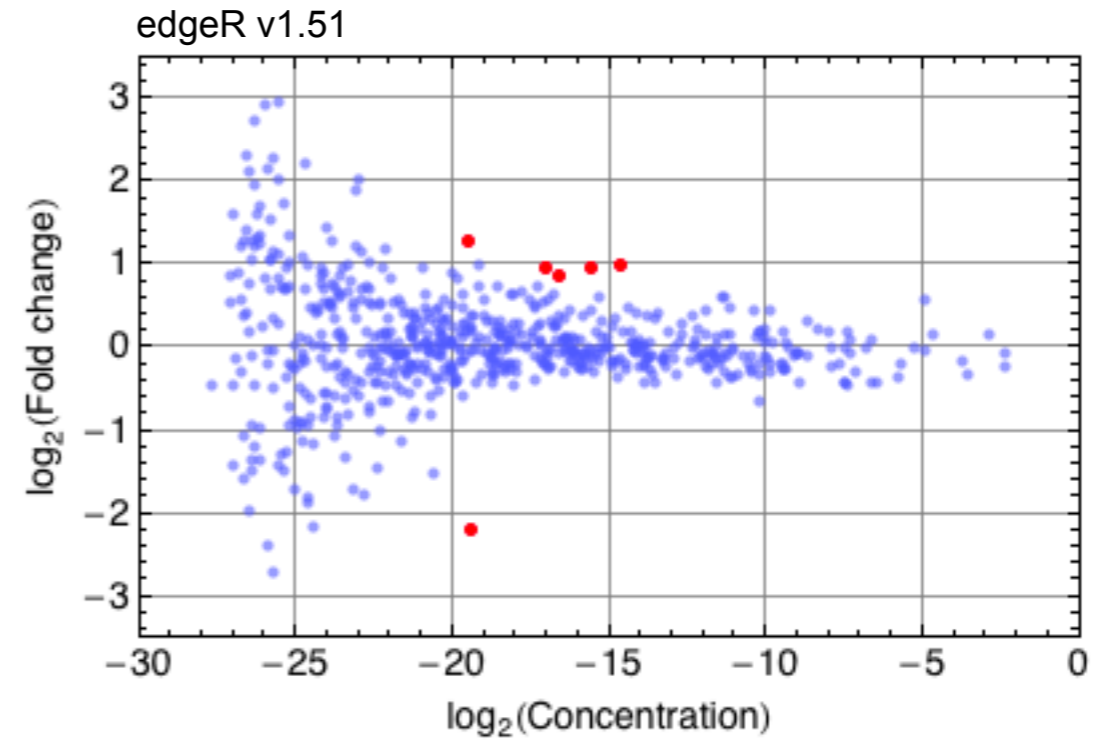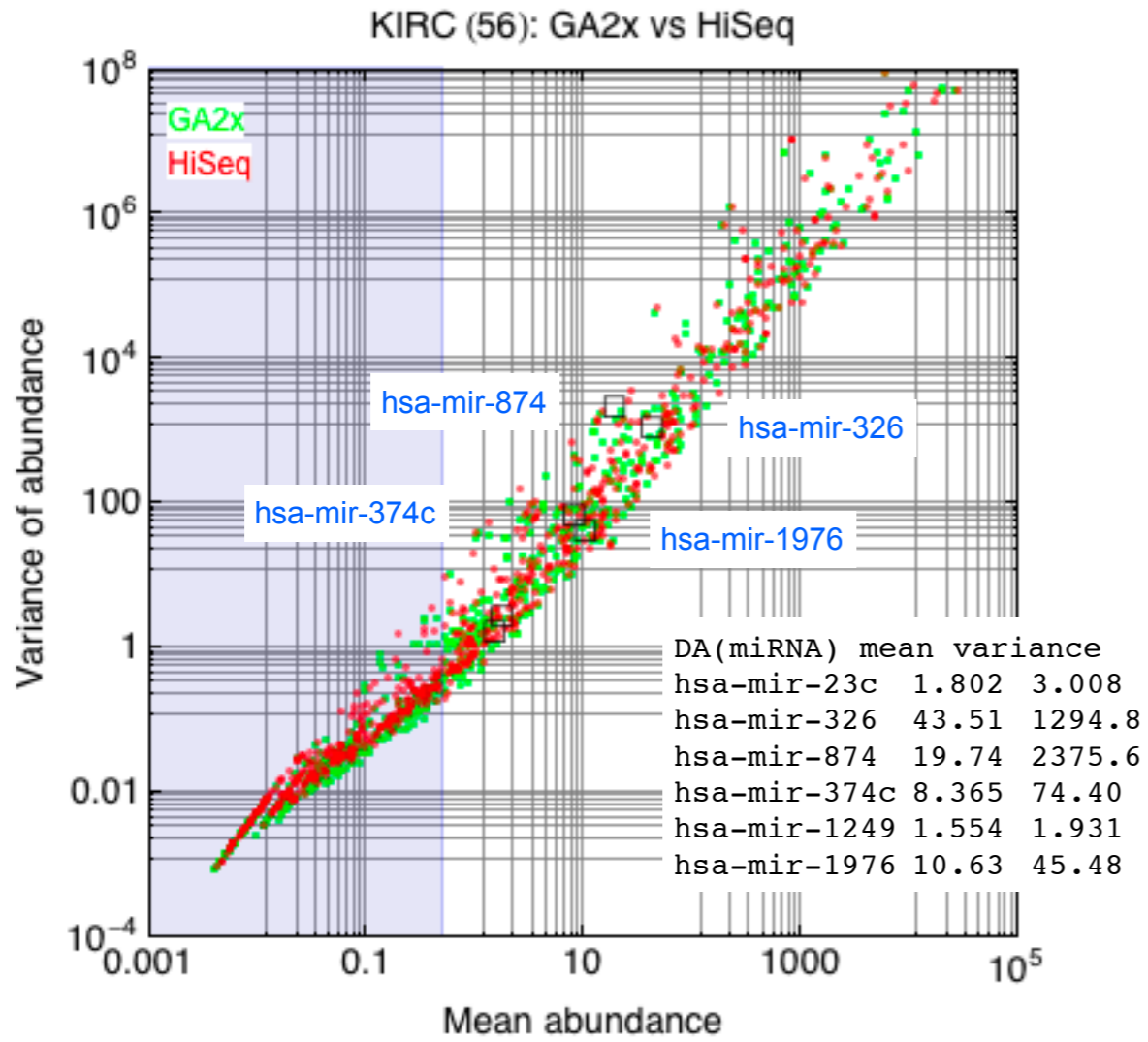# Differentially abundant KIRC/P miRNAs between GA2x and HiSeq

G. Robertson, Andy Chu, Richard Corbett, Samuel Wu
25 May 2011, 17h10

1. Adjusted data: GA2x vs. subsampled HiSeq.
2. Original data: GA2x vs. non-subsampled HiSeq
   GC content of miRNAs

KIRC/P GA2x vs HiSeq, subsample 5, 738 miRNAs

KIRC (56): GA2x vs HiSeq

GA2x
HiSeq

hsa-mir-874
hsa-mir-326
hsa-mir-374c
hsa-mir-1976

```
DA(miRNA) mean variance
hsa-mir-23c   1.802  3.008
hsa-mir-326   43.51  1294.8
hsa-mir-874   19.74  2375.6
hsa-mir-374c  8.365  74.40
hsa-mir-1249  1.554  1.931
hsa-mir-1976  10.63  45.48
```

