Figure 1

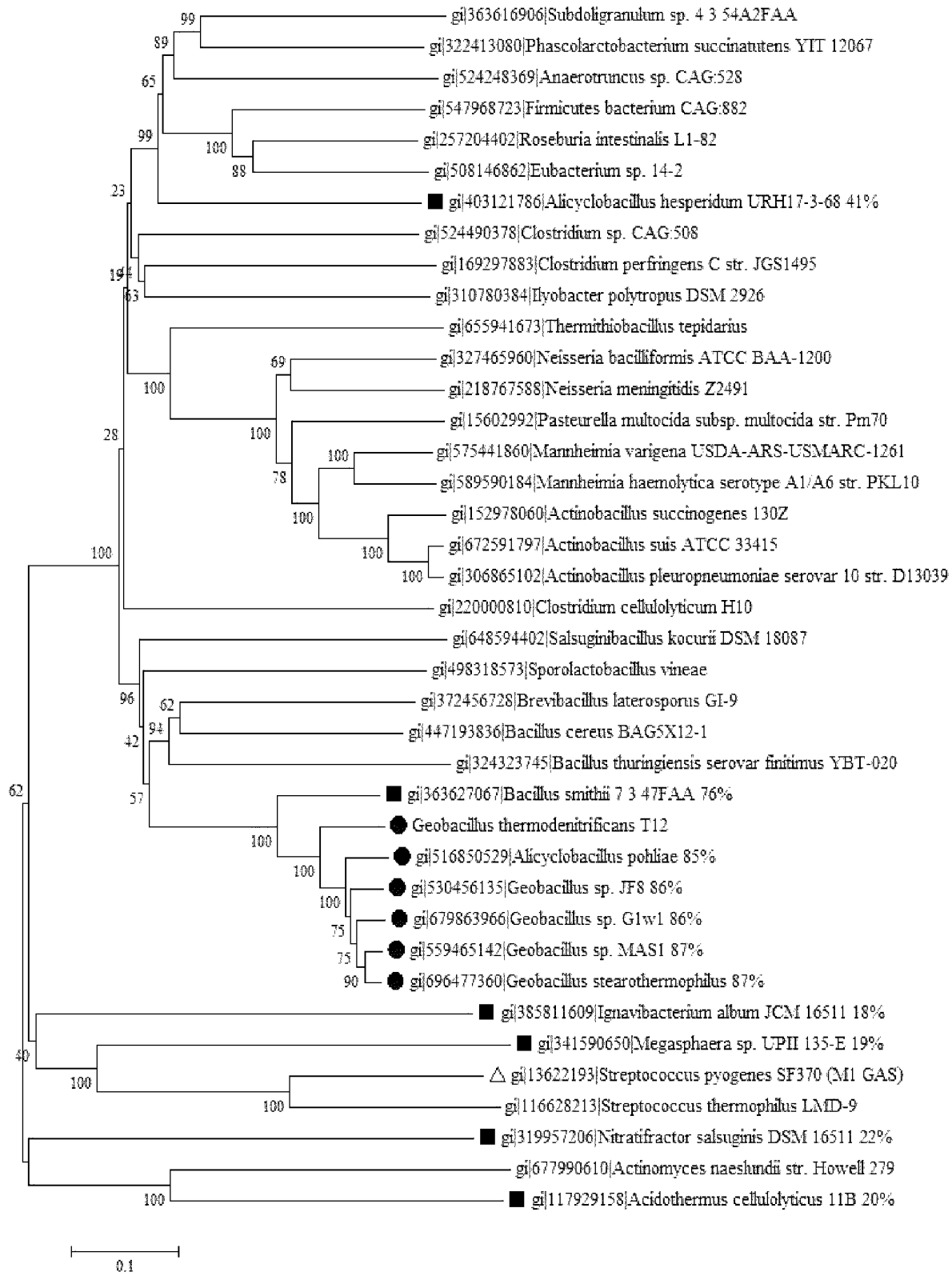

gi|363616906|Subdoligranulum sp. 4 3 54A2FAA
gi|322413080|Phascolarctobacterium succinatutens YIT 12067
gi|524248369|Anaerotruncus sp. CAG:528
gi|547968723|Firmicutes bacterium CAG:882
gi|257204402|Roseburia intestinalis L1-82
gi|508146862|Eubacterium sp. 14-2
gi|403121786|Alicyclobacillus hesperidum URH17-3-68 41%
gi|524490378|Clostridium sp. CAG:508
gi|169297883|Clostridium perfringens C str. JGS1495
gi|310780384|Ilyobacter polytropus DSM 2926
gi|655941673|Thermithiobacillus tepidarius
gi|327465960|Neisseria bacilliformis ATCC BAA-1200
gi|218767588|Neisseria meningitidis Z2491
gi|15602992|Pasteurella multocida subsp. multocida str. Pm70
gi|575441860|Mannheimia varigena USDA-ARS-USMARC-1261
gi|589590184|Mannheimia haemolytica serotype A1/A6 str. PKL10
gi|152978060|Actinobacillus succinogenes 130Z
gi|672591797|Actinobacillus suis ATCC 33415
gi|306865102|Actinobacillus pleuropneumoniae serovar 10 str. D13039
gi|220000810|Clostridium cellulolyticum H10
gi|648594402|Salsuginibacillus kocurii DSM 18087
gi|498318573|Sporolactobacillus vineae
gi|372456728|Brevibacillus laterosporus GI-9
gi|447193836|Bacillus cereus BAG5X12-1
gi|324323745|Bacillus thuringiensis serovar finitimus YBT-020
gi|363627067|Bacillus smithii 7 3 47FAA 76%
Geobacillus thermodenitrificans T12
gi|516850529|Alicyclobacillus pohliae 85%
gi|530456135|Geobacillus sp. JF8 86%
gi|679863966|Geobacillus sp. G1w1 86%
gi|559465142|Geobacillus sp. MAS1 87%
gi|696477360|Geobacillus stearothermophilus 87%
gi|385811609|Ignavibacterium album JCM 16511 18%
gi|341590650|Megasphaera sp. UPII 135-E 19%
gi|13622193|Streptococcus pyogenes SF370 (M1 GAS)
gi|116628213|Streptococcus thermophilus LMD-9
gi|319957206|Nitratifractor salsuginis DSM 16511 22%
gi|677990610|Actinomyces naeslundii str. Howell 279
gi|117929158|Acidothermus cellulolyticus 11B 20%
0.1

