atSNP: affinity tests for regulatory SNP detection

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Contents

1 Introduction

This document provides an introduction to the affinity test for large sets of SNP-motif interactions using the *atSNP* package(**a**ffinity **t**est for regulatory **SNP** detection) [**?**]. *atSNP* implements in-silico methods for identifying SNPs that potentially may affect binding affinity of transcription factors. Given a set of SNPs and a library of motif position weight matrices (PWMs), *atSNP* provides three main functions for analyzing SNP effects:

1. Computing the binding affinity score for each allele and each PWM.
2. Computing the p-values for allele-specific binding affinity scores.
3. Computing the p-values for affinity score changes between the two alleles for each SNP.

atSNP implements the importance sampling algorithm in [**?**] to compute the p-values. Compared to other bioinformatics tools, such as FIMO [**?**] and is-rSNP [**?**] that provide similar functionalities, *atSNP* avoids computing the p-values analytically. In one of our research projects, we have used atSNP to evaluate interactions between 26K SNPs and 2K motifs within 5 hours. We found no other existing tool can finish the analysis of such a scale.

2 Installation

We are working to make the package available through bioconductor. The developing version can be installed from the Github repository:

```
> library(devtools)
> install_github("chandlerzuo/atSNP")
```

atSNP depends on the following *R* packages:

- *data.table* is used for formatting results that are easy for users to query.
- *motifStack* is relied upon to draw sequence logo plots.
- *doMC* is used for parallel computation.
- *Rcpp* interfaces the C++ codes that implements the importance sampling algorithm.

In addition, users also need to install the annotation package from `www.bioconductor.org/packages/3.0/data/annotation/` that corresponds to the species type and genome version. Our example SNP data set in the subsequent sections corresponds to the hg19 version of human genome. To repeat the sample codes in this vignette, the *BSgenome.Hsapiens.UCSC.hg19* package is required. To install it from the *Bioconductor* repository,

```
> source("http://bioconduc.org/biocLite.R")
> biocLite("BSgenome.Hsapiens.UCSC.hg19")
```

Notice that the annotation package is usually large and this installation step may take a substantial amout of time.

3 Example

3.1 Load motif and SNP data

atSNP provides a default motif library downloaded from `compbio.mit.edu/encode-motifs/motifs.txt`. This library contains 2065 known and discovered motifs from ENCODE TF ChIP-seq data sets. The following commands allows to load this motif library:

```
> library(atSNP)
> data(encode_motif)
> length(motif_encode)
[1] 2065
> motif_encode[seq(3)]
$SIX5_disc1
             [,1]       [,2]     [,3]        [,4]
 [1,] 8.51100e-03 4.2550e-03 0.987234 1.00000e-10
 [2,] 9.02127e-01 1.2766e-02 0.038298 4.68090e-02
 [3,] 4.55319e-01 7.2340e-02 0.344681 1.27660e-01
 [4,] 2.51064e-01 8.5106e-02 0.085106 5.78724e-01
 [5,] 1.00000e-10 4.6809e-02 0.012766 9.40425e-01
 [6,] 1.00000e-10 1.0000e-10 1.000000 1.00000e-10
 [7,] 3.82980e-02 2.1277e-02 0.029787 9.10638e-01
 [8,] 9.44681e-01 4.2550e-03 0.051064 1.00000e-10
 [9,] 1.00000e-10 1.0000e-10 1.000000 1.00000e-10
[10,] 1.00000e-10 1.0000e-10 0.012766 9.87234e-01

$MYC_disc1
             [,1]        [,2]        [,3]        [,4]
 [1,] 1.73516e-01 1.05023e-01 7.21461e-01 1.00000e-10
 [2,] 1.00000e-10 1.00000e-10 1.00000e-10 1.00000e+00
 [3,] 1.00000e-10 1.00000e+00 1.00000e-10 1.00000e-10
 [4,] 1.00000e+00 1.00000e-10 1.00000e-10 1.00000e-10
 [5,] 1.00000e-10 9.58904e-01 1.00000e-10 4.10960e-02
 [6,] 5.93610e-02 1.00000e-10 9.40639e-01 1.00000e-10
 [7,] 1.00000e-10 1.00000e-10 1.00000e-10 1.00000e+00
 [8,] 1.00000e-10 1.00000e-10 1.00000e+00 1.00000e-10
 [9,] 1.00000e+00 1.00000e-10 1.00000e-10 1.00000e-10
[10,] 1.00000e-10 7.26028e-01 1.14155e-01 1.59817e-01

$SRF_disc1
             [,1]  [,2]  [,3]        [,4]
 [1,] 1.00000e-10 1e+00 1e-10 1.00000e-10
 [2,] 1.00000e-10 1e+00 1e-10 1.00000e-10
 [3,] 4.95495e-01 1e-10 1e-10 5.04505e-01
 [4,] 2.61261e-01 1e-10 1e-10 7.38739e-01
 [5,] 1.00000e+00 1e-10 1e-10 1.00000e-10
 [6,] 1.00000e-10 1e-10 1e-10 1.00000e+00
 [7,] 7.29730e-01 1e-10 1e-10 2.70270e-01
 [8,] 5.04505e-01 1e-10 1e-10 4.95495e-01
 [9,] 1.00000e-10 1e-10 1e+00 1.00000e-10
[10,] 1.00000e-10 1e-10 1e+00 1.00000e-10
```

