

Supplementary Material for  
**Differential Expression in SAGE: Accounting for  
Normal Between-Library Variation**

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January 6, 2003

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## Unconditional Mean and Var of $X_i/n_i$

Here,

$$p_i \sim \text{Beta}(\alpha, \beta), \quad E(p_i) = \frac{\alpha}{\alpha + \beta}, \quad V(p_i) = \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)}.$$

The second part of our model says that given the true proportion in a sample, the corresponding count will have a binomial distribution with the true proportion as a parameter:

$$X_i|p_i \sim \text{Binomial}(n_i, p_i).$$

to get the unconditional mean and variance of the estimated proportion  $\hat{p}_i = X_i/n_i$  we make use of the tower property of conditional expectation, that  $E(X) = E(E(X|Y))$ . Here, this yields

$$\begin{aligned} E(X_i) &= E[E(X_i|p_i, n_i)] \\ &= E(n_i p_i) \\ &= n_i \frac{\alpha}{\alpha + \beta} \\ E(X_i/n_i) &= \frac{\alpha}{\alpha + \beta} \\ E(X_i^2) &= E[E(X_i^2|p_i, n_i)] \\ &= E(n_i p_i(1 - p_i) + (n_i p_i)^2) \\ &= n_i \frac{\alpha}{\alpha + \beta} + n_i(n_i - 1) \left[ \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)} + \frac{\alpha^2}{(\alpha + \beta)^2} \right] \\ V(X_i) &= n_i \frac{\alpha}{\alpha + \beta} + n_i(n_i - 1) \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)} - n_i \frac{\alpha^2}{(\alpha + \beta)^2} \\ &= n_i^2 \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)} + n_i \left[ \frac{\alpha}{\alpha + \beta} - \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)} - \frac{\alpha^2}{(\alpha + \beta)^2} \right] \\ &= \frac{\alpha}{\alpha + \beta} \left\{ n_i^2 \frac{\beta}{(\alpha + \beta)(\alpha + \beta + 1)} + n_i \left[ 1 - \frac{\beta}{(\alpha + \beta)(\alpha + \beta + 1)} - \frac{\alpha}{\alpha + \beta} \right] \right\} \\ &= \frac{\alpha}{\alpha + \beta} \left\{ n_i^2 \frac{\beta}{(\alpha + \beta)(\alpha + \beta + 1)} + n_i \left[ \frac{\alpha^2 + 2\alpha\beta + \beta^2 + \alpha + \beta - \beta - \alpha^2 - \alpha\beta - \alpha}{(\alpha + \beta)(\alpha + \beta + 1)} \right] \right\} \\ &= \frac{\alpha}{\alpha + \beta} \left\{ n_i^2 \frac{\beta}{(\alpha + \beta)(\alpha + \beta + 1)} + n_i \left[ \frac{\beta(\alpha + \beta)}{(\alpha + \beta)(\alpha + \beta + 1)} \right] \right\} \\ &= \frac{\alpha\beta}{(\alpha + \beta)(\alpha + \beta + 1)} \left[ n_i^2 \frac{1}{\alpha + \beta} + n_i \right] \\ V(X_i/n_i) &= \frac{\alpha\beta}{(\alpha + \beta)(\alpha + \beta + 1)} \left[ \frac{1}{\alpha + \beta} + \frac{1}{n_i} \right]. \end{aligned}$$

## Derivation of $\hat{V}_{umb}$

We recall that the general form of the variance of a single proportion is

$$V(X_i/n_i) = \sigma_1^2 + \frac{\sigma_2^2}{n_i},$$

with the first piece (which doesn't change with  $n_i$ ) coming from the variation between libraries and the second (which changes with  $n_i$ ) from the sampling variability within a library. The explicit values of  $\sigma_1^2$  and  $\sigma_2^2$  can be found by matching terms with the value for  $V(X_i/n_i)$  found in the previous section; we have simply found this notation a convenient shorthand. Given this form, the variance of a weighted combination of proportions is of the form

$$V(\sum w_i(X_i/n_i)) = \sum w_i^2 \sigma_1^2 + \sum w_i^2 \frac{\sigma_2^2}{n_i}.$$

