Cella Technologies

yeast two-hybrid system services

Peptide Protein–Protein Interaction Inhibitor Development

Protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) that is involved in PPI between (fictional) Protein 1 (PRTN1) and a protein that interacts with PRTN1 may be prevented specifically by an appropriate short linear peptide. Such short linear peptides / peptide protein–protein interaction inhibitors are highly specific (Figure 1) and have many applications in research—using these tools can lead to new insights.

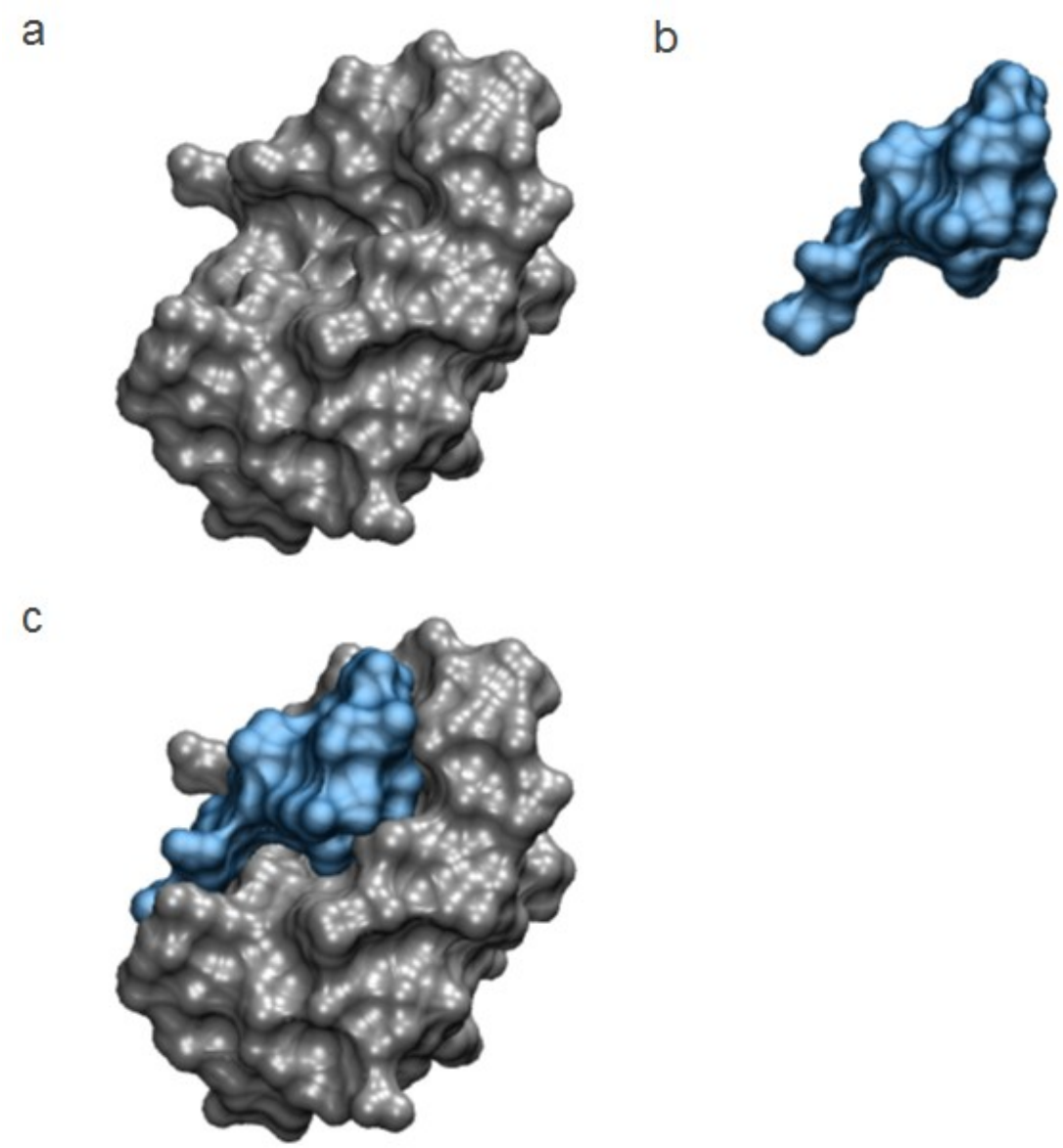

Figure 1. Protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) prevented specifically. **(a)** *Target*—a representation of the tertiary structure of a PPID of MDM2 protein that has strong protein–protein interaction activity towards a PPID of p53 protein (freely interpreted from PDB ID: 1YCR). **(b)** *Tool*—a PPID-fragment (short linear peptide) of p53 protein that has strong PPI activity towards a PPID of MDM2 protein (freely interpreted from PDB ID: 1YCR). **(c)** *Tool bound to target*—PPI function of a specific PPID can be prevented by an appropriate short linear peptide (freely interpreted from PDB ID: 1YCR). Note: the representations of the protein structures were made with VMD (http://www.ks.uiuc.edu/Research/vmd/).

