Protocol for Generating Stably Expressed Mammalian Cell Lines Using Lentivirus

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APR 14, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn2qo6g5d/v1

Protocol Citation: creative-biogene 2023. Protocol for Generating Stably Expressed Mammalian Cell Lines Using Lentivirus. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2qo6g5d/v1

Protocol status: Working
We use this protocol and it's working

Created: Apr 14, 2023

Last Modified: Apr 14, 2023

PROTOCOL integer ID: 80503

ABSTRACT

Stable cell lines generated using lentivirus exhibit long term protein expression and the system is highly reproducible. In this protocol, we describe the use of the 3rd generation lentiviral system which uses three different plasmids for generating stable cell lines.

MATERIALS

1. 10 cm^2tissue culture treated plates
2. Low protein binding 0.45 µm PDVF filters
3. 10 ml plastic syringes sterile
4. Sterile Glass jar
5. Disposable plastic pipettes (5 ml and 10 ml)
6. Sterile 1.5 ml tubes
7. Pasteur pipettes
8. HEK293T (293T)
9. Target cell line (any human cancer cell line)
10. Xtreme gene 9 transfection reagents
11. Packaging plasmids:pCMV delta R8.2andpCMV-VSV-Gand your plasmid of interest
12. 10% bleach
13. Polybrene solution: 8 mg/ml in water filter sterilized, made fresh every time
14. Opti-MEM reduced serum medium
15. DMEM complete medium with 10% FBS and pen-strep
16. Selection antibiotic
17. Dry ice or liquid nitrogen
18. DMSO
19. Liquid nitrogen

Experiment Summary

1 Stable cell lines generated using lentivirus exhibit long term protein expression and the system is

highly reproducible. In this protocol, we describe the use of the 3rd generation lentiviral system which uses three different plasmids for generating stable cell lines. First plasmid contains your gene of interest usually flanked by Long Terminal Repeat (LTR) sequences, that are integrated into the host genome. Second is the pCMV Delta R8.2 plasmid that encodes all components necessary for packaging the lentivirus viz. HIV-1 Gag, Pol, Tat and Rev. Third and the final plasmid is the pCMV VSVG that encodes the viral envelope protein.

2

Equipment

3 1. Cell culture incubators (37 °C, 5% $CO_2$and 32 °C, 5% CO_2)
2. Hemocytometer (Standard)
3. Laminar hood BSL-2 type
4. 37 °C water bath
5. Autoclave
6. Micropipettes (1,000 µl, 200 µl)
7. -80 °C freezer

Procedure

4 A. Preparation of lentivirus in HEK293T cells

1. Day 0: Seed about 2-3 million HEK293T cells in a 10 cm^2plate.
2. Day 1: HEK293T cells should be about 70-80% confluent. If for some reason they seem less you may wait for another 8-10 h.
3. Prepare transfection mix using the following proportions in a sterile 1.5 ml Eppendorf tube as per manufacturer's instructions: