PRODUCT INFORMATION & MANUAL

Retroviral Expression System

NBP2-29499

For research use only. Not for diagnostic or therapeutic procedures.

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TABLE OF CONTENTS

I. COMPONENTS

Store at -20° C		
Cat. No.	Description	Quantity
NBP2-29500	pCLXSN Expression Vector (0.5 mg/ml)	20 µl
NBP2-29502	pCLNCX Expression Vector (0.5 mg/ml)	20 µl
NBP2-29538	pCLNRX Expression Vector (0.5 mg/ml)	20 µl
NBP2-29539	pCLNDX Expression Vector (0.5 mg/ml)	20 µl
NBP2-29540	pCL-Eco Packaging Vector (0.5 mg/ml)	20 µl
NBP2-29541	pCL-Ampho Packaging Vector (0.5 mg/ml)	20 µl
NBP2-29542	pCL-10A1 Packaging Vector (0.5 mg/ml)	20 µl
NBP2-29543	pCLMFG-LacZ Retrovirus Reporter Vector (0.5 mg/ml)	20 µl

Store at 4° C		
Description		Quantity
Retromax	Buffer (2x)	10 ml
2.0 M $CaCl_2$		2 ml

Additional Materials Required:

- 293 Cells may be ordered from ATCC
- G418 selection antibiotic
- Cell culture reagents: DMEM, Fetal bovine serum, penicillin-streptomycin
- Trypsin-EDTA
- Phosphate buffered saline
- Cloning cylinders (for picking up stably transfected colonies)
- Polybrene for retroviral infection.

II. INTRODUCTION

Retrovirus vectors are very efficient tools for stably introducing genes into dividing cells. RetroMax™ retrovirus vector system is based on the pCL vector system developed by Naviaux et al. (1). The vectors used in this system have been designed to maximize recombinant-retrovirus titers in a simple, efficient, and flexible experimental system. All members of the RetroMax expression vector family (pCLXSN, pCLNCX, pCLNRX, and pCLNDX) have an extended packaging signal (y+) and are derived from safety-modified retrovirus vectors in which the gag open reading frame has been stopped by a point mutation, thereby minimizing the opportunity for replication-competent retrovirus production by recombination with packaging genome. The 5'-enhancer of Moloney murine sarcoma virus long terminal repeat (LTR) which is inhibited by E1A has been deleted and fused at the TATA box of the human CMV immediate early region (1). This results in initiation of viral RNA at or near the +1 position in the R region of the naturally programmed retrovirus. This results in transient-retrovirus titers in the range of 2-5 x 10^6 CFU/ml when 293 cells are used.

All three members of the RetroMax packaging vectors (pCL-Eco, pCL-Ampho, and pCL-10A1) have also been safety modified by deleting the packaging signal and the 3' LTR enhancer. This makes the RNAs of the helper genome virtually un-packageable. The advantage of these pCL packaging plasmids is a high level expression of gag, pol, and env proteins with a balanced stoichiometry that is not achieved with either transiently or stably expressed split-genome packaging constructs. Inclusion of these three packaging plasmids in the RetroMax kit allows the choice of expressing ectotropic, amphotropic, or 10A1 envelopes which leads to greater experimental flexibility.

The RetroMax system is designed for maximal virus titer in 293 cells. It takes advantages of two properties of 293 cells, i) high level of transfectibility, ii) strong E1A-mediated stimulation of CMV promoter controlled transcription. 293 cells are of nonmurine origin, hence the problem of selective packaging and transfer of VL30 genomes (present in all murine packaging cells) is avoided. Vector supernatants are free of helper virus and are of sufficiently high titer within 2 days of transient transfection in 293 cells to permit infection of more than 50% of dividing target cells in culture.

By introducing a retroviral vector into a cell expressing retroviral proteins, retroviral articles (virions) are shed into the culture medium at the rate of about one infectious particle/cell/day. Retrovirus tropism is determined at three levels. The first is simply a function of viral envelope protein, gp70. The envelope determines which cells the virus will enter. gp70 comes in three different flavors for gene therapy. A fourth one 10A1 is still experimental.

1. Ectotropic (usually (MoMuLV) mouse and rat cells only (not human)
2. Amphotropic (from 4070A MuLV) most mammalian cells (but not hamster)
3. Gibbon Ape Leukemia virus (GALV) many mammalian cells (including hamster)
4. 10A1 (MuLV) most mammalian cells (including hamster)

The second level of tropism is nuclear translocation and integration. This is defined by structural features of p30CA (but requires the full 160S nucleoprotein pre-integration complex, comprised of all the gag proteins and viral RNA and/or DNA). Naked DNA in the cytoplasm after retrovirus un-coating and reverse transcription is never seen.

The product of the FV-1 locus in murine cells interacts with p30CA, and can reduce the efficiency of translocation and integration (and thus apparent titer) 20-100 fold. Fortunately, the common Moloney-based packaging cells supply a p30CA form (NB tropic) that avoids this problem. The human equivalent of FV-1 has not yet been identified.

