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# Microbial diagnostic microarrays

## **Aim:**

To develop a technology for the high-throughput detection and quantification of microorganisms from any sample

## **Methodology:**

Oligonucleotide microarrays + Molecular ecology

## **Application potential:**

**Agriculture** - Detection of human and veterinary pathogens throughout the production of food- and feedstuff; Detection of plant pathogens; Analysis of the effect of land management practices on soil microbial diversity => soil quality); Analysis of the effect of transgenic plants on soil microbial communities; Evaluation of the impact of phytosanitary or other pollutants inputs, of agricultural practices or of other relevant ecological factors on community diversity

**Environmental microbiology** - Environmental analysis, ecotoxicology

**Clinical microbiology** - Detection of human pathogens in clinical samples and wastewaters; Detection of animal pathogens in veterinary samples; Food quality control; Detection of food-borne pathogens

# Advantages of diagnostic microbial microarrays:

**Fast detection** - with the current technology results can be obtained within 8 hours from the arrival of the clinical, environmental, etc. sample

**High throughput** - hundreds to thousands of different microbial species or strains can be targeted by a single array

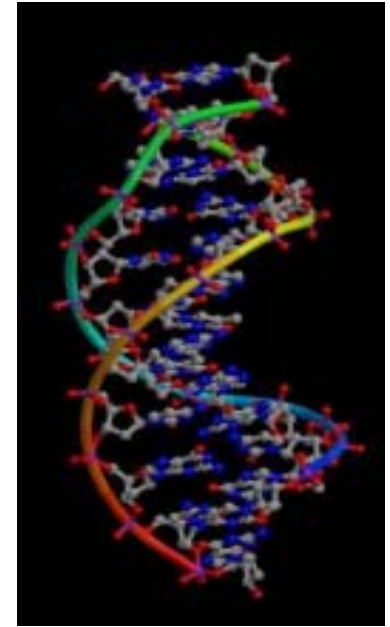
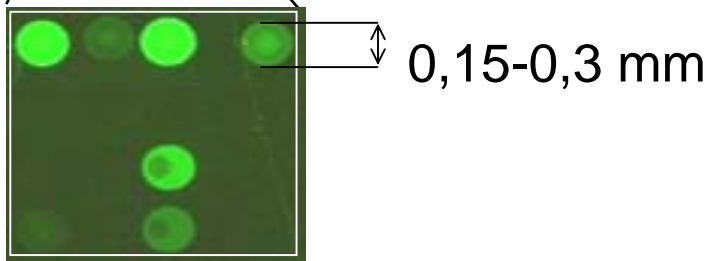
**Quantification** - it is possible to quantify the abundance of the different microbes detected (thus understanding the composition of microbial communities or obtain information on the level of infection by pathogens)

**Multiple probes** - multiple probes targeting the same species or strain can be applied onto the same array thus significantly increasing the reliability of the results

**Nested probe approach** - by applying probes targeting higher taxonomic groups of bacteria it becomes possible to obtain a more general picture of the bacterial community composition. It also enables the detection of novel species or strains which are not targeted by any of the species or strain specific probes

# Oligonucleotide microarrays are:

sets of oligonucleotides  
(typically 10 to 100 bp in length)  
immobilised onto a glass carrier  
in a highly parallel, addressable format.



Reminder: it's all about:  
**hybridisation!**

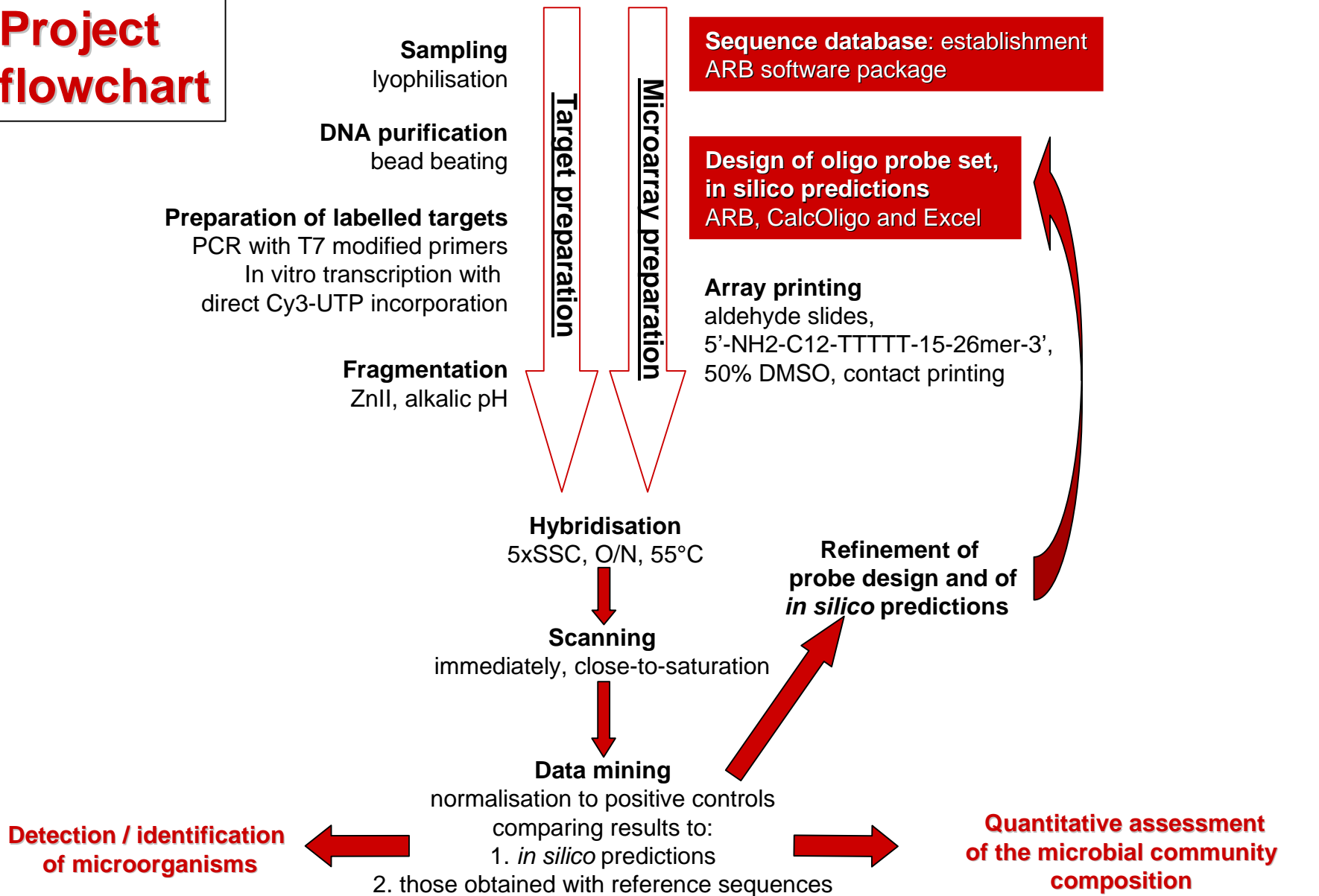
# Target group of bacteria for the pilot array: Methanotrophs

Methanotrophs are...

- ... a unique group of bacteria using methane as sole source for carbon and energy
- ... very important in mitigating the greenhouse effect
- ... a taxonomically well defined group of bacteria suiting the requirements for a test group
- ... the *pmoA* gene can be used as a (“functional”) phylogenetic marker (over 500 sequences available)



# Project flowchart



**PROBE DESIGN**

This module searches for specific oligonucleotides in the database.  
 The PT\_SERVER's (not the current) database is used searching probe targets

PT\_SERVER: localhost: proaFull.arb

Enter some parameters (press help to get more information)

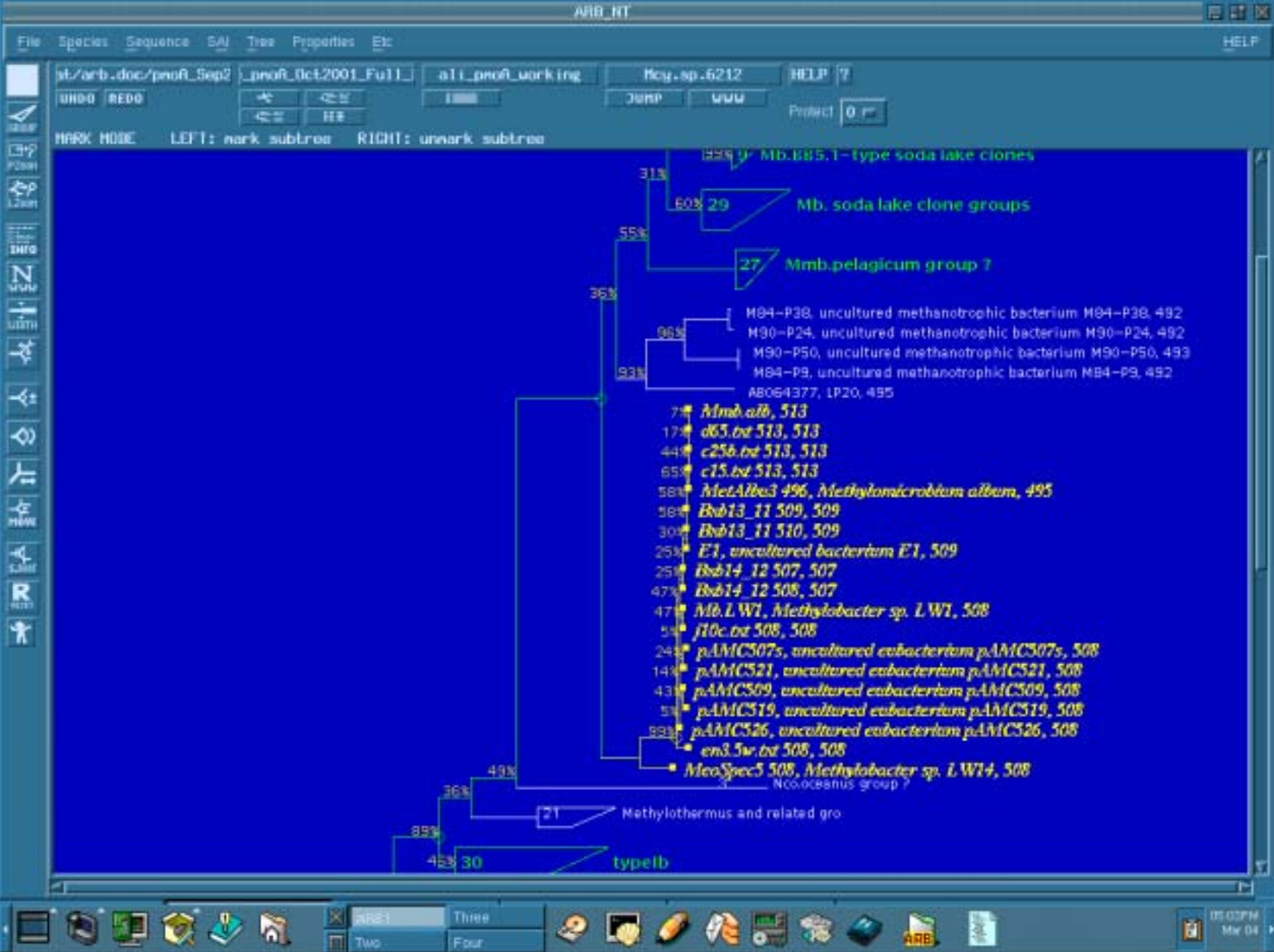
	MIN	MAX
Length of output	50	
Length of probe	22	
Max. non group hits	2	
Temperature	55	65
Max. hairpin bonds	4	
G+C-content	40	100
Min group hits (%)	80	
ECOLI-position	0	10000

