diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME **BirA and Avitag plasmids - Plasmids for *in vivo* biotin tagging in yeast**		
Catalog #: tag-001 to tag-004 tag-007 to tag-009	**Type:** Low copy BirA expression plasmid	**Size:** 3 µl (3 µg)
Catalog #: *tag-005 to tag-006*	**Type:** Tagging cassette	**Size:** 3 µl (3 µg)
Lot #: See above	**Source:** Competent E.coli DH5Ð	**Concentration:** 1 µg/µl

Available plasmids and tagging cassettes:

- pRS313-BirA-NLS cat. No.: tag-001 lot No.: DA-001
- pRS314-BirA-NLS cat. No.: tag-002 lot No.: DA-001
- pRS315-BirA-NLS cat. No.: tag-003 lot No.: DA-001
- pRS316-BirA-NLS cat. No.: tag-004 lot No.: DA-001
- pUG6-Myc-C-Avitag cat. No.: tag-005 lot No.: DA-002
- pUG6-Myc-N-Avitag cat. No.: tag-006 lot No.: DA-002
- pRS316-BirA cat. No.: tag-007 lot No.: DA-001
- pRS313-BirA cat. No.: tag-008 lot No.: DA-002
- pRS315-BirA cat. No.: tag-009 lot No.: DA-001

Description:
- Yeast low copy **BirA** expression plasmids are designed for in vivo biotinylation of proteins in yeast for highly sensitive detection of protein-DNA complexes using chromatin immunoprecipitation (ChIP). The constructs contain an HA tag, as well as the HIS3 marker and a nuclear localisation signal (NLS, tag-001 to tag-004 only).
- Biotinylation tagging cassette for C- and N- terminal tagging includes an **Avitag** affinity tag (GLNDIFEAQKIEWHW) as biotin acceptor (K in bold in the sequence). Biotinylation of the Avitagged recombinant target protein is dependent on BirA expression.

Specificity: Yeast

Cat. No.:	Application	Suggested dilution	Reference
tag-001 to tag-004 tag-007 to tag-009	In vivo biotinylation of Avitag	200 ng per transformation	(1)
tag-005 to tag-006	Chromosomal tagging with biotinylation tag (Avitag)	1 ng per PCR reaction	(1)

Format: In solution in 10 mM Tris- HCl and 1 mM EDTA

Storage: Store at -20°C. Avoid multiple freeze-thaw cycles

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures

References citing this antibody:

(1) Van Werven FJ and Timmers HT (2006) The use of biotin tagging in Saccharomyces cerevisiae improves the sensitivity of chromatin immunoprecipitation. Nucleic Acids Res 34:e33.

Last data sheet update: April 29, 2011

Biotin tagging in yeast quick overview

Affinity tagging has been used in many global studies towards protein function. Yeast strains with chromosomally integrated versions of tagged transcription factor genes were generated using N- or C-terminal biotin-tagging cassettes (1). This in vivo biotinylation of transcription factors in the yeast Saccharomyces cerevisiae, which is based on the bacterial BirA biotin ligase, is a highly efficient system. Due to the strength of the biotin-streptavidin interaction, biotin tagging in Saccharomyces cerevisiae improves the sensitivity of chromatin immunoprecipitation (ChIP), resulting in a better in vivo detection of protein-DNA complexes.

In comparison with other affinity tags (TAP, 170 residues; 3xHA, 36 residues; 13xMyc, 156 residues) the 15-amino acid Avitag is small, reducing the risk of interference with protein function. Multiple essential transcription factor genes have been successfully tagged with Avitag and no interference with protein function has been observed as determined by phenotypic analysis. Moreover, quantitative biotinylation of the Avitagged target protein was observed in all cases.

ChIP assays are performed on formaldehyde cross-linked chromatin from cells expressing both the BirA-NLS protein and the Avitagged-target protein. Capturing biotinylated transcription factors by streptavidin-coated beads allows stringent washing conditions which results in high signal to noise ratios and improved detection of cross-linked protein–DNA complexes.