Here, the motif library is represented by motif$_encode, whichisalistofpositionweightmatrices.Thecodesbelowshowsthecont$e

```
> motif_encode[[1]]
             [,1]       [,2]     [,3]        [,4]
 [1,] 8.51100e-03 4.2550e-03 0.987234 1.00000e-10
 [2,] 9.02127e-01 1.2766e-02 0.038298 4.68090e-02
 [3,] 4.55319e-01 7.2340e-02 0.344681 1.27660e-01
 [4,] 2.51064e-01 8.5106e-02 0.085106 5.78724e-01
 [5,] 1.00000e-10 4.6809e-02 0.012766 9.40425e-01
 [6,] 1.00000e-10 1.0000e-10 1.000000 1.00000e-10
 [7,] 3.82980e-02 2.1277e-02 0.029787 9.10638e-01
 [8,] 9.44681e-01 4.2550e-03 0.051064 1.00000e-10
 [9,] 1.00000e-10 1.0000e-10 1.000000 1.00000e-10
[10,] 1.00000e-10 1.0000e-10 0.012766 9.87234e-01
> GetIUPACSequence(motif_encode[[1]])
[1] "GARWTGTAGT"
```

The data object encode$_motifalsocontainsacharactervector$

The binding affinity scores for all pairs of SNP and PWM can be computed by the ComputeMotifScore function. It returns a list of two fields: 'snp.tbl' is a *data.table* containing the nucleotide sequences for each SNP; 'motif.scores' is a *data.table* containing the binding affinity scores for each SNP-motif pair.

```
> motif_score <- ComputeMotifScore(motif_library, snpInfo, ncores = 2)
> motif_score$snp.tbl
        snpid                                                         ref_seq
 1: rs10910078 TGATGCCAGGTGGTCAGTGGGTTTTTGCCATCCGCCAGGAGCTTCACTGGGCCTCCCGTTG
 2:  rs4486391 ATGGAGAATTCCACAGCTGATTGGAACCTAAACGAGAGAACCAAATGGACATCCCAGGGCT
 3:  rs3748816 TTGGAGTACTCCTCGTCCAGGCGCCTGTTCATCTCCTCCAGGATGTAGTCAGGGTGCCCGA
 4:  rs2843401 TCCTCCACCATTGTGCCAAACAGCGCCTGGTGGGGCCACCCGATCATCCCACGGGCCCCCA
 5:  rs2843402 CACCTTCTGGGCTGCAGGACTTCCTGCCCTTTAGGAAAGGGAGGCAGCCCTTTCTTCCTCC
 6:  rs2843403 CCCCCTAGGGCCTCCCTGCGGTTCCTTGTCTCCACCCTCACCCCAGCCCTGGAGCAGCCAC
 7:  rs2843404 AAATGGAATATTTAATTTGAAACTTTCCAATAAAGAAATTTCCAGACCCATTTGGCTTCAC
 8:  rs2985855 ACCTGATAAAGGAAATGTATGAAGCAGCAGAAGCAACAAAAACAACTCCATAGCAAACATA
 9:  rs2296442 CCGCTTCCTCGTCTGGGACCACGATCCCATCGGGCGTGACTTCATTGGCCAGAGGACGCTG
10: rs10797432 GACTCACAGGTGGGAGACAGGAGTTCCGACCGCCAGGGGGAGAGTCCTGGAGGATCCTGGG
11:  rs6667605 TCCCACAAATGCAGAAAGCTCAACAGACCCCAAGAGGGGTAAATAGAGAGGCATGCACTGC