The third level of retrovirus tropism is determined by the transcriptional activity of the LTR (and/or internal promoter) in the transfected cell. In general, the Moloney (and MSV) LTR is active in most mammalian cell types, with the distinct exception of embryonic stem cells and teratocarcinoma cell lines (like F9), in which it is silenced. It is also potentially inhibited by E1A/p300 in 293 cells.

Ping-pong amplification is sometimes used to increase retrovirus vector titers, by co-culturing vector-producing ectotropic and amphotropic cell lines. This can increase vector titers 10 fold, but often at a heavy cost:

1. Frequent truncation, deletions, and point mutations may occur in the inserted cDNA.
2. You may generate helper virus if you are not using a safety-modified system.

III. OVERVIEW

Retrovirus particles are fragile. They are easily inactivated by 0.1% detergent, chloroform, phenol, 1% bleach, 70% ethanol, at 65°C for 30 min, pH <6.5 or >9.0, UV light, and autoclaving.

Simple high-speed centrifugation (100,000g x 90 min) produces enough hydrodynamic shear to strip many virions of their gp70, and thus infectivity (although reverse transcriptase activity is preserved).

Virus can be stored in culture medium (with 10% serum) at -70°C indefinitely. One freeze-thaw cycle reduces the titer about 2-3 fold compared to the fresh virus. The second freeze-thaw drops the titer another 5-10 fold. **Aliquot your virus for storage at -70°C.**

When filter sterilizing retrovirus, be sure to use non-detergent treated 0.22 or 0.45 um filters. Any trace of detergent will strip virus envelope and reduce your titers. Filter before freezing, and not after in order to avoid loses due to aggregation.

All murine retrovirus vectors produced in either mouse cells (like the NIH 3T3-based packaging cell lines) or primate cells (COS and 293) are rapidly inactivated by human serum complement, with kill kinetics of 2-3 logs in 5 min at 37°C. Human C1q initiates the cascade by binding p15ETM at the virion surface. This is an antibody-independent process.

Murine retroviruses are heat labile. They have an infectious half-life of only 6 h in culture medium at 4°C.

OUTLINE OF RETROMAX PROCEDURE

Day 0 Seed 293 cells and grow overnight.

Day 1 Transfect with retroviral vector containing gene of interest and an appropriate packaging vector.

Day 2 Replace medium.

Day 3 Harvest virus-containing supernatant. Virus may be stored at -70°C at this stage. Infect target cells, either for titer determination or for gene expression.

Day 4 Split infected target cells and grow for selecting stable virus-producing cell lines. For transient expression experiments, you may harvest the cells at this stage.

Day 5 Start selection by replacing the medium with G418 containing medium.

Day 9 Change medium and continue selection.

Day 14 Count antibiotic resistant colonies and calculate titer.

Note: If you are using retroviral expression system for the first time, we strongly recommend using the LacZ control plasmid included in the kit. The β-galactosidase expression can be monitored using β-gal staining kit (Cat.# NBP2-29546) or any other standard protocol.

IV. PROTOCOL

One Day Before Transfection

1. Seed 1 x 10^6 293 cells in 6 cm tissue culture plates. This should yield a cell density of about 30% confluence on the day of the experiment.

2. Incubate overnight at 37°C in DMEM supplemented with 10% fetal bovine serum, 1% pen-strep.

TRANSFECTION OF 293 CELLS WITH RETROVIRAL VECTOR

Day 1

1. Add 0.25 ml of RetroMax transfection buffer (previously tested for optimum transfection efficiency) to the required number of sterile 15 ml polypropylene tubes. Lipofectamine may have certain advantages in reproducibility, but this has not been tested extensively by us for overall virus titers.

2. Dilute 2 M $CaCl_2$ to final concentration of 0.25 M in sterile distilled water (30 µl of 2 M $CaCl_2$ and 220 µl of sterile water). Add the following to 1.5 ml sterile Eppendorf tube:

0.25 M $CaCl_2$ - 30 µl
pCL-ECO, pCL-Ampho, or pCL-10A1 - 10 µg
Retroviral vector containing your gene - 10 µg
Mix by vortexing.

3. Add the DNA/$CaCl_2$ mix drop-wise to the transfection buffer tubes (step 1) while lightly vortexing. Incubate at room temperature for 20 min.

4. During this incubation aspirate the transfection media from plates to be transfected and add 2 ml DMEM containing 10% FBS/1% P/S and place back in the incubator until step 3 is completed.

5. Add the DNA/$CaPO_4$ mix drop-wise to 293 cells on 6 cm T.C. plates. Place in humidified CO_2 incubator for 3-4 h. (Longer times may result in cells coming off the plates).

6. Carefully aspirate medium. Add 2 ml of warm PBS-15% Glycerol-no serum (glycerol shock medium) for 2 min. This step is optional. In some cases, it may increase the transfection efficiency by 2-fold.

7. Aspirate glycerol shock medium. Carefully add 4 ml DMEM containing 10% FBS, 1% P/S along the side of the dish. Incubate for 12 h.

