



Post-Transfection Analysis of Cells

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Analysis of Transfected Cells

The advancement of transfection technologies has enabled scientists to investigate protein function and gene regulation in a variety of cell types, tissues, and organisms. Transfection is generally achieved by three different methods: chemical, physical, and biological. The choice of the method will depend on the application, the transfected molecule, and the cell type because various cells may respond differently to a particular method. Once cells have been transfected, various methods can be used for analysis post-transfection and for assessing transfection efficiency.

There are many factors that can influence transfection efficiency, a number of which are specific to the target cell. Cell-related factors affecting transfection include cell density, cell size, replication state, passage number, health of cells, biomolecule type, and concentration. Some factors are method specific, for example, electroporation parameters such as voltage, capacitance, and resistance strongly affect transfection efficiency. Therefore, to obtain high efficiencies, all relevant factors should be considered when planning transfection experiments.

Frequently, after any transfection experiment, it is important to assess the efficiency of transfection and the impact of transfection on the cells. Analysis of transfection efficiency can be as simple as confirming the expression of your gene of interest. However, in many cases assessment of transfection involves determining the total expression level of your gene of interest, determining the number of

positive cells within a transfected population (% positive cells), and/or visual confirmation of your protein of interest (Jordan et al. 2007).

Most methods for measuring protein expression level of your transfected cell will determine the total expression from a population of transfected cells. Measurement of total gene expression can be done through real-time quantitative PCR (real-time qPCR), western blot analysis, molecular imaging, and fluorometry. Determining the number of positive cells within a transfected cell population can be done through microscopy and flow cytometry. Finally, confirming localization of your protein of interest can be done by microscopy.

Reporter genes, such as green fluorescent protein (GFP), luciferase, or β -galactosidase can be used to analyze transfection efficiency because their expression can be easily monitored. They can also be used to standardize transfection efficiencies between different transfection experiments by comparing the expression levels of their products. Reporter genes can be used alone or fused to a gene of interest to determine the protein expression level, the number of positive cells, or the location of the protein being studied.

We present here general guidelines and methods that you can use for the analysis of transfected cells. The methods can be used to determine transfection efficiencies and to perform more in-depth analyses of the expression of your favorite gene/protein. However, depending on your cells, reagents, or equipment availability these methods should be modified to fit your laboratory needs.

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Flow Cytometry

A flow cytometer can determine the number of positive cells within a transfected cell population (% positive cells). In addition, a flow cytometer with sorting abilities can enrich for positive cell populations. However, flow cytometry requires that the cells either express a fluorescent protein, such as GFP, or your protein of interest must be labeled with a fluorescent molecule.

For more information about flow cytometry, please visit <http://flowcyt.sourceforge.net/>.

Requirements or Limitations

- Expensive
- Can only detect fluorescence (often requires labeling protein with a fluorescent molecule)
- Time consuming

Advantages

- Determines percentage of positive cells (quantitative measurement)
- Can enrich for positive cell population with sorting ability

Fluorometry

A fluorometer can detect a wide range of fluorescence. Extracts from cells expressing fluorescent protein can be used to measure the expression level of your gene in the transfected cells.

For more information about fluorescent protein detection in cell extracts, please see Measuring Intracellular Enhanced Green Fluorescent Protein With the VersaFluor™ Fluorometer, Bio-Rad Bulletin 2368.

Requirements or Limitations

- Moderately expensive
- Can only detect a fluorescent molecule or protein

Advantages

- Able to perform a quick and easy sample detection (direct analysis of lysate)
- Can perform quantitative analysis for total expression

Laser-Scanning Molecular Imaging

Laser-scanning molecular imagers can detect a wide range of fluorescence. With the use of appropriate fluorophores, western blots or gels can be quantitatively analyzed with a scanner. In addition to analyzing western blots or gels, scanners can also directly detect and quantify lysate from a population of cells expressing a fluorescent protein, such as GFP. The ability to detect fluorescence allows the scanner to directly analyze cell lysate without running a gel.

For more information about laser-scanning molecular imaging, please see Applications for Molecular Imager FX™ Systems: Instrument Settings, Bio-Rad Bulletin 2752.

Requirements or Limitations

- Expensive
- Can only detect a fluorescent molecule or protein
- For quantitative analysis, need an internal control and densitometer

Advantages

- Able to perform a quick and easy sample detection (direct analysis of lysate)
- Can perform quantitative analysis for total expression

Luminometry

A luminometer is an instrument that measures light intensity. Expression or co-expression of luciferase, which emits light after interacting with its substrate, can be used for quantitative analysis of total protein expression.

