TECHNICAL MANUAL

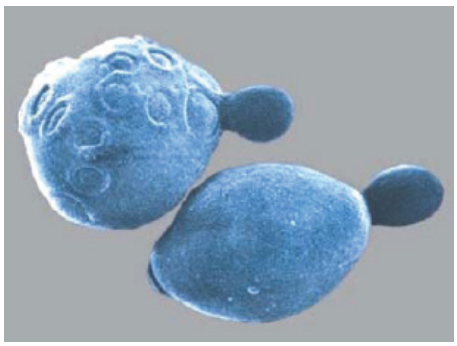
horizon™
INSPIRED CELL SOLUTIONS

Yeast ORF collection

Cat. #YSC3867 (clone,*E. coli*), #YSC3868 (collection, *E. coli*)
Cat #YSC3869 (clone, yeast), #YSC3870 (collection, yeast)

Introduction

Developed by Eric Phizicky (University of Rochester) and Mike Snyder (Yale University)[1], the Yeast ORF Collection enables robust protein expression and purification for over 4,900 *S. cerevisiae* genes. Each plasmid construct is available in yeast or *E. coli* format as a glycerol stock.



Yeast opens reading frames (ORFs) have been cloned into Gateway™ destination vectors, fully sequenced, and transformed into and yeast hosts. Each ORF is under the control of a GAL1 promoter and fused on the C-terminus to a tandem affinity tag that includes a hemagglutination (HA) tag. Successful yeast transformants have been verified to express protein of the correct length (including the 19-kDA tandem fusion tag) by western blotting.

Product description

E. coli

Ampicillin resistant
DH5α F^- Φ80 *lac*ZΔM15Δ(lacZYA-*arg*F) U169 deoR recA1 endA1 hsdR17 (r_K^- mK^+) *pho*A supE44 λ^- thi-1 *gyr*A96 *rel*A1

Yeast

URA3 (multicopy 2-micron) identical to yeast ChrV 116011-117048. The URA3 ORF in BG1805 is yeast ChrV:116167 to 116970. This means the vector also contains 155 base pairs upstream of the ATG translation start of URA3 and + 78 base pairs downstream of the translation termination site.

Y258 is MATa, pep4-3, his4-580, ura3-52, leu2-3,112

The vector is derived from pRSAB1234 from Erin O'Shea.

Their vector was modified to BG1805 by Beth Grayhack as follows:

1. The C-terminal fusion tag was modified.
2. The vector was converted to a Gateway™ destination vector

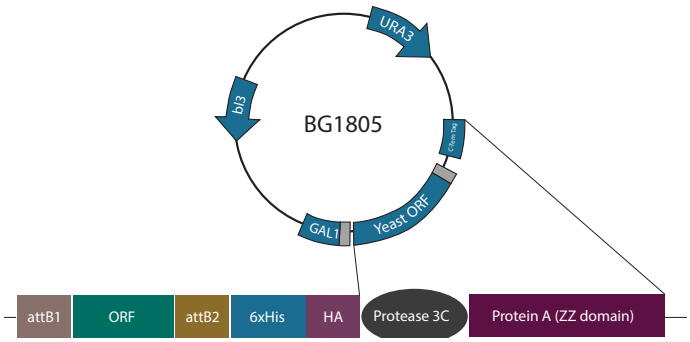


Figure 1. Schematic map for vector BG1805.
Note: When an individual ORF replaces the Gateway™ cassette, the size of the resulting vector will vary depending on the size of the ORF.

***GAL*1 promoter in BG1805 is identical to yeast *Chr*II bp 278,565-279048.**

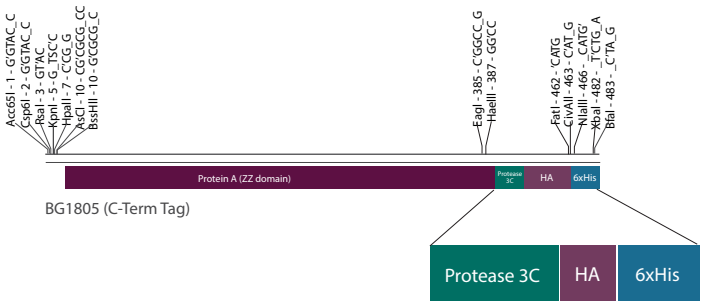


Figure 2. Information on the C-terminal fusion tag. Translation: A C-terminal fusion tag is present in the BG1805 vector The Protein A ZZ domain is derived from *Staphylococcus aureus*. The amino acid sequence of the fusion tag is shown below: His6, HA epitope, protease 3C site, ZZ domain.
Note: This is a translation of the reverse complement of the vector sequence.

6xHis — HA-Epitope — Protease 3C

H H H H H H G R I F Y P Y D V P D Y A G L E V L F Q G P G P S A V D N K

F N K E Q Q N A F Y E I L H L P N L N E E Q R N A F I Q S L K D D P S Q

ZZ-Domain

S A N L L A E A K K L N D A Q A P K V D N K F N K E Q Q N A F Y E I L H

L P N L N E E Q R N A F I Q S L K D D P S Q S A N L L A E A K K L N D A

Q A P K V D A N H Q Stop

Storage

- 4 °C for up to one week
- –80 °C indefinitely

Growth medium

Yeast ORF expression ready clones should be grown in SD–ura medium without any antibiotics. Long-term storage of strains should be stored in SD-ura medium containing 15% glycerol.

Grow a liquid culture in SD-ura overnight at 30 °C with shaking. Then, the cells are centrifuged:

- SS34 rotor: 5,000 rpm for 5–10 minutes
- Microcentrifuge: 2 minutes at top speed 3
- Sorval 96-well deep-well plates: 1500–2000 for 10 minutes
- The clear medium is removed and the cells are resuspended in SD-ura. The expression plasmid is selected for by growth in the absence of uracil. The URA3 gene is present on the plasmid and not in the chromosome. Thus, the yeast will not grow in medium lacking uracil if the plasmid is lost. Furthermore, expression of the ORF-fusion protein is repressed in glucose (D in SD and in YPD), so there should be little selection against any clone due to the identity of the ORF in the clone.
- Make a concentrated mixture of solids including yeast nitrogen base, ammonium sulfate and selected amino acids and purine or pyrimidine bases.

Table 1. S powder-ura.

Component	15 LIters	60 Liters
YNB w/o a.a, w/o A.S.	25.1 g	100.4 g
Ammonium sulfate	75.4 g	301.6 g
Isoleucine	450 mg	1.8 g
Valine	2.25 g	9.0 g
Adenine	300 mg	1.2 g
Arginine	300 mg	1.2 g
Histidine	300 mg	1.2 g
Leucine	450 mg	1.8 g
Lysine	450 mg	1.8 g
Methionine	300 mg	1.2 g
Phenylalanine	750 mg	3.0 g
Tryptophan	300 mg	1.2 g
Tyrosine	450 mg	1.8 g

YNB w/o a.a, w/o A.S = yeast nitrogen base without amino acids and without ammonium sulfate Difco: Ref 233520. All aminoacids are L amino acids and obtained from Sigma (98–99% purity).

SD-ura medium, 1 liter

S powder–ura	7.14 g
Dextrose	20 g
dH_2O	to 1 L

YPD medium, 1 liter

Yeast extract	10 g
Peptone	20 g
Dextrose	20 g
dH_2O	to 1 L
Agar (for plates)	20 g

The yeast ORF *E. coli* clones are grown in Terrific Broth (TB), with 0.1 mg/mL ampicillin, 30 °C with shaking. For long term storage, strains should be placed in TB medium containing 8% glycerol and stored at –80 °C.

TB medium, 1 liter

Tryptone	12 g
Yeast extract	24 g
Glycerol	4 mL
KH_2PO_4	2.31 g
K_2HPO_4	12.54 g
dH_2O	to 1 L

Western blotting

Table 2. Growth & western blot of PGAL- att-ORF-att-His6 HA 3C site ZZ clones.

Material	Vendor	Cat #
ECL Plus Western Blotting	Amersham Biosciences	RPN2132
0.5-mm Zirconia/Silica Beads	Biospec Products, INC.	11079105z
Calf Serum	Invitrogen (Gibco)	1601-0159
Goat anti-rabbit	Thermo Scientific	SAB1003
Rabbit anti-HA	Thermo Scientific	CAB3872
Complete Mini, EDTA-free Protease Inhibitor	Roche	1873580

3x YP + 6% galactose: per liter

Yeast extract	30 g
Peptone	60 g
H_2O	700 mL

Autoclave

Add 300 mL sterile 20% Galactose

Western blot lysis buffer

Tris-Cl pH 7.5	50 mM
EDTA	1 mM
Triton X- 100	0.5%
DTT	1 mM
NaCl	1 M
1X Complete Mini	pH 7
Pepstatin	2.5 ug/mL

YP galactose induction MORF growth protocol

1. Set up 5 mL SD-URA culture from single colony for overnight growth at 30 °C, shaking.
2. Inoculate a 25 mL -URA, 2% raffinose media with 1 mL of 5 mL overnight growth culture. Grow overnight at 30 °C shaking.
3. Dilute into 200 mL -URA, 2% raffinose media at starting OD_{600} = 0.3. Grow at 30 °C, shaking.
4. At OD_{600} = 1.2, add 100 mL 3x YP, 6% galactose, for a final concentration of 1x YP, 2% galactose.
5. Harvest after 6 hours*.
   - Spin 5 K rpm for 10 minutes at 4 °C.
   - Take off supernatant and wash the pellets with 5 mL cold water.
   - Transfer to a new 14-mL tube and spin 5K for 10 minutes at 4 °C.
   - Take off supernatant, quick freeze, and store in the -80 °C freezer.

   ** For Western Blot, harvest 2 mL culture separately.*

Protocol can be adjusted to 96-well format using a 1–2 mL culture.

Western blot—lysis buffer -> SDS loading buffer method

Method based on 96-well plate format

1. Add zirconium beads to a tube with cell pellets from 2 mL harvested cells.
2. Add 50 µL Lysis buffer.
3. Vortex at 4 °C for 10 × 30 sec with 1–minute ice water bath between vortex.
4. At room temp, add 50 µL boiling SDS 2x loading dye + BME.
5. Boil for 5 minutes at 100 °C.
6. Spin at 3 K at room temp for 5 minutes.
7. Load 10 µL on a SDS-PAGE gel.
8. Transfer proteins and Western Blot using Rabbit anti-HA (1˚) and Goat anti-Rabbit (2˚).
9. Detected with ECL Plus kit

Note

We provide certain clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other commercial entities. In order to provide these as a public resource, we depend on the contributing academic laboratories for quality control.

Therefore, these are distributed in the format provided by the contributing institution "as is" with no additional product validation or guarantee. We are not responsible for any errors or performance issues. Additional information can be found in the product manual as well as in associated published articles (if available). Alternatively, the source academic institution can be contacted directly for troubleshooting.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

horizon™
INSPIRED CELL SOLUTIONS

V30818