

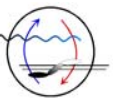
Using the ARB-Tool for Identification of Microorganisms

Frank Oliver Glöckner

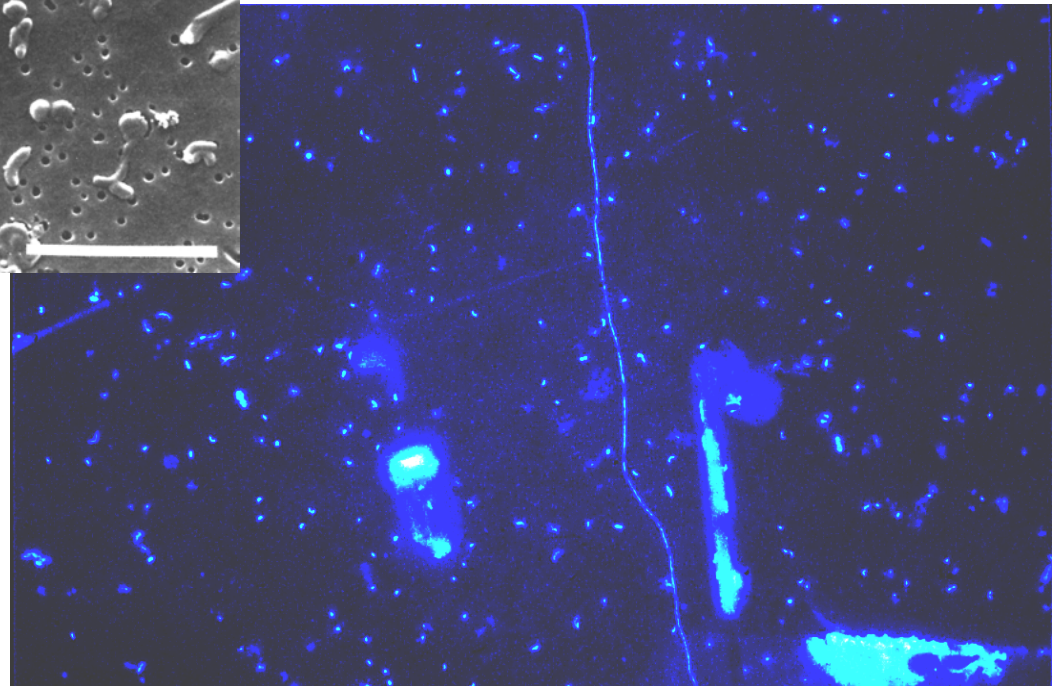
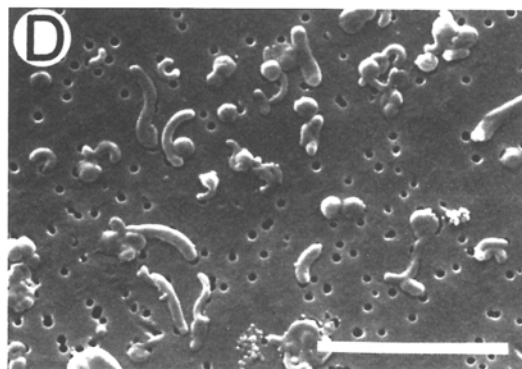
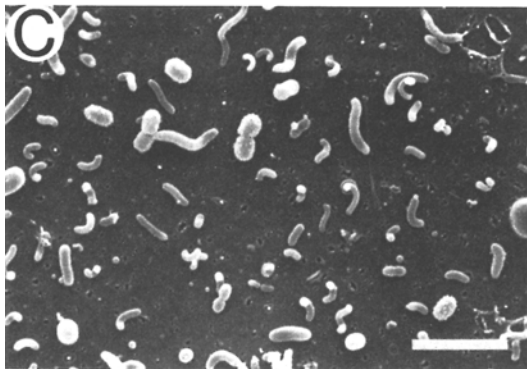
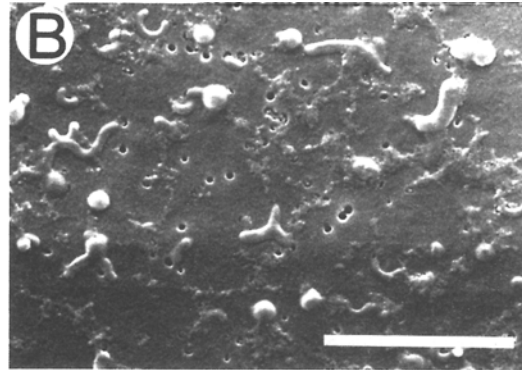
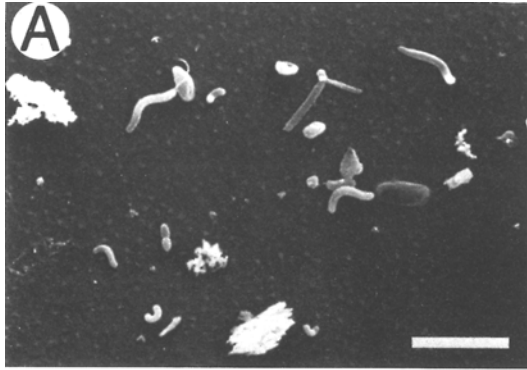
Wolfgang Ludwig

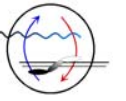
Max Planck Institute for Marine Microbiology Bremen

