22625

22625

National Foreign Assessment Center

Confidential

Soviet Recombinant DNA Research: Status and Trends

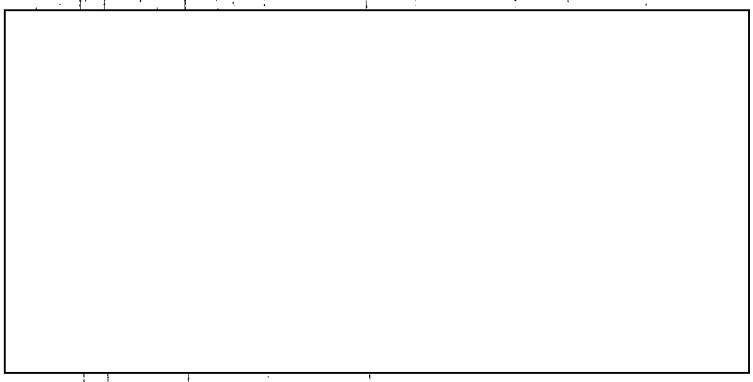
An Intelligence Assessment

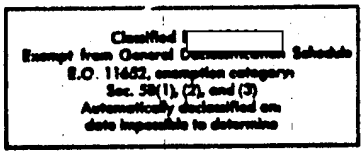
Confidential

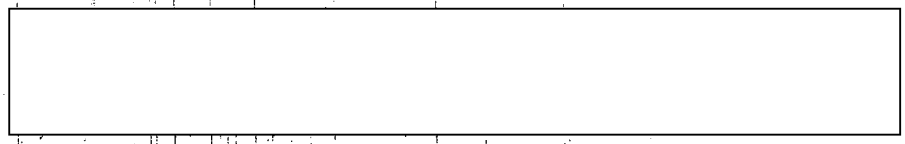
51 78-10092

September 1978

NATIONAL SECURITY INFORMATION
Unauthorized Disclosure Subject to Criminal Sanctions







Soviet Recombinant DNA Research: Status and Trends

Central Intelligence Agency
National Foreign Assessment Center

September 1978

Key Judgments

- Soviet recombinant DNA research capabilities have improved rapidly, but still lag Western state-of-the-art by several years. The scope of Soviet recombinant DNA research is limited because of the small number of qualified molecular geneticists to initiate research projects and a shortage of adequately equipped laboratories. The Soviet research enjoys high-level support, and a continuing high interest in the field is expected.
- Taking advantage of continuing access to virtually all advanced Western recombinant DNA research which is neither classified nor proprietary, the Soviets should be able to establish within two to five years a basic research program using recombinant techniques that is of moderate size and high quality; it is improbable, however, that they will challenge the leadership of the best Western laboratories.
- Soviet recombinant DNA research appears to be directed primarily toward agricultural, biomedical, and commercial applications. The Soviets probably will experience difficulties in adapting laboratory techniques to achieve these goals because of the still limited scope of their program and inadequate support technologies in the biomedical sciences.
- Some military-sponsored recombinant DNA work has been reported, but it is improbable that this work is directed toward or is capable of achieving significant biological warfare applications.

BLANK PAGE

PREFACE

The recent development of recombinant DNA technology has raised concern that the USSR will acquire recombinant capabilities from the West as well as through its own efforts and use them to the disadvantage of the United States. This paper assesses current Soviet research capabilities, likely trends in the scope and quality of future Soviet research, the importance to the Soviets of the various means through which they can obtain detailed information about advanced Western recombinant research techniques, and the likelihood that the Soviets will derive meaningful benefits from recombinant DNA techniques.

BLANK PAGE

CONTENTS

Soviet Recombinant DNA Research: Status and Trends

Introduction

The term "genetic engineering" often is used interchangeably with the term "recombinant DNA," but the two are not synonymous. *Genetic engineering* refers to advanced basic and applied research techniques that have evolved from molecular biology and related research in biochemical genetics and cell physiology. These techniques impart the capability to modify or manipulate purposefully the genetic characteristics or the developmental sequences of cells or organisms at the molecular or cellular level. Included within this restricted definition of genetic engineering are such things as "test tube babies" as well as more exotic research directed toward producing hybrid (chimeric) organisms or cloning human beings or other mammals. At times genetic engineering has been used somewhat inappropriately to refer to all applied genetic research, including such traditional areas of genetics as livestock breeding and developing new hybrid varieties of plants.

