

Ed DeLong

Diversity and variability of microbes in the environment

Unculturable microorganisms

- ① obligate symbionts
- ② free living marine

rRNA - small subunit

invariant regions serve to align & seq. primers

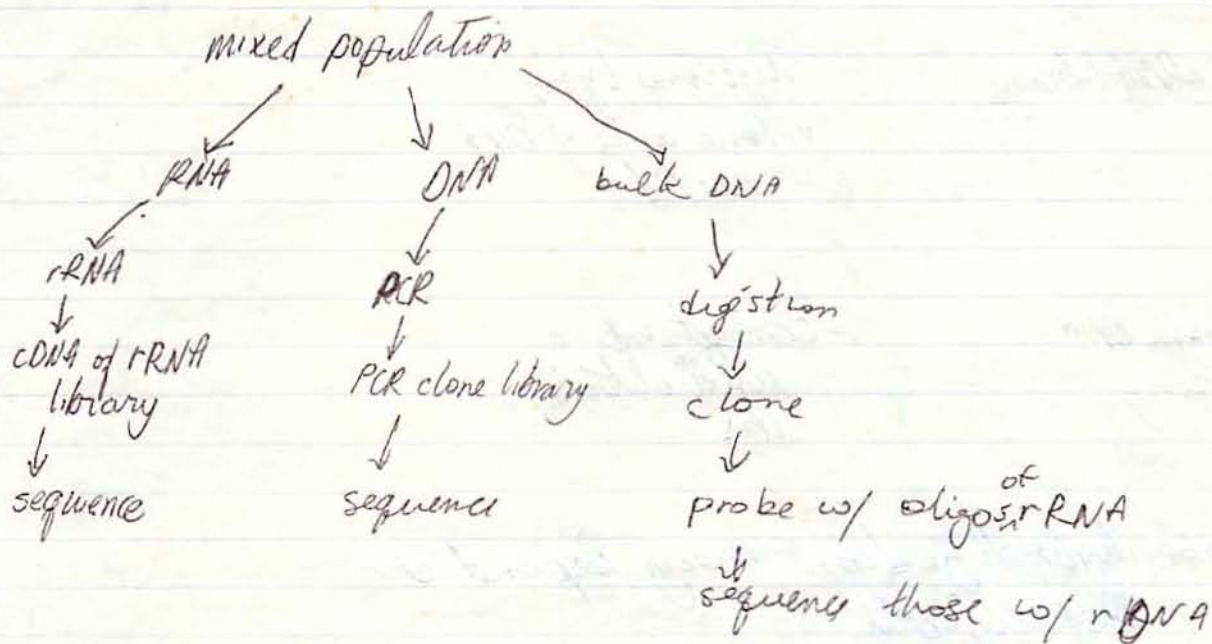
① align

② count differences

③ calc. evol. distance

④ find tree topology that best fits all pairwise comparisons

Retrieving RNA genes from natural populations



Nature 345:63

Weller/Ward AEM 55:1818. 1989.

(A)

① RNA extraction

② cDNA library of rRNA

③ vector --- sequence

but only gave ~500 bp
sec. ---

(B) Giovannoni - PCR library

Nature 345:602.

rCDNA

+
- can use strong lysis protocol
- all clones should be
rDNA --- don't have to
screen

PCR DNA library

rigorous lysis
v. low amts of DNA
needed

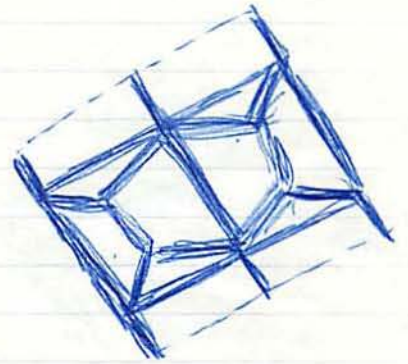
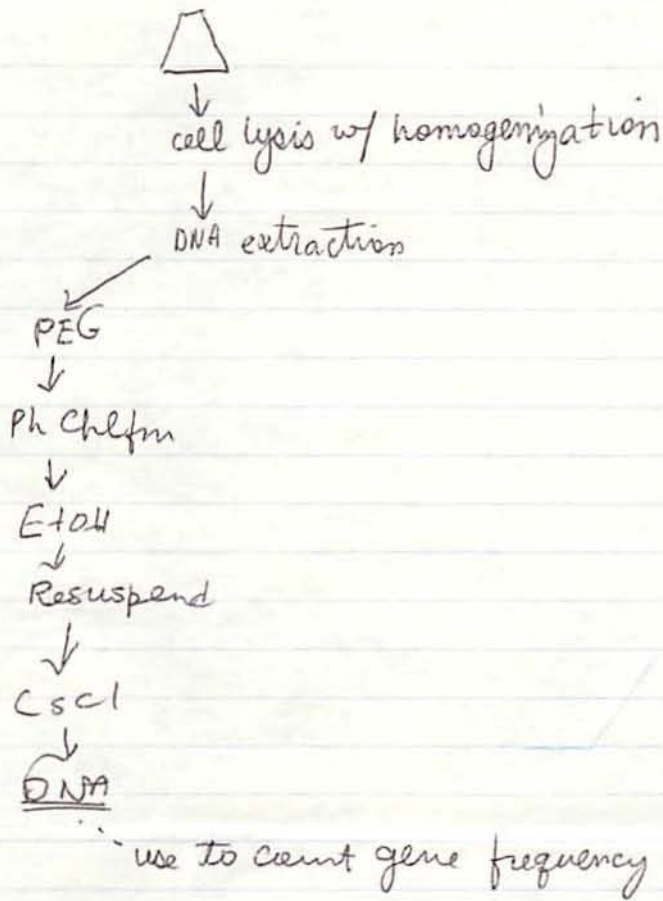
shotgun DNA
library

