“Cooking From Scratch: Development of Inquiry Based Activities for the General Microbiology Laboratory”

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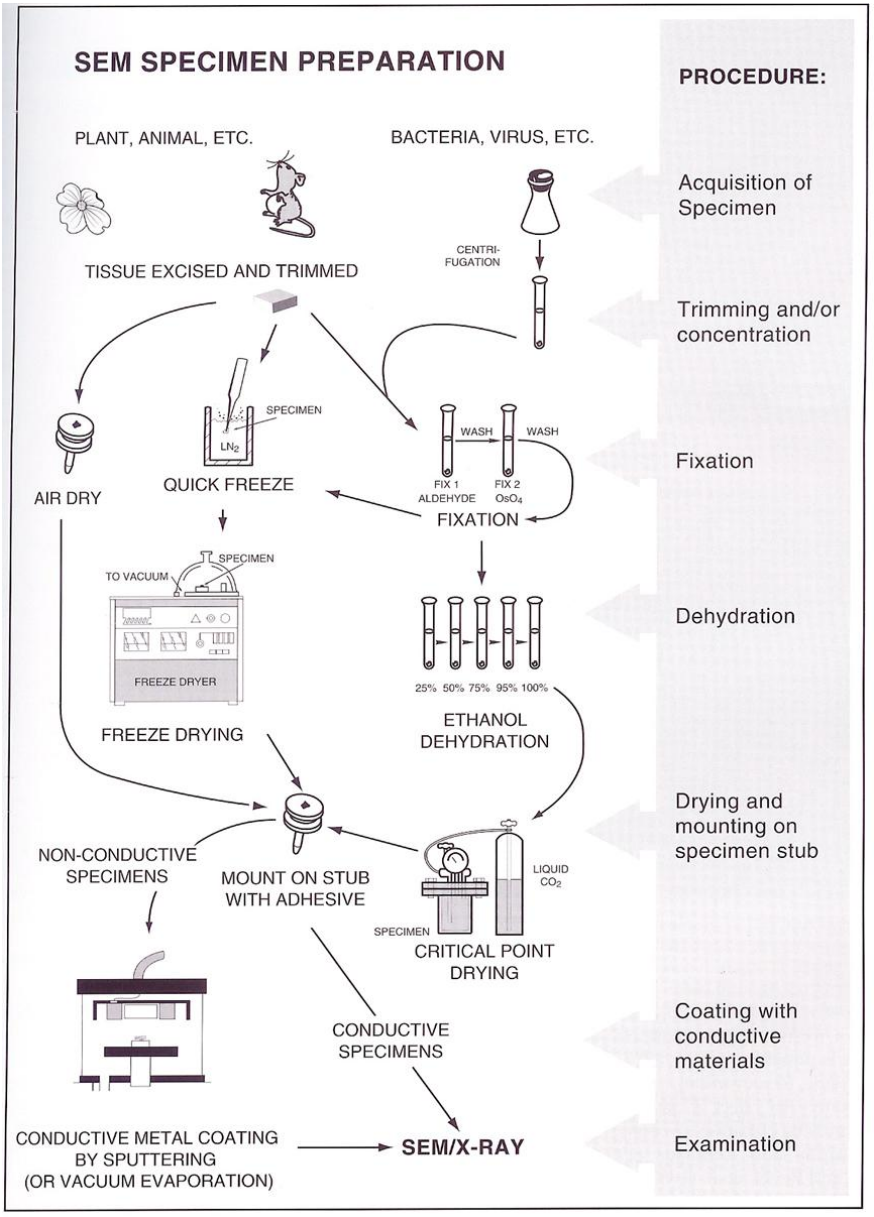
Introduction

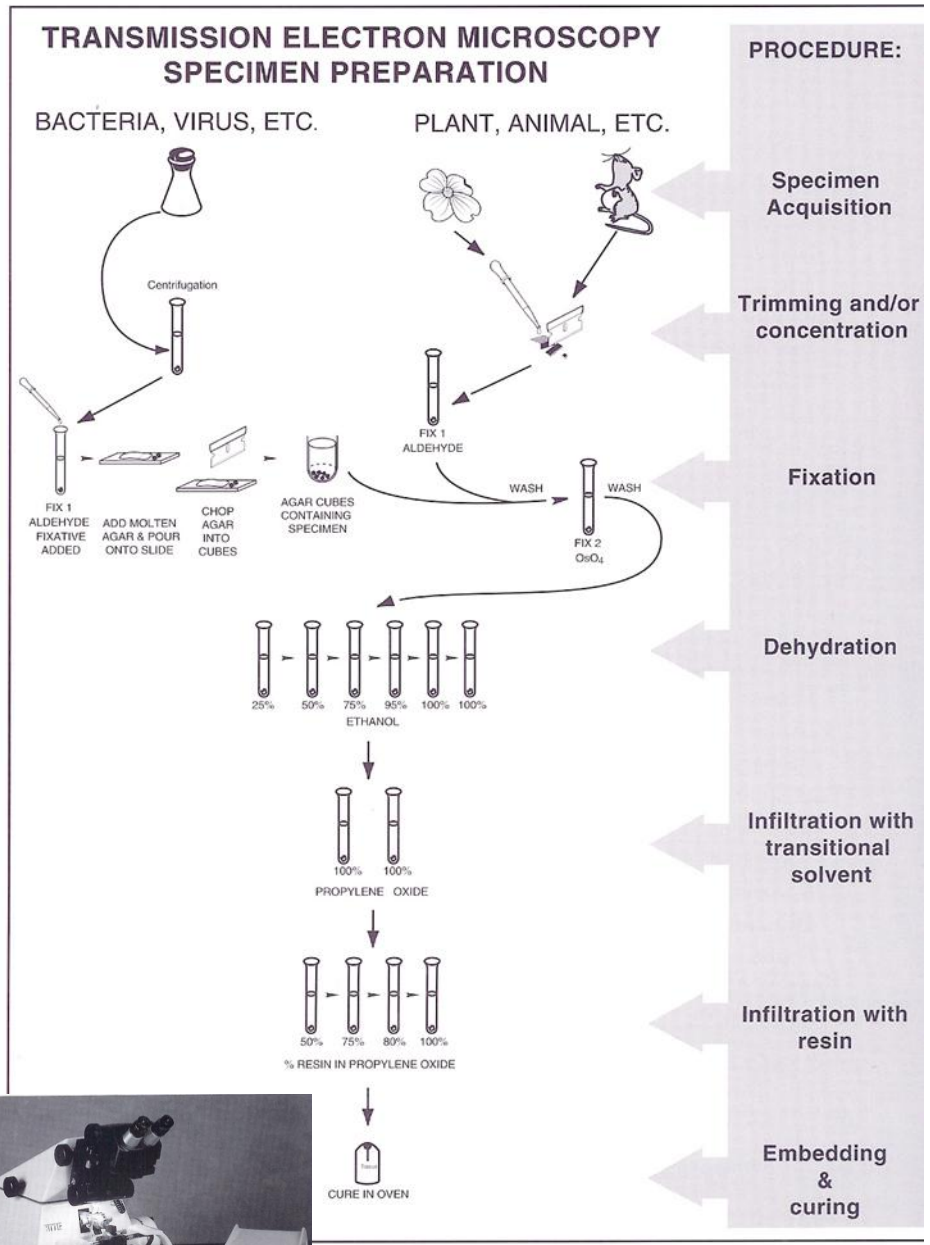
- The Problem
  - Undergraduate microbiology students lack direct exposure to the variety of research tools used in the field.
- The Project
  - Develop hands on investigative activities.
    - Electron microscopy
    - Transformation
- Goals
  - Increased understanding of applications of the technology.
  - Recruitment for advanced courses.