edgeR v1.51



```
logConc       logFC P.Value      adj.P.Val
hsa-mir-23c  -19.43      -2.20 8.01e-12      5.91e-09
hsa-mir-326  -14.64       0.991 2.39e-06      0.00088
hsa-mir-874  -15.61       0.961 9.25e-06      0.0023
hsa-mir-374c       -17.01  0.963 2.23e-05      0.00411
hsa-mir-1249       -19.48  1.30  4.74e-05      0.0070
hsa-mir-1976       -16.60  0.854 0.00014       0.017
...
```
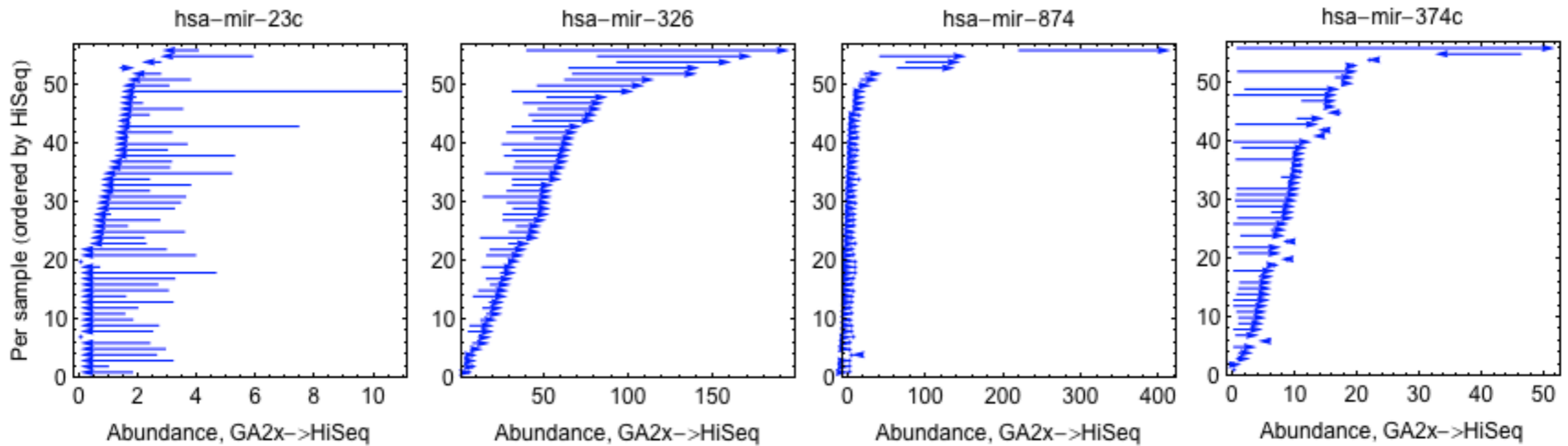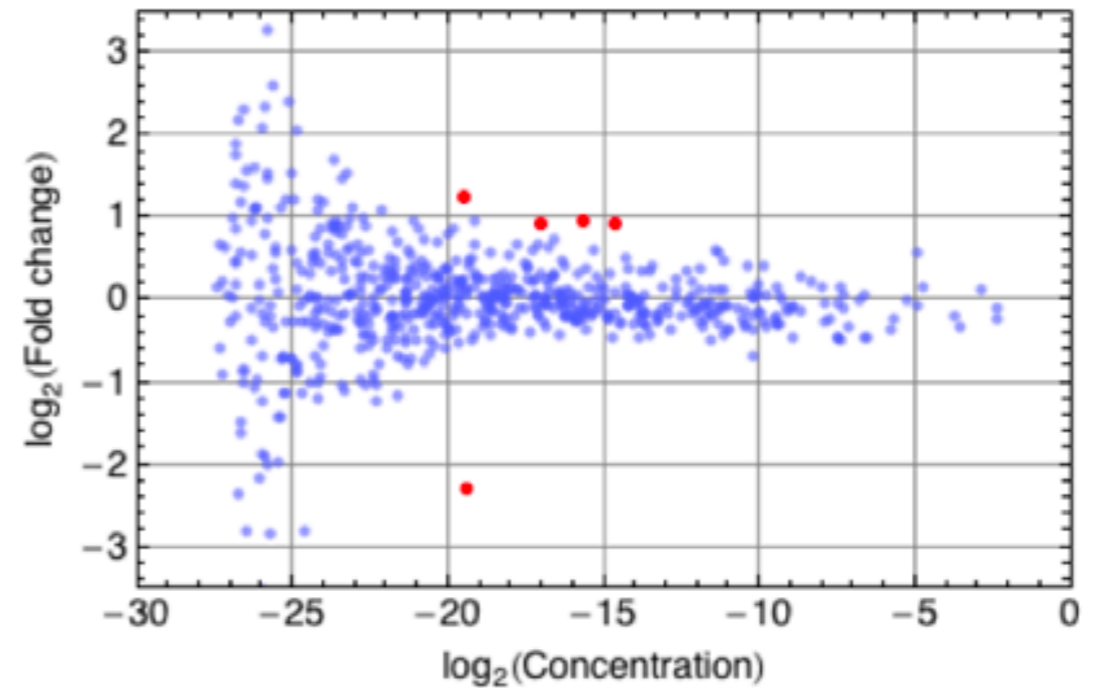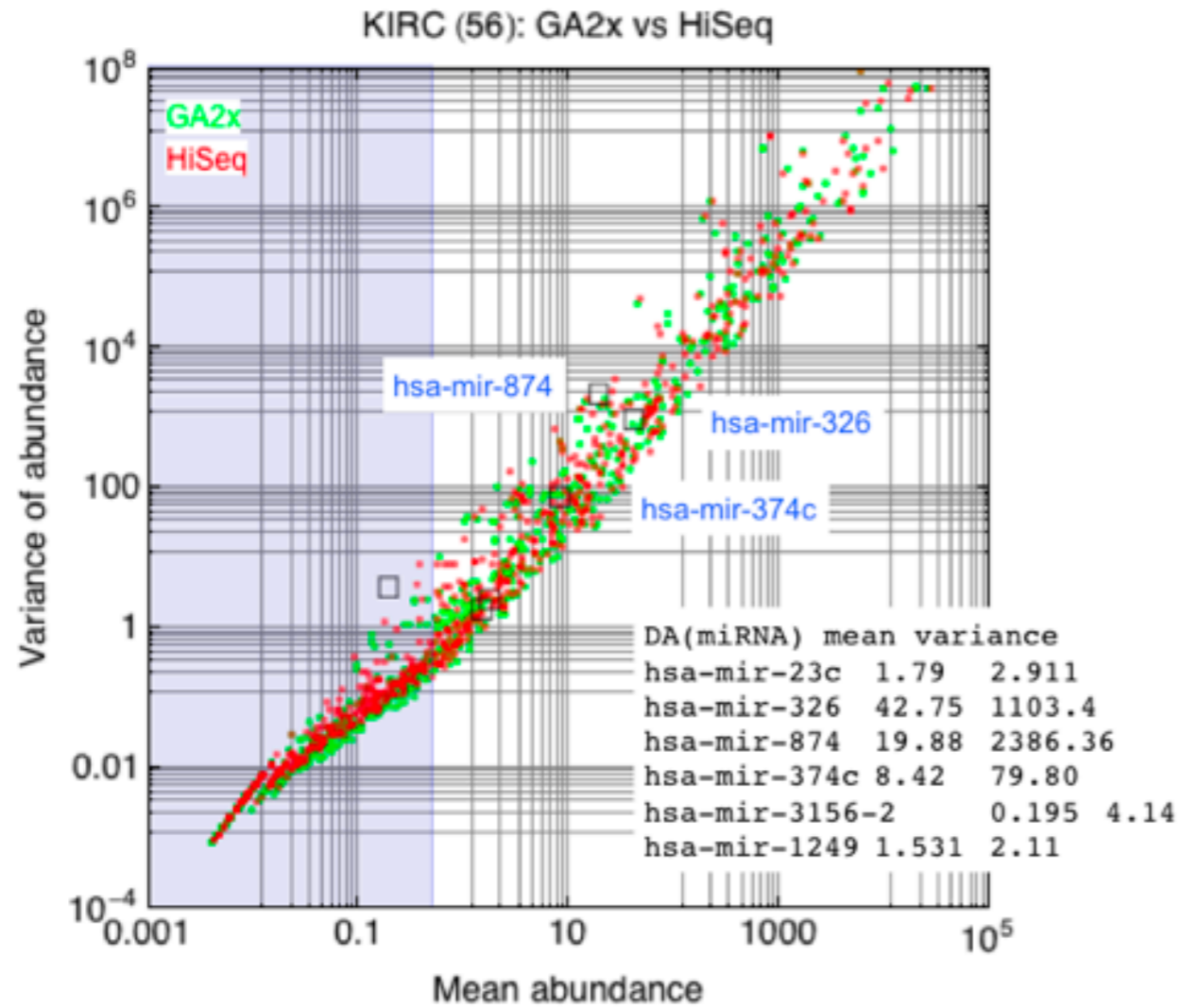
Differential abundance analysis, edgeR v1.5.1
A larger set of miRNAs should estimate
background variance more effectively.

