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Therefore, this United States

Patent

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Katherine Kelly Vidal

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If the application for this patent was filed on or after June 8, 1995, the term of this patent begins on the date on which this patent issues and ends twenty years from the filing date of the application or, if the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121, 365(c), or 386(c), twenty years from the filing date of the earliest such application ("the twenty-year term"), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b), and any extension as provided by 35 U.S.C. 154(b) or 156 or any disclaimer under 35 U.S.C. 253.

If this application was filed prior to June 8, 1995, the term of this patent begins on the date on which this patent issues and ends on the later of seventeen years from the date of the grant of this patent or the twenty-year term set forth above for patents resulting from applications filed on or after June 8, 1995, subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b) and any extension as provided by 35 U.S.C. 156 or any disclaimer under 35 U.S.C. 253.

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Martinez et al.

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(54) **SINGLE-VECTOR TYPE I VECTORS**

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(58) **Field of Classification Search**

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See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to the production and use of Cas-encoding sequences and vectors comprising these. Aspects of the invention provide products, vectors, delivery vehicles, uses and methods for producing Cas-encoding sequences in bacterial or archaeal cells.

18 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

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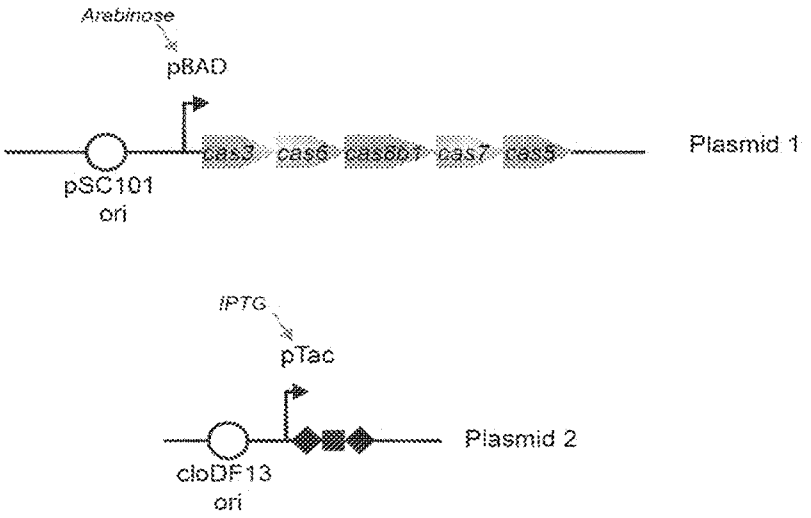

FIG. 1A

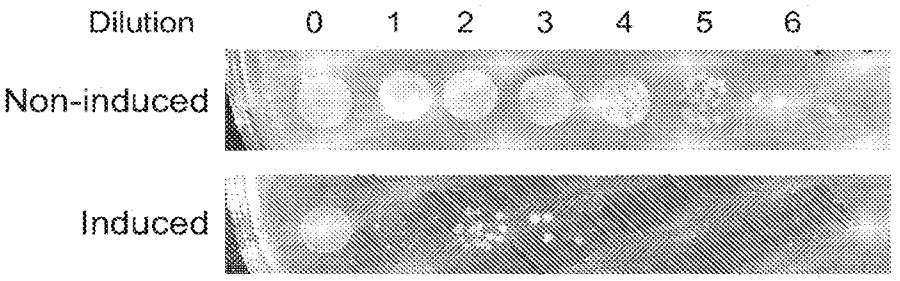

FIG. 1B

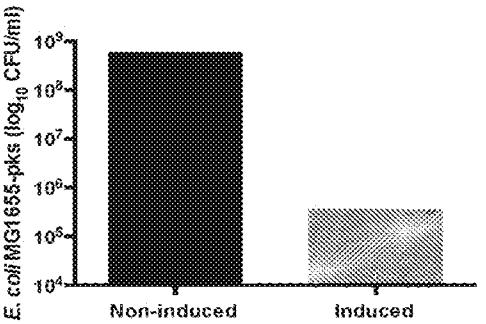

FIG. 1C

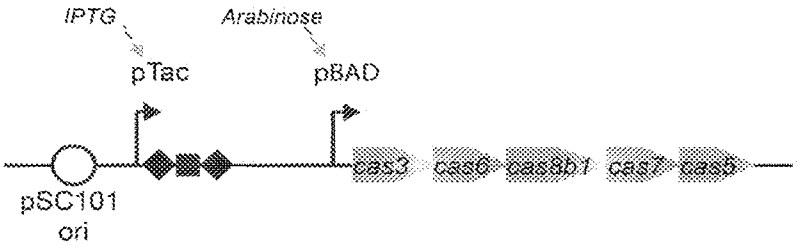

FIG. 2A

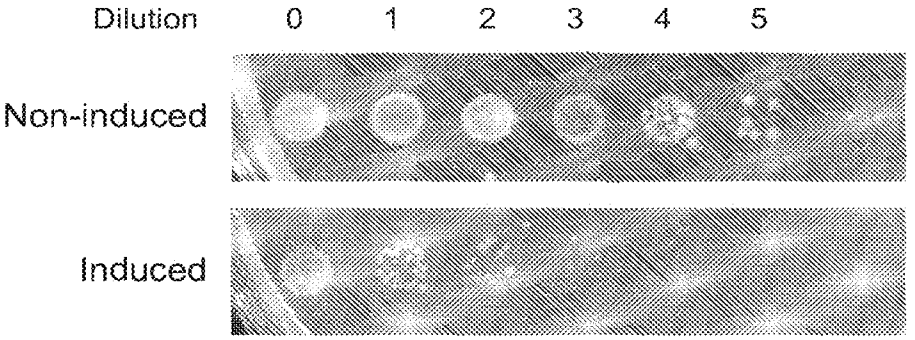

FIG. 2B

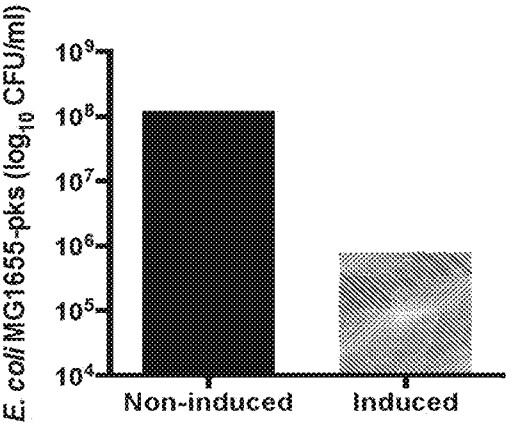

FIG. 2C

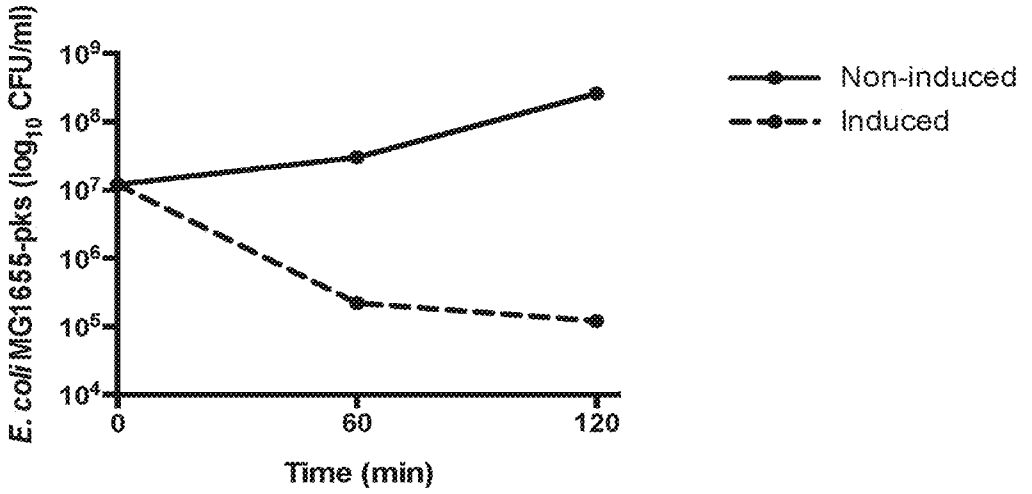
E. coli MG1655-pks (log_{10} CFU/ml)
10^9
10^8
10^7
10^6
10^5
10^4
0
60
120
Time (min)
Non-induced
Induced

FIG. 3A

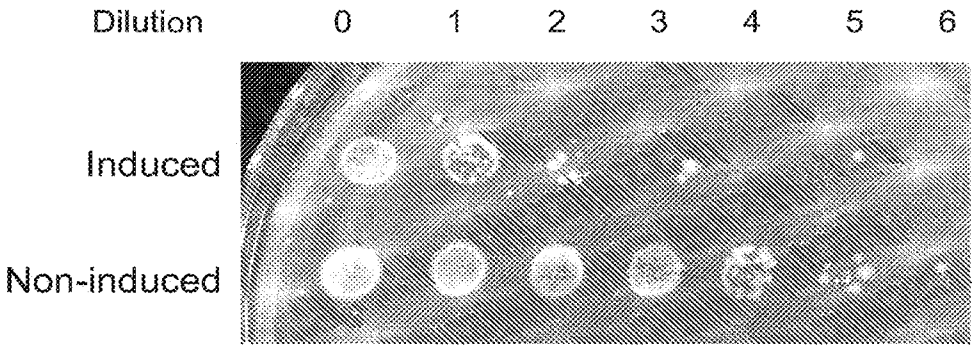
Dilution
0
1
2
3
4
5
6
Induced
Non-induced

FIG. 3B

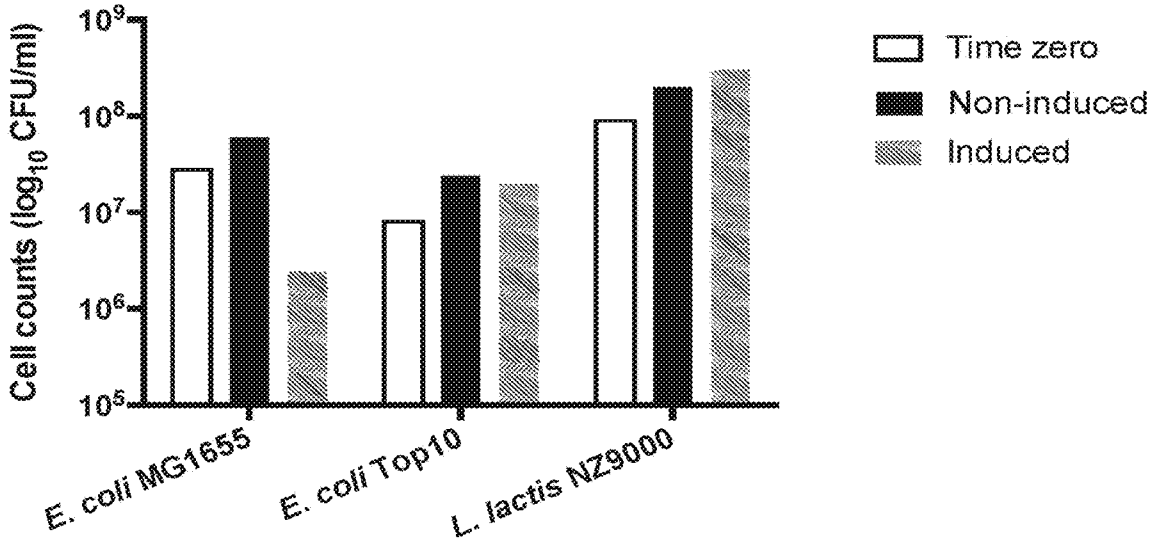
Cell counts (log_{10} CFU/ml)
10^9
10^8
10^7
10^6
10^5
Time zero
Non-induced
Induced
E. coli MG1655
E. coli Top10
L. lactis NZ9000

FIG. 4A

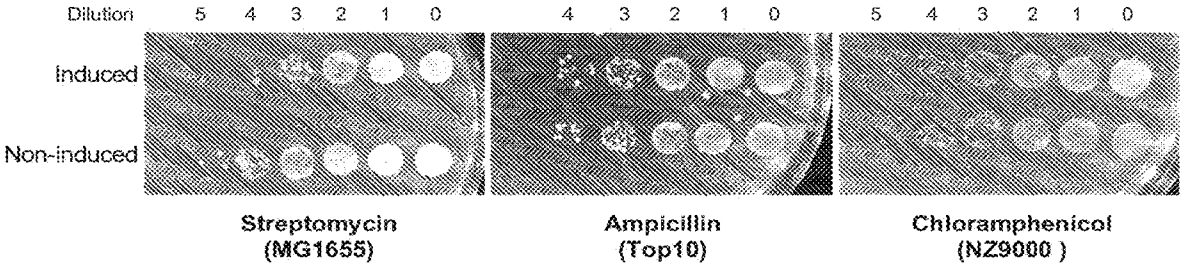
Dilution
5 4 3 2 1 0
4 3 2 1 0
5 4 3 2 1 0
Induced
Non-induced
Streptomycin (MG1655)
Ampicillin (Top10)
Chloramphenicol (NZ9000)

FIG. 4B

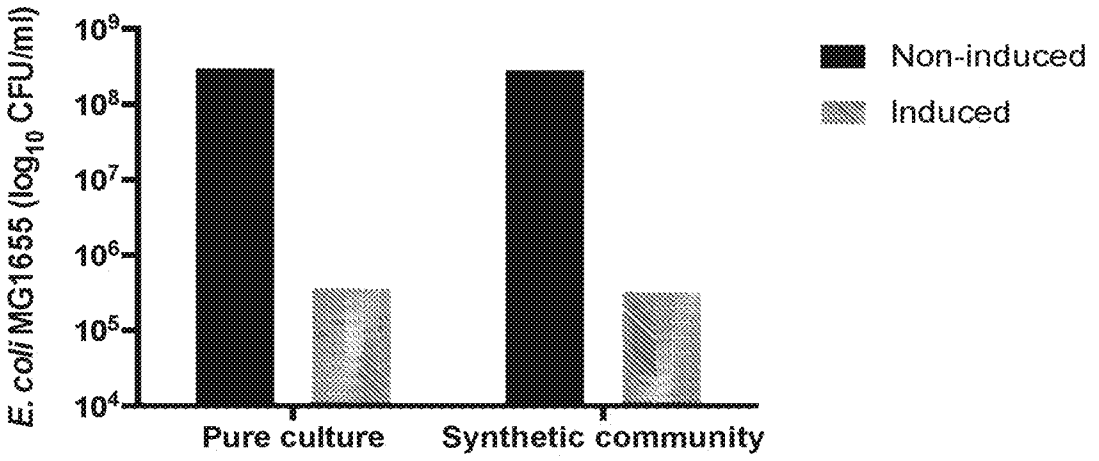
E. coli MG1655 (log_{10} CFU/ml)
10^9
10^8
10^7
10^6
10^5
10^4
Non-induced
Induced
Pure culture
Synthetic community

FIG. 5A

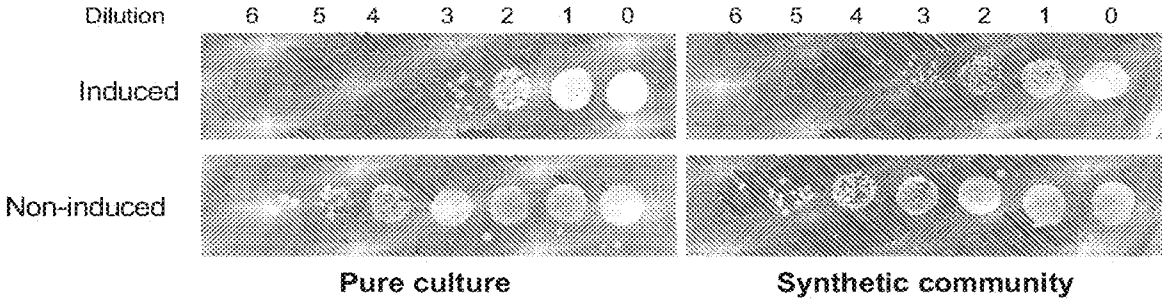
Dilution
6 5 4 3 2 1 0
6 5 4 3 2 1 0
Induced
Non-induced
Pure culture
Synthetic community

FIG. 5B

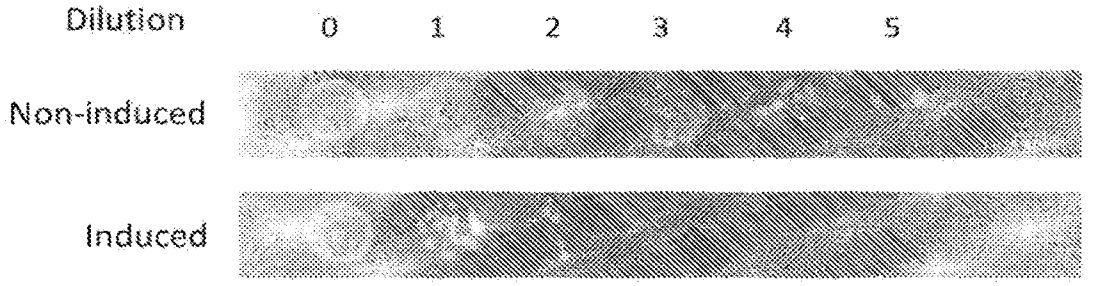
Dilution
0
1
2
3
4
5
Non-induced
Induced

FIG. 6A

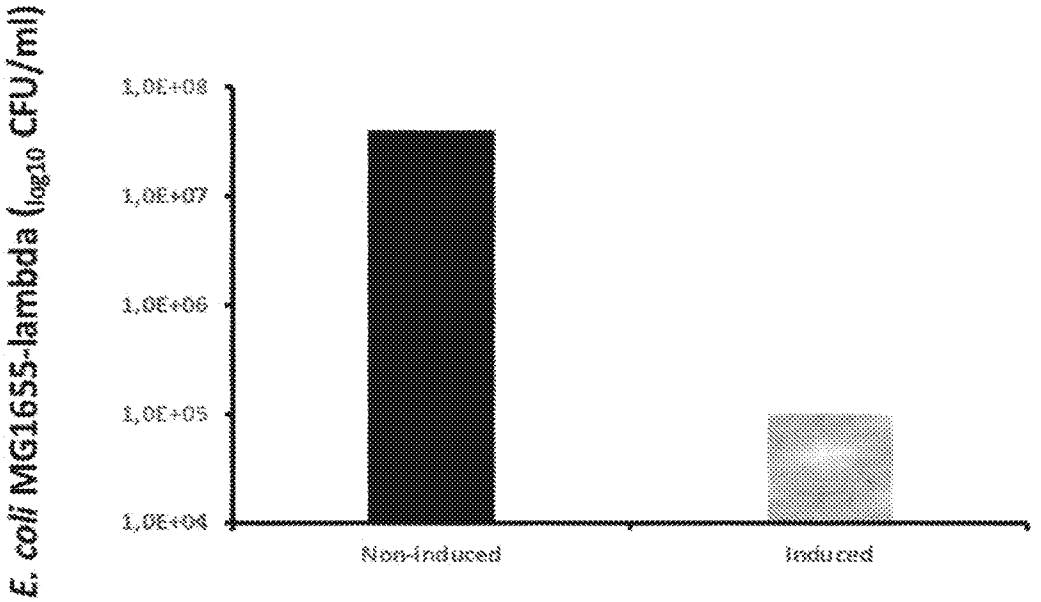
E. coli MG1655-lambda (log10 CFU/ml)
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
Non-induced
Induced

FIG. 6B

SINGLE-VECTOR TYPE I VECTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/201,736, filed Nov. 27, 2018 which claims priority to Great Britain Patent Application No. 1816700.7, filed Oct. 14, 2018, and Great Britain Patent Application No. 1817509.1, filed Oct. 27, 2018, the contents of each of which are hereby incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 786212000602SEQLIST.TXT, date recorded: Feb. 10, 2021, size: 6 KB).

TECHNICAL FIELD

The invention relates to the production and use of Cas-encoding sequences and vectors comprising these. Aspects of the invention provide products, vectors, delivery vehicles, uses and methods for producing Cas-encoding sequences in bacterial or archaeal cells.

BACKGROUND

The state of the art describes vectors and uses of these that employ CRISPR/Cas systems. For example, reference is made to WO2017/118598, US20180140698, US20170246221, US20180273940, US20160115488, US20180179547, US20170175142, US20160024510, US20150064138, US20170022499, US20160345578, US20180155729, US20180200342, WO2017112620, WO2018081502, PCT/EP2018/066954, PCT/EP2018/066980, PCT/EP2018/071454 and U.S. Ser. No. 15/985,658 and equivalent publications by the US Patent and Trademark Office (USPTO) or WIPO, the disclosures of which are incorporated herein by reference.

SUMMARY OF THE INVENTION

The invention provides the following configurations.

In a First Configuration

A nucleic acid vector for introduction into a host cell, the vector comprising a first nucleotide sequence encoding a Type I Cas3 and a second nucleotide sequence encoding one or more Cascade proteins, wherein the first and second sequences are under the control of one or more promoters comprised by the vector for expression of the proteins in the cell.

In an example, the vector comprises an operon for expression in the cell of the Cas3 and Cascade proteins from a Cas module, the module comprising the nucleotide sequences encoding the Cas3 and

Cascade proteins, and the operon comprising the Cas module under the control of a promoter for controlling the expression of both the Cas3 and Cascade proteins.

The invention also provides a delivery vehicle comprising the vector, as well as a pharmaceutical composition comprising the vector or vehicle and a pharmaceutically acceptable diluent, excipient or carrier.

The invention also provides a method of treating or reducing the risk of a disease or condition in a human or animal subject, the method comprising administering the vector, vehicle or composition to the subject, and introducing the vector into target host bacterial or archaeal cells in the subject (eg, in a gut microbiota, lung, eye or blood of the subject), wherein the Cas cuts (or otherwise modifies) one or more target sequences in the target cells and the cells are killed or growth or proliferation of the cells is reduced.

In a Second Configuration

A method of amplifying copies of a DNA encoding a functional Cas protein (optionally a Cas nuclease) in a bacterial or archaeal production strain of cells, the method comprising

(a) Providing production strain cells, each cell comprising a copy of said DNA, wherein each DNA comprises a nucleotide sequence encoding said Cas, wherein the nucleotide sequence is under the control of a promoter for controlling the expression of the Cas in the production strain cell, the DNA comprising an origin of replication that is operable in the cell for replication of the DNA;

(b) Culturing the cells to allow replication of the DNA, whereby the DNA is amplified; and

(c) Optionally isolating copies of the DNA,

Optionally wherein the promoter is an attenuated constitutive promoter.

In a Third Configuration

Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for enhancing the yield of amplified DNA produced by the production host cells.

In a Fourth Configuration

Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing toxicity of the Cas in the production strain.

