Cella Technologies

yeast two-hybrid system services

Yeast Two-Hybrid Screening Service

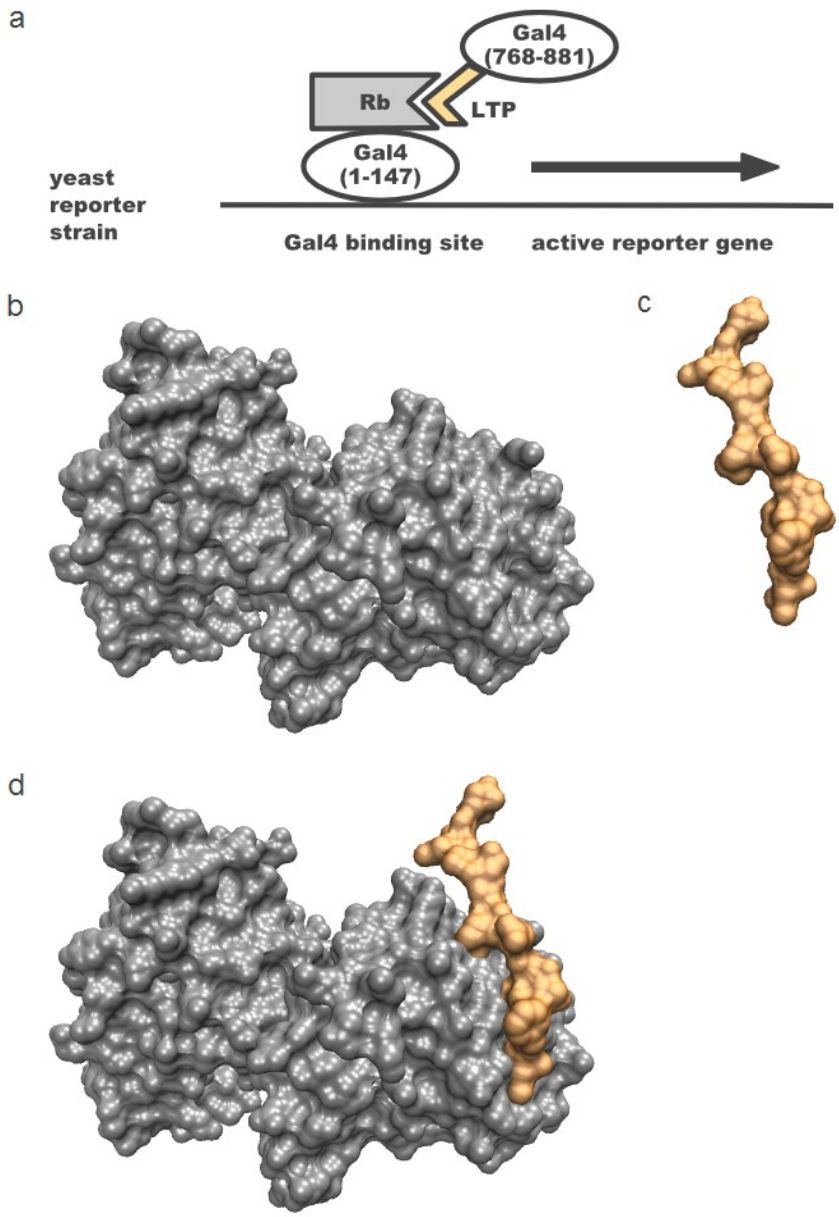

Figure 1. Protein–protein interaction between retinoblastoma protein and large T antigen protein. **(a)** Binding between Rb (the 'bait' protein) and LTP (the 'prey' protein) results in an active reporter gene in the Gal4 yeast two-hybrid (Y2H) system / assay. Data acquired and diagram adapted from NAR 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 Identification by Yeast Two-Hybrid Screening

The yeast two-hybrid (Y2H) system (Figure 1) can be used to screen for (novel) protein–protein interactions. Physical binding between a protein of interest (the bait) and proteins of a library (the preys) can be detected. When using sophisticated strategies / systems, relatively easy millions of 'library prey's' can be tested to see whether they bind to the bait protein of interest in the Y2H system.

In a yeast two-hybrid screening project a large number of cotransformants (yeast cells containing bait and cDNA library plasmids) are generated (optionally using yeast mating), which are subsequently grown on appropriate agar plates. In many of these yeast cells potential protein–protein interaction (PPI) between a known 'bait' and an unknown 'prey' (encoded by a cDNA from a library) is tested. On agar plates a single yeast cell can grow out to a colony – consisting of a large number of clones of the original cell. The dots on the illustrated agar plate (Figure 2) represent such colonies. The activation of a specific Gal4 reporter gene in yeast cells (through bait–prey binding, for example, see binding between Rb and LTP in Figure 1) leads to blue coloring of the corresponding colony. Thus in the three blue colored colonies (Figure 2) potentially physical binding between bait and prey occurs, and in most or all of the white colored colonies bait–prey binding (with strong enough affinity) does not occur. Next steps could be (1) isolation of (prey encoding) plasmid DNA from cells of the three blue colored colonies; (2) use this DNA to perform control assays; and (3) identify the prey's through sequencing of the corresponding DNA. See Table 1 to learn about our yeast two-hybrid screening service (Cat. No. C100).

