

Epi-fluorescence Wide-Field Microscopy

Bacterial culture and Manipulation:

4mL of LB broth with Ampicillin was inoculated from colonies off a master plate of positive clones and grown overnight at 37°C in a shaking incubator. 2mL of each bacterial culture was then used to inoculate 25mL of LB broth with Ampicillin and grown for \pm 3 hours in a shaking incubator at 37°C until the cells reached an OD of 0.55 (mid-log phase). 2 x 5mL of each sample culture was aliquoted into separate 15mL falcon tubes. One tube was left untreated; 150 μ L of 15mM theophylline was added to the other (to make a concentration of 1.5mM theophylline). These 5mL aliquots were placed in a shaking incubator at 37°C for 30 min each to allow for activation. 200 μ L of each treated and untreated sample was then used in a microscope plate for imaging.

Equipment:

Zeiss Axioscope

- Andor EMCCD Camera
- Till Photonics Polychrome V

Imaging:

Widefield fluorescence microscopy was used to assess the expression of the Venus protein. The bacterial samples were excited at 500nm using the polychrome as an excitation source. The fluorescence was then observed by capturing light in the emission spectrum of Venus (528nm). The figure below represents a schematic of the imaging setup. Corresponding bright-field images (using white light) were also captured for each experiment.

Microscope Setup:

