S.cerevisiae β-GAL assays

Filter and liquid assays

Note 1: The LacZ gene of *E.coli* encodes b-Galactosidase, capable of the hydrolysis of a variety of β-D-galactosides including chromogenic substrates (colorless compounds that yield a colored compound when hydrolyzed). Because of the ease and sensitivity of the assays, yeast genes are often tagged with a functional portion of the lacZ gene to monitor the regulation of expression of the gene under test. The liquid assay **(#1)** is sensitive and quantitative and generally used to accurately monitor the kinetics of gene expression. The filter assay **(#2)** is less sensitive and provides only a qualitative assessment of β-Gal activity. However it is useful for simultaneously and rapidly analyzing a large number of colonies.

Note 2: Yeast plasmid pRS416-Gal1-LacZ (F644/b2938) contains a GAL1 promoter driving lacZ, URA3 and CEN/ARS. Used to determine the efficiency and kinetics of GAL induction in different deletion backgrounds. Prior to induction strains, will be grown in synthetic complete medium + 2% Raffinose / 0.1% Glucose until induction by addition of Galactose to 2% (see the ***Media* protocol**).

1. Liquid β-gal assay

Background: Cells are permeabilized and the chromogenic substrate *o*-Nitro phenoyl-β-D-Galactoside (ONPG) added in excess. After incubation at pH 7 and 30°C the enzyme is inactivated by raising the pH to 11, stopping the reaction. Product formation is determined spectrophotometrically.

1.1 Innoculate yeast into 5ml appropriate media, generally YPD (with some exceptions, as in **Note 2**). Grow overnight at 30°C (or as appropriate).

1.2 Sub 1/20 into 10ml appropriate media (see **Note 2**). Grow 4-6 hours (nice healthy, exponentially growing culture) to mid-log ($OD_{600} \approx 0.5$).

Note 3: If the goal is to investigate the activity of a promoter (such as pRS416-Gal1-LacZ) under inducing conditions (here, by addition of galactose), a kinetic analysis is usually performed, with samples removed at relatively frequent intervals (≥9 sample timecourse such as: $T_0, T_{5m}, T_{10m}, T_{15m}, T_{30m}, T_{45m}, T_{60m}, T_{90m}, T_{120m}$) – calculate the starting culture volume accordingly.

1.3 Collect 1ml cells to a cuvette (*usually in duplicate*). Determine OD_{600}. Transfer to an ependorff tube and collect by flash spin centrifugation (flash spin to 10K). Resuspend to 1ml with *Z-buffer* and place on ice. Add one drop (20µl) 0.1% SDS and two drops (15µl) $CHCl_3$ (permeabilizes the cells). Vortex vigorously 15secs and place at 30°C for 15 mins to equilibrate the termperature.

1.4 Add 200µl ONPG (4mg/ml) and vortex five secs. Place at 30°C and note the time. When a medium yellow color has developed (**Note 4**) stop the reaction by addition of 500µl Stop Solution (0.1M Na_2CO_3). Note the time elapsed.

Note 4: For accuracy the OD_{420} should be between 0.3 - 0.7. With practice the color correlating with this range can be easily recognized.

1.5 Pellet cells by centrifugation (6K, 2min, RT°). Determine OD_{420} (color change) and OD_{550} (light scattering by cell debris; should be 0, so generally don't bother) of the supernatant. Calculate β-GAL units with the following formula.

$$\text{UNITS} = \frac{1000 \times [(OD_{420}) - (1.65 \times OD_{550})]}{(t) \times (v) \times (OD_{600})}$$

t	=	reaction time (min)
v	=	culture volume used (ml)
OD_{600}	=	cell density at start of assay
OD_{420}	=	absorbance by yellow *o*-nitrophenyl product
OD_{550}	=	light scattering by cell debris (generally 0)

Note 5: Samples are usually examined in duplicate, and these are generally very tight. For a strong gene promoter the required (t) is generally < 30mins (>4h and you have problems or a negative).

2. Yeast Filter β-gal assay

Background: Based on the blue-white assay as used for color-screening many *E.coli* cloning plasmids. The chromogenic substrate is in this case is X-Gal. Can be quantitative (more intense blue color = higher expression), but not in the same league as the liquid assay above. This approach is generally used to screen Yeast-2-Hybrid plates or in genetic screens for mutants affecting the expression of a specific reporter construct. Always use **(+)** and **(–)** control strains in this assay.

2.1 Spot or streak yeast colonies onto a plate containing appropriate selection (or use primary screen plates). Grow cells until optimal colony size (equivalent to ≈ 40h / WT / YPD / 30°C). Place a Whatman #5 (S+S #576; 8.26cm diameter) filter paper onto the surface of the plate and press gently to ensure all colonies are transferred. If required remove bubbles with a spreader (but avoid this if at all possible).

2.2 Carefully remove the filter and place it for 30seconds into liquid nitrogen (**Safety**: careful to avoid shattering of the membrane and splashing onto yourself). Carefully remove the membrane to a sheet of dry 3MM to thaw (five minutes).

2.3 Place a circular piece of 3MM (cut to fit) into a Petri dish and add 4ml *buffer Z + X-GAL* (enough to soak the paper without flooding it: per 10ml Z buffer + 150µl 2% X-gal in DMSO). Place the thawed nitrocellulose onto the 3MM and allow the buffer to absorb slowly.

2.4 Incubate 0.5 hrs - O/N, covered to prevent evaporation. Stop reaction be removing test filters and letting them dry. Strong positives will give an intense blue color within minutes.

Buffers

Z Buffer (per L)
- 16.1g Na_2HPO_4 $7H_2O$ (60 mM final)
- 5.5g NaH_2PO_4 H_2O (40 mM final)
- 0.75g KCl (10mM final)
- 0.246g $MgSO_4$ $7H_2O$ (1mM final)

Adjust to pH 7.0 & bring to 1 liter

Before use add β-ME to 50mM (14.25M stock)

ONPG
o-Nitrophenoyl-β-D-Galactoside
4mg/ml in 0.1M Potassium Phosphate, pH 7.0

Stop Solution 0.1M Na_2CO_3