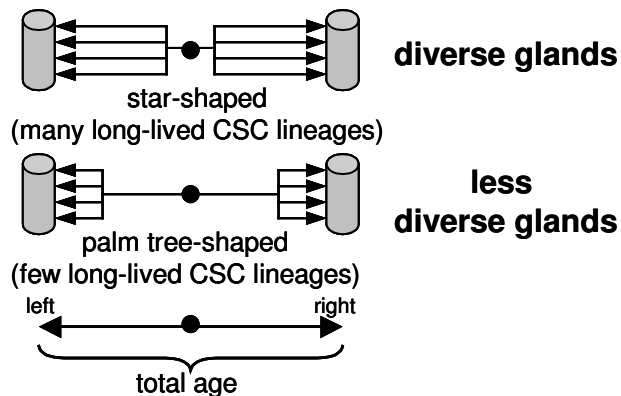


SI Appendix

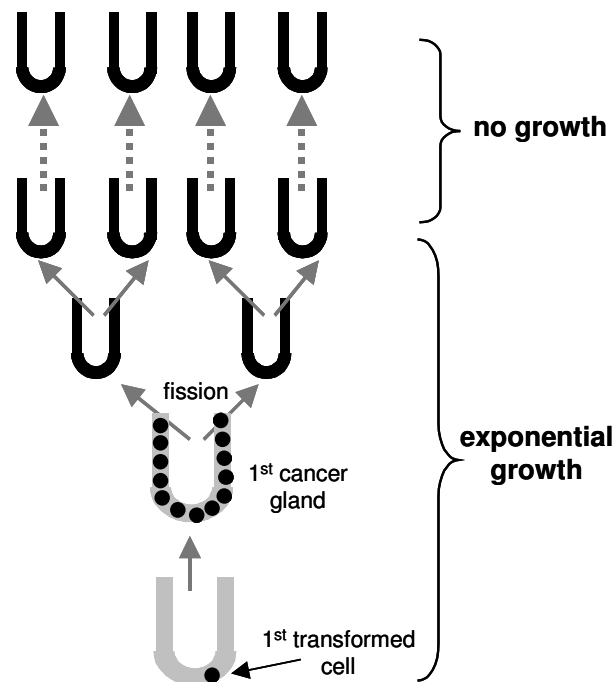
Basic Approach:

We compare epigenetic pairwise distances between cells isolated from different parts of the same tumor. Experimentally, cells are sampled from individual cancer glands, and therefore the simulations follow the ancestries of both cells and their glands. Whereas adjacent cells within a gland may or may not be closely related, cells on opposite sides of a tumor (separated by several centimeters) cannot be closely related and likely last shared a common ancestor around the time of transformation. Therefore, average pairwise tag distances between opposite cancer sides are used to estimate cancer mitotic age (total numbers of divisions since transformation). Tag pairwise distances within glands are also used to estimate relative numbers of long-lived or cancer stem cell (CSC) lineages per gland. Cancer glands likely contain many CSCs if average pairwise tag distances within a gland are similar to the distances between tags from opposite cancer sides:



Simulating Tumor Growth:

Rapid initial exponential growth was simulated with BGN and LOC tags (available at <http://www-hsc.usc.edu/~kims/SupplementaryInfo.html>). Starting from a first transformed cell and single tag, 32 exponential doubling divisions were simulated to produce about four billion cells. Cells were grouped within glands, with gland fission or replication occurring whenever gland size exceeded a threshold:



After exponential growth and the end of gland fission or duplication, cell division is balanced by cell death (i.e. cell numbers are constant). Whereas all cancer cells are “stem” cells during exponential growth (because no cells die), the onset of cancer cell death after exponential growth allows for a stem cell hierarchy---non-CSCs die whereas CSC lineages persist. This no-growth phase is essentially the same as simulating normal colon crypts (1), with cancer glands consisting of long-lived CSC lineages and shorter-lived non-CSCs. There are two different types of potential CSCs---deterministic stem cells (100% asymmetric divisions) and stochastic stem cells (50% asymmetric and 50% symmetric divisions, balanced such that CSC numbers per gland remain constant):

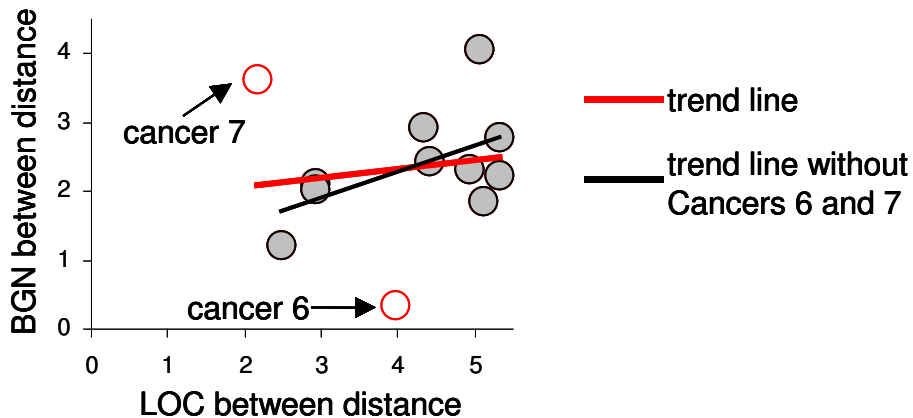
Simulated CSC Scenarios For Cancer Glands With 8,192 Total Cells

Scenario	CSCs per gland	Division	non-CSCs per gland	numbers of non-CSC divisions
rare CSCs	1	100% asymmetric	8191	12
all CSCs	4096	50% asymmetric 50% symmetric	4096	0
multiple deterministic CSCs	2-2048*	100% asymmetric	6144-8190	11-1
multiple stochastic CSCs	2-2048*	50% asymmetric 50% symmetric	6144-8190	11-1

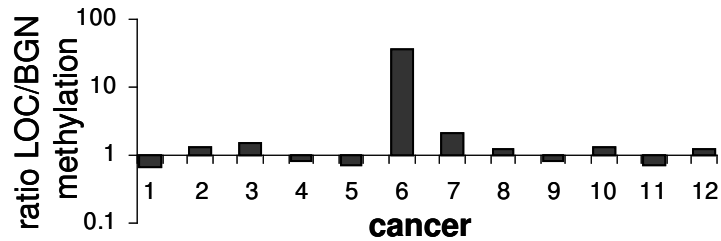
*either 2, 4, 8,.....,2048 CSCs per gland

Estimating Cancer Mitotic Ages With LOC and BGN Tag Data:

To improve cancer mitotic age estimates, BGN and LOC tag data are combined. Average pairwise distances between BGN and LOC tags from opposite cancer sides should correlate:



The correlation of LOC and BGN pairwise distances between opposite sides is poor, and Cancer 6 and Cancer 7 appear to be outliers. One reason for a lack of correlation may be cancer specific differences in error rates. It has been previously noted that error rates for the BGN tag are smaller when the tag is unmethylated (2), indicating that methylation error rates are not constant but may vary as a function of tag methylation. Plotted are the ratios of average LOC to BGN tag methylation for each cancer:

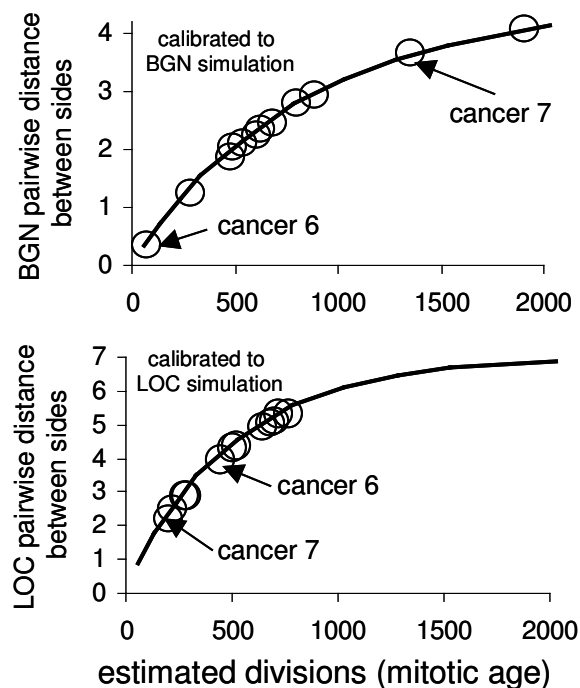


This plot reveals that Cancers 6 and 7 have the greatest discordances between LOC and BGN percent methylation. Cancer 6 has extremely low average BGN tag methylation (2%) compared to its LOC methylation (76%). Cancer 7 has relatively high average LOC tag methylation (90%) compared to its BGN methylation (43%). Overall for the 12 cancers, average LOC methylation is 70% and average BGN methylation is 59%. These data indicate that some of the discordances between LOC and BGN tag distances may be due to variations in error rates when tag methylation is either very low or very high. This observation is used to justify the cancer specific changes in methylation error rates noted below.

Estimating Cancer Mitotic Ages:

Estimating the mitotic age of an individual cancer is problematic. However, it is easier to estimate the average mitotic age of a group of cancers because most cancers are unlikely to arise suddenly within a few months or remain asymptomatic or undetected for many years. As a first approximation, we assume that most cancers are removed within two years after transformation, which yields estimated error rates per CpG site per division of 0.0003 for the BGN tag and 0.0005 for the LOC tag (both methylation and demethylation changes are possible). These error rates give similar estimated LOC and BGN mitotic ages for each tumor (see below). For the 12 cancers in this study, estimated mitotic ages were 250 to 1,130 divisions (average of 642 divisions), which translates to about 8 to 37 months (average of 21 months) assuming one division per day.

Tag pairwise distances between cancer sides were simulated for the different CSC scenarios. Similar average curves were generated regardless of the number of CSCs per gland, consistent with the idea that pairwise distances between opposite sides measure the overall mitotic age of the cancer. Mitotic ages were estimated by curve fitting, with the overall estimated cancer mitotic age based on both tag estimates:



BGN and LOC cancer mitotic age estimates were fairly close for 10 cancers, with greater discordances with Cancers 6 and 7. For Cancer 6, most BGN tags were unmethylated (average 2%) and a prior analysis (2) indicated that BGN error rates are lower until some CpG sites become methylated. The error rate for BGN was therefore changed to from 0.0003 to 0.00005 to better match the mitotic age estimated from the LOC data. For Cancer 7, average LOC tag methylation was relatively high (90%) and the demethylation error rate was changed to 0.0001 per division per CpG site. These changes in error rates also allowed simulations to better fit other experimental data for these cancers (unique tags per gland, intragland tag distances). Mitotic age estimates were also adjusted for Cancers 4, 8, and 9, by simulations that modeled the 8 instead of 9 CpG sites in their BGN tags. Adjusted cancer mitotic age estimates are provided below:

Table of Mitotic Age Estimates

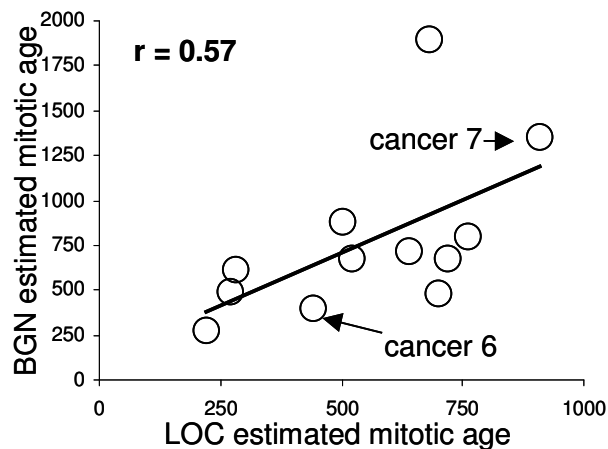
Cancer	BGN distance between sides	BGN estimated divisions	LOC distance between sides	LOC estimated divisions	overall estimated mitotic age
1	1.9	480	5.1	700	590
2	1.2	280	2.5	220	250
3	2.4	680	4.4	520	600
4	2.3*	720	4.9	640	660
5	2.9	880	4.3	500	690
6	0.4	400 [†]	4.0	440	420
7	3.6	1350	2.2	910 [‡]	500
8	2.1*	620	2.9	280	400
9	2.2*	680	5.3	720	700
10	2.0	490	2.9	270	380
11	2.8	800	5.3	760	780
12	4.1	1900	5.1	680	1100

* BGN tag had 8 CpG sites in these patients whereas 9 CpG sites were present in the other 9 patients

[†] The error rate for BGN was changed to from 0.0003 to 0.00005 because most BGN tag were unmethylated and a prior analysis (2) indicated that BGN error rates are lower until some CpG sites become methylated.

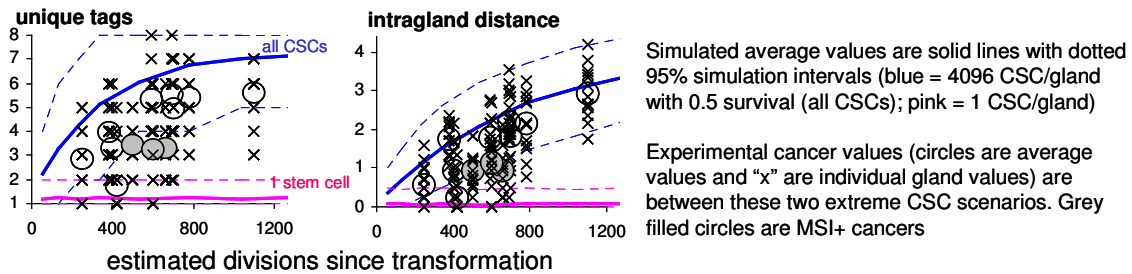
[‡] The demethylation error rate for LOC was changed to 0.0001 from 0.0005 because average tag methylation was relatively high in this cancer.

