

APPENDIX 1.

SEQUENCES AND PRIMERS.

(i) pDS49

pDS49 is a 362 bp fragment of AR10 DNA inserted into the *Hind* III site of the pUC18 multiple cloning site of pHS4

```
CTATTTATAA GAATAAGGGC AAAAAAAGTCCTTATAGCTCG CGCGATACAT ACAGAGCGGC 60
DS49top
GCAACGGAGC AATTAAAGGA GTGGGCTGAC AGAGCCGGAA CCTCCG ATCA TTTGCGGAAA 120
AGAAGGCGGA GATCCCGG AA GCGTCATTGA TGATGCAGCA TTCGCATTGA AGTCAAGGGA 180
CTGCGACATT CATAACAAGA AGAATCTTAT GGATGAGTTG TCCAAGATGA ACAGGATCCT 240
AACAAACGGGC CGATAAGGAA CTTGGTDSGGATC TCTGCAGGAG AATTTTATTG TTCTTGACG 300
DSS
AGAACGCTAT AATGCAGGCA AGAGAATTCTG GTGAGGCCGC A GATCTTACC GGTATTATAC 360
DS49PE DSS & DSG
362
TT
```

Primer sequences are underlined.

ITALICS show complementary sequence sections which form stem loops.

D G for stem loop = -15.8 Kcal/mole.

(ii) pDSS

pDSS is a deletant of pDS49(above). Nucleotides are numbered as for the parent plasmid (pDS49) sequence as far as the deletion at the centre of the primer DSS.

60

G ACC TTATAGCTCG CGCGATACAT ACAGAGCGGC
DS49top

120

GCAACGGAGC AATTAAAGGA GTGGGCTGAC AGAGCCGGAA *CCTCCG* ATCA TTTGCGGAAA

180

AGAAGGCGGA *GATCCCGGAA* G CGTCATTTA TGATGCAGCA TTCGCATTTA AGTCAAGGGA

240

CTGCGACATT CATAACAAGA AGAATCTTAT GGATGAGTTG TCCAAGATGA ACAGGATCCT

280

CGATAAGGAA CTTGGTGATC TCTGCAGGGA TCT TACCGGT
DSS

Primer sequences are underlined.

ITALICS show complementary sequence sections which form stem loops.

D G for stem loop = -15.8 Kcal/mole.

(iii). pJW4

This shows part of the sequence, including target primer sites of the plasmid pJW4 which encodes the *celA* gene of *Prevotella ruminicola* sequenced by Vercoe (Vercoe and Gregg, 1994).

810
GGCTCGGATG GAATCTGGGT AACCAGCTTC ATGCCCATGT AAAGCAAATA GCCGAGCAAA
MTP1A

870
CCTCATGGGG CAACGGCAAA GTTACACAAC AAACACTCTT GAAAGTAAAA CAAGCCGGAT

930
TTACATCAGT CCGCATCCCC GTTACCTGGT TGGGAAAAGT AGGCGAGGCC CCACACTATC

990
ACATCGACAC GGA CTGGATG AACCGCCTGG CAGAAGTAGT GGAATATGCT GAAAAAGCCG
GA3I

1050
GACTGAAAGC CATTATCAAC ATCCATCATG ACGGACACCG CCACATCCAC GAAGACGGT

1110
TTGACGAGCA TCGCTGGCTT GACATCGTAG GAGCAGCACA AAACAAGGAC ATGAATACCG
GA1B

1170
CTATCAAAGA GCAACTCAAA TCCATGTGGA CACAAATAGC CCAACGTTTC GAGAACAAAG
GA1B

1230
GCGAGTTCCT CATTTTTGAA **GGCCT**CAATG AAATCCATGA CGGAAGATCG GGCTCTGGCA
Stu I

1290
CCAATACCAC CGACGGAGGC AAA CAATATG CCATACTCAA CGAATGGCAA CAAGTTTTTG
G2D2 GA1B

1350
TAGATGCCGT CCGTGCCACC GCGGCAACA ATGCAACCCG CTACTTGGGT ATAGCCGGCT
PVP2ex

1370
ATTGCACAAG CCCTAATCTT
PVP2ex

Primer sequences are underlined.

BOLD shows *Stu I* recognition sequence.

There are no stem loops in the sequence.

(IV). pJD6

810
GGCTCGGATG GAATCTGGGT AACCAGCTT ATGCCCATGT AAAGCAAATA GCCGAGCAAA
MTP1A

870
CCTCATGGGG CAACGGCAAA GTTACACAAG AAACACTCTT GAAAGTAAAA CAAGCCGGAT

930
TTACATCAGT CCGCATCCCC GTTACCTGGT TGGGAAAAGT AGGCGAGGCC CCACACTATC

990
ACATCGACAC GGACTGGATG AACCGCCTGG CAGAAGTAGT GGAATATGCT GAAAAAGCCG
GA3I

1050
GACTGAAAGC CATTATCAAC ATCCATCATG ACGGACACCG CCACATCCAC GAAGACGGTT

1110
TTGACGAGCA TCGCTGGCTT GACATCGTAG GAGCAGCACA AAACAAGGAC ATGAATACCG
GA1B

1170
CTATCAATAT GCCATACTCA ACGAATGGCA ACAAGTTTTT GTAGATGCCG TCCGTGCCAC
GA1B

1230
CGGCGGCAAC AATGCAACCC GCTACTTGGG TATAGCCGGC TATTGCACAA GCCCTAATCT
PVP2ex

Primer sequences are underlined.

There are no stem loops in the sequence.

v). pGAR11

This represents part of the sequence of an approximately 500 bp fragment of AR29 genomic DNA cloned into the *Bam*H I site of the pUC18 multiple cloning site

```

                                                                 60
GGATCCGGTA GGCCTGTNC GTCATANAGG CACAGATACT TGGTGGGGTG AGAAAAACGA
                                                                 GARF
                                                                 120
AGAACCGGTT ATGTCCTGA AAGCATTGCA GGATTTCATT GACAAACGTC ACGAAGAAAT
                                                                 180
GCCCCAAGGT TCTTACACCA CCAGNCTGTT CGAATCCGGA ATCAACAAGA TCGTCAGAA
                                                                 240
ATGAGGTGAG GAAGCTGTCG AAACAGTGAT CGAAGCTACG AATGGAACAA ACGAACGTCT
                                                                 GARI2
                                                                 300
GATCTATGAA GGAGNCGACC NGATTTATCA CATGATTGTA TTATTAACTT CAANAGGCTA
                                                                 GARI
TCGGATCC
```

Primer sequences are underlined.

ITALICS show complementary sequence sections which form stem loops.

N shows unidentified nucleotides.

D G stem loop = -22.4 Kcal/mole.

(vi) pGOB5

This represents part of the sequence of an approximately 750 bp fragment of OB156 genomic DNA cloned into the *Bam*H I site of the pUC18 multiple cloning site

GGATCCATAT AGATTGTGAA TATTTAAGAT AAATTAGGAC TCCACGGAAAA TCGTGGAGCC (1) (1) 60
GOBF2

*CCGATTT*TGN ACGACACCAT ATAAAGTAAA AACTTTNAAAT GATTTTCTGN CATGCTAGGC 120
GOBF4

TACAATGCCA AAITCCTATA CAAGGTTACT CGCCATTGG GTAATCGCTT TCAGCGATGG 180
GOBF4

GGTCGGGCTC GTCATGTTCT *CGATTTTCAA* *AGTCAAGCTT* *TGAAAATCAA* GAAATTAATT (2) (2) 240

ATCCAAGAAT TGAAAGAAC G CCCTGAGNTA GACTGGTCAG CTTGAGCTAG CATNGTCTGA 300

NCTGCCTGAG CAAGAATGTT ATTCTNGCTG GNATGTAACC ACCTCATCAG CCATGCTCTGT 360
GOBI

ATCGCGAATA CGAGATCCAG CAGGAATTGG TGTTTT CAA CNTACGTTAT CANCNCCTGG 420

GGATTGTGTG CTCTCGNCGA CTCNGACTNG GGGCCCANAN GANCCCANAN GANCCCCCCT GAGN 474

Primer sequences are underlined.

ITALICS show complementary sequence sections which form stem loops.

N shows unidentified nucleotides.

