Sampling and Source Tracking of *Cryptosporidium* spp. Oocysts

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Kristen L. Jellison, Ph.D.

Department of Civil & Environmental Engineering
Lehigh University
Bethlehem, Pennsylvania

Ultimate Goal of Source Tracking

- Identify important watershed sources of *Cryptosporidium* spp. oocysts
- Develop watershed management policies to prevent *Cryptosporidium* spp. contamination of surface waters

Methods

Surface Water Filtration **Fecal Sample Collection**

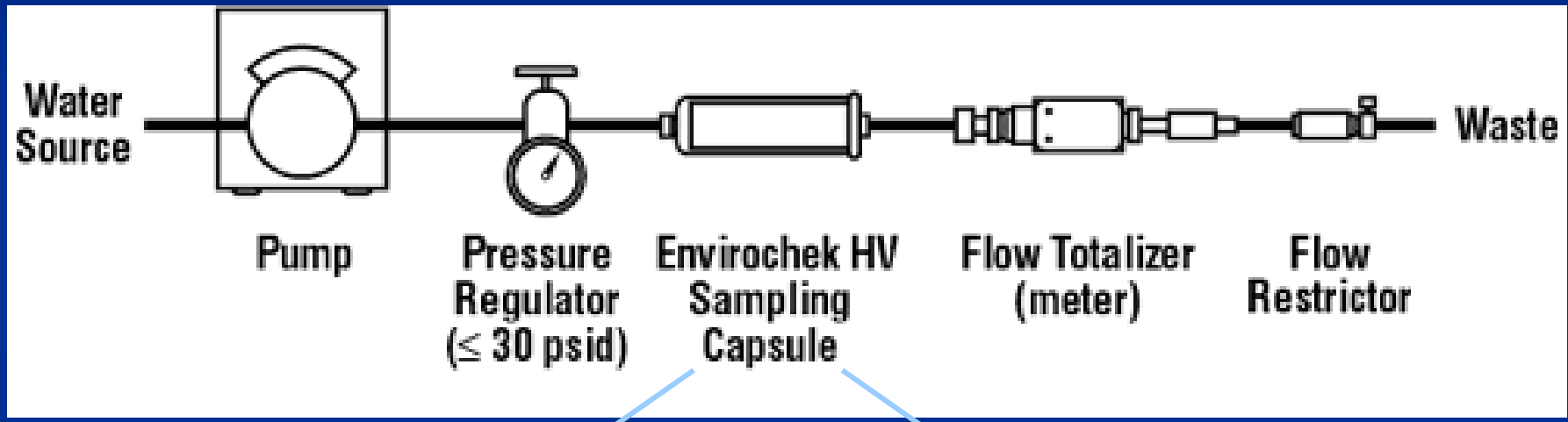
Immunomagnetic Separation

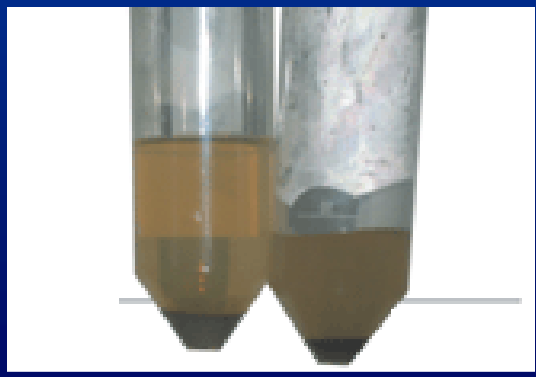
DNA Extraction

Nested PCR

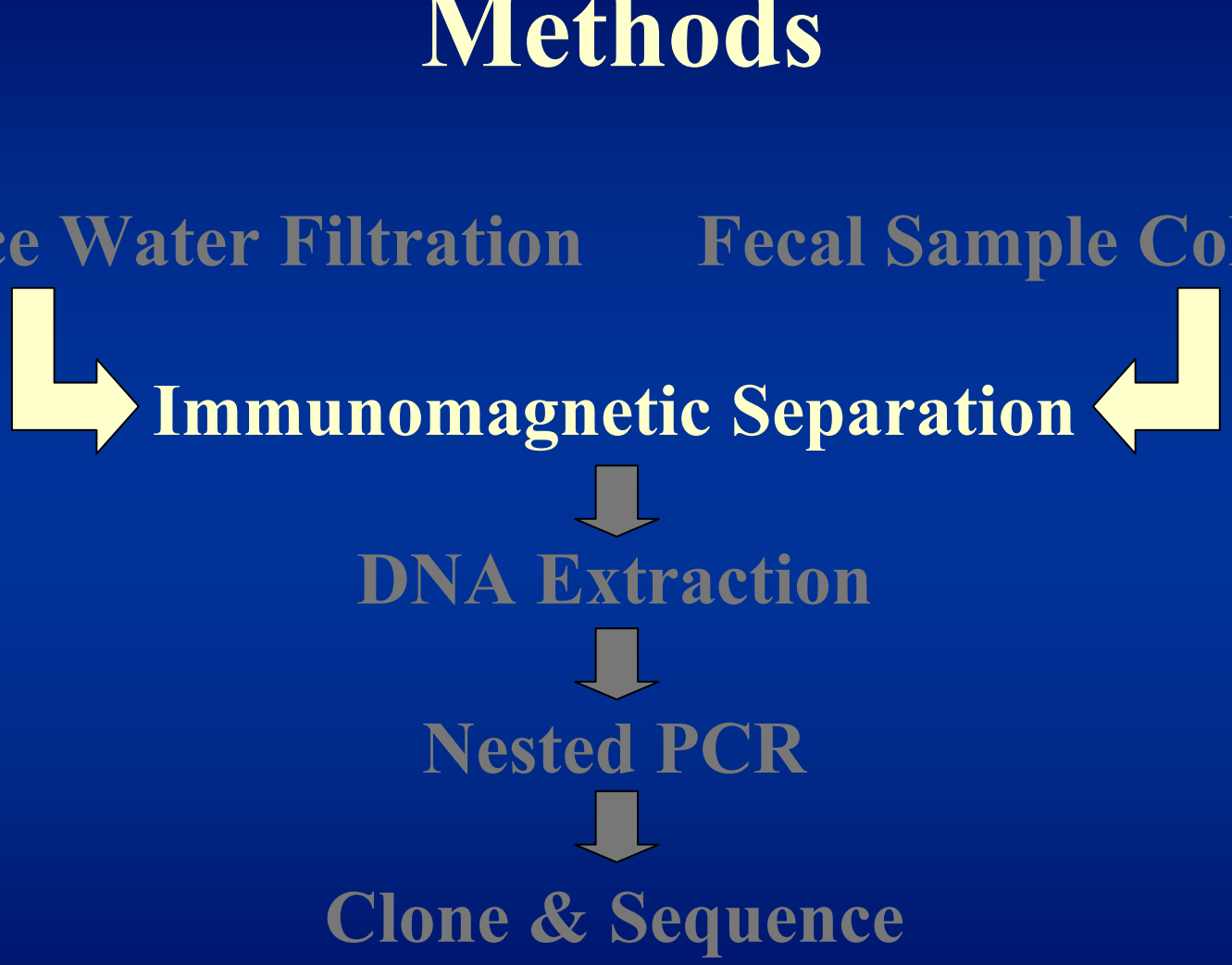
Clone & Sequence

Surface Water Filtration: Gelman Envirochek Filter Capsules





Methods


Surface Water Filtration
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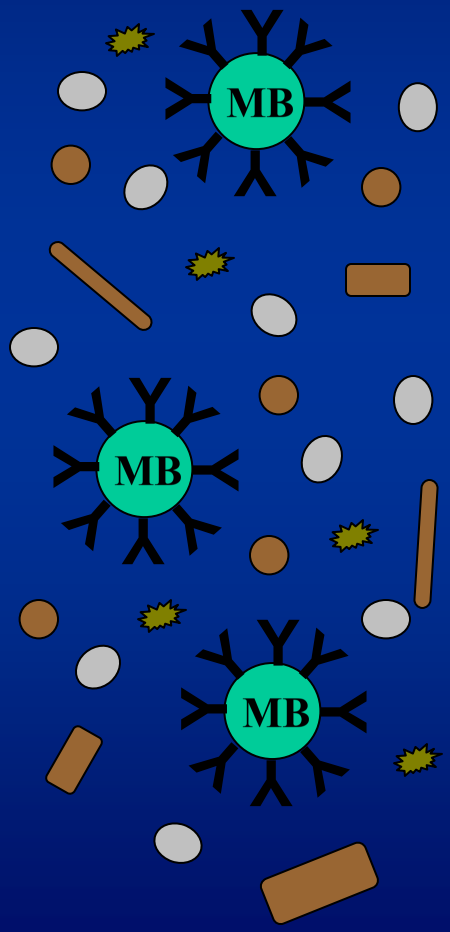
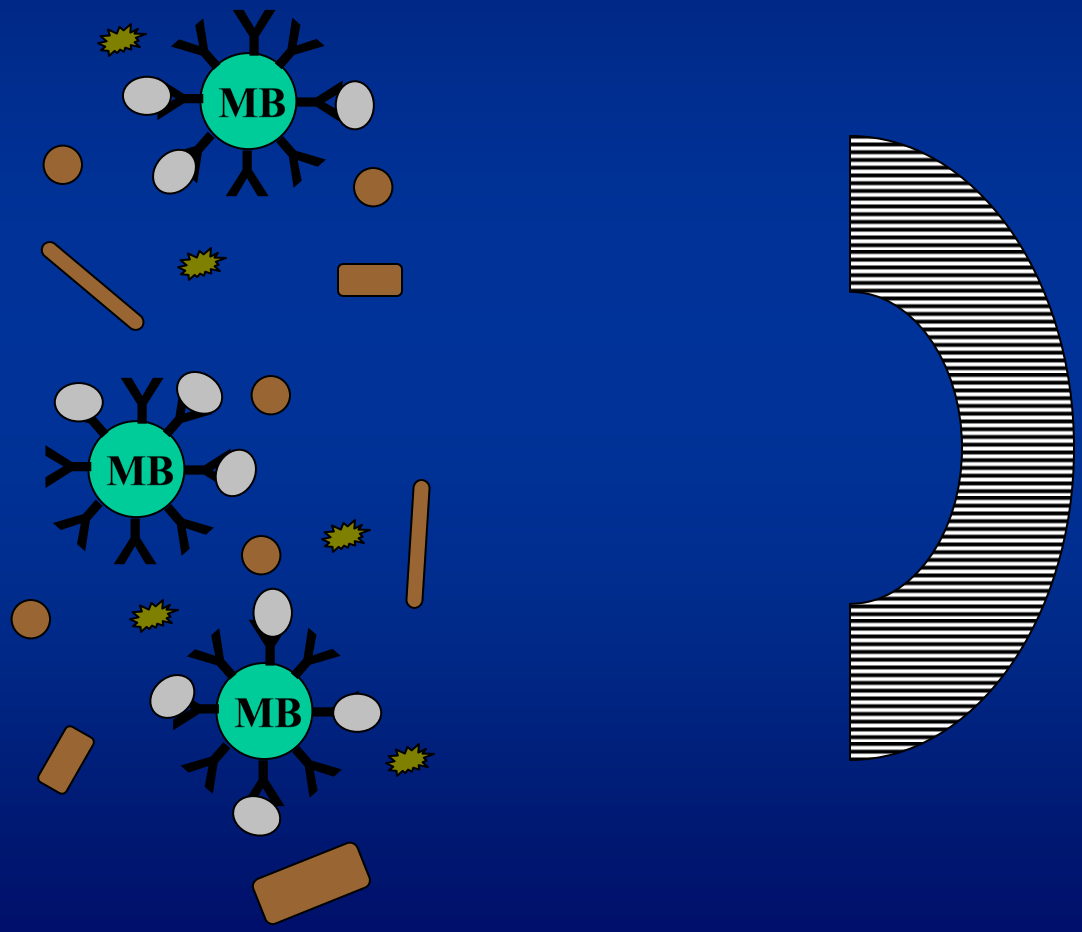
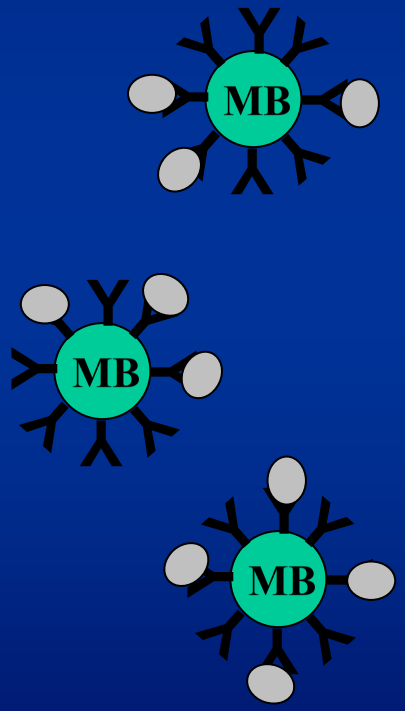
Immunomagnetic Separation