28 Peptide PRTN1–PRTN2 Interaction Inhibitor Candidates

One or more of the twenty-eight peptides that are indicated in Table 6 below may be able to prevent protein–protein interaction (PPI) function of a protein–protein interaction domain (PPID) of PRTN1 that has PPI activity towards (fictional) Protein 2 (PRTN2).

Using the yeast two-hybrid (Y2H) system (for references and explanation, see our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system) it is possible to demonstrate binding between Rb protein and LTP (a linear peptide of 13 amino acid residues) experimentally (Figure 2a (related tertiary structures are shown in Figures 2b-d))—this showed that the Y2H system / assay can be used to map short protein–protein interaction domains (PPIDs) experimentally.

A hypothetical example: in Table 1 to Table 5 hypothetical PPI between PRTN1(1-500) and PRTN2(1-600) (or a part thereof) is used to demonstrate a straightforward strategy to identify the primary structure of a segment of PRTN2 that contains a protein–protein interaction domain that is involved in PRTN1–PRTN2 interaction; from that segment short linear peptides can be derived (Table 6) that potentially can prevent protein–protein interaction between PRTN1 and PRTN2.

If yeast / bacterial two-hybrid system assay A1 turns out positive and control assays A2 and A3 turn out negative (Project 1) (Table 1), then hypothetical PRTN1(1-500)–PRTN2(1-600) interaction is confirmed. In Project 2 (Table 2) three fragments of PRTN2(1-600) are used as prey and, in this example, positive assays A4 and A5 (Table 2) suggest that PRTN2(151-300) contains a PPID that is involved in binding between PRTN1 and PRTN2—it is less likely that PRTN2(1-150) and PRTN2(301-450) both contain such a PPID. Segments of this putative positive region can be tested in similar assays and by repeating this procedure several times, a short region of PRTN2 that seems to be able to significantly bind to PRTN1 can be systematically identified (Table 3 to Table 5). In Project 6, based on the primary structure of PRTN2(226-244) (Table 5), twenty-eight inhibitor tool candidates are generated (Table 6).