D G stem loop (1) = -17.2 Kcal/mole.

(2) = -15.2 Kcal/mole

(vii). pDS63.

pDS63 is a 333 bp fragment of AR10 DNA inserted into the *Hind* III site of the pUC18 multiple cloning site of pHS4

```
AAGCTTACGT ATTTGCCGTA GCTAAGCTNT GTGGCTTATG GGATAATTTT CCTTGCGCAT 60
                                                    DS63top

ATTTCGATTT TCCAGACGGA AGATCAGTAC CTTGATGCTT ATCNGGATGT CACTTTC AAT 120
DS63top

CGCACGGTAC GNINNNCGTT TATCGTTGCG GGGTGTATCT TTTGGCCGGG GTATTGTATA 180

ATGAGTCGGG TGTATTA AAAA AATAGAAAGG CCGCCTTCGG CGCTTTTCTT GTATTGTCT 240

TTTGACTGC GGATTATAAAA GGTTTTTCTT TTTTATATAAACGGATAGA GATGTTGCGG 300

AGAAAAACAG GGCTTACATA GAGGATATGA TCG 333
DS63PE
```

Primer sites are underlined.

There are no stem loops in the sequence.

Table A.1.1.**Primer Sequences and Annealing Temperatures.**

| Primer | Sequence | Target | Annealing Temp °C | ΔG 65°C |
|---------|---------------------------|------------|-------------------|-----------------|
| DS49top | GTCCTTATAGCTCGCGGATACAT | AR10 | 73.48 | -22.3 |
| DS49PE | TGCGGCCTCACCGAATTCCTTGC | AR10 | 76.34 | -27.2 |
| DSS | ACCGGTAAGATCCCTGCAACACATC | Control | 74.91 | -22.7 |
| DS63top | CTTGCGCATATTTTCGATTTCCAG | AR10 | 70.62 | 22.9 |
| DS63PE | CGATCATATCCTCTATGTAAGCCC | AR10 | 72.05 | -19.5 |
| MTP1A | GCTCGGATGGAATCTGGGTTAACC | AR20 | 74.91 | -24.2 |
| GA3I | TGGCAGAAGTAGTGGAATATGCTG | AR20 | 72.05 | -23.2 |
| GA1B | TTGAGTATGGCATATTGATAGCTG | Control | 70.62 | -20.6 |
| G2D2 | TTGCCTCCGTCCGGTGGTATT | AR20 | 66.33 | -20.6 |
| PVP2ex | CATAGTCGCCGATAACGCTTCGGG | AR20 | 74.91 | -24.1 |
| PRRA | TCTTCAGCAGCAAAGAT | AR67 | 63.47 | -17.4 |
| PRA6 | AAATATCCCTGACTTCGJCG | AR67 | 64.9 | -19.1 |
| CC5 | TGCGAGGCTATGGCGATTCGGACA | Dehal gene | 76.34 | N.D. |
| CC6.2I | CTGACCGATCATGTGCTCGGGGAA | Dehal gene | 76.34 | N.D. |
| GOBF4 | ATGCTAGGCTACAATGCCAAATTC | OB156 | 70.62 | -21.1 |
| GOBI | AGACACGGCTGATGAGGTTTAC | OB156 | 67.76 | -20.7 |
| GARF | AGGCACAGATACTTGGTTTAC | AR29 | 69.19 | -18.0 |
| GARI2 | AGAGCTTCGTTTGTTCATTCGTA | AR29 | 70.62 | -21.3 |
| JUPF | ATCTGCCTTACAGATAAGCCAACC | Control | 72.05 | -20.5 |
| JUPI | CGAAGACGACGTCTACGTCCGTTC | Control | 76.34 | -23.5 |
| | | | | |

ΔG is taken from the JROligos Program (Rozas, 1991)

Annealing temperature is calculated from the formula of Wu *et al*, 1991: $-T = 22 = 1.43Ln$

Table A1.2.

PCR Fragment Lengths.

| Primer Pair | Plasmid | Use | Length bp |
|--------------------|----------------|---------------|------------------|
| MTPIA/PVP2ex | pJW4 | AR20 probe | 591 |
| GA3I/G2D2 | pJW4 | AR20 probe | 295 |
| GA3I/GA1B | pJD6 | AR20 control | 174 |
| DS49top/DS49PE | pDS49 | AR10 probe | 314 |
| DS49top/DSS | pDSS | AR10 control | 254 |
| DS63top/ DS63PE | pDS63 | AR10 probe | 282 |
| GARF/GARI2 | pGAR | AR29 probe | 213 |
| GOBF4/GOBI | pGOB | OB156 control | 331 |

APPENDIX 2.

GENBANK SEQUENCES HOMOLOGOUS TO pGOB5

(Nucleotide) FASTA of: pgob from: 1 to: 454 February 15, 1994 19:59

TO: genbank:* Sequences: 162,946 Symbols: 173,261,500 Word Size: 6

```
Score Init1 Initn
< 4 2003 2003:=====
  8 10 10:=====
 12 18 18:=====
 16 60 60:=====
 20 526 526:=====
 24 12637 12637:=====
 28 50050 50050:=====
 32 84500 84500:=====
 36 74798 74798:=====
 40 42914 42914:=====
 44 25570 25570:=====
 48 16058 16058:=====
 52 8913 8816:=====
 56 4254 4046:=====
 60 1997 1842:=====
 64 968 895:=====
 68 348 321:=====
 72 161 139:=====
 76 48 43:=====
 80 26 34:=====
 84 17 161:=====
 88 4 130:=====
 92 0 135:=====
 96 2 65:=====
100 0 25:=====
104 0 30:=====
108 0 8:++++
112 0 6:+++
116 3 7:===+
120 0 8:++++
124 0 7:++++
128 0 7:++++
132 0 4:++
136 0 5:+++
140 0 1:+
144 0 0:
148 0 0:
152 0 1:+
156 0 0:
160 0 2:+
>160 7 10:====+
mean initn score: 34.0 (5.91)
mean initl score: 34.0 (5.91)
```

The best scores are:

initl initn opt..

```
genbank:rosflah /rev R.cecicola flagellin gene, complete... 182 257 383
genbank:s62780 /rev hag=flagellin {3' region, clone 5, p... 225 225 260
genbank:s62773 /rev hag=flagellin {3' region, clone 1, p... 225 225 260
genbank:s62775 /rev hag=flagellin {3' region, clone 2, p... 225 225 260
genbank:s62783 /rev hag=flagellin {3' region, clone 9, p... 225 225 260
genbank:s62779 /rev hag=flagellin {3' region, clone 4, p... 225 225 260
genbank:s62776 /rev hag=flagellin {3' region, clone 3, p... 225 225 260
genbank:bacflaga /rev B.subtilis flagellin gene, complet... 114 178 280
genbank:bachagfa /rev B.subtilis flagellin gene (hag), c... 114 178 280
```

