The pPAL8 Vector Map and Plasmid Sequence

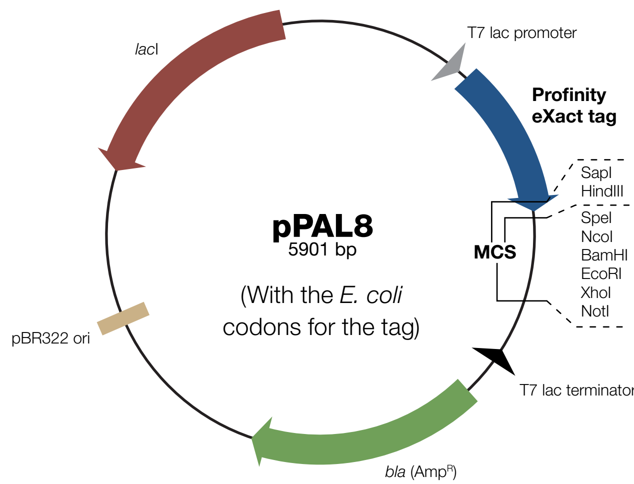
(pPAL8 is different from pPAL7 only in the Profinity eXact™ tag codon sequence. The amino acid sequence of the tag and the cloning sites remain the same as pPAL7.)

The Profinity eXact pPAL8 Vector Enables *E. coli* Expression of Fusion Proteins with the Profinity eXact Tag

Precise fusions of the Profinity eXact tag to a protein of interest may be constructed with either the HindIII or SapI site. When using the HindIII site to insert a target gene, it is essential that the insertion contains the Profinity eXact tag's TG bases immediately downstream of the HindIII recognition site to restore the tag's terminal leucine. Similarly, if SapI is used, the tag must be fully restored by including AAGCTTTG upstream of the first codon of the target gene. A Thr-Ser spacer sequence may be created by cloning into the SpeI site; a longer spacer would be created by cloning into the other sites downstream of SpeI. RIC (restriction-independent cloning)–ready vector is available for precise fusion and high-throughput cloning. Finally, inserted genes must contain their own stop codon.

Table 1. Features of pPAL7 expression vector.

Feature	Position
T7*lac* promoter	1–17
Profinity eXact tag	92–316
MCS	
SpeI	318
NcoI	325
BamHI	333
EcoRI	345
XhoI	351
NotI	358
T7 terminator	413–460
bla (Amp^R)	881–1,738
Ori	2,499
*lac*I	4,436–5,515



Profinity eXact pPAL7 vector.

Cloning and Expression Region

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           T7 Promoter                  lac Operator             XbaI
GCGAAATTAA  TACGACTCAC  TATAGGGGAA  TTGTGAGCGG  ATAACAATTC  CCCTCTAGAA  ATAATTTTGT  TTAACTTTAA  GAAGGAGATA

                                      Profinity eXact Tag
  NdeI
TACAT ATG GGC GGC AAA TCT AAC GGT GAG AAG AAG TAC ATT GTT GGT TTC AAG CAA GGC TTT AAG AGC TGT GCG AAG
      Met Gly Gly Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Gly Phe Lys Ser Cys Ala Lys

                                      Profinity eXact Tag
AAA GAG GAT GTG ATC TCC GAG AAA GGT GGT AAG CTG CAG AAA TGC TTC AAA TAC GTC GAC GCG GCG AGC GCA ACC CTG
Lys Glu Asp Val Ile Ser Glu Lys Gly Gly Lys Leu Gln Lys Cys Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr Leu

                                      Profinity eXact Tag
                                                                                       SapI    HindIII
AAT GAG AAA GCA GTG GAA GAA CTG AAG AAA GAT CCG AGC GTT GCC TAT GTC GAA GAG GAC AAG CTC TTC AAA GCT TTG
Asn Glu Lys Ala Val Glu Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp Lys Leu Phe Lys Ala Leu
                                                                    Cleavage Recognition Sequence
                                                                    Profinity eXact Cleavage Site (FKAL)

 SpeI    NcoI     BamHI            EcoRI   XhoI     NotI
ACT AGT ACC ATG GCG GGA TCC GGC TGC GAA TTC CTC GAG GCG GCC GCA TAA GCCCGAAAGG AAGCTGAGTT GGCTGCTGCC
Thr Ser Thr Met Ala Gly Ser Gly Cys Glu Phe Leu Glu Ala Ala Ala Stop

                                  T7 Terminator
ACCGCTGAGC  AATAACTAGC  ATAACCCCTT  GGGGCCTCTA  AACGGGTCTT  GAGGGGTTTT  TTGCTGAAAG  GAGGAACTAT
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pPAL7 5901 bp

