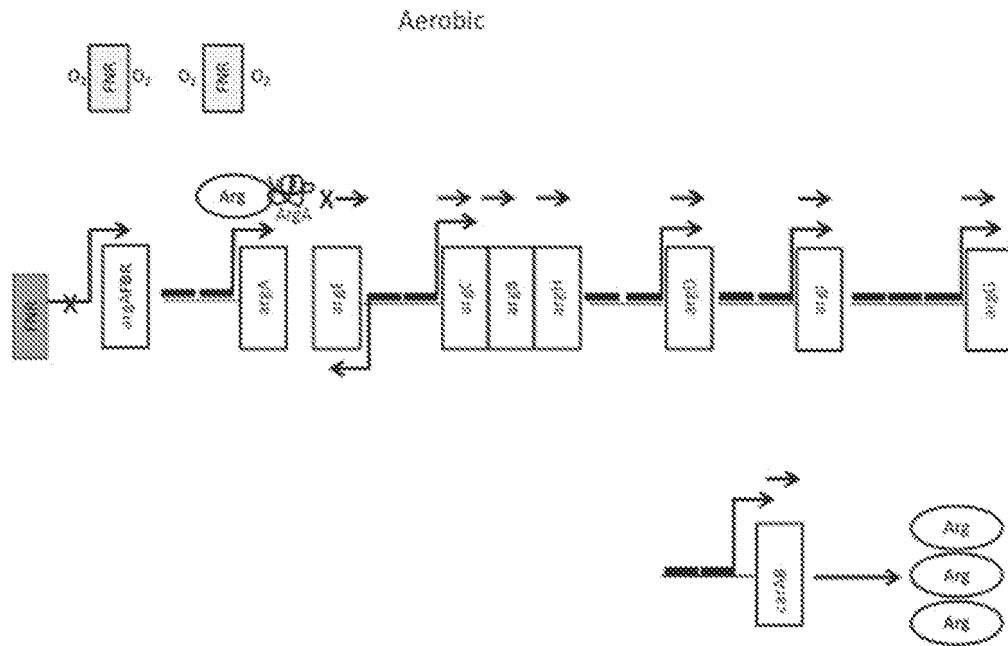


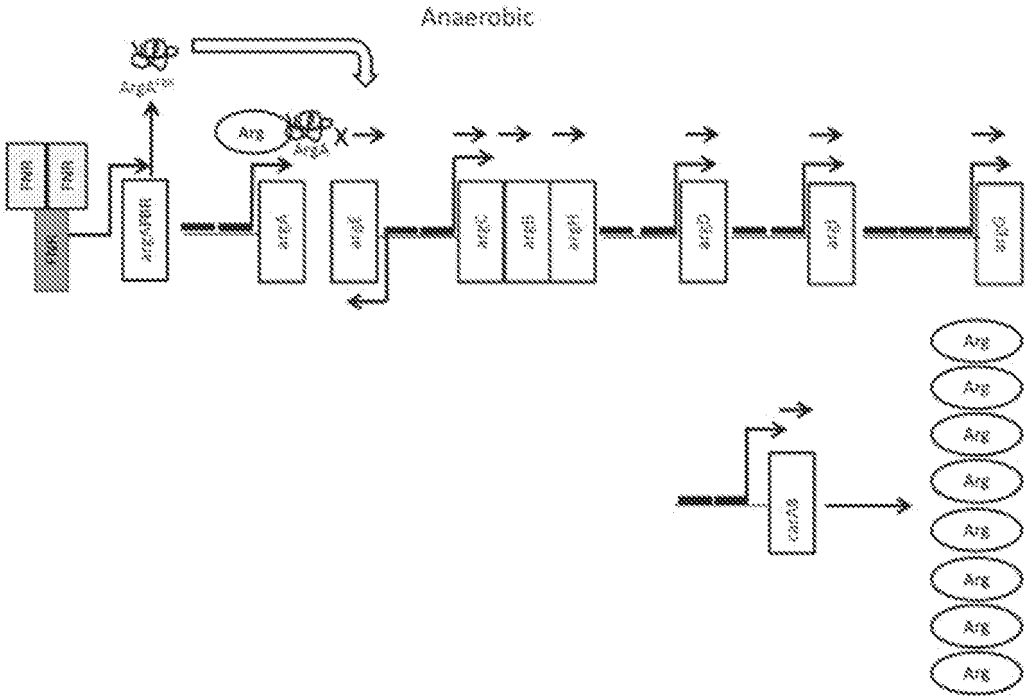
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FIG. 1A



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FIG. 1B



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FIG. 2A

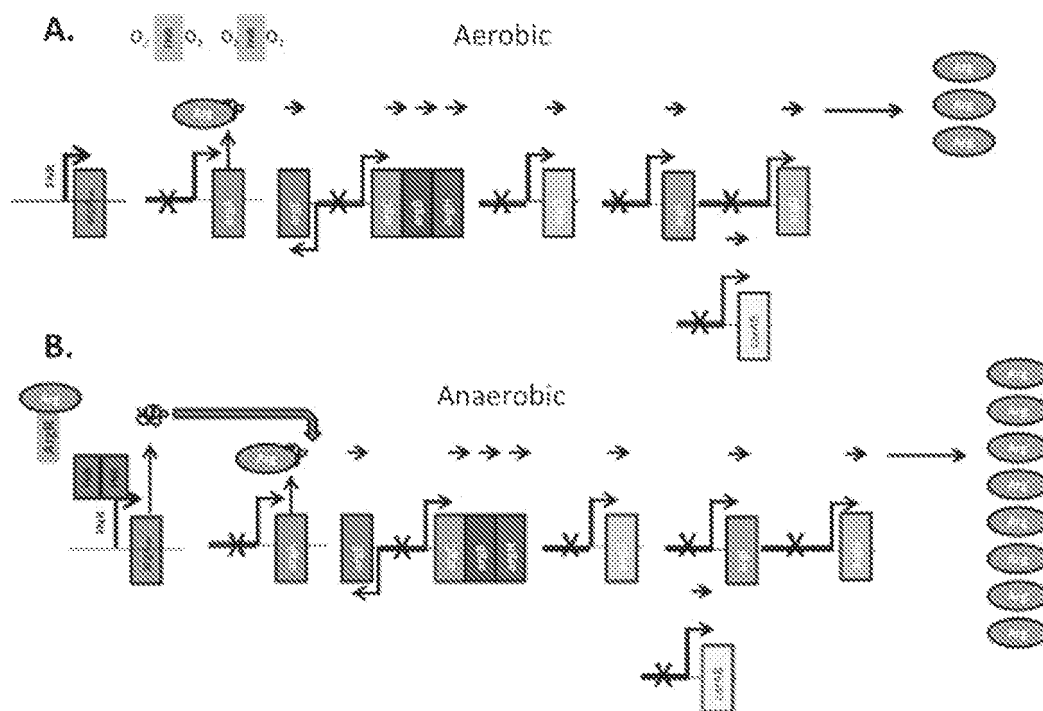
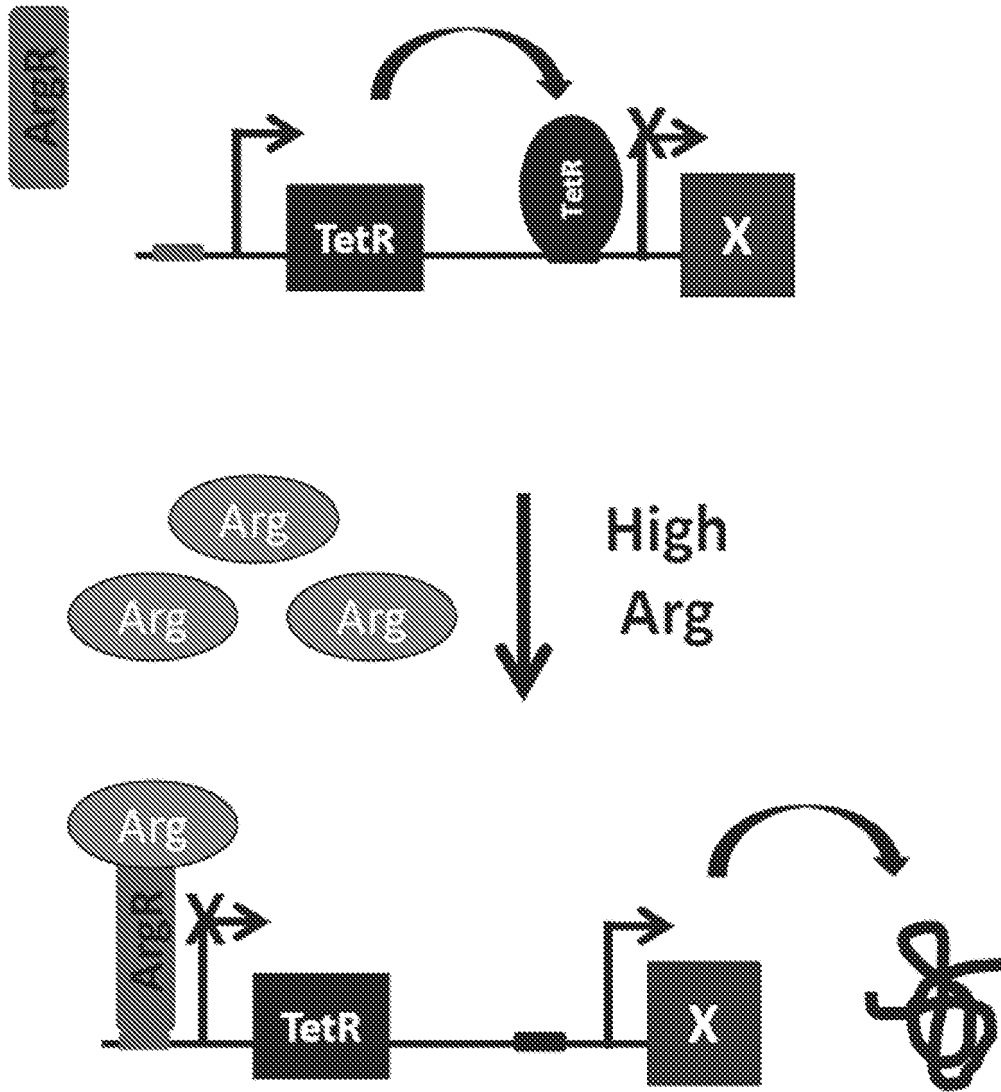


FIG. 2B

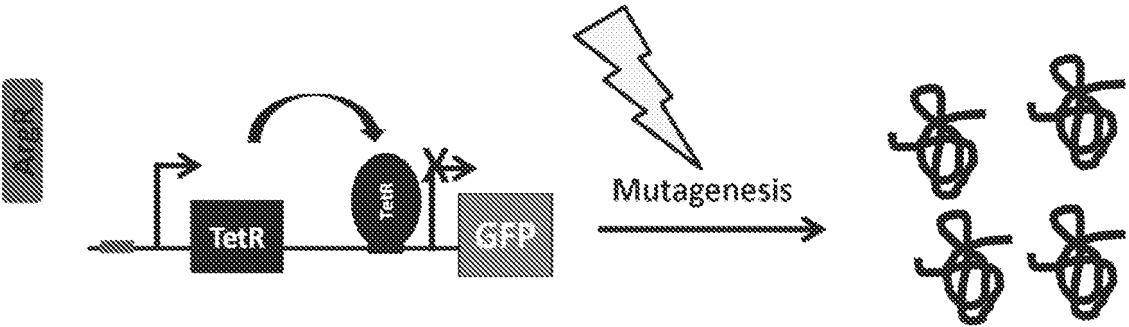
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FIG. 3



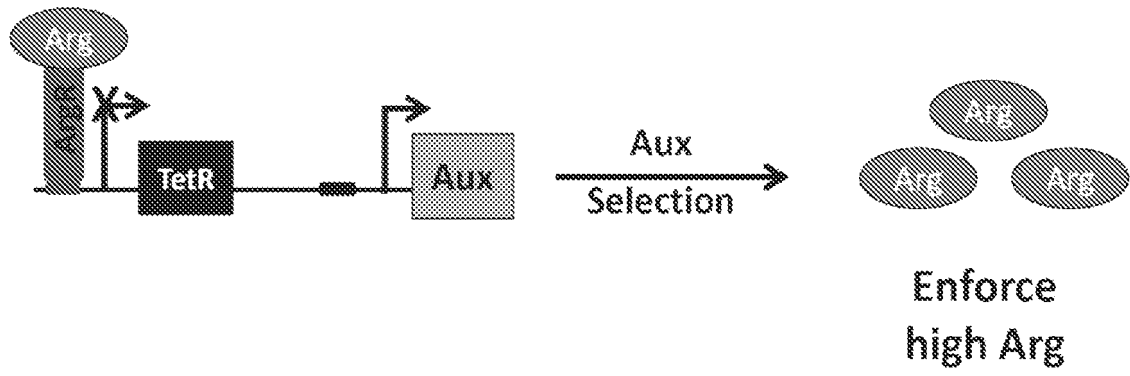
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FIG. 4



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FIG. 5



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FIG. 6

Regulatory region	123456789012345678901234567890
argA WT (SEQ ID NO: 1)	GCAAAAAAACAGAAATAAAATCAATAATT TCAATAATCATGCCAAAGAGGTGTACCGTG
argA mutant (SEQ ID NO: 2)	gcaaaaaaacactttaaaaaacttaataatt tcctttaatcacttaaagaggtgtaccgtg
argI WT (SEQ ID NO: 3)	AGACTTGCAAATGAATAATCATCCATATAG ATTGAATTTTAATTCAATTAAGGCGTTAGCC ACAGGAGGGATCTATG
argI mutant (SEQ ID NO: 4)	agacttgcaaacttatacttatccatataag attttgttttaatttgtaaggcgtttagcc acaggagggatctatg
argCBH WT (SEQ ID NO: 5)	TCATTGTTGACACACCTCTGGTCATGATAG TATCAATATTCATGCCAGTATTTATGAATAA AAATACACTAACGTTGAGCGTAATAAAACC CACCAGCCGTAAGGTGAATGTTTTACGTTT AACCTGGCAACCAGACATAAGAAGGTGAAT AGCCCCGATG
argCBH mutant (SEQ ID NO: 6)	tcattgttgacacacctctggtcatgatag tatcaaacttcatgggatatttatctttaa aaatacttgaacgttgagcgtaataaaaacc caccagccgtaagggtgaatgttttacgttt aacctggcaaccagacataagaaggtgaat agccccgatg

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Regulatory region	123456789012345678901234567890
argE WT (SEQ ID NO: 7)	CATCGGGGCTATTACCTTCTTATGTCTGG TTGCCAGGTAAACGTAAAACATTACCTT ACGGCTGGTGGGTTTTATTACGCTCAACGT T AGT <u>GTATTTTTATT</u> <u>CATAAA</u> TACT <u>GCATG</u> AATATT GATACTATCATGACCAGAGGTGTG TCAACA ATGA
argE mutant (SEQ ID NO: 8)	catcggggctattcaccttcttatgtctgg ttgccaggttaaacgtaaaacattcacctt acggctggtgggtttttattacgctcaacgt tcaagtattttttaagataaatatcccatg aagtttgatactatcatgaccagaggtgtg tcaacaatga
carAB WT (SEQ ID NO: 9)	AGCAGATTTGCATTGATTTACGTCATCAT T GTGAAT <u>TAATATG</u> <u>CAAATAA</u> AGT <u>GAGTGAA</u> TATT <u>CTCTGGAGGGTGT</u> <u>TTG</u>
carAB mutant (SEQ ID NO: 10)	agcagatttgcattgatttacgtcatcatt gtcttttaatatcttaataactggagtgac gtttctctgagggtgttttg
argD WT (SEQ ID NO: 11)	TTTCTGATTGCCATT CAGT <u>GATTTTTATG</u> <u>CATATTT</u> <u>TGTGATTATAATTT</u> <u>CATATTTAT</u> TTATGCGTAACAGGGTGATCATGAGATG
argD mutant (SEQ ID NO: 12)	tttctgattgccattcagtcctttttttact tatattttgtctttataatcttatatttat ttatgcgtaacagggtgatcatgagatg

FIG. 6 continued

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Regulatory region	123456789012345678901234567890
argG WT (SEQ ID NO: 13)	CTAATCACGTTGAATGAATATCCAGTTCACT TTCATTTGTTGAATACTTTTACCTTCTCCT GCTTTCCCTTAAGCGCATTATTTTACAAAA AACACACTAAACTCTTCCTGTCTCCGATAA AAGATGATTAAATGAAAACTCATTTATTTT GCATAAAAATTGAGTGAAAGCAGAAATCCA GGCTCATCATCAGTTAATTAAGCAGGGTGT TATTTTATG
argG mutant (SEQ ID NO: 14)	ctaatcaccttaaatgaatcttcagttcact ttcatttggtgaatacttttaccttctcct gctttcccttaagcgcattattttacaaaa aacacactaaactcttcctgtctccgataa aagatgatcttatgaaaacctttttatctt ttataaaaaatcttggtgaaagcagaaatcca ggctcatcatcagttaattaagcaggggtgt tattttatg
argG mutant (SEQ ID NO: 15)	cctgaaacgtggcaaattctactcgttttg ggtaaaaaatgcaaatactgctgggatttg gtgtaccgagacgggacgtaaaaatctgcag gcattatagtgatccacgccacattttgtc aacgtttattgctaatacttgacggctagc tcagtcctaggtacagtgctagcACCCGTT TTTTTGGGCTAGAAATAATTTTGTTTAACT TTAAGAAGGAGATATACATACCC

FIG. 6 continued

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FIG. 7

FNR-responsive regulatory region	12345678901234567890123456789012345678901234567890
SEQ ID NO: 16	ATCCCCATCACTCTTGATGGAGATCAATTCCCCAAGCTGCTAGAGCGTTA CCTTGCCCTTAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCT CCCACAGGAGAAAACCG
SEQ ID NO: 17	CTCTTGATCGTTATCAATTCCCACGCTGTTTCAGAGCGTTACCTTGCCCT TAAACATTAGCAATGTCGATTTATCAGAGGGCCGACAGGCTCCCACAGGA GAAAACCG
<i>nirB1</i> SEQ ID NO: 18	GTCAGCATAACACCCTGACCTCTCATTAATTGTTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCCAAAA
<i>nirB2</i> SEQ ID NO: 19	CGGCCCGATCGTTGAACATAGCGGTCCGCAGGCGGCACTGCTTACAGCAA ACGGTCTGTACGCTGTGCTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTC AGCCGTCACCGTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCC GGACGGCACTATCGTCGTCCGGCCTTTTCCTCTCTTCCCCGCTACGTGC ATCTATTTCTATAAACCCGCTCATTTTGTCTATTTTTTGCACAAACATGA AATATCAGACAAATCCGTGACTTAAGAAAAATTTATACAAATCAGCAATAT ACCCATTAAGGAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGG GTTGCTGAATCGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCCAAAA atgtttgtttaactttaagaaggagatatatacat
<i>nirB3</i> SEQ ID NO: 20	GTCAGCATAACACCCTGACCTCTCATTAATTGCTCATGCCGGACGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTCCCCGCTACGTGCATCTATTTCT ATAAACCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGAC AATTCCGTGACTTAAGAAAAATTTATACAAATCAGCAATATACCCATTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCCAAAA
<i>ydfZ</i> SEQ ID NO: 21	ATTTCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCGACTTATGGC TCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAACAAAAATATT TCACTCGACAGGAGTATTTATATTGCGCCCGTTACGTGGGCTTCGACTGT AAATCAGAAAGGAGAAAAACACCT

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<i>nirB</i> +RBS SEQ ID NO: 22	GTCAGCATAACACCCTGACCTCTCATTAAATTGTTTCATGCCGGGCGGCACT ATCGTCGTCCGGCCTTTTCCTCTCTTACTCTGCTACGTACATCTATTTCT ATAAATCCGTTCAATTTGTCTGTTTTTGCACAAACATGAAATATCAGAC AATTCGGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCCTTAAG GAGTATATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAAT CGTTAAG GGATCC CTCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATA TACAT
<i>ydfZ</i> +RBS SEQ ID NO: 23	CATTCCTCTCATCCCATCCGGGGTGAGAGTCTTTTCCCCGACTTATGG CTCATGCATGCATCAAAAAAGATGTGAGCTTGATCAAAAAACAAAAATAT TTCACCTCGACAGGAGTATTTATATTGCGCC GGATCC CTCTAGAAATAAT TTTGTTTAACTTTAAGAAGGAGATATACAT
<i>fnrS1</i> SEQ ID NO: 24	AGTTGTTCTTATTGGTGGTGTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTCCGGCTGTCTGTATACAAAACGCCGTAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT GGATCC CTCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACAT
<i>fnrS2</i> SEQ ID NO: 25	AGTTGTTCTTATTGGTGGTGTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTCCGGCTGTCTGTATACAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCTT GGATCCAAAGTGAACCTCTAGAAATAATTTGTTTAACTTTAAGAAGGAGA TATACAT
<i>nirB</i> + <i>crp</i> SEQ ID NO: 26	TCGTCTTTGTGATGTGCTTCCTGTTAGGTTTCGTACGCCGTCACCGTCAG CATAACACCCTGACCTCTCATTAAATTGCTCATGCCGGACGGCACTATCGT CGTCCGGCCTTTTCCTCTCTTCCCCCGCTACGTGCATCTATTTCTATAAA CCCGCTCATTTTGTCTATTTTTTGCACAAACATGAAATATCAGACAATTC CGTGACTTAAGAAAATTTATACAAATCAGCAATATACCCATTAAGGAGTA TATAAAGGTGAATTTGATTTACATCAATAAGCGGGGTTGCTGAATCGTTA AGGTAGaaatgtgatctagttcacatttGCGGTAATAGAAAAGAAATCGA GGCAAAAtgtttgtttaactttaagaaggagatatatacat
<i>fnrS</i> + <i>crp</i> SEQ ID NO: 27	AGTTGTTCTTATTGGTGGTGTGCTTTATGGTTGCATCGTAGTAAATGGT TGTAACAAAAGCAATTTTCCGGCTGTCTGTATACAAAACGCCGCAAAG TTTGAGCGAAGTCAATAAACTCTCTACCCATTCAGGGCAATATCTCTCaa atgtgatctagttcacattttttgtttaactttaagaaggagatatatacat

FIG. 7 continued

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FIG. 8A

Nucleotide sequence of exemplary *argA*^{fb} sequence

(SEQ ID NO: 28)

ATGGTAAAGGAACGTAAAAACCGAGTTGGTCGAGGGATTCCGCCATTTCGGTTCCTGTGTA
TCAATACCCACCGGGGAAAAACGTTTGTTCATCATGCTCGGCGGTGAAGCCATTGAGCA
TGAGAATTTCTCCAGTATCGTTAATGATATCGGGTTGTTGCACAGCCTCGGCATCCGT
CTGGTGGTGGTCTATGGCGCACGTCCGCAGATCGACGCAAATCTGGCTGCGCATCACC
ACGAACCGCTGTATCACAAGAATATACGTGTGACCGACGCCAAAACACTGGAAGTGGT
GAAGCAGGCTGCGGGAACATTGCAACTGGATATTACTGCTCGCCTGTGATGAGTCTC
AATAACACGCCGCTGCAGGGCGCGCATATCAACGTCGTCAGTGGCAATTTTATTATTG
CCCAGCCGCTGGGCGTCGATGACGGCGTGGATTACTGCCATAGCGGGCGTATCCGGCG
GATTGATGAAGACGCGATCCATCGTCAACTGGACAGCGGTGCAATAGTGCTAATGGGG
CCGGTCGCTGTTTCAGTCACTGGCGAGAGCTTTAACCTGACCTCGGAAGAGATTGCCA
CTCAACTGGCCATCAAAGTGAAGCTGAAAAGATGATTGGTTTTTGCTCTTCCCAGGG
CGTCACTAATGACGACGGTGATATTGTCTCCGAACTTTCCCTAACGAAGCGCAAGCG
CGGGTAGAAGCCCAGGAAGAGAAAAGGCGATTACAACCTCCGGTACGGTGCGCTTTTTGC
GTGGCGCAGTGAAAGCCTGCCGCAGCGGCGTGCGTCGCTGTCATTTAATCAGTTATCA
GGAAGATGGCGCGCTGTTGCAAGAGTTGTTCTCACGCGACGGTATCGGTACGCAGATT
GTGATGGAAAGCGCCGAGCAGATTTCGTCGCGCAACAATCAACGATATTGGCGGTATTC
TGGAGTTGATTGCGCCACTGGAGCAGCAAGGTATTCTGGTACGCCGTTCTCGCGAGCA
GCTGGAGATGGAAATCGACAAATTACCATTTATTCAGCGCGATAACACGACTATTGCC
TGCGCCGCGCTCTATCCGTTCCCGGAAGAGAAGATTGGGGAAATGGCCTGTGTGGCAG
TTCACCCGGATTACCGCAGTTCATCAAGGGGTGAAGTTCTGCTGGAACGCATTGCCGC
TCAGGCTAAGCAGAGCGGCTTAAGCAAATTGTTTGTGCTGACCACGCGCAGTATTAC
TGTTCCAGGAACGTGGATTTACCCAGTGGATATTGATTTACTGCCCAGAGCAAAA
AGCAGTTGTACAACCTACCAGCGTAAATCCAAAGTGTTGATGGCGGATTTAGGGTAA

