

# Estrogen 2×2 Factorial Design

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## Experimental Data

In this vignette, we demonstrate how to use linear models and the package *factDesign* to analyze data from a factorial designed microarray experiment. When careful attention is paid to the biological interpretation of the linear model parameters, multifactor experiments can be particularly useful for disentangling complex biological systems. These methods are also more generally applicable to any microarray experiment to which linear modeling applies. For small experiments, investigators may want to consider moderating variance estimates using the techniques available in packages such as *limma*, *siggenes*, *LPE*, and *EBarrays*.

In this package, an `exprSet` called `estrogen` contains gene expression levels for 500 genes from Affymetrix HGU95av2 chips for eight samples from a breast cancer cell line. The results of the analysis of the full data set (12,625 probes, 32 samples) are discussed in Scholtens, et al. Analyzing Factorial Designed Microarray Experiments. *Journal of Multivariate Analysis*. (To appear). The expression estimates were calculated using the `rma` method after quantile normalization from the *affy* package. The expression values are reported in log base 2 scale as returned by `rma` (Irizarry et al, 2003).

```
> library(Biobase)
> library(affy)
> library(stats)
> library(factDesign)
```

The investigators in this experiment were interested in the effect of estrogen on the genes in ER+ breast cancer cells over time. After serum starvation of all eight samples, they exposed four samples to estrogen, and then measured mRNA transcript abundance after 10 hours for two samples and 48 hours for the other two. They left the remaining four samples untreated, and measured mRNA transcript abundance at 10 hours for two samples, and 48 hours for the other two. Since there are two factors in this experiment (*estrogen* and *time*), each at two levels, (*estrogen: absent or present, time: 10 hours or 48 hours*), this experiment is said to have a 2×2 factorial design. Table 1 shows the correspondence of the sample names in `estrogen` with the experimental conditions.

```
> data(estrogen)
> estrogen
```

```
An object of class "exprSet"
Slot "exprs":
```

Table 1: Experimental Conditions for .cel Files

time	estrogen	
	absent	present
10 hours	et1	Et1
	et2	Et2
48 hours	eT1	ET1
	eT2	ET2

	et1.CEL	et2.CEL	Et1.CEL	Et2.CEL	eT1.CEL
33598_r_at	4.970288	4.652992	4.450018	4.718027	4.760855
39859_r_at	9.145143	9.055006	9.189090	9.169929	9.328266
35114_at	7.232742	7.116724	7.345879	7.254973	7.359185
41041_s_at	6.538163	6.646323	6.608893	6.510601	7.503181
41759_at	10.286364	10.116122	9.999341	10.043443	9.846629
1532_g_at	7.436714	7.424547	7.061846	7.135486	7.930144
36601_at	7.978883	7.963313	8.008160	8.164599	7.769837
41155_at	11.819258	11.626182	11.345942	11.301453	11.510576
36892_at	7.567243	7.717751	7.526438	7.454821	7.411874
35706_at	5.428676	5.400391	5.069292	5.352490	5.085719
505_at	10.406275	10.353465	10.446062	10.640110	10.200179
35665_at	5.988568	6.051763	5.883811	5.781307	5.973252
38467_at	8.300965	8.308773	8.690797	8.569640	8.362930
37155_at	7.357752	7.539195	7.447979	7.278215	7.668269
293_at	9.402392	9.701194	9.572019	9.485111	9.719416
41240_at	4.928643	4.942358	4.995556	4.883394	4.878421
33075_at	4.855748	5.111703	4.984265	5.022695	4.913980
1491_at	4.998913	4.847238	4.670994	4.857900	5.001759
34097_at	4.904656	4.945787	4.826071	4.890654	4.790175
467_at	6.980601	6.819131	7.527414	7.953618	6.411550
41107_at	6.961095	7.042236	7.041169	6.843951	7.201602
38651_at	9.314938	9.249487	8.851031	8.815931	9.271247
37997_r_at	8.631416	8.657571	8.175735	8.658201	9.170318
968_i_at	7.750437	7.599703	7.663498	7.557983	7.484008
38185_at	6.361552	6.498700	6.199686	6.454859	6.759783
38844_at	7.625933	7.606120	7.696984	7.664659	7.356092
36790_at	10.556211	10.363827	11.149453	10.895733	10.597071
37415_at	5.742533	5.952363	6.005328	5.755890	5.805610
33609_at	7.854395	7.989175	8.064037	7.972029	8.556093
31838_at	4.487766	4.409834	4.582326	4.695803	4.795614
566_at	8.189731	8.105651	7.945615	7.942056	8.207552
41257_at	4.679297	4.809743	4.801395	4.670938	4.788435
38617_at	7.850688	7.942119	7.994160	7.843856	8.194784

39923_at	7.146702	7.131945	6.659479	6.810034	6.789936
1287_at	9.244252	9.089225	9.857992	9.796468	8.355748
35959_at	4.888715	4.873592	5.034444	4.908688	5.055040
39459_at	8.895531	8.944760	8.723910	8.871594	9.301454
32700_at	7.128567	7.044443	7.241352	6.996534	7.855390
33968_at	4.781838	4.696228	4.711794	4.714198	4.715042
32890_at	4.716813	4.871944	5.055796	4.829605	5.264269
31783_at	6.380184	6.555268	6.633053	6.290060	6.655529
35168_f_at	5.123504	4.850075	5.077339	5.324760	5.206889
35157_at	4.797974	4.847844	4.930997	4.974396	4.619670
34598_at	6.080008	6.025588	5.968558	5.807942	6.169266
31901_at	8.764136	8.685934	8.501990	8.674400	8.532642
31732_at	5.221401	5.384583	5.502749	5.635290	5.097321
32905_s_at	6.886915	7.304335	7.570045	6.895309	7.061549
35805_at	8.299936	8.294497	8.333216	8.471175	7.919863
33315_at	7.045928	7.028337	6.870911	6.882174	6.878856
40688_at	8.582645	8.750651	8.659600	8.505579	9.013112
1534_at	4.963282	4.995038	5.036907	4.950046	4.989774
35508_at	9.873679	10.061145	9.911090	10.002710	9.823620
39951_at	8.233340	8.051479	8.087094	7.936472	7.807482
31520_at	7.026339	7.222799	7.901664	7.283828	7.512777
36986_at	11.638770	11.564372	11.501736	11.364349	10.972251
32574_at	7.469520	7.543834	7.149472	7.241375	7.309511
31590_g_at	9.289738	9.207711	8.775010	9.092451	9.499859
39379_at	6.418296	6.275485	6.396587	6.507564	6.190068
41143_at	11.414288	11.380561	11.817183	11.714598	10.468317
34662_at	6.550305	6.621861	6.463183	6.364406	6.289717
38279_at	6.661349	6.839765	7.287322	7.145905	6.860265
2075_s_at	9.023294	9.014346	8.841500	9.009791	9.037403
34779_at	8.543712	8.766038	8.448134	8.500937	8.596640
33415_at	12.605295	12.632743	12.855410	13.039199	12.228118
40137_at	9.862920	9.654962	9.676150	9.738056	9.566452
35581_at	4.587558	4.608945	4.794966	4.564204	4.720281
40079_at	8.282810	7.995803	7.499557	7.577245	10.060174
40106_at	10.667784	10.644271	10.629442	10.759162	10.452599
39882_at	5.162723	5.077224	5.173207	5.764223	5.327677
35471_g_at	5.848706	5.800421	5.887828	5.707159	5.844071
39384_at	4.625786	4.940410	4.965135	4.883566	4.883672
37825_at	7.044339	6.765157	6.965767	7.166824	6.671194
38831_f_at	9.969966	9.835984	9.714463	9.718783	9.351946
1007_s_at	11.903294	11.744474	11.527160	11.408763	11.606915
35909_at	8.240290	8.094940	8.649730	8.658063	8.999305
32738_at	8.605304	8.653614	8.572132	8.607994	8.319327
34722_at	8.150657	8.195811	8.289292	8.045654	8.497210
37617_at	6.759178	6.473472	6.244674	6.579477	6.606029

34374_g_at	11.644884	11.270676	10.625574	10.525063	11.619944
31384_at	8.127089	8.350207	8.469426	8.260202	8.383407
33029_at	8.076609	8.259453	8.403838	8.082913	8.399333
38301_at	5.148442	4.897045	4.970874	5.063934	4.777211
35190_at	8.708525	8.732896	8.752398	8.655312	9.284159
39793_at	6.401641	6.335806	6.139760	6.426522	5.551397
286_at	13.779465	13.779060	13.094700	13.361393	13.529532
35402_at	7.383135	7.167207	7.558215	7.287597	7.660190
789_at	8.703428	8.646853	8.664539	8.419823	8.881025
41093_at	5.412683	5.463020	5.525233	5.374224	5.622053
41009_at	4.718415	4.597322	4.639168	4.630605	4.697312
32176_at	8.934395	8.991345	9.083678	8.856348	9.295755
31625_at	5.157976	5.116584	5.480449	5.171648	5.301576
38108_at	7.636342	7.614968	7.907841	7.756023	8.123009
34266_at	5.138681	5.251264	5.133451	4.941384	5.456289
38153_at	5.095949	5.121765	5.014638	4.899572	5.331845
33978_at	6.206496	6.031280	5.962216	5.864592	6.005952
32331_at	6.892743	6.749522	7.533555	7.375121	6.622532
39709_at	12.388671	12.403177	12.463425	12.468450	12.088515
2058_s_at	10.357516	10.091901	10.375895	10.483348	10.319759
35793_at	8.597158	8.437330	8.381226	8.621172	7.992547
36347_f_at	10.496625	10.471665	10.046875	10.323380	10.726426
37444_at	8.811735	8.856793	9.079907	8.765629	9.077684
34130_at	7.436739	7.225584	7.342999	7.048754	7.346131
36061_at	5.919046	6.008810	6.054759	5.978947	6.022354
41840_r_at	5.596402	5.466852	5.077118	5.280005	5.051869
35990_at	7.684509	7.666578	7.473288	7.339712	7.659566
41043_at	7.691893	8.007206	7.991713	7.499685	7.975560
41341_at	7.353501	7.107716	7.273078	6.998386	7.372273
37549_g_at	6.344341	6.392109	6.039821	6.182514	6.130574
35093_at	9.583930	9.620578	9.684763	9.510298	9.824783
31826_at	9.176990	9.112975	9.105956	9.069884	9.333190
41597_s_at	9.081495	9.026768	9.046475	9.122980	8.557405
33735_at	5.559309	5.702579	5.686162	5.451108	5.681816
1678_g_at	5.315207	5.232677	5.387135	5.462833	5.508052
41747_s_at	7.278867	7.281313	6.728079	7.182464	7.302486
38931_at	5.436352	4.772612	4.811039	4.993965	4.797734
31766_s_at	7.284643	7.393000	7.466288	7.237622	7.492562
36806_at	7.014555	7.203604	6.924937	6.899638	7.287165
41822_at	8.459628	8.391374	8.282737	8.412865	8.645961
37254_at	7.382947	7.345420	7.369565	7.268238	7.301370
35011_at	5.424899	5.504772	5.505856	5.348219	5.603058
38025_r_at	5.097418	4.976963	5.055840	5.160543	5.157463
33963_at	8.779536	8.976976	8.990733	8.752927	8.845228
33812_at	7.408567	7.289623	7.366606	7.267041	7.153898