Culture vessel	Surface area/well (cm^2)	Volume of plating medium (ml)	Transfection buffer (ml)	Packaging vector	Retro-vector	2 M $CaCl_2$(µl) and dil. vol (µl)
6 well	10	2	0.125	5 µg	5 µg	15 µl + 110 µl
6 cm	20	4	0.25	10 µg	10 µg	30 µl + 220 µl
10 cm	60	10	0.5	20 µg	20 µg	60 µl + 440 µl

Day 2

1. Aspirate medium and add fresh medium in the morning and incubate for 24 to 72 h.

Day 3 (24 h after addition of fresh medium).

1. Filter sterilize (0.45 mm syringe filters are convenient) the virus-containing supernatant to remove any cells in suspension. The virus can now be used directly, or stored at -70°C until needed.

2. Infect the desired target cells with 1 ml to 4 ml of 293 supernatant in 8 µg/ml Polybrene. The amount of supernatant you use depends on whether you are titering virus or want to infect the maximum number of target cells possible. Do not forget the polybrene. Omission of polybrene will drop your apparent titers 100-1000 fold.

DETERMINING THE VIRAL TITER

Remember that for titering, you must dilute the transfected supernatant at least 50 fold to stay in the linear part of the dilution curve. If you just want the maximum number of cells infected, then as little as a 2-fold dilution (equal volume mix) with the medium of the intended target cells is usually enough to prevent significant cell cycle inhibition.

1. For tittering, prepare serial dilutions (four 10-fold dilutions) of vector supernatant in order to be sure that you are in the linear part of the titration curve (ie, out of the Poisson region). Infections for accurate titering must be done at effective MOI is **0.1**. Target cells must be growing exponentially and only 30-50% confluent for maximum infection efficiencies.

2. Total virus-cell contact time should be a minimum of 12-24 h. This is because cycling cells are continuously entering and exiting the window of infectability. Even though the infective half life of the murine retrovirus particle is just 6-8 h at 37ºC the rate of new cells entering the window is greater for the first 24 h, so longer contact times means more infected target cells.

3. Always test your titers on a standard control cell line (we use NIH 3T3) in parallel with infections of other desired target cells. Intrinsic infectability of many target cells can vary widely from 0.01-100% of the titers on NIH 3T3 cells.

   - Virus titers on NIH 3T3 cells for empty RetroMax vectors are typically 2-3 x 10^6 CFU/ml for ecotropic virus and 1 x 10^6 for amphotropic virus, assuming a typical 293 transfection efficiency of 30-50%.

   - When tittering virus on NIH 3T3 cells, infect 2 x 10^5 cells on a 6 cm plate (in 4 ml medium), overnight (16 h) with 1, 3, and 10 ml of pCL vector supernatant. You will need larger volumes for lower titer vectors, or cells that are more refractory to infection than NIH 3T3.

   - If virus stock is limiting: the most efficient use can be made by using 0.5-1 ml volumes to serially infect target cells in 6 cm plates (or 2-3 ml in 10 cm plates), and adding fresh virus every 4-6 h for 3-4 infection cycles. Continuous exposure to virus for about 24 h is necessary in order to ensure that all cells have cycled through their receptivity window (S-G2) for retroviral infection. Be sure to add polybrene to 8 μg/ml.

4. Check your transfection efficiency by drawing a 1 cm square on the bottom of the plate of transfected 293 cells. Scrape harvest all the cells outside of this square (if desired) for RNA or protein analysis (CAT assays, ONPG-LacZ, Westerns, Northerns, Hirts, etc.). Fix and stain the transfected cells remaining inside the 1 cm square with X-Gal to determine the transfection efficiency (TXE). Typical transfection efficiencies are 30-50% in this subline of 293 cells. The same DNA and reagents will give TXEs of 2-15% on COS cells.

SELECTION FOR STABLE CELL LINES

Day 4 (12-24 h after infection)

1. If using a vector that confers G418 resistance, split the infected target cells at various dilutions (1:20 to 1:200) into 10 cm T.C. plates. A 1:20 dilution is about 10^5 NIH 3T3 cells. If 0.1% of the cells were infected, you will get about 100 colonies after 8-12 days of selection.

When infecting primary cells:

Accurate titers cannot be obtained when infecting primary fibroblasts, bone marrow or tumor cells because these cell types display density-dependent growth and typically have low plating efficiencies of 0.01%. This means that if 1000-10,000 cells are plated, only 1-100 colonies will actually clone out, *even if they are all infected and G418-resistant.* Therefore when infecting these cells, do not split them more than they will tolerate and only if they are >80% confluent (this is usually only a 1:2 to 1:4 dilution).

If you are selecting primary cells in G418, you will need to trypsinize and concentrate the cells by replating on sequentially smaller dishes until sufficient G418-resistant cells have grown out that you can begin expanding the infected pool of cells. This process can take 2 weeks. Effective titers for a particular primary cell type and vector will be a constant percentage of the titer observed on NIH 3T3 cells.

If using vectors that do not confer antibiotic resistance (like LacZ or GFP), simply change the medium today. Primary bone marrow cells should always be infected by co-cultivation of autochthonous stromal cells and virus producer cells in the presence of IL-3 (or WEHI-conditioned medium) and GM-CSF (a potent stromal cell growth factor). **Never select them in G418.**

Day 5 (2 days after infection)

1. Begin selection of cells infected with virus vectors conferring antibiotic resistance by adding 100 ml of a 100x stock to a 10 cm dish containing 10 ml of medium.

