Singer

Titrate Down E. coli DNA with RNA polymerase
(also ψU and check on BSA in incubation)

(see 2-19-63 for inhibition by more than the usual 0.075 ml ~~per~~ ~~fo~~ per 0.25 ml reaction

Each tube: 0.05 ml Tris mixture ✓ X — 10 minute incubation
0.03 ml β-ME ✓ X
0.04 ml triphosphate mixture X
0.12 ml

Stock soln 40 01,8 cpm/.01 ml

	1	2	3	4	5	6	Blank
Diluting fluid 1.27 μmols/ml	.010	.010	—	.010	.010	.010 ✓	
E. coli DNA, 8.39 OD/ml	—	.075	.075	.050	.025	.075 ✓	
DEAE #46	.005	.005	.005	.005	.005	.005	
ψUDP? 47-61 Jan 63 10 μmoles/ml						.010	
H_2O to 0.25 ml	.115	.040	.050	.065	.090	.030 ✓ X	
cpm (10 min)	11.9	109.7	100.8	117.8	108.4	128.0	11.2
− Bl		98	89	106	96	116	
μmoles DNA per assay		95		63.5	32		

Enzyme has lost activity!
.025 ml DNA about as good as .075 so would get relatively more RNA than DNA which would have its advantages.
ψU seems to increase activity somewhat.

Proteins on Methylase
N-91

Singer
3-14-63

Tube	Sole	ml H_2O	Abs_{660}		- Bl	mg	mg/ml
1	Bl	.500	059	059			
2	0.01 ml 7.9 mg/ml stand	.490	419	419	.360	.079	7.9
3	0.005 Methylase	.495	333	333	274	.060	12.10 > 11.4 mg/ml
4	0.010 Methylase	.490	551	551	492	.108	10.8

3-15-63

Cold triphosphate mixture.

	ml
UTP 21.7 μm/ml	.046 ✓
ATP 22.8 μm/ml	.044 ✓
CTP 24.3 μm/ml	.041 ✓
GTP 24.4 μm/ml	.041 ✓
	.172
H_2O	.228 ✓
	.400

New Hot Triphosphate Mix.

	ml
	.23 ✓
	.22 ✓
	.2ϕ ✓
	.2ϕ ✓
	.64
hot CTP^{C14} Schwarz 6206, 5 μc/μ	.50
	2.01 M

"Preincubation."

	Tri P-cold	Tris-Mg/Mn	0.1 Flyid	0.1M β-Me ✓	DNA E. coli ✓	H_2O ✓	Vf
1	.040	.050	.010	0.03	0.025	.095	.250
2						.250	.250
3	.040	.050	.010	.03	025	.095	.250
4						.250	.250

Heat 2 min at 70°

"Incubation. 30 min @ 37° ✓"

	Preinc Sup ✓	Methylase	AmeH^3 10.6 μ/l	Methyl cRNA	Mg	Vf
1	.200	.150	.015	.030	.005	.400
2	.200	.150	.015	.030	.005	.400
3	.200	.150	.015	—	.035	.040
4	.200	.150	.015	—	.035	.400

Add 2 ml 5% TCA. Wash on millipore 3x ē 1% TCA.
Put millipore in counting vial ē

Tap 9, 10-75.

Tube	cpm (3 min)	−Bl
1	950	711
2	1000	694
3	239	
4	306	
Blgd Bag	23.6	

Polymerase reaction mixture has little or no effect on methylase reaction. Can go ahead with this.

Mr 11, 12

Singer
3-12-63

Sup from alcohol precipitation

Concentrate to 1 ml. Count 0.1 ml as usual (~~c~~ 1 N 1-10 NH_4OH) in Scintillator. M 11 (#8) M 12 (#9)

Precipitate (insoluble after alcohol precipitation.
add 0.1 ml 0.01 N Tris. Still did not dissolve completely. Add 1 ml 1 -> 10 NH_4OH. # 10, # 11

Blkgd = 11.2
10 min counts

	8	9	10	11
cpm	230.8	158.0	234.8	230.0
dupl.	197.9	148.2	231.3	225.0

~~Check on~~ quenching
to #10 was added
0.01 ml of triP. Rx Mix:
4163.9 cpm.
0.01 ml alone, 4001.8
4001.8 + 234 = 4235.8
so have $\frac{4163.9}{4235.8}$ = 98.4%

Then very few counts left in what didn't dissolve for sucrose gradient.
But what are counts that were soluble ~~after~~ in EtOH after extensive dialysis?
Plans concentrate down, chromatograph in phosphate - Amsulf.
Tremendous amount of salt - little hope for chromatography.
Even difficult to put on sample. Discard.

