20.109
Laboratory Fundamentals in Biological Engineering

Module 1
Nucleic Acid Engineering
Lecture 2

Module 1

Explore Microsporidia Primer design (2 strains)

→ Sensitivity

→ Specificity

Evaluate & compare with class

Sample(s)

Length
Base composition
Salt/ion
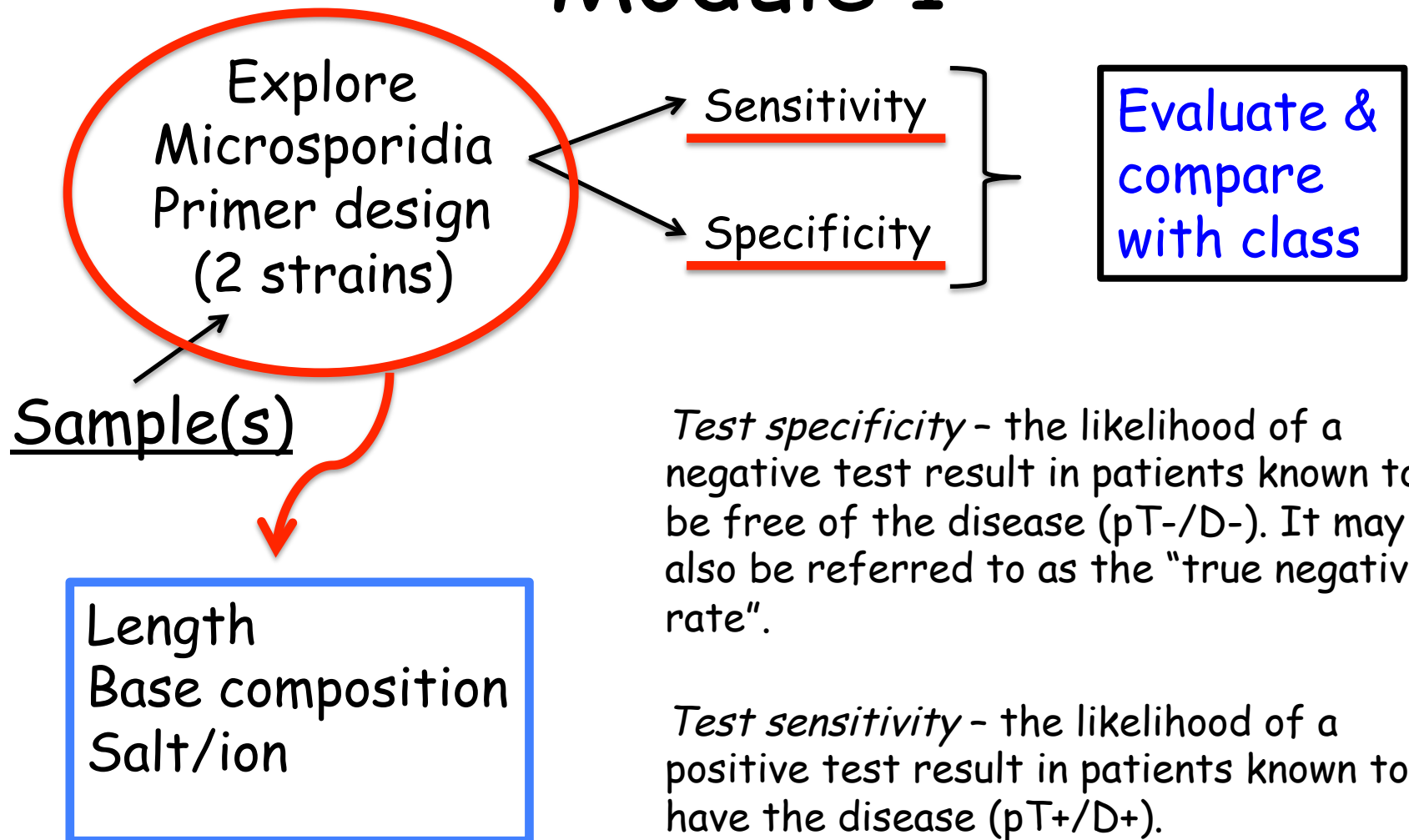
Test specificity – the likelihood of a negative test result in patients known to be free of the disease (pT-/D-). It may also be referred to as the "true negative rate".

Test sensitivity – the likelihood of a positive test result in patients known to have the disease (pT+/D+).
Also called the "true-positive rate" or "operational sensitivity".

