Determination of Transformation Efficiency of Competent Cells

1. Prepare two dilutions of supercoiled DNA at 1 ng/ul and 5 ng/ul.
2. Place 1 ul of each DNA dilutions in eppendorff tubes on ice.
3. Add 50 ul of competent cells (thawed on ice) to each tube.
4. After 30 min, incubate the tubes at 42 C for 30 seconds.
5. Remove tubes from water bath and add 1 ml of LB to each tube.
6. Incubate the tubes at 37 C for 1 hour.
7. After 1 hour, prepare serial dilutions of cells as follow:

   For 1 ng DNA used,

   - 10-1 dilution -- add 20 ul of cells to 180 ul LB
   - 10-2 dilution -- add 20 ul of 10-1 dilution to 180 ul LB
   - 10-3 dilution -- add 20 ul of 10-2 dilution to 180 ul LB

   For 5 ng DNA used,

   - 10-1 dilution -- add 20 ul of cells to 180 ul LB
   - 10-2 dilution -- add 20 ul of 10-1 dilution to 180 ul LB
   - 10-3 dilution -- add 20 ul of 10-2 dilution to 180 ul LB
   - 10-4 dilution -- add 20 ul of 10-3 dilution to 180 ul LB
8. Plate 100 ul of each dilution on LB + Amp plates.
9. After overnight incubation at 37 C, count the number of colonies on each plate.

Number of colonies expected

Transformation Efficiency	**1ng DNA**			**5ng DNA**			
	10-1	**10-2**	**10-3**	**10-1**	**10-2**	**10-3**	**10-4**
109/ug	10000	1000	100	50000	5000	500	50
108/ug	1000	100	10	5000	500	50	5
107/ug	100	10	1	500	50	5	0.5
106/ug	10	1	0	50	5	0.5	0

Note: The number of colonies at each dilution and at each DNA dose should be consistent to each other. That is, if at 1 ng DNA dose, 10-2 dilution plate gives 10 colonies. Then 10-3 dilution at 5 ng DNA dose should give 5 colonies.