Singer
3-19-63

Sephadex G-100 Lot No To-33
140-400 mesh.
Bed volume (ml bed per g dry gel) 15-20
water regain (g water per g dry gel) 10 ± 1

$V_t = V_o + V_i + V_g = 20$ ml

$20 = V_o + 10 + V_g$

$10 = V_o + V_g$

a 1 gm column is probably OK for 0.1 ml aliquots.

Suspend 1 gm in 0.01 M Tris, pH 7.8, 0.005 M NaCl

Pour column.

500 ml Tris NaCl

0.5 ml 5M NaCl
5 ml 1 M Tris pH 7.83
} = 500

$0.5(5) = 2.5 = .005(500) = 2.5$

$5(.1) = 500(.01)$

Plan Wash c̄ Tris-NaCl.

Det'm V_o with DNA 9-2-60 Lot 592 10 mg/ml in 0.005 N NaCl

Apply 0.1 ml. diluted to 1 ml c̄ above buffer Wash c̄ above buffer. Collect 1 ml fractions about 20.

4-3-63 Wash column with some of salt-buffer.
Apply sample as in plan.

Singer
4-3-63

Sephadex G-100

Vo detm (cont)

Fraction	Vol	Com vol	OD_{260}
1	1.3	1.3	2
2	1.3	2.6	2
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Why so ch high ODs?

Wash further ē buffer-salt
It is something from tubing or mixer flash.

Repour column. Change to rubber tubing. Dov OD down to 0.045 with about 200 ml wash.

4-4 AM OD about 0.1 wash some, down to .045 change to ~2 cm OD Kontes column. Continue washing. Change to pH 8.2.

4-6 Wash again. Switch to pH 8.2 buffer.
Wash continuously get down to Abs_{260} = ~.025. only.
Apply DNA as above.

Actual bed vol = $\pi \times 8.3(h) \cdot 0.73^2 = 13,85$ ml
1 gm column, little less. 13.9 ml = $V_o + 10 + V_g$, $V_o = 5.0$, so must have amount less than 1 gm lost some in repouring.

4-18-63

Check activity of DNA polymerase.

Each tube ✓ 0.05 ml Tris mixture
✓ 0.03 ml 0.1 M βME (.068 14M → 10 ml)
✓ 0.04 ml triphosphate mix 1-23-63
✓ 0.01 ml diluting fluid (~~0.01 M~~ /ml)
0.13 0.1M βME/ml.

10 min. incubation
Add .02 ml 60 mg/l BSA
3 ml 3.5% PCA.
Wash 2x ℄ 3 ml 3.5% PCA

	1	2	3	4
E. coli DNA 1.27 μmole/ml 8.39 OD/ml	—	.050	.050	.050 ✓
DEAE 46	.010	.010		
45			.010	
49				.020
H2O to 0.25 ml	0.110	.060	.060	.050 ✓
cpm. (10 min)	10.6	112.5	43.1	33.2

Blank = ~~103~~ 10.3 cpm.
Stand = 14,834

Enzyme quite dead.

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Sephadex G-100

Vo Detm 4-17-63

Tube	Vol	OD_{260}	
1	1.00	024	} variation due to cells.
2	1.08	045	
3	0.91	025	
4	0.98	052	
5	1.10	400	} variations may be due to lack of washing of cells in between samples.
	5.07		
6	1.18	1.02	
7	1.40	.184	
8	1.19	1.03	
9	1.00	.995	
10	1.00	.873	
11	~1	692	
12	~1	668	
13	1.7	670	
14	(15.54 ~~25~~ 2.5)	625	
15	1.45	695	
16	3.5	448	
17	1.8	313	
18	3.4	232	
19	4.2	164	
20	0.9	148	
21	2.0	148	
22	2.5	127	

4-19 a.m.

