Motif Discovery from SELEX-seq data

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1 Background

This package provides a new and efficient implementation of an approach for analyzing SELEX-seq data. The underlying methodology was developed by Todd Riley and Harmen Bussemaker with significant input from Matthew Slattery and Richard Mann, and described in detail in two papers listed in the References section below: Slattery *et al.* (2011) and Riley *et al.* (2014). We request that you cite both papers when you use this software. This tutorial will walk you through a example SELEX analysis using (down-sampled) data from Slattery *et al.* (2011).

2 Installation

Before installing the `SELEX` package, you will need to install `rJava`, which is available from CRAN. If Java is properly installed on your machine, typing

```
install.packages('rJava')
```

should properly install the package. If you encounter any difficulty installing `rJava`, please refer to its documentation at `http://www.rforge.net/rJava/`. You can continue the rest of the tutorial after installing the `SELEX` package.

3 Initializing the SELEX Workspace

Before you load the SELEX package, you need to set the maximum Java memory usage limit:

```
> options(java.parameters="-Xmx1500M")
> library(SELEX)
```

Workflow in the SELEX package is centered around the workspace. The
workspace consists of samples and data files you are currently working with,
and the saved outputs of the various analyses you have run on these data.
The workspace has a physical location on disk, and can be configured at any
time:

```
> workDir = "./cache/"
> selex.config(workingDir=workDir, maxThreadNumber=4)
```

Before any analyses can be performed, samples must be made 'visible' to the
current SELEX session, which can be performed with the selex.loadAnnotation
or selex.defineSample commands. Loading a sample lets the current SE-
LEX session know the experimental setup of your data. You are required
to provide round, barcode, variable region, and file path information for
each sample you load. selex.defineSample is convenient when one needs
to quickly analyze new data, but the XML-based database used by se-
lex.loadAnnotation can be very useful for long-term storage and cataloging
of data.

```
> # Extract example data from package, including XML annotation
> exampleFiles = selex.exampledata(workDir)
> # Load all sample files using XML database
> selex.loadAnnotation(exampleFiles[3])
```

You can use selex.sampleSummary to see the currently available datasets:

```
> selex.sampleSummary()
```

```
        seqName     sampleName rounds leftBarcode rightBarcode
1 R0.libraries R0.barcodeCG       0         TGG      CCACGTC
2 R0.libraries R0.barcodeGC       0         TGG      CCAGCTG
3 R2.libraries     ExdHox.R2      2         TGG      CCAGCTG
                    leftFlank                        rightFlank
1 GTTCAGAGTTCTACAGTCCGACGATCTGG CCACGTCTCGTATGCCGTCTTCTGCTTG
2 GTTCAGAGTTCTACAGTCCGACGATCTGG CCAGCTGTCGTATGCCGTCTTCTGCTTG
3 GTTCAGAGTTCTACAGTCCGACGATCTGG CCAGCTGTCGTATGCCGTCTTCTGCTTG
            seqFile
```

```
1 ./cache/R0.fastq.gz
2 ./cache/R0.fastq.gz
3 ./cache/R2.fastq.gz
```

Sample handles are an easy way to address visible data, and are used by most package functions to allow easy manipulation of your datasets.

```
> r0train = selex.sample(seqName="R0.libraries",
+           sampleName="R0.barcodeGC", round=0)
> r0test = selex.sample(seqName="R0.libraries",
+           sampleName="R0.barcodeCG", round=0)
> r2 = selex.sample(seqName="R2.libraries",
+        sampleName="ExdHox.R2", round=2)
```

At this point, you are ready to analyze your data.

4   Building the Markov Model

In order to properly identify motifs from a SELEX experiment, one needs to be able to characterize the non-randomness of the initial pool of oligomers. This non-randomness can be represented with a Markov model. In order to choose the optimal Markov model, models of various orders will be evaluated, and the one with the greatest cross-validated predictive capability will be chosen. This requires a testing dataset and finding the longest oligonucleotide length k such that all K-mers within this dataset are found at least 100 times. You can find this value using `selex.kmax`:

```
> kmax.value = selex.kmax(sample=r0test)
```

Now, the optimal Markov model can be found, built, and stored:

```
> mm = selex.mm(sample=r0train, order=NA,
+        crossValidationSample=r0test, Kmax=kmax.value)
```

