Exploring Protein-DNA Binding Sites from Paired-End ChIP-Seq Data

This demonstration performs a genome-wide analysis of a transcription factor in the *Arabidopsis Thaliana* (Thale Cress) model organism.

Contents

For enhanced performance, it is recommended that you run this demo on a 64-bit platform, because the memory footprint is close to 2 Gb. On a 32-bit platform, if you receive "`Out of memory`" errors when running this demo, try increasing the virtual memory (or swap space) of your operating system or try setting the `3GB` switch (32-bit Windows® XP only). These techniques are described in this document.

Introduction

ChIP-Seq is a technology that is used to identify transcription factors that interact with specific DNA sites. First chromatin immunoprecipitation enriches DNA-protein complexes using an antibody that binds to a particular protein of interest. Then, all the resulting fragments are processed using high-throughput sequencing. Sequencing fragments are mapped back to the reference genome. By inspecting over-represented regions it is possible to mark the genomic location of DNA-protein interactions.

In this demonstration, short reads are produced by the paired-end Illumina® platform. Each fragment is reconstructed from two short reads successfully mapped, with this the exact length of the fragment can be computed. Using paired-end information from sequence reads maximizes the accuracy of predicting DNA-protein binding sites.

Data Set

This demo explores the paired-end ChIP-Seq data generated by Wang *et.al.* [1] using the Illumina® platform. The data set has been courteously submitted to the Gene Expression Omnibus repository with accession number GSM424618. The unmapped paired-end reads can be obtained from the NCBI FTP site.

This demonstration assumes that you:

(1) downloaded and uncompressed the unmapped paired-end reads (`SRR054715_1.fasta.bz2` and `SRR054715_2.fasta.bz2`),

(2) produced a SAM formatted file by mapping the short reads to the Thale Cress reference genome, using a mapper such as BWA [2], Bowtie, or SSAHA2 (which is the mapper used by authors of [1]), and,

(3) ordered the SAM formatted file by reference name first, then by genomic position.

For the published version of this demo, 8,653,488 paired-end short reads are mapped using the BWA mapper [2]. BWA produced a SAM formatted file (`aratha.sam`) with 17,306,976 records (8,653,488 x 2). Repetitive hits were randomly chosen, and only one hit is reported, but with lower mapping quality. The SAM file was ordered using SAMtools [3] before being loaded into MATLAB.

The last part of the demo also assumes that you downloaded the reference genome for the Thale Cress model organism (which includes five chromosomes). Uncomment the following lines of code to download the reference from the NCBI repository:

```
% getgenbank('NC_003070','FileFormat','fasta','tofile','ach1.fasta');
% getgenbank('NC_003071','FileFormat','fasta','tofile','ach2.fasta');
% getgenbank('NC_003074','FileFormat','fasta','tofile','ach3.fasta');
% getgenbank('NC_003075','FileFormat','fasta','tofile','ach4.fasta');
% getgenbank('NC_003076','FileFormat','fasta','tofile','ach5.fasta');
```

Indexing the SAM File and Creating a MATLAB® Interface to Access it

Use `BioIndexedFile` to index the SAM formatted file. This allows quick and efficient access to the short reads from MATLAB without having to load the whole content of the file into memory. Display a small number of the short reads. Observe that each short read is mapped to one of the five reference chromosomes or it appears as unmapped (*).

```
bif = BioIndexedFile('sam',which('aratha.sam'),'indexedbykeys',false)
i = 1:1000000:17000000;  % some random sequences
disp(getEntryByIndex(bif,i))
```

```
Source File: aratha.sam
   Path: C:\demos\chipsepedemo
   Size: 3.463918e+009 bytes
   Date: 20-Oct-2010 07:24:59
Index file exists.
Index File: aratha.sam.idx
   Path: C:\demos\chipsepedemo
   Size: 655444776 bytes
   Date: 20-Oct-2010 09:20:13
Mapping object to aratha.sam.idx ...
Done.

bif =
 BioIndexedFile

 Properties:
           FileFormat: 'sam'
            InputFile: 'C:\demos\chipsepedemo\aratha.sam'
            IndexFile: 'C:\demos\chipsepedemo\aratha.sam.idx'
           NumEntries: 17306976
        IndexedByKeys: 0
    MemoryMappedIndex: 1
          Interpreter: @samread


SRR054715.3174659       163     Chr1    1       18      8S32M   =       205
        244     ACACTAAACCCTAAACCCTAAACCCTAAACCCTAAACCTC        BBBBB;-;BBBC?-
BBBBB=B?BBBB<8BBBBB0@,69CB      XT:A:M  NM:i:0  SM:i:18 AM:i:18 XM:i:0  XO:i:0
        XG:i:0  MD:Z:32
SRR054715.5925556       147     Chr1    9555568 60      40M     =       9555377 -
231     TTCACCTCATACAAAAATGTCATGATTTGAATACAATTAA        B?BBBBBBBBBB;BBBBB-
;BBBBB;?61;CBCBBCBBBC   XT:A:U  NM:i:0  SM:i:37 AM:i:37 X0:i:1  X1:i:0  XM:i:0
        XO:i:0  XG:i:0  MD:Z:40
SRR054715.7954729       83      Chr1    18823581        60      40M     =
        18823372        -249    AGTTCCTAACGTAACAGTTAATCTCTTTAACACATTTCTC
        6'6?+<7<?7<BBBBBBBBCBB?B=BBBBCBBBBBBBBBB        XT:A:U  NM:i:0  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:40
SRR054715.3770683       163     Chr1    29012143        60      40M     =
        29012357        254     GCAACGTGAAAAGATCATGTGAGATATTTTAGGGATTACA
        B<,30==BB8<9B8BBB;B=BC=.==BB6,?B;BB-B-;-        XT:A:U  NM:i:2  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:2  XO:i:0  XG:i:0  MD:Z:37T1T0
SRR054715.2025934       147     Chr2    3367512 60      40M     =       3367293 -
259     GATCACTCTTCCACTCTTGCACTTGCCTTCATTCGGTTCT        ;B?CB-
CBBB?9,0;BBBB8B9448B8B=CBBBBBB@2=B      XT:A:U  NM:i:2  SM:i:37 AM:i:37 X0:i:1
        X1:i:0  XM:i:2  XO:i:0  XG:i:0  MD:Z:5A20A13
SRR054715.3782337       147     Chr2    7449564 60      40M     =       7449359 -
245     ACCGCATAAGCAATGTCGGGTCTAGTTATCGTCAAATATA
        BB=@4A4AB@8+BB?=2@BB60<BBB@>@??<?<BBBBBB        XT:A:U  NM:i:0  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:40
SRR054715.8370121       147     Chr2    17510273        60      40M     =
        17510062        -251    GCAAAGGAGAAGGAAGCATGTGATGAGCAACATGATAAAA
        B*29*?;8BBBB;BC@''.=+=BBB-=67+BBCBBB4+@.        XT:A:U  NM:i:2  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:2  XO:i:0  XG:i:0  MD:Z:4T21A13
SRR054715.1558535       163     Chr3    7144734 60      40M     =       7144936
        242     TCCTATTACATGATGAATTCCCCATGAAAAATGGTCTAGT
        BBCBBCBBBB?=BB?BBBBBCBCCCBBBBB8???=BBB--        XT:A:U  NM:i:0  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:40
SRR054715.6448030       99      Chr3    14193252        0       40M     =
        14193488        276     CCGCAACGCTCGCAAAGGTGGATAGTGAGAATAATAAGGG
        BBBB?BBBBBBCBB6BBB?BBBB=B99BBBBBB=BBC3'.        XT:A:R  NM:i:2  SM:i:0
        AM:i:0  X0:i:2  X1:i:1  XM:i:2  XO:i:0  XG:i:0  MD:Z:20C17T1
        XA:Z:Chr3,+14193252,40M,2;Chr2,+9690,40M,3;
SRR054715.2685734       147     Chr3    20725298        60      40M     =
        20725096        -242    GGTGTAAGAGACTTGAGAGTTGACCAATCTGCGAACATCA
        ;'9BBBBB?B>4@>@BBCBBBBBBBBBB?6@9@CCBCBCC        XT:A:U  NM:i:0  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:0  XO:i:0  XG:i:0  MD:Z:40
SRR054715.4718782       163     Chr4    3998835 0       40M     =       3999038
        243     TACACATAAAATCAAGTCATATTCGACTCCAAAACACTAA
        BCBCBCCBBBB?CCBBCCBBBCBBBCBBBBBBCBB?C8BB        XT:A:R  NM:i:2  SM:i:0
        AM:i:0  X0:i:3  X1:i:248        XM:i:2  XO:i:0  XG:i:0  MD:Z:7G1C30
SRR054715.2395180       99      Chr4    13639101        60      40M     =
        13639300        239     TGATGTTAACAAAAGATTGGTGGATGTACAATACTTGAGT
        BBBB@99,66>?2?<<,'9BBBBBB4,,6?4<''3B<0'3        XT:A:U  NM:i:2  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:2  XO:i:0  XG:i:0  MD:Z:17C20A1
SRR054715.1661718       147     Chr5    4622109 60      40M     =       4621897 -
252     ACATCTAATATCAATCTCTTTGCTTATAAATATATTTTTT        ;139?C-BBBBBB-
BB8@ABBB?@49?,2+=BBBBBBBBB      XT:A:U  NM:i:1  SM:i:37 AM:i:37 X0:i:1  X1:i:0
        XM:i:1  XO:i:0  XG:i:0  MD:Z:16A23
SRR054715.6278779       147     Chr5    11732526        15      40M     =
        11732321        -245    ACGATCTTATAAGCCTAAGTAGTGTTTCCTTGTTAGAAGA
        BBB9990<6BBBBBBB=BBBBBBB8@4>B?A9@BCBB;B8        XT:A:U  NM:i:0  SM:i:15
        AM:i:15 X0:i:1  X1:i:14 XM:i:0  XO:i:0  XG:i:0  MD:Z:40
SRR054715.3699144       147     Chr5    20778416        60      40M     =
        20778189        -267    TTTATACAACAAACCGTCTTAAGAAACCATATATTAAAAC
        ?B-B1'0-+8<=6--;?=<6.BB0=BB;6=BBBBBBBC-B        XT:A:U  NM:i:2  SM:i:37
        AM:i:37 X0:i:1  X1:i:0  XM:i:2  XO:i:0  XG:i:0  MD:Z:2C2C34
SRR054715.1186676       77      *       0       0       *       *       0       0       ACCAGC
SRR054715.4370803       77      *       0       0       *       *       0       0       TGTAAT
```