Consensus clustering, Spearman, k-means, 200 iterations
The two platforms are mixed, i.e. do not clearly separate.

miRNA-13

consensus matrix k=7
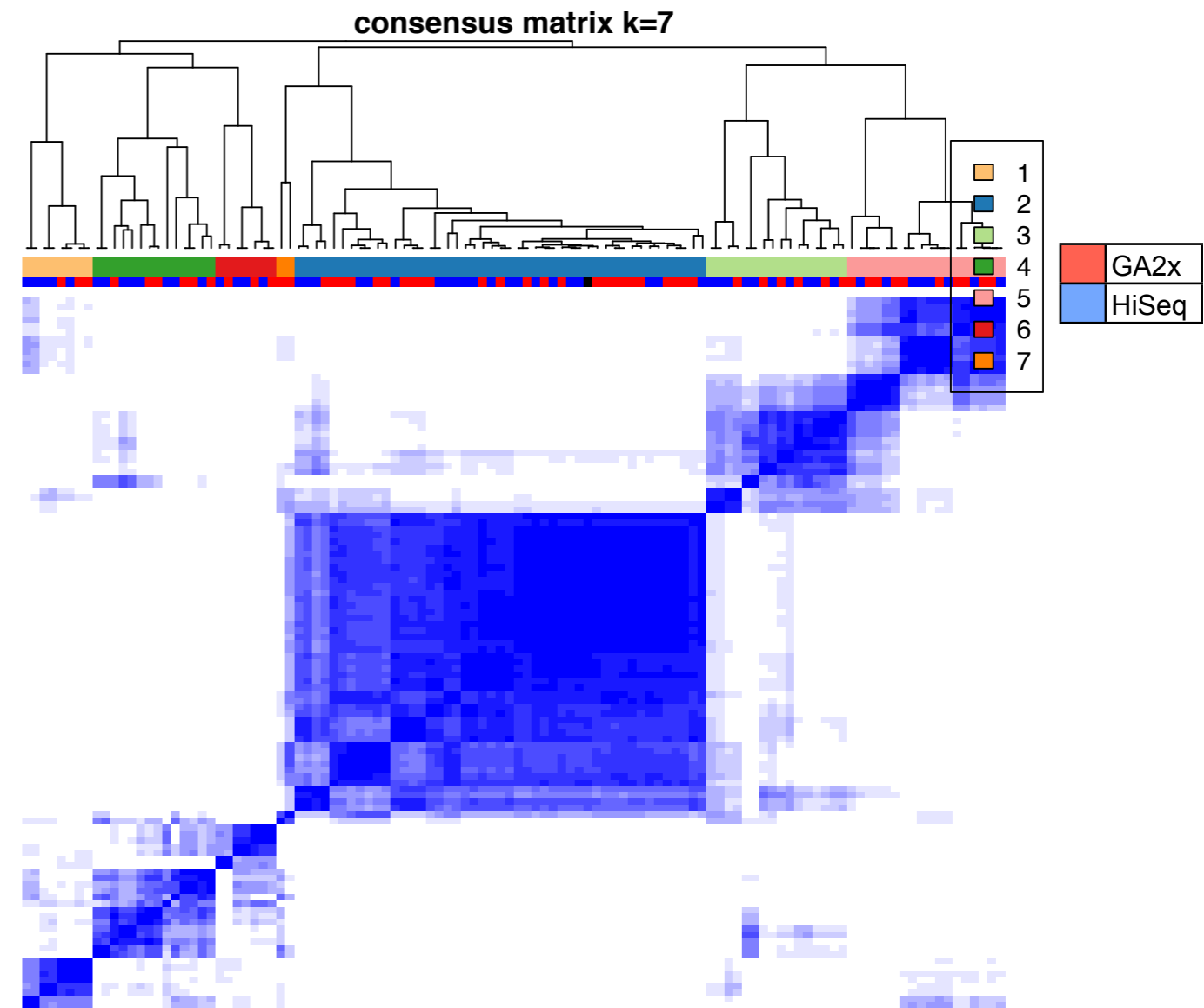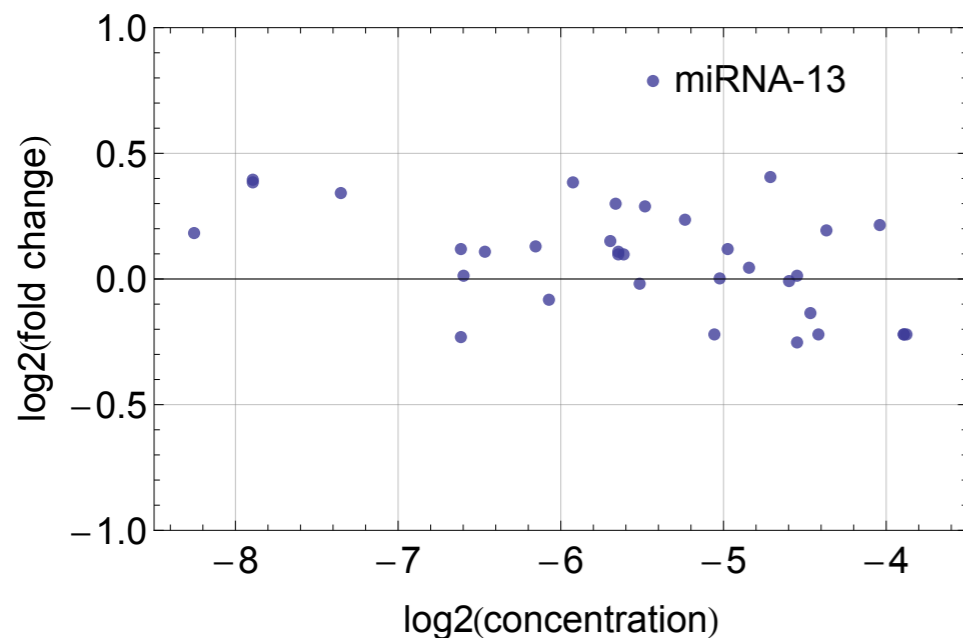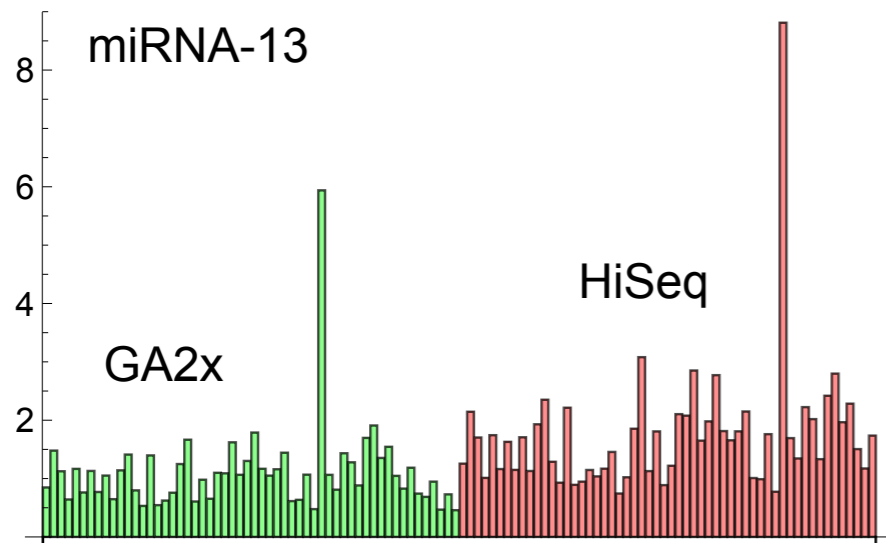
gene	logConc	logFC	P.Value	adj.P.Val
mi-13	-5.4255	0.783499	0.000945101	0.0330785
mi-23	-4.70622	0.406441	0.0291534	0.510185
mi-1	-3.89152	-0.221841	0.120596	0.751885
mi-2	-3.89345	-0.220216	0.120596	0.751885
mi-3	-3.87952	-0.220564	0.122239	0.751885
mi-34	-4.04362	0.211961	0.152072	0.751885
...				

