

Development of DNA-Microarrays for Monitoring Phytoplankton Composition

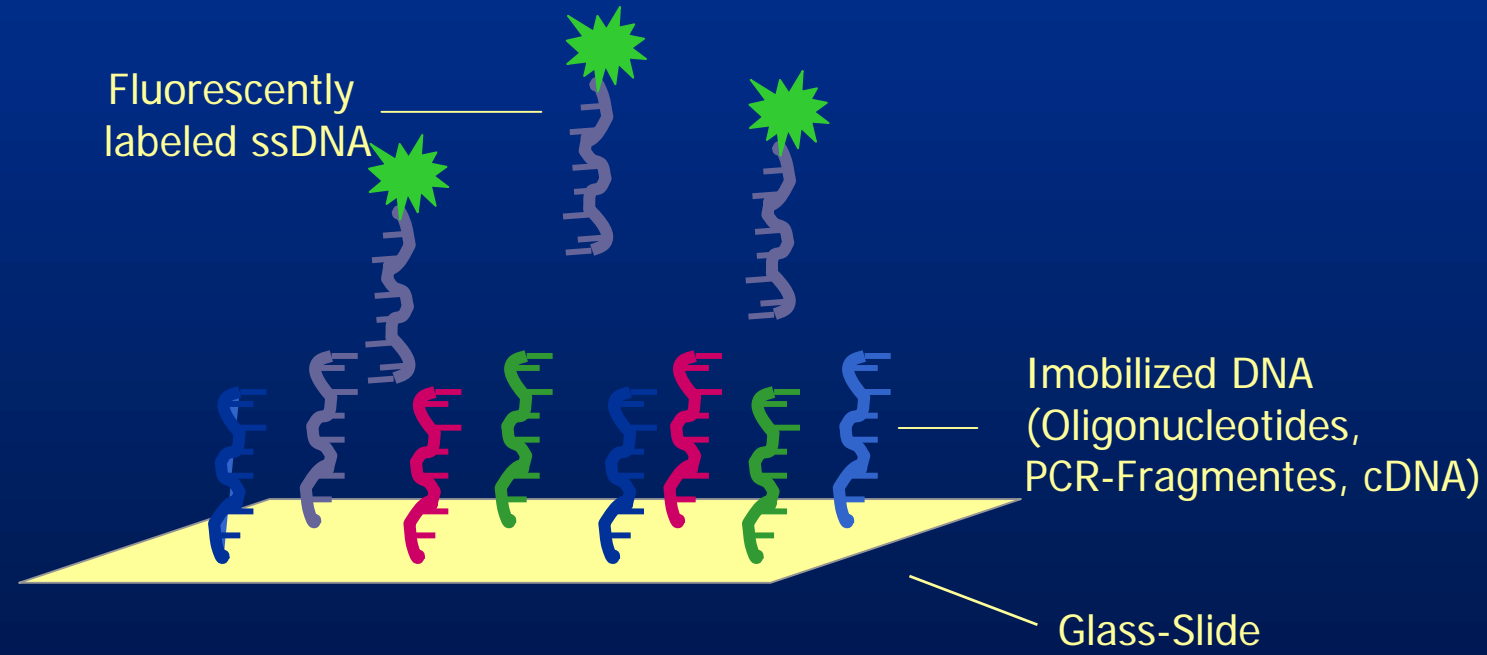
Katja Kerkmann

Linda Medlin

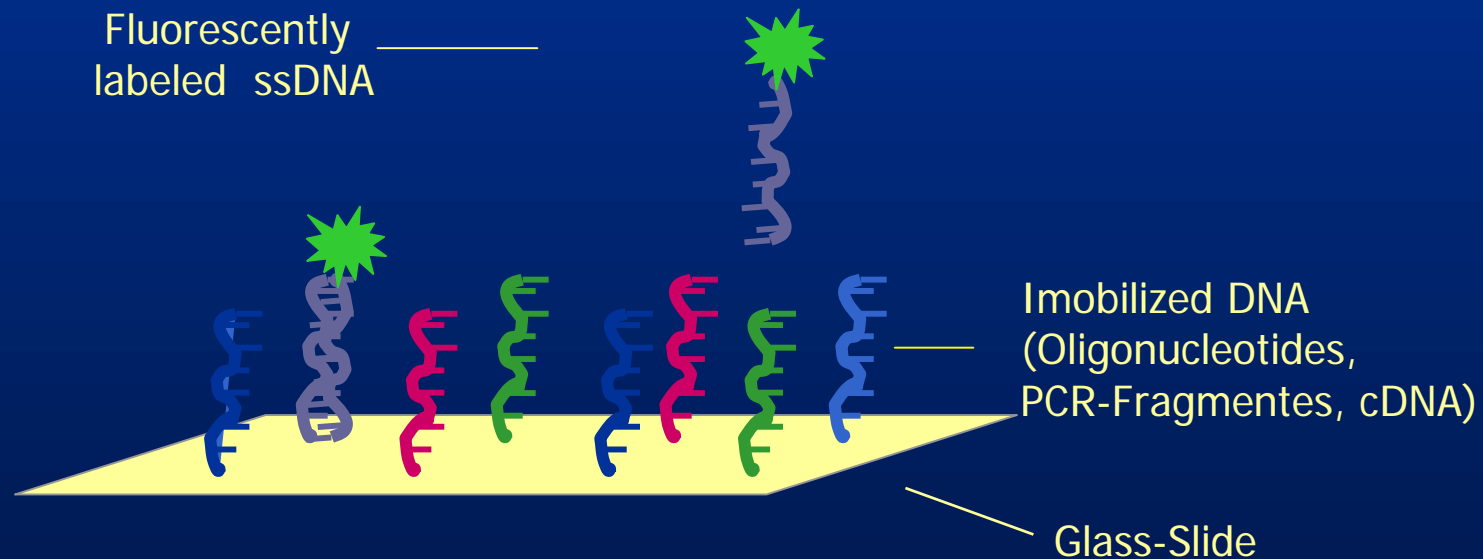
Alfred Wegener Institute for Polar- and Marine Research
Bremerhaven