To Look at Phosphorolysis of demethylSRNA 4-18-63

Blcgd = 13.7

New reaction mixture. 4-18-63

2 ml 0.5 M Tris pH 8.2
~~0.2 M~~ 0.05 M EDTA
~~0.025 ml 0.2 M~~ EDTA
0.03 ml P^{32} 27.6 μm/ml 3/63 a.c.
~~2.055~~ 2.023
~~.945~~ .977 H_2O
3.000

Dilute count 4-19, .05 → 5.00 ml.

Plate ml	cpm	- Blcgd	cpm/ml
.05	560	546	10.92×10^5
.10	1085	1071	10.71×10^5

10.8×10^5

sa = $\frac{10.8 \times 10^5 \times 0.03}{1 + .008}$ = ~~32~~ 32,200 cpm/μm

4-19-63

Each tube: 0.03 ml AK mix. ✓
0.01 ml 0.05 M $MgCl_2$ ✓
~~0.01~~ 0.005 ml 2 mg/ml BSA ✓
0.01 ml 0.1 M K_2HPO_4

usual 15' assay.

	polyA 5 mg/ml Taka NEM 12-19 ✓	poly U JV272 5 mg/ml ✓	demethyl SRNA 3250D/ml ✓	E coli SRNA Ap16163 NEU 3-28-63 pooled Sep. 3250D/ml ✓	H_2O ✓	cpm	- Bl	μmoles incorp.	u/l	approx μmoles Substrate
1	.010				~~.030~~ .035	40.8				
2	.010			.005	.030	851	810	.0252	504	0.1
3		.010			.035	43.7				
4		.010		.005	.030	908	864	.0268		0.1
5			~~.010~~ .005		.040	46.3				
6			~~.010~~ .005	.005	.035	150	104	.0032		0.163
7				.005	.040	44.9				
8				.005 .005	.035	72.4	28	.0009		0.163

Add u/ml of poly A. Expect about 9 u/ml, see 4-16-63, HM II.
Pk mix used 4-16 had 1/2 as much EDTA as here. That is only apparent difference.

.03 .12 .018 .036

Expt #2 on Phosphorolysis of demethyl SRNA

4-19-63 pm

Look at extent of phosphorolysis.

~~#~~ 6x enzyme concentration

start 2^09 pm

Rx Mixture:	①	②	Remove 0.1 ml at 1 hr, 90 m, 2 hr.
Rx Mix P^{32}	0.12	0.12 ✓	usual nuclease assay
$MgCl_2$ .05 M	0.04	0.04 ✓	
2 mg/ml BSA	0.02	0.02 ✓	
0.1 M K_2HPO_4	0.04	0.04 ✓	
demethyl SRNA	0.02	0.02 ✓	
Ap-16163 label	0.12	~~0.12~~	
H2O to 0.4 ml	0.04	0.16 ✓	

#	Descrip'	cpm
1	1, 1 hr	Lost
2	2 1 hr	35.9
3	1 90'	59.2
4	2 90'	39.4
5	1 2 hr	60.3
6	2 2 hr.	

This does not repeat experience of 4-~~18~~ 19 am where demethyl seemed ~~#~~ go ~~faster~~ even more in 15 min than here. Recheck 15' expt ~~c~~ poly A as well

4-23-63

To try out our cells and sonicator for RNA polymerase prep.

1.4 gm. batch fermentor E. coli B. 3/13/63.
Thaw some at room temperature.
Cream well
In small amounts add & suspend well in 3 ml buffer A.
Stir 10 min, in ice bath & magnetic stirrer.

① Take 1.5 ml, try out in sonicator. Position 6, probe completely down, −10° bath. 45 sec. Cells darkened somewhat main problem, heated up at least to 40°. Use small cellulose nitrate tube.

⑤ Treated remainder - about 2.5 ml. Used dry ice - alcohol bath. Did not dip probe all the way. Same conditions as above. Looked broken up. Temp = ~ 6° C.

Centrifuge both ① & ⑤, Servall, 100. 30'.
① clear yellow sup, dusty ppt
② hazy sup, fairly turbid & murky.

