



Protein–Protein Interaction Testing Service

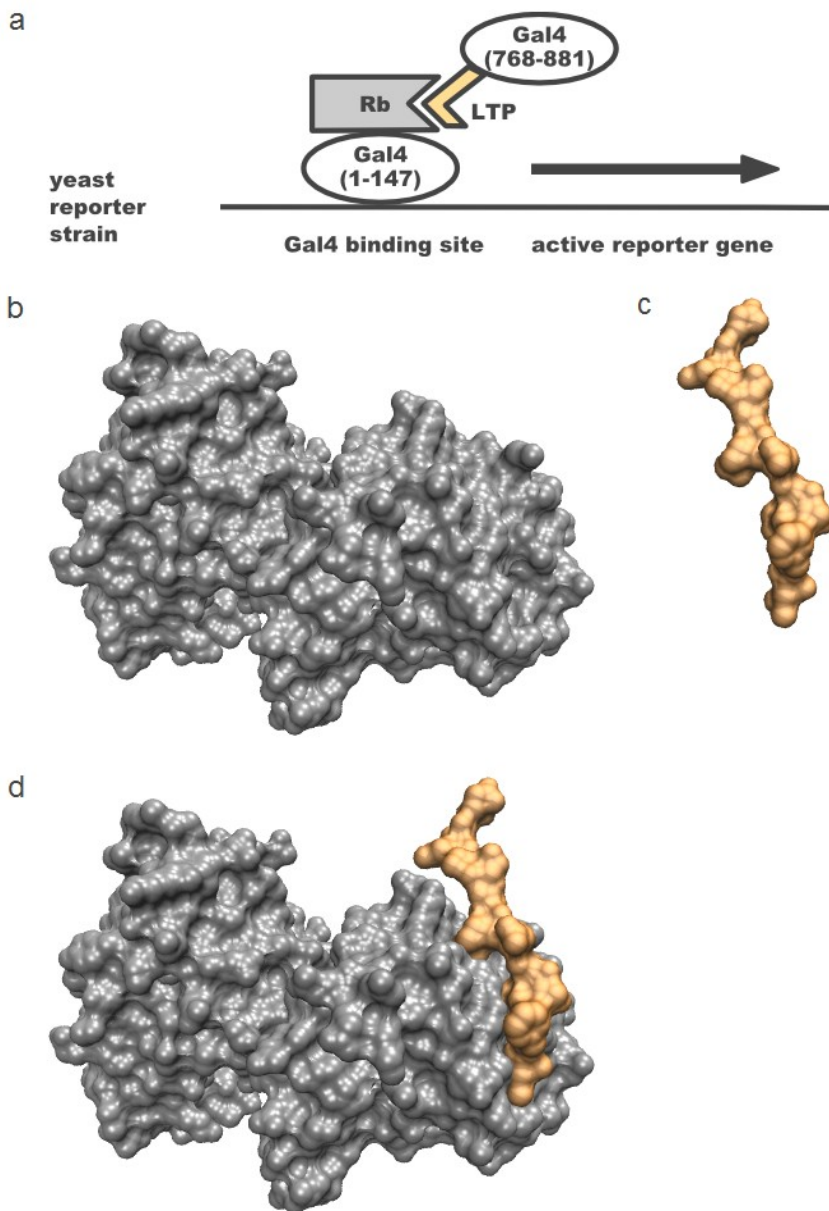


Figure 1. Protein–protein interaction between retinoblastoma protein and large T antigen protein. **(a)** Binding between Rb (the ‘bait’ protein) and LTP (the ‘prey’ protein) results in an active reporter gene in the Gal4 yeast two-hybrid (Y2H) system / assay. Data acquired and diagram adapted from NAR 23:1152, Oxford Journals; Rb: Retinoblastoma protein (301-918); LTP (for Large T antigen Peptide): SV40 Large T Antigen (residues 103-115). VMD (<http://www.ks.uiuc.edu/Research/vmd/>) and coordinates from PDB ID: 1GH6 were used to generate representations of: **(b)** Retinoblastoma protein (residues 378-772 minus residues 578-644); **(c)** SV40 Large T Antigen (residues 103-115); and **(d)** Retinoblastoma protein (residues 378-772 minus residues 578-644) and SV40 Large T Antigen (residues 103-115). Note: for references and further explanation of this Figure and the Y2H system please refer to our yeast two-hybrid system web page at <https://cellatechnologies.com/tech/yeast-two-hybrid-system>.

