20.109
Laboratory Fundamentals in Biological Engineering

Module 1
Nucleic Acids

Infectious Disease

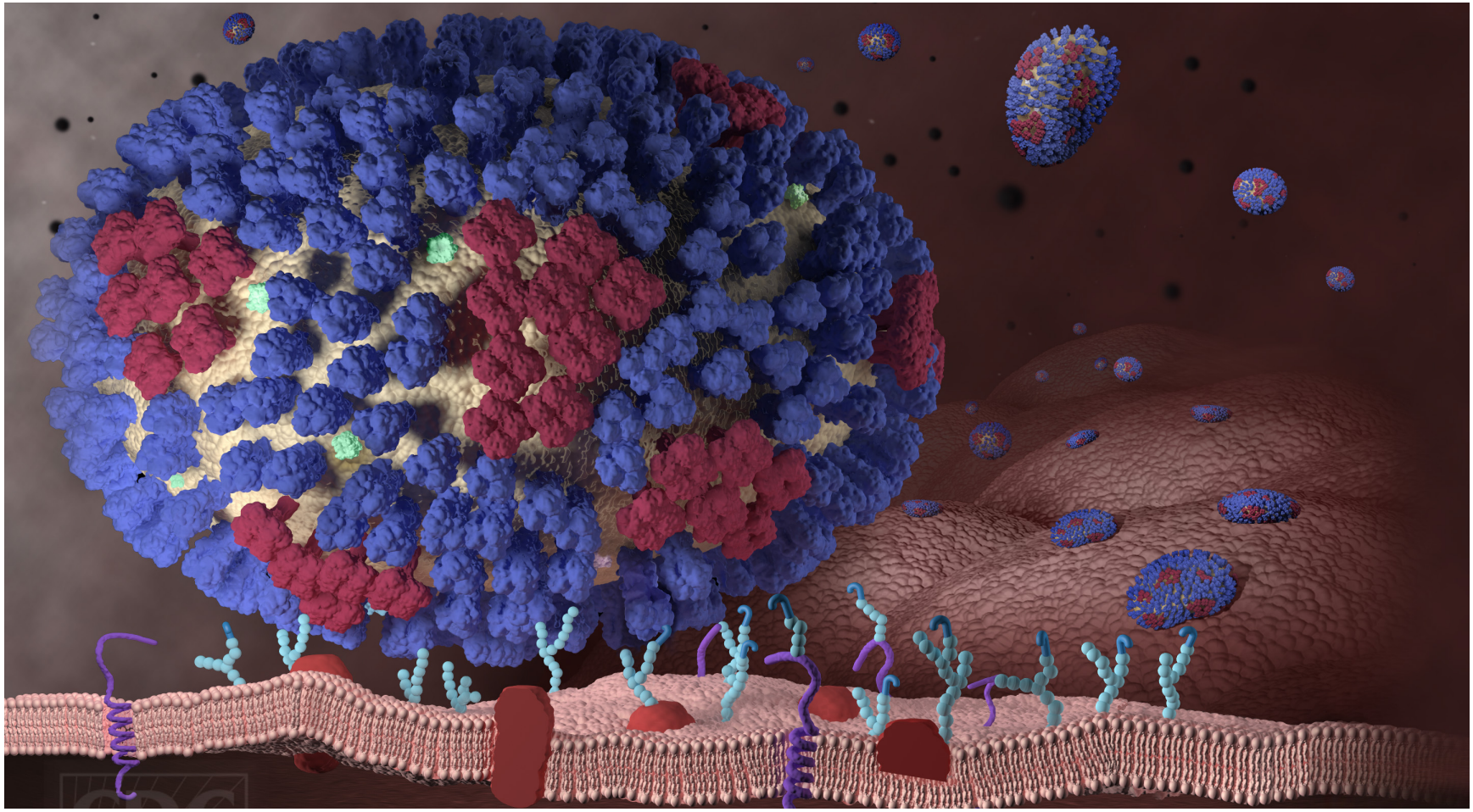
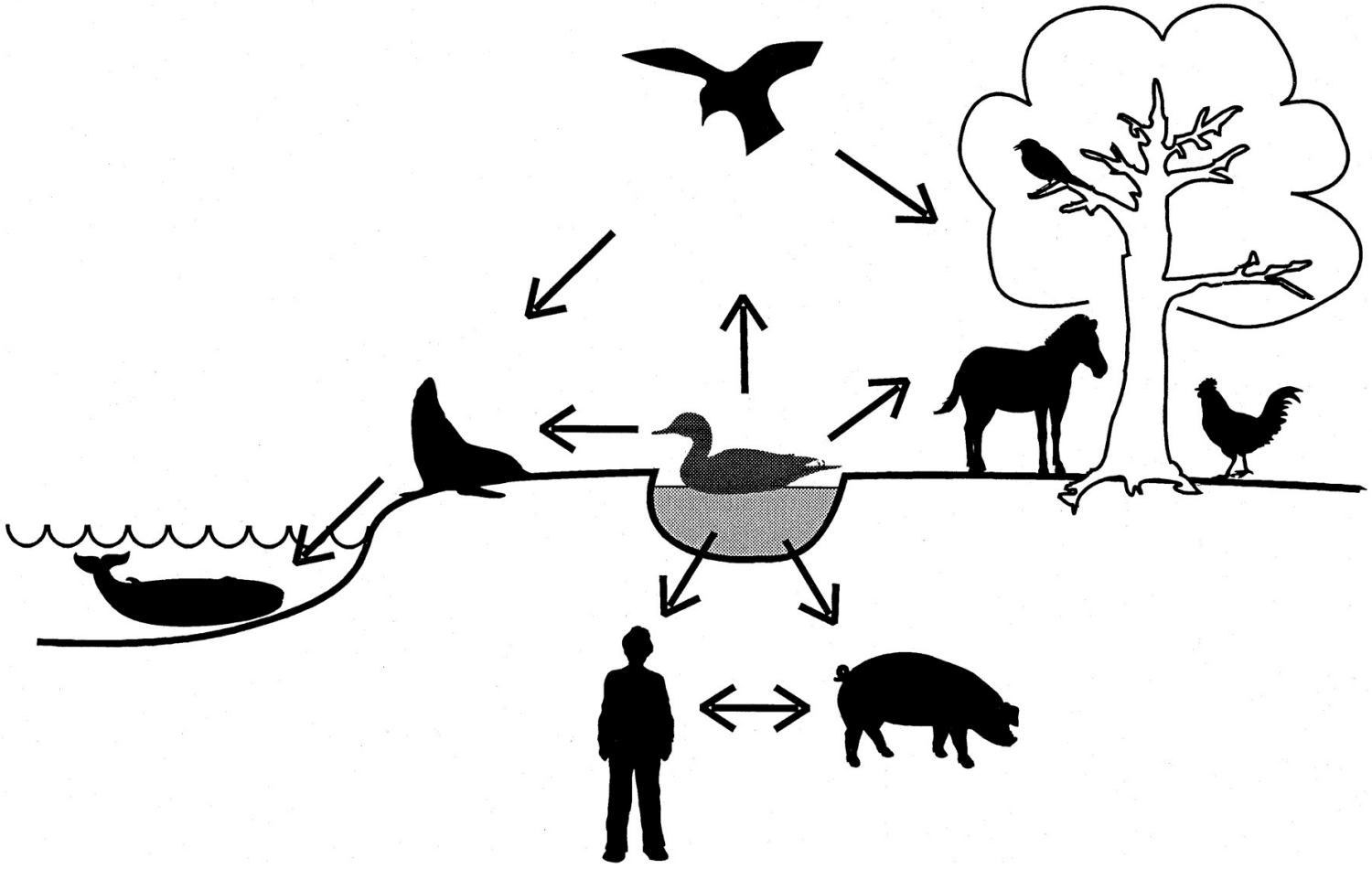


