# Analysing cultures by SSCP

Problem 1: Judge whether a culture is unialgal

Problem 2: Identify culture

Methods on hand:

Light microscopy - allows to judge whether a culture is NOT unialgal

(Electron microscopy) - Very labor-intense ... for Wenche, only few cells can be checked

Flow cytometry – will identify different forms (i.e. cysts, sexual stages …) as different species

Pigment analysis (HPLC) – can not identify mixed culture

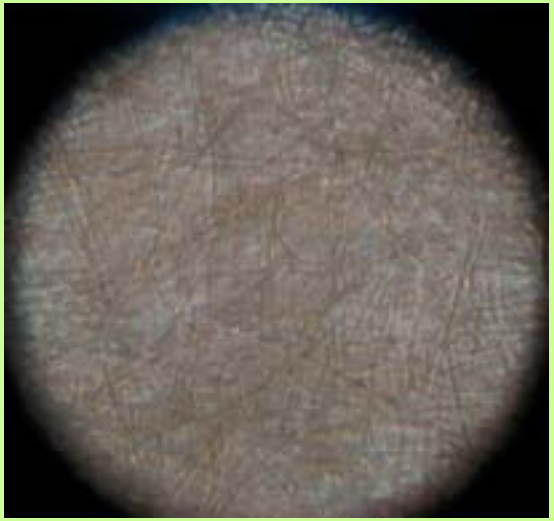
18S sequencing - too expensive

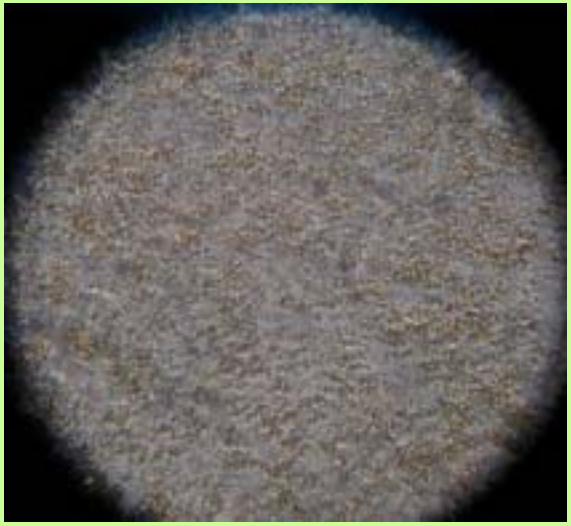
Fingerprinting using SSCP and subsequent sequencing - may miss some unialgals

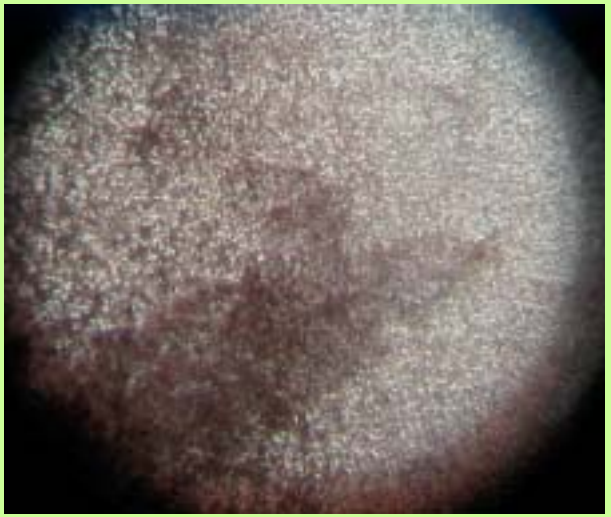
# Protocol currently established

Screen cultures by eye:

Screen cultures by invers light microscopy:







# Protocol

- Harvest 1.5 ml of culture by centrifugation

- Resuspend pellet in 20 µl water, shock-freeze in $N_2$fl.

- Boil for 15 - 20‘, store @ -20°C or use immediately

- centrifuge again and use 5 µl of supernatant for PCR

# Protocol established

- Run SSCP PCR, check on gel

  (typically 50% of cultures produce bands)

