$APC^{Min/+}$ Expression Profiling

Re-analysis of Affymetrix Array Study, March, 2006

Analysis Methodology

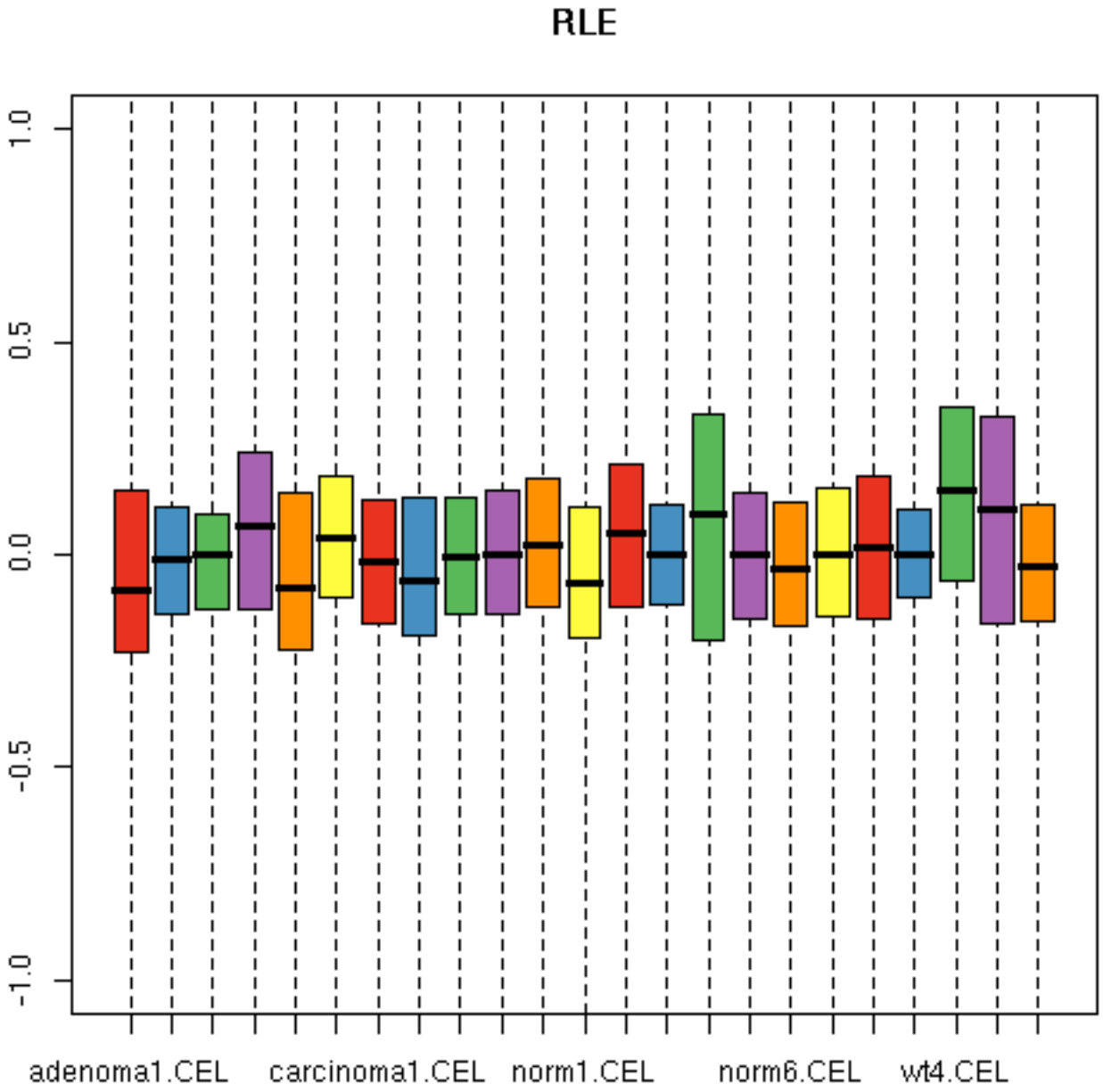
- Do a quality assessment
- Exclude the poor quality arrays
- Normalize with gcrma
- Use a modified t-test for differing expression levels; not an ANOVA. An ANOVA isn’t reliable with few samples.