Use the method `getDictionary` to obtain a list of all the reference names present in the source file of `BioIndexedFile`.

```
getDictionary(bif)
```

```
ans = 

    'Chr1'
    'Chr2'
    'Chr3'
    'Chr4'
    'Chr5'
```

To create local alignments and look at the coverage we need to construct a `BioMap`. `BioMap` can use the `BioIndexedFile` as the interface to the actual data, thus minimizing the amount of data that is actually loaded to the workspace. The remainder of this demonstration focuses on the analysis of one of the five chromosomes, `Chr1`. Create a `BioMap` to access the short reads mapped to the first chromosome.

```
tic
bm1 = BioMap(bif,'SubsetReference','Chr1');
toc
```

```
Elapsed time is 4.810797 seconds.
```

Further inspection of `BioMap` can indicate the range of the mapped short reads and how many short reads are mapped to the first chromosome.

```
x1 = min(getStart(bm1));
x2 = max(getStop(bm1));
fprintf('Chromosome 1 Range: %d-%d\n',x1,x2)
fprintf('Chromosome 1 Number of Reads: %d\n',bm1.NSeqs)
```

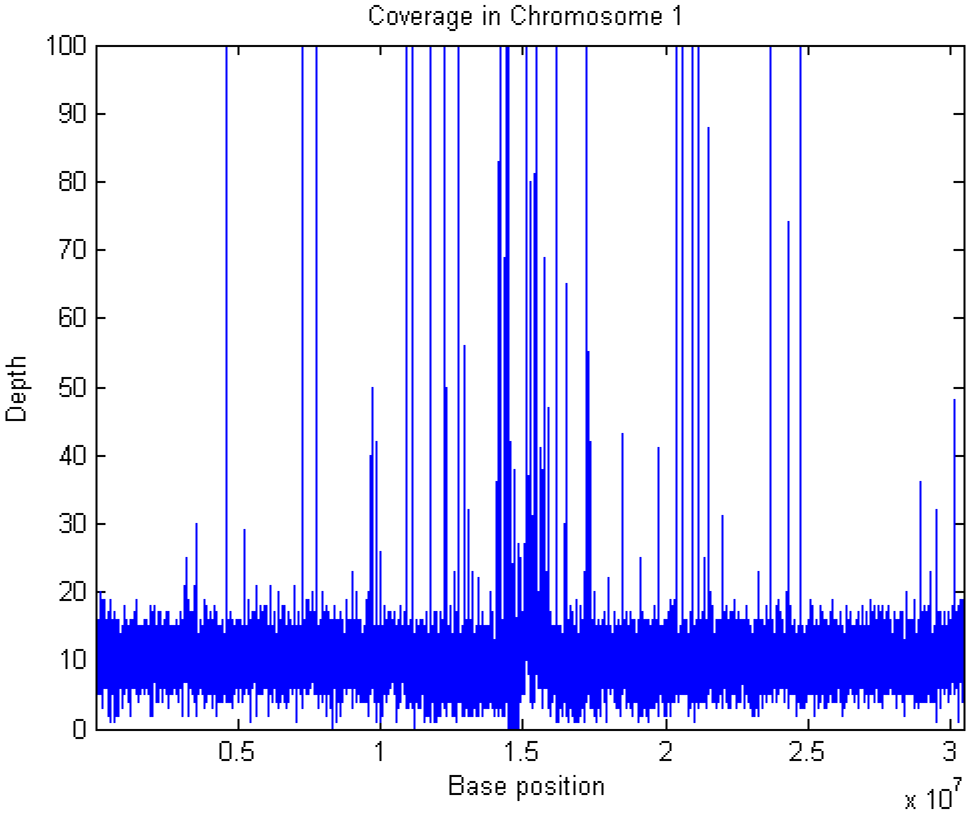
```
Chromosome 1 Range: 1-30427671
Chromosome 1 Number of Reads: 3151047
```

Exploring the Coverage at Different Resolutions

To explore the coverage for the whole range of the chromosome, a binning algorithm is required. The `getBaseCoverage` method produces a coverage signal based on effective alignments. It also allows you to specify a bin width to control the size (or resolution) of the output signal. However internal computations are still performed at the base pair (bp) resolution. This means that despite setting a large bin size, narrow peaks in the coverage signal can still be observed. Once the coverage signal is plotted you can program the figure's data cursor to display the genomic position when using the tooltip. You can zoom and pan the figure to determine the position and height of the ChIP-Seq peaks.

