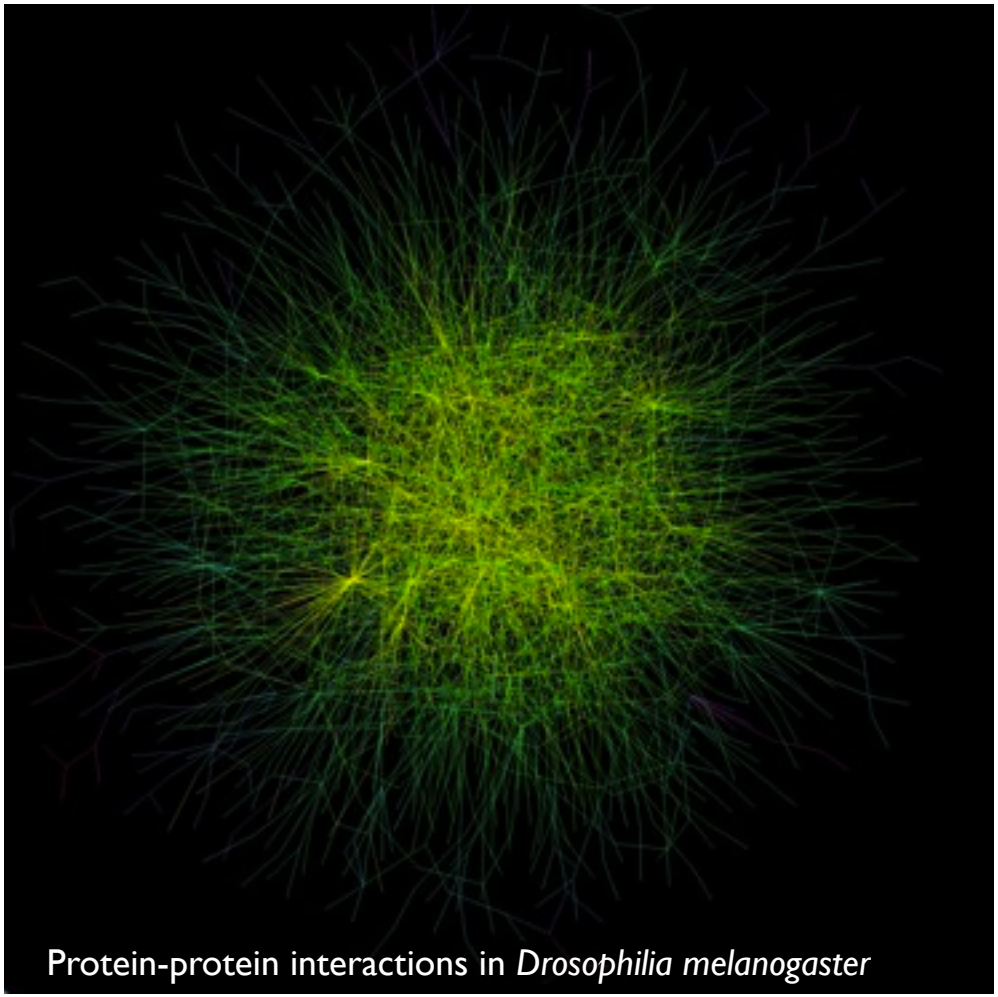
The bacterial two-hybrid system based on adenylate cyclase reconstitution in *Escherichia coli*