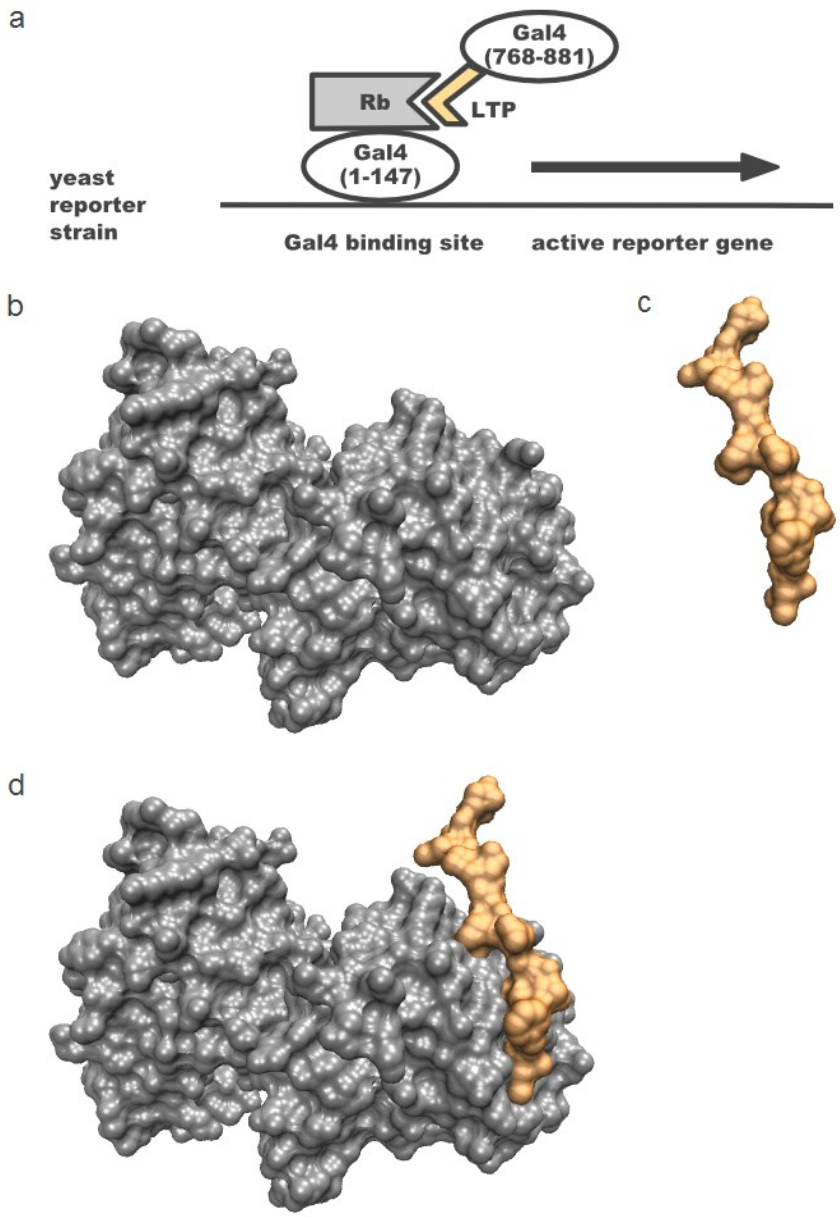

Figure 2. Protein–protein interaction between retinoblastoma protein and large T antigen protein. **(a)** Binding between Rb (the 'bait' protein) and LTP (the 'prey' protein) results in an active reporter gene in the Gal4 yeast two-hybrid (Y2H) system / assay. Data acquired and diagram adapted from NAR 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.

Table 1. Project 1. Testing of protein–protein interaction between PRTN1(1-500) and PRTN2(1-600) in the yeast / bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C4 expresses System Domain 2 (SD2) fused to PRTN2(1-600) (accession.version N/A). **(b)** A1 is a bait–prey interaction assay; A2 is a prey-dependency control assay; A3 is a bait-dependency control assay.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C2	pSD2	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	This Project	290 EUR	
	C4	pSD2-PRTN2(1-600)	This project	320 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A1	Cell + C3 & C4	SD1-PRTN1(1-500) & SD2-PRTN2(1-600)	65 EUR	Active
	A2	Cell + C3 & C2	SD1-PRTN1(1-500) & SD2	65 EUR	Inactive
	A3	Cell + C1 & C4	SD1 & SD2-PRTN2(1-600)	65 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our PPI Testing web page at https://cellatechnologies.com/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast / bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active / inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active / inactive.

Table 2. Project 2. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(1-600) in the yeast / bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C5/C6/C7 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle 50% / C-terminal 50% of PRTN2(1-600) (accession.version N/A). **(b)** A4 to A6 are bait–prey interaction assays; A7 to A9 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C5	pSD2-PRTN2(1-300)	This project	220 EUR	
	C6	pSD2-PRTN2(151-450)	This project	220 EUR	
	C7	pSD2-PRTN2(301-600)	This project	220 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A4	Cell + C3 & C5	SD1-PRTN1(1-500) & SD2-PRTN2(1-300)	45 EUR	Active
	A5	Cell + C3 & C6	SD1-PRTN1(1-500) & SD2-PRTN2(151-450)	45 EUR	Active
	A6	Cell + C3 & C7	SD1-PRTN1(1-500) & SD2-PRTN2(301-600)	45 EUR	Inactive
	A7	Cell + C1 & C5	SD1 & SD2-PRTN2(1-300)	45 EUR	Inactive
	A8	Cell + C1 & C6	SD1 & SD2-PRTN2(151-450)	45 EUR	Inactive
	A9	Cell + C1 & C7	SD1 & SD2-PRTN2(301-600)	45 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our PPI Testing web page at https://cellatechnologies.com/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast / bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active / inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active / inactive.