Buttons: GO, RESULT, EXPERT

**Phylogenetic Tree:**

- 89%
  - 89%
    - 58% lsk18508, LOPB13.2, 508
    - 89% Hb.885.1-type soda lake clones
  - 50% 29 Hb. soda lake clone groups
  - 27 Mmb.pelagicum group ?
  - 84-92
    - M84-P38, uncultured methanotrophic bacterium M84-P38, 492
    - M90-P24, uncultured methanotrophic bacterium M90-P24, 492
    - M90-P50, uncultured methanotrophic bacterium M90-P50, 493
    - M84-P9, uncultured methanotrophic bacterium M84-P9, 492
    - AB064377, LP20, 495
  - 513
    - Mmb.alb, 513
    - d65.bt 513, 513
    - 44% c25b.bt 513, 513
    - 65% c15.bt 513, 513
    - 58% MetAlb3 496, *Methylomicrobium album*, 495
    - 58% Hb13\_11 509, 509
    - 30% Hb13\_11 510, 509
    - 25% E1, uncultured bacterium E1, 509
    - 25% Hb14\_12 507, 507
    - 47% Hb14\_12 508, 507
    - 47% Mb.L.WI, *Methylobacter* sp. L.WI, 508
    - 54% j10c.bt 508, 508
    - 24% pAMC507s, uncultured eubacterium pAMC507s, 508
    - 14% pAMC521, uncultured eubacterium pAMC521, 508
    - 43% pAMC509, uncultured eubacterium pAMC509, 508
    - 54% pAMC519, uncultured eubacterium pAMC519, 508
    - 89% pAMC526, uncultured eubacterium pAMC526, 508
    - em3.5w.bt 508, 508
  - MeoSpec550B, *Methylobacter* sp. LW14, 508
  - 3 Nococeanus group ?
  - 36%
    - 49%
      - 21 Methylothermus and related gro