   The correct concentration of G418 (or any antibiotic) varies widely for different cell types. You must determine the concentration empirically. For NIH 3T3 cells this is 400-1000 mg/ml (active) G418. For other cell types, the right concentration is that which results no observable death at Day 2 and about-30-50% on Day 4. Complete G418 selection is usually achieved in 7-10 days.

2. If using a virus vector that does not contain a selectable marker (e.g., pCL-LacZ, MFG-GM-CSF, GFP), or if you would like a rapid assessment of gene expression in the infected target cells (for vectors expressing CAT, Luciferase, GFP, or LacZ), this can be tested today: b-gal staining of fixed cells in situ, (you can calculate the LacZ titer of your virus from this); CAT, Luciferase, or ONPG assays are done from cell lysates.

3. Because of the natural kinetics of retroviral infection, integration, and expression, no selection pressure (antibiotics) or assessment of gene expression should be made until 48 h after infection, i.e., if cells are infected on Day 3, gene expression cannot be accurately tested until Day 5.

Day 9 (4 days after starting selection).

1. Add fresh medium (and antibiotic) to cells under selection.

2. If infected cells were primary fibroblasts or primary tumor cells, you may need to increase the cell density (that has fallen due the death of uninfected cells under selection) by one of two methods, in order to avoid cell death due to densities falling below that tolerated by your particular primary cell type: concentrate the infected cells by trypsinization and plating on a smaller dish, or add uninfected primary cells (of the same type) to bring the density up to 50%, and continue selection. You must let the added (non-G418 resistant) cells attach to the plates for 3-4 h before adding G418 again.

3. Most primary cells will not grow as isolated clones because of density-dependent growth requirements. Attempts to pick clones frequently result in the loss of all infected cells.

Day 14 (10-13 days after infection)

1. Count the antibiotic resistant colonies, and calculate the titer (e.g., Neo titer) in your virus supernatants.

Example: Let us say you count 125 G418-resistant colonies on a 10 cm plate. If you infected (5×10^5) NIH 3T3 cells with 1 ml of virus supernatant, then split out the infected cells 1:20, your calculated titer is $125 \times 1000 \times 20 = 2.5 \times 10^6$ CFU/ml.

Note: *Many cDNAs of interest may have either cytostatic or cytotoxic effects on infected cells, so that stable colony formation under G418 selection does not actually reflect the true number of cells initially infected. Only growing cells make colonies.*

V. MAXIMIZING RETROVIRUS TITERS

1. The principal determinant of retrovirus titer is the abundance of packageable RNA, and not the abundance of viral proteins. Viral pro teins are typically made in 20-fold stoichiometric excess. In fact, too much gp85 env can actual lower your titers because of impaired glycoprotein processing and assembly.

2. The RetroMax (pCL) system generates the highest abundance of packageable viral RNA of any known transient system by exploiting the power of the CMV IE enhancer-promoter in E1A-expressing 293 cells. The natural enhancer of the unmodified MuLV LTR is inhibited by E1A-p300 in 293 cells, so attempts to use non-pCL retroviral vectors in 293 cells will yield 20-50 fold lower titers, even with the same transfection efficiencies.

3. If you are studying cDNAs that do not have cytostatic or cytotoxic phenotypes, it may be possible to generate higher titer virus using traditional retrovirus packaging cells. This process takes 2 months (instead of 2 days for pCL). The highest titers are always obtained from stably infected (not transfected), cloned (not pooled) packaging cell lines. This is because transfected sequences are often inactivated by methylation, and because pro-virus integration position effects can influence gene expression from the same retrovirus vector in different clones of infected cells can vary over a 100-fold range (i.e., integration into heterochromatic regions of the genome gives poor expression, while integration into euchromatic regions gives high expression).

4. In deciding whether to go through the process of selecting and characterizing clones of packaging cells or simply preparing virus by the rapid pCL system, one must consider the intended applications. If you need a rapid test for the stable expression properties of a battery of mutant cDNAs that you have prepared, the pCL system is often adequate, or in the case of cytostatic and cytotoxic cDNAs, it is often the only way to produce usable amounts of virus.

   Sometimes producing the virus (with a toxic or static cDNA) in cells from a different species can overcome the titer problems that result from cell growth inhibition.

   If on the other hand, you plan to use the virus produced as a reagent that you can go back to many times over the next few years, then you need to pick clones of stable packaging cells.

5. pCL vectors reproducibly produce titers of 0.5-5 x 10^6 CFU/ml with good transfections, independent of phenotype and size (less than 4 kb) of the cDNA.

6. Typical retrovirus titers from cloned packaging cells are 10^4-10^6 CFU/ml (sometimes you can get 107), depending profoundly on the size and toxic properties of the cDNA expressed in these mouse fibroblast cell lines. cDNAs that are 2-4 kb long lead to modest reductions in titer because of packaging constraints. **cDNAs larger than 4 kb are subject to frequent spontaneous deletions and truncation during retroviral reverse transcription, and show large reductions in virus titers, and frequent non-expressing clones.**

Scaling Up:

1. Transfect 10 cm plates of 293 cells with 30-40 mg of pCL vector containing your gene of interest in 1 ml of $CaCl_2$-HBS.