This is the quantity that we wish to estimate. Now, to check the bias of certain estimators, we need some expectations. Specifically, we note that

$$\begin{aligned} E(X_i/n_i) &= \mu \\ E((X_i/n_i)^2) &= \sigma_1^2 + \frac{\sigma_2^2}{n_i} + \mu^2. \end{aligned}$$

Again, the value for  $\mu$  can be found by plugging in the value from the previous section. Note that the expectation of the quadratic term is different for different proportions. Starting with the estimate

$$\hat{V} = \sum w_i(X_i/n_i)^2 - \left(\sum w_i(X_i/n_i)\right)^2$$

and taking expectations yields

$$\begin{aligned} E(\hat{V}) &= \sum w_i \left( \sigma_1^2 + \frac{\sigma_2^2}{n_i} + \mu^2 \right) - \left( \mu^2 + \sum w_i^2 \left( \sigma_1^2 + \frac{\sigma_2^2}{n_i} \right) \right) \\ &= \sigma_1^2 \left( \sum w_i(1 - w_i) \right) + \sigma_2^2 \left( \sum w_i(1 - w_i)/n_i \right). \end{aligned}$$

Now, we want the multiplier for  $\sigma_1^2$  to be  $\sum w_i^2$ , so we could multiply  $\hat{V}$  by the ratio

$$\frac{\sum w_i^2}{\sum w_i(1 - w_i)}$$

to achieve this. Unfortunately, we want the multiplier for  $\sigma_2^2$  to be  $\sum w_i^2/n_i$ , and the appropriate multiplication factor for achieving this is

$$\frac{\sum w_i^2/n_i}{\sum w_i(1 - w_i)/n_i},$$

which is different than the factor suggested above. Thus, we cannot get an unbiased estimate of the quantity we want as a scalar multiple of an actual sample variance (which is guaranteed to be positive).

This problem has a few fixes. The fix we propose is associated with the idea of estimating variance components – essentially, finding unbiased estimates of  $\sigma_1^2$  and  $\sigma_2^2$  and combining them. Now, these estimates start from the estimate of the two combined, and involve some subtraction steps, so we can wind up with negative estimates. The result of one such excursion (based on trial and error) is

$$\hat{V}_0 = \sum w_i^2 (X_i/n_i)^2 - (\sum w_i^2) \left( \sum w_i (X_i/n_i) \right)^2.$$

Taking expectations here gives

$$\begin{aligned} E(\hat{V}_0) &= \sum w_i^2 \left( \sigma_1^2 + \frac{\sigma_2^2}{n_i} + \mu^2 \right) - (\sum w_i^2) \left[ \mu^2 + \sum w_i^2 \left( \sigma_1^2 + \frac{\sigma_2^2}{n_i} \right) \right] \\ &= \left( 1 - \sum w_i^2 \right) * \sigma_1^2 \sum w_i^2 + \left( 1 - \sum w_i^2 \right) * \sigma_2^2 \sum w_i^2 / n_i \end{aligned}$$

so that the quantity

$$\hat{V}_{unb} = \hat{V}_0 / (1 - \sum w_i^2)$$

is actually an unbiased estimate of the variance that we want. Empirically, this estimator does give rise to negative variances in some situations, which is one reason we couple it with a functional floor in the paper.

## Some Simulation Results

In order to assess the performance of our new statistic,  $t_w$ , we ran some small simulations comparing it with the two-sample  $t$ -test (with no assumption of equal variances) and with the  $\chi^2$  test (as a representative member of the class of pooled tests). For the simulations, we generated data as follows. First, the true mean proportions for group  $A$ ,  $p_A$ , was generated. Proportions (as counts out of 50K) of 1, 2, 5, 10, and 100 were tried. Then, the mean proportion for group  $B$ ,  $p_B$ , was specified as a multiple of  $p_A$ . Multiples of 1, 2, 3, 5, 10 were tried. Null proportions for each of the individual libraries in a group were then generated from a beta distribution, with the parameters of the beta chosen so as to yield a fixed multiple of overdispersion relative to the straight binomial. The number of libraries in both groups was taken to be 4, and the overdispersion multipliers were taken to be 2, 5, 10, or 50. Given the library proportions, counts of the tag of interest were then generated. This was done using Poisson sampling rather than binomial because of the faster average run time for scarce observations. This process was repeated 10000 times for each combination. In each case, we computed the  $t_w$ ,  $t$  and  $\chi^2$  values, converted