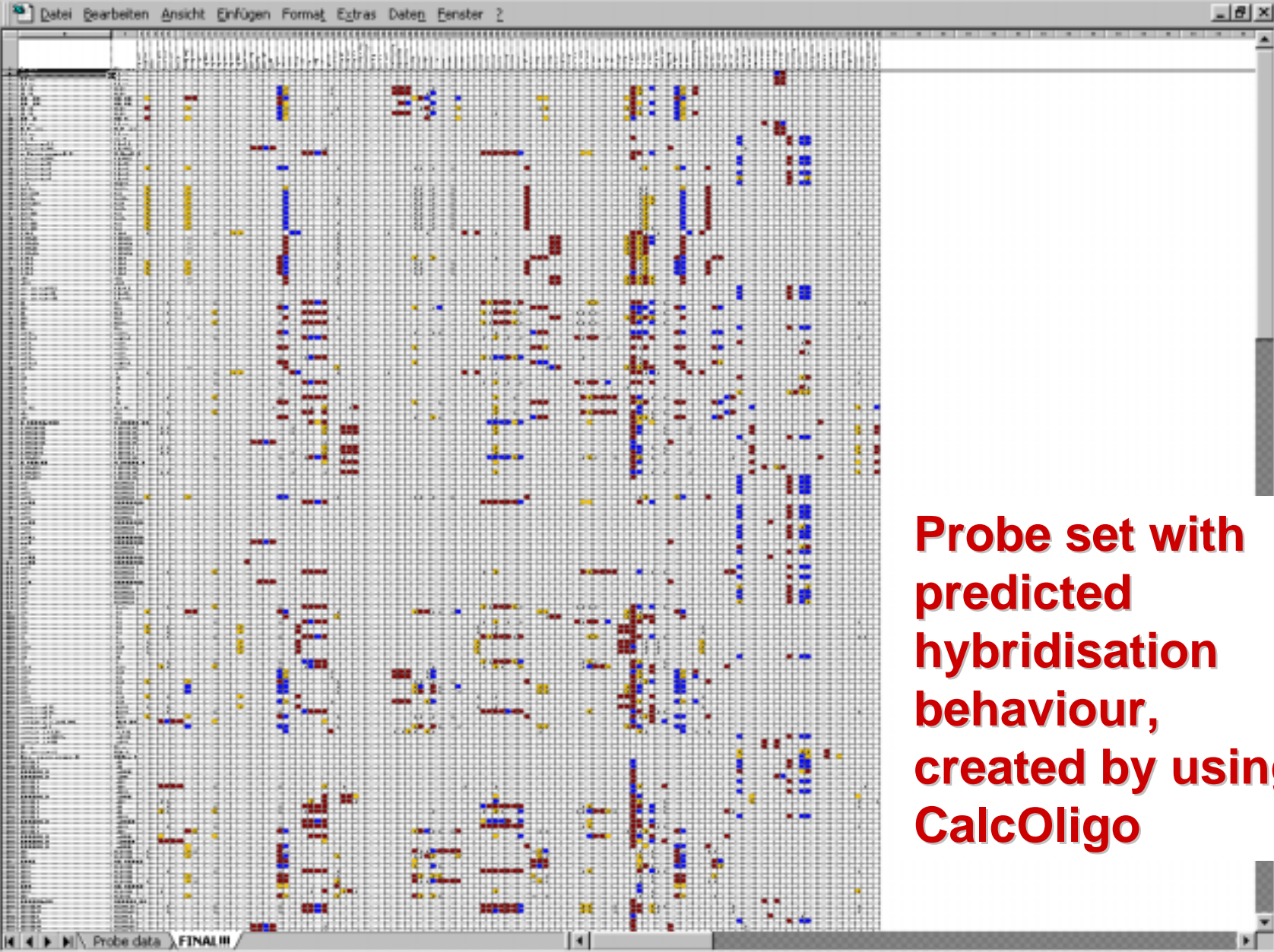
Probe design - selecting target group



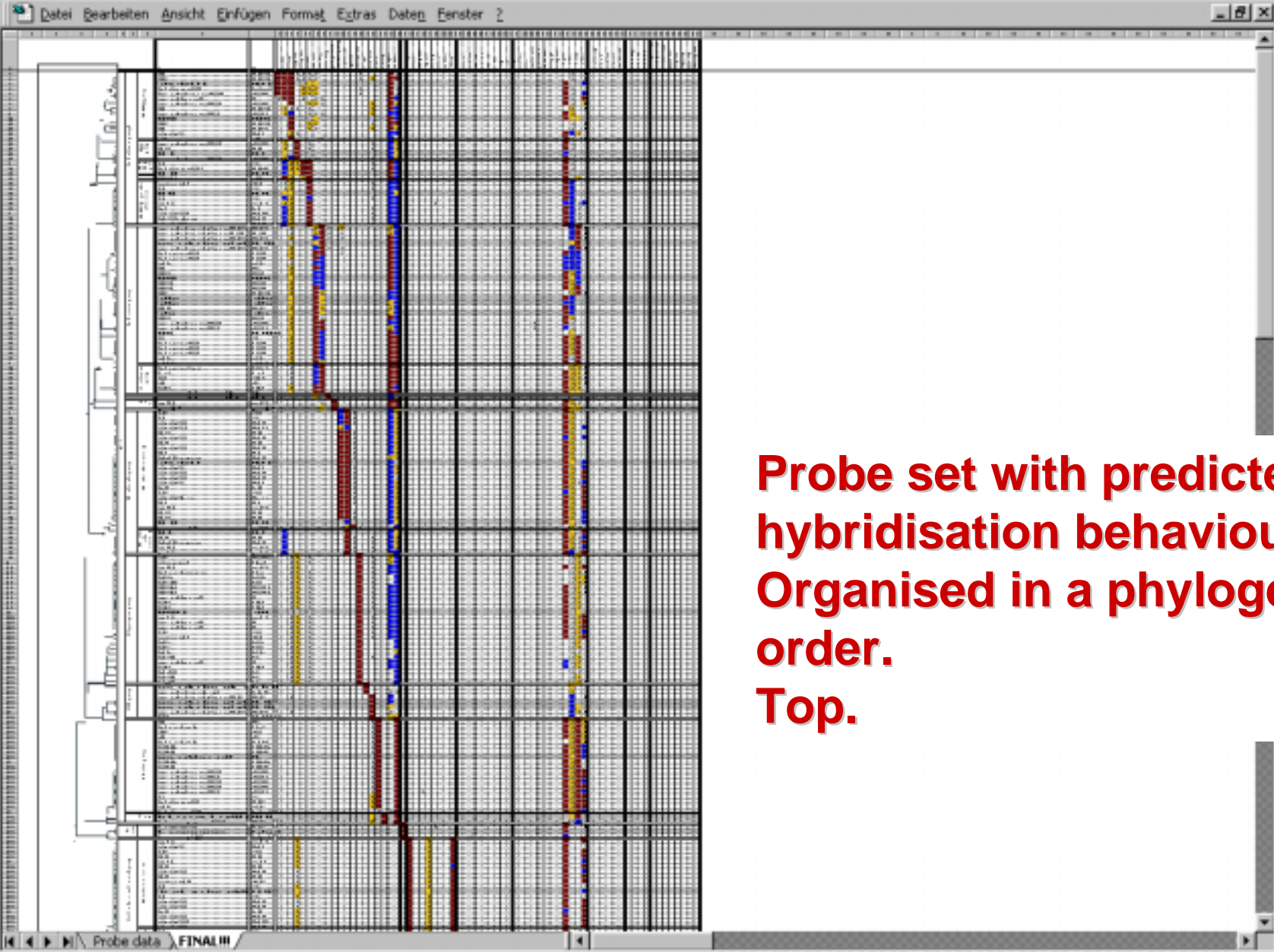
Probe match - 3 MM allowed, results

# Probe match - 3 MM results into Excel

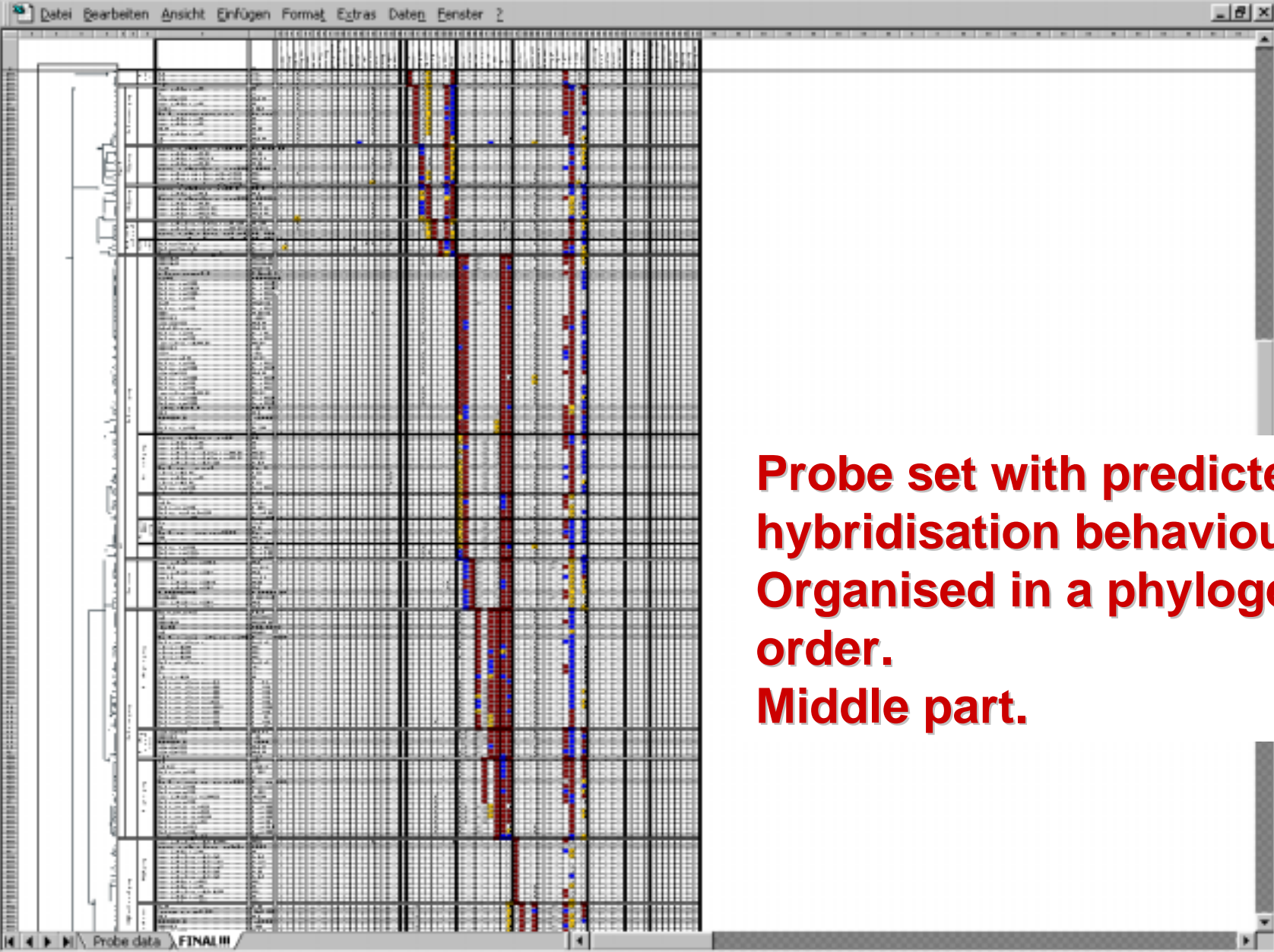
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Searched	for	cgctgcacgtgccggtg			Mc533							
2													
3	Short name	Long name	mis	N_mis	wmis	pos	ecoli	rev	'CGCUGCACGUGCCGGUG'				
4	B4	uncultured_bacterium_B4	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
5	Q.txt	Q	0	0	0	361	361	0	UCAUCGCGC=====	GAAAACAAC			
6	SL_5_28	soda_lake_5-28	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
7	B5	uncultured_bacterium_B5	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
8	Bxb1_1	Bxb1_1	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
9	Mc.cap	Methylococcus_capsulatus	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
10	B3	uncultured_bacterium_B3	0	0	0	361	361	0	UCAUCGCGC=====	GAAUGCAAC			
11	B1	uncultured_bacterium_B1	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
12	B2	uncultured_bacterium_B2	0	0	0	361	361	0	UCAUCGCGC=====	GAAUACAAC			
13	Mcl.gra	Methylocaldum_gracile	0	0	0	361	361	0	UGAUUGCGC=====	GAAUACAAC			
14	Mcl.tep	Methylocaldum_tepidum	0	0	0	361	361	0	UCAUUGCGC=====	GAAUACAAC			
15	Mcl.sze	Methylocaldum_szegediense	0	0	0	361	361	0	UGAUUGCCC=====	GAAUACAAC			
16	RC1	uncultured_putative_methanotr	0	1	0	361	361	0	UCAUCGCGC==N=====	GAAUACAAC			
17	SP-64	SP-64	1	0	0.6	361	361	0	UCAUCGCGC=====g=	GAAUACAAC			
18	FL_DIKO	p2	1	0	1.5	361	361	0	UCAUCGCGC=====U=	GAAUACAAC			
19	pAMC501	uncultured_eubacterium_pAMC50	1	0	1.5	361	361	0	UCAUCGCC=====A=====	GAAUUAAC			
20	RC4	uncultured_putative_methanotr	1	0	1.5	361	361	0	UGAUUGCCC=====U=	GAAUACAAC			
21	J3.9	J3.9	2	0	2.1	361	361	0	UUAUUGCGC=U=====	GAAUACAAC			
22	J3.10	J3.10	2	0	2.1	361	361	0	UUAUUGCGC=U=====	GAAUACAAC			
23	J3.12	J3.12	2	0	2.1	361	361	0	UUAUUGCGC=U=====	GAAUACAAC			
24	FW-36	uncultured_bacterium_FW-36	2	0	2.1	361	361	0	UCAUCGCGC==u=====A=====	GAAUACAGC			
25	WC306-17	uncultured_bacterium_WC306-17	2	0	2.1	361	361	0	UCAUCGCGC==u=====A=====	GAAUACAGC			
26	WC301-37	uncultured_bacterium_WC301-37	2	0	2.1	361	361	0	UCAUCGCGC==u=====A=====	GAAUACAGC			
27	FW-1	uncultured_bacterium_FW-1	2	0	2.5	361	361	0	UCAUUGCAC==g=U=====	GAAUACAGC			
28	FW-50	uncultured_bacterium_FW-50	2	0	2.5	361	361	0	UCAUUGCAC==g=U=====	GAAUACAGC			
29	WC306-5	uncultured_bacterium_WC306-5	2	0	3	361	361	0	UCAUCGCC=====A=====U=	GAAUACAAC			
30	PS-49	uncultured_bacterium_PS-49	2	0	3	361	361	0	UCAUCGCC=====A=====U=	GAAUACAAC			
31	M84-P105	uncultured_bacterium_M84-P105	3	0	2.7	361	361	0	UUUUCGGCA-u=u=U=====	GAGU AUGGC			
32	M80-P69	uncultured_bacterium_M80-P69	3	0	2.7	361	361	0	UUUUCGGCA-u=u=U=====	GAGU AUGGC			
33	pAMC512	Uncultured_eubacterium_pAMC51	3	0	3.2	361	361	0	UCAUUGCGC==u=====AC=====	GAGUACAGU			
34	peat20-1	peat20-1	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
35	JB-66	JB-66	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
36	RB-13	RB-13	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
37	peat1-1	peat1-1	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
38	SP-12	SP-12	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
39	SL_5_32	soda_lake_5-32	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
40	RB-66	RB-66	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
41	JY_6.110	japan_yumoto_6-110	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			
42	J2.F	J2.F	3	0	3.6	361	361	0	UCAUUGCGC=U=====A=====	GAAUACA AU			



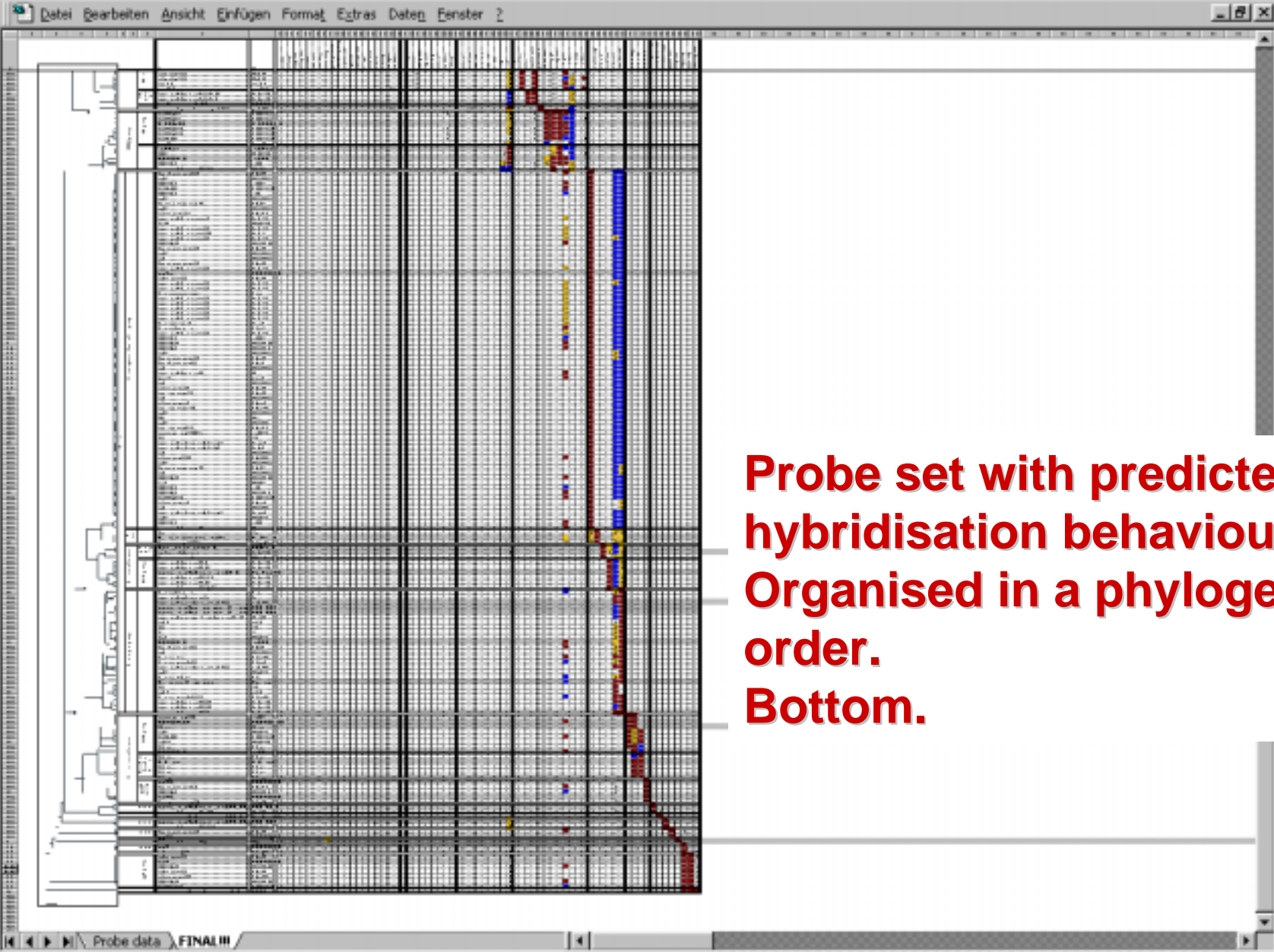
**Probe set with predicted hybridisation behaviour, created by using CalcOligo**



