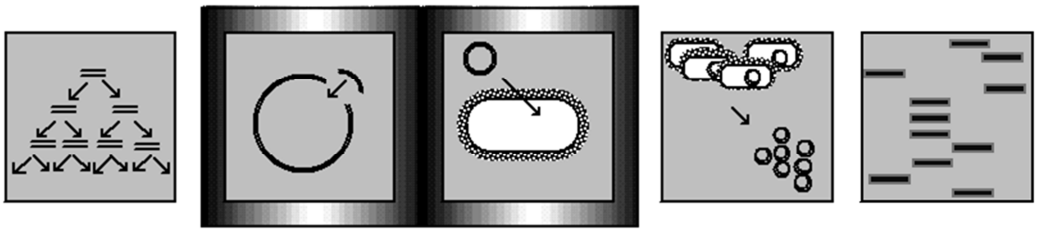
DAY 3 - ANALYSING YOUR TRANSFORMED BACTERIAL CELLS



Introduction

In the process described in the previous section, transformation efficiency is very poor and only a small percentage of the bacteria will actually take up the plasmid. Bacteria that have been transformed must be selected, or isolated. The plasmid is too small to be seen, so it is useful for it to contain a gene that expresses a characteristic that can be seen or interpreted, for example, antibiotic resistance. Plasmids can have multiple genes inserted and these can be used to select for bacterial cells containing a plasmid. In the case of this lab, the cells containing plasmid with your ligated gene will grow on agar plates that contain the antibiotic ampicillin and a sugar known as X-Gal. The colonies of bacteria on your plates should be either white or blue in colour.

1. Analysing your transformations

With luck, you should have colonies on at least some of your plates. This section will guide you through their significance.

Q2. Briefly, explain the significance of the difference between a blue and a white colony.

Before analysing your own plates, think about the following table, considering the **expected results** first.

Q3. Complete the Table below, explaining what you expect to see, and what you actually see, on each of the plates. Score as "none", "few" (1-10), "some" (10-100) or "lots" (100+).

Plate Description	Expected Result		Your Result		Overall Class Result	
	Blue	White	Blue	White	Blue	White
1) plasmid: PCR product ligation						
2) Plasmid only ligation						
3) Ligase control						
4) Teacher's ligation						
5) (a) Uncut pBluescript						
(b) Cells only (without DNA)						
(c) Double cut, unligated plasmid						
(d) Cells only – no ampicillin selection						

Discuss the following questions, **explaining how you arrive at your conclusions**:

Q4. Which of the control plate(s) tell you that the antibiotic selection worked? Did this selection work on your own plate(s) and for the majority of the class?

Q5. Did the blue/white screening work in your plates?

Q6. Note the appearance of control plate grown in absence of ampicillin (d). You should have seen a bacterial lawn, or that the entire plate was covered with bacteria. Why does a bacterial lawn appear on this plate while other plates with growth have distinct, punctate colonies?

Q7. Which of the control plate(s) tell you that the cells survived the transformation procedure? Did your own cells survive this process?

Q8. One of the controls you set up for the ligation reaction was to test whether the DNA ligase was working or not. What was your result for this control, following the transformation reaction? Comment on the importance of including this control as one of the ligation reactions you carried out.

Q9. In a restriction digest it is not uncommon for a few DNA molecules to escape digestion (cutting). One of the transformation controls ((c) double cut, unligated plasmid) set up by the class will tell us whether our plasmid preparation contained any residual circular (uncut) plasmid, which had not been cut by either enzyme. Was the pre-cut pBluescript plasmid vector completely cut – comment on your own result and the class as a whole? If not, can you think why that might be?

2. Calculating Transformation Efficiency

Aim

To determine how well your *E.coli* competent cells took up and expressed plasmid vector.

Background

Your next task is to determine the extent to which you genetically transformed *E.coli* bacterial cells. This quantitative measurement is referred to as the **transformation efficiency**. In many experiments it is important to genetically transform as many cells as possible. The transformation efficiency is calculated to help scientists determine how well the transformation is working.

Transformation efficiency is a number. It represents the total number of bacterial cells that express a plasmid vector, divided by the amount of DNA used in the experiment. It tells us the total number of bacterial cells transformed by one microgram of DNA. To work this out in this lab, use the control plate that was transformed with uncut pBluescript plasmid (control plate (a)).

To calculate the transformation efficiency, you require to know the following information:

10pg/μl = concentration of uncut pBluescript plasmid DNA

2μl = volume of the above plasmid solution used for transformation

100μl = volume spread on the agar plate

250μl = total volume of transformation reaction (50μl of bacterial cells plus 200μl L-Broth)

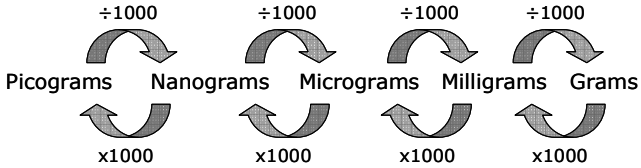
A. Determine the Concentration of DNA in μg

In molecular biology you often use extremely small measures, such as picogram or nanogram amounts of DNA. It is useful to be able to convert between different units of measurement.

