

Attempted Synthesis of Transforming Principle

9/2/52

| | 1 | 2 | 3 | 4 |
|--|-----------------|-----|-----|-----------------|
| TPC ¹⁴ $0.5 \mu\text{M}$ $1.2 \times 10^6 \text{ cpm}$ | .02 → | | | |
| DGTP- $0.59 \mu\text{M}$ /ml | .02 → | | | |
| DCTP- $0.50 \mu\text{M}$ /ml | .02 → | | | |
| DATP- $0.50 \mu\text{M}$ /ml | .02 → | | | |
| H/I Oligo, 9.2 | .02 → | | | |
| MgCl ₂ -0.1M | .01 → | | | |
| H.I. DNA- $0.5 \mu\text{g}/\text{ml}$ | .02 | - | .02 | 0.02 |
| H.I. DNA - " " 3 PWB | 0.02 | .02 | | .02 |
| AS-2 P. Exp. 9/22 | .02 | .02 | - | - |
| water | .15 | .15 | .17 | .17 |

90' 37° V_f = .30 →

Dilute 0.1/1.0 in extract saline for transformation assay.
To remainder add 0.2 ml 1% PCA and 0.4 ml carrier. Assay for incorporation in usual way.

Big: 2/2, 13/6 mean = 10
SA: 1075, 1146

| | | | | |
|-----------------|------|------|-----|-----|
| Cp min: | 2561 | 2097 | 177 | 119 |
| Cp corr: | 1270 | 1038 | 65 | |
| corr for plate: | 1205 | 973 | | |

In tube #1 $0.96 \mu\text{M}$ TTP incorp $\times 4 = 3.83 \times \frac{3}{2} = 5.7 \mu\text{M}$ of all four bases
incorp per 0.3 ml $\times 1.5$ self absorption correction = 8.7 μM . incorporates total.

H.I. DNA = 12 U/ml at 260 = $1 \mu\text{g}/\text{ml}$; in 0.02 ml there are 20 μg N
Then there is a net increase of 9/10 or 40-50%.

See over for T.P. assay.

T.P.: 9/14

cells: 9/14 overnight, freeze. (cells for T.P. assay grown to c.r. = .42)

Dilution of T.P. (0.1ml in assay)
constant T.P. dil $10^2/30$, then $0.1/1.0$

Dilution of Cells for Plating (1.0ml plates)

Tube#

| | | |
|---|-----------------|--------------|
| 1 | 10^2 | $\cdot 1/10$ |
| 2 | 5×10^2 | $\cdot 2/10$ |
| 3 | 10^3 | $\cdot 1/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 5×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

header T.P. dil $10^2/30$ then $0.1/1.0$

| | | |
|---|-----------------|--------------|
| 4 | 10^1 | $\cdot 1/10$ |
| 5 | 5×10^1 | $\cdot 2/10$ |
| 6 | 10^2 | $\cdot 1/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 2×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

Preinc 1 dil $1/1.0$

| | | |
|---|-----------------|--------------|
| 7 | 10^2 | $\cdot 1/10$ |
| 8 | 5×10^2 | $\cdot 2/10$ |
| 9 | 10^3 | $\cdot 1/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 5×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

Preinc 2 dil $1/1.0$

| | | |
|----|-----------------|--------------|
| 10 | 2×10^1 | $\cdot 1/10$ |
| 11 | 10^2 | $\cdot 1/10$ |
| 12 | 5×10^2 | $\cdot 2/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 2×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

Preinc 3 dil $1/1.0$

| | | |
|----|-----------------|--------------|
| 13 | 10^2 | $\cdot 1/10$ |
| 14 | 5×10^2 | $\cdot 2/10$ |
| 15 | 10^3 | $\cdot 1/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 5×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

Preinc 4 dil $1/1.0$

| | | |
|----|-----------------|--------------|
| 16 | 10^1 | $\cdot 1/10$ |
| 17 | 5×10^1 | $\cdot 2/10$ |
| 18 | 10^2 | $\cdot 1/10$ |

| | |
|-----------------|--------------|
| 10^2 | $\cdot 1/10$ |
| 2×10^1 | $\cdot 2/10$ |
| 10^1 | $\cdot 1/5$ |

19. no T.P.

