Resume of DNA synthesis in various passages
expts O-132 and N111, M14172

| Infecting virus | HIRT DNA: Total cpm in I | II | I+II | I/II | pfu/ml. | total cpm / pfu/ml $\times 10^{-3}$ | Total cpm / pfu/ml % homology (16) | Days to full CPE |
|---|---|---|---|---|---|---|---|---|
| X 8 | $2.2\times10^6$ | $1.6\times10^6$ | $3.8\times10^6$ | 1.4 | | | | |
| O-132 | | | | | | | | |
| 777 CV/1 B | $2.8\times10^6$ | $2.5\times10^6$ | $5.3\times10^6$ | 1.1 | $1\times10^9$ | 5.3 | 0.3 | 4 |
| 777 CV/1 G | $5.6\times10^5$ | $4.6\times10^5$ | $1.0\times10^6$ | 1.2 | $1\times10^8$ | 10 | 0.85 | 5 |
| 777 CV/1 H | $7.8\times10^5$ | $1.4\times10^6$ | $2.2\times10^6$ | 0.56 | $3\times10^6$ | 730 | 0.72 | >9 |
| 777 CV/1 I | $6.8\times10^5$ | $5.8\times10^5$ | $1.3\times10^6$ | 1.2 | $2.1\times10^5$ | 6500 | 1.6 | >11 |
| O132, | | | | | | | | |
| 776 CV/8 B | $2.0\times10^6$ | $1.0\times10^6$ | $3.0\times10^6$ | 2.0 | $5\times10^8$ | 6 | 0.8 | 4 |
| 776 CV/8 F | $5.1\times10^5$ | $1.2\times10^6$ | $1.7\times10^6$ | 0.43 | $5\times10^6$ | 340 | 1.4 | >14 |
| 776 CV/8 G | $8.6\times10^4$ | $1.3\times10^5$ | $2.2\times10^5$ | 0.66 | $2\times10^6$ – 110 | | 3.7 | 8 |
| 776 CV/8 H | $0.9\times10^6$ | $2.1\times10^6$ | $3.0\times10^6$ | 0.43 | $4\times10^7$ | 75 | 2.3 | 4 |
| 776 CV/8 J | $7.2\times10^4$ | $1.2\times10^5$ | $1.9\times10^5$ | 0.60 | $0.9\times10^6$ | 210 | 15 | ? n.d. |
| N-111 | | | | | | | | |
| 777 $NP_2$ | $3.3\times10^6$ | $7.2\times10^5$ | $4.0\times10^6$ | 4.6 | | | 3.3 | 4 |
| 777 $NP_4$ | $1.3\times10^6$ | $1.5\times10^6$ | $2.8\times10^6$ | 0.9 | | | 25 | >12 |
| 777 $NP_5$ | $1.2\times10^4$ | $1.0\times10^5$ | $1.1\times10^5$ | 0.12 | | | 40 | n.d. |
| N111 | | | | | | | | |
| 777 CV/B B | $6.8\times10^6$ | $1.4\times10^6$ | $8.2\times10^6$ | 4.9 | $0.9\times10^9$ | | 0.32 | 3 |
| 777 CV/B E | $1.7\times10^6$ | $2.3\times10^6$ | $4\times10^6$ | 0.7 | $1.1\times10^9$ | | 75 | 4 |
| 777 CV/B D | | | | | $2\times10^9$ | | 35.3 | |
| 777 CV/B $E_2$ (Lavic) | | | | | $5\times10^8$ – $1\times10^9$ | | 81 | |
| 777 CV/B F | | | | | $9\times10^7$ | | 71 | |
| 777 CV/B G | | | | | $4\times10^6$ | | 78 | |

Prep of BSC DNA. D 71.

See methods book.

