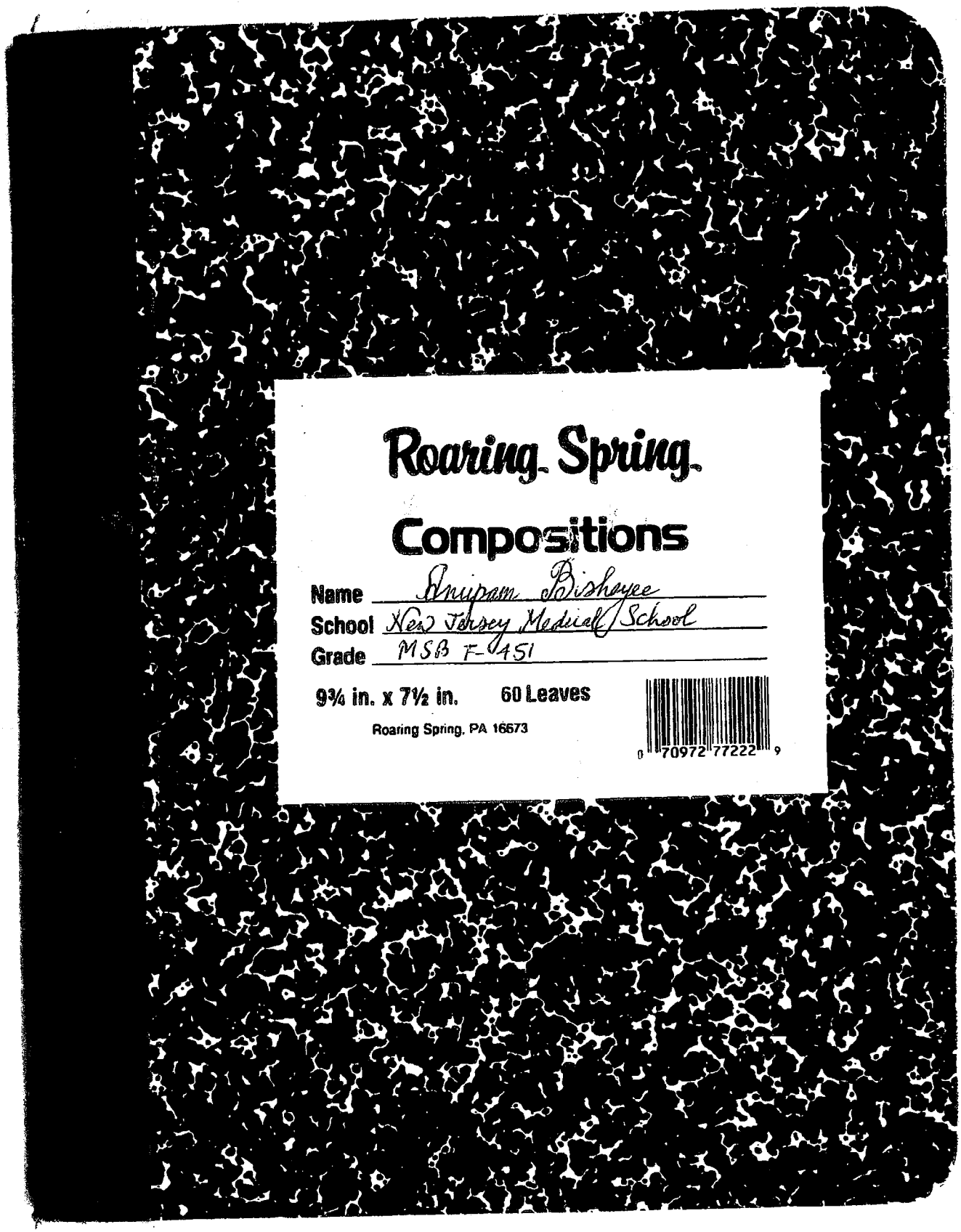

Roaring Spring
Compositions
Name Anupam Bishayee
School New Jersey Medical School
Grade MSB F-451
9¾ in. x 7½ in. 60 Leaves
Roaring Spring, PA 16673
0 70972 77222 9

02/03/98.