10^0 use home.

assay as before

9/24/82

Results

| Tube # | cont cells | transformed/ml | μDNA added | cells transformed / μDNA |
|--------------|------------|-------------------|------------|--------------------------|
| control T.P. | | | .00035 | |
| 1 | 171 | 1.7×10^5 | .0035 | 4.9×10^7 |
| 2 | 61 | 3.1×10^4 | .00070 | 4.3×10^7 |
| 3 | 182 | 1.8×10^4 | .00035 | 5.1×10^7 |
| | | | | } 4.8×10^7 |
| Healy, T. P. | | | | |
| 4 | 105 | 1.1×10^5 | .035 | 3.1×10^6 |
| 5 | 140 | 2.8×10^4 | .070 | 4.0×10^6 |
| 6 | 98 | 9.8×10^3 | .0035 | 2.8×10^6 |
| | | | | } 3.3×10^6 |
| prairie 1 | | | | |
| 7 | 0 | | .0035 | |
| 8 | 0 | | .00070 | |
| 9 | 0 | | .00035 | |
| prairie 2 | | | | |
| 10 | 0 | | .017 | |
| 11 | 0 | | .0035 | |
| 12 | 1 | | .0007 | |
| prairie 3 | | | | |
| 13 | 124 | 1.2×10^5 | .0035 | 3.4×10^7 |
| 14 | 63 | 3.2×10^4 | .00070 | 4.6×10^7 |
| 15 | 83 | 1.8×10^4 | .00035 | 5.1×10^7 |
| | | | | } 4.4×10^7 |
| prairie 4 | | | | |
| 16 | 136 | 1.4×10^5 | .035 | 4.0×10^6 |
| 17 | 159 | 3.2×10^4 | .0070 | 4.6×10^6 |
| 18 | 176 | 1.8×10^4 | .0035 | 5.2×10^6 |
| | | | | } 5.4×10^6 |
| no T.P. | | | | |
| 19 | 0 | 0 | 0 | 0 |

Attempted Synthesis of H.I. Transforming Principle

9/24/52

Incubation

| | 1 | 2 | 3 | 4 |
|---|-------|-----|-----|-----|
| TTP (1.2 x 10 ⁸ cpm) } 14 .54 μm/ml | .02 | - | .02 | .02 |
| DATP - 0.5 μm/ml | .02 | - | .02 | - |
| DCTP - 0.5 μm/ml | .02 | - | .02 | .02 |
| DGTP - 0.5 μm/ml | .02 | - | .02 | .02 |
| Glycine M/1, 9.2 | .02 → | | | |
| HgCl ₂ - 0.1M | .01 → | | | |
| H.I. DNA - 0.5 μg/ml | .02 → | | | |
| AS-2 (1 μg. 9/22) | .02 | .02 | - | .02 |
| water | .15 | .23 | .17 | .17 |

V.F. = .30

90' 37°

0.1 ml removed from each for transformation assay. (Remains 0.2 ml 7% PCL + 0.05 ml carrier added. assay for incorporation as usual.)

Big: 1 1/2, 2 1/2 mean = 10 cpm
 St: 1071, 1041

| | | | | |
|----------|------|----|-----|-----|
| cpm min | 2974 | 12 | 141 | 228 |
| cpm corr | 1477 | | | |
| - Blank | 147 | 0 | 0 | 44 |

1.1 μm x 1/2 x 4 x 1.5 = 10.0 μm total incorp or 50% incorp. in DNA.
 see one for Transformation essay.

cells overnight 9/19 frozen.

T.P. 9/14.