Image from CDC

Influenza Disease Ecology (measure, model)



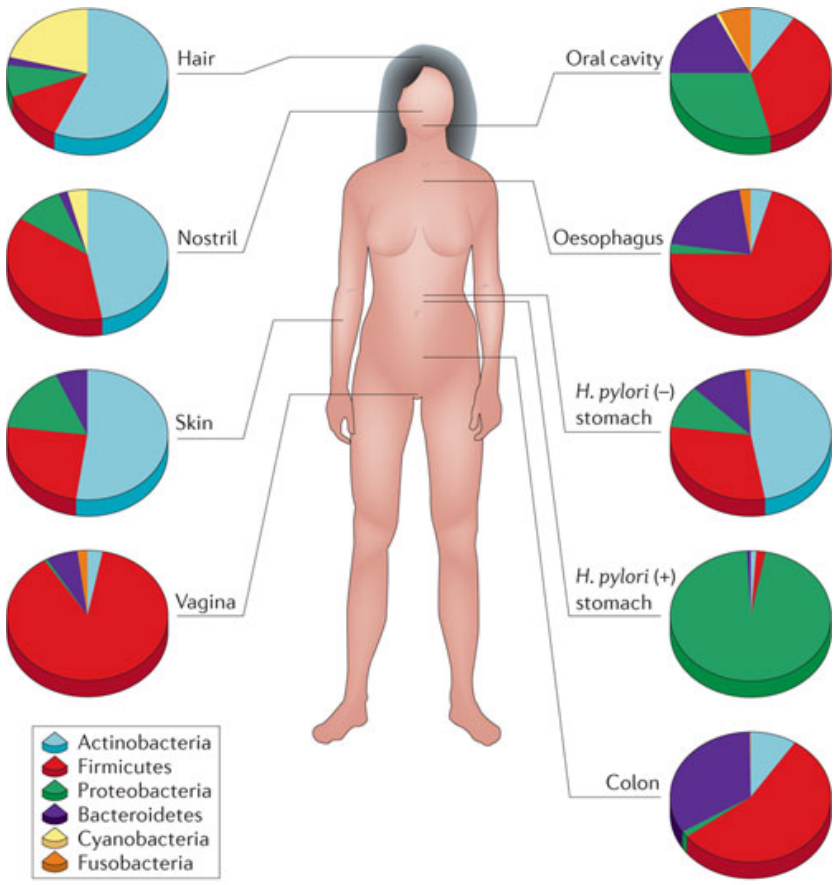
Clin. Microbiol. Rev. January 2001 vol. 14 no. 1 129-149

The microbial environment

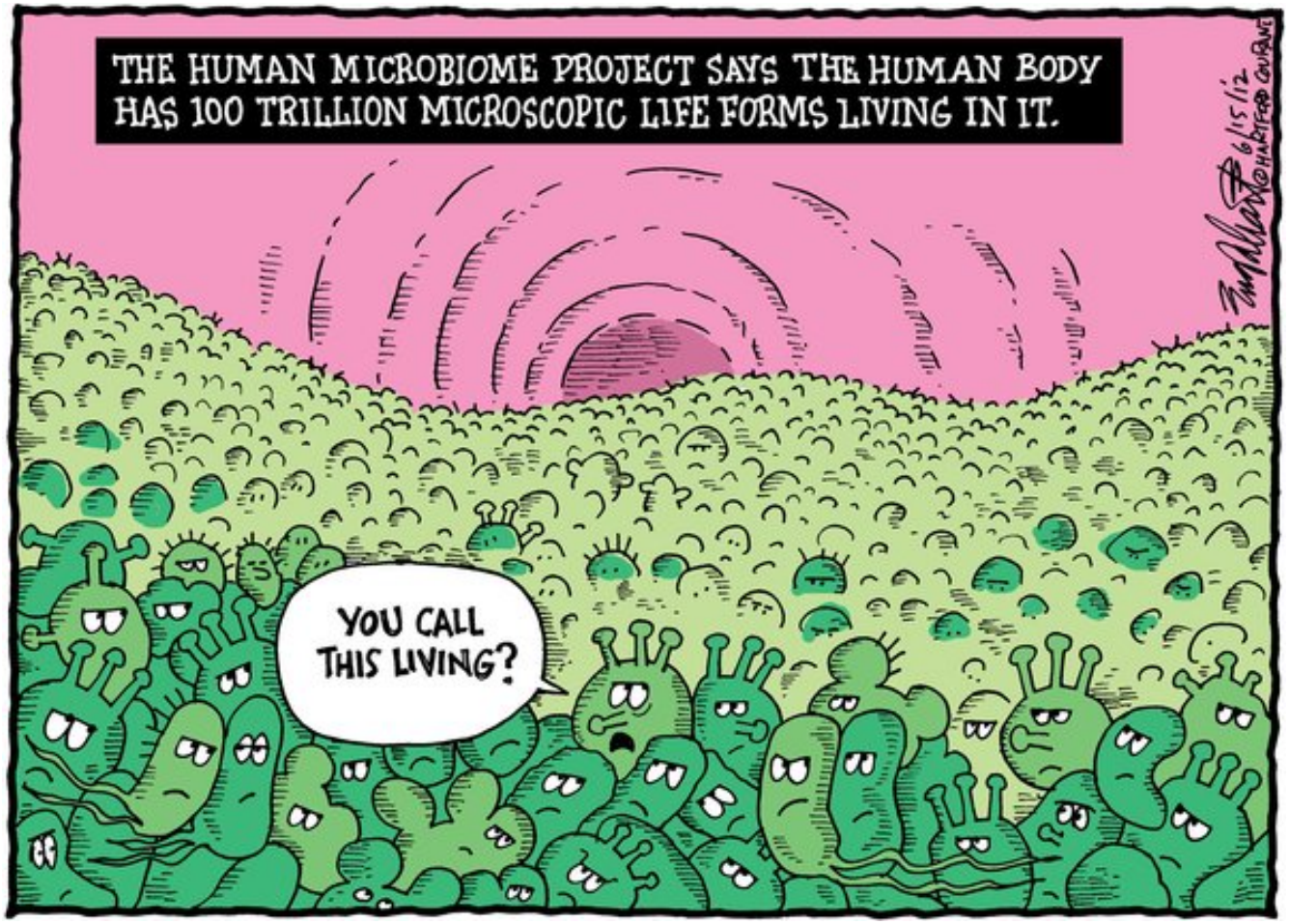
Scientific American interactive microbiome

Scitechdaily.com

A world of environments



Nature Reviews | Genetics


THE HUMAN MICROBIOME PROJECT SAYS THE HUMAN BODY HAS 100 TRILLION MICROSCOPIC LIFE FORMS LIVING IN IT.
YOU CALL THIS LIVING?
6/15/12
©HARTFORD COURANT

Identifying what's there
What is the best test?

- Morphology, imaging, culture?

- Molecular?

