Fast and efficient *Drosophila melanogaster* gene knock-ins using MiMIC transposons

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Protocol S1: Bioinformatics protocol to generate Figure 1.

1 Analysis of all genes within 70k of a MiMIC

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This document is created with iPython (http://ipython.org/) and makes use of the Python Pandas (http://pandas.pydata.org/) and Matplotlib (http://matplotlib.org/) libraries.

1.1 Approach

The analysis starts with a list of MiMIC elements and a list of genes. Subsequently, we check how many genes fall within a predefined range around each MiMIC element. We search for two types of overlap: one requires a gene to completely fall within the range; the other requires a gene to overlap with at least one nucleotide.

1.2 Load python libraries

```
In [23]: #Pandas dataframes
         import pandas as pd
         #Graphs
         import matplotlib.pyplot as plt
```

1.3 Download the MiMIC list

The following list was downloaded: http://flypush.imgen.bcm.tmc.edu/pscreen/files/MI-list-2014-06-13.xls and converted this (using openoffice) to tsv. A minor adaptation of the column names was applied for ease of processing.

This list has 6507 entries.

```
In [9]: #milist = pd.read_csv("MiList.csv", sep="₩t")
        milist = pd.read_csv("MI-list-2014-06-13.csv", sep="₩t")

        # clean up column names
        milist.columns = [x.lower().strip() for x in milist.columns]

        # extract location into a separate chromosome and position field
        splitup = milist['location'].str.split(':')
        milist['chrom'] = splitup.str[0]
```

Extract position information from the list of MiMIC elements:

```
In [10]: def get_pos(x):
             s = x.split('[')
             p = s[0].strip()
             o = s[1].replace(']', '').strip()
```

```
            try:
                return int(p), o
            except:
                return -1, o

        _pos, _ori = zip(*splitup.str[1].map(get_pos))
        milist['pos'] = _pos
        milist['orient'] = _ori

        print "Removing %d entries with unspecified positions" % len(milist[milist['pos'] == -1])
        milist = milist[milist['pos'] != -1]
        print "Kept %d records" % len(milist[milist['pos'] != -1])
```

```
Removing 139 entries with unspecified positions
Kept 6367 records
```

```
In [11]: # making a few shortcuts for later
         mchrom = milist['chrom'] #shortcut for later
         mpos = milist['pos'] #and another one

         # create two emtpy fields to contain the genes and CDSs
         # within 70k of each element
         milist['gene_in70k'] = [set() for i in range(len(milist))]
         milist['cds_in70k'] = [set() for i in range(len(milist))]
```

1.4 Load gene annotations

The gene annotation is available as a big GFF file from Flybase. Used version 5.50.

The file was downloaded using:

```
In [45]: !wget ftp://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r5.57_FB2014_03/gff/dmel-all-

--2014-06-17 10:33:21--  ftp://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r5.57_FB2014_03/gff/
           => ‘dmel-all-r5.57.gff.gz’
Resolving ftp.flybase.net... 149.165.226.140
Connecting to ftp.flybase.net|149.165.226.140|:21... connected.
Logging in as anonymous ... Logged in!
==> SYST ... done.    ==> PWD ... done.
==> TYPE I ... done.  ==> CWD (1) /genomes/Drosophila_melanogaster/dmel_r5.57_FB2014_03/gff ... done.
==> SIZE dmel-all-r5.57.gff.gz ... 377738814
==> PASV ... done.    ==> RETR dmel-all-r5.57.gff.gz ... done.
Length: 377738814 (360M) (unauthoritative)

100%[======================================>] 377,738,814  3.06MB/s   in 2m 1s

2014-06-17 10:35:24 (2.98 MB/s) - ‘dmel-all-r5.57.gff.gz’ saved [377738814]
```

Subsequently unzipped the file and extracted all genes using the following command - extracting only the GFF records which have the word “gene” inbetween two spaces

```
In [46]: !gunzip dmel-all-r5.57.gff.gz
         !egrep "₩tgene₩t" dmel-all-r5.57.gff > dmel.57.gene.gff
```

To remove all miRNA and snoRNA records (assuming we are only interested in protein coding genes):

```
In [15]: !grep -v 'mir-' dmel.57.gene.gff | grep -v 'snoRNA' ₩
             > dmel.57.gene.norg.gff
         !wc -l dmel.57.gene.norg.gff
```

```
16771 dmel.57.gene.norg.gff
```

