5: Consistency of OD diluting every doubling time.

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**Overview and goals:**

The goal of this experiment is to see how well a 2:1 dilution every doubling time approximates back diluting at a specific OD. We want to know this information because the robot cannot read OD, and thus will have to dilute the stable culture at a specific rate.

At the end of this experiment, we should:

* Know how much the OD diverges after diluting every doubling time vs at OD of 0.4

**Three days before Experiment(4/11):**

* Streak LP\_HS\_GFP and LP\_HS\_mCherry from Glycerol on plates of MSR+CAM. Grow up to 24 hours. Also streak LP\_WT as a control
* Prepare MSR+CAM
* Prepare PBS+CAM

**One day before the experiment (4/13):**

* Grow 3 tubes of LP\_WF\_GFP and LP\_WF\_mCherry from 3 separate colonies from plates in 3 mL MSR+CAM. Grow a tube of LP\_WT as a control.

**Day 1 (4/14):**

* Dilute LP replicates in MSR+CAM to an OD of 0.5. Read the OD using the cuvette reader in lab
* Further dilute this OD of 0.5 culture to an OD of 0.05. Grow until the culture reaches log-phase around an OD of 0.4. Check OD on plate reader
* Remove Plate from plate reader and dilute 1:2.
* Repeat every doubling time for 3-4 cycles.
* Measure divergence from predicted OD (OD at first dilution)

**Improvements for next time (on robot)**

* Use 150uL not 200uL to make sure robot does not overflow well.
* Try more frequent doubling time dilution on robot.
* Can we do fixative to maintain OD so I don’t have to come and take readings every 90 minutes?