Samuel Wu

25 May 2011, 17h00

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KIRC/P GA2x vs HiSeq, subsample 3, 731 miRNAs

KIRC (56): GA2x vs HiSeq

GA2x
HiSeq

hsa-mir-874
hsa-mir-326
hsa-mir-374c

```
DA(miRNA) mean variance
hsa-mir-23c  1.79   2.911
hsa-mir-326  42.75  1103.4
hsa-mir-874  19.88  2386.36
hsa-mir-374c 8.42   79.80
hsa-mir-3156-2     0.195 4.14
hsa-mir-1249 1.531 2.11
```



```
logConc       logFC P.Value      adj.P.Val
hsa-mir-23c -19.46      -2.278       2.19e-12    1.604e-09
hsa-mir-326 -14.65      0.944 6.76e-06    0.00194
hsa-mir-874 -15.65      0.964 7.95e-06    0.0019
hsa-mir-374c     -17.00      0.939 3.33e-05    0.0061
hsa-mir-3156-2   -24.36      5.884 4.54e-05    0.0066
hsa-mir-1249     -19.49      1.247 8.14e-05    0.0099
...
```


hsa-mir-23c

hsa-mir-326

hsa-mir-874
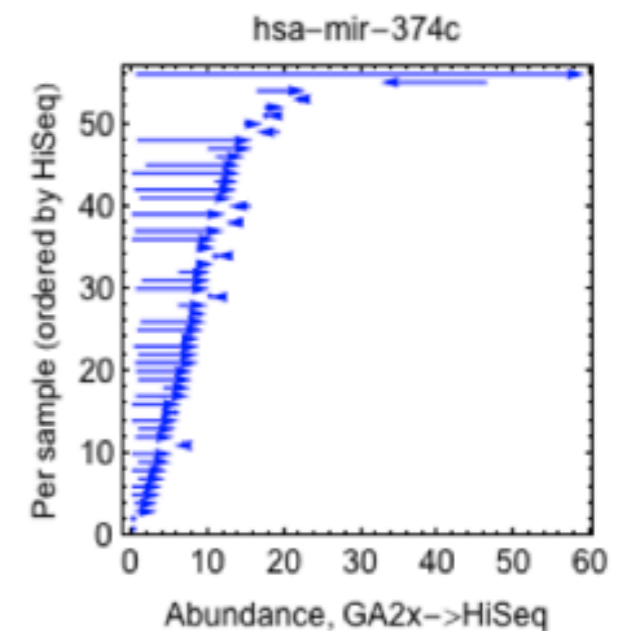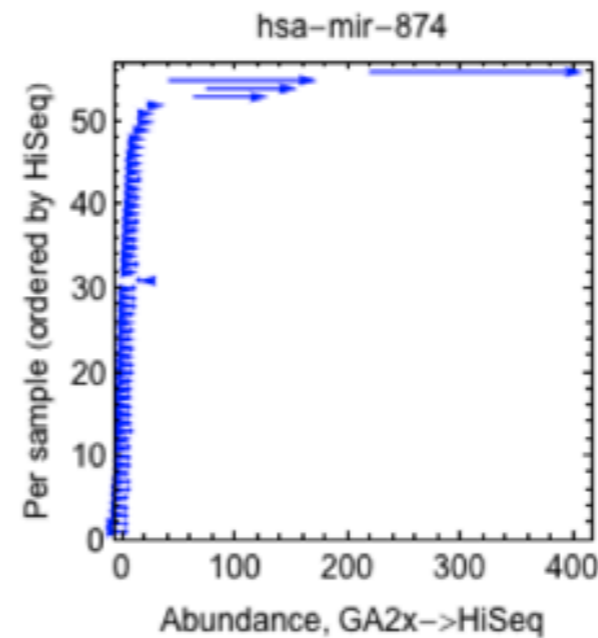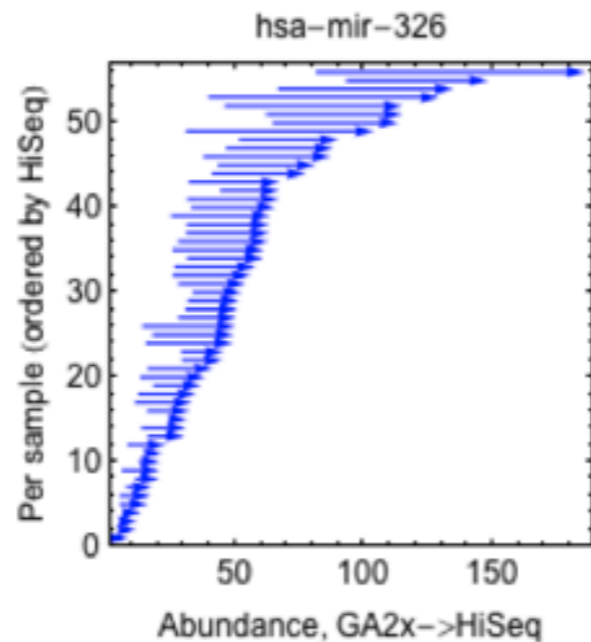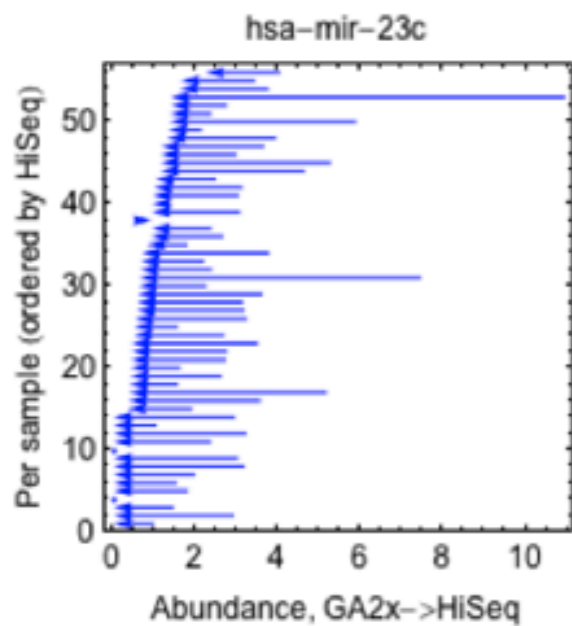
hsa-mir-374c

