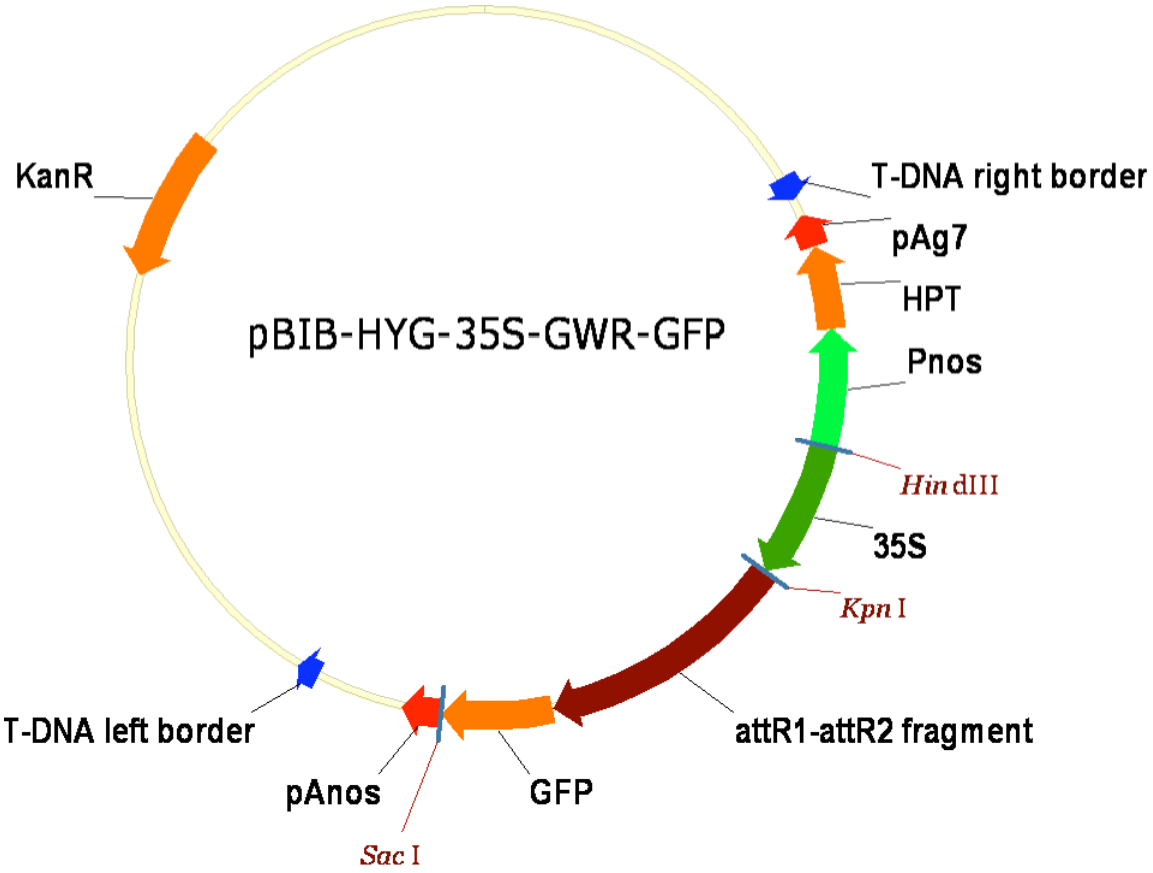
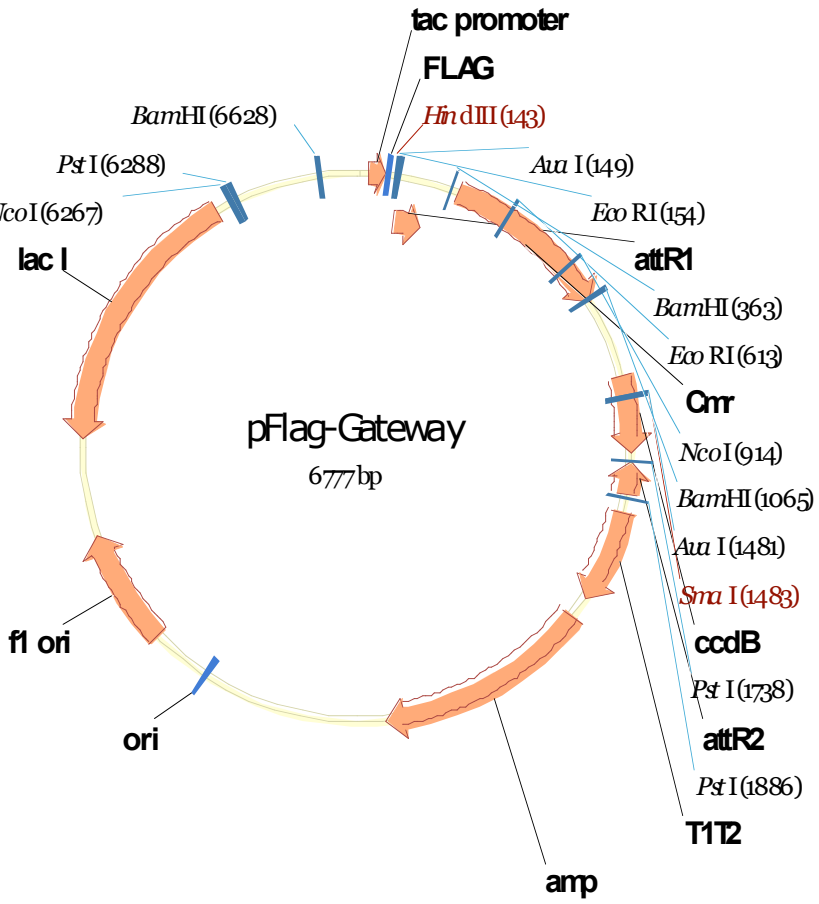
GATEWAY CONSTRUCTS FOR FUNCTIONAL ANALYSIS OF ARABIDOPSIS LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASES

This collection of constructs is a product of the National Science Foundation Arabidopsis 2010 project MCB-0419819, "Functional Analysis and Phosphorylation Site Mapping of Leucine-Rich Repeat Receptor-Like Kinases (LRR RLKs) in Arabidopsis". For more detailed information please visit http://www4.ncsu.edu/~sclouse/Clouse2010.htm. Full-length cDNAs for over 170 LRR RLKs are available in the Gateway donor vector pDONR/zeo (Invitrogen). Cytoplasmic kinase domains (beginning with the first amino acid after the putative transmembrane domain) are available in the same vector for 179 LRR RLKs. A collection of new Gateway-compatible plant transformation vectors and a set of full-length constructs for *in planta* analysis of LRR RLK function in transgenic plants has been generated by Xiaoping Gou and Jia Li, University of Oklahoma (contact lij@ou.edu for details of vector construction). A second set of bacterial expression constructs with N-terminal Flag epitope tags for biochemical analysis of kinase domain function has been generated by Murali Dhundaydham and Steve Clouse at North Carolina State University (contact steve_clouse@ncsu.edu for details of vector construction). All entry clones were fully sequenced to verify accuracy. In most cases our sequence matches that in TAIR 8 exactly, but in several clones it does not and these differences are noted for each case. Vector maps for pH35GWG, pFlag-Gateway and pB35GWF are attached as well as information on Invitrogen's pDONR/Zeo obtained from www.invitrogen.com.


