EZ-YEAST TRANSFORMATION KIT

30 min protocol

Cat# 2100-200 **200 preps**

- **Simple and fast with minimal hands-on time**
- **No need to make cells competent prior to transformation**
- **High throughput: can be easily adapted to 96 well format**
- **Ideal for simultaneous transformation of library and bait vectors into yeast two-hybrid reporter strains**
- **Transformation efficiency up to $1x10^3$ colonies per ug DNA**

Shipping and Storage:

The EZ-YEAST Transformation kit is shipped and stored at room temperature. Carrier DNA is stored at 4°C

January 2000

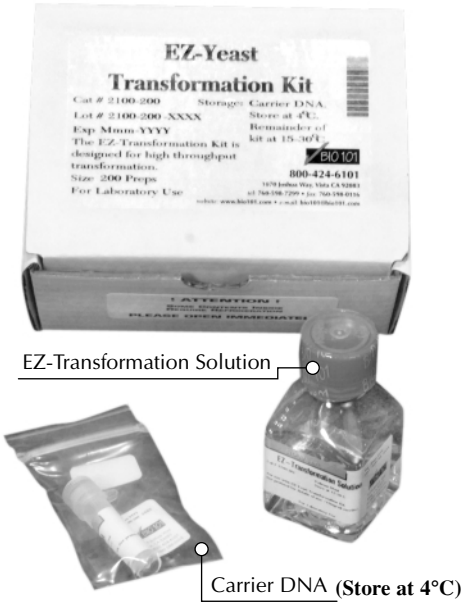
Revision # 2100-200-9K18

kit name

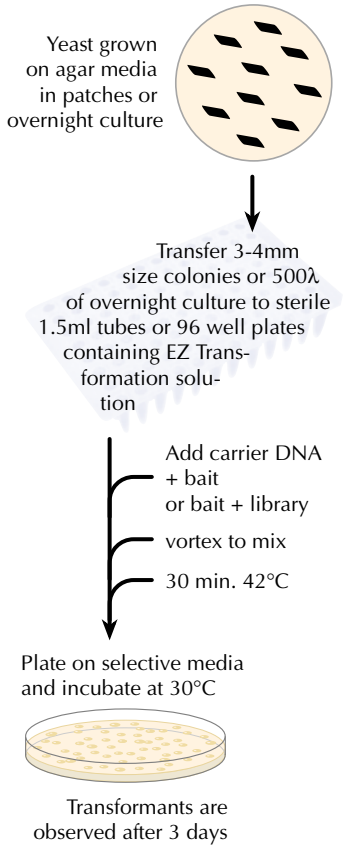
Kit Components
2100-200: 200 preps

Name	Volume	Catalog #
EZ-Transformation Solution	**28 ml**	**2100-201**
Carrier DNA*	**1.1 ml**	**2100-202**

* Stored at 4°C. All items in kit are shipped at ambient temperature.



EZ-Yeast Transformation Schematic Protocol



Introduction

The EZ-Yeast Transformation kit is designed for high throughput transformations. It is ideal for cases where a large number of transformations must be performed, but only a few transformants are needed. The EZ-Yeast Transformation kit does not require preparing competent cells prior to transformation. Simply resuspend yeast cells from an overnight culture or a fresh plate in EZ-Transformation solution. Add vector and carrier DNA. Incubate at 42°C for 30 min and plate on selective media. Lower temperatures can also be used (see Table 1).