Electron Microscopy Lab

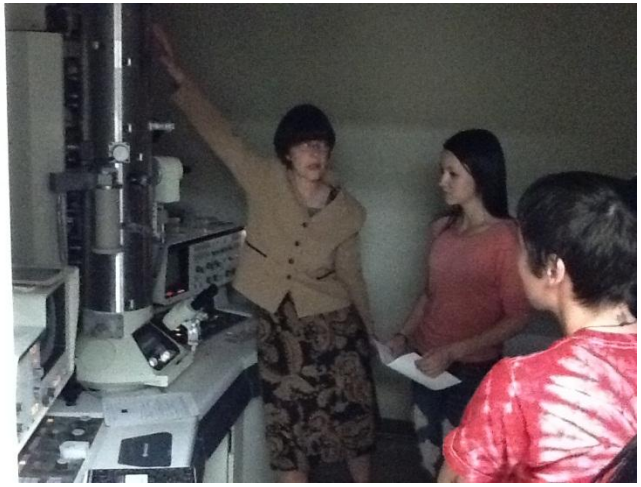
Approach

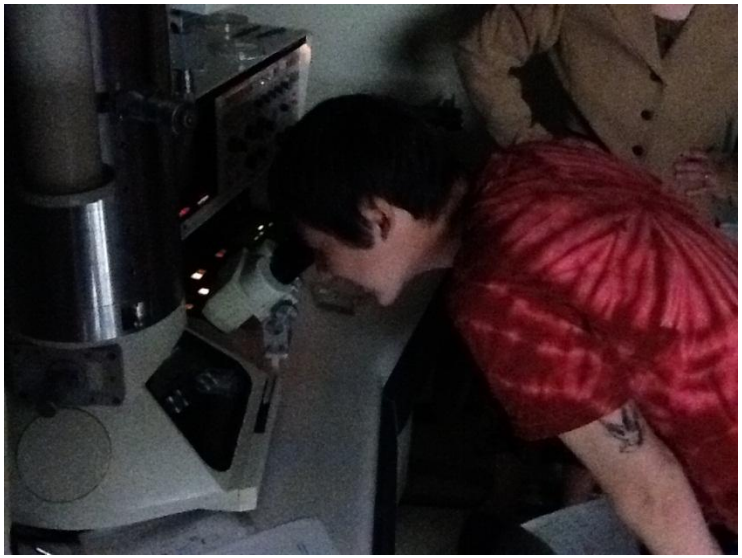
- Prior exposure to compound light microscope
  - Viewed yeast and bacteria
- Introductory lecture on electron microscopy
- Hands-on activities
  - Specimen preparation
  - Time on transmission and scanning electron microscopes
  - Micrograph stations
- Evaluation
  - Pre/post test
  - Student survey

