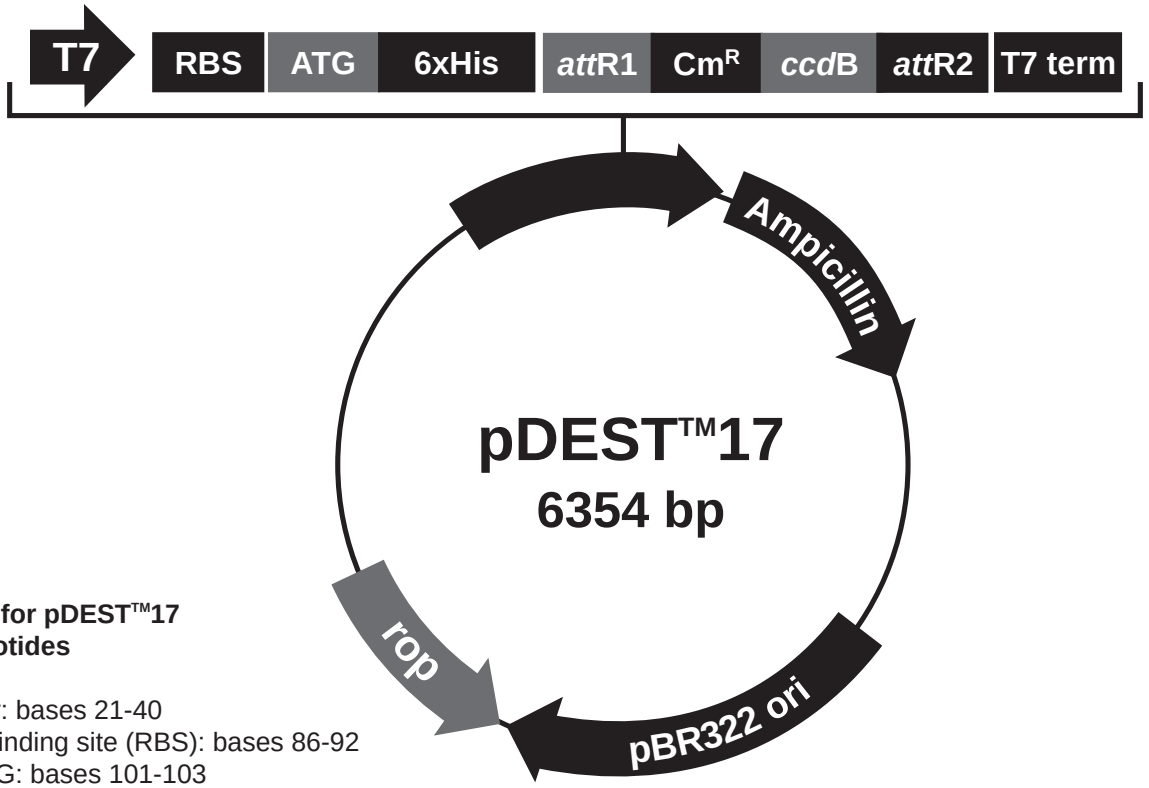


Comments for pDEST™17
6354 nucleotides

T7 promoter: bases 21-40
Ribosome binding site (RBS): bases 86-92
Initiation ATG: bases 101-103
6xHis tag: bases 113-130
*att*R1: bases 140-264
Chloramphenicol resistance gene (Cm^R): bases 373-1032
*ccd*B gene: bases 1374-1679
*att*R2: bases 1720-1844
T7 transcription termination region: bases 1855-1983
bla promoter: bases 2471-2569
Ampicillin (*bla*) resistance gene: bases 2570-3430
pBR322 origin: bases 3575-4248
ROP ORF: bases 4619-4810 (C)

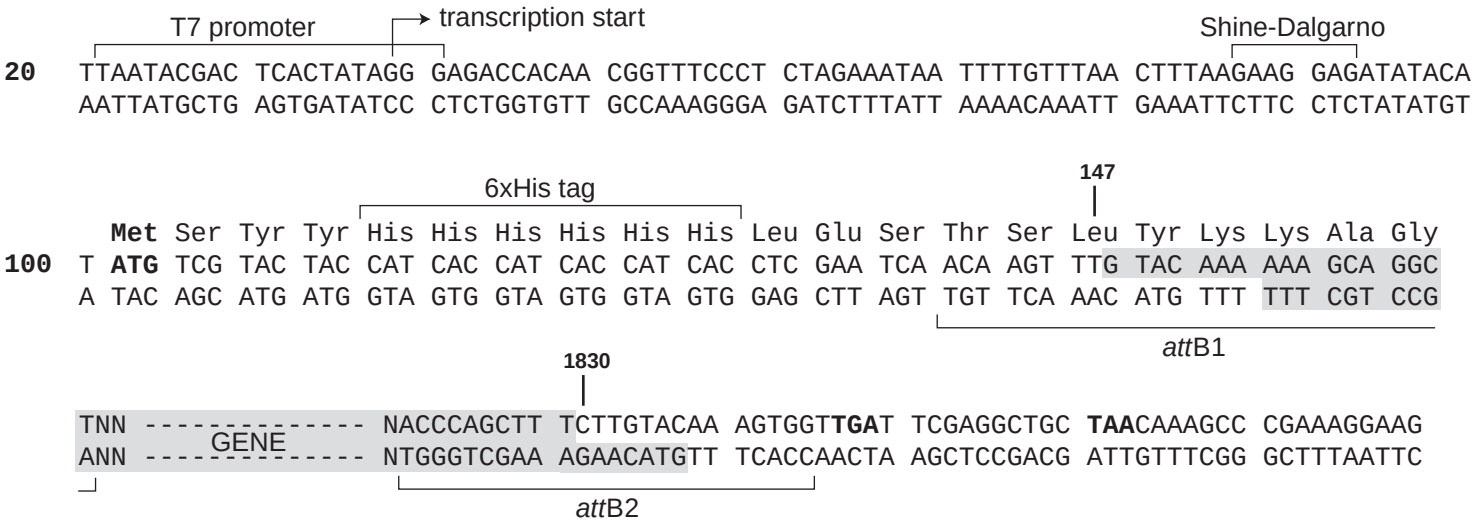
C=complementary strand

Invitrogen™
life technologies

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAAT
TTTGTTTAACTTTAAGAAGGAGATATACATATGTCGTACTACCATCACCATCACCATCACCTCGAATCAA
CAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTT
GCATAAAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGCATTAGGCA
CCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGAGTTAGGATCCGTCGAGATT
TTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGG
CATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCTGG
ATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCT
TGCCCGCCTGATGAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGAT
AGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACC
ACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTA
TTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTT
GATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAG
GCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTCTGTGATGGCTTCCATGTCGGCAG
AATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAAGATCTGGATCCGGCTTA
CTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGT
ATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGC
TATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGC
CCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAGGTCGCCCGGTTTATT
GAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAA
AGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGG
TGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATAT
CGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAA
GTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAA
TGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGT
ATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCG
TTCAGCTTTCTTGTACAAAGTGGTTGATTCGAGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTG
CTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCT
GAAAGGAGGAACTATATCCGGATATCCACAGGACGGGTGTGGTCGCCATGATCGCGTAGTCGATAGTGGC
TCCAAGTAGCGAAGCGAGCAGGACTGGGCGGCGGCCAAAGCGGTCGGACAGTGCTCCGAGAACGGGTGCG
CATAGAAATTGCATCAACGCATATAGCGCTAGCAGCACGCCATAGTGACTGGCGATGCTGTCGGAATGGA
CGATATCCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGT
GCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTTAGCAATTTAACTGT
GATAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGC
CTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTT
TTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCAT
GAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGT
GTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAG
TAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGAT
CCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG
GTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGG
TTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGC

CATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACC
GCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCA
TACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGG
CGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCA
CTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTC
GCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAG
TCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA
CTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCT
AGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTC
AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAA
ACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGG
TAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
CAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGC
GATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAA
CGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGA
ACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCC
ACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAA
CGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCT
GATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGC
GCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGG
TATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATA
CACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCC
TGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTC
AGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAG
CGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGG
CTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGG
GATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGA
TGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGA
AAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGC
ATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAAC
ACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTT
CGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACG
ACAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGAGATGCGCCGCGTGCGGCTGCT
GGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTTGCGCATTCACAGTTCTCCGCAAGAAT
TGATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGTCGAGGT
GGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCAT
GCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATCGAAG
TTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAG
CATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAG
CCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCGGCGATAATGGCCTGCT
TCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATAC
CGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGCT

GCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATGCCCC
GCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGATCGACGCTCTCCCTTAT
GCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGG
TGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAA
GCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAA
CCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCG


T7 promoter
transcription start
Shine-Dalgarno
20
TTAATACGAC TCACTATAGG GAGACCACAA CGGTTTCCCT CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA
AATTATGCTG AGTGATATCC CTCTGGTGTT GCCAAAGGGA GATCTTTATT AAAACAAATT GAAATTCTTC CTCTATATGT
6xHis tag
147
Met Ser Tyr Tyr His His His His His His Leu Glu Ser Thr Ser Leu Tyr Lys Lys Ala Gly
100
T ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC CTC GAA TCA ACA AGT TTG TAC AAA AAA GCA GGC
A TAC AGC ATG ATG GTA GTG GTA GTG GTA GTG GAG CTT AGT TGT TCA AAC ATG TTT TTT CGT CCG
attB1
1830
TNN ---------- NACCCAGCTT TCTTGTACAA AGTGGTTGAT TCGAGGCTGC TAACAAAGCC CGAAAGGAAG
ANN ---------- NTGGGTCGAA AGAACATGTT TCACCAACTA AGCTCCGACG ATTGTTTCGG GCTTTAATTC
GENE
attB2

General Description

DNA Plasmid pDEST17_verA
Entire molecule length: 6354 bp

Restriction Map

Enzyme	# of cuts	Positions
AatII	1	2439
AccI	3	1121 1716 4478
AccIII	3	583 1978 5057
AciI	77	13 266(c) 270 565 929 1023(c) 1095(c) 1290(c) 1629 1898 2059(c) 2062(c) 2071(c) 2179 2321 2467(c) 2514 2613(c) 2722(c) 2799(c) 2843 2964(c) 3010 3201(c) 3292(c) 3654 3663(c) 3798 3908(c) 4029(c) 4048(c) 4175(c) 4203(c) 4294 4315 4322 4365(c) 4382(c) 4408(c) 4421 4461 4486 4528 4538 4577 4662(c) 4718 4773(c) 4789(c) 4945(c) 4956(c) 4959(c) 5088 5226 5307 5313(c) 5329(c) 5382 5436 5478 5490(c) 5514(c) 5559(c) 5584(c) 5617 5685(c) 5700 5785 5832(c) 5881 5921(c) 6000(c) 6020 6143 6146 6149 6302
AcsI	2	587 2360
AcyI	6	2436 2818 5517 6178 6292 6313
AflIII	1	4246
AluI	26	159 359 368 487 616 1190 1553 1826 1879 2331 2346 3005 3068 3168 3689 3946 3992 4082 4308 4591 4610 4659 4670 4727 5635 6038
Alw44I	4	1487 2686 3932 4432
AlwI	17	3(c) 4 332(c) 345 1035(c) 1048 1467(c) 2719 2723(c) 3040

		3503(c) 3504 3600(c) 3602 3688 5049(c) 5619(c)
AlwNI	2	1366 3837
AosI	4	2100 3135 5269 5367
ApaLI	4	1487 2686 3932 4432
ApoI	2	587 2360
AseI	2	21 3183
AsnI	2	21 3183
Asp700	2	2758 4692
AspEI	1	3358
AspHI	9	1491 2086 2690 2775 3936 4436 5260 5551 6142
AspI	1	4504
AvaI	2	1456 5296
AvaII	8	2994 3216 4962 5241 5283 5586 5835 5923
AviII	4	2100 3135 5269 5367
BamHI	2	337 1040
BanI	10	278 2239 2282 3405 5432 5516 5955 6177 6291 6312
BanII	2	6244 6258
BbsI	3	2375 5128 5991
BbvI	25	1170 1842(c) 1875(c) 1878(c) 2145 2924(c) 3126 3315 3618(c) 3824(c) 3827(c) 3917 4336 4354 4500(c) 4597(c) 4646(c) 4668 5049 5172 5175 5279(c) 5303(c) 5936(c) 6123
BcgI	3	2820(c) 4675 6030
BfaI	7	61 1912 2130 3165 3500 3753 5234
BglI	3	3240 5559 5793
BglII	2	2 1034
BmyI	11	1491 2086 2690 2775 3936 4436 5260 5551 6142 6244 6258
BpmI	5	709 3289 4725(c) 5341 5895(c)
Bpu1102I	1	1901
BpuAI	3	2375 5128 5991
BsaAI	1	4498
BsaBI	5	1 7 1039 1471 5053
BsaHI	6	2436 2818 5517

