

Name _____ Lab Section (day) _____

1. (3 points) Define bacterial transformation.

Bacterial transformation is the process by which bacterial cells take up naked DNA molecules.

2. (6 points) Name two methods of bacterial transformation.

1) Chemical transformation.

2) Electroporation.

3. (3 points) What component of the transformation buffer TSS used last week increases cell permeability?

Dimethyl sulfoxide (DMSO).

4. (4 points) Why do we recover the transformed bacteria in LB medium for one hour before plating on LB+Ampicillin+Tetracycline?

To give the bacteria time to express the antibiotic resistance genes encoded in the newly acquired plasmid DNA.

5. (3 points) Why do we include Ampicillin in the plates?

Ampicillin selects for the pTriplex vector we transformed.

6. (3 points) Two weeks ago we transformed our cDNA clones into the bacterial strain HT115 rather than the strain used previously DH5 α . What characteristic of HT115 is necessary for our upcoming experiments?

HT115 carries an inducible T7 RNA polymerase gene. It also lacks RNase III activity that could degrade double stranded RNAs.

7. (3 points) Why do we add IPTG to the nematode growth plates used for RNAi experiments?

IPTG binds to lac repressor, leading to expression from the lac promoter. In HT115 this induces plus strand synthesis from the pTriplex lac promoter and leads to expression of T7 RNA polymerase necessary for minus strand synthesis in pTriplex. Together these RNAs form a double-stranded molecule that can induce RNAi when ingested by *C. elegans*.