

A

A

```
CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....10.....20.....30.....40.....50.....60.....70.....80.....90
ATGAAAAAATTATTAGCAGCTATGTTAATGACCTTTTTCTTGACTCCTTTGACCGCTTATTAGCACAGAAAAACAGGACATTTTCTAA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....100.....110.....120.....130.....140.....150.....160.....170.....180
GCAACTATTTACCAATTGCGTCAAGATATTGTTCAATCAACAGCTACTATATCAGCTTCTGTTAATCCTAACCTTTATCAAGAAACA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....190.....200.....210.....220.....230.....240.....250.....260.....270
CAAACTTATAAAGACGCTGAACTAACTTTACCAGCGCAACCCCTAGGACCTAATCATAGTTAGATATCAAGTCACCTTGTGATAAATGAA
.....
AGGACCTAATCATAGTTAGATATCAAGTCACCTTGTGATAAATGAA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....280.....290.....300.....310.....320.....330.....340.....350.....360
CAAGCAAAACCAATTTTGAACTTGCAGATGGTACTTACCCTGAAGCAAAATCGCGAAGTAGTTTATGAGGATATAGTTCTGAGTCAAGTA
CAAGCAAAACCAATTTTGAACTTGCAGATGGTACTTACCCTGAAGCAAAATCGCGAAGTAGTTTATGAGGATATAGTTCTGAGTCAAGTA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....370.....380.....390.....400.....410.....420.....430.....440.....450
GAGCGCGACTCTCTTTTTTGGACACAAAACAAAGTGACTCTCTACTCTGAACCCCTATGTGTTAGGCACTAAGGCAATTCATCTCCATTT
GAGCGCGA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....460.....470.....480.....490.....500.....510.....520.....530.....540
ACCTTTGCCAAAAAGTTTCATGCTAGTCAATGGCACAACCCGACATGGGATTTATCTFAATTGATCAGAAAGGTTGGGATCACAA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....550.....560.....570.....580.....590.....600.....610.....620.....630
GAGGATCTATCTGTTTTGATAATCGCATGCAAAAGTCCCAAGAGATGCTGTACAAAAATACAAATATGCAAATTTATCTATCTTTGT
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....640.....650.....660.....670.....680.....690.....700.....710.....720
AAACAACCTTGATACCGAGGCAAGTGCTGGAAATTAATGCAGATAAAGAAATGATGCAGCTAGTATTGCAAAAGTTAGCCCCCTTTTACAGT
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....730.....740.....750.....760.....770.....780.....790.....800.....810
GTTCAAAAACCACTTCAAGAAAGAAAGTGACAGAAACCAATTTCTCAGATATATCGATGATGTGAATCATTTTTATGGGGATTACAGT
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....820.....830.....840.....850.....860.....870.....880.....890.....900
CCATCTGGAAGTGGTAAAAATTAGTAAAAAGCTGATAAAGAAAGCATATAAGTTAGTACCTTATTGAAGAGCTGTTGCTCAAAATCTCAGAT
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....910.....920.....930.....940.....950.....960.....970.....980.....990
AATGTTGCCACCATAATTTTATAGGATATTATTTATGCCATCAGTATGACCAAGCTTTTCAACAGAAATAAAAGCCTTGTTCAGGAAGTAAC
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....1000.....1010.....1020.....1030.....1040.....1050.....1060.....1070.....1080
TGGGATATGAAAAACGATTTCTTATCTTCTCGTGCAAGCTAATGTGATGGAAGCATTTTACTATCAAAAGGGACACATATTTTCTTAT
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....1090.....1100.....1110.....1120.....1130.....1140.....1150.....1160.....1170
CTTTCCAATACCTGATTTTATGCAACACGCAATTTCAAAAAATATGCTGTCCTCTGGGCCCAATAAAATGGTGATGCCATGGAATTCACAG
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....1180.....1190.....1200.....1210.....1220.....1230.....1240.....1250.....1260
CATGATGTTGCTATCATTTATGGTGATATCCCTTTTGTCTTACTATCTTTACAGATAAAGCAACCTATGATGACATCACAGCTATTGCA
.....

CP068478.1:173816-172530
S. dysgalactiae (Sanger seq.)
.....1270.....1280
GATGATGTTTATGCTATTTTAAAAATGA
.....
