BABS UNSW iGEM Lab Protocol

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| Procedure | Name | Mammalian cell invasion assay | | | | |
| | Description | Testing of the IPTG induced invasion+LLO+GFP system across all mammalian and bacterial cells. | | | | |
| Document | Name | Kristen Perry | Date | 20/08/15 | Version | 1 |

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| Requirements | Time | Induction and incubation – 2 days<br>Spec. prep. – 30 mins + 24 hr incubation<br>Microscopy – 1 day |
| | PPE | Gloves, lab coats (experiments)<br>Gloves, gowns (microscopy) |
| | Equipment | 210 x small culture dishes<br>210 x 0.17 mm round coverslips<br>105 x microscope slides<br>Pipettes, tips<br>Centrifuge<br>OD reader |
| | Materials | Mammalian cells<br>Bacterial cells transformed with IPTG+INV+LLO+GFP<br><br>L-15 media<br>L-15 media + 100 µg/ml gentamicin<br>IPTG<br>PBS<br><br>Fixing and mounting materials – 0.3 M glycine, 3-4% paraformaldehyde, Prolon gold |
| Step 1 | Prepare 210 small cell culture dishes with mammalian cells in L-15 media, with coverslips inserted in the base<br><br>**I know this is a lot, and that's just for one mammalian cell type – this would be ideal in my opinion, but if we need to cut down, we could remove the 10:1 and/or 8:1 ratio, or the 18 hr time point** | |
| Step 2 | Measure concentration/confluence of mammalian cells and calculate cell population | |
| Step 3 | Pellet transformed bacteria, gently wash in PBS and resuspend | |

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| Step 4 | Dilute to desired OD (measured per 100 ul) such that ratios range from <1:1 to 10:1 |
| Step 5 | Gently wash mammalian cells in L-15 media (and resuspend (EDTA/trypsin)?) in L-15<br><br>**Unsure as to whether we should resuspend monolayer to provide more cell surface area at this step** |
| Step 6 | Add 100 uL of bacterial solution to each well (100 uL PBS for control) and add IPTG to induce gene expression |
| Step 7 | Incubate at 37 degrees in atmospheric CO2 as follows:<br><br>**Synthetic chloroplast paper incubated their cyanobacteria cultures at 30 degrees, unsure whether this is a good idea? Optimal temp. seems to be 30-33 for Synechocystis**<br><br>e.g. HeLa cells |

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| E. coli |
| Lactococcus |
| Synechocystis |

| | RATIO OF BACTERIA TO HOST | | | | | | |
|---|---|---|---|---|---|---|---|
| INCUBATION TIME | 3 hour incubation | 0 | <1:1 | 2:1 | 4:1 | 6:1 | 8:1 | 10:1 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | 6 hour incubation | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | 9 hour incubation | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | 18 hour incubation | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |

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|---|---|---|---|---|---|---|---|---|
| | 24 hour incubation | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |
| | | 0 | <1 | 2 | 4 | 6 | 8 | 10 |

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| Step 8 | Following incubation, wash cells three times with PBS and replace media with L-15 containing 100 ug/mL gentamycin (does not permeate mammalian cell membrane) |
| Step 9 | Rinse cells in PBS and remove supernatant |
| Step 10 | Fix immediately in 3-4% paraformaldehyde for 10-20 minutes, with 0.3M glycine added. Rinse briefly with PBS. |
| Step 11 | Mount with Prolon gold and leave for 24 hours. |
| Step 13 | Image with brightfield Zeiss microscope. Before imaging, dab coverslip clean of PBS with Kimiwipe and water to avoid crystal artefact |
| Notes | Need to confirm whether IPTG in cultures will autofluoresce within GFP emission or otherwise affect imaging |
| Version History | First version – up for correction and annotations |