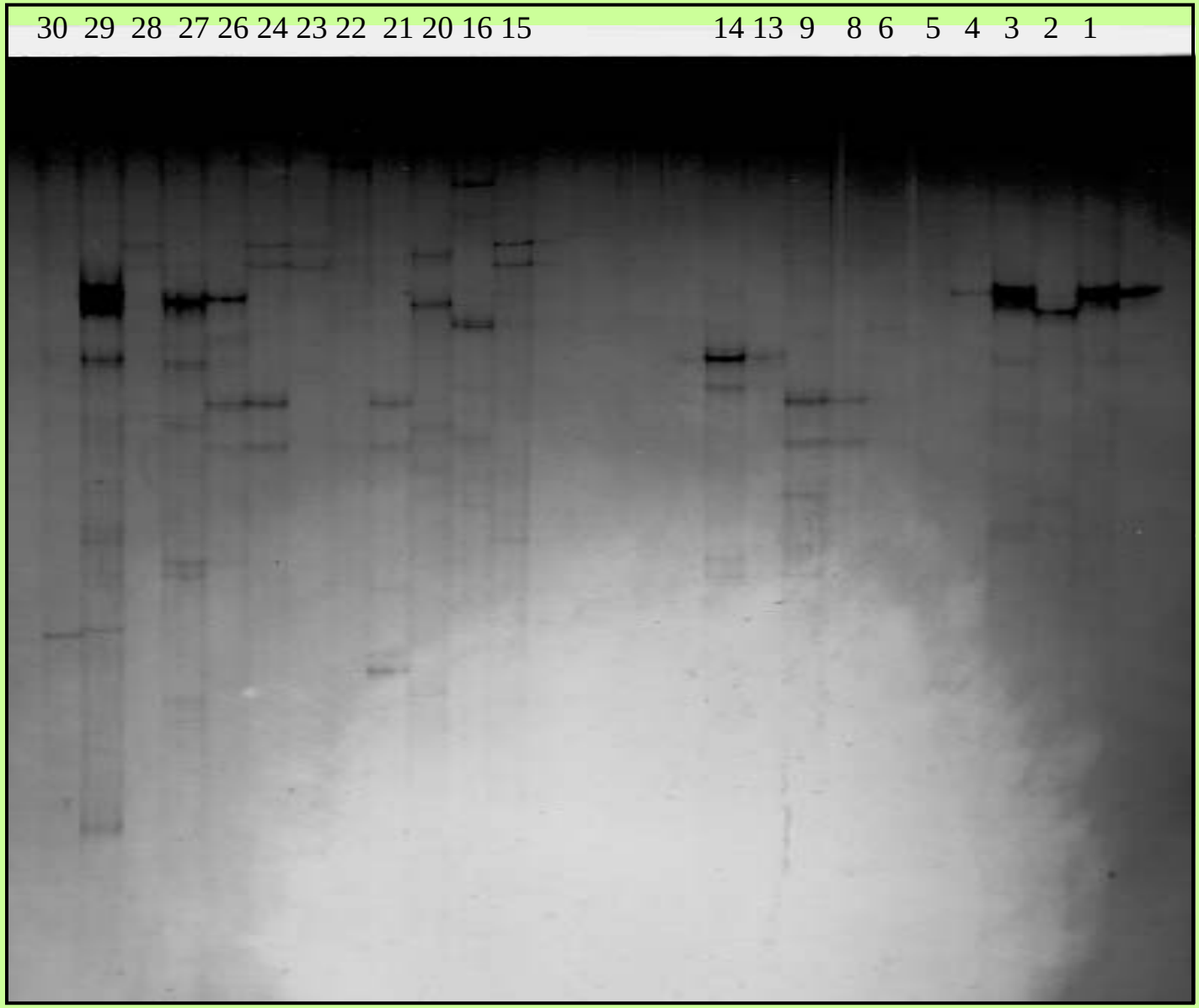
- Purify SSCP PCR product on Qiagen column

- Elute in 50 µl and divide in half, store one half @ -20° for later sequencing

- Use the other half for SSCP digest

- Purify SSCP digest on Qiagen mini columns, adjust elution volume (7 - 25 µl) according to yield of initial SSCP PCR

# SSCP of May 5, 2002;  30 Cultures



- Chose singular Bands.
  (potentially 20 - 50%of the SSCP products)

- Send second half of SSCP PCR for sequencing, keep 5 µl.

- Do BLAST search and send to Daniel for inclusion into the ARB tree.

- Go back to Culture and check by microscope, send culture to Wenche and Daniel.

# Results

## 1. Internal controls

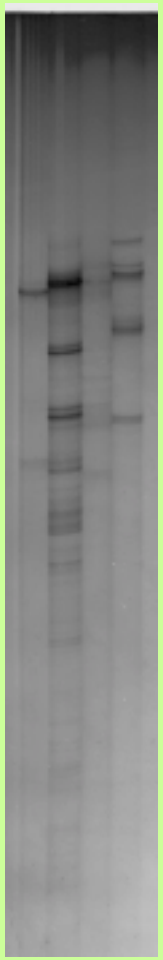
(Partly done by Dominique :-)

### A - Run SSCP analysis with *Skeletonema*

bad: produces multiple bands on SSCP gel
god: SSCP product gave a nice clean sequence
100% identical with *Skeletonema*

### B - Analyse samples provided by Dominique

bad: supposingly clean culture produced multiple bands on SSCP gel
good: *Thalassiosira* (?) culture resulted in a single band and a clean sequence identical with *T*.