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FIG. 8B

Nucleotide sequence of exemplary FNR promoter-driven *argA^{fbr}* plasmid

(SEQ ID NO: 29)

GTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCATCCCCATCACTCTTGATGGAGATCAA
 TTCCCCAAGCTGCTAGAGCGTTACCTTGCCCTTAAACATTAGCAATGTCGATTTATCAGAGG
 GCGACAGGCTCCACAGGAGAAAACCGATGGTAAAGGAACGTAAAACCGAGTTGGTCGAGG
 GATTCGCCCATTCGGTTCCCTGTATCAATACCCACCGGGGAAAAACGTTTGTTCATCATGCTC
 GGCGGTGAAGCCATTGAGCATGAGAATTTCTCCAGTATCGTTAATGATATCGGGTTGTTGCA
 CAGCCTCGGCATCCGTCTGGTGGTGGTCTATGGCGCACGTCCGCAGATCGACGCAAATCTGG
 CTGCGCATCACCAAGAACCGCTGTATCACAAGAATATACGTGTGACCGACGCCAAAACACTG
 GAACTGGTGAAGCAGGCTGCGGGAACATTGCAACTGGATATTACTGCTCGCCTGTGATGAG
 TCTCAATAACACGCGCGCTGCAGGGCGCGCATATCAACGTCGTCAGTGGCAATTTTATTATTG
 CCCAGCGCGCTGGGCGTTCGATGACGGCGTGGATTACTGCCATAGCGGGCGTATCCGGCGGATT
 GATGAAGACGCGATCCATCGTCAACTGGACAGCGGTGCAATAGTGCTAATGGGGCCGGTCCG
 TGTTCAGTCACTGGCGAGAGCTTTAACCTGACCTCGGAAGAGATTGCCACTCAACTGGCCA
 TCAAACCTGAAAGCTGAAAAGATGATTGGTTTGTGCTCTTCCCAGGGCGTCACTAATGACGAC
 GGTGATATTGTCTCCGAACCTTTCCCTAACGAAGCGCAAGCGCGGGTAGAAGCCAGGAAGA
 GAAAGGCGATTACAACCTCCGGTACGGTGGCCTTTTGGCTGGCGCAGTGAAAGCCTGCCGCA
 GCGGCGTGGCTCGCTGTCAATTAATCAGTTATCAGGAAGATGGCGCGCTGTTGCAAGAGTTG
 TTCTCAGCGCAGGTATCGGTACGCAGATTGTGATGGAAAGCGCCGAGCAGATTCTGTCGCGC
 AACAATCAACGATATTGGCGGTATTCTGGAGTTGATTGCGCCACTGGAGCAGCAAGGTATTCT
 TGGTACGCCGTTCTCGCGAGCAGCTGGAGATGGAAATCGACAAATTCACCATTATTCAGCGC
 GATAACACGACTATTGCCTGCGCCGCGCTCTATCCGTTCCCGGAAGAGAAGATTGGGGAAAT
 GGCTGTGTGGCAGTTCACCCGGATTACCGCAGTTCATCAAGGGGTGAAGTTCTGCTGGAAC
 GCATTGCCGCTCAGGCTAAGCAGAGCGGCTTAAGCAAAATGTTTGTGCTGACCACGCGCAGT
 ATTCAGTGGTTCCAGGAACGTGGATTTACCCAGTGGATATTGATTTACTGCCCGAGAGCAA
 AAAGCAGTTGTACAACCTACCAGCGTAAATCCAAAGTGTTGATGGCGGATTTAGGGTAAACAG
 AATAAAAATACAATAATTTGAATAATCATGCAAAGCTTGGCGTAATCATGGTCATAGCTGT
 TTCTGTGTGAAATTGTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATGTAC
 GGGTTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTGCTAGTTTGTATCAGAATCGCAGAT
 CCGGCTTCAGGTTGCCGGCTGAAAGCGCTATTTCTTCCAGAATTGCCATGATTTTTTCCCC
 ACGGGAGGCGTCACTGGCTCCCGTGTGTGCGCAGCTTTGATTCGATAAGCAGCATCGCCTG
 TTTCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTTCAT
 GTTCTAGTTGCTTTGTTTTACTGGTTTTACCTGTTCTATTAGGTGTTACATGCTGTTTCATCT
 GTTACATTGTGATCTGTTTCATGGTGAACAGCTTTAAATGCACCAAAAACTCGTAAAAGCTC
 TGATGTATCTATCTTTTTTACACCGTTTTTCATCTGTGCATATGGACAGTTTTCCCTTTGATA
 TCTAACGGTGAACAGTTGTTCTACTTTTTGTTTGTAGTCTTGATGCTTCACATGATAGATACA
 AGAGCCATAAGAACCCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGTGTGGTTCGTT
 GTTTTTGCGTGAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCATGTCACCTCAA
 AATTTTGCCTCAAAACTGGTGAGCTGAATTTTGCAGTTAAAGCATCGTGTAGTGTGTTTTCT
 TAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCAACATTCATTT
 TTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACTTGGAAA
 ATCAACGTATCAGTCGGGCGGCCTCGCTTATCAACCACCAATTTTCATATTGCTGTAAGTGTT
 TAAATCTTTACTTATTTGGTTTCAAAACCCATTGGTTAAGCCTTTTAACTCATGGTAGTTAT
 TTCAAGCATTAACATGAACCTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAGTT
 TTCTTTTGTGTTAGTTCTTTTAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAA
 AGACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATA
 TCTCTTCACTAAAACTAATTCTAATTTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGG

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AAAATCTCAAAGCCTTTAACCAAAGGATTCTGATTTCCACAGTTCTCGTCATCAGCTCTCT
GGTTGCTTTAGCTAATACACCATAAGCATTTCCTTACTGATGTTTCATCATCTGAGCGTATT
GGTTATAAGTGAACGATAACCGTCCGTTCTTTCCCTGTAGGGTTTTCAATCGTGGGGTTGAGT
AGTGCCACACAGCATAAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGC
TAGTTTCATTTGCTTTGAAAACAATAATTAGACATACATCTCAATTGGTCTAGGTGATTTT
AATCACTATAACCAATTGAGATGGGCTAGTCAATGATAAATTACTAGTCCTTTTCCCTTTGAGTT
GTGGGTATCTGTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAATTCTGCTAGACCCCTC
TGTAATTCCGCTAGACCTTTGTGTGTTTTTTTTGTTTTATATCAAGTGGTTATAATTTATA
GAATAAAGAAAGAATAAAAAAGATAAAAAAGAAATAGATCCCAGCCCTGTGTATAACTCACTA
CTTTAGTCAGTTCGCGAGTATTACAAAAGGATGTCGCAACGCTGTTTGCTCCTCTACAAAA
CAGACCTTAAAACCCTAAAGGCTTAAGTAGCACCCCTCGCAAGCTCGGGCAAATCGCTGAATA
TTCCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTTCGTGACATTCAGTTCGC
TGCGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGATTCT
ATGCAAGGAAACTACCCATAATAACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTATG
GCGGGTCTGCTATGTGGTGTCTATCTGACTTTTTGCTGTTTCAGCAGTTCCTGCCCTCTGATTT
TCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCAATCAGACTGGCTAATGCACCCAGT
AAGGCAGCGGTATCATCAACAGGCTTACCCGTCTTACTGTCTTTTCTACGGGGTCTGACGCT
CAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCAC
CTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTT
GGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGT
TCATCCATAGTTGCCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCATC
TGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAA
TAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATC
CAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTTCGCA
CGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTC
GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTT
AGCTCCTTCGGTCCCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGT
TATGGCAGCACTGCATAATTCTCTTACTGTGATGCCATCCGTAAGATGCTTTTCTGTGACTG
GTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAA
ACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAAC
CCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCA
AAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACT
CATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAA
GTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTAT
CACGAGGCCCTTTTCGTCTCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGC
TCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGC
GCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGT
ACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCA
TCAGGCGCCATTGCGCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCT
TCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCC
AGGGTTTTCCAGTCACGACGTT

FIG. 8B continued

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FIG. 9

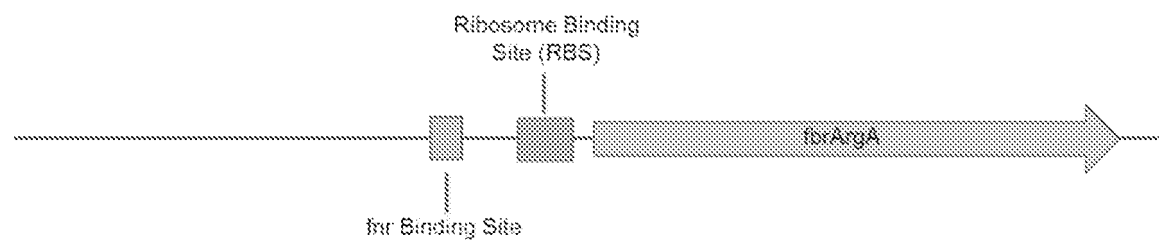
Nucleotide sequence of exemplary FNR promoter-driven *argA*^{fbr} sequence

(SEQ ID NO: 30)

AGTTGTTCTTATTGGTGGTGTGCTTTATGGTTGCATCGTAGTAAATGGTTGTAACAA
AAGCAATTTTTCCGGCTGTCTGTATACAAAACGCCGCAAAGTTTGAGCGAAGTCAAT
AAACTCTCTACCCATTTCAGGGCAATATCTCTCTTggatccaaagtgaactctagaaat
aatTTTTgtttaactttaagaaggagatatacatATGGTAAAGGAACGTAAAACCGAG
TTGGTCGAGGGATTCCGCCATTCGGTTCCCTGTATCAATACCCACCGGGGAAAAACG
TTTGTCATCATGCTCGGCGGTGAAGCCATTGAGCATGAGAATTTCTCCAGTATCGTT
AATGATATCGGGTTGTTGCACAGCCTCGGCATCCGTCTGGTGGTGGTCTATGGCGCA
CGTCCGCAGATCGACGCAAATCTGGCTGCGCATCACCACGAACCGCTGTATCACAAG
AATATACGTGTGACCGACGCCAAAACACTGGAAGTGGTGAAGCAGGCTGCGGGGAACA
TTGCAACTGGATATTACTGCTCGCCTGTGATGAGTCTCAATAACACGCCGCTGCAG
GGCGCGCATATCAACGTCGTCAGTGGCAATTTTATTATTGCCAGCCGCTGGGCGTC
GATGACGGCGTGGATTACTGCCATAGCGGGCGTATCCGGCGGATTGATGAAGACGG
ATCCATCGTCAACTGGACAGCGGTGCAATAGTGCTAATGGGGCCGGTCGCTGTTTCA
GTCAGTGGCGAGAGCTTTAACCTGACCTCGGAAGAGATTGCCACTCAACTGGCCATC
AAACTGAAAGCTGAAAAGATGATTGGTTTTTGCTCTTCCCAGGGCGTCACTAATGAC
GACGGTGATATTGTCTCCGAACTTTTCCCTAACGAAGCGCAAGCGCGGTAGAAGCC
CAGGAAGAGAAAAGGCGATTACAACCTCCGGTACGGTGCGCTTTTTGCGTGCGCGAGTG
AAAGCCTGCCGCAGCGGCGTGCGTGCCTGTCATTTAATCAGTTATCAGGAAGATGGC
GCGCTGTTGCAAGAGTTGTTCTCACGCGACGGTATCGGTACGCAGATTGTGATGGAA
AGCGCCGAGCAGATTGTCGCGCAACAATCAACGATATTGGCGGTATTCTGGAGTTG
ATTCGCCCACTGGAGCAGCAAGGTATTCTGGTACGCCGTTCTCGCGAGCAGCTGGAG
ATGGAAATCGACAAATTCAACATTATTCAGCGCGATAACACGACTATTGCCTGCGCC
GCGCTCTATCCGTTCCCGGAAGAGAAGATTGGGGAAATGGCCTGTGTGGCAGTTCAC
CCGATTACCGCAGTTCATCAAGGGGTGAAGTTCTGCTGGAACGCATTGCCGCTCAG
GCTAAGCAGAGCGGCTTAAGCAAATTGTTTGTGCTGACCACGCGCAGTATTCAGTGG
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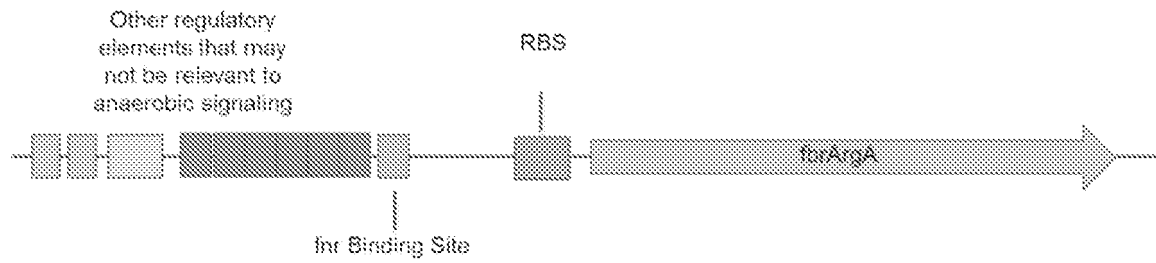
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FIG. 10



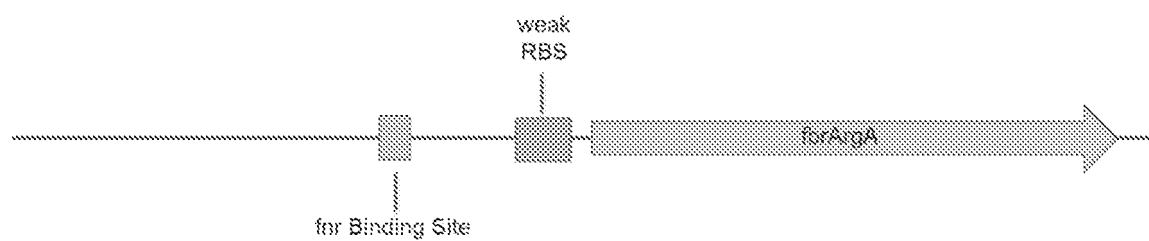
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FIG. 11



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FIG. 12



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FIG. 13A

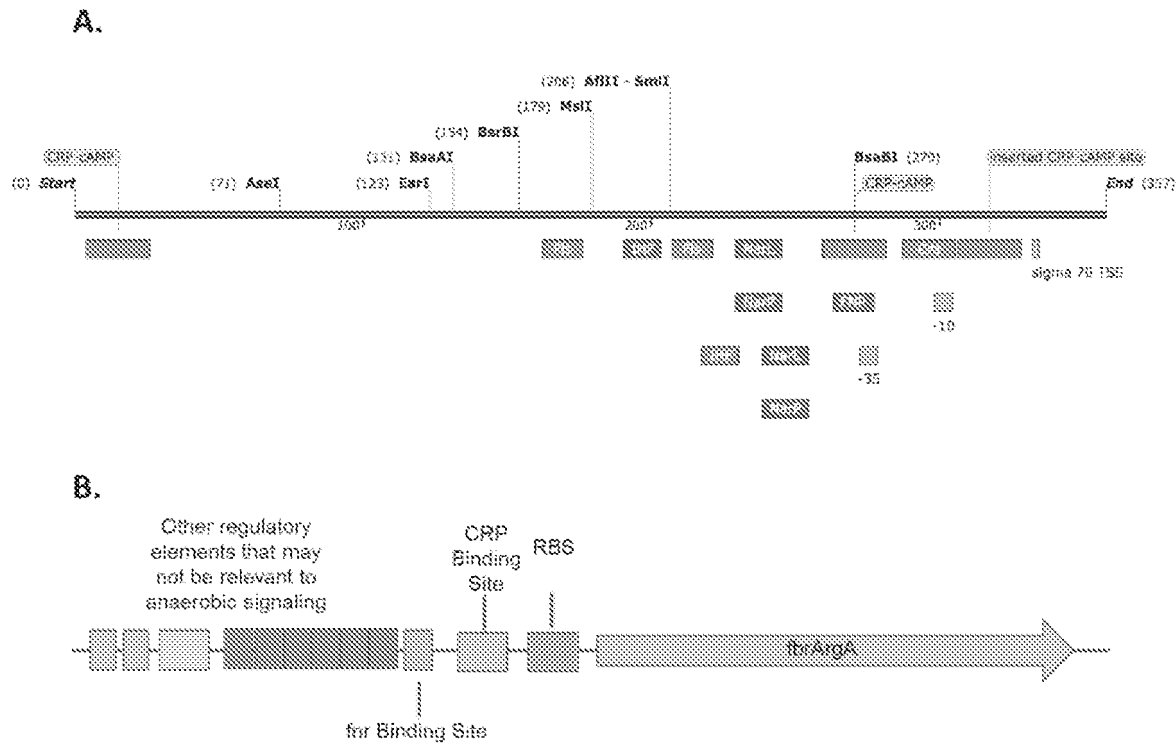
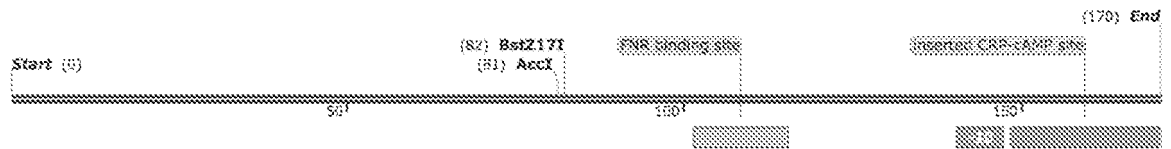


FIG. 13B

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FIG. 14A

A.



B.

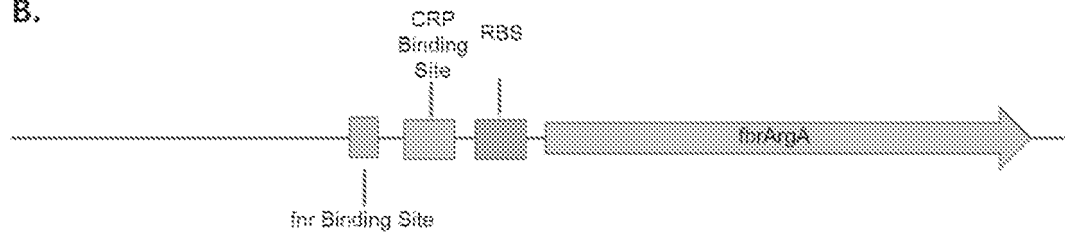


FIG. 14B

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FIG. 15

Wild-type argG (SEQ ID NO: 31)	gtgatccacgccacattttgtcaacgtttattgctaatacaCGTG AATGAATATCCAGTtcacttttcatttgttgaataacttttacctt ctcctgctttcccttaagcgcattattttacaaaaaacacacta aactcttctctgtctccgataaaaagatgATTAAATGAAAACTCAT TtatTTTGCATAAAAATTCAGTgaaagcagaaatccagggtcat catcagttaattaagcagggtgttattttatgacgacgattct caagcatctcccggtagggtcaacgtattggtatcgctttttcc ggcgggtctggacaccagtgcgcactgctgtggatgcgacaaa agggagcgggttccttatgcatatactgcaaacctgggccagcc agacgaagaggattatgatgcatccctcgtcgtgccatggaa tacggcgcgaggagaacgcacgtctgatcgactgcgcaaacaaac tggtggccgaaggatttgcgcctattcagtgtggcgcatttca taacaccactgggtggactgacctatttcaacacgacgcgctg ggcgcgcgcgtgacccggcaccatgctggttgcctgctatgaaag aagatggcgtgaatatctgggggtgacggcagcacctataaagg aaacgatatcgaaacgtttctaccgttacgggtctgctgaccaat gctgaactgcagattttacaaaccgtggcttgatactgacttta ttgatgaactgggtggccgtcatgagatgtctgaatttatgat tgctgcgggtttcgactacaaaatgtctgtcgaaaaagcttac tccacggactccaacatgcttggtgcaacgcatgaagcgaagg atctggaatacctcaactccagcgtcaaaatcgtcaacccaat tatgggcgtgaagttttgggatgagagcgtgaaaaatcccgga gaagaagtcacagtagctttgagcaaggatcccggtggcgc tgaacggtaaaacctttagcgacgacgtagaaatgatgctgga agctaaccgcacgccc
Constitutive argG (SEQ ID NO: 31)	ttgacggctagctcagtcctaggtacagtgcctagcACCCGTTTT TTTGGGCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATA CATACCCatgacgacgattctcaagcatctcccggtagggtcaa cgtattggtatcgctttttccggcgggtctggacaccagtgcgc

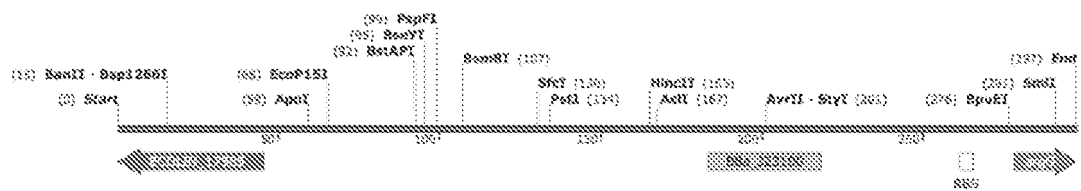
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	caatgctgtggatgcgacaaaagggagcggttcottatgcata
	tactgcaaacctgggccagccagacgaagaggattatgatgcg
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	tgatcgactgcgcgcaaacaaactgggtggccgaaggattatgcgcg
	tattoagtgtggcgcaatttcataaacaccactgggtggactgacc
	tatttcaacacgacgcgcgctggggccgcgccgtgaccggcacca
	tgctgggttgctgctatgaaagaagatggcgtgaatatctgggg
	tgacggcagcacctataaaggaaacgatatcgaaacgtttctac
	cgttacggctctgctgaccaatgctgaactgcagatttacaaac
	cgtggcttgatactgactttattgatgaactgggtggccgctca
	tgagatgtctgaatttatgattgcctgcggtttcgactacaaa
	atgtctgtcgaaaaagcttactccacggactccaacatgcttg
	gtgcaacgcatgaagcgaaggatctggaatacctcaactccag
	c

FIG. 15 continued

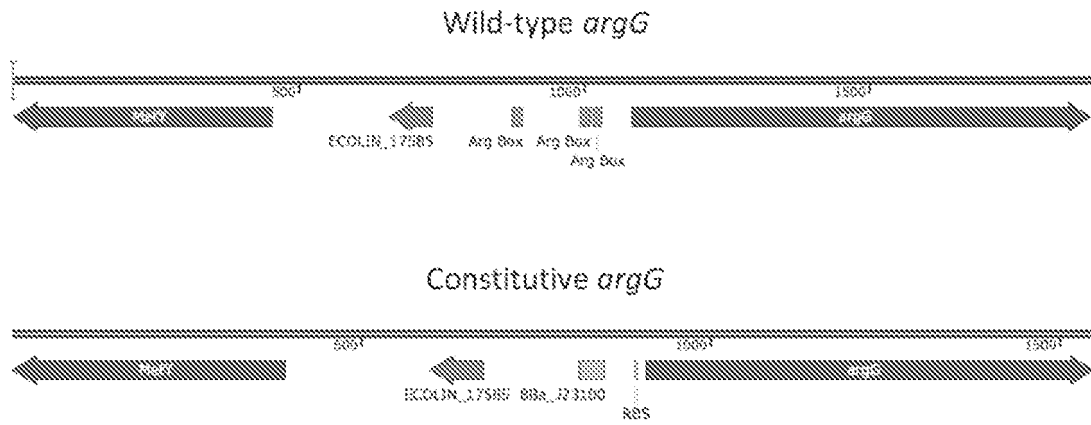
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FIG. 16