the values to p-values using the approximate asymptotics, and recorded the fraction of the observations found to be significant at the 10%, 5%, 1%, and 0.5% levels. The results are summarized in several tables below.

Qualitatively, we find that  $t_w$  and the two-sample  $t$ -test perform quite similarly throughout, with  $t_w$  exhibiting very slightly less power. The  $\chi^2$  test has higher power throughout, so the sensitivity is good, but it suffers from an abysmal type I error rate, so the specificity is very bad. The specificity of the  $t$  and  $t_w$  tests is ok, though the asymptotic bounds we are using here are evidently conservative: We pick up a fraction smaller than the nominal type I error rate as significant when there is in fact no difference, particularly when the counts are low.

Given that the  $t$  and  $t_w$  tests are quite similar as far as coarse measures of power are concerned, we checked several plots of the simulation results to suggest what differences did exist. In general, the range of  $t_w$  is smaller than that of  $t$ . This is due in large part to the presence of “explosive”  $t$  values where the two groups exhibit differences but the sampling variance is less than the binomial variance. In many of the cases where this occurs, the test statistic value is reduced from one extreme value (eg,  $t = 30$ ) to another extreme value (eg  $t_w = 12$ ) so that the overall power of the tests is largely unaffected. Such corrections can, however, serve to reorder the list of the most significantly expressed genes. We have seen this type of correction in real data, with the most dramatic corrections occurring when the number of libraries is small and the improperly low variance estimate is associated with the higher overall proportion. This correction for explosion also happens with low-count genes, and there it often shifts the  $t$  values from “significant” to “not significant”. This can reduce the perceived power, but it may be more realistic, in that 4 counts of 1 in one group are not persuasively different from 4 counts of 0 in the other group.

It is possible for  $t_w$  to be “more significant” than  $t$ ; this can occur if the different variance estimates give rise to different assessments of the degrees of freedom to use. There are other cases we have seen in real data where  $t_w$  actually gives a larger value than  $t$ . These occur when the library sizes are different, and the most outlying values within a group are associated with the smallest libraries.

$p_B = p_A$													
		$\chi^2$				$t$				$t_w$			
	$p_A$	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005
$k = 2$	1	2318	1391	392	241	550	182	12	5	210	23	0	0
	2	2400	1557	597	423	771	343	56	33	504	106	0	0
	5	2430	1680	674	483	854	395	67	30	632	213	3	1
	10	2467	1653	642	443	835	370	64	34	628	221	12	0
	100	2456	1666	695	455	914	433	83	36	715	277	21	5
$k = 5$	1	3973	3073	1534	1235	229	47	5	4	126	17	0	0
	2	4451	3549	2082	1709	447	159	20	7	363	83	0	0
	5	4571	3770	2445	2001	678	290	49	31	635	237	12	3
	10	4590	3786	2391	2014	782	336	66	30	751	307	31	10
	100	4588	3757	2497	2118	917	425	84	32	897	402	71	28
$k = 10$	1	4531	3741	2313	2002	83	9	1	0	45	5	0	0
	2	5530	4747	3293	2912	251	54	10	7	208	39	5	1
	5	5939	5250	3951	3536	503	196	23	12	492	182	10	3
	10	5989	5337	4091	3665	626	291	54	30	625	283	46	14
	100	6036	5308	4110	3717	860	368	63	32	857	364	62	30
$k = 50$	1	3140	2786	2158	2005	3	0	0	0	2	0	0	0
	2	5017	4537	3586	3362	23	1	0	0	17	1	0	0
	5	7261	6758	5759	5467	109	14	2	1	95	13	1	0
	10	7892	7528	6730	6485	269	64	9	4	265	61	8	4
	100	8224	7872	7195	6967	783	339	51	26	783	340	52	27

$p_B = 2 * p_A$													
		$\chi^2$				$t$				$t_w$			
	$p_A$	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005
$k = 2$	1	3975	2858	1391	1056	1411	633	107	66	1003	204	0	0
	2	5081	4176	2344	1879	2296	1194	232	139	1977	783	7	0
	5	7618	6856	5125	4435	4545	2827	732	408	4323	2502	302	50
	10	9301	8948	7875	7361	7109	5254	1863	1137	7001	5100	1524	651
	100	10000	10000	10000	10000	10000	9999	9814	9342	10000	9999	9841	9447
$k = 5$	1	5132	4205	2514	2128	630	186	37	25	479	85	0	0
	2	5754	4993	3534	3090	1108	471	75	37	1039	380	9	0
	5	7079	6503	5288	4843	2427	1266	259	135	2389	1238	199	50
	10	8350	7946	6972	6583	3900	2321	554	302	3875	2311	527	253
	100	10000	10000	10000	10000	9969	9794	7476	5888	9969	9795	7523	5969
$k = 10$	1	5685	4884	3304	2895	237	40	5	2	186	28	0	0
	2	6465	5776	4466	4091	629	193	35	14	584	161	10	1
	5	7150	6632	5596	5232	1391	573	98	63	1387	566	78	27
	10	7957	7550	6773	6428	2383	1222	266	146	2379	1221	263	134
	100	9990	9987	9971	9968	9316	8202	4142	2720	9316	8204	4164	2744
$k = 50$	1	4307	3864	3045	2845	4	0	0	0	3	0	0	0
	2	6241	5716	4755	4501	75	11	1	0	57	8	1	0
	5	7964	7561	6782	6529	266	51	5	0	250	47	4	0
	10	8380	8070	7442	7210	600	161	32	16	598	160	30	15
	100	9501	9411	9259	9187	4010	2428	601	326	4010	2428	601	327

$p_B = 3 * p_A$													
		$\chi^2$				$t$				$t_w$			
	$p_A$	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005
$k = 2$	1	6380	5349	3423	2851	2996	1585	341	208	2671	959	2	0
	2	8266	7645	5917	5224	5179	3227	901	552	4971	2832	158	2
	5	9840	9705	9248	8985	8553	6876	2891	1871	8513	6828	2536	1104
	10	9998	9996	9974	9957	9841	9269	5821	4187	9843	9276	5921	4213
	100	10000	10000	10000	10000	10000	10000	9999	9990	10000	10000	9999	9990
$k = 5$	1	6393	5620	4026	3568	1284	463	74	47	1149	306	3	0
	2	7564	7043	5816	5365	2610	1247	244	127	2565	1152	81	11
	5	9212	8953	8347	8047	5325	3322	958	531	5314	3310	909	430
	10	9870	9816	9653	9556	7885	5933	2231	1380	7882	5935	2262	1383
	100	10000	10000	10000	10000	10000	10000	9866	9437	10000	10000	9869	9448
$k = 10$	1	6644	5975	4474	4070	585	147	18	9	508	113	2	0
	2	7547	7055	5948	5567	1312	476	80	43	1275	436	32	7
	5	8595	8269	7595	7324	3112	1555	356	206	3110	1553	339	165
	10	9502	9396	9108	8964	5266	3250	888	505	5267	3250	901	509
	100	10000	10000	10000	10000	9998	9975	8763	7383	9998	9975	8767	7393
$k = 50$	1	5216	4774	3843	3628	37	2	0	0	30	0	0	0
	2	7221	6770	5740	5465	141	17	1	0	118	13	0	0
	5	8533	8250	7655	7424	562	150	20	8	554	146	15	6
	10	8890	8689	8228	8066	1358	494	69	33	1357	493	65	30
	100	9978	9972	9962	9954	7993	6050	2229	1363	7993	6050	2229	1365



