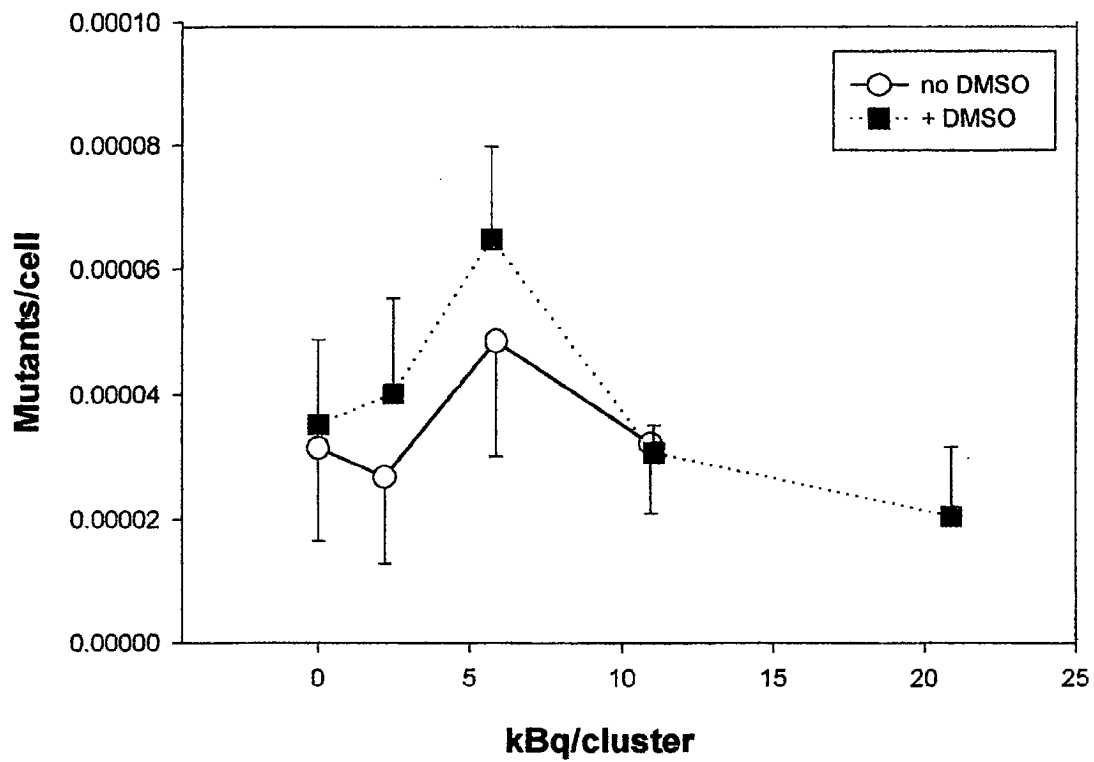
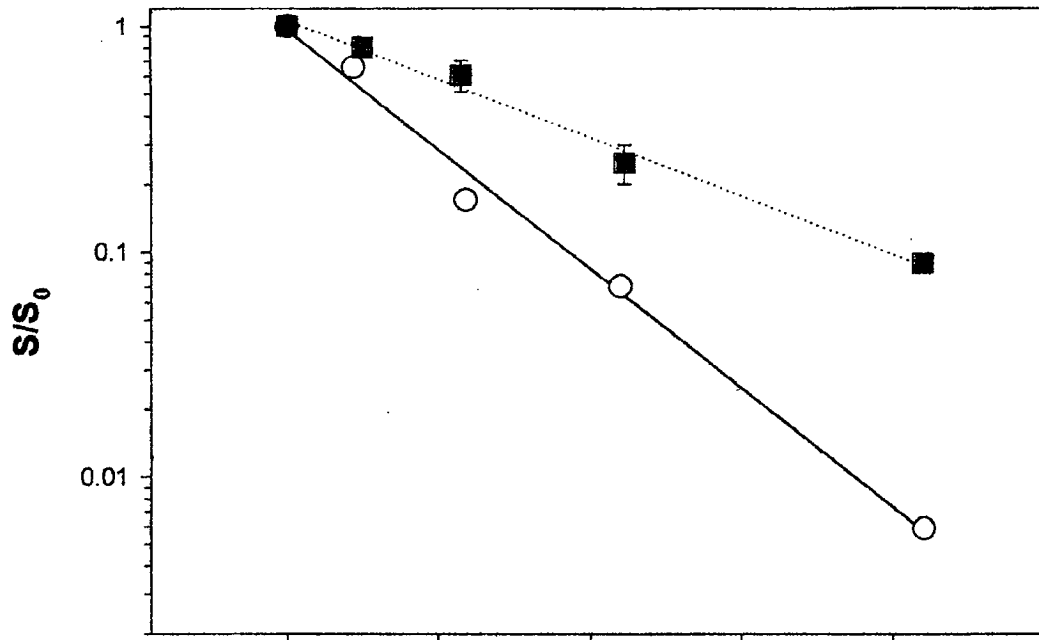


