Protocol

Observation of Fluorescent Proteins in Living Cells

Richard Behringer, Marina Gertsenstein, Kristina Vintersten Nagy, and Andras Nagy

Frequently, there is a need for fluorescent protein detection in mouse cell cultures, including embryonic stem cells or their differentiated derivatives, primary and transformed cells. Here, cells expressing green fluorescent protein-labeled proteins are observed using fluorescent microscopy.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Cell culture (live cells growing in tissue culture)
Culture medium appropriate for the cells used
Phenol Red-free cell growth medium (GIBCO) or phosphate-buffered saline (PBS) <R>
If using PBS, supplement with Ca^{2+} and Mg^{2+} as directed and adjust to pH 7.2.

Equipment

Microscope (either dissecting or inverted compound) with fluorescent visualization capabilities
Tissue culture dishes or glass-bottomed tissue culture dishes (MatTek Corporation) or multichamber culture plates (Nalgene Lab-Tek 2 Chambers)

METHOD

1. Grow the cells to the desired stage, confluence, or time.
2. Replace the culture medium with Phenol Red-free medium or PBS with Ca^{2+} and Mg^{2+} .
3. Place the chamber or tissue culture plate on the microscope stage and use appropriate filters to visualize green fluorescent protein (GFP).
4. Replace the Phenol Red-free medium with culture medium, and place the chamber or tissue culture plate back into the incubator.

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DISCUSSION

The prototype GFP was isolated from the jellyfish *Aequorea victoria*. Mutagenesis studies of the wild-type GFP have resulted in variants with increased thermostability, such as enhanced GFP (EGFP) (Cormack et al. 1996), which has at least one order of magnitude higher quantum yield and an elevated resistance to photobleaching than wild-type GFP. In addition, novel spectral variants have been generated, such as enhanced cyan (ECFP) and yellow (EYFP) fluorescent proteins (Tsien 1998; Yang et al. 1998). Subsequent cloning of the red fluorescent protein-encoding gene from an anemone, *Discosoma striata* (dsRED), has provided a further red-shifted fluorescent protein. The original protein has slow maturation and aggregation-associated cellular toxicity in certain cell types (Baird et al. 2000) including ES cells and early embryonic cell types (K Vintersten and A Nagy, unpubl.). A huge effort has been concentrated on deriving FPs with different spectra (Shaner et al. 2005).

In general, caution should be taken in planning the use of fluorescent proteins. There are a few areas of applications where expression of certain variants might interfere with the biological functions in question.

The basic principle of visualizing fluorescent proteins is the same as for the fluorophores used in fluorescent microscopy. The fluorescence elicited by an excitation light source of a specific wavelength and the resulting emitted light is observed through a specific filter set, which cuts off the excitation wavelength. Depending on the FP, the excitation and emission wavelengths are different, therefore necessitating different optimal filter sets for visualization. Microscope manufacturers provide basic sets with their products.

If fluorescent protein observation takes place outside a sterile hood, care should be taken to retain the sterility of the cell culture.

It is essential to observe fluorescent protein expression in Phenol Red-free conditions because Phenol Red causes significant background fluorescence.

If a low-power dissecting microscope is used, be aware that dust particles attached to the bottom of the plate or chamber frequently autofluoresce and could be mistaken for a positive cell or colony. The best way to avoid being misled is to check frequently whether the microscope is perfectly focused on the cell layer by turning the normal light on and off.

RECIPE

Phosphate-Buffered Saline (PBS)

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na ₂ HPO ₄	1.44 g	10 mM	14.4 g	100 mM
KH ₂ PO ₄	0.24 g	1.8 mM	2.4 g	18 mM

If necessary, PBS may be supplemented with the following:

CaCl ₂ ·2H ₂ O	0.133 g	1 mM	1.33 g	10 mM
MgCl ₂ ·6H ₂ O	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1× solution or as a 10× stock. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H₂O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H₂O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle or by filter sterilization. Store PBS at room temperature.

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