REVIEW

Commonly used tag combinations for tandem affinity purification

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TAP (tandem affinity purification) allows rapid and clean isolation of a tagged protein along with its interacting partners from cell lysates. Initially developed in yeast, the TAP method has subsequently been adapted to other cells and organisms. In combination with MS analysis, this method has become an indispensable tool for systematic identification of target-associated protein complexes. The key feature of TAP is the use of a dual-affinity tag, which is fused to the protein of interest. The original TAP tag consisted of two IgG-binding units of Protein A of *Staphylococcus aureus* and the calmodulin-binding peptide. As the technique has been widely exploited, a number of alternative TAP tags based on other affinity handles have been developed. The present review gives an overview of the various tag combinations for TAP with a highlight on those alternatives that result in improved yields or unique features. The information provided should assist in the selection and development of TAP tags for specific applications.

TAP (tandem affinity purification)

TAP is a methodology developed to purify protein complexes under native conditions [1,2]. It involves fusion of the TAP tag to the target protein, which is introduced into the host cell or organism as a bait to trap endogenous interacting partners. The TAP tag, which is composed of two affinity modules, allows the target protein along with its binding partners to be isolated in two consecutive purification steps. This sequential purification ensures highly specific isolation with low background, which simplifies the subsequent identification and validation of proteins co-purified with the target. In addition, the TAP tag allows the protein complex to be eluted under mild conditions, which favour the maintenance of complex integrity and the native conformations of protein components, facilitating downstream composition and functional analyses. TAP has proved to be superior to the yeast two-hybrid approach because of its higher sensitivities, lower error rates and the ability to disclose multi-component interactions. Although the TAP method was initially developed in yeast, it has been successfully adapted to various cells and organisms [3–6]. TAP followed by MS analysis has become a standard approach for identification and characterization of protein complexes [7], and has allowed the systematic study of protein assemblies on a genome-wide scale [8].

As its name implies, TAP is a dual-affinity approach, a common practice that was used up to 10 years before the development of TAP. Although the original dual-affinity system employed sequential purification as in TAP, it was developed for a different purpose [9]. Heterologous proteins expressed in a bacterial host may suffer proteolytic degradation and incomplete translation, resulting in non-full-length products. Separation of the intact protein from the undesired fragments can be cumbersome and time-consuming. To overcome this, Hammarberg et al. [10] developed the dual-affinity approach. Instead of using a single-affinity tag, they fused two different tags to the target protein, one at each end. This design allowed the target protein to be purified 'from both ends' and ensured that only the full-length protein was recovered after two successive affinity chromatography steps. The dual-affinity approach has proved useful for expression and purification of proteins that are susceptible to proteolysis [11–14]. In addition to flanking dual-affinity tags, an N-terminal multipartite tag consisting of multiple affinity domains has been described to improve the flexibility of binding and elution, and to allow a multitude of purification and detection assays [15]. In particular, Cocca et al. [16] developed a tandem affinity tag for sequential purification of recombinant antibodies

Key words: affinity tag, dual-affinity, protein complex, tag combination, tandem affinity purification (TAP).

Abbreviations used: CBD, chitin-binding domain; CBP, calmodulin-binding peptide; DTT, dithiothreitol; GFP, green fluorescent protein; GS, Protein G–SBP; GST, glutathione transferase; HA, haemagglutinin; HB, histidine and biotin; IMAC, immobilized metal affinity chromatography; MBP, maltose-binding protein; Ni-NTA, Ni^{2+}-nitrilotriacetate; ProtA, Protein A of *Staphylococcus aureus*; ProtC, Protein C epitope; SBP, streptavidin-binding peptide; TAP, tandem affinity purification; TEV, tobacco etch virus.

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Table 1 Some commonly used affinity tags for protein purification

Affinity tag	Sequence or size (kDa)	Affinity matrix	Elution strategy
Z domain*	VDNKFNKEQQNAFYEILHLPNLNEEQRNAFI QSLKDDPSQSANLLAEAKKLNDAQAPK	IgG	IgG or low pH
CBP	KRRWKKNFIAVSAANRFKKISSSGAL	Calmodulin	2 mM EGTA
His tag	HHHHHH	Ni^{2+}, Co^{2+}	150–500 mM imidazole
FLAG	DYKDDDDK	Antibody	FLAG peptide or low pH
HA	YPYDVPDYA	Antibody	HA peptide or low pH
Myc	EQKLISEEDL	Antibody	Low pH
V5	GKPIPNPLLGLDST	Antibody	V5 peptide or low pH
Strep II	WSHPQFEK	StrepTactin	2.5–5 mM desthiobiotin
SBP	MDEKTTGWRGGHVVEGLAGELEQLRARLEH HPQGQREP	Streptavidin	2 mM biotin
S-peptide	KETAAAKFERQHMDS	S-protein	Denaturant or low pH
CBD	TNPGVSAWQVNTAYTAGQLVTYNGKTYKCL QPHTSLAGWEPSNVPALWQLQ	Chitin	Thiol reagents or pH and temperature shift (when fused with intein)
GST	26	Glutathione	10 mM reduced glutathione
MBP	40	Maltose	10 mM maltose

*Z domain is a synthetic Fc-region-binding domain derived from the B domain of ProtA.

expressed in *Escherichia coli* and showed that the second purification step removed most of the contaminants that had not been removed by a single-step purification. The paper published by Pathak and Imperiali in 1997 [17] is probably the first report to use dual-affinity purification for the isolation of a protein complex. Using FLAG–His-tagged oligosaccharyl transferase Nlt1p subunit as the bait protein, the authors purified the other four subunits of the enzyme from yeast. However, TAP, as a generic method for protein-complex characterization and proteome exploration, was developed by Séraphin and co-workers [1]. In their pioneering work, they constructed a fusion cassette containing two IgG-binding units (double Z domain) of ProtA (Protein A of *Staphylococcus aureus*) and the CBP (calmodulin-binding peptide), which they named the TAP tag, and showed that the TAP tag allowed rapid purification of target-associated protein complexes under native conditions [1]. As the TAP technique has been widely exploited, a number of alternative TAP tags have been developed and proved effective in isolating protein complexes. The present review gives an overview of various tag combinations that have been used for TAP and will start with a brief review of some of the most commonly used affinity tags for protein purification.

Commonly used affinity tags

Affinity tagging enables rapid purification of diverse proteins using generalized protocols. The first use of an affinity tag for protein purification was reported by Uhlén et al. in 1983 [18]. In that original invention, the affinity tag, which was based on ProtA, allowed the fused protein to be purified by a one-step IgG-affinity chromatography. Since then, a variety of tags employing different modes of affinity interactions have been developed and affinity-based separation methods have proved to be the most efficient approaches for capturing recombinant proteins. A selection of some commonly used affinity tags, together with their respective affinity matrices and elution strategies, are listed in Table 1. Based on their sizes, affinity tags can be divided into small peptides and large peptides/proteins. Tags such as FLAG, HA (haemagglutinin), Myc and V5 are examples of short peptide tags. These peptide epitopes are recognized by tag-specific antibodies. For all commonly used epitope tags, antibody resins for immunoaffinity purification and immunodetection of the tagged proteins are commercially available. An advantage of small peptide tags is that they are less likely to disrupt the structure or interfere with the function of the target protein. GST (glutathione transferase) and MBP (maltose-binding protein) are examples of large protein tags, and they have a dual role. In addition to serving as affinity tags, they serve as fusion partners that enhance the solubility and/or promote the proper folding of the tagged proteins [19]. Affinity tags differ in specificity, solubility and conditions for binding and elution. Moreover, different tags vary significantly in their effectiveness of purification and associated costs [20]. As many have pointed out, none of these tags is ideal from every standpoint. A particular tag can be a good or bad choice depending on the application. Properties of the target protein itself (e.g. stability and solubility), production scale and desired purity are the major factors that need to be considered when choosing an affinity tag for a particular project. Usually, the most appropriate tag can only be determined on an empirical basis. Among the commonly used tags, GST and MBP are rarely employed for TAP because of their large size. In the following sections, several small tags that have been used to construct various TAP tags are briefly introduced. Comprehensive reviews of individual affinity tags can be found elsewhere [21–23].

