aniel Vaulot:

Progress on Probe Development until June 2002

Project PICODIV

Gundula Eller & Linda Medlin

Summary results until December 2001

New probes and targets in dot blots

Whole cell hybridisations

Detection by laser scanning microscopy

Dual labelling of cells in one hybridisation

Summary

Plans for the next half year period

Summary of results achieved until December 2001

- Several probes for different taxonomic levels designed and tested in silico
- First in vitro tests by dot blot and FISH:

Probename	Target Group	Dot Blot	FISH	Specific?
Hetero01	Heterokonta	+	-	in dot blots
Prym03	Prymnesiophyta	+	First tests	Yes
PrymGl01A	*Prymnesium*	+	+	Yes
PrymGl02B	*Prymnesium*	+	+	Yes
PrymParv	*Prym parvum*	tested	-	No!
Cocco01	Coccolithes	+	-	in dot blots
OliGr01	Clones in OLI-Clade	+	No cells	in dot blots
OliGr04	Clones in OLI-Clade	+	No cells	in dot blots
			-	No!

- Probes unspecific in dot blots (PrymParv) were redesigned

Progress until June 2002

Dot blot hybridisations

Redesigned probes:

PrymParvAll: Tested unspecific

=> Realignment of sequences and design of a third probe

Tests with further targets

Hetero01: Tested with additional target DNA

=> increased stringency necessary

Progress until June 2002

In situ Hybridisations (FISH)

Comparison of **signal intensity** between

- cells hybridised without probe (autofluorescence)
- universal eukaryotic probe
- new probe in test

Comparison of **suitability of cells** for FISH, fixed...

- in exponential growth phase: good signal as expected
- in stationary phase: many cells / particles with strong autofluorescence

=> dormant cells, low (?) ribosome content,

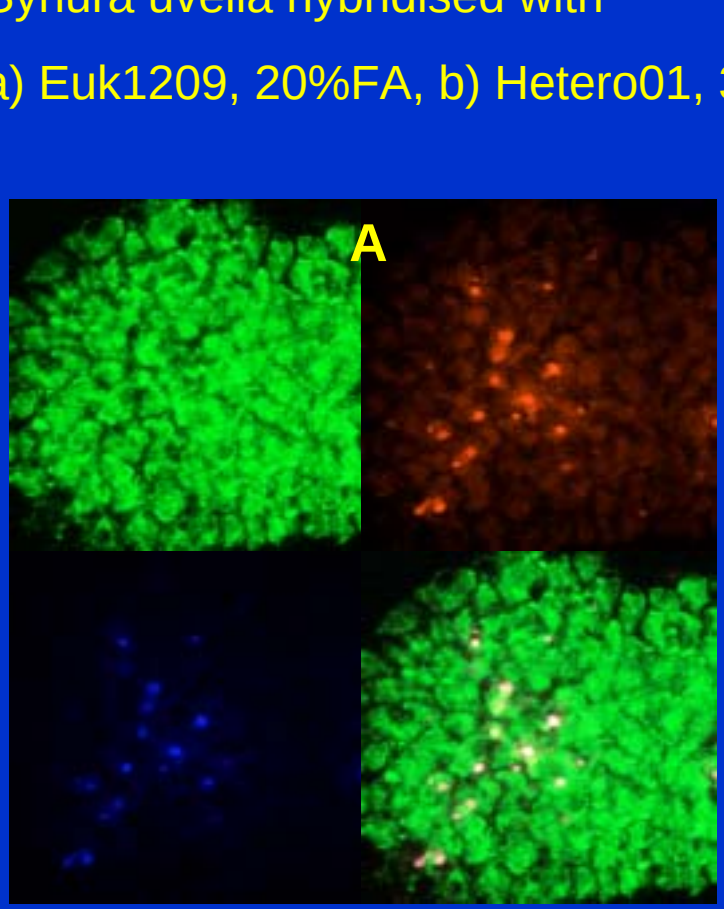
changes in cell permeability, reserve material

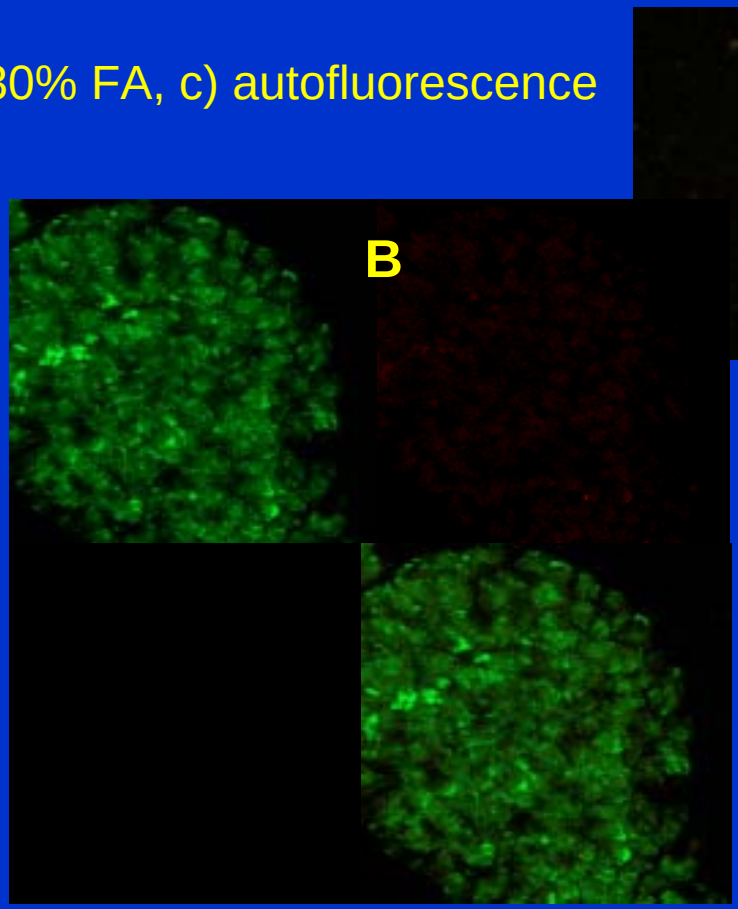
Progress until June 2002

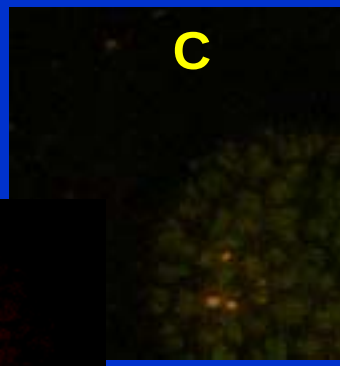
FISH with probe Hetero01 and CLSM detection