		6178 6292 6313
BsaI	3	35(c) 1596 3292
BsaJI	15	283 818 819 888 1456 1478 1923 2229 2243 4086 5274 5352 5554 6191 6197
BsaWI	8	583 1227 1978 3062 3893 4040 5057 6028
BseAI	3	583 1978 5057
BsgI	1	5092
BsiEI	9	270 2075 2840 2989 3912 4336 5785 6071 6075
BsiHKAI	9	1491 2086 2690 2775 3936 4436 5260 5551 6142
BsiYI	25	819 1282 1463 1601 1937 1993 2093 2184 3768 4047 4213 4231 4604 4784 4826 4917 5280 5358 5407 6032 6104 6122 6152 6198 6199
BslI	25	819 1282 1463 1601 1937 1993 2093 2184 3768 4047 4213 4231 4604 4784 4826 4917 5280 5358 5407 6032 6104 6122 6152 6198 6199
BsmAI	7	35(c) 811 1515 1596 2516(c) 3292 4605
BsmFI	5	1377 4975 5623(c) 5848 6173(c)
BsmI	3	580 987 5368(c)
Bsp1286I	11	1491 2086 2690 2775 3936 4436 5260 5551 6142 6244 6258
BspDI	1	2336
BspEI	3	583 1978 5057
BspHI	4	2413 2518 3526 6236
BspMI	2	1702(c) 5667
BspWI	40	285 365 928 1317 1864 1885 1897 2012 2046 2068 2126 2133 2199 3120 3240 3628 4200 4314 4379 5146 5169 5234

		5276 5301 5310 5559 5568 5668 5677 5682 5686 5784 5793 5802 5927 5953 6098 6289 6310 6321
BsrBI	2	2516(c) 4317(c)
BsrDI	3	606 3124 3298(c)
BsrFI	8	1587 2198 3273 5438 5792 5952 6315 6324
BsrGI	3	148 1431 1833
BsrI	27	254(c) 394 834(c) 1371 1484(c) 1580(c) 1704(c) 1733 2059 2154 2713 2883(c) 3152 3195 3313 3719 3831(c) 3844(c) 4474(c) 4507 4922 4946 5212(c) 5589(c) 5838(c) 6047 6116(c)
BssHII	1	1081
Bst1107I	2	1122 4479
BstNI	10	285 764 820 1480 2230 4087 4100 4221 5281 5664
BstUI	26	15 1083 1631 2015 2467 2799 3292 3622 4203 4546 4649 4651 4720 5090 5187 5309 5335 5480 5490 5619 5685 5746 5751 5778 5907 6022
BstXI	1	1575
BstYI	11	2 337 1034 1040 2711 2728 3496 3508 3594 3605 5054
CelII	1	1901
CfoI	34	1083 1085 1559 2101 2129 2261 2467 2799 3136 3229 3622 3731 3905 4005 4072 4342 4375 4518 4548 4651 4997 5080 5270 5306 5368 5519 5778 5909 5949 6024 6180 6234 6294 6315
Cfr10I	8	1587 2198 3273 5438 5792 5952

		6315 6324
ClaI	1	2336
Csp6I	9	107 149 465 1003 1432 1834 2196 2876 4443
DdeI	14	360 808 1312 1621 1880 1901 2431 2857 3397 3563 3972 4439 4979 5141
DpnI	30	4 10 339 1036 1042 1474 1619 2012 2677 2713 2730 2988 3034 3052 3393 3498 3510 3588 3596 3607 3682 5056 5263 5580 5595 5626 5898 6074 6261 6352
DpnII	30	2 8 337 1034 1040 1472 1617 2010 2675 2711 2728 2986 3032 3050 3391 3496 3508 3586 3594 3605 3680 5054 5261 5578 5593 5624 5896 6072 6259 6350
DraI	5	507 846 2780 3472 3491
DraII	5	1928 2378 5241 5283 6202
DrdI	2	4144 4559
DsaI	3	888 5274 6197
DsaV	24	283 762 818 1320 1455 1456 1478 1526 2188 2228 2820 3171 3867 4085 4098 4219 4568 4603 4909 5237 5279 5463 5662 6191
EaeI	10	267 852 1481 1577 2063 2965 5277 5782 6194 6326
EagI	2	267 5782
Eam1105I	1	3358
EarI	2	2559(c) 4363(c)
EclXI	2	267 5782
Eco47III	4	2128 4996 5948 6233
Eco57I	2	2692 3704(c)
EcoNI	1	6102

EcoO109I	5	1928 2378 5241 5283 6202
EcoRI	2	587 2360
EcoRII	10	283 762 818 1478 2228 4085 4098 4219 5279 5662
EcoRV	2	1984 2175
Esp3I	2	811 4605
EspI	1	1901
Fnu4HI	49	267 270 929 1159 1856 1889 1892 2060 2063 2134 2614 2843 2938 2965 3115 3304 3632 3838 3841 3906 4049 4204 4322 4325 4343 4461 4514 4611 4657 4660 4957 5038 5161 5164 5293 5307 5314 5317 5436 5515 5560 5617 5700 5785 5950 6001 6112 6146 6149
FnuDII	26	15 1083 1631 2015 2467 2799 3292 3622 4203 4546 4649 4651 4720 5090 5187 5309 5335 5480 5490 5619 5685 5746 5751 5778 5907 6022
FokI	17	567(c) 1319 1441 1479 1558 2212(c) 2260 2919 3206 3387 4560(c) 4701(c) 4887 4965 5027(c) 5676(c) 5721(c)
FspI	4	2100 3135 5269 5367
HaeII	11	2130 4006 4376 4998 5081 5520 5950 6181 6235 6295 6316
HaeIII	30	269 500 545 767 854 1483 1579 1930 2065 2188 2380 2967 3234 3314 3772 4206 4224 4235 4777 5279 5463 5676 5733 5784 5805 5894 6132 6196 6204

		6328
HgaI	11	2826 3556(c) 4134(c) 4552 4709(c) 5341 5491 5735(c) 5767(c) 6086 6325(c)
HgiAI	9	1491 2086 2690 2775 3936 4436 5260 5551 6142
HhaI	34	1083 1085 1559 2101 2129 2261 2467 2799 3136 3229 3622 3731 3905 4005 4072 4342 4375 4518 4548 4651 4997 5080 5270 5306 5368 5519 5778 5909 5949 6024 6180 6234 6294 6315
HinP1I	34	1081 1083 1557 2099 2127 2259 2465 2797 3134 3227 3620 3729 3903 4003 4070 4340 4373 4516 4546 4649 4995 5078 5268 5304 5366 5517 5776 5907 5947 6022 6178 6232 6292 6313
HincII	3	1179 1717 2816
HindII	3	1179 1717 2816
HindIII	1	2329
HinfI	14	28 135 937 1847 3359 3876 4272 4347 4693 5197 5418 5716 5870 6094
HpaII	37	306 542 584 712 1044 1228 1322 1457 1528 1588 1979 2190 2199 2821 3063 3173 3240 3274 3678 3868 3894 4041 4570 4604 4911 5058 5238 5439 5465 5703 5793 5953 6029 6193 6316 6325 6340
HphI	24	108(c) 114(c) 120(c) 623 629(c) 631 767 823(c) 835 877(c)

		1381 1482 2245 2631(c) 2666 2872(c) 3288 3515 4622(c) 4631(c) 5206 5427 6285 6330
ItaI	49	267 270 929 1159 1856 1889 1892 2060 2063 2134 2614 2843 2938 2965 3115 3304 3632 3838 3841 3906 4049 4204 4322 4325 4343 4461 4514 4611 4657 4660 4957 5038 5161 5164 5293 5307 5314 5317 5436 5515 5560 5617 5700 5785 5950 6001 6112 6146 6149
KasI	4	5516 6177 6291 6312
Ksp632I	2	2559(c) 4363(c)
MaeI	7	61 1912 2130 3165 3500 3753 5234
MaeII	15	171 674 849 1491 1812 2436 2756 3129 3545 4497 4923 5153 5177 5766 5822
MaeIII	22	256 644 749 1170 1724 2145 2233 2697 2885 3038 3096 3427 3710 3826 3889 4498 4593 4806 4890 4913 5573 5840
MamI	5	1 7 1039 1471 5053
MboI	30	2 8 337 1034 1040 1472 1617 2010 2675 2711 2728 2986 3032 3050 3391 3496 3508 3586 3594 3605 3680 5054 5261 5578 5593 5624 5896 6072 6259 6350
MboII	13	862(c) 1618 2380 2576 2685 2763 3518 3589(c) 4380 5133 5725 5996 6254(c)
McrI	9	270 2075 2840 2989

		3912 4336 5785 6071 6075
MluNI	4	854 1483 1579 5279
MnlI	35	68 141 433(c) 1136(c) 1307(c) 1845(c) 1940(c) 1941 1960(c) 2178(c) 2238(c) 2391 2985(c) 3191(c) 3338 3419 3819 4069(c) 4143 4352(c) 4616(c) 4646(c) 4827 4865(c) 4923(c) 5254 5423(c) 5450(c) 5488(c) 5549(c) 5752 5851(c) 5936 6122(c) 6341(c)
MroI	3	583 1978 5057
MscI	4	854 1483 1579 5279
MseI	25	21 77 83 198 506 845 987 1382 1655 1785 2304 2326 2407 2779 3144 3183 3418 3471 3485 3490 3542 4469 4751 4783 5003
MslI	9	1245 1573 2587 2946 3105 4677 5068 5263 5694
MspA1I	9	487 931 1900 2722 3663 3908 4540 4659 5584
MspI	37	306 542 584 712 1044 1228 1322 1457 1528 1588 1979 2190 2199 2821 3063 3173 3240 3274 3678 3868 3894 4041 4570 4604 4911 5058 5238 5439 5465 5703 5793 5953 6029 6193 6316 6325 6340
MvaI	10	285 764 820 1480 2230 4087 4100 4221 5281 5664
MvnI	26	15 1083 1631 2015 2467 2799 3292 3622 4203 4546 4649 4651 4720 5090 5187 5309 5335 5480 5490 5619 5685 5746 5751 5778 5907

		6022
MwoI	40	285 365 928 1317 1864 1885 1897 2012 2046 2068 2126 2133 2199 3120 3240 3628 4200 4314 4379 5146 5169 5234 5276 5301 5310 5559 5568 5668 5677 5682 5686 5784 5793 5802 5927 5953 6098 6289 6310 6321
NaeI	4	5440 5794 5954 6326
NarI	4	5517 6178 6292 6313
NciI	14	1322 1457 1458 1528 2190 2822 3173 3869 4570 4605 4911 5239 5465 6193
NcoI	1	888
NdeI	1	100
NdeII	30	2 8 337 1034 1040 1472 1617 2010 2675 2711 2728 2986 3032 3050 3391 3496 3508 3586 3594 3605 3680 5054 5261 5578 5593 5624 5896 6072 6259 6350
NgoMI	4	5438 5792 5952 6324
NheI	1	2129
NlaIII	32	662 892 953 974 1250 1563 2011 2358 2417 2522 2915 2951 3029 3039 3530 4250 4512 4617 4782 4845 4909 5134 5268 5475 5532 5547 5675 5792 5978 6017 6167 6240
NlaIV	28	280 339 1042 1689 1929 2031 2241 2284 2471 3061 3272 3313 3407 4179 4218 4963 5242 5285 5399

		5434 5469 5518 5836 5957 6179 6203 6293 6314
NotI	1	267
NruI	1	5751
NspI	4	4250 4617 4909 6167
PflMI	3	819 5358 5407
PleI	5	22(c) 3367 3870(c) 4355 6088(c)
PpuMI	2	5241 5283
Psp1406I	5	674 2756 3129 4923 5822
PstI	2	1713 3116
PvuI	2	2989 6075
PvuII	2	487 4659
RcaI	4	2413 2518 3526 6236
RsaI	9	108 150 466 1004 1433 1835 2197 2877 4444
SalI	1	1715
SapI	1	4363(c)
Sau3AI	30	2 8 337 1034 1040 1472 1617 2010 2675 2711 2728 2986 3032 3050 3391 3496 3508 3586 3594 3605 3680 5054 5261 5578 5593 5624 5896 6072 6259 6350
Sau96I	16	1928 2187 2378 2994 3216 3233 3312 4775 4962 5241 5283 5462 5586 5835 5923 6202
ScaI	2	1004 2877
ScrFI	24	285 764 820 1322 1457 1458 1480 1528 2190 2230 2822 3173 3869 4087 4100 4221 4570 4605 4911 5239 5281 5465 5664 6193
SfaNI	24	430 915(c) 2121 2145(c) 2234 2657(c) 2906 3097(c) 4149(c) 4369(c) 4409 4447(c) 4582 4803(c) 4865(c) 4943(c) 5039(c) 5049 5291(c) 5686(c) 5698