- Probe Testing
- Enhancement of the Signal-Intensity
- Testing the DNA-Chips

Scheme of a DNA-Chip Experiment



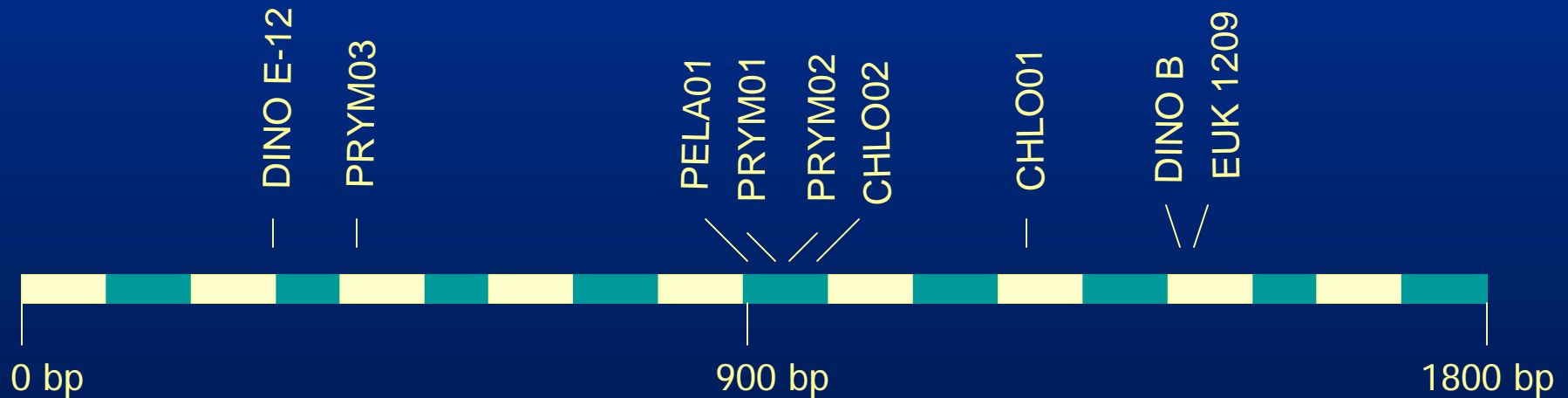
Scheme of a DNA-Chip Experiment



Probes and Targets used for preliminary Chip-Experiments

Class	Probe	Source	Species
Dinophyceae	DINO B	Groben, John and Medlin, unpublished	▪ <i>Alexandrium tamarense</i>
	DINO E-12	Groben, John and Medlin, unpublished	▪ <i>Prorocentrum minimum</i>
Prymnesiophyceae	PRYM01	Lange et al., 1996	▪ <i>Prymnesium patelliferum</i>
	PRYM02	Smon et al., 2000	
	PRYM03	Eler und Medlin, unpublished	
Chlorophyceae	CHLO01	Smon et al., 1995	▪ <i>Dunaniella salina</i>
	CHLO02	Smon et al., 2000	▪ <i>Pyramimonas obovata</i>
Pelagophyceae	PELA01	Smon et al., 2000	▪ <i>Cocoid pelagophyte</i> ▪ <i>Pulvinaria spec.</i>
Bolidophyceae	BOLI01	Guillou et al., 1999	▪ <i>Clone. No. 151 PICODIV</i>
	BOLI02	Guillou et al., 1999	