Time is money

Virus	Subtype
Influenza A	Non-specific influenza A
	H1
	H3
Influenza B	
Respiratory Syncytial Virus (RSV)	A
	B
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Metapneumovirus	
Rhinovirus	
Adenovirus	

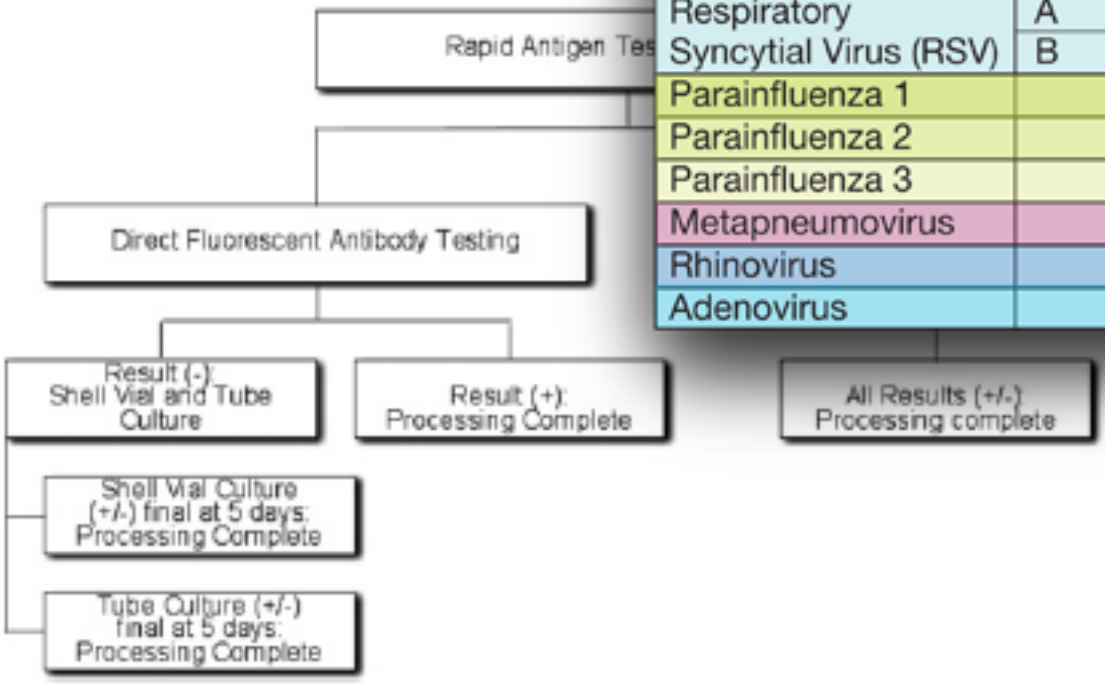


Figure 1. Respiratory viral testing procedure. Workflow of respiratory viral testing samples with negative rapid antigen results.

J Mol Diag 13:175-9 (2011)

Time is money

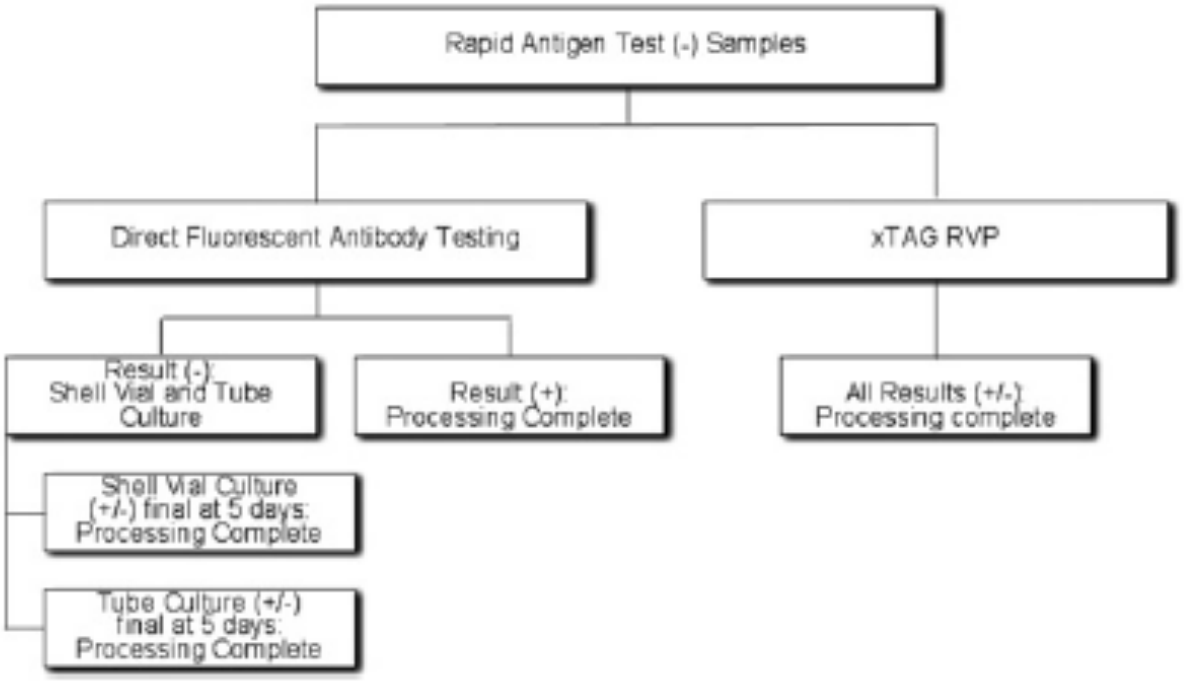


Figure 1. Respiratory viral testing procedure. Workflow of respiratory viral testing samples with negative rapid antigen results.

J Mol Diag 13:175-9 (2011)

Time is money

Table 3. Cumulative Operational Analysis Results Traditional versus Molecular Methodology

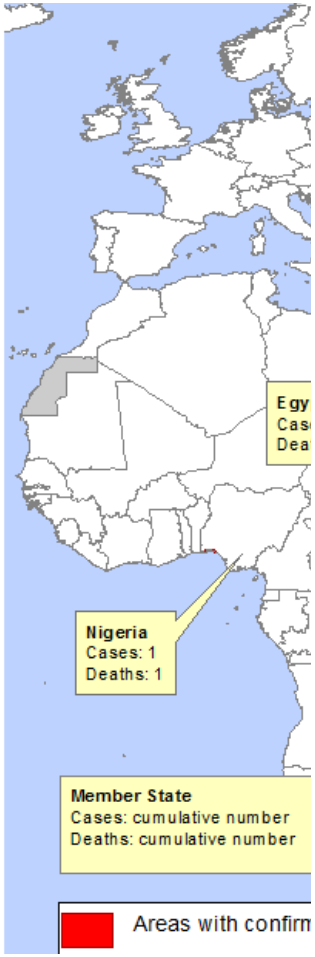
Component	Hands-on time (hours)	Operator steps
RVP	80	52,780
Traditional	503	342,775