Protein–Protein Interaction Testing in the Yeast Two-Hybrid Assay

The yeast two-hybrid (Y2H) system / assay (Figure 1) is a well-known technique that can be used to demonstrate physical binding between two proteins of interest experimentally. The system is often very suitable to verify putative / potential protein–protein interactions (PPIs) that were identified using affinity purification-mass spectrometry (AP-MS) or any other method to identify PPIs, including PPI prediction methods. Once a PPI has been confirmed in the Y2H system, involved binding sites can be mapped relatively easily (learn more at <https://cellatechnologies.com/tech/yeast-two-hybrid-system>). Once a binding site has been identified, potential peptide protein–protein interaction inhibitors can be derived (learn more at <https://cellatechnologies.com/tech/peptide-protein-protein-interaction-inhibitors>). See Table 1 to learn about our protein–protein interaction testing service (Cat. No. C200).

Table 1. Protein–protein interaction testing service (Cat. No. C200). Physical binding (direct and or indirect) between the bait protein and the prey protein is confirmed if the bait–prey interaction / binding assay (Step 2a) turns out positive and the prey-dependency control assay (Step 2b) as well as the bait-dependency control assay (Step 2c) turn out negative, for explanation, see our yeast two-hybrid (Y2H) system web page.^[1]

Step 1a	Bait Plasmid Construction – DNA that encodes the bait (protein of interest (or fragment / mutant form thereof)) will be appropriately cloned as insert into empty bait vector. <i>Starting from 170 euro for a single construct.</i> ^[2]
Step 1b	Prey Plasmid Construction – DNA that encodes the prey (protein of interest (or fragment / mutant form thereof)) will be appropriately cloned as insert into empty prey vector. <i>Starting from 170 euro for a single construct.</i> ^[2]
Step 2a	Bait–Prey Binding Assay – cells of a yeast two-hybrid system reporter strain will be cotransformed with the bait plasmid (Step 1a) and the prey plasmid (Step 1b) and subsequently reporter activity in generated cotransformants will measured. <i>Starting from 85 euro for a single assay.</i> ^[2]
Step 2b	Prey-Dependency Assay – cells of a yeast two-hybrid system reporter strain will be cotransformed with the bait plasmid (Step 1a) and empty prey vector and subsequently reporter activity in generated cotransformants will measured. <i>Starting from 85 euro for a single assay.</i> ^[2]
Step 2c	Bait-Dependency Assay – cells of a yeast two-hybrid system reporter strain will be cotransformed with empty bait vector and the prey plasmid (Step 1b) and subsequently reporter activity in generated cotransformants will measured. <i>Starting from 85 euro for a single assay.</i> ^[2]

[1] <https://cellatechnologies.com/tech/yeast-two-hybrid-system> [2] See also Table 2.

Table 2. Yeast Two-Hybrid System Plasmids and Assays. **(a)** Bait / prey plasmid construction. **(b)** Bait–prey binding / prey-dependency / bait-dependency / bait autoactivation and toxicity assay. This table relates to Steps 1a, 1b, 2a, 2b and 2c of Table 1. For details, please contact us.

(a)	#^[1]	Price^[2]	#^[1]	Price^[2]	#^[1]	Price^[2]	(b)	#^[3]	Price per Assay^[3]
	1	€170	6	€145	11	€120		1	€85
	2	€165	7	€140	12	€115		2	€75
	3	€160	8	€135	13	€110		3	€65
	4	€155	9	€130	14	€105		4	€55
	5	€150	10	€125	15+	€100		5+	€45

[1] The number of plasmids (with identical backbone) of which the to be subcloned insert can be generated using the same template DNA / source vector determines the price per plasmid. [2] The price per custom-made plasmid; these prices apply if the protein is up to 100 amino acid residues in size; for a protein of 101-200 amino acid residues in size an additional €30 is charged, for a protein of 201-300 amino acid residues in size an additional €60 is charged, and so on (additional charges may apply if the DNA sequence of the template is not yet fully known); to the extent applicable, bait / prey protein-encoding template DNA has to be provided to us; in case you don't have that DNA available, obtaining it through DNA synthesis might be an option, please contact us to learn more. [3] The number of assays to be conducted in the project determines the (starting from) price per assay. The actual price per assay may be higher than indicated, this depends on several factors, including the way the activity of the reporter gene(s) has (have) to be measured.

Notes

- ◆ Our services can be purchased at Scientist.com, which is a platform where research services can be sold and purchased very conveniently and securely. For quotes (possible in EUR, USD, GBP, CHF and SEK) please contact us via our Scientist.com profile (<https://app.scientist.com/providers/cella-technologies>), or contact us directly. A Scientist.com transaction fee surcharge applies, taxes and or other charges may apply.
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- ◆ To the extent applicable, on request we will review project-related text and figures of your manuscript-in-progress; we will be glad to do this (free of additional charges).

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