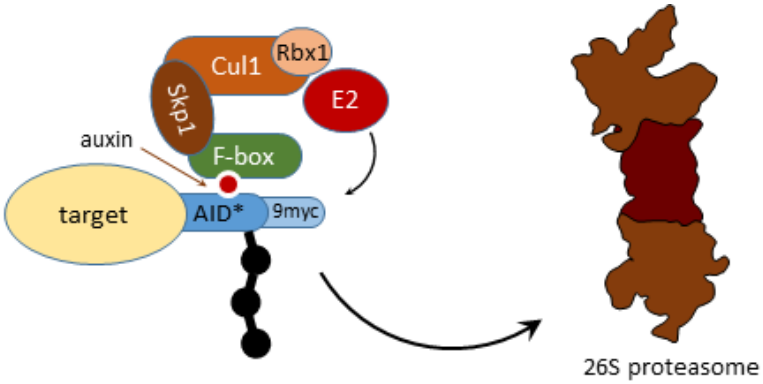
The AID* tool kit for degron tagging in S. cerevisiae

Reference:

M. Morawska & H.D. Ulrich (2013) An expanded tool kit for the auxin-inducible degron system in budding yeast. *Yeast* **30**, 41-51; doi: 10.1002/yea.2967.

Degradation of the AID*-tagged target protein is induced by addition of auxin to the growth medium, which mediates the interaction of the AID* tag with the SCF ubiquitin ligase. The plant-derived auxin-responsive F-box protein is constitutively expressed in the host strain.



Protocol:

The "AID*" tag [Morawska & Ulrich (2013) *Yeast* **30**, 41-51] is a minimised version of the original "AID" degron [Nishimura *et al.* (2009) *Nat Methods* **6**, 917-922]. For the purpose of detection by commercial antibodies or fluorescence microscopy, common epitope tags have been appended to the AID* sequence (9myc, 6HA, 6FLAG, GFP). The use of the kit is described here for C-terminal and the N-terminal tagging strategies. Further experimental details are given in the publication.

Step 1: Amplification of the tagging cassette

Use the following primers:

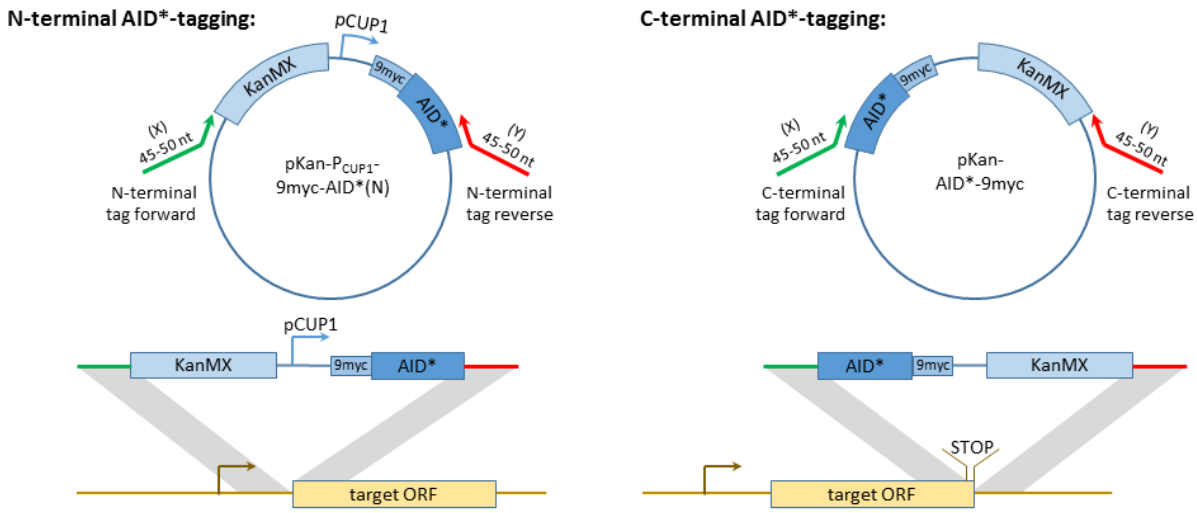
N-terminal tag forward: 5'-$(X)_{45\text{-}50}$CGTACGCTGCAGGTCGAC-3'
or 5'-$(X)_{45\text{-}50}$TAAGGCGCGCCAGATCTG-3'