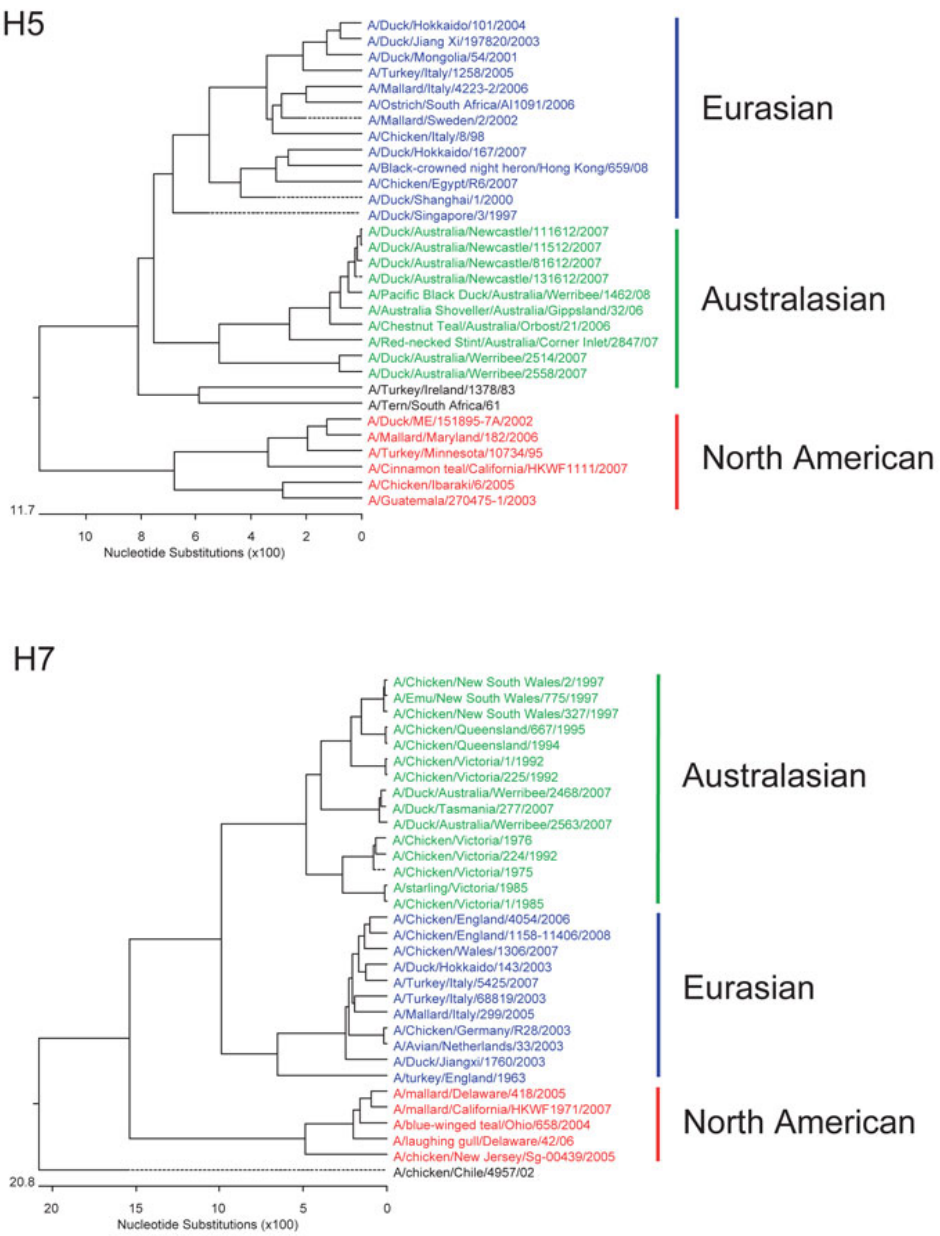
Figure 1. Respiratory viral testing procedure. Workflow of respiratory viral testing samples with negative rapid antigen results.

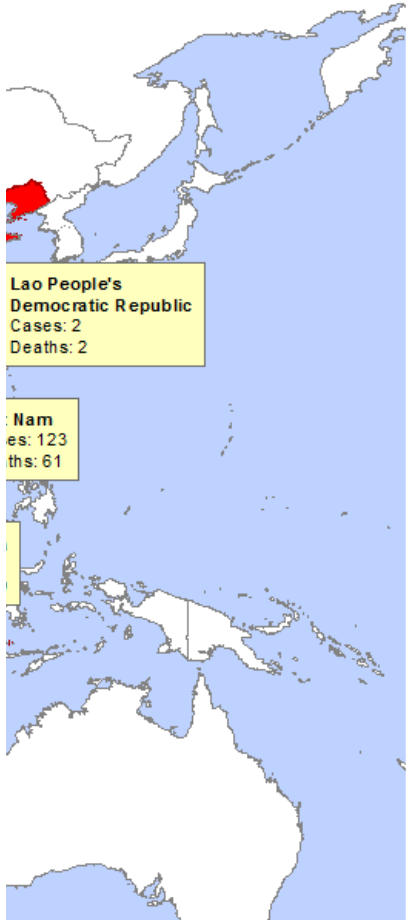
J Mol Diag 13:175-9 (2011)

Dic tical

Figure 2. Phylogenetic tree of the haemagglutinin gene sequences from H5 and H7 viruses showing the distinct genetic clade formed by Australian AIV strains.







WHO

Hansbro & Hurt, 2011

What is the best test?

Diagnostics choices are often not straight forward

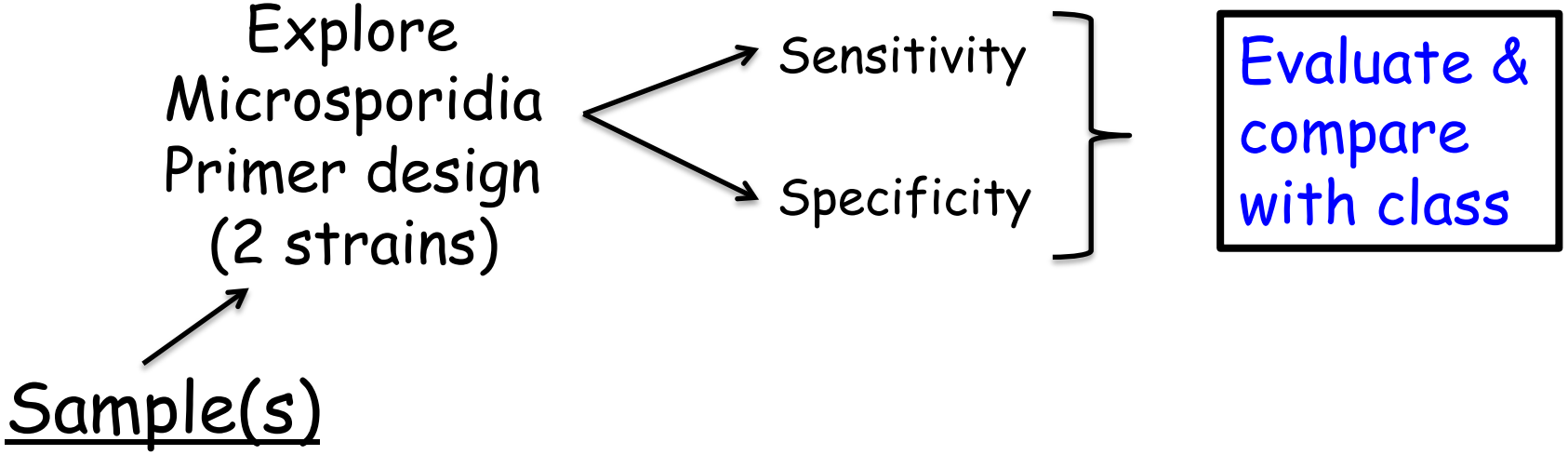
		Egg Isolation +	Egg Isolation -
PCR	+	22	52
	-	20	408

What other utility is there for microbe testing?

