterman
- 2006 and 2007 BioQUEST workshops – Arlin Toro, Rafael Tosado-Acevedo, Chi-cheng Lin, Dinitra White
- 2008 and 2009 SCOPE workshops – Ethel Stanley and Sam Donovan
- 2012 The Science Case Network (RCN-UBE)
- 2012, 2013 BioQUEST workshops
- 2016 Collaboration with EvoEd project – Jim Smith, Peter White

Overview of Case It Project

- Electronic framework for analyzing and discussing case studies in molecular biology
- Genetic and infectious diseases and associated ethical issues
- Students gather background information on cases
- Analyze DNA and protein sequences using the Case It simulation (v6.07)
- Can extend case analysis using MEGA bioinformatics software (megasoftware.net)
- We have used online poster sessions and role-playing, but there are many other ways to use software and cases

Features of Case It v6.07 for PC and Mac

- DNA and protein electrophoresis
- Restriction enzyme digestion and mapping
- Southern, Dot and Western blotting
- Polymerase Chain Reaction (single and multiplex)
  - qPCR under development
- ELISA
- Microarrays (SNP and expression)
- BLAST, alignments and tree-building (in conjunction with MEGA software (PC) or MABL web site (PC and Mac))
- Used to analyze case studies in genetic and infectious diseases and other biology topics

Case It! Project

Case It! Home Page: www.caseitproject.org

- Includes tutorials, downloads, case descriptions, suggestions for class use, link to Science article

Case It! is part of ScienceCaseNet.org network for case and problem-based learning, funded by RCN-UBE program of NSF

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Case It! funding was provided by the CCD/CCLI/TUES programs of the National Science Foundation

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Honey bee cases

- Collaborators:
  - Kim Mogen and Brad Mogen, UWRF Biology
  - Marla Spivak, UM Bee Lab
- Incorporating research on honey bee health and colony collapse disorder in first-year biology classes
  - Virus detection by RT-PCR (qPCR)
  - Effects of pesticide exposure, mite levels, etc.

Case scenario

Honey bees are commonly exposed to pesticides as they forage for pollen and nectar. Some pesticides are known to affect the central nervous system of bees and thus impact their behavior. Sub-lethal exposures of some pesticides are considered possible contributing factors to Colony Collapse Disorder (CCD). Dr. Muskiver was curious if pesticide exposure was linked to virus infection, another possible contributing factor to CCD.

To test this question, Dr. Muskiver set up test colonies, and fed the honey bees either with untreated pollen or pollen treated with sub-lethal doses of pesticides. She then tested the bees for the presence of several viruses using multiplex PCR on cDNA isolated from the bees.

DNA samples to test

Negative control – bee sample with no viruses present

Positive control – bee sample containing all four viruses

Hive 1 – exposed to pesticides

Hive 2 – exposed to pesticides

Hive 3 – no pesticides exposure

Hive 4 – no pesticide exposure

Multiplex PCR primers

Primers	PCR product size
Actin	120 bp
Deformed wing virus (DWV)	203 bp
Black queen cell virus (BQCV)	322 bp
Sac brood virus (SBV)	487 bp
Israeli acute paralysis virus (IAPV)	719 bp

