THE AUTHOR FILE

Carl Hansen

A million picoliter PCR chambers give quick, precise answers.

When Carl Hansen says the strategy he takes in his laboratory is one of "divide and conquer," he is not referring to the scientists who work with him at the Center for High-Throughput Biology at the University of British Columbia in Vancouver. Rather, he is referring to the samples they work with. Last month in *Nature Methods,* Hansen described a microfluidic chip that is used to deposit single hematopoetic stem cells into nanoliter chambers where medium exchange can occur and which can be imaged extensively (Lecault *et al.*, 2011).

Jens Hueft and Tim Leaver

Carl Hansen

This month, he uses picoliter chambers to make highly accurate measurements of specific molecules of DNA (Heyries *et al.*, 2011). In a technique called digital PCR, a sample is spread across many compartments, allowing DNA amplification to occur separately in each one. The presence and original concentration of a transcript can then be assessed by counting the chambers in which a reaction occurs. The dynamic range, precision and sensitivity of measurements improve with the number of chambers.

In a chance meeting with Hansen's former graduate advisor, Stephen Quake, the two scientists speculated how many reactions would be necessary for a particularly difficult application: identifying extra or missing chromosomes by measuring fetal DNA in maternal blood. Fetal DNA is present in tiny amounts compared with maternal DNA, and detecting very small differences in the ratios of sequences was beyond any available measurement technique. "It came out to about a million chambers," recalls Hansen, "and that challenge captured my imagination."

There was no way to make valves at a sufficiently high density to create a million-reaction chip. As a workaround, Hansen began creating tiny depressions in one layer that would be sealed off by another layer, but the first attempts were not promising. As he was playing with some soon-to-be-discarded prototypes, Hansen came up with a viable alternative. Oil could take the place of valves. Researchers in his lab could make a device that would load a chip with reagents and then use a final push of oil that would break off liquid at the entrance to every chamber, creating sealed-off individual reactors.

"One of the most frustrating things was that we knew from the beginning that it would work," says Hansen, "but it wouldn't be valuable unless we could make it perform with high precision." What followed were three years of optimization: accommodating fast-evaporating solutions, optimizing device geometries and reagent properties, writing scripts that could handle data acquisition, conducting round after round of testing, troubleshooting, tweaking and testing again. Some of the most obstinate problems are trivial in hindsight. At one point, the device seemed to be over- and under-estimating dilution factors. In fact, the measurement was accurate, but DNA was getting stuck in the microcