CELL BIOLOGY

FACS-on-a-chip

A new microfluidic device allows fluorescence-activated cell sorting (FACS) of as few as 1,000 mammalian cells to high purity and with efficient yields. The high-throughput FACS-on-a-chip may not be so far away.

Many a researcher has experienced that uncomfortable shaking of the hand at the moment of placing a precious cell sample in the flow cytometer. Many have also come to the painful realization that if the sample has less than a few hundred thousand cells, FACS is just not an option. For those frustrated cell sorters, help is likely to come from miniaturization, and the 'FACS-on-a-chip' dream has started to crystallize thanks to microfluidics.

Microfluidic techniques take advantage of the laminar flows adopted by microliters of liquid moving in micron-scale channels. Several groups have already succeeded in sorting cells in such devices by using electric fields or hydrodynamic valves to divert the cell flow into different microchannels. However, these devices remain mostly impractical for the manipulation of fragile mammalian cells. In a recent report in *Nature Biotechnology*, a group led by William Butler at the San Diego–based company Genoptix announces that they have achieved a big step forward by sorting HeLa cells on a microfluidic chip using the forces of a focused light beam (Wang *et al.*, 2005). In the new device, a narrow stream of cells in a microchannel goes through an analysis region, then through an 'optical switch' region situated just before a Y-shaped junction, where the flow splits between two microchannels. If a fluorescent cell is detected in the analysis region, the optical switch is turned on and the cell is moved within the flow so that it is directed down the 'collection' branch of the Y junction.

The main advantage of this optical switch is the rapid response as the cells are moved within the laminar flow, relative to approaches relying on transient changes to the fluid flow. The result is a major improvement in purity and yields. To make the optical switch efficient, Butler explains, they had to use a 20-watt laser and this high power, in turn, necessitated the use of glass microfluidic devices rather than simpler plastic devices. Obviously, implementation remains difficult, but Genoptix intends to commercialize the technology. "[We will] most likely [be] commercializing instruments for sale to the research, industrial and clinical communities as various applications are developed," says Butler. So steady that shaking hand—help is on the way.

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RESEARCH PAPERS
Wang, M.M. *et al.* Microfluidic sorting of mammalian cells by optical force switching. *Nat. Biotechnol.* **23**, 83–87 (2005).

GENE REGULATION

WHEN DNA ALONE JUST IS NOT ENOUGH

DNA oligonucleotides can form a triplex with their target DNA and induce mutations, but their poor uptake by the cell limits their use. Now hybrids made of a triplex-forming oligonucleotide (TFO) and a translocation peptide are shown to effectively penetrate the plasma and nuclear membranes of a cell and trigger the formation of a DNA triplex leading to mutagenesis or site-directed recombination.

It began as a curious observation by physical chemists in 1957: a double-stranded DNA can bind another short molecule of DNA to form a triple helix. However, not until the synthesis of short DNA oligonucleotides became routine did this finding capture the imaginations of biologists; now it became possible to make TFOs that targeted specific genomic sequences and subsequently study the effect of this triplex DNA on, for example, transcription, mutagenesis or recombination.

Peter Glazer at Yale University has long been interested in the ability of TFOs to induce mutations and initiate DNA recombination. However, his and other researchers' studies were hampered by the fact that TFOs are not easily taken up by the cell. Glazer realized that without the ability to efficiently deliver these molecules to the nucleus of a cell, their use would be very limited. A recent article in *Nucleic Acids Research* describes his method to improve nuclear TFO delivery (Rogers *et al.*, 2004).

His team started with a translocation peptide, previously known to transport molecules into the cytosol of a cell and covalently linked it to a TFO. They found that the mutation frequency in the targeted gene in cells treated with the TFO-peptide hybrid was 20 times higher than in cells treated with the TFO alone. Glazer attributes this effect to the dual function of the peptides: "They shuttle the attached DNA through the plasma membrane into the cell and then into the nucleus. Their positive charge helps neutralize the negative charge of the DNA double helix and so aids the binding of the TFO to form the triplex."

By adding a nuclear localization signal to the translocation peptide, Glazer hopes to improve the nuclear delivery of TFO-peptide hybrids even more. This improvement in the efficiency of uptake is likely to usher in a bright future for TFOs in applications such as targeted gene knock out.

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RESEARCH PAPERS
Rogers, F.A. *et al.* Peptide conjugates for chromosomal gene targeting by triplex-forming oligonucleotides. *Nucleic Acids Res.* **32**, 6595–6604 (2004).