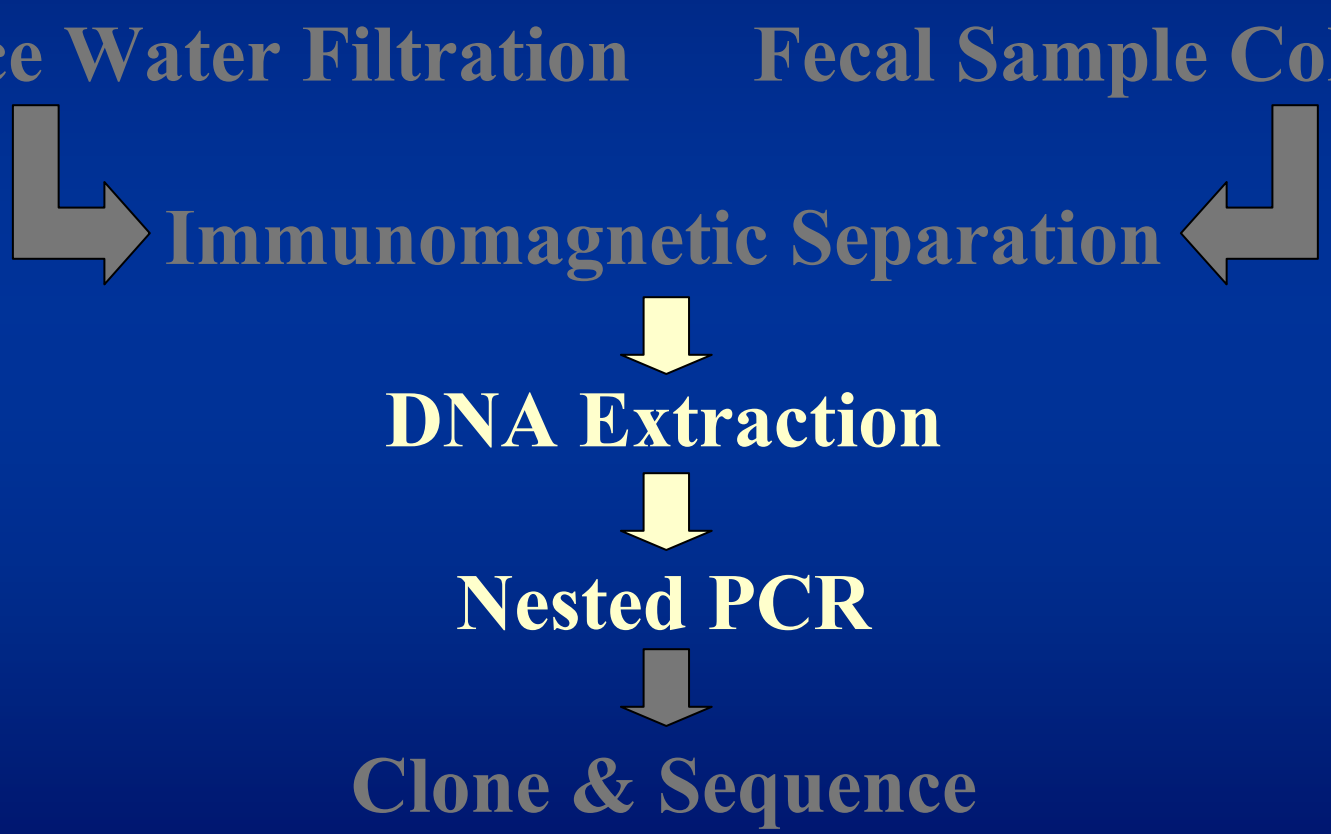
Immunomagnetic Separation



Immunomagnetic Separation



Methods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