GENEEXPRESS: A COMPUTER SYSTEM FOR DESCRIPTION, ANALYSIS, AND RECOGNITION OF REGULATORY SEQUENCES IN EUKARYOTIC GENOME

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Abstract

GeneExpress system has been designed to integrate description, analysis, and recognition of eukaryotic regulatory sequences. The system includes 5 basic units: (1) **GeneNet** contains an object-oriented database for accumulation of data on gene networks and signal transduction pathways and a Java-based viewer that allows an exploration and visualization of the GeneNet information; (2) **Transcription Regulation** combines the database on transcription regulatory regions of eukaryotic genes (***TRRD***) and ***TRRD Viewer;*** (3) **Transcription Factor Binding Site Recognition** contains a compilation of transcription factor binding sites (**TFBSC**) and programs for their analysis and recognition; (4) **mRNA Translation** is designed for analysis of structural and contextual features of mRNA 5'UTRs and prediction of their translation efficiency; and (5) **ACTIVITY** is the module for analysis and site activity prediction of a given nucleotide sequence. Integration of the databases in the **GeneExpress** is based on the Sequence Retrieval System (**SRS**) created in the European Bioinformatics Institute.

GeneExpress is available at ***http://wwwmgs.bionet.nsc.ru/systems/GeneExpress/.***

Introduction

The eukaryotic gene expression is one of the most complex biological phenomena involving a number of molecular events. It may start with reception of a definite stimulus by the cell, which is then conveyed via the particular signal transduction pathway to initiate transcription of the relevant genes. Their pre-mRNAs are processed by 3' cutting/polyadenylation, capping, splicing, and finally the corresponding proteins are translated from these mature mRNAs. This totality of molecular events forms the particular gene network that provides the cell response to the stimulus. The cellular and organismic homeostases as well as cell/tissue differentiation and development are maintained by their gene networks. That is the reason why molecular biologists investigating the gene expression should be able to access databases on all the stages of gene expression as well as the relevant programs for their analysis. Thus, investigation of the gene expression is an integrative problem of biology.

Currently, various experimental data on genomic regulatory sequences controlling the eukaryotic gene expression are being rapidly accumulated. The transcription regulatory regions have been sequenced for thousands of genes. A great number of transcription regulatory elements have been localized including transcription factor binding sites, enhancers, promoters, etc. (Kel' A.E. et al., 1997; Peter et al., 1998; Wingender et al., 1996). A wide range of functional sites controlling other stages of gene expression (splicing, processing-polyadenylation, and translation) have been isolated and studied. A considerable volume of the experimental data on the activity of various types of functional sites controlling the gene expression has been generated (Kolchanov et al., 1998). The experimental data on the gene networks, the ensembles of coordinately functioning

genes (Kolpakov et al., 1998), is growing. Computer analysis of the genomic regulatory sequences becomes even more important in case of functional interpretation of newly sequenced genomic fragments as well as for study of the molecular mechanisms of gene expression regulation. The current number of databases on various genomic regulatory regions is considerable. In addition to the general databases, such as EMBL and GenBank, a number of specialized databases on gene expression regulation are available: EPD (Peter et al., 1998), TRANSFAC (Wingender et al., 1996), TRRD (Kel', A.E. et al., 1997), COMPEL (Kel, O.V. et al., 1995b), EpoDB (Salas et al., 1998), etc. Many computer methods for recognition of regulatory genomic sequences (Waterman et al, 1984; Lawrence et al., 1993; Chen et al., 1995; Ulyanov & Stormo, 1995; Quandt et al., 1995; Fickett & Hatzigeorgiou, 1997 (review); Kel, A.E. et al., 1995; Prestridge, 1995; Pedersen et al., 1996; Solovyev & Salamov, 1997; Salamov & Solovyev, 1997) have been developed. Thus, the challenging problem is to create a WWW-based environment capable of integrating the information coming from various databases on expression regulation and make this information accessible by software for investigation and prediction of regulatory sequences.

We started this integration from cross-linking the **TRANSFAC, TRRD,** and **COMPEL** databases through introduction of a common format table for all of them (Wingender et al., 1996). Appearance of **SRS** query system (Etzold and Argos, 1993) opened a new era of web-integration. It provides unification of queries to various databases concealing any specific details of their realization; unified representation of the queried information; flexible format of information representation (for example, FASTA, PIR, etc.); a possibility to include additional modules for graphic representation; a powerful reference and help systems for each of the databases; and a possibility of linkage with the other databases and computer systems.

Using **SRS,** we have developed **GeneExpress,** the **SRS**-based integrator for the databases and programs supporting investigation of the gene expression. The database **GeneNet** on molecular events forming gene networks was assigned its integrative core. To study transcription, this core was supplemented with the database **TRRD** on transcription regulatory regions and the compilation **TFBSC** of the sequence sets of transcription factor binding sites. The **TRRD** and **TFBSC** were linked to the system **RgScan,** recognizing the sites in DNA sequences. For translation, the database **LeaderRNA** on mRNA leaders was included and linked to the program predicting the High/Low translation levels from a given mRNA sequence. The gene expression is also quantitatively described by the system **ACTIVITY** compiling the functional site activity magnitudes and linked with the programs predicting the activities from site sequences. Thus, the **GeneExpress** system is designed to integrate description, analysis, and recognition of eukaryotic genomic sequences. The modular and hierarchical organization of regulatory genomic sequences and the network-organized regulation of gene expression were taken into consideration during the system development. **GeneExpress**, is WWW-available at http://wwwmgs.bionet.nsc.ru/systems/GeneExpress/.

