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Modified two-hybrid

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The yeast two-hybrid system is one of the most widely used functional genomic tools for studying protein-protein interactions. But transcriptional activators cannot be used as 'bait' proteins in the assay as they can activate the reporter gene, usually used as an indicator of protein interaction, even in the absence of protein interactions. In the July 17 Proceedings of the National Academy of Sciences, Hirst *et al.* describe a modified two-hybrid assay that is based on transcriptional repression (*Proc Natl Acad Sci USA* 2001, **98:**8726-8731). In their repressed transactivator (RTA) system the activator 'bait' is fused to the GAL4 DNA-binding domain, and the 'prey' is fused to the repressor domain of the yeast TUP1 protein. Protein-protein interactions are detected by the repression of GAL4-dependent reporter gene transcription. Hirst *et al.* validated their RTA system by demonstrating its ability to detect interactions between the mammalian basic helix-loop-helix proteins MyoD and E12, and between the c-Myc oncoprotein and the Bin1 tumour suppressor. They also used the RTA assay to screen for novel proteins interacting with the activation domain of the VP16 transcriptional activator. This system should prove useful for identifying coactivators and regulators of transcription.

References

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