Technical University Munich

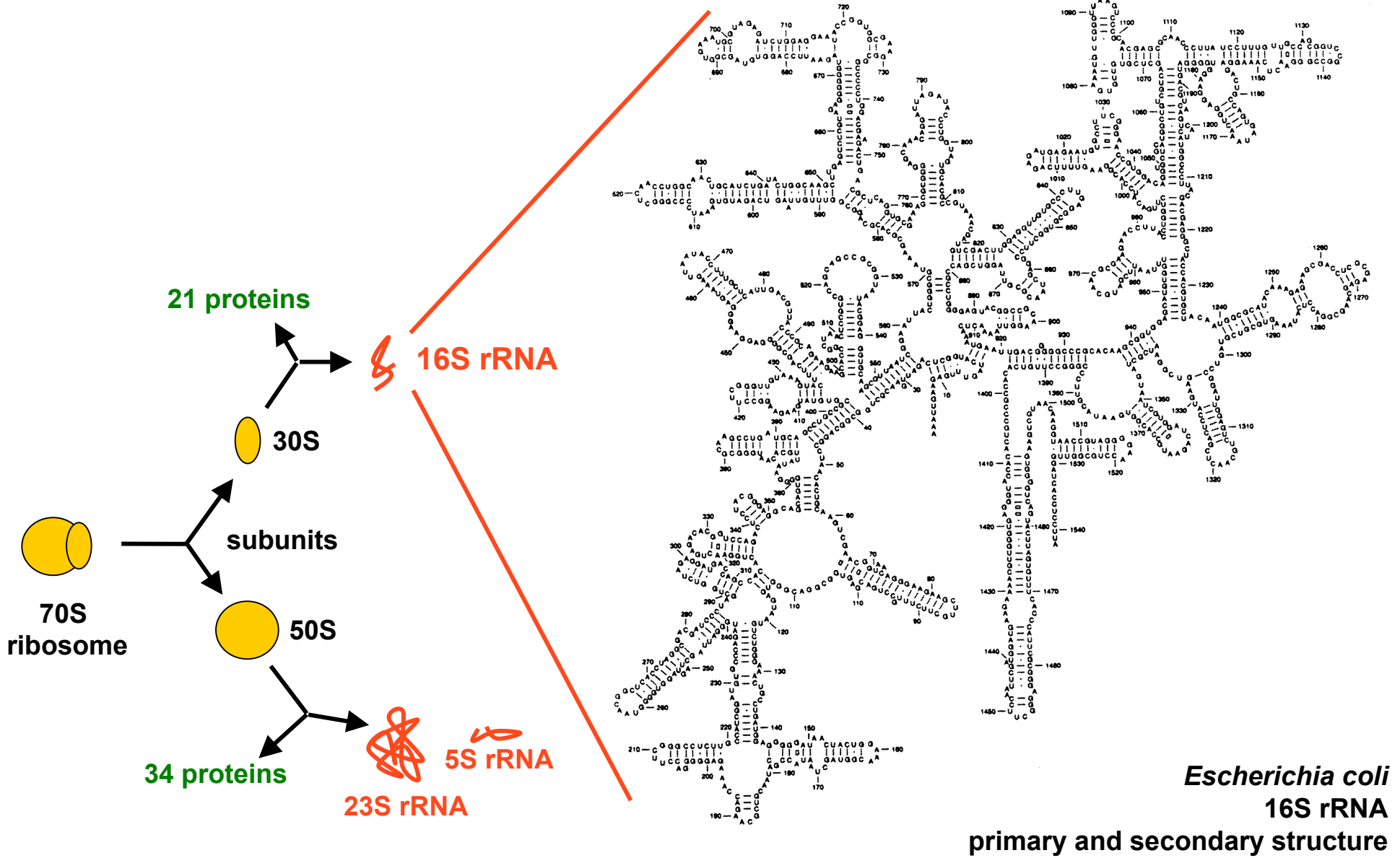


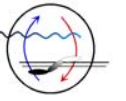
Identification?