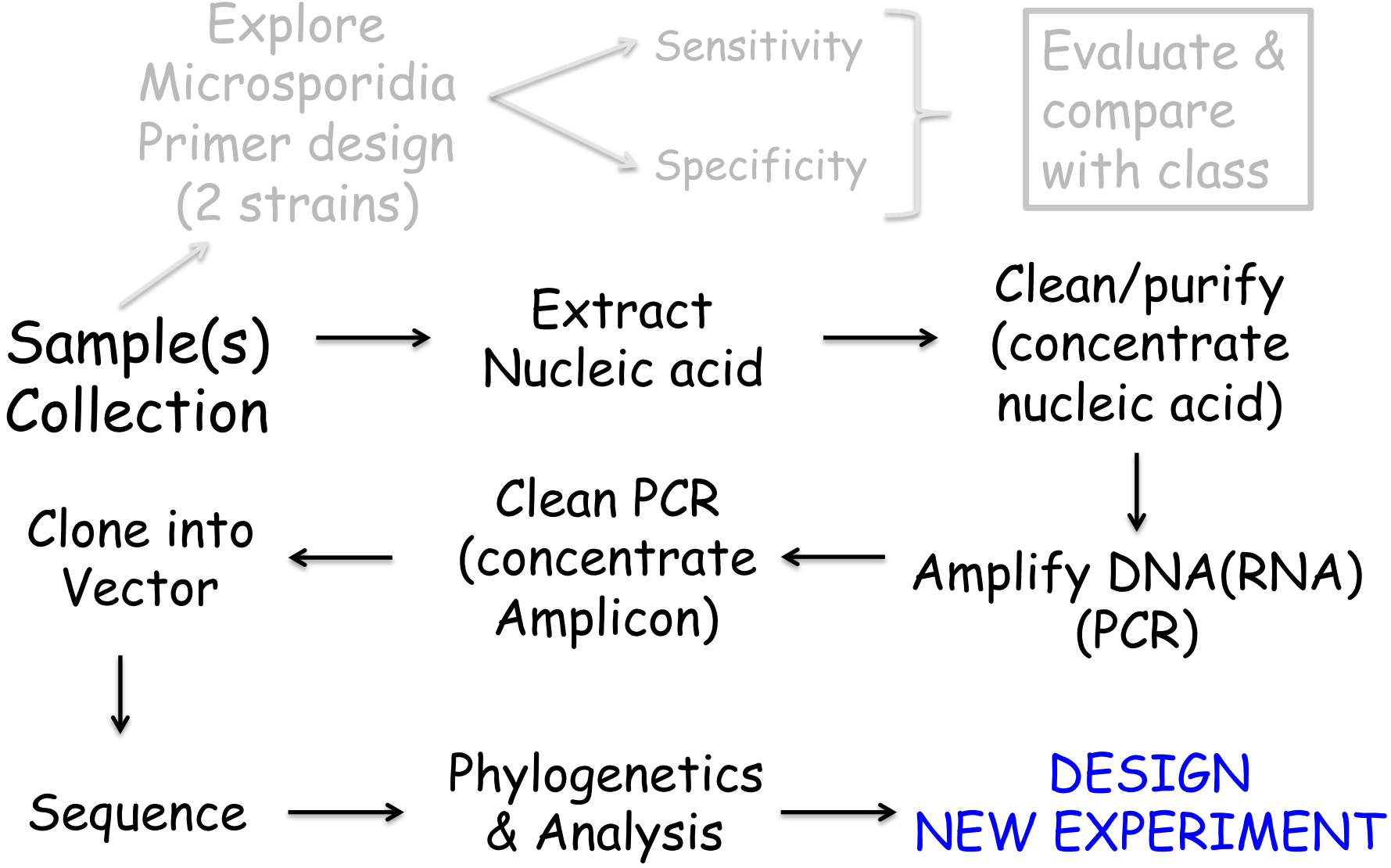
Module 1

- Learn techniques
- Design, analysis, interpretation
- Communication

Module 1

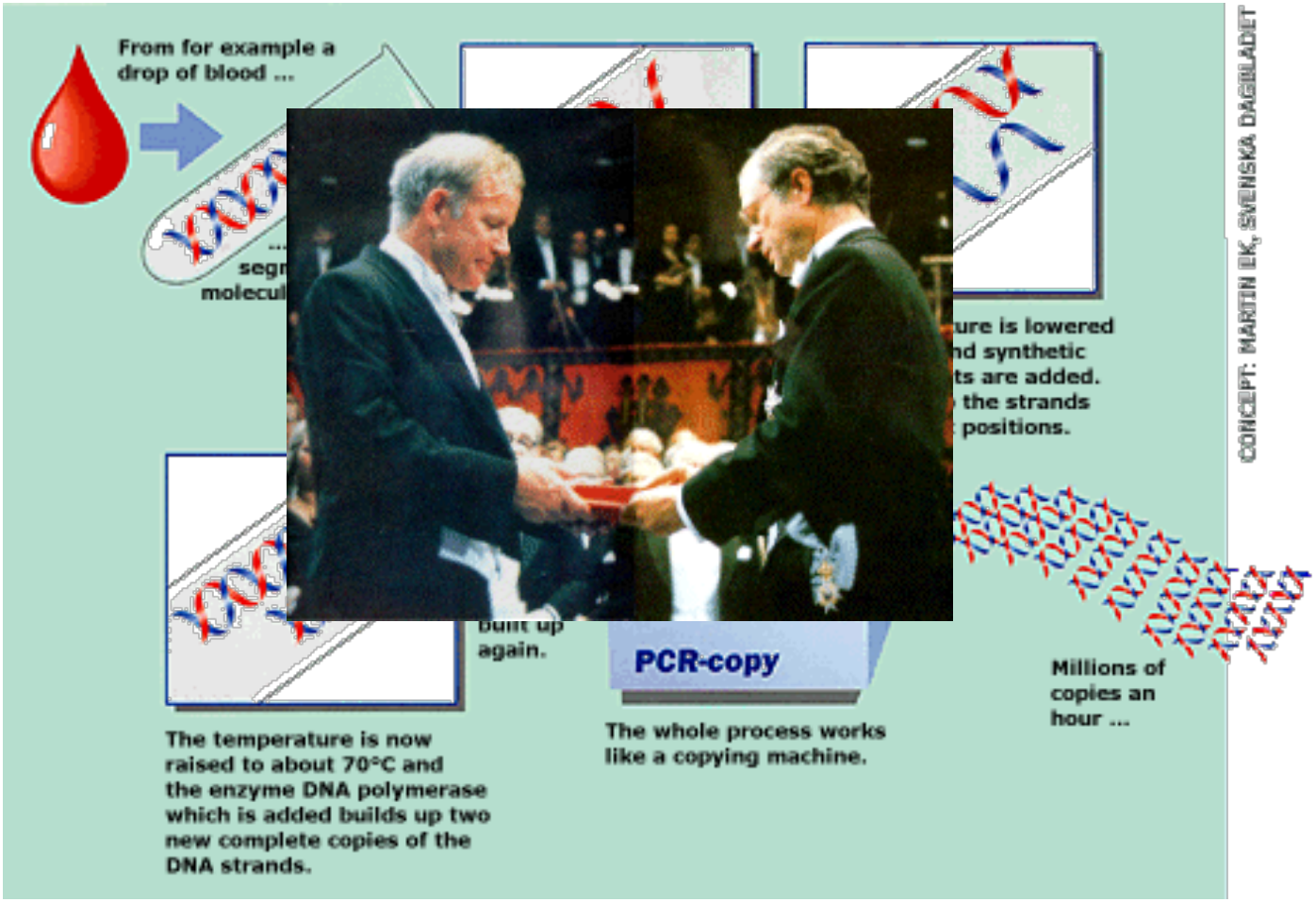

Explore
Microsporidia
Primer design
(2 strains)
Sensitivity
Specificity
Evaluate & compare with class
Sample(s)

Module 1


Explore Microsporidia Primer design (2 strains)
Sensitivity
Specificity
Evaluate & compare with class
Sample(s) Collection
Extract Nucleic acid
Clean/purify (concentrate nucleic acid)
Amplify DNA(RNA) (PCR)
Clean PCR (concentrate Amplicon)
Clone into Vector
Sequence
Phylogenetics & Analysis
DESIGN NEW EXPERIMENT

