V79 COLONY FORMING ASSAY

Experiment Name : ^{3}HTdR toxicity (cluster, 50% labeling); **Exp. # :** 2

Investigator: **Date:** 4/19/2001

1. Set the rocker-roller at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 175 cm^2 flasks, sub-cultured 1:2, 2 days 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 ~2,000,000 cells/ml in MEMB [Actual count : 2.0×10^6 cells/ml) Flasks 95% confluent
3. Transfer 1 ml of cell suspension into 20 12 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_2 **Date/Time:** 3:15 pm
5. Prepare MEMB containing radioactivity in hood

   60 µl ^{3}HTdR (Stock : 1 µCi/µl on) + 2.94 ml MEMB
6. After 3-4 h, remove first set of 10 test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 6:00 pm

Add 1 ml of MEMB to the second set of tubes.

Tube #	^{3}HTdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{3}HTdR (ml) [20uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.1	1.0	0.99	0.01
4	0.5	1.0	0.95	0.05
5	0.75	1.0	0.925	0.075
6	1	1.0	0.9	0.1
7	2	1.0	0.8	0.2
8	4	1.0	0.6	0.4
9	5	1.0	0.5	0.5
10	10	1.0	0	1

Prepare 3 ml ($\frac{20 \mu Ci}{ml}$) = 60 µCi

9 ml
+10 ml for 2nd set

1,2
2.2
3 2
4.2 4.3
5.3 5.4
6.3 6.4
7.3 7.4
8.3 8.4
9.3 9.4
10.3 10.4

15/54

7. Return test tubes to roller for 12 h . **Date/Time:** 6:17pm
8. Next day, while test tubes are in roller label 10 gamma-tubes (13 X 100 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:** 7:35 am 4/20/2001
10. Remove buckets from centrifuge and carefully remove 150 µl of supernatant 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 7 5 ml of MEMA
18. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
19. ~~Decant supernatant, click tubes, vortex~~
20. Add 10 ml of wash MEMA to second set of tubes containing 2,000,000 cells, and follow steps 11-17, suspend in 7 5 ml of MEMA and transfer cells to the corresponding tubes containing 2,000,000 cells in step 19 ← wash second set with 2 ml MEMA and transfer that too to 1st set
21. Centrifuge tubes for 10 min at 2000 rpm, 4°C
22. Decant supernatant, click tubes, vortex
23. Transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using 200 ul pipet tips
24. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul) ← had difficulty with transfers due to inconsistent decanting. Leftover volumes different. Recommend correcting cell only counts as per Marek's idea programmed into spreadsheet.
25. Centrifuge tubes for 5 min at 1000 rpm, 4°C 4/20/2001
26. Transfer tubes at 10°C for 72 h. **Date/Time:** 10:40am
27. Transfer 30 ul supernatant in three sets of 20 ml scintillation vials containing 6 ml liquid scintillation cocktail (~~Aquasol~~ Ecolume) from 150 ul supernatant removed earlier (Step 10) and count them for radioactivity **Date/Time:**
28. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 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 **Date/Time:** 11:30 am 4/23/2001
29. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell

suspensions in 12 ml tubes

30. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
31. Labeling and preparation of dilution tubes and colony dishes
    - load 66, 60 mm petri dishes with 4 ml MEMA
    - load 40 sterile 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.
32. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
33. Centrifuge tubes for 10 min at 2000 rpm, 4°C
34. ~~Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA~~
35. ~~Centrifuge tubes for 10 min at 2000 rpm, 4°C~~
36. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
37. Determine cell concentration by transfering 100 µl to Coulter cup
38. Vortex tube, transfer 0.5 ml into dilusion 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. Keep tubes on ice.
39. 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.
40. Transfer 200 µl of cell suspension (in triplicate) to 20 ml scintillation vial containing 6 ml cocktail (Aquasol)
41. Incubate petridishes for 1 week
42. Count vials for radioactivity **Date/Time :**
43. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with 0.05% crystal violet
44. Count colonies. There must be between 25 and 250 colonies for the dish to be a valid data point.