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FIG. 17



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FIG. 18

Nucleotide sequence of exemplary BAD promoter-driven *argA^{fbr}*
(SEQ ID NO: 33)

cgacggtggcgatagggcatccgggtggtgctcaaaagcagcttcgcctgactgatgc
gctggtcctcgcgccagcttaatacgttaactccctaactgctggcggaacaaatgcg
acagacgcgacggcgacagggcagacatgctgtgcgacgctggcgatatcaaaattac
tgtctgccaggtgatcgctgatgtactgacaagcctcgcgtagccgattatccatcg
gtggatggagcgactcggttaatcgcttccatgcgcccgcagtaacaattgctcaagca
gatttatcgccagcaattccgaatagcgcccttccccttgctccggcattaatgattt
gcccacacaggtcgctgaaatgcggctggtgcttccatccgggcgaaagaaaccgg
tattggcaaatatcgacggccagtttaagccattcatgccagtaggcgcgcggacgaa
agtaaaccacactggtgataccattcgctgagcctccggatgacgacgtagtgatgaa
tctctccagggcggaacagcaaaaatatcaccggctcggcagacaaattctcgctcct
gatttttaccacccccctgaccgcgaatggtgagattgagaatataacctttcattc
ccagcggctcggtcgataaaaaaatcgagataaccgttggcctcaatcggcggttaaac
ccgccaccagatgggcggttaaacgagtatcccggcagcaggggatcattttgcgctt
cagccatacttttcatactcccgcattcagagaagaaaccaattgtccatattgca
tcagacattgcogtcaactgcgtcttttactggctcttctcgctaaccocaaccggtaa
ccccgcttattaaaagcattctgttaacaaagcgggaccaaagccatgacaaaaacgc
gtaacaaaagtgtctataatcacggcagaaaagtcacattgattatttgacggcg
tcacacttttctatgccatagcatttttatccataagattagcggatccagcctgac
gctttttttcgcaactctctactgtttctccatacccggttttttggatggagtga
acg[ATGGTAAAGGAACGTAAAACCGAGTTGGTCGAGGGATTCCGCCATTCGGTTCCT
TGTATCAATAACCCACCGGGGAAAAACGTTTGTTCATCATGCTCGGCGGTGAAGCCATT
GAGCATGAGAATTTCTCCAGTATCGTTAATGATATCGGGTTGTTGCACAGCCTCGGC
ATCCGTCTGGTGGTGGTCTATGGCGCACGTCCGCAGATCGACGCAAATCTGGCTGCG
CATCACCACGAACCGCTGTATCACAAGAATATACGTGTGACCGACGCCAAAACACTG
GAACTGGTGAAGCAGGCTGCGGGAACATTGCAACTGGATATTACTGCTCGCCTGTCTG
ATGAGTCTCAATAACACGCCGCTGCAGGGCGCGCATATCAACGTCGTCAGTGGCAAT
TTTATTATTGCCAGCCGCTGGGCGTCGATGACGGCGTGGATTACTGCCATAGCGGG
CGTATCCGGCGGATTGATGAAGACGCGATCCATCGTCAACTGGACAGCGGTGCAATA
GTGCTAATGGGGCCGGTCGCTGTTTCAGTCACTGGCGAGAGCTTTAACCTGACCTCG

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GAAGAGATTGCCACTCAACTGGCCATCAAAGCTGAAAGCTGAAAAGATGATTGGTTTT
 TGCTCTTCCCAGGGCGTCACTAATGACGACGGTGATATTGTCTCCGAACTTTTCCCT
 AACGAAGCGCAAGCGCGGGTAGAAGCCCAGGAAGAGAAAGGCGATTACAACCTCCGGT
 ACGGTGCGCTTTTTGCGTGGCGCAGTGAAAGCCTGCCGCAGCGGCGTGCGTCGCTGT
 CATTTAATCAGTTATCAGGAAGATGGCGCGCTGTTGCAAGAGTTGTTCTCACGCGAC
 GGTATCGGTACGCAGATTGTGATGGAAAGCGCCGAGCAGATTTCGTGCGCAACAATC
 AACGATATTGGCGGTATTCTGGAGTTGATTGCGCCACTGGAGCAGCAAGGTATTCTG
 GTACGCCGTTCTCGCGAGCAGCTGGAGATGGAAATCGACAAATTCACCATTATTCTAG
 CGCGATAACACGACTATTGCCTGCGCCGCGCTCTATCCGTTCCCGGAAGAGAAGATT
 GGGGAAATGGCCTGTGTGGCAGTTCACCCGGATTACCGCAGTTCATCAAGGGGTGAA
 GTTCTGCTGGAACGCATTGCCGCTCAGGCTAAGCAGAGCGGCTTAAGCAAATTGTTT
 GTGCTGACCACGCGCAGTATTCAGTGGTTCAGGAACGTGGATTTACCCAGTGGAT
 ATTGATTTACTGCCCGAGAGCAAAAAGCAGTTGTACAATACCAGCGTAAATCCAAA
 GTGTTGATGGCGGATTTAGGGTAAATGGGAATTAGCCATGGTCCATATGAATATCCTC
 CTTAGTTCCTATTCC gaagttcctattccgaagttcctattctctagaaagtatag
 gaacttc GAAGCAGCTCCAGCCTACACAATCGCTCAAGACGTGTAATGCTGCAATC
 TGCATGCAAGCTTGGCACTGGCCACGCAAAAAGGCCATCGCTCAGGATGGCCTTCTG
 CTTAATTTGATGCCTGGCAGTTTATGGCGGGCGTCTGCCCCGCCACCCTCCGGGCCG
 TTGCTTCGCAACGTTCAAATCCGCTCCCGGCCGATTGTGCTTACTCAGGAGAGCGTT
 CACCGACAAACACAGATAAAACGAAAGGCCCGAGTCTTTCGACTCAGCCTTTCGTTT
 TATTTGATGCCTGGCAGTTCCTACTCTCGCATGctcgagccatgggacgtc**caggt**
 attagaagccaacctggcgctgcaaaaacacaacctggtcacgctcacctggggcaa
 tgtcagcgccggttgatcgcgggcgcggtcctggtgatcaaaccttcggcgctcga
 ctacagcatcatgaccgctgacgatatggtcgtggtcagcatcgaaaccggtgaagt
 ggttgaaggtagcaaaaagccctcctccgacacgccaactcaccgggtgctctatca
 ggcattcccgctctattggcggtcattgtgcacacacactcgcgccacgccaccatctg
 ggcgagggcgggccagtcgattccagcagccggcaccacccacgcogactatttota
 cggcaccattccctgcacccgcaaatgaccgacgcagaaatcaacgggtgaatatga
 gtgggaaaccggtaacgtcatcgtagaaaccttcgaaaaacagggtatcaatgcagc
 gcaaatgcccggcggtgctggtccattctcacggcccatttgcatggggaaaaaacgc
 cgaagatgcggtgcataacgccatcggtgctggaagaagtcgcttatatggggatatt

FIG. 18 continued

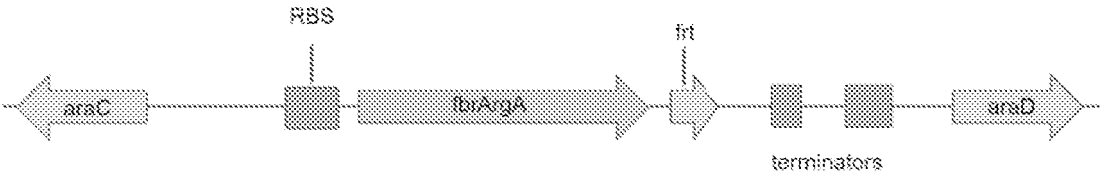
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ctgccgtcagttagcgccgcagttaccggatatgcagcaaacgctgctggataaaca  
ctatctgcgtaagcatggcgcggaaggcatattacgggcagtaa
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FIG. 18 continued

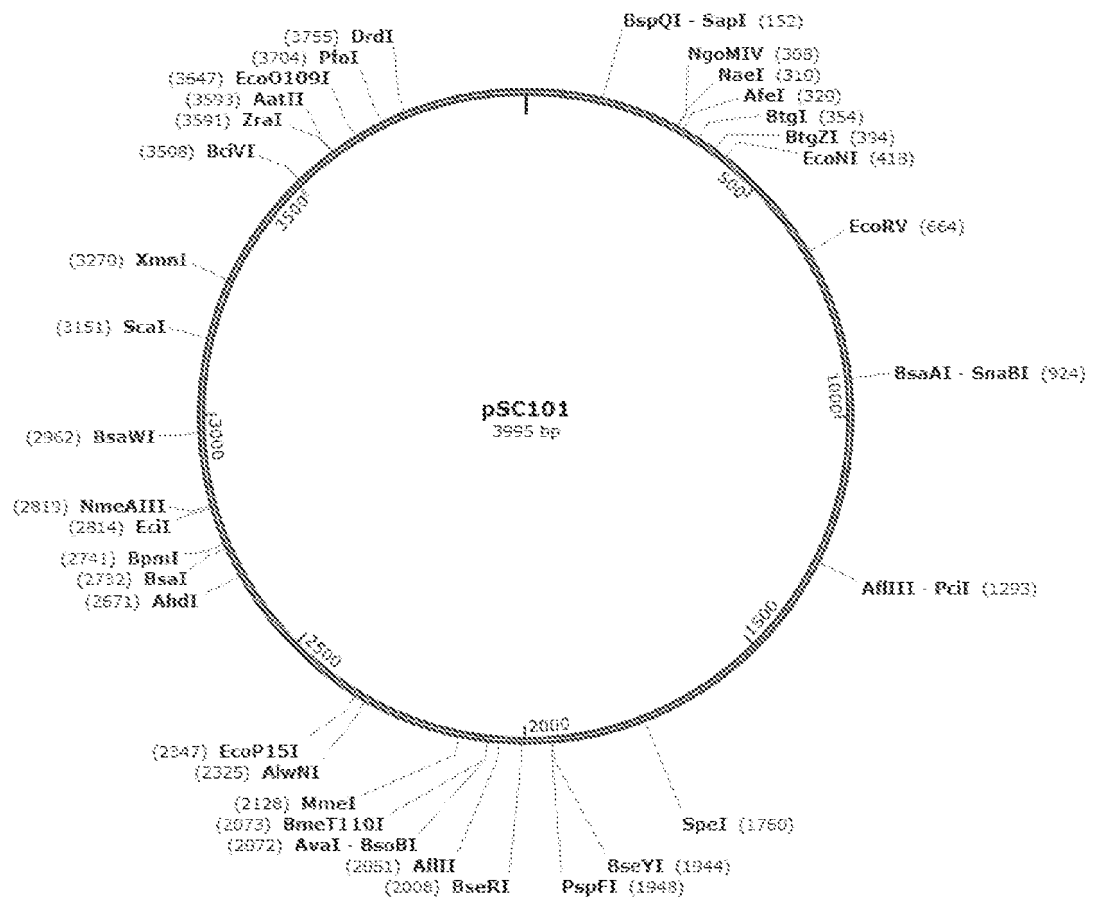
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FIG. 19



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FIG. 20



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FIG. 21A

pSC101 plasmid (SEQ ID NO: 34)
ATTAAGTTGGGTAACGCCAGGGTTTTTCCAGTCACGACGTTATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGTTGCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAGTACGGGTTTTGCTGCCCGCAAACGGGCTGTTCTGGTGTGCTAGTTTGTATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAGCGCTATTTCTCCAGAATTGCCATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGTTGTGCGGCAGCTTTGATTCGATAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTTCATGTTCTAGTTGCTTTGTTTACTGGTTTTACCTGTTCTATTAGGTGTTACATGCTGTTTCATCTGTTACATTGTGCTGCTGTTTCATGGTGAACAGCTTTAAATGCACCAAAAAGCTCGTAAAAGCTCTGATGTATCTATCTTTTTCACCGTTTTTCATCTGTGCATATGGACAGTTTTTCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTTTTTGTTTGTAGTCTTGATGCTTCAGTATAGATACAAGAGCCATAAGAACCCTCAGATCCTTCCGTATTTAGCCAGTATGTTCTCTAGTGTGGTTCGTTGTTTTTGGCTGAGCCATGAGAACGAACCATTGAGATCATGCTTACTTTGCATGTCACTCAAAAATTTTGCCTCAAAAAGTGAGCTGAATTTTTTGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTGATGTAATGGTTGTTGGTATTTTGTCAACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTACGAGATCCATTTGTCTATCTAGTTCAACTTGGAATCAACGTATCAGTCGGCGGGCTCGCTTATCAACCACCAATTTTCATATTGCTGTAAAGTGTATAAATCTTTACTTATTGGTTTCAAAACCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAACATGAACTTAAATTCATCAAGGCTAATCTCTATATTTGCCTTGAGTGTTCCTTTTGTGTTAGTTCCTTTAATAAACCCTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAACATGTTCCAGATTATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAACTAATCTAATTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAATCTCAAAGCCTTTAACCAAAGGATTCCTGATTTCCACAGTTCCTCGTCATCAGCTCTCTGGTTGCTTTAGCTAATACACCATAAGCATTTTCCCTACTGATGTTTCATCATCTGAGCGTATTGGTTATAAGTGAACGATACCGTCCGTTCTTTTCTTGTAGGGTTTTTCAATCGTGGGGTTGAGTAGTGCCACACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTTCATTTGCTTTGAAACAATAAATTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTATACCAATTGAGATGGGCTAGTCAATGATAATTACTAGTCCTTTTCCCTTTGAGTTGTGGGTATCTGTAAATCTGCTAGACCTTTGCTGGAAAACCTTGTAATTTCTGCTAGACCTCTGTAAATTTCCGCTAGACCTTTGTGTGTTTTTTTTTGTATTATTTCAAGTGGTTATAATTTATAGAATAAAGAAAGAATAAATAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTCACTACTTTAGTCACTTCCGCACTATTACAAAAGGATGTGCAAAACGCTGTTTGTCTCTACAAAACAGACCTTAAACCCCTAAAGGCTTAAGTAGCACCCCTCGCAAGCTCGGGCAATCGCTGAATATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTTCTGTGACATTCAGTTCGCTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGATTTCATGCAAGGAACTACCCATAATACAAGAAAAGCCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTTTGTGTTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCAATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTTACCCGTTCTTACTGTCTTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTTGGTTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCAGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA

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CAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
TCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCCTCC
GATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATA
ATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAG
TCATTCTGAGAAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAA
TACCGCGCCACATAGCAGAACTTTAAAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAA
AACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCAAC
TGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAA
TGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATT
TAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTA
AGAAACCATTTATTATCATGACATTAACTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC
TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACA
GCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGG
CGGGTGTGGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATA
TGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCCATTGCGCA
TTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCT
GGCGAAAGGGGGATGTGCTGCAAGGCG

FIG. 21A continued

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FIG. 21B

Nucleotide sequence of exemplary <i>fnrS</i> promoter-driven <i>argA</i> ^{EBF} pSC101 plasmid (SEQ ID NO: 35)	
ggtacc	AGTTGTTCTTATTGGTGGTGGTGGCTTTATGGTTGCATCGTAGTAAATGGTTGTAAC
AAAAGCAATTTTTCGGCTGTCTGTATACAAAAACGCCGCAAAGTTTGAGCGAAGTCAATAA	
ACTCTCTACCCATTTCAGGGCAATATCTCTCTT	ggatccaaagtgaactctagaaataatttt
gtttaactttaagaaggagatatatacat	ATGGTAAAGGAACGTAAAACCGAGTTGGTTCGAGGG
ATTCCGCCATTTCGGTTCCCTGTATCAATACCCACCGGGGAAAAACGTTTGTTCATCATGCTCG	
GCGGTGAAGCCATTGAGCATGAGAATTTCTCCAGTATCGTTAATGATATCGGGTTGTTGCAC	
AGCCTCGGCATCCGTCTGGTGGTGGTCTATGGCGCACGTCCGCAGATCGACGCAAATCTGGC	
TGCGCATCACCAAGAACCGCTGTATCACAAGAATATACGTGTGACCGACGCCAAAACTGG	
AACTGGTGAAGCAGGCTGCGGGAACATTGCAACTGGATATTACTGCTCGCCTGTGATGAGT	
CTCAATAACACGCCGCTGCAGGGCGCGCATATCAACGTCGTCAAGTGGCAATTTTATTATTGC	
CCAGCCGCTGGGCGTGCATGACGGCGTGGATTACTGCCATAGCGGGCGTATCCGGCGGATTG	
ATGAAGACGCGATCCATCGTCAACTGGACAGCGGTGCAATAGTGCTAATGGGGCCGGTCTGCT	
GTTTCAGTCACTGGCGAGAGCTTTAACCTGACCTCGGAAGAGATTGCCACTCAACTGGCCAT	
CAAAGTGAAGCTGAAAAGATGATTGGTTTTTGGTCTTCCAGGGCGTCACTAATGACGACG	
GTGATATTGTCTCCGAACCTTTTCCCTAACGAAGCGCAAGCGCGGGTAGAAGCCAGGAAGAG	
AAAGGCGATTACAACCTCCGGTACGGTGGCGCTTTTGGCGTGGCGCAGTGAAAGCCTGCCGCAG	
CGGCGTGGCTCGCTGTCAATTAATCAGTTATCAGGAAGATGGCGCGCTGTTGCAAGAGTTGT	
TCTCACGCGACGGTATCGGTACGCAGATTGTGATGGAAAGCGCCGAGCAGATTCTGTCGCGCA	
ACAATCAACGATATTGGCGGTATTCTGGAGTTGATTGCCCCACTGGAGCAGCAAGGTATTCT	
GGTACGCCGTTCTCGCGAGCAGCTGGAGATGGAAATCGACAAATTCACCATTATTCAGCGCG	
ATAACACGACTATTGCTGCGCGCGCTCTATCCGTTCCCGGAAGAGAAGATTGGGGAAATG	
GCCTGTGTGGCAGTTCACCCGGATTACCGCAGTTCATCAAGGGGTGAAGTTCTGCTGGAACG	
CATTGCCGCTCAGGCTAAGCAGAGCGGCTTAAGCAAATTTGTTTGTGCTGACCACGCGCAGTA	
TTCACTGGTTCCAGGAACGTGGATTTACCCAGTGGATATTGATTTACTGCCCGAGAGCAAA	
AAGCAGTTGTACAACCTACCAGCGTAAATCCAAAGTGTGATGGCGGATTTAGGGTAA	GGAAG
TTTGTCTAGATCTCAGGCGTGGATGGCTTGGCGTAATCATGGTCATAGCTGTTTCTCTGTGTG	
AAATTGTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCT	
GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCCAG	
TCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTT	
CGGTATTGGGCGCTCTTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGGCTGC	
GGCGAGCGGTATCAGCTCACTCAAAGGCGGTAGTACGGGTTTTGCTGCCCCGAAACGGGCTG	
TTCTGGTGTGCTAGTTTGTATCAGAATCGCAGATCCGGCTTCAGGTTTGCCGGCTGAAAG	
CGCTATTTCTTCCAGAATTGCCATGATTTTTTCCCCACGGGAGGCGTCACTGGCTCCCGTGT	
TGTCGGCAGCTTTGATTCGATAAGCAGCATCGCCTGTTTCAGGCTGTCTATGTGTGACTGTT	
GAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTTCATGTTCTAGTTGCTTTGTTTTACTGGTT	
TCACCTGTTCTATTAGGTGTTACATGCTGTTTCATCTGTTACATTGTCGATCTGTTTCATGGTG	
AACAGCTTTAAATGCACCAAAAACCTCGTAAAGCTCTGATGTATCTATCTTTTTTACACCGT	
TTTCATCTGTGCATATGGACAGTTTTCCCTTTGATATCTAACGGTGAACAGTTGTTCTACTT	
TTGTTTGTAGTCTTGATGCTTCACTGATAGATACAAGAGCCATAAGAACCTCAGATCCTTC	
CGTATTTAGCCAGTATGTTCTCTAGTGTGGTTGTTGTTTTGCGTGAGCCATGAGAACGAA	
CCATTGAGATCATGCTTACTTTGCATGTCACTCAAAAATTTTGCCTCAAACTGGTGAGCTG	
AATTTTTGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTACGTAGGTAGGAATCTG	
ATGTAATGGTTGTTGGTATTTTGTACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTT	
ACGAGATCCATTTGTCTATCTAGTTCAACTTGGAAAATCAACGTATCAGTCGGGCGGCCCTCG	
CTTATCAACCACCAATTTTCATATTGCTGTAAGTGTTTAAATCTTTACTTATTGGTTTTCAAAA	

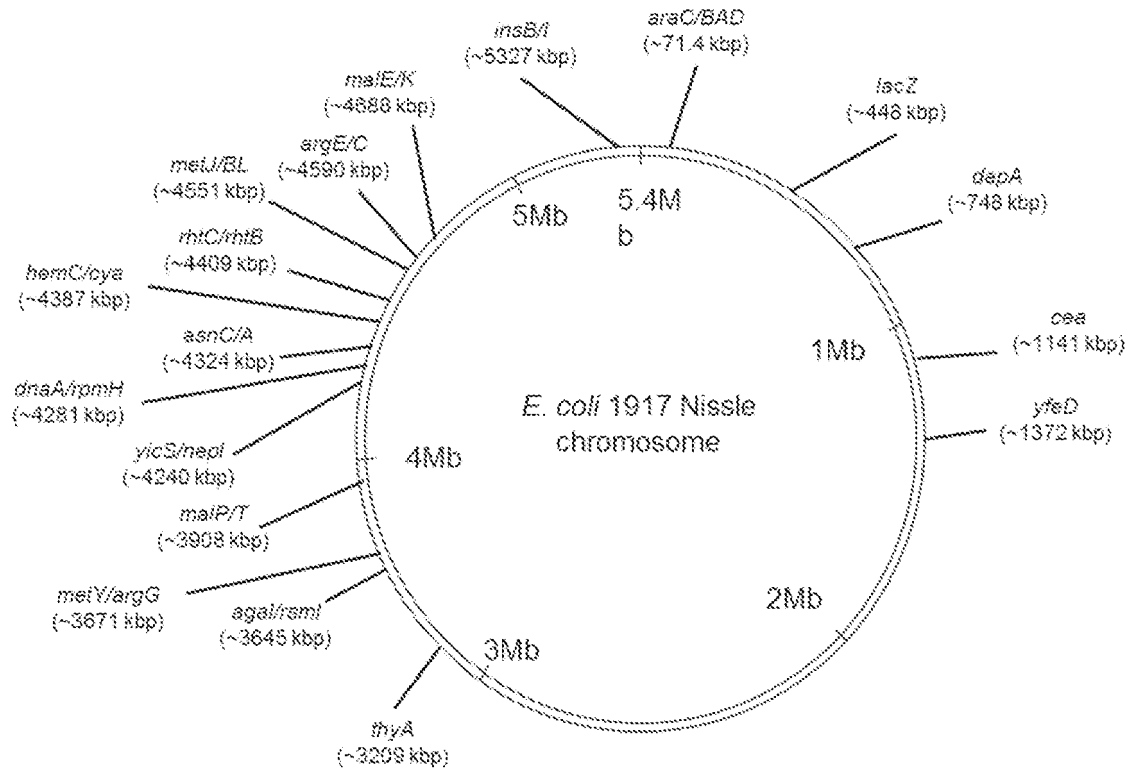
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CCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAAACATGAACTTAAAT
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CCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAAACATGTTCCAGATTATATT
TTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAACTAATTCTAAT
TTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAAATCTCAAAGCCTTTAAACCAAAGG
ATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTGCTTTAGCTAATACACCATAAG
CATTTTCCCTACTGATGTTTCATCATCTGAGCGTATTGGTTATAAGTGAACGATAACCGTCCGT
TCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCCACACAGCATAAAATTAGCTT
GGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTCATTTGCTTTGAAAACAATA
ATTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATCACTATAACCAATTGAGATGGGCT
AGTCAATGATAATTACTAGTCCTTTTCCCTTGTAGTTGTGGGTATCTGTAAATTCTGCTAGAC
CTTTGCTGGAAAACCTTGTAATTCTGCTAGACCCTCTGTAAATTCCGCTAGACCTTTGTGTG
TTTTTTTTTGTATTATATTCAAGTGGTTATAATTTATAGAATAAAGAAAGAATAAAAAAAGATA
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AAGGATGTCGCAAACGCTGTTTGCTCCTCTACAAAACAGACCTTAAACCCTAAAGGCTTAA
GTAGCACCCCTCGCAAGCTCGGGCAAATCGCTGAATATTCTTTTGTCTCCGACCATCAGGCA
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GGGGTAAATGGCACTACAGGCGCCTTTTATGGATTTCATGCAAGGAACTACCCATAATACAA
GAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTG
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CGTGACAGGTCAATCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTT
ACCCGTCTTACTGTCTTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGG
GATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAA
GTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATC
AGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCCTGACTCCCCGT
CGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGC
GAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAG
CGCAGAAGTGGTCTGCAACTTTATCCGCCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC
TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC GCAACGTTGTTGCCATTGCTACAGGCATCG
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GTTACATGATCCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATCGTTGT
CAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTA
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GAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCC
ACATAGCAGAACTTTAAAGTGCTCATCATTTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAA
GGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCA
GCATCTTTTACTTTTACCAGCGTTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAA
AAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTTCAATATTATT
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TATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTT
TCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTG
TAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCG
GGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTAAGTACTGAGAGTGCACCATATGCGGTGTG
AAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCCATTGCGCATTCAGGCTG
CGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGG
GGGATGTGCTGCAAGGCG