In a Fifth Configuration

Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain.

In a Sixth Configuration

Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for promoting production cell viability during the amplification of the DNA.

In a Seventh Configuration

Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing the occurrence of Cas cutting of DNA.

In an Eighth Configuration

A method for enhancing the yield of amplified copies of a DNA construct in a population of bacterial or archaeal production strain cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

In a Ninth Configuration

A method for reducing toxicity of a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

In a Tenth Configuration

A method for reducing mutation of a DNA construct encoding a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

In an Eleventh Configuration

A method for promoting production cell viability of a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct comprised by the cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

In a twelfth Configuration A method for reducing the occurrence of Cas nuclease cutting of a DNA construct in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A-**1**C. Type I CRISPR-Cas system of *C. difficile* targeting *E. coli* MG1655. (FIG. **1**A) Layout of the CRISPR Guided Vector™, CGV™. Plasmid 1: pSC101 ori, pBAD promoter (induced by arabinose), cas3 and cascade genes. Plasmid 2: pCloDF13 or pTac promoter (induced by IPTG), CRISPR array. (FIG. **1**B) Dilution series (10^1-10^6) of drop spots (5 μl) of *E. coli* MG1655 harboring the CGV on LB agar plates with and without inducers. (FIG. **1**C) CRISPR induction killed 99.9% of the population (grey bar). Growth in absence of induction is shown in black. CGV™ refers to a CRISPR Guided Vector™, which is a nucleic acid vector comprising nucleotide sequences encoding CRISPR/Cas components.

FIGS. **2**A-**2**C. Type I CRISPR-Cas system of *C. difficile* targeting *E. coli* MG1655. (FIG. **2**A) Layout of the CRISPR Guided Vector™, CGV™. pSC101 or pTac promoter (induced by IPTG), CRISPR array, pBAD promoter (induced by arabinose), cas3 and cascade genes. (FIG. **2**B) Dilution series (10^1-10^6) of drop spots (5 μl) of *E. coli* MG1655 harboring the CGV on SM agar plates with and without inducers. (FIG. **2**C) CRISPR induction killed 99% of the population (grey bar). Growth in absence of induction is shown in black. CGV™ refers to a CRISPR Guided Vector™, which is a nucleic acid vector comprising nucleotide sequences encoding CRISPR/Cas components.

FIGS. **3**A-**3**B. Time-kill curves for *E. coli* MG1655 harboring the CGV. (FIG. **3**A) CRISPR induction killed 99% of the population in 60 minutes (dashed line). Growth in absence of induction is shown in black lines. CRISPR/Cas was induced at time-point 0 and monitored until 120 minutes. (FIG. **3**B) Dilution series (10^1-10^6) of drop spots (5 μl) on SM agar plates of *E. coli* MG1655 after 60 minutes of induction.

FIGS. **4**A-**4**B. Specific killing of *E. coli* MG1655 with type I-B CRISPR-Cas system of *C. difficile* in a synthetic microbial consortium. (FIG. **4**A) Bacteria count of a synthetic population composed of three different strains. CRISPR was induced at time-point 0 and monitored for 60 minutes. Growth in absence of induction is shown in black. CRISPR induction prompted 1-$\log_{10}$ reduction in viable cells of target strain *E. coli* MG1655, while leaving *E. coli* Top10 and *L. lactis* NZ9000 populations intact (dark grey bars). (FIG. **4**B) Dilution series (10^1-10^6) of drop spots (5 μl) of the bacterial community mixture after 60 minutes of induction. *E. coli* MG1655 grows selectively on BHI+ streptomycin, *E. coli* Top10 on ampicillin, and *L. lactis* NZ9000 on chloramphenicol.

FIGS. **5**A-**5**B. Killing of *E. coli* MG1655 with type I-B CRISPR-Cas system of *C. difficile* in a synthetic microbial consortium compared to a pure culture of *E. coli* MG1655. (FIG. **5**A) CRISPR induction generated 4-$\log_{10}$ reductions in viable cells of target strain *E. coli* MG1655, both in the pure culture and in the community mixture (grey bars). Growth in absence of induction is shown in black. (FIG. **5**B) Dilution series of a pure culture of *E. coli* MG1655 and the bacterial community mixture on streptomycin plates with and without inducers.

FIGS. **6**A-**6**B. Type I CRISPR-Cas system of *E. coli* targeting *E. coli* MG1655. (FIG. **6**A) Dilution series (10^1-10^6) of drop spots (5 μl) of *E. coli* MG1655 harboring the CGV on SM agar plates with and without inducers. (FIG. **6**B) CRISPR induction killed 99% of the population (grey bar). Growth in absence of induction is shown in black. CGV™ refers to a CRISPR Guided Vector™ which is a nucleic acid vector comprising nucleotide sequences encoding CRISPR/Cas components.

DETAILED DESCRIPTION

The invention relates to the production and use of Cas-encoding sequences and vectors comprising these. Aspects

of the invention provide products, vectors, delivery vehicles, uses and methods for producing Cas-encoding sequences in bacterial or archaeal cells.

An aspect of the invention provides for the control of expression of Cas and optionally also Cascade proteins from single vectors, such as by regulated use of Cas modules in an operon and/or using attenuated promoters.

Concepts:

An aspect of the invention provides nucleic acid vectors that are useful for introducing into target host cells of any eukaryotic or prokaryotic species (eg, ex vivo or in vitro) for expressing Type I Cas and optionally other components of a Type I CRISPR/Cas system. Usefully, the vector may in some examples therefore provide a single-vector means for introducing a complete exogenous Type I CRISPR/Cas system into a target cell for modification (eg, cutting by Cas3) of DNA in the target cell. In an example, a chromosomal target sequence (ie, protospacer that is cognate with the Cas3) is modified. In another example, an episomal DNA sequence is modified, for example a plasmid sequence or a DNA that has been introduced into the cell. The latter may be useful in a recombineering method of the invention wherein exogenous DNA in the target cell is cut by the Cas3 and optionally this produces one or more recombinogenic ends for recombination of the cut DNA with a further DNA of interest, thereby producing a recombination product in the cell. For example, in such a recombineering method, the target cell is a recombinongenic *E coli* cell, eg, comprising a red/ET system. In another example, the target cell is an undesired cell (eg, a cell of a species or strain that is pathogenic to humans or animals, such as a bacterial disease-causing species or strain) and the cutting by Cas3 kills the cell. This may be useful for treating or preventing an infection in a human or animal harbouring target cells. The provision of single-vector means that express minimally a Cas endonuclease (eg, Cas3), cognate accessory proteins (eg, Cascade proteins) and at least one CRISPR array (or nucleotide sequence encoding a guide RNA (eg, a single guide RNA)), wherein the Cas, accessory proteins and array (or nucleotide sequence) comprise a functional CRISPR/Cas system is more convenient and the inventors believe more efficient for introducing into a target cell and effecting CRISPR/Cas modification of a target sequence therein than the use of 2 or 3 or more separate vectors (eg, a vector encoding the Cas nuclease and a different vector encoding the accessory proteins, and possibly a further vector comprising the array (or gRNA-encoding nucleotide sequence) which all need to transform the target cell for the system to function). This may provide one or more benefits, therefore, such as simplifying delivery (and thus the design of delivery vehicles), simplifying construction of the vector and vehicle and/or providing for better cutting or killing efficiencies. Conveniently, an example of the invention therefore uses an operon for the coordinated expression in the target cells of the Cas and accessory proteins (and optionally also the array or gRNA-encoding sequence(s)). Whilst not wishing to be bound by any particular theory, the introduction of a single vector (eg, using an operon) as per the invention may advantageously coordinate the expression of the Cas and accessory proteins (and optionally production of cRNAs or gRNAs) so that these are available to operate together without undue delay in the target cell. This may be important to tip the balance between, on the one hand the target cell using its endogenous anti-restriction, endogenous Cas or other endogenous mechanisms that seek out and degrade invading phage and DNA, and on the other hand efficient cell killing or deactivation of such mechanisms by the invading CRISPR components of the vector of the invention. In such an arms race, concerted and early operation of the CRISPR components in the cell are likely to be important to gain the upper hand and effect cell killing. The invention provides means to assist this.

By way of example, the invention thus provides the following Concepts:—

1. A nucleic acid vector for introduction into a host cell, the vector comprising a first nucleotide sequence encoding a Type I Cas3 and a second nucleotide sequence encoding one or more Cascade proteins, wherein the first and second sequences are under the control of one or more promoters comprised by the vector for expression of the proteins in the cell.
2. The vector of concept 1, wherein the vector comprises an operon for expression in the cell of the Cas3 and Cascade proteins from a Cas module, the module comprising the nucleotide sequences encoding the Cas3 and Cascade proteins, and the operon comprising the Cas module under the control of a promoter for controlling the expression of both the Cas3 and Cascade proteins.
3. The vector of concept 2, wherein
   (a) the first sequence is between the promoter and the second sequence in the operon;
   (b) the operon comprises no Cas-encoding nucleotide sequences between the promoter and the first nucleotide sequence; and/or
   (c) the operon comprises (in 5' to 3' direction) the promoter, the first sequence and the second sequence.
4. The vector of any preceding concept, wherein each promoter is a constitutive promoter.
5. The vector of any one of concepts 1 to 3, wherein the promoter is repressible (optionally repressible by a tetracycline repressor or lac repressor).
6. The vector of any one of concepts 1 to 3, wherein the promoter is inducible.
7. The vector of any preceding concept, wherein the first sequence is under the control of a medium strength promoter.
8. The vector of any preceding concept, wherein the first sequence is under the control of a promoter that has an Anderson Score (AS) of 0.5>AS>0.1.
9. The vector of any preceding concept, wherein the first sequence (and optionally the second sequence) is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.
10. The vector of concept 9, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.
11. The vector of any preceding concept, wherein the vector comprises an origin of replication that is operable in the host cell.
12. The vector of any preceding concept, wherein the vector comprises an origin of replication that is operable in a bacterial cell of a vector production strain, wherein the

Cas3 is not operable in the production strain cell to target and cut a chromosomal sequence thereof.

13. The vector of concept 12, wherein the first sequence is under the control of a promoter that is capable of controlling expression of the Cas3 at a level that is not toxic to the production strain cell.
14. The vector of any preceding concept, wherein the vector is a high copy number vector.
15. The vector of any preceding concept, wherein the first nucleotide sequence or operon is comprised by a mobile genetic element.
16. The vector of any preceding concept, wherein the vector is devoid of a Cas adaption module.
17. The vector of any preceding concept, wherein the vector is devoid of nucleotide sequence encoding one, more or all of a Cas1, Cast, Cas4, Cas6, Cas7 and Cas8.
18. The vector of any preceding concept, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas11, Cas7 and Cas8a1.
19. The vector of concept 18, wherein the vector comprises nucleotide sequence encoding Cas3' and/or Cas3".
20. The vector or concept 19, wherein the nucleotide sequences encoding the Cas3' and/or Cas3" are between the promoter and the sequence(s) recited in concept 18.
21. The vector of any one of concepts 18 to 20, wherein the host cell comprises a Type IA CRISPR array that is cognate with the Cas3.
22. The vector of any one of concepts 18 to 20, wherein the host cell comprises an endogenous Type TB, C, U, D, E or F CRISPR/Cas system.
23. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8b1, Cas7 and Cas5.
24. The vector of concept 23, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in concept 23.
25. The vector of concept 23 or 24, wherein the host cell comprises a Type IB CRISPR array that is cognate with the Cas3.
26. The vector of concept 23 or 24, wherein the host cell comprises an endogenous Type IA, C, U, D, E or F CRISPR/Cas system.
27. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas5, Cas8c and Cas7.
28. The vector of concept 27, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in concept 27.
29. The vector of concept 27 or 28, wherein the host cell comprises a Type IC CRISPR array that is cognate with the Cas3.
30. The vector of concept 27 or 28, wherein the host cell comprises an endogenous Type IA, B, U, D, E or F CRISPR/Cas system.
31. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8U2, Cas7, Cas5 and Cas6.
32. The vector of concept 31, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in concept 31.
33. The vector of concept 31 or 32, wherein the host cell comprises a Type IU CRISPR array that is cognate with the Cas3.
34. The vector of concept 31 or 32, wherein the host cell comprises an endogenous Type IA, B, C, D, E or F CRISPR/Cas system.
35. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas10d, Cas7 and Cas5.
36. The vector of concept 35, wherein the vector comprises a nucleotide sequence encoding Cas3' and/or Cas3".
37. The vector of concept 36, wherein the nucleotide sequences encoding the Cas3' and/or Cas3" are between the promoter and the sequence(s) recited in concept 35.
38. The vector of any one of concepts 35 to 37, wherein the host cell comprises a Type ID CRISPR array that is cognate with the Cas3.
39. The vector of any one of concepts 35 to 37, wherein the host cell comprises an endogenous Type IA, B, C, U, E or F CRISPR/Cas system.
40. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8e, Cas11, Cas7, Cas5 and Cas6.
41. The vector of concept 40, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in concept 40.
42. The vector of concept 40 or 41, wherein the host cell comprises a Type IE CRISPR array that is cognate with the Cas3.
43. The vector of concept 40 or 41, wherein the host cell comprises an endogenous Type IA, B, C, D, U or F CRISPR/Cas system.
44. The vector of any one of concepts 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8f, Cas5, Cas7 and Cas6f.
45. The vector of concept 44, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in concept 44, wherein the vector is devoid of nucleotide sequence encoding further Cas between the promoter and the sequence encoding the Cas3.
46. The vector of concept 44 or 45, wherein the host cell comprises a Type IF CRISPR array that is cognate with the Cas3.
47. The vector of concept 44 or 45, wherein the host cell comprises an endogenous Type IA, B, C, D, U or E CRISPR/Cas system.
48. The vector of any one of concepts 1 to 17, wherein the Cas and Cascade are
    (a) Type IA Cas and Cascade proteins;
    (b) Type IB Cas and Cascade proteins;
    (c) Type IC Cas and Cascade proteins;
    (d) Type ID Cas and Cascade proteins;
    (e) Type IE Cas and Cascade proteins;
    (f) Type IF Cas and Cascade proteins; or
    (g) Type IU Cas and Cascade proteins.
49. The vector of any preceding concept, wherein the Cas and Cascade are *E coli* (optionally Type IE or IF) Cas and Cascade proteins.
50. The vector of concept 49, wherein the *E coli* is ESBL-producing *E. coli* or *E. coli* ST131-O25b:H4.
51. The vector of any preceding concept, wherein the Cas and Cascade are
    (a) *Clostridium* (eg, *C difficile*) Cas and Cascade proteins, optionally *C difficile* resistant to one or more antibiotics selected from aminoglycosides, lincomycin, tetracy-

clines, erythromycin, clindamycin, penicillins, cephalosporins and fluoroquinolones;

(b) *Pseudomonas aeruginosa* Cas and Cascade proteins, optionally *P aeruginosa* resistant to one or more antibiotics selected from carbapenems, aminoglycosides, cefepime, ceftazidime, fluoroquinolones, piperacillin and tazobactam; or

(c) *Klebsiella pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*) Cas and Cascade proteins.

52. The vector of any preceding concept, wherein the Cas and Cascade are *E coli, C difficile, P aeruginosa, K pneumoniae, P furiosus* or *B halodurans* Cas and Cascade proteins.

53. The vector of any preceding concept, wherein the Cas3 is a Cas3 of a CRISPR/Cas locus of a first bacterial or archaeal species, wherein the distance between the Cas3-encoding sequence of the locus and its cognate promoter is further than the distance between the Cas3-encoding sequence and the respective promoter comprised by the vector.

54. The vector of any preceding concept, wherein the distance between the promoter and the Cas3-encoding sequence and/or Cascade protein-encoding sequence(s) is shorter than in a corresponding wild-type Type I locus.

55. The vector of any preceding concept, wherein the vector comprises (i) a CRISPR array for producing crRNAs in the host cell and/or (ii) one or more nucleotide sequences encoding one or more guide RNAs (gRNAs or single gRNAs), wherein the crRNAs or gRNAs are cognate to the Cas3 (and optionally cognate to the Cascade proteins).

56. The vector of concept 55 when dependent from concept 2, wherein the array or gRNA-encoding sequence(s) are comprised by the operon and under the control of the promoter.

57. The vector of concept 56, wherein the array or gRNA-encoding sequence(s) are under the control of a promoter that is different from the promoter that controls the expression of the Cas3.

58. The vector of concept 56 or 57, wherein one or more of the crRNAs or gRNAs comprises a spacer sequence that is capable of hybridising to a target nucleotide sequence of the host cell, wherein the target sequence is adjacent a PAM, the PAM being cognate to the Cas3.

59. The vector of concept 58, wherein the target sequence is a chromosomal sequence of the host cell.

60. The vector of concept 58 or 59, wherein the Cas3 is operable to cut the target sequence.

61. The vector of any preceding concept, wherein the vector is a plasmid or phagemid.

62. A delivery vehicle comprising the vector of any preceding concept, wherein the delivery vehicle is capable of delivering the vector into the host cell.

63. The vehicle of concept 62, wherein the delivery vehicle is a phage, non-replicative transduction particle, nanoparticle carrier, bacterium or liposome.

64. The vector or vehicle of any preceding concept, wherein the host cell is a bacterial or archaeal cell, optionally, the host cell is a *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), *H pylori, S pneumoniae* or *S aureus* cell.

65. The vector or vehicle of any preceding concept for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

66. The vector or vehicle of concept 65, wherein the disease or condition is an infection of the subject with host cells (eg, bacterial cells), or wherein the disease or condition is mediated by host cells (eg, bacterial cells).

67. A pharmaceutical composition comprising the vector or vehicle of any preceding concept and a pharmaceutically acceptable diluent, excipient or carrier.

68. A method of treating or reducing the risk of a disease or condition in a human or animal subject, the method comprising administering the vector, vehicle or compostion of any preceding concept to the subject, and introducing the vector into target host bacterial or archaeal cells in the subject (eg, in a gut microbiota, lung, eye or blood of the subject), wherein the Cas cuts (or otherwise modifies) one or more target sequences in the target cells and the cells are killed or growth or proliferation of the cells is reduced.

69. The method of concept 68, wherein the target cells are cells of a disease pathogen species.

70. The method of concept 68 or 69, wherein the targt cells are *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), H *pylori, S pneumoniae* or *S aureus* cells.

EMBODIMENTS

An aspect of the invention provides improved ways of amplifying DNA constructs in bacterial and archaeal production strain cells. For example, the DNA may be a high copy number plasmid or phagemid comprising a constitutive promoter for controlling the expression of one or more Cas proteins when the DNA has been introduced into a target host bacterial or host cell. It is desirable, according to an aspect of the invention, to consider attenuating the promoter activity during amplification of the DNA in the production strain. This is useful, since the inventors have found that Cas expression in production strains may be toxic to production strain cells, thereby reducing the yield of amplified DNA. Toxicity may be due, for example, to off-target cutting of the production strain chromosomal DNA when the Cas is a nuclease (such as Cas9 or Cas3) and/or due to relatively high levels of expression of the Cas in the cells. Additionally or alternatively, undesirably the Cas expression or activity may impose a selective pressure that favours mutation and propagation of mutated DNA constructs (such as mutation in one more or all of a CRISPR/Cas operon, Cas-encoding gene, Cascade-encoding gene, CRISPR array and gRNa-encoding sequence of the DNA construct) in production cells, thereby reducing the yield of desired amplified constructs and imposing an undesired step of separating desired from mutated DNA constructs for further formulation into useful compositions. Such compositions may be pharmaceutical compositions, herbicides, pesticides, environmental remediation compositions etc. In one example, the promoter attenuation in production strains is achieved by using a medium strength (not high or low) promoter to control the Cas-encoding nucleotide sequence of the DNA constructs. A medium level of Cas expression may be tolerable in the production strains, and yet once the DNA is subsequently introduced into target host cells the Cas is expressed at sufficiently high levels to produce desired activity to modify (eg, cut) target sequences in target cells. In an alternative, the

invention uses a repressible promoter, wherein the promoter is repressed in production strain, but not repressed in target host cells. For example, aspects of the invention use a tetracycline repressor (tetR) expressed in production strain cells that represses the promoter.

Thus, the yield can be enhanced by one or more of

(a) reducing toxicity of the Cas in the production strain;

(b) reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain;

(c) promoting production cell viability during the amplification of the DNA; and

(d) reducing the occurrence of Cas cutting of DNA (optionally cutting of production host cell chromosomal DNA or said DNA construct).

To this end, the invention provides Embodiments as follows:—

1. A method of amplifying copies of a DNA encoding a functional Cas protein (optionally a Cas nuclease) in a bacterial or archaeal production strain of cells, the method comprising
   (a) Providing production strain cells, each cell comprising a copy of said DNA, wherein each DNA comprises a nucleotide sequence encoding said Cas, wherein the nucleotide sequence is under the control of a promoter for controlling the expression of the Cas in the production strain cell, the DNA comprising an origin of replication that is operable in the cell for replication of the DNA;
   (b) Culturing the cells to allow replication of the DNA, whereby the DNA is amplified; and
   (c) Optionally isolating copies of the DNA, wherein the promoter is an attenuated constitutive promoter.

In an example, promoter is a medium strength promoter. In another example, the promoter is repressed in the production strain cell. Hence, the promoter is an attenuated promoter in these examples.

2. Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for enhancing the yield of amplified DNA produced by the production host cells.
3. The use of paragraph 2, wherein the use is for enhancing said yield by
   (a) reducing toxicity of the Cas in the production strain;
   (b) reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain;
   (c) promoting production cell viability during the amplification of the DNA; and/or
   (d) reducing the occurrence of Cas cutting of DNA (optionally cutting of production host cell chromosomal DNA or said DNA construct).
4. Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing toxicity of the Cas in the production strain.
5. Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain.
6. Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for promoting production cell viability during the amplification of the DNA.
7. Use of an attenuated promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing the occurrence of Cas cutting of DNA.
8. A method for enhancing the yield of amplified copies of a DNA construct in a population of bacterial or archaeal production strain cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.
9. A method for reducing toxicity of a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.
10. A method for reducing mutation of a DNA construct encoding a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.
11. A method for promoting production cell viability of a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct comprised by the cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.
12. A method for reducing the occurrence of Cas nuclease cutting of a DNA construct in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises

a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated promoter.

13. The use of paragraph 5 or 7, or the method of paragraph 10 or 12, wherein the mutation or cutting is mutation or cutting of host cell chromosomal DNA or the construct DNA.
14. The method or use of any one of paragraphs 2 to 13, wherein the promoter is a constitutive promoter.
15. The method or use of any preceding paragraph, wherein the promoter is repressed in the production strain cells (optionally repressed by a tetracycline repressor or a lac repressor).
16. The method or use of paragraph 15, wherein the promoter is $P_{LtetO\text{-}1}$, $P_{LlacO\text{-}1}$ or a repressible homologue thereof.

