SV 40 prep.

BSC-1 or CV-1 or 1° Monkey kidney
(some claim higher yield ∈ 1°MK, but expensive)
BSC-1 develop resistance ∈ ↑ passage.
Earnest uses oldest stock, grows + freezes most of it.
From Sabin. Will test Flow Lab. line.
Can get ATCC line.
Passage at high cell dens. in desr. EM ∈ 10% calf ser.
— can use basal EM. (1 → 5 in passage)
3 stocks in culture + then go back to freezer

100 large plates needed ∈ 10^6 cells/plate
6 days growth or just ∫ confluence.
Remove medium + repl. ∈ fresh med. cont. 2% calf ser + stock virus (3×10^8/ml) dil 1:1000.
i.e. 3×10^5 dil in maint. med. i.e. moi = ~1/20
Can use moi 1/10,000 (Yoshiiki Kup. to use very low multipl. to avoid defectives)
Maintain cultures until full CPE (f. 7 d. — complete in ~10-12 d. dep on moi). Controls can do well in this med. for 3 days.

Freeze + thaw 3X - entire cells + med.
This is crude stock. Should be $1-5 \times 10^8$ pfu/ml
Store at −20° - quite stable.
Plaque assay BSC-1
V Ag assay - Ab fr. Flow Labs.

SV40 labile at 37° - ½ time of ~2 days. (PV ~7 days)

To conc. virus, pellet crude lysate 30,000 rpm
×2 hrs + resuspend.

DOC ½% final
Trypsin .01% final

750 ml crude lysate
35000 rpm 2½ hrs. - MSE
5 ml super left in each tube + pellet scraped into it (45 ml)
Sonicated 3 min. Overnite 4°
Homog by hand to break up clumps

45 ml
+2.25 ml 0.25% trypsin
+2.25 ml 10% DOC (final 0.5%)
37° - 30'

Sorvall 10,000 rpm - 30'
Super at 30,000 rpm 2½ hrs.
Drained + 5 ml CsCl pH 7.9 ρ = 1.304
into each of 2 tubes 3' cent to clarify
Homog by hand + centrifuge to 12 ml = CsCl soln
SW 39 32K 19½ hrs

Dialyz. 1 x SSC

Yield: 90 plates → 740 μg

Strain here is a) 777 - greater tend. to stick to cell debris
Others: b) 776 - virus release better
c) Koch's strain
d) small pl mutant - Takemoto at NIH