KanR
T-DNA right border
pAg7
HPT
Pnos
pBIB-HYG-35S-GWR-GFP
Hin dIII
35S
Kpn I
attR1-attR2 fragment
T-DNA left border
pAnos
GFP
Sac I

pH35GWG

pFlag-Gateway Bacterial Expression Vector with N-terminal Flag Epitope Tag



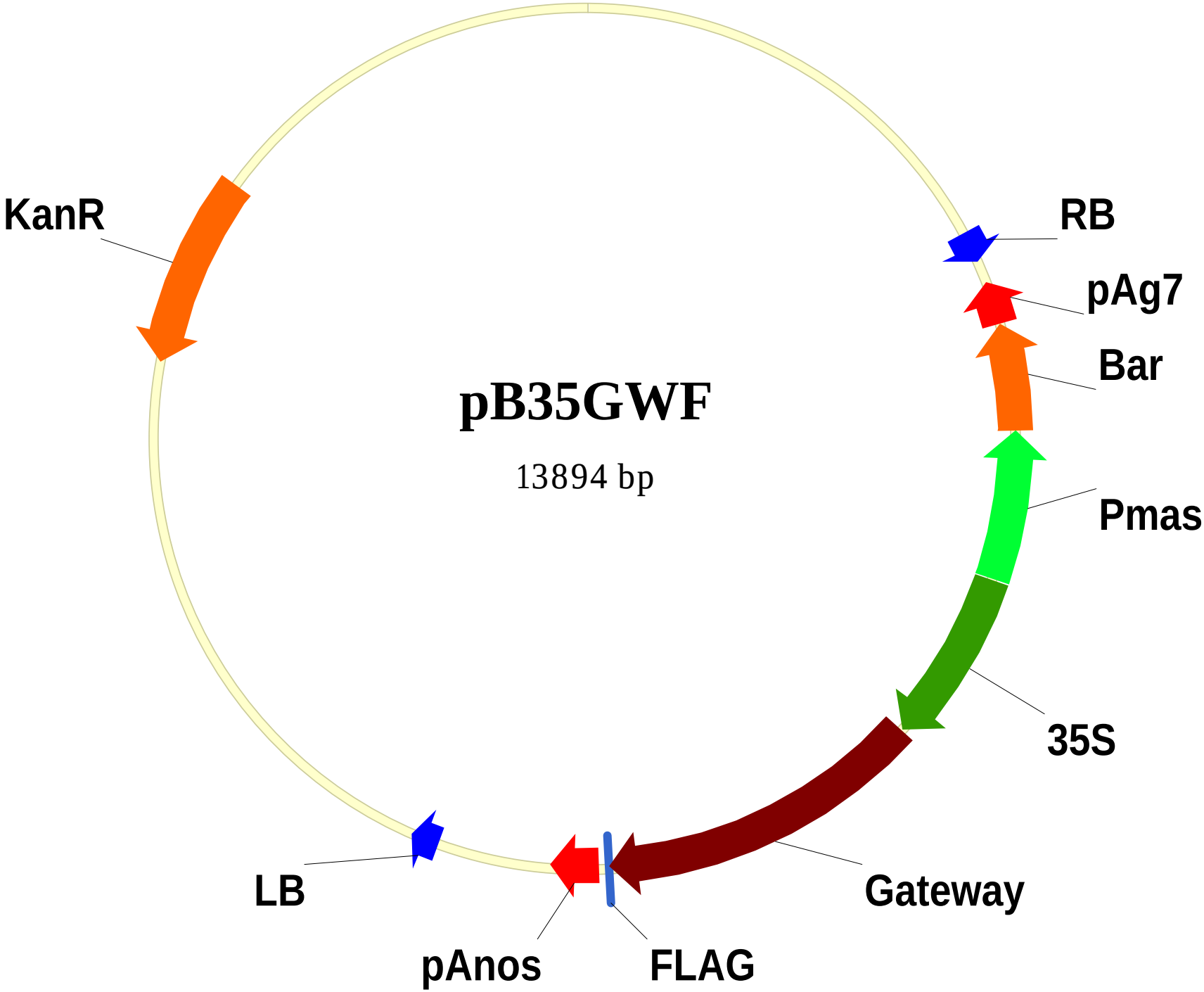
pFlag-Gateway was generated by digesting pFlag-Mac (Sigma Cat. # E5644) with SmaI followed by ligation with the Gateway DNA reading frame cassette C.1 containing attR sequences, chloramphenicol resistance gene and ccdB lethal gene (Invitrogen Cat. # 11828-029). The vector accepts inserts from any Gateway entry clone via the LR clonase reaction. The protein expressed from this vector in *E. coli* will have the following additional N-terminal sequences:

Flag Peptide

```
ATG GAC TAC AAG GAC GAC GAT GAC AAA GTC AAG CTT CTC GAG AAT
Met Asp Tyr Lys Asp Asp Asp Asp Lys Val Lys Leu Leu Glu Asn
```

***att*B1**

```
TCC CAT CAA ACA AGT TTG TAC AAA AAA GCA GGC TTC – INSERT - STOP
Ser His Gln Thr Ser Leu Tyr Lys Lys Ala Gly Phe
```


KanR
RB
pAg7
Bar
pB35GWF
13894 bp
Pmas
35S
Gateway
FLAG
pAnos
LB

Summary of pB35GWF Gateway Plant Expression Vector Features

CaMV 35S promoter sequence:

```
                                  AAGCTTG CATGCCTGCA GGTCAACATG
                                  Hind III Sph I    Pst I
GTGGAGCACG ACACACTTGT CTACTCCAAA AATATCAAAG ATACAGTCTC
AGAAGACCAA AGGGCAATTG AGACTTTTCA ACAAAGGGTA ATATCCGGAA
ACCTCCTCGG ATTCCATTGC CCAGCTATCT GTCACTTTAT TGTGAAGATA
GTGGAAAAGG AAGGTGGCTC CTACAAATGC CATCATTGCG ATAAAGGAAA
GGCCATCGTT GAAGATGCCT CTGCCGACAG TGGTCCCAAA GATGGACCCC
CACCCACGAG GAGCATCGTG GAAAAAGAAG ACGTTCCAAC CACGTCTTCA