12:  rs4648648 CAGGTCCTGCGATCTCCCCACGCCCTGACAGTGACCTATCTTTGTGCACACACGTGTGTTT
13:   rs734999 CCACTGAAATACCCGTGGGAAAGAAAAGCACAACAGAGAACAGGAGACTTATGTGACTCCG
14:  rs2764845 CACCATGGCCAAGCCTGTCACCTCACCTGGGTGACCACATCGGCCTCCATGCTGACCCCGC
15:  rs2764841 CTGTTTCTGCTCCCGGGAAATCACCCCGCCGCCTCTTCAGGCCTTTAAGGTCTCAAATGTC
16:  rs2985857 CACTCTTGAAGAACAAAGTTGAAATATATACTCTATTGACTATCAAGACATTATAAAGCTG
17:  rs6424092 ATCTCACTGTCCATTAAAAAAATCAACTCACAGTAGATTGTAGACCTAAGCAAACCTGAGG
                                                          snp_seq
 1: TGATGCCAGGTGGTCAGTGGGTTTTTGCCACCCGCCAGGAGCTTCACTGGGCCTCCCGTTG
 2: ATGGAGAATTCCACAGCTGATTGGAACCTATACGAGAGAACCAAATGGACATCCCAGGGCT
 3: TTGGAGTACTCCTCGTCCAGGCGCCTGTTCGTCTCCTCCAGGATGTAGTCAGGGTGCCCGA
 4: TCCTCCACCATTGTGCCAAACAGCGCCTGGCGGGGCCACCCGATCATCCCACGGGCCCCCA
 5: CACCTTCTGGGCTGCAGGACTTCCTGCCCTCTAGGAAAGGGAGGCAGCCCTTTCTTCCTCC
 6: CCCCCTAGGGCCTCCCTGCGGTTCCTTGTCCCCACCCTCACCCCAGCCCTGGAGCAGCCAC
 7: AAATGGAATATTTAATTTGAAACTTTCCAACAAAGAAATTTCCAGACCCATTTGGCTTCAC
 8: ACCTGATAAAGGAAATGTATGAAGCAGCAGCAGCAACAAAAACAACTCCATAGCAAACATA
 9: CCGCTTCCTCGTCTGGGACCACGATCCCATTGGGCGTGACTTCATTGGCCAGAGGACGCTG
10: GACTCACAGGTGGGAGACAGGAGTTCCGACTGCCAGGGGGAGAGTCCTGGAGGATCCTGGG
11: TCCCACAAATGCAGAAAGCTCAACAGACCCTAAGAGGGGTAAATAGAGAGGCATGCACTGC
12: CAGGTCCTGCGATCTCCCCACGCCCTGACAATGACCTATCTTTGTGCACACACGTGTGTTT
13: CCACTGAAATACCCGTGGGAAAGAAAAGCATAACAGAGAACAGGAGACTTATGTGACTCCG
14: CACCATGGCCAAGCCTGTCACCTCACCTGGTTGACCACATCGGCCTCCATGCTGACCCCGC
```

```
15: CTGTTTCTGCTCCCGGGAAATCACCCCGCCACCTCTTCAGGCCTTTAAGGTCTCAAATGTC
16: CACTCTTGAAGAACAAAGTTGAAATATATATTCTATTGACTATCAAGACATTATAAAGCTG
17: ATCTCACTGTCCATTAAAAAAATCAACTCAAAGTAGATTGTAGACCTAAGCAAACCTGAGG
                                                      ref_seq_rev
 1: CAACGGGAGGCCCAGTGAAGCTCCTGGCGGATGGCAAAAACCCACTGACCACCTGGCATCA
 2: AGCCCTGGGATGTCCATTTGGTTCTCTCGTTTAGGTTCCAATCAGCTGTGGAATTCTCCAT
 3: TCGGGCACCCTGACTACATCCTGGAGGAGATGAACAGGCGCCTGGACGAGGAGTACTCCAA
 4: TGGGGGCCCGTGGGATGATCGGGTGGCCCCACCAGGCGCTGTTTGGCACAATGGTGGAGGA
 5: GGAGGAAGAAAGGGCTGCCTCCCTTTCCTAAAGGGCAGGAAGTCCTGCAGCCCAGAAGGTG
 6: GTGGCTGCTCCAGGGCTGGGGTGAGGGTGGAGACAAGGAACCGCAGGGAGGCCCTAGGGGG
 7: GTGAAGCCAAATGGGTCTGGAAATTTCTTTATTGGAAAGTTTCAAATTAAATATTCCATTT