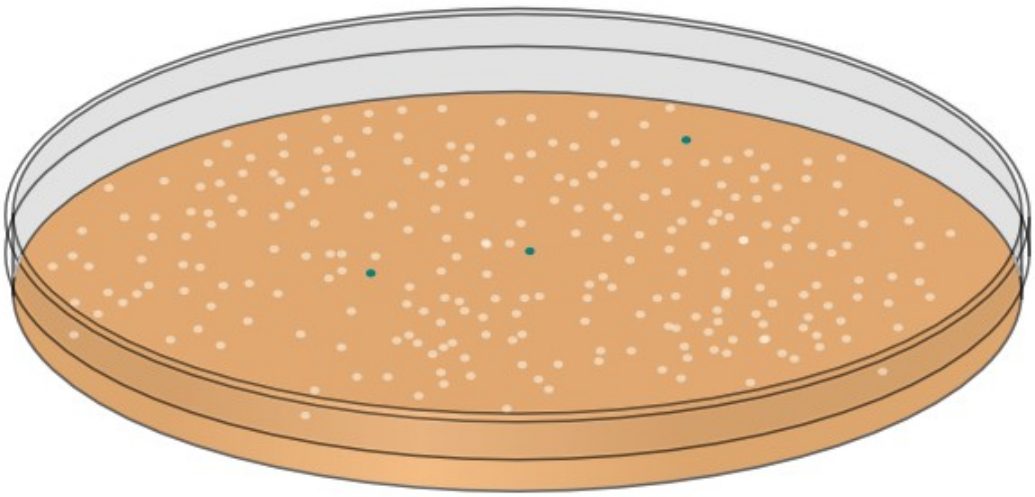

Figure 2. An illustration of a yeast two-hybrid (Y2H) screen. Two-hybrid technology can be used to identify novel protein–protein interactions. Note: for references and further explanation of this Figure and the Y2H system please refer to our yeast two-hybrid system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

Table 1. Yeast Two-Hybrid Screening Service (Cat. No. C100). Steps 5-10 depend on obtained results in Step 4 and are all optional. Indicated prices apply to a single sub-project within a single yeast two-hybrid screening project. Sub-projects from different steps / Y2H screening projects cannot be combined, unless agreed otherwise.

Step 1	**Bait Plasmid Construction** – DNA that encodes the bait (protein or peptide of interest) will be appropriately cloned as insert into empty bait vector. *Starting from 170 euro for a single construct.*[1]
Step 2	**Bait Autoactivation, Bait Toxicity and Prey-Dependency Tests** – Several tests will be performed to investigate whether or not the bait protein of interest is appropriate to be used as bait in the yeast two-hybrid system. *Starting from 85 euro for a single assay.*[1]
Step 3	**Yeast Two-Hybrid Library** – a yeast two-hybrid cDNA library contains a large number of prey plasmids with varying (prey encoding) inserts. The cDNA libraries that can be chosen from are Arabidopsis Universal (Normalized), Drosophila Universal (Normalized), HeLa S3 (Normalized), Human Brain (Normalized) / Heart / Liver / Ovary / Testis / Universal (Normalized), Mouse Brain (Normalized) / Embryo 11-day / Embryo 17-day / Embryonic Stem Cell / Universal (Normalized). Custom libraries are also possible. *For further information and prices, please refer to Table 3.*
Step 4	**Yeast Two-Hybrid Library Screening** – binding between the bait and a prey can result in activation of reporter genes (see also Figure 1), which will be visible on the screening plates (see also Figure 2). *For screening options and prices, please refer to Table 3.*
Step 5	**High Stringency Screening of Potential Interactors** – positive colonies obtained in Step 4 are streaked on higher stringency screening plates. *40 euro for every up to 20 colonies (clones).*
Step 6a	**Yeast Colony PCR Analysis I** – prey vector insert amplification and analysis of PCR products by gel electrophoresis. *180 euro for the first up to 12 colonies, and 120 euro for every additional up to 12 colonies.*
Step 6b	**Yeast Colony PCR Analysis II** – restriction enzyme analysis of PCR products (Step 6a) to see whether or not similar sized bands (Step 6a) contain the same insert. *180 euro for every up to 12 PCR products / clones.*
Step 6c	**Yeast Colony PCR Analysis III** – DNA sequence analysis (see also Step 10) of (prey-encoding) inserts (partly or completely) / PCR products (Step 6a), where applicable, insert-specific sequence primers will be made and used. *75 euro for every up to 2 sequence reactions.*
Step 6d	**Yeast Colony PCR Analysis IV** – assaying bait–prey binding and or bait-dependency using linear empty prey vector, PCR product (Step 6a) and gap repair cloning. *Starting from 85 euro for a single assay.*[1]
Step 6e	**Yeast Colony PCR Analysis V** – subcloning a specific fragment of the PCR product (Step 6a) into empty prey vector and subsequently assaying bait–prey binding and or bait-dependency. *Starting from 170 euro for a single construct*[1] *and starting from 85 euro for a single assay.*[1]
Step 7	**Segregation of Library Plasmid in Yeast** – it can happen that a single yeast cell contains prey plasmids with varying inserts, and through this segregation step the chance on a false negative in Step 9 decreases. *180 euro for the first up to 12 colonies, and 15 euro for every additional clone / colony.*

