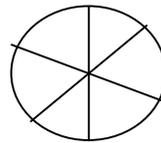


## $\beta$ -GAL FILTER ASSAYS

1. Prepare yeast colonies containing plasmid(s) of interest.
2. Pour 9cm plates containing appropriate selection media
3. Cut some Whatman Number 1 round filter papers to fit onto a 9cm petri dish. Try and use some sterile filter paper, as fungal and bacterial contaminants often have endogenous  $\beta$ -GAL activity.
4. Place filter paper onto media
5. Grid the bottom of the plate so that several clones can be streaked on the same dish. Eg:



6. Pick a yeast colony using a pipette tip. Streak onto the filter paper in a wavy pattern:  

7. Repeat for all clones to be assayed. Then place plates at 30°C for 1-2 days. Until you can clearly see the yeast growing on the filter paper.
8. Prepare some Z-Buffer/X-GAL solution:  
100ml **Z-buffer**  
0.27ml  $\beta$ -mercaptoethanol  
1.67ml **X-GAL stock**  
  
(Probably won't need 100 ml).
9. Cut some Whatman 3MM paper and place in a 20cm<sup>2</sup> square Petri dish.  
Soak with Z-Buffer/X-GAL solution, but not so much that there is excess liquid submerging the paper.

10. Mark the filter paper with yeast growing so you can re-orientate the paper back onto the plate and then remove the paper with forceps.
11. While still gripping the filter paper, place it colony side up into a flask of liquid nitrogen for 5 sec.
12. Remove filter paper and allow it to defrost at room temp for a couple of minutes.
13. Place the filter paper COLONY SIDE UP onto the Z-Buffer/X-GAL solution soaked whatman paper.
14. Cover the petri dish and seal with parafilm. Place at 30°C until blue colour develops. This can take 30' for strong expression up to overnight for weaker.
15. Store the filter paper by drying in the fume-hood overnight then wrapping in saran-wrap.

#### **Z-Buffer (1L)**

Na <sub>2</sub> HPO <sub>4</sub> .7H <sub>2</sub> O	16.1 g
NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	5.5 g
KCl	0.75 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.246 g

Check pH is 7.0 then autoclave.

#### **X-GAL stock solution**

X-GAL 20 mgml<sup>-1</sup>

Dissolve in DiMethyFormamide (DMF)

Store in foil at -20°C.