 8: TATGTTTGCTATGGAGTTGTTTTTGTTGCTTCTGCTGCTTCATACATTTCCTTTATCAGGT
 9: CAGCGTCCTCTGGCCAATGAAGTCACGCCCGATGGGATCGTGGTCCCAGACGAGGAAGCGG
10: CCCAGGATCCTCCAGGACTCTCCCCCTGGCGGTCGGAACTCCTGTCTCCCACCTGTGAGTC
11: GCAGTGCATGCCTCTCTATTTACCCCTCTTGGGGTCTGTTGAGCTTTCTGCATTTGTGGGA
12: AAACACACGTGTGTGCACAAAGATAGGTCACTGTCAGGGCGTGGGGAGATCGCAGGACCTG
13: CGGAGTCACATAAGTCTCCTGTTCTCTGTTGTGCTTTTCTTTCCCACGGGTATTTCAGTGG
14: GCGGGGTCAGCATGGAGGCCGATGTGGTCACCCAGGTGAGGTGACAGGCTTGGCCATGGTG
15: GACATTTGAGACCTTAAAGGCCTGAAGAGGCGGCGGGGTGATTTCCCGGGAGCAGAAACAG
16: CAGCTTTATAATGTCTTGATAGTCAATAGAGTATATATTTCAACTTTGTTCTTCAAGAGTG
17: CCTCAGGTTTGCTTAGGTCTACAATCTACTGTGAGTTGATTTTTTTAATGGACAGTGAGAT
                                                      snp_seq_rev
 1: CAACGGGAGGCCCAGTGAAGCTCCTGGCGGGTGGCAAAAACCCACTGACCACCTGGCATCA
 2: AGCCCTGGGATGTCCATTTGGTTCTCTCGTATAGGTTCCAATCAGCTGTGGAATTCTCCAT
 3: TCGGGCACCCTGACTACATCCTGGAGGAGACGAACAGGCGCCTGGACGAGGAGTACTCCAA
 4: TGGGGGCCCGTGGGATGATCGGGTGGCCCCGCCAGGCGCTGTTTGGCACAATGGTGGAGGA
 5: GGAGGAAGAAAGGGCTGCCTCCCTTTCCTAGAGGGCAGGAAGTCCTGCAGCCCAGAAGGTG
 6: GTGGCTGCTCCAGGGCTGGGGTGAGGGTGGGGACAAGGAACCGCAGGGAGGCCCTAGGGGG
 7: GTGAAGCCAAATGGGTCTGGAAATTTCTTTGTTGGAAAGTTTCAAATTAAATATTCCATTT
 8: TATGTTTGCTATGGAGTTGTTTTTGTTGCTGCTGCTGCTTCATACATTTCCTTTATCAGGT
 9: CAGCGTCCTCTGGCCAATGAAGTCACGCCCAATGGGATCGTGGTCCCAGACGAGGAAGCGG
10: CCCAGGATCCTCCAGGACTCTCCCCCTGGCAGTCGGAACTCCTGTCTCCCACCTGTGAGTC
11: GCAGTGCATGCCTCTCTATTTACCCCTCTTAGGGTCTGTTGAGCTTTCTGCATTTGTGGGA
12: AAACACACGTGTGTGCACAAAGATAGGTCATTGTCAGGGCGTGGGGAGATCGCAGGACCTG
13: CGGAGTCACATAAGTCTCCTGTTCTCTGTTATGCTTTTCTTTCCCACGGGTATTTCAGTGG
14: GCGGGGTCAGCATGGAGGCCGATGTGGTCAACCAGGTGAGGTGACAGGCTTGGCCATGGTG
15: GACATTTGAGACCTTAAAGGCCTGAAGAGGTGGCGGGGTGATTTCCCGGGAGCAGAAACAG
16: CAGCTTTATAATGTCTTGATAGTCAATAGAATATATATTTCAACTTTGTTCTTCAAGAGTG
17: CCTCAGGTTTGCTTAGGTCTACAATCTACTTTGAGTTGATTTTTTTAATGGACAGTGAGAT
> motif_score$motif.scores[, list(snpid, motif, log_lik_ref,
+                                 log_lik_snp, log_lik_ratio)]
         snpid      motif log_lik_ref log_lik_snp log_lik_ratio
 1: rs10910078  MYC_disc1   -95.57417   -92.79201    -2.7821535