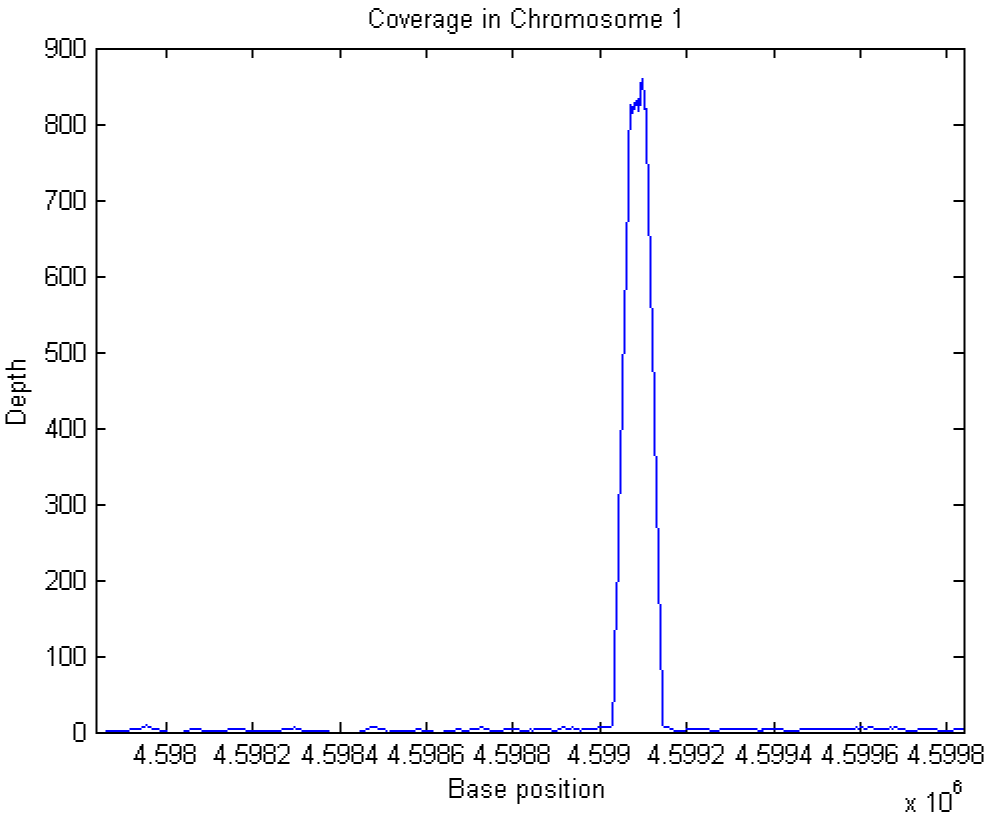
```
[cov,bin] = getBaseCoverage(bm1,x1,x2,'binWidth',1000,'binType','max');
figure
plot(bin,cov)
xlim([x1,x2])
ylim([0 100])
xlabel('Base position')
ylabel('Depth')
title('Coverage in Chromosome 1')

mdc = @(h,e)  {['Position: ',num2str(get(e,'Position')*[1;0])], ['Y:
',num2str(get(e,'Position')*[0;1])]}
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
```

```
mdc =

    @(h,e){['Position: ',num2str(get(e,'Position')*[1;0])],['Y:
',num2str(get(e,'Position')*[0;1])]}
```



It is also possible to explore the coverage signal at the bp resolution (also referred to as the *pile-up* profile). Explore one of the large peaks observed in the data at position 4598837.

```
p1 = 4598837-1000;
p2 = 4598837+1000;

figure
plot(p1:p2,getBaseCoverage(bm1,p1,p2))
xlim([p1,p2])
xlabel('Base position')
ylabel('Depth')
title('Coverage in Chromosome 1')
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
```



Identifying and Filtering Regions with Artifacts

Observe the large peak with coverage depth of 800+ between positions 4599029 and 4599145. Investigate how these reads are aligning to the reference chromosome. You can retrieve a subset of these reads enough to satisfy a coverage depth of 25, since this is sufficient to understand what is happening in this region. Use `getIndex` to obtain indices to this subset. Then use `getCompactAlignment` to display the corresponding multiple alignment of the short-reads.

```
i = getIndex(bm1,4599029,4599145,'depth',25);
bmx = getSubset(bm1,i,'indexed',false)
getCompactAlignment(bmx,4599029,4599145)
```

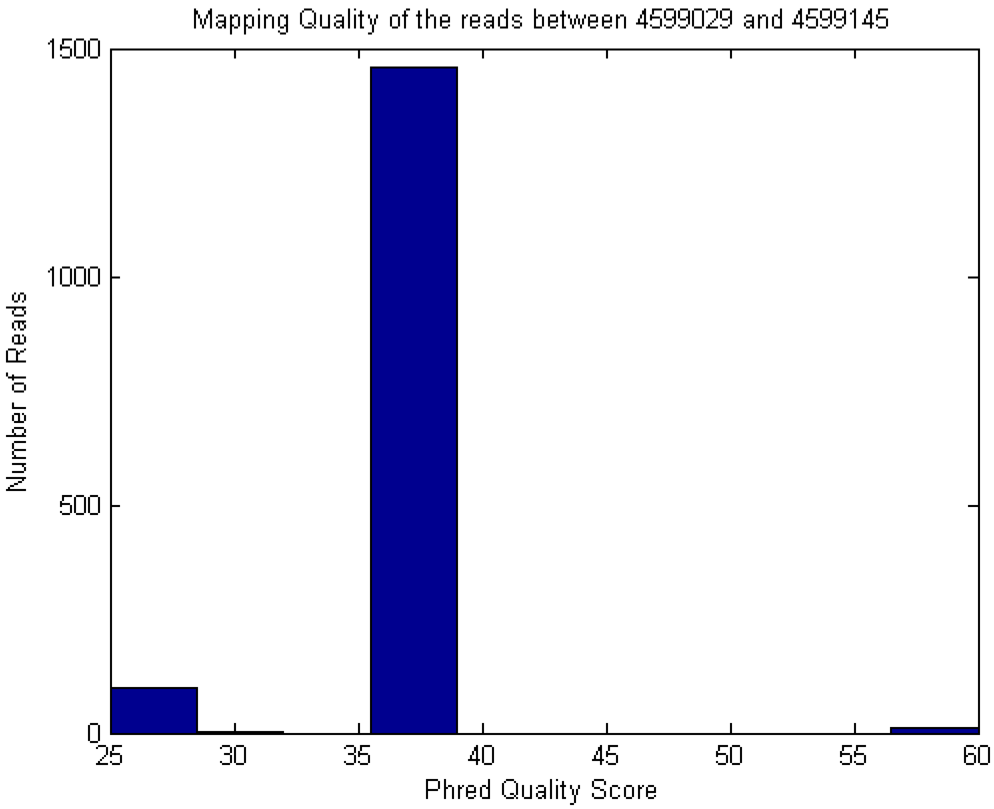
```
bmx = 

  BioMap

  Properties:
         Reference: 'Chr1'
         Signature: {62x1 cell}
             Start: [62x1 uint32]
    MappingQuality: [62x1 uint8]
              Flag: [62x1 uint16]
      MatePosition: [62x1 uint32]
           Quality: {62x1 cell}
          Sequence: {62x1 cell}
            Header: {62x1 cell}
```

```
             NSeqs: 62
              Name: ''



ans =

AGTT AATCAAATAGAAAGCCCCGAGGGCGCCATATCCTAGGCGC
AAACTATGTGATTGAATAAATCCTCCTCTATCTGTTGCGG   GAGGACTCCTTCTCCTTCCCCTTTTGG
AGTGC  TCAAATAGAAAGCCCCGAGGGCGCCATATTCTAGGAGCCC
GAATAAATCCTCCTCTATCTGTTGCGGGTCGAGGACTCCT CTCCTGCCCCTTTTGG
AGTTCAA             CCCGAGGGCGCCATATTCTAGGAGCCCAAACTATGTGATT
TATCTGTTGCGGGTCGAGGACTCCTTCTCCTTCCCCTTCT
AGTTCAATCAAATAGAAAGC               TTCTAGGAGCCCAAACTATGTGATTGAATAAATCCTCCTC
AGGACTCCTTCTCCTTCCCCTTTTGG
AGTT
AAGGAGCCCAAAATATGTGATTGAATAAATCCACCTCTAT
GGACTCCTTCTCCTTCCCCTTTTGG
AGTACAATCAAATAGAAAGCCCCGAGGGCGCCATA
TAGGAGCCCAAACTATGTGATTGAATAAATCCTCCTCTAT
CCTTCACCTTCCCCTTTTGG
CGTACAATCAAATAGAAAGCCCCGAGGGCGCCATATTC
GGAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCT                         TTCCCCTTTTGG
CGTACAATCAAATAGAAAGCCCCGAGGGCGCCATATTC
GGAGCCCAAGCTATGTGATTGAATAAATCCTCCTCTATCT
CGTACAATCAAATAGAAAGCCCCGAGGGCGCCATATTC
GGAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCT
CGTACAATCAAATAGAAAGCCCCGAGGGCGCCATATTC
GGAGCCCAAGCTATGTGATTGAATAAATCCTCCTCTATCT
AGTTCAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTA
GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG
GATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTA
GAGCCCAAACTATGTGATTGAATAAATCCTCATGTATCTG
GATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTA
GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG
GATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTA
GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG
GATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTA
GAGCCCAAATTATGTGATTGAATAAATCCTCCTCTATCTG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
CCCAAACTATGTGATTGAATAAATCCTCCTCTATCTGTTG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
CACAAACTATGTGATTGAATAAATCCTCCTCTATCTGTTG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
CCAAACTATGTGATTGAATAAATCCTCCTCTATCTGTTGC
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
 ATACAATCAAATAGAAAGCCCCGGGGGCGCCATATTCTAG
 ATTGAGTCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
 ATACAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAG
    CAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAGGAG
    CAATCAAATAGAAAGCCCCGAGGGCGCCATATTCTAGGAG

TAGGAGCCCAAACTATGTGATTGAATAAATCCTCCTCTAT
```

```
TAGGAGCCCAAACTATGCCATTGAATAAATCCTCCGCTAT

GGTGCACAAACTATGTGATTGAATAAATCCTCCTCTATCT

GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG

GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG

GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG

GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG

GAGCCCAAACTATGTGATTGAATAAATCCTCCTCTATCTG
```

