IMPORTANT:
This note is intended to provide supplemental information only.
For detailed information about a specific product, or for product specific protocols, please refer to the respective product manual.

Tip:
1. **To search the note, press CTRL-F keys in combination**
2. **To navigate the note, use the links.**

Mammalian Expression Vectors

TABLE OF CONTENTS

PRODUCT DESCRIPTION

(back to Table of Content)

Vectors-General description
Promoters
Fusion Tags
Selection Markers
Nomenclature of vectors

Vectors-General description

(back to Table of Content)
(back to Product Description)

- Vectors for constitutive, high level expression in mammalian cells
- Choice of promoter for constitutive, high-level expression, choice of epitope tag for easy detection and polyhistidine (6xHis) or GST tag for rapid purification (with the exception of the 6 pCDNA3.1 untagged vectors) and choice of selection marker for generating stable cell lines, thus allowing for expression of more than one gene in a cell line
- Available as restriction enzyme cloning (three reading frames provided for most tagged vectors), TOPO TA, Directional TOPO and Gateway versions.
- To study protein expression by transient transfection as well as by stable integration into the genome
- Useful for studying protein expression involving post-translational modifications, not seen in E.coli, yeast and insect cells

Promoters

(back to Table of Content)
(back to Product Description)

- CMV: Immediate-early Cytomegalovirus virus promoter for high-level expression in a wide variety of mammalian cell lines
- EF1-1α: Human elongation factor 1α-subunit promoter for high-level expression
- Ubc: Human ubiquitin C promoter for high-level expression that is equivalent across a broad range of species and tissue types
- SV40: Simian virus 40 promoter for high -level expression. Permits replication in cell lines expressing the large T antigen
- PGK: Murine Phosphoglycerate Kinase-1 promoter for long-term persistent expression in cells that are susceptible to promoter silencing from methylation or histone deacetylation , such as undifferentiated embryonic stem (ES) cells

Fusion Tags

(back to Table of Content)
(back to Product Description)

See also Epitope-tagged antibodies application note for additional details on applications involving Myc, 6xHis, V5, and Xpress epitopes

- V5: Low background, efficient Western blot detection, immunoprecipitation and immunostaining. The V5 epitope is derived from the V protein of SV5 virus, a simian paramyxovirus, that causes flu-like and pneumonia-like symptoms in primates. Full length V protein causes STAT degradation and may impact immune response. However the V5-epitope is non-pathogenic, does not constitute a virus and is in fact harmless.
- Xpress: Efficient Western blot detection and immunoprecipitation
- myc: Efficient Western blot detection, immunoprecipitation and immunostaining
- 6xHis: Efficient Western blot detection, immunoprecipitation and protein purification
- GST: Efficient Western blot detection, immunoprecipitation and protein purification. The origin of GST is Schistosoma japonicum.
- BioEase: Efficient Western blot detection, immunostaining, immunoprecipitation and protein purification

- capTEV (6xHis-TEV-TEV-BioEase): Efficient Western blot detection, immunostaining, immunoprecipitation and protein purification
- Lumio: Small (six amino acid) tag for in vivo and in vitro protein detection and localization. The small size reduces the likelihood that the tag will interfere with protein function.
- Fluorescent protein: Cycle 3 Green fluorescent protein (Cycle 3 GFP), EmGFP (Emerald Green fluorescent protein) or YFP (Yellow fluorescent protein) for fluorescence detection and localization in living or fixed cells.

Selection Markers
(back to Table of Content)
(back to Product Description)

- Neomycin: Reliable and convenient selection using widely used selection agent, Geneticin
- Blasticidin: Rapid selection
- Zeocin: Efficient, high potency selection
- Hygromycin: Different mode of action than Geneticin or Zeocin, hence useful for dual selection

Nomenclature of vectors
(back to Table of Content)
(back to Product Description)

Promoter	Selection Marker	Fusion Tag
pcDNA = CMV	3.1, 3.2 or EF1 = Geneticin resistance	/His = N-terminal His tag
pEF = EF-1α	4 = Zeocin resistance	/myc-His = C-terminal myc-His tag
pUB = Ubiquitin	6, 6.2 = Blasticidin resistance	/V5-His = C terminal V5-His tag
		/His-Xpress = N-terminal His-Xpress tag

SHIPPING CONDITIONS
(back to Table of Content)

Lyophilized vectors are shipped at room temperature; Expression kits are shipped on dry ice.

STORAGE CONDITIONS
(back to Table of Content)

Lyophilized vectors are stable at room temperature until reconstituted, however after reconstitution (in TE) store at 4°C for short term and -20°C for long-term storage. Kits should be stored at -20°C for the vector box, whereas competent cells should be stored at -80°C upon receipt.

STABILITY
(back to Table of Content)

All vectors and kits are guaranteed stable for six months.

QC SPECIFICATIONS
(back to Table of Content)

All supercoiled vectors are qualified by restriction endonuclease digestion using enzymes described in vector-specific manuals. All TOPO vectors are qualified using the control reagents in the kit to ensure cloning efficiencies as stated in the vector-specific manuals. All Gateway-adapted DEST vectors are qualified for functionality in an LR recombination assay using Gateway LR Clonase Enzyme Mix. The ccdB gene is assayed by transformation using an appropriate E.coli strain. The control vectors are qualified by restriction endonuclease digestion.