Dilution of T.P. @ 1ml used

Dilution of cells (incl plates)

| <u>Tube#</u> | <u>Preinc 1 dil %/vol</u> | | |
|---|----------------------------------|--|--------------------------------|
| 1 | 10^0 use 0.1ml | | 10^1 $\frac{1}{10}$ |
| 2 | 10^1 $\frac{1}{2}$ | | 10^1 " |
| 3 | 10^2 $\frac{1}{10}$ | | 10^1 " |
| <u>preinc 2 dil $\frac{1}{10}$</u> | | | |
| 4 | 10^2 $\frac{1}{10}$ | | 10^2 $\frac{1}{10}$ |
| 5 | 5×10^2 $\frac{1}{10}$ ↓ | | 5×10^1 $\frac{1}{10}$ |
| 6 | 10^3 $\frac{1}{10}$ ↓ | | 10^1 $\frac{1}{10}$ |
| <u>preinc 3 dil $\frac{1}{10}$</u> | | | |
| 7 | 10^2 $\frac{1}{10}$ | | 10^2 $\frac{1}{10}$ |
| 8 | 5×10^2 $\frac{1}{10}$ ↓ | | 5×10^1 $\frac{1}{10}$ |
| 9 | 10^3 $\frac{1}{10}$ ↓ | | 10^1 $\frac{1}{10}$ |
| <u>preinc 4 dil $\frac{1}{10}$</u> | | | |
| 10 | 10^2 $\frac{1}{10}$ | | 10^2 $\frac{1}{10}$ |
| 11 | 5×10^2 $\frac{1}{10}$ ↓ | | 5×10^1 $\frac{1}{10}$ |
| 12 | 10^3 $\frac{1}{10}$ ↓ | | 10^1 $\frac{1}{10}$ |
| 13 | no T.P. | | 10^0 use 1ml. |

Results

| <u>Tube#</u> | <u>count</u> | <u>cells transformed/ml</u> | <u>xDNA added</u> | <u>cells transformed/xDNA</u> |
|--------------|--------------|-----------------------------|-------------------|-------------------------------|
| 1 | 10 | 1×10^3 | .35 | 2.9×10^3 |
| 2 | 1 | - | - | - |
| 3 | 1 | - | - | - |
| 4 | 0 | - | - | - |
| 5 | 0 | - | - | - |
| 6 | 0 | - | - | - |
| 7 | 403 | 4.0×10^5 | .0025 | 1.6×10^8 |
| 8 | 171 | 8.6×10^4 | .00070 | 1.2×10^8 |
| 9 | 388 | 3.9×10^4 | .00035 | 1.1×10^8 |
| 10 | 0 | | | |
| 11 | 0 | | | |
| 12 | 0 | | | |
| 13 | 0 | | | |

} 1.1×10^8

Attempted synthesis of transforming principle

9/25/66

Preincubation

| | 1 | 2 | 3 | 4 |
|---------------------|-------|------|------|------|
| * DNase 18/ml | .01 | - | .01 | - |
| * " 0.18/ml | - | .01 | - | .01 |
| * Anti-DNase 3/1 | .02 | .02 | - | - |
| Normal Rabbit Serum | - | - | .02 | .02 |
| water | .175 | .175 | .175 | .175 |
| NaCl - 1.0M | .04 → | - | - | - |

30' 37°

then add:

| | | | | |
|------------------------|-------|---|---|---|
| H.I. DNA 5000/ml | .02 → | - | - | - |
| 0.1M MgCl ₂ | .01 → | - | - | - |
| Glycerol 4/1, 9.2 | .02 → | - | - | - |

V.F. = .295

80' 37°

then dilute 0.1/1.0 in citrate-saline for Transformation assay.

| | 5 | 6 |
|--|-------|-----|
| TTP (C ¹⁴) 0.52 μm/ml (1.76 x 10 ⁶ cpm) | .02 → | - |
| DATP - 0.5 μm/ml | .02 → | - |
| DCTP - 0.5 μm/ml | .02 → | - |
| DGTP - 0.5 μm/ml | .02 → | - |
| Glycerol 4/1, 9.2 | .02 → | - |
| H.I. DNA 0.1 μm/ml | .02 | .02 |
| Spiraea Sup 8/28 | - | .20 |
| water | .17 | - |
| V.F. | .30 | .33 |

90' 37°

then dil 0.1/1.0 in saline, assay remains for incorporation.