		6073 6311(c) 6323(c)
SfcI	6	34 1709 2220 3112 3790 3981
SgrAI	1	6315
SmaI	1	1458
SnoI	4	1487 2686 3932 4432
SphI	1	6167
SspBI	3	148 1431 1833
SspI	2	899 2553
StyI	3	888 1923 5352
TaqI	14	7 133 344 1716 1850 2021 2336 2704 4148 5455 5596 6071 6075 6353
TfiI	9	135 937 1847 4272 4693 5197 5418 5716 5870
ThaI	26	15 1083 1631 2015 2467 2799 3292 3622 4203 4546 4649 4651 4720 5090 5187 5309 5335 5480 5490 5619 5685 5746 5751 5778 5907 6022
Tru9I	25	21 77 83 198 506 845 987 1382 1655 1785 2304 2326 2407 2779 3144 3183 3418 3471 3485 3490 3542 4469 4751 4783 5003
Tsp509I	14	18 68 200 587 992 1781 2107 2300 2360 2925 3180 3486 5388 5402
Tth111I	1	4504
Van91I	3	819 5358 5407
XbaI	1	60
XhoII	11	2 337 1034 1040 2711 2728 3496 3508 3594 3605 5054
XmaI	1	1456
XmaIII	2	267 5782
XmnI	2	2758 4692

No cuts: AatI, Acc65I, AflII, AgeI, ApaI, AscI, Asp718, AsuII, AvrII, BbrPI, BclI, BfrI, BlnI, BsiWI, Bsp120I, BstBI, BstEII, Bsu36I, Csp45I, DraIII, Ecl136II, HpaI, KpnI, KspI, MfeI, MluI, MunI, NsiI, NspV, PacI, PaeR7I, PinAI, PmaCI, PmeI, PmlI, Ppu10I, RsrII, SacI, SacII, SexAI, SfiI, SfuI, SnaBI, SpeI, StuI, SwaI, XcmI, XhoI

Invitrogen™
life technologies

Instruction Manual

E. coli Expression System with with Gateway® Technology

Gateway®-adapted destination vectors for cloning and high-level expression of native or tagged recombinant proteins in *E. coli*

Catalog nos. 11824-026, 11801-016, 11802-014, 11803-012, 12216-016

Version E
19 October 2004
25-0517

Table of Contents

Kit Contents and Storage

Types of Products

This manual is supplied with the following products listed below.

Product	Catalog no.
E. coli Expression System with Gateway® Technology	11824-026
Gateway® pDEST™14 Vector	11801-016
Gateway® pDEST™15 Vector	11802-014
Gateway® pDEST™17 Vector	11803-012
Gateway® pDEST™24 Vector	12216-016

Kit Components

Each product contains the following components. For a detailed description of the contents of each component, see pages vi-vii.

Component	Catalog no.				
	11824-026	11801-016	11802-014	11803-012	12216-016
pDEST™14 Vector	√	√			
pDEST™15 Vector	√		√		
pDEST™17 Vector	√			√	
pDEST™24 Vector	√				√
Gateway® LR Clonase™ II Enzyme Mix	√				
Library Efficiency® DH5α Competent *E. coli*	√				
BL21-AI™ One Shot® Chemically Competent *E. coli*	√				

Shipping/Storage

The *E. coli* Expression System with Gateway® Technology is shipped as described below. Upon receipt, store each item as detailed below.

Box	Item	Shipping	Storage
1	pDEST™ Vectors	Room temperature	-20°C
2	Gateway® LR Clonase™ II Enzyme Mix	Dry ice	-20°C
3	Library Efficiency® DH5α™ Competent *E. coli* Kit	Dry ice	-80°C
4	BL21-AI™ One Shot® Chemically Competent *E. coli* Kit	Dry ice	-80°C

Note: The individual Gateway® pDEST™ vectors (Catalog nos. 11801-016, 11802-014, 11803-012, 12216-016) are shipped at room temperature. **Upon receipt, store at -20°C.**

continued on next page

Kit Contents and Storage, continued

Destination Vectors

The following destination vectors (Box 1) are supplied with the *E. coli* Expression System with Gateway® Technology. **Store the vectors at -20°C.**

Note: Catalog nos. 11801-016, 11802-014, 11803-012, and 12216-016 contain 6 µg of the appropriate lyophilized pDEST™ vector **only**.

Reagent	Composition	Amount
pDEST™14 Vector	Lyophilized in TE Buffer, pH 8.0	6 µg
pDEST™15 Vector	Lyophilized in TE Buffer, pH 8.0	6 µg
pDEST™17 Vector	Lyophilized in TE Buffer, pH 8.0	6 µg
pDEST™24 Vector	Lyophilized in TE Buffer, pH 8.0	6 µg

LR Clonase™ II Enzyme Mix

The following reagents are included with the Gateway® LR Clonase™ II Enzyme Mix (Box 2). **Store Box 2 at -20°C for up to 6 months.** For long-term storage, store at -80°C.

Reagent	Composition	Amount
LR Clonase™ II Enzyme Mix	Proprietary	40 µl
Proteinase K solution	2 µg/µl in: 10 mM Tris-HCl, pH 7.5 20 mM $CaCl_2$ 50% glycerol	40 µl
pENTR™-gus Positive Control	50 ng/µl in TE Buffer, pH 8.0	1 µg

DH5α™ Competent *E. coli*

The Library Efficiency® DH5α™ Competent *E. coli* kit (Box 3) includes the following items. Transformation efficiency is ≥ 1 x 10^8 cfu/µg DNA. **Store Box 3 at -80°C.**

Item	Composition	Amount
S.O.C. Medium (may be stored at room temperature or +4°C)	2% tryptone 0.5% yeast extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	2 x 6 ml
Library Efficiency® Chemically Competent DH5α	--	5 x 200 µl
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

continued on next page

Kit Contents and Storage, continued

Genotype of DH5α™

Use this strain to propagate and maintain your expression clone.

Genotype: F- *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1

BL21-AI™ One Shot® Competent *E. coli*

The BL21-AI™ One Shot® Chemically Competent *E. coli* Kit (Box 4) includes the following items. Transformation efficiency is ≥ 1 x 10^8 cfu/µg DNA. **Store Box 4 at -80°C.**

Item	Composition	Amount
S.O.C. Medium (may be stored at room temperature or +4°C)	2% tryptone 0.5% yeast extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 ml
20% L-arabinose	20% L-arabinose in sterile water	1 ml
BL21-AI™ chemically competent cells	--	21 x 50 µl
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype of BL21-AI™

Use this strain for expression only. Do not use these cells to propagate or maintain your expression clone.

Genotype: F- *ompT hsdS*$_B$ ($r_B^-m_B^-$) *gal dcm araB*::*T7RNAP-tetA*

The BL21-AI™ strain is an *E. coli* B/r strain and does not contain the *lon* protease. It is also deficient in the outer membrane protease, OmpT. The lack of these proteases reduces degradation of heterologous proteins expressed in this strain.

The strain carries a chromosomal insertion of a cassette containing the T7 RNA polymerase (T7 RNAP) gene in the *ara*B locus, allowing expression of the T7 RNAP to be regulated by the *araBAD* promoter (see page 22 for more information). The presence of the *tet*A gene confers resistance to tetracycline and permits verification of strain identity using tetracycline.

Accessory Products

Introduction

The products listed in this section may be used with the *E. coli* Expression System with Gateway® Technology. For more information, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 30).

Additional Products

Many of the reagents supplied in the *E. coli* Expression System with Gateway® Technology as well as other products suitable for use with the kit are available separately from Invitrogen. Ordering information for these reagents is provided below.

Item	Quantity	Catalog no.
LR Clonase™ II Enzyme Mix	20 reactions	11791-020
	100 reactions	11791-100
Library Efficiency® DH5α Competent Cells	5 x 0.2 ml	18263-012
BL21-AI™ One Shot® Chemically Competent *E. coli*	20 x 50 µl	C6070-03
Gateway® pDEST™14 Vector	6 µg	11801-016
Gateway® pDEST™15 Vector	6 µg	11802-014
Gateway® pDEST™17 Vector	6 µg	11803-012
Gateway® pDEST™24 Vector	6 µg	12216-016
Ampicillin	20 ml (10 mg/ml)	11593-019
Carbenicillin	5 g	10177-012

Purification of Recombinant Protein

The presence of the polyhistidine (6xHis) tag in pDEST™17 allows purification of your recombinant fusion protein using a nickel-charged agarose resin such as ProBond™ or Ni-NTA. Ordering information is provided below.

Item	Quantity	Catalog no.
ProBond™ Nickel-Chelating Resin	50 ml	R801-01
	150 ml	R801-15
ProBond™ Purification System	6 purifications	K850-01
Ni-NTA Agarose	10 ml	R901-01
	25 ml	R901-15
	100 ml	R901-10
Ni-NTA Purification System	6 purifications	K950-01

Introduction

Overview

Introduction

The *E. coli* Expression System with Gateway® Technology contains a series of Gateway®-adapted destination vectors designed to facilitate high-level, inducible expression of recombinant proteins in *E. coli* using the pET system. Depending on the vector chosen, the pDEST™ vectors allow production of native, N-terminal, or C-terminal-tagged recombinant proteins (see table below).

Vector	Fusion Peptide	Fusion Tag
pDEST™14	---	---
pDEST™15	N-terminal	Glutathione *S*-transferase (GST) (Smith *et al.*, 1986)
pDEST™17	N-terminal	6xHis
pDEST™24	C-terminal	Glutathione *S*-transferase (GST) (Smith *et al.*, 1986)

For more information about the Gateway® Technology, see the next page.

The pET Expression System

The pET system was originally developed by Studier and colleagues and takes advantage of the high activity and specificity of the bacteriophage T7 RNA polymerase to allow regulated expression of heterologous genes in *E. coli* from the T7 promoter (Rosenberg *et al.*, 1987; Studier and Moffatt, 1986; Studier *et al.*, 1990). For more information about T7-regulated expression, see the next page.

Features of the Vectors

pDEST™14, pDEST™15, pDEST™17, and pDEST™24 contain the following elements:

- T7 promoter for high-level, T7 RNA polymerase regulated expression of the gene of interest in *E. coli* (Studier and Moffatt, 1986; Studier *et al.*, 1990)
- N- or C-terminal fusion tags for detection and purification of recombinant fusion proteins (choice of tag depends on the particular vector; see above)
- Two recombination sites, *att*R1 and *att*R2, downstream of the T7 promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene (Cm^R) located between the two *att*R sites for counterselection
- The *ccd*B gene located between the *att*R sites for negative selection
- Ampicillin resistance gene for selection in *E. coli*
- pBR322 origin for low-copy replication and maintenance of the plasmid in *E. coli*

Overview, continued

The Gateway® Technology

The Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest in *E. coli* using the Gateway® Technology, simply:

1. Clone your gene of interest into a Gateway® entry vector of choice to create an entry clone.
2. Perform an LR recombination reaction between the entry clone and a Gateway® destination vector (*e.g.* pDEST™14, pDEST™15, pDEST™17, pDEST™24).
3. Transform Library Efficiency® DH5α *E. coli* and select for an expression clone.
4. Purify plasmid and transform your expression construct into BL21-AI™. Induce expression of your recombinant protein with L-arabinose.

For more detailed information about Gateway® Technology, refer to the Gateway® Technology with Clonase™ II manual. To generate an entry clone, refer to the manual for the entry vector you are using. The Gateway® Technology with Clonase™ II manual and entry vector manuals are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).

LR Recombination Reaction

You will perform an LR recombination reaction between the entry clone and your destination vector of choice to generate an expression clone. The LR recombination reaction is mediated by LR Clonase™ II Enzyme Mix, a mixture of the bacteriophage λ Integrase (Int) and Excisionase (Xis) proteins, and the *E. coli* Integration Host Factor (IHF) protein. For more information about the LR recombination reaction, see the Gateway® Technology with Clonase™ II manual.

The Basis of T7-Regulated Expression

The pET expression system uses elements from bacteriophage T7 to control expression of heterologous genes in *E. coli*. In the pDEST™14, pDEST™15, pDEST™17, and pDEST™24 vectors, expression of the gene of interest is controlled by a strong bacteriophage T7 promoter. In bacteriophage T7, the T7 promoter drives expression of gene 10 (ϕ10). T7 RNA polymerase specifically recognizes this promoter. To express the gene of interest, it is necessary to deliver T7 RNA polymerase to the cells by inducing expression of the polymerase or infecting the cell with phage expressing the polymerase. In the *E. coli* Expression System with Gateway® Technology, T7 RNA polymerase is supplied by the BL21-AI™ host *E. coli* strain in a regulated manner (see the next page for more information about the strain).

The BL21-AI™ *E. coli* Strain

Description of the Strain

The BL21-AI™ *E. coli* strain is included in the kit and is intended for use as a host for expression of T7 RNA polymerase-regulated genes. The BL21-AI™ strain is derived from the BL21 strain (Grodberg and Dunn, 1988; Studier and Moffatt, 1986) and contains a chromosomal insertion of the gene encoding T7 RNA polymerase (T7 RNAP) into the *ara*B locus of the *araBAD* operon, placing regulation of the T7 RNAP gene under the control of the *araBAD* promoter. The *ara*B gene is deleted in this strain.