For more information about luminometry and luciferase assays, please see Sambrook J and Russell D (2001). Chapter 17: Analysis of gene expression in cultured mammalian cells. In *Molecular Cloning: A Laboratory Manual*, 3rd ed. (Woodbury, NY: Cold Spring Harbor Laboratory Press), 17.96.

Requirements or Limitations

- Moderately expensive
- Luminometer needed
- Requires expression of luciferase gene (when luciferase assays are used)
- Only measures total gene expression from population of cells

Advantages

- Able to perform a quick and easy sample detection (direct analysis of lysate with luminometer after addition of substrate)
- Can perform quantitative analysis for total expression

Microscopy

Microscopy allows direct visualization of transfected cells. Microscopy can be used to count the number of transfected cells for estimation of positive cell numbers. Microscopy can also be used to confirm correct localization of your protein of interest. Visualization of cells transfected with your gene of interest can be accomplished in several ways. Expression or co-expression of a fluorescent protein, such as GFP, will allow direct identification of your transfected cells. However, a non-fluorescent protein must be labeled for visualization. Immunofluorescence, chemical

labeling, and immunohistochemistry techniques can be used to label proteins. In general, microscopy cannot be used for quantitative measurement of transfection efficiency unless you have image analysis software that can count and quantitate positively transfected cells.

For more information about chemical and immunofluorescent labeling, fluorescence microscopy, and immunohistochemistry techniques, please visit:

- http://www.ihcworld.com/_protocols/immunofluorescence/immunofluorescence.htm
- <http://www.mwrn.com/microscopy/light/fluorescence.aspx>

Requirements or Limitations

- Expensive
- Epifluorescence microscope needed
- Must be able to visualize protein (often requires labeling protein)
- Time consuming if labeling protein is required

Advantages

- Visual (direct observation of your protein of interest)
- Allows examination of individual cells
- Microscopy is the only method that can confirm correct localization of your protein of interest after transfection

Real-Time Quantitative PCR

Real-time qPCR can quantify the expression level of a transgene or measure the degree to which a gene silencing (knockdown) experiment has been effective. It is fast and, because it requires little starting material, it is applicable when transfected cells are available in limited amounts.

Using real-time qPCR, one can determine the relative abundance of specific messenger RNA (mRNA) compared to a control. Additionally, real-time qPCR can be used to assess how the knockdown of one gene may affect the expression of other genes in the pathway. However, it does not directly measure or identify the presence of protein. Therefore, any problems post-mRNA synthesis, such as problems during protein translation, will not be identified with this method. As a result, you may observe mRNA production where there may not be any protein synthesis.

For more information about real-time qPCR, please see the Real-Time PCR Applications Guide, Bio-Rad Bulletin 5279 (catalog #170-9799).

Requirements or Limitations

- Moderately expensive
- Real-time PCR system needed
- Protein is not directly measured or identified

Advantages

- Requires little starting material
- Commonly used and widely accepted technique

Spectrophotometry

A spectrophotometer is an instrument that measures light intensity as a function of color or wavelength. β -galactosidase assays can be used to determine transfection efficiency by measuring colorimetric changes associated with β -galactosidase activity.

For more information about spectrophotometry and β -galactosidase assays, please see:

- Nielsen DA et al. (1983). Expression of a preproinsulin-beta-galactosidase gene fusion in mammalian cells. *Proc Natl Acad Sci USA* 80, 5198-5202
- Sambrook J, Fritsch EF, Maniatis T, eds. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Woodbury, NY: Cold Spring Harbor Laboratory Press)
- http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=1240

Requirements or Limitations

- Moderately expensive
- Expression of β -galactosidase (when β -galactosidase assays are used)

Advantages

- Able to perform a quick and easy sample detection (direct analysis of lysate with spectrophotometer after addition of substrate)
- Can perform quantitative analysis for total expression

Western Blot Analysis

Western blot analysis can be used to identify or quantitate the total expression of your transfected gene from a population of cells. With western blot analysis, you can determine the relative expression level of your transfected protein in a population of cells. This method of quantitation requires the use of an internal control, such as *GAPDH* or β -actin.

For more information about western blot analysis, please see:

- Increase Western Blot Throughput With Multiplex Fluorescent Detection, Bio-Rad Bulletin 5723
- <http://www.westernblotting.org/>

Requirements or Limitations

- Must be able to label and detect protein
- Time consuming due to running and transfer of gels and labeling protein for detection
- For quantitative analysis, need an internal control and densitometer

Advantages

- Inexpensive
- Commonly used and widely accepted technique
- Can be used for quantitative analysis for total expression

References

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Sambrook J, Fritsch EF, Maniatis T, eds. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Woodbury, NY: Cold Spring Harbor Laboratory Press).

Western Blot Analysis

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