— Precision of analysis which ensures detecting a change of a single chromosome in the cell flow karyotype.

We suppose, the method will be effective in studying chromosomal aberrations and classification of cell karyotypes, and in other tasks of biology and medicine.

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DISCRETE PREPARATIVE ELECTROFRACTIONATION OF COMPLEX PROTEIN MIXTURE

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The purification of homogeneous protein is the main task in its study. The excellent resolution of SDS-PAGE in analysis of proteins stimulated the creation of various preparative devices in which bands emerging from the bottom of electrophoresis gels are swept away to fraction collector [1-2]. Though the efficiency of this technique was reported as being high, some problems still exist. Among them, strong dilution of protein zones by the eluting buffer, complexity of elution chamber and high cost of commercial devices. The described method and apparatus allow to overcome all these problems. The essence of the method is that separation is carried out in a gel cylinder and fractionation is accomplished by new patented manner which is distinguished by overturning of the gel cylinder and up-side discrete protein fractions collection in maximum concentration by means of pipette. It's necessary to emphasize the concentrating effect of this method which allows to find a faint residual enzyme activity after its SDS-denaturation. The efficiency of this technique was demonstrated by the purification of one of protein kinases and error-correcting 3'-5'exonuclease from rat liver.

The schematic view of the electrophoresis system is shown in Fig.1. The separation is carried out in a gel cylinder according to Laemmli (3). The glass tube with a gel is fixed in the apparatus, standard electrophoresis buffer is poured into the both reservoirs (B) and sample is loaded onto the gel. Electrophoresis is carried out until the electrophoresis front (bromphenol blue) reachs the lower edge of the resolving gel (Position 1, Fig 1). Power is turned off and instrument is overturned. Then power is switched on again and electrophoresis is going on. Now protein zones are migrating from bottom to top (F) and gathering in section E where they are being drawn with a pipette (Position 2, Fig.1). For this case a buffer level (C) is lowered (D) and section E is separated from the main reservoir. This is done by means of unit G.

Electrofractionation results showing a distribution of protein zones in eluted fractions are represented in Fig.2. The eluted fractions were analyzed by standard SDS-PAGE [3] and stained by silver.

