RPU Measurement Method

1. Grow cell culture overnight (Incubate 37°C and shake for 15 hours) in falcon tubes with M9 minimal medium
2. Prepare M9 medium with desired volume in a 96 Deep Well plate. The positive control for the experiment is BBa_I20260, which contains constitutive promoter J23100, and GFP generator.
3. Take out 20-30 μl of overnight cell culture and mix it with M9 medium in the 96 Deep Well plate
4. Incubate in 37°C and shake for 3-4 hours.
5. Take out 200ul of cells from the 96 well plates, and put it on a micro test plate 96 well flat bottom.
6. Measure the OD_{600} and green fluorescence protein expression of the cells plate reader.
7. Measure every 30 minutes span, for 2 - 3 hours. Make sure that the cells are in the mid log phase (OD_{600} = 0.3-0.5). In between measurements, keep incubating the cells in 37°C while shaking.
8. Calculate the RPU (Relative Promoter Unit) based on the paper “Measuring the activity of BioBrick promoters using an in vivo reference standard” by Kelly et al.

$$RPU = \frac{S^{SS}_{cell,\phi}}{S^{SS}_{cell,J23101}} = \frac{(dF_{\phi}/dt)/ABS_{\phi}}{(dF_{J23101}/dt)/ABS_{J23101}}$$