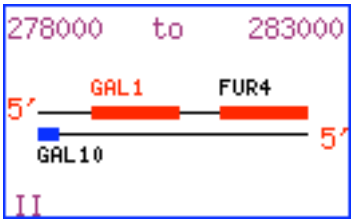
S.cerevisiae galactose inductions

NOTE 1: When we clone a constitutively expressed gene we usually maintain under its own promoter and 3' flanking sequences. There are some exceptions: (i) when we add a 3' epitope tag and also provide a polyA site, (ii) when we 5' epitope tag and provide a constitutive promoter of a strength appropriate to the gene of interest (eg. *ADH1p* (YOL086c), 125.6 mRNAs/hr; *TFA1p* (YKL028w), 4.6mRNAs/hr) (relative expression frequency from http://web.wi.mit.edu/young/expression/). Note that the *ADH1p* is generally used for overexpression: very few yeast genes have expression levels anywhere near this.

NOTE 2: Many inducible expression systems are in common use, including galactose, copper, heat shock and PHO5. The transient expression level derived from some of these elements is usually orders of magnitude higher than the usual promoter.

The GAL1-10 promoter is usually used (see **SGD** for locus information) as the regulatory element to drive GAL-inducible expression of a cassette.



Background: The galactose structural genes (*GAL1*, *GAL10*, *GAL7*, *GAL2*) are co-ordinately regulated at the transcriptional level in response to galactose by Gal4, Gal80, and Gal3. Regardless of carbon source, the Gal4p transcriptional activator binds as a dimer to upstream activation sites found in the promoters of these GAL genes. In the presence of galactose, Gal3 sequesters the transcriptional repressor Gal80 in the cytoplasm, thereby relieving inhibition of Gal4 and resulting in GAL gene expression. In the absence of galactose, Gal80 remains bound as a dimer, to Gal4, preventing Gal4 from recruiting other factors of the Pol II transcription machinery. During growth on 2% glucose these genes are subject to additional active repression by changes in chromatin structure: it is NOT recommended to do a Gal induction straight from growth on this carbon source (see **NOTE 3**).

Galactose induction time course

1. Transform strain of choice with plasmid containing a gene of interest under the *GAL1-10p* (or replace an endogenous promoter with *GAL1-10p* by homologous recombination *in situ*). Perform initial clone selection and sector-streaking on glucose-containing medium. Innoculate yeast into 10 - 100ml (vol dependent on the experiment planned – see **NOTE 4**) appropriate media to maintain selection but with 2% Raffinose / 0.1% Glucose as the carbon source (see **NOTE 3**). Grow overnight at 30°C (or as appropriate).

NOTE 3: *GAL1-10p* expression on different carbon sources -
Glucose – actively repressed, takes a while to switch back on

Raffinose – not expressed but quickly activated by addition of Galactose
Galactose – highly expressed

2. Sub 1/10 into 10 - 1000ml (vol dependent on the experiment planned – see **NOTE 4**) appropriate media + 2% Raffinose / 0.1% Glucose. Grow ≈ 4 hours (nice healthy, exponentially growing culture) to mid-log ($OD_{600} \approx 0.4$).

> **NOTE 4:** In this experiment, the plan is to investigate the response of a yeast strain to induced high-level expression of *protein X*. Samples can be taken at any number of time points but a relatively frequent time-course would cover: T_0, T_{10m}, T_{15m}, T_{30m}, T_{45m}, T_{60m}, T_{90m}, T_{120m}, T_{150m}, T_{180m}. The frequency and time-points used are obviously determined by the experiment, but you should calculate the starting culture volume in **Step 1** based on your needs.

4. Determine OD_{600} when ready to begin and remove desired volume of T_0 sample. Add Galactose to 2% final (and recalculate OD_{600} to account for dilution). Transfer all samples to an appropriate container (ependorff tube - 50 ml universal) and collect by centrifugation. Wash pellets in *pre-chilled* ddH_2O and keep on ice (see **NOTE 5**). At each time point also collect 1ml cells to a cuvette and determine OD_{600} (see **NOTE 6**).

> **NOTE 5:** When analyzing multiple closely-spaced time points it is **essential** to quickly wash out the galactose and chill the samples on ice. Collect a sufficient number of time-points and then process up to a safe -20ºC step in tandem: eg. when collecting samples suitable for Protein Immunoprecipitations wash the samples collected in every 30min period with lysis buffer, snap-freeze on dry-ice and move to -20ºC. All are then lysed and analysed simultaneously on a later occasion.

> **NOTE 6:** Use the OD_{600} values to determine the relative pellet volume at each time-point and normalize accordingly.

5. Further steps are obviously as appropriate and dependent on the experiment being performed (eg. protein-stability analyses by westerns, complex formation by co-immunoprecipation, gene-expression by Chromatin immunoprecipitation, etc). Specific protocols for all of these and also for mating type switching or DNA double-strand break induction with *pGAL-HO*, LoxP recombination with *pGAL-Cre* or LacZ induction with *pGAL-LacZ* are available at http://mckeogh.googlepages.com/protocols.

Sugar Stocks -

<u>40% Glucose</u> 400g Glucose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L and autoclave

<u>30% Galactose</u> 300g Galactose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L and autoclave

<u>20% Raffinose</u> 200g Raffinose, ddH_2O to 600 ml and mix well to dissolve
ddH_2O to 1L **and 0.2µ sterilize**