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(54) **PROCEDE DE REGROUPEMENT DE SEQUENCES PAR FAMILLES**
(54) **METHOD FOR CLUSTERING SEQUENCES IN GROUPS**

(57) Pour le regroupement de séquences par familles biologiques, on appelle en mode itératif les programmes traditionnels de survol des banques de données afin de convertir plusieurs séquences apparentées en une séquence protéinique déterminée. La méthode proposée permet une répartition entièrement automatique d'un grand nombre de séquences protéiniques en groupes. La majeure partie de ces groupes sont dissociés les uns des autres et représentent donc un regroupement de données rationnel et valable. La méthode en question se caractérise en ce qu'elle requiert un temps de calcul extrêmement court du fait qu'il n'est plus nécessaire de comparer chaque séquence séparément avec chaque autre.

(57) In order to cluster sequences to biological groups, the conventional databank search programs are iteratively called in with a view to clustering various related sequences to one determined protein sequence. The inventive method enables full automatic distribution of a high number of protein sequences in groups. The major part of such groups are segregated, so that they represent a meaningful and valid grouping of data.

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(54) Title: METHOD FOR CLUSTERING SEQUENCES IN GROUPS

(54) Bezeichnung: VERFAHREN ZUR EINGRUPPIERUNG VON SEQUENZEN IN FAMILIEN

(57) Abstract

In order to cluster sequences to biological groups, the conventional databank search programs are iteratively called in with a view to clustering various related sequences to one determined protein sequence. The inventive method enables full automatic distribution of a high number of protein sequences in groups. The major part of such groups are segregated, so that they represent a meaningful and valid grouping of data.

(57) Zusammenfassung

Zur Eingruppierung von Sequenzen in biologische Familien werden traditionelle Datenbanksuchprogramme iterativ aufgerufen, um zu einer gegebenen Proteinsequenz eine Menge von verwandten Sequenzen zu finden. Mit dem Verfahren kann ein großer Satz von Proteinsequenzen vollautomatisch in Gruppen aufgeteilt werden. Der Großteil der Gruppen ist zueinander disjunkt und stellt daher eine sinnvolle und gültige Clusterung der Daten dar. Das Verfahren zeichnet sich durch eine extrem kurze Rechenzeit aus, da nicht mehr jede Sequenz separat mit jeder anderen verglichen werden muß.

METHOD FOR CLUSTERING SEQUENCES IN GROUPS

This invention concerns a method of grouping sequences in families.

Large quantities of protein sequence data are generated today in molecular biology. A major problem here is how to group such protein sequence data logically in biological families. Since families are not defined exactly, but instead the diversity of different gene families varies, this involves a problem in data grouping which is not at all trivial.

In the past, biological information could only be of assistance for human experts who would thoroughly research the output of database searching programs and would create a grouping according to families. This method is time-consuming, labor-intensive and not very reproducible.

Therefore, the object of this invention is to find a method with which a large set of protein sequences can be divided into groups fully automatically.

This object is achieved with the features of Patent Claim 1.

The method described here is based on the finding that rapid grouping can be achieved when traditional database searching programs are run iteratively to find a quantity of sequences related to a given protein sequence.

It is advantageous if the method described here is carried out for each sequence in the database, removing clusters that occur repeatedly except for one cluster each, removing clusters that are contained in other clusters; of the remaining quantity of clusters, outputting the clusters that do not overlap with other clusters as partitioning of the database and outputting the remaining portion of the

overlapping clusters as groups whose clusters are linked together by overlapping.

In this way, database clustering is achieved, resulting in the fact that most of the clusters are disjunctive to one another, and therefore a valid and reasonable clustering of data is achieved.

This method permits a much faster and more objective analysis of new sequence data than has been possible in the past. The paired disjunctive part of the clustering no longer requires any checking from a practical standpoint, and it forms the ideal basis for automatic annotations and more extensive analyses. The residue, i.e., the remaining portion of overlapping clusters, is the portion that must be studied by human experts. This portion is extremely reduced and is also prestructured due to the overlapping clusters.