The EZ-Yeast transformation kit is ideal for the simultaneous transformation of two-hybrid reporter strains with bait and library vectors when processing putative positive clones (Table 2). It is not designed for high efficiency transformation. However it is capable of achieving efficiencies of up to 1000 colonies per ug DNA. For high efficiency transformation use the Alkali Cation Yeast Transformation kit (cat# 2200-200) or the yeast spheroplast Transformation Kit (Cat # 2210-200)

Data

Table 1

		# of yeast transformants following incubation in EZ-Transformation solution:			
		Overnight	30 minutes		
Vector	**Media**	**25°C**	**42°C**	**37°C**	**30°C**
Bait	SD-trp	38	300	100	40
Library	SD-leu	4	100	25	19
Bait + Library	SD-trp-leu	0	21	8	2

The yeast two-hybrid strain SFY526 (Clontech) was transformed with bait (2 ug pVA3, 6.4 kb), library (2 ug pTDI, 15 kb) or both vectors (2 ug each) by incubating in EZ-Transformation solution in the presence of carrier DNA at the indicated conditions. Cells were plated on selective media and the number of transformants was recorded after 3 days incubation at 30°C.

Table 2

2a

		# of yeast transformants following incubation in EZ-Transformation solution for 30 min at 42°C	
Vector	**Media**	**HF7c**	**Y190**
Bait	SD-trp	100	100
Library	SD-leu	60	30
Bait + Library	SD-trp-leu	34	16

2b

Vector	**Media**	**EGY48**
Bait	SD-his	100
Library	SD-trp	150
Bait + Library	SD-his-trp	13

The yeast two-hybrid strains HF7c, Y190 (Clontech; 2a) and EGY48 (Mobitech; 2b) were transformed as in Table 1. Bait and library for HF7C and Y190 are pVA3 and pTDI respectively. Bait and library for EGY48 are pEG202-P53 (10 kb) and pJG-4-5-LTA (8 kb).

Protocol

Single vector transformation

Starting material: liquid culture

! = read me first

1. Inoculate a single isolated yeast colony into 1 ml of rich media (YPD) or minimal dropout media (SD-amino acid) and grow at 30°C with shaking until OD_{600} >1.

- OD_{600} > 1: Overnight culture if using YPD. 1-3 days culture if using minimal media inoculated with a single colony; increase initial inoculum to get a denser overnight culture for slow growers.

! Use BIO 101 Molecular Biology Certified™ Yeast media to improve growth and transformation efficiency (see page 12).

2. Transfer 500 ul of cells to sterile 1.5 ml centrifuge tubes or deep 96 well plates. Spin 10 seconds if using a microcentrifuge and 2-3 minutes if spinning 96 well plates to pellet the cells. Decant the supernate and shake once or twice to remove the majority of the media.

! Effective removal of culture media improves transformation efficiency.

3. Add 125 ul **EZ-Transformation solution,** 2 ug of plasmid DNA and 5 ul **Carrier DNA**. Resuspend the cells by vortexing at moderate to maximum speed.

4. Incubate at 42°C for 30 minutes. Incubation can also be performed at 37°C and 30°C for 30 minutes or overnight at room temperature (see Table 1 on page 5 for more details)

5. Transfer the contents to selective media plates, spread the cells and incubate at 30°C until transformants are observed (typically 2-3 days)

Protocol

Single vector transformation

Starting material: Cells grown on agar plate

! = read me first

1. Patch yeast cells on agar media and grow for few days at 30°C until patches are dense.

> **!** Higher transformation efficiency is obtained with fresh cultures. Growth on Molecular Biology Certified™ Yeast Media (page 12), however, allows the use of 2-3 week old culture plates for transformation.

2. Scoop the equivalent of a 3-4 mm size colony with a sterile toothpick or pipet tip and transfer to sterile tubes or 96 well plates containing 125 ul **EZ-Transformation solution**.

3. Add 2 ug of plasmid DNA and 5 ul **Carrier DNA**. Resuspend the cells by vortexing at moderate to maximum speed.

4. Incubate at 42°C for 30 minutes. Incubation can also be performed at 37°C and 30°C for 30 minutes or overnight at room temperature (see Table 1 on page 5 for more details)

5. Transfer the contents to selective media plates, spread the cells and incubate at 30°C until transformants are observed (typically 2-3 days)

Protocol

Simultaneous transformation of two vectors

i.e, cotransformation of bait and library vectors into yeast two-hybrid reporter strains.