GENE NETWORKS

The **GeneNet** database is designed for accumulation of formalized description of gene networks and signal transduction pathways. Using the object-oriented approach, the following components are included in the description of a gene network: entities (any material objects), relations between the entities, and processes connected with them (for example, viral infection, anemia, or erythrocyte differentiation). Four classes of entities are distinguished: (1) Cell (tissue, organ) entity, regarded as a definite compartment containing a certain set of entities of other classes; (2) Protein; (3) Gene; and (4) Substance (a nonprotein regulatory substance, for example, metabolite). Two classes of relations between the entities are described: (1) reaction of interaction between entities yielding a new entity or process; and (2) regulatory event as the effect of an entity on a certain reaction. Instances of Cell (tissue, organ), Gene, Protein, Substance, State, and Relation classes are described in the separate tables CELL, GENE, PROTEIN, STATE, and RELATION, respectively. The database is also supplemented with the SCHEME table. Thus, the database contains eight tables in the EMBL-like text format: (1) CELL (information on the cell types and lines, including also the description of tissues and organs); (2) GENE (genes and their regulatory features based on the information from the TRRD database); (3) PROTEIN (proteins and protein complexes); (4) SUBSTANCE (regulatory substances and metabolites); (5) PROCESS (physiological process and the organismic state during the gene network functioning); (6) RELATION (relations between the gene network components); and (7) SCHEME (description of the gene network graph).

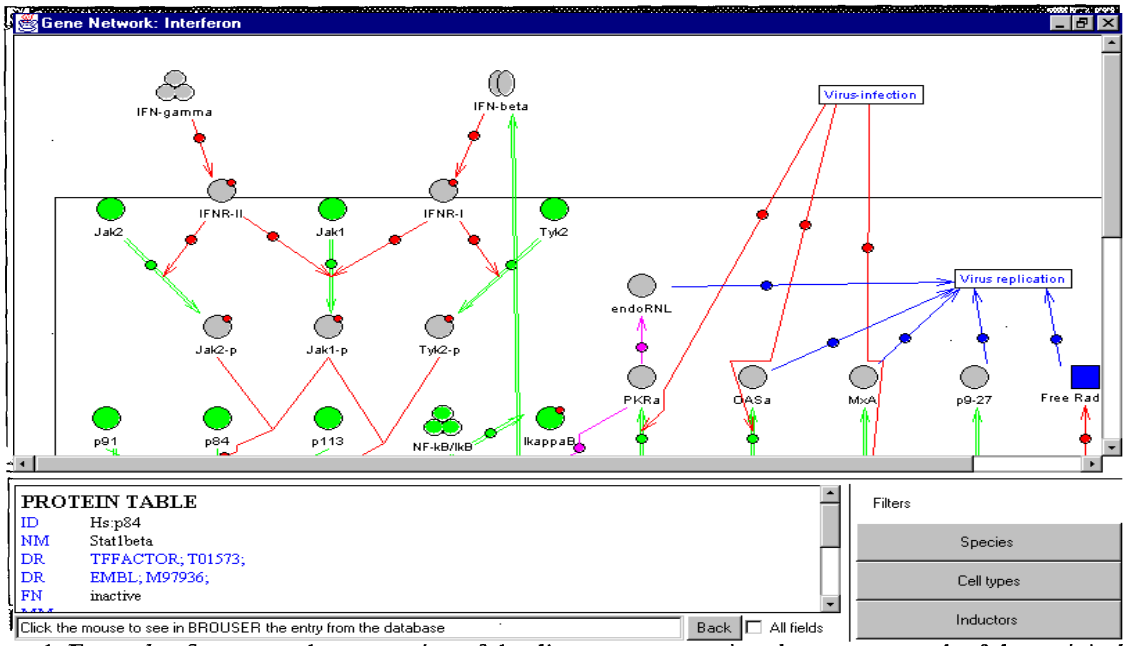


Figure 1. Example of automated construction of the diagram representing the gene network of the antiviral response at the cell level

The **GeneNet** database is also developed using the **SRS**. It supports the cross-references within the **GeneNet** database and with EMBL, SWISS-PROT, TRRD, TRANSFAC, and EPD databases. The current version of the **GeneNet** database contains the descriptions of gene networks of antiviral response (Ananko et al., 1997) and erythropoiesis (Podkolodnaya and Stepanenko, 1997).

The **GeneNet** includes automatic construction of a gene network diagram. The diagram is presented as a graph with the nodes corresponding to entities or states and the edges reflecting the relations between the gene network components. Information on the graph structure is taken from the SCHEME table. Each gene network component has its own image on the diagram, showing its features (Fig. 1). The **GeneNet** system takes into account that the gene network components can belong to different organs, tissues, cells, and cell compartments. The three following hierarchical levels are considered: (1) **organism level**, at which such entities as organs, tissues, cell types, and various substances affect other organs, tissues, and cells; (2) **the single cell level**, where four compartments are distinguished: the intercellular space, cell membrane, cytoplasm, and nucleus; and (3) **the single gene level**, where the description of transcription regulation employs the data from the TRRD database. Each level can be displayed in a separate window. The gene level is visualized via the TRRD Viewer described above.