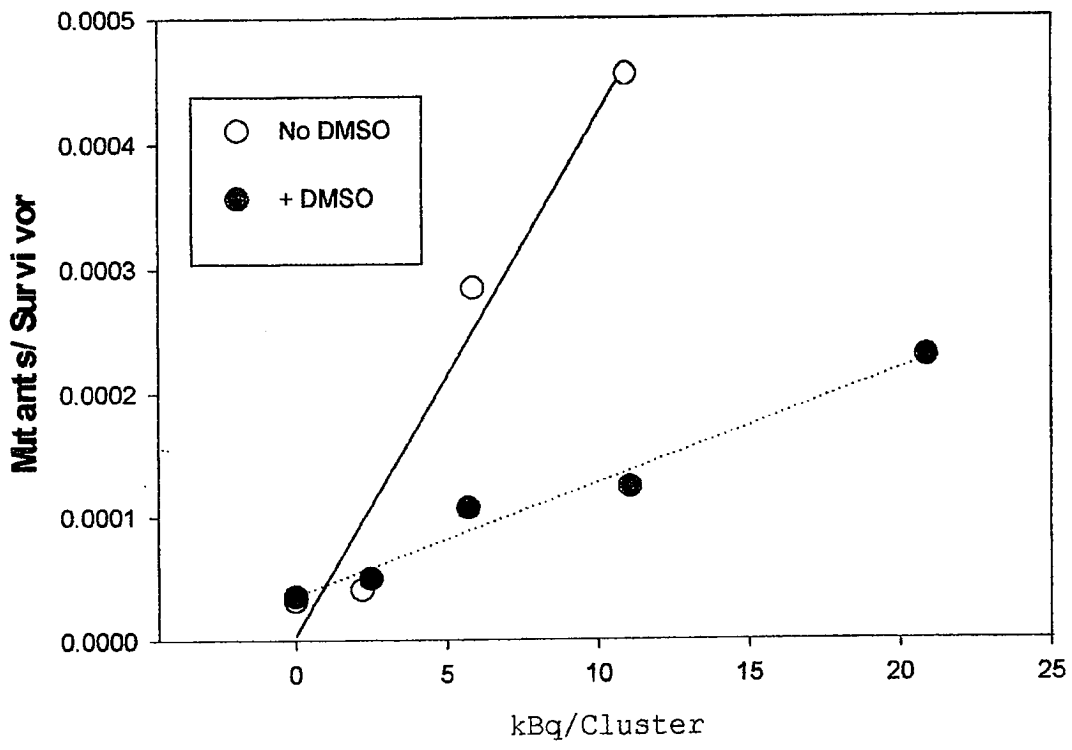
# <sup>3</sup>HdThd Incorp into V79 Clusters: Survival and Mutation with and without DMSO



