LacZalpha-ccdB

张寅良　何宗校

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   1 tagctgttca agtttgtgtt tcaactgttc tcgtcgtttc cgcaacaagt
  51 cctcttcaga aatgagcttt tgctcctctg cttggacgga caggatgtat
 101 gctgtggctt ttttaaggat aactaccttg ggggcctttt cattgttttc
 151 caactccggg atctggtcac gcagggcaaa aaagctccgt tttagctcgt
 201 tcctcctctg gcgctccaag acgttgtgtg ttcgcctctt gacattctcc
 251 tcggtgtccg agggccctgt gtgaaattgt tatccgctca caattccaca
 301 cagacgtcag tcctttgatt ctaataaatt ggatttttgt cacactattg
 351 tatcgctggg aatacaatta cttaacataa gcacctgtag gatcgtacag
 401 gtttacgcaa gaaaatggtt tgttatagtc gaataaacgc aagggaggtt
 451 ggtatgacca tgattacgcc aagctattta ggtgacacta tagaatactc
 501 aagctatgca tcaagcttgg taccgagctc ggatccacta gtaacggccg
 551 ccagtgtgct ggaattctgc agatatccat cacactggcg gccgctcgag
 601 catgcatcta gagggcccaa ttcgccctat agtgagtcgt attacaattc
 651 actggccgtc gttttacaac gtcgtgactg ggaaaaccct ggcgttaccc
 701 aacttaatcg ccttgcagca catccccctt tcgccagctg gcgtaatagc
 751 gaagaggccc gcaccgatcg cccttcccaa cagttgcgca gcctatacgt
 801 acggcagttt aaggtttaca cctataaaag agagagccgt tatcgtctgt
 851 ttgtggatgt acagagtgat attattgaca cgccggggcg acggatggtg
 901 atccccctgg ccagtgcacg tctgctgtca gataaagtct cccgtgaact
 951 ttacccggtg gtgcatatcg gggatgaaag ctggcgcatg atgaccaccg
1001 atatggccag tgtgccggtc tccgttatcg gggaagaagt ggctgatctc
1051 agccaccgcg aaaatgacat caaaaacgcc attaacctga tgttctgggg
1101 aatataaatg tcaggcgacg tcggaattgc cagctggggc gccctctggt
1151 aaggttggga agccctgcaa agtaaactgg atggctttct tgccgccaag
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                 391       400       410       420       430       440       450       460       470       480       490       500       510       520
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  GATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGAATAAACGCAAGGGAGGTTGGTATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATC---------
        2  GATCGTACAGGTTTACGCAAGAAAATGGTTTGTTATAGTCGAATAAACGCAAGGGAGGTTGGTATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCAAGCTTGG
        3                                                              ATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCAAGCTTGG
 Consensus  gatcgtacaggtttacgcaagaaaatggtttgttatagtcgaataaacgcaagggaggttggtATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCaagcttgg

                 521       530       540       550       560       570       580       590       600       610       620       630       640       650
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  -----------------------------------------------------------------------------------------------------TAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC
        2  TACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC
        3  TACCGAGCTCGGATCCGCTAGTAACGGCCGCCAGTGTGCTGGAGTTCTGTAGATATCCATCACACTGGAGACCGCTCGAGCATGCATCCAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC
 Consensus  taccgagctcggatcc.ctagtaacggccgccagtgtgctgga.ttctg.agatatccatcacactgg.g.ccgctcgagcatgcatctAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC

                 651       660       670       680       690       700       710       720       730       740       750       760       770       780
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAA
        2  ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAA
        3  ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAA
 Consensus  ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAA

                 781       790       800       810       820       830       840       850       860       870       880       890       900       910
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  CAGTTGCGCAGCCTATACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGG
        2  CAGTTGCGCAGCCTATACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGG
        3  CAGTTGCGCAGCCTATACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGG
 Consensus  CAGTTGCGCAGCCTATACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGG

                 911       920       930       940       950       960       970       980       990       1000      1010      1020      1030      1040
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  CCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGT
        2  CCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGT
        3  CCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGT
 Consensus  CCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGT

                 1041 1050      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  GGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCGACGTCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAA
        2  GGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCGACGTCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAA
        3  GGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAA
 Consensus  GGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAatgtcaggcgacgtcggaattgccagctggggcgccctctggtaaggttgggaagccctgcaa

                 1171 1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
                 |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        1  AGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCT
        2  AGTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCT
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" So, I can imagine that it'd still work even if you remove more sequences from the MCS. But, less sequences before the native ccdB would likely to give higher toxicity which makes cloning process more difficult (native ccdB is very toxic) "

------Yu Tanouchi

" with regard to the in-frame deletion of the multi-cloning sites, I deleted ~102 bps and the gene became more toxic. This version was what we used for the predator-prey system (Balaggadde et al 2008). My guess is that, as long as you don't touch the CcdB, longer in-frame deletion will still work. "

------Lingchong You

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            1        10        20        30        40        50        60        70        80        90       100       110       120       130
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
form_plasmid                                        TTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
BBa_I732017 AAAGAGGAGAAATACTAGATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
BBa_I732018 AAAGAGGAGAAATACTAGATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
Consensus   aaagaggagaaatactagatgaccatgattacggaTTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC

            131      140       150       160       170       180       190       200       210       220       230       240       250       260
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
form_plasmid GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTATACGT
BBa_I732017 GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGA
BBa_I732018 GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTAATAA
Consensus   GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTgaAtGgcgaatggcgctttgcctggtttccggcaccagaagcggtgccggaaagctggctggagt...a.........

            261      270       280       290       300
            |--------+---------+---------+---------|
form_plasmid
BBa_I732017 GGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCAC
BBa_I732018
Consensus   .......................................
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