Recombinant DNA encompasses only one specialized area of genetic engineering; namely, specific biochemical and microbiological techniques to alter, in a relatively controlled and reasonably predictable manner, the molecules that encode the genetic characteristics of an organism, and to introduce specifically selected traits—often new to the organism—into an organism's set of genetic instructions.

The specific molecular manipulations that are made and the exact methods used to make them can vary widely from one recombinant DNA experiment to another. The basic process, however, usually involves the initial isolation or synthesis of a specific set of chemically identical nucleic acid molecules (usually Deoxyribonucleic Acid--DNA). These molecules are then chemically bonded or recombined—hence recombinant DNA—into specially prepared vectors (carriers) that usually are plasmids, bacteriophages, or other virus-like infectious entities that can self-replicate in appropriate host cells. After the bonding has been completed, the modified vectors can be inserted into bacteria or other cells that have been prepared biochemically to accept them. If the vectors have been "constructed" appropriately, the piece of initially selected DNA can give the recipient cell a new genetic characteristic. For instance, the host cell might be given the capability to synthesize an enzyme that it previously could not make. This enzyme in turn could permit the host cell to metabolize a previously undigestible food source or to become immune to some previously lethal drug or parasite. Alternatively, these same procedures can be used to study the chemical structure (or other molecular biological properties) of the inserted DNA. In this case, the host cell is simply allowed to propagate, which in turn causes many copies of the inserted DNA sequence to be made (cloned) also. These cloned sequences then can be isolated biochemically in quantities and purities not possible by other means.

Status of Soviet Recombinant DNA Research

Most Soviet studies using recombinant DNA techniques have been initiated only within the past few years. The Soviets have advanced their recombinant DNA capabilities primarily by attempting to exploit experimental techniques developed in the West (mostly in the United States) and by acquiring specially modified vectors and host cell-lines from Western colleagues. These Soviet efforts to obtain Western recombinant technologies appear to be continuing. [1, 2, 3, 4]

Soviet recombinant research is conducted by competent scientists, but has not progressed suf-

ficiently for their researchers to have specialized knowledge to offer in return for advanced experimental techniques obtained from the West. They have contributed somewhat to recombinant technology, however, [5] by devising a few methods to change the chemical recognition specificity of several enzymes (restrictases) used during some recombinant procedures. [6, 7]

The Soviets are believed to have only about five research groups that have demonstrated reasonably advanced recombinant capabilities (see table 1), and these Soviet groups are from one to two years behind most of the several hundred US groups. The best Soviet research also trails that in numerous West European laboratories, which now rival the US in sophistication. [8] Other Soviet groups, some of which are listed in table 2, have conducted related (support or preparatory) research and could begin recombinant experiments soon.

A group headed by academician Aleksandr A. Bayev at the Institute of Biochemistry and Physiology of Microorganisms (IBPM), Pushchino, has investigated the fundamental processes involved in recombination phenomena using *E. coli* as a host cell and several well-studied virus vectors. This work has included the study of molecular specificity and normal physiological

Table 1

Recombinant Research and Facilities in the USSR

Institute/Research Leader	Research
Institute of Biochemistry and Physiology of Microorganisms/Bayev	Hybrid plasmids *Lambda-III—E. coli E1* and *phage Lambda-T4A* have been constructed. Investigations have isolated Eco-RI restructase, studied restrictase action, and mapped DNA hydrolysis products. Systems studied include only the well known bacterial and virus host vectors.
All-Union Scientific Research Institute of Genetics and Selection of Industrial Microorganisms/Debabov	Hybrid plasmids E1-R6k and RP4-ColE1 (pAS8) of *E. coli* have been constructed by restruction and ligation, transferred by method of transformation in CaC'_s-treated *E. coli* C600, and clones were isolated by antibiotic resistance. *E. coli* and several phage strains were obtained from US scientists.
Ivanovskiy Institute of Virology/Tikhonenko	The Glactose operon from Lambda p Gal has been isolated using Bam Hi endonuclease and transferred into *E. coli* by way of a vector (hybrid) plasmid pMB9. Clones were selected by antibiotic resistance and ability to use galactose as the only source of carbon. Methods and techniques had been established several years earlier by Western researchers.
Institute of Molecular Biology/Georgiyev Institute of Atomic Energy/Ananiyev	*Drosophila melanogaster* genes have been incorporated into Lambda gt-Lambda C phage using restriction and ligation techniques and selected by nucleic acid hybridization; resulting hybrid clones are the subject of further investigation. The vector phage was provided by a Western scientist.
Institute of General Genetics Institute of Botany/Andrianov	Pea chloroplast DNA in *E. coli* C600 plasmids (pSC101 or RSF 2124) has been transferred to *E. coli* C600 (rk-mk-). Transformants were selected by antibiotic resistance. *E. coli* NM182, used in isolating Eco RI restrictase, originally was obtained from a Western scientist.