PROTOCOL AND APPLICATION NOTES
(back to Table of Content)

General information
(back to Table of Content)
(back to Protocol and Application Notes)

Common vector elements
(back to Table of Content)
(back to Protocol and Application Notes)
(back to General Information)

Elements important for efficient eukaryotic translation
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

- Both the 5' cap structure and the spacing between the cap and the initiation ATG codon affect ribosome binding and initiation of eukaryotic translation.
- In particular, the sequence immediately surrounding the initiation codon may be important for efficient translation initiation.

Kozak sequence
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Elements important for efficient eukaryotic translation)

- Kozak analyzed 699 5' untranslated sequences from vertebrate mRNAs and derived a consensus sequence that is believed to be important for efficient initiation of translation (1). It is G/ANNATGG, where ATG is the initiation codon.. Point mutations in the nucleotides surrounding the ATG have been shown to modulate translation efficiency (2). A purine (A/G) in -3 position has a dominant effect - expression levels can be reduced upto 95% when the -3 position is changed from a purine to a pyrimidine (C/T) and further, with a pyrimidine in -3 position, translation becomes more sensitive to changes in -1, -2 and +4 positions. The +4 position has less influence on expression levels where approximately 50% reduction is seen. Of the 699 vertebrate mRNAs analyzed at position +4:
    - G...occurs 46% of the time in the open reading frame.
    - A...occurs 23% of the time in the open reading frame.
    - C...occurs 16% of the time in the open reading frame.
    - T...occurs 15% of the time in the open reading frame.
- Note: Yeast do not follow this rule (3). The optimal Kozak sequence for Drosophila differs slightly (C/AAAA/CATG) (4).
- For expression of a novel portion of a gene, a non-eukaryotic gene or a gene with an undefined eukaryotic initiation sequence, optimizing with Kozak may help (1,5,6). If concerned about expression, it is advisable to test two constructs, one with the native start site and the other with a consensus Kozak,
- In general, eukaryotic translation initiation at non-ATG codons is extremely rare and even if it does occur, the initiation is inefficient.

Promoters
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

CMV promoter
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

- The immediate-early CMV (Cytomegalovirus) promoter is used in our vectors. In addition to the CAAT and TATA boxes, the promoter contains sequence homologous to base pairs 137 to 724 of the sequence submitted by Boshart et al (GenBank Accession # K03104). The complete enhancer region is contained between base pairs 214 and 620 of this sequence. Therefore, by this definition, the promoter could be said to be complete. Please note that the promoter does not contain an intron (some people may believe that a complete promoter must contain an intron). This has not been demonstrated to be needed in all cases of expression.
- The CMV promoter in our vectors does not contain a chimeric or other intron. Although some reports suggest that the presence of an intron increases expression levels, our data show higher expression levels with pcDNA3.1 than from a similar vector containing a chimeric intron (Expressions 5.3, page 10).
- Repression of CMV promoter - Typically the CMV promoter is not repressed in 293 cells due to the presence of the adenovirus E1a gene. CMV repression is observed in other cell types (e.g. 3T3) when it integrates in a genomic location that causes it to shutdown.
- Comparison to other CMV promoters - We have made many comparisons of our CMV promoter to others on the market and ours either out-performs or is equivalent to the others. Additional sequences between the TATA box and the transcription start site) typically do not contain any regulatory or transcription factor binding sites, so their actual base composition is probably irrelevant. They are more just for spacing, with typically ~25 bp of sequence between the TATA box (where the transcription machinery is assembled) and where they actually touch down and begin transcription.
- Cryptic E. coli promoter - likely to be somewhere in the CMV promoter. When the gene of interest is in the correct orientation for eukaryotic expression, the cryptic E. coli promoter will often express the gene in E. coli. The level of toxicity to E. coli will depend upon the particular gene product and upon the efficiency of translation initiation. One way of getting around this problem is by engineering a prokaryotic transcription terminator , upstream of the gene of interest. This will result in reduced expression in E. coli without altering eukaryotic expression.
- CMV promoter in yeast - The CMV promoter works in S. pombe, but is essentially non-functional in S. cerevisiae (anything with a TATA box that isn't specifically repressed will give some level of expression in S. cerevisiae, but not enough that one can actually detect, say, with a GFP tag). There are yeast "tricks" that involve co-tranformation and selection for homologous recombination between vectors that can be used to try to express a library created in a mammalian vector, in S. cerevisiae, but in the general case, it would be much better to subclone the gene of interest in a yeast expression vector.
- The CMV promoter has a broad host range that includes a wide variety of mouse tissue and cell types (7,8,9) and several human cell lines including B-lymphoblastoma (10), a lung cancer cell line (11), embryonic kidney cells (12), breast cancer cell lines (13) and placenta cells (14). Expression using CMV promoter-based vectors has also been observed in a canine thymus cell line (15), Xenopus oocytes (16), quail embryo fibroblasts and avian bursal lymphomas (17), D. melanogaster (insect) cells (18) and yeast (19). The CMV promoter does not seem to work in T24 and HCV29 human bladder urethelium carcinoma cell lines but SV40 exhibits high activity in these cell lines. Both T24 and HCV29 cell lines originated from human transitional cell carcinoma of bladder urethelium.
- The CMV promoter is weak in B cells and so is the SV40 promoter. This may pose a problem if selection is under this promoter, resulting in poor expression of the protein of interest. Recommend EF-1α promoter or UbC promoter.

Augmenting or altering expression from CMV promoter

- Sodium butyrate in cell culture increases transcription of genes driven by the CMV promoter (and certain other viral promoters). One of the activities of sodium butyrate is inhibition of deacetylation.

