pCMV-ProLink™

Mammalian Cloning Vectors Datasheet

Vectors for expressing ProLink-tagged fusion proteins in EA parental cell-lines

Vector	Part Number
pCMV-ProLink Cloning Vector Bundle (Includes pCMV-ProLink 1 Vector, pCMV-ProLink 2 Vector, pCMV-ARMS1-ProLink 2 Vector and pCMV-ARMS2-ProLink 2 Vector)	93-0491
pCMV-ProLink 1 Vector	93-0167
pCMV-ProLink 2 Vector	93-0171
pCMV-ARMS1-ProLink 2 Vector	93-0489
pCMV-ARMS2-ProLink 2 Vector	93-0490

Read the entire product insert fully before beginning the assay.

For additional information or Technical Support, contact info@discoverx.com or visit www.discoverx.com.

Corporate Headquarters
42501 Albrae Street
Fremont, CA 94538 USA
Tel: 510-771-3500 or 866-448-4864
Fax: 510-979-9930
Email: techsupport@discoverx.com
Web: www.discoverx.com

European Regional Headquarters
DiscoverX Corporation Ltd
Faraday Wharf, Holt Street
Aston Science Park, Birmingham, B7 4BB UK
Phone: +44 121 260 6142
Fax: +44 121 260 6143

Overview

Technology Principle

DiscoverX cell-based products feature the powerful *in vivo* application of the established Enzyme Fragment Complementation technology pioneered by DiscoverX. In this approach, two complementing fragments (**ProLink**, a small enzyme donor fragment, and **EA**, a larger enzyme acceptor fragment) of the β-galactosidase (β-gal) enzyme are expressed as protein fusions in stably transfected, clonally derived cells. pCMV-ProLink™ cloning vectors are intended for cloning a GPCR or other protein of interest with ProLink as a C-terminal tag, and subsequently transfecting into a parental cell line containing an EA-tagged protein. The ProLink tag is similar to the ProLabel™ tag used in other EFC technologies from DiscoverX, however ProLink has been optimized to detect protein-protein interactions.

In the GPCR β-arrestin system, upon activation by a compound, the tagged GPCR is phosphorylated, providing a binding site for β-Arrestin:EA, which is expressed in the PathHunter® β-Arrestin Cell Line. The interaction of β-Arrestin and the GPCR forces the interaction of ProLink and EA, thus allowing complementation of the two fragments of β-gal to form a functional enzyme capable of hydrolyzing a substrate molecule and generating a chemiluminescent signal.

ProLink2 tag has higher affinity for EA than the original ProLink tag (~3-fold greater) and is useful for weak protein:protein interactions.

G-protein Receptor Kinase (GRK) phosphorylates GPCRs; facilitating recruitment of Arrestins. The Arrestin Recruitment Modifying Sequence (ARMS*) is a GRK consensus phosphorylation site deduced from analysis of GPCRs that generate a high signal to background in the PathHunter assay. The addition of ARMS can improve assays that have a low signal-to-background ratio.

ProLink Vectors

Vector Name	Prod. No.	Vector Features	Application
pCMV-ProLink 1	93-0167	Low Affinity PK1 tag	Standard protein:protein interaction assays
pCMV-ProLink 2	93-0171	High Affinity PK2 tag	Weak protein:protein interactions
pCMV-ARMS1-ProLink 2	93-0489	High affinity PK2 tag; ARMS1 sequence	Improve signal-to-background (S:B) ratios by addition of an ARMS1 sequence
pCMV-ARMS2-ProLink 2	93-0490	High affinity PK2 tag; ARMS2 sequence	Improve S:B ratios by addition of ARMS2

*ARMS is an 18-21 amino acid spacer between the GPCR and the PK tag that has been shown to enhance β-Arrestin recruitment thus improving S:B in the PathHunter assays. ARMS1 and ARMS2 are different variants of the 18-21 amino acid spacer.

