ACCEGEN
BIOTECHNOLOGY

Reporter Stable Cell Lines
Product Manual

Reporter Cell Lines Product Information

1. Description

In reporter cell lines, the reporter gene is inserted into the target cell using genetic engineering technology to achieve stable expression, so as to track the location and expression of the target gene in the target cell. Lentivirus transduction is mainly used in most cases. The reporter genes include GFP, RFP, YFP, luciferase, etc. We offer customized service for the development of dual luciferase reporter systems and other types of reporter gene functional cell lines based on customer needs.

With our customized service, you can get our high-quality customer service experience. Reporter stable cell lines are widely used in detecting target gene expression, localization of protein functions, and measuring the production of proteins. It can realize the visual tracking of target genes, enabling researchers to monitor the production and location of proteins in living cells in real time. At the same time, it facilitates continuously monitoring of the expression levels of endogenous recombinant proteins through reporter genes. In addition, the reporter stable cell line can be used to study the transcriptional activity of gene promoters.

2. Reporter Stable Cell Line Generation Service

Service	Technical methods	Gene expression evaluation	Application	Delivery
Reporter Stable Cell Line Generation	Lentivirus/CRISPR (GFP / RFP / luciferase /mCherry)	qPCR Flow Cytometry	Gene function research Gene expression detection Monitor protein localization and production	Polyclonal cell line Monoclonal cell line

3. Technical Advantages

Our lentiviral vector has high infection efficiency and can quickly and efficiently integrate the target gene into the host cell genome. The expression efficiency of the reporter gene will also be significantly improved to achieve long-term, stable and significant expression effects. We have a variety of reporter genes and host cells for customers to choose from. In addition, we can provide services such as cell gene expression regulation and cell function verification. Finally, professional laboratory reports and quality inspection reports will be delivered.

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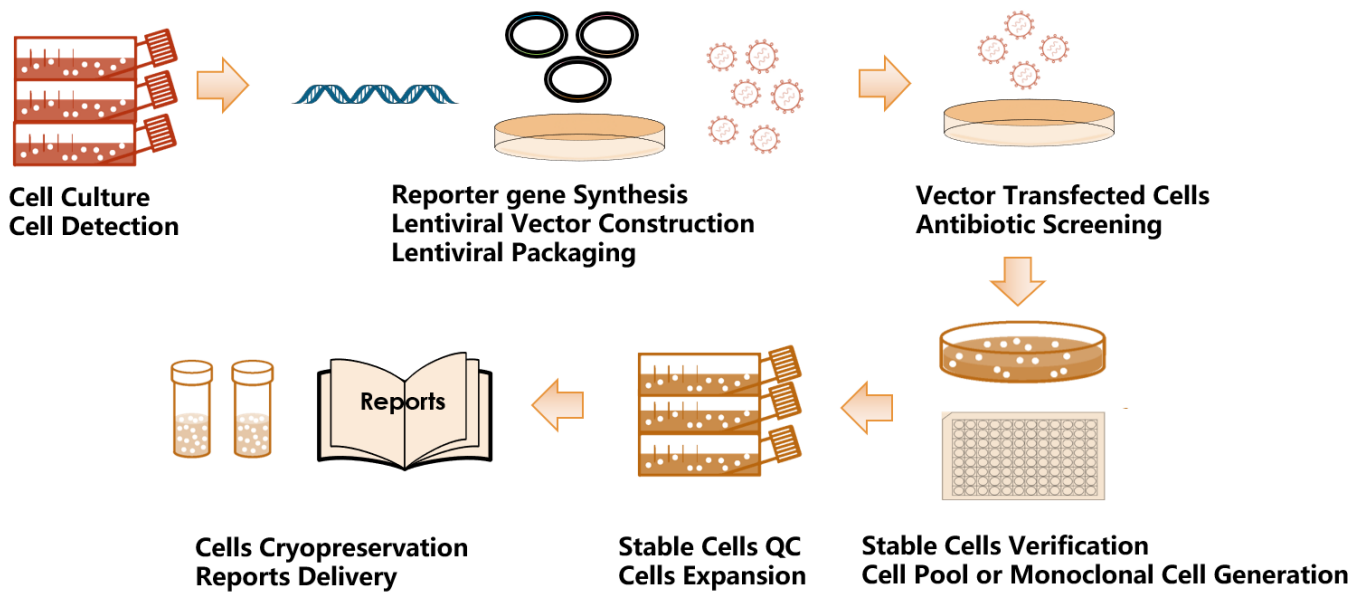
4. Experimental Processes

Lentivirus transduction:

Cell Culture and Detection (2-3 weeks)	Vector Construction and Lentivirus packaging (3-5weeks)	Lentivirus-mediated cell transduction (3-6 weeks)	Stable cell line QC (1 week)
• Bacteria and mycoplasma detection • The genotype of the target cells detection	• Reporter gene synthesis; • Lentiviral vector construction • Lentiviral packaging and lentiviral titer determination	• Drug-resistance selection • Reporter cell generation(cell pool or monoclonal cell)	• QPCR & stability test • Cell lines and reports Delivery



5. Application Operations and Detection Methods

Target gene function discovery and drug-resistance selection

a) Construct a gene reporter stable cell line

b) Culture and expand the reporter stable cell line with the suitable medium and serum

c) Group cells according to experimental requirements

d) Study the expression level of genes on cells or in vivo tracking through various experimental methods in terms of phenotype or gene function.

RT-qPCR The process of extracting cellular RNA can be operated according to the nucleic acid extraction kit, and

the reporter gene product can be obtained after reverse transcription. You can use SYBR Green or TaqMan Probe method to detect the expression level of the reporter gene or target gene.

Western blotting Cells were lysed using RIPA or other reagents, and the concentration of the target protein was determined by obtaining the cell supernatant after centrifugation. Then the reporter gene expression between reporter cells and control cells can be obtained.

Flow cytometry Inoculate cells (5×10^5 cells/ml) into 6-well plates and culture them for 24 hours. Add antibodies or stimulating factors required for the experiment to the cells and incubate for several hours. Finally, a flow cytometer can be used to detect cell cycle and apoptosis, etc.

Construction of animal disease models and in vivo imaging experiments First, we need to count the reporter cells and make a single cell suspension (1×10^7 cells/ml). We use the mouse as the representative. Second, the mouse is injected subcutaneously or intravenously with 0.2ml cells (approximately 2×10^6 cells). Third, the mouse is injected with anesthesia and then injected with luciferin (intraperitoneal injection at a concentration of D-luciferin/body weight of 150 mg/kg). Finally, the mouse is put into an in vivo imager for observation, and over the course of several days, the experimental observations allow us to explore cell migration and establish a tumor metastasis model.

Statistical analysis Experimental data can be analyzed using software such as SPSS, GraphPad Prism, Flow Jo, and Excel.

6. Delivery of Cell Lines and Report

1 vial of stable cell line (1×10^6 cells/vial) and qPCR test report will be delivered to the customer. If the project requires the customer to provide a host cell, we will request the customer to fill in AcceGen Custom Stable Cell Line Service Requisition Form.