Other examples of suitable repressible promoters are Ptac (repressed by lad) and he Leftward promoter (pL) of phage lambda (which repressed by the λcI repressor). In an example, the promoter comprises a repressible operator (eg, tetO or lacO) fused to a promoter sequence. The corresponding repressor is encoded by a nucleic acid in the production strain (eg, a chromosomally-integrated sequence or a sequence comprised by an episome) and the repressor is expressed during the DNA or vector amplification method of the invention, whereby the promoter controlling Cas expression is repressed. In delivery vehicles that are subsequently produced from isolated amplified DNA/vector, the vehicle is devoid of an expressible nucleotide sequence encoding the repressor, whereby the promoter is functional when the DNA/vector is introduced into a target host cell. For example, in the absence of the repressor the promoter is consitutively ON for expression of the Cas. The system is therefore primed to work once the DNA/vector is introduced into the host cells, and this effect can be enhanced further by using a high copy number DNA/vector comprising an origin of replication that is operable in the host cell. A high copy number vector or DNA is also desirable in the production strain cells for enhancing yield of the DNA/vector, and by use of an attenuated promoter as described herein (eg, medium strength promoter and/or repressed promoter in the production strain cells) one can minimise Cas toxicity whilst culturing to maximise amplification and thus yield of the DNA/vector.

17. The method or use of any preceding paragraph, wherein the promoter is a medium strength promoter.
18. The method or use of any preceding paragraph, wherein the promoter has an Anderson Score (AS) of 0.5>AS>0.1.
19. The method or use of any preceding paragraph, wherein the nucleotide sequence encoding said Cas is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.
20. The method or use of paragraph 19, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.
21. The method or use of any preceding paragraph, wherein the nuclease is Cas3 and optionally the DNA or cell encodes cognate Cascade proteins.
22. The method or use of any one of paragraphs 1 to 20, wherein the Cas is a Cas9.
23. The method or use of any preceding paragraph, wherein the production strain cells comprise a helper phage genome that is inducible to produce phage coat proteins in the cells, wherein the method further comprises inducing production of the phage proteins and causing packaging of the amplified DNA into phage particles or non-self-replicative transduction particles, and further isolating the phage or transduction particles and optionally formulating the particles into a pharmaceutical composition for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.
24. The method or use of paragraph 23, wherein the particles are capable of infecting target host cells in the subject and transducing the cells with the DNA, wherein the Cas and crRNAs (or guide RNAs, gRNAs) encoded by the DNA are expressed in the cells, the crRNAs or (gRNAs) being operable to guide the Cas to a target nucleotide sequence (optionally a chromosomal sequence) comprised by the cells, wherein the Cas cuts the target sequences in the cells, thereby killing host cells and treating or reducing the risk of the disease or condition.
25. The method or use of paragraph 24, wherein the host cells are bacterial or archaeal cells, optionally, the host cells are *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), *H pylori, S pneumoniae* or *S aureus* cells.
26. The method or use of any preceding paragraph, wherein each DNA is comprised by a high copy number plasmid or phagemid.
27. The method or use of any preceding paragraph, wherein the DNA construct comprises one or more nucleotide sequences for producing crRNAs or gRNAs that are operable for Cas nuclease targeting in target host cells.

PARAGRAPHS & GENERALLY APPLICABLE FEATURES

The invention provides the following Paragraphs, which are supported by the Examples below. Any features of the Concepts are combinable with any features of the Embodiments. Any features of the Concepts are combinable with any features of the Embodiments. Any features of the Paragraphs are combinable with any features of the Embodiments.

Any cell herein (eg, a production strain cell or target host cell) may be a bacterial cell, archaeal cell, algal cell, fungal cell, protozoan cell, invertebrate cell, vertebrate cell, fish cell, bird cell, mammal cell, companion animal cell, dog cell, cat cell, horse cell, mouse cell, rat cell, rabbit cell, eukaryotic cell, prokaryotic cell, human cell, animal cell, rodent cell, insect cell or plant cell. Preferably, the cell is a bacterial cell. Alternatively, the cell is a human cell. Optionally, the production strain cell(s) and target host cell(s) are of the same phylum, order, family, genus, species or strain.

1. A nucleic acid vector for introduction into a host cell, the vector comprising a first nucleotide sequence encoding a

Type I Cas3, wherein the sequence is under the control of a promoter comprised by the vector for expression of the Cas3 in the cell.

In an example, the vector is a DNA vector, eg, ssDNA vector or dsDNA vector.

2. The vector of paragraph 1, wherein the vector comprises a second nucleotide sequence encoding one or more Cascade proteins, wherein the first and second sequences are under the control of one or more promoters comprised by the vector for expression of the proteins in the cell.
3. The vector of paragraph 2, wherein the Cascade protein(s) are cognate with the Cas3.

In an example, the Cas3 is cognate with Cascade proteins encoded by the host cell and/or encoded by a second operon. Optionally, the second operon is comprised by the vector. Optionally, the second operon is comprised by a second vector that is capable of introducing the second operon into the host cell, whereby the Cas3 and Cascade proteins are expressed from the operons in the host cell and are operable with crRNA or gRNA to target the Cas to a host cell target sequence, wherein the Cas3 is capable of modifying the target sequence.

4. The vector of paragraph 2 or 3, wherein the vector comprises an operon for expression in the cell of the Cas3 and Cascade proteins from a Cas module, the module comprising the nucleotide sequences encoding the Cas3 and Cascade proteins, and the operon comprising the Cas module under the control of a promoter for controlling the expression of both the Cas3 and Cascade proteins.

The term "operon" is known to the skilled person such as relating to a functioning unit of DNA containing at least expressible 2 nucleotide sequences respectively encoding for an expression product (eg, a respective translatable mRNA), wherein the sequences are under common promoter control.

5. The vector of paragraph 4, wherein the first sequence is between the promoter and the second sequence in the operon.
6. The vector of paragraph 4 or 5, wherein the operon comprises no Cas-encoding nucleotide sequences between the promoter and the first nucleotide sequence.

Optionally, the Cas3 is a Cas3 encoded by a CRISPR/Cas locus of a first bacterial or archaeal species, wherein in the locus the Cas3-encoding sequence is 3' of Cascade protein-encoding sequences (ie, the latter are between the Cas3 and the 5'-most promoter of the locus).

Optionally, the Cas3 is a ygcB protein (eg, wherein the production strain cell and/or host target cell is an *E coli*).

Optionally, the Cascade proteins comprise or consist of

cas5 (casD, csy2)

cas6 (cas6f, cse3, casE)

cas7 (csc2, csy3, cse4, casC)

cas8 (casA, cas8a1, cas8b1, cas8c, cas10d, cas8e, cse1, cas8f, csy1).

Optionally herein the promoter and the Cas3-encoding sequence are spaced no more than 150, 100, 50, 40, 30, 20 or 10 bp apart, eg, from 30-45, or 30-40, or 39 or around 39 bp apart.

Optionally herein a ribosome binding site and the Cas3-encoding sequence are spaced no more than

20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 4 or 3 bp apart, eg, from 10-5, 6 or around 6 bp apart.

7. The vector of any one of paragraphs 4 to 6, wherein the operon comprises (in 5' to 3' direction) the promoter, the first sequence and the second sequence.
8. The vector of any preceding paragraph, wherein each promoter is a constitutive promoter.
9. The vector of any one of paragraphs 1 to 7, wherein the promoter is repressible (optionally repressible by a tetracycline repressor or lac repressor).
10. The vector of any one of paragraphs 1 to 7, wherein the promoter is inducible.
11. The vector of any preceding paragraph, wherein the first sequence is under the control of a weak promoter.
12. The vector of any one of paragraphs 1 to 7, wherein the first sequence is under the control of a medium strength promoter.
13. The vector of any one of paragraphs 1 to 7, wherein the first sequence is under the control of a strong promoter.

In an example, the promoter is in combination with a Shine-Dalgarno sequence comprising the sequence 5'-aaagaggagaaa-3' (SEQ ID NO: 5) or a ribosome binding site homologue thereof.

14. The vector of any one of paragraphs 1 to 7, wherein the first sequence is under the control of a promoter that has an Anderson Score (AS) of AS>0.5.

See Table 2 for more information on Anderson Scores in relation to promoters.

15. The vector of any one of paragraphs 1 to 7, wherein the first sequence is under the control of a promoter that has an Anderson Score (AS) of 0.5>AS>0.1.
16. The vector of any one of paragraphs 1 to 7, wherein the first sequence is under the control of a promoter that has an Anderson Score (AS) of ≤0.1.
17. The vector of any one of paragraphs 1 to 7, wherein the first sequence (and optionally the second sequence) is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.
18. The vector of paragraph 17, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.

For example, fluorescence using the first EOU is 0.5 to X times the fluorescence using the second EOU, wherein X is from 3.0 to 1.0, eg, 3, 2.5, 2, 1.5 or 1, wherein fluorescence is determined using excitation at 481 nm and emission at 507 nm. Optionally, *E coli* cultures at OD600 of 0.3-0.5 in the exponential growth phase are used.

For example, the upstream insulator, the nucleotide sequence encoding GFP, 3' UTR, transcription terminator and downstream insulator of each EOU are as disclosed in Mutalik et a1 (2013). For example, the upstream insulator, the nucleotide sequence encoding GFP, 3' UTR, transcription terminator and downstream insulator of each EOU are corresponding sequences of SEQ ID NO: 4. For example, the *E coli* is *E. coli* BW25113 is grown in MOPS EZ Rich Medium (Teknova) supplemented with 50 μg/ml kanamycin (kan) at 37° C., shaken at 900 r.p.m. For example, each EOUs is comprised by a medium copy plasmid, eg, a plasmid derived from pFAB217 comprising a p15A replication origin and a kan resistance gene.

19. The vector of any preceding paragraph, wherein the vector comprises an origin of replication that is operable in the host cell.

20. The vector of any preceding paragraph, wherein the vector comprises an origin of replication that is operable in a bacterial cell of a vector production strain, wherein the Cas3 is not operable in the production strain cell to target and cut a chromosomal sequence thereof.

An example of a production strain cell is an *E coli* cell. A production strain cell is a cell that is used to amplify DNA encoding Cas (and optionally other components of a CRISPR/Cas system). Usefully, the strain may package the amplified DNA into transduction particles that are may be isolated to produce a composition that can be contacted with a population of target host cells (eg, bacterial, archaeal, prokaryotic, eukaryotic, human, animal, mammal, rodent, mouse, rat, rabbit, *Xenopus*, fish, bird, amphibian, insect, plant, amoeba or algae cells) wherein the DNA is introduced into the cells for expression of the Cas (and optional other CRISPR/Cas system components), wherein the Cas is guided to a protospacer target sequence in the host cells and modifies (eg, cuts) the sequence. In another example, the amplified DNA isolated from a population of production strain cells and is combined with a delivery vehicle (eg, a carrier bacterium, nanoparticle or liposome), wherein the delivery vehicle can be contacted with a population of target host cells (eg, bacterial, archaeal, prokaryotic, eukaryotic, human, animal, mammal, rodent, mouse, rat, rabbit, *Xenopus*, fish, bird, amphibian, insect, plant, amoeba or algae cells) wherein the DNA is introduced into the cells for expression of the Cas (and optional other CRISPR/Cas system components), wherein the Cas is guided to a protospacer target sequence in the host cells and modifies (eg, cuts) the sequence.

21. The vector of paragraph 20, wherein the first sequence is under the control of a promoter that is capable of controlling expression of the Cas3 at a level that is not toxic to the production strain cell.

In an example, substantially no production strain cells are killed when the Cas3-encoding sequence is amplified therein. In another example, no more than 40, 30, 20, 10, 5, 4, 3, 2, or 1% of production strain cells are killed when the Cas3-encoding sequence is amplified therein. For example this is in a

1, 2, 3, 4, 5, 6, 7, 8 9 10, 12 or 24 hour period of culturing the cells.

22. The vector of paragraph 20, wherein the first sequence is under the control of a promoter that controls expression of the Cas3 in the production strain cell such that the cell is capable of growth and propagation sufficient to produce at least 1000 copies of the vector.

For example this is in a 1, 2, 3, 4, 5, 6, 7, 8 9 10, 12 or 24 hour period of culturing the cells. For example, at least 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15}, 10^{16}, 10^{17} or 10^{18} copies of the vector are produced per 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15}, 10^{16}, 10^{17} production strain cells respectively.

23. The vector of any one of paragraphs 20 to 22, wherein the cell is capable of at least 2 or 3 logs of expansion when the vector is comprised therein.

For example, this is in a 1, 2, 3, 4, 5, 6, 7, 8 9 10, 12 or 24 hour period of culturing the cells.

24. The vector of any preceding paragraph, wherein the vector is a high copy number vector.

25. The vector of any preceding paragraph, wherein the first nucleotide sequence or operon is comprised by a mobile genetic element.

Suitable mobile genetic elements, eg, transposons, are disclosed in WO2016177682 and US20170246221, the disclosures of which are explicitly incorporated herein for possible use in the invention and for providing one or more features for the claims herein.

26. The vector of any preceding paragraph, wherein the vector is devoid of a Cas adaption module.

For example, the vector is devoid of nucleotide sequences encoding a Cas1, Cas2 and/or Cas4.

27. The vector of any preceding paragraph, wherein the vector is devoid of nucleotide sequence encoding one, more or all of a Cas1, Cas2, Cas4, Cas6 (optionally Cas6f), Cas7 and Cas8 (optionally Cas8f).

28. The vector of any preceding paragraph, wherein the vector is devoid of a sequence encoding a Cas6 (optionally a Cas6f).

29. The vector of any one of paragraphs 1 to 27, wherein the module encodes a Cas6 (optionally a Cas6f).

30. The vector of any preceding paragraph, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas11, Cas7 and Cas8a1.

31. The vector of paragraph 30, wherein the vector comprises nucleotide sequence encoding Cas3' and/or Cas3'' (optionally wherein the nucleotide sequences encoding the Cas3' and/or Cas3'' are between the promoter and the sequence(s) recited in paragraph 30).

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3 (eg, Cas3' and/or Cas3''), Cas11, Cas7 and Cas8a1. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas11 sequence. Optionally, the vector comprises a Type IA CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

32. The vector of paragraph 30 or 31, wherein the host cell comprises a Type IA CRISPR array that is cognate with the Cas3.

33. The vector of paragraph 30 or 31, wherein the host cell comprises an endogenous Type IB, C, U, D, E or F CRISPR/Cas system.

34. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8b1, Cas7 and Cas5.

35. The vector of paragraph 34, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in paragraph 34.

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas8b1, Cas7 and Cas5. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas8b1 sequence. Optionally, the vector comprises a Type IB CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the

vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

36. The vector of paragraph 34 or 35, wherein the host cell comprises a Type IB CRISPR array that is cognate with the Cas3.
37. The vector of paragraph 34 or 35, wherein the host cell comprises an endogenous Type IA, C, U, D, E or F CRISPR/Cas system.
38. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas5, Cas8c and Cas7.
39. The vector of paragraph 38, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in paragraph 38.

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas5, Cas8c and Cas7. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas5 sequence. Optionally, the vector comprises a Type IC CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

40. The vector of paragraph 38 or 39, wherein the host cell comprises a Type IC CRISPR array that is cognate with the Cas3.
41. The vector of paragraph 38 or 39, wherein the host cell comprises an endogenous Type IA, B, U, D, E or F CRISPR/Cas system.
42. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8U2, Cas7, Cas5 and Cas6.
43. The vector of paragraph 42, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in paragraph 42.

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas8U2, Cas7, Cas5 and Cas6. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas8U2 sequence. Optionally, the vector comprises a Type IU CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

44. The vector of paragraph 42 or 43, wherein the host cell comprises a Type IU CRISPR array that is cognate with the Cas3.
45. The vector of paragraph 42 or 43, wherein the host cell comprises an endogenous Type IA, B, C, D, E or F CRISPR/Cas system.
46. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas10d, Cas7 and Cas5.
47. The vector of paragraph 46, wherein the vector comprises a nucleotide sequence encoding Cas3' and/or Cas3" (optionally wherein the nucleotide sequences encoding the Cas3' and/or Cas3" are between the promoter and the sequence(s) recited in paragraph 46).

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas10d, Cas7 and Cas5. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas10d sequence. Optionally, the vector comprises a Type ID CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

48. The vector of paragraph 46 or 47, wherein the host cell comprises a Type ID CRISPR array that is cognate with the Cas3.
49. The vector of paragraph 46 or 47, wherein the host cell comprises an endogenous Type IA, B, C, U, E or F CRISPR/Cas system.
50. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8e, Cas11, Cas7, Cas5 and Cas6.
51. The vector of paragraph 50, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in paragraph 50.

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas8e, Cas11, Cas7, Cas5 and Cas6. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas11 sequence. Optionally, the vector comprises a Type IE CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify

(eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

52. The vector of paragraph 50 or 51, wherein the host cell comprises a Type IE CRISPR array that is cognate with the Cas3.
53. The vector of paragraph 50 or 51, wherein the host cell comprises an endogenous Type IA, B, C, D, U or F CRISPR/Cas system.
54. The vector of any one of paragraphs 1 to 29, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8f, Cas5, Cas7 and Cas6f.
55. The vector of paragraph 54, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in paragraph 54, wherein the vector is devoid of nucleotide sequence encoding further Cas between the promoter and the sequence encoding the Cas3.

In one embodiment, the vector comprises nucleotide sequences (in 5' to 3' direction) that encode a Cas3, Cas8f, Cas5, Cas7 and Cas6f. Optionally, a nucleotide sequence encoding Cas6 is between the Cas3 sequence(s) and the Cas8f sequence. Optionally, the vector comprises a Type IF CRISPR array or one or more nucleotide sequences encoding single guide RNA(s) (gRNA(s)), wherein the array and each gRNA comprises repeat sequence that is cognate with the Cas3. Thus, the array is operable in a host cell when the vector has been introduced into the cell for production of guide RNAs, wherein the guide RNAs are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell. Similarly, the single guide RNAs encoded by the vector in one embodiment are operable with the Cas and Cascade proteins to target and modify (eg, cut) a target nucleotide sequence in the host cell, optionally thereby killing the host cell.

56. The vector of paragraph 54 or 55, wherein the host cell comprises a Type IF CRISPR array that is cognate with the Cas3.
57. The vector of paragraph 54 or 55, wherein the host cell comprises an endogenous Type IA, B, C, D, U or E CRISPR/Cas system.
58. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IA Cas and Cascade proteins.
59. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IB Cas and Cascade proteins.
60. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IC Cas and Cascade proteins.
61. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type ID Cas and Cascade proteins.
62. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IE Cas and Cascade proteins.
63. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IF Cas and Cascade proteins.
64. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are Type IU Cas and Cascade proteins.
65. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are *E coli* (optionally Type IE or IF) Cas and Cascade proteins, optionally wherein the *E coli* is ESBL-producing *E. coli* or *E. coli* ST131-O25b:H4.
66. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are *Clostridium* (eg, *C difficile*) Cas and Cascade proteins, optionally *C difficile* resistant to one or more antibiotics selected from aminoglycosides, lincomycin, tetracyclines, erythromycin, clindamycin, penicillins, cephalosporins and fluoroquinolones.
67. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are *Pseudomonas aeruginosa* Cas and Cascade proteins, optionally *P aeruginosa* resistant to one or more antibiotics selected from carbapenems, aminoglycosides, cefepime, ceftazidime, fluoroquinolones, piperacillin and tazobactam.
68. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are *Klebsiella pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*) Cas and Cascade proteins.
69. The vector of any one of paragraphs 1 to 29, wherein the Cas and Cascade are *E coli, C difficile, P aeruginosa, K pneumoniae, P furiosus* or *B halodurans* Cas and Cascade proteins.
70. The vector of any preceding paragraph, wherein the Cas3 is a Cas3 of a CRISPR/Cas locus of a first bacterial or archaeal species, wherein the distance between the Cas3-encoding sequence of the locus and its cognate promoter is further than the distance between the Cas3-encoding sequence and the respective promoter comprised by the vector.

The cognate promoter here is the one that controls expression of Cas3 in the wild-type locus.

71. The vector of any preceding paragraph, wherein the distance between the promoter and the Cas3-encoding sequence and/or Cascade protein-encoding sequence(s) is shorter than in a corresponding wild-type Type I locus.

A corresponding locus is a wild-type locus of a bacterial or archaeal species or strain that comprises an endogenous CRISPR/Cas system encoding the Cas3 and/or Cascade proteins of the type that are also encoded by the vector. Thus, when the vector comprises an operon, the operon may comprise Cas3- and Cascade-encoding nucleotide sequences that are not in a natural configuration.

72. The vector of any preceding paragraph, wherein the vector comprises (i) a CRISPR array for producing crRNAs in the host cell and/or (ii) one or more nucleotide sequences encoding one or more single guide RNAs (gRNAs), wherein the crRNAs or gRNAs are cognate to the Cas3 (and optionally cognate to the Cascade proteins).
73. The vector of paragraph 72 when dependent from paragraph 4, wherein the array or gRNA-encoding sequence(s) are comprised by the operon and under the control of the promoter.
74. The vector of paragraph 72, wherein the array or gRNA-encoding sequence(s) are under the control of a promoter that is different from the promoter that controls the expression of the Cas3.
75. The vector of any one of paragraphs 72 to 74, wherein one or more of the crRNAs or gRNAs comprises a spacer sequence that is capable of hybridising to a target nucleotide sequence of the host cell, wherein the target sequence is adjacent a PAM, the PAM being cognate to the Cas3.

Thus, the spacer hybridises to the protospacer to guide the Cas3 to the protospacer. Optionally, the Cas3 cuts the protospacer, eg, using exo- and/or endonuclease activity of the Cas3. Optionally, the Cas3 removes a plurality (eg, at least 2, 3, 4, 5, 6, 7, 8, 9 or 10) nucleotides from the protospacer.

76. The vector of paragraph 75, wherein the target sequence is a chromosomal sequence of the host cell.
77. The vector of paragraph 75 or 76, wherein the Cas3 is operable to cut the target sequence.
78. The vector of any preceding paragraph, wherein the vector is a plasmid or phagemid.

79. A delivery vehicle comprising the vector of any preceding paragraph, wherein the delivery vehicle is capable of delivering the vector into the host cell.

80. The vehicle of paragraph 79, wherein the delivery vehicle is a phage, non-replicative transduction particle, nanoparticle carrier, bacterium or liposome.

The phage or particles comprise phage coat proteins encapsidating DNA, wherein the DNA comprises the vector. Suitable examples of phage and particles are disclosed in U.S. Ser. No. 15/985,658 (and its equivalent publication by USPTO) the disclosures of which are incorporated herein by reference for possible use in the invention and for providing one or more features that may be included in the claims herein. Phage or particle is capable of infecting the cell, thereby introducing the vector into the cell.