Primer Design – Additional Thoughts

- ## Hairpins
  - Particularly bad at 3' end
  - $\Delta G < -3$ kcal/mol may be tolerated

- ## Self-dimers
  - Homologous to self, 3' end worse
  - $\Delta G < -5$ kcal/mol may be tolerated

- ## Cross-dimers
  - Sense/antisense pair

→ All reduce primer availability, annealing

Primer Design – Additional thoughts

- Degenerate Oligos may be useful
  - When amino acid only known
  - When only reference is known
  - When variation is known
- Oligo synthesis is not perfect
 - Unless HPLC or PAGE purified, 98% efficient

Primer Design – Additional thoughts

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- Oligo synthesis is not perfect
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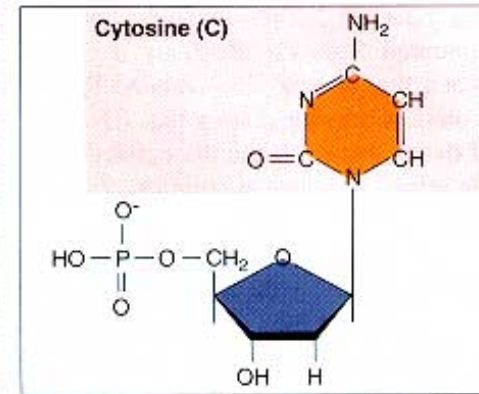 - $(0.98)^{oligolength}$ = % with correct sequence
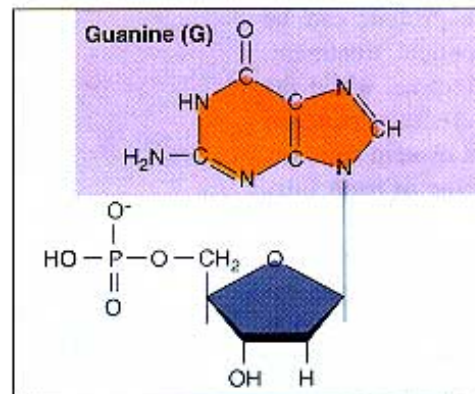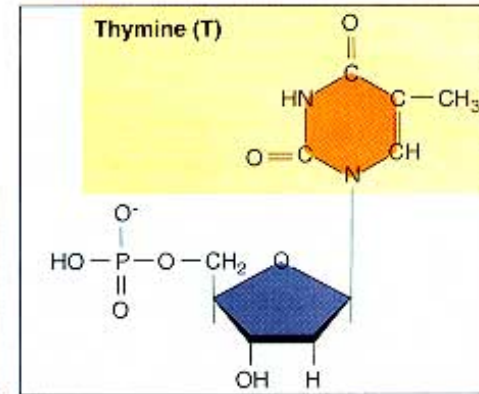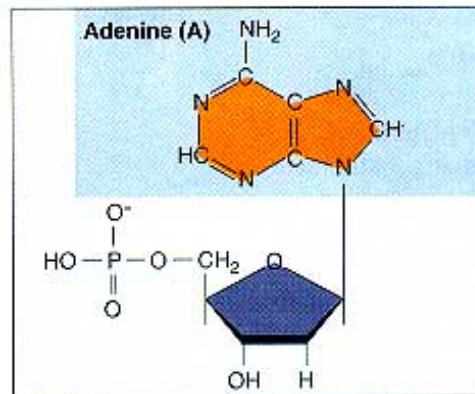 - 20mer = 66.7%

