GENERATION OF REPORTER CELL LINES TO THE STUDY OF PLURIPOTENCY MODULATION

Lima IMS[1,4], Fráguas MS[2,4], Pacheco DCS[3,4], Covas DT[2,4], Zago MA[2,4], Panepucci RA[4]

[1]Department of Genetics - FMRP/USP; [2]Department of Clinical Medicine – FMRP/USP; [3]Human and Medical Genetics Laboratory – UFPA; [4]Hemotherapy Center of Ribeirão Preto – FMRP/USP.

BACKGROUND

Pluripontency is the ability that some cells hold to generate all the cells lines from the germ layers (ectoderm, mesoderm and endoderm). The embryonic stem cells (ESC) are the most well known pluripotent cells, those which present high capacity of cell differentiation and self-renewal. Those properties make ESC potential tools for regenerative medicine, but using ESC in fact is unfeasible, because in addition to ethical questions, it is necessary immunological compatibility with the receiver. Induced pluripotent stem cells (iPS) were developed to solve some problems like those presented before. The iPS cells are obtained by ectopic expression of some pluripotency related transcription factors resulting in a nuclear-reprogramming. Initially, those iPS cells were generated by using the Yamanaka factors (Oct4, Sox2, c-Myc e Klf4). Oct4, Sox2 and Nanog transcription factors are critical for the maintaining of pluripotency in ESC as well as in iPS cells, because they generate a network of self-regulation and modulate others important components involved in cell differentiation and self-renewal capacities of those stem cells. Reporter cell lines containing the luciferase gene for gene expression studies have been widely used with distinct aims.

AIM

The present study aims to generate different luciferase reporter cell lines specific to detect the expression of pluripotency related transcription factors (Oct4 and Nanog) and the expression of retinoic acid receptor (RAR) by lentiviral transduction.

METHODS

The original cell lines which will be used for lentiviral transduction are BJ (human fibroblasts), HCT116 (human colorectal cancer cells), HCT116-DKO (human colorectal cancer cells, double-knockout for DNMT 1 and 3b), N-Tera2 (human embryonic carcinoma cells). Initially, it was determined the ideal concentration of the antibiotics (Hygromycin B and Puromycin) used to select the transduced cells by XTT assay in some cell lines. After determining antibiotics ideal concentration, it will be generated an initial cell line containing renilla-luciferase control. The cells will be seeded in 96-well plates containing complete culture medium (with 10% FBS, Penicillin and Streptomycin) and incubated for 24 hours (37ºC, 5% CO_2). Then, medium will be suited for a medium without antibiotics and cells will be exposed to lentiviral particles (renilla-luciferase control) and SureEntry® (8µg/mL) for 20 hours. Transduction medium will be again suited by complete medium. After 24 hours, cells will be submitted to selection with medium containing Hygromycin B and selected cells will be submitted to a single-cell cloning selection. The selected clone with the better expression of renilla-luciferase will be submitted to a similar process of transduction and selection, to generate the cell lines containing firefly-luciferase reporter

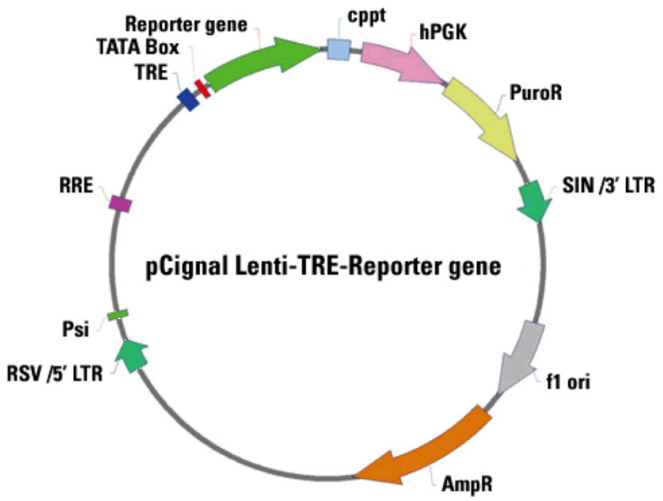

Figure 1. Lentivirus construction scheme containing reporter and antibiotic resistance genes among others (figure obtained from Sabioscience).

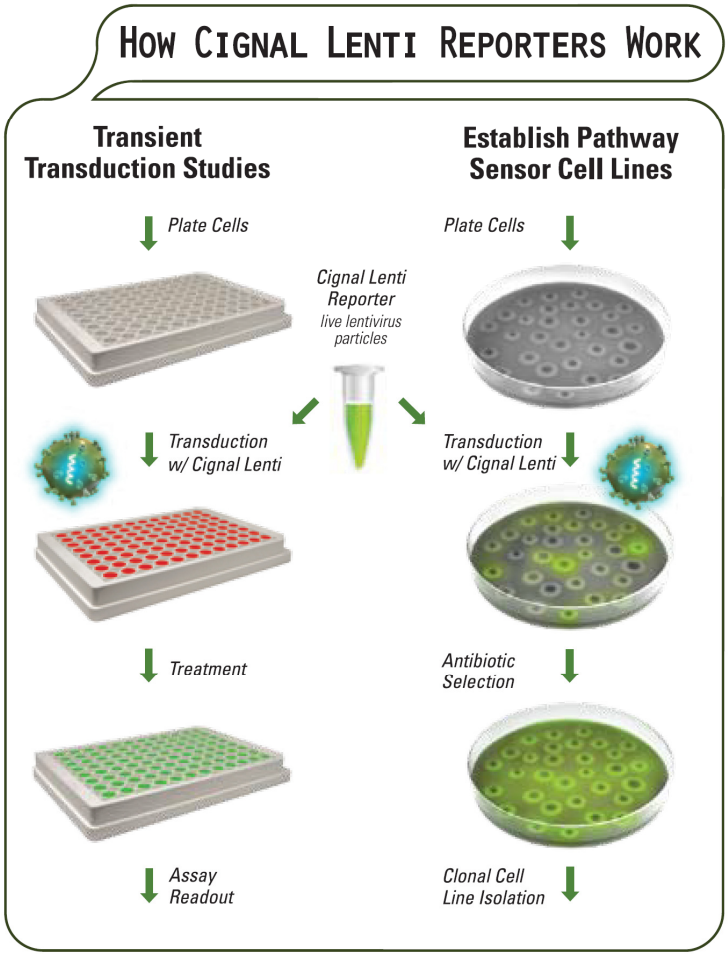

Figure 2. Experiment design for reporters clonal cell lines establishment (figure obtained from Sabioscience).

Author email: ildercilio_lima@yahoo.com.br

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