! = read me first

1. Inoculate a single isolated yeast colony into 1 ml of rich media (YPD) or minimal dropout media (SD-amino acid) and grow at 30°C with shaking until OD_{600} >1.

> • OD_{600} > 1: Overnight culture if using YPD. 1-3 days culture if using minimal media inoculated with a single colony; increase initial inoculum to get a denser overnight culture for slow growers.

2. Transfer 500 ul of cells to sterile 1.5 ml centrifuge tubes or deep 96 well plates. Spin 10 seconds if using a microcentrifuge and 2-3 minutes if spinning 96 well plates to pellet the cells. Completely remove the growth media by pipetting or aspiration.

3. Add 125 ul **EZ-Transformation solution,** 2 ug of each vector and 5 ul **Carrier DNA**. Resuspend the cells by vortexing at moderate to maximum speed.

4. Incubate at 42°C for 30 minutes.

5. Transfer the content to selective media plates to select for transformants containing both vectors. Spread the cells and incubate at 30°C until transformants are observed (typically 2-3 days)

If processing putative positive cells following a yeast two-hybrid screen, replica plate onto screening media to determine interaction between the expressed bait and library proteins.

> **!** Plating cells following the transformation process directly on screening media greatly reduces the cotransformation efficiency.

Yeast two-hybrid support kits /reagents /tools

Kits:

Alkali Cation Yeast transformation kit. For high efficiency transformation of libraries (10^4-10^5 transformants per ug DNA). Cat # 2200-200

Whole Cell yeast PCR kit. For direct PCR amplification from yeast cells. Cat # 2016-200

RPM® yeast kit. For the isolation of plasmid DNA from yeast cells. Cat # 2069-200.

Fast Protein Red. For the preparation of total protein extract from yeast for SDS PAGE, western analysis and immunoprecipitation. Cat # 6550-600.

Reagents:

	*Quantity**	*Cat#*
X-GAL	1 g	4063-102
5-FOA	1 g	4066-102
3-AT	500 g	4061-742

Tools:

Replica Plating Apparatus	1 x 100 mm	5000-001
	1 x 150 mm	5000-004
Velvet pad	1 x 6 inch^2	5000-006
	1 x 9 inch^2	5000-008

* Available in larger sizes.

Bacterial minimal media to rescue yeast vectors

Recovery of library vectors carrying TRP marker

Bacterial host cell: KC8
Media content per liter:
11.3 g M9 minimal salt 1 part formulation (cat# 3037-012) + 17 g agar + 990 ml H_2O. Autoclave and cool to 50°C
Add 0.74 g CSM-trp (cat # 4511-012)
Add 10 ml 20% sterile glucose
Add 1 ml 1 mg/ml sterile filtered thiamine-HCl
Add the appropriate antibiotic
Pour plates

Recovery of Library vectors carrying the LEU marker

Bacterial hosts: KC8, HB101, RR1, JA226, C600
11.3 g M9 minimal salt 1 part formulation (cat# 3037-012) + 17 g agar + 990 ml H_2O. Autoclave and cool to 50°C
Add 069 g CSM-leu (cat # 4510-512)
Add 10 ml 20% sterile glucose
Add 1 ml 1 mg/ml sterile filtered thiamine-HCl
Add 4 ml of 10 mg/ml sterile filtered proline (RR1 and C600 only)
Add 4 ml of 10 mg/ml sterile filtered threonine (C600 only)
Add the appropriate antibiotic
Pour plates

Useful Information about the yeast *S. cerevisiae*.

Methods in Enzymology vol 194 (1991). Current protocols in molecular biology 13.01-13.210. See BIO 101 web site for more details.

