Creative Proteomics

Protein-Protein Interactions

Co-Immunoprecipitation vs Pull-Down

INTRODUCTION OF CO-IP AND PULL-DOWN

Many cellular events, such as cell proliferation, cell differentiation, and cell death, are controlled by protein-protein interactions. Some dynamic characteristics of the intracellular proteins can be changed through the interactions. For example, substrate binding characteristics and catalytic activity can be changed; new binding sites are created and the specificity of a protein for a substrate is changed; some proteins can be inactivated to regulate the expression of other genes. Only when the protein-protein interactions are regulated can the normal cellular activities be carried out. Co-immunoprecipitation (Co-IP) and pull-down methods are related methods that are used to determine the stable protein-protein interactions.

Co-Immunoprecipitation

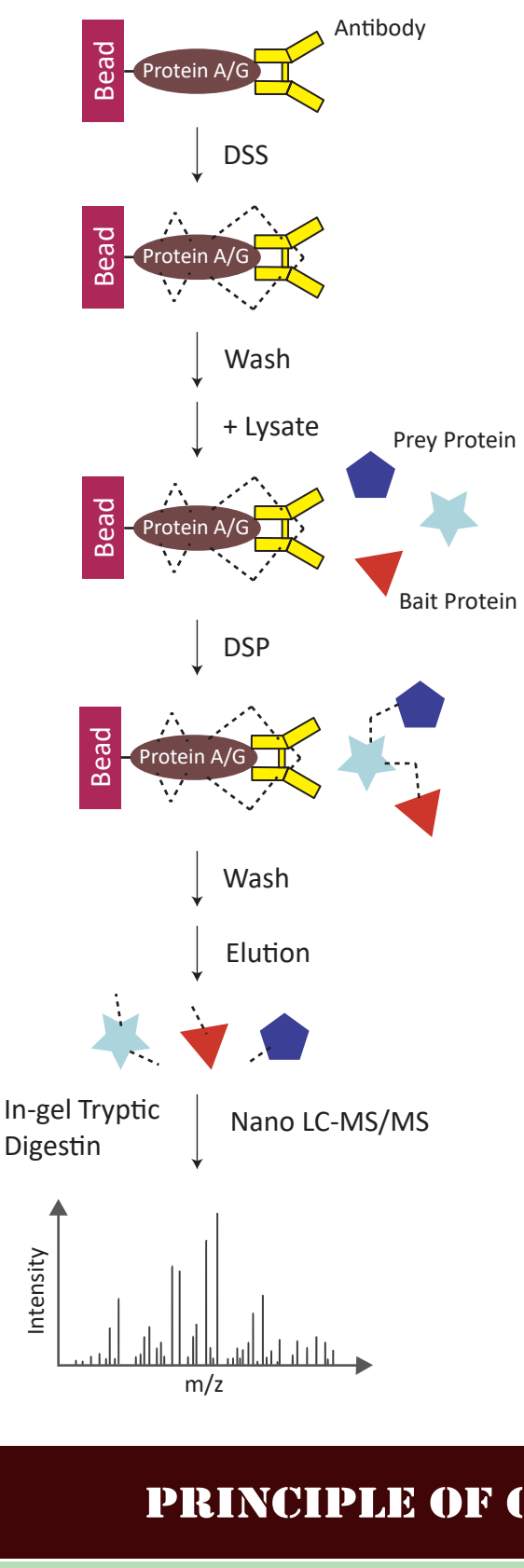
Co-immunoprecipitation detects the presence of specific interactions between two proteins *in vitro*. The principle of CO-IP is as follows: When cells are lysed under non-denaturing conditions, many intracellular protein interactions are preserved. If protein X is immunoprecipitated by an antibody, protein Y can also be precipitated if it's binding to protein X. By studying protein Y, an interaction between proteins X and Y can be confirmed.

Pull-Down

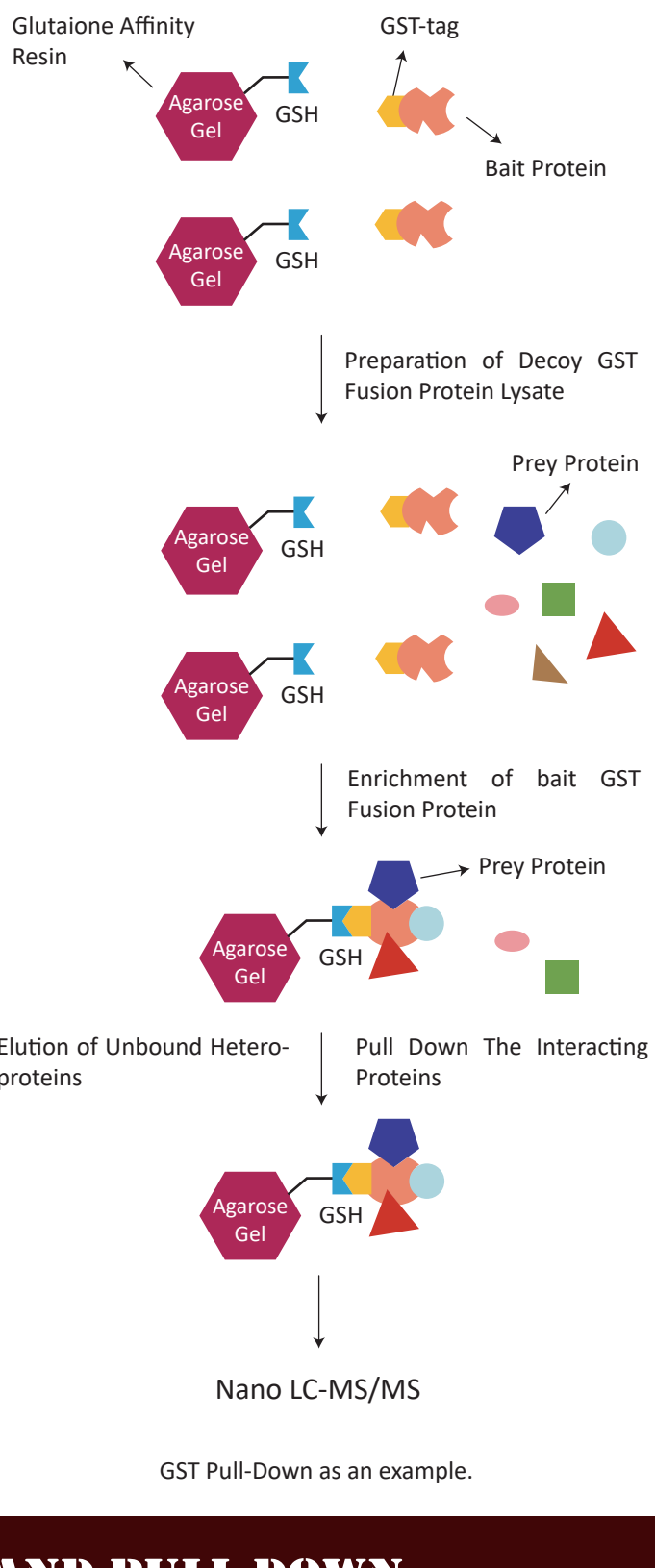
Protein pull-down assay is an *in vitro* affinity purification method that uses a bait protein to enrich proteins that interact with the bait protein. The basic principle of pull down assay is to use a protein fused with a tag (such as GST-tag, His-tag and biotin-tag) immobilized to affinity resin as the bait protein. The prey protein binding to the bait protein can be captured and "pulled-down" when the target protein or cell lysate flows through. By subsequent elution and analysis using Western Blot or Mass Spectrometry, a predicted interaction can be confirmed or previously unknown interactions can be discovered.

PRINCIPLE OF CO-IP AND PULL-DOWN

Co-IP



Pull-Down



GST Pull-Down as an example.

PRINCIPLE OF CO-IP AND PULL-DOWN

	Co-IP	Pull-Down
Advantages	1) Bait protein and prey protein are in natural conformation in CO-IP analysis. 2) The interaction between bait and prey protein occurs in the body with little external influence. 3) Does not require cloning and heterologous expression. Rapid if antibody is available.	1) Generic ability to purify low-abundant protein complexes. 2) The pull-down assay is generally used for in vitro transcription or translation systems.
Disadvantages	1) Low affinity or transient interactions between proteins may not be detected. 2) Co-IP results cannot determine whether the interaction is direct or indirect, as the possibility of other proteins participating cannot be ruled out. 3) Not generic-requires access to specifics antibodies.	1) The presence of a protein tag may influence results competition with the endogenous complex. 2) It doesn't really reflect the interactions between proteins, because they don't necessarily meet spatially in the body, so it doesn't mean that they're bound physiologically.

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