32723_at	5.910021	5.900353	5.821141	6.181985	5.600248
34576_at	5.061792	5.054009	4.949311	4.754419	5.015458
739_at	5.201412	5.216524	5.096920	5.196600	5.180359
31911_at	7.175775	7.160169	7.311579	7.055111	7.484233
2026_at	5.034902	5.016826	5.159047	4.998273	4.870630
31759_at	7.949341	8.052135	8.321604	8.065408	7.603769
38542_at	12.798297	12.693698	13.133367	13.110223	12.514898
37665_at	5.324090	5.369112	5.466988	5.179297	5.577796
41033_at	6.513487	6.178622	6.136610	6.453373	6.028006
38328_at	6.292445	6.013784	6.429973	6.337861	6.255250
39121_at	6.742279	6.825735	6.681016	6.654868	6.893644
36448_at	6.610807	6.346525	6.450614	6.417308	5.926399
38164_at	5.738329	5.987768	5.680917	5.628249	5.444309
40168_at	5.567465	5.640527	5.778285	5.511703	5.778666
39727_at	8.704436	8.041374	8.175839	8.352746	7.696304
672_at	7.214869	7.338292	7.140576	7.063471	7.636534
35435_s_at	7.215061	7.121819	7.732836	7.938600	6.668230
33856_at	11.159392	11.018522	10.747858	10.927720	11.103772
35064_at	5.910484	5.951906	6.168752	5.798646	6.042786
1119_at	8.505242	8.371545	8.904403	8.932208	8.094564
32275_at	7.004181	7.162810	6.985287	6.985777	6.890773
40420_at	8.579060	8.331943	8.074226	8.319302	8.306851
32859_at	6.819248	6.375526	5.956028	6.173659	6.319955
32957_g_at	7.152246	6.889396	6.659775	6.750700	7.115821
1859_s_at	5.250203	5.350753	5.208927	5.359407	4.832839
33038_at	6.196819	6.370786	6.319483	6.076820	6.499533
39049_at	7.865286	8.011817	8.187339	7.922005	8.058478
40473_at	9.282693	8.819897	9.386553	9.514449	8.744618
36148_at	8.454432	8.643407	8.592417	8.442186	9.017852
34794_r_at	4.983377	4.834184	4.872541	4.876095	5.222831
33046_f_at	7.254670	7.270350	7.381796	7.344720	7.381667
31375_at	7.424947	7.474517	7.525079	7.386504	7.579249
34818_at	5.647744	5.854727	5.536611	5.791584	5.640135
37065_f_at	5.062410	4.843119	4.860958	5.037572	5.371287
32681_at	10.253695	10.233421	9.887731	9.962126	10.706248
35493_at	4.643412	4.644183	4.422747	4.529457	4.845811
32694_at	5.335571	5.227831	5.094631	5.186919	5.188712
36202_at	4.869310	4.750750	4.935706	4.911475	4.618929
34371_at	7.491076	7.384423	7.309332	7.481901	7.540697
38024_at	6.613411	6.578835	6.322718	6.306972	6.641458
34626_at	8.123147	8.330542	8.367397	8.172682	8.473832
34389_at	5.829901	5.830340	5.797365	5.738536	5.923954
36587_at	12.920246	12.792634	12.839583	12.819629	12.614849
39137_at	10.011675	9.884737	9.397686	9.414500	9.834964
32730_at	9.168860	9.009589	9.168717	9.135784	8.211777

35094_f_at	7.655393	7.726485	7.764418	7.580865	7.773648
33280_r_at	5.451532	5.257387	5.444472	5.383350	5.382480
37773_at	4.641767	4.656805	4.472184	4.629481	4.576515
32270_g_at	5.939744	6.648743	6.613003	6.321798	6.564576
40654_at	6.816812	6.541244	6.667815	6.742474	5.965154
39209_r_at	4.537192	4.601898	4.400869	4.552369	4.646413
38242_at	6.453461	6.197265	5.435737	5.450943	6.311774
37622_r_at	4.859975	4.547420	4.616265	5.105081	5.096554
34591_at	7.900225	8.180415	8.319066	8.043415	8.482454
36533_at	5.442943	5.459702	5.560423	5.224614	5.765528
40786_at	6.161292	6.047884	5.833158	6.101684	5.752135
37173_at	5.291193	5.410884	5.316117	5.672542	5.469115
40353_at	8.189778	8.090870	8.121116	8.289728	7.703283
33853_s_at	7.677422	7.562622	7.801226	7.725870	7.834262
38731_at	5.781450	6.015335	6.026429	5.943916	5.835615
33268_at	9.494311	9.549058	9.397353	9.039929	10.191594
37325_at	9.319949	9.147104	10.175096	10.409006	8.164050
35931_at	5.708449	5.764342	5.676123	5.852905	5.828755
33267_at	6.338429	6.323259	5.774369	5.881369	6.164061
36956_at	7.820790	7.542837	7.581044	7.365079	7.610981
41251_at	8.664351	8.551047	8.393115	8.573140	8.575336
41110_at	5.010577	4.922338	4.930139	4.894522	4.886422
33379_at	4.791891	4.644261	4.657667	4.680165	4.661222
33961_at	6.920192	7.332096	7.216670	7.087266	7.355230
691_g_at	12.147669	12.223757	12.294010	12.319133	12.373635
37635_at	5.062645	5.041665	5.337722	4.965665	5.145868
41543_at	9.110452	9.270565	9.284702	9.203600	9.432403
35745_f_at	11.516729	11.256795	11.441836	11.443758	10.585940
35009_at	4.710024	4.737223	4.655904	4.661292	4.876610
38115_at	8.751617	8.613524	8.623443	8.744989	8.020952
31878_at	5.531298	5.197344	5.302436	5.293515	5.746593
37052_at	6.094524	6.302899	6.125080	6.239995	5.925142
32940_at	5.339671	5.429523	5.481128	5.252495	5.700524
41845_at	8.330889	8.468036	8.329331	8.203540	8.466024
37400_at	8.344358	8.297200	8.378121	8.334430	8.198542
36337_at	9.116129	9.297816	9.816303	9.341427	9.674206
34954_r_at	4.611243	4.650665	4.676791	4.556937	4.838123
40830_at	8.290321	8.443827	8.269256	8.286557	8.486291
34480_at	8.430367	8.734402	8.862478	8.617100	8.830785
33547_i_at	4.617035	4.540013	4.527914	4.608987	4.768410
32100_r_at	10.908110	11.026969	11.005400	10.860535	11.382851
32119_at	5.374388	5.118952	4.946626	4.771966	5.003255
35928_at	6.011143	6.079231	6.255108	6.220554	6.141714
33112_at	4.946307	5.009718	5.071589	4.972025	4.989706
32661_s_at	7.816348	7.742681	8.005298	8.208203	7.779883

38292_at	9.087766	9.204424	9.468147	9.298753	9.261232
1548_s_at	5.926930	5.883962	5.693337	5.843225	5.716376
38616_at	7.333000	7.128378	7.200087	6.925264	7.461377
34326_at	7.749208	7.672301	7.378916	7.789014	7.024826
35027_at	8.152997	8.060576	7.985807	8.041652	8.053497
40091_at	6.062076	6.086483	5.866955	5.703984	5.952008
41293_at	8.252754	8.541235	8.491031	8.432211	8.756360
33473_at	4.802123	4.851111	4.638781	4.775853	4.584588
41810_at	5.810713	5.886225	5.912742	5.826263	6.035207
38950_r_at	8.781672	8.984050	8.898028	8.818778	9.359226
38433_at	5.133602	5.248500	5.280196	5.297688	5.299033
160026_at	6.368558	6.377702	6.364843	6.384174	6.427891
34855_at	8.177933	8.142002	7.994680	8.145509	7.803173
35869_at	5.895448	5.711324	5.900677	5.680524	5.810648
36881_at	9.964031	9.836116	9.853764	10.047656	9.833667
38576_at	9.837711	9.801950	8.582584	8.847503	9.358895
41244_f_at	5.959066	6.181712	5.995961	6.107072	6.069578
37593_at	6.184951	5.890399	5.578821	5.715797	5.657360
35260_at	8.580720	8.606566	8.548783	8.697517	9.033386
36655_at	5.656077	5.531231	5.242593	5.287467	5.476590
1646_at	5.566478	5.620834	5.433360	5.582938	5.678101
33203_s_at	7.167866	7.293869	7.353052	7.357643	7.370011
34875_r_at	4.877279	4.748971	4.621326	4.805195	4.762642
32428_at	6.354282	6.620105	6.695911	6.230231	6.630203
40209_at	6.018807	5.947080	6.202750	6.162611	6.638242
865_at	6.530993	6.679571	6.668475	6.669867	6.723452
AFFX-YEL021w/URA3_at	4.972595	4.997817	5.158548	4.935422	5.550304
36708_at	5.181230	4.809721	5.154532	4.750124	5.075722
38125_at	6.662927	6.483893	6.013347	6.296359	7.182400
32108_at	9.552141	9.386269	9.758330	9.773099	9.636557
37765_at	7.591268	7.908705	7.772825	7.659577	8.018628
33826_at	9.417782	9.382574	9.381774	9.308945	9.410677
38886_i_at	4.695338	4.697814	4.604120	4.567223	4.953777
34893_at	9.577759	9.671076	9.672898	9.967226	9.191497
432_s_at	4.858931	4.870105	5.076422	4.968401	5.258501
41862_at	6.204166	6.291644	6.649816	6.823502	6.529665
38909_at	6.868142	6.847269	7.067201	6.947600	6.984939
33155_at	8.374884	8.361594	8.370943	8.354681	8.567470
32720_at	6.195268	6.362156	5.954924	6.331904	5.833369
37269_at	7.307652	7.112275	7.362977	7.170874	7.717042
894_g_at	11.142055	11.132810	11.600913	11.738474	10.463821
36507_at	9.256305	9.287443	8.964527	9.124855	9.359314
39623_at	5.668330	5.608000	4.930906	5.314163	5.741399
32061_at	6.767144	6.540888	6.807246	6.684418	6.438740
34955_at	4.650731	4.780818	4.816342	4.580822	4.728824

35777_at	9.108668	9.056381	9.138277	9.215466	8.999975
40635_at	11.483981	11.546249	11.074397	11.013988	11.620683
41340_at	8.821891	8.755120	8.823593	8.631886	8.875103
40802_at	5.229397	5.037524	4.855910	5.166516	4.849764
38256_s_at	6.821541	6.798189	6.895626	7.101083	6.927814
41636_at	8.347239	8.350509	8.431230	8.390321	8.953773
36323_at	5.282887	5.339127	5.353454	5.273177	5.548312
37059_at	6.525012	6.581900	6.391499	6.420342	6.588551
34118_at	5.284804	5.142372	5.208121	5.043240	5.219853
34253_at	6.055160	5.965787	6.107706	6.463808	5.718696
1228_s_at	7.867580	7.877642	7.587835	7.718219	7.074044
36154_at	10.134418	10.102104	9.839917	9.986242	9.711988
40661_at	7.313507	7.374891	7.590858	7.243381	7.356901
1070_at	6.000628	5.979949	5.868718	5.892179	6.017959
1797_at	7.519240	7.429599	7.319302	7.424426	7.662896
32011_g_at	7.316652	7.307644	7.469121	7.328834	7.155023
39425_at	9.174530	9.016934	9.031590	9.281168	9.264071
38995_at	7.997446	8.108547	7.863491	8.080366	7.900384
435_g_at	8.151464	8.294677	8.480010	8.141320	8.162223
AFFX-ThrX-3_at	5.748200	6.126162	6.584884	5.899371	6.215511
1822_at	7.340313	7.224242	8.760383	9.092731	6.223216
37935_at	8.116096	7.993852	8.380309	8.525008	7.879642
39277_at	6.417645	6.403490	6.309017	6.338992	6.639090
31679_at	5.181546	5.233710	5.168936	4.894604	5.221037
34850_at	7.958166	7.898177	8.018972	8.302638	7.944569
32752_at	10.130098	10.080176	9.780313	10.023781	10.058779
1638_at	6.002359	6.076525	6.129564	5.936957	6.182886
34840_at	6.983197	6.629131	6.350932	6.751900	6.022908
1716_at	7.206373	7.091716	7.094872	7.164574	7.281981
32762_i_at	6.142181	6.044645	6.312576	5.944761	6.452827
160044_g_at	9.102038	9.038358	9.043948	9.031691	8.863374
38191_at	7.719098	7.768319	7.947133	7.633918	8.067246
33639_g_at	7.189758	7.274921	7.133154	6.801199	7.219324
238_at	6.406144	6.657909	6.355918	6.661752	6.153364
1793_at	7.745781	7.832262	7.542917	7.529837	7.812716
40756_at	8.971810	8.919399	9.037605	9.148159	8.808105
39213_at	4.631157	4.618122	4.600708	4.564543	4.731657
39975_at	6.626158	6.545520	6.276210	6.383459	5.470150
545_g_at	8.574622	8.679364	8.886410	8.373688	9.173291
273_g_at	5.417680	5.353278	5.126045	5.374425	5.468553
216_at	9.395321	9.474208	9.395020	9.426446	9.630495
39754_at	9.481705	9.256958	9.581738	9.594333	9.221936
39579_at	5.711733	5.804655	5.825485	5.772984	5.802030
1106_s_at	7.600855	7.738858	7.622819	7.601307	7.418882
39510_r_at	4.669309	4.672794	4.581165	4.709882	4.679153



38903_at	7.894371	7.865996	8.061746	7.852818	8.063775
37691_at	9.067702	9.171623	9.124819	9.038488	9.169644
837_s_at	8.964986	8.728042	8.958977	8.951899	7.690266
31913_at	6.093802	6.197537	6.672774	5.987634	6.733211
1092_at	9.976273	10.021810	10.090216	9.955615	10.159698
35520_at	9.035965	9.018330	8.937071	8.694899	9.363154
36006_at	6.665597	6.717609	6.584620	6.743045	6.913593
34753_at	9.040111	8.930431	8.822650	8.851613	8.501225
35668_at	7.544462	7.221749	8.853395	8.884988	6.992925
38700_at	11.030733	11.059230	10.803357	10.892978	11.367163
1606_at	7.699784	7.610901	7.434341	7.523222	8.944769
34327_at	6.712883	6.371325	6.537322	6.751637	6.021009
39790_at	7.193318	7.043826	7.372008	7.378832	7.295332
40191_s_at	5.989614	5.774925	5.865818	6.127850	5.776148
32900_at	9.207560	9.418383	9.456004	9.319608	9.677986
34316_at	10.017932	9.922475	9.884351	10.128898	10.069901
40446_at	10.240020	10.115903	9.463301	9.633330	10.036681
40407_at	9.545634	9.093629	9.728440	9.753128	7.846160
33607_at	6.868833	6.873574	6.903568	6.837203	7.151858
31795_at	5.858949	5.908529	5.990725	5.746365	5.983663
38580_at	7.389434	7.303416	7.007977	7.197412	6.909762
272_at	6.687326	6.922381	6.641168	6.647265	7.500555
1385_at	6.302767	6.385349	6.457697	6.555469	6.847967
728_at	5.682799	5.765564	5.674733	5.619549	5.988522
1398_g_at	9.973885	9.969161	9.822086	9.855065	10.215751
34123_at	4.542895	4.477835	4.457639	4.602800	4.779346
41146_at	8.248696	8.184007	8.906425	8.913270	7.666184
37517_at	9.201565	9.183872	9.018854	9.132977	9.475028
33488_at	4.803886	4.667582	4.648239	4.700040	4.661980
38985_at	6.553388	6.253329	6.256644	6.592458	6.156131
40157_s_at	4.879697	4.953366	5.036059	5.010159	4.856543
33827_at	5.371693	5.538775	5.291570	5.382357	5.460064
35997_g_at	8.911681	9.022213	8.714515	8.820875	8.789947
38491_at	7.578306	7.686116	7.462323	7.468143	7.715282
37808_at	5.616394	5.520666	5.441257	5.613125	5.407876
37837_at	6.782468	6.498425	6.572910	6.445031	6.613337
448_s_at	9.106440	8.929153	9.228958	9.143812	8.794723
35048_at	5.561271	5.483320	5.344447	5.440914	5.563945
31455_r_at	4.617194	4.531069	4.439800	4.503847	4.560463
36400_at	4.672865	4.567117	4.640842	4.666419	4.920692
32740_at	5.330865	5.204771	5.054786	5.120082	5.270378
35917_at	9.314226	9.482422	9.252556	9.440657	9.528937
34504_at	5.430949	5.626907	5.432372	5.572160	5.765332
509_at	5.428435	5.662574	5.418260	5.613559	5.352311
34723_at	8.084559	8.054483	8.060878	8.221951	7.746048

32790_at	7.077629	6.890884	6.753157	6.931568	6.521190
37580_at	5.821848	5.825163	5.553752	5.541238	5.478780
31663_at	8.123699	8.281176	8.092191	8.150253	8.447217
41764_at	6.252657	6.203681	6.335468	6.216567	6.192222
36262_at	8.516370	8.098060	7.827590	7.993492	7.786517
31985_at	5.881062	6.060382	5.961080	5.573861	6.184117
37560_at	5.592950	5.799154	5.813848	5.643873	5.686419
533_g_at	7.412122	7.655110	8.082808	7.458021	7.753757
38128_at	7.052635	7.152593	7.012594	7.180887	7.351803
35092_at	8.308852	8.488030	8.530029	8.430888	8.478962
33930_at	4.993110	5.015462	4.799895	4.965324	4.861996
40150_at	7.392145	7.247402	7.425280	7.299397	7.250607
31749_f_at	9.350914	9.501740	9.401000	9.251023	9.389914
32344_r_at	4.616597	4.550378	4.446848	4.732398	4.878643
40894_at	5.221269	4.992587	5.042234	5.134904	5.096219
33744_at	8.386912	8.142862	8.568018	8.604011	8.430149
34413_at	8.146746	7.925560	7.827914	7.999927	8.102178
39521_at	7.350705	7.050078	7.276597	6.857975	7.747327
32350_at	5.069698	4.985904	4.816323	4.909152	4.773638
1198_at	4.779664	4.668691	4.468245	4.654340	4.577439
34937_at	5.382455	5.565582	5.298952	5.375665	5.673490
35866_at	4.996200	5.068867	5.001593	5.114655	5.021034
35121_at	7.992410	8.078250	8.099079	7.996558	7.989470
34162_at	9.426457	9.361948	9.105715	8.824854	8.296802
40518_at	5.134267	5.295800	5.010589	5.390477	5.439237
35523_at	6.241249	6.209831	6.513197	6.124009	6.319740
36478_at	6.351936	6.277529	6.179106	6.222411	6.194801
41698_at	8.079786	8.033362	8.110849	7.957359	8.114009
39399_at	8.567517	8.537117	8.708868	8.535400	8.469014
32536_at	9.849866	9.721037	10.648758	10.787468	9.198465
33747_s_at	7.062552	7.029105	7.222345	7.227311	6.829282
41785_at	12.062739	11.891753	12.058916	12.001071	11.364488
37096_at	5.826029	6.083948	6.224953	5.992633	6.284973
39228_at	5.744601	5.678656	6.132398	5.737792	5.976216
34521_at	5.891633	5.936001	5.981567	5.814085	5.840794
39328_at	7.705910	7.520064	8.026050	7.780944	7.426409
36519_at	8.743232	8.819485	8.357680	8.271931	8.566832
41083_at	8.634192	8.725061	8.659654	8.642311	8.532768
32248_at	7.721018	7.549478	7.682907	7.683528	7.959843
33422_at	8.766816	8.715781	8.804681	8.819226	7.959130
2049_s_at	9.635940	9.678346	8.940772	8.797628	10.474072
32553_at	12.586414	12.557262	12.708809	12.584304	12.594300
41843_r_at	4.522426	4.654837	4.373483	4.579839	4.611890
33592_at	6.751279	6.694593	6.797357	6.531654	6.962860
38798_s_at	9.446141	9.380412	9.389620	9.595840	9.862366