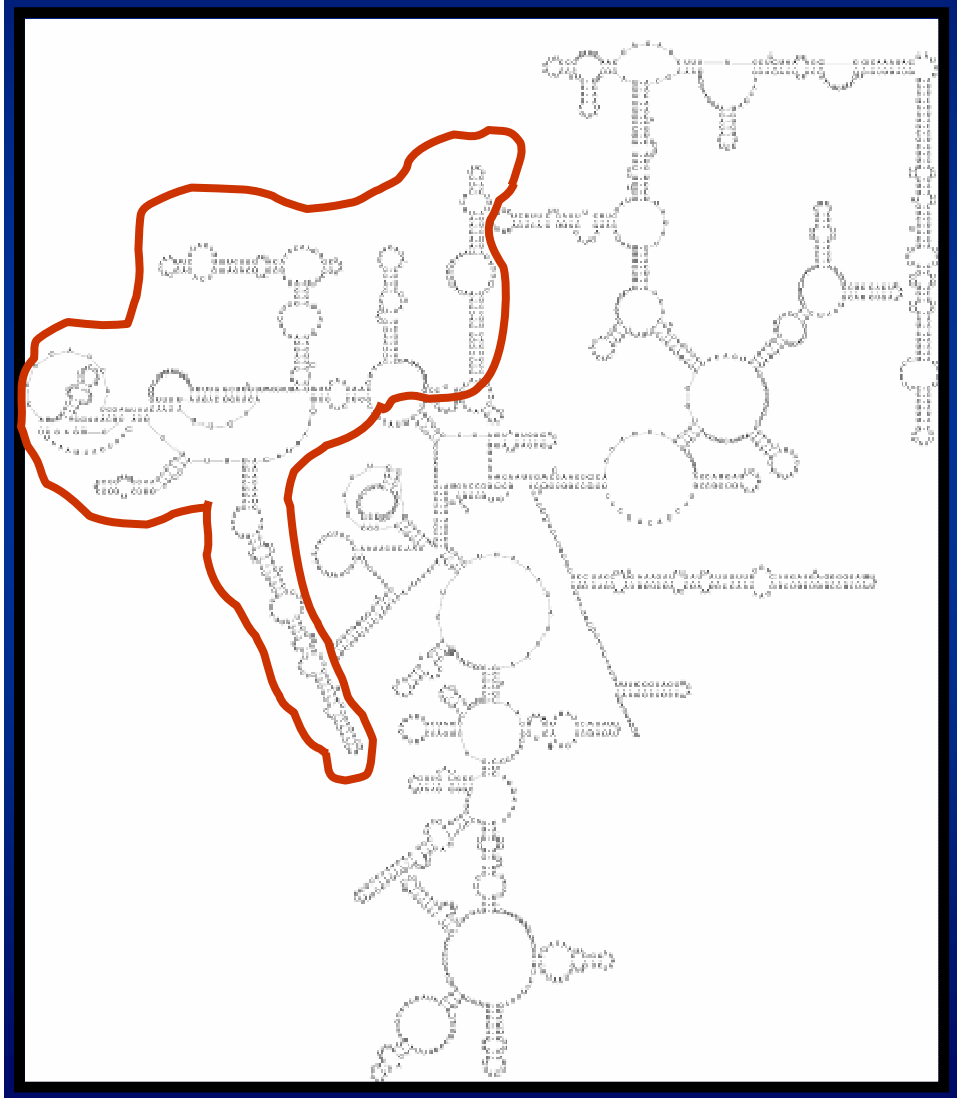
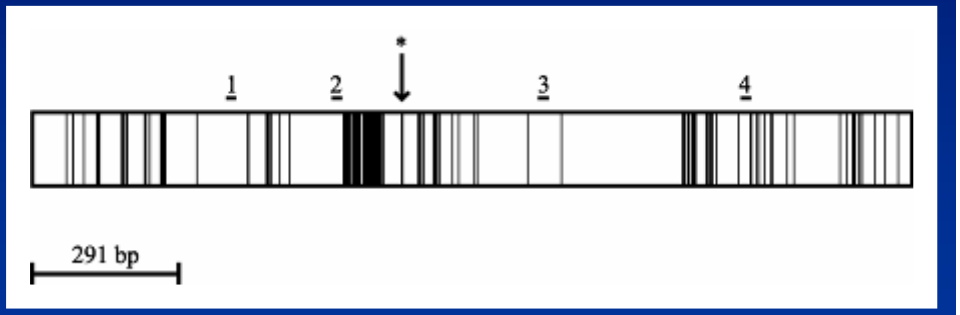
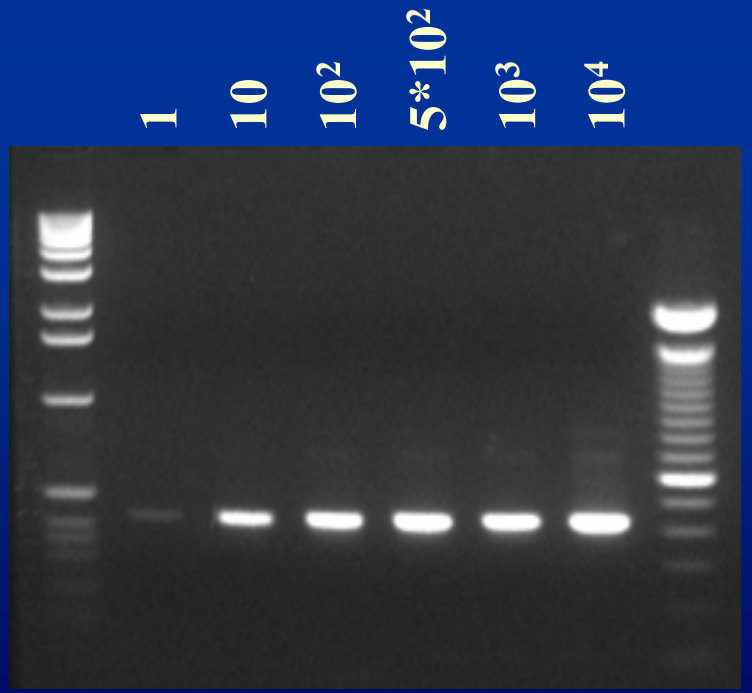
Nested PCR: 18S rRNA







