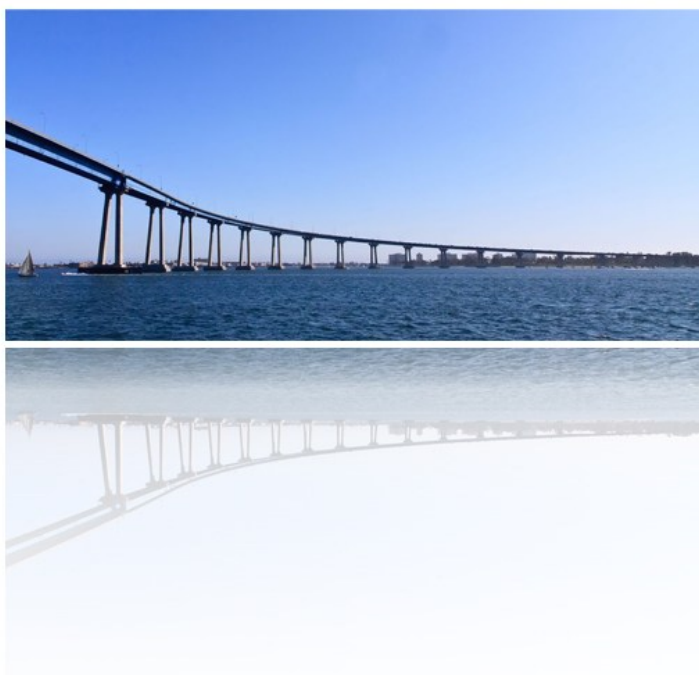




EBY100 Yeast Cells

INSTRUCTION MANUAL

EBY100 Yeast Cells for Yeast Display

Catalog #: YC001

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Description

Product Description

EBY100 is a specialized *Saccharomyces cerevisiae* yeast strain engineered specifically for yeast surface display and protein engineering. Derived from the BJ5465 background, it features a stable integration of the *AGA1* gene under control of the galactose-inducible *GAL1* promoter. This genetic architecture allows for the high-density display of proteins on the cell surface upon induction, providing a robust platform for high-throughput screening and directed evolution via fluorescence-activated cell sorting (FACS).

The display mechanism utilizes the yeast Aga1p-Aga2p mating adhesion system. In this system, the integrated *AGA1* gene of the strain produces the Aga1p protein, which is covalently anchored to the yeast cell wall. When a display vector expressing an Aga2p-fusion protein is introduced (i.e., via yeast display vectors such as the pYDL series vectors), the Aga2p subunit forms two disulfide bonds to the surface-anchored Aga1p during secretion, effectively tethering the target protein to the exterior of the cell, which remains accessible for binding assays and kinetic analysis.

To ensure selection and maintenance of genetic payloads, EBY100 utilizes tryptophan and leucine auxotrophy by a *trp1* mutation and *leu2* deletion. Transformants, either clonal lines or combinatorial libraries, are isolated by their ability to grow on synthetically defined (SD) media lacking tryptophan or leucine. This selective pressure ensures that only cells harboring the *TRP1* or *LEU2*-containing display plasmid survive, which aids in maintaining stringency and genetic stability throughout induction and screening phases of yeast display.

Components

PRODUCT:	EBY100 yeast cells <i>MATa aga1::GAL1-AGA1::URA3 ura3-52 trp1 leu2-delta200 his3-delta200 pep4::HIS3 prb1-delta1.6R can1 GAL</i>
CATALOG NUMBER:	YC001
QUANTITY:	1 x 0.5 ml
TITER:	50 OD ₆₀₀ /ml, >2E8 cfu/ml
DOUBLING TIME:	90 min at 30°C and 250 rpm in YPD medium

Reagents, consumables, and equipment to be supplied by user

<i>Reagents:</i>	YPD medium, YPD agar plates, SD dropout medium and agar depending on preferred selection marker(s).
<i>Consumables:</i>	5-ml culture tubes, 125-ml baffled-bottom shake flasks, sterile pipettes, microfuge tubes, cuvettes, sterile tips with air barrier.
<i>Equipment:</i>	Personal protection equipment (e.g., lab coat, gloves, goggles), Bunsen burner, manual pipettors, spreaders, a microcentrifuge, spectrophotometer.

