BACILLUS SUBTILIS TRANSFORMATION :

Make an overnight pre-culture in ~5ml LB + Can

D (-1)
Incubate overnight at 30°C with agitation.

D-DAY
**make sure to label tubes as you proceed with performing the assay!

1. Pipette 1000 µl of overnight culture **1** into two 2 mL tube; repeat this step for overnight culture **2**. Also, dispense 1000µl into an additional tube as a control.
2. Spin culture-containing tubes for 3-4min at 5000 rpm.
3. Pipette 1000 µl of solution **I** into each tube ****and spin; dispense of supernatant
4. Re-suspend each tube in 100 µl of solution **I** .
5. Next, transfer the contents of each *eppendorf* tube into its own, separate Falcon tube.
6. Again, pipette 900 µl of solution **I** into each Falcon tube.
7. Place the 3 Falcon tubes into the incubator at 37°C.
8. After 1hr, check the OD. Continue this process until it measures at ~0.5

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 9. Add 900 µl of solution **II** to each Falcon tube to dilute by 10 fold.
10. Pipette 500 µl of each Falcon dilution into 2 individual *eppendorf* tubes.
11. Add 2.5 µl of **s.020** into a tube containing overnight culture**.**
12. Next repeat this step, instead with **s.021** in a separate.
13. Incubate for 1h30 at 37°C.
14. Plate on chloramphenicol plates and grow overnight at 37°C.