Figure 2

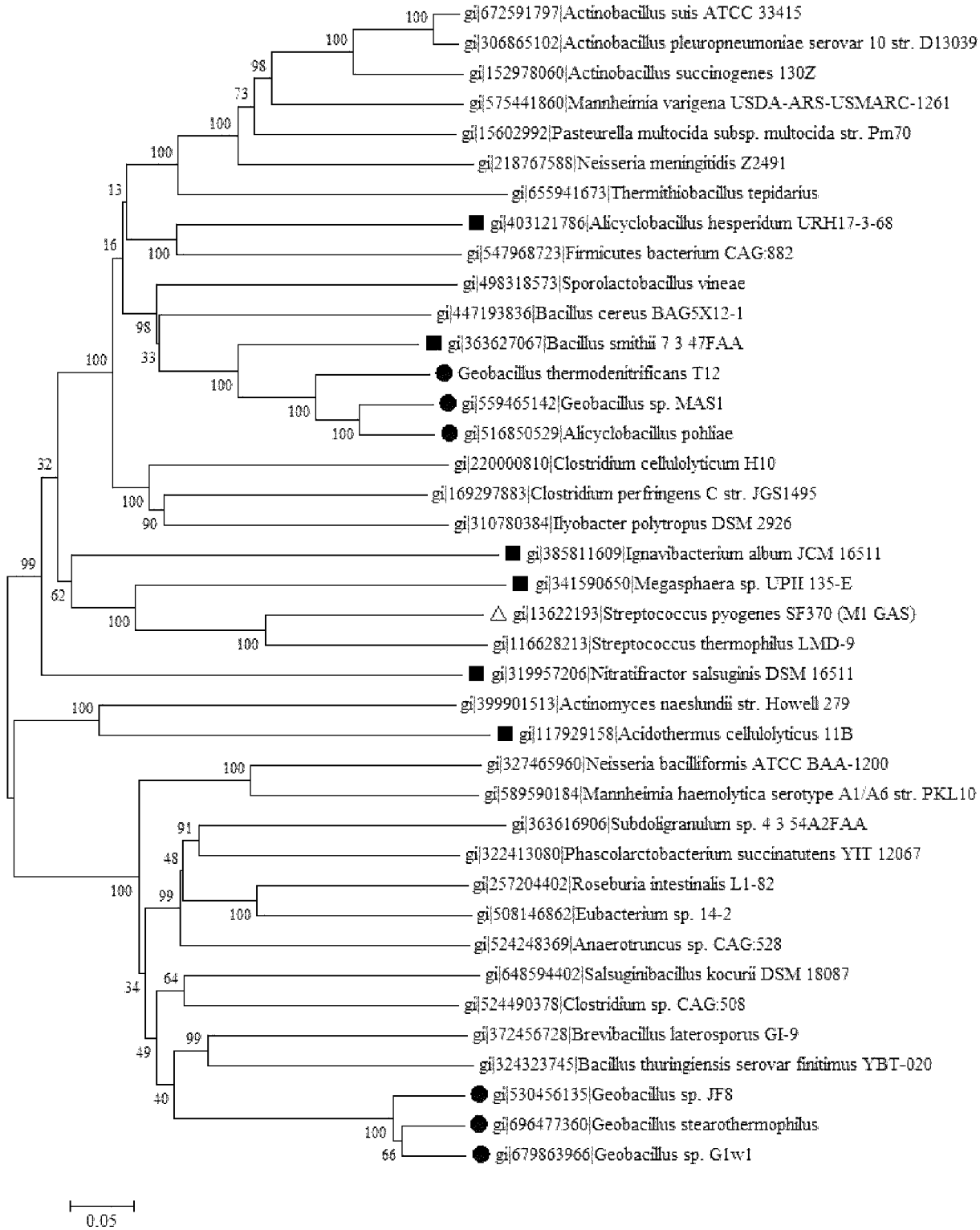

gi|672591797|Actinobacillus suis ATCC 33415
gi|306865102|Actinobacillus pleuropneumoniae serovar 10 str. D13039
gi|152978060|Actinobacillus succinogenes 130Z
gi|575441860|Mannheimia varigena USDA-ARS-USMARC-1261
gi|15602992|Pasteurella multocida subsp. multocida str. Pm70
gi|218767588|Neisseria meningitidis Z2491
gi|655941673|Thermithiobacillus tepidarius
gi|403121786|Alicyclobacillus hesperidum URH17-3-68
gi|547968723|Firmicutes bacterium CAG:882
gi|498318573|Sporolactobacillus vineae
gi|447193836|Bacillus cereus BAG5X12-1
gi|363627067|Bacillus smithii 7 3 47FAA
Geobacillus thermodenitrificans T12
gi|559465142|Geobacillus sp. MAS1
gi|516850529|Alicyclobacillus pohliae
gi|220000810|Clostridium cellulolyticum H10
gi|169297883|Clostridium perfringens C str. JGS1495
gi|310780384|Ilyobacter polytropus DSM 2926
gi|385811609|Ignavibacterium album JCM 16511
gi|341590650|Megasphaera sp. UPII 135-E
gi|13622193|Streptococcus pyogenes SF370 (M1 GAS)
gi|116628213|Streptococcus thermophilus LMD-9
gi|319957206|Nitratifractor salsuginis DSM 16511
gi|399901513|Actinomyces naeslundii str. Howell 279
gi|117929158|Acidothermus cellulolyticus 11B
gi|327465960|Neisseria bacilliformis ATCC BAA-1200
gi|589590184|Mannheimia haemolytica serotype A1/A6 str. PKL10
gi|363616906|Subdoligranulum sp. 4 3 54A2FAA
gi|322413080|Phascolarctobacterium succinatutens YIT 12067
gi|257204402|Roseburia intestinalis L1-82
gi|508146862|Eubacterium sp. 14-2
gi|524248369|Anaerotruncus sp. CAG:528
gi|648594402|Salsuginibacillus kocurii DSM 18087
gi|524490378|Clostridium sp. CAG:508
gi|372456728|Brevibacillus laterosporus GI-9
gi|324323745|Bacillus thuringiensis serovar finitimus YBT-020
gi|530456135|Geobacillus sp. JF8
gi|696477360|Geobacillus stearothermophilus
gi|679863966|Geobacillus sp. G1w1
0.05

