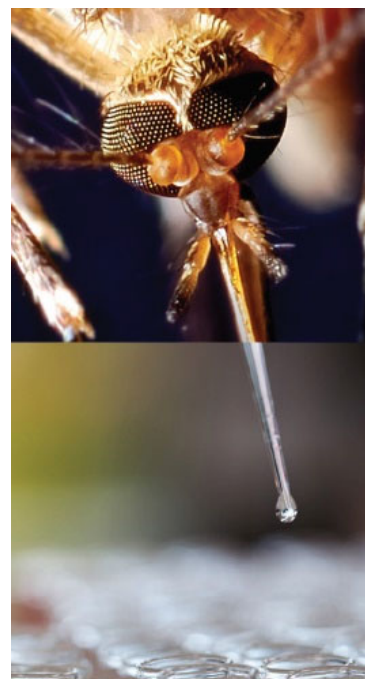
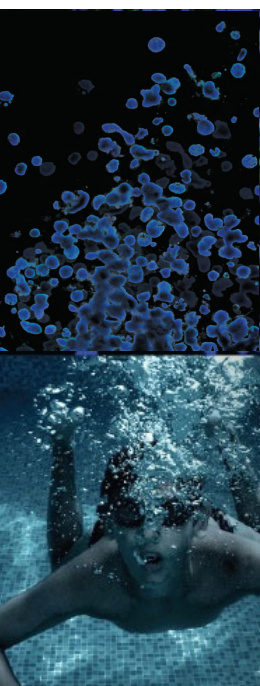




The Protistology Collection at ATCC: Scientific Relevance and Best Practices

Kathleen Glaser, BSAg
Senior Biologist, ATCC

Credible Leads to Incredible™

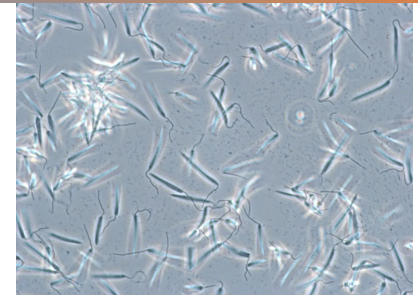


About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the “*gold standard*”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

Protists

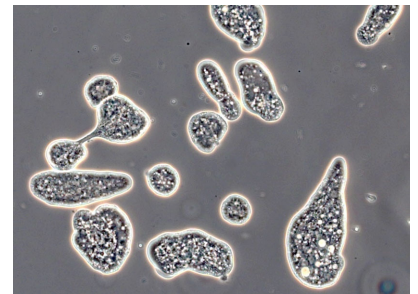
- Definition of a protist
- Examples
- Relevance to biomedical research and the wider community
- Current and potential uses in industry
- Techniques/applications



Leptomonas costaricensis (ATCC® PRA-186™)



Jakoba bahamiensis (ATCC® 50695™)



Entamoeba histolytica (ATCC® 30458™)

What is a Protist?

Protist

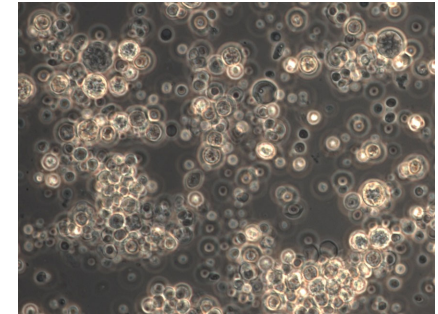
noun

pro·tist | \ 'prō-(.)tist \

--any of a diverse taxonomic group and especially a kingdom (Protista synonym Protoctista) of eukaryotic organisms that are unicellular and sometimes colonial or less often multicellular and that typically include the protozoans, most algae, and often some fungi (such as slime molds)

Source: Merriam-Webster.com

- Catch-all category
- Highly diverse



Prototheca wickerhamii (ATCC® 16529™)



Treponomas agilis (ATCC® 50286™)

The Protistology Collection at ATCC

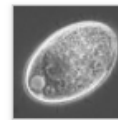
- The only general service collection of taxonomically diverse living stock protists in the United States
- The only large service repository of parasitic protozoa in the world



Amoebozoa

ATCC holdings include genera such as Entamoeba, Acanthamoeba,

Hyperamoeba, Hartmannella, and Spongomonas.



Chromalveolata

ATCC holdings include genera such as Plasmodium, Toxoplasma,

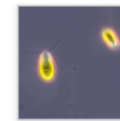
Tetrahymena, Paramecium, Adriaemonas, and Goniomonas.