action of DNA modification enzymes and basic capabilities have been established for the future use of recombinant DNA techniques. No intended applications have been apparent. [9, 10-16, 17] Two other laboratories at IBPM (see table 2) under the direction of Ya. I. Buryanov and A. M. Boronin, respectively, have investigated the specific action of methylases during natural recombination and have used classical genetic manipulations involving plasmids to produce bacterial strains that oxidize napthalene at an enhanced rate. No attempt has been made to use recombinant techniques in Boronin's commercially oriented research. [18-23]

At the All-Union Scientific Research Institute of Genetics and Selection of Industrial Microorganisms (VII Genetika), Moscow, a laboratory under the direction of V. G. Debabov has used several *E. coli* host-vector systems to transfer specific genes between differing strains of *E. coli*. [24-29] Some of this research has been done in collaboration with personnel from the Institute of Bioorganic Chemistry, Moscow. [24] Recombinant related research also has been performed by another laboratory at VII Genetika, headed by S. I. Alikhanyan. This group has mapped the location of specific genes on plasmids by using restriction enzymes to generate deletion mutants. [30, 31]

Under the direction of T. I. Tikhonenko, a laboratory at the Ivanovskiy Institute of Virology, Moscow has transferred a gene that confers the ability to utilize the sugar galactose as a source of carbon. This was accomplished by isolating the gene from a lambda virus and transferring it via a laboratory-modified plasmid. Clones of *E. coli* containing the transferred gene were selected by antibiotic resistance. [32, 33] The techniques used represent only slight modifications of methods developed several years earlier in the West.

Table 2

Recombinant-Related Research and Facilities in the USSR

Institute/Research Leader	Research
Institute of Biochemistry and Physiology of Microorganisms/Boronin	Investigations of napthalene catabolism include production of plasmid mutants by classical means with the intent of producing *Pseudomonas* strains capable of a high degree of napthalene turnover.
/Buryanov	Research centers on DNA methylases in *E. coli* MRE 600.
All-Union Scientific Research Institute of Genetics and Selection of Industrial Microorganisms/Alikhanlyan	Hybrid plasmid pAS8, constructed by Debabov's group, was used in genetic analysis of plasmid RP4. Other related research centers on realtionship between plasmids and spore formation in *Bacillus* strains.
All-Union Institute of Synthesis of Protein Subsances/Domaradskiy [55]	Investigations focus on the genetic exchange between unrelated microorganisms. To date, plasmid DNA from *E. coli* (RI-19) has been transferred to *B. subtilis* (SB25) and transformants selected by antibiotic resistance.
N.I. Pirogov Second Moscow Medical Institute/Gnedoy [34]	Investigation to optimaze conditions for absorbing transforming vectors in several *E. coli* strains. Transformability achieved for homologous (*E coli* C600) strains; heterologous strains exhibited low transformability.
Gamaleya Institute of Epidemiology and Microbiology/Rudchenko [35]	Investigations involve the restrictase capability of R factors (R-Eco RII and R-Eco RIII) *in vivo* on different phage strains (ØX 174 and S13) in study of antibiotic resistance.

The only examples of Soviet experiments that have transferred genes from higher organisms have come from the Institute of Molecular Biology (IMB), Moscow—an offshoot of the Kurchatov Institute of Atomic Energy, Moscow—and from a joint project of the Institute of General Genetics in Moscow, and the Institute of Botany in Alma Ata. [40, 41] G. P. Georgiyev's group at IMB transferred genes from the fruit fly *Drosophila* to *E. coli* and then isolated specific clones using standard nucleic acid hybridization techniques. [36-39] In the joint study, [40, 41] V. M. Andrianov *et al* transferred a pea chloroplast gene to *E. coli*. Standard antibiotic-resistance techniques were used to select host cells carrying the inserted gene.