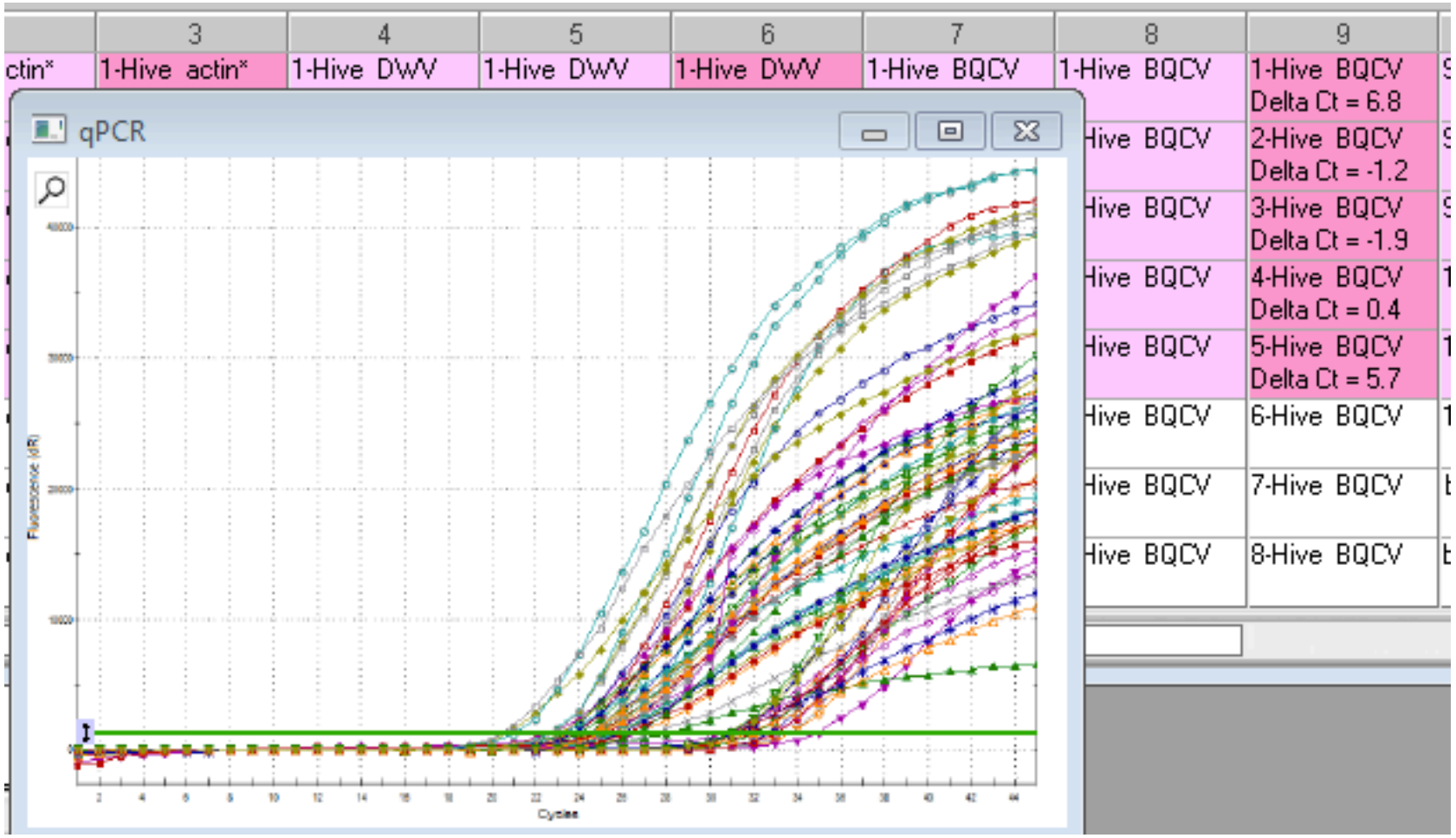
PCR Result

1.0 % agarose runtime = 60 100 volts	1. 100 bp ladder 2. neg control 3. pos control all viruses 4. Hive 1 pesticide-treated 5. Hive 2 pesticide-treated 6. Hive 3 no pesticide 7. Hive 4 no pesticide

Analyze the data

- Do the control sample produce the results you expected?
- What are the results for each experimental hive, in terms of viruses that are detected?
- Is there any correlation between pesticide exposure and viruses detected?
- How would you explain these results to Dr. Muskiver?
- What changes would you make to the experiment design if this is repeated?
- What would you suggest that these researchers do next?
- What are some other tests that could be done to address this question?

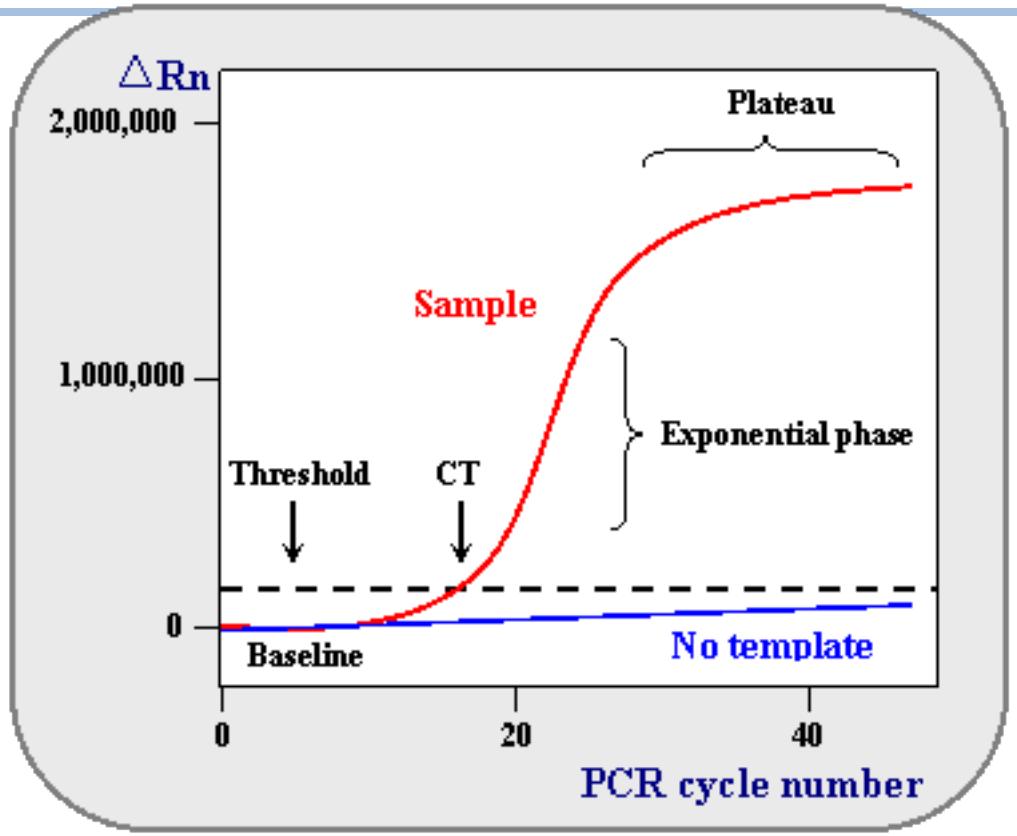
qPCR module


3
4
5
6
7
8
9
ctin*
1-Hive actin*
1-Hive DWV
1-Hive DWV
1-Hive DWV
1-Hive BQCV
1-Hive BQCV
1-Hive BQCV
Delta Ct = 6.8
2-Hive BQCV
Delta Ct = -1.2
3-Hive BQCV
Delta Ct = -1.9
4-Hive BQCV
Delta Ct = 0.4
5-Hive BQCV
Delta Ct = 5.7
6-Hive BQCV
7-Hive BQCV
8-Hive BQCV
qPCR
Fluorescence (dR)
Cycles

Quantitative PCR (qPCR)

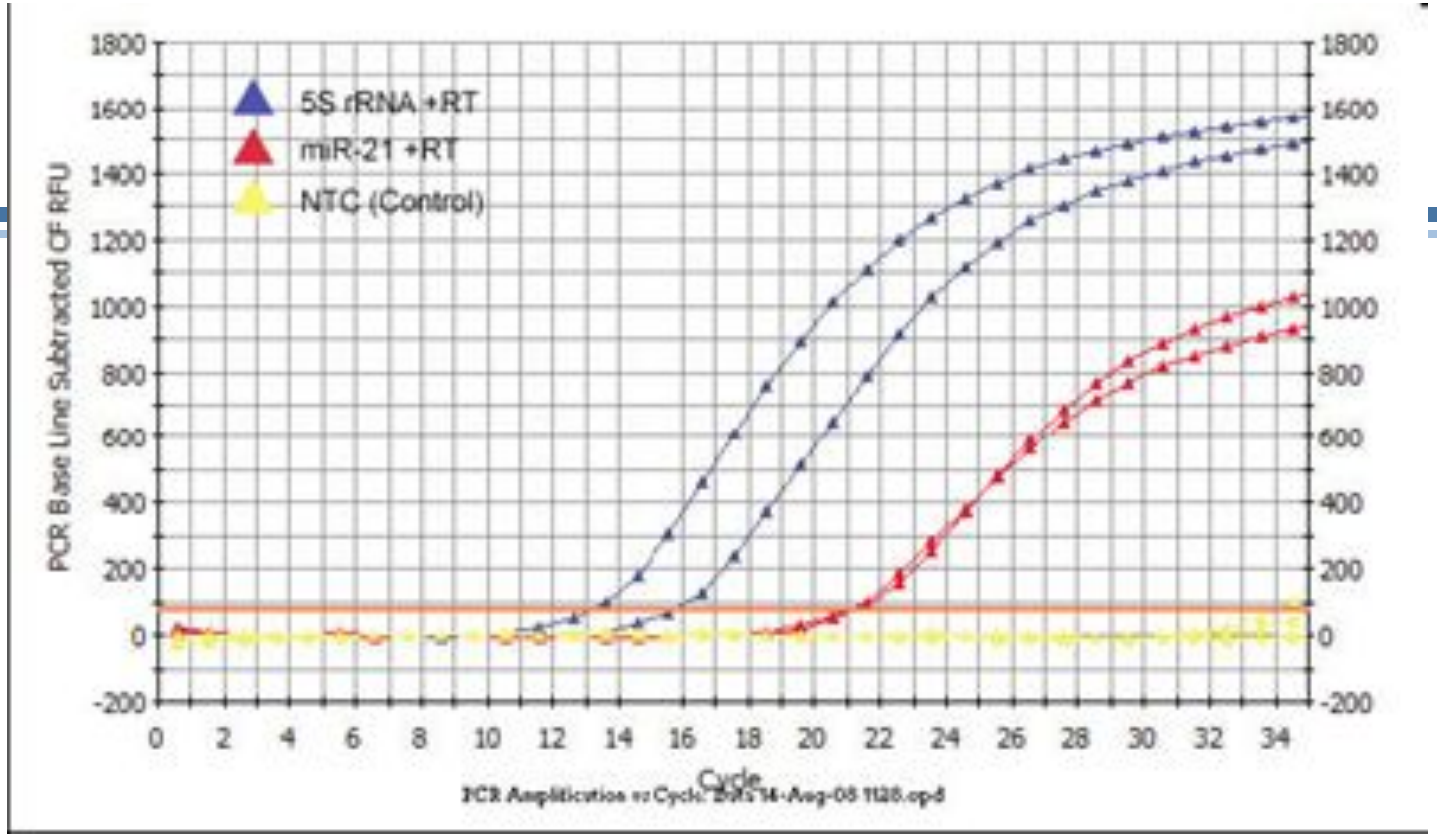
- a.k.a. Real-time PCR (RT-PCR)
- Measures the amount of double-stranded DNA copied during each cycle of PCR
- Based on a fluorescent dye (SYBR green) that binds to dsDNA
- Samples are evaluated based on the cycle number where detectable DNA is generated
  - (**threshold cycle**, or **Ct**)

