

FISH, Probe Protocol
September 2006, Updated August 20th, 2013

Filtering Samples

- 1) Seawater samples were killed with 2% paraformaldehyde or 10% formalin.
- 2) The seawater was filtered through 0.2 μ M white polycarbonate membrane filter 25mm (Osmonics). Typically 10mls of BATS water or 1:100 dilution of culture with filtered seawater.
- 3) The filter is fixed to a slide with a tough spot at each end.
- 4) These can be stored at -20°C with dessicant until ready to proceed. (~2 months- 1 year).

Probing Filters

- 1) Cut the filter into quarters. Fix a quarter onto a new slide using two tough spots and label the slide with the probe, sample, tower and mls filtered.
- 2) Wash the filters in 95% Ethanol
- 3) Probe the sample with 40 μ l of 2ng/ μ l probe in hybridization solution. To dilute the probe (10ng/ μ l), use a 1 in 10 dilution, adding the hybridization solution first.
- 4) The hybridization solution to use depends on the probe.
- 5) Place a cover slip (22mm) on top of the two tough spots. This makes it easier to remove later and ensures most of the probe remains on top of the filter.
- 6) Place in a hybridisation oven set at 37°C for 16 hours.

Washing the Filters

- 1) Set up a water bath at required temperature check with a thermometer.
- 2) Temperature depends on the probe used.
- 3) Place two coplin jars with hybridization wash solution in the water bath.
- 4) Remove the cover slip by hand and place in the wash for 10 min.
- 5) Place in the second wash for 10 mins.

Mounting the Filters

- 1) Cut off the filter quarter from the tough spots. Dry the filters on Whatman filter paper (paper towel or kimwipe).
- 2) Remove the tough spots and clean the slide with kim wipe.
- 3) Place the filter correct side up onto the slide and cover with a coverslip (22mm) containing a drop of 20 μ l DAPI/citiflour (1:2) mixture. Press down to remove excess mountant and dry using a kimwipe.
- 4) Seal with nail polish and place at 4°C to reduce floaters.

Probe	Hyb Sln SET %Formamide	Hyb Temp °C	Hyb Wash SET NaCl Conc	Wash Temp °C
Negative 338F	Any	37	Any	Any
Roseobacter 536R-Cy3	SET 001 35	37	SET 001 0.07M	52
Alteromonas AC137R-Cy3	SET 001 35	37	SET 001 0.07M	52
SAR11 152R-Cy3 441R-Cy3 542R-Cy3 732R-Cy3	SET 002 15	37	SET 002 0.15M	55
Eubac 27R-Cy3 338Rpl-Cy3 700R-Cy3 700Ral-Cy3 1522R-Cy3	SET 002 15	37	SET 002 0.15M	50
Flavobacteria I CF319aR-Cy3 CF319bR-Cy3	SET 001 35	37/46	SET 001/005 0.07M/0.08M	48
Flavobacteria II CF563-Cy5	SET 003 20	46	SET 006 0.225M	48
Vibrio 127R-Cy3	SET 001 35	37	SET 001 0.07M	45
SAR 202 103R-Cy3 311R-Cy3	SET 001 35	37	SET 001 0.07M	57.5
SAR 86 60R -Cy3 735R-Cy3 1092R-Cy3 1247R-Cy3	SET 002 15	37	SET 002 0.15M	55

Probe	Hyb Sln SET %Formamide	Hyb Temp °C	Hyb Wash SET NaCl Conc	Wash Temp °C
Archaea Arch344-Cy3 Arch915-Cy3	SET 003 20	46	SET 002 0.15M	48
Archaea Eury806-Cy3	SET 003 20	35	SET 002 0.15M	37
Archaea Cren537-Cy3	SET 003 20	35	SET 002 0.15M	45
Chlorobium Chlorob441-Cy3	SET 002 15	37	SET 002 0.15M	52
E.coli Colinsitu-Cy3	SET 004 22	46	SET 004 0.1M	48
Endozoicomonas Endozoi663-Cy3 Endozoi736-Cy3	SET005 40	46	SET 005 0.08M	50
Chloroflexi GNSB-941 CFX1223	SET 001 35	46	Try SET001	48

Hybridisation Solutions and Washes

0.2 μ M filter sterilized

SET 01

Hyb Solution 0.9 M NaCl
20mM Tris/HCl pH 7.4
35% formamide
0.01% SDS

For 20 mls
9mls 2m NaCl
0.4mls 1ml Tris/HCl pH 7.4
7mls formamide
20 μ l 10% SDS
3.58 mls QW

Hyb Wash 70mM NaCl
20mM Tris/HCl pH 7.4
5mM EDTA
0.01% SDS

For 1 L
35mls 2M NaCl
20mls 1M Tris/HCl pH 7.4
10mls 0.5M EDTA
1ml 10% SDS
934mls QW

SET 02

Hyb Solution 0.9 M NaCl
20mM Tris/HCl pH 7.4
15% formamide
0.01% SDS

For 20 mls
9mls 2m NaCl
0.4mls 1ml Tris/HCl pH 7.4
3mls formamide
20 μ l 10% SDS
7.58 mls QW

SET 02

Hyb Wash 150mM NaCl
20mM Tris/HCl pH 7.4
5mM EDTA
0.01% SDS

For 1 L
75mls 2M NaCl
20mls 1M Tris/HCl pH 7.4
10mls 0.5M EDTA
1ml 10% SDS
894mls QW

SET 03

Hyb Solution 0.9 M NaCl
20mM Tris/HCl pH 7.4
20% formamide
0.01% SDS

For 20 mls
9mls 2m NaCl
0.4mls 1ml Tris/HCl pH 7.4
4mls formamide
20 μ l 10% SDS
6.58 mls QW

