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(54) **METHOD FOR ANALYZING SAMPLES BY MEANS OF HYBRIDIZATION**

(76) Inventors: **Lei Zhang**, Bremen (DE); **Barbara Reinhold-Hurek**, Bremen (DE); **Thomas Hurek**, Bremen (DE)

Correspondence Address:
RANKIN, HILL & CLARK LLP
925 EUCLID AVENUE, SUITE 700
CLEVELAND, OH 44115-1405

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G01N 33/566 (2006.01)

(52) **U.S. Cl.** **435/6**; 436/501; 536/23.1

(57) **ABSTRACT**

The invention relates to a method of analysing samples by means of a ligand binding, in which duplexes or complexes are created and analysed.

The invention is distinguished by the fact that the target molecules have a detectable marking in proximity to a target sequence and/or a detectable marking is incorporated in the target sequence. By this means, significantly higher signal intensities are obtained than with conventional methods.

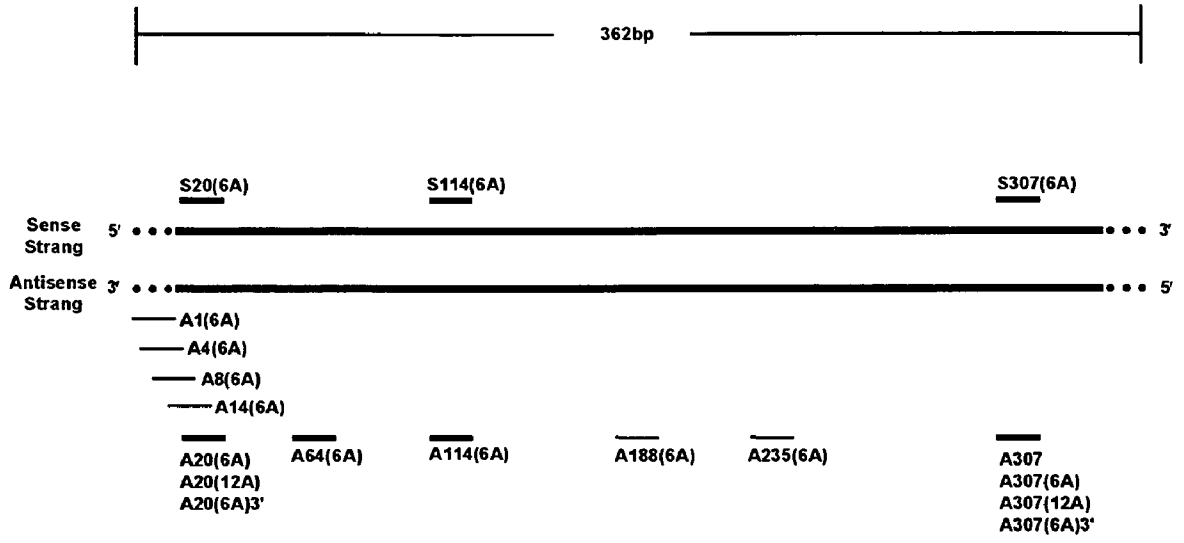


Fig. 1A

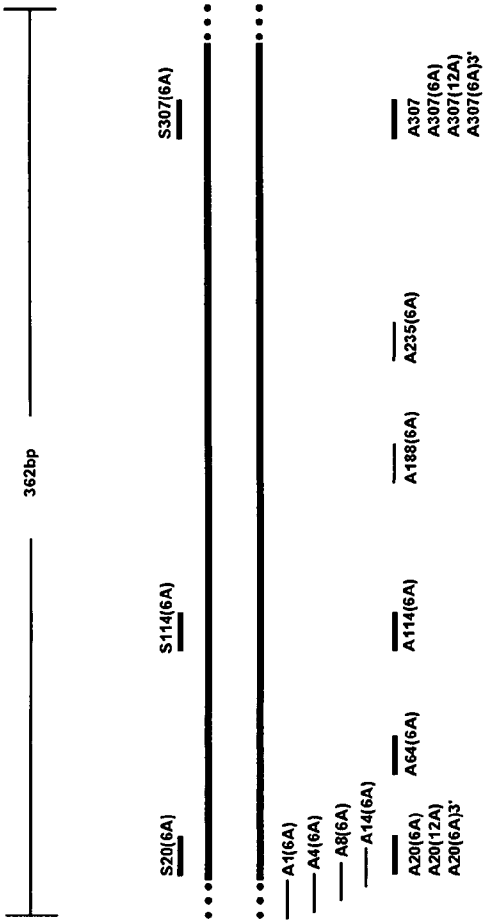


Fig. 1B

Modifikation	Fängerol.	Sequenz (5'-3')	Tm(°C)	Länge(nt)	Position
5'Aminolink (kein Spacer)	A289	GGGCCTTGTTTTCGCG	65	16	207-322
5'Aminolink (6A Spacer)	A1(6A)	AAAAAA TGCGACCCGAAGGCTGA	68	17	1-17
	A4(6A)	AAAAAA GAGTCAGCCTTCGGGTC	68	17	4-20
	A8(6A)	AAAAAA GGTGGAGTCAGCCTTCG	68	17	8-24
	A14(6A)	AAAAAA CAGACGGGTGGAGTCAG	68	17	14-30
	A20(6A)	AAAAAA GAGGATCAGACGGGTGG	68	17	20-36
	A64(6A)	AAAAAA CTTCAGCGGCCAGGTG	68	16	64-79
	A114(6A)	AAAAAA GAAGCCGACCGACAGG	68	16	114-129
	A188(6A)	AAAAAA GGCGGTGATAACGCCAC	67	18	235-252
	A235(6A)	AAAAAA GAAGTCGAGTTCGTCGTC	65	16	307-322
	A307(6A)	AAAAAA GGGCCTTGTTTTCGCG	65	16	207-322
	S20(6A)	AAAAAA CCACCCGTCTGATCCTC	68	17	327-343
	S114(6A)	AAAAAA CCTGTCGGTCGGCTTC	68	16	234-249
	S307(6A)	AAAAAA CGCGAAAACAAGGCCC	65	16	41-56
5'Aminolink (12A Spacer)	A20(12A)	AAAAAAAAAAAA GAGGATCAGACGGGTGG	68	17	20-36
	A307(12A)	AAAAAAAAAAAA GGGCCTTGTTTTCGCG	65	16	207-322
3'Aminolink (6A Spacer)	A20(6A)3'	GAGGATCAGACGGGTGG AAAAAA	68	17	20-36
	A307(6A)3'	GGGCCTTGTTTTCGCG AAAAAA	65	16	207-322

Fig. 1C: Primer for the amplification by PCR

Sequence (5'-3')	Tm(°C)	Length	Description	Reference	
TGYGAYCCNAARGCNGA		17	nifH PCR forward primer	Zehr and McReynolds, 1989	
ADNGCCATCATYTCNCC		17	nifH PCR reverse primer	Zehr and McReynolds, 1989	
TGCGACCCGAAGGCTGA	68	17	nifH PCR forward primer specific for *Azoarcus sp.* BH72	this test	
CCTGTCGGTCGGCTTC	68	16	nifH PCR forward primer specific for *Azoarcus sp.* BH72	this test	
GGGCCTTGTTTTCGCG	65	16	nifH PCR reverse primer specific for *Azoarcus sp.* BH72	this test	

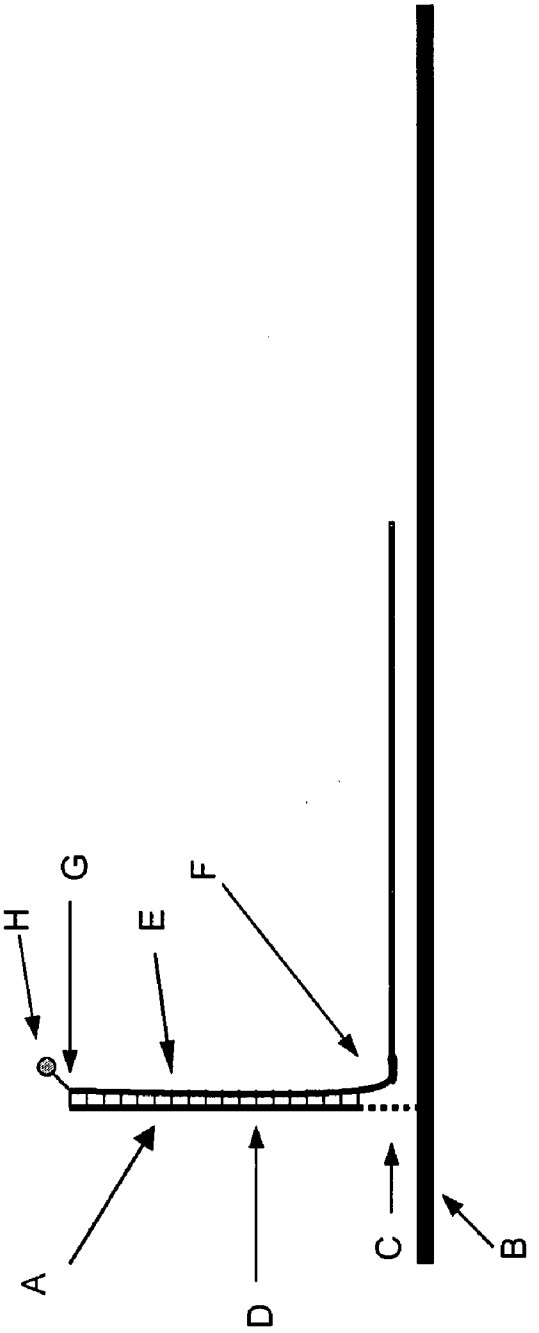


Fig. 1D

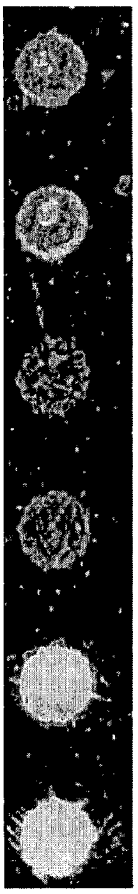


Fig. 2A

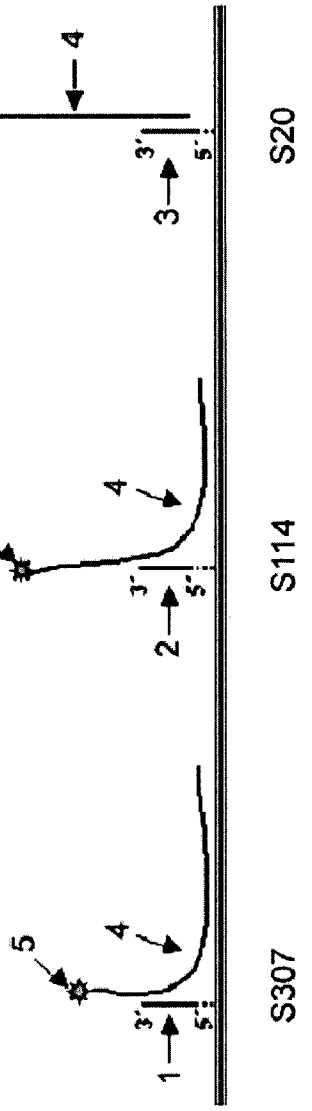


Fig. 2B

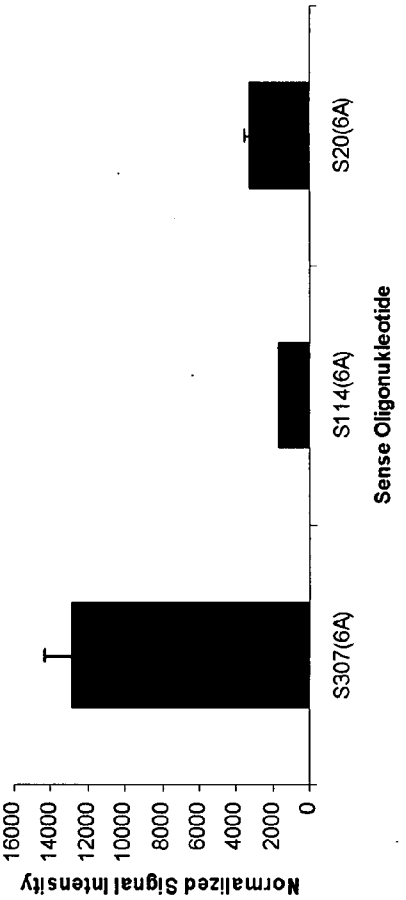


Fig. 2C

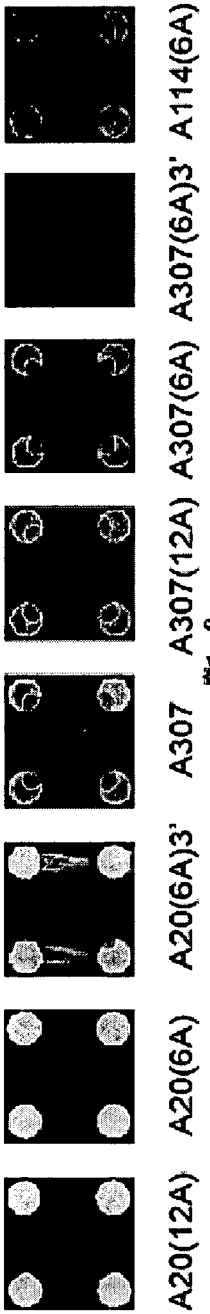


Fig. 3A

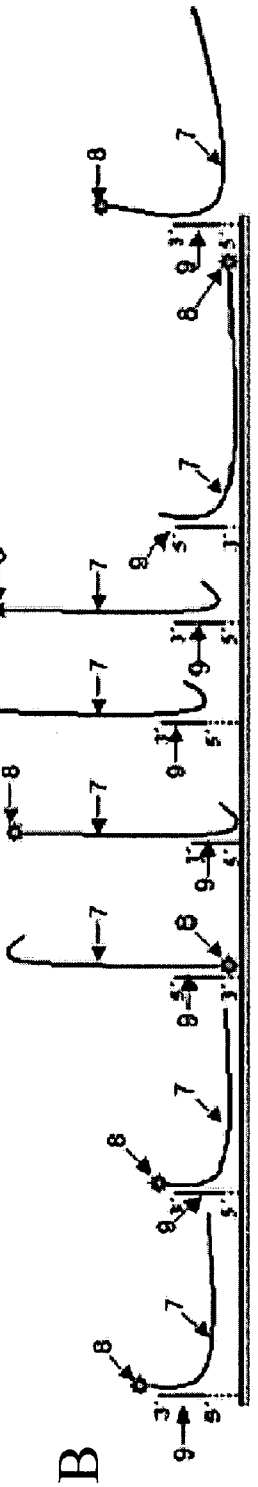


Fig. 3B

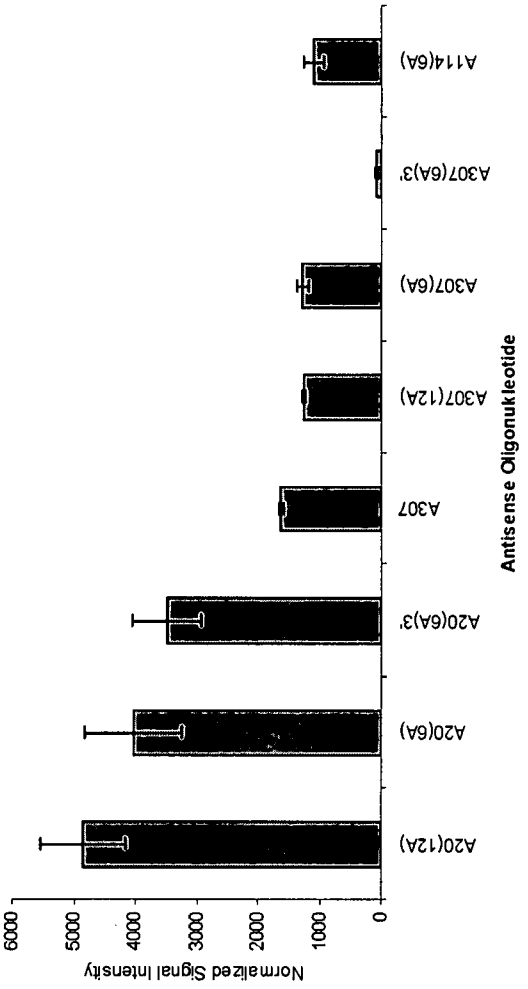


Fig. 3C

Fig. 4A

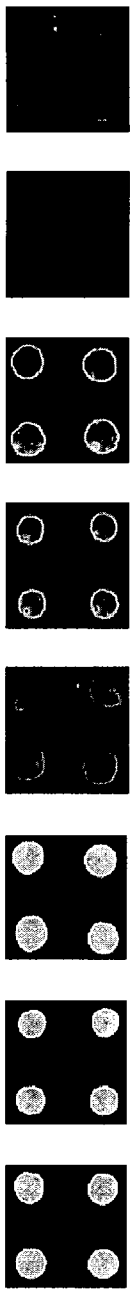


Fig. 4B

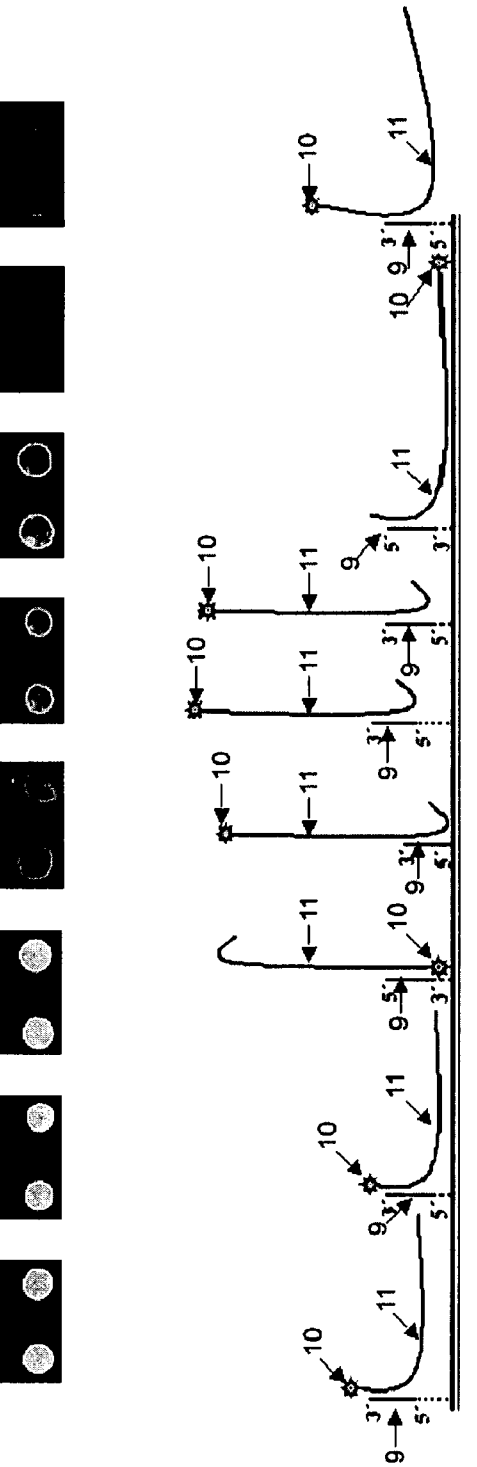


Fig. 4C

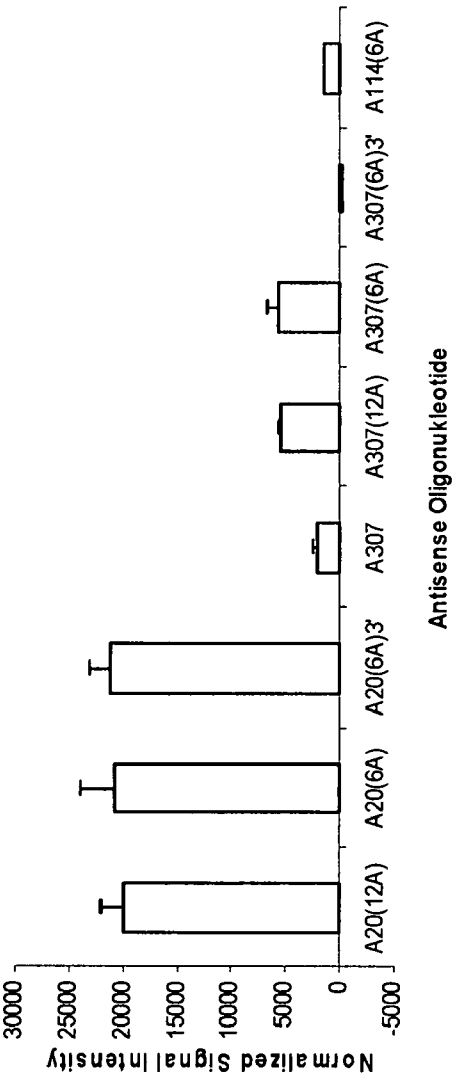


Fig. 5A

Fig. 6A

Fig. 5B

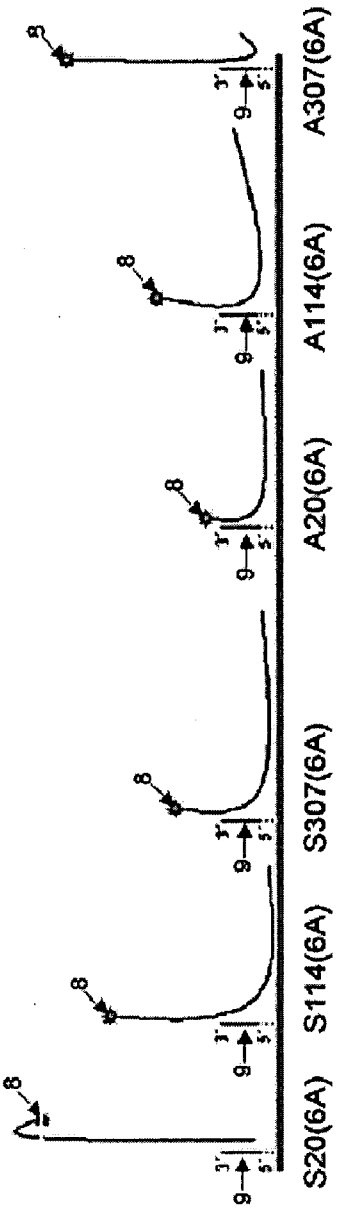
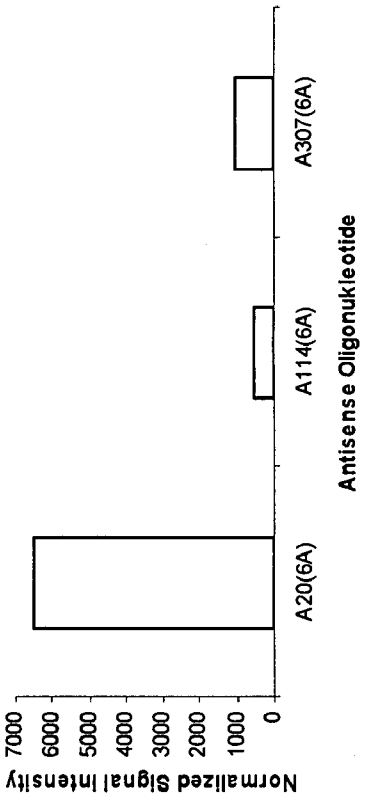


