

Galactose Induction Protocol

1. Grow cells from a single colony overnight in 2 mls at 30°. Selective medium should be used if a plasmid needs to be maintained. For some strains that grow slowly it may be necessary to use a larger inoculum. In these cases use the 2 mls to inoculate 50-100 mls of medium in Erlenmeyer flasks. Sometimes this process requires 2 days of growth to get to a sufficiently high cell density. During this time it may be convenient to obtain an estimate of the strain's doubling time in YEP-lactate medium.

Although *GAL::HO* is normally repressed in glucose media, sometimes recombination events can be observed, which is attributed to leakiness of the *GAL1-GAL10* repression. Normally this occurs only at a low level (about 1% of the total). Depending on the substrate, these events can sometimes be minimized or eliminated by appropriate selection of the selective media during this pre-growth step.

2. Spin down cells and discard supernatant. Resuspend the pellet in an equal or larger volume of YEP-lactate medium. Spin the cells down again and resuspend in 4 mls of YEP-lactate medium. Shake the cells at 30° for 3 to 10 hours or until the cells begin to grow again. Strains undergo a lag phase upon the shift to lactate medium. The pre-growth in lactate medium in this step is intended to overcome the lag phase and make it easier to obtain the proper cell density of the final culture.
3. Inoculate YEP-lactate medium with enough cells to give 10^7 cells/ml the next day. Shake the culture vigorously overnight at 30° using a flask large enough to provide sufficient aeration (for example, 0.5 liters in a 2 liter flask with a foam plug). Most strains have a doubling time of about 3 hours in YEP-lactate. In YEP-raffinose, which is sometimes used as a substitute for YEP-lactate, the doubling time is about 5 hr.
4. When cells have grown to a density of 10^7 cells/ml take a sample (usually 50 mls) for the uninduced control and then add 1/10 volume of 20% (w/v) galactose (~2% final concentration) to the remaining culture. The culture should not be removed from the incubator if possible; sometimes it is convenient to use a 50 ml pipet to withdraw samples. Continue shaking culture at 30° and take 50 ml samples at the necessary time points. For

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experiments studying mating type switching, add 1/10th volume of 20% glucose to down-regulate the HO induction after 30 min (or 60 min) to prevent re-cutting of the HO-cut site. Process samples for DNA extraction as given in the glass bead DNA prep protocol.

At the initial time point and at the desired time points, dilute a sample of the cultures by 10^{-4} and plate on YPD. Usually 100 ul per plate (3 plates) is adequate; although more should be plated if viability is expected to be low due to inability to repair the break. After the colonies grow they can be replica-plated to selective media to determine 1) the fraction of colonies with recombined substrates 2) the viability of the strain after HO induction or 3) the stability of the *GAL::HO* plasmid or other plasmids.

YEP-lactate:

37 g 85% lactic acid
1% yeast extract
2% peptone
1 liter total

Add 37 g of 85% lactic acid to 0.9 liters of distilled water and add 10 M NaOH (about 30 mls) until the pH is about 5.5. Alternatively, dissolve 12 g of NaOH pellets in 0.9 liters and adjust the pH to 5.5 with 85% lactic acid. Dissolve 10 g yeast extract and 20 g peptone and bring volume up to 1 liter. Add 8 mls of adenine solution (0.5% adenine in 0.05 M HCl) if desired.

Galactose:

Dissolve galactose in water and filter sterilize. Do not autoclave. Galactose will dissolve in water at room temperature but it takes an hour or longer. Galactose isomerizes at elevated temperatures. Galactose stock contains less than 0.01% glucose (Sigma).

Visit the Haber Lab web page (<http://www.bio.brandeis.edu/haberlab/jehsite/protocol.html>) for the most recent version of this protocol.