81. The vector or vehicle of any preceding paragraph, wherein the host cell is a bacterial or archaeal cell, optionally, the host cell is a *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), *H pylori, S pneumoniae* or *S aureus* cell.

82. The vector or vehicle of any preceding paragraph for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

83. The vector or vehicle of paragraph 82, wherein the disease or condition is an infection of the subject with host cells (eg, bacterial cells), or wherein the disease or condition is mediated by host cells (eg, bacterial cells).

84. A pharmaceutical composition comprising the vector or vehicle of any preceding paragraph and a pharmaceutically acceptable diluent, excipient or carrier.

85. A method of amplifying copies of a DNA encoding a functional Cas protein (optionally a Cas nuclease) in a bacterial or archaeal production strain of cells, the method comprising

(a) Providing production strain cells, each cell comprising a copy of said DNA, wherein each DNA comprises a nucleotide sequence encoding said Cas, wherein the nucleotide sequence is under the control of a promoter for controlling the expression of the Cas in the production strain cell, the DNA comprising an origin of replication that is operable in the cell for replication of the DNA;

(b) Culturing the cells to allow replication of the DNA, whereby the DNA is amplified; and

(c) Optionally isolating copies of the DNA,

86. The method of paragraph 85, wherein the promoter is a constitutive promoter.

87. The method of paragraph 85, wherein the promoter is repressible (optionally repressible by a tetracycline repressor or a lac repressor).

88. The method of paragraph 85, wherein the promoter is inducible.

89. The method of any one of paragraphs 85 to 88, wherein the promoter is a medium strength promoter.

90. The method of any one of paragraphs 85 to 89, wherein the promoter has an Anderson Score (AS) of 0.5>AS>0.1.

91. The method of any one of paragraphs 85 to 90, wherein the nucleotide sequence encoding said Cas is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.

92. The method of paragraph 91, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.

93. The method of any one of paragraphs 85 to 92, wherein the nuclease is Cas3 and optionally the DNA or cell encodes cognate Cascade proteins and/or one or more crRNAs that are operable for Cas nuclease targeting.

For example, the targeting is targeting of the Cas to a protospacer sequence comprised by a host cell chromosome or an episome thereof. In another example the targeting is in a recombineering method and the Cas is targeted to a protospacer sequence of a DNA that has been introduced into or amplified in the host cell. In an example of such recombineering, the host cell is an *E coli* cell.

94. The method of any one of paragraphs 85 to 92, wherein the Cas is a Cas9.

95. The method of any one of paragraphs 85 to 92, wherein the Cas is a Type IIIA csm protein or a Type IIIB cmr protein.

96. The method of any one of paragraphs 85 to 92, wherein the Cas is a Csf1.

97. The method of any one of paragraphs 85 to 92, wherein the Cas is a Cpf1.

98. The method of any one of paragraphs 85 to 92, wherein the Cas is a Cas13 (optionally Cas13a or Cas13b).

99. The method of any one of paragraphs 85 to 92, wherein the Cas is selected from a Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, GSU0054, Cas10, Csm2, Cmr5, Cas10, Csx11, Csx10, Csf1, Cas9, Csn2, Cas4, Cpf1, C2c1, C2c3, Cas13a, Cas13b and Cas13c.

100. The method of any one of paragraphs 85 to 99, wherein the production strain cells comprise a helper phage genome that is inducible to produce phage coat proteins in the cells, wherein the method further comprises inducing production of the phage proteins and causing packaging of the amplified DNA into phage particles or non-self-replicative transduction particles, and further isolating the phage or transduction particles and optionally formulating the particles into a pharmaceutical composition for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

101. The method of paragraph 100, wherein the particles are capable of infecting target host cells in the subject and transducing the cells with the DNA, wherein the Cas and crRNAs (or gRNAs) encoded by the DNA are expressed in the cells, the crRNAs or (gRNAs) being operable to guide the Cas to a target nucleotide sequence (optionally a chromosomal sequence) comprised by the cells, wherein the Cas cuts the target sequences in the cells, thereby killing host cells and treating or reducing the risk of the disease or condition.

102. The method of paragraph 101, wherein the host cells are bacterial or archaeal cells, optionally, the host cells are *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), *H pylori, S pneumoniae* or *S aureus* cells.

103. The method of any one of paragraphs 85 to 102, wherein each DNA is comprised by a high copy number vector, optionally a high copy number plasmid (an optionally the promoter is a constitutive promoter).

104. The method of any one of paragraphs 85 to 103, wherein each DNA is comprised by a vector or vehicle according to any one of paragraphs 1 to 83.

105. Use of an attenuated strength promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for enhancing the yield of amplified DNA produced by the production host cells.

Thus, said enhancing may be relative to the yield produced using a strong promoter, eg, a strong constitutive promoter (for example a promoter having an Anderson Score (AS) of AS>0.5). In another example, the strong promoter is a promoter comprised by a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of >4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.

106. The use of paragraph 105, wherein the use is for enhancing said yield by

(d) reducing toxicity of the Cas in the production strain;

(e) reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain;

(f) promoting production cell viability during the amplification of the DNA; and/or (g) reducing the occurrence of Cas cutting of DNA (optionally cutting of production host cell chromosomal DNA or said DNA construct).

107. Use of an attenuated strength promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing toxicity of the Cas in the production strain.

108. Use of an attenuated strength promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing mutation of the DNA (optionally the Cas-encoding sequence) in the production strain.

109. Use of an attenuated strength promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for promoting production cell viability during the amplification of the DNA.

110. Use of an attenuated strength promoter in a DNA construct comprising a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of the promoter, in a method of amplifying copies of the DNA in a population of bacterial or archaeal production strain cells, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, for reducing the occurrence of Cas cutting of DNA.

111. A method for enhancing the yield of amplified copies of a DNA construct in a population of bacterial or archaeal production strain cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) that is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated strength promoter.

112. A method for reducing toxicity of a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated strength promoter.

113. A method for reducing mutation of a DNA construct encoding a functional Cas protein (optionally a Cas nuclease) in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated strength promoter.

114. A method for promoting production cell viability of a population of bacterial or archaeal production strain cells in a process of amplifying copies of a DNA construct comprised by the cells, wherein the construct comprises a nucleotide sequence encoding a functional Cas protein (optionally a Cas nuclease) and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated strength promoter.

115. A method for reducing the occurrence of Cas nuclease cutting of a DNA construct in a population of bacterial or archaeal production strain cells in a process of amplifying copies of the construct, wherein the construct comprises a nucleotide sequence encoding the Cas and the sequence is under the control of a promoter, the method comprising culturing the cells to allow replication of the DNA thereby amplifying the DNA in the cells, wherein the promoter is an attenuated strength promoter.

116. The use of paragraph 108 or 110, or the method of paragraph 113 or 115, wherein the mutation or cutting is mutation or cutting of host cell chromosomal DNA or the construct DNA.

117. The use or method of any one of paragraphs 105 to 116, wherein the promoter is a constitutive promoter.

118. The use or method of any one of paragraphs 105 to 117, wherein the promoter is repressible (optionally repressible by a tetracycline repressor or a lac repressor).

In an example, the promoter is a constitutive promoter and optionally the DNA is comprised by a high copy number plasmid or phagemid.

119. The use or method of any one of paragraphs 105 to 118, wherein the promoter is $P_{LtetO-1}$, $P_{LlacO-1}$ or a repressible homologue thereof.

$P_{LlacO-1}$ is repressed by lac repressor (LacR). Pueto-1 is repressed by tet repressor (TetR).

120. The use or method of any one of paragraphs 105 to 119, wherein the promoter is a medium strength promoter.

121. The use or method of any one of paragraphs 105 to 120, wherein the promoter has an Anderson Score (AS) of 0.5>AS>0.1.

122. The use or method of any one of paragraphs 105 to 121, wherein the nucleotide sequence encoding said Cas is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.

123. The use or method of paragraph 122, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.

124. The use or method of any one of paragraphs 105 to 123, wherein the nuclease is Cas3 and optionally the DNA construct encodes cognate Cascade proteins.

125. The use or method of any one of paragraphs 105 to 123, wherein the Cas is a Cas9.

126. The use or method of any one of paragraphs 105 to 123, wherein the Cas is a Type IIIA csm protein or a Type IIIB cmr protein.

127. The use or method of any one of paragraphs 105 to 123, wherein the Cas is a Csf1.

128. The use or method of any one of paragraphs 105 to 123, wherein the Cas is a Cpf1.

129. The use or method of any one of paragraphs 105 to 123, wherein the Cas is a Cas13 (optionally Cas13a or Cas13b).

130. The use or method of any one of paragraphs 105 to 123, wherein the Cas is selected from a Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, GSU0054, Cas10, Csm2, Cmr5, Cas10, Csx11, Csx10, Csf1, Cas9, Csn2, Cas4, Cpf1, C2c1, C2c3, Cas13a, Cas13b and Cas13c.

131. The use or method of any one of paragraphs 105 to 130, wherein the DNA construct comprises one or more nucleotide sequences for producing crRNAs or gRNAs that are operable for Cas nuclease targeting.

132. The use or method of any one of paragraphs 105 to 131, wherein the production strain cells comprise a helper phage genome that is inducible to produce phage coat proteins in the cells, wherein the method further comprises inducing production of the phage proteins and causing packaging of the amplified DNA into phage particles or non-self-replicative transduction particles, and further isolating the phage or transduction particles and optionally formulating the particles into a pharmaceutical composition for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

133. The method of paragraph 132, wherein the particles are capable of infecting target host cells in the subject and transducing the cells with the DNA, wherein the Cas and crRNAs (or gRNAs) encoded by the DNA are expressed in the cells, the crRNAs or (gRNAs) being operable to guide the Cas to a target nucleotide sequence (optionally a chromosomal sequence) comprised by the cells, wherein the Cas cuts the target sequences in the cells, thereby killing host cells and treating or reducing the risk of the disease or condition.

134. The method of paragraph 133, wherein the host cells are bacterial or archaeal cells, optionally, the host cells are *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b: H4), *H pylori, S pneumoniae* or *S aureus* cells.

135. The use or method of any one of paragraphs 105 to 134, wherein each DNA is comprised by a high copy number vector, optionally a high copy number plasmid (an optionally the promoter is a constitutive promoter).

136. The use or method of any one of paragraphs 105 to 135, wherein each DNA is comprised by a vector according to any one of paragraphs 1 to 78 and 81 to 83.

CLAUSES

The invention provides, by way of example, the following Clauses; the features of these are combinable with any other disclosure herein.

1. A nucleic acid vector for introduction into a host cell, the vector comprising a first nucleotide sequence encoding a Type I Cas3 and a second nucleotide sequence encoding one or more Cascade proteins, wherein the first and second sequences are under the control of one or more promoters comprised by the vector for expression of the proteins in the cell.
2. The vector of Clause 1, wherein the vector comprises an operon for expression in the cell of the Cas3 and Cascade proteins from a Cas module, the module comprising the nucleotide sequences encoding the Cas3 and Cascade proteins, and the operon comprising the Cas module under the control of a promoter for controlling the expression of both the Cas3 and Cascade proteins.
3. The vector of Clause 2, wherein
   (a) the first sequence is between the promoter and the second sequence in the operon;
   (b) the operon comprises no Cas-encoding nucleotide sequences between the promoter and the first nucleotide sequence; and/or
   (c) the operon comprises (in 5' to 3' direction) the promoter, the first sequence and the second sequence.
4. The vector of any preceding Clause, wherein each promoter is a constitutive promoter.
5. The vector of any one of Clauses 1 to 3, wherein the promoter is repressible (optionally repressible by a tetracycline repressor or lac repressor).
6. The vector of any one of Clauses 1 to 3, wherein the promoter is inducible.
7. The vector of any preceding Clause, wherein the first sequence is under the control of a medium strength promoter.

8. The vector of any preceding Clause, wherein the first sequence is under the control of a promoter that has an Anderson Score (AS) of 0.5>AS>0.1.
9. The vector of any preceding Clause, wherein the first sequence (and optionally the second sequence) is under the control of a promoter and translation initiation site (TIS) combination that is capable of producing expression of green fluorescent protein (GFP) from a first expression operating unit (EOU) in *E. coli* strain BW25113 cells with a fluorescence of from 0.5 to 4 times the fluorescence produced in *E. coli* strain BW25113 cells using a second EOU comprising a P10 promoter (SEQ ID NO: 1) combined with a BCD14 TIS (SEQ ID NO: 2), wherein the EOUs differ only in their promoter and TIS combinations, wherein each EOU comprises (in 5' to 3' direction) an upstream initiator, the respective promoter, the respective TIS, a nucleotide sequence encoding GFP, a 3' UTR, a transcription terminator and a downstream insulator.
10. The vector of Clause 9, wherein fluorescence using the first EOU is 0.5 to 2 times the fluorescence using the second EOU.
11. The vector of any preceding Clause, wherein the vector comprises an origin of replication that is operable in the host cell.
12. The vector of any preceding Clause, wherein the vector comprises an origin of replication that is operable in a bacterial cell of a vector production strain, wherein the Cas3 is not operable in the production strain cell to target and cut a chromosomal sequence thereof.
13. The vector of Clause 12, wherein the first sequence is under the control of a promoter that is capable of controlling expression of the Cas3 at a level that is not toxic to the production strain cell.
14. The vector of any preceding Clause, wherein the vector is a high copy number vector.
15. The vector of any preceding Clause, wherein the first nucleotide sequence or operon is comprised by a mobile genetic element.
16. The vector of any preceding Clause, wherein the vector is devoid of a Cas adaption module.
17. The vector of any preceding Clause, wherein the vector is devoid of nucleotide sequence encoding one, more or all of a Cas1, Cast, Cas4, Cas6, Cas7 and Cas8.
18. The vector of any preceding Clause, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas11, Cas7 and Cas8a1.
19. The vector of Clause 18, wherein the vector comprises nucleotide sequence encoding Cas3' and/or Cas3".
20. The vector or Clause 19, wherein the nucleotide sequences encoding the Cas3' and/or Cas3" are between the promoter and the sequence(s) recited in Clause 18.
21. The vector of any one of Clauses 18 to 20, wherein the host cell comprises a Type IA CRISPR array that is cognate with the Cas3.
22. The vector of any one of Clauses 18 to 20, wherein the host cell comprises an endogenous Type IB, C, U, D, E or F CRISPR/Cas system.
23. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8b1, Cas7 and Cas5.
24. The vector of Clause 23, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in Clause 23.
25. The vector of Clause 23 or 24, wherein the host cell comprises a Type IB CRISPR array that is cognate with the Cas3.
26. The vector of Clause 23 or 24, wherein the host cell comprises an endogenous Type IA, C, U, D, E or F CRISPR/Cas system.
27. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas5, Cas8c and Cas7.
28. The vector of Clause 27, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in Clause 27.
29. The vector of Clause 27 or 28, wherein the host cell comprises a Type IC CRISPR array that is cognate with the Cas3.
30. The vector of Clause 27 or 28, wherein the host cell comprises an endogenous Type IA, B, U, D, E or F CRISPR/Cas system.
31. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8U2, Cas7, Cas5 and Cas6.
32. The vector of Clause 31, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in Clause 31.
33. The vector of Clause 31 or 32, wherein the host cell comprises a Type IU CRISPR array that is cognate with the Cas3.
34. The vector of Clause 31 or 32, wherein the host cell comprises an endogenous Type IA, B, C, D, E or F CRISPR/Cas system.
35. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas10d, Cas7 and Cas5.
36. The vector of Clause 35, wherein the vector comprises a nucleotide sequence encoding Cas3' and/or Cas3".
37. The vector of Clause 36, wherein the nucleotide sequences encoding the Cas3' and/or Cas3" are between the promoter and the sequence(s) recited in Clause 35.
38. The vector of any one of Clauses 35 to 37, wherein the host cell comprises a Type ID CRISPR array that is cognate with the Cas3.
39. The vector of any one of Clauses 35 to 37, wherein the host cell comprises an endogenous Type IA, B, C, U, E or F CRISPR/Cas system.
40. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8e, Cas11, Cas7, Cas5 and Cas6.
41. The vector of Clause 40, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in Clause 40.
42. The vector of Clause 40 or 41, wherein the host cell comprises a Type IE CRISPR array that is cognate with the Cas3.
43. The vector of Clause 40 or 41, wherein the host cell comprises an endogenous Type IA, B, C, D, U or F CRISPR/Cas system.
44. The vector of any one of Clauses 1 to 17, wherein the vector comprises (optionally in 5' to 3' direction) nucleotide sequence encoding one, more or all of Cas8f, Cas5, Cas7 and Cas6f.
45. The vector of Clause 44, wherein the vector comprises a nucleotide sequence encoding Cas3 between the promoter and the sequence(s) recited in Clause 44, wherein

the vector is devoid of nucleotide sequence encoding further Cas between the promoter and the sequence encoding the Cas3.

46. The vector of Clause 44 or 45, wherein the host cell comprises a Type IF CRISPR array that is cognate with the Cas3.

47. The vector of Clause 44 or 45, wherein the host cell comprises an endogenous Type IA, B, C, D, U or E CRISPR/Cas system.

48. The vector of any one of Clauses 1 to 17, wherein the Cas and Cascade are
(a) Type IA Cas and Cascade proteins;
(b) Type IB Cas and Cascade proteins;
(c) Type IC Cas and Cascade proteins;
(d) Type ID Cas and Cascade proteins;
(e) Type IE Cas and Cascade proteins;
(f) Type IF Cas and Cascade proteins; or
(g) Type IU Cas and Cascade proteins.

49. The vector of any preceding Clause, wherein the Cas and Cascade are *E coli* (optionally Type IE or IF) Cas and Cascade proteins.

50. The vector of Clause 49, wherein the *E coli* is ESBL-producing *E. coli* or *E. coli* ST131-O25b:H4.

51. The vector of any preceding Clause, wherein the Cas and Cascade are
(a) *Clostridium* (eg, *C difficile*) Cas and Cascade proteins, optionally *C difficile* resistant to one or more antibiotics selected from aminoglycosides, lincomycin, tetracyclines, erythromycin, clindamycin, penicillins, cephalosporins and fluoroquinolones;
(b) *Pseudomonas aeruginosa* Cas and Cascade proteins, optionally *P aeruginosa* resistant to one or more antibiotics selected from carbapenems, aminoglycosides, cefepime, ceftazidime, fluoroquinolones, piperacillin and tazobactam; or
(c) *Klebsiella pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*) Cas and Cascade proteins.

52. The vector of any preceding Clause, wherein the Cas and Cascade are *E coli, C difficile, P aeruginosa, K pneumoniae, P furiosus* or *B halodurans* Cas and Cascade proteins.

53. The vector of any preceding Clause, wherein the Cas3 is a Cas3 of a CRISPR/Cas locus of a first bacterial or archaeal species, wherein the distance between the Cas3-encoding sequence of the locus and its cognate promoter is further than the distance between the Cas3-encoding sequence and the respective promoter comprised by the vector.

54. The vector of any preceding Clause, wherein the distance between the promoter and the Cas3-encoding sequence and/or Cascade protein-encoding sequence(s) is shorter than in a corresponding wild-type Type I locus.

55. The vector of any preceding Clause, wherein the vector comprises (i) a CRISPR array for producing crRNAs in the host cell and/or (ii) one or more nucleotide sequences encoding one or more guide RNAs (gRNAs or single gRNAs), wherein the crRNAs or gRNAs are cognate to the Cas3 (and optionally cognate to the Cascade proteins).

56. The vector of Clause 55 when dependent from Clause 2, wherein the array or gRNA-encoding sequence(s) are comprised by the operon and under the control of the promoter.

57. The vector of Clause 56, wherein the array or gRNA-encoding sequence(s) are under the control of a promoter that is different from the promoter that controls the expression of the Cas3.

58. The vector of Clause 56 or 57, wherein one or more of the crRNAs or gRNAs comprises a spacer sequence that is capable of hybridising to a target nucleotide sequence of the host cell, wherein the target sequence is adjacent a PAM, the PAM being cognate to the Cas3.

59. The vector of Clause 58, wherein the target sequence is a chromosomal sequence of the host cell.

60. The vector of Clause 58 or 59, wherein the Cas3 is operable to cut the target sequence.

61. The vector of any preceding Clause, wherein the vector is a plasmid or phagemid.

62. A delivery vehicle comprising the vector of any preceding Clause, wherein the delivery vehicle is capable of delivering the vector into the host cell.

63. The vehicle of Clause 62, wherein the delivery vehicle is a phage, non-replicative transduction particle, nanoparticle carrier, bacterium or liposome.

64. The vector or vehicle of any preceding Clause, wherein the host cell is a bacterial or archaeal cell, optionally, the host cell is a *C difficile, P aeruginosa, K pneumoniae* (eg, carbapenem-resistant *Klebsiella pneumoniae* or Extended-Spectrum Beta-Lactamase (ESBL)-producing *K pneumoniae*), *E coli* (eg, ESBL-producing *E. coli*, or *E. coli* ST131-O25b:H4), *H pylori, S pneumoniae* or *S aureus* cell.

65. The vector or vehicle of any preceding Clause for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

66. The vector or vehicle of Clause 65, wherein the disease or condition is an infection of the subject with host cells (eg, bacterial cells), or wherein the disease or condition is mediated by host cells (eg, bacterial cells).

67. A pharmaceutical composition comprising the vector or vehicle of any preceding Clause and a pharmaceutically acceptable diluent, excipient or carrier.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine study, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims. All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications and all US equivalent patent applications and patents are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Reference is made to WO2017/118598, US20180140698, US20170246221, US20180273940, US20160115488, US20180179547, US20170175142, US20160024510, US20150064138, US20170022499, US20160345578, US20180155729, US20180200342, WO2017112620, WO2018081502, PCT/EP2018/066954, PCT/EP2018/066980, PCT/EP2018/071454 and U.S. Ser. No. 15/985,658 and equivalent publications by the US Patent and Trademark Office (USPTO) or WIPO, the disclosures of which are incorporated herein by reference for providing disclosure that may be used in the present invention and/or to provide

one or more features (eg, of a vector) that may be included in one or more claims herein.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps

The term "or combinations thereof" or similar as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

Any part of this disclosure may be read in combination with any other part of the disclosure, unless otherwise apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

The present invention is described in more detail in the following non-limiting Examples.

EXAMPLES

The examples illustrate fast and precision killing of *Escherichia coli* strains. As a model programmable nuclease system, we used a CRISPR guided vector (CGV™) to specifically target *Escherichia coli* MG1655.