FIG. 21B continued

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FIG. 22

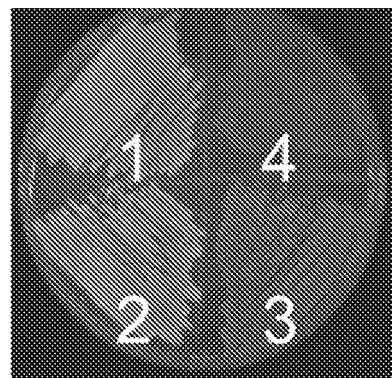


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FIG. 23

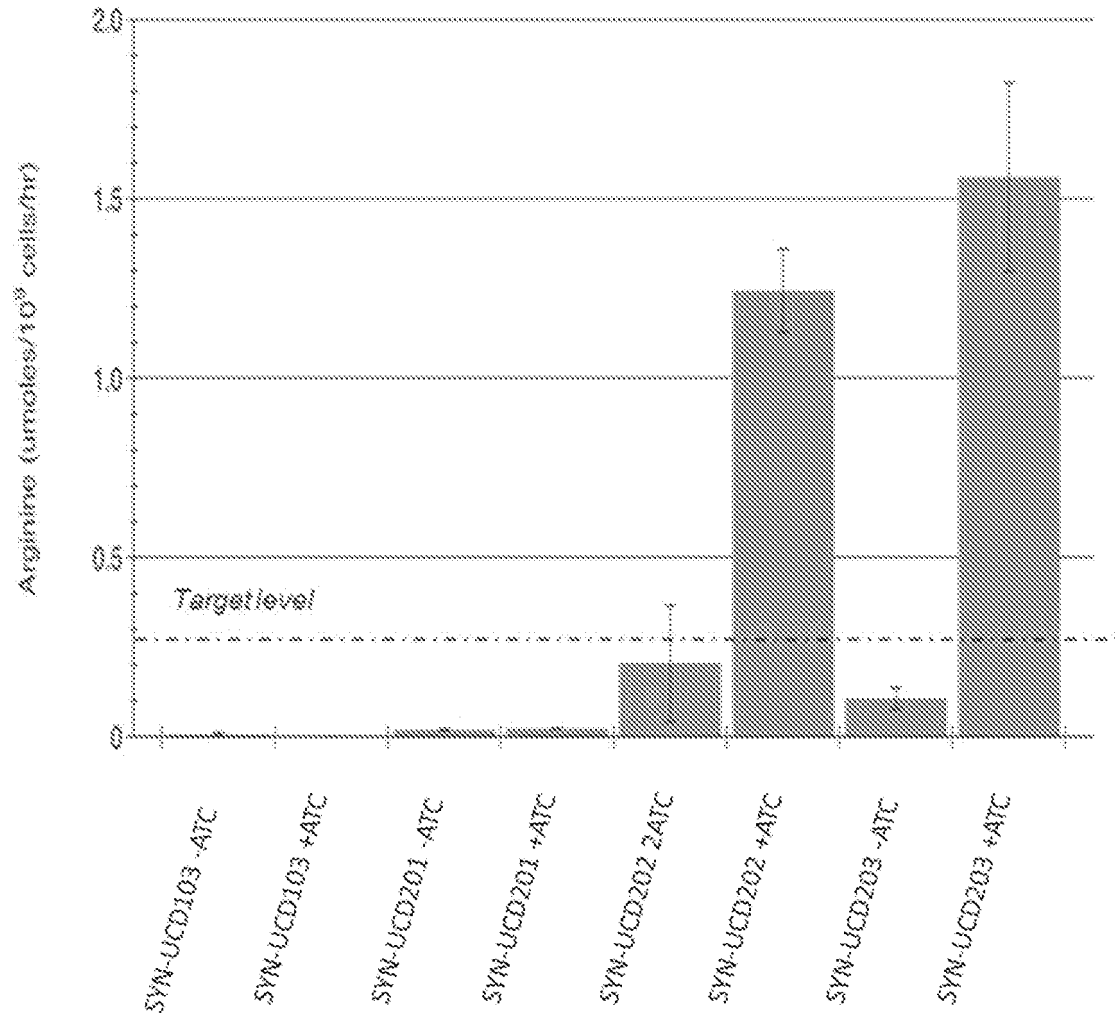
Brightness of constitutive RFP integrated in
three locations:

1. AraB/C
2. MalE/K
3. MetY/ArgG
4. Nissle (non-fluorescent)



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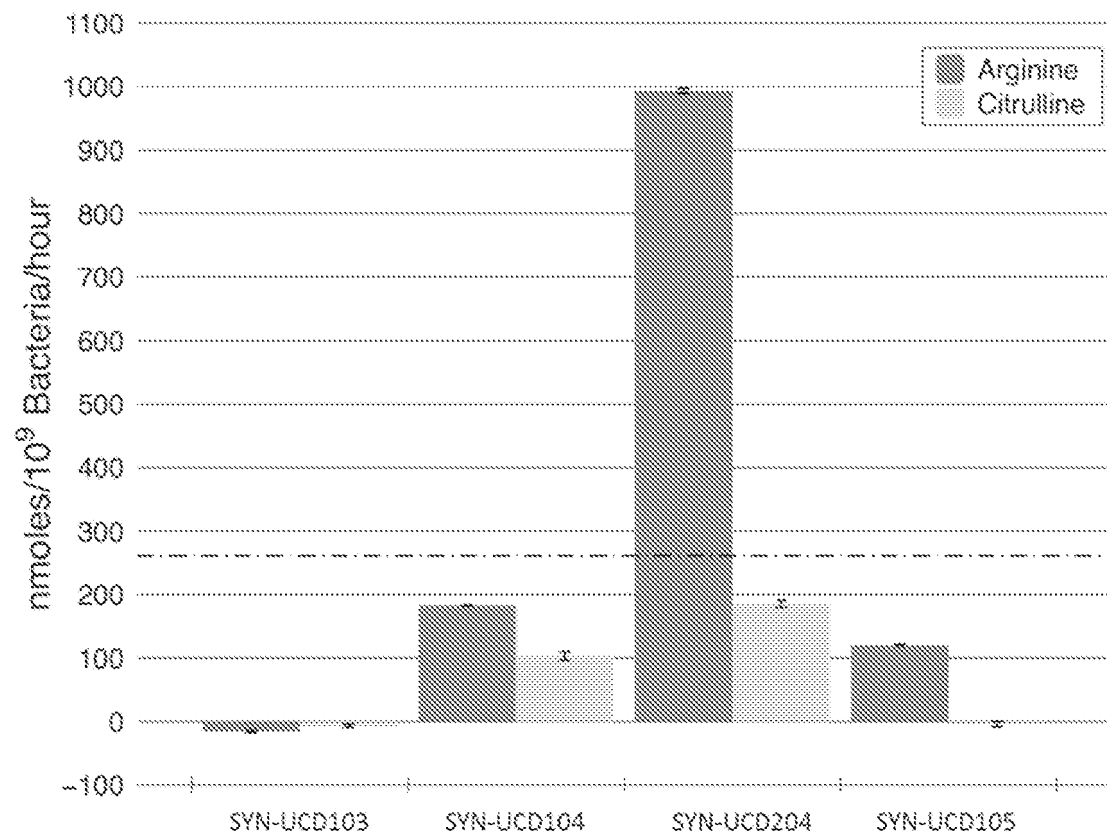
FIG. 24

**Day 5 blood ammonia (BA) comparisons**

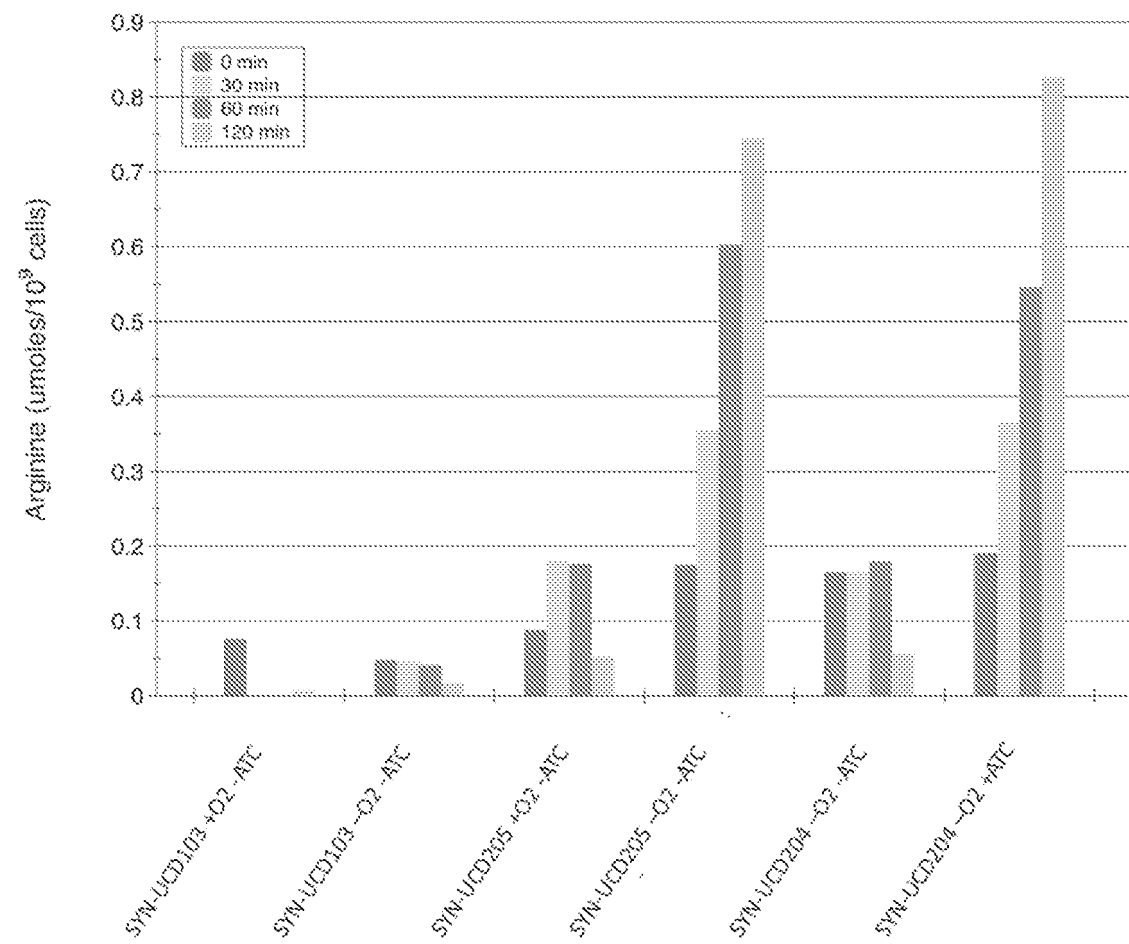
- Nissle - 220 mM BA
- Syn016 - 105 mM BA
- Difference = 115 mM
- Average mouse blood volume ~1.5 mL
- **SYN016 prevented absorption of 172.5 μ moles**

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FIG. 25

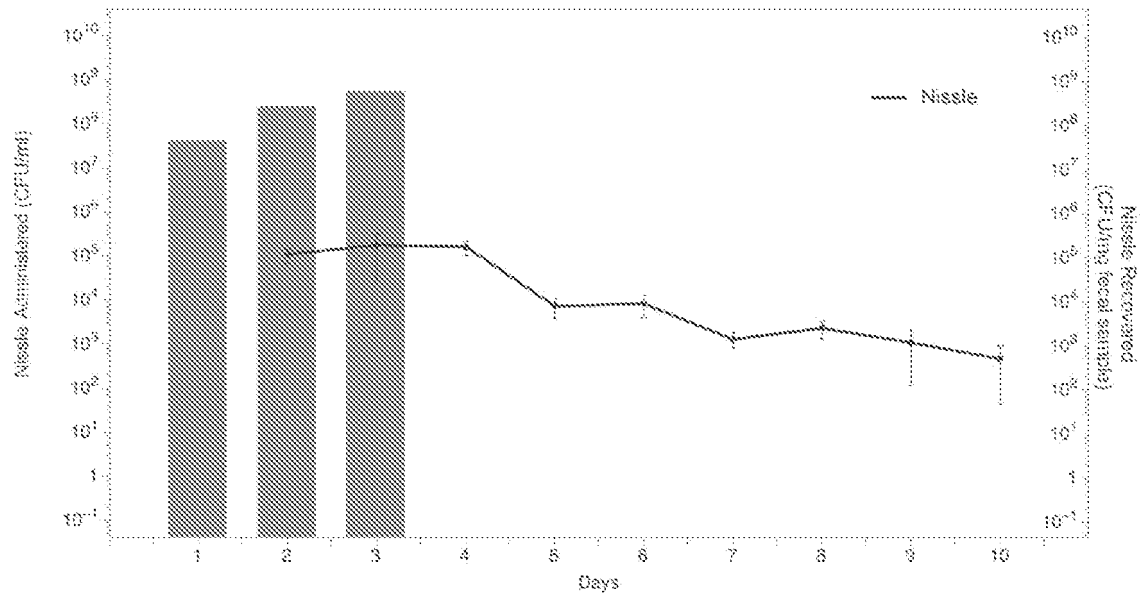


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FIG. 26



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FIG. 27



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FIG. 28A

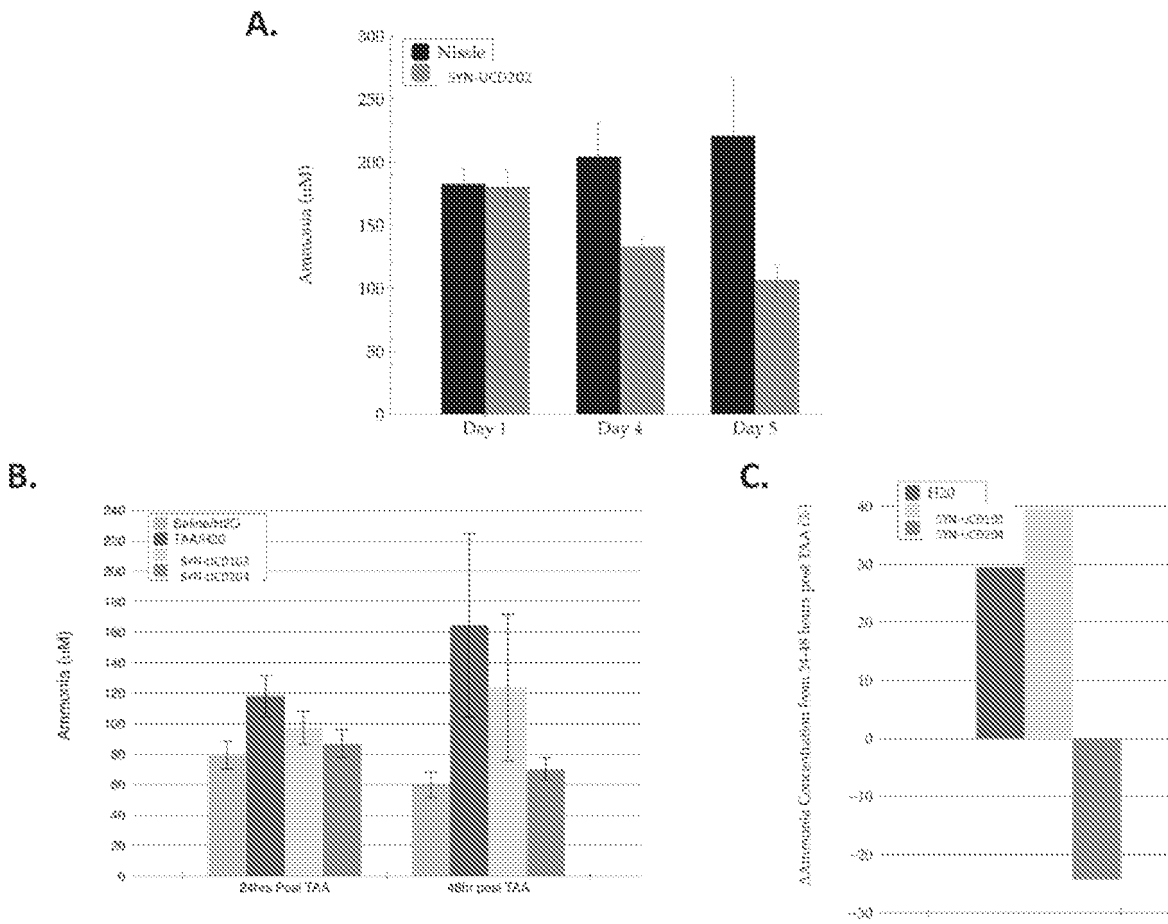
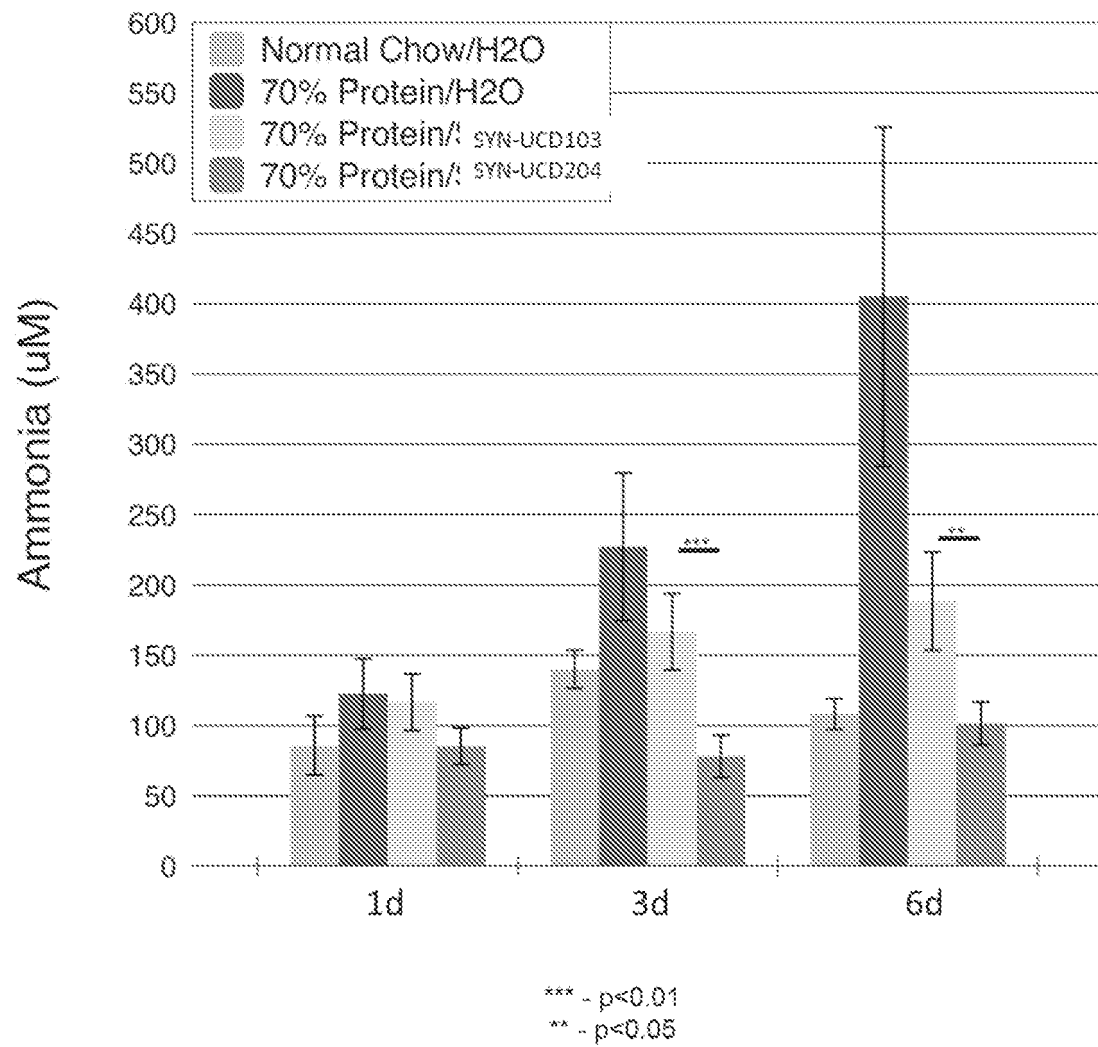


FIG. 28B

FIG. 28C

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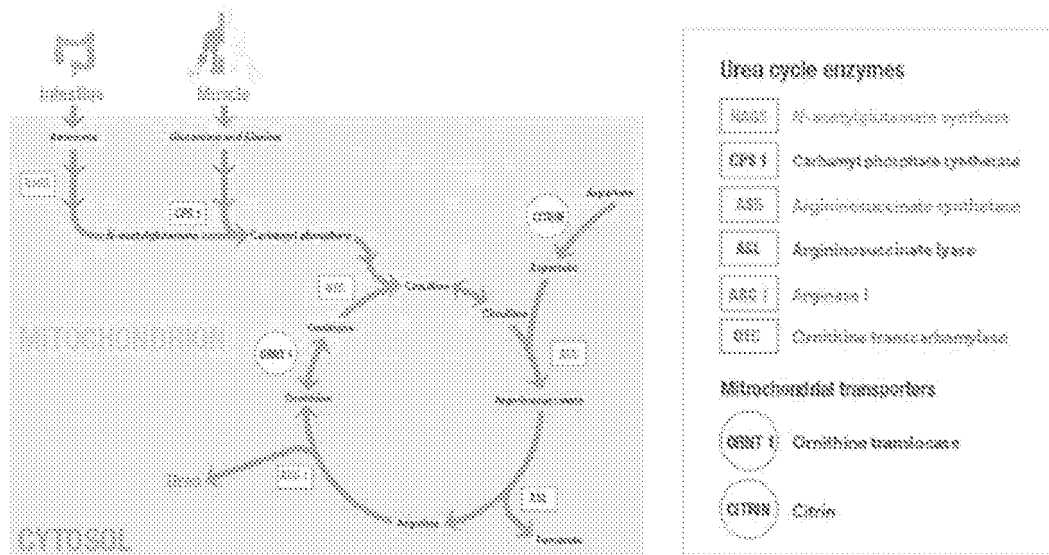
FIG. 29



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FIG. 30

- Urea Cycle Disorders (UCD) result in hyperammonemia and CNS pathology
- Large proportion of ammonia in UCD patients produced in gut (> 70%)
- Complementary, treatable hepatic encephalopathy population
- In United States:
 - Urea cycle disorders = 6,200 patients
 - Inborn errors of metabolism with hyperammonemia = 33,000 patients
 - Hepatic encephalopathy/cirrhosis = 917,800 patients



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FIG. 31

Parameter	Value
Maximum burden of NH_4^+ (in blood) in hyperammonemic patients (UCD<HE)	800 μmol s total excess NH_4^+ Total blood ammonia levels: ~1000 μmol s
Arginine production target: NH_4^+ burden	267 $\mu\text{mol/day}$ (1 Arg = 3 NH_4^+)
Target arginine production rate: NH_4^+ burden	267 $\mu\text{mol/day}/10^{11}$ bacteria
Lab assay target: NH_4^+ burden	0.11 $\mu\text{mol/hr}/10^9$ bacteria
Current arginine production rate	1.25 $\mu\text{mol/hr}/10^9$ bacteria
Maximum flux of NH_4^+ (in blood from colon); in healthy individuals>UCD	800 $\mu\text{mol/hr}$
Arginine production target: NH_4^+ flux	267 $\mu\text{mol/hr}$
Target arginine production rate: NH_4^+ flux	267 $\mu\text{mol/hr}/10^{11}$ bacteria
Lab assay target: NH_4^+ flux	2.67 $\mu\text{mol/hr}/10^9$ bacteria

