Improvements to pFLIP and Lentiviral Transgenesis Vectors

Technology #12557

Applications

FLIP cassette-containing plasmid vectors (pFLIP), which is suitable for lentiviral transgenesis, can be used to transduce Cre mice and cells, creating constitutive and inducible lines expressing miR30-based RNAi constructs that knock down a gene of interest. This targeted knockdown is commonly used to investigate the role of particular genes in development and disease progression. The FLIP cassette can also contain reporter genes such as Thy1.1, eGFP, or RFP to test for viral titer.

Problem Addressed

Many proprietary lentivirus transfer vectors that confer the expression of micro-RNA (miRNA) for gene knockdown via RNA interference (RNAi) either are not inducible, inducible but not expressed in a cell-type-specific manner, or only inducible and cell-type specific with both the additional expression of Cre recombinase and the administration of doxycycline or tetracycline. A more elegant method to create transgenic lines with cell-type-specific knockdown would improve upon current methods.

Technology

This technology takes advantage of the Cre-lox system. The enzyme Cre recombinase can catalyze the site-specific recombination of DNA strands encoding corresponding LoxP sequences. Recombinations can lead to either the deletion or the flipping of the DNA sequence between the two LoxP sites. In this invention, two LoxP pairs along a DNA insert allow for Cre-induced recombination to occur, which simultaneously deletes a strand of DNA encoding an antibiotic resistance gene and the Thy1.1 cell surface marker protein and flips another antisense strand of DNA into the sense orientation. This strand of DNA contains a selectable marker, such as a gene for a fluorescent marker, as well as a 3' UTR (untranslated region) that encodes a micro-RNA which can knock down a gene of interest. The sequence is engineered with self-cleavage at the C-terminal of the fluorescent marker protein upon protein translation. The 3' UTR, which encodes the miRNA sequence, is engineered for high levels of transcription. This elegant system can be customized with different selectable markers, different antibiotic resistance genes, different miRNA knockdown sequences, and different promoters for gene expression. Moreover, this method leaves the doxycycline/tetracycline channel open, introducing the possibility of creating a gene knockdown system that is both cell-type-specific and time-dependent.

Advantages

- Elegant method to create cell-type-specific and time-dependent gene knockdown
- Selection marker for determining viral titer and cell sorting
- 3'UTR region encoding miRNA engineered for high levels of transcription
- Easily customizable for specific purposes

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Categories For This Invention:

Life Sciences
Research Tools
Animal Models
Cell Line
DNA
Expression Systems
Screening Assays
Transgenic
Vector & Plasmid

Intellectual Property:

Cre-Lox based gene knockdown constructs and methods of use thereof
Issued US Patent
9,043,994

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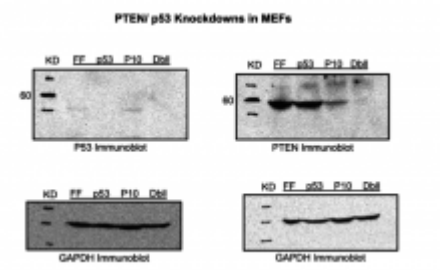
Publications:

A System for Cre-Regulated RNA Interference in Vivo
PNAS
Sep 16, 2008, p. 13895-13900

External Links:

Hynes Lab
http://hynes-lab.mit.edu/

Image Gallery:



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