AAGCAAGTGG ATTGATGTGA TAACATGGTG GAGCACGACA CACTTGTCTA
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Cauliflower mosaic virus dual 35S promoter

```
CTCCAAAAAT ATCAAAGATA CAGTCTCAGA AGACCAAAGG GCAATTGAGA
CTTTTCAACA AAGGGTAATA TCCGGAAACC TCCTCGGATT CCATTGCCCA
GCTATCTGTC ACTTTATTGT GAAGATAGTG GAAAAGGAAG GTGGCTCCTA
CAAATGCCAT CATTGCGATA AAGGAAAGGC CATCGTTGAA GATGCCTCTG
CCGACAGTGG TCCCAAAGAT GGACCCCCAC CCACGAGGAG CATCGTGGAA
AAAGAAGACG TTCCAACCAC GTCTTCAAAG CAAGTGGATT GATGTGATAT
CTCCACTGAC GTAAGGGATG ACGCACAATC CCACTATCCT TCGCAAGACC
CTTCCTCTAT ATAAGGAAGT TCATTTCATT TGGAGAGGAC CTCGAGAATT
                                             Xho I  EcoR I
CTCAACACAA CATATACAAA ACAAACGAAT CTCAAGCAAT CAAGCATTCT
```

Translational enhancer; 5'UTR from tobacco etch virus

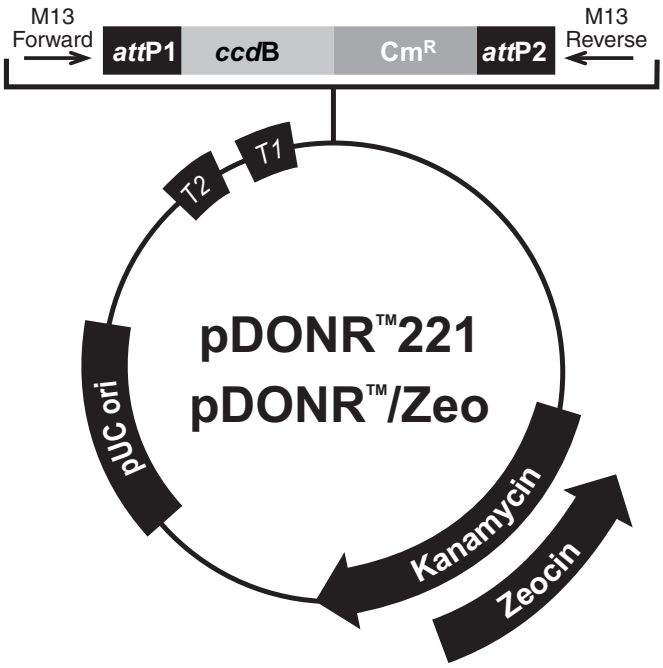
```
ACTTCTATTG CAGCAATTTA AATCATTTCT TTTAAAGCAA AAGCAATTTT
CTGAAAATTT TCACCATTTA CGAACGATAG CCGGTACC
                                 Kpn I
```

Cloned sample sequence (BAK1/At4g33430)-attB1 is next to *Kpn* I site:

```
ACAAGTTTGTACAAAAAAGCAGGCTTCATGGAACGAAGATTAATGATCCCTT---
        attB1                          BAK1 sequence
-CGAAAACGAATACCCCTCGGGTCCAAGAGACCCAGCTTTCTTGTACAAAGTGGT
                                                attB2
GGACTACAAAGACGATGACGACAAATGACTAGGTGAGGAGCTCGAATTTCCCCGAT
          FLAG          *          Vector sequence
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGC
GATGATTAT
```

Notes:

1. An additional nucleotide (such as G here) attached to 5' primer is required to keep the cloned gene in frame with the Flag epitope tag.
