Title: Yeast transformation with the OEP2

Date

14062019

Objective

To remove the ura3 gene from ylic132= ade2- ura3+ by inserting an empty sequence in the ade2 locus , w here the ura3 gene is in ylic132.

Method

Yeast transformation protocol

Homology arms:

- left homology arm: 252 bp

> PCR of primer_1_new _upstream_forw ard and primer_7_upstream
>
> - right homology arm: 271 bp
>   PCR of primer_6_new _dow nstream_reverse_NO_Rga1 and primer_8_dow nstream

DNA concentration

7ul of 600 ng/ul OEP2 = 4.2 ug

Selection plates

- **-URA + 3x ADE** (for ylic132 positive control- no transformed cells) and for the transformed cells as a negative control
- **5FOA plates + 3x ADE** , in w hich only cells lacking URA3 are capable of grow . (positive control for the transformed cells)

Results

No growth in 5FOA plates on 17062019 ☹

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-> The reason could be that I made the protocol using the same amount of lithium acetate for a volume of 50ml instead of
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Patterned growth of pink and lighter colonies in the -URA +3xADe2 plates

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-> Actually it was a gradient of adenine when I dropped. After some days that spot became pink :)
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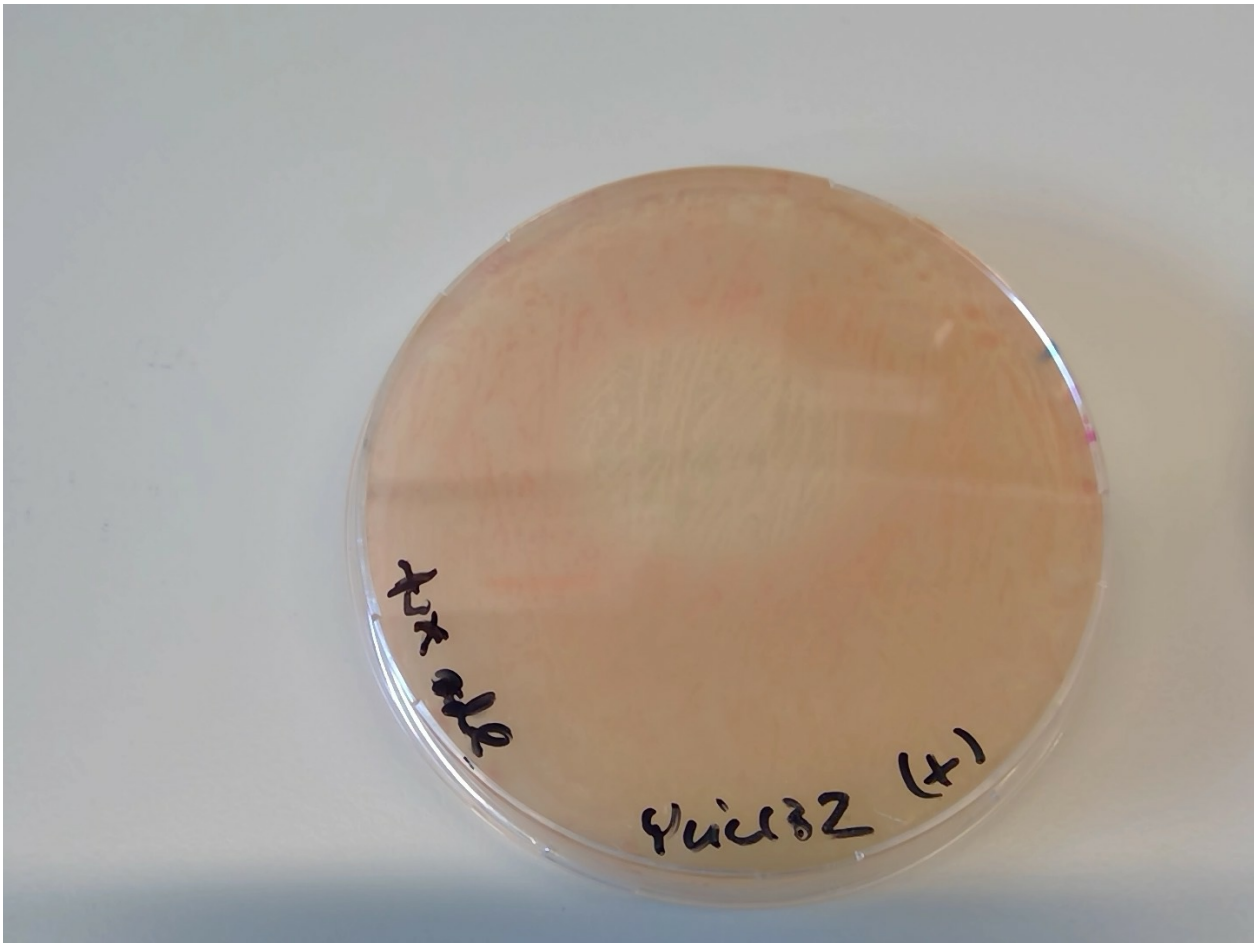
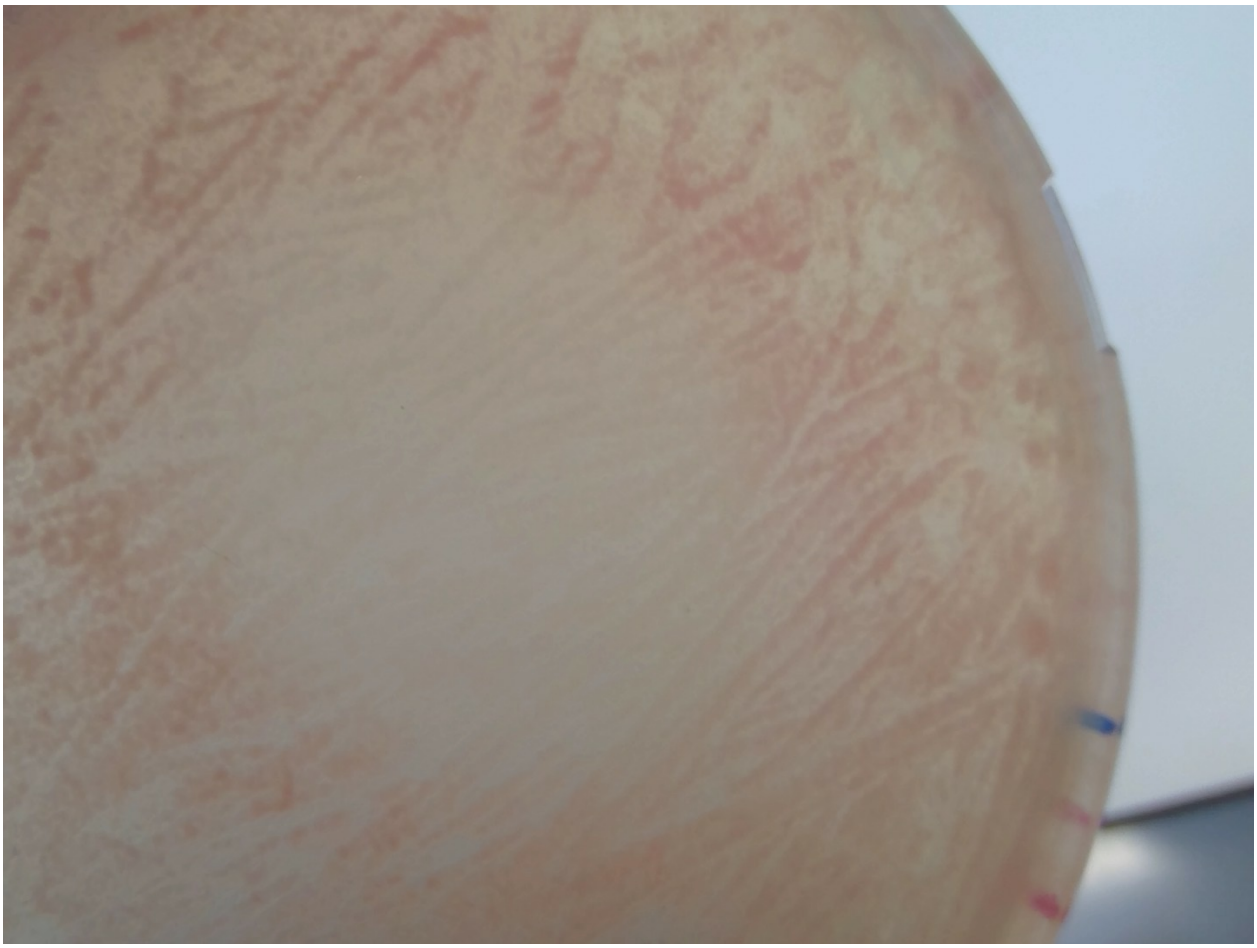

Yuc32 (+)



Next steps

- [ ] Prepare more OEP2 to have more concentration to transform.
- [ ] Repeat the transformation.