HOW TO MONITOR A SPECIFIC CELL STATE?

Flash Therapeutics provides custom and premade lentiviral vectors with specific promoters for theexpression of genes of interest during specific cell states.

Cell-state specific promoters:

Flash Therapeutics has designed and optimized all cell-state specific promoters available below, in order to make the construction compatible with the lentiviral particle encapsidation capability. If the specific promoter you need is not in our list, do not hesitate to contactour experts.

Cell-state specific promoters allow the expression of genes of interest under specific cell conditions such as:

- Cell-senescence pathway and DNA damages (Genotoxicity)
- Cell-cycle phases (G0, proliferation state)
- Inflammatory response (Oxydative stress, NFκB pathway activity)

All designed promoters come from bibliographic data and bio-informatics analysis. For more details, please contact us.

1. Monitoring of cell senescence pathway

- CDKN2A promoter (P16) and CDKN1A promoter (P21) validation in H9C2 cells (rat cardiomyocytes)

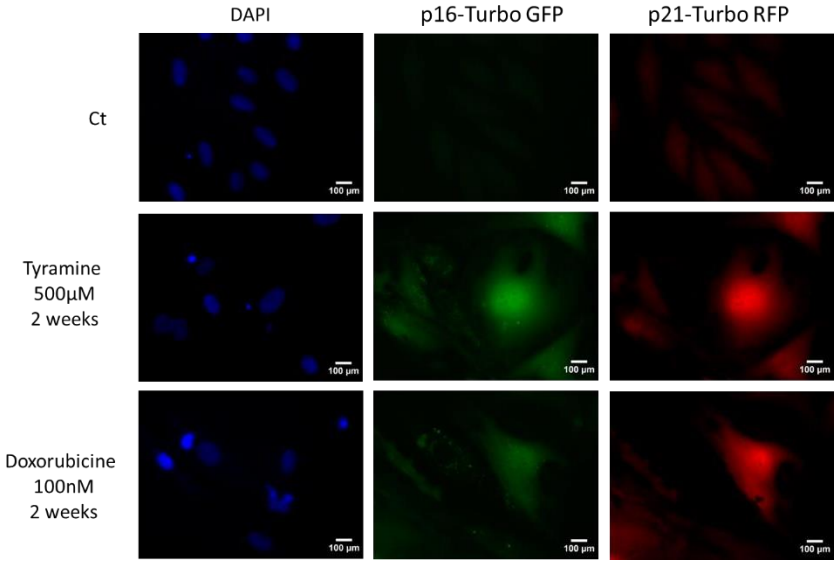

Fig.1: Transduction of H9C2 cells with two lentiviral vector batches. The first batch carried TurboGFP driven by a cell senescence specific promoter (P16) and the second batch carried TurboRFP driven by a cell senescence specific promoter (p21). Cell senescence was induced with an addition of 500µM of Tyramine or 100 nM of Doxorubicine.

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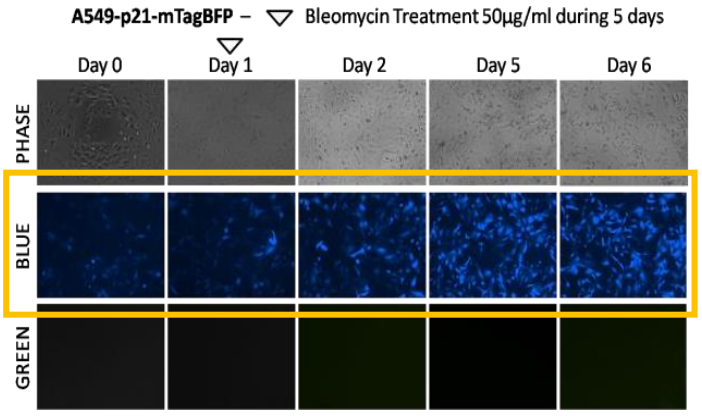
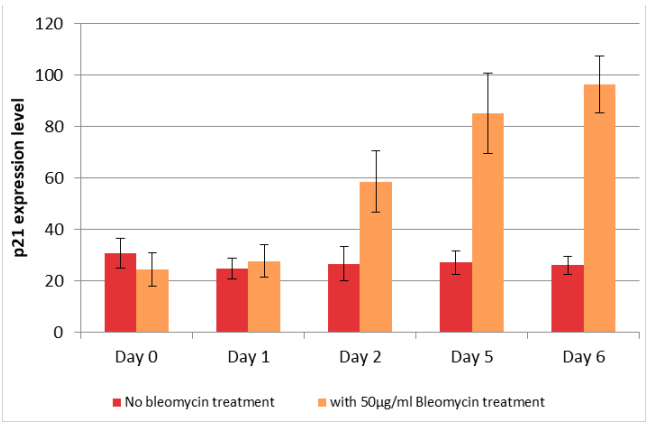
Figure 1 shows the specific expression of both TurboGFP under the control of CDKN2A promoter (P16) and TurboRFP under the control of CDKN1A promoter (P21), after cell senescence induction. Vectalys demonstrates that these two promoters are specifically activated during cell senescence. These two promoters can be used for the expression of a gene of interest specifically during cell senescence.