Opisthokonta

ATCC holdings include genera such as Sphaerothecum, Encephalitozoon,

Nosema, and Salpingoeca.



Archaeplastida

ATCC holdings include genera such as Prototheca, Chlorella,

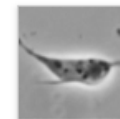
Chlamydomonas, Polythoma, and Cephaleuros.



Excavata

ATCC holdings include genera such as Crithidia, Leishmania,

Trypanosoma, Leptomonas, Euglena, Naegleria, Trichomonas, and Giardia.



Rhizaria

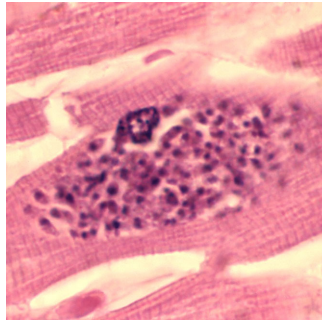
ATCC holdings include genera such as Heteromita, Cercomonas,

Thaumatomonas, and Biomyxa.

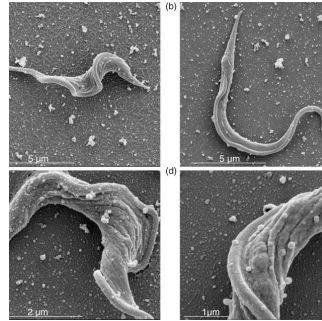
Highest-Demand Cultures/Reagents (last 5 years)

ATCC® No.	Description	Rank (Unit Distribution)
PRA-2154™	LYI Entamoeba Medium	1
30001™	<i>Trichomonas vaginalis</i>	2
327-X™	Modified PYNFH Medium	3
PRA-2695™	Keister's Modified TYI-S-33 Giardia Medium	4
PRA-405D™	Genomic DNA from <i>Plasmodium falciparum</i>	5
PRA-67DQ™	Quantitative Genomic DNA from <i>Cryptosporidium parvum</i>	6
30001D™	Genomic DNA from <i>Trichomonas vaginalis</i>	7
30888™	<i>Giardia intestinalis</i>	8
30010™	<i>Acanthamoeba castellanii</i>	9
50174D™	Genomic DNA from <i>Toxoplasma gondii</i>	10
30459DQ™	Quantitative Genomic DNA from <i>Entamoeba histolytica</i>	11
30459™	<i>Entamoeba histolytica</i>	12
30459D™	Genomic DNA from <i>Entamoeba histolytica</i>	13
30957™	<i>Giardia intestinalis</i>	14
50608D™	Genomic DNA from <i>Blastocystis hominis</i>	15
50143™	<i>Trichomonas vaginalis</i>	16
22662™	<i>Pseudokirchneriella subcapitata</i>	17
30888D™	Genomic DNA from <i>Giardia intestinalis</i>	18
30221D™	Genomic DNA from <i>Babesia microti</i>	19
PRA-1171™	TYGM-9 Medium	20

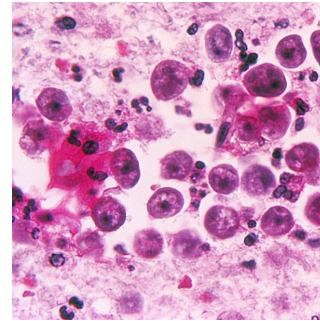
Clinically Significant Protists



Toxoplasma gondii in heart muscle



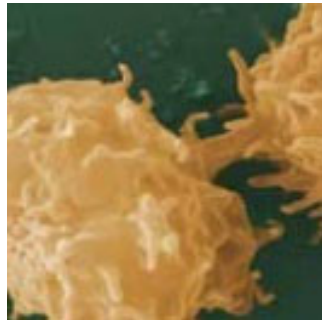
Trypanosoma cruzi courtesy of PM Nogueira, Universidade Federal de Minas Gerais



Naegleria fowleri in brain tissue



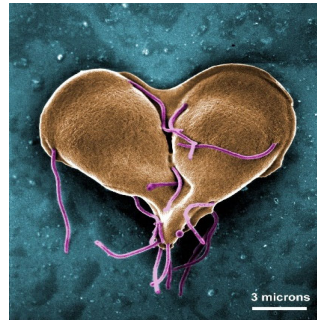
Trichomonas vaginalis



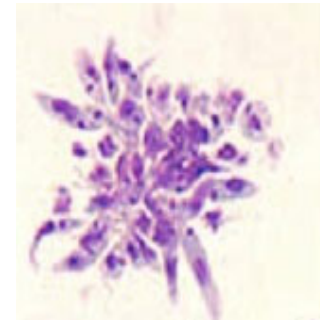
Acanthamoeba sp.