Blg: 20, 27, mean = 12.5

| | | |
|----------|----|------|
| cp 7 min | 92 | 1038 |
| cp cont | 34 | 505 |

St: 1073

* Rabbit Serum - 3rd bleeding after 2 injections of DNase.

* Freshly diluted in 0.1/1.0 Tris 7.5

cells: - 9/15 frozen
 T.P. - 9/14 - 2500x/ml.

Dilution for T.P. assay

Dilution of T.P. (0.1ml used)

Dilution of Cells (use 1ml)

| Tube# | preinc 1 | | |
|-------|-----------------|----------------|--------------------------------|
| 1 | 10^0 | | |
| 2 | 10^2 | $\frac{1}{10}$ | $10^1 - \frac{1}{5}$ |
| | | | " " |
| | <u>preinc 2</u> | | |
| 3 | 10^0 | | |
| 4 | 10^2 | $\frac{1}{10}$ | $10^1 - \frac{1}{5}$ |
| | | | " " |
| | <u>preinc 3</u> | | |
| 5 | 10^0 | | |
| 6 | 10^2 | $\frac{1}{10}$ | $10^1 - \frac{1}{5}$ |
| | | | " " |
| | <u>preinc 4</u> | | |
| 7 | 10^0 | | |
| 8 | 10^2 | $\frac{1}{10}$ | $10^1 - \frac{1}{5}$ |
| | | | " " |
| | <u>preinc 5</u> | | |
| 9 | 10^2 | $\frac{1}{10}$ | |
| 10 | 5×10^2 | $\frac{2}{10}$ | 10^2 $\frac{1}{10}$ |
| 11 | 10^3 | $\frac{1}{10}$ | 5×10^1 $\frac{1}{10}$ |
| | | | 10^1 $\frac{1}{5}$ |
| | <u>preinc 6</u> | | |
| 12 | 10^2 | $\frac{1}{10}$ | |
| 13 | 5×10^2 | $\frac{2}{10}$ | 10^2 $\frac{1}{10}$ |
| 14 | 10^3 | $\frac{1}{10}$ | 5×10^1 $\frac{1}{10}$ |
| 15 | no T.P. | | 10^1 $\frac{1}{5}$ |
| | | | 10^0 |

Results

| Tube# | count | cell transformed/ml | sDNA added | cell transformed/sDNA |
|----------|----------|---------------------|------------|-----------------------|
| preinc 1 | too many | 2 | .35 | |
| | 197 | 2.0×10^4 | .0035 | 5.7×10^6 |
| preinc 2 | too many | — | .35 | — |
| | 334 | 3.3×10^4 | .0035 | 9.4×10^6 |
| preinc 3 | 3 | 3×10^2 (?) | .35 | $\sim 10^3$ |
| | 0 | | .0035 | |
| preinc 4 | 3 | 3×10^2 (?) | .35 | $\sim 10^3$ |
| | 5 | | .0035 | |
| preinc 5 | 159 | 1.6×10^5 | .0035 | 4.6×10^7 |
| | 67 | 3.4×10^4 | .00070 | 4.8×10^7 |
| | 264 | 2.6×10^4 | .00035 | 7.4×10^7 |
| preinc 6 | 116 | 1.2×10^5 | .0035 | 3.4×10^7 |
| | 58 | 2.9×10^4 | .00070 | 4.1×10^7 |
| | 250 | 2.5×10^4 | .0035 | 7.1×10^7 |
| | 0 | 0 | 0 | |

$\left. \begin{matrix} 4.6 \times 10^7 \\ 4.8 \times 10^7 \\ 7.4 \times 10^7 \end{matrix} \right\} 5.6 \times 10^7$
 $\left. \begin{matrix} 3.4 \times 10^7 \\ 4.1 \times 10^7 \\ 7.1 \times 10^7 \end{matrix} \right\} 4.9 \times 10^7$