Distributions of HIV integration sites after TNPO3 knockdown and rescue with siRNA insensitive allele

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Contents

1 Introduction

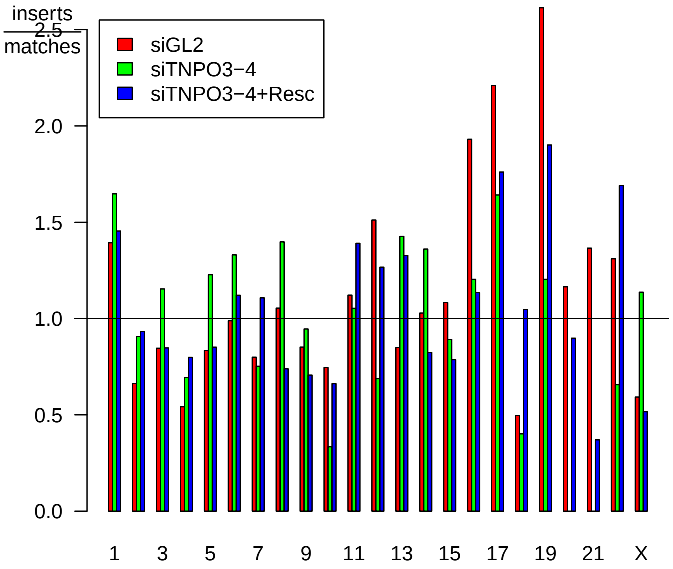
In this document, I examine the association of integration sites with various genomic features.

The data consist of both actual integration sites and sets of control sites, each set chosen to match the spacing (in bases) from the nearest restriction site (according to the direction in which the sequence was read) to an integration site. The numbers of insertion and matching sites for several data sets are shown below:

```
                  type
Origin.of.data.set insertion match
    siGL2               1226  2856
    siTNPO3-4            314   756
    siTNPO3-4+Resc      1492  3589
```

The advantage of choosing 'control' sites that match the spacing from the nearest restriction site is that biases due to location and density of restriction sites are eliminated by applying the classical multinomial logit model (reviewed in [2]). This model allows regression procedures to be applied to the study of integration intensity as a function of genomic features. The `clogit` function of the R `survival` library) implements estimation and fitting for such models along with the usual likelihood ratio and Wald tests.

The distribution of relative frequency of insertions across the chromosomes is given in this barplot:

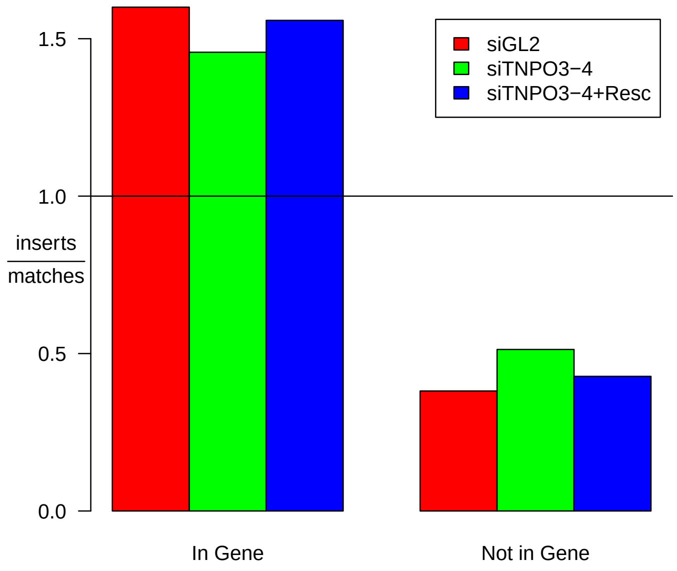


It seems evident that there are some chromosomes that are particularly favored for integration. This is reinforced by a test of statistical significance. The test performed used the likelihood ratio statistic for the multinomial logit model (reviewed in [2]) as implemented by the `clogit` function of the R `survival` library). The null hypothesis tested is that the ratio of true integration events to matched control sites is constant across all chromosomes. This test attains a p-value of $< 2.22e - 16$.

2 Preference for Genes

2.1 Acembly Genes

Here we examine the preference that integration events have for genes. In the following plot we show the relative frequency of integrations in genes according to the 'Acembly' annotation. The bars grouped over the label "In Gene" give the relative frequency of integration events (compared to control sites) between bases located within Acembly gene annotations, while the label "Not in Gene" give the relative frequency of integration events (compared to control sites) between bases not located within Acembly gene annotations.

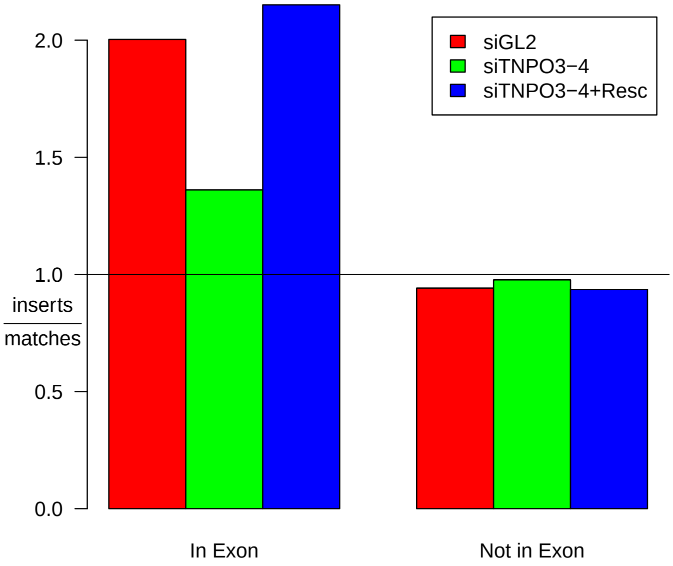


It seems evident that there is a strong tendency for insertions to occur in genes. A formal test of significance bears this out with a p-value of $< 2.22e-16$. Also, it appears that the tendency of different viruses to integrate into genes varies, and a test for this hypothesis attains 0.037701. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
               coef     se     z        p
siGL2          1.48 0.0876 16.90 9.75e-64
siTNPO3-4      1.03 0.1540  6.68 2.41e-11
siTNPO3-4+Resc 1.30 0.0744 17.50 1.58e-68
```

As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

In the following plot we show the relative frequency of insertions in exons according to the 'Acembly' annotation. The bars grouped over the label “In Exon” give the relative frequency of integration events (compared to control sites) between bases located in exons according to the Acembly annotation, while the label “Not in Exon” give the relative frequency of integration events (compared to control sites) between bases not located in exons according to the Acembly gene annotation.



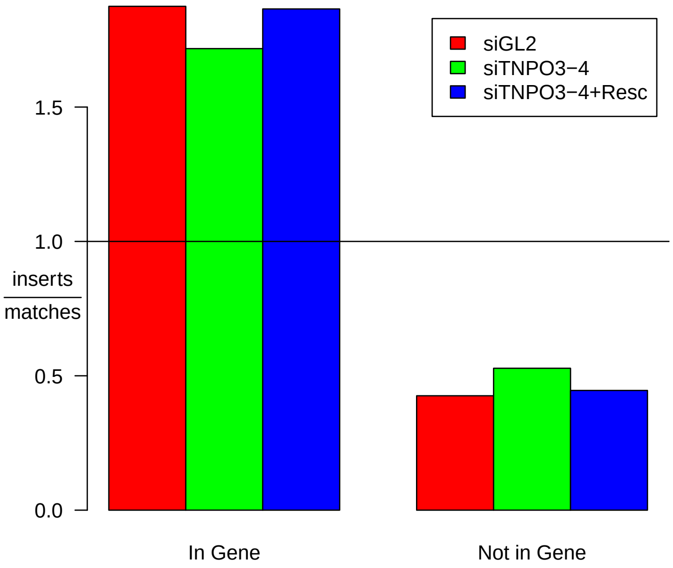
Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                  coef    se      z       p
siGL2            0.254 0.129  1.970 0.04840
siTNPO3-4       -0.110 0.265 -0.414 0.67900
siTNPO3-4+Resc   0.338 0.115  2.950 0.00316
```

The model on which these coefficients are based include terms for whether the site is in a gene or not. Thus, the effect shown as ”In Exon” is net of that due to being in a gene. Note that in the barplot above the 'Not in Exon' bars include both the introns and intergenic regions, so the impression given by the table may differ from that for the barplot.

2.2 refGenes

Here we examine the preference that insertions have for genes. In the following plot we show the relative frequency of insertions in genes according to the 'refGene' annotation.

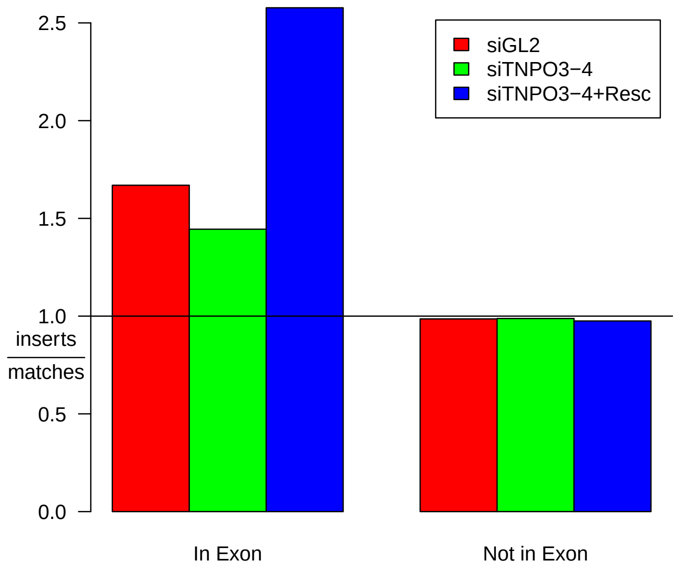


It seems evident that there is a strong tendency for insertions to occur in genes. A formal test of significance bears this out with a p-value of $< 2.22e-16$. Also, it appears that the tendency of different viruses to integrate into genes varies, and a test for this hypothesis attains 0.094082. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
               coef     se     z        p
siGL2          1.48 0.0798 18.50 2.04e-76
siTNPO3-4      1.13 0.1430  7.89 3.02e-15
siTNPO3-4+Resc 1.45 0.0714 20.20 3.70e-91
```

As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

In the following plot we show the relative frequency of insertions in exons according to the 'refGene' annotation.



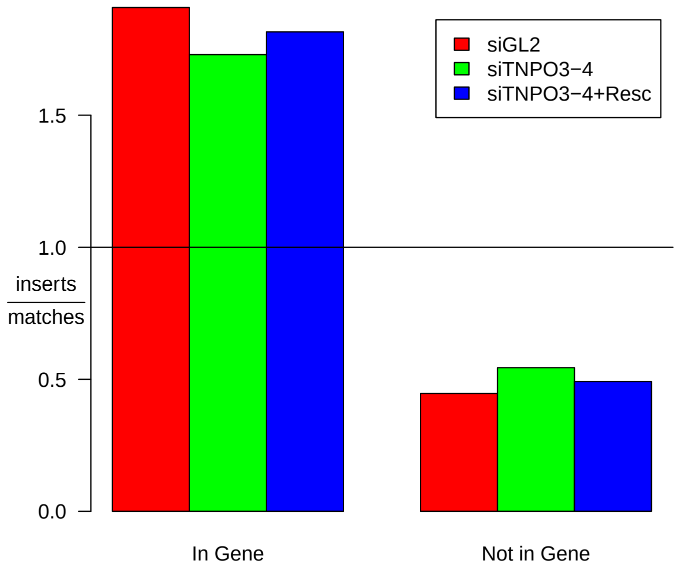
Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                    coef    se      z      p
siGL2            -0.0813 0.208 -0.391 0.6960
siTNPO3-4        -0.2430 0.384 -0.632 0.5270
siTNPO3-4+Resc    0.3410 0.197  1.730 0.0835
```

The model on which these coefficients are based include terms for whether the site is in a gene or not. Thus, the effect shown as "In Exon" is net of that due to being in a gene.

2.3 ensGenes

Here we examine the preference that insertions have for genes. In the following plot we show the relative frequency of insertions in genes according to the 'ensGene' annotation.

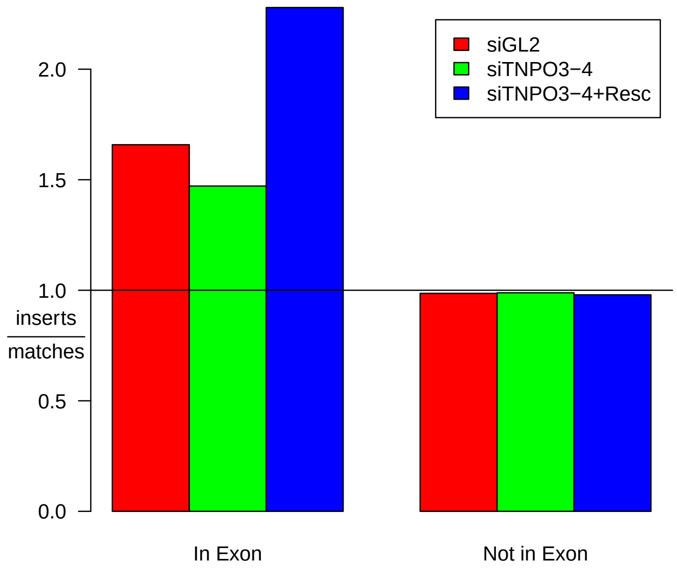


It seems evident that there is a strong tendency for insertions to occur in genes. A formal test of significance bears this out with a p-value of $< 2.22e-16$. Also, it appears that the tendency of different viruses to integrate into genes varies, and a test for this hypothesis attains 0.083195. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
               coef     se     z        p
siGL2          1.46 0.0793 18.50 3.19e-76
siTNPO3-4      1.12 0.1420  7.85 4.33e-15
siTNPO3-4+Resc 1.32 0.0695 19.00 1.92e-80
```

As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

In the following plot we show the relative frequency of insertions in exons according to the 'ensGene' annotation.



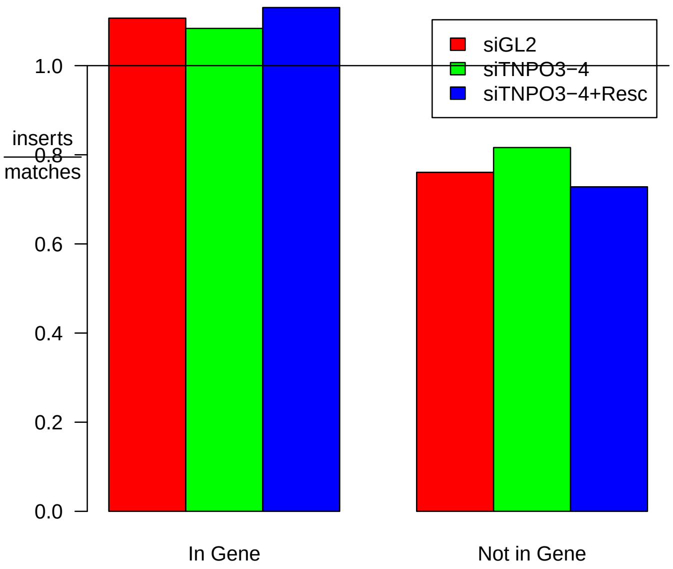
Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                  coef    se      z     p
siGL2           -0.105 0.211 -0.497 0.619
siTNPO3-4       -0.179 0.396 -0.451 0.652
siTNPO3-4+Resc   0.210 0.200  1.050 0.293
```

The model on which these coefficients are based include terms for whether the site is in a gene or not. Thus, the effect shown as ”In Exon” is net of that due to being in a gene.

2.4 genScan Genes

Here we examine the preference that insertions have for genes. In the following plot we show the relative frequency of insertions in genes according to the ’genScan’ annotation.

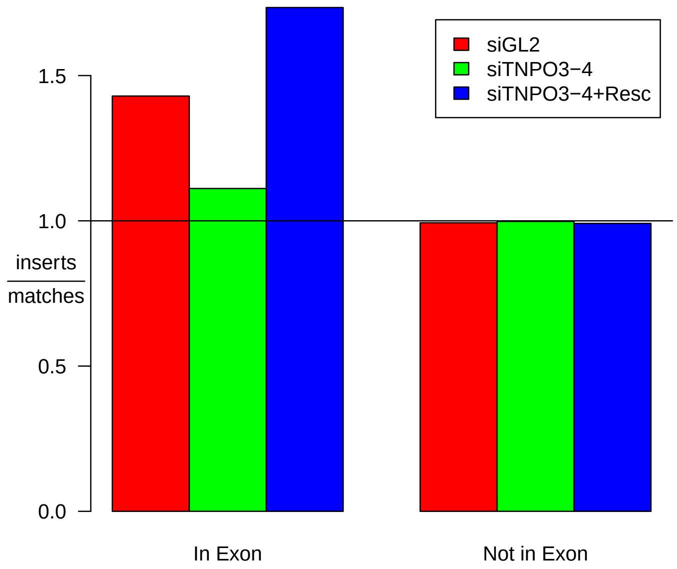


It seems evident that there is a strong tendency for insertions to occur in genes. A formal test of significance bears this out with a p-value of $3.0965e-16$. Also, it appears that the tendency of different viruses to integrate into genes varies, and a test for this hypothesis attains 0.55462. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef     se    z        p
siGL2          0.374 0.0791 4.73 2.29e-06
siTNPO3-4      0.284 0.1530 1.86 6.32e-02
siTNPO3-4+Resc 0.450 0.0716 6.29 3.09e-10
```

As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siTNPO3-4+Resc data set, while the smallest is seen in the siTNPO3-4 data set.

In the following plot we show the relative frequency of insertions in exons according to the 'genScan' annotation.



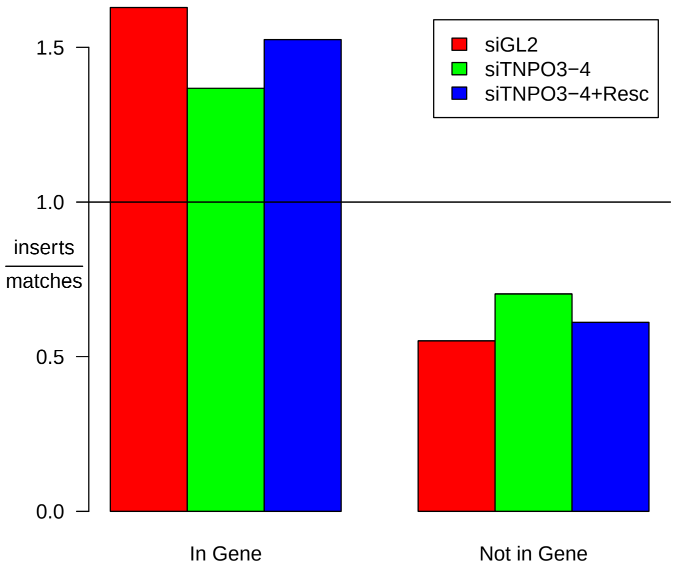
Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                  coef    se      z      p
siGL2           0.2230 0.247  0.905 0.3650
siTNPO3-4      -0.0618 0.497 -0.124 0.9010
siTNPO3-4+Resc  0.4210 0.241  1.740 0.0814
```

The model on which these coefficients are based include terms for whether the site is in a gene or not. Thus, the effect shown as ”In Exon” is net of that due to being in a gene.

2.5 uniGenes

Here we examine the preference that insertions have for genes. In the following plot we show the relative frequency of insertions in genes according to the ’uniGene’ annotation.