Localization of the Class-level probes in the 18S-Sequence

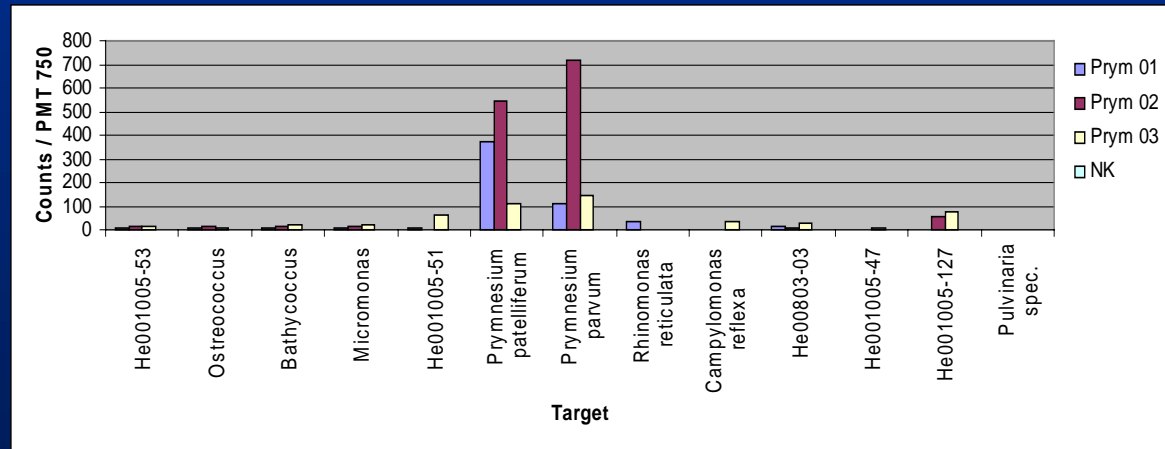


18S-DNA ~ 1800 bp

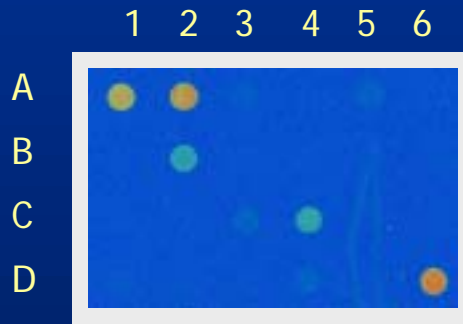
Prymnesiophyceae



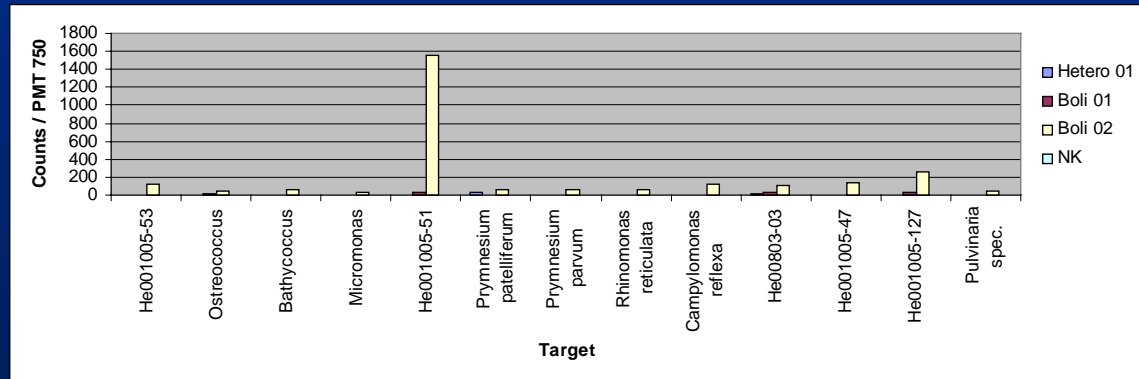
- | | |
|---------------|---------------|
| A1- PC | C1- Pela 02 |
| A2- Euk 328 | C2- Dino B |
| A3- Euk 1209 | C3- Dino E-12 |
| A4- Chlo 01 | C4- NS 03 |
| A5- Chlo 02 | C5- NS 04 |
| A6- Hetero 01 | C6- Pras 04 |
| B1- Boli 01 | D1- Bathy 01 |
| B2- Boli 02 | D2- Micro 01 |
| B3- Prym 01 | D3- Ostreo 01 |
| B4- Prym 02 | D4- Crypto B |
| B5- Prym 03 | D5- NC |
| B6- Pela 01 | D6- PC |



Bolidophyceae



- | | |
|---------------|---------------|
| A1- PC | C1- Pela 02 |
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| A3- Euk 1209 | C3- Dino E-12 |
| A4- Chlo 01 | C4- NS 03 |
| A5- Chlo 02 | C5- NS 04 |
| A6- Hetero 01 | C6- Pras 04 |
| B1- Boli 01 | D1- Bathy 01 |
| B2- Boli 02 | D2- Micro 01 |
| B3- Prym 01 | D3- Ostreo 01 |
| B4- Prym 02 | D4- Crypto B |
| B5- Prym 03 | D5- NC |
| B6- Pela 01 | D6- PC |



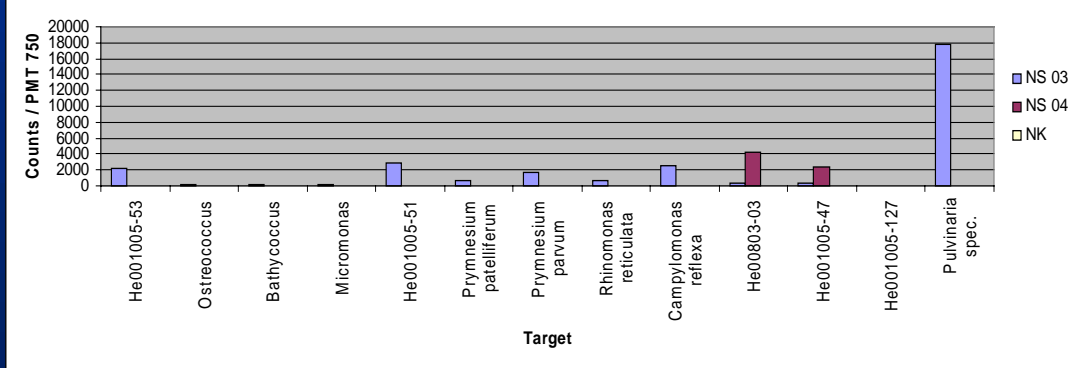
New Probes on the DNA-Chip

Probe	Source	Target	Location
NS 03	Ramon Massana	New Stramenopiles	~ 400 bp
NS 04	Ramon Massana	New Stramenopiles	~ 850 bp
Pras 04	Fabrice Not	Prasinophytes	~ 300 bp
Bathy 01	Fabrice Not	Bathycoccus	~ 650 bp
Ostreo 01	Fabrice Not	Ostreococcus	~ 600 bp
Micro 01	Fabrice Not	Micromonas	~ 200 bp
Crypto B	Linda Medlin	Cryptophytes	~ 830 bp

New Stramenopiles



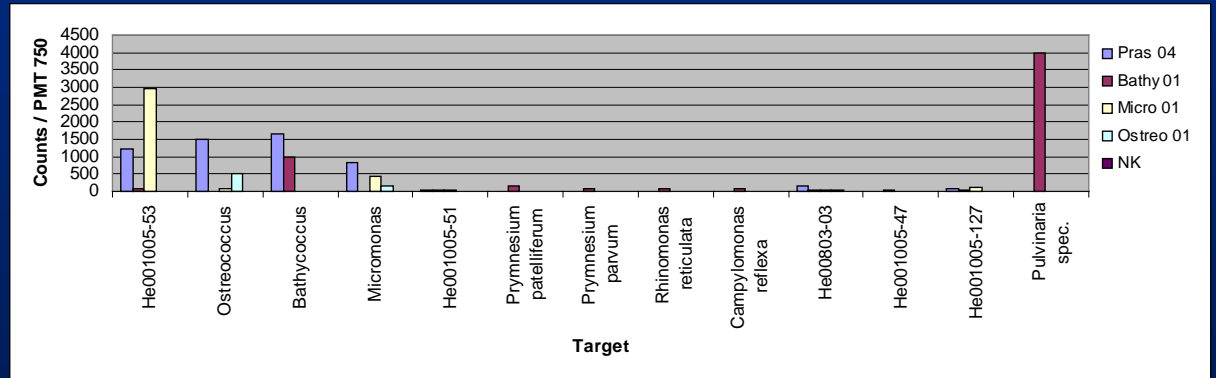
- A1- PC
- A2- Euk 328
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- B1- Boli 01
- B2- Boli 02
- B3- Prym 01
- B4- Prym 02
- B5- Prym 03
- B6- Pela 01
- C1- Pela 02
- C2- Dino B
- C3- Dino E-12
- C4- NS 03
- C5- NS 04
- C6- Pras 04
- D1- Bathy 01
- D2- Micro 01
- D3- Ostreo 01
- D4- Crypto B
- D5- NC
- D6- PC



Prasinophyceae



- | | |
|---------------|---------------|
| A1- PC | C1- Pela 02 |
| A2- Euk 328 | C2- Dino B |
| A3- Euk 1209 | C3- Dino E-12 |
| A4- Chlo 01 | C4- NS 03 |
| A5- Chlo 02 | C5- NS 04 |
| A6- Hetero 01 | C6- Pras 04 |
| B1- Boli 01 | D1- Bathy 01 |
| B2- Boli 02 | D2- Micro 01 |
| B3- Prym 01 | D3- Ostreo 01 |
| B4- Prym 02 | D4- Crypto B |
| B5- Prym 03 | D5- NC |
| B6- Pela 01 | D6- PC |

