



Swiss Institute of
Bioinformatics



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

École Polytechnique Fédérale de Lausanne

Swiss Institute of Bioinformatics

TEMPLE v.1.0

Maria Litovchenko^{1,2} and Stefan Laurent^{1,2}

¹School of Life Sciences, École Polytechnique Fédérale
de Lausanne, 1015 Lausanne, Switzerland

²Swiss Institute of Bioinformatics, 1015 Lausanne,
Switzerland

Contents

1	Installation	3
1.1	Requirements	3
1.2	Download	3
1.3	Support	3
2	Graphical version of TEMPLE	4
2.1	Structure of input files in the graphical mode	4
2.1.1	The sequence file	4
2.1.2	The region file	5
2.1.3	Position Weight Matrix (PWM) file	6
2.2	Specifying sequence and region sets	6
2.2.1	Sequence sets	6
2.2.2	Region sets	7
2.3	Saving and loading workspaces	9
2.4	Single population analysis	9
2.5	Two population analysis	9
2.6	Visualization	9
2.7	Diversity analysis	10
3	Command-line version of TEMPLE	11
3.1	Structure of input files	11
3.1.1	The sequence file	11
3.1.2	The region file	12
3.1.3	The PWM file	13
3.2	Execution	13
4	The output files	15
4.1	The Mutation output file	15
4.2	The Transcription Factor Binding Site (TFBS) output	18
4.3	The region output	19

5	Appendix	20
5.1	Algorithm description	20
5.1.1	Transformation of alignment matrices into weight matrices	21
5.1.2	Scoring function	21
5.1.3	Calculation of p-values and determination of the cutoff score	22
5.2	List of Abbreviations	23
5.3	Citation and information	23
	Bibliography	24

Chapter 1

Installation

1.1 Requirements

Temple can run on any operating system provided that java runtime environment (JRE) 1.7 or higher is installed.

1.2 Download

1. Download the compressed (rar) file at the following link: <https://github.com/gTEMPLE>
2. Uncompress the rar file and enter the folder.
3. Double click on the file called TEMPLE.jar to launch the graphical version (make sure to make TEMPLE.jar executable).

1.3 Support

Please direct your questions to the gTEMPLE support group: <https://groups.google.com/forum/?hl=en#!forum/gtemple-support>.

Chapter 2

Graphical version of TEMPLE

2.1 Structure of input files in the graphical mode

2.1.1 The sequence file

The file containing the DNA alignment has to be uploaded with "File/Open sequence(s)". The alignment should be in the FASTA format but the following information has to be added to the sequence names.

Chromosome	e.g. X, 2L, 19,...
Start coordinate	This can be the start coordinate of the sequence in an appropriate reference genome or simply set to "1" if a reference genome is not available.
Stop coordinate	Coordinate of the last nucleotide of the sequence in the coordinate system of an appropriate reference sequence. Alternatively this can be set to the length of the sequence if an appropriate reference genome is not available.
ID	ID of the sequence.

These four information fields have to be specified in the following way: CHR.START_STOP_ID. Here are two examples of a valid sequence name:

```
>X_1200000_1205000_Pop1seq35
```

```
>chromosome_1_10000_PopSouthIndividual23.
```

The sequences themselves can be written on one line or on multiple lines. If sequences are longer than 250kb please consider splitting them over multiple lines in order to speed up the execution time of the program. Note that TEMPLE does not recognize the International Union of Pure and Applied Chemistry ([IUPAC](#)) ambiguity code. The only valid states in the FASTA sequence are A,T,G,C,N,-, where "N" and "-" represents missing data and gaps, respectively. Please note that TEMPLE will not align the sequences. The user has to make sure that the DNA sequences in the sequence file represent a valid alignment. Allowed file extensions for the sequence file are .fas, .fasta., and, .afa.

Important note: names of the sequences must be unique

2.1.2 The region file

The "region file" can be uploaded from the menu by clicking on "File/Open region(s) file". This file contains the information on the genomic regions in which [TFBS](#) will be predicted and analyzed. Several regions can be defined on successive rows. The first line of the file has to be a header containing the following column names separated by tabulations or semicolons: chr, start, stop, id, pwms. All columns have to be present and specified. Description of each column can be found below. Table [2.1](#) illustrates an example of a possible region file.

chr	The name of the chromosome.
start	Start coordinate of the region in the alignment.
stop	Stop coordinate of the region in the alignment.
id	Name of the region.
pwms	This column tells TEMPLE which PWM will be used to score the region(s). Possible values are: all, or a list of PWMs names separated by commas (e.g. pho, zeste).