Table 3. Project 3. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(151-300) in the yeast / bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C8/C9/C10 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle ≈50,67% / C-terminal 50% of PRTN2(151-300) (accession.version N/A). **(b)** A10 to A12 are bait–prey interaction assays; A13 to A15 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C8	pSD2-PRTN2(151-225)	This project	160 EUR	
	C9	pSD2-PRTN2(188-263)	This project	160 EUR	
	C10	pSD2-PRTN2(226-300)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A10	Cell + C3 & C8	SD1-PRTN1(1-500) & SD2-PRTN2(151-225)	45 EUR	Inactive
	A11	Cell + C3 & C9	SD1-PRTN1(1-500) & SD2-PRTN2(188-263)	45 EUR	Active
	A12	Cell + C3 & C10	SD1-PRTN1(1-500) & SD2-PRTN2(226-300)	45 EUR	Inactive
	A13	Cell + C1 & C8	SD1 & SD2-PRTN2(151-225)	45 EUR	Inactive
	A14	Cell + C1 & C9	SD1 & SD2-PRTN2(188-263)	45 EUR	Inactive
	A15	Cell + C1 & C10	SD1 & SD2-PRTN2(226-300)	45 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our PPI Testing web page at https://cellatechnologies.com/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast / bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active / inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active / inactive.

Table 4. Project 4. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(188-263) in the yeast / bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C11/C12/C13 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle 50% / C-terminal 50% of PRTN2(188-263) (accession.version N/A). **(b)** A16 to A18 are bait–prey interaction assays; A19 to A21 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C11	pSD2-PRTN2(188-225)	This project	160 EUR	
	C12	pSD2-PRTN2(207-244)	This project	160 EUR	
	C13	pSD2-PRTN2(226-263)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A16	Cell + C3 & C11	SD1-PRTN1(1-500) & SD2-PRTN2(188-225)	45 EUR	Inactive
	A17	Cell + C3 & C12	SD1-PRTN1(1-500) & SD2-PRTN2(207-244)	45 EUR	Active
	A18	Cell + C3 & C13	SD1-PRTN1(1-500) & SD2-PRTN2(226-263)	45 EUR	Inactive
	A19	Cell + C1 & C11	SD1 & SD2-PRTN2(188-225)	45 EUR	Inactive
	A20	Cell + C1 & C12	SD1 & SD2-PRTN2(207-244)	45 EUR	Inactive
	A21	Cell + C1 & C13	SD1 & SD2-PRTN2(226-263)	45 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our PPI Testing web page at https://cellatechnologies.com/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast / bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active / inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active / inactive.

Table 5. Project 5. Testing of protein–protein interaction between PRTN1(1-500) and fragments of PRTN2(207-244) in the yeast / bacterial two-hybrid system. **(a)** C3 expresses System Domain 1 (SD1) fused to PRTN1(1-500) (accession.version N/A); C14/C15/C16 expresses System Domain 2 (SD2) fused to the N-terminal 50% / middle ≈52,63% / C-terminal 50% of PRTN2(207-244) (accession.version N/A). **(b)** A22 to A24 are bait–prey interaction assays; A25 to A27 are bait-dependency control assays.

(a)	**Construct**	**Plasmid**	**Source**[1]	**Price**[2]	
	C1	pSD1	Empty vector	N/A	
	C3	pSD1-PRTN1(1-500)	Project 1 (Table 1A)	N/A	
	C14	pSD2-PRTN2(207-225)	This project	160 EUR	
	C15	pSD2-PRTN2(216-235)	This project	160 EUR	
	C16	pSD2-PRTN2(226-244)	This project	160 EUR	
(b)	**Assay**[3]	**Cotransformant**[4]	**Expressed Constitutively**[5]	**Price**[2]	**Reporter**[6]
	A22	Cell + C3 & C14	SD1-PRTN1(1-500) & SD2-PRTN2(207-225)	45 EUR	Inactive
	A23	Cell + C3 & C15	SD1-PRTN1(1-500) & SD2-PRTN2(216-235)	45 EUR	Inactive
	A24	Cell + C3 & C16	SD1-PRTN1(1-500) & SD2-PRTN2(226-244)	45 EUR	Active
	A25	Cell + C1 & C14	SD1 & SD2-PRTN2(207-225)	45 EUR	Inactive
	A26	Cell + C1 & C15	SD1 & SD2-PRTN2(216-235)	45 EUR	Inactive
	A27	Cell + C1 & C16	SD1 & SD2-PRTN2(226-244)	45 EUR	Inactive

(1) Compatible hybrid-encoding plasmids that were generated under a previous agreement between Client and Supplier can be used in the assays.