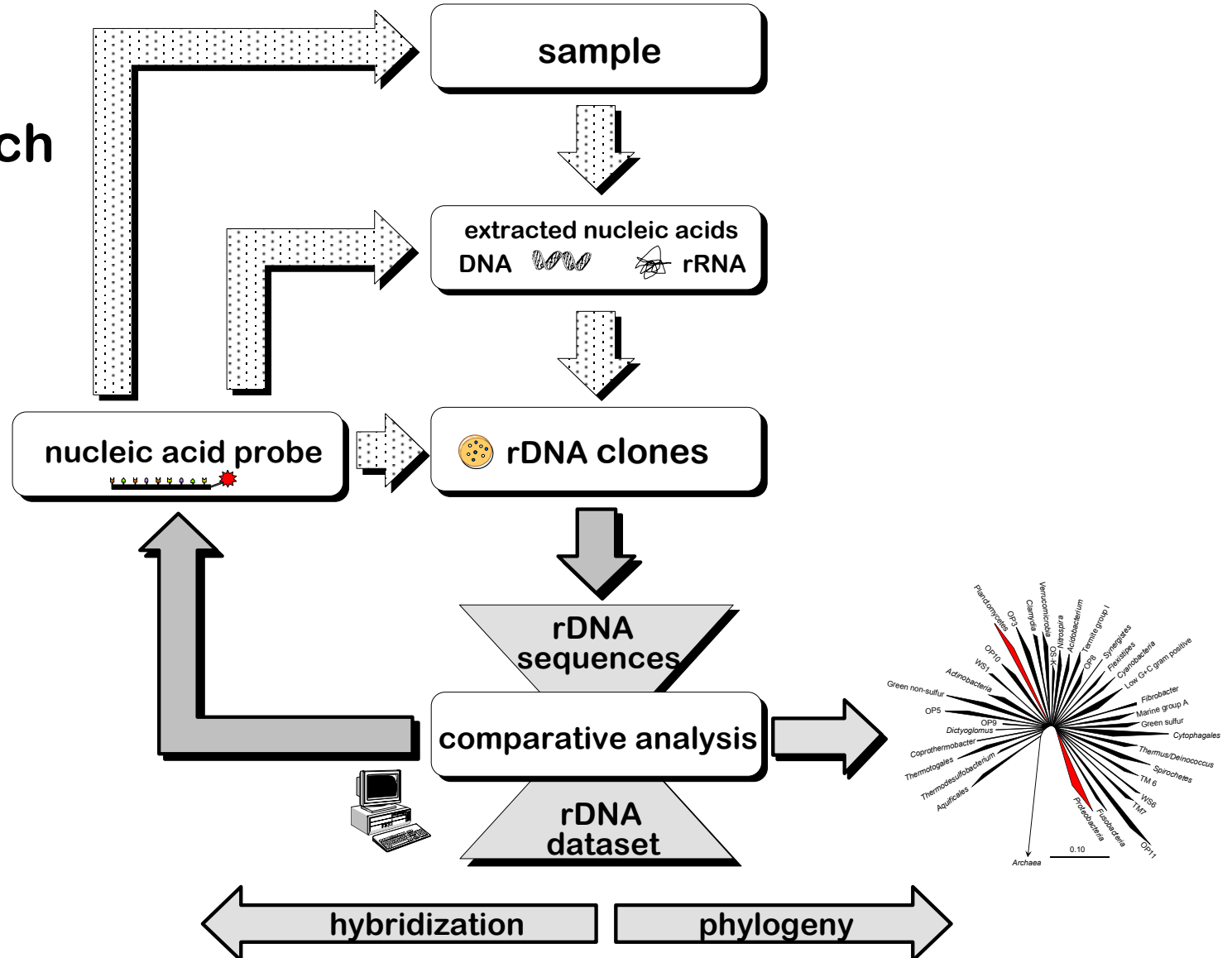
The 16S ribosomal RNA

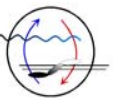




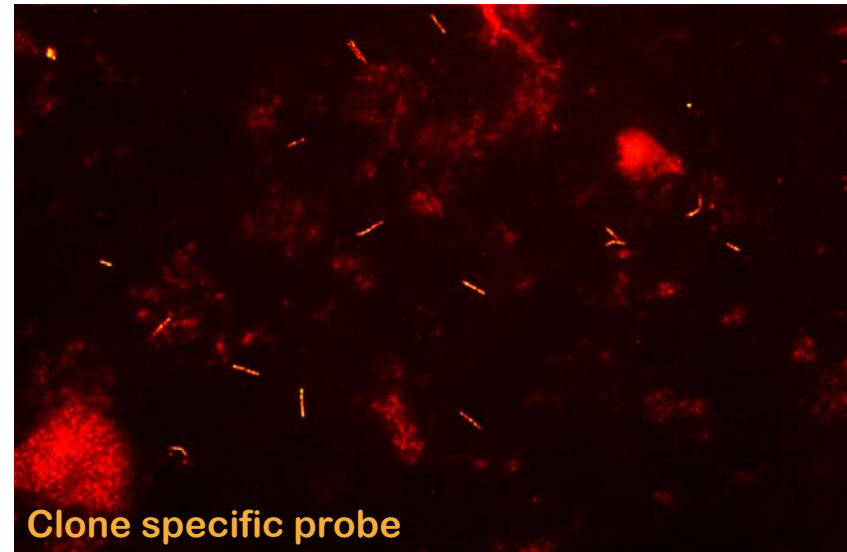
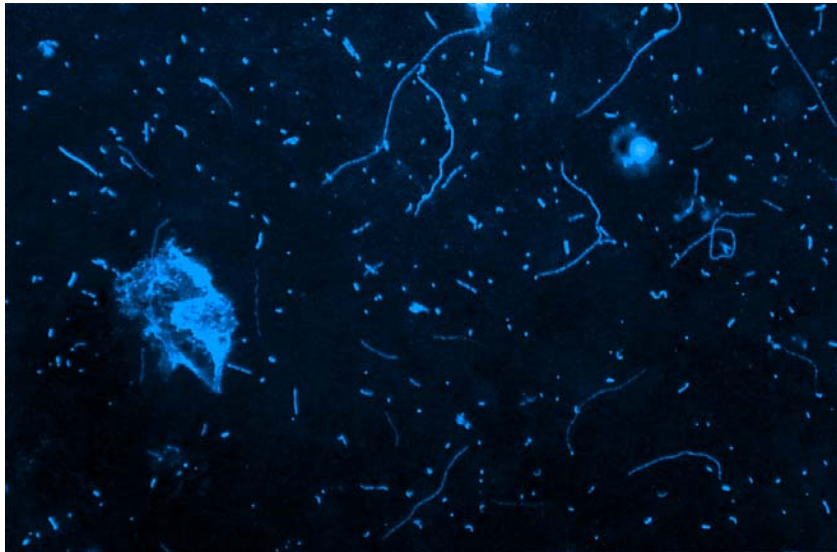
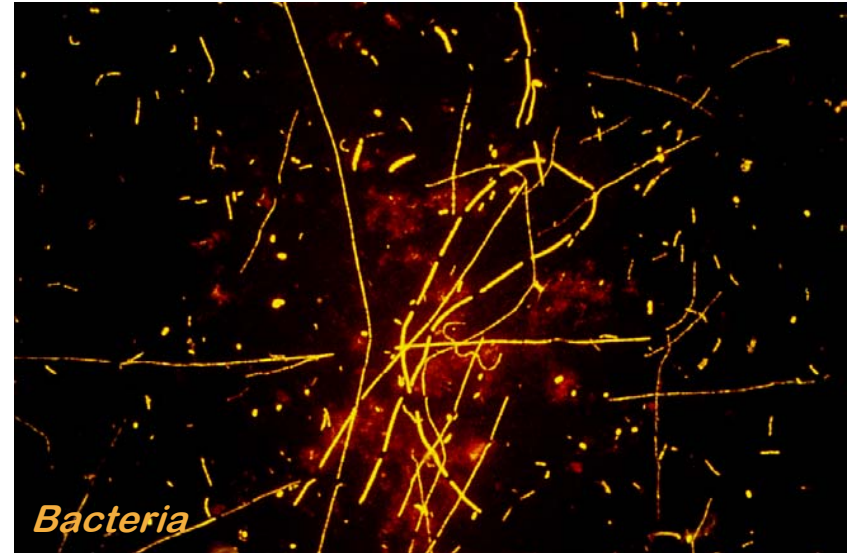
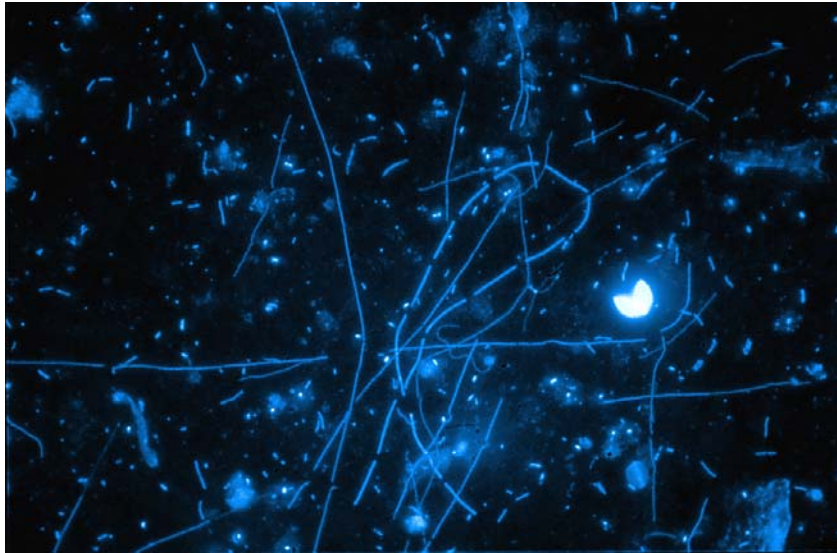
Linking Diversity with Community Composition

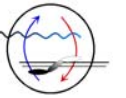
Full cycle rRNA-approach



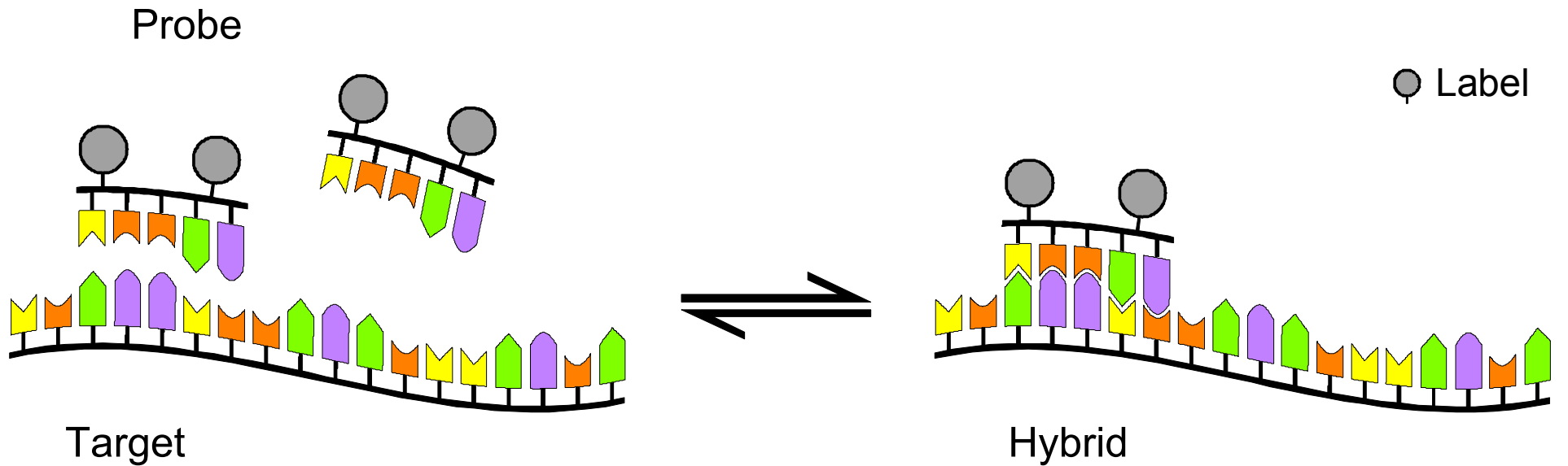


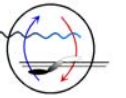
Gossenköllesee





Probe Design





Probe Design

▶ Criteria for probe design

- Dissociation temperature

- ◊ G+C content

- ◊ $T_d = 4N_{(G+C)} + 2N_{(A+T)}$

Suggs, 1981

- Number of diagnostic mismatches

- Quality of mismatches

- ◊ Destabilizing:

A-A, A-C, T-T, T-C, C-C

- ◊ Slightly destabilizing:

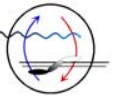
G-T, G-A, G-G

- Position of mismatches

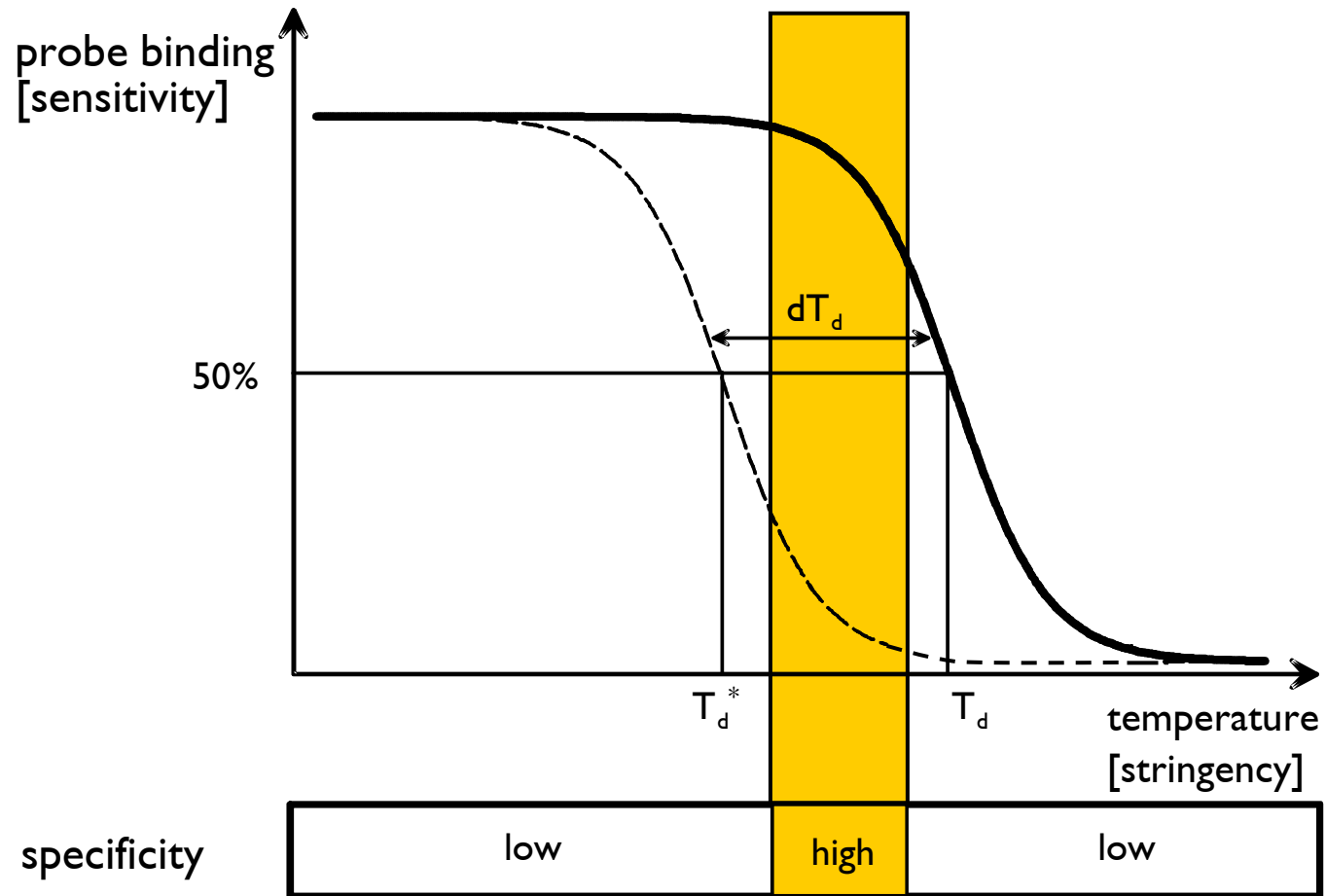
- Neighborhood bases

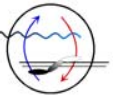
- Intramolecular base pairing

- Method of hybridization



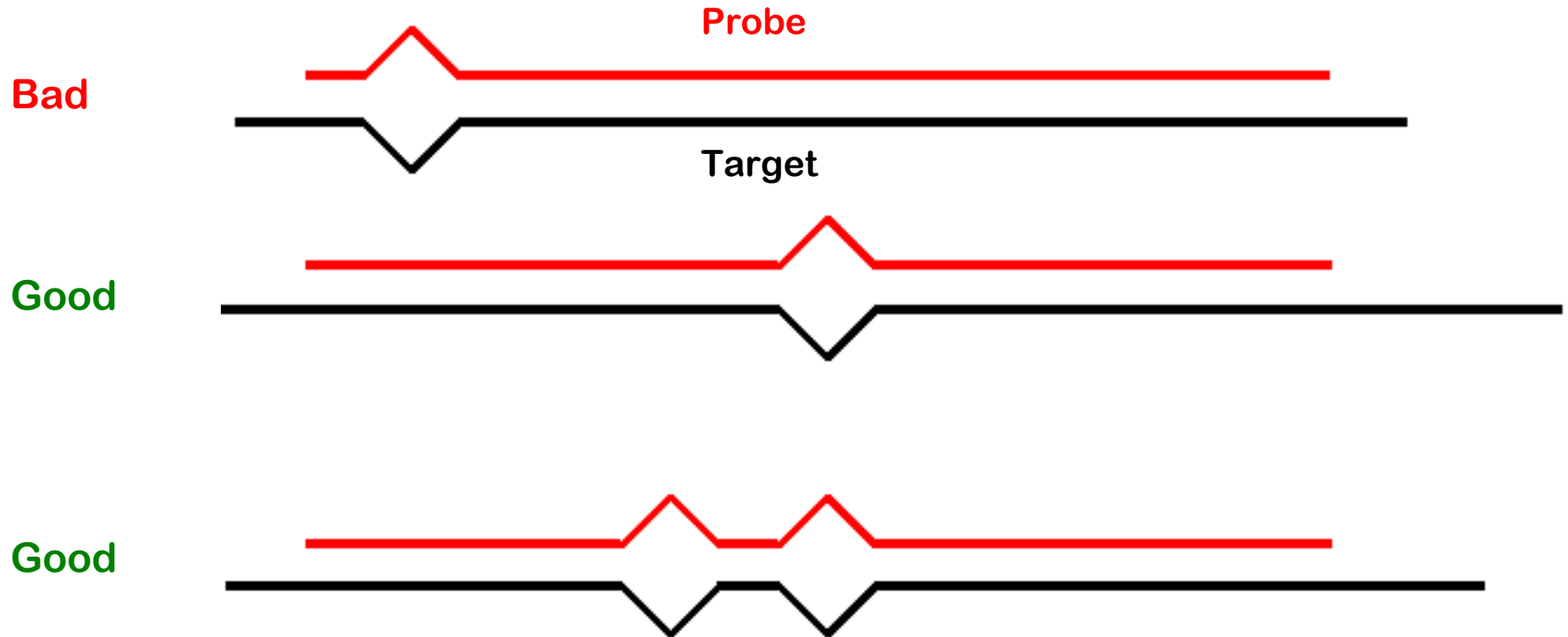
Optimizing the Hybridization Conditions

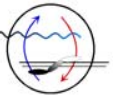




Probe Design

▶ Position of mismatches