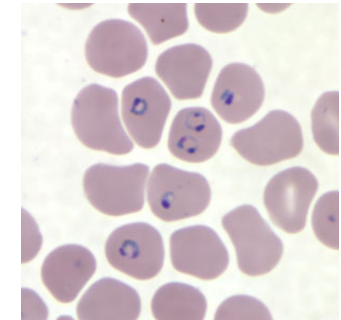
Entamoeba histolytica



Giardia intestinalis



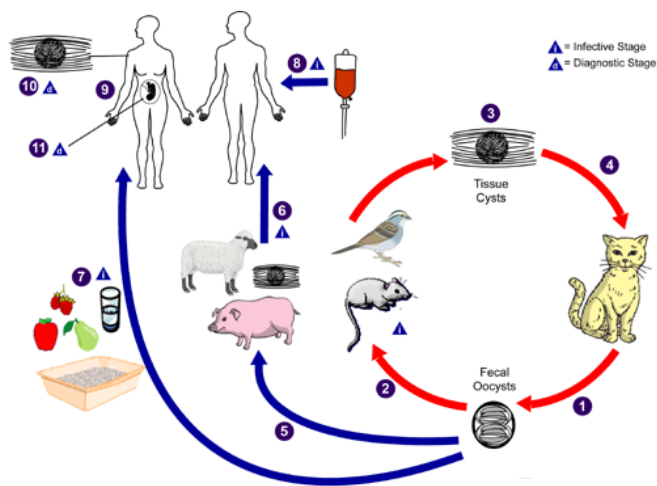
Leishmania sp. courtesy of CDC



Plasmodium falciparum courtesy of CDC

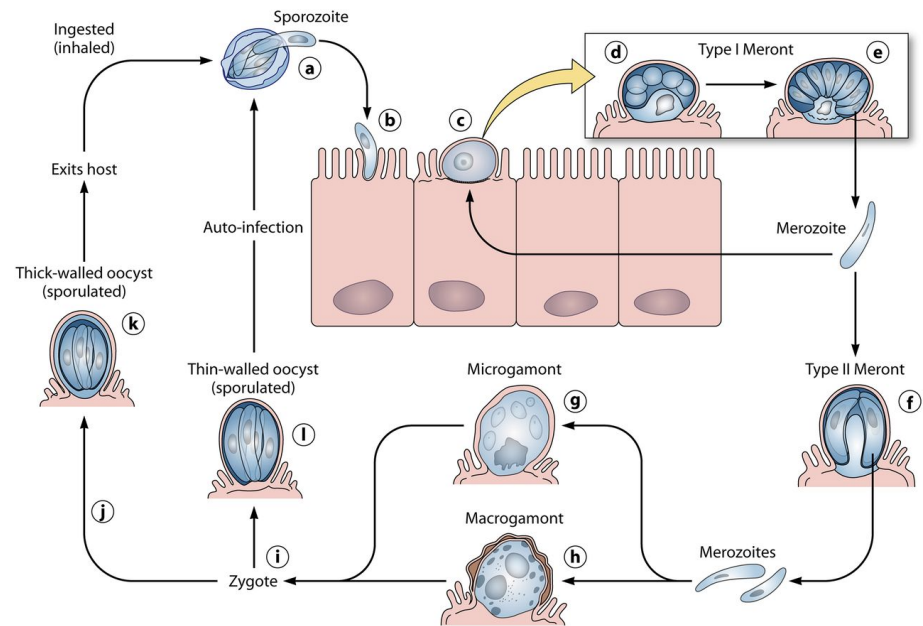
Life Cycles

- Life cycles may involve one or more hosts



Toxoplasma

Source: CDC.gov

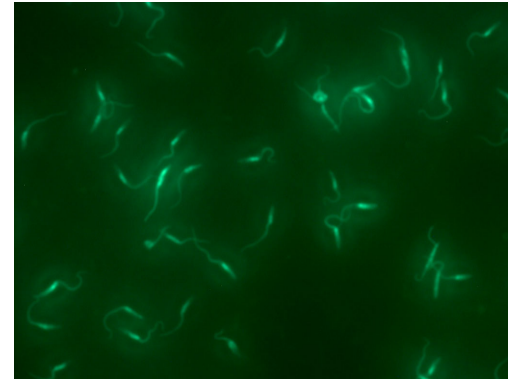


Cryptosporidium

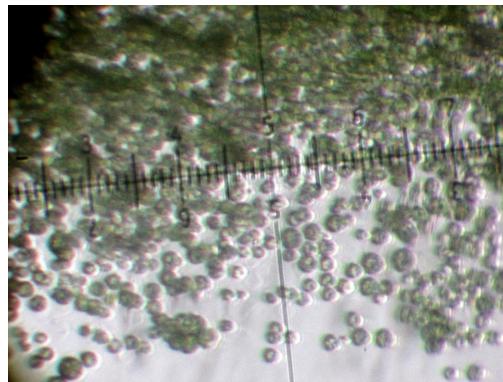
Bouzid, et al. *Cryptosporidium* Pathogenicity and Virulence. *Clin Micro Rev* 26(1):115, 2013.

Scientific Applications

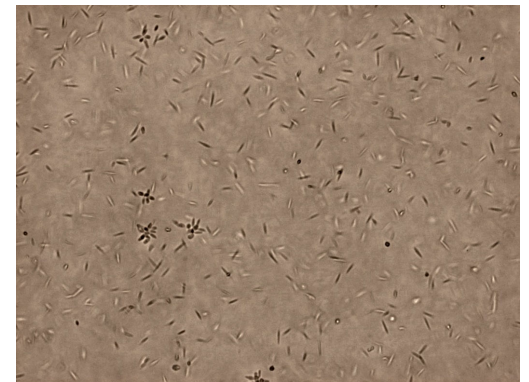
- Bioassays
- Molecular models
- Diagnostics
- Biofuels
- Fatty acid production



Trypanosoma cruzi (ATCC® 50832GFP™)



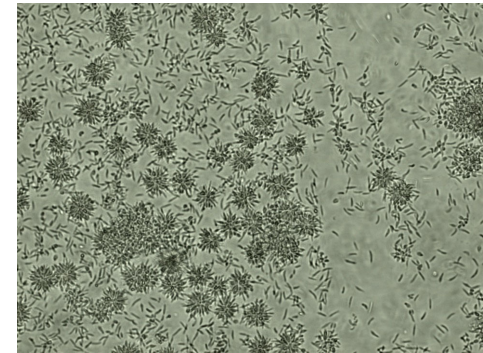
Chlorella vulgaris (ATCC® 9765™)



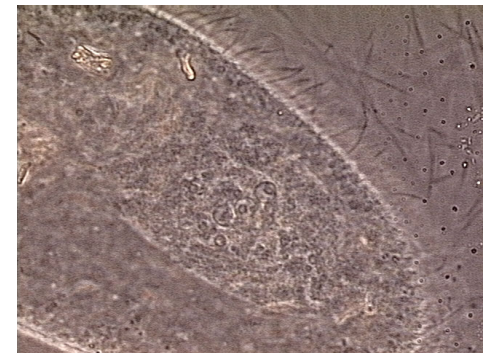
Crithidia fasciculata (ATCC® 50083™)

Propagation of Protists

- ATCC Product Sheet is the standard
- Wide variety of techniques
- Most items grown in broth, some on agar plates
- Recovery from cryopreservation varies



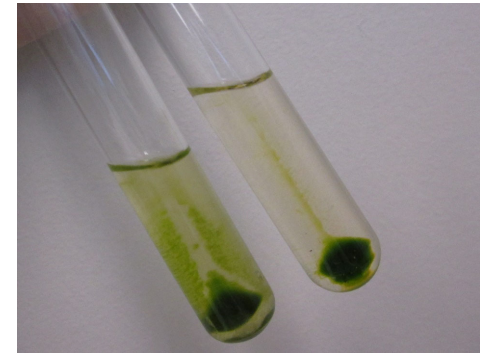
Leishmania amazonensis (ATCC® 50159™)



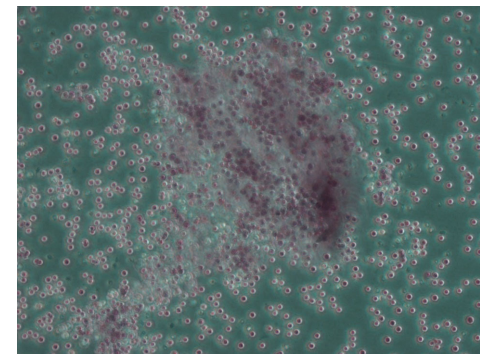
Paramecium octaurelia ATCC® 30699™)