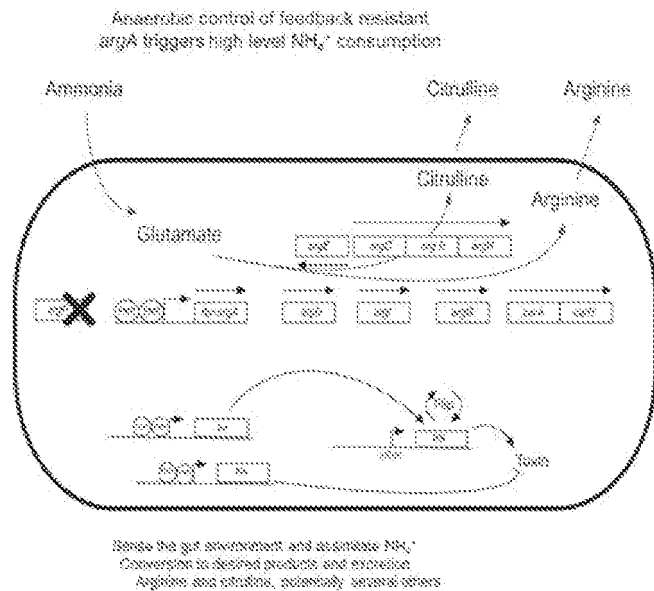
Total arginine production: 0.11 g/day if based on flux rate (267 $\mu\text{mol/day}/10^{11}$ bacteria), or 0.26 g/day if based on the maximum production rate of the strain 1.25 $\mu\text{mol/hr}/10^9$ bacteria

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Fig. 32

Synthetic Biotic Targeting UCD

- Engineered probiotic bacterial therapeutic to consume excess ammonia and produce beneficial byproducts to improve patient outcomes
 - Many UCD patients are also administered arginine or citrulline as adjunctive co-therapy with ammonia scavenging treatment.
 - Eliminates protein restriction
- Highly controllable kill switch and genomic auxotrophy to ensure safety



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Fig. 33A

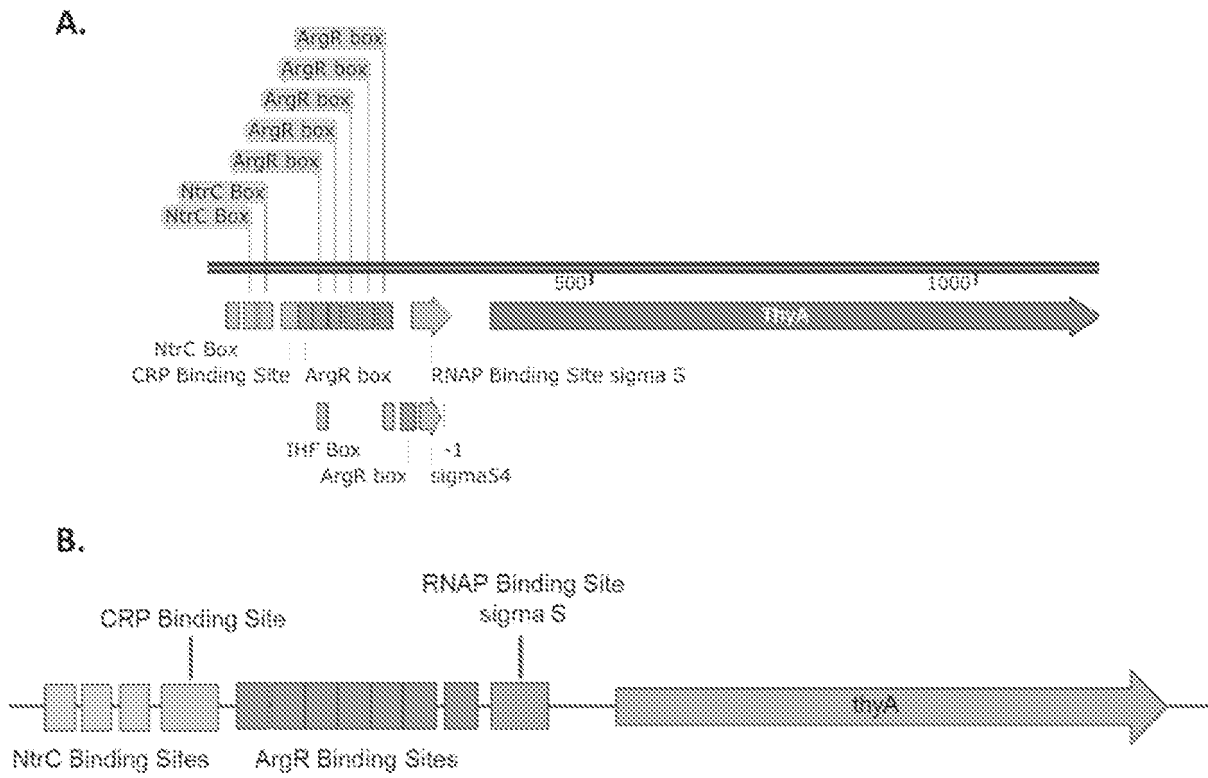


Fig. 33B

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Fig. 34

Amino Acid	Oligonucleotide	Cell wall
cysE	thyA	dapA
glnA	uraA	dapB
ilvD		dapD
leuB		dapE
lysA		dapF
serA		
metA		
glyA		
hisB		
ilvA		
pheA		
proA		
thrC		
trpC		
tyrA		

Fig. 35

Gene	argA	cysE	glnA	glyA	hisB	ilvA	leuB	lysA	metA
AA									
Auxotroph	Arginine	Cysteine	Glutamine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine
Pre-Gavage									
24 hours									
48 hours									

Gene	pheA	proA	serA	thrC	trpC	tyrA	ilvD	thyA	uraA	flhD
AA										
Auxotroph	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine/Isoleucine/Leucine	Thiamine	Uracil	FlhD
Pre-Gavage										
24 hours										
48 hours										



Fig. 36

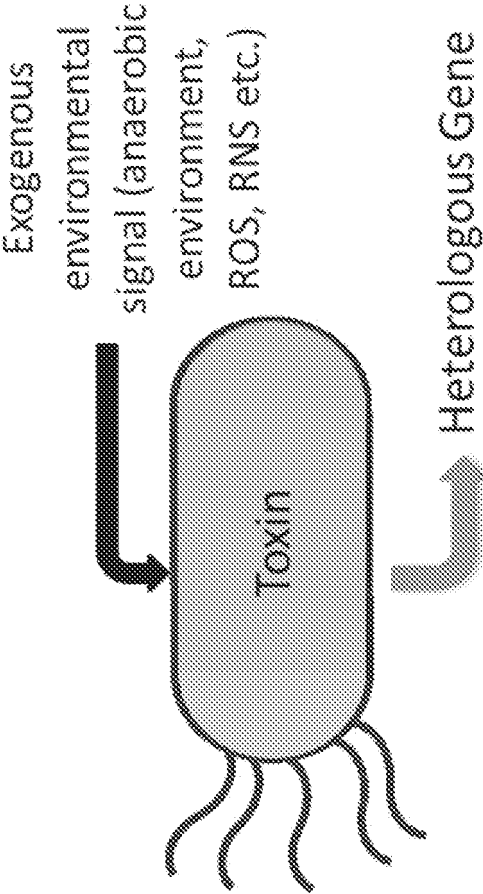
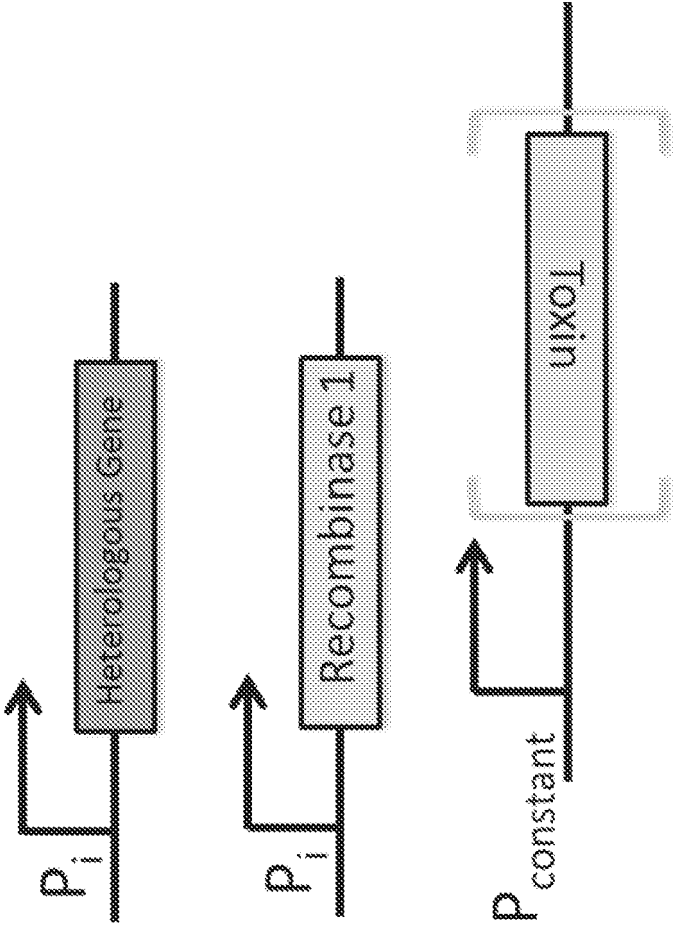


Fig. 37

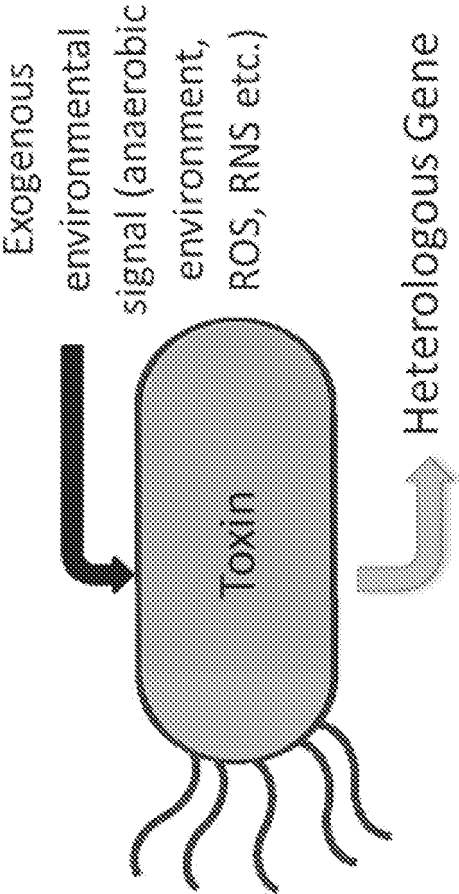
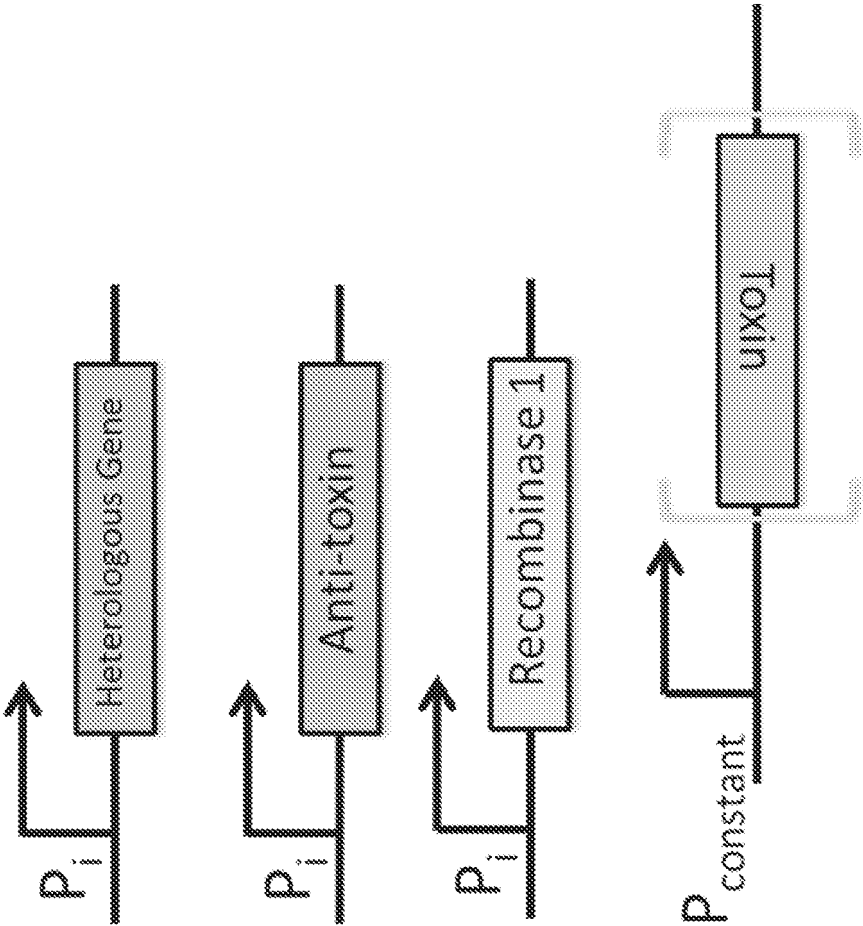


