List of Protocols Stability

Temperature Stress

Establish overnight cultures of each strain by inoculating a colony of the strain in 5 mL of LB (if the cells are transformed with a Biobrick add the corresponding antibiotic or selective factor in the LB) and growing overnight.

Dilute the cultures grown overnight to an $O.D._{600}$ of 0.1.

Fill in PCR tubes with 75 µL of diluted culture and place the tubes in the thermal cycler (in our case the thermal cycler used was the ***Mastercycler – Eppendorf***) previously programmed with the gradient, which starts at 30ºC and ends at 50ºC.

Incubate the tubes for 3 hours, recollect the tubes, resuspend the culture in each tube carefully with a pipet and measure $O.D._{600}$ in a microplate reader (in our case the device used was the ***Multiskan Ascent 96 well Plate Reader)*** or manually.

Measuring Fluorescence

To measure fluorescence follow the same procedure described above, but apart from $O.D._{600}$, the samples have to be taken to a fluorometer. In our case the flurorometer used was the ***Hitachi F-7000 Fluorescence Spectrophotometer, 96 Well.*** Setting the corresponding wavelengths of GFP (λ_{ex} = 493 nm; λ_{em} = 505 nm) or RFP (λ_{ex} = 572 nm; λ_{em} = 592nm) fluorescence is to be measured and the data obtained normalized to the values of $O.D._{600}$.

Material Fatigue

Establish overnight cultures of each strain by inoculating a colony of the strain in 5 mL of LB (if the cells are transformed with a Biobrick add the corresponding antibiotic or selective factor in the LB) and growing overnight.

Dilute the cultures grown overnight to an $O.D._{600}$ of 0.1

Fill in PCR tubes with 75 µL of diluted culture and place the tubes in the thermal cycler previously programmed with the temperature oscillation setting (one

minute at 37ºC and the next at 41ºC, continuous change every minute) for 3 hours.

It is indispensable to set controls, so the same numbers of tubes, with 75 µL of diluted culture each, are to be placed in the thermo cycler, but this time programmed to establish a single uniform temperature: 37ºC. This is to be repeated with a temperature of 41ºC. Run the program for 3 hours just like the oscillation.

Recollect the tubes and measure $O.D._{600}$ as well as fluorescence just like the previous experiment (temperature stress).

pH & Salinity

Establish overnight cultures of each strain by inoculating a colony of the strain in 5 mL of LB (if the cells are transformed with a Biobrick add the corresponding antibiotic or selective factor in the LB) and growing overnight.

Dilute the cultures grown overnight to an $O.D._{600}$ of 0.2.

Pellet the cells by centrifugation (14000 r.p.m, 3 minutes), discard supernatant and resuspend the pellet (cells) in different Eppendorf tubes tubes containing LB with pH ranging from 5 to 8.

Incubate the tubes in a shaker (200 r.p.m.) at 37ºC for 90 minutes.

After the incubation period recollect the tubes and measure $O.D._{600}$ as well fluorescence using the same methodology and instrumentation as the abovementioned experiments.

The salinity experiment follows the same design as the pH but instead of using LB with different pH, use LB with salt concentration ranging from 0% to +2% salts maintaining the normal pH of the LB medium for all the tubes

Ultraviolet

Establish overnight cultures of each strain by inoculating a colony of the strain in 5 mL of LB (if the cells are transformed with a Biobrick add the corresponding antibiotic or selective factor in the LB) and growing overnight.

Dilute the cultures grown overnight to an $O.D._{600}$ of 0.03.

Spot plate the cultures (20 µL) on LB agar (if the cells are transformed with Biobricks, use LB agar with corresponding antibiotic or selective factor).

Subject the spots to timed pulses of UV radiation (intensity of 340 $\mu W/cm^2$), i.e. each strain should receive a pulse of 2, 4, 6, 8 and 10 seconds, with a control that receives no UV irradiation at all.

*Each pulse is given to a different spot plated Petri dish, so in total, 6 Petri dishes per strain (one per pulse of UV radiation + control) are needed to carry out the experiment.

Incubate the Petri dishes for 48 hours at 37ºC to allow bacterial growth.

After the incubation period, effects of UV on bacterial growth can be discerned with the naked eye on the plate by counting the number of colonies that survived. But to assay the Biobrick behaviour, fluorescence is to be measured and normalized to $O.D._{600}$. These measurements should be carried out as in the experiments described above.

Vacuum

Establish overnight cultures of each strain by inoculating a colony of the strain in 5 mL of LB (if the cells are transformed with a Biobrick add the corresponding antibiotic or selective factor in the LB) and growing overnight.

Dilute the cultures grown overnight to an $O.D._{600}$ of 0.1

Spot plate the cultures (20 µL) on LB agar (if the cells are transformed with Biobricks, use LB agar with corresponding antibiotic or selective factor).

Incubate the plates in deep vacuum and 37ºC for 48 hours to enable bacterial growth and additionally maintain an identical control set of plates that must grow at 37ºC for 48 hours but at atmospheric pressure.

After the incubation period, effects of vacuum on bacterial growth can be discerned by measuring colony diameter. But to assay the Biobrick behaviour, fluorescence is to be measured and normalized to $O.D._{600}$. These measurements should be carried out as in the experiments described above.

To establish the growth limit under vacuum, instead of measuring $O.D._{600}$, triple streak LB-agar plates with the wildtype strains and incubate for 48 hours at 37ºC in vacuum, as well as the control set of plates in atmospheric pressure.

After the incubation period, select three representative colonies (individual, isolated colonies) from each plate and measure their diameter.