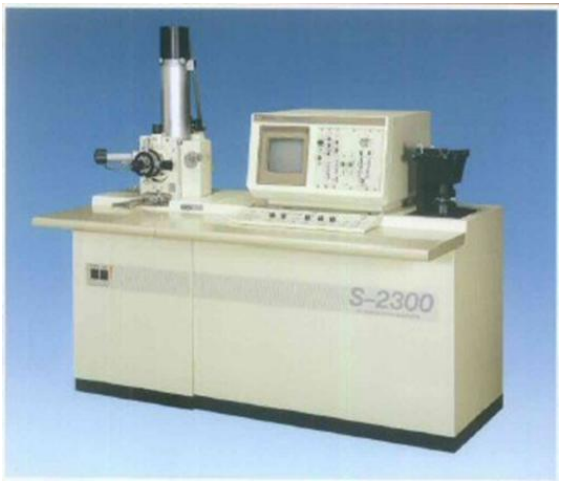




Electron Microscopy, 2nd edition
Bozzola and Russell 1999

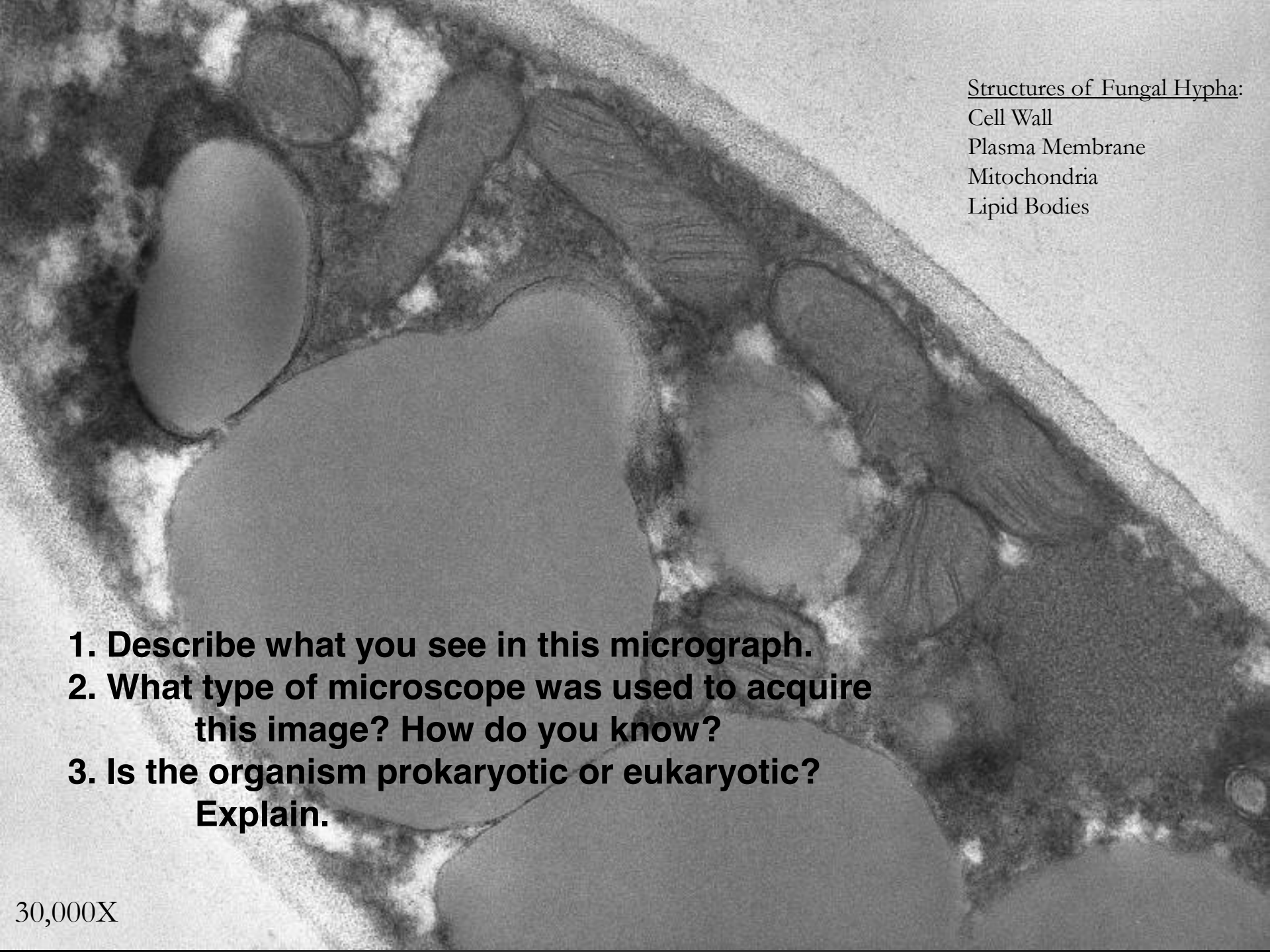






Viewing Specimens with TEM and SEM

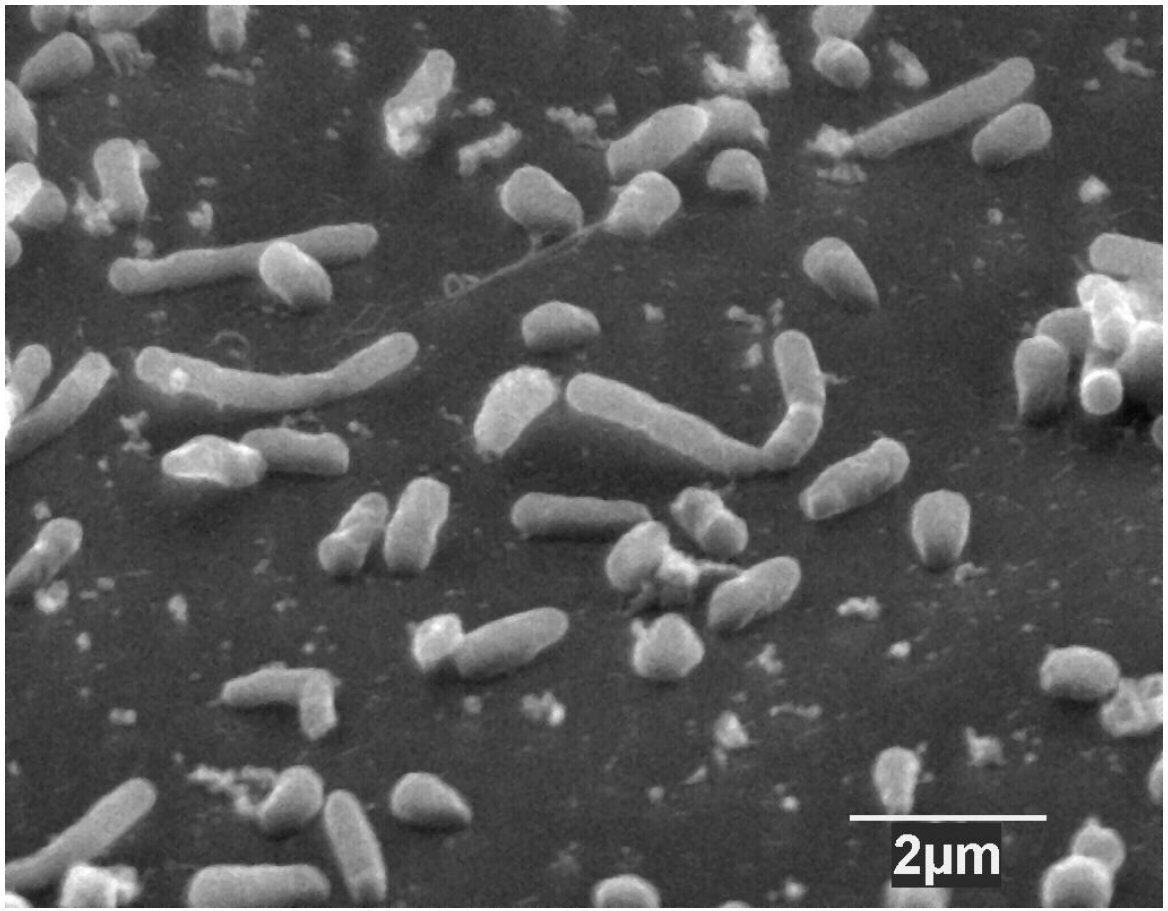
Micrograph stations



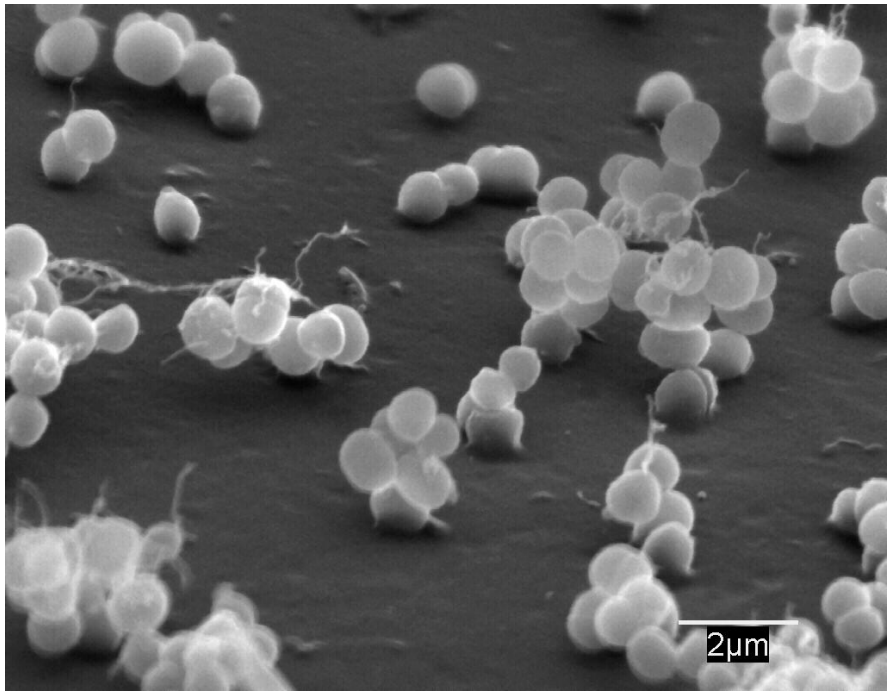
Structures of Fungal Hypha:
Cell Wall
Plasma Membrane
Mitochondria
Lipid Bodies