**Probe set with predicted hybridisation behaviour, Organised in a phylogenetic order. Top.**

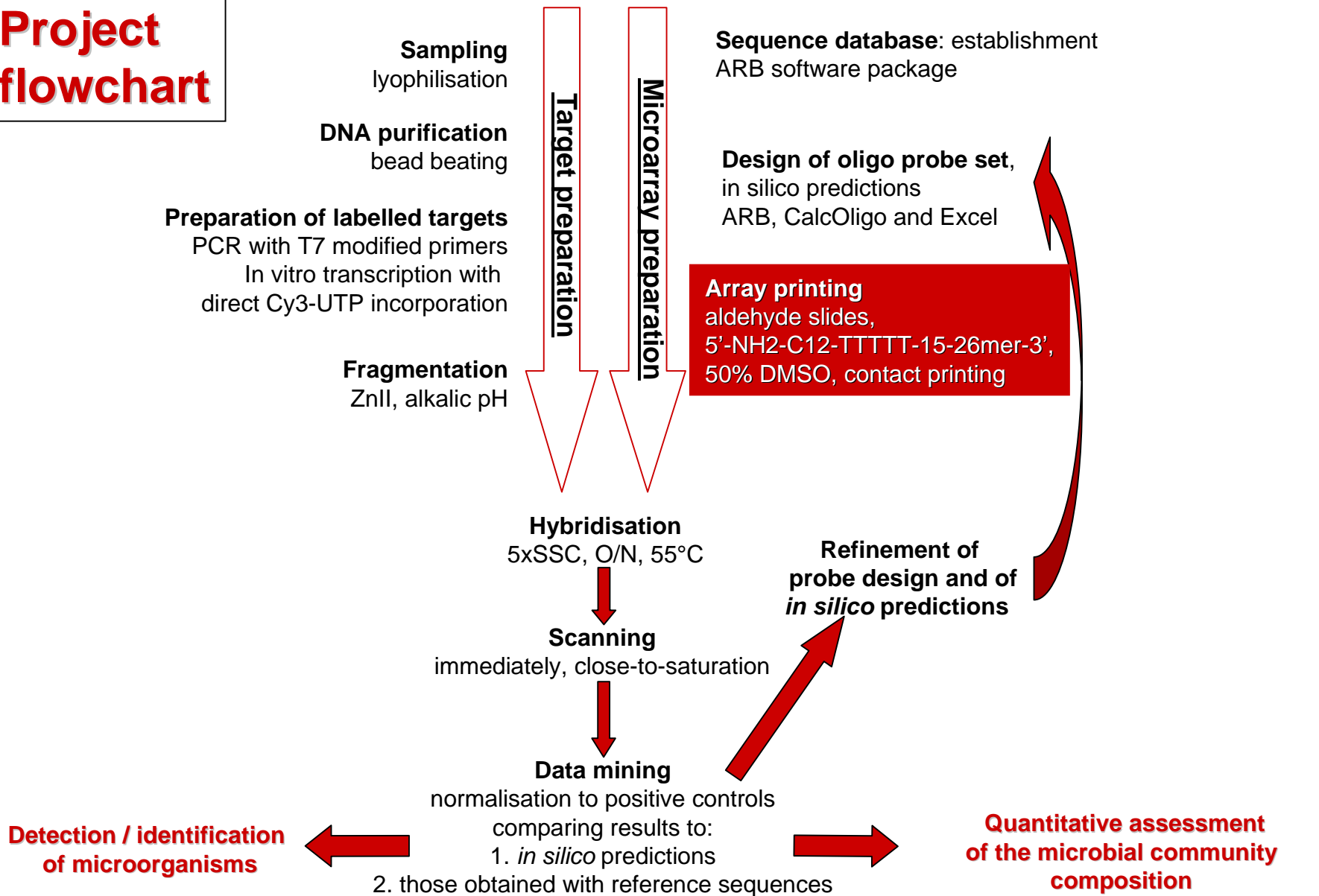


**Probe set with predicted hybridisation behaviour, Organised in a phylogenetic order. Middle part.**



**Probe set with predicted hybridisation behaviour, Organised in a phylogenetic order. Bottom.**

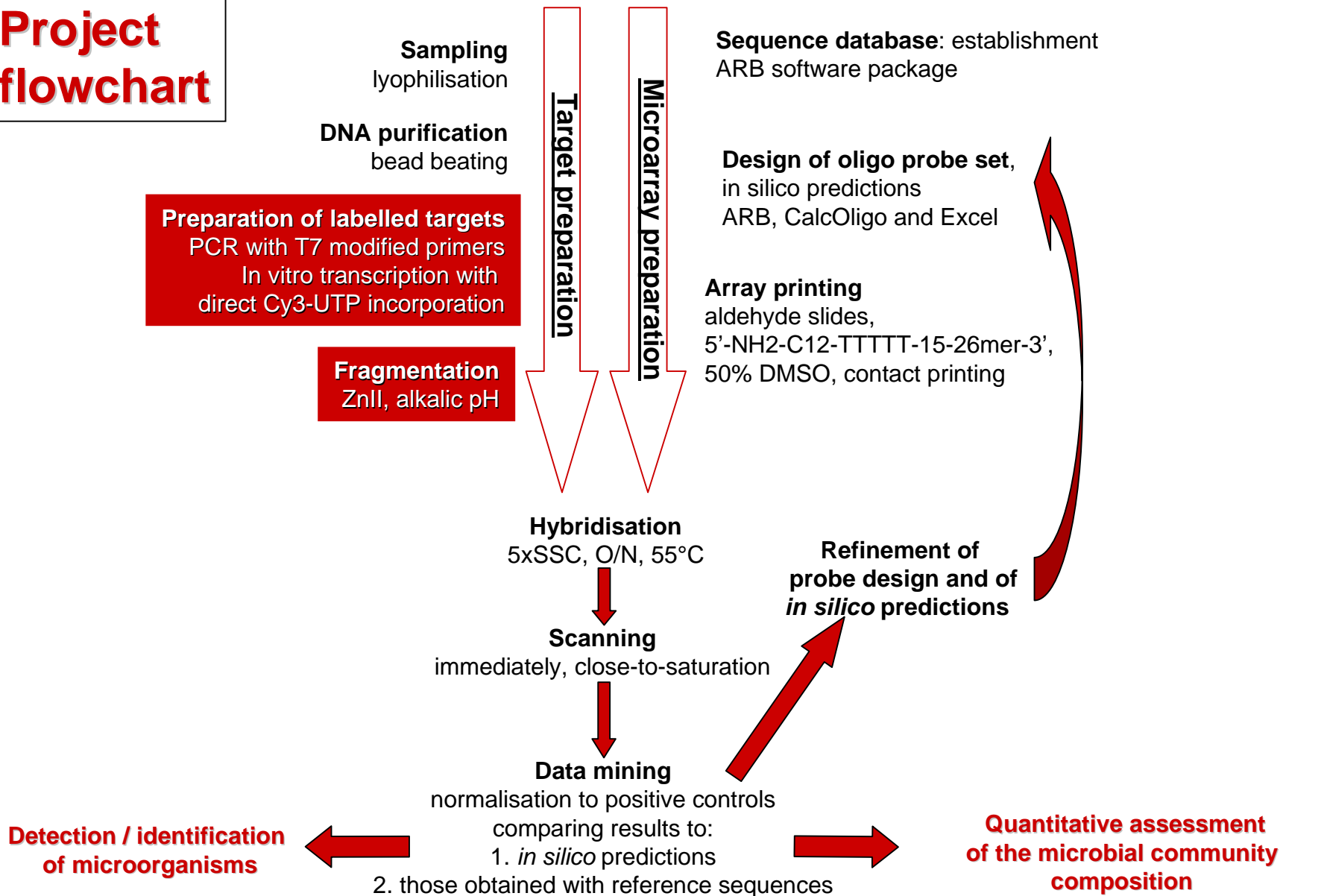
# Project flowchart



## Array printing

1. Prepare a 384 well plate with 30  $\mu$ l 50  $\mu$ M oligonucleotide solutions in 50% DMSO.
  2. Spot samples with an OmniGrid spotter (1 TeleChem .... pin) at 50% relative humidity, 22°C.
  3. Incubate spotted slides overnight at room temperature in a closed sandwich box containing saturated NaCl solution in the bottom (taking up about 10% of the volume of the box). This provides optimal humidity for the formation of Schiff bases between the aldehyde groups of the slide and the 5' amino group of the oligos.
  4. Rinse slides twice in 0.2% SDS for 2 min at room temperature with vigorous agitation to remove the unbound DNA.
  5. Rinse slides twice in dH<sub>2</sub>O for 2 min at room temperature with vigorous agitation.
  6. Transfer slides into dH<sub>2</sub>O at 95-100°C for 2 min to denature the DNA.
  7. Allow slides to cool at room temperature (~5 min).
  8. Treat slides in a freshly (right before use) prepared sodium borohydride solution for 5 min at room temperature to reduce free aldehydes. Sodium borohydride solution: Dissolve 0.5 g NaBH<sub>4</sub> in 150 ml phosphate buffered saline (PBS), then add 44 ml 100% ethanol to reduce bubbling.
  9. Rinse slides three times in 0.2% SDS for 1 min each at room temperature.
  10. Rinse slides once in dH<sub>2</sub>O for 1 min at room temperature.
  11. Dry slides one by one using an airgun with a cottonwool filter inside (to keep oil microdroplets away from the slide surface). Apply a modest stream to the area containing the array first to blow the drops down on the slide, rather than drying them onto it. Dried slides can be stored at room temperature in the dark for several months.
- 50% DMSO is used as printing buffer for the following advantages:
    - It doesn't dry in during long spotting rounds (a routine spotting of 100 oligos onto 100 slides takes about 6 hours) as opposed to aqueous solutions, like 3xSSC or phosphate buffers.
    - It provides uniform spots on the slides applied. Standard deviation between replicate spots is 10-15% as opposed to 20-30% for 3xSSC.
  - 384 well plates are used because of the smaller evaporation rate and smaller volume required.
  - 1 pin is applied to avoid variations inherent in the use of multiple spotting pins.
  - 50% humidity and 22°C provides optimal conditions in our hands to yield uniform, homogenous spots from 50% DMSO.
  - Reduction of the free aldehydes makes prehybridisation with BSA or other aminated compounds (which then serve to block free amino groups) unnecessary.