Aurélia Battesti, Emmanuelle Bouveret, 2012

Bo Heinz
Cellular Machines, Fundamentals
Diez, Schlierf

Introduction



Protein-protein interactions in *Drosophilia melanogaster*

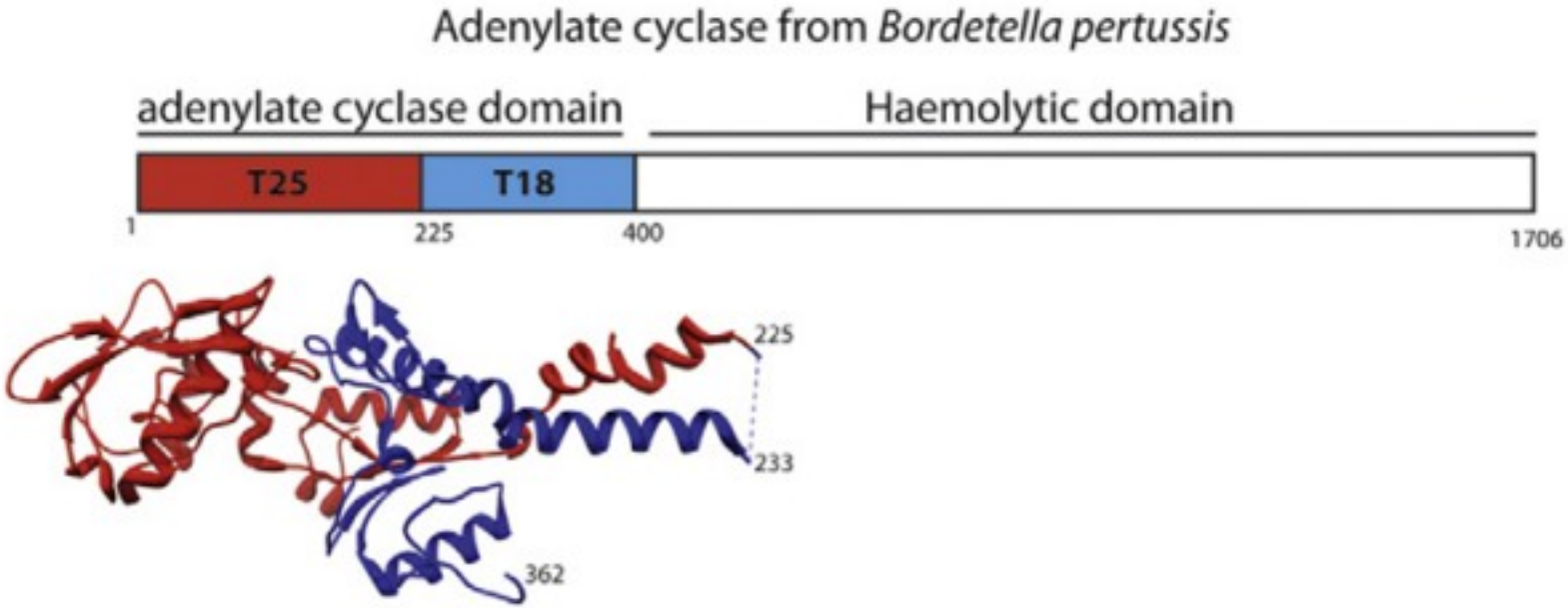
Protein-protein interactions are intrinsic to all biological processes, ranging from metabolism to structure.

Discovering new interacting partners can provide insight into actual function, beyond what is possible with sequence-based predictions.

It can also provide a platform for future research.

"Modeling Dynamics of Information Networks." *Modeling Dynamics of Information Networks*. N.p., n.d. Web. 01 June 2015.

Introduction



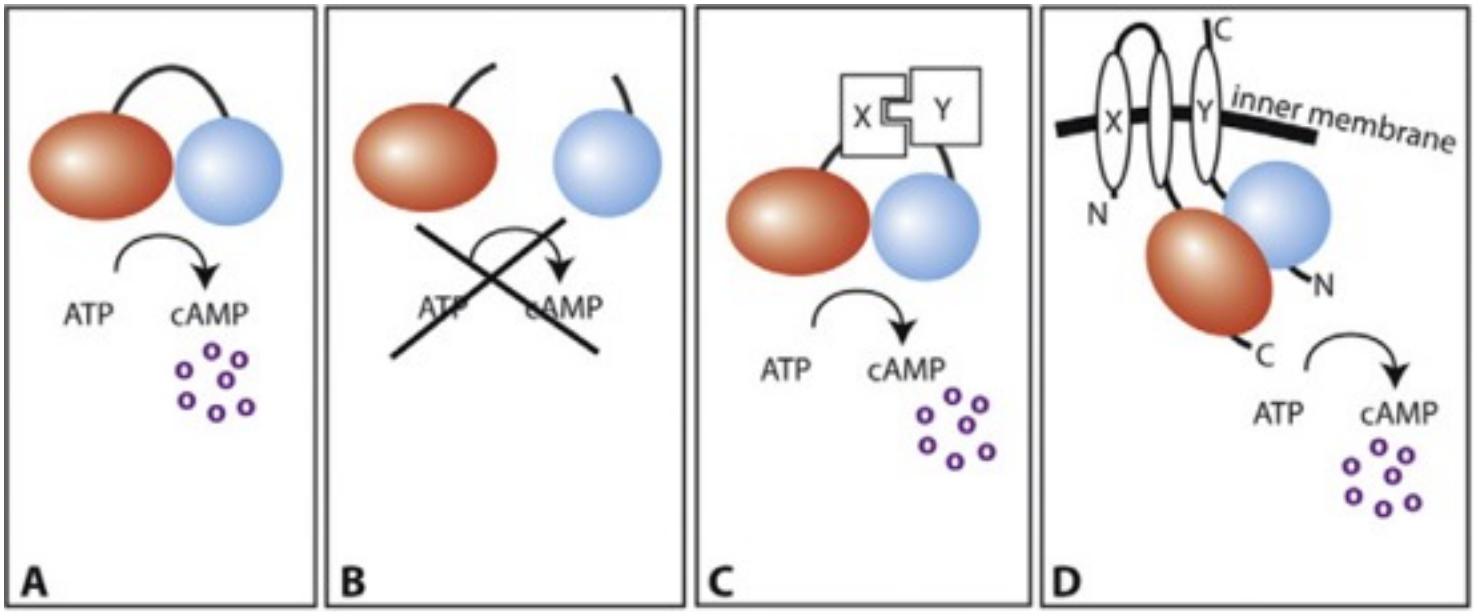
Adenylate cyclase is an enzyme from *B. pertussis*, the agent of whooping cough, and acts as a toxin that binds to calmodulin in eukaryotes elevating the level of cAMP in the organism.