Oligonucleotide synthesis

- But first, structure

- What is the structure of a nucleotide base? Can you draw it?
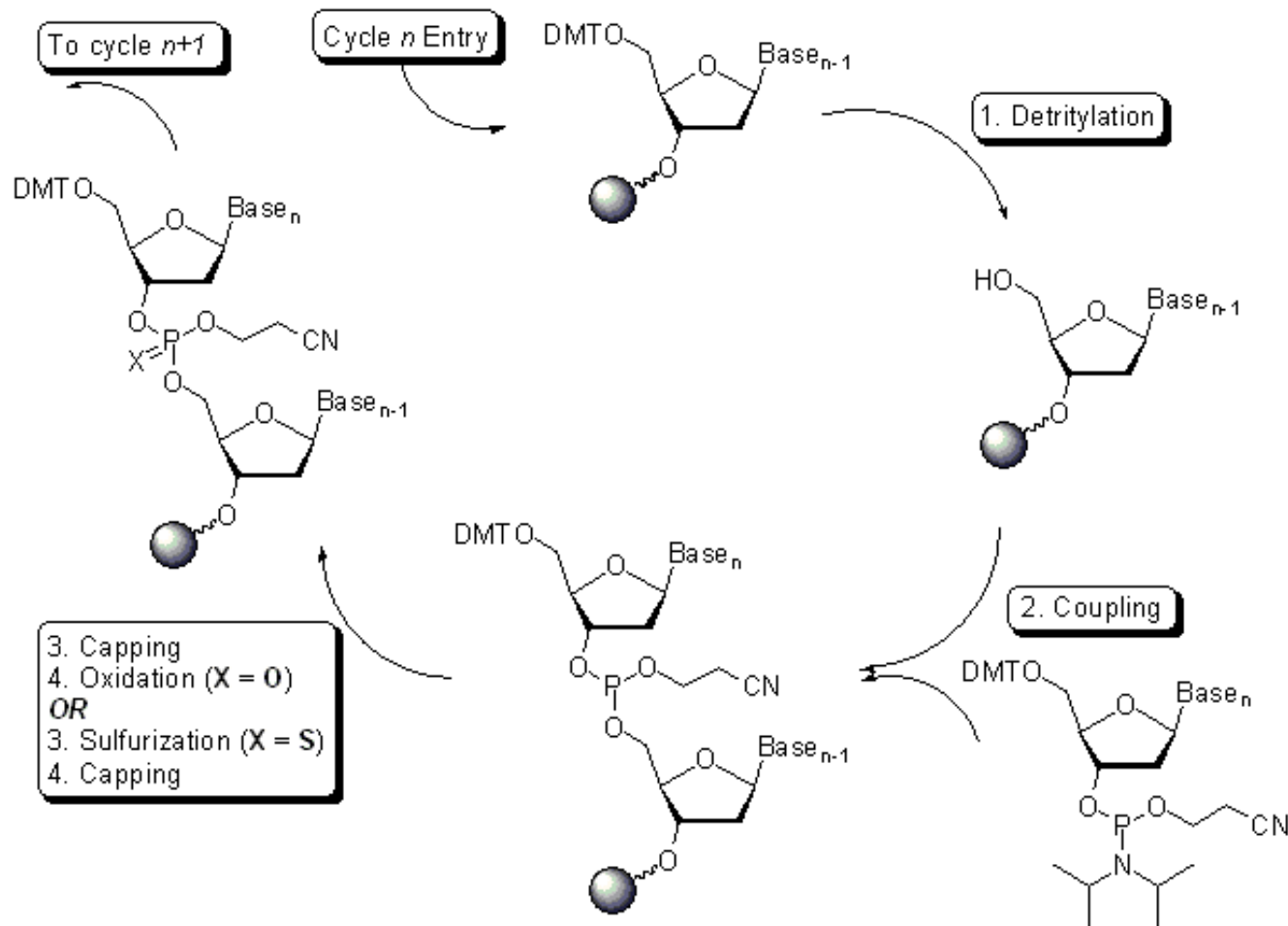