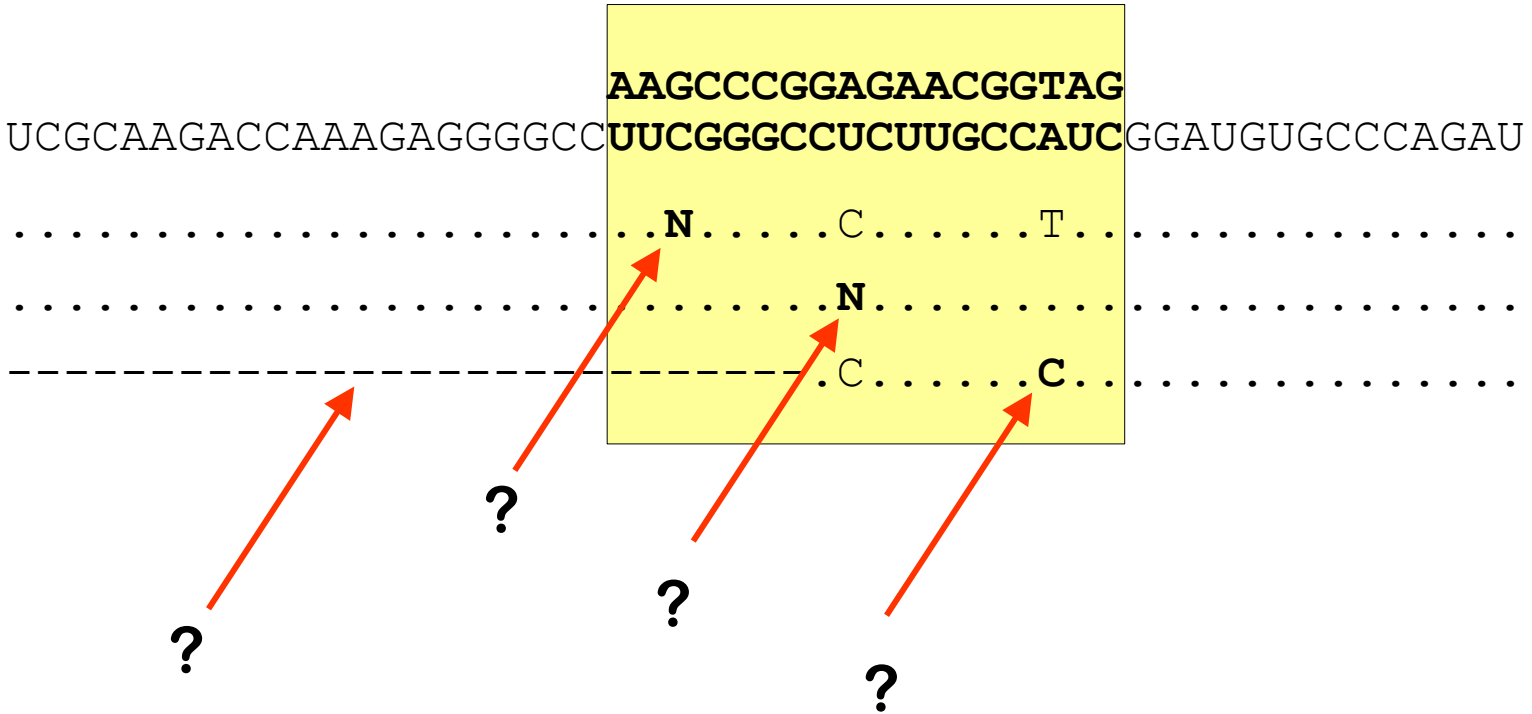


Problems - Data

Probe 3' - 5'

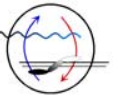
Target 5' - 3' UCGCAAGACCAAAGAGGGGCC **AAGCCCGGAGAACGGTAG** UUCGGGCCUCUUGCCAUC GGAUGUGCCCAGAU

Non-Target ?

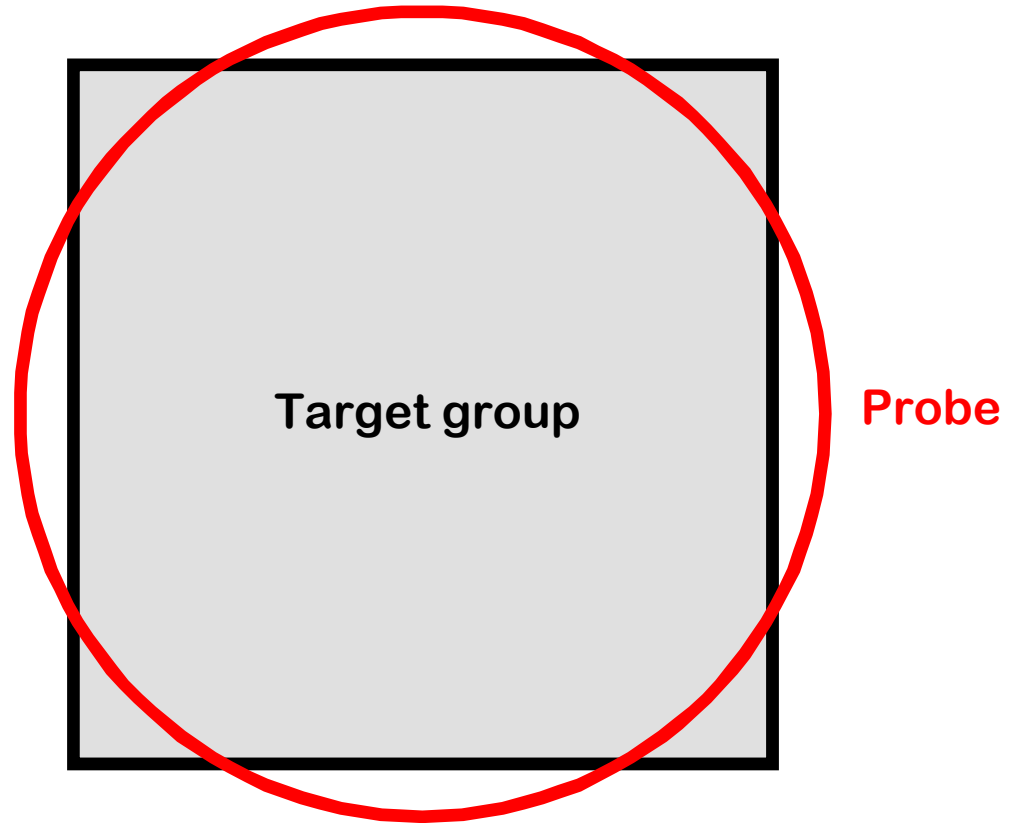


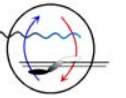
▶ Microheterogeneities

- Multiple operons
- Cloning – expressed operon?
- Direct sequencing – ambiguities

