Growth curve with OD_{600} measurements with dilutions(v2)

This version of the growth curve measurement protocol has been designed after troubleshooting the previous version. In fact, the previous version present the following issues:

1. Comparing the growth rate of single transformed *E. coli* DH5-α to the double transformed cells introduce an uncertainty due to the difference of antibiotics pressure. Namely, the single transformed *E. coli* DH5-α grow on LB-CAM or LB-AMP, while the double transformed cell grow with both antibiotics present. This issue was addressed by creating an empty pdCas9 vector (that is, a pdCas9 with the coding sequence (CDS) for dCas9 truncated) and working with the following *E. coli* DH5-α strains which all grow in LB-AMP-CAM:
    - 3xFLAG-pdCas9 and pgRNA-bacteria;
    - EpdCas9 and pgRNA-bacteria; (we did not manage to obtain this strain)
    - 3xFLAG-pdCas9 and pgRNA1;
    - 3xFLAG-pdCas9 and pgRNA2;
    - 3xFLAG-pdCas9 and pgRNA3.
2. If the DNA replication is successfully stopped by dCas9-gRNA the cell are expected to enlarge in volume (Wiktor et al., 2016). Since the optical density is highly sensible to cell size (Sutton, 2006) we decided to repeatedly dilute the cell to wash out the non-replicating cell and measure the optical density only at the end point of growth (4 hours).
3. To compare the growth curve of different culture, their OD should be as close as possible at t_0. To do so, we inoculated a volume of cell in 10 mL LB-AMP-CAM calculated to give an OD_{600} = 0.1

Materials:

- Optical neutral cuvettes (1 mL capacity)
- Spectrophotometers
- LB broth
    - with with ampicillin 100 µg/mL
    - with chloramphenicol 37 µg/mL
    - with both antibiotics
- Anhydrotetracycline (aTc)

Procedure:

1. Prepare 10 mL liquid culture in 12 mL round-bottom tubes and incubate them overnight (ON) at 37 °C, 220 rpm. Working strains are:
    a. *E. coli* DH5-α pdCas9 pgRNA-bacteria;
    b. *E. coli* DH5-α pdCas9 pgRNA1;
    c. *E. coli* DH5-α pdCas9 pgRNA2;
    d. *E. coli* DH5-α pdCas9 pgRNA3.

2. The day after measure the OD_{600} of each culture.
3. Calculate the dilution factor needed to obtain OD_{600} = 0.1 in a final volume of 10 mL.

   e.g. If the OD_{600} results 1.645, the volume of cell should be $V_{cell} = (OD_f * V_f)/OD_i = (0.1*10$ mL)/1.645 = 0.608 mL. Hence 0.608 mL should be inoculated in (10 - 0.608) mL = 9.392 mL ≃ 9.4 mL of LB-AMP-CAM broth.

4. Inoculate the calculated volume of cell in LB-AMP-CAM broth for a final volume of 10 mL
5. After vigorous mixing, transfer 1 mL of cell in optical neutral cuvettes. Do NOT label or touch the cuvettes' smooth side. Immediately put the flasks back in the incubator.
6. Set the spectrophotometer on absorbance and the wavelength (λ) at 600 nm.
7. Mix the sample in the cuvettes by pipetting up and down and measure the OD_{600} using LB-AMP-CAM broth as blank. The OD_{600} should be ~ 0.1 for all the liquid cultures.
8. Incubate the tubes at 37 °C, 220 rpm for 1 hour.
9. Add 200 ng/mL anhydrotetracycline (aTc) to the liquid cultures in the incubator.
10. After 4-8 hours dilute 200 µL of cell in fresh LB-AMP-CAM **with 200 ng/mL aTc** for a final volume of 1 mL (This is to maintain the OD measurement within the linear interval 0.1 - 0.6)
11. Measure the OD_{600} of the 5-times diluted cultures as described (steps 5-7).
12. Calculate back the original OD by multiplying 5 times.
13. Dilute each liquid culture 10-times in LB-AMP-CAM **with 200 ng/mL aTc** for a final volume of 10 mL.
14. Measure the OD_{600} of each new liquid culture.
15. Incubate at 37 °C, 220 rpm.
16. Repeat steps 10-15 two times more. The total time of the experiment is ~ 3 days.
17. Transfer the OD_{600} measurements (**N.B.** Always multiply by the OD by the dilution factor) to an electronic sheet, calculate the $\log_{10}$ of each data and draw a growth curve.

References:

Sutton, S. (2006, August). Measurement of Cell Concentration in Suspension by Optical Density. Pharmaceutical Microbiology Forum Newsletter – Vol. 12 (8)
http://www.microbiologyforum.org/content/file/PMFNews.12.08.0608.pdf