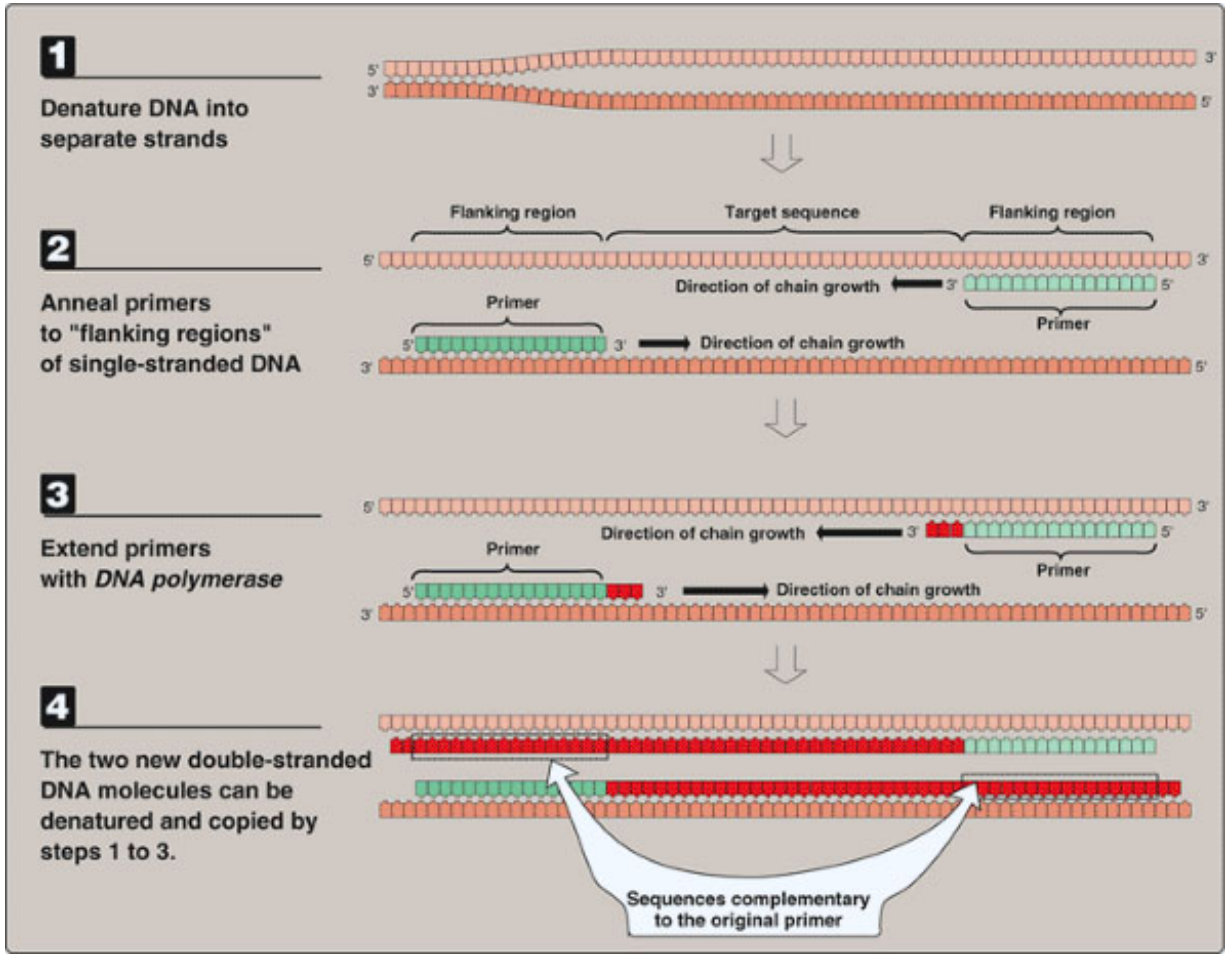
What other ways might you identify a pathogen/microbe?