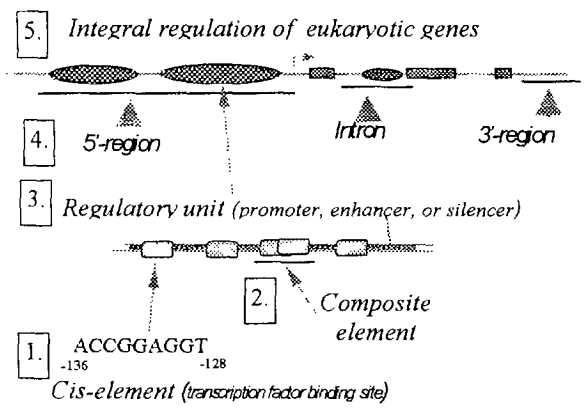


Figure 2. **Structural and functional organization of eukaryotic genes.**

The **GeneNet Viewer** is a Java applet. It includes the above-described generation of the gene network diagram and some tools for data navigation, on-line help, interactive cross-references within the **GeneNet** database, and references to other databases. All images on the diagram are interactive, i.e., if a user clicks the image, the textual description of the corresponding entry is displayed in the special text window under the diagram (Fig. 1). Double clicking the gene image starts the **TRRD Viewer**, and the regulatory map of the gene is visualized. The text window contains a formatted text with hypertext references of three types: (1) the reference explaining the type of information described in the field; (2) cross-references within the **GeneNet**

database; and (3) references to other databases (EMBL, SWISS-PROT, TRRD, TRANSFAC, and EPD).

TRANSCRIPTION REGULATION

Transcription Regulatory Regions Database (TRRD)

The model of functional organization of eukaryotic gene regulatory regions (Kel', A.E. et al., 1997; Kel, O.V. et al., 1995a) was used as the basis for the **TRRD** database. It takes into account a great diversity of the elements controlling gene transcription, their modular organization, and the hierarchy of these elements, essential for their functioning. The TRRD format

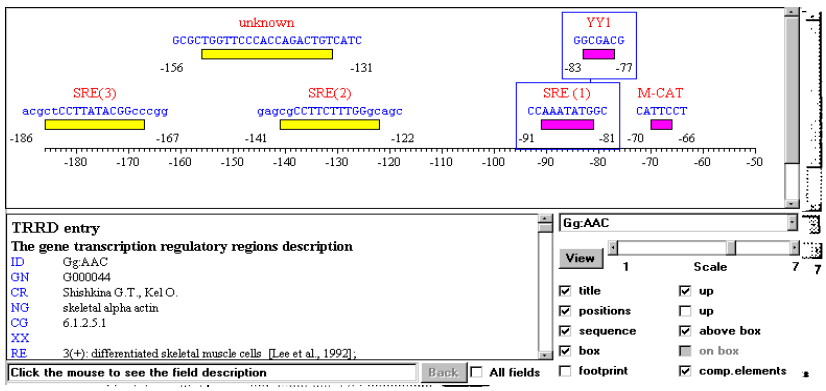


Figure 3. Example of visualization of gene regulatory map by TRRD Viewer. Boxes represent binding sites of transcription factors; the line

allows describing the modular structure of transcription regulatory regions and the hierarchy of their constituent regulatory units. The hierarchy of the following elements has been implemented (Fig. 2): (1) ***Cis-elements*** provide the interaction of transcription factors with DNA (Wingender, 1993); (2) ***Composite elements*** support the interactions between DNA sites and the protein factors or the protein-protein interactions, causing either synergistic or antagonistic regulatory effects (Kel, O.V. et al., 1995b; Kel', A.E. et al., 1997); (3) ***Promoters, enhancers, and silencers*** at this level of the hierarchy provide the transcription regulation under certain conditions; (4) ***Transcription regulatory regions*** are represented by continuous regions of genomic DNA containing the regulatory elements of the levels described above (Kel, O.V. et al., 1995a; Kolchanov, 1997) and located in the gene 3'- and 5'-flanking regions or introns; and (5) ***The system of integral regulation of gene transcription*** comprises all these regulatory elements (Kolchanov, 1997; Kel', A.E. et al., 1997).

The **TRRD** database has three interconnected tables: **TRRDGENES** (description of genes), **TRRDSITES** (description of sites), and **TRRDBIB** (references).

TRRDGENES contains a general description of genes, peculiarities of their transcription regulation (dependence on the cell cycle stage, developmental stage, tissue-specificity, or effects of external factors), chromosomal location, description of regulatory units (promoters, enhancers, or silencers), composite elements, and free text comments. This table is linked to the **TRANSFAC_GENE** (Wingender et al., 1996), **COMPEL** (Kel, O.V., 1995b), and **GeneNet** (Kolpakov et al., 1998) databases.

TRRDSITES accumulates information on transcription factor binding sites: nucleotide sequences and their location within the gene, the list of the relevant cell lines and codes of experiments, and free text comments. This table is linked to the following computer systems: **ACTIVITY** for predicting site activity (Kolchanov et al., 1998) and programs for site recognition (Kondrakhin et al., 1998). The table also contains the references to **TRANSFAC_SITE**, **TRANSFAC_FACTOR** (Wingender et al., 1996), and EMBL databases.

TRRDBIB is linked with the site and gene description tables and contains the complete references to the original articles and to **MEDLINE** references.