Storage conditions

Store immediately at -80°C in the original container.

For research use only; not intended for any animal or human therapeutic or diagnostic use.

Protocols

Procedures

Working with yeast cells

Always keep the cells at -80°C in their original packaging. When needed, take out a tube, incubate at 30°C in a water bath until just thawed and immediately transfer a small amount (e.g., 50 µl up to all) of the contents to a plate or culture flask with 1/5 volume of YPD (e.g., 25 mL in 125 mL flask). Incubate inoculated media at 30°C with agitation at 250 rpm (shake flasks) or 300 rpm (culture tubes). When done, put the cells back in their original packaging at -80°C. Continue incubation until visible growth after 1-2 days of incubation. Colonies on YPD appear as round white colonies, and cells are ovoidal in shape with visible budding daughter cells. Repeated freeze-thaw cycles will significantly reduce cell viability. Plates and cultures can be stored at 4°C up to a month.

Biopanning by yeast surface display typically involves successive rounds of selection and enrichment using FACS. The process begins by inducing the expression of the variant library, allowing the target proteins to be displayed on the yeast cell surface. The induced library is then incubated with a specific target under defined stringency conditions. Yeast cells that successfully bind the target are then labeled with fluorophores and isolated from the broader population using FACS. The sorted cells are subsequently expanded in culture to create a sublibrary for the next round of enrichment.

The following procedures detail the methods for handling EBY100 for the purpose of yeast display.

Procedure 1. Yeast Transformation

Ensure the correct, in-frame insertion of your gene of interest into a yeast display vector (e.g., pYDL series) prior to transforming the EBY100 yeast strain. Incorrect cloning will result in failed display. The user should also be familiar with yeast molecular biology and microbiological techniques.

For detailed methodologies on general yeast techniques, refer to *Budding Yeast: A Laboratory Manual* (Andrews et al., 2016). For comprehensive protocols on yeast display applications, consult *Yeast Surface Display: Methods, Protocols, and Applications* (Traxlmayr, 2023). Transform EBY100 with commercial kits or common transformation yeast transformation methods (i.e., lithium Acetate). We recommend Frozen-EZ Yeast Transformation II Kit (Zymo Research) to prepare competent yeast for transformation. Competent cells can also be frozen for future use.

Procedure

1. Inoculate 5 ml of LB supplemented with carbenicillin with single *E. coli* colonies containing pYDL vector with sequence of interest and incubate at 37°C with shaking overnight.
2. Isolate plasmid DNA for yeast transformation by Miniprep of *E. coli* culture following method of choice.
3. Prepare fresh competent EBY100 yeast following the Zymo Research Frozen-EZ yeast Transformation II Kit protocol or thaw vials of cells from a previously prepared frozen stock on ice.
4. Transform competent cells following the manufacturer's protocol. Incubation time can be increased from 45 min to 2-3 h for increased transformation efficiency.
5. Plate transformation on SDCAA plate (see recipe in appendix) without tryptophan for selection of transformants. Incubate plate at 30°C for 2 to 4 days until single colonies appear.
6. Isolate transformants to test display of fusion proteins after induction.

Procedure 2. Yeast Library Generation

Yeast libraries are generated via homologous recombination, an efficient naturally occurring DNA repair mechanism in yeast. EBY100 is made electrocompetent by treatment with lithium Acetate and DTT to condition the cells. Linearized vector backbone and library inserts are then transformed into the electrocompetent yeast via electroporation.

