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Biosafety Information Document
Stable Cell Lines and Cell Pools

Catalog Number	93-1000C3
Product Name	PathHunter® U2OS IL4R/IL13RA1 Dimerization Cell Line

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Section I: Product Information

Catalog Number: 93-1000C3

Product Name: PathHunter® U2OS IL4R/IL13RA1 Dimerization Cell Line

	Overexpressed Targets
Target 1	IL4R(1-261)-PK
Target 2	IL13RA1(1-371)-EA
Target 3	Not Present

Stable Cell Format: Clone

Host Cell Line: U2OS

Host Cell Species: Human

Section II: Retroviral Transduction System

Target gene(s) of interest are transduced into a host cell line via a replication incompetent Moloney Murine Leukemia Virus (MMLV). The viral vectors are produced by co-transfecting Phoenix helper-free retrovirus producer cells with a retroviral plasmid containing the target gene of interest (which may also be fused to an Enzyme Acceptor [EA] or Enzyme Donor [ED] enzyme fragment complementation [EFC] tag), as well as separate vectors containing genes for the gag-pol and envelope proteins required for producing the virus (see References below). The retrovirus is pseudotyped with the amphotropic envelope protein VSV-G, which enables the virus to infect any mammalian cell type. Infection of a host cell results in delivery of the target gene of interest, which is then integrated into the host cell's genome. Expression of the transduced target gene is driven either by a cytomegalovirus (CMV) promoter or by a hybrid MMLV/Murine Sarcoma Virus retroviral LTR promoter.

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Section III: Overview of Transduction Protocol

The following is a brief protocol for generating virus containing the retroviral plasmid construct (including the target gene), transducing it into the host cell, and generating stable pools. Complete information (e.g. Target and antibiotic selection marker) for each vector construct transduced into the host cells is included in Section IV: Vector Information. When generating cell lines overexpressing multiple target genes, each target gene-carrying retroviral vector is transduced into the host cells via a series of independent infections (i.e. infection cycles). Infected cells undergo antibiotic selection to generate a stable pool, thus completing the infection cycle, before moving on to the next infection cycle (if required).

Protocol:

1. Phoenix Cells (see References for details) were transfected with an MMLV-derived retroviral vector containing the target gene of interest (which may be fused to an EFC tag). Cells are incubated for 48 hours to allow for production of infectious, but replication-incompetent retrovirus.

2. Following the 48-hour incubation, the supernatant from the transfected Phoenix Cells was collected and used to infect the recipient host cells. Infected cells were cultured in selection antibiotic-free culture media for 48 hours to allow for expansion and protein expression.

3. Following the 48-hour incubation, the infected cells were transferred to a new culture vessel containing fresh culture media supplemented with the selection antibiotic that matches the antibiotic resistance marker contained in the transduced vector, plus any selection antibiotic that may be required for maintaining expression of any overexpressed genes previously transduced into the recipient host cell. The cells were then incubated under antibiotic selection pressure for 5 – 10 days to generate a stable pool.

4. For cell lines that overexpress multiple genes, steps 1 – 3 are repeated until all vector constructs have been transduced into the recipient host cells. Refer to Number of Overexpressed Targets in Section I: Product Information for a list of targets transduced in to the cells.

5. Upon completion of all infection cycles and generation of the final stable pool, cell assay development proceeds to the final product development and production stages (see Section V: Additional Information).