Wash both precipitates & 0.5 ml of buffer A. Centrifuge 30' as above. Combine wash sups.
① 1.3 ml
② 2.3 ml

Protein determ

			H2O	Abs 660	-Bl	mg	mg/ml
1				.059			
1	Blank		.500	.049			
2	Stand, 7.9 mg/l	.010	.490	412	.353	.079	
3	① dil 1-10	.010	.490	075	.016	.0036	3.6
4	① "	.050	.450	160	.101	.0226	4.5
5	⑤ dil 1-10	.005	.495	135	.076	.017	33
6	⑤ "	.020	.480	305	.246	.055	27.5

Singer.
4-19-63

G-100 Sephadex Column

Wash ⊂ 0.01 N Tris, 8.2 } until UV_{260} = 0.049 and steady
0.005 M NaCl

Yeast SRNA (Martin-Singer 6-29-61) 9.3 mg/1.86 ml, 5 mg/4 8-14-61
Mix 0.1 ml + 0.4 ml above buffer. Apply to column. Start collecting.
Wash in with 2 @ 0.5 ml washes. At first collect every 4 min.
switch to every 6.
~ 1000 units or less.

Fraction	Vol	cum vol	OD 260	Total OD.
1	0.9		042	
2	1.0	1.9	038	
3	0.85	2.75	038	
4	0.80	3.55	071	
5	0.80	4.35	1.83	1.46
6	0.75	5.10	2.5	1.88
7	0.7	5.80	2.15	1.5
8	0.75	6.55	0.96	.72
9	0.80	7.35	0.460	
10	1.10	8.45	.224	
11	1.15	9.60	.126	
12	1.0	10.60	084	
13	1.0	11.60	063	
14	1.05	12.65	049	
15	1.18	13.83	042	
16	1.10	14.93	034	
17	1.09	16.02	027	
18	1.05	17.07	029	

Fract	Vol	Cumul	OD 260
19	1.2	18.27	031
20	1.08	19.35	016
21	1.1	20.45	012
22	1.05	21.50	027
23	1.1	22.60	032
24	0.98	23.58	028
25	1.00	24.58	033
26	0.99	26.57	035

Count C^{14} at: Tap 7
10-75

4-23-63
Senger

Activity Assays (RNA polymerase) on E. coli extracts

Each tube: 0.05 Tris mix ✓
0.04 Tri P mixture 3-15-63 ✓
0.03 ml βME 0.1 M ✓
0.01 ml dilution fluid ✓
0.13 ml

C^{14} Standard - 21,100
Added 0.01 of Tri P mix to #2,
recount - 8150, 8180 cpm,
expect ~~8415 ± 5~~ 8390 ± 34
8424
so ~~100~~ is 97%

	1	2	3	4	5	6		
E. coli DNA 8.39 OD/ml	—	.050	—	—	.050	—		
①	~~.060~~	.060						
②				.050	.050	.025		
H_2O	.060	.010	.12	.070	.020	.095		

Vial	1	2	3	4	5	6	7	8	
							Bray Bl	0.01 of Tri P mix	
cpm (5 min)	67.6	58	53.4	~~344~~ 445	~~254~~ 377	344	25.2	8450	83.56×10^4 cpm/ml
cpm (5 min)	65.3	59	46	~~343~~ 454	388	343	22.4	8380	8356×10^2 = cpm
— Blank = 24	41	35	22	430	364	319	—	8356	2,500 mμmole
×6 (per hr)				2580	2160				334 cpm/mμmole
mμmoles/hr = units				7.73	6.47				
u/ml				155					

4-25-63

Reassay Crude Polymerase (sonicate) after storage in deep freeze see 4-23

10-15, tap 7

Bkgd = ~~24.1~~ 26.2

C14 stand 21,100

Each tube 0.05 ml Tris mixture ✓

0.04 ml Tris P mix 3-15-63 ✓

0.03 ml βME, 0.1 M ✓

0.01 ml dilution fluid ✓

10' as usual.

	1	2 (2nd time)	3	4
E coli DNA @ 3900/ml	—	—	—	—
Sample 2 of 4-23	—	.050	.050	.025
H_2O	0.12	.070	.070	.095 ✓
	mixed in vial			
cpm (5 mn)	273		352 (2.6)	313 (2.6)
− Bkgd			326	287

This extract after storing frozen 2 day has ~~lost~~ retained about 80 % of its activity, or more.

