PROCEDURES FOR TRANSFORMATION EXPERIMENTS

Preparation of Media:

H.I. grows well in a Levinthal medium. The medium used in this laboratory has been modified slightly from the original description. It is prepared the following way:

(1) 37 gms. brain heart infusion media in 1000 liters of distilled H_2O brought to a boil.

(2) 100 ml. definbrinated sheeps blood added a little at a time to prevent excess frothing.

(3) Allow mixture to settle one or two minutes, then filter through a fluted filter (Whatman # 12).

(4) Refilter ~~just~~ first 50 ml. of the filtrate.

(5) ~~Filter sterilely with Seitz filters.~~ Autoclave 120°C 20-25' (or longer)

(6) This constitutes Levinthal stock and is used 1:1 with sterile Eugonbroth as Levinthal broth (B.B.L. Eugonbroth 30 gms./1. of H_2O).

(7) For plates add 2.5% agar to Eugonbroth, sterilze in autoclave 20 mins. at 120°C. and upon cooling to 45-50°C. mix 1:1 with Levinthal stock (45°C) for Levinthal agar (1.25%).

(8) Levinthal stock is usually kept in 200 ml. quantities and mixed with 200 ml. 2.5% Eugonbroth for platings. Levinthal stock is kept in 50 ml. quantities and mixed with 50 ml. of Eugonbroth for use as a liquid growth medium.

(9) Two (2) & 1ml DPN (sterile) is added to all media before use

Dilutions:

(1) Dilutions are always made with Eugonbroth or some diluent containing protein.

(2) 2×10^2 dilutions are usually made with 9.9 ml. of broth and 10^1 dilutions with 9 ml. of broth.

Procedures for Transformation Experiments (Cont.)

Platings:

(1) 20 ml. of media per plate is normally used for pour plates.

(2) If an agar layer technique is employed, two 10 ml. layers are used.

Handling of T.P.:

(1) Always dilute transformation preparations (T.P.) in saline-citrate buffer (.15 M NaCl + .014 M Na citrate).

(2) Dilute preparations of T.P. should be stored frozen.

Cells:

Transformations depend to a large extent upon the phsyiological state of the organism at the time at which DNA is added to the cells. Cells to be transformed are shaken gently for aeration--not vigorously since the cells are broken up by this process-aat 35-37°C. to give a concentration of approximately ~~3~~ 6x10⁸ organisms/ml. (Coleman reading of 34 at 650 mμ ~~with side-arm adapter~~).

TRANSFORMATION EXPERIMENTS

One may define transformation as the transfer of genetic characteristics to a host cell by means of DNA prepared from a donor population. Transformations have been described for ~~two~~ organisms and perhaps one or two more questionable cases. Hemophilus influenza (H.I.) will be used to demonstrate transformation in the experiments to be discussed below.

DEMONSTRATION OF TRANSFORMATION AND TITRATION CURVE OF ~~THE~~ A TRANSFORMING ~~PRINCIPLE~~ FACTOR.

One of the easiest methods of demonstrating transforming activity utilizes the characteristic of streptomycin resistance. Rough H.I. type d, or simply HI. Rd, or Rd is suceptible to ~~10~~ 10^3 gamma/ml. or less of streptomycin while the mutant "strep resistent" is capable of growth in media containing greater than 2000 gamma/ml. of streptomycin. DNA prepared from cells resistant to streptomycin transform susceptible cells to streptomycin resistence with a frequency 6 or more orders of magnitude greater than the spontaneous mutation frequency.

In this experiment also the frequency of transformation will be related to the concentration of DNA added, i.e., serial dilutions of transforming principle (T.P.) will be added to a constant number of cells and the frequency of transformation determined.

Experiment XXII

(1) To 7 test tubes (size 22 x 175 mm) add .8 ml. of Levinthal broth. Number the tubes 1-7.

(2) To tube #1 add .1 ml. of saline buffer. To tube #2 add .1 ml. of 10^2 dilution of T.P., to #3 add .1 ml. of 10^3 dilution of T.P., etc. To tube #7 add .1 ml. of 10^2 dilution of T.P. and add .05 ml. of a DNAse preparation calculated to give 1 gamma/ml.

(3) After 1 minute add to each tube .1 ml. of H.I. cells ~~grown from an inoculum of 1 x 10^8 cells/ml. to approximately 3.5 x 10^9/ml.~~ (the Instructor will provide cells).

(4) Incubate tubes for 20 minutes at 35-37°C, with gentle shaking.

(5) Plate cells according to the following schedule with 10 ml. of Levinthal agar cooled to 37-40°C:

(Schedule depends on competence of cells)

Tube	Dilution
1	Undiluted
2 & 3	10^2
4	10^1
5	10^0
6	10^0

(6) Incubate plates 2 hrs. at 35-37°C.

(7) Layer plates with 10 ml. of Levinthal agar containing 500 gamma/ml. of streptomycin. Incubate at 35-37°C.