Synura uvella hybridised with

a) Euk1209, 20%FA, b) Hetero01, 30% FA, c) autofluorescence



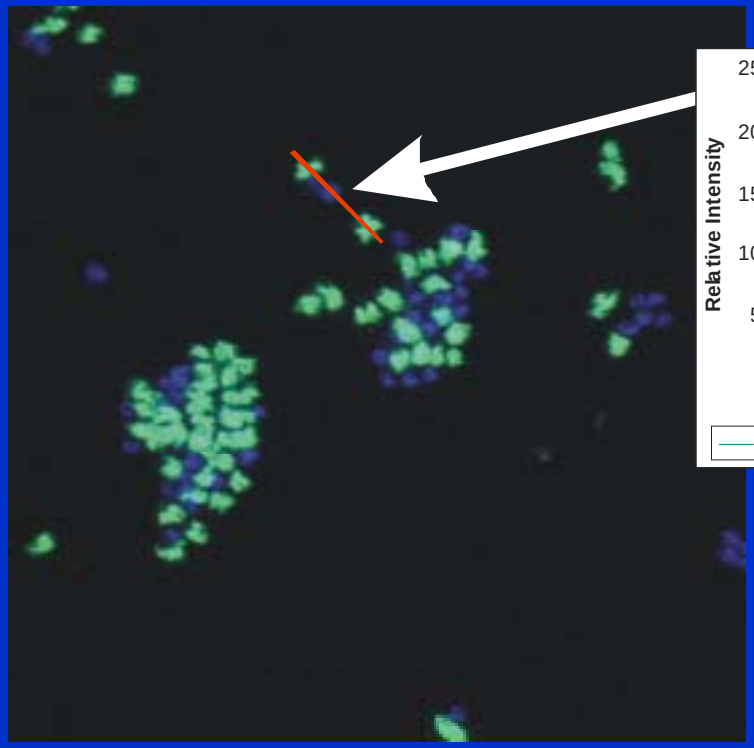


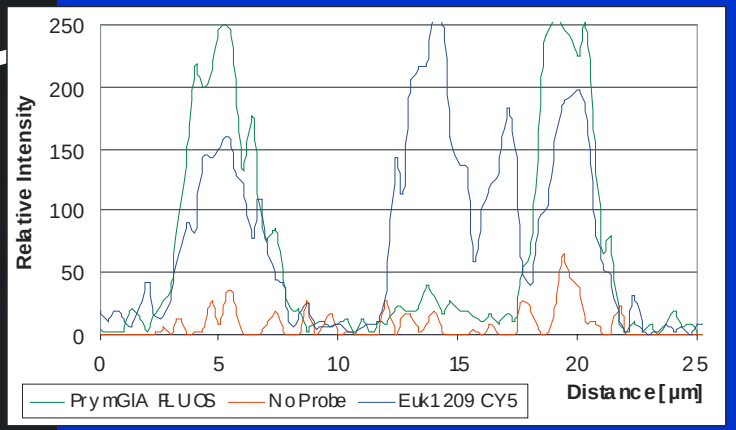


Progress until June 2002

Differentiation of *Prymnesium nemamethecum* and *Emiliania huxleyi* by FISH: Dual labelling with optimal results

Cell mixture labelled with universal eukaryotic probe EUK1209, blue and *Prymnesium* genus specific probe (PrymGl01A), green; CLSM detection