# Results

20 cultures (out of ca. 100) could be identified, belonging to the following clades:

- Prasinophytes (8)

- Chlorophytes (3)

- Stramenopiles (*Caecitellus*, 4)

- Diatom (1-2)

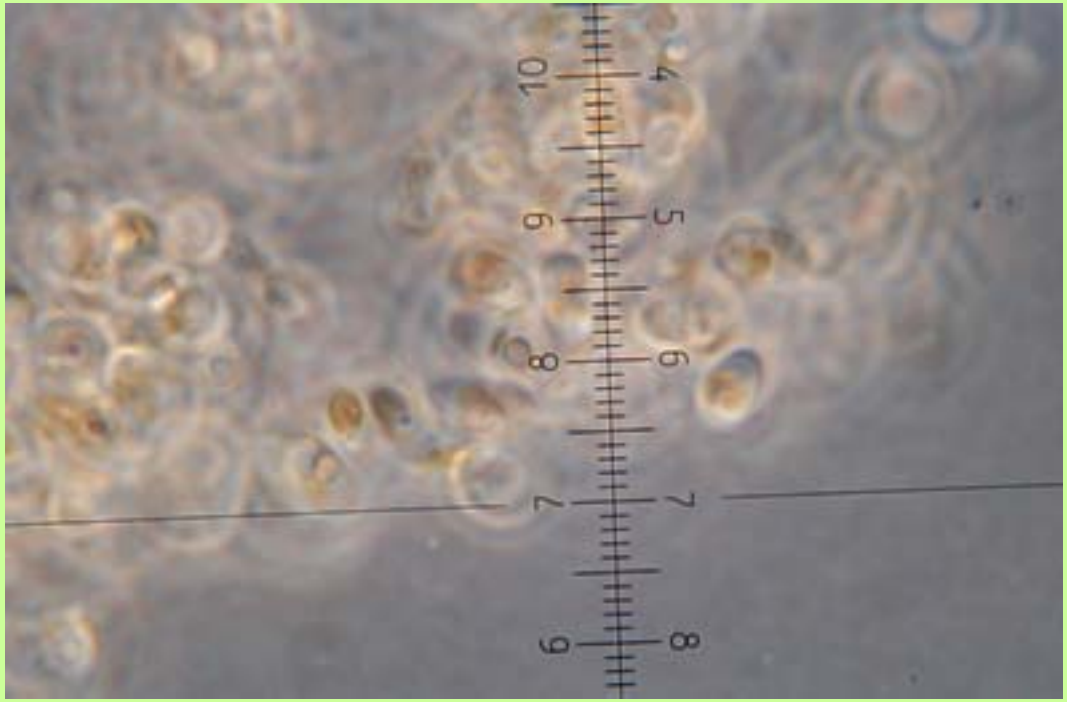
- Chrysophytes (1)

- Dictyochiophytes (1)

- Alveolates (*Cafeteria*, 1)

# Results

Examples:
clone: He010322 D2 B5
best match: *Phaeodactylum* (99%/506)

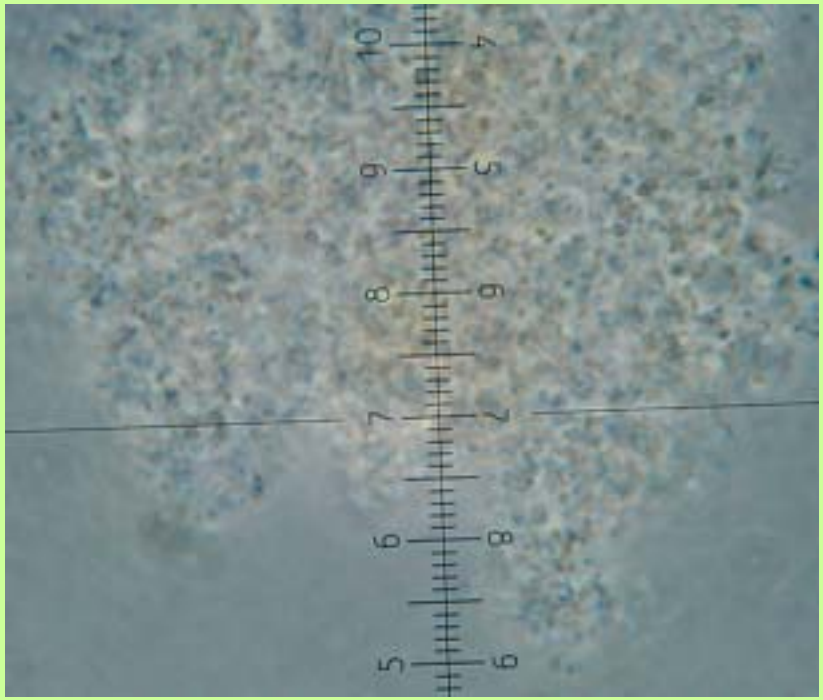


# Results

Examples:
clone:He001206I1 D1
best match: *Caecitellus parvulus* (89%/397)

