PUBLISHED IN ASSOCIATION WITH
COLD SPRING HARBOR LABORATORY

Detection of protein-protein interactions using the GST fusion protein pull-down technique

Glutathione-S-transferase (GST) fusion proteins have had a range of applications since their introduction as tools for synthesis of recombinant proteins in bacteria[1]. Typically, GST pull-down experiments are used to identify interactions between a probe protein and unknown targets and to confirm suspected interactions between a probe protein and a known protein[2,3]. The probe protein is a GST fusion, whose coding sequence is cloned into an isopropyl-β-D-thiogalactoside (IPTG)-inducible expression vector. This fusion protein is expressed in bacteria and purified by affinity chromatography on glutathione-agarose beads. Target proteins are usually lysates of cells, either labeled with [^{35}S]methionine or unlabeled, depending on the method used to assay the interaction between the target and the probe. The cell lysate and the GST fusion protein are incubated together with glutathione-agarose beads. Complexes recovered from the beads are resolved by SDS-PAGE and analyzed by western blotting, autoradiography or staining.

PROCEDURE

Precleaning the cell lysate

1| Incubate the cell lysate with 50 µl of a 50% slurry of glutathione-agarose beads and 25 µg of GST for 2 h at 4 °C with end-over-end mixing. The amount of lysate needed to detect an interaction is highly variable. Start with a volume of lysate equivalent to 1×10^6–1×10^7 cells.
Because the aim of the experiment is to compare GST with a GST fusion protein, it is necessary to prepare enough precleared lysate for each reaction. Efficient mixing of reagents is the key to success and is best achieved if the reaction is carried out in a reasonable volume: 500–1,000 µl is a good starting point. Typically a cell lysate is used in which the proteins are labeled with ^{35}S. It is, however, possible to use unlabeled cell lysates, depending on the goals of the experiment and the desired detection method.

2| Centrifuge the mixture at maximum speed for 2 min at 4 °C in a microcentrifuge.

3| Transfer the supernatant (the precleared cell lysate) to a clean microcentrifuge tube.

Probing the cell lysate

4| Set up two microcentrifuge tubes, each containing equal amounts of precleared cell lysate and 50 µl of glutathione-agarose beads. To one tube add ~10 µg of GST protein; to the other tube add ~10 µg of the GST fusion probe protein.
The amount of probe and control protein added should be equimolar in the two reactions (that is, the final molar concentration of GST should be the same as that of the GST fusion probe protein).

5| Incubate the tubes for 2 h at 4 °C with end-over-end mixing, and centrifuge the samples at maximum speed for 2 min in a microcentrifuge.

6| Save the supernatants at 4 °C in clean microcentrifuge tubes. These samples will be analyzed by SDS-PAGE in step 9.

7| Wash the beads four times with 1 ml of ice-cold GST lysis buffer. Centrifuge the tubes at maximum speed for 1 min in a microcentrifuge. Discard the supernatants.

GST lysis buffer consists of 20 mM Tris-Cl (pH 8.0), 200 mM NaCl, 1 mM EDTA (pH 8.0), 0.5% Nonidet P-40, 2 μg/μl aprotinin, 1 μg/μl leupeptin, 0.7 μg/ml pepstatin and 25 μg/ml phenylmethylsulfonyl fluoride (PMSF).

8| *Optional*: Elute the GST fusion protein and any proteins bound to it by adding 50 μl of 20 mM reduced glutathione in 50 mM Tris-Cl (pH 8.0) to the beads. Centrifuge the tubes at maximum speed for 2 min in a microcentrifuge.

Detecting interacting proteins

9| Analyze the beads (from **step 7**) or the eluted proteins (from **step 8**) by SDS-PAGE. Detect the proteins associated with the GST fusion protein by one of the following means.

The method of detecting proteins associated with the GST fusion protein will depend on whether or not the cell lysate was radiolabeled and on the goal of the experiment.

If the goal is to detect all of the ^{35}S-labeled proteins associated with the fusion protein:

Dry the gel on a gel dryer and expose it to X-ray film to produce an autoradiograph.

If the goal is to detect specific associated proteins:

Transfer the proteins from the SDS-polyacrylamide gel to a membrane and perform immunoblotting.

If the goal is to determine the sizes and abundance of proteins associated with the fusion protein from a nonradioactive lysate:

Stain the gel with Coomassie blue or silver nitrate.

SOURCE

This protocol was adapted from **"**Detection of protein-protein interactions using the GST fusion protein pull-down technique," in *Molecular Cloning: A Laboratory Manual* (eds. Sambrook, J. & Russell, D.W.) Chapter 18 Protocol 3, pp. 18.55–18.59 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 2001). This protocol was provided by Margret B. Einarson (Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA).

1. Smith, D.B. & Johnson, K.S. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions of glutathione-S-transferase. *Gene* **67,** 31–40 (1988).
2. Kaelin, W.G. Jr, Pallas, D.C., DeCaprio, J.A., Kaye, F.J. & Livingston, D.M. Identification of cellular proteins that can interact specifically with the T/E1A-binding region of the retinoblastoma gene product. *Cell* **64,** 521–532 (1991).
3. Orlinick, J.R. & Chao, M.V. Interactions of the cellular polypeptides with the cytoplasmic domain of the mouse Fas antigen. *J. Biol. Chem.* **271,** 8627–8632 (1996).