Regulating Expression of T7 RNA Polymerase

Because the T7 RNAP gene is inserted into the *ara*B locus of the *araBAD* operon, expression of T7 RNA polymerase can be regulated by the sugars, L-arabinose and glucose.

- To induce expression from the *araBAD* promoter, use L-arabinose (Lee, 1980; Lee *et al.*, 1987). To modulate expression, simply vary the concentration of L-arabinose added.
- To repress basal expression from the *araBAD* promoter, use glucose.

  Note: In the absence of glucose, basal expression from the *araBAD* promoter is generally low (Lee, 1980; Lee *et al.*, 1987). Adding glucose further represses expression from the *araBAD* promoter by reducing the levels of 3´, 5´-cyclic AMP (Miyada *et al.*, 1984).

For more information on the mechanism of expression and repression of the *ara* regulon, see the **Appendix**, page 22 or refer to Schleif, 1992.

Experimental Outline

Experimental Outline

The table below outlines the steps required to express your gene of interest in *E. coli* from pDEST™14, pDEST™15, pDEST™17, or pDEST™24.

Step	Action	Page
1	Design an appropriate scheme and clone your gene of interest into the Gateway® entry vector of choice to generate an entry clone.	5-6
2	Perform an LR recombination reaction by mixing the entry clone and the appropriate pDEST™ vector with Gateway® LR Clonase™ II Enzyme Mix.	7-13
3	Transform the recombination reaction into competent Library Efficiency® DH5α™ cells and select for expression clones.	14
4	Analyze transformants for the presence of insert by restriction enzyme digestion or colony PCR.	15
5	**Optional:** Sequence to confirm that the gene of interest is cloned in frame with the appropriate N-terminal or C-terminal tag	15
6	Prepare purified plasmid DNA of the expression clone and transform into BL21-AI™ One Shot® cells.	16-17
7	Pick a transformant and perform a pilot expression study. Add L-arabinose to induce expression of your recombinant protein.	18-19
8	Purify your recombinant protein, if desired.	20

Methods

Generating an Entry Clone

Introduction

To recombine your gene of interest into pDEST™14, pDEST™15, pDEST™17, or pDEST™24, you will need an entry clone containing the gene of interest. Many entry vectors are available from Invitrogen to facilitate generation of entry clones (see table below). For more information about each vector, see our Web site or contact Technical Service (see page 30).

Entry Vector	Catalog no.
pENTR™/D-TOPO®	K2400-20
pENTR™/SD/D-TOPO®	K2420-20
pENTR™1A	11813-011
pENTR™2B	11816-014
pENTR™3C	11817-012
pENTR™4	11818-010
pENTR™11	11819-018

Once you have selected an entry vector, refer to the manual for the specific entry vector you are using for instructions to construct an entry clone. All entry vector manuals are available for downloading from our Web site or by contacting Technical Service.

Points to Consider Before Recombining into pDEST™14

Your gene of interest in the entry clone must:

- Contain an ATG initiation codon and a Shine-Dalgarno sequence (RBS) with optimal spacing for proper translation initiation in *E. coli* (Shine and Dalgarno, 1975).

  Note: If you clone your gene of interest into an entry vector that supplies a Shine-Dalgarno RBS (*e.g.* pENTR/SD/D-TOPO® or pENTR™11), then your gene of interest need only include an ATG initiation codon.

- Contain a stop codon.

Refer to the diagram of the recombination region of pDEST™14 on page 8 to help you design a strategy to generate your entry clone.

continued on next page

Generating an Entry Clone, continued

Points to Consider Before Recombining into pDEST™15 and pDEST™17

pDEST™15 and pDEST™17 are N-terminal fusion vectors and contain an ATG initiation codon upstream of the GST and 6xHis tags, respectively. In each vector, a Shine-Dalgarno RBS is included upstream of the initiation ATG to ensure optimal spacing for proper translation initiation in *E. coli*. Your gene of interest in the entry clone must:

- Be in frame with the N-terminal tag after recombination.
- Contain a stop codon.

Refer to the diagram of the recombination region of pDEST™15 or pDEST™17 on pages 9 and 10, respectively to help you design a strategy to generate your entry clone.

Points to Consider Before Recombining into pDEST™24

pDEST™24 is a C-terminal fusion vector. Your gene of interest in the entry clone must:

- Contain an ATG initiation codon and a Shine-Dalgarno RBS with optimal spacing for proper translation initiation in *E. coli.*

  Note: If you clone your gene of interest into an entry vector that supplies a Shine-Dalgarno RBS (*e.g.* pENTR/SD/D-TOPO® or pENTR™11), then your gene of interest need only include an ATG initiation codon.
- Be in frame with the C-terminal GST tag after recombination.
- **NOT** contain a stop codon.

Refer to the diagram of the recombination region of pDEST™24 on page 11 to help you design a strategy to generate your entry clone.

Creating an Expression Clone

Introduction

After you have generated an entry clone, you will perform the LR recombination reaction to transfer the gene of interest into the pDEST™ vector to create your expression clone. To ensure that you obtain the best possible results, we recommend that you read this section and the next section entitled **Performing the LR Recombination Reaction** (pages 12-13) before beginning.

Experimental Outline

To generate an expression clone, you will:

1. Perform an LR recombination reaction using the *att*L-containing entry clone and the *att*R-containing pDEST™ vector. **Note:** Both the entry clone and the destination vector should be supercoiled (see **Important Note** below).
2. Transform the reaction mixture into a suitable *E. coli* host (see page 14).
3. Select for expression clones (see pages 8-11 for illustrations of the recombination region of expression clones in pDEST™14, pDEST™15, pDEST™17, or pDEST™24.

Important

The pDEST™14, pDEST™15, pDEST™17, and pDEST™24 vectors are supplied as supercoiled plasmids. Although we have previously recommended using a linearized destination vector for more efficient recombination, further testing at Invitrogen has found that linearization of the destination vector is **NOT** required to obtain optimal results for any downstream application.

Resuspending the Vectors

Each pDEST™ vector is supplied as 6 µg of plasmid, lyophilized in TE, pH 8.0. To use, simply resuspend the pDEST™ plasmid in 40 µl of sterile water to a final concentration of 150 ng/µl.

Propagating the Vectors

If you wish to propagate and maintain the pDEST™14, pDEST™15, pDEST™17, or pDEST™24 vectors prior to recombination, we recommend using One Shot® *ccd*B Survival T1^R Chemically Competent *E. coli* from Invitrogen (Catalog no. C7510-03) for transformation. The *ccd*B Survival T1^R *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene. To maintain the integrity of the vector, select for transformants in media containing 50-100 µg/ml ampicillin and 15-30 µg/ml chloramphenicol.

Note: Do not use general *E. coli* cloning strains including TOP10 or DH5α™ for propagation and maintenance as these strains are sensitive to CcdB effects.

continued on next page

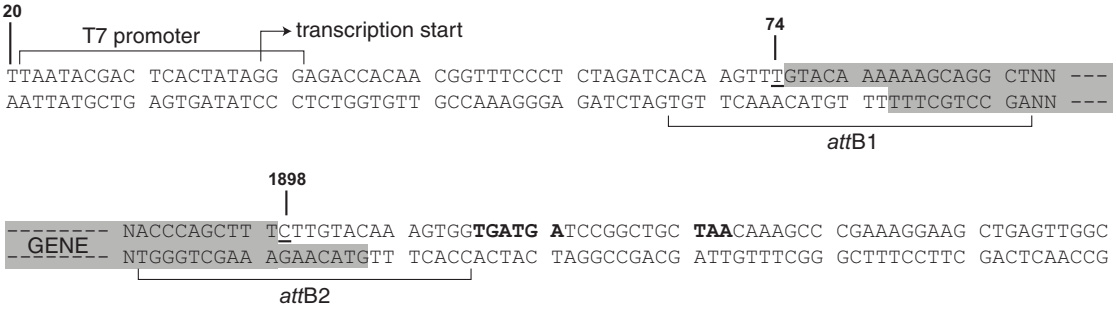
Creating an Expression Clone, continued

Recombination Region of pDEST™14

The recombination region of the expression clone resulting from pDEST™14 x entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pDEST™14 vector by recombination. Non-shaded regions are derived from the pDEST™14 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 74 and 1898, respectively, of the pDEST™14 vector sequence.
- Potential stop codons are indicated in bold.



continued on next page

Creating an Expression Clone, continued

Recombination Region of pDEST™15

The recombination region of the expression clone resulting from pDEST™15 x entry clone is shown below.

Features of the Recombination Region:

- The glutathione S-transferase (GST) gene is marked to help you determine if your gene will be in frame with the GST tag after recombination.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pDEST™15 vector by recombination. Non-shaded regions are derived from the pDEST™15 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 799 and 2482, respectively, of the pDEST™15 vector sequence.
- Potential stop codons are indicated in bold.

continued on next page

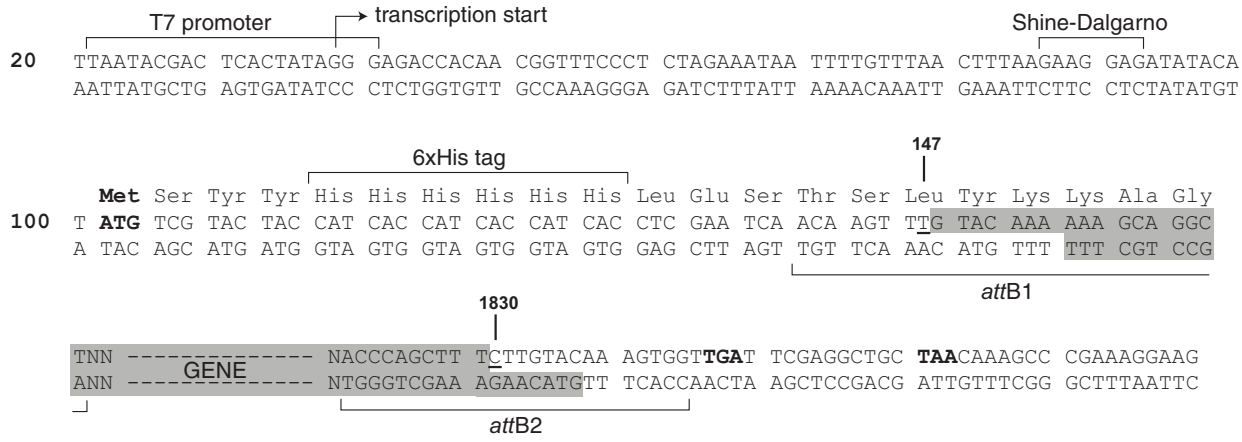
Creating an Expression Clone, continued

Recombination Region of pDEST™17

The recombination region of the expression clone resulting from pDEST™17 x entry clone is shown below.

Features of the Recombination Region:

- The location of the 6xHis tag is indicated to help you determine if your gene will be in frame with the 6xHis tag after recombination.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pDEST™17 vector by recombination. Non-shaded regions are derived from the pDEST™17 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 147 and 1830, respectively, of the pDEST™17 vector sequence.
- Potential stop codons are indicated in bold.



continued on next page

Recombination Region of pDEST™24

The recombination region of the expression clone resulting from pDEST™24 x entry clone is shown below.

Features of the Recombination Region:

- The glutathione S-transferase (GST) gene is marked to help you determine if your gene will be in frame with the GST tag after recombination.
- Shaded regions correspond to those DNA sequences transferred from the entry clone into the pDEST™24 vector by recombination. Non-shaded regions are derived from the pDEST™24 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 78 and 1761, respectively, of the pDEST™24 vector sequence.

```
                T7 promoter      transcriptional start                          78
21    AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC ACAAGTTTGT ACAAAAAAGC AGGC TNN---
                                                 GGAGATCTCG TGTTCAAACA TGTTTTTTCG TCCG ANN---
                                                                              attB1
                          1761                                  Glutathione S-transferase
                Pro Ala Phe Leu Tyr Lys Val Val Ile Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile
      ------ NAC CCA GCT TTC TTG TAC AAA GTG GTG ATT ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT
       GENE
      ------ NTG GGT CGA AAG AAC ATG TTT CAC CAC TAA TAC
                         attB2

      Lys Gly Leu Val Gln Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
1810  AAG GGC CTT GTG CAA CCC ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG

      Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu Gly Leu Glu Phe Pro Asn
1876  TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG GGT TTG GAG TTT CCC AAT

      Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala
1942  CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT

      Asp Lys His Asn Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu Gly Ala
2008  GAC AAG CAC AAC ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA GGA GCG

      Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val
2074  GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT AAA GAC TTT GAA ACT CTC AAA GTT

      Asp Phe Leu Ser Lys Leu Pro Glu Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr
2140  GAT TTT CTT AGC AAG CTA CCT GAA ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT

      Leu Asn Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp Val Val Leu Tyr
2206  TTA AAT GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT GTT GTT TTA TAC

      Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala Ile
2272  ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC

      Pro Gln Ile Asp Lys Tyr Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
2338  CCA CAA ATT GAT AAG TAC TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC

      Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg Pro Trp Gly Ser Gly Cys
2404  ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT CCA TGG GGA TCC GGC TGC

      ***
2470  TAA CAAAGCCC GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA
```

Performing the LR Recombination Reaction

Introduction

Once you have produced an entry clone containing your gene of interest, you are ready to perform an LR recombination reaction between the entry clone and the appropriate pDEST™ vector, and to transform the reaction mixture into Library Efficiency® DH5α™ to select for an expression clone. It is important to have everything you need set up and ready to use to ensure that you obtain the best results. We suggest that you read this section and the one entitled **Transforming Library Efficiency® DH5α™ Cells**, page 14 before beginning. We also recommend that you include a positive control (see below) and a negative control (no LR Clonase™) in your experiment.

