SUPPLEMENTAL DOCUMENT 2

In recent semesters, we have been mutating yeast in collaboration with a lab in our department (mutating certain proteins they are interested in). In our first iteration of this project, we used the same process described to mutate the Ade2 gene in yeast. Ade2 is a gene in the adenine biosynthetic pathway. When intact, yeast are white in color. However, when Ade2 is mutated, a red product is accumulated in the yeast causing them to change color to a red phenotype. The CRISPR process described was used, however, the gene targeted was Ade2. Therefore, pML104_Ade2 was cloned and verified. The DRC produced by PCR amplified the full Leu2 gene with 50 bp of the Ade2 flanking it on either side (50 bp 5'UTR Ade2::Full Length Leu2 gene::50 bp 3'UTR Ade2). Therefore, when the transformants were selected, we plated onto com-ura, com- leu, and com-ura-leu media to identify the ones that successfully incorporated the DRC into the Ade2 locus. Instead of spot plating, we patch colonies onto a master plate using a template. We allowed the yeast to grow and then screened for % of colonies that had been successfully mutated red indicating that the CRISPR reaction had occurred

Ade2 Biosynthetic Pathway and Master Plate Template Adenine Biosynthetic Pathway

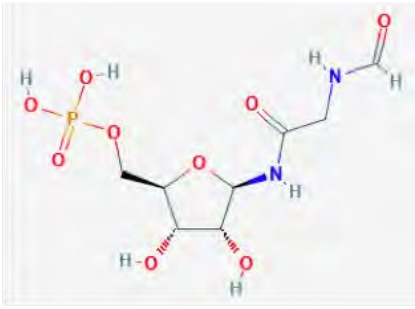
Yeast cells use a fairly complex enzymatic pathway to produce adenosine monophosphate (AMP). When adenine is present in the growth media, the cells take it up and convert it to AMP in a single enzymatic step by adding the adenine to phophoribosylpyrophosphate (PRPP). When adenine is NOT present in the growth media, cells use a 12-step enzymatic pathway to first synthesize their own adenine by converting P-ribosyl-PP into adenine and then adenine into AMP. The first 7 steps of this pathway are shown below.

P-ribosyl-PP
↓ ***ADE4***
P-ribosylamine
↓ ***ADE5***
P-ribosyl glycinamide
↓ ***ADE8***
P-ribosylformyl glycinamide
↓ ***ADE6***
P-ribosylformyl glycinamidine
↓ ***ADE7***
P-ribosylamino imidazole
↓ ***ADE2***
P-ribosylamino imidazolecarboxylate
↓ ***ADE1***
P-ribosylsuccino carboxamide aminoimidazole
↓
↓
adenine

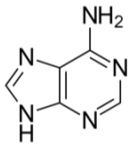
Yeast cell colonies are typically white (a phenotype). However, if *ADE2* or *ADE1* are mutated and no longer functional, the cells will develop a red phenotype. This is caused by the buildup and oxidation of P-ribosylamino imidazole.

When there is excess adenine available to the cell from its medium, the adenine synthetic pathway shuts down. Without the pathway, there is no production of the P-ribosylamino imidazole and subsequently no red phenotype.

Typically, synthetic (complete) media have an excess of adenine. However, over time, the red phenotype of *ADE* mutants will develop in YEPD (yeast extract, peptone, dextrose) medium. Through experimentation, we have found that synthetic plates with 5 µg/mL adenine allow for rapid development of the red phenotype in mutant cells.



P-ribosylamino Imidazole



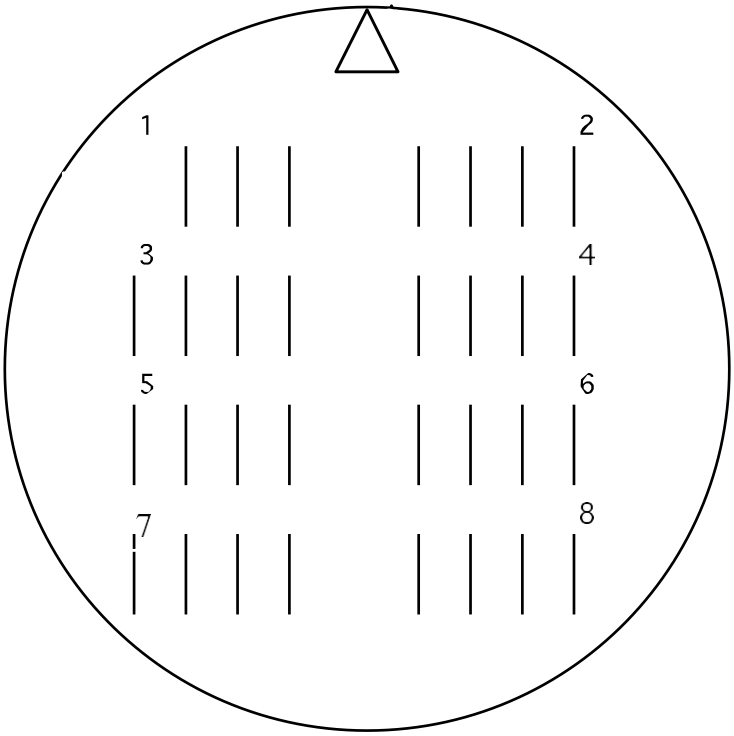
Adenine

Adapted from:
McIntosh, L. Adenine Biosynthetic Pathway in Yeast and Observation of Adenine- mutants. Pbworks.com [Accessed 2012]. http://mityeast.pbworks.com/w/page/55679061/Adenine Biosynthetic Pathway in Yeast and Observation of Adenine-mutants

Plate Templates

Grid Pattern for Phenotype Tests

Place your plate agar-side-up on this template and trace the lines onto the plastic bottom of the plate. Be sure to number each set of lines and draw the arrow at the top of the plate. Also remember to fully label your plate with what is on it, the date, and your initials along the edge of the plastic.



Pattern for Streak Plating

The pattern to the right indicates how you should streak for single colonies on a plate. Trace this pattern on the bottom of a plate or recreate it free-hand. The top bold line is the first streak. Then, sterilize your loop or get a new toothpick and follow the pattern of the solid zig-zag, careful not to cross the first streak more than once. Finally, sterilize your loop or get another new toothpick and follow the dashed zig-zag. This will deposit single cells on the plate, leading to single, genetically-pure colony growth.