Inventing PCR



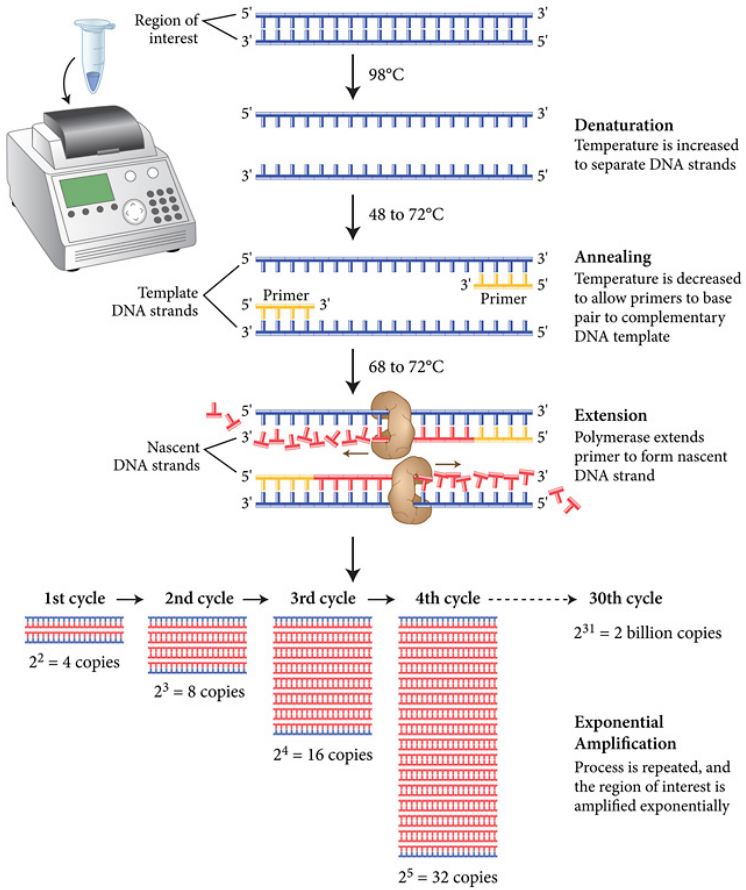
www.nobelprize.org

In a bit more detail…



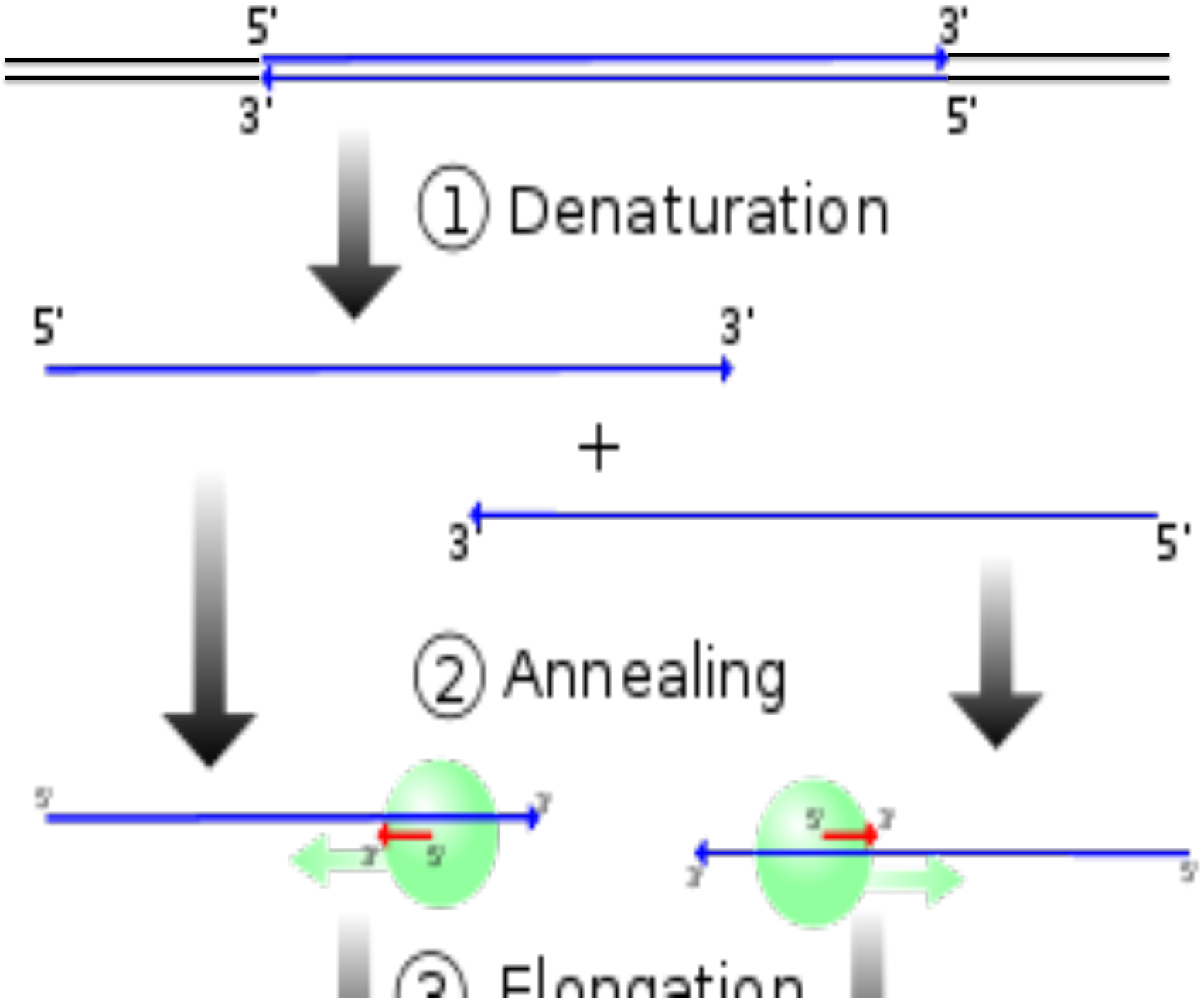
www.medicinexplained.blogspot.com

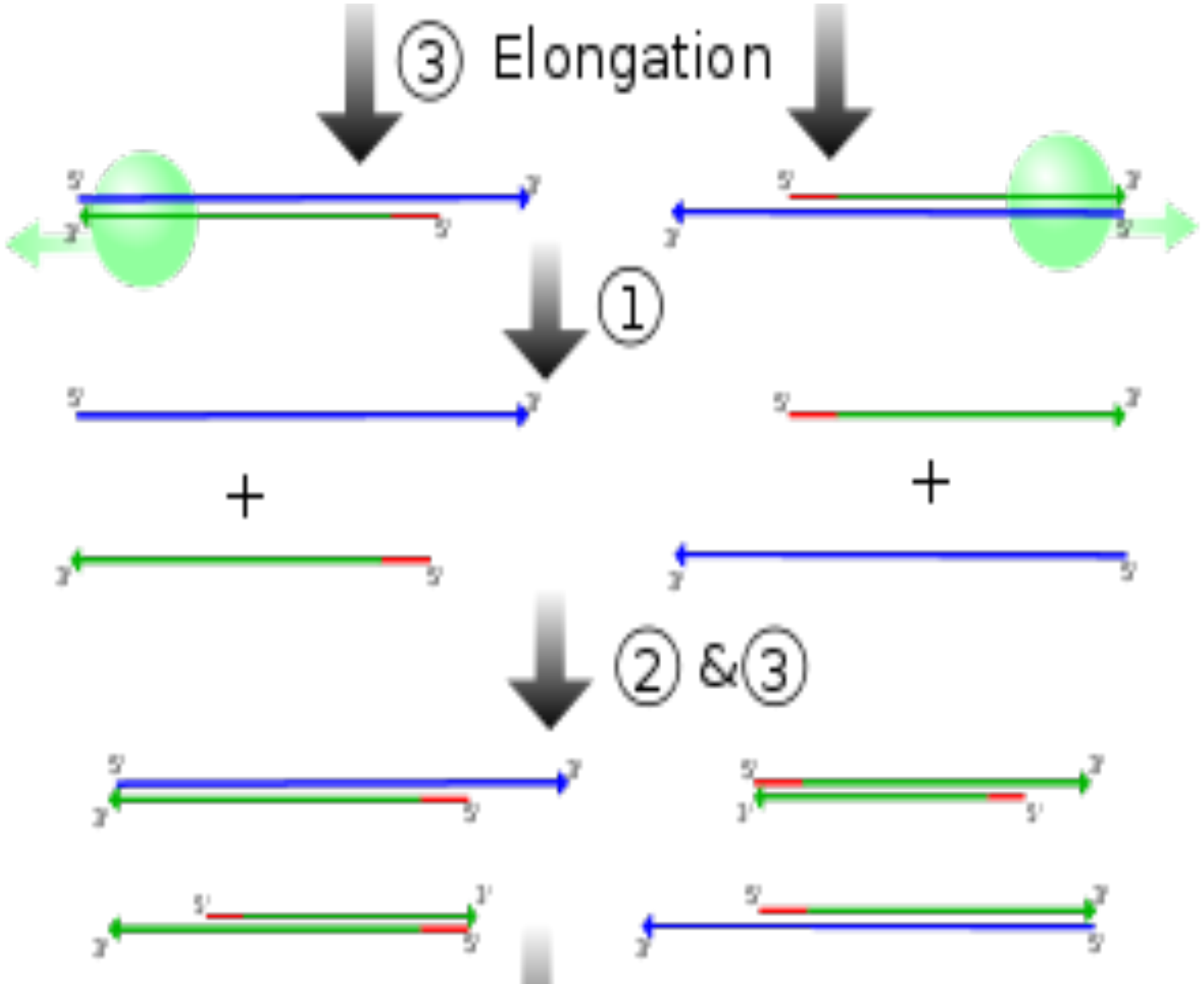
With temperature now…

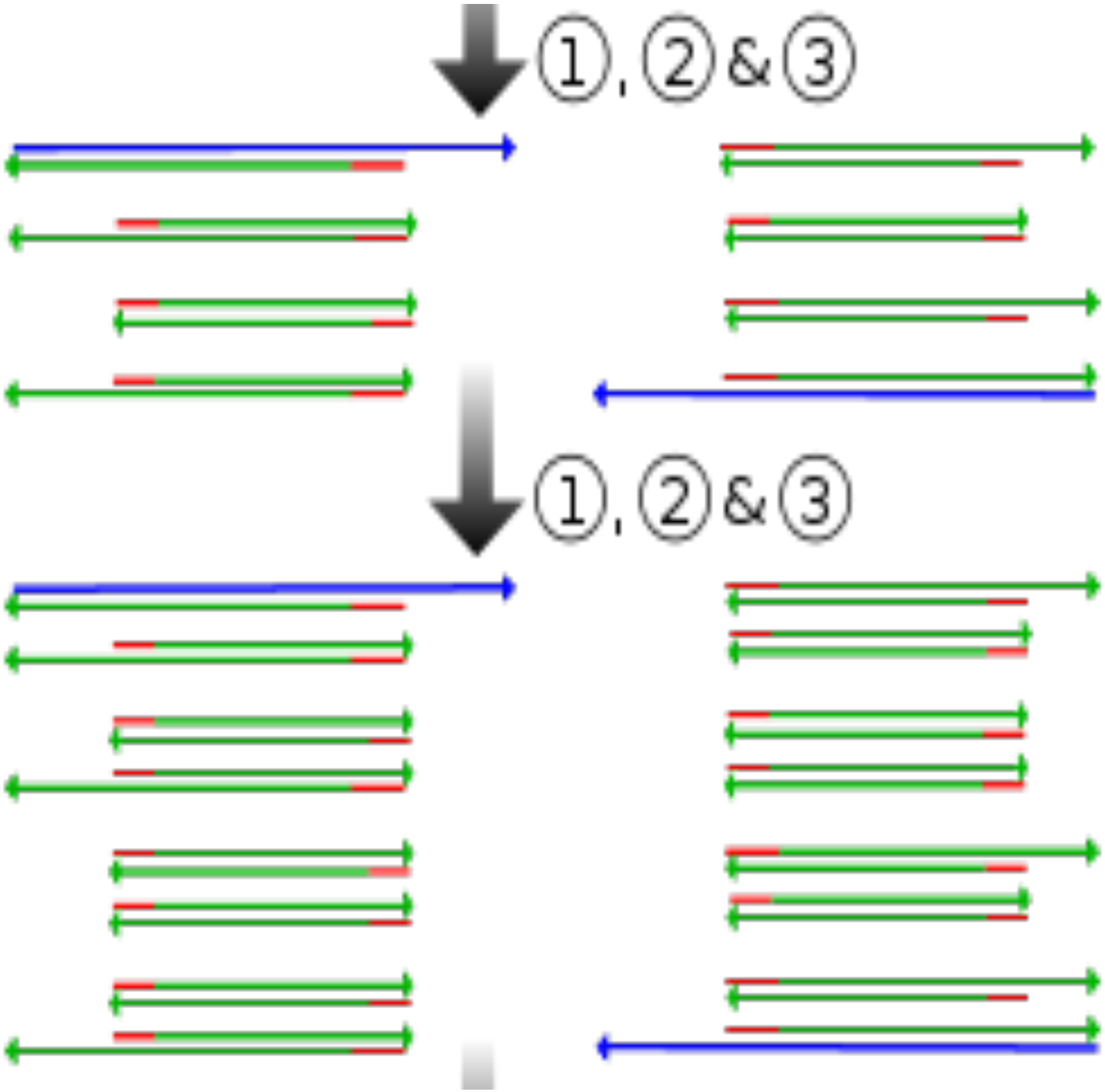


www.neb.com

Wikipedia.com




③ Elongation
①
②&③


①, ② & ③
①, ② & ③

Primer Design

Specificity

Yield

Length, Tm, Annealing, GC content,
GC clamp, 2ary structure, 3' end

Primer Design - length

- Hybridization stability or Tm
  - Itakura or Wallace rule
  - Tm = 2(A+T) + 4(G+C)
  - Good from ~45-70 F
  - Location of mismatches matter!
- Template complexity
  - If each base is equally likely, then for 12mer $(1/4)^{12}$ ~ 6e-8
  - How likely in bacteria? Human? Messy sample?

- Possible mismatching
  - Not every sequence target is known.

Primer Design – 2° structure

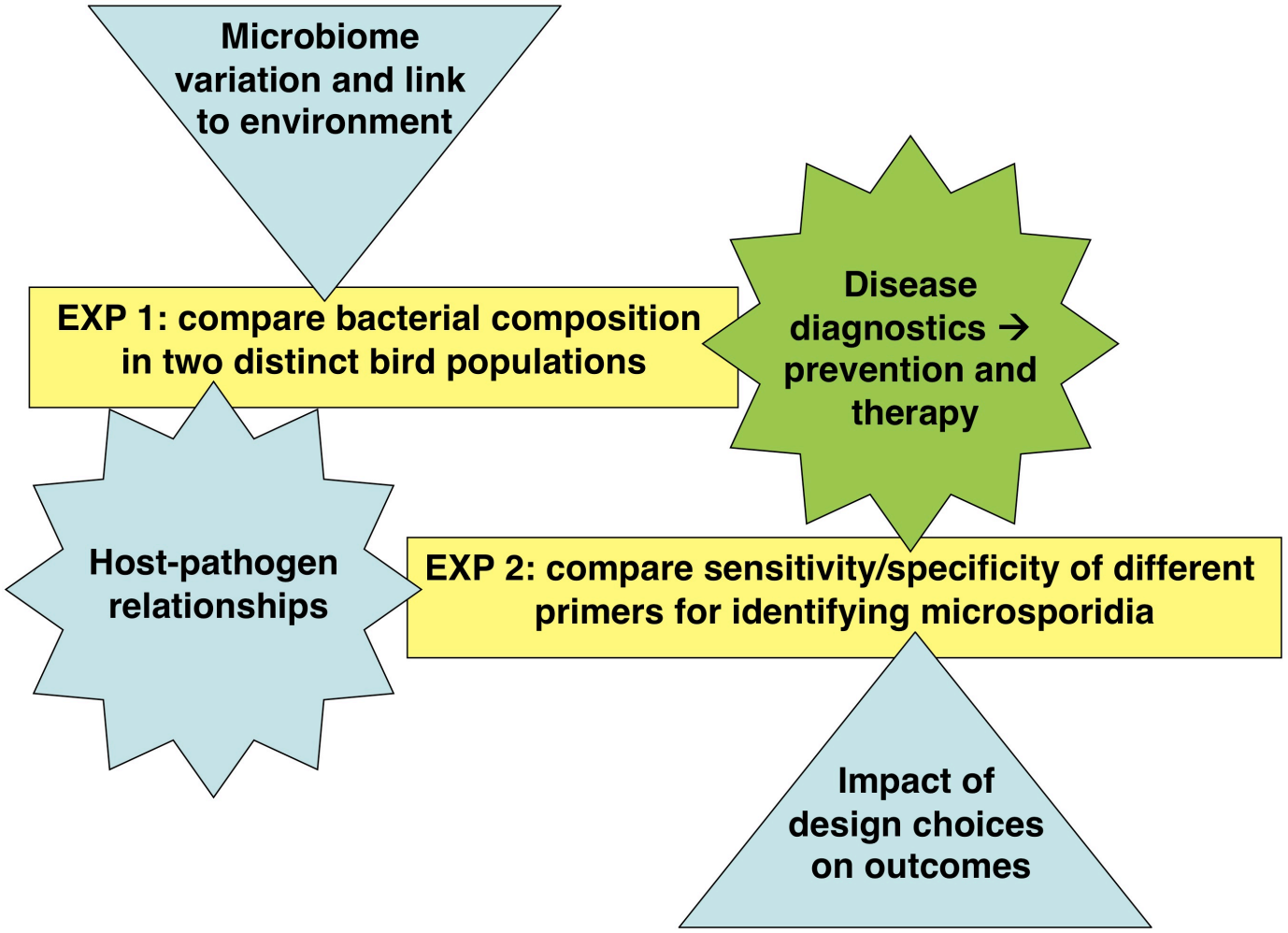
- Hairpins
  - Particularly bad at 3' end
  - ΔG < -3 kcal/mol may be tolerated
- Self-dimers
  - Homologous to self, 3' end worse
  - ΔG < -5 kcal/mol may be tolerated
- Cross-dimers
  - Sense/antisense pair

→ All reduce primer availability, annealing

Primer Design – Other considerations

- Things to aim for:
  - 52 < Ta < 58 (Tm 55-80)