Fig. 6B



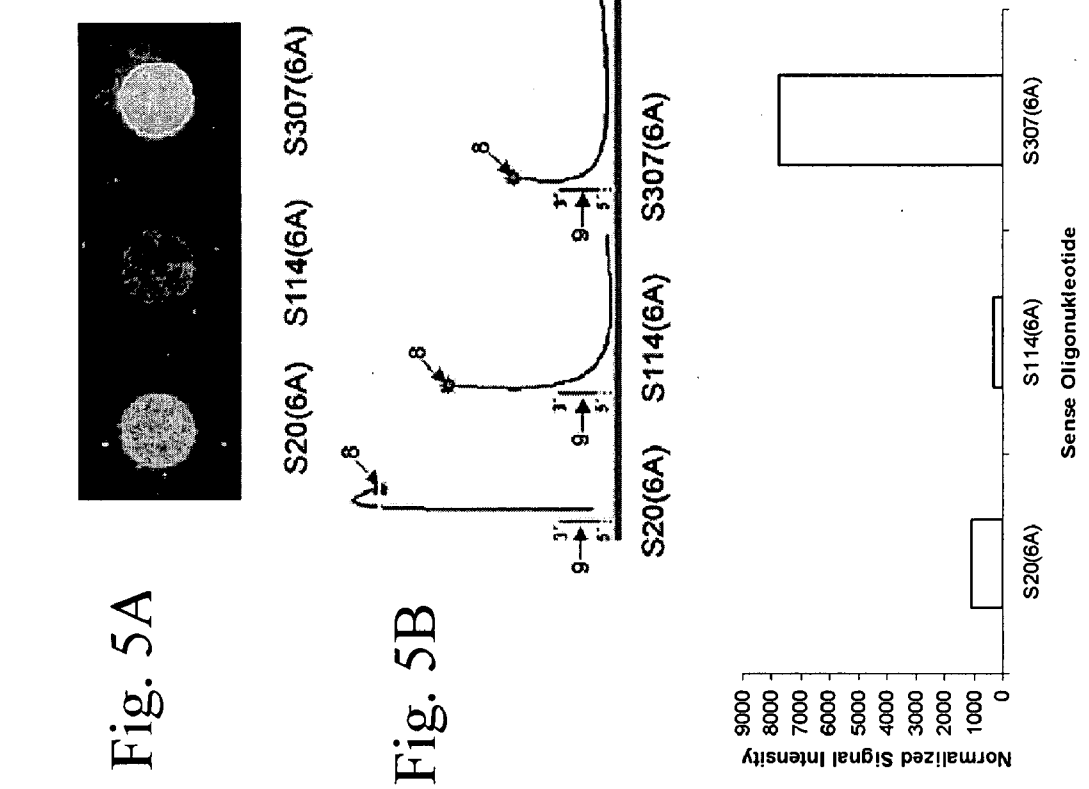


Fig. 5C

Fig. 6C

Fig. 7A

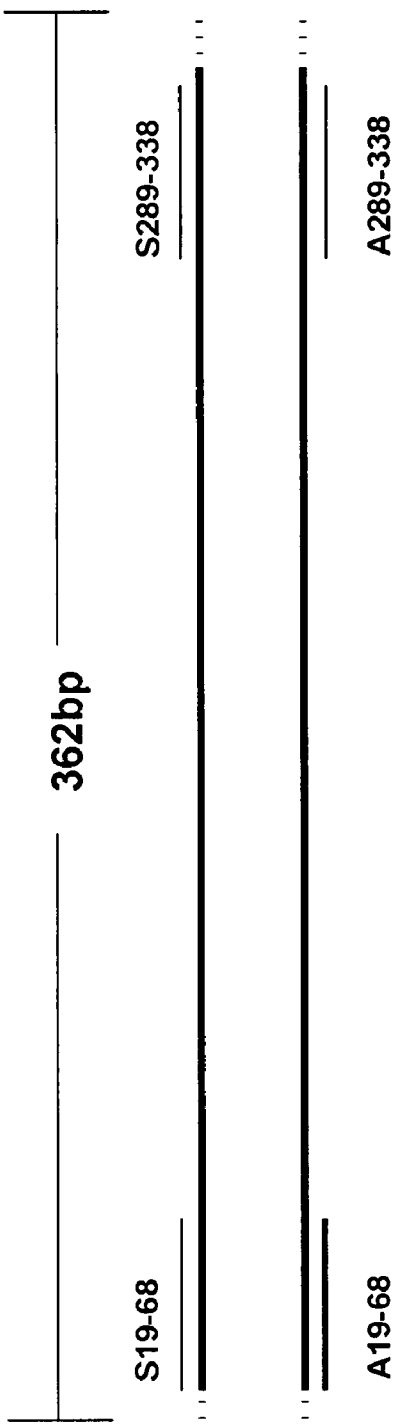
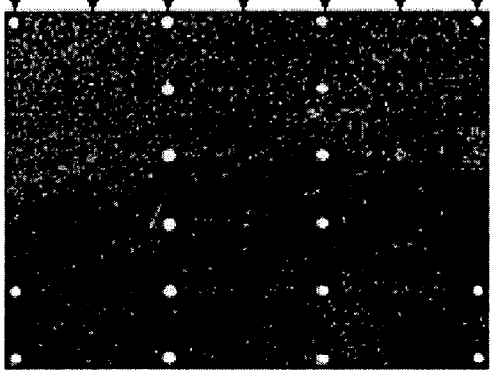


Fig. 7B

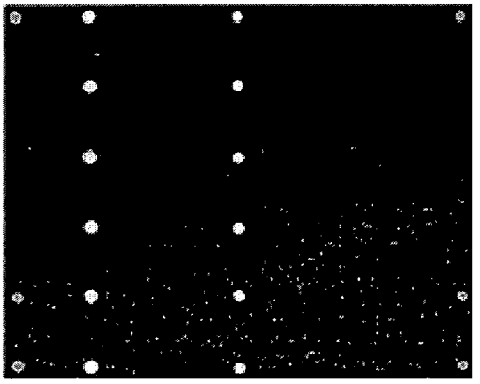
Modification	Catcher	Sequence (5'-3')	Tm(℃)[f]	Length (bp)
5'Aminolink (no+A1spacer)	A19-68	AGGTGCATCACGGTGGTCTGGGCCTTGCTGTGGAGGATCAGACGGGTGGA	91	50
	A289-338	ACGATGTAGATTTCCTGGGCCTTGTTTTCGCGAATCGGCATCGCGAAGCC	87	50
	S19-68	TCCACCCGTCTGATCCTCCACAGCAAGGCCCAGACCACCGTGATGCACCT	91	50
	S289-338	GGCTTCGCGATGCCGATTCGCGAAAACAAGGCCCAGGAAATCTACATCGT	87	50

Fig. 8A



Sense-Strang Probe

Fig. 9A



Antisense-Strang Probe

Fig. 8B

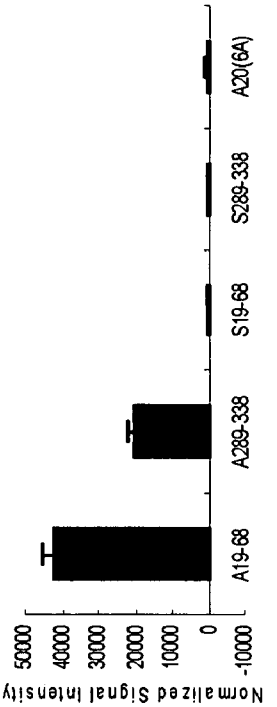
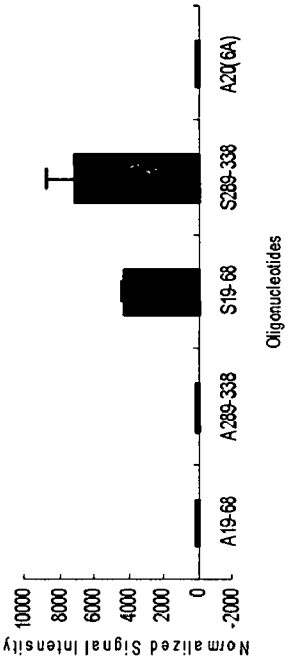


Fig. 9B



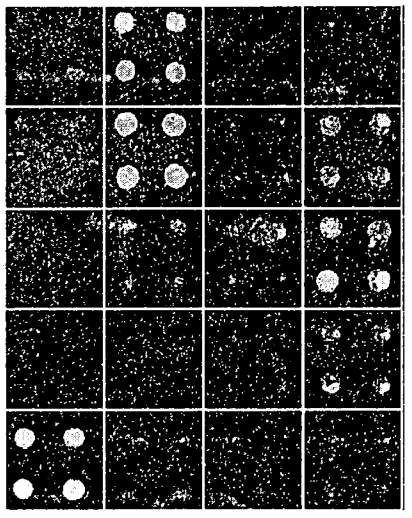


Fig. 10A

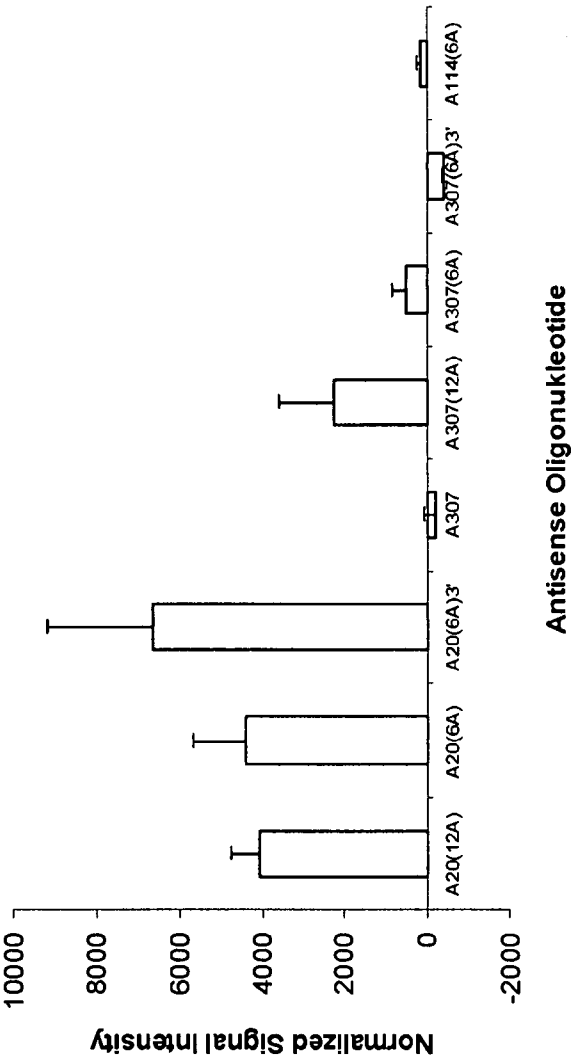


Fig. 10B

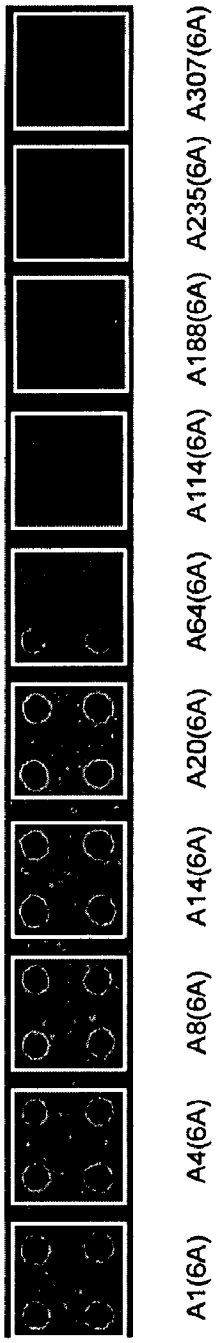


Fig. 11A

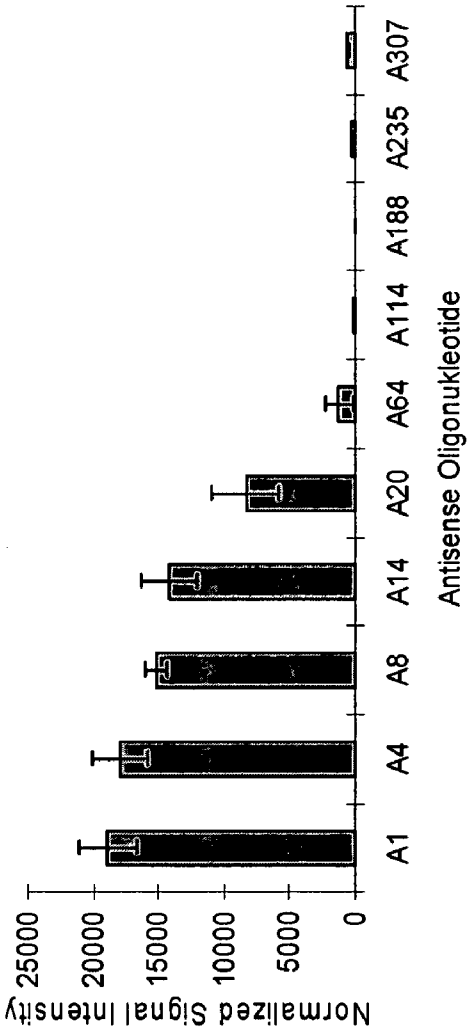


Fig. 11A

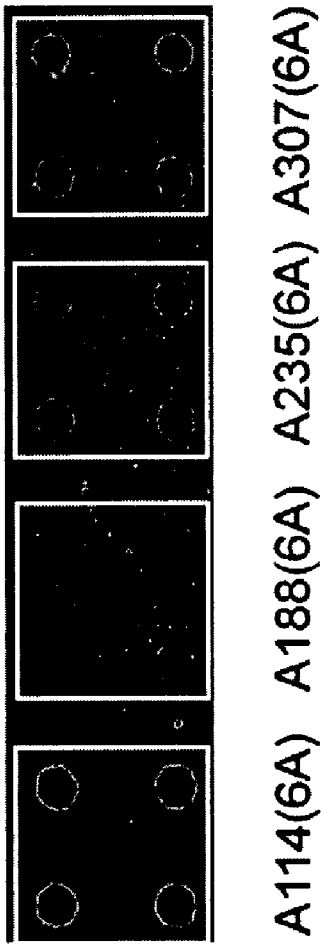


Fig. 12A

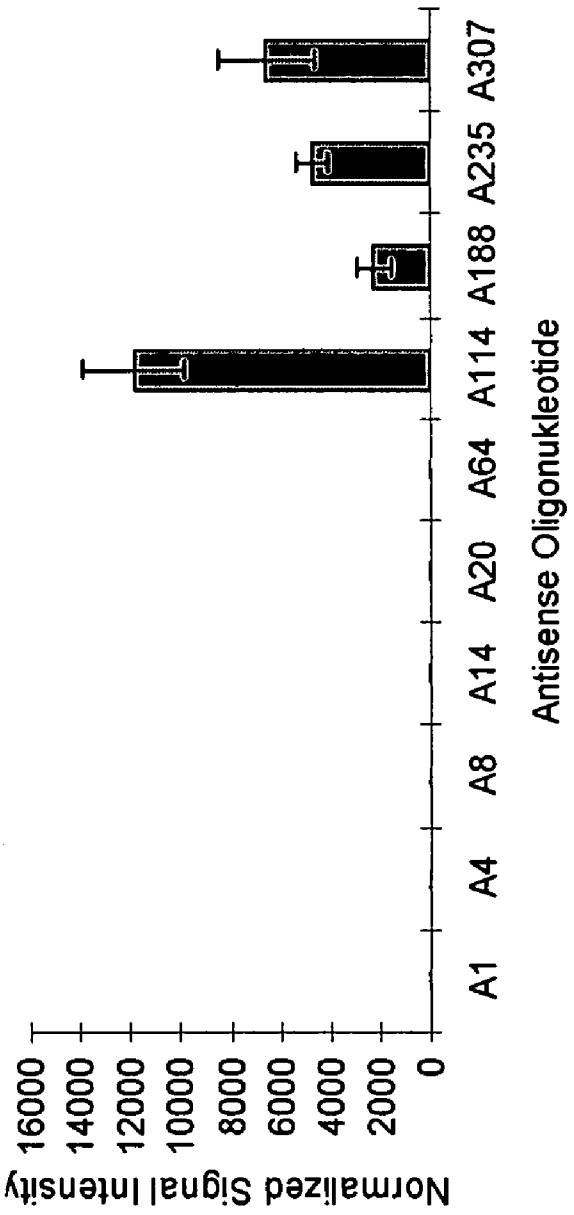
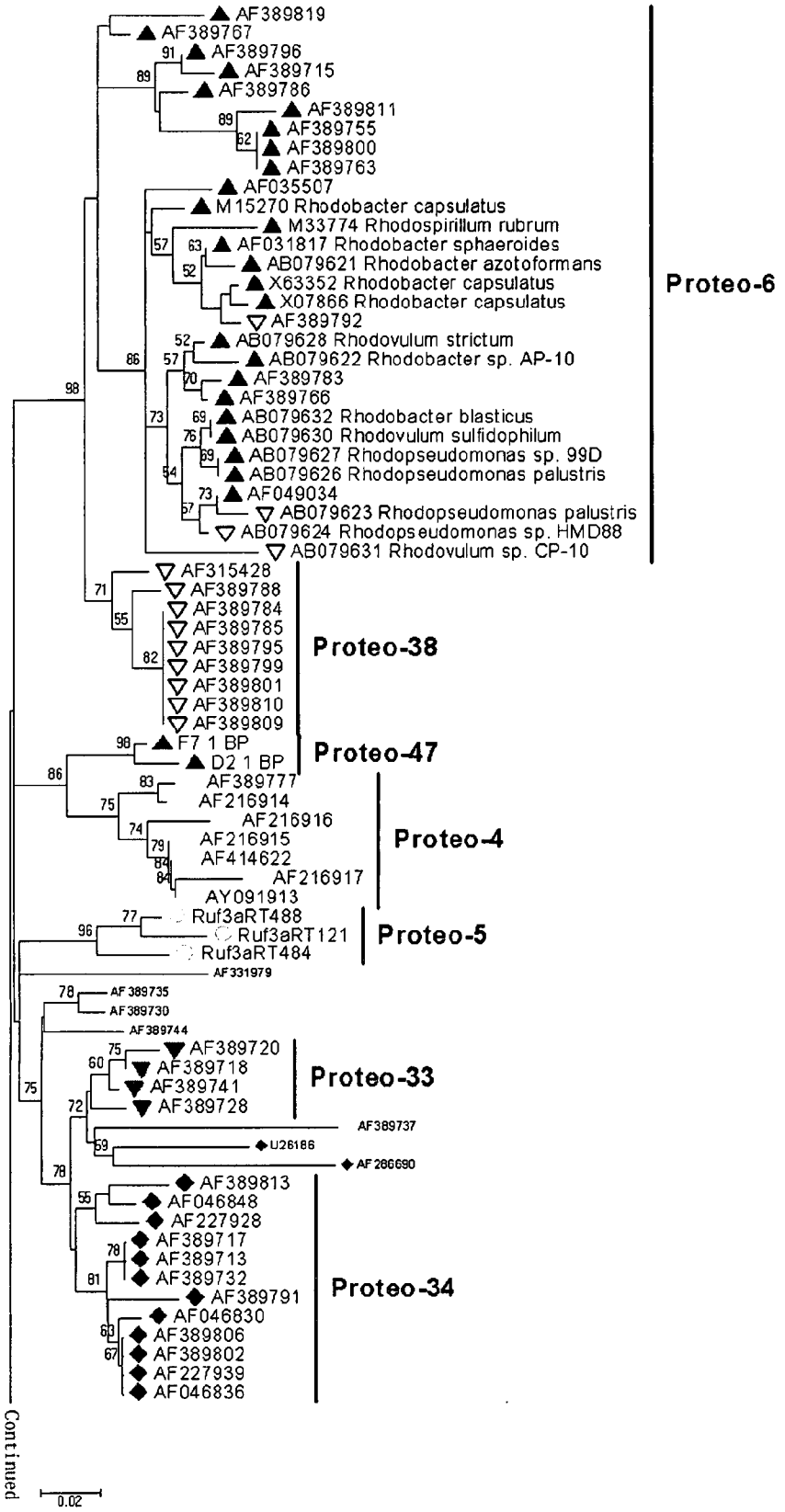


