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

Prep of BSC DNA. D 71.

See methods book.

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

Thaw at rm temp. Add 1 ml/5 plates = 12 ml of SDS — Saline (see methods) (S Lauic). ~~Vortex~~, Wash into long t.t. Vortex. ~~It~~ 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-Q(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 C ~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 ~~tip~~ 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) O-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 $A_{260} = 0.210$

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

60 plates = 15 OD

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

If 1 OD = 50 µg DNA/µl (Felsenfeld, quoted by Hurt)

then, 10.7 $A_{260}/\mu l$ = ~~530~~ 535 µg/µl = 0.54 mg/µl

£ 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, QD. 10 mg/ml NAD.
Boehringer Control # 645441 (663) Free acid.
free acid. for weeks. ($C_{21}H_{27}O_{14}N_7P_2$)
Stable cold neutral, (Kornberg, Meth Enz 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.
1 N = 1 mmole/ml.
~ 0.27 M.

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

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

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

* BHI-Agar $\frac{37.5}{4}$ = 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°.
2.5/100 0.5/10

* 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 - ~~c~~ 0.2 ml glycerol

* Sterilize by autoclave, 15 min.

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

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 c #1, 37° stab
Inoculate 50 ml BHI - DPN - Hemin c #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 c sterile water. Filter thru millipore. Add 50 mg Hemin (E.K. from Nathans.) 15' at 67°.

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 Nathans.
Store 37 #1 in cold room
Start 4:15 pm.

Plates from 37 #1. (37°.)

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

	1	2	3
6:52	0.097	0.110	0.072
6:52 – 2010	0.028	0.041	0.003
10^02 – 2010	1.665 (4.568)	1.832	0.117. 0.045
11^10 – 2010 – 2010			0.156 0.084

Stop 1 to 2 at 10^02 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

21-12-

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 ד"ר קלקר
ד"ר קליין 4
רחובות

D-211 71.

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 2×10^5, 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
5×10^{-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, 42×10^{-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 steule.

24-12 #2. #4. Remove medium. Replace ē hot (2) & cold (4) fresh medium ~~et~~ made exactly as 23-12, using dilution of ^{3}H dT. make 23.12: Med~~ium 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: ~~4~~ 2 plates at 24°. ~~2~~ days. Then label at 5×10^{-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, 5.1×10^{-7} M.
6	– no label, no dT.
7	10 μCi/ml. 5.1×10^{-6} M dT 2.37 Ci/mmole
8	~~5×10^{-6}~~ no label, 5.1×10^{-6} M dT.
9	10 μCi/ml 5.1×10^{-5} M dT 0.237 Ci/mmole
10	no label, 5.1×10^{-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) ~~85~~ 87
^{3}H-C: 5 ml F + 0.2 ml 1→33 dT ~~87~~
D: 5 ml G + 0.2 ml 1→3.3 dT (0.25 μmols) 90
^{3}HD: 5 ml F + 0.2 ml 1→3.3 dT. 9 to
(dilutions in MEM.

12 12 25-12 Nbrs 1 → 10 or at all look like close to 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 1x c̄ 4 ml PBS

1x c̄ 2 ml PBS.

Prior to wash ~~Add 2 ml 0.5~~. 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 ml.

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

a & b, filter & wash as per methods. ~~Dry + 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 filters in hotwire

Background ~~= 20~~, included on blank: for C, total cpm = x 20.
for exp't. under. for Other ~~C, D~~) total = cpm x 4

		cpm	cpm repeat	cpm − Bl	Total cpm/plate	A_{260}
C	/ control	59	51			
	1	3181	3120	3065	61,300	2.06
	2 contrd	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	3,609,000	> 1.54
	5	~~900,000~~ ~~514,000~~	899,036	899,000	3,600,000	
	7	514,000 ~~2,255,000~~	~~255,700~~ 514,000	514,000	2,056,000	
	2	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	~~138,000~~	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	2.27

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 dilution)

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

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Summary - Prelabel conditions. p5

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

Gustav Stern Symposium Virology

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