

The development of a cell line capable to repress the expression of the transgene carried by an adenoviral vector during the production process may prevent the negative effects of the transgene overexpression. Therefore, the system is based on a cell line expressing high levels of TetR and by Ad vectors carrying a transgene under control of human cytomegalovirus promoter or other strong promoters such as CASI promoter, CAG promoter, EF1 α promoter with two or more copies of TetR binding sites inserted downstream the promoter TATA box.

Summary

In one aspect, the present disclosure refers to a cell line, wherein the cells of the cell line are HEK293 cells, and wherein a tetracycline repressor (Tet-R) is expressed under the control of the CAG promoter.

In one example, the present disclosure provides a cell line, wherein a tetracycline repressor (Tet-R) is expressed under the control of a cellular or partly cellular promoter.

In a further aspect, the disclosure relates to a use of the cell line as described herein for producing infectious viral particles. In yet another aspect, the present disclosure refers to a method for producing infectious viral particles comprising the steps of: (i) growing the cells of the cell line as described herein *in vitro* in the presence or absence of tetracycline or a tetracycline analog; or (ii) infecting the cells of the cell line as described herein with a viral vector, preferably an adenoviral vector; and (iii) recovering the viral particles.

In another example, the disclosure relates to a method for producing infectious viral particles comprising the steps of:

- (i) growing the cells of the cell line of the first example of the invention further comprising a viral genome capable of assembling into an infectious viral particle *in vitro* in the absence of tetracycline or a tetracycline analog; or
- (ii) infecting the cells of the cell line of the first example of the invention with a viral vector, preferably an adenoviral vector; and
- (iii) recovering the viral particles.

Claims

1. A cell line, wherein the cells of the cell line are HEK293 cells, and wherein a tetracycline repressor (Tet-R) is expressed under the control of the CAG promoter.
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2. The cell line according to claim 1, wherein a tetracycline repressor (Tet-R) is expressed in at least 90% of the cells of the cell line.
3. The cell line according to any of claims 1 to 2, wherein the expression is stable over
10 time.
4. The cell line according to any of claims 1 to 3, wherein the Tet-R represses transcription of genes comprising in their promoters one or more Tetracycline Operators (TetOs) in the presence or absence of tetracycline or a tetracycline analog.
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5. The cell line according to any of claims 1 to 4, wherein the gene encoding Tet-R is codon optimized for expression in eukaryotic cells, preferably in mammalian cells.
6. The cell line according to any of claims 1 to 5, further comprising
20 (i) in its genome a mutation, deletion or insertion that reduces or prevents expression of a functional protein selected from the group consisting of one or more stimulator of an IFN gene, preferably cGas and STING, one or more JAK/STAT activator of transcription and one or more IFN-stimulated gene (ISG), preferably PKR, MX1, OAS1, APOBEC3G, TRIM5, ZAP, ISG15, ADAR, IFITM1/2/3, BST2, and/or
25 RSAD2, and/or
(ii) a gene encoding a selection marker, preferably a G418, hygromycin B, puromycin, blasticidin, or zeocin resistance gene in genetic linkage to the gene encoding Tet-R.
- 30 7. The cell line according to any of claims 1 to 6, comprising stably integrated into its genome a gene encoding tetracycline repressor (Tet-R).
8. The cell line according to any of claims 1 to 7, wherein the cells of the cell line express at least one viral gene that is required for the formation of infectious virus particles,

preferably wherein the at least one viral gene is selected from the group consisting of E1, preferably comprising a gene with the nucleotide sequence of SEQ ID NO: 1.

9. The cell line according to any of claims 1 to 8, wherein said cell line is the cell line deposited under accession number 17080901 with the ECACC.
10. The cell line according to any of claims 1 to 9, further comprising a viral genome capable of assembling into an infectious viral particle and comprising at least one heterologous gene under the control of a promoter comprising one or more Tetracycline Operators (TetO), preferably wherein
 - (i) the viral genome is selected from the group consisting of a herpes virus genome, a modified vaccinia Ankara genome and an adenoviral genome, preferably, the viral genome is an adenoviral genome and/or
 - (ii) the heterologous gene is toxic to the cell or interferes with the viral replication cycle.
11. The cell line according to claim 10, wherein the heterologous gene is selected from the group consisting of self antigens, viral genes, genes of cellular origin, synthetic strings encoding for combination of tumor-associated neo-antigens and strings encoding for neo-epitopes, preferably wherein
 - (i) the self antigen is selected from the group consisting of HER2/neu, CEA, Hepcam, PSA, PSMA, Telomerase, gp100, Melan-A/MART-1, Muc-1, NY-ES0-1, Survivin, Stromelysin 3, Tyrosinase, MAGE3, CML6S, CML66, OY-TES-1, SSX-2, SART-1, SART-2, SART-3, NYC0-5S, NY-BR-62, hKLP2 and VEGF,
 - (ii) the viral gene is selected from the group consisting of HCV E1-E2 protein, rabies glycoprotein, and HIV-1 GP 160, and/or
 - (iii) the gene of cellular origin is selected from the group consisting of Tim-3, Her2 and E2 F-1.
12. Use of the cell line according to any one of claims 1 to 11 for producing infectious viral particles.
13. A method for producing infectious viral particles comprising the steps of:

- (i) growing the cells of the cell line of claim 10 or 11 *in vitro* in the presence or absence of tetracycline or a tetracycline analog; or
- (ii) infecting the cells of the cell line of any of claims 1 to 9 with a viral vector, preferably an adenoviral vector; and
- 5 (iii) recovering the viral particles.