Description

EGFR (Exon19del) Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses result in the expression of EGFR (epidermal growth factor receptor) (NM_005228.5), with an Exon19 deletion, driven by an EF1a promoter. The lentiviruses also contain a puromycin selection marker (Figure 1).

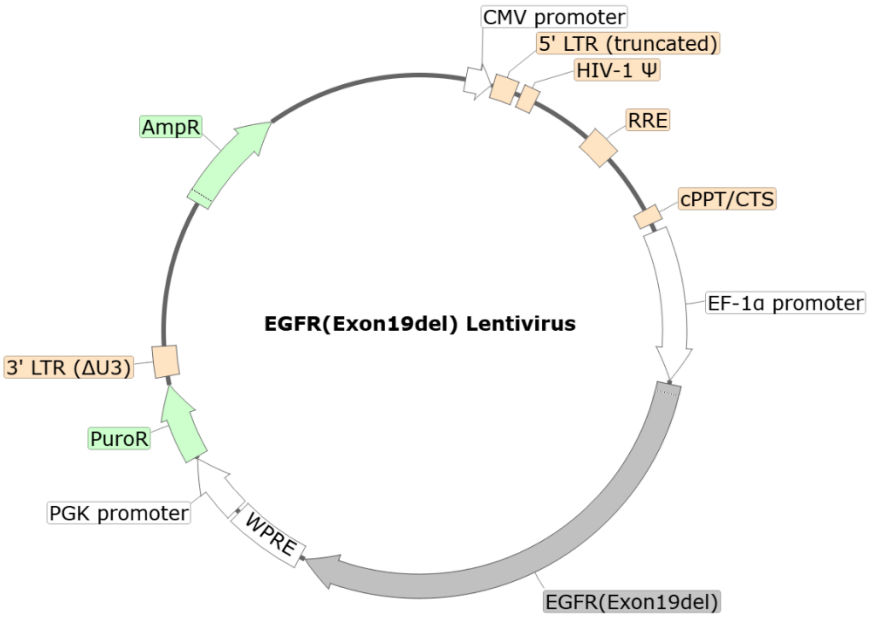

Figure 1. Schematic of the lenti-vector used to generate EGFR (Exon19del) Lentivirus.

Background

EGFR (epidermal growth factor receptor), also known as ERBB-1 and HER1, is the cell-surface tyrosine kinase receptor for members of the epidermal growth factor family. Its ligands include EGF, TGFα (transforming growth factor alpha), HB-EGF (heparin-binding EGF), betacellulin, amphiregulin, epiregulin, and epigen. EGFR exists in basal states as an inactive monomer. Upon ligand binding it forms an asymmetric dimer, for instance with HER2 (human epidermal growth factor receptor 2), which induces autophosphorylation, creating binding sites for adaptor proteins such as GRB2 (growth factor receptor-bound protein 2) and/or CBL (Casitas B-lineage lymphoma). EGFR can bind to several adaptor proteins simultaneously and thus activate multiple positive and negative signaling pathways. Overexpression and/or hyperactivation of EGFR kinase is associated with several human cancers such as lung, glioblastoma (GBM), and epithelial tumors of the neck and head. Mutations in EGFR can result in constitutive activation, allowing tumor cell proliferation and development of resistance to therapy. One of the most common mutations in NSCLC (non-small cell lung cancer) is an exon 19 deletion. Its role in cancer has led to the development of anticancer therapeutics targeting EGFR. There are several clinically approved inhibitors, such as Erlotinib and Gefitinib, for the treatment of NSCLC (non-small cell lung cancer) and pancreatic cancer. In addition, several monoclonal antibodies have been approved, such as Cetuximab. Patients that respond to anti-EGFR therapy tend to develop resistance, highlighting the need for further studies and new therapeutic avenues.

Application(s)

- Expression of human EGFR (Exon19del) in cells of interest.
- Generate human EGFR (Exon19del) expressing cell pools or stable cell lines by puromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells. They are supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Notes

To generate a EGFR (Exon19del) stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, https://bpsbioscience.com/kill-curve-protocol), for antibiotic selection of transduced cells, followed by clonal selection.

Validation Data

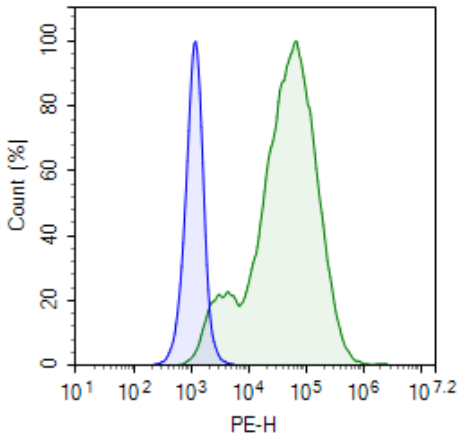

Figure 2. Expression of EGFR in EGFR Knockout A549 cells transduced with EGFR (Exon19del) Lentivirus.
Approximately 100,000 EGFR Knockout A549 cells (BPS Bioscience #83541) were transduced with 1 x 10^6 TU (100 µl of 10^7 TU/ml) of EGFR (Exon19del) Lentivirus in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (#78939). 48 hours post-transduction, the cells were selected with 1 µg/ml of puromycin, and the puromycin resistant cell pool was stained with PE anti-human EGFR Antibody (BioLegend #352904) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity. Blue, EGFR knockout A549 cells; Green, EGFR knockout A549 cells transduced with EGFR lentivirus (Exon19del).

Data is representative.

Sequence

Human EGFR (Exon19del) sequence

MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLK
TIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVES
IQWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCCHNQCA
AGCTGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYVVTDHGSCVRACGAD
SYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILK
TVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWK
KLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQ
CHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGL
EGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFMRRRHIVRKRTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIK
VLGSGAFGTVYKGLWIPEGEKVKIPVAIKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHK
DNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALE
SILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELII
EFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNS
TVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHY
QDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLRVAP
QSSEFIGA

References

Nakamura J.L., 2007 *Expert Opin. Ther. Targets* 11(4):463-472.
Uribe M.K., *et al.*, 2021 *Cancers (Basel)* 13(11):2748.
Huang L.-T., *et al.*, 2022 *Lung Cancer* 166:9016.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
EGFR Lentivirus	82459	500 µl x 2
EGFR (T790M, C797S, L858R) Lentivirus	82437	500 µl x 2
EGFR (D770-N771insNPG) Lentivirus	82461	500 µl x 2
EGFR Knockout A549 Cell Line	83541	2 vials

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