Figure 3

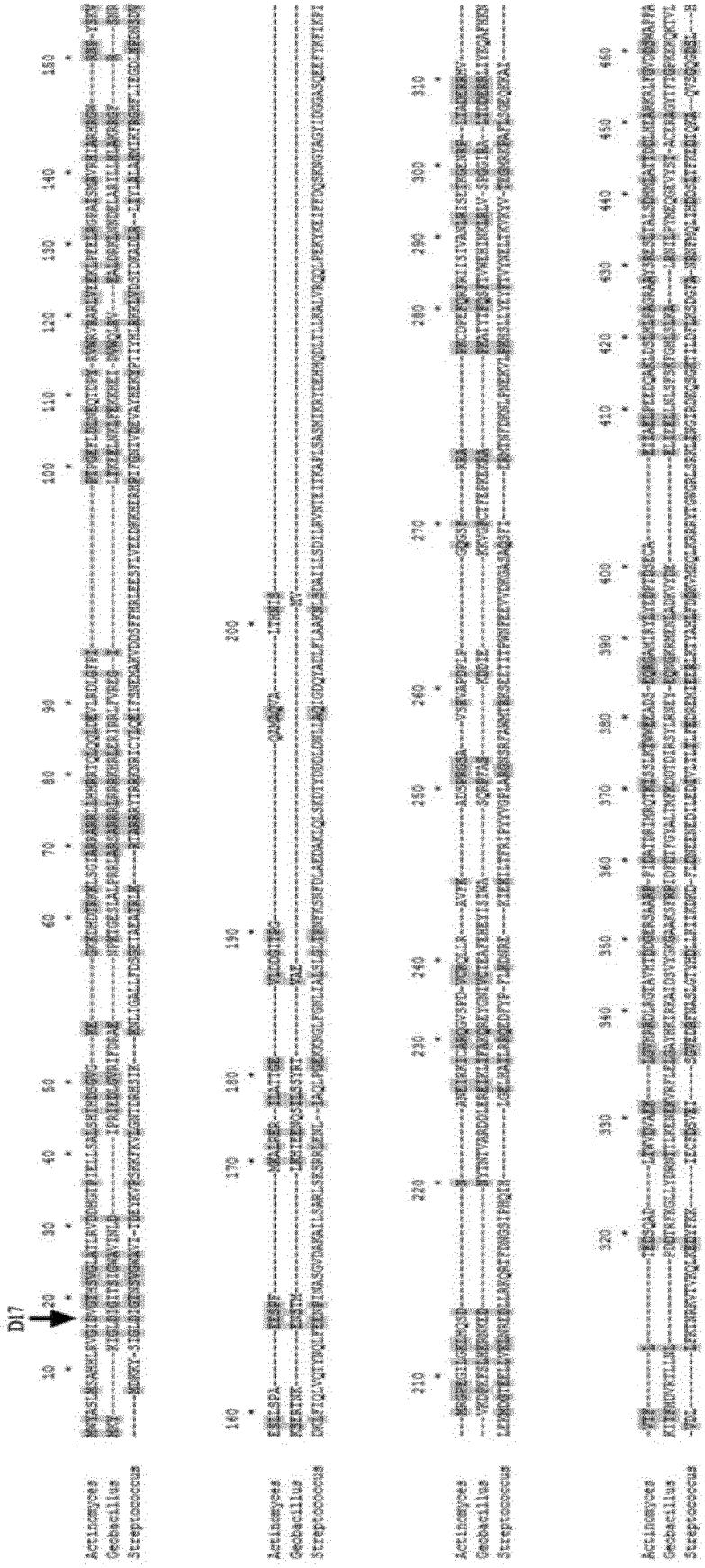


Figure 3 (continued)

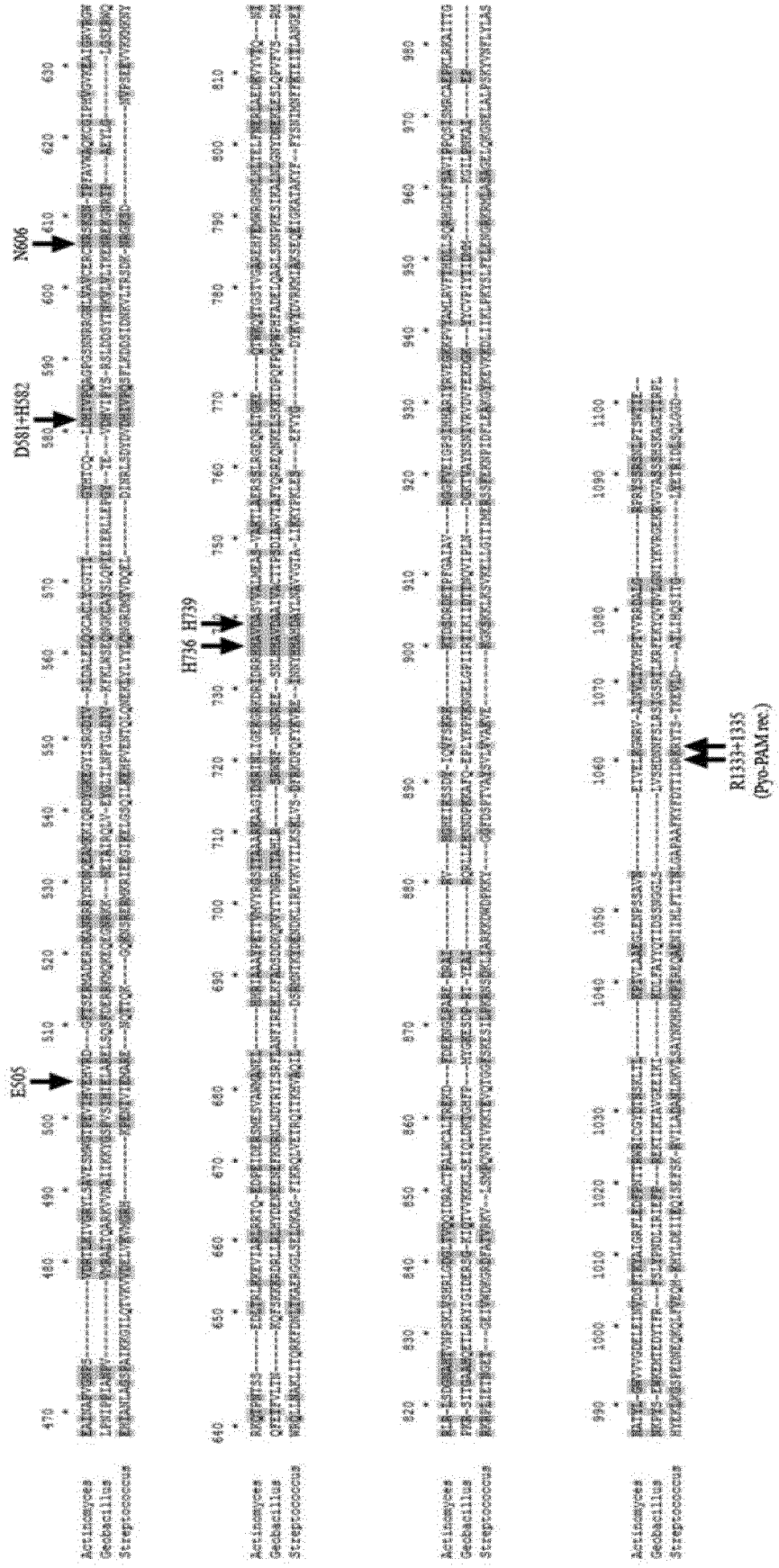
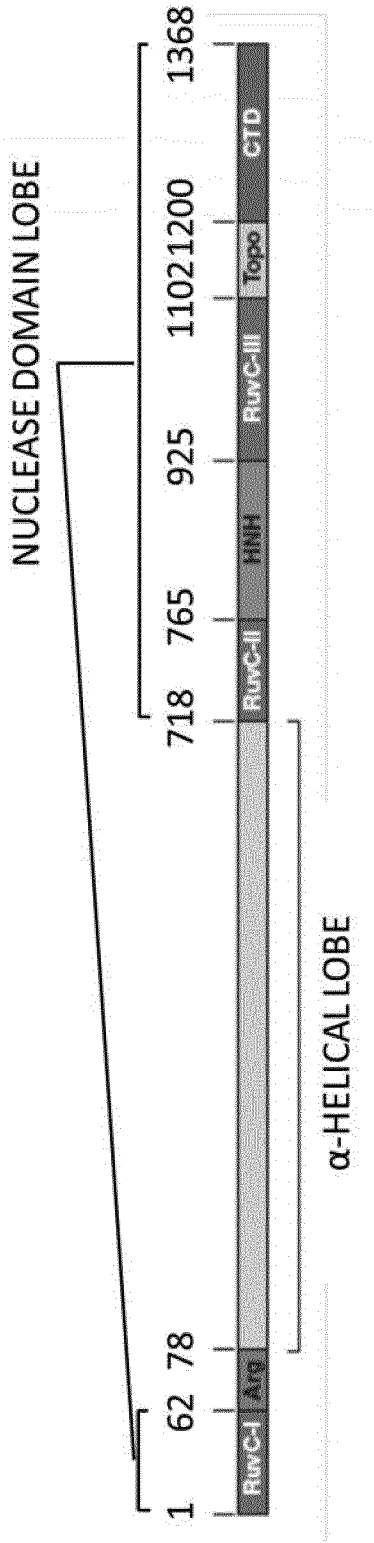
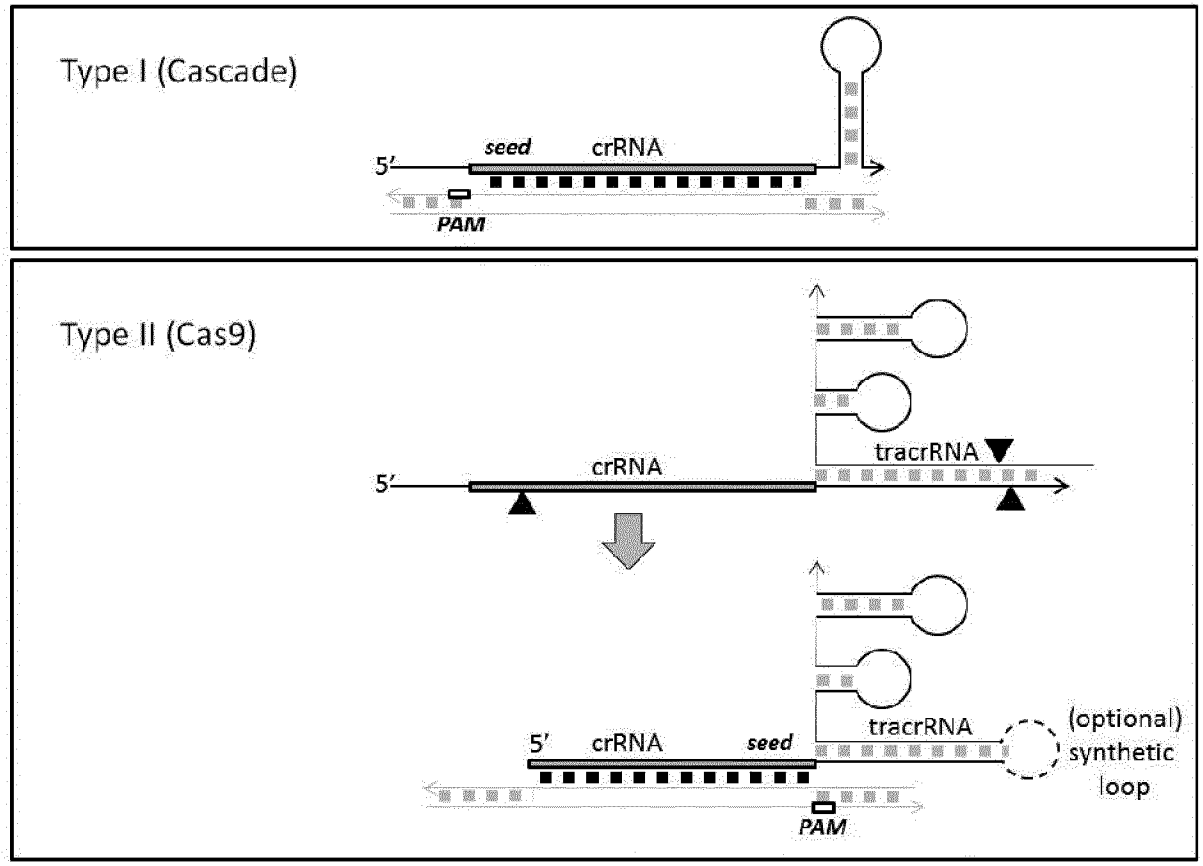


Figure 4


NUCLEASE DOMAIN LOBE
α-HELICAL LOBE
1
62
78
718
765
925
1102
1200
1368
RuvC-I
Arg
RuvC-II
HNH
RuvC-III
Topo
CTD

5

Figure 5


Type I (Cascade)
5′
seed
crRNA
PAM
Type II (Cas9)
crRNA
5′
tracrRNA
5′
crRNA
seed
tracrRNA
(optional)
synthetic
loop
PAM

5

Figure 6

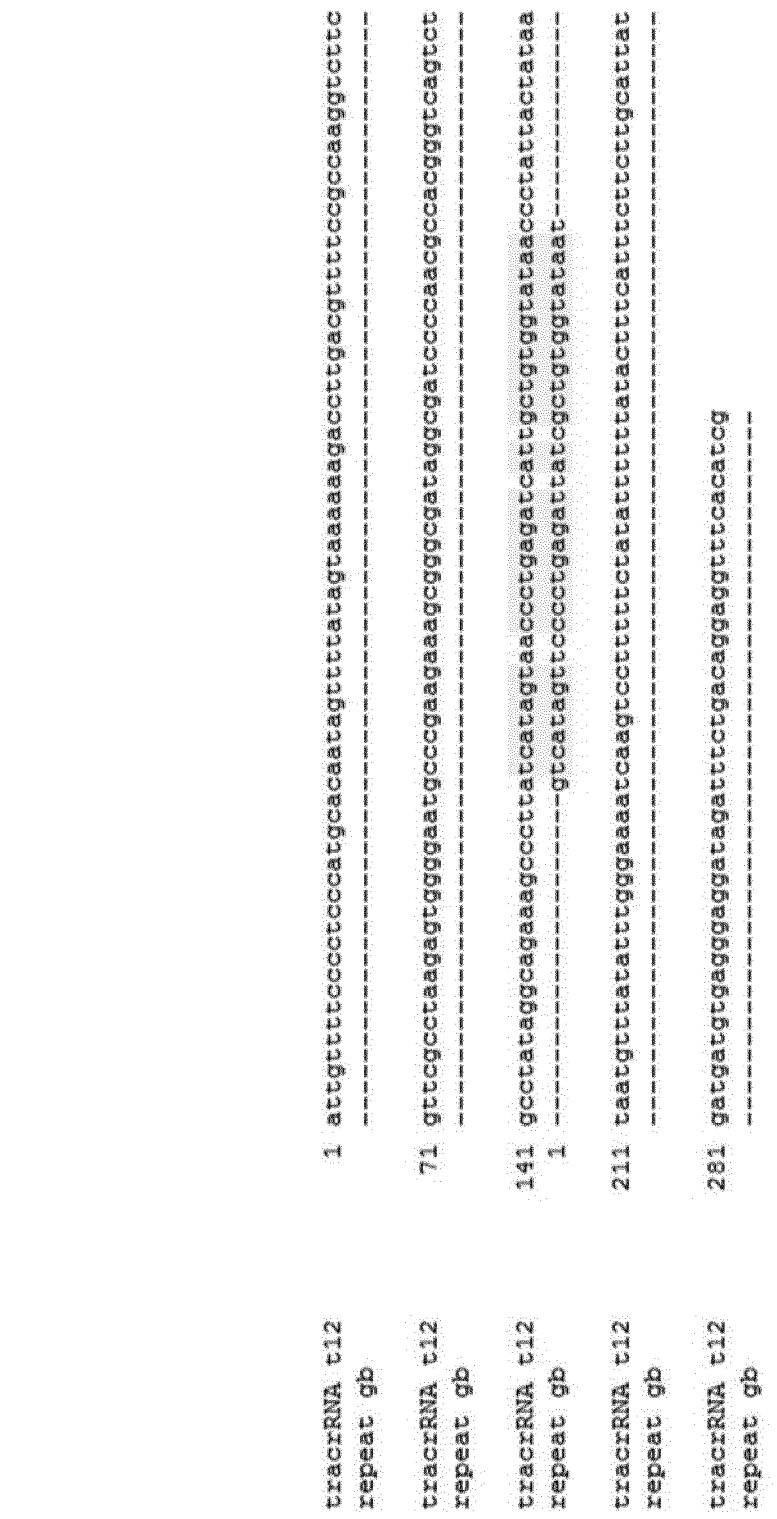

tracrRNA t12
repeat gb
tracrRNA t12
repeat gb
tracrRNA t12
repeat gb
tracrRNA t12
repeat gb
tracrRNA t12
repeat gb
1 attgtttcccctcccatgcacaatagtttatagtaaaaaagaccttgacgttttccgccaaggtcttc
71 gttcgcctaagagtgggggaatgcccgaagaaagcgggcgataggcgatccccaacgccacgggtcagtct
141 gcctatagggcagaaagccctatcatagtaaccctgagatcattgctgtggtataaccctattactataa
1 ----------gtcatagttcccctgagattatcgcctgtggtataat----------
211 taatgtttatatttgggaaaatcaagtccttttctatattttttatacttttcattcttcttgcattat
281 gatgatgtgagggaggatagatttctgacaggaggttttcacatcg

