

# LacZalpha-ccdB

张寅良 何宗校

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1 tagctgttca agtttgtgtt tcaactgttc togtogtttc cgcaacaagt
51 cctcttcaga aatgagcttt tgctcctctg ctgggacgga caggatgtat
101 gctgtggcct ttttaaggat aactaccttg ggggcctttt cattgttttc
151 caactcoggg atctggtcac gcagggcaaa aaagctcogt ttagctogt
201 tctcctctg gcgctccaag acgttgtgtg ttgcctctt gacattctcc
251 togtgtcog agggcctgt gtgaaattgt tatcogtca caattccaca
301 cagacgtcag tcttttgatt ctaataaatt ggatttttgt cacactattg
351 tatcogtggg aatacaatta cttaacataa gcacctgtag gatcgtacag
401 gtttacgcaa gaaaatggtt tgttatagtc gaataaacgc aaggagggtt
451 ggtatgacca tgattacgcc aagctattta ggtgacacta tagaatactc
501 aagctatgca tcaagcttgg taccgagctc ggatccacta gtaacggccg
551 ccagtgtgct ggaattctgc agatatccat cacactggcg gcogctogag
601 catgcatcta gagggcccaa ttgcocctat agtgagtcgt attacaattc
651 actgqccctc qttttacaac gtctgtactg qaaaaaccct ggcqttacc
701 aacttaatcg ccttgcagca catccccctt tcqccagctg qcqtaataq
751 qaagapqccc qcaccqatcg cccctcccaa cagttgcqca qcctatacgt
801 acggcagttt aaggtttaca cctataaaag agagagccgt tatcgtctgt
851 ttgtggatgt acagagtgat attattgaca cgccggggcg acggatggtg
901 atccccctgg ccagtgcacg tctgctgtca gataaagtct ccogtgaact
951 ttaccoggtg gtgcatatcg gggatgaaag ctggcgcatg atgaccaccg
1001 atatggccag tgtgcccgtc tccgttatcg gggagaagt ggctgatctc
1051 agccaccgcg aaaatgacat caaaaacgcc attaacctga tgttctgggg
1101 aatataaatg tcaggcgacg tcggaattgc cagctggggc gccctctggt
1151 aaggttggga agccctgcaa agtaaactgg atggctttct tgccgccaag
```

Click to Highlight:

Current: **lacZ\_a**

[M13 pUC ... @ 288..266](#)  
[Sp6 prim... @ 476..493](#)  
[EcoRV, cuts before 575](#)  
[T7 promo... @ 644..626](#)  
[M13 forw... @ 667..651](#)  
[M13 pUC ... @ 682..660](#)  
[lacZ\\_a @ 648..791](#)  
[ORF fram... @ 415..1107](#)  
[ApaLI, cuts before 915](#)  
[ccdB @ 814..1107](#)  
[NEOKAN p... @ 1133..1170](#)  
[BclI, cuts before 1224](#)  
[BclI, cuts before 1229](#)  
[ORF fram... @ 1259..2053](#)  
[NeoR/Kan... @ 1262..2050](#)  
[NcoI, cuts before 1820](#)  
[BstBI, cuts before 2363](#)  
[p15A ori... @ 2983..2192](#)  
[AclI, cuts before 2688](#)  
[SacII, cuts before 2852](#)  
[rrnB T1 ... @ 3090..3047](#)  
[ORF fram... @ 4509..3757](#)  
[Mud... cuts before 4200](#)

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391 400 410 420 430 440 450 460 470 480 490 500 510 520

1 |-----|  
 1 GATCGTACAGGTTTACGCAAGAAATGGTTTGTATAGTCGARTAACGCCAAGGGAGGTTGGTATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATC-----  
 2 GATCGTACAGGTTTACGCAAGAAATGGTTTGTATAGTCGARTAACGCCAAGGGAGGTTGGTATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCAGAGCTTGG  
 3 ATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCAGAGCTTGG

Consensus gatcgtacaggtttacgcaagaaaatggtttgttatagtcgaataaacgcaagggaggttggatgacccatgattacgccaaagctatTTAGGTGACACTATAGAATACTCAAGCTATGCATCaagcttgg

521 530 540 550 560 570 580 590 600 610 620 630 640 650

1 |-----|  
 1 TAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC  
 2 TACCAGCTCGGATCCACTAGTAAACGGCCGCCAGTGTGCTGGAAATCTGCGAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC  
 3 TACCAGCTCGGATCCGCTAGTAAACGGCCGCCAGTGTGCTGGAGTTCTGTAGATATCCATCACACTGGAGACCCTCGAGCATGCATCCAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC

Consensus taccagctcggatcc.ctagtaaacggccgccagtgctgga.ttctg.agat.atccat.cacactgg.g.ccgctcgagcatgcat.ctAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTC

651 660 670 680 690 700 710 720 730 740 750 760 770 780

1 |-----|  
 1 ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGGCCCGACCGATCGCCCTTCCCA  
 2 ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGGCCCGACCGATCGCCCTTCCCA  
 3 ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGGCCCGACCGATCGCCCTTCCCA

