**Exercise 8-2: Yeast Transformation**

**Streaking out transformants for GFP expression**

In Exercise 3, you isolated plasmid DNA from bacteria (plasmids: pREP3X, pSGP9, pSGP10, or pNC1).
In Exercise 7, you digested these plasmid DNAs with restriction enzymes and analyzed the pattern of linear DNA fragments separated out by agarose gel electrophoresis.
In Exercise 8-1, you transformed these plasmid DNAs into fission yeast cells and selected for the growth of cells carrying copies of the plasmids.

Here, each team will "streak out" yeast transformants (yeast cells now carrying a foreign plasmid DNA) onto agar plates. The cells growing on the agar plates should express the jellyfish protein: Green Fluorescent Protein (GFP).

\[ \text{nmt promoter} = \text{“no message in thiamine” promoter} \]

<table>
<thead>
<tr>
<th>media</th>
<th>GFP expression?</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ thiamine (Vitamin B1)</td>
<td>NO</td>
</tr>
<tr>
<td>- thiamine (Vitamin B1)</td>
<td>YES</td>
</tr>
</tbody>
</table>

**Materials needed:**

- SP7 genotype: \( h^+ \ cdc24-M38 \ ura4-D18 \ leu1-32 \)  \[ts\]

**Transformed strains**

<table>
<thead>
<tr>
<th>Set</th>
<th>Strain</th>
<th>plasmids</th>
<th>Selection plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set A</td>
<td>SP7</td>
<td>pREP3X, pSGP9, pSGP10</td>
<td>Leu- + thiamine</td>
</tr>
<tr>
<td>Set B</td>
<td>SP7</td>
<td>pSGP572a, pNC1</td>
<td>Ura- + thiamine</td>
</tr>
</tbody>
</table>

EMM/Leu- and EMM/Leu- + thiamine OR EMM/Ura- agar plates (Note: Absence of thiamine allows GFP gene to be expressed)

Sterile toothpicks

Sharpie

Petri dish sector template

Each group consists of two teams. Each individual in each group should participate in streaking out the transformants onto the appropriate selective agar plates.

1. Use a sharpie to draw 3 sectors on a Leu- plate or 3 sectors on a Ura – plate.
2. Label each sector with the plasmid type
   (Plate 1 EMM/Leu-, Plate 2 EMM/Leu- + thiamine) pREP3X, pSGP9, pSGP10 OR
   (Plates 3 & 4 EMM/Ura-) pSGP572a, pNC1, pNC1 (yes, 2 sectors with pNC1)

**BEFORE streaking out on your selective EMM/Leu- or EMM/Ura- plate, practice streaking out cells on a YES plate.**
3. To streak out a colony on the pREP3X or pSGP572a sector:
*Keep the toothpick flat edge on the plate surface as your streak*
• Use a toothpick and touch a pREP3X or pSGP572a colony on the transformation plate and draw one streak across the top of the sector (along edge of plate). To transfer the cells to a media plate begin the streak at one edge of the plate. Press the side of the toothpick containing the cells to the agar plate’s surface and quickly streak the toothpick across part of the plate’s surface
• Use a second toothpick for the next streak. Start by crossing over the first streak and draw the cells toward the center of the plate, making a zig zag pattern without crossing over any previous streak
• Use a third toothpick for the third set of streaks. Start by crossing over the second streak and draw the cells toward the center of the plate, making a zig zag pattern without crossing over any previous streak

Figure 1. Streaking out colony

4. Repeat this with pSGP9 and pSGP10 OR pNC1 for each remaining sector.
5. Incubate the cells with the agar plate inverted for several days at 25°C (or 34°C, Plate 4) to allow colonies to form.

Plates 1 and 2: all EMM/Leu- and EMM/Leu- + thiamine plates at 25°C.
Plates 3 and 4: all EMM/Ura- plates – Plate 1 incubates at 25°C, the Plate 2 incubates at 34°C.

Each GROUP will have 4 plates:

1) EMM/Leu- with pREP3X, pSGP9, pSGP10
2) EMM/Leu- + Thiamine with pREP3X, pSGP9, pSGP10
3) EMM/Ura- with pSGP572a and pNC1 (two different pNC1 colonies, pNC1-colony A and pNC1-colony B)
4) EMM/Ura- with pSGP572a and pNC1 (two different pNC1 colonies, pNC1-colony A and pNC1-colony B, the same colonies as on Plate 3)