N-terminal tag reverse: 5'-$(Y)_{45\text{-}50}$/AAG/CTG/GTA/CCG/AGC/TCT/GG-3' (in frame with the ORF)
or 5'-$(Y)_{45\text{-}50}$/GGC/ACC/CGC/TCC/AGC/GCC/TG-3'

C-terminal tag forward: 5'-$(X)_{45\text{-}50}$/CGT/ACG/CTG/CAG/GTC/GAC-3' (in frame with the ORF)

C-terminal tag reverse: 5'-$(Y)_{45\text{-}50}$ATCGATGAATTCGAGCTCG-3'

Slashes (/) indicate the open reading frame. X and Y correspond to sequences complementary to the insertion site as illustrated below.



Use the following templates:		*for tagging:*	*selection:*
#99513	pHyg-AID(1-114)	C-terminal	hygromycin
#99514	pHyg-AID(31-114)	C-terminal	hygromycin
#99515	pHyg-AID*	C-terminal	hygromycin
#99516	pHyg-AID(1-114)-8myc	C-terminal	hygromycin
#99517	pHyg-AID(31-114)-9myc	C-terminal	hygromycin
#99518	pHyg-AID*-9myc	C-terminal	hygromycin
#99519	pHyg-AID*-6FLAG	C-terminal	hygromycin
#99520	pHyg-AID*-6HA	C-terminal	hygromycin
#99521	pHyg-AID*-GFP	C-terminal	hygromycin
#99522	pKan-AID*-9myc	C-terminal	G418
#99523	pNat-AID*-9myc	C-terminal	nourseothricin
#99524	pHis-AID*-9myc	C-terminal	histidine drop-out
#99525	pKan-PCUP1-AID*(N)	N-terminal	G418
#99526	pKan-PCUP1-AID*(N)-9myc	N-terminal	G418
#99527	pKan-PCUP1-9myc-AID*(N)	N-terminal	G418
#99528	pKan-PRFA1-9myc-AID*(N)	N-terminal	G418
#99529	pHyg-PCUP1-AID*(N)	N-terminal	hygromycin

Amplify the tagging cassette by standard PCR and use a clean-up procedure to isolate the product. The reaction should yield a single major product; there is no need to gel-purify this.

Step 2: Integration of the tagging cassette

Use the PCR product for a standard yeast transformation, followed by selection for the appropriate marker (as shown above). Verify successful fusion of the tagging cassette by colony PCR (e.g. using one primer inside the tagging cassette and the other inside your gene of interest) and by subjecting total protein extracts to western blotting with an antibody against the appropriate tag (as indicated in the vector name). The *CUP1* promoter affords a basal expression of N-terminally tagged constructs that can be further enhanced by addition of 100 µM $CuSO_4$ to the growth medium.

Step 3: Integration of the vector encoding the F-box protein (TIR1 or AFB2)

Use one of the following vectors:		*digest with:*	*for integration into:*
#99530	pRS303-pADH-AFB2	NheI	HIS3
#99531	YIp204-pADH-AFB2	EcoRV	TRP1
#99532	YIp204-pADH-atTIR1-9myc	EcoRV	TRP1

TIR1-9myc expression can be monitored by western blotting (anti-myc). For AFB2, it is advisable to test multiple clones for degradation of the protein of interest (see below). Alternatively, the F-box protein expression construct can be integrated first, and a verified clone that has been shown previously to express AFB2 is then used for tagging the protein of interest.

Step 4: Induction of protein degradation

Induce degradation of the protein of interest by addition of 1 mM auxin (indole-3-acetic acid, IAA) to the culture for 1 h and collect samples for total protein extraction before and after auxin addition. Verify degradation by blotting against the tag. It is possible to vary the auxin concentration or use analogs such as 1-naphthaleneacetic acid (NAA).