Example 1. Single-Vector Cas3 & Cascade: Type I CRISPR-Cas System Targeting *E. coli*

A plasmid (which we call a CRISPR Guided Vector™, CGV™) was constructed comprising an operon with nucleotide sequences encoding a Type I Cas3 and Cascade proteins under the control of a common promoter. *C. difficile* Type IB Cas3 and Cascade was used. A cognate CRISPR array comprising *C. difficile* repeat sequences and spacer sequence for targeting an *E. coli* host cell chromosome was also introduced into target cells. An adaptation module containing Cast, Cast and Cas4 was omitted in the vector (see FIG. **1**A). In the wild-type *C. difficile* Type IB CRISPR/Cas locus, the cas3 gene is 3' of the Cascade genes (cas8b1, cas7 and cas5) and thus spaced away from the promoter upstream of the Cascade genes. When we tried this arrangement, we found killing of *E. coli* cells, but surprisingly when we changed to a synthetic operon arrangement (in 5' to 3' orientation) of promoter, cas3, cas8b1, cas7 and cas5 we saw significantly higher killing of the target *E. coli* cells.

Results using this synthetic operon arrangement are shown in FIGS. **1**A-**1**C. In FIG. **1**B there is shown a dilution series (10^1-10^6) of drop spots (5 μl) of target *E. coli* MG1655 cells harboring the CGV on LB agar plates with and without inducers. CRISPR/Cas induction surprisingly killed 99.9% of the population (FIG. **1**C, grey bar). Growth in absence of induction is shown in black. CGV™ refers to a CRISPR Guided Vector™, which is a nucleic acid vector comprising nucleotide sequences encoding CRISPR/Cas components.

We also managed to achieve desirable targeted killing of *E coli* cells using a similar set-up, except that *E coli* Type IE Cas and Cascade were used, together with a cognate array targeting host cell *E coli* chromosomal DNA (data not shown). In this case, a vector was used comprising (in 5' to 3' direction) a promoter controlling the expression of Cas3, Cas8e, Cas11, Cas7, Cas5 and Cas6 in an operon.

Materials and Methods

E. coli MG1655 was grown in lysogeny broth (LB) with shaking (250 rpm) at 37° C. When necessary, cultures were supplemented with tetracycline (10 μg/mL), and spectinomycin (400 Kg/mL).

To construct a plasmid containing *C. difficile* CRISPR system under arabinose inducible pBAD promoter, cas3, cas6, cas8b, cas7 and cas5 genes from *C. difficile* were amplified and cloned in a low copy number plasmid (pSC101 ori). cas3 was located in the beginning of the operon followed by cas6, cas8b, cas7 and cas5. The adaptation module (consisting of cas1, cas2, and cas4) was omitted in the vector (FIG. **1**A). A second plasmid containing an IPTG inducible single-spacer array targeting a chromosomal intergenic region in *E. coli* MG1655 was constructed (FIG. **1**A). The spacer was cloned under control of the IPTG-inducible Ptrc promoter, in a CloDF13 ori backbone. It contains 37 nucleotides from the genome of *E. coli* MG1655 (ctttgccgcgcgcttcgtcacgtaattctcgtcgcaa) (SEQ ID NO: 26). Additionally, the 3'-CCT protospacer adjacent motif (PAM) is located adjacent to the selected target sequence in the genome of *E. coli* MG1655 (FIG. **1**A).

To perform killing assays, both plasmids were transformed into *E. coli* MG1655 by electroporation. Transformants were grown in liquid LB with antibiotics to mid-log phase, and the killing efficiency was determined by serial dilution and spot plating onto LB, and LB+inducers (0.5 mM IPTG and 1 arabinose). Viability was calculated by counting colony forming units (CFUs) on the plates and data were calculated as viable cell concentration (CFU/ml).

Example 2. Single-Vector Cas3-Cascade & Array: Type I CRISPR-Cas System Targeting *E. coli*

A plasmid (which we call a CRISPR Guided Vector™, CGV™, which is a nucleic acid vector comprising nucleo-

tide sequences encoding CRISPR/Cas components) was constructed comprising an operon with nucleotide sequences encoding a Type I Cas3 and Cascade proteins under the control of a common promoter. *C. difficile* Type IB Cas3 and Cascade was used. Adaptation module containing Cas1, Cas2 and Cas4 was omitted in the vector. A cognate CRISPR array comprising *C. difficile* repeat sequences and spacer sequence for targeting an *E. coli* host cell chromosome was also cloned in the vector (see FIG. **2**A). Similarly we also constructed a plasmid comprising of an operon with nucleotide sequences encoding *E. coli* Type IE Cas3 and Cascade proteins under control of a common promoter. The *E. coli* adaption module containing Cas1 and Cas2 was omitted, in the vector. A cognate CRISPR array comprising *E. coli* repeat sequences and spacer sequence for targeting an *E. coli* host cell chromosome was also cloned in the vector.

The CGV containing the *C. difficile* CRISPR-Cas system was transformed into *E. coli* MG1655 which contains a pks sequence incorporated into the genome. Results using this synthetic operon arrangement are shown in FIGS. **2**A-**2**C. In FIG. **2**B there is shown a dilution series (10^1-10^5) of drop spots (5 μl) of target *E. coli* MG1655 cells harboring the CGV on synthetic medium (SM) agar plates with and without inducers. CRISPR/Cas induction resulted in more than 2-$\log_{10}$ reductions in viable cells of *E. coli* MG1655 (FIG. **2**C, grey bar). Growth in absence of induction is shown in black. CGV™ refers to a CRISPR Guided Vector™.

The survival of *E. coli* MG1655 upon induction was followed over time by plating the cultures in serial dilutions every 60 minutes, for 2 h (FIG. **3**A). Killing curves revealed that CRISPR/Cas induction mediated rapid killing of *E. coli* MG1655, generating a two-$\log_{10}$ reduction in *E. coli* by the first 60 minutes. FIG. **3**B shows a dilution series (10^1-10^6) of drop spots (5 μl) of induced and non-induced cultures of target *E. coli* MG1655 on SM agar plates.

The CGV containing the *E. coli* CRISPR-Cas system was transformed into other *E. coli* MG1655 cells which contain a lambda sequence incorporated into the genome. Results using this synthetic operon arrangement are shown in FIGS. **6**A-**6**B. In FIG. **6**A there is shown a dilution series (10^1-10^5) of drop spots (5 μl) of target *E. coli* MG1655 cells harboring the CGV on synthetic medium (SM) agar plates with and without inducers. CRISPR/Cas induction resulted in more than 2-$\log_{10}$ reductions in viable cells of *E. coli* MG1655 (FIG. **6**B, grey bar). Growth in absence of induction is shown in black. In a repeat experiment (not shown) we saw a 3-$\log_{10}$ reductions in viable cells of *E. coli* MG1655 with CRISPR/Cas induction.

Materials and Methods

E. coli MG1655 was grown in synthetic medium (SM) with shaking (250 rpm) at 37° C. Cultures were supplemented with 10 μg/mL tetracycline when required.

To construct a plasmid containing *C. difficile* CRISPR system under arabinose inducible pBAD promoter, cas3, cas6, cas8b, cas7 and cas5 genes from *C. difficile* were amplified and cloned in a low copy number plasmid (pSC101 ori). cas3 was located in the beginning of the operon followed by cas6, cas8b, cas7 and cas5. Additionally, an IPTG inducible single-spacer array targeting a chromosomal intergenic region in *E. coli* MG1655 was included in the vector under control of the IPTG-inducible Ptrc promoter (FIG. **2**A). It contains 37 nucleotides from the PKS gene (previously integrated into the genome of *E. coli* MG1655) (gtttggcgatggcgcgggtgtggttgtgcttcggcgt) (SEQ ID NO: 27). Additionally, the 3'-CCT protospacer adjacent motif (PAM) is located adjacent to the selected target sequence in the genome of *E. coli* MG1655 (FIG. **2**A).

To construct a plasmid containing *E. coli* CRISPR system under arabinose inducible pBAD promoter, cas3, cse1, cse2, cas7, cas5 and cas6 genes from *E. coli* were amplified and cloned in a low copy number plasmid (pSC101 ori). The operon comprised (in 5' to 3' direction) cas3 followed by cse1 cse2, cas7, cas5 and cas6. Additionally, an IPTG inducible single-spacer array targeting a chromosomal intergenic region in *E. coli* MG1655 was included in the vector under control of the IPTG-inducible Ptrc promoter. It contained 32 nucleotides from the lambda sequence (previously integrated into the genome of *E. coli* MG1655) (tgggatgcctaccgcaagcagcttggcctgaa) (SEQ ID NO: 28) and found to efficiently target in Brouns et al., 2008 (Science. 2008 Aug. 15; 321(5891):960-4. doi: 10.1126/science.1159689; "Small CRISPR RNAs guide antiviral defense in prokaryotes"). Additionally, the 3'-ATG protospacer adjacent motif (PAM) is located adjacent to the selected target sequence in the genome of *E. coli* MG1655.

The CGVs were transformed into *E. coli* MG1655 by electroporation. Transformants were grown in liquid SM with antibiotics to mid-log phase, and the killing efficiency was determined by serial dilution and spot plating onto LB, and LB+inducers (0.5 mM IPTG and 1% arabinose). Viability was calculated by counting colony forming units (CFUs) on the plates and data were calculated as viable cell concentration (CFU/ml).

To perform killing curves, *E. coli* MG1655 harboring the CGV was grown in liquid SM with antibiotics to mid-log phase. The culture was divided into two tubes and either inducers (0.5 mM IPTG and 1% arabinose) or PBS were added. Survival of the strain was followed over time by plating the cultures in serial dilutions (10^1-10^6) of drop spots (5 μl) every 60 minutes, for 2 h, on SM plates with antibiotics. Survival frequency was calculated by counting colony forming units (CFUs) on the plates and data were calculated as viable cell concentration (CFU/ml).

Example 3. Precision Killing of Target Strain *E. coli* MG1655 in a Microbiome

An artificial microbial consortium was constructed to study the efficiency of the CGV carrying the CRISPR-Cas system of *C. difficile*, to specifically target *E. coli* MG1655 in the presence of other microbes, mimicking the human microbiome.

The synthetic consortium consisted of three strains (two different species) with differential antibiotic resistance profiles: a streptomycin-resistant *E. coli* MG1655 (target strain), an ampicillin-resistant *E. coli* Top10, and a chloramphenicol-resistant *Lactococcus lactis* NZ9000. To create the consortium, bacterial cultures were grown separately in Brain Heart Infusion broth (BHI, optimal growth medium for *L. lactis*) to mid-log phase and mixed in fresh BHI broth with and without inducers. After 1 h induction at 30° C., the composition of the consortium was determined by counting viable colonies on selective plates. Induction of the CRISPR system in the mixed community, resulted in >10-fold killing of target *E. coli* MG1655, while leaving *E. coli* Top10 and *L. lactis* NZ9000 cell populations unharmed (FIG. **4**A). In FIG. **4**B there is shown a dilution series (10^1-10^5) of drop spots (5 μl) of the synthetic consortium after 1 h induction on BHI agar plates.

Additionally, CRISPR killing of target strain *E. coli* MG1655 in the synthetic microbial consortium was compared to a pure culture (ie, target strain *E. coli* MG1655 that

is not mixed with another strain or species). Unexpectedly, in both conditions, killing of 3 logs was achieved when plated on BHI agar plates with inducers (FIG. **5**A). Thus, surprisingly the killing in the microbiome setting was as efficient as the killing in pure culture. In FIG. **5**B there is shown a dilution series (10^1-10^5) of drop spots (5 μl) of the synthetic consortium and *E. coli* MG1655 in pure culture on BHI agar plates with and without inducers.

Materials and Methods

E. coli MG1655, *E. coli* Top10, and *Lactococcus lactis* NZ9000 were grown in BHI broth with shaking (250 rpm) at 30° C. Cultures were supplemented with 1000 μg/mL streptomycin, 100 μg/mL ampicillin, or 10 μg/mL chloramphenicol, respectively.

To create the consortium, bacterial cultures were grown in BHI with appropriate antibiotics to mid-log phase. Cultures were washed twice in PBS to remove the antibiotics and mixed in fresh BHI broth. The mixed culture was spotted onto BHI plates with streptomycin, ampicillin or chloramphenicol to quantify the initial concentration of *E. coli* MG1655, *E. coli* Top10 and *L. lactis* NZ9000, respectively. The mixed culture was divided into two tubes and either inducers (0.5 mM IPTG and 1% arabinose) or PBS were added. After 1 h induction at 30° C., the composition of the consortium was calculated by counting colony forming units (CFUs) on selective plates and data were calculated as viable cell concentration (CFU/ml).

Example 4. Use of Promoter Repression in Vector Amplification Strains

We engineered an *E coli* Top10 production strain cell population comprising plasmid CGV DNA and an expressible sequence encoding a Tet repressor (TetR). The DNA comprised a Cas9-encoding nucleotide sequence under the control of a Tet promoter (pLtetO-1 promoter). The promoter is normally constitutively ON, but it was repressed by TetR in our cells. Thus, in this way we could successfully culture the cells and amplify the CGV without observing adverse toxicity due to Cas9 expression.

In an experiment in the absence of repression, we did not observe any colonies of production strain bacteria, and we surmise that this was due to Cas9 toxicity. We believe, in addition to providing a way of increasing CGV yield (eg, for subsequent packaging into phage or non-self-replicative transduction particles), our method using repression can minimize selection for mutations in the DNA that would otherwise be forced by higher Cas9 expression and cutting (eg, due to CGV cutting).

REFERENCES

Mutalik et a1, Nat Methods. 2013 April; 10(4):354-60. doi: 10.1038/nmeth. 2404. Epub 2013 Mar. 10, "Precise and reliable gene expression via standard transcription and translation initiation elements".

TABLE 1

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Abiotrophia	*Acidocella*	*Actinomyces*	*Alkalilimnicola*	*Aquaspirillum*
Abiotrophia defectiva	*Acidocella aminolytica*	*Actinomyces bovis*	*Alkalilimnicola ehrlichii*	*Aquaspirillum polymorphum*
Acaricomes	*Acidocella facilis*	*Actinomyces denticolens*	*Alkaliphilus*	*Aquaspirillum*
Acaricomes phytoseiuli	*Acidomonas*	*Actinomyces europaeus*	*Alkaliphilus oremlandii*	*putridiconchylium*
Acetitomaculum	*Acidomonas methanolica*	*Actinomyces georgiae*	*Alkaliphilus transvaalensis*	*Aquaspirillum serpens*
Acetitomaculum ruminis	*Acidothermus*	*Actinomyces gerencseriae*	*Allochromatium*	*Aquimarina*
Acetivibrio	*Acidothermus cellulolyticus*	*Actinomyces*	*Allochromatium vinosum*	*Aquimarina latercula*
Acetivibrio cellulolyticus	*Acidovorax*	*hordeovulneris*	*Alloiococcus*	*Arcanobacterium*
Acetivibrio ethanolgignens	*Acidovorax anthurii*	*Actinomyces howellii*	*Alloiococcus otitis*	*Arcanobacterium*
Acetivibrio multivorans	*Acidovorax caeni*	*Actinomyces hyovaginalis*	*Allokutzneria*	*haemolyticum*
Acetoanaerobium	*Acidovorax cattleyae*	*Actinomyces israelii*	*Allokutzneria albata*	*Arcanobacterium pyogenes*
Acetoanaerobium noterae	*Acidovorax citrulli*	*Actinomyces johnsonii*	*Altererythrobacter*	*Archangium*
Acetobacter	*Acidovorax defluvii*	*Actinomyces meyeri*	*Altererythrobacter ishigakiensis*	*Archangium gephyra*
Acetobacter aceti	*Acidovorax delafieldii*	*Actinomyces naeslundii*	*Altermonas*	*Arcobacter*
Acetobacter cerevisiae	*Acidovorax facilis*	*Actinomyces neuii*	*Altermonas haloplanktis*	*Arcobacter butzleri*
Acetobacter cibinongensis	*Acidovorax konjaci*	*Actinomyces odontolyticus*	*Altermonas macleodii*	*Arcobacter cryaerophilus*
Acetobacter estunensis	*Acidovorax temperans*	*Actinomyces oris*	*Alysiella*	*Arcobacter halophilus*
Acetobacter fabarum	*Acidovorax valerianellae*	*Actinomyces radingae*	*Alysiella crassa*	*Arcobacter nitrofigilis*
Acetobacter ghanensis	*Acinetobacter*	*Actinomyces slackii*	*Alysiella filiformis*	*Arcobacter skirrowii*
Acetobacter indonesiensis	*Acinetobacter baumannii*	*Actinomyces turicensis*	*Aminobacter*	*Arhodomonas*
Acetobacter lovaniensis	*Acinetobacter baylyi*	*Actinomyces viscosus*	*Aminobacter aganoensis*	*Arhodomonas aquaeolei*
Acetobacter malorum	*Acinetobacter bouvetii*	*Actinoplanes*	*Aminobacter aminovorans*	*Arsenophonus*
Acetobacter nitrogenifigens	*Acinetobacter calcoaceticus*	*Actinoplanes auranticolor*	*Aminobacter niigataensis*	*Arsenophonus nasoniae*
Acetobacter oeni	*Acinetobacter gerneri*	*Actinoplanes brasiliensis*	*Aminobacterium*	
Acetobacter orientalis	*Acinetobacter haemolyticus*	*Actinoplanes consettensis*	*Aminobacterium mobile*	*Arthrobacter*
Acetobacter orleanensis	*Acinetobacter johnsonii*	*Actinoplanes deccanensis*	*Aminomonas*	*Arthrobacter agilis*
Acetobacter pasteurianus	*Acinetobacter junii*	*Actinoplanes derwentensis*	*Aminomonas paucivorans*	*Arthrobacter albus*
Acetobacter pornorurn	*Acinetobacter lwoffi*	*Actinoplanes digitatis*	*Ammoniphilus*	*Arthrobacter aurescens*
Acetobacter senegalensis	*Acinetobacter parvus*	*Actinoplanes durhamensis*	*Ammoniphilus oxalaticus*	*Arthrobacter chlorophenolicus*
Acetobacter xylinus	*Acinetobacter radioresistens*	*Actinoplanes ferrugineus*	*Ammoniphilus oxalivorans*	*Arthrobacter citreus*
Acetobacterium	*Acinetobacter schindleri*	*Actinoplanes globisporus*	*Amphibacillus*	*Arthrobacter crystallopoietes*
Acetobacterium bakii	*Acinetobacter soli*	*Actinoplanes humidus*	*Amphibacillus xylanus*	*Arthrobacter cumminsii*
Acetobacterium carbinolicum	*Acinetobacter tandoii*	*Actinoplanes italicus*	*Amphritea*	*Arthrobacter globiformis*
Acetobacterium dehalogenans	*Acinetobacter tjernbergiae*	*Actinoplanes liguriensis*	*Amphritea balenae*	*Arthrobacter*
Acetobacterium fimetarium	*Acinetobacter towneri*	*Actinoplanes lobatus*	*Amphritea japonica*	*histidinolovorans*
Acetobacterium malicum	*Acinetobacter ursingii*	*Actinoplanes missouriensis*	*Amycolatopsis*	*Arthrobacter ilicis*
Acetobacterium paludosum	*Acinetobacter venetianus*	*Actinoplanes palleronii*	*Amycolatopsis alba*	*Arthrobacter luteus*
Acetobacterium tundrae	*Acrocarpospora*	*Actinoplanes philippinensis*	*Amycolatopsis albidoflavus*	*Arthrobacter methylotrophus*
Acetobacterium wieringae	*Acrocarpospora corrugata*	*Actinoplanes rectilineatus*	*Amycolatopsis azurea*	*Arthrobacter mysorens*
Acetobacterium woodii	*Acrocarpospora*	*Actinoplanes regularis*	*Amycolatopsis coloradensis*	*Arthrobacter nicotianae*
Acetofilamentum	*macrocephala*	*Actinoplanes*	*Amycolatopsis lurida*	*Arthrobacter nicotinovorans*
Acetofilamentum rigidum	*Acrocarpospora pleiomorpha*	*teichomyceticus*	*Amycolatopsis mediterranei*	*Arthrobacter oxydans*
Acetohalobium	*Actibacter*	*Actinoplanes utahensis*	*Amycolatopsis rifamycinica*	*Arthrobacter pascens*
Acetohalobium arabaticum	*Actibacter sediminis*	*Actinopolyspora*	*Amycolatopsis rubida*	*Arthrobacter*
Acetomicrobium	*Actinoalloteichus*	*Actinopolyspora halophila*	*Amycolatopsis sulphurea*	*phenanthrenivorans*
Acetomicrobium faecale	*Actinoalloteichus*	*Actinopolyspora mortivallis*	*Amycolatopsis tolypomycina*	*Arthrobacter*
Acetomicrobium flavidum	*cyanogriseus*	*Actinosynnema*	*Anabaena*	*polychromogenes*
Acetonema	*Actinoalloteichus*	*Actinosynnema mirum*	*Anabaena cylindrica*	*Atrhrobacter protophormiae*

