Assay for β-galactosidase activity of *B. subtilis* transformed with *lacZ* reporter plasmid

Growth assay:

1. streak onto TBAB, incubate 37°C overnight
2. inoculate 10 ml LB supplemented with 5% glucose & 0.2% glutamine
3. incubate at 37°C with shaking until ~ 100 Klett
4. dilute 1:50 in fresh LB/glucose/glutamine (**t=0**)
5. incubate at 37°C with shaking
6. for the following timepoints: every hour from t=0 to t=8h
    a. remove 2 x 1.5 ml culture
    b. centrifuge
    c. discard supernatant
    d. resuspend pellet in 0.5 ml Z buffer
    e. centrifuge
    f. discard supernatant
    g. freeze pellet (-80°C)

β-galactosidase assay:

1. add β-mercaptoethanol to the Z buffer
2. resuspend pellet in 1 ml Z buffer (work on ice)
    a. dilute a portion 1:5 in Z buffer (before t=3h, do not dilute)
    b. read absorbance at OD_{600} (blank with Z buffer)
3. aliquot 0.1 and 0.2 ml of the resuspended pellet in a 10 ml glass test tube and make up to 1 ml with Z buffer (0.9 and 0.8 ml, respectively)
4. add 2 drops of chloroform and 1 drop of 0.1% SDS using pasteur pipette, vortex 10 s
5. incubate in 28°C waterbath for 5 min

6. begin timing, add 0.2 ml ONPG (4 mg/ml) to each tube (record the exact time of addition for each tube)

7. after development of yellow colour, stop the reaction by adding 0.5 ml 1 M Na_2CO_3 (record the exact time of addition for each tube)

8. transfer to a microcentrifuge tube (avoid transferring chloroform layer)

9. centrifuge

10. transfer supernatant to microtitre plate

11. read absorbance at OD_{420} (aim for 0.6 to 0.8)