| | | | | |
|-----------------------|---|-----|-----|-----|
| genbank:i01816 /rev | Sequence 1 from patent US 4801536 | 114 | 178 | 274 |
| genbank:yscmtcg | <i>S.cerevisiae</i> mitochondrion DNA, complete... | 58 | 160 | 60 |
| genbank:chmpxx /rev | Liverwort <i>Marchantia polymorpha</i> chlo... | 78 | 157 | 94 |
| genbank:chmpxx | Liverwort <i>Marchantia polymorpha</i> chloropla... | 77 | 149 | 80 |
| genbank:hehcmvcg /rev | Human Cytomegalovirus Strain AD169... | 70 | 139 | 70 |
| genbank:mpomtcg /rev | <i>Marchantia polymorpha</i> mitochondrion... | 67 | 134 | 68 |
| genbank:clegcga | <i>E.gracilis</i> chloroplast complete genome | 65 | 134 | 65 |
| genbank:asfv55kb | African swine fever virus 55kb from the... | 63 | 133 | 63 |
| genbank:chntxx | Tobacco chloroplast genome DNA | 62 | 133 | 68 |
| genbank:pgncp37 /rev | <i>Columba livia</i> annexin I (cp37) gene... | 71 | 133 | 76 |
| genbank:hsfgfrbe /rev | Human bek mRNA for fibroblast grow... | 61 | 132 | 65 |
| genbank:humksami /rev | Human fibroblast growth factor rec... | 61 | 132 | 65 |
| genbank:celzc21 /rev | <i>C. elegans</i> cosmid ZC21 | 68 | 131 | 68 |
| genbank:ecouw89 | <i>E. coli</i> chromosomal region from 89.2 to ... | 60 | 130 | 64 |
| genbank:humneurof | Human oligodendrocyte myelin glycoprot... | 59 | 128 | 59 |
| genbank:cec07a9 | <i>Caenorhabditis elegans</i> cosmids C07A9 and... | 61 | 128 | 67 |
| genbank:cer107 | <i>Caenorhabditis elegans</i> cosmid R107 | 60 | 127 | 60 |
| genbank:crplast | <i>C.reflexa</i> plastid psbA, trnH, trnL genes... | 62 | 126 | 76 |
| genbank:hivoyi /rev | Human immunodeficiency virus type 1 ... | 56 | 126 | 58 |
| genbank:humhprt | Human hypoxanthine phosphoribosyltransf... | 58 | 125 | 61 |
| genbank:clegcga /rev | <i>E.gracilis</i> chloroplast complete genome | 62 | 125 | 65 |
| genbank:cet05g5 /rev | <i>Caenorhabditis elegans</i> cosmid T05G5 | 60 | 124 | 62 |
| genbank:musfosb /rev | <i>Mus musculus</i> FosB protein (fosB) ge... | 59 | 123 | 59 |
| genbank:cer107 /rev | <i>Caenorhabditis elegans</i> cosmid R107 | 57 | 123 | 57 |
| genbank:celb0303 | <i>C.elegans</i> cosmid B0303 | 73 | 122 | 78 |
| genbank:yepsercaro | <i>Y.enterocolitica</i> 5-enolpyruvylshikima... | 56 | 122 | 58 |
| genbank:cec38c10 /rev | <i>Caenorhabditis elegans</i> cosmid C38C10 | 62 | 122 | 73 |
| genbank:celr05d3 | <i>C. elegans</i> cosmid R05D3 | 58 | 122 | 58 |
| genbank:celzk370 /rev | <i>C. elegans</i> cosmid ZK370 | 54 | 120 | 57 |
| genbank:rabas1cg /rev | <i>Oryctolagus cuniculus</i> as1-casein g... | 58 | 120 | 58 |
| genbank:misc13 | oxi3 locus in yeast mitochondrion (strain... | 56 | 120 | 59 |

pgob /rev
genbank:rosflah

LOCUS ROSFLAH 1031 bp ds-DNA BCT 15-MAR-1989
DEFINITION *R.cecicola* flagellin gene, complete cds.
ACCESSION M20983
KEYWORDS flagellin.
SOURCE *R.cecicola* DNA.
ORGANISM *Roseburia cecicola* . .

SCORES Init1: 182 Initn: 257 Opt: 383
72.3% identity in 195 bp overlap

```

pgob          10      20      30      40
              TGGGCCCCNAGTCNGAGTCGNCGAGAGCACACAATCCCCAA
              ||:|| | |: ||| ||| ||| ||| |||
rosfla ATCTGAGCAGAGATCAGCACTTGGTGCAGTTCAGAACCGTTTAGAGCACACTATCGCTAA
              740      750      760      770      780      790

pgob          50      60      70      80      90      100
              GNGNTGATAACGTANGTTGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAG
              : : || ||| | : | ||| || || | ||| | ||| ||| ||| ||| |||
rosfla --CTTAGACAACATTTCTGAGAATAC-TCAGT--CTGCAGAGTCTCGTATCCGTGATACAG
              800      810      820      830      840

pgob          110      120      130      140      150      160
              ACATGGCTGATGAGGTGGTTCCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAG
              | ||| ||| ||| ||| | : |||: ||| || | ||| ||| ||| ||| |||
rosfla ATATGGCTGAAGAGATGGTT-GAGTACAGCAAGAACAATATCCTTGCTCAGGCAGGACAG
              850      860      870      880      890      900

pgob          170      180      190      200      210      220
              ACNATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTCTTTCAATTCTTGATAATT

```

```

|:||||| ||||| ||||| ||||| ||||| ||||| |||||
rosfla TCTATGCTTGCTCAGGCTAACCAGTCTA-CTCAGGGTGTACTTTCTCTTACAGTAATG
      910      920      930      940      950      960
pgob   230      240      250      260      270      280
AATTTCTTGATTTTCAAAGCTTGACTTTGAAAATCGAGAACATGACGAGCCCGACCCCAT
rosfla TAAGATTACAGTAGAAGATATACTACCAATAAAAACAGAGCCGAAATATTTCCGGCTCTGT
      970      980      990      1000     1010     1020

```

pgob /rev
genbank:s62780

LOCUS S62780 191 bp mRNA BCT 11-AUG-1993
DEFINITION hag=flagellin {3' region, clone 5, polyadenylation site}
[Bacillus subtilis, mRNA Partial, 191 nt]
ACCESSION S62780
KEYWORDS .
SOURCE Bacillus subtilis . . .

SCORES Init1: 225 Initn: 225 Opt: 260
68.9% identity in 148 bp overlap

```

      50      60      70      80      90      100
pgob   NTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAGACA
s62780                                     ||||| ||||| || || |||
                                     GAAAACTTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGACA
                                     10      20      30      40

      110     120     130     140     150     160
pgob   TGGCTGATGAGGTGGTTCCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAGACN
s62780 ||||| ||||| ||||| ||||| :||| :||| ||||| ||||| ||||| :||| |:
TGGCTAAAGAGATGAGCGAATTCCAAAGAACAACATTCTTCTCAGGCTTCTCAAGCT
      50      60      70      80      90     100

      170     180     190     200     210     220
pgob   ATGCTAGCTCAAGCTGACCAGTCTAAYCTCAGGGCGTTCTTCAATTCTT-GGATAATTAA
s62780 ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
ATGCTTGCTCAAGCAAACCA-ACAGGCGCAAACGTAC-TTCAATTATTACGTTAATTTT
      110     120     130     140     150     160

      230     240     250     260     270     280
pgob   TTTCTTGATTTTCAAAGCTTGACTTTGAAAATCGAGAACATGACGAGCCCGACCCCATCG
s62780 AAAAAAAAAAAAAAAAAAAACCCGGGGATC
      170     180     190

```

pgob /rev
genbank:s62773

LOCUS S62773 200 bp mRNA BCT 11-AUG-1993
DEFINITION hag=flagellin {3' region, clone 1, polyadenylation site}
[Bacillus subtilis, mRNA Partial, 200 nt]
ACCESSION S62773
KEYWORDS .
SOURCE Bacillus subtilis . . .

SCORES Init1: 225 Initn: 225 Opt: 260
68.9% identity in 148 bp overlap

```

      50      60      70      80      90      100
pgob   NTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAGACA
s62773                                     ||||| ||||| || || |||
                                     GAAAACTTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGACA

```


KEYWORDS .
SOURCE Bacillus subtilis . . .

SCORES Init1: 225 Initn: 225 Opt: 260
68.9% identity in 148 bp overlap

```

50          60          70          80          90          100
pgob  NTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAGACA
s62783                ||||| ||||| ||||| |||||
                GAAAACCTTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGACA
                10          20          30          40

110        120        130        140        150        160
pgob  TGGCTGATGAGGTGGTTCCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAGACN
s62783 ||||| | ||| || ||: ||| : |||| ||||| ||||| ||||| : ||| |:
TGGCTAAAGAGATGAGCGAATTCACAAAGAACAACATTCTTTCTCAGGCTTCTCAAGCT
50          60          70          80          90          100

170        180        190        200        210        220
pgob  ATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTTCTTTCAATTCTT-GGATAATTAA
s62783 ||||| ||||| ||||| | : ||| ||| | ||||| ||| | |||||
ATGCTTGCTCAAGCAAACCA-ACAGCCGCAAACGTAC-TTCAATTATTACGTTAATTTT
110        120        130        140        150        160

230        240        250        260        270        280
pgob  TTTCTTGATTTTCAAAGCTTGACTTTGAAAATCGAGAACATGACGAGCCCGACCCCATCG
s62783 AAAAAAAAAAAAAAAAAAAAAAAAAACCCGGGGATC
170        180        190

```

pgob /rev
genbank:s62779

LOCUS S62779 191 bp mRNA BCT 11-AUG-1993
DEFINITION hag=flagellin (3' region, clone 4, polyadenylation site)
[Bacillus subtilis, mRNA Partial, 191 nt]

ACCESSION S62779

KEYWORDS .