(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our PPI Testing web page at https://cellatechnologies.com/services/protein-protein-interaction-testing-in-the-yeast-two-hybrid-assay.

(3) The assays are explained on our Yeast two-hybrid (Y2H) system web page at https://cellatechnologies.com/tech/yeast-two-hybrid-system.

(4) A cell of a yeast / bacterial two-hybrid system reporter strain cotransformed with the two indicated constructs.

(5) Note: in hybrids of most two-hybrid systems SD1 and SD2 are located N-terminally (with regard to the bait/prey), however, placing them C-terminally may be possible.

(6) For the purpose of this example, hypothetical results are shown; active / inactive means that the observed phenotype suggests that a specific reporter in the cotransformant is active / inactive.

Table 6. Project 6. Construction of plasmids that encode GFP-linker-peptide. All possible 13- to 19-residue peptides that can be derived from PRTN2(226-244) (accession.version N/A) are covered.

Plasmid	Fusion Protein	Potential Effect[1]	Price[2]
1	GFP-linker-PRTN2(226-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
2	GFP-linker-PRTN2(226-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
3	GFP-linker-PRTN2(226-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
4	GFP-linker-PRTN2(226-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
5	GFP-linker-PRTN2(226-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
6	GFP-linker-PRTN2(226-239)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
7	GFP-linker-PRTN2(226-238)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
8	GFP-linker-PRTN2(227-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
9	GFP-linker-PRTN2(227-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
10	GFP-linker-PRTN2(227-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
11	GFP-linker-PRTN2(227-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
12	GFP-linker-PRTN2(227-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
13	GFP-linker-PRTN2(227-239)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
14	GFP-linker-PRTN2(228-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
15	GFP-linker-PRTN2(228-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
16	GFP-linker-PRTN2(228-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
17	GFP-linker-PRTN2(228-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
18	GFP-linker-PRTN2(228-240)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
19	GFP-linker-PRTN2(229-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
20	GFP-linker-PRTN2(229-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
21	GFP-linker-PRTN2(229-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
22	GFP-linker-PRTN2(229-241)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
23	GFP-linker-PRTN2(230-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR

Table 6. (continued)

24	GFP-linker-PRTN2(230-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
25	GFP-linker-PRTN2(230-242)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
26	GFP-linker-PRTN2(231-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
27	GFP-linker-PRTN2(231-243)	Loss of PPI function of targeted PPID of PRTN1	100 EUR
28	GFP-linker-PRTN2(232-244)	Loss of PPI function of targeted PPID of PRTN1	100 EUR

(1) If the fusion protein is expressed in cells, then the PRTN2-part of the fusion protein may bind to the targeted PRTN2-binding protein–protein interaction domain (PPID) of endogenous PRTN1 and cause loss of protein–protein interaction (PPI) function of the targeted PPID. Note: with these plasmids it may also be possible to confirm this peptide–protein interaction by co-immunoprecipitation analysis using anti-GFP antibodies and antibodies against PRTN1.
(2) Mentioned prices are subject to change and excluding possible additional charges and applicable taxes. For further information, please refer to our Plasmid Construction Service web page at https://cellatechnologies.com/services/plasmid-construction-service.

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.

♦ Our services can be purchased on Science Exchange, which is a platform where research services can be sold and purchased very conveniently and securely. For quotes (possible in EUR, USD, GBP, JPY, CAD, AUD and CHF) please contact us via our Science Exchange profile (https://www.scienceexchange.com/labs/cella-technologies), or contact us directly. A Science Exchange fee surcharge applies, taxes and or other charges may apply.

♦ Proteins that bind to (and work with) your protein of interest (to facilitate a specific cellular process) may be identified through a yeast two-hybrid screen, see also our Y2H screening service web page at https://cellatechnologies.com/services/yeast-two-hybrid-screening.

♦ 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).

Please note, some PDF-readers have difficulties with URLs, if clicking on an underlined URL results in an error message in your web browser, then please complete the URL in the URL bar of your web browser through copy-paste or by typing.

Cella Technologies
Molengraaffsingel 12-14
2629 JD Delft
The Netherlands

www.cellatechnologies.com
contact@cellatechnologies.com
Phone Number +31 85 876 89 56

VAT Number NL 853249349B01

Cella Technologies (statutory name Cella Biotech B.V.), is registered under number 58945539 in the Trade Register of the Dutch Chamber of Commerce.

Document Version 20190429