Fig. 38

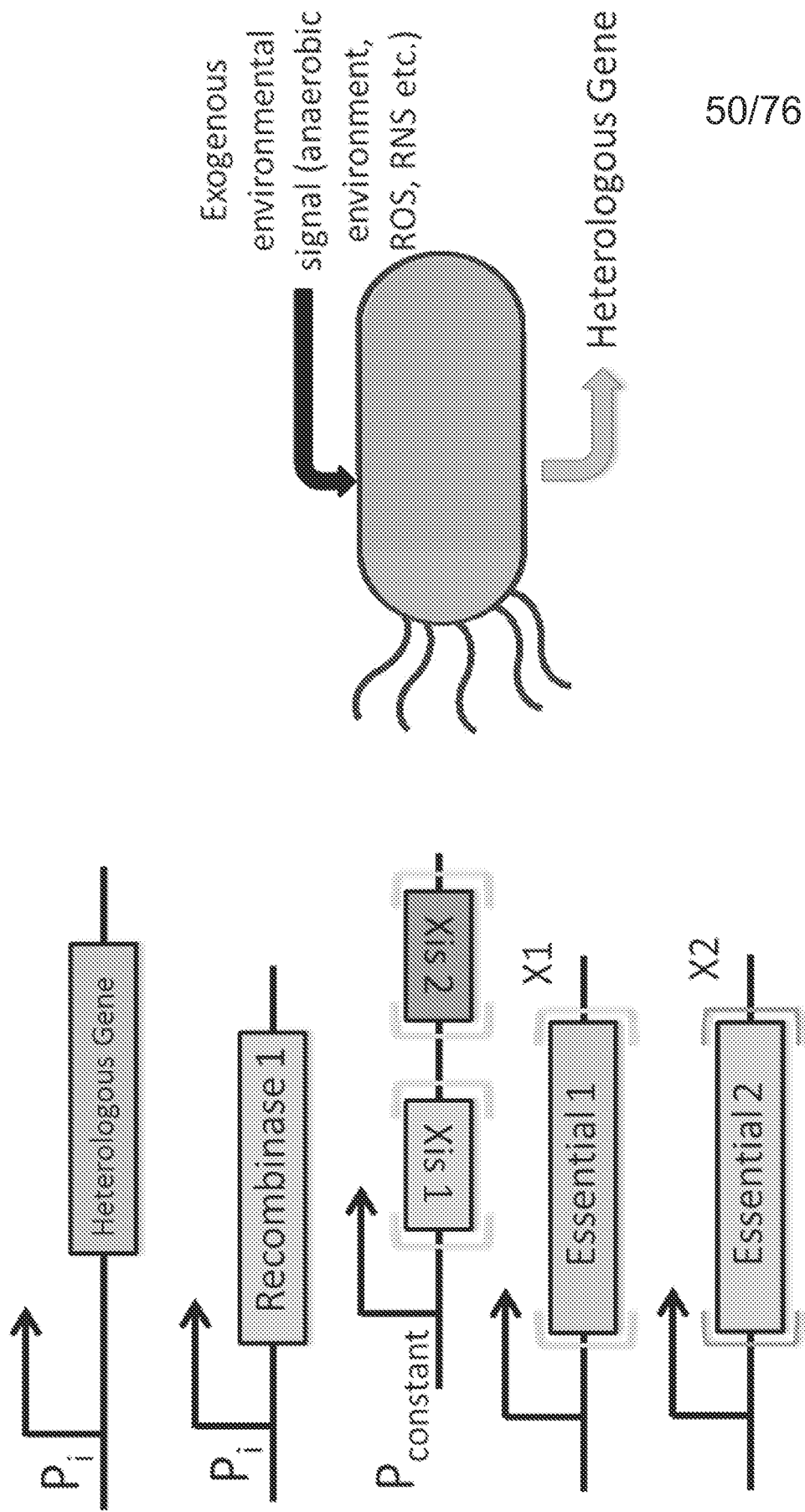


Fig. 39

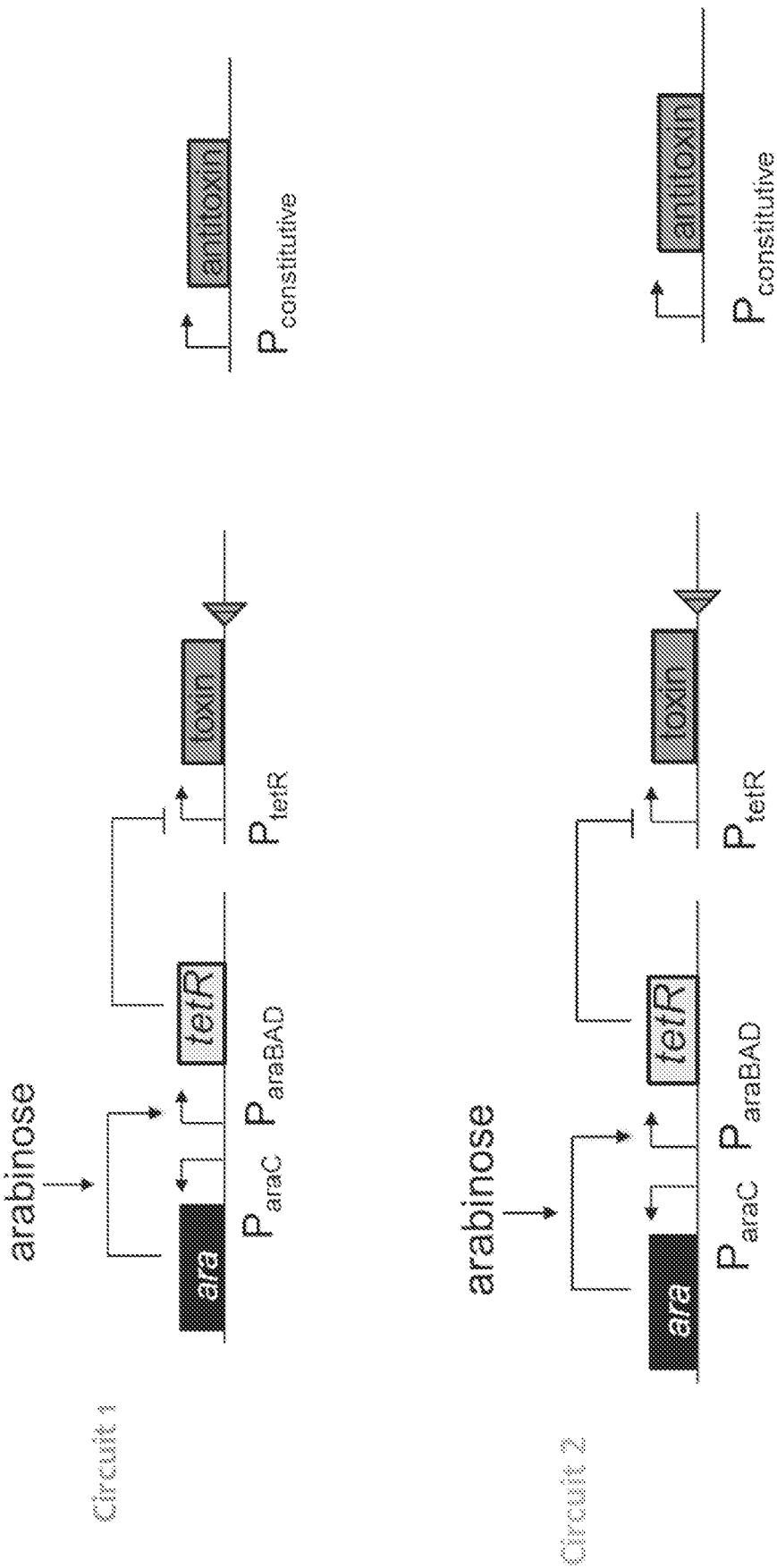


Fig. 40

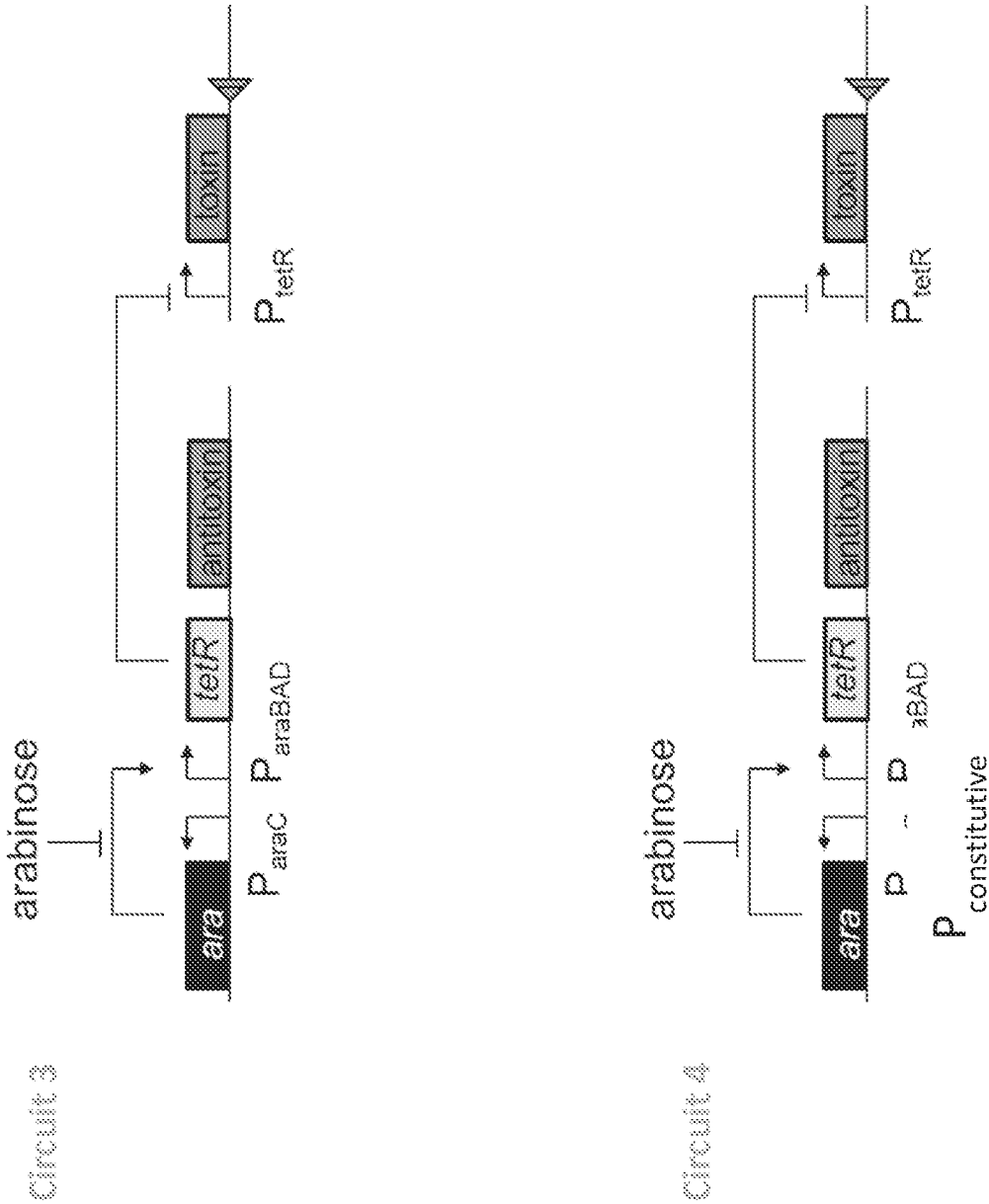


Fig. 41

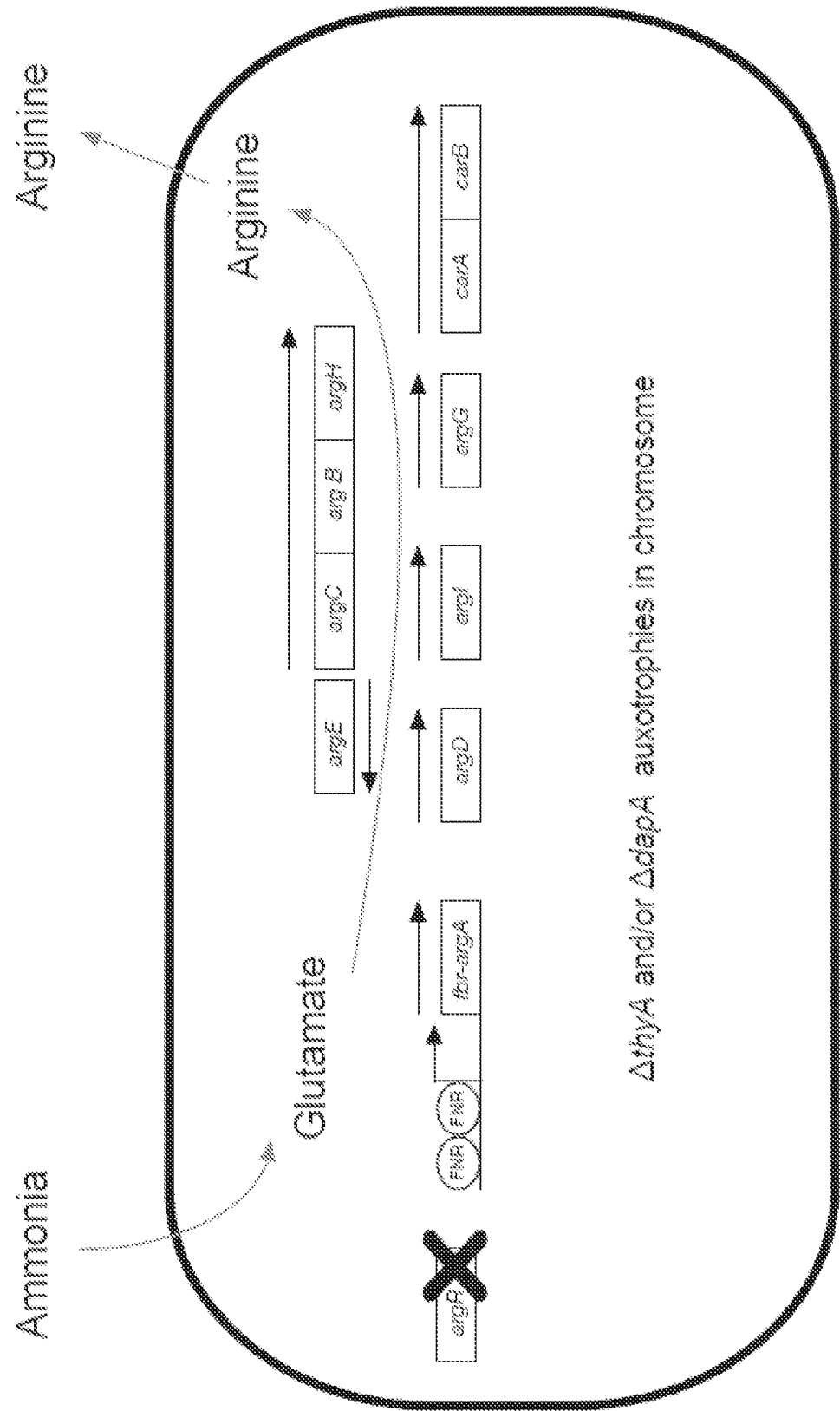


Fig. 42

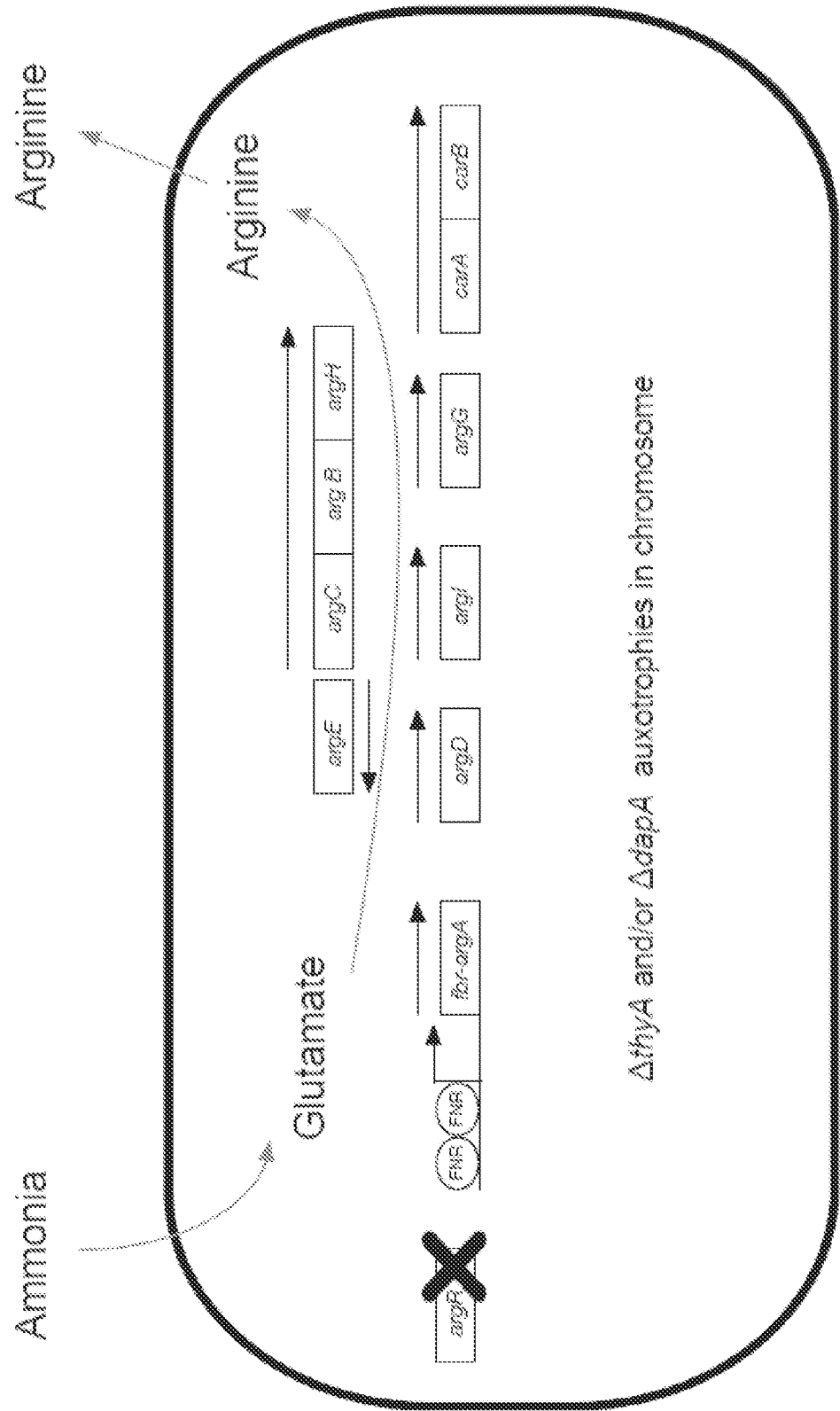


Fig. 44

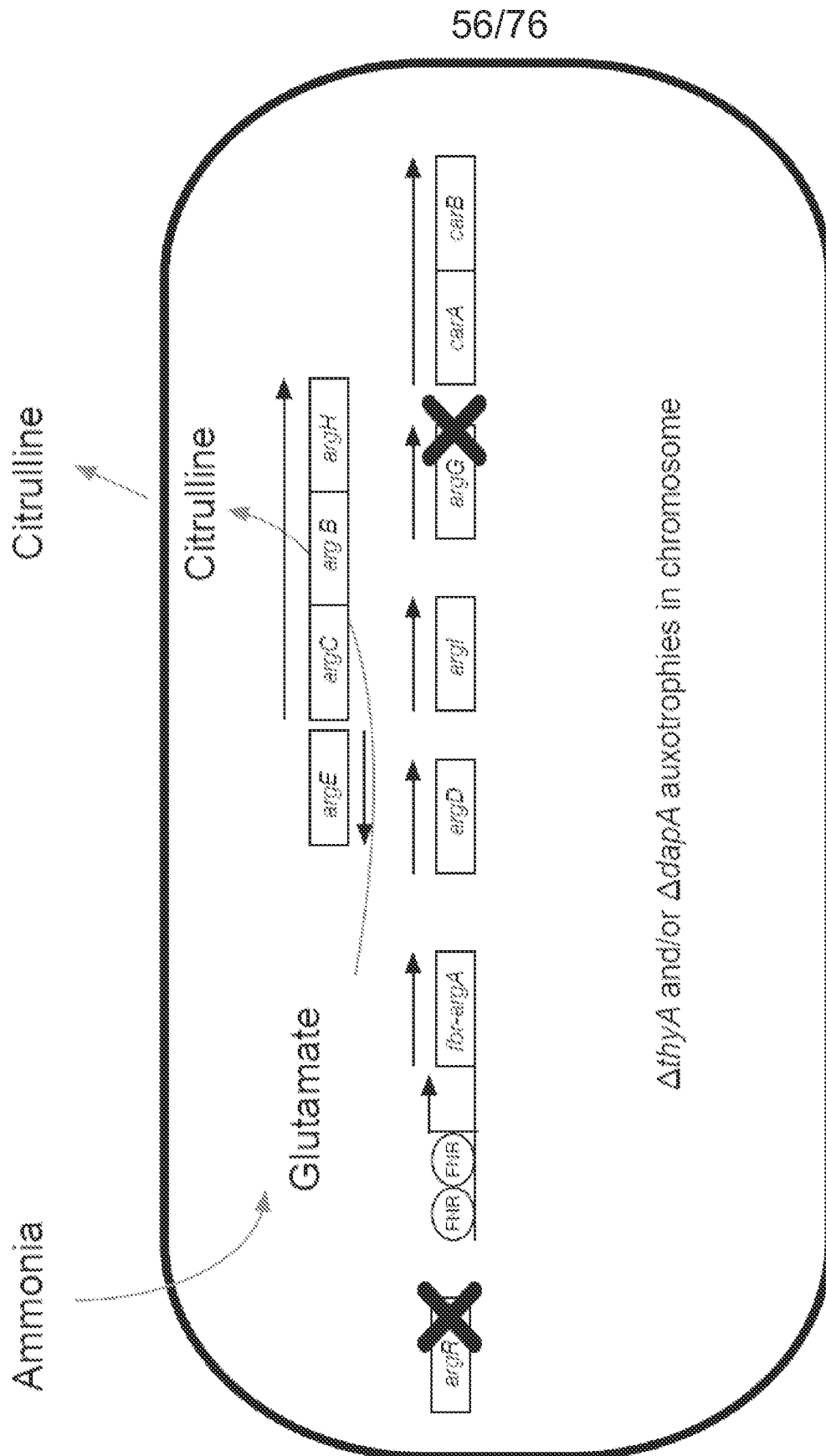
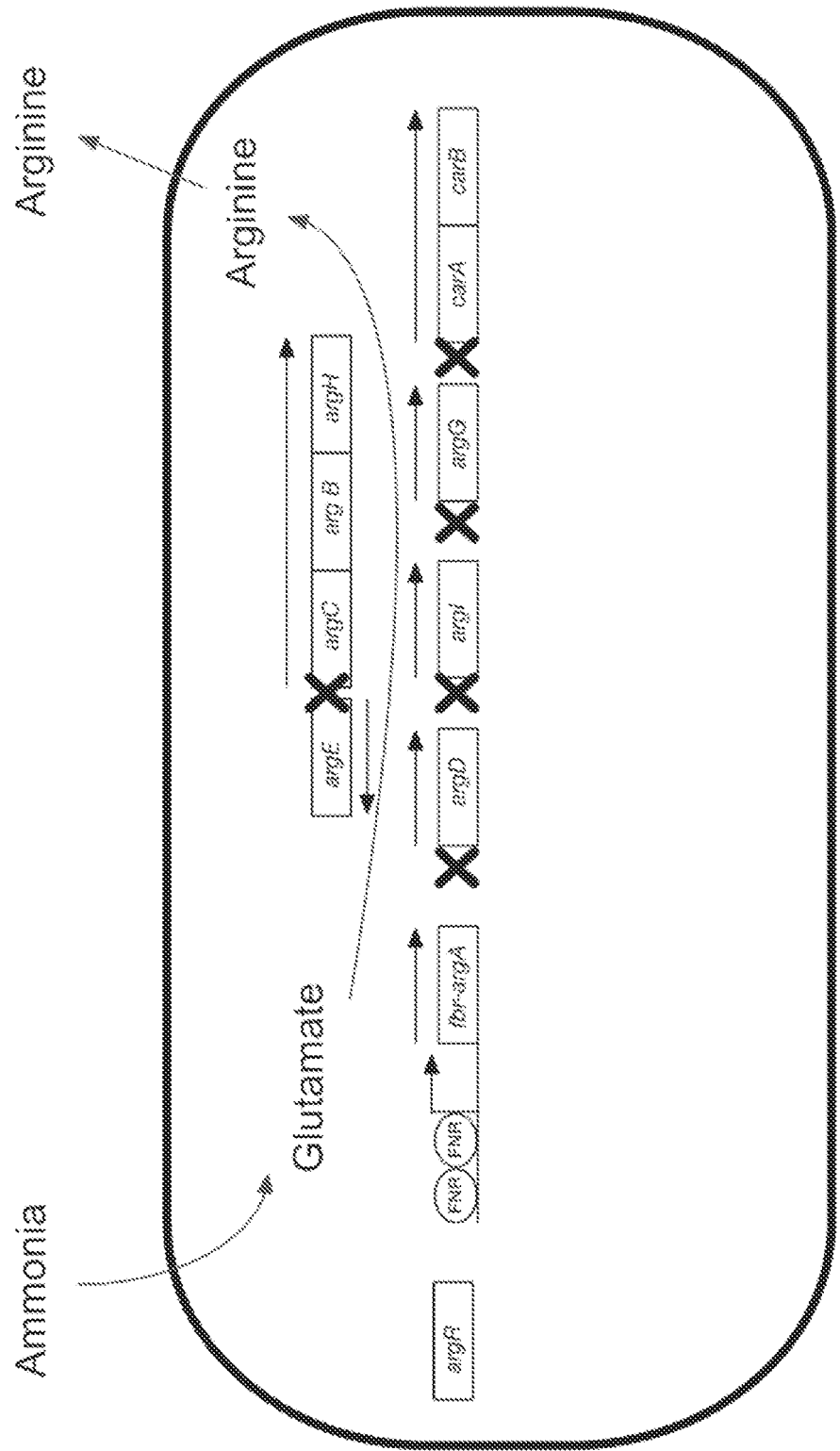


Fig. 45



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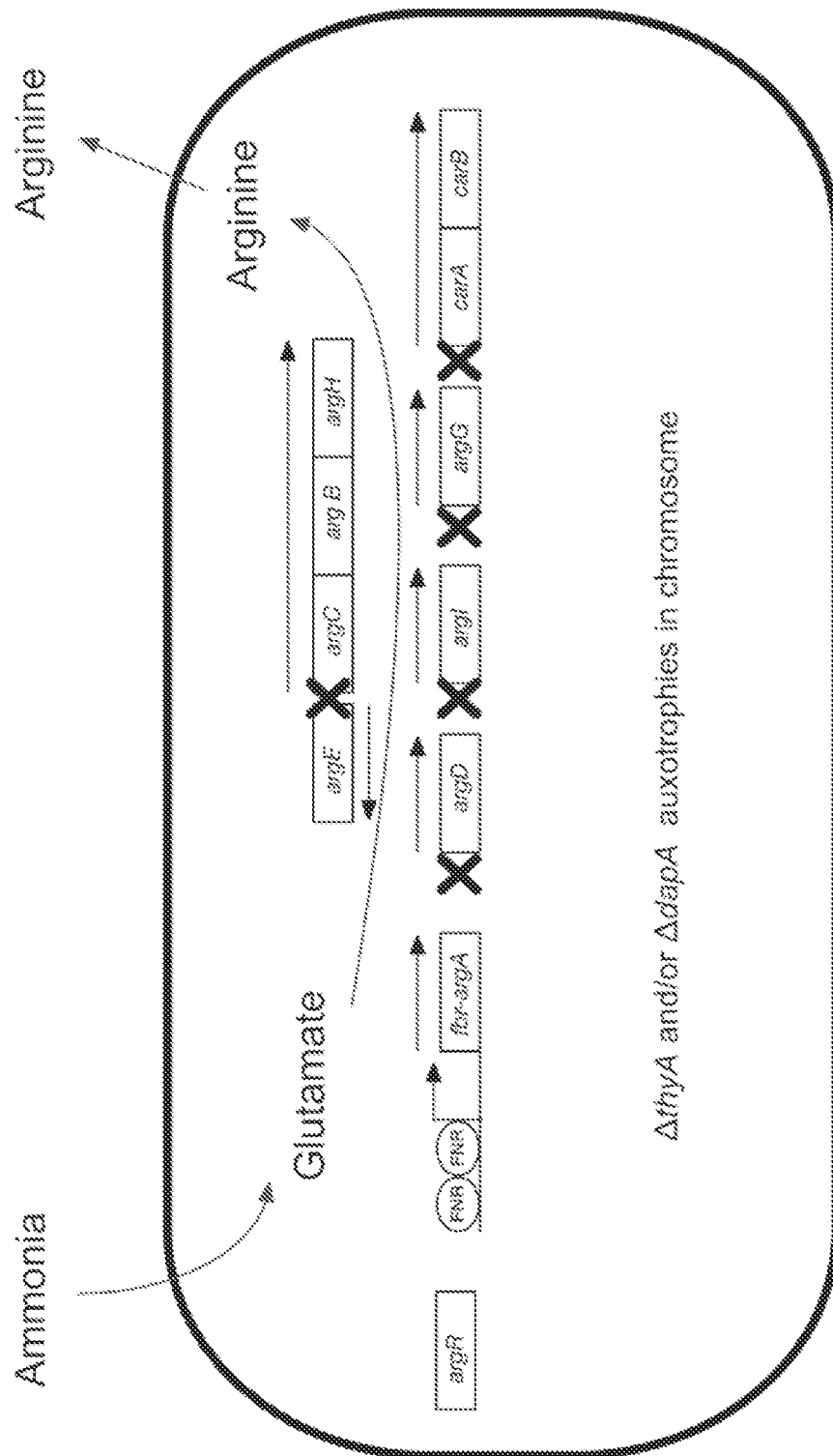
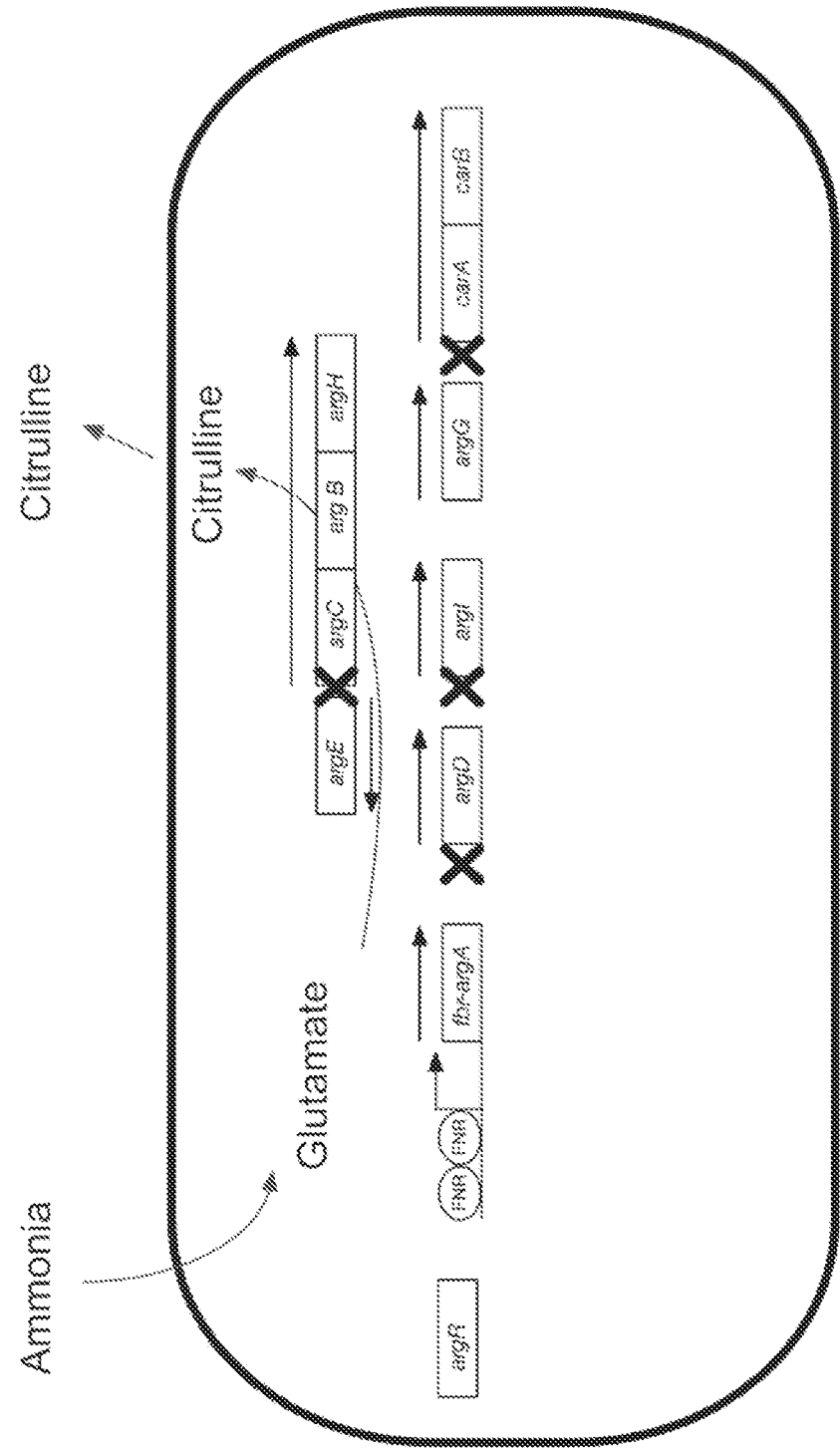