Model of real time quantitative PCR plot



The **Ct** value is the important data!

Ct = cycle number where fluorescence (red line) crosses threshold value



Samples with lower Ct values (blue) had more starting template cDNA and therefore generated detectable fluorescence (double stranded DNA) sooner

- More specific RNA in these samples

Data analysis

If you want to compare the relative amounts of a specific DNA generated in two samples (**e.g. bees infected with nosema vs healthy bees**), the data should be normalized using a reference gene

- Control for having different amounts or quality of RNA in samples
- Then you can assume that more DNA generated means more starting material (RNA)

We used **actin** as a reference gene, since all bee cells should contain constant amounts of actin RNA

Data analysis

Example data:

Does pesticide treatment affect DWV levels in bees?

	Ct values	
Sample	**Actin (reference gene)**	**DWV (target gene)**
Untreated bees	21.3	28.7
IMD-treated bees	20.1	25.2

Steps in calculation (ΔCt method)

	Ct values	
Sample	**Actin (reference gene)**	**DWV (target gene)**
Untreated bees	21.3	28.7
IMD-treated bees	20.1	25.2

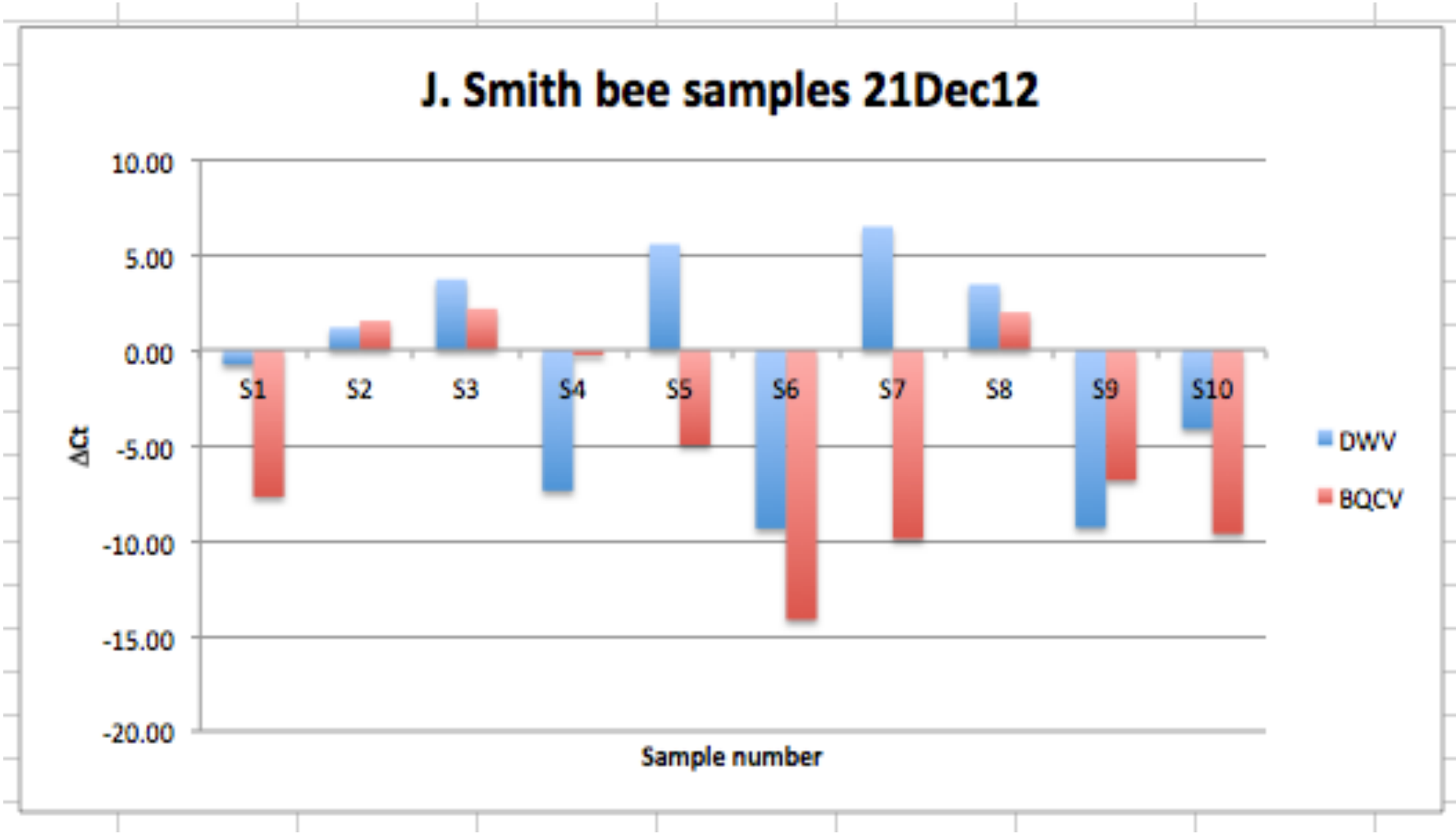
1. Normalize target gene values for each sample