```

Supplementary Figure 3: Alignment of the amplicon sequence (Sanger sequencing) generated from the primers designed in the present study and the target region in the reference genomes of each pathogen. A) *Streptococcus dysgalactiae*, B) *Streptococcus uberis*

B

```

      10      20      30      40      50      60      70      80      90
LS483408.1:c1704429-1702096  ATGAATCAAAAAATTTTAGAACAAATTAGAAATTTCAAAAAGTTAAAGAACAAATCCAAACCTATCTGCAGACAGCCCAAGGAATCCAGGAA
S. uberis (Sanger seq.)

      100     110     120     130     140     150     160     170     180
LS483408.1:c1704429-1702096  TTACGTTCTTCTTGAACCAAGCAACCACTATGACAAAGATTTCAGATTACTTTAAAGAAATGCTGAATGGCAACCACTTTTGTGGAAAT
S. uberis (Sanger seq.)

      190     200     210     220     230     240     250     260     270
LS483408.1:c1704429-1702096  CATCATTTTCAATAGGGAATCTTCGCGATGTCCTCCGAGAGTATGCGTCGCTTGGAACTAGAAGCAGACTTGAAATCCCAAGAGTTGTTA
S. uberis (Sanger seq.)

      280     290     300     310     320     330     340     350     360
LS483408.1:c1704429-1702096  GATATCAAAAAATGCTCTTAGTTTCTGGGGAAATCAGTCGTTTTTACCAGAGTTTAGAAATGTGGACCTTCAGGTTTTAAACGAATA
S. uberis (Sanger seq.)

      370     380     390     400     410     420     430     440     450
LS483408.1:c1704429-1702096  TTTGAAAAAATAGAAGTCTTTCCACAATTACCAAGGAAGTTTACAGGCTGTTAATGATGCAGCCTTATTGAAAAATTTTGCAGTCCAGAA
S. uberis (Sanger seq.)

      460     470     480     490     500     510     520     530     540
LS483408.1:c1704429-1702096  TTGGATCGCCTTAGACGTCAAATAGCAGAAATGAACGTTATAGTCGCCAATTGCTACAAGACATCCTAAGAGAAATCCAGATTATTG
S. uberis (Sanger seq.)

      550     560     570     580     590     600     610     620     630
LS483408.1:c1704429-1702096  TCTGAATCATTTAGTTGCTAGTCTGTAATGGTAGAGTGTCTTACCTGTTAAGAAATACCTTTTCGTACACAGAGTTTACAGGGTTGTCATGAT
S. uberis (Sanger seq.)

-----640-----650-----660-----670-----680-----690-----700-----710-----720
LS483408.1:c1704429-1702096  ATCTCAGCATCTGGAATACCGTTTACATTGAACCTCGTGCCTTAGTGCAAGTCAATGAAGAAATGACGCAATTACTAGCAGATGAAAGG
S. uberis (Sanger seq.)
                    -TCATTGAACCTCGTGCCTTAGTGCAAGTCAATGAAGAAATGACGCAATTACTAGCAGATGAAAGG

      730     740     750     760     770     780     790     800     810
LS483408.1:c1704429-1702096  CATGAAATTCGAAGGATACTCAAAGAGTTGTACAGATGTTACGTCCTCATAGTCGTGCCGATAGCAAAATATGATGGCTTTTGGGACAT
S. uberis (Sanger seq.)
CATGAAATTCGAAGGATACTCAAAGAGTTGTACAGATGTTACGTCCTCATAGTCGTGCCGATAGCAAAATATGATGGCTTTTGGGACAT

      820     830     840     850     860     870     880     890     900
LS483408.1:c1704429-1702096  CTAGATTTTGTAGAGCAAGATATCATATCTTGTTAATAACAAAGCAACCATCCCTAAAAATTAGCAAGATAAAAATATCCAGCTATTA
S. uberis (Sanger seq.)
CTAGA

-----910-----920-----930-----940-----950-----960-----970-----980-----990
LS483408.1:c1704429-1702096  AATGTTAGACACCCCTTTGTAAAAAATCCTGTGGCCAATGATTTGCATTTTGCAGATGATTTAGCCGTGATTTGATTACGGGCCCTAAT
S. uberis (Sanger seq.)

      1000    1010    1020    1030    1040    1050    1060    1070    1080
LS483408.1:c1704429-1702096  ACAGGTGGTAAGACCAATATGCTTAAAAACACTGGGATTAGCACAATTAAATGGGTCAGTCAGGTCCTCCCAATATTAGCAGATGAAGGCTCA
