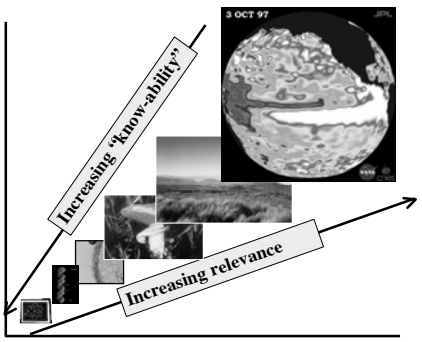
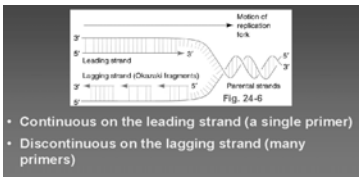
Molecular ecology

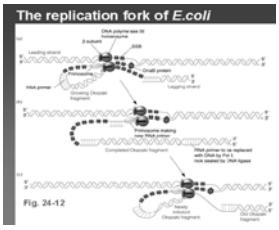
Tools for population, community, and ecosystem studies

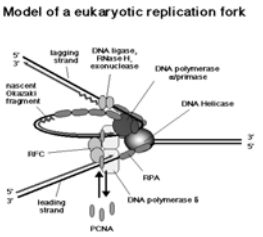


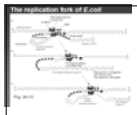
DNA replication in the cell



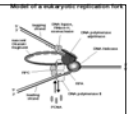
Proteins required for DNA replication





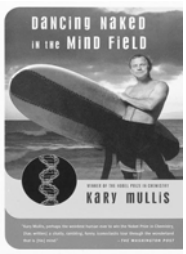


Proteins required for DNA replication



Protein	Function
DNA polymerases	Chain elongation
Helicases	Unwinding of dsDNA
Topoisomerases	Initiates synthesis of RNA primers
Single-stranded binding proteins (SSB)	Prevents premature reannealing of ds DNA
DNA ligase	Seals nicks in phosphodiester backbone

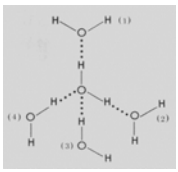
Woah Dude!!!! …. I bet it will work!!

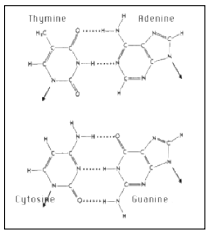


PCR got Mullis the Noble Prize in Chemistry!

- Peoples Republic of China??
- Pulmonary Cardiovascular Resuscitation??
- Polymerase Chain Reaction!!!

The double helix is formed by hydrogen bonds





PCR reaction mix

- Template DNA
- TAQ polymerase
- A buffer to keep TAQ happy
- Nucleotides
  - dGTC, dATP, dTTP, dCTP
- Primer (10 - 20bp in length of DNA)

TAQ polymerase

- *Thermus aquaticus,* a hot spring bacterium
- Proteins are relatively stable at 95°C