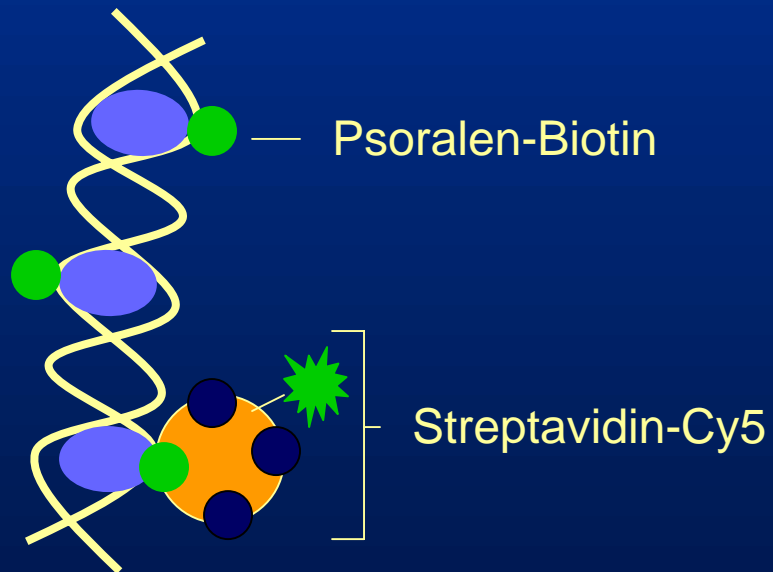


Summary of the Probe-Testing

Probe	Target	Location
Micro 01	Micromonas	~ 200 bp
Pras 04	Prasinophyta	~ 300 bp
Boli 02	Bolidophyta	~ 300 bp
Dino E-12	Dinophyta	~ 350 bp
Prym 03	Prymnesiophyta	~ 450 bp
Ostreo 01	Ostreococcus	~ 600 bp
NS 04	New	~ 850 bp
	Stramenopiles	
Prym 01	Prymnesiophyta	~ 900 bp
Prym 02	Prymnesiophyta	~ 950 bp
Chlo 02	Chlorophyta	~ 950 bp
Bathy 01	Bathycoccus	~ 650 bp
Crypto B	Cryptophyta	~ 830 bp
NS 03	New	~ 400 bp
	Stramenopiles	
Pela 01	Pelagophyta	~ 900 bp
Chlo 01	Chlorophyta	~ 1350 bp
Euk 1209	Eukaryotes	~ 1400 bp
Dino B	Dinophyta	~ 1400 bp
Boli 01	Bolidophyta	~ 1450 bp
Hetero 01	Stramenopiles	~ 1700 bp

Enhancement of Signal-Intensities

Labelling DNA with Psoralen-Biotin



BrightStar Psoralen-Biotin
Nonisotopic Labeling Kit
Ambion, Austin, Texas

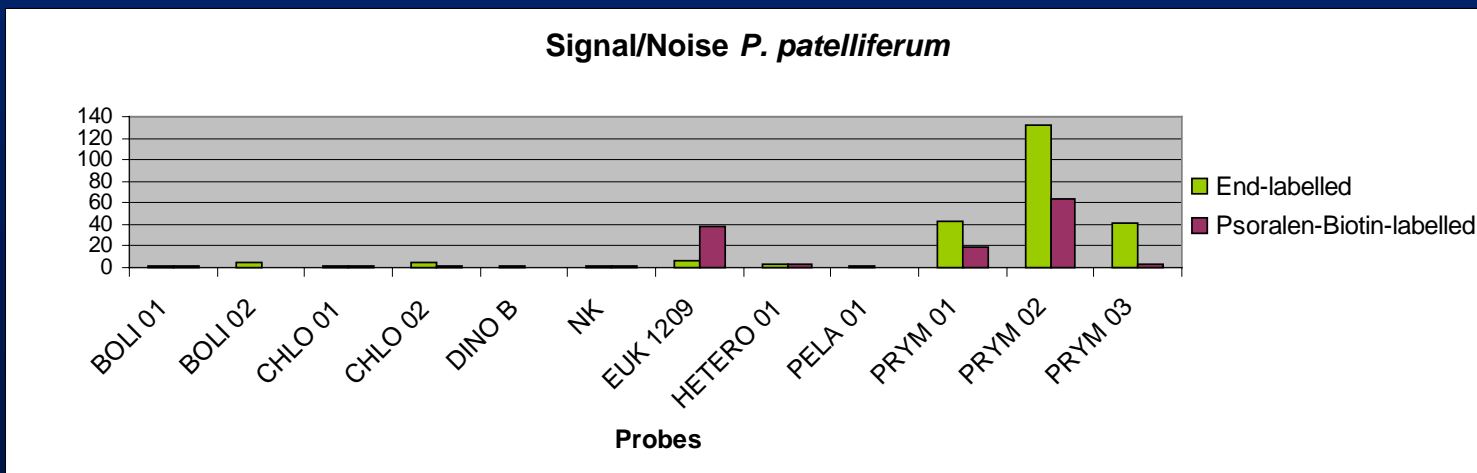
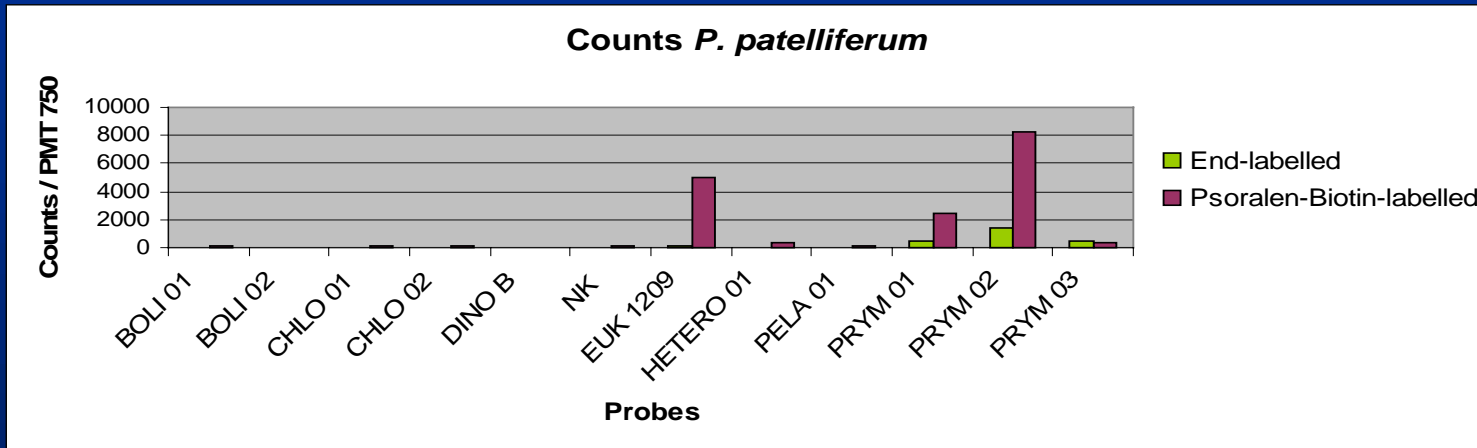