Procedure

Preparation of electrocompetent cells:

1. Inoculate a single colony of EBY100 into 50 mL of YPD medium in a 250 mL baffled shake flask and grow the culture overnight at 30°C with shaking at 250 rpm.
2. Seed 500 mL of fresh YPD with the overnight culture to reach an initial OD₆₀₀ of 0.2-0.3.
3. Incubate the flask at 30°C until the cells reach log phase growth at an OD₆₀₀ of 1.6-1.8.
4. Harvest the cells by centrifugation at 3,000 x g for 5 min at 4°C.
5. Wash cells by resuspending in 100 mL of ice-cold sterile water.
6. Centrifuge as before and wash cells with 100 mL of ice-cold electroporation buffer (1 M Sorbitol, 1 mM CaCl₂)
7. Pellet and resuspend washed cells in 50 mL of conditioning buffer (100 mM lithium Acetate, 10 mM DTT).
8. Incubate cell suspension for 30 min at 30°C with gentle shaking at 225 rpm to condition cells.
9. Pellet conditioned cells and wash them with 100 mL of ice-cold electroporation buffer.
10. Resuspend final pellet in electroporation buffer to an OD₆₀₀ of 160 (~1.6 x 10⁹ cells/mL) and keep on ice until ready for electroporation.

Electroporation

1. For each electroporation, mix 400 µL of electrocompetent yeast cells with 12 µg of insert DNA and 4 µg of linearized vector backbone (3:1 insert to backbone mass ratio) in a pre-chilled 0.2 cm gap electroporation cuvette and incubate on ice for 5 min.
2. Place the cuvette into an electroporator and deliver a pulse at 2.5 kV, 25 µF, and 200 Ω. The time constant for the electroporation pulse should range between 3.0 and 4.5 ms.
3. Add 1 mL of ice-cold 1 M Sorbitol to the cuvette immediately and transfer to flask containing 1:1 mixture of 1 M Sorbitol and YPD medium.
4. Incubate the cells at 30°C for 1 h with gentle shaking at 250 rpm to allow cells to recover.
5. Pellet culture and resuspend in SDCAA (SD-trp) medium for library expansion.
6. Remove an aliquot of the culture for serial dilutions on SDCAA to estimate library size and calculate total transformation efficiency,
7. Passage library in SDCAA, ensuring 10-fold diversity number of viable yeast cells are transferred to the new culture, to remove dead non-transformed yeast and enrich the culture for transformants before cryopreservation or induction for subsequent sorting.

Notes

- ❖ EBY100 doubling time in rich YPD media is approximately 90 min under ideal conditions, approximately 5 h of incubation is required to reach the target OD₆₀₀.
- ❖ Incubating yeast with the conditioning buffer longer than 30 min significantly decreases transformation efficiency.
- ❖ Ensure high-quality purified DNA fragments are used in electroporation by performing an ethanol precipitation to remove residual salts that could otherwise interfere with electroporation.

Procedure 3. Induction Conditions & Control of Expression

The expression of both Aga1p and the Aga2p fusion protein on common yeast display vectors (e.g., pYDL, pCTcon2) in EBY100 is regulated by the *GAL1* promoter. The *GAL1* promoter is a highly regulated genetic switch in yeast, governing the expression of genes based on available carbon source. This promoter is tightly repressed in the presence of glucose in the medium and strongly activated by galactose (in the absence of glucose). EBY100 transformants are grown using glucose as a carbon source before switching to galactose for induction.