2. Replace the medium on Day 2.

3. Harvest and replace the medium every 24 h on Days 3, 4, and 5. This should give you 30 ml of virus supernatant from each transfected plate. The titers in supernatants harvested on Days 3 and 4 are equivalent. We suspect that Day 5 will be almost the same.

VI. THE SAFE USE OF MURINE RETROVIRUS VECTORS AND SAFETY PRECAUTIONS

Replication competent retroviruses (RCR) are called helper virus, or simply "Helper".

They require 3 trans- (gag, pol, and env), and 7 major, cis-active control elements (U3, R, U5, PBS, SD, y, and SA) in order to replicate.

The most common retrovirus vectors are based on the Moloney Murine Leukemia Virus (MoMuLV), encoding only the 7 cis elements.

These vectors are defective and cannot replicate without picking up 7.1 kb of sequence by homologous recombination with a helper genome (while simultaneously deleting your cDNA). Modern vectors are now "safety modified" by including a stop mutation early in "gag" (or a frame-shift) that pre-

vents gag translation and limits the sequence window available for productive recombination with helper genomes.

Packaging cells supply the 9 processed proteins encoded by gag, pol, and env (p15MA, p12 p30CA, p10NC, p14PRO, p85RT, p40IN, gp70SU, and p15ETM) necessary for virion assembly.

Modern packaging cells are safety-modified by dividing the gag-pol genes, and the env gene on two separate plasmids. These two plasmids are serially transfected (not co-transfected) into NIH 3T3 cells. The resulting safety modifications yield the modern split genome packaging cells.

Current evidence suggests that in order to initiate a pathogenic infection in primates with amphotropic murine retroviral vectors, three requirements must be met:

1. The infected host must be immunocompromised.
2. The vector preparation must contain helper virus.
3. Direct body fluid contact, e.g., intravenous inoculation is required for transfer.

However, for safe use of the RetroMax system, the user is strongly advised to follow the following guidelines:

1. According to NIH guidelines all retroviral production and transduction work must be done in a Biosafety Level 2 (BL2) facility.
2. Work in laminar flow, HEPA filtered hoods that receive annual maintenance and recertification.
3. Use sterile technique (flaming is not necessary and not recommended because of convection disturbances to airflow patterns).
4. Aspirate all liquid waste into flasks containing 5-10% (v/v) of a microbiocidal agent.
5. Discard spent plasticware in biohazard bags and autoclave before discarding.
6. Dispose spent glassware in detergent containers for cleaning and autoclaving.
7. Clean all surfaces with 70% ethanol at the end of the work.
8. Switch on the UV light immediately after work in laminar flow

Note: Retroviruses are not spread by aerosols.

VI. REFERENCES

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2. Cornetta K, Blaese RM, Anderson WF. Amphotropic leukemia retrovirus is not an acute pathogen for primates. Human Gene Therapy, 1: 15-30 (1990).
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4. Miller AD, Miller DG, Garcia JV, and Lynch C. Use of retroviral vectors for gene transfer and expression. Methods in Enzymology 217: 581-599 1993).
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6. Ott D, Friedrich R and Rein A. Sequence analysis of amphotropic and 10A1 murine leukemia viruses: close relationship to mink cell focus-inducing viruses. J. Virol. 64: 757-766 (1990).

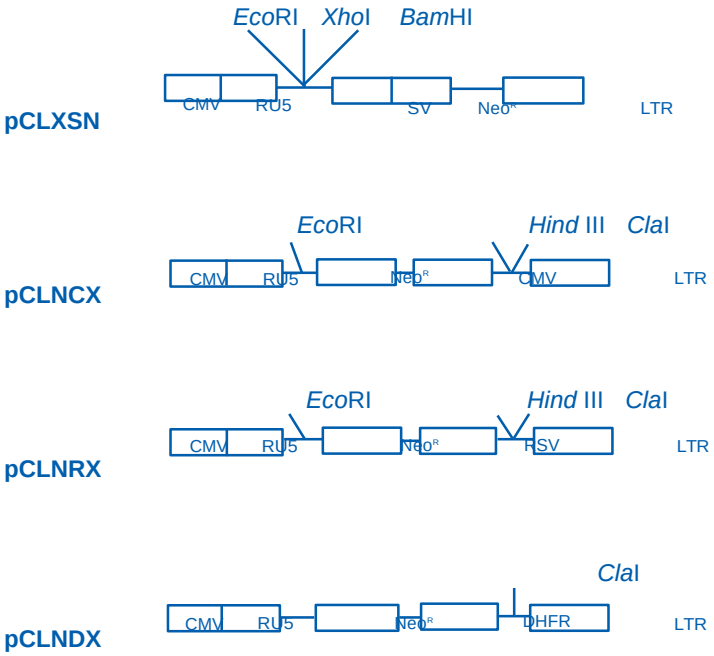
VII. APPENDIX

A. Expression Vectors

Choice of Vectors

The RetroMax expression vectors are designed to maximize recombinant-retrovirus titers in a simple, efficient, and flexible experimental system. All members of the RetroMax expression vector family (pCLXSN, pCLNCX, pCLNRX, and pCLNDX) have an extended packaging signal (y+) and are derived from safety-modified retrovirus vectors in which the gag open reading frame has been stopped by a point mutation (1), thereby minimizing the opportunity for replication competent retrovirus production by recombination with packaging genome. Four expression vectors are provided in the kit. Clone your gene of interest into one of these vectors:

Schematic Diagram of pCL Vectors



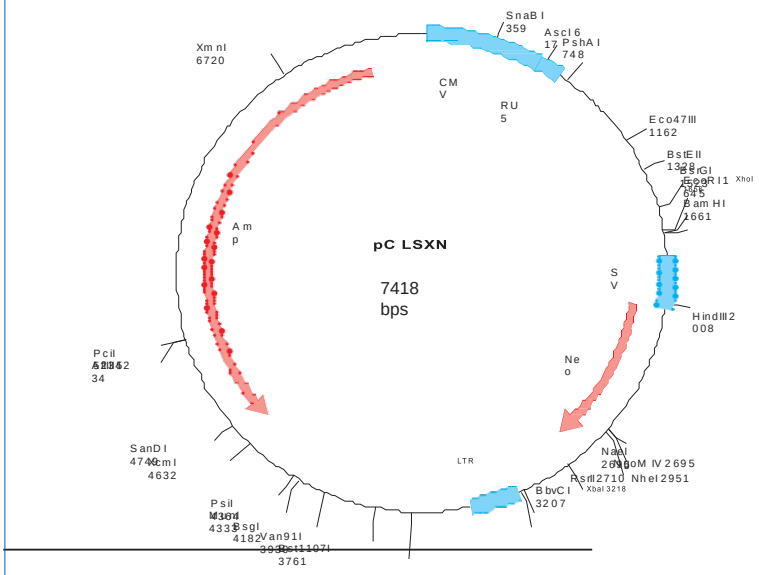
PCLXSN Retrovirus Expression Vector

Catalog No.: NBP2-29500

Quantity: 10 μg in 20 μl 1x TE (10 mM Tris, pH 7.5, 1 mM EDTA)

The inserted cDNA is under the control of LTR. The gene of interest can be cloned in *Eco* RI, *Xho* I and Bam HI cloning sites. In this case permanent cell lines can be selected. The complete vector sequence is available online at http://www.novusbio.com/.

Schematic presentation of pCLXSN vector



CMV immediate early enhancer-promoter allows high-efficiency transcription in 293 cells. However, this is lost during viral replication. Hence, the gene of interest cloned into Eco RI, Xho I, or Bam HI cloning site is driven by Moloney MLV and murine sarcoma virus LTR (RU5).

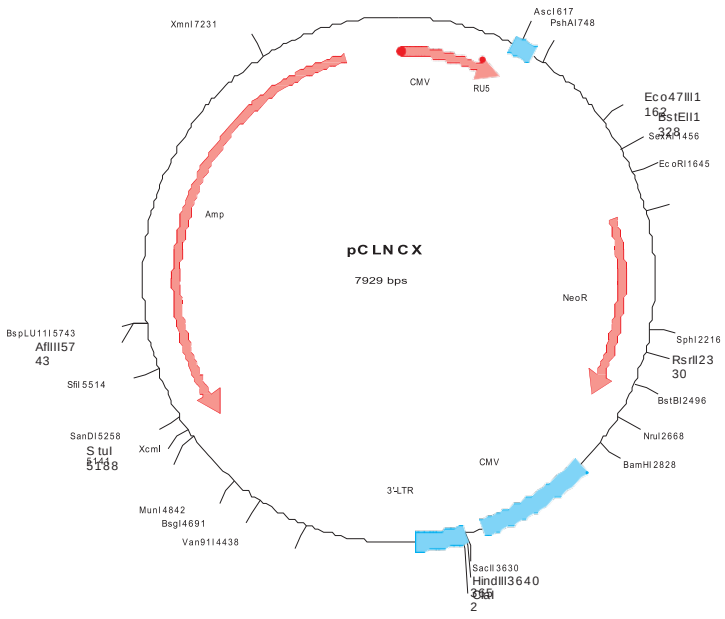
pCLNCX Retrovirus Expression Vector

Catalog No.: NBP2-29502

Quantity: 10 μg in 20 μl 1x TE (10 mM Tris, pH 7.5, 1 mM EDTA)

The gene of interest is cloned into *Hind* III and *Cla* I sites and is expressed under CMV promoter control. A second gene can be cloned into *Eco*RI site located upstream of the Neo^R gene. However, in this case permanent cell lines cannot be selected. Combined size of two inserts should not be more than 4 kb. The complete vector sequence is available online at http://www.novusbio.com/.

Schematic presentation of pCLNCX vector



CMV immediate early promoter/enhancer allows high-efficiency transcription in 293 cells. However, this is lost during viral replication. Hence, the gene of interest cloned into Eco RI cloning site is driven by Moloney MLV and murine sarcoma virus LTR (RU5). However, in this case Neo^R gene will not be expressed. For expression under the control of CMV early promoter/enhancer, the gene of interest should be cloned into Hind III or Cla I site.