2. The backbone of pB35GWF is from the vector pBIB (Becker D. 1990. Binary vectors which allow the exchange of plant selectable markers and reporter genes. Nuc. Ac. Res. 18: 203).
3. The Gateway module and the Flag epitope tag in pB35GWF are from pEarleyGate 302 (ABRC stock CD3-693).
4. The Bar gene and the Pmas promoter are from the vector pSKI015 and are mutated to remove specific restriction sites (http://www.salk.edu/labs/pbio-w/pSKI015.html).



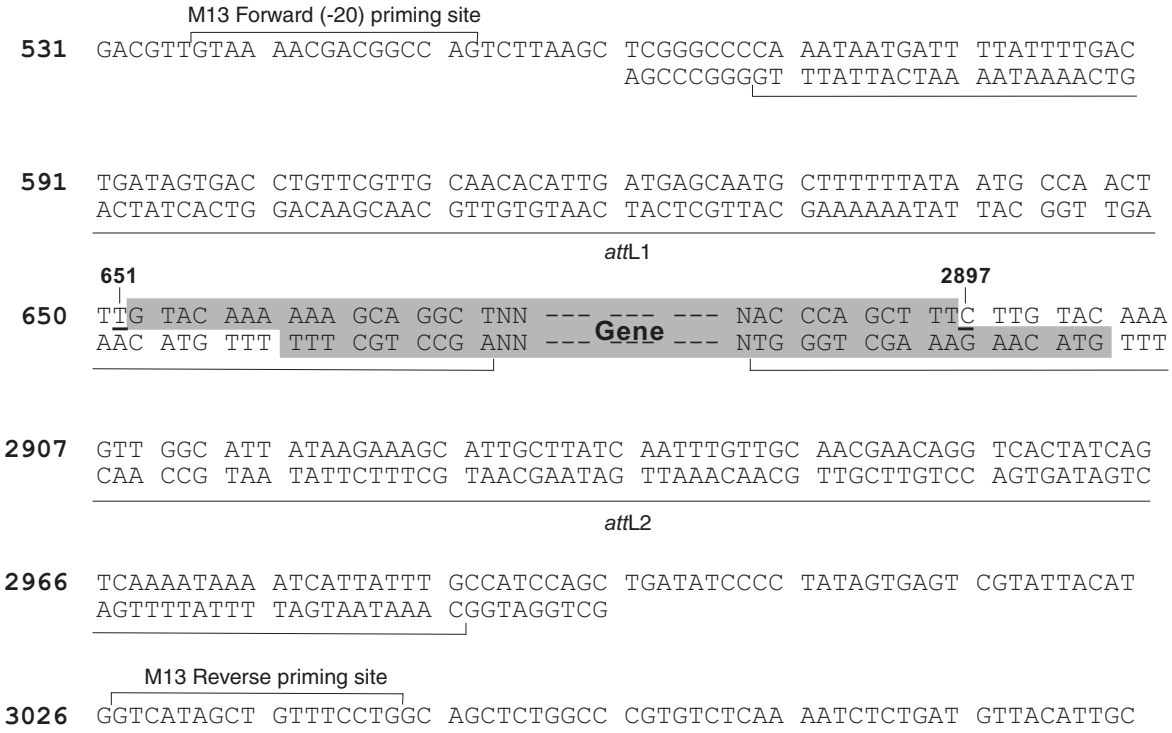
Comments for:	**pDONR™221 4762 nucleotides**	**pDONR™/Zeo 4291 nucleotides**
*rrn*B T2 transcription termination sequence (c):	268-295	268-295
rrnB T1 transcription termination sequence (c):	427-470	427-470
M13 Forward (-20) priming site:	537-552	537-552
*att*P1:	570-801	570-801
*ccd*B gene (c):	1197-1502	1197-1502
Chloramphenicol resistance gene (c):	1847-2506	1847-2506
*att*P2 (c):	2754-2985	2754-2985
T7 Promoter/priming site (c):	3000-3019	3003-3022
M13 Reverse priming site:	3027-3043	3027-3043
Kanamycin resistance gene:	3156-3965	---
EM7 promoter (c):	---	3486-3552
Zeocin resistance gene (c):	---	3111-3485
pUC origin:	4086-4759	3615-4288

invitrogen™

The recombination region of the expression clone resulting from pDONR™221 × entry clone or pDONR™/Zeo × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to DNA sequences transferred from the *att*B substrate into pDONR™221 or pDONR™/Zeo by recombination. Non-shaded regions are derived from the pDONR™221 or pDONR™/Zeo vector.