This method can be carried out in an extremely short computation time because it is no longer necessary to compare each sequence separately with every other sequence in order to cluster an entire database.

In an advantageous embodiment, the threshold value is between 10^{-20} and 10^{-35}. In practice, a value of 10^{-30} has proven feasible. In addition to clustering protein sequences, this method is also suitable for DNA sequences, where it may be appropriate to relax the threshold value beyond 10^{-20}.

A further refinement of the method according to this invention consists of the fact that of the positive quantity of sequences found in one iteration step, not only the sequence weighted as worst, is used for another database search, but also all sequences of this quantity serve as a search sequence in additional searches. Due to the larger number of searches to be performed, this alternative is not

as fast as the method described originally for an individual search. In conjunction with clustering, however, this does not cause any time loss. However, since the sequence space around the initial sequence is searched more thoroughly, the resulting cluster has a high probability of already including all the sequences belonging to this protein family.

The "BLASTP" program is very suitable as a database searching program. This program is described in greater detail by S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic Local Alignment Search Tool," J. Mol. Biol., 215: 403-410, 1990.

As an alternative, however, the "FASTA" database search program may also be used as described, for example, in the following literature citation: W. R. Pearson and D. J. Lipman, "Improved tools for biological sequence comparison," Proc. Natl. Acad. Sci. USA, 85: 2444-2448, 1988.

In addition to the "BLASTP" and "FASTA" database search programs, any other database search program may also be used. For example, the "gapped BLAST" program (described by S. F. Altschul, T. L. Madden, A. A. Schaeffer, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Research, 25 (17): 3389-3402, 1997, is also suitable.

Using the "BLASTP" program, the PIR1 database, release 51, has been clustered by quantitative theory. This database contains 13,489 protein sequences and is described in detail by David G. George, Richard J. Dodson, John S. Garavelli, Daniel H. Haft, Lois T. Hunt, Christopher R. Marzee, Bruce C. Orcutt, Kathryn E. Sidman, Geetha Y. Srinivasarao, Lai-Su L. Yeh, Lieslie M. Arminski, Robert S. Ledley, Akira Tsugita and Winoma Barker, "The Protein Information Resource (PIR)

and the PIR international protein sequence database," Nucleic Acids Research, 25 (1): 24-27, 1997. Grouping this database required approximately one day of computation time, and 91 % of the database sequences were grouped into disjunctive clusters in a fully automatic procedure. The residue includes only approximately 9 % of the database sequences.

The SWISS-PROT database, whose 34th release contains 59,021 sequences, is a much larger database. This database is described by Amos Bairoch and Rolf Apweiler, "The SWISS-PROT protein sequence data bank and its supplement TrEMBL," Nucleic Acids Research, 25 (1):31-36, 1997. Within approximately five days of computation time, 80 % of the sequences were classified in disjunctive classes.

These examples show that extreme savings in terms of computation time are possible with the method according to this invention in comparison with traditional methods, and the cluster results are excellent.

An algorithm for the method described here is presented below:

```
cluster ← empty quantity
search sequence ← inquiry sequence
as long as      (search sequence defined)
                database searched with the search sequence
                positive quantity ← all found sequences below
                                    the threshold level with
                                    a probability value
                search sequence ← undefined
                if (first search) then
                reference quantity ← positive quantity
            end if
                if (sequence exists in a positive quantity that
                is not contained in the cluster) and
```

(intersecting quantity between the positive quantity and reference quantity is not blank) then

search sequence ← sequence weighted as worst in the positive quantity not contained in the cluster

cluster ← combined quantity of cluster and positive quantity

end if

end as long as

The sequence with which the search method begins is called the initial sequence, and the quantity of found sequences is called the cluster belonging to this initial sequence. First, a database search program such as "BLASTP" or "FASTA" is started with the initial sequence, and all sequences from the database that have a significant similarity with the initial sequence are accepted. We call this quantity of sequences a positive quantity, and we include it in the cluster as related sequences. There is a significant similarity between two sequences when the probability that this similarity occurs randomly is very low, i.e., below a given threshold. From the positive quantity thus obtained, we now use the sequence weighted as worst (i.e., the sequence having the highest probability) as a search sequence for another database search. This process is repeated as long as sequences below the threshold value are found which are not contained in the cluster and as long as there is an intersection quantity between the positive quantity of the initial sequence and the positive quantity of the instantaneous search sequence.