   ΔCt = Ct(actin) – Ct(DWV)

   Untreated: ΔCt = 21.3 – 28.7 = -7.4

   IMD-treated: ΔCt = 20.1 – 25.2= -5.1

qPCR module


J. Smith bee samples 21Dec12
10.00
5.00
0.00
-5.00
-10.00
-15.00
-20.00
ΔCt
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Sample number
DWV
BQCV

Steps in calculation (ΔCt method)

	Ct values	
Sample	**Actin (reference gene)**	**DWV (target gene)**
Untreated bees	21.3	28.7
IMD-treated bees	20.1	25.2

1. Normalize target gene values for each sample

$2^{(\Delta Ct)}$ = relative RNA quantity

$\Delta Ct = Ct(actin) - Ct(DWV)$

Untreated: $\Delta Ct = 21.3 - 28.7 = -7.4$

$2^{(-7.4)} = 0.00592$

IMD-treated: $\Delta Ct = 20.1 - 25.2 = -5.1$

$2^{(-5.1)} = 0.029$

Steps in calculation (ΔCt method)

2. Determine ratio of values for treated and untreated samples

$$\text{Ratio} = \frac{\text{relative quantity (IMD-treated)}}{\text{relative quantity (untreated)}}$$

$$= 0.029/0.00592 = 4.92$$

- 4.92-fold increase in DWV RNA quantity for treated bees

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Case scenario - bioinformatics

Recent declines in honey bee populations have given rise to the syndrome named Colony Collapse Disorder (CCD). Several potential stressors have been identified. A team of research scientists, funded by the North American Honey Bee Council, decide to survey colonies from around North America for two of the notable stressors – Deformed Wing Virus (DWV), a virus that causes wing deformation, and *Varroa destructor*, a parasitic mite that feeds on the bee.

