

For next time

Questions 1 and 2 are theoretical but they should help prepare you to interpret the results you will collect next time.

1. You have purchased some supercompetent bacteria that are provided at a transformation efficiency of 10^9 colony forming units/ug of DNA. You transform the cells with 1 ng of plasmid DNA and plate 1/1000th of the cells. How many colonies do you expect? Next you transform another aliquot of cells, also at 10^9 colony forming units/ug of DNA, with 2 μ l of plasmid DNA. You spread 1/100th of the cells and find 50 colonies growing on the plate after 24 hours at 37°C. What is the concentration of plasmid?

2. To illustrate your understanding and the importance of the controls you performed today, please write a one-sentence interpretation for each of the following transformation outcomes. There may be more than one valid interpretation for some of the data (only one answer for each is required for the assignment).

	# colonies/plate			
	Outcome 1	Outcome 2	Outcome 3	Outcome 4
No DNA	>1000	0	0	0
50 ng plasmid DNA	>1000	0	>1000	>1000
bkb+ligase	>1000	0	100	0
bkb+insert+ligase	>1000	0	100	500

3. Next time you will prepare DNA from four transformants and begin to characterize the plasmids in these bacteria. Using the plasmid map you drew after Module 1 Day 1, plan at least two restriction digests that will confirm the presence of the PCR insert. It will help to read the introduction for Module 1 Day 5 before you complete this part of the assignment. Be sure to predict the size of the fragments you expect when the plasmid does and doesn't have the PCR insert. Also include reaction conditions such as buffer and temperature. Use the NEB website for details on various enzymes and reaction conditions (http://www.neb.com/nebecomm/tech_reference/default.asp?).

Diagnostic digest 1	plasmid with insert	plasmid no insert
Enzyme(s) used		
Buffer used		
Temperature		
Predicted fragments		
Diagnostic digest 2		

Enzyme(s) used		
Buffer used		
Temperature		
Predicted fragments		