1.2	92, 111, 119
2.2	78, 85, 74
3.2	142, 126, 120
4.2	120, 129, 121
5.2	64, 68, 79
6.2	92, 101, 78
7.2	74, 62, 94
8.2	89, 69, 67
9.2	85, 87, 97
10.2	71, 58, 55

Preparation of Cells for Roll

4/19/2001

2080 50 ml
2162
2182
2004

$\bar{x}$: 2107 x 4000 = 8,428,000 /ml

Need 2×10^6/ml (~~22~~ 44 ml) = ~~44~~ x 10^6 cells

#ml stock = ~~44~~ 22 / 8.428 = ~~5.22~~ 5.27 ml stock cells

Need 1.4ml more

+ ~~12.2 ml~~ MEMB 16.8

4.6ml MEDIA

500ml Coulter scaling

5281
5291
5363
5311.7 ⟹ 2.12×10^6/ml

5556
5609

5094
~~4986~~
4918

$\bar{x}$: 4999 x 400 = 1,999,733

bgd: 3

Exp. #2 4/23/2001

Coulter Counts after resuspending cluster
500Ml Coulter setting, 100Ml cells in 20ml Istore

1) 7967, 7945, 8022
2) 7404, 7497, 7513
3) 8321, 8426, 8221
4) 7644, 7486, 7622
5) 7535, 7595, 7432
6) 7009, 7128, 7026
7) 7540, 7458, 7458
8) 7117, 7107, 7177
9) 7204, ~~6821~~, 7236, 7144
10) 6896, 6826, 6875

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 24 APR 2001 13:01
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H[redacted]1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
1	29- 1	1	8.00	70.71	1.00	1.42	99.0		1C
2	29- 2	1	6.00	81.65	1.00	2.99	100.0		1C
3	29- 3	1	5.00	89.44	1.00	4.61	98.0		1C
4	29- 4	1	11.00	60.30	1.00	6.18	99.0		2C
5	29- 5	1	10.00	63.25	1.00	7.75	97.0		2C
6	29- 6	1	7.00	75.59	1.00	9.32	99.0		2C
7	29- 7	1	617.00	8.05	1.00	10.94	96.0		3C
8	29- 8	1	818.00	6.99	1.00	12.51	97.0		3C
9	29- 9	1	909.00	6.63	1.00	14.13	98.0		3C
10	29-10	1	3005.00	3.65	1.00	15.70	98.0		4C
11	29-11	1	2931.00	3.69	1.00	17.27	98.0		4C
12	29-12	1	3001.00	3.65	1.00	18.82	99.0		4C
13	29-13	1	5252.00	2.76	1.00	20.39	98.0		5C
14	29-14	1	4961.00	2.84	1.00	21.96	97.0		5C
15	29-15	1	5269.00	2.76	1.00	23.52	100.0		5C
16	29-16	1	8084.00	2.22	1.00	25.11	99.0		6C
17	29-17	1	7048.00	2.38	1.00	26.68	100.0		6C
18	29-18	1	6832.00	2.42	1.00	28.31	100.0		6C
[redacted]	**- 1	1	11877.65	1.99	0.85	29.77	97.0		7C
20	**- 2	1	~~7565.00~~	2.30	1.00	31.35	95.0		7C
21	**- 3	1	11457.78	1.97	0.90	32.82	96.0		7C
22	**- 4	1	28545.00	1.87	0.40	33.77	97.0		8C
23	**- 5	1	26127.50	1.96	0.40	34.78	98.0		8C
24	**- 6	1	25097.50	2.00	0.40	35.78	100.0		8C
25	**- 7	1	42776.00	1.93	0.25	36.58	97.0		9C
26	**- 8	1	43272.00	1.92	0.25	37.43	99.0		9C
27	**- 9	1	40664.00	1.98	0.25	38.24	100.0		9C
28	**-10	1	68466.66	1.97	0.15	38.95	100.0		10
29	**-11	1	60605.00	1.82	0.20	39.70	101.0		10
30	**-12	1	79920.00	1.83	0.15	40.40	99.0		10
31	**-13	1	29020.00	1.98	0.35	41.36	1.0		std.