Procedure

The general induction protocol is as follows:

1. Inoculate a single yeast colony into 4 ml of SDCAA containing 2% glucose in a culture tube and grow overnight at 30°C with shaking until OD₆₀₀ reaches 6-8. Alternatively, passage 10-fold the diversity of the library number of yeast cells into sufficient volume of media in a baffled shake flask as to not exceed an OD₆₀₀ of 0.5.
2. Pellet cells at 2,000 x g for 3 min and resuspend cells in fresh SDCAA to an OD₆₀₀ of 1. Continue growing the culture at 30°C with shaking until OD₆₀₀ reaches 2-5, approximately two doubling events. If inducing a library, ensure 10-fold the library in yeast cells are passaged to prevent loss of variants during passaging.
3. Centrifuge cells as before, aspirate supernatant and wash cells with PBS to remove traces of glucose.
4. Pellet cells again and resuspend cells in SGCAA (see recipe in appendix) to an OD₆₀₀ of 1. Induce the culture by incubating at 20°C or 30°C (see notes) while shaking overnight.
5. Induced cells can be analyzed immediately by surface staining or stored at 4°C for several weeks.

Notes

- ❖ One OD₆₀₀ yeast is approximately 1×10^7 cells/mL, when measuring a yeast, it is important to dilute the culture to an OD₆₀₀ of < 1.0 for an accurate measurement. Diluting an overnight culture grown in YPD 1:10-20 is recommended.
- ❖ Aeration in yeast culture is essential. Baffled-bottom shake flasks should be filled to 1/5 maximum volume and agitated at 250 rpm to ensure adequate oxygen exchange. Culture tubes should be agitated at 300 rpm.
- ❖ If bacterial contamination is a concern, yeast media can be supplemented with antibiotics (e.g., 100 µg/mL carbenicillin) to select for yeast growth.
- ❖ Growth conditions and type of scaffold affect expression and display on yeast. Induction at 20°C aids the surface expression of less thermally-stable fusions. However, induction incubation temperature can be raised to 25-37°C to select for stable clones.
- ❖ Induction time can be increased up to 72 h with optimal time between 12-48 h. We recommend assaying display over 48 h by aliquoting and staining 1 OD₆₀₀ equivalent number of induced cells at each time point.

Procedure 4. Detection of Displayed Proteins

Display of the Aga2p fusion protein on the yeast surface is achieved through antibody staining, utilizing the specific epitope tag encoded by display vector (e.g., HA for pYDL-12 and pYDL-22 or c-Myc for pYDL-13 and pYDL-23). These tags can be detected with fluorophore-conjugated secondary antibodies.

For proper experimental controls, we recommend including uninduced cells as background control, and untransformed yeast as a negative control for non-specific staining.

To analyze display:

1. Pellet aliquoted yeast in a microcentrifuge tube at 2,000 x *g* for 3 min. Aspirate supernatant and wash with 1 ml of PBS supplemented with 1 mg/ml BSA (PBSA). Repeat wash a second time.
2. Resuspend in 250 µl of PBSA supplemented with 1 µg/ml anti-HA or anti-c-Myc primary antibody.
3. Incubate cells with primary antibody for 0.5 – 1 h at room temperature with periodic mixing or gentle rotation.
4. Pellet cells and wash three times with PBSA as before.
5. Resuspend cells in 250 µl of PBSA supplemented with 2 µg/ml secondary antibody conjugated with fluorescein isothiocyanate (FITC) or any other suitable fluorophore.
6. Incubate cells with secondary antibody for 15-30 min at 4°C with periodic mixing or gentle rotation.
7. Pellet cells and wash three times with PBSA as before.
8. Resuspend cells in 500 µl PBSA for analysis by fluorescent microscopy or flow cytometry.

Notes

- ❖ Keep fluorescently-labeled cells in the dark on ice prior to analysis to minimize photobleaching and remove clumps by filtering cells through cell-strainer capped round-bottom tubes compatible with the flow cytometer.
- ❖ In optimal conditions, up to 70% of the total cells are easily detectable as displaying the fusion protein by flow cytometry. However, display varies greatly and is highly dependent on the fusion protein and induction conditions. Little to no fluorescence should be observed in uninduced or untransformed yeast.
- ❖ Alternatively, perform all incubation steps at 4°C if stability of displayed fusion protein is a concern.

Procedure 5. Equilibrium Sort by FACS

An equilibrium sort is a high-throughput selection method used to isolate specific yeast clones displaying proteins (e.g., antibodies) with high binding affinity to a target antigen.