ProtA and Z domain

ProtA is a bacterial protein that specifically binds to the Fc portion of immunoglobulins [24]. Based on the IgG-binding property of ProtA, Uhlén et al. [18] developed the first gene-fusion approach for affinity purification of recombinant proteins. The Z domain is a synthetic IgG-binding fragment based on the B domain of ProtA [25]. It has a molecular mass of 7 kDa, which is about one-fifth the size of ProtA. Since its development, this Z domain, in single or double form, has replaced the full-length protein as an affinity handle in most cases [1,26,27]. A disadvantage of the ProtA-based system is that elution of the fusion requires denaturing conditions at low pH [28].

CBP

The CBP is a 26-amino-acid peptide derived from the C-terminus of rabbit skeletal-muscle myosin light-chain kinase [29]. It binds to calmodulin in a calcium-dependent manner, and hence the addition of chelating agents, such as EGTA, allows elution of the tagged protein. Both binding and elution can be carried out under moderate conditions. The CBP was used in conjunction with the double Z domain to construct the original TAP tag [1]. However, when used in mammalian cells, its specificity might be compromised due to the existence of many endogenous calmodulin-binding proteins in these cells.

His tag

The His tag is the most commonly used affinity tag for protein purification. It binds to metal ions, allowing the tagged protein to be purified by IMAC (immobilized metal affinity chromatography). Commonly used IMAC resins [e.g. Ni-NTA (Ni^{2+}-nitrilotriacetate) agarose from Qiagen, Valencia, CA, U.S.A., and TALON® resin from Clontech Laboratories, Mountain View, CA, U.S.A.] have a high binding capacity (i.e. $>$5 mg of His-tagged protein per ml of resin). In addition, these resins are relatively inexpensive and can be regenerated. The His tag has several advantages. First, its small size makes it less likely to interfere with the structure or activity of the target protein. Secondly, it works under both native and denaturing conditions. Thirdly, elution can be accomplished under mild conditions by adding imidazole as a competitor. Non-specific binding of endogenous histidine-containing proteins can be reduced by washing with a low concentration of imidazole (e.g. 5–10 mM). It is worthwhile to point out that strong reducing and chelating agents can compromise IMAC purification, as they will reduce and strip the immobilized metal ions. According to the user's manual, Ni-NTA and TALON® resins allow 2-mercaptoethanol to be used at concentrations of up to 20 and 10 mM respectively. Whereas the Ni^{2+} resin can withstand up to 10 mM DTT (dithiothreitol), the TALON® cobalt resin is incompatible with DTT and DTE (dithioerythritol) at any concentration.

Epitope tags

Epitope tags are short peptides that can be recognized by readily available tag-specific antibodies. Thus tagged proteins can be purified using immunoaffinity chromatography on immobilized monoclonal antibodies. The FLAG tag was the first epitope tag [30]. Other epitope tags subsequently developed include the HA tag, the Myc tag and the V5 tag, to name a few. Similar to the His tag, these small epitope tags generally do not interfere with protein folding or function. Among them, Myc is mainly used for detection rather than purification [22]. Epitope-tagged proteins can be eluted with an excess of the corresponding peptide and several rounds of elution may be needed for maximum recovery. Alternatively, elution can be achieved using denaturing conditions (e.g. low pH). A disadvantage of antibody-based purification is its relatively high cost.

Strep II and SBP (streptavidin-binding peptide)

The Strep II tag is an 8-amino-acid peptide that is capable of binding to streptavidin [31]. It overcomes the restriction associated with the original Strep tag that required the tag to be on the C-terminus of the target protein. The Strep II tag can be fused to either end of the target or placed within the protein sequence. In addition, it binds with enhanced affinity to a streptavidin mutant termed StrepTactin [32]. Fusion proteins can be eluted with biotin derivatives under gentle conditions. StrepTactin–Sepharose (GE Healthcare, Pittsburgh, PA, U.S.A.) can be regenerated with 0.5 M NaOH. Avidin, which has no affinity toward the Strep II tag, can be added to the extraction buffer to suppress co-purification of biotinylated proteins [33]. Witte et al. [34] more recently showed that the Strep II tag allowed one-step isolation of target protein from plant material to the same degree of purity as achieved by TAP. The SBP tag, consisting of 38 residues, is selected using mRNA display technology [35]. Compared with Strep II, it has a substantially stronger binding to streptavidin. Despite its high affinity, SBP-tagged protein can be eluted under mild conditions (e.g. 2 mM biotin).

S-peptide

The S-peptide is 15 amino acids in length and exhibits high-affinity interaction with S-protein. Both the carrier and the ligand are derived from RNase A [36,37]. S-peptide is highly soluble and bears little net charge at neutral pH. It is unlikely for the peptide to interfere with the proper folding or function of the target protein. Elution of the S-peptide requires harsh conditions (e.g. 3 M guanidinium thiocyanate or 0.2 M potassium citrate buffer, pH 2) [37]. If purification

under native conditions is preferred, fusion proteins bound to the matrix can be released by enzymatic cleavage at an engineered recognition site between the tag and the target.

CBD (chitin-binding domain)

The CBD is derived from the C-terminal region of *Bacillus circulans* chitinase A1 and consists of 51 amino acids. It binds tightly to chitin substrate and elution requires harsh denaturing conditions. Consequently, the CBD is typically used in conjunction with self-splicing inteins [38]. More recently, a modified CBD that is capable of reverse binding under native conditions has been developed [39]. The CBD was one of the several candidate tags tested during the development of the original TAP tag [1].

Practical tag combinations for TAP

In theory, the TAP strategy can make use of any two affinity tags. In practice, however, certain factors may limit the number of possible combinations. For example, when sequential purification with the Ni^{2+} resin and the calmodulin resin was attempted, it was found that in either order the elution buffer for the first purification was incompatible with binding to the second resin [40]. Although buffer exchange can be performed to remedy this problem, it not only introduces an extra step but may also cause significant loss of yield. Nevertheless, a number of TAP tags, using different combinations of single-affinity tags, have been developed (Table 2). In these TAP-tag variants, His tag, FLAG, HA, SBP and Strep II tag are frequently used in place of ProtA and/or CBP (Figure 1). The original TAP tag and its various modified versions are briefly discussed in the following sections.