① Weigh ~10 mg MEA in Argon bag.
Add ~ 500 µl PBS to make 20 mg/ml, filter it.

② Dilute 1:10 to have 2 mg/ml

Wt of glass tube + MEA = 3.54984 g
Wt of glass tube = 3.57745 g
Wt of MEA = 0.02239 g

20 mg ≡ 1 ml
22 mg = $\frac{1}{20}$ × 22 = 1.11 ml.

02/05/98

Wipe test

		cpm	mR/hr
Background	=	1	0·1
Area 1	=	2	0·2
Area 2	=	2	0·2
Area 3	=	3	0·4
Area 4	=	6	0·4

02/06/98

Tube #	Coulter Count (100ul; MS · 500 ul)
1	679, 688, 644
2	480, 458, 469
3	554, 539, 513
4	~~676~~, 586, 588, 611
5	~~650~~, ~~672~~, 514, 564, 549
6	650, 625, 638
7	451, 456, 440
8	494, 486, 527
9	440, 432, 443
10	573, 580, 595

12-15 p.m.
02/10/98

Tube #	cpm for 10uL medium
1	1, 2, 0
2	2, 0, 1
3	1086, 1107, 1067
4	2105, 2135, 2290
5	2914, 2957, 3310
6	3, 1, 0
7	3, 0, 1
8	1096, 1215, 1211
9	2215, 2200, 2349
10	3131, 3410, 3597, 3454

-15 p.m.
10/98

Expt # 2-2

Tube #	Coulter Count (100 μl cells) MS = 50	02/13/98
1	578, 548, 552	
2	579, 551, 585	
3	641, 624, 654	
4	591, 586, 591	
5	536, 550, 533	
6	585, 588, 550	
7	483, 469, 487	
8	609, 604, 617	
9	590, 582, 550	
10	558, 581, 544	

Tube No. (Dilution)	Colony Count	Avg	02/13/98
		118	
1·2	137, 125, 129	130·33	124·16
2·2	129, 134, 129	130·66	
4·2	117, 118, 124	~~104~~ 120	
5·2	116, 128, 127	123	
6·2	107, 102, 105	104·66	
5·2	111, 107, 113	110·33	
7·2	102, 102, 101	101·66	
8·2	64, 53, 58	58	
9·2	25, 26, 30	27·66	
10·2	110, 128, 108	118	

198

14-16

2/13/98

Expt # 2·3

Tube #	Medium Count (cpm)
1	3, 3, -1
2	2, 1, 0
3	1012, 1069, 1039
4	2068, 2181, 2099
5	2942, 3062, 3146
6	1, 0, 1
7	0, 0, 1
8	1012, 1167, 1043
9	1911, 2192, 2039
10	2803, 2714, 3015

5-30 p.m.

Expt # 2·2

Tube #	Cpm for 300 ml cell
1	2, 0, 1
2	1, 1, 0
3	5239, 5328, 5206
4	9748, 9272, 9875
5	15500, 15674, 15812
6	3, 0, 2
7	2, 2, 1
8	5040, 5069, 5117
9	10930, 10855, 10802
10	14349, 14157, 13695

6-15 pm

Expt #2-3

02/16/98

Tube #	Coulter Count (100 ul Cell.)
1	616, 658, 615
2	632, 638, 606
3	581, 590, 620
4	548, 536, 517
5	509, 525, 496
6	519, 528, 514
7	455, 498, 444
8	506, 520, 531
9	537, 545, 558
10	577, 572, 542

Tube #	cpm for 300 ul cells 4-30 p.m.
1	0, 4, 2
2	2, 4, 1
3	5796, 5959, 5627
4	9639, 9700, 10013
5	16160, 16869, 16707
6	2, 1, 0
7	2, 1, 1
8	6666, 6564, 6693
9	12008, 12215, 11919
10	18130, 19154, 19048

02/16/98

7797, 7666, 7671

7711

$7711 \times 400 = 3084533.333$

1.42 ml Cells + 9.57 ml MEMB = 11 ml

1076, 993, 1008,

10 p.m.