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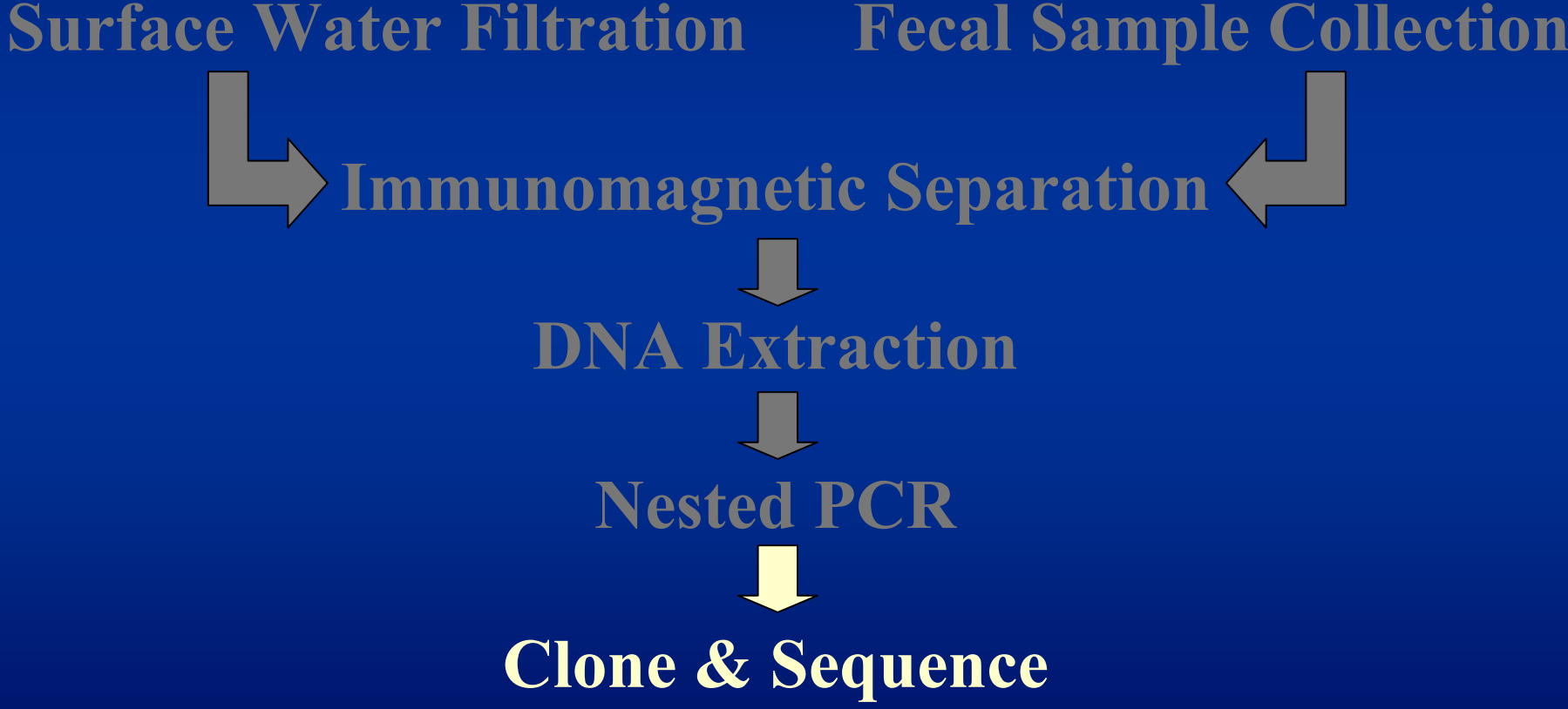
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