Original TAP tag

The classical TAP tag consists of two IgG-binding units of ProtA and the CBP, separated by a TEV (tobacco etch virus) protease cleavage site. ProtA and CBP were chosen because it was found that among all the tags originally tested, which also included the FLAG tag, the Strep tag, the His tag and the CBD, only these two tags allowed efficient recovery (approx. 80% for ProtA and 50% for CBP) of the fusion protein. Both C- and N-terminal tags have been described and in either case the CBP is adjacent to the protein of interest, whereas the ProtA module is located at the extreme terminus of the fusion. An antibody against the TAP tag for fusion-protein detection is commercially available (e.g. Open Biosystems, Huntsville, AL, U.S.A.). Sequential purification of the target protein and its interacting partners is carried out using an IgG matrix and a calmodulin resin respectively. It has been estimated that the overall yield of this approach is approx. 20–30% of the starting material [41]. When applied to yeast, the two purification steps can be performed in either order. However, using the streptavidin resin after the calmodulin resin will leave the final purified fraction contaminated with TEV protease [2]. Using the calmodulin resin first is not recommended for purification of protein complexes from mammalian cells owing to the existence of many endogenous calmodulin-binding proteins in these cells. The reason that a TEV cleavage site is needed is that ProtA can only be released from matrix-bound IgG under denaturing conditions and this site allows proteolytic release of the bound material under native conditions. TEV protease is highly specific and very few cellular proteins contain its recognition sequence. Therefore cleavage of the target or its associated proteins by this enzyme is expected to be rare. TAP tags harbouring two TEV cleavage sites have been shown to allow more efficient cleavage and thus higher recovery of the IgG-bound protein complexes [42].

Despite its strength, the original TAP tag has limitations and shortcomings. For example, when the standard TAP tag is applied to higher eukaryotes, the recovery of the fusion is found to be much lower than that in yeast (i.e. 1% in mammalian and insect cells compared with 20–30% in yeast) [43–47]. As mammalian and insect cells cannot grow to the same density as yeast, in many cases the protein yield is not sufficient for subsequent MS-based identification, which limits the use of the TAP method in such systems. In particular, the calmodulin affinity chromatography step has proved to be inefficient [46,47]. As mentioned previously, there are a considerable number of endogenous proteins in mammalian cells that bind to calmodulin with high affinity. In addition, endogenous calmodulin could bind to the CBP moiety and prevent its binding to the calmodulin resin [2,46]. Both instances interfere with binding of the target and probably contribute to poor recovery. Besides a low efficiency, the chelating agent (e.g. EGTA) used for CBP elution may affect certain cation-dependent proteins or interfere with downstream functional analysis. In addition to the disadvantages regarding the use of the CBP, the relatively large size of the original TAP tag ($\sim$20 kDa) is considered to be a drawback, as it increases the chance that the tag will interfere with the target protein. In fact, Gavin et al. [8] found that 18% of C-terminal tagged essential yeast proteins gave rise to non-viable strains, suggesting that the tag impairs protein function. In another study, the expression level of a ProtA–CBP-tagged protein was found to be significantly lower than that of the same protein fused to a smaller tag, further indicating that the conventional TAP tag may have a negative effect on protein stability owing to its large size [48].

TAP tags with CBP replaced

As the CBP in the original TAP tag imposes certain disadvantages, it has been substituted in several modified

Table 2 Tag combinations for tandem affinity purification found in the literature

HRV, human rhinovirus; N.r., not required; S, S-peptide.

Tag combination	Approximate size (kDa)	Cleavage site	Systems and references
ProtA–CBP	20	TEV protease	Yeast [1], mammalian cells [3], bacteria [4], insects [5], plants [6]
ProtA–S	19	TEV protease	Yeast [49–51]
ProtA–FLAG	18	TEV protease	Mammalian cells [52]
ProtA–2 × FLAG	19	TEV protease	Mammalian cells [53]
ProtA–His-9 × Myc*	32	HRV 3C protease	Plants [54], yeast [55]
ProtA–His	18	TEV protease	Plants [56], mammalian cells [57,58]
ProtA–biotinylation tag	20+2‡	TEV protease	Mammalian cells [43]
ProtA–ProtC	18	TEV protease	Parasites [46,59–62], mammalian cells [63]
Protein G–SBP	19	TEV protease	Mammalian cells [44], insects [64], plants [65]
ProtA-3 × HA	20	TEV protease	Mammalian cells [66,67]
ProtA–Strep II	18	TEV protease	Mammalian cells [68]
CBP–His–3 × HA§	8	N.r.	Yeast [40]
CBP–2 × His–3 × HA‖	14	TEV protease	Yeast [69]
CBP–3 × FLAG	8	TEV protease/N.r.**	Mammalian cells [70–72], bacteria [73]
CBP–2 × FLAG	6	TEV protease	Mammalian cells [74]
CBP–FLAG	5	TEV protease	Bacteria [75]
CBP–SBP	9	TEV protease	Mammalian cells [76,77]
His–HA	2	N.r.	Yeast [91], plants [92]
His–FLAG	2	N.r.	Yeast [93], mammalian cells [94]
His–3 × FLAG	3	N.r.	Insects [45], yeast [95]
HAT–3 × FLAG	5	TEV protease	Mammalian cells [96]
His–9 × Myc	14	PreScission protease	Yeast [97]
His–Myc	2	N.r.	Mammalian cells [98]
His–S	3	TEV protease/N.r.	Mammalian cells [99–101]
His–V5	2	N.r.	Bacteria [102]
His–calmodulin	19	Thrombin†	Bacteria [103]
His–Strep II	3	TEV protease	Mammalian cells [104]
His–ProtC	3	N.r.	Yeast [105]
His–SBP	5	N.r.	Mammalian cells [106]
2 × His–biotinylation tag	10	N.r.	Yeast [107,108]
His–biotinylation tag	9	N.r.	Mammalian cells [109,110]
FLAG–Strep II	2	N.r.	Bacteria [113]
FLAG–2 × Strep II	5	N.r.	Mammalian cells [48,114,115]
S–Strep II	4	HRV 3C protease	Mammalian cells [116]
3 × FLAG–biotinylation tag	6	PreScission protease	Mammalian cells [117]
FLAG–2xHA	3	N.r.	Mammalian cells [118,119]
HA–His–Myc¶	4	TEV protease	Nematodes [121]
Myc–FLAG	3	TEV protease	Mammalian cells [122,123]
HA–biotinylation tag	11	PreScission protease	Plants [124]
SBP–HA	5	N.r.	Mammalian cells [125]
GFP–S††	29	TEV protease	Mammalian cells [126,127]

*ProtA and His tag are used for affinity purification.
‡The original TAP tag is fused to one end of the target protein and the biotinylation tag is attached to the other end. The CBP is present, but not used.
§Initial purification is done with either a calmodulin or a Ni^{2+} resin, and the second purification is achieved by immunoprecipitation.
‖CBP and His tag are used for affinity purification. Because the buffers for calmodulin and IMAC are incompatible, buffer exchange is needed between the two purifications.
**Certain constructs do not contain the TEV protease cleavage site, as the initial binding can be released under mild conditions.
†Thrombin-mediated cleavage is used for tag removal rather than for protein elution.
¶HA and Myc epitopes are used for affinity purification.
††This is the N-terminal tag. The C-terminal tag consists of GFP and the His tag, separated by two PreScission protease cleavage sites.