By incubating a yeast library with biotinylated antigen and allowing the reaction to reach a steady state (equilibrium), binders are identified and can be separated by fluorescent labeling. This process enables the precise enrichment of full-length, high-affinity clones from a diverse population.

Before you begin:

- ❖ Confirm the yeast display library or sublibrary is $\leq 1 \times 10^7$ in size before proceeding with FACS. Libraries with higher diversity should be sorted by magnetic-assisted cell sorting (MACS) in order to reduce the diversity to a smaller size suitable for analysis and sorting by FACS.
- ❖ Select a target antigen concentration, using 1 µM as a standard starting point for naïve libraries where the K_D is unknown. Alternatively, use the K_D of the original protein (in the case of a maturation library) as a starting point to enrich for binders equal to or better than the original. Rounds of sorting commonly proceed with a 10-fold dilution of the concentration used in the previous round to select for higher-affinity binders.
- ❖ Calculate the minimum incubation volume using the following formula to ensure the antigen remains in a 10-fold molar excess relative to the estimated displayed binders (assuming 10^5 copies of the fusion protein on the surface of each yeast cell).

$$\circ \quad V_{min}(ml) = \frac{10 \cdot 10^5 \cdot N_{yeast}}{C_{Ag} \cdot N_A} \cdot 1000$$

- N_{yeast} = number of total yeast cells (equivalent to 10-fold diversity of library/sublibrary)
- C_{Ag} = Molar concentration of antigen
- N_A = Avogadro's number (6.022×10^{23})

Procedure

1. Transfer 10-fold the maximum library or sublibrary diversity of induced cells to a microcentrifuge tube and pellet cells by centrifugation at $2,000 \times g$ for 3 min.
2. Aspirate supernatant and resuspend in 1 mL of 0.1% w/v BSA in PBS (PBSA). Repeat PBSA wash an additional time.
3. Resuspend cells in PBSA supplemented with anti-epitope tag antibody (e.g., murine anti-HA, or anti-c-Myc) at a final concentration of $1 \mu\text{g/ml}$ and the desired concentration of biotinylated antigen.
4. Incubate the cells at room temperature, or 4°C if antigen stability is a concern, with gentle rotation for a sufficient time to reach equilibrium. Three hours is usually sufficient for naïve libraries, for antigen concentrations $< 10 \text{ nM}$ or below 1 nM , incubate 16-20 h to reach equilibrium.
5. Pellet and wash cells as three times with PBSA to remove unbound labeling reagents.
6. Resuspend cells in PBSA supplemented with secondaries for detection, including anti-murine IgG antibody conjugated with FITC or Alexa Fluor 488 to detect display, and a fluorophore-conjugated streptavidin (e.g., Alexa Fluor 647), each at a final concentration of $2 \mu\text{g/mL}$.
7. Incubate cells with secondaries for 15-30 min at 4°C with gentle rotation protected from light.
8. Pellet and wash cells as three times with PBSA to remove unbound detection reagents.
9. Resuspend in $500 \mu\text{L}$ PBSA for immediate sorting, filter through a $40 \mu\text{m}$ cell-strainer to remove any clumps.
10. Analyze cells by flow cytometry and sort double positive events using a diagonal gate (to include all clones with demonstrated binding regardless of the level of surface expression. Sort cells directly into collection tubes containing SDCAA media. The number of events collected is the maximum diversity of the sublibrary.
11. After the sort, transfer collected cells to 25 mL of SDCAA in a 125 mL baffled flask, rinsing the collection tube with additional media to ensure adequate transfer.
12. Allow the sorted cells to recover by incubating at 30°C with shaking at 250 rpm until saturation, approximately 1-2 days.
13. Passage cells in fresh SDCAA media, ensuring 10-fold the maximum diversity of the sublibrary count of cells are transferred to the new culture.
14. Induce sublibrary and repeat sorting as desired.

Recipes

Yeast Peptone Dextrose medium (YPD)

1. Dissolve the following in 900 mL deionized water:
 - a. 10 g yeast extract
 - b. 20 g peptone
 - c. 20 g agar (optional, for preparing solid media)
2. Autoclave solution and cool to 50-60°C.
3. Separately, dissolve 20 g glucose in 100 mL deionized water, sterilize solution by filtration.
4. Gently mix sterilized liquid media with filter-sterilized 20% glucose.
5. Store at 4°C for 1 – 2 months.

Conditioning Buffer

1. Prepare 1 M Lithium Acetate stock (10X Stock)
 - a. Dissolve 10.2 g of Lithium Acetate in 80 mL of deionized water.
 - b. Adjust to final volume of 100 mL with deionized water.
 - c. Filter sterilize the solution and store at room temperature.
2. Prepare 1 M DTT (100X Stock)
 - a. Dissolve 1.54 g Dithiothreitol (DTT) in 10 mL of sterile deionized water.
 - b. Aliquot solution into microcentrifuge tubes to avoid repeated freeze-thaw cycles.
 - c. Store aliquots at -20°C for up to a month.
3. Combine the following in a 50 mL conical tube:
 - a. 44.5 mL of sterile deionized water
 - b. 5 mL of 1 M Lithium Acetate stock
 - c. 0.5 mL of 1 M DTT, thawed on ice
4. Mix well and use buffer within 1-2 h.

Synthetic Dextrose medium with CasAmino Acids (SDCAA), pH 4.5

6. Dissolve the following in 1 L deionized water:
 - a. 20 g dextrose
 - b. 6.7 g yeast nitrogen base
 - c. 5 g casamino acids
 - d. 10.4 g sodium citrate
 - e. 7.4 g citric acid monohydrate
7. Sterilize by filtration
8. Store at 4°C for 1 – 2 months.

SDCAA agar plates, pH 6.0

1. Prepare an agar solution by dissolving the following in 900 ml deionized water:
 - a. 182 g sorbitol
 - b. 15 g agar
 - c. 8.56 g sodium phosphate monobasic monohydrate
 - d. 5.4 g sodium phosphate dibasic anhydrous
2. Autoclave and cool the agar solution to 60°C.
3. Prepare a nutrient mixture by dissolving the following in 100 ml deionized water.
 - a. 20 g dextrose
 - b. 6.7 g yeast nitrogen base
 - c. 5 g casamino acids
4. Sterilize nutrient solution by filtration.
5. Gently mix nutrient solution into the warmed agar solution and pour plates.
6. Store at 4°C for 1 – 2 months.

Synthetic Galactose medium with CasAmino Acids (SGCAA)

1. Dissolve the following in 1 L deionized water:
 - a. 20 g galactose
 - b. 2 g dextrose
 - c. 6.7 g yeast nitrogen base
 - d. 5 g casamino acids
 - e. 8.56 g sodium phosphate monobasic monohydrate
 - f. 5.4 g sodium phosphate dibasic anhydrous
2. Sterilize by filtration
3. Store at 4°C for 1 – 2 months.

Appendix

MSDS information

MSDSs (Material Safety Data Sheets) are available on **Antibody Design Labs** website at the corresponding product page.

Quality Control

Specifications are detailed on the online product page. **Antibody Design Labs** certifies that the product will perform according to these specifications.

Limited Product Warranty

This warranty limits our liability to the replacement of this product. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Antibody Design Labs. Antibody Design Labs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Technical Support

Visit **Antibody Design Labs** website at www.abdesignlabs.com for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

For more information or technical assistance, call, write, fax, or email us at:

Antibody Design Labs
4901 Morena Blvd, Suite 203
San Diego, CA 92117

Email: support@abdesignlabs.com
Phone: 1-877-223-3104 (Toll Free)
Fax: 1-858-272-6007 (24 hour)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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