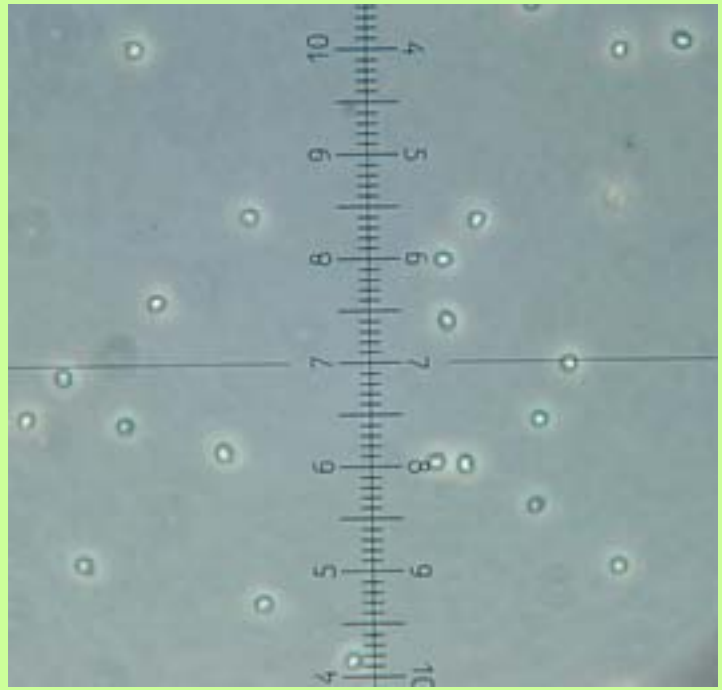
# Results

Examples:
clone: He010516 I1 B6
best match: *Micromonas pusilla* (92%/527)



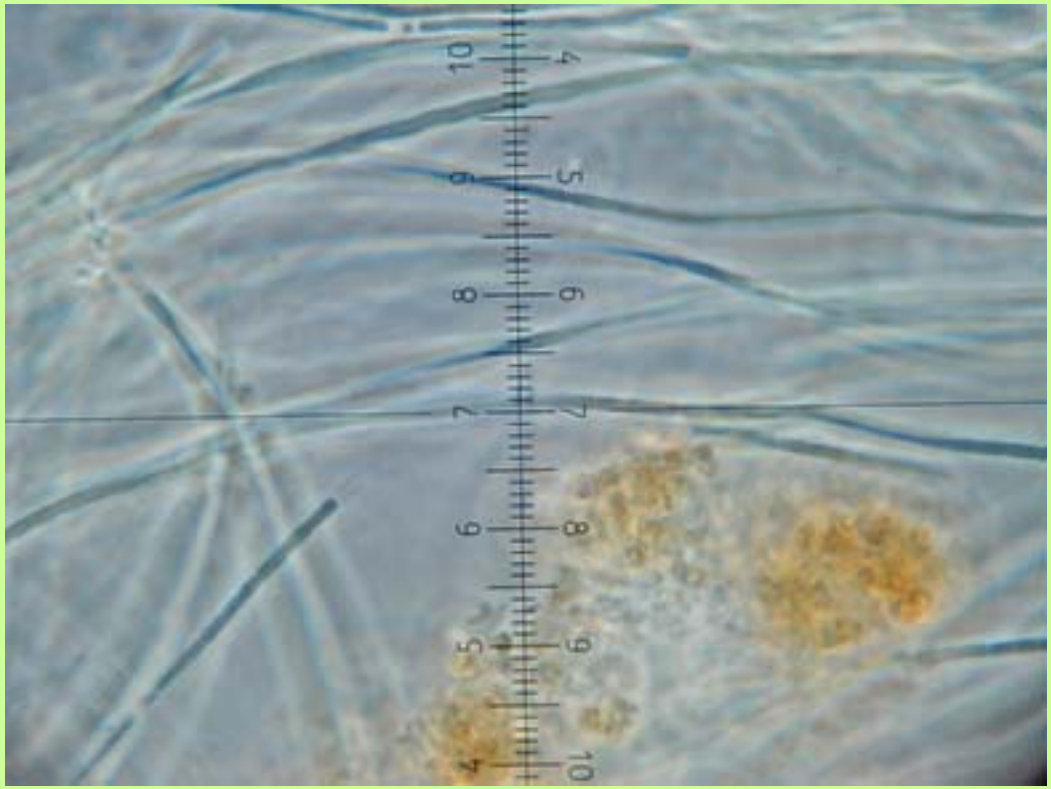
# Results

Examples:
clone: He010516 I1 B6
best match: *Mantoniella sq*. (98%/486)