Table 2.1: An example of region file.

chr	start	stop	id	pwms
chrX	1	1000	region1	pho
scaffold34	150	950	geneX	all

2.1.3 PWM file

The "PWM" file has to be uploaded from the menu by clicking on "File/Open PWM file". This file has to contain horizontal or vertical count position weight matrices. The format is the same as the one provided by the "Fly Factor Survey" database (<http://pgfe.umassmed.edu/ffs/>). Similarly to the FASTA format the first line has to start with the "greater than" sign (">") followed by the name of the PWM. The next four lines (or columns in the vertical format) contain the matrix itself. The PWM file can be used to upload multiple PWMs. Table 2.2 illustrates an example of a valid PWM.

2.2 Specifying sequence and region sets

Grouping sequences into sequence sets can be done from the menu by clicking on "Data/Specify sequence sets".

2.2.1 Sequence sets

Example:

1. Specify a name for the new set (see Figure 2.1)
2. Select sequences in the left panel using the mouse. Multiple sequences can be selected with the use of the "ctrl" and "shift" keys. You can also search for sequences whose names share a common substring (for

>pho															
A	142	111	134	299	244	221	419	0	0	0	37	12	81	139	77
C	75	159	204	26	59	52	0	0	0	0	383	44	62	208	146
G	96	88	58	80	6	90	0	0	429	429	0	340	243	30	66
T	84	64	33	24	120	66	10	429	0	0	9	33	43	49	115

Table 2.2: An example of PWM file.

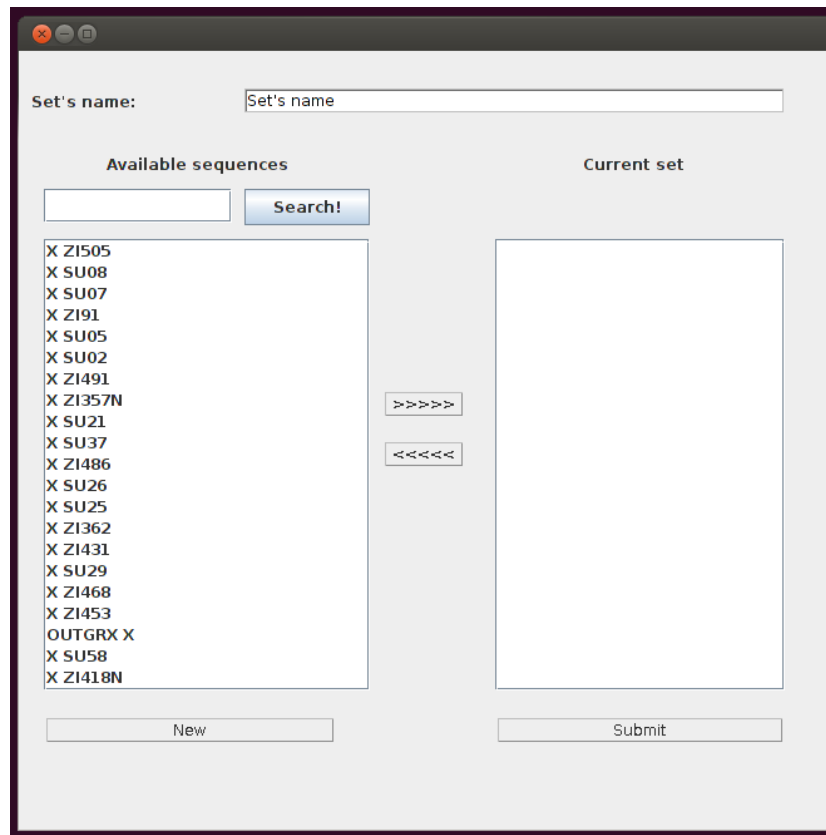


Figure 2.1: The sequence set definition window.

example "ZI" in figure 2.2). Just type in the string of interest in the search box and press "search!" (see Figure 2.2).

