



University of Melbourne iGEM 2014 Lab Procedure

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|-----------------|---|--|-------|----------|
| Procedure | Name: | Protein Expression in <i>E. coli</i> | | |
| | Version: | 1 | | |
| | Description: | Inducing protein expression in <i>E. coli</i> | | |
| | Trigger: | | | |
| Last updated | Name: | Elizabeth Brookes | Date: | 29.07.14 |
| You will need | Time: | | | |
| | PPE: | Gloves Lab coat | | |
| | Equipment: | Centrifuge 37°C Shaking Incubator 2L Flask | | |
| | Materials: | LB Broth Antibiotic IPTG Solution | | |
| Step 1 | Pick a single colony from a freshly streaked plate of the expression host containing the recombinant vector. | | | |
| Step 2 | Inoculate the picked colony in 4mL of LB containing the appropriate antibiotic (use 1μL of antibiotic for 1 mL of LB). | | | |
| Step 3 | Grow the culture for 4-8 hours at 37°C with shaking (200 rpm). | | | |
| Step 4 | After incubating, transfer 1mL of the culture to 25mL of LB with the appropriate antibiotic added. Grow the culture overnight at 37°C with shaking. Don't leave the culture for any longer than 16 hours. | | | |
| Step 5 | <p>Ensure that the cold room incubator is available for overnight expression. Note that you need to use the cold room incubator to get the temperature down to 16°.</p> <p>Spin the overnight cultures to remove β-lactamase and resuspend the pellet in 800mL fresh medium (with antibiotic added) in a 2L flask. Do not vortex cells.</p> | | | |
| Step 6 | <p>Grow the cells at 37°C with shaking and monitor the OD. When the OD reaches 0.6, cool the culture to 16°C in ice water.</p> <p><i>Note: For more detailed instructions for this procedure see 'A4 Measuring OD Protocol'.</i></p> | | | |
| Step 7 | Add 800μL of 1M IPTG solution (filter sterilized) to the culture and induce overnight at 16°, 200 RPM. Add the Aerotop seals if using Ultra Yield Flasks. | | | |
| Step 8 | Harvest the cell pellet using a centrifuge for 20 minutes at high speed and store the cell pellet at -20°C. Do not vortex cells. | | | |
| Version history | <i>Describe the changes made in each new version of the protocol here.</i> | | | |