Algae

- Different types by pigment
- Methanol cryoprotectant
- Pigment loss after thawing
- Light sources
- As food supplements
 - Chlorella
 - Brown algae (kelp)
 - Red algae



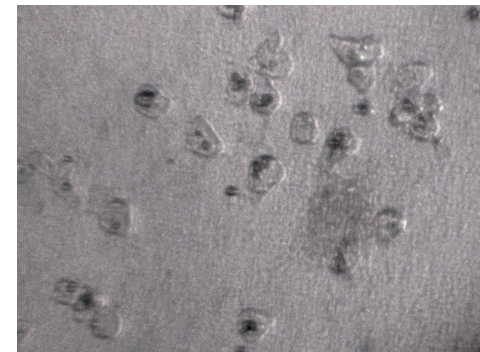
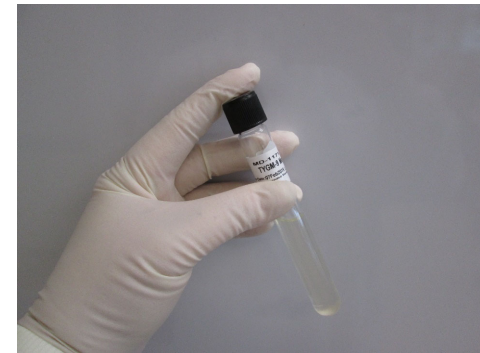
Chlorella kessleri (ATCC® 11468™)



Porphyridium purpureum (ATCC® 50161™)

Bacterized Entamoeba strains

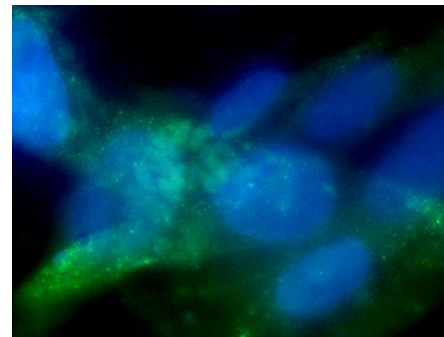
- Media (ATCC® PRA-1171™)
- Specialized culture techniques
 - Rice starch titration
 - Control of bacterial overgrowth
- Microscopy



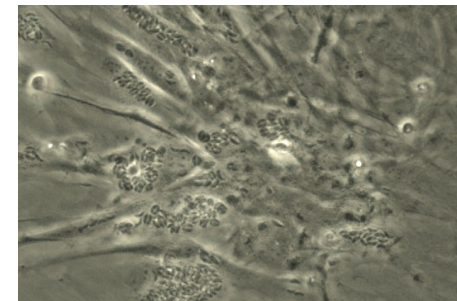
Entamoeba histolytica (ATCC® 30925™)

Intracellular Parasites

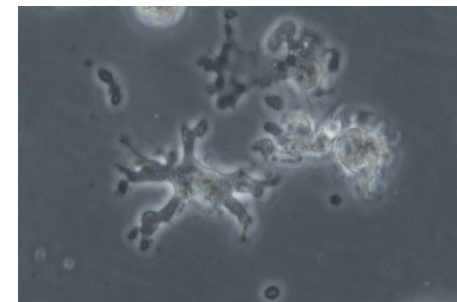
- Cell culture
- Cell lines used
 - Vero (ATCC® CCL-81™)
 - BALB/3T3 clone A31 (ATCC® CCL-163™)
 - Hs27 (ATCC® CRL-1634™)
- Immunofluorescence assays (IFA)
- Mycoplasma testing
 - Mycoplasma PCR Testing Service (ATCC® 136XV™)
 - Mycoplasma Direct & Indirect Culture Testing Service (ATCC® 119-X™)
 - Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)



Toxoplasma gondii (ATCC® PRA-426™)



Neospora hughesi (ATCC® 209622™)