3. Move all selected sequences to the right panel by clicking on the ">>>>>" button.
4. Save the sequence set by clicking on the "Submit" button.
5. Close the window or create a new sequence set by clicking on the "New" button. (A sequence set should not be created for the outgroup sequence)

2.2.2 Region sets

Region sets can be defined from the menu by clicking on "Data/Specify region sets", and are created in the same way as sequence sets. Region sets can be

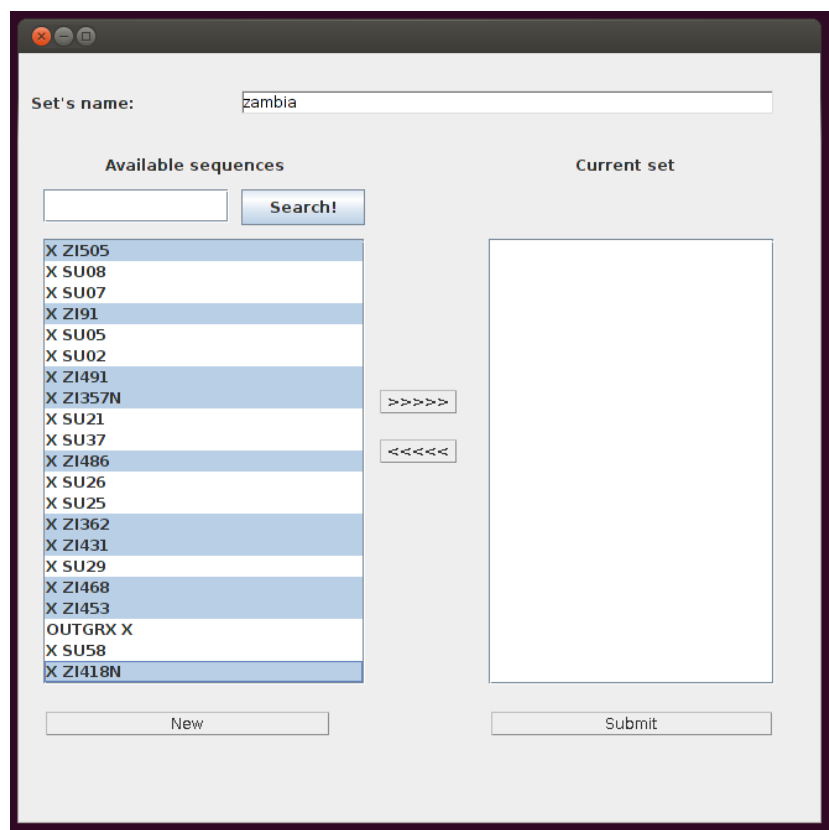


Figure 2.2: Creating a new sequence set.

used to restrict the analyses to a subset of the regions contained in the region file.

2.3 Saving and loading workspaces

Temple saves all settings relative to the sequence and region sets, the uploaded PWMs, as well as an index of the sequence file in workspaces. Saving and loading workspaces can be done with "File/Save workspace" and "File/Load workspace". For large alignments (>250kb), the use of workspaces is highly recommended.

2.4 Single population analysis

A single population analysis can be launched from the menu by clicking on "Analysis/Single set". Five independent analyses using different sequence and region sets can be specified and conducted in parallel. Note that depending on the size of the uploaded datasets the time needed for the calculation to complete can vary from a few minutes to several hours. It is also possible to include p-values for the difference in scores in the output by checking box "calculate difference in scores p-value".

Calculation of p-values for the difference in scores can be time-consuming.

2.5 Two population analysis

A two-population analysis can be launched from the menu by clicking on "Analysis/Double set". The settings are similar to the case of one population.

2.6 Visualization

After TEMPLE has successfully finished the calculations the program stores all results in the result folder (whose path was specified by the user). Results can be visualized by clicking on "View/View results". This will open a window in which the user is prompted to specify the samples and the regions for visualization. (In the case of a single population analysis the user is required to explicitly set the second sequence set to "empty"). If the analysis was conducted with an outgroup sequence the name of the outgroup can be defined at this stage. The user also has to choose which genomic region to visualize. Finally, the user has to specify the location of the .tfbs file (located

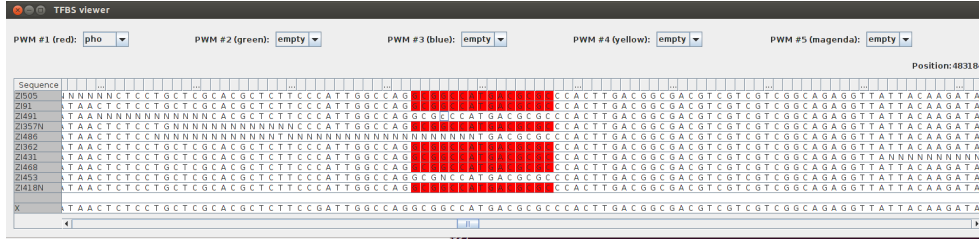


Figure 2.3: The visualization window

in the result folder). Pressing on proceed should open the visualization window (Figure 2.3, this can take several minutes). The user can highlight DNA regions for which a significant score was calculated by the **TFBS** prediction function. This can be done for up to five different **PWMs** using the different drop-down menus available on the upper part of the screen.