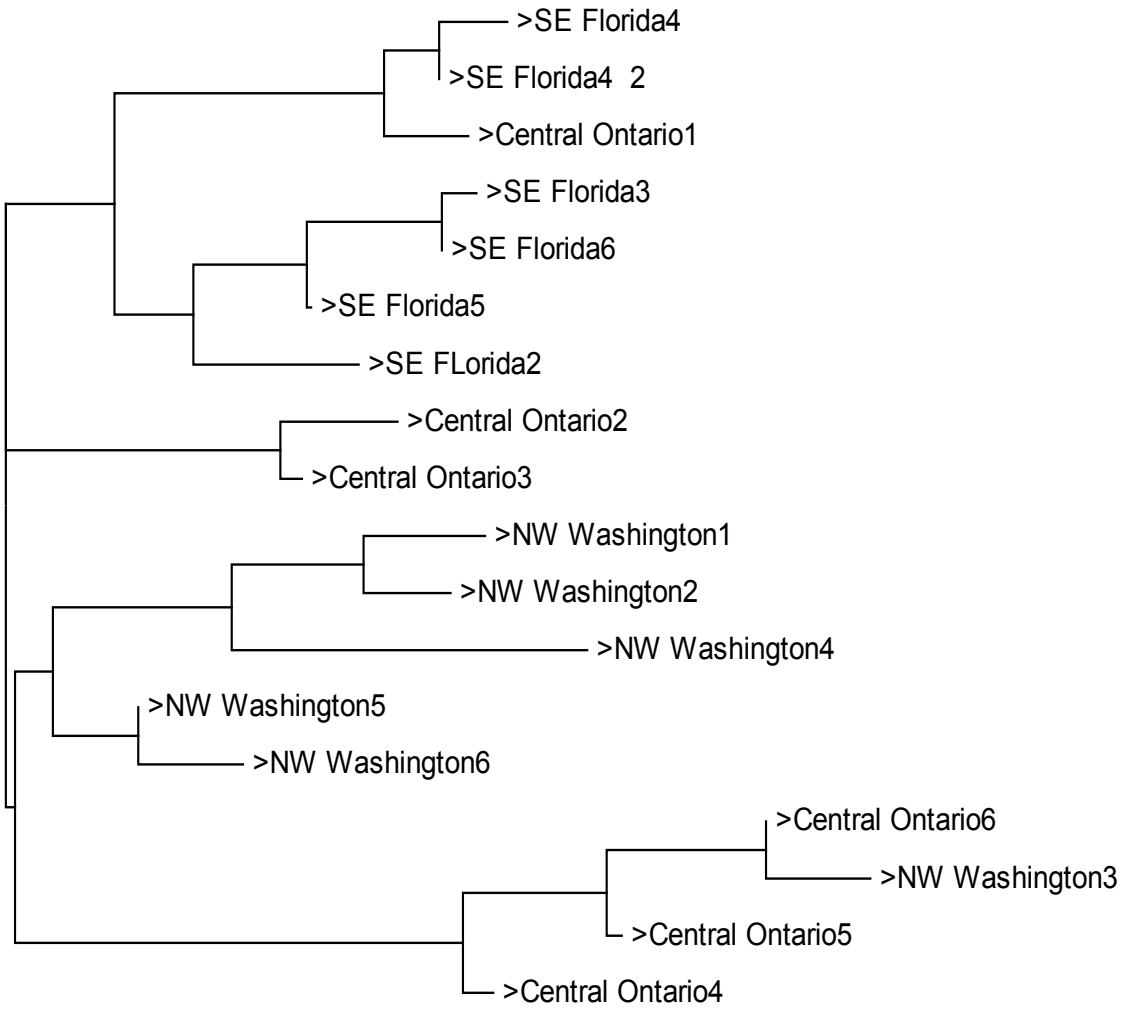
It has recently been reported that *V. destructor* transmits certain strains of DWV more effectively, and that long-term mite infection reduces virus diversity and leads to the prevalence of more pathogenic viruses (Martin *et al.* 2012). The scientists are interested in testing the relationship between DWV strains and the *Varroa* mite in North America.