Histone deacetylation is a frequently used endogenous method for silencing genes. It is likely that sodium butyrate increases transcriptional activation at the CMV promoter by inhibiting deacetylation of histones. One cannot guarantee that sodium butyrate will increase transcription but it may if it makes the site of integration of the CMV promoter - gene of interest more accessible. The drawback is that the transcriptional increase is not specific to the CMV promoter making the results a little suspect (20,21,22,23,24).

- Retinoic acid - Cells transfected with CMV promoter-containing vectors and subsequently treated with retinoic acid to induce differentiation show up-regulation of expression of the target gene (25).
- PHA and PMA - The CMV promoter is constitutively active in many cell types. However, in T-cell derived cell lines such as Jurkat, KGI and K562, activation of the cells is required for expression. In Jurkat cells, maximum expression is achieved with the lectin PHA-L (phytohemagglutinin) and the phorbol ester PMA (phorbol myristate acetate) whereas in KGI and K562 cells, PMA alone is sufficient. In Jurkat cells, addition of PHA and PMA to the "post-transfection replacement medium" at a final concentration of 1 ug/ml and 50 ng/ml, respectively, induces promoter activity and gene expression (probably mediated by protein kinase C). PHA/PMA are not absolutely required, but expression levels may be 5X-20X lower if they are not added (FOCUS 18:2, p.45).The activation is general and will probably affect many promoters. Other T-cells may also need this stimulation.

SV40 promoter

(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

- The SV40 (Simian Virus 40) promoter in our vectors contains the SV40 enhancer promoter region and origin of replication for high-level expression and replication in cell lines expressing the large T antigen. It contains two 72bp SV40 enhancer repeats (26).

SV40 ori/Episomal replication/Large T Antigen/Stability of cell lines

- Mammalian vectors that contain the SV40 polyoma ori (usually part of the SV40 early promoter in our vectors) replicate episomally, but only in cells that express the SV40 large T antigen (e.g. COS-7 and 293T cells). They will not replicate episomally in the absence of the SV40 large T antigen.
- Generating stable lines in cells expressing the SV40 large T antigen is very difficult, if not impossible, with any plasmid that contains the SV40 origin/promoter. This is because the SV40 large T antigen induces DNA replication at the SV40 ori in the vector sequence. If this occurs after the vector has integrated into the genome, it will result in isolated regions of the chromosome trying to replicate illegitimately, leading to chromosomal duplications and generally messing up the cell's genome. The best recommendation would be to use a cell line that does not express the SV40 large T antigen. Deleting the origin could be an option, (50), however, this may affect promoter activity. Transient transfection of SV40 ori-containing plasmids into cells expressing the SV40 large T antigen results in replication of the plasmid (without hurting the host cell's chromosomes) and therefore an amplification of the gene of interest and subsequently very high levels of expression. Hence, we recommend the SV40 ori-containing vectors for transient use only in SV40 large T antigen-containing cells, but they can be used both transiently and stably in cells that do not have the SV40 large T antigen.
- There is a sequence discrepancy for the SV40 ori/promoter region between pcDNA3.1 (also pcDNA3) and its parent vector pRcCMV. pRcCMV has the essential sequences for SV40 ori including the Sfi I site which is critical for activity. The SV40 ori in pcDNA3.1differs in sequence at this exact location (the Sfi I site) - John Comiskey mutated that Sfi I site a couple of years ago (with a single base change) when pcDNA3 was made from pRcCMV and it has been sequenced and tested in CHO cells. As far as we have seen, the SFi I site, per se, is not essential for replication in CHO cells as long as the spacing within the origin isn't changed too much.
- To extract episomal plasmids, use a standard HIRT prep..
- The SV40 promoter is weak in B cells. The same applies to the CMV promoter. This may pose a problem if selection is under this promoter, resulting in poor expression of the protein of interest. Recommend EF-1α promoter or UbC promoter.
- The SV40 promoter expresses fairly well in Jurkat cells under transient conditions and also when stably integrated into a ‘good’ locus (transcriptionally active locus).

EF-1α promoter
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

- EF-1α (Elongation Factor-1α) promoter is a strong, constitutive non-viral promoter (27,28,29,30,31). The EF-1α gene encodes EF-1α enzyme that catalyzes the GTP-dependent binding of aminoacyl-tRNA to ribosomes. EF-1 α is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The EF-1 α promoter exhibits a strong activity, higher than that of viral promoters such as SV40 and RSV (32) and, on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo (33). In our experience, EF-1α and CMV express nearly equivalently in 293 cells when plasmids are transiently transfected and gene expression is measured 2-3 days later.
- All our EF-1α promoter based vectors contain intron 1 of the EF-1α gene. In the vector maps, intron 1 runs from base 706 to 1644. The transcriptional start site is not yet determined, but in general starts about 20-25 bases downstream of the TATA box, which in this case is located at bases 645 to 649.
- Developmental expression from the EF1α promoter (34,35)

Ubc promoter
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

- Ubc (Human Ubiquitin C) promoter provides high-level expression that is equivalent across a broad range of species and tissue types (36,37)
- The activity of the UbC promoter is typically about 50% the activity of CMV and EF-1α promoters in 293 cells. Other weaker promoters (that we don't sell) include HSV TK and cellular PGK promoters.

PGK promoter
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

- PGK (Murine Phosphoglycerate Kinase-1) promoter is a ubiquitous housekeeping promoter that has been shown to promote long-term persistent expression (53), and may provide consistent expression in cells that are susceptible to promoter silencing from methylation (54) or histone deacetylation (55), such as undifferentiated embryonic stem (ES) cells (53, 56).

