1.89, Environmental Microbiology
Prof. Martin Polz
Lecture 15

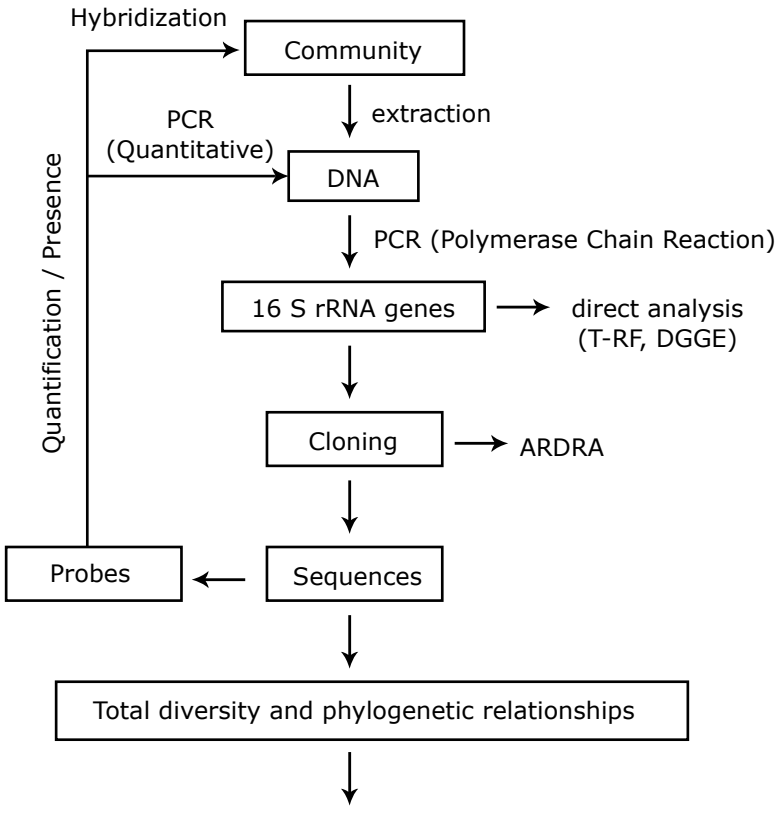
A. How many Microbes are there? → Direct Counts
→ average cell concentration × volume of habitat > 10^{30} prokaryotic cells

B. Biomass of plants ~ equal to biomass of prokaryotes

C. Diversity: 1980s: Carl Woese → used sequence similarities to determine phylogenetic relationships among microorganisms.

→ Carl proposed the 3 domain idea, separating prokaryotes into: Bacteria and Archaea.

Norman Pace → application to environment "phylogenetic framework"

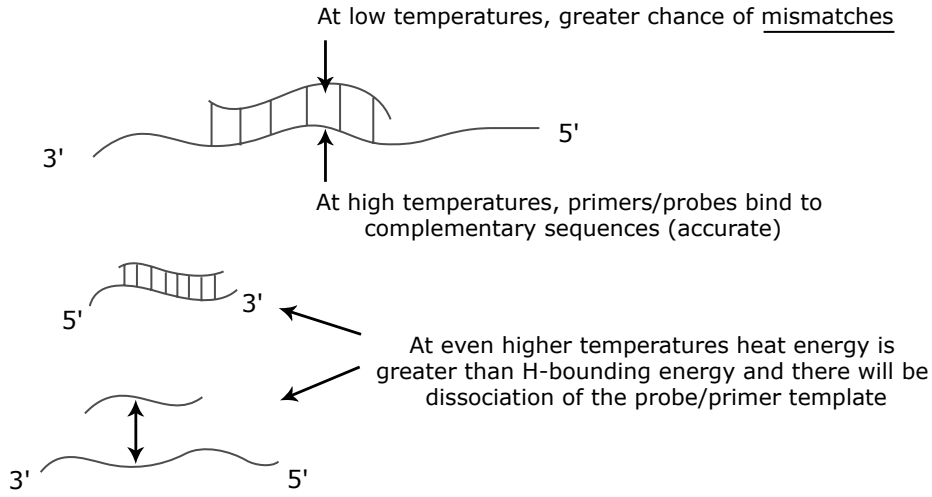


See examples handout: Acinas et al.

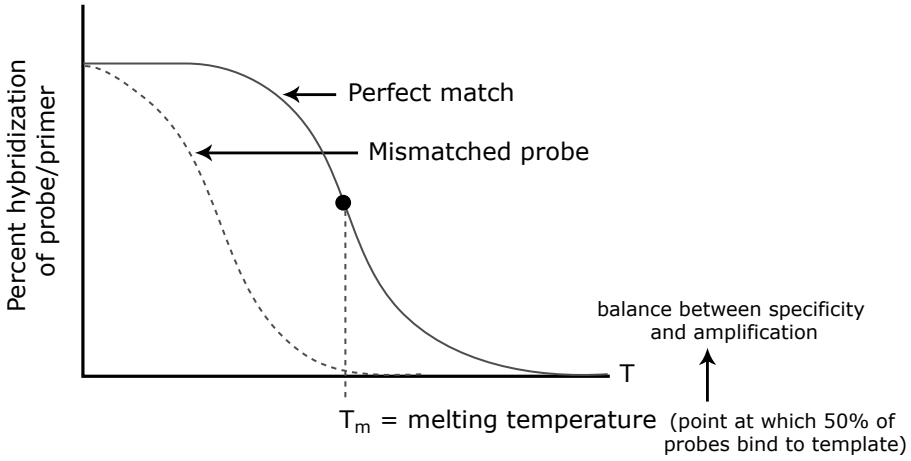
Probes and Primers = single–stranded pieces of DNA that hybridize to target sequence