Fig. 47



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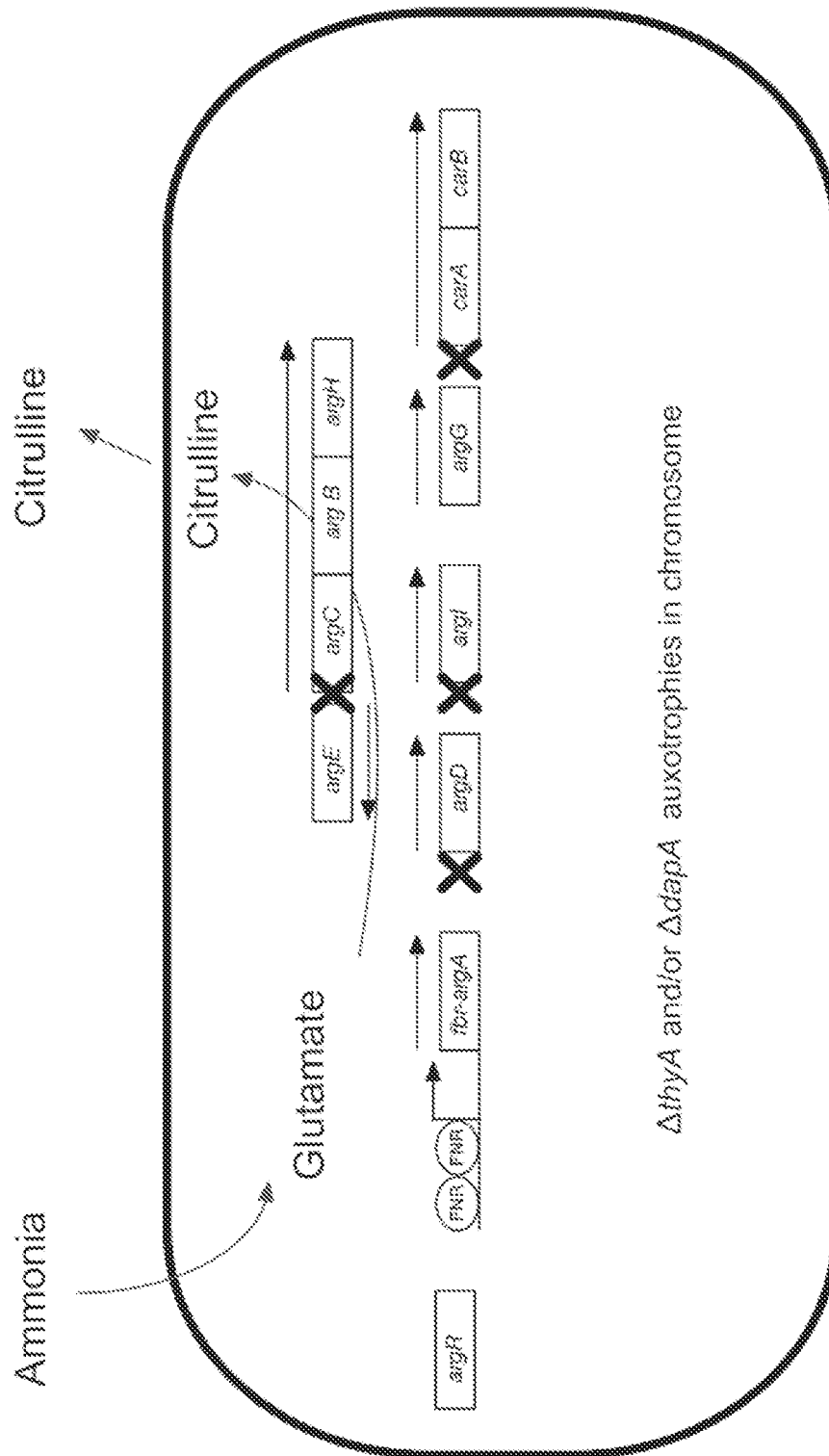


Fig. 49

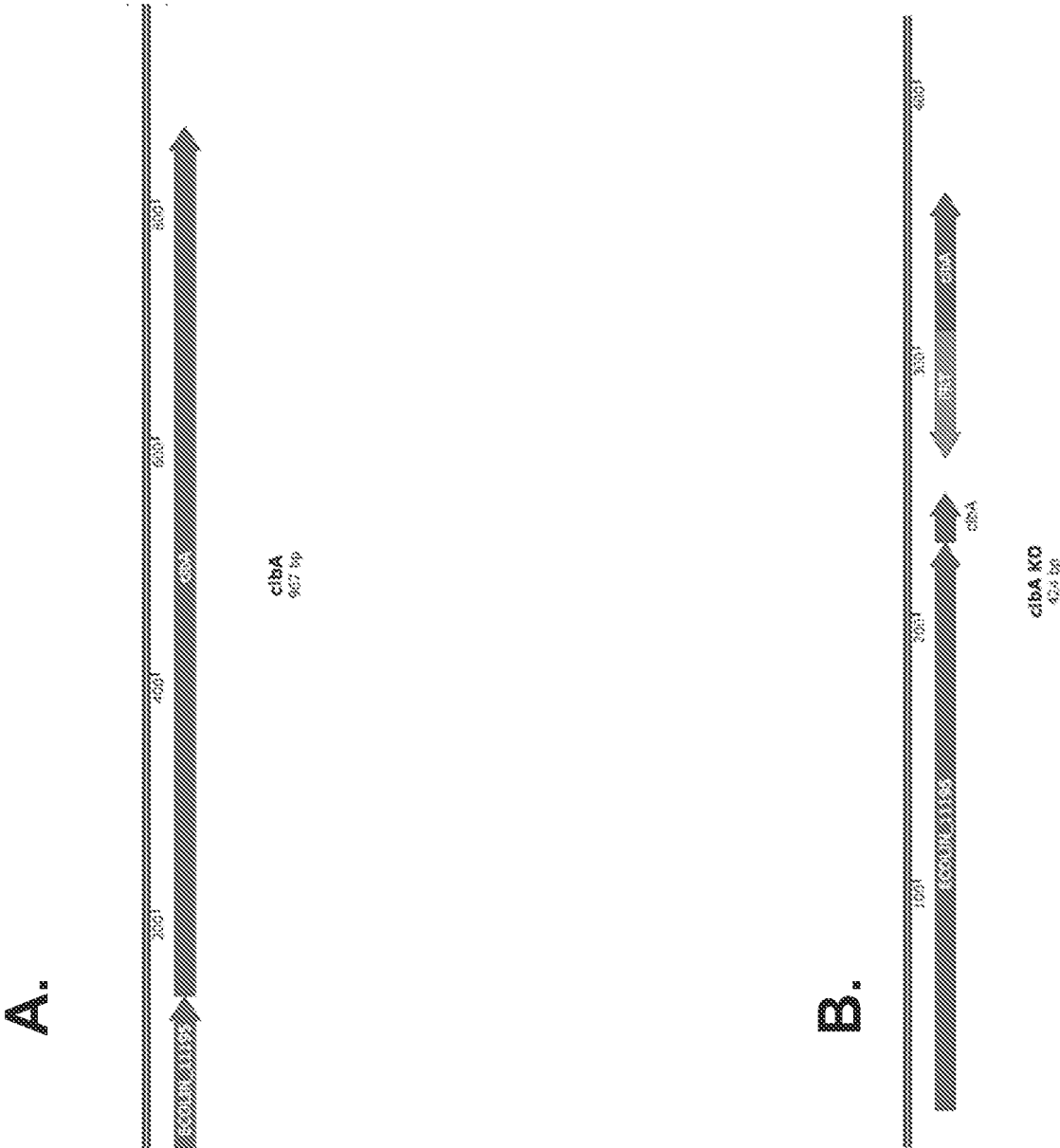
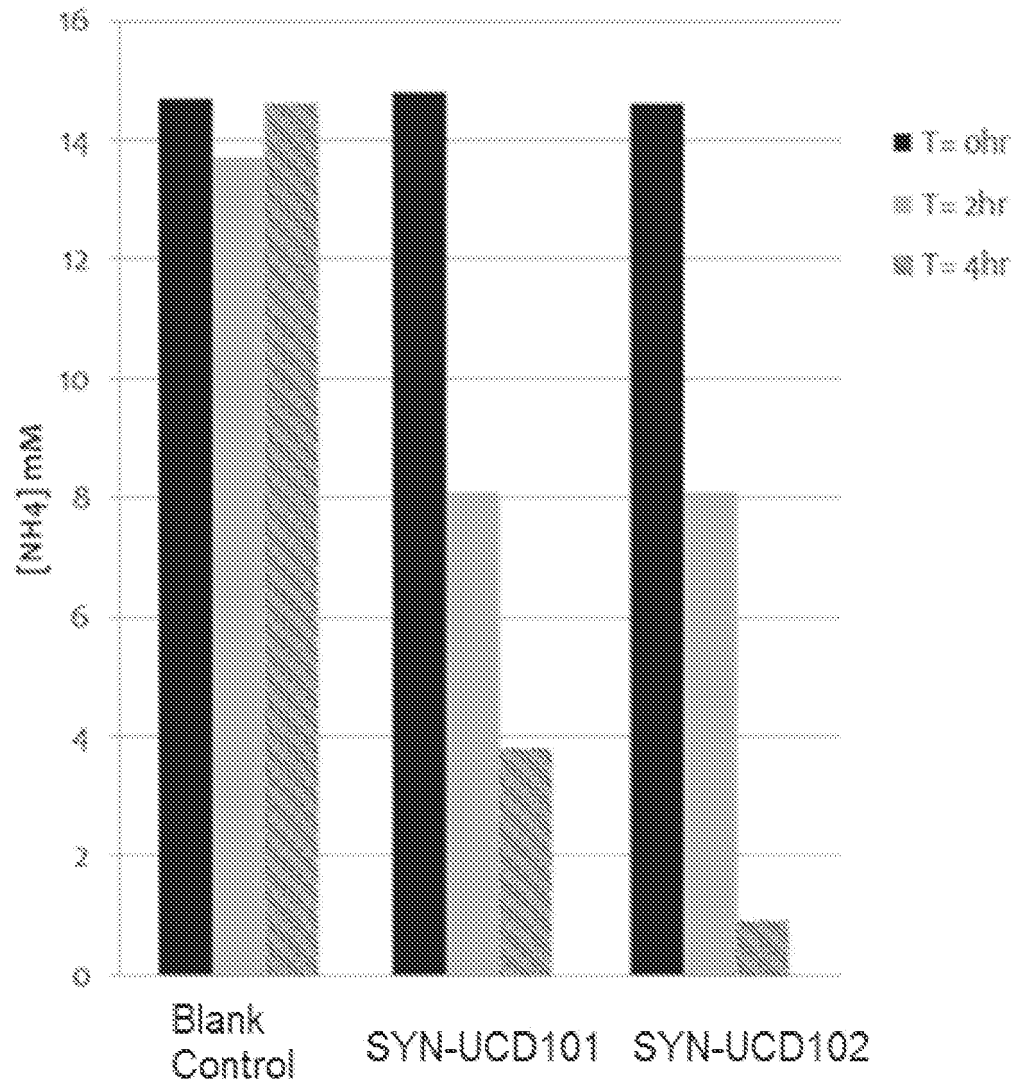


Fig. 50

Wild-type clbA (SEQ ID NO: 36)	clbA knockout (SEQ ID NO: 37)
caaatatcacataaatcttaacatatcaataaacacagctaaagtttcatgtgaaaaaacatcaaacataaaatacaagctcggaatacgaatcagctatacaacattgctaaacaggaatgagattatctaaatgaggattgatatataattaattggacatactagtgttttttcatcaaaccca gtagagataaacttccttcactatctcaatgaggagaataaaacgctatgatcagtttcattttgtgagtgaataaaagaactctatatatttaagccgtatcctgctcaaaaacagcactaaaaagatatcaacccgtgatgtctcattacaatcatggcaattttagtagcgtgcacaaatatgg caaacatttatagtttttccctcagttggcaaaaaagatttttttttaacccctttcccatactatagatcacagtagccgtttgctatttagttctcaactgcgagcttggtgtcgatatattgaacaaaataagagattttagacaaactcttatactgaatatcagtcagcaatttttttactccacag gaagctactaaacatagtttccacttccctcgttatgaaggctcaatttcttttggaaaaatgtggacgctcaaaagaagcttagcatcaaaata tgcagggtaaaggccctatcttttaggactggattgtatttgaatttcatttaacaaaataaaaaactaaactcaaaaatatagaggttcacctgt tttatttctctcaatggaaaaatatgtaactcattttctcgcattagcctctccactcatcccccctaaaaataaactatttgagctatttctct atgcagctcccaactttatcaccacgactatcagctaatctcattcgtcaaatgggcagaatttgaatcgccacggataatctagacacttc tgagccgtcgataaatattgatttttcataattccgtcgtgtgtaagtatcccgcataaatcgtgccattccacatttag	ggatggggggaacatgggataaagttcaaaagaaaaaaacccggttatctctgcgtgaaagacaaagtattgcgcattgctggcacaaggtgat gagtactctcaaaatatcacataaatctttaacatatcaataaaacacagtaaaagtttccatgtgaaaaaacatcaaacataaaaaatacaagctcg gaatacgaatcacgctatacacacattgctaaacagggaatgagattatctaaatgaggatttgaTGTGTAGGCTGGAGCTGCTTCGAAGTTCC TATACTTTCTAGAGAATAGGAAC TTCCGGAATAGGAAC TTAGGAATAGGAAC TTAGGAATAGGAATATTCATATGtcgtcaaatgggcagaattt gaatcgccacgggataatctagacacttctgagccgtcgataaatattgatttttcataattccgctcggtgg

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Fig. 51



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Fig. 52

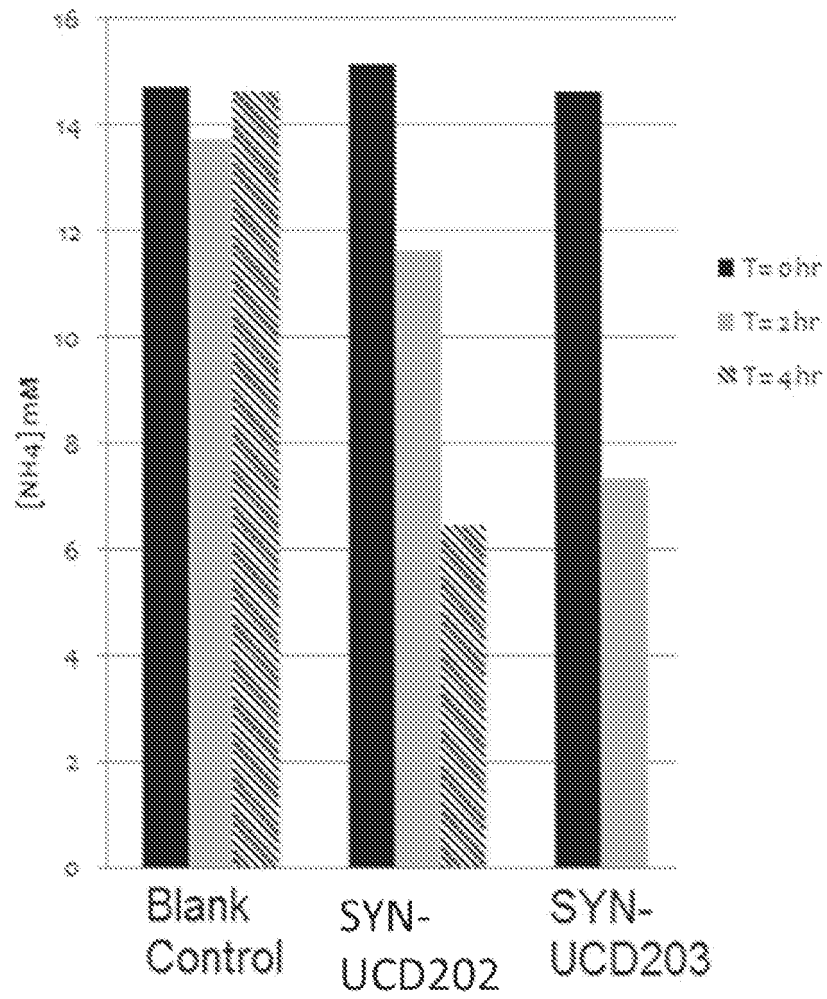
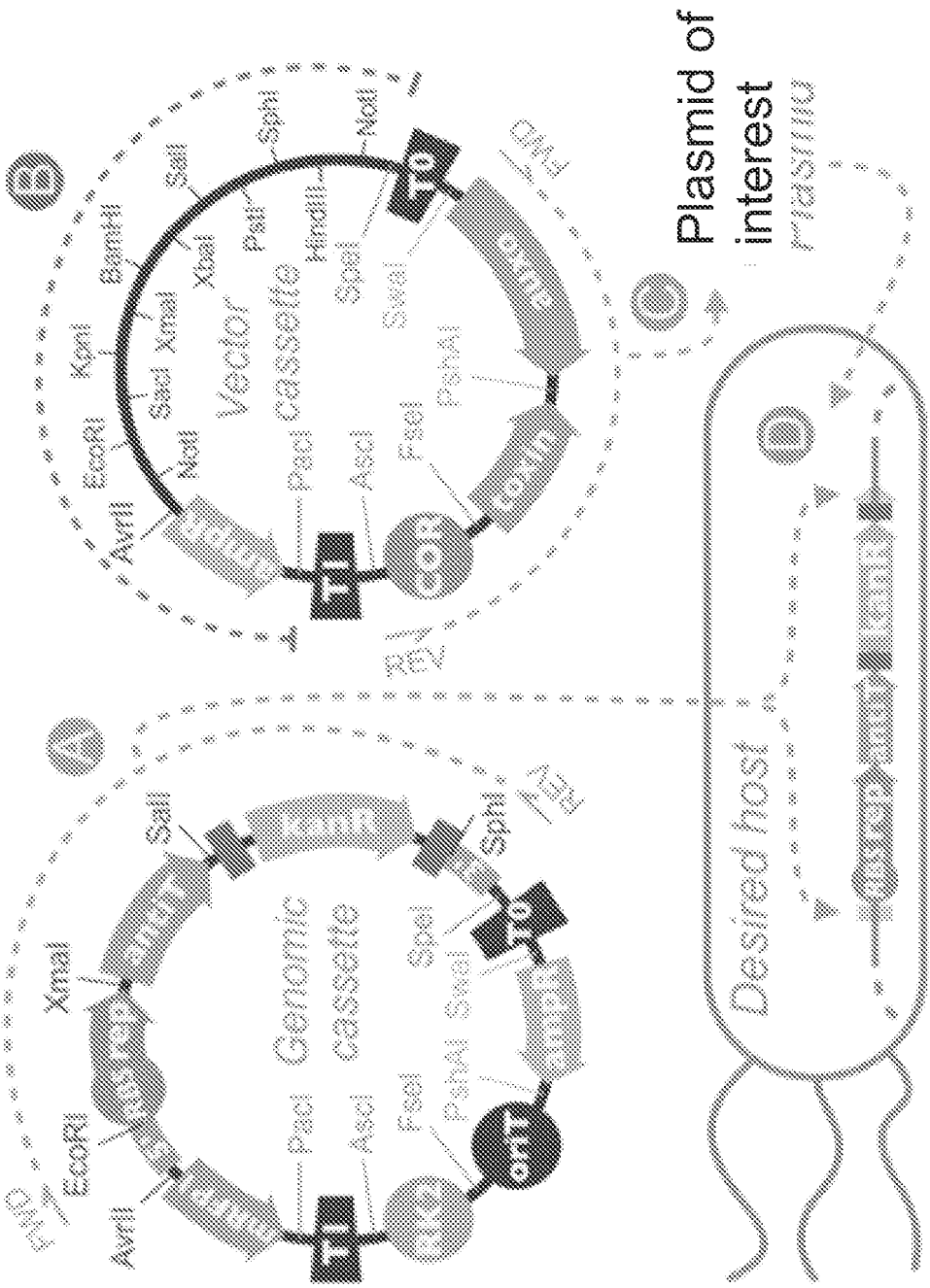
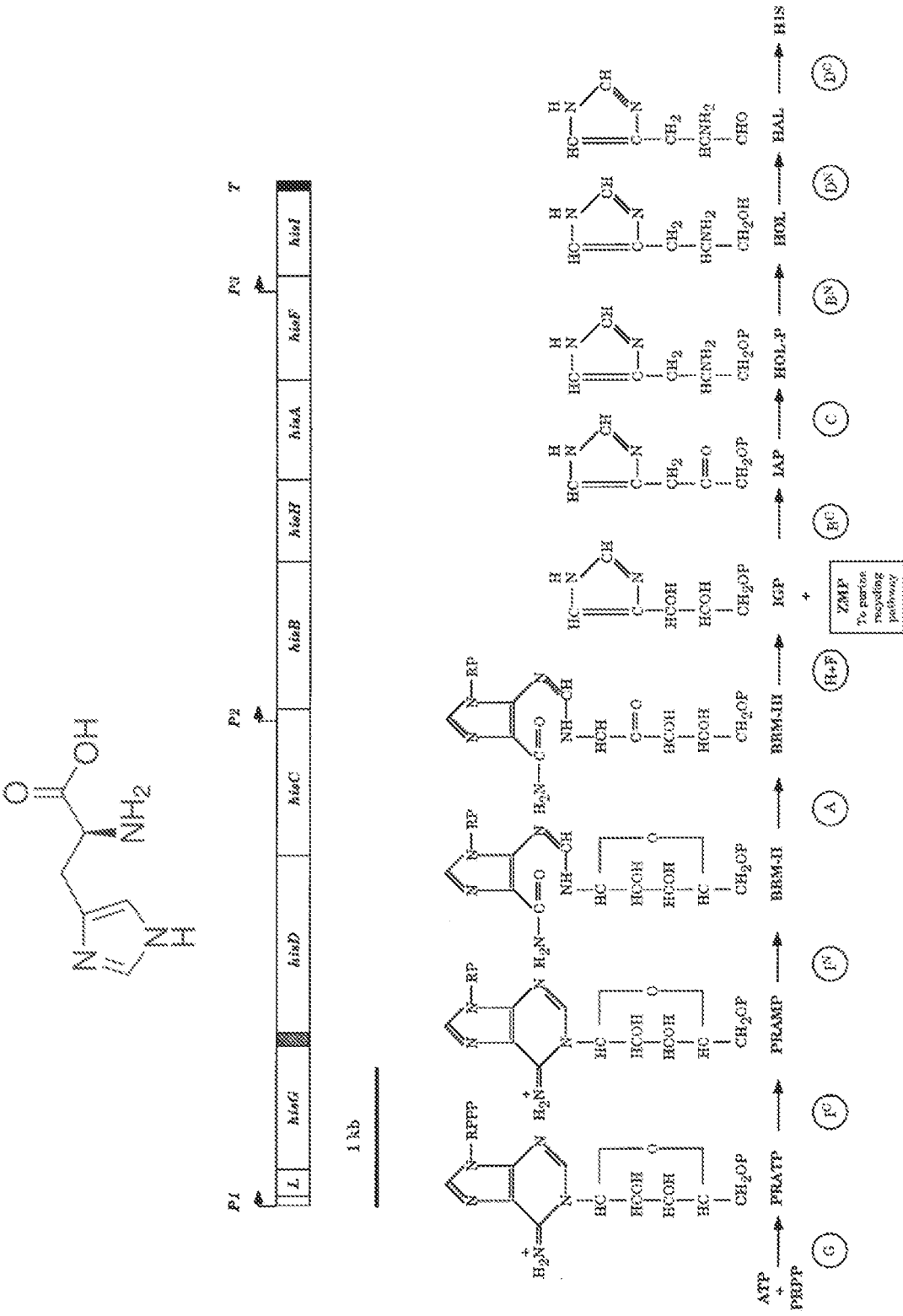