Positive Control

The pENTR™-gus plasmid is included in the *E. coli* Expression System with Gateway® Technology for use as a positive control for LR recombination and expression. Use of the pENTR™-gus entry clone in an LR recombination reaction with a pDEST™ vector will allow you to generate an expression clone containing the gene encoding β-glucuronidase (*gus*).

LR Clonase™ II Enzyme Mix

LR Clonase™ II enzyme mix is supplied with the kit (Catalog no. 11824-026 only) or available separately from Invitrogen to catalyze the LR recombination reaction. The LR Clonase™ II enzyme mix combines the proprietary enzyme formulation and 5X LR Clonase™ Reaction Buffer previously supplied as separate components in LR Clonase™ enzyme mix into an optimized single-tube format for easier set-up of the LR recombination reaction. Use the protocol provided on page 13 to perform the LR recombination reaction using LR Clonase™ II enzyme mix.

Note: You may perform the LR recombination reaction using LR Clonase™ enzyme mix, if desired. To use LR Clonase™ enzyme mix, follow the protocol provided with the product. **Do not** use the protocol for LR Clonase™ II enzyme mix provided in this manual as reaction conditions differ.

Materials Needed

You should have the following materials on hand before beginning:

- Purified plasmid DNA of your entry clone (50-150 ng/µl in TE, pH 8.0)
- pDEST™ vector (150 ng/µl in TE, pH 8.0)
- LR Clonase™ II Enzyme Mix (Box 2, keep at -20°C until immediately before use)
- TE Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Proteinase K solution (supplied with the LR Clonase™ II Enzyme Mix; thaw and keep on ice until use)
- pENTR™-gus positive control (50 ng/µl in TE, pH 8.0)

continued on next page

Performing the LR Recombination Reaction, continued

Setting Up the LR Recombination Reaction

Follow this procedure to perform the LR recombination reaction between your entry clone and the destination vector. If you want to include a negative control, set up a separate reaction but omit the LR Clonase™ II enzyme mix.

1. Add the following components to 1.5 ml microcentrifuge tubes at room temperature and mix.

Component	Sample	Positive Control
Entry clone (50-150 ng/reaction)	1-7 µl	--
Destination vector (150 ng/µl)	1 µl	1 µl
pENTR™-gus (50 ng/µl)	--	2 µl
TE Buffer, pH 8.0	to 8 µl	5 µl

2. Remove the LR Clonase™ II Enzyme Mix from -20°C and thaw on ice (~ 2 minutes).
3. Vortex the LR Clonase™ II Enzyme Mix briefly twice (2 seconds each time).
4. To each sample above, add 2 µl of LR Clonase™ II Enzyme Mix. Mix well by pipetting up and down.

   Reminder: Return LR Clonase™ II Enzyme Mix to -20°C immediately after use.
5. Incubate reactions at 25°C for 1 hour.

   Note: For most applications, 1 hour will yield a sufficient number of colonies for analysis. Depending on your needs, the length of the recombination reaction can be extended up to 18 hours. For large plasmids (≥ 10 kb), longer incubation times will yield more colonies.
6. Add 1 µl of Proteinase K solution to each reaction. Incubate for 10 minutes at 37°C.
7. Proceed to **Transforming Library Efficiency® DH5α™ Cells**, next page.

   Note: You may store the LR reaction at -20°C for up to 1 week before transformation, if desired.

Transforming Library Efficiency® DH5α™ Cells

Introduction

Once you have performed the LR recombination reaction, you will transform competent *E. coli*. Library Efficiency® DH5α™ chemically competent *E. coli* (Box 3) are included with the *E. coli* Expression System to facilitate transformation.

Materials Needed

You should have the following materials on hand before beginning:

- LR recombination reaction (from Step 7, previous page)
- Library Efficiency® DH5α™ chemically competent cells (supplied with the kit, Box 3; thaw on ice before use)
- S.O.C. medium (supplied with the kit, Box 3; warm to room temperature)
- pUC19 control (supplied with the kit, Box 3; use as a control for transformation, if desired)
- LB plates containing 100 µg/ml ampicillin (two for each transformation; warm at 37°C for 30 minutes)
- 42°C water bath
- 37°C shaking and non-shaking incubator

Note

Library Efficiency® DH5α™ competent cells are supplied in 5 tubes containing 0.2 ml of competent cells each. Each tube contains enough competent cells to perform 4 transformations using 50 µl of cells per transformation. Once you have thawed a tube of competent cells, discard any unused cells. **Do not** re-freeze cells as freezing and thawing of cells will result in the loss of transformation efficiency.

Transformation Protocol

1. For each transformation, aliquot 50 µl of Library Efficiency® DH5α™ competent cells into a sterile microcentrifuge tube.
2. Add 1 µl of the LR recombination reaction (from **Setting Up the LR Recombination Reaction**, Step 7, previous page) into the tube containing 50 µl of Library Efficiency® DH5α™ competent cells and mix gently. **Do not mix by pipetting up and down.**
3. Incubate on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Immediately transfer the tubes to ice.
6. Add 450 µl of room temperature S.O.C. medium.
7. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
8. Spread 20 µl and 100 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We generally plate 2 different volumes to ensure that at least 1 plate has well-spaced colonies.
9. An efficient LR recombination reaction should produce hundreds of colonies (> 5000 colonies if the entire LR reaction is transformed and plated).

Analyzing Transformants

Analyzing Positive Clones

1. Pick 5 colonies and culture them overnight in LB or SOB medium containing 100 µg/ml ampicillin.
2. Isolate plasmid DNA using your method of choice. We recommend using the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01) or the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01) available from Invitrogen.

   Note: Since pDEST™14, pDEST™15, pDEST™17, and pDEST™24 are low-copy number plasmids, you may need to increase the amount of bacterial culture to obtain enough plasmid DNA for sequencing or analysis purposes. Use extra care during purification to obtain plasmid DNA of sufficiently pure quality for sequencing (see below).
3. Analyze plasmids by restriction analysis to confirm the presence of the insert.

Analyzing Transformants by PCR

You may also analyze positive transformants using PCR. For PCR primers, use a primer that hybridizes within the vector (*e.g.* T7 Promoter Primer; Invitrogen, Catalog no. N560-02) and one that hybridizes within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, you may want to perform restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol below is provided for your convenience. Other protocols are suitable.

Materials Needed:

PCR SuperMix High Fidelity (Invitrogen, Catalog no. 10790-020)

Appropriate forward and reverse PCR primers (20 µM each)

Procedure:

1. For each sample, aliquot 48 µl of PCR SuperMix High Fidelity into a 0.5 ml microcentrifuge tube. Add 1 µl each of the forward and reverse PCR primer.
2. Pick 5 colonies and resuspend them individually in 50 µl of the PCR SuperMix containing primers (remember to make a patch plate to preserve the colonies for further analysis).
3. Incubate reaction for 10 minutes at 94°C to lyse cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at +4°C.
6. Visualize by agarose gel electrophoresis.

Confirming the Expression Clone

The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccdB* gene will be ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 µg/ml chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Sequencing

Optional: To confirm that your gene of interest is in frame with the appropriate tags (if any), you may sequence your expression construct.

General Guidelines for Expression

Introduction

BL21-AI™ One Shot® *E. coli* are included with the *E. coli* Expression System with Gateway® Technology (Box 4) for use as the host for expression. You will need purified plasmid DNA of your pDEST™ expression construct to transform into BL21-AI™. Since each recombinant protein has different characteristics that may affect expression, we recommend performing a time course of expression to determine the best conditions to express your protein.

BL21-AI™ Strain

The BL21-AI™ *E. coli* strain is specifically designed for recombinant protein expression from any T7-based expression vector. Because T7 RNA polymerase levels can be tightly regulated by L-arabinose, the BL21-AI™ strain is especially suited to express genes that may be toxic to other BL21 strains where basal expression of T7 RNA polymerase is leakier.

Each time you perform an expression experiment, you will transform your plasmid into BL21-AI™. **Do not use this strain for propagation and maintenance of your plasmid. Use a general cloning strain (*e.g.* DH5α™) instead.**

Plasmid Preparation

Prepare plasmid DNA using your method of choice. We recommend using the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01) or the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01) for isolation of plasmid DNA. Note that since you are purifying a low-copy number plasmid, you should increase the amount of bacterial culture used to prepare your plasmid construct.

Choosing a Selection Agent

For most purposes, ampicillin works well for selection of transformants and expression experiments. However, if you find that your expression level is low, you may want to use carbenicillin instead. The resistance gene for ampicillin encodes a protein called β-lactamase. This protein is secreted into the medium where it hydrolyzes ampicillin, inactivating the antibiotic. Since β-lactamase is catalytic, ampicillin is rapidly removed from the medium, resulting in non-selective conditions. If your plasmid is unstable, this may result in the loss of plasmid and low expression levels.

Using Carbenicillin

Carbenicillin is generally more stable than ampicillin, and studies have shown that using carbenicillin in place of ampicillin may help to increase expression levels by preventing loss of the pDEST™ expression plasmid. If you wish to use carbenicillin, perform your transformation and expression experiments in LB containing 50 µg/ml carbenicillin.

Note: If your gene is highly toxic, increasing the concentration of carbenicillin used from 50 µg/ml to 200 µg/ml may help to increase expression levels.

Transforming BL21-AI™ One Shot® Cells

Modulating Gene Expression

To modulate expression of your gene of interest in BL21-AI™ cells, use:

- L-arabinose to induce expression of T7 RNA polymerase. L-arabinose is supplied with the BL21-AI™ cells, but is also available from Sigma (Catalog no. A3256).
- Glucose to repress **basal** transcription of T7 RNA polymerase and thereby, your gene of interest (optional). Add to plates and/or media (to a final concentration of 0.1% glucose), if needed.

Materials to Have on Hand

Be sure to have the following solutions and equipment on hand before starting the transformation procedure:

- Purified DNA of your pDEST™ expression clone (1-10 ng/µl)
- BL21-AI™ One Shot® chemically competent cells (supplied with the kit, Box 4; use one vial per transformation)
- pUC19 control (supplied with the kit, Box 4; use as a control for transformation if desired)
- S.O.C. Medium (supplied with the kit, Box 4; warm to room temperature)
- LB plates containing 100 µg/ml ampicillin or 50 µg/ml carbenicillin (2 plates for each transformation; prewarm to 37°C for 30 minutes
- 37°C incubator (shaking and nonshaking)
- 42°C water bath

BL21-AI™ One Shot® Transformation Procedure

Follow the instructions below to transform your expression construct into BL21-AI™ One Shot® cells. If you are including the pUC19 control, transform 10 pg of DNA. You will need one vial of cells per transformation.

1. Thaw on ice, one vial of BL21-AI™ One Shot® cells per transformation.
2. Add 5-10 ng DNA, in a volume of 1-5 µl, into each vial of BL21-AI™ One Shot® cells and mix by tapping gently. **Do not mix cells by pipetting up and down.**
3. Incubate on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Immediately transfer the tubes to ice.
6. Add 250 µl of room temperature S.O.C. Medium.
7. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 30 minutes.
8. Spread 20 µl and 100 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We generally plate 2 different volumes to ensure that at least 1 plate has well-spaced colonies.
9. Select a transformant and proceed to **Pilot Expression**, next page. **Note:** Expression can vary between clones. You may wish to characterize additional transformants.

continued on next page

Expressing Your Recombinant Protein

Materials to Have on Hand

Be sure to have the following solutions and equipment on hand before starting the expression experiment:

- LB media containing 100 µg/ml ampicillin or 50 µg/ml carbenicillin
- 37°C shaking incubator
- 20% L-arabinose (supplied with the kit, Box 4)
- 20% glucose (if needed; prepare in sterile, deionized water)
- Lysis Buffer (see page 29 for a recipe)
- Liquid nitrogen
- 1X and 2X SDS-PAGE sample buffer (see page 29 for a recipe)
- Reagents and apparatus for SDS-PAGE gel (see the next page)
- Boiling water bath
- Sterile water

Pilot Expression

1. Pick 3 or 4 transformants from **BL21-AI™ One Shot® Transformation Procedure**, Step 8, page 17 and culture them in 5 ml of LB medium containing 100 µg/ml ampicillin or 50 µg/ml carbenicillin. Grow at 37°C with shaking until the OD_{600} reaches 0.6 to 1.0.
2. Use these cultures to inoculate fresh LB medium containing 100 µg/ml ampicillin or 50 µg/ml carbenicillin to an OD600 of 0.05-0.1 (~1:20 dilution of the initial culture). This dilution allows the cells to quickly return to logarithmic growth and reach the appropriate cell density. Use a volume appropriate for taking time points, if desired.
3. Grow the cultures until they reach mid-log phase (OD_{600}=~0.4, 2 to 3 hours).
4. Split each culture into two cultures. Add L-arabinose to a final concentration of 0.2% to one of the cultures. You will now have two cultures: one induced, one uninduced.
5. Remove a 500 µl aliquot from **each** culture, centrifuge at maximum speed in a microcentrifuge for 30 seconds, and aspirate the supernatant.
6. Freeze the cell pellets at -20°C. These are the zero time point samples.
7. Continue to incubate the cultures at 37°C with shaking. Take time points for each culture every hour for 2 to 4 hours.
8. For each time point, remove 500 µl from the induced and uninduced cultures and process as described in Steps 5 and 6. Proceed to the next section.

continued on next page

Expressing Your Recombinant Protein, continued

Preparing Samples

Before starting, prepare SDS-PAGE gels or use one of the pre-cast polyacrylamide gels available from Invitrogen (see below) to analyze the samples you collected. If you wish to analyze your samples for soluble protein, see the next section.