2.7 Diversity analysis

The main function of TEMPLE is to report and analyze the genetic diversity that occurs at **TFBS** loci. For TEMPLE, a **TFBS** loci is a loci at which at least one sequence has generated a significant **PWM** score. The genetic diversity identified at **TFBS** loci is reported in three different output files that represent three hierarchical levels at which diversity can be observed: the mutation, **TFBS**, and region level. In this three output files each row represents, a mutation, a **TFBS**, and a region, respectively. The three files have essentially the same format. The first lines provide a short summary of the dataset that was analyzed and are followed by the "results" section. Whose three files are comprehensively described below.

Chapter 3

Command-line version of TEMPLE

TEMPLE can also be run in the command line mode. The only differences with graphical user interface ([GUI](#)) mode described above are the arguments that are passed on the command line and the additional information that needs to be added to the alignments and region files.

3.1 Structure of input files

3.1.1 The sequence file

Overall, the input file containing sequences in the case of one population analysis should be in a commonly accepted fasta format. However, following changes should be made to the file:

- The user should indicate to TEMPLE which sequences belong to which populations. This is done by grouping the sequences according to which population they belong to in the alignment file then separating the two groups with a double slash symbol (//). Populations identifier can be specified after the “//”. Listing 4.1 provides an example:

```
5 //pop_north
>chrX_12000_20000_northIndividual1
AGTGTGTGTA
>chrX_12000_20000_northIndividual2
AGTGTGTGTA
//pop_south
>chrX_12000_20000_southIndividual1
AGTGTGTGTA
```

```

10 >chrX_12000_20000_southIndividual2
    AGTGTGTGTA
    //

```

Listing 3.1: Example of sequence file for command line version.

Important note: file must end with //

- The sequence names should follow the same pattern as described in [2.1.1](#)
- If an outgroup sequence is included, the name of the sequence should start with the letters “OUTGR”. For the two population analysis, outgroup sequence should always be located in the second group. Listing 4.2 provides an example:

```

//pop_north
>chrX_12000_20000_northIndividual1
AGTGTGTGTA
>chrX_12000_20000_northIndividual2
5 AGTGTGTGTA
//pop_south
>chrX_12000_20000_southIndividual1
AGTGTGTGTA
>chrX_12000_20000_southIndividual2
10 AGTGTGTGTA
>OUTGRchrX_12000_20000_sisterSpecies
AGTTTATGTA
//

```

Listing 3.2: Example of outgroup sequence set-up

3.1.2 The region file

An additional column (“set”) is needed in the region file when using the command-line version. In the one population analysis, this column should contain the id of the sequence set that has to be analyzed for a given region (e.g. pop_north in the example above). The ID should be the same as the one used in the alignment file. In the two population analysis the sequence set IDs have to be combined with the ampersand symbol (“&”, Table [3.1](#)).

set	chr	start	stop	id	pwms
pop_north&pop_south	chrX	1	1000	region1	pho
pop_south	scaffold34	150	950	geneX	all

Table 3.1: An example of region file for command line version.

3.1.3 The PWM file

The format of the PWM file is the same as in the [GUI](#) mode, but information about the significance threshold used for the motif prediction can be specified next to the line with the name of the sequence with use of key-words "pval" and "score", see [table 3.2](#) for example. If no information is specified TEMPLE will assumes that the maximum $\ln(\text{p-value})$ for printing scores equals the negative of the sample-size adjusted information content (-li option in patser).

>phol	pval=0.001													
A	142	111	134	299	244	221	419	0	0	0	37	12	81	139
C	75	159	204	26	59	52	0	0	0	0	383	44	62	208
G	96	88	58	80	6	90	0	0	429	429	0	340	243	30
T	84	64	33	24	120	66	10	429	0	0	9	33	43	49
>phol2	score=100													
A	142	111	134	299	244	221	419	0	0	0	37	12	81	139
C	75	159	204	26	59	52	0	0	0	0	383	44	62	208
G	96	88	58	80	6	90	0	0	429	429	0	340	243	30
T	84	64	33	24	120	66	10	429	0	0	9	33	43	49

Table 3.2: An example of [PWM](#) file.