The current version, **TRRD 3.5**, comprises the description of 427 genes, 607 regulatory units (promoters, enhancers, and silencers), and 2147 transcription factor binding sites. Over 1500 scientific publications have been processed to obtain these data. The **TRRDGENES** database includes information on human (185 entries), mouse (126), rat (69), chicken (29), and other genes. Most of the tissue-specific genes are expressed in liver (120), blood (67), or muscle cells (37). The major part of the genes compiled in **TRRD** are either interferon-induced (62) or glucocorticoid-regulated genes (30), or belong to lipid metabolism (41), erythroid differentiation (37), or cell cycle regulation (23) functional systems. **TRRD** is installed under the **SRS** to provide easy information retrieval and integration with other databases and computer systems for information processing.

TRRD Viewer. The Java applet, **TRRD-Viewer,** allows to visualize the data on location of transcription factor binding sites in a map form (Fig. 3) and overlook their textual description. While working with this applet, the user selects a gene identifier from the list, and the textual description of the gene (from

TRRDGENES), its sites (from **TRRDSITES**), and the relevant references (from **TRRDBIB**) appears in the text window. Transcription factor binding sites and composite elements are presented graphically. If the user clicks the site image, the description from the **TRRDSITES** table is displayed in the text window. Clicking the field title provides comments on the information described in the field. Several options allow a number of different site representations.

TRANSCRIPTION FACTOR BINDING SITE RECOGNITION

This module includes two blocks: the database on transcription factor binding site compilations (**TFBSC**) and programs for site analysis and recognition SiteGroup and SiteScan (Kondrakhin et al., 1998). The training samples from **TFBSC**, containing experimentally determined sequences of a particular site have been used in developing the recognition methods. The data include samples of 41 transcription factor binding sites (from 6 to 199 sequences for each factor; 1496 sequences totally) in EMBL-like format.

Table 1. A set of realizations for AP-1 binding site

N	Weight [$)]	Realization
0	26	tgactca
1	10	tgactAa
2	5	tgaAtca
3	4	tgacGca
4	2	tAactca
5	2	tgacAca
6	2	tgactGa
7	1	tCactca
8	1	TgGctca
9	1	TgactcG
10	1	TgactcC

$) Realization weight is the number of binding sites from U_0 containing a given realization.

A simple recognition method is based on the representation of transcription factor binding sequences as a set of site realizations $\mathbf{R}=\{\mathbf{R_0}, \mathbf{R_1}, ..., \mathbf{R_{k-1}}\}$. In other words, the set of realizations in the form of oligonucleotides τ bases long coded in the IUPAC-IUB 15 single-letter based codes is created by our algorithm for any particular site. This approach avoids the averaging of the nucleotide composition, as occur while describing the functional site by the weight matrix or consensus. The program SiteGroup uses a set $\mathbf{U_0}=\{\mathbf{u_1},...,\mathbf{u_m}\}$ of experimentally determined binding site sequences extracted from the **TFBSC** as the initial information to create the set of realizations **R**. It is determined by two parameters: (1) the length of the oligonucleotide τ and (2) the maximally allowable difference (distance) $\mathbf{t^{(miss)}}$ between these oligonucleotides. The main realization is the oligonucleotide $\mathbf{R_0}$ of length τ having the highest frequency in the sample $\mathbf{U_0}$. Then, all sequences $\mathbf{u_i}$ containing $\mathbf{R_0}$ are removed from the sample $\mathbf{U_0}$, producing the sample $\mathbf{U_1}$ and so on. Analysis of the sample $\mathbf{U_r}$, during which the rth realization $\mathbf{R_r}$ is searched for, is performed at the rth step. In this iterative process, all oligonucleotide words of length τ are considered. The distance t from the word $\mathbf{R_0}$ is estimated for each word. The word $\mathbf{R_r}$ that has the least distance from the $\mathbf{R_0}$ word is selected. If a number of words have the same minimal distance, the word with the maximal frequency in the set $\mathbf{U_r}$ is selected. The iteration is stopped when the set $\mathbf{U_r}$ either becomes empty or contains only the words that differ from $\mathbf{R_0}$ by the value exceeding $\mathbf{t^{(miss)}}$. Each set of realizations created by the method described may be characterized by parameter $\mathbf{f_{ij}}$: the proportion of the sequences from the initial set $\mathbf{U_0}$ represented in this set of realizations (covering of the set $\mathbf{U_0}$). The set of realizations

Table 2. Examples of the accuracy of binding site recognition by SiteScan program

No	Binding site	Errors [*)] for RGScan method		Errors for matrix method	
		α_1	α_2	α_1	α_2
1	AP1	0.188	0.004303	0.156	0.007421
2	AP2	0.125	0.000872	0.063	0.032477
3	ATF/CREB	0.147	0.000207	0.118	0.000964
4	C/EBP	0.060	0.023392	0.096	0.021284
5	COUP/RAR	0.025	0.003936	0.050	0.057874
6	ETF	0.000	0.002229	0.333	$<10^{-5}$
7	GATA	0.127	0.000491	0.072	0.015774
8	GR	0.118	0.003092	0.039	0.033286
9	NF-1	0.038	0.000620	0.099	0.013694
10	NF-kB	0.138	$<10^{-5}$	0.069	0.002014
11	NF-Y	0.045	0.001466	0.409	0.000258
12	OCT	0.163	0.000505	0.571	$<10^{-5}$
13	Pit-1	0.176	0.000522	0.059	0.009959
14	Sp1	0.029	0.008347	0.194	0.001999