Promoter strength comparison
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Promoters)

In most adherent mammalian cells, eg. CHO cells, promoter strength of CMV > EF-1α > UbC. EF-1α is almost always stronger than UbC, but sometimes only a little bit stronger

- **Comparative expression levels**

Vector	ng CAT/mg total protein (2 experiments each)
pCDNA3.1	432, 715
pCR3.1	129,139
pRC/CMV2	374,218
pCDNA1.1	27,40

Expression in pCR3.1 appears to be about 3-4 fold lower than that in pCDNA3.1.

- **Summary of promoter activities as a percent relative to activity of SV40 immediate early promoter**

This table represents a summary of the data found in the papers listed below under references. The data shown below is intended as a general guide only. The CAT assay values for each set of data have been converted to percent relative to SV40 promoter activity (SV40 promoter activity is assumed to be 100%). For some cell lines, results from several papers have been averaged. Not every paper tested all promoters with each cell line. In addition, there are reports from researchers that the EF-1α promoter offers sustained expression in some cell lines that tend to turn the CMV promoter down over time, such as HeLa cells.

Cell Line	Source	CMV	RSV	SV40	EF-1α	Reference
Cos-7	Monkey kidney	1404	80	100	205	38,39,41,42,45
HeLa	Human cervical carcinoma	232	67	100	170	39,40,41,42,43,45
NIH-3T3	Mouse fibroblast	150	160	100	215	39,40,44,45
CHO	Hamster ovary	643	71	100		39,41,42
CV1	Mouse kidney	1295	9	100		39
Bowes Melanoma	Human melanoma	1936	48	100		39
CEF	Chick fibroblast	1660	1280	100		40
BHK-21	Baby Hamster kidney	50	100	100		41
HEK-293	Human embryonic kidney	2130	1584	100		41,43
IVEC	Human umbilical vascular endothelium*	1429	4571	100		41
HBEC-90	Human brain endothelium*	2500	1750	100		41
TR2	Mouse oligodendritic*	263	368	100		41
REMC	Rat mammary epithelium	90	100	100		44
L929	Mouse		143	100	396	45
CHU-2	Human		53	100	167	45
PU5-1.8	Mouse macrophage	200	128	100	195	45
LTK	Mouse fibroblast	800		100		42
N2A	Mouse neuroblastoma	200		100		42
C6	Rat astrocytoma	225		100		42
33.1.1	Mouse pre-B cell†	1500		100		42
K46	Mouse B-lymphoma	50		100		42
J558L	Mouse plasmacytoma	100		100		42
IC11	Mouse teratocarcinoma*	200		100		42

* Transformed with SV40 Large T antigen
† Transformed with Abelson virus

- **In-house evidence that CMV and SV40 promoters function with different strengths in 3T3 cells and S2 cells**
  3T3 cells and S2 cells were transfected with pTracer-CMV2 and pTracer-SV40 vectors. In 3T3 cells, good fluorescence was seen within 24 hours with pTracer-SV40. Fluorescence was barely discernible in pTracer-CMV transfected 3T3 cells even after 4 days. However, good fluorescence was seen in dying 3T3 cells (transfected with pTracer-CMV2) as early as 24 hours post-transfection. In S2 cells, pTracer-SV40 fluoresced quite nicely, indicating that the CMV promoter is functional in this cell type, which is contrary to popular belief. pTracer-CMV was again just barely visible, more so in dying cells. This indicates that the EF1-α promoter is less funtional in this cell line.

Polyadenylation and Transcription termination
(back to Table of Content)
(back to Protocol and Application Notes)

(back to Common vector elements)

- In our mammalian expression vectors, transcription termination is provided by the BGH (Bovine growth hormone) polyA, SV40 polyA, or TK (Thymidine kinase) polyA site downstream of the multiple cloning sequence (MCS).
- There is evidence of specific termination factors involved with the polyA polymerase in transcription termination, though none have been well-characterized (46). The primary transcript for many mRNAs extends well beyond the polyadenylation site.
- Polyadenylation in eukaryotes occurs at a specific consensus sequence, AAUAAA, in the mRNA. The polyA polymerase cleaves after the U residue and adds a string of adenine residues, typically 50-250 in higher eukaryotes (46).
- Eukaryotic transcription termination signals are poorly defined whereas in prokaryotes, distinct transcription termination signals are recognized by the cell machinery.
- Transcription in yeast appears to terminate at AT-rich regions and is thought to be due to the formation of secondary structure which inhibits further transcription (47).
- If there is evidence for a single transcriptional product from a vector, it usually indicates that there is a transcription termination signal in the vector. If not, a smear of many different bands would be obtained.
- It is common for plasmids to have some gratuitous sequence (unpredictable a priori) that functions as a termination signal. It is possible to get expression of a cloned fragment in CMV-containing vectors from the Amp promoter, an observation that suggests that there isn't a transcription termination signal in the CMV promoter itself.

BGH (Bovine growth hormone) polyA

- The BGH polyA signal (bases 1019-1250) is the sequence that allows for polyadenylation.
- There is no intron in the BGH polyA sequence.

