

Sequence Analysis on Microcomputers

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In recent years molecular biology has undergone a revolution that can be partially attributed to the advent of facile DNA sequencing methods. The amount of available sequence data is such that the accurate handling and analysis of these data represent a significant effort for the average molecular biologist. Since all but the simplest analyses are impossible to do manually, computer techniques have been devised for the storage, comparison, and searching of sequences. Microcomputer versions of these programs have been developed, many of which exceed the performance of their mainframe counterparts. This review will consider three packages of analysis programs that are commercially available for microcomputers with the Microsoft disk operating system (MS-DOS). These packages differ in their exact implementation of various analytic procedures and in their flexibility. DNASTAR (1) is a large set of programs that is essentially a nucleic acid workstation fully capable of accessing the GenBank (2) and Protein Information Resource (PIR) (3) databases, as well as performing most of the analytical functions usually associated with mainframe computers. The other two analysis packages, MicroGenie (4) and IBI-Pustell (5), are not as comprehensive as DNASTAR but offer a less expensive way to perform many of the common sequence-handling techniques.

Because the packages represent a wide range of capabilities and collectively contain more than 75 individual programs or functions, I have reviewed those functions that I consider to be most useful to the average molecular biologist and have grouped them into four classes as follows:

1) *Sequence mapping.* These functions list and number a sequence in a convenient, user-defined manner, as well as search the sequence and indicate the location of important landmarks such as restriction endonuclease recognition sites. Mapping includes functions that search for short, variable, or ill-defined subsequences of interest such as polyadenylation sites, promoters, and splice sites, and for physical arrangements such as inverted repeats.

2) *Protein analysis.* The investigator may need to translate the six possible protein reading frames of a DNA sequence, to predict actual protein coding regions, and to analyze the putative proteins in terms of chemical and physical characteristics. This class of functions includes programs that predict coding regions, translate DNA or RNA sequences, and analyze such chemical and physical parameters as charge, hydrophilicity, and secondary structure of the resultant protein.

3) *Homology analysis.* These functions compare two sequences in order to identify regions of similarity and present these homologous regions graphically or numerically for evaluation.

4) *Database handling.* Such functions provide access to and easy extraction of information from sequence databases such as GenBank or PIR. Also included are functions that allow the creation of local or personal databases for the organization and analysis of raw sequencing data.

For this review I have examined how each of the program sets performs the functions described in the above four classes. Not all of the features of each package are discussed; this is particularly true for DNASTAR.

Implementation and Discussion

General operation. The specifications of the packages evaluated are listed in Table 1; some of the analytical functions they perform are shown in Table 2. Each package can be thought of as a group of individual programs connected by a user-accessible environment or "shell." The DNASTAR and IBI-Pustell systems use direct menu access, in that an analysis is chosen from a menu and the user is then presented with a submenu of selections for that individual analysis function. MicroGenie has data entry and analysis functions grouped into two modules that are selected from an initial menu. More than one analytical function can be selected for a given sequence, and more than one sequence can be listed for analysis at once. Although both the IBI-Pustell and MicroGenie packages allow use of a floppy disk system, only the hard disk versions will be discussed here. It is strongly recommended that a hard disk system, rather than a floppy disk, be used with these

programs. Before any analysis can be performed, the sequence of interest must be entered into the computer. All of the packages have facilities for direct entry of sequence data from gel digitizers; only the manual entry method was evaluated. MicroGenie and DNASTAR provide simple text editors that are tailored specifically for use with sequence data. Character-by-character editing is allowed in both systems. The IBI-Pustell editor does not allow direct cursor control for modification of existing sequences. Instead, positioning is accomplished by scrolling a number of lines entered from the console. Incorporation of a simple editor capable of direct cursor control would greatly facilitate use of this system. All three programs create unique file formats, so that the use of files created by other systems requires some editing by the user. Both DNASTAR and IBI-Pustell include automated programs for converting some foreign formats to those that are usable by the package. MicroGenie will accept and process files from other sources if they are strictly ASCII compatible and contain no characters prior to the beginning of the sequence.

All three program systems work with commonly available dot matrix printers. No alternative peripherals such as a plotter are supported. Output to letter quality printers is possible for the production of slides or publication quality tables. However, none of the packages provides an output editor that permits the user to conveniently manipulate the data, so that most presentation grade output must be generated by the user on a word processor.

Sequence mapping. In general, to map a sequence with any of the program sets, a file containing the sequence of interest is searched for the recognition sites of restriction endonucleases stored in a separate file of sites. The DNA sequence is then printed across the page, base numbers are marked at convenient intervals, and the name of the restriction enzyme is printed above its cleavage site in the sequence (Fig. 1). In each of the programs the list of enzymes used for the analysis can be modified by the user. All three program sets provide a means of representing restriction enzyme sites in tables as well as producing graphic representations of the fragments formed upon cleavage. To further map the sequence for short regions of interest that may be less well defined than restriction enzyme sites (for example, control regions, splice junctions, or promoters), each of the systems contains a subsequence search function.

DNASTAR permits searches of this type through use of a program called "Matseek." Literal searches are permitted for subse-

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