"probes" → hybridization techniques
"primers" → PCR analysis

- DNA/RNA hybridizes in a temperature dependent fashion

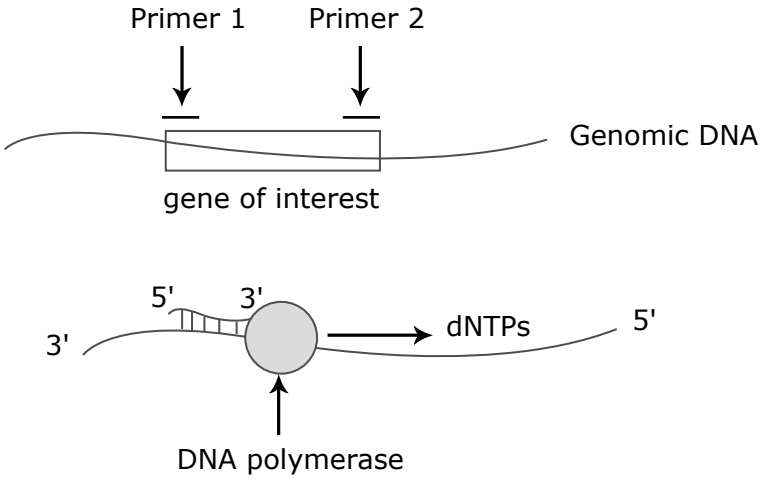


Melting Curves



PCR

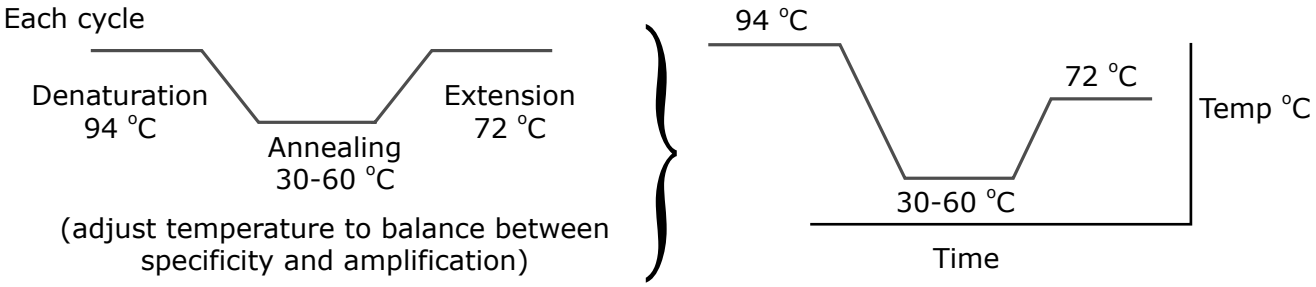
Allows for the amplification of specific genes to million–billion fold.



PCR reaction contains

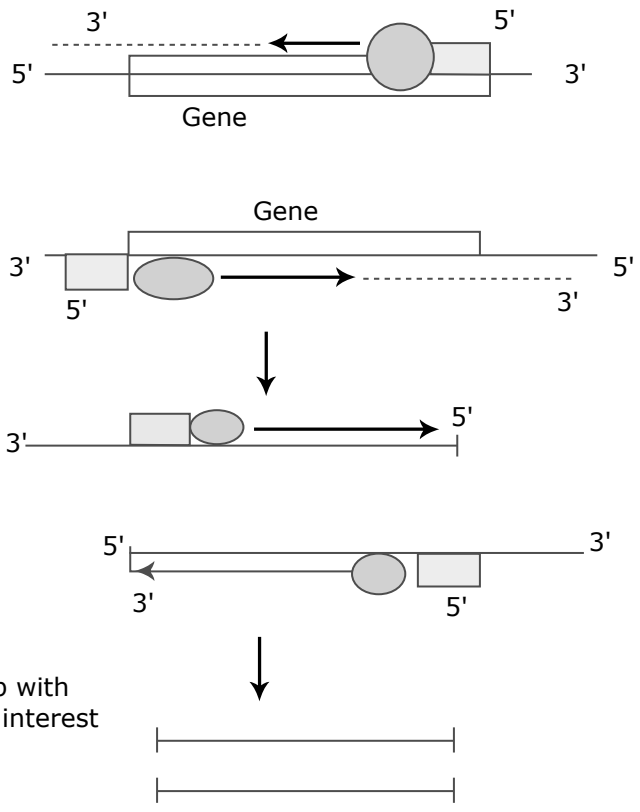
- Target DNA (example: environmental DNA)
- 2 primers (20-30 nts long)
- Thermostable DNA polymerase
- Nucleotides (dNTPs)
- Mg^{2+} (cofactor for DNA polymerase)

