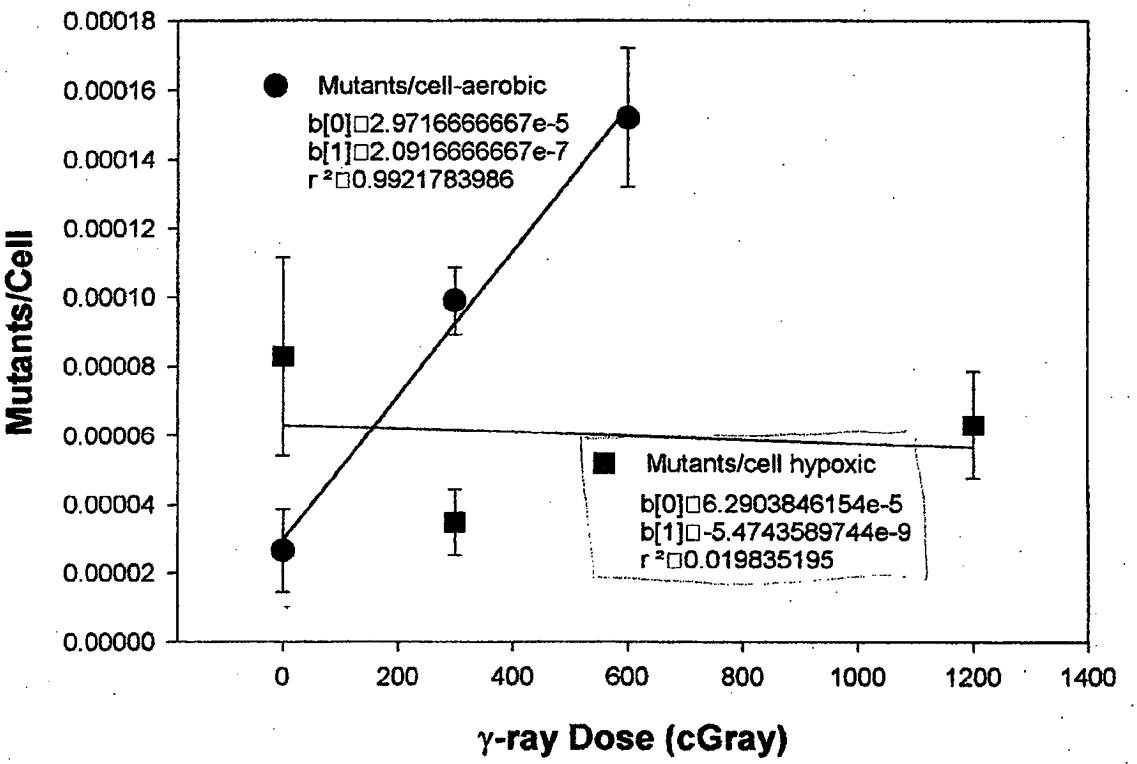
V79 HPRT Mutants/Cell
Hypoxic versus Aeobic Clusters



9/28/99

B020145

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
9/10 12. After 72 h, for tubes 1-5, carefully remove the supernatant, resuspend the pellet in 400 ~~ml~~ µl 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

Resus/p

left in pellet

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.

ZM 3616
U.S.A.

9/10/99

	Dox(Gy)		9/13/99 d3 hemo 70x5=	0.5ml Coulter		Av	cells/ml x400	10^6	Rel incr.	x4 Total/P100	#doublings
Resusp.	0	1	350	8282 8815 5987 8221		7701	3.08×10^6/ml →	0.32	1.00	1.2×10^7	3.6
	0	2		7355 7404		7380	2.95	0.34		1.2	3.6
	3	3		7962 7852		7907	3.16	0.32	1.08	1.3	3.6
	6	4		5530 5520		5525	2.21	0.45	0.73	0.88	3.1
~~12~~	~~5~~			5541 5494		5518	2.21	0.45	0.73	0.88	3.1
~~No loss~~	0	6		8409 8765		8287	3.31	0.30	1.00	1.3	3.6
	0	7		8760 8755		8758	3.50	0.29		1.4	3.8
	3	8		7748 (5295)	7689 7726	7139	2.86	0.35	0.81	1.1	3.5
	~~6~~	~~9~~		5731 5987	5871 6426	6004	2.40	0.42	0.71	0.96	3.3
	12	10	60x5= 825	6484 6240	6474 6203	5850	2.34	0.43	0.70	0.94	3.2
	Bk			3539 2273							