SV40 polyA

- The SV40 early polyA signal is used in our vectors. The SV40 early and late polyA signals are actually the same chunk of DNA - early on one strand and late on the other. So it is the orientation of the sequence relative to the ORF being polyadenylated that determines whether it is the early or late SV40 polyA.
- The SV40 polyA sequence in our vectors is derived from the Genbank SV40 genome sequence. The SV40 polyA is a region of the SV40 genome where transcripts coming from both directions terminate - small and large T antigens from counterclockwise direction and VP1, VP2 and VP3 from clockwise direction. Hence, it functions as a transcription terminator and poly A signal in either orientation.
- Critical sequences for efficient function of the SV40 early polyA - Efficient cleavage at the early site requires an element between 5 and 18 nucleotides downstream of the cleavage site, but removal of sequences upstream of the early AAUAAA hexanucleotide has no effect on cleavage efficiency (48). For example, the AAUAAA in pcDNA3 is at 3149-3155. This would put the required sequences no further downstream than 3173. BsmI cuts at 3200 so presumably there will be a limited impact on the efficiency of the poly A sequence. There could still be a diffuse effect due to the loss of a less critical downstream sequence.
- In some of our early vectors (now discontinued), the SV40 ori/promoter contains a splice site (49).
  If labelled as "SV40 intron/pA" : element contains an SV40 intron.
  If labelled as "SV40" :element does not contain an SV40 intron.
  If labelled as "SV40 ori" : element does not contain an SV40 intron.
  If labelled as "SV40 pA" : element does not contain an SV40 intron.

  For example:

pcDNA1	SV40 intron/pA
pcDNA1/amp	SV40 intron/pA
pCDM8	SV40 intron/pA
pcDNA3	none
pRc/CMV	none
pRc/RSV	none
pcDNA3.1-derived vectors	none

Origin of Replication: pUC ori and pBR322 ori
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

- Our mammalian expression vectors contain either the pUC ori (high copy number) or pBR322 ori (low copy number). However, the vectors with pBR322 ori have been discontinued and all of our currently available mammalian expression vectors contain the pUC ori.
- Both our low copy and high copy plasmids are derived from ColE1. The high copy plasmids, however, are missing the ROP protein which normally serves to reduce copy number. One could say that a pUC ori is derived from a mutated ColE1.

Untranslated regions (UTRs)
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

- Kozak surveyed 699 eukaryotic 5' untranslated regions and revealed that most are 50-150 bp in length (1). However, in some cases, these 5' untranslated regions are longer due to the presence of an intron which is subsequently removed before translation initiation (1). The fact that only 4 of these 699 eukaryotic transcripts do not have 5' untranslated regions indicates that these 5' untranslated regions may be needed for proper translation initiation. Since translation initiation in eukaryotes is not well characterized, it is recommended that the 5' untranslated region be kept to 50-100 base pairs in length and that mRNA secondary structure formation be minimized in this region (51).
- Untranslated sequences (including introns, 3' and 5' untranslated regions) do not, as a general rule, improve expression. However, if there is evidence to suggest that certain gene-specific sequences improve the efficiency of ectopic expression of specific genes, then it may be worthwhile to include those sequences during cloning. However, in nearly all cases, no untranslated sequences are necessary for high level expression from our vectors.

Enhancers
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

- Enhancers are genetic elements that, although not absolutely necessary for expression, act to increase the expression of a gene from a promoter through interactions with different transcription factors. An enhancer can be located upstream, downstream, or even overlapping the promoter, as long as it is in cis with the promoter. The effect of a promoter/enhancer combination on expression of a given gene depends upon the cell line (52).
- Invitrogen's CMV and SV40 promoters contain enhancer regions. See sections in this note above describing specific promoter characteristics

Antibiotic Selection Markers for Eukaryotic Selection and for Cloning in Bacteria
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Common vector elements)

Eukaryotic Selection Markers

- In bacteria, Neomycin resistance genes are dominant and are located on transposons Tn601 (Tn903) and Tn5. The neomycin resistance gene from Tn601 (Tn903) is referred to as aminoglycoside phosphotransferase 3' (I) or APH (3') I and that from Tn5 is referred to as aminoglycoside phosphotransferase 3' (II) or APH (3') II. APH (3') I and II encode the enzymes neomycin phosphotransferase I and II respectively, which confer resistance to various aminoglycoside antibiotics, including kanamycin and neomycin, in bacteria.

- The Neomycin resistance gene in our mammalian expression vectors is bacterial in origin (derived from Tn5 Neo) but is efficiently expressed in eukaryotic cells. Introduction of the Neomycin resistance gene into eukaryotic cells confers resistance to Geneticin (G418 Sulfate), enabling the cells to grow in media containing Geneticin.
- Geneticin (G418 Sulfate) is an aminoglycoside related to gentamycin, neomycin, and kanamycin. It is toxic to normal prokaryotic and eukaryotic cells.
- Neomycin sulfate is an antibiotic that is active against gram positive and gram negative bacteriaIt is typically used at a concentration of 50 ug/ml. It is generally stable in media stored at 37C for 5 days. Neomycin sulphate cannot be used instead of Geneticin in mammalian cells since Neomycin sulphate does not cross the cell membrane and hence does not enter the cell.
- The Neomycin resistance gene in our mammalian expression vectors is not functional in E coli because the bacterial promoter has been completely removed.
- All of Invitrogen's antibiotics (G418, Geneticin, Zeocin, Hygromycin and Blasticidin) can in theory be used together. Puromycin belongs to the same familiy as Blasticidin, hence, puromycin and blasticidin may not be compatible, however this has not been tested in-house.

Selection markers for cloning in bacteria

- Promoter for Ampicillin resistance sequence below is the sequence upstream of the ATG that contains the promoter. The promoter and Shine-Delgarno sequence are shown underlined.
  GTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAA ATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG AGT
- Promoter for Kan resistance: The kanamycin gene is driven by the ampicillin promoter.
  Ampicillin cannot be used for selection of eukaryotic cells if put under control of a eukaryotic/viral promoter. B-lactamase is targeted to (and secreted through) specific linkages in the bacterial cell wall. A eukaryotic cell lacks a cell wall and, therefore, cannot be selected using ampicillin.