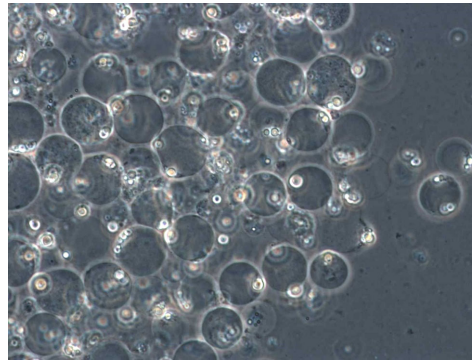
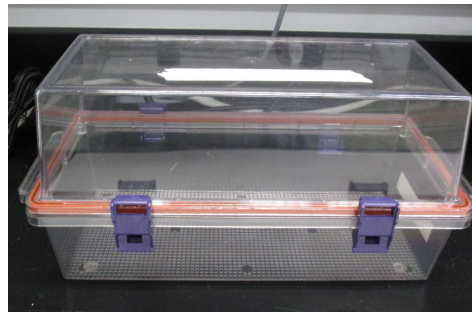


Balamuthia mandrillaris (ATCC® PRA-290™)

Anaerobic Cultures

Equipment

- Incubator chambers
- Instrumentation
- Anaerobe chamber
- Anaerobic gas generators



Blastocystis hominis (ATCC® 50177™)



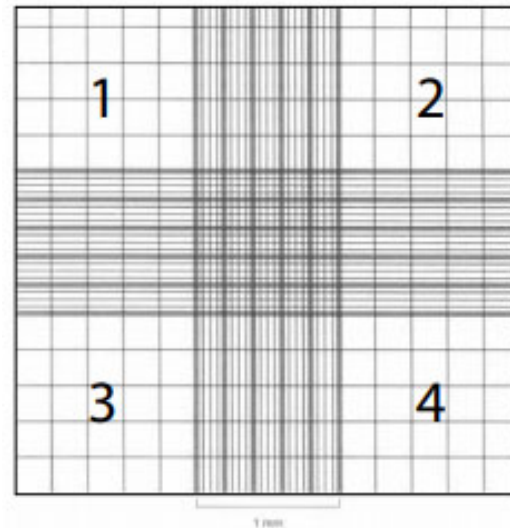
Culture Initiation

- Liquid nitrogen storage
- Rapid thawing at 35°C
- Prompt culture initiation
- Careful handling
- Proper incubation conditions



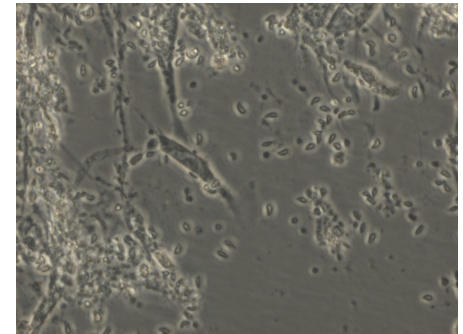
Cell Counting

- Hemacytometer used
- Different techniques
- Estimation
- Counts not always performed

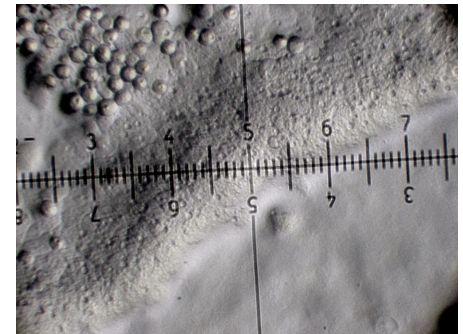


Techniques in Protistology

- Slow hydration of freeze dried cultures
- Reduction of bacterial density
- Picking of individual cells
- For intracellular parasites growing in cell lines:
 - Propagation of cell culture
 - Maintenance
- In vivo propagation



Toxoplasma gondii ATCC 40050



Acanthamoeba castellanii ATCC 30011

Special Growth Considerations

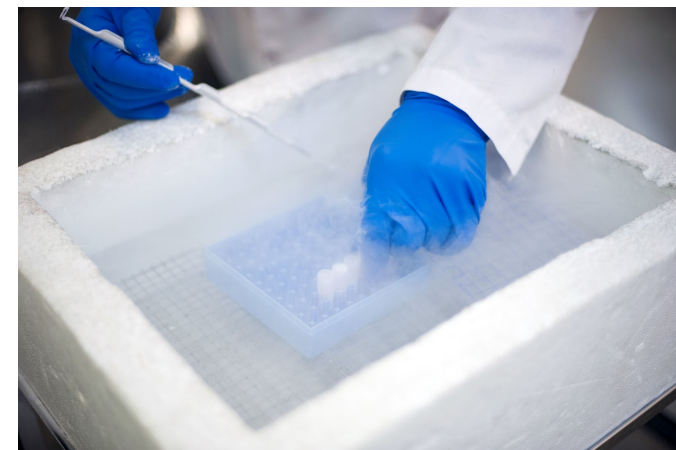
- Starvation of free-living protists before freezing
- Differences in medium component lots
- Types of light sources for photosynthetic organisms
- Depleted media/"X-factor"