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Acetonema longum
Acetothermus
Acetothermus paucivorans
Acholeplasma
Acholeplasma axanthum
Acholeplasma brassicae
Acholeplasma cavigenitalium
Acholeplasma equifetale
Acholeplasma granularum
Acholeplasma hippikon
Acholeplasma laidlawii
Acholeplasma modicum
Acholeplasma morum
Acholeplasma multilocale
Acholeplasma oculi
Acholeplasma palmae
Acholeplasma parvum
Acholeplasma pleciae
Acholeplasma vituli
Achromobacter
Achromobacter denitrificans
Achromobacter insolitus
Achromobacter piechaudii
Achromobacter ruhlandii
Achromobacter spanius
Acidaminobacter
Acidaminobacter hydrogenoformans
Acidaminococcus
Acidaminococcus fermentans
Acidaminococcus intestini
Acidicaldus
Acidicaldus organivorans
Acidimicrobium
Acidimicrobium ferrooxidans
Acidiphilium
Acidiphilium acidophilum
Acidiphilium angustum
Acidiphilium cryptum
Acidiphilium multivorum
Acidiphilium organovorum
Acidiphilium rubrum
Acidisoma
Acidisoma sibiricum
Acidisoma tundrae
Acidisphaera
Acidisphaera rubrifaciens
Acidithiobacillus
Acidithiobacillus albertensis
Acidithiobacillus caldus
hymeniacidonis
Actinoalloteichus spitiensis
Actinobaccillus
Actinobacillus capsulatus
Actinobacillus delphinicola
Actinobacillus hominis
Actinobacillus indolicus
Actinobacillus lignieresii
Actinobacillus minor
Actinobacillus muris
Actinobacillus
pleuropneumoniae
Actinobacillus porcinus
Actinobacillus rossii
Actinobacillus scotiae
Actinobacillus seminis
Actinobacillus succinogenes
Actinobaccillus suis
Actinobacillus ureae
Actinobaculum
Actinobaculum massiliense
Actinobaculum schaalii
Actinobaculum suis
Actinomyces urinale
Actinocatenispora
Actinocatenispora rupis
Actinocatenispora
thailandica
Actinocatenispora sera
Actinocorallia
Actinocorallia aurantiaca
Actinocorallia aurea
Actinocorallia cavernae
Actinocorallia glomerata
Actinocorallia herbida
Actinocorallia libanotica
Actinocorallia longicatena
Actinomadura
Actinomadura alba
Actinomadura atramentaria
Actinomadura
bangladeshensis
Actinomadura catellatispora
Actinomadura chibensis
Actinomadura chokoriensis
Actinomadura citrea
Actinomadura coerulea
Actinomadura echinospora
Actinomadura fibrosa
Actinomadura formosensis
Actinotalea
Actinotalea fermentans
Aerococcus
Aerococcus sanguinicola
Aerococcus urinae
Aerococcus urinaeequi
Aerococcus urinaehominis
Aerococcus viridans
Aeromicrobium
Aeromicrobium erythreum
Aeromonas
Aeromonas
allosaccharophila
Aeromonas bestiarum
Aeromonas caviae
Aeromonas encheleia
Aeromonas
enteropelogenes
Aeromonas eucrenophila
Aeromonas ichthiosmia
Aeromonas jandaei
Aeromonas media
Aeromonas popoffii
Aeromonas sobria
Aeromonas veronii
Agrobacterium
Agrobacterium
gelatinovorum
Agrococcus
Agrococcus citreus
Agrococcus jenensis
Agromonas
Agromonas oligotrophica
Agromyces
Agromyces fucosus
Agromyces hippuratus
Agromyces luteolus
Agromyces mediolanus
Agromyces ramosus
Agromyces rhizospherae
Akkermansia
Akkermansia muciniphila
Albidiferax
Albidiferax ferrireducens
Albidovulum
Albidovulum inexpectatum
Alcaligenes
Alcaligenes denitrificans
Alcaligenes faecalis
Alcanivorax
Anabaena flos-aquae
Anabaena variabilis
Anaeroarcus
Anaeroarcus burkinensis
Anaerobaculum
Anaerobaculum mobile
Anaerobiospirillum
Anaerobiospirillum
succiniciproducens
Anaerobiospirillum thomasii
Anaerococcus
Anaerococcus hydrogenalis
Anaerococcus lactolyticus
Anaerococcus prevotii
Anaerococcus tetradius
Anaerococcus vaginalis
Anaerofustis
Anaerofustis stercorihominis
Anaeromusa
Anaeromusa acidaminophila
Anaeromyxobacter
Anaeromyxobacter
dehalogenans
Anaerorhabdus
Anaerorhabdus furcosa
Anaerosinus
Anaerosinus glycerini
Anaerovirgula
Anaerovirgula multivorans
Ancalomicrobium
Ancalomicrobium adetum
Ancylobacter
Ancylobacter aquaticus
Aneurinibacillus
Aneurinibacillus aneurinilyticus
Aneurinibacillus migulanus
Aneurinibacillus
thermoaerophilus
Angiococcus
Angiococcus disciformis
Angulomicrobium
Angulomicrobium tetraedrale
Anoxybacillus
Anoxybacillus pushchinoensis
Aquabacterium
Aquabacterium commune
Aquabacterium parvum
Arthrobacter
psychrolactophilus
Arthrobacter ramosus
Arthrobacter sulfonivorans
Arthrobacter sulfureus
Arthrobacter uratoxydans
Arthrobacter ureafaciens
Arthrobacter viscosus
Arthrobacter woluwensis
Asaia
Asaia bogorensis
Asanoa
Asanoa ferruginea
Asticcacaulis
Asticcacaulis biprosthecium
Asticcacaulis excentricus
Atopobacter
Atopobacter phocae
Atopobium
Atopobium fossor
Atopobium minutum
Atopobium parvulum
Atopobium rimae
Atopobium vaginae
Aureobacterium
Aureobacterium barkeri
Aurobacterium
Aurobacterium liquefaciens
Avibacterium
Avibacterium avium
Avibacterium gallinarum
Avibacterium paragallinarum
Avibacterium volantium
Azoarcus
Azoarcus indigens
Azoarcus tolulyticus
Azoarcus toluvorans
Azohydromonas
Azohydromonas australica
Azohydromonas lata
Azomonas
Azomonas agilis
Azomonas insignis
Azomonas macrocytogenes
Azorhizobium
Azorhizobium caulinodans
Azorhizophilus
Azorhizophilus paspali
Azospirillum
Azospirillum brasilense

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Acidithiobacillus ferrooxidans	*Actinomadura hibisca*	*Alcanivorax borkumensis*		*Azospirillum halopraeferens*
Acidithiobacillus thiooxidans	*Actinomadura kijaniata*	*Alcanivorax jadensis*		*Azospirillum irakense*
Acidobacterium	*Actinomadura latina*	*Algicola*		*Azotobacter*
Acidobacterium capsulatum	*Actinomadura livida*	*Algicola bacteriolytica*		*Azotobacter beijerinckii*
	Actinomadura luteofluorescens	*Alicyclobacillus*		*Azotobacter chroococcum*
		Alicyclobacillus disulfidooxidans		*Azotobacter nigricans*
	Actinomadura macra			*Azotobacter salinestris*
	Actinomadura madurae	*Alicyclobacillus sendaiensis*		*Azotobacter vinelandii*
	Actinomadura oligospora			
	Actinomadura pelletieri	*Alicyclobacillus vulcanalis*		
	Actinomadura rubrobrunea	*Alishewanella*		
	Actinomadura rugatobispora	*Alishewanella fetalis*		
	Actinomadura umbrina	*Alkalibacillus*		
	Actinomadura verrucosospora	*Alkalibacillus haloalkaliphilus*		
	Actinomadura vinacea			
	Actinomadura viridilutea			
	Actinomadura viridis			
	Actinomadura yumaensis			
Bacillus	*Bacteroides*	*Bibersteinia*	*Borrelia*	*Brevinema*
[see below]	*Bacteroides caccae*	*Bibersteinia trehalosi*	*Borrelia afzelii*	*Brevinema andersonii*
	Bacteroides coagulans	*Bifidobacterium*	*Borrelia americana*	*Brevundimonas*
Bacteriovorax	*Bacteroides eggerthii*	*Bifidobacterium adolescentis*	*Borrelia burgdorferi*	*Brevundimonas alba*
Bacteriovorax stolpii	*Bacteroides fragilis*	*Bifidobacterium angulatum*	*Borrelia carolinensis*	*Brevundimonas aurantiaca*
	Bacteroides galacturonicus	*Bifidobacterium animalis*	*Borrelia coriaceae*	*Brevundimonas diminuta*
	Bacteroides helcogenes	*Bifidobacterium asteroides*	*Borrelia garinii*	*Brevundimonas intermedia*
	Bacteroides ovatus	*Bifidobacterium bifidum*	*Borrelia japonica*	*Brevundimonas subvibrioides*
	Bacteroides pectinophilus	*Bifidobacterium boum*	*Bosea*	*Brevundimonas vancanneytii*
	Bacteroides pyogenes	*Bifidobacterium breve*	*Bosea minatitlanensis*	*Brevundimonas variabilis*
	Bacteroides salyersiae	*Bifidobacterium catenulatum*	*Bosea thiooxidans*	*Brevundimonas vesicularis*
	Bacteroides stercoris	*Bifidobacterium choerinum*	*Brachybacterium*	*Brochothrix*
	Bacteroides suis	*Bifidobacterium coryneforme*	*Brachybacterium*	*Brochothrix campestris*
	Bacteroides tectus	*Bifidobacterium cuniculi*	*alimentarium*	*Brochothrix thermosphacta*
	Bacteroides thetaiotaomicron	*Bifidobacterium dentium*	*Brachybacterium faecium*	*Brucella*
	Bacteroides uniformis	*Bifidobacterium gallicum*	*Brachybacterium*	*Brucella canis*
	Bacteroides ureolyticus	*Bifidobacterium gallinarum*	*paraconglomeratum*	*Brucella neotomae*
	Bacteroides vulgatus	*Bifidobacterium indicum*	*Brachybacterium rhamnosum*	*Bryobacter*
	Balnearium	*Bifidobacterium longum*	*Brachybacterium*	*Bryobacter aggregatus*
	Balnearium lithotrophicum	*Bifidobacterium*	*tyrofermentans*	*Burkholderia*
	Balneatrix	*magnumBifidobacterium*	*Brachyspira*	*Burkholderia ambifaria*
	Balneatrix alpica	*merycicum*	*Brachyspira alvinipulli*	*Burkholderia andropogonis*
	Balneola	*Bifidobacterium minimum*	*Brachyspira hyodysenteriae*	*Burkholderia anthina*
	Balneola vulgaris	*Bifidobacterium*	*Brachyspira innocens*	*Burkholderia caledonica*
	Barnesiella	*pseudocatenulatum*	*Brachyspira murdochii*	*Burkholderia caryophylli*
	Barnesiella viscericola	*Bifidobacterium*	*Brachyspira pilosicoli*	*Burkholderia cenocepacia*
	Bartonella	*pseudolongum*		*Burkholderia cepacia*
	Bartonella alsatica	*Bifidobacterium pullorum*	*Bradyrhizobium*	*Burkholderia cocovenenans*
	Bartonella bacilliformis	*Bifidobacterium ruminantium*	*Bradyrhizobium canariense*	*Burkholderia dolosa*
	Bartonella clarridgeiae	*Bifidobacterium saeculare*	*Bradyrhizobium elkanii*	*Burkholderia fungorum*
	Bartonella doshiae	*Bifidobacterium subtile*	*Bradyrhizobium japonicum*	*Burkholderia glathei*

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

	Bartonella elizabethae	*Bifidobacterium*	*Bradyrhizobium liaoningense*	*Burkholderia glumae*
	Bartonella grahamii	*thermophilum*	*Brenneria*	*Burkholderia graminis*
	Bartonella henselae	*Bilophila*	*Brenneria alni*	*Burkholderia kururiensis*
	Bartonella rochalimae	*Bilophila wadsworthia*	*Brenneria nigrifluens*	*Burkholderia multivorans*
	Bartonella vinsonii	*Biostraticola*	*Brenneria quercina*	*Burkholderia phenazinium*
	Bavariicoccus	*Biostraticola tofi*	*Brenneria quercina*	*Burkholderia plantarii*
	Bavariicoccus seileri	*Bizionia*	*Brenneria salicis*	*Burkholderia pyrrocinia*
	Bdellovibrio	*Bizionia argentinensis*	*Brevibacillus*	*Burkholderia silvatlantica*
	Bdellovibrio bacteriovorus	*Blastobacter*	*Brevibacillus agri*	*Burkholderia stabilis*
	Bdellovibrio exovorus	*Blastobacter capsulatus*	*Brevibacillus borstelensis*	*Burkholderia thailandensis*
	Beggiatoa	*Blastobacter denitrificans*	*Brevibacillus brevis*	*Burkholderia tropica*
	Beggiatoa alba	*Blastococcus*	*Brevibacillus centrosporus*	*Burkholderia unamae*
	Beijerinckia	*Blastococcus aggregatus*	*Brevibacillus choshinensis*	*Burkholderia vietnamiensis*
	Beijerinckia derxii	*Blastococcus saxobsidens*	*Brevibacillus invocatus*	*Buttiauxella*
	Beijerinckia fluminensis	*Blastochloris*	*Brevibacillus laterosporus*	*Buttiauxella agrestis*
	Beijerinckia indica	*Blastochloris viridis*	*Brevibacillus parabrevis*	*Buttiauxella brennerae*
	Beijerinckia mobilis	*Blastomonas*	*Brevibacillus reuszeri*	*Buttiauxella ferragutiae*
	Belliella	*Blastomonas natatoria*	*Brevibacterium*	*Buttiauxella gaviniae*
	Belliella baltica	*Blastopirellula*	*Brevibacterium abidum*	*Buttiauxella izardii*
	Bellilinea	*Blastopirellula marina*	*Brevibacterium album*	*Buttiauxella noackiae*
	Bellilinea caldifistulae	*Blautia*	*Brevibacterium aurantiacum*	*Buttiauxella warmboldiae*
	Belnapia	*Blautia coccoides*	*Brevibacterium celere*	*Butyrivibrio*
	Belnapia moabensis	*Blautia hansenii*	*Brevibacterium epidermidis*	*Butyrivibrio fibrisolvens*
	Bergeriella	*Blautia producta*	*Brevibacterium*	*Butyrivibrio hungatei*
	Bergeriella denitrificans	*Blautia wexlerae*	*frigoritolerans*	*Butyrivibrio proteoclasticus*
	Beutenbergia	*Bogoriella*	*Brevibacterium halotolerans*	
	Beutenbergia cavernae	*Bogoriella caseilytica*	*Brevibacterium iodinum*	
		Bordetella	*Brevibacterium linens*	
		Bordetella avium	*Brevibacterium lyticum*	
		Bordetella bronchiseptica	*Brevibacterium mcbrellneri*	
		Bordetella hinzii	*Brevibacterium otitidis*	
		Bordetella holmesii	*Brevibacterium oxydans*	
		Bordetella parapertussis	*Brevibacterium paucivorans*	
		Bordetella pertussis	*Brevibacterium stationis*	
		Bordetella petrii		
		Bordetella trematum		
Bacillus				
B. acidiceler	*B. aminovorans*	*B. glucanolyticus*	*B. taeanensis*	*B. lautus*
B. acidicola	*B. amylolyticus*	*B. gordonae*	*B. tequilensis*	*B. lehensis*
B. acidiproducens	*B. andreesenii*	*B. gottheilii*	*B. thermantarcticus*	*B. lentimorbus*
B. acidocaldarius	*B. aneurinilyticus*	*B. graminis*	*B. thermoaerophilus*	*B. lentus*
B. acidoterrestris	*B. anthracis*	*B. halmapalus*	*B. thermoamylovorans*	*B. licheniformis*
B. aeolius	*B. aquimaris*	*B. haloalkaliphilus*	*B. thermocatenulatus*	*B. ligniniphilus*
B. aerius	*B. arenosi*	*B. halochares*	*B. thermocloacae*	*B. litoralis*
B. aerophilus	*B. arseniciselenatis*	*B. halodenitrificans*	*B. thermocopriae*	*B. locisalis*
B. agaradhaerens	*B. arsenicus*	*B. halodurans*	*B. thermodenitrificans*	*B. luciferensis*
B. agri	*B. aurantiacus*	*B. halophilus*	*B. thermoglucosidasius*	*B. luteolus*
B. aidingensis	*B. arvi*	*B. halosaccharovorans*	*B. thermolactis*	*B. luteus*
B. akibai	*B. aryabhattai*	*B. hemicellulosilyticus*	*B. thermoleovorans*	*B. macauensis*
B. alcalophilus	*B. asahii*	*B. hemicentroti*	*B. thermophilus*	*B. macerans*

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

B. algicola
B. alginolyticus
B. alkalidiazotrophicus
B. alkalinitrilicus
B. alkalisediminis
B. alkalitelluris
B. altitudinis
B. alveayuensis
B. alvei
B. amyloliquefaciens
B.
a. subsp. *amyloliquefaciens*
B. a. subsp. *plantarum*

B. dipsosauri
B. drentensis
B. edaphicus
B. ehimensis
B. eiseniae
B. enclensis
B. endophyticus
B. endoradicis
B. farraginis
B. fastidiosus
B. fengqiuensis
B. firmus
B. flexus
B. foraminis
B. fordii
B. formosus
B. fortis
B. fumarioli
B. funiculus
B. fusiformis
B. galactophilus
B. galactosidilyticus
B. galliciensis
B. gelatini
B. gibsonii
B. ginsengi
B. ginsengihumi
B. ginsengisoli
B. globisporus (eg, *B.*
g. subsp. *Globisporus*; or *B.*
g. subsp. *Marinus*)

B. atrophaeus
B. axarquiensis
B. azotofixans
B. azotoformans
B. badius
B. barbaricus
B. bataviensis
B. beijingensis
B. benzoevorans
B. beringensis
B. berkeleyi
B. beveridgei
B. bogoriensis
B. boroniphilus
B. borstelensis
B. brevis Migula
B. butanolivorans
B. canaveralius
B. carboniphilus
B. cecembensis
B. cellulosilyticus
B. centrosporus
B. cereus
B. chagannorensis
B. chitinolyticus
B. chondroitinus
B. choshinensis
B. chungangensis
B. cibi
B. circulans
B. clarkii
B. clausii
B. coagulans
B. coahuilensis
B. cohnii
B. composti
B. curdlanolyticus
B. cycloheptanicus
B. cytotoxicus
B. daliensis
B. decisifrondis
B. decolorationis
B. deserti

B. herbersteinensis
B. horikoshii
B. horneckiae
B. horti
B. huizhouensis
B. humi
B. hwajinpoensis
B. idriensis
B. indicus
B. infantis
B. infernus
B. insolitus
B. invictae
B. iranensis
B. isabeliae
B. isronensis
B. jeotgali
B. kaustophilus
B. kobensis
B. kochii
B. kokeshiiformis
B. koreensis
B. korlensis
B. kribbensis
B. krulwichiae
B. laevolacticus
B. larvae
B. laterosporus
B. salexigens
B. saliphilus
B. schlegelii
B. sediminis
B. selenatarsenatis
B. selenitireducens
B. seohaeanensis
B. shacheensis
B. shackletonii
B. siamensis
B. silvestris
B. simplex
B. siralis
B. smithii
B. soli
B. solimangrovi
B. solisalsi
B. songklensis
B. sonorensis
B. sphaericus
B. sporothermodurans
B. stearothermophilus

B. thermoruber
B. thermosphaericus
B. thiaminolyticus
B. thioparans
B. thuringiensis
B. tianshenii
B. trypoxylicola
B. tusciae
B. validus
B. vallismortis
B. vedderi
B. velezensis
B. vietnamensis
B. vireti
B. vulcani
B. wakoensis
B. weihenstephanensis
B. xiamenensis
B. xiaoxiensis
B. zhanjiangensis
B. peoriae
B. persepolensis
B. persicus
B. pervagus
B. plakortidis
B. pocheonensis
B. polygoni
B. polymyxa
B. popilliae
B. pseudalcalophilus
B. pseudofirmus
B. pseudomycoides
B. psychrodurans
B. psychrophilus
B. psychrosaccharolyticus
B. psychrotolerans
B. pulvifaciens
B. pumilus
B. purgationiresistens
B. pycnus
B. qingdaonensis
B. qingshengii
B. reuszeri
B. rhizosphaerae
B. rigui
B. ruris
B. safensis
B. salarius

B. macquariensis
B. macyae
B. malacitensis
B. mannanilyticus
B. marisflavi
B. marismortui
B. marmarensis
B. massiliensis
B. megaterium
B. mesonae
B. methanolicus
B. methylotrophicus
B. migulanus
B. mojavensis
B. mucilaginosus
B. muralis
B. murimartini
B. mycoides
B. naganoensis
B. nanhaiensis
B. nanhaiisediminis
B. nealsonii
B. neidei
B. neizhouensis
B. niabensis
B. niacini
B. novalis
B. oceanisediminis
B. odysseyi
B. okhensis
B. okuhidensis
B. oleronius
B. oryzaecorticis
B. oshimensis
B. pabuli
B. pakistanensis
B. pallidus
B. pallidus
B. panacisoli
B. panaciterrae
B. pantothenticus
B. parabrevis
B. paraflexus
B. pasteurii
B. patagoniensis

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

B. stratosphericus
B. subterraneus
B. subtilis (eg, *B.*
s. subsp. *Inaquosorum*, or *B.*
s. subsp. *Spizizenr*, or *B.*
s. subsp. *Subtilis*)
Cardiobacterium
Cardiobacterium hominis
Carnimonas
Carnimonas nigrificans
Carnobacterium
Carnobacterium alterfunditum
Carnobacterium divergens
Carnobacterium funditum
Carnobacterium gallinarum
Carnobacterium maltaromaticum
Carnobacterium mobile
Carnobacterium viridans
Caryophanon
Caryophanon latum
Caryophanon tenue
Catellatospora
Catellatospora citrea
Catellatospora methionotrophica
Catenococcus
Catenococcus thiocycli

Caenimonas
Caenimonas koreensis
Caldalkalibacillus
Caldalkalibacillus uzonensis
Caldanaerobacter
Caldanaerobacter subterraneus
Caldanaerobius
Caldanaerobius fijiensis
Caldanaerobius polysaccharolyticus
Caldanaerobius zeae
Caldanaerovirga
Caldanaerovirga acetigignens
Caldicellulosiruptor
Caldicellulosiruptor bescii
Caldicellulosiruptor kristjanssonii
Caldicellulosiruptor owensensis

Campylobacter
Campylobacter coli
Campylobacter concisus
Campylobacter curvus
Campylobacter fetus
Campylobacter gracilis
Campylobacter helveticus
Campylobacter hominis
Campylobacter hyointestinalis
Campylobacter jejuni
Campylobacter lari
Campylobacter mucosalis
Campylobacter rectus
Campylobacter showae
Campylobacter sputorum
Campylobacter upsaliensis
Capnocytophaga
Capnocytophaga canimorsus
Capnocytophaga cynodegmi
Capnocytophaga gingivalis
Capnocytophaga granulosa
Capnocytophaga haemolytica
Capnocytophaga ochracea
Capnocytophaga sputigena

Catenuloplanes
Catenuloplanes atrovinosus
Catenuloplanes castaneus
Catenuloplanes crispus
Catenuloplanes indicus
Catenuloplanes japonicus
Catenuloplanes nepalensis
Catenuloplanes niger
Chryseobacterium
Chryseobacterium balustinum
Citrobacter
C. amalonaticus
C. braakii
C. diversus
C. farmeri
C. freundii
C. gillenii
C. koseri
C. murliniae
C. pasteurii[1]
C. rodentium
C. sedlakii
C. werkmanii
C. youngae
Clostridium
(see below)
Coccochloris
Coccochloris elabens
Corynebacterium
Corynebacterium flavescens
Corynebacterium variabile