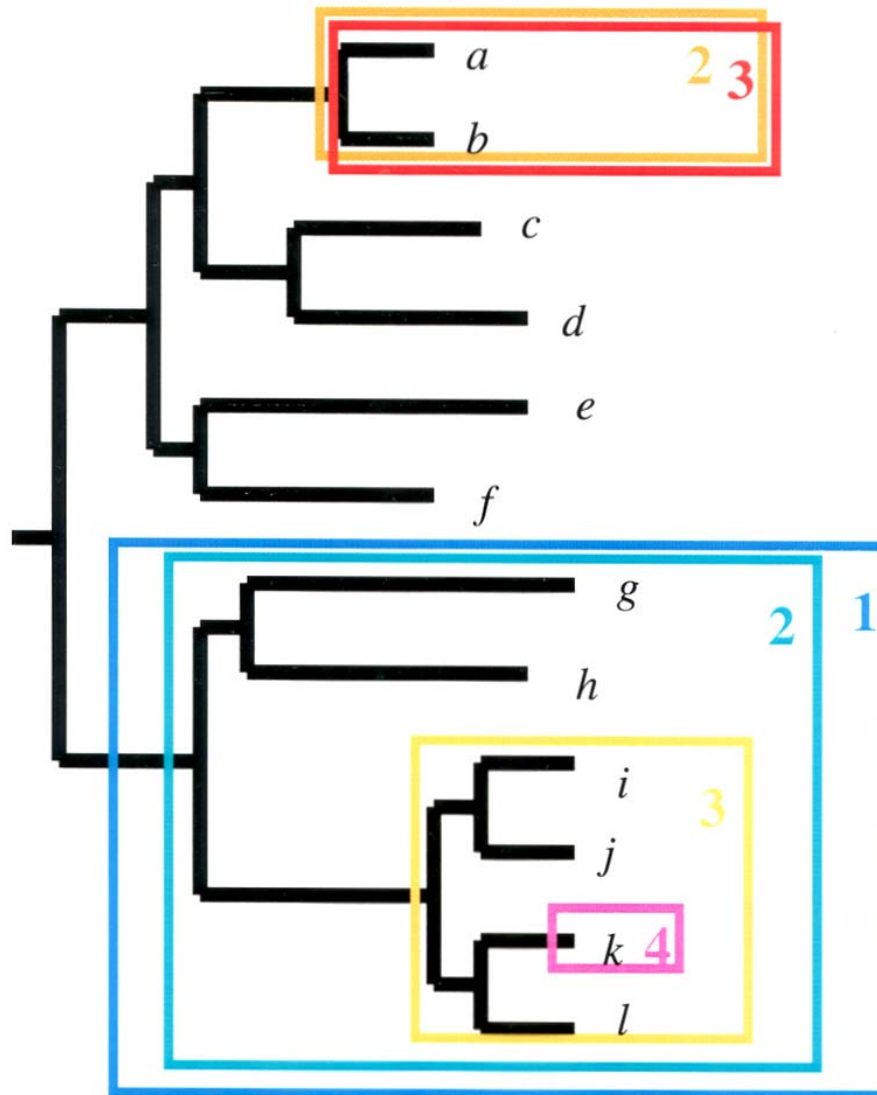


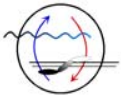
Problems - Coverage





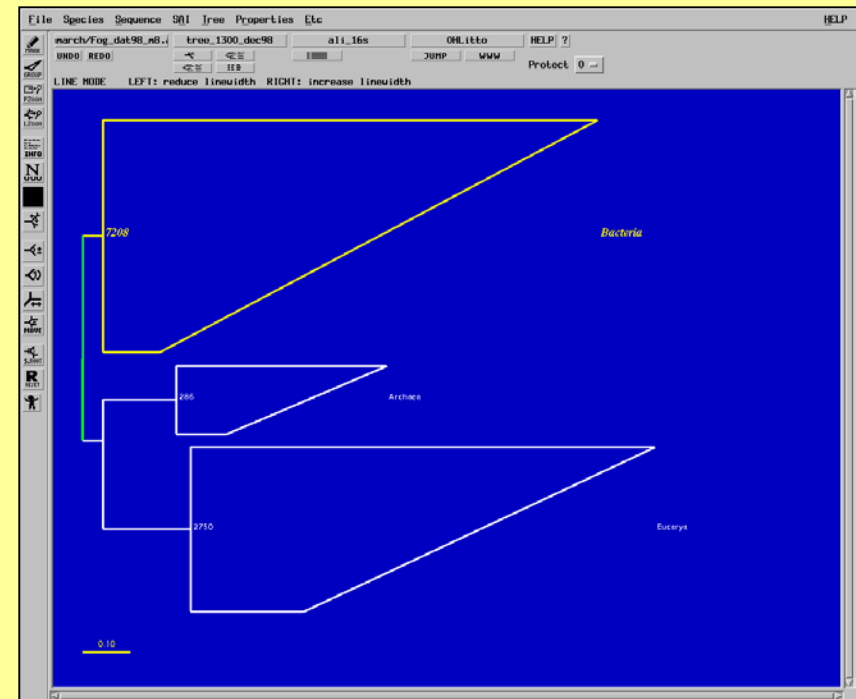
Multiple Probes Concept

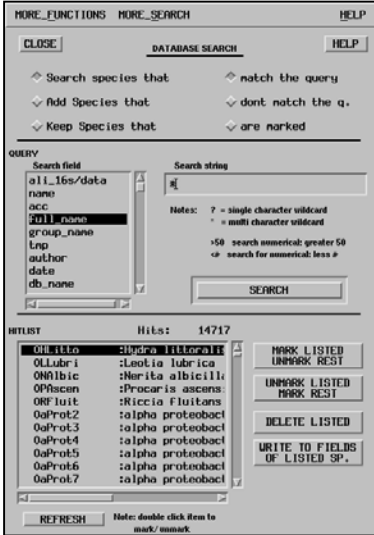
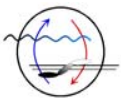




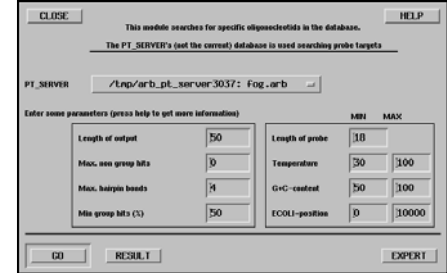
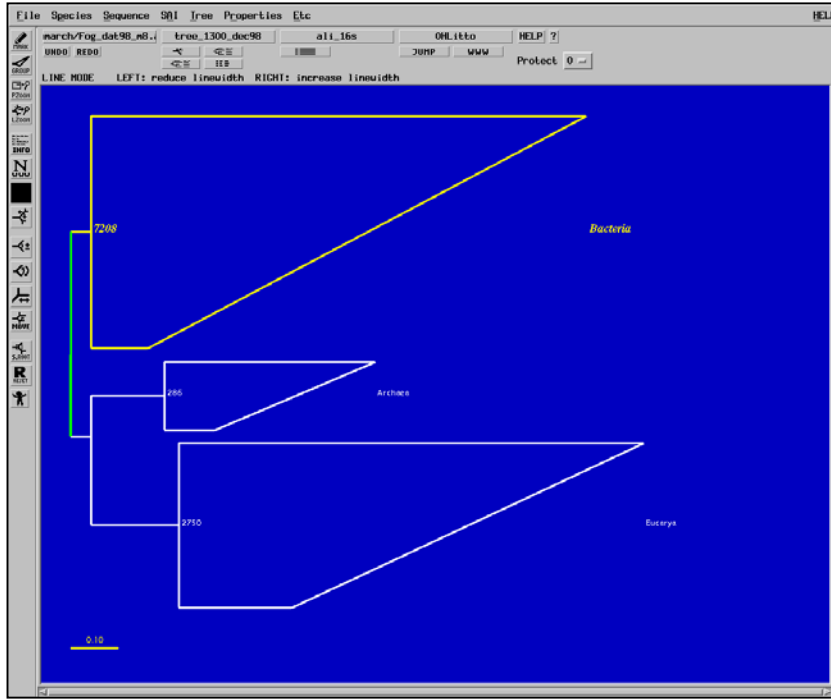
A Software Environment for Sequence Data