39520_at	6.960094	7.018213	6.895664	7.026446	7.175864
285_g_at	5.681474	5.537218	5.166080	5.322532	5.063036
32904_at	9.182521	9.430452	9.074260	9.163947	9.483220
33279_s_at	6.007573	5.983286	6.429525	5.769493	5.732184
231_at	7.118217	7.416125	7.142209	7.023276	7.351233
31430_at	5.161138	4.931130	4.950954	4.838932	5.016544
555_at	6.252736	6.292151	5.935412	6.119792	6.079456
489_at	8.466712	8.623957	8.674298	8.627096	8.694987
31662_at	8.288894	8.167778	8.276907	8.043193	8.957807
40962_s_at	6.985718	6.742064	6.640735	7.006375	6.444688
36978_at	5.912770	5.823974	6.004727	6.061931	5.444935
34788_at	9.795492	9.938067	10.412049	10.239386	9.806886
35445_at	7.183817	7.167550	7.481426	7.058887	7.897639
38552_f_at	6.084084	6.025226	5.785069	6.016954	6.266849
1908_at	7.171327	7.495653	7.329172	7.270637	7.222646
34107_at	5.300714	4.937451	4.836977	5.178390	5.355117
38238_at	5.131960	5.096333	5.325922	4.869824	4.892017
33836_at	7.624724	7.541545	7.620684	7.671287	9.386438
40062_s_at	5.554651	5.512592	6.046825	5.533476	5.997667
32604_s_at	6.213924	6.293449	6.326589	6.330628	5.998815
36023_at	8.814412	8.488383	8.977534	9.073204	8.303511
33340_at	7.786905	7.654343	7.113049	7.638798	6.676976
32324_at	11.414187	11.266928	11.417174	11.444044	11.203879
41499_at	6.292747	6.001541	6.063785	5.983627	6.180879
33892_at	4.842360	4.962020	4.527347	4.642261	4.727859
35786_at	8.416430	8.357433	8.351163	8.484246	8.276709
1318_at	9.391611	9.392606	9.949977	9.922003	8.721036
36618_g_at	9.133171	8.978264	8.248726	8.233269	8.492557
37789_at	4.896087	4.935782	4.929698	4.929153	4.836985
37208_at	4.623984	4.711724	4.813500	5.045123	4.678018
33893_r_at	4.895448	4.961517	4.592307	4.883585	4.876341
40771_at	5.580545	5.811116	5.682777	5.616283	6.124819
37569_at	10.730112	10.322017	10.417093	10.559189	10.848470
33662_at	4.771274	4.895336	4.995780	4.810705	4.893778
34692_r_at	9.307551	9.346001	9.167328	9.288195	9.579691
31941_s_at	4.956192	4.734622	4.756157	4.891960	5.036935
40421_at	8.039623	8.119775	8.145619	8.332634	7.574889
41515_at	5.177133	4.972350	5.057031	5.232020	5.013697
36808_at	5.489725	5.634076	5.499906	5.652074	5.926075
33982_f_at	6.398434	6.160706	6.501092	6.342597	5.813371
587_at	4.653048	4.663049	4.560749	4.681560	4.929805
32837_at	10.338250	10.512199	10.321515	10.340555	11.018670
37399_at	5.467918	5.562576	5.427679	5.373471	5.472311
31308_at	5.200747	5.635688	5.631536	5.210201	5.434456
35880_at	7.539220	7.741541	7.581769	7.208343	7.471253

2093_s_at	9.802067	9.782667	10.124299	10.137792	9.673596
31947_r_at	12.520100	12.578376	12.483455	12.507655	12.748244
33417_at	7.924000	7.785007	7.883655	7.738488	7.794361
37047_at	8.194995	8.194956	8.307832	8.244200	7.674530
993_at	8.060243	8.060595	8.037542	8.141639	8.227303
319_g_at	11.800506	11.690384	12.064975	11.865627	11.526599
40456_at	5.549254	5.762090	7.270563	7.661323	5.026918
38550_at	8.597038	8.760805	8.766060	8.507226	9.004437
39174_at	9.029419	9.037159	9.395530	9.496284	8.144690
31777_at	6.193008	6.516813	6.668294	6.275064	6.727206
34320_at	9.579740	9.597306	9.813888	9.568020	9.683127
38121_at	9.341202	9.511126	9.494917	9.507116	9.647457
36317_at	7.557477	7.465826	6.702609	6.635716	7.566588
34219_at	5.650688	5.813952	5.505959	5.687352	5.673672
32901_s_at	7.881089	7.800345	7.686088	7.666362	8.224178
1631_at	7.606109	8.168957	8.160706	7.863114	7.966099
32854_at	6.613859	6.420007	5.686245	6.358970	5.456334
40731_at	7.789471	7.949470	8.092053	8.112341	7.458547
39787_at	8.308357	8.264818	8.575181	8.541409	8.304810
39010_at	12.029538	11.866705	12.024493	11.880977	12.050577
31869_at	6.057965	5.673766	5.644380	5.872322	5.857860
31505_at	14.387275	14.346250	14.371246	14.400673	14.408941
950_at	5.494000	5.593917	5.293810	5.557752	5.332697
35256_at	9.592575	9.654022	9.384690	9.654951	9.800330
35591_at	6.121873	5.972327	6.167041	5.990357	6.054588
31994_at	5.448850	5.653002	5.759732	5.580655	6.213504
620_at	8.045273	8.247047	8.220667	8.166126	8.265006
33263_at	7.912847	7.818765	7.587063	7.701754	8.043011
32773_at	6.494515	6.582405	6.430435	6.439684	6.546634
38324_at	9.827168	9.491523	9.166336	9.641847	9.579053
33407_at	5.507385	5.468851	5.417186	5.502791	5.456765
39759_at	5.904746	5.814625	5.526258	6.095102	5.153680
202_at	5.965660	5.889342	5.904665	6.243003	5.702416
39898_at	4.773011	4.639004	4.728071	4.749846	4.579801
35146_at	7.773004	7.906940	8.241661	7.852951	8.594620
390_at	4.906756	5.198826	5.100984	5.115511	5.393144
37973_at	6.559428	6.619695	6.269052	6.341862	6.103938
35351_at	10.925899	10.782071	10.701254	10.704121	10.403062
37548_at	5.321804	5.358392	5.107153	5.401579	5.405635
1005_at	9.206827	8.993848	8.338061	8.237932	9.173212
32615_at	7.172887	6.971517	7.145982	7.374883	6.469300
38482_at	10.369110	10.360721	10.604179	10.506996	11.155463
32591_at	9.682015	9.601937	9.558869	9.762639	9.447112
39792_at	7.785619	7.554671	7.792175	8.167640	6.793780
33703_f_at	4.831589	4.853263	4.920614	4.727208	4.716036

38272_at	7.817712	7.757859	7.554900	7.612415	7.497055
41217_at	5.049969	4.953150	4.759041	5.104716	5.049955
31395_i_at	6.038301	5.841180	6.094141	5.897041	6.111883
31538_at	14.023865	14.013151	14.028277	14.031891	13.884187
38155_at	6.315125	6.294934	6.366129	6.477187	6.016454
35158_at	5.060659	5.030125	4.968258	5.196504	5.226916
40032_at	6.789958	6.670936	7.070212	7.190335	6.680520
41679_at	5.324493	5.445822	5.471512	5.279444	5.418709
1765_at	6.107999	6.132261	5.976489	5.921839	6.139147
35526_at	7.401737	7.558831	7.640453	7.605873	7.512714
38432_at	11.586386	11.755469	11.281175	11.416958	11.759083
194_at	4.762908	4.693969	4.598020	4.583370	4.538329
479_at	5.323431	5.264057	5.320964	5.187602	5.277864
33042_r_at	4.998370	5.008771	4.866084	4.976725	5.068288
1556_at	7.881078	7.932963	7.690084	7.693281	8.243397
38245_i_at	4.741346	4.721125	4.500526	4.599647	4.773613
39453_at	5.293902	5.486888	5.533448	5.499716	5.442538
	eT2.CEL	ET1.CEL	ET2.CEL		
33598_r_at	4.883518	4.697013	4.568902		
39859_r_at	9.470384	9.250283	9.663969		
35114_at	7.545388	7.302415	7.745817		
41041_s_at	8.143311	7.173642	8.131253		
41759_at	9.478198	9.813761	9.489163		
1532_g_at	7.493236	7.502159	7.053151		
36601_at	7.518949	7.810517	7.457067		
41155_at	11.116919	11.302171	10.738177		
36892_at	7.537353	7.534161	7.413570		
35706_at	5.553208	5.358180	5.104939		
505_at	10.111407	10.386093	10.096908		
35665_at	5.764129	5.930370	5.595601		
38467_at	8.398776	8.784625	8.477533		
37155_at	7.662369	7.222011	7.636735		
293_at	10.281736	9.779979	10.304022		
41240_at	4.927627	4.924196	4.776095		
33075_at	4.764387	4.850981	4.946187		
1491_at	4.735592	4.630627	4.890838		
34097_at	4.729341	4.710009	4.850891		
467_at	6.375991	7.066461	6.893920		
41107_at	7.131507	6.749076	7.162625		
38651_at	9.207648	8.729270	8.243492		
37997_r_at	9.638153	8.849091	9.775964		
968_i_at	7.781496	7.503551	7.575065		
38185_at	6.642384	6.578486	6.702875		
38844_at	7.651773	7.448243	8.040328		
36790_at	9.858803	10.846502	10.357288		

37415_at	5.969290	5.963656	6.102958
33609_at	9.011029	8.230950	9.051061
31838_at	4.574110	4.805341	4.712763
566_at	8.264233	7.892156	8.077518
41257_at	4.849890	4.679059	4.788253
38617_at	8.018083	7.786704	8.067131
39923_at	6.501877	6.455455	6.354310
1287_at	8.476732	10.181359	9.316795
35959_at	5.410721	4.919100	4.721720
39459_at	9.260177	8.968055	9.414008
32700_at	7.502795	7.395991	7.429489
33968_at	4.894051	4.601490	4.660449
32890_at	5.271570	5.023852	5.336599
31783_at	7.048878	6.574902	6.760811
35168_f_at	4.903464	4.805728	4.797124
35157_at	4.557002	4.799753	4.657474
34598_at	6.063323	5.741536	5.971066
31901_at	8.437228	8.482676	8.462516
31732_at	5.128359	5.510132	5.070144
32905_s_at	7.390903	7.108259	7.174432
35805_at	7.705363	8.401201	7.802099
33315_at	6.940284	6.704204	6.475294
40688_at	9.001557	8.726209	8.893780
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31520_at	7.261744	7.143923	7.510491
36986_at	11.178194	10.733719	10.689333
32574_at	7.115902	7.182194	6.942054
31590_g_at	9.685467	9.254673	9.716072
39379_at	5.963738	7.170717	6.260116
41143_at	10.190861	11.393271	10.891542
34662_at	6.592755	6.378261	6.187914
38279_at	6.840036	7.310094	7.094850
2075_s_at	9.093515	8.945285	9.008095
34779_at	8.531868	8.542043	8.895718
33415_at	12.063704	12.831589	12.276403
40137_at	9.055993	9.447347	9.181020
35581_at	5.031457	4.983349	5.204250
40079_at	9.598514	7.589340	7.241078
40106_at	10.362193	11.008530	10.731571
39882_at	5.301840	5.315204	5.528370
35471_g_at	6.066646	5.807044	5.806552
39384_at	5.091980	4.947292	4.999759
37825_at	6.883702	6.863198	6.475198