No Soviet groups have reported the successful transfer via recombinant techniques of any DNA sequence that has immediate commercial or other applied use. The types of material being transferred (for example, chloroplast genes), however, are consistent with reported Soviet intentions to use recombinant techniques in agriculture to produce new nitrogen-fixing plants and in the food industry to improve the nutritional quality of single cell protein concentrates. [36, 37]

Although these Soviet researchers are highly capable and technically competent individuals, their efforts to develop recombinant capabilities rapidly have been hampered by a lack of: (1) high-quality modern laboratory equipment; (2) a readily available commercial (or other dependable) source of needed restriction enzymes; (3) a reliable source of high-quality biochemicals; and (4) a large cadre of qualified molecular biologists (many still are attempting to recover from the effects of the Lysenko era). [4, 42-45] They have been able to give postdoctoral and graduate-level training to a few individuals domestically, but they do not have a sufficient number of personnel or facilities to develop independently and quickly a large, highly advanced program. [2]

Manpower and space limitations also severely restrict current Soviet capabilities to exploit more than a few potential applications of recombinant techniques. It probably will take several years for this situation to change significantly, even though the Soviets already are reported to be building several new laboratories—at least one of which is to be a P-4 (high containment) facility—and to be upgrading existing laboratories. [42] Any successful applications of recombinant DNA techniques achieved by the Soviets probably would not be accomplished quickly enough to place their researchers either realistically or apparently at the forefront of recombinant DNA research worldwide.

Soviet Military-Sponsored Recombinant Research

Several military-funded, closed, microbiological research institutes [redacted] reportedly were established during the early 1970s. The missions of these institutes included conducting research directed toward possible defense applications of recombinant DNA techniques. [46, 47] Nonetheless, the Soviet military is not known to be using recombinant techniques for biological warfare (BW) purposes. Military-funded Soviet research institutes probably would use recombinant techniques for basic biomedical studies. For instance, projects could be oriented toward developing prophylactic agents suitable for use against infectious diseases. Such work probably would include the development of improved vaccines for dysentery and typhoid fever, as well as vaccines for more exotic viral diseases—such as those in Africa.

Soviet military-sponsored recombinant DNA research probably is not advanced technically. Except for Nikolay Matvienko, who reportedly has transferred to a military institute, [46] the most capable Soviet recombinant DNA researchers are believed to be conducting unclassified basic research and to have published freely in the open literature. The research sophistication of even these prominent researchers, who have been working in the best Soviet laboratories—that is, those that are reasonably well-equipped and fully staffed—still lags that of most Western groups by a year or more. Newly established military research institutes probably could not have assembled staffs of equal or superior competence. Furthermore, these institutes probably could not

have successfully initiated advanced research projects on topics lacking both domestic and foreign sources of directly relevant research data.

Information regarding Soviet recombinant DNA research by military-funded institutes has been meager. One such institute was reported to be in or near Protvino (a town near Moscow) and another in or near Pushchino. [46] The Pushchino institute could be a misidentification of a high containment facility, reportedly being built for IBP at Pushchino. No such [redacted] facility is known to exist near Protvino.

Nikolay Matvienko, who conducted recombinant DNA research previously at IMB in Moscow and at IPBM in Pushchino, [10-13, 17] is believed to be the deputy director of the Protvino Institute. The quality of the researchers at Protvino is not known, but Matvienko is the only technically competent bacterial geneticist known to have been hired out of the academy community. A second individual, a graduate student who was judged by a source to have rather poor capabilities, reportedly was hired to work at Protvino also. [46]

It is not yet clear if recombinant techniques can be useful for BW research. Some US experts have argued that traditional techniques to develop BW agents are more cost effective. Also, they felt that recombinant techniques probably could not be used to enhance meaningfully the virulence of existing agents or to create a new "super" bug. [47] Others have argued that the development of a "super" bug is not necessary. The simple transfer of debilitating diseases, such as dysentery, to normally nonpathogenic bacteria, such as *E. coli*—a common inhabitant of the human digestive tract—would be sufficient to make identification of the causative agent and effective treatment of the induced pathologies extremely difficult. The counter argument to this has been that several decades of testing usually is required to understand sufficiently the ecology and epidemiology of any new "bug" to sanction its use as a predictable military weapon.

Two basic categories of Western recombinant DNA information which are potentially relevant to the Soviet military include: recombinant techniques involving the transfer of factors that are pathogenic to man or that can increase in a novel way the virulence of organisms infectious to humans; and procedures and equipment used to support biologically hazardous recombinant DNA research.