- ▶ Phylogeny
 - Ribosomal RNA
 - Functional genes
- ▶ Probe design

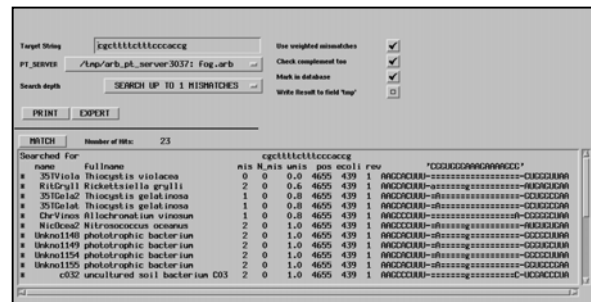




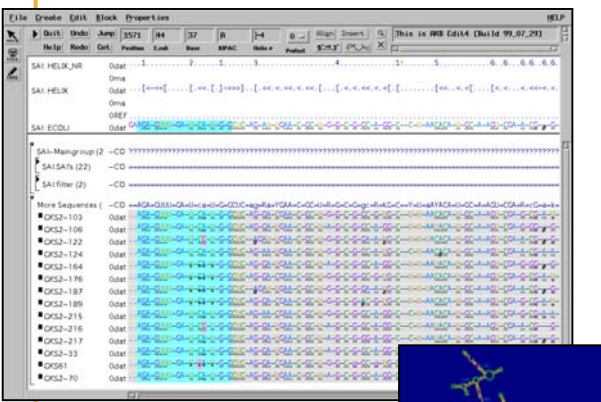
Database-management



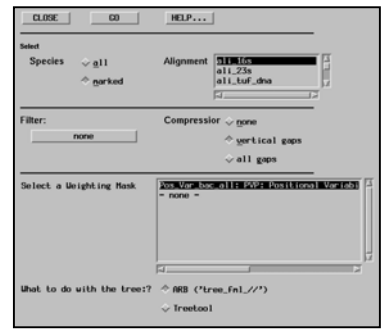
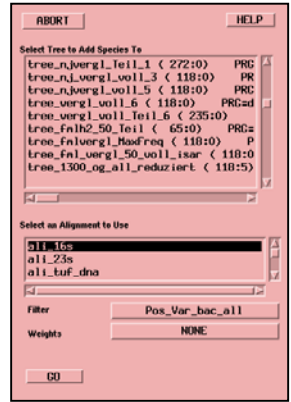
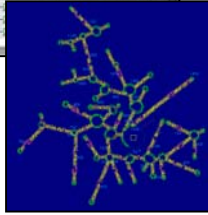
Probe-functions



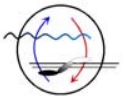
The concept of ARB



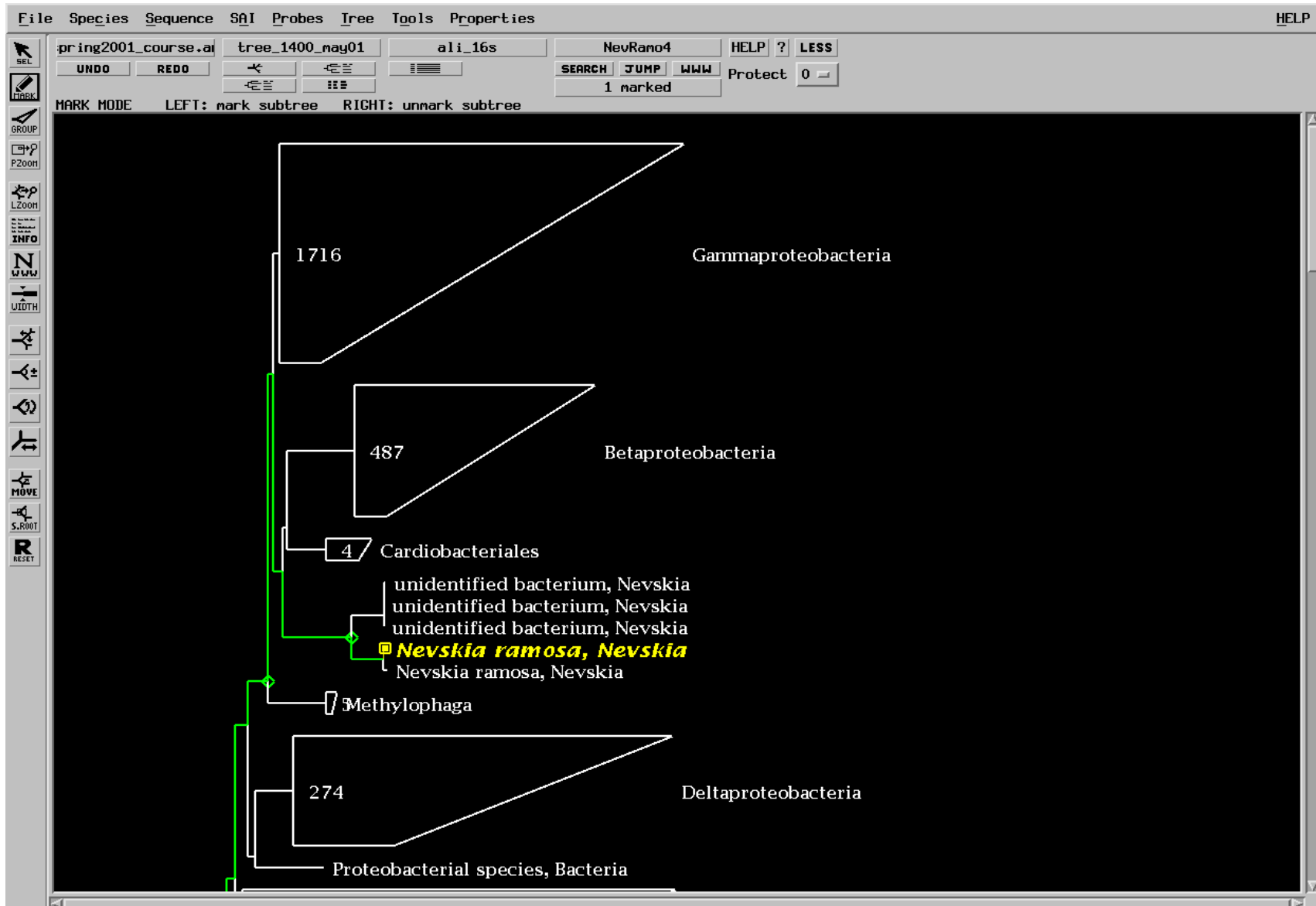
Sequence-alignment

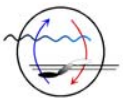


Phylogenetic reconstructions



ARB: Selection of Organisms





ARB: Probe_Design

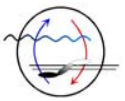
This module searches for specific oligonucleotids in the database.

The PT_SERVER's (not the current) database is used searching probe targets

PT_SERVER

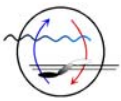
Enter some parameters (press help to get more information)

		MIN	MAX
Length of output	<input type="text" value="50"/>		
Max. non group hits	<input type="text" value="0"/>		
Max. hairpin bonds	<input type="text" value="0"/>		
Min group hits (%)	<input type="text" value="100"/>		
Length of probe	<input type="text" value="18"/>		
Temperature	<input type="text" value="30"/>	<input type="text" value="100"/>	
G+C-content	<input type="text" value="50"/>	<input type="text" value="100"/>	
ECOLI-position	<input type="text" value="0"/>	<input type="text" value="10000"/>	