38831_f_at	9.235179	9.688342	9.071732
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32738_at	8.077730	8.615022	8.236200
34722_at	8.840044	8.326761	8.709686
37617_at	6.337825	6.358842	6.241089
34374_g_at	11.326656	10.475292	10.210713
31384_at	8.563581	8.405574	8.485678
33029_at	8.455467	8.226595	8.335843
38301_at	4.610486	4.591642	4.602953
35190_at	9.574960	9.049553	9.098185
39793_at	5.524049	6.435352	5.775248
286_at	13.364249	12.392652	12.052024
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789_at	8.884321	8.371599	8.476222
41093_at	6.043221	5.739214	5.460804
41009_at	4.851183	4.769246	4.739819
32176_at	9.356632	9.262384	9.424414
31625_at	5.418817	5.351008	5.369109
38108_at	7.939990	7.811868	8.417849
34266_at	5.075304	5.076998	5.177654
38153_at	5.087500	4.897811	5.189378
33978_at	6.541900	5.715335	6.205289
32331_at	6.446183	7.007531	6.946397
39709_at	11.770482	12.386640	12.147658
2058_s_at	9.828476	10.770092	10.282561
35793_at	7.682557	8.230815	7.621044
36347_f_at	10.551038	10.131503	10.077489
37444_at	9.441688	9.013071	9.458217
34130_at	7.289460	7.298381	7.501635
36061_at	5.980082	5.742814	5.804383
41840_r_at	5.269405	5.052123	5.171925
35990_at	7.568081	7.388835	7.435166
41043_at	8.461551	7.850348	8.270621
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37549_g_at	6.078765	6.149293	6.086317
35093_at	9.896537	9.665452	9.954466
31826_at	9.367171	9.281120	9.237804
41597_s_at	8.140800	8.696248	8.080245
33735_at	5.992380	5.827612	5.959562
1678_g_at	6.354953	5.413139	5.944566
41747_s_at	6.714932	6.788545	6.481012
38931_at	4.634422	4.873878	4.752261
31766_s_at	7.660683	7.435099	7.541669
36806_at	7.492056	7.363537	7.205241

41822_at	8.453489	8.551267	8.463779
37254_at	7.598890	7.543233	7.349078
35011_at	5.526761	5.447732	5.444510
38025_r_at	5.026817	5.037167	5.080495
33963_at	9.161856	8.689099	9.208725
33812_at	7.259488	7.288867	7.325526
32723_at	5.401692	6.223131	5.583154
34576_at	5.123586	4.917669	5.244636
739_at	5.115749	5.239764	5.132401
31911_at	7.922657	7.178470	7.706296
2026_at	4.922013	4.903668	5.103028
31759_at	7.760064	7.860346	7.943241
38542_at	11.986763	12.873351	12.459217
37665_at	5.399739	5.466260	5.626004
41033_at	6.210437	5.968550	5.837947
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39121_at	7.095620	6.822256	6.894131
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38164_at	5.417907	5.411389	5.295518
40168_at	6.003578	5.708668	5.897323
39727_at	7.179991	7.859086	6.876949
672_at	7.664490	7.297039	7.312901
35435_s_at	6.897628	7.666477	7.505057
33856_at	10.745264	10.763354	10.057760
35064_at	6.122910	5.932498	6.128422
1119_at	7.791194	8.765989	8.241933
32275_at	7.199997	6.949380	7.055516
40420_at	8.165262	8.233943	7.814284
32859_at	5.893490	5.646335	5.294326
32957_g_at	6.901767	6.914510	6.751010
1859_s_at	4.941307	4.899785	5.014732
33038_at	6.615330	6.251003	6.360028
39049_at	8.517683	8.105425	8.338234
40473_at	8.153504	9.048410	8.269033
36148_at	9.167959	8.798775	8.895342
34794_r_at	5.619992	5.202165	5.308943
33046_f_at	7.794930	7.490306	7.855473
31375_at	7.760042	7.357160	7.702816
34818_at	5.610760	5.682122	5.612469
37065_f_at	4.859958	4.969187	5.018000
32681_at	10.583069	10.140759	10.015657
35493_at	4.499183	4.804758	4.545718
32694_at	5.035637	5.129677	5.113945
36202_at	4.762388	4.818655	4.929654
34371_at	7.355718	7.449394	7.384127



38024_at	6.322837	6.298226	6.421134
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39137_at	9.905145	9.125003	9.429151
32730_at	7.961657	8.672006	7.746445
35094_f_at	7.886512	7.928284	7.747885
33280_r_at	5.153574	5.085240	4.953901
37773_at	4.668168	4.609005	4.817642
32270_g_at	7.239904	6.427672	6.900103
40654_at	5.461233	6.348074	5.881445
39209_r_at	4.914553	4.608220	4.578263
38242_at	5.984858	5.388259	5.054592
37622_r_at	4.573502	4.793512	4.559290
34591_at	8.656709	8.164602	8.675799
36533_at	5.544165	5.357898	5.428567
40786_at	5.581632	6.059594	5.638660
37173_at	5.220982	5.731113	5.465947
40353_at	7.485284	7.940137	7.306496
33853_s_at	8.103487	7.790551	8.382135
38731_at	6.076355	5.807313	5.810033
33268_at	9.714333	9.597316	9.504375
37325_at	7.929937	9.607316	8.819029
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33267_at	5.635343	5.858616	5.543187
36956_at	7.424126	7.212424	7.246201
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41110_at	4.698827	4.989018	4.743241
33379_at	4.687440	4.662576	4.673217
33961_at	7.645214	7.022559	7.306326
691_g_at	12.202069	12.673023	12.453313
37635_at	5.456359	5.214053	5.453419
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38115_at	7.408378	8.030783	7.278744
31878_at	5.487286	5.384374	5.675870
37052_at	5.970509	6.045975	5.980677
32940_at	5.586174	5.439220	5.736564
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37400_at	8.313257	8.174724	8.250270
36337_at	10.632261	9.683845	10.517556
34954_r_at	4.578364	4.525306	4.762666
40830_at	8.587564	8.311917	8.714345
34480_at	9.286107	8.611693	9.440739

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32100_r_at	11.605246	11.032834	11.434576
32119_at	4.804210	4.837018	4.624202
35928_at	6.352402	5.955547	6.630461
33112_at	5.023707	4.993622	4.938682
32661_s_at	7.690534	8.600764	7.846106
38292_at	9.248357	9.356315	9.272471
1548_s_at	6.169040	5.913334	6.073286
38616_at	7.630506	7.190080	7.419274
34326_at	6.820891	7.354378	6.930030
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40091_at	5.673057	6.226631	5.709507
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33473_at	4.663142	4.764401	4.849826
41810_at	5.654252	5.787158	5.896442
38950_r_at	9.486678	8.854443	9.299862
38433_at	5.147484	5.015607	5.380068
160026_at	6.440726	6.137820	6.158771
34855_at	7.239284	7.608423	7.406412
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36881_at	9.697136	10.602043	10.063323
38576_at	8.981296	8.976030	8.358915
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37593_at	5.469228	5.396687	5.456219
35260_at	8.842289	8.766271	8.845748
36655_at	5.099621	5.242517	5.152913
1646_at	5.565041	5.477265	5.534547
33203_s_at	7.482151	7.329609	7.600083
34875_r_at	5.083317	4.769869	5.096837
32428_at	7.185860	6.373223	6.529433
40209_at	7.117516	6.459683	7.310304
865_at	6.525965	6.478605	6.775071
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32108_at	9.371227	9.834491	9.464862
37765_at	8.163278	7.983139	8.436647
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38886_i_at	4.758975	4.699468	4.456960
34893_at	9.014232	9.735580	9.271056
432_s_at	5.241727	4.914260	4.812385
41862_at	6.347728	6.867104	6.862112
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33155_at	8.467093	8.433903	8.372123
32720_at	5.786401	5.985566	5.503978

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40802_at	5.028926	4.714327	4.841386
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41636_at	9.163478	8.776966	9.131170
36323_at	5.397621	5.256939	5.402799
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34118_at	5.480028	5.182603	5.421970
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40661_at	7.594014	7.288280	7.678916
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32011_g_at	7.135211	7.144422	7.157339
39425_at	8.812826	9.116950	8.403801
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Et1.CEL  P  10h
Et2.CEL  P  10h
eT1.CEL  A  48h
eT2.CEL  A  48h
ET1.CEL  P  48h
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> pData(estrogen)

      ES TIME
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et2.CEL  A  10h
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```

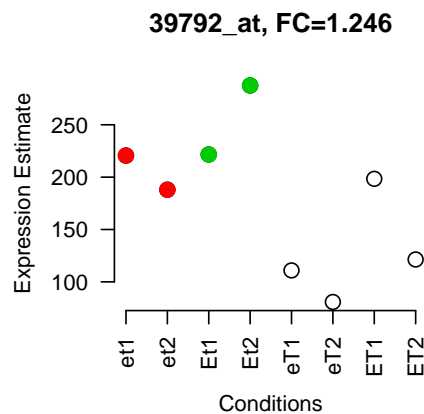
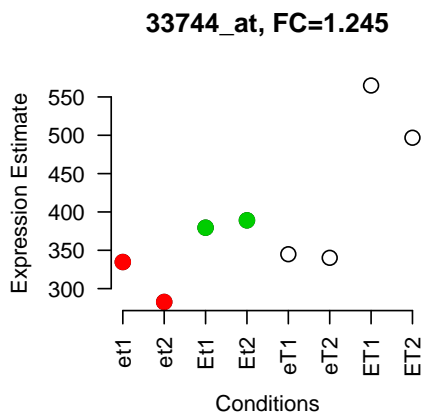
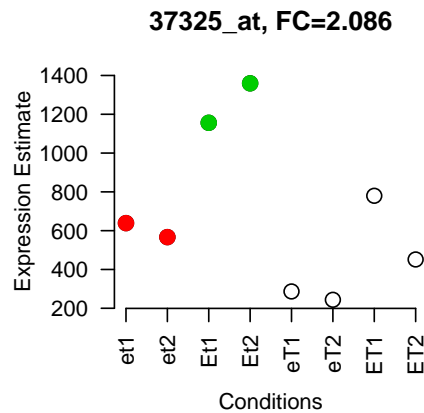
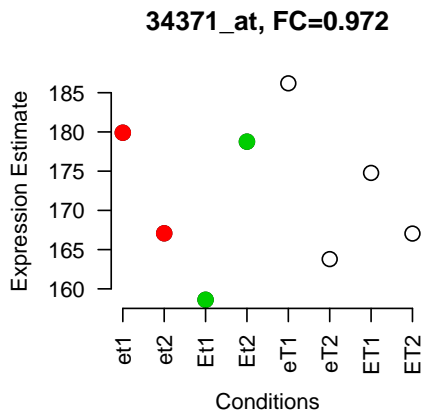
```
eT2.CEL  A  48h
ET1.CEL  P  48h
ET2.CEL  P  48h
```

## Analysis Using Fold Change Criteria

A simple method for finding estrogen-affected genes would be to form a ratio of the mean expression levels of the estrogen-treated samples to the mean of the expression levels for the untreated samples. Suppose we consider only the 10-hour time point, calculate fold change (FC) values for the estrogen-treated vs. untreated samples, and select genes for which we observe  $FC > 2$ . In the plots below, absence/presence of estrogen is represented by e/E and the 10/48 hour time point is represented by t/T on the horizontal axis. The proposed FC criteria at 10 hours would compare the mean of the green dots to the mean of the red dots.