- CDKN1A promoter (P21) validation in A549 cells



Picture of fluorescent protein expression (mTagBFP) after senescence induction



Quantification of mTagBFP expression after senescence induction

Fig.2: Transduction of A549 cells with a lentiviral vector batch carrying mTagBFP driven by a cell senescence specific promoter (p21). To induce cell senescence, a Bleomycin solution was added at 50µg per ml and cells were incubated 5 days at 37°C 5%CO2.

After senescence induction (day 1), the increase of fluorescence in figure 2 confirms the senescence-specificity of the CDKN1A promoter (P21). The low fluorescence level (day 0) is due to a background expression of P21 transactivator in A549 cells.

Cell-senescence pathway and DNA damages specific promoters		
CDKN1A (p21)	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli involved in proliferation, apoptosis, differentiation, stem cell self-renewal and senescence monitoring of DNA damages, genotoxicity assays
TP53-RE	Synthetic promoter based on TP53 response element from p21 promoter	Biosensor for detection of p53 activation (in response to DNA damage, genotoxic stress...)
CDKN2A (p16)	Cyclin-dependent kinase inhibitor 2A	Ageing, senescence, cell cycle regulation (also implicated in tumorigenesis as a sensor of oncogenic stress)
MAOA	Monoamine oxidase A	Allows monitoring of MAOA mitochondrial enzyme expression - MAOA activity in cardiomyocytes is a source of oxidative stress (H_2O_2 byproduct production) inducing cardiac senescence – MAOA is also expressed in brain

Each specific promoters above are available for custom lentiviral vector projects. Premade lentiviral particles with fluorescent proteins are also available for testing.