7.2.71 Pellet of 12.11.71, BSC Flow 60 plates. Washed 2x c̄ PBS.

Thaw at rm temp. Add 1 ml/5 plates = 12 ml of SDS – Saline (see methods) (S Lauc). ~~Vortex~~, Wash into long t.t. Vortex. ~~##~~ Very viscous, not really homogeneous, see lumps. Add 6 ml (½ SDS-saline) 1 M NaTCA, pH 7.4. Inc. 37° 30 min. Still some light tan lumps. Add 19 ml. Tris-ϕ(OH) Shake on rotary, high speed, 30 m. Spin 15' @ 8,000 rpm Sorvall. Collect upper, aqueous. Difficult to get last bits, got only 10.8 ml. Therefore, decided to wash c̄ ~6 ml (1 ml/centr tube) of 1x SSC, 0.05 M Tris pH 7.4. Quick vortex. ~~Cen~~ Spin as above. Collect total of 20 ml aqueous. Add 1 ml 20x SSC. Add 42 ml cold 95% EtOH along side of 100 ml beaker. Stir with rod winding DNA onto rod. Dip 3-4x in fresh EtOH tube. Press against ~~top~~ wall to drain. Dry 5 min at rm temp. Dissolve in 3 ml 0.01 SSC 1 hr rm temp. Sit overnight. refrig.

8.12.71 Add 1/20 vol 20 SSC (0.15 ml) and 0.2 ml of heated 1 mg/ml RNase. 8:25 am. 37°. to 9 am. Add equal volume (3.35 ml) ϕ-OH - Tris. Vortex 15', "medium" in PYREX Sorvall tube. Centr. as above. Take off sup. Store several hrs. refrig. 32 ml
Add equal vol 99% $CHCl_3$ 1% isoamyl alc.
shake on hand vortex. Spin Clinical Centr.
~10 min #5. (all in 12 ml conical). Remove sup. 2.8 ml. Only tiniest speck of protein at interface.

Place 2.8 ml in 40 ml conical tube. Add 1/20th vol, 0.14 ml 20 x SSC. Add 2 vol as to EtOH, cold as before, wind DNA, rinse in EtOH. Dry in air. Put to dissolve in 1 ml. 0.01 SSC. 30 min. on shaker. Refrig. overnight

9.12.71 Dialyse vs 1 l 0.1 SSC start noon. Cold.

10-12-71. Change to 1 l 0.01 SSC.

11-12-71 Change fluid.

12-12-71. Stop, 11.30 pm. 1.5 ml.

0.02 ml + 1.00 1/100 SSC $A_{260} = 0.210$

$\frac{.210\ (1.02)}{.02} = 10.7\ A_{260}/ml$,

60 plates = 15 OD

$\frac{15}{60}$ 0.25 OD/plate

if 1 OD = 50 µg DNA/ml (Felsenfeld, quoted by Hurt)

then, 10.7 $A_{260}$/ml = ~~530~~ 535 µg/ml = 0.54 mg/ml

£ 0.81 mg yield.

Store frozen

50 µg/filter, ~~0.81~~ $\frac{810\ \mu g}{50}$ = 16 filters

$\frac{810}{40}$ = 20 filters

$\frac{810}{35}$ = 23 filters

$\frac{810}{30}$ = 27 filters

# Growth of H. influenzae strain Rd. D-917,

9.12.71 ~~100~~ 90 mg DPN in ~~10~~ 9 ml $H_2O$, 10 mg/ml NAD.
Boehringer Control # 645441 (663) Free acid.
free acid. Stable cold neutral, for weeks. ($C_{21}H_{27}O_{14}N_7P_2$)
(Kornberg, Methods III p878.)
very labile in alkali.
$\epsilon_{260} = 18 \times 10^6$ cm²/mole Observed.

| | |
|---|---|
| 21 × 12 | 252 |
| 27 × 1 | 27 |
| 14 × 16 | 224 |
| 7 × 14 | 98 |
| 2 × 31 | 62 |
| | 663 = MW. |

Add 5 ml $H_2O$. Then add 1 N NaOH to pH ~ 6.8. Then $H_2O$ to 9 ml.
Took ~ 0.2 ml 1 N NaOH. pH 6.0.
1N = 1 mmole/l. ~ 0.27%.