In addition to visually confirming the alignment, you can also explore the mapping quality for all the short reads in this region, as this may hint to a potential problem. In this case, less than one percent of the short reads have a Phred quality of 60, indicating that the mapper most likely found multiple hits within the reference genome, hence assigning a lower mapping quality.

```
figure
i = getIndex(bm1,4599029,4599145);
hist(double(getMappingQuality(bm1,i)))
title('Mapping Quality of the reads between 4599029 and 4599145')
xlabel('Phred Quality Score')
ylabel('Number of Reads')
```



Most of the large peaks in this data set occur due to satellite repeat regions or due to its closeness to the centromere [4], and show characteristics similar to the example just explored. You may explore other regions with large peaks using the same procedure.

To prevent these problematic regions, two techniques are used. First, given that the provided data set uses paired-end sequencing, by removing the reads that are not aligned in a proper pair reduces the number of potential aligner errors or ambiguities. You can achieve this by exploring the `flag` field of the SAM formatted file, in which the second less significant bit is used to indicate if the short read is mapped in a proper pair.

```
i = find(bitget(getFlag(bm1),2));
bm1_filtered = getSubset(bm1,i)
```

```
bm1_filtered = 

  BioMap

  Properties:
         Reference: 'Chr1'
         Signature: [3040722x1 File indexed property]
             Start: [3040722x1 File indexed property]
    MappingQuality: [3040722x1 File indexed property]
              Flag: [3040722x1 File indexed property]
      MatePosition: [3040722x1 File indexed property]
           Quality: [3040722x1 File indexed property]
          Sequence: [3040722x1 File indexed property]
            Header: [3040722x1 File indexed property]
             NSeqs: 3040722
              Name: ''
```

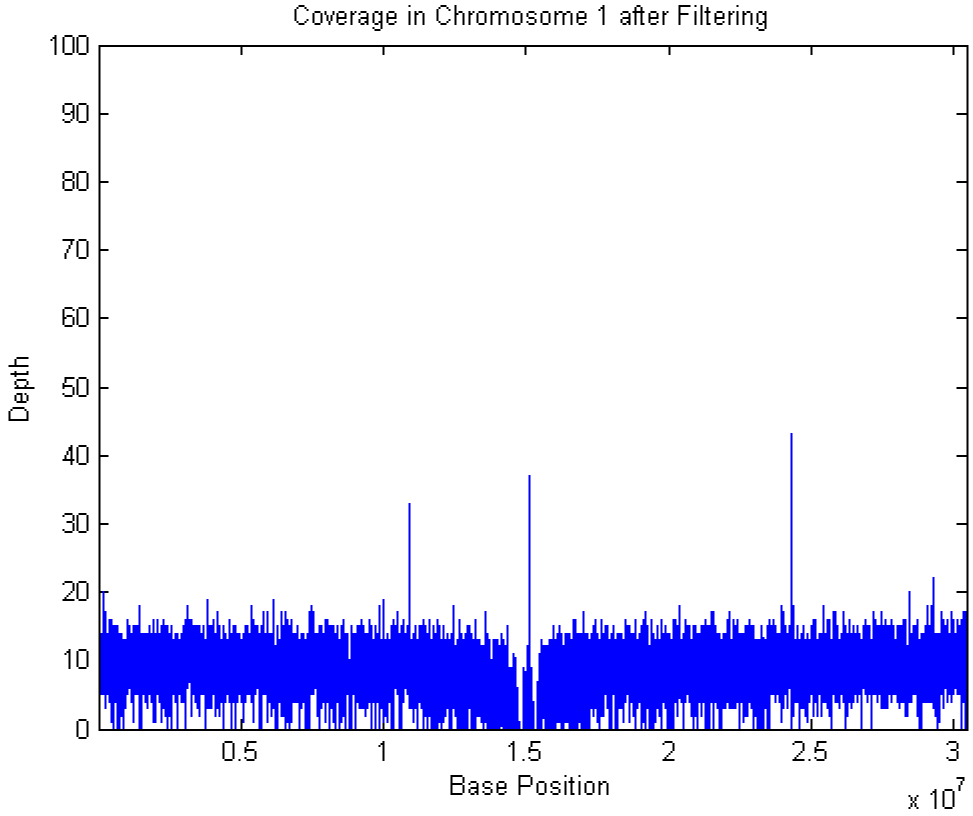
Second, consider only uniquely mapped reads. You can detect reads that are equally mapped to different regions of the reference sequence by looking at the mapping quality, because BWA assigns a lower mapping quality (less than 60) to this type of short read.

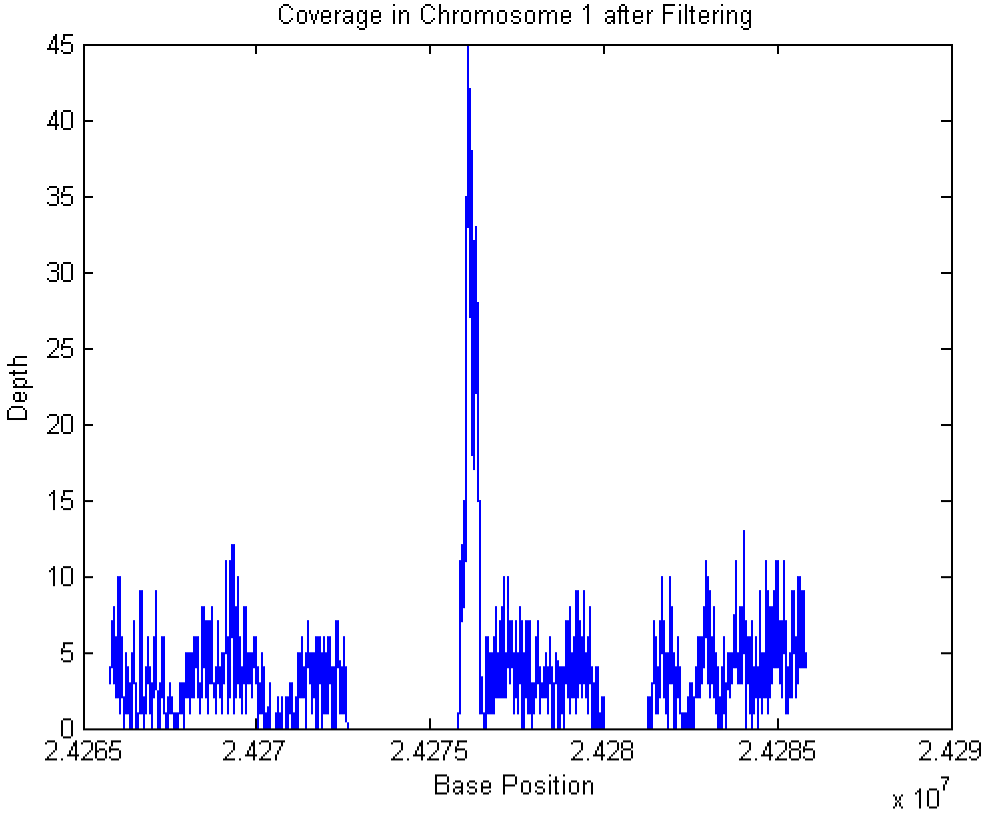
```
i = find(getMappingQuality(bm1_filtered)==60);
bm1_filtered = getSubset(bm1_filtered,i)
```

```
bm1_filtered = 

  BioMap

  Properties:
         Reference: 'Chr1'
         Signature: [2313252x1 File indexed property]
             Start: [2313252x1 File indexed property]
    MappingQuality: [2313252x1 File indexed property]
              Flag: [2313252x1 File indexed property]
      MatePosition: [2313252x1 File indexed property]
           Quality: [2313252x1 File indexed property]
          Sequence: [2313252x1 File indexed property]
            Header: [2313252x1 File indexed property]
             NSeqs: 2313252
              Name: ''
```

