Extension Resources

Gram staining bacteria links

Medpedia, Inc. 2007-2009. Gram Stain - Includes information on why gram stains are done and how they are done.
http://wiki.medpedia.com/Gram_Stain

Gadget Science Gram Staining Bacteria 2007. This site gives the protocol for performing a gram stain.
http://www.gadgetscience.com/gram-staining-bacteria/

Colony PCR – see attached protocol

Gene Sequencing and Bioediting

GenBank Overview, National Center for Biotechnology Information 2009.
http://www.ncbi.nlm.nih.gov/Genbank/index.html
An example of a sequence for Saccharomyces cerevisiae, a species of budding yeast, can be viewed.

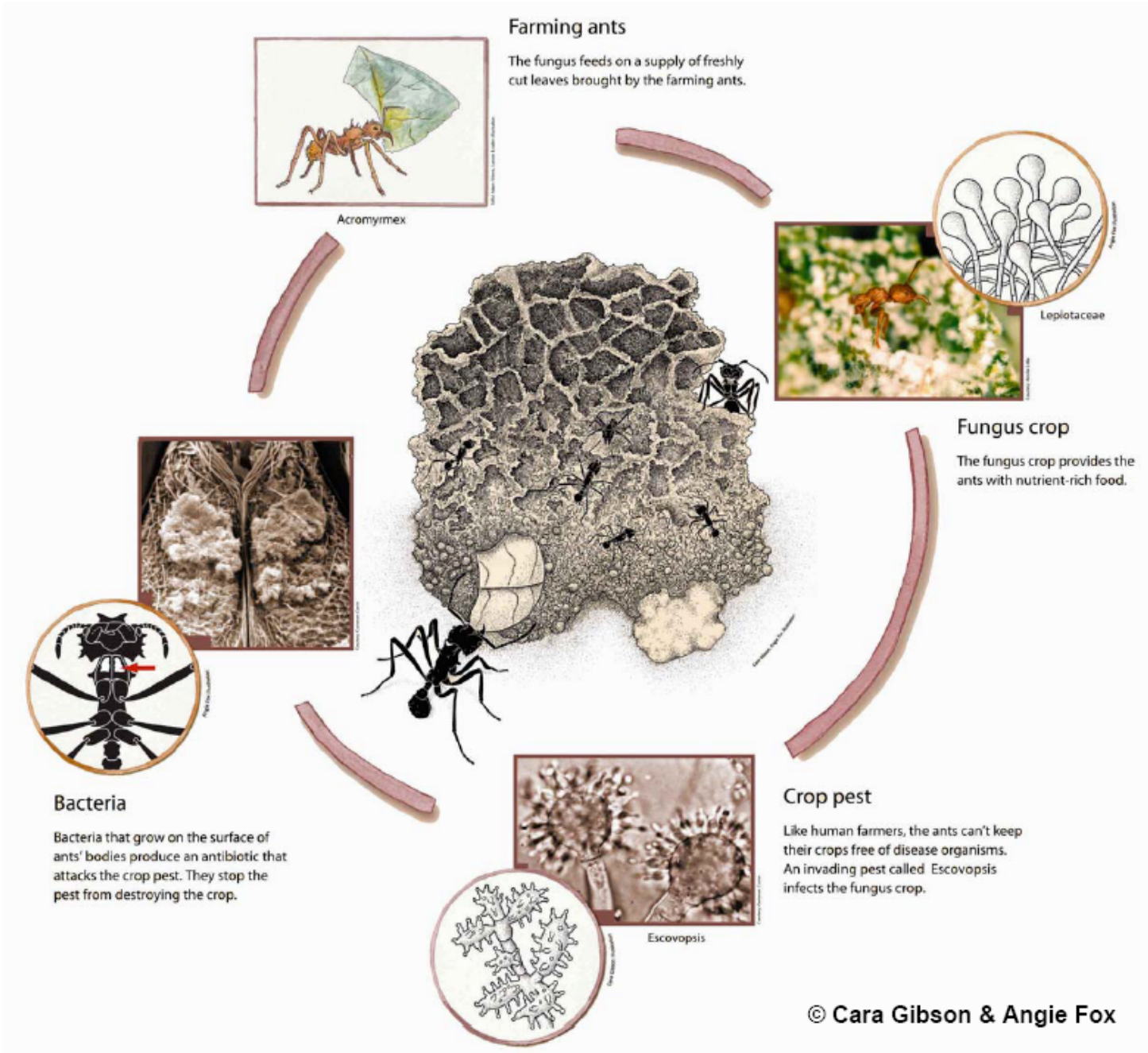
Green Genes – a 16S ribosomal RNA database. This site has a tutorial showing how bio-editing and chromatograms are used in biotechnology. Appropriate for upper level high school students and higher education students. There are sample chromatograms that students can see.
http://greengenes.lbl.gov/cgi-bin/JD_Tutorial/nph-Fasta_Files.cgi

Colony PCR Protocol

Materials

- Thermocycler
- PCR tubes – two per sample to be tested
- Distilled/Deionized Water – 29.5 uL per sample
- Lysis buffer- protease K (optional)- 1uL per sample
- Monoculture of isolates to be tested
- PCR Master Mix – dNTPs, Taq DNA Polymerase with buffers, and MgCl2 – 12.5 uL per sample
- + primer – 0.5uL per sample
- - primer – 0.5uL per sample

1. Aliquot 19uL of water and 1uL of lysis buffer into each PCR tube. Lysis buffer, protease K, will help to break up proteins and allow for better binding of the primers.
2. Using a sterile pipette tip, pick a colony from the plate and mix it into the solution in the PCR tube.
3. Place in the thermocycler
   30min – 50° C
   20min – 60° C
   10min – 65° C
   10min – 96° C
   Lysis buffer works best at 50-65° C, and 95-97° C will kill anything remaining and help to lyse the cells to expose the DNA.
4. At this point they can be placed in the fridge for storage, until the next class period.
5. Centrifuge 2400 rpm for 10 min
6. Aliquot into a second set of PCR tubes: 12.5uL PCR Master Mix, 0.5uL +primer, 0.5uL – primer, 10.5uL water, and 1uL of the supernatant from the tube that was centrifuged.
7. Place in the thermocycler
   5min – 95° C
   Then 25-30 cycles of the following temperatures.
   1min – 94° C (Denature)
   1min – 58° C (Anneal)
   2min – 72° C (Extend)
8. At this point they can be placed in the fridge for storage, until the next class period.
9. The PCR reactants can be run through electrophoresis at this time, load 5-10uL of the PCR product into each well. This ensures that your PCR reaction was successful.
10. The PCR Products can then be sent in for sequencing. Follow the protocol for the specific sequencing service being used.



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