Case scenario - bioinformatics

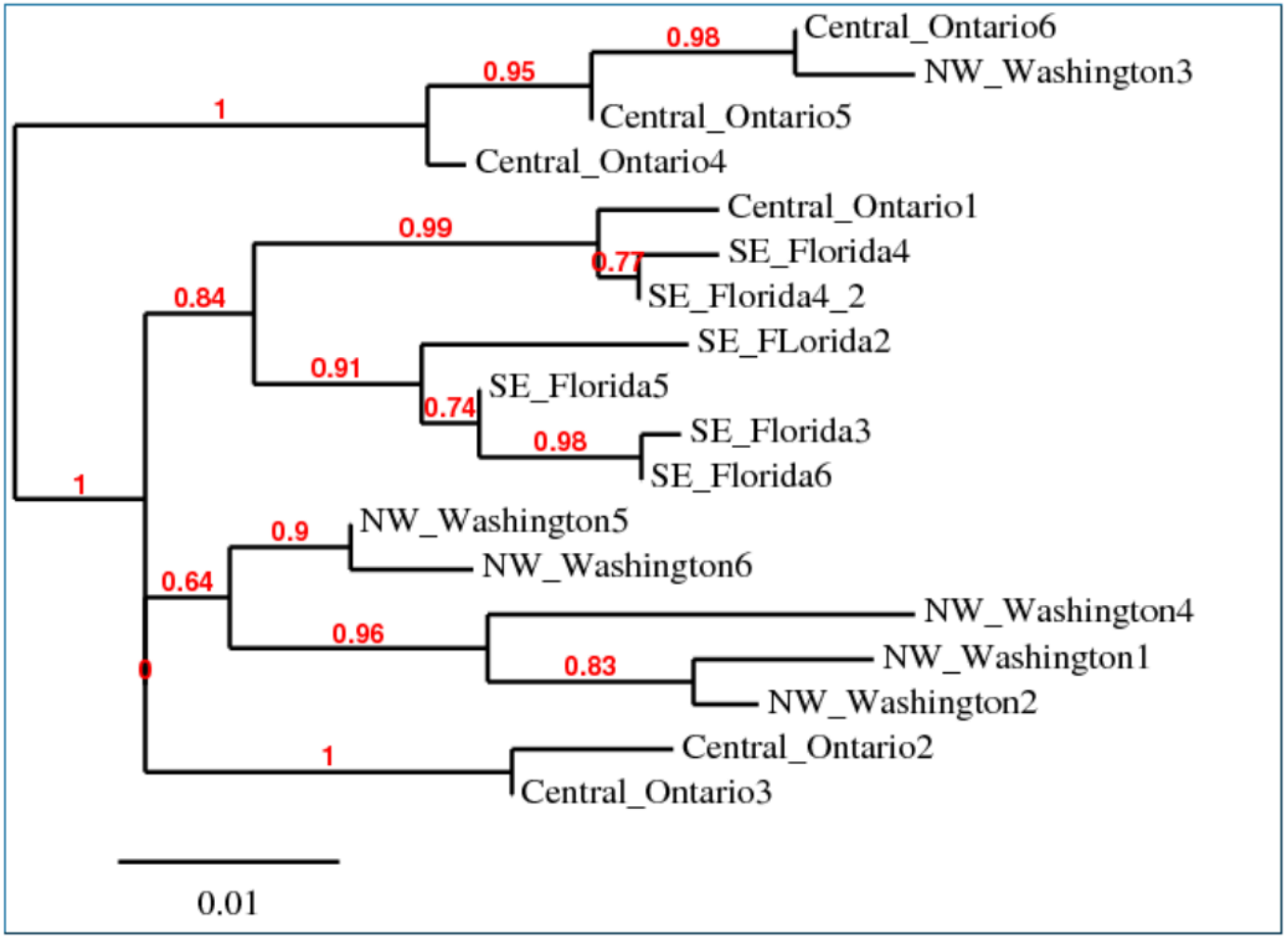
Bees tested from:

- Central Ontario - low mite levels
- Northwestern Washington - low mite levels
- Southeast Florida - high mite levels
- Oahu, Hawaii - high mite levels
- Northern Arizona - moderate mite levels
- Southern British Columbia - moderate mite levels

DNA sequence analysis using MEGA



DNA sequence analysis using MABL site



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Case It Mobile

- Case It v6.06 is a PC application that can also be run on Macs running Windows, via Parallels or Bootcamp
- Case It Mobile is a collection of screen-capture videos of the Case It simulation in action, that can be run using a web browser on a laptop, smart phone, or tablet
- Useful when either time is not available to run the actual simulation, or PCs (or Macs running Windows) are not available
- Examples: http://www.caseitproject.org/mobile/

Case It! Project

Additional Collaborators

- Mary Lundeberg, Biology Department, University of Wisconsin-River Falls
- Chi-Cheng Lin, Computer Science Department,Winona State University
- Arlin Toro, Biology Department, Inter American University of Puerto Rico-San German campus
- Rafael Tosado, Medical Technology Program, Inter American University of Puerto Rico-Metropolitan Campus
- C. Dinitra White, Biology Department, North Carolina A&T State University

Authentic research for first-year students: HHMI SEA-PHAGES Project

Fall semester

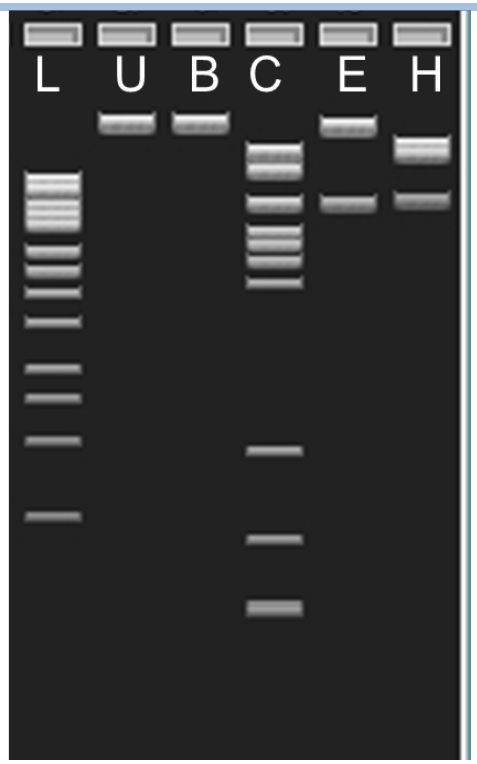
- Isolate mycobacteriophages from soil
- Isolate phage DNA and analyze by restriction enzyme digestion
- Select one phage to send for sequencing