Visualize again the filtered data set using both, a coarse resolution with 1000 bp bins for the whole chromosome, and a fine resolution for a small region of 20,000 bp. Most of the large peaks due to artifacts have been removed.

```
[cov,bin] =
getBaseCoverage(bm1_filtered,x1,x2,'binWidth',1000,'binType','max');
figure
plot(bin,cov)
xlim([x1,x2])
ylim([0 100])
xlabel('Base Position')
ylabel('Depth')
title('Coverage in Chromosome 1 after Filtering')
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on

p1 = 24275801-10000;
p2 = 24275801+10000;

figure
plot(p1:p2,getBaseCoverage(bm1_filtered,p1,p2))
xlabel('Base Position')
ylabel('Depth')
title('Coverage in Chromosome 1 after Filtering')
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
```


Coverage in Chromosome 1 after Filtering
Depth
100
90
80
70
60
50
40
30
20
10
0
0.5
1
1.5
2
2.5
3
Base Position
x 10^7


Coverage in Chromosome 1 after Filtering
Depth
45
40
35
30
25
20
15
10
5
0
2.4265
2.427
2.4275
2.428
2.4285
2.429
Base Position
x 10^7

Recovering Sequencing Fragments from the Paired-End Reads

In Wang's paper [1] it is hypothesized that paired-end sequencing data has the potential to increase the accuracy of the identification of chromosome binding sites of DNA associated proteins because the fragment length can be derived accurately, while when using single-end sequencing it is necessary to resort to a statistical approximation of the fragment length, and use it indistinctly for all putative binding sites.

Use the paired-end reads to reconstruct the sequencing fragments. First, get the indices for the forward and the reverse reads in each pair. This information is captured in the fifth bit of the `flag` field, according to the SAM file format.

```
fow_idx = find(~bitget(getFlag(bm1_filtered),5));
rev_idx = find(bitget(getFlag(bm1_filtered),5));
```

SAM-formatted files use the same header strings to identify pair mates. By pairing the header strings you can determine how the short reads in `BioMap` are paired. To pair the header strings, simply order them in ascending order and use the sorting indices (`hf` and `hr`) to link the unsorted header strings.

```
[~,hf] = sort(getHeader(bm1_filtered,fow_idx));
[~,hr] = sort(getHeader(bm1_filtered,rev_idx));
mate_idx = zeros(numel(fow_idx),1);
mate_idx(hf) = rev_idx(hr);
```

Use the resulting `fow_idx` and `mate_idx` variables to retrieve pair mates. For example, retrieve the paired-end reads for the first 10 fragments.

```
for j = 1:10
  disp(getInfo(bm1_filtered, fow_idx(j)))
  disp(getInfo(bm1_filtered, mate_idx(j)))
end
```

```
SRR054715.6849385      163     20     60      40M
        AACCCTAAACCTCTGAATCCTTAATCCCTAAATCCCTAAA
        BBBBBBBBBBCBCB?2?BBBBB@7;BBC?7=7?BCC4*)3
SRR054715.6849385      83      229    60      40M
        CCTATTTCTTGTGGTTTTCTTTCCTTCACTTAGCTATGGA
        06BBBB=BBBBBBBBBBBBBBA6@@@9<*9BBA@>BBBBB
SRR054715.6992346      99      20     60      40M
        AACCCTAAACCTCTGAATCCTTAATCCCTAAATCCCTAAA
        =B?BCB=2;BBBBB=B8BBCCBBBBBBBC66BBB=BC8BB
SRR054715.6992346      147     239    60      40M
        GTGGTTTTCTTTCCTTCACTTAGCTATGGATGGTTTATCT
        BBCBB6B?B0B8B<'.BBBBBBBB=BBBBB6BBBBB;*6@
SRR054715.8438570      163     47     60      40M
        CTAAATCCCTAAATCTTTAAATCCTACATCCATGAATCCC
        BC=BBBBCBB?==BBB;BB;?BBB8BCB??B-BB<*<B;B
SRR054715.8438570      83      274    60      40M
        TATCTTCATTTGTTATATTGGATACAAGCTTTGCTACGAT
        BBBBB=;BBBBBBBBB;6?=BBBBBBBB<*9BBB;8BBB?
```

```
SRR054715.1676744       163     67      60      40M
        ATCCTACATCCATGAATCCCTAAATACCTAATCCCCTAAA
        BBCB>4?+<BB6BB66BBC?77BBCBC@4ABB-BBBCCBB
SRR054715.1676744       83      283     60      40M
        TTGTTATATTGGATACAAGCTTTGCTACGATCTACATTTG
        CCB6BBB93<BBBB>>@B?<<?BBBBBBBBBBBBBBBBBB
SRR054715.6820328       163     73      60      40M
        CATCCATGAATCCCTAAATACCTAATTCCCTAAACCCGAA
        BB=08?BB?BCBBB=8BBB8?CCB-B;BBB?;;?BB8B;8
SRR054715.6820328       83      267     60      40M
        GTTGGTGTATCTTCATTTGTTATATTGGATACGAGCTTTG
        BBBBB646;BB8@44BB=BBBB?C8BBBB=B6.9B8CCCB
SRR054715.1559757       163     103     60      40M
        TAAACCCGAAACCGGTTTCTCTGGTTGAAACTCATTGTGT
        BBBBBCBBBBBBBBBBBCBBBB?BBBB<;?*?BBBBB7,*
SRR054715.1559757       83      311     60      40M
        GATCTACATTTGGGAATGTGAGTCTCTTATTGTAACCTTA
        <?BBBBB?7=BBBBBBBBBBBBBB@;@>@BBBBBBBBBBB
SRR054715.5658991       163     103     60      40M
        CAAACCCGAAACCGGTTTCTCTGGTTGAAACTCATTGTGT        7?BBBBBB;=BBBB?8B;B-
;BCB-B<49<6B8-BB?+?B
SRR054715.5658991       83      311     60      40M
        GATCTACATTTGGGAATGTGAGTCTCTTATTGTAACCTTA        3,<-
BBCBBBBBB?=BBBBA<ABBBBBBBBB?79BBB?BB
SRR054715.4625439       163     143     60      40M
        ATATAATGATAATTTTAGCGTTTTTATGCAATTGCTTATT
        BBBBB@,*<8BBB++2B6B;+6B8B;8+9BB0,'9B=.=B
SRR054715.4625439       83      347     60      40M
        CTTAGTGTTGGTTTATCTCAAGAATCTTATTAATTGTTTG        +BB8B0BBB?BBBB-
BBBB22?BBB-BB6BB-BBBBBB?B
SRR054715.1007474       163     210     60      40M
        ATTTGAGGTCAATACAAATCCTATTTCTTGTGGTTTGCTT
        BBBBBBBB;.>BB6B6',BBBCBB-08BBBBB;CB9630<
SRR054715.1007474       83      408     60      40M
        TATTGTCATTCTTACTCCTTTGTGGAAATGTTTGTTCTAT
        BBB@AABBBCCCBBBBBBB=BBBCB8BBBBB=B6BCBB77
SRR054715.7345693       99      213     60      40M
        TGAGGTCAATACAAATCCTATTTCTTGTGGTTTTCTTTCT
        B>;>BBB9,<6?@@BBBBBBBBBBBBBB7<9BBBBBB6*'
SRR054715.7345693       147     393     60      40M
        TTATTTTTGGACATTTATTGTCATTCTTACTCCTTTGGGG        BB-?+?C@>9BBBBBB6.<BBB-
BBB94;A4442+49';B
```

