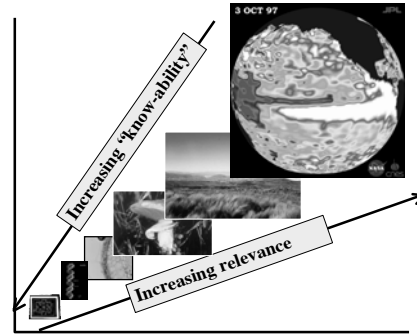
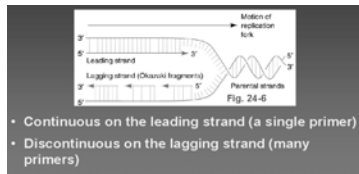


Molecular ecology

Tools for population, community,
and ecosystem studies

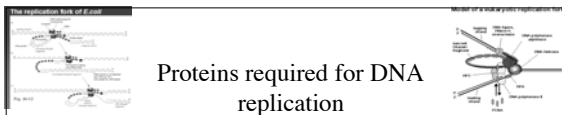
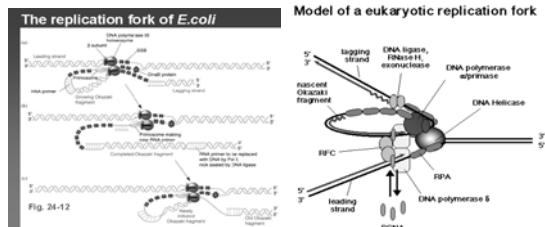


DNA replication in the cell



- Continuous on the leading strand (a single primer)
- Discontinuous on the lagging strand (many primers)

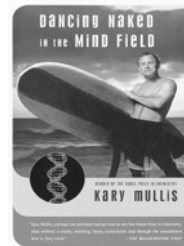
Proteins required for DNA replication



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

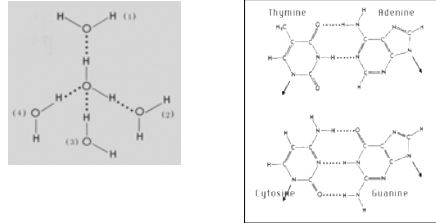
Wash Dudette!!! I bet it will work!!



