9/20/57

Lets Look at Some Ways of Precipitating DNA. (or separating it from Triphosphates) which do not involve procedures injurious to hydrogen bonds.

1- Alcohol. To 3.0 ml of distilled water add 0.20 ml DNA (2.5 mg/ml). Remove 0.02 ml and dilute to 1.0. Read at 260; = .305
.305 × 25 = 7.5 O.D. total.
To remainder of solution add 2.0 ml of Cold Abs. Alcohol. Turbidity but no obvious precip. Sit 5' 0°. Spin top speed International for 5'. Decant supe. Jelly-like material present along side and bottom of tube. Add 0.95 ml. of H_2O. Mix ē glass rod. Jelly appears to dissolve. Add 0.05 ml of Tris 8.0, mix. Dilute 0.5 → 1.0 and read at 260 = 0.100
0.100 × 20 = 2.0 O.D. $\frac{2.0}{7.5} \times 100$ = 27% recovery

2- Acid Alcohol - To 0.30 ml of H_2O add 0.20 ml DNA (2.5 mg/ml). Total O.D. = 6.20
Add 2.0 ml Acid Alcohol (0.5 ml 2M Formate, 5.0 → 10 ml Abs. EtOH)
Ppte forms immediately. Spin after 5' 0° (Top Speed International 5').
Dissolve ppte in 1.0 ml M/10 Tris. 7.5 (Use pestle. Turbid soln). Dilute 1→10.
Read @ 260; = 0.610 × 10 = 6.10 total OD $\frac{6.1}{6.2} \times 100$ = 98% recov.

(6.2 O.D. total)

3- Norit To 0.30 ml H_2O add 0.20 ml DNA (2.5 mg/ml). Add 0.40 ml H_2O.
Add 0.10 ml Norit. Mix (Norit seems to clump) 5' 0°. Spin. Read supe 0.5 → 1.0 λ 260 O.D. = 0.310 × 20 = 6.20 = ~~[illegible]~~ 100% recovery.
To see if dCTP is adsorbed to Norit under these conditions (Neutral pH) add 0.10 ml Norit to 1.0 ml dCTP solution at neutral pH. OD before Norit = 2.350. O.D. after Norit = 0.000. 100% adsorption.

4- Remove Alcohol ✗

5- Higher DNA conc ✗

6- Acid in 0.1M NaCl - To 0.30 ml of H_2O add 0.20 ml DNA (2.5 mg/ml) + 0.05 ml ~~1M NaAc pH 4.0~~ 2M NaCl and 0.05 ml 1M NaAc pH 5.0.
No ppte.

9/23/57

Are Oligonucleotides Formed When 3 triphosphates are omitted?

	1	2*	3	4*	5	6*
✓ DCTP[32] .5 μ M/ml	.01 →					
✓ Thymus DNA 2.5 mg/ml	.02 →					
✓ $MgCl_2$ 0.1 M	.02 →					
✓ KPO_4 1M 7.4	.02 →					
✓ DEAE frac 4 9/17 refractionated	—	—	.04	.04	.04	.04
✓ H_2O	.23	.23	.19	.19	.19	.19
V_f	.30 →					
37°	30'	30'	30'	30'	60'	60'

Work up #1, 3, 5 as follows: chill, Add 0.20 ml Carrier DNA (2.5 mg/ml) then 2.0 ml Acid alcohol (2.5 ml 2M NaAc pH 5.0 → 50 c Abs. EtOH). 3' 0°, mix up. Spin. Dissolve ppte in 0.5 ml $\frac{M}{10}$ Tris pH 7.5 (ppte is very difficult to dissolve!). Add 2.0 ml acid-alcohol, mix 3' 0°. Spin. Decant supe, redissolve and reprecipitate. Try to dissolve ppte in 0.03 ml $\frac{M}{2}$ NaOH + 0.17 ml H_2O. It will not dissolve. Plate anyhow.

std = 790

Bkg = 23 cpm

	1	2	3	4	5	6
c/2 min	~~249~~ 3569	~~314~~ 279	2615	314	2909	681
c/m corr	1770	115	1293	132	1445	315
m μmoles inc	0.93	.09	0.69	0.10	0.76	0.24

Tubes 2, 4, 6 — At indicated time, add 0.40 ml H_2O (ice cold), 0.015 ml. $\frac{M}{2}$ HCl (this brings pH to ~6.8-7) and add 0.20 ml "Carrier" DNA. Mix thoroughly and add 0.10 ml Norit (20% P.V.). Mix 5' 0°. (Norit clumps somewhat). Spin. Plate 0.70 ml.

Sp Activity of DCTP[32]

Dilute 1:20 O.D. 280 (acid) = .340 = 6.8 at ml = 0.53 μ M/ml

1000 cpm × 50 × 20 = 1 × 10⁶ cpm/ml

9/24/57

Use of Dialysis To See if Oligonucleotides are Formed.

	1	2
✓ $DCTP^{32}$ .53 μM/ml	.02 →	
✓ Thymus DNA .5 mg/ml	.04 →	
✓ $MgCl_2$ 0.1 M	.04 →	
✓ M/1 KPO_4 7.4	.04 →	
✓ DEAE 9/5 fr 4 (~~Thymus DNA~~)	—	.05 (~~.10~~)
✓ H_2O	.41 (~~.36~~)	.41 (~~.36~~)
	.50	.60

1 hr 37°

Add 0.5 ml 4 M NaCl. Heat 60° 3 minutes (before heating #1 add .05 ml enzyme).

Std 740
Bkg = 20/min

Dialyze complete reaction mixtures against 6 liters of 0.2 M NaCl for 16 hrs.
Plate 0.8 ml. of bag contents.

c/2m	290	300
c/m corr	125	130

Conclusion – If ~~po~~ oligonucleotides are formed during the limit reaction, they are dialyzable under these conditions.

594
7
4158

10/1/57

Determination of Type of Acid-Soluble Products Formed in Limit Reaction:

	1	2
✓ DCTP 0.5 μm/ml (37 $\times 10^6$/μm)	.05	.01
✓ KPO_4 1M 7.4	.10	.02
✓ $MgCl_2$ 0.1M	.10	.02
✓ Thymus DNA .5 γ/ml	.10	.02
✓ DEAE	.10	—
✓ H_2O	1.05	.23
	1.50	.30 ← work up #2 in usual assay procedure (3 precipitations) (omit)

37° 30'

To # 1 add 1.0 ml Carrier DNA (2.5 mg/ml) + 0.25 ml 35% PCA. Mix. Add 1.0 ml H_2O. Mix. Spin. Decant and save supe (S_1). ~~Dissolve ppte in 0.3 ml of 0.1 N NaOH~~ Add 3.0 ml H_2O to ppte. Rub up. (pH < 5 BCG). Spin. Combine supe ē "S_1". Volume = 6.5 ml. Neutralize ē 10 N NaOH. Add 2 μMoles of DG 5P and 2 μ moles of DGTP. Volume = 7.5 ml.

Column — Dow 1-Cl (fast running) 7×1 cm. Put on 7.0 ml of above soln. at rate of 0.5 ml/min. (Although column was well packed before addition of solution, a large bubble formed about 1/3 way down disturbing even distribution of resin. The upper part of column was stirred ē a glass rod to redistribute the resin evenly. I hope it doesn't distort the chromatogram.)

Tube	Vol	E_{250}	E_{260}	E_{280}	ΣE_{260}	μM	Vol plated	cpm.	Σ cpm
Original	7.0						.01	554	4.2×10^5
PT + wash	18	.055	.035	.010			.10	10	
0.01 N HCl 1	10.0	.010	.010	.005			↓	0	
2	12.0	.020	.015	.007				10	1200
3	"		.007	.000				19	2280
4	"		.000	.000				23	2760
5	13	.030	.029	.020	.377			19	2470
6	13	.110	.105	.070	1.365			31	4020
7	13	.512	.495	.340	6.450	1.74 μM G5P		42	5460
8	13	.710	.700	.480	9.100			38	4940
9	13	.200	.193	.128	2.510			20	2600
10	13.5	.031	.027	.014	.310			9	1350
					20,152				