KIRC/P GA2x vs HiSeq, subsample 2, 735 miRNAs

edgeR v1.51

hsa-mir-23c

hsa-mir-326

hsa-mir-326

KIRC (56): GA2x vs HiSeq
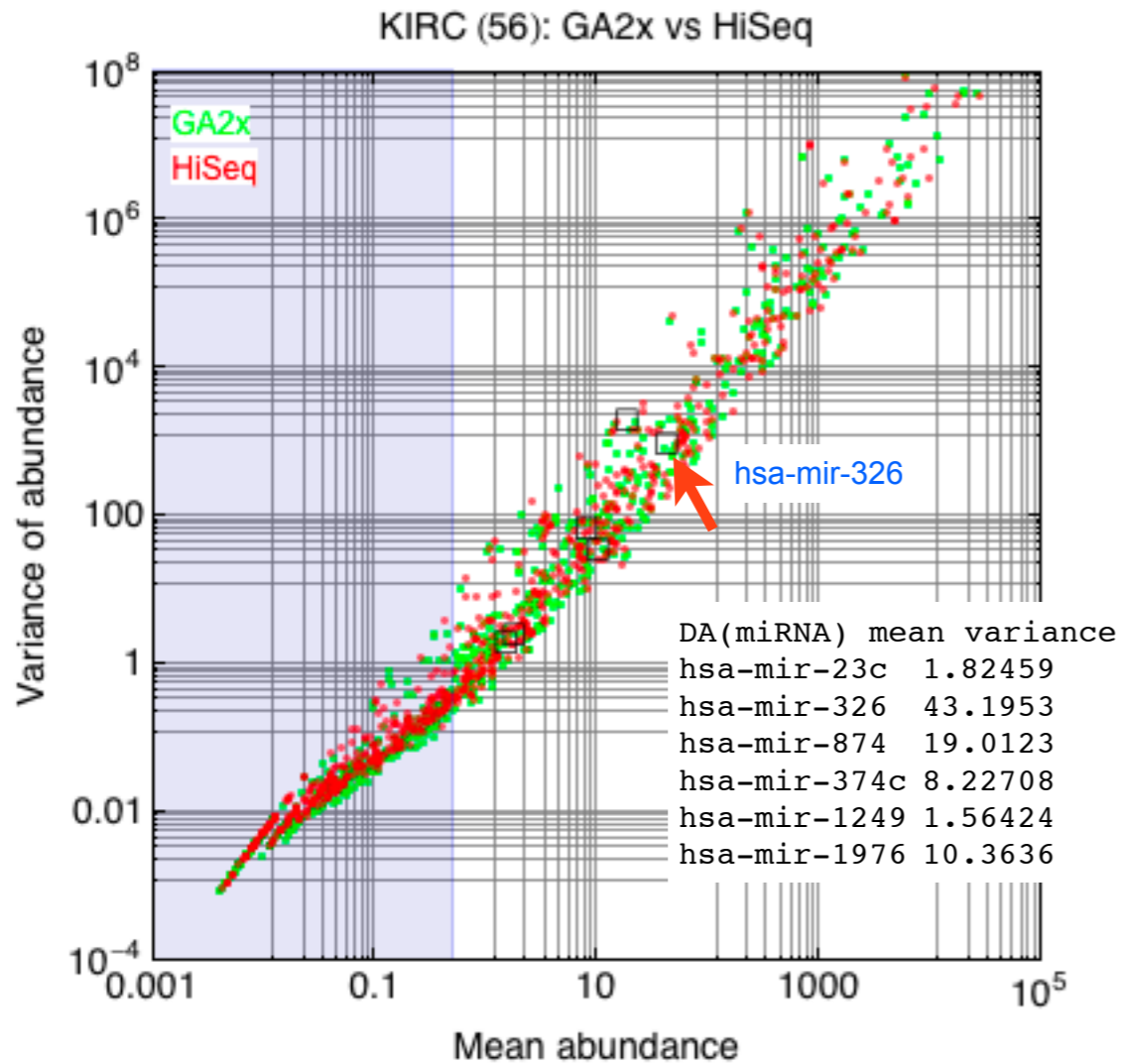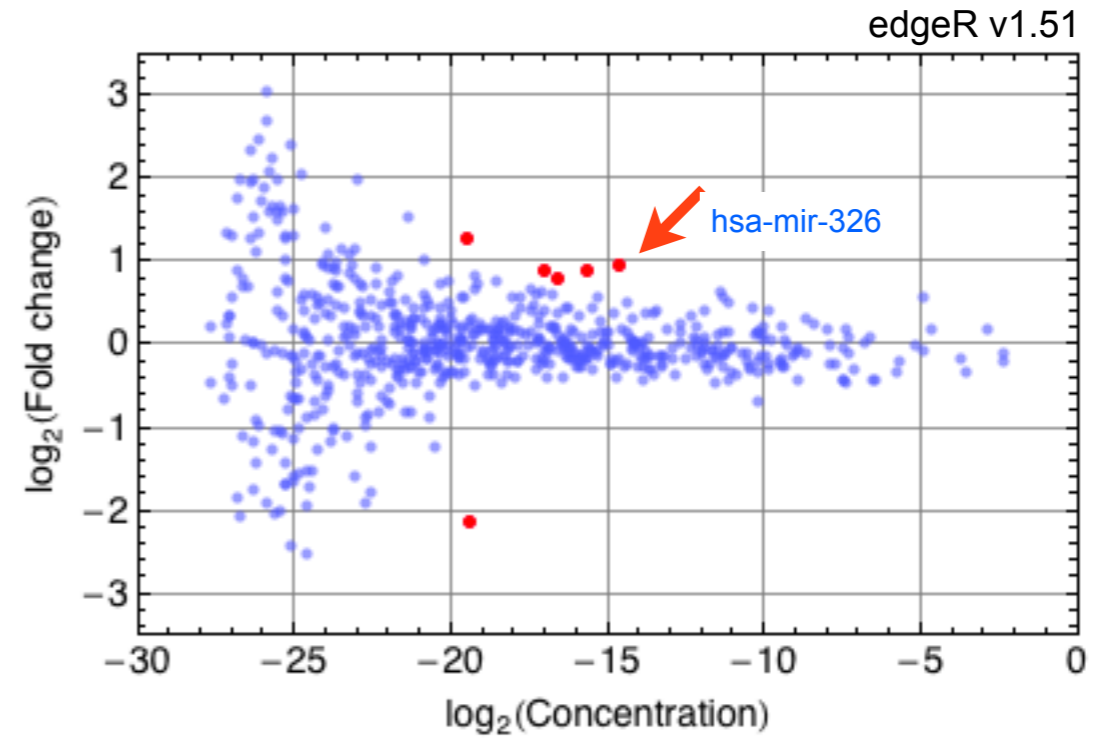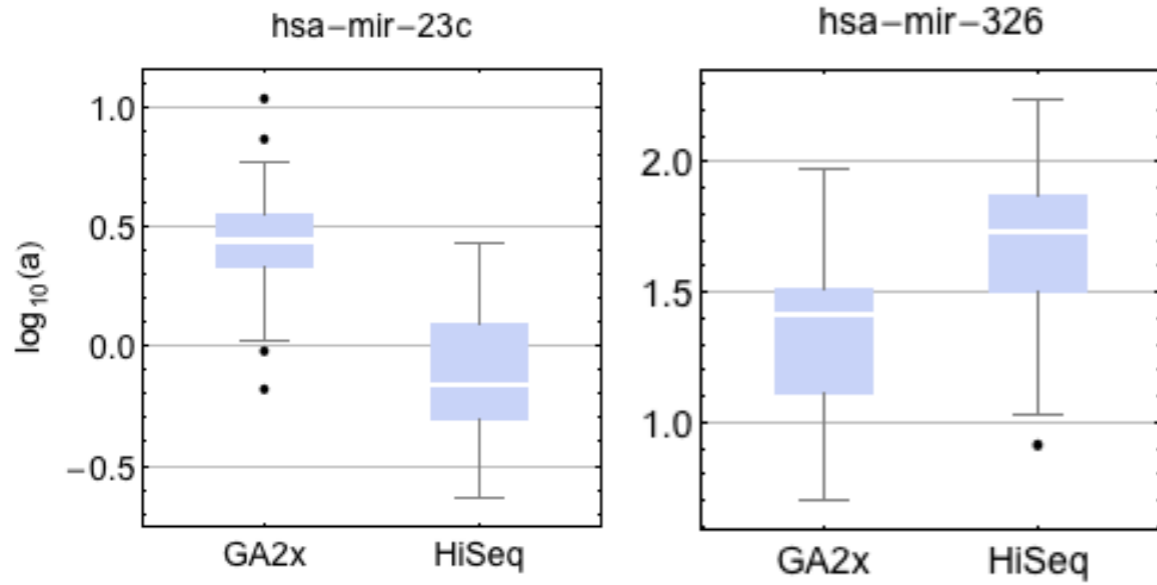
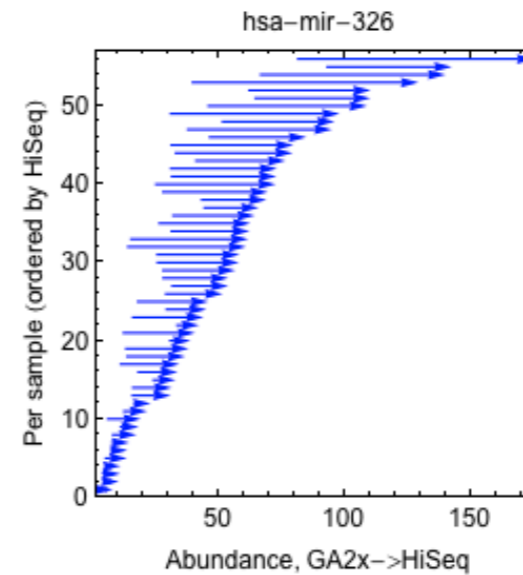| gene	logConc	logFC	P.Value	adj.P.Val
hsa-mir-23c	−19.40	−2.133	1.69e-11	1.24e-08
hsa-mir-326	−14.65	0.975	3.10e-06	0.00114
hsa-mir-874	−15.701	0.906	2.55e-05	0.00625
hsa-mir-374c	−17.033	0.911	5.31e-05	0.00813
hsa-mir-1249	−19.485	1.280	5.53e-05	0.00813
hsa-mir-1976	−16.611	0.797	0.00037	0.0450