7. Experimental Cases

Case1: GFP Reporter Cell Line-HEK293

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

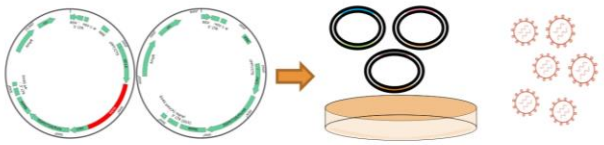


Fig.1a Lentiviral preparation

3. Lentivirus-mediated cell transduction and drug-resistance selection
4. Stable cell line QC (qPCR results)

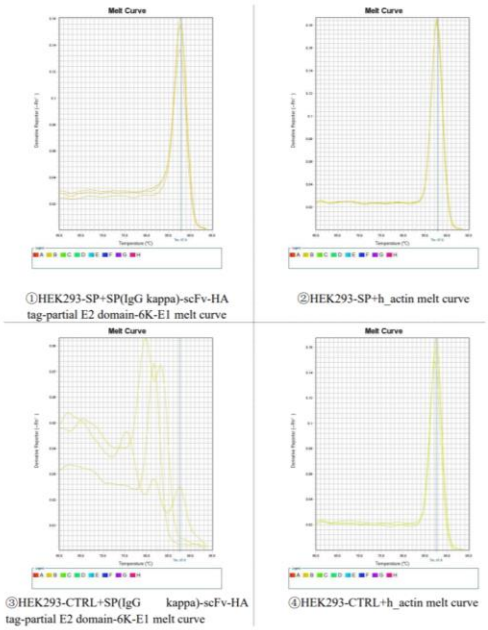


Fig.1b Melting curve

Expression rate

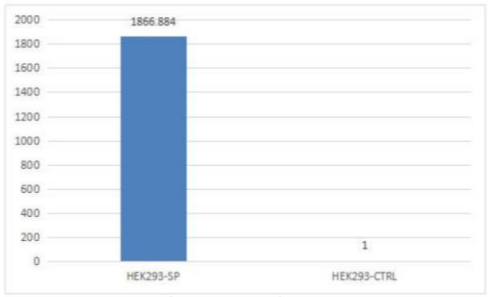


Fig.1c qPCR results

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

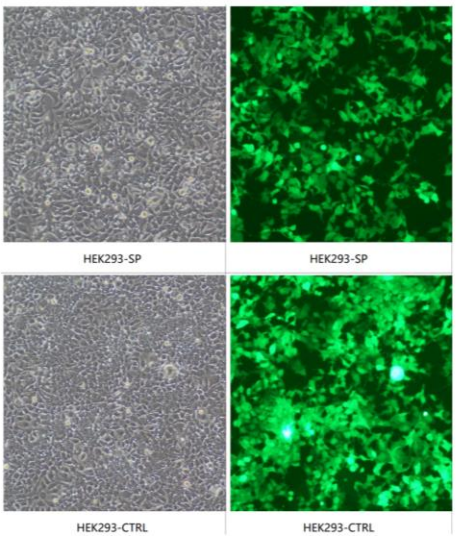


Fig.1d Reporter cells generation

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Case2: EGFP Reporter Cell Line-HEK293

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

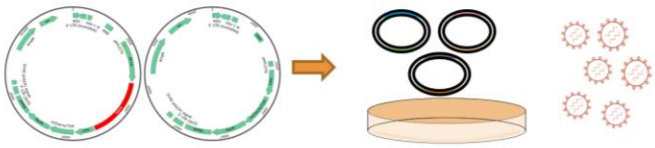

Fig.2a Lentiviral preparation

3. Lentivirus-mediated cell transduction and Drug-resistance selection
4. Stable cell line QC (qPCR results)

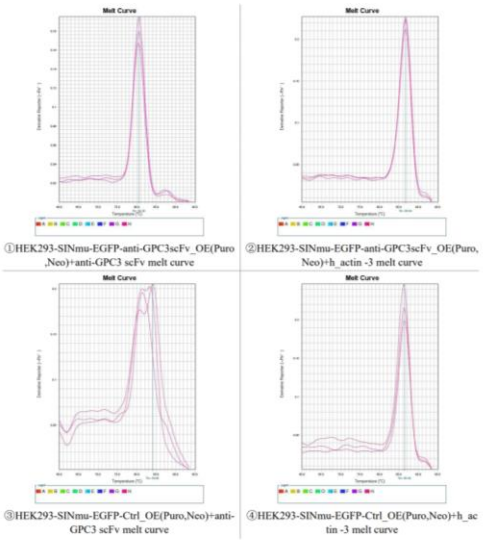

Fig.2b Melting curve

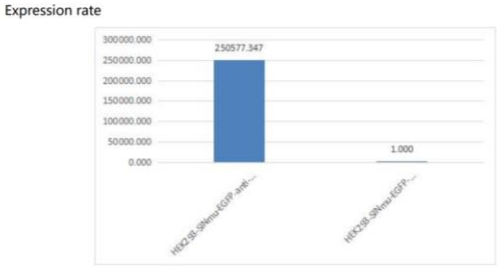

Fig.2c qPCR results

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

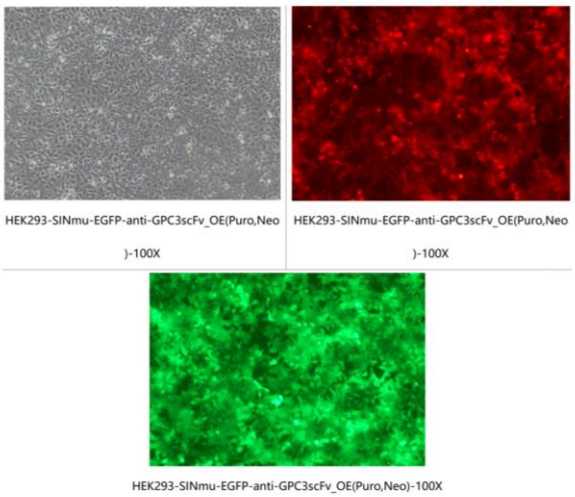

Fig.2d Reporter cells generation

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Case3: EGFP Reporter Cell Line-HEK293