3.2 Execution

Execution of command line version of the program is achieved by the following command:

```
java -jar gTemple.jar "path/to/pwm/file" "path/to/fasta/
file" "path/to/region/file" "number/of/threads" "
threshold/for/missing/data" "analysisMode" "
withPvalue"
```

Listing 3.3: Example of outgroup sequence set-up

where

- path/to/pwm/file is a location of the file containing the position weight matrices
- path/to/fastq/file is a location of the file containing the sequence alignments
- path/to/region/file is a location of the file containing the description of the regions to be analyzed
- number/of/threads is a number of java multi-threads requested for the analysis
- threshold/for/missing/data is a proportion of valid DNA bases (i.e. A,T,G,C) at a given polymorphic site below which the site is not analyzed
- analysisMode equals 1 for a one population analysis and 2 for a two-population analysis.
- withPvalue OPTIONAL, if added p-values for difference in scores will be calculated. The calculations can be time consuming.

Chapter 4

The output files

As a result, TEMPLE produces three output file: `_MUTATION.csv`, `_TFBSUnique.csv` and `_REGION.csv`. The results found in the files are described below.

4.1 The Mutation output file

1. Region
The name of the region in which the mutation has been found
2. Chr
The chromosome on which the mutation has been identified
3. Region_start
The absolute position of the first nucleotide in the region
4. Region_stop
The absolute position of the last nucleotide in the region.
5. PWM
The name of the [PWM](#) as it is specified in the [PWM](#) file.
6. Position
Position of the mutation in the coordinate system of the uploaded DNA sequence (as specified in the name of the sequence in the fasta file)
7. Pos_in_tfbs
Position of the mutation within it's [TFBS](#) on the forward strand.

8. Reverse
Indicates whether the [TFBS](#) has been predicted on the forward (TRUE) or reverse strand (FALSE).
9. Type
Type of the genetic variant. Possible values are “snp” or “indel”.
10. NumOfAlleles
Number of alleles. The number of distinct allelic states observed at this position.
11. Length
Length of the genetic variant. If the identified variant is an single nucleotide polymorphism ([SNP](#)), the length is always one. For an indel it is the length of the indel.
12. Polarization
Possible values are -1, 0, and 1.”1” means that TEMPLE was able to use a valid outgroup sequence at this position to infer which of the two segregating alleles is the ancestral and which is the derived one.”0” means that no valid outgroup sequence was available (i.e. missing data, or allelic state not present in ingroup) for this site in which case TEMPLE considers that the derived allele is the allele with the lowest frequency. Finally, ”-1” means that the site was not polarizable (e.g. more than 2 allelic states are present).
13. A1,A2,A3,A4
The state of the observed alleles. If polarization is equal to 1 then A1 is the ancestral state (as inferred by the outgroup) and A12 is the derived state. If polarization was not possible A12 is the minor allele. If more than 2 alleles are present at a site, the order has no specific meaning.
14. A11_pop1, A12_pop1, A13_pop1, A14_pop1
The absolute frequencies of the allelic states reported in A11,A12,A13, and A14 in population 1 (population1 will have the name of the first sequence set that was defined). These column will be printed for the second population as well in a double population analysis.
15. NumbMisLines_pop1

The number of sequences in the alignment with missing data at this position in the first population (A second column is present in a double population analysis).

16. π

Nucleotide diversity calculated as $2pq$, where p and q are the relative frequencies of the derived and ancestral allele, respectively. (Note that in a double analysis π will be calculated on the pooled sequence sets)

17. F_{st}

The fixation index as comprehensively discussed in [Holsinger and Weir \[2009\]](#). Only for two populations-analyses.

18. EffectOnNucleotide

The difference in [TFBS](#) scores caused by the derived allele at the nucleotide level. This is calculated as $\Delta S = M(i, x_{derived}) - M(i, x_{ancestral})$, where M is the logodds pwm, i is the position of the [SNP](#) in the [TFBS](#), and $x_{derived}$ and $x_{ancestral}$ are the allelic states of the derived and ancestral alleles. If polarization is not possible the minor allele is considered to be the derived allele.