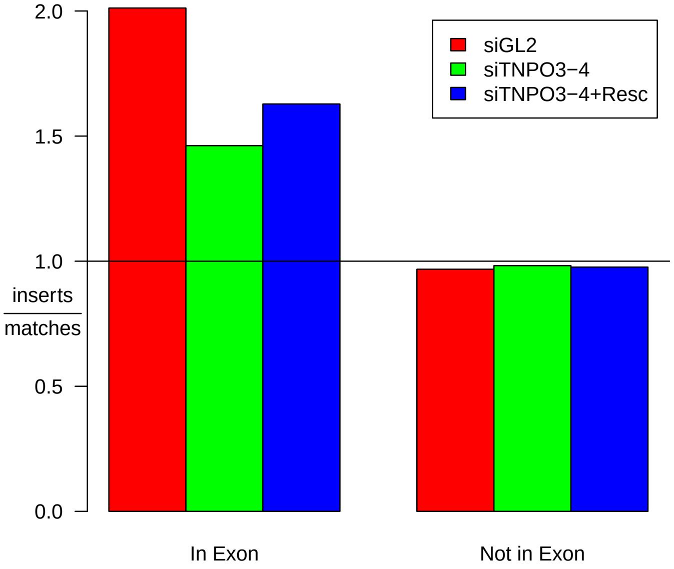


It seems evident that there is a strong tendency for insertions to occur in genes. A formal test of significance bears this out with a p-value of $< 2.22e-16$. Also, it appears that the tendency of different viruses to integrate into genes varies, and a test for this hypothesis attains 0.015377. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef     se     z        p
siGL2          1.090 0.0746 14.60 3.28e-48
siTNPO3-4      0.644 0.1380  4.67 2.98e-06
siTNPO3-4+Resc 0.931 0.0661 14.10 4.32e-45
```

As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

In the following plot we show the relative frequency of insertions in exons according to the 'uniGene' annotation.



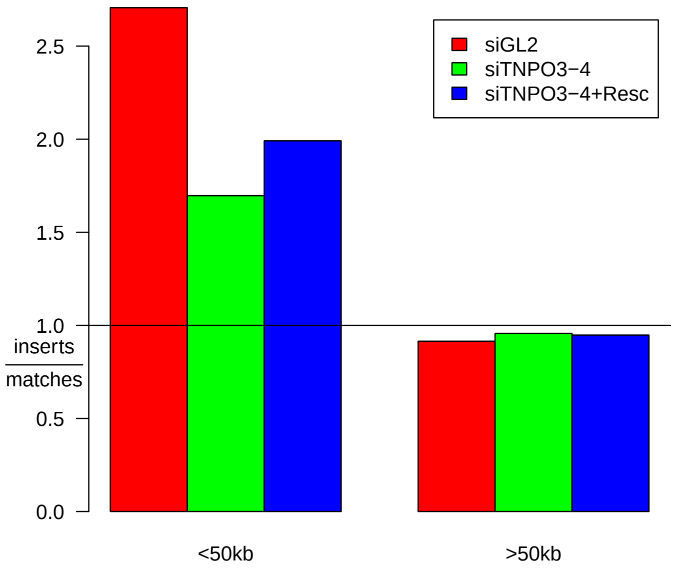
Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                  coef    se     z     p
siGL2           0.2760 0.168 1.640 0.100
siTNPO3-4       0.0366 0.326 0.112 0.911
siTNPO3-4+Resc 0.0752 0.147 0.512 0.609
```

The model on which these coefficients are based include terms for whether the site is in a gene or not. Thus, the effect shown as ”In Exon” is net of that due to being in a gene.

2.6 oncogenes

Here we examine the preference that insertions have for oncogenes. In the following plot we show the relative frequency of insertions with 50kb of an oncogene 5' end.



A formal test of oncogenic insertion returns p-value of $< 2.22e - 16$. The tendency of different viruses to integrate near oncogenes may vary, and a test for this hypothesis attains 0.035113. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                 coef    se     z        p
siGL2          -1.110 0.127 -8.75 2.04e-18
siTNPO3-4      -0.576 0.251 -2.30 2.15e-02
siTNPO3-4+Resc -0.716 0.116 -6.17 6.93e-10
siGL2              NA 0.000    NA       NA
siTNPO3-4          NA 0.000    NA       NA
siTNPO3-4+Resc     NA 0.000    NA       NA
```

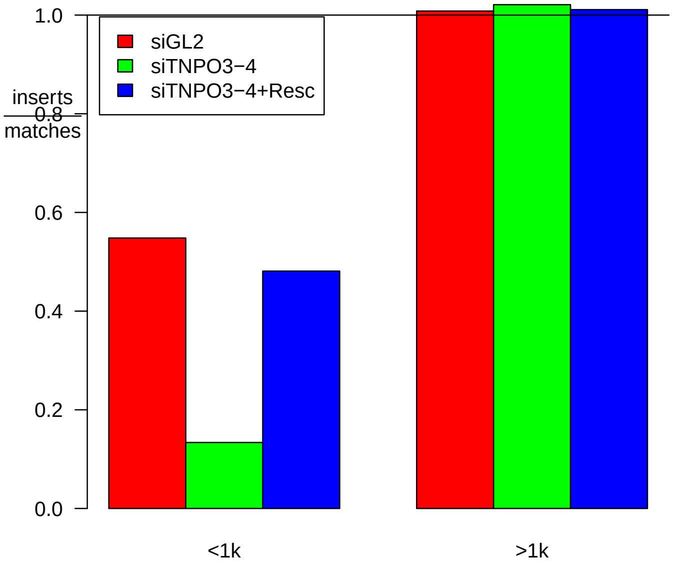
As is evident, there are some differences in the coefficients. The largest coefficient is seen in the siTNPO3-4 data set, while the smallest is seen in the siGL2 data set.

3 CpG Island Neighborhoods

Here we study the effect of being in the neighborhood of CpG Islands. Following Wu et al [3], who found that the neighborhoods within ±1kb of CpG islands are enriched for MLV insertions, we study such neighborhoods.

3.1 1 kilobase neighborhoods

The following plot shows the effect of being in or within ±1kb of a CpG island:



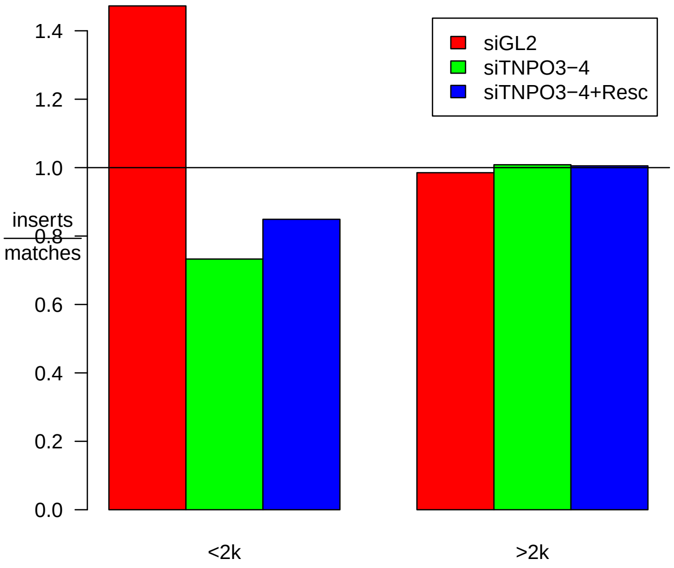
A formal test of significance comparing the difference attains a p-value of $4.7283e-05$. A test for differences between viruses attains 0.33821. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                 coef    se     z       p
siGL2          -0.611 0.322 -1.90 0.05750
siTNPO3-4      -1.960 1.030 -1.90 0.05690
siTNPO3-4+Resc -0.745 0.284 -2.63 0.00861
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

3.2 2 kilobase neighborhoods

The following plot shows the effect of being in or within ±2kb of a CpG island:



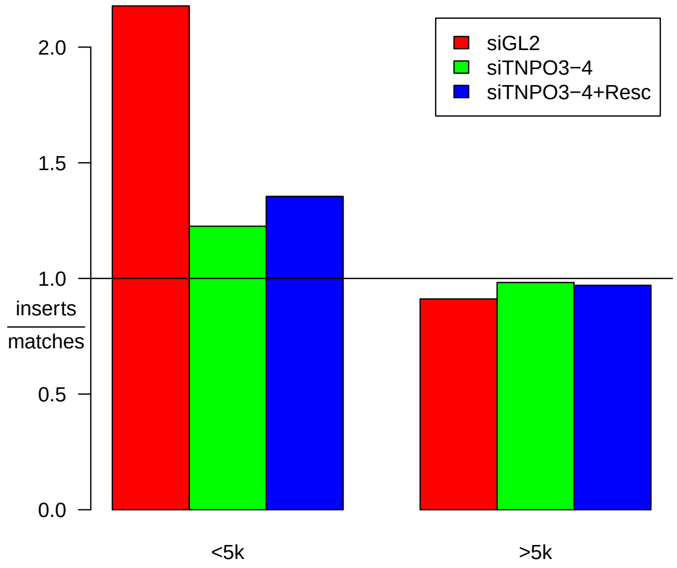
A formal test of significance comparing the difference attains a p-value of 0.57773. A test for differences between viruses attains 0.044963. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                  coef    se      z      p
siGL2            0.396 0.176  2.250 0.0244
siTNPO3-4       -0.305 0.433 -0.703 0.4820
siTNPO3-4+Resc -0.188 0.183 -1.030 0.3050
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

3.3 5 kilobase neighborhoods

The following plot shows the effect of being in or within ±5kb of a CpG island:



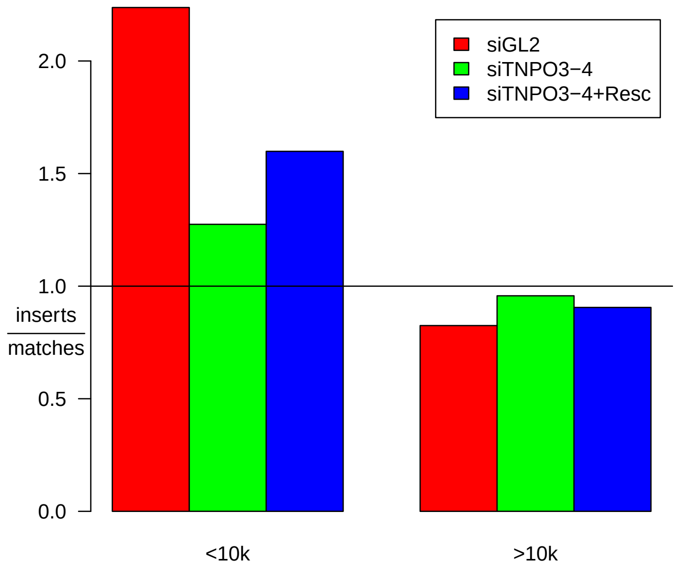
A formal test of significance comparing the difference attains a p-value of $3.6569e - 14$. A test for differences between viruses attains 0.00016490. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef    se    z        p
siGL2          0.895 0.110 8.15 3.63e-16
siTNPO3-4      0.205 0.241 0.85 3.95e-01
siTNPO3-4+Resc 0.308 0.105 2.95 3.19e-03
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

3.4 10 kilobase neighborhoods

The following plot shows the effect of being in or within ±10kb of a CpG island:



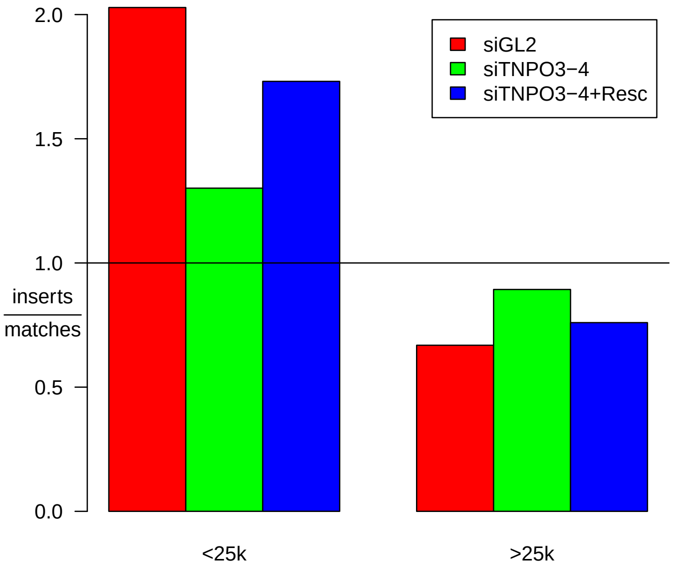
A formal test of significance comparing the difference attains a p-value of $< 2.22e - 16$. A test for differences between viruses attains $1.7387e - 05$. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef     se     z        p
siGL2          1.000 0.0875 11.50 2.29e-30
siTNPO3-4      0.268 0.1840  1.46 1.45e-01
siTNPO3-4+Resc 0.536 0.0796  6.73 1.66e-11
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

3.5 25 kilobase neighborhoods

The following plot shows the effect of being in or within ±25kb of a CpG island:



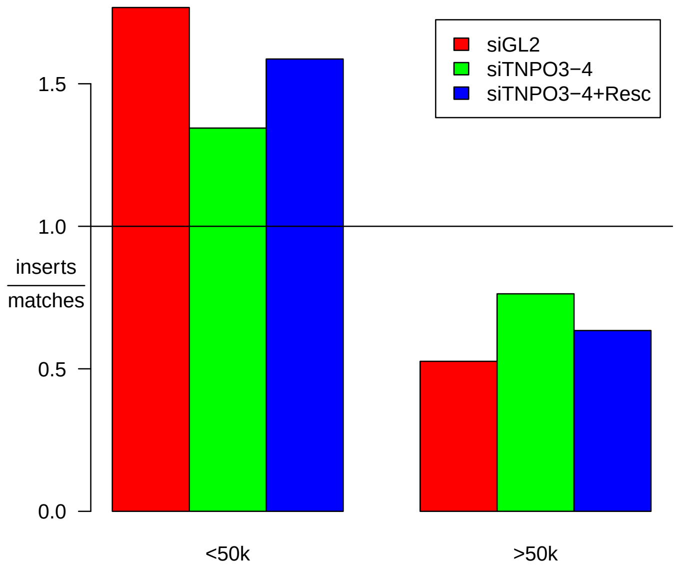
A formal test of significance comparing the difference attains a p-value of $< 2.22e - 16$. A test for differences between viruses attains $8.0664e - 06$. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef     se     z        p
siGL2          1.100 0.0745 14.80 1.09e-49
siTNPO3-4      0.364 0.1460  2.49 1.28e-02
siTNPO3-4+Resc 0.790 0.0658 12.00 3.76e-33
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

3.6 50 kilobase neighborhoods

The following plot shows the effect of being in or within ±50kb of a CpG island:



A formal test of significance comparing the difference attains a p-value of $< 2.22e - 16$. A test for differences between viruses attains $2.2027e - 05$. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites along with their standard errors, z statistics, and p-values for each data set:

```
                coef     se     z        p
siGL2          1.210 0.0753 16.10 2.95e-58
siTNPO3-4      0.542 0.1380  3.94 8.11e-05
siTNPO3-4+Resc 0.892 0.0644 13.90 1.05e-43
```

The largest coefficient is seen in the siGL2 data set, while the smallest is seen in the siTNPO3-4 data set.

4 Gene Density, Expression 'Density', and CpG Island Density

In this section the association with gene density is examined. For expression analysis, the 'genes' that are counted are the genes represented on the microarray. In addition, we the number of such genes expressed at various levels. The levels are

low.ex Count genes whose expression is in the upper half and divide by number of bases

med.ex Count genes whose expression is in the upper $1/8^{th}$ and divide by number of bases

high.ex Count genes whose expression is in the upper $1/16^{th}$ and divide by number of bases

The bolded terms are used as abbreviations in what follows. The abbreviation **dens** is used to indicate gene density as number of genes per base.

4.1 25 kilobase Window

In the barplot that follows we examine the association of insertion sites with gene density in a 25 kilobase window surrounding each locus. More such plots will follow and the method of their construction is always to try to divide the data according to the deciles of density. However, it often happens that there is a very skewed distribution of density and even the 90^{th} percentile is zero. In that case, the barplots simply show the sites for which the density is zero and those for which it is non-zero. If there are fewer than ten groups of bars, the groupings contain ten percent of the sites each except for the leftmost grouping which will contain all of the remaining sites.

Also note that the title of the plot contains clues as to its content; the prefix indicates the type of variable studied while the suffix indicates the window width in the number of bases. The p-value given is the result of fitting a cubic polynomial to the gene density values.

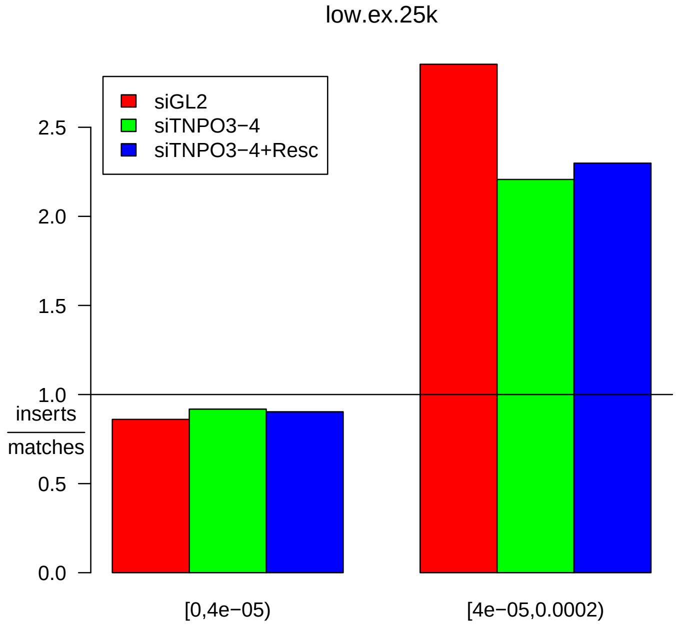
The following expression data and probe set were used for this report:

```
[1] "ledgf293TS-HU133Plus2"
```

```
[1] "HG-U133"
```

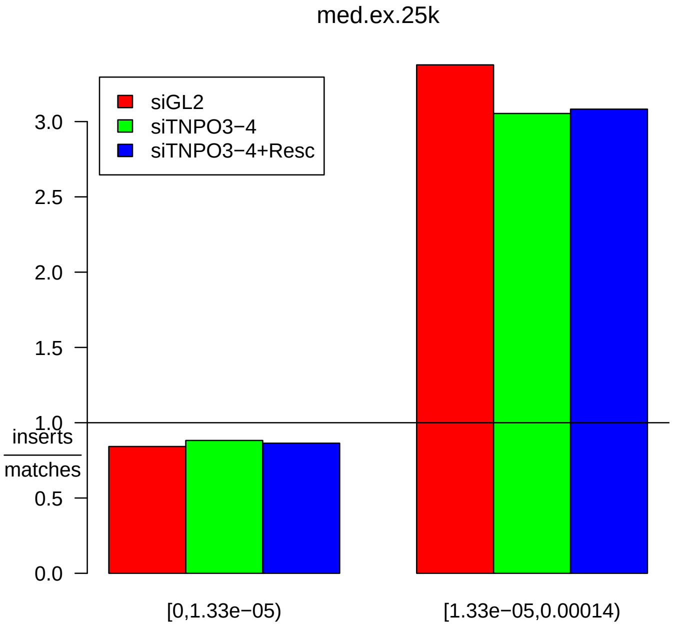
```
                 coef     se     z        p
siGL2           1.090 0.0737 14.80 1.15e-49
siTNPO3-4       0.587 0.1420  4.13 3.57e-05
siTNPO3-4+Resc 0.940 0.0669 14.00 7.92e-45
```

Here are the results for expression density. First, we count just genes that are in the upper half.