2. To monitor the inflammatory response:

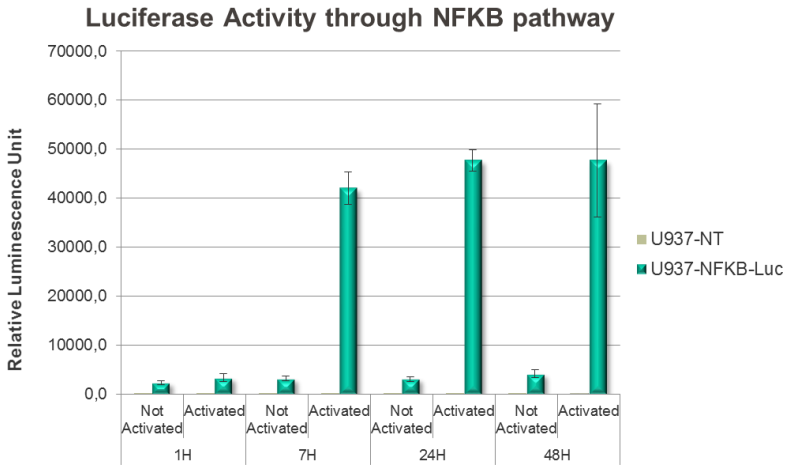


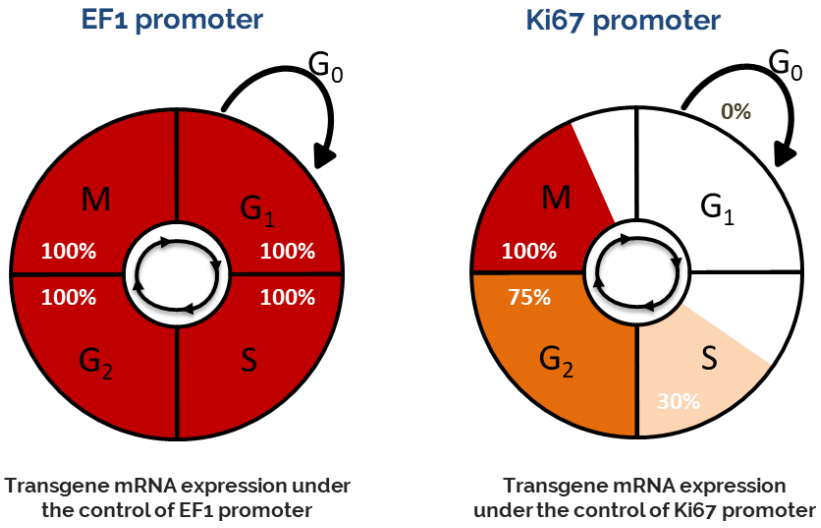
Fig.4: Cells were transduced by a luciferase expressing-lentiviral vector, driven by a NFKB Response element. One week after transduction, U937 cells were activated by LPS/PMA for 1h. Luciferase activity was detected at 1h, 7h, 24h and 48h after activation in 96-well multiplate in triplicate (data gathered by Vectalys).

Figure 4 demonstrates the high specificity of NFKB promoter to be activated and to express a gene of interest during inflammatory response.

Inflammatory response specific promoters		
NFκB-RE	Synthetic promoter based on NFκB response elements	Biosensor for detection of NFκB pathway activity

3. Monitor and track the cell cycle stages of a cell population

- Validation of KI67 promoter in HCT116 cell line



Transgene mRNA expression under the control of EF1 promoter

Transgene mRNA expression under the control of Ki67 promoter

Fig.3: variation of mRNA expression during cell cycle using our KI67 specific promoter compared to mRNA expression driven by a constitutive EF1a promoter (data gathered by Flash Therapeutics). To see raw data, please contact us at tech@flashtherapeutics.com.

Cell line were maintained in Dulbecco's modified eagle medium containing 10% fetal bovine serum at 37°C in 5% CO2. To obtain a synchronous population of cells, cells were grown until 80% confluence. The medium was removed and washed with 1X PBS and incubated in medium containing 1% fetal bovine serum for 72h. After 72h of serum starvation, cells were considered to be in a quiescent state (G0 stage). Cells were stimulated by adding Dulbecco's modified eagle medium containing 10% fetal bovine serum and harvested at 0, 8, 16, 24 and 32h after stimulation.

Data figure 3 show the high specificity of Ki67 promoter to express a gene of interest during specific cell cycle phases (higher expression during M phase regarding the KI67 promoter). The diagram on the left side figure 3 shows the constitutive expression of the mRNA of interest with the constitutive promoter EF1a. The diagram on the right side of figure 3 shows the cell cycle phases specific expression of the mRNA of interest with the KI67 promoter. This kind of specific promoter is ideal for the monitoring and the tracking of specific cell cycle phases.

Cell-cycle phases specific promoters		
PCNA	Proliferating Cell Nuclear Antigen	Proliferating cells
MKI67	Marker of proliferation Ki-67	Proliferating cells (expressed in all active cell cycle phases and absent in G0)

The two cell cycle stages specific promoters above are available for custom lentiviral vectors projects. Premade lentiviral particles with fluorescent proteins are also available for testing.

You can also find more data in the following reference: Ref. Annette M. Sysel and al.(2014) Immunohistochemical quantification of the cobalamin transport protein, cell surface receptor and Ki-67 in naturally occurring canine and feline malignant tumors and in adjacent normal tissues. Oncotarget;6(4):2331-48.

For more information or to request a quote, please contact us.