Remember:

NAME	ABBREVIATION	EQUIVALENT
1 gram	g	1000mg
1 milligram	mg	1000μg
1 microgram	μg	1000ng
1 nanogram	ng	1000pg
1 picogram	pg	1/1000ng

To convert from smaller units to larger ones, we need to divide by 1000, or to convert from a larger unit to a smaller one, we need to multiply by 1000.



As seen in the table above, each smaller unit has 1000 of them to equate to the larger unit. For instance, 1 mg is equivalent to 1000μg. To convert to a smaller unit you need more of them so multiply.

For example:

Micrograms to *picograms (μg to pg):* 1,000,000 x μg = pg

For example, if μg = 143 then pg = 1,000,000 x 143 = 143,000,000pg or 1.43×10^8 pg

Picograms to *micrograms (pg to μg): pg* ÷ 1,000,000 = μg

For example, if pg = 143 then μg = 143÷ 1,000,000 = 0.000143 μg or 1.43×10^{-4} μg

Q10. Given the concentration of uncut pBluescript plasmid DNA is 10pg/μl, convert this to μg/μl

B. Determine the Total Number of Transformed Cells

Have a look at a control plate representing the cells transformed with uncut pBluescript plasmid (control plate (a)). Each colony on the plate can be assumed to be derived from a single cell. As individual cells reproduce, more and more cells are formed and develop into what is termed a colony. The most direct way to determine the total number of cells containing uncut pBluescript is to count the colonies on the plate. Enter the number of colonies below:

#Colonies =

C. Determine the Fraction of pBluescript DNA in the Bacterial Cells spread on the L-agar plate.

A. Total amount of pBluescript plasmid DNA:

(DNA in μg) = (concentration of DNA in μg/μl) x (volume of plasmid in μl)

B. Fraction of pBluescript plasmid DNA (in the bacteria) that actually got spread onto the L-agar plate:

Fraction of DNA used = Volume spread on L-agar plate (μl) ÷Total volume of the transformation reaction (μl)

Therefore, pBluescript DNA spread (in μg) = A x B

D. Transformation Efficiency

Based on the calculations from Step 2 and Step 3, determine the transformation efficiency:

Transformation Efficiency = Total number of colonies growing on the agar plate ÷ amount of DNA spread on the agar plate (in μg)

Enter that number →

Transformation efficiency = transformants/μg

E. Transformation Efficiency – How Successful was Your Transformation?

Questions

Q11. The transformation protocol you used generally has a transformation efficiency of $2x10^8$ to 10^{11} transformants/μg plasmid DNA. How does this compare with your own result?

Q12. Note below the transformation efficiency of several others in the class – how does your result compare with theirs?

Q13. Thinking about the transformation protocol, what factors do you think might influence transformation efficiency?

3. Setting up an 'Overnight' Culture

Introduction

E.coli grows well either on nutrient agar (as last time) or in vigorously shaken liquid culture. It is this latter form of bacteria that we need for tomorrow. Liquid cultures are used to prepare large amounts of recombinant plasmid DNA, and until the advent of PCR, this was the *only* way of amplifying DNA. Because it is cheaper, can handle larger DNA fragments and is less prone to introducing sequence errors, it is still the method of choice for many applications. In this section, we choose single colonies from your plates, and add them to nutrient L-broth containing antibiotic, and grow them overnight with shaking.

CAUTION: If the experiment has succeeded, you are handling recombinant bacteria. Observe good microbiological practice. Dispose of all solutions into disinfectant. Clear up and disinfect any spills.

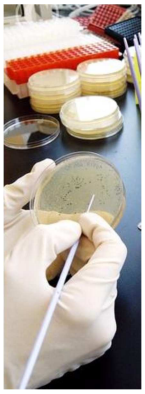
Method

Set up 2 overnight cultures:

1. You are provided with 20 ml L-broth, to which you must add ampicillin to a final concentration of 50 μg/ml (stock solution is 50 mg/ml). To do so, use the following calculation:

$$\frac{\text{Final concentration}}{\text{Stock concentration}} \times \text{final volume} = \text{volume to add to solution}$$

- Check with a demonstrator that you have calculated the amount correctly.
- Put approx. 10 ml into each of 2 culture tubes, labelled clearly on the tube, **not the lid**, with your initials and "blue" or "white".



2. Inoculate a blue colony as a control into the first tube (these bacteria should carry plasmid vector with no insert). To pick the bacteria, use the sterile plastic loop provided and touch an isolated, single blue colony, making certain that some of the cells have been transferred to the loop. Drop it into the liquid L-broth and shake the loop a bit. Replace the tube's cap as soon as the inoculation is complete.
3. Inoculate a white colony (which should be bacteria carrying recombinant plasmid [vector + insert]) into the second tube using the same method as above. If you didn't get any white colonies, the demonstrator will provide you with a plate.

The cultures will be grown overnight in a shaking incubator at 37°C.

Questions

Q14. Suppose you have a stock concentration of ampicillin at 100mg/ml and you want to make 5ml of L-broth having 50μg/ml. What volume of stock ampicillin should you add?

Q15. If ampicillin was prepared as a 500X stock solution, how many μl would you have to add to 25ml to make it a final 1x concentration?