9/15 #s 1 & 9 are contaminated. (3)

9/16 #s 5 & 6 "

9/17 switched to Gibco MEM + 5% FBS (Gen 1567A)

											Total do
Coulter 9/17 d7	2	3327 5110	4678 3497	6607	4644 ± 1334	1.86×10^6/ml →	0.54		7.4×10^6	2.9	6.5
	3	8254 4838	8354 4756	4875 3112	5698 ± 2125	2.28	0.44		9.12	3.2	6.8
	4	7797 4053	6087 4060	5858 7204	5843 ± 1556	2.34	0.43		9.36	3.2	6.3
	7	8752 5577	4007 5604	4013 5640	4748 ± 909	1.90	0.53		7.6	2.9	6.7
	8	8598 4435	4525 6666	4683 6113	5840 ± 1633	2.34	0.43		9.36	3.2	6.7
	10	9784 4144	6314 8415	4755 6735	6358 ± 1968	2.54	0.39		10.16	3.3	6.5
	Bk	11 3 11 6									

9/20 d10 Harvest, dilute $\frac{1}{10}$ ($\frac{0.1}{1.0ml}$ isoton x 4ml) count by hemocytometer

					#doublings	Total doublings
	2	221/4 235/4 (2d)	5.7×10^6/ml	2.3×10^7 cells/P100	4.5	11
	3	139/4 144/4	3.5×10^6	1.4×10^7 "	3.8	10.6
	4	121/4 109/4	2.9×10^6	1.2×10^7 "	3.6	9.9
	7	179/4 177/4	4.5×10^6	1.8×10^7 "	4.2	10.9
175/4 196/4	8	214/4 261/4	4.6×10^6	1.8×10^7 "	4.2	10.9
	10	175/4 181/4	4.5×10^6	1.8×10^7 "	4.2	10.9

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J. S. A.

9/20/99
d10

2	5.7	$\times 10^6$	0.035 →	2×10^5 /200 × 5			
3	3.5		0.057 →				
4	2.9		0.069 →				
7	4.5		0.044 → 0.69				
8	4.6		0.043 →				
10	4.5		0.044 →				
2	5.7	$\times 10^6$	0.0088 / 1 →	5×10^4	0.0046 / 7 →	33	2ml → 66 | P35 ×3
3	3.5	$\times 10^6$	0.014 / 1 →		0.0046 / 7 →	33	
4	2.9	$\times 10^6$	0.017 / 1 →		→		
7	4.5	$\times 10^6$	0.011 / 1 →		→		
8	4.6	$\times 10^6$	0.0100 / 1 →		→		
10	4.5	$\times 10^6$	0.011 / 1 →		→		

GTG 2.5mg FW 167.2 Add 25ml MEMS to vial = 100µg/ml

100µg/ml → 5.4ml / 329ml MEMS → 167µg/ml = 10µM

B020150

RM 3616
U. S. A.

Plate counts 9/27/99 = day 17

Sample	P35's			Av	std	P100's					Av	std	parcell/pl
2	80	74	70	74.7	5.03	3	4	7	10	6	6.0	2.74	2.65E-5 ±
3	65	53	64	60.7	6.66	17	18	20	16	20	18.2	1.79	9.89E-5 ±
4	64	56	65	61.7	4.93	28	35	25	27	27	28.4	3.85	15.2E-5
7	64	67	53	61.3	7.37	11	16	24	15	11	15.4	5.34	8.29E-5
8	74	86	69	76.3	8.74	5	9	8	7	11	8.0	2.24	3.46E-5
10	65	65	65	65.0	0	10	11	10	14	17	12.4	3.05	6.30E-5
		overall	av	66.61	8.40								