

Microfluidizer Processor Helps Increase The Odds of Drug Discovery at Tularik

It's been said that to make an omelet, you have to break a few eggs, and the same can be said for genetically engineered pharmaceuticals. The difference is that, in the pharmaceutical business, the eggs are the bacterium *Escherichia coli* (*E. coli*), used as hosts to grow recombinant proteins that are naturally used by pathogenic viruses to reproduce. Find a way to disable the protein, and you may have found a way to disable the virus. You may even have found a cure for a disease like Hepatitis C. But first you have to break open the bacteria's "shell" in order to extract the protein for study. And unlike an egg you'll cook for breakfast, when you break open an *E. coli*, you don't want to scramble what's inside.

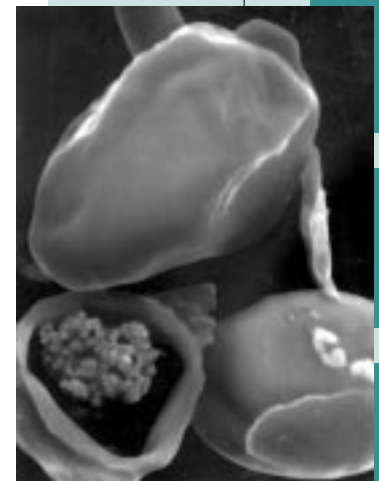
That's why pharmaceutical companies use Microfluidizer technology. It can apply 16,000 psi of steady pressure, break open any number of bacteria in a continuous batch, and yet leave the cell's contents intact for harvesting and research.

Tularik Inc. (South San Francisco, CA) uses the Microfluidizer as a critical part of its protein harvesting operation. The company was recently profiled in the *New York Times* as representative of a new trend in drug discovery: to perform tests, called assays, that show how target substances, like proteins, respond to different chemical compounds in environments that mimic the conditions of a living cell. Robots are used to assay thousands of different compounds in a few hours. In the case of certain viruses, the target protein is an enzyme necessary for the replicative processes in the virus.

Once the assay is developed, the search is on to find the right compound, from the thousands in Tularik's library, that might prevent the protein from doing its work. Once there's a "hit," that doesn't mean it's a drug candidate, however. Further tests must be done to show the compound is potent, specific to the target, and nontoxic. Of 450,000 assay tests, perhaps 450 will produce hits, and fewer than one of those will yield a potential drug. That's a lot of *E. coli* to break open. Hence, the need for the Microfluidizer Processor, a fast, effective, and above all controlled way to disrupt cells.

High Volume Production

Gabrielle Heilek-Snyder, Ph.D., established one of Tularik's viral programs. "My responsibility," says Heilek-Snyder, "is partially basic research, trying to understand what the requirement of a certain protein is, and also to develop effective assays that apply that knowledge to find a drug." Using *E. coli* as a host is necessary, she says, because it makes mass production of the protein possible. High volume production of proteins using the infectious virus is not possible due to the absence of a cell culture system. Similar technical issues apply for the assay. Rather than viral RNA which is tedious to produce, the harvested protein is tested against an artificial nucleic acid substrate. The assay works if the harvested protein successfully binds and unwinds the nucleic acid, as it would the natural substrate in the virus. Once that happens, Tularik scientists can introduce compounds that might stop the protein.



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But first they have to get the protein out of the E. coli. "The harvest and yield of the protein, needed for the assay, depends in a large part on how efficiently we crack the E. coli host cells," says Heilek-Snyder. "But it's not just getting the protein out that's important, the protein must still be active. The advantage of the Microfluidizer Processor is that it allows us to break the E. coli fast, with minimal impact on the cell's internal compartments. Additionally, the efficiency of cell-breakage needs to be consistent for different batches of E. coli."

To do that, Heilek-Snyder suspends the bacteria in a buffer that maintains the correct pH and protects the recombinant protein from degradation by host enzymes or exposure to oxidization. The suspended bacteria are then passed through a Model 110S Microfluidizer Processor, at 16,000 psi, two to four times until a 90% cell breakage is achieved.

Easy to Scale

Heilek-Snyder uses a Microfluidizer Processor because it is fast, easy to scale, easy to use and maintain, and does a good job of both cracking open the E. coli and minimizing any impact on the activity of the protein. "The advantage of the Microfluidizer Processor is that we can do anything from a small volume like 25 milliliters, up to anything in the 10 to 20 liter range, which gives us about 250 to 500 milliliters of cell lysate [suspended, processed bacteria]. The machine processes either volume with ease and great speed." Some alternatives to the Microfluidizer Processor, like using chemicals instead of mechanical action to break open the bacteria, would affect the activity of the protein. Others, like sonification, are much slower or, like the French Press, are more labor intensive to use.

"Clean up with the Microfluidizer Processor is not problematic," Heilek-Snyder says. "You don't have to take anything apart, which is different from the French press, which you do have to disassemble for cleaning. Additionally, the French Press is limited in the volume that can be processed at a time, depending on the chamber size of the model."

Tularik's "innovative approach" to creating high quality assays in high volumes is a major reason the company capitalization is estimated at \$400 million, states the New York Times. If so, it is because scientists like Heilek-Snyder have both the knowledge and the tools to make that approach work.



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