versions with other affinity tags such as S-peptide [49–51], FLAG [52,53], His tag [54–58], biotinylation peptide [43], ProtC (Protein C epitope) [46,47,59–63], SBP [44,64,65], triple HA [66,67] or Strep II [68]. Except for biotinylation peptide and ProtC, all other substitutes have been introduced in the earlier sections. The biotinylation tag is a 15-amino-acid sequence that can be specifically biotinylated by *E. coli* biotin holoenzyme synthetase. Therefore, when the tag is used in mammalian cells, the target cell needs to be co-transfected with the *BirA* gene, which encodes the biotin holoenzyme synthetase. Biotin enzymatically added to the tag allows the target protein to be purified using avidin resin. Biotin and avidin bind with a dissociation constant of approx. 10^{-15} M, making it one of the

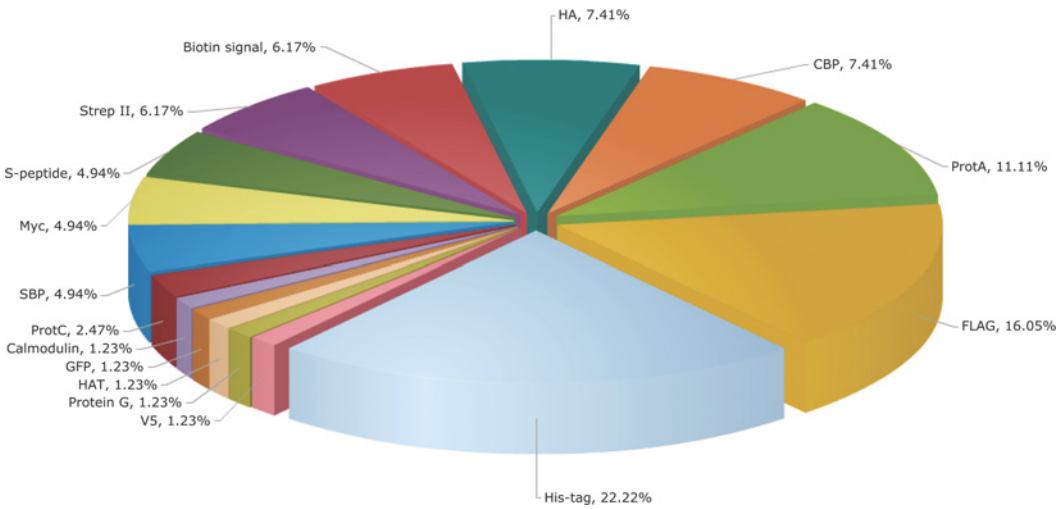

Figure 1 The relative popularity of individual affinity tags as a handle in non-traditional TAP tags

The calculation is based on the 40 non-traditional TAP tags in Table 2.

strongest non-covalent interactions. Tagged protein can be eluted using free biotin or 0.1 M glycine (pH 2.8). ProtC is a 12-amino-acid peptide derived from human Protein C. ProtC-tagged protein binds with high affinity to anti-Protein-C antibody in the presence of calcium and elution can be achieved with either Protein C peptide or chelating agents. In the TAP-tag variant where CBP is replaced by SBP, ProtA is actually replaced with two IgG-binding units of Protein G from *Streptococcus* spp. However, since Protein G is an immunoglobulin-binding protein that is similar to ProtA but has slightly higher affinity, this GS (Protein G–SBP) tag is included in this group. The GS tag is optimized for use in mammalian cells. Compared with the original TAP tag, use of the GS tag resulted in a 10-fold increase in protein yield and higher specificity, allowing successful purification and identification of the protein complex from only 5×10^7 HEK-293 cells (human embryonic kidney cells) [44]. Consistent with its superior performance in mammalian cells, the GS tag has been shown to outperform the original TAP tag with respect to both yield and specificity in insects and plants [64,65]. Because the ProtA (Protein G in the case of the GS tag) moiety is maintained in this group of TAP-tag variants, proteolytic elution is required to release the protein complex from the IgG matrix.

TAP tags with ProtA replaced

In several modified TAP tags, the CBP is maintained, whereas the ProtA moiety is replaced by HA [40], His tag [69], FLAG [70–75] or SBP [76,77]. In particular, Stratagene (La Jolla, CA, U.S.A.) has patented the use of the CBP–SBP tandem tag and created the commercial InterPlay™ mammalian TAP system based on it [78]. The major advantage of this system as claimed by the manufacturer is that it supports a purification protocol without the need for a proteolytic enzyme, as both tags can be eluted from their respective resins with gentle washing. It is noteworthy that when this system is utilized to isolate protein complexes from mammalian cells the order in which the two purification steps are performed is critical for the results. If the calmodulin resin is used first, many endogenous calmodulin-binding proteins other than the tagged target will also bind, adding burdens to the second purification. Therefore it is highly recommended to purify the protein complex using the streptavidin resin first. Stratagene provides expression vectors that facilitate both N- and C-terminal tagging. A purification kit containing the corresponding affinity resins and required buffers is also available. As the only commercially available TAP-tagging system, Stratagene's CBP–SBP tandem tag has been used to isolate various protein complexes [79–90]. However, the overall recovery rate of this tag is absent from most studies. Considering the fact that a high positive charge is critical for CBP binding and approx. 20% of the residues in SBP are negatively charged, a concern would be that the uncleaved SBP may interfere with CBP binding and therefore worsen this already problematic purification step.

Polyhistidine-containing TAP tags

Besides being used in ProtA–His and CBP–His to replace CBP and ProtA respectively, the polyhistidine tag has been used in combination with various other affinity tags, including HA [40,91,92], FLAG [45,93–96], Myc [97,98], S-peptide [99–101], V5 [102], calmodulin [103], Strep II [104], ProtC [105] and SBP [106]. Except for one case in which the

19-amino-acid HAT (histidine-affinity tag) was used [95], all polyhistidine sequences contain 6–10 histidine residues. A unique polyhistidine-containing TAP tag worth further mention is the HB (histidine and biotin) tag, which consists of one or two hexahistidine sequences and a peptide serving as an *in vivo* biotinylation signal [107–110]. The biotinylation signal peptide is a 75-amino-acid sequence containing a specific lysine residue that can be efficiently biotinylated in yeast and mammalian cells by endogenous biotin ligase. The HB tag was original developed to allow cross-linked protein complexes to be purified sequentially by Ni^{2+} resin and streptavidin resin under fully denaturing conditions (e.g. 8 M urea) [107,108]. However, this tag can also be used for purification of protein complexes under native conditions [109]. Similarly to the HB tag, hexahistidine-tagged ubiquitin has been used to isolate ubiquitylated proteins in yeast and plants using a combination of ubiquitin-affinity and IMAC [111,112]. In most cases, polyhistidine-containing TAP tags are considerably smaller than the original TAP tag and usually do not contain a TEV site because competitive elution is applicable to all tags except for the S-peptide. For all polyhistidine-containing TAP tags, chelating and reducing agents need to be used with caution.