This is a large protein with 1706 amino acids, however its catalytic activity residing in the first 400 amino acids.

The catalytic domain is divided further into two sub-domains:
25 kDa (residues 1-224) catalytic site and18 kDa (residues 225-399) calmodulin binding site.

Battesti, Aurélia, and Emmanuelle Bouveret. "The Bacterial Two-hybrid System Based on Adenylate Cyclase Reconstitution in Escherichia Coli." *Methods* 58.4 (2012): 325-34. Web.

Introduction



When the adenylate cyclase domain is expressed in *E. coli*, there is some residual cAMP produced.

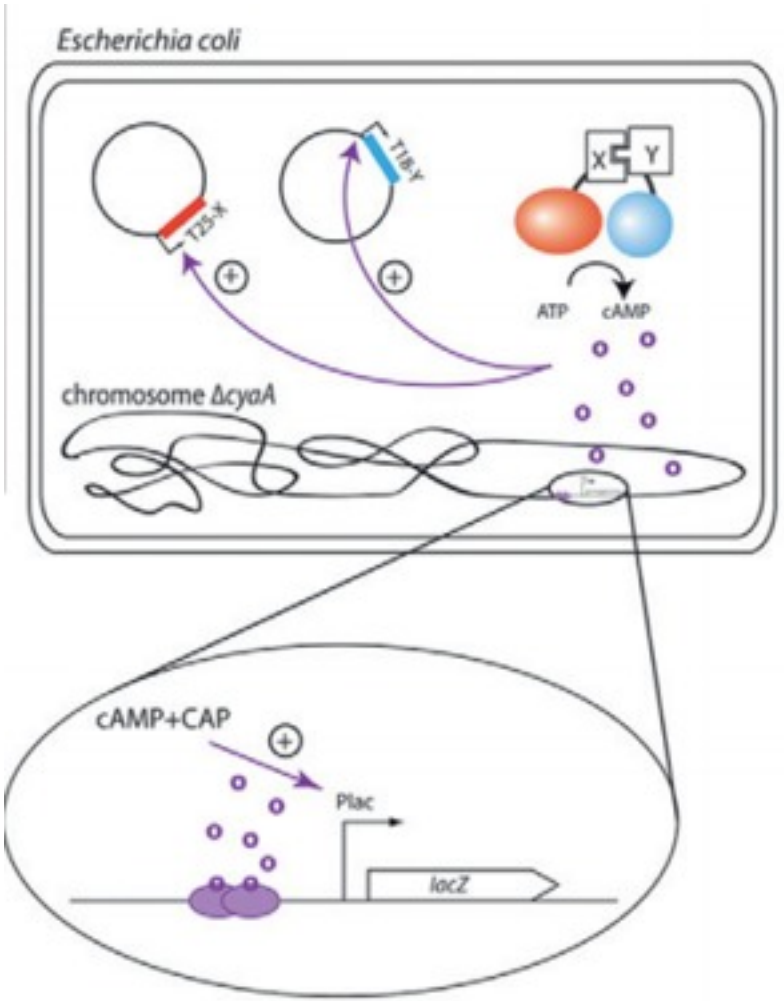
When T18 and T25 domains are expressed separately, no cAMP is produced.