- Bases 651 and 2897 of the pDONR™221 or pDONR™/Zeo vector sequence are marked.



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CTGGCCTTTTGCTCACATGTT

General Description

DNA pDONR™/Zeo

Entire molecule length: 4291 bp

Restriction/Methylation Map

Enzyme	# of cuts	Positions
AatII	1	3443
AccI	4	804 852 1754 2750
AccIII	1	2293
AciI	32	43 64 71 114(c) 138 148 204(c) 238 324(c) 337 375 384(c) 1246(c) 1585 1780 1841(c) 1855 1949(c) 2313(c) 3175 3278 3330 3429 3449(c) 3694 3703(c) 3838 3948(c) 4069(c) 4088(c) 4215(c) 4243(c)
AcsI	1	2289
AcyI	3	328 3221 3440
AflII	1	555
AflIII	4	231 3551 3590 4286
AluI	19	57 175 560 663 1324 1687 2264 2393 2512 2521 2893 2995 3034 3048 3267 3729 3986 4032 4122
Alw44I	3	1386 3136 3972
AlwI	6	1409 1828(c) 1841 3640(c) 3642 3728
AlwNI	2	1514 3877
ApaI	1	568
ApaLI	3	1386 3136 3972
ApoI	1	2289
AseI	3	167 226 3541
AsnI	3	167 226 3541
Asp700	1	1053
AspEI	3	550 800 2757
AspHI	4	1390 3140 3458 3976
AvaI	4	561 1417 3393 3403
AvaII	4	3321 3327 3360 3415
BamHI	1	1833
BanI	1	3311
BanII	1	568
BbrPI	1	3554
BbsI	1	438(c)
BbvI	12	85 103 184 826(c)

		1703(c) 2724(c) 3057 3173(c) 3658(c) 3864(c) 3867(c) 3957
BclI	2	3109 3559
BfaI	4	241 507 1015 3793
BfrI	1	555
BglI	1	3183
BmyI	5	568 1390 3140 3458 3976
BpmI	1	2173(c)
BpuAI	1	438(c)
BsaAI	2	2600 3554
BsaBI	1	1406
BsaHI	3	328 3221 3440
BsaI	1	1277(c)
BsaJI	14	1395 1417 1988 2057 2058 2636 2637 3123 3181 3291 3297 3393 3483 4126
BsaWI	5	1646 2293 3458 3933 4080
BseAI	1	2293
BsiEI	7	85 808 856 3153 3413 3429 3952
BsiHKAI	4	1390 3140 3458 3976
BsiYI	15	154 346 1279 1417 1598 2064 2631 3292 3297 3298 3324 3808 4087 4253 4271
BslI	15	154 346 1279 1417 1598 2064 2631 3292 3297 3298 3324 3808 4087 4253 4271
BsmAI	5	1277(c) 1358(c) 2065(c) 2618 3065
BsmFI	4	2673 3208(c) 3340(c) 3376(c)
BsmI	2	1895(c) 2302(c)
Bsp120I	1	564
Bsp1286I	5	568 1390 3140 3458 3976
BspEI	1	2293
BspHI	2	3106 3562
BspMI	1	1171
BspWI	12	63 130 214 1563 1955 2518 2722 3051 3183 3453 3668 4240
BsrBI	3	66(c) 377(c) 3431(c)
BsrDI	4	633 2276(c) 2925(c)

		3080(c)
BsrFI	5	1286 3148 3209 3367 3408
BsrGI	3	652 1442 2900
BsrI	15	201 442(c) 524(c) 550(c) 758(c) 1175 1299 1395 2048 2488(c) 2800 3474 3759 3871(c) 3884(c)
BssHII	2	1792 3445
Bst1107I	1	1755
BstNI	17	246 311 479 1396 2059 2115 2638 2666 3042 3274 3293 3298 3319 3325 4127 4140 4261
BstUI	16	150 152 233 498 1246 1794 1841 3160 3280 3332 3445 3447 3449 3592 3662 4243
BstXI	1	1306
BstYI	3	1833 3634 3645
CfoI	18	91 124 152 217 1320 1794 1796 2718 3162 3282 3334 3447 3449 3662 3771 3945 4045 4112