2/03/00

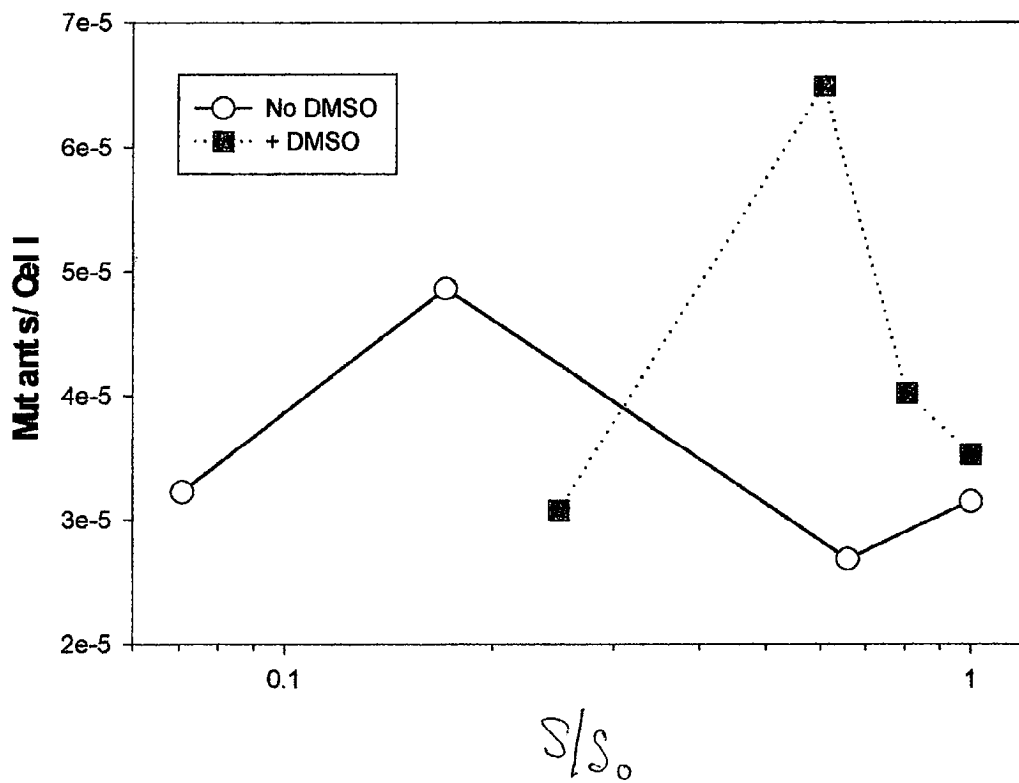
# <sup>3</sup>HdThd Incorp into V79 Clusters: Survival and Mutation with and without DMSO

## <sup>3</sup>HdThd Incorp into V79 Clusters: Survival and Mutation with and without DMSO



2/03/00

2D Graph 4



V79 COLONY FORMING ASSAY, Mutation, Comet an

Experiment Name : <sup>3</sup>HTdR + 0 or 10% DMSO (cluster, 100% labeling); Exp.# {

Experiment performed by : A. Bishayee

Date: 02/03/00

1. Set the rocker-roller at 37°C incubator with 5% CO<sub>2</sub>, set the Coulter Counter, wash cells (from two 150 cm<sup>2</sup> flasks, subcultured 1:2, 24h before) with PBS, trypsinize cells, each resuspend in 9 ml MEMB, pool, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)

2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 3,976,000 cells/ml]

3. Transfer 1 ml of cell suspension into 10 14-ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall

4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO<sub>2</sub>

Date/Time: 02/03/00  
2-30 PM

5. Prepare MEMB containing radioactivity in hood  
40 µl <sup>3</sup>HTdR (Stock : 1 µCi/µl on 01/31/00) + 4.6 ml MEMB

6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below.

Date/Time: 02/03/00; 7-0

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6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below.

Tube #	<sup>3</sup> HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>3</sup> HTdR [8uCi/ml] (ml)
1	0	1.0	1.0	0
2	0.5	1.0	0.875	0.125
3	1	1.0	0.75	0.25
4	2	1.0	0.5	0.5
5	4	1.0	0	1
6	0	1.0	1.0	0
7	0.5	1.0	0.875	0.125
8	1	1.0	0.75	0.25
9	2	1.0	0.5	0.5
10	4	1.0	0	1

A. 96  
MEMB = 4.5 ml  
3H = 0.04 ml  

---

5.00 ml

7. Return test tubes to roller for 12 h. Date/Time: 02/03/00, 7-30 pm.
8. Next day, while test tubes are in roller label 10 gamma-tubes (12 X 75 mm VWR glass test tube)
9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (*precooled centrifuge*). Date/Time: 02/04/00, 9-30 a.m.
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant from each tube and place in prelabeled gamma-tube.
11. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
12. Centrifuge tubes for 10 min at 2000 rpm, 4°C
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 8 ml of MEMA with (Tube# 6-10) or without (Tube# 1-5) 10% DMSO
18. Centrifuge tubes for 5 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips = 200 X 2