```
                coef     se     z        p
siGL2          1.440 0.0857 16.80 2.63e-63
siTNPO3-4      0.964 0.1670  5.77 8.01e-09
siTNPO3-4+Resc 1.090 0.0753 14.50 1.48e-47
```

Now we count genes in the upper $1/8^{th}$:



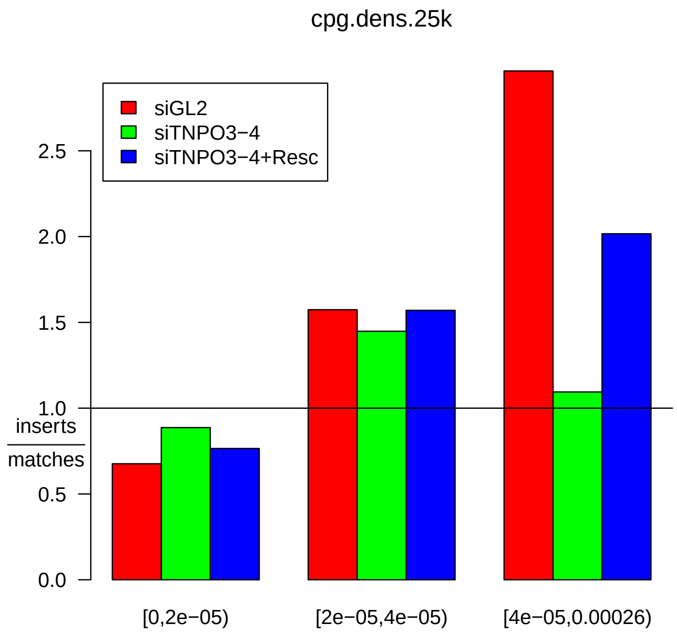
```
               coef     se     z         p
siGL2          1.57 0.1040 15.10 1.96e-51
siTNPO3-4      1.21 0.2060  5.86 4.51e-09
siTNPO3-4+Resc 1.36 0.0917 14.80 9.73e-50
```

And here we count genes in the upper $1/16^{th}$:

```
Density data too sparse for barplot

               coef    se     z         p
siGL2          1.45 0.128 11.30 9.30e-30
siTNPO3-4      1.24 0.263  4.73 2.30e-06
siTNPO3-4+Resc 1.29 0.115 11.20 2.74e-29
```

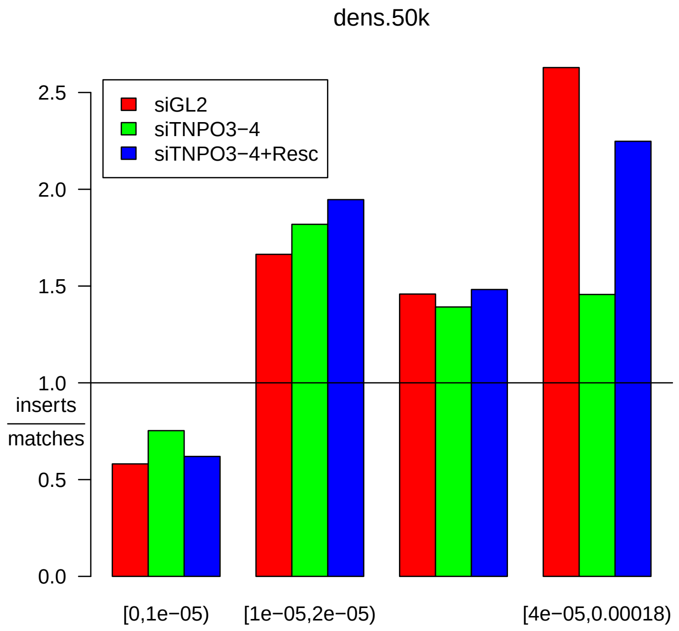
Here the effect of density of CpG islands is studied:



```
                coef     se     z        p
siGL2          1.090 0.0744 14.60 3.30e-48
siTNPO3-4      0.389 0.1460  2.66 7.76e-03
siTNPO3-4+Resc 0.780 0.0659 11.80 2.82e-32
```

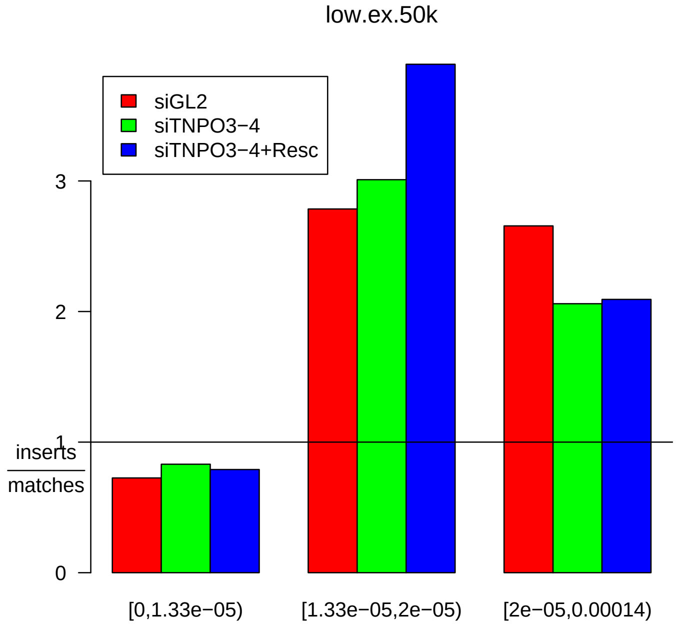
4.2 50 kilobase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 50 kilobase window surrounding each locus. First, we count just the number of genes represented on the chip.



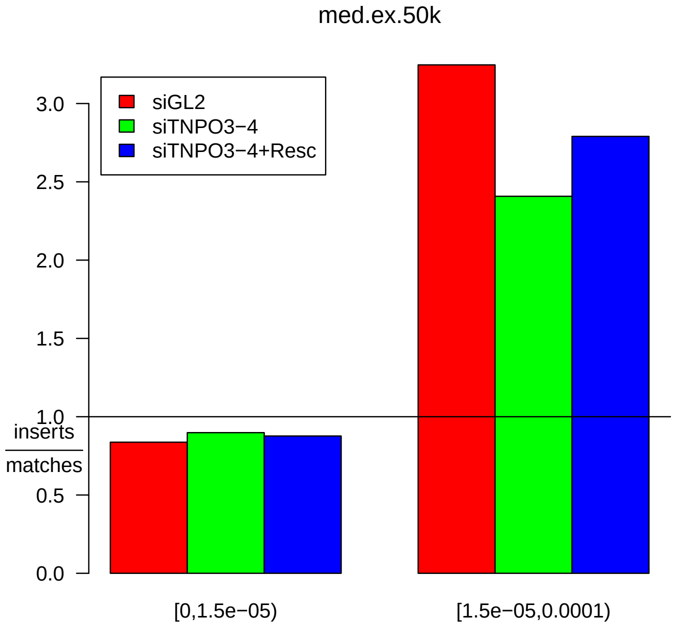
```
                   coef     se     z         p
siGL2             1.220 0.0744 16.40 2.47e-60
siTNPO3-4         0.767 0.1370  5.58 2.42e-08
siTNPO3-4+Resc    1.090 0.0660 16.60 7.04e-62
```

Here are the results for expression density. First, we count just genes that are in the upper half.



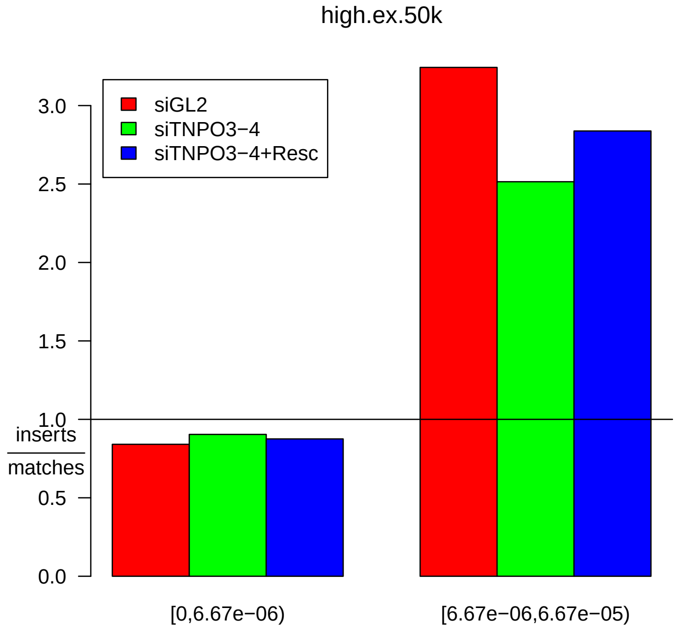
```
               coef     se     z        p
siGL2          1.50 0.0778 19.30 4.84e-83
siTNPO3-4      1.01 0.1470  6.82 9.13e-12
siTNPO3-4+Resc 1.14 0.0670 17.10 2.06e-65
```

Now we count genes in the upper $1/8^{th}$:



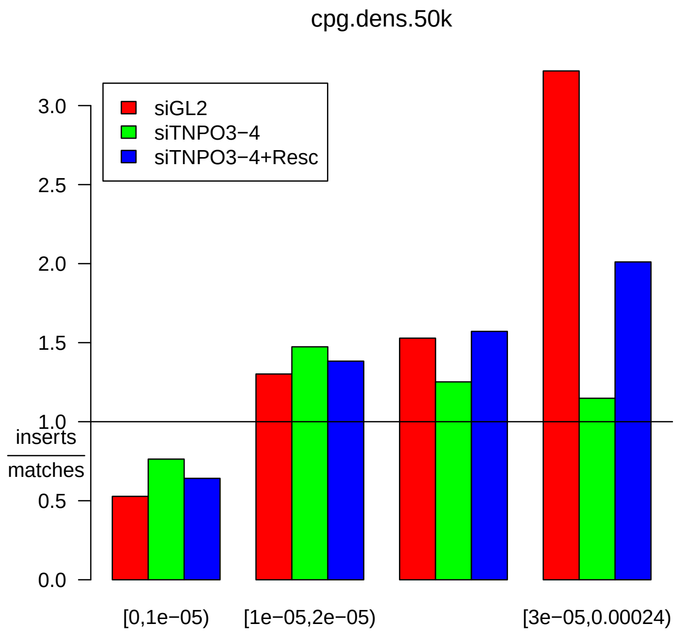
```
               coef     se     z        p
siGL2          1.59 0.0882 18.00 2.12e-72
siTNPO3-4      1.15 0.1680  6.85 7.29e-12
siTNPO3-4+Resc 1.28 0.0749 17.00 3.79e-65
```

And here we count genes in the upper $1/16^{th}$:



```
                coef     se     z        p
siGL2          1.410 0.0999 14.10 2.92e-45
siTNPO3-4      0.945 0.2060  4.59 4.37e-06
siTNPO3-4+Resc 1.190 0.0894 13.30 2.56e-40
```

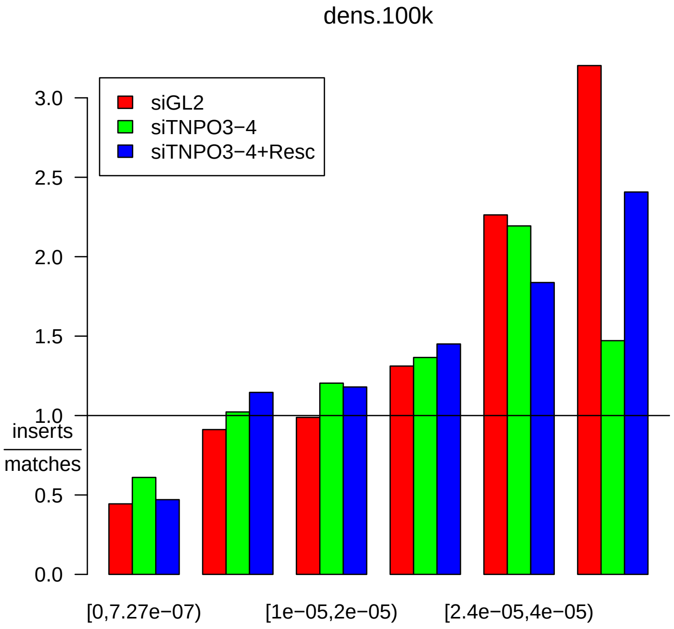
Here the effect of density of CpG islands is studied:



```
                coef     se     z         p
siGL2          1.220 0.0753 16.20 1.04e-58
siTNPO3-4      0.543 0.1370  3.96 7.54e-05
siTNPO3-4+Resc 0.878 0.0643 13.70 1.80e-42
```

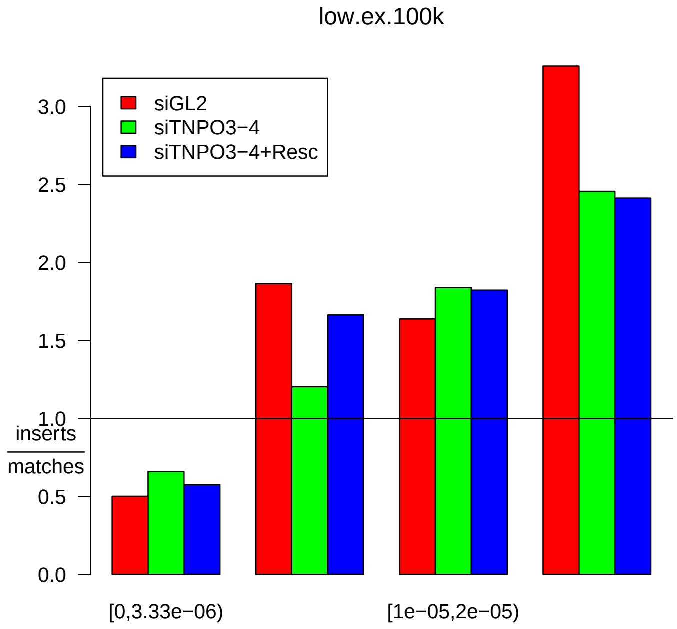
4.3 100 kilobase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 100 kilobase window surrounding each locus. First, we count just the number of genes represented on the chip.



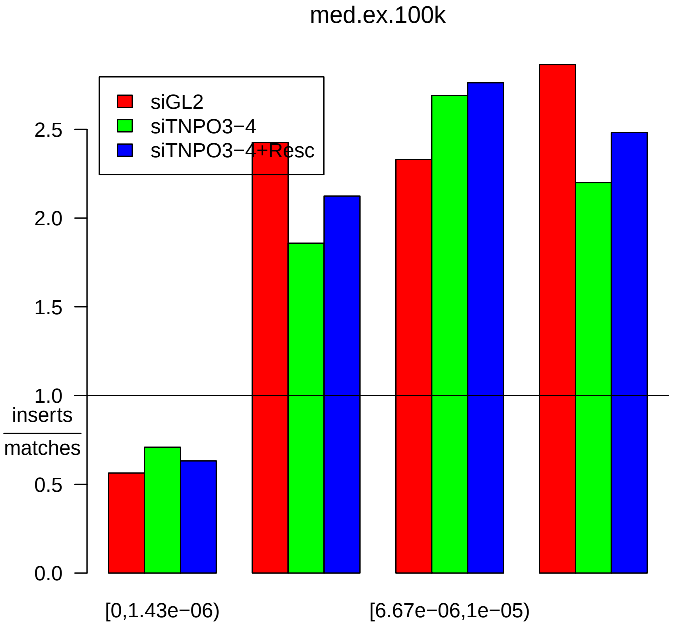
```
                 coef     se     z        p
siGL2           1.260 0.0741 17.00 9.92e-65
siTNPO3-4       0.855 0.1470  5.81 6.31e-09
siTNPO3-4+Resc 0.962 0.0647 14.90 6.21e-50
```

Here are the results for expression density. First, we count just genes that are in the upper half.



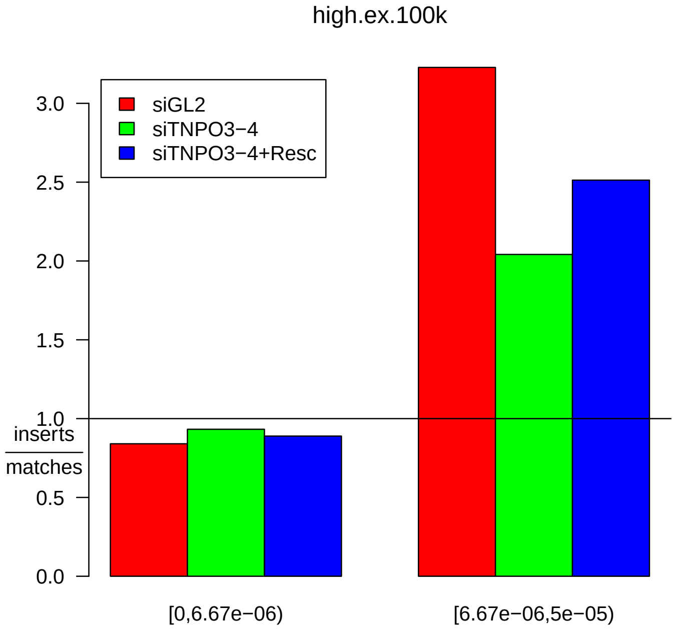
```
                coef     se     z        p
siGL2           1.49 0.0773 19.30 1.22e-82
siTNPO3-4       1.06 0.1430  7.38 1.64e-13
siTNPO3-4+Resc  1.23 0.0663 18.50 2.03e-76
```

Now we count genes in the upper $1/8^{th}$:



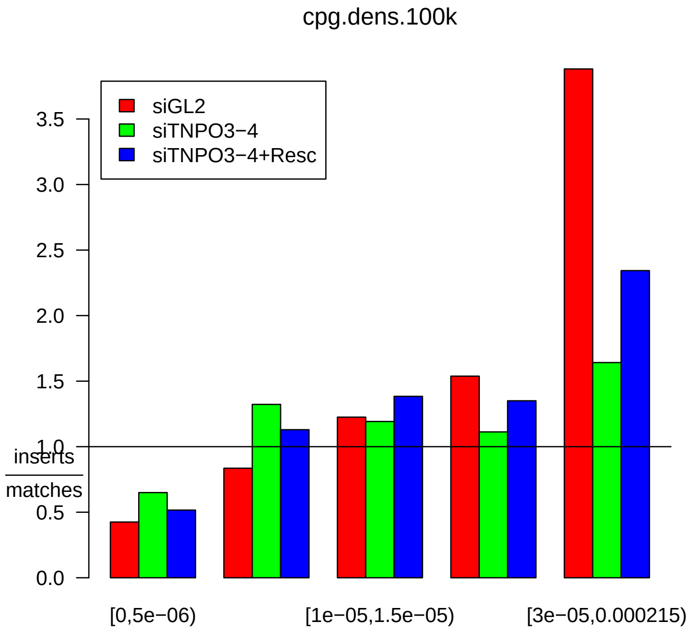
```
               coef     se     z        p
siGL2          1.59 0.0802 19.90 9.42e-88
siTNPO3-4      1.15 0.1530  7.51 5.70e-14
siTNPO3-4+Resc 1.31 0.0686 19.10 2.90e-81
```

And here we count genes in the upper $1/16^{th}$:



```
              coef     se     z        p
siGL2         1.44 0.0863 16.70 1.01e-62
siTNPO3-4     1.00 0.1780  5.64 1.68e-08
siTNPO3-4+Resc 1.18 0.0760 15.60 1.05e-54
```

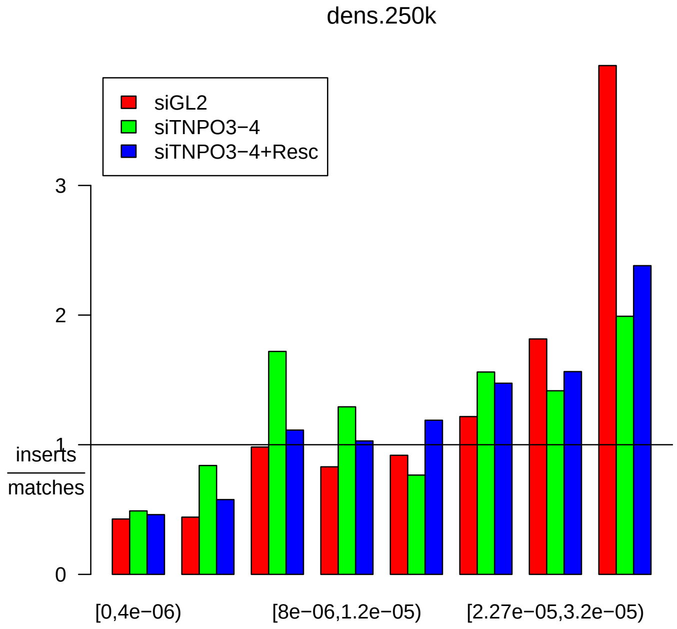
Here the effect of density of CpG islands is studied:



```
                 coef     se     z        p
siGL2           1.260 0.0754 16.70 1.70e-62
siTNPO3-4       0.344 0.1410  2.45 1.45e-02
siTNPO3-4+Resc 0.801 0.0640 12.50 6.78e-36
```

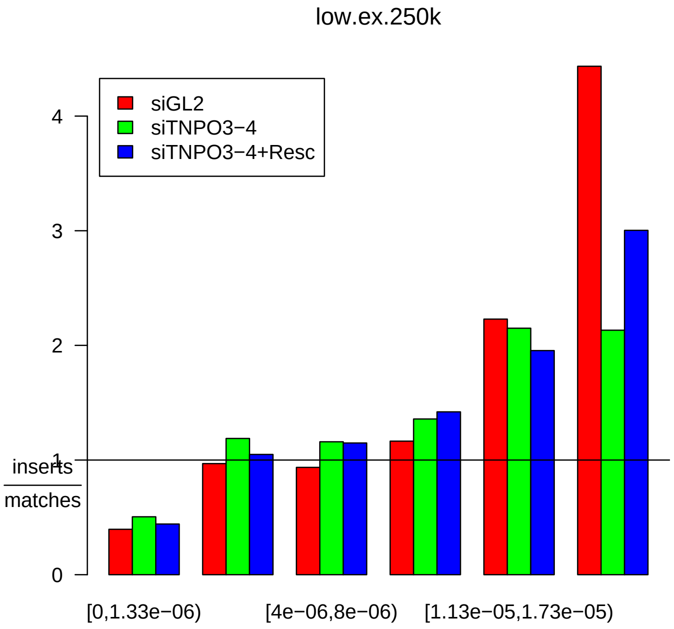
4.4 250 kilobase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 250 kilobase window surrounding each locus. First, we count just the number of genes represented on the chip.



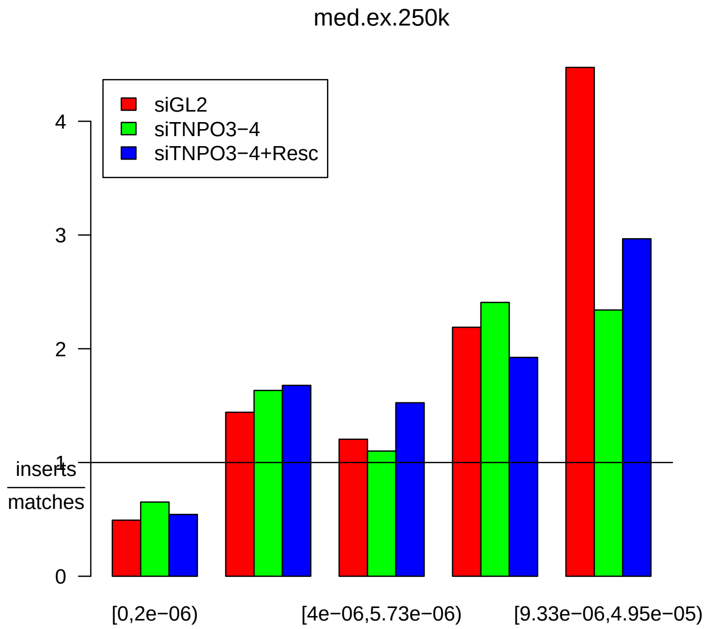
```
                coef     se     z        p
siGL2          1.180 0.0752 15.70 2.11e-55
siTNPO3-4      0.598 0.1340  4.46 8.12e-06
siTNPO3-4+Resc 0.964 0.0648 14.90 5.47e-50
```

Here are the results for expression density. First, we count just genes that are in the upper half.



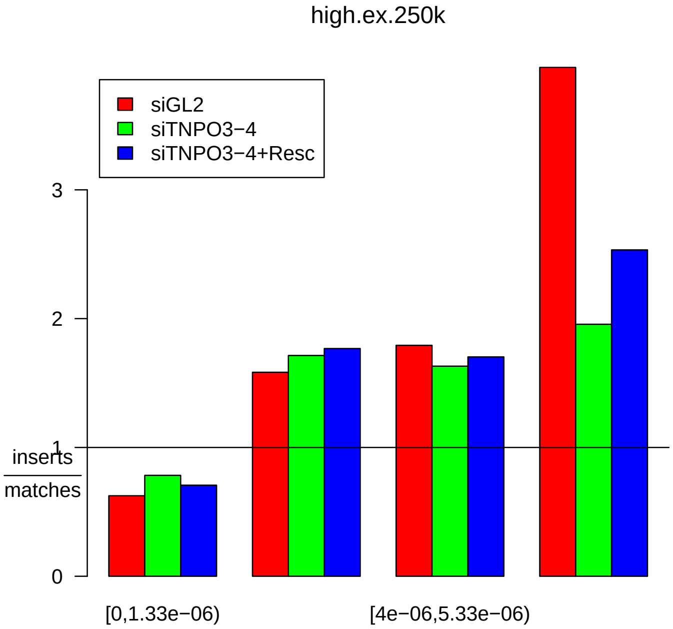
```
                 coef     se     z        p
siGL2           1.340 0.0745 18.00 4.35e-72
siTNPO3-4       0.827 0.1360  6.06 1.33e-09
siTNPO3-4+Resc 1.030 0.0645 16.00 2.21e-57
```

Now we count genes in the upper $1/8^{th}$:



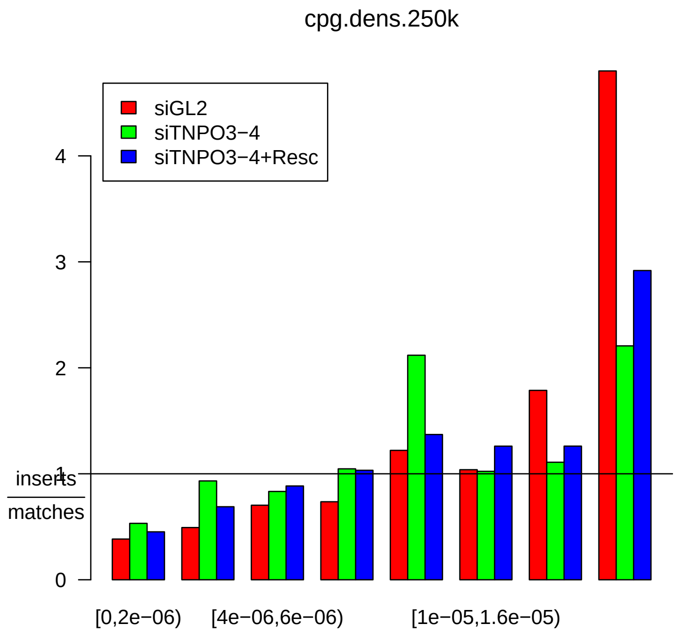
```
                coef     se     z        p
siGL2          1.480 0.0802 18.50 4.37e-76
siTNPO3-4      0.906 0.1420  6.39 1.67e-10
siTNPO3-4+Resc 1.320 0.0681 19.40 4.95e-84
```

And here we count genes in the upper $1/16^{th}$:



```
                coef     se     z         p
siGL2          1.380 0.0765 18.00 1.60e-72
siTNPO3-4      0.818 0.1430  5.72 1.05e-08
siTNPO3-4+Resc 1.110 0.0665 16.60 4.04e-62
```

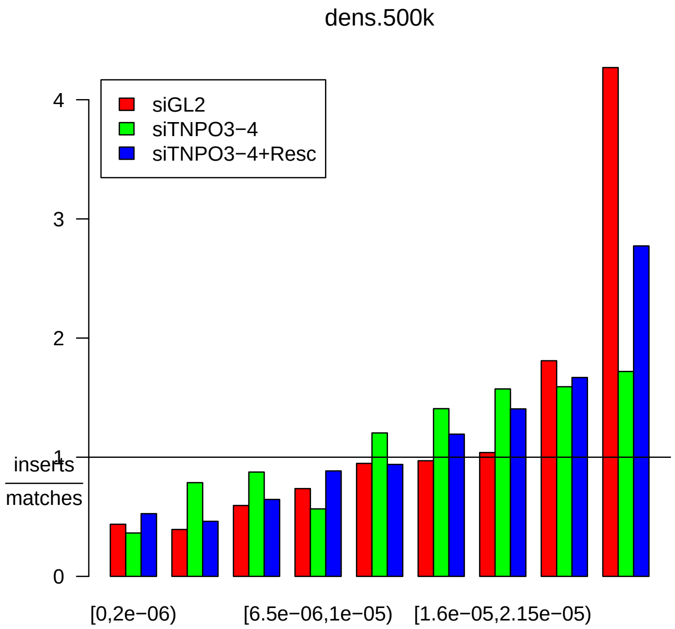
Here the effect of density of CpG islands is studied:



```
                 coef     se     z         p
siGL2           1.290 0.0766 16.80 1.33e-63
siTNPO3-4       0.591 0.1380  4.27 1.96e-05
siTNPO3-4+Resc 0.805 0.0639 12.60 1.95e-36
```

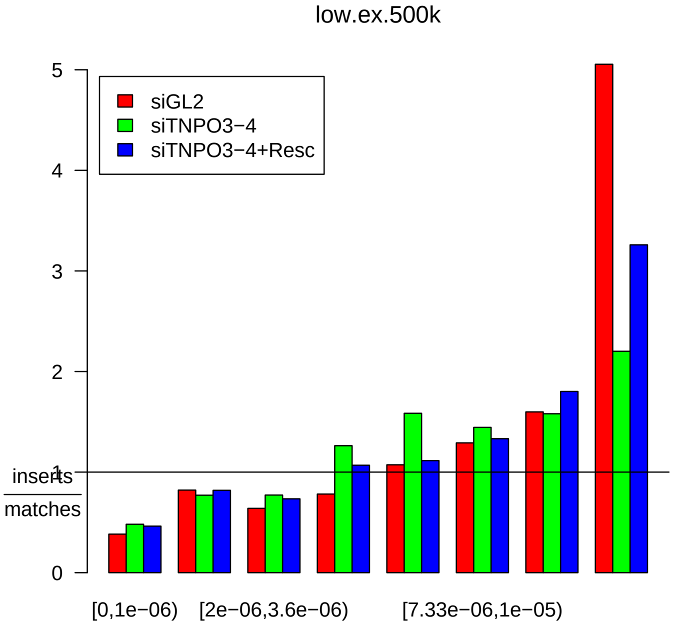
4.5 500 kilobase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 500 kilobase window surrounding each locus. First, we count just the number of genes represented on the chip.



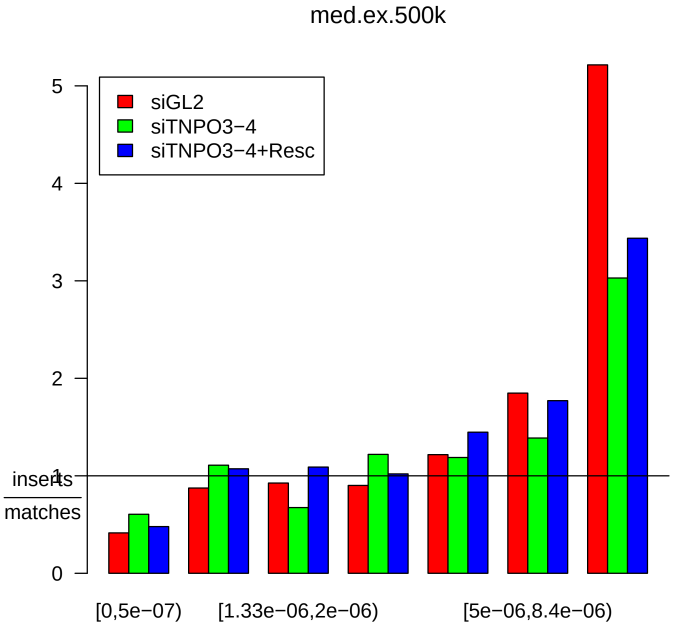
```
                   coef     se     z        p
siGL2             1.160 0.0755 15.30 6.86e-53
siTNPO3-4         0.863 0.1390  6.22 4.88e-10
siTNPO3-4+Resc    0.910 0.0647 14.10 5.94e-45
```

Here are the results for expression density. First, we count just genes that are in the upper half.



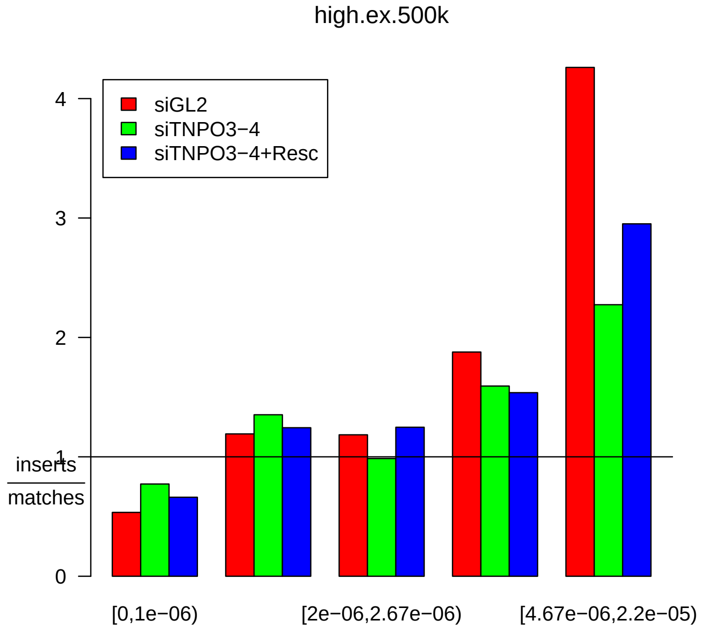
```
                coef     se    z         p
siGL2          1.200 0.0763 15.7 2.28e-55
siTNPO3-4      0.931 0.1430  6.5 7.81e-11
siTNPO3-4+Resc 0.971 0.0659 14.7 4.41e-49
```

Now we count genes in the upper $1/8^{th}$:



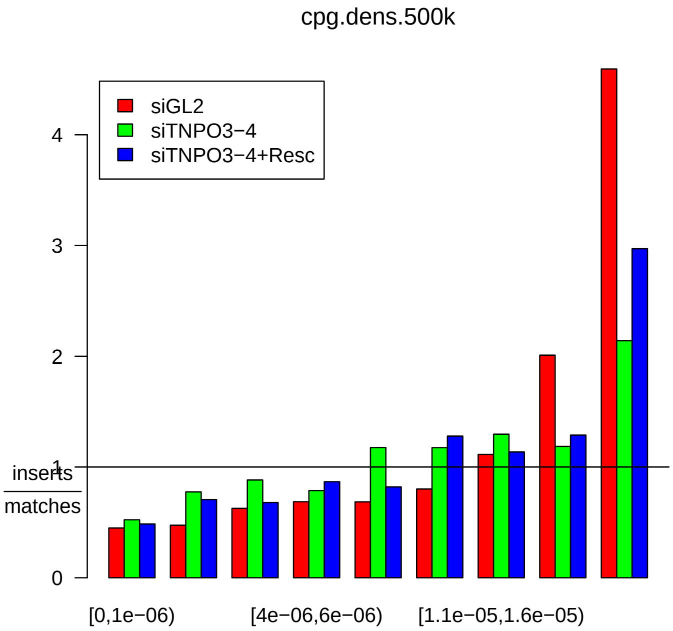
```
                coef     se     z        p
siGL2          1.250 0.0775 16.20 6.71e-59
siTNPO3-4      0.641 0.1380  4.66 3.23e-06
siTNPO3-4+Resc 0.978 0.0663 14.80 2.39e-49
```

And here we count genes in the upper $1/16^{th}$:



```
                coef     se     z        p
siGL2          1.280 0.0768 16.60 4.87e-62
siTNPO3-4      0.600 0.1390  4.33 1.51e-05
siTNPO3-4+Resc 0.912 0.0654 14.00 2.92e-44
```

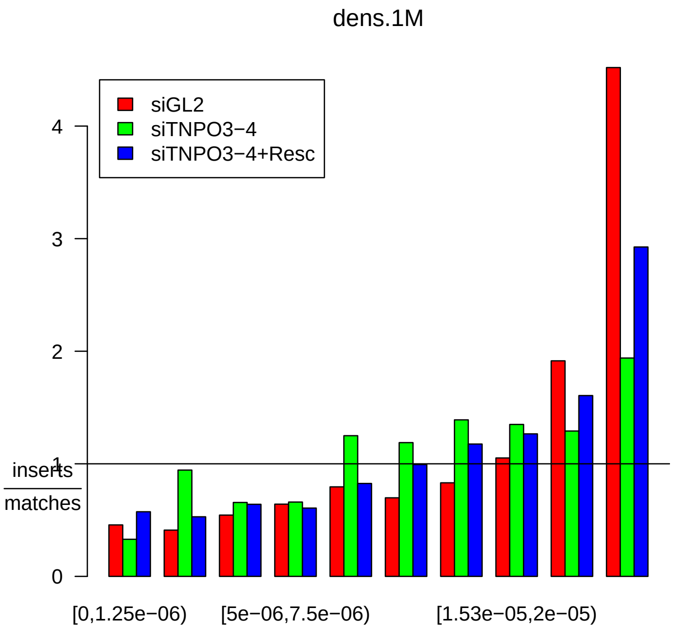
Here the effect of density of CpG islands is studied:



```
                coef     se    z        p
siGL2          1.070 0.0740 14.5 8.71e-48
siTNPO3-4      0.542 0.1390  3.9 9.67e-05
siTNPO3-4+Resc 0.741 0.0637 11.6 2.72e-31
```

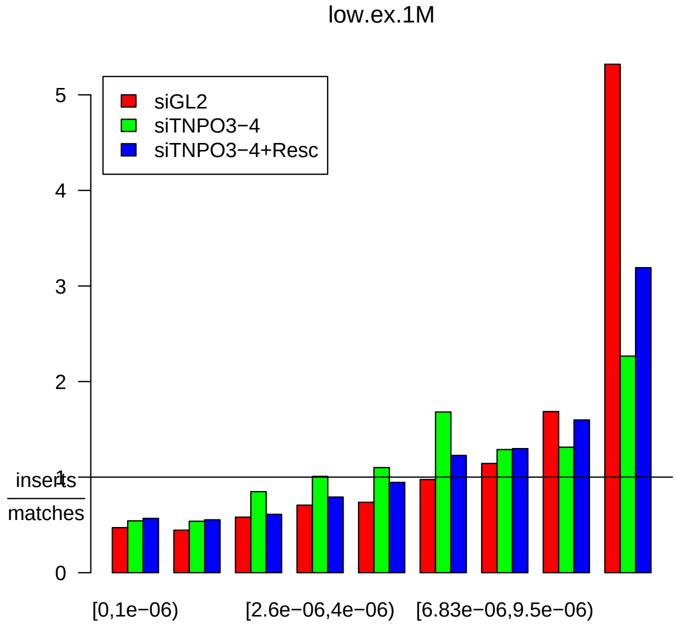
4.6 1 megabase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 1 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



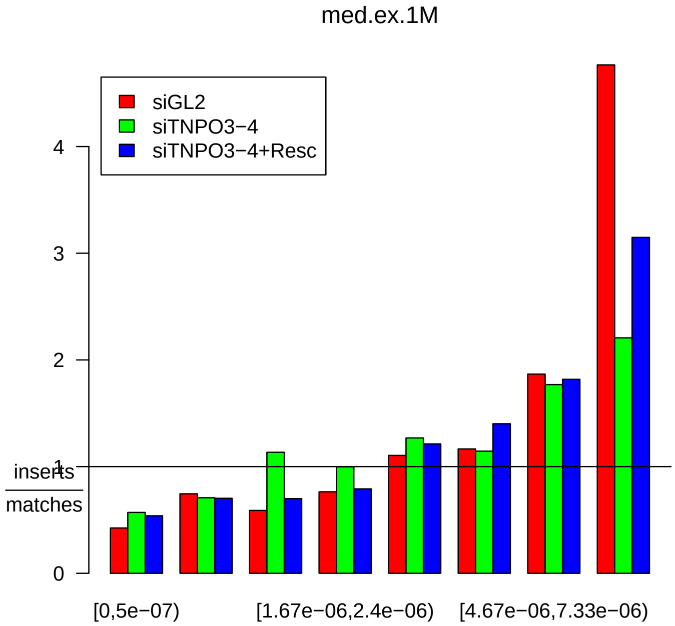
```
                 coef     se    z        p
siGL2           0.966 0.0731 13.2 7.98e-40
siTNPO3-4       0.660 0.1400  4.7 2.57e-06
siTNPO3-4+Resc  0.831 0.0639 13.0 1.11e-38
```

Here are the results for expression density. First, we count just genes that are in the upper half.



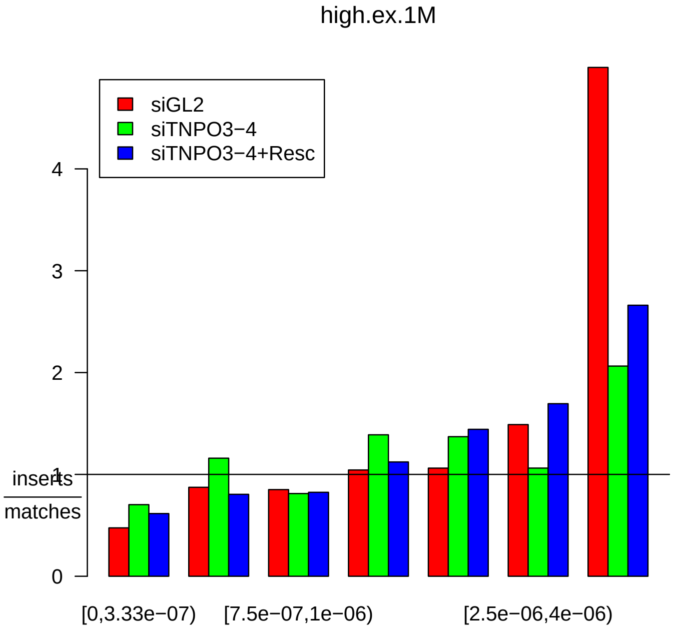
```
                coef     se     z        p
siGL2          1.100 0.0752 14.60 3.62e-48
siTNPO3-4      0.726 0.1410  5.15 2.56e-07
siTNPO3-4+Resc 0.877 0.0652 13.40 3.38e-41
```

Now we count genes in the upper $1/8^{th}$:



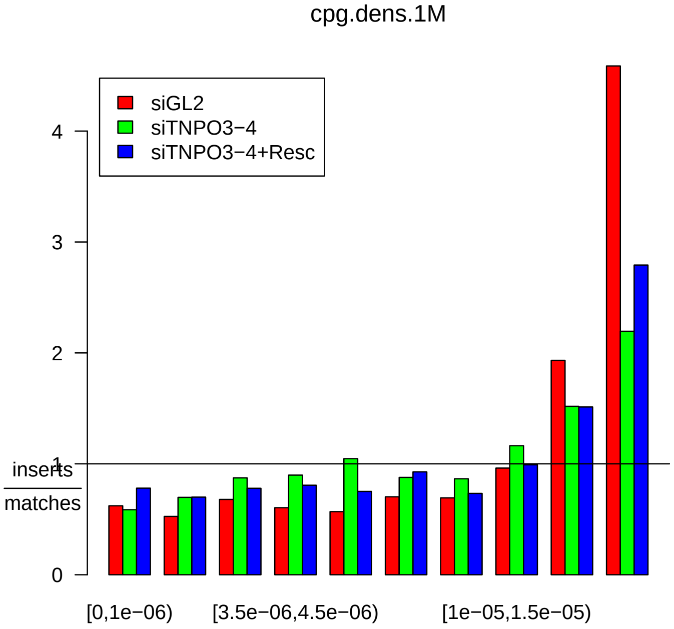
```
                 coef     se     z        p
siGL2           1.160 0.0759 15.30 3.94e-53
siTNPO3-4       0.601 0.1390  4.33 1.46e-05
siTNPO3-4+Resc  0.923 0.0662 13.90 3.39e-44
```

And here we count genes in the upper $1/16^{th}$:



```
                 coef     se     z         p
siGL2           1.060 0.0749 14.20 1.76e-45
siTNPO3-4       0.465 0.1360  3.43 6.04e-04
siTNPO3-4+Resc  0.776 0.0646 12.00 2.98e-33
```

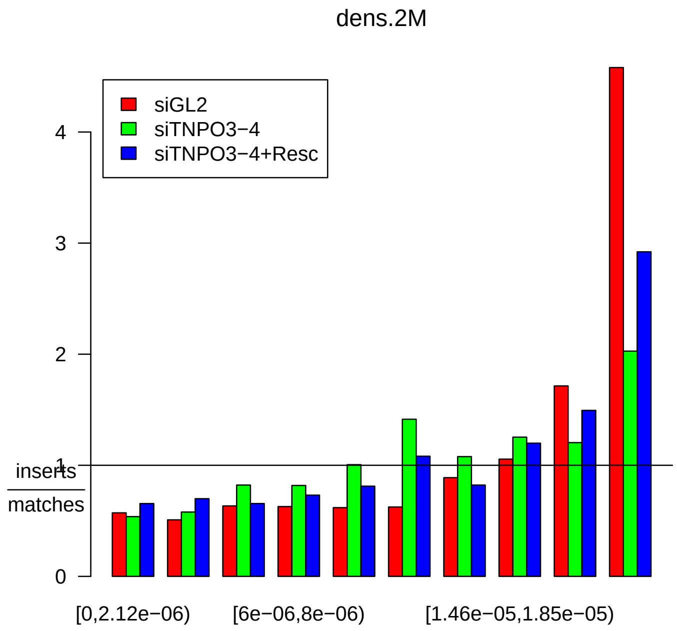
Here the effect of density of CpG islands is studied:



```
               coef     se     z        p
siGL2          0.910 0.0726 12.50 5.04e-36
siTNPO3-4      0.349 0.1390  2.51 1.20e-02
siTNPO3-4+Resc 0.521 0.0631  8.26 1.44e-16
```

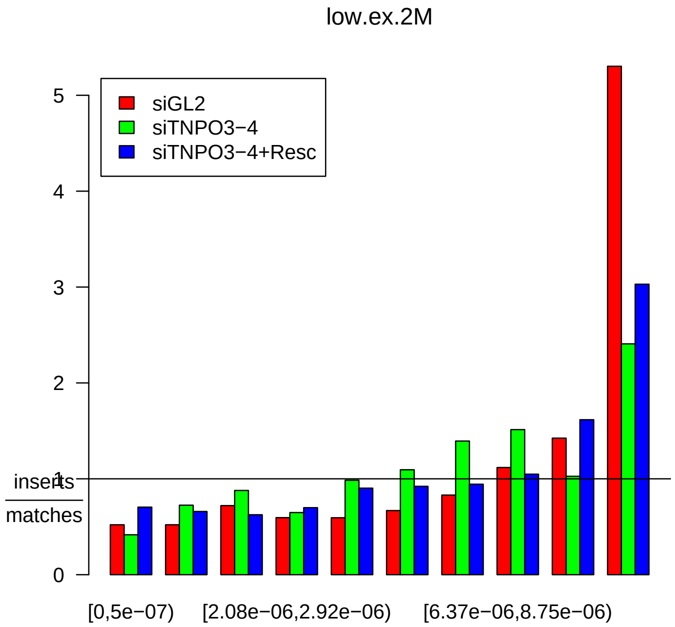
4.7 2 megabase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 2 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



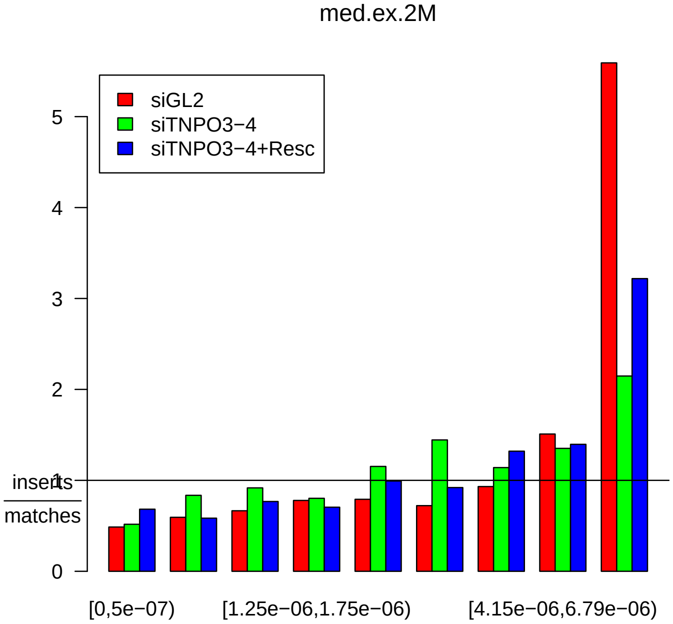
```
                coef     se     z        p
siGL2          0.944 0.0745 12.70 7.25e-37
siTNPO3-4      0.601 0.1420  4.22 2.42e-05
siTNPO3-4+Resc 0.646 0.0638 10.10 4.37e-24
```

Here are the results for expression density. First, we count just genes that are in the upper half.



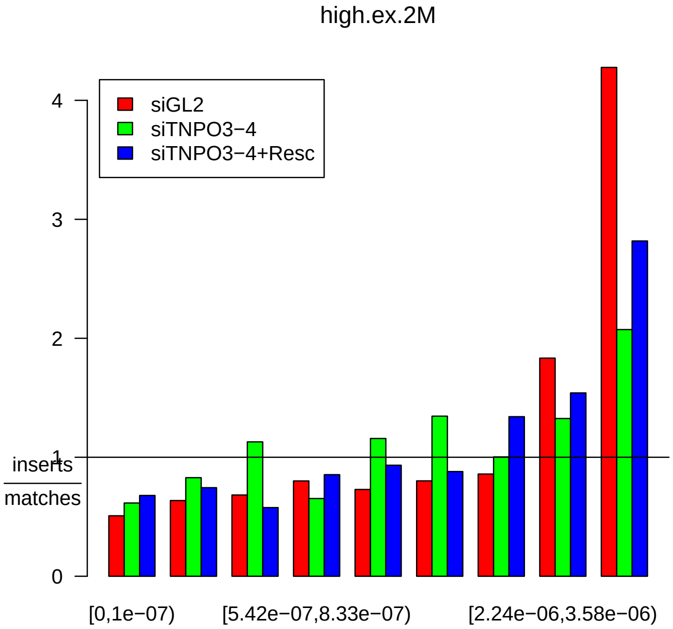
```
                coef     se     z        p
siGL2          0.955 0.0745 12.80 1.09e-37
siTNPO3-4      0.653 0.1410  4.63 3.66e-06
siTNPO3-4+Resc 0.620 0.0637  9.73 2.20e-22
```

Now we count genes in the upper $1/8^{th}$:



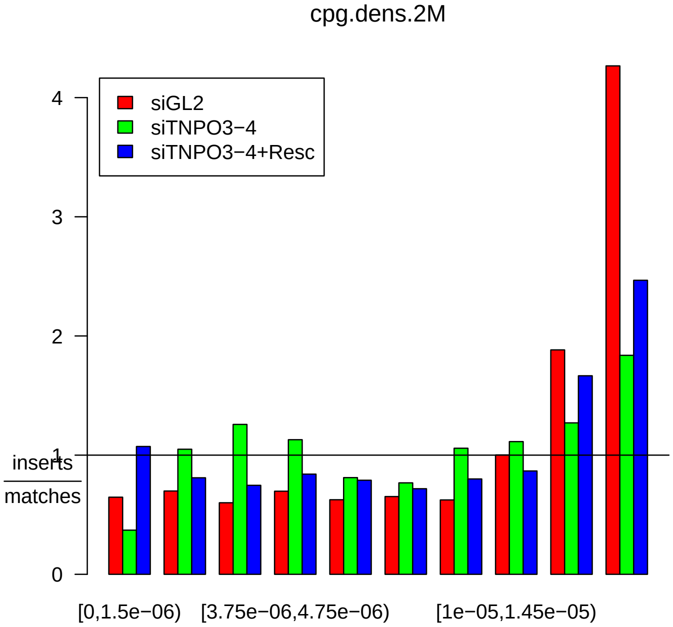
```
                coef     se     z        p
siGL2          0.915 0.0743 12.30 7.27e-35
siTNPO3-4      0.638 0.1400  4.55 5.24e-06
siTNPO3-4+Resc 0.729 0.0642 11.40 6.48e-30
```

And here we count genes in the upper $1/16^{th}$:



```
                coef     se     z        p
siGL2          0.861 0.0740 11.60 3.05e-31
siTNPO3-4      0.490 0.1370  3.59 3.33e-04
siTNPO3-4+Resc 0.642 0.0642 10.00 1.49e-23
```

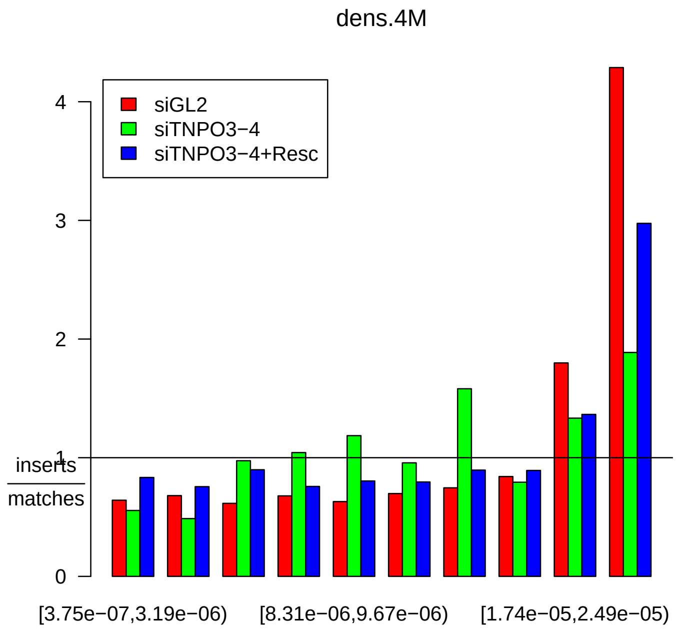
Here the effect of density of CpG islands is studied:



```
                coef     se     z         p
siGL2          0.784 0.0727 10.80 4.21e-27
siTNPO3-4      0.217 0.1360  1.60 1.10e-01
siTNPO3-4+Resc 0.335 0.0631  5.31 1.09e-07
```

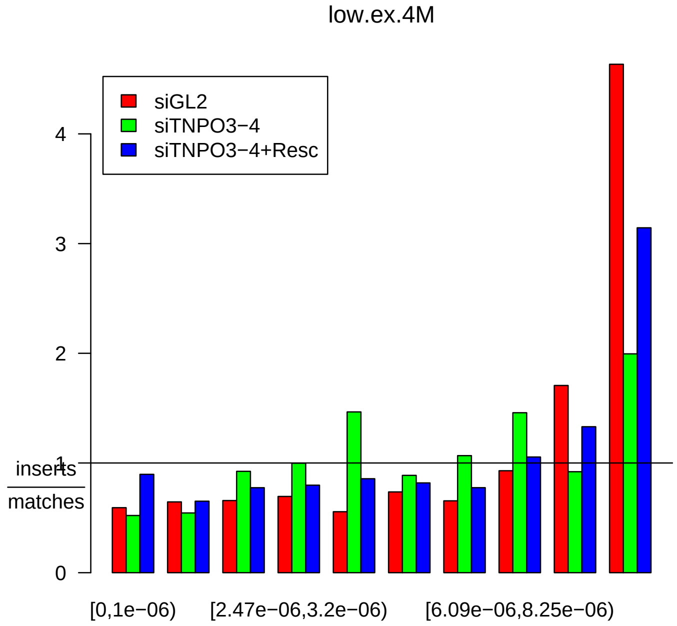
4.8 4 megabase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 4 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



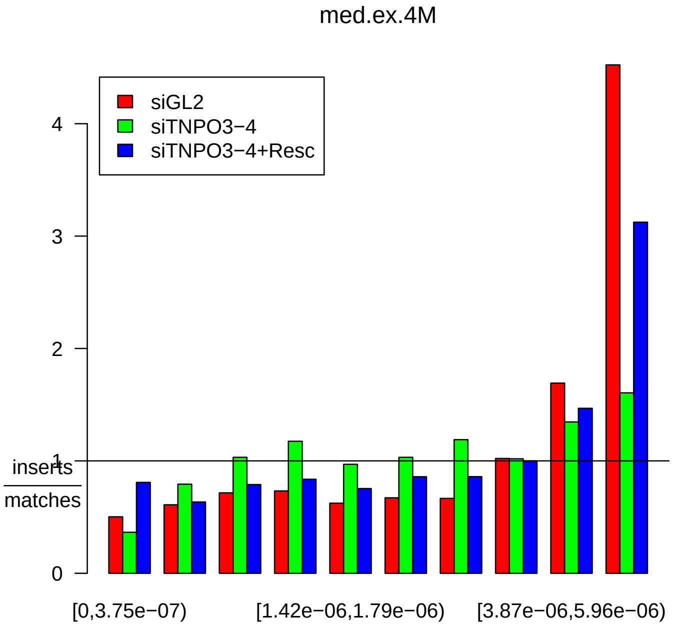
```
                 coef     se     z         p
siGL2           0.780 0.0730 10.70 1.24e-26
siTNPO3-4       0.380 0.1360  2.80 5.13e-03
siTNPO3-4+Resc 0.397 0.0629  6.31 2.79e-10
```

Here are the results for expression density. First, we count just genes that are in the upper half.



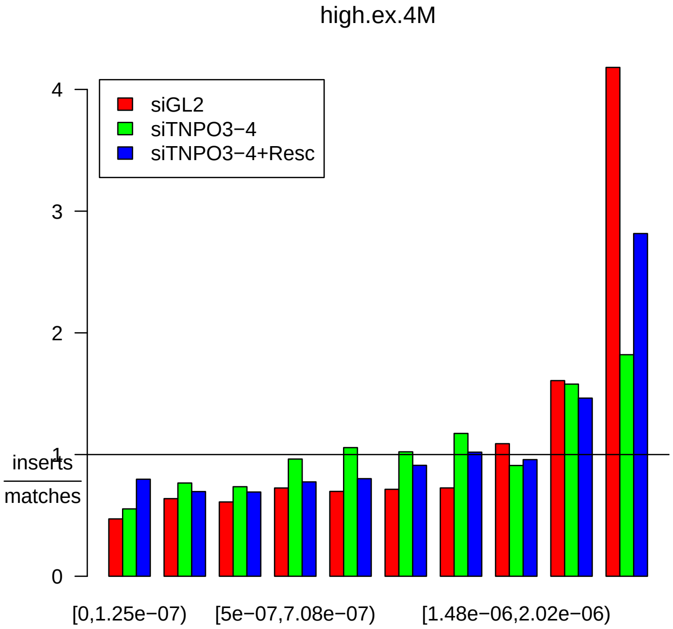
```
                 coef     se     z        p
siGL2           0.841 0.0736 11.40 2.68e-30
siTNPO3-4       0.317 0.1380  2.29 2.19e-02
siTNPO3-4+Resc 0.424 0.0626  6.77 1.33e-11
```

Now we count genes in the upper $1/8^{th}$:



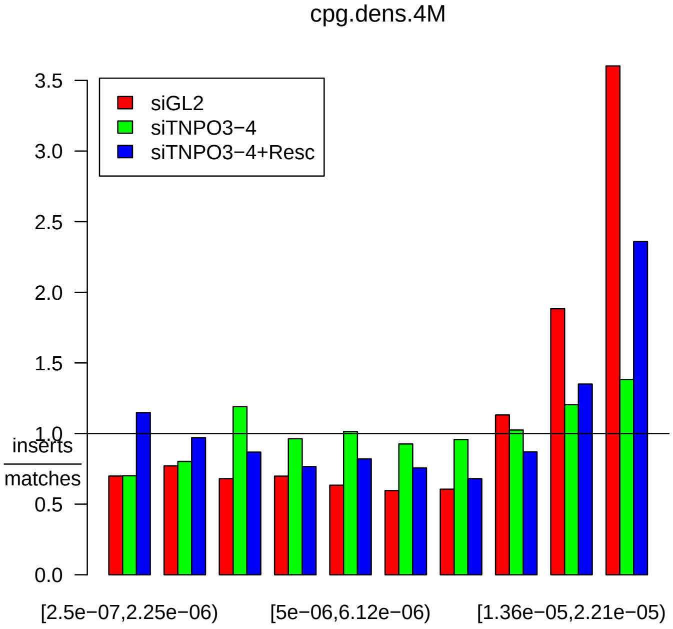
```
                coef     se     z        p
siGL2          0.838 0.0740 11.30 1.06e-29
siTNPO3-4      0.336 0.1330  2.52 1.18e-02
siTNPO3-4+Resc 0.502 0.0634  7.92 2.29e-15
```

And here we count genes in the upper $1/16^{th}$:



```
                  coef     se     z        p
siGL2            0.858 0.0740 11.60 4.13e-31
siTNPO3-4        0.394 0.1340  2.95 3.21e-03
siTNPO3-4+Resc 0.530 0.0634  8.36 6.39e-17
```

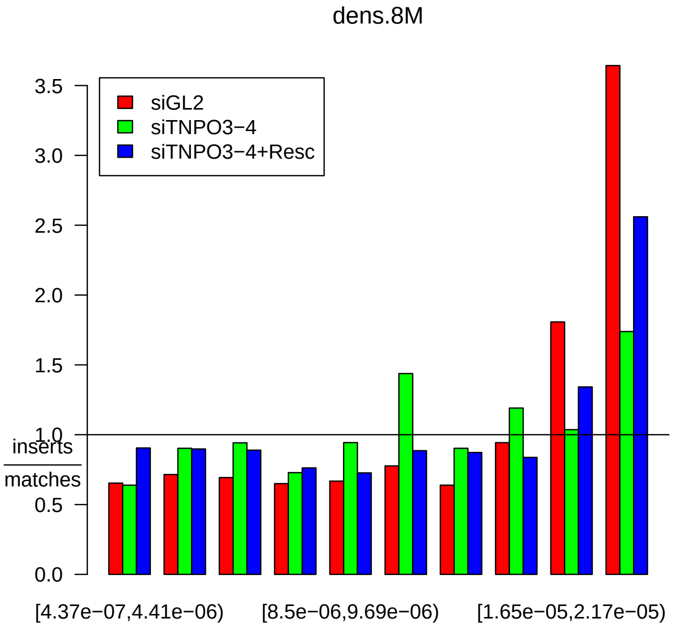
Here the effect of density of CpG islands is studied:



```
                coef     se     z        p
siGL2          0.663 0.0719 9.230 2.71e-20
siTNPO3-4      0.101 0.1360 0.745 4.56e-01
siTNPO3-4+Resc 0.180 0.0622 2.900 3.75e-03
```

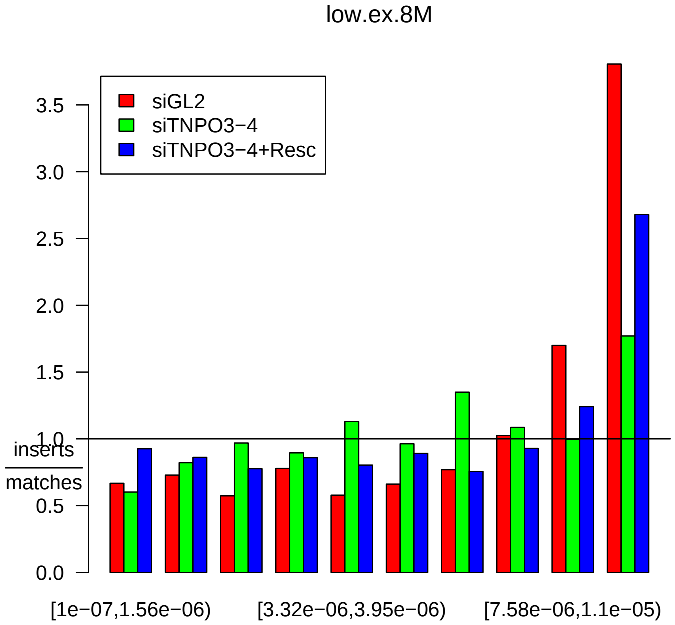
4.9 8 megabase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 8 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



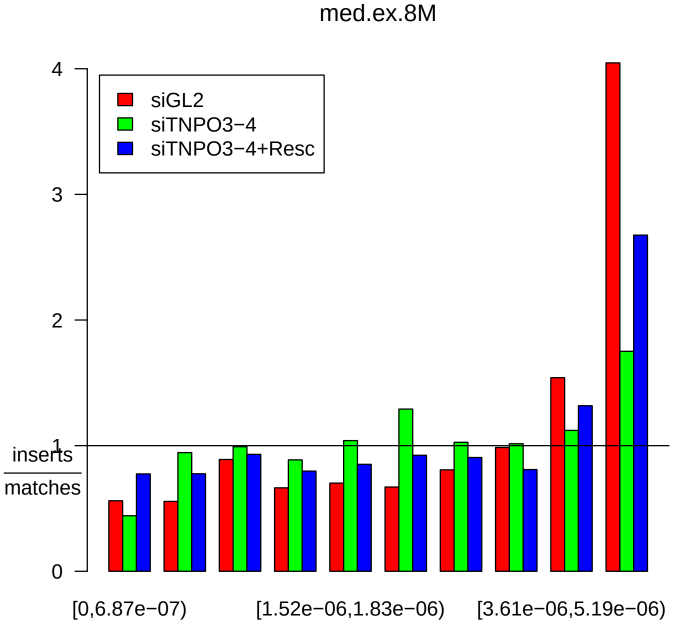
```
                coef     se     z        p
siGL2          0.729 0.0724 10.10 7.33e-24
siTNPO3-4      0.388 0.1370  2.83 4.62e-03
siTNPO3-4+Resc 0.339 0.0624  5.43 5.58e-08
```

Here are the results for expression density. First, we count just genes that are in the upper half.



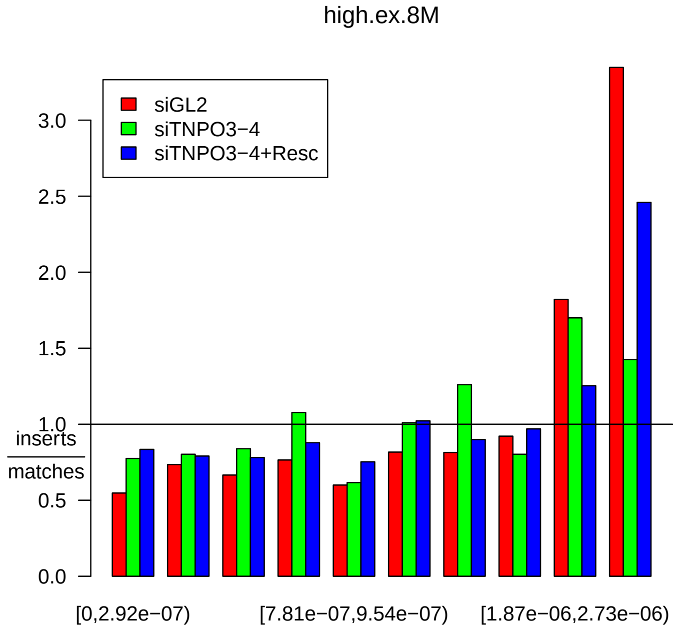
```
                coef     se     z        p
siGL2          0.738 0.0724 10.20 2.21e-24
siTNPO3-4      0.286 0.1380  2.08 3.74e-02
siTNPO3-4+Resc 0.312 0.0622  5.02 5.26e-07
```

Now we count genes in the upper $1/8^{th}$:



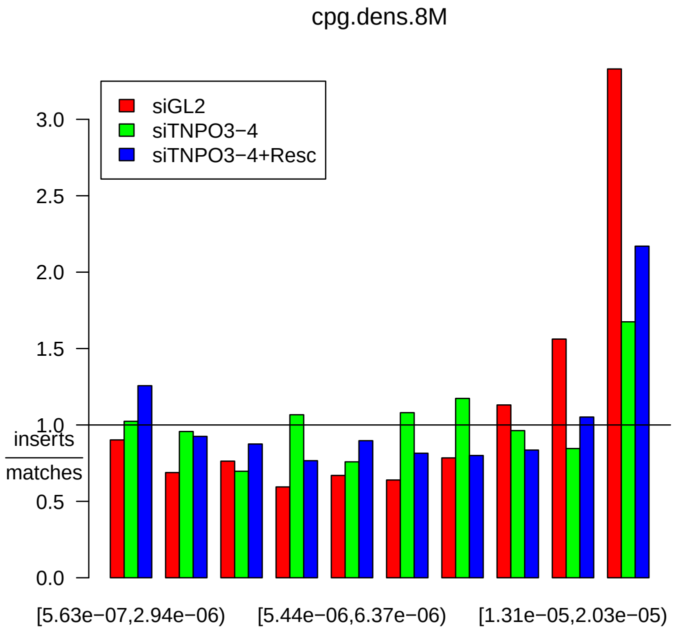
```
                  coef     se     z        p
siGL2            0.749 0.0728 10.30 8.11e-25
siTNPO3-4        0.333 0.1340  2.48 1.31e-02
siTNPO3-4+Resc   0.357 0.0630  5.66 1.52e-08
```

And here we count genes in the upper $1/16^{th}$:



```
               coef     se     z        p
siGL2         0.773 0.0725 10.70 1.71e-26
siTNPO3-4     0.400 0.1350  2.96 3.10e-03
siTNPO3-4+Resc 0.410 0.0629  6.52 7.02e-11
```

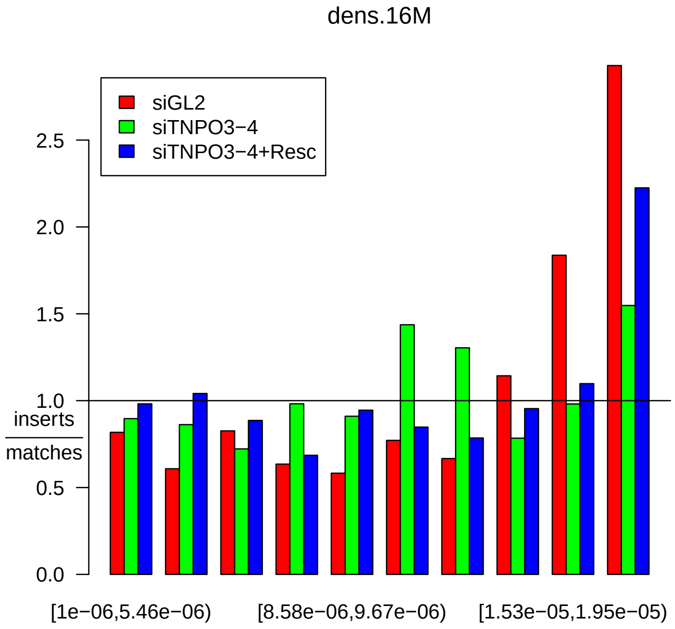
Here the effect of density of CpG islands is studied:



```
               coef     se    z        p
siGL2         0.625 0.0714 8.75 2.17e-18
siTNPO3-4     0.188 0.1360 1.38 1.67e-01
siTNPO3-4+Resc 0.128 0.0624 2.06 3.94e-02
```

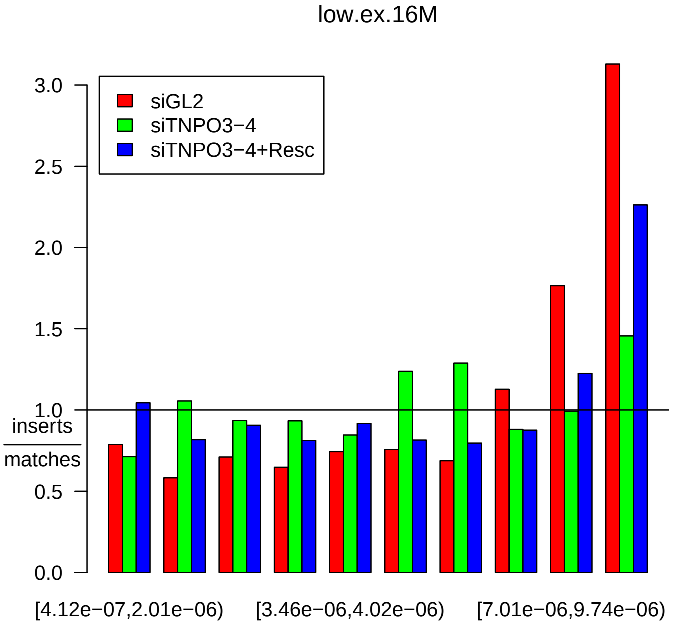
4.10 16 megabase Window

In the barplot that follows we examine the association of insertion sites with expression density in a 16 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



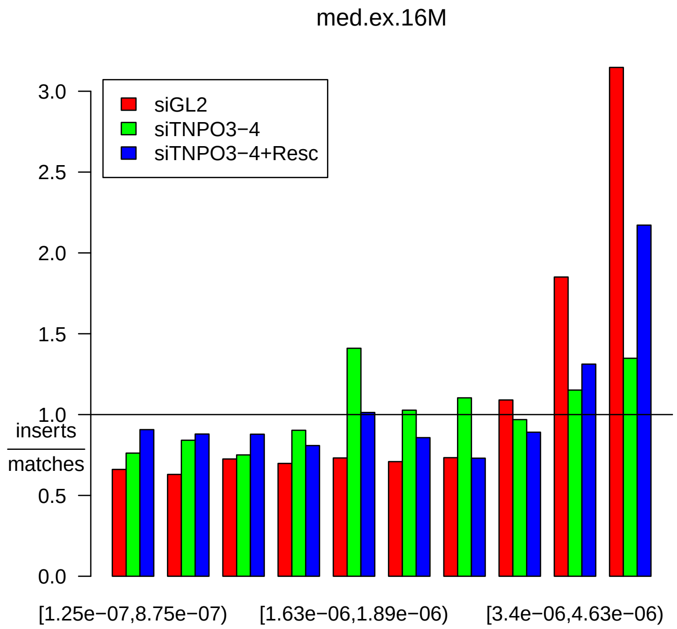
```
                coef     se    z        p
siGL2          0.683 0.0723 9.45 3.33e-21
siTNPO3-4      0.265 0.1370 1.93 5.33e-02
siTNPO3-4+Resc 0.190 0.0624 3.04 2.37e-03
```

Here are the results for expression density. First, we count just genes that are in the upper half.



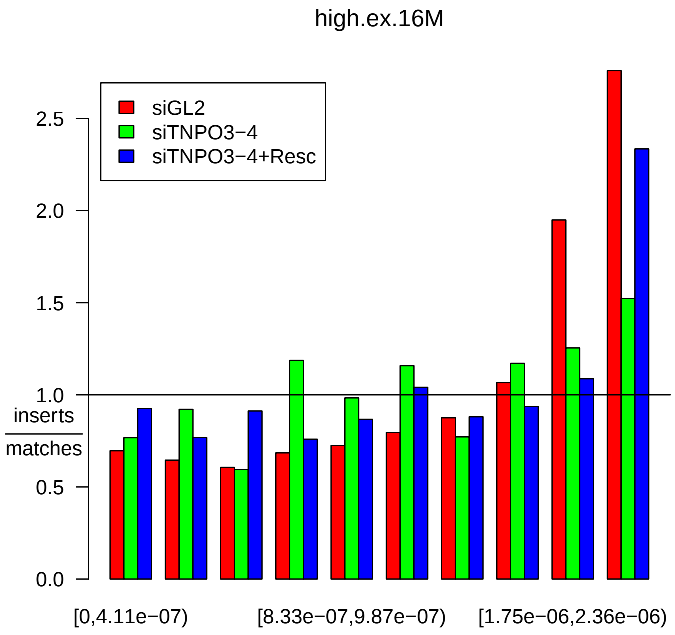
```
                 coef    se    z         p
siGL2           0.683 0.072 9.49 2.41e-21
siTNPO3-4       0.247 0.137 1.80 7.13e-02
siTNPO3-4+Resc 0.208 0.063 3.30 9.67e-04
```

Now we count genes in the upper $1/8^{th}$:



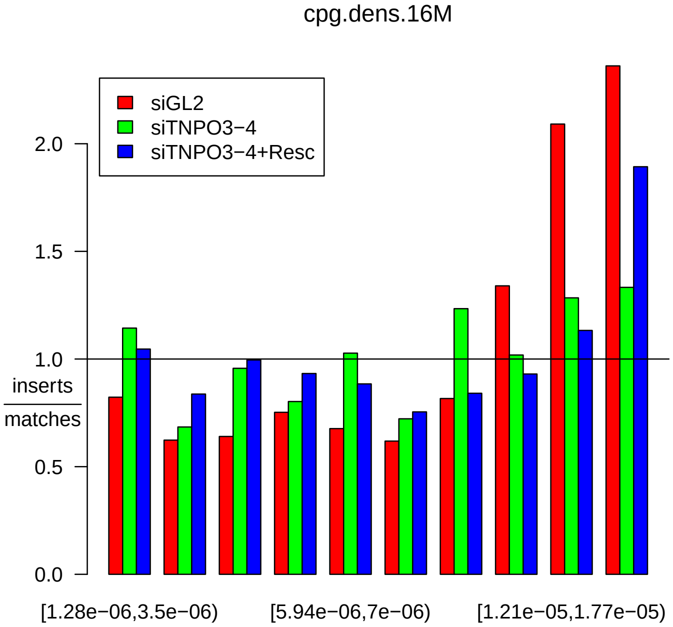
```
               coef     se    z        p
siGL2          0.687 0.0716 9.60 8.32e-22
siTNPO3-4      0.181 0.1350 1.34 1.81e-01
siTNPO3-4+Resc 0.197 0.0625 3.15 1.66e-03
```

And here we count genes in the upper $1/16^{th}$:



```
                 coef     se     z         p
siGL2           0.722 0.0713 10.10 4.15e-24
siTNPO3-4       0.267 0.1340  1.99 4.70e-02
siTNPO3-4+Resc  0.314 0.0627  5.01 5.52e-07
```

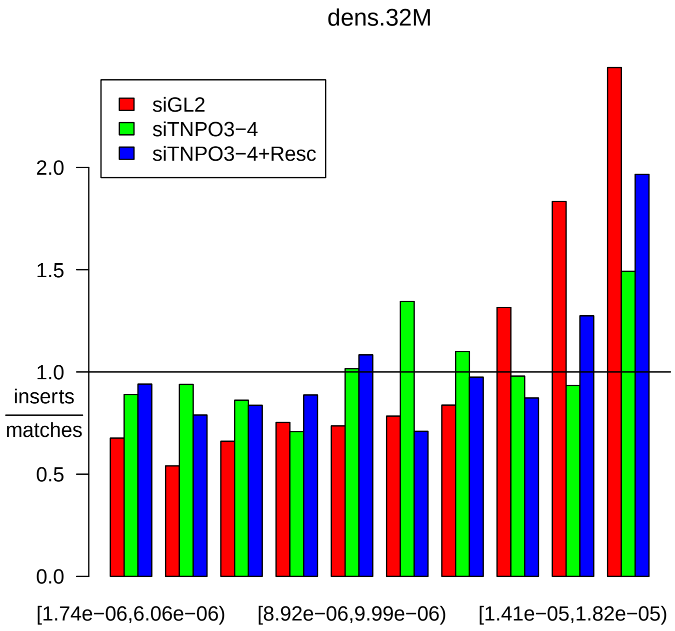
Here the effect of density of CpG islands is studied:



```
                  coef     se    z        p
siGL2          0.654 0.0716 9.13 7.14e-20
siTNPO3-4      0.171 0.1350 1.26 2.08e-01
siTNPO3-4+Resc 0.116 0.0621 1.86 6.28e-02
```

4.11 32 megabase Window

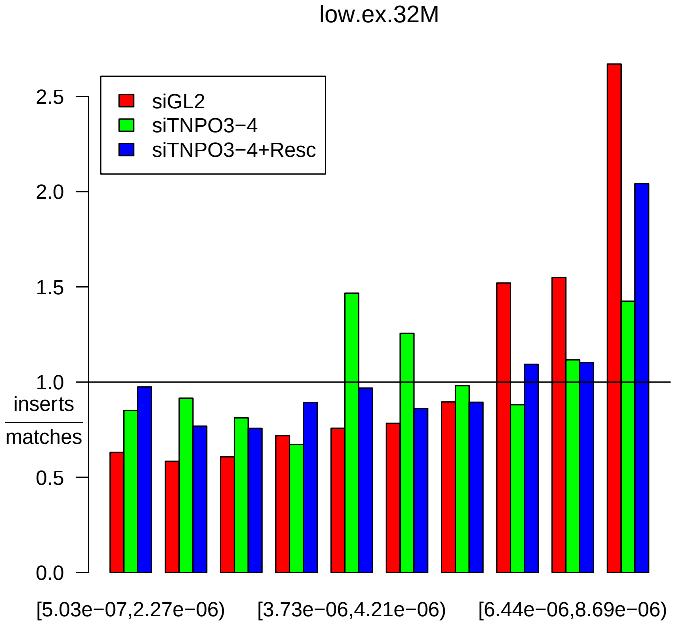
In the barplot that follows we examine the association of insertion sites with expression density in a 32 megabase window surrounding each locus. First, we count just the number of genes represented on the chip.



dens.32M

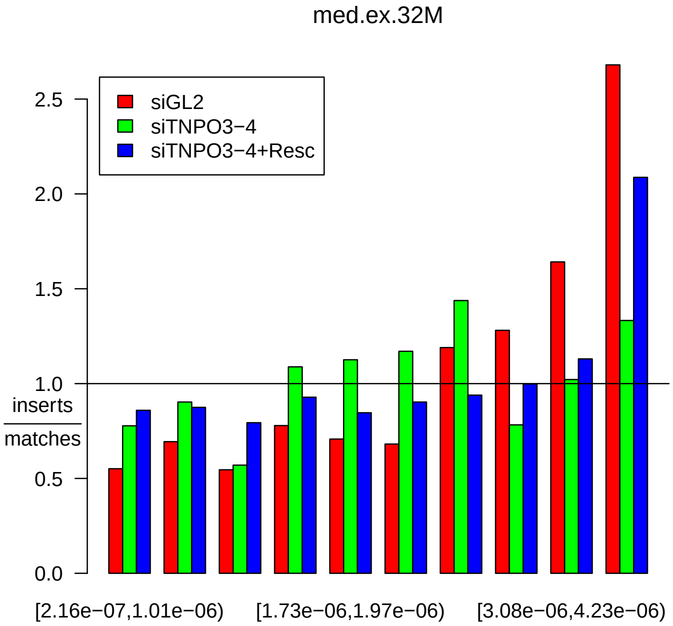
```
                coef     se     z        p
siGL2          0.747 0.0727 10.30 8.85e-25
siTNPO3-4      0.257 0.1370  1.88 6.00e-02
siTNPO3-4+Resc 0.184 0.0624  2.94 3.26e-03
```

Here are the results for expression density. First, we count just genes that are in the upper half.



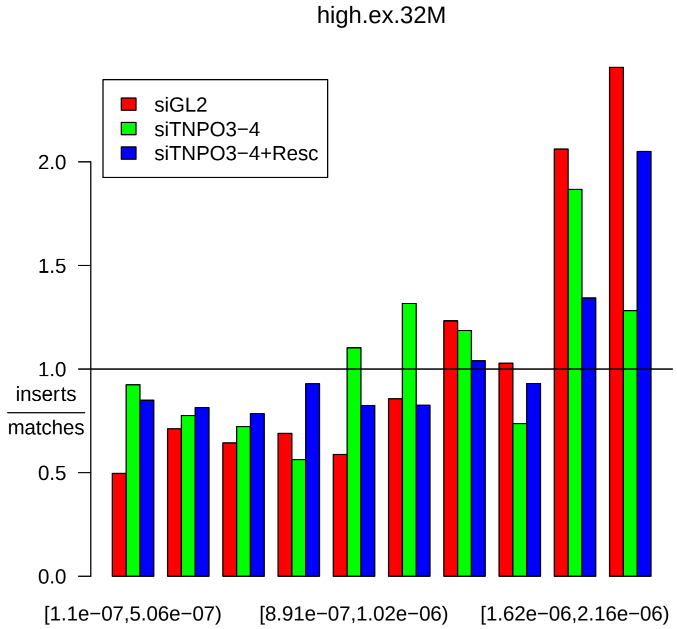
```
               coef     se     z        p
siGL2         0.778 0.0730 10.70 1.68e-26
siTNPO3-4     0.180 0.1360  1.32 1.86e-01
siTNPO3-4+Resc 0.267 0.0627  4.27 1.98e-05
```

Now we count genes in the upper $1/8^{th}$:



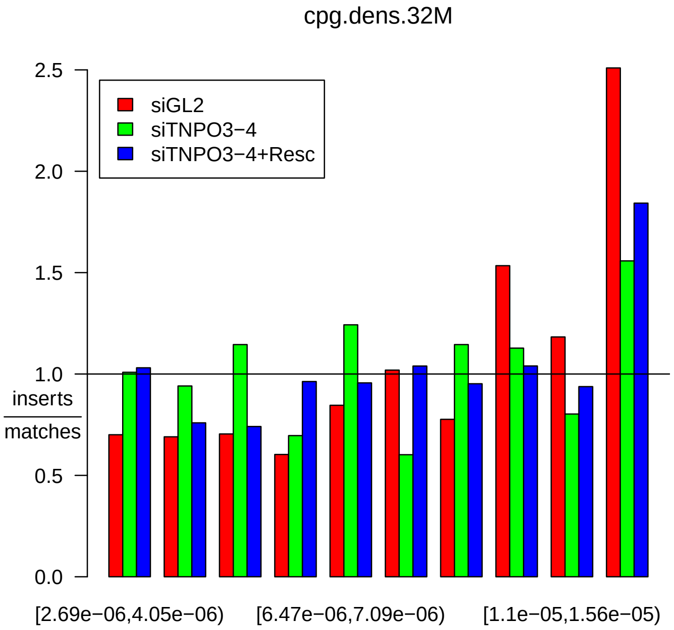
```
                coef     se     z        p
siGL2          0.775 0.0720 10.80 5.32e-27
siTNPO3-4      0.237 0.1360  1.74 8.12e-02
siTNPO3-4+Resc 0.295 0.0627  4.70 2.64e-06
```

And here we count genes in the upper $1/16^{th}$:



```
                coef     se     z        p
siGL2          0.859 0.0730 11.80 6.15e-32
siTNPO3-4      0.388 0.1370  2.83 4.59e-03
siTNPO3-4+Resc 0.332 0.0626  5.30 1.13e-07
```

Here the effect of density of CpG islands is studied:

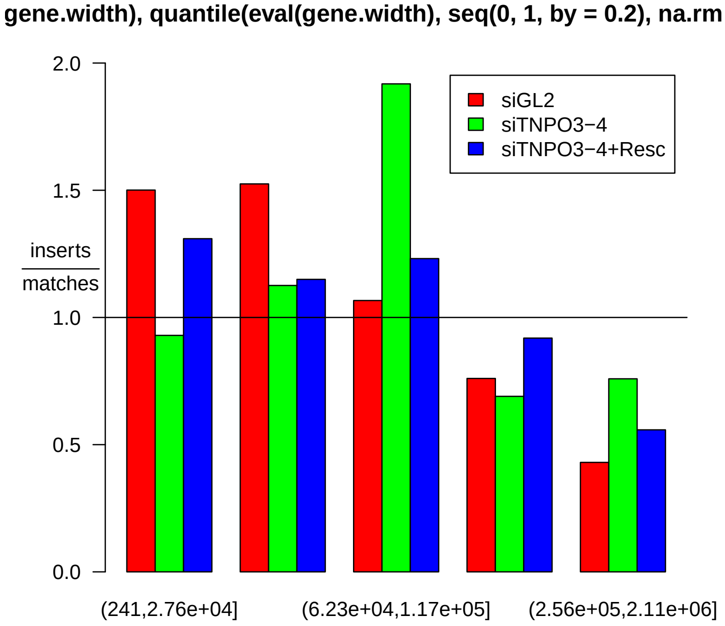


```
                coef     se      z        p
siGL2         0.63900 0.0711 8.9900 2.43e-19
siTNPO3-4     0.00622 0.1370 0.0455 9.64e-01
siTNPO3-4+Resc 0.21400 0.0615 3.4800 4.98e-04
```

5 Juxtaposition with Gene Start and End Positions

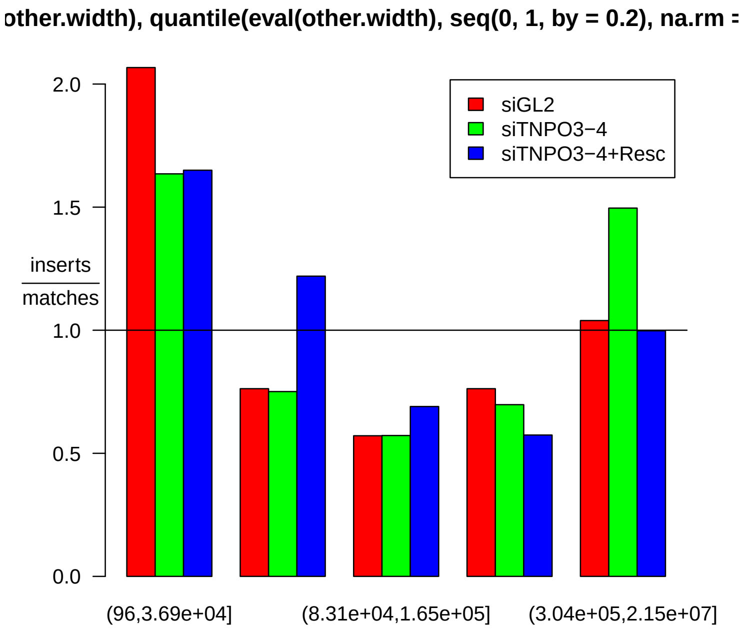
5.1 Acembly Annotations

In this section we study the effect of juxtaposition in terms of gene start and end positions. The first barplot shows the effect of gene width for those insertions that are located within an Acembly gene. The table following the barplot shows the p-values for a test of the hypothesis that the proportions in each of the categories that define the bars are equal in the insertions and their matches. This p-value is obtained from the $5 \times 2 \times k$ table of counts defined by gene width category, insertion/match status, and stratum (consisting of an insertion and its matched sites) using a likelihood ratio test for the hypothesis of no association between gene width category and insertion/match status. The test used compared the log-linear model [1] with all two-way configurations to that with no gene width category and insertion/match status configuration.



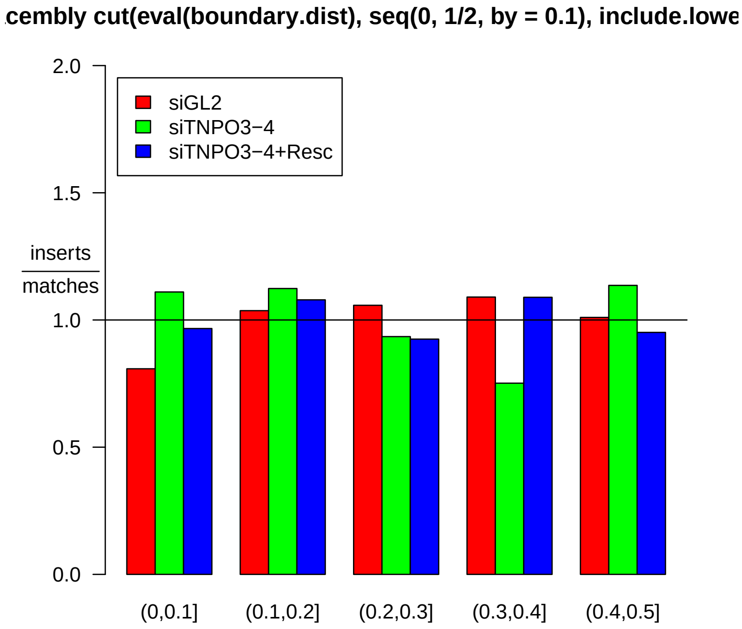
```
   siGL2      siTNPO3-4 siTNPO3-4+Resc
1.39e-28       2.95e-05       6.84e-15
```

The next plot uses the width of a non-gene region for insertions that fall into such regions.



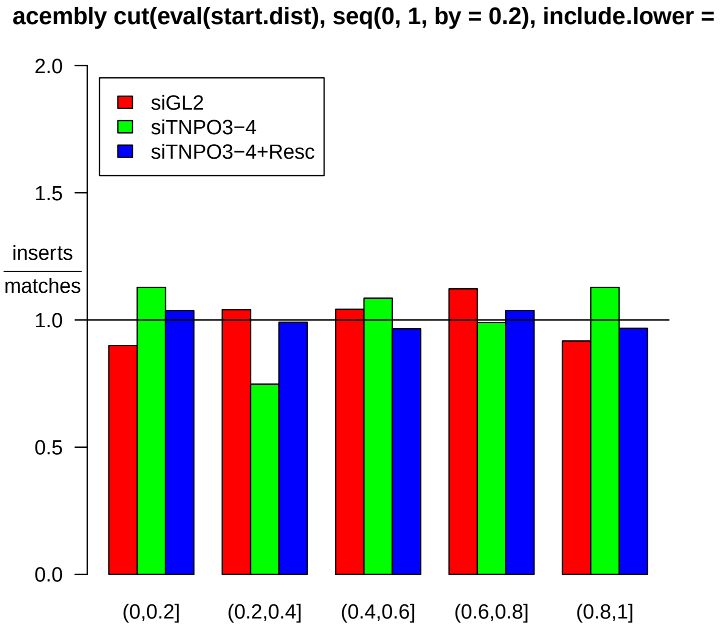
```
    siGL2      siTNPO3-4 siTNPO3-4+Resc
 4.36e-14       1.06e-02       2.07e-06
```

The next plot studies the distance to the nearest boundary between a gene and a non-gene region. The distance is expressed as a fraction of the length of the region. Thus, '0.25' refers to one quarter of the distance from the site to nearest boundary divided by the total width of the region.



```
 siGL2      siTNPO3-4 siTNPO3-4+Resc
0.0216         0.0556         0.1690
```

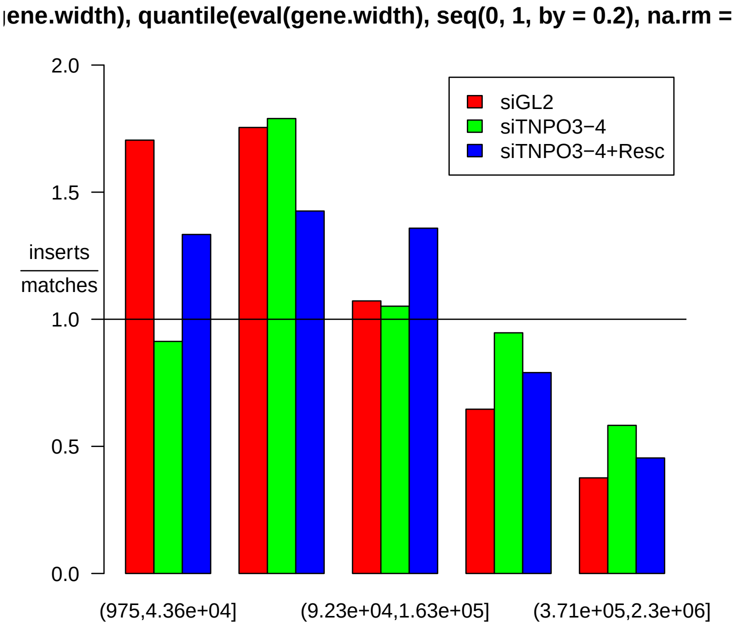
This plot studies the effect of nearness to the beginning of a transcript. For sites in genes, it is the distance to the start of the gene divided by the width of the gene. For other sites it is the distance from the site to the nearer gene if that gene boundary is also a transcription starting point. Locations near '0' are relatively near the beginning of transcription, while those near '1' are near the termination of the transcript.



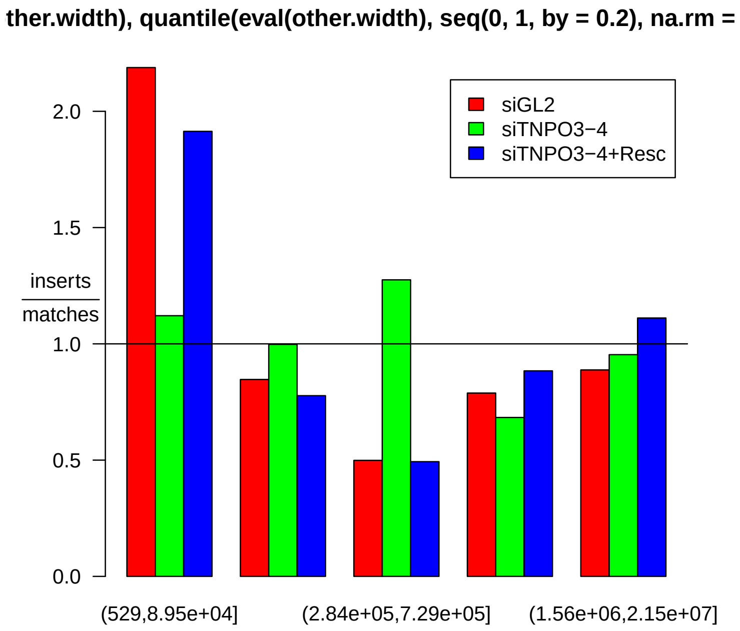
acembly cut(eval(start.dist), seq(0, 1, by = 0.2), include.lower =

