Reverse two-hybrid the mammalian way

The yeast two-hybrid screen has been a tool of such paramount importance for the study of protein interactions that it now comes in a variety of flavors: forward and reverse, double and triple, yeast, bacterial and mammalian... So far, however, reverse two-hybrid screens, in which the disruption of a given protein-protein interaction provides a positive readout (that is, the expression of a reporter gene), were only possible in yeast. The group of Jan Tavernier now proposes an elegant strategy, exploiting the JAK-STAT signal transduction pathway, to perform such screens in the cytoplasm of intact mammalian cells, an environment of utmost relevance for drug screens.

Article p427, News and Views p412

Step by step to maintain stemness

Human embryonic stem cell culture is the subject of intense research to understand the cellular mechanisms that govern pluripotency and to determine conditions amenable to clinical applications. Beyond these objectives, all research on stem cells relies on the ability to cultivate them while maintaining their pluripotency. In this month’s protocol, Gerald Schatten, Roger Pedersen and their colleagues, detail a tried-and-tested procedure to achieve this goal by using mouse embryonic fibroblasts as a source of the necessary components to cultivate pluripotent progenitor cells.

Protocol p455

Rescuing the gatekeeper

Following the actions of a particular kinase amidst the more than 500 kinases in a cell was a challenge Kevan Shokat’s group recently took on. In 2000, they developed a chemical genetic screen that promised to selectively target any kinase in vivo: a mutation in the active site of the kinase, termed a gatekeeper mutation, increased the size of the ATP-binding pocket and thus allowed the kinase to accept specific inhibitors and ATP analogs. As the wild type kinases cannot bind these analogs, only the activity of the gatekeeper mutant kinase is highlighted. Unfortunately, some kinases lose activity when the gatekeeper mutation is introduced. Shokat’s group now presents a way to overcome this limitation. They successfully identified a region that, when mutated, restored activity in formerly ‘dead’ gatekeeper mutant kinases. This second site mutation will greatly enhance the power and versatility of the original gatekeeper chemical genetic approaches.

Article p435, News and Views p411

Transgenic bacteria—stable without antibiotics

More than 150 bacterial genomes have been sequenced, but genetic engineering of many of these organisms has been challenging. Schweizer and colleagues have now developed an optimized, broad-host-range cloning and expression system that does not require continuous antibiotic selection. This system is based on the Tn7 transposon, a mobile DNA segment that stably integrates at a specific site present in most bacterial genomes. Tn7-based vectors have great potential to be an effective means of transgenic expression in a wide range of bacteria, especially in environments such as biofilms and animal and plant models, in which antibiotic selection is not always possible.

Article p443

Cell ablation made easy

Targeted ablation of cells in vivo is a powerful method for elucidating cell function. Cell-specific expression of a diphtheria toxin receptor (DTR) in mice can be used for targeted ablation of cells after administration of diphtheria toxin. Unfortunately, the production and characterization of a new transgenic mouse strain for ablation of each cell type of interest is very difficult. Waismann and colleagues report the creation of a mouse strain they call iDTR that promises to radically simplify this process. These mice express a DTR, which is only activated by Cre-mediated excision of a STOP cassette. Because a large resource of cell-specific Cre mouse strains already exists, creation of mice designed for diphtheria toxin-mediated ablation of a desired cell type just requires breeding with an appropriate Cre mouse.

Article p419