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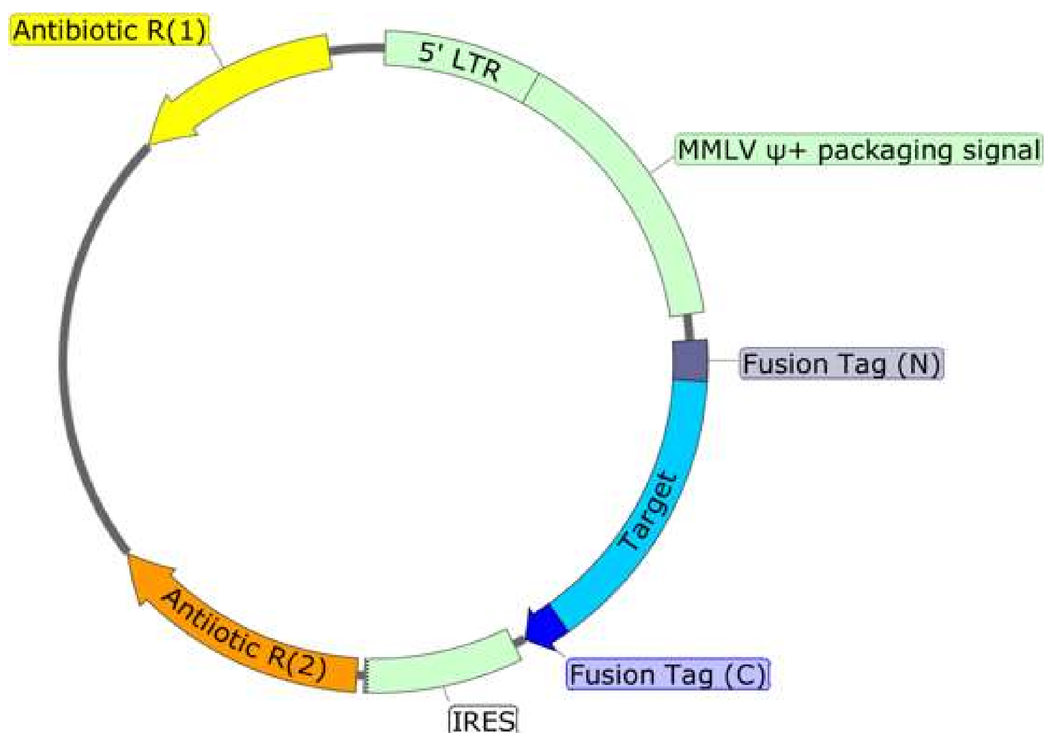
Section IV: Vector Information

The following section provides information for each of the retroviral vectors transduced into the cell assay. Note: some vector map elements may not be to scale. Please refer to the tables to determine the specific elements included in each vector.

Target 1 Plasmid Elements	
Element Label	**Element Description**
5' LTR	MMLV-MMSV hybrid LTR
3' LTR	MMLV 3' long terminal repeat
Packaging Signal	MMLV Ψ+ Packaging Signal
Antibiotic Resistance 1	Ampicillin resistance gene (β-lactamase)
Antibiotic Resistance 2	G418
Promoter_for Target	5' LTR
Fusion Tag (N-terminal)	Not Present
Fusion Tag (C-terminal)	PK
Fusion Tag Species	E. coli K12
Target Name	IL4R(1-261)
Target Species	Human
Target Accession #	NM_000418