Expression yields of specific proteins

(back to Table of Content)
(back to Protocol and Application Notes)
(back to General Information)

Cells were transiently transfected using the Calcium Phosphate Transfection Kit. Expression data is as follows:

Vector	COS Cells (ng CAT/mg Total Protein)	HeLa Cells (ng CAT/mg Total Protein)
pcDNA3/CAT	261 304	434 343
pRc/CMV/CAT	72 63	165 145
pSV/CAT	5 5	98 145
pB/CAT	0.1	0.4 0.5
Cells only	0.6	0.1 0.2

CAT protein levels were determined using an enzyme linked immunosorbent assay (ELISA) kit. The colorimetric assay is capable of detecting the fully native form as well as the denatured, enzymatically inactive form of the CAT protein. This method is comparable to the radioisotopic methods. This assay does not determine enzyme activity.

Molecular weights of Epitope tags and Control proteins

(back to Table of Content)
(back to Protocol and Application Notes)
(back to General Information)

Epitope tags

V5 :	1.5 kDa
V5-His :	3 kDa
HisG :	1 kDa
His (C-term) :	1 kDa
Xpress :	1 kDa
His-Xpress :	5 kDa
c-myc :	1.1 kDa

Control proteins

LacZ :	117 kDa, 3 kb
CAT :	32 kDa, 800 bp
GFP :	7 kDa
HSA :	67 kDa, 2 kb

Vector proteins

she-ble (zeocinR) :	13.7 kDa
kdRxR subunit :	56 kDa
Thioredoxin :	11.7 kDa
sFv :	34 kDa
PDGF :	42 kDa

Vector specific information
(back to Table of Content)
(back to Protocol and Application Notes)

Detailed information for all vectors including sequence, restriction map and polylinker information can be found on the Invitrogen website at http://www.invitrogen.com/content.cfm?pageid=94
pCDM8
pcDNA1, pcDNA1.1, pcDNA1/Amp, pcDNA1.1/Amp and pcDNA1/Neo
pcDNA3 and pcDNA3.1 Difference between pcDNA3 and pcDNA3.1
pCDNA3.2 and pCDNA6.2
pDEST26
pDEST27
pCR3.1 pCDNA3.1 His
pCDNA3.1 myc-His
pcDNA3.1/V5-His pcDNA3.1/V5-His-TOPO pcDNA3.1D/V5-His-TOPO
pCDNA3.2/V5/GW/D-TOPO and pCDNA6.2/V5/GW/D-TOPO vectors
pcDNA6/BioEase-DEST
pcDNA4/HisMax
pcDNA6.2/nGeneBLAzer-DEST
pcDNA6.2/nGeneBLAzer-GW/D-TOPO
pRc/CMV and pRc/CMV2
pRc/RSV pZeoSV
pEF
pBudCE4.1
NativePure pcDNA Gateway Vectors

pCDM8 (back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This vector has been discontinued.

- The vector is different from the one that was sent to use by Brian Seed in that an Ssp I to Ssp I fragment has been deleted in the SV40 poly A. The same deletion is present in pcDNA1and pcDNA1/Neo, in the SV40 polyA after the MCS, but not in the SV40 polyA after the Neomycin gene. The vector that was sent to us by Brian Seed does not have the deletion, but whether his sequence was wrong from the start or whether the deletion came later is unknown.
- SV40 ori is enhancer-less.
- pCDM8 splice site and polyA were derived from pSV2. pSV2 carried the SV40 Small T antigen which provided less efficient splicing than the SV40 Large T antigen or β-globin large T antigen..

pcDNA1, pcDNA1.1, pcDNA1/Amp, pcDNA1.1/Amp and pcDNA1/Neo (back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- These vectors have been discontinued.
- A restriction enzyme digest using Apa I will be able to differentiate between the vectors. Expected results are as follows: pcDNA1: 4033bp (linear) pcDNA1/Amp: 3621bp and 1180bp
- The pCDM8 reverse primer (Cat. #N570-02, discontinued) will work as a 3' primer for the pcDNA1 vectors. pCDM8 Reverse Primer: 5' TAAGGTTCCTTCACAAAG 3'.
- Low yields are common : pCDNA1/Neo expresses kanamycin resistance very weakly and the plasmid usually forms dimers which run very high on the gel. Also, the plasmid is grown in MC1061/P3 cells, and the residual P3 plasmid itself is kanamycin resistant, and will transform alone. Individual colonies should be picked and checked.
- Putative transcriptional start site in pcDNA1.1/Amp : the transcriptional start site has not been determined experimentally. It is based on the start site for the native CMV promoter or rather assumed to be at the same relative position. It is not likely that the transcription start site is affected by the insert in the MCS.
- The SV40 origin in these vectors is the same as that in pCDM8 (enhancerless, 163 bp)
- SV40 intron and polyA start and stop positions are the same in these vectors as they are in pcDM8.
- pcDNA I has an early SV40 pA.