Curtobacterium
Curtobacterium albidum
Curtobacterium citreus

Clostridium

Clostridium absonum, *Clostridium aceticum*, *Clostridium acetireducens*, *Clostridium acetobutylicum*, *Clostridium acidisoli*, *Clostridium aciditolerans*, *Clostridium acidurici*, *Clostridium aerotolerans*, *Clostridium aestuarii*, *Clostridium akagii*, *Clostridium aldenense*, *Clostridium aldrichii*, *Clostridium algidicarni*, *Clostridium algidixylanolyticum*, *Clostridium algifaecis*, *Clostridium algoriphilum*, *Clostridium alkalicellulosi*, *Clostridium aminophilum*, *Clostridium aminovalericum*, *Clostridium amygdalinum*, *Clostridium amylolyticum*, *Clostridium arbusti*, *Clostridium arcticum*, *Clostridium argentinense*, *Clostridium asparagiforme*, *Clostridium aurantibutyricum*, *Clostridium autoethanogenum*, *Clostridium baratii*, *Clostridium barkeri*, *Clostridium bartlettii*, *Clostridium beijerinckii*, *Clostridium bifermentans*, *Clostridium bolteae*, *Clostridium bornimense*, *Clostridium botulinum*, *Clostridium bowmanii*, *Clostridium bryantii*, *Clostridium butyricum*, *Clostridium cadaveris*, *Clostridium caenicola*, *Clostridium caminithermale*, *Clostridium carboxidivorans*, *Clostridium carnis*, *Clostridium cavendishii*, *Clostridium celatum*, *Clostridium celerecrescens*, *Clostridium cellobioparum*, *Clostridium cellulofermentans*, *Clostridium cellulolyticum*, *Clostridium cellulosi*, *Clostridium cellulovorans*, *Clostridium chartatabidum*, *Clostridium chauvoei*, *Clostridium chromiireducens*, *Clostridium citroniae*, *Clostridium clariflavum*, *Clostridium clostridioforme*, *Clostridium coccoides*, *Clostridium cochlearium*, *Clostridium colletant*, *Clostridium colicanis*, *Clostridium colinum*, *Clostridium collagenovorans*, *Clostridium cylindrosporum*, *Clostridium difficile*, *Clostridium diolis*, *Clostridium disporicum*, *Clostridium drakei*, *Clostridium durum*, *Clostridium estertheticum*, *Clostridium estertheticum estertheticum*, *Clostridium estertheticum laramiense*, *Clostridium fallax*, *Clostridium felsineum*, *Clostridium fervidum*, *Clostridium fimetarium*, *Clostridium formicaceticum*, *Clostridium frigidicarnis*, *Clostridium frigoris*, *Clostridium ganghwense*, *Clostridium gasigenes*, *Clostridium ghonii*, *Clostridium glycolicum*, *Clostridium glycyrrhizinilyticum*, *Clostridium grantii*, *Clostridium haemolyticum*, *Clostridium halophilum*, *Clostridium hastiforme*, *Clostridium hathewayi*, *Clostridium herbivorans*, *Clostridium hiranonis*,

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Clostridium histolyticum, *Clostridium homopropionicum*, *Clostridium huakuii*, *Clostridium hungatei*, *Clostridium hydrogeniformans*, *Clostridium hydroxybenzoicum*, *Clostridium hylemonae*, *Clostridium jejuense*, *Clostridium indolis*, *Clostridium innocuum*, *Clostridium intestinale*, *Clostridium irregulare*, *Clostridium isatidis*, *Clostridium josui*, *Clostridium kluyveri*, *Clostridium lactatifermentans*, *Clostridium lacusfryxellense*, *Clostridium laramiense*, *Clostridium lavalense*, *Clostridium lentocellum*, *Clostridium lentoputrescens*, *Clostridium leptum*, *Clostridium limosum*, *Clostridium litorale*, *Clostridium lituseburense*, *Clostridium ljungdahlii*, *Clostridium lortetii*, *Clostridium lundense*, *Clostridium magnum*, *Clostridium malenominatum*, *Clostridium mangenotii*, *Clostridium mayombei*, *Clostridium methoxybenzovorans*, *Clostridium methylpentosum*, *Clostridium neopropionicum*, *Clostridium nexile*, *Clostridium nitrophenolicum*, *Clostridium novyi*, *Clostridium oceanicum*, *Clostridium orbiscindens*, *Clostridium oroticum*, *Clostridium oxalicum*, *Clostridium papyrosolvens*, *Clostridium paradoxum*, *Clostridium paraperfringens* (Alias: *C. welchii*), *Clostridium paraputrificum*, *Clostridium pascui*, *Clostridium pasteurianum*, *Clostridium peptidivorans*, *Clostridium perenne*, *Clostridium perfringens*, *Clostridium pfennigii*, *Clostridium phytofermentans*, *Clostridium piliforme*, *Clostridium polysaccharolyticum*, *Clostridium populeti*, *Clostridium propionicum*, *Clostridium proteoclasticum*, *Clostridium proteolyticum*, *Clostridium psychrophilum*, *Clostridium puniceum*, *Clostridium purinilyticum*, *Clostridium putrefaciens*, *Clostridium putrificum*, *Clostridium quercicolum*, *Clostridium quinii*, *Clostridium ramosum*, *Clostridium rectum*, *Clostridium roseum*, *Clostridium saccharobutylicum*, *Clostridium saccharogumia*, *Clostridium saccharolyticum*, *Clostridium saccharoperbutylacetonicum*, *Clostridium sardiniense*, *Clostridium sartagoforme*, *Clostridium scatologenes*, *Clostridium schirmacherense*, *Clostridium scindens*, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium sphenoides*, *Clostridium spiroforme*, *Clostridium sporogenes*, *Clostridium sporosphaeroides*, *Clostridium stercorarium*, *Clostridium stercorarium leptospartum*, *Clostridium stercorarium stercorarium*, *Clostridium stercorarium thermolacticum*, *Clostridium sticklandii*, *Clostridium straminisolvens*, *Clostridium subterminale*, *Clostridium sufflavum*, *Clostridium sulfidigenes*, *Clostridium symbiosum*, *Clostridium tagluense*, *Clostridium tepidiprofundi*, *Clostridium termitidis*, *Clostridium tertium*, *Clostridium tetani*, *Clostridium tetanomorphum*, *Clostridium thermaceticum*, *Clostridium thermautotrophicum*, *Clostridium thermoalcaliphilum*, *Clostridium thermobutyricum*, *Clostridium thermocellum*, *Clostridium thermocopriae*, *Clostridium thermohydrosulfuricum*, *Clostridium thermolacticum*, *Clostridium thermopalmarium*, *Clostridium thermopapyrolyticum*, *Clostridium thermosaccharolyticum*, *Clostridium thermosuccinogenes*, *Clostridium thermosulfurigenes*, *Clostridium thiosulfatireducens*, *Clostridium tyrobutyricum*, *Clostridium uliginosum*, *Clostridium ultunense*, *Clostridium villosum*, *Clostridium vincentii*, *Clostridium viride*, *Clostridium xylanolyticum*, *Clostridium xylanovorans*

Dactylosporangium	*Deinococcus*	*Delftia*	*Echinicola*
Dactylosporangium aurantiacum	*Deinococcus aerius*	*Delftia acidovorans*	*Echinicola pacifica*
Dactylosporangium fulvum	*Deinococcus apachensis*	*Desulfovibrio*	*Echinicola vietnamensis*
Dactylosporangium matsuzakiense	*Deinococcus aquaticus*	*Desulfovibrio desulfuricans*	
Dactylosporangium roseum	*Deinococcus aquatilis*	*Diplococcus*	
Dactylosporangium thailandense	*Deinococcus caeni*	*Diplococcus pneumoniae*	
Dactylosporangium vinaceum	*Deinococcus radiodurans*		
	Deinococcus radiophilus		
Enterobacter	*Enterobacter kobei*	*Faecalibacterium*	*Flavobacterium*
E. aerogenes	*E. ludwigii*	*Faecalibacterium prausnitzii*	*Flavobacterium antarcticum*
E. amnigemis	*E. mori*	*Fangia*	*Flavobacterium aquatile*
E. agglomerans	*E. nimipressuralis*	*Fangia hongkongensis*	*Flavobacterium aquidurense*
E. arachidis	*E. oryzae*	*Fastidiosipila*	*Flavobacterium balustinum*
E. asburiae	*E. pulveris*	*Fastidiosipila sanguinis*	*Flavobacterium croceum*
E. cancerogenous	*E. pyrinus*	*Fusobacterium*	*Flavobacterium cucumis*
E. cloacae	*E. radicincitans*	*Fusobacterium nucleatum*	*Flavobacterium daejeonense*
E. cowanii	*E. taylorae*		*Flavobacterium defluvii*
E. dissolvens	*E. turicensis*		*Flavobacterium degerlachei*
E. gergoviae	*E. sakazakii Enterobacter soli*		*Flavobacterium*
E. helveticus	*Enterococcus*		*denitrificans*
E. hormaechei	*Enterococcus durans*		*Flavobacterium filum*
E. intermedius	*Enterococcus faecalis*		*Flavobacterium flevense*
	Enterococcus faecium		*Flavobacterium frigidarium*
	Erwinia		*Flavobacterium mizutaii*
	Erwinia haponticl		*Flavobacterium*
	Escherichia		*okeanokoites*
	Escherichia coli		
Gaetbulibacter	*Haemophilus*	*Ideonella*	*Janibacter*
Gaetbulibacter saemankumensis	*Hlaemophilus aegyptius*	*Ideonella azotifigens*	*Janibacter anophelis*
Gallibacterium	*Hlaemophilus aphrophilus*	*Idiomarina*	*Janibacter corallicola*
Gallibacterium anatis	*Haemophilus felis*	*Idiomarina abyssalis*	*Janibacter limosus*
Gallicola	*Haemophilus gallinarum*	*Idiomarina baltica*	*Janibacter melonis*
Gallicola barnesae	*Haemophilus haemolyticus*	*Idiomarina fontislapidosi*	*Janibacter terrae*
Garciella	*Haemophilus influenzae*	*Idiomarina loihiensis*	*Jannaschia*

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Garciella nitratireducens
Geobacillus
Geobacillus thermoglucosidasius
Geobacillus stearothermophilus
Geobacter
Geobacter bemidjiensis
Geobacter bremensis
Geobacter chapellei
Geobacter grbiciae
Geobacter hydrogenophilus
Geobacter lovleyi
Geobacter metallireducens
Geobacter pelophilus
Geobacter pickeringii
Geobacter sulfurreducens
Geodermatophilus
Geodermatophilus obscurus
Gluconacetobacter
Gluconacetobacter xylinus
Gordonia
Gordonia rubripertincta
Kaistia
Kaistia adipata
Kaistia soli
Kangiella
Kangiella aquimarina
Kangiella koreensis

Kerstersia
Kerstersia gyiorum
Kiloniella
Kiloniella laminariae
Klebsiella
K. gramilomatis
K. oxytoca
K. pneumoniae
K. terrigena
K. variicola
Kluyvera
Kluyvera ascorbata
Kocuria
Kocuria roasea
Kocuria varians

Haemophilus paracuniculus
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrohaemolyticus
Haemophilus parasuis
Haemophilus pittmaniae
Hafnia
Hafnia alvei
Hahella
Hahella ganghwensis
Halalkalibacillus
Halalkalibacillus halophilus
Helicobacter
Helicobacter pylori

Labedella
Labedella gwakjiensis
Labrenzia
Labrenzia aggregata
Labrenzia alba
Labrenzia alexandrii
Labrenzia marina
Labrys
Labrys methylaminiphilus
Labrys miyagiensis
Labrys monachus
Labrys okinawensis
Labrys portucalensis

Lactobacillus
[see below]
Laceyella
Laceyella putida
Lechevalieria
Lechevalieria aerocolonigenes
Legionella
[see below]

Idiomarina ramblicola
Idiomarina seosinensis
Idiomarina zobellii
Ignatzschineria
Ignatzschineria larvae

Ignavigranum
Ignavigranum ruoffiae
Ilumatobacter
Ilumatobacter fluminis
Ilyobacter
Ilyobacter delafieldii
Ilyobacter insuetus
Ilyobacter polytropus
Ilyobacter tartaricus

Listeria ivanovii
L. marthii
L. monocytogenes
L. newyorkensis
L. riparia
L. rocourtiae
L. seeligeri
L. weihenstephanensis
L. welshimeri
Listonella
Listonella anguillarum
Macrococcus
Macrococcus bovicus
Marinobacter
Marinobacter algicola
Marinobacter bryozoorum
Marinobacter flavimaris
Meiothermus
Meiothermus ruber
Methylophilus
Methylophilus methylotrophus
Microbacterium

Jannaschia cystaugens
Jannaschia helgolandensis
Jannaschia pohangensis
Jannaschia rubra

Janthinobacterium
Janthinobacterium agaricidamnosum
Janthinobacterium lividum
Jejuia
Jejuia pallidilutea
Jeotgalibacillus
Jeotgalibacillus alimentarius
Jeotgalicoccus
Jeotgalicoccus halotolerans

Micrococcus
Micrococcus luteus
Micrococcus lylae
Moraxella
Moraxella bovis
Moraxella nonliquefaciens
Moraxella osloensis
Nakamurella
Nakamurella multipartita
Nannocystis
Nannocystis pusilla
Natranaerobius
Natranaerobius thermophilus
Natranaerobius trueperi
Naxibacter
Naxibacter alkalitolerans
Neisseria
Neisseria cinerea
Neisseria denitrificans
Neisseria gonorrhoeae
Neisseria lactamica

Nesterenkonia
Nesterenkonia holobia
Nocardia
Nocardia argentinensis
Nocardia corallina
Nocardia otitidiscaviarum

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Kurthia	*Listeria*	*Microbacterium*	*Neisseria mucosa*	
Kurthia zopfii	*L. aquatica*	*ammoniaphilum*	*Neisseria sicca*	
	L. booriae	*Microbacterium arborescens*	*Neisseria subflava*	
	L. cornellensis	*Microbacterium liquefaciens*	*Neptunomonas*	
	L. fleischmannii	*Microbacterium oxydans*	*Neptunomonas japonica*	
	L. floridensis			
	L. grandensis			
	L. grayi			
	L. innocua			
Lactobacillus				
L. acetotolerans	*L. catenaformis*	*L. mali*	*L. parakefiri*	*L. sakei*
L. acidifarinae	*L. ceti*	*L. manihotivorans*	*L. paralimentarius*	*L. salivarius*
L. acidipiscis	*L. coleohominis*	*L. mindensis*	*L. paraplantarum*	*L. sanfranciscensis*
L. acidophilus	*L. collinoides*	*L. mucosae*	*L. pentosus*	*L. satsumensis*
Lactobacillus agilis	*L. composti*	*L. murinus*	*L. perolens*	*L. secaliphilus*
L. algidus	*L. concavus*	*L. nagelii*	*L. plantarum*	*L. sharpeae*
L. alimentarius	*L. coryniformis*	*L. namurensis*	*L. pontis*	*L. siliginis*
L. amylolyticus	*L. crispatus*	*L. nantensis*	*L. protectus*	*L. spicheri*
L. amylophilus	*L. crustorum*	*L. oligofermentans*	*L. psittaci*	*L. suebicus*
L. amylotrophicus	*L. curvatus*	*L. oris*	*L. rennini*	*L. thailandensis*
L. amylovorus	*L. delbrueckii* subsp. *bulgaricus*	*L. panis*	*L. reuteri*	*L. ultunensis*
L. animalis	*L. delbrueckii* subsp.	*L. pantheris*	*L. rhamnosus*	*L. vaccinostercus*
L. antri	*delbrueckii*	*L. parabrevis*	*L. rimae*	*L. vaginalis*
L. apodemi	*L. delbrueckii* subsp. *lactis*	*L. parabuchneri*	*L. rogosae*	*L. versmoldensis*
L. aviarius	*L. dextrinicus*	*L. paracasei*	*L. rossiae*	*L. vini*
L. bifermentans	*L. diolivorans*	*L. paracollinoides*	*L. ruminis*	*L. vitulinus*
L. brevis	*L. equi*	*L. parafarraginis*	*L. saerimneri*	*L. zeae*
L. buchneri	*L. equigenerosi*	*L. homohiochii*	*L. jensenii*	*L. zymae*
L. camelliae	*L. farraginis*	*L. iners*	*L. johnsonii*	*L. gastricus*
L. casei	*L. farciminis*	*L. ingluviei*	*L. kalixensis*	*L. ghanensis*
L. kitasatonis	*L. fermentum*	*L. intestinalis*	*L. kefiranofaciens*	*L. graminis*
L. kunkeei	*L. fornicalis*	*L. fuchuensis*	*L. kefiri*	*L. hammesii*
L. leichmannii	*L. fructivorans*	*L. gallinarum*	*L. kimchii*	*L. hamsteri*
L. lindneri	*L. frumenti*	*L. gasseri*	*L. helveticus*	*L. harbinensis*
L. malefermentans			*L. hilgardii*	*L. hayakitensis*
Legionella				
Legionella adelaidensis	*Legionella drancourtii*	*Candidatus Legionella jeonii*	*Legionella quinlivanii*	
Legionella anisa	*Legionella dresdenensis*	*Legionella jordanis*	*Legionella rowbothamii*	
Legionella beliardensis	*Legionella drozanskii*	*Legionella lansingensis*	*Legionella rubrilucens*	
Legionella birminghamensis	*Legionella dumoffii*	*Legionella londiniensis*	*Legionella sainthelensi*	
Legionella bozemanae	*Legionella erythra*	*Legionella longbeachae*	*Legionella santicrucis*	
Legionella brunensis	*Legionella fairfieldensis*	*Legionella lytica*	*Legionella shakespearei*	
Legionella busanensis	*Legionella fallonii*	*Legionella maceachernii*	*Legionella spiritensis*	
Legionella cardiaca	*Legionella feeleii*	*Legionella massiliensis*	*Legionella steelei*	
Legionella cherrii	*Legionella geestiana*	*Legionella micdadei*	*Legionella steigerwaltii*	
Legionella cincinnatiensis	*Legionella genomospecies*	*Legionella monrovica*	*Legionella taurinensis*	
Legionella clemsonensis	*Legionella gormanii*	*Legionella moravica*	*Legionella tucsonensis*	
Legionella donaldsonii	*Legionella gratiana*	*Legionella nagasakiensis*	*Legionella tunisiensis*	
	Legionella gresilensis	*Legionella nautarum*	*Legionella wadsworthii*	
	Legionella hackeliae	*Legionella norrlandica*	*Legionella waltersii*	

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Oceanibulbus
Oceanibulbus indolifex
Oceanicaulis
Oceanicaulis alexandrii
Oceanicola
Oceanicola batsensis
Oceanicola granulosus
Oceanicola nanhaiensis
Oceanimonas
Oceanimonas baumannii
Oceaniserpentilla
Oceaniserpentilla haliotis
Oceanisphaera
Oceanisphaera donghaensis
Oceanisphaera litoralis
Oceanithermus
Oceanithermus desulfurans
Oceanithermus profundus
Oceanobacillus
Oceanobacillus caeni
Oceanospirillum
Oceanospirillum linum

Legionella impletisoli
Legionella israelensis
Legionella jamestowniensis

Paenibacillus
Paenibacillus thiaminolyticus
Pantoea
Pantoea agglomerans

Paracoccus
Paracoccus alcaliphilus
Paucimonas
Paucimonas lemoignei
Pectobacterium
Pectobacterium aroidearum
Pectobacterium atrosepticum
Pectobacterium betavasculorum
Pectobacterium cacticida
Pectobacterium carnegieana
Pectobacterium carotovorum
Pectobacterium chrysanthemi
Pectobacterium cypripedii
Pectobacterium rhapontici
Pectobacterium wasabiae
Pianococcus
Pianococcus citreus
Planomicrobium
Planomicrobium okeanokoites
Plesiomonas
Plesiomonas shigelloides
Proteus
Proteus vulgaris

Legionella oakridgensis
Legionella parisiensis
Legionella pittsburghensis
Legionella pneumophila
Legionella quateirensis
Prevotella
Prevotella albensis
Prevotella amnii
Prevotella bergensis
Prevotella bivia
Prevotella brevis
Prevotella bryantii
Prevotella buccae
Prevotella buccalis
Prevotella copri
Prevotella dentalis
Prevotella denticola
Prevotella disiens
Prevotella histicola
Prevotella intermedia
Prevotella maculosa
Prevotella marshii
Prevotella melaninogenica
Prevotella micans
Prevotella multiformis
Prevotella nigrescens
Prevotella oralis
Prevotella oris
Prevotella oulorum
Prevotella pallens
Prevotella salivae
Prevotella stercorea
Prevotella tannerae
Prevotella timonensis
Prevotella veroralis
Providencia
Providencia stuartii
Pseudomonas
Pseudomonas aeruginosa
Pseudomonas alcaligenes
Pseudomonas anguillispetica
Pseudomonas fluorescens
Pseudoalteromonas haloplanktis
Pseudomonas mendocina
Pseudomonas pseudoalcaligenes
Pseudomonas putida
Pseudomonas tutzeri
Pseudomonas syringae

Legionella worsleiensis
Legionella yabuuchiae

Quadrisphaera
Quadrisphaera granulorum
Quatrionicoccus
Quatrionicoccus australiensis

Quinella
Quinella ovalis

Ralstonia
Ralstonia eutropha
Ralstonia insidiosa
Ralstonia mannitolilytica
Ralstonia pickettii
Ralstonia pseudosolanacearum
Ralstonia syzygii
Ralstonia solanacearum
Ramlibacter
Ramlibacter henchirensis
Ramlibacter tataouinensis

Raoultella
Raoultella ornithinolytica
Raoultella planticola
Raoultella terrigena
Rathayibacter
Rathayibacter caricis
Rathayibacter festucae
Rathayibacter iranicus
Rathayibacter rathayi
Rathayibacter toxicus
Rathayibacter tritici
Rhodobacter
Rhodobacter sphaeroides
Ruegeria
Ruegeria gelatinovorans