(8) At the next period count the number of colonies per plate.

(9) Plot the number of colonies transformed in each tube as a function of DNA dilution.

(10) What significance can be attributed to the shape of the curve.

If you assume that 10 per cent of the DNA in the T.P. was taken up by the cells and the molecular weight of the DNA and T.P. is 6 x 10^6, then calculate the ratio of T.P. molecules to DNA molecules.

ISOLATION OF T.P. BY THE LYSOZYME TECHNIQUE AND CALCULATION OF T.P. PER DONOR CELL.

Experiment XXIII

This experiment illustrates the fact that the T.P. introduced into the cell is transmitted by cell growth as genetic material, i.e., it is not only responsible for the streptomycin resistence, but it also replicates.

(1) Isolate a colony of H.I. transformed to streptomycin resistant ~~[illegible]~~ 3 ml. of Levinthal broth and grow culture to a turbid suspension. This operation will be performed by the instructor in cases of conflict of time since it will be necessary to have the cells available at the start of the period.

(2) Dilute cells 1/3 with Levinthal broth, 1.2 ml. cells + 2.4 ml. broth. Make a viable cell count at a dilution of 2×10^7.

(3) To 3 ml. of suspension add .1 ml. of lysozyme (final concentration to be 100 gamma/ml) and shake at 37°C for 10 minutes. } lysozyme not needed.

(4) Add .2 ml. of 1 N NaOH. This should produce a pH of about 10.3.

(5) When the cells lyse as judged by a clearing of the suspension--usually 1 minute is sufficient--neutralize the NaOH by adding .2 ml. of 1 N HCl. This material is a crude preparation containing T.P. activity for streptomycin resistence and will be used to transform Rd cells.

5a) Heat 65° for 5' →

(6) Dilute crude T.P. 10^3 with citrate-saline.

(7) To 3 tubes add 1.8 ml. Levinthal broth + .1 ml. of crude T.P. (10^3 dil.). Number these tubes 1-3. To Tube #1 add .05 ml. DNAse (1 gamma).
To tube #2 add .05 ml. RNAse (10 gamma).
To tube #3 add .05 ml. saline buffer.

(8) To each tube add .1 ml. of Rd cells, ~~grown to a concentration of $\simeq 3.5 \times 10^9$ (turbidity = .20).~~

(9) Incubate cells at 35-37° for 20 minutes and plate at 10^0 dilution $\approx$ 10 ml. of Levinthal agar.

(10) As a control plate .1 ml. of T.P. (10^3 dil.).

(11) Incubate plates 2 hrs. at 35-37°C. and layer plates with 10 ml. of Levinthal agar with 500 gamma/ml. of streptomycin.

(12) Incubate at 35-37°C. and count at the next period.

Assuming no variation in transformation from Experiment XXII calculate the amount of DNA-T.P. present in the lysozymed culture from the curve obtained in Experiment XXII, given the value of 200 gamma/ml. of DNA for the T.P. (undiluted) used in the previous experiment.

The amount of T.P. taken up by the cells may be obtained by centrifuging the cells and then titering the supernatant for loss of T.P.. Assuming a value of 10% uptake of T.P. by the cells in this experiment, calculate the number of units of T.P. contrifuged per lysozymed cell.

Experiment XXIV

DNA prepared from various sources will interfere with transforming DNA if present in sufficiently high concentration. In this experiment DNA extracted from host and donor cells will be used to test for direct interference. The T.P. used will be T.P. obtained from streptomycin resistant cells, interfering DNA will have come from Rd streptomycin sensitive cells.

(1) To 4 tubes add .7 ml. Levinthal broth + .1 ml. of T.P. diluted 10^2.
Number tubes 1-4. To tube #1 add .1 ml. saline buffer.
To tube #2 add .1 ml. DNA 10^1 dilution.
To tube #3 add .1 ml. DNA 10^2 dilution.
To tube #4 add .1 ml. DNA 10^3 dilution.
To an empty tube (#5) add .8 ml. Levinthal broth + .1 ml. of DNA 10^1 dilution. To another tube (#6) add .7 ml. Levinthal broth + .1 ml. of DNA 10^2 dilution.

(2) To each tube add .1 ml. Rd ~~(turbidity = .20)~~.

(3) Shake for 20 minutes at 35-37°C. However, at 2 min. add .1 ml. of 10^2 dilution of T.P. to tube #6.

(4) Plate cells according to the following schedule:

#1 - 10^2	#4 - 10^2
#2 - 10^1	#5 - 10^0
#3 - 10^2	#6 - 10^1

(5) Incubate plates at 35-37°C. for 2 hours and layer with streptomycin agar containing 500 gamma/ml of streptomycin. Incubate.

From the previous experiment you calculated that T.P. preparations from Sd cells consist of 1 T.P. molecule for every 350 molecules of DNA of 6×10^6 molecular weight. Assuming this to be true, can you arrive at a figure from the above experiment for the number of DNA molecules that interfere with T.P. uptake by cells?