...

```
DA(miRNA)    mean      variance
hsa-mir-23c   1.82459    2.88598
hsa-mir-326   43.1953    1102.68
hsa-mir-874   19.0123    2158.44
hsa-mir-374c  8.22708    78.6303
hsa-mir-1249  1.56424    2.24081
hsa-mir-1976  10.3636    41.0126
```

hsa-mir-326

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KIRC/P GA2x vs HiSeq, subsample 1, 735 miRNAs

hsa-mir-23c

edgeR v1.51

KIRC (56): GA2x vs HiSeq
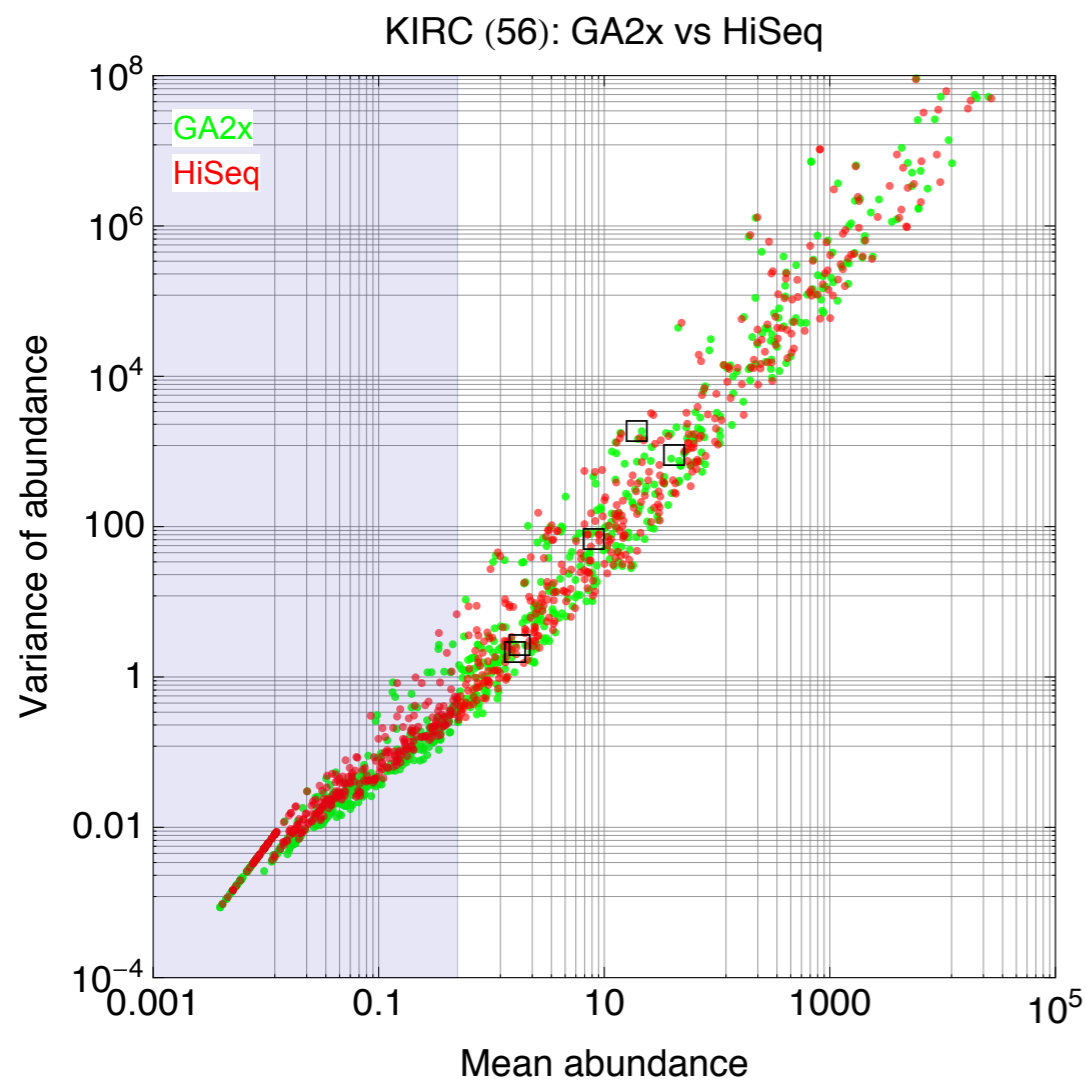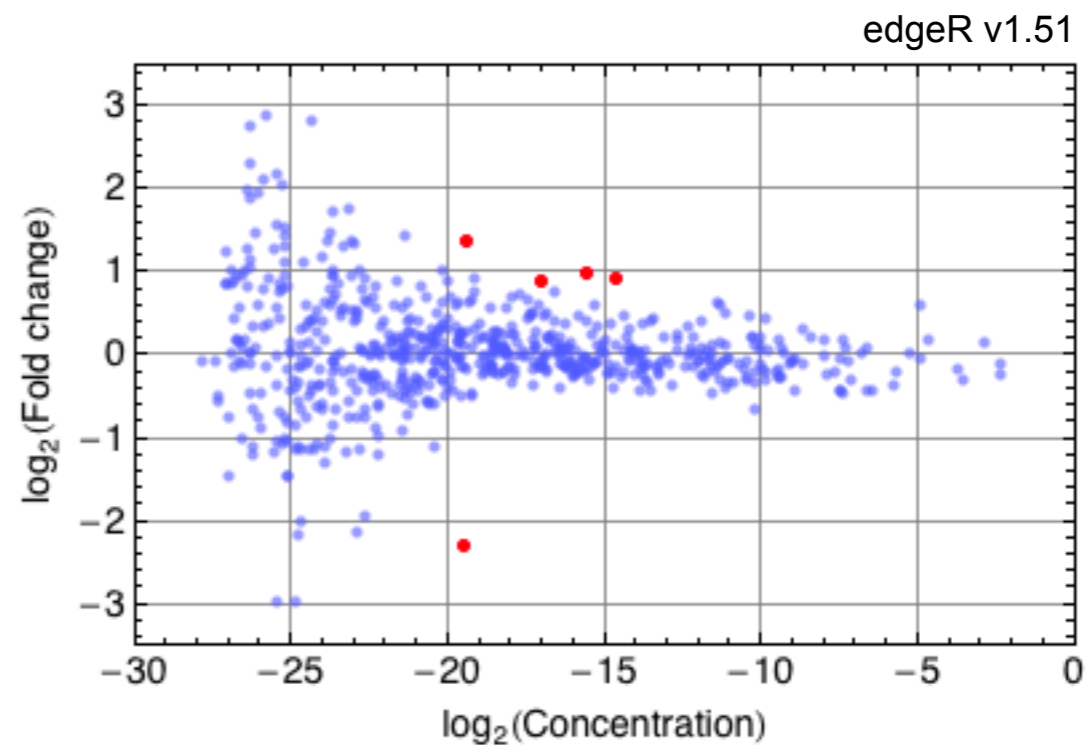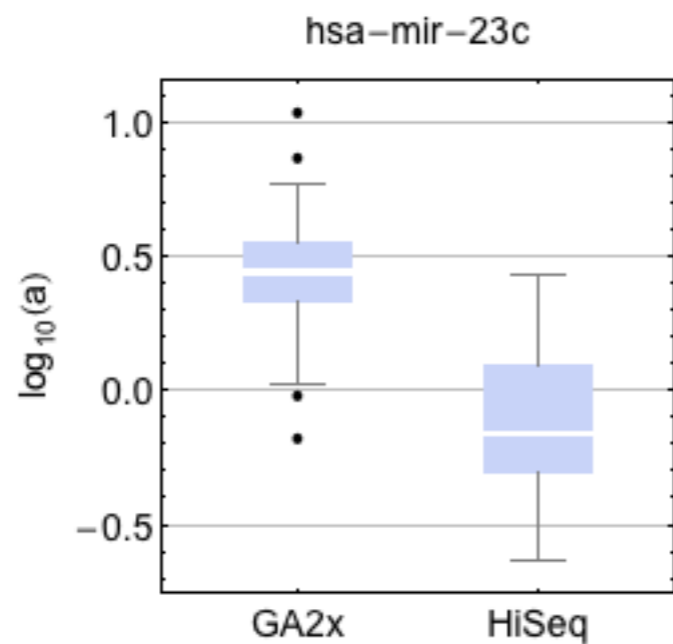
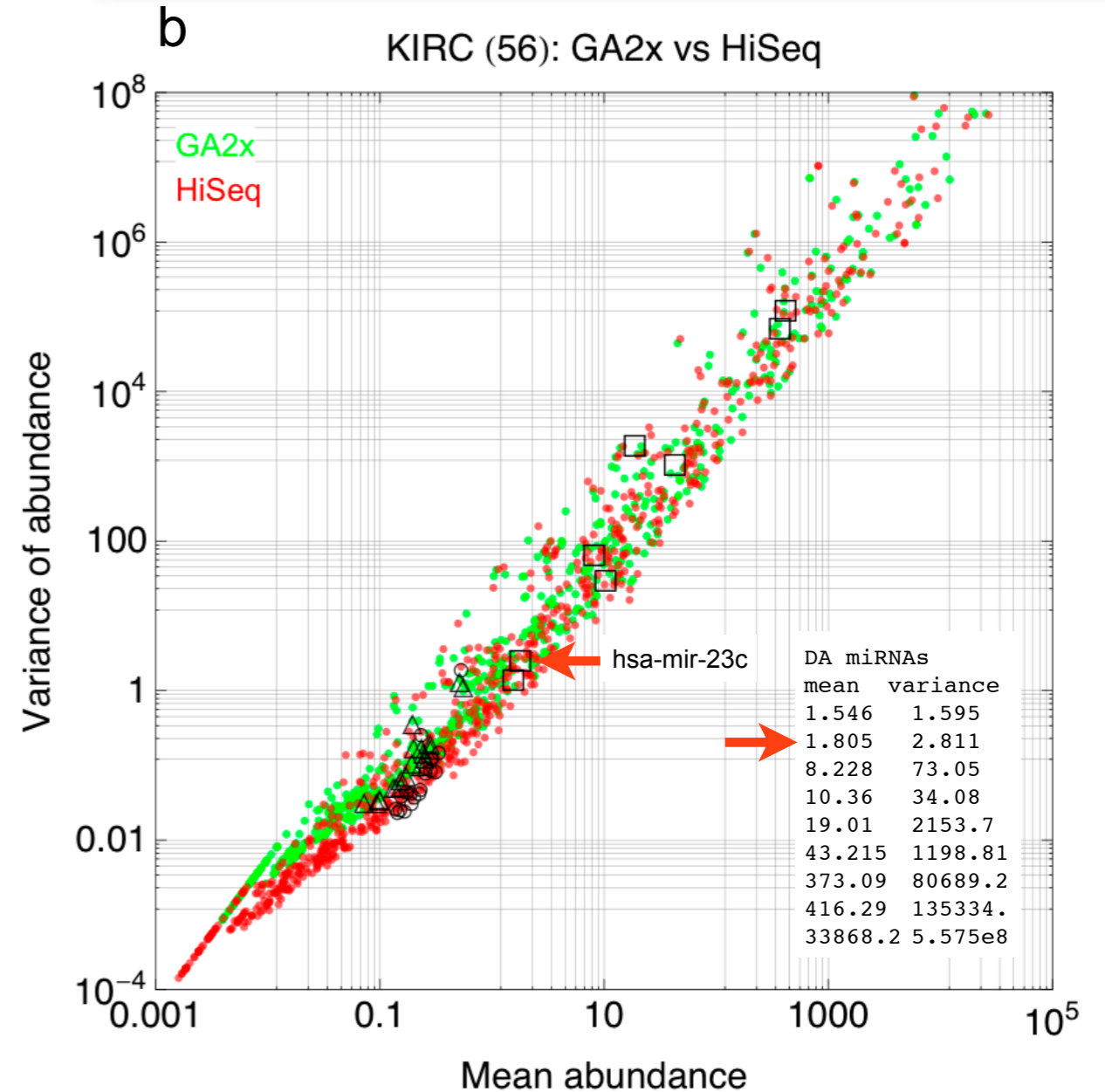| gene	logConc	logFC	P.Value	adj.P.Val
hsa-mir-23c	−19.477	−2.29	2.88e-12	2.12e-09
hsa-mir-874	−15.620	0.984	5.62e-06	0.0021
hsa-mir-326	−14.679	0.923	1.13e-05	0.0024
hsa-mir-1249	−19.440	1.376	1.32e-05	0.0024
hsa-mir-374c	−17.047	0.884	0.000101	0.0148