Fig. 12B

Fig. 13 (page1)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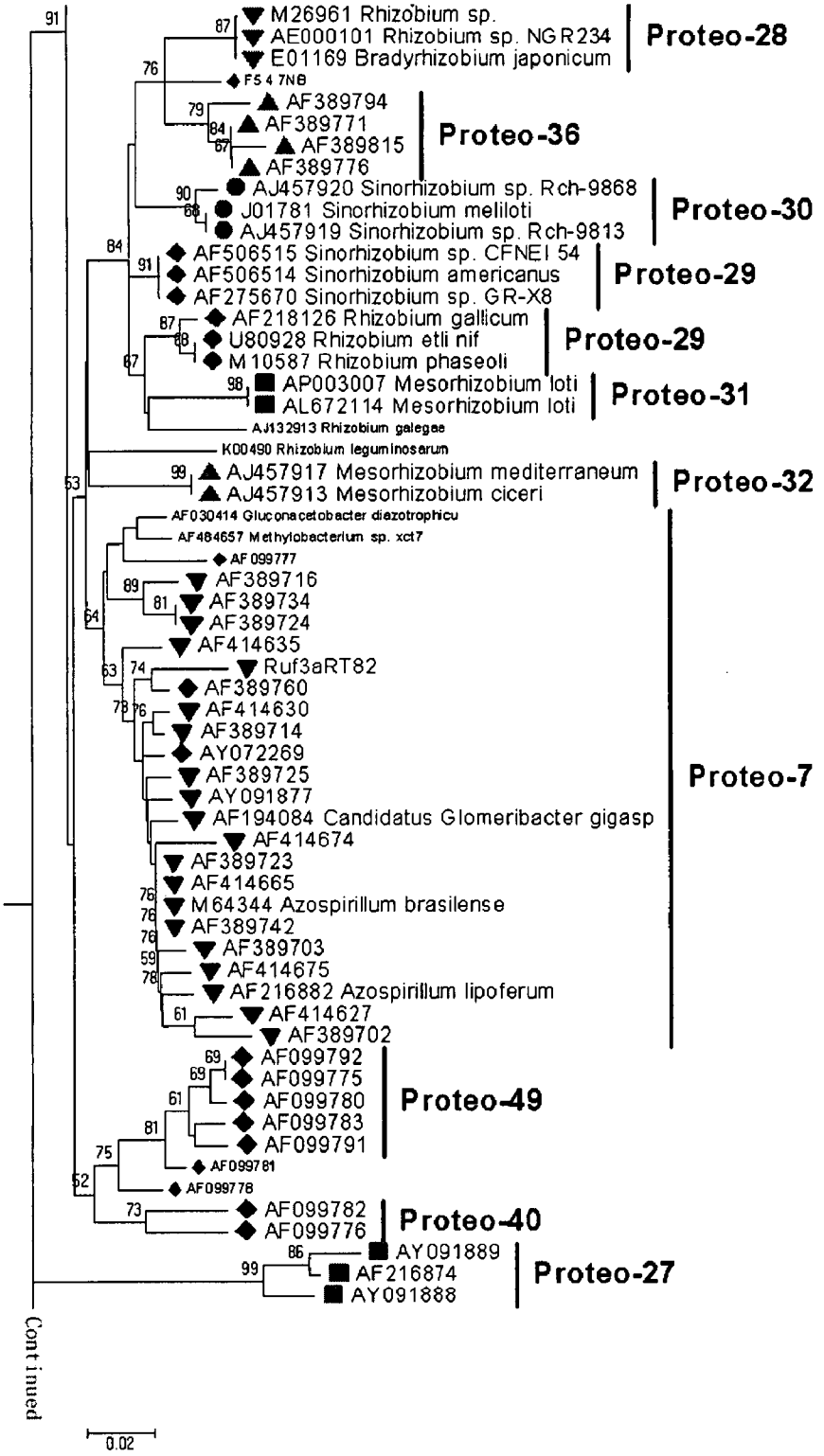
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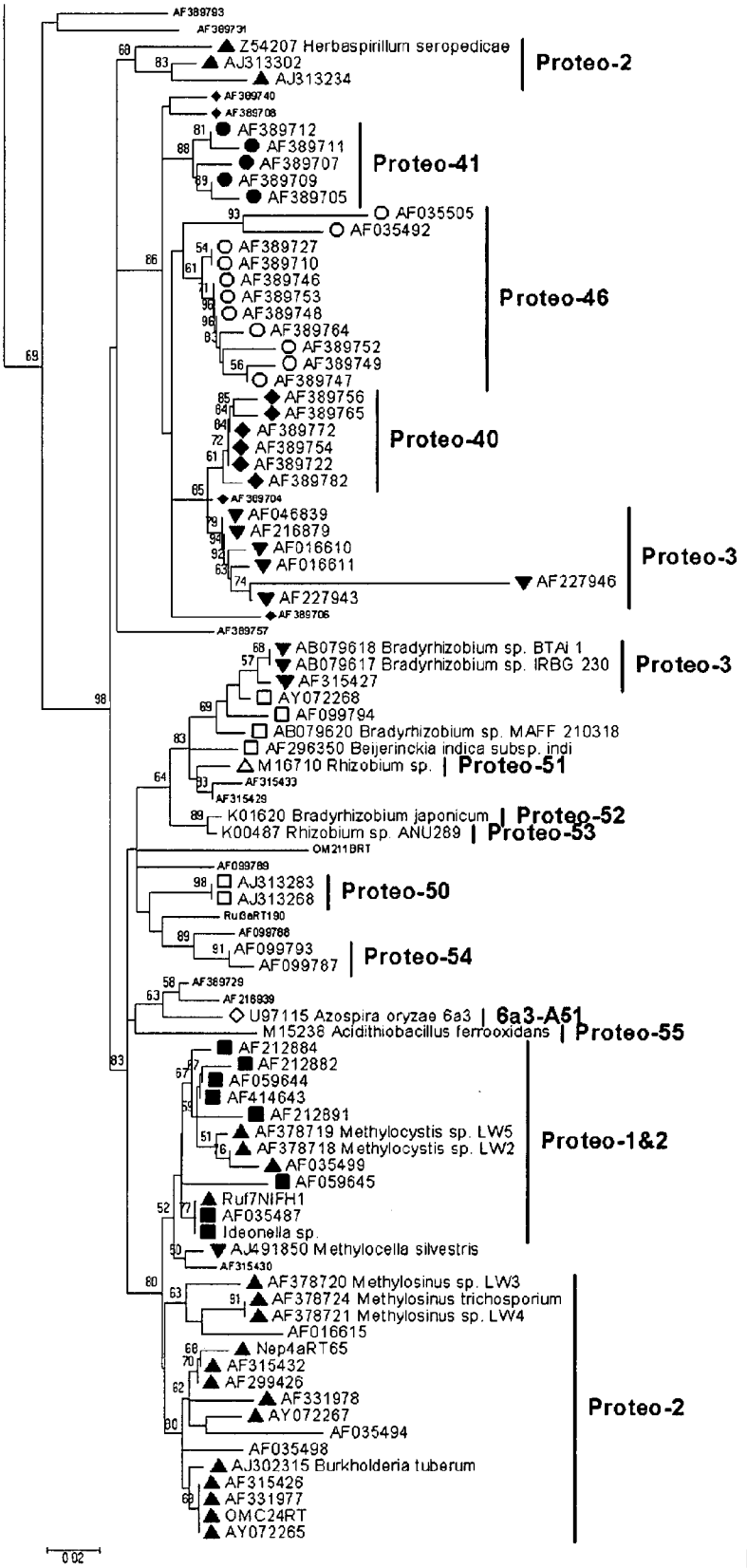
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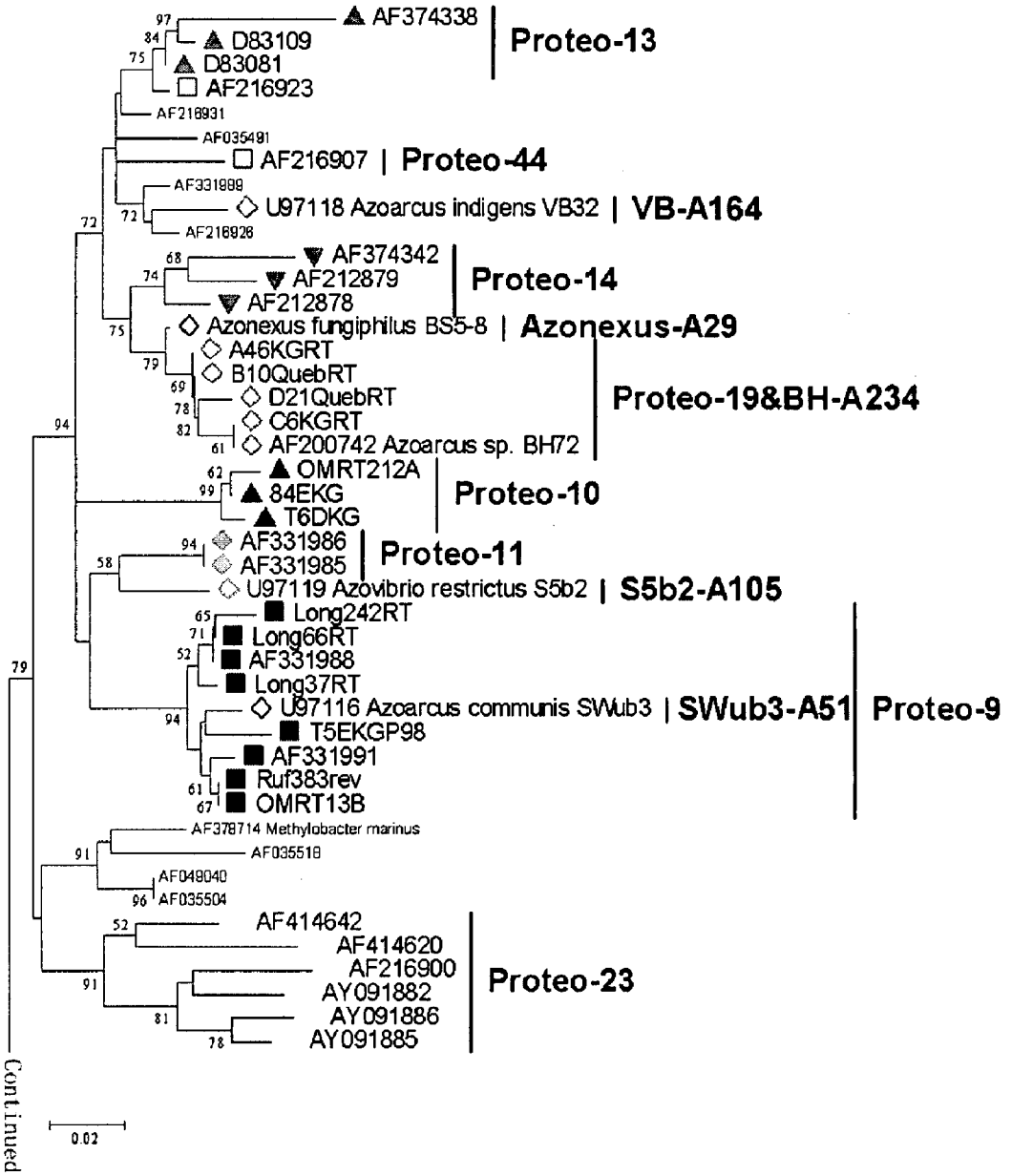
Fig. 14 (page 1)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Ruf383rev
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Proteo-9
AF378714 Methylobacter marinus
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Proteo-23
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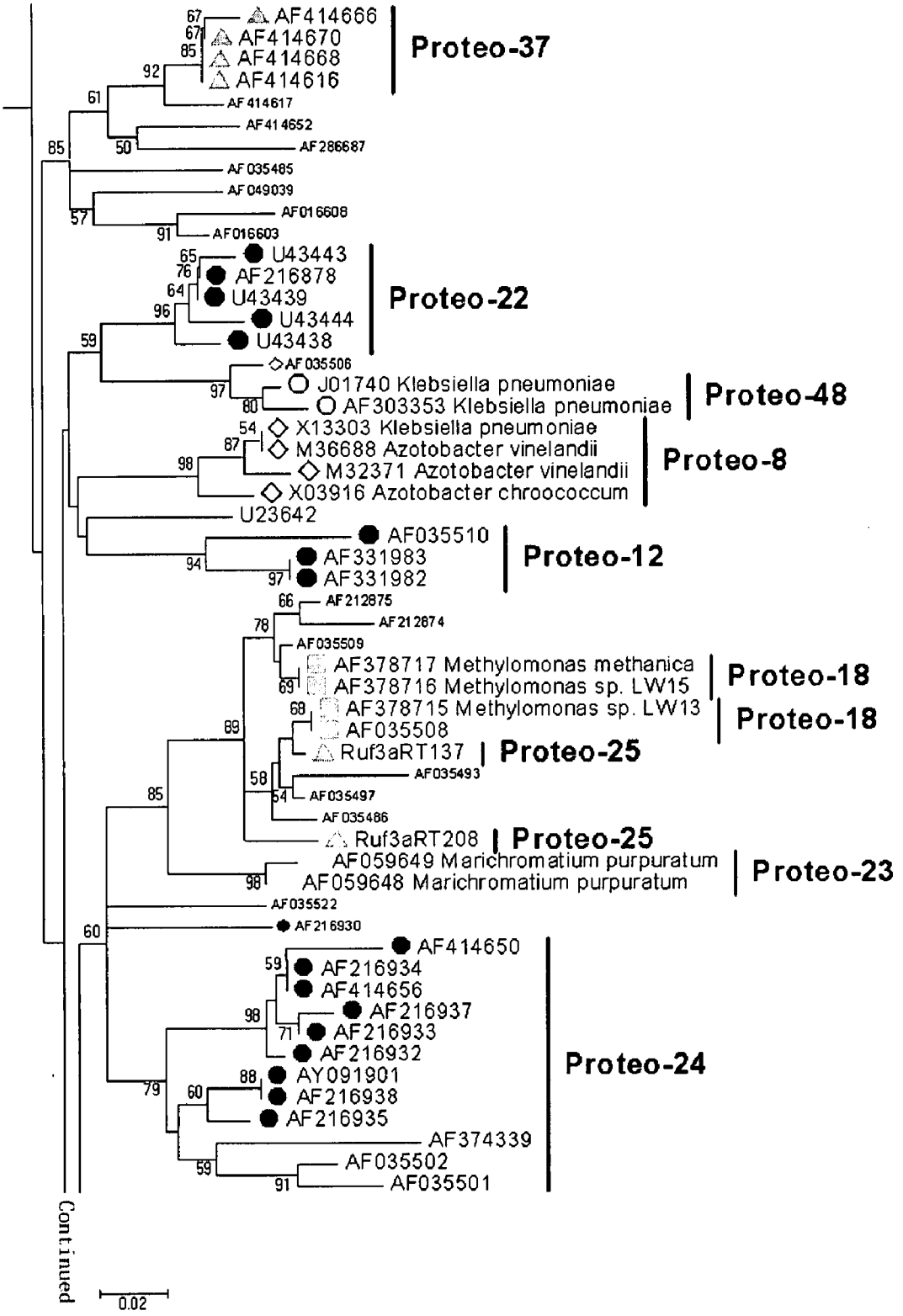
Fig. 14 (page 2)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AF016603
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AF216878
U43439
U43444
U43438
Proteo-22
AF035506
J01740 Klebsiella pneumoniae
AF303353 Klebsiella pneumoniae
Proteo-48
X13303 Klebsiella pneumoniae
M36688 Azotobacter vinelandii
M32371 Azotobacter vinelandii
X03916 Azotobacter chroococcum
Proteo-8
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AF035510
AF331983
AF331982
Proteo-12
AF212875
AF212874
AF035509
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AF378716 Methylomonas sp. LW15
Proteo-18
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AF035508
Ruf3aRT137
Proteo-18
Proteo-25
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AF035497
AF035486
Ruf3aRT208
Proteo-25
AF059649 Marichromatium purpuratum
AF059648 Marichromatium purpuratum
Proteo-23
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AF216930
AF414650
AF216934
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AF216937
AF216933
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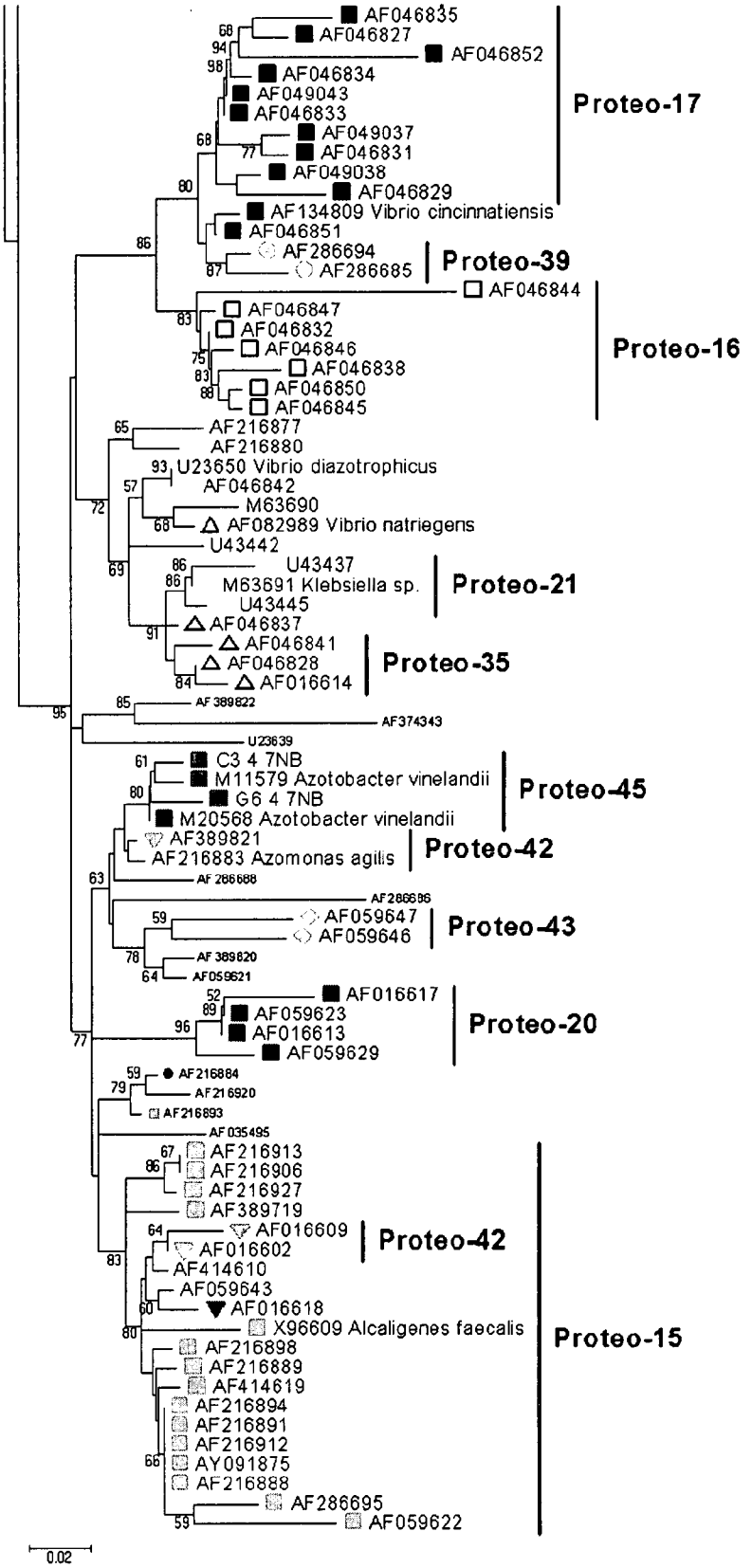
Fig. 15 (page 1)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AF046832
AF046846
AF046838
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AF046845
Proteo-16
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AF216880
U23650 Vibrio diazotrophicus
AF046842
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U43442
U43437
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Proteo-20
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AF216920
AF216893
AF035495
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AF216927
AF389719
AF016609
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AF059643
AF016618
X96609 Alcaligenes faecalis
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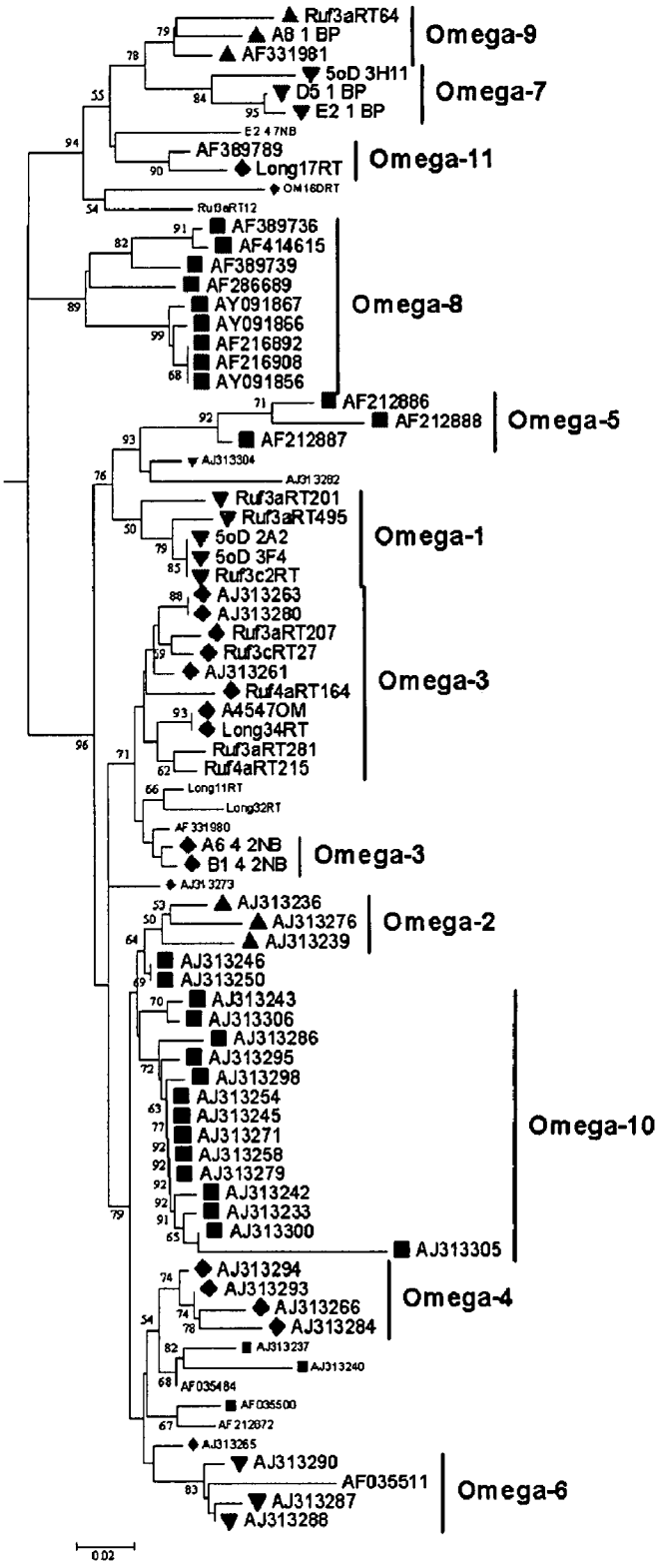
0.02