Protocol for transformation in Yeast

Material:

- YPD
- Selective Plates
- 50% PEG (w/v)
- 1 M LiAc (pH 7.5)
- Herring sperm DNA (10 mg/ml)
- plasmid DNA 200 ng for each transformation

Method:

1. Inoculate culture of 10 ml overnight.
2. Measure the cell density of the culture (OD between 0.5 – 1)
3. Transfer 5 ml in 100 ml culture medium (1:20 dilution, 5x10^6 cells/ml),
4. Incubate for 3 - 5 hours to obtain an OD between 0.5 – 1 (100 ml is enough for 20 transformations).
5. Prepare tubes with 200 ng of each plasmid in a total volume of 5 µl
6. Spin down the cells by centrifugation at 3000 rpm for 4 minutes
7. Resuspend in 25 ml H20 by vortexing, and spin down at 2000 rpm for 4 minutes
8. Resuspend the cells in 1.0 ml of 100 mM LiAc and transfer to 1.5 ml tube.
9. Spin down for 10 seconds and remove LiAc.
10. Resuspend the cells to a final volume of 500 µl in 100 mM LiAc. Sup plus 400 µl LiAc.
11. Boil Herring sperm DNA for 5 minutes and put on ice.
12. Prepare the following transformation mix

240 µl of PEG (50% w/v)
36 µl of 1.0 M LiAc
5 µl of Herring sperm DNA (10 mg/ml)
65 µl of H20

13. Add 50 µl cell solution per tube per transformation
14. Spin down and remove supernatant.
15. Add 350 µl of transformation mix to the cells and mix gently
16. Transfer the mixture to 1.5 ml tube with 200 ng of each plasmid DNA and mix
17. Incubate for 30 minutes at 30°C
18. Heat Shock for 20 seconds at 42°C
19. Spin down at 6000 rpm for 30 seconds and remove transformation mix
20. Resuspend in 100 µl H20 and plate out on selective plates

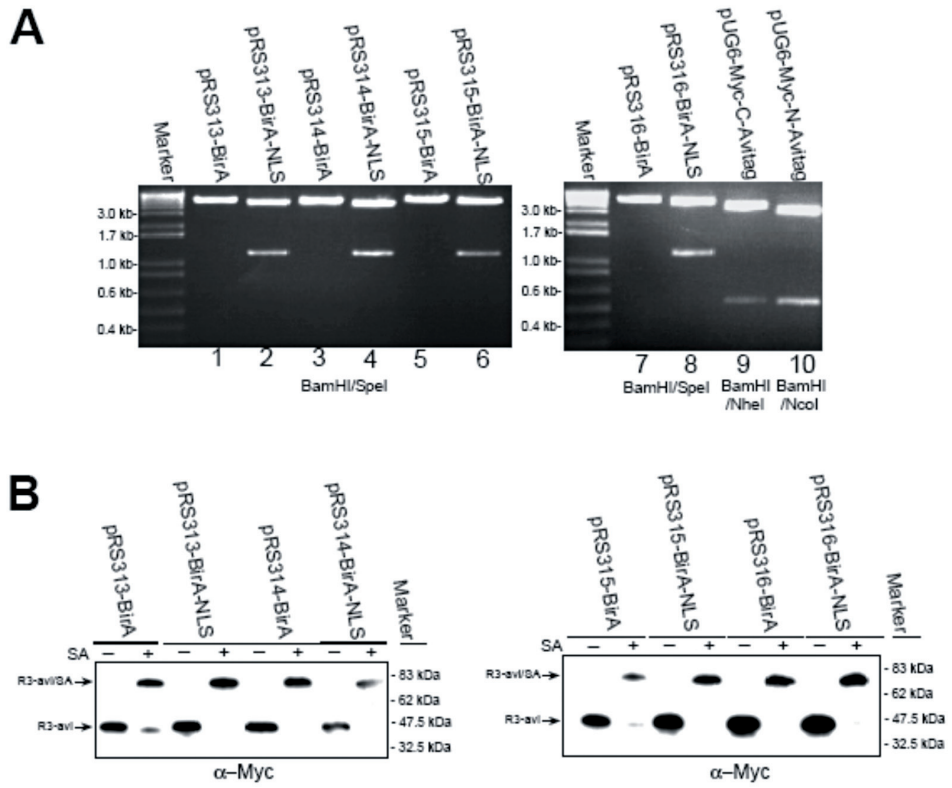

Figure 1

Quality control analysis of the BirA and Avitag plasmids.