Spring semester – phage genomics (www.phagesdb.org)

- Annotate genes
- Comparative genomics
- Research projects on phage biology

Abrogate lab gel

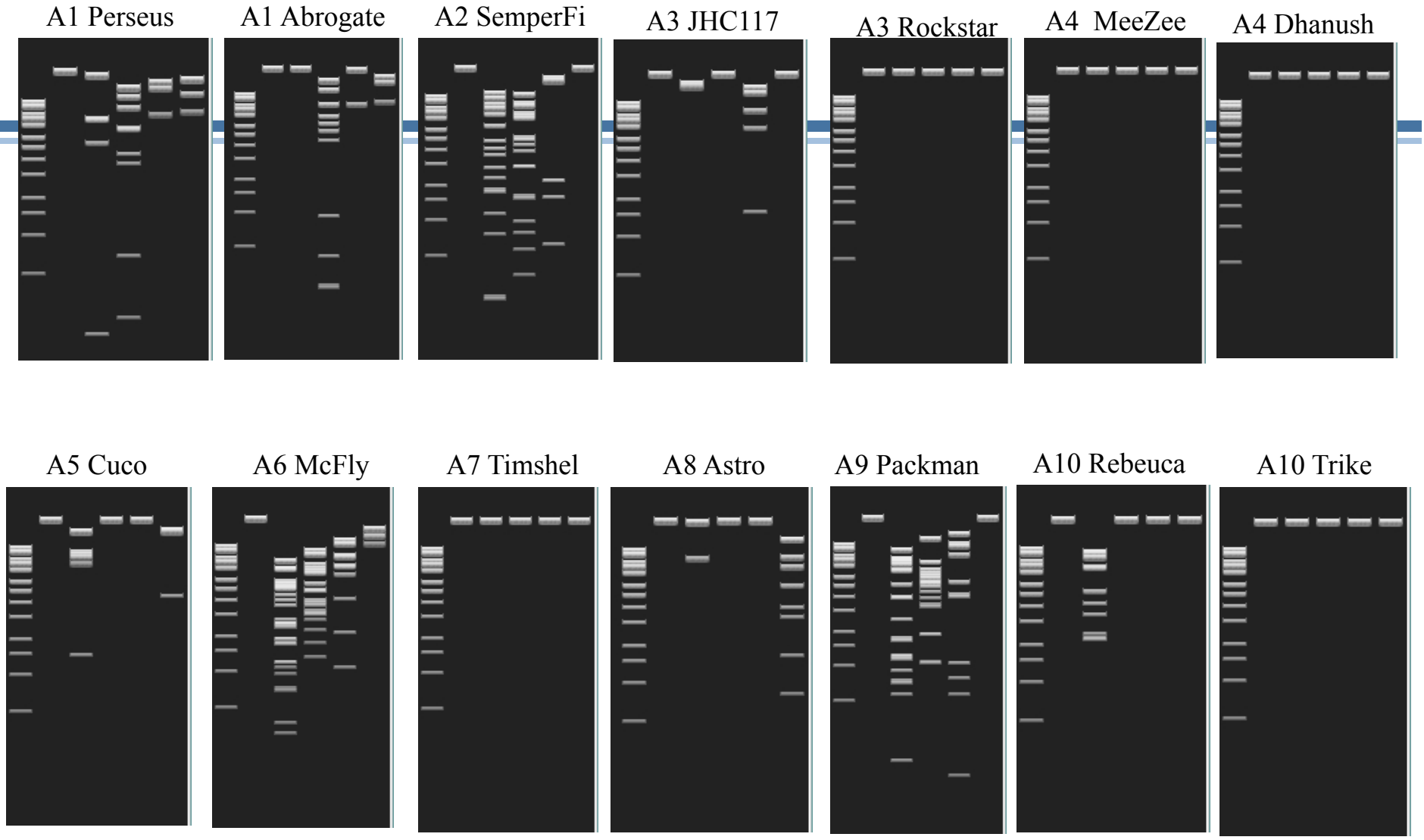
Abrogate virtual gel



Bxb1 virtual gel

L=1 kb ladder; U=undigested; B=BamHI; C=ClaI; E=EcoRI H=HindIII

Cluster A phages, listed by subcluster



Left to right: 1 kb ladder, undigested, BamHI, ClaI, EcoRI, HindIII