When two interacting proteins of interest , either cystolic or membrane, are fused to T18 and T25, the domains are brought into close proximity and restore adenylate cyclase activity.

Battesti, Aurélia, and Emmanuelle Bouveret. "The Bacterial Two-hybrid System Based on Adenylate Cyclase Reconstitution in Escherichia Coli." *Methods* 58.4 (2012): 325-34. Web.

Method

Bacterial Two-Hybrid System



The gene coding for endogenous adenylate cyclase is deleted on an *E. coli* strain (*cya*$^-$).

The *cya*$^-$ strain is then transformed with both plasmids containing the T25 and T18 hybrids.

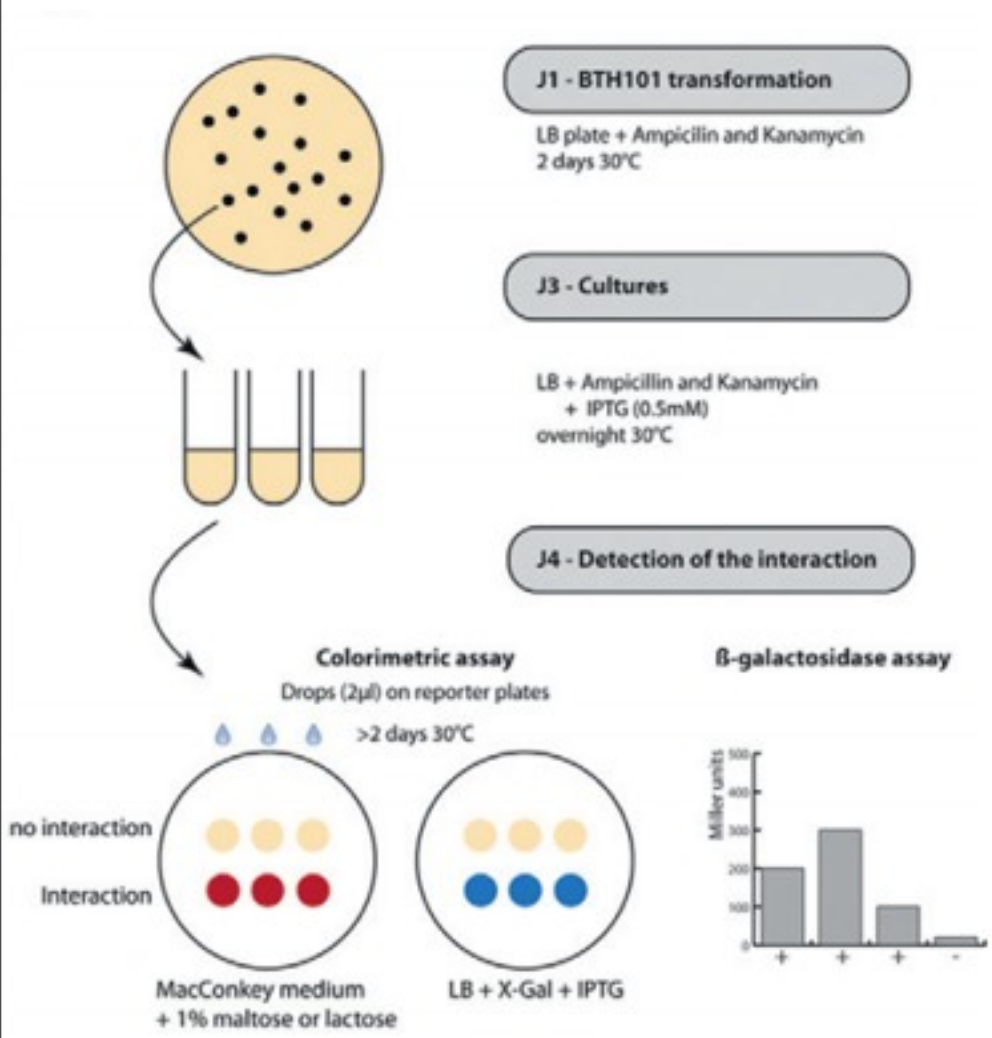
A positive interaction between two proteins of interest (X and Y) restores adenylate cyclase activity of T25 and T18.