*) α_1, false negatives; α_2; false positives

```
**********************************************
 * Prediction of potential binding site
 * of transcription factors in a given
 * DNA sequence on the basis of
 * recognition groups.
 **********************************************
The name of sequence: XLACTA2 standard;
The number of all predicted binding sites =  14
 Name      Position    Site
 APF         5        agtaac
 NFIII      52        tcattt
 GT-2B      81          cagctg
 MLTF      107          gtcact
 AP-1      108        tcactca
 SRF       221        ccatgtaagg
 GATA-1    262          cttatca
 SRF       272        ccaaatatgg
 NFIII     288        aaatga
 AR        301        tcttct
 AR        356        agagca
 NFIII     361        aaatga
 Pit-1     378        atgaata
 TFIID     385        tataaa
```

Figure 4. Prediction of transcription factor binding sites in the promoter region of *Xenopus laevis* alpha2 gene (sarcomeric actin) by SiteScan program.

providing maximization of the functional is searched for by exhaustion of the pairs $(t^{(miss)},\tau)=(i,j)$.

$$q_{i,j}=f_{i,j}\times[(f_{i,j}-f_{i-1,j})+(f_{i,j}-f_{i,j+1})]. \quad (1)$$

Examples of the first and second type errors in recognition of several transcription factor binding sites are listed in Table 2. For assessing the second type errors, the compilation of eukaryotic non-first exons was used. Small first and second type errors were observed for the SiteScan recognition. It is especially important for analysis of transcription factor binding sites in long genomic sequences.

COMPUTER SYSTEM FOR PREDICTING mRNA TRANSLATION ACTIVITY

This part of the **GeneExpress** system is designed for prediction of mRNA translation efficiency basing on analysis of structural and contextual features of 5' untranslated regions (5'UTRs). It has a program (Leader) for mRNA translation rate prediction and the database containing 5'UTR sequences (Leader_Sq) and some information on the effect of several 5'UTR features on mRNA translation efficiency.

Eukaryotic mRNAs differ considerably in their translation efficiency. This has been attributed to different efficiency of translation initiation. The contextual and structural features of 5'UTRs have strong effect on translation initiation. To reveal these characteristics, we have compared the mRNA sequences of several house-keeping gene groups, highly expressed in eukaryotic cells, and some groups of regulatory genes, whose expression is low and under stringent control. The group of highly expressed mRNAs consists of mRNAs of highly abundant proteins such as actins, tubulins, ribosomal proteins, histones, hsp70, etc.

Low expression mRNAs include mRNAs of transcription factors, protein kinases, growth factors, protooncogenes, etc. We have found several features that are different for these two groups (Fig. 5): 5'UTR length, nucleotide composition, context of start AUG codon, and presence of AUGs within 5'UTRs (Ischenko et al., 1996; Kochetov A.V. et al., 1998). These 5'UTR features affect the 40S ribosomal subunit movement along the leader and, therefore, the efficiency of the translation initiation.

Expert Weights (0-10 are valid; 5 employed automatically)

1. *Translation increases with decreasing the Leader length*
2. *Translation increases with decreasing the G/C ratio*
3. *Translation increases with increasing the G/C-imbalance*
4. *Translation increases with decreasing the alt-AUG content*
5. *Translation increases with decreasing the framed AUG content*
6. *Translation increases depending on the "-3 position" rule*
7. *Translation increases with decreasing the AUG inside leader*
8. *Translation increases with increasing the [C] content*
9. *Translation increases with increasing the [YM] content*
10. *Translation increases with increasing the[CnY] content*
11. *Comparison with the weight matrices for nucl. content in 5'UTRs of high expression mRNAs.*
12. *Comparison with complex (high to low) weight matrices for nucl. content in 5'UTRs of high and low expression mRNAs*

Figure 5. List of 5'UTR mRNA characteristics important for predicting the level of gene expression. Parameters 8-12 were determined for the (-35;-1) 5'UTR fragment.

The difference in these features was used for discrimination between high and low expressed genes The program calculates the 5'UTR features and evaluates the translation activity of mRNA. We create a simple discrimination function based on Penrose distance. The discrimination between the control samples of the high and low expressed mRNAs of dicot plants showed that 84% of the high and 76% of the low expressed mRNAs were classified correctly (Fig. 6).

SEQUENCE-BASED PREDICTION OF FUNCTIONAL SITE ACTIVITY

Initial postulates. It is suggested that the site activity **F** is determined by context-dependent properties of its nucleotide sequence **S**: statistical, physical, and conformational (Ponomarenko et al., 1997a, Kolchanov et al., 1998). These properties are of two types (Kel, A.E. et al., 1993): (1) obligatory, which are invariant for all sequences $\mathbf{S}_n$ of the site and

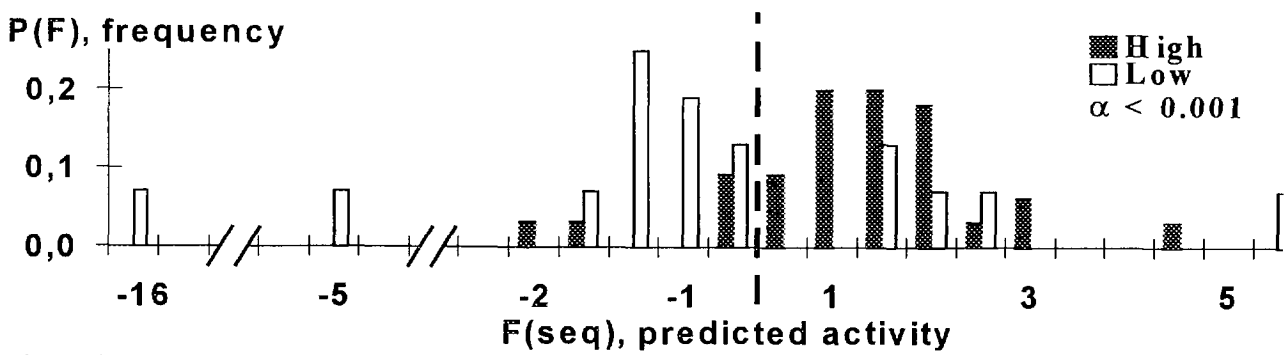


Figure 6. The control results were obtained using a set of independent data. Broken line is the selected threshold to separate low and high expressed mRNA.