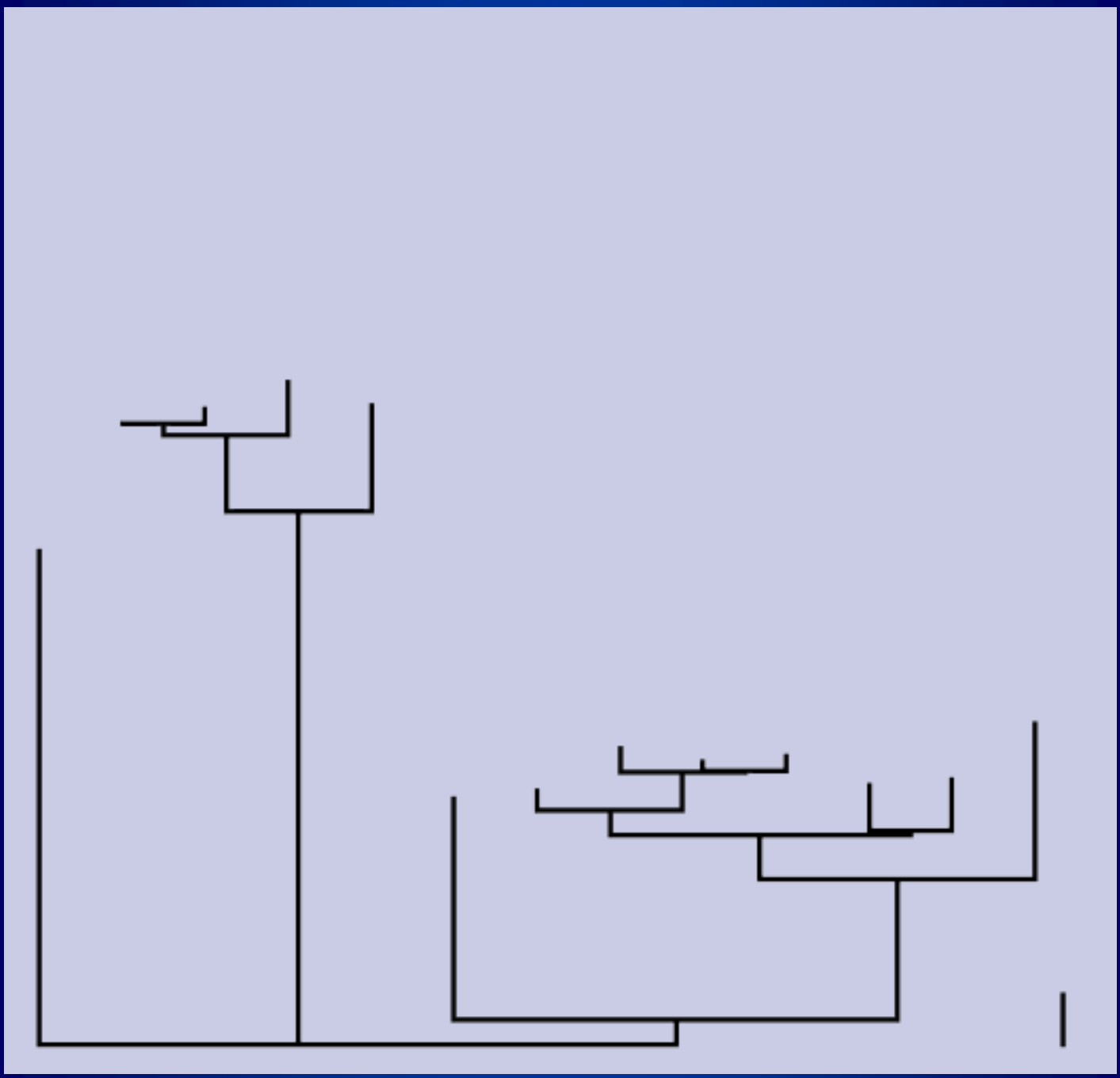
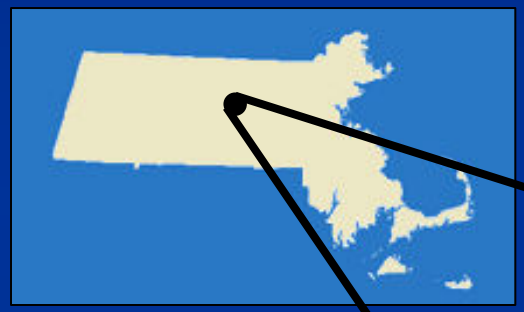
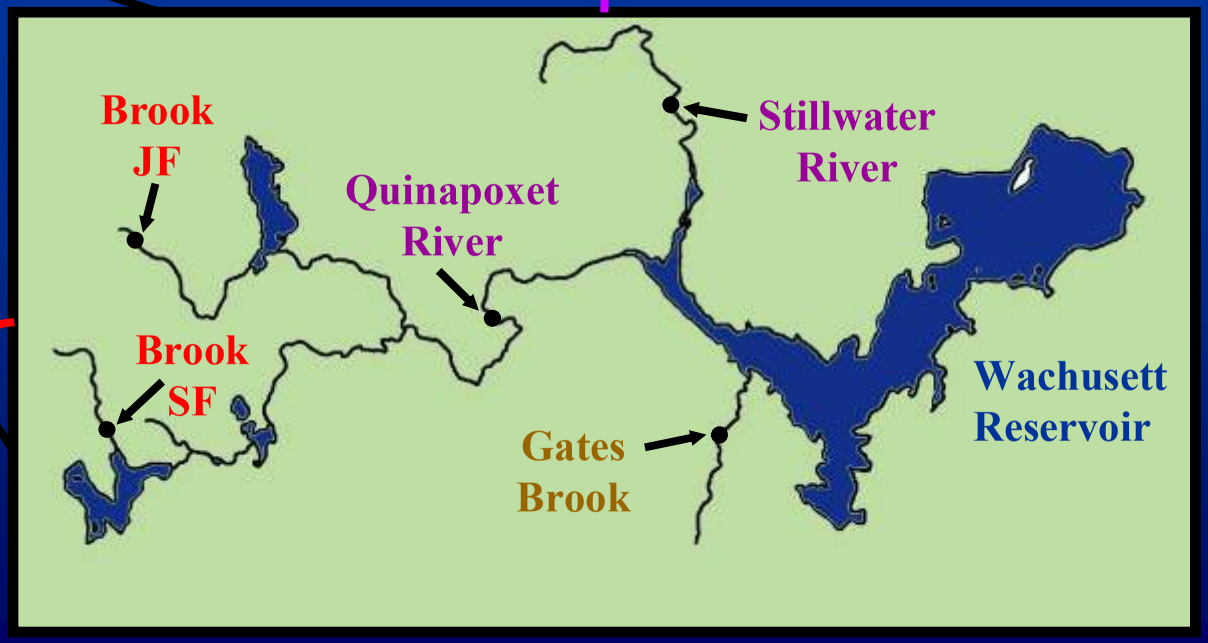
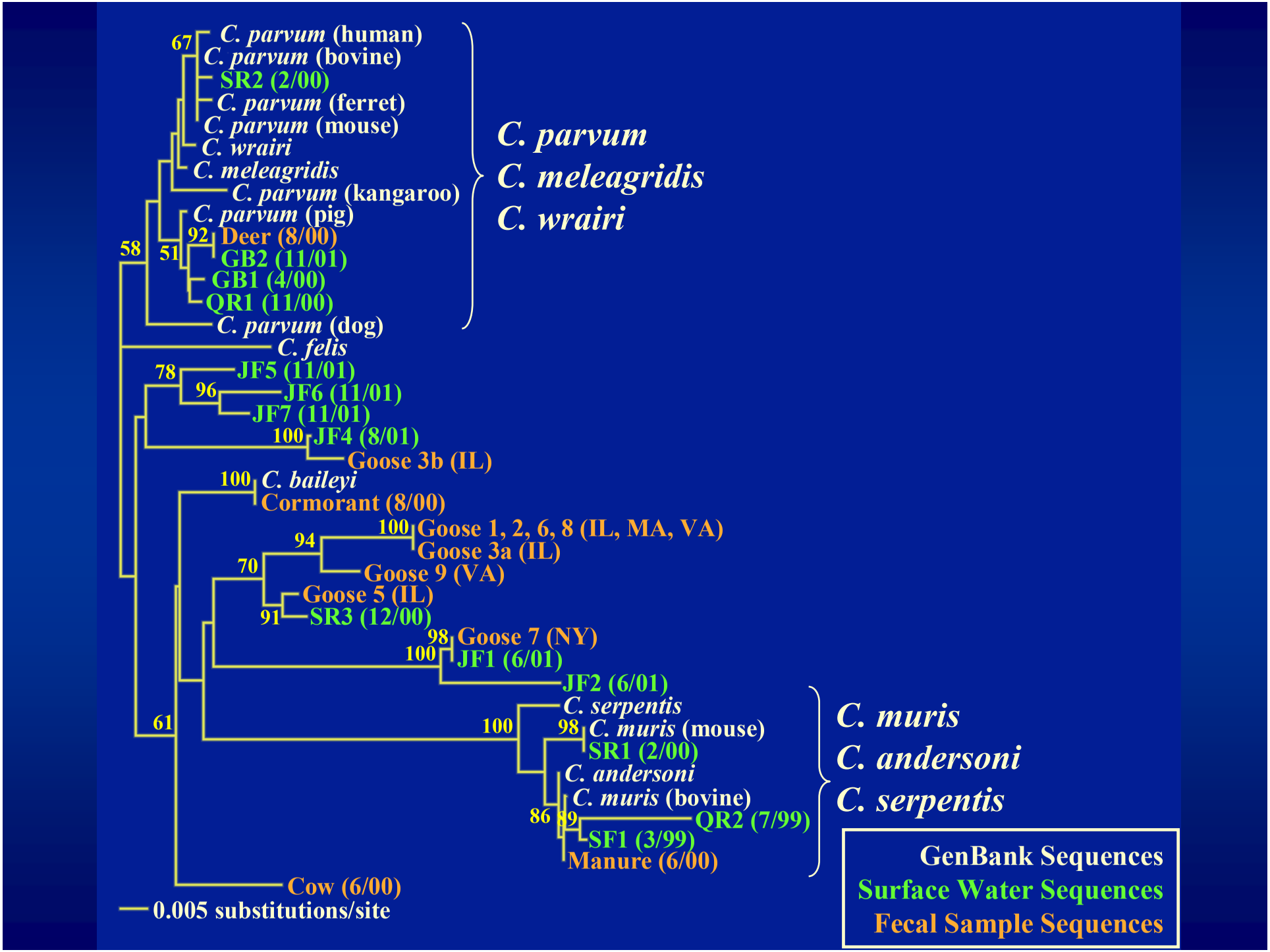


