

Chapter Five: Co-localization/FRET/BRET

Contents	Page
Co-localization overview	17
Co-localization assay formats	
Immunofluorescence	18
FRET	18
BRET	19
When to use co-localization	19
Reagent requirements for immunofluorescence	19
Reagent requirements for FRET	19
Reagent requirements for BRET	19

Co-localization overview

Methods such as co-immunoprecipitation and pull-downs require the preparation of cell extracts which may not preserve the physiological conditions under which proteins may interact in a true cellular environment. Using various co-localization techniques protein:protein interactions may be characterized directly in the cell without the need to create cell lysates or isolate complexes from a cell.

5 CHAPTER

CO- LOCALIZATION/ FRET/BRET



Co-localization assay formats

Immunofluorescence

Immunofluorescence can be used to determine whether two proteins share the same location in a cell. Two primary antibodies that recognize the two specific proteins are added simultaneously to the sample. Two secondary antibodies with different fluorescent tags are then added. The sample is then analyzed under a confocal microscope to determine whether the two fluorescent signals overlap. If both fluorescent signals are in the same location then the proteins are located in the same cellular region. This is an indirect way of determining whether two proteins may interact; if they are located in the same region there is a possibility that they may bind to each other.

When available, immunofluorescent antibodies to endogenously expressed proteins should be used. However, when antibodies are not available, or when the cell line does not express the protein at sufficient levels for immunofluorescence detection, cells may be transfected with recombinant vectors encoding tagged proteins. Antibodies to the tag can then be used to localize the protein in the cell.

REFERENCES

Key original reference for FRET

1. Gordon, G. *et al.* (1998) *Biophys. J.* **74**, 2702–13.

Key original reference for BRET

1. Xu, Y. *et al.* (1999) *Proc. Natl. Acad. Sci.* **96**, 151–56.

Use of immunofluorescence

1. Snabaitis, A. *et al.* (2006) *J. Biol. Chem.* **281**, 20252–62.
2. Vandermoere, F. *et al.* (2006) *J. Biol. Chem.* **281**, 14307–13.
3. Burgess, A. *et al.* (2006) *J. Gen. Virol.* **87**, 789–93.

Use of FRET

1. Sohn, H-W. *et al.* (2006) *Proc. Natl. Acad. Sci.* **103**, 8143–48.
2. Treanor, B. *et al.* (2006) *J. Cell. Biol.* **174**, 153–61.
3. Hunger, K. *et al.* (2006) *J. Bact.* **188**, 240–8.
4. Herrick-Davis, K. (2006) *J. Biol. Chem.* **281**, 27109–16.
5. Kramer, J. *et al.* (2006) *J. Immunol.* **176**, 711–15.

FRET

Fluorescence Resonance Energy Transfer (FRET, Figure 9) can be used to measure interactions between two proteins in vivo. Using this technique, two different fluorescent molecules (donor and acceptor fluorophores) are used to label the suspected protein partners. FRET is observed by exciting the sample at the donor excitation wavelength and measuring fluorescence intensities emitted at the wavelengths corresponding to the emission peaks of the donor compared to those of the acceptor. When the donor and acceptor are in close proximity (1–10nm) due to the interaction of the two proteins, the acceptor emission is predominantly observed because of the intermolecular FRET from the donor to the acceptor.

Another option to labeling proteins with fluorescent dyes is to express both partners as fusion proteins with different GFP (green fluorescent protein) tags. The most popular FRET pair for biological use is a cyan fluorescent protein (CFP)-yellow fluorescent protein (YFP) pair. Both are color variants of GFP.

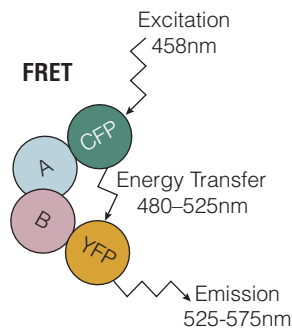


Figure 9. Overview of FRET for the analysis of protein:protein interactions. Protein partner A is cloned into a vector containing cyan fluorescent protein (CFP, donor). The second partner B is cloned into a vector containing a yellow fluorescent protein (YFP, acceptor). The vectors should also contain the appropriate elements for protein expression in mammalian cells. Both recombinant vectors are then transfected into mammalian cells. FRET can be observed by exciting the sample at the donor excitation wavelength while measuring fluorescence intensities emitted at the wavelengths corresponding to the emission peaks of the donor compared to that of the acceptor. When the donor and acceptor are in close proximity, acceptor emission is predominantly observed because of the intermolecular FRET from the donor to the acceptor.

TO ORDER

Phone
1-800-356-9526

Fax
1-800-356-1970

Online
www.promega.com



Promega
www.promega.com

5 CHAPTER

CO- LOCALIZATION/ FRET/BRET

BRET

Bioluminescence Resonance Energy Transfer (BRET) involves the transfer of energy from a donor enzyme to suitable acceptor molecule. Using this method one protein partner is expressed as a fusion with GFP and the other is expressed as fusion with *Renilla*. BRET technology is based on the transfer of resonant energy from a bioluminescent donor protein to a fluorescent acceptor protein using *Renilla* luciferase (*Rluc*) as the donor and a GFP mutant as the acceptor molecule. BRET technology is analogous to FRET, but eliminates the need for an excitation light source and its associated problems such as high background caused by autofluorescence.

When to use co-localization

Co-localization is used in conjunction with other techniques to characterize protein:protein interactions in a true mammalian environment. This technique is typically not used for screening large numbers of prey proteins.

Reagent requirements for immunofluorescence

- Confocal laser scanning microscope (and appropriate filters)
- Tissue culture equipment
- Mammalian cells
- Transfection reagents
- Fluorescently labeled antibodies

Reagent requirements for FRET

- Confocal laser scanning microscope (and appropriate filters)
- Tissue culture equipment
- Mammalian cells
- Transfection reagents
- Fluorescent dyes
- Mammalian expression vectors containing donor tag (e.g., CFP) and coding sequences for one protein partner
- Mammalian expression vectors containing acceptor tag (e.g., YFP) and coding sequences for the other protein partner

Reagent requirements for BRET

- Microplate reader capable of luminescent and fluorescent detection
- Tissue culture equipment
- Mammalian cells
- Transfection reagents
- Mammalian expression vectors containing donor tag (e.g., *Renilla*) and coding sequences for one protein partner
- Mammalian expression vectors containing acceptor tag (e.g., YFP) and coding sequences for the other protein partner

REFERENCES (CONTINUED)

Use of BRET

1. Shimizu, T. *et al.* (2006) *Pro. Natl. Acad. Sci.* **103**, 2093–97.
2. Whittard, J. *et al.* (2006) *Mol. Biol. Cell* **17**, 2696–706.
3. Goin, J. *et al.* (2006) *J. Biol. Chem.* **281**, 5416–25.
4. Rebois, R-V. *et al.* (2006) *J. Cell. Sci.* **119**, 2807–18.
5. Koshimizu, T. *et al.* (2006) *Mol. Pharmacol.* **69**, 1588–98.
6. Sirokmany, G. *et al.* (2006) *J. Biol. Chem.* **281**, 6096–105.

TO ORDER

Phone
1-800-356-9526

Fax
1-800-356-1970

Online
www.promega.com



Promega
www.promega.com