ORIGIN BASE COUNT 1413 A 1535 C 1610 G 1343 T

Profinity eXact tag 92–316

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1    TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA TTCCCCTCTA GAAATAATTT TGTTTAACTT TAAGAAGGAG ATATACATAT GGGCGGCAAA TCTAACGGTG
111  AGAAGAAGTA CATTGTTGGT TTCAAGCAAG GCTTTAAGAG CTGTGCGAAG AAAGAGGATG TGATCTCCGA GAAAGGTGGT AAGCTGCAGA AATGCTTCAA ATACGTCGAC
221  GCGGCGAGCG CAACCCTGAA TGAGAAAGCA GTGGAAGAAC TGAAGAAAGA TCCGAGCGTT GCCTATGTCG AAGAGGACAA GCTCTTCAAA GCTTTGACTA GTACCATGGC
331  GGGATCCGGC TGCGAATTCC TCGAGGCGGC CGCATAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA ACCCCTTGGG GCCTCTAAAC
441  GGGTCTTGAG GGGTTTTTTG CTGAAAGGAG GAACTATATC CGGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA AGCCTATGCC TACAGCATCC AGGGTGACGG
551  TGCCGAGGAT GACGATGAGC GCATTGTTAG ATTTCATACA CGGTGCCTGA CTGCGTTAGC AATTTAACTG TGATAAACTA CCGCATTAAA GCCTATCGAT GATAAGCTGT
661  CAAACATGAG ATTTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA
771  ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
881  ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA
991  GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC
1101 TATGTGGCGC GGTATTATCC CGTGTTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT
1211 CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC
1321 CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA
1431 TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC
1541 TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT
1651 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
1761 CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC
1871 CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT
1981 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT
2091 TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG
2201 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
2301 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT
2421 ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC
2531 CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG
2641 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGGGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATATAT GGTGCACTCT
2751 CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
2861 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC
2970 TGCGGTAAAG CTCATCAGCG TGGTCGTGAA GCGATTCACA GATGTCTGCC TGTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA
3081 AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAGGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT
3191 CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT
3301 GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA
3411 CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA
3521 ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCCTCAAC GACAGGAGCA CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC
3631 TGGAGATGGC GGACGCGATG GATATGTTCT GCCAAGGGTT GGTTTGCGCA TTCACAGTTC TCCGCAAGAA TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG
3741 AGGTGCCGCC GGCTTCCATT CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT AGGGCGGCGC CTACAATCCA TGCCAACCCG
3851 TTCCATGTGC TCGCCGAGGC GGCATAAATC GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT TGAAGCTGTC CCTGATGGTC
2961 GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA GCCTCGCGTC GCGAACGCCA
4071 GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT AATGGCCTGC TTCTCGCCGA AACGTTTGGT GGCGGGACCA GTGACGAAGG CTTGAGCGAG GGCGTGCAAG
4181 ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA GCGGTCCTCG CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCCTACGA GTTGCATGAT
4291 AAAGAAGACA GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT GACTGGGTTG AAGGCTCTCA AGGGCATCGG TCGAGATCCC GGTGCCTAAT
4401 GAGTGAGCTA ACTTACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT
4511 TTGCGTATTG GGCGCCAGGG TGGTTTTTCT TTTCACCAGT GAGACGGGCA ACAGCTGATT GCCCTTCACC GCCTGGCCCT GAGAGAGTTG CAGCAAGCGG TCCACGCTGG
4621 TTTGCCCCAG CAGGCGAAAA TCCTGTTTGA TGGTGGTTAA CGGCGGGATA TAACATGAGC TGTCTTCGGT ATCGTCGTAT CCCACTACCG AGATATCCGC ACCAACGCGC
4731 AGCCCGGACT CGGTAATGGC GCGCATTGCG CCCAGCGCCA TCTGATCGTT GGCAACCAGC ATCGCAGTGG GAACGATGCC CTCATTCAGC ATTTGCATGG TTTGTTGAAA
4841 ACCGGACATG GCACTCCAGT CGCCTTCCCG TTCCGCTATC GGCTGAATTT GATTGCGAGT GAGATATTTA TGCCAGCCAG CCAGACGCAG ACGCGCCGAG ACAGAACTTA
4951 ATGGGCCCGC TAACAGCGCG ATTTGCTGGT GACCCAATGC GACCAGATGC TCCACGCCCA GTCGCGTACC GTCTTCATGG GAGAAAATAA TACTGTTGAT GGGTGTCTGG
5061 TCAGAGACAT CAAGAAATAA CGCCGGAACA TTAGTGCAGG CAGCTTCCAC AGCAATGGCA TCCTGGTCAT CCAGCGGATA GTTAATGATC AGCCCACTGA CGCGTTGCGC
5231 GAGAAGATTG TGCACCGCCG CTTTACAGGC TTCGACGCCG CTTCGTTCTA CCATCGACAC CACCACGCTG GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA
5171 TTTGCGACGG CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT TGCCCGCCAG TTGTTGTGCC ACGCGGTTGG GAATGTAATT CAGCTCCGCC
5391 ATCGCCGCTT CCACTTTTTC CCGCGTTTTC GCAGAAACGT GGCTGGCCTG GTTCACCACG CGGGAAACGG TCTGATAAGA GACACCGGCA TACTCTGCGA CATCGTATAA
5501 CGTTACTGGT TTCACATTCA CCACCCTGAA TTGACTCTCT TCCGGGCGCT ATCATGCCAT ACCGCGAAAG GTTTTGCGCC ATTCGATGGT GTCCGGGATC TCGACGCTCT
5611 CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTAGTA GGTTGAGGCC GTTGAGCACC GCCGCCGCAA GGAATGGTGC ATGCAAGGAG ATGGCGCCCA ACAGTCCCCC
5721 GGCCACGGGG CCTGCCACCA TACCCACGCC GAAACAAGCG CTCATGAGCC CGAAGTGGCG AGCCCGATCT TCCCCATCGG TGATGTCGGC GATATAGGCG CCAGCAACCG
5831 CACCTGTGGC GCCGGTGATG CCGGCCACGA TGCGTCCGGC GTAGAGGATC GAGATCTCGA TCCCGCGAAA T
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