The correlation between LOC and BGN estimated cancer mitotic ages was improved after these adjustments:

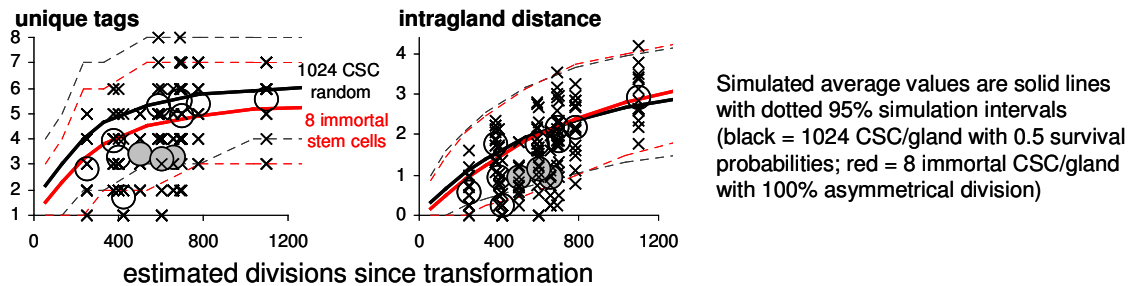


Estimating CSCs Per Cancer Gland:

It is possible to infer numbers of long-lived lineages or CSCs per cancer gland by comparing tag diversity between cancer sides with tag diversity within cancer glands. Simulations with rapid initial exponential growth can be performed with different numbers of CSCs per gland. Two simple extreme scenarios are a single CSC per gland, or that all cells within a gland effectively function as CSCs (stochastic survival with a 0.5 probability of offspring survival). Neither scenario appeared to fit the experimental BGN tag data for unique tags per gland or intragland tag distances, which fell between these two extreme scenarios:

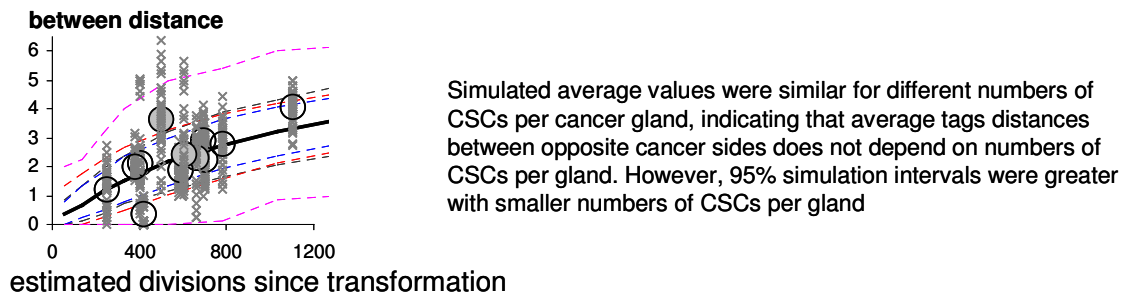


Scenarios with intermediate numbers of CSCs per gland better fit the experimental data:

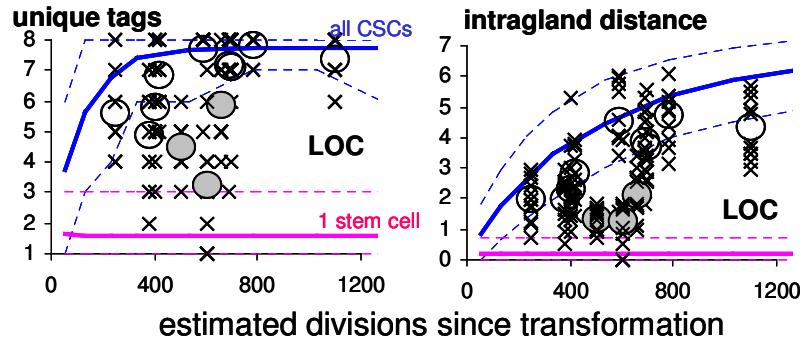


One can better match the experimental data by either increasing numbers of immortal or deterministic CSCs per gland, or decreasing numbers of stochastic CSCs per gland. A single scenario does not appear to fit all the cancers, so cancer specific simulations were performed to infer likely numbers of CSCs per gland with respect to the experimental data from each cancer (see below).

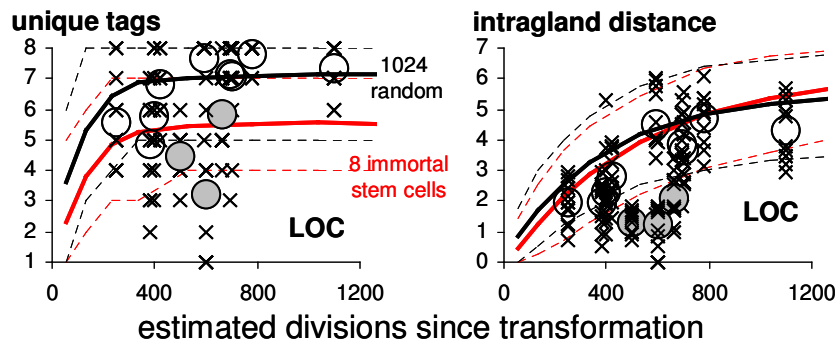
Of note, simulations indicated that average tag distances between opposite cancer sides did not change with different numbers of CSCs per gland, which indicates that cancer mitotic age estimates do not depend on CSC number estimates:



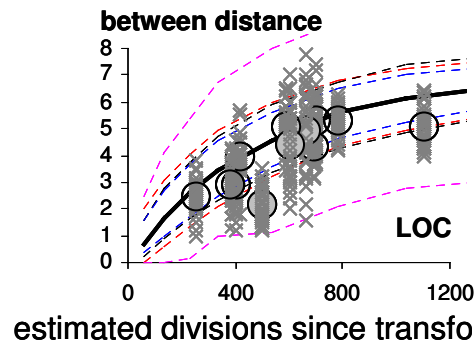
Similar results are seen with LOC tags, with the experimental data intermediate between the extreme simulated scenarios of one CSC per gland or with all cells effectively CSCs:



Simulations with intermediate numbers of either deterministic or stochastic CSCs per gland better fit the experimental LOC tag data:



As before, simulations with different numbers of CSCs per gland did not substantially change average LOC tag distances between opposite cancer sides, although 95% simulation intervals were greater with smaller numbers of CSCs per gland:



Experimental cancer tag values were intermediate between simulations with 1 CSC per gland or 4,096 CSCs per 8,196 cell gland (i.e. all CSCs). A single CSC scenario does not appear to fit the experimental data from all of the cancers. Therefore, cancer specific simulations were performed to ascertain which CSC scenarios best fit both their LOC and BGN experimental data, using stem cell multiples of two (ie 2, 4, ..., 2048 CSCs per gland) with either 100% asymmetric (deterministic) or 50% asymmetric (stochastic) division. The following Tables indicates the number of individual experimental values (unique tags per gland and intragland pairwise distances) that fell within 95% simulation intervals:

BGN Data				Unique Tags/Gland		Intragland Pairwise Distance		Summary			comments
	specimen	divisions	simulated stem cells per gland	survival	total sampled tags	tags within 95% simulation intervals	total sampled intragland distances	intragland distances within 95% simulation intervals	total comparisons	within 95% simulation intervals	
cancer 1	590	1024	0.5	13	13	13	12	26	25	0.962	
		1	1	13	0	13	0	26	0	0	
		4096	0.5	13	13	13	12	26	25	0.962	
		16	1	13	13	13	12	26	25	0.962	
cancer 2	250	1024	0.5	14	14	14	14	28	28	1	
		1	1	14	10	14	8	28	18	0.643	
		4096	0.5	14	12	14	12	28	24	0.857	
		8	1	14	14	14	14	28	28	1	
cancer 3	600	64	0.5	14	12	14	12	28	24	0.857	
		1	1	14	5	14	3	28	8	0.286	
		4096	0.5	14	6	14	7	28	13	0.464	
		4	1	14	10	14	11	28	21	0.75	
cancer 4 8 CpG sites	660	128	0.5	14	13	14	14	28	27	0.964	
		1	1	14	6	14	5	28	11	0.393	
		4096	0.5	14	6	14	7	28	13	0.464	
		4	1	14	13	14	10	28	23	0.821	
cancer 5	690	1024	0.5	14	13	14	13	28	26	0.929	
		1	1	14	1	14	1	28	2	0.071	
		4096	0.5	14	13	14	13	28	26	0.929	
		8	1	14	12	14	12	28	24	0.857	
cancer 6	420	512	0.5	14	13	14	13	28	26	0.929	0.00005 error rate
		1	1	14	12	14	11	28	23	0.821	
		4096	0.5	14	13	14	13	28	26	0.929	
		8	1	14	12	14	13	28	25	0.893	
cancer 7	1130	256	0.5	14	14	14	14	28	28	1	
		1	1	14	3	14	3	28	6	0.214	
		4096	0.5	14	3	14	0	28	3	0.107	
		4	1	14	14	14	14	28	28	1	
cancer 8 8 CpG sites	400	256	0.5	14	14	14	14	28	28	1	
		1	1	14	4	14	2	28	6	0.214	
		4096	0.5	14	10	14	12	28	22	0.786	
		4	1	14	13	14	14	28	27	0.964	
cancer 9 8 CpG sites	700	1024	0.5	14	13	14	13	28	26	0.929	
		1	1	14	1	14	1	28	2	0.071	
		4096	0.5	14	12	14	12	28	24	0.857	
		16	1	14	13	14	12	28	25	0.893	
cancer 10	380	128	0.5	14	14	14	14	28	28	1	
		1	1	14	2	14	0	28	2	0.071	
		4096	0.5	14	14	14	12	28	26	0.929	
		8	1	14	14	14	14	28	28	1	
cancer 11	780	1024	0.5	14	12	14	13	28	25	0.893	
		1	1	14	0	14	0	28	0	0	
		4096	0.5	14	10	14	11	28	21	0.75	
		8	1	14	14	14	13	28	27	0.964	
cancer 12	1100	1024	0.5	14	12	14	13	28	25	0.893	
		1	1	14	0	14	0	28	0	0	
		4096	0.5	14	11	14	13	28	24	0.857	
		8	1	14	13	14	13	28	26	0.929	
						TOTALS					
						multiple stochastic		334	316	0.946	
						one CSC per gland		334	78	0.234	
						all CSCs		334	247	0.74	
						multiple deterministic		334	307	0.919	

LOC data				Unique Tags/Gland		Intragland Pairwise Distance		Summary			comments
	specimen	divisions	simulated stem cells per gland	survival	total sampled tags	tags within 95% simulation intervals	total sampled intragland distances	intragland distances within 95% simulation intervals	total comparisons	within 95% simulation intervals	
cancer 1	590	1024	0.5	10	10	10	7	20	17	0.85	
		1	1	10	0	10	0	20	0	0	
		4096	0.5	10	9	10	9	20	18	0.9	
		16	1	10	10	10	8	20	18	0.9	
cancer 2	250	1024	0.5	12	12	12	11	24	23	0.958	
		1	1	12	0	12	1	24	1	0.042	
		4096	0.5	12	9	12	9	24	18	0.75	
		8	1	12	10	12	11	24	21	0.875	
cancer 3	600	64	0.5	12	8	12	8	24	16	0.667	
		1	1	12	7	12	4	24	11	0.458	
		4096	0.5	12	2	12	1	24	3	0.125	
		4	1	12	7	12	2	24	9	0.375	
cancer 4	660	128	0.5	12	11	12	12	24	23	0.958	
		1	1	12	0	12	0	24	0	0	
		4096	0.5	12	6	12	0	24	6	0.25	
		4	1	12	8	12	5	24	13	0.542	
cancer 5	690	1024	0.5	11	10	11	10	22	20	0.909	
		1	1	11	1	11	0	22	1	0.045	
		4096	0.5	11	10	11	6	22	16	0.727	
		8	1	11	5	11	10	22	15	0.682	
cancer 6	420	512	0.5	10	10	10	9	20	19	0.95	
		1	1	10	0	10	0	20	0	0	
		4096	0.5	10	9	10	6	20	15	0.75	
		8	1	10	7	10	9	20	16	0.8	
cancer 7	1130	256	0.5	12	12	12	12	24	24	1	0.0005 demethylation error rate
		1	1	12	0	12	0	24	0	0	
		4096	0.5	12	12	12	8	24	20	0.833	
		4	1	12	12	12	12	24	24	1	
cancer 8	400	256	0.5	12	12	12	10	24	22	0.917	
		1	1	12	1	12	0	24	1	0.042	
		4096	0.5	12	5	12	2	24	7	0.292	
		4	1	12	8	12	10	24	18	0.75	
cancer 9	700	1024	0.5	12	11	12	8	24	19	0.792	
		1	1	12	0	12	0	24	0	0	
		4096	0.5	12	11	12	5	24	16	0.667	
		16	1	12	11	12	8	24	19	0.792	
cancer 10	380	128	0.5	12	12	12	12	24	24	1	
		1	1	12	3	12	1	24	4	0.167	
		4096	0.5	12	5	12	7	24	12	0.5	
		8	1	12	11	12	8	24	19	0.792	
cancer 11	780	1024	0.5	10	10	10	10	20	20	1	
		1	1	10	0	10	0	20	0	0	
		4096	0.5	10	10	10	8	20	18	0.9	
		8	1	10	2	10	10	20	12	0.6	
cancer 12	1100	1024	0.5	12	12	12	12	24	24	1	
		1	1	12	0	12	0	24	0	0	
		4096	0.5	12	12	12	6	24	18	0.75	
		8	1	12	6	12	12	24	18	0.75	
				TOTALS							
				multiple stochastic				274	251	0.916	
				one CSC per gland				274	18	0.066	
				all CSCs				274	167	0.609	
				multiple deterministic				274	202	0.737	

Simulations with either small numbers of deterministic CSCs (4 to 16 per gland) or greater numbers of stochastic CSCs (64 to 1,024 per gland) were more consistent with the experimental data (see Table 3 in the manuscript and the Table below). In addition, it appears that the numbers of CSCs per cancer gland can differ between cancers (see Table 2 the manuscript).