1. When all the samples have been collected from Steps 5 and 7, previous page, resuspend each cell pellet in 80 µl of 1X SDS-PAGE sample buffer.
2. Boil 5 minutes and centrifuge briefly.
3. Load 5-10 µl of each sample on an SDS-PAGE gel and electrophorese. Save your samples by storing them at -20°C.

Preparing Samples for Soluble/Insoluble Protein

1. Thaw and resuspend each pellet in 500 µl of Lysis Buffer (see **Recipes**, page 29).
2. Freeze sample in dry ice or liquid nitrogen and then thaw at 42°C. Repeat 2 to 3 times. **Note:** To facilitate lysis, you may need to add lysozyme or sonicate the cells.
3. Centrifuge samples at maximum speed in a microcentrifuge for 1 minute at +4°C to pellet insoluble proteins. Transfer supernatant to a fresh tube and store on ice.
4. Mix together equivalent amounts of supernatant and 2X SDS-PAGE sample buffer and boil for 5 minutes.
5. Add 500 µl of 1X SDS-PAGE sample buffer to the pellets from Step 3 and boil 5 minutes.
6. Load 10 µl of the supernatant sample and 5 µl of the pellet sample onto an SDS-PAGE gel and electrophorese.

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your recombinant fusion protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. In addition, Invitrogen also carries a large selection of molecular weight protein standards and staining kits. For more information about the appropriate gels, standards, and stains to use to visualize your recombinant protein, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 30).

Analyzing Samples

To determine the success of your expression experiment, you may want to perform the following types of analyses:

1. Stain the polyacrylamide gel with Coomassie blue and look for a band of increasing intensity in the expected size range for the recombinant protein. Use the uninduced culture as a negative control.
2. Perform a western blot to confirm that the overexpressed band is your desired protein. You will need to have an antibody to your protein of interest. **Note:** If you are expressing your protein from pDEST™15 or pDEST™24, you may use an antibody to GST to detect your protein.

continued on next page

Expressing Your Recombinant Protein, continued

Note

Expression of your protein with the N- or C-terminal tags will increase the size of your recombinant protein. The table below lists the increase in the molecular weight of your recombinant fusion protein that you should expect from the tag in each pDEST™ vector. Be sure to account for any additional amino acids between the fusion tag and the start of your protein.

Vector	Fusion Tag	Expected Size Increase (kDa)
pDEST™15	N-terminal	27.7
pDEST™17	N-terminal	2.6
pDEST™24	C-terminal	27.9

Purifying Recombinant Protein

- The presence of the N-terminal 6xHis tag in pDEST™17 allows affinity purification of recombinant fusion protein using a nickel-chelating resin such as ProBond™ or Ni-NTA. ProBond™ and Ni-NTA resin are available separately from Invitrogen (see page viii for ordering information). Refer to the ProBond™ or Ni-NTA manual, as appropriate, for guidelines to purify your protein. Both manuals are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).
- The presence of the N-terminal or C-terminal GST tag in pDEST™15 and pDEST™24, respectively allows purification of recombinant fusion protein using glutathione agarose. Refer to the manufacturer's instructions to purify your protein.

Troubleshooting Expression

Introduction

Use the information below to troubleshoot your expression experiment.

No Expression

Sequence your construct and make sure it is in frame with the N-terminal or C-terminal tag, as appropriate.

Low Expression Due to Plasmid Instability

If you are using ampicillin for selection in your expression experiments and see low levels of expression, you may be experiencing plasmid instability due to the absence of selective conditions. This occurs as the ampicillin is destroyed by β-lactamase or hydrolyzed under the acidic media conditions generated by bacterial metabolism. You may want to substitute carbenicillin for ampicillin in your transformation and expression experiments (see page 16 for more information).

Low Expression Due to Toxicity

When expressing recombinant proteins in the BL21-AI™ strain, one can generally assume that the recombinant protein is toxic to bacterial cells when any of the following occurs:

- No transformants are obtained after following the **BL21-AI™ One Shot® Transformation Procedure**, page 17 **or** a combination of large and small, irregular colonies appears on the plate
- The initial culture does not grow (see Step 1 of **Pilot Expression**, page 18)
- It takes longer than 5 hours after a 1:20 dilution of the initial culture for the fresh culture to reach an OD_{600}=0.4 (see Steps 2 and 3 of **Pilot Expression**, page 18)
- The cells lyse after induction with L-arabinose (see Step 4 of **Pilot Expression**, page 18)

Precautions

Several precautions may be taken to prevent problems resulting from basal level expression of a toxic gene of interest (see below). These methods all assume that the T7-based expression plasmid has been correctly designed and created.

- Propagate and maintain your expression plasmid in a strain that does not contain T7 RNA polymerase (*i.e.* DH5α).
- Perform a fresh transformation of BL21-AI™ cells before each expression experiment.
- After following the transformation protocol on page 17, plate the transformation reaction on LB plates containing 100 µg/ml ampicillin and 0.1% glucose. The presence of glucose represses basal expression of T7 RNA polymerase.
- Following transformation of BL21-AI™ cells using the protocol on page 17, pick 3 or 4 transformants and inoculate directly into fresh LB medium containing 100 µg/ml ampicillin or 50 µg/ml carbenicillin (and 0.1% glucose, if desired). When the culture reaches OD_{600}=0.4, induce expression of the recombinant protein by adding L-arabinose to a final concentration of 0.2%.
- When performing expression experiments, supplement the growth medium with 0.1% glucose in addition to 0.2% arabinose.

Appendix

Regulation by L-Arabinose

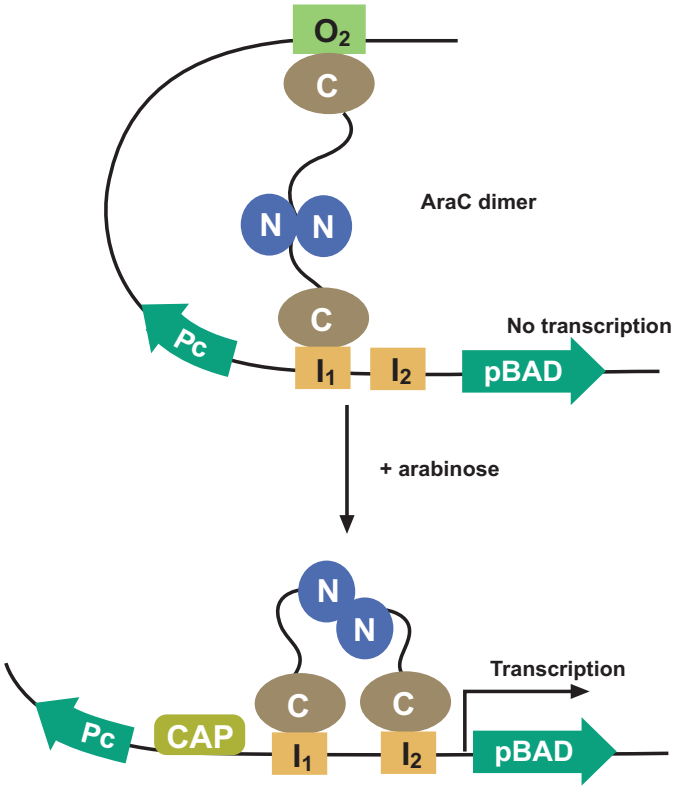
Introduction

The L-arabinose regulatory circuit is briefly described below.

Regulation of the *araBAD* (P_{BAD}) Promoter

The *ara*BAD promoter (P_{BAD}) used to control expression of T7 RNA polymerase in BL21-AI™ is both positively and negatively regulated by the product of the *ara*C gene (Ogden *et al.*, 1980; Schleif, 1992). AraC is a transcriptional regulator that forms a complex with L-arabinose. In the absence of L-arabinose the AraC dimer contacts the O_2 and I_1 half sites of the *ara*BAD operon, forming a 210 bp DNA loop (see figure below). For maximum transcriptional activation two events are required.

- L-Arabinose binds to AraC and causes the protein to release the O_2 site and bind the I_2 site that is adjacent to the I_1 site. This releases the DNA loop and allows transcription to begin.
- The cAMP activator protein (CAP)-cAMP complex binds to the DNA and stimulates binding of AraC to I_1 and I_2.



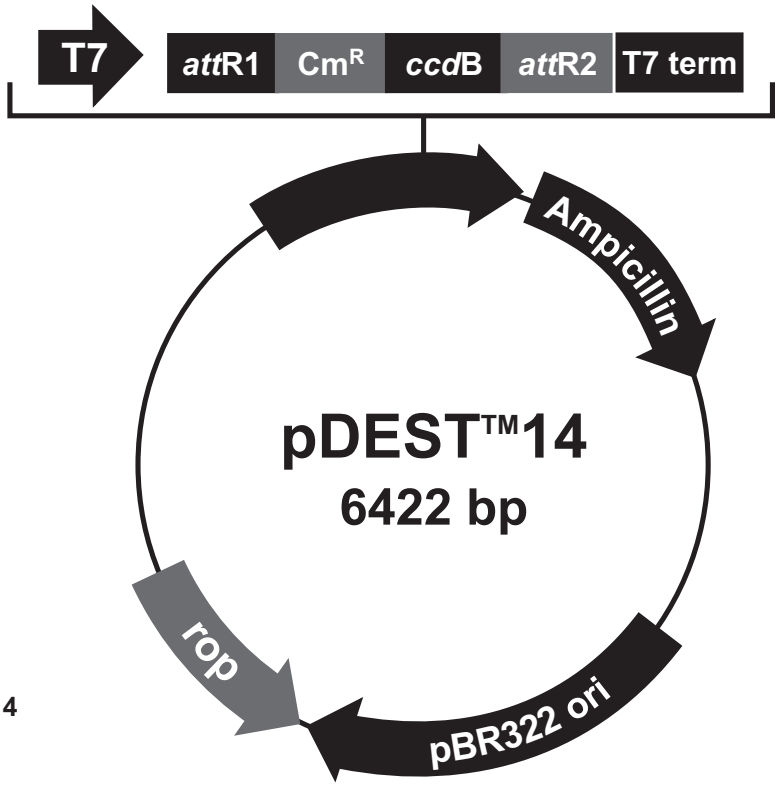
Glucose Repression

Basal expression levels can be repressed by introducing glucose to the growth medium. Glucose acts by lowering cAMP levels, which in turn decreases the binding of CAP. As cAMP levels are lowered, transcriptional activation is decreased.