 2:  rs4486391  MYC_disc1   -94.37676   -79.51729   -14.8594729
 3:  rs3748816  MYC_disc1   -96.67901   -99.39326     2.7142529
 4:  rs2843401  MYC_disc1   -94.66127   -94.21702    -0.4442544
 5:  rs2843402  MYC_disc1  -117.34142  -117.34142     0.0000000
 6:  rs2843403  MYC_disc1  -115.81786  -115.81786     0.0000000
 7:  rs2843404  MYC_disc1   -95.73058  -118.75643    23.0258509
 8:  rs2985855  MYC_disc1  -116.88074  -120.49201     3.6112717
 9: rs10910078 SIX5_disc1   -46.06943   -38.82055    -7.2488780
10:  rs4486391 SIX5_disc1   -41.21034   -41.21034     0.0000000
11:  rs3748816 SIX5_disc1   -51.99542   -40.50007   -11.4953572
12:  rs2843401 SIX5_disc1   -19.33735   -23.09387     3.7565188
13:  rs2843402 SIX5_disc1   -20.74899   -23.90834     3.1593576
14:  rs2843403 SIX5_disc1   -38.21561   -41.13338     2.9177676
15:  rs2843404 SIX5_disc1   -43.06925   -38.31571    -4.7535476
16:  rs2985855 SIX5_disc1   -58.35713   -58.35713     0.0000000
```

```
17:  rs2296442  MYC_disc1   -71.67739   -75.86532     4.1879305
18: rs10797432  MYC_disc1  -117.72909   -99.39326   -18.3358300
19:  rs6667605  MYC_disc1   -75.86532   -79.01520     3.1498802
20:  rs4648648  MYC_disc1   -92.79201   -94.21702     1.4250085
21:   rs734999  MYC_disc1   -75.86532   -79.01520     3.1498802
22:  rs2764845  MYC_disc1   -50.51744   -50.03458    -0.4828589
23:  rs2764841  MYC_disc1   -94.64204   -94.23625    -0.4057914
24:  rs2985857  MYC_disc1   -71.57423   -92.42357    20.8493426
25:  rs6424092  MYC_disc1   -53.34156   -76.32544    22.9838866
26:  rs2296442 SIX5_disc1   -23.43206   -21.51514    -1.9169281
27: rs10797432 SIX5_disc1   -24.19256   -21.27479    -2.9177676
28:  rs6667605 SIX5_disc1   -18.25728   -41.28313    23.0258509
29:  rs4648648 SIX5_disc1   -44.20012   -36.50922    -7.6909014
30:   rs734999 SIX5_disc1   -36.30181   -56.49106    20.1892485
31:  rs2764845 SIX5_disc1   -41.43849   -41.69843     0.2599394
32:  rs2764841 SIX5_disc1   -17.46570   -16.16641    -1.2992901
33:  rs2985857 SIX5_disc1   -54.58632   -54.58632     0.0000000
34:  rs6424092 SIX5_disc1   -38.05012   -37.46235    -0.5877710
         snpid      motif log_lik_ref log_lik_snp log_lik_ratio
```

