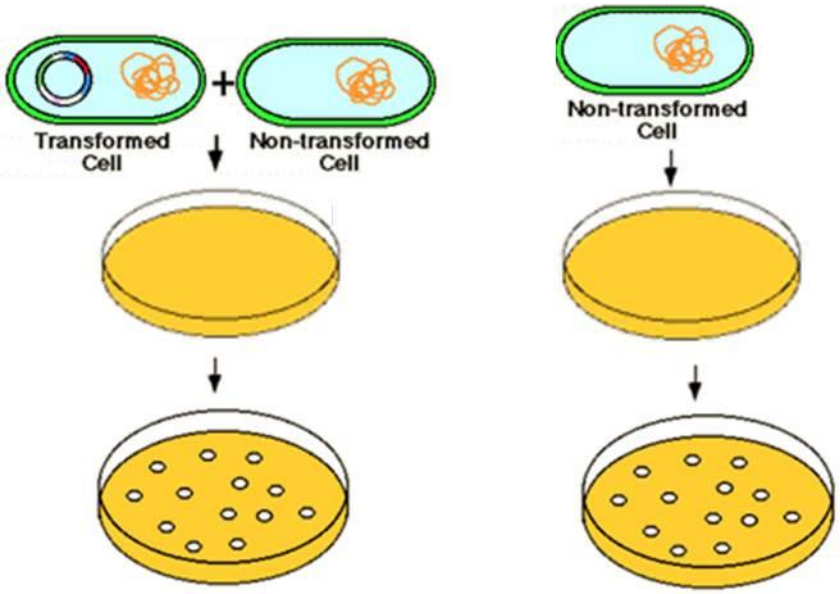
Chemistry 4055 (Spring 2013)
Biochemistry I- Introduction to the Chemistry of the Animal Cell
Chapter 9 HW Assignment

1. What is the difference between DNA sticky ends and blunt ends produced by restriction enzymes?

2. Say you have been diligently working on inserting a DNA fragment into a plasmid with a gene for ampicillin resistance and after transforming your cells you are ready to determine which cells contain the DNA fragment. You check the refrigerator to look for your agar plates containing ampicillin but notice you do not have any more left. You decide to see if Professor Tinoco has any and find that he has a stack of them but he made them when he was a grad student…many years ago! You decide "what the heck" and go ahead and use them. On one plate you load your cells that you transformed and on another plate you load cells that you did not transform. The next morning you find that both plates have cells on them as shown below. What does this data tell you?



What would have been the correct way to do this experiment? How would you determine whether a particular cell colony has your desired DNA fragment?

3. The following DNA duplex sequence is a modified form of a gene that you are interested in

```
5′-GATATCAGGAGGTATGXXXTAAGATATC-3′
3′-CTATAGTCCTCCATACxxxATTCTATAG-5′
```

where XXX is the `5′-3′` sequence of your gene and `xxx` is the complementary `3′-5′` strand.

a. What would be the mRNA sequence transcribed from the intact DNA duplex present in a bacterial expression vector?

b. If you obtained your gene from a commercially available plasmid, what forward and reverse primers should you use to obtain the modified gene presented above by PCR?

4. A gene is inserted into the pCMV6-AC-His expression vector (see below) using the NOT I restriction enzyme. What is the most efficient way to purify the protein that is expressed? What would happen if your insert contained a stop codon?