# Project flowchart



## PCR amplification of DNA sequence for in vitro transcription

1. Design PCR primers to amplify the gene of interest. The primer of the strand to be labelled has to contain the T7 promoter site as well: 5'-TAATACGACTCACTATAG – ACTUAL PRIMER-3'. In our case this is always the reverse primer.
2. Per target start 3 PCR reactions of 50µl volume each. Each 50µl reaction contains: 5µl 10x PCR buffer, 4µl dNTP mixture (2.5mM for each dNTP), 1.5µl 50 mM MgCl<sub>2</sub>, 1-1µl of both primers (15pmol/µl ≈ 100ng/µl), 1U Taq polymerase (Gibco Life Sciences / Invitrogen), template DNA (1 ng for gDNA or 0.1 ng for plasmid DNA), ultrapure water to 50µl.
3. Design PCR primers to amplify the gene of interest. The primer of the strand to be labelled has to contain the T7 promoter site as well: 5'-TAATACGACTCACTATAG – ACTUAL PRIMER-3'. In our case this is always the reverse primer.
4. Per target start 3 PCR reactions of 50µl volume each. Each 50µl reaction contains: 5µl 10x PCR buffer, 4µl dNTP mixture (2.5mM for each dNTP), 1.5µl 50 mM MgCl<sub>2</sub>, 1-1µl of both primers (15pmol/µl ≈ 100ng/µl), 1U Taq polymerase (Gibco Life Sciences / Invitrogen), leave space for template DNA (10 ng for environmental DNA, 1 ng for gDNA or 0.1 ng for plasmid DNA), ultrapure water to 50µl.
5. 95°C, 5 mins. Pause @95°C. Add template DNA ("hot start" to minimise mispriming). 32 cycles of 1 min @ 95°C; 1 min at the annealing temperature; 1 min @72°C for every 1000 bp to be amplified. A final 10 mins @72°C to allow the completion of all amplifications.
6. Pool parallel PCR products (3x50µl) and purify with a commercial PCR purification kit according to manufacturer's instructions (we use the HighPure PCR purification kit from Macherey-Nagel). Dissolve or elute purified DNA in ultrapure water keeping in mind that the final concentration has to be adjusted to 50 ng/µl.
7. Measure the concentration of purified DNA by spectrophotometry (Concentration of dsDNA = (A<sub>260</sub> x Dilution rate)/0.02 [ng/µl]). Adjust concentration to 50ng/µl with ultrapure water. Store @-20°C.

## *In vitro transcription*

Work under RNase free conditions.

Into an RNase-free Eppendorf tube add:

- 8 µl 50 ng/µl purified PCR product;
- 4 µl 5x T7 RNA polymerase buffer;
- 2 µl 100mM DTT;
- 0.5 µl 40 U/µl RNasin (Promega);
- 1 µl each of 10mM ATP, CTP, GTP;
- 0.5 µl 10mM UTP;
- 1 µl 40U/µl T7 RNA polymerase (Gibco BRL);
- 1 µl 5mM Cy3-UTP

Incubate @37°C, for 4 hours.

Purify labelled RNA immediately.

## *RNA purification*

We use the RNeasy kit from Quiagen.  
To remove unincorporated nucleotides, DNA template, T7 polymerase and salts.

Work under RNase free conditions.

1. Add 80µl DEPC treated water to the IVT mix.
2. Add 350µl RLT; mix thoroughly.
3. Add 250µl EtOH; mix thoroughly.
4. Sample (700µl) into an RNeasy mini column.  
15 secs @>10.000 rpm.
5. Transfer column into a new 2ml collection tube.  
Add 500µl RPE, 15 secs @>10.000 rpm.
6. Add 500µl RPE, 2 minutes @>10.000 rpm.
7. Transfer column into a 1.5 ml collection tube.  
Add 50µl RNase free water. 1 minute @>10.000 rpm.  
Transfer the eluate into a new 1.5 ml Eppendorf tube.

Proceed to fragmentation.

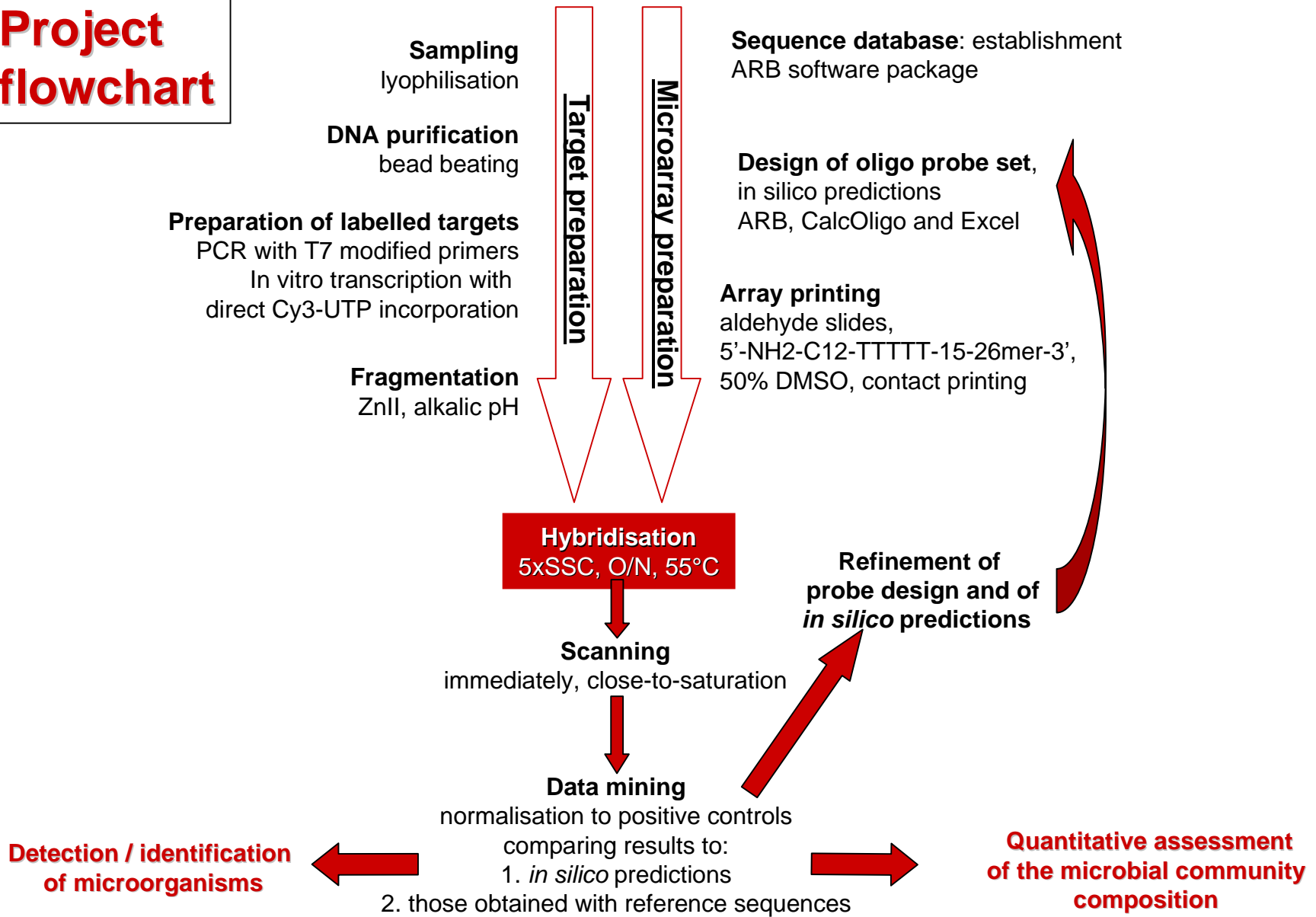
## Zn<sup>2+</sup> fragmentation of RNA

Work under RNase free conditions.

1. To 50  $\mu$ l, purified RNA add:  
1.43  $\mu$ l, 1M Tris.Cl pH 7.4  
5.71  $\mu$ l, 100mM ZnSO<sub>4</sub>
2. Mix; incubate at 60°C, 30 minutes.  
Note: Use dry block and do not mix during the incubation because the condensation on the lid of the tube is also included in the optimisation of the protocol (it causes gradual concentrating of the reaction thus influencing efficiency).
3. Add 1.43  $\mu$ l 500mM EDTA to stop the reaction (by chelating Zn<sup>2+</sup>).
4. Put onto ice for 1 min, add 1  $\mu$ l 40 U/ $\mu$ l RNasin.

Fragmented, labelled RNA target can now be stored at -20°C for several months.