You can use `selex.mmSummary` to see the cross-validated R^2 values of these models:

```
> selex.mmSummary()
```

	Sample	Order	MarkovModelType	R
1	R0.libraries.R0.barcodeGC.0	0	DIVISION	0.9800940
2	R0.libraries.R0.barcodeGC.0	1	DIVISION	0.9892230
3	R0.libraries.R0.barcodeGC.0	2	DIVISION	0.9891462
4	R0.libraries.R0.barcodeGC.0	3	DIVISION	0.9889096
5	R0.libraries.R0.barcodeGC.0	4	DIVISION	0.9837154

|	CVSample	CVLength
1	R0.libraries.R0.barcodeCG.0	5
2	R0.libraries.R0.barcodeCG.0	5
3	R0.libraries.R0.barcodeCG.0	5
4	R0.libraries.R0.barcodeCG.0	5
5	R0.libraries.R0.barcodeCG.0	5
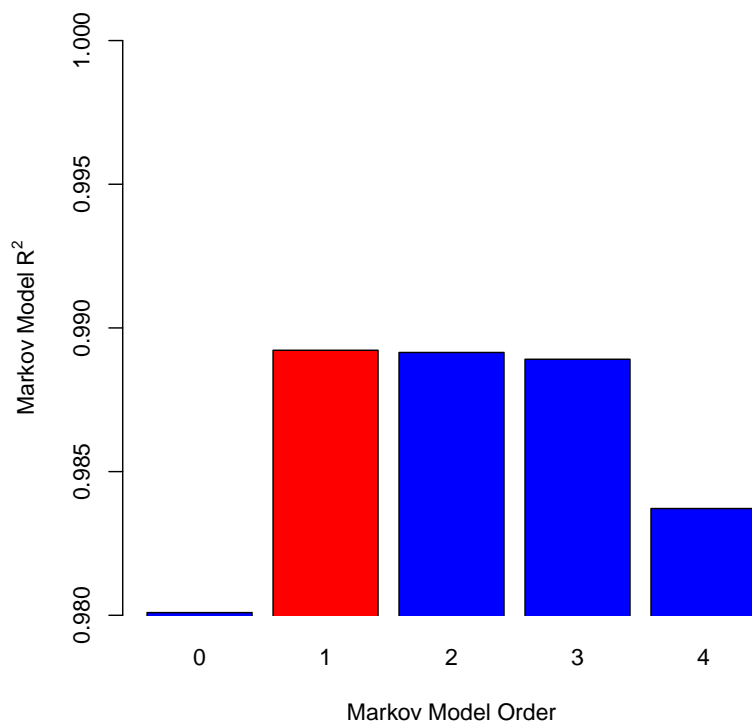
5 Finding the Optimal Motif Length

By observing how concentrated the distribution of later round K-mers frequences vs. previous round K-mer frequencies becomes, we can find the optimal binding site length. The Kullback-Leibler divergence metric can be used to measure this concentration for a variety of lengths using `selex.infogain`:

```
> selex.infogain(sample=r2,markovModel=mm)
```