Figure 7

1. Match to: **DQ453159 DQ453159 Geobacillus virus E2, complete genome**(DQ453159) position: 39980-39951, with: **spacer4** CRISPR No.4 Spacer No.1 (spacer4_4_1) position: 1-30, Strand: +

```
 5' --------UUGGCGGUGCGAAUUCUAACCGUCCCGGAA-------- 3'     <- CRISPR
spacer                                                      RNA
            ||||||||||||||||||||||||||||||
 3' TAGCGTACAACCGCCACGCTTAAGATTGGCAGGGCCTTCGTGGTGT 5'     <-
Protospacer                                                 Sequence
    ||||||||                                    ||||||||
 5' ATCGCATGTTGGCGGTGCGAATTCTAACCGTCCCGGAAGCACCACA 3'     <-
[Entrez Nucleotide]
```

Score: 30

2. Match to: **Bacillus alveayuensis strain 24KAM51 LG50_053, whole genome shotgun sequence**(NZ_JYCE01000053) position: 110918-110889, with: **spacer9** CRISPR No.9 Spacer No.1 (spacer9_9_1) position: 1-30, Strand: +

```
 5' --------UUCUACCUCUACUCUCGAUUCACGAAUCGG-------- 3'     <- CRISPR
spacer                                                      RNA
            ||||||||||||||||||||||||||||||
 3' CGACGAGAAAGATGGAGATGAGAGCTAAGTGCTTAGCCAAAAGGTT 5'     <-
Protospacer                                                 Sequence
    ||||||||                                    ||||||||
 5' GCTGCTCTTTCTACCTCTACTCTCGATTCACGAATCGGTTTTCCAA 3'     <-
[Entrez Nucleotide]
```

Score: 30

3. Match to: **Anoxybacillus flavithermus WK1, complete genome**(CP000922) position: 701422-701450, with: **spacer5** CRISPR No.5 Spacer No.1 (spacer5_5_1) position: 1-29, Strand: -

```
 5' --------UCACGGAGCUUUACACAAAUAAAGCCGGA-------- 3'     <-  CRISPR
spacer                                                      RNA
            |||||||||||||||||||||||||||||
 3' GATGTTCTAGTGCCTCGAAATGTGTTTATTTCGGCCTCGCTGCTT 5'     <-
Protospacer                                                 Sequence
    ||||||||                                    ||||||||
 5' CTACAAGATCACGGAGCTTTACACAAATAAAGCCGGAGCGACGAA 3'     <- [Entrez
Nucleotide]
```

Score: 29

4. Match to: **Geobacillus kaustophilus strain Et23 LG51_086, whole genome shotgun sequence**(NZ_JYCF01000086) position: 15565-15537, with: **spacer5** CRISPR No.5 Spacer No.1 (spacer5_5_1) position: 1-29, Strand: +

```
 5' --------UCACGGAGCUUUACACAAAUAAAGCCGGA-------- 3'     <-  CRISPR
spacer                                                      RNA
            |||||||||||||||||||||||||||||
 3' CATGTTCTAGTGCCTCGAAATGTGTTTATTTCGGCCTCGTTGCTT 5'     <-
Protospacer                                                 Sequence
    ||||||||                                    ||||||||
```

Figure 7 (continued)

```
 5' GTACAAGATCACGGAGCTTTACACAAATAAAGCCGGAGCAACGAA 3'     <- [Entrez
Nucleotide]
```

Score: 29

5. Match to: **DQ453159 DQ453159 Geobacillus virus E2, complete genome**(DQ453159) position: 6492-6463, with: **spacer6** CRISPR No.6 Spacer No.1 (spacer6_6_1) position: 1-30, Strand: +

```
 5' --------CAACACCUUCCGCGCUGUCUCGUCUACUUU-------- 3'     <- CRISPR
spacer                                                        RNA
            |||||||||||||||||||||||||||||||
 3' CGAAGAAAGTTGTGGAAGGCACGACAGAGTAGATGAAAAAATGCTT 5'     <-
Protospacer                                                  Sequence
    ||||||||                                    ||||||||
 5' GCTTCTTTCAACACCTTCCGTGCTGTCTCATCTACTTTTTTACGAA 3'     <-
[Entrez Nucleotide]
```

Score: 26

6. Match to: **Pasteurella bettyae CCUG 2042 contig00003, whole genome shotgun sequence**(NZ_AJSX01000041) position: 137780-137808, with: **spacer7** CRISPR No.7 Spacer No.1 (spacer7_7_1) position: 1-30, Strand: -

```
 5' --------UUGAUUAGCAAUUUGACUUGGGAAUUUAGC-------- 3'     <- CRISPR
spacer                                                        RNA
            |||||||||||||||||||||||||||||||
 3' TAATTGTAAACTAATCGTTAAACTGACGCCTTAAATCATTACGGTT 5'     <-
Protospacer                                                  Sequence
    ||||||||                                    ||||||||
 5' ATTAACATTTGATTAGCAATTTGACTGCGGAATTTAGTAATGCCAA 3'     <-
[Entrez Nucleotide]
```

