β-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 β-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 β-mercaptoethanol
   1.67ml X-GAL stock

   (Probably won't need 100 ml).

9. Cut some Whatman 3MM paper and place in a 20cm² 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)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Na₂HPO₄·7H₂O</td>
<td>16.1 g</td>
</tr>
<tr>
<td>NaH₂PO₄·H₂O</td>
<td>5.5 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.75 g</td>
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<tr>
<td>MgSO₄·7H₂O</td>
<td>0.246 g</td>
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Check pH is 7.0 then autoclave.

**X-GAL stock solution**

X-GAL 20 mg/ml

Dissolve in DiMethyFormamide (DMF)

Store in foil at -20°C.