1. Adjusted data: GA2x vs. subsampled HiSeq.

2. Original data: GA2x vs. non-subsampled HiSeq
   GC content of miRNAs

Differential abundance between HiSeq and GA2x

hsa-mir-23c, chrX:20,035,181-20,035,330

We identified differentially abundant miRNAs between HiSeq and GA2x (1/0) using edgeR v2.2.3 and an expression matrix of 767 miRNAs that had nonzero sums across 2x56 samples. Nine miRNAs had BH-corrected p-val < 0.05 (red in 'a', black squares in 'b', table). As expected, the FC plot ('smearPlot') identified no low-abundance miRNAs as differentially abundant. **hsa-mir-23c** was flagged as having a negative PC1 loading (slide 9), when all other 8 loadings had positive values.


GC%

a
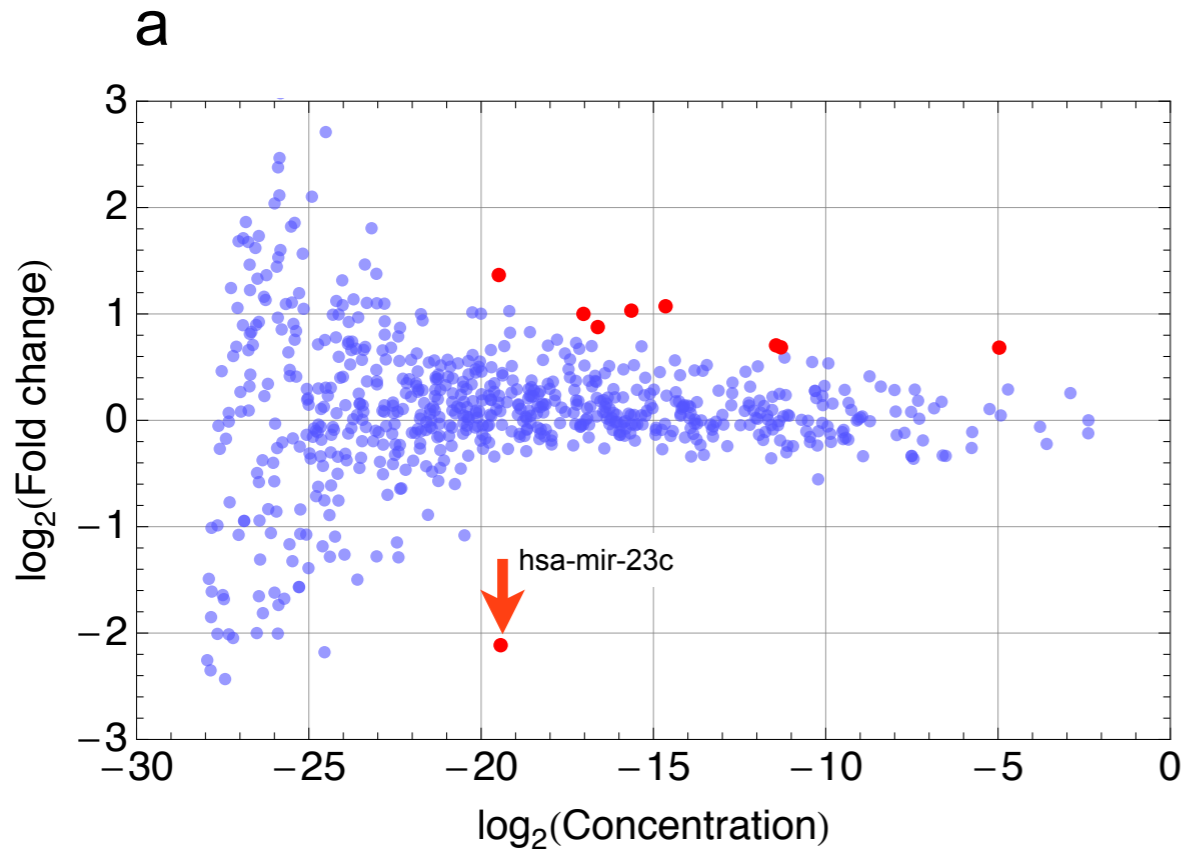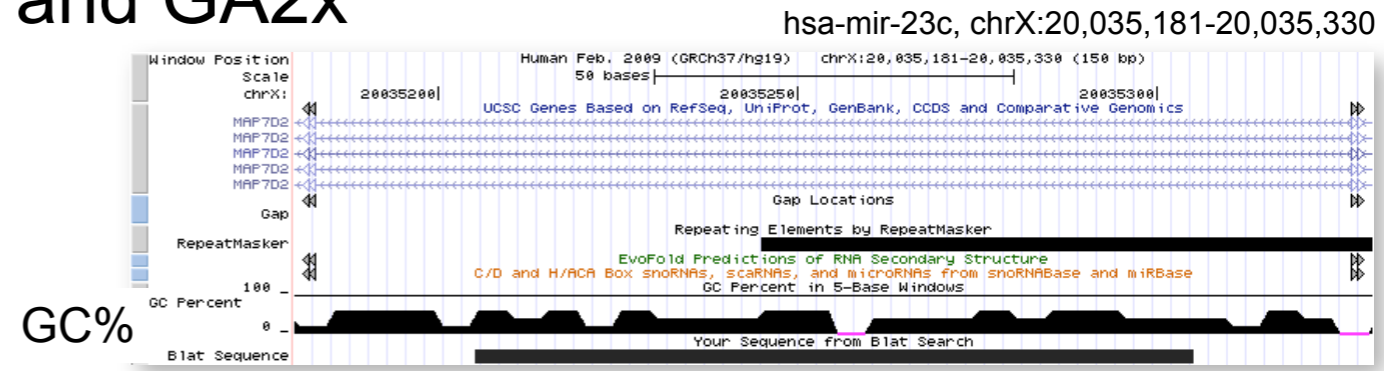
gene	logConc	logFC	P.Value	adj.P.Val
hsa-mir-23c	-19.4337	-2.114	2.88E-11	2.21E-08
hsa-mir-326	-14.6473	1.072	1.52E-07	0.0000583
hsa-mir-874	-15.6407	1.031	1.01E-06	0.000257
hsa-mir-374c	-17.0304	1.000	6.31E-06	0.00121
hsa-mir-1249	-19.488	1.366	0.0000124	0.00190
hsa-mir-1976	-16.6154	0.8766	0.0000653	0.00833
hsa-mir-204	-11.4417	0.7045	0.000394	0.0431
hsa-mir-150	-11.2998	0.6859	0.000556	0.0481
hsa-mir-99b	-4.96516	0.6833	0.000565	0.0481

b

KIRC (56): GA2x vs HiSeq

DA miRNAs	
mean	variance
1.546	1.595
1.805	2.811
8.228	73.05
10.36	34.08
19.01	2153.7
43.215	1198.81
373.09	80689.2
416.29	135334.
33868.2	5.575e8