Robustness of the Simulations:

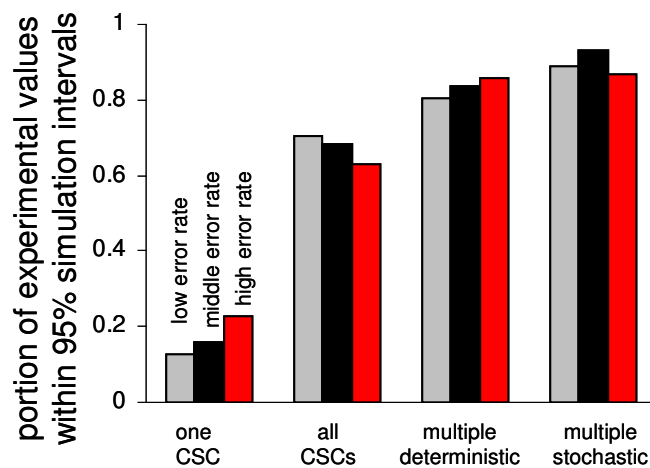
To test whether changes in parameter values markedly change our conclusions about numbers of CSCs per cancer gland, simulations were performed with different methylation error rates. The conclusion that cancer glands are maintained by a hierarchy with intermediate numbers of CSCs was unchanged when error rates were increased two-fold higher (with average cancer ages of 10.5 months) or decreased two-fold lower (with average cancer ages of 42 months).

Portions of intragland experimental values within 95 percent simulation intervals

CSC Scenario	BGN tags low/middle/high error rates*	LOC tags low/middle/high error rates*	Both low/middle/high error rates*
one CSC per gland	0.19/0.23/0.31	0.05/0.07/0.12	0.13/0.16/0.23
all CSCs	0.72/0.74/0.68	0.69/0.61/0.56	0.70/0.68/0.63
multiple deterministic CSCs	0.89/0.92/0.90	0.71/0.73/0.80	0.81/0.84/0.86
multiple stochastic CSCs	0.87/0.95/0.90	0.92/0.92/0.83	0.89/0.93/0.87
total comparisons	334	274	608

*BGN low/middle/high error rates are 0.00015, 0.0003, and 0.0006 changes per CpG site per division. LOC low/middle/high error rates are 0.00025, 0.0005, and 0.001 changes per CpG site per division.

The proportions of BGN and LOC experimental data consistent with the simulations are illustrated:



1. Yatabe Y, Tavaré S, Shibata D (2001) Investigating stem cells in human colon by using methylation patterns. Proc Natl Acad Sci USA 98:10839-10844.

0.00
0.00
0.00
0.75
0.63
0.00
0.00
0.00
0.13
0.13
0.88
0.75
0.13
0.13
0.00
0.00
0.75
0.63
0.00
0.00
0.00

average 0.36

Cancer 7

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	43	CRA-1	0.40	3.00	0.68		0.50	1.13	3.06
	43	CRA-2	0.44	3.00	0.50		0.59	1.91	3.00
	43	CRA-3	0.40	5.00	1.82		0.63	2.03	3.47
	43	CRA-4	0.47	3.00	0.50		0.63	2.13	3.00
	43	CRA-6	0.32	4.00	1.82		0.63	2.13	3.34
	43	CRA-7	0.29	2.00	0.75		0.69	2.13	3.09
	43	CRA-5	0.46	3.00	0.68		1.22	2.25	3.66
							1.31	2.59	5.63
							1.31	2.63	5.38
							1.31	2.66	4.63
side 2	43	CRB-1	0.33	4.00	0.93		1.34	2.75	5.25
	43	CRB-2	0.61	2.00	0.57		1.38	2.75	5.88
	43	CRB-3	0.35	5.00	1.25		1.41	2.88	5.38
	43	CRB-4	0.57	2.00	0.25		1.44	2.88	6.38
	43	CRB-5	0.50	5.00	1.21		1.47	2.97	2.97
	43	CRB-6	0.40	4.00	1.18		1.50	3.13	2.91
	43	CRB-7	0.40	3.00	0.82		1.59	3.31	2.63
							1.63	3.50	2.91
Side 1	cra	ave	0.40	3.29	0.96		1.75	3.88	3.22
Side 2	crb	ave	0.45	3.57	0.89		1.78	4.25	2.84
Both	cr	all ave	0.43	3.43	0.93		2.09	4.50	3.56
						average	1.25	2.78	1.50
						overall	2.01		1.34

2.00
1.13
1.50
2.25
2.50
3.88
3.53
3.69
3.69
4.13
3.91
4.72
4.22
4.16
3.53
4.16
4.47
3.88
4.72
3.75
3.63
4.09
3.59
4.00
3.75
4.50

average 3.64

Cancer 8

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	98	CZA-1	0.78	3.00	0.79		0.34	0.75	1.63
	98	CZA-2	0.75	3.00	0.50		0.38	1.13	1.38
	98	CZA-3	0.70	2.00	0.75		0.47	1.41	1.50
	98	CZA-4	0.77	2.00	0.25		0.47	1.44	1.25
	98	CZA-5	0.75	5.00	1.43		0.50	1.47	1.38
	98	CZA-6	0.72	2.00	0.50		0.53	1.63	1.34
	98	CZA-7	0.73	4.00	0.75		0.59	1.97	1.50
							0.59	2.13	5.06
							0.59	2.25	4.88
side 2	98	CZB-1	0.86	2.00	0.25		0.63	2.47	4.59
	98	CZB-2	0.42	3.00	1.68		0.66	2.66	4.97
	98	CZB-3	0.75	3.00	0.50		0.72	2.69	4.44
	98	CZB-4	0.80	3.00	1.04		0.75	2.78	4.84
	98	CZB-5	0.63	3.00	0.86		0.75	2.88	5.00
	98	CZB-6	0.69	5.00	1.71		0.84	3.13	0.72
	98	CZB-7	0.53	6.00	2.14		0.84	3.41	0.50
							0.94	3.69	0.59
							0.97	3.84	0.38
Side 1	98	ave A	0.74	3.00	0.71		1.03	3.94	0.91

Side 2	98 ave B	0.67	3.57	1.17	1.06	4.03	0.50
Both	98 ave all	0.71	3.29	0.94	1.13	4.66	0.59
					average	0.70	2.59
					overall	1.65	1.88
							1.53
							1.66
							1.50
							1.59
							1.53
							1.72
							1.75
							1.69
							1.56
							1.63
							1.81
							1.69
							1.63
							2.97
							2.56
							2.63
							2.63
							2.50
							2.56
							2.75
							2.22
							1.84
							1.81
							1.88
							2.19
							1.75
							2.09
						average	2.11

Cancer 9

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	85	CP1-1	0.63	5.00	2.07		1.00	1.44	2.50
	85	CP1-2	0.70	6.00	2.04		1.19	1.84	2.22
	85	CP1-3	0.78	4.00	1.07		1.50	1.88	1.69
	85	CP1-4	0.78	6.00	2.36		1.53	1.91	2.25
	85	CP1-5	0.78	5.00	1.86		1.59	1.97	1.91
	85	CP1-6	0.88	2.00	0.50		1.75	2.03	1.31
	85	CP1-7	0.73	7.00	2.96		2.00	2.06	2.81
							2.09	2.13	3.50
							2.16	2.13	2.66
side 2	85	CP2-1	0.80	5.00	1.96		2.34	2.16	2.28
	85	CP2-2	0.78	7.00	2.86		2.38	2.28	2.63
	85	CP2-3	0.75	7.00	2.29		2.44	2.31	2.47
	85	CP2-4	0.61	4.00	1.32		2.59	2.38	2.06
	85	CP2-5	0.78	4.00	1.71		2.59	2.53	2.94
	85	CP2-6	0.84	3.00	1.86		2.69	2.56	2.75

