X-α-Gal
Protocol-at-a-Glance
(PT3353-2)

Indicator plates, containing the chromogenic substrate X-α-Gal (5-Bromo-4-chloro-3-indolyl-α-D-galactopyranoside), can be used to rapidly detect protein interactions when using any Matchmaker™ GAL4-based System or Library. X-α-Gal indicator plates provide a convenient alternative to β-galactosidase liquid and filter-lift assays. X-α-Gal can be either included in the medium prior to pouring plates or spread on top of the medium prior to plating liquid cultures.

A. Preparing X-α-Gal

1. Dissolve X-α-Gal at 20 mg/ml in dimethylformamide (DMF). Store X-α-Gal solutions in glass or polypropylene bottles at –20°C in the dark.

B. Pouring X-α-Gal indicator plates

1. Prepare and autoclave 1.0 L of the appropriate dropout agar medium. Let cool to 55°C.
2. Add 2 ml of X-α-Gal (20 mg/ml).
3. Pour plates and allow medium to harden at room temperature.
4. Plate cells and incubate at the appropriate temperature until blue colonies form (3 days).

C. Spreading X-α-Gal onto premade plates

1. Dilute X-α-Gal to 4 mg/ml in DMF.
2. Pour appropriate dropout plates and allow medium to harden at room temperature.
3. Spread 200 µl of X-α-Gal onto a 15-cm plate or 100 µl onto a 10-cm plate using glass beads.
4. Allow plates to dry for 15 min at room temperature.
5. Plate cells and incubate at the appropriate temperature until blue colonies form (3 days).

D. Testing for *MEL1*-responsive yeast strains

MEL1, which encodes α-galactosidase, is regulated by GAL4. Because not all yeast strains contain *MEL1*, several commonly used two-hybrid yeast strains were tested for a *MEL1* response. To determine the genotype of additional yeast strains, simply transform the strain with either pCL1, a control plasmid expressing wild-type GAL4, or two GAL4-based plasmids expressing known interacting proteins (e.g., pGADT7-T + pGBKT7-53).

Strain	*MEL1* response
HF7c	–
CG1945	–
SFY526	–
YRG-2	–
Y190	+
AH109	+
Y187	+
PJ69-2A	+
PJ69-4A	+
J692*	+
J693*	+

* Courtesy of Dr. R. Rothstein.

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