# Bioinformatics and Bioengineering Summer Institute (2003)

Introduction to the Institute – Dilution Problems

**1.** You want to dilute a culture of *E. coli* at a concentration of $2 \cdot 10^9$ cells/ml to a point where you can spread 100 µl on a plate and get a countable number of colonies (define "countable" as 50 to 300).

**1a.** What volume of the original culture would you need to pipette into 100 µl fresh growth medium in order to get a countable number of colonies?

**1b.** Considering that the smallest volume you can pipet accurately is about 2 µl, can you perform the dilution calculated in **4a**?

**1c.** Fill in the blanks in the dilution scheme below, designed to achieve the desired objective:

| Culture | Operation | Concentration | Cells in 100 µl (= cells on plate) |
|---|---|---|---|
| Culture **0** | (original culture) | $2 \cdot 10^9$ cells/ml | $2 \cdot 10^8$ cells |
| Culture **A** | 1 ml medium + 10 µl of Culture **0** | $2 \cdot 10^7$ cells/ml | $2 \cdot 10^6$ cells |
| Culture **B** | 1 ml medium + 10 µl of Culture **A** | | |
| Culture **C** | 1 ml medium + 5 µl of Culture **B** | | |

**2.** You want to dilute a culture of *E. coli* at a concentration of $1 \cdot 10^9$ cells/ml to a point where you can spread 200 µl on a plate and get a countable number of colonies (define "countable" as 50 to 300).

**2a.** What volume of the original culture would you need to pipette into 100 µl fresh growth medium in order to get a countable number of colonies?

**2b.** Considering that the smallest volume you can pipet accurately is about 2 µl, can you perform the dilution calculated in **4a**?

**2c.** Fill in the blanks in the dilution scheme below, designed to achieve the desired objective:

| Culture | Operation | Concentration | Cells in 100 µl (= cells on plate) |
|---|---|---|---|
| Culture **0** | (original culture) | $1 \cdot 10^9$ cells/ml | $2 \cdot 10^8$ cells |
| Culture **A** | 1 ml medium + 10 µl of Culture **0** | $1 \cdot 10^7$ cells/ml | $2 \cdot 10^6$ cells |
| Culture **B** | 1 ml medium + 10 µl of Culture **A** | | |
| Culture **C** | 1 ml medium + 5 µl of Culture **B** | | |

**3.** You wish to grow *E. coli* in 5 ml of liquid medium containing 15 μg/ml of the antibiotic tetracycline. You have 5 ml of liquid medium without antibiotic and you have a stock solution of tetracycline at a concentration of 15 mg/ml.

**3a.** By what factor must the 15 mg/ml stock solution of tetracycline be diluted to reach the final concentration of 15 μg/ml? Call that factor the "dilution factor".

**3b.** What volume of the stock solution must you add to 5 ml in order for the tetracycline to be diluted by the dilution factor?

**4.** You wish to grow *E. coli* in 10 ml of liquid medium containing 50 μg/ml of the antibiotic kanamycin. You have 10 ml of liquid medium without antibiotic and you have a stock solution of kanamycin at a concentration of 25 mg/ml.

**4a.** By what factor must the 25 mg/ml stock solution of kanamycin be diluted to reach the final concentration of 50 μg/ml? Call that factor the "dilution factor".

**4b.** What volume of the stock solution must you add to 10 ml in order for the kanamycin to be diluted by the dilution factor?

**5.** **Person #1:**Using a micropipettor, pipet 150 μl of water 3 times to a weighing dish.
**Person #2:** Using the same micropipettor, pipet 75μl of water 6 times from the weighing dish. (you should exactly exhaust the water).

**6.** **Person #1 and #2:**Using a glass pipet, suck up 6.6 ml water and distribute 2.2 ml to each of three weiging dishes.