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

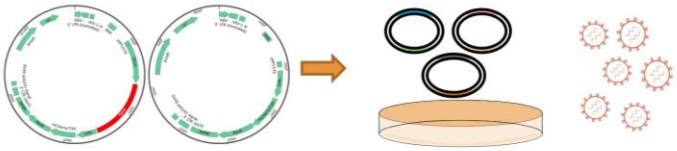

Fig.3a Lentiviral preparation

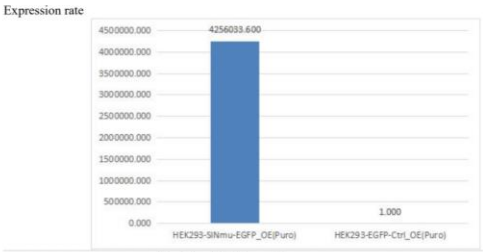

Fig.3c qPCR results

3. Lentivirus-mediated cell transduction and Drug-resistance selection
4. Stable cell line QC (qPCR results)

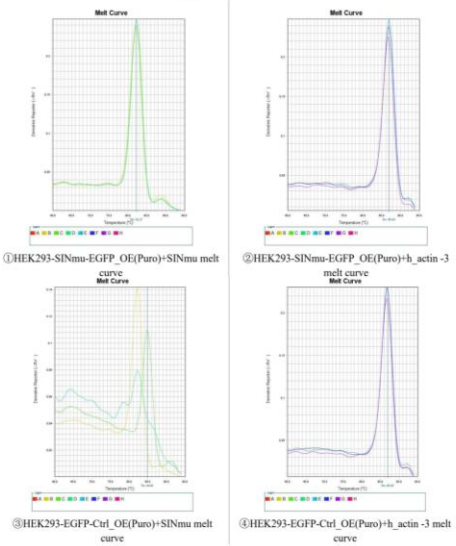

Fig.3b Melting curve

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

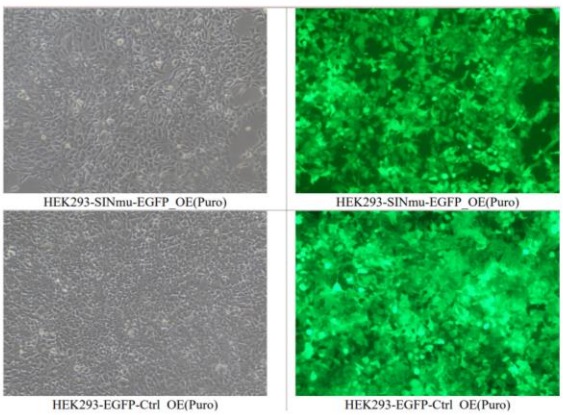

Fig.3d Reporter cells generation

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Case4: EGFP Reporter Cell Line-MBT2

1. Target gene synthesis and codon optimization
2. Lentiviral vector construction and lentiviral packaging

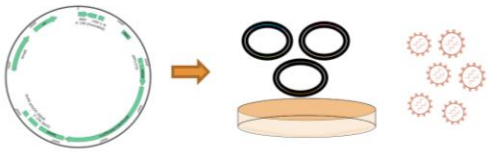

Fig.4a Lentiviral preparation

3. Lentivirus-mediated cell transduction and Drug-resistance selection
4. Stable cell line QC (qPCR results)

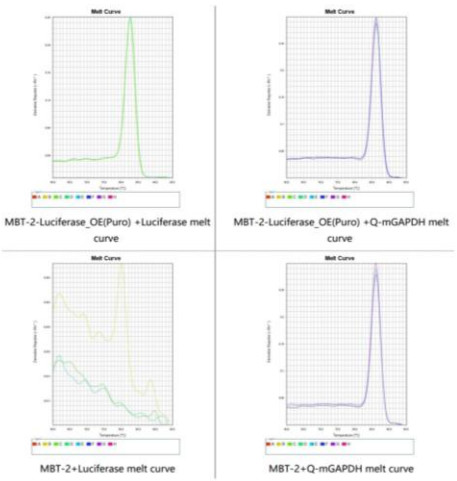

Fig.4b Melting curve

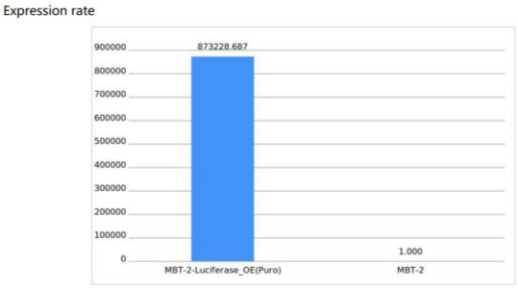

Fig.4c qPCR results

5. Stable cell expansion (cell pool or monoclonal cells) and stability test
6. Delivery of cell lines and reports

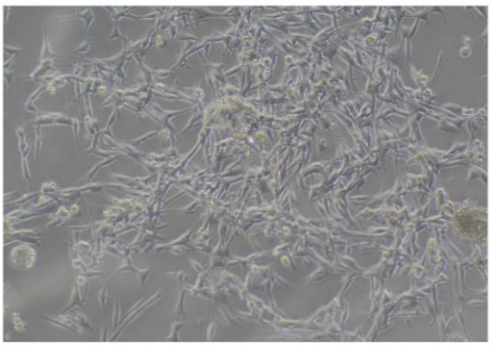

Fig.4d Reporter cells generation

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