1. Open black circles/triangles show the 20 PCA 'driver' miRNAs. Squares show nine differentially abundant miRNAs for BH-pval<0.05.

2. Red and green point clouds appear to separate for mean abundances below ~2.

19 May 2011, 16h45

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GC content of differentially abundant miRNA mature|star strands

GC content of 1733 mature or star strands (v17,blue) and 939 primary transcripts (v15, green)
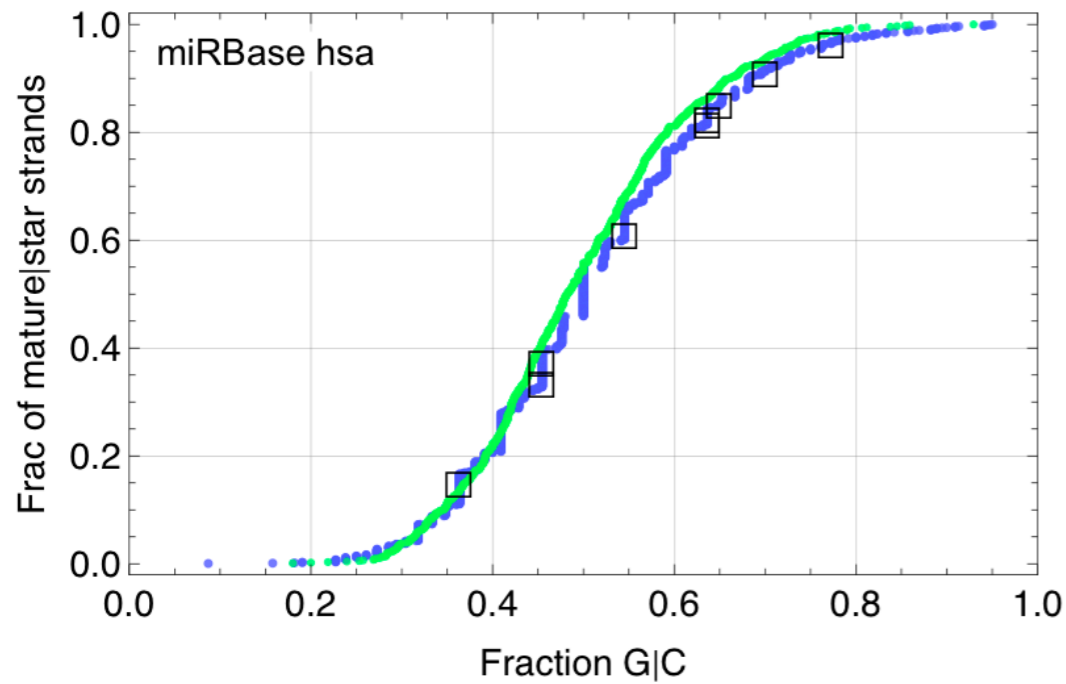
miRNA	MIMAT	sequence	f(GC)
hsa-miR-874	MIMAT0004911	CUGCCCUGGCCCGAGGGACCGA	0.773
hsa-miR-326	MIMAT0000756	CCUCUGGGCCCUUCCUCCAG	0.700
hsa-miR-1976	MIMAT0009451	CCUCCUGCCCUCCUUGCUGU	0.650
hsa-miR-1249	MIMAT0005901	ACGCCCUUCCCCCCCUUCUUCA	0.636
hsa-miR-99b	MIMAT0000689	CACCCGUAGAACCGACCUUGCG	0.636
hsa-miR-150	MIMAT0000451	UCUCCCAACCCUUGUACCAGUG	0.545
hsa-miR-23c	MIMAT0018000	AUCACAUUGCCAGUGAUUACCC	0.454
hsa-miR-204	MIMAT0000265	UUCCCUUUGUCAUCCUAUGCCU	0.454
hsa-miR-374c	MIMAT0018443	AUAAUACAACCUGCUAAGUGCU	0.364

/Users/grobertson/GENEREG/Data/miRNA/mirBase/v16/mature.hsa.fraction-GC.txt

Nine differentially abundant miRNAs (BH-pval<0.05, red) for 767 miRNAs (blue) in GA2x-HiSeq KIRC/KIRP test
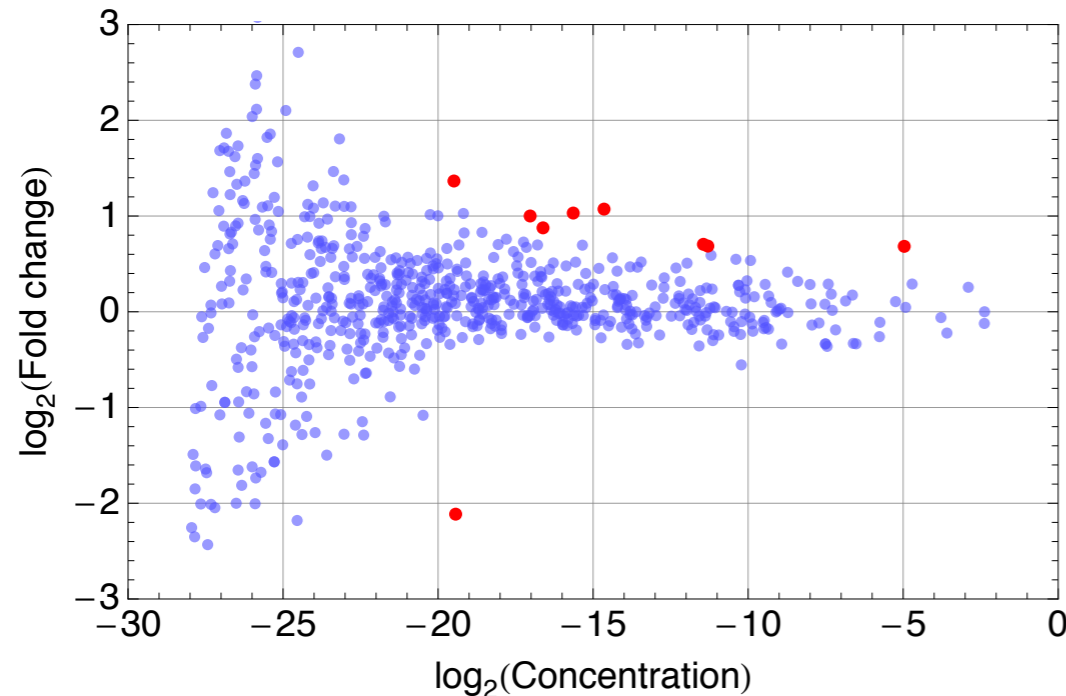
gene	logConc	logFC	P.Value	adj.P.Val
hsa-mir-23c	-19.43	-2.114	2.88E-11	2.21E-08
hsa-mir-326	-14.64	1.072	1.52E-07	0.0000583
hsa-mir-874	-15.640	1.031	1.01E-06	0.000257
hsa-mir-374c	-17.030	1.000	6.31E-06	0.00121
hsa-mir-1249	-19.488	1.366	0.0000124	0.00190
hsa-mir-1976	-16.615	0.877	0.0000653	0.00833
hsa-mir-204	-11.442	0.705	0.000394	0.0431
hsa-mir-150	-11.30	0.686	0.000556	0.0481
hsa-mir-99b	-4.965	0.683	0.000565	0.0481

19 May 2011, 10h36