Similarly - to extract all coding (parts of) exons:
Now loading these files into memory.

```
In [12]: #loading the gff with protein coding genes
         gff_cols = ['chr', 'src', 'type', 'start', 'stop',
                     'score', 'strand', 'frame', 'attr']

         gff_gene = pd.read_csv("dmel.57.gene.norg.gff", sep="₩t", names=gff_cols)
         #convert start & stop to integers (not certain if this is necessary)
         gff_gene['start'] = gff_gene['start'].map(lambda x: int(x))
         gff_gene['stop'] = gff_gene['stop'].map(lambda x: int(x))

         #extract the gene name from the attribute list
         gff_gene['gene'] = gff_gene['attr'].map(lambda x:x.split(';')[1].split('=')[1])

         #how many genes do we have?
         print len(gff_gene)
```

```
16771
```

1.5 Map genes to MiMIC elements

A function (`registerDist`) is defined to assess and register for each gene which MiMIC element is nearby.

```
In [13]: def registerDist(distance, gene, gchrom, gstart,
                                   gstop, column, touch_or_include="touch"):
             gstart, gstop = sorted([gstart, gstop])
             # 'touch' means - 70k touches an element
             if touch_or_include == "touch":
                 # the element is on the same chromosome
                 # AND MIS + distance is bigger than gene/cds start
                 # AND MIS - distance is smaller than gene/cds stop
                 milist[ (mchrom == gchrom) &
                         (mpos >= gstart - distance) &
                         (mpos <= gstop + distance)
                        ][column].map(lambda x:x.add(gene))
             else:
             # 'within' means gene is fully within 70k
                 milist[ (mchrom == gchrom) &
                         (mpos >= gstop - distance) &
                         (mpos <= gstart + distance)
                        ][column].map(lambda x:x.add(gene))
```

```
In [14]: # Map genes to the MiMic elements with differing window sizes
         for distance in [0, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90, 100]:
             # create two emtpy fields to contain the genes that either
             # touch the 'distance' window, and are completely within.
             milist['gene_touch_%dk' % distance] = ₩
                 [ set() for i in range(len(milist))]
             milist['gene_within_%dk' % distance] = ₩
```

```
            [set() for i in range(len(milist))]

        # Map gene/cds to mitos elements within/touch
        gff_gene.apply(lambda x: registerDist(
                            distance * 1000,
                            x.name, x['chr'],
                            x['start'], x['stop'],
                            'gene_touch_%dk' % distance,
                            "touch"),
                       axis=1)
        gff_gene.apply(lambda x: registerDist(
                            distance * 1000,
                            x.name, x['chr'],
                            x['start'], x['stop'],
                            'gene_within_%dk' % distance,
                            "within"),
                       axis=1)
```

In [16]:
```
# Count how many genes are in the neighbourhood of each
# MiMIC element
for x in [0, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90, 100]:
    milist['gene_count_within_%dk' % x] = ₩
        milist['gene_within_%dk' % x].map(lambda x: len(x))
    milist['gene_count_touch_%dk' % x] = ₩
        milist['gene_touch_%dk' % x].map(lambda x: len(x))
```

1.5.1 Export to disk

In [17]:
```
milist.to_csv('MiMIC_to_gene.tsv', sep="₩t", index=False)
```

1.5.2 Summarize results

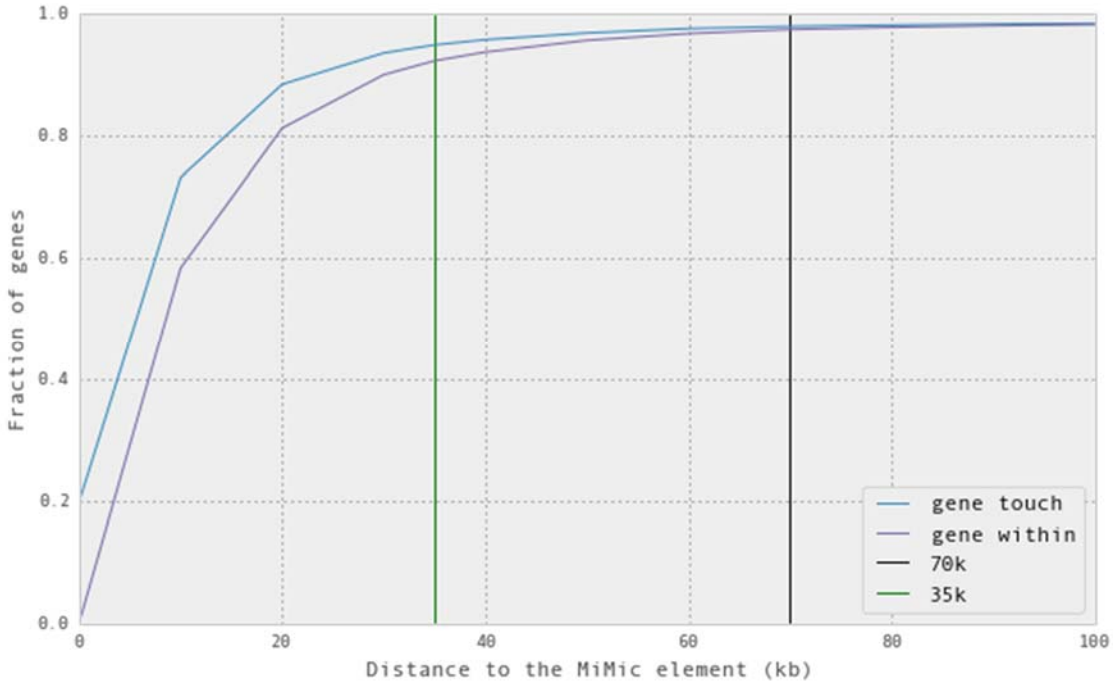
In [18]:
```
results = pd.DataFrame(
    index=[0, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90, 100])
results['gene_within'] = [0] * 12
results['gene_touch'] = [0] * 12
```

In [19]:
```
#calculate numbers of unique genes per category
def get_unique_sum(cat):
    allgenes = set()
    milist[cat].map(lambda x: allgenes.update(x))
    return len(allgenes)

for x in [0, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90, 100]:
    for c2 in ['within', 'touch']:
        results['gene_%s' % (c2)][x] = get_unique_sum('gene_%s_%dk' % (c2, x))
        results['gene_%s_frac' % (c2)] = results['gene_%s' % c2] / float(len(gff_gene))

print results
```

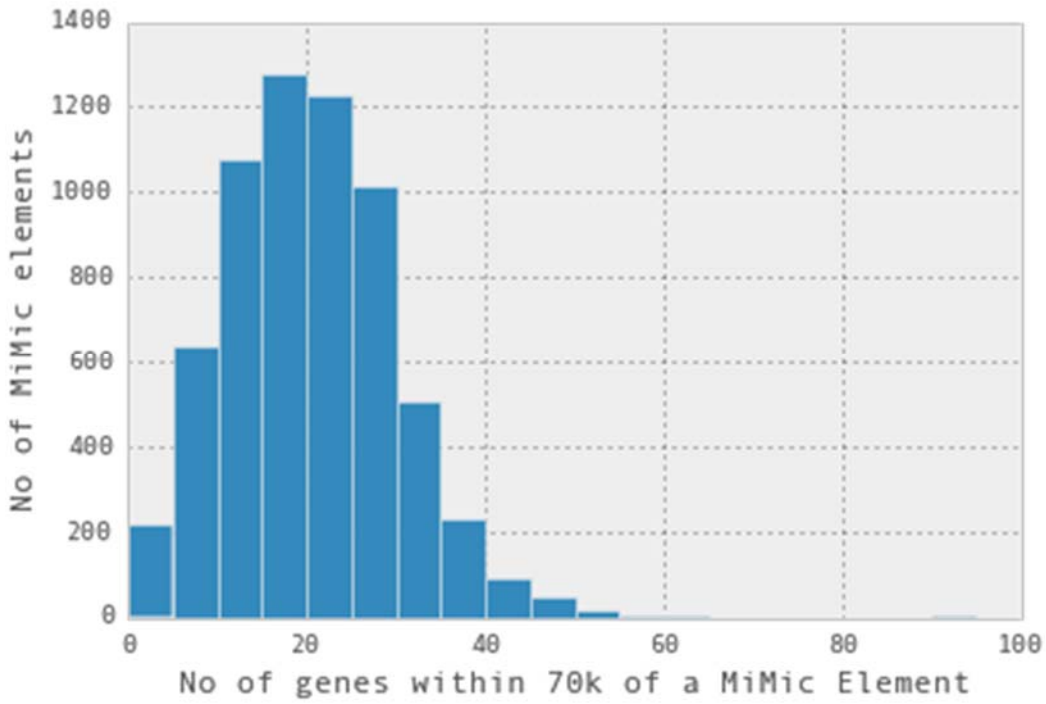
	gene_within	gene_touch	gene_within_frac	gene_touch_frac
0	0	3316	0.000000	0.197722
10	9784	12270	0.583388	0.731620
20	13618	14824	0.811997	0.883907
30	15088	15686	0.899648	0.935305

35	15471	15908	0.922485	0.948542
40	15710	16051	0.936736	0.957069
50	16033	16236	0.955995	0.968100
60	16220	16357	0.967146	0.975315
70	16333	16417	0.973883	0.978892
80	16391	16453	0.977342	0.981039
90	16437	16482	0.980085	0.982768
100	16470	16500	0.982052	0.983841

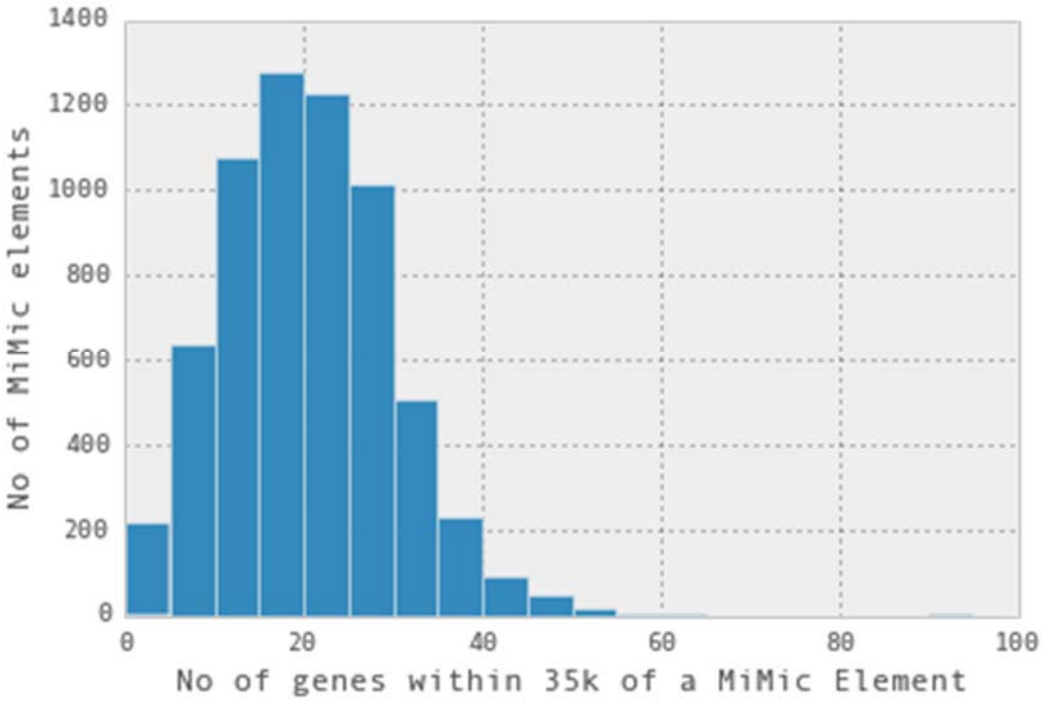
In [31]:
```
%matplotlib inline
plt.figure(figsize=(10,6))
plt.plot(results.index, results['gene_touch_frac'], label="gene touch")
plt.plot(results.index, results['gene_within_frac'], label="gene within")
plt.xlabel("Distance to the MiMic element (kb)")
plt.ylabel("Fraction of genes")
plt.vlines(70, 0, 1, label="70k")
plt.vlines(35, 0, 1, "green", label="35k")

plt.legend(loc=4)
plt.savefig("MiMic.take2.genes.vs.distance.png", dpi=300)
```



In [29]:
```
milist['genecount_within_70k'] = milist['gene_within_70k'].map(len)
x = milist['genecount_within_70k'].hist(bins=range(0,100,5))
x.set_xlabel("No of genes within 70k of a MiMic Element")
x.set_ylabel("No of MiMic elements")
x.set_xlim(0, 100)
plt.savefig("MiMICDistributionGene.70k.png", dpi=300)
```




```
In [30]: milist['genecount_within_35k'] = milist['gene_within_70k'].map(len)
         x = milist['genecount_within_35k'].hist(bins=range(0,100,5))
         x.set_xlabel("No  of genes within 35k of a MiMic Element")
         x.set_ylabel("No  of MiMic elements")
         x.set_xlim(0,  100)
         plt.savefig("MiMicDistributionGene.35k.png", dpi=300)
```


1400
1200
1000
800
600
400
200
0
No of MiMic elements
0
20
40
60
80
100
No of genes within 35k of a MiMic Element