	85 CP2-7	0.75	4.00	0.93		2.72	2.59	2.53
						2.75	2.72	1.81
Side 1	85 CP1-AVE	0.75	5.00	1.84		2.81	2.75	2.53
Side 2	85 CP2-AVE	0.76	4.86	1.85		2.84	2.81	2.19
Both	85 ave all	0.76	4.93	1.84		3.22	2.91	1.69
						3.66	2.97	3.00
					average	2.28	2.30	3.09
					overall	2.34		2.19
								1.72
								2.81
								2.47
								2.28
								3.19
								2.56
								1.81
								1.41
								2.09
								1.66
								1.19
								2.81
								2.75
								2.19
								2.16
								2.22
								2.13
								1.56
								2.78
								2.06
								1.53
								1.53
								2.28
								1.56
								1.25
								3.03
							average	2.25

Cancer 10

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
		HA-1	0.61	4.00	1.71		4.50	2.41	2.13
		HA-2	0.51	3.00	1.32		1.88	2.47	2.38
		HA-3	0.72	4.00	1.36		2.47	2.75	1.88
		HA-4	0.53	2.00	1.07		1.50	2.41	2.13
		HA-5	0.53	4.00	1.36		1.06	2.88	2.00
		HA-6	0.57	3.00	1.32		2.34	2.19	1.75
		HA-7	0.65	4.00	1.89		1.63	2.00	2.25
							1.22	1.88	1.69
							2.31	2.38	1.91
		HC-1	0.53	6.00	2.14		1.09	2.38	2.03
		HC-2	0.44	6.00	2.21		1.63	2.25	2.16

HC-3	0.67	5.00	2.36	1.28	2.50	1.63
HC-4	0.69	4.00	2.07	2.03	2.13	1.84
HC-5	0.50	2.00	1.71	1.19	2.38	1.81
HC-6	0.58	5.00	2.21	1.28	2.13	2.59
HC-7	0.47	3.00	1.86	1.63	2.22	3.19
				1.91	1.88	2.13
HA AVE	0.59	3.43	1.43	1.84	2.63	2.06
HC AVE	0.56	4.43	2.08	1.78	2.72	2.63
ave all	0.57	3.93	1.76	1.88	1.63	2.13
				1.84	2.34	3.06
				average	1.82	2.31
				overall	2.07	1.66
						1.78
						1.91
						2.03
						1.50
						1.75
						1.69
						1.78
						1.88
						2.00
						2.03
						1.63
						1.88
						1.84
						1.63
						2.09
						1.97
						1.84
						1.63
						1.84
						1.94
						2.38
						2.66
						1.91
						2.13
						2.25
						1.88
						2.53
					average	2.03

Cancer 11

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1		83 COC1-1	0.79	7.00	2.11		1.28	1.75	2.41
		83 COC1-2	0.75	7.00	2.86		1.94	1.75	2.75
		83 COC1-3	0.89	4.00	1.50		2.16	1.78	1.75
		83 COC1-4	0.94	3.00	0.79		2.25	2.28	1.38
		83 COC1-5	0.65	7.00	3.25		2.38	2.31	2.84
		83 COC1-6	0.76	6.00	2.96		2.44	2.44	2.22
		83 COC1-7	0.74	4.00	2.18		2.44	2.50	2.06

						2.47	2.50	2.75
						2.50	2.59	3.34
side 2	83 COC2-1	0.90	3.00	1.11		2.56	2.69	2.53
	83 COC2-2	0.78	6.00	2.71		2.63	2.81	2.22
	83 COC2-3	0.69	5.00	2.14		2.66	2.94	3.09
	83 COC2-4	0.78	6.00	2.86		2.66	2.94	3.03
	83 COC2-5	0.89	5.00	1.71		2.81	2.94	2.75
	83 COC2-6	0.68	7.00	2.68		2.88	3.19	2.75
	83 COC2-7	0.90	5.00	1.61		3.06	3.22	2.59
						3.06	3.44	2.53
						3.41	3.50	3.03
Side 1	83 ave1	0.79	5.43	2.23		3.53	3.69	4.44
Side 2	83 ave2	0.80	5.29	2.12		3.69	4.00	3.13
Both	83 ave all	0.80	5.36	2.18		3.97	4.34	3.56
					average	2.70	2.84	3.13
					overall	2.77		3.06
								2.44
								2.06
								3.31
								3.06
								3.13
								2.59
								2.97
								1.88
								1.50
								3.69
								2.78
								3.06
								3.28
								4.09
								3.25
								3.28
								3.78
								3.56
								3.16
								2.22
								2.44
								1.53
								1.22
								3.44
								2.56
								2.84
							average	2.79

Cancer 12

BGN	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	44	CXA1	0.39	6.00	3.43		2.44	2.44	4.34
	44	CXA2	0.31	7.00	3.36		2.56	2.50	4.81
	44	CXA3	0.44	6.00	2.36		2.81	2.63	3.34

	44 CXA4	0.18	4.00	1.75		2.91	2.66	4.97
	44 CXA5	0.43	3.00	2.18		2.97	2.66	3.94
	44 CXA6	0.49	7.00	4.18		3.06	2.78	4.19
	44 CXA7	0.31	7.00	3.14		3.09	3.00	4.97
						3.09	3.16	4.09
						3.13	3.19	4.53
						3.16	3.19	2.75
						3.22	3.25	4.03
side 2	44 CXB1	0.67	5.00	2.64		3.28	3.28	3.38
	44 CXB2	0.58	6.00	2.57		3.31	3.31	4.06
	44 CXB3	0.47	7.00	3.79		3.56	3.34	4.59
	44 CXB4	0.53	5.00	2.64		3.66	3.41	4.59
	44 CXB5	0.58	3.00	2.64		3.75	3.53	4.53
	44 CXB6	0.67	6.00	2.86		3.84	3.75	4.38
	44 CXB7	0.50	6.00	3.50		3.94	3.78	4.41
						3.97	3.88	4.56
Side 1	44 AVE A	0.36	5.71	2.91		4.00	3.97	4.44
Side 2	44 AVE B	0.57	5.43	2.95		4.03	4.00	4.56
Both	44 AVE ALL	0.47	5.57	2.93	average	3.32	3.22	3.66
					overall	3.27		3.81
								3.28
								4.06
								3.25
								4.28
								3.75
								3.75
								4.25
								2.78
								4.09
								3.28
								4.13
								4.47
								4.00
								4.50
								3.19
								4.78
								3.50
								4.03
								4.63
								3.94
								4.16
								3.31
								4.13
								3.84
								4.53
								4.53
						average		4.07

