Transformation of competent cells with plasmid DNA:

1. When needed, remove a tube of competent cells from the -70 ºC freezer. Thaw the cells by holding the tube in the palm of your hand. Just as the cells thaw, transfer the tube to an ice bath. Store the cells on ice for 10 minutes.

2. Using a chilled, sterile pipette tip, transfer the competent cells chilled, sterile poly-propylene tubes. Store the cells on ice.

3. Add the DNA to the competent cells, and swirl the tubes gently several times to mix their contents. Store the tubes on ice for 30 minutes.

For optimal results, the volume of the DNA solution should not exceed 5% of the volume of the competent cells. The number of transformants increases in proportion to the amount of DNA added until the system becomes saturated. Although there is some variation from one batch of competent cells to the next, 50-µl aliquots of competent cells are usually saturated by approximately 1 ng of supercoiled plasmid DNA. Although more DNA can be added without affecting the total yield of transformants, the efficiency of the system (measured in transformants per microgram of DNA) is reduced when excess DNA is used. This becomes important when the transforming DNA is in short supply (e.g., cDNA synthesized from mRNA extracted from relatively inaccessible sources). To obtain the maximum number of transformed colonies, divide the available DNA among several aliquots of competent cells so that the system never becomes saturated.

Be sure to include the following controls in your experiment:

- Competent bacteria that receive a known amount of the standard preparation of supercoiled plasmid DNA
- Competent bacteria that receive no plasmid DNA at all

4. Transfer the tubes to a rack placed in a circulating water bath that has been preheated to 42 ºC. Leave the tubes in the rack for exactly 90 seconds. Do not shake the tubes.

5. Rapidly transfer the tubes to an ice bath. Allow the cells to chill for 1-2 minutes.

6. Add 800 µl of TSB medium (should not contain any antibiotic!) to each tube. Warm the cultures to 37 ºC in a water bath, and then transfer the tubes to a shaking incubator set at 37 ºC. Incubate the cultures for 45 minutes to allow the bacteria to recover and to express the antibiotic resistance marker encoded by the plasmid. To maximize the efficiency of transformation, the cells should be gently agitated (225 cycles/minute or less) during the recovery period.

7. Transfer the appropriate volume (up to 200 µl per 90-mm plate) of transformed competent cells onto McConkey agar plates with ampicillin (50 µg/ml). Add additional broth if small volumes of culture (<10 µl) are transferred. Using a sterile bent glass rod, gently spread the transformed cells over the surface of the agar plate. If more than 200 µl of transformed competent cells are to be plated on a single 90-mm plate, the cells should be concentrated by centrifugation (4000 rpm for 10 minutes at room temperature in a Sorvall SS34 rotor or its equivalent) and gently resuspended in an appropriate volume of TSB. The entire transformation mixture may be spread on a single plate. However, if resistance to ampicillin is selected, only a portion of the culture (empirically determined) should be spread on a single plate. The number of ampicillin-resistant colonies does not increase in linear proportion to the number of cells applied to the plate, perhaps because of growth-inhibiting substances released from the cells killed by the antibiotic.

8. Leave the plates at room temperature until the liquid has been absorbed.

9. Invert the plates and incubate at 37 ºC. Colonies should appear in 12-16 hours. When selecting for resistance to ampicillin, transformed cells should be plated at low density ($<10^4$ colonies per 90-mm plate) and the plates should not be incubated for more than 20 hours at 37 ºC. ß-Lactamase, secreted into the medium from ampicillin-resistant transformants, rapidly inactivates the antibiotic in regions surrounding the colonies. Thus, plating cells at high density or incubating them for long periods results in the appearance of ampicillin-sensitive satellite colonies. This problem is ameliorated, but not completely eliminated, by using carbenicillin rather than ampicillin in selective media and increasing the concentration of antibiotic from 50 µg/ml to 100 µg/ml.