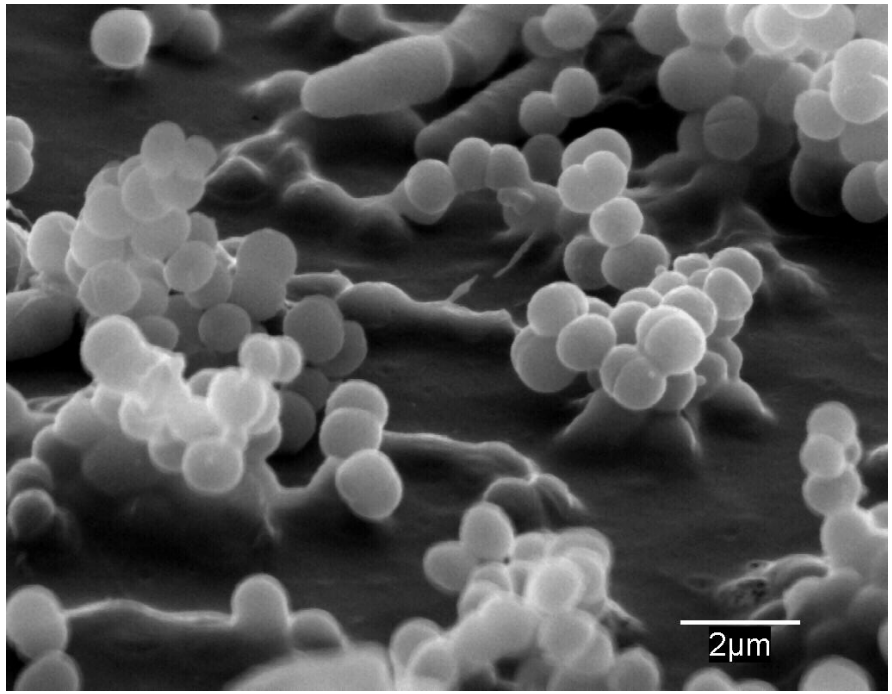
1. Describe what you see in this micrograph.
2. What type of microscope was used to acquire this image? How do you know?
3. Is the organism prokaryotic or eukaryotic? Explain.

30,000X



1. Describe what you see in this micrograph.
2. What type of microscope was used to acquire this image? How do you know?
3. Based on the structures depicted, is the organism a bacterium or a fungus? Explain.



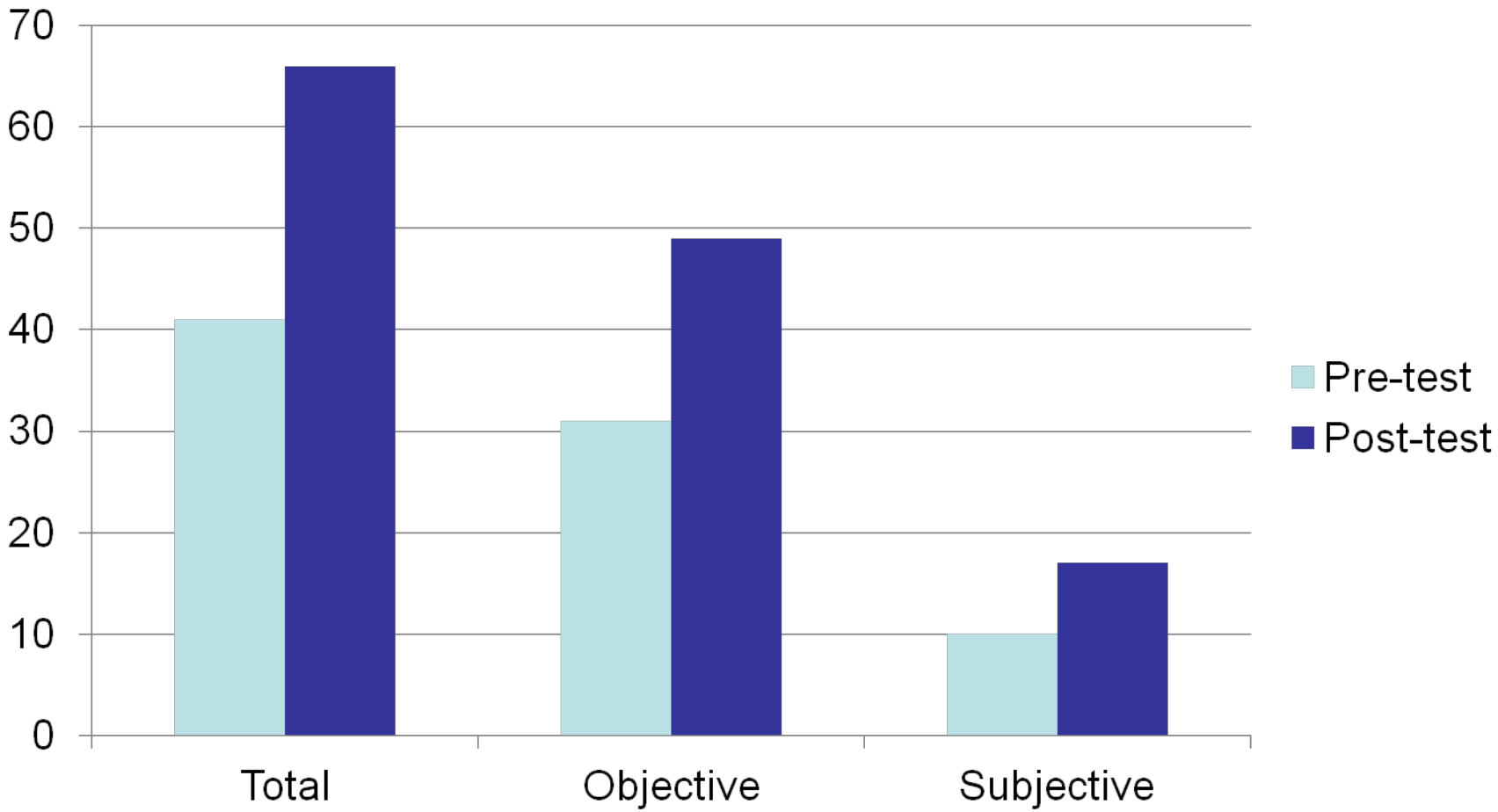


These micrographs depicts spherical shaped bacterial cells. Approximate the size of these cells. Explain your method for making this estimation.

Pre/post Test – Electron Microscopy

- Objective and subjective questions; N = 17
- Pre-test
  - Mean % correct = 41; high = 56
- Post-test
  - Mean % correct = 66; high = 88
- All students improved in level of knowledge.
  - Range of improvement 11 – 37%; mean = 25%

Electron Microscopy Lab Scores



An electron microscope uses _____ to focus the source of illumination.

a. Mirrors
b. Glass lenses
c. Magnets
d. a and b
e. a, b and c

15/17 (88%) answered correctly on post-test.

True or **False**

Denser regions of a specimen scatter more electrons and therefore appear lighter in the image projected onto the screen of a transmission electron microscope.

12/17 (71%) answered correctly on post-test.

