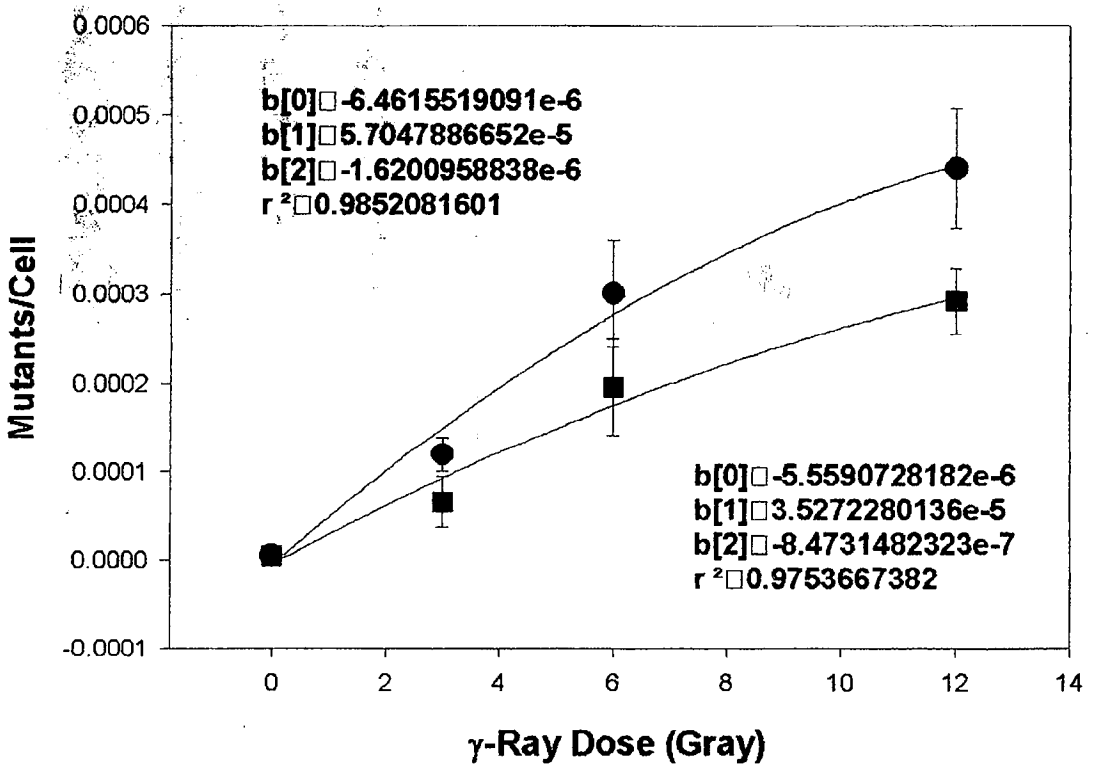
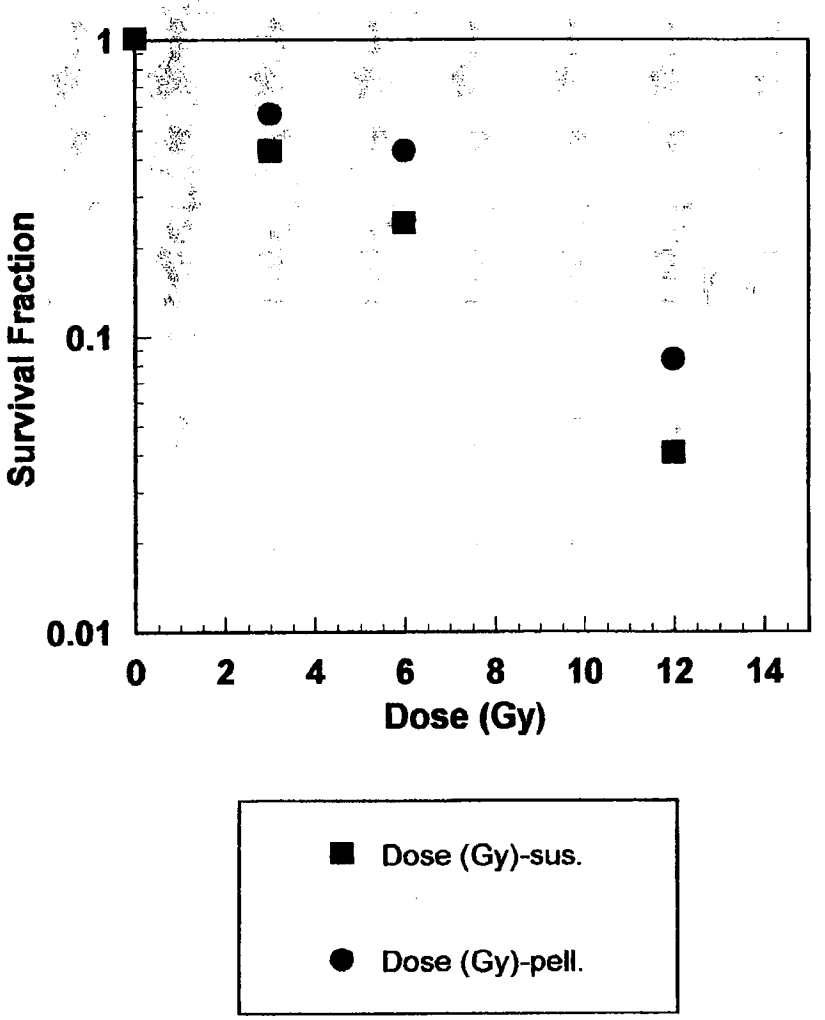
V79 HPRT Mutants/Cell
Hypoxic versus Aerobic Clusters




Survival Fraction
1
0.1
0.01
0
2
4
6
8
10
12
14
Dose (Gy)
Dose (Gy)-sus.
Dose (Gy)-pell.