Other TAP tags

In addition to the above-mentioned TAP tags, several other tag combinations have also been developed. Fodor et al. [113] used the FLAG–Strep II combination to isolate protein complexes from bacteria. A slightly modified version containing two copies of Strep II instead of one was also developed to purify protein complexes from mammalian cells [48,114,115]. Since both FLAG and Strep II tags can be eluted by competition, proteolytic cleavage is not required to release the bound protein complex. Another advantage of this tag combination is that the entire purification can be performed in only one buffer system [48]. Recently, two tandem tags that are similar to FLAG–Strep II were developed, in which the FLAG and Strep II tags were replaced by an S-peptide and biotinylation signal respectively [116,117]. In 2004, Ye et al. [118,119] reported the use of a FLAG–double-HA tag to purify protein complexes from HeLa cells. Recently, Sigma–Aldrich (St Louis, MO, U.S.A.) filed a patent application covering the use of FLAG–HA for TAP [120]. The company provides a commercial kit that allows rapid generation of N-terminal FLAG–HA fusion proteins. A purification kit containing the anti-FLAG M2 and the anti-HA affinity resins is also available from the same company. The small size and non-eukaryotic nature of the FLAG–HA tandem tag will probably minimize its interference with complex assembly or protein functions. Similar to the FLAG–Strep II tagging system, the FLAG–HA combination eliminates the need for TEV protease for elution. Other small TAP tags that allow tandem immunoaffinity purification include the HA–Myc tag [121] and the Myc–FLAG tag [122,123]. Very recently, two other TAP tags have been developed. Qi and Katagiri [124] utilized HA and an 80-amino-acid biotinylation signal peptide and demonstrated that this tag is suitable for the purification of low-abundance membrane-protein complexes in plants. On the other hand, Glatter et al. [125] used HA and the SBP and showed that purification using this tag allows 30–40% of the bait protein present in the cell lysate to be recovered. In addition to these single-purpose tags, Cheeseman and Desai [126] developed a dual-functional TAP tag that contains GFP (green fluorescent protein) coupled to either S-peptide (N-terminal tag) or His tag (C-terminal tag). GFP is used as the first purification tag and allows the fusion protein to be purified using antibodies against it. This GFP-containing TAP tag facilitates both affinity purification and protein localization. It has been successfully applied to the purification and identification of BBS4 (Bardet–Biedl syndrome type 4)-associated proteins [127].

Conclusions

The key advantage of TAP compared with single-step purification is the highly improved sample purity. The two sequential purifications enabled by the dual-affinity tag significantly reduce non-specific background and hence sample complexity, which in turn greatly reduces the amount of work needed for identification and validation of the isolated proteins as true interacting partners.

Despite its application in other cells or organisms, the original TAP tag is predominantly used in yeast (Figure 2). Its 20–30% overall recovery in yeast cells allows a sufficient amount of protein complexes for subunit identification by MS to be purified from 2 litres of culture in most cases [128], although sometimes more starting material is required. However, when applied in mammalian and insect cells, the original TAP tag suffers from low yields [43–47]. Bürckstümmer et al. [44] claimed that their GS tag was 10 times more efficient than the original TAP tag in bait recovery and retrieved approx. 5% of the bait present in the cell lysate, implying that the overall recovery of the original tag is less than 1% in mammalian cells. Similar low recovery rate (i.e. 1%) of the original TAP tag has also been reported in insect cells [45]. The original TAP typically requires 5×10^8–1×10^9 mammalian cells (i.e. ~50–100 15-cm-diameter culture dishes), the cultivation and handling of which can be labour-intensive and costly. To address this issue, many alternative TAP tags have been developed to improve protein yields. Whereas the application of

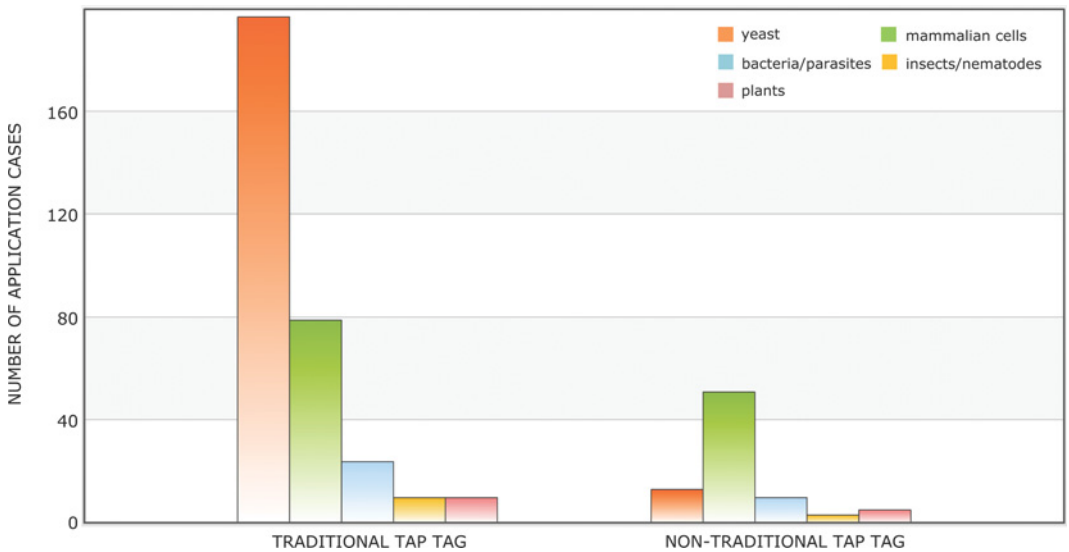

Figure 2 The relative applicability of traditional and non-traditional TAP tags

Individual cases were found through an extensive literature search and only those in which both purification steps were employed to fully purify protein complexes were counted. The specific number is probably not accurate, but it nevertheless gives a rough idea of the relative applicability of the original TAP tag and its modified versions towards various cells and organisms.

the traditional TAP tag outnumbers that of the non-traditional tags in every system, the newly developed TAP tags are predominantly used in mammalian cells (Table 2 and Figure 2).

Several non-traditional TAP tags have shown improved protein recovery or provide unique features. For example, in addition to the GS tag, the FLAG–His combination allows purification from *Drosophila* tissues with a 10–20% recovery, which is approx. 10–40 fold higher than that of the original TAP tag [45]. Recently, Glatter et al. [125] reported that the SBP–HA dual-affinity tag retrieves 30–40% of various bait proteins from mammalian cell lysates, making it one of the most efficient tag combinations for mammalian cells. Whereas most TAP tags are designed to facilitate mild binding and elution in favour of the preservation of protein-complex integrity, the HB tag was specifically developed to allow purification under fully denaturing conditions [107,108]. As a trade-off for high sample purity, weak and transient interactions are typically lost during standard TAP procedures. *In vivo* cross-linking prior to TAP is a commonly used strategy to capture weakly and transiently interacting proteins, and purification under denaturing conditions can substantially reduce cross-linking-introduced non-specific background [108].

As summarized in Table 2, various TAP tags have been developed by different laboratories. All of these tags have proved successful at isolating at least one protein complex. However, except for a few cases, the recovery rate of the specific TAP tag being used was not provided and a systematic comparison of different TAP tags is generally lacking. Nevertheless, the available results suggest that ProtA/Protein G, His tag, FLAG, HA, SBP and Strep II tag are good candidates for TAP-tag components, and it is likely that a reasonable combination of two of them will result in a TAP tag that is more efficient than the original one in systems other than yeast. To reduce the numbers of tags in use and make the method more efficient and generic, a systematic study in the future is necessary to find the best TAP tags, which yield more protein and cost less.