The affinity scores for the reference and the SNP alleles are represented by the 'log_lik_ref' and 'log_lik_snp' columns in '$motif.scores'. The affinity score change is included in the 'log_lik_ra column. These three affinity scores are tested in the subsequent steps. '$motif.scores' also include other columns for the position of the best matching subsequence on each allele. For a complete description on all these columns, users can look up the help documentation.

After we have computed the binding affinity scores, they can be tested using the ComputePValues function. The result is a *data.table* extending the affinity score table by six columns:

- 'pval_ref': p-value for the reference allele affinity score.
- 'pval_snp': p-value for the SNP allele affinity score.
- 'pval_cond_ref' and 'pval_cond_snp': conditional p-values for the affinity scores of the reference and SNP alleles.
- 'pval_diff': p-value for the affinity score change between the two alleles.
- 'pval_rank': p-value for the rank test between the two alleles.

We recommend using 'pval_ref'and 'pval_snp' for assessing the significance of allele specific affinity; and using 'pval_rank' for assessing the significance of the SNP effect on the affinity change.

```
> motif.scores <- ComputePValues(motif.lib = motif_library,
+                                snp.info = snpInfo,
+                                motif.scores = motif_scores$motif.scores,
+                                ncores = 7)
> motif.scores[, list(snpid, motif, pval_ref, pval_snp, pval_rank, pval_diff)]
         snpid      motif   pval_ref   pval_snp  pval_rank   pval_diff
 1: rs10910078  MYC_disc1 0.55250823 0.34976728 0.49731358 0.599057634
 2:  rs4486391  MYC_disc1 0.42467126 0.32482700 0.61817128 0.531919133
 3:  rs3748816  MYC_disc1 0.62428125 0.78352831 0.63538138 0.613347967
 4:  rs2843401  MYC_disc1 0.47098190 0.39017206 0.66297624 0.809006055
 5:  rs2843402  MYC_disc1 0.92392287 0.92392287 1.00000000 1.000000000
 6:  rs2843403  MYC_disc1 0.84071552 0.84071552 1.00000000 1.000000000
 7:  rs2843404  MYC_disc1 0.56662380 0.97929975 0.33680154 0.150135756
 8:  rs2985855  MYC_disc1 0.85727211 0.99400000 0.69437460 0.572437051
 9: rs10910078 SIX5_disc1 0.87876479 0.62172720 0.45687986 0.280402306
10:  rs4486391 SIX5_disc1 0.77460249 0.77460249 1.00000000 1.000000000
11:  rs3748816 SIX5_disc1 0.90275130 0.74334730 0.62512531 0.206882124
12:  rs2843401 SIX5_disc1 0.08081194 0.23011782 0.01654064 0.434515202
13:  rs2843402 SIX5_disc1 0.13659677 0.32832950 0.14789201 0.490855902
14:  rs2843403 SIX5_disc1 0.60110887 0.77253940 0.57145169 0.618352633
15:  rs2843404 SIX5_disc1 0.84323561 0.61067088 0.48140291 0.354767558
16:  rs2985855 SIX5_disc1 0.97623554 0.97623554 1.00000000 1.000000000
```

```
17:  rs2296442  MYC_disc1 0.11261743 0.26841651 0.29890362 0.553813438
18: rs10797432  MYC_disc1 0.95726548 0.78177027 0.64089998 0.457487238
19:  rs6667605  MYC_disc1 0.27303296 0.29517812 0.74665067 0.592775953
20:  rs4648648  MYC_disc1 0.33416921 0.37746258 0.71484150 0.716330202
21:   rs734999  MYC_disc1 0.26841651 0.29517812 0.73348109 0.591312146
22:  rs2764845  MYC_disc1 0.02476245 0.02225671 0.72498353 0.803576225
23:  rs2764841  MYC_disc1 0.44985448 0.40610585 0.73131633 0.816179825
24:  rs2985857  MYC_disc1 0.09264310 0.32482700 0.14064803 0.367260674
25:  rs6424092  MYC_disc1 0.05381326 0.28884471 0.02528970 0.171276172
26:  rs2296442 SIX5_disc1 0.30747596 0.17194308 0.30722405 0.688903343
27: rs10797432 SIX5_disc1 0.33296184 0.14606464 0.17415016 0.621522278
28:  rs6667605 SIX5_disc1 0.06436599 0.77764949 0.01572935 0.002994372
29:  rs4648648 SIX5_disc1 0.87216144 0.48674767 0.26849187 0.268226489
30:   rs734999 SIX5_disc1 0.44673064 0.95825787 0.21057043 0.102711064
31:  rs2764845 SIX5_disc1 0.80012831 0.80656654 0.93486937 0.915061559
32:  rs2764841 SIX5_disc1 0.05984278 0.04457300 0.53680487 0.751367323
33:  rs2985857 SIX5_disc1 0.92853988 0.92853988 1.00000000 1.000000000
34:  rs6424092 SIX5_disc1 0.59650110 0.54347923 0.73416105 0.866256977
         snpid      motif   pval_ref   pval_snp  pval_rank   pval_diff
```