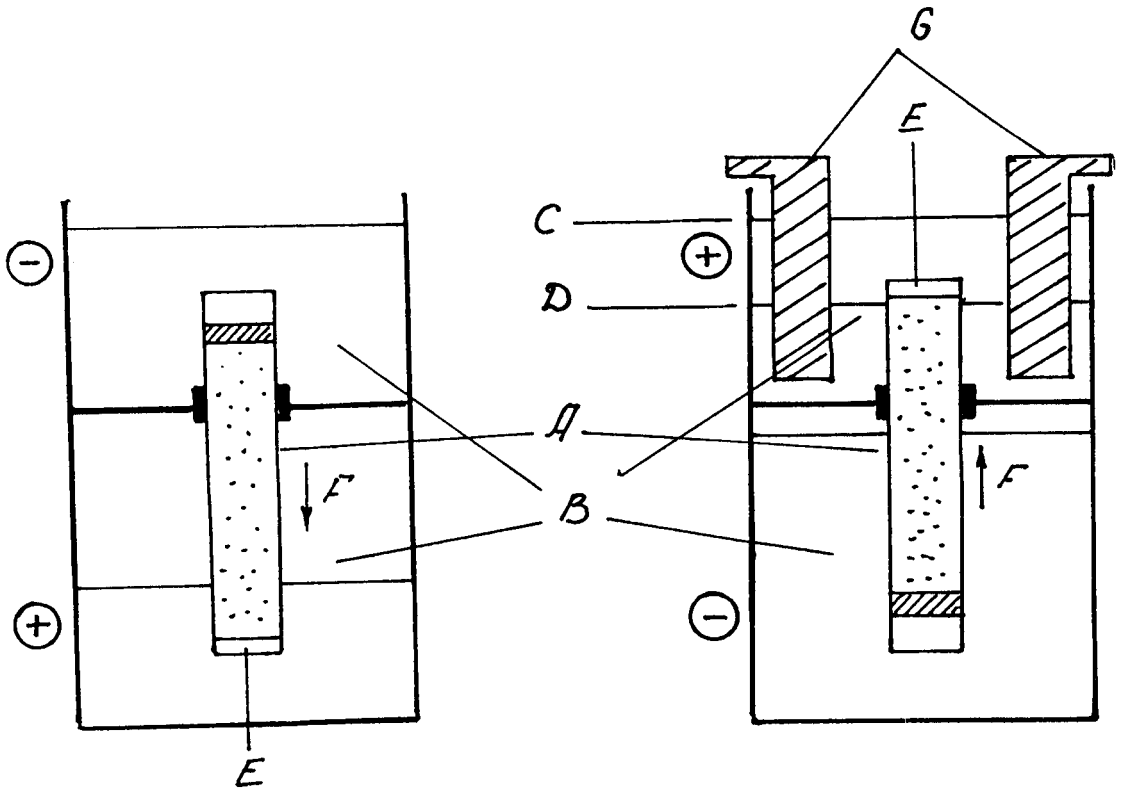

Fig.1.

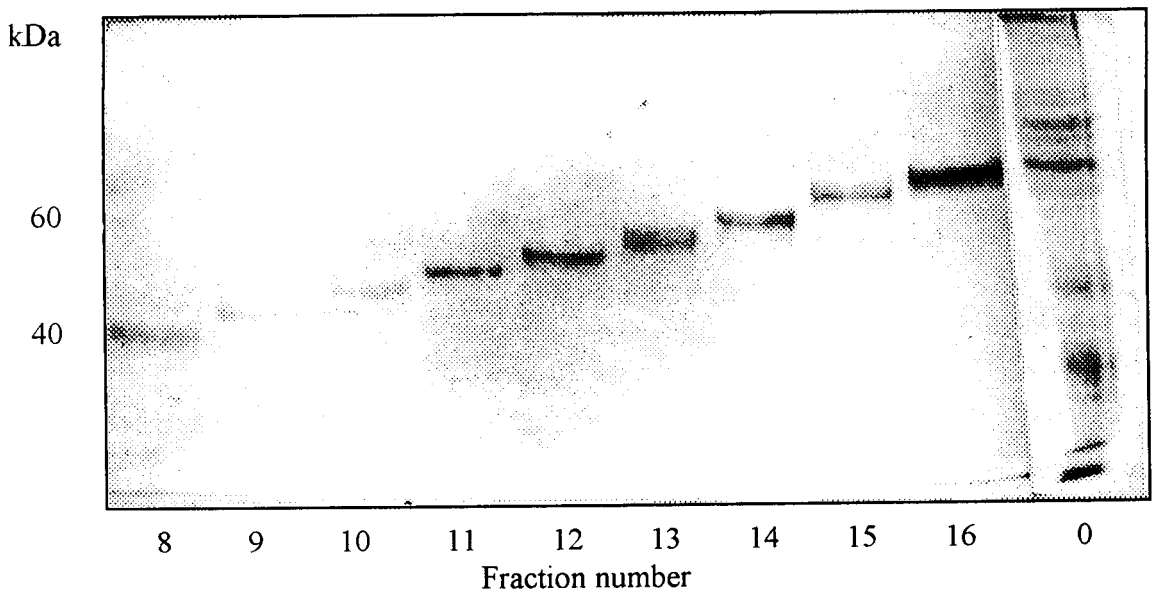

Fig.2.

The described method and device gives a chance to obtain necessary protein in preparative quantities enough for sequence determination or immunization and to decide the main task of reverse genetics - to go from protein to its gene. Especially it gives good possibilities in purification of proteins and enzymes whose quantities in cell are very low and usual chromatographic purification procedures are unsuccessful because of strong aggregation. As far as classic enzymatic investigation of homogeneous proteins, there are some difficulties because of high enzyme inactivation after denaturation. In conclusion, there are no reason not to use the described method and device to fractionate other biomolecules, not only proteins, in various buffer systems and gels.

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