↘	Label	A	B	C	D
Label		Dose (Gy)-sus.	SF	Dose (Gy)-pell.	SF
1		0	1	0	1
2		3	0.42	3	0.56
3		6	0.24	6	0.42
4		12	0.04	12	0.083

Effect of hypoxia on cluster against external γ-ray

V79 COLONY FORMING ASSAY

Experiment Name : ^{137}Cs toxicity (acute, cluster, suspension); **Exp. # :** 2;
Experiment performed by: A. Bishayee **Date:** 09/20/99

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 : 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:** 09/20/99 ; 6-00 pm
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:** 09/21/99; 9-30 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:** 09/21/99; 11-00 a.m.
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

Date/Time: 09/24/99; 11:00 a.m.

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	~~387~~ 239.8	~~0.81~~ 1.55	X-0
5	1200	~~387~~ 239.8	~~1.62~~ 3.09	X-0
6	0	0	0	0
7	0	0	0	0
8	300	97.3	3.08	X-10
9	600	~~387~~ 239.8	~~0.81~~ 1.55	X-0
10	1200	~~387~~ 239.8	~~1.62~~ 3.09	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.

V79 mutation Assay

Date	Day	
09/24/99	0	Plate 10^6 cells from each tube in P100 & 10 ml MEM10
09/27/99	3	Plate 10^6 cells from each plate in P100 & 10 ml MEM10
10/01/99	7	Plate 10^6 cells from each plate in P100 & 10 MEM10
10/04/99	10	i) count and plate 200,000 cells from each p100 to another P100 x 5 & 10 ml MEM5 & 10 µM serum
		ii) Plate 200 cells in P60 x 3 in MEM5
10/11/99	17	Colony count

10/01/99

Cells suspended in 6ml MEM-A

1.	772,	761,	756
2	666,	655,	677
3	701,	711,	722
4	656,	631,	634
5	732,	745,	739
6	741,	756,	762
7	635,	659,	662
8	672,	657,	659
9	713,	732,	742
10	699,	710,	729

10/04/99

Cells suspended in 6 ml of MEM-A

1.	499,	488,	502
2	436,	456,	462
3	522,	532,	542
4	536,	542,	539
5	561,	572,	585
6	437,	452,	462
7	501,	533,	529
8	490,	471,	478
9	522,	535,	542
10	531,	555,	563

600/.05 = 12,000/ml
200
2400000
4.8×10^6

Cells suspended in 2ml MEMA, 09/24/99
For counting, take 100µl in 20ml

1.	589,	598,	571 in 50µl
2	611,	627,	631
3	541,	559,	561
4	629,	642,	629
5	667,	656,	672
6	542,	561,	559
7	620,	635,	642
8	529,	549,	557
9	607,	598,	622
10	511,	509,	507

Cells suspended in 6ml MEMA 09/24/99

1	411,	431,	435
2	471,	461,	459
3	389,	362,	372
4	332,	321,	341
5	441,	456,	465
6	432,	444,	456
7	409,	422,	436
8	381,	392,	401
9	356,	365,	369
10	403,	372,	385

Plating Efficiency

10/11/99

200 cells were plated for each tube

Tube #	# of colonies	Avg # of colonies
1	150, 166, 149	147.16
2	137, 129, 152	
3	121, 137, 145	134.33
4	152, 130, 119	133.66
5	117, 125, 139	127
6	165, 147, 155	151
7	141, 159, 139	
8	129, 125, 138	130.6
9	147, 152, 118	139.0
10	167, 145, 149	153.6

Mutant colonies

200,000 cells were plated for each tube

Tube #	# of colonies	Avg. # of colonies	Mutants/cell
1	1, 2, 1, 1, 1	0.8	0.00000544
2	0, 1, 0, 1, 0		
3	12, 17, 19, 15, 17	16	0.000119
4	38, 45, 29, 50, 39	40.2	0.000300
5	60, 62, 59, 41, 58	54	0.000425
6	2, 0, 0, 1, 0	0.7	0.0000046
7	2, 1, 1, 0, 0		
8	11, 6, 5, 7, 14	8.6	0.000065
9	29, 31, 19, 37, 20	27.2	0.000195
10	36, 39, 47, 40, 48	42	0.000273

TABLE-4

Expt # : 2 **Date :** 10/01/99

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1.2	140	166	152	149.8	
2.2	135	149	157		
3.2	52	63	73	62.6	0.4183
4.2	36	41	31	36	0.2403
5.3	53	60	68	6.03	0.0402
6.2	121	115	139	134.6	
7.2	137	152	144		
8.2	69	75	82	75.33	0.5594
9.2	47	56	66	56.33	0.4183
10.3	100	114	124	11.06	0.0822