The conduct of biologically hazardous experiments involves traditional microbiological safety equipment and techniques that have been used in the biomedical handling and study of pathogenic organisms. Although some of the techniques for constructing advanced protection equipment (or "high containment" facilities in general) have become somewhat of an art, most of the techniques and equipment designs have been published openly. Much of the equipment is available commercially.

Recombinant DNA research involving factors pathogenic to humans has been severely limited by the safety restrictions governing most Western research. Thus, little information currently is available. A substantial amount of information should become available in the future as the number of high containment facilities—those that permit experiments to be undertaken safely—increases. Such information probably will be made available in the open literature.

Technology Acquisition Channels

The Soviets can acquire information useful to their recombinant DNA research efforts from a number of sources. Virtually all US and most foreign research data and techniques have been published or are being prepared for publication in the open literature. Even the details of a procedure recently submitted for US patent rights have been published openly. [48] Two of the coauthors on this patented research project are now conducting research in West European laboratories.

Most advanced Western scientists are free to communicate research activities and procedures to foreign scientists both through written correspondence and at international meetings. [49] The distribution of samples and supplies of newly developed vectors or host cells also has been left

to the discretion of the scientists possessing them, for non-Communist nations have neither enforced regulations that exist (such as item 5799D of the US unilateral Commodity Control List) nor issued guidelines concerning the dissemination of research information or specimen transfers.

Although the US remains at the forefront of recombinant research worldwide, many foreign researchers, particularly in Europe are capable of providing, and in some cases have provided, the Soviets with experimental supplies that were both adequate and as good as could have been obtained from US sources (see table 1). Many of the specialized enzymes also are now available commercially from numerous Western suppliers. As the number of researchers and research groups conducting advanced recombinant DNA studies in West European countries increases—a substantial number exist already—the easier it should become for Soviet scientists to obtain needed technical data from professional contacts.

International scientific meetings also have provided a channel for the rapid, multilateral exchange of experimental data. Except for representatives of industrial firms, most scientists attending international meetings have exchanged information and answered questions freely. [2, 36, 50]

Although no formal agreement exists between the United States and the USSR to exchange researchers or research information either on genetic engineering in general or on recombinant DNA specifically, [57] the Soviets have begun to propose numerous exchanges involving recombinant DNA techniques under the auspices of existing microbiological, health, and medical programs. [50] Some exchange visits and experimental collaboration also can be expected between Soviet researchers and scientists in Europe and Japan. [46, 49]

All of these channels for information transfer have been supported by Soviet intelligence collection efforts, which have been directed toward acquiring advanced Western recombinant technologies to support Soviet research capabilities. [46, 58] At least some Soviets who have international contacts, including those who would be involved in recombinant DNA-related exchanges, will have been prebriefed and can be expected to attempt to service Soviet intelligence requirements.

Soviet Capabilities To Assimilate Foreign Recombinant Techniques

The Soviets traditionally have had little success at converting microbiological and biochemical laboratory techniques into useful commercial-scale operations. This probably will be the case with Soviet attempts to apply recombinant DNA techniques. For instance, the Soviets are considered to be relatively unsophisticated in several fields of biological research (such as the genetic manipulation of plant cell protoplasts in tissue culture) that probably will be needed in conjunction with recombinant techniques to achieve certain industrial or agricultural applications. Furthermore, despite the extremely high quality of Soviet organic chemists, few consistently high-quality laboratory reagents have been produced in the USSR. [47-49] It appears improbable that the use of recombinant techniques suddenly would enable Soviet biochemical or pharmaceutical industries either to develop or to market a sufficiently large number of high-quality products to compete meaningfully with Western industries. Soviet capabilities to apply recombinant DNA techniques to military-oriented research projects should face similar constraints.

Despite the problems anticipated with Soviet attempts to adapt recombinant techniques to commercial uses, over the next two to five years they should be able to establish a cadre of competent, basic research scientists fully trained in the use of advanced recombinant DNA procedures. The ready availability of Western research data already has saved Soviet researchers several years of developmental research effort. The rate at which the Soviets are able to continue improving their recombinant capabilities should remain moderately—although decreasingly—dependent on the acquisition of advanced Western work for at least a few years more.

The number of laboratories that the Soviets are building or refurbishing to accommodate

recombinant experiments [42, 53] indicates that sufficient support will be provided to establish and maintain a moderate size recombinant research program of from 20-30 technically sound research groups. The research efforts of these groups appear unlikely to be hampered by strictly enforced research restrictions designed to ensure that research activities do not pose inadvertant health hazards, despite the fact that research guidelines have been adopted officially. [36, 52, 58, 53] The Soviets are thus expected to attain recombinant research capabilities comparable to many Western laboratories, but they are not likely to challenge seriously the leadership of the most advanced Western laboratories.

If Soviet access to Western expertise were suddenly to be restricted, it would delay noticeably—perhaps by as much as an additional five years—the establishment of a sophisticated Soviet research program. Sufficient capabilities have been established by the Soviets already, however, for them to proceed on their own. Currently, Soviet recombinant DNA research dependence on the West probably is most tightly linked to Western biochemical supplies and advanced laboratory equipment—such as ultracentrifuges and scintillation counters. [45, 58, 51, 49] Although inconvenienced by the need to anticipate laboratory supply requirements at least a year in advance because of the long lag time frequently experienced between requesting and receiving material, Soviet biomedical research would be dealt an even greater blow if only Soviet biochemicals and precision equipment were available. Soviet biochemical industries produce only about a dozen laboratory reagents of a consistently high quality (for example, free from contaminants), and Soviet-built precision laboratory equipment has been habitually poor in quality. [58, 51, 49]

Western scientific literature has been and continues to be important to the advancement of Soviet recombinant DNA capabilities. [4, 58] It has provided a rather extensive list of those experimental techniques known to work, others that have not worked, and has detailed the procedures used to obtain the numerous enzymes, vectors, and mutant cell lines found to be useful for recombinant procedures. Information of this type has permitted the Soviets—as well as new laboratory groups elsewhere—to select experimental procedures previously demonstrated to be appropriate and to avoid procedures already found to present difficulties or to be less efficient than other methods. A substantial volume of these types of generally applicable recombinant techniques should continue to appear in the Western literature for the next year or two. Thereafter, an increasingly greater portion of new recombinant techniques probably will become relatively project specific.

As Soviet laboratories begin increasingly to narrow the focus of their research projects, however, they probably will establish unique projects of their own choosing rather than simply duplicating or attempting to compete with advanced Western work. At this point the relative importance of personal contacts should increase relative to the information available in the open literature because the experimental problems that are encountered during specialized research projects usually are best solved through informal consultations with colleagues, and because the West will continue to have a substantially greater number of technially advanced scientists than will the USSR.

In the long term, when Soviet research problems have become quite specific, informal contacts, such as at international meetings, probably will be the most useful form of personal contact to the Soviets, since such contacts provide access to a broad cross-section of Western scientific expertise. Currently, however, formal research exchanges probably provide a more useful form of personal contact to the Soviets. Exchange visits bring technically competent people together *in the laboratory*, where equipment and experimental techniques can be observed first hand and where tutored, practical experience can be obtained. These factors increase the value of the technology transferred, particularly in cases where a problem in experimental procedure has been encountered by a Soviet laboratory (such as difficulty in replicating the work of another laboratory) or where an individual who is basically untrained in recombinant techniques de-

sires a formal training program. In the former case, taking an individual through a lengthy procedure on a step-by-step basis probably would identify the problem (that is, the difference in procedure, such as using a different brand of buffer or resuspending a precipitate too vigorously) much more quickly than could be accomplished through written correspondence or through trial and error deviations in laboratory procedures. In the latter case, the Soviets could benefit appreciably by getting a graduate student or postdoctoral level scientist trained in a suitable Western laboratory. Where the trainee probably would be tutored by and be exposed to a much larger number of competent, recombinant qualified researchers than would be the case in the USSR and where the still limited Soviet laboratory space and experimental supplies would not be strained further.

Nonetheless, it is improbable that research exchanges will provide a significant contribution to Soviet recombinant DNA capabilities in the near term. After returning to the USSR, a Soviet researcher trained during an exchange visit probably would spend several months to a year reestablishing a research project, equipping a laboratory, or attempting to integrate any US-acquired skills into an already ongoing research project. The Soviets probably will benefit more over the long term from the personal contacts established during an exchange visit. These contacts should facilitate Soviet abilities to acquire rapidly a specialized reagent needed unexpectedly or to get a prompt reply to a technical inquiry.

The authors of this paper are

. Comments and queries are welcome and should be directed to

confidential