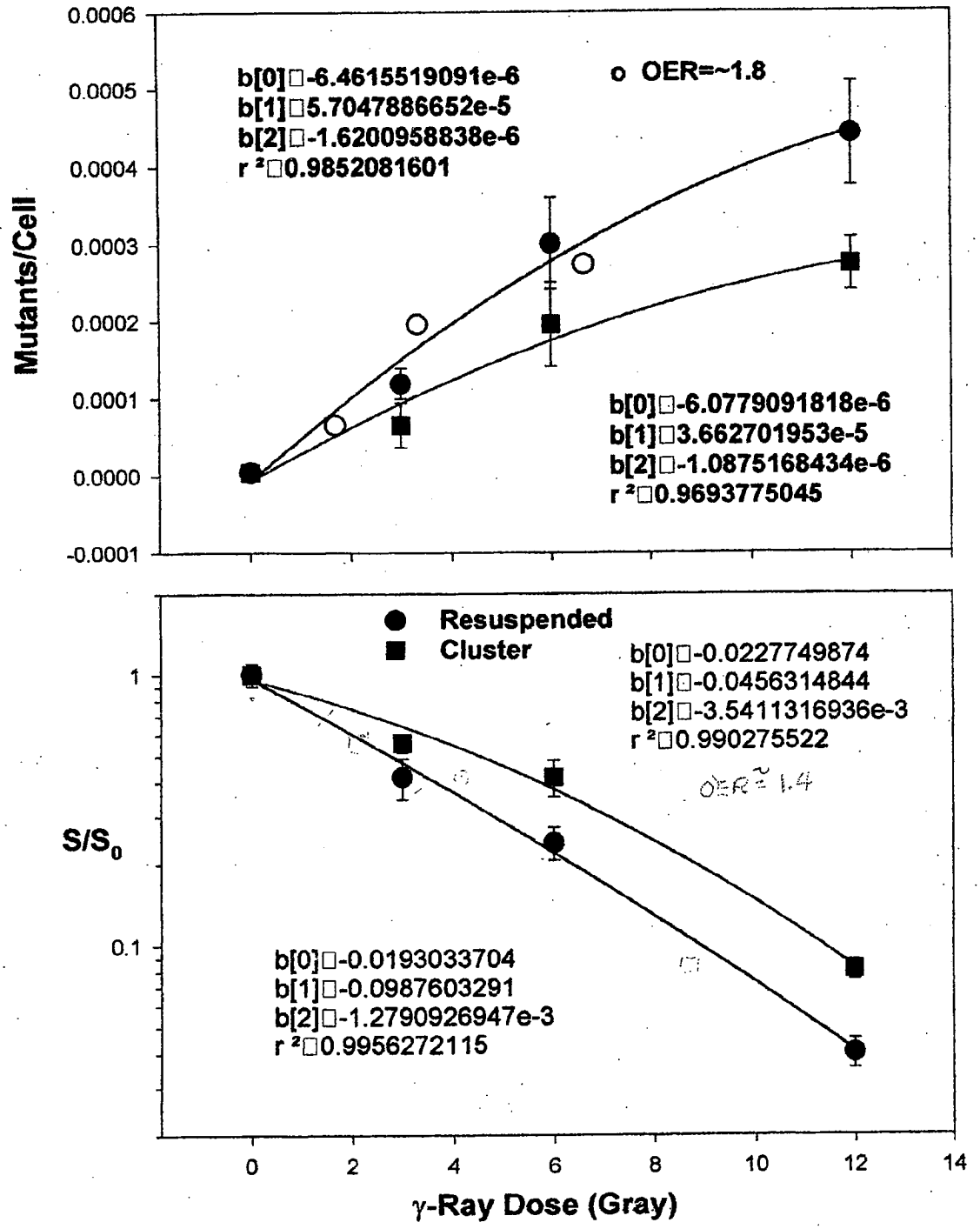
V79 HPRT Mutants/Cell
Hypoxic versus Aerobic Clusters