The Primers

- A primer is a 15 to 20 base pair DNA strand that is complimentary to a site in the template DNA.
- Usually, 2 different primers are used that flank the *site of interest*

```
  Primer 1

  ctagtagatc…  ——————————————————————→
3′gatcatctag…tacctcatgactblahblahtctatgtctc 5′

5′ctagtagatcatggagtactgahalbhalb…agatacagag 3′
  ←——————————————————————  …tctatgtctc
                              Primer 2
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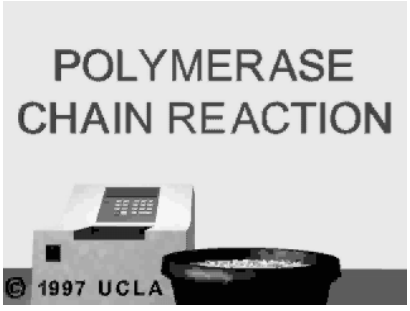
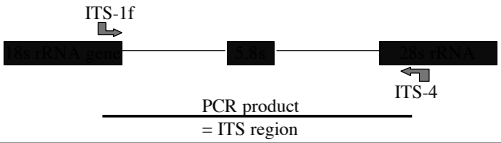


PCR products can be used for identification at all taxonomic levels

	Pop.Eco	Comm.Eco	Ecosystems
• Individuals	√		
• Species	√	√	√
• Families		√	√
• On up the hierarchy		√	√

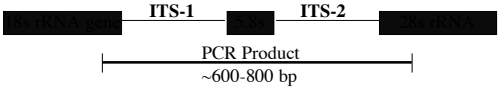
Nuclear ribosomal DNA

- RNA genes are relatively conserved regions
  - Good for primer design
- ITS (internal transcribed spacer) region is highly variable
  - Good for species identification in fungi

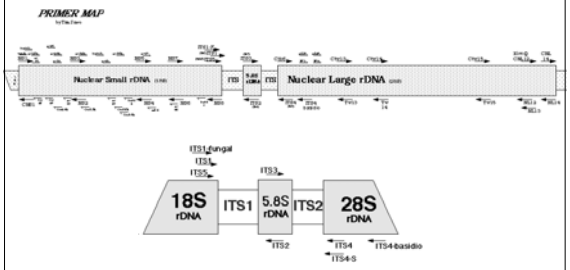


Internal Transcribed Spacer

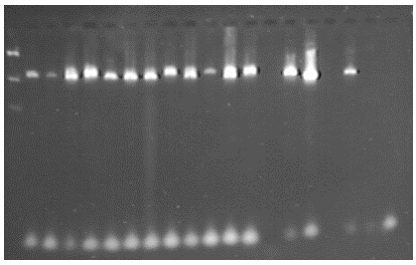
- rDNA gene sequence is repeated up to 200 times
  - under concerted evolution….A change in one repeat copy is duplicated in all the others
- ITS regions are spliced out during ribosome processing
  - Mutations are neutral (no selection)



Nuclear rDNA Primer Maps



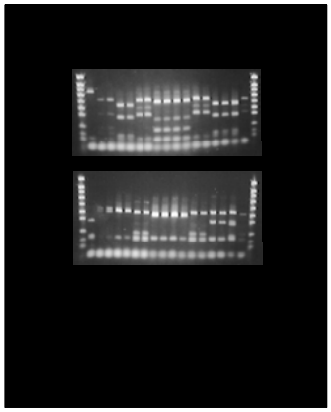