MWRA Water Supply System

Wachusett Reservoir Watershed: Surface Water Sampling Sites






C. parvum (human)
C. parvum (bovine)
SR2 (2/00)
C. parvum (ferret)
C. parvum (mouse)
C. wrairi
C. meleagridis
C. parvum (kangaroo)
C. parvum (pig)
Deer (8/00)
GB2 (11/01)
GB1 (4/00)
QR1 (11/00)
C. parvum (dog)
C. parvum
C. meleagridis
C. wrairi
C. felis
JF5 (11/01)
JF6 (11/01)
JF7 (11/01)
JF4 (8/01)
Goose 3b (IL)
C. baileyi
Cormorant (8/00)
Goose 1, 2, 6, 8 (IL, MA, VA)
Goose 3a (IL)
Goose 9 (VA)
Goose 5 (IL)
SR3 (12/00)
Goose 7 (NY)
JF1 (6/01)
JF2 (6/01)
C. serpentis
C. muris (mouse)
SR1 (2/00)
C. andersoni
C. muris (bovine)
QR2 (7/99)
SF1 (3/99)
Manure (6/00)
C. muris
C. andersoni
C. serpentis
Cow (6/00)
0.005 substitutions/site
GenBank Sequences
Surface Water Sequences
Fecal Sample Sequences

Observations: Wachusett Reservoir Watershed

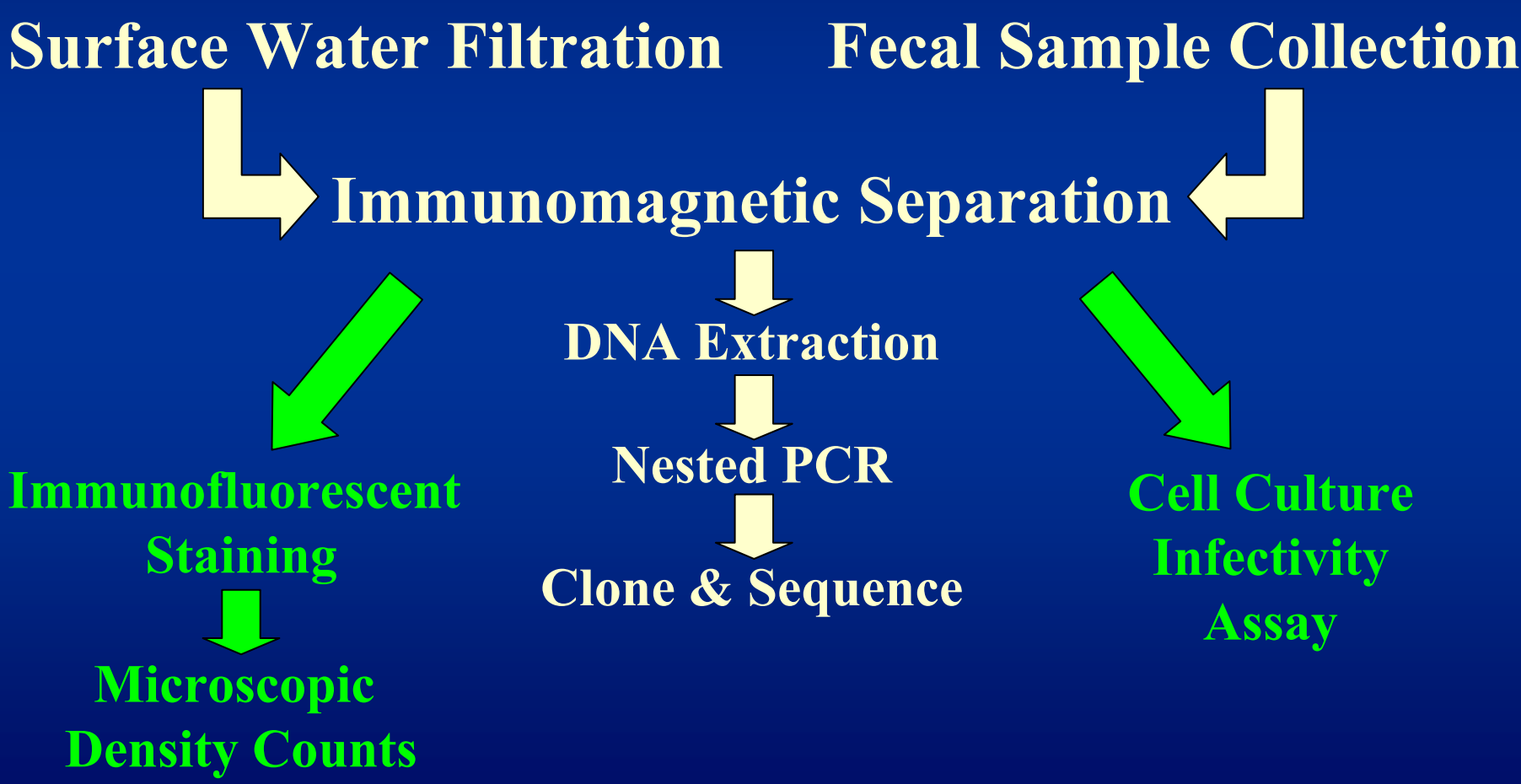
- The molecular detection method is sensitive and specific for *Cryptosporidium* spp. oocysts in water and fecal samples
- Nested PCR is required for detection of *Cryptosporidium* spp. in baseline environmental samples
- Multiple species of *Cryptosporidium* are detectable in environmental samples
- Suspected oocyst source is not always the observed source
  - Evidence of wildlife, not human, impacts on GB
  - Birds seem to be a bigger influence on JF than dairy farm activity