pcDNA3 and pcDNA3.1 (back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- pcDNA3 has been discontinued and has been replaced by pcDNA3.1.
- pcDNA3.1 is derived from pcDNA3 which in turn is derived from pRc/CMV.
- There is a unique BsaBI site in pcDNA3.1, right before the Neomycin resistance gene, that is not present in pcDNA3.1/Zeo. This site can be used to distinguish between these two vectors by restriction analysis.
- In pcDNA3.1(+), pUC origin (according to KO, Harry, and PNAS 71:2260-2264) is unidirectional. Alignment of bp 3618-4291 of pcDNA3.1 with pBR322 (we know which way the origin runs in pBR322 as per NEB catalog Pg 230) results in a perfect match, indicating that the pUC origin runs counterclockwise toward the SV40 polyA.
- In pcDNA3.1, the SV40 poly A recognition site for the Neomycin resistance gene is AAATAAA and is located at 3148 and 3177 in the SV40 Poly A.
- pcDNA3.1 has no pBR322 sequences. The Ampicillin resistance gene and ori in pcDNA3.1 come from pUC.
- Splicing sites in pcDNA3 vector: None that have been experimentally mapped.
- The putative transcription start site from the CMV promoter is around 30 nt 5' of the starting site of the T7 promoter priming site. The putative transcription start site from the T7 promoter (if customers want to use the vector for in vitro transcription) is the first G at the 3' end of the T7 promoter priming site.
- pcDNA3.1/Zeo vectors have the bacterial EM7 promoter for expression of the zeocin resistance gene in E. coli, however, the EM7 promoter is not shown on the vector map.

Difference between pcDNA3 and pcDNA3.1
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- pcDNA3 is no longer available. pcDNA3.1 is a direct replacement of this mammalian expression vector. pcDNA3.1 was derived from pcDNA3 as follows. The middle of the original pcDNA3 polylinker contained

homology to a hairpin mRNA structure which involved the Eag I, Not I, and both BstXI sequences. The hairpin would only have affected expression of genes cloned downstream of the Not I site, if at all.

pcDNA3.2 and pcDNA6.2
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- These vectors have a TK polyadenylation site after the cloning site, as indicated by.2 in the names of the vectors.

pCDNA3.3 TOPO TA
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- The human cytomegalovirus immediate-early (HCMV IE1) gene promoter in the pcDNA3.3-TOPO vector is 680 bp and contains the native transcriptional start site (Hennighausen & Fleckenstein, 1986). This sequence results in high levels of transgene expression.

- The pcDNA3.3-TOPO vector is ideal for use with the FreeStyle MAX System and the OptiCHO Antibody Express Kit. OptiCHO Antibody Express Kit contains two vectors, pOptiVEC TOPO and pcDNA3.3 TOPO.

pDEST26
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This is an original LTI vector and is not derived from pcDNA. It has an N-terminal 6xHis tag which is not a HisG tag.

pDEST27
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This is an original LTI vector and is not derived from pcDNA. It has an N-terminal GST tag.
- In this vector, the ClaI site at 3632 is dam methylated, whereas the ClaI site at 5650 is not. This will explain single cutting by ClaI when DNA is propagated in dam+ cells.

pCR3.1
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- Unidirectional version was discontinued March 2004. Bidirectional version was discontinued August 2004
- The kanamycin resistance gene is driven by the Ampicillin promoter somewhere in the region of 4470-4586. There is also a weak promoter in the region 3535-3566 just before the SV40 promoter. This was tested in the lab and pCR3.1 is Ampicillin and Kanamycin resistant.

pCDNA3.1 His
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- Some anti-T7 antibodies (e.g. one is available from Novagen) recognize the sequence MASMTGGQQMG which is found immediately after the His-tag cleavage site in pcDNA3.1His A,B,C and pRSET. The DNA position is 953-985 bp in pcDNA3.1HisA. The antibody is capable of immunoprecipitating any protein carrying this tag.

- An easy way to remove the neo cassette from pcDNA3.1/His is to use Pvu II. This leaves a blunt ended backbone ready to receive another selection cassette. When the selection swap is done this way you also lose the f1 origin. It usually doesn't matter but be aware of it.

pCDNA3.1 myc-His
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- 3 aa spacing between the two tags is probably not functionally important the space was for a restriction site, and having the tags adjacent should work
- distinguish between the A, B, and C versions of pcDNA3.1(+)/myc-his

	ORF A	ORF B	ORF C
ApaI:	one cut	one cut	no
SacII:	no	one cut	no
BstEII:	no	no	one cut

So if one cuts A, B and C with SacII and BstEII separately, the B would be the one linearized by SacII, the C would be the one linearized by C alone, and the A would be the one that did not linearize with either.

pcDNA3.1/V5-His pcDNA3.1/V5-His-TOPO pcDNA3.1D/V5-His-TOPO
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- early stop codon is present In A version between BamHI and BstXI (in the MCS) presenting cloning difficulties to some customers. If not careful about the cloning strategy, one could stop translation before the V5His tag..

pCDNA3.2/V5/GW/D-TOPO and pCDNA6.2/V5/GW/D-TOPO vectors
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- Tested in house: HLA and CAT genes were cloned into vector, transfected into CHO cells, and expression detected with V5 antibody.

pcDNA6/BioEase-DEST
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- BioEase vectors include 72 amino acid sequence from K. pneumoniae that directs in vivo biotinylation of a specific lysine residue. Proteins produced in the BioEase vectors are expressed as fusions to this sequence.
- The entire 72 aa biotinylation sequence is necessary for efficient biotinylation in vivo.
- In E. coli, the K. pneumoniae tag is recognized and efficiently biotinylated by the enzyme biotin protein ligase (BPL) encoded by the birA gene (1). A similar system exists in mammalian cells
- This system is designed for in vivo biotinylation. This means that the cell does the work, and the protein tag is recognized by the cells internal machinery and is biotinylated. Biotin does not have to be added to the medium.
- applications with SA-conjugates for visualization.
- Vector backbone was prepared by digesting pcDNA6 His A with HindIII and KpnI, removing the His6, Xpress epitope, and EK cleavage site. Biotag epitope was cloned in at HindIII and Kpn1.
- GW version prepared by digesting pcDNA6/Biotag A vector with Acc65I and XbaI. The ends were blunted with Klenow and the vector treated with CIAP. The vector was ligated with the prepared Gateway Cassette RFC.1.