Paramecium caudatum

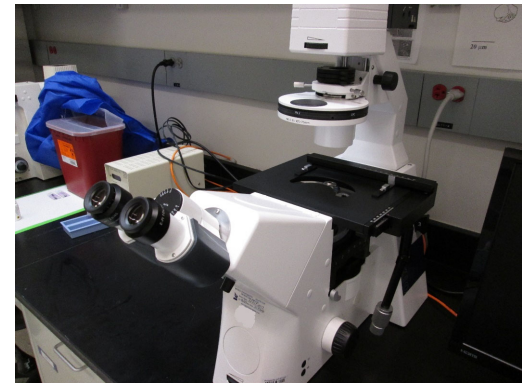
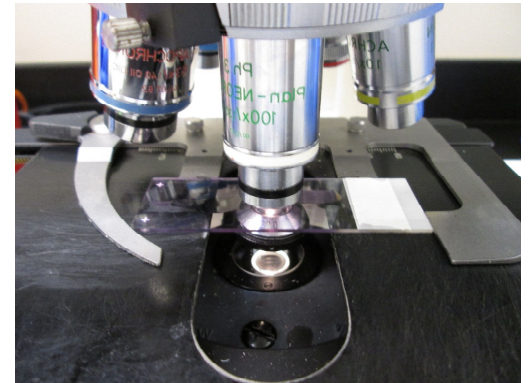
Preservation

- Most protist cultures are stored in liquid nitrogen vapor
- Slow freezing to -70°C
- CoolCell[®] LX Alcohol-Free Cryopreservation Container (ATCC[®] ACS-6000[™])
- Cryoprotectants
- Some protist cultures are delivered freeze-dried or growing in test tubes



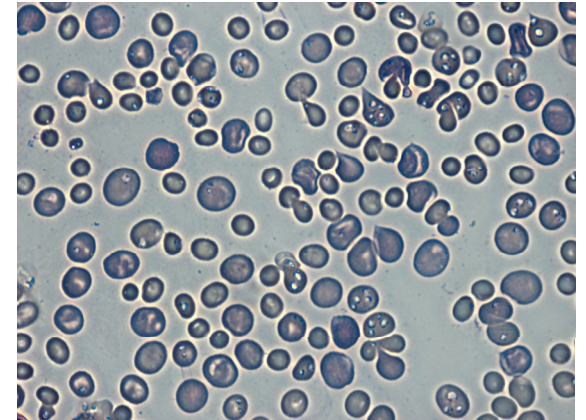
QC of Protist Items

- Parameters follow product sheet/Bioproduction guidelines
- Morphology and behavior observed
- Manual sterility testing for axenic cultures
- Specialized testing
 - Biochemical tests
 - Antibiotic susceptibility
 - Special stains

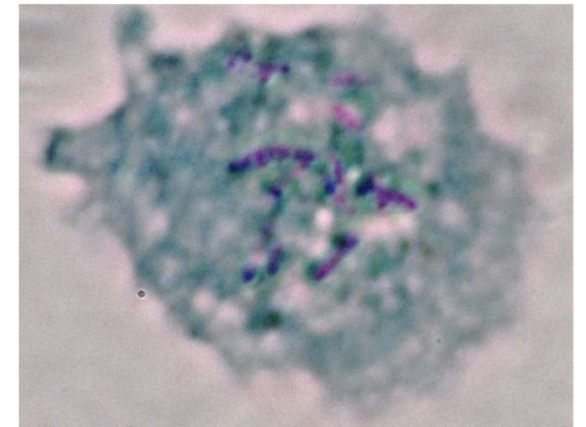


Staining

- Giemsa stain
- Acid-fast (Kinyoun) stain
- Gram stain



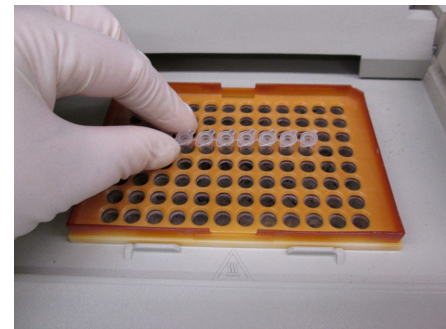
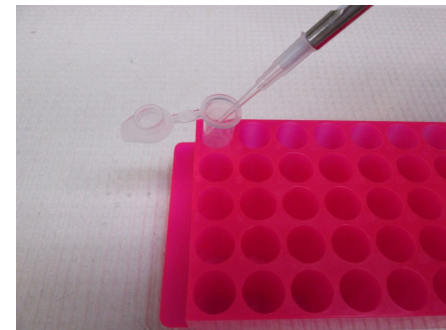
Babesia microti (ATCC® 30221™)