Exp 2

USER: 6 ID:H3 HOWELL PRESET TIME: 1.00 TUE 24 APR 2001 12:26
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 1 AQC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:@ 1.00000
HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVG H#	ERR	
1	**- 1	1	12.00	57.74	1.00	1.43	81.0		1M
2	**- 2	1	11.00	60.30	1.00	3.00	80.0		1M
3	**- 3	1	10.00	63.25	1.00	4.57	80.0		1M
4	**- 4	1	10.00	63.25	1.00	6.14	80.0		2M
5	**- 5	1	7.00	75.59	1.00	7.71	81.0		2M
6	**- 6	1	10.00	63.25	1.00	9.28	80.0		2M
7	**- 7	1	2309.00	4.16	1.00	10.90	81.0		3M
8	**- 8	1	2276.00	4.19	1.00	12.52	82.0		3M
9	**- 9	1	2490.00	4.01	1.00	14.08	81.0		3M
10	**-10	1	10712.63	1.98	0.95	15.62	81.0		4M
11	**-11	1	10708.42	1.98	0.95	17.19	81.0		
12	**-12	1	10422.00	1.96	1.00	18.82	80.0		
13	**-13	1	16584.62	1.93	0.65	20.02	82.0		5M
14	**-14	1	15967.69	1.96	0.65	21.24	81.0		
15	**-15	1	15457.14	1.92	0.70	22.56	79.0		
16	**-16	1	20740.00	1.96	0.50	23.67	81.0		6M
17	**-17	1	21212.00	1.94	0.50	24.72	80.0		
18	**-18	1	20938.00	1.95	0.50	25.78	80.0		
19	**- 1	1	42412.00	1.94	0.25	26.69	79.0		7M
20	**- 2	1	44056.00	1.91	0.25	27.49	82.0		
21	**- 3	1	42976.00	1.93	0.25	28.29	82.0		
22	**- 4	1	84780.00	1.77	0.15	28.99	81.0		8M
23	**- 5	1	83973.33	1.78	0.15	29.69	82.0		
24	**- 6	1	83833.33	1.78	0.15	30.39	81.0		
25	**- 7	1	102080.00	1.98	0.10	31.09	80.0		9M
26	**- 8	1	103486.66	1.61	0.15	31.80	80.0		
27	**- 9	1	102959.99	1.61	0.15	32.51	80.0		
28	**-10	1	222746.66	1.09	0.15	33.22	82.0		10M
29	**-11	1	223886.66	1.09	0.15	33.93	80.0		
30	**-12	1	223680.00	1.34	0.10	34.64	81.0		

Parameters

Date	4/19/01		
Experiment No.	2		
Investigator	R. Howell		
Cell Line	V79		
Modifier	None		
Radionuclide	H-3		
Half-life (days)	4500.45	I-125=59.408, H-3=4500.45, Po-210=138.376, I-131=8.02	
Radiation Yield	1	I-125=1.47, H-3=1.0, Po-210=1.0, I-131=8.02	
Radiochemical	H-3 thymidine		
Manufacturer/Lot	NEN/3106-421		
Original Calibration Date/Time	4/16/01 12:00	Original Activity Concentration (MBq/ml)	37
Present Calibration Date/Time	4/16/01 12:00	Time Elapsed Since Original Calibration (d)	3
Fraction of Cells Labeled	0.5	Present Activity Concentration (MBq/ml)	36.98
Liquid Scintillation Cocktail	Ecolume		
Volume of LSC Cocktail (ml)	6		
Volume/Type Counting Vial	7		
Model of Counter	Beckman 5800		
Counting Efficiency	0.65		
Activity Added (Date/Time)	4/19/01 18:00		
Cells Washed (Date/Time)	4/20/01 7:35	Time Elapsed Between Add and Wash (hr)	13.50
Medium Tubes Counted (Date/Time)	4/24/01 12:26	Time Elapsed Between Add and Count (hr)	114.50
Cell Tubes Counted (Date/Time)	4/24/01 12:26	Time Elapsed Between Wash and Count (hr)	91.00
Vol. Supernatant Counted (µl)	30		
Vol. Suspension Counted Cell Activity (µl)	200		
Vol. Suspension Coulter (µl)	100		
Coulter Manometer Volume (µl)	500		
Average Coulter Background Counts	2		
Coulter Calibration Parameter	400		
Hemocytometer Counting (Yes or No)?			