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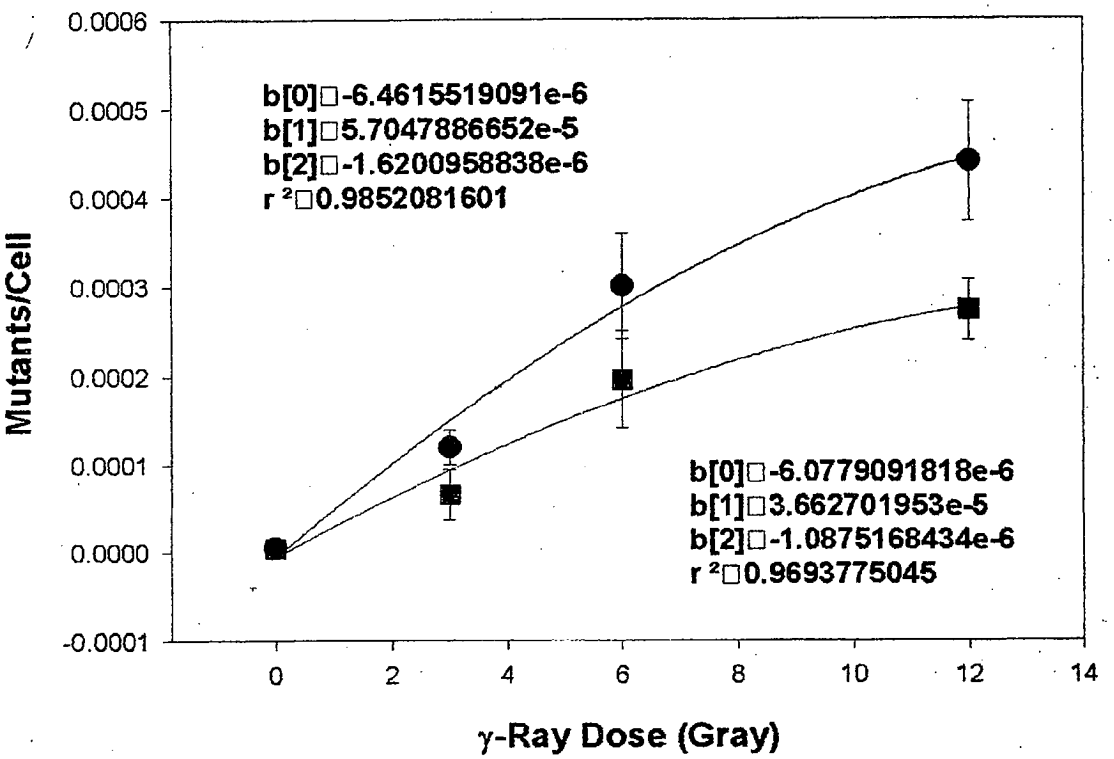

	colonies			av col	std col	mutants					av muts	std mut	mut/cell	std mut/cell	Survival			PE	S/S0	StdS/S0
1	150	166	149	155	9.53939	1	2	1	1	1	0.8	0.632456	5.16129E-06	4.08036E-06	140	166	152	0.7	1	0.0752
2	137	129	152	139.33	11.6762	0	1	0	1	0					135	149	157			
3	121	137	145	134.33	12.2202	12	17	19	15	17	16	2.645751	0.000119107	1.96954E-05	52	63	73	0.3	0.4182	0.0701
4	152	130	119	133.67	16.8028	38	45	29	50	39	40.2	7.918333	0.000300748	5.92394E-05	36	41	31	0.2	0.2403	0.03337
5	117	125	139	127	11.1355	60	62	59	41	58	56	8.514693	0.000440945	6.70448E-05	5.3	6	6.8	0	0.0403	0.00501
6	165	147	155	155.67	9.0185	2	0	0	1	0	0.7	0.823273	4.49679E-06	5.28869E-06	121	115	139	0.7	1	0.09369
7	141	159	139	146.33	11.0151	2	1	1	0	0					137	152	144			
8	129	125	138	130.67	6.65833	11	6	5	7	14	8.6	3.781534	6.58163E-05	2.89403E-05	69	75	82	0.4	0.5594	0.04342
9	147	152	118	139	16.3578	29	31	19	37	20	27.2	7.626892	0.000195683	5.48841E-05	47	56	66	0.3	0.4183	0.06343
10	167	145	149	153.67	11.7189	36	39	47	40	48	42	5.244044	0.000273319	3.41261E-05	10	11	12	0.1	0.0817	0.00887