# Project flowchart

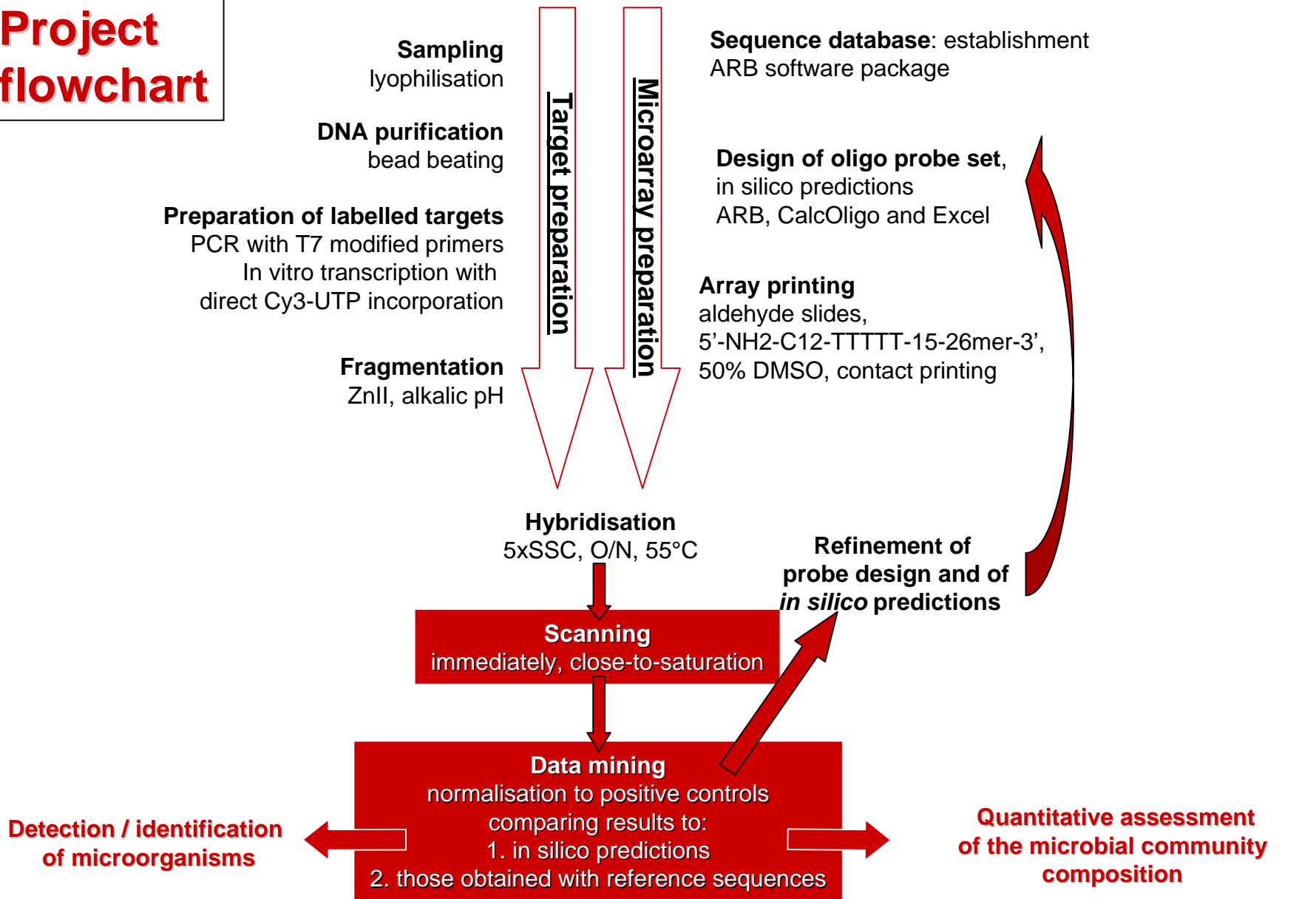


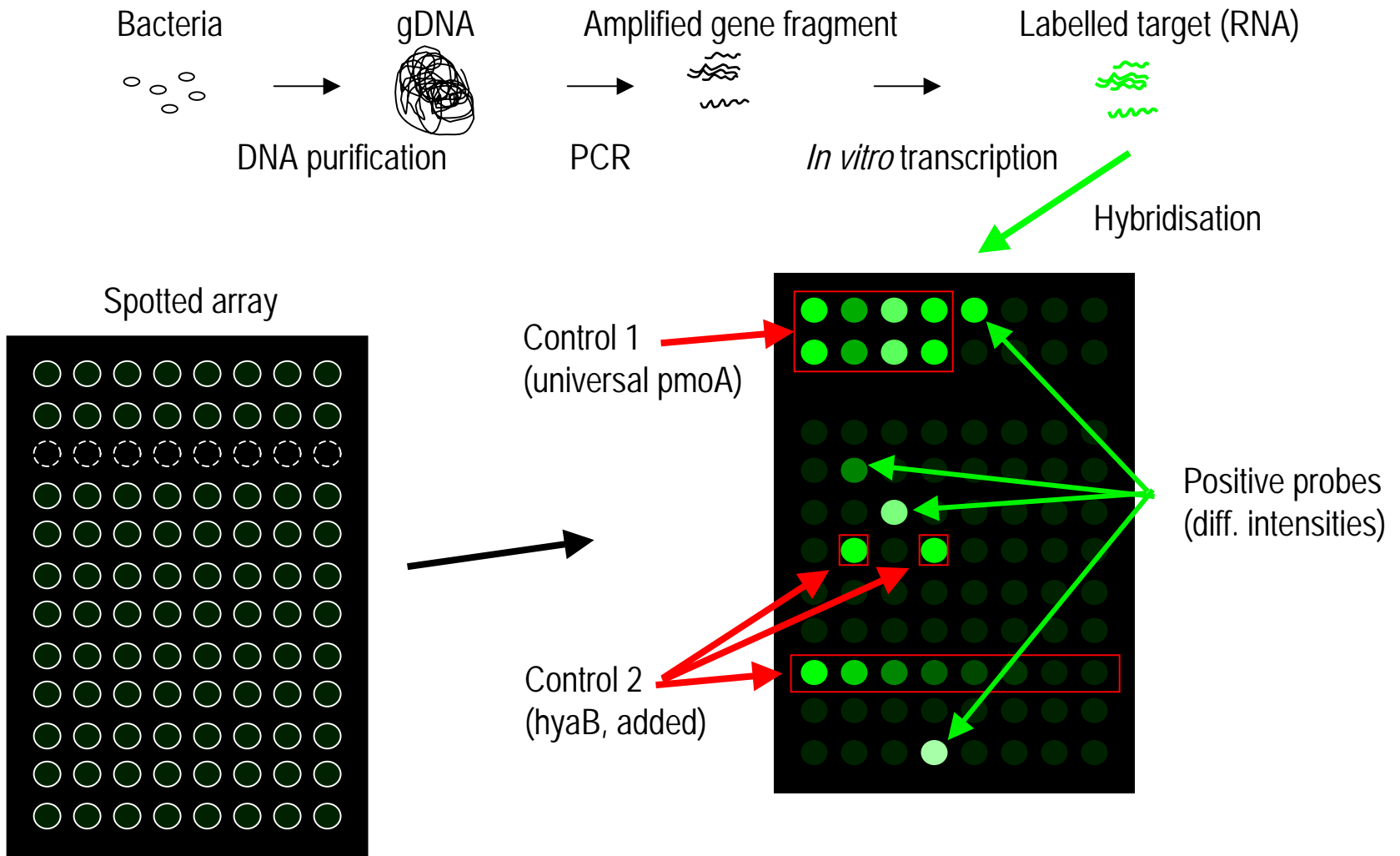
## Hybridisation

To ensure a good control over hybridisation temperature a custom tailored aluminium block is used with holes of 11mm in depth for 14 slides. The block is used as an insert for a Belly Dancer. 1-2 mm spacing between the block and the wall of the water bath chamber allows for the addition of about 20 ml water which is required for reliable heat transfer to the aluminium block.

1. Preheat hybridisation block to 55°C. Allow for at least 30 minutes for the temperature to stabilise.
2. Preheat an Eppendorf incubator (dry block) to 66°C.
3. Apply 200 µl HybriWell (.....) chambers onto the slides containing the arrays. Preheat assembled slides on top of the hybridisation block. The BellyDancer should be set to the maximum bending.
4. Per hybridisation:  
Add to a 1.5 ml Eppendorf tube:
  - 124 µl DEPC-water
  - 2 µl 10% SDS
  - 4 µl 50x Denhardt's reagent
  - 60 µl 20x SSC
  - 10 µl target RNAIncubate at 65°C for 1 - 15 mins.
5. Apply preheated hybridisation mixtures onto assembled slides via the lower (closer to ground) port. Seal chambers with seal spots.
6. Incubate overnight at 30-40rpm circulation at maximum bending.
7. Take slides one by one, remove sticky chamber and put them immediately into 2xSSC, 0.1% SDS at RT.
8. Wash slides by shaking at RT for: 5 mins in 2xSSC, 0.1% SDS; 2x5 mins in 0.2x SSC; finally for 5 mins in 0.1x SSC
9. Dry slides one by one using an airgun with a cottonwool filter inside (to keep oil microdroplets away from the slide surface). Apply a modest stream to the area containing the array first to blow the drops down on the slide, rather than drying them onto it. Dried slides can be stored at room temperature in the dark for several months.
10. Scan slides the same day.

# Project flowchart

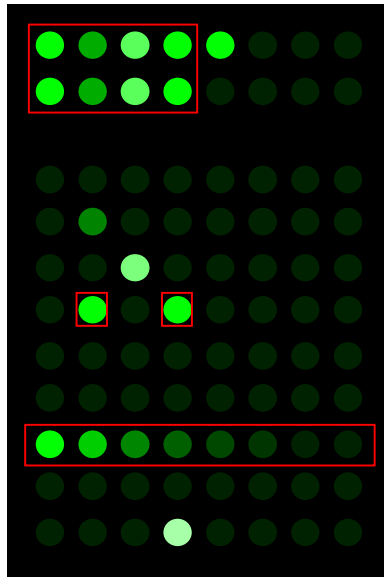
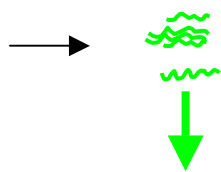
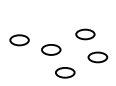




## Hybridisation with a single strain #1

Bacteria

Labelled target (RNA)



mtrof173	13526
mtrof362	8652
mtrof661	22513
mtrof662	14018
<b>hyaBp</b>	<b>10257</b>
Jpn284	4895
LP20-644	19820
la193	24315
la577	11968