11 ml of 100 µg/ml = 1100 µg = 1.1 mg

= 0.0011 g

Stock 200 mg/ml

~~to~~ 200 mg = 1 ml

1 mg = $\frac{1}{200}$

1.1 mg = $\frac{1.1}{200}$ = 5.5 ml

Take 5.5 ml Stock 200 mg/ml MEA + 10.99 ml MEMA

2/17/93

Expt # 4

11-30 a.m.

Tube #	Medium Count for 10ul (cpm)
1	2, 0, 1
2	0, 0, 1
3	443, 481, 417
4	821, 812, 926
5	1342, 1381, ~~1281~~ 1328
6	2, 2, 1
7	3, 2, 1
8	419, ~~349~~ 451, 405
9	857, 916, 895
10	1309 ~~1094~~, 1385, 1373

04/14/98

~~Effo~~

P2

Tube	1-30	Media Count Expt 1 (MEM)
Tube	31-56	Media " Expt 1 (CO)
Tube	57-86	Cell Count Expt 1 (MEM)
Tube	87-97	Wipe test

Effi = 50.52

Yield = 1.47

Irradiation of V-79 cells ⓒ ^{137}Cs

05/11/98

Tube #	Total Dose	Dose rate	Time	Attenuator	
1.	0 rad	0	0	0	
2.	0 rad	0	0	0	
✓3	100 rad	101.4 R/min	0.98 min	X-10	*
✓4	200 rad	101.4 R/min	1.97 min	X-10	*
* ✓5	300 rad	101.4 R/min	2.95 min	X-10	*
✓6	400 rad	101.4 R/min	3.94 min	X-10	*
✓7	500 rad	101.4 R/min	4.93 min	X-10	*
✓8	750 rad	169.6 R/min	4.42 min	X-5	**
✓9	1000 rad	169.6 R/min	5.89 min	X-5	**
✓10	1250 rad	169.6 R/min	7.37 min	X-5	**

* uniform dose Height from turntable 4.6" – 9.4" wide – uniform at any radius

** 4.2" – 9.8", at any radius

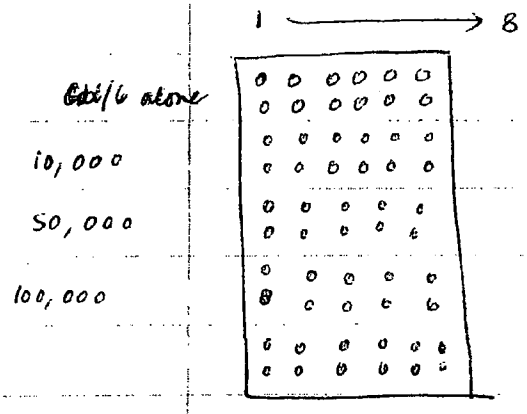
237.8 R/min 2" – 8"

3.4" radius from center

A547

10,000 cells/0.2 ml in each well

Total # of rats = 15



05/29/98

Falcon 2 X 150 cm² ABI/6 70-80% confluence

Suspend in 248 ml

Cell Count = 912, 909, 886

Avg = 902.3

Cell Conc = 902.3 x 400

= 360933.3 cells/ml

Total volume = 30 ml

Total # of cells from 2 flask = 7218666 cells

cells/flask = 3,609,333

= 3.6 million

06/18/98

4 x 150 cm² Falcon flask, ABI/6

80% confluent

Total volume = 32 ml

Cell count = 1233, 1257, 1166

Cell conc. = 487466 cells/ml

Total # of cells from 4 flask = 15598933

= 15.59 million

cells/flask = 3,899,733

= 3.8 million

4-30 pm
6/19/98

1
2
3
4
5 0
6 1938, 1977, 2002
7 2145, 2140, 2030
8 2183, 2114, 2002
9 1998, 1942, 1995
10 1854, 1964, 1822

