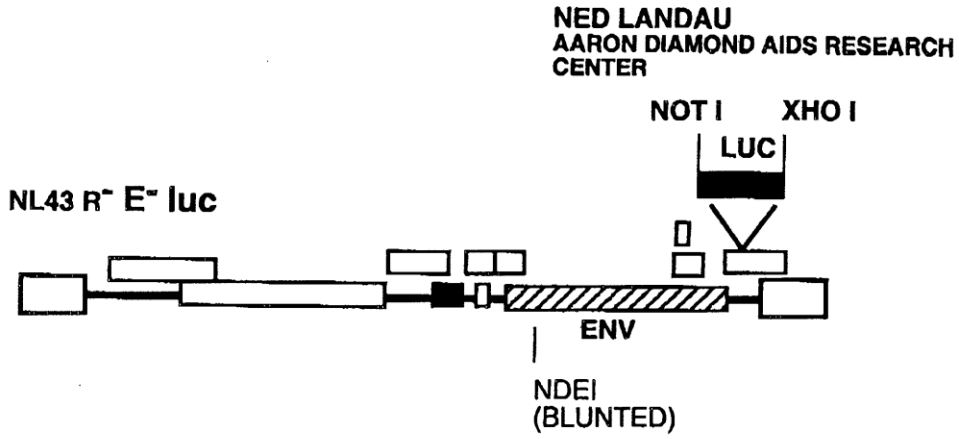
Centre For AIDS Reagents

Data Sheet

NAME:	pNL4-3.Luc.R-E-
REPOSITORY REFERENCE:	**ARP2128**
DESCRIPTION:	Firefly luciferase gene was inserted into the pNL4-3 nef gene. Two frameshifts (5′ Env and Vpr aa 26) render this clone Env^- and Vpr^-.
SPECIAL CHARACTERISTICS:	Competent for a single round of replication. Requires cotransfection with *env* expression vector to produce infectious virus. Virus can be produced by transfecting 2 x 10^6 293 or 293T cells with 20 µg of the NL4-3 DNA, or with 10 µg NL4-3 DNA and 10 µg *env* expression vector DNA. Transfections can be performed in a 10 cm^2 tissue culture dish using standard calcium phosphate protocols. Virus is typically harvested 48 hours post-transfection. Infections should be performed in a total volume of 0.5 ml. Amphotropic pseudotypes generally have much higher infectivity than those bearing HIV-1 Env. Cultures infected with the luciferase viruses can be lysed 2–5 days post-infection and assayed using commercial lysis buffer and luciferase reagents (Promega).
PRESENTATION:	5 µl purified plasmid DNA at 1µg/µl.
STORAGE:	Store at –70 °C or less. Avoid multiple freeze-thaw cycles as product degradation may occur.
CLONING VECTOR:	pUC-19.
SOURCE:	Dr. Nathaniel Landau, Aaron Diamond AIDS Research Center, The Rockefeller University.
REFERENCES:	He J, Choe S, Walker R, Di Marzio P, Morgan DO, Landau NR. *J Virol* **69**: 6705–6711, 1995.Connor RI, Chen BK, Choe S, Landau NR. *Virology* **206**: 935–944, 1995.

NOTE: **Patent pending. Requests from commercial organizations must be directed to the New York University Medical Center, ATTN: Office of Industrial Liaison, 550 First Avenue, New York, NY 10016, Tel: 212-263-8178.**

PLASMID MAP.



LUCIFERASE REPORTER VIRUSES

PNL-LUC-R-ENV- AND PNL-LUC-R+-ENV- ARE PLASMIDS BASED ON THE HIV-1 PROVIRAL CLONE PNL4-3. BOTH PLASMIDS CONTAIN THE FIREFLY LUCIFERASE GENE IN THE *nef* position, are *env*- due to a frameshift near its 5'-end. pNL-Luc-R-Env- has a frameshift in *vpr* that prevents its production. The plasmids can be grown in E. coli with ampicillin selection.

TO MAKE INFECTIOUS VIRUS, 293 OR 293T CELLS ARE TRANSFECTED WITH A MIXTURE OF THE REPORTER VIRUS DNA AND ENV EXPRESSION VECTOR DNA. WE GENERALLY USE A RATIO OF REPORTER TO ENV DNA OF 10µg:10µg. Virus is harvested 48 hr. after transfection, titred by p24 ELISA, aliquoted and frozen at -80°. Cells can be infected in virtually any format. Typically we infect 1 X 10^5 cells with 10 ng p24 in about 0.5 ml total volume. Amphotropic pseudotypes generally have a much higher infectivity than viruses bearing HIV-1 Env. Cultures are lysed in 100 µl two to five days postinfection and luciferase activity in about 20 µl is measured using commercial reagents (Promega). Construction and use of these vectors is described in Connor et al., (1995)Virology 206:935-944.

ACKNOWLEDGEMENTS: Publications should acknowledge the donor of the reagent and the Centre for AIDS Reagents. Suggested wording can be found on our website in the "Acknowledgement" section at:-

www.nibsc.org/science_and_research/virology/centre_for_aids_reagents.aspx

Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR, this can be by e-mail or printed copy