Date	Cell Doublings Counts			Vol(ml)					Doublings	Sample	Total Doublings
9/24/99	0 589	598	571	2	4688000	483.5	1.0			1	11.12182
	0 611	627	631		4984000		0.91				
	3 541	559	561		4429333		1.04				
	6 629	642	629		5066667		1.100				
	7 667	656	672		5320000		0.92				
	9 542	561	559		4432000	474.5	1.02				
	9 620	635	642		5058667		0.86				
	12 529	549	557		4360000						
	12 607	598	622		4872000						
	12 511	509	507		4072000						
9/29/99	411	431	435	6	1E+07	3.35276	1.05	1.00		1	11.12182
	471	461	459		1.1E+07	3.47612	0.90	0.86		2	10.91181
	389	362	372		8984000	3.16736	0.80	0.76		4	10.62649
	332	321	341		7952000	2.99132	1.10	1.05		6	11.37441
	441	456	465		1.1E+07	3.44573	1.05	1.00		6	11.02331
	432	444	456		1.1E+07	3.41359				7	10.95364
	409	422	436		1E+07	3.34142	0.95	0.89		8	10.74782
	381	392	401		9392000	3.23143	0.87	0.83		9	10.93045
	356	365	369		8720000	3.12433	0.93	0.89		10	11.03198
	403	372	385		9280000	3.21412					
10/1/99	772	761	756	6	1.8E+07	4.19472					
	666	655	677		1.6E+07	3.99856					
	701	711	722		1.7E+07	4.09356					
	656	631	634		1.5E+07	3.94186					
	732	745	739		1.8E+07	4.14796					
	741	756	762		1.8E+07	4.17568					
	635	659	662		1.6E+07	3.96791					
	672	657	659		1.6E+07	3.99132					
	713	732	742		1.7E+07	4.12695					
	699	710	729		1.7E+07	4.09626					
10/4/99	499	488	502	6	1.2E+07	3.57434					
	436	456	462		1.1E+07	3.43723					
	522	532	542		1.3E+07	3.67448					
	536	542	539		1.3E+07	3.69332					
	561	572	585		1.4E+07	3.78073					
	437	452	462		1.1E+07	3.43403					
	501	533	529		1.3E+07	3.64432					
	490	471	478		1.2E+07	3.52507					
	522	535	542		1.3E+07	3.67717					
	531	555	563		1.3E+07	3.72159					

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;

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	~~239.8~~	~~0.81~~ 155	X-0
5	1200	~~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	~~239.8~~	~~0.81~~ 1.55	X-0
10	1200	~~239.8~~	~~1.62~~ 3.09	X-0

aerobic

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

1. Determine cell concentration by transfering 100 µl to Coulter cup
2. 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.
3. 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.
4. Incubate petridishes for 1 week
5. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.05% crystal violet
6. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

mutation Assay

ate	Day	
24/99	0	Plate 10^6 cells from each tube in P100 & 10 ml MEM10
27/99	3	Plate 10^6 cells from each plate in P100 & 10ml MEM10
31/99	7	Plate 10^6 cells from each plate in P100 & 10 MEM10
4/99	10	4) count and plate 200,000 cells from each p100 to another P100 X5 & 10 ml MEM5 & 10 µM sGma
		11) Plate 200 cells in ~~(illegible)~~ P60 X3 in MEM5
99	17	Colony count

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

09/24/99

Cells suspended in 2ml MEMA,
dan: For countup, take 100µl in 20ml

589, 598, 571 in 50µl
601, 627, 631
541, 559, 561
629, 642, 629
667, 656, 672
542, 561, 559
620, 635, 642
529, 549, 557
607, 598, 622
511, 509, 507

Cells suspened in 6ml MEMA 09/27/99

0	411,	431,	435	426± 12.9	426/425 = 1.00
0	471,	461,	459	463± 6.4	
3	389,	362,	372	374± 13.7	0.84
6	332,	321,	341	331 ± 10.0	0.74
12	441,	456,	465	454 ± 12.1	1.02
0	432,	444,	456	444 ± 12.0	433 = 1.00
0	409,	422,	436	422 ± 13.5	
3	381,	392,	401	391 ± 10.0	0.90
6	356,	365,	369	363 ± 6.66	0.84
12	403,	372,	385	387 ± 15.0	0.89

10/01/99

Cells suspended in 6ml MEM-A

772, 761, 756
666, 655, 677
701, 711, 722
656, 631, 634
732, 745, 739
741, 756, 762
635, 659, 662
672, 657, 659
713, 732, 742
699, 710, 729

10/04/99

Cells suspended in 6 ml of MEM-A

499, 488, 502
436, 456, 462
522, 532, 542
536, 542, 539
561, 572, 585
437, 452, 462
501, 533, 529
490, 471, 478
522, 535, 542
531, 555, 563

Plating Efficiency

200 cells were plated for each tube

10/11/99

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, 129	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