Newly synthesized cAMP interacts with the catabolite activator protein (CAP); the cAMP/CAP complex binds to promoters and regulates transcription of several genes.

Quantitative assays can directly measure the level of cAMP synthesized.

Battesti, Aurélia, and Emmanuelle Bouveret. "The Bacterial Two-hybrid System Based on Adenylate Cyclase Reconstitution in Escherichia Coli." *Methods* 58.4 (2012): 325-34. Web.

Method



Following heat-shock transformation, cells are incubated for two days.

Triplicates are then picked from each colony and grown overnight with shaking.

Each culture is dropped on LB-X-Gal plates, MacConkey/maltose, or lactose plates for detection.

Positive controls are composed of T25-zip and T18-zip plasmids.

Negative controls are composed of proteins that do not interact, two empty T18 and T25 plasmids, or each protein of interest against an empty T18 or T25 plasmid.

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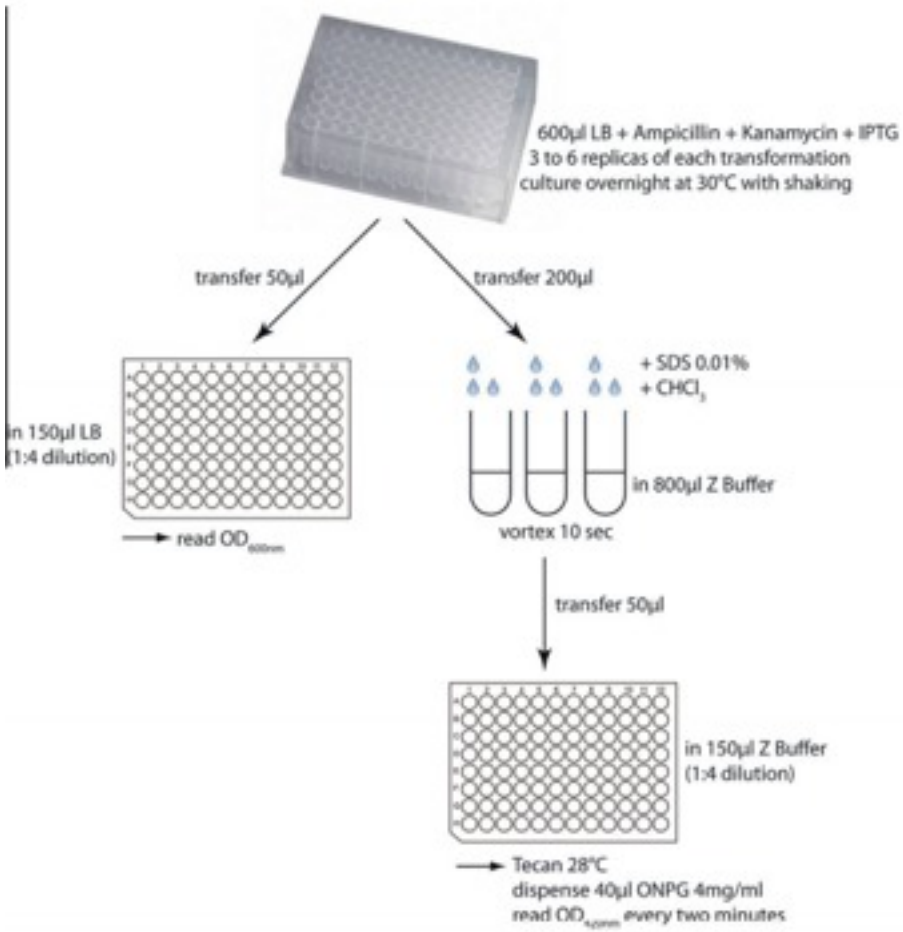
Beta-galactosidase assay



This is a high throughput assay with 3,4, or 6 replicates to account for variability in detecting interactions.

Indicator plates often display higher sensitivity than the beta-galactosidase assay.

A microplate spectrophotometer is required for this protocol.