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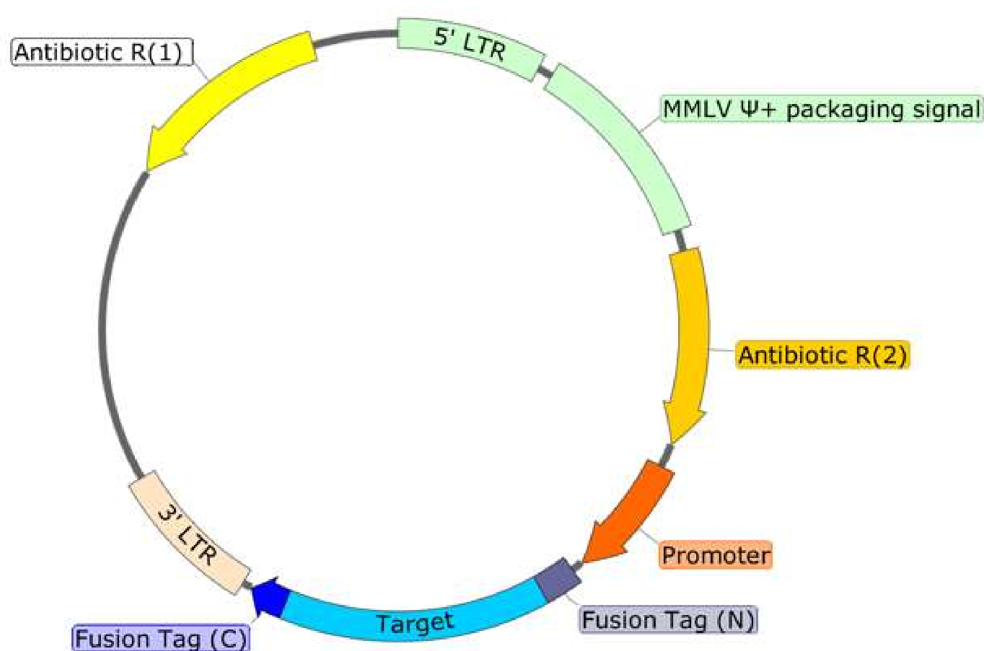
Target 2 Plasmid Elements	
Element Label	**Element Description**
5' LTR	MMSV 5' long terminal repeat
3' LTR	MMLV 3' long terminal repeat
Packaging Signal	MMLV Ψ+ Packaging Signal
Antibiotic Resistance 1	Ampicillin resistance gene (β-lactamase)
Antibiotic Resistance 2	Hygromycin
Promoter_for Target	CMV (cytomegalovirus) promoter/enhancer
Fusion Tag (N-terminal)	Not Present
Fusion Tag (C-terminal)	EA
Fusion Tag Species	E. coli K12
Target Name	IL13RA1(1-371)
Target Species	Human
Target Accession #	NM_001560.2



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Section V: Additional Information

Following the development of the stable pool, a clonal cell line was isolated from the pool (via limiting dilution method) and a master lot of cryopreserved cells was generated using standard cell-cryopreservation techniques. The finished product is a clonal cell line overexpressing the transduced genes described in Section IV: Vector Information.

Cell assay lot production was initiated by thawing cells from the cryopreserved master lot, and then culturing the thawed cells in an appropriate growth media to expand the population of cells. Aliquots of the cells were transferred to cryovials and then frozen using standard cell-cryopreservation techniques. The resulting production lot of the cell assay was confirmed to be free of Mycoplasma (using a commercially available mycoplasma detection kit) before the lot was released to inventory. Each production lot of cells represents at least 5 passages from the final viral transduction stage.

References:

Pear, W., Scott, M., and Nolan, G.P. (1997) Generation of high titre, helper-free retroviruses by transient transfection. In Methods in Molecular Medicine: Gene Therapy Protocols, (P. Robbins, ed.), Humana Press, Totowa, NJ), pp. 41-57.

See https://web.stanford.edu/group/nolan/_OldWebsite/retroviral_systems/phx.html for additional information about the Phoenix retrovirus producer cells.

Glossary of Terms:

Term	Definition
ARMS	Arrestin Recruitment Modulating Sequence: if present on a target fusion, it is an element designed to enhance or enable β-arrestin recruitment
CMV	Cytomegalovirus
EA	Enzyme Acceptor: An inactive fragment of β-Galactosidase enzyme.
ED	Enzyme Donor: An inactive fragment of β-Galactosidase enzyme. ED is a generic term for the ProLabel, ProLink and ProLink2 fragments.
EFC	Enzyme Fragment Complementation: the recombination of the complementary, but enzymatically inactive EA and ED fragments to form active β-Galactosidase enzyme.
LTR	Viral Long Terminal Repeat
MMLV	Moloney Murine Leukemia Virus
MMSV	Moloney Murine Sarcoma Virus
PK	ProLink
PK2	ProLink2
PL or ePL	ProLabel

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