Labelling with Psoralen-Biotin enhances the Signal-Intensity

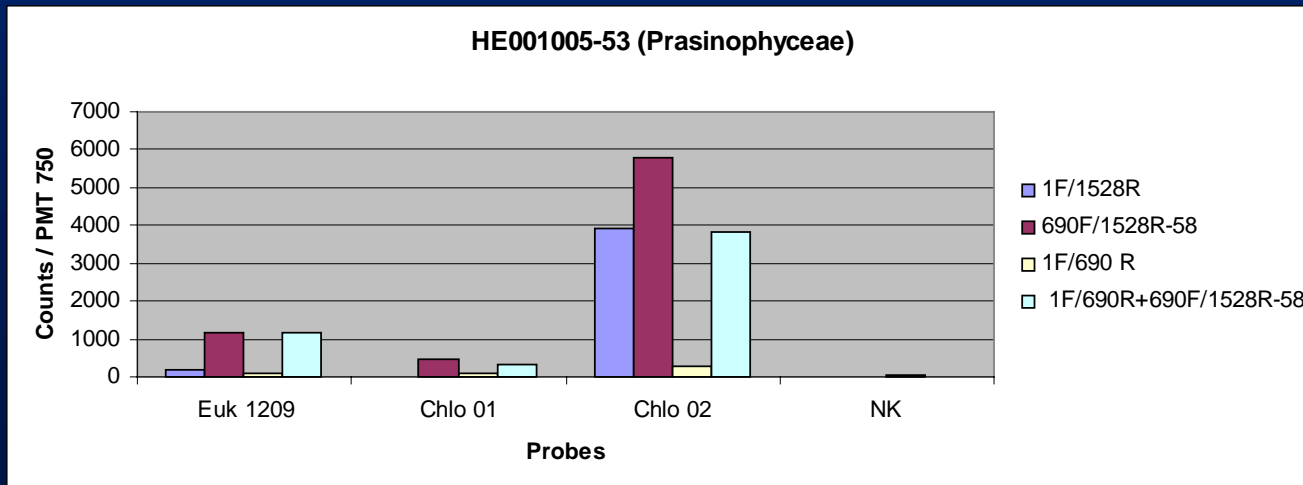
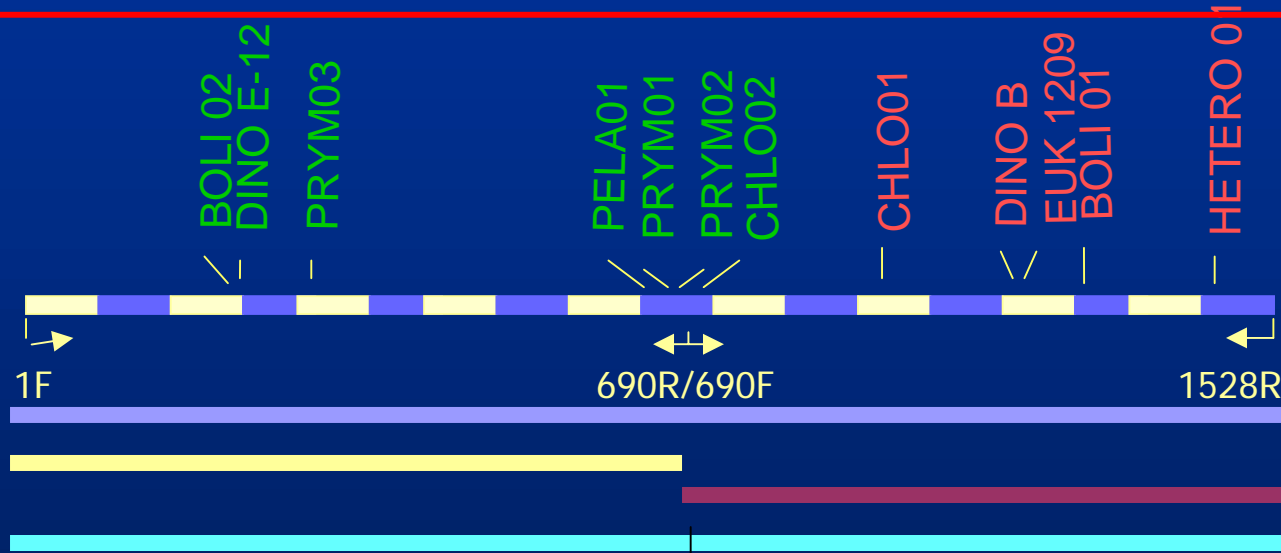


- The end-labelling was carried out via a biotin-labelled PCR-primer
- The concentration of the target-DNA was in both experiments 5 ng/ μ l
- A hybridisation with Psoralen-Biotin labeled target-DNA generates higher signal-intensities
- Psoralen-Biotin generates a relatively high background
- The probe Euk 1209 does get bound by Psoralen-Biotin labeled target-DNA