The relative beta-galactosidase activity in each of the 96 samples is calculated with this formula:

$$\frac{((OD420nm\ at\ T2 - OD420nm\ at\ T1)/(T2 - T1))}{(OD600nm)}$$

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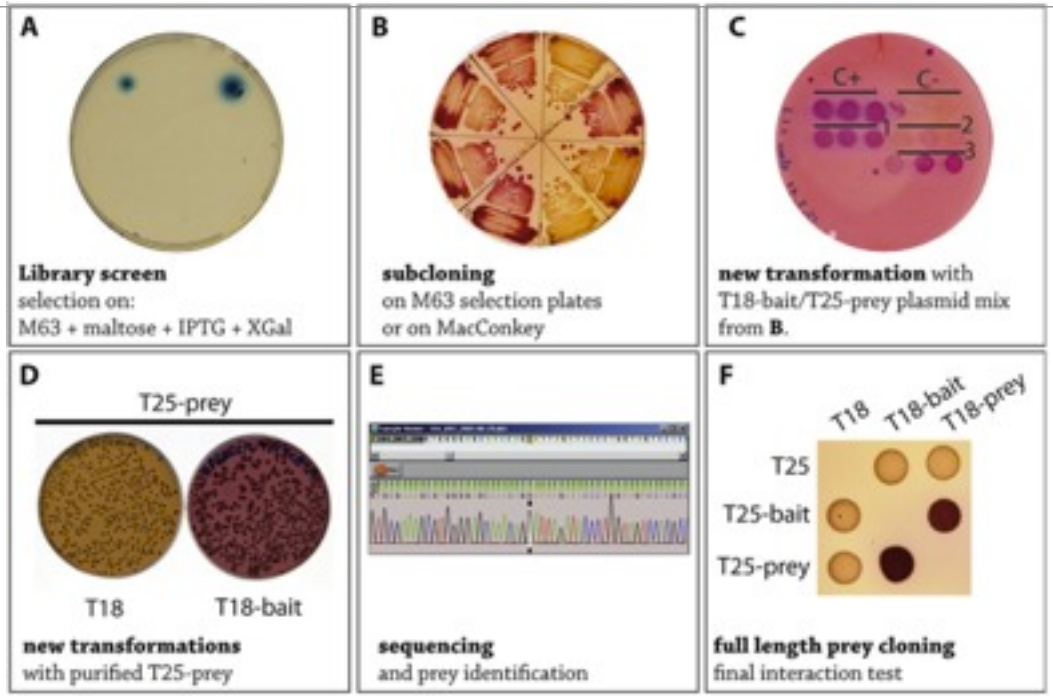
Method

Library screening



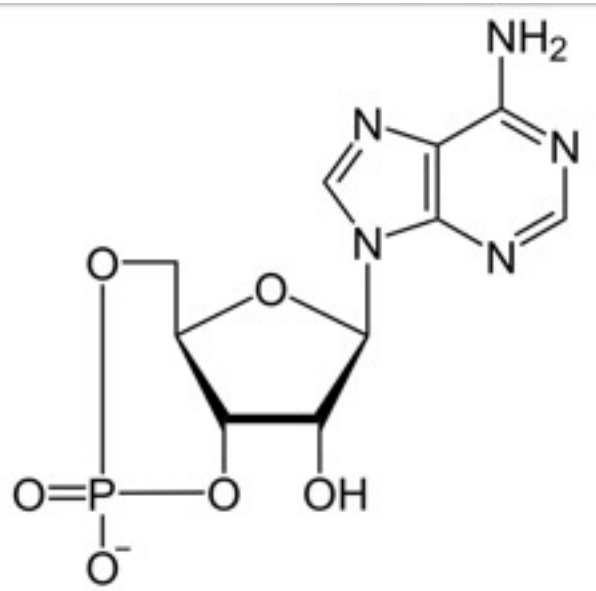
BACTH is a simple and rapid tool allowing for the generation of T18 and T25 libraries.