$\frac{90\text{mg}}{663\text{mg/mmole}} = 0.136$ mmoles. 0.27 mmole.

Sterilize in 2 lots thru Swinney into sterile tubes.
Freeze.

12.12.71 *Brain Difco ~~Beef~~ Heart Infusion: 37 gm dissolved in 1 liter deionized $H_2O$. Dispense 50 ml/each of 6 250 ml flasks.

* BHI-Agar. $\frac{37}{4}$ = 9.25 gm. BHI + $\frac{15.0}{4}$ = 3.75 gm Difco agar, in 250 ml deionized $H_2O$. After autoclave, cool to 55° in water bath. Add, 2.5 ml hemin, 0.05 ml DPN. Pour plates. Cool. Keep overnight 37°. 2.5/250, 0.5/50

* Nutrient Agar, Difco, 5.75 gm + 250 ml $H_2O$. Heat to boil to dissolve. Autoclave. Cool to 55°. Pour plates. Cool. Keep overnight 37°.

* Vials - ~ 0.2 ml glycerol

* Sterilize by autoclave, 15 min.

Equine hemin. 2x recryst. Mann 3054, Batch V3032.
from J Gressel.

9.8 mg into 9.8 ml triethanolamine in 50 ml sterile Erlenmeyer. Much dissolves. ↖ WRONG
15 min 65°. Store at 4°. see below

12.12. Received from Nathans. (sent 6.12.71)
4 vials, stabs, incubated 37°. Growth visible
4 vials, stabs. not incubated. No visible growth.

Inoculate 50 ml BHI - DPN - Hemin c̄ #1, 37° stab
Inoculate 50 ml BHI - DPN - Hemin c̄ #1 25° stab
Shake - 37° - start 11 pm.

Put #2, 25° stab in 37° incubator. 11 pm

13.12. 25° #2 Stab - no signs of growth. 9 am. Remove from incubator.

37° #1, 25° #1, no signs of growth in waterbath. 9 am noon see growth.

Media for these 2 and BHI plates made up 12-12 ~~from~~ wrong since hemin should have been in 4% triethanolamine.

Make 2ml triethanolamine → 50 ml c̄ sterile water. Filter thru millipore. Add 50 mg Hemin (E.K. from Nathans.) 15' at 67°.

20 | 12
4 | 12

From 37° #1
Streak on ~~Blo~~ Choco Agar
Streak on BHI (12.12 plate)
Rest into liquid medium.

13 12.71 Cool incubated media 2:30 pm
Read Abs 650 vs H2O.

| | |
|---|---|
| medium | 0.015 |
| 25° #1 | 0.016 — Discard |
| 37° #1 | 0.174. |

Add Hemin • DPN to 50 ml media aliquots using new hemin solution.

Inoculate 2 flasks (50 ml media) with #1 0.50 and #2 1.00 ml of 37° #1 above. (1 and 2%). – Zero time of media = 0.069 = 4:15 pm.
Inoculate a third ē stab from 37° #2 – #3 stab of Nathan.
Store 37 #1 in cold room
Plates from 37 #1 (37°.)

BHI – Hemin – DPN
Blood Agar
Nutrient Agar.
Choc Agar

Start 4:15 pm.

| | 1 | 2 | 3 |
|---|---|---|---|
| 6:52 | 0.097 | 0.110 | 0.072 |
| 6:52 −2010 | 0.028 | 0.041 | 0.003 |
| 10°2 −2010 | 1.665 | 1.832 | 0.117. 0.045 |
| 11 −2010 | | | 0.156 0.084 |

Stop 1 to 2 at 10°2 pm. cool.
14.12 71, #3 – Dense cells.

Control #4.
Medium minus Hemin minus DPN. $A_{650}$ = 0.035.
Add 1 ml of 37 #1 (note have opened this many times now) at 10:30 pm. Put @ 37° Shake.
$A_{650}$ = 0.085 at 3:30, 14.12. Replace at 37°; $A_{650}$ = 0.085 on 15.12.
O.K. Essentially No Growth.

From #1. 1 ml of #1 / vial containing 0.2 ml. glycerol.
Mark: Vial 1/D 91
~~10~~ 15 vials. mark 1 → 15

From 37#1, original culture - of 12.12 to 12.13.
10 vials. mark 2/D 91. (Direct from Stab)
mark 1–10

Store −20° overnight
Then next day put at −60°.

D-91
P3

For antibiotic test

20-12-

Vial 1/Dai #2.

21-12-71.

Streak, 2 BHI plates
1 chocolate agar. } 37° noon.

Store vial @ -20°.

22-12-71.

Good colonies on choc. agar.
Nothing on BHI.
Take Choc Agar plate to lab-

951 224 86
ד"ר קלינר
ד"ר קליין 4
תזונה

Trials for Best prelabeling conditions

21-12-71

large plates- $d = 8.6$ cm $r = 4.3$, area $= \pi(4.3)^2 = 58\ cm^2$.
small plates $d = 4.7$ cm $r = 2.35$ area $= \pi(2.35)^2 = 17.4\ cm^2$
ratio of area of large/small $= 58/17.4 = 3.33$.

Small plates, seed $2 \times 10^5$, grow to $10^6$ in 6 days.
Usually 4 ml of medium. 8 ml in large plates.
Seed 16 plates ē BSC today.

22-12- Spause cells.

23-12-

day 3 or 4 - ~50% perhaps more
Trials for long term labeling at low level of $^3H$-dT.
4 plates: Replace medium ē MEM + 10% calf serum
$5 \times 10^{-5}$ M in dT (discregard dT in hot).
2 μCi/ml. dT-$^3H$ in 2. 2 cold.
1 mCi/ml, 23.7 Ci/mmole,

D-211. 1 $^3H$
2 $^3H$
3 no $^3H$
4 no $^3H$.

Plans #2 & #4, replace medium ē hot and cold, respechively, after 24 hr. Save medium to count.

For 20 ml medium.
18 ml MEM
1.5 ml calf serum.
0.25 ml. Stock cold dT, 1 mg/ml, $42 \times 10^{-4}$ M. 4.2 μmoles/ml (end of 0.1+ 4 ml MEM)
Add 4 ml. to 3 & 4. Have 12 ml left, need 24 μCi add. 0.025 ml $^3H$ dT.
Count medium A

23.12 Save 0.1 ml of $^3$HdT + 4 ml MEM, frozen stock.

24-12 #2 & #4. Remove medium. Replace ~~c~~ hot (2) & cold (4) fresh medium ~~et~~ made exactly as 23-12, using dilution of $^3$H dT. make 23.12: Med ~~from E~~ (9 ml MEM + 1 ml CS + ~~0.5 ml of~~ 0.13 M cold stock dT. Add 4 ml to #4. ~~To~~ Then add 0.5 ml of diluted $^3$H-dT, add 4 ml to #2. (Medium E).

Plan: ~~at~~ 60 - 70% confluence: 2 plates at 24°. ~~2~~ days. Then label at $5 \times 10^{-5}$ M dT, 10 μCi/ml. 12 hr (use warm medium). Chase 2 days in maintenance medium.

24-12.

Plan at 60 - 70% confluence. Actually 75-80%.

| | |
|---|---|
| 5 | 10 μCi/ml 23.7 Ci/mmole, $5.1 \times 10^{-7}$ M. |
| 6 | – no label, no dT. |
| 7 | 10 μCi/ml. $5.1 \times 10^{-6}$ M dT 2.37 Ci/mmole |
| 8 | ~~$5 \times 10^{-6}$~~ no label, $5.1 \times 10^{-6}$ M dT. |
| 9 | 10 μCi/ml $5.1 \times 10^{-5}$ M dT 0.237 Ci/mmole |
| 10 | no label, $5.1 \times 10^{-5}$ M dT. |

Media for 5-10.