Assay E. coli alkaline phosphatase. 4-25-63

Unit - μmoles/hr

	1	2	3	4
Tris, 0.5 M pH 8.2 2-19-63 Neutral	0.01	0.01	0.01	0.01
5'-AMP 94.5 μm/ml	0.05	0.05	0.05	0.01
Enzyme, del. 1-100, ~ 30 u/l	~~.005~~ .001	~~.010~~ .002	.005	.020
H_2O	0.08	0.075	.070	.060
	0.40	0.375 .079	0.350 .075	0

20', 37°.

Add 0.9 ml 2.5% PCA

take 0.3 for Amer P.

20 minute incubation.

	1	2	3	4	5	Bl	.102 μm Pi
Tris, 0.5 M pH 8.2 2-19-63 neutral	.01	.01	.01	.01	.01		
5'-AMP 94.5 μm/ml del 1-100 7 mg/l stored Frozen	.01	.01	.01	.01	.01		
Stock sample 6120A 5-16-62 del 1-100 5-16-62	—	.001	.005	~~.001~~	~~.005~~		100 10 mm/l .0005
Use sample 6120A 2984 u/l	—	—	—	.001	.005		
H_2O	.08	.079	.075	.079	.075		
Abs 820	014	090	322	044	107		477
μmoles Pi	.0006	.0038	.0135	.0018	.0045		
— Bl		.0032	.0129	.0012	.0039		
μmoles/ml mix		.0107	.0430	.0040	.0130		
μmoles/ml enzyme		1070	860	400	260		
μmoles/hr/ml enzyme		3210	2580	1200	780		

~~Plan to use stock sample, about 1000 μm~~

Plan to use stock material, in excess for alkaline digests.

4-25-63

Test Ap16163 for nuclease

Blank, 0.1 ml NH_4OH + 0.3 ml 2.5% PCA 24.1 cpm

Stand " " + .005 C^{14} RNA 9291.9 cpm

Cold nuc mix:

2 ml 0.5 M Tris 8.2 ✓
0.2 ml 0.05 M EDTA ✓
0.5 ml 0.1 M $MgCl_2$ ✓
0.3 ml H_2O ✓
3.0 ml.

Plan 1, 2, 5, 8, take 0.3 ml of acid soluble + 0.1 ml 1→10 NH_4OH, 10 ml Brays Count Tap 7, 10-75

Others read OD at 260 μ 3, 6, 9, 10
at 257 μ 1, 4, 7.

SRNA, .005 → 1.0 ml ē .005 M KPO_4, 0.01 N NaCl
Abs_{260} = .440, Undel = 88

Each

	1	2	3	4	5	6	7	8	9	10
✓ Rx Mix	.030	.030	.030	.030	.030	.030	.030	.030	.030	.030
✓ polyA 5 mg/ml Talca 1279	.010			.010			.010			
✓ RNA C^{14} F19163 34.8 μg/ml	~~0~~	.005			.005			.005		
Ap-16163 Whel 8800/ml 6 mg/l	—	—	—	.010	.010	.010	.010	.010	.010	.020
✓ SRNA, yeast, ~~32500~~/ml Maxton Scnqu 8-14-61			.010 ~~.005~~			.010 ~~.005~~			.010 ~~.005~~	.010 ~~.005~~
✓ H_2O	.060	.065	.065	.050	.055	.055	.050	.055	.055	.045
time	1 hr	1 hr	1 hr	0	0	0	1 hr	1 hr	1 hr.	1 hr
ml 2.5% PCA	0.9	0.4	0.9	0.9	0.4	0.9	0.9	0.4	0.9	0.9
OD 260			.048			.116			.084?	042
OD 257	056			.161 *	~~.116~~ *	~~.116~~ *	035			
cpm		51.5			39.3			32.5		

Why high UV for zero times.
In general no nuclease here. Must extend test ē SRNA.

* Slow in taking zero time.