pm

28

Irradiation of 96-well plate E ABL/6 6/24/98

Marked	Total Dose	Dose rate	Time	AH
unmarked	0	0		
B/head	100 R	101.4 R/min	0.98 min	X-10
GB/head	200 R	101.4 R/min	1.97 min	X-10
B/Body	400 R	101.4 R/min	3.94 min	X-10
G/Body	600 R	101.4 R/min	5.918 min	X-10

Falcon GM/6; 7 days following 1:5 subculturing; 80% confluent

MS = 500 ml

Count vol. = 100 ml

6/25/98

Cell count = 1469; 1502, 1443

Cell = 588533 Cells/ml

Total volume = 10ml

Total # of cells from two 150 cm^2 flask

= 5885333

Cells/ flask = 2,942,666

= 2.94 million

07/02/98

GBC/6 ; 7 days following 1:5 subculturing ; 80-85% confluent.

MS = 500 ml

Total volume =

Cell count = 3617, 3499, 3515

Cell conc = 1,417,466 cells/ml

Total Volume = 11 ml

Total # of cells from 5 x 150 cm² flask

= 15,592,133

Cells/flask = 3,113,426 Cells/f

= 3.1 million

DMSO = 100%

~~100 x V1 = 5~~

~~100 x 50 = 5~~

$S_1V_1 = S_2V_2$

$100 \times V_1 = 5 \times 1$

$V_1 = \frac{5}{100} = 0.05 \text{ ml} = 50 \mu l$.

1. Take 10 x 1ml cryo vial.
2. Take ~~0.95~~ 0.95 ml of cell suspension in each
3. Add ~~50~~ ml of 100% DMSO, Vortex, place on ice.
4. Transfer to -70°C

V79 07/02/98

Initial cell count = 7263, 7372, 7301

Cell conc. = 2924800 cells/ml

Total volume = 10 ml

Total # of cells/flask = 29,248,000

= 29 million

vol required = $\frac{4400000}{29248000}$ = 1.5 ml

Take 1.5ml Cells + 9.5ml MEMB = 11mL

Final count = ~~1239~~, 1193, 1117, 1172

= 4,64,266 Cells/ml

GM-CSF

0.408 ml contains 1200 U

= ~ 2941 U/ml

Previous Lot # 5000 units / mg | proliferation

25000 unit / 5 mg | EC_{50}

ml = 25000 ÷ 2941 = ~ 8.5

Present Lot # 3570 unit / mg

= 17850 unit / 5 mg

ml = 17850 ÷ 2941 = ~ 6.0

07/21/98

1. L- Asperagine
Stock 50 mg.
~~Add~~ Inject 10 ml of sterile water into a vial. containg 50 mg (50 mg/10 ml or 5 mg/ml or 5 ug/ml)

2. Dextran: 1 ug/ml or 1 mg/ml
Prepare: 10 ml
Weighe: 10 mg
Add 10 ml sterile water

3. $NaHCO_3$: weight 3.7 g.

07/23/98

Prepare 65 ml of 50,000 cells/ml GBL/6

= 3,250,000 Cells

Vol. of Cell suspension required = $\frac{3,250,000}{553333}$

= 5.87

Initial count = 151, ~~126~~, 135, 129

Cell conc = 138.33 X 4000

= 553333.3 Cells/ml

Take ~6 ml Cell + 59 ml DMEM = 65 ml.

Final count = 172, 185, 164, 191

Avg = 179

Cell Conc = 179 X 400

= 71,733 Cells/ml

CAFC Array 07/28/98

Collection of bone marrow cells.