If we used a  $FC > 2$  criteria to identify ES-affected genes in the **estrogen** data set, we would successfully eliminate genes like 34371\_at and select genes like 37325\_at; however, we could miss several interesting genes. For example, 33744\_at has a lower fold change value of 1.245, but the replicates are very consistent, leading us to believe that this smaller effect might be real. We would want to distinguish this from other genes like 39792\_at which has a similar fold change value of 1.246, but quite variable observations.

```
> par(mfrow = c(2, 2))
> par(las = 2)
> for (i in c("34371_at", "37325_at", "33744_at", "39792_at")) {
+   expvals <- 2^exprs(estrogen)[i, ]
+   plot(expvals, axes = F, cex = 1.5, xlab = "Conditions", ylab = "Expression Estimate")
+   points(1:2, expvals[1:2], pch = 16, cex = 1.5, col = 2)
+   points(3:4, expvals[3:4], pch = 16, cex = 1.5, col = 3)
+   axis(1, at = 1:8, labels = c("et1", "et2", "Et1", "Et2",
+     "eT1", "eT2", "ET1", "ET2"))
+   axis(2)
+   FC <- round(mean(expvals[3:4])/mean(expvals[1:2]), 3)
+   title(paste(i, ", FC=", FC, sep = ""))
+ }
```



We would like to find genes with consistent expression estimates between replicate samples that are either up- or down-regulated by estrogen, for example 32536\_at and 40446\_at. We would also like to find genes like gene 32901\_at for which the magnitude of the effect of estrogen changes over time. Furthermore, we would like to exclude genes like 31826\_at that demonstrate change primarily over time, and not necessarily due to estrogen.

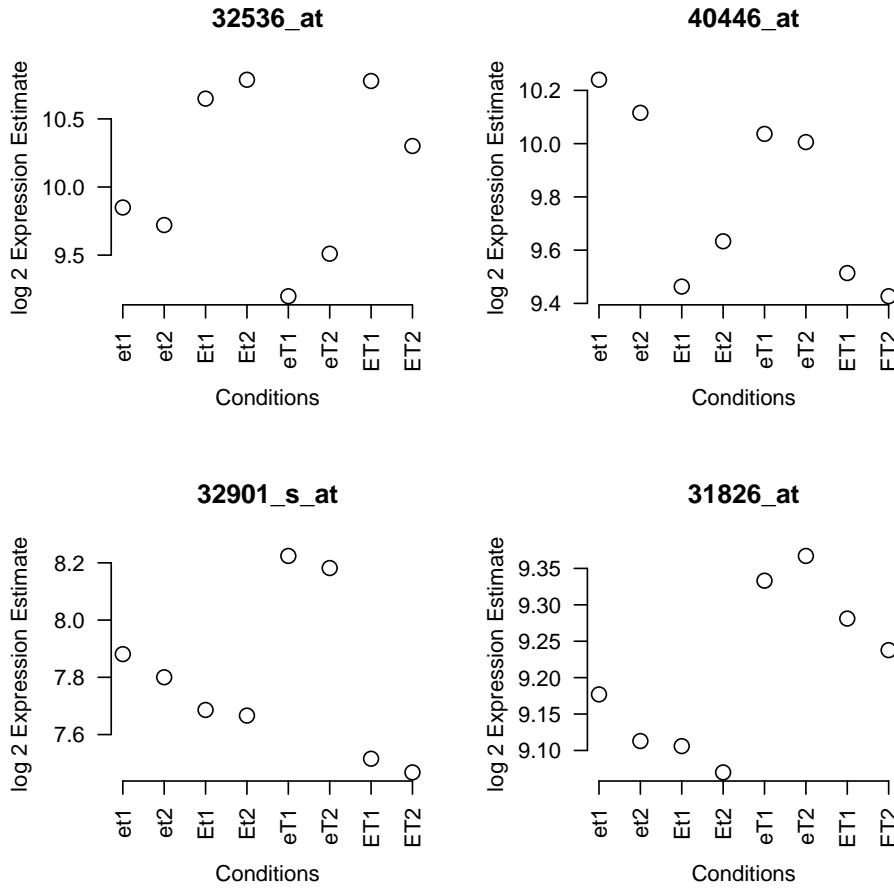
Selecting genes according to fold change estimates alone does not take advantage of the measure of variability in gene expression offered by the replicate samples. Furthermore, we cannot attach statistical significance (i.e., a  $p$ -value) to the fold change estimates computed in this manner. It is also difficult to quantify any change in estrogen effect over time. Classical statistical linear modeling with thoughtful biological interpretation of the parameters offers a natural paradigm for the analysis of factorial designed microarray experiments.

```
> par(mfrow = c(2, 2))
> par(las = 2)
> for (i in c("32536_at", "40446_at", "32901_s_at", "31826_at")) {
+   expvals <- exprs(estrogen)[i, ]
+   plot(expvals, axes = F, cex = 1.5, xlab = "Conditions", ylab = "log 2 Expression Estima
+   axis(1, at = 1:8, labels = c("et1", "et2", "Et1", "Et2",
+     "eT1", "eT2", "ET1", "ET2"))
```

```

+ axis(2)
+ title(i)
+ }

```



## Removing Outliers

Before defining the linear model for this particular experiment, we may want to remove observations that might be single outliers in the data set. The test we used is based on the differences between replicates and is appropriate for small factorial experimental designs. First, we identify replicate pairs with differences that are significantly larger than expected, and then we can apply a median absolute deviation filter to make sure one of the observations is indeed the single outlier. For example, 728\_at has a replicate pair with a large difference, but we wouldn't want to label either observation as the single outlier. 33379\_at has one observation that indeed appears to be a single outlier.

Removing single outliers from small factorial designed experiments does assume that the changes in expression across experimental conditions are small compared to the outlier effects. For probe 33379\_at, it could very well be the second observation which is the outlier if true

expression happens to be higher at the earlier time in the presence of estrogen. Users should consider whether or not single outlier elimination is appropriate in their particular experimental setting. Here we have commented out the code that could be used to replace single outliers with "NA" values.

```

> op1 <- outlierPair(exprs(estrogen)["728_at", ], INDEX = pData(estrogen),
+   p = 0.05)
> print(op1)

$test
[1] TRUE

$pval
[1] 0.01432178

$whichPair
[1] 7 8

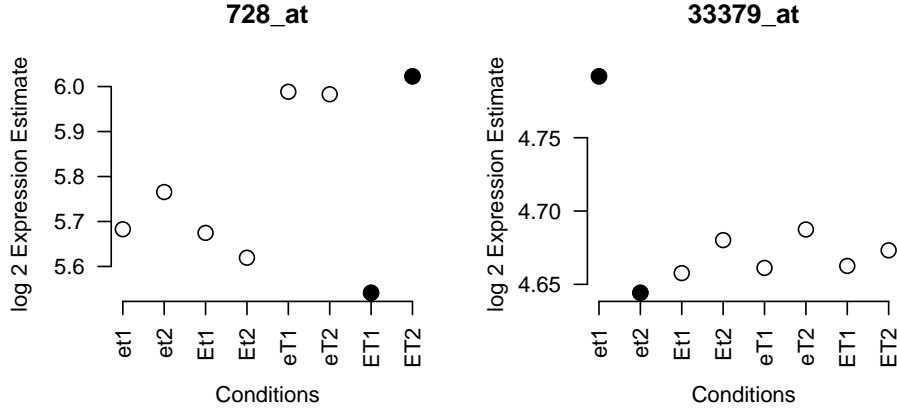
> madOutPair(exprs(estrogen)["728_at", ], op1[[3]])

[1] NA

> par(mfrow = c(2, 2))
> par(las = 2)
> for (i in c("728_at", "33379_at")) {
+   expvals <- exprs(estrogen)[i, ]
+   plot(expvals, axes = F, cex = 1.5, xlab = "Conditions", ylab = "log 2 Expression Estima
+   if (i == "728_at")
+     points(7:8, expvals[7:8], pch = 16, cex = 1.5)
+   if (i == "33379_at")
+     points(1:2, expvals[1:2], pch = 16, cex = 1.5)
+   axis(1, at = 1:8, labels = c("et1", "et2", "Et1", "Et2",
+     "eT1", "eT2", "ET1", "ET2"))
+   axis(2)
+   title(i)
+ }

```





## Describing the Linear Model

The  $2 \times 2$  factorial design of this experiment allows us to use a statistical linear model to measure the effects of estrogen and time on gene expression. In equation (1),  $y_{full,ij}$  is the observed expression level for gene  $i$  in sample  $j$  ( $j = 1, \dots, 8$ ).  $x_{ESj} = 1$  if estrogen is present and 0 otherwise;  $x_{TIMEj} = 1$  if gene expression was measured at 48 hours and 0 otherwise.  $\mu_i$  is the expression level of untreated gene  $i$  at 10 hours.  $\beta_{ESi}$  and  $\beta_{TIMEi}$  represent the effects of estrogen and time on the expression level of gene  $i$ , respectively.  $\beta_{ES:TIMEi}$  is called an interaction term for gene  $i$ ; this allows us to quantify any change in estrogen effect over time for probes like 1700\_at.  $\epsilon_{ij}$  represents random error for gene  $i$  and sample  $j$ , and is assumed to be independent for each gene and sample, and normally distributed with mean 0 and variance  $\sigma_i^2$ . The biologically independent replicates of the experimental conditions in this study allow us to estimate  $\sigma_i^2$ .

$$y_{ij} = \mu_i + \beta_{ESi}x_{ESj} + \beta_{TIMEi}x_{TIMEj} + \beta_{ES:TIMEi}x_{ESj}x_{TIMEj} + \epsilon_{ij} \quad (1)$$

To proceed with the analysis, we estimate the  $\beta$  parameters for every gene using least squares, and call the estimates  $\hat{\beta}_{ESi}$ ,  $\hat{\beta}_{TIMEi}$ , and  $\hat{\beta}_{ES:TIMEi}$ . For gene  $i$ , the samples that were not

treated with estrogen and were measured at 10 hours will have estimated expression values of  $\hat{\mu}_i$ . The estrogen-treated, 10-hour samples will have estimates  $\hat{\mu}_i + \hat{\beta}_{ESi}$ . The untreated, 48-hour samples will have estimates  $\hat{\mu}_i + \hat{\beta}_{TIMEi}$ . The estrogen-treated, 48-hour samples will have estimates  $\hat{\mu}_i + \hat{\beta}_{ESi} + \hat{\beta}_{TIMEi} + \hat{\beta}_{ES:TIMEi}$ .

We will also form a reduced model with only an effect for time (2), and use this to decide if a model including estrogen is appropriate for the gene of interest.

$$y_{ij} = \mu_i + \beta_{TIMEi}x_{TIMEj} + \epsilon_i \quad (2)$$

```
> lm.full <- function(y) lm(y ~ ES + TIME + ES:TIME)
> lm.time <- function(y) lm(y ~ TIME)
> lm.f <- esApply(estrogen, 1, lm.full)
> lm.t <- esApply(estrogen, 1, lm.time)
> lm.f[[1]]
```

Call:

```
lm(formula = y ~ ES + TIME + ES:TIME)
```

Coefficients:

(Intercept)	ESP	TIME48h	ESP:TIME48h
4.81164	-0.22762	0.01055	0.03839

```
> lm.t[[1]]
```

Call:

```
lm(formula = y ~ TIME)
```

Coefficients:

(Intercept)	TIME48h
4.69783	0.02974

## Selecting Genes of Interest using the Linear Model

We are only interested in genes which are affected by estrogen. One way to select such genes is to compare the full linear model (`lm.f`) to the linear model consisting of only a term for time (`lm.t`) using an ANOVA  $F$ -test. If the full model `lm.f` fits better than the reduced model `lm.t`, then we know the gene must be affected by estrogen.

Since we have so many genes to consider, multiple comparisons is an obvious problem. The R package *multtest* contains many functions that are suitable for multiple comparisons adjustment for microarrays. Here, the  $p$ -values from the ANOVA  $F$ -tests are adjusted according to the Benjamini and Hochberg (1995) False Discovery Rate method with an FDR of .15.

```
> Fpvals <- rep(0, length(lm.f))
> for (i in 1:length(lm.f)) {
+   Fpvals[i] <- anova(lm.t[[i]], lm.f[[i]])$P[2]
+ }
```

```

> library(multtest)
> procs <- c("BH")
> F.res <- mt.rawp2adjp(Fpvals, procs)
> F.adjps <- F.res$adjp[order(F.res$index), ]
> Fsub <- which(F.adjps[, "BH"] < 0.15)
> estrogen.Fsub <- estrogen[Fsub]
> lm.f.Fsub <- lm.f[Fsub]
> estrogen.Fsub

```

An object of class "exprSet"

Slot "exprs":

	et1.CEL	et2.CEL	Et1.CEL	Et2.CEL	eT1.CEL	eT2.CEL
40079_at	8.282810	7.995803	7.499557	7.577245	10.060174	9.598514
34374_g_at	11.644884	11.270676	10.625574	10.525063	11.619944	11.326656
286_at	13.779465	13.779060	13.094700	13.361393	13.529532	13.364249
32331_at	6.892743	6.749522	7.533555	7.375121	6.622532	6.446183
672_at	7.214869	7.338292	7.140576	7.063471	7.636534	7.664490
35435_s_at	7.215061	7.121819	7.732836	7.938600	6.668230	6.897628
32681_at	10.253695	10.233421	9.887731	9.962126	10.706248	10.583069
39137_at	10.011675	9.884737	9.397686	9.414500	9.834964	9.905145
38242_at	6.453461	6.197265	5.435737	5.450943	6.311774	5.984858
160026_at	6.368558	6.377702	6.364843	6.384174	6.427891	6.440726
432_s_at	4.858931	4.870105	5.076422	4.968401	5.258501	5.241727
41862_at	6.204166	6.291644	6.649816	6.823502	6.529665	6.347728
894_g_at	11.142055	11.132810	11.600913	11.738474	10.463821	9.968144
39213_at	4.631157	4.618122	4.600708	4.564543	4.731657	4.741143
38700_at	11.030733	11.059230	10.803357	10.892978	11.367163	11.663329
40446_at	10.240020	10.115903	9.463301	9.633330	10.036681	10.005929
37580_at	5.821848	5.825163	5.553752	5.541238	5.478780	5.407607
33744_at	8.386912	8.142862	8.568018	8.604011	8.430149	8.410366
32536_at	9.849866	9.721037	10.648758	10.787468	9.198465	9.510483
2049_s_at	9.635940	9.678346	8.940772	8.797628	10.474072	10.123041
36618_g_at	9.133171	8.978264	8.248726	8.233269	8.492557	8.406378
36317_at	7.557477	7.465826	6.702609	6.635716	7.566588	7.667485
32901_s_at	7.881089	7.800345	7.686088	7.666362	8.224178	8.181943
39787_at	8.308357	8.264818	8.575181	8.541409	8.304810	8.300011
1005_at	9.206827	8.993848	8.338061	8.237932	9.173212	9.040521
31538_at	14.023865	14.013151	14.028277	14.031891	13.884187	13.890177
40032_at	6.789958	6.670936	7.070212	7.190335	6.680520	6.684240
38432_at	11.586386	11.755469	11.281175	11.416958	11.759083	11.665397
	ET1.CEL	ET2.CEL				
40079_at	7.589340	7.241078				
34374_g_at	10.475292	10.210713				
286_at	12.392652	12.052024				
32331_at	7.007531	6.946397				

672_at	7.297039	7.312901
35435_s_at	7.666477	7.505057
32681_at	10.140759	10.015657
39137_at	9.125003	9.429151
38242_at	5.388259	5.054592
160026_at	6.137820	6.158771
432_s_at	4.914260	4.812385
41862_at	6.867104	6.862112
894_g_at	12.202302	11.857381
39213_at	4.645703	4.612490
38700_at	10.638621	10.611983
40446_at	9.513993	9.427049
37580_at	5.362758	5.414473
33744_at	9.141964	8.956593
32536_at	10.778387	10.301206
2049_s_at	8.638922	8.797647
36618_g_at	8.215679	8.004585
36317_at	6.651701	6.932811
32901_s_at	7.515624	7.467901
39787_at	8.655906	8.520160
1005_at	7.926145	8.069757
31538_at	13.987350	13.983279
40032_at	7.131127	7.088095
38432_at	11.103805	10.886529

Slot "se.exprs":

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]
[1,]	NA	NA	NA	NA	NA	NA	NA	NA
[2,]	NA	NA	NA	NA	NA	NA	NA	NA
[3,]	NA	NA	NA	NA	NA	NA	NA	NA
[4,]	NA	NA	NA	NA	NA	NA	NA	NA
[5,]	NA	NA	NA	NA	NA	NA	NA	NA
[6,]	NA	NA	NA	NA	NA	NA	NA	NA
[7,]	NA	NA	NA	NA	NA	NA	NA	NA
[8,]	NA	NA	NA	NA	NA	NA	NA	NA
[9,]	NA	NA	NA	NA	NA	NA	NA	NA
[10,]	NA	NA	NA	NA	NA	NA	NA	NA
[11,]	NA	NA	NA	NA	NA	NA	NA	NA
[12,]	NA	NA	NA	NA	NA	NA	NA	NA
[13,]	NA	NA	NA	NA	NA	NA	NA	NA
[14,]	NA	NA	NA	NA	NA	NA	NA	NA
[15,]	NA	NA	NA	NA	NA	NA	NA	NA
[16,]	NA	NA	NA	NA	NA	NA	NA	NA
[17,]	NA	NA	NA	NA	NA	NA	NA	NA
[18,]	NA	NA	NA	NA	NA	NA	NA	NA
[19,]	NA	NA	NA	NA	NA	NA	NA	NA

```

[20,] NA NA NA NA NA NA NA NA
[21,] NA NA NA NA NA NA NA NA
[22,] NA NA NA NA NA NA NA NA
[23,] NA NA NA NA NA NA NA NA
[24,] NA NA NA NA NA NA NA NA
[25,] NA NA NA NA NA NA NA NA
[26,] NA NA NA NA NA NA NA NA
[27,] NA NA NA NA NA NA NA NA
[28,] NA NA NA NA NA NA NA NA

```

Slot "description":

An object of class "MIAME"

Slot "name":

```
[1] ""
```

Slot "lab":

```
[1] ""
```

Slot "contact":

```
[1] ""
```

Slot "title":

```
[1] ""
```

Slot "abstract":

```
[1] ""
```

Slot "url":

```
[1] ""
```

Slot "samples":

```
list()
```

Slot "hybridizations":

```
list()
```

Slot "normControls":

```
list()
```

Slot "preprocessing":

Slot "preprocessing":\$filenames

```
[1] "et1.CEL" "et2.CEL" "Et1.CEL" "Et2.CEL" "eT1.CEL" "eT2.CEL" "ET1.CEL"
```

```
[8] "ET2.CEL"
```

Slot "preprocessing":\$affyversion

```
[1] "1.4.18"
```

Slot "other":

```
list()
```

Slot "annotation":

```
[1] "hgu95av2"
```

Slot "notes":

```
[1] ""
```

Slot "phenoData":

An object of class "phenoData"

```

Slot "pData":
      ES TIME
et1.CEL  A  10h
et2.CEL  A  10h
Et1.CEL  P  10h
Et2.CEL  P  10h
eT1.CEL  A  48h
eT2.CEL  A  48h
ET1.CEL  P  48h
ET2.CEL  P  48h
Slot "varLabels":
Slot "varLabels":$ES
[1] "presence or absence of estrogen"

Slot "varLabels":$TIME
[1] "length of exposure to treatment (hours)"