Map and Features of the pDEST™ Vectors

pDEST™14 Map

The map below shows the elements of pDEST™14. DNA from the entry clone replaces the region between bases 74 and 1898. **The complete sequence for pDEST™14 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).**



Comments for pDEST™14
6422 nucleotides

T7 promoter: bases 21-40
*att*R1: bases 67-191
Chloramphenicol resistance gene (Cm^R): bases 441-1100
*ccd*B gene: bases 1442-1747
*att*R2: bases 1788-1912
T7 transcription termination region: bases 1923-2051
bla promoter: bases 2539-2637
Ampicillin (*bla*) resistance gene: bases 2638-3498
pBR322 origin: bases 3643-4316
ROP ORF: bases 4687-4878 (C)

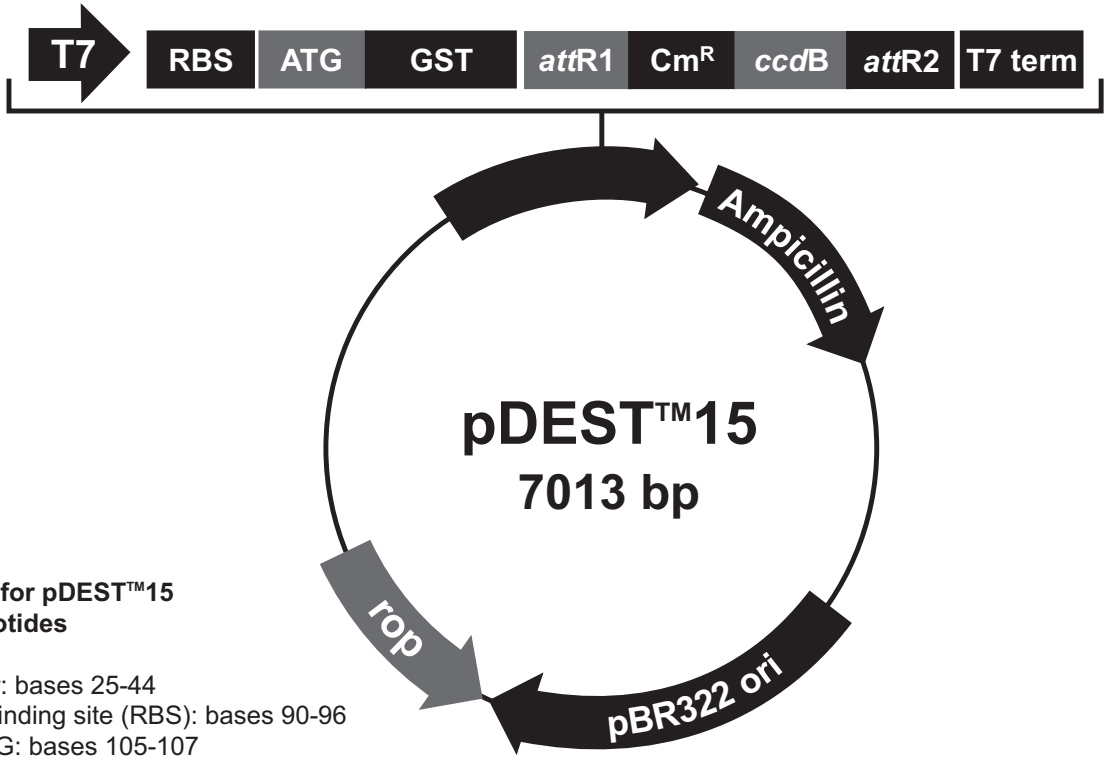
C=complementary strand

continued on next page

Map and Features of the pDEST™ Vectors, continued

pDEST™15 Map

The map below shows the elements of pDEST™15. DNA from the entry clone replaces the region between bases 799 and 2482. **The complete sequence for pDEST™15 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).**



Comments for pDEST™15
7013 nucleotides

T7 promoter: bases 25-44
Ribosome binding site (RBS): bases 90-96
Initiation ATG: bases 105-107
GST tag: bases 108-776
*att*R1: bases 792-916
Chloramphenicol resistance gene (Cm^R): bases 1025-1684
*ccd*B gene: bases 2026-2331
*att*R2: bases 2372-2496
T7 transcription termination region: bases 2518-2646
bla promoter: bases 3134-3232
Ampicillin (*bla*) resistance gene: bases 3233-4093
pBR322 origin: bases 4238-4911
ROP ORF: bases 5282-5473 (C)

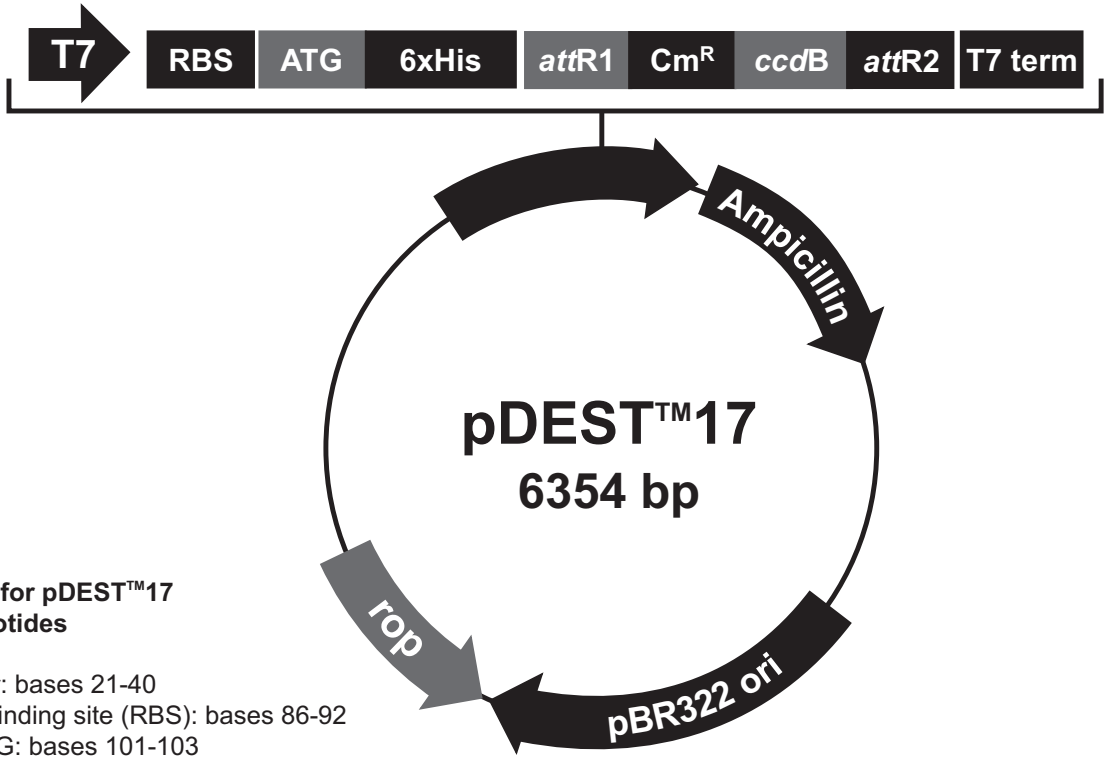
C=complementary strand

continued on next page

Map and Features of the pDEST™ Vectors, continued

pDEST™17 Map

The map below shows the elements of pDEST™17. DNA from the entry clone replaces the region between bases 147 and 1830. **The complete sequence for pDEST™17 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).**



Comments for pDEST™17
6354 nucleotides

T7 promoter: bases 21-40
Ribosome binding site (RBS): bases 86-92
Initiation ATG: bases 101-103
6xHis tag: bases 113-130
*att*R1: bases 140-264
Chloramphenicol resistance gene (Cm^R): bases 373-1032
*ccd*B gene: bases 1374-1679
*att*R2: bases 1720-1844
T7 transcription termination region: bases 1855-1983
bla promoter: bases 2471-2569
Ampicillin (*bla*) resistance gene: bases 2570-3430
pBR322 origin: bases 3575-4248
ROP ORF: bases 4619-4810 (C)

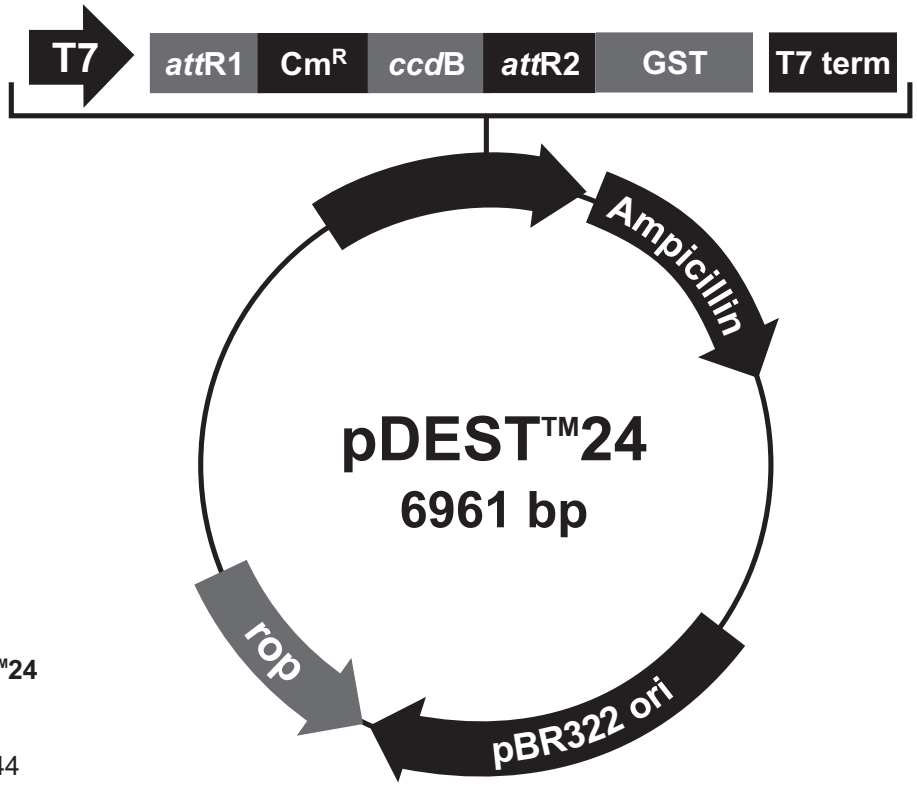
C=complementary strand

continued on next page

Map and Features of the pDEST™ Vectors, continued

pDEST™24 Map

The map below shows the elements of pDEST™24. DNA from the entry clone replaces the region between bases 78 and 1761. **The complete sequence for pDEST™24 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).**



Comments for pDEST™24
6961 nucleotides

T7 promoter: bases 25-44
*att*R1: bases 71-195
Chloramphenicol resistance gene (Cm^R): bases 304-963
*ccd*B gene: bases 1305-1610
*att*R2: bases 1651-1775
GST tag: bases 1783-2451
T7 transcription termination region: bases 2466-2594
bla promoter: bases 3082-3180
Ampicillin (*bla*) resistance gene: bases 3181-4041
pBR322 origin: bases 4186-4859
ROP ORF: bases 5230-5421 (C)

C=complementary strand

continued on next page

Map and Features of the pDEST™ Vectors, continued

Features of the Vectors

pDEST™14 (6422 bp), pDEST™15 (7013 bp), pDEST™17 (6354 bp), and pDEST™24 (6961 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
T7 promoter	Permits high-level, IPTG-inducible expression of your recombinant protein in *E. coli* strains expressing the T7 RNA polymerase.
Ribosome binding site (*i.e.* Shine-Dalgarno sequence) **(in pDEST™15 and pDEST™17 only)**	Optimally spaced from the initiation ATG in the N-terminal tag for efficient translation of the PCR product.
N-terminal glutathione *S*-transferase (GST) tag **(in pDEST™15 only)**	Allows affinity purification of recombinant fusion protein using glutathione agarose
N-terminal 6xHis tag **(in pDEST™17 only)**	Permits affinity purification of recombinant fusion protein using a metal-chelating resin such as ProBond™ or Ni-NTA
*att*R1 and *att*R2 sites	Bacteriophage λ-derived DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Landy, 1989).
Chloramphenicol resistance gene (Cm^R)	Allows counterselection of the plasmid.
*ccd*B gene	Permits negative selection of the plasmid.
C-terminal glutathione *S*-transferase (GST) tag **(in pDEST™24 only)**	Allows affinity purification of recombinant fusion protein using glutathione agarose
T7 transcription termination region	Sequence from bacteriophage T7 that permits efficient transcription termination.
bla promoter	Allows expression of the ampicillin resistance gene.
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*.
pBR322 origin of replication (*ori*)	Permits replication and maintenance in *E. coli*.
ROP ORF	Interacts with the pBR322 origin to facilitate low-copy replication in *E. coli.*

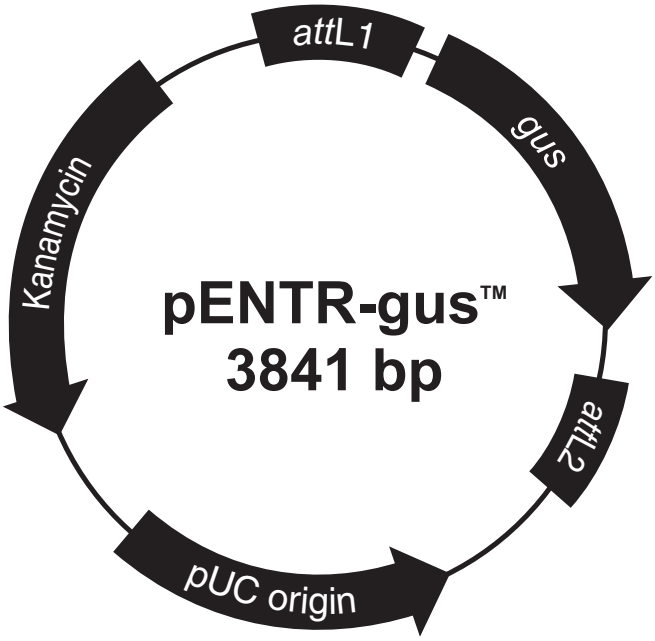
Map of pENTR™-gus

Description

pENTR™-gus is a 3841 bp entry clone containing the *Arabidopsis thaliana* gene for β-glucuronidase (*gus*) (Kertbundit *et al.*, 1991). The *gus* gene was amplified using PCR primers containing *att*B recombination sites. The amplified PCR product was then used in a BP recombination reaction with pDONR201™ to generate the entry clone. For more information about the BP recombination reaction, refer to the Gateway® Technology with Clonase™ II manual.