Table 1 (continued).

Step 8a	**Isolation of Library Plasmid DNA** – a small amount of plasmid DNA will be isolated from a yeast clone and used to transform E. coli cells. Subsequently, library plasmid DNA will be isolated from a cultured E. coli transformant. *750 euro for the first up to 6 isolation's, and 250 euro for every additional up to 2 isolation's.*
Step 8b	**Analysis of Isolated Library Plasmid DNA** – Restriction enzyme analysis of isolated library plasmid DNA (Step 8a). *180 euro for the analysis of every up to 12 plasmid isolation's.*
Step 9	**Bait–prey binding / bait-dependency assay** – assaying bait–prey binding and or bait-dependency using isolated library plasmid DNA (Step 8a). These assays show whether or not the prey protein that is expressed by the isolated prey plasmid binds to the bait protein (see also Table 1 on our PPI Testing web page[2]). *Starting from 85 euro for a single assay.*[1]
Step 10	**DNA Sequence Analysis** – per sequence reaction an as large as possible part of the DNA sequence of the (prey encoding) insert of an isolated library plasmid (Step 8a) will be determined and analyzed, where applicable, insert-specific sequence primers will be made and used. *75 euro for every up to 2 sequence reactions.*

[1] See also Table 2. [2] https://cellatechnologies.com/index.php/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

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 1, 2, 6d, 6e and 9 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 DNA template / 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.

Table 3 Yeast Two-Hybrid System cDNA Libraries. **(a-c)** cDNA library and screening options. This table relates to Steps 3 and 4 of Table 1. For details, please contact us.

(a)	**cDNA Library[1]**	**First Screen**	**Second Screen[2]**
	Human Universal (Normalized)	€1934	€1200
	Mouse Universal (Normalized)	€1934	€1200
(b)	**cDNA Library[1]**	**First Screen**	**Second to Fifth Screen[2]**
	Arabidopsis Universal (Normalized)	€2412	€1000 per screen
	Drosophila Universal (Normalized)	€2412	€1000 per screen
	HeLa S3 (Normalized)	€2412	€1000 per screen
	Human Brain (Normalized)	€2412	€1000 per screen
	Human Heart	€2412	€1000 per screen
	Human Liver	€2549	€1000 per screen
	Human Ovary	€2372	€1000 per screen
	Human Testis	€2412	€1000 per screen
	Human Universal (Normalized)	€2412	€1000 per screen
	Mouse Brain (Normalized)	€2412	€1000 per screen
	Mouse Embryo 11-day	€2412	€1000 per screen
	Mouse Embryo 17-day	€2412	€1000 per screen
	Mouse Embryonic Stem Cell	€2468	€1000 per screen
	Mouse Universal (Normalized)	€2412	€1000 per screen
(c)	**cDNA Library**	**First Screen**	**Second and Up Screen[2,3]**
	Tissue of Choice[3]	€1000[3]	€1000 per screen

[1] Costs for the commercial ready-to-use cDNA library (enough for two or five screens) are charged in the first screen. Storage of leftover library material is guaranteed until one year after the start of the first screen screen, after that year, storage takes place on a best effort basis for as long as needed. [2] The price for an additional screening project (optional) with leftover library material and, for example, a fragment of the bait protein, will be as indicated (this price is exclusive of, to the extent applicable, costs for another bait plasmid and applies at least until one year after the start of the first screen). [3] Custom libraries can be obtained through our cDNA Library Construction Service (Cat. No. C110), costs per library is 3500 euro, this amount includes quality control and storage. A sample of your RNA of interest has to be supplied to us. By estimate, enough library will be produced to perform 50 to 100 screens. Storage of the library is guaranteed until one year after production, after that year, storage takes place on a best effort basis for as long as needed.

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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