5

Figure 8

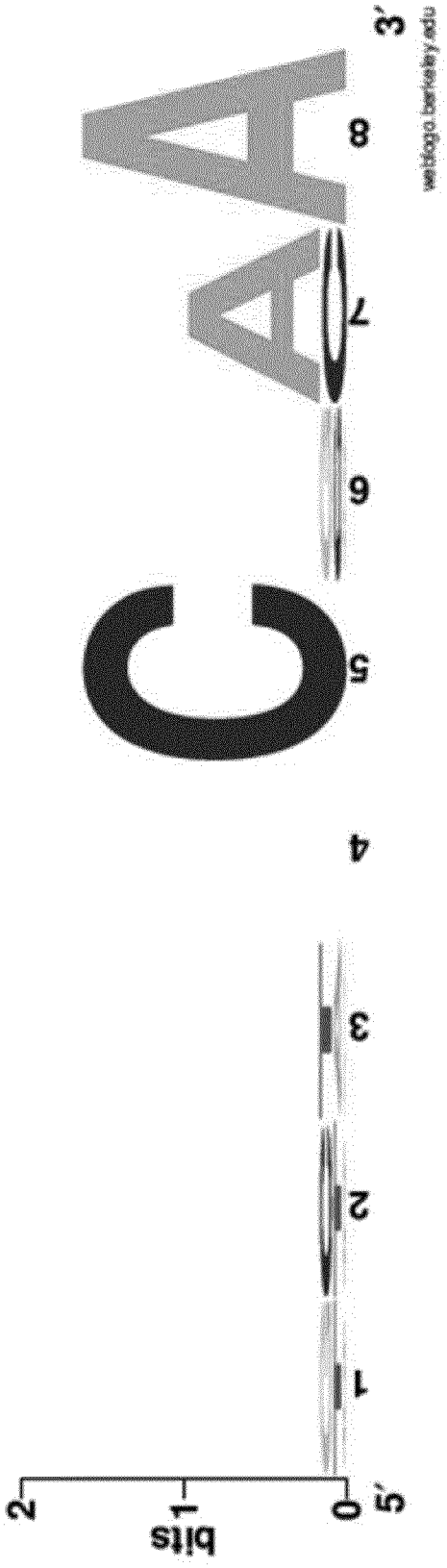


Figure 9

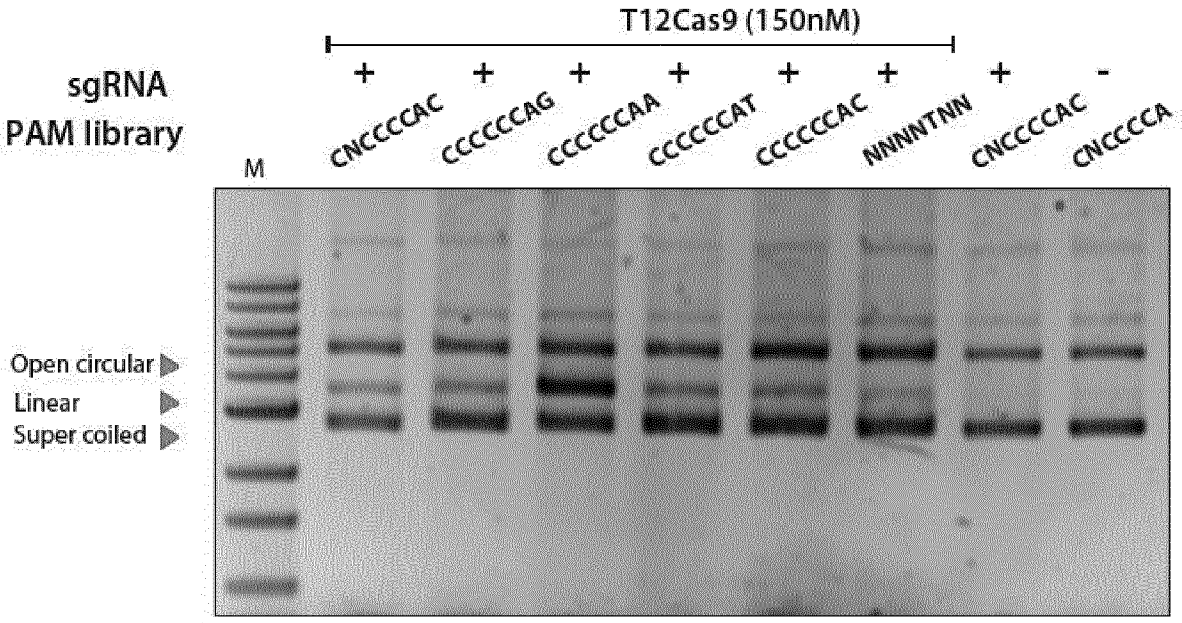

T12Cas9 (150nM)
sgRNA
PAM library
+ + + + + + + -
CNCCCCAC
CCCCCCAG
CCCCCCAA
CCCCCCAT
CCCCCCAC
NNNNTNN
CNCCCCAC
CNCCCCA
M
Open circular
Linear
Super coiled

Figure 10

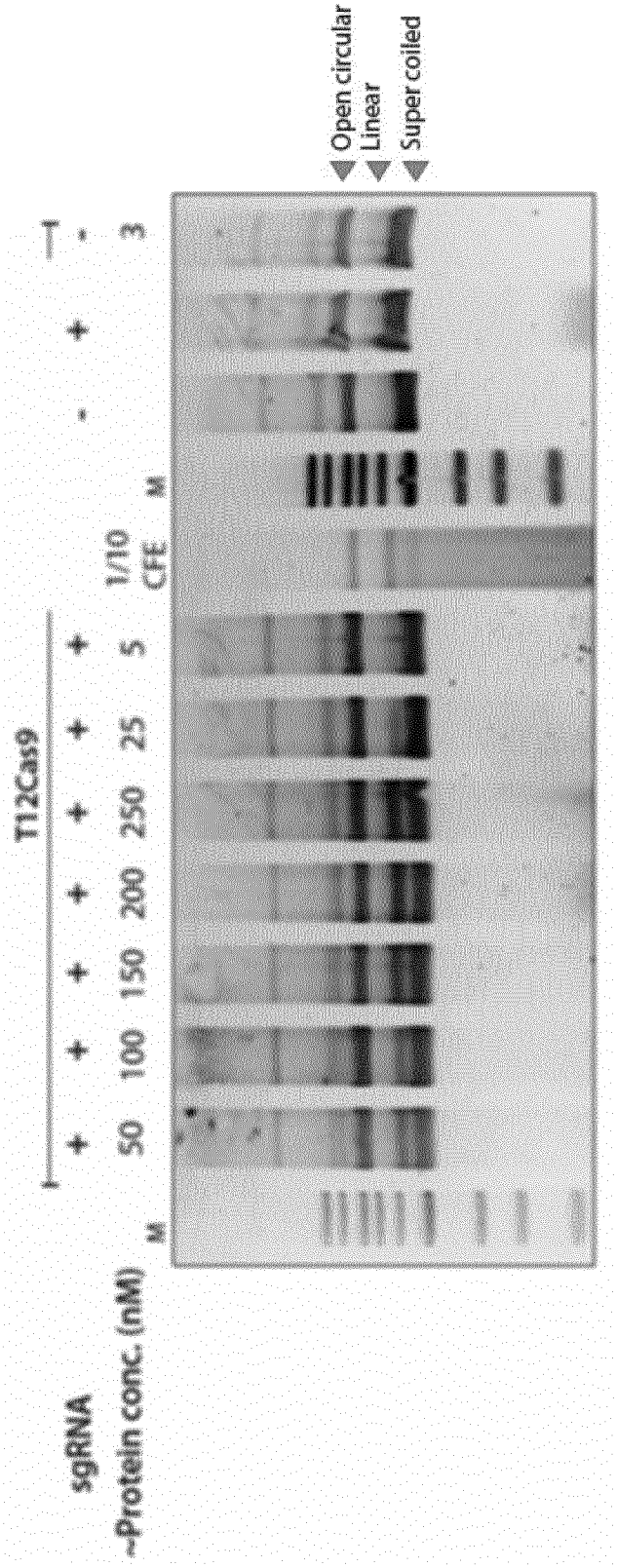

Open circular
Linear
Super coiled
sgRNA
-Protein conc. (nM)
M
50
100
150
200
250
25
5
T12Cas9
1/10 CFE
M
3