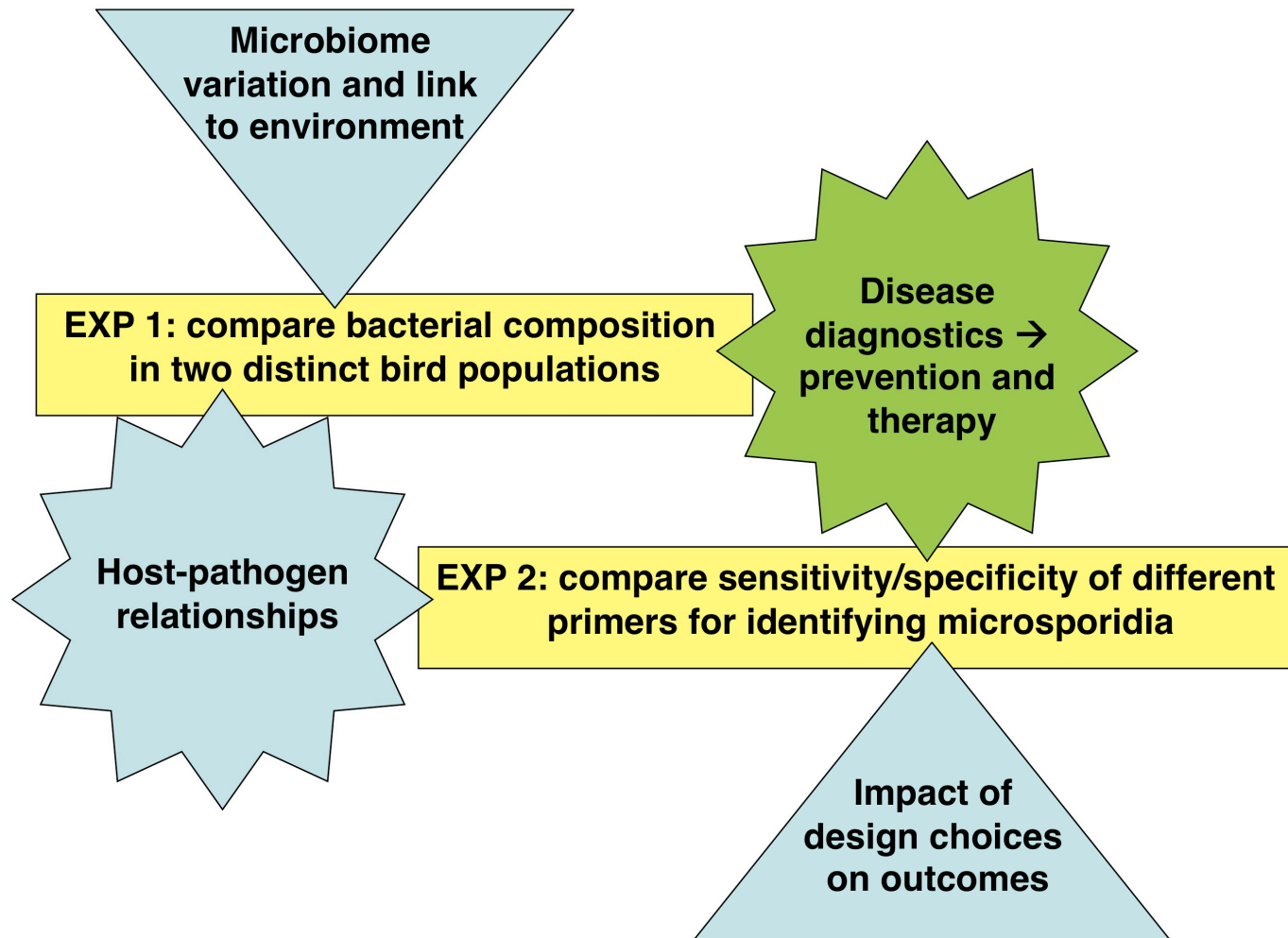# Nucleotide structure
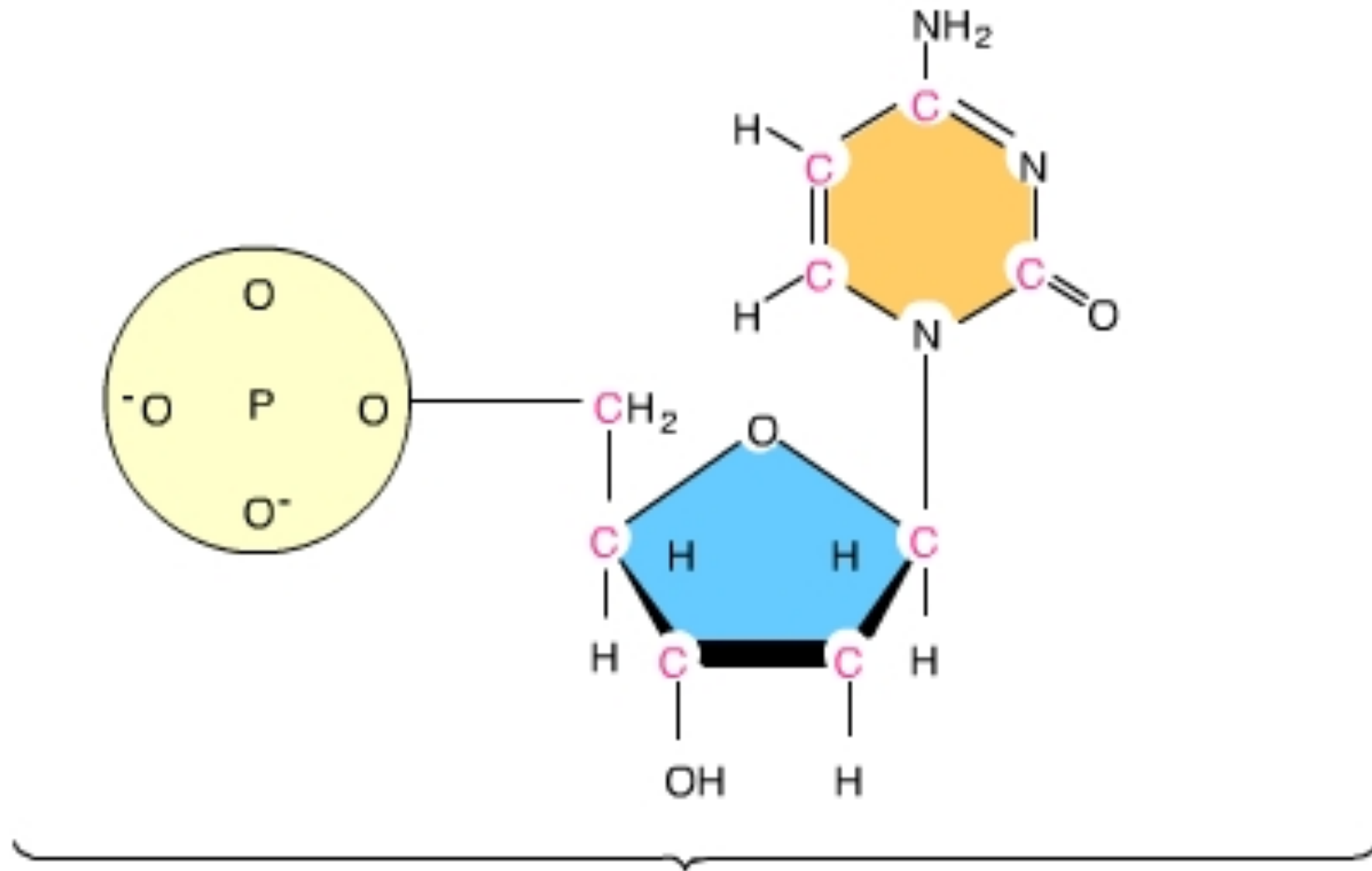
Nucleotide structure