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

		Psychrobacter		
		Psychrobacter faecalis		
		Psychrobacter phenylpyruvicus		
Saccharococcus	*Sagittula*	*Sanguibacter*	*Stenotrophomonas*	*Tatlockia*
Saccharococcus thermophilus	*Sagittula stellata*	*Sanguibacter keddieii*	*Stenotrophomonas maltophilia*	*Tatlockia maceachernii*
Saccharomonospora	*Salegentibacter*	*Sanguibacter suarezii*	*Streptococcus*	*Tatlockia micdadei*
Saccharomonospora azurea	*Salegentibacter salegens*	*Saprospira*		*Tenacibaculum*
Saccharomonospora cyanea	*Salimicrobium*	*Saprospira grandis*		*Tenacibaculum amylolyticum*
Saccharomonospora viridis	*Salimicrobium album*	*Sarcina*	[also see below]	*Tenacibaculum discolor*
Saccharophagus	*Salinibacter*	*Sarcina maxima*	*Streptomyces*	*Tenacibaculum gallaicum*
Saccharophagus degradans	*Salinibacter ruber*	*Sarcina ventriculi*	*Streptomyces achromogenes*	*Tenacibaculum lutimaris*
Saccharopolyspora	*Salinicoccus*	*Sebaldella*	*Streptomyces cesalbus*	*Tenacibaculum mesophilum*
Saccharopolyspora erythraea	*Salinicoccus alkaliphilus*	*Sebaldella termitidis*	*Streptomyces cescaepitosus*	*Tenacibaculum skagerrakense*
Saccharopolyspora gregorii	*Salinicoccus hispanicus*		*Streptomyces cesdiastaticus*	*Tepidanaerobacter*
Saccharopolyspora hirsuta	*Salinicoccus roseus*	*Serratia*	*Streptomyces cesexfoliatus*	*Tepidanaerobacter syntrophicus*
Saccharopolyspora hordei	*Salinispora*	*Serratia fonticola*	*Streptomyces fimbriatus*	*Tepidibacter*
Saccharopolyspora rectivirgula	*Salinispora arenicola*	*Serratia marcescens*	*Streptomyces fradiae*	*Tepidibacter formicigenes*
Saccharopolyspora spinosa	*Salinispora tropica*	*Sphaerotilus*	*Streptomyces fulvissimus*	*Tepidibacter thalassicus*
Saccharopolyspora taberi	*Salinivibrio*	*Sphaerotilus natans*	*Streptomyces griseoruber*	*Thermus*
Saccharothrix	*Salinivibrio costicola*	*Sphingobacterium*	*Streptomyces griseus*	*Thermus aquaticus*
Saccharothrix australiensis	*Salmonella*	*Sphingobacterium multivorum*	*Streptomyces lavendulae*	*Thermus filiformis*
Saccharothrix coeruleofusca	*Salmonella bongori*	*Staphylococcus*	*Streptomyces phaeochromogenes*	*Thermus thermophilus*
Saccharothrix espanaensis	*Salmonella enterica*	[see below]	*Streptomyces thermodiastaticus*	
Saccharothrix longispora	*Salmonella subterranea*		*Streptomyces tubercidicus*	
Saccharothrix mutabilis	*Salmonella typhi*			
Saccharothrix syringae				
Saccharothrix tangerinus				
Saccharothrix texasensis				
Staphylococcus				
S. arlettae	*S. equorum*	*S. microti*	*S. schleiferi*	
S. agnetis	*S. felis*	*S. muscae*	*S. sciuri*	
S. aureus	*S. fleurettii*	*S. nepalensis*	*S. simiae*	
S. auricularis	*S. gallinarum*	*S. pasteuri*	*S. simulans*	
S. capitis	*S. haemolyticus*	*S. petrasii*	*S. stepanovicii*	
S. caprae	*S. hominis*	*S. pettenkoferi*	*S. succinus*	
S. carnosus	*S. hyicus*	*S. piscifermentans*	*S. vitulinus*	
S. caseolyticus	*S. intermedius*	*S. pseudintermedius*	*S. warneri*	
S. chromogenes	*S. kloosii*	*S. pseudolugdunensis*	*S. xylosus*	
S. cohnii	*S. leei*	*S. pulvereri*		
S. condimenti	*S. lentus*	*S. rostri*		
S. delphini	*S. lugdunensis*	*S. saccharolyticus*		
S. devriesei	*S. lutrae*	*S. saprophyticus*		
S. epidermidis	*S. lyticans*			
	S. massiliensis			
Streptococcus				
Streptococcus agalactiae	*Streptococcus infantarius*	*Streptococcus orisratti*	*Streptococcus thermophilus*	
Streptococcus anginosus	*Streptococcus iniae*	*Streptococcus parasanguinis*	*Streptococcus sanguinis*	
Streptococcus bovis	*Streptococcus intermedius*	*Streptococcus peroris*	*Streptococcus sobrinus*	

TABLE 1-continued

Example Bacteria

Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Streptococcus canis
Streptococcus constellatus
Streptococcus downei
Streptococcus dysgalactiae
Streptococcus equines
Streptococcus faecalis
Streptococcus ferus
Uliginosibacterium

Uliginosibacterium gangwonense
Ulvibacter
Ulvibacter litoralis
Umezawaea
Umezawaea tangerina
Undibacterium
Undibacterium pigrum
Ureaplasma
Ureaplasma urealyticum

Ureibacillus
Ureibacillus composti
Ureibacillus suwonensis
Ureibacillus terrenus
Ureibacillus thermophilus
Ureibacillus thermosphaericus

Streptococcus lactarius
Streptococcus milleri
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus tigurinus

Vagococcus
Vagococcus carniphilus
Vagococcus elongatus
Vagococcus fessus
Vagococcus fluvialis
Vagococcus lutrae
Vagococcus salmoninarum
Variovorax
Variovorax boronicumulans
Variovorax dokdonensis
Variovorax paradoxus
Variovorax soli
Veillonella
Veillonella atypica
Veillonella caviae
Veillonella criceti
Veillonella dispar
Veillonella montpellierensis
Veillonella parvula
Veillonella ratti
Veillonella rodentium
Venenivibrio
Venenivibrio stagnispumantis

Verminephrobacter
Verminephrobacter eiseniae

Verrucomicrobium
Verrucomicrobium spinosum

Streptococcus pneumoniae
Streptococcus pseudopneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivariu

Vibrio
Vibrio aerogenes
Vibrio aestuarianus
Vibrio albensis
Vibrio alginolyticus
Vibrio campbellii
Vibrio cholerae
Vibrio cincinnatiensis
Vibrio coralliilyticus
Vibrio cyclitrophicus
Vibrio diazotrophicus
Vibrio fluvialis
Vibrio furnissii
Vibrio gazogenes
Vibrio halioticoli
Vibrio harveyi
Vibrio ichthyoenteri
Vibrio mediterranei
Vibrio metschnikovii
Vibrio mytili
Vibrio natriegens
Vibrio navarrensis
Vibrio nereis
Vibrio nigripulchritudo
Vibrio ordalii
Vibrio orientalis
Vibrio parahaemolyticus
Vibrio pectenicida
Vibrio penaeicida
Vibrio proteolyticus
Vibrio shilonii
Vibrio splendidus
Vibrio tubiashii
Vibrio vulnificus

Streptococcus suis
Streptococcus uberis
Streptococcus vestibularis
Streptococcus viridans
Streptococcus zooepidemicus

Virgibacillus
Virgibacillus halodenitrificans
Virgibacillus pantothenticus
Weissella
Weissella cibaria
Weissella confusa
Weissella halotolerans
Weissella hellenica
Weissella kandleri
Weissella koreensis
Weissella minor
Weissella paramesenteroides
Weissella soli
Weissella thailandensis
Weissella viridescens
Williamsia
Williamsia marianensis
Williamsia maris
Williamsia serinedens
Winogradskyella
Winogradskyella thalassocola
Wolbachia
Wolbachia persica

Wolinella
Wolinella succinogenes

Zobellia
Zobellia galactanivorans
Zobellia uliginosa
Zoogloea
Zoogloea ramigera
Zoogloea resiniphila

Xanthobacter
Xanthobacter agilis
Xanthobacter aminoxidans
Xanthobacter autotrophicus
Xanthobacter flavus
Xanthobacter tagetidis
Xanthobacter viscosus
Xanthomonas
Xanthomonas albilineans
Xanthomonas alfalfae
Xanthomonas arboricola
Xanthomonas axonopodis
Xanthomonas campestris
Xanthomonas citri
Xanthomonas codiaei
Xanthomonas cucurbitae
Xanthomonas euvesicatoria
Xanthomonas fragariae
Xanthomonas fuscans
Xanthomonas gardneri
Xanthomonas hortorum
Xanthomonas hyacinthi
Xanthomonas perforans
Xanthomonas phaseoli
Xanthomonas pisi
Xanthomonas populi
Xanthomonas theicola
Xanthomonas translucens
Xanthomonas vesicatoria
Xylella
Xylella fastidiosa
Xylophilus
Xylophilus ampelinus

TABLE 1-continued

Example Bacteria
Optionally, the target host cells are cells of a genus or species selected from this Table and/or the production strain cells are cells of a genus or species selected from this Table

Xenophilus	*Yangia*	*Yersinia mollaretii*	*Zooshikella*	*Zobellella*
Xenophilus azovorans	*Yangia pacifica*	*Yersinia philomiragia*	*Zooshikella ganghwensis*	*Zobellella denitrificans*
Xenorhabdus	*Yaniella*	*Yersinia pestis*	*Zunongwangia*	*Zobellella taiwanensis*
Xenorhabdus beddingii	*Yaniella flava*	*Yersinia pseudotuberculosis*	*Zunongwangia profunda*	
Xenorhabdus bovienii	*Yaniella halotolerans*	*Yersinia rohdei*	*Zymobacter*	*Zeaxanthinibacter*
Xenorhabdus cabanillasii	*Yeosuana*	*Yersinia ruckeri*	*Zymobacter palmae*	*Zeaxanthinibacter*
Xenorhabdus doucetiae	*Yeosuana aromativorans*	*Yokenella*	*Zymomonas*	*enoshimensis*
Xenorhabdus griffiniae	*Yersinia*	*Yokenella regensburgei*	*Zymomonas mobilis*	*Zhihengliuella*
Xenorhabdus hominickii	*Yersinia aldovae*	*Yonghaparkia*	*Zymophilus*	*Zhihengliuella*
Xenorhabdus koppenhoeferi	*Yersinia bercovieri*	*Yonghaparkia alkaliphila*	*Zymophilus paucivorans*	*halotolerans*
Xenorhabdus nematophila	*Yersinia enterocolitica*	*Zavarzinia*	*Zymophilus raffinosivorans*	*Xylanibacterium*
Xenorhabdus poinarii	*Yersinia entomophaga*	*Zavarzinia compransoris*		*Xylanibacterium ulmi*
Xylanibacter	*Yersinia frederiksenii*			
Xylanibacter oryzae	*Yersinia intermedia*			
	Yersinia kristensenii			

TABLE 2

Sequences
Nucleic acid sequences herein are written in 5' to 3' direction; amino acid sequences are written in N- to C-terminal direction.
SEQ ID NO: 1 (P10)
TTTCAATTTAATCATCCGGCTCGTATAATGTGTGGA
SEQ ID NO: 2 (BCD14)
GGGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTA CTGAAACATCTTAATCATGCGGTGGAGGGTTTCTAATG
SEQ ID NO: 3 (gfp)
ATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTC
SEQ IDs NO: 4 & 29 (example Expression Operating Unit, EOU)
The EOU is (in 5' to 3' direction):-
[SEQ ID NO: 4]-[promoter]-[TIS]-[GFP-encoding nucleotide sequence]-[SEQ ID NO: 29]
Where
SEQ ID NO: 4 is
GAATTCAAAAGATCTTAAGTAAGTAAGAGTATACGTATATCGGCTAATAA CGTATGAAGGCGCTTCGGCGCCTTTTTTTATGGGGGTATTTTCATCCCAA

TABLE 2-continued

Sequences
TCCACACGTCCAACGCACAGCAAACACCACGTCGACCCTATCAGCTGCGT GCTTTCTATGAGTCGTTGCTGCATAACTTGACAATTAATCATCCGGCTCG TATAATGTGTGGAA
SEQ ID NO: 29 is
GGATCCAAACTCGAGTAAGGATCTCCAGGCATCAAATAAAACGAAAGGCT CAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGC TCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTT ATA
SEQ ID NO: 5 (Example Shine Dalgarno Sequence)
AAAGAGGAGAAA
SEQ ID NO: 26 (Spacer sequence)
CTTTGCCGCGCGCTTCGTCACGTAATTCTCGTCGCAA
SEQ ID NO: 27 (Spacer sequence)
GTTTGGCGATGGCGCGGGTGTGGTTGTGCTTCGGCGT
SEQ ID NO: 28 (Spacer sequence)
TGGGATGCCTACCGCAAGCAGCTTGGCCTGAA

TABLE 3

Anderson Promoter Collection

SEQ ID NO:	Identifier	Sequence[a]	Measured Strength[b]
6	BBa_J23119	TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC	n/a
7	BBa_J23100	TTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGC	1
8	BBa_J23101	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC	0.7
9	BBa_J23102	TTGACAGCTAGCTCAGTCCTAGGTACTGTGCTAGC	0.86
10	BBa_J23103	CTGATAGCTAGCTCAGTCCTAGGGATTATGCTAGC	0.01
11	BBa_J23104	TTGACAGCTAGCTCAGTCCTAGGTATTGTGCTAGC	0.72
12	BBa_J23105	TTTACGGCTAGCTCAGTCCTAGGTACTATGCTAGC	0.24
13	BBa_J23106	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC	0.47
14	BBa_J23107	TTTACGGCTAGCTCAGCCCTAGGTATTATGCTAGC	0.36
15	BBa_J23108	CTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC	0.51
16	BBa_J23109	TTTACAGCTAGCTCAGTCCTAGGGACTGTGCTAGC	0.04
17	BBa_J23110	TTTACGGCTAGCTCAGTCCTAGGTACAATGCTAGC	0.33
18	BBa_J23111	TTGACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC	0.58
19	BBa_J23112	CTGATAGCTAGCTCAGTCCTAGGGATTATGCTAGC	0
20	BBa_J23113	CTGATGGCTAGCTCAGTCCTAGGGATTATGCTAGC	0.01
21	BBa_J23114	TTTATGGCTAGCTCAGTCCTAGGTACAATGCTAGC	0.1
22	BBa_J23115	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC	0.15
23	BBa_J23116	TTGACAGCTAGCTCAGTCCTAGGGACTATGCTAGC	0.16

TABLE 3-continued

Anderson Promoter Collection

SEQ ID NO:	Identifier	Sequence[a]	Measured Strength[b]
24	BBa_J23117	TTGACAGCTAGCTCAGTCCTAGGGATTGTGCTAGC	0.06
25	BBa_J23118	TTGACGGCTAGCTCAGTCCTAGGTATTGTGCTAGC	0.56

aalso shown in the Anderson Catalog, see <http://parts.igem.org/Promoters/Catalog/Anderson>
bStrength is the Anderson Score (AS), e.g., a strength of 1 is a AS of 1. Reported activities of the promoters are given as the relative fluorescence of plasmids in strain TG1 grown in LB media to saturation. A suitable plasmid is EX-Ptet-S-rbsRFP-P "RFP reporter" as described at <http://parts.igem.org/Part:BBa_J61002>; insertion of a promoter element between XbaI and SpeI sites results in a RFP reporter.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 29

<210> SEQ ID NO 1
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 1

tttcaattta atcatccggc tcgtataatg tgtgga 36

<210> SEQ ID NO 2
<211> LENGTH: 88
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 2

gggcccaagt tcacttaaaa aggagatcaa caatgaaagc aattttcgta ctgaaacatc 60

ttaatcatgc ggtggagggt ttctaatg 88

<210> SEQ ID NO 3
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 3

atgagcaaag gagaagaact tttcactgga gttgtc 36

<210> SEQ ID NO 4
<211> LENGTH: 214
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 4

gaattcaaaa gatcttaagt aagtaagagt atacgtatat cggctaataa cgtattaagg 60

cgcttcggcg cctttttttta tgggggtatt ttcatcccaa tccacacgtc caacgcacag 120

caaacaccac gtcgacccta tcagctgcgt gctttctatg agtcgttgct gcataacttg 180

acaattaatc atccggctcg tataatgtgt ggaa 214

<210> SEQ ID NO 5
<211> LENGTH: 12
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 5

aaagaggaga aa 12

<210> SEQ ID NO 6
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 6

ttgacagcta gctcagtcct aggtataatg ctagc 35

<210> SEQ ID NO 7
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 7

ttgacggcta gctcagtcct aggtacagtg ctagc 35

<210> SEQ ID NO 8
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 8

tttacagcta gctcagtcct aggtattatg ctagc 35

<210> SEQ ID NO 9
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 9

ttgacagcta gctcagtcct aggtactgtg ctagc 35

<210> SEQ ID NO 10
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 10

ctgatagcta gctcagtcct agggattatg ctagc 35

<210> SEQ ID NO 11
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 11

ttgacagcta gctcagtcct aggtattgtg ctagc 35

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<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 12

tttacggcta gctcagtcct aggtactatg ctagc 35

<210> SEQ ID NO 13
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 13

tttacggcta gctcagtcct aggtatagtg ctagc 35

<210> SEQ ID NO 14
<211> LENGTH: 35
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 14

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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<210> SEQ ID NO 16
<211> LENGTH: 35
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 16

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<210> SEQ ID NO 17
<211> LENGTH: 35
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<220> FEATURE:
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<400> SEQUENCE: 17

tttacggcta gctcagtcct aggtacaatg ctagc 35

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<210> SEQ ID NO 18
<211> LENGTH: 35
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 18

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<211> LENGTH: 35
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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ctgatagcta gctcagtcct agggattatg ctagc 35

<210> SEQ ID NO 20
<211> LENGTH: 35
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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ctgatggcta gctcagtcct agggattatg ctagc 35

<210> SEQ ID NO 21
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 21

tttatggcta gctcagtcct aggtacaatg ctagc 35

<210> SEQ ID NO 22
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 22

tttatagcta gctcagccct tggtacaatg ctagc 35

<210> SEQ ID NO 23
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 23

ttgacagcta gctcagtcct agggactatg ctagc 35

<210> SEQ ID NO 24
<211> LENGTH: 35
<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 24

ttgacagcta gctcagtcct agggattgtg ctagc 35

<210> SEQ ID NO 25
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 25

ttgacggcta gctcagtcct aggtattgtg ctagc 35

<210> SEQ ID NO 26
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 26

ctttgccgcg cgcttcgtca cgtaattctc gtcgcaa 37

<210> SEQ ID NO 27
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 27

gtttggcgat ggcgcgggtg tggttgtgct tcggcgt 37

<210> SEQ ID NO 28
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 28

tgggatgcct accgcaagca gcttggcctg aa 32

<210> SEQ ID NO 29
<211> LENGTH: 153
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 29

ggatccaaac tcgagtaagg atctccaggc atcaaataaa acgaaaggct cagtcgaaag 60

actgggcctt tcgttttatc tgttgtttgt cggtgaacgc tctctactag agtcacactg 120

gctcaccttc gggtgggcct ttctgcgttt ata 153

The invention claimed is:

1. A method for enhancing the yield of amplified copies of a first DNA construct in a population of *Escherichia coli* (*E. coli*) production strain cells,

wherein the first DNA construct comprises a nucleotide sequence encoding a Cas nuclease that is under the control of a promoter for controlling the expression of the Cas nuclease in the production strain cells,

wherein the promoter comprises a nucleotide sequence that is capable of binding to a repressor;

wherein production strain cells comprise a nucleic acid sequence encoding the repressor,

wherein the nucleic acid sequence encoding the repressor is a chromosomally-integrated sequence or comprised by a second DNA construct, and the amplified copies of the first DNA construct do not comprise the nucleic acid sequence encoding the repressor;

the method comprising culturing the cells to allow replication of the first DNA construct thereby amplifying the first DNA construct in the cells and to allow expression of the repressor in the cells,

wherein the promoter is repressed by the repressor in the production strain cells,

whereby the yield of amplified copies of the first DNA construct is enhanced relative to the yield produced using a constitutive promoter having an Anderson Score (AS) of AS≥0.5 to control the expression of the Cas nuclease in the production strain cells,

wherein the production strain cell does not comprise a crRNA or gRNA operable with the Cas nuclease to target and cut a chromosomal sequence of the production strain cell.

2. The method of claim **1**, wherein enhancing said yield is by

(a) reducing toxicity of the Cas in the production strain;

(b) reducing mutation of the first DNA construct or production strain cell chromosomal DNA in the production strain;

(c) promoting production cell viability during the amplification of the first DNA construct; and/or

(d) reducing the occurrence of Cas cutting of production strain cell chromosomal DNA or of the first DNA construct.

3. The method of claim **1**, wherein the promoter that is capable of binding to a repressor has a strength less than the Anderson Score promoter BBa_J23108.

4. The method of claim **1**, wherein the nucleotide sequence that is capable of binding to the repressor is a tetO or lacO.

5. The method of claim **4**, wherein the promoter is $P_{LtetO\text{-}1}$, $P_{LlacO\text{-}1}$ or a repressible homologue thereof.

6. The method of claim **1**, wherein the repressor is a tetracycline repressor (TetR) or a lac repressor (LacR).

7. The method of claim **1**, wherein the nuclease is Cas9.

8. The method of claim **1**, wherein the nuclease is Cpf1.

9. The method of claim **1**, wherein the nuclease is Cas3 and the first DNA construct or cell encodes Cascade proteins that are cognate with the Cas3.

10. The method of claim **1**, wherein the first DNA construct comprises one or more nucleotide sequences for producing crRNAs or gRNAs that are operable for Cas nuclease targeting in target host cells.

11. The method of claim **1**, wherein the first DNA construct is comprised by a high copy number plasmid or phagemid.

12. A method of making phage particles or non-self replicative transduction particles comprising amplified copies of a first DNA construct, the method comprising:

(a) culturing a population of *Escherichia coli* (*E. coli*) production strain cells, wherein the production strain cells comprise:

(i) the first DNA construct, wherein the first DNA construct comprises a nucleotide sequence encoding a Cas nuclease that is under the control of a promoter for controlling the expression of the Cas nuclease in the production strain cells, and wherein the promoter comprises a nucleotide sequence that is capable of binding to a repressor,

(ii) a nucleic acid sequence encoding the repressor, wherein the nucleic acid sequence encoding the repressor is a chromosomally-integrated sequence or comprised by a second DNA construct, and

(iii) a nucleotide sequence that is inducible to produce phage coat proteins in the cells;

wherein the cultured production strain cells express the repressor in the cells and replicate the first DNA construct to produce the amplified copies of the first DNA construct, wherein the amplified copies of the first DNA construct do not comprise the nucleic acid sequence encoding the repressor; and

(b) inducing production of the phage proteins and causing packaging of the amplified DNA into phage particles or non-self-replicative transduction particles, and further isolating the phage or transduction particles,

wherein the phage particles or non-self-replicative transduction particles are devoid of the nucleic acid sequence encoding the repressor.

13. The method of claim **12**, further comprising formulating the particles into a pharmaceutical composition for administration to a human or animal subject for treating or reducing the risk of a disease or condition in the subject.

14. The method of claim **13**, wherein the particles are capable of infecting target host cells in the subject and transducing the cells with the first DNA construct, wherein the Cas nuclease encoded by the first DNA construct is expressed in the target host cells, wherein the Cas nuclease is operable with one or more crRNAs or gRNAs to modify a target sequence in the target host cells.

15. The method of claim **14**, wherein the Cas nuclease cuts the target sequence thereby killing the target host cells and treating or reducing the risk of the disease or condition.

16. The method of claim **14**, wherein the target host cells are bacterial cells selected from the group consisting of *C. difficile, P. aeruginosa, K. pneumonia, E. coli, H. pylori, S. pneumoniae* and *S. aureus* cells.

17. The method of claim **13**, wherein the disease or condition is an *E. coli* infection.

18. The method of claim **12**, wherein the production strain cell does not comprise a crRNA or gRNA operable with the Cas nuclease to target and cut a chromosomal sequence of the production strain cell.

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