Method Comparison

	EPA Method 1622	Molecular Detection Method
Quantitative estimate of oocyst density	Yes	No*
Identification of oocyst infectivity	No	No*
Detection of low oocyst densities (e.g., baseline environmental samples)	No	**Yes**
Identification of oocyst genotype	No	**Yes**

*But could be determined with method modifications

Methods


Surface Water Filtration
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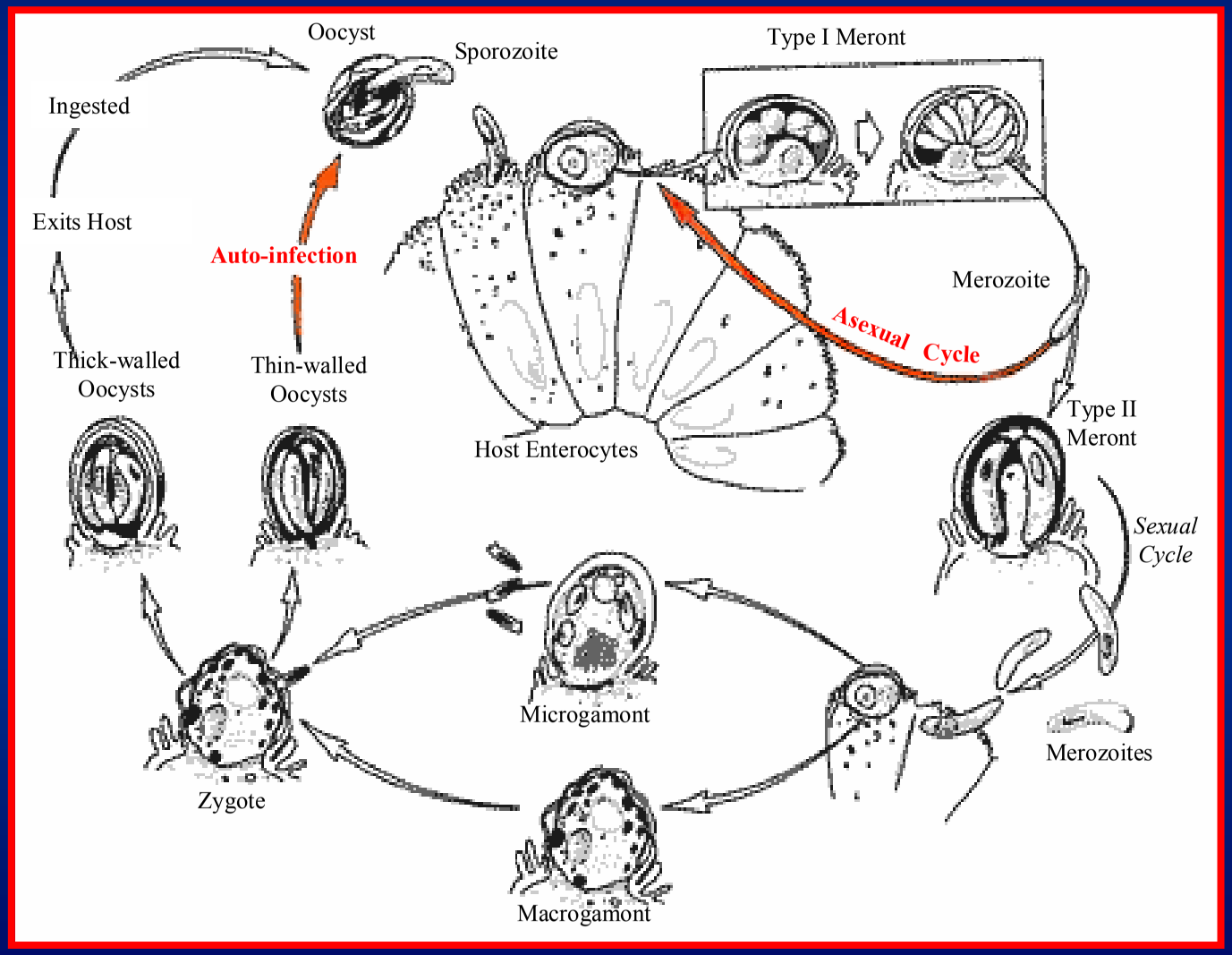
Immunomagnetic Separation
DNA Extraction
Nested PCR
Clone & Sequence
Immunofluorescent Staining
Microscopic Density Counts
Cell Culture Infectivity Assay

Kristen Jellison, Ph.D.
Fritz Engineering Lab
Lehigh University
13 E. Packer Avenue
Bethlehem, PA 18015
kjellison@lehigh.edu
(610) 758-3555

Cryptosporidium Life Cycle



(Adapted from Current & Blagburn, 1990)