# Results

Examples:
clone: He010322 I3A3
best match: *Caecitellus parvulus*. (92%/397)

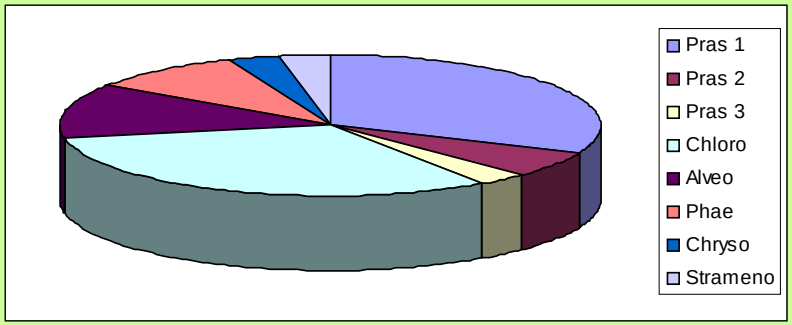


# New cultures

- 150 well grown new cultured analysed
- 84 produced an SSCP PCR product
- 48 sent for sequencing
- 39 sequences obtained
- 8 sequences were ambigous, i.e. consisted of more than 1 sequence

# Identities of cultures

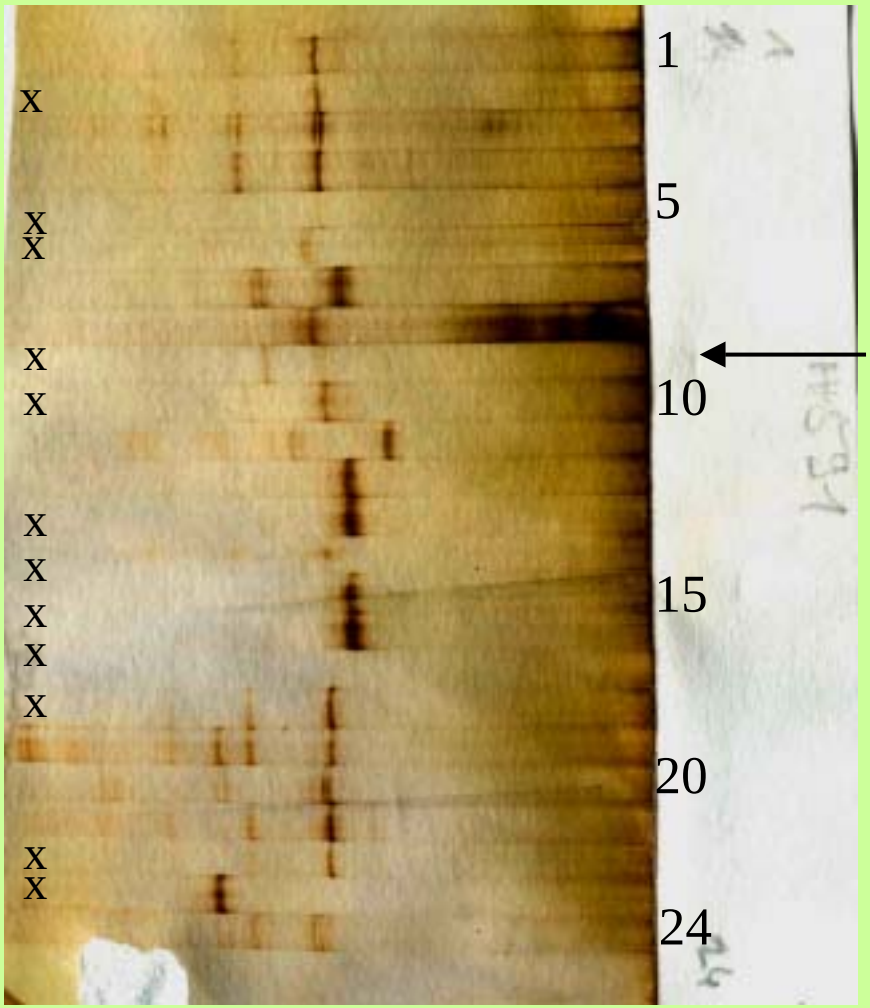
- 10 cultures ~ 93% similar to *Micromonas*
- 9 cultures ~ 97% similar to C3 E009/*Nanochloron*
- 4 cultures ~ 98% similar to *Methanochrys sinensis* (alveolate/ciliate)
- 3 cultures 99-100% similar to *Phaeocystis*
- 2 cultures ~ 99% similar to *Ostreococcus*
- 1 culture 99% similar to *Spumella* (chrysophyta)
- 1 culture 98% identical to ANT37-3/*Mantoniella*
- 1 culture 96% similar to *Chlorella*
- 1 culture 93% similar to *Developayella elegans* (stramenopile)