A. Restriction digest analysis of pRS313-BirA, pRS313-BirA-NLS, pRS314-BirA, pRS314-BirA-NLS, pRS315-BirA, pRS315-BirA-NLS, pRS316-BirA, pRS316-BirA-NLS, pUG-myc-C-avitag and pUG-myc-N-avitag plasmids. The BirA containing plasmids were digested with BamHI and SpeI enzymes. This results in a second band in the plasmids containing the NLS of approximately 1.2 kb corresponding to the BirA fragment (lanes 2, 4, 6 and 8). Since the plasmids without the NLS also lack the BamHI restriction site, only a single band is generated (lanes 1, 3, 5, and 7). pUG-myc-C-avitag and pUG-myc-N-Avitag were digested with NheI/BamHI or NcoI/BamHI, respectively, which results in a fragment of approximately 0.5 kb (lanes 9 and 10).

B. Biotinylation efficiency analysis of the BirA containing plasmids. Each of the BirA plasmids was transformed into Rpb3-myc-C-Avitag (R3-avi) yeast strain. Transformed plasmids were selected for the auxotrophic marker and grown on synthetic complete media lacking the corresponding amino acid (HIS, TRP, LEU or URA). Protein extracts were incubated with streptavidin (SA) as indicated and analysed by immunoblotting using anti-Myc antibodies.

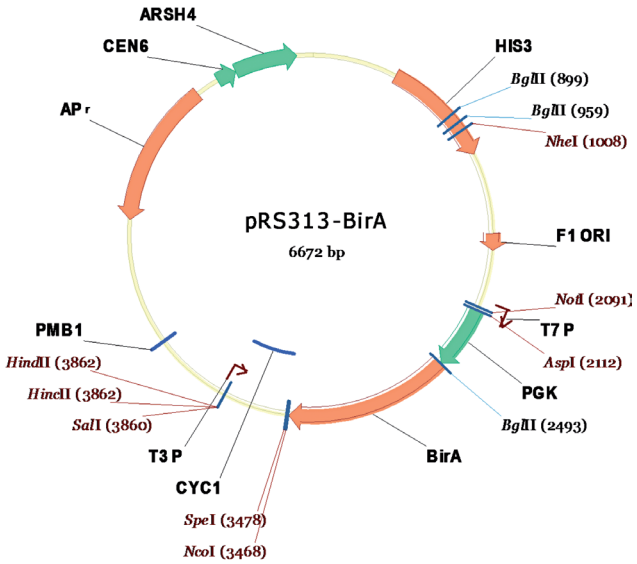

Figure 2
Plasmid map of pRS313-BirA (Diagenode cat. No.: tag-008)

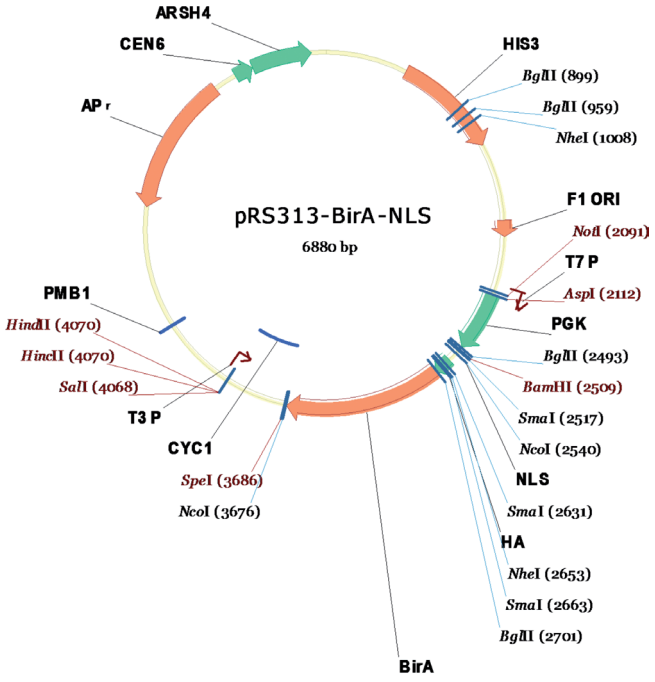

Figure 3
Plasmid map of pRS313-BirA-NLS (Diagenode cat. No.: tag-001)