BGN Data: Summary

Cancer	Side	side methylation	average methylation	unique tags	average tags	intragland distance	average intragland	distance within side	average side distance	distance between sides
1	1	0.86	0.87	5.71	5.31	1.95	1.78	1.73	1.89	1.86
1	2	0.88		4.83		1.57		2.06		
2	1	0.70	0.70	3.29	2.86	0.75	0.59	1.46	1.26	1.23
2	2	0.70		2.43		0.42		1.06		
3	1	0.48	0.50	2.43	3.21	0.60	1.15	2.43	2.45	2.44
3	2	0.52		4.00		1.70		2.47		
4	1	0.80	0.78	3.86	3.29	1.09	0.94	2.04	1.65	2.33
4	2	0.75		2.71		0.80		1.26		
5	1	0.70	0.75	5.71	5.50	2.43	2.19	3.28	2.84	2.93
5	2	0.81		5.29		1.94		2.40		
6	1	0.02	0.02	1.57	1.71	0.22	0.24	0.39	0.38	0.36
6	2	0.02		1.86		0.27		0.36		
7	1	0.40	0.43	3.29	3.43	0.96	0.93	1.25	2.01	3.64
7	2	0.45		3.57		0.89		2.78		
8	1	0.74	0.71	3.00	3.29	0.71	0.94	0.70	1.65	2.11
8	2	0.67		3.57		1.17		2.59		
9	1	0.75	0.76	5.00	4.93	1.84	1.84	2.28	2.29	2.25
9	2	0.76		4.86		1.85		2.30		
10	1	0.59	0.57	3.43	3.93	1.43	1.76	1.82	2.07	2.03
10	2	0.56		4.43		2.08		2.31		
11	1	0.79	0.80	5.43	5.36	2.23	2.18	2.70	2.77	2.79
11	2	0.80		5.29		2.12		2.84		
12	1	0.36	0.47	5.71	5.57	2.91	2.93	3.32	3.27	4.07
12	2	0.57		5.43		2.95		3.22		
overall averages			0.59		3.92		1.43		2.06	2.38

LOC Cancer Gland Data

Cancer 1

LOC	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1		65CWA1	0.55	8.00	5.93		5.19	5.31	5.47
		65CWA2	0.59	8.00	4.00		5.09	7.84	5.56
		65CWA4	0.67	8.00	3.39		5.06	4.72	5.84
		65CWA5	0.69	8.00	4.04		5.75	5.50	5.19
		65CWA6	0.46	8.00	5.75		3.91	5.78	5.34
		65CWA7					4.16	5.06	4.88
		65CWA8					5.28	5.38	5.03
							3.53	5.28	5.63
						5.28	5.13	4.28	
						5.75	4.72	4.91	
side 2		65CWB4	0.84	5.00	1.57	average	4.90	5.47	3.44
		65CWB5	0.57	8.00	6.00	overall	5.19		4.94
		65CWB7	0.30	8.00	4.07				6.19
		65CWB8	0.58	8.00	4.61				4.00
		65CWB9	0.51	8.00	5.46				4.72
		65CWB10							3.66
								5.06	
								6.44	
Side 1	65 ave A	0.59	8.00	4.62					4.41
Side 2	65 ave B	0.56	7.40	4.34					5.09
Both	65 ave all	0.58	7.70	4.48					6.50
									5.81
									5.19
									5.25
									5.22
								average	5.12

Cancer 2

LOC	age	gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1		79CNC1-2	0.84	6.00	2.71		2.84	3.00	2.31
		79CNC1-3	0.89	6.00	1.71		2.50	2.59	3.53
		79CNC1-4	0.81	4.00	1.18		2.81	2.31	2.88
		79CNC1-5	0.85	8.00	2.82		3.28	2.47	2.75
		79CNC1-6	0.88	8.00	2.96		2.50	2.09	2.94
		79CNC1-7	0.97	4.00	0.75		2.75	2.28	2.28
							2.53	2.28	2.41
side 2		79CNC2-1	0.88	5.00	2.14		2.53	2.28	2.91
		79CNC2-2	0.88	4.00	2.43		1.66	2.84	2.28
		79CNC2-3	0.92	7.00	1.96		2.25	1.63	2.00
		79CNC2-4	0.96	5.00	1.25		3.53	1.78	2.09
		79CNC2-5	0.94	5.00	1.75		2.91	2.09	2.47
		79CNC2-7	0.90	5.00	1.96		3.09	1.44	2.66
							2.38	1.84	3.81
						1.88	2.16	2.75	

Cancer 5								
LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	53 CTA-1	0.47	8.00	3.96		4.19	3.63	3.88
	53 CTA-2	0.49	8.00	4.82		4.59	4.47	4.44
	53 CTA-3	0.54	7.00	4.86		3.88	3.81	4.94
	53 CTA-4	0.49	7.00	2.96		5.03	3.94	4.16
	53 CTA-5	0.60	3.00	1.82		4.78	3.44	4.56
						4.06	4.78	4.13
						5.09	4.19	3.97
						3.94	4.00	4.28
side 2	53 CTB-1	0.55	7.00	3.64		5.06	3.59	4.94
	53 CTB-2	0.65	8.00	3.54		3.28	4.69	4.38
	53 CTB-3	0.47	8.00	5.32	average	4.39	5.06	4.50
	53 CTB-4	0.51	8.00	3.54			4.41	4.22
	53 CTB-5	0.57	8.00	4.50			4.75	4.31
	53 CTB-6	0.54	7.00	3.36			3.75	4.63
						4.22		5.34
Side 1	53 ave CTA	0.52	6.60	3.69	average		4.18	4.38
Side 2	53 ave CTB	0.55	7.67	3.98	overall	#REF!		4.88
Both	53 ave all CT	0.54	7.18	3.85				4.53
								3.63
								4.03
								4.38
								3.13
								4.38
								3.69
								4.31
								4.59
								4.81
								3.25
								5.03
								4.16
							average	4.33
Cancer 6								
LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	51 CSA-1	0.75	8.00	3.71		4.00	4.03	3.72
	51 CSA-2	0.74	8.00	3.89		3.88	3.84	3.72
	51 CSA-4	0.67	6.00	3.25		5.44	3.78	3.78
	51 CSA-6	0.70	7.00	2.36		4.16	4.22	3.91
	51 CSA-3	0.92	6.00	1.96		3.88	3.94	3.72
						4.47	4.06	3.50
side 2	51 CSC-1					4.06	2.88	3.47
	51 CSC-2	0.73	6.00	2.43		4.47	4.78	4.13
	51 CSC-3	0.74	7.00	2.61		4.03	4.00	3.78

