3-9-71 Transformation of Balb 3T3

Balb 3T3 in MEM 20-3A in 50 mm dishes - 9 dishes Plated on 3-6-71

SV40-4A stock (being titred) - assume 10 pfu/ml (7.5 x 10^7)

Remove med & rinse cells c̄ MEM s̄ FBS x2, Infect:

1.	1 dish	-	1.0 ml med	6	1 dish	1:100
2.	1 dish	-	1.0 ml undil SV40	7	1 "	1:100
3.	1 dish	-	" "	8	1 "	10^{-3}
4.	1 dish	-	" 1:10	9	1 "	10^{-4}
5.	1 dish	-	" 1:10			

Incubate 3 hrs., mixing Q ½ hr. at 37° in CO_2 inc.
Remove SV40, wash x2 c̄ MEM s̄ FBS & add 5 ml MEM 20 3A
Next day (15 hrs.) trypsinize c̄ 2 ml .1% trypsin & transfer to 4 new dishes ~~2 at 1:10 + 2 at 1:100 (0.5 + 0.05/5)~~ containing 5 ml pre warmed MEM 20-3A after 2 wks. pick the colonies.

Scored on 3/21 over

		Cell dil.	Dense Col. (unstained)			Dense Col.			
1-10	(A)	.5/5	0	5A	.5	~30	8A	.5	0
1-10	"	.5/5	0	5A	.2	~15	A	.2	0
1-100	(B)	.05/5	0	5B	.05	~?	B	.05	0
1-100	"	.05/5	0	5B	.02	~3	B	.02	0
2-A		.5/5	Confluent	6A	.5	1	9A	.5	0
2-A		.2/5	>110	A	.2	~0	A	.2	0
2-B		.05/5	?	6B	.05	0	B	.05	0
2-B		.02/5	~25	B	.02	0	B	.02	0
3 A		.5/5	~Confl, ~100	7A	.5	0			
3 B		.2	~20, ~10	A	.2	0			
4 A		.05	~40	7B	.05	0			
4 B		.02	~6	B	.02	0			

~~272/.1 cc mm~~ (2.7 × 10^6/ml)

On 3/20 many dense colonies on plates 2, 3, 4

As noted on other side. These col. were clearly piled up except occ. one ē rounder, epithelial-like cells.

Very striking that in dishes ē high moi & large no. cells inoculated, ep. 2 A's + 3 A's, but also to less extent in 4 A's + 5 A's, normal cells were floating in large clumps & very few normal cells were growing — almost entirely clones of tr. cells! In contrast in 1 & 7, 8, 9 many normal cells in colonies & in #1 at highest conc. cells inoc. there was nearly confluent monolayer.

Interesting that ē ↑ no. tr. cells on dish CPE for normal cells is ↑.

3/21 Picking tr. col. from 3/9 exper.

Transformed col. picked by using glass cylinder + 1 drop trypsin. Glass cyl. had silicone vac. grease. All selected col checked by micro. + were piled up, except 1 marked E wh. looked epithelioid. Trypsinization at rm. temp. × ~30' then tr. to petri dish c̄ 5 ml MEM 20-3A

2 large dishes used to "spot" cells in MEM 20,3A to get single cells. } no growth 3/27

3/27 Most dishes have good gr. of tr. cells c̄ typical piling up.
These were trypsinized + plated in small dishes c̄ ~~[illegible]~~ pieces of cover slips. 2 dishes c̄ diff. conc. cells (1/10, 1/1000) in MEM 20-3A. Cont SV40 antiserum 1/1000.

5/5 After carrying 2, 3, 4, 5, 6 (1 for each) for several passages, cells were diluted + plated at < 1 cell/well of microtiter plate. (~20 for each tr. line) — ā trypsin, inc 3 hrs c̄ 1/100 anti SV40 rabbit serum (titer = 1/160)

2	– no colonies	/20		
3	– 3 colonies	/20	– 2 picked	
4	– 0 "	/20		
5	– 3 "	/20	2 "	
6	– 2 "	/20	2 "	

#2 + #4 plated at <100 cells/petri dish. Single well isolated col. picked for cloning (these had been inc. ē anti-SV 40 AS as above)

10⁸/

Finally

cl 2B } from isol. col on dishes - both from same orig tr. colony
cl 2C }

cl 3A } from wells of microtiter plates — total of 1 clone/well
cl 3C }

cl 4B - from isol. col. on dish
4C - from well of microdish

cl 5A } from wells of microtiter
cl 5C }

cl 6A } "
cl 6C }

2 thru 6 - diff. letters designate different clones of same tr. colony.