Figure 11

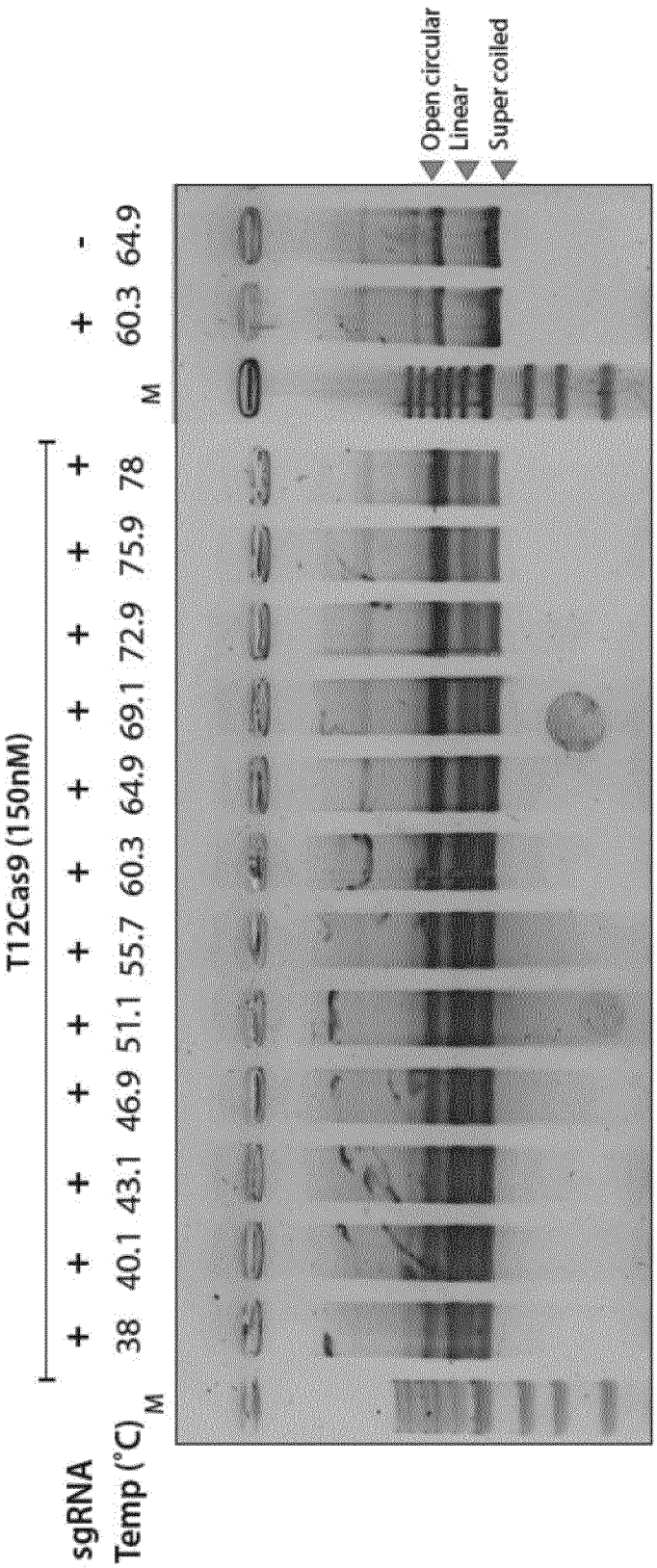
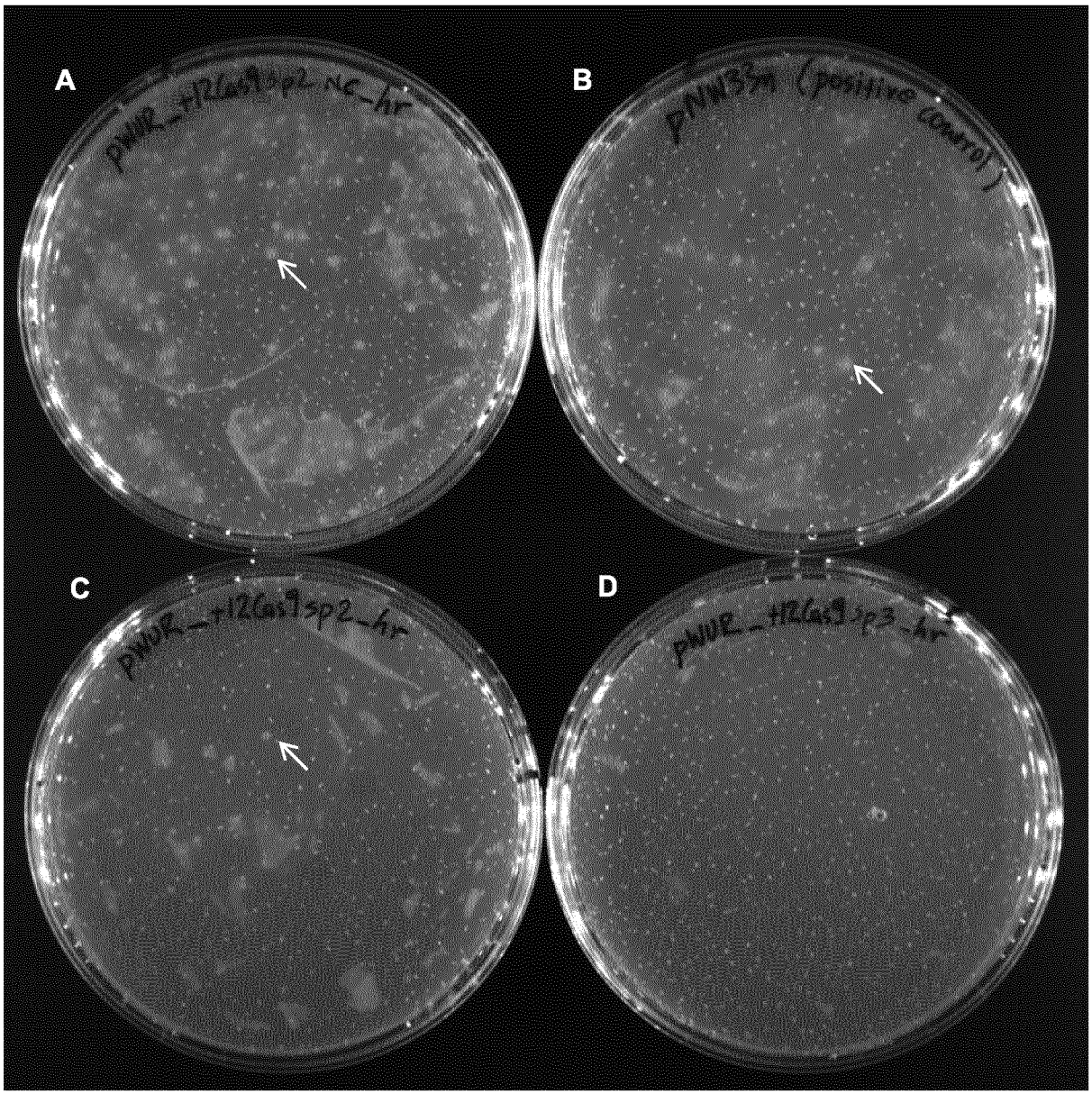
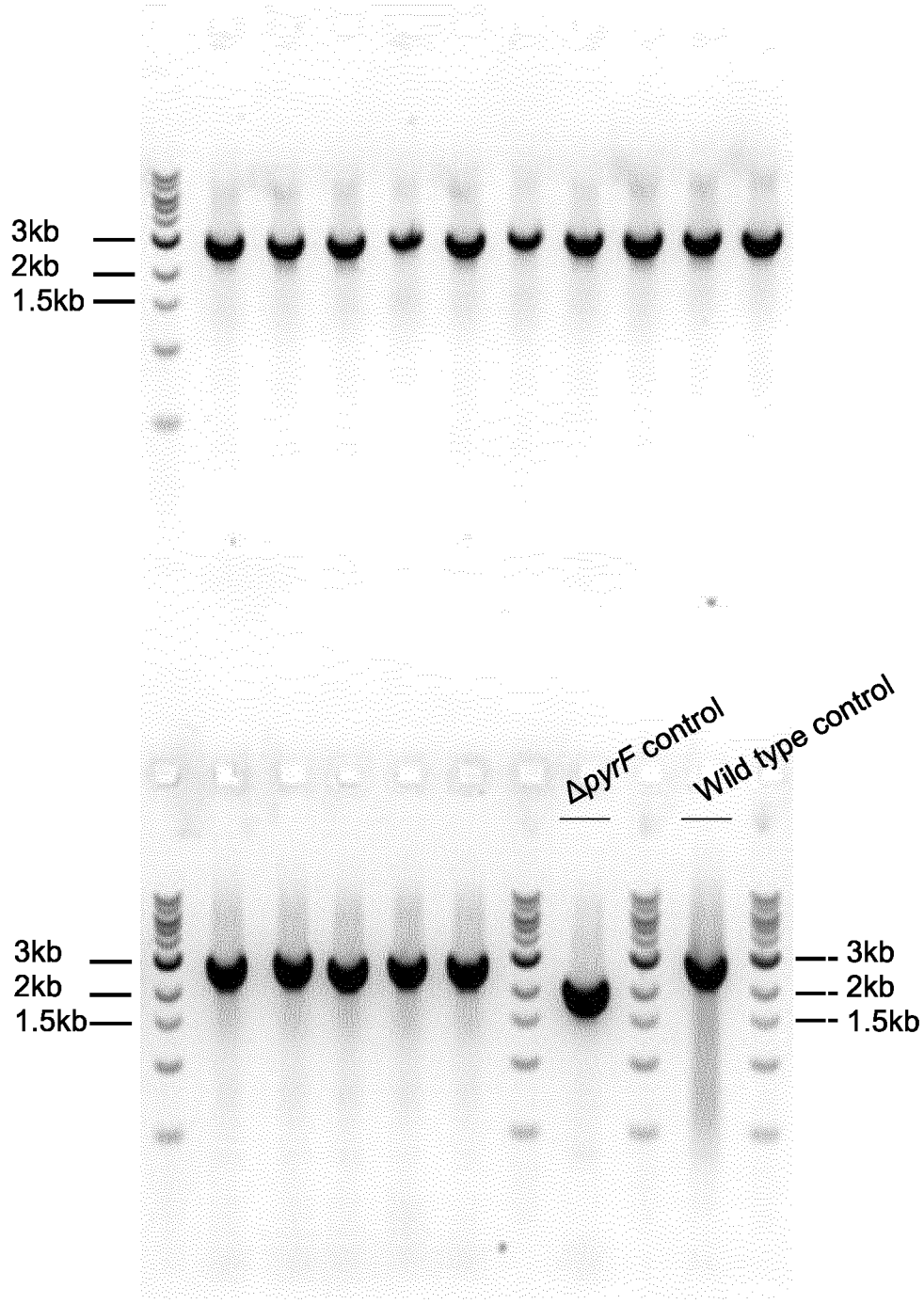

