

## Alternative protocol for experiment 2

- Grow transformed cells in LB until they reach an  $OD_{600}$  of 0.4 – 0.5.
- Spin down 1ml of the culture at 8000rpm for 5 minutes.
- Resuspend cells in 0.5ml 3% BSA and leave to stand at room temperature for 20 minutes.
- Spin down cells.
- Resuspend in PBS and add 0.5ml of the diluted primary antibody (antiFLAG tag antibody).
- Leave to stand at room temperature for 10 minutes.
- Spin down the cells, and wash in 0.5ml PBS.
- Resuspend the cells in 0.5ml 3% BSA, and add 0.5ml of the diluted secondary antibody (anti-mouse antibody). Leave to stand at room temperature for 15 minutes.
- Spin down the cells and complete 2 PBS.
- Resuspend the cells in 20 $\mu$ l PBS.
- Place 10 $\mu$ l PBS onto a glass slide (and seal with vacuum grease).
- Visualise under a fluorescent microscope.