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Joint Human Genome Program Between Argonne National Laboratory and the Engelhardt Institute of Molecular Biology

Andrei Mirzabekov,^{1,2} G. Yershov,^{1,2} Y. Lysov,² V. Barsky,² V. Shick,² and S. Bavikin¹

¹Argonne National Laboratory; Argonne, IL 60439
630/252-3161 or -3361, Fax: /252-3387

amir@everest.bim.anl.gov

²Engelhardt Institute of Molecular Biology; 117984 Moscow, Russia

In 1996, more than thirty U.S. and Russian research workers participated in the joint Human Genome Program between Argonne National Laboratory and Engelhardt Institute of Molecular Biology on the development of sequencing by hybridization with oligonucleotide microchips (SHOM).

During this year, about twenty Russian scientists have been working from 3 months to 1 year in ANL. In this period, 3 papers have been published and 5 papers accepted for publication, 3 more papers are submitted for publication.

The main research efforts of the group have been concentrated in three directions:

- I. Improvement of SHOM technology.
- II. Development of SHOM for the needs of Human Genome Program.

III. Development of new approaches based on SHOM technology.

I. Improvement of SHOM technology

As a major result of the work in this direction, simple, reliable and effective methods of microchip manufacturing, sample preparations, and quantitative hybridization analysis by fluorescence microscopy have been developed or improved.

1. Photopolymerization technique for production of micromatrices of polyacrylamide gel pads on hydrophobicized glass surface was improved to become a simple, highly reproducible and inexpensive procedure (7).
2. New and cheaper chemistry of the oligonucleotide immobilization has been developed and introduced for production of more durable microchips. It is based on the use of amino-oligonucleotides and aldehyde-gels instead of 3-methyluridine-oligonucleotides and hydrazide-gels (3).
3. Four-pin robot has been constructed with computer control of every microchip element production. High quality microchips with 4100 immobilized oligonucleotides have been manufactured and the complexity of the microchips can easily be scaled up to a few tens of thousand elements.
4. Two-color fluorescence microscope has been equipped for regular use with proper mechanics and software. It allows investigators to regularly use the automatic quantitative monitoring of the hybridization on the whole microchip and to measure the kinetics of hybridization as well as the melting curves of duplexes formed with all microchip oligonucleotides (1,2,8).
5. Four-color fluorescence microscope was manufactured and four proper fluorescence dyes are at present under selection.
6. Chemical methods of introduction of several fluorescence dyes into DNA and RNA with or without fragmentation have been developed and regularly used in SHOM experiments (4).
7. A theory describing the kinetics of hybridization with gel-immobilized oligonucleotides has been developed (5).
8. Simple and relatively inexpensive equipment (around \$10,000 per set) has been produced for manual manufacturing of microchips and fluorescence measurement of hybridization, which will enable every laboratory to produce and practically use microchips containing up to 100 immobilized oligonucleotides or other compounds.

II. Application of SHOM

Although the main goal of our SHOM development is to produce a simple de novo sequencing procedure, a number