F: 14 ml MEM + 1.5 ml CS + 0.15 ml $^3$HdT (150 μCi) (4 ml plate 5)

G: Same but minus $^3$H dT to #6. (4 ml).

C: 5 ml G + 0.2 ml 1→33 dT (0.025 μmols) ~~#5~~ #7

$^3$H-C: 5 ml F + 0.2 ml 1→33 dT #7

D: 5 ml G + 0.2 ml 1→3.3 dT (0.25 μmols) #9

$^3$HD: 5 ml F + 0.2 ml 1→3.3 dT. 9 to

(dilutions in MEM.

12 12 25-12 Nbrs 1 → 10 all look like close to or at full monolayer.

~~also 26-12-71~~

26 12.71, All good monolayer. #7 may be slightly less dense.

26 12.71 Do chase on all plates #1 → 10.

Stocks are 1 mg/ml dT and dC. want 50 µg/ml dT, 10 µg/ml dC.

10 plates: wash 2x c̄ 4 ml warm chase medium, then add 4 ml for maintenance (2% calf serum).

Make ~~100~~ 150. ml medium

150 ml MEM

3 ml CS.

7.5 ml dT stock cold.

1.5 ml dC stock

28-12-71. (Tues) 10 am. #7 still looks not quite so good, also #5.

Wash each plate 1 x c̄ 4 ml PBS

1 x c̄ 2 ml PBS.

~~Add 2 ml 0.5~~ Prior to wash. From labeled plates: 1, 2, 5, 7, 9, add ~~ch~~ overlying medium (chase medium) to one of plates that was untreated (control on cpm), Remove immediately. Remove all media. Wash.

Add. 2 ml 0.5 M NaOH. as per methods.

Remove 2 0.5 ml aliquots, to each cold, 0.5 ml cold 20% TCA. 9 %.

a: count 0.1 ml by drying onto GF/C for total cpm.

a & b, filter & wash as per methods. ~~Dry 1 hr.~~

Dry a, b, c, 1 hr 80°. Toluene — count.

For all plates, hot, cold, controls, take 0.1 ml to 1 ml for Abs. reading.

Count dry fillers in software

Background = 20, included in blank: for C, total cpm = x 20.
for exp't. other undil. for A, total = cpm x 4

| | | cpm | cpm repeat | cpm – Bl | total cpm/plate | A 260 |
|---|---|---|---|---|---|---|
| C | 1 control | 59 | 51 | | | |
| | 1 | 3181 | 3120 | 3065 | 61,300 | 2.06 |
| | 2 control | 27 | 22 | | | |
| | 2 | 3271 | 3245 | 3233 | 64,660 | 2.01 |
| | 5 control | 120 | 103 | | | |
| | 5 | 221,600 | 220,700 | 221,040 | 9,420,800 | |
| | 7 control | 56 | 64 | | | |
| | 7 | 73,360 | 74,631 | 73,304 | 1,466,080 | |
| | 9 control | 41 | 35 | | | |
| | 9 | 25,320 | 25,080 | 25,280 | 505,600 | |
| a+b | 1 control | 194 | 191 | 173 | | |
| | 1 control | 168 | 159 | 144 | | |
| | 2 | 34 | 34 | 14 | | |
| | 2 | 16 | 28 | 2 | | |
| | 5 | 181 | 206 | 170 | | |
| | 5 | 179 | 177 | 158 | | |
| | 7 | 123 | 140 | 112 | | |
| | 7 | 159 | 156 | 138 | | |
| | 9 | 130 | 112 | 101 | | |
| | 9 | 115 | 120 | 98 | | |
| | 1 | 15,200 | 15,341 | 15,300 | 61,200 | > 2.06 |
| | 1 | 13,500 | 13,260 | 13,400 | 53,600 | |
| | 2 | 13,100 | 13,060 | 13,100 | 52,400 | > 2.01 |
| | 2 | 13,562 | 13,250 | 13,400 | 53,606 | |
| | 5 | ~~900,000~~ | 898,945 | 899,000 | 3,600,000 | > 1.54 |
| | 5 | 900,000 ~~514,000~~ | 899,036 | 899,000 | 3,600,000 | |
| | 7 | ~~514,000~~ ~~255,700~~ | 514,000 | 514,000 | 2,056,000 | |
| | 7 | 255,000 ~~107,600~~ | 254,000 | 254,500 | 1,020,000 | 1.68 |
| | 9 | 107,000 | 107,990 | 108,000 | 436,000 | |
| | 9 | 114,000 | ~~118,000~~ | 114,000 | 456,000 | 1.88 |