The four nucleotide subunits that make up DNA

The nucleotide subunits of DNA are composed of three elements: a central five-carbon sugar, a phosphate group, and an organic, nitrogen-containing base.

Oligonucleotide synthesis

www.wikipedia.org

DNA engineering: investigating pathogens

Microbiome variation and link to environment

EXP 1: compare bacterial composition in two distinct bird populations

Disease diagnostics → prevention and therapy

Host-pathogen relationships

EXP 2: compare sensitivity/specificity of different primers for identifying microsporidia

Impact of design choices on outcomes

Microsporidia are a new disease

- Obligate intracellular
- Fungi
- Diverse taxonomic distribution (insects, fish, mammals, other?)
- Unknown impact overall
- 15-45% of AIDS with unknown diarrheal etiology
- 90% are E. bieneusi, other mostly E. intestinalis
- Varied infection strategies

Microsporidia life cycle

Intracellular development of *E. bieneusi* and *E. intestinalis* spores.

Enterocytozoon bieneusi

*Encephalitozoon intestinalis**

www.cdc.gov
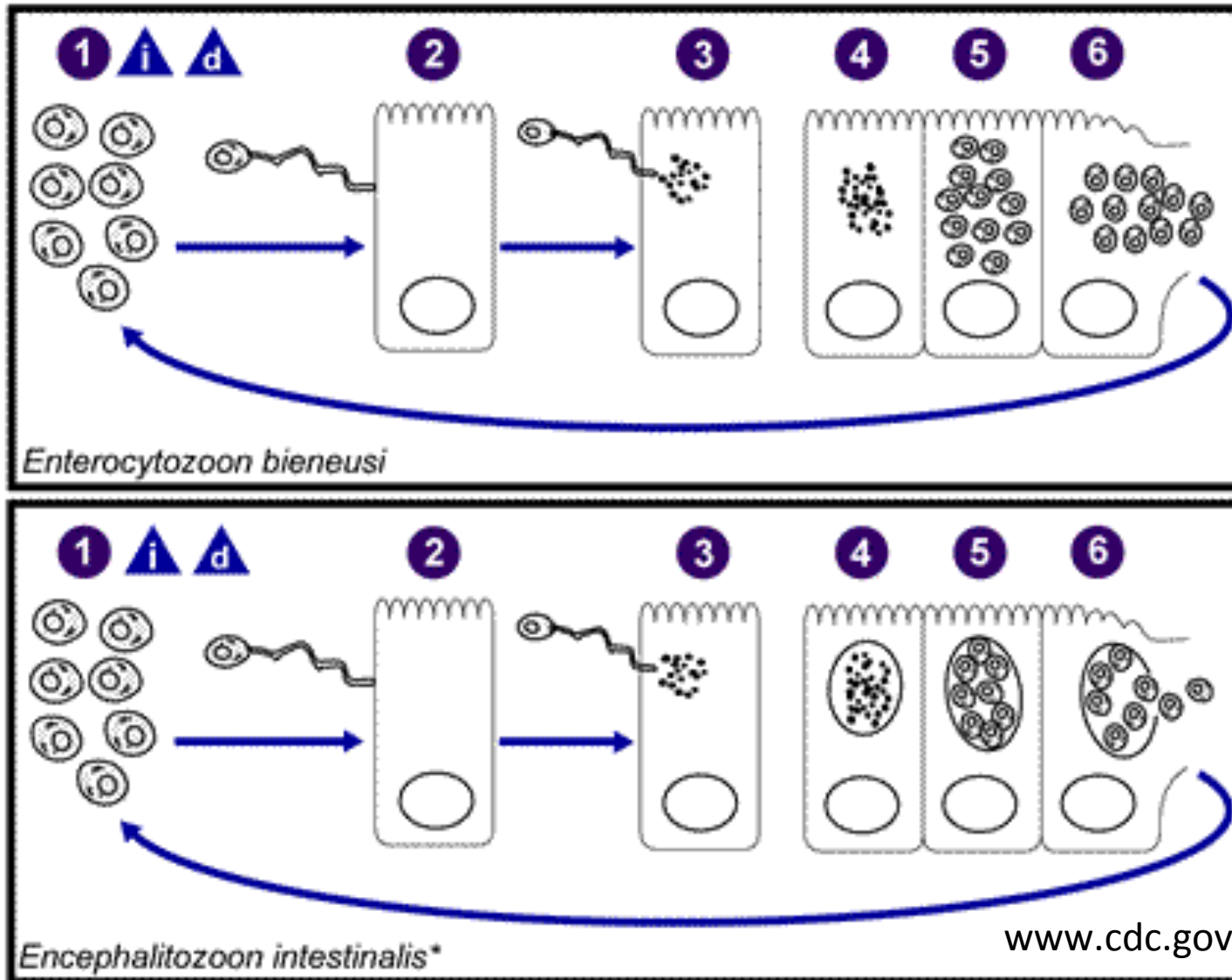
*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.
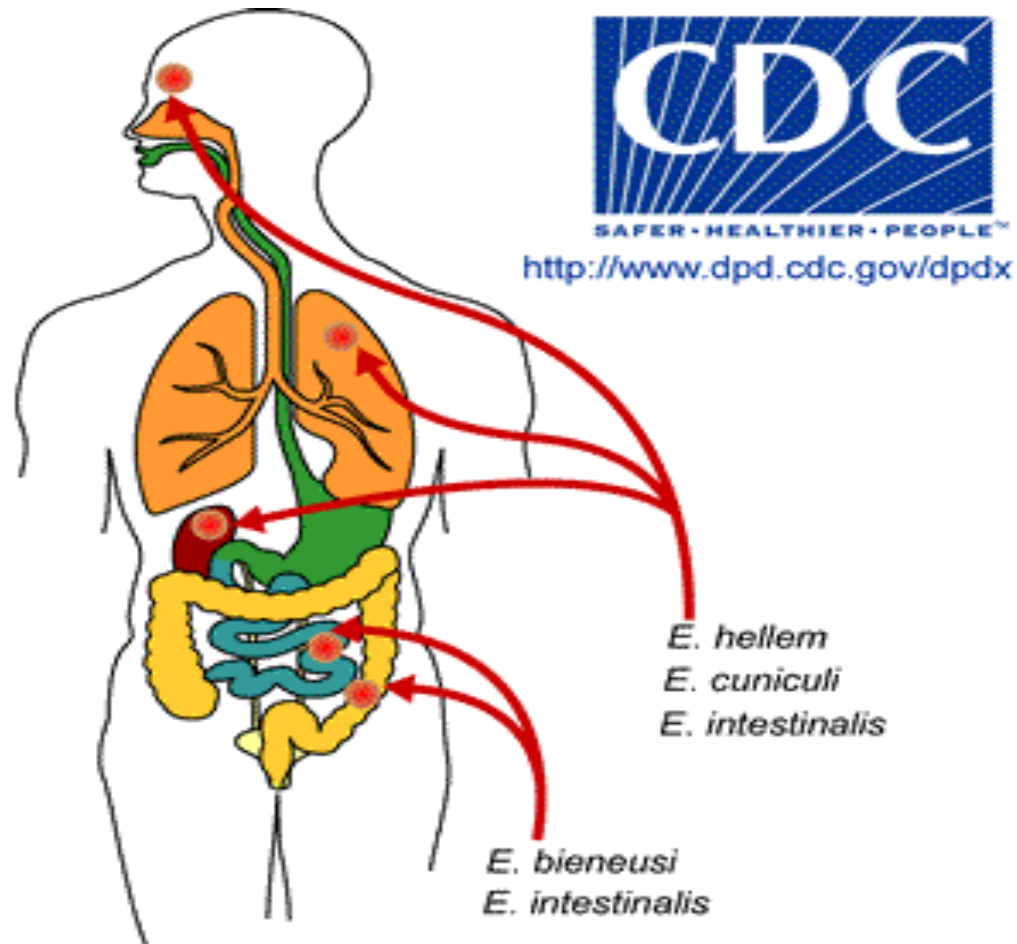
Microsporidia – human infection