Fig. 15 (page 2)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E2 1 BP
Omega-7
E2 4 7NB
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Long17RT
Omega-11
OM16DRT
Ruf3aRT12
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AF286689
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AF216908
AY091856
Omega-8
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AF212887
Omega-5
AJ313304
AJ313282
Ruf3aRT201
Ruf3aRT495
5oD 2A2
5oD 3F4
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Omega-1
AJ313263
AJ313280
Ruf3aRT207
Ruf3cRT27
AJ313261
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Ruf4aRT215
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Long32RT
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AJ313276
AJ313239
Omega-2
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AJ313243
AJ313306
AJ313286
AJ313295
AJ313298
AJ313254
AJ313245
AJ313271
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AJ313279
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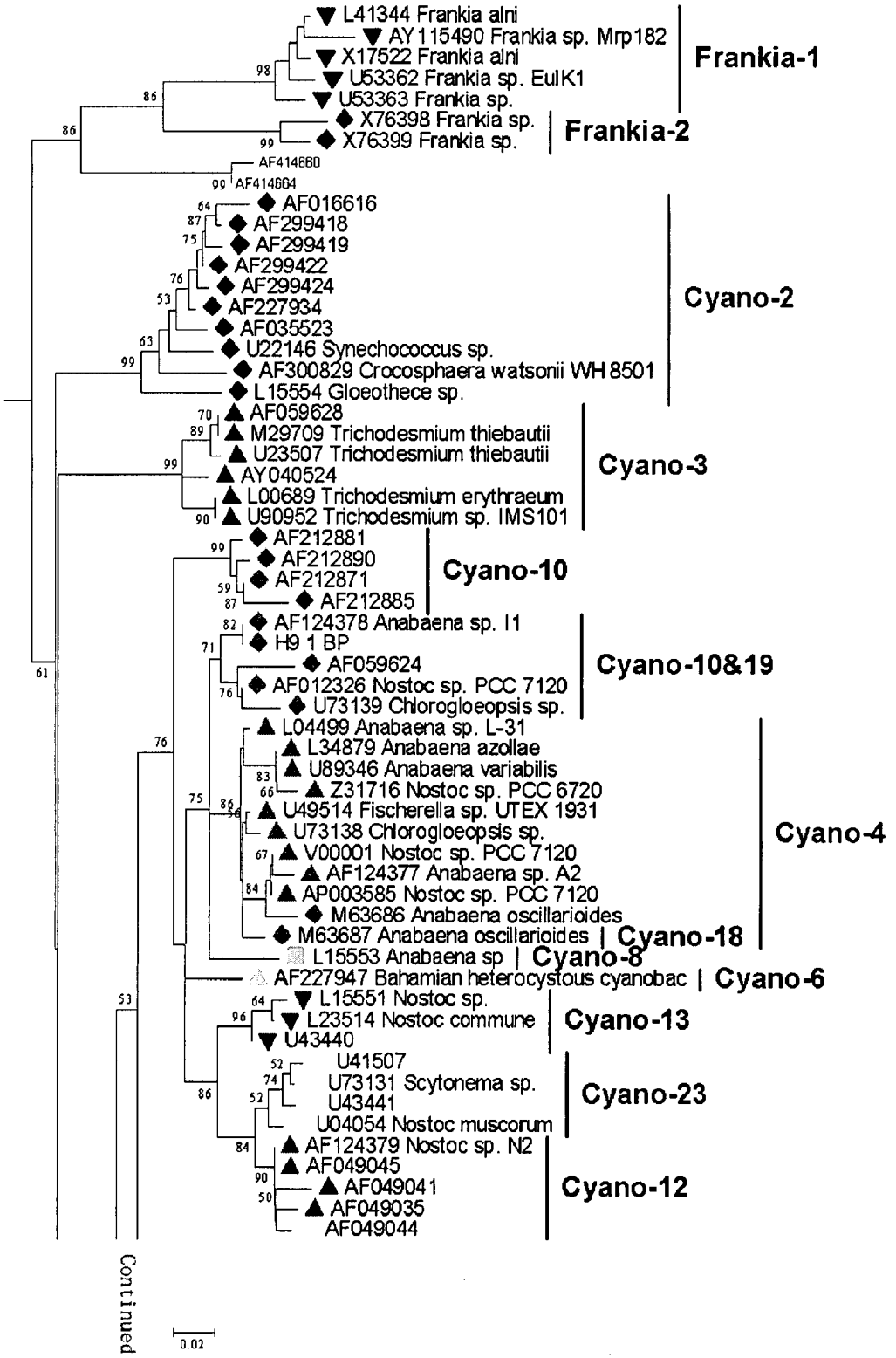
Fig. 15 (page 3)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X76398 Frankia sp.
X76399 Frankia sp.
Frankia-1
Frankia-2
AF414660
AF414664
AF016616
AF299418
AF299419
AF299422
AF299424
AF227934
AF035523
U22146 Synechococcus sp.
AF300829 Crocosphaera watsonii WH 8501
L15554 Gloeothece sp.
Cyano-2
AF059628
M29709 Trichodesmium thiebautii
U23507 Trichodesmium thiebautii
AY040524
L00689 Trichodesmium erythraeum
U90952 Trichodesmium sp. IMS101
Cyano-3
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AF212885
Cyano-10
AF124378 Anabaena sp. I1
H9 1 BP
AF059624
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Cyano-10&19
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U73138 Chlorogloeopsis sp.
V00001 Nostoc sp. PCC 7120
AF124377 Anabaena sp. A2
AP003585 Nostoc sp. PCC 7120
M63686 Anabaena oscillarioides
M63687 Anabaena oscillarioides
Cyano-4
Cyano-18
L15553 Anabaena sp
Cyano-8
AF227947 Bahamian heterocystous cyanobac
Cyano-6
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L23514 Nostoc commune
U43440
Cyano-13
U41507
U73131 Scytonema sp.
U43441
U04054 Nostoc muscorum
Cyano-23
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Cyano-12
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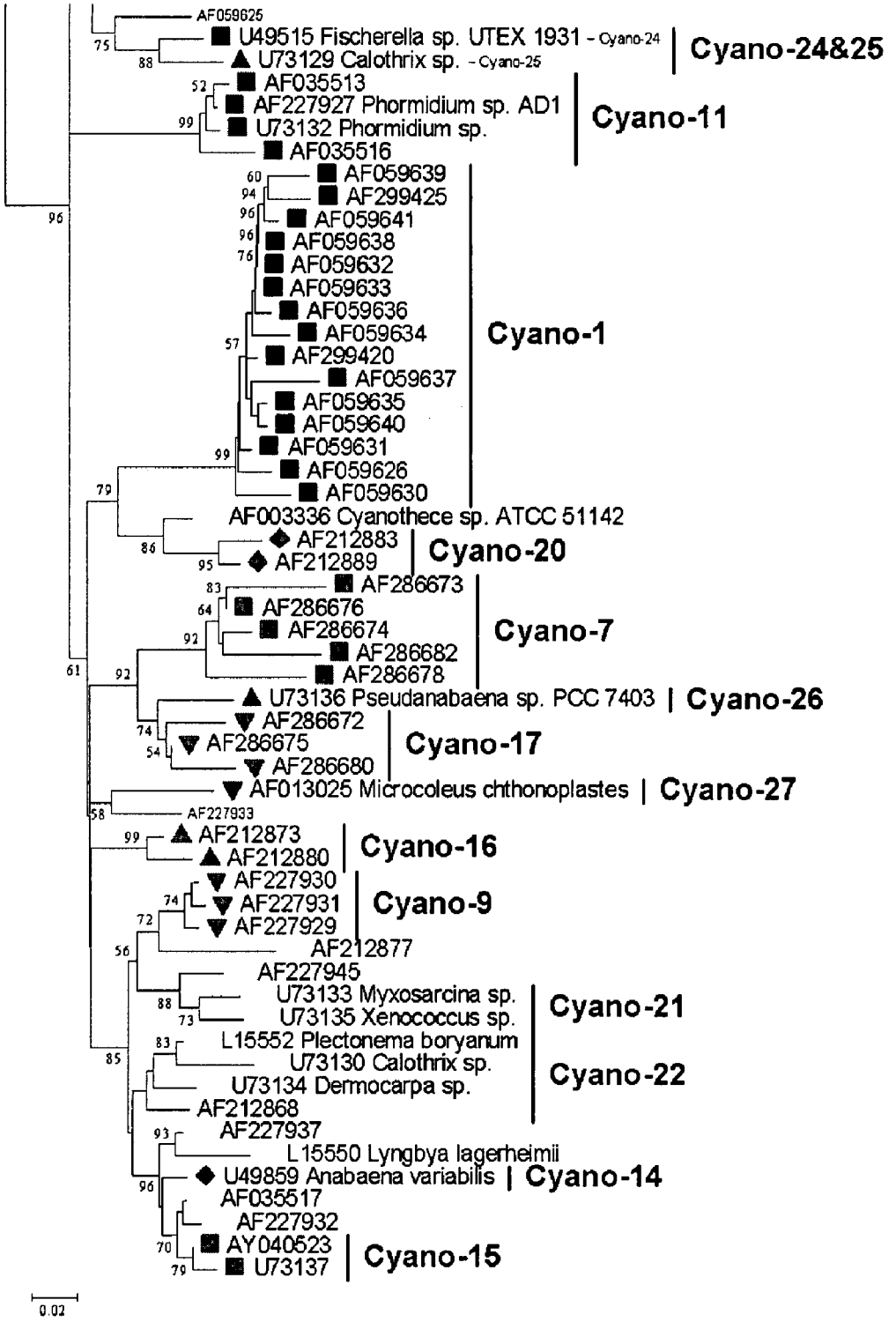
Fig. 16 (page 1)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Cyano-24&25
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U73132 Phormidium sp.
AF035516
Cyano-11
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AF059633
AF059636
AF059634
AF299420
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AF059635
AF059640
AF059631
AF059626
AF059630
Cyano-1
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AF212889
Cyano-20
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AF286676
AF286674
AF286682
AF286678
Cyano-7
U73136 Pseudanabaena sp. PCC 7403
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AF286675
AF286680
Cyano-17
AF013025 Microcoleus chthonoplastes
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AF212880
Cyano-16
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AF227931
AF227929
Cyano-9
AF212877
AF227945
U73133 Myxosarcina sp.
U73135 Xenococcus sp.
Cyano-21
L15552 Plectonema boryanum
U73130 Calothrix sp.
U73134 Dermocarpa sp.
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AF227937
Cyano-22
L15550 Lyngbya lagerheimii
U49859 Anabaena variabilis
Cyano-14
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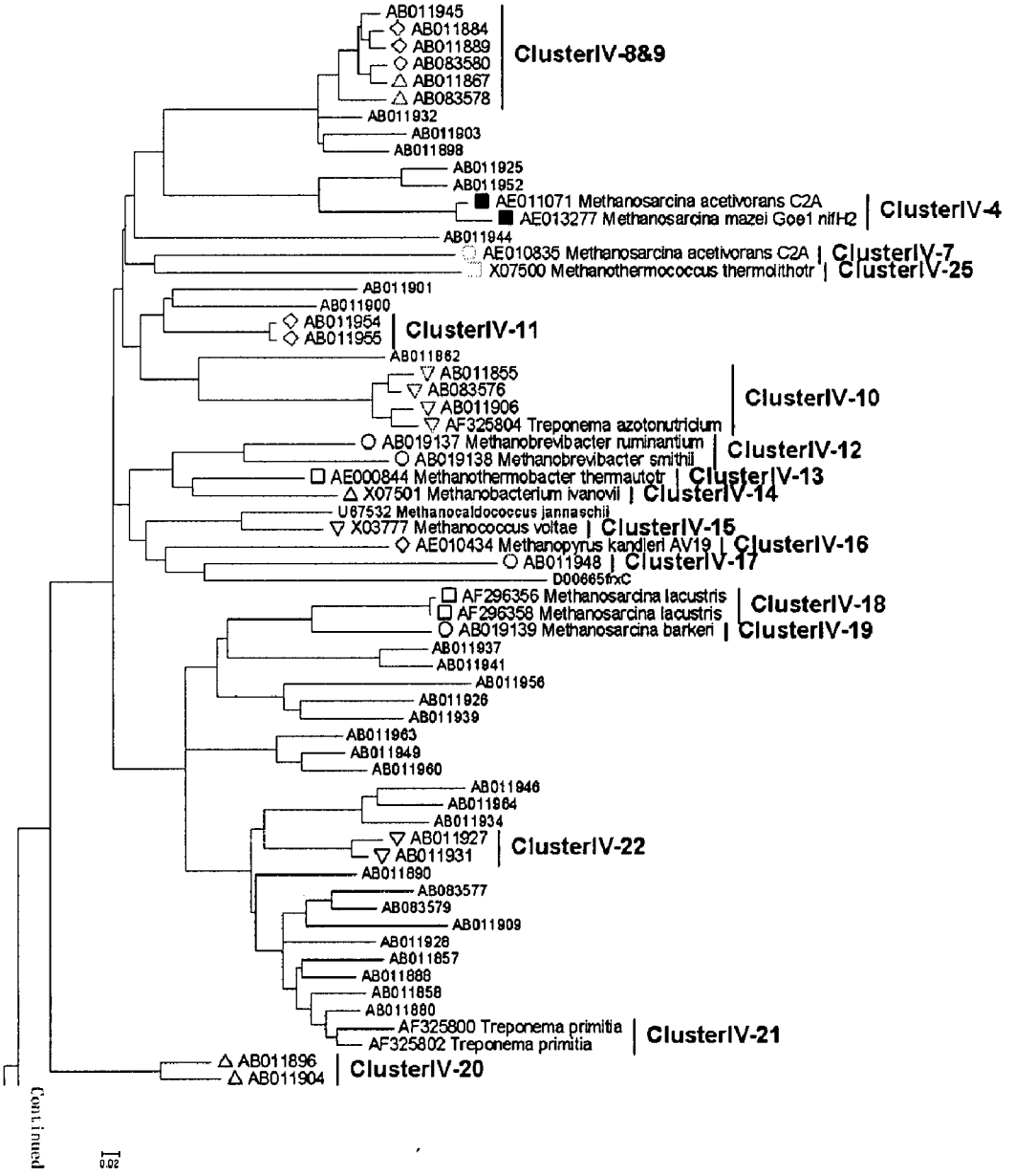
AY040523
U73137
Cyano-15
0.02

Fig. 16 (page 2)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AB083578
ClusterIV-8&9
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AB011898
AB011925
AB011952
AE011071 Methanosarcina acetivorans C2A
AE013277 Methanosarcina mazei Goe1 nifH2
ClusterIV-4
AB011944
AE010835 Methanosarcina acetivorans C2A
ClusterIV-7
X07500 Methanothermococcus thermolithotr
ClusterIV-25
AB011901
AB011900
AB011954
AB011955
ClusterIV-11
AB011862
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AB083576
AB011906
AF325804 Treponema azotonutricium
ClusterIV-10
AB019137 Methanobrevibacter ruminantium
AB019138 Methanobrevibacter smithii
ClusterIV-12
AE000844 Methanothermobacter thermautotr
ClusterIV-13
X07501 Methanobacterium ivanovii
ClusterIV-14
U67532 Methanocaldococcus jannaschii
X03777 Methanococcus voltae
ClusterIV-15
AE010434 Methanopyrus kandleri AV19
ClusterIV-16
AB011948
ClusterIV-17
D00665fixC
AF296356 Methanosarcina lacustris
AF296358 Methanosarcina lacustris
AB019139 Methanosarcina barkeri
ClusterIV-18
ClusterIV-19
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AB011941
AB011956
AB011926
AB011939
AB011963
AB011949
AB011960
AB011946
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AB011931
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AB083577
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AB011909
AB011928
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AB011880
AF325800 Treponema primitia
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ClusterIV-20
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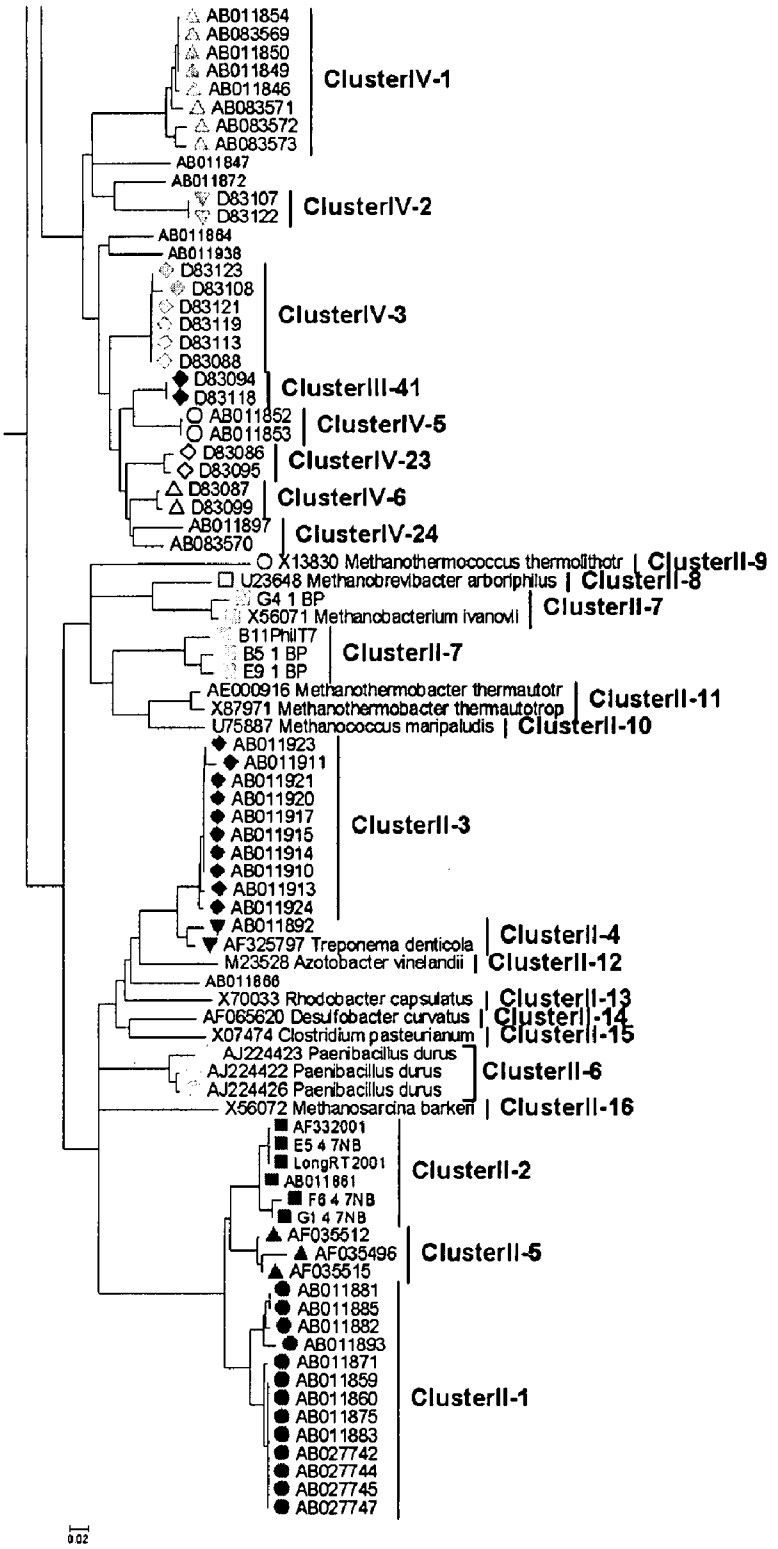
Fig. 17 (page 1)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ClusterIV-2
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D83121
D83119
D83113
D83088
ClusterIV-3
D83094
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ClusterIII-41
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AB011853
ClusterIV-5
D83086
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ClusterIV-23
D83087
D83099
ClusterIV-6
AB011897
AB083570
ClusterIV-24
X13830 Methanothermococcus thermolithotr
ClusterII-9
U23648 Methanobrevibacter arboriphilus
ClusterII-8
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X56071 Methanobacterium ivanovii
ClusterII-7
B11PhiT7
B5 1 BP
E9 1 BP
ClusterII-7
AE000916 Methanothermobacter thermautotr
X87971 Methanothermobacter thermautotrop
ClusterII-11
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ClusterII-10
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AB011921
AB011920
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AB011915
AB011914
AB011910
AB011913
AB011924
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AF325797 Treponema denticola
ClusterII-4
M23528 Azotobacter vinelandii
ClusterII-12
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X70033 Rhodobacter capsulatus
ClusterII-13
AF065620 Desulfobacter curvatus
ClusterII-14
X07474 Clostridium pasteurianum
ClusterII-15
AJ224423 Paenibacillus durus
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ClusterII-6
X56072 Methanosarcina barkeri
ClusterII-16
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AB011861
F6 4 7NB
G1 4 7NB
ClusterII-2
AF035512
AF035496
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ClusterII-5
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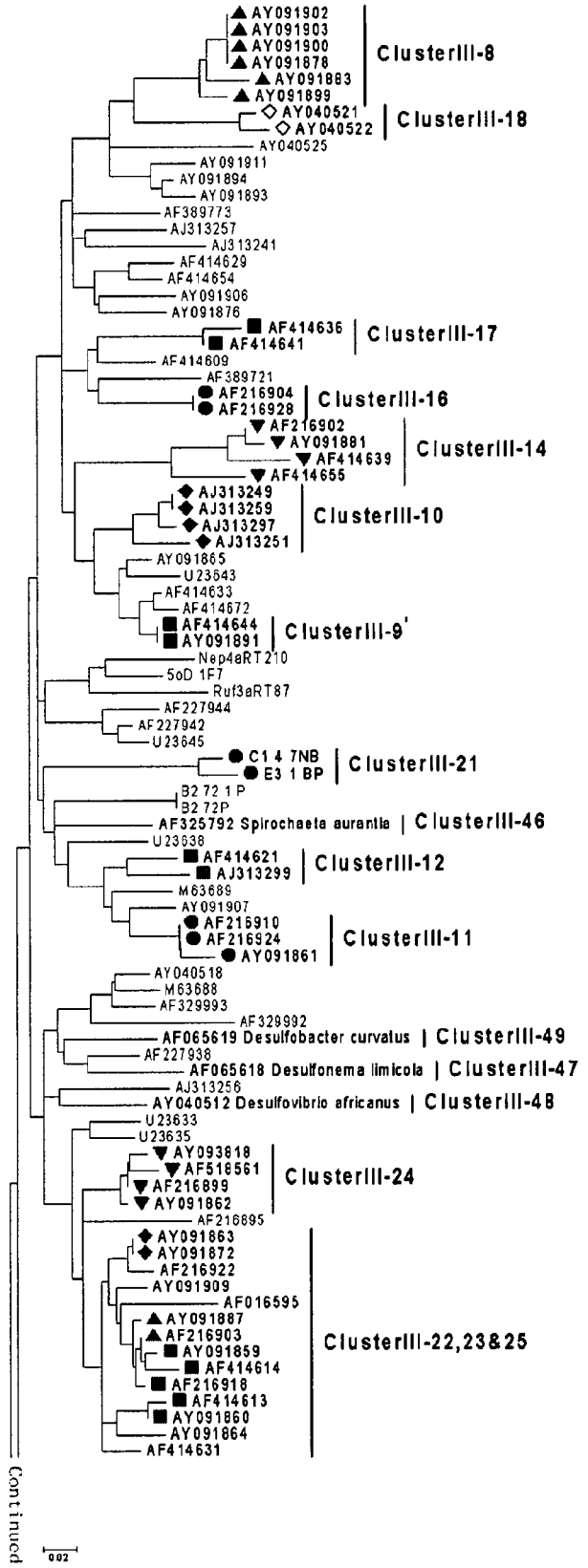
Fig. 17 (page 2)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ClusterIII-10
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Ruf3aRT87
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B2 72P
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ClusterIII-46
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AJ313299
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ClusterIII-47
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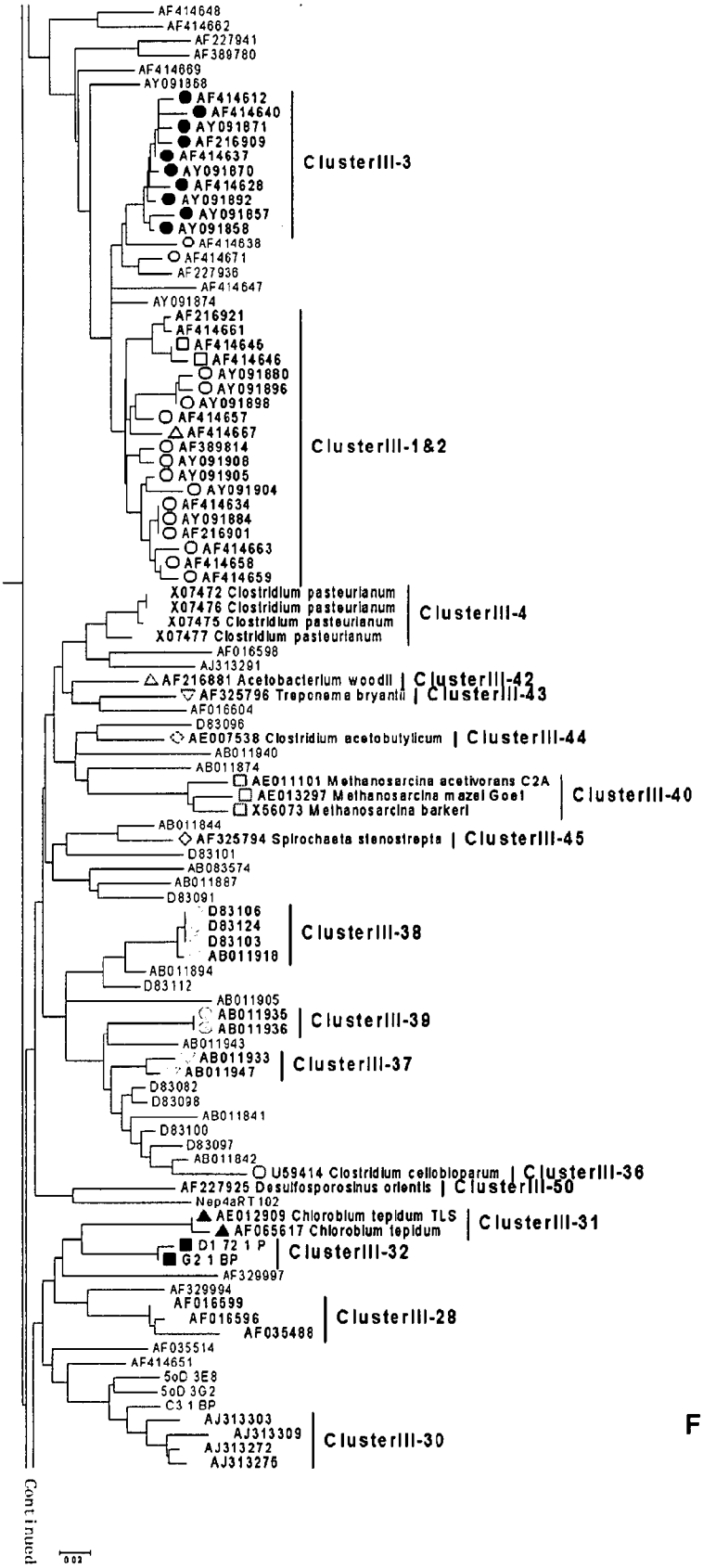
Fig. 18 (page 1)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X07477 Clostridium pasteurianum
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AF325796 Treponema bryantii
AF016604
D83096
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AE013297 Methanosarcina mazei Goe1
X56073 Methanosarcina barkeri
AB011844
AF325794 Spirochaeta stenostrepta
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AB083574
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D83106
D83124
D83103
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AB011894
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AB011936
AB011943
AB011933
AB011947
D83082
D83098
AB011841
D83100
D83097
AB011842
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AF227925 Desulfosporosinus orientis
Nep4aRT102
AE012909 Chlorobium tepidum TLS
AF065617 Chlorobium tepidum
D1 72 1 P
G2 1 BP
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AF329994
AF016599
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AF035488
AF035514
AF414651
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5oD 3G2
C3 1 BP
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AJ313272
AJ313276
ClusterIII-3
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ClusterIII-4
ClusterIII-42
ClusterIII-43
ClusterIII-44
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ClusterIII-45
ClusterIII-38
ClusterIII-39
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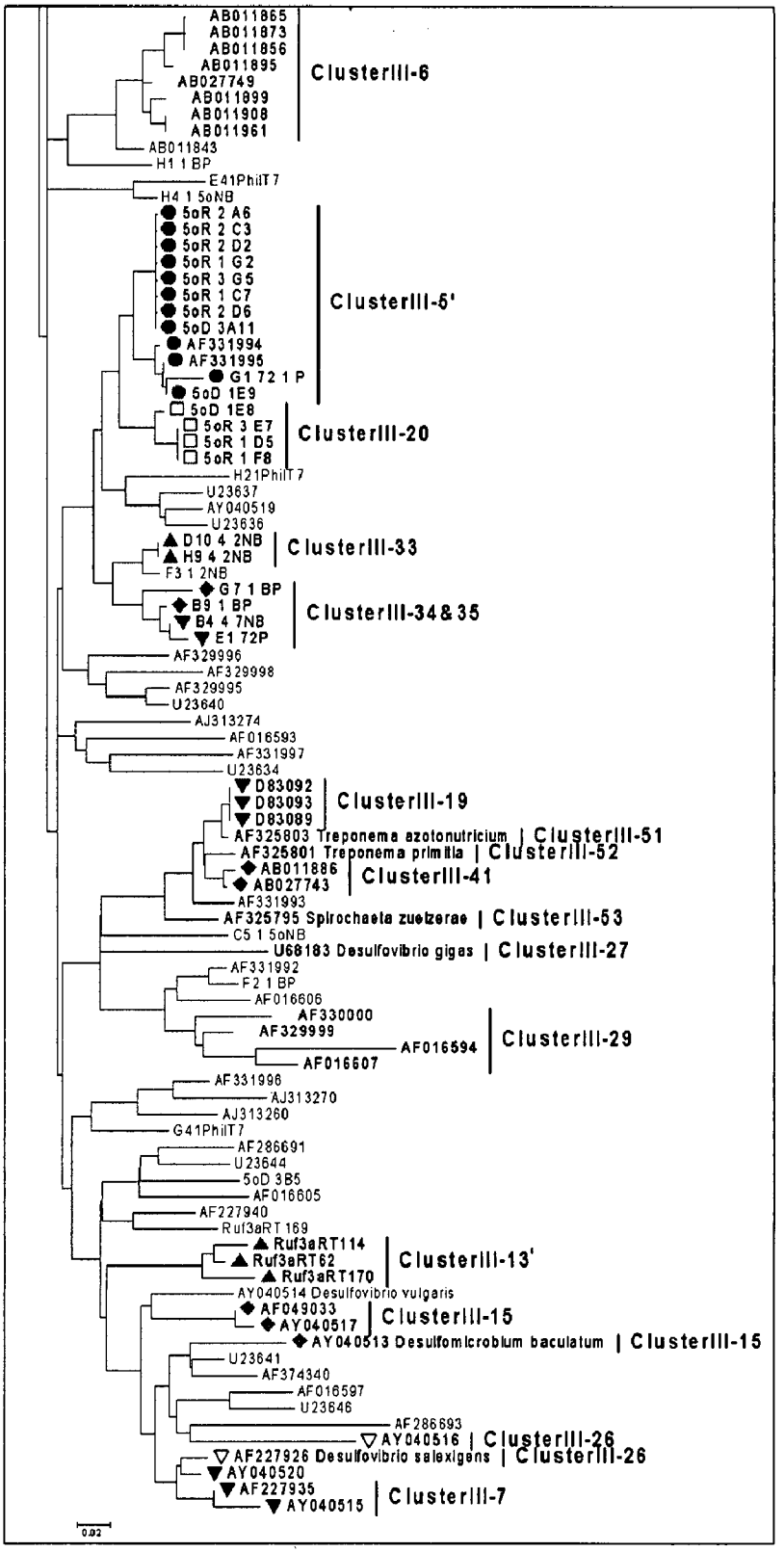
Fig. 18 (page 2)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5oR 2 D2
5oR 1 G2
5oR 3 G5
5oR 1 C7
5oR 2 D6
5oD 3A11
AF331994
AF331995
G1 72 1 P
5oD 1E9
ClusterIII-5'
5oD 1E8
5oR 3 E7
5oR 1 D5
5oR 1 F8
ClusterIII-20
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AY040519
U23636
D10 4 2NB
H9 4 2NB
ClusterIII-33
F3 1 2NB
G7 1 BP
B9 1 BP
B4 4 7NB
E1 72P
ClusterIII-34&35
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AF329998
AF329995
U23640
AJ313274
AF016593
AF331997
U23634
D83092
D83093
D83089
ClusterIII-19
AF325803 Treponema azotonutricium | ClusterIII-51
AF325801 Treponema primitia | ClusterIII-52
AB011886
AB027743
ClusterIII-41
AF331993
AF325795 Spirochaeta zuelzerae | ClusterIII-53
C5 1 5oNB
U68183 Desulfovibrio gigas | ClusterIII-27
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F2 1 BP
AF016606
AF330000
AF329999
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AF016607
ClusterIII-29
AF331996
AJ313270
AJ313260
G41PhilT7
AF286691
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5oD 3B5
AF016605
AF227940
Ruf3aRT169
Ruf3aRT114
Ruf3aRT62
Ruf3aRT170
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AF049033
AY040517
ClusterIII-15
AY040513 Desulfomicrobium baculatum | ClusterIII-15
U23641
AF374340
AF016597
U23646
AF286693
AY040516 | ClusterIII-26
AF227926 Desulfovibrio salexigens | ClusterIII-26
AY040520
AF227935
AY040515
ClusterIII-7
0.02