Open circular
Linear
Super coiled
T12Cas9 (150nM)
sgRNA
Temp (°C)
M
38
40.1
43.1
46.9
51.1
55.7
60.3
64.9
69.1
72.9
75.9
78
M
60.3
64.9
+
-

Figure 12



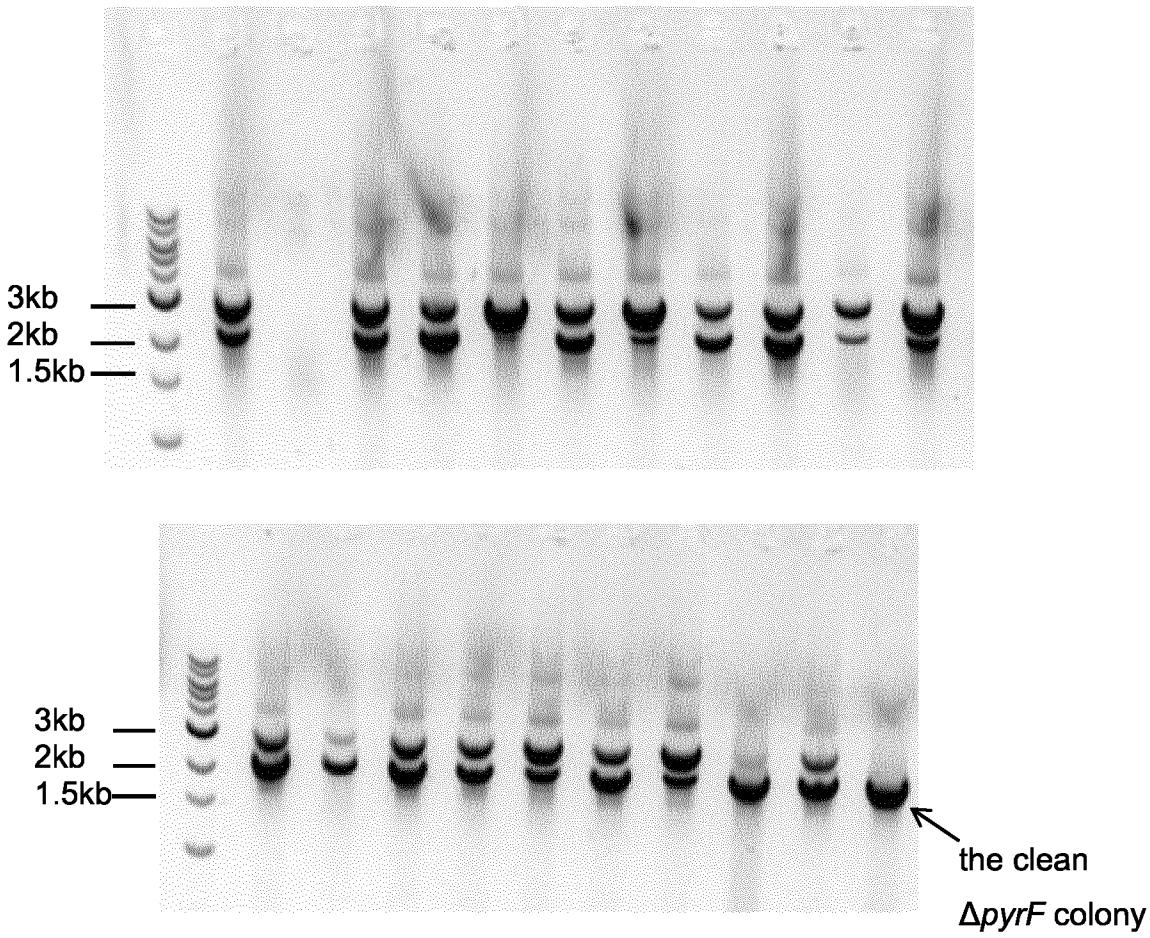
Colonies are indicated with arrows.

Figure 13

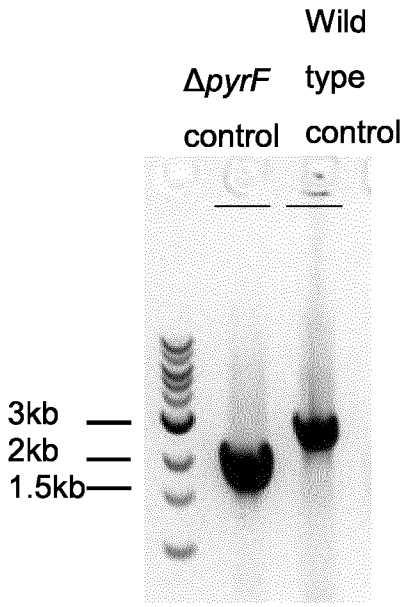

3kb
2kb
1.5kb
ΔpyrF control
Wild type control
3kb
2kb
1.5kb
3kb
2kb
1.5kb

5

Figure 14


3kb
2kb
1.5kb
3kb
2kb
1.5kb
the clean
ΔpyrF colony

5


ΔpyrF control
Wild type control
3kb
2kb
1.5kb