V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (acute, cluster, suspension); **Exp. # :** 1;
Experiment performed by: A. Bishayee **Date:** 09/06/99

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB for each flusk, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~4,000,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 3,997,333 Cells/ml cells/ml)
3. Transfer 1 ml of cell suspension into ten 14 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 16 h at 37°C, 5% CO_2 **Date/Time:** 09/06/99; 4-00 P.m.
5. After ~16 h incubation period, remove tubes, add 8 ml wash MEMA, vortex and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:** 09/07/99; 10-00 a.m
6. Decant supernatant, click tubes, vortex, resuspend in 3 ml wash MEMA
7. Centrifuge tubes for 10 min at 2000 rpm, 4°C
8. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA,transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
9. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
10. Centrifuge tubes for 5 min at 1000 rpm, 4°C
11. Transfer tubes at 10°C for 72 h. **Date/Time:** 09/07/99 ; 12-00 noon
12. After 72 h, for tubes 1-5, carefully remove the supernatant, resuspend the pellet in 400 ml MEMA and place all tubes on the perforated plate of Rainin pipet tip box containing ice (to maintain ~ 10.5°C)

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), two tube (one tube for pellet and one for the suspension) at a time for a single dose-point, while placing onto a Rainin pipet tip box containing ice as per the Table below

Tube #	Total Dose (R)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	300	97.3	3.08	X-10
4	600	739.8	0.81	X-0
5	1200	739.8	1.62	X-0
6	0	0	0	0
7	0	0	0	0
8	300	97.3	3.08	X-10
9	600	739.8	0.81	X-0
10	1200	739.8	1.62	X-0

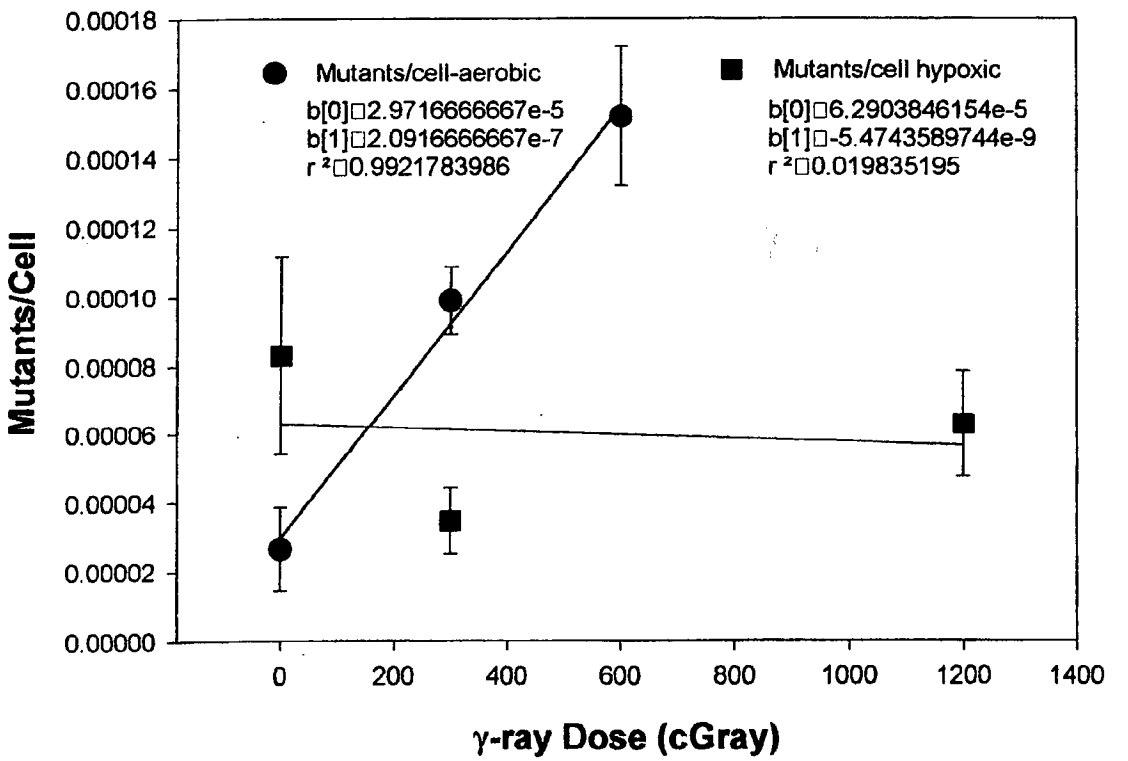
14. After irradiation, carefully remove the supernatant from the top for tubes 6-10, resuspend pellet in 200 ul wash MEMA and transfer the content from all tubes to ten 14 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
15. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 14 ml tubes
16. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
17. Labeling and preparation of dilution tubes and colony dishes
    - load 60 mm petri dishes with 4 ml MEMA
    - load T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
18. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
19. Centrifuge tubes for 10 min at 2000 rpm, 4°C
20. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle

21. Determine cell concentration by transfering 100 µl to Coulter cup
22. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
23. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
24. Incubate petridishes for 1 week
25. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.05% crystal violet
26. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

This Survival experiment was terminated due to contamination

However, the mutation assay survived.

V79 HPRT Mutants/Cell
Hypoxic versus Aeobic Clusters



9/28/99

18 P60 / 200 cm

120 ~~50~~

200,000 cells P100

120,000 60

$\frac{60^{W}}{120,000} =$

09/06/99

Initial cell count = 1496, 1523, ~~1436~~, 1508
Avg cell count = 1509
Cell conc. = 6,036,000 cells/ml.

Vol. required = $\frac{44,000,000}{6,036,000}$ = 7.28

≈ 7.3 ml

Take 7.3 ml cells + 3.7 ml MEMB = 11 ml

Final count = 990, 999, 1009
Avg. count = 999
Cell conc. = 3,997,333 cells/ml

09/1d/99

1.	643,	666,	652
2.	601,	636,	645
3	622,	649,	619
4	645,	662,	639
5	685,	672,	679
6	561,	586,	592
7	595,	611,	622
8	695,	707,	687
9.	669,	639,	652
10	631,	663,	622

Some

0.39 ml (10^5) cell was plated for mutation assay

2-
5-2- 1

13. The tubes were irradiated using Mark I irradiator (^{137}Cs gamma-ray), two tube (one tube for pellet and one for the suspension) at a time for a single dose-point, while placing onto a ~~separate~~ Rainin pipet tip box containing ice as per the Table below

Tube #	Total Dose (R)	Dose rate (Rad/min)	Time (min)	Attenuat.
1	0	0	0	0
2	0	0	0	0
3	300	~~101.4~~ 99.7	~~2.95~~ 3.08	X-10
4	600	~~101.4~~ 199.9	~~5.9~~ 6.08	X-10
5	1200	739.8	1.62	X-0
6	0	0	0	0
7	0	0	0	0
8	300	~~101.4~~ 99.7	~~2.95~~ 3.08	X-10
	600	~~101.4~~ 199.8	~~5.9~~ 6.08	X-10
			1.62	X-0

natant fro 10 suspend

V79 Mutagenesis

Date **Day**

0 Plate 10^6 cells/P100 from each experimental condition (=10 P100's) in 10 ml of MEM10.
Count and replate each P100 on days 2,4,6 or 2,5 or 3,5 @ 10^6 cells/P100 in 10 ml MEM10.

8 Count and replate each dish @ 2 x 10^5 cells/P100 x 5 in MEM10 + 10 μM sGua and @ 65 cells/P35 x 3 in MEM10.
200 60 5

15 Fix and stain dishes. Count mutant colonies on the P100's and survivors on the P35's.
60

4 million/ 2ml → 0.5ml for dilution for colony assay
3 million/ 1.5 ml → 0.5 ml for mutagenesis assay

8.68 ml + 511 ml MEM → store

200000