Yeast size and composition

cell	*haploid*	*diploid*
size	4 um	5 x 6 um
Shape	Spheroid	Ellipsoid
Volume	70 um^3	120 um^3
DNA content	0.017 pg	0.034 pg
RNA content	1.2 pg	1.9 pg
Protein content	6 pg	8 pg

OD_{600} vs cell concentration

	OD_{600}	*Cells/ml*
Early-log phase	<0.4	$<10^7$
Mid-log phase	0.4-1.7	$1\text{-}5x10^7$
Late-log phase	1.7-6.6	$5x10^7\text{-}2x10^8$
Stationary phase	>6.6	$>2x10^8$

Storage	*Short*	*Medium*	*Long*
Time	1-3 months	up to 2 years	> 2years
Storage	Agar plates*	Agar stabs / Glycerol*2	Glycerol*2
Temperature	4°C or RT	RT/-20°C	-70°C

* Seal with parafilm to prevent plate desiccation.

*2 20-50% glycerol

Yeast growth

Yeast cells are manipulated essentially as are *E. coli*. Culturing is simple, economical, rapid and non-hazardous. Yeast grows non-selectively on rich media (YPD) and selectively, i.e. with vectors expressing selective markers, such as amino acids, on synthetic defined minimal media (SD) containing the appropriate dropout nutritional supplement (CSM). Wild type strains of *S. cerevisiae* have a doubling time of ~90 min in rich media and ~140 min in minimal media.

Carbon sources

Wild type yeast cells can utilize a variety of carbon sources glucose being dextrose is the most common. Galactose, maltose, fructose, raffinose can also be used. These fermentable carbon sources are added to the media to a final concentration of 2%. Glucose-free galactose is used to induce transcription of yeast two-hybrid reporter genes fused to the GAL1/GAL 10 promoters. Non-fermentable carbon sources can also be used (glycerol, ethanol and acetate)

Yeast Media

Only BIO 101 offers Molecular Biology Certified™ Media for all yeast two-hybrid systems, genetic screens and the manipulation of *S. pombe and S. cerevisiae*

YPD. Content per L: 20 g peptone, 10 g yeast extract, 20 g glucose pH 6.5. Available in agar format.

	*Quantity**	*Cat #*
Solution	500 ml	4001-024
Large capsules	227 g	4001-016
Capsules	227 g	4001-011
Powder	227 g	4001-012
0.5 L pouch	10	4001-065
1L pouch	10	4001-075

* Available in larger sizes.

Minimal media

= [YNB* + glucose] + [Complete Supplement Mixture (CSM)]

= [DOB] + [CSM] DOB + agar = DOBA

= [SD] SD + agar = SDA

* Yeast Nitrogen Base with Ammonium Sulfate without amino acids.

SD media (available in 0.5/1 L pouches). SD = broth. SDA = agar media

Media	*Cat #**	*Media*	*Cat #**
SDA-leu	4811-175	SDA-leu-ura	4824-175
SDA-his	4810-175	SDA-trp-ura	4825-175
SDA-trp	4812-175	SDA-his-leu-trp	4830-175
SDA-ura	4813-175	SDA-his-leu-ura	4831-175
SDA-his-leu	4820-175	SDA-his-trp-ura	4832-175
SDA-his-trp	4821-175	SDA-leu-trp-ura	4833-175
SDA-his-ura	4822-175	SDA-his-leu-trp-ura	4840-175
SDA-leu-trp	4823-175		

* Cat # for 10x 1 L pouches. Available in different sizes.

DOB: Drop-Out Base	Cat #*
DOB = DOB 2% glucose	4025-012
DOB 2% galactose (glucose-free)	4025-512
DOB 2% galactose, 1% raffinose (glucose-free)	4025-912
DOB 2% Raffinose (glucose-free)	4025-712
DOB w/ Succinate	4025-612

* Cat # for the 227 g size. Larger sizes available. Call or see web site.

