# Fluorescence Microscopy – Smeared Culture Technique (Fixed Bacteria)

- 4mL of LB broth with Ampicillin (the antibiotic resistance of the pSB1A3 backbone, into which the constructs were cloned) was inoculated from colonies off a master plate (or a scrape off a frozen glycerol stock) of positive clones and grown overnight at 37°C in a shaking incubator.

- 1mL of each bacterial culture was then used to inoculate 25mL of LB broth with Ampicillin and grown for ± 2 hours in a shaking incubator at 37°C until the cells reached mid-log phase.

- 2 x 5mL of each sample culture was aliquoted into separate 50mL falcon tubes (to have enough aeration during induction).

- One tube was left untreated; 150µL of 15mM theophylline was added to the other (to make a concentration of 1.5mM theophylline – to induce activation of the theophylline riboswitches).

- These 5mL aliquots were placed in a shaking incubator at 37°C for 90 min each to allow for activation.

- 1mL of each culture was then aliquoted into a 1.5mL Eppendorf tube and centrifuged for 10min at 4000rpm.

- Supernatant was poured off.

- The bacterial pellet was re-suspended in 50µL of LB broth.

- 5µL of these re-suspended cultures were then mixed with 5µL of an aqueous mounting medium on normal glass microscope slides.

- This mixture was smeared across the glass slide with a long cover slip, before placing the cover slip on top of this smear.

- Slide is ready for viewing under a fluorescent microscope using a 100X objective.