Fig. 18 (page 3)

METHOD FOR ANALYZING SAMPLES BY MEANS OF HYBRIDIZATION

[0001] The present invention relates to a method of analysing samples by means of a ligand binding method, target molecules which may be used in such methods, and kits for the provision of such target molecules and/or implementation of the aforementioned method.

BACKGROUND OF THE INVENTION

[0002] Nucleic acids are normally present in the form of double stranded molecules, also described as heteroduplexes and comprised of two nucleic acid molecules. If duplexes are subjected to a rise in temperature, they dissociate into two single-stranded nucleic acid molecules. With a lowering of temperature, these may re-associate to form a heteroduplex. The direct reaction to duplexes is described as hybridization or re-hybridization. Duplexes are also described as hybrids.

[0003] Nucleic acids are polymers of the nucleotides adenine, cytosine, guanine, thymin, uracil, inosin (a, c, g, t, u, i), synthetic or modified nucleotides, arranged next to one another in the polymer like pearls in a chain of pearls. The sequence of the nucleotides in a nucleic acid molecule is specific for that particular nucleic acid molecule. Double strands are formed through hydrogen bonds, which may develop for example between individual nucleotides a and t, also c and g, if on a single strand there are sufficient nucleotides in succession meeting a particular counter-nucleotide on another single strand—and there in the corresponding sequence. In nature, nucleic acids in the form of such complementary single strands occur as double strands.

[0004] One is frequently faced with the problem that the existence of a specific nucleic acid in a reaction mixture or a biological sample (hereafter combined under the term "reaction mixture") needs to be detected. This is generally done by using catcher molecules, which have single-stranded nucleic acids which hybridise to form duplexes with a single-stranded or partly single-stranded nucleic acid molecule, hereafter known as the target molecule. The detection of the duplex permits a statement on the presence of the target molecule. Since in nucleic acids certain sequences can and do occur several times, the catcher molecule is so chosen that it reacts preferably with a target sequence which represents a section of the total sequence of the nucleic acid to be detected, and is as specific as possible for it. In so doing, an attempt is generally made to ensure the most rigorous possible proof of the catcher-target sequence hybrids or duplexes, and the greatest possible specificity or uniqueness of the target sequence for the nucleic acid concerned.

[0005] The detection of nucleic acids using catcher-target sequence hybrids has been known for many years. It has developed from the so-called Southern Blot through many variants to oligonucleotide chips or DNA micro-arrays, in which on a few cm^2 thousands of oligonucleotide sequences are provided as physically separated spots at a fixed phase, and function as catcher molecules. Many of these spots allow, parallel in time, a qualitative and to a limited extent quantitative statement on the presence or absence of certain sequences in a reaction mixture. In addition, there is a multiplicity of variations of the catcher-target sequence hybrid detection principle, which are generally known to the experts in this field.

[0006] To make possible the detection of a catcher-target sequence hybrid, an adequate number of detectable hybrids are needed.

[0007] Frequently one is confronted with small sample quantities, which must be amplified by a PCR-reaction over several cycles. Each cycle of a PCR-reaction is equivalent to a copying process, in which the number of copies increases exponentially with each step. Each step costs time and material and, the longer the copying process is continued, the more likely the copies are to be defective, The fewer cycles are needed to produce a detectable sample quantity, the more advantageous and reliable will be the hybridization experiment conducted with a sample prepared in this way.

[0008] In the quantification of hybrids there are considerable imponderables. The maximum number of hybrids in a probe spot or spot of a micro-array may not exceed the number of catcher molecules in the spot. This is generally known. It is however difficult to make reliable statements as to how many catcher molecules of a spot will form catcher-target sequence molecules in an experiment. Quite a few of the factors which affect the efficiency of hybridization are already known (Southern et al., 1999), such as e.g. the distance between the sequence of the catcher oligonucleotide complementary to the target molecule and the glass surface. A worsening of efficiency when the duplex leads to an excessive overhang of the target molecule in the direction of the glass surface has also been described as a steric effect (Peplies et al. 2003). The intensity of the detection signals used to detect the presence of hybrids does indeed seem to be roughly proportional to the number of hybrids present in a probe spot, but the proportionality factor is apparently not identical from one probe spot to another, when different hybrids are present in different probe spots.

[0009] EP 0721016 A2 describes a method for the discrimination of perfectly complementary hybrids from those which differ in one or more bases. Through enzymatic decomposition of single-stranded polynucleotides after hybridization reaction, discrimination is made between perfectly complementary hybrids and those with incorrect pairings. Only perfectly complementary and double stranded hybrids are still present after decomposition and may be detected with the aid of their fluorescent marking. Marked RNA target molecules may be produced by means of standard PVR or in vitro transcription, with approximately 10% of the uracil of the target sequence fluorescein being marked at random. A second method relates to the detection of perfect hybrids through ligation of marked oligonucleotides, after hybridization with the target molecule has taken place. After ligation, the target molecule is once again separated from the catcher molecule and washed. Detection of the remaining single strands then takes place.

[0010] A similar method is described in WO 98/23776. Here, target molecules should be hybridized by a varying number of repetitions with catcher molecules of known length. After digestion by exonuclease, only perfect hybrids remain, so that the number of repetitions may be determined. Once again only the perfectly complementary double strand may be detected with the aid of its marking. The target molecule is marked. This marking may be inserted during the PCR with marked primer or marked nucleotides or by

other methods. The marking is applied at any desired position and preferably at the 5' or 3' end.

[0011] WO 98/53103 describes DNA arrays with various polynucleotides within individual spots, and kits with such DNA arrays, together with their production and use. Each of the spots on a solid substrate belongs to a specific gene type (e.g. heavily regulated gene, or gene associated with specific stages of sickness). The target molecules to be hybridized may be produced by all known methods, with the use of primers specific for the gene to be analysed being proposed. The target molecules are marked, and the marking may be located in the gene-specific primer or in the dNTPs. Here the length of the catcher molecules in the array typically amounts to 120-800 bases, which represents only a portion of the overall length of the cDNA (target molecules) to be analysed.

[0012] WO 01/23600 A2 on the other hand is concerned with a method for the quantification of relative specificities of the hybridization reaction with the aid of dissociation curves. At least a portion of the detectable marked target molecules is in this case at least partly complementary to the samples. The dissociation curve of a perfectly complementary sequence may be used e.g. as reference. The difference in the integral of the dissociation curve to be analysed to the reference curve is a function of specificity and is used as the measure. Detectable marked target molecules are here hybridized to the samples and subsequently washed in stages, with the dissociation curve resulting from the signal intensity. The method may be used with all types of marked polynucleotides.

[0013] The publication Peplies et al., Applied and Environmental Microbiology (69), 3, pp. 1397-1407, 2003 describes a study which systematically investigates the applicability of arrays to questions of microbiological ecology. In order to decide which factors influence the specific recognition of sections of the 16SrRNA gene and lead to false positive and false negative results, the authors use twenty different catcher molecules of 15-20 bases in length, in which it is known that the 16SrRNA gene of different species differs. The target molecules are produced by amplification the 16SrRNA gene of six typical bacterial strains with the aid of marked gene-specific primers. The hybridization between the target molecules and the corresponding catcher molecules then takes place in different areas of the target molecules marked at the 5' end. The marking therefore has sharply varying distances from the catcher molecule in the different spots on the array. It should be located outside the hybridization areas (5' end base **8**: see *Materials and Methods*—preparation of fluorescently labelled target single-stranded DNA: "5'-indocarbocyanine-labelled forward primer 8*f*" and Table 1: "16SrRNA binding site and length").

[0014] U.S. Pat. No. 5,871,928 A describes methods of sequencing, fingerprinting and plotting biological macromolecules. Since the position and sequence of a probe on an array is known, sequencing of marked target molecules may be undertaken. In this, e.g. overlapping probe nucleotides of five bases in length are used. The marked target molecule binds to different probes and the sequence of the target molecule may be determined through overlapping of the probe sequence. The marking of the signal molecule is achieved by standard methods. The marked signal molecule may be fragmented to enhance the signal. The signal enhancement effect results from a higher concentration of marked hybridized fragments per probe sequence. A relatively long target molecule is also detectable with a relatively small number of markings per unit length since, on account of the length, many markings are available.

[0015] U.S. Pat. No. 6,027,889 A relates to a method for the detection of nucleic acid sequences through the coupling of ligase with PCR-reactions. Two target sequences, which bind next to one another at a sequence to be analysed and also contain an overhang, are ligated. After ligation, the overhangs are used in a PCR-reaction for the hybridization of a marked ZIP code primer. The amplified DNA may be analysed by various methods (gel filtration, arrays, etc.). The marking is introduced by means of PCR, with one primer carrying the marking and the other primer being linked to the hybridisable sequence, so that hybridization and marking are spatially separate from one another. Ligation reaction and PCR-reaction may be combined in different ways for various applications.

[0016] The problem of the present invention is therefore to provide a method for the analysis of samples by means of hybridization, in which even very small sample quantities may be detected more reliably and clearly than before, and in which the detected signals may be better correlated with one another than is the case with known methods.

[0017] The problem is solved by a method of analysing samples by means of a ligand binding method in which, through the binding of target sequences of target molecules to catcher sequences of probes, duplexes or complexes are generated and/or duplexes or complexes thus generated are analysed, wherein the target sequence is a partial sequence of the target molecule and the duplexes or complexes have at least one detectable marking or an accumulation of markings in proximity to and/or within the target sequence.

[0018] Surprisingly it has been found that the use of catcher sequences complementary to target sequences of the target molecules which have a detectable marking or an accumulation of markings in proximity to and/or within the target sequence lead, in methods according to the invention, to signal intensities considerably higher than those obtained in comparable methods using catcher sequences not selected according to the invention, so that the marking is not in proximity to the target sequence.

[0019] Preferably the detectable marking or the accumulation of markings is located only in proximity to and/or within the target sequence.

[0020] Target molecules may be marked with a single marking. In principle, though, it is also customary to provide target molecules with several markings. Here the accumulation of markings is to be provided in proximity to and/or within the target sequence.

[0021] For the purposes of the present invention, an accumulation of markings is understood to mean preferably the maximum accumulation of markings on the target molecule, to be found within an area which is not longer than the target sequence and permits a specific duplex formation. In principle it is possible for the target molecule to be provided with further markings or accumulations of markings which however do not always lie in areas which are specific for the target molecule and are therefore not suitable as target sequence.

[0022] Due to the fact that the target sequence is limited to areas specific to the target molecule, it is often not freely variable, for which reason according to the invention at least one marking is provided in proximity to or within an area

specific to the target molecule, and the catcher sequence is determined complementary to this area, and then represents the target sequence.

[0023] In a method according to the invention, several samples are analysed simultaneously by means of ligand binding, wherein all target molecules have either at least one detectable marking or an accumulation of markings in proximity to the relevant target sequence.

[0024] In another method according to the invention, all target molecules have the same number of markings.

[0025] In methods according to the invention, at least ten samples, preferably at least one hundred samples or at least a thousand samples are analysed simultaneously.

[0026] In methods according to the invention, the marking may be a fluorescent marking.

[0027] In methods according to the invention, the fluorescent marking is obtained by means of one or more of the following marking agents: Cy3, Cy5, fluorescein, Texas red, Alexa fluor dyes and other fluorescent dyes.

[0028] The problem is also solved by a method for the production of target molecules which have at least one marking only in proximity to the relevant target sequence or within the relevant target sequence, wherein the target sequences are partial sequences of the relevant target molecule.

[0029] Used in the invention are target molecules which comprise and/or have target sequences and a marking in proximity to or within the target sequence concerned.

[0030] These target molecules may be used in the methods according to the invention described above and lead to catcher-target sequence hybrids with a greater signal intensity than hybrids produced with the assistance of target molecules which have target sequences and a marking for the relevant target sequence which is not in proximity to the latter.

[0031] The marking of the target molecules may be effected by means of enzymatic end marking, reverse transcription, indirect marking and/or "sandwich" methods.

[0032] The problem is also solved by a method for the generation of target molecules involving a PCR method in which at least one primer provided with a marking agent is used.

[0033] In the method according to the invention, at least one further primer provided with an identical or another marking agent may be used. By this means, different types of signal may be provided, in order to verify results in methods according to the invention, but also to match to one another the intensity of different spots containing varying numbers of hybrids, so that the intensities lies in the same order of magnitude. The spot containing more hybrids may have for example the marking agent with the lower signal intensity. Since the signals may be distinguished from one another on the basis of different marking agents, and the factor by which the signal intensities differ from one another is known, such a method makes possible quantification and a better comparison of the intensities in both spots. This is of particular benefit when the use of a marking agent with a greater signal intensity would lead to a saturation value in the detection unit, for example a photo-multiplier. In such a case, quantification would be impossible or at least more difficult due to factors relating to the apparatus used.

[0034] If in a PCR-method, use is made of primers which are marked and dNTPs which have no markings, then the PCR-product is marked at the end formed by the primers, and unmarked at other points. If the primers are selected so that the target sequence of the PCR-product is in proximity to the primer, then the PCR-product represents a target molecule which has a marking according to the invention only in proximity to the target sequence. This is an extremely simple and uncomplicated method of obtaining target molecules according to the invention. By using defined primers of a specific length, it is possible to make a more accurate statement as to how much marking agent has been incorporated into each target molecule than if a mixture of marked and unmarked dNTPs is used to produce a marked PCR-product; for if individual dNTPs are marked it is not always clear how many marked and how many unmarked dNTPs are incorporated in a PCR-product, or else the PCR-products differ in respect of the marked nucleotides incorporated in them. This is also dependent on the particular sequence amplified. Usually one of the four bases to be incorporated is marked. If this appears less often in an amplification product, then the amplification product contains less marking agent than other amplification products. Because of this fact it is considerably more difficult to make quantitative statements on the basis of a micro-array experiment. With suitable choice of primer it is possible to ensure that each primer used in an experiment has roughly the same amount of marking agent. These amounts no longer vary on the basis of a target sequence to be amplified, but depend solely on the composition and form of the particular primer used. Apart from the enhanced signal intensities which improve considerably the sensitivity of micro-array experiments, methods according to the invention also represent, for the reasons given above, significantly more accurate methods than methods known to date from the prior art.

[0035] In a method according to the invention for the production of target molecules according to the invention, target molecules are generated by means of a PCR method in which at least one type of dNTP provided with a marking agent is used, and in which the marked dNTPs are incorporated in the target molecule in direct proximity to the target sequence and/or in the target sequence itself.

[0036] In contrast to the prior art, such a method according to the invention ensures that the marking agent is actually present in direct proximity to the target sequence. In this connection, the target sequence represents only a section or a partial sequence of the target molecule. The length of the target molecule is generally considerably more than that of the target sequence, and in particular 2, 3, 4 or 5 times the length of the target sequence. In target molecules of such length, a random distribution of markings leads to significantly lower signal intensities than is the case when the markings are positioned according to the invention. This is shown very impressively by the examples explained below. In this context, direct proximity to the target sequence means that marking agent is bound to nucleotides incorporated in the target sequence or to nucleotides incorporated adjacent to the target sequence. For the purposes of the invention, direct proximity to the target sequence means that the marking or the marking agent is no further than 100 or 60, and preferably no further than 0-20 bases distant from the target sequence. Significant positive effects (factors 2-146) may still be detected at a distance of 100 bases. Preferably the marking agent is located only in direct proximity to the target sequence.

[0037] Other methods according to the invention comprise the fluorescent marking of nucleic acids (DNA or RNA) by

means of other methods, e.g. by enzymatic end marking (e.g. using terminal transferase, polynucleotide kinase, poly(a)-polymerase or other enzymes or by reverse transcription with fluorescent-marked primers, or by indirect marking methods.

[0038] A further method according to the invention for the production of target molecules according to the invention is one in which the target molecules are generated by means of a PCR method and in which at least one type of dNTP provided with a marking agent is used, and in which the marked dNTPs or an accumulation thereof are incorporated in direct proximity to the target sequence and/or in the target sequence itself, and in which one or more primers is provided with the same or other marking agents .

[0039] Other methods according to the invention for the production of target molecules according to the invention are methods in which marking agents are incorporated in direct proximity to the target sequence and/or in the target sequence itself. Here many methods are possible, e.g. incorporation of marked dNTPs in cDNA through reverse transcription, or post-labelling protocols which make use of the incorporation of aminoallyl-nNTP in cDNA and then a chemical coupling with fluorescent dyes, or direct non-enzymatic marking of the RNA with fluorescent dyes.

[0040] Such a method leads to target molecules according to the invention which are still easily detected even with hybrids formed at very low concentrations since, due to the incorporation of marking agent in the target sequence, a comparably large amount of marking agent may be incorporated. At least with target molecules present in normal concentrations, the result in one spot may also be verified with the aid of the additional marking agent in the primer used.

[0041] The problem is also solved by target molecules, for use in a method according to the invention, which have a marking or an accumulation of markings in proximity to or within the relevant target sequence, with the target sequence forming a section or a partial sequence of the target molecule.

[0042] Target molecules according to the invention provided by methods according to the invention are preferred.

[0043] The problem is finally solved by a method according to the invention in which target molecules according to the invention are used, which also have been or may be produced by a method according to the invention for the production of such molecules.

[0044] A set of catcher molecules according to the invention for the implementation of methods according to the invention comprises:

[0045] a predetermined number of different catcher molecules comprising in each case different catcher sequences, each designed to from ligand bonds with target sequences of target molecules in such a way that they are complementary to the relevant sections of the target sequences to be found in proximity to a marking or an accumulation of markings.

[0046] A set of catcher molecules according to the invention has at least 10, 30, 50, 100, 1000 or 5000 different catcher sequences. Preferably all catcher molecules of the set of catcher molecules are designed so that they are complementary to the relevant sections of the target sequences which are in proximity to a marking or an accumulation of markings.

[0047] In a set according to the invention, the catcher sequences are constituents of the probes of a DNA array.

[0048] According to a method according to the invention, the catcher molecules are so determined and calculated that they are complementary to the relevant sections of the target sequences which are in proximity to a marking or an accumulation of markings.

[0049] Here a target sequence is defined as an area of the target molecule which is specific to the target molecule.

[0050] In one embodiment, the method according to the invention may be used for the identification of N_2-fixing organisms by means of sequence analysis. N_2-fixing organisms contain nitrogenases for the reduction of atmospheric nitrogen to ammonium, the coded genes of which are suitable for phylogenetic sequence analysis. For this purpose it is now possible to use diagnostic arrays containing catcher molecules according to the invention, which bind to regions of the nitrogenase gene which are specific for certain nitrogenase groups. Here the specificity of a catcher oligonucleotide may cover larger phylogenetic or other groups, and smaller groups such as e.g. species, types or individual strains.