Tube #	<sup>3</sup> HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ <sup>3</sup> HTdR [8uCi/ml] (ml)
1	0	1.0	1.0	0
2	0.5	1.0	0.875	0.125
3	1	1.0	0.75	0.25
4	2	1.0	0.5	0.5
5	4	1.0	0	1
6	0	1.0	1.0	0
7	0.5	1.0	0.875	0.125
8	1	1.0	0.75	0.25
9	2	1.0	0.5	0.5
10	4	1.0	0	1

20. Again add 200 ul ice cold MEMA with or without 10% DMSO, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time: 02/04/00; 11-00 a.m.**
23. Transfer 30 ul supernatant from from 150 ul supernatant removed earlier (Step 10) in triplicate in 6 ml scintillation vials containing 6 ml liquid scintillation cocktail (Ecolume, ICN) and count them for radioactivity **Date/Time:**
24. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the contents by using pasteur pipets to 10 corresponding 14-ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA in each **Date/Time: 02/07/00; 10-00 a.m.**
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in corresponding 14 ml tubes
26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C (precooled centrifuge)
27. Labeling and preparation of dilution tubes and colony dishes
  - load 60 mm tissue culture dishes with 4 ml MEMA
  - load 40 sterile tubes with 4.5 ml wash MEMA in each 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.
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C
32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by transferring 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex.
35. 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.
36. Transfer 200 µl of cell suspension (in triplicate) to 6 ml scintillation vial containing 6 ml cocktail.
37. Incubate petridishes for 1 week
38. Count vials for radioactivity **Date/Time : 02/07/00; 5-30 pm.**
39. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.5% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

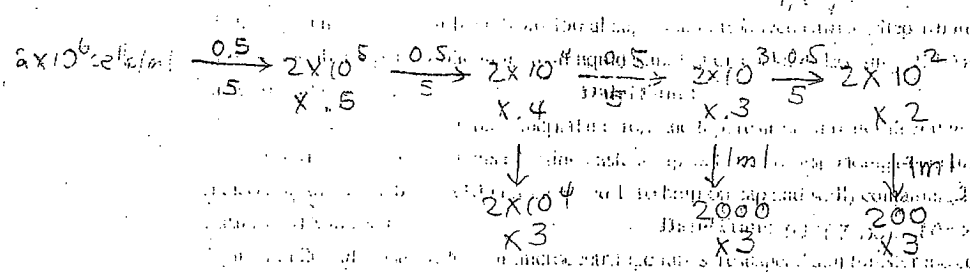


TABLE-3

Expt. # : (

Date/Time : 02/07/00

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 4000]	pCi/cell [ $\mu\text{Ci/ml} \times 10^6$ Cells/ml]
1			3,344,000	
2			2,894,666	0.0148
3			2,938,666	0.0397
4			3,128,000	0.0790
5			2,897,333	0.1419
6			3,332,000	
7			3,394,666	0.0168
8			2,929,333	0.0386
9			2,960,000	0.0748
10			2,793,833	0.1413

*KBq/  
cluster*  
[*pCi/cell*  
*x*  
*1.48*]

2.20  
5.88  
10.96  
20.99  
2.49  
5.72  
11.07  
20.91

*100  
ul  
MSO*

*0.1  
DMSO*

TABLE-4

Expt #: 1

Date: 02/14/00

0%  
DMSO

10%  
DMSO

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1-2	157	139	142	146	
2-2	88	96	105	98.33	0.6598
3-2	23	25	27	25	0.1712
4-3	95	103	112	103.33	0.0708
5-4	83	92	87	87.33	0.0059
6-2	132	141	129	134	
7-2	99	108	118	108.33	0.8084
8-2	71	78	96	81.66	0.6094
9-2	27	33	40	33.33	0.2487
10-3	130	120	109	119.66	0.0893