# Identities of cultures – sampling time

- ***Micromonas*-like cultures**: He010322 IMR (3x), He010619 Dr (2x), He010418 Dr (2x), He010518 Dr (2x)
- ***Nanochloron*-like cultures**: He001206 Dr , He000428 (2x), He010619 (2x), He010418 (3x), He010322 (2x)
- **dto. *Methanochrys***: He010619 (3x), He010322

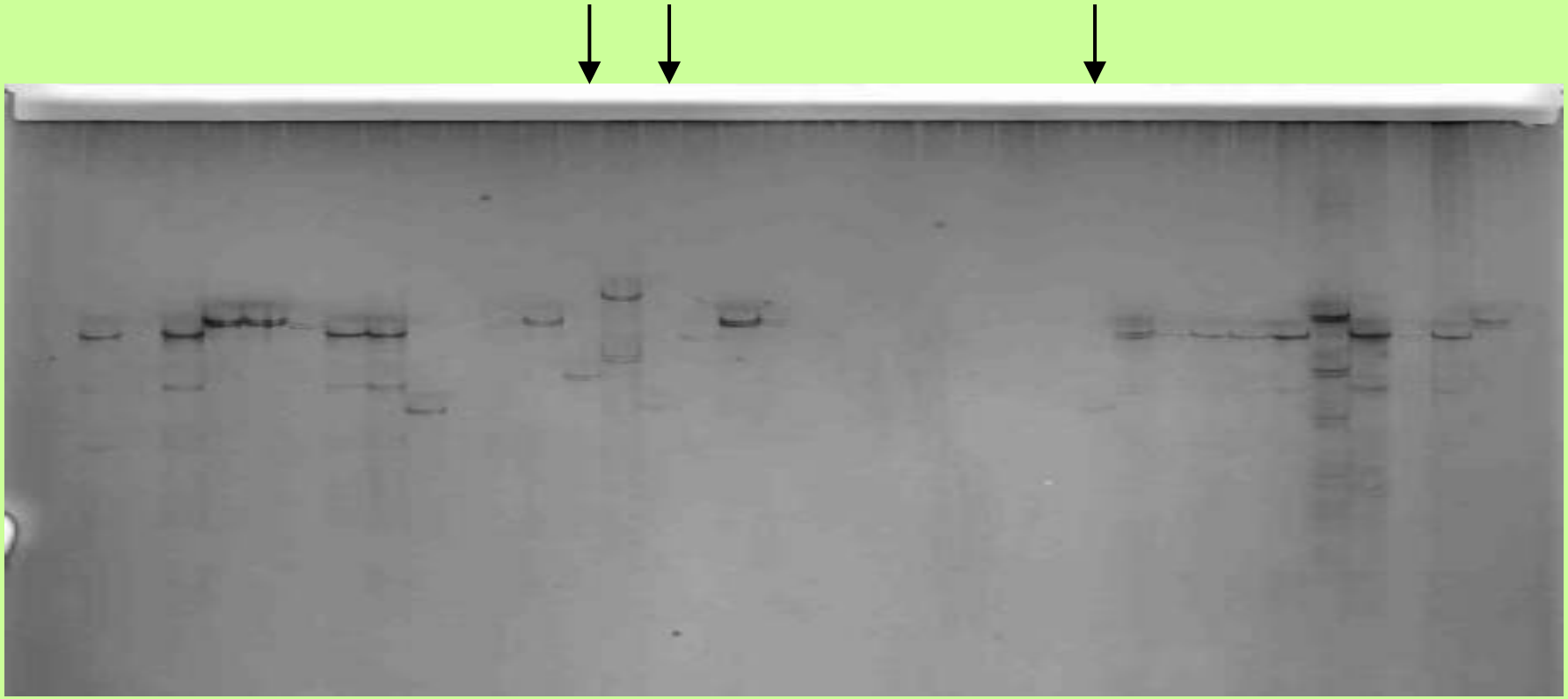
# Details – (1) multiple band = multiple species?




Yes!?