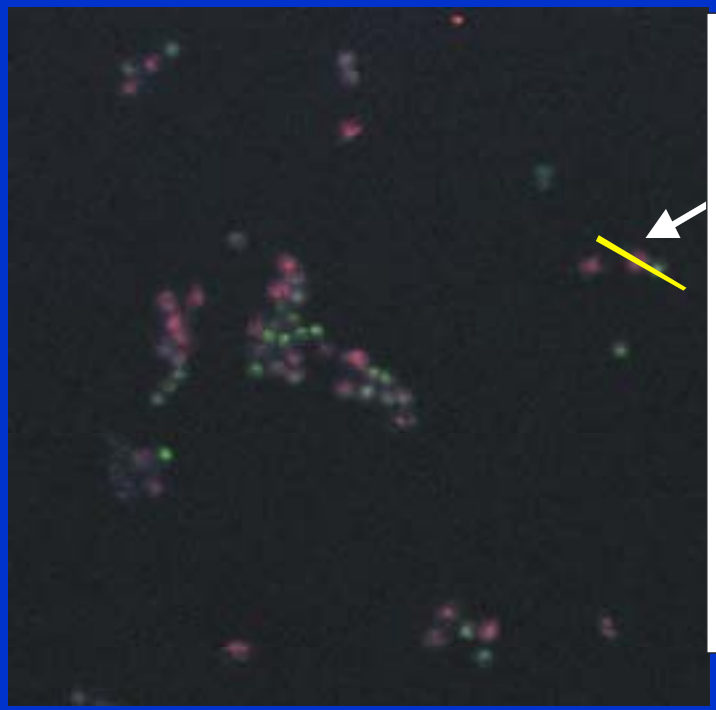


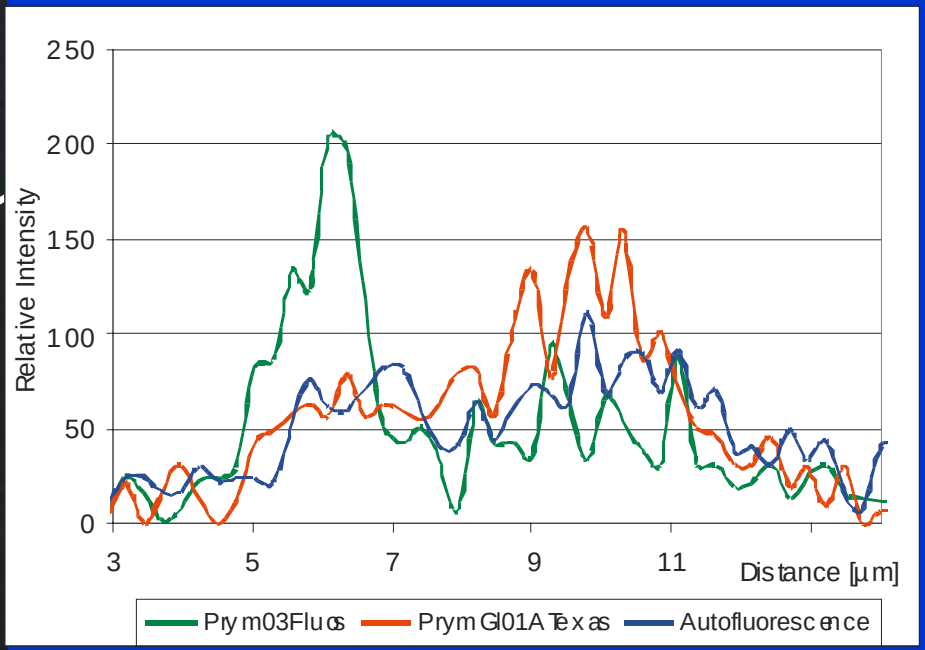


Progress until June 2002

Differentiation of *Prymnesium nemamethecum* and *Emiliania huxleyi* by FISH: Dual labelling with sub-optimal results

Cell mixture labelled with Prymnesiophyceae probe (Prym03), green and *Prymnesium* genus specific probe (PrymGl01A), red; CLSM detection





Summary

Dot Blots

- More / less stringent hybridisation conditions needed for different probes

FISH

- Use of actively growing cells for probe tests necessary
- Detection of probe signal by laser scanning methods possible
- Dual labelling of algal cells possible, combination of dyes FITC & Cy5 better than FITC & Texas red
- Application of dual labelling would reduce time needed for routine characterisation of samples

Plans for second half year period 2002

Dot Blot

- further tests with the different **Prymnesium parvum** probes, including the third probe designed
- continuation of dot blot tests for the **OLI** probes and the **COCCO1** clade level probes

FISH

Cocco01 and **Prym03:** additional target and non-target species

addition of the competitor for Cocco01

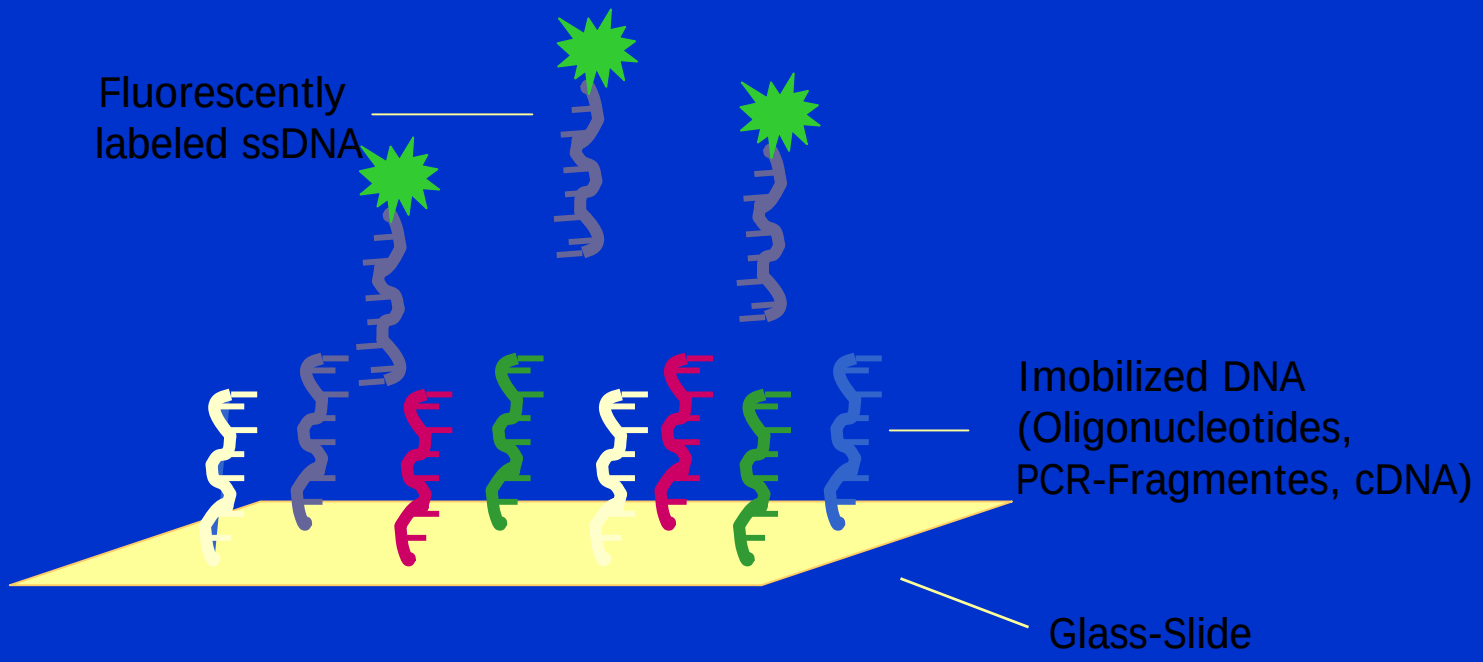
Hetero01: Optimization of signal intensity, addition of competitor