[0051] Especially suitable for such a phylogenetic sequence analysis is the enzyme nitrogenase, in particular the subunit coded by the gene nifH (Hurek et al., 1997; Hurek et al., 2002). Through sequence analyses of nifH—including genes for alternative nitrogenase anfH and vnfH—it is possible to detect and identify nitrogen-fixing procaryotes without cultivation in environmental samples in DNA preparations. The subunit nifH, for example, is highly suitable for simultaneous amplification of all phylogenetically different nifH variants by means of PCR (Tan, Hurek, Reinhold-Hurek 2003) owing to the relatively high degree of conservation and the very well matured primer systems. Even the most active N_2-fixing bacteria may be detected if the analyses are made on the mRNA level (Hurek et al., 2002). The analysis of the diversity of nitrogenase genes may be significantly reduced, in comparison with sequencing, if diagnostic micro-arrays are used. Thus, the invention also covers the use of a diagnostic micro-array, with the catcher molecules binding to regions of nifH/anfH/vnfH genes which are specific for certain nitrogenase gene groups.

[0052] Suitable catcher molecules for this sequence analysis according to the invention may be obtained by firstly arranging (alignment) for known genes the nifH, anfH or vnfH gene areas which are amplified by the PCR primers used. Here the protein sequence is taken into account, i.e. the bases lie in a position which in each case codes the amino acid concerned. With the aid of this alignment and after creating a phylogenetic pedigree, it is possible from this to select as target sequences the sequences which are identical in a target gene group and may be delimited from all other known sequences, with the delimitation being effected through the presence of mismatch positions.

[0053] Preferably the target sequences are so selected that there are at least two mismatch positions, one of which may lie centrally in the target sequence which delimits the target group of nitrogenase genes from other nitrogenase genes, while at least one other mismatch position is to be found in a non-target sequence.

[0054] It is also advantageous for the target sequences to have a Tm value which is as similar as possible to the other target sequences, preferably 60-70° C., with 62-69° C. being especially preferred.

[0055] The sequences with reverse complementarity to the above are then selected as catcher sequences for the catcher molecules.

[0056] To obtain specific detection with high phylogenetic resolution, in particular at the species or type level, catcher molecules of up to 35 mer, preferably up to 30 mer with 17-25 mer being especially preferred, may be used. The short catcher molecules increase the rigorousness of the hybridization, making small sequence variations such as occur among different types and/or species detectable.

[0057] Examples of suitable catcher molecules (SEQ ID NO: 1-189) for the method of sequence analysis referred to above are listed in Table 1. The specificities of the catcher molecules (SEQ ID NO: 1-189) of Table 1 are to be found in FIGS. **13** to **18**. The catcher molecules of SEQ ID NO: 1-189 have been selected so that they bind as close as possible to the marked end of the target molecule and at the same time have the desired specificity (FIGS. **13** to **18**). Besides the oligonucleotides (SEQ ID NO: 1-189) listed in Table 1, according to the invention oligonucleotides are also suitable as catcher molecules if they differ in their length by one or more nucleotides from the oligonucleotides of SEQ ID NO: 1-189, or if their nucleotide sequence differs at one or more positions from the corresponding oligonucleotides of SEQ ID NO: 1-189, so long as they have the same specificity as the corresponding oligonucleotides of SEQ ID NO: 1-189, i.e. they bind at the same respective nitrogenase gene regions listed in FIGS. **13** to **18** as the corresponding oligonucleotide of Table 1.

[0058] To produce the arrays for sequence analysis according to the invention, nitrogenase gene-specific catcher molecules are immobilised on a matrix (slide).

PRECISE DESCRIPTION OF THE INVENTION

[0059] The invention is explained in detail below with the aid of the figures and a number of embodiments. The figures show in:

[0060] FIG. **1***a* a 362 bp long target molecule according to one of the embodiments, in schematic form

[0061] FIG. **1***b* a table listing oligonucleotides as used in the embodiments

[0062] FIG. **1***c* a table listing oligonucleotides as used in the embodiments as primers for amplification by means of PCR

[0063] FIG. **1***d* the model system in schematic form

[0064] FIG. **2***a* fluorescence signals of the hybridization of a Cy5-marked antisense strand with sense oligonucleotides

[0065] FIG. **2***b* a schematic representation of the hybrid molecules

[0066] FIG. **2***c* a graphical representation of determined signal intensities

[0067] FIG. **3***a* fluorescence signals of the hybridization of a Cy5-marked sense strand with antisense oligonucleotides

[0068] FIG. **3***b* a schematic representation of the hybrid molecules belonging to FIG. **3***a*

[0069] FIG. **3***c* a graphical representation of the signal intensities of the fluorescence signals of FIG. **3***a*

[0070] FIG. **4***a* fluorescence signals of a hybridization of a Cy3-marked sense strand with antisense nucleotides

[0071] FIG. **4***b* a schematic representation of the hybrid molecules of FIG. **4***a*

[0072] FIG. **4***c* a graphical representation of the signal intensities of the fluorescence signals of FIG. **4***a*

[0073] FIG. **5***a* fluorescence signals of the hybridization of a Cy3-marked antisense strand with sense oligonucleotides

[0074] FIG. **5***b* a schematic representation of the hybrid molecules which lead to the fluorescence signals in FIG. **5***a*

[0075] FIG. **5***c* a graphical representation of the signal intensities of the fluorescence signals of FIG. **5***a*

[0076] FIG. **6***a* fluorescence signals of a hybridization of a Cy3-marked sense strand with antisense nucleotides

[0077] FIG. **6***b* a schematic representation of the hybrid molecules which lead to the fluorescence signals of FIG. **6***a*

[0078] FIG. **6***c* a graphical representation of the signal intensities of the fluorescence signals of FIG. **6***a*

[0079] FIG. **7***a* a schematic representation of 50 mer oligonucleotides according to the embodiments

[0080] FIG. **7***b* a table of the 50 mer oligonucleotides of FIG. **7***a*

[0081] FIG. **8***a* fluorescence signals of a hybridization of a Cy3-marked sense strand with 50 mer antisense oligonucleotides

[0082] FIG. **8***b* a graphical representation of the signal intensities of the fluorescence signals of FIG. **8***a*

[0083] FIG. **9***a* fluorescence signals of a hybridization of a Cy3-marked sense strand with 50 mer sense oligonucleotides

[0084] FIG **9***b* a graphical representation of the signal intensities of the fluorescence signals of FIG. **9***a*

[0085] FIG. **10***a* fluorescence signals of a hybridization of a sense strand, marked with fluorescein-12-dUTP, with short antisense oligonucleotides, wherein the strands are not end-marked, but are uniformly marked internally through the incorporation of fluorescein-12-dUTP

[0086] FIG. **10***b* a graphical representation of the signal intensities of the fluorescence signals of FIG. **10***a*

[0087] FIG. **11***a* fluorescence signals of the hybridization of a Cy5-end-marked sense strand with short antisense oligonucleotides as in FIG. **3**

[0088] FIG. **11***b* a graphical representation of the signal intensities of the fluorescence signals of FIG. **11***a*

[0089] FIG. **12***a* fluorescence signals of the hybridization of a Cy5-end-marked sense strand, shortened at the 5' end, with short antisense oligonucleotides as in FIG. **11***a,* and

[0090] FIG. **12***b* a graphical representation of the signal intensities of the fluorescence signals of FIG. **12***a.*

[0091] FIG. **13** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes from bacteria of the alpha and beta sub-group of the proteobacteria. The names of the catcher molecules are shown next to the lines which indicate the sequence coverage. The precise sequence coverage is shown by symbols of various types. The nitrogenase genes (nifH, anfH, vnfH) from data banks are identified by their file number.

[0092] FIG. **14** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes from bacteria of the beta and gamma sub-group of the proteobacteria. The names of the catcher molecules and the sequence coverage are as shown in FIG. **13**.

[0093] FIG. **15** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes from bacteria of the omega sub-group of the proteobacteria. The names of the catcher molecules and the sequence coverage are as shown in FIG. **13**.

[0094] FIG. **16** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes

from frankia, cyanobacteria and similar bacteria. The names of the catcher molecules and the sequence coverage are as shown in FIG. **13**.

[0095] FIG. **17** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes of clusters II and IV. The names of the catcher molecules and the sequence coverage are as shown in FIG. **13**.

[0096] FIG. **18** shows the specificities of the catcher molecules of Table 1 which cluster with nitrogenase genes of cluster III. The names of the catcher molecules and the sequence coverage are as shown in FIG. **13**.

[0097] The invention is based on the surprising finding that the signal yield from fluorescent marked target molecules forming hybrids or duplexes with catcher sequences in a partial section is greater when the fluorescent marking lies close to the hybrid formed. Unexpectedly this effect is independent of strand and therefore of sequence.

[0098] Surprisingly the observed effect is also independent of the chemical nature of the fluorescent marking. The effect occurs with the use of both long (e.g. 50 mer) and short catcher oligonucleotides (e.g. 16-17 mer). Surprisingly this effect is also independent of the glass micro-array surface or coating used to immobilise the catcher oligonucleotides.

[0099] The invention may now be better explained with the aid of the following embodiments. The embodiments make it clear that the invention leads to a dramatic improvement in signal yield and in the informative value of micro-array hybridization experiments.

[0100] FIG. **1**D shows schematically the structure of a duplex or hybrid complex A created by a method according to the invention: at the surface B of a DNA array, for example a glass surface, a catcher sequence or a catcher oligonucleotide D is bound via a spacer C, for example a poly-adenin section of an oligonucleotide. Complexed to the former via a target sequence E is a target molecule F. As may be inferred from FIG. **1**D, the target sequence E is only a section or a partial sequence of the target molecule F. In the case of such target molecules with an overhang formed at the duplex, the markings may in principle be provided at any desired point and may also be positioned away from the duplex. Only in the case of such duplexes does the problem on which the invention is based occur, namely that the signal intensities measured, in particular with small sample quantities, are so unstable that no quantitative statement can be made.

[0101] A position **1** of the hybrid complex is designated G in FIG. **1**D. This is the first base of the hybrid complex A from catcher sequence D and target sequence E. At this point there is a fluorescent marking H. The schematically depicted hybrid complex thus represents an embodiment of the invention, in which the marking H is incorporated within the target sequence F of the target molecule E.

[0102] The invention may be better explained with the aid of the following embodiments.

[0103] The embodiments which follow show that the invention considerably improves signal intensity in micro-array experiments. This is shown with the aid of specific fluorescent markings. The embodiments demonstrate that the important factor is the position of the marking agent relative to the hybrid to be detected. Here it is completely irrelevant, what specific kind of marking agent is involved. All that matters is the position of the marking agent relative to the hybrid or duplex to be detected, while of course mixed effects involving other factors which may influence the efficiency of the hybridization (e.g. length of the oligonucleotide spacer, steric effects, effects of the secondary structure) occur. The following embodiments should therefore be understood only as explanatory examples; in particular the following embodiments and the experiment also explained do not restrict the teaching of the invention in respect of sequences to be detected, and markings or similar to be used.

Embodiments

[0104] The embodiments are parts of a typical experiment, which was set out as follows:

[0105] The target molecule in the typical experiment is a single-stranded DNA, 5'-end-marked or marked with fluorescein-12-dUTP by random labelling, and specifically in the fragment of the nitrogenase gene nifH (Hurek et al., 1995) from the bacterium *Azoarcus* sp. strain BH72. The fragment was amplified by means of PCR by the primers Zehr-nifH from chromosomal DNA of the strain BH72 (Hurek et al., 2002), with one of the primers being marked with Cy3/Cy5, and the other with biotin. Fluorescent marked single-stranded DNA could thus be isolated from the PCR-product. In connection with the use of fluorescein-12-dUTP for random labelling, only biotinylated primer was used. For all experiments, the primers had the sequences listed in FIG. **1**C, primers Z-nifH-f and Z-nifH-r (Zehr and McReynolds, 1989), up to experiments in connection with sequences (Zehr and McReynolds, 1989) following FIG. **11** (primers Z-nifH-f-BH72-Cy5 and Z307-nifH-r-BH72-biotin) and FIG. **12** (primer Z114-nifH-f-BH72-Cy5 and Z307-nifH-r-BH72-biotin):

[0106] Biotin-marked strands were separated (Niemayer et al., 1999) by means of streptavadin-coated paramagnetic spheres (Roche). The concentration of the remaining single-stranded DNA was determined by spectral photometry. Before each hybridization, the single-stranded DNA was denatured for 10 minutes at 95° C. and then incubated on ice for at least three minutes.

[0107] The oligonucleotides used in this experiment and acting as catcher molecules all bind to the nifH gene fragment of the strain BH72 referred to above. The relevant sequences and their characteristics are set out in table 1, FIG. **1***b* and table 2, FIG. **7***b*. A schematic representation of a possible duplex after hybridization is shown in FIG. **1**D. Oligonucleotides with either 5' amino-modifications (amino link c6) or 3' modifications, some with poly-A spacers of 6-12 nucleotides, were synthesised by the company Thermoelectron (Ulm, Germany).

[0108] To conduct hybridization experiments, DNA micro-arrays were created on standard microscopic glass slides made by Menzel of Braunschweig, Germany. Chemicals and solvents came from the company Fluka (Neu-Ulm, Germany). To create the micro-arrays, the glass substrates were cleaned, silylated and activated, as described by Bentas et al (2002). The activated surfaces were used directly for the immobilisation of either 5' or 3' amino-modified catcher oligonucleotides by means of covalent binding.

[0109] The application of the probes to slide surfaces activated in this way was made using a piezo-driven Spotter Robodrop BIAS, Bremen, Germany) or else a MicroGrid II Compact 400 from the firm of BioRobotics, United Kingdom. The concentration of the oligonucleotides was around 10 μm per ml water. The water used contained 1% glycerol.

In each spot of the micro-array approx. 250 pl was applied, corresponding to a spot diameter of around 200 μm.

[0110] The slides were incubated overnight at room temperature in a water-saturated atmosphere, in order to effect the covalent binding. Blocking of the micro-arrays was effected by means of 6-amino-1-hexanol (50 mM) and diisopropylethylamine (150 mM) in dimethyl formit after Beier et al (1999). The slides were then washed with deionised, particle-free water, air-dried and stored under N_2 at 4° C.

[0111] The hybridization of the target molecules to the probe of the micro-arrays, and washing, took place in a Personal Hyb oven of the company Stratagene, United States of America. Hybridization lasted for 1-16 hours. Unless otherwise stated, hybridization took place at room temperature with 50% formamide, at 46° C. with 50% formamide, and 10 nM single-stranded DNA was used in the process. The hybridization buffer used contained 4×SET, 10×Denhardt's. During hybridization, the slide was covered by a cover glass. After hybridization, washing took place with 2×SET (0.1% SDS) for 5 min. and 1×SET (0.1% SDS) for 10 min. at room temperature, or with 1×(0.1% SDS) for 5 min. and 0.1×SET (0.1% SDS) for 10 min. at 46° C. The dried micro-arrays were analysed at a resolution of 10 μm by a GenePix 4000 Micro-array Scanner from Avon, Union City, Calif., at constant laser strength and constant photomultiplier sensitivity. For this reason the signal intensities determined in the respective embodiments may be compared.

Embodiment 1

[0112] The reverse complementary strand or antisense strand of the nifH gene fragment of strain BH72 referred to above was hybridised with the sense oligonucleotides (catcher molecules) S**307** (**6**A), S**114** (**6**A) and S**20** (**6**A). The antisense strand is shown schematically in FIG. **1**A. The Cy5-marking was introduced into the strand by using a Cy5-marked primer to generate the strand. Shown schematically in FIG. **1**A are the binding points of the sense oligonucleotide to the antisense strand, with the respective distance of these binding points from the 3' and 5' end respectively of the antisense strand also being shown. The antisense strand represents the target molecule, and the sense oligonucleotides represent the catcher oligonucleotides and the catcher sequences, which have been applied to a micro-arrays in the form of probes. The hybridization of these probes by the target molecule leads to different signal intensities in the respective spots on the micro-array, as shown in FIG. **2**A. From left to right these spots contain, in each case in pairs, catcher oligonucleotides with the sequence S**307** (**6**A), S**114** (**6**A) and S**20** (**6**A). The antisense strand target molecule is marked only at the 5' end. Closest to the 5' end is the binding point and target sequence S**307**(**6**A). Hybrids formed at this point are detected in the first two spots shown in FIG. **2**A. There the signal intensity is highest. Further removed from the marking is the sequence section S**114**(**6**A), which is detected in the next two spots in FIG. **2**A. Here the signal is noticeably weaker. Somewhat stronger, but still weak, is the signal given by hybrids into which the binding point S**20**(**6**A) enters.

[0113] Shown schematically in FIG. **2**B are the catcher oligonucleotides **1**, **2** and **3**, together with the target molecule **4** in the form of hybrid molecules comprised of **1** and **4**, **2** and **4**, and **3** and **4**, and the resultant position of the marking **5** on the target molecule **4** in the respective hybrid. In this case catcher oligonucleotide **1** corresponds to S**307** (**6**A), catcher molecule **2** to S**114** (**6**A) and catcher molecule **3** to S**20**(**6**A).

[0114] The corresponding signal intensities are shown graphically in FIG. **2**C. It may be clearly seen that the use of the catcher oligonucleotide S**307**(**6**A) or **1** leads to hybrids in which the marking is in close proximity to the hybrid, so that a 3 to 7 times greater signal intensity is obtained as compared to the other hybrids represented. It is also evident that there is an especially large reduction in signal yield when the fragment to be hybridised is bound to the catcher nucleic acid (S**114**(**6**A))at a great distance from the marked primer.

Embodiment 2

[0115] This embodiment shows that the effect described in embodiment 1 is independent of strand and therefore of sequence. Cy5-marked counter-strands (sense strand) were hybridised with the corresponding antisense oligonucleotides. The same effect was observed as in example 1 (cf. FIGS. **3**A-**3**C). Shown in FIG. **3**A from left to right in each case are four spots with identical catcher oligonucleotides, namely from left to right spots with the catcher oligonucleotides A**20**(**12**A), A**20**(**6**A), R**20**(**6***a*)**3'**, A**307**, A**307**(**12**A), A**307**(**6**A), A**307**(**6**A)**3'**, and A**114**(**6**A). Shown schematically in FIG. **3**B in the same sequence is how the target molecule **7**, which has a marking **8** at its end, binds to the relevant oligonucleotide, and the resultant location of the marking relative to the hybrid **9** formed is discernible in FIG. **3**B.

[0116] As may be seen with the aid of the graphical representation of the signal intensities in FIG. **3**C, but is also evident from the brightness of the spots in FIG. **3**A, the signal intensities are always at their highest when the marking is in proximity to the respective hybrid formed, and specifically in comparison with the other hybrids by twice to around 4.5 times, in extreme cases well above this level (cf. the values for A**307**(**6**A)**3'** in FIG. **3**C). The designation **6**A or **12**A (of the catcher oligonucleotides) denotes the length of the respective spacer. This embodiment shows that the difference in length of the spacer, i.e. the spacer between the glass surface of a micro-array and the binding zone of the catcher oligonucleotide, has a comparatively limited effect on signal intensity. Consequently the difference between A**20**(**6**A) and A**20**(**12**A) is not especially marked, while between A**307**(**12**A) and A**307**(**6**A) there is virtually no difference at all. A slight reduction of the signal for A**20**(**6**A)**3'** in comparison with A**20**(**6**A) could be due to quenching effects from the close proximity of the fluorescent dye to the glass surface.

[0117] In this connection it should be noted that, in micro-array hybridization experiments, false negative results may occur due to a marking lying in an unfavourable position leading to an excessively low signal intensity, as for example in the case of the catcher oligonucleotide A**307**(**6**A)**3'**. Similar steric effects of hybridization have been described by Peplies et al. (2003). This oligonucleotide supplies a

signal intensity which is 48 times less than that of A**20**(**6**A). The adverse position is far removed from the target sequence.

Embodiment 3

[0118] This embodiment shows that the effect observed in the preceding embodiments may be even further strengthened by greater proximity of the marking to the target sequence. For this purpose an antisense catcher oligonucleotide was used, A**1** (**6**A) in FIG. **11**, which is complementary to the primer oligonucleotide with which the end-marked probe was generated by means of PCR. As a result, the fluorescent marking is already positioned at the first nucleotide of the heteroduplex which is formed in hybridization. In this embodiment the fluorescence signal is increased by a factor of 2.3 as compared with a catcher oligonucleotide, A**20** (**6**A), which is shifted by 20 nucleotides. As is evident from FIGS. **11**A and **11**B, for the intermediate positions (A**4** (**6**A)-A**14** (**6**A)), the signal strengths are progressively weaker than for A**1** (**6**A). In comparison with the most unfavourable position from embodiment 2, A**114** (**6**A), a signal which is as much as 146 times stronger is observed for the most advantageous position A**1** (**6**A). An even further removed but still relatively central position A**188** (**6**A) leads to an even lower signal intensity (factor **357**). The embodiment confirms that relatively high signal yields may be obtained when the marking is less than 64 nucleotides from the target sequence forming the heteroduplex; in this embodiment the signal is 15 times weaker.

[0119] The positive effect of position is also made clear in FIG. **12**. If a shortened marked probe is used (here by 113 nucleotides, so that the primer used for the PCR is complementary to the catcher oligonucleotide A**114**), a similar position effect is revealed for other catcher oligonucleotides. Oligonucleotide A**114** (**6**A), which in FIG. **11** with a central position gave only low signal strength levels (130 rel. intensity), shows in FIG. **12** as end-placed catcher oligonucleotide high signal strength (11,800 rel. intensity). In this example too, a sharp drop in signal intensity (factor 5.2) is to be observed when the marking is at a greater distance (e.g. 74 nucleotides for A **188** (A**6**), FIG. **12**A, B) from the formed hybrid.

Embodiment 4

[0120] This embodiment shows that the effect observed in the preceding embodiments is independent of the chemical nature of the marking. The same experiments as in embodiment 2 were conducted with a Cy3-marked sense strand instead of a Cy5-marked sense strand, and supplied substantially the same results as described in example 2. These results are set out in FIGS. **4**A-**4**C, in which the markings in FIG. **4**B are provided with reference number **10**, and the accordingly marked target molecule with reference number **11**. Shown from left to right in FIG. **4**A in each case are four spots in which catcher oligonucleotides as described in embodiment 2 are present. FIG. **4**B shows from left to right in each case schematically how the respective catcher oligonucleotide is hybridised with the target molecule, and in which position the marking is to be found in each case, relative to the hybrid formed. FIG. **4**C shows the signal intensities of the spots shown in FIG. **4**A, in the same sequence.

[0121] As already indicated, the results correspond substantially to those discussed in embodiment 2. The fact that a different marking leads to substantially the same results confirms that it does not matter what type of fluorescent marking is used in implementing the invention.

Embodiment 5

[0122] The experiment described in embodiment 1 was repeated with Cy3-marked sense strand and Cy3-marked antisense strand. The results in the case of the Cy3-marked sense strand are shown in FIGS. **5**A-**5**C. The results of the experiment conducted with the Cy3-marked antisense strand are depicted in FIGS. **6**A-**6**C. In each case the signal intensity is greatest where the marking **8** is in direct proximity to the respectively formed hybrid **9**. When the Cy3-marked sense strand is used as target molecule this is the case in the spot with the catcher sequence for S**307** (**6**A), and for the Cy3-marked antisense strand it is in the spot with the catcher sequence for A**20**(**6**A) (see FIGS. **5**A-**5**C, FIGS. **6**A-**6**C).

[0123] These experiments comparable with embodiment 1 confirm that both the Cy3-marked sense strand and also the Cy3-marked antisense strand are detectable with high signal yields when the hybrids formed with catcher sequences have the marking in direct proximity. In the case of probes A**20**(**6**A) or S**307**(**6**A), signal intensity is increased by a factor of 22 as compared with other hybrids.

Embodiment 6

[0124] This embodiment confirms that the invention also functions when longer oligonucleotides are used as catcher molecules. In this case 50 mer oligonucleotides were used, binding in each case at the outer ends of the target molecule. Cy3-marked sense strand shows the stronger signal when the marking lies close to the duplex (A**19**-**68**). In this case signal intensity was increased by a factor of 2 over the other signals.

[0125] The designation and sequences of the 50 mer oligonucleotide catchers and target sequences used may be taken from table 2 in FIG. **7**B. The position of the relevant binding points on the sense or antisense strand of the 362 bp long nifH gene fragment are shown schematically in FIG. **7**A.