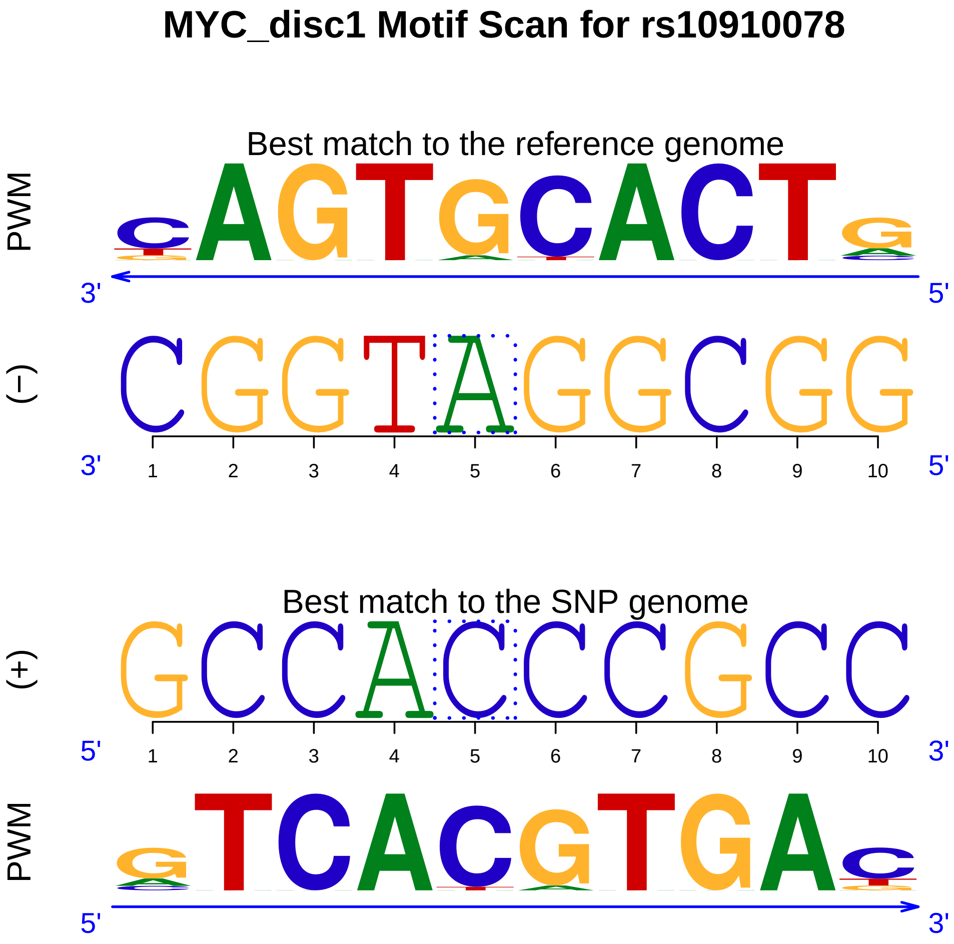
3.2 Additional analysis

atSNP provides additional functions to extract the matched nucleotide subsequences that match to the motifs. Function MatchSubsequence adds the subsequence matches to the affinity score table by using the motif library and the SNP set. The subsequences matching to the motif in the two alleles are returned in the 'ref_match_seq' and 'snp_match_seq' columns. The 'IUPAC' column returns the IUPAC letters of the motifs. Notice that if you have a large number of SNPs and motifs, the returned table can be very large.

```
> match_result <- MatchSubsequence(snp.tbl = motif_scores$snp.tbl,
+                                  motif.scores = motif.scores,
+                                  motif.lib = motif_library,
+                                  snpids = c("rs10910078", "rs4486391"),
+                                  motifs = names(motif_library)[1:2],
+                                  ncores = 2)
> match_result[, list(snpid, motif, IUPAC, ref_match_seq, snp_match_seq)]
        snpid      motif      IUPAC ref_match_seq snp_match_seq
1: rs10910078  MYC_disc1 GTCACGTGAC    GGCGGATGGC    GCCACCCGCC
2: rs10910078 SIX5_disc1 GARWTGTAGT    CTGGCGGATG    GGCGGGTGGC
3:  rs4486391  MYC_disc1 GTCACGTGAC    TAAACGAGAG    CTCGTATAGG
4:  rs4486391 SIX5_disc1 GARWTGTAGT    CTCGTTTAGG    CTCGTATAGG
```

To visualize how each motif is matched to each allele using the plotMotifMatch function:

```
> plotMotifMatch(snp.tbl = motif_scores$snp.tbl,
+                motif.scores = motif_scores$motif.scores,
+                snpid = motif_scores$snp.tbl$snpid[1],