PE  
0.7

0.67

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1-2	157	139	142	146	
2-2	88	96	105	98.33	0.6598
3-2	23	25	27	25	0.1712
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6-2	132	141	129	134	
7-2	99	108	118	108.33	0.8084
8-2	71	78	96	81.66	0.6094
9-2	27	33	40	33.33	0.2487
10-3	130	120	109	119.66	0.0893



$1 \text{ kBq/cell} = 10^{-6} \text{ kBq/cell} = 1 \text{ mBq/cell} = 10 \text{ dpm} \times 3600 \text{ sec/hr} = 3.24 \text{ decays/cell}$   
 $1 \text{ kBq/cell} = 3.24 \text{ decays/cell}$

cellStr	pClCell	kBq/culture	std	P35's	Average	SD	P100's	Average	SD	Mut10e5	SD				
3.24E+06	0	0	1	0.036052	53	50	55	54.33333	4.041452	3	5	5.8	2.774887	3.15133E-05	1.31E-05
2.89E+06	0.0148	2.2	0.66	0.058269	59	55	61	58.33333	3.05505	8	3	4.6	2.408319	2.68333E-05	1.4E-05
2.94E+06	0.0397	5.88	0.171	0.01368	48	58	46	50.66667	6.428101	5	12	9.6	3.646917	0.00004864	1.85E-05
3.13E+06	0.074	10.96	0.0708	0.005827	42	39	37	39.33333	2.516611	6	5	8.2	2.863564	3.22533E-05	1.13E-05
2.90E+06	0.1419	20.99	0.0059	0.000305											
3.33E+06	0	0	1	0.046604	49	54	44	44	49	6	7	7.2	2.774887	0.00003528	1.36E-05
3.39E+06	0.0168	2.49	0.808	0.070888	45	45	44	44.66667	0.57735	7	5	9	3.391185	0.00000402	1.51E-05
2.83E+06	0.0366	5.72	0.609	0.096175	60	58	59	59	59	13	14	11	2.54851	0.00000949	1.5E-05
2.86E+06	0.0748	11.07	0.249	0.048603	52	53	54	54	53	5	6	5.8	0.83666	0.00003074	4.43E-06
2.79E+06	0.1413	20.91	0.0893	0.007838	41	38	44	44	41	3	5	5	2.738613	0.0000205	1.12E-05

mutsurvivor  
 3.15133E-05  
 4.06566E-05  
 0.000284444  
 0.000455556  
 0.00003528  
 4.97525E-05  
 0.000106568  
 0.000123454  
 0.000229563

Sample	Colonies	Average	SD
1	157	142	146
2	88	96	96.33333
3	23	25	25
4	95	103	103.3333
5	83	87	87.33333
6	132	129	134
7	99	108	108.3333
8	71	96	81.66667
9	27	40	33.33333
10	130	109	119.6667

No DMSO  
 s/s<sub>0</sub> mut (x10<sup>5</sup>)  
 1.0 3.15  
 0.66 2.69  
 0.17 4.86  
 0.071 3.23  
 + DMSO  
 1.0 3.5  
 0.81 4.0  
 0.61 6.5  
 0.25 3.1  
 0.589 2.1

Mutagenesis assay  
 10 P100's received fr. Anupama 2/8/00 (or maybe 2/7)

2/11/00 Replate  $10^6$  cells / P100

How Harvest w. trypsin. Transfer ~ 1 ml to new tubes, syringe & count

Replate fr. orig. tube (not syringed)

Count					X 3.5 ml	Doublings
1	109 x 5	$5.5 \times 10^6$	$\frac{0.18}{\rightarrow}$	$10^6$	$1.9 \times 10^7$	4.2
2	$\frac{49 \times 5}{269 \times 1}$ $53 \times 5$	$2.6 \times 10^6$	$\frac{0.38}{\rightarrow}$	}	0.91	3.2
3	127 x 5	$6.4 \times 10^6$	$\frac{0.16}{\rightarrow}$		2.2	4.4
4	$\frac{56 \times 5}{367 \times 1}$ $74 \times 5$	$3.3 \times 10^6$	$\frac{0.30}{\rightarrow}$		1.2	3.6
5	C	—	—		—	—
6	130 x 5	$6.5 \times 10^6$	$\frac{0.15}{\rightarrow}$		2.3	4.5
7	89 x 5 109 x 5	$5.0 \times 10^6$	$\frac{0.20}{\rightarrow}$		1.8	4.2
8	116 x 5	$5.8 \times 10^6$	$\frac{0.17}{\rightarrow}$		2.0	4.3
9	107 x 5	$5.4 \times 10^6$	$\frac{0.19}{\rightarrow}$		1.9	4.2
10	115 x 5 98 x 5	$5.3 \times 10^6$	$\frac{0.19}{\rightarrow}$		1.9	4.2