Then the following algorithm is carried out:

input: database

```
output: partitioning of the database and groups with
        overlapping clusters

perform the above database search method for all (sequences
     in the database)
cluster quantity ← all clusters generated for all
                   (identical clusters in the cluster
                   quantity) remove identical clusters
                   except for one representative
cluster quantity ← clusters without identical clusters for
                   all (clusters in the cluster quantity
                   contained completely in another cluster)
                   remove the smaller cluster of this
                   cluster pair
cluster quantity ← clusters without identical clusters and
                   without inclusions
partitioning ← all clusters in the cluster quantity that do
               not overlap
overlapping ← all overlapping clusters in the cluster
              quantity are combined in groups
```

To obtain database clustering, the method described above is carried out for all sequences in the database, i.e., each sequence is assigned a cluster of sequences to which it is related. From this quantity of clusters, all identical clusters are removed except for one example, because they do not contain any additional information. The remaining cluster quantity is then examined for inclusions, and clusters that are contained completely in other clusters are removed until no more inclusions are present. Of this cluster quantity, the clusters that do not overlap with others can now be regarded as a logical partitioning of the database. The remaining small number of overlapping clusters are combined in groups whose clusters are linked together among one another by overlapping.

An embodiment of this invention is described in greater detail below.

We use the sequence of the human homeobox engrailed-1 protein (HME1_HUMAN) as the inquiry sequence and then search the Swissprot database (release 34) with it for related sequences. The search is performed with the BLASTP program, and we select a threshold with a probability of 10^{-30}. The result of this search looks approximately as follows (excerpts):

Sequences found:		Probability:
SPR|Q05925|HME1_HUMAN	HOMEOBOX PROTEIN ENGRAILED-1 (HU-E...	2.4e-279
SPR|P09065|HME1_MOUSE	HOMEOBOX PROTEIN ENGRAILED-1 (MO-E...	4.7e-189
SPR|Q05916|HME1_CHICK	HOMEOBOX PROTEIN ENGRAILED-1 (GG-E...	4.6e-132
SPR|Q05917|HME2_CHICK	HOMEOBOX PROTEIN ENGRAILED-2 (GG-E...	3.6e-95
SPR|P19622|HME2_HUMAN	HOMEOBOX PROTEIN ENGRAILED-2 (HU-E...	8.0e-95
SPR|P09066|HME2_MOUSE	HOMEOBOX PROTEIN ENGRAILED-2 (MO-E...	5.6e-92
SPR|P09015|HME2_BRARE	HOMEOBOX PROTEIN ENGRAILED-2 (ZF-E...	5.2e-70
SPR|P31538|HMEB_XENLA	HOMEOBOX PROTEIN ENGRAILED-1B (EN-...	1.5e-⊗
SPR|P52729|HMEC_XENLA	HOMEOBOX PROTEIN ENGRAILED-2A (EN-...	2.1e-66
SPR|P52730|HMED_XENLA	HOMEOBOX PROTEIN ENGRAILED-2B (EN-...	2.1e-65
SPR|P31533|HME3_BRARE	HOMEOBOX PROTEIN ENGRAILED-3 (ZF-E...	6.1e-64
SPR|Q04896|HME1_BRARE	HOMEOBOX PROTEIN ENGRAILED-1	1.0e-61
SPR|P09145|HMEN_DROVI	SEGMENTATION POLARITY PROTEIN ENGR...	9.1e-59
SPR|P05527|HMIN_DROME	INVECTED PROTEIN.	4.5e-57
SPR|P27609|HMEN_BOMMO	SEGMENTATION POLARITY PROTEIN ENGR...	1.5e-55
SPR|P27610|HMIN_BOMMO	INVECTED PROTEIN.	1.1e-52
SPR|P09532|HMEN_TRIGR	HOMEOBOX PROTEIN ENGRAILED (SU-HB...	4.0e-44
SPR|Q05640|HMEN_ARTSF	HOMEOBOX PROTEIN ENGRAILED.	1.7e-42
SPR|P09076|HME3_APIME	HOMEOBOX PROTEIN E30 (FRAGMENT).	2.1e-41
SPR|P09075|HME6_APIME	HOMEOBOX PROTEIN E60 (FRAGMENT).	1.0e-40
SPR|P14150|HMEN_SCHAM	HOMEOBOX PROTEIN ENGRAILED (G-EN...	2.3e-40
SPR|P23397|HMEN_HELTR	HOMEOBOX PROTEIN HT-EN (FRAGMENT).	1.3e-38
SPR|P31537|HMEA_XENLA	HOMEOBOX PROTEIN ENGRAILED-1A (EN-...	7.9e-33
SPR|P31535|HMEA_MYXGL	HOMEOBOX PROTEIN ENGRAILED-LIKE A...	1.1e-27
SPR|P34326|HM16_CAEEL	HOMEOBOX PROTEIN ENGRAILED-LIKE CE...	7.1e-27