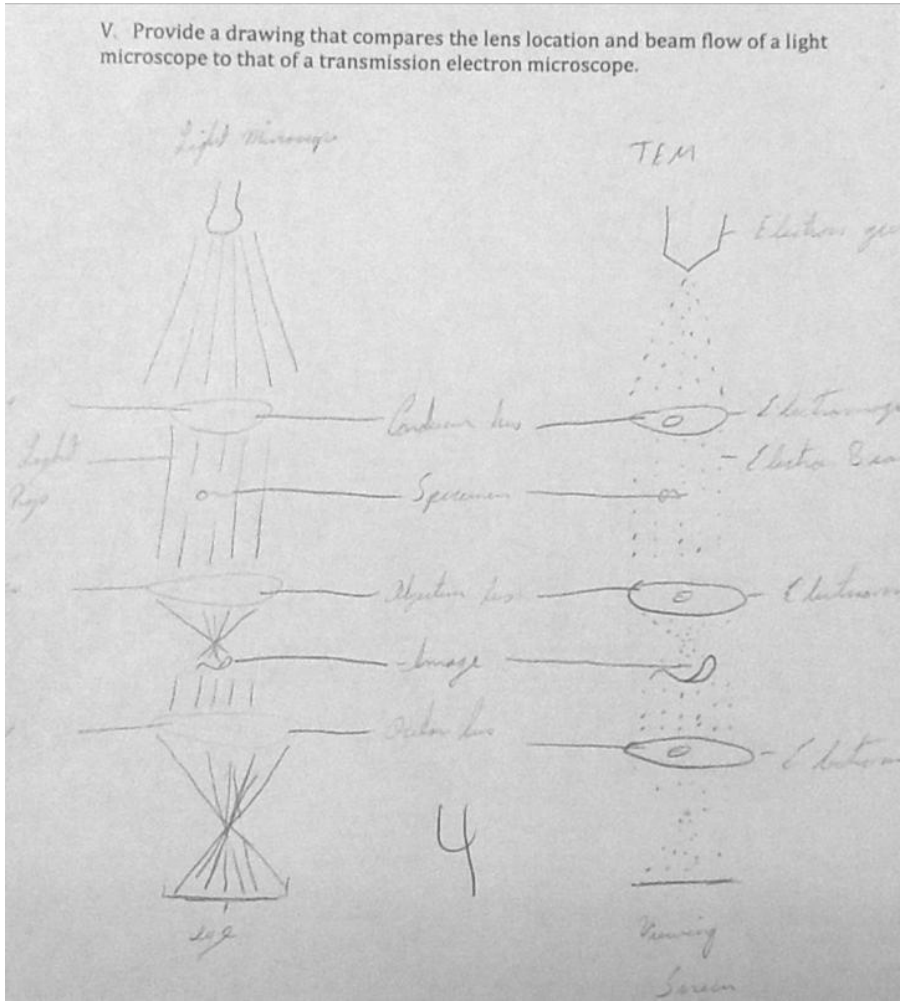
Choose the best microscope to use for visualizing the specimen described in each situation.

Small morphological structures within a cell's interior. **(TEM)**

15/17 (88%) answered correctly on post-test.

Smallpox virus. **(TEM)**

7/17 (41%) answered correctly on post-test.



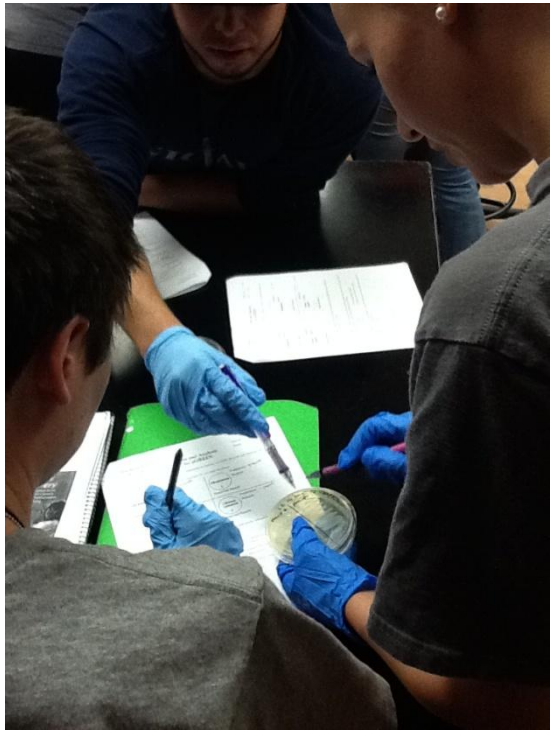
11/17 (65%) scored 3 or 4 (max) on post-test.

Transformation Lab

Approach

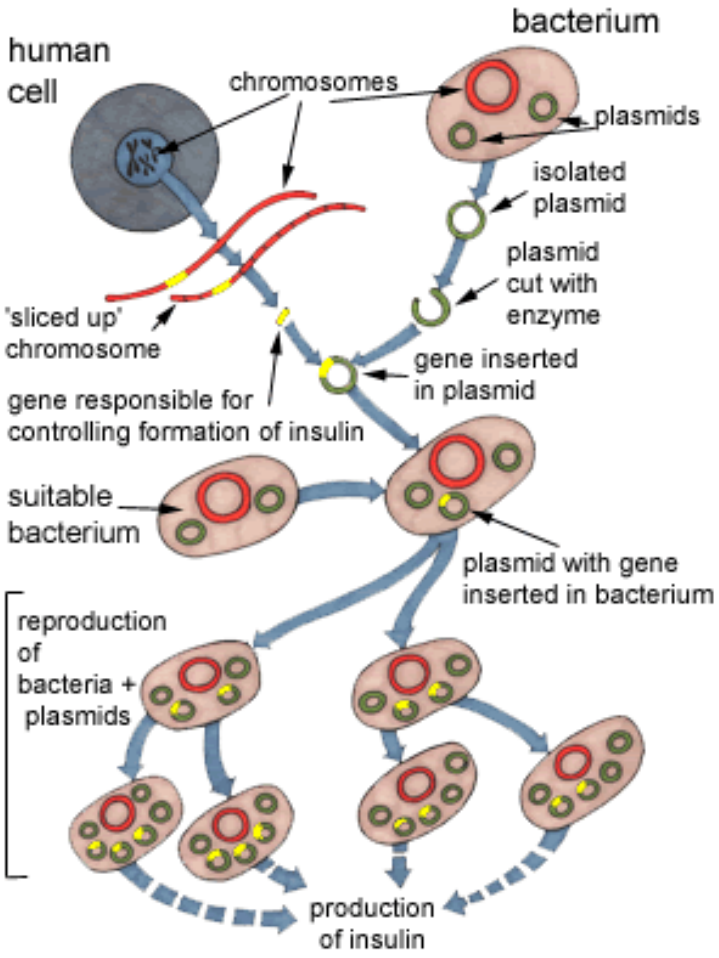
- Introductory lecture on biotechnology
- Hands-on activities
  - Transformation of *E. coli* cells to produce green fluorescent protein
    - Students conducted the procedure.
  - "The Life and Times of Lydia Villa-Komaroff"
    - Transformation of bacteria to produce human insulin.
      - Group time-line stations, followed by class discussion.
- Evaluation
  - Pre/post test
  - Student survey

E. coli transformation



Observing transformed cells after 48 hrs.