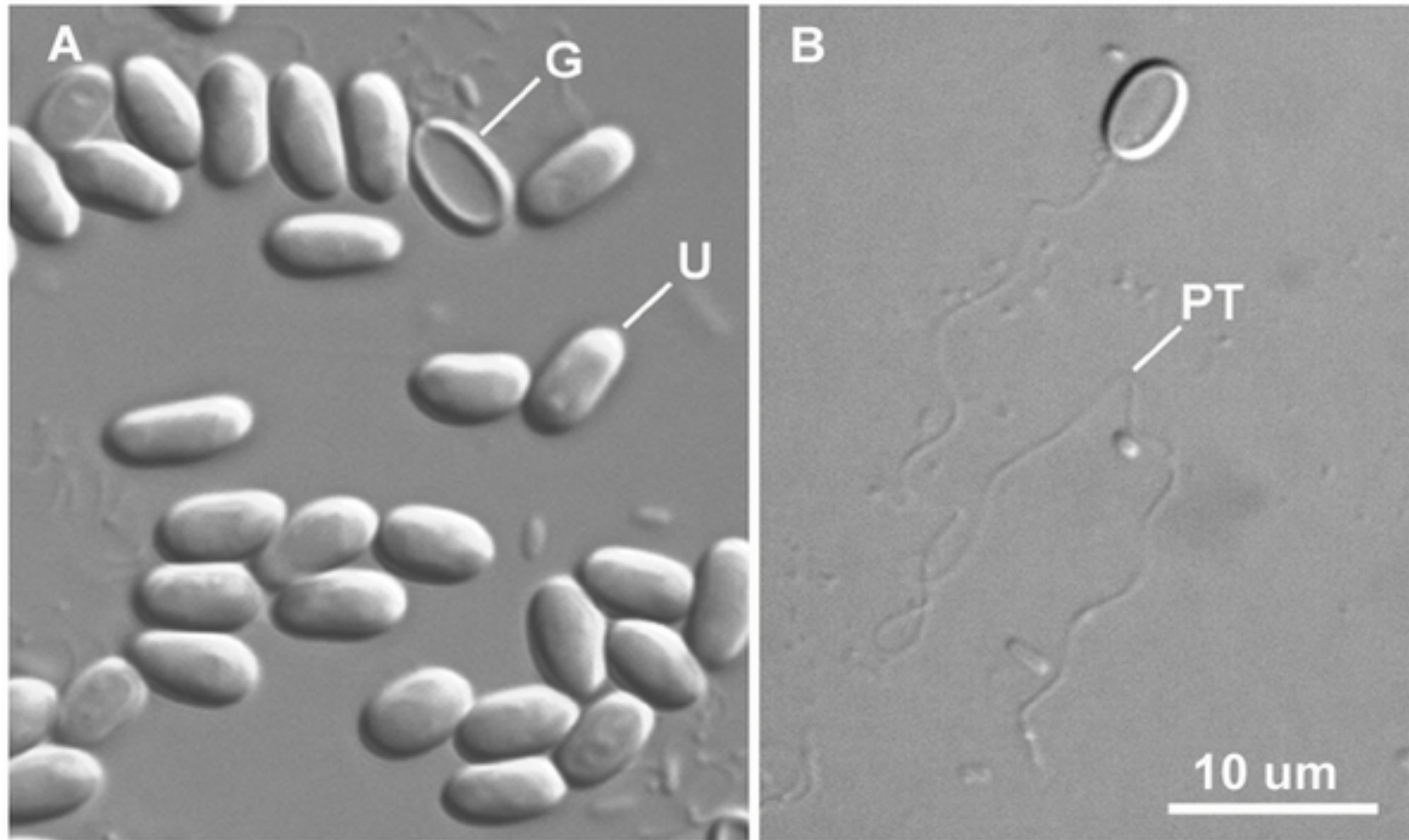**Figure 1. Light micrograph of Antonospora locustae with pressure-induced polar tube eversion.**

Table 2. Estimates of the number of N. ceranae genes that are homologous or orthologous to genes in the microsporidian E. cuniculi and yeast.