Background		
Coulter 1	Coulter 2	Coulter 3
2	1	3

MediumActivity

Experiment: 2
Date: 4/19/2001

Tube #	Medium count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	At µCi/ml on counting	Ao µCi/ml at addition [At/e-0.693t/T]	Ao kBq/ml at addition
1	*12*	*11*	*10*	*10*	*0*	*0*	*0*	*0*	*0*
2	*10*	*7*	*10*		*0*	*0*	*0*	*0*	*0*
3	2309	2276	2490	2358	2348	3613	0.0542	0.0543	2.0086
4	10712	10708	10422	10614	10604	16314	0.2450	0.2451	9.0699
5	16584	15967	15457	16003	15993	24604	0.3694	0.3697	13.6790
6	20740	21212	20938	20963	20953	32236	0.4840	0.4844	17.9220
7	42412	44056	42976	43148	43138	66366	0.9965	0.9972	36.8972
8	84780	83973	83833	84195	84185	129516	1.9447	1.9461	72.0062
9	102080	103486	102959	102842	102832	158203	2.3754	2.3772	87.9549
10	222746	223886	223680	223437	223427	343734	5.1612	5.1650	191.1039

CellSuspension

Experiment: 2
Date: 04/19/01

Tube #	Suspension count (CPM) 1st	2nd	3rd	CPM Average	CPM corrected for control	DPM CPM/(y e)	A_i µCi/ml on counting	A_o µCi/ml after uptake	A_o kBq/ml after uptake
1	*8*	*6*	*5*	*8*	*0*	*0*	*0.00000*	*0*	*0.0000*
2	*11*	*10*	*7*		*0*	*0*	*0.00000*	*0*	*0.0000*
3	617	818	909	781	774	1190	0.00268	0.00268	0.0992
4	3005	2931	3001	2979	2971	4571	0.01030	0.01030	0.3811
5	5252	4961	5269	5161	5153	7927	0.01785	0.01787	0.6610
6	8084	7048	6832	7321	7314	11252	0.02534	0.02536	0.9382
7	11877	11457		11667	11659	17937	0.04040	0.04042	1.4956
8	28545	26127	25097	26590	26582	40895	0.09211	0.09216	3.4099
9	42776	43272	40664	42237	42230	64968	0.14633	0.14641	5.4172
10	68466	60605	79920	69664	69656	107163	0.24136	0.24150	8.9355

CoulterSurvival

riment: 2
e/Time: 4/19/01

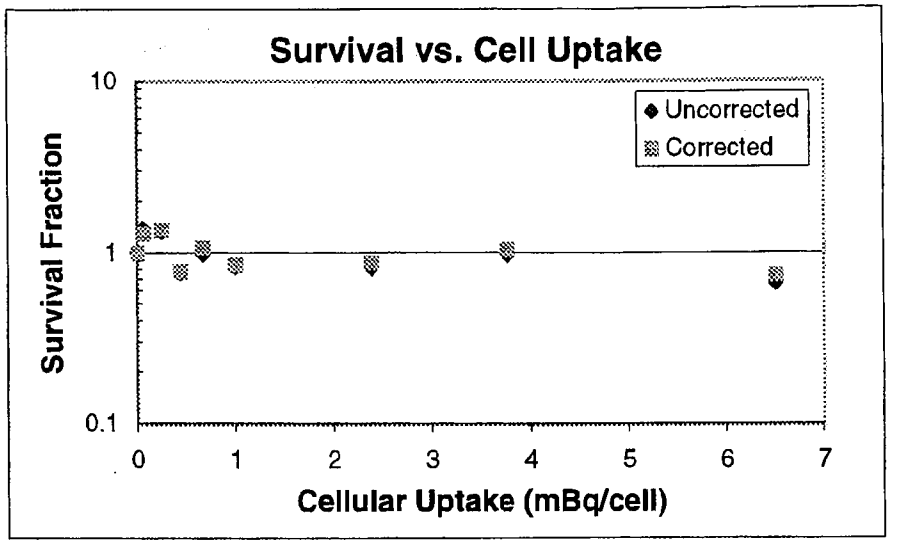
Tube #	Coulter count 1st	2nd	3rd	Average	Cells/ml	Hemocytometer Count in Grid 1st	2nd	3rd	4th
1	*7967*	*7945*	*8022*	*7978*	*3190400*				
2	*7404*	*7497*	*7513*	*7471*	*2987733*				
3	8321	8426	8221	8323	3328267				
4	7644	7486	7622	7584	3032800				
5	7535	7595	7432	7521	3007467				
6	7009	7128	7026	7054	2820933				
7	7540	7458	7458	7485	2993333				
8	7117	7107	7177	7134	2852667				
9	7204	7236	7144	7195	2877067				
10	6896	6826	6875	6866	2745467				

