

## Introduction

FACS stands for "Fluorescence Activated Cell Sorting." The FACS machine has revolutionized biology by allowing researchers to isolate cells based on their spectral qualities. For example, if you have a fluorescently tagged antibody that binds to a certain cell type, you can isolate a pure sample of this cell type from a complex mixture by using a FACS machine. In addition to purification, the FACS machine can count the number of cells that have a certain spectral quality. If a FACS machine is used just for counting and not for separating subpopulations of cells, then the procedure is called "flow cytometry," and this is what you will be doing today.

"Image of flow cytometer removed due to copyright reasons."

### Flow cytometer

Before there were FACS machines, there were Coulter counters. Coulter counters are automated cell counting machines developed in the 1950s that count cells as they flow in a liquid stream. In an ingenious conceptual leap, Mack Fulwyler combined the technology of ink jet printers with that of Coulter counters to develop the first FACS machine. The ink jet printer head works by vibrating a nozzle so that a spray of discrete droplets is formed. Similarly, in a FACS machine, a liquid suspension of cells is forced at high pressure through a vibrating nozzle to create tiny charged droplets, each containing a single cell. The stream of droplets pass in front of a laser beam, and the scattered light is analyzed by a series of filters and photomultiplier tubes that convert the light signal into electrical impulses. Thus, each cell is "interrogated."

For FACS, the spectral qualities of the cell are analyzed nearly instantaneously and the machine "decides" whether or not the cell matches your desired spectral qualities. For example, if you have a mixture of green fluorescent cells and non-fluorescent cells, you can ask the machine to isolate the green cells. If a cell registers as green, an electrical charge deflects the cell to make it fall into a collection chamber.

"Image of how FACS works removed due to copyright reasons."

### **How FACS works**

FACS is technically challenging and most FACS machines are only run by experts. In contrast, biologists are often trained to perform flow cytometry in order to analyze the proportion of their sample that has particular spectral qualities. You will be using flow cytometry to measure the percentage of cells that are fluorescent. You have lipofected cells with two non-functional EGFP genes. Recombination between these two genes can restore the full length EGFP coding sequence so that cells express EGFP. By measuring the percentage of cells that fluoresce green, you will have some measure of the frequency of homologous recombination within mammalian cells.