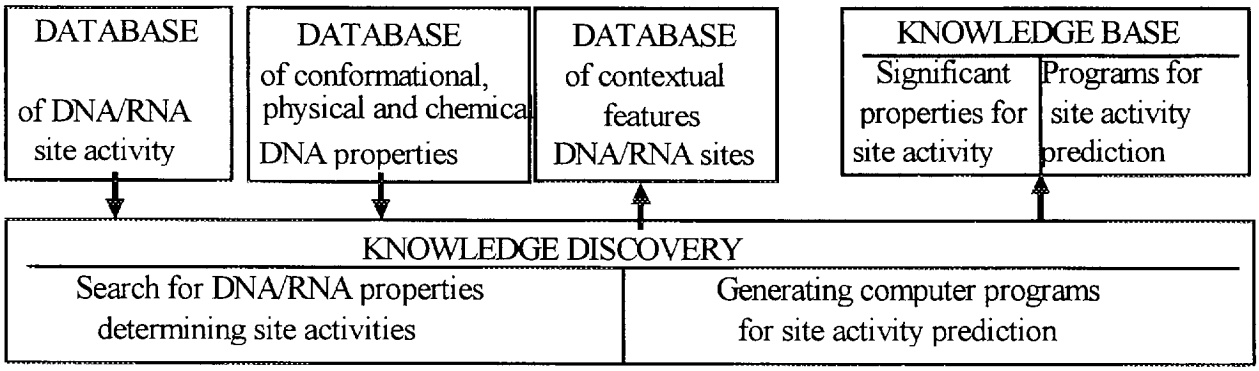


Figure 7. Principal scheme of the **ACTIVITY** computer system.

determine its basal activity; and (2) facultative, which are individual in terms of their "number, size, and location" for each sequence of the site and modulate the site activity with respect to the basal level. Hence, within the framework of the linear-additive approximation, the activity of the site with sequence S may be described by the following equation:

$$\mathbf{F(S_n) = F_0(S_n) + \sum_{k=1}^{K} F_k \times X_k(S_n);} \quad \mathbf{(2)}$$

where $\mathbf{F_0(S)}$ is the basal activity level determined by the occurrence of the obligatory properties of this site in the sequence $\mathbf{S_n}$; $\mathbf{\{X_k\}_{k=1,K}}$ are the facultative properties; and $\mathbf{F_k}$ is the contribution of the facultative property $\mathbf{X_k}$ to the site activity **F**. The principal scheme of the **ACTIVITY** system is shown in Fig. 7.

MI P0000001
MN Conformational
MD B-DNA
ML dinucleotide step
HN SCI00001
RN RF000012
RN RF000017
PN Twist
PM Calculated
PV TwistCalc
PU Degree
DINUCLEOTIDE
AA 38.90
AT 33.81
AG 32.15
AC 31.12
TA 33.28
TT 38.90
TG 41.41
TC 41.31
GA 41.31
GT 31.12
GG 34.96
GC 38.50
CA 41.41
CT 32.15
CG 32.91
CC 34.96

Figure 8. Description o conformational property "Helical twist angle of B DNA" in the ACTIVITY system database.

Database on functional site activities compiles the available data on functional sites with the experimentally measured activities: over 240 site samples of different types, such as promoters and binding sites for *E. coli* regulatory proteins, TATA boxes and binding sites for eukaryotic transcription factors, translation starts, splicing and 3'-processing sites, etc. Site activity characteristics include the association/dissociation rates of DNA-protein complexes, their lifetimes, equilibrium constants, transcription and translation efficiencies, etc.

Database on conformational and physical/chemical DNA properties compiles the information on context-dependent properties which may play a significant role in DNA-protein interactions. The format of this database is illustrated in Fig. 8. The database currently contains over 40 conformational parameters, determined either computationally or by X-ray analysis. Over 10 physical DNA properties -- melting temperature, persistent length, bending-rigidity, entropy, etc. -- are also included. The system for knowledge discovery on site activity contains two blocks. The first is responsible for revealing any site properties significant for predicting the site activity and the second provides generation of C-code programs to predict the activity of a given sequence.

One example of the context characteristics of the sequence S is the positional weighted concentration $\mathbf{X_{zmw}(S)}$ of mono-, di-, tri-, and tetranucleotides (Ponomarenko et al., 1997b):

$$\mathbf{X_{Z,m,w}(S) = \sum_{i=1}^{L-m+1} w(i) \times \delta_Z(s_i\, s_{i+1} \ldots s_{i+m-1}),} \quad \mathbf{(3)}$$

where δ_Z is the "1" or "0" indicator function depending on the match or mismatch between the sequence **S** and the oligonucleotide **Z**; **w(i)** is the function of position effect determined according to the rule: "the more important is the position **i** for the site activity, the higher its assigned weight **w(i)**". The activity prediction employs 180 various weight functions **w(i)**. They are stored in the database for contextual characteristics of the **ACTIVITY** system (Fig. 9). Three examples of weight functions **w(i)** are shown in Fig. 10. The nucleotide composition of any oligonucleotide is presented in the 15 single-letter based code.