+                motif.lib = motif_library,
+                motif = motif_scores$motif.scores$motif[1])
```


MYC_disc1 Motif Scan for rs10910078
Best match to the reference genome
PWM
3'
5'
(−)
CGGTAGGCGG
1 2 3 4 5 6 7 8 9 10
Best match to the SNP genome
(+)
GCCACCCGCC
PWM
5'
3'

4 Session Information

```
R version 3.1.1 (2014-07-10)
Platform: x86_64-redhat-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=zh_TW.UTF-8       LC_NUMERIC=C               LC_TIME=en_US.UTF-8
 [4] LC_COLLATE=en_US.UTF-8     LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C                  LC_ADDRESS=C
[10] LC_TELEPHONE=C             LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] grid      parallel  stats     graphics  grDevices utils     datasets  methods
[9] base

other attached packages:
 [1] BSgenome.Hsapiens.UCSC.hg19_1.3.19 BSgenome_1.30.0
 [3] Biostrings_2.30.1                  GenomicRanges_1.14.4
 [5] XVector_0.2.0                      IRanges_1.20.7
 [7] atSNP_1.0                          motifStack_1.6.5
 [9] ade4_1.6-2                         MotIV_1.18.0
[11] BiocGenerics_0.8.0                 grImport_0.9-0
[13] XML_3.98-1.1                       testthat_0.9.1
[15] doMC_1.3.3                         iterators_1.0.7
```

```
[17] foreach_1.4.2                       data.table_1.9.4
[19] Rcpp_0.11.4

loaded via a namespace (and not attached):
 [1] BiocStyle_1.0.0 chron_2.3-45    codetools_0.2-8 compiler_3.1.1  lattice_0.20-29
 [6] plyr_1.8.1      reshape2_1.4.1  rGADEM_2.10.0   seqLogo_1.28.0  stats4_3.1.1
[11] stringr_0.6.2   tools_3.1.1
```