Stable Transfection

Unlike transient transfection, in which introduced DNA persists in cells for several days, stable transfection introduces DNA into cells long-term. Stably transfected cells pass the introduced DNA to their progeny, typically because the transfected DNA has been incorporated into the genome, but sometimes via stable inheritance of nongenomic DNA.

How does stable transfection work?

Successful stable transfection requires both effective DNA delivery and a way to select cells that have acquired the DNA. Approximately one in 10^4 transfected cells will stably integrate DNA, although the efficiency varies with cell type and whether linear or circular DNA is used. Integration is most efficient when linear DNA is used.

One of the most reliable ways to select cells that stably express transfected DNA is to include a selectable marker on the DNA construct used for transfection or on a separate vector that is co-transfected into the cell, and then apply the appropriate selective pressure to the cells after a short recovery period. When the selectable marker is expressed from the co-transfected vector, the molar ratio of the vector carrying the gene of interest to the vector carrying the selectable marker should be in the range of 5:1 to 10:1 to ensure that any cell that contains the selectable marker also contains the gene of interest.

Frequently used selectable markers are genes that confer resistance to various selection drugs or genes that compensate for an essential gene that is defective in the cell line to be transfected. When cultured in selective medium, cells that were not transfected or were transiently transfected will die, and those that express the antibiotic resistance gene at sufficient levels or those that can compensate for the defect in the essential gene will survive.

Recommended transfection reagents

- **Lipofectamine 3000** reagent leverages our most advanced lipid nanoparticle technology to enable superior transfection performance and reproducible results. It delivers exceptional transfection efficiency into the widest range of difficult-to-transfect and common cell types with improved cell viability.
- **Lipofectamine 2000** has been the most-cited transfection reagent for over a decade.

Selection antibiotics for eukaryotic cells

We offer high-quality selection reagents to complement our wide variety of selectable eukaryotic expression vectors. Geneticin (G418 sulfate), Zeocin™, hygromycin B, puromycin, and blasticidin antibiotics are the most commonly used selection antibiotics for stable cell transfection. These antibiotics provide unique solutions for your research needs, such as dual selection and rapid, stable cell line establishment.

- View all the **Gibco Selection Antibiotics**

Geneticin Selection Antibiotic

Zeocin™ Selection Antibiotic

Hygromycin B Selection Antibiotic

Puromycin Dihydrochloride Selection Antibiotic

Blasticidin S HCl Selection Antibiotic

Selection of stable transfectants

Selection of stably transfected cells begins with successful transient transfection with a plasmid containing a selectable marker, such as an antibiotic resistance gene. As a negative control, cells should be transfected using DNA that does not contain the selectable marker.

Before starting

- Ensure that the cell line you are using can produce colonies from isolated cells as some cells require contact with one another to grow. For such cells, adapted or conditioned medium may be beneficial.

- Choose an appropriate selectable marker.
- Select a transfection procedure suitable for your cell type.
- Determine the selective conditions for your cell type by establishing a dose-response curve (kill curve) (Ausubel et al. 1995).

Kill curve

A kill curve should be established for each cell type and each time a new lot of the selective antibiotic is used.

1. Split a confluent dish of cells at approximately 1:5 to 1:10 (depending on the cell type and cell density post-transfection) into medium containing various concentrations of the antibiotic.
2. Incubate the cells for 10 days replacing selective medium every 4 days (or as needed).
3. Examine the dishes for viable cells using the desired method (e.g., Countess II Automated Cell Counter, hemocytometer with trypan blue staining).
4. Plot the number of viable cells versus antibiotic concentration to establish a kill curve to determine the most appropriate selective drug concentration required to kill untransfected cells

Selection workflow

Step 1 : Transfect cells

Transfect the cells using the desired transfection method. If the selectable marker is on a separate vector, use a 5:1 to 10:1 molar ratio of plasmid containing the gene of interest to plasmid containing the selectable marker.

Note: Perform control transfections with a vector containing the selectable marker but not the gene of interest. If colonies are obtained from cells transfected with the control plasmid but not from cells transfected with plasmid containing the gene of interest, indicating that the gene of interest may be toxic. It is also important to perform replicate transfections in case the transfection fails or the cultures become contaminated.

Step 2 : Passage cells with antibiotic

Step 3 : Monitor for cell "islands"

Step 4 : Isolate colonies

Step 5 : Transfer single cells

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ICP备案号：沪ICP备11003924号-4