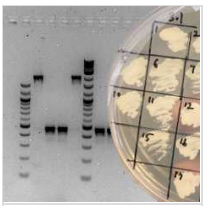

Feb 22, 2022

Colony PCR for screening transgenic yeast

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dx.doi.org/10.17504/protocols.io.b5g2q3ye

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dx.doi.org/10.17504/protocols.io.b5g2q3ye

Anbarasu Karthikaichamy 2022. Colony PCR for screening transgenic yeast . **protocols.io** https://dx.doi.org/10.17504/protocols.io.b5g2q3ye

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Wear safety equipments when handling sodium hydroxide (NaOH) and microwave.

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Prepare [M] **10 millimolar (mM)** sodium hydroxide (NaOH)

1 Pick single yeast colony from the transformation plate and re-streak to a fresh plate with appropriate selection marker.

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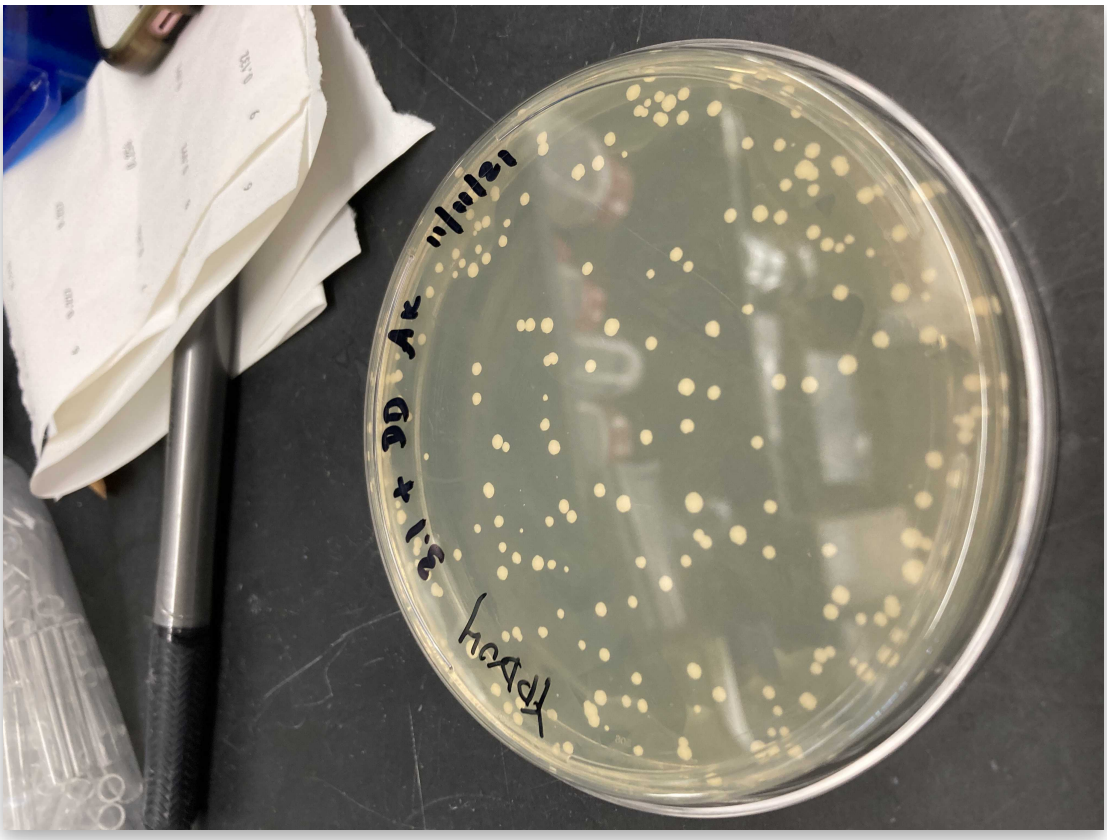
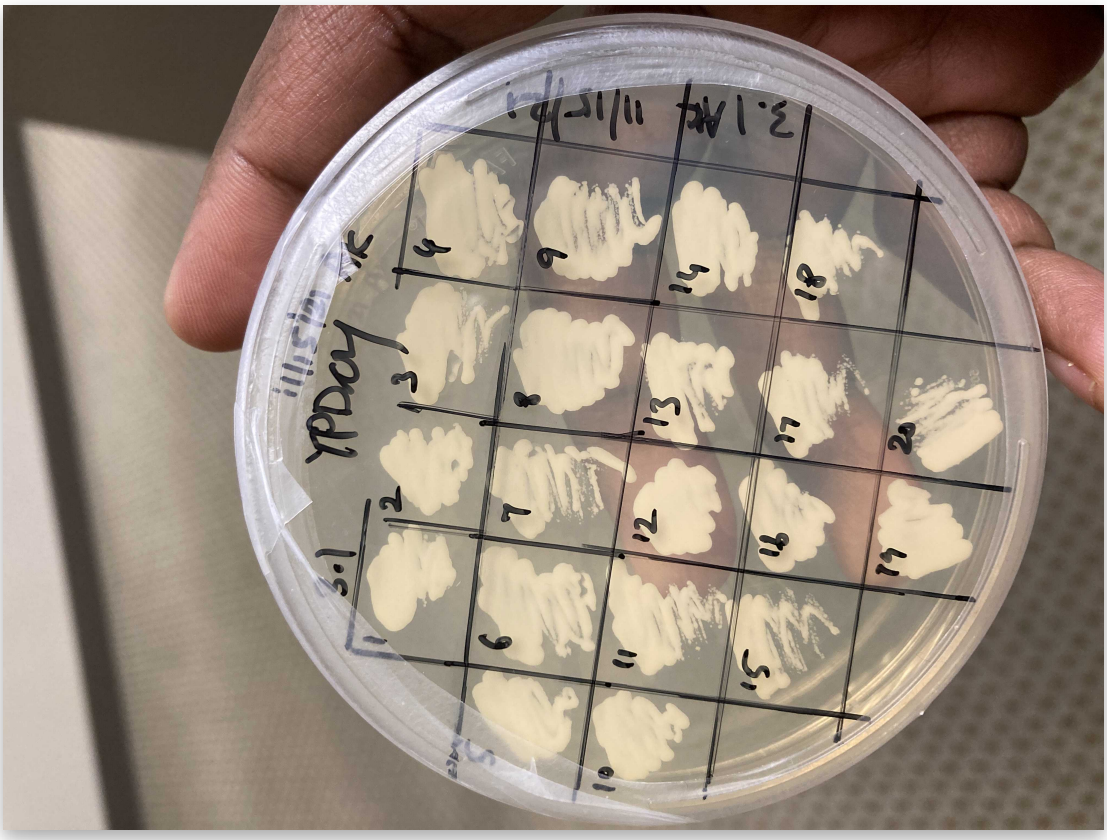


Individual yeast colonies on the transformation plate.



Re-streaked (master) plate containing 20 representative colonies from the original transformation plate.

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2 Incubate the plate in a **30 °C** incubator for 2 days.

3 After 2 days, pick around 8 colonies from the re-streaked plate using the smallest micropipette tip or sterile wooden toothpick.

4 Re-suspend the cells into **20 µL** of **10 millimolar (mM)** sodium hydroxide (NaOH) by twirling the pipette tip or wooden toothpick inside the NaoH solution.

5 Microwave the PCR tubes at the highest power setting for **00:05:00** . 5m

The tubes will be hot. Care should be taken while handling the tubes after microwaving.

6 Take the PCR tubes out of the microwave oven and briefly spin to pellet down the cells using a bench-top centrifuge.

7 Without disturbing the pellet, carefully aspirate **2 µL** out of the tubes and use it as template for PCR.

8 The PCR reaction mix and the primer annealing temperatures can be adjusted depending on the polymerase and the primer pair used.

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