| # | $A_{260}$ |
|---|---|
| 3 | 2.35 |
| 4 | 2.34 |
| 6 | 2.32 |
| 8 | 2.37 |
| 10 | 2.31 |
| control | 2.25 |
| 1 cont | 2.34 |
| 2 conts | 2.13 |
| 5 conts | 2.07 |
| 7 cont | 2.25 |
| 9 cont | 2.21 |

Controls indicate less than 2% contamination by medium in all cases. Much less than that in some.

Ignore:

Counts on hot media (0.1 ml of 1→10 dilut)

| | cpm (dupl) |
|---|---|
| 23-12-71 | 5100 |
| "C" | 47,000 |
| "D" | 46,000 |
| "E" | 4500 |
| "F" | 51,000 |

D911 71

# Summary - Prelabel conditions. p5

| Experiment | $^3H$ dT | Input conc. | μCi/ml media | S.a. μCi/mmole | Determined cpm/ml media | Total cpm per plate after chase | Total acid insol cpm per plate after chase | Total Abs per plate | Acid insol cpm per Abs. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | + | $5\times10^{-5}$ | 2 | | $5.1\times10^5$ | $6.1\times10^4$ | $5.7\times10^4$ | 4.1 | $1.4\times10^4$ |
| 2 | + | $5\times10^{-5}$ | 2+2 | | $5.1\times10^5$ $4.5\times10^5$ | $6.5\times10^4$ | $5.3\times10^4$ | 4.0 | $1.3\times10^4$ |
| 3 | − | $5\times10^{-5}$ | | | | | | 4.7 | |
| 4 | − | $5\times10^{-5}$ | | | | | | 4.7 | |
| 5 | + | $5.1\times10^{-7}$ M | 10 | | $2\times10^7$ | $5.1\times10^6$ | $4.4\times10^6$ | $3.6\times10^6$ | 3.1 | $1.2\times10^6$ |
| 6 | − | $5.1\times10^{-7}$ | | | | * | | 4.6 | |
| 7 | + | $5.1\times10^{-6}$ | 10 | | $2\times10^6$ | $4.7\times10^6$ | $1.5\times10^6$ | $1.5\times10^6$ | 3.3 | $4.6\times10^5$ |
| 8 | − | $5.1\times10^{-6}$ | | | | | | | 4.7 | |
| 9 | + | $5.1\times10^{-5}$ | 10 | | $2\times10^5$ | $4.6\times10^6$ | $0.5\times10^6$ | $0.45\times10^6$ | 3.8 | $1.2\times10^5$ |
| 10 | − | $5.1\times10^{-5}$ | | | | | | | 4.6 | |

Compare 5 & 9 - s.a. of $^3H$ dT decreases 100x from 5 → 7, but total counts incorporated decreased less than 10x.
~~Partly~~ Also, have more cells in #9 - since Abs 3.8 compared to 3.1 in #5.
Use 5 & 9. Do infection in chase medium.
Use X8

phenol? → sevag or equivalent → EtBr ↓ → hybridize
alk CsCl → hybridize

HIRT - 5-10 plates pooled maximum
think about using virions.
20 plates - good banding

fragmentation.

Gustafson Stem Symposium Virology

1/16/87 12