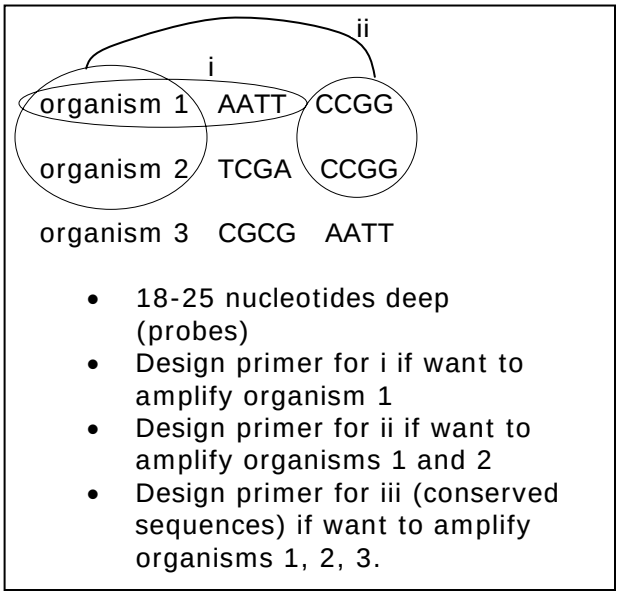
Mix is subjected to temperature cycling



Almost pure sample!

Repeat cycle! → Allows for exponential increase of target gene to the point at which genomic DNA has been diluted out.





- 18-25 nucleotides deep (probes)
- Design primer for i if want to amplify organism 1
- Design primer for ii if want to amplify organisms 1 and 2
- Design primer for iii (conserved sequences) if want to amplify organisms 1, 2, 3.

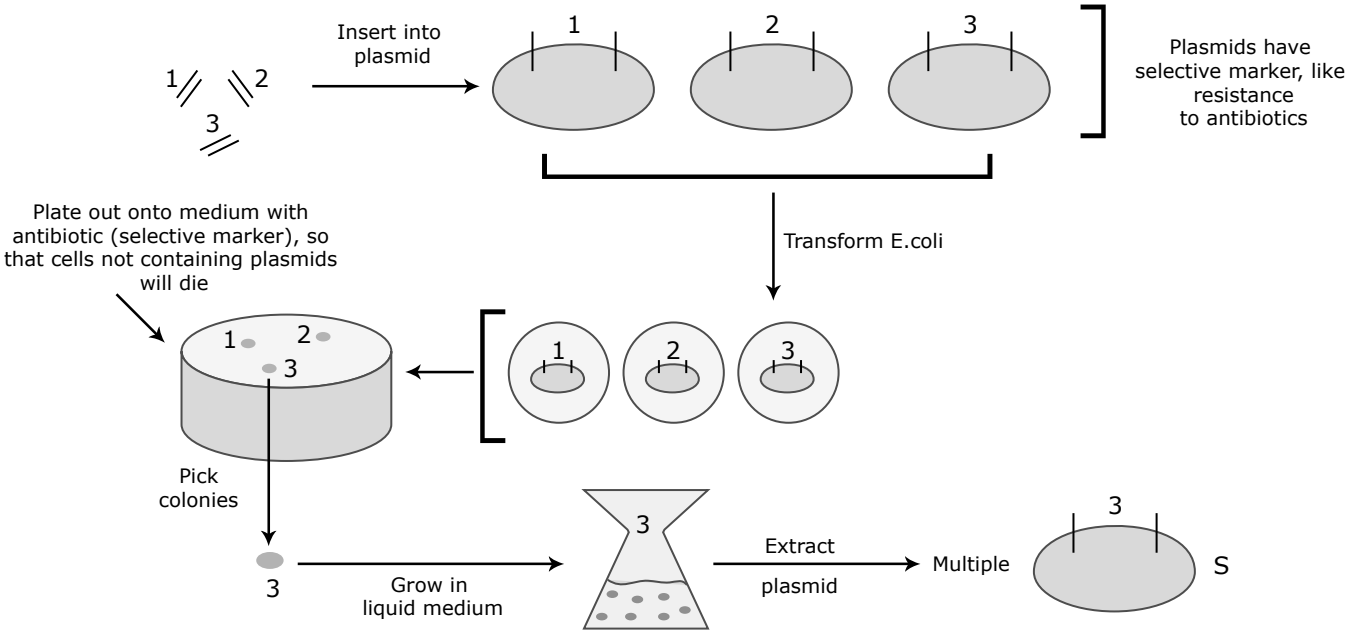
⇒ **PCR Primer Design**

1) Specific Primers – uniquely match a certain sequence

2) Universal Primers – recognize for example all bacteria

3) Group Specific Primers – recognizes sequences specific to certain groups

Cloning



Example: Ocean bacterioplankton

Most abundant organisms have eluded cultivation. We only know of their existence through cloning.

Plate Count (CFU)
Direct Count (DAPF)
Cloning “Great Plate Count Anomaly”