Use the paired-end indices to construct a new `BioMap` with the minimal information needed to represent the sequencing fragments. First, calculate the insert sizes.

```
J = getStop(bm1_filtered, fow_idx);
K = getStart(bm1_filtered, mate_idx);
L = K - J - 1;
```

Obtain the new signature (or CIGAR string) for each fragment by using the short read original signatures separated by the appropriate number of skip CIGAR symbols (`N`).

```
n = numel(L);
cigars = cell(n,1);
```

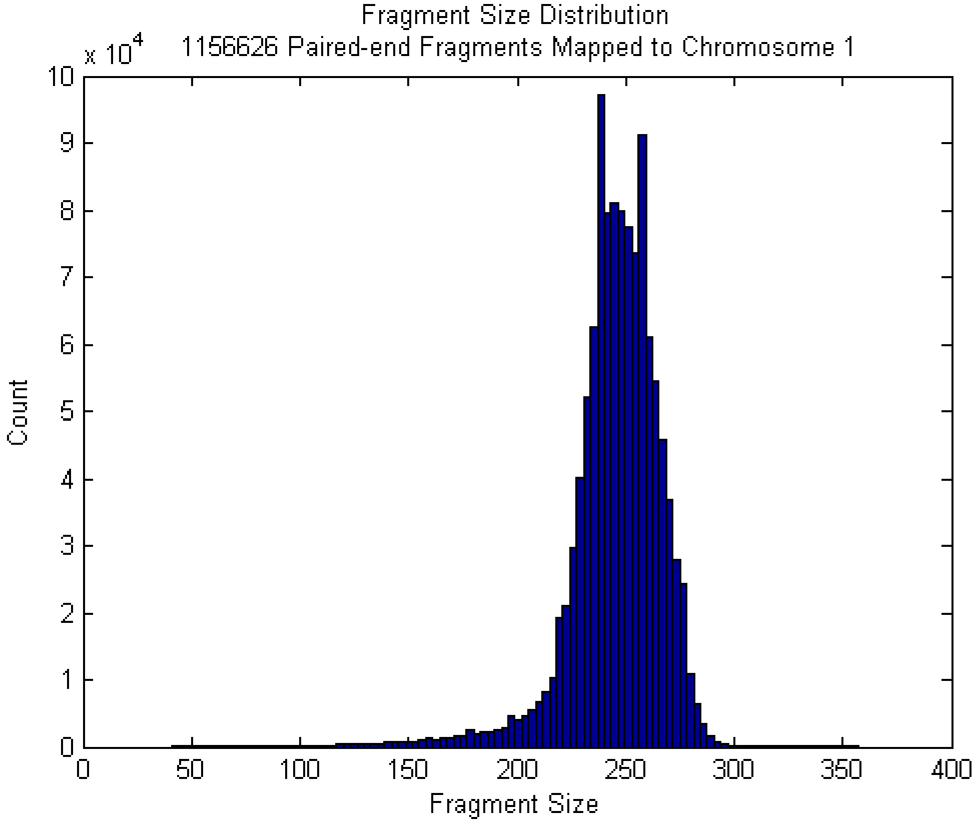
```
for i = 1:n
   cigars{i} = sprintf('%dN' ,L(i));
end
cigars = strcat( getSignature(bm1_filtered, fow_idx),...
                 cigars,...
                 getSignature(bm1_filtered, mate_idx));
```

Reconstruct the sequences for the fragments by concatenating the respective sequences of the paired-end short reads.

```
seqs = strcat( getSequence(bm1_filtered, fow_idx),...
               getSequence(bm1_filtered, mate_idx));
```

Calculate and plot the fragment size distribution.

```
J = getStart(bm1_filtered,fow_idx);
K = getStop(bm1_filtered,mate_idx);
L = K - J + 1;
figure
hist(double(L),100)
title(sprintf('Fragment Size Distribution\n %d Paired-end Fragments Mapped to Chromosome 1',n))
xlabel('Fragment Size')
ylabel('Count')
```



Construct a new `BioMap` to represent the sequencing fragments. With this, you will be able explore the coverage signals as well as local alignments of the fragments.

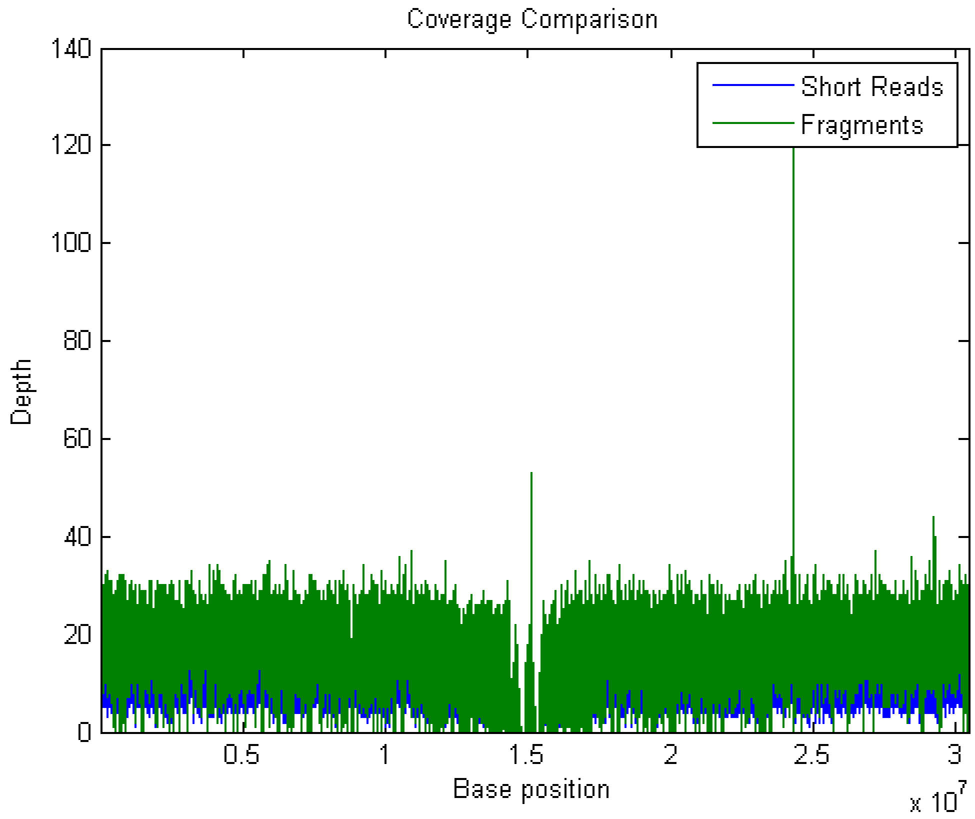
```
bm1_fragments = BioMap('Sequence',seqs,'Signature',cigars,'Start',J)
```

```
bm1_fragments = 

  BioMap

  Properties:
         Reference: ''
         Signature: {1156626x1 cell}
             Start: [1156626x1 uint32]
    MappingQuality: [0x1 uint8]
              Flag: [0x1 uint16]
      MatePosition: [0x1 uint32]
           Quality: {0x1 cell}
          Sequence: {1156626x1 cell}
            Header: {0x1 cell}
             NSeqs: 1156626
              Name: ''
```

Exploring the Coverage Using Fragment Alignments

Compare the coverage signal obtained by using the reconstructed fragments with the coverage signal obtained by using individual paired-end reads. Notice that enriched binding sites, represented by peaks, can be better discriminated from the background signal.