19. EffectOnTfbs

This is the average effect of the derived allele on the [TFBS](#) scores. This statistic is useful when more than one mutation occurs at a [TFBS](#). It is calculated as the difference between the average scores of the [TFBS](#) carrying the derived and the ancestral alleles, respectively. Note that all non-significant scores, as well as [TFBS](#) with one or more missing site(s) (N) are set to 0 before calculation of the averages so that this statistic will always differ from the "EffectOnNucleotide".

20. EffectOnRegion

This is similar to the EffectOnTFBS column, but here the score of a single [TFBS](#) is replaced by the sum of all scores across the complete region. Currently, only sequences with no missing data are included in the analysis. This statistic can be used to identify mutations that contribute to an increase or decrease of binding affinity over the whole region (as defined in the region file), taking into account potential compensatory mutations.

21. DiffInScorePval

p-value for the observed difference in score. The statistics is calculated according to [Macintyre et al. \[2010\]](#).

4.2 The TFBS output

Columns that have the same name as in the mutation output file contain the same information and are not re-described here.

1. Threshold

This is the cutoff value used by the PWM scoring method to identify significant TFBS. It is calculated following . Temple assumes that the maximum $\ln(\text{p-value})$ for printing scores equals the negative of the sample-size adjusted information content.

2. Len

The length of the predicted TFBS.

3. NumMut

The number of polymorphic sites at this TFBS locus

4. SumPi

Nucleotide diversity calculated as the sum of $2pq$ across all mutations within the TFBS.

5. mean_S_pop1

The average of the distribution of TFBS score in population 1. Non-significant scores and scores of TFBS containing missing data are set to 0 in the distribution of scores.

6. sd_S_pop1

The standard deviation of the distribution of TFBS score in population 1

7. S_Qst

The equivalent of the F_{st} statistic for a continuous phenotype. Here we consider the PWM score at the TFBS level as a truncated continuous phenotype for which values below the PWM threshold are set to zero. The $S_{Q_{st}}$ correspond to proportion of the total variance in truncated scores that is explained by the population effect. (The truncation is there to avoid that variation among non-significant scores affects the Q_{st} statistic). Scores of TFBS affected by missing data are set to 0 as well.

4.3 The region output

1. SumPiTFBS

The nucleotide diversity measured at **TFBS** site in this region. Calculated as the sum of 2pq across all polymorphic sites in all **TFBS** loci in this region.

2. LenTFBS

The sum of the length of all **TFBS** in this region. This can be used to measure the per site nucleotide diversity.

3. NumTFBS

The number of **TFBS** loci that have been predicted in this region

4. NumPolyTFBS

The number of polymorphic **TFBS**_loci predicted in this region

5. mean_RS_pop1

The mean of the distribution of region scores in sequence set 1. Region scores are calculated by summing over all significant **TFBS** scores in a region for a given sequence. Sequences for which one or more **TFBS** contain missing data ("N") will not be taken into account in the distribution of scores. (This is done to avoid that missing data affects the variation in score).

6. sd_RS_pop1

The standard deviation of the distribution of region scores (RS) in sequence set 1.

7. RS_ Q_{st}

This statistic is similar to the $S_{Q_{st}}$ value but is applied to region scores instead of **TFBS** score. This statistic can be used to identify regions that have large differences in score between populations.

Chapter 5

Appendix

5.1 Algorithm description

Bioinformatic predictions of [TFBS](#) rely on the assumption that all sequences that bind to a given DNA-binding protein share a certain level of similarity. Investigating the degree of similarity within a set of functionally related sequences starts by collecting DNA sequences that have been empirically shown to bind a given DNA-binding protein (e.g. genome-wide profiling experiments). The second step is to create an alignment of these sequences that maximizes sequence conservation. This alignment is then used to identify a sub-region characterized by a statistically significant high level of conservation. This region is then defined as the motif to which the DNA-binding protein binds. Although motifs consist more or less of conserved DNA sequences they usually contain some variation, such that these motifs cannot be fully represented by a single consensus sequence. A more appropriate way of representing such a motif is an alignment matrix (or count matrix). An example of such a matrix is given in [table 2.2](#). This matrix contains the number of times, n_{xi} , that a letter x is observed at position i in the alignment. In this matrix, for example, the positions seven to ten are highly conserved. This is an example matrix for input to the program. When searching for occurrences of TFBS in a genome or in a sub-genomic region, these matrices can be used to identify substrings that are similar to the motif described in the matrix. In practice, this is done by transforming the alignment matrix into a weight matrix, whose elements are the weights used to construct a similarity score to the motifs for every position of the investigated DNA sequence. Scores that are higher than a previously defined statistical threshold are reported and considered as bioinformatically predicted [TFBS](#). In the following section we describe how the program transforms alignment matrices into weight

matrices, how weight matrices are used to calculate a similarity score, how the probability distributions of score are calculated for every weight matrix, and which options are provided to the user to define a statistical threshold for [TFBS](#) predictions.