SOURCE Bacillus subtilis . . .

SCORES Init1: 225 Initn: 225 Opt: 260
68.9% identity in 148 bp overlap

```

50          60          70          80          90          100
pgob  NTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAGACA
s62779                ||||| ||||| ||||| |||||
                GAAAACCTTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGACA
                10          20          30          40

110        120        130        140        150        160
pgob  TGGCTGATGAGGTGGTTCCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAGACN
s62779 ||||| | ||| || ||: ||| : |||| ||||| ||||| ||||| : ||| |:
TGGCTAAAGAGATGAGCGAATTCACAAAGAACAACATTCTTTCTCAGGCTTCTCAAGCT
50          60          70          80          90          100

170        180        190        200        210        220
pgob  ATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTTCTTTCAATTCTT-GGATAATTAA
s62779 ||||| ||||| ||||| | : ||| ||| | ||||| ||| | |||||
ATGCTTGCTCAAGCAAACCA-ACAGCCGCAAACGTAC-TTCAATTATTACGTTAATTTT
110        120        130        140        150        160

230        240        250        260        270        280
pgob  TTTCTTGATTTTCAAAGCTTGACTTTGAAAATCGAGAACATGACGAGCCCGACCCCATCG
s62779 AAAAAAAAAAAAAAAAAAAAAAAAAACCCGGGGATC

```

pgob /rev
genbank:s62776

LOCUS S62776 191 bp mRNA BCT 11-AUG-1993
DEFINITION hag=flagellin {3' region, clone 3, polyadenylation site}
[Bacillus subtilis, mRNA Partial, 191 nt]
ACCESSION S62776
KEYWORDS .
SOURCE Bacillus subtilis . . .

SCORES Init1: 225 Initn: 225 Opt: 260
68.9% identity in 148 bp overlap

```

      50      60      70      80      90      100
pgob  NTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGGATACAGACA
s62776                GAAAACCTTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGACA
                        10      20      30      40

      110     120     130     140     150     160
pgob  TGGCTGATGAGGTGGTTCCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAGACN
s62776 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      50      60      70      80      90      100
pgob  ATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTTCTTTCAATTCTT-GGATAATTAA
s62776 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      110     120     130     140     150     160
pgob  ATGCTTGCTCAAGCAAACCA-ACAGCCGCAAACGTAC-TTCAATTATTACGTTAATTTT
s62776 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      170     180     190     200     210     220
pgob  ATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTTCTTTCAATTCTT-GGATAATTAA
s62776 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      110     120     130     140     150     160
pgob  TTTCTTGATTTTCAAAGCTTGACTTIGAAAATCGAGAACATGACGAGCCCGACCCCATCG
s62776 AAAAAAAAAAAAAAAAAAAAAACCCGGGATC
      170     180     190
```

pgob /rev
genbank:bacflaga

LOCUS BACFLAGA 1474 bp ds-DNA BCT 15-SEP-1989
DEFINITION B.subtilis flagellin gene, complete cds.
ACCESSION M26947
KEYWORDS H-antigen; flagellar organelle; flagellin; motility.
SOURCE B.subtilis (strain 1168) DNA.
ORGANISM Bacillus subtilis . . .

SCORES Init1: 114 Initn: 178 Opt: 280
64.3% identity in 210 bp overlap

```

      10      20      30      40
pgob  TGGGCCCGNAGTCNGAGTCGNGCAGAGCACACAATCCCCAAG
bacfla | : | | | : | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      870     880     890     900     910     920
pgob  NGNTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGGATACAGA
bacfla | : | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
      930     940     950     960     970
```


LOCUS I01816 1473 bp ss-DNA PAT 05-MAR-1993
 DEFINITION Sequence 1 from patent US 4801536
 ACCESSION I01816
 KEYWORDS .
 SOURCE .
 ORGANISM Unclassified. . . .

SCORES Init1: 114 Initn: 178 Opt: 274
 64.2% identity in 201 bp overlap

```

                                10      20      30      40
pgob          TGGGCCCCNAGTCNAGTTCGNCGAGAGCACACAATCCCCAAG
i01816 TCTTCTCAACGTGCTAAGCTTGGTTCGGTACAAAATCGTCTAGAGCACACAATTAACAA-
                                870      880      890      900      910

                                50      60      70      80      90      100
pgob  NGNTGATAACGTANGTTGGAAAACACCAATTCCTGCTGGATCTCGTATTCGCGATACAGA
i01816 -CTTAAGCGCTTCTGGT-GAAAAC-TTGACAGCTGCTGAGTCTCGTATCCGTGACGTTGA
                                920      930      940      950      960      970

                                110     120     130     140     150     160
pgob  CATGGCTGATGAGGTGGTTCATNCCAGCNAGAATAACATTCTTGCTCAGGCAGNTCAGA
i01816 CATGGCTAAAGAGAT-GAGCGAATTCACAAAGAACAACATTCTTCTCAGGCTTCTCAAG
                                980     990     1000    1010    1020    1030

                                170     180     190     200     210     220
pgob  CNATGCTAGCTCAAGCTGACCAGTCTAYCTCAGGGCGTTCTTTCAATTCTTGATAATTA
i01816 CTATGCTTGTCTCAAGCAAACCA-ACAGCCGCAAACGTAC-TTCAATTATTACGTTTATT
                                1040    1050    1060    1070    1080    1090

                                230     240     250     260     270     280
pgob  ATTTCTTGATTTTCAAAGCTTGACTTTGAAAATCGAGAACATGACGAGCCCCGACCCCATC
i01816 TTAAAAAAGACCTTGGCGTTGCCAGGGTCTTTTAATTTAAATTTCTATCTCCTAATCATT
                                1100    1110    1120    1130    1140    1150

```

pgob
 genbank:yscmtcg

LOCUS YSCMTCG 78520 bp ds-DNA Circular PLN 08-FEB-1993
 DEFINITION S.cerevisiae mitochondrion DNA, complete genome, compiled from man
 strains and entries, with a few gaps.
 ACCESSION M62622
 KEYWORDS 15S ribosomal RNA; 21S ribosomal RNA; 9S ribosomal RNA;
 complete genome; cytochrome oxidase; origin of replication; . . .

SCORES Init1: 58 Initn: 160 Opt: 60
 81.8% identity in 22 bp overlap

```

                                10      20      30      40
pgob          TCGATAAGATTGTGAATATTTAAGATAAATTAGGACTCCA
yscmtc ATAAATTATTTTGTATTATTATTAATTTATATTAATATTTTATATAAATTATTTATTTA
                                5670    5680    5690    5700    5710    5720

                                50      60      70      80      90      100
pgob  CGGAAATCGTGGAGCCCCGATTTTGNACGACACCATATAAAGTAAAACTTNAATGATT
yscmtc ATCTTTCATTATATATTTAATATATTTAATATTTAATTAATATTTTATAATAAATAAAT
                                5730    5740    5750    5760    5770    5780

```

APPENDIX 3.

DENSITOMETER SCANS AND CALCULATIONS.

Table A3.1.