Complete Supplement mixture: CSM*

Media	*Cat#*	*Media*	*Cat #*
CSM	4500-012	CSM-leu-ura	4520-212
CSM-leu	4510-512	CSM-trp-ura	4520-512
CSM-his	4510-312	CSM-his-leu-trp	4530-112
CSM-trp	4511-012	CSM-his-leu-ura	4531-212
CSM-ura	4511-212	CSM-his-trp-ura	4530-812
CSM-his-leu	4520-412	CSM-leu-trp-ura	4530-912
CSM-his-trp	4520-112	CSM-his-leu-trp-ura	4540-012
CSM-his-ura	4520-312	CSM-his-leu-trp-ura-lys	4550-012
CSM-leu-trp	4520-012		

* Supports vigorous growth of all two hybrid yeast reporter strains and virtually all strains of *S. cerevisiae*. Components verified by HPLC analysis (Table 3). Additional CSM dropouts are available. Other supplement mixtures include: BSM, HSM and SC.

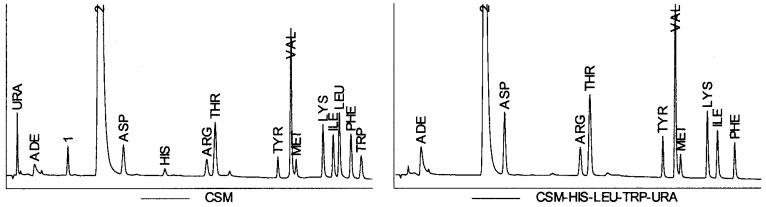


Table 3. HPLC chromatograms. CSM and CSM-his-leu-trp-ura were derivatized with the AccQ-Fluor Reagent kit (Waters), analyzed by reverse phase HPLC on a C18 column and detected by absorbance at 554 nm.

Pre-poured plates*

SDA-leu	4811-124	SDA-leu-ura	4824-124
SDA-his	4810-124	SDA-trp-ura	4825-124
SDA-trp	4812-124	SDA-his-leu-trp	4830-124
SDA-ura	4813-124	SDA-his-leu-ura	4831-124
SDA-his-leu	4820-124	SDA-his-trp-ura	4832-124
SDA-his-trp	4821-124	SDA-leu-trp-ura	4833-124
SDA-his-ura	482-124	SDA-his-leu-trp-ura	4840-124
SDA-leu-trp	4823-124		

* Available in sleeves of 10 plates or cases of 10 sleeves. Available with different concentrations of 3-AT or cycloheximide. Available with 2% galactose that is glucose-free. Cat # for one10 plate sleeves are shown.

Slant

	*Quantity**	*Cat #*
YPAD*2	25 slants	4015-023
RCY*3	25 slants	4016-023

* Available in larger quantity.

*2 Content per L: YPD agar + 40 mg Adenine sulfate

*3 Content per L: 20 g peptone, 10 g yeast extract, 30 g glucose, 1 g CSM, 20 g agar.

Yeast media ingredients

	*Quantity**	*Cat #*
Yeast extract*2	227 g	4018-012
Peptone-Y	227 g	4018-512
Peptone-YM (meat)	227 g	4018-612
Agar-Y*2	227 g	4019-011
YNB*3	227 g	4027-012
Dextrose	227 g	4014-012
D-Sorbitol	1 kg	4020-032
Sterile 80% glycerol	50 ml	3055-024
Sterile 20% glucose	100 ml	3055-534

Individual amino acids. See web site.

* Available in larger sizes.

*2 Available in powder and capsules format. Cat # for 227 g of powder is shown.

*3 Available in a wide variety of component dropouts. See web site for details

Yeast specialty media. Call or check web site for details.

YEP. Content per L: 20 g peptone, 10 g yeast extract, pH 6.5.

Mock YEPD. No ingredients derived from yeast.

YPL broth w/ lactose. Similar to YPD with lactose replacing dextrose.

LP-YPD. For labeling cells to high efficiency with [^{32}P].

***S. pombe* Media**. Call or check web site.

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BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the GENECLEAN® Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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(760) 929-1700 (Outside USA)
(760) 918-9313 (Fax line)
technical@bio101.com
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Office Hours:

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