5.1.1 Transformation of alignment matrices into weight matrices

Let's consider a position weight matrix M indexed by $(1...m) \times 4$, where m is the length of the motif. The coefficients $M(i, x)$ give the scores at position i in $[1, m]$ for the letter x in A,T,G,C. TEMPLE follows [\[Hertz and Stormo, 1999\]](#) and calculates $M(i, x)$ as follows:

$$M(i, x) = \ln \frac{(n_{xi} + p_x)/(N + 1)}{p_x} \approx \ln \frac{f_{xi}}{p_x} \quad (5.1)$$

where n_{xi} is the number of times a letter x is observed in the alignment matrix, p_x is the *a priori* probability of the letter x , and N is the total number of sequences that was used to create the alignment matrix. The users should refer [\[Hertz and Stormo, 1999\]](#) for more detailed information. TEMPLE calculates p_x from the local base-pair composition of the currently analyzed genomic region.

5.1.2 Scoring function

Scoring DNA sequences given a position weight matrix is done using a sliding-window approach. We start at the first base pair of the sequence and calculate

$$Score(u, M) = \sum_{i=1}^m M(i, u_i) \quad (5.2)$$

Where u is a substring of size m , M is a position weight matrix, and u_i denotes the nucleotide found at position i in u . Once the score is calculated we calculate the next scores at each successive position in the sequence smaller or equal to $L-m+1$, where L is the length of the analyzed region. Note that when $u_i = \text{"N"}$ (i.e. missing information), $M(i, u_i) = 0$. Scores associated with a significant p-value are reported in the output files. The reverse strand is scored by scoring the forward strand with a reversed-complemented [PWM](#).

5.1.3 Calculation of p-values and determination of the cutoff score

To assess the significance of a given score, it is necessary to calculate the probability that a score equal or greater than occurs under a background model characterized by a randomized DNA sequence with the same *a priori* probabilities for the different nucleotidic states (p_x). To do this, we followed [Staden, 1989] and used probability-generating functions to calculate the complete probability distribution across all possible scores given a position weight matrix (M) and p_x . We recommend the users to read [Staden, 1989] as well as for a complete description of the algorithm.

Significance thresholds for motif predictions can be defined from the menu in "Data/Specify thresholds/pvalue for PWM". Here the user has access to three alternatives: set a threshold on the p-value, set a threshold on the score value itself, or let TEMPLE calculate a threshold automatically (default behavior, "Auto"). When the automatic threshold calculation is chosen, TEMPLE assumes that the maximum $\ln(p - value)$ for printing scores equals the negative of the sample-size adjusted information content (-li option in patser).

5.2 List of Abbreviations

GUI graphical user interface

JRE java runtime environment

IUPAC International Union of Pure and Applied Chemistry

TFBS Transcription Factor Binding Site

PWM Position Weight Matrix

SNP single nucleotide polymorphism

5.3 Citation and information

TEMPLE: a bioinformatic tool to analyze population genetic diversity at transcription factor binding sites. Contact: maria.litovchenko@gmail.com and stefan.laurent@epfl.ch

Bibliography

- Kent E Holsinger and Bruce S Weir. Genetics in geographically structured populations: defining, estimating and interpreting f_{st} . Nature Reviews Genetics, 10(9):639–650, 2009.
- Geoff Macintyre, James Bailey, Izhak Haviv, and Adam Kowalczyk. is-rsnp: a novel technique for in silico regulatory snp detection. Bioinformatics, 26(18):i524–i530, 2010.
- Gerald Z Hertz and Gary D. Stormo. Identifying dna and protein patterns with statistically significant alignments of multiple sequences. Bioinformatics, 15(7):563–577, 1999.
- Rodger Staden. Methods for calculating the probabilities of finding patterns in sequences. Computer applications in the biosciences: CABIOS, 5(2): 89–96, 1989.