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References

1 Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Séraphin, B. (1999) Nat. Biotechnol. **17**, 1030–1032
2 Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M. and Séraphin, B. (2001) Methods **24**, 218–229
3 Cox, D. M., Du, M., Guo, X., Siu, K. W. and McDermott, J. C. (2002) Biotechniques **33**, 267–270
4 Gully, D., Moinier, D., Loiseau, L. and Bouveret, E. (2003) FEBS Lett. **548**, 90–96

5 Forler, D., Köcher, T., Rode, M., Gentzel, M., Izaurralde, E. and Wilm, M. (2003) Nat. Biotechnol. **21**, 89–92
6 Rivas, S., Romeis, T. and Jones, J. D. (2002) Plant Cell **14**, 689–702
7 Bauer, A. and Kuster, B. (2003) Eur. J. Biochem. **270**, 570–578
8 Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A. M., Cruciat, C. M. et al. (2002) Nature **415**, 141–147
9 Ståhl, S., Nygren, P. A. and Uhlén, M. (1997) Methods Mol. Biol. **62**, 37–54
10 Hammarberg, B., Nygren, P. A., Holmgren, E., Elmblad, A., Tally, M., Hellman, U., Moks, T. and Uhlén, M. (1989) Proc. Natl. Acad. Sci. U. S. A. **86**, 4367–4371
11 Jansson, B., Palmcrantz, C., Uhlén, M. and Nilsson, B. (1990) Protein Eng. **3**, 555–561
12 Murby, M., Cedergren, L., Nilsson, J., Nygren, P. A., Hammarberg, B., Nilsson, B., Enfors, S. O. and Uhlén, M. (1991) Biotechnol. Appl. Biochem. **14**, 336–346
13 Kim, J. S. and Raines, R. T. (1994) Anal. Biochem. **219**, 165–166
14 Panagiotidis, C. A. and Silverstein, S. J. (1995) Gene **164**, 45–47
15 Nilsson, J., Larsson, M., Ståhl, S., Nygren, P. A. and Uhlén, M. (1996) J. Mol. Recognit. **9**, 585–594
16 Cocca, B. A., Seal, S. N. and Radic, M. Z. (1999) Protein Expr. Purif. **17**, 290–298
17 Pathak, R. and Imperiali, B. (1997) Arch. Biochem. Biophys. **338**, 1–6
18 Uhlén, M., Nilsson, B., Guss, B., Lindberg, M., Gatenbeck, S. and Philipson, L. (1983) Gene **23**, 369–378
19 Waugh, D. S. (2005) Trends. Biotechnol. **23**, 316–320
20 Lichty, J. J., Malecki, J. L., Agnew, H. D., Michelson-Horowitz, D. J. and Tan, S. (2005) Protein Expr. Purif. **41**, 98–105
21 Hearn, M. T. and Acosta, D. (2001) J. Mol. Recognit. **14**, 323–369
22 Terpe, K. (2003) Appl. Microbiol. Biotechnol. **60**, 523–533
23 Kimple, M. E. and Sondek, J. (2004) Curr. Protoc. Protein Sci. Chapter 9, Unit 9.9
24 Langone, J. J. (1982) Adv. Immunol. **32**, 157–252
25 Nilsson, B., Moks, T., Jansson, B., Abrahmsén, L., Elmblad, A., Holmgren, E., Henrichson, C., Jones, T. A. and Uhlén, M. (1987) Protein Eng. **1**, 107–113
26 Moks, T., Abrahamsen, L., Österlöf, B., Josephson, S., Östling, M., Enfors, S. O., Persson, I., Nilsson, B. and Uhlén, M. (1987) Biotechnology (N.Y.) **5**, 379–382
27 Jansson, M., Li, Y. C., Jendeberg, L., Anderson, S., Montelione, B. T. and Nilsson, B. (1996) J. Biomol. NMR **7**, 131–141
28 Uhlén, M. and Moks, T. (1990) Methods Enzymol. **185**, 129–143
29 Stofko-Hahn, R. E., Carr, D. W. and Scott, J. D. (1992) FEBS Lett. **302**, 274–278
30 Hopp, T. P., Prickett, K. S., Price, V. L., Libby, R. T., March, C. J., Cerretti, D. P., Urdal, D. L. and Conlon, P. J. (1988) Biotechnology (N. Y.) **6**, 1204–1210
31 Schmidt, T. G., Koepke, J., Frank, R. and Skerra, A. (1996) J. Mol. Biol. **255**, 753–766
32 Voss, S. and Skerra, A. (1997) Protein Eng. **10**, 975–982
33 Skerra, A. and Schmidt, T. G. (2000) Methods Enzymol. **326**, 271–304
34 Witte, C. P., Noël, L. D., Gielbert, J., Parker, J. E. and Romeis, T. (2004) Plant Mol. Biol. **55**, 135–147
35 Wilson, D. S., Keefe, A. D. and Szostak, J. W. (2001) Proc. Natl. Acad. Sci. U. S. A. **98**, 3750–3755
36 Kim, J. S. and Raines, R. T. (1993) Protein Sci. **2**, 348–356
37 Raines, R. T., McCormick, M., Van Oosbree, T. R. and Mierendorf, R. C. (2000) Methods Enzymol. **326**, 362–376
38 Chong, S., Mersha, F. B., Comb, D. G., Scott, M. E., Landry, D., Vence, L. M., Perler, F. B., Benner, J., Kucera, R. B., Hirvonen, C. A. et al. (1997) Gene **192**, 271–281
39 Xu, M. Q., Ferrandon, S. M., Taron, C. H. and Colussi, P. A. (2006) Modified chitin binding domain and uses thereof. U.S. Pat. 7,060,465
40 Honey, S., Schneider, B. L., Schieltz, D. M., Yates, J. R. and Futcher, B. (2001) Nucleic Acids Res. **29**, E24
41 Séraphin, B., Puig, O., Bouveret, E., Rutz, B. and Caspary, F. (2002) in Protein–Protein Interactions: A Molecular Cloning Manual, Chapter 17, pp. 313–328, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
42 Mayer, D., Baginsky, S. and Schwemmle, M. (2005) Proteomics **5**, 4483–4487
43 Drakas, R., Prisco, M. and Baserga, R. (2005) Proteomics **5**, 132–137
44 Bürckstümmer, T., Bennett, K. L., Preradovic, A., Schütze, G., Hantschel, O., Superti-Furga, G. and Bauch, A. (2006) Nat. Methods **3**, 1013–1019
45 Yang, P., Sampson, H. M. and Krause, H. M. (2006) Proteomics **6**, 927–935
46 Schimanski, B., Nguyen, T. N. and Günzl, A. (2005) Eukaryot. Cell **4**, 1942–1950
47 Günzl, A. and Schimanski, B. (2009) Curr. Protoc. Protein Sci. Chapter 19, Unit 19.19
48 Gloeckner, C. J., Boldt, K., Schumacher, A., Roepman, R. and Ueffing, M. (2007) Proteomics **7**, 4228–4234
49 Cheeseman, I. M., Brew, C., Wolyniak, M., Desai, A., Anderson, S., Muster, N., Yates, J. R., Huffaker, T. C., Drubin, D. G. and Barnes, G. (2001) J. Cell Biol. **155**, 1137–1145
50 Sanchatjate, S. and Schekman, R. (2006) Mol. Biol. Cell **17**, 4157–4166
51 Copic, A., Starr, T. L. and Schekman, R. (2007) Mol. Biol. Cell **18**, 1803–1815
52 Knuesel, M., Wan, Y., Xiao, Z., Holinger, E., Lowe, N., Wang, W. and Liu, X. (2003) Mol. Cell. Proteomics **2**, 1225–1233