S. uberis (Sanger seq.)

      1090    1100    1110    1120    1130    1140    1150    1160    1170
LS483408.1:c1704429-1702096  AAAATAGCTGTTTTGTATGCCATTTATGCGCAGCATAGGCGATGAGCAATCCATCGAACAAAGTCTATCAACCTTCTCAAGTCATATGACA
S. uberis (Sanger seq.)

      1180    1190    1200    1210    1220    1230    1240    1250    1260
LS483408.1:c1704429-1702096  CATATCGTTGACATTTTAGAGGAATCAAAATAGTAACAGTTTAGTCCTTTTGTATGAACCTGGTGCAGAACAGATCCACAAGAGGTGCC
S. uberis (Sanger seq.)

      1270    1280    1290    1300    1310    1320    1330    1340    1350
LS483408.1:c1704429-1702096  AGTCTTGCTATGGCAATCTTAGAGCAATTACGCTTAACCAATATCAAAACCATGGCAACACTCATATTCCAGAGCTCAAGGCCTATGGT
S. uberis (Sanger seq.)

      1360    1370    1380    1390    1400    1410    1420    1430    1440
LS483408.1:c1704429-1702096  ATTGAAAGTGACTATGTCGAAATGCTAGCATGGGAATTTGATAGTCAAGCCCTAAAACCCACTTATCGCTTTATGCAAGGTGTTCTGGT
S. uberis (Sanger seq.)

      1450    1460    1470    1480    1490    1500    1510    1520    1530
LS483408.1:c1704429-1702096  CGATCCAAACGCTTTGAATTTGCTCGTCGACTTGGTTTAGCTGAACGATTTGAAAGAAAGCGGAGCAGATGACAGATACCGATAGTGAC
S. uberis (Sanger seq.)

      1540    1550    1560    1570    1580    1590    1600    1610    1620
LS483408.1:c1704429-1702096  GTCAATCGTATCATAGAAGAGTTAGAACGACAGACGCTTTCTAGCCGGCGACGCTTGATCATATCAGAGAAGTTGAGCAGGAAATATA
S. uberis (Sanger seq.)

      1630    1640    1650    1660    1670    1680    1690    1700    1710
LS483408.1:c1704429-1702096  AAATTCAATCGTGTCTCAAGAACTCTATATGAATTTCTCTTGGCGAAAGACAAAGAAATTTGAGAAAGCTAGTCAAGAAAGCGCAAGCT
S. uberis (Sanger seq.)

      1720    1730    1740    1750    1760    1770    1780    1790    1800
LS483408.1:c1704429-1702096  ATTGTTGAGTTAGCCTTAAACCGAAAGTGAAGAGATTTTGTAGTAAGCTCCATGAAAAAGCAGAGCTTAAACACATGAATATTATTGAAGCA
S. uberis (Sanger seq.)

      1810    1820    1830    1840    1850    1860    1870    1880    1890
LS483408.1:c1704429-1702096  AAATCAAAACTGAAGTCACTGGTTCCTCAAGAGATTTATCTAAGAATAAAGTCTGAAAAAAGCTAAGAAATTAAGAGAGCCTAGGATA
S. uberis (Sanger seq.)

      1900    1910    1920    1930    1940    1950    1960    1970    1980
LS483408.1:c1704429-1702096  GGTGATGATATATTATTGTTCTGATATGGTCAACCGGAACTTAATTTGGTCAAGTGAAGGGGAATTAAGTGGGAAGCTCAAGTTGGACTG
S. uberis (Sanger seq.)

      1990    2000    2010    2020    2030    2040    2050    2060    2070
LS483408.1:c1704429-1702096  ATTAAGATGACTTTAAAGAGGAGTGAATTTCCAACTCGTAAAGTTGGAGCGGAAGCACAAGAAACCTAAGAAACCAAGTATTAAACATGGTT
S. uberis (Sanger seq.)

      2080    2090    2100    2110    2120    2130    2140    2150    2160
LS483408.1:c1704429-1702096  AAAAAAGCCAATCAAAATGGTCTCTGGGCAGCTTTAGATCTTCTGTGGAAACCTTATGAAGAACCATGCAAGAACTAGATGCTCTTTATA
S. uberis (Sanger seq.)

      2170    2180    2190    2200    2210    2220    2230    2240    2250
LS483408.1:c1704429-1702096  GACCAAGCATGGTTAATAATATGAGCCAGGTTGATATCATCCATGGTATTGGAACAGGTGTCTATTAGAGAAGCTGTATCCAAATATCTT
S. uberis (Sanger seq.)
```