Probe / Species	Prym03	Prym Gl01A	Prym Gl02B	Cocco01	OliGr01	OliGr04	Prym Parv01	Pryparv AllA
Emiliania huxleyi	+	-	-	+	-	-	(+)	-
Isochrysis galbana	+	-	-	+	-	-	+	+
Pleurochrysis carterae	+	-	n.t.	+	n.t.	n.t.	n.t.	
Pleurochrysis elongata	n.t.	n.t.	n.t.	n.t.	-	-	(+)	-
Imantonia spec.	+	n.t.	-	-	-	-	+	+
Prymnesium patelliferum	+	+	+	-	n.t.	n.t.	K0252: +	K0252: +
Prymnesium parvum	+	+	+	-	n.t.	n.t.	PCC94: +	PCC94: +
Prymnesium nemamethecum	+	+	+	-	-	-	(-)	-
Chrysochromulina hirta	+	-	-	-	n.t.	n.t.	+	+
Chrysochromulina throndsenii	+	-	-	-	-	-	(-)	-
Chrysochromulina campanulifera	+	-	-	-	n.t.	n.t.	-	+
Chrysochromulina polylepis Klon B 11	n.t.	-	-	n.t.	n.t.	n.t.	n.t.	+
OLI11007	n.t.	n.t.	n.t.	n.t.	+	n.t.	n.t.	n.t.
OLI11019	n.t.	n.t.	n.t.	n.t.	n.t.	+	n.t.	n.t.
Phaeocystis antarctica	+	-	-	-	n.t.	n.t.	(-)	-
Phaeocystis globosa	(-)	-	-	n.t.	n.t.	n.t.	n.t.	n.t.
Phaeocystis pouchettii	n.t.	n.t.	-	n.t.	n.t.	n.t.	n.t.	n.t.
Pavlova lutheri	-	n.t.	n.t.	-	-	-	-	-
Alexandrium tamarense 31/4 W. Europe	-	-	-	-	-	-	(+)	(+)
Alex tam GTPP01 America	-	n.t.	-		n.t.	n.t.	n.t.	n.t.
Alexandrium fundyense GT-7	-	n.t.	n.t.		n.t.	n.t.	n.t.	n.t.
Alexandrium minutum AL1	-	-	n.t.		n.t.	n.t.	n.t.	(+)
Fragellariopsis sublimata	-	-	-	-	-	-	-	(+)

