*Development of Intracellular Flow Karyotype Analysis

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Instrumentation for univariate fluorescent flow analysis of chromosome sets has been developed for human cells. A new method of cell preparation and intracellular staining of chromosome with different dyes was developed and improved. Cells suspension for flow analysis must satisfy the following requirements: minimal amount of free chromosomes and debris (dead cells, cell fragments etc.); chromosomes structure must be stabilized inside mitotic cells; chromosomes must be stained inside the cells up to saturation with the used dyes; chromosomes must be able to release from cells with minimal possible mechanical treatment. The method includes enzyme treatment (chymotrypsin), incubation with saponin and separation of prestained cells from debris on sucrose gradient. The developed protocol was tested and improved in the course of several months of work and allows us to obtain a well stained sample with a minimal amount of contaminates [2].

A special magnetic mixing/stirring device was constructed to perform cell membrane breaking. It was placed inside the flow chamber of a serial flow cytometer ATC-3000 equipped with additional electronic card for time-gated data acquisition [1]. The rupturing of prestained mitotic cells is performed by means of a small magnetic rod vibrating in an alternative magnetic field. The efficiency of mitotic cells breaking with electromagnetic cell breaking device was tested using different human cell lines[2,3].

The device works in a stepwise mode: a defined volume of sample is delivered to the breaking chamber for rupturing mitotic cell (cells) for a defined time period, followed by buffer wash to move the released chromosomes from the breaking chamber to the point of the analysis. The information about the chromosomes appearing at the point of analysis is accumulated in list mode files, making it possible to resolve chromosome sets arising from single cells on the basis of time gating. The concentration of cells in the sample must be kept low to ensure that only one cell at a time enters the breaking device.

The developed software classifies chromosome sets according to different criteria: total number of chromosomes, overall DNA content in the set, and the number of chromosomes of certain type [2,3]. In addition it's possible to determine the presence of extra chromosomes or loss of chromosome types. Thus this approach combines the high performance of flow cytometry (quantitation and high throughput) with the advantages of image analysis (cell to cell karyotype analysis and skills of trained cytogeneticist). The data analysis capabilities offer extensive flexibility in determining important features of the karyotypes under study. This development offers the potential to duplicate most of what is determined by clinical cytogeneticists. The results now obtained are in good accordance with goals of the project formulated before [4].

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Mapping and Sequencing with BACs and Fosmids

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BACs and fosmids are stable, nonchimeric, and highly representative cloning systems. BACs maintain large-fragment genomic inserts (100 to 300 kb) that are easily prepared for most types of experiments, including DNA sequencing.

We have improved the methods for generating BACs and developed extensive BAC libraries. We have constructed human BAC libraries with more than 175,000 clones from male fibroblast and sperm, and a mouse BAC library with more than 200,000 clones. We are currently expanding human library with the aim of achieving total 50X coverage human genomic library using sperm samples from anonymous donors.