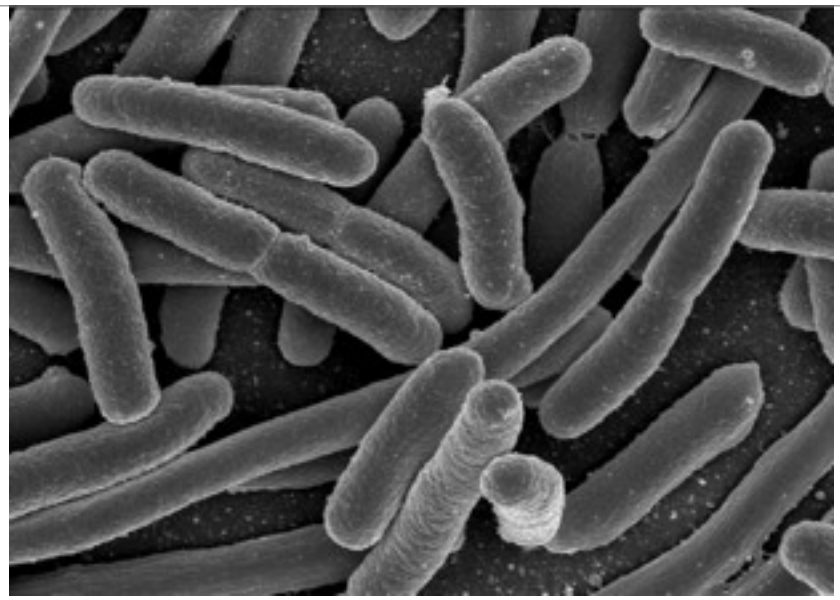
The screen is based on the ability of cells to grow on minimal medium containing 0.2% maltose or lactose.

Blue positives undergo isolation, interaction testing, purification, sequencing and repeated interaction tests for verification.

Battesti, Aurélia, and Emmanuelle Bouveret. "The Bacterial Two-hybrid System Based on Adenylate Cyclase Reconstitution in Escherichia Coli." *Methods* 58.4 (2012): 325-34. Web.

Advantages





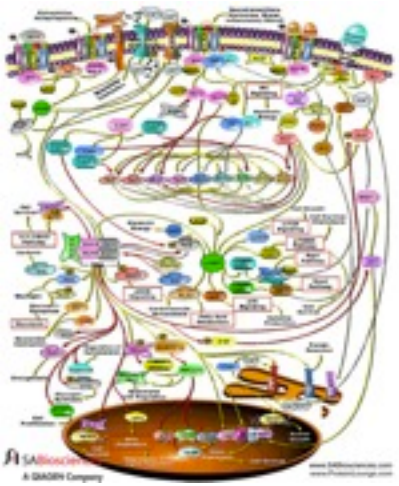
Since the output is a diffusible cAMP, interactions can take place anywhere in the cell, making it possible to study a large variety of proteins including membrane proteins.

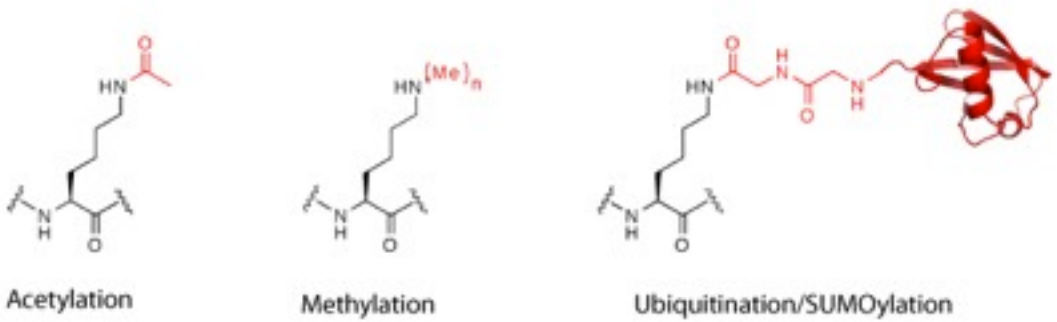
Using an indirect method to detect protein interaction based on a complicated signal cascade allows for the use of minimal media consisting of lactose or maltose for positive selection, a powerful tool for library screening.

BACTH is composed in E.coli and the proteins of interest are therefore isolated from indirect interactions otherwise existing in their host microenvironments.

"Escherichia Coli." *Wikipedia*. Wikimedia Foundation, n.d. Web. 01 June 2015.

Disadvantages





"Post-translational Modifications." *ChinLab MRC LMB*. N.p., n.d. Web. 01 June 2015.

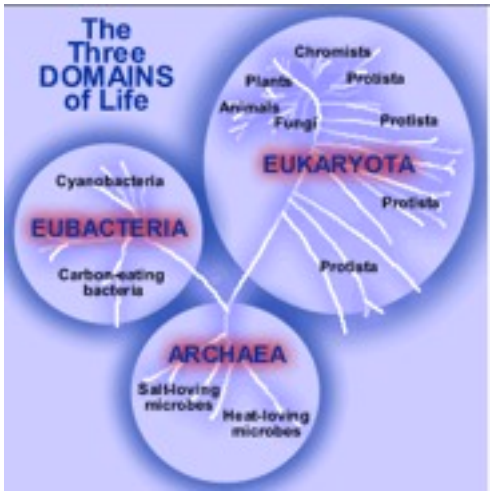
With different origins of replications but the same promoter, optimizing stoichiometry of Protein X and Y to insure an interaction becomes challenging.

