URA transformation in ADE2 locus

Insert:

- PCR product of an Overlap Extension PCR that glue together, the homology arms around the ADE2 gene and the URA gene + native URA promotor. The fragment has a total length of 1984bp. The URA gene + promotor has a length of 1019 bp.

Homology arms:

- left homology arm: 215 bp
- right homology arm: 439bp
- No overlap with other sequences on the S. cerevisae genome Result of blasting

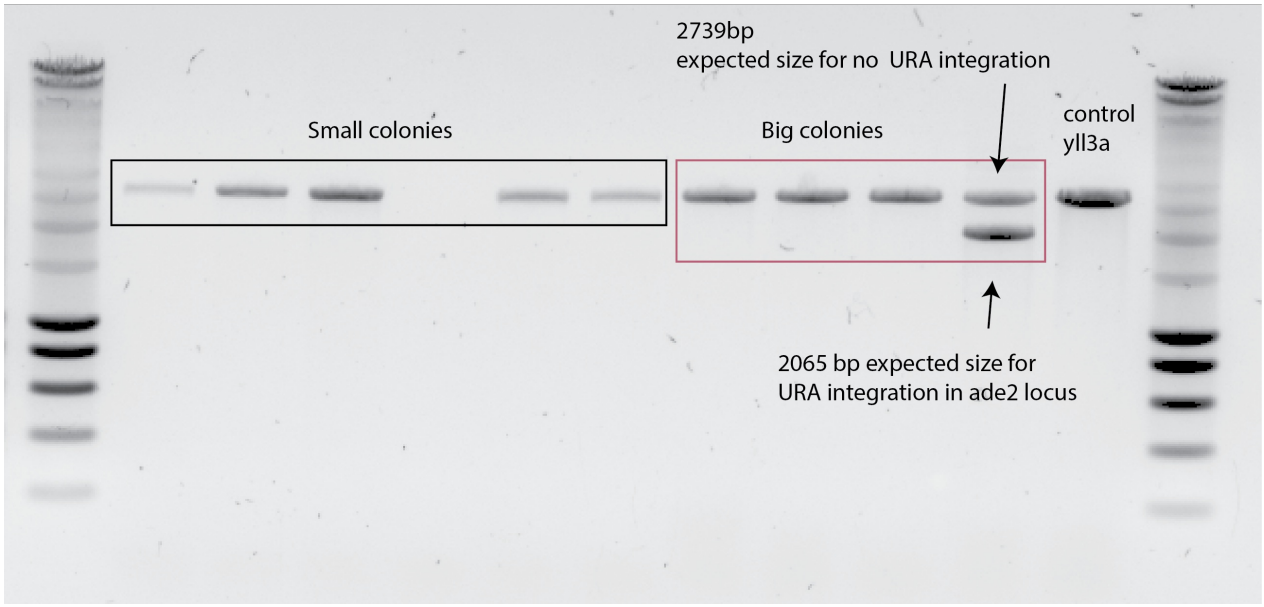
Transformation Protocol

- 2,5ng of the PCR product was used for it.
- OD starting colony : 3

Results

- The negative control, did not grow on the selection plate. The negative control is the reference strain with any insert on the mixture. They did not grow on -URA plates
- I have more than 100 transformats colonies growing on -URA. However, after 5 days of incubation none of them turned pink, as expected if they dont produce ADE2 gene.
- High variability in colony sizes, indicating different genetic backgrounds (?)

Colony PCR



Results:

1. The small colonies that grew on -URA has the same band size as the control strain yll3a, meaning, that they didnt insert the URA gene + promoter on the ADE2 locus.
2. One big colony show two bands, one in the control size and the other one in the correct size, if the URA gene is integrated in ADE2 locus. This is puzzling.

Things we dont understand:

1. **Where does the URA gene was inserted during transformation?** Since the homology arms of the ADE2 we are using has no overlap anywhere else in the genome there is no way the URA gene, in between the homology arms, can be inserted somewhere else. The bands at the same heigh as the control indicates the ADE2 is intacted,as the yll3a.
2. **Why there is one colony showing the right and the wrong band at the same time?** This means that the URA gene + promotor was inserted in the ADE2 locus, replacing it , and at the same time not replacing it ??????????????

Next experiments

1. Sequencing the ADE2 gene of yll3a, and one small and big colony, to see if the ADE2 we are taking from SGD is indeed the same sequence as the one we have in yll3a.