Cell Type	Coulter count for cone	Cell conc (cells/ml)	Total # of cells (x 2)	Cells/femur
C57	3329, 3257, 3268	13138666	26277333	4379555
SW	3121 ~~3245~~, 3243, 3214 ~~3421~~ ✓	12770666	25541333	4,256,888

Max # of cells / well (0.2 ml) = $\frac{1}{10} \times 4379555$

= 437955.5

Dil. #
400,000
200,000
100,000
50,000
25,000
12,500

100ml, 50ml

C57	2696, 2537, 2605
SW	2401, 2454, 2514, 2437

C57

① we need 10 ml of 400,000 Cells/ml

= 4,000,000 Cells.

vol required = $\frac{4000,000}{13138666}$ = 0.30 ml

Take 9.7 ml Fisher + 0.3 ml cells = 10 ml

② 5ml Fisher's + 5ml ①

③ 3ml " + " ②

④ 3ml " + " ③

⑤ 3ml " + " ④

⑥ 3ml " + " ⑤

SW ① vol. requ = $\frac{4000,000}{12770666}$ = 0.31 ml

Cell Type	Coulter count for 100ml (MS = 50ml)	Cell conc (cells/ml)	Total # of cell	cells/fem
C57	2646, 2537, 2605	10384000	20768000	3,461,333
SW	2401, 2454, 2437	9722666	19445333	3,240,888

(1) ∴ Max # of cells $= \frac{1}{10} \times 3{,}461{,}333$

$= 346133$

(1) 300,000

(2) 150,000

(3) 75,000

(4) 37,500

(5) 18,750

(6) 9,375

10 ml of 300,000 cells/ml

= 3000,000 cells

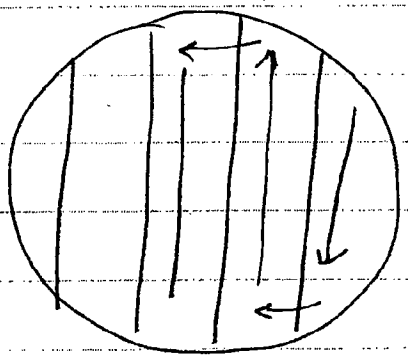
1/Jeme

C57 vol. required = $\frac{3000,000}{10384000}$ = 0·28 ml

,333

SW " " = $\frac{300,000}{9722666}$ = 0·30

0888



GM Colony Count

8/4/98

Dilution		# of colonies			
1,000,000	~~3~~A	31, 43, 50	41.33		
300,000	3B	~~15~~ 50, ~~27~~ 8, 7	~~14.33~~ 8.33	~~20~~ 0.11	
	3C	4, 3, 2	3		
	2B	23, 21, 27	23.6	0.33	
	2C	7, 8, 5	6.66		
	1A	187, 192, 179	186		
	1B	Denis 82, Anupa 82, Rory 74			
		82, ~~96~~, ~~74~~	70.66		
	1C	10, 12, 15	12.33		

~~82~~

186

77.8 0.41

41.33 0.22

8/10/98

Prepare 1X DMEM & 2% horse Serum

1X DMEM = 410 ml.

Horse Serum = x ml.

$$\frac{x}{410+x} = \frac{2}{100}$$

$$\therefore 100x = 820 + 2x$$

$$\therefore 98x = 820$$

$$\therefore x = 8.36 \text{ ml}.$$

Take 410 ml 1X DMEM

Add 8.5 ml Horse Serum

Coulter Count for 20 ml Cells 10/14/98

1	3830, 3833, 3740
2	5729, 5546, 5881, 5876
3	2568, 2444
4	5224, 5234, 5053
5	3412, 3363, 3379
6	3116, 3086, 3028

Main menu → goto windows → enter

MS DOS Executive → goto S100

goto → MCA.EXE → OK

Display → Expanse

E-Cal → ON

Menu, Collect Stat Enter

Menu, Compute, area, 3 X back gr

enter