Vector

The pCMV-ProLink vectors are proprietary protein expression vectors ready for sub-cloning your GPCR or other protein of interest. The resulting plasmid pCMV-protein-ProLink, when expressed in a mammalian cell, will have a ProLink tag on the C-terminus of the protein.

For the text sequence of the vector(s), please address an email with your request and the vector's product code to techsupport@discoverx.com.

Materials Provided

- 10 µg of pCMV-ProLink plasmid DNA (frozen) as specified on the tube label. Store at –20°C until use.
- Product insert containing vector map.

Materials Not Provided

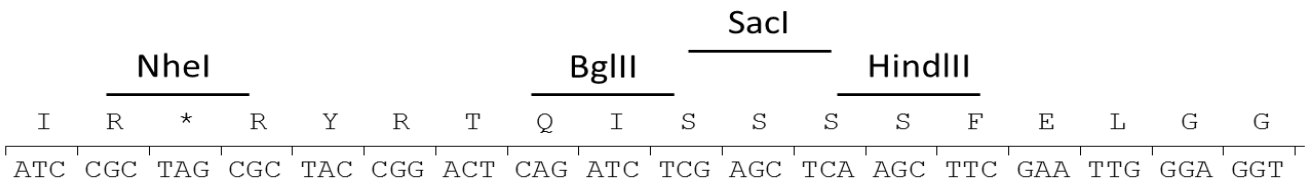
- PathHunter Parental Cell Lines (DiscoverX, Cat. # Many)
- PathHunter Detection Kit (DiscoverX, Cat. # 93-0001 Series)

Cloning Notes

Cloning Information

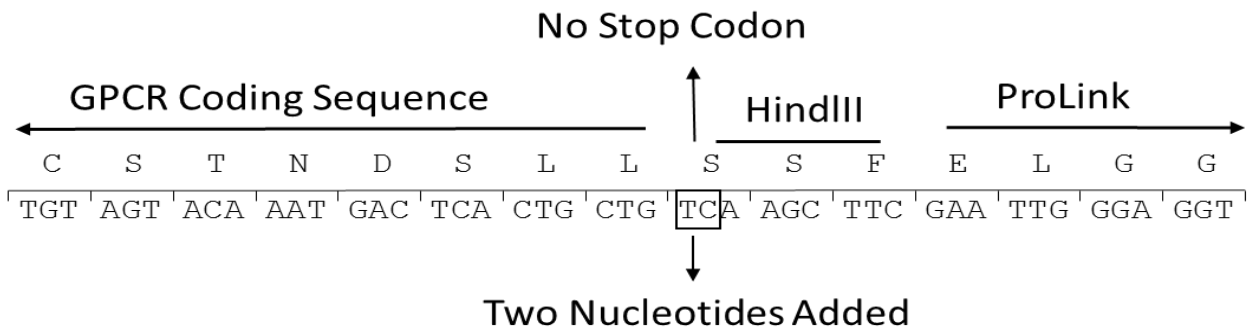
- ProLink sequence does not contain a start codon.
- A proper start sequence and Kozak sequence should be present in the GPCR or other protein of interest. DiscoverX uses ACC (Kozak sequence) immediately prior to the ATG start codon to enhance expression.
- The GPCR or other protein of interest must be in frame with ProLink peptide and not contain a stop codon. Restriction sites may require nucleotide additions to maintain reading frame integrity. See example below for *Hind III* site.
- Using *Lac Z* as Gene-X is not recommended.
- Use 50 µg/mLKanamycin for propagating plasmid DNA in *E. coli* cells.