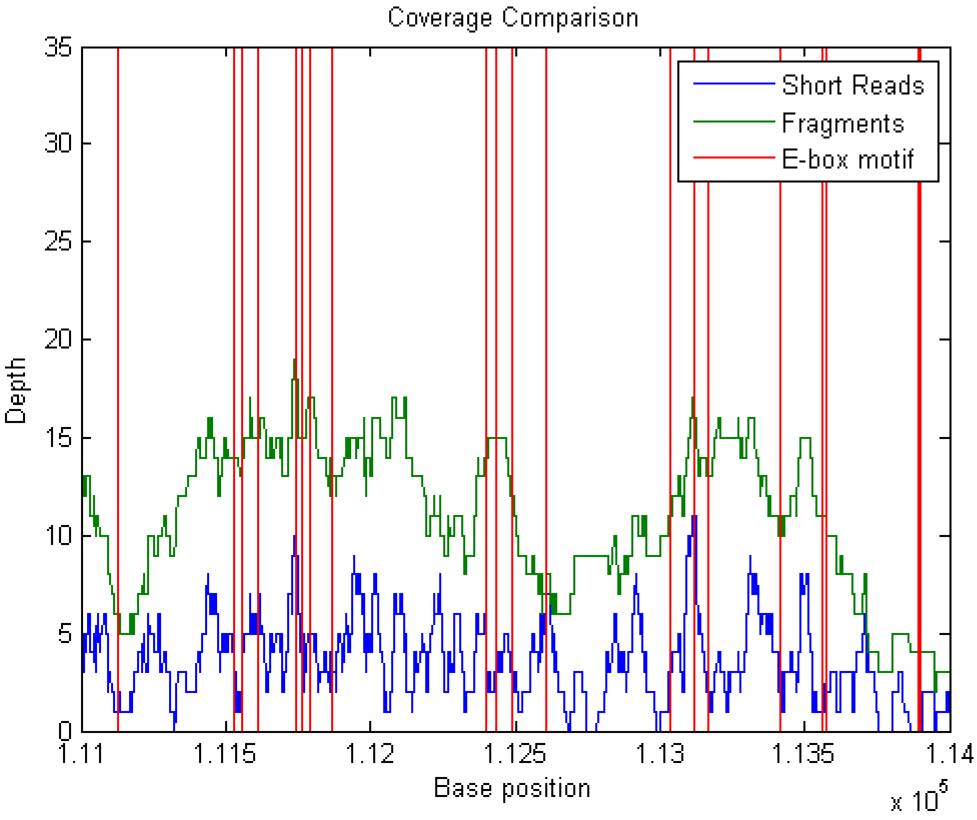
```
cov_reads =
getBaseCoverage(bm1_filtered,x1,x2,'binWidth',1000,'binType','max');
[cov_fragments,bin] =
getBaseCoverage(bm1_fragments,x1,x2,'binWidth',1000,'binType','max');

figure
plot(bin,cov_reads,bin,cov_fragments)
xlim([x1,x2])
xlabel('Base position')
ylabel('Depth')
title('Coverage Comparison')
legend('Short Reads','Fragments')
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
```



Perform the same comparison at the bp resolution. In this dataset, Wang et.al. [1] investigated a basic helix-loop-helix (*bHLH*) transcription factor. *bHLH* proteins typically bind to a consensus sequence called an *E-box* (with a `CANNTG` motif). Use `fastaread` to load the reference chromosome, search for the *E-box* motif in the 3' and 5' directions, and then overlay the motif positions on the coverage signals. This example works over the region 1-200,000, however the figure limits are narrowed to a 3000 bp region in order to better depict the details.

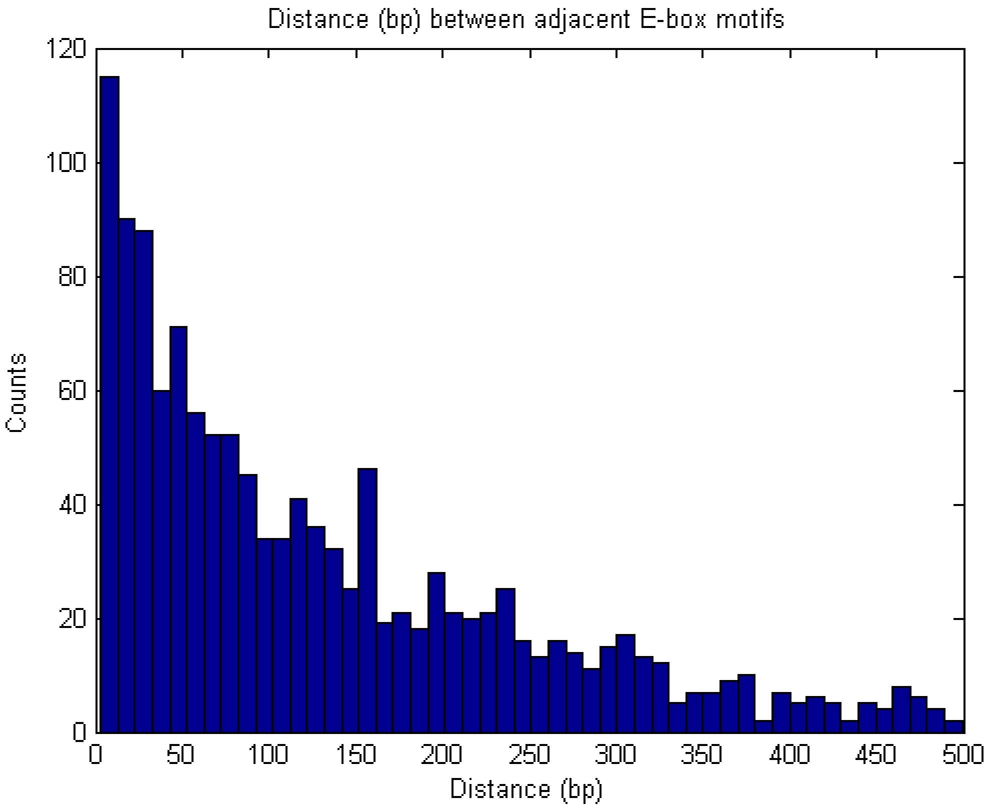
```
p1 = 1;
p2 = 200000;

cov_reads = getBaseCoverage(bm1_filtered,p1,p2);
[cov_fragments,bin] = getBaseCoverage(bm1_fragments,p1,p2);

chr1 = fastaread('ach1.fasta');
mp1 = regexp(chr1.Sequence(p1:p2),'CA..TG')+3+p1;
mp2 = regexp(chr1.Sequence(p1:p2),'GT..AC')+3+p1;
motifs = [mp1 mp2];

figure
plot(bin,cov_reads,bin,cov_fragments)
hold on
plot([1;1;1]*motifs,[0;max(ylim);NaN],'r')
xlabel('Base position')
ylabel('Depth')
title('Coverage Comparison')
legend('Short Reads','Fragments','E-box motif')
```

```
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
xlim([111000 114000])
```



Observe that it is not possible to associate each peak in the coverage signals with an *E-box* motif. This is because the length of the sequencing fragments is comparable to the average motif distance, blurring peaks that are close. Plot the distribution of the distances between the *E-box* motif sites.