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
    
```

POLYMERASE CHAIN REACTION

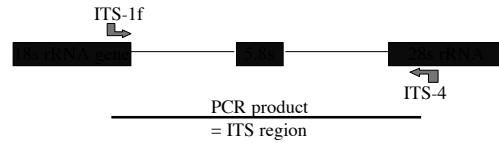


PCR products can be used for identification at all taxonomic levels

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

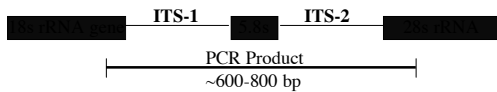
Nuclear ribosomal DNA

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

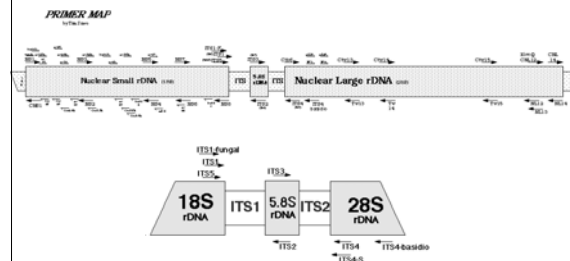


Internal Transcribed Spacer

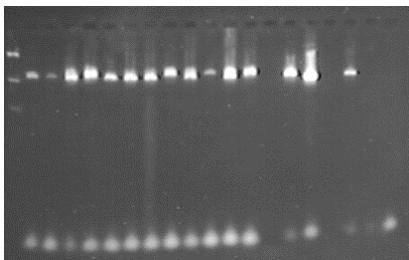
- rDNA gene sequence is repeated up to 200 times
 - under concerted evolution....A change in one repeat copy is duplicated in all the others
- ITS regions are spliced out during ribosome processing
 - Mutations are neutral (no selection)



Nuclear rDNA Primer Maps

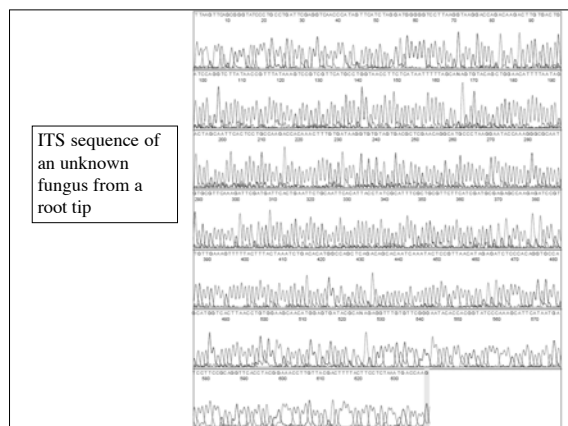
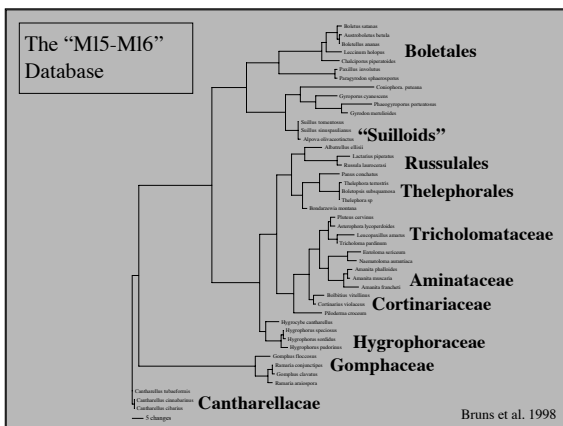
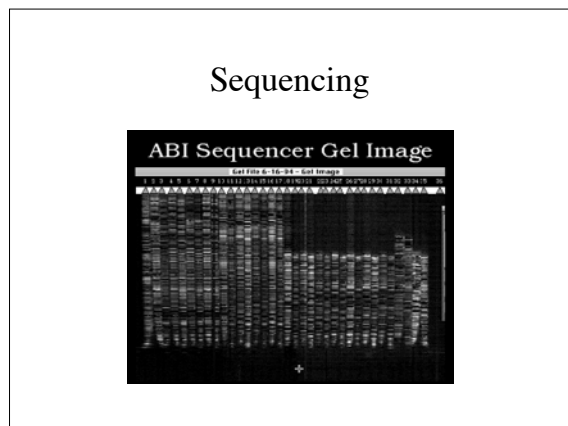
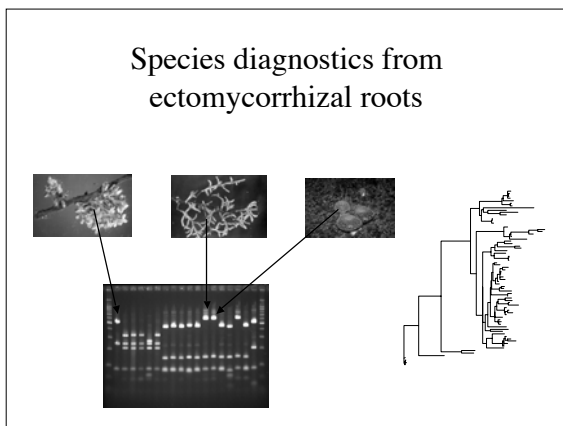
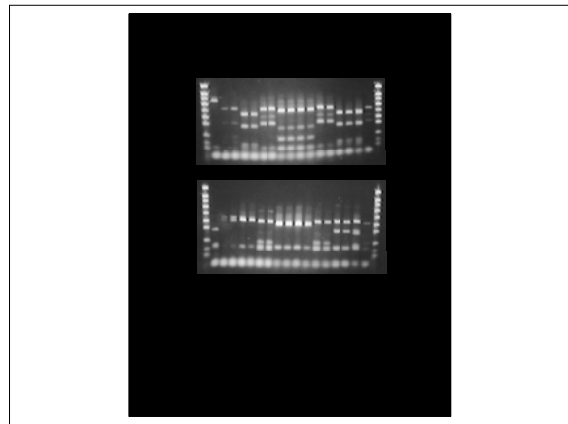
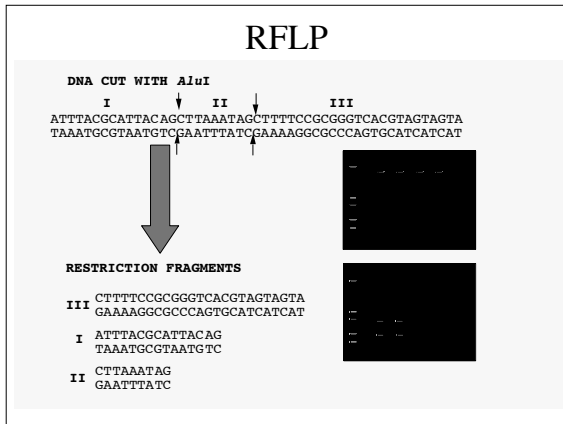


Checking for Successful PCRs

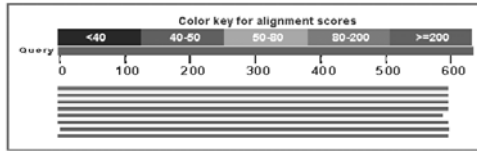


After PCR...

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



8 of 100 top Blast Hits in Genbank



6 of 100 top Blast Hits

NCBI Blast Me/Dark1_Tuberolomus (538 letters) <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>

