TAN TAO UNIVERSITY
FROM KNOWLEDGE TO THE STARS

Final Examination
Subject: Lab Practices 2 (BT_AB205)
Time: 90min

Name:_____

Student's number:_____

Questions:

1. Generation time of *E. coli* is 20 min/generation. If 100 cells growing for 5 hours, how many cells were produced? (10p)
2. Describe how to prepare 1L **Solution:** 50 mM glucose, 10 mM EDTA, 25 mM Tris, pH 8.0. (15p)
   (Glucose: 180 g/mol, EDTA 292 g/mol, Tris 121 g/mol)
3. Composition of Luria-Bertani (LB) medium for bacteria culture:
   (For 1 litter)
   - 10 g tryptone
   - 5 g yeast extract
   - 10 g NaCl
   - pH 7.4

   What is the role of each component in LB medium? (15p)
4. Write the protocol for preparing *E. coli* competent cell and plasmid transformation. (30p)
5. Write the protocol for plasmid isolation. (30p)

Answers:

1. 5h = 300 min
   Number of generation: 300 : 20 = 15 generation
   Number of bacteria after 5h: 100 x 2^{15} = 3276800 cells
2. Weight 9g glucose and dissolve in 80ml dH_2O, after that adjust total volume to 100ml with dH_2O. Final concentration of solution is 500mM glucose.

Weight 14.6g EDTA and dissolve in 80ml dH_2O, add NaOH to increase solubility of EDTA, after that adjust total volume to 100ml with dH_2O. Final concentration of solution is 500mM EDTA.
Weight 12.1g Tris and dissolve in 80ml dH_2O, after that adjust total volume to 100ml with dH_2O. Final concentration of solution is 1M Tris.

Add 100ml 500mM glucose, 20ml 500mM EDTA, and 25ml 1M Tris to 700ml dH_2O. Mix solution very well and adjust pH to 8.0, after that adjust total volume to 1000ml with dH_2O.

3. 10 g tryptone: provide amino acid
   5 g yeast extract: provide amino acid and carbohydate
   10 g NaCl; Provide osmolality pressure
   pH 7.4: pH suitable for wide range of bacteria.

4.

- Inoculate a single colony into 5mL Lb in 50mL falcon tube. Grow
- O/N at 37°C.
- Use 1mL to inoculate 100mL of LB in 250mL bottle the next morning.
- Shake at 37°C for 1.5-3hrs.

Or

- Inoculate a single colony into 25mL LB in a 250 mL bottle in the morning.
- Shake at 37°C for 4-6 hrs.
- Put the cells on ice for 10 mins (keep cold form now on).
- Collect the cells by centrifugation in the big centrifuge for 3 mins at 6000rpm
- Decant supernatant and gently resuspend on 10 mL cold 0.1M $CaCl_2$ (cells are susceptible to mechanical disruption, so treat them nicely).
- Incubate on ice x 20 mins
- Centrifuge as in 2
- Discard supernatant and gently resuspend on 5mL cold
- 0.1MCaCl
- Dispense in microtubes (300µL/tube). Freeze in -80°C.
- Put 1µL of circular plasmid or all of a ligation reaction of plasmid
- DNA in a microtube. Gently add ~100µL of competent cells. Do NO
- DNA control tube with cells and no DNA.
- Incubate for 30 mins on ice.
- Heat shock for 2 mins @ 42°C. Put back on ice.
- Add 900 µL of LB to tubes. Incubate @ 37°C for 30 mins.
- Plate 100-1000 µL of the cells in LBAmp (100µg/ml) plates.
- Plate 100 µL of the NO DNA control in a plate (to check for quality of cells).

5.

- 5 ml LB medium containing proper antibiotics were inoculated with a single bacterial colony. The tube was incubated at 37 °C overnight with vigorous shaking at 360 rpm.
- Pellet bacteria from the culture at 10,000 x g for 5 minutes at room temperature.
- Discard the supernatant**.**
- Resuspend bacterial pellet in a total of 1 ml ice-cooled solution I (50 mM). Pipet up and down or vortex as necessary to fully resuspend the bacteria.
- Add 2 ml room temperature 0.2 N NaOH/1.0% SDS to the suspension. Mix thoroughly by repeated gentle inversion. **Do not vortex.**
- Add 1.5 ml ice-cold Solution III to the lysate. Mix thoroughly by repeated gentle inversion. **Do not vortex.**
- Centrifuge at 15,500 x g for 30 minutes at 4C.
- Recover resulting supernatant.
- Add 2.5 volume isopropanol to precipitate the plasmid DNA. Mix thoroughly by repeated gentle inversion. **Do not vortex.**
- Centrifuge at 15,500 x g for 30 minutes at 4C.
- Removal of resulting supernatant. The pellet is plasmid DNA.
- Rinse the pellet in ice-cold 70% EtOH and air-dry for about 10 minutes to allow the EtOH to evaporate.
- Add ddH_2O or TE to dissolve the pellet. After addition of 2ul RNase A (10mg/ml), the mixture was incubated for 20 minutes at room temperature to remove RNA.