Checking for Successful PCRs



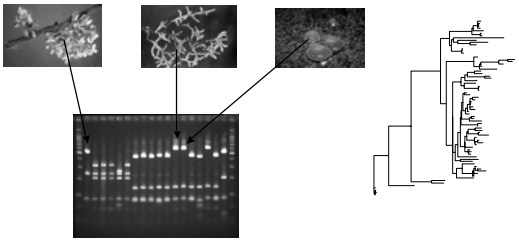
After PCR…

- Variation in the DNA sequence is analyzed using
  - RFLP analysis
    - Restriction Fragment Length Polymorphism
  - Direct sequence analysis
    - Blast search in Genbank
    - Phylogenetic placement

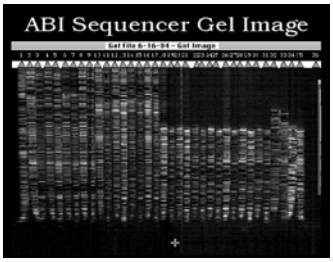
RFLP



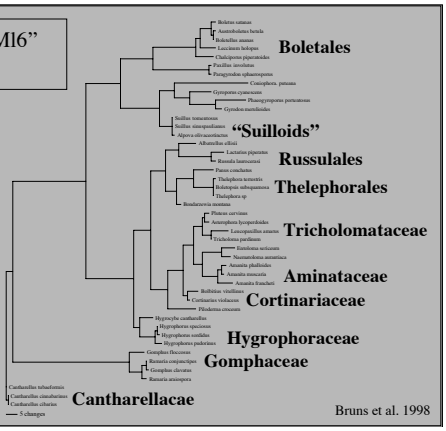
Species diagnostics from ectomycorrhizal roots



Sequencing

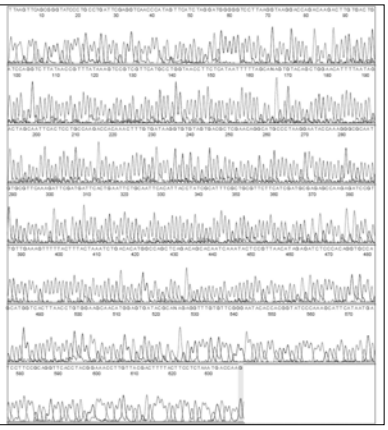


The "M15-M16" Database

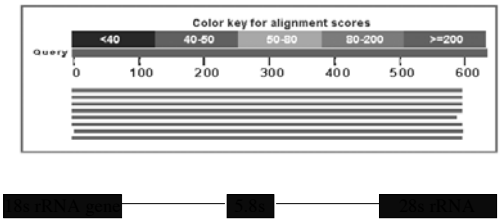


Bruns et al. 1998

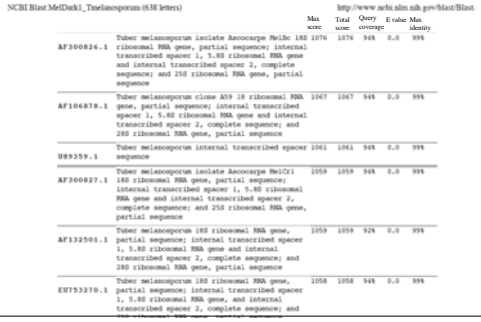
ITS sequence of an unknown fungus from a root tip



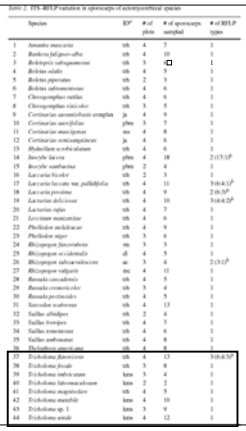
8 of 100 top Blast Hits in Genbank



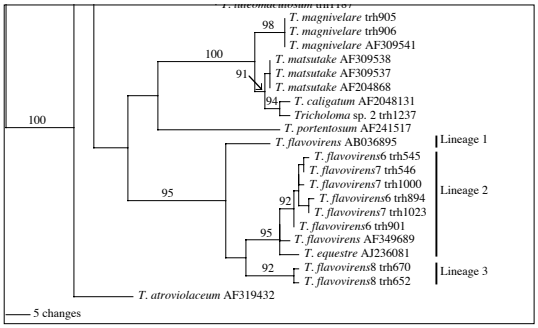
6 of 100 top Blast Hits



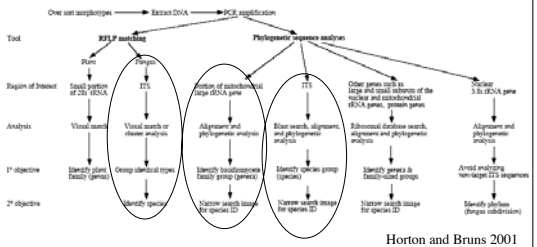
Sand dune mushroom RFLP survey



Horton 2002



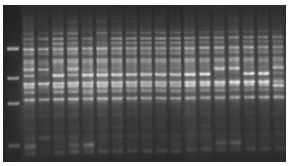
Flow Chart



Horton and Bruns 2001

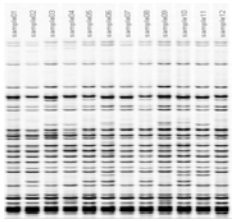
Identifying individuals

RAPD



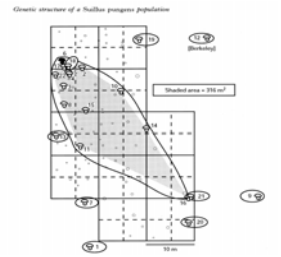
Randomly Amplified Polymorphic DNA

AFLP



Amplified Fragment Length Polymorphism

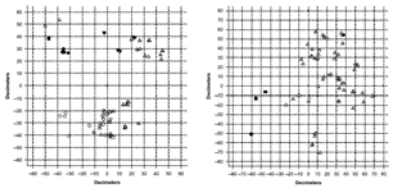
Not Quite a Humungous Fungus



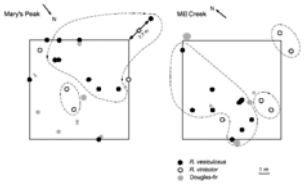
Suillus pungens - Bonello et al. 1996

Chanterelles

Dunham et al. 2003



Kretzer et al. 2003



Rhizopogon