```

Suppose we want to identify genes that are affected by estrogen at 10 hours. In our linear model, this corresponds to testing a null hypothesis  $H_{0ES} : \beta_{ES} = 0$ , and if the hypothesis is rejected, concluding that the gene has a main estrogen effect.

```

> betaNames <- names(lm.f[[1]][["coefficients"]])
> lambda <- par2lambda(betaNames, c("ESP"), c(1))
> mainES <- function(x) contrastTest(x, lambda, p = 0.05)[[1]]
> mainESgenes <- sapply(lm.f.Fsub, FUN = mainES)
> sum(mainESgenes == "REJECT")

[1] 22

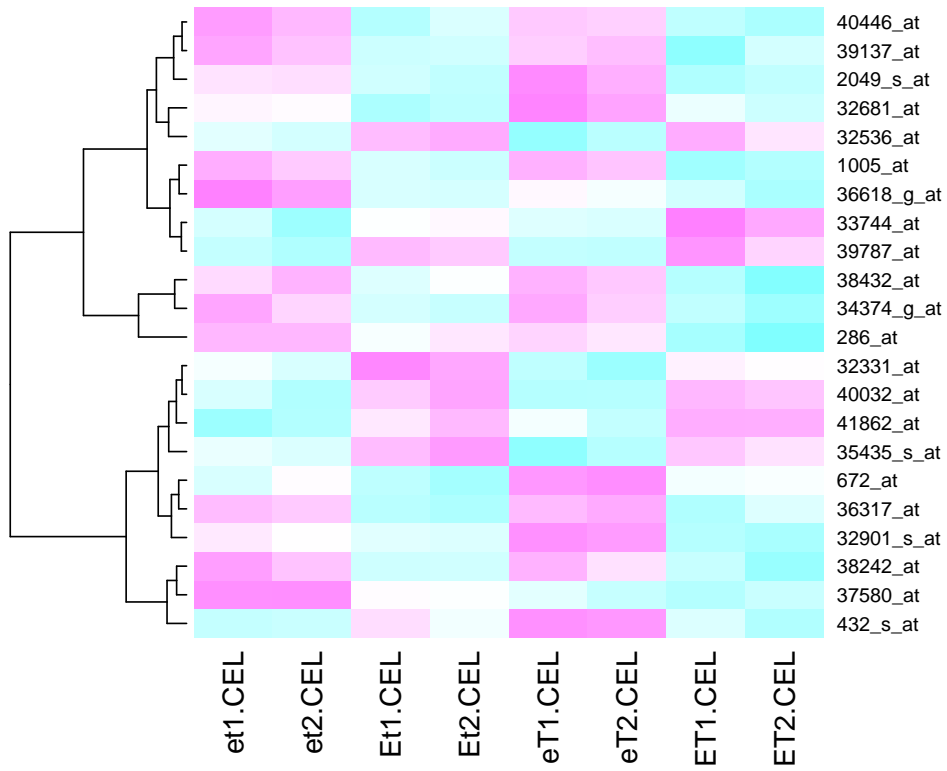
```

Heatmaps can be a useful way to visualize genes that are selected according to a certain criteria. In the first heatmap that follows, we see genes for which the null hypothesis  $H_{0ES}$  was rejected at a 0.05 significance level. In the second heatmap, we see the genes for which the main estrogen effect was not statistically significant; it appears that estrogen affected these genes only after 48 hours.

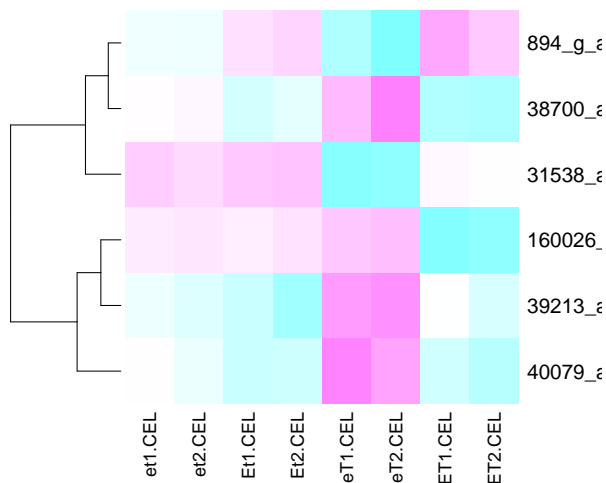
```

> heatmap(exprs(estrogen.Fsub)[mainESgenes == "REJECT", ], Colv = NA,
+         col = cm.colors(256))

```



```
> heatmap(exprs(estrogen.Fsub)[mainESgenes == "FAIL TO REJECT",
+      ], Colv = NA, col = cm.colors(256))
```



Selecting genes according to  $p$ -value can produce some possibly misleading results. For example, 32901\_s\_at had a main ES effect with  $p$ -value for  $\beta_{ES}$  less than 0.01, but the estimate of fold change suppression at 10 hours is only .8922. While this small effect is statistically significant, it may not be biologically interesting. Selecting genes with significant contrast test  $p$ -values as well as fold change values above a certain threshold can give a good approximation to more sophisticated variance moderating analyses.

```
> lambdaNum <- par2lambda(betaNames, list(c("(Intercept)", "ESP")),
+   list(c(1, 1)))
> lambdaDenom <- par2lambda(betaNames, list(c("(Intercept)")),
+   list(c(1)))
> FCval <- findFC(lm.f.Fsub[["32901_s_at"]], lambdaNum, lambdaDenom,
+   logbase = 2)
> print(FCval)
```

```
      [,1]
[1,] 0.8922424
```

```
> FCvals <- lapply(lm.f.Fsub, FUN = findFC, lambdaNum, lambdaDenom,
+   logbase = 2)
> largeFC <- unlist(FCvals > 1.4 | FCvals < 0.7)
> estrogen.Fsub.FC <- estrogen.Fsub[largeFC & mainESgenes == "REJECT"]
> heatmap(exprs(estrogen.Fsub.FC), Colv = NA, col = cm.colors(256))
```

Now suppose we want to find genes that are affected by estrogen after both 10 and 48 hours. By testing for the main estrogen effect, we have already found genes with an estrogen effect at 10 hours. To select genes with an estrogen effect at 48 hours, we want to compare the gene expression levels of the untreated samples that were measured at 48 hours with the



estrogen-treated samples at 48 hours. In terms of our linear model, for each gene, we want to test the null hypothesis  $H_{0ES,TIME}$  in (3).

$$H_{0ES,TIME} : \mu + \beta_{TIME} = \mu + \beta_{ES} + \beta_{TIME} + \beta_{ES:TIME} \quad (3)$$

Testing the null hypothesis  $H_{0ES,TIME}$  is equivalent to testing the linear contrast  $H_{0ES,TIME*}$  in (4).

$$H_{0ES,TIME*} : \beta_{ES} + \beta_{ES:TIME} = 0 \quad (4)$$

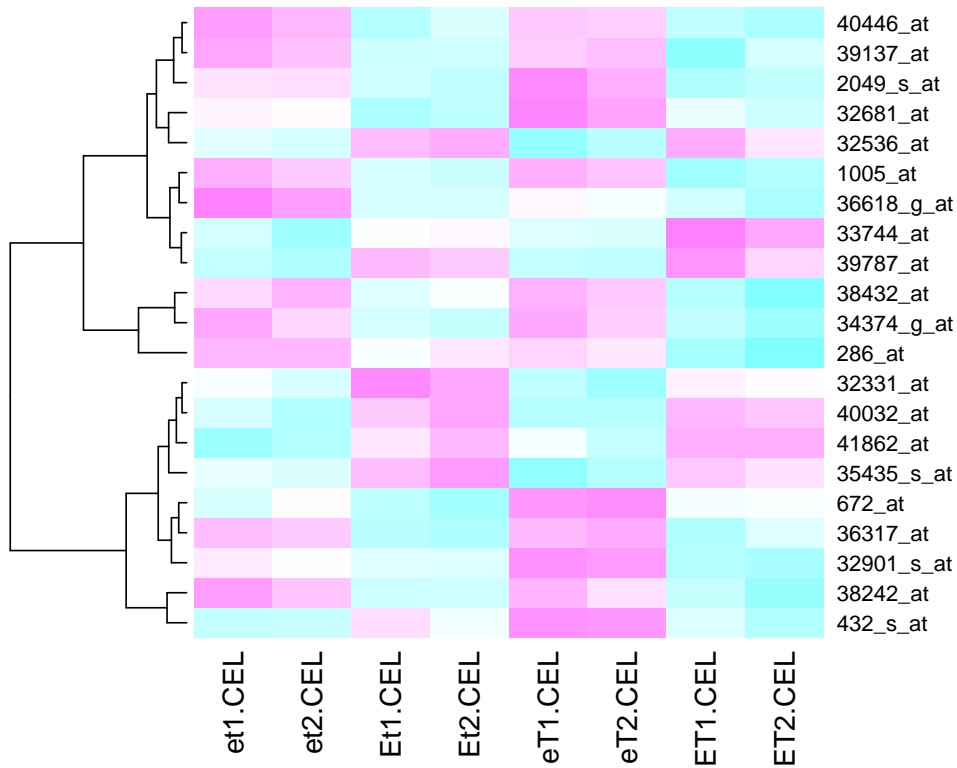
The technique for testing this linear contrast follows from straightforward linear model theory. The `par2lambda` function helps set up the appropriate matrix for testing sets of linear contrasts.

```
> lambdaEST <- par2lambda(betaNames, list(c("ESP", "ESP:TIME48h"),
+   list(c(1, 1)))
> ESTcontrast <- function(x) contrastTest(x, lambdaEST, p = 0.1)[[1]]
> ESTgenes <- sapply(lm.f.Fsub, FUN = ESTcontrast)
> sum(ESTgenes == "REJECT")
```

```
[1] 27
```

Again, we can use a heatmap to look at genes for which we rejected both  $H_{0ES}$  and  $H_{0ES,TIME*}$ .

```
> heatmap(exprs(estrogen.Fsub)[mainESgenes == "REJECT" & ESTgenes ==
+   "REJECT", ], Colv = NA, col = cm.colors(256))
```



After genes are selected according to contrast tests of interest, the annotation information available in other Bioconductor packages allows for more in-depth research on specific genes.

Using linear models for factorial designed microarray experiments enables investigators to extend analyses beyond basic gene filtering according to fold change. Genes can be selected in a high-throughput manner with biologically interpretable parameters and quantifiable measures of confidence. This lab investigated the effects of estrogen on breast cancer cells, but the principles behind this specific example are applicable to any carefully designed microarray study.