SPR|P31536|HMEB_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE B... 5.0e-26

On the basis of the threshold at 10^{-30}, our cluster now contains the following sequences:

HME1_HUMAN, HME1_MOUSE, HME1_CHICK, HME2_CHICK, HME2_HUMAN, HME2_MOUSE, HME2_BRARE, HMEB_XENLA, HMEC_XENLA, HMED_XENLA, HME3_BRARE, HME1_BRARE, HMEN_DROVI, HMIN_DROME, HMEN_BOMMO, HMEN_DROME, HMIN_BOMMO, HMEN_TRIGR, HMEN_ARTSF, HME3_APIME, HME6_APIME, HMEN_SCHAM, HMEN_HELTR, HMEA_XENLA.

The next run through the BLASTP program is then carried out with the sequence weighted as worst in this quantity, namely with the engrailed-1A homeobox protein of the horned toad (HMEA_XENLA). The result of this search looks as follows (excerpts):

Sequences found:		Probability:
SPR|P31538|	HMEB_XENLA HOMEOBOX PROTEIN ENGRAILED-1B (EN-...	2.8e-36
SPR|P31537|	HMEA_XENLA HOMEOBOX PROTEIN ENGRAILED-1A (EN-...	3.2e-36
SPR|P09015|	HME2_BRARE HOMEOBOX PROTEIN ENGRAILED-2 (ZF-E...	1.1e-34
SPR|Q05925|	HME1_HUMAN HOMEOBOX PROTEIN ENGRAILED-1 (HU-E...	1.3e-33
SPR|P09065|	HME1_MOUSE HOMEOBOX PROTEIN ENGRAILED-1 (MO-E...	1.4e-33
SPR|Q05916|	HME1_CHICK HOMEOBOX PROTEIN ENGRAILED-1 (GG-E...	1.5e-33
SPR|P52729|	HMEC_XENLA HOMEOBOX PROTEIN ENGRAILED-2A (EN-...	9.9e-33
SPR|P52730|	HMED_XENLA HOMEOBOX PROTEIN ENGRAILED-2B (EN-...	5.9e-32
SPR|Q05917|	HME2_CHICK HOMEOBOX PROTEIN ENGRAILED-2 (GG-E...	1.3e-31
SPR|P09066|	HME2_MOUSE HOMEOBOX PROTEIN ENGRAILED-2 (MO-E...	1.8e-31
SPR|P19622|	HME2_HUMAN HOMEOBOX PROTEIN ENGRAILED-2 (HU-E...	2.0e-31
SPR|Q04896|	HME1_BRARE HOMEOBOX PROTEIN ENGRAILED-1.	8.1e-31
SPR|P31535|	HMEA_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE A...	4.3e-30
SPR|P31533|	HME3_BRARE HOMEOBOX PROTEIN ENGRAILED-3 (ZF-E...	6.7e-30
SPR|P31536|	HMEB_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE B...	1.8e-28
SPR|P09532|	HMEN_TRIGR HOMEOBOX PROTEIN ENGRAILED (SU-HB-...	8.8e-28
SPR|P31534|	HMEN_LAMPL HOMEOBOX PROTEIN ENGRAILED-LIKE (E...	8.8e-28
SPR|P09075|	HME6_APIME HOMEOBOX PROTEIN E60 (FRAGMENT).	2.1e-26
SPR|P23397|	HMEN_HELTR HOMEOBOX PROTEIN HT-EN (FRAGMENT).	2.3e-26

