3-31-69 SV40 prep.

BSC-1 cells
SV40 str 777
grown by Eliahu in 1968 & stored frozen
ppt made CPE & scraping of cells fr. plates

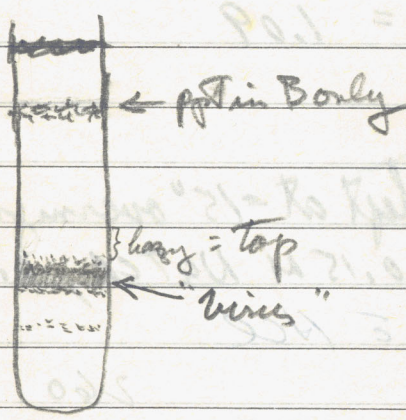
A. 800 ml - Cent at 30,000 rpm in #30 Spinco rotor - 2 hrs.

B. 800 ml treated in ice bath c 1/3 vol. of abs MeOH c stirring. Stirring continued for 45' Cent. at 8000 rpm in large Sorvall rotor for 30'. Super poured off & stirred overnight to check for more ppt.

Each ppt sonicated in few ml high speed super (A) or Tris .05 M pH 7.4 (B).
& incubated at 37° for 30' c 0.01% trypsin & 1% DOC.
Cent. at 10,000 g x 30' & super at 30,000 rpm for 3½ hrs.
Pellet left in CsCl 8 for 2 days at 4° & homogenized in CsCl ρ = 1.30 g/ml & cent in #40 at 40,000 rpm for 20 hrs. (Tris pH 8.5 0.01 M)

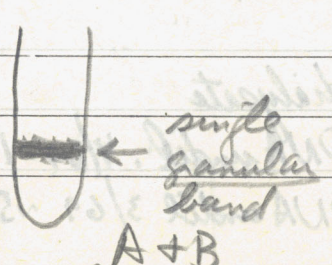
4-5-69

A. + B.



Each "virus" band collected in SW39 tube & made up to vol. c CsCl ρ = 1.31.
Top fr. A & B combined for 3rd tube

2nd spin:



Top → not collected, too little

A+B combined + dialyzed against .01 M Tris pH 7.9, EDTA .002 M.

4-8

DNA prep

1.5 ml virus sol'n (cloudy) + 1/10 0.15 ml NaTCA 1.5 M
+ 1/10 0.15 ml EDTA 0.1 M pH 8.4
{Then equal vol 90% phenol (phenol + 1/10 vol 0.5 M Tris pH 7.9)
To decrease conc. ionic (as per EW)
3.5 ml water cont. 1/10 vol EDTA + 1/10 vol NaTCA
+ 3.5 ml phenol ≅ Tris added.

Entire mix agitated for 15' at rm temp
Cent at 9000 rpm in Sorvall x 15'
~~Aqueous + 1 wash go dialyzed ag. 1/10 SSC~~
Too much ppt at interface. ∴ 8 ml more of aq. sol'n + 8 ml phenol-tris added + mixture re-agitated 10'. Cent in plastic tubes at 14000 rpm x 15'
Super + 1 wash dial ag 1/10 SSC x 3
Vol of DNA sol'n = 16 ml

	230	260	280 mμ
dialysate	.045	−.002	−.002
DNA undil	.640	.068	.043

O.D. units = .068 x 16 = 1.09

Yield very low!

Re-extract phenol residue (kept at −15° overnight) ≅ equal vol: 0.15 M NaCl, 0.15 M NaTCA, 0.025 M Tris 7.9 0.01 M EDTA, adj. to pH 7.9 ≅ HCl

		260	280		260	280
dialysate						
DNA undil. 4/69	15 ml	1.42	.94	1:10	.145	.110
DNA undil 3/69	~5.5 ml	.97	.65			