Cfr10I	5	1286 3148 3209 3367 3408
Csp6I	7	235 653 1443 1875 2413 2901 3262
DdeI	13	298 398 454 779 847 1253 1562 2069 2517 2745 2774 3603 4012
DpnI	13	1258 1403 1835 2564 2607 2691 3111 3197 3561 3628 3636 3647 3722
DpnII	13	1256 1401 1833 2562 2605 2689 3109 3195 3559 3626 3634 3645 3720
DraI	2	2034 2373
DraII	1	565
DraIII	1	3135
DrdI	1	4184
DsaI	3	1988 3181 3483
DsaV	32	244 309 346 379 477

		1346 1394 1416 1417 1552 2057 2113 2553 2636 2645 2664 3040 3152 3213 3224 3272 3291 3296 3317 3323 3356 3392 3393 3907 4125 4138 4259
EaeI	9	156 547 1296 1392 2024 3126 3150 3207 3480
EagI	1	3150
Eam1105I	3	550 800 2757
EarI	2	112(c) 244(c)
EclXI	1	3150
Eco57I	1	3744(c)
EcoO109I	1	565
EcoRI	1	2289
EcoRII	17	244 309 477 1394 2057 2113 2636 2664 3040 3272 3291 3296 3317 3323 4125 4138 4259
EcoRV	1	3000
Esp3I	2	2065(c) 2618
Fnu4HI	18	71 74 92 173 840 1717 1842 1950 2738 3046 3187 3278 3672 3878 3881 3946 4089 4244
FnuDII	16	150 152 233 498 1246 1794 1841 3160 3280 3332 3445 3447 3449 3592 3662 4243
FokI	9	262(c) 298 527 1315(c) 1394(c) 1432(c) 1554(c) 2309 2975(c)
HaeII	2	125 4046
HaeIII	26	158 274 290 351 440 549 566 1298 1394 2026 2113 2335 2380 2635 3054 3128 3152 3209 3213 3277 3296 3482 3812 4246 4264 4275
HgaI	6	317(c) 2714(c) 3210(c) 3581(c) 3596(c) 4174(c)
HgiAI	4	1390 3140 3458

		3976
HhaI	18	91 124 152 217 1320 1794 1796 2718 3162 3282 3334 3447 3449 3662 3771 3945 4045 4112
HinP1I	18	89 122 150 215 1318 1792 1794 2716 3160 3280 3332 3445 3447 3660 3769 3943 4043 4110
HincII	7	502 805 853 1698 2751 3475 3549
HindII	7	502 805 853 1698 2751 3475 3549
HinfI	9	21 96 161 769 887 1940 2784 3013 3916
HpaI	1	502
HpaII	29	347 381 1287 1347 1418 1553 1647 1831 2166 2294 2336 2555 2647 3149 3153 3210 3215 3226 3309 3358 3368 3394 3409 3434 3459 3718 3908 3934 4081
HphI	15	403(c) 845 1396(c) 1497(c) 2004 2046(c) 2058 2114(c) 2250(c) 2252 2258(c) 2591 2743 3463 3490(c)
ItaI	18	71 74 92 173 840 1717 1842 1950 2738 3046 3187 3278 3672 3878 3881 3946 4089 4244
Ksp632I	2	112(c) 244(c)
MaeI	4	241 507 1015 3793
MaeII	14	365 533 675 1128 1384 2029 2204 2599 2611 2879 3350 3440 3553 3581
MaeIII	18	526 596 760 792 979 1702 2126 2231 2697 2759 2791 2955 3076 3346 3586 3750 3866 3929

MamI	1	1406
MboI	13	1256 1401 1833 2562 2605 2689 3109 3195 3559 3626 3634 3645 3720
MboII	10	129 261 438(c) 819(c) 867(c) 1260(c) 2019 2684(c) 3629(c) 3781(c)
McrI	7	85 808 856 3153 3413 3429 3952
MluI	2	231 3590
MluNI	4	1298 1394 2026 3482