Consensus ACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGGCCCGACCGATCGCCCTTCCCA

781 790 800 810 820 830 840 850 860 870 880 890 900 910

1 |-----|  
 1 CAGTTGCGCAGCCTATACGTACGGCAGTTTAAAGTTTACACCTATAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCAGCGGATGGTGATCCCCTGG  
 2 CAGTTGCGCAGCCTATACGTACGGCAGTTTAAAGTTTACACCTATAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCAGCGGATGGTGATCCCCTGG  
 3 CAGTTGCGCAGCCTATACGTACGGCAGTTTAAAGTTTACACCTATAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCAGCGGATGGTGATCCCCTGG

Consensus CAGTTGCGCAGCCTATACGTACGGCAGTTTAAAGTTTACACCTATAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCAGCGGATGGTGATCCCCTGG

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040

1 |-----|  
 1 CCAGTGACGCTCTGCTGTACAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGATGAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGARAAGT  
 2 CCAGTGACGCTCTGCTGTACAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGATGAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGARAAGT  
 3 CCAGTGACGCTCTGCTGTACAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGATGAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGARAAGT

Consensus CCAGTGACGCTCTGCTGTACAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGATGAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGARAAGT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170

1 |-----|  
 1 GGCTGATCTCAGCCACCGCGAARAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAAATGTCAGGCGACGTCGGAAATGCCAGCTG6GGCGCCCTCTGGTAAAGTTGGGAAGCCCTGCA  
 2 GGCTGATCTCAGCCACCGCGAARAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAAATGTCAGGCGACGTCGGAAATGCCAGCTG6GGCGCCCTCTGGTAAAGTTGGGAAGCCCTGCA  
 3 GGCTGATCTCAGCCACCGCGAARAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAA

Consensus GGCTGATCTCAGCCACCGCGAARAATGACATCAAAAACGCCATTAACTGATGTTCTGGGGAATATAAatgt.caggcgacgtcggaattgccagct.gggcgccct.ct.ggtaaggttgggaagccctgcaa

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300

1 |-----|  
 1 AGTAAACTGGATGGCTTTCTTCCGCCAAGGATCTGATGGCGCAGGGGATCAGATCTGATCAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAGATGGATTGCACGCAGGTTCTCCGGCCGCT  
 2 AGTAAACTGGATGGCTTTCTTCCGCCAAGGATCTGATGGCGCAGGGGATCAGATCTGATCAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAGATGGATTGCACGCAGGTTCTCCGGCCGCT

“ 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

1 10 20 30 40 50 60 70 80 90 100 110 120 130  
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|

form\_plasmid TTCACTGGCCGTCGTTTTACACGTCGTGACTGGGAAACCCTGGCGTTACCCACTTAATCGCCTTGACGACATCCCCTTTCGCCAGCTGGC  
BBa\_I732017 AAAGAGGAGAACTAGATGACCATGATTACGGATTCACTGGCCGTCGTTTTACACGTCGTGACTGGGAAACCCTGGCGTTACCCACTTAATCGCCTTGACGACATCCCCTTTCGCCAGCTGGC  
BBa\_I732018 AAAGAGGAGAACTAGATGACCATGATTACGGATTCACTGGCCGTCGTTTTACACGTCGTGACTGGGAAACCCTGGCGTTACCCACTTAATCGCCTTGACGACATCCCCTTTCGCCAGCTGGC  
Consensus aaagaggagaaatactagatgaccatgattacggaTTCACTGGCCGTCGTTTTACACGTCGTGACTGGGAAACCCTGGCGTTACCCACTTAATCGCCTTGACGACATCCCCTTTCGCCAGCTGGC

131 140 150 160 170 180 190 200 210 220 230 240 250 260  
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|

form\_plasmid GTAATAGCGAAGAGGCCCGCACCGATCGCCCTCCCAACAGTTGCGCAGCCTATACGT  
BBa\_I732017 GTAATAGCGAAGAGGCCCGCACCGATCGCCCTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTCCGGCACCAGAAGCGGTGCCGGAAGCTGGCTGGAGTGCATCTTCCTGTA  
BBa\_I732018 GTAATAGCGAAGAGGCCCGCACCGATCGCCCTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTCCGGCACCAGAAGCGGTGCCGGAAGCTGGCTGGAGTAAATAA  
Consensus GTAATAGCGAAGAGGCCCGCACCGATCGCCCTCCCAACAGTTGCGCAGCCTgaAtGgcgaatggcgctttgcctgggttccggcaccagaagcggtgccggaaagctggctggagt...a.....

261 270 280 290 300  
|-----+-----+-----+-----|

form\_plasmid  
BBa\_I732017 GGCCGATACTGTCGTCGTCCCTCAACTGGCAGATGCAC  
BBa\_I732018  
Consensus .....