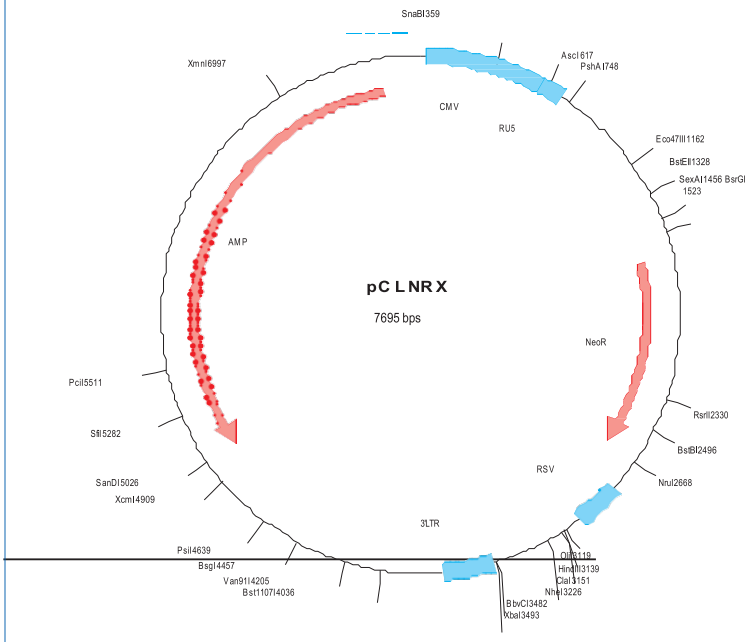
pCLNRX Retrovirus Expression Vector

Catalog No.: NBP2-29538

Quantity: 10 μg in 20 μl 1x TE (10 mM Tris, pH 7.5, 1 mM EDTA)

Similar to the pCLNCX vector, it differs only in that the foreign gene is cloned downstream of RSV promoter into *Hind*III or *Cla*I. A second gene can be cloned into *Eco*RI site located upstream of the Neo^R gene. However, in this case permanent cell lines cannot be selected. Combined size of two inserts should not be more than 4 kb. The complete vector sequence is available online at http://www.novusbio.com/.

Schematic presentation of pCLNRX vector



CMV immediate early promoter/enhancer allows high-efficiency transcription in 293 cells. However, this is lost during viral replication. Hence, the gene of interest cloned into Eco RI cloning site is driven by Moloney MLV and murine sarcoma virus LTR (RU5). However, in this case Neo^R gene will not be expressed. For expression under the control of RSV the gene of interest should be cloned into Hind III or Cla I site.

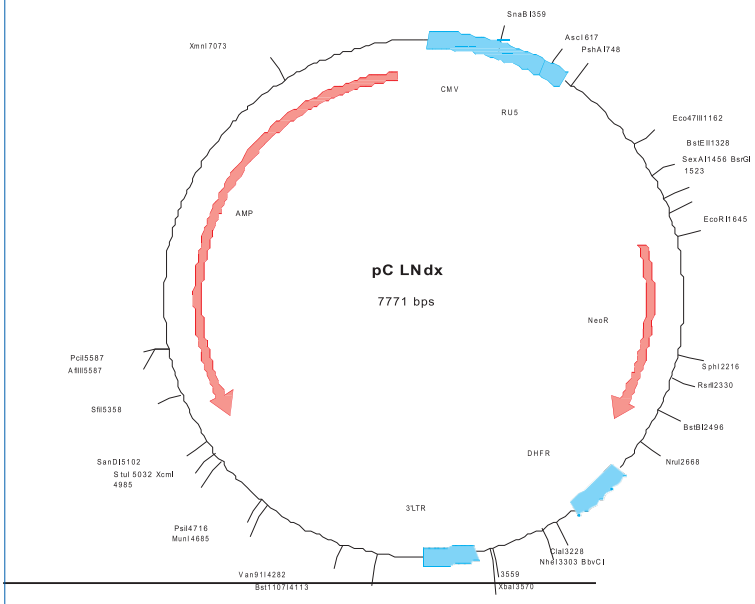
pCLNDX Retrovirus Expression Vector

Catalog No.: NBP2-29539

Quantity: 10 μg in 20 μl 1x TE (10 mM Tris, pH 7.5, 1 mM EDTA)

The gene of interest can be cloned into the single cloning site, *Cla* I, which puts it under the control of the DHFR promoter. However, in this case permanent cell lines cannot be selected. The complete vector sequence is available online at http://www.novusbio.com/.