  - TaOpt = 0.3 x (Tm primer/pr) + 0.7 (Tm product) – 14.9
  - Matched primer Tm (within 5 degrees)
  - Mixed content, *GC* clamp?
- Things to avoid:
  - Repeats
  - Runs
  - Excess 3' end stability, avoid 3+ *Gs*, *Cs*
    - Mispriming in *GC* rich zones, stability

DNA engineering: investigating pathogens



Lecture

- Jim Collins
- Dr. Arup Chakraborty is launching the new IMES speaker series and is hosting our first guest lecturer in early February.
- **Thursday, February 7, 2013**
- **Pre-lecture reception: 5-5:30 pm**
- **Lecture: 5:30-6:30 pm**
- **E25-111**
- **Guest Lecturer: Professor J.J. Collins**
- Howard Hughes Medical Institute; Dept. of Biomedical Engineering, Boston University; Wyss Institute for Biologically Inspired Engineering, Harvard University
-
- **Topic: "Network Biology Approaches to Microbial Threats"**
- In this talk, we will highlight recent work in synthetic biology and systems biology aimed at elucidating the mechanisms of action of antibacterials and bacterial responses to antibiotic treatment. We discuss how the insights arising from these studies can be harnessed to create more effective antibiotics and innovative antibacterial therapies to treat resistant and persistent infections.