Quantitative Analysis of two different Labelling-Methods



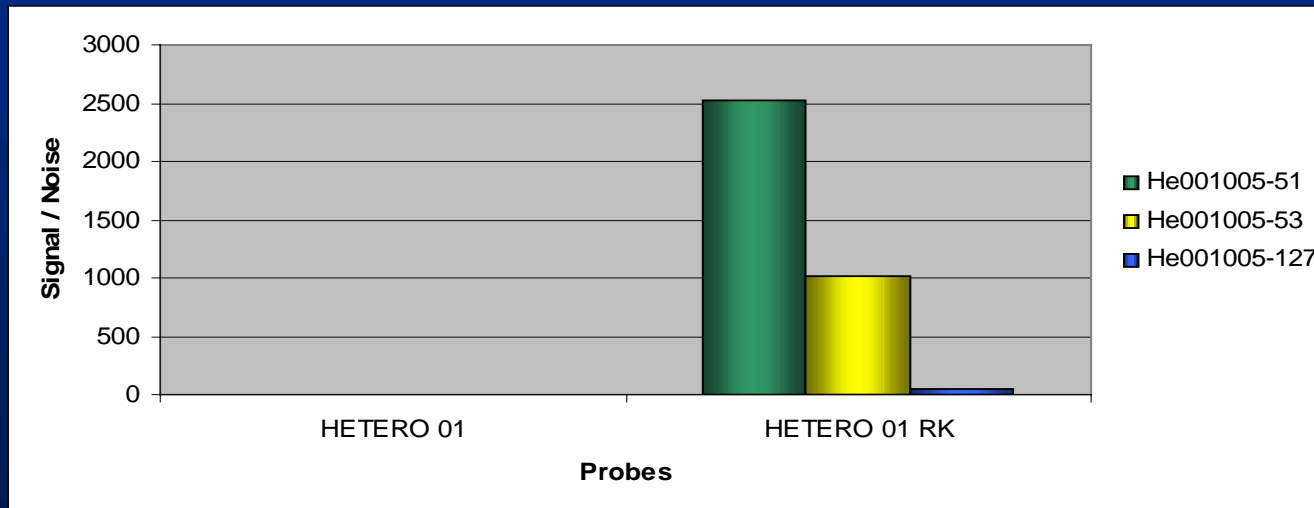
Fragmentation of the 18S-DNA by PCR-Amplification



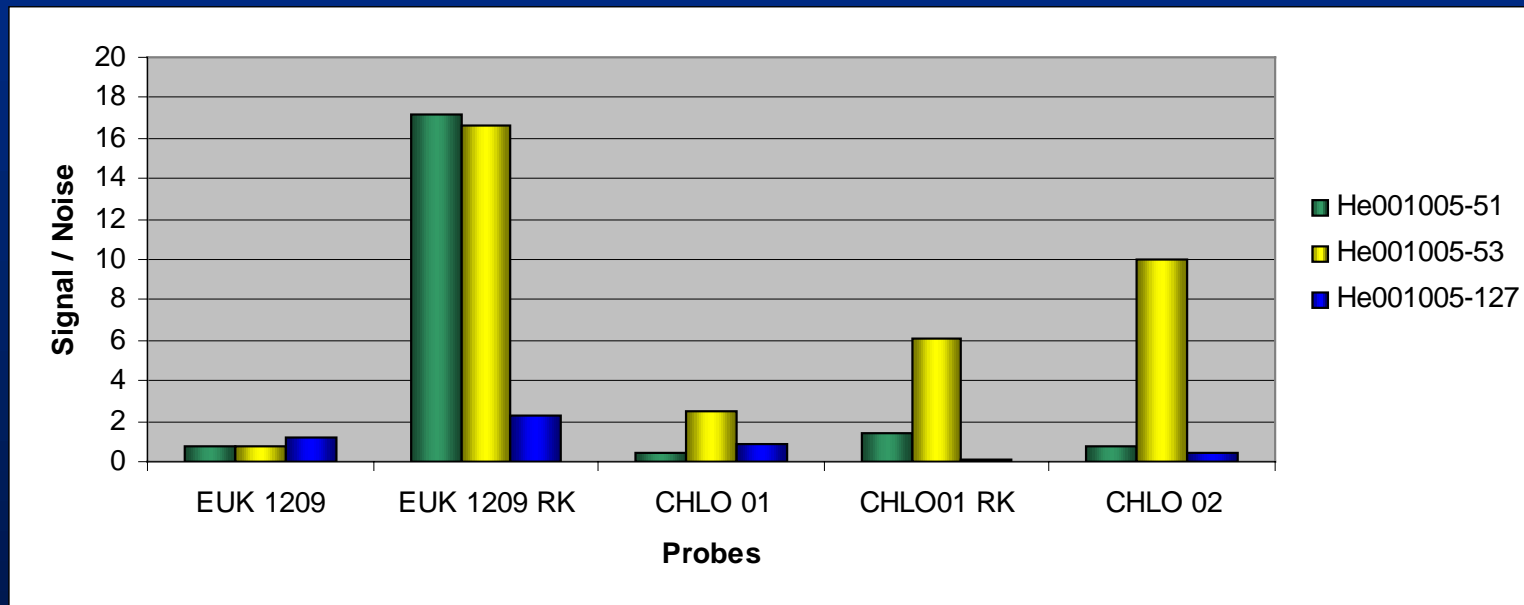
Enhanced Signals by the Use of a Reverse Complementary Probe?



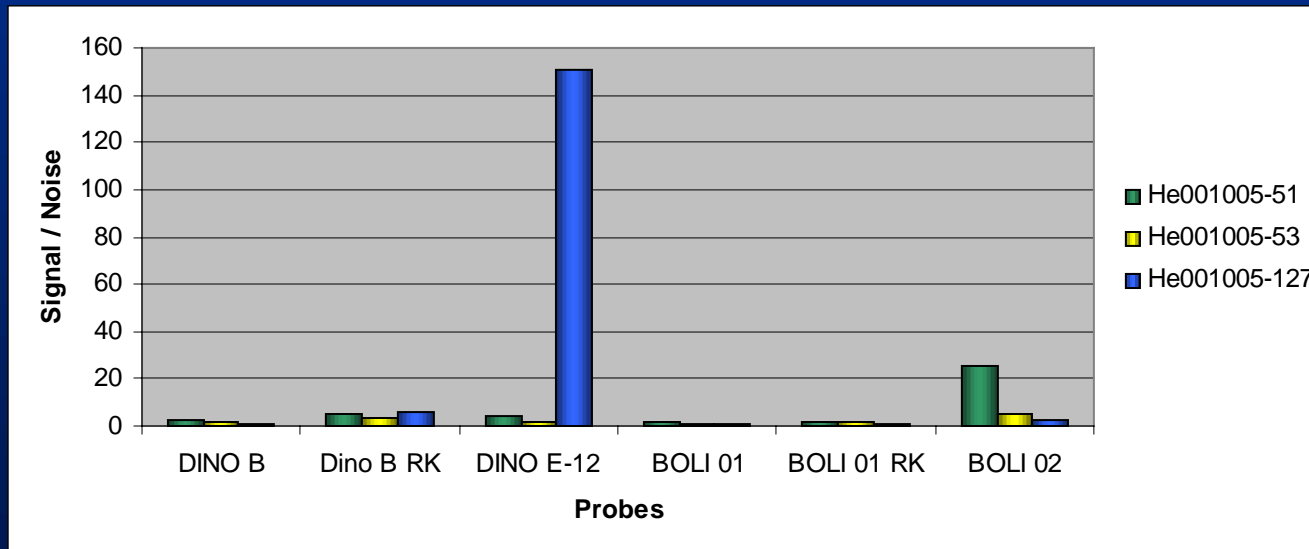
Enhancement of the Signal-Intensity of Probe Hetero 01