Acanthamoeba castellanii (ATCC® 50370™)

DNA Extraction/PCR

- Commercially-available kits
- Several methods
- Special techniques
 - Enzymatic digestion
 - Mechanical disruption
- Primers
 - Universal eukaryotic (18S rRNA)
 - Genus-specific (ITS regions)
 - Specific primers for transgenes in genetically-modified strains



Authentication

- Molecular authentication (Sanger sequencing)
- 18S nuclear rRNA gene sequenced
- Sequences compared with NCBI and other online databases



General representation of eukaryotic 18S, 5.8S, and 28S rRNAs. Genes are flanked by the external transcribed spacers (5' and 3' ETS) and separated by the internal transcribed spacers (ITS1 and ITS2).

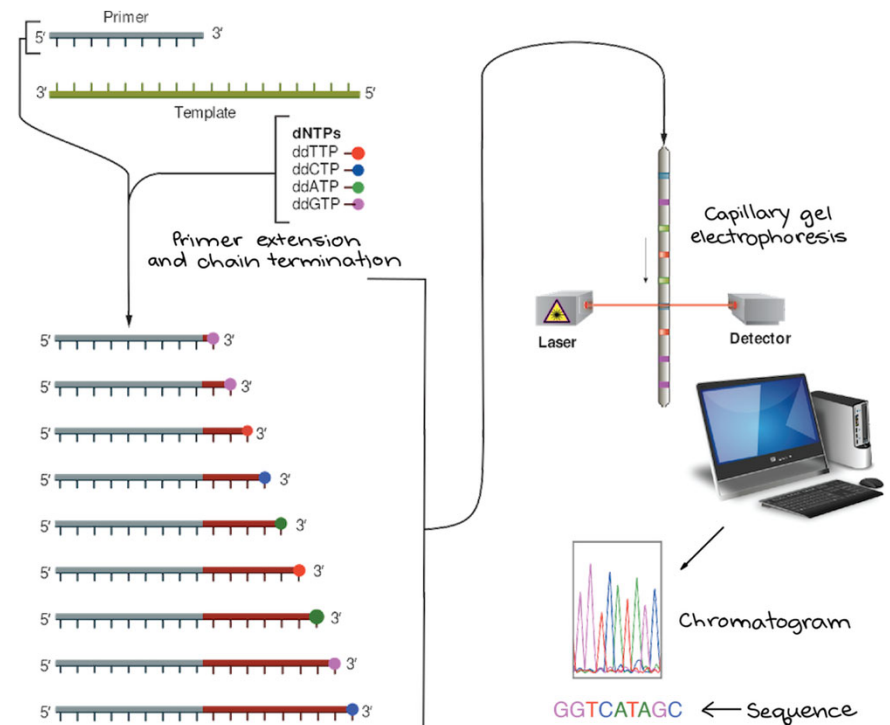


Image modified from "Sanger sequencing," by Estevezj ([CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)). The modified image is licensed under a ([CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)) license

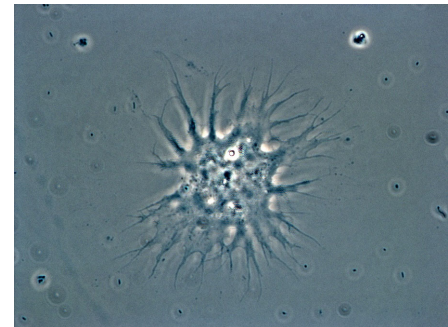
In Conclusion

Protists:

- Are a highly-diverse group of eukaryotes
- Have applications in the fields of research, nutrition, and industry
- Are extensively studied as models to investigate particular biological phenomena or diseases of humans and animals



Salpingoeca macrocollata (ATCC® 50938™)



Tlaeopolella tubasferens (ATCC® 50593™)

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Safety Tips for Returning to the Lab

To help you get back to work, we have put together a free kit containing best practices to help keep your lab infection-free:

- Poster
- Infographic
- Decals

Order the kit here:

www.atcc.org/backtothelab



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