4-26-63

Repeat on phosphorolysis of demethyl SRNA

Each tube

0.03 ml MK Mix 4-18-63. ✓ plan usual 15' assay

0.01 ml 0.05 M $MgCl_2$ ✓

0.005 ml 2 mg/ml BSA ✓ Sa - 32,200 × .612 = 19,700

0.01 ml 0.1 M K_2HPO_4 ✓

	polyA 5mg/ml Tatum 12-19	demethyl SRNA 32500/1	$Ap_{16}I_{63}$ tube #1	H_2O	0-134 #4 of 4	cpm	- Bl	μmoles	u/ml
1	.010	—	—	.035		32			
2	.010	—	.005	.030		435	403	.0205	4.1
3		.005	—	.040		33	—		
.163 μmoles 4		.005	.020	.020		45.2	12		
5	.010	—	—	.025	.010	147	115	.0058	0.58
6	—	.005	—	.010	.030	93	60	.0030	

0-134, last assayed was 0.7 u/l ≈ FB polyA 5 mg/l

Expt 4-18 must have been a freak. This exp't agrees with that of 4-19, essentially no phosphorolysis with $Ap_{16}I_{63}$

But 0-134 again is tantalizing. Recheck with longer incubation.

RNA Polymerase Prep II p. 1 4-30-63

55.6 gm E. coli B (Benzer) Fermentor Batch, 3-13-63.
Take out about 9 am from freezer. Thaw with aid of cold H_2O bath and mechanical chopping (~ 1/2-3/4 L). Mix into good paste. Seems somewhat more liquid than Leder's cells. Slowly mix in 119 ml Buffer A to make good suspension. ~~Stirred~~ Cool down as this was done. Mix 10' ē magnet in cold room.
Sonifier on 15 ml batches, setting 6 (or 5) 30 sec. blasts, 3x on each. Looks pretty good.
Centrifuge Sorvall 30 min, 120 set (> 20,000 x g).
Murky ~~sn~~ sup Recentrifuge, looks better.
Wash ppt with ~ 30 ml buffer A as last time. Centrifuge.
Pool wash + sup. Ap 301 165 ml. Ppt to Neu. 1 ml saved.
Spinco, 30,000 x g, #30 head. Plan 5 h. had only about 3 1/2 no brake. Get 140 ml. after filtering through glass wool. Renumber 14 Ap 302

Protein

		ml	ml H_2O	OD_{660}	- Bl	mg	mg/ml	
#1	Blank	—	.500	053	—			
2	St .005	.005	.495	252	199	.0395		
3	.010	.010	.490	401	348	.079		
4	Ap 301 dil-10	.010	.490	182	129	.0256	25.6	24.2
5	"	.020	.480	283	230	.0456	22.8	
6	Ap 302 dil-10	.010	.490	138	.085	.0169	16.9	16.2
7		.025	.475	249	.196	.0388	15.5	

Ap 302 16.2 mg/ml, have 139 ml = 2250 mg $\frac{2250}{V} = \boxed{12}$ V = 187.5
Adjust to 187.5 ml by adding $\frac{187.5}{139.0}$ $\frac{}{48.5}$ ml buffer A.

Add 14.7 M βME → 0.01 M V(14.7) = 187.5 (.01) V = .128 ml. Add 9.38 ml 10 % to Streptomycin Sulfate (Squibb, 2.5 gm / 25 ml) 6E55845

over ~ 15 min. Never scew any strings. Then stir cold 15 min more.
Spin ~ 14.000×g VRA 15'. Tight white ppt Pale yellow sup.
My- 1163. Remove 0.25 ml

Add 7.5 ml 1% Protamine sulfate (Lilly, 0.25 gm/25 ml)(0048-545662)
Sit 15' Spin 15' 10,000×g VRA. Give sup to Tolbert My 1263.
Small white precipitate. Make 75 ml Buf A 0.01 N in βME & 0.05 ml
14.7 M. Wash precipitate with this as before. Centrifuge 10,000×g
9" Lourdes.
Put ppt in homogenizer ex, using total of about 20 ml Buffer A- $(NH_4)_2SO_4$
-βME. Centrifuge. Obtain 20.5 ml supernatant. My-1363 Store cold room

5-2-63 To 20 ml of sup above, add 8.54 ml A.S (satd 25°, pH 7)
Spin 15' Top speed, Servall
Small yellow-white button
To sup add 8.75 ml A.S. as above.
Centrifuge 25 min Take up ppt in 1 ml Buffer B. My 2163
Store in −70 Box
My-11 11.2 mg/ml
My 12 10.4 mg/ml

