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Colony Lift Colorimetric Assay for β-Galactosidase Activity

Juan I. Fuxman Bass[1], **John S. Reece-Hoyes**, and **Albertha J.M. Walhout**[1]

Program in Systems Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Abstract

In this protocol, we present a qualitative assay for monitoring the level of expression of β-galactosidase, an enzyme encoded by the *LacZ* gene, in yeast. This is useful both for determining autoactivity of *LacZ* expression in yeast DNA "bait" strains and for assessing *LacZ* reporter gene activation mediated by a transcription factor "prey" interaction with a DNA bait of interest in yeast one-hybrid (Y1H) assays. In this colorimetric assay, yeast are lysed in liquid nitrogen and then assayed for β-galactosidase expression using the colorless compound X-gal, which turns blue in the presence of this enzyme.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

β-mercaptoethanol

Liquid nitrogen

X-gal (4%, w/v, in dimethyl formamide)

YAPD plates (each containing a nitrocellulose filter) <R>

Z-buffer <R>

Equipment

Camera

Forceps

Fume hood

Incubator (set at 37°C)

[1]Correspondence: juan.fuxmanbass@umassmed.edu; marian.walhout@umassmed.edu.

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Liquid nitrogen bath

Petri dishes (150-mm)

Tubes (plastic, 15-mL)

Waste bottle (glass)

Whatman filters (125-mm diameter, hardened; Sigma-Aldrich 1452125)

METHOD

1. For each nitrocellulose filter-YAPD plate to be analyzed, place two Whatman filters in an empty 150-mm Petri dish.
2. Move to a fume hood for Steps 3–5.
3. For each plate, in a 15-mL plastic tube, set up a reaction mix at room temperature containing:

Z-buffer	6 mL
β-mercaptoethanol	11 µL
X-gal (4% solution)	100 µL

Use this entire mix to completely soak the Whatman filters in the Petri dish from Step 1. Remove any air bubbles using forceps to lift the filters and squeeze the bubbles to the sides, and then remove excess liquid into a waste bottle by tipping the plate.

It is important to work in the fume hood with gloves at all times as β-mercaptoethanol and dimethyl formamide are toxic, irritants, and permeators. Once made, Z-buffer can be stored for up to 1 yr at room temperature.

4. Lift the nitrocellulose filter from the YAPD plate using forceps and place the filter, yeast side up, in a liquid nitrogen bath for 10 sec. Discard the YAPD plate.
5. Use the forceps to place the frozen nitrocellulose filter, with the yeast facing up, onto the wet Whatman filters, and use forceps (or a needle) to remove air bubbles under the nitrocellulose filter quickly as the filter (and yeast lysate) thaws.
6. Use an incubator to maintain each plate at 37°C.

Check for blue coloring regularly (every hour if necessary) over a maximum 24 h period. Timing is important as yeast expressing high levels of β-galactosidase can produce a saturating intensity of blue within just a few hours. See Troubleshooting.

7. Take pictures with a digital camera to show the amount of blue compound generated by each yeast lysate.

This is a qualitative assay, and so intensities of blue should be compared only within a plate and within a bait: either between different strains for the same DNA bait in autoactivation assays, or between a yeast DNA

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*bait strain transformed with empty vector versus the same strain transformed with an AD-prey clone. Pictures should be taken at different time points to better capture the differences in blue intensities. For quantitative measures of β-galactosidase production, a liquid assay using ortho-nitrophenyl-β-galactoside can be performed (*Deplancke et al. 2004; Pruneda-Paz et al. 2009).

TROUBLESHOOTING

Problem (Step 6): No blue compound is generated.

Solution: If, after 24 h at 37°C, no blue compound has been generated by any yeast lysate, it is possible that one of the reagents has been omitted or incorrectly prepared. The most important considerations regarding ingredients are that the Z-buffer should be pH 7.0 and that the X-gal is at the correct concentration. The easiest way to ensure that the reagents are in good order is to test the assay using yeast control strains that are known to express differing levels of β-galactosidase.

RECIPES

YAPD Plates

Reagent	Quantity (for 2 L)
Peptone	40 g
Yeast extract	20 g
Adenine hemisulfate dehydrate	0.32 g
Glucose (40%, w/v) in water, sterile	100 mL
Agar	35 g

Dissolve the first three powders in 950 mL of water in a 2-L flask and add a stir bar. In a second 2-L flask, add the agar to 950 mL of water (do not add a stir bar as it will cause the agar to boil over in the autoclave). Autoclave for 40 min at 15 psi on liquid cycle. Immediately pour the contents of the first flask, including the stir bar, into the agar-containing flask. Add the glucose, mix well on a stir plate, and cool to 55°C. Pour into 150-mm sterile Petri dishes (~80 mL per dish), dry for 3–5 d at room temperature, wrap in plastic bags, and store for up to 6 mo at room temperature

.Z-Buffer

60 mM Na_2HPO_4 (anhydrous)

60 mM NaH_2PO_4

10 mM KCl

1 mM $MgSO_4$

Adjust the pH to 7.0 with 10 M NaOH.

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