ARB: Probe Design Results

Probe design Parameters:																				Probe sequence			
Length of probe	18																						
Temperature	[30.0 -100.0]																						
GC-Content	[50.0 -100.0]																						
E.Coli Position	[0 -100000]																						
Max Non Group Hits	0																						
Min Group Hits	100%																						
Target	le apos	ecol grps	G+C	4GC+2AT	I	Decrease	T	by	n*	.3C	->	probe	matches	n	non	group	species	Probe sequence					
AUGUGGCUUCGGGAACUU	18	A=17775 1021	1	50.0 54.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	AAGUCCCGAAGCCACAU				
GAUCAGCACUUGUGGGCG	18	B=24854 1355	1	61.1 58.0	 	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	CGCCACAGUCUGAUC				
GAUCGGGAUGUGGCUUC	18	A- 17 1014	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	CGAGCCACAUCCGAUC				
GAUGUGGCUUCGGGAACU	18	A- 2 1020	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	AGUCCCGAAGCCACAU				
GGCGAACGUGAUCCAGC	18	C=3946 378	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	GCUGGAUCACGUUCCGC				
UGUGGCUUCGGGAACUUG	18	A+ 2 1022	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	CAAGUCCCGAAGCCACA				
GGCAACGUGAUCCAGCA	18	D=4048 379	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	UGCUGGAUCACGUUCCGC				
GAUGUGGCUUCGGGAAC	18	A- 5 1019	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	3;	GUCCCGAAGCCACAUCC			
AUCAGCACUUGUGGGCGU	18	B+ 1 1356	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	1;	ACCGCCACAGUCUGAU			
GUGGCUUCGGGAACUUGA	18	A+ 11 1023	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	2;	UCAAGUCCCGAAGCCAC			
GAGAUGCGGAUGUGGCUU	18	E=17755 1012	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	2;	6;	AAGCCACAUCCGAUCUC		
UCAGCACUUGUGGGCGUG	18	B+ 4 1357	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	2;	5;	CACCGCCACAGUCUGA			
AGAUGCGGAUGUGGCUUC	18	E+ 1 1013	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	1;	1;	2;	GAGCCACAUCCGAUCU		
CAGCACUUGUGGGCGUGA	18	B+ 5 1358	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	2;	4;	4;	4;	UCACCGCCACAGUCUG		
ACUUGUGGGCGUGAUAAC	18	B+ 12 1361	1	50.0 54.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	3;	6;	6;	12;	GUUUCACCGCCACAGU		
CGAUCAGCACUUGUGGC	18	B- 8 1353	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	4;	4;	5;	9;	GCCACAGUCUGAUCCG		
UGGCUUCGGGAACUUGAA	18	A+ 14 1024	1	50.0 54.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	3;	7;	17;	18;	UUCAGUCCCGAAGCCA		
AGAGAUGCGGAUGUGGCU	18	E- 2 1011	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	2;	5;	5;	8;	11;	AGCCACAUCCGAUCUCU		
AUGCGGAUGUGGCUUCGC	18	A- 15 1015	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	1;	1;	1;	2;	3;	CCGAGCCACAUCCGCAU	
GAUCAGCACUUGUGGGC	18	B- 3 1354	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	34;	36;	43;	64;	101;	103;	CGCCACAGUCUGAUC
UGCGGAUGUGGCUUCGGC	18	A- 13 1016	1	66.7 60.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	1;	2;	2;	2;	3;	3;	CCCGAAGCCACAUCCGA	
CACUUGUGGGCGUGAAUA	18	B+ 11 1361	1	50.0 54.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	2;	2;	3;	5;	6;	8;	10;	UAUUCACCGCCACAGUC	
CGAUGUGGCUUCGGGA	18	A- 8 1018	1	61.1 58.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	3;	3;	3;	4;	4;	7;	7;	UUCCGAAGCCACAUCCG	
CCUUCGGGAACUUGAAC	18	A+ 16 1025	1	55.6 56.0		0;	0;	0;	0;	0;	0;	0;	0;	0;	1;	1;	1;	9;	20;	20;	24;	29;	CUUUCAGUCCCGAAGCC



ARB: Probe_Match

Target String Use weighted mismatches

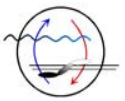
PT_SERVER Check complement too

Search depth Mark in database

Write Result to field 'tmp'

Number of Hits: 147

Searched for		GAUCAGCACUUGUGGCGG							'GAUCAGCACUUGUGGCGG'	
name	fullname	mis	N_mis	wmis	pos	ecoli	rev			
* NevRamo4	Nevskia ramosa	0	0	0.0	24854	1355	0	GUAUCCGG-=====	=====UGAAUACGU	
* UniBa108	unidentified bacterium	1	0	1.7	24854	1355	0	GUAUCCGG-=====	====C====UGAAUACGU	
* UniBa109	unidentified bacterium	1	0	1.7	24854	1355	0	GUAUCCGG-=====	====C====UGAAUACGU	
* UniBa174	unidentified bacterium	1	0	1.7	24854	1355	0	GUAUCCGG-=====	====C====UGAAUACGU	
* NevRamo3	Nevskia ramosa	1	0	1.7	24854	1355	0	GUAUCCGG-=====	====C====UGAAUACGU	
* UniArc30	unidentified archaeon	4	0	2.1	6831	538	1	GUAUAACU-G=====	====g====G====g====UCGAUUAUU	
* PlaBrass	Plasmodiophora brassicae	4	0	2.4	26325	1542	1	CGUCGGCGA-====u====g====h====g====	====CUUCCACAC	
* PbeLeio2	Photobacterium leiognathi	5	1	2.5	19797	1125	0	AUCCUUGUU-UgC=====	====Ng====u====GAACUCCAG	
* PbeHista	Photobacterium histaminum	5	1	2.5	19797	1125	0	AUCCUUGUU-UgC=====	====Ng====u====GAACUCCAG	
* UniArc38	unidentified archaeon	4	0	2.5	6831	538	1	GUAACACCG-G=====	====g====Gg====GCGUUUAUU	
* CrmATCC5	Cercomonas ATCC50316	7	0	2.8	9054	621	1	ACGGUCGUA-aG====g====g====u====g====G-UGCGCCUGN		
* MnyKand1	Methanopyrus kandleri	5	0	2.8	6831	538	1	GUAUAGCG-G====g====G====g====G-CUUUUUAUU		
* MttFerv2	Methanothermus fervidus	7	0	2.9	962	0	1	GAUACAUAG-GuC====g====g====g====a-AAGUCCCAA		
* HrnMolli	Heronimus mollis	7	0	2.9	9482	651	1	CCGUGUCUG-auUu====g====g====g====u-GGGUUGAUC		
* UclEub60	uncultured eubacterium WCHB1-	4	0	2.9	19801	1127	1	AUUUAGUUG-==A====g====a====g====CUGUGAGCA		
* TyhNata3	Typhlonectes natans	6	1	3.0	24825	1341	1	ACGAGGAU-u=C=Ngu====g====g====AUAAGCUNG		



ARB: Weighted Mismatches

Relative Strength of Base Pairings

Target

	A	C	G	U/T
A	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0.5"/>	<input type="text" value="1.1"/>
C	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="1.5"/>	<input type="text" value="0"/>
G	<input type="text" value="0.5"/>	<input type="text" value="1.5"/>	<input type="text" value="0.4"/>	<input type="text" value="0.9"/>
U/T	<input type="text" value="1.1"/>	<input type="text" value="0"/>	<input type="text" value="0.9"/>	<input type="text" value="0"/>

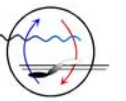
Probe

Experimental Parameters (Do not change):

Treat base pair as a mismatch when its strength is less than max strength minus

Edge misval

single misval



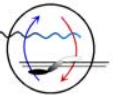
ARB: Secondary Structure Editor

File Properties HELP

Close HELP ? Undo Redo Center Fit To Window

ZOOM MODE : CLICK or SELECT an area to ZOOM IN (LEFT) or ZOOM OUT (RIGHT)

1350



Fluorescence *in situ* hybridization of *Nevskia ramosa*

