New method takes analysis of genetic libraries to next level

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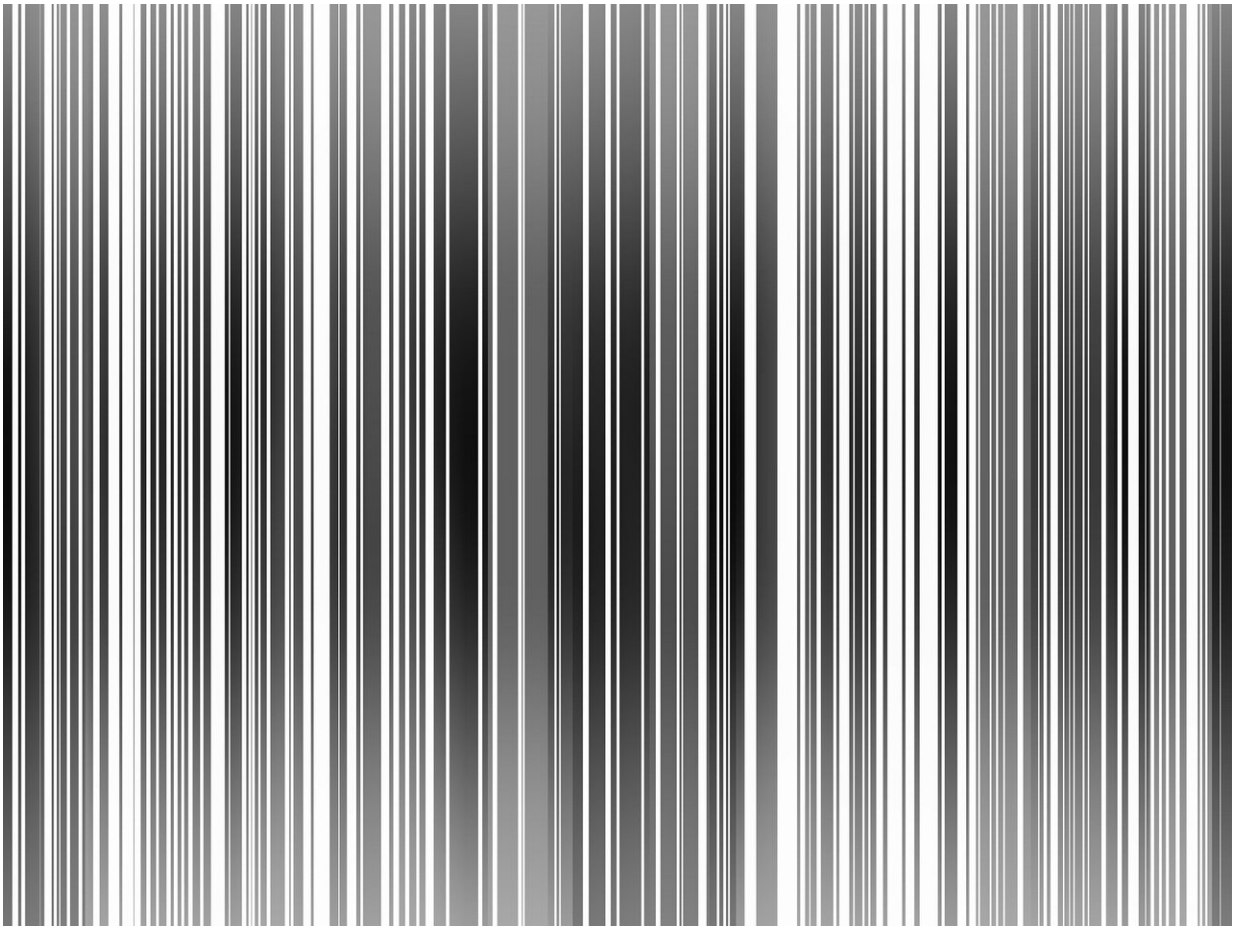

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Uppsala researchers have developed a new method for investigating

dynamic processes in large genetic libraries. By using this method to study cell cycle regulation,researchers can paint a clearer picture of the elusive control mechanism. The study is published in the journal *Nature Methods*.

Modern gene technology makes it possible to quickly and inexpensively introduce thousands of DNA modifications in human cells or bacteria to create genetic libraries. The CRISPR/Cas9 system, a.k.a. the "gene scissors," can be modified and used to alter the expression of thousands of proteins. By labeling each modification with a genetic barcode, it is possible to keep track of which cell carries which change.

Recent developments in optics and image analysis have made it possible to investigate the chemical processes inside the cell with exceedingly high precision. In principle, it is possible to observe basic biological processes such as protein expression or cell division at the molecular level inside a living cell.

Combining these advanced optical methods with large-scale genetic engineering is highly desirable. Researchers could, in theory, identify all the genes involved in a given process by observing the biology in a genetic library. Studies that have so far taken several years could be conducted in a single experiment, in theory.

The challenges that have previously prevented scientists from putting theory into practice have primarily been technical. How do you keep track of thousands of cells so that you can first examine their biology and then read the genetic barcode?

A group of Uppsala researchers rose to the challenge, and now presents the DuMPLING method (Dynamic u-fluidic Microscopy-based Phenotyping of a Library before IN situ Genotyping). This method enables the examination of an entire library of living cells in a single

microfluidic chip.

"The method is exceptionally potent and allows us to link genetic information to complex cell behavior at an entirely new level," says Johan Elf, professor of physical biology, who led the study.

Among other things, Elf and his team study the bacterial cell cycle. In all cells,it is vital that all DNA is copied exactly once before each cell division. If this is not the case, the cell is at risk of losing genetic material or accumulating DNA with equally devastating consequences. Although cell cycle regulation has been studied for decades, it is still unclear how cells achieve the strict control that is required.

"We can develop models that can reproduce the mechanism, but since we don't know all the players yet, it's hard to test if the models are biologically relevant. With this new method, it will be possible to identify the unknown components," says Daniel Camsund, researcher in molecular cell biology at Uppsala University.

The researchers created a genetic library where they decreased the expression of various known cell cycle regulators as well as some unknown genes and then used the DuMPLING method to study how the cell cycle was affected by these modifications. The next step is the game-changer. When all cell cycle data is collected, the nutrient solution in the chip is replaced with a solution that preserves the cells and fixes them in their positions. The genetic barcode can now be read using microscopy and color-coded pieces of DNA.

"It's fascinating to see how the color code develops, but fortunately, we're not decoding it manually. We have software that makes the identification," says Jimmy Larsson, researcher in molecular cell biology at Uppsala University

The results are encouraging. From the data, the researchers can identify most of the known regulatory elements, which means that the method works. Since the DuMPLING produces time-resolved data, it is also possible to tell how the cell cycle is affected by the various modifications. In the next phase, the team plans to expand the library to include all genes in the bacterial genome. Hopefully, this will take research one step closer to a complete description of the cell cycle control mechanism.

More information: Time-resolved imaging-based CRISPRi screening, *Nature Methods* (2019). DOI: 10.1038/s41592-019-0629-y , nature.com/articles/s41592-019-0629-y

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