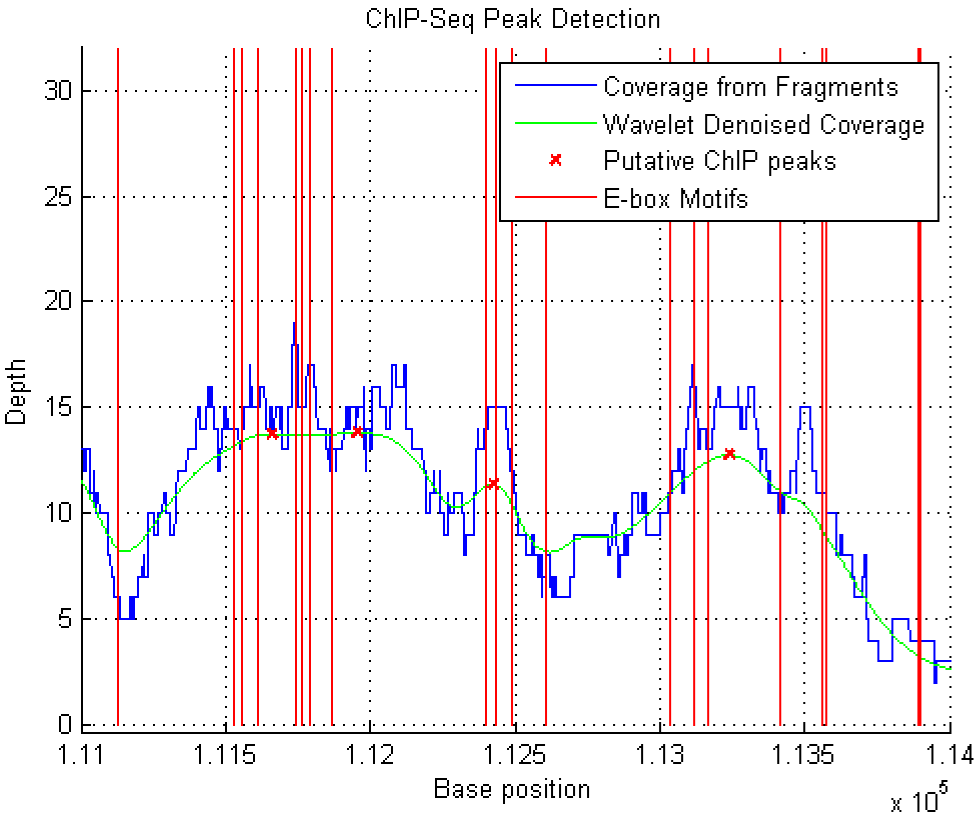
```
motif_sep = diff(sort(motifs));
figure
hist(motif_sep(motif_sep<500),50)
title('Distance (bp) between adjacent E-box motifs')
xlabel('Distance (bp)')
ylabel('Counts')
```



Finding Significant Peaks in the Coverage Signal

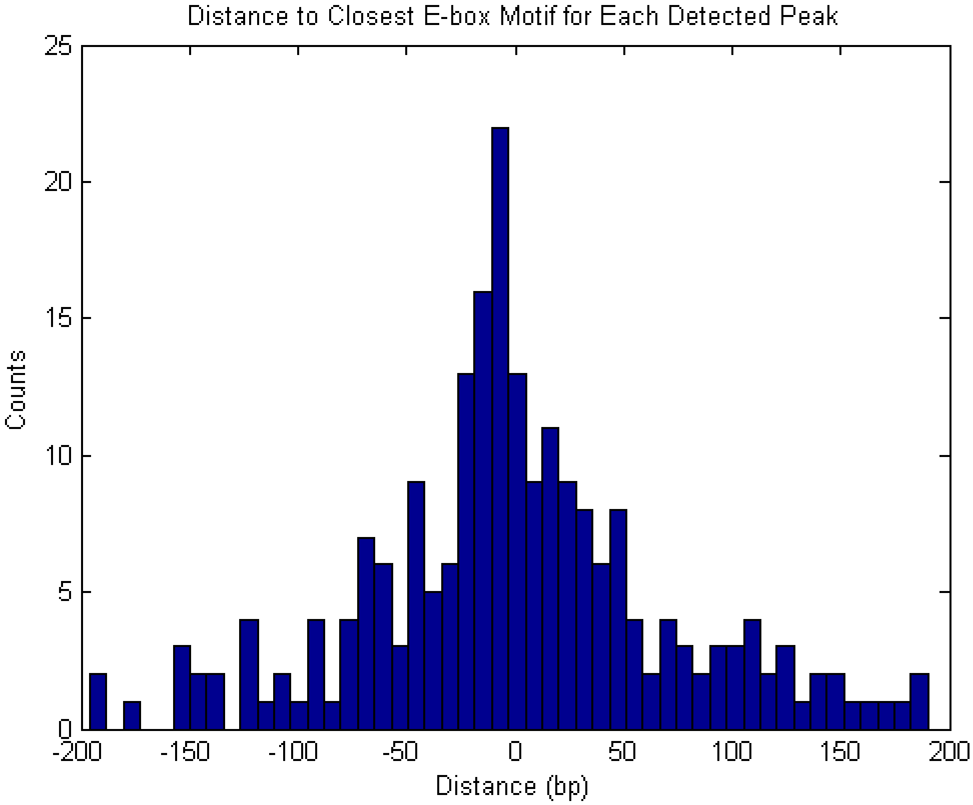
Use the function `mspeaks` to perform peak detection with Wavelets denoising on the coverage signal of the fragment alignments. Filter putative ChIP peaks using a height filter to remove peaks that are not enriched by the binding process under consideration.

```
putative_peaks = mspeaks(bin,cov_fragments,'noiseestimator',20,...
                         'heightfilter',10,'showplot',true);
hold on
plot([1;1;1]*motifs(motifs>p1 & motifs<p2),[0;max(ylim);NaN],'r')
legend('Coverage from Fragments','Wavelet Denoised Coverage','Putative ChIP
peaks','E-box Motifs')
xlabel('Base position')
ylabel('Depth')
title('ChIP-Seq Peak Detection')
set(datacursormode(gcf),'UpdateFcn',mdc)
datacursormode on
xlim([111000 114000])
```



Use the `knnsearch` function to find the closest motif to each one of the putative peaks. As expected, most of the enriched ChIP peaks are close to an *E-box* motif [1]. This reinforces the importance of performing peak detection at the finest resolution possible (bp resolution) when the expected density of binding sites is high, as it is in the case of the *E-box* motif. It also demonstrates that for this type of analysis, paired-end sequencing should be considered over single-end sequencing [1].

```
h = knnsearch(motifs',putative_peaks(:,1));
distance = putative_peaks(:,1)-motifs(h(:))';
figure
hist(distance(abs(distance)<200),50)
title('Distance to Closest E-box Motif for Each Detected Peak')
xlabel('Distance (bp)')
ylabel('Counts')
```



References

[1] Wang C., Xu J., Zhang D., Wilson Z.A., and Zhang D. "An effective approach for identification of in vivo protein-DNA binding sites from paired-end ChIP-Seq data", BMC Bioinformatics, 11:81, Feb 9, 2010.

[2] Li H. and Durbin R. "Fast and accurate short read alignment with Burrows-Wheeler transform", Bioinformatics, 25, pp 1754-60, 2009.

[3] Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. and 1000 Genome Project Data Processing Subgroup "The Sequence Alignment/map (SAM) Format and SAMtools", Bioinformatics, 25, pp 2078-2079, 2009.

[4] Jothi R, Cuddapah S, Barski A, Cui K, Zhao K. "Genome-wide identification of in vivo protein-DNA binding sites from ChIP-Seq data", Nucleic Acids Research, 36(16), pp 5221-31, Sep 2008.

[5] Hoofman B.G., and Jones S.J.M. "Genome-wide identification of DNA–protein interactions using chromatin immunoprecipitation coupled with flow cell sequencing", Journal of Endocrinology 201, pp 1-13, 2009.

[6] Ramsey SA, Knijnenburg TA, Kennedy KA, Zak DE, Gilchrist M, Gold ES, Johnson CD, Lampano AE, Litvak V, Navarro G, Stolyar T, Aderem A, Shmulevich I. "Genome-wide histone acetylation data improve prediction of mammalian transcription factor binding sites", Bioinformatics, 26(17), pp 2071-5, Sep 1, 2010.

Provide feedback for this demo.