SPR|P09076|HME3_APIME HOMEOBOX PROTEIN E30 (FRAGMENT). 3.9e-26

Let us again consider all sequences having a probability lower than 10^{-30} we and find that except for HMEA_MYXGL, all sequences are contained in the cluster. This sequence is now included in the cluster, and the next BLASTP search is started with it. This search yields the following result (excerpts):

Sequences found:		Probability:
SPR|P31535|	HMEA_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE A...	3.8e-36
SPR|P31534|	HMEN_LAMPL HOMEOBOX PROTEIN ENGRAILED-LIKE (E...	1.5e-30
SPR|P31538|	HMEB_XENLA HOMEOBOX PROTEIN ENGRAILED-1B (EN-...	1.8e-30
SPR|P31537|	HMEA_XENLA HOMEOBOX PROTEIN ENGRAILED-1A (EN-...	3.8e-30
SPR|P52729|	HMEC_XENLA HOMEOBOX PROTEIN ENGRAILED-2A (EN-...	4.9e-29
SPR|P09015|	HME2_BRARE HOMEOBOX PROTEIN ENGRAILED-2 (ZF-E...	1.4e-28
SPR|Q05925|	HME1_HUMAN HOMEOBOX PROTEIN ENGRAILED-1 (HU-E...	1.7e-28
SPR|P09065|	HME1_MOUSE HOMEOBOX PROTEIN ENGRAILED-1 (MO-E...	1.8e-28
SPR|P09066|	HME2_MOUSE HOMEOBOX PROTEIN ENGRAILED-2 (MO-E...	3.1e-28
SPR|P19622|	HME2_HUMAN HOMEOBOX PROTEIN ENGRAILED-2 (HU-E...	3.3e-28
SPR|Q05916|	HME1_CHICK HOMEOBOX PROTEIN ENGRAILED-1 (GG-E...	4.6e-28
SPR|P52730|	HMED_XENLA HOMEOBOX PROTEIN ENGRAILED-2B (EN-...	2.1e-27
SPR|Q05917|	HME2_CHICK HOMEOBOX PROTEIN ENGRAILED-2 (GG-E...	2.2e-27
SPR|P09075|	HME6_APIME HOMEOBOX PROTEIN E60 (FRAGMENT).	2.9e-27
SPR|P23397|	HMEN_HELTR HOMEOBOX PROTEIN HT-EN (FRAGMENT).	4.4e-27
SPR|Q04896|	HME1_BRARE HOMEOBOX PROTEIN ENGRAILED-1.	4.9e-27
SPR|P09076|	HME3_APIME HOMEOBOX PROTEIN E30 (FRAGMENT).	5.4e-27
SPR|P31533|	HME3_BRARE HOMEOBOX PROTEIN ENGRAILED-3 (ZF-E...	2.0e-26
SPR|P31536|	HMEB_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE B...	8.8e-26

This time we add HMEN_LAMPL to our cluster, and we start the next BLASTP search with this sequence, yielding the following result (excerpt):