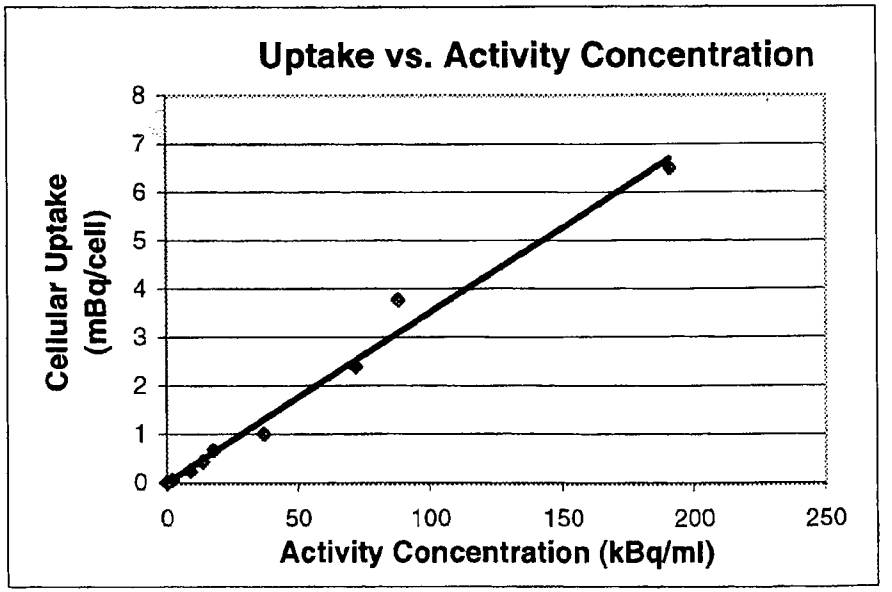
Tube #	Predicted # Cells Seeded	Actual # Cells Seeded	Colony count 1st	2nd	3rd	Average	PE (%)	SF Uncorrected	SF Corrected
1	200	*319*	*92*	*111*	*119*	*93*	*30.160*	*1.00*	*1.0000*
2	200	*299*	*78*	*85*	*74*				
3	200	333	142	126	120	129	38.859	1.3882	1.2884
4	200	303	120	129	121	123	40.666	1.3238	1.3484
5	200	301	64	68	79	70	23.386	0.7549	0.7754
6	200	282	92	101	78	90	32.022	0.9696	1.0617
7	200	299	74	62	94	77	25.612	0.8229	0.8492
8	200	285	89	69	67	75	26.291	0.8050	0.8717
9	200	288	85	87	97	90	31.166	0.9624	1.0334
10	200	275	71	58	55	61	22.340	0.6583	0.7407

Summary

Experiment:
Date/Time: 4/19/01

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*1.0000*	*1.0000*
2	*0.000*	*0.000*		
3	2.009	0.060	1.3882	1.2884
4	9.070	0.251	1.3238	1.3484
5	13.679	0.440	0.7549	0.7754
6	17.922	0.665	0.9696	1.0617
7	36.897	0.999	0.8229	0.8492
8	72.006	2.391	0.8050	0.8717
9	87.955	3.766	0.9624	1.0334
10	191.104	6.509	0.6583	0.7407





B007394

Experiment:
Date/Time: 4/19/01

Tube #	Activity Conc. (kBq/ml)	Activity/Cell (mBq/cell)	Survival Uncorrected	Survival Corrected
1	*0.000*	*0.000*	*1.0000*	*1.0000*
2	*0.000*	*0.000*		
3	2.009	0.060	1.3882	1.2884
4	9.070	0.251	1.3238	1.3484
5	13.679	0.440	0.7549	0.7754
6	17.922	0.665	0.9696	1.0617
7	36.897	0.999	0.8229	0.8492
8	72.006	2.391	0.8050	0.8717
9	87.955	3.766	0.9624	1.0334
10	191.104	6.509	0.6583	0.7407