"New promoters and new lentiviral vectors: efficient and coordinated expression of multiple genes"

Background and Description of Invention. The present invention describes new promoters designed and constructed for multiple gene expression that have been incorporated into state-of-the-art, self-inactivating lentiviral vectors to reach stable integration and efficient, coordinated expression in all transduced cells. The cassette includes a minimal promoter joined upstream to an efficient promoter in the opposite orientation. The rationale behind this design is the sharing of orientation-independent enhancer activity contributed from the efficient promoter between the two closely linked basal promoters acting in opposite directions. The bi-directional promoter then mediates the coordinated, divergent transcription of two mRNAs *(Amendola M., Venneri MA., Biffi A, Vigna E., Naldini L., "Coordinate dual-gene transgenesis by lentiviral vectors carrying synthetic bidirectional promoters" Nat. Biotechnol.* 2005*).*

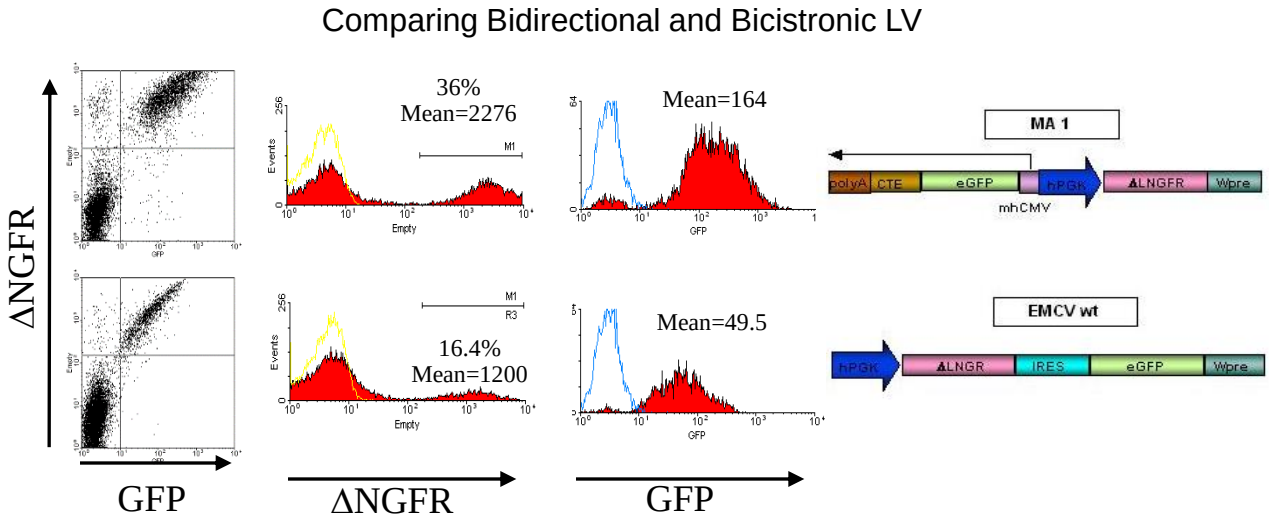

Figure 1. Efficient and coordinated gene expression in human hematopoietic progenitor cells by bidirectional lentiviral vectors. CD34+ hematopoietic progenitors were purified from cord blood and transduced with normalized amounts of the indicated vectors ($5x10^7$ TU/ml) expressing a truncated form of the human low-affinity NGF receptor (DLNGFR) and GFP (Green Fluorescent Protein). The bidirectional MA-1 vector was compared to the best performing IRES bicistronic vector. The bidirectional vector reached a higher frequency of DLNGFR+ cells that also displayed a higher expression level and this was even more true for GFP. In the left panels the dot plot analysis for both DNGFR and GFP expression are shown; in the center panels the histograms for DNGFR expression are shown; in the right panels the histograms for GFP expression of the DNGFR expressing cells are shown.

Patent information. The international patent application was published as WO2004094642.
Patents granted in Europe (EP1616012), US (US8501464) and Canada (CA2523138). Available for outlicensing in specific fields.

Stage of Development. Murine transplantation studies are in evaluation, to verify the advantages of such vectors for the amplification or selection of polyclonal population of engineered hematopoietic stem cells. We are also performing vector transgenesis experiments to prove efficient and coordinated expression of two exogenous genes regardless of tissue type.

Potential Applications and Competitive Advantages. Expression of two or more exogenous genes in an efficient and coordinated manner within the same cell is a difficult but important task to achieve. Our system allows the coordinated expression of two or more genes and can find several applications in:

- Gene function and in vitro and in vivo target validation studies
- Gene therapy
- Expression of multiple genes in animal cells
- Generation of transgenic animals and\or knock down of multiple genes
- Manufacturing of medicaments

Our system compared with the use of two separate expression vectors, use of two expression cassettes driven by different promoters in a single vector, or with IRES bicistronic vectors shows:

- More efficient expression
- Coordinated expression of both genes in virtually all transduced cells
- Efficient integration and robust expression by lentiviral vector delivery
- Cell-type independent applications

We seek a potential commercial partner with a strong pipeline in human gene therapy for the treatment of genetic and acquired diseases or should be a biotech company selling research reagents for creation of animal models and for in vitro target validation.

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