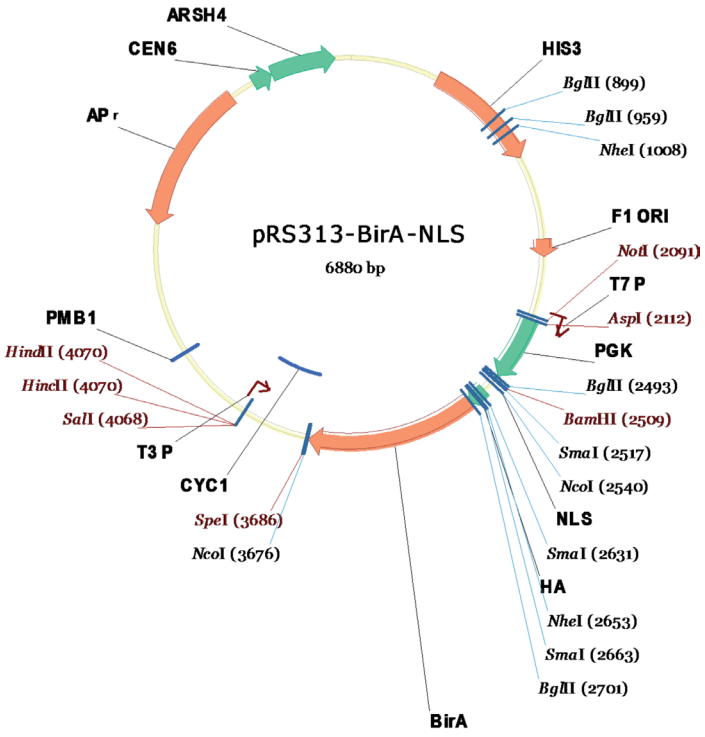

Figure 4
Plasmid map of pRS314-BirA

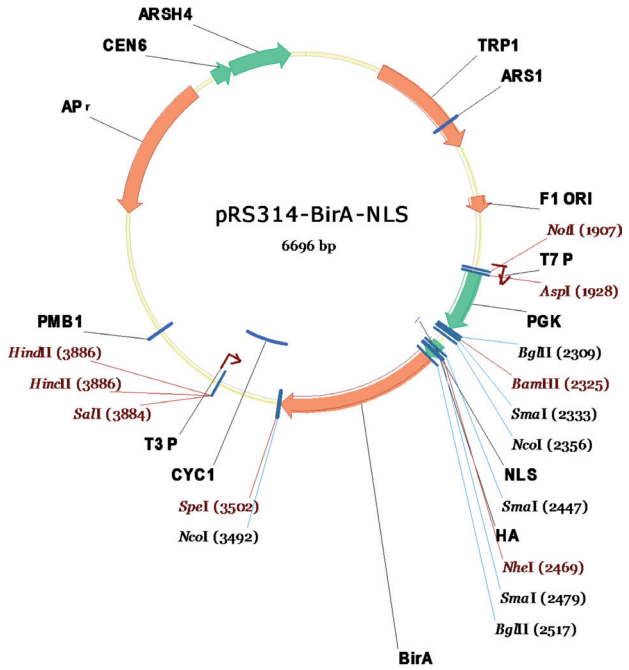

Figure 5
Plasmid map of pRS314-BirA-NLS (Diagenode cat. No.: tag-002)

