Growth Experiments

Design:
Chose a type of container you want to use for your experiments; some examples: sealed/anaerobic bottles, Falcon tubes, Erlenmeyers, 96-wells plates. Always use three biological replicates of your strain. During the experiments, we recommend using a minimal medium (e.g., M9). It is recommended to also add a positive control (e.g., the WT strain of your engineered strain) and a negative control (e.g., minimal medium without culture). During sampling, treat the controls in the same way as the strain you are testing. Chose the time you want to grow and sample your cultures (e.g., every 6 hours for 24 hours, or only at the starting and ending point of the growth experiment). Measure OD_{600} and possibly other components in the medium or headspace (e.g., product formation or consumption) when sampling.
NOTE: it is of great importance to work sterilely in the preparation of the growth experiment, and during sampling.

Materials:

- Sterile containers
- Minimal medium without carbon source
- Minimal medium with carbon source
- Cuvettes
- Liquid pre-culture of the strain you want to test and of your control

Steps:

1. Prepare your mode of incubation: e.g., for a 96-wells plate a platereader is recommended, and for bigger flasks an incubator.
2. Add your minimal medium with carbon source to your containers (this step can also be performed after step 5)
3. Wash your precultures (2mL) in minimal medium without carbon source (twice) and leave them in this medium (1 mL)
4. Measure OD_{600} (in cuvette)
   > blank with minimal medium without carbon source
   > use 20x dilution of microbial culture for measurements
5. Calculate how much culture to add to in the containers; we recommend staring experiment with OD_{600}=0.1 ($C_0*V_0= C*V$ → where the concentration of your washed cells multiplied with the original volume you need to add to your container equals the volume of the liquid in the container multiplied by the starting OD_{600})
6. Add the calculated volume of the culture to the containers (except for the negative controls)
7. Take samples t=0 and measure the OD_{600}
8. Centrifuge the liquid samples for at least 5 minutes at maximum speed and save the supernatant if you want to examine the supernatant.
9. Repeat steps 7&8 at all sampling timepoints