[0126] Shown in FIG. **8**A are the spots of a corresponding micro-array, with in each case six identical spots in one row. The target sequences or catcher sequences detectable in the respective spots are plotted on the right-hand side of the represented micro-array surface in the representation of FIG. **8**A. In FIG. **8**B, the correspondingly determined signal intensities are shown in the form of a graphical presentation.

[0127] The same experiment was conducted with Cy3-marked antisense strand. In this case too, the strongest signal is obtained when the marking is close to the duplex (S**289**-**338**), see FIGS. **9**A, **9**B. Here too the signal intensity with use of a method according to the invention was increased by a factor of 2 over other signal intensities, at any rate distinctly improved, as shown by FIG. **9***b* for the sequence S**289**-**338**.

Embodiment 7

[0128] This embodiment confirms that other marking strategies may also be used to produce target molecules. Using unmarked PCR primer and the random incorporation

of fluorescein-12-dUTP ("random labelling"), a suitably marked sense strand was created, and hybridised with short antisense oligonucleotides. On the left of FIG. **10**A, in a grid comprised of rows and columns, in each case four probe spots or spots are shown. The rows are numbered consecutively from I to IV, and the columns are designated a-e. Next to this on the right is a schematic reproduction of the same grid, with the designation of the catcher oligonucleotides located in the respective spots entered in the corresponding grid areas. Thus for example field IIa contains spots with the catcher oligonucleotides A**307**(**6**A) etc. In FIG. **10**E the signal intensities determined for the respective spots are presented graphically. The highest are the signal intensities in spot A**20**(**6**A)**3'**, followed by spot A**20**(**6**A) and A**20** (**12**A).

[0129] Table I in FIG. **1**B discloses that the catcher oligonucleotide A**20**(**6**A)**3'** binds at four positions to T and U respectively, whereas the catcher oligonucleotide **307** binds to T and U respectively at **0** positions, so that there is a higher incorporation rate of fluorescent marked nucleotides in the A**20** target sequence. Through fluorescent marking on these bases there is in consequence a greater fluorescent intensity in the duplex A **20** than at A**307**, resulting in a noticeably higher signal intensity (see FIG. **10**B).

[0130] In addition to the glass slides described above, commercial supports for micro-arrays were also used, for example aldehyde slides and amine slides, plus slides from the company Genetics, QMT® aldehyde slides from Peqlab, and Pan ® amine slides from MWG Biotech. With these micro-arrays the same results were obtained as described above with the aid of embodiments 1-7.

[0131] This confirms that the present invention may be used in conjunction with any type of micro-array.

[0132] It has been shown above, with the aid of target sequence marked according to the invention and applied to DNA arrays in solution, that the invention is independent of strand and therefore of sequence.

[0133] Within the scope of the invention, this procedure may easily be reversed, i.e. target molecules to be analysed may be provided on an array, to which catcher molecules in solution and marked according to the invention are added, so that duplexes or complexes with high signal intensities are created. In other words, the term "target molecule" is to be understood as being interchangeable with the term "catcher molecule" and vice-versa. At the same time the term "target sequence" should then be understood as being interchangeable with the term "catcher sequence" and vice-versa.

[0134] Also, within the scope of the invention, the entire ligand binding reaction may in principle be effected in solution.

TABLE 1

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
Proteo-1	1	GACAAGATGGTGTCCTGAG	67	19	52	29-47
Proteo-2	2	GACAGGATGGTGTCCTG	66	17	58	31-47
Proteo-3	3	GGCTCAGGATGGTGTCC	68	17	64	33-49
Proteo-4	4	AGGACGGTGTCCTGGG	68	16	68	29-44
Proteo-5	5	TTCCGCGGCAAGGGAGA	68	17	64	44-60
Proteo-6	6	GTGTCCTGCAGCTTGGT	66	17	58	22-38
Proteo-7	7	AGCCGATCTTGAGAACGTC	67	19	52	91-109
Proteo-8	8	AGCCGATCTGCAGCACGT	69	18	61	92-109
Proteo-9	9	CAGTTCCATCACGGAGTTC	67	19	52	33-51
Proteo-10	10	TGCATGACGCTGGTTTGC	67	18	55	30-47
Proteo-11	11	TACCCGATGGCCAGCACAT	69	19	57	92-110
Proteo-12	12	ATGACGGTGTTCTGCATCTT	66	20	45	25-44
Proteo-13	13	CACGGTCGTTTGCGCCTT	69	18	61	25-42
Proteo-14	14	TCTGAGCCTTTGAGTGCAG	67	19	52	16-34
Proteo-15	15	TTGATGTCGCCGTAGCCG	69	18	61	105-122
Proteo-16	16	TTTAATGCCGCTGTAACCAGT	66	21	42	103-123
Proteo-17	17	CCAGTCAGTAGTACATCTTCTA	66	22	40	86-107
Proteo-18	18	TTTTGCGCTTTCGCATGCAG	68	20	50	16-35

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
Proteo-19	19	CTTGCTGTGGAGGATCAG	67	18	55	10-27
Proteo-20	20	CTCCATTATTGTGTTTTGCGC	66	21	42	28-48
Proteo-21	21	GTGTTTTGCGCTTTAGAGTG	66	20	45	19-38
Proteo-22	22	GGAAGTTAATCGCTGTGATAAC	66	20	40	175-196
Proteo-23	23	ATGGTCTCCTGGGCCTT	66	17	58	25-41
Proteo-24	24	CACTTGACGTCGCCGTA	66	17	58	109-125
Proteo-25	25	TTCCAAATCTTCAACGCTACC	66	21	42	64-84
Proteo-26	26	GTGACAGCACCGACGTC	67	18	64	33-49
Proteo-27	27	ACGGTCTGCTGGGCTTT	66	17	58	25-41
Proteo-28	28	ATGCAGGACCGTGTCCTG	69	18	61	31-48
Proteo-29	29	AGATGCAGAACCGTGTCCT	67	19	52	32-50
Proteo-30	30	GAACCTTCGGTTGCCGC	68	17	64	52-68
Proteo-31	31	GCGGCGAGATGCAGAAC	68	17	64	40-56
Proteo-32	32	ATCCAGGACGGTATCCTG	67	18	55	31-48
Proteo-33	33	GATGTCCTGATAGCCGAC	67	18	55	103-120
Proteo-34	34	TTGATGTCCTTGAAGCCGA	65	19	47	104-122
Proteo-35	35	GCCTGATTCAACACAACGAAC	68	21	47	118-138
Proteo-36	36	CCAGCGAAAGGACGGTG	68	17	64	36-52
Proteo-37	37	AATATCTTTAAAACCGGCTTTCAG	66	24	33	97-120
Proteo-38	38	GTGTCCTGCATCTTGGTG	67	18	55	21-38
Proteo-39	39	GGTGTTTTGCATTTTAGTGTG	64	21	38	19-39
Proteo-40	40	CCAGCGAGAGGATGGTG	68	17	64	36-52
Proteo-41	41	CTTGGCATGCAGCATCAG	67	18	55	10-27
Proteo-42	42	ATGCAGAATCAGTCGAGTAGA	66	21	42	1-21
Proteo-43	43	GTGTTCTGTGCTTTAGAGTGAAG	69	23	43	16-38
Proteo-44	44	GTGCATTACAGTAGTCTG	62	18	44	31-48
Proteo-45	45	GTAGCCAGCCTTCAGCAC	69	18	61	94-111
Proteo-46	46	AGGCTGAGGATCGTGTCC	69	18	61	33-50
Proteo-47	47	CAGCAGCAAGGTGCAGC	68	17	64	42-58
Proteo-48	48	GCCATCTCCATAATGGTGT	65	19	47	35-53
Proteo-49	49	CCTTGCAGCCGAGGATC	68	17	64	12-28
Proteo-50	50	AGGCTGAGGATGGTGTC	66	17	58	34-50
Proteo-51	51	CTCGAGGTCTTCGACGCT	69	18	61	67-84
Proteo-52	52	CTCGCGGCAAGACTCAAA	67	18	55	42-59

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
Proteo-53	53	CTCGCTGCAAGGCTCAAA	67	18	55	42-59
Proteo-54	54	CGTGTAGGATCAGCCGTGT	69	19	57	4-22
Proteo-55	55	AGACTAAGCACGGTGTCTTG	68	20	50	31-50
Omega-1	56	TGTCTGAGCCTTGGCATGA	67	19	52	18-36
Omega-2	57	CTTCATAACGTCCTTCAGTTC	66	21	42	79-99
Omega-3	58	GGTCCATAACCGTTGACTG	67	19	52	31-49
Omega-4	59	CCATTACCGTGTTCTGTGC	67	19	52	28-46
Omega-5	60	GGTGTCGCCATAGCCGA	68	17	64	104-120
Omega-6	61	CAACCTTCATCACGGATTCG	68	20	50	87-106
Omega-7	62	CGGATGAGGTCCATCAC	66	17	58	40-56
Omega-8	63	GAACCTTGTCCATAACCGT	64	19	47	37-55
Omega-9	64	CTCGCGGATCAGGTCCAT	69	18	61	43-60
Omega-10	65	GACCTTCATGACATCTTCCAG	68	21	47	85-105
omega-11	66	ACGTCCGAGAGCTCCAGA	69	18	61	78-95
Cyano-1	67	CAGTGGTTTGTGCTTTACA	63	19	42	22-40
Cyano-2	68	AGTGAAGAACTGTTACCTGTGC	68	22	45	28-49
Cyano-3	69	GTTTGAGCTTTAGCGTTTAAGATTA	66	25	32	11-35
Cyano-4	70	AAACCGGTCAACATTACTTCGTG	69	23	43	88-110
Cyano-5	71	GCACCTGGTTTACATACATC	66	20	45	91-110
Cyano-6	72	GCTTAGTACGGTTGTTTGTG	66	20	45	29-48
Cyano-7	73	TCTACACATCGGATACCTTTGTA	67	23	39	109-131
Cyano-8	74	CTGCACCTTTTTCAGCAGC	67	19	52	52-70
Cyano-9	75	TACAGTGGAGCATTAAGCGAG	68	21	47	5-25
Cyano-10	76	CAGCCAAGTGAAGAACGGT	67	19	52	37-55
Cyano-11	77	CACTTAACGCCGCGATAGC	69	19	57	107-125
Cyano-12	78	ATTACTTCTTCGAGTTCAATATCTT	65	25	28	74-98
Cyano-13	79	ACGAACGTTACGGAAACC	64	18	50	106-123
Cyano-14	80	CTAAGTGGAGAATGGTAGTTTG	66	22	40	31-52
Cyano-15	81	GGTGAAGAATCGTGGTTTG	65	19	47	31-49
Cyano-16	82	GGTGTAGAATTGTGGTTTGG	66	20	45	30-49
Cyano-17	83	AGCTAGGTGCAGAACTGTG	67	19	52	36-54
Cyano-18	84	GCAGCCAAGGAAAGAACG	67	18	55	39-56
Cyano-19	85	CTTCACACCACGGAAGCC	69	18	61	106-123
Cyano-20	86	CGAGCAATACTTCCGAGAGT	68	20	50	84-103

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
Cyano-21	87	ACGTTGTTATAGCCAGTGAGTA	66	22	40	98-119
Cyano-22	88	GTGCAGAATGGTGGTTTG	64	18	50	31-48
Cyano-23	89	CAGCAGCCAATTGGAGAAC	67	19	52	40-58
Cyano-24	90	CTTTTCTGCTGCCAGGTG	67	18	55	46-63
Cyano-25	91	AGCTTTACAGTGAAGAATCAAGC	67	23	39	8-30
Cyano-26	92	GAGCTTCATCAAGTTCCAC	65	19	47	79-97
Cyano-27	93	CTGGATGCCAGCGTAGC	68	17	64	107-123
Frankia-1	94	ATGCCCCACTGGCCCTC	71	17	70	103-119
Frankia-2	95	GCCTTCTCGATGACGGTG	69	18	61	36-53
ClusterII-1	96	CCCAGAATCATGCGGGTA	67	18	55	3-20
ClusterII-2	97	GTTCATTCCGCCTAAAATCATAC	67	23	39	8-30
ClusterII-3	98	GTTTTGATGACCTTCTCGTTG	66	21	42	81-101
ClusterII-4	99	ACATCCATCAGGGTTTCCTG	68	20	50	31-50
ClusterII-5	100	TTGATTCATCCCTCCCAAAA	64	20	40	14-33
ClusterII-6	101	TATCCATCAATGTTTCTTGCGG	66	22	40	28-49
ClusterII-7	102	CCATCATGGTGGTCTGCA	67	18	55	29-46
ClusterII-8	103	GCATTTTACCTCTAAGAATCATAC	66	24	33	8-31
ClusterII-9	104	GTTTTCCGTGGAGAATCATTC	66	21	42	8-28
ClusterII-10	105	CCGTGCAAGATTAACCTTG	65	19	47	5-23
ClusterII-11	106	GTTGTCTGCATTTTACCGC	65	19	47	20-38
ClusterII-12	107	GGTCTCCTCAGGTTTGC	66	17	58	23-39
ClusterII-13	108	ATCACCGTCTGCTGCGG	68	17	64	28-44
ClusterII-14	109	CCAGGATCAGCCGATTTGA	67	19	52	1-19
ClusterII-15	110	CACCAAGGATAAGACGAGTT	66	20	45	3-22
ClusterII-16	111	CGAGCACAAGACGAGTACA	67	19	52	1-19
ClusterIII-1	112	CCTTCTTCGCGGAGCGT	68	17	64	49-65
ClusterIII-2	113	ACTCGGTGCACCGGCAA	68	17	64	111-127
ClusterIII-3	114	CCTTCCTCGCGGAGTGT	68	17	64	49-65
ClusterIII-4	115	GAAGTGATTATTCCTCTTCC	64	20	40	160-179
ClusterIII-5	116	CGCCTAAGAGAAGACGAGT	67	19	52	4-22
ClusterIII-6	117	TCGTCGCGAAGGGTATCCA	69	19	57	44-62
ClusterIII-7	118	CCGCCTTTACGGATATC	63	17	52	85-101
ClusterIII-8	119	CCGCAAGGTCCACGTC	68	16	68	70-85
ClusterIII-9'	120	CTTCCCGGCGGATGTC	68	16	68	85-100

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
ClusterIII-10	121	GCAGCGTATCCAATACGGT	67	19	52	37-55
ClusterIII-11	122	GTCTTCCCCTTCCGACC	68	17	64	56-72
ClusterIII-12	123	TCTTCCAGATCCACGTCTTC	68	20	50	67-86
ClusterIII-13'	124	CTTTTCTGCGCAAGACCAC	67	19	52	20-38
ClusterIII-14	125	ACCCTGTCCGGCGGATA	68	17	64	87-103
ClusterIII-15	126	GGGTATCCAGAACGGACTT	67	19	52	34-52
ClusterIII-16	127	ACGGTCCGTTGACTCAAAC	67	19	52	23-41
ClusterIII-17	128	GAGCCAAGCCATGCAACA	67	18	55	14-31
ClusterIII-18	129	GTATCTAACACACTCTTTTGGT	65	22	36	29-50
ClusterIII-19	130	GTTCTGAGTGTATCCAACAC	66	20	45	40-59
ClusterIII-20	131	CCAAGGAGAAGACGGGTG	69	18	61	3-20
ClusterIII-21	132	GTCCAAGACGGTGCTTTG	67	18	55	31-48
ClusterIII-22	133	TCGAGAAGATTGATGGAGG	65	19	47	176-194
ClusterIII-23	134	TATCCAGGACAGTGCGCT	67	18	55	32-49
ClusterIII-24	135	TCCAAAACGGTCCGCTG	66	17	58	31-47
ClusterIII-25	136	CCGAAGCCCTGTTTGCG	68	17	64	91-107
ClusterIII-26	137	AACCGGGTTTACGGATATC	65	19	47	85-103
ClusterIII-27	138	AAAGCCGGGCGACACGA	68	17	64	89-105
ClusterIII-28	139	CACCTAAAAGTAGACGTGTTGA	66	22	40	1-22
ClusterIII-29	140	CCAACAACAATCGGGTTGA	65	15	47	1-19
ClusterIII-30	141	CACAGCGAACACCTTTAAAAC	66	21	42	101-121
ClusterIII-31	142	CGGTTTTCTGCTGAAGACC	67	19	52	22-40
ClusterIII-32	143	GTCTTTTGTGCTAAACCACC	66	20	45	19-38
ClusterIII-33	144	CACCTTCCGCGAGTGTAT	67	18	55	47-64
ClusterIII-34	145	AGTACGGTTTTCTGGGCTAA	66	20	45	25-44
ClusterIII-35	146	CTCCCAATAAGAGACGGG	67	18	55	5-22
ClusterIII-36	147	GTATCCTACCTTCAGAACCG	68	20	50	86-105
ClusterIII-37	148	TCAATGTCATCGCCTTCTTC	66	20	45	58-77
ClusterIII-38	149	ATGCCGGAAAAGCCGGG	68	17	64	97-113
ClusterIII-39	150	CCAGCTCTATGTCGTCGC	69	18	61	65-82
ClusterIII-40	151	GTCTTCTGGTTCAGGCC	66	17	58	22-38
ClusterIII-41	152	CGTATCAAGCACCGTTTTC	65	19	47	33-51
ClusterIII-42	153	CAAAATGGCATCCAATTCAATTTC	66	24	33	70-93
ClusterIII-43	154	CATGATACGGTCGAGTTCAAC	68	21	47	73-93

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
ClusterIII-44	155	CTGAGCTAATCCTCCTAAAAG	66	21	42	13-33
ClusterIII-45	156	GGTGAAGACCACCAAGAAG	67	19	52	13-31
ClusterIII-46	157	TGCTGGAGTCCTCCCAAC	69	18	61	15-32
ClusterIII-47	158	CAAGTTTTCGCACATCATCCAG	68	22	45	79-99
ClusterIII-48	159	GCCGAGCTTGATGATGTC	67	18	55	85-102
ClusterIII-49	160	CGACTTTTCTAACGTCTTCAAG	66	22	40	79-100
ClusterIII-50	161	GTACGGTCTTTTGGCTCAAAC	68	21	47	23-43
ClusterIII-51	162	CGGTTCGCAATGTATCCAG	67	19	52	43-61
ClusterIII-52	163	CAGGCCGTTCAGCAAAAG	67	18	55	10-27
ClusterIII-53	164	GCCTCCCAGAAGCAAAC	66	17	58	8-24
ClusterIV-1	165	CCCTGATGGCGTCAAGC	68	17	64	42-58
ClusterIV-2	166	AGTACCGTTTCCTGGTGCA	67	19	52	26-44
ClusterIV-3	167	CGGTTCGGTTTTGCCGTC	69	18	61	58-75
ClusterIV-4	168	CCTCTGAGAAGGGTTAT	61	17	47	7-23
ClusterIV-5	169	GATGCCTTTGTAGCCGGT	67	18	55	109-126
ClusterIV-6	170	CTGCGTGTTGTCCCGTAT	67	18	55	52-69
ClusterIV-7	171	TTCTGCAAGGATCCGCGT	67	18	55	4-21
ClusterIV-8	172	CACCGCGGTGATGATCC	68	17	64	164-180
ClusterIV-9	173	TCATGGGCATTGACCG	63	16	56	68-83
ClusterIV-10	174	CCGTCCCTCATGCTGTC	68	17	64	46-62
ClusterIV-11	175	TTGCCGCCTGTCAGGTT	66	17	58	10-26
ClusterIV-12	176	CCTCCGCTTTCAACACAT	64	18	50	114-131
ClusterIV-13	177	GCCTCTCACCATAGAGTG	67	18	55	14-31
ClusterIV-14	178	CAATATCAAGGATAGTAGGCAATC	67	24	37	29-52
ClusterIV-15	179	CTTTCCGTGCATTAATGTCC	66	20	45	8-27
ClusterIV-16	180	AATCCTACGTCCAGCGAG	67	18	55	13-30
ClusterIV-17	181	CTGTATTTTGTGTCCGCATAC	66	21	42	13-33
ClusterIV-18	182	ACCGTGGGGATCTTCCTC	69	18	61	24-41
ClusterIV-19	183	ACTGTGGGAATGTCAGCC	67	18	55	24-41
ClusterIV-20	184	TAATATCCTGACCCTTTTGC	64	20	40	57-76
ClusterIV-21	185	AGGTAATCCAGTACCGT	61	17	47	37-53
ClusterIV-22	186	CCGTGCAACAACAGTTTC	64	18	50	6-23
ClusterIV-23	187	GCCAAGGCTTTCCAGCAT	67	18	55	187-204

TABLE 1-continued

Sequences of catcher oligonucleotides for nitrogenase gene detection. Proximity to the hybrid is shown in the table („Position" column).

Sample[a]	SEQ ID NO	Sequence (5'-3')[b]	Tm[c]	Length (nt)	GC%	Position[d]
ClusterIV-24	188	TGACCTCCTGGACGTTATC	67	19	52	58-76
ClusterIV-25	189	CCGCCCCTTAAATTTGAAG	65	19	47	5-23

[a]catcher oligonucleotides have been denoted with the aid of distribution in the clusters. The number of catcher oligonucleotides in the clusters does not represent their importance.
[b]oligonucleotide sequences shown have reverse complementarity to the relevant sequences of the sense nifH/anfH/vnfH strands. All oligonucleotides are bound to the micro-arrays by the 5'end.
[c]T_m values were calculated using the program MELT 1.1.0 (Jo P. Sanders).
[d]the position of the oligonucleotides represents the position at which the target sequence is bound [Here, other than as shown in the other figures, the region of the PCR primer (18 nt) has not been included].