My21, on 5-3 Thaw, put up 8 0.1 ml aliquots
+ lemeerala ~~deep~~ −70 Box

Assays on RNA polymerase Prep II. 5-2-63

Each tube 0.05 ml Tris mix ✓ 10° as usual
0.04 ml TriP ✓ (334 cpm/mμmole). 10.75, tap 7.
0.03 ml 0.1 M βME ✓ Blkgd cpm = 25.5
0.01 ml diluting fluid ✓ C^{14} stand 20,950

	1	2	3	4	5	6	7	8	12
✓Ecoli 8.3900/ml DNA	.050	.050	–	.050	.050	.050	.050	–	
Ap 301	–	.050 ~~.025~~	.050	–	–	–	–	–	
Ap 302	–	–	–	.050	.050	–	–	–	
My 1362 del 1-10	–	–	–	–	.010	.010	.020	–	
My 2162	–	–	–	–	–	.010	.020	.020	
H_2O	.070	.020	.070	.020	.070	.060	.050	.060	.050	.100	.070
cpm (30 min)	29.9	352	~~652~~	652 ~~970~~		970	1470	1442	2440	42.8	.040
Sample #2 of 4-23	4	326		626		944	1444	1416	2414	17	
– Blkgd.											
– Bl = 4		322		622		940	1440	1412	2410	13	
× 6 (pu/hr)		1930		3730		5640	8640	8490	14500		
mμmoles/hr (units)		5.78		11.2		16.9	25.8	25.4	43.5		
u/ml		115		224		1690	1290	25,400	21,700		
Sa		4.75		13.8		563	430	1260	1080		
Vol		165		140		20.5	20.5	/	/		
Total units		19,000		31,200		34,600	26,400	25,400	21,700		

overall $\frac{1100}{5}$ = 220 fold

Berg $\frac{6100}{48}$ 152 with DEAE

5-3-63

Proteins for RNA Polymerase Prep 2

#		Spl	ml CO_2 NaOH		– Bl	mg	mg/spl ×6/5	mg/ml
1	Blank		.500	.052				
2	Prot Stand 7.9 mg/ml	.005	.495	.249	.197	.0395		
3	My 13_{61}	.020	~~.49~~ .500	.308	.256	.0514	.0616	~~13.2~~ 3.08 → 3.0
4		.040	.500	.518	.466	.0935	.112	2.8
5	My 21_{63}	.005		518	466	.0935	.112	22.4
6		.010		790	738	.148	.178	17.8
							402	
							20.1	

to 3-6, add 1 ml ~~3~~ 3.5% PCA & precipitate Sit 10', spin 10' take up as indicated in ~~CO_2-NaOH~~. 0.6 ml CO_2-NaOH. Use 0.500 ml for sample.

4-30-63

Ap 30363

SRNA E coli (Zubay) from Peterkofsky.

2 ml, OD = ~ 350.

Total OD = ~ 700

1 ml / 5 mg

700 OD = 70 μmols = 35 mg. Want 7 ml.

~~V (0.3) =~~ $K(1) = (2+K)0.3$

$K = 0.6 + 0.3K$

$0.7K = 0.6$

$K = 0.86$ ml.

$K(1) = 7(0.3)$

K =	2.1 ml	1 N KOH
	2.0 ml	SRNA
	2.9 ml	H_2O
	7.0	

$2.1(1) = 7(0.3)$

2.1

5 PM.

5-1-63 Wash (in a column) AG 50W-X2, 50-100 mesh, H^+, ē distilled H_2O. # 5158-41 B1099. to negligible O.D.

Make 1:1 suspension. 0.7 meq/ml. 7 ml, 0.3 N KOH =

Make up dummy, 2.1 ml 1 N KOH, 4.9 ml H_2O.

3.5 ml resin volume to pH 6.5

,

Sample Transfer to grad cylinder. Total vol = 12.8. Take to pH just about 9.5 ē ~ 2 ml resin volume. Filter & wash. Volume now is 31 ml. Add 0.31 ml 0.5 M Tris pH 8.2. PH checks OK at 8.2.

Dilute .01 → 1.00 ē H_2O. $OD_{260} = 0.344$. OD/ml = 34.4

Total OD = 34.4 × 31.31 = 1077 ODs. = ~ 90 μmoles / 31.3 ml = ~~2.9~~ 2.9 μm/l

Ash P on 0.005, 0.010 samples. Store Cold Room