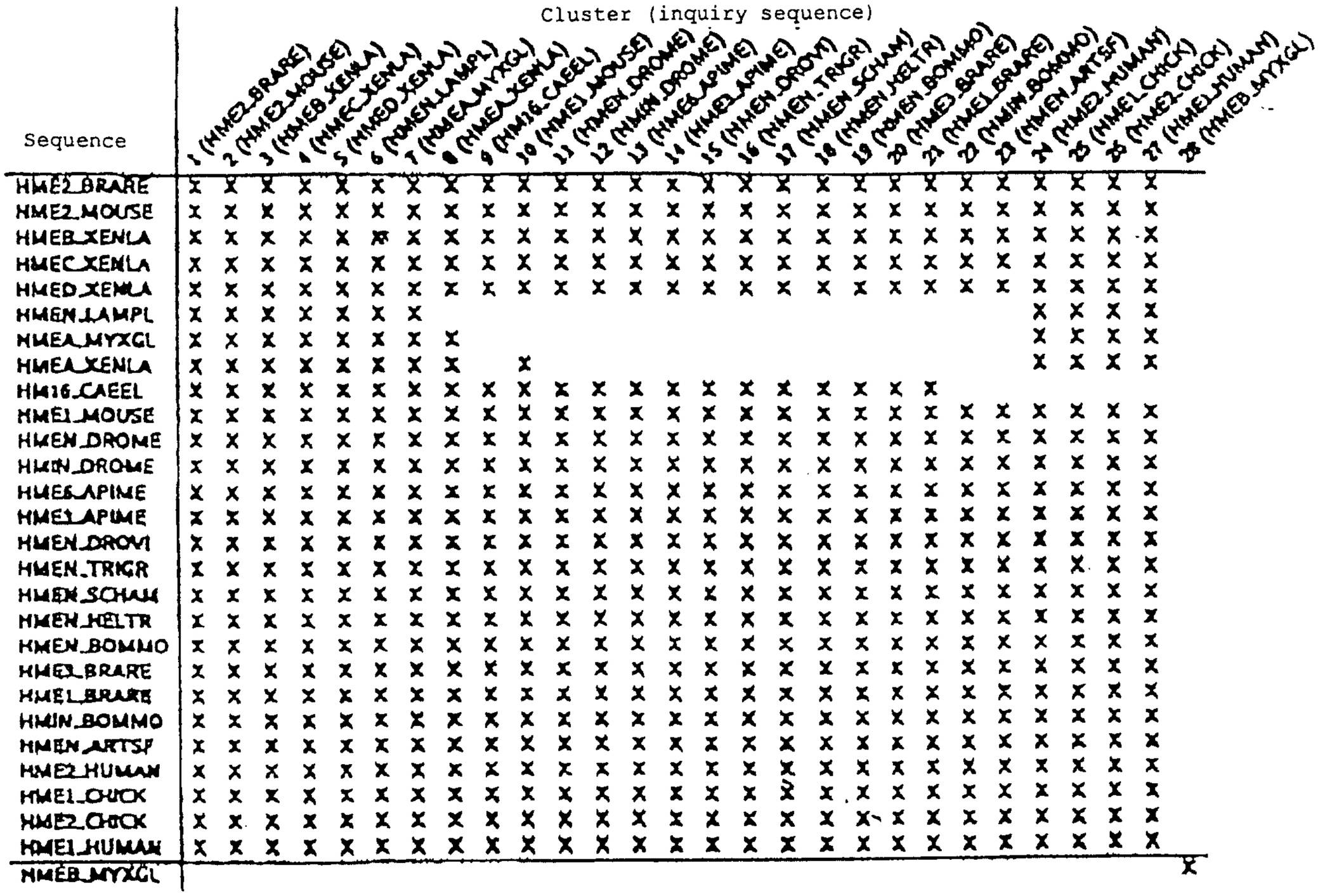
Sequences found:		Probability:
SPR|P31534|	HMEN_LAMPL HOMEOBOX PROTEIN ENGRAILED-LIKE (E...	5.7e-37
SPR|P31535|	HMEA_MYXGL HOMEOBOX PROTEIN ENGRAILED-LIKE A...	5.0e-31