```
 siGL2       siTNPO3-4 siTNPO3-4+Resc
0.1580          0.0841         0.9050
```

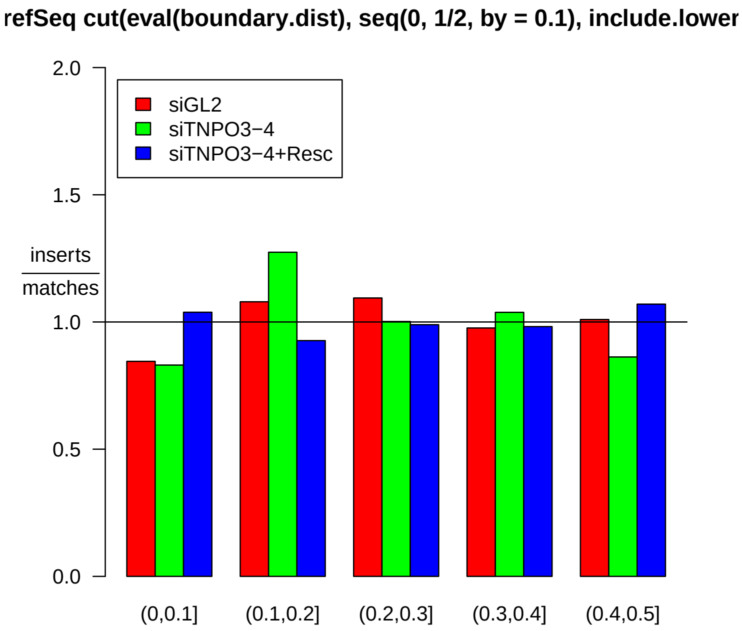
5.2 RefSeq Annotations



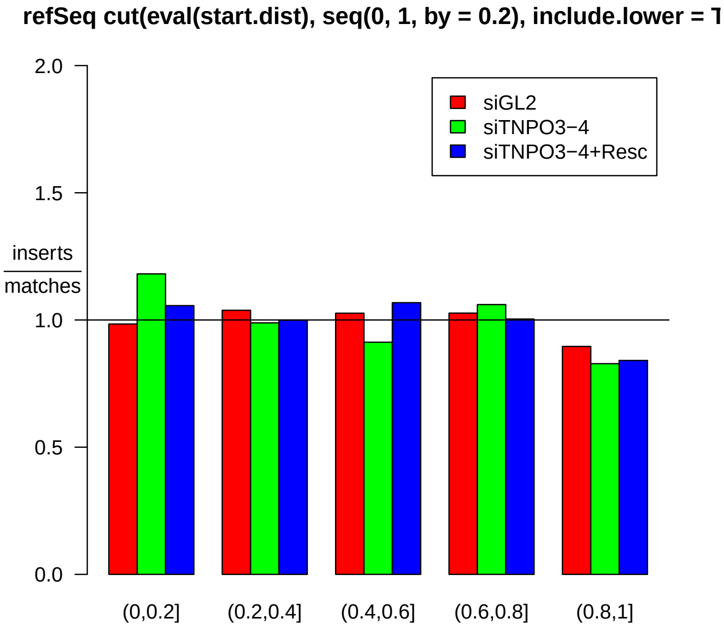
```
    siGL2      siTNPO3-4 siTNPO3-4+Resc
 6.03e-34       2.93e-04       2.93e-26
```



```
   siGL2       siTNPO3-4 siTNPO3-4+Resc
4.33e-15       4.95e-01       6.93e-14
```

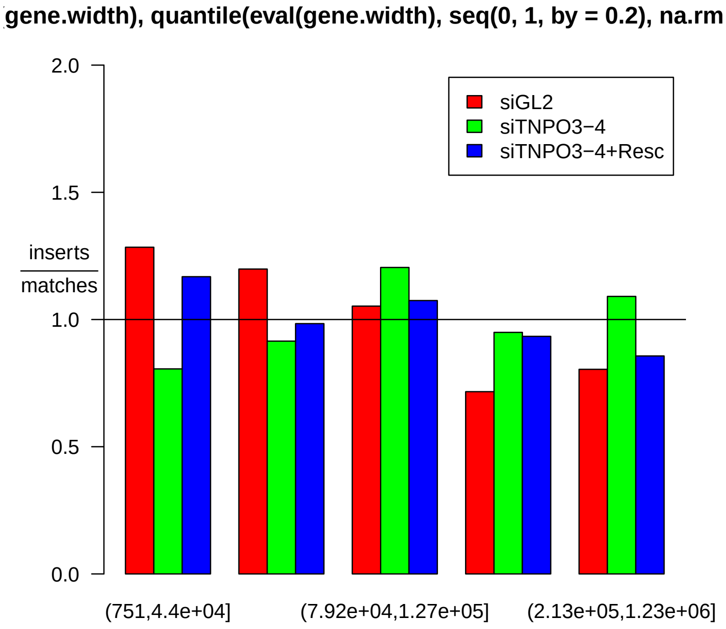


```
 siGL2      siTNPO3-4 siTNPO3-4+Resc
0.0541         0.0971         0.3980
```

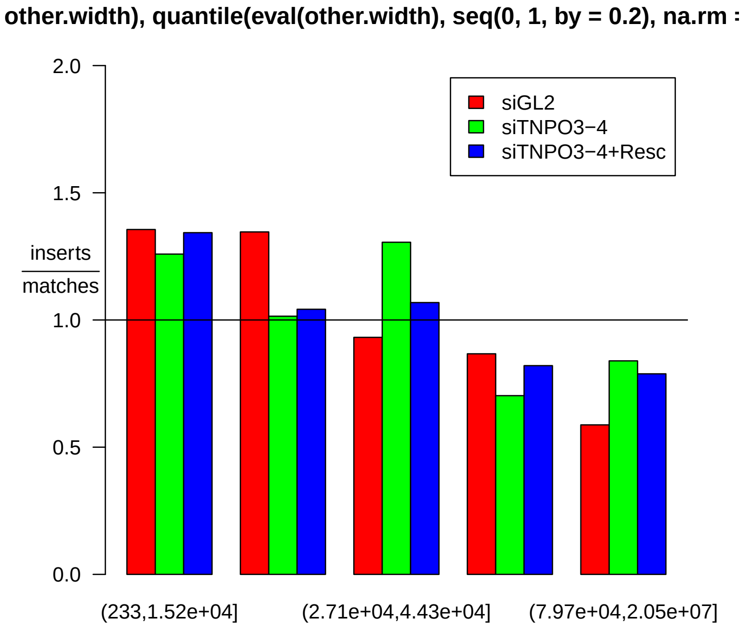


```
 siGL2       siTNPO3-4 siTNPO3-4+Resc
0.7580          0.4380         0.0421
```

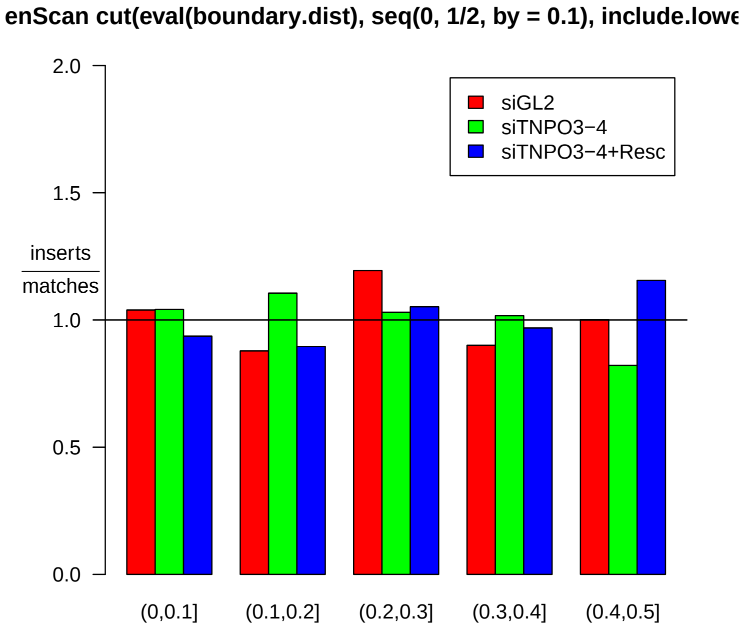
5.3 genScan Annotations



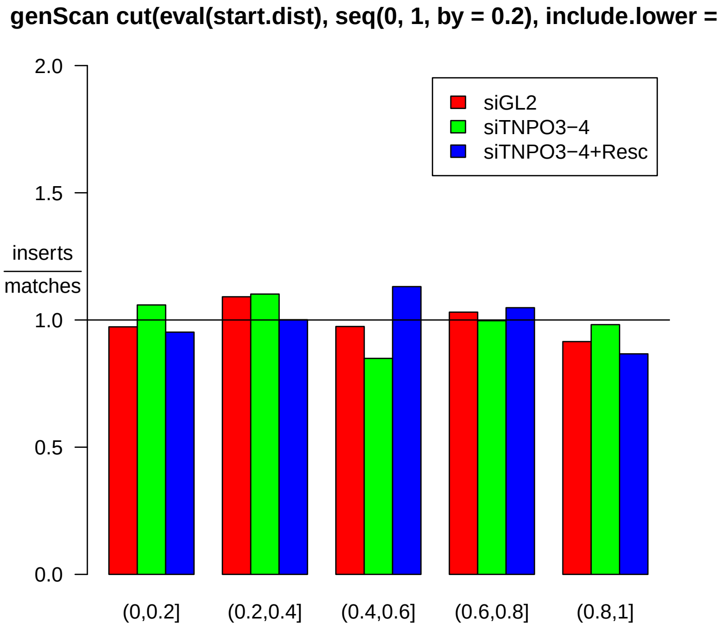
```
    siGL2       siTNPO3-4 siTNPO3-4+Resc
 9.84e-08        1.10e-01       9.99e-02
```



```
     siGL2       siTNPO3-4 siTNPO3-4+Resc
  1.06e-05        9.89e-02       1.54e-01
```

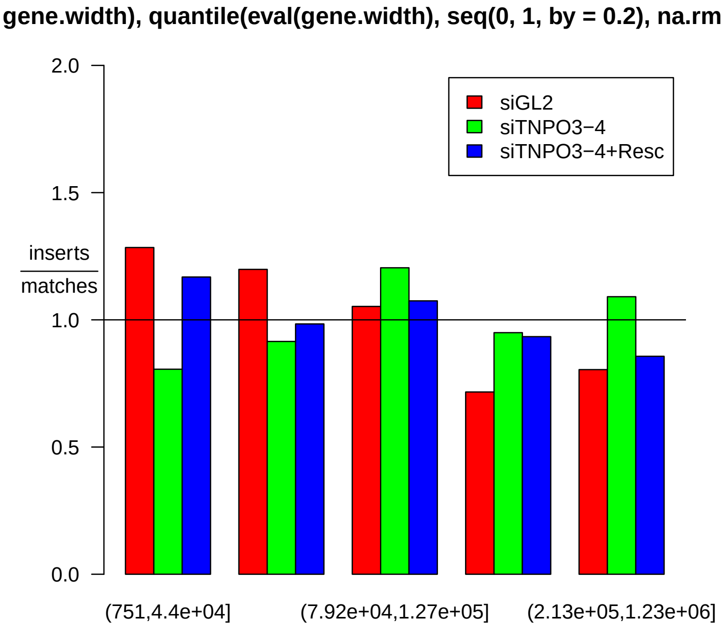


```
  siGL2       siTNPO3-4 siTNPO3-4+Resc
0.00641         0.60000        0.01670
```

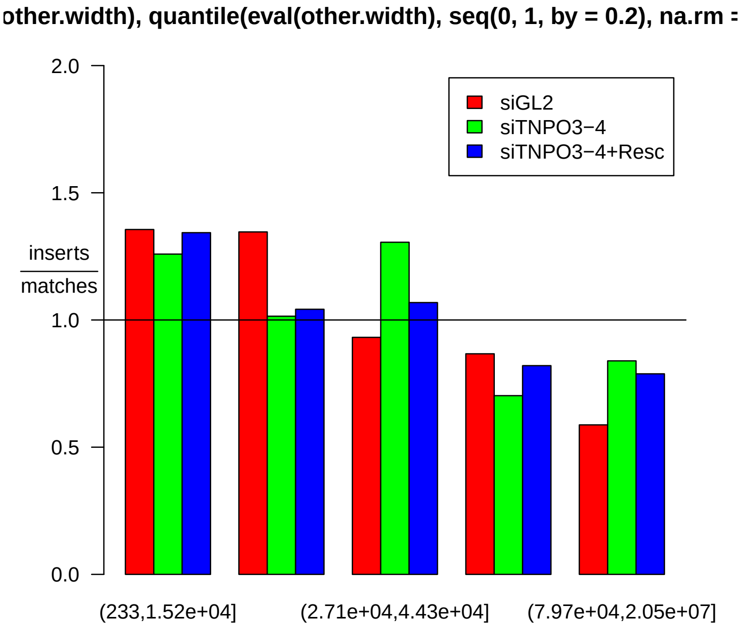


```
siGL2       siTNPO3-4 siTNPO3-4+Resc
0.204           0.818          0.010
```

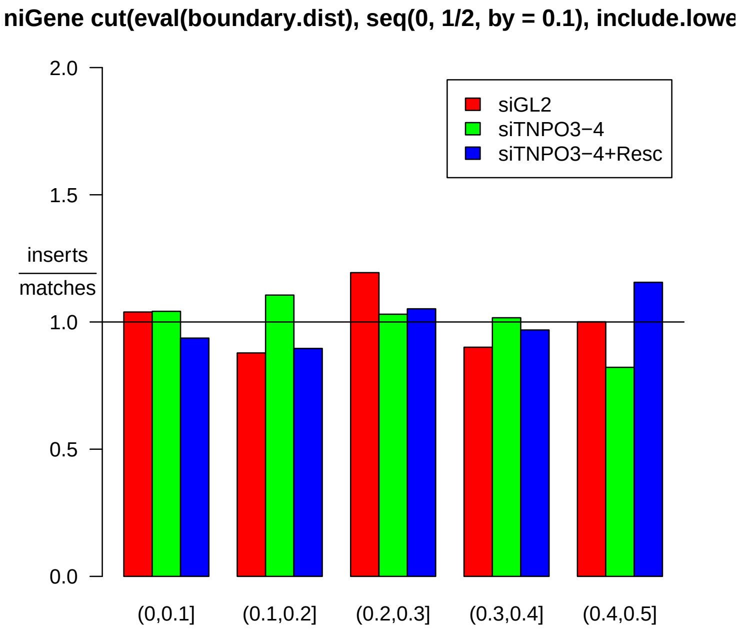
5.4 uniGene Annotations



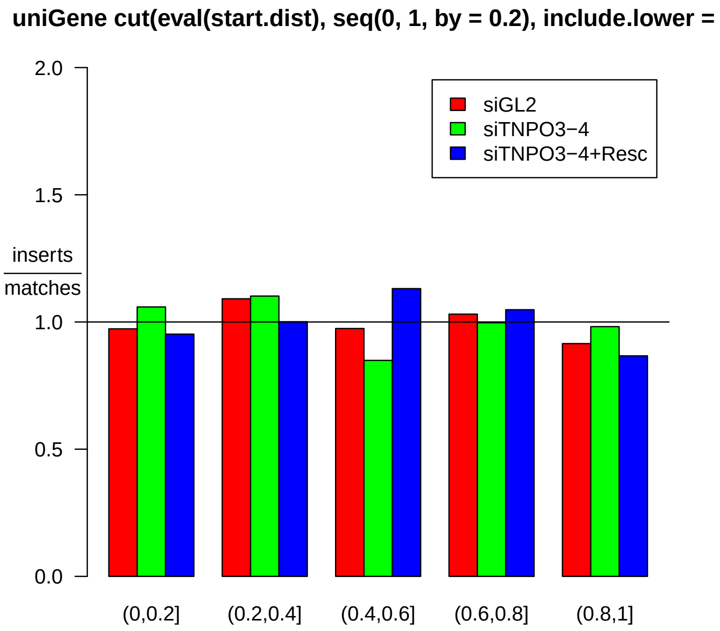
```
    siGL2      siTNPO3-4 siTNPO3-4+Resc
 9.84e-08       1.10e-01       9.99e-02
```



```
   siGL2      siTNPO3-4 siTNPO3-4+Resc
1.06e-05       9.89e-02       1.54e-01
```



```
  siGL2       siTNPO3-4 siTNPO3-4+Resc
0.00641         0.60000        0.01670
```

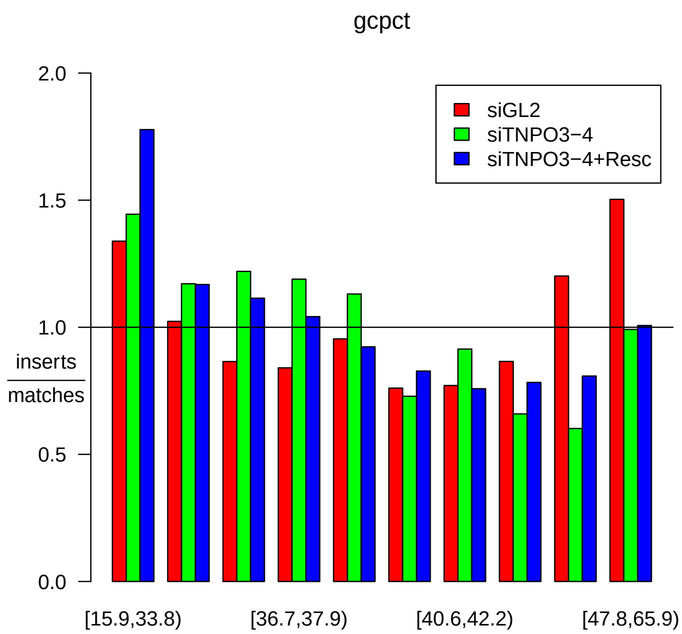


```
siGL2      siTNPO3-4 siTNPO3-4+Resc
0.204          0.818          0.010
```

6 GC content

Here we study the effect of GC content on insertion. The GC content is taken from the Human Genome Draft at GoldenPath from the table `http://genome.ucsc.edu/goldenPath/hg18/database/gc5Base.txt.gz`.

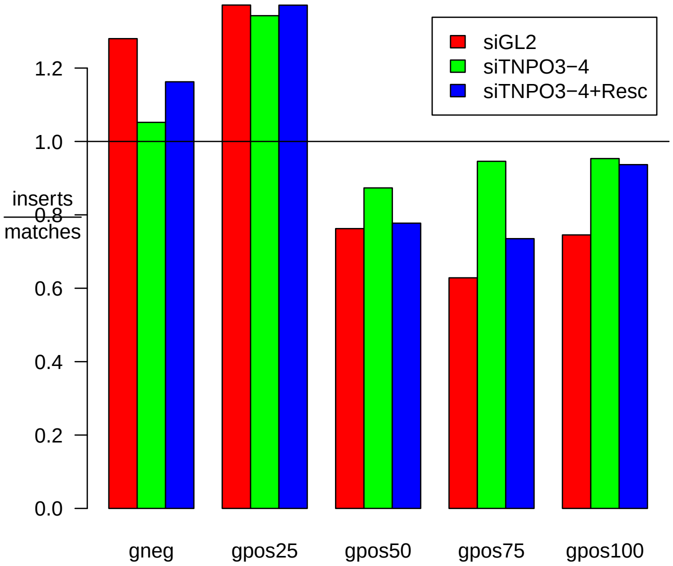
Following the plot is a table of fitted coefficients based on splitting the GC percent data at the median.



```
                   coef     se      z        p
siGL2           -0.0133 0.0707 -0.188 8.51e-01
siTNPO3-4       -0.5040 0.1380 -3.650 2.64e-04
siTNPO3-4+Resc  -0.3880 0.0629 -6.170 6.88e-10
```

7 Cytobands

Here we study the association of cytoband with insertion intensity. The data are obtained from
`http://genome.ucsc.edu/goldenPath/hg18/database/cytoBand.txt.gz`.



A formal test of significance attains a p-value of $< 2.22e - 16$. Here is the table of coefficients of the log ratio of intensities for true insertion sites versus control insertion sites (comparing each category of Giemsa staining to 'gneg') along with their standard errors, z statistics, and p-values:

```
                   coef     se     z        p
cyto.typeacen        NA 0.0000    NA       NA
cyto.typegpos100 -0.323 0.0612 -5.27 1.35e-07
cyto.typegpos25   0.124 0.0839  1.48 1.39e-01
cyto.typegpos50  -0.438 0.0708 -6.18 6.47e-10
cyto.typegpos75  -0.509 0.0703 -7.24 4.43e-13
cyto.typegvar        NA 0.0000    NA       NA
```

References

[1] Yvonne M.M. Bishop, Stephen E. Fienberg, and Paul W. Holland. *Discrete multivariate analyses: Theory and practice* (MIT Press, 1975).

[2] P. McCullagh and John A. Nelder. *Generalized linear models.* (Chapman & Hall ltd, 1999).

[3] Xiaolin Wu, Yuan Li, Bruce Crise, Shawn M. Burgess "Transcription Start Regions in the Human Genome Are Favored Targets for MLV Integration," *Science,* **300**(5626), (June 2003): 1749-1751.