Fig. 53



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Fig. 55



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Fig. 56

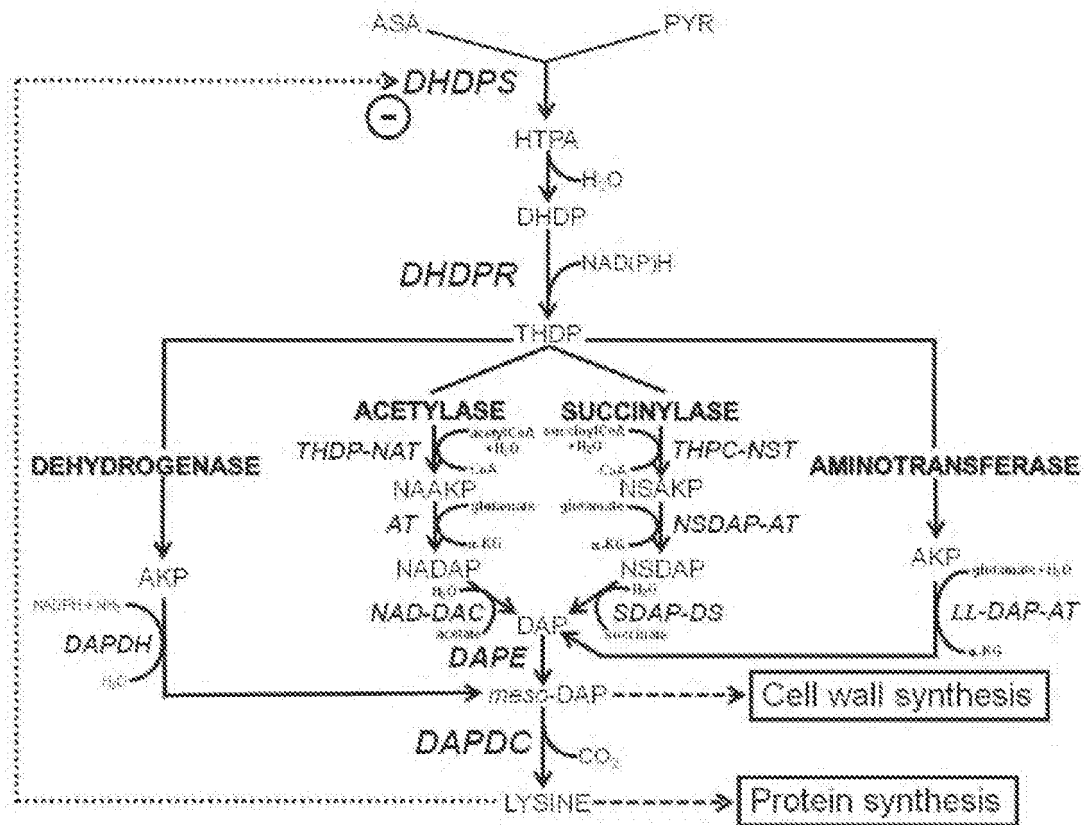


Fig. 57

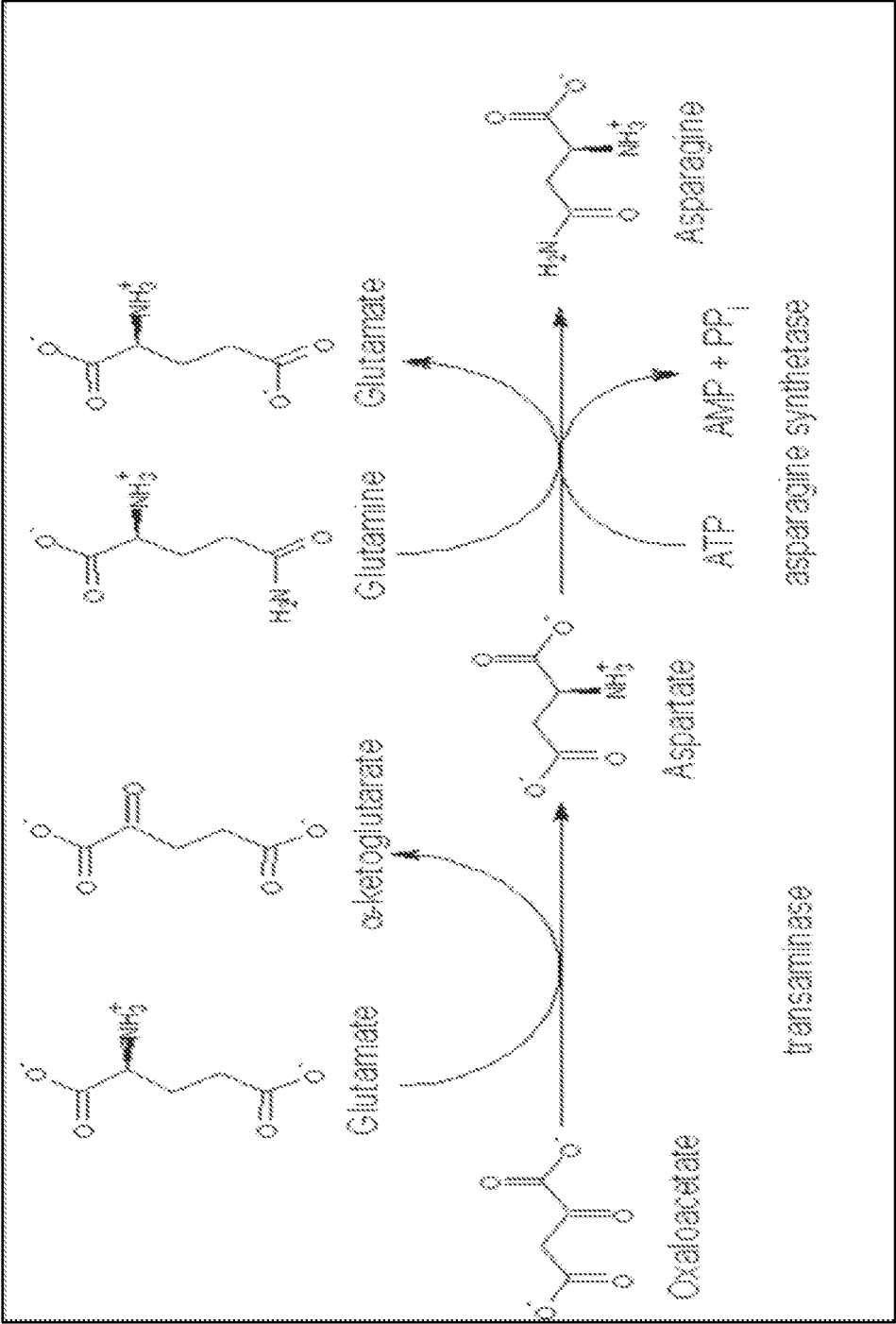
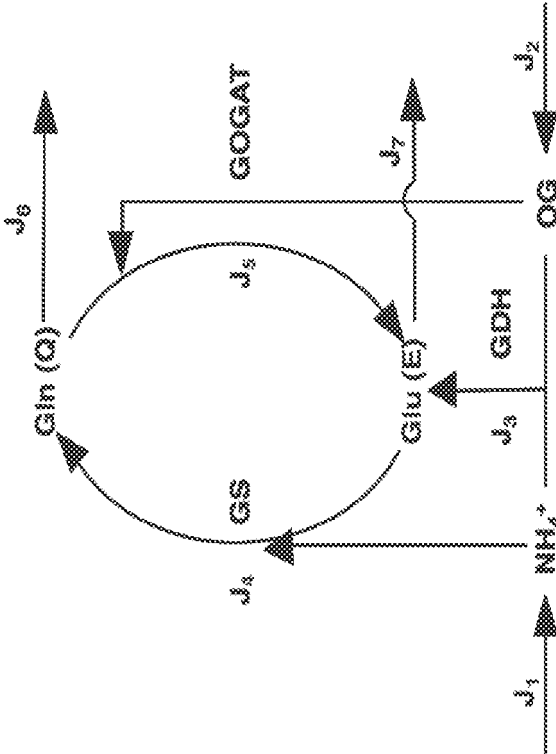
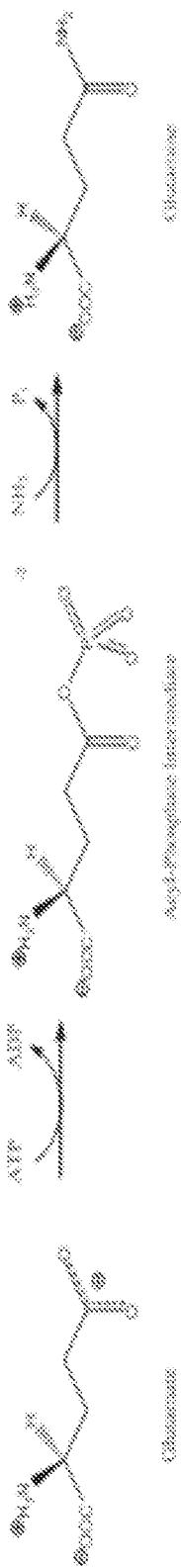
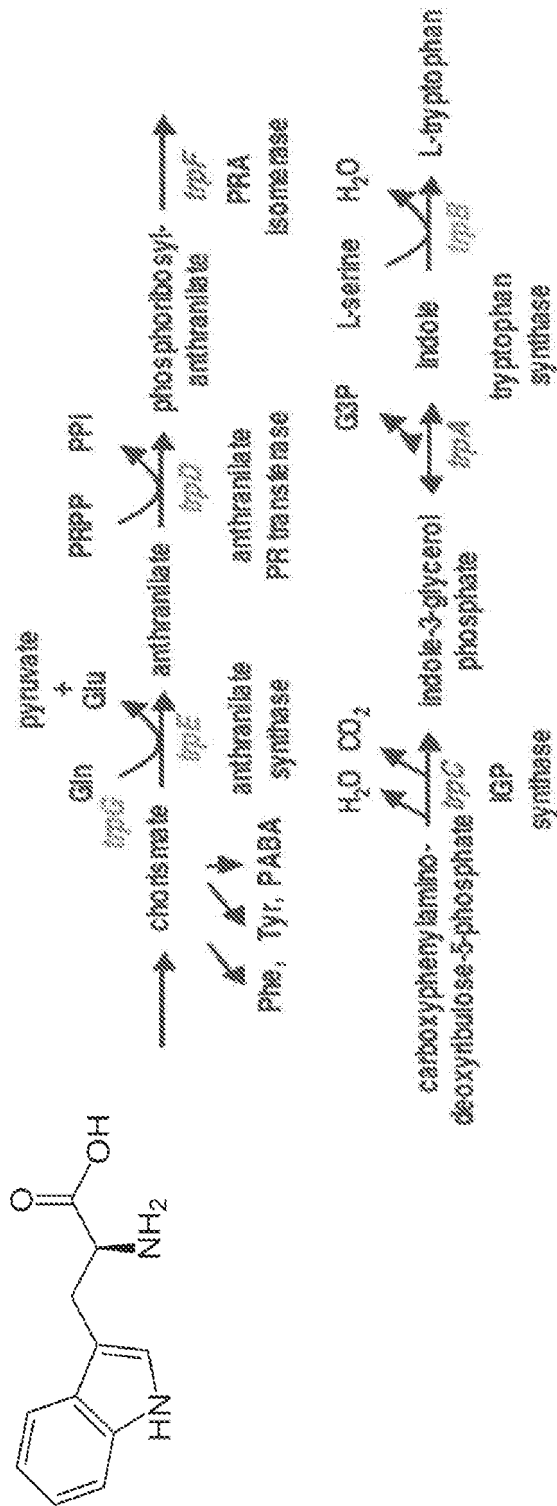


Fig. 58

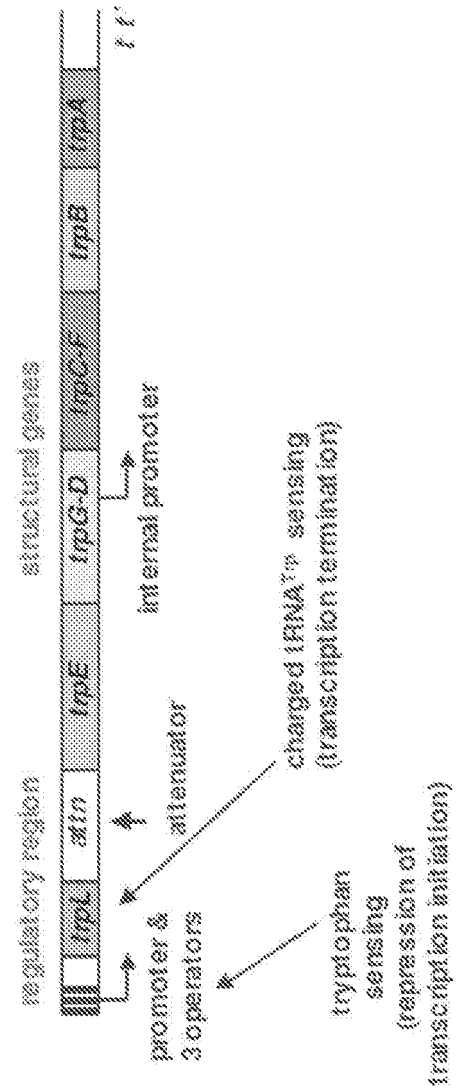


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Fig. 59



trp operon



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Fig. 60

Time-Delay Shut-off Switch (Toxin Producer)

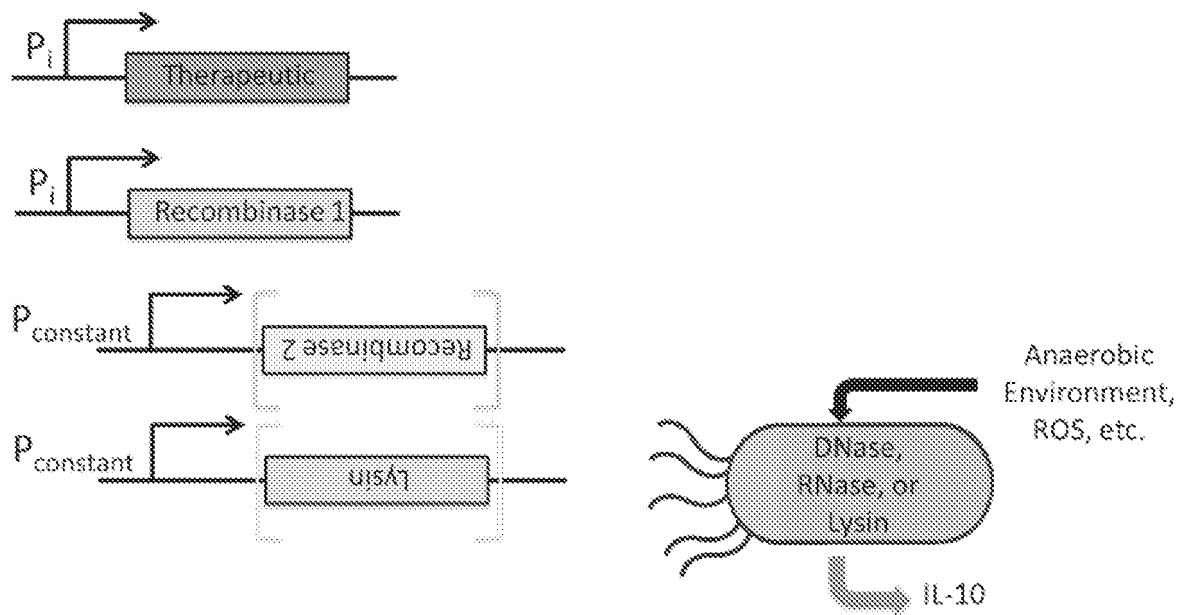


Fig. 61

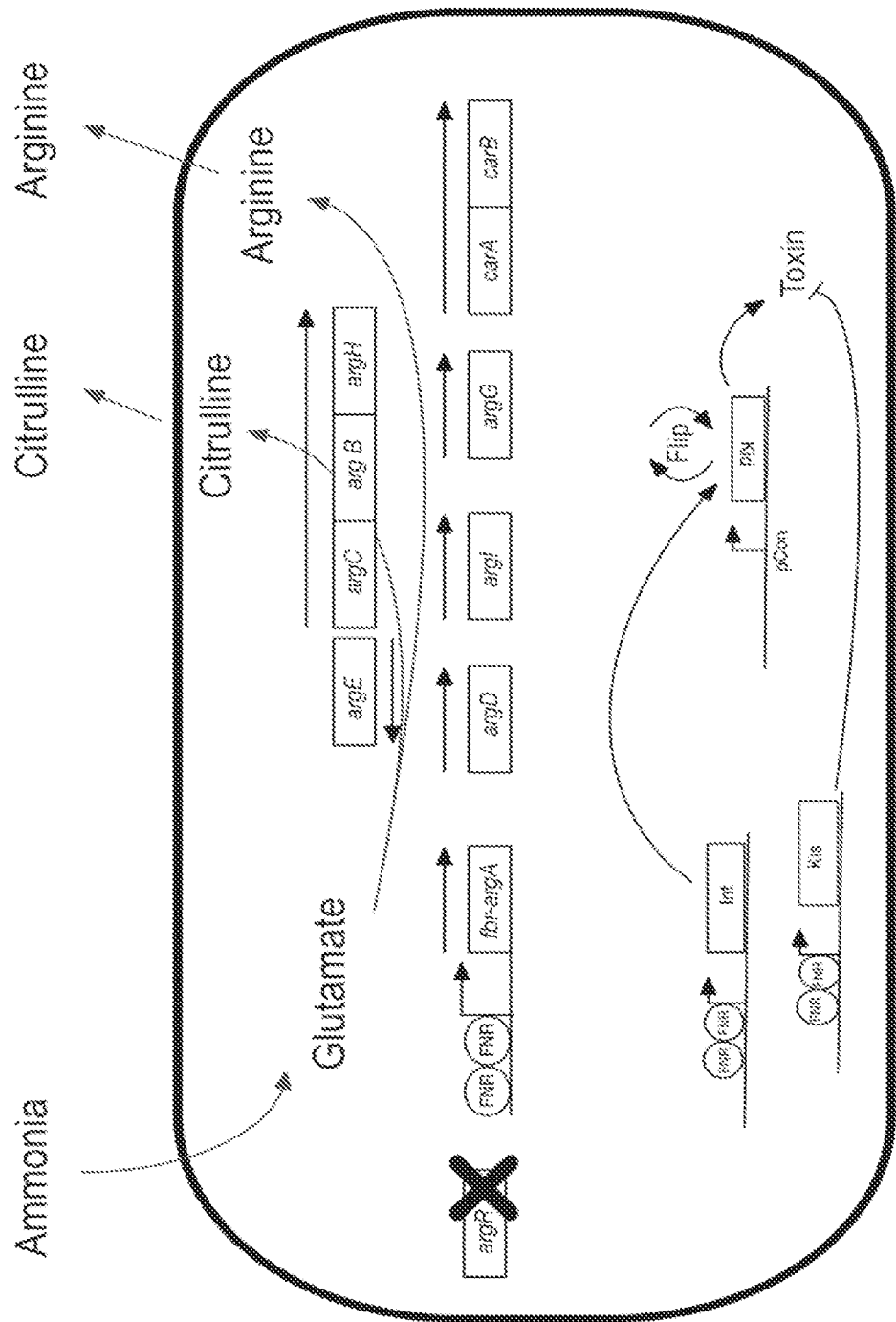


Fig. 62

“Dead Man” Kill Switch: Cells viable only in the presence of arabinose (or other sugar)

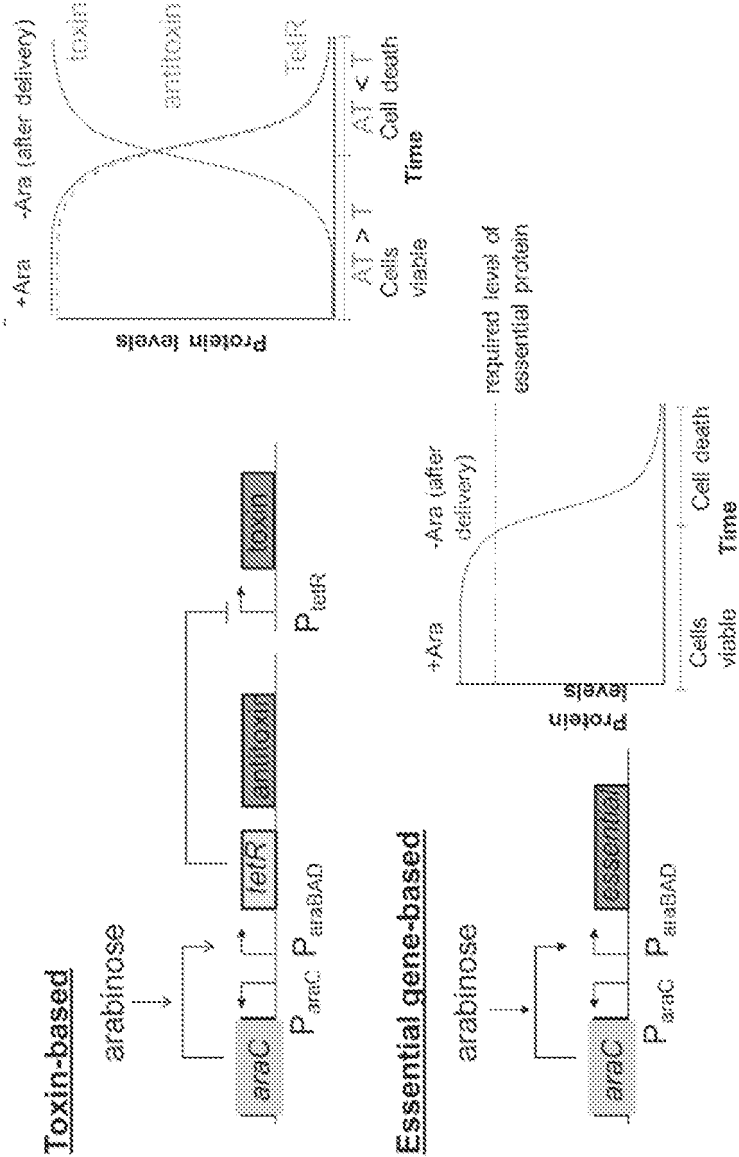


Fig. 63

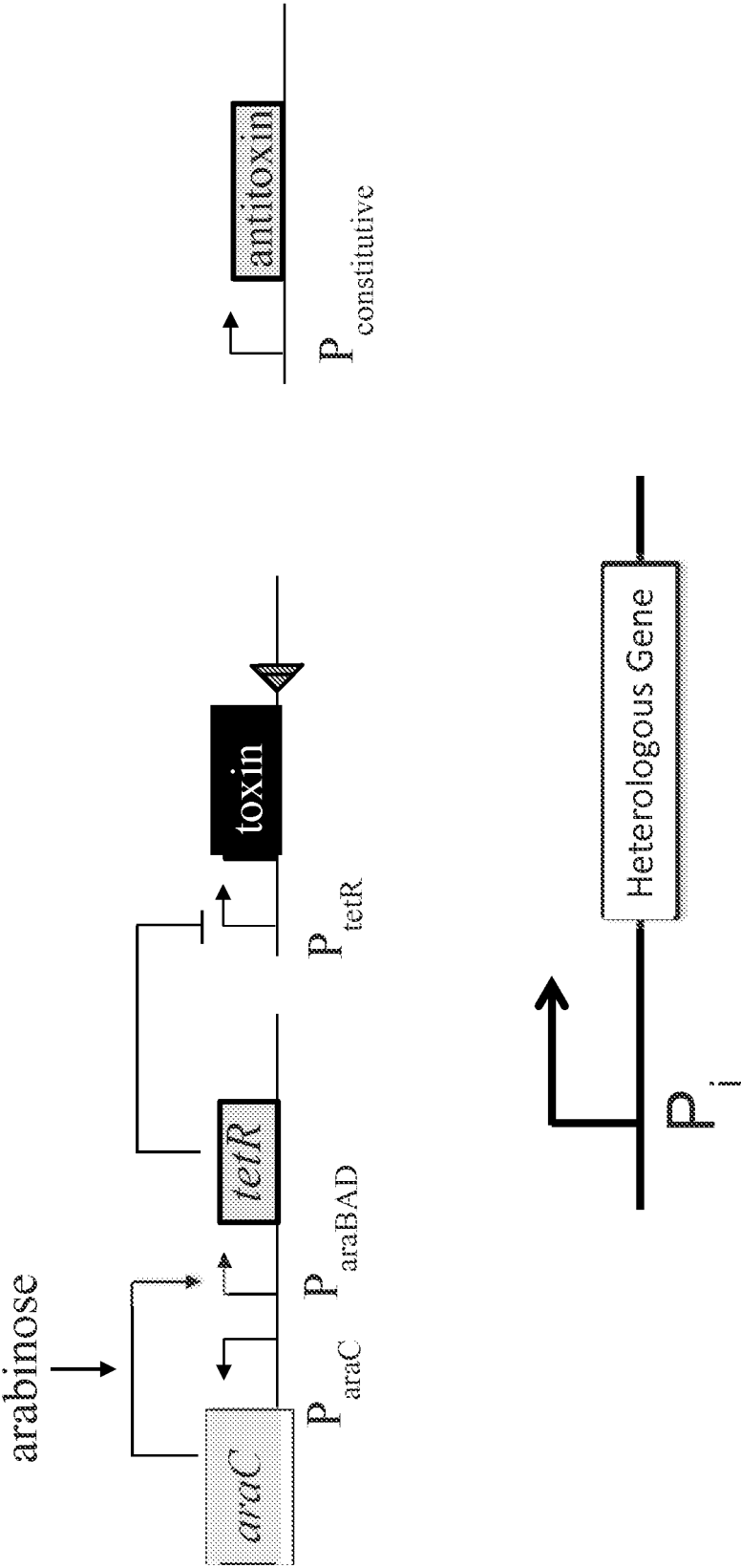


Fig. 64

I. Inherent Safety

Waste Management

II. Engineered Safety-

- Nissle -background chassis is a naturally occurring probiotic widely used
 - Isolated from human microbiome
 - Extensive human safety profile
- Genes being refactored are derived from human genome or commensal microorganism
 - Transient: non-colonizing probiotic well characterized

Auxotrophy

- *thyA* (DNA synthesis)
- *dapA* (cell wall synthesis)
- *serA* and *metA* (amino acid auxotrophs)

Kill Switch