SPR|P31538|HMEB_XENLA	HOMEOBOX PROTEIN ENGRAILED-1B (EN-...	1.4e-28
SPR|P31537|HMEA_XENLA	HOMEOBOX PROTEIN ENGRAILED-1A (EN-...	2.9e-28
SPR|P23397|HMEN_HELTR	HOMEOBOX PROTEIN HT-EN (FRAGMENT).	1.2e-27
SPR|P31536|HMEB_MYXGL	HOMEOBOX PROTEIN ENGRAILED-LIKE B...	1.4e-27
SPR|Q04896|HME1_BRARE	HOMEOBOX PROTEIN ENGRAILED-1.	1.5e-27
SPR|P09015|HME2_BRARE	HOMEOBOX PROTEIN ENGRAILED-2 (ZF-E...	6.9e-27
SPR|Q05925|HME1_HUMAN	HOMEOBOX PROTEIN ENGRAILED-1 (HU-E...	1.5e-26
SPR|P09065|HME1_MOUSE	HOMEOBOX PROTEIN ENGRAILED-1 (MO-E...	1.6e-26
SPR|P09075|HME6_APIME	HOMEOBOX PROTEIN E60 (FRAGMENT).	1.9e-26
SPR|Q05916|HME1_CHICK	HOMEOBOX PROTEIN ENGRAILED-1 (GG-E...	4.5e-26

Above the threshold, we do not find any sequences that would not already be contained in our cluster, so the SYSTERS search for this inquiry sequence is now concluded, and the cluster contains the following 26 sequences:

HME1_HUMAN, HME1_MOUSE, HME1_CHICK, HME2_CHICK, HME2_HUMAn, HME2_MOUSE, HME2_BRARE, HMEB_XENLA, HMEC_XENLA, HMED_XENLA, HME3_BRARE, HME1_BRARE, HMEN_DROVI, HMIN_DROME, HMEN_BOMMO, HMEN_DROME, HMIN_BOMMO, HMEN_TRIGR, HMEN_ARTSF, HME3_APIME, HME6_APIME, HMEN_SCHAM, HMEN_HELTR, HMEA_XENLA, HMEA_MYXGL, HMEN_LAMPL.

If this procedure is performed for all 28 sequences annotated as homeobox engrailed in the Swissprot database, this yields 28 clusters at first. The clusters thus found are plotted in the following table against the sequences, where the columns represent the clusters belonging to the inquiry sequence listed at the head of the table and the line indicate the clusters in which the sequence listed at the left is contained (marked with an X). In this case, there are seven clusters having 27 sequences each, five clusters having 26 sequences each, etc.



After removing identical clusters and solving for inclusions, the homeobox engrailed proteins are distributed among two clusters - one with 27 sequences and the other with only the HMEA_MYXGL sequence.

CLAIMS

1. A method of grouping sequences in families, wherein

- all sequences similar to an inquiry sequence for which the probability that the similarity is random is below a predetermined threshold level are determined as a positive quantity from a sequence database using a database search program,

- at least one sequence is selected as a search sequence from this positive quantity,

- then the search method described here is repeated with the search sequence thus determined being used as the inquiry sequence as long as the positive quantity just determined still contains sequences that are not contained in the positive quantities determined previously and as long as there is an intersection quantity between the positive quantity and the inquiry sequence and the positive quantity of the instantaneous search sequence, and

- all the different sequences contained in the calculated positive quantities are output as clusters.

2. A method according to claim 1, characterized in that

- the method is carried out according to Claim 1 for each sequence in the database,

- clusters that occur more than once are removed except for one cluster each time,

- clusters that are contained in other clusters are removed,

- of the remaining cluster quantity, those clusters that do not overlap with other clusters are output as partitioning of the database, and

- the remaining portion of overlapping clusters is output as groups whose clusters are linked together by overlapping.

3. A method according to one of the preceding claims, characterized in that the threshold value is between 10^{-20} and 10^{-35}.

4. A method according to one of the preceding claims, characterized in that the sequence having the highest probability is selected as the positive quantity.

5. A method according to one of claims 1 through 3, characterized in that all the sequences of the positive quantity serve as a search sequence.

6. A method according to one of the preceding claims, characterized in that the BLASTP program is used as the database search program.

7. A method according to one of claims 1 through 3, characterized in that the FASTA program is used as a database search program.

8. A method according to one of claims 1 through 3, characterized in that the "gapped BLAST" program is used as the database search program.