Transfection with pTK-neo

Description

pTK-neo DNA	20 µg	71284-3

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The pTK-neo selection plasmid is designed to establish stable integration of plasmid into mammalian cells. To establish stable transfectants, mammalian cells are cotransfected with a transient expression vector containing the gene of interest and the pTK-neo plasmid (1, 2) and selected in the presence of G 418. A minimal thymidine kinase promoter controls expression of the neomycin resistance gene (see pTK-neo vector map, TB375). This promoter facilitates selection for stable integration of both the selection plasmid and a cotransfected expression plasmid, such as the pTandem™-1, pTriEx™, pMLuc, or any mammalian expression vector.

Components

- 20 µg pTK-neo DNA

Storage

Store DNA at –20°C.

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Establishing Stably-transfected Cells with pTK-neo

General Guidelines

DNA preparation

It is necessary to begin with plasmid DNA preparations that do not contain contaminants that interfere with transfection. Although standard miniprep DNA may work for transfections, results are often variable among different plasmids and different preparations of the same plasmid. Novagen's Mobius™ and UltraMobius™ Plasmid Kits (Cat. Nos. 70853 and 70906) produce DNA of consistent quality for transfection. Alternatively, transfection quality plasmid DNA may be prepared by using a CsCl/EtBr protocol. The final DNA preparation should be suspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or TlowE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8) at a concentration of 0.5–1 µg/µl.

Neomycin activity

The pTK-neo vector contains the neomycin resistance gene for selection of stable cell lines. Neomycin is an aminoglycoside that is similar in structure to G 418, gentamycin, and kanamycin. In mammalian cells, neomycin blocks protein synthesis by binding to the small subunit of ribosomes. Expression of the bacterial aminoglycoside phosphotransferase gene (APH) in mammalian cells detoxifies neomycin by phosphorylation, thus conferring resistance to neomycin. G 418 is available as a solid (Cat. No. 345810) or as a sterile-filtered solution (Cat. No. 345812).

Selection of stable cells

Unless there is special need for the use of a clonal cell line, the mixed population of drug-resistant cells can be used directly for experimental analysis. To generate clonal cell lines, it is necessary to dilute the resistant cells and plate for single cells in 96-well plates.

Determination of antibiotic sensitivity

In order to successfully generate stable cell lines expressing the gene of interest, it is important to determine the minimum concentration of G 418 needed to kill nontransfected cells (kill curve). The following protocol describes a simple procedure to quickly test a range of concentrations of G 418 to determine the minimum concentration needed:

1. Plate cells at 25% confluency in all 6 wells of a 6-well plate. Incubate cells overnight.
2. The next day, aspirate the growth medium and replace with freshly prepared growth medium containing the following concentrations of G 418: 0, 100, 250, 500, 750, and 1000 µg/ml.
3. Every 3–4 days, aspirate the old medium and replace with freshly prepared selective growth medium, and observe the percentage of surviving cells.
4. The minimum antibiotic concentration to use is the lowest concentration that kills 100% of host cells in 10–12 days from the start of antibiotic selection.

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Generation of stable cell lines

The following procedure describes introduction of plasmid DNA into mammalian adherent cultures using Novagen's GeneJuice® Transfection Reagent in a T-75 culture flask. Alternative formats require adjustment of the amount of GeneJuice Transfection Reagent and cell seeding densities. For more information on GeneJuice see Technical Bulletin 289.

1. The day before transfection, plate 1–5 × 10^6 cells in 5 ml complete growth medium per T-75 flask. Incubate at 37°C (5% CO_2) overnight. Cells should be 50–80% confluent before transfection.
2. For each T-75 flask to be transfected, place 800 µl of serum-free medium (for example, GIBCO RPMI 1640 or Opti-MEM) into a sterile tube. Add 49.5 µl of GeneJuice® Transfection Reagent (3 µl/ug DNA) dropwise directly to the serum-free medium and mix thoroughly by vortexing. The volumes can be scaled up for transfection of multiple dishes with the same DNA.

Note: For most cell lines the optimal ratio of GeneJuice reagent to DNA is 3 µl GeneJuice to 1 µg DNA, but the ratio can be varied from 2–6 µl per µg of DNA during optimization.

3. Incubate at room temperature for 5 min.
4. For each T-75 flask to be transfected, combine 15 µg recombinant expression plasmid and 1.5 µg pTK-neo plasmid DNA (10:1 to 20:1 ratio is recommended) and add to the GeneJuice/serum-free medium mixture.
   Optional: Include the following negative control transfections:
   a) no DNA
   b) recombinant expression plasmid only, no pTK-neo DNA.
5. Incubate the DNA/GeneJuice mixture at room temperature for 5–15 min.
6. Add the entire volume of DNA/GeneJuice mixture dropwise to the cells in complete growth medium. Distribute the drops all over the surface of the flask, and gently rock the flask to ensure even distribution. Do not swirl the plate, as this will concentrate the transfection mixture in the center of the plate.
7. Incubate the cells for 48–72 h at 37°C (5% CO_2).
8. Subculture cells (1:10 to 1:20 dilution recommended) with fresh growth medium containing the desired concentration of G 418.
9. For one week, replace the growth medium every 2–3 days with fresh growth medium containing selective antibiotic, splitting the cells as needed.
10. Continue to passage the cells in the presence of full strength antibiotic for two additional passages. At this point the antibiotic-resistant colonies should be visible and can be isolated either by using cloning cylinders or sterile toothpicks.

References

1. Wigler, M., Sweet, R., Sim, G.K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. and Axel, R. (1979) *Cell* **16**, 777–785.
2. Colbere-Garapin, F., Horodniceanu, F., Kourilsky, P. and Garapin, A.C. (1981) *J. Mol. Biol.* **150**, 1–14.