# Hybridisation with a single strain #2 Normalisation of results

Reference set #1 - universal pmoA probes

Average := 100%

Reference option #2 - hyaB probe; externally added DNA

Average := 100%

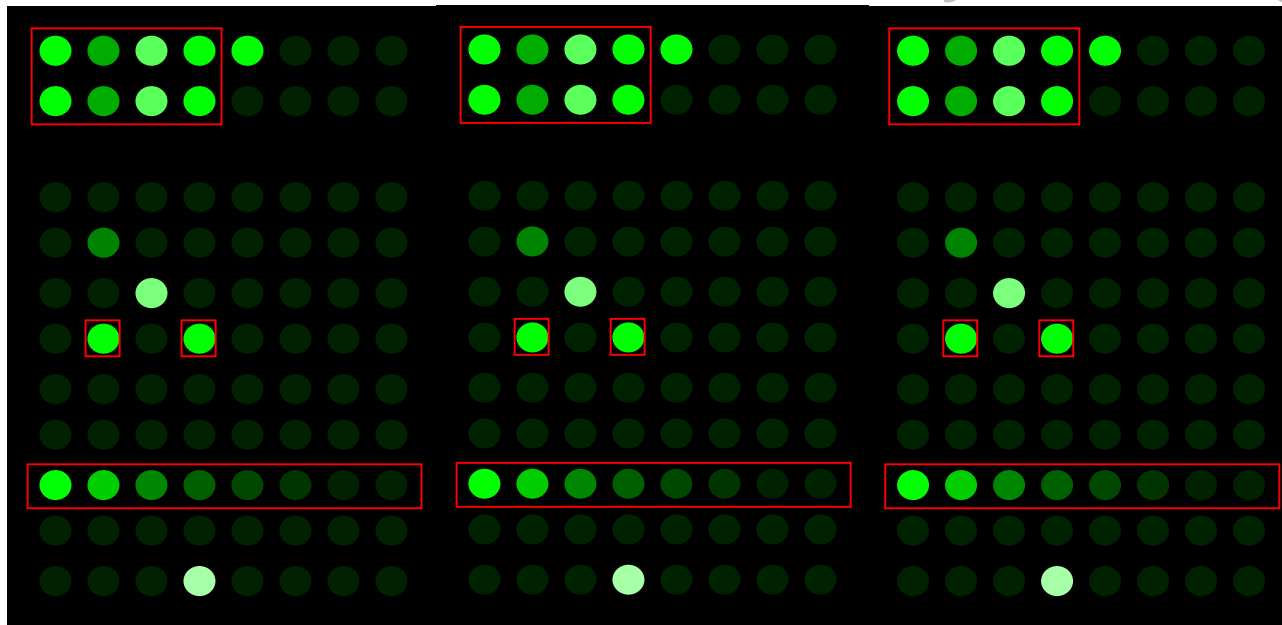
Jpn284	41%
LP20-644	112%
la193	145%
la577	78%

Bacteria

Labelled target (RNA)



# Hybridisation with a single strain #3 Parallel arrays on a single chip

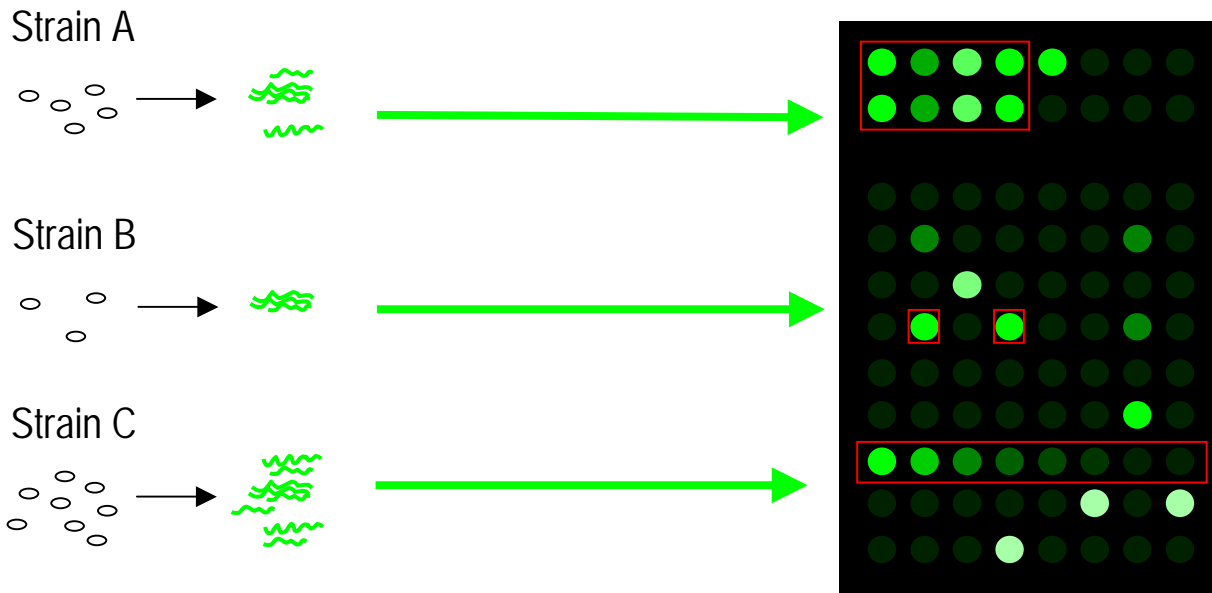


Jpn284	41%
LP20-644	112%
la193	145%
la577	78%

Jpn284	39%
LP20-644	125%
la193	135%
la577	87%

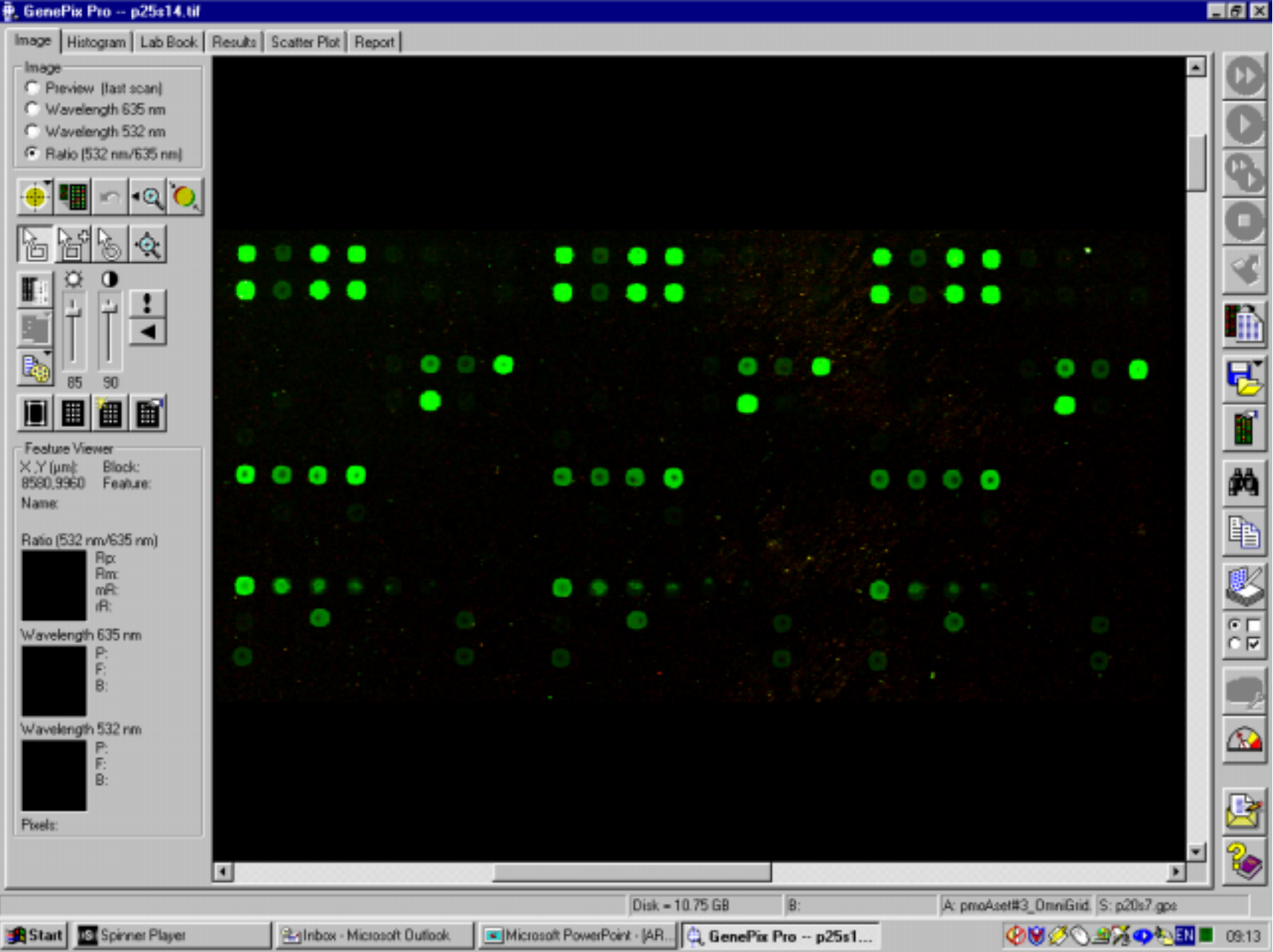
Jpn284	48%
LP20-644	118%
la193	139%
la577	72%

<b>Jpn284</b>	<b>43%</b>
<b>LP20-644</b>	<b>118%</b>
<b>la193</b>	<b>139%</b>
<b>la577</b>	<b>79%</b>



Probe	Max%	Read	Rel.abundance	
Jpn284	41%	13%	0.32	} ~30% <i>Methylobacter</i> sp. BB5.1
LP20-644	112%	41%	0.35	
la193	145%	52%	0.35	
la577	78%	28%	0.38	
B2-400	72%	18%	0.25	} ~20% <i>Methylocapsa acidophila</i> - related
B2all	61%	12%	0.18	
Msi270	172%	87%	0.52	} ~50% <i>Methylosinus sporium</i> - related
II510	228%%	114%	0.58	
II630	310%%	158%	0.49	

**Hybridisation with an environmental mixture  
=> single colour quantification potential**



# Scanning

26/09/2002

[www.diagnostic-arrays.com](http://www.diagnostic-arrays.com)  
[www.arcs.ac.at/ul/ulb/bt](http://www.arcs.ac.at/ul/ulb/bt)



# Deviations in the hyb. behaviour of the probes

Hybridisation between an oligonucleotide and any target is a reversible process!

Binding efficiency in turn is influenced by:

- 1., T<sub>m</sub> of the oligos; this is however also a very rough guide, see 2. and 3.
- 2., Steric effects - distance from the surface / bulk of the coating polymer; crowding → nts close to the surface play a lesser role
- 3., End nucleotides in general influence binding less → also nts at the free end play a less important role
- 4., 2<sup>nd</sup>ary structure of the probe
- 5., 2<sup>nd</sup>ary structure of the target → fragmented ssRNA is the preferential choice
- 6., Overhanging nts of the target molecules
- 7., Further, not fully understood effects

Application of group specific probes and target mixes → further complications:

Multiple targets with different hyb. efficiencies (see 5.-7.) can bind to the same probe →

- 8., Targets with 1 or 2 MM can also hybridise, at a lower efficiency than PM targets

# Deviations in the hyb. behaviour - results of the probe set

Unexpected results (expected: PM and 1MM yields positive signal, 2MM and above: no signal):

84% of the probes (42 of 50 analysed) displayed hybridisation behaviour as predicted.

Hybridisation behaviour of 44% could be predicted based solely on their  $T_m$ .

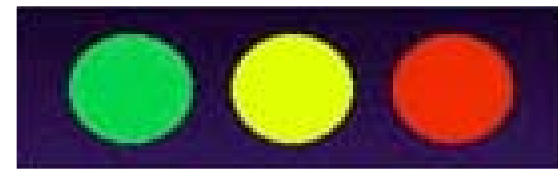
For 40% further points had to be considered (high GC but too short; strong hairpin structure; MM at end positions; most of the GC content in the end positions; “neutral” mismatches).

**CalcOligo** software under development: generates Excel table  
instead of MM numbers, it predicts  $T_m$   
will enable accounting for microarray-specific effects  
will enable refining of the prediction parameters to fit  
predictions to results

## Two colour hybridisations:

A mixture of two DNA targets is hybridised to the chip

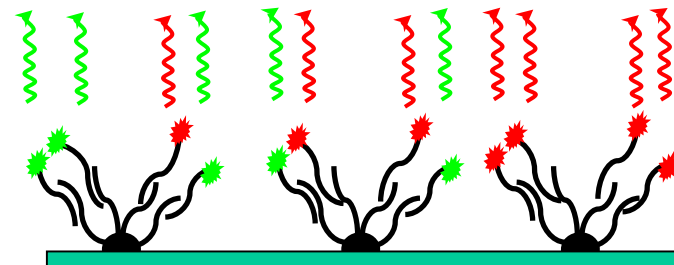
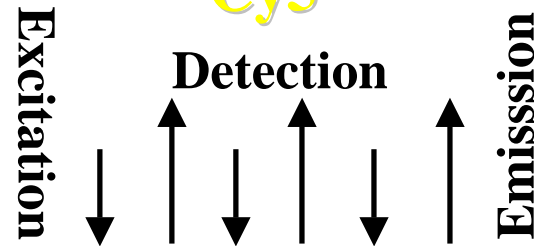
One is used as an internal control to enable more exact quantification



Cy3 Cy3 Cy5

+

Cy5

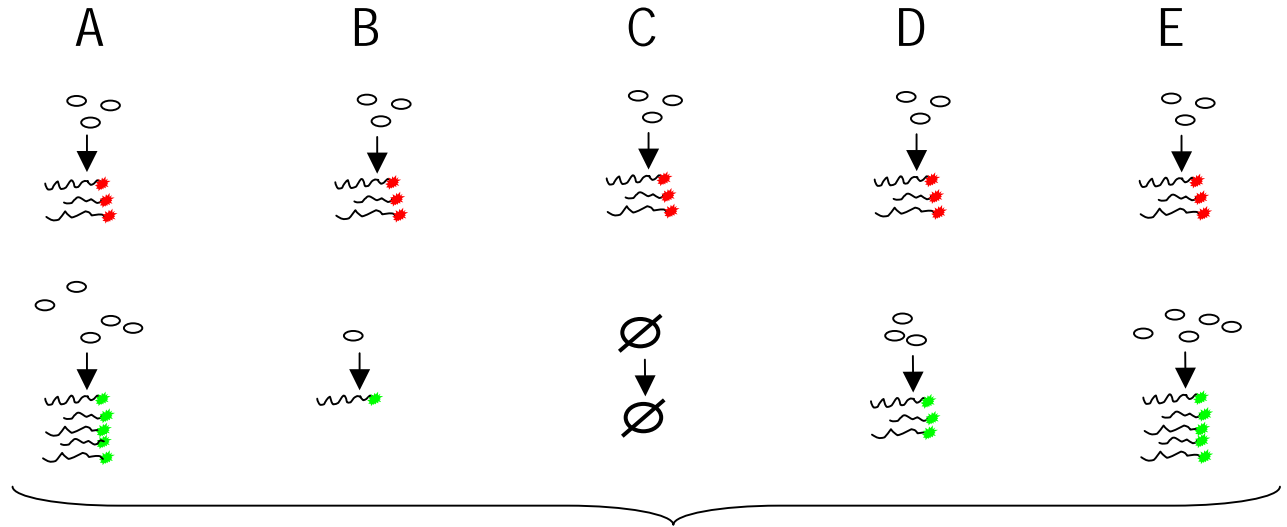


# Two-colour quantification:

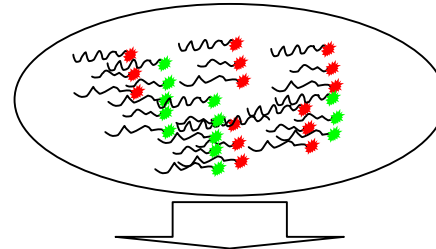
Strains:

Internal control {  
Bacteria  
Labelled target

Actual sample {

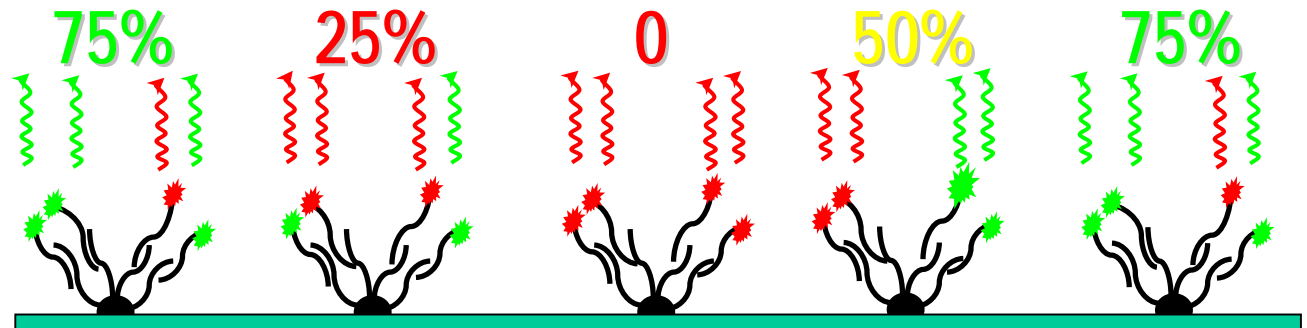


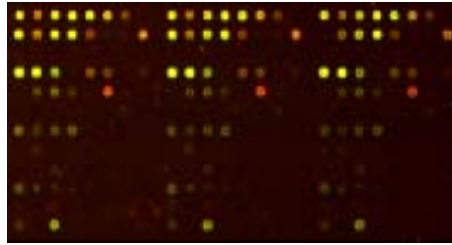
Mixture of labelled target



Hybridisation onto the chip

Chip after hybridisation

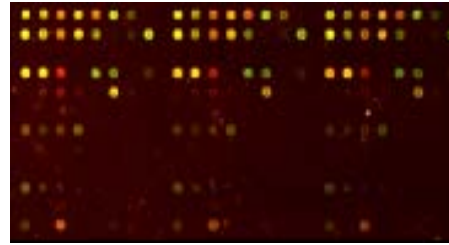




Artificial Mixture #1

Ratios (%) Measured ratios (%)

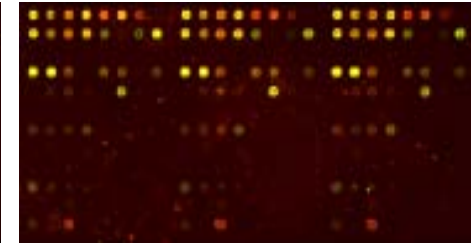
Clone SL-5.102	60	68
<i>Mcl. szegediense</i> OR2	30	21
Clone SL-5.70	10	6.1
Clone rbp46	3	4.0
Clone JY-6.48	1	1.8



Artificial Mixture #2

Ratios (%) Measured ratios (%)

	1	2.1
	3	4.7
	60	36
	30	43
	10	12



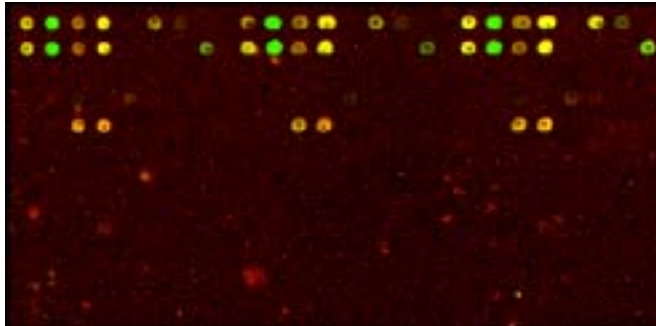
Artificial Mixture #3

Ratios (%) Measured ratios (%)

	10	20
	1	2.3
	3	3.6
	60	51
	30	22

Standard deviations are in the range of 0.4-17.2 %

## Two-colour quantification: Testing its potential with artificial PCR mixtures



Community structure:	
<i>Methylobacter</i>	5%
<i>Methylomonas</i>	40%
<i>Methylococcus</i> or <i>Methylocaldum</i>	5%
<i>Methylocystis</i>	50%

Analysis of the methanotroph community of Movile cave,  
a closed methane- and sulphur-dependent ecosystem.  
Results are in good agreement with those of independent community analyses.

## Two-colour quantification: Testing against an environmental sample

## Summary:

- A comprehensive set of methods and protocols for microbial diagnostic microarrays - easily adaptable to a wide range of upcoming novel technology platforms (bead arrays, capillary bead arrays, cantilevers, electronic detection, etc.).
- High resolution, semi-quantitative picture of the bacterial community from any samples / environments.
- *In silico* oligo design: only 16% of the oligos display false positive / negative hybridisations. This will be further improved by the upgrade of CalcOligo.
- Demonstration array: Set of 59 oligo probes covering the whole known diversity of (cultivated and uncultivated) methanotrophs
- Please visit our website: [www.diagnostic-arrays.com](http://www.diagnostic-arrays.com) ==> [www.arcs.ac.at](http://www.arcs.ac.at)

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**[www.diagnostic-arrays.com](http://www.diagnostic-arrays.com) ==> [www.arcs.ac.at](http://www.arcs.ac.at)**