We also use the mean values of site S properties within the region **[a;b]** as site characteristics:

$$X_{q,a,b}(S) = \frac{\sum_{i=a}^{b-1} P_q(s_i s_{i+1})}{b-a}. \quad (4)$$

Search for statistically significant characteristics is implemented as analysis of conformational and physical characteristics for all oligonucleotides with lengths from **1** to **M**, checking each of the 180 position effect functions **w(i)**. The total number of combinations <**Z, m, w**> is about 10^7 for **m=4**. Similarly, for every conformational or physical property **q**, the analysis of all possible locations of the region **[a, b]** within the site sequence is performed. $\mathbf{X_{qab}(S_n)}$ is calculated for a fixed combination <**q, a, b**> for each sequence of the site. The total number of the combinations <**q, a, b**> is 10^5.

The significance of a property for site activity prediction is estimated (Ponomarenko et al., 1997b) within the frames of the Utility Theory for Decision Making (Fishburn et al., 1970). Let's calculate the fixed property $\mathbf{X_{Zmw}(S_n)}$ for each sequence $\mathbf{S_n}$ with the known activity $\mathbf{F_n}$. If the resulting pairs $\{\mathbf{X_{Zmw}(S_n)}, \mathbf{F_n}\}$ meet all the necessary conditions of the linear regression applicability, then the activity **F** is predictable from an arbitrary sequence **S** via the feature $\mathbf{X_{Zmw}}$. To test these conditions of linear regression applicability, a simple regression is optimized for the pair $\{\mathbf{X_{Zmw}(S_n)}, \mathbf{F_n}\}$:

$$F_{Z,m,w}(S_n) = f_0 + f_1 \times X_{Z,m,w}(S_n); \quad (5)$$

where $\mathbf{f_0}$ and $\mathbf{f_1}$ are the regression optimized coefficients.

To ensure the reliability of the regression between $\mathbf{X_{Zmw}(S_n)}$ and $\mathbf{F_n}$, 22 conditions of regression analysis are tested (the presence of linear, sign, and rank correlations between the predicted $\mathbf{F_{Zmw}(S_n)}$ and the experimental $\mathbf{F_n}$ activities; the equality of $\mathbf{X_{Zmw}(S_n)}$ and $\mathbf{F_n}$ distributions, etc.).The significance level α_{rt} at which the **r**th condition is met is estimated. Then, the partial utility of the feature $\mathbf{X_{Zmw}}$ in predicting the activity **F** is calculated as follows:

$$U(X_{Z,m,w}, F) = \frac{\sum_{t=1}^{2}\sum_{r=1}^{11} u_{rt}(X_{Z,m,w}, F)}{22}. \quad (5)$$

$\mathbf{u_{rt}}$ in the Utility Theory for Decision Making is determined as:

$$u_{rt}(X_{Zmw},F)=\begin{cases} 1, \text{ if } \alpha_{rt} < 0.01; \\ 1.3 - 28.3 \times \alpha_{rt} + 55.6 \times \alpha_{rt}^2, \text{ if } 0.01 \le \alpha_{rt} \le 0.1; \\ -1, \text{ if } \alpha_{rt} > 0.1 \end{cases} \quad (6)$$

Only the properties with $U(X_{Zmw},F)>0$ are selected for the activity prediction and used to choose a limited set of linearly independent properties

Knowledge base on functional site activity. All selected characteristics are stored in the knowledge base of the **ACTIVITY** system. The format of the conformational property description is illustrated in Fig. 9. Using these properties, we generate the program to predict the site activity from its nucleotide sequence through optimization of equation (2). C-code programs for such prediction are also stored in the knowledge base (Ponomarenko et al., 1997b).

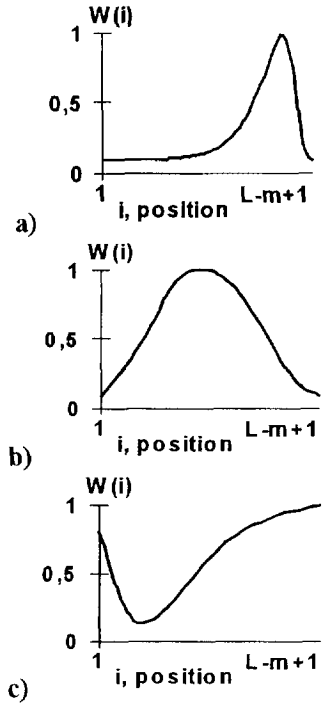
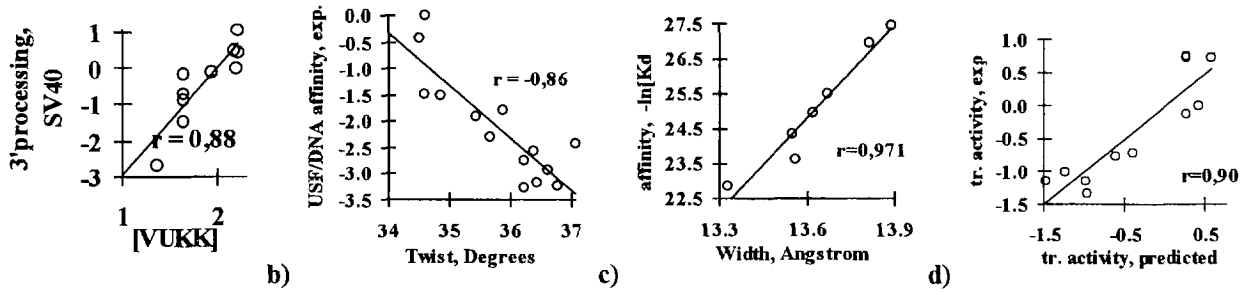


Figure 10. Examples of weight functions **w(i)**.