Schematic presentation of pCLNDX vector



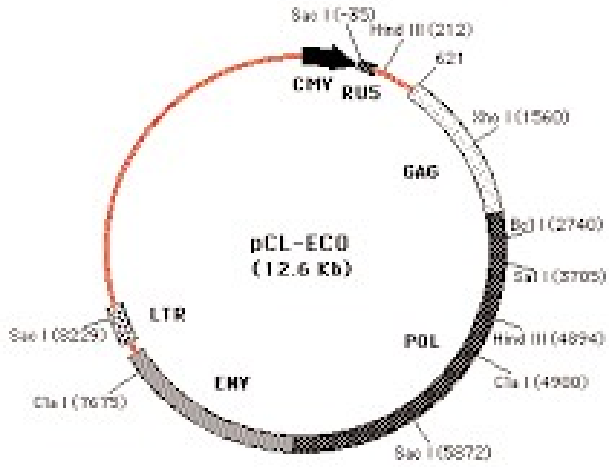
CMV immediate early promoter/enhancer allows high-efficiency transcription in 293 cells. However, this is lost during viral replication. Hence, the gene of interest cloned into Eco RI cloning site is driven by Moloney MLV and murine sarcoma virus LTR (RU5). However, in this case Neo^R gene will not be expressed. For expression under the control of DHFR the gene of interest should be cloned into Cla I site.

B. Packaging Vectors

The pCL packaging vectors is a part of the RetroMax Expression System (Cat# NBP2-29499) and has been designed to maximize recombinant retrovirus titers in a simple, efficient, and flexible experimental system. By introducing a retroviral vector into a cell expressing retroviral proteins, retroviral particles (virions) are shed into the culture medium at the rate of about 1 infectious particle/cell/day. Retrovirus tropism is determined at 3 levels. The first is simply a function of viral envelope protein, gp70. The envelope determines which cells the virus will enter. gp70 comes in three different flavors for gene therapy. Retroviruses obtained by co-transfection with pCL-Eco vector will infect mouse and rat cells, but not human cells.

Sequencing information is not available for any of our packaging vectors.

Schematic presentation of one of three packaging vectors: pCL-Eco



The gene coding for envelope protein was replaced with envelop gene from 4070A and 10A1 strain of MuLV to create pCL-Ampho and pCL-10A1 packaging vectors, respectively.

pCL-Eco Retrovirus Packaging Vector

Catalog No.: NBP2-29540

Quantity: 20 μg in 20 μl 1x TE (10 mM Tris, pH 7.5, 1 mM

Ectotropic (usually) (MoMuLV)	Mouse and rat cells only (not human)

pCL-Ampho Retrovirus Packaging Vector

Catalog No.: NBP2-29541

Quantity: 20 μg in 20 μl (10 mM Tris, pH 7.5, 1 mM EDTA)

Amphotropic (from 4070A MuLV)	Most mammalian cells (but not hamster)

pCL-10A1 Retrovirus Packaging Vector

Catalog No.: NBP2-29542

Quantity: 20 μg in 20 μl (10 mM Tris, pH 7.5, 1 mM EDTA)

10A1 (MuLV)	Most mammalian cells (including hamster)

Storage:
For long-term storage, store the Packaging at -20°C.

References:

1. Naviaux, RK, Costanzi, E, Haas, M and Verma, I. The pCL vector system: Rapid production of helper-free, high titer, recombinant retoviruses. J. Virol 70: 5701-5705 (1996).

Note: *Packaging Vectors are for research use only. Not for use in humans. Use of this by commercial entities for any commercial purpose requires the user to obtain a commercial license.*

C. LacZ Retroviral Reporter Vector

pCL-MFG-LacZ Retrovirus Reporter Vector

Catalog No.: NBP2-29543

Quantity: 20 μg in 20 μl (10 mM Tris, pH 7.5, 1 mM EDTA)

Sequencing information is not available for any of our packaging vectors.

Background:

The pCL-MFG-LacZ reporter vector is a part of the RetroMax expression system (Cat# NBP2-29499) and has been designed to assay beta-galactosidase activity. This plasmid has an ampicillin resistance gene and should be grown in LB-ampicillin media before use.

Novus also provides Beta-Galactosidase Staining Kit (Catalog no: NBP2-29546) as well as Beta-Galatosidase Quantitation Kit (Catalog no: NBP2-29547).

Storage:
For long-term storage, store at -20°C.

References:

1. Naviaux, RK, Costanzi, E, Haas, M and Verma, I. The pCL vector system: Rapid production of helper-free, high titer, recombinant retro viruses. J. Virol 70: 5701-5705 (1996).

Note: *For research use only. Not for use in humans. Use of this by commercial entities for any commercial purpose requires the user to obtain a commercial license.*

D. Nucleotide Sequence

Nucleotide Sequence pCLXSN

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CMV - 21-584
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SV- 1770-2030
NeoR – 2030- 2880
3’LTR- 3230 – 3471
AMP- 7135-4666C

Nucleotide Sequence pCLNCX

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Molecule Features:

Name	Start	End
CMV prom	21	584
RU5 and packaging signal	585	700
NeoR	1645	2645
3’-LTR	3637	3878
AmpR	7624	5155

Nucleotide Sequence pCLNRX

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CMV - 21-584
RU5 – 584 -700
NeoR – 1645 – 2645
RSV- 2827 – 3039
3’LTR- 3502 – 3743
AMP- 7495-5026C

Nucleotide Sequence pCLNDX

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CMV – 21- 584 bp
RU5 – 584- 700 bp
Neo – 1645 – 2643
DHFR – 2832 – 3615
3’LTR – 3637 – 3878
AMP – 7624 – 5155C