Scan Readings for PDS49/pDSS Linearity.

| Template Ratio pDS49/pDSS | pDS49 Scan Au x mm | pDss Scan Au x mm | Scan Ratio | Mean Scan Ratio |
|------------------------------|-----------------------|----------------------|------------|--------------------|
| 2/4 | 0.138 | 0.436 | 0.317 | |
| 2/4 | 0.164 | 0.399 | 0.411 | |
| 2/4 | 0.148 | 0.352 | 0.420 | 0.383 |
| 3/4 | 0.189 | 0.402 | 0.470 | |
| 3/4 | 0.189 | 0.392 | 0.482 | 0.476 |
| 4/4 | 0.234 | 0.363 | 0.645 | |
| 4/4 | 0.228 | 0.361 | 0.6321 | 0.638 |
| 5/4 | 0.316 | 0.394 | 0.802 | |
| 5/4 | 0.303 | 0.389 | 0.779 | |
| 5/4 | 0.270 | 0.339 | 0.796 | 0.792 |
| 6/4 | 0.328 | 0.358 | 0.916 | |
| 6/4 | 0.296 | 0.329 | 0.900 | |
| 6/4 | 0.309 | 0.349 | 0.885 | 0.900 |
| 7/4 | 0.352 | 0.356 | 0.989 | |
| 7/4 | 0.324 | 0.328 | 0.988 | |
| 7/4 | 0.310 | 0.348 | 0.891 | 0.956 |
| 8/4 | 0.390 | 0.390 | 1.000 | |
| 8/4 | 0.329 | 0.329 | 1.000 | |
| 8/4 | 0.373 | 0.298 | 1.252 | 1.084 |
| 9/4 | 0.397 | 0.367 | 1.082 | |
| 9/4 | 0.361 | 0.293 | 1.2321 | |
| 9/4 | 0.322 | 0.294 | 1.095 | 1.136 |
| 10/4 | 0.354 | 0.293 | 1.208 | |
| 10/4 | 0.373 | 0.327 | 1.141 | |
| 10/4 | 0.389 | 0.323 | 1.204 | 1.184 |
| 11/4 | 0.389 | 0.304 | 1.280 | |
| 11/4 | 0.432 | 0.346 | 1.249 | |
| 11/4 | 0.420 | 0.341 | 1.232 | 1.253 |
| 12/4 | 0.273 | 0.198 | 1.379 | |
| 12/4 | 0.262 | 0.194 | 1.351 | 1.365 |

Table A3.2.

Scan Readings for AR10 Genome Standards.

| pDS49 ng | Scan Readings Au mm x 1000 | Scan Ratio | Mean | Duplicate Mean |
|-----------------|---------------------------------------|-------------------|-------------|---------------------------|
| 0.04 | 240/443 | 0.54 | | |
| 0.04 | 208/401 | 0.52 | | |
| 0.04 | 193/351 | 0.55 | 0.54 | |
| 0.04 | 315/363 | 0.59 | | |
| 0.04 | 285/498 | 0.57 | 0.58 | 0.56 |
| 0.06 | 242/266 | 0.92 | | |
| 0.06 | 257/294 | 1.05 | | |
| 0.06 | 242/256 | 1.05 | 1.01 | |
| 0.06 | 242/266 | 0.91 | | |
| 0.06 | 257/294 | 0.87 | | |
| 0.06 | 242/256 | 0.85 | 0.88 | 0.94 |
| 0.08 | 310/262 | 1.18 | | |
| 0.08 | 307/256 | 1.20 | | |
| 0.08 | 243/206 | 1.18 | 1.19 | |
| 0.08 | 385/344 | 1.12 | | |
| 0.08 | 350/347 | 1.01 | 1.06 | 1.13 |
| 0.10 | 415/342 | 1.21 | | |
| 0.10 | 385/269 | 1.43 | 1.37 | |
| 0.10 | 302/245 | 1.23 | | |
| 0.10 | 351/261 | 1.34 | | |
| 0.10 | 280/234 | 1.20 | 1.27 | 1.32 |

pDss concentration was 0.05ng/tube throughout

Table A3.3.

AR10 Genome Size: Scan Readings and Calculations.

| AR10 DNA ng | Scan Readings Au mm x 1000 | Scan Ratio | Mean Scan Ratio | Equip ng pDS49 | Genome Size Mb |
|--------------------|---------------------------------------|-------------------|----------------------------|---------------------------|---------------------------|
| 10.00 | 235/420 | 0.56 | | | |
| 10.00 | 232/487 | 0.48 | 0.52 | 0.031 | 1.54 |
| 15.00 | 286/407 | 0.70 | | | |
| 15.00 | 298/426 | 0.70 | | | |
| 15.00 | 269/411 | 0.72 | 0.71 | 0.047 | 1.53 |
| 20.00 | 254/283 | 0.87 | | | |
| 20.00 | 289/305 | 0.95 | | | |
| 20.00 | 291/321 | 0.91 | 0.91 | 0.064 | 1.50 |
| 20.00 | 247/284 | 0.87 | | | |
| 20.00 | 300/326 | 0.92 | | | |
| 20.00 | 295/324 | 0.91 | 0.90 | 0.063 | 1.52 |

Mean Genome Size 1.52 Mb

Standard Deviation 0.017

Standard Error 0.009g

Table A3.4.

Scan Readings for AR20 Genome Standards.

| pJW4 n | Scan Ratio | Mean Scan Ratio | Duplicate Mean |
|---------------|-------------------|------------------------|-----------------------|
| 0.025 | 0.473 | | |
| 0.025 | 0.557 | | |
| 0.025 | 0.474 | 0.501 | |
| 0.025 | 0.441 | | |
| 0.025 | 0.459 | 0.450 | 0.476 |
| 0.05 | 0.768 | | |
| 0.05 | 0.857 | | |
| 0.05 | 0.894 | 0.840 | |
| 0.05 | 1.038 | | |
| 0.05 | 1.053 | | |
| 0.05 | 1.168 | 1.086 | 0.963 |
| 0.075 | 1.402 | | |
| 0.075 | 1.411 | | |
| 0.075 | 1.458 | 1.424 | |
| 0.075 | 1.604 | | |
| 0.075 | 1.621 | | |
| 0.075 | 1.749 | 1.658 | 1.541 |
| 0.1 | 2.599 | | |
| 0.1 | 2.929 | | |
| 0.1 | 2.950 | 2.826 | |
| 0.1 | 2.52 | | |
| 0.1 | 2.367 | | |
| 0.1 | 2.0382 | 2.423 | 2.624 |

Table A3.5

AR20 Genome Scan Readings and Calculations.

| AR20 DNA ng | Scan Ratio | Mean | Equiv ng pJW4 | Genome Size Mb | |
|------------------------|-------------------|-------------|--------------------------|---------------------------|---------------------------------------|
| 36.0 | 1.048 | | | | |
| 36.0 | 0.964 | | | | |
| 36.0 | 0.946 | | | | |
| 36.0 | 1.237 | | | | Mean Genome Size = 3.47 Mb |
| 36.0 | 1.221 | 1.098 | 0.052 | 3.30 | |
| 48.0 | 1.567 | | | | St'd Devciation =0.15 |
| 48.0 | 1.490 | | | | |
| 48.0 | 1.342 | 1.466 | 0.065 | 3.51 | |
| 60.0 | 1.855 | | | | Std Error =0.086 |
| 60.0 | 1.645 | | | | |
| 60.0 | 1.541 | | | | |
| 60.0 | 12.265 | | | | |
| 60.0 | 2.074 | | | | |
| 60.0 | 1.831 | 1.868 | 0.079 | 3.59 | |

Table A3.6.

Bacterial Cell Number Estimates

(i). Standard Curve.

| PJW4 ng | Scan Ratio |
|---------|------------|
| 0.02 | .407 |
| 0.04 | 1.004 |
| 0.06 | 1.133 |
| 0.08 | 1.547 |
| 1.0 | 2.309 |

(ii). AR20 Copy Numbers.

| Template | Scan Ratio | Equiv.pg PJW4 | Copy No $\times 10^{-6}$ | Copies/ml $\times 10^{-8}$ | Mean | Std Error |
|-----------|------------|------------------|-----------------------------|-------------------------------|-------|-----------|
| 1 x DNA | 0.163 | 8.36 | 1.6 | 8.0 | | |
| 2 x DNA | 0.352 | 17.24 | 3.30 | 8.25 | | |
| 4 x DNA | 0.939 | 44.82 | 8.57 | 11.45 | 9.23 | 1.1 |
| | | | | | | |
| 1 x cells | 0.194 | 9.82 | 1.88 | 18.8 | 24.83 | 3.7 |
| 2 x cells | 0.689 | 33.07 | 6.32 | 31. | | |
| 4 x cells | 1.057 | 50.36 | 9.63 | 24.1 | | |

Table A3.8.