Effect of the Use of the Reverse Complement Sequence on Probes Euk 1209 and Chlo 01

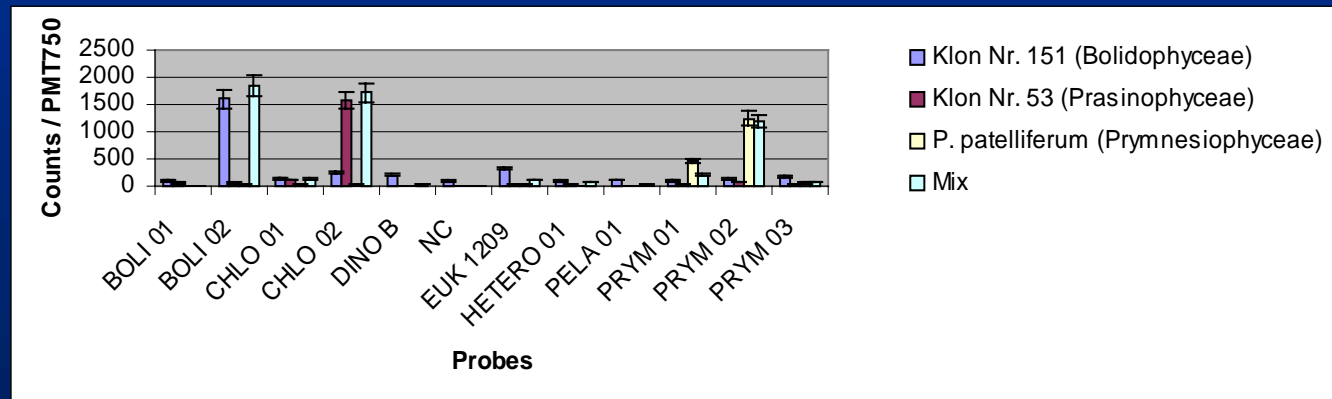
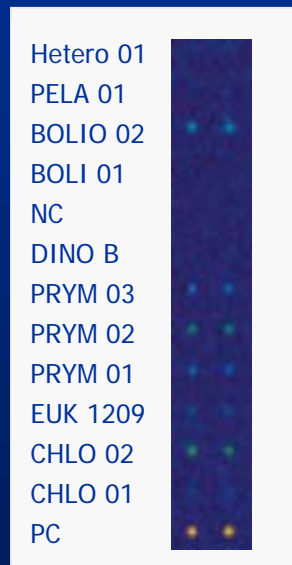


Effect of the Use of the Reverse Complement Sequence on Probes Dino B and Boli 01



Testing the DNA-Chips

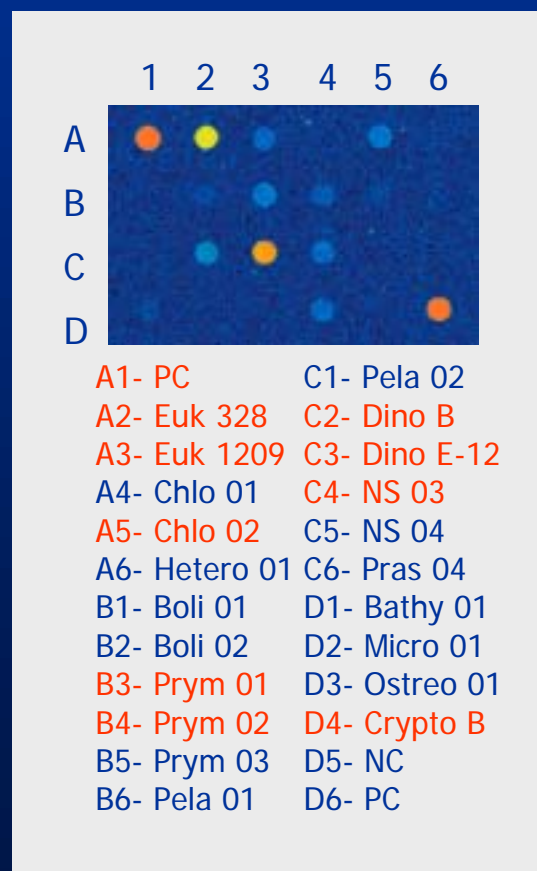
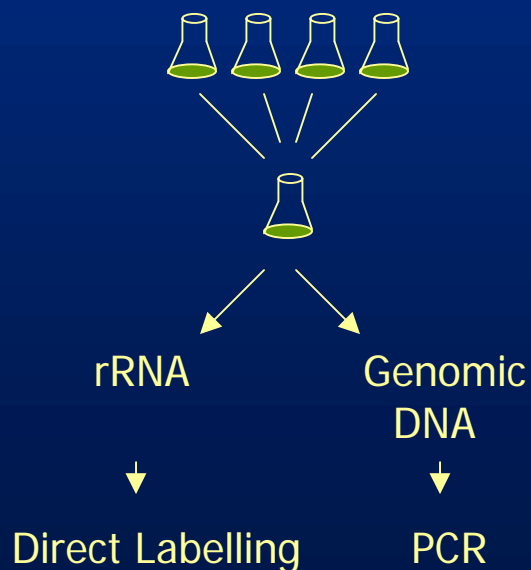
Hybridization of several PCR-Fragments from PICODIV-Clones to a DNA-Chip



Bolidophyceae HE001005-51 [30 nM]
 Prasinophyceae HE001005-53 [30 nM]
 Prymnesiophyceae *P. patelliferum* [10 nM]

Use of a DNA-Chip for the Analysis of a Mix of Laboratory Strains

- *Alexandrium ostenfeldii*
- *Plagiomonas prolunga*
- *Pyramimonas parkae*
- *Prymnesium parvum*



Falsification of a DNA-Chip Result caused by PCR-Amplification

- *Alexandrium andersonii*
- *Chrysochromulina ericina*
- *Dunaliella salina*
- *Guillardia theta*