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<400> SEQUENCE: 55

agactaagca cggtgtcttg 20

<210> SEQ ID NO 56
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Omega-1 Oligonucleotide

<400> SEQUENCE: 56

tgtctgagcc ttggcatga 19

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Omega-2 Oligonucleotide

<400> SEQUENCE: 57

cttcataacg tccttcagtt c 21

<210> SEQ ID NO 58
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Omega-3 Oligonucleotide

<400> SEQUENCE: 58

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ggtccataac cgttgactg 19

<210> SEQ ID NO 59
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Omega-4 Oligonucleotide

<400> SEQUENCE: 59

ccattaccgt gttctgtgc 19

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Omega-5 Oligonucleotide

<400> SEQUENCE: 60

ggtgtcgcca tagccga 17

<210> SEQ ID NO 61
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Omega-6 Oligonucleotide

<400> SEQUENCE: 61

caaccttcat cacggattcg 20

<210> SEQ ID NO 62
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<223> OTHER INFORMATION: Omega-7 Oligonucleotide

<400> SEQUENCE: 62

cggatgaggt ccatcac 17

<210> SEQ ID NO 63
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Omega-8 Oligonucleotide

<400> SEQUENCE: 63

gaaccttgtc cataaccgt 19

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<223> OTHER INFORMATION: Omega-9 Oligonucleotide

<400> SEQUENCE: 64

ctcgcggatc aggtccat 18

<210> SEQ ID NO 65

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<211> LENGTH: 21
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Omega-10 Oligonucleotide

<400> SEQUENCE: 65

gaccttcatg acatcttcca g 21

<210> SEQ ID NO 66
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Omega-11 Oligonucleotide

<400> SEQUENCE: 66

acgtccgaga gctccaga 18

<210> SEQ ID NO 67
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-1 Oligonucleotide

<400> SEQUENCE: 67

cagtggtttg tgctttaca 19

<210> SEQ ID NO 68
<211> LENGTH: 22
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<220> FEATURE:
<223> OTHER INFORMATION: Cyano-2 Oligonucleotide

<400> SEQUENCE: 68

agtgaagaac tgttacctgt gc 22

<210> SEQ ID NO 69
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-3 Oligonucleotide

<400> SEQUENCE: 69

gtttgagctt tagcgtttaa gattagtttg agctttagcg tttaagatta 50

<210> SEQ ID NO 70
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-4 Oligonucleotide

<400> SEQUENCE: 70

aaaccggtca acattacttc gtg 23

<210> SEQ ID NO 71
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Cyano-5 Oligonucleotide

<400> SEQUENCE: 71

gcacctggtt tacatacatc 20

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-6 Oligonucleotide

<400> SEQUENCE: 72

gcttagtacg gttgtttgtg 20

<210> SEQ ID NO 73
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-7 Oligonucleotide

<400> SEQUENCE: 73

tctacacatc ggataccttt gta 23

<210> SEQ ID NO 74
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-8 Oligonucleotide

<400> SEQUENCE: 74

ctgcaccttt ttcagcagc 19

<210> SEQ ID NO 75
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-9 Oligonucleotide

<400> SEQUENCE: 75

tacagtggag cattaagcga g 21

<210> SEQ ID NO 76
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-10 Oligonucleotide

<400> SEQUENCE: 76

cagccaagtg aagaacggt 19

<210> SEQ ID NO 77
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-11 Oligonucleotide

<400> SEQUENCE: 77

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cacttaacgc cgcgatagc 19

<210> SEQ ID NO 78
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-12 Oligonucleotide

<400> SEQUENCE: 78

attacttctt cgagttcaat atctt 25

<210> SEQ ID NO 79
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-13 Oligonucleotide

<400> SEQUENCE: 79

acgaacgtta cggaaacc 18

<210> SEQ ID NO 80
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-14 Oligonucleotide

<400> SEQUENCE: 80

ctaagtggag aatggtagtt tg 22

<210> SEQ ID NO 81
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-15 Oligonucleotide

<400> SEQUENCE: 81

ggtgaagaat cgtggtttg 19

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-16 Oligonucleotide

<400> SEQUENCE: 82

ggtgtagaat tgtggtttgg 20

<210> SEQ ID NO 83
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-17 Oligonucleotide

<400> SEQUENCE: 83

agctaggtgc agaactgtg 19

<210> SEQ ID NO 84

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<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-18 Oligonucleotide

<400> SEQUENCE: 84

gcagccaagg aaagaacg 18

<210> SEQ ID NO 85
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-19 Oligonucleotide

<400> SEQUENCE: 85

cttcacacca cggaagcc 18

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-20 Oligonucleotide

<400> SEQUENCE: 86

cgagcaatac ttccgagagt 20

<210> SEQ ID NO 87
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-21 Oligonucleotide

<400> SEQUENCE: 87

acgttgttat agccagtgag ta 22

<210> SEQ ID NO 88
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-22 Oligonucleotide

<400> SEQUENCE: 88

gtgcagaatg gtggtttg 18

<210> SEQ ID NO 89
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-23 Oliognukleotid

<400> SEQUENCE: 89

cagcagccaa ttggagaac 19

<210> SEQ ID NO 90
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Cyano-24 Oligonucleotide

<400> SEQUENCE: 90

cttttctgct gccaggtg 18

<210> SEQ ID NO 91
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-25 Oligonucleotide

<400> SEQUENCE: 91

agctttacag tgaagaatca agc 23

<210> SEQ ID NO 92
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-26 Oligonucleotide

<400> SEQUENCE: 92

gagcttcatc aagttccac 19

<210> SEQ ID NO 93
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cyano-27 Oligonucleotide

<400> SEQUENCE: 93

ctggatgcca gcgtagc 17

<210> SEQ ID NO 94
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Frankia-1 Oligonucleotide

<400> SEQUENCE: 94

atgccccact ggccctc 17

<210> SEQ ID NO 95
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Frankia-2 Oligonucleotide

<400> SEQUENCE: 95

gccttctcga tgacggtg 18

<210> SEQ ID NO 96
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-1 Oligonucleotide

<400> SEQUENCE: 96

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cccagaatca tgcgggta 18

<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-2 Oligonucleotide

<400> SEQUENCE: 97

gttcattccg cctaaaatca tac 23

<210> SEQ ID NO 98
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-3 Oligonucleotide

<400> SEQUENCE: 98

gttttgatga ccttctcgtt g 21

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-4 Oligonucleotide

<400> SEQUENCE: 99

acatccatca gggtttcctg 20

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-5 Oligonucleotide

<400> SEQUENCE: 100

ttgattcatc cctcccaaaa 20

<210> SEQ ID NO 101
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-6 Oligonucleotide

<400> SEQUENCE: 101

tatccatcaa tgtttcttgc gg 22

<210> SEQ ID NO 102
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-7 Oligonucleotide

<400> SEQUENCE: 102

ccatcatggt ggtctgca 18

<210> SEQ ID NO 103

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<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-8 Oligonucleotid

<400> SEQUENCE: 103

gcattttacc tctaagaatc atac 24

<210> SEQ ID NO 104
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-9 Oligonucleotide

<400> SEQUENCE: 104

gttttccgtg gagaatcatt c 21

<210> SEQ ID NO 105
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-10 Oligonucleotide

<400> SEQUENCE: 105

ccgtgcaaga ttaaccttg 19

<210> SEQ ID NO 106
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-11 Oligonucleotid

<400> SEQUENCE: 106

gttgtctgca ttttaccgc 19

<210> SEQ ID NO 107
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-12 Oligonucleotide

<400> SEQUENCE: 107

ggtctcctca ggtttgc 17

<210> SEQ ID NO 108
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-13 Oligonucleotide

<400> SEQUENCE: 108

atcaccgtct gctgcgg 17

<210> SEQ ID NO 109
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: ClusterII-14 Oligonucleotide

<400> SEQUENCE: 109

ccaggatcag ccgatttga 19

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-15 Oligonucleotide

<400> SEQUENCE: 110

caccaaggat aagacgagtt 20

<210> SEQ ID NO 111
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterII-16 Oligonucleotide

<400> SEQUENCE: 111

cgagcacaag acgagtaca 19

<210> SEQ ID NO 112
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-1 Oligonucleotide

<400> SEQUENCE: 112

ccttcttcgc ggagcgt 17

<210> SEQ ID NO 113
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-2 Oligonucleotid

<400> SEQUENCE: 113

actcggtgca ccggcaa 17

<210> SEQ ID NO 114
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-3 Oligonucleotide

<400> SEQUENCE: 114

ccttcctcgc ggagtgt 17

<210> SEQ ID NO 115
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-4 Oligonucleotide

<400> SEQUENCE: 115

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gaagtgatta ttcctcttcc 20

<210> SEQ ID NO 116
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-5'Oligonucleotide

<400> SEQUENCE: 116

cgcctaagag aagacgagt 19

<210> SEQ ID NO 117
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-6 Oligonucleotide

<400> SEQUENCE: 117

tcgtcgcgaa gggtatcca 19

<210> SEQ ID NO 118
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-7 Oligonucleotide

<400> SEQUENCE: 118

ccgcctttac ggatatc 17

<210> SEQ ID NO 119
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-8 Oligonucleotide

<400> SEQUENCE: 119

ccgcaaggtc cacgtc 16

<210> SEQ ID NO 120
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-9'Oligonucleotide

<400> SEQUENCE: 120

cttcccggcg gatgtc 16

<210> SEQ ID NO 121
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-10 Oligonucleotide

<400> SEQUENCE: 121

gcagcgtatc caatacggt 19

<210> SEQ ID NO 122

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<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-11 Oligonucleotide

<400> SEQUENCE: 122

gtcttcccct tccgacc 17

<210> SEQ ID NO 123
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-12 Oligonucleotide

<400> SEQUENCE: 123

tcttccagat ccacgtcttc 20

<210> SEQ ID NO 124
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-13'Oligonucleotide

<400> SEQUENCE: 124

cttttctgcg caagaccac 19

<210> SEQ ID NO 125
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-14 Oligonucleotide

<400> SEQUENCE: 125

accctgtccg gcggata 17

<210> SEQ ID NO 126
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-15 Oligonucleotide

<400> SEQUENCE: 126

gggtatccag aacggactt 19

<210> SEQ ID NO 127
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-16 Oligonucleotide

<400> SEQUENCE: 127

acggtccgtt gactcaaac 19

<210> SEQ ID NO 128
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: ClusterIII-17 Oligonucleotide

<400> SEQUENCE: 128

gagccaagcc atgcaaca 18

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-18 Oligonucleotide

<400> SEQUENCE: 129

gtatctaaca cactcttttg gt 22

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-19 Oligonucleotide

<400> SEQUENCE: 130

gttctgagtg tatccaacac 20

<210> SEQ ID NO 131
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-20 Oligonucleotide

<400> SEQUENCE: 131

ccaaggagaa gacgggtg 18

<210> SEQ ID NO 132
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-21 Oligonucleotide

<400> SEQUENCE: 132

gtccaagacg gtgctttg 18

<210> SEQ ID NO 133
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-22 Oligonucleotide

<400> SEQUENCE: 133

tcgagaagat tgatggagg 19

<210> SEQ ID NO 134
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-23 Oligonucleotide

<400> SEQUENCE: 134

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tatccaggac agtgcgct 18

<210> SEQ ID NO 135
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-24 Oligonucleotide

<400> SEQUENCE: 135

tccaaaacgg tccgctg 17

<210> SEQ ID NO 136
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-25 Oligonucleotide

<400> SEQUENCE: 136

ccgaagccct gtttgcg 17

<210> SEQ ID NO 137
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-26 Oligonucleotide

<400> SEQUENCE: 137

aaccgggttt acggatatc 19

<210> SEQ ID NO 138
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-27 Oligonucleotide

<400> SEQUENCE: 138

aaagccgggc gacacga 17

<210> SEQ ID NO 139
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-28 Oligonucleotide

<400> SEQUENCE: 139

cacctaaaag tagacgtgtt ga 22

<210> SEQ ID NO 140
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-29 Oligonucleotide

<400> SEQUENCE: 140

ccaacaacaa tcgggttga 19

<210> SEQ ID NO 141

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<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-30 Oligonucleotide

<400> SEQUENCE: 141

cacagcgaac acctttaaaa c 21

<210> SEQ ID NO 142
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-31 Oligonucleotide

<400> SEQUENCE: 142

cggttttctg ctgaagacc 19

<210> SEQ ID NO 143
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-32 Oligonucleotide

<400> SEQUENCE: 143

gtcttttgtg ctaaaccacc 20

<210> SEQ ID NO 144
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-33 Oligonucleotide

<400> SEQUENCE: 144

caccttccgc gagtgtat 18

<210> SEQ ID NO 145
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-34 Oligonucleotide

<400> SEQUENCE: 145

agtacggttt tctgggctaa 20

<210> SEQ ID NO 146
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-35 Oligonucleotide

<400> SEQUENCE: 146

ctcccaataa gagacggg 18

<210> SEQ ID NO 147
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: ClusterIII-36 Oligonucleotide

<400> SEQUENCE: 147

gtatcctacc ttcagaaccg 20

<210> SEQ ID NO 148
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-37 Oligonucleotide

<400> SEQUENCE: 148

tcaatgtcat cgccttcttc 20

<210> SEQ ID NO 149
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-38 Oligonucleotide

<400> SEQUENCE: 149

atgccggaaa agccggg 17

<210> SEQ ID NO 150
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-39 Oligonucleotide

<400> SEQUENCE: 150

ccagctctat gtcgtcgc 18

<210> SEQ ID NO 151
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-40 Oligonucleotide

<400> SEQUENCE: 151

gtcttctggt tcaggcc 17

<210> SEQ ID NO 152
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-41 Oligonucleotide

<400> SEQUENCE: 152

cgtatcaagc accgttttc 19

<210> SEQ ID NO 153
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-42 Oligonucleotide

<400> SEQUENCE: 153

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caaaatggca tccaattcaa tttc 24

<210> SEQ ID NO 154
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-43 Oligonucleotide

<400> SEQUENCE: 154

catgatacgg tcgagttcaa c 21

<210> SEQ ID NO 155
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-44 Oligonucleotide

<400> SEQUENCE: 155

ctgagctaat cctcctaaaa g 21

<210> SEQ ID NO 156
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-45 Oligonucleotide

<400> SEQUENCE: 156

ggtgaagacc accaagaag 19

<210> SEQ ID NO 157
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-46 Oligonucleotide

<400> SEQUENCE: 157

tgctggagtc ctcccaac 18

<210> SEQ ID NO 158
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-47 Oligonucleotide

<400> SEQUENCE: 158

caagttttcg cacatcatcc ag 22

<210> SEQ ID NO 159
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-48 Oligonucleotide

<400> SEQUENCE: 159

gccgagcttg atgatgtc 18

<210> SEQ ID NO 160

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<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-49 Oligonucleotide

<400> SEQUENCE: 160

cgacttttct aacgtcttca ag 22

<210> SEQ ID NO 161
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-50 Oligonucleotide

<400> SEQUENCE: 161

gtacggtctt ttggctcaaa c 21

<210> SEQ ID NO 162
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-51 Oligonucleotide

<400> SEQUENCE: 162

cggttcgcaa tgtatccag 19

<210> SEQ ID NO 163
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-52 Oligonucleotide

<400> SEQUENCE: 163

caggccgttc agcaaaag 18

<210> SEQ ID NO 164
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIII-53 Oligonucleotide

<400> SEQUENCE: 164

gcctcccaga agcaaac 17

<210> SEQ ID NO 165
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-1

<400> SEQUENCE: 165

ccctgatggc gtcaagc 17

<210> SEQ ID NO 166
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: ClusterIV-2 Oligonucleotidee

<400> SEQUENCE: 166

agtaccgttt cctggtgca 19

<210> SEQ ID NO 167
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-3

<400> SEQUENCE: 167

cggttcggtt ttgccgtc 18

<210> SEQ ID NO 168
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-4 Oligonucleotide

<400> SEQUENCE: 168

cctctgagaa gggttat 17

<210> SEQ ID NO 169
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-5 Oligonucleotide

<400> SEQUENCE: 169

gatgcctttg tagccggt 18

<210> SEQ ID NO 170
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-6 Oligonucleotide

<400> SEQUENCE: 170

ctgcgtgttg tcccgtat 18

<210> SEQ ID NO 171
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-7 Oligonucleotide

<400> SEQUENCE: 171

ttctgcaagg atccgcgt 18

<210> SEQ ID NO 172
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-8 Oligonucleotide

<400> SEQUENCE: 172

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caccgcggtg atgatcc 17

<210> SEQ ID NO 173
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-9 Oligonucleotide

<400> SEQUENCE: 173

tcatgggcat tgaccg 16

<210> SEQ ID NO 174
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-10 Oligonucleotide

<400> SEQUENCE: 174

ccgtccctca tgctgtc 17

<210> SEQ ID NO 175
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-11 Oligonucleotide

<400> SEQUENCE: 175

ttgccgcctg tcaggtt 17

<210> SEQ ID NO 176
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-12 Oligonucleotide

<400> SEQUENCE: 176

cctccgcttt caacacat 18

<210> SEQ ID NO 177
<211> LENGTH: 18
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<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-13 Oligonucleotide

<400> SEQUENCE: 177

gcctctcacc atagagtg 18

<210> SEQ ID NO 178
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-14 Oligonucleotide

<400> SEQUENCE: 178

caatatcaag gatagtaggc aatc 24

<210> SEQ ID NO 179

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-15 Oligonucleotide

<400> SEQUENCE: 179

ctttccgtgc attaatgtcc 20

<210> SEQ ID NO 180
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-16 Oligonucleotide

<400> SEQUENCE: 180

aatcctacgt ccagcgag 18

<210> SEQ ID NO 181
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-17 Oligonucleotide

<400> SEQUENCE: 181

ctgtattttg tgtccgcata c 21

<210> SEQ ID NO 182
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-18 Oligonucleotide

<400> SEQUENCE: 182

accgtgggga tcttcctc 18

<210> SEQ ID NO 183
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Cluster-IV-19 Oligonucleotide

<400> SEQUENCE: 183

actgtgggaa tgtcagcc 18

<210> SEQ ID NO 184
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-20 Oligonucleotide

<400> SEQUENCE: 184

taatatcctg acccttttgc 20

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<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: ClusterIV-21 Oligonucleotide

<400> SEQUENCE: 185

aggtaatcca gtaccgt 17

<210> SEQ ID NO 186
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-22 Oligonucleotide

<400> SEQUENCE: 186

ccgtgcaaca acagtttc 18

<210> SEQ ID NO 187
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-23 Oligonucleotide

<400> SEQUENCE: 187

gccaaggctt tccagcat 18

<210> SEQ ID NO 188
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-24 Oligonucleotide

<400> SEQUENCE: 188

tgacctcctg gacgttatc 19

<210> SEQ ID NO 189
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ClusterIV-25 Oligonucleotide

<400> SEQUENCE: 189

ccgcccctta aatttgaag 19

<210> SEQ ID NO 190
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A289

<400> SEQUENCE: 190

gggccttgtt ttcgcg 16

<210> SEQ ID NO 191
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A1(6A)

<400> SEQUENCE: 191

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aaaaaatgcg acccgaaggc tga 23

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A4(6A)

<400> SEQUENCE: 192

aaaaaagagt cagccttcgg gtc 23

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A8(6A)

<400> SEQUENCE: 193

aaaaaaggtg gagtcagcct tcg 23

<210> SEQ ID NO 194
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 194

aaaaaacaga cgggtggagt cag 23

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Oligonucleotide A20(6A)

<400> SEQUENCE: 195

aaaaaagagg atcagacggg tgg 23

<210> SEQ ID NO 196
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A64(6A)

<400> SEQUENCE: 196

aaaaaacttc agcggccagg tg 22

<210> SEQ ID NO 197
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A114(6A)

<400> SEQUENCE: 197

aaaaaagaag ccgaccgaca gg 22

<210> SEQ ID NO 198

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<211> LENGTH: 23
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A188(6A)

<400> SEQUENCE: 198

aaaaaaggcg gtgataacgc cac 23

<210> SEQ ID NO 199
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<212> TYPE: DNA
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<400> SEQUENCE: 199

aaaaaagaag tcgagttcgt cgtc 24

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<220> FEATURE:
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aaaaaagggc cttgttttcg cg 22

<210> SEQ ID NO 201
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide S20(6A)

<400> SEQUENCE: 201

aaaaaaccac ccgtctgatc ctc 23

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<223> OTHER INFORMATION: Oligonucleotide S114(6A)

<400> SEQUENCE: 202

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide S307(6A)

<400> SEQUENCE: 203

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<210> SEQ ID NO 204
<211> LENGTH: 29
<212> TYPE: DNA
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<220> FEATURE:

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<223> OTHER INFORMATION: Oligonucleotide A20(12A)

<400> SEQUENCE: 204

aaaaaaaaaa aagaggatca gacgggtgg 29

<210> SEQ ID NO 205
<211> LENGTH: 28
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A307(12A)

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aaaaaaaaaa aagggccttg ttttcgcg 28

<210> SEQ ID NO 206
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A20(6A)3'

<400> SEQUENCE: 206

gaggatcaga cgggtggaaa aaa 23

<210> SEQ ID NO 207
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A307(6A)3'

<400> SEQUENCE: 207

gggccttgtt ttcgcgaaaa aa 22

<210> SEQ ID NO 208
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nifH PCR forward primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 208

tgygayccna argcnga 17

<210> SEQ ID NO 209
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: nifH reverse primer
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<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)

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<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 209

adngccatca tytcncc 17

<210> SEQ ID NO 210
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nifH PCR forward primer

<400> SEQUENCE: 210

tgcgacccga aggctga 17

<210> SEQ ID NO 211
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nifH PCR forward primer

<400> SEQUENCE: 211

cctgtcggtc ggcttc 16

<210> SEQ ID NO 212
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nifH reverse primer

<400> SEQUENCE: 212

gggccttgtt ttcgcg 16

<210> SEQ ID NO 213
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A19-68

<400> SEQUENCE: 213

aggtgcatca cggtggtctg ggccttgctg tggaggatca gacgggtgga 50

<210> SEQ ID NO 214
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide A289-338

<400> SEQUENCE: 214

acgatgtaga tttcctgggc cttgttttcg cgaatcggca tcgcgaagcc 50

<210> SEQ ID NO 215
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide S19-68

<400> SEQUENCE: 215

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tccacccgtc tgatcctcca cagcaaggcc cagaccaccg tgatgcacct 50

<210> SEQ ID NO 216
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide S289-338

<400> SEQUENCE: 216

ggcttcgcga tgccgattcg cgaaaacaag gcccaggaaa tctacatcgt 50

1. Method of analyzing samples by means of a ligand binding method with a high phylogenetic resolution in which, through the binding of target sequences of target molecules to catcher sequences of probes, duplexes or complexes are generated and/or duplexes or complexes thus generated are analyzed, wherein the target sequences are partial sequences of the target molecule concerned, and the catcher sequences have a length of 17 mer to 25 mer, and the length of the target molecules is at least four times the length of the target sequence,

wherein within the target sequence, the duplexes or complexes have at least one detectable marking or an accumulation of markings, and at least ten samples are analyzed simultaneously.

2-4. (canceled)

5. Method according to claim **1**,

wherein all target molecules have the same number of markings.

6. Method according to claim **1**,

wherein at least one hundred samples are analyzed simultaneously.

7. Method according to claim **1**,

wherein the marking is a fluorescent marking.

8. Method according to claim **7**,

wherein the fluorescent marking is obtained by means of one or more of the following marking agents:

Cy3, Cy5, fluorescein, Texas red, Alexa fluor dyes and other fluorescent dyes.

9-23. (canceled)

24. Method for the determination of catcher sequences, in each case determined for the development of ligand bonds with target sequences each forming a partial sequence of a longer target molecule in such a way that they are complementary to the relevant sections of the target sequences which are to be found in proximity to a marking or an accumulation of markings.

25. Method according to claim **24**,

wherein the catcher sequences are determined in such a way that the marking or accumulation of markings is located less than 100 bases from the target sequence or is within the target sequence.

26. Method according to claim **25**,

wherein the catcher sequences are calculated by means of a computer program.

27. Set of catcher molecules wherein the catcher molecules have the specificity of that catcher molecule which in each case comprises a sequence selected from SEQ ID NO: 1-189.

28-29. (canceled)

30. Method according to claim **1**,

wherein the target molecules are 5'-end-marked.

31. Method of analyzing samples by means of a ligand binding method with a high phylogenetic resolution in which, through the binding of target sequences of target molecules to catcher sequences of probes, duplexes or complexes are generated and/or duplexes or complexes thus generated are analyzed, wherein the target sequences are partial sequences of the target molecule concerned, and the length of the target molecules is at least two times the length of the target sequence, wherein within the target sequence, the duplexes or complexes have at least one detectable marking or an accumulation of markings, and at least ten samples are analyzed simultaneously.

32. Method according to claim **31**,

wherein the target molecules are 5'-end-marked.

33. Method according to claim **31**, wherein the catcher sequences have a length of up to 35 mer and constituents of the probes of a DNA array, and the length of the target molecules are at least four times the length of the target sequence.

34. Method according to claim **33**,

wherein said at least one detectable marking is no further than 0-20 bases distant from the target sequence.

35. Method according to claim **34**,

wherein the target molecules are 5'-end-marked.

36. Method according to claim **1** in which target molecules are used having a marking in proximity to or within a target sequence which is a partial sequence of the relevant target molecule, wherein the target molecules are produced by means of a PCR method in which at least one type of dNTP provided with a marking agent is used, and in which the marked dNTPs are incorporated in direct proximity to the target sequence and/or in the target sequence itself, and in which one or more primers is provided with the same or other marking agents.

37. Set according to claim **19**,

wherein the catcher molecules are oglionucleotides.

38. Set according to claim **37**,

wherein it has at least 10 different catcher sequences.

39. Method according to claim **1**, wherein the catcher molecules are at least in part catcher molecules which in each case have the specificity of a catcher molecule comprising a sequence selected from SEQ ID NO: 1-189.

40. Method of analyzing variations of a gene by means of a ligand binding method with a high phylogenetic resolution in which, through the binding of target sequences of target molecules to catcher sequences of probes, duplexes or complexes are generated and/or duplexes or complexes thus generated are analyzed, wherein each target sequence is

specific for a certain variation of said gene, and, wherein in the proximity to and/or within the target sequence, the duplexes or complexes have at least one detectable marking or an accumulation of markings.

41. Method according to claim **40**, wherein at least ten variations are analyzed simultaneously.

* * * * *