pER-NH Vector

Source	Constructed by Farrell MacKenzie
Company	Structural Genomics Consortium, Toronto

Description	pER-NH is a mammalian expression vector derived from the pCMV/myc/ER vector (Invitrogen). It has an immediate-early cytomegalovirus (CMV) promoter that drives expression of recombinant proteins with the addition of an N-terminal endoplasmic reticulum (ER) signal peptide, 6xHis-tag and TEV cleavage site. A stop codon in the reverse cloning primer prevents the expressed protein from incorporating the C-terminal myc epitope and ER retention signal found in pCMV/myc/ER, thus targeting the protein for secretion rather than retention in the ER.

Antibiotic resistance	Ampicillin (resistance in *E. coli*) Neomycin (resistance in mammalian cells)
Promoter	CMV
Cloning Methods	Insertion of a DNA sequence into the cloning/expression region is performed using Clontech's In-fusion enzyme-mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and vector linearized with BmtI & HindIII. Insertion of a target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
N – terminal fusion sequence	MGWSCIILFLVATATGAHSQVQLHHHHHH-DYDIPENLYFQG
5' primer tail for amplification of insert	5' CTGTATTTCCAGGGG --- 3'
3' primer tail for amplification of insert	5' CTGCGGCCTAAAGCTA --- 3'
5' sequencing primer pCMV-fwd	5' CGCAAATGGGCGGTAGGCGTG 3'
3' sequencing primer BGH -rev	5' TAGAAGGCACAGTCGAGG 3'

pER-NH sequence (7,090 bp):

GTACCGAATTCACATTGATTATTGAGTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTC
ATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG
GACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATC
AAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGG
CATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGGTTAGTCA
TCGCTATTACCATAGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAC
TCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAA
TCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGG
CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTTTCTGGCTAACTAGAGAACCCACTGCTTA
CTGGCACGTGGAAATTAATACGACGTGGCCACCATGGGATGGAGCTGTATCATCCTCTTCTT
GGTAGCAACAGCTACAGGTAAGGGGTTAACAGTAGCAGGCTTGAGGTCTGGACATATATAT
GGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGCGCGCACTCCCAGGTCCAACTG
CATCACCATCACCATCACGATTACGATATCCCAGAAAACCTGTATTTCCAGGGGCTAGCCTG
AAAGATCCATAACTTCGTATAGCATACATTATACGAAGTTATGCGGCCGCGACGTCCACATAT
ACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTGAATTGTGATTAAAA
AGGCAACTTTATGCCCATGCAACAGAAACTATAAAAAATACAGAGAATGAAAAGAAACAGATA
GATTTTTTAGTTCTTTAGGCCCGTAGTCTGCAAATCCTTTTATGATTTTCTATCAAACAAAAGA
GGAAAATAGACCAGTTGCAATCCAAACGAGAGTCTAATAGAATGAGGTCGAAAAGTAAATCG
CGCGGGTTTGTTACTGATAAAGCAGGCAAGACCTAAAATGTGTAAAGGGCAAAGTGTATACT
TTGGCGTCACCCCTTACATATTTTAGGTCTTTTTTTATTGTGCGTAACTAACTTGCCATCTTCA
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ATCGTCTTTGCATTAGCCGGAGATCCTAAAAATGCGGATGACACATCGATTTACATGTTCTAT
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CGACAAATTCGATGCAAATGATTCTATCCTAAAAGACCAAACACAAGAATGGTCAGGTTCAG
CCACATTTACATCTGACGGAAAAATCCGTTTATTCTACACTGATTTCTCCGGTAAACATTACG
GCAAACAAACACTGACAACTGCACAAGTTAACGTATCAGCATCAGACAGCTCTTTGAACATC
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GCAGTTCATCGATGAAGGCAACTACAGCTCAGGCGACAACCATACGCTGAGAGATCCTCAC
TACGTAGAAGATAAAGGCCACAAATACTTAGTATTTGAAGCAAACACTGGAACTGAAGATGG
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