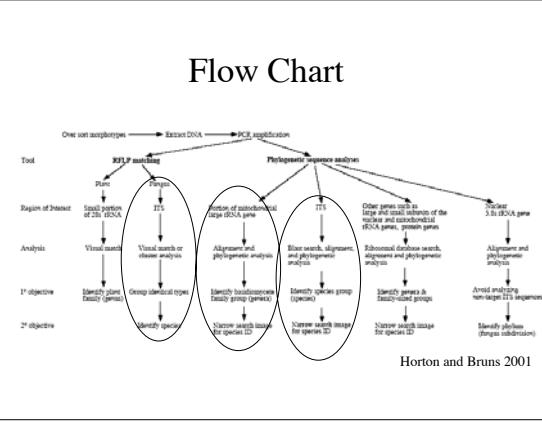
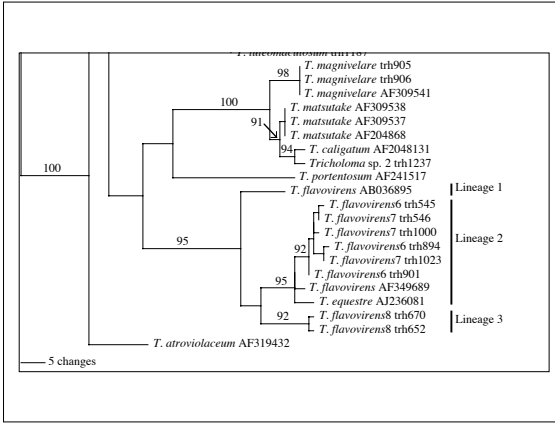
Accession	Match	Query	E-value	Max. score	Coverage	Identity
AF300824.1	Tuber melanosporum isolate Ancocharge MelC1	180	2176	1076	948	0.0 99%
AF106878.1	Tuber melanosporum clone A59 18	180	1047	1047	948	0.0 99%
U89259.1	Tuber melanosporum internal transcribed spacer 1	191	1041	1041	948	0.0 99%
AF300823.1	Tuber melanosporum isolate Ancocharge MelC1	180	1059	1059	948	0.0 99%
AF122501.1	Tuber melanosporum 18S ribosomal RNA gene	1059	1059	928	0.0 99%	
EU55270.1	Tuber melanosporum 18S ribosomal RNA gene	1058	1058	948	0.0 99%	

Table 2. 18S rDNA RFLP variation in a group of *Tuberolomus* species

Species	Genotype	# of genotypes	# of RFLP types
1. <i>Tuberolomus</i> sp. 1	18	4	1
2. <i>Tuberolomus</i> sp. 2	18	4	1
3. <i>Tuberolomus</i> sp. 3	18	4	1
4. <i>Tuberolomus</i> sp. 4	18	4	1
5. <i>Tuberolomus</i> sp. 5	18	4	1
6. <i>Tuberolomus</i> sp. 6	18	4	1
7. <i>Tuberolomus</i> sp. 7	18	4	1
8. <i>Tuberolomus</i> sp. 8	18	4	1
9. <i>Tuberolomus</i> sp. 9	18	4	1
10. <i>Tuberolomus</i> sp. 10	18	4	1
11. <i>Tuberolomus</i> sp. 11	18	4	1
12. <i>Tuberolomus</i> sp. 12	18	4	1
13. <i>Tuberolomus</i> sp. 13	18	4	1
14. <i>Tuberolomus</i> sp. 14	18	4	1
15. <i>Tuberolomus</i> sp. 15	18	4	1
16. <i>Tuberolomus</i> sp. 16	18	4	1
17. <i>Tuberolomus</i> sp. 17	18	4	1