- can get info a
little outside
lbs

probe hybridization may depend on

① specificity

② membrane permeability

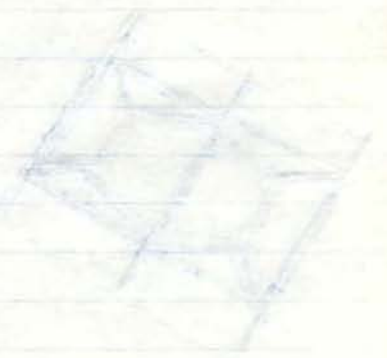


Isolating DNA from soil

⊙ need to remove humic material by hydroxyapatite

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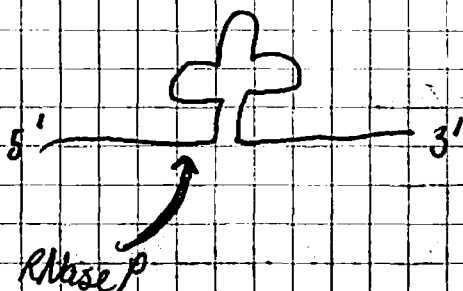
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Diversity can be invisible.

- ① align homologous sequences
- ② count differences in each pair of sequences
- ③ calculate the evolutionary distance between the sequences
- ④ define the tree that best fits ALL distances

Structural Analysis



- RNase P. cuts 5' end of tRNA
- v. similar in *E. coli* & *B. subtilis*
- RNA and protein
- RNA is catalytic center

- what is structure of RNA subunit of RNase P

20
30

- difficulties
 - ① short binding regions (~5 BP) yet only 4 diff. bases so very many possibilities

②

3 methods of working out 2^o structure

①

② minimum energy

③ phylogeny

- compare E. coli & B. subtilis

since NOT v. similar can

pick a closer relative

(based on RFLP phylogeny)

- see where similar complement
occur in closely related
organisms.

After get general 2^o structure

③ deletion experiments

- mould "gaps" across molecularly
and definitively removed.

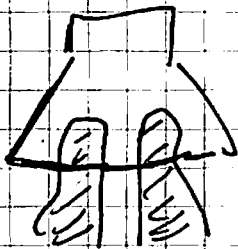
③ see what deletions
lead to changes

③ see what deletions
do nothing

wherever structure that should be
the region of conserved function
So can remove other sequences

Upper Temperature Limits of life

- ① put contact slides down
- ② vent caps

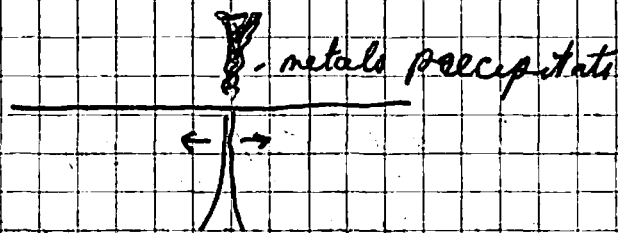


May be able to do phylogeny on single cells through oligonucleotide probes.

- ① probes for all life
- ② kingdom level probes
- ③ species " "

Growth rate determines # of ribosomes
(or is it vice versa)

Hydrothermal Vents



but

can't cultivate symbionts

- phylogeny may tell a great deal
about general biochem of symbionts

Methods of Characterizing Microbial World

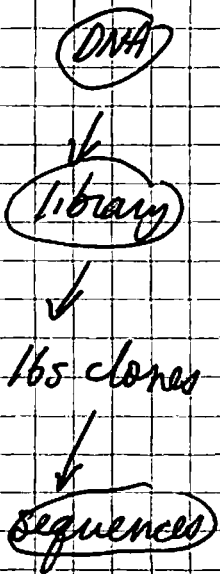
Problems

① Cultivation difficult

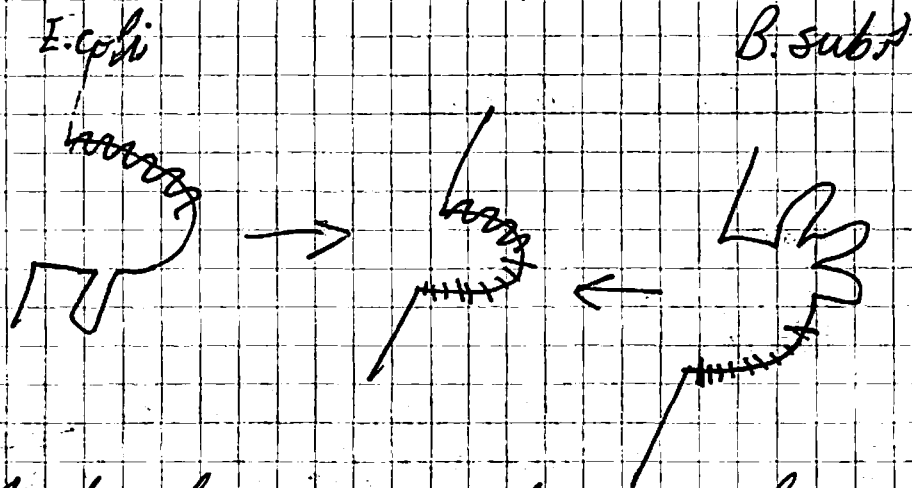
So what use --

① Phylogeny

- phylogeny can help guide future research (such as was used for RNase P 2^o structure.)



but there is a problem because you don't know what to learn



make chimera - combine simpler regions of two molecules.