Species	Gene category*	Relative to E. cuniculi	Relative to S. cerevisiae
N. ceranae	Homologous	1,366	700
	Orthologous	1,252	466
E. cuniculi	Homologous		740
	Orthologous		511

*See text for assignment method.
doi:10.1371/journal.ppat.1000466.t002

PLOS | PATHOGENS

Clinical Signs of Microsporidiosis
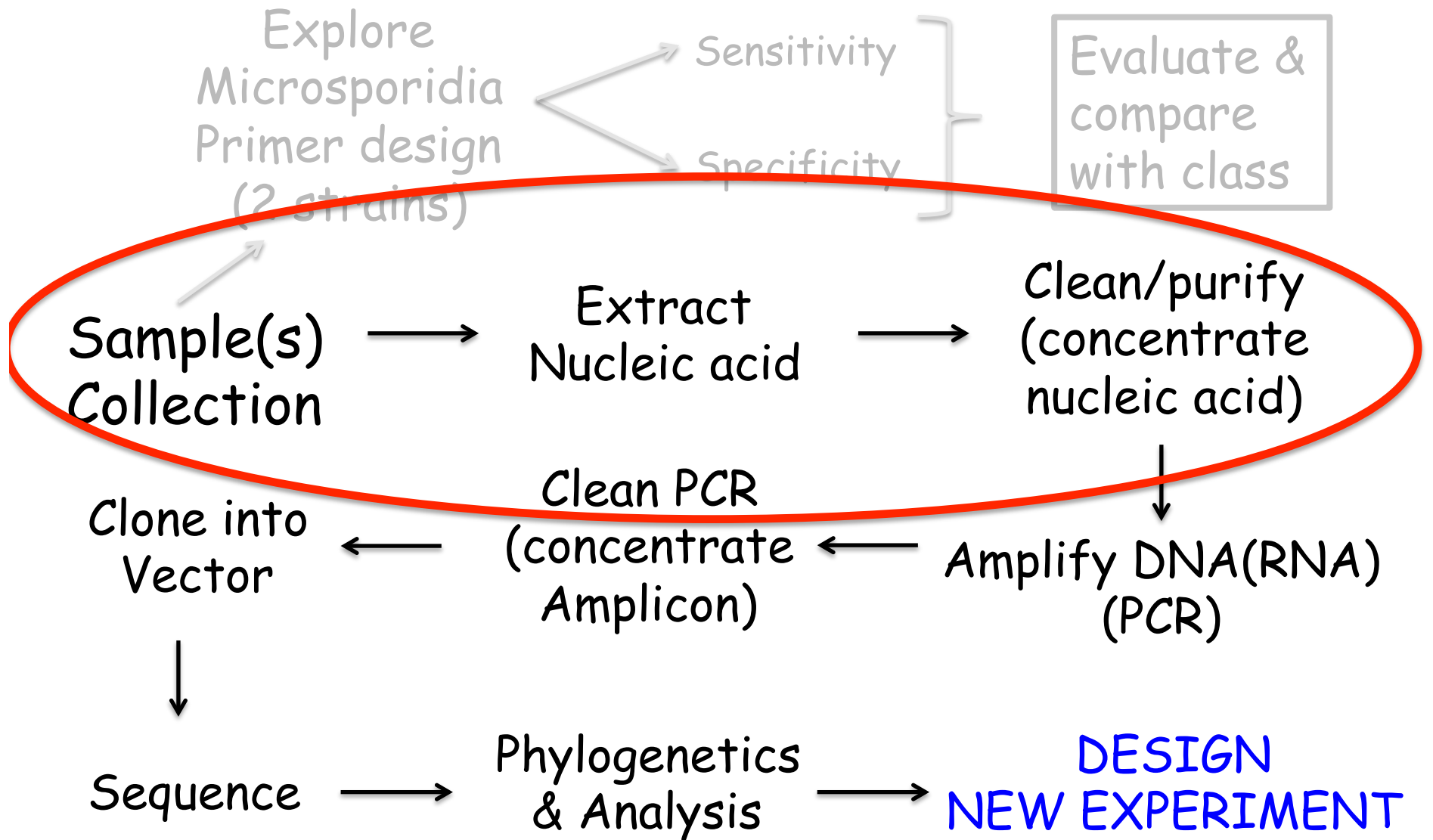
- Chronic diarrhea and wasting
  but different species invade different sites (the cornea, urinary track and muscles).

- Disseminated - cholecystitis (inflammation of the gallbladder), renal failure, respiratory infection, headache, nasal congestion, ocular pain and sinus involvement.

- Infections of the brain or other nervous tissue - seizures, headache and other symptoms.

Discovering the microbial environment

"Meet your microbes"

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)

Clone into Vector ← Clean PCR (concentrate Amplicon) ← Amplify DNA(RNA) (PCR)

Sequence → Phylogenetics & Analysis → DESIGN NEW EXPERIMENT

Microbial community profiling

- What criteria make a good molecular target?

Community profiling

- # unique reads vs # total reads

- # sequences vs taxonomic units (OTUs)