MnlI	19	101(c) 151 354 928 951(c) 978 1122 1571 1742 2448 2603 3133 3211(c) 3256 3391 3506 3859 4109(c) 4183
MroI	1	2293
MscI	4	1298 1394 2026 3482
MseI	14	167 226 501 556 702 931 1220 1493 1891 2033 2372 2852 3541 3578
MslI	4	619 1304 1632 2577
MspA1I	6	175 1949 2393 2995 3703 3948
MspI	29	347 381 1287 1347 1418 1553 1647 1831 2166 2294 2336 2555 2647 3149 3153 3210 3215 3226 3309 3358 3368 3394 3409 3434 3459 3718 3908 3934 4081
MvaI	17	246 311 479 1396 2059 2115 2638 2666 3042 3274 3293 3298 3319 3325 4127 4140 4261
MvnI	16	150 152 233 498 1246 1794 1841 3160 3280 3332 3445 3447 3449 3592 3662 4243
MwoI	12	63 130 214 1563 1955 2518 2722 3051 3183 3453

		3668 4240
NaeI	2	3150 3211
NciI	15	348 381 1348 1418 1419 1554 2555 2647 3154 3215 3226 3358 3394 3395 3909
NcoI	2	1988 3483
NdeII	13	1256 1401 1833 2562 2605 2689 3109 3195 3559 3626 3634 3645 3720
NgoMI	2	3148 3209
NheI	2	240 506
NlaIII	13	514 1318 1631 1910 1931 1992 2222 3027 3110 3208 3487 3566 4290
NlaIV	8	566 567 1188 1835 2591 3313 4219 4258
NspI	1	4290
PflMI	2	2064 2631
PleI	3	104 3021 3910(c)
PmaCI	1	3554
PmlI	1	3554
Psp1406I	2	365 2204
PstI	1	1168
PvuII	3	175 2393 2995
RcaI	2	3106 3562
RsaI	7	236 654 1444 1876 2414 2902 3263
SalI	3	803 851 2749
SapI	1	112(c)
Sau3AI	13	1256 1401 1833 2562 2605 2689 3109 3195 3559 3626 3634 3645 3720
Sau96I	11	349 439 564 565 2634 3053 3212 3321 3327 3360 3415
ScaI	1	1876
ScrFI	32	246 311 348 381 479 1348 1396 1418 1419 1554 2059 2115 2555 2638 2647 2666 3042 3154 3215 3226 3274 3293 3298 3319 3325 3358 3394 3395 3909

		4127 4140 4261
SexAI	1	3317
SfaNI	8	296(c) 464(c) 826(c) 1051 1961 2446(c) 2724(c) 4189(c)
SfcI	6	798 1164 2754 3006 3830 4021
SgrAI	1	3367
SmaI	2	1419 3395
SnoI	3	1386 3136 3972
SspBI	3	652 1442 2900
SspI	1	1981
StyI	2	1988 3483
TaqI	7	804 852 1159 2534 2750 3425 4188
TfiI	6	21 161 769 887 1940 2784
ThaI	16	150 152 233 498 1246 1794 1841 3160 3280 3332 3445 3447 3449 3592 3662 4243
Tru9I	14	167 226 501 556 702 931 1220 1493 1891 2033 2372 2852 3541 3578
Tsp509I	9	223 704 1052 1145 1884 2289 2848 2936 3542
Van91I	2	2064 2631
XhoII	3	1833 3634 3645
XmaI	2	1417 3393
XmaIII	1	3150
XmnI	1	1053

No cuts: AatI, Acc65I, AgeI, AosI, AscI, Asp718, AspI, AsuII, AviII, AvrII, BcgI, BglII, BlnI, Bpu1102I, BsgI, BsiWI, BspDI, BstBI, BstEII, Bsu36I, CelII, ClaI, Csp45I, Ecl136II, Eco47III, EcoNI, EspI, FspI, HindIII, KasI, KpnI, KspI, MfeI, MunI, NarI, NdeI, NotI, NruI, NsiI, NspV, PacI, PaeR7I, PinAI, PmeI, Ppu10I, PpuMI, PvuI, RsrII, SacI, SacII, SfiI, SfuI, SnaBI, SpeI, SphI, StuI, SwaI, Tth111I, XbaI, XcmI, XhoI