Analysis of several site samples has demonstrated applicability of this simple approach. For all these

```
MI K0000039
CF SEQUENCE-DEPENDENT CONFORMATIONAL FEATURE
CT PROPERTY AVERAGED FOR REGION [A;B]
DP P0000001
PV Twist
AB 10 18
UT 0.234
LC -0.859
FG http://wwwmgs.bionet.nsc.ru/.../USFTWIST.htm
C-CODE
/*USF/DNA-binding increases with Twist decrease*/
double TwistCalc_for_SynthUSFbind (char *s){
double X; char *seq; int i,k, SiteLength=9;
double DinucPar[16]={ 38.90, ....., 34.96 };
seq=&s[0];
if(strlen(seq) < SiteLength+1)return(-1001.);
for (i=0, X=0.;i < SiteLength-1;i++) {
switch (seq[i ]) { case 'A': k= 0; break;
.....
switch (seq[i+1]) { case 'A': k+=0; break;
.....
if (k > 15) return(-1004.); X+=DinucPar[k]; }
return (X/(double)(SiteLength-1));}
```

Figure 9. Description of a conformational property Helical twist for USF-binding site in the ACTIVITY.



a) b) c) d)

Figure 11. (a) Dependence of the mature DNA yield in the 3'-processing of SV40 virus pre-mRNA on the weighted concentration of VUKK tetranucleotid downstream of the mRNA cleavage point; (b) the dependence of the USF/DNA affinity on the helical twist angle of B-DNA; (c) the dependence of the Cr repressor/DNA affinity on the major groove width of B-DNA; (d) correlation of the experimental and calculated transcription activity for TATA/PE1B containing promoter region of mouse αA-crystalline gene.

samples, the significant features have been identified and the linear-additive approximation for predicting the site activity has been derived. For example, the weighted concentration of the tetranucleotide VUKK downstream of the SV40 pre-mRNA cleavage point was found to be responsible for the 3'-processing efficiency (Fig. 11a). The USF/DNA factor affinity correlates very well with the helical twist of B-DNA (Fig. 11b). The major groove width determines the Cro repressor/DNA affinity (Fig. 11c). The identified characteristics provide a first approximation in predicting the value of the specific activity of functional sites. These properties were used to generate the method for predicting the transcription activity of mouse αA-crystalline gene by analyzing its promoter sequence; the prediction shows a good agreement with the experimental data (Fig. 11d).

Reliability of the functional site activity values predicted by **ACTIVITY** from their sequences was studied by the authors earlier (Ponomarenko et al., 1997b) as well as **ACTIVITY** was compared with weight matrices (Stormo et al., 1986) and neural networks (Jonson et al., 1993).

CONCLUSION

The first step in interpreting a human genome sequence involves finding and annotation of the all genes it contains. The second step consists in characterizing the biological function of the individual genes, the way they are controlled, and their possible involvement in human disease. Significant success has been made in predicting and annotating protein coding regions (exons), although the exons account for only a few percents of the genomic sequence. A considerable part of the genome is occupied by regulatory sequences, which specify the tissue, developmental stage, or biochemical context of gene expression. Recognition, interpretation, and annotation of genome regulatory sequences should be one of the major tasks in the future progress of Human Genome Project. We designed the **GeneExpress** computer system as a first attempt to integrate the variety of information on genomic regulatory sequences and to use this information in developing and running software for their analysis and recognition. **GeneExpress** integrates **TRRD** and **GeneNet** databases and provides references to external databases, such as **TRANSFAC, COMPEL,** and **EMBL** using the **SRS** query system.

It is essential that the **GeneExpress** can and have to progress and expand continuously to update and integrate new resources for investigating other molecular events of the gene expression, such as splicing, DNA/protein interactions, etc. In the nearest future, a number of new basic modules will be added to the system including programs for recognition of eukaryotic promoters (Solovyev & Salamov, 1997) and composite regulatory elements (Kel, O.V. et al., 1995). A great number of software and information resources on various aspects of gene expression regulation, developed by the bioinformatics community, are currently existing. However, representation diversity of the data and the results of the data processing hinders the access to these resources. This diversity is and will be the natural trait of bioinformatics, inherent for its development. Hence, the problem is not to develop uniform data formats but to succeed in integration of the already available software and information resources in the formats developed by their authors to make these resources maximally convenient for experimenters. The advent of the SRS (Etzold and Argos, 1993) opens a way to solve this problem. In addition, the users should be provided with the possibility to arrange complex scenarios of step-by-step running programs in the course of data analysis using the integrated WWW resources. This may be realized, for example, by creating a virtual knowledge base, so that the user can accumulate the results of analysis, visualize them, and compare to both one another and the information available in the integrated databases. In the project AUTOGENE (Ptitsyn et al, 1996), the

authors have already tried to integrate the analyzing programs into flexible scenarios with input/output transfer of the results using the virtual knowledge base and demonstrated that the approach is promising.

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