`selex.infogainSummary` can be used to view the results:

```
> selex.infogainSummary()[,1:3]
```

```
                      Sample  K InformationGain
1  R2.libraries.ExdHox.R2.2  2         0.1448297
2  R2.libraries.ExdHox.R2.2  3         0.3623032
3  R2.libraries.ExdHox.R2.2  4         0.6695950
4  R2.libraries.ExdHox.R2.2  5         1.0660545
5  R2.libraries.ExdHox.R2.2  6         1.5104582
6  R2.libraries.ExdHox.R2.2  7         1.9133892
7  R2.libraries.ExdHox.R2.2  8         2.2302201
8  R2.libraries.ExdHox.R2.2  9         2.4158743
9  R2.libraries.ExdHox.R2.2 10         2.4143849
10 R2.libraries.ExdHox.R2.2 11         2.0598432
11 R2.libraries.ExdHox.R2.2 12         1.1818942
12 R2.libraries.ExdHox.R2.2 13         0.1063632
13 R2.libraries.ExdHox.R2.2 14         0.0000000
14 R2.libraries.ExdHox.R2.2 15         0.0000000
15 R2.libraries.ExdHox.R2.2 16         0.0000000
```
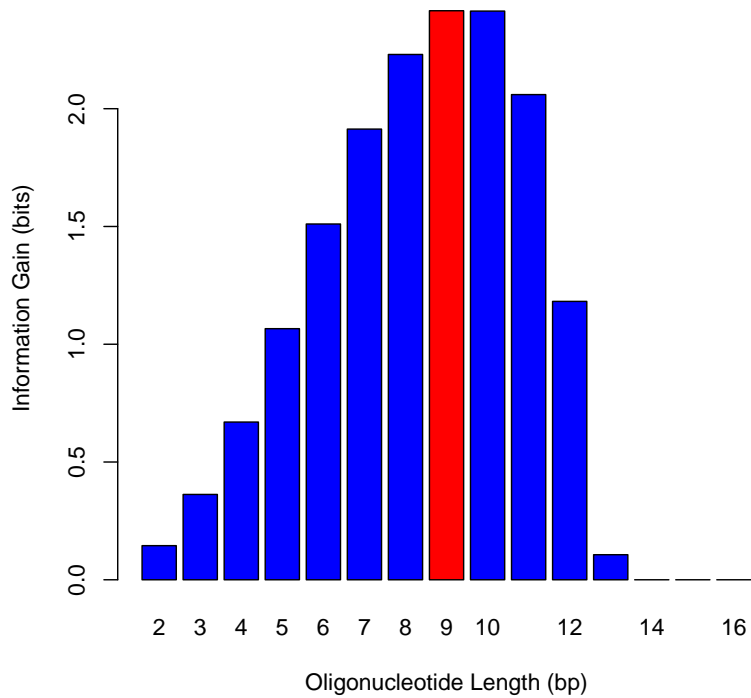
To see what the K-mer count tables look like for the optimal length, use selex.counts:

```
> table = selex.counts(sample=r2, k=optimalLength,
+           markovModel=mm)

> head(table)

      Kmer ObservedCount  Probability ExpectedCount
1 ATGATTGAT          7118 7.741514e-06      3.093261
2 TGATTGATT          6068 9.665983e-06      3.862217
3 TGATTGATG          4310 7.506314e-06      2.999283
4 GATTGATTA          3996 7.916402e-06      3.163141
5 ATCAATCAT          3870 4.642924e-06      1.855164
6 TTGATTGAT          3451 9.665982e-06      3.862217
```

6 Calculating Affinity and Error

With the optimal binding length, you can estimate the affinity and the standard error of the estimate with `selex.affinities` and a Markov Model:

```
> aff = selex.affinities(sample=r2, k=optimalLength,
+          markovModel=mm)

> head(aff)[,1:4]

      Kmer ObservedCount  Probability ExpectedCount
1 ATGATTGAT          7118 7.741514e-06      3.093261
2 TGATTGATT          6068 9.665983e-06      3.862217
3 TGATTGATG          4310 7.506314e-06      2.999283
4 GATTGATTA          3996 7.916402e-06      3.163141
5 ATCAATCAT          3870 4.642924e-06      1.855164
6 TTGATTGAT          3451 9.665982e-06      3.862217

   Affinity         SE
1 1.0000000 0.01676239
2 0.8262924 0.01500120
3 0.7902404 0.01702298
4 0.7409396 0.01657620
5 0.9521244 0.02164478
6 0.6231369 0.01500120
```

References

[1] Slattery, M.*, Riley, T.*, Liu, P., Abe, N., Gomez-Alcala, P., Dror, I., Zhou, T., Rohs, R.†, Honig, B.†, Bussemaker, H.J.†,and Mann, R.S.†". (2011) *Cofactor binding evokes latent differences in DNA binding specificity between Hox proteins.* **Cell** 147:1270–1282. [PMID:22153072]

[2] Riley, T.R.*, Slattery, M.*, Abe, N., Rastogi, C., Liu, D., Mann, R.S.†, and Bussemaker, H.J.†. (2014) *SELEX-seq: a method for characterizing the complete repertoire of binding site preferences for transcription factor complexes.* **Methods Mol. Biol.** 1196:255–278. [PMID:25151169]