The Life and Times of Lydia Villa-Komaroff



Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3727–3731, August 1978
Biochemistry

A bacterial clone synthesizing proinsulin

(rat preproinsulin/cDNA cloning/solid-phase radioimmunoassay/DNA sequence/fused proteins)

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Contributed by Walter Gilbert, June 9, 1978

ABSTRACT **We have cloned double-stranded cDNA copies of a rat preproinsulin messenger RNA in *Escherichia coli* χ1776, using the unique *Pst* endonuclease site of plasmid pBR322 that lies in the region encoding amino acids 181–182 of penicillinase. This site was reconstructed by inserting the cDNA with an oligo(dG)-oligo(dC) joining procedure. One of the clones expresses a fused protein bearing both insulin and penicillinase antigenic determinants. The DNA sequence of this plasmid shows that the insulin region is read in phase; a stretch of six glycine residues connects the alanine at position 182 of penicillinase to the fourth amino acid, glutamine, of rat proinsulin.**

Can the structural information for the production of a higher cell protein be inserted into a plasmid in such a way as to be expressed in a transformed bacterium? To attack this problem, we used as a model rat insulin, an interesting protein that can be identified by immunological and biological means.

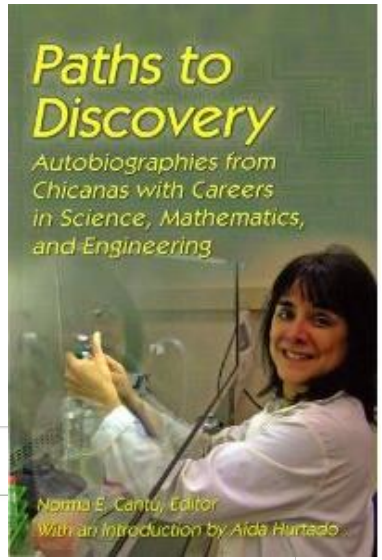
Although mature insulin contains two chains, A and B, it is the product of a single longer polypeptide chain. The hormone is initially synthesized as a preproinsulin structure (1, 2). A hydrophobic leader sequence of 23 amino acids at the amino terminus of the nascent chain is cleaved off, presumably as the polypeptide chain moves through the endoplasmic reticulum (2–4), producing a proinsulin molecule. The proinsulin chain folds up and then the C peptide is cleaved from its middle (5). Thus each of the two (nonallelic) insulin genes in the rat (6–8)

minA1, supE42, Δ40[*gal-uvrB*], λ⁻, *minB2, rfb-2, nalA25, oms-2, thyA57, metC65, oms-1,* Δ29[*bioH-asd*], *cycB2, cycA1, hsdR2*) was provided by R. Curtiss.

DNA and Enzymes. pBR322 DNA, a gift from A. Poteete, was used to transform *E. coli* HB101. Plasmid DNA was purified according to the procedure of Clewell (16). Avian myeloblastosis virus reverse transcriptase (RNA-dependent DNA polymerase), *E. coli* DNA polymerase I, and terminal transferase were gifts from T. Papas, M. Goldberg, and J. Wilson, respectively. Restriction enzymes were purchased from Bethesda Research Labs and New England BioLabs.

RNA Purification. An x-ray-induced, transplantable rat beta cell tumor (10) was used as source of preproinsulin mRNA. Tumor slices (20 g per preparation) were homogenized, and a cytoplasmic RNA (about 2 mg/g of tissue) was purified from a postnuclear supernatant by Mg^{2+} precipitation (17), followed by extraction with phenol and chloroform, and enriched for poly(A)-containing RNA by oligo(dT)-cellulose chromatography (18). About 4% of the material binds to the column (data from eight preparations). Further purification of the oligo(dT)-cellulose-bound material by sucrose gradient centrifugation and/or polyacrylamide gel electrophoresis showed that the preproinsulin mRNA was a minor component of the preparation.

Autobiographical Resources



Lydia Villa-Komaroff

MAKERS PROFILE

MOLECULAR BIOLOGIST

Share these MAKERS Moments

Lydia Villa-Komaroff on wanting to be a scientist, defying prejudices as a young Latina, and her breakthrough in diabetes research.

[Lydia Villa-Komaroff | MAKERS]

Hispanic Heritage

Lydia Villa-Komaroff

Born August 7, 1947
Molecular Biologist

Lit Kit
- Literary Index
- Glossary of Literary Terms
- How to Write a Term Paper
- Citing Information from Gale Databases

For molecular biologist Lydia Villa-Komaroff, the secret to success was in the genes. In a 1995 interview for the book *Journeys of Women in Science and Engineering: No Universal Constraints* (*JWSE*), Villa-Komaroff said, "I am interested in the question of development: how you get from a single cell the fertilized egg to a person, where all of the stissues are in the right place and each organ knows what to do and when to do it." Villa-Komaroff spent more than 20 years studying genes, mainly concentrating on protein synthesis, cell development, and growth mutations. She gained international recognition in 1978 as one of the pioneers in the emerging field of cloning. From 1985 to 1995, the award-winning scientist was on the faculty at Harvard University Medical School.

Villa-Komaroff now serves as the vice president for research and graduate studies at Northwestern University in Evanston, IL. "I haven't left science," she said of her new job in a 1998 telephone interview, "I've just left the bench." In a school publication from that same year, she summed up her office's primary function: "Research occurs in every corner of the university, and we're here to expedite all of it."

Credits Strong Family History for Success

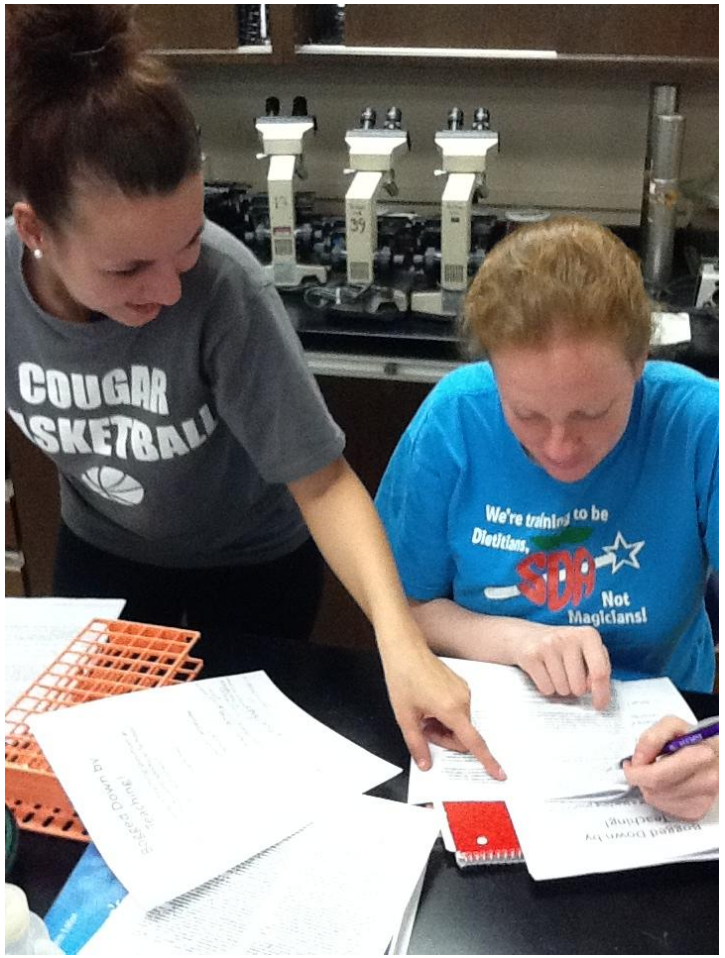
Villa-Komaroff partially credits her own genetic makeup for her personal development and professional success, both as a manager and a scientist. Her family history includes many tales of victory over adversity. *Invisible Frontiers: The Race to Synthesize a Human Gene* recounts one such family legend: While trying to escape the Mexican Revolution, her grandfather, Encarnación Villa, came face-to-face with the revolutionaries. The rebels had captured his northbound getaway train and ordered all passengers to disembark. They were given three choices: join the fight, donate valuables to the cause, or be shot. The young Encarnación stood firm, refusing to abandon his family yet holding nothing of value. Just then the revolutionary leader Pancho Villa rode up. When he heard the prisoner's surname, the general freed Encarnación and ordered him: "Have many sons with that name."