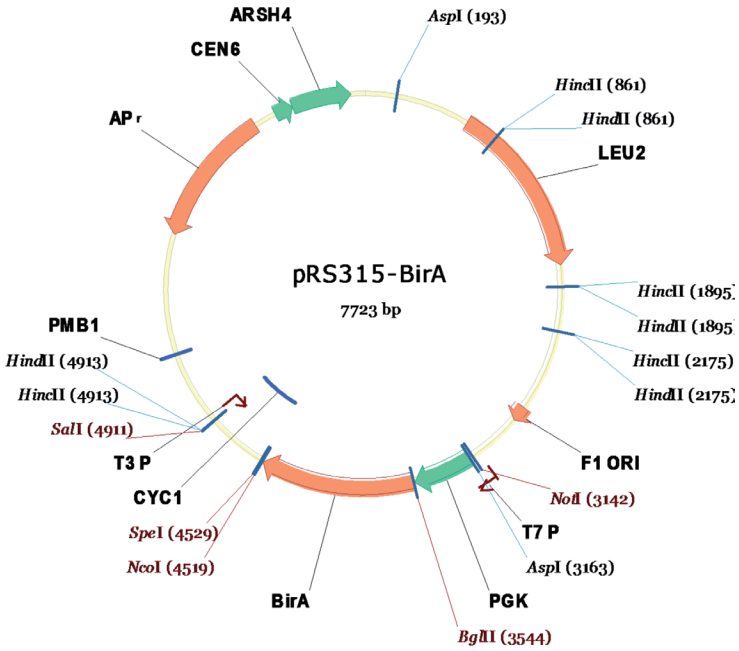

Figure 6
Plasmid map of pRS315-BirA (Diagenode cat. No.: tag-009)

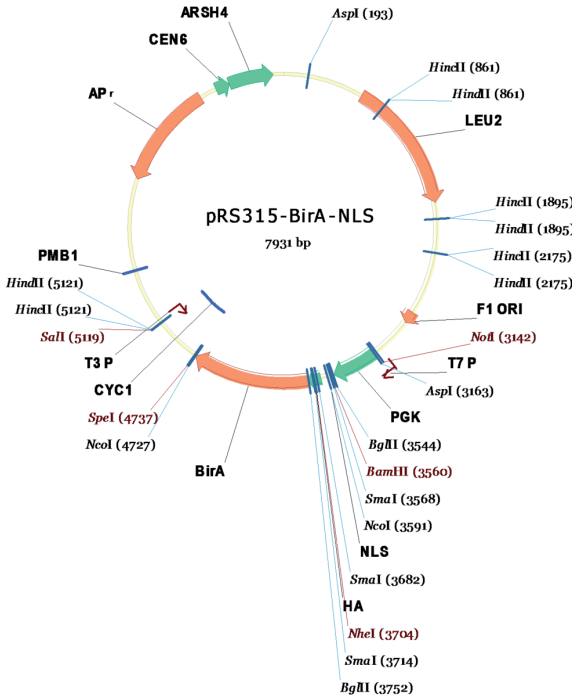

Figure 7
Plasmid map of pRS315-BirA-NLS (Diagenode cat. No: tag-003)

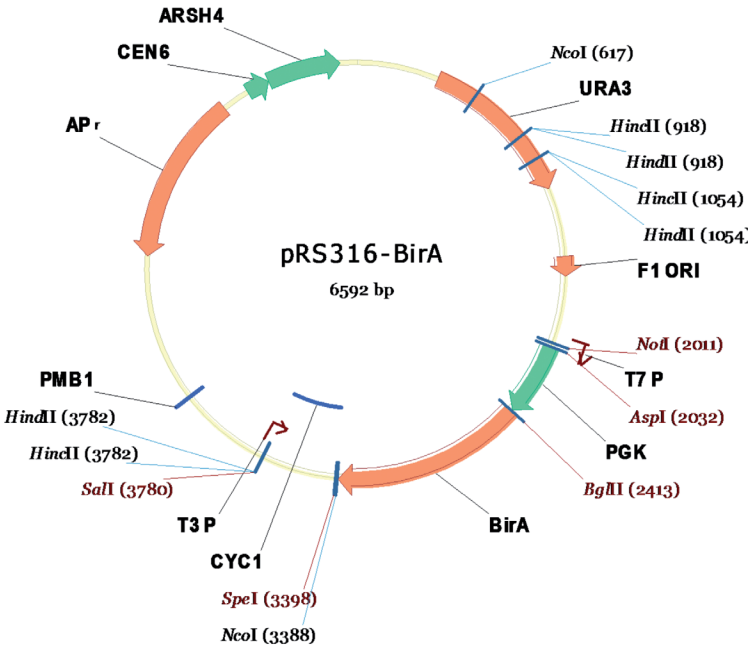

Figure 8
Plasmid map of pRS316-BirA (Diagenode cat. No.: tag-007)

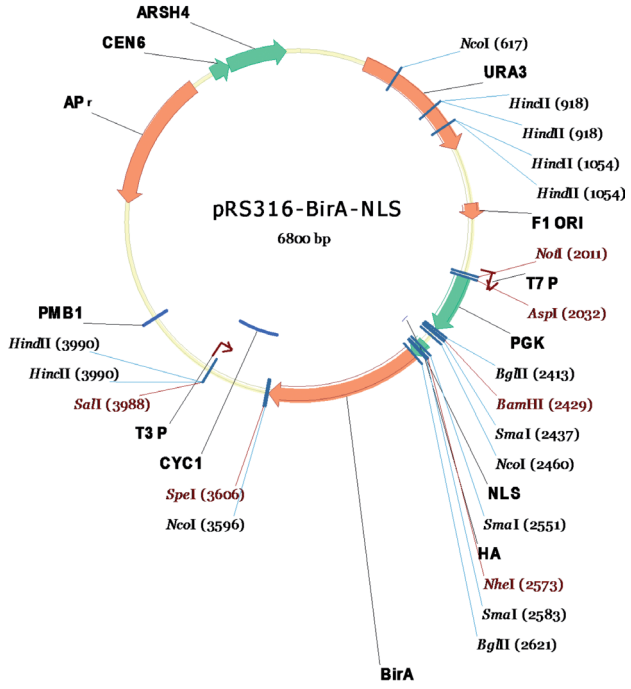

Figure 9
Plasmid map of pRS316-BirA-NLS (Diagenode cat. No.: tag-004)

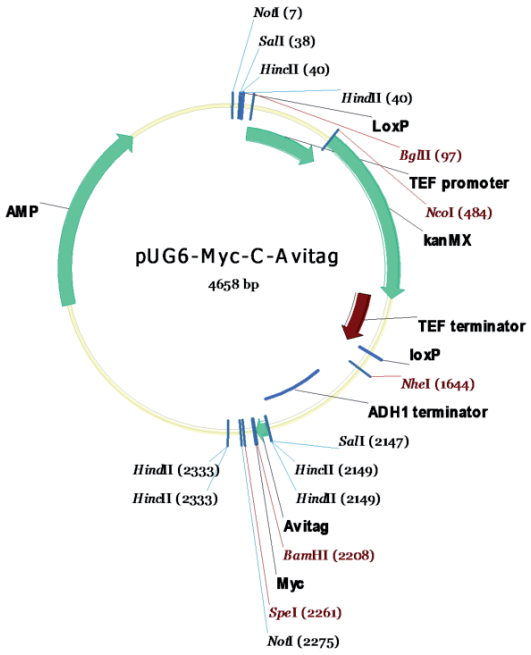

Figure 10
Plasmid map of pUG6-Myc-C-Avitag (Diagenode cat. No.: tag-005)

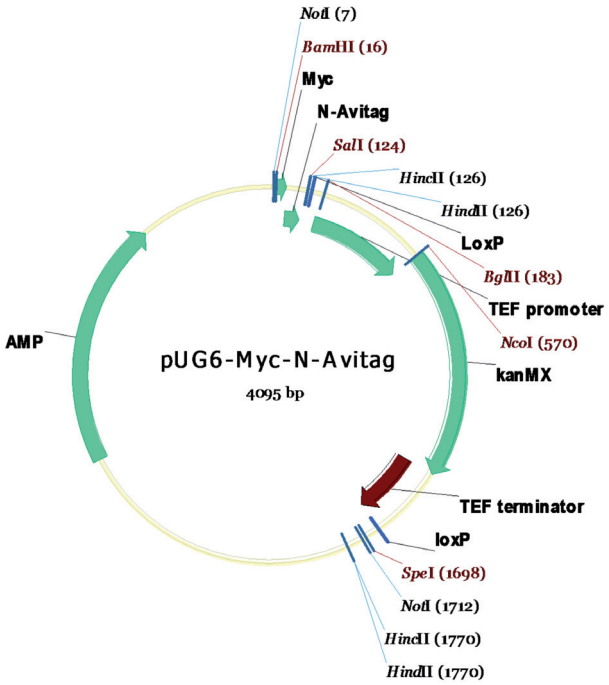

Figure 11
Plasmid map of pUG6-Myc-N-Avitag (Diagenode cat. No.: tag-006)