

## **Cultivation *Escherichia coli* for RNA purification**

The following protocol was used to cultivate *E. coli* for the RNA purification for tRNA quantification and for the bio brick characterisation.

1. Pick single colony from agar plate and inoculate a glass test tube containing 5 mL 2YT medium. Add antibiotic if necessary (see tables below).
2. Incubate the culture at 37 °C and 130 rpm.
3. Take samples depending on the experiment.
4. Pellet the cells via centrifugation and store the pellets at -70 °C.

Table 1: Strains and required antibiotic.

| Strain                       | Antibiotic      |
|------------------------------|-----------------|
| <i>E. coli</i> BL21DE3       | -               |
| <i>E. coli</i> BL21DE3 pRARE | Chloramphenicol |
| <i>E. coli</i> Rosetta       | Chloramphenicol |
| <i>E. coli</i> MG1655        | -               |
| <i>E. coli</i> MG1655 J61002 | Ampicillin      |

Table 2: Applied antibiotics and their concentrations.

| Antibiotic      | Stock concentration<br>[mg/mL] | Working<br>concentration<br>[µg/mL] |
|-----------------|--------------------------------|-------------------------------------|
| Chloramphenicol | 20                             | 20                                  |
| Tetracyclin     | 10                             | 10                                  |
| Kanamycin       | 50                             | 50                                  |
| Ampicillin      | 100                            | 100                                 |