Villa-Komaroff was born on August 7, 1947, and grew up in Sante Fe, New Mexico. As the eldest of six children, she developed teamwork- and consensus-building skills out of necessity; these skills would later serve her well in the lab and the workplace. Taking cues from those above her, Villa-Komaroff had many relatives who served as strong role models. Her mother worked as a teacher and social worker. Villa-Komaroff's paternal grandmother had been a *curandera,* or a healer, and her maternal grandmother, a lone breadwinner with three children, had sold chemical toilets on horseback up in the mountains. Villa-Komaroff's parents encouraged their offspring to follow their dreams. In *JWSE,* Villa-Komaroff spoke at length of her father, a schoolteacher and musician: "I remember when I was five he brought home the *World Book Encyclopedia,* and he said that everything I wanted to know was in those books. I was very excited by that notion. He bought the books and my mother read to us — that's one of my earliest and warmest childhood memories."

http://www.gale.cengage.com/free_resources/chh/bio/villa_l.htm

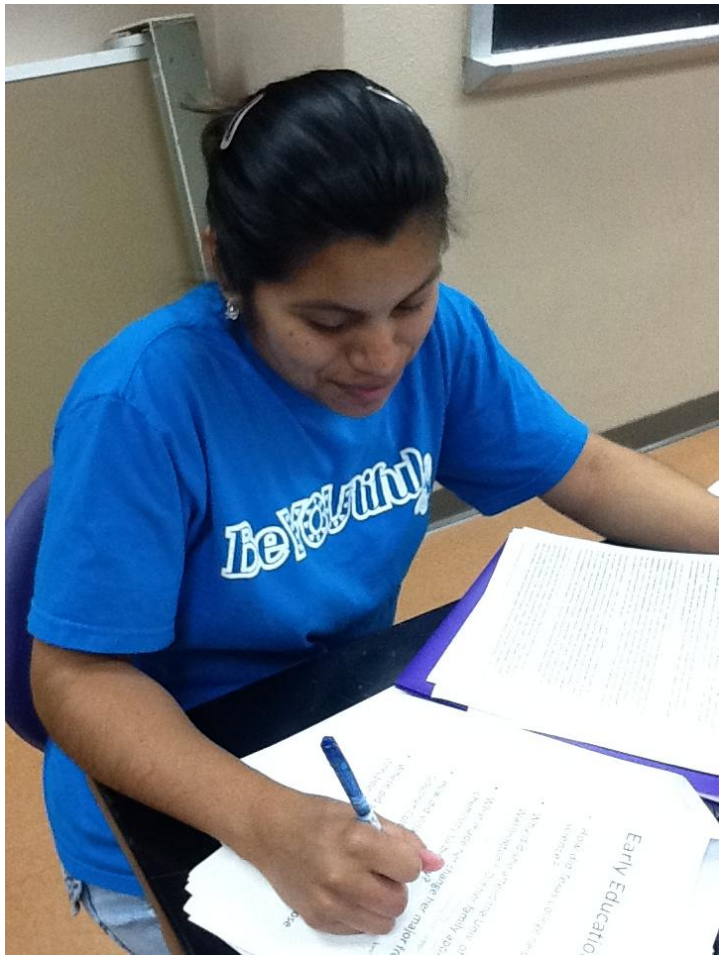
Family History

- When and where was she born?
- How is her family connected to Pancho Villa?
- Which relatives shared as good role models? In what ways?
- How did her father help by bringing home a copy of the World Book encyclopedia?



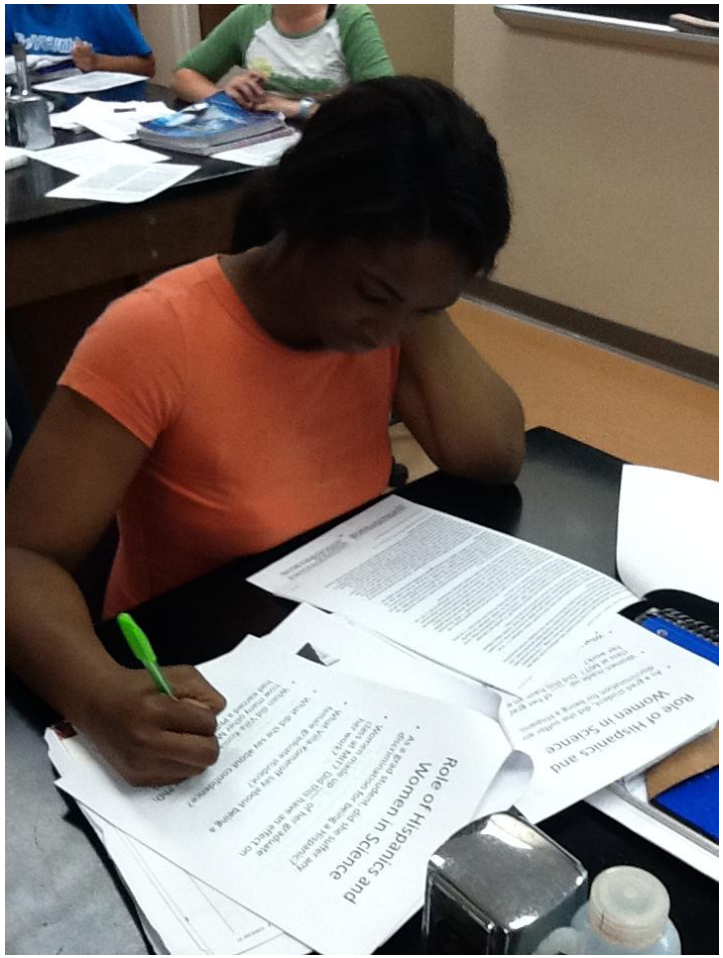
Education

- How did Texas College help her get into science?
- What made her change her major from chemistry to biology?
- Women made up ___ of her graduate class at MIT. Did this have an effect on her work?
- What did Villa-Komaroff say about being a female graduate student?