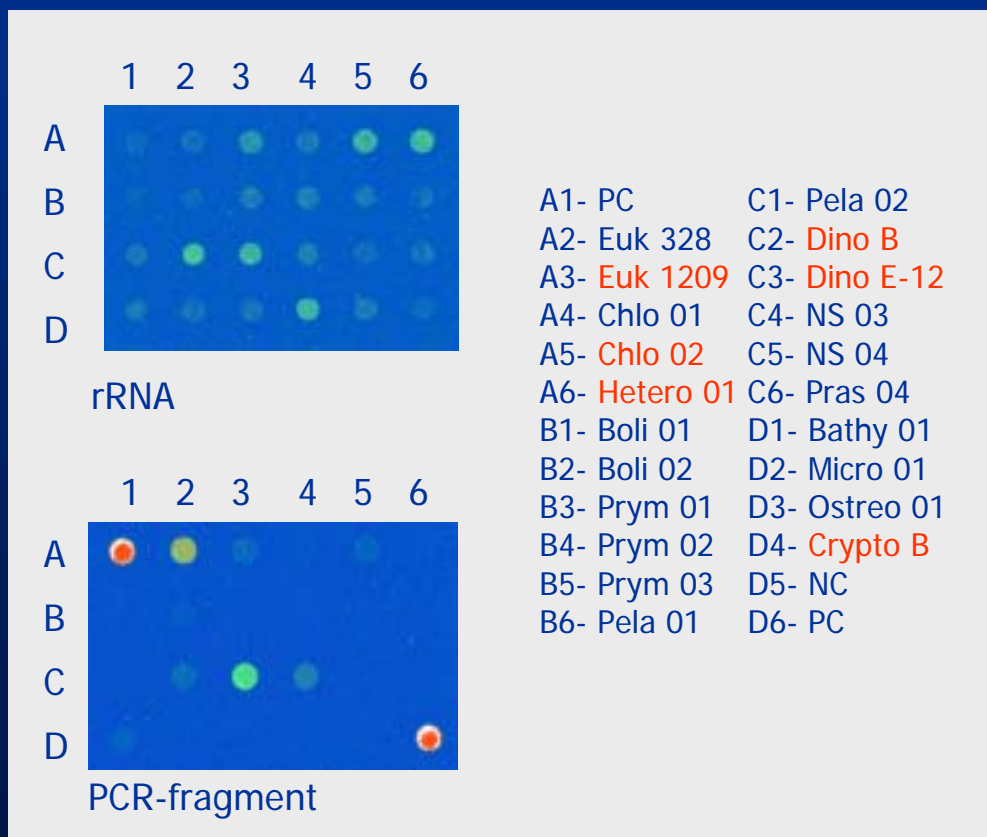
rRNA

Genomic
DNA

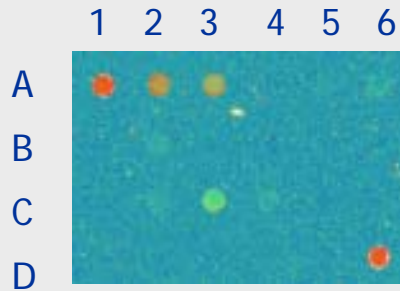


Direct Labelling

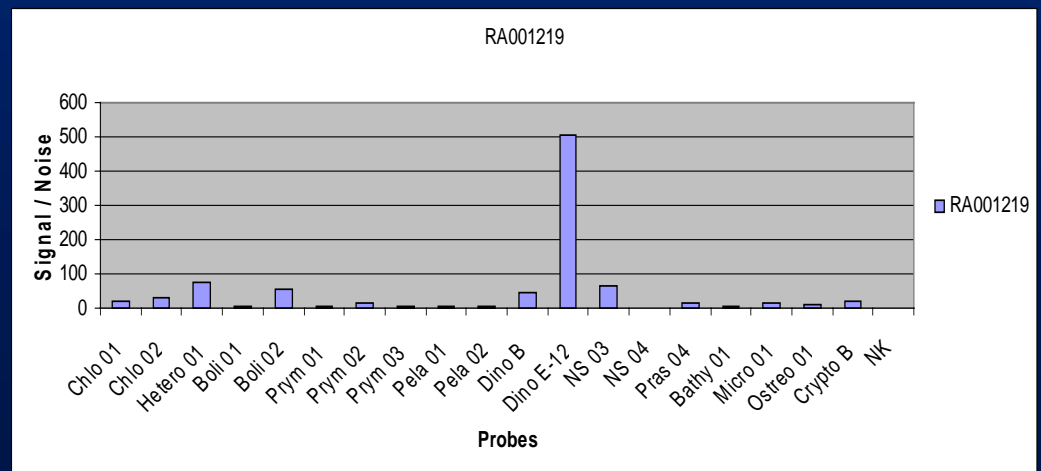
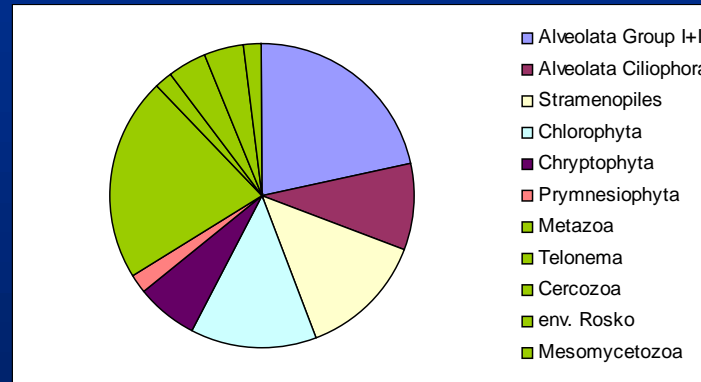
PCR



Comparison of a Clone-Library with a DNA-Chip Experiment



A1- PC	C1- Pela 02
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B5- Prym 03	D5- NC
B6- Pela 01	D6- PC



Summary

- It was possible to show that a set of probes that was initially developed for other hybridisation-techniques works on DNA-chips
- Probes work best, if they are located in an area of up to 900 bp from the 5'-end of the 18S-molecule
- There is no patent remedy for the enhancement of probes that are located further downstream of the molecule
- The signal-intensities on the DNA-chip are reproducible
- PCR-amplification can falsify the result of the analysis of samples
- DNA-chips have been used to analyse samples

Special Thanks to....

Alfred Wegener Institute:

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Centrum für Angewandte Gensensorik, Bremen:

Prof. Dr. Blohm