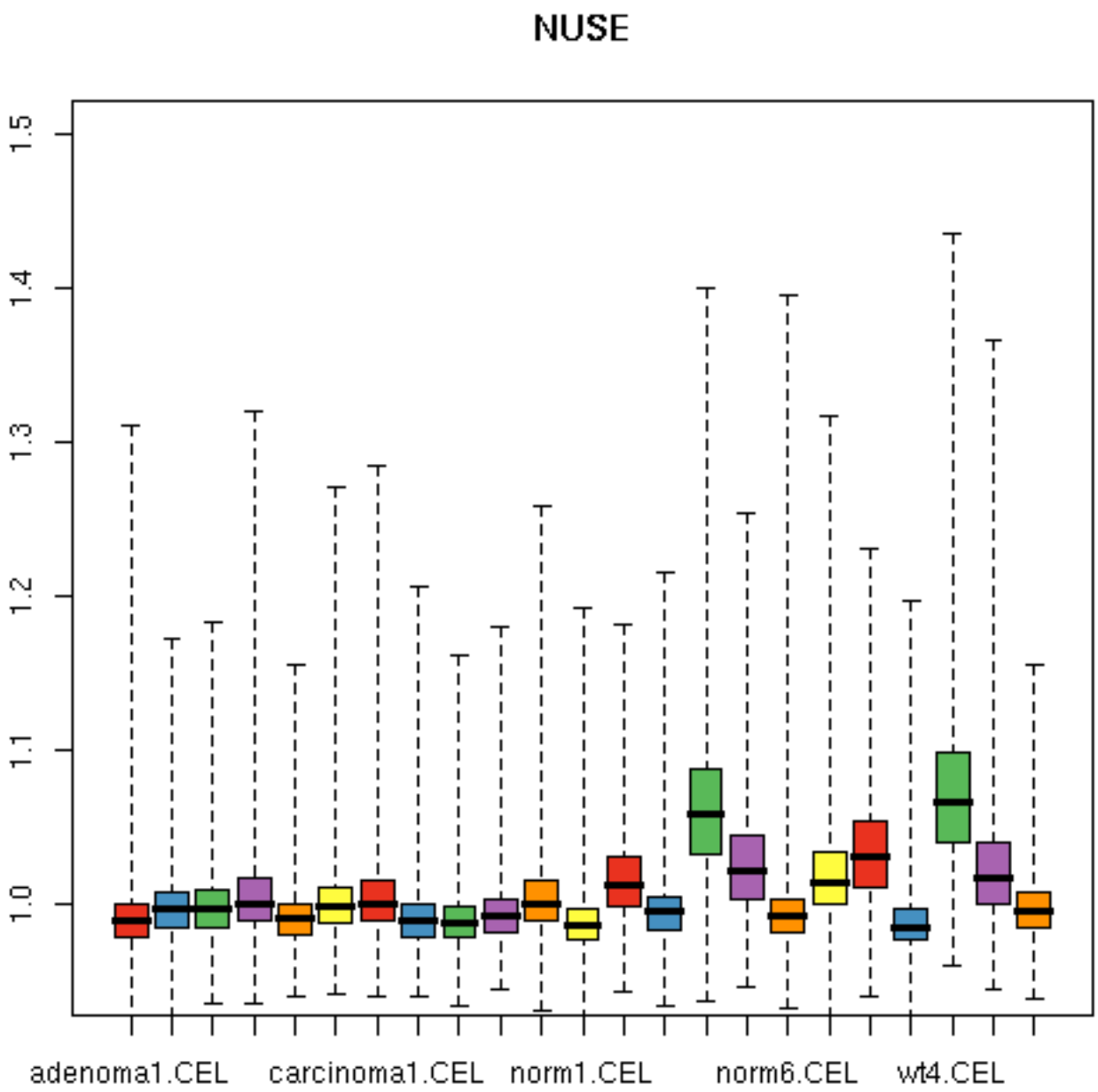
Quality Assessment

- Methodology is to check for scale factor, average background, percent present, 3’/5’ ratios, and the two plots: RLE and NUSE.

Quality Parameters

Array	Scale Factor	Avg Bkgnd	Percent Present	3'/5' ratios are OK
adenoma1.CEL	1.18	37.44	44.40	
adenoma2.CEL	2.31	50.71	37.08	
adenoma3.CEL	2.32	48.86	35.68	
adenoma4.CEL	4.79	44.35	30.53	
adenoma5.CEL	1.85	44.31	42.56	
adenoma6.CEL	3.74	50.13	30.96	
carcinoma1.CEL	3.29	45.67	31.55	
carcinoma2.CEL	1.74	40.69	42.06	
carcinoma3.CEL	1.40	39.31	40.78	
carcinoma4.CEL	2.22	50.61	37.04	
carcinoma5.CEL	4.02	44.67	32.66	
norm1.CEL	1.59	35.35	41.73	
norm2.CEL	3.92	50.63	30.88	
norm3.CEL	2.71	48.56	35.81	
norm4.CEL	12.29	37.12	21.84	
norm5.CEL	5.82	48.00	29.89	
norm6.CEL	1.67	60.53	39.56	
wt1.CEL	5.04	44.17	29.92	
wt2.CEL	7.85	37.00	27.15	
wt3.CEL	1.73	49.72	37.16	
wt4.CEL	7.91	30.58	28.50	
wt5.CEL	7.32	32.04	26.12	
wt6.CEL	2.63	54.92	36.80	


RLE
1.0
0.5
0.0
-0.5
-1.0
adenoma1.CEL
carcinoma1.CEL
norm1.CEL
norm6.CEL
wt4.CEL


NUSE
1.0
1.1
1.2
1.3
1.4
1.5
adenoma1.CEL
carcinoma1.CEL
norm1.CEL
norm6.CEL
wt4.CEL

Quality Conclusions

- From the scale factors, norm4 is bad and wt2, wt4, wt5 are marginal at best. Other parameters are OK.
- RLE and NUSE plots reinforce that the following are poor in this order: norm4, wt4, wt5, wt3.

Planned Analyses

- Analysis1: Exclude norm4 and all wild types. This leaves 6 adenoma, 5 carcinoma, 5 normal arrays to compare.
- Analysis2: Exclude norm4, wt4, wt5 and analyze remaining four sample types: 6 adenoma, 5 carcinoma, 5 normal, and 4 wild type. Be conservative in conclusions involving wild types

Summary of Both

- The two analyses lead to the following number of differentially expressed genes.

Pairs	C-N	A-N	C-A	C-W	A-W	N-W
#Genes	144	0	2	533	146	0

Points of Emphasis

- In differential expression between adenoma and carcinoma only two genes (Serpine2, Saa3) pass the tests.
- Some comparison possible using the common reference of wild type. Of the 146 genes differentially expressed in A-W, all but 10 are differentially expressed in C-W.

Points of Emphasis, II

- Comparisons between adenoma and wild type yield different results than comparison with normal. (Same for carcinoma). While no genes pass the test in comparing normal and wild type, there are clear differences.