pcDNA4/HisMax
(back to Table of Content)
(back to Protocol and Application Notes)

(back to Vector specific information)

- A 2-5 fold increase in expression of GFP, LacZ, and Luciferase in pcDNA4/HisMax over the same vectors lacking the SP163 sequence was observed. This increase in GFP expression was seen by western blot in HeLa, CHO, 293 and NIH3T3 cells. No absolute numbers were calculated for the amount of protein or RNA expressed from these vectors in comparison to the controls.
- spacing between the end of the SP163 sequence (.....AAACC) and the ATG start codon is crucial. The last ACC acts like a triplet repeat codon and needs to be in the same 'triplet frame' as the start codon [ie. AA ACC (NNN)xN ATG...]. The number of triplets can be up to about 5-6 (15-18 bases), but any further away or a change of frame severely perturbs the enhancement activity of SP163. Because of the variability surrounding different cloning strategies, it was decided to provide an endogenous ATG in the best possible location relative to SP163.

pcDNA6.2/nGeneBLAzer-DEST
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This vector is compatible with Tag on Demand technology

pcDNA6.2/nGeneBLAzer-GW/D-TOPO
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This vector is compatible with Tag on Demand technology

pRc/CMV and pRc/CMV2
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- These vectors have been discontinued.
- pRc/CMV is a parental plasmid of pRcCMV/2 which contained both SP6 and T7 promoters for in vitro transcription.
- pRc/CMV2, pRc/RSV, pZeo/SV2 designed for high-level stable and transient expression in eukaryotic hosts. pRc/CMV is a parental plasmid of pRcCMV/2 which contained both SP6 and T7 promoters for in vitro transcription.
- contains the lac promoter: pCRII bases 1-200 (which contain lac promoter) align exactly with pRc/CMV bases 3589-3390. The orientation of this alignment is the opposite in the two plasmids relative to the graphic maps, meaning that the lacZ promoter directs transcription in a clockwise direction in pcRII (1->200) and counter clockwise in pRc/CMV (3589->3390) relative to the plasmid maps.
- CMV sequence in pRc/CMV stops 15 bases short of the 5' start site of the mRNA in the native CMV. This is an intronless version of the immediate early human cytomegalovirus promotor.
- pRcCMV/CAT (5.5kb) positive control plasmid Constuction details: An 800bp HindIII fragment encoding CAT was cloned into the HindIII site of pRC/CMV. The first ATG in this fragment is the correct ATG for the CAT open reading frame. The sequence of the 5' end of CAT fragment between the HindIII site and ATG is: AAGCTTCGACGAGATTTTCAGGAGCTAAGGAAGCTAAA ATG GAG AAA AAA ATC.....
- The 3' end of the BGH mRNA (to which the polyA tail is added) is position 1156 on the pRc/CMV map

pRc/RSV (back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- This vector has been discontinued.

- pRc/CMV2, pRc/RSV, pZeo/SV2 designed for high-level stable and transient expression in eukaryotic hosts. pRc/CMV is a parental plasmid of pRcCMV/2 which contained both SP6 and T7 promoters for in vitro transcription.

PZeoSV
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- pRc/CMV2, pRc/RSV, pZeo/SV2 designed for high-level stable and transient expression in eukaryotic hosts. pRc/CMV is a parental plasmid of pRcCMV/2 which contained both SP6 and T7 promoters for in vitro transcription.

pEF
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- All the pEF vectors contain intron 1 of the EF gene. In the maps, the intron runs from base 706 to 1644 (inclusive). The transcriptional start site is not yet determined, but in general starts about 20-25 bases downstream of the TATA box, which in the EF vectors is located at bases 645 to 649.

pBudCE4.1
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- For expression of two separate C-terminal tagged proteins from the same vector**.**
- There currently is no information available comparing the relative promoter strengths from this vector. The QC data indicate that both promoters express well but how each promoter expresses relative to the other in a given cell line should be determined by the investigator.
- The earlier version of this vector, pBudCE4 (discontinued) has an ATG present at position 674 in the MCS. Although this did not appear to affect translation of a cloned insert or the lacZ control, it has been changed to an ATT in the replacement vector, pBudCE4.1.

NativePure pcDNA Gateway Vectors
(back to Table of Content)
(back to Protocol and Application Notes)
(back to Vector specific information)

- Gateway-adapted vectors for expression of N- and C-terminal biotinylated fusion proteins.
- These vectors contain an N- or C-terminal capTEV tag (6xHis-TEV-TEV-BioEase) which directs in vivo biotinylation of the protein of interest. The biotinylated protein and associated protein complexes are then purified under native conditions by binding to streptavidin agarose using the NativePure Affinity purification kit. The TEV sites allow removal of the bound biotinylated proteins/complexes of interest while endogenous biotinylated proteins remain bound to the streptavidin agarose column. After TEV cleavage, the 6xHis tag on the protein allows purification of the protein/protein complexes under denaturing conditions using a Nickel column.
- Upon TEV cleavage, the BioEase tag is lost and the protein can be detected using the V5 epitope tag.

PRODUCT DOCUMENTATION
(back to Table of Content)

Brochures	**Citations**	**Cell lines**
COA	**FAQ**	**Licensing**

Manuals **MSDS** **Newsletters**

Vector Data

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(back to Table of Content)

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