Av 4.1 ± 0.42

2/14 Replate  $10^6$  cells / P100 as above

Count					X 3.5 ml	Doublings	Total Doublings
1	111 x 5	$5.5 \times 10^6$	$\frac{0.18}{\rightarrow}$	$10^6$	$1.9 \times 10^7$	4.2	8.4
2	124 x 5	$6.2 \times 10^6$	$\frac{0.16}{\rightarrow}$	}	2.2	4.5	7.7
3	156 x 5	$7.8 \times 10^6$	$\frac{0.13}{\rightarrow}$		2.7	4.8	9.2
4	134 x 5	$6.7 \times 10^6$	$\frac{0.15}{\rightarrow}$		2.3	4.5	8.1
6	111 x 5	$5.5 \times 10^6$	$\frac{0.18}{\rightarrow}$		1.9	4.2	8.7
7	149 x 5	$7.5 \times 10^6$	$\frac{0.13}{\rightarrow}$		2.6	4.7	8.9
8	100 x 5	$5.0 \times 10^6$	$\frac{0.20}{\rightarrow}$		1.8	4.2	8.5
9	90 x 5	$4.5 \times 10^6$	$\frac{0.22}{\rightarrow}$		1.6	4.0	8.2
10	122 x 5	$6.1 \times 10^6$	$\frac{0.16}{\rightarrow}$		2.1	4.4	8.6

Av 4.39 ± 0.26

2/17 00

Count

1 72x5  
2 72x5  
3 73x5  
4 98x5  
6 88x5  
7 85x5  
8 79x5  
9 98x5  
10 85x5

$3.6 \times 10^6 \xrightarrow{0.056} 2 \times 10^5$   
 $3.6 \times 10^6 \xrightarrow{0.056}$   
 $3.7 \times 10^6 \xrightarrow{0.054}$   
 $4.9 \times 10^6 \xrightarrow{0.041}$   
 $4.4 \times 10^6 \xrightarrow{0.045}$   
 $4.3 \times 10^6 \xrightarrow{0.047}$   
 $4.0 \times 10^6 \xrightarrow{0.050}$   
 $4.9 \times 10^6 \xrightarrow{0.041}$   
 $4.3 \times 10^6 \xrightarrow{0.047}$

$\xrightarrow{0.0097} 3.5 \times 10^4 \xrightarrow{0.005} 25$   
 $\xrightarrow{0.0097}$   
 $\xrightarrow{0.0095}$   
 $\xrightarrow{0.007}$   
 $\xrightarrow{0.0080}$   
 $\xrightarrow{0.0081}$   
 $\xrightarrow{0.00875}$   
 $\xrightarrow{0.007}$   
 $\xrightarrow{0.0081}$

X3.5ml Doubl. Total Doubl  
 $1.3 \times 10^7$  3.7 12.1  
 $1.3 \times 10^7$  3.7 11.4  
 $1.4 \times 10^7$  3.8 13.0  
 $1.7 \times 10^7$  4.1 12.2  
 $1.5 \times 10^7$  3.9 12.6  
 $1.5 \times 10^7$  3.9 12.8  
 $1.4 \times 10^7$  3.8 12.3  
 $1.7 \times 10^7$  4.1 12.3  
 $1.5 \times 10^7$  3.9 12.5  
Av 3.9 ± 0.15

2/23 F+S

P35's

1 58 50 55  
2 59 55 61  
3 48 58 46  
4 42 39 37  
6 49 54 44  
7 45 45 44  
8 60 58 59  
9 52 53 54  
10 41 38 44

P100's

3 7 4 10 5  
8 3 6 4 2  
5 12 10 7 14  
6 5 8 10 12  
6 7 6 12 5  
7 5 13 8 12  
9 13 14 11 8  
5 6 5 6 7  
5 3 9 2 6

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