Scan Ratios for pJW4/pJD6 Amplification Efficiency.

| pJW4 ng | Scan Ratio | Mean Scan Ratio |
|----------------|-------------------|------------------------|
| 0.025 | 0.473 | |
| 0.025 | 0.557 | |
| 0.025 | 0.474 | |
| 0.025 | 0.441 | |
| 0.025 | 0.459 | |
| 0.025 | 0.763 | 0.526 |
| 0.05 | 0.768 | |
| 0.05 | 0.857 | |
| 0.05 | 0.894 | |
| 0.05 | 1.038 | |
| 0.05 | 1.053 | |
| 0.05 | 1.168 | 0.963 |
| 0.075 | 1.402 | |
| 0.075 | 1.411 | |
| 0.075 | 1.458 | |
| 0.075 | 1.604 | |
| 0.075 | 1.621 | |
| 0.075 | 1.749 | 1.607 |
| 0.10 | 2.599 | |
| 0.10 | 2.929 | |
| 0.10 | 2.950 | |
| 0.10 | 2.520 | |
| 0.10 | 2.367 | |
| 0.10 | 2.382 | 2.426 |

Table A3.9.

Scan Ratios for pDS49/pDSS Amplification Efficiency.

| pDS49 ng | Scan Ratio | Mean Scan Ratio |
|-----------------|-------------------|------------------------|
| 0.04 | 0.542 | |
| 0.04 | 0.519 | |
| 0.04 | 0.550 | |
| 0.04 | 0.588 | |
| 0.04 | 0.605 | 0.558 |
| 0.06 | 0.917 | |
| 0.06 | 1.052 | |
| 0.06 | 0.846 | |
| 0.06 | 0.808 | |
| 0.06 | 0.891 | 0.941 |
| 0.08 | 1.183 | |
| 0.08 | 1.199 | |
| 0.08 | 1.180 | |
| 0.08 | 1.119 | |
| 0.08 | 0.951 | |
| 0.08 | 0.933 | 1.126 |
| 0.10 | 1.213 | |
| 0.10 | 1.431 | |
| 0.10 | 1.233 | |
| 0.10 | 1.345 | |
| 0.10 | 1.197 | 1.318 |

BIBLIOGRAPHY.

Amman, R., Zarda, B., Stahl, D.A. and Schleifer, K-H. 1992. Identification of individual procaryotic cells by using enzyme labelled, rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology* **58** 3007-3011.

Attwood, G.T., Lockington, R.A., Xue, Gang-Ping, Brooker, J.D. 1988. Use of a unique gene sequence to enumerate a strain of *Bacteroides ruminicola* introduced into the rumen. *Applied and Environmental Microbiology* **54** 534-539.

Avgustin, G., Wright, F., Flint, H.J. 1994. Genetic diversity and phylogenetic relationships among strains of *Prevotella (Bacteroides) ruminicola* from the rumen. *International Journal of Systematic Bacteriology*. **44** 246-255.

Bej, A.J., Stefan, R.J., DiCesare, J., Haff, L. and Atlas, R.M. 1990. Détection of coliform bacteria in water by polymerase chain reaction and gene probes. *Applied and Environmental Microbiology* **56** 307-314.

Bourgeois, P., Mata, M., Ritzenthaler, P. 1989. Genome comparison of *Lactobacillus*. *FEMS Microbial Letters*. **50** 65-69.

Briesacher, S.L., May, T., Grigsby, K.N., Kerley, M.S., Anthony, R.V., Paterson, J.A. 1992. Use of DNA probes to monitor nutritional effects on rumen procaryotes and *Fibrobacter succinogenes* S85. *Journal of Animal Science*. **70** 289-295.

Brooker, J.D., Stokes, B. 1990. Monoclonal antibodies against the ruminal bacterium *Selenomonas ruminantium*. *Applied and Environmental Microbiology* **56** 2193-2199.

- Brooker, J.D., Lum, D.K. 1993. Use of transposon TN916 as a genetic marker in the rumen. *Letters in Applied Microbiology* **17** 224-2127.
- Cecava, M.J., Merchan, N.R., Gay, L.C., Berger, L.L. 1990. Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. *Journal of Dairy Science*. **73** 2480-2488.
- Chaudry, G.R., Toranzos, G.A., Bhatti, A.R. 1989. Novel Method for monitoring genetically engineered organisms in the Environment. *Applied and Environmental Microbiology* **55** 1301-1304.
- Chelly, J., Kaplan, J-C, Maire, P., Gauron, S. and Kahn, A. 1988. Transcription of the dystrophin gene in human muscle and non-muscle tissue. *Nature* **333** 858-860.
- Chou, J.L., Russell, M., Birch, D., Raymond, J., Bloch, W. 1992. Prevention of pre-PCR mispriming and primer dimerisation improves low-copy-number amplifications. *Nucleic Acids Research*, **20** 1717-1723.
- Cimino, G.C., Metchette, K. C., Tessman, J.W., Hearst, J.E. and Isaacs, S.T. 1991. Post-PCR sterilization: a method to control carry over contamination for the polymerase chain reaction. *Nucleic Acids Research* **191** 99-107.
- Cooper, C.L., Vercoe, P.E., Ford, M., Gregg, K. 1993. Single-step amplification sequencing reactions (ASR) on genomic DNA. *Asia Pacific Journal of Molecular Biology and Biotechnology*. **1** 122-125
- DeFelippes, F.R. 1991. Decontaminating the Polymerase Chain Reaction. *Biotechniques* **10** 26-30

Flint, H.J., Bisset, J., Webb, J. 1989. Use of antibiotic resistance mutations to track strains of obligately anaerobic bacteria introduced into sheep. *Journal of Applied Bacteriology*. **67** 177-183.

Flint, H.J., Bisset, J. 1991. Genetic diversity in *Selenomonas ruminantium* isolated from the rumen. *FEMS Microbiology Ecology* **73** 351-360.

Forsberg, C.W., Crosby, R. and Thomas, D.Y. 1986. Potential for manipulation of the rumen ecosystem through the use of recombinant DNA techniques. *Journal of Animal Science*. **63** 310.

Forster, R.J., Teather, R.M., Gong, J., Deng, S-J. 1996. 16S rDNA analysis of *Butyrivibrio fibrisolvens* phylogenetic position and relationship to the butyrate-producing anaerobic bacteria from the rumen of white-tailed deer. *Letters in Applied Microbiology*. **23** 218-222.

Fox, G.E., Stackebrandt, E., Hespell, R.B., Gibson, J., Maniloff, J., Dyer, T., Wolfe, R., Gupta, R., Bonen, L., Lewis, B., Stahl, D., Luehrson, K., Chen, K. and Woese, C. 1980. The phylogeny of procaryotes. *Science*. **209** 457-463.

Frutos, R., Pages, M., Bellis, M., Roizes, G., Bergoin, M. 1989. Pulsed field electrophoresis determination of the genome size of obligate intra cellular bacteria belonging to the genera *Chlamydia*, *Rickettsia* and *Porochlamydia*. *Journal of Bacteriology*. **171** 4511-4513.

Gilliland, G., Perrin, S. and Bunn, H.F. 1990. Competitive PCR for quantitation of mRNA. In PCR Protocols: a Guide to Methods and Applications. Ed. Innis, M.A. *et al.* Academic Press Inc. San Diego. p60-69.

Gregg, K., Bauchop, T., Hudman, J.F., Vercoe, P., Ware, C.E., Woods, J.R., Leng, R.A. 1987. Application of recombinant DNA methods to rumen bacteria. *Proceedings of Recent Advances in Animal Nutrition in Australia*. ed D. Farrell. University of New England. Pp 112-120

Gregg, K., Bauchop, T., Leng, R.A. 1989. Genetic Engineering of rumen microorganisms. In Biotechnology for Livestock Production. An FAO/Plenum Publication, Plenum Publishing Corporation. pp 263-75.

Gregg, K. and Ware, C. 1990. Importance of genetic diversity in manipulation of rumen bacteria. In Microbial and Plant Opportunities to Improve Lignocellulose Utilisation by Ruminants. Ed Akin, D.E., Ljungdahl, L.G., Wilson, J.R., Harris, P.J. Elsevier Science Publishers, London. pp 357-66.

Gregg, K. 1991. State of the art in rumen biotechnology. *Proceedings of Recent Advances in Animal Nutrition in Australia*. ed D. Farrell. University of New England. pp 1-11.