53 Tsai, A. and Carstens, R. P. (2006) Nat. Protoc. **1**, 2820–2827
54 Rubio, V., Shen, Y., Saijo, Y., Liu, Y., Gusmaroli, G., Dinesh-Kumar, S. P. and Deng, X. W. (2005) Plant J. **41**, 767–778
55 Ma, X., Yang, C., Alexandrov, A., Grayhack, E. J., Behm-Ansmant, I. and Yu, Y. T. (2005) EMBO J. **24**, 2403–2413
56 Chang, I. F., Curran, A., Woolsey, R., Quilici, D., Cushman, J. C., Mittler, R., Harmon, A. and Harper, J. F. (2009) Proteomics **9**, 2967–2985
57 Francesconi, A., Kumari, R. and Zukin, RS. (2009) J. Neurochem. **108**, 1515–1525
58 Crawford, N. P., Yang, H., Mattaini, K. R. and Hunter, K. W. (2009) J. Biol. Chem. **284**, 28660–28673
59 Schimanski, B., Nguyen, T. N. and Günzl, A. (2005) Mol. Cell. Biol. **25**, 7303–7313
60 Palfi, Z., Schimanski, B., Günzl, A., Lücke, S. and Bindereif, A. (2005) Nucleic Acids Res. **33**, 2493–2503
61 Nguyen, T. N., Schimanski, B., Zahn, A., Klumpp, B. and Günzl, A. (2006) Mol. Biochem. Parasitol. **149**, 27–37
62 Takebe, S., Witola, W. H., Schimanski, B., Günzl, A. and Mamoun, C. B. (2007) Eukaryot. Cell **6**, 584–591
63 Kamil, J. P. and Coen, D. M. (2007) J. Virol. **81**, 10659–10668
64 Kyriakakis, P., Tipping, M., Abed, L. and Veraksa, A. (2008) Fly **2**, 229–235
65 Van Leene, J., Witters, E., Inzé, D. and De Jaeger, G. (2008) Trends Plant Sci. **13**, 517–520
66 Venteicher, A. S., Meng, Z., Mason, P. J., Veenstra, T. D. and Artandi, S. E. (2008) Cell **132**, 945–957
67 Venteicher, A. S., Abreu, E. B., Meng, Z., McCann, K. E., Terns, R. M., Veenstra, T. D., Terns, M. P. and Artandi, S. E. (2009) Science **323**, 644–648
68 Forsman, A., Rüetschi, U., Ekholm, J. and Rymo, L. (2008) J. Proteome Res. **7**, 2309–2319
69 Auty, R., Steen, H., Myers, L. C., Persinger, J., Bartholomew, B., Gygi, S. P. and Buratowski, S. (2004) J. Biol. Chem. **279**, 49973–49981
70 Zeghouf, M., Li, J., Butland, G., Borkowska, A., Canadien, V., Richards, D., Beattie, B., Emili, A. and Greenblatt, J. F. (2003) J. Proteome Res. **3**, 463–468
71 Cochrane, A., Murley, L. L., Gao, M., Wong, R., Clayton, K., Brufatto, N., Canadien, V., Mamelak, D., Chen, T., Richards, D. et al. (2009) Virology **388**, 103–111
72 Wakabayashi, T., Craessaerts, K., Bammens, L., Bentahir, M., Borgions, F., Herdewijn, P., Staes, A., Timmerman, E., Vandekerckhove, J., Rubinstein, E. et al. (2009) Nat. Cell Biol. **11**, 1340–1346
73 Butland, G., Zhang, J. W., Yang, W., Sheung, A., Wong, P., Greenblatt, J. F., Emili, A. and Zamble, D. B. (2006) FEBS Lett. **580**, 677–681
74 Liang, S., Yu, Y., Yang, P., Gu, S., Xue, Y. and Chen, X. (2009) J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **877**, 627–634
75 Hervey, IV, W. J., Khalsa-Moyers, G., Lankford, P. K., Owens, E. T., McKeown, C. K., Lu, T. Y., Foote, L. J., Asano, K. G., Morrell-Falvey, J. L., McDonald, W. H. et al. (2009) J. Proteome Res. **8**, 3675–3688
76 Gustafson, M. P., Welcker, M., Hwang, H. C. and Clurman, B. E. (2005) Biochem. Biophys. Res. Commun. **338**, 1359–1367
77 Angers, S., Thorpe, C. J., Biechele, T. L., Goldenberg, S. J., Zheng, N., MacCoss, M. J. and Moon, R. T. (2006) Nat. Cell Biol. **8**, 348–357
78 Braman, J. C., Carstens, C. P., Novoradovskaya, N., Bagga, R. and Basehore, L. S. (2007) Compositions and methods for protein isolation. U.S. Pat. 7,238,478
79 Chiu, Y. L., Witkowska, H. E., Hall, S. C., Santiago, M., Soros, V. B., Esnault, C., Heidmann, T. and Greene, W. C. (2006) Proc. Natl. Acad. Sci. U. S. A. **103**, 15588–15593
80 Breese, E. H., Uversky, V. N., Georgiadis, M. M. and Harrington, M. A. (2006) DNA Cell Biol. **25**, 704–714
81 Gallois-Montbrun, S., Kramer, B., Swanson, C. M., Byers, H., Lynham, S., Ward, M. and Malim, M. H. (2007) J. Virol. **81**, 2165–2178
82 Wei, X., Shimizu, T. and Lai, Z. C. (2007) EMBO J. **26**, 1772–1781
83 Wiederschain, D., Chen, L., Johnson, B., Bettano, K., Jackson, D., Taraszka, J., Wang, Y. K., Jones, M. D., Morrissey, M., Deeds, J. et al. (2007) Mol. Cell. Biol. **27**, 4968–4979
84 Bradley, C. M., Jones, S., Huang, Y., Suzuki, Y., Kvaratskhelia, M., Hickman, A. B., Craigie, R. and Dyda, F. (2007) Structure **15**, 643–653
85 Kitagawa, H., Yamaoka, I., Akimoto, C., Kase, I., Mezaki, Y., Shimizu, T. and Kato, S. (2007) Genes Cells **12**, 1281–1287
86 Medina-Palazon, C., Gruffat, H., Mure, F., Filhol, O., Vingtdeux-Didier, V., Drobecq, H., Cochet, C., Sergeant, N., Sergeant, A. and Manet, E. (2007) J. Virol. **81**, 11850–11860
87 Conner, S. L. and Wang, M. (2008) Scientific Research and Essays **3**, 143–153
88 Gallois-Montbrun, S., Holmes, R. K., Swanson, C. M., Fernández-Ocaña, M., Byers, H. L., Ward, M. A. and Malim, M. H. (2008) J. Virol. **82**, 5636–5642
89 Alazard-Dany, N., Nicolas, A., Ploquin, A., Strasser, R., Greco, A., Epstein, A. L., Fraefel, C. and Salvetti, A. (2009) PLoS Pathog. **5**, e1000340
90 Ahlstrom, R. and Yu, A. S. (2009) Am. J. Physiol. Renal Physiol. **297**, F685–F692
91 Shou, W., Verma, R., Annan, R. S., Huddleston, M. J., Chen, S. L., Carr, S. A. and Deshaies, R. J. (2002) Methods Enzymol. **351**, 279–296
92 Hafrén, A. and Mäkinen, K. (2008) J. Gen. Virol. **89**, 1509–1518
93 Kaneko, A., Umeyama, T., Hanaoka, N., Monk, B. C., Uehara, Y. and Niimi, M. (2004) Yeast **21**, 1025–1033
94 Saade E, Mechold U, Kulyyassov A, Vertut D and Lipinski M, Ogryzko V. (2009) Proteomics **9**, 4934–4943