$p_B = 5 * p_A$													
		$\chi^2$				$t$				$t_w$			
	$p_A$	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005
$k = 2$	1	9111	8717	7289	6724	6224	3954	1070	648	6054	3531	58	0
	2	9916	9837	9560	9356	8816	6982	2829	1780	8796	6905	2133	424
	5	10000	10000	9999	9997	9971	9723	6756	5101	9971	9729	6829	5204
	10	10000	10000	10000	10000	10000	9996	9142	7875	10000	9996	9205	7987
	100	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000
$k = 5$	1	8343	7918	6781	6385	3063	1382	250	143	2990	1235	53	1
	2	9379	9168	8607	8359	5519	3231	824	468	5512	3204	629	185
	5	9972	9963	9913	9891	8830	7094	2806	1788	8831	7099	2823	1786
	10	10000	10000	10000	9999	9909	9414	5769	4097	9909	9416	5794	4137
	100	10000	10000	10000	10000	10000	10000	10000	9982	10000	10000	10000	9982
$k = 10$	1	8023	7551	6497	6103	1465	492	64	31	1387	424	21	5
	2	8933	8678	8087	7854	3185	1393	270	155	3173	1374	205	59
	5	9789	9739	9569	9491	6420	4010	1091	648	6418	4011	1095	636
	10	9985	9981	9963	9953	8852	7119	2796	1760	8951	7119	2807	1775
	100	10000	10000	10000	10000	10000	10000	9925	9585	10000	10000	9925	9586
$k = 50$	1	6598	6120	5132	4877	121	8	0	0	95	5	0	0
	2	8359	8021	7260	7028	458	85	8	3	428	78	5	1
	5	9195	9037	8719	8570	1503	503	75	41	1499	497	68	34
	10	9545	9464	9266	9193	3266	1511	316	171	3266	1510	317	170
	100	10000	10000	10000	10000	9930	9457	5785	4120	9930	9457	5785	4122

$p_B = 10 * p_A$													
		$\chi^2$				$t$				$t_w$			
	$p_A$	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005	0.1	0.05	0.01	0.005
$k = 2$	1	9990	9982	9937	9899	9533	8121	3453	2254	9531	8116	2854	497
	2	10000	10000	10000	10000	9980	9748	6552	4744	9980	9754	6613	4803
	5	10000	10000	10000	10000	10000	9999	9461	8315	10000	9999	9478	8365
	10	10000	10000	10000	10000	10000	10000	9981	9804	10000	10000	9982	9813
	100	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000
$k = 5$	1	9809	9730	9518	9411	6802	4122	1023	580	6793	4116	747	180
	2	9992	9989	9975	9968	9210	7399	2793	1675	9210	7403	2800	1640
	5	10000	10000	10000	10000	9987	9795	6575	4752	9987	9797	6580	4780
	10	10000	10000	10000	10000	10000	9996	9070	7610	10000	9996	9073	7624
	100	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000	10000
$k = 10$	1	9520	9389	9075	8926	4051	1846	335	168	4037	1819	233	59
	2	9914	9879	9792	9745	6867	4226	1049	587	6866	4229	1044	536
	5	10000	9998	9996	9996	9583	8263	3540	2181	9583	8265	3545	2183
	10	10000	10000	10000	10000	9990	9787	6584	4814	9990	9787	6587	4822
	100	10000	10000	10000	10000	10000	10000	10000	9999	10000	10000	10000	9999
$k = 50$	1	8639	8308	7559	7342	529	96	8	4	479	84	4	2
	2	9455	9348	9059	8964	1523	462	54	26	1506	456	49	17
	5	9818	9783	9698	9664	4251	1903	355	179	4251	1903	356	180
	10	9969	9961	9940	9933	7059	4354	1051	594	7059	4354	1051	594
	100	10000	10000	10000	10000	10000	9997	9112	7601	10000	9997	9112	7601