Gregg, K. and Sharpe, H. 1991. Enhancement of rumen microbial detoxification by gene transfer. In Physiological Aspects of Digestion and Metabolism in Ruminants. Ed Tsuda, T., Sasaki, Y., Kawashima, R. Academic Press, San Diego. Pp 719-735.

Gregg, K., 1992. Developments in rumen bacterial biotechnology. *AgBiotech News and Information*. 4 375N-380N.

Gregg, K., Allen, G., Bauchop, T., Klieve, A. and Lincoln, M. 1993. Practical genetic engineering of rumen bacteria. *Proceedings of Recent Advances in Animal Nutrition in Australia*. ed D. Farrell. University of New England. pp 13-21.

- Gregg, K., Cooper, C.L., Schafer, D.J., Sharpe, H., Beard, C.E., Allen, G., Xu, J. 1994. Detoxification of the plant toxin fluoracetate by a genetically modified rumen bacterium. *Bio/Technology*. **12** 1361-1365.
- Gregg, K. 1995. Engineering gut flora of ruminant livestock to reduce forage toxicity: progress and problems. *Trends in Biotechnology*. **13** 418-421.
- Gregg, K., Allen, G., Beard, C. 1996. Genetic manipulation of rumen bacteria: from potential to reality. *Australian Journal of Agricultural Research* **47** 247-256.
- Hammond, A.C., Allison, M.J., Williams, M.J., Prine, G.M., Bates, D.B. 1989. Prevention of leucaena toxicosis of cattle in Florida by ruminal inoculation with 2-hydroxy-4-(1H)-pyridone -degrading bacteria. *American Journal of Veterinary Research*. **50** 2176-2180.
- Hespell, R.B. 1989. Biotechnology and modification of the rumen ecosystem. *Proc. Nutrition Society*. **46** 407-413
- Higuchi, R. 1990. Recombinant PCR. In PCR Protocols: a Guide to Methods and Applications. Ed. Innis, M.A. *et al.*. Academic Press Inc. New York. pp.177-183
- Hoover, W.H., Miller, T.K. 1991. Rumen digestive physiology and microbial ecology. *Veterinary and Clinical North American Food Animal Practices*. **7** 311-325.
- Hudman, F.A. and Gregg, K.G. 1989. Genetic diversity among strains of bacteria from the rumen. *Current Microbiology* **19** 313-318.
- Hung, T., Mak, K. and Fong, K. 1990. A specificity enhancer for the polymerase chain reaction. *Nucleic Acids Research*. **18** 4953.

- Innes, M.A. 1990. In PCR Protocols: a Guide to Methods and Applications. Ed. Innis, M.A. *et al.* Academic Press Inc., San Diego. pp 15-33
- Jain, R.K., Saylor, G.S., Wilson, J.T., Houston, L., Pacia, D. 1987. Maintenance and stability of introduced genotypes in groundwater aquifer material. *Applied and Environmental Microbiology* **53** 996-1002.
- Jones, R.J. and McGarrity, R.G. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of *Leucaena*. *Australian journal of Veterinary Science.* **63** 259-264.
- Joshi, A.K., Baichwal, V., Ames, G.F-L. 1991. Rapid polymerase chain reaction amplification using intact bacterial cells. *BioFeedback.* **10** 42-44.
- Kamman, M., Laufs, J., Schell, J., Groneborn, B. 1989. Rapid insertional mutagenesis of DNA by polymerase chain reaction. *Nucleic Acids Research* **17** 5404.
- Kawasaki, H., Yahara, H. and Tonomura, K. 1984. Cloning and expression in *E. coli* of the haloacetate dehalogenase genes from *Moraxella* plasmid pUO1. *agricultural and Biological Chemistry.* **48** 3627-3632.
- Kellogg, D.E., Sninsky, J.J., Kwok, S. 1990. Quantitation of HIV-1 proviral DNA relative to cellular DNA by the polymerase chain reaction. *Analytical Biochemistry* **189** 202-208.
- Klieve, A.V., Gregg, K., Bachope, T. 1991. Isolation and characterisation of lytic phages from *Bacteroides rumenicola* ss *brevis*. *Current Microbiology* **23** 180-183.

- Klijn, N., Weerkamp, A.H., de Vos, W.M. 1995. Detection and characterisation of lactose using *Lactobacillus* spp. in natural ecosystems. *Applied and Environmental Microbiology* **62** 788-792
- Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* **339** 237-238.
- Kwok, S., Kellogg, D.E., McKinney, M., Spasic, D., Goda, L., Levenson, C., Sninsky, J.J. 1990. Effects of primer-template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies. *Nucleic Acids Research* **18** 999-1005.
- Lampel, K.A., Sagow, J.A., Trucksess, M., Hill, W.E. 1990. Polymerase chain reaction for the detection of *Shigella flexneri* in food. *Applied and Environmental Microbiology* **56** 1536-1540.
- Lane, D.J. 1991. 16S/23S rRNA sequencing. in Nucleic Acid Techniques in Bacterial Systematics. Ed. Stackenbrandt, E. & Goodfellow, M. Chichester: Academic Press.
- Li, H., Cui, X., Arnheim, N. 1991. Eliminating primers from completed polymerase chain reactions with exonuclease III. *Nucleic Acids Research* **19** 3139.
- Lin, C., Flesher, B., Capman, W.C., Anman, R.I., Stahl, D.A. 1994. Taxon specific hybridisation probes for fiber-digesting bacteria suggest novel gut-associated *Fibrobacter*. *Systematic Applied Microbiology*. **17** 413-424.
- Ludwig, W. and Schleifer, K.H., 1994. Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiological Reviews* **15** 155-173.

McSweeney, C.S., Mackie, R.I., Odenyo, A.A., Stahl, D.A. 1993. Development of an oligonucleotide probe targeting 16s rRNA and its application for detection and quantitation of the ruminal bacterium *Synergistes jonesii* in a mixed population chemostat. *Applied and Environmental Microbiology*. **59** 1607-1612.

Mannarelli, B.M. 1988. Deoxyribonucleic acid relatedness among strains of the species *Butyrivibrio fibrisolvens*. *International Journal of Systemic Bacteriology*. **38** 340-347.

Mannarelli, B.M., Ericsson, L.D., Lee, D., Stack, R.J. 1991. Taxonomic relationships among strains of the anaerobic bacterium *Bacteroides ruminicola* determined by DNA and extracellular polysaccharide analysis. *Applied and Environmental Microbiology*. **57** (10) 2975-2980.

Marmur, J. 1963. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *Methods in Enzymology* **6** 726-738.

Miyagi, T., Kaneichi, K., Aminov, R.I., Koboyashi, Y., Sakka, K., Hoshino, S., Omiya, K. 1995. Enumeration of transconjugated *Ruminococcus albus* and its survival in the goat rumen microcosm. *Applied and Environmental Microbiology*. **61** 2030-2032.

Morgan, A.J., Winstanley, C., Pickup, R.W., Jones, G., Saunders, J.R. 1989. Direct phenotypic and genotypic detection of a recombinant pseudomonad population released into lake water. *Applied and Environmental Microbiology* **55** 2537-2544.

Moyer, C.L., Dobbs, F.C., Karl, D.M. 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16s rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology* **60** 871-879.

- Mullis, K.B., and Faloona, F. 1987. Specific synthesis of DNA *in Vitro* via a Polymerase-Catalysed Chain reaction. *Methods in Enzymology* **155** 335-350.
- Murphy, L.D., Herzog, C.E., Rudick, J.B., Fojo, A.J., Bates, S.E. 1990. Use of the polymerase chain reaction in the quantitation of *mdr-1* gene expression. *Biochemistry* **29** 10351-10356.
- Odenyo, A.A., Mackie, R.I., Stahl, D.A., White, B.A. 1994. The use of 16s rRNA targeted oligonucleotide probes to study competition between rumen fibrolytic bacteria: pure culture studies with cellulose and wheat straw. *Applied and Environmental Microbiology*. **60** 3697-3703.
- Olive, D.M. 1989. Detection of enterotoxic *Escherichia coli* after polymerase chain amplification with a thermostable DAN polymerase. *Journal of Clinical Microbiology* **27** 261-265.
- Oluboboken, J.A., Craig, W.M. 1990. Quantity and characteristics of microorganisms associated with ruminal fluid or particles. *Journal of Animal Science*. **68** 3360-3370.
- Pace, N.R., Stahl, D.A., Lane, D.J. and Olsen, G.J. 1986. The use of rRNA sequences to characterise natural microbial populations. *Advances in Microbial Ecology*. **9** 1-55.
- Paster, B.J., Dewhirst, F.E., Olsen, I., Fraser, G.J. 1994. Phylogeny of *Bacteroides*, *Prevotella* and *Porphyomonas* ssp and related bacteria. *Journal of Bacteriology*. **176** 725-732.
- Pickup, R.W. 1991. Development of molecular methods for the detection of specific bacteria in the environment. *Journal of General Microbiology* **137** 1009-1019.