DNA Microchip - Progress to Date

Katja Kerkmann and Linda Medlin

Scheme of a DNA-Chip Experiment



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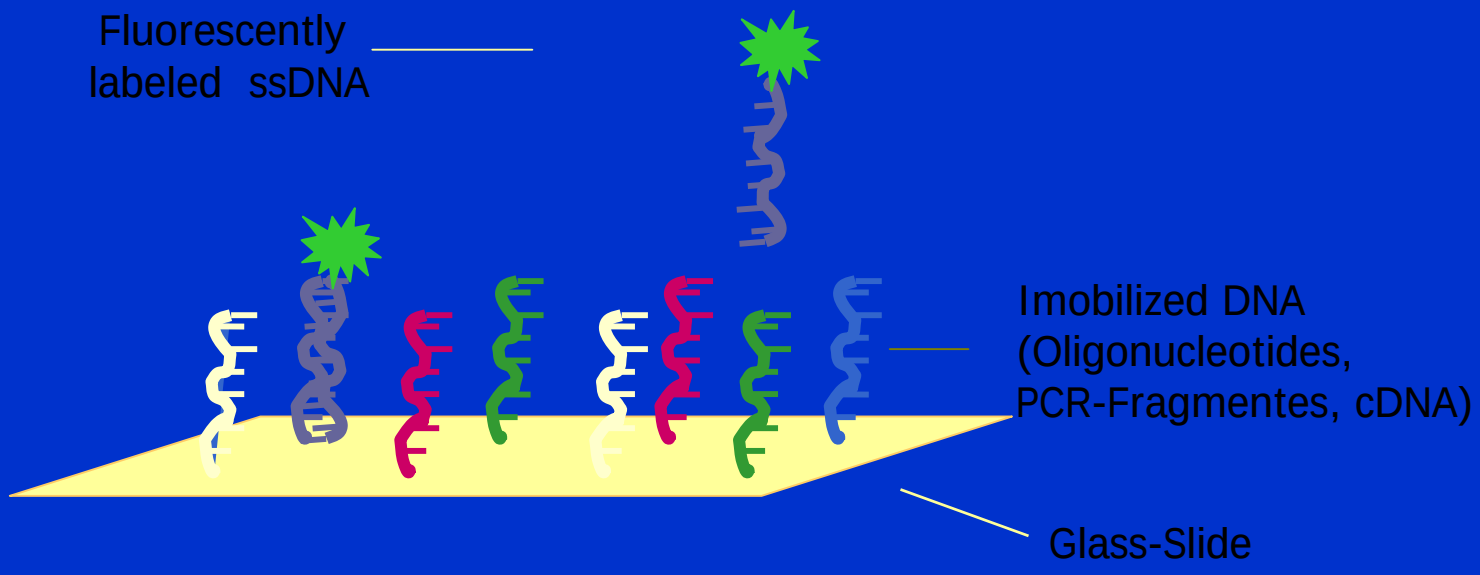


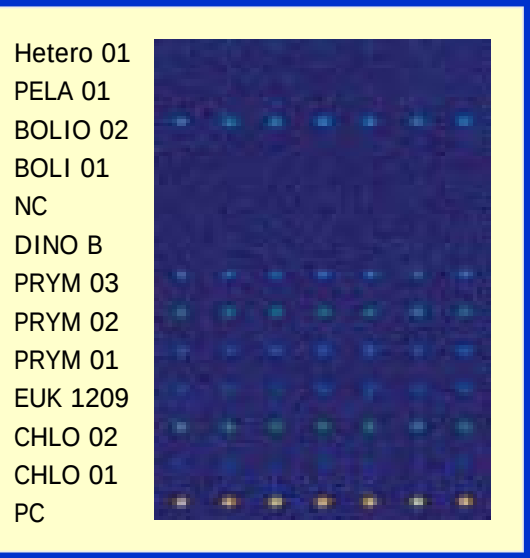
Tested to Date

- Length of C spacer linker from slide to probe
- Helper oligonucleotide
- FITC vs CY3

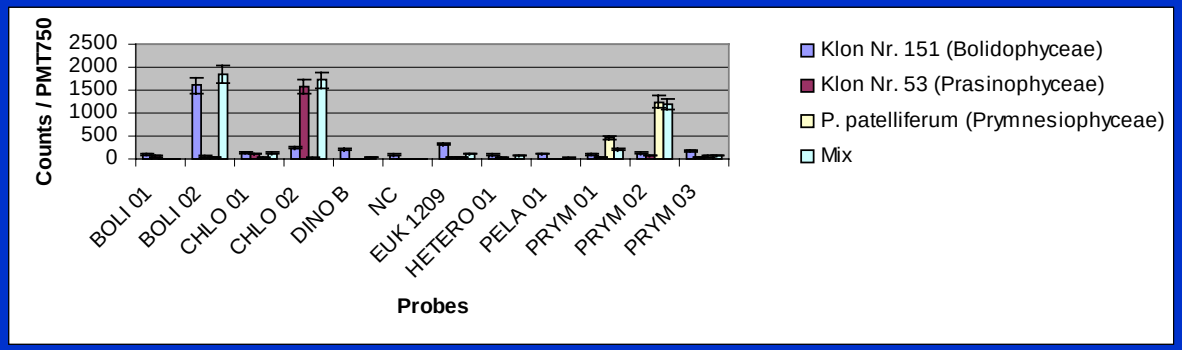
New Items to be Tested

- TSA enhancement for CY3
- Multiple hierarchical probes per spot
- double the probe length