Fusion to T25 or T18 can mis-fold the protein of interest, make it unstable, or disallow its interaction with its partner.

Using an indirect measure to determine protein interactions has constrains as the signaling cascade is complicated and the output is not only regulated by cAMP.

BACTH is composed in E.coli and therefore any post-translational modification of proteins of interest can be an issue.

Applications



Dimerization and interactions of *Brucella suis* VirB8 with VirB4 and VirB10 are required for its biological activity

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Edited by Patricia C. Zambryski, University of California, Berkeley, CA, and approved March 21, 2006 (received for review February 1, 2006)

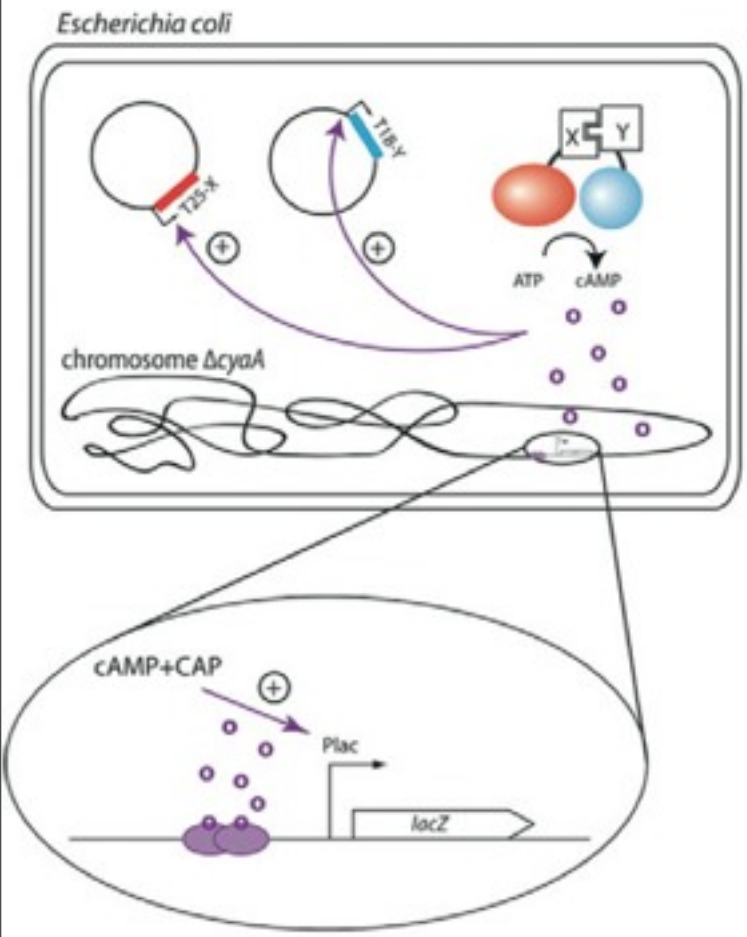
BACTH crosses Domain boundaries and investigates protein interactions of Bacterial, Eukaryotic, and Viral systems.

BACTH investigates cell division, two-component system proteins, small proteins forming transmembrane segments, and *E. coli* and *M. tuberculosis* genomic libraries.

Paschos. et al. used BACTH for screening a 30,000 compound collection in order to find molecules that inhibit the dimerization of VirB8.

Additionally, BACTH can test the effects of gene deletions, characterization of residues or domains involved in an interaction of interest.

Conclusion



BACTH identifies a positive interaction between two proteins of interest (X and Y) when adenylate cyclase activity of T25 and T18 is restored in *cya*$^{-}$ strains.

Newly synthesized cAMP binds to promoters and regulates transcription of several genes, used for measurable readouts of successful protein-protein interactions.

BACTH is a simple and rapid tool allowing for generation of high throughput assays and library screening.

Certain characteristics of BACTH that can be both seen as advantageous and disadvantageous.

BACTH has a multitude of applications and can study interactions in a variety of protein types.

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