Rainey, F.A. and Janssen, P.H. 1995. Phylogenetic analysis by 16S ribosomal DNA sequence comparison reveals two unrelated groups of species within the genus *Ruminococcus*. *FEMS Microbiological Letters*. **129** 69-73.

Rozas, J. 1991. A programme to optimise the design of oligonucleotides for PCR amplification. *The Journal of Heredity*. **62** 84.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Ehrlich, H.H. 1988. Primer-directed enzymic amplification of DNA with a thermostable DNA polymerase. *Science* **239** 487-491.

Saint, C.P. 1993. Molecular developments for the tracking of microorganisms in the environment. *Bio/Technology* **3** 76-80

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular cloning: A laboratory manual. Cold Springs Harbor Laboratory Press.

Sarkar, G., Kapeiner, S. and Sommer, S.S. 1990. Formamide can dramatically improve the specificity of the polymerase chain reaction. *Nucleic Acids Research* **18** 7465.

Sayler, G.S., Layton, A.C. 1990. Environmental application of nucleic acid hybridisation. *Annual review of Microbiology*. **44** 625-648.

Shah, H.N and Collins, D.M. 1990. *Prevotella*, a new genus to include *bacteroides melangogenus* and related species formerly classified in the genus *Bacteroides*. *International Journal of Systematic Bacteriology*. **40** 205-208.

Sharp, R., Hazelwood, G.P., Gilbert, H.J., O'Donnell, A.G. 1994. Unmodified and recombinant strains of *Lactobacillus planarum* are rapidly lost from the rumen by protozoal predation. *Journal of Applied Bacteriology* **76** 110-17.

- Siebert, P.D., Larrick, J.W. 1992. Competitive PCR. *Nature* **359** 557-558.
- Smith, C.J. and Hespell. R.B. 1983. Prospects for development and use of recombinant deoxyribonucleic acid techniques with ruminal bacteria. *Journal of Dairy Science*. **66** 1536-1556.
- Sommer, R., Tautz, D. 1989. Minimal homology requirements for PCR primers. *Nucleic Acids Research* **17** 6749.
- Stahl, D.A., Flesher, B., Mansfield, H.R., Montgomery, L. 1988. Use of phylogenically based hybridisation probes for studies in ruminal microbial ecology. *Applied and Environmental Microbiology*. **54** 1079-1084.
- Starnbach, M.N., Falkow, S., Tompkins, L.S. 1989. Species specific detection of *Legionella pneumophila* in water by DNA amplification and hybridisation. *Journal of Clinical Microbiology* **27** 1257-1261.
- Steffan, R.J., Atlas, R.M. 1988. DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. *Applied and Environmental Microbiology* **54** 2185-2191.
- Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., Painter, P.R. General microbiology. 5th Ed. 1987. Table 10.1, p. 236.
- Steffan, R.J., Atlas, R.M. 1991. Polymerase chain reaction: applications in environmental microbiology. *Annual Review of Microbiology* **45** 137-162.
- Stewart, C.S. and Bryant, M.F. 1988. The rumen bacteria. In The rumen microbial ecosystem. Ed. Hobson, P.N. Elsevier, Earking, Essex, UK.

Szabo, E.A., Pemberton, J.M., Gibson, A.M., Eyles, M.J., Demarchellier, P.M. 1994. Polymerase chain reaction for detection of *Chlostridial botulinum* types A, B and E in food. *Journal of Applied Bacteriology* . **76** 539-554.

Teather, R.M. 1985. Application of gene technology to rumen microflora. *Canadian Journal of Animal Science*. **43** 563-574

Vercoe, P. and Gregg, K. 1992. DNA sequence and transcription of an endoglucanase gene from *Prevotella (Bacteroides) ruminicola*. *Molecular Genes and Genetics*. **233** 284-292.

Vercoe, P. and Gregg, K. 1995. Sequence and transcriptional analysis of an endoglucanase gene from *R. albus* AR6^T. *Animal Biotechnology*. **61** 59-71.

Wallace, R.J. 1992. Rumen microbiology, biotechnology and ruminant nutrition: the application of research findings to the complex ruminal ecosystem. *FEMS Microbiology Letters* **79** 529-534.

Wallace, R.J. 1994. Rumen microbiology, biotechnology and ruminant nutrition: progress and problems. *Journal of Animal Science*. **72** 2992-3003

Wang, A.M., Mark ,D. 1990. Quantitative PCR. In PCR Protocols: a Guide to Methods and Applications. Ed. Innis,M.A. *et al.* Academic Press Inc., San Diego. pp 70-75.

Wang, R-F., Cao, W-W., Johnson, M.G. 1992. 16s rRNA probes and polymerase chain reaction method to detect *Listeria monocytogenes* cells added to foods. *Applied and Environmental Microbiology*. **58** 2827-2831.

- Wang, R-F., Cao, W-W., Cerniglia, C.E. 1996. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Applied and Environmental Microbiology*. **62** 1242-1247.
- Ware, C.E., Bauchop, T. and Gregg, K. 1989. Isolation and comparison of cellulase genes from two strains of *Ruminococcus albus*. *Journal of General Microbiology*. **135** 921-930.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D. 1991. 16s ribosomal RNA amplification for phylogenetic study. *Journal of Bacteriology* **172** 697-703.
- Welch, D., Lee, Chao-Hung, Larsen, S.H. 1990. Detection of plasmid DNA from all *Chlamydia trachomatis* serovars with a twostep polymerase chain reaction. *Applied and Environmental Microbiology* **56** 494-498.
- West, Suzanne. 1995. Method of improving nutrition by ruminant or ruminant-like animals. International Patent Application WO/95 11041 (AU 79845/94). W.I.P.O., Geneva.
- Whitehouse, N.L., Olson, V.M., Schwab, C.G., Chesbro, W.R., Cunningham, K.D., Lykos, T. 1994. Improved techniques for dissociating particle-associated mixed ruminal microbes from ruminal digesta solids. *Journal of Animal Science*. **72** 1355-1343.
- Willems, A., Amat-Marco, M., Collins, M.D. 1996. Phylogenetic analysis of *Butyrivibrio* strains reveals three distinct groups of species within the *Chlostridium* subphylum of the gram-positive bacteria. *Journal of Systematic Bacteriology*. **46** 195-199.
- Williams, A.G. and Coleman, G.S. 1988. The rumen bacteria In The Rumen Microbial Ecosystem. Elsevier, Barking, UK.

Williams, A.G. and Bryant, R.A. 1988. The rumen protozoa. In The Rumen Microbial Ecosystem. Elsevier, Barking, UK.

Woese, C.R., Stackebrandt, E., Macke, T.J. and Fox, G.E. 1985. A phylogenetic definition of the major eubacterial taxa. *Systematic and Applied Microbiology*. **6** 143-151.

Woods, J.R., Hudman, F. and Gregg, K. 1989. Isolation of an endoglucanase gene from *Bacteroides rumenicola* subsp. *Brevis*. *Journal of General Microbiology*. **135** 2543-2549.

Wu, D.Y., Ugozzoli, L., Pal, B.K., Qian J., Wallace, B. 1991. The effect of temperature and oligonucleotide primer length on the specificity and efficiency of amplification by the polymerase chain reaction. *DNA and Cell Biology* **10** 233-238.

Yolov, A.A., Zabara, Z.A. 1990. Constructing DNA by polymerase recombination. *Nucleic Acids Research* **18** 3983-86.

Zhu, S.Y., Isaacs, S.T., Cimino, G.D., Hearst, J.E. 1991. The use of exonuclease III for polymerase chain reaction sterilisation. *Nucleic Acids Research* **19** 250.