SET 04

Hyb Solution 0.9 M NaCl
20mM Tris/HCl pH 7.4
22% formamide
0.1% SDS
0.2% BSA

For 20 mls
9mls 2M NaCl
0.4mls 1ml Tris/HCl pH 7.4
4.4mls formamide
200 μ l 10% w/v SDS
400 μ l 10% w/v BSA
3.58 mls QW

Hyb Wash
100mM NaCl
20mM Tris/HCl pH 7.4
5mM EDTA
0.01% SDS

For 1 L
50mls 2M NaCl
20mls 1M Tris/HCl pH 7.4
10mls 0.5M EDTA
1ml 10% SDS
919mls QW

SET 05

Hyb Solution
0.9 M NaCl
20mM Tris/HCl pH 7.4
40% formamide
0.01% SDS

For 20 mls
9mls 2m NaCl
0.4mls 1ml Tris/HCl pH 7.4
8mls formamide
20 μ l 10% SDS
2.58 mls QW

Hyb Wash
80mM NaCl
20mM Tris/HCl pH 7.4
5mM EDTA
0.01% SDS

For 1 L
40mls 2M NaCl
20mls 1M Tris/HCl pH 7.4
10mls 0.5M EDTA
1ml 10% SDS
929mls QW

SET 06

Hyb Wash
225mM NaCl
20mM Tris/HCl pH 7.4
5mM EDTA
0.01% SDS

For 1 L
112.5mls 2M NaCl
(OR 45mls 5M NaCl)
20mls 1M Tris/HCl pH 7.4
10mls 0.5M EDTA
1ml 10% SDS

Sequences

Probe	DNA Sequence 5' to 3'	Probe Base (RDP) or SILVA
Negative 338F-Cy3	TGAGGATGCCCTCCGTCG	0% RDP 0% SILVA
SAR11 152R-Cy3 441R-Cy3 542R-Cy3 732R-Cy3	ATTAGCACAAGTTTCCYCGTGT TACAGTCATTTTCTTCCCCGAC TCCGA ACTACGCTAGGTC GTCAGTAATGATCCAGAAAGYTG	97.9% SAR11 on SILVA 99.3% SAR11 on SILVA 99.6% SAR11 on SILVA 99.8% SAR11 on SILVA
Eubac 27R-Cy3 338Rpl-Cy3 700R-Cy3 700Ral-Cy3 1522R-Cy3	CTGAGCCAKGATCRAACTCT GCWGCCWCCCGTAGGWGT CTAHGCATTTACACYGCTACAC CTACGAATTTACCTCTACAC AAGGAGGTGATCCANCCVCA	90% Bacteria
Roseo 536R-Cy3	CAACGCTAACCCCTCCG	99.3% <i>Rhodobacteraceae</i> (RDP) 99.1% <i>Rhodobacteracea</i> (SILVA)
Alteromonas AC-137R-Cy3	TGTTATCCCCCTCGCAA	93.5% <i>Alteromonas</i> on SILVA 97.9% <i>Alteromonas</i> on RDP
SAR 202 103R-Cy3 311R-Cy3	GTTACTCAGCCGTCTGCC TGCTCAGTCCCCCTCTG	
SAR 86 60R -Cy3 735R-Cy3 1092R-Cy3 1247R-Cy3	GATACTTTCTCGCACGAC TCAGTACAGATCCAGGAG TGCGCTCGTTATCCGACT GCTTAGCGTCCGTCTGTA	
Vibrio VibSpl-127-Cy3	CCCCACATCAAGGCAATTTTC	99.1% <i>Vibrio</i> on SILVA 97.5% <i>Vibrio</i> on RDP
CFB CF319aR-Cy3 CF319bR-Cy3	TGGTCCGTGTCTCAGTAC TGGTCCGTATCTCAGTAC	96.9% <i>Bacteroidetes</i> (98.4% RDP) 97.9% <i>Bacteroidetes</i> (99.6% RDP)
CFB CF563-Cy5	GGACCCTTTAAACCCAAT	97.7% <i>Flavobacteriales</i> on RDP 92.6% <i>Flavobacteriaceae</i> on RDP

Probe	DNA Sequence 5' to 3'	Probe Match
Archaea Arch344-Cy3 Arch915-Cy3	TCGCGCCTGCTGCICCCCGT GTGCTCCCCCGCCAATTCCT	
Archaea Eury806-Cy3	CACAGCGTTTACACCTAG	100% <i>Euryarchaea</i> on SILVA 99.9% <i>Euryarchaea</i> on RDP
Archaea Cren537-Cy3	TGACCACTTGAGGTGCTG	99.1% <i>Thaumarchaea</i> on SILVA
Chlorobium Chlorob441-Cy3	AAAACAGGATTCTTCCTCTAC	90.6% <i>Chlorobiaceae</i> on SILVA 96.9% <i>Chlorobiaceae</i> on RDP
Colinsitu – Cy3	GAGACTCAAGATTGCCAGTATCAG	
Endozoicomonas Endozoi663-Cy3 Endozoi736-Cy3	AGGAGUGUGGAAUUUCC CUCUGGUCUGACACUGAC	
Chloroflexi GNSB-941 CFX1223	AAACCACACGCTCCGCT CCATTGTAGCGTGTGTGTMG	Both probes 99.7% <i>Chloroflexi</i> 98.9% <i>Chloroflexi</i> on RDP 99.1% <i>Chloroflexi</i> on RDP