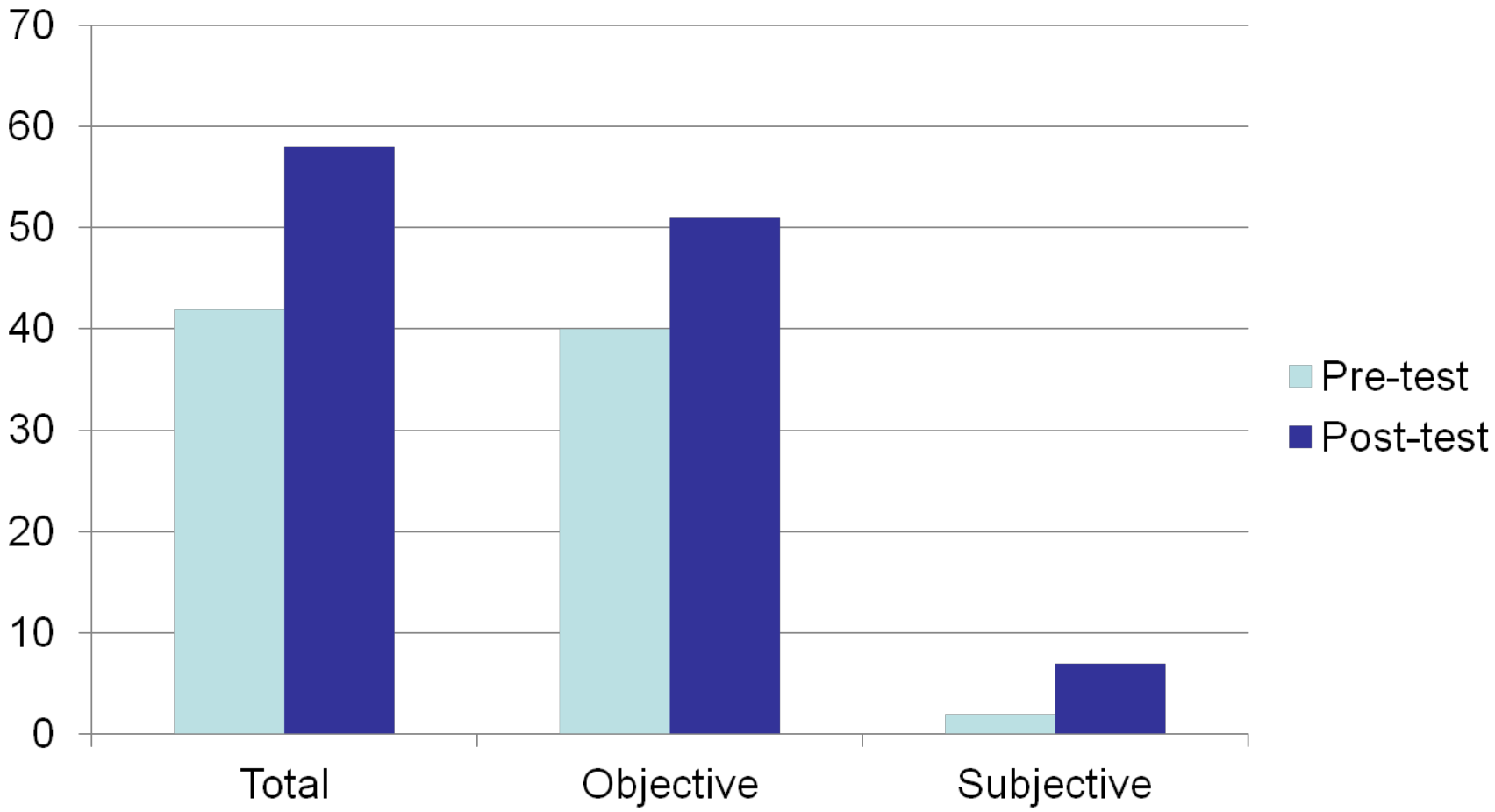


Groundbreaking Research!

- Where did Villa-Komaroff spend 3 years as a post-doctorate?
- How did the city of Cambridge impact her studies; what was the fear?
- Describe the situation and precautions she had to take when she worked on Gilbert's recombinant DNA team.
- What major breakthrough did she make in 1978?



Transformation Lab Scores



Transformation is best described as:

a. The uptake of DNA from a medium and the incorporation of this DNA into the host chromosome.

b. The transfer of DNA from a donor to a recipient cell by direct cell to cell contact.

c. The transfer of DNA into a cell by viruses.

d. **All of the above are examples of transformations.**

Which of the following was the first commercial product of recombinant DNA technology?

a. human growth hormone
b. interferon
c. hepatitis B vaccine
d. penicillin
e. insulin

Subjective questions on transformation

- Briefly outline the steps that are typically carried out to genetically engineer a bacterium so that it contains and expresses (a) gene(s) normally found in human cells.
- What role did Lydia Villa-Komaroff play in the story of bacterial production of proinsulin? What role did Lydia's successes and failures during her educational experience play in her life as a scientist?

Student Survey Results

Student Opinions – Electron Microscopy Lab

Question	Rating range	Rating average
1. This activity brought pictures and text in the book to life and helped me understand operation and use of electron microscopes.	4 - 5	4.6
2. This activity helped me prepare for laboratory and lecture assessments concerning microscopy.	3 - 5	4.1
3. This activity encouraged me to consider taking upper division courses that focus on electron microscopy.	2 - 5	3.2
4. This activity expanded my horizons for careers in biology that involve the use of microscopy.	2 - 5	3.9
5. This activity should be repeated during subsequent semesters.	4 - 5	4.6

Student participants answered each question using the following scale:
1 = strongly disagree
2 = disagree
3 = neutral
4 = agree
5 = strongly agree

Student Opinions – Transformation Lab

Question	Rating range	Rating average
1. This activity brought pictures and text in the book to life and helped me understand the process and uses of transformation.	3 - 5	4.3
2. This activity helped me prepare for laboratory and lecture assessments concerning biotechnology.	3 - 5	4.3
3. This activity encouraged me to consider taking upper division courses that focus on molecular biology.	2 - 5	3.4
4. This activity expanded my horizons for careers in biology that involve the molecular biology.	2 - 5	4.0
5. This activity should be repeated during subsequent semesters.	4 - 5	4.6
6. This activity helped me understand the important contributions that minority scientists like Lydia Villa-Komaroff made to science.	2 - 5	4.3

Student participants answered each question using the following scale:
1 = strongly disagree
2 = disagree
3 = neutral
4 = agree
5 = strongly agree

Conclusions

- An effective method to introduce students to research tools and their applications.
  - Test results revealed areas needing additional emphasis.
- Future goals
  - More hands-on time at the EMs and support equipment (2 labs).
  - Emphasize connections between primary research (PNAS article) and the transformation procedure.