Map of Control Vector

The figure below summarizes the features of the pENTR™-gus vector. **The complete sequence for pENTR™-gus is available from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).**



Comments for pENTR-gus™
3841 nucleotides

*att*L1: bases 99-198 (complementary strand)
gus gene: bases 228-2039
*att*L2: bases 2041-2140
pUC origin: bases 2200-2873 (C)
Kanamycin resistance gene: bases 2990-3805 (C)

C = complementary strand

Recipes

Lysis Buffer

50 mM potassium phosphate, pH 7.8
400 mM NaCl
100 mM KCl
10% glycerol
0.5% Triton X-100
10 mM imidazole

1. Prepare 1 M stock solutions of KH_2PO_4 and K_2HPO_4.
2. For 100 ml, dissolve the following reagents in 90 ml of deionized water:

   0.3 ml KH_2PO_4
   4.7 ml K_2HPO_4
   2.3 g NaCl
   0.75 g KCl
   10 ml glycerol
   0.5 ml Triton X-100
   68 mg imidazole
3. Mix thoroughly and adjust pH to 7.8 with HCl. Bring the volume to 100 ml.
4. Store at +4°C.

2X SDS-PAGE Sample Buffer

1. Combine the following reagents:

0.5 M Tris-HCl, pH 6.8	2.5 ml
Glycerol (100%)	2.0 ml
β-mercaptoethanol	0.4 ml
Bromophenol Blue	0.02 g
SDS	0.4 g

2. Bring the volume to 10 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

1X SDS-PAGE Sample Buffer

1. Combine the following reagents:

0.5 M Tris-HCl, pH 6.8	1.25 ml
Glycerol (100%)	1.0 ml
β-mercaptoethanol	0.2 ml
Bromophenol Blue	0.01 g
SDS	0.2 g

2. Bring the volume to 10 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

Corporate Headquarters:

Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
USA

Tel: 1 760 603 7200

Tel (Toll Free): 1 800 955 6288

Fax: 1 760 602 6500

E-mail: tech_service@invitrogen.com

Japanese Headquarters:

Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park
Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi

Tel: 81 3 3663 7972

Fax: 81 3 3663 8242

E-mail: jpinfo@invitrogen.com

European Headquarters:

Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100

Tech Fax: +44 (0) 141 814 6117

E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

continued on next page

Technical Service, continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Introduction

Use of the *E. coli* Expression System with Gateway® Technology is covered under the licenses detailed below.

Information for European Customers

The BL21-AI™ *E. coli* strain is genetically modified and carries a chromosomal insertion of a cassette containing the T7 RNA polymerase (T7 RNAP) gene. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Limited Use Label License No. 19: Gateway® Cloning Products

This product and its use is the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, and 6,277,608 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for profit entity). The purchase of this product does not convey a license under any method claims in the foregoing patents or patent applications, or to use this product with any recombination sites other than those purchased from Invitrogen Corporation or its authorized distributor. The right to use methods claimed in the foregoing patents or patent applications with this product for research purposes only can only be acquired by the use of Clonase™ purchased from Invitrogen Corporation or its authorized distributors. The buyer cannot modify the recombination sequence(s) contained in this product for any purpose. The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the employment of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Notwithstanding the preceding, any buyer who is employed in an academic or government institution may transfer materials made with this product to a third party who has a license from Invitrogen under the patents identified above to distribute such materials. Transfer of such materials and/or information to collaborators does not convey rights to practice any methods claimed in the foregoing patents or patent applications. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that none of (i) this product, (ii) any of its components, or (iii) a method claim of the foregoing patents, was used in the manufacture of such product. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the use of this product to manufacture a protein for sale, provided that no method claim in the above patents was used in the manufacture of such protein. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to use this product for purposes other than those permitted above, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200.

continued on next page

Purchaser Notification, continued

Gateway® Clone Distribution Policy

For additional information about Invitrogen's policy for the use and distribution of Gateway® clones, see the section entitled **Gateway® Clone Distribution Policy**, page 34.

Limited Use Label License No. 22: Vectors and Clones Encoding Histidine Hexamer

This product is licensed under U.S. Patent Nos. 5,284,933 and 5,310,663 and foreign equivalents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

Limited Use Label License No. 23: GUS Control Vector

The GUS positive control vector in these products is claimed in patents and patent applications (See U.S. Patent No. 5,599,670 and Great Britain Patent No. 2,197,653) licensed to Invitrogen by Cambia Biosystems, L.L.C. ("CBL"). Use of the GUS gene is restricted to use as a positive control. Any other use may require a license from CBL.

Limited Use Label License No. 30: T7 Expression System

The composition and/or use of this product may be claimed in one or more patents U.S. Patent Nos. 4,952,496 and 5,693,489 and 5,869,320 licensed to Invitrogen Corporation, by Brookhaven Science Associates, LLC. The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy, and is the subject of patents and patent applications assigned to Brookhaven Science Associates, LLC (BSA,). By provisions of the Distribution License Agreement granted to Invitrogen covering said patents and patent applications, Invitrogen grants you a non-exclusive sub-license under patents assigned to BSA for the use of this technology, including the enclosed materials, based upon the following conditions: 1 – these materials are to be used for non-commercial research purposes only. A separate license under patents owned by BSA is required for any commercial use, including the use of these materials for research purposes or production purposes by any commercial entity. Information about commercial license may be obtained from The Office of Technology Transfer, Brookhaven National Laboratory, Bldg. 475D, P.O. Box 5000, Upton, New York 11973-5000. Phone (516) 344-7134. 2 - No materials that contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this sub-license and agrees to be bound by its terms. This limitation applies to strains BL21(DE3), BL21(DE3)pLysS and BL21(DE3)pLysE, CE6, BL21-SI Competent Cells and any derivatives that are made of them. You may refuse this sub-license by returning this product unused in which case Invitrogen accept return of the product with a full refund. By keeping or using this product, you agree to be bound by the terms of this license.

Limited Use Label License No. 48: *ara*B Promoter

Products containing the *araB* promoter are the subject of U.S. Patent No. 5,028,530 and foreign equivalents and sold under patent license for research purposes only and are non-transferable. Inquiries for any commercial use, including production of material to be sold commercially or used in production or in product development efforts, which includes efforts toward regulatory approval, should be made directly to Xoma Corporation, 2910 Seventh Street, Berkeley, CA 94710, Tel: 1-510-644-1170 Fax: 1-510-649-7571.

Limited Use Label License No. 125: GST

This product is the subject of WIPO patent WO8809372 and foreign equivalents to be used for scientific investigation and research and for no other purpose whatsoever. Licenses for commercial use of the above mentioned patents must be negotiated directly with Amrad Corporation, 576 Swan Street, Richmond, Victoria Australia 3121, Telephone: 61 3 9208 4000.

Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Invitrogen's policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Invitrogen's commercially available Gateway® Technology.

Gateway® Entry Clones

Invitrogen understands that Gateway® entry clones, containing *att*L1 and *att*L2 sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by non-profit organizations and by for-profit organizations without royalty payment to Invitrogen.

Gateway® Expression Clones

Invitrogen also understands that Gateway® expression clones, containing *att*B1 and *att*B2 sites, may be generated by academic and government researchers for the purpose of scientific research. Invitrogen agrees that such clones may be distributed for scientific research by academic and government organizations without royalty payment to Invitrogen. Organizations other than academia and government may also distribute such Gateway® expression clones for a nominal fee ($10 per clone) payable to Invitrogen.

Additional Terms and Conditions

We would ask that such distributors of Gateway® entry and expression clones indicate that such clones may be used only for research purposes, that such clones incorporate the Gateway® Technology, and that the purchase of Gateway® Clonase™ from Invitrogen is required for carrying out the Gateway® recombinational cloning reaction. This should allow researchers to readily identify Gateway® containing clones and facilitate their use of this powerful technology in their research. Use of Invitrogen's Gateway® Technology, including Gateway® clones, for purposes other than scientific research may require a license and questions concerning such commercial use should be directed to Invitrogen's licensing department at 760-603-7200.

Product Qualification

Introduction

This section describes the criteria used to qualify the components of the *E. coli* Expression System with Gateway® Technology.

Vectors

The structure of each vector is verified by restriction enzyme digestion. In addition, the functionality of each destination vector is qualified in a recombination assay using Gateway® LR Clonase™ II Enzyme Mix. The *ccd*B gene is assayed by transformation using an appropriate *E. coli* strain.

LR Clonase™ II Enzyme Mix

Gateway® LR Clonase™ II Enzyme Mix is functionally tested in a one hour recombination reaction followed by a transformation assay.

Chemically Competent *E. coli*

1. All competent cells are tested for transformation efficiency using the control plasmid included in the kit. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be:
   - Greater than 1 x 10^8 cfu/µg plasmid DNA for Library Efficiency® DH5α
   - Greater than 1 x 10^8 cfu/µg plasmid DNA for BL21-AI™
2. To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.
3. Untransformed cells are plated on LB plates containing 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

References

Grodberg, J., and Dunn, J. J. (1988). *ompT* Encodes the *Escherichia coli* Outer Membrane Protease that Cleaves T7 RNA Polymerase During Purification. J. Bacteriol. *170*, 1245-1253.

Kertbundit, S., Greve, H. d., Deboeck, F., Montagu, M. V., and Hernalsteens, J. P. (1991). *In vivo* Random β-glucuronidase Gene Fusions in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA *88*, 5212-5216.

Landy, A. (1989). Dynamic, Structural, and Regulatory Aspects of Lambda Site-specific Recombination. Ann. Rev. Biochem. *58*, 913-949.

Lee, N. (1980) Molecular Aspects of *ara* Regulation. In *The Operon*, J. H. Miller and W. S. Reznikoff, eds. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory), pp. 389-410.

Lee, N., Francklyn, C., and Hamilton, E. P. (1987). Arabinose-Induced Binding of AraC Protein to $araI_2$ Activates the *ara*BAD Operon Promoter. Proc. Natl. Acad. Sci. USA *84*, 8814-8818.

Miyada, C. G., Stoltzfus, L., and Wilcox, G. (1984). Regulation of the *ara*C Gene of *Escherichia coli*: Catabolite Repression, Autoregulation, and Effect on *ara*BAD Expression. Proc. Natl. Acad. Sci. USA *81*, 4120-4124.

Ogden, S., Haggerty, D., Stoner, C. M., Kolodrubetz, D., and Schleif, R. (1980). The *Escherichia coli* L-Arabinose Operon: Binding Sites of the Regulatory Proteins and a Mechanism of Positive and Negative Regulation. Proc. Natl. Acad. Sci. USA *77*, 3346-3350.

Rosenberg, A. H., Lade, B. N., Chui, D.-S., Lin, S.-W., Dunn, J. J., and Studier, F. W. (1987). Vectors for Selective Expression of Cloned DNAs by T7 RNA Polymerase. Gene *56*, 125-135.

Schleif, R. S. (1992). DNA Looping. Ann. Rev. Biochem. *61*, 199-223.

Shine, J., and Dalgarno, L. (1975). Terminal-Sequence Analysis of Bacterial Ribosomal RNA. Correlation Between the 3'-Terminal-Polypyrimidine Sequence of 16-S RNA and Translational Specificity of the Ribosome. Eur. J. Biochem. *57*, 221-230.

Smith, D. B., Davern, K. M., Board, P. G., Tiu, W. U., Garcia, E. G., and Mitchell, G. F. (1986). Mr 26,000 Antigen of *Schistosoma japonicum* Recognized by Resistant WEHI 129/J Mice is a Parasite Glutathione S-transferase. Proc. Natl. Acad. Sci. USA *83*, 8703-8707.

Studier, F. W., and Moffatt, B. A. (1986). Use of Bacteriophage T7 RNA Polymerase to Direct Selective High-Level Expression of Cloned Genes. J. Mol. Biol. *189*, 113-130.

Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990). Use of T7 RNA Polymerase to Direct Expression of Cloned Genes. Meth. Enzymol. *185*, 60-89.

Notes

Notes

United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, California 92008
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 603 7229
Email: tech_service@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
Email: eurotech@invitrogen.com

International Offices:
Argentina 5411 4556 0844
Australia 1 800 331 627
Austria 0800 20 1087
Belgium 0800 14894
Brazil 0800 11 0575
Canada 800 263 6236
China 10 6849 2578
Denmark 80 30 17 40

France 0800 23 20 79
Germany 0800 083 0902
Hong Kong 2407 8450
India 11 577 3282
Italy 02 98 22 201
Japan 03 3663 7974
The Netherlands 0800 099 3310
New Zealand 0800 600 200
Norway 00800 5456 5456

Spain & Portugal 900 181 461
Sweden 020 26 34 52
Switzerland 0800 848 800
Taiwan 2 2651 6156
UK 0800 838 380
For other countries see our website

www.invitrogen.com