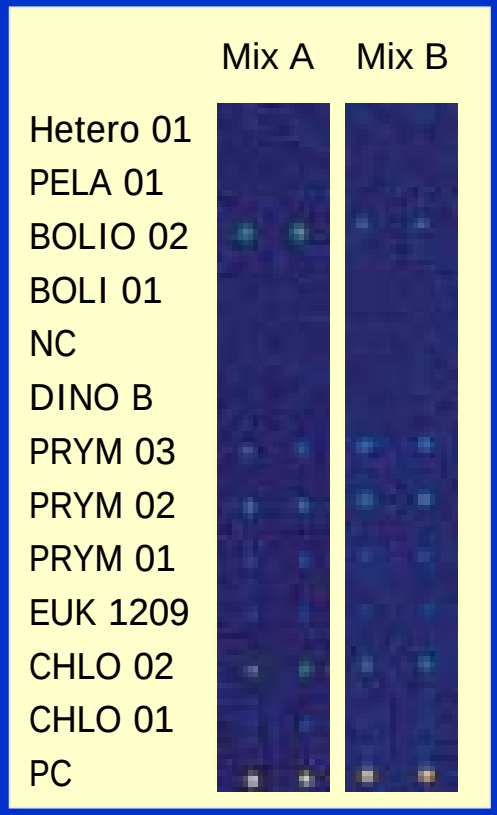
Hybridization of PCR-Fragments from PICODIV-Clones to a DNA-Chip



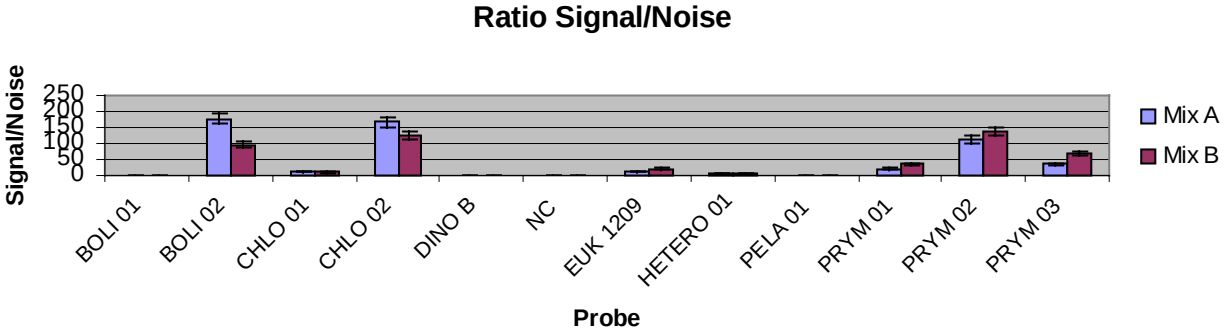
Bolidophyceae Clone # 151 [30 nM]
Prasinophyceae Clone # 53 [30 nM]
Prymnesiophyceae *P. patelliferum* [10 nM]



Comparison of two Hybridizations with different Target-Concentrations



	Mix A	Mix B	Probe	Ratio A/B
Clone # 151 (Bolidophyceae)	30 nM	15 nM	BOLI 02	-1.8
Clone # 53 (Prasinophyceae)	30 nM	15 nM	CHLO 02	-1.4
P. patelliferum (Prymnesiophyceae)	10 nM	20 nM	PRYM 01	1.8
			PRYM 02	1.3
			PRYM 03	1.9



Outlook for Year 3 Samples

- Class level probes will be ready for routine use: Dino B, DinoE+12, Prym01, 02, 03, Bolido01, Chloro02, Hetero01
- Supplemented with dot blots