95 Blethrow, J. D., Tang, C., Deng, C. and Krutchinsky, A. N. (2007) PLoS One **2**, e358

96 Fernández, E., Collins, M. O., Uren, R. T., Kopanitsa, M. V., Komiyama, N. H., Croning, M. D., Zografos, L., Armstrong, J. D., Choudhary, J. S. and Grant, S. G. (2009) Mol. Syst. Biol. **5**, 269

97 Graumann, J., Dunipace, L. A., Seol, J. H., McDonald, W. H., Yates, III, J. R., Wold, B. J. and Deshaies, R. J. (2004) Mol. Cell. Proteomics **3**, 226–237

98 Brown, K. A., Ham, A. J., Clark, C. N., Meller, N., Law, B. K., Chytil, A., Cheng, N., Pietenpol, J. A. and Moses, H. L. (2008) J. Cell. Biochem. **105**, 596–611

99 Ozaki, K., Inoue, K., Sato, H., Iida, A., Ohnishi, Y., Sekine, A., Sato, H., Odashiro, K., Nobuyoshi, M., Hori, M., Nakamura, Y. and Tanaka, T. (2004) Nature **429**, 72–75

100 Rosas-Acosta, G., Russell, W. K., Deyrieux, A., Russell, D. H. and Wilson, V. G. (2005) Mol. Cell. Proteomics **4**, 56–72

101 Kwak, H. I., Mendoza, E. A. and Bayless, K. J. (2009) Matrix Biol. **28**, 470–479

102 Connelly, H. M., Pelletier, D. A., Lu, T. Y., Lankford, P. K. and Hettich, R. L. (2006) Anal. Biochem. **357**, 93–104

103 McCluskey, A. J., Poon, G. M. and Gariépy, J. (2007) Protein Sci. **16**, 2726–2732

104 Giannone, R. J., McDonald, W. H., Hurst, G. B., Huang, Y., Wu, J., Liu, Y. and Wang, Y. (2007) Biotechniques **43**, 296–302

105 Claypool, S. M., Oktay, Y., Boontheung, P., Loo, J. A. and Koehler, C. M. (2008) J. Cell Biol. **182**, 937–950

106 Kobayashi, T., Morone, N., Kashiyama, T., Oyamada, H., Kurebayashi, N. and Murayama, T. (2008) PLoS One **3**, e3822

107 Guerrero, C., Tagwerker, C., Kaiser, P. and Huang, L. (2006) Mol. Cell. Proteomics **5**, 366–378

108 Tagwerker, C., Flick, K., Cui, M., Guerrero, C., Dou, Y., Auer, B., Baldi, P., Huang, L. and Kaiser, P. (2006) Mol. Cell. Proteomics **5**, 737–748

109 Wu, F., Wang, P., Young, L. C., Lai, R. and Li, L. (2009) Am. J. Pathol. **174**, 361–370

110 Wang, P., Wu, F., Ma, Y., Li, L., Lai, R. and Young, L. C. (2010) J. Biol. Chem. **285**, 95–103

111 Mayor, T. and Deshaies, R. J. (2005) Methods Enzymol. **399**, 385–392

112 Saracco, S. A., Hansson, M., Scalf, M., Walker, J. M., Smith, L. M. and Vierstra, R. D. (2009) Plant J. **59**, 344–358

113 Fodor, B. D., Kovács, A. T., Csáki, R., Hunyadi-Gulyás, E., Klement, E., Maróti, G., Mészáros, L. S., Medzihradszky, K. F., Rákhely, G. and Kovács, K. L. (2004) Appl. Environ. Microbiol. **70**, 712–721

114 Gloeckner, C. J., Boldt, K., Schumacher, A. and Ueffing, M. (2009) Methods Mol. Biol. **564**, 359–372

115 Gloeckner, C. J., Boldt, K. and Ueffing, M. (2009) Curr. Protoc. Protein Sci. Chapter 19, Unit 19.20

116 Lehmann, R., Meyer, J., Schuemann, M., Krause, E. and Freund, C. (2009) Proteomics **9**, 5288–5295

117 Haas, T. L., Emmerich, C. H., Gerlach, B., Schmukle, A. C., Cordier, S. M., Rieser, E., Feltham, R., Vince, J., Warnken, U., Wenger, T. et al. (2009) Mol. Cell **36**, 831–844

118 Ye, J. Z., Hockemeyer, D., Krutchinsky, A. N., Loayza, D., Hooper, S. M., Chait, B. T. and de Lange, T. (2004) Genes Dev. **18**, 1649–1654

119 Ye, J. Z., Donigian, J. R., van Overbeek, M., Loayza, D., Luo, Y., Krutchinsky, A. N., Chait, B. T. and de Lange, T. (2004) J. Biol. Chem. **279**, 47264–47271

120 Zenser, N., Bettinger, K. and Song, K. (2008) Tandem affinity purification systems and methods utilizing such systems. U.S. Pat. application 20080039616

121 Polanowska, J., Martin, J. S., Fisher, R., Scopa, T., Rae, I. and Boulton, S. J. (2004) Biotechniques **36**, 778–782

122 Ichimura, T., Yamamura, H., Sasamoto, K., Tominaga, Y., Taoka, M., Kakiuchi, K., Shinkawa, T., Takahashi, N., Shimada, S. and Isobe, T. (2005) J. Biol. Chem. **280**, 13187–13194

123 Tanaka, Y., Kanai, F., Ichimura, T., Tateishi, K., Asaoka, Y., Guleng, B., Jazag, A., Ohta, M., Imamura, J., Ikenoue, T. et al. (2006) Oncogene **25**, 633–642

124 Qi, Y. and Katagiri, F. (2009) Plant J. **57**, 932–944

125 Glatter, T., Wepf, A., Aebersold, R. and Gstaiger, M. (2009) Mol. Syst. Biol. **5**, 237

126 Cheeseman, I. M. and Desai, A. (2005) Sci. STKE. **2005**, pl1

127 Nachury, M. V. (2008) Methods Enzymol. **439**, 501–513

128 Dziembowski, A. and Séraphin, B. (2004) FEBS Lett. **556**, 1–6

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