18. <i>Tuberolomus</i> sp. 18	18	4	1
19. <i>Tuberolomus</i> sp. 19	18	4	1
20. <i>Tuberolomus</i> sp. 20	18	4	1
21. <i>Tuberolomus</i> sp. 21	18	4	1
22. <i>Tuberolomus</i> sp. 22	18	4	1
23. <i>Tuberolomus</i> sp. 23	18	4	1
24. <i>Tuberolomus</i> sp. 24	18	4	1
25. <i>Tuberolomus</i> sp. 25	18	4	1
26. <i>Tuberolomus</i> sp. 26	18	4	1
27. <i>Tuberolomus</i> sp. 27	18	4	1
28. <i>Tuberolomus</i> sp. 28	18	4	1
29. <i>Tuberolomus</i> sp. 29	18	4	1
30. <i>Tuberolomus</i> sp. 30	18	4	1
31. <i>Tuberolomus</i> sp. 31	18	4	1
32. <i>Tuberolomus</i> sp. 32	18	4	1
33. <i>Tuberolomus</i> sp. 33	18	4	1
34. <i>Tuberolomus</i> sp. 34	18	4	1
35. <i>Tuberolomus</i> sp. 35	18	4	1
36. <i>Tuberolomus</i> sp. 36	18	4	1
37. <i>Tuberolomus</i> sp. 37	18	4	1
38. <i>Tuberolomus</i> sp. 38	18	4	1
39. <i>Tuberolomus</i> sp. 39	18	4	1
40. <i>Tuberolomus</i> sp. 40	18	4	1
41. <i>Tuberolomus</i> sp. 41	18	4	1
42. <i>Tuberolomus</i> sp. 42	18	4	1

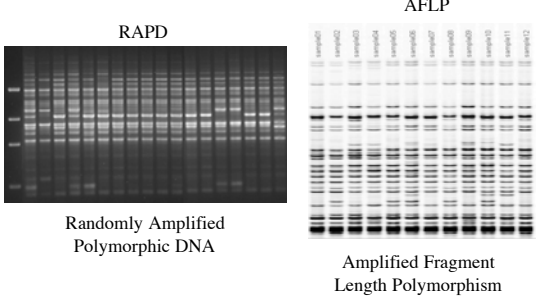
Sand dune mushroom RFLP survey

Horton 2002

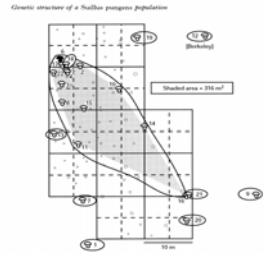


Horton and Bruns 2001

Identifying individuals



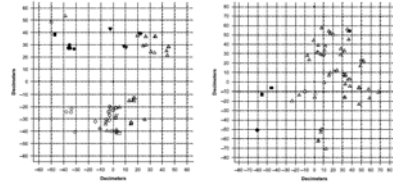
Not Quite a Humungous Fungus



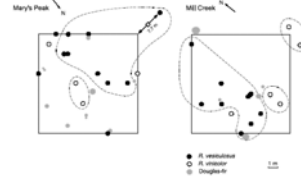
Suillus pungens - Bonello et al. 1996

Chanterelles

Dunham et al. 2003



Kretzer et al. 2003



Rhizopogon