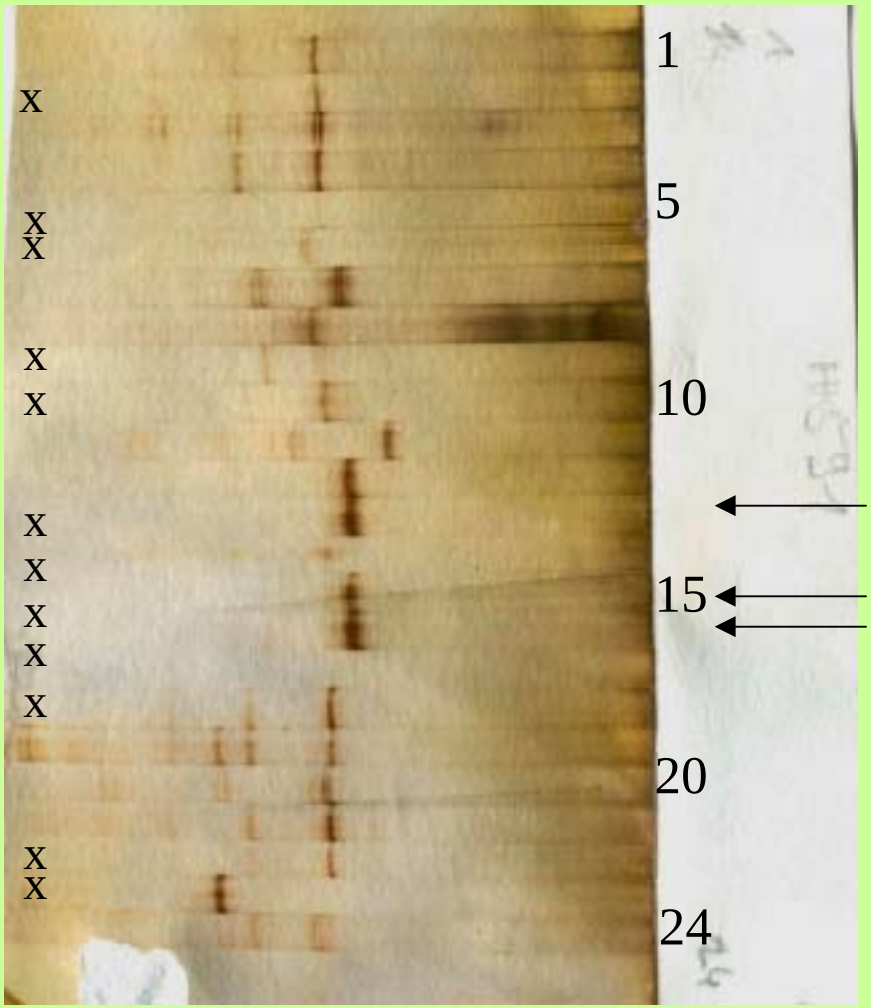
X = chosen for sequencing

# Details – (1) multiple band = multiple species?



Difficult to judge when bands are weak

# Details – (2) similar band = identical species?

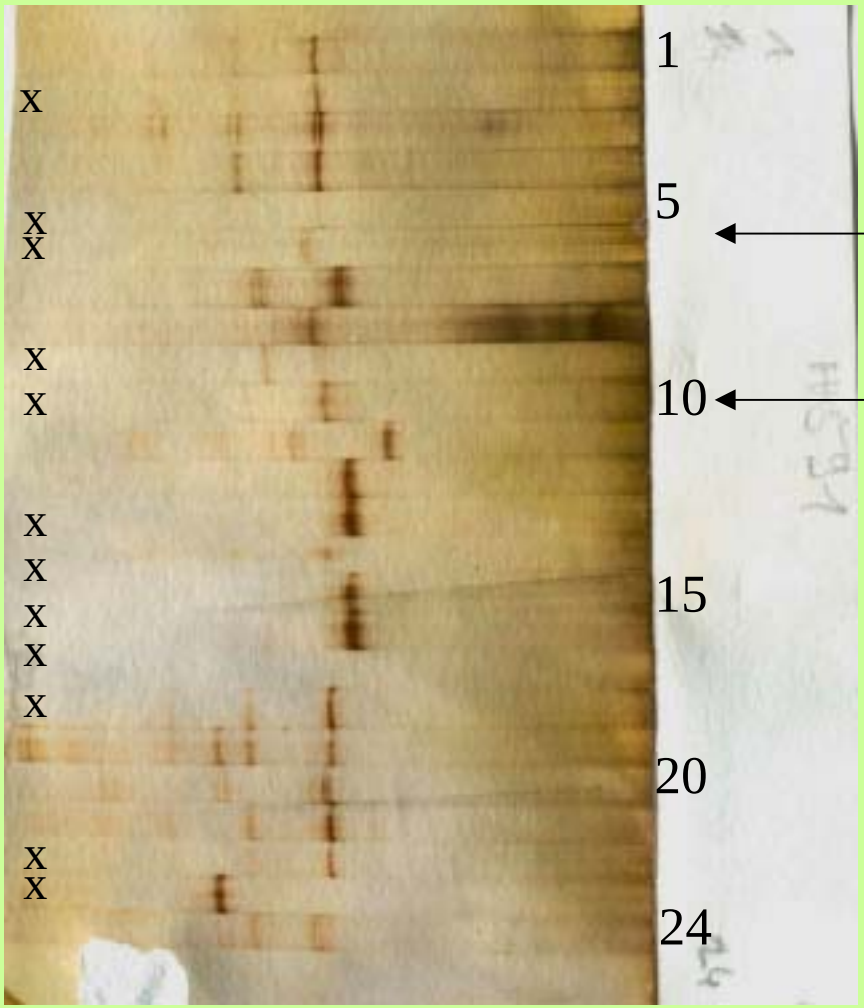



euc. Isolate C3 E009/*Nanochloron*

Yes

X = chosen for sequencing

# Details – (2) similar band = identical species?



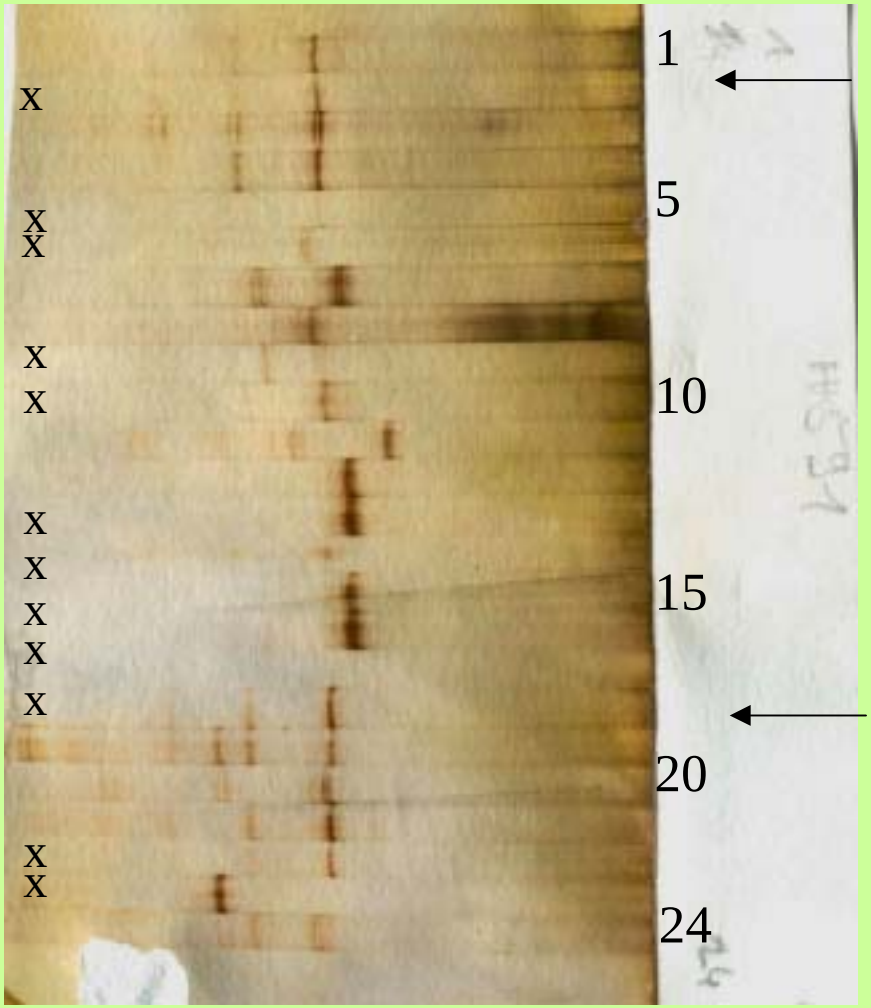

*Ostreococcus*

*Ostreococcus*

No?

X = chosen for sequencing

# Details – (2) similar band = identical species?




*Micromonas pusilla*

Yes, but sometimes difficult to judge, even on one gel

X = chosen for sequencing

# Summary

- Screening of cultures with SSCP allows to determine the nature of a culture and whether it is unialgal
- Large numbers of cultures can be screened, i.e. for PICODIV screening of > 250 cultures led to the identification of ca. 50 of them.
- Sometimes presence of multiple bands in SSCP gels is difficult to judge.
- Closely related sequences can migrate at slightly different speed.