Multiple Cloning Site Sequence*:



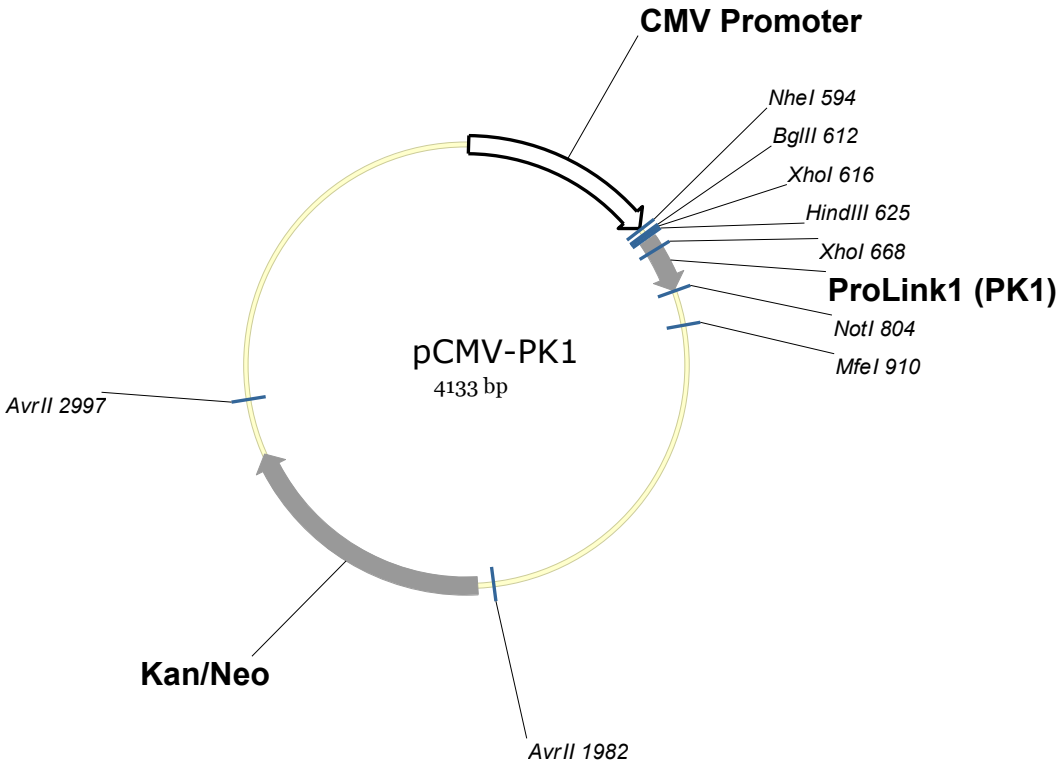
*For the entire ProLink tag text sequence, please address an email with your request and the product code 93-1067 to info@discoverx.com.

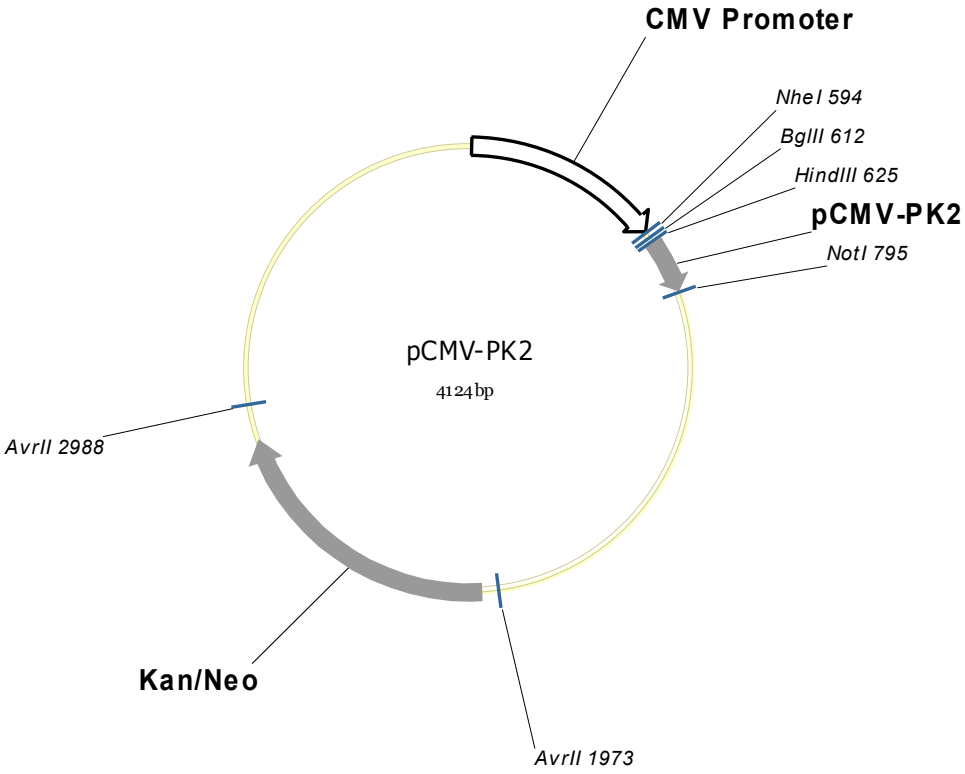
Example of GPCR Properly Inserted In-Frame with ProLink Tag:

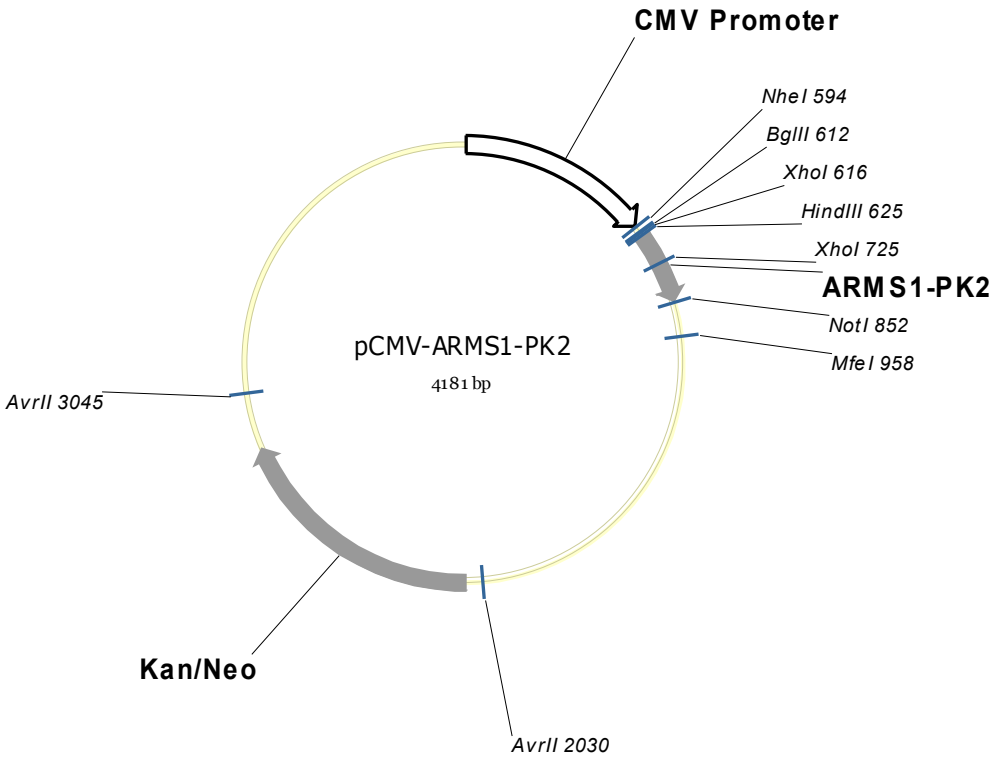


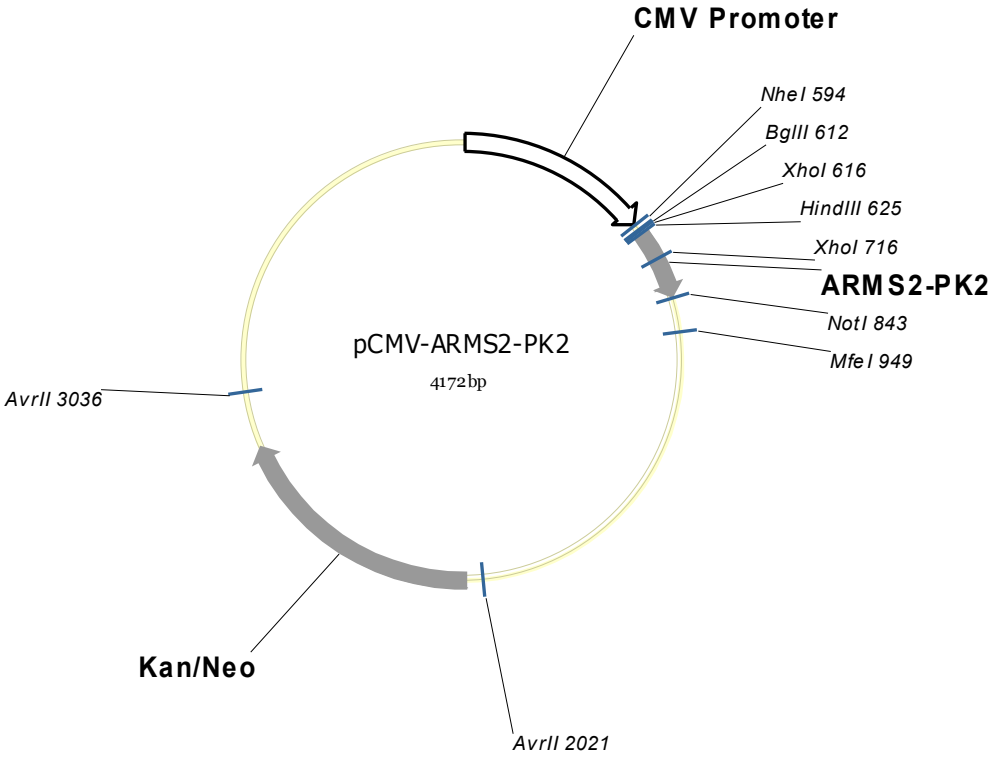
Important Note: When designing a PCR primer for the 3' end of the GPCR, the GPCR stop codon should be removed. Primers must also be designed for in-frame fusion with ProLink. For example, when using the *Hind III* site, add two nucleotides before the AAGCTT to ensure that the reading frame is maintained between the GPCR and ProLink. Other restriction sites require nucleotide additions as well to maintain reading frame integrity.

Vector Maps


CMV Promoter
NheI 594
BglII 612
XhoI 616
HindIII 625
XhoI 668
ProLink1 (PK1)
NotI 804
MfeI 910
pCMV-PK1
4133 bp
AvrII 2997
Kan/Neo
AvrII 1982


CMV Promoter
NheI 594
BglII 612
HindIII 625
pCMV-PK2
NotI 795
pCMV-PK2
4124 bp
AvrII 2988
Kan/Neo
AvrII 1973


CMV Promoter
NheI 594
BglII 612
XhoI 616
HindIII 625
XhoI 725
ARMS1-PK2
NotI 852
MfeI 958
pCMV-ARMS1-PK2
4181 bp
AvrII 3045
Kan/Neo
AvrII 2030


CMV Promoter
NheI 594
BglII 612
XhoI 616
HindIII 625
XhoI 716
ARMS2-PK2
NotI 843
MfeI 949
pCMV-ARMS2-PK2
4172bp
AvrII 3036
Kan/Neo
AvrII 2021

Detection

Detection Reagents

For ProLink-tagged fusion protein detection in PathHunter® β-Arrestin Parental Cell Lines (expressing EA), follow the step-by-step protocol in the PathHunter Detection Reagents (93-0001 Kit Series) product insert.

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