Cancer 8								
LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	98 CZA-1	0.88	7.00	2.04		1.75	3.78	4.00
	98 CZA-2	0.87	5.00	1.32		1.97	3.69	2.97
	98 CZA-3	0.90	8.00	1.96		2.09	3.75	2.25
	98 CZA-4	0.86	5.00	1.93		2.75	3.91	2.13
	98 CZA-5	0.81	8.00	3.75		2.16	4.16	2.56
	98 CZA-6	0.95	5.00	1.43		1.72	2.25	3.41
						1.72	2.06	4.03
						2.63	2.34	2.69
side 2	98 CZB-1	0.76	8.00	5.32		2.34	2.47	2.53
	98 CZB-2	0.88	6.00	2.11		1.97	1.38	2.38
	98 CZB-3	0.94	5.00	1.61		2.84	1.94	2.78
	98 CZB-4	0.96	4.00	0.93		1.91	2.84	3.22
	98 CZB-5	0.92	3.00	2.11		2.91	1.59	4.03
	98 CZB-7	0.84	5.00	3.00		2.38	2.63	2.59
						2.97	2.81	2.09
Side 1	98 AVE CZA	0.88	6.33	2.07	average	2.27	2.77	1.84
Side 2	98 AVE CZB	0.88	5.17	2.51	overall	2.52		2.38
Both	98 AVE ALL	0.88	5.75	2.29				3.13
								4.25
								3.16
								2.47
								2.50
								3.03
								3.69
								4.56
								3.63
								3.16
								3.00
								3.53
								4.00
								3.81
								2.28
								1.53
								1.22
								1.81
								2.91
							average	2.93

Cancer 9

LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	85 CP1-2	0.70	7.00	2.57		4.53	4.41	6.31
	85 CP1-3	0.40	8.00	3.39		4.41	4.66	5.75
	85 CP1-4	0.63	7.00	4.21		4.16	5.94	5.13
	85 CP1-5	0.64	6.00	2.86		5.22	4.25	6.25
	85 CP1-6	0.63	8.00	5.57		5.09	4.91	5.44
	85 CPA1-1	0.57	8.00	4.21		4.25	6.91	4.53
						3.91	5.75	6.69
side 2	85 CP2-1	0.65	8.00	4.61		4.88	6.66	4.84
	85 CP2-2	0.41	7.00	2.64		4.47	4.69	4.31
	85 CP2-3	0.78	7.00	3.25		4.69	5.25	6.19
	85 CP2-4	0.60	7.00	3.25		5.31	4.09	3.75
	85 CP2-5	0.61	8.00	4.93		3.69	4.38	6.22
	85 CP2-6	0.70	4.00	2.64		4.72	4.16	4.53
						4.50	5.41	7.19
Side 1	85 AVE1	0.59	7.33	3.80	average	4.56	5.10	6.06
Side 2	85 AVE2	0.62	6.83	3.55	overall	4.83		7.38
Both	85 ave CP all	0.61	7.08	3.68				6.19
								4.78
								5.06
								4.75
								4.84
								5.88
								4.34
								4.03
								4.66
								4.31
								3.97
								5.50
								3.66
								5.47
								5.84
								5.22
								5.50
								6.34
								5.34
							average	5.10

Cancer 10

LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	61 HA-1	0.79	6.00	2.57		2.78	2.03	2.84
	61 HA-4	0.82	5.00	1.50		3.53	2.66	3.31
	61 HA-5	0.69	7.00	2.32		2.19	3.31	3.75
	61 HA-6	0.84	3.00	1.64		3.03	1.41	3.06
	61 HA-7	0.73	6.00	2.36		2.13	2.66	3.75
	61 HA-8	0.74	7.00	2.39		3.56	3.16	4.31
						2.50	2.50	1.25
side 2	61 HC-1	0.82	3.00	0.93		2.78	3.16	2.25
	61 HC-2	0.72	6.00	2.54		3.38	3.66	2.53

Cancer 12								
LOC	age gland	gland methylation	unique tags per gland	intradistance	interdistances	side 1	side 2	between sides
side 1	44 CXA-1	0.66	8.00	4.93		5.44	3.78	4.34
	44 CXA-2	0.61	8.00	5.71		5.56	5.41	5.06
	44 CXA-3	0.47	8.00	4.89		4.78	3.41	6.09
	44 CXA-5	0.54	8.00	4.75		5.16	3.13	4.31
	44 CXA-6	0.67	8.00	5.32		4.56	4.06	4.72
	44 CXA-7	0.56	7.00	3.46		5.72	5.72	5.31
						5.31	3.84	5.13
						5.66	3.66	6.13
side 2	44 CXB-1	0.60	7.00	3.46		4.72	4.06	6.22
	44 CXB-2	0.59	6.00	3.79		4.94	5.47	5.41
	44 CXB-3	0.45	7.00	5.50		5.63	5.13	5.13
	44 CXB-4	0.66	8.00	3.64		4.75	5.00	5.81
	44 CXB-5	0.63	6.00	2.93		5.59	3.38	4.44
	44 CXB-7	0.49	7.00	3.18		4.66	4.19	5.19
						4.97	3.78	5.53
Side 1	44 ave cxa	0.59	7.83	4.85	average	5.16	4.27	4.94
Side 2	44 ave cxb	0.57	6.83	3.75	overall	4.71		4.38
Both	44 ave all cx	0.58	7.33	4.30				5.31
								4.09
								4.69
								5.44
								4.34
								4.28
								4.31
								4.66
								5.34
								6.44
								4.66
								4.81
								6.38
								3.94
								5.47
								5.66
								4.56
								4.28
								5.25
							average	5.06

LOC Data: Summary

Cancer	Side	side methylation	average methylation	unique tags	average tags	intragland distance	average intragland	distance within side	average side distance	distance between sides
1	1	0.59	0.58	8.00	7.70	4.62	4.48	4.90	5.19	5.12
1	2	0.56		7.40		4.34		5.47		
2	1	0.88	0.89	6.00	5.58	2.02	1.97	2.63	2.42	2.48
2	2	0.91		5.17		1.92		2.21		
3	1	0.79	0.75	2.67	3.25	1.05	1.26	3.75	3.67	4.41
3	2	0.72		3.83		1.46		3.60		
4	1	0.71	0.63	5.83	5.83	2.27	2.10	3.47	3.66	4.92
4	2	0.56		5.83		1.93		3.85		
5	1	0.52	0.54	6.60	7.18	3.69	3.85	4.39	4.29	4.33
5	2	0.55		7.67		3.98		4.18		
6	1	0.76	0.76	7.00	6.80	3.04	2.83	4.40	4.20	3.95
6	2	0.76		6.60		2.62		4.01		
7	1	0.93	0.90	4.67	4.50	1.42	1.34	1.75	1.71	2.18
7	2	0.87		4.33		1.26		1.66		
8	1	0.88	0.88	6.33	5.75	2.07	2.29	2.27	2.52	2.93
8	2	0.88		5.17		2.51		2.77		
9	1	0.59	0.61	7.33	7.08	3.80	3.68	4.54	4.86	5.31
9	2	0.62		6.83		3.55		5.17		
10	1	0.77	0.75	5.67	4.83	2.13	1.96	2.81	2.87	2.92
10	2	0.74		4.00		1.78		2.92		
11	1	0.59	0.58	7.80	7.80	4.51	4.69	5.11	5.37	5.31
11	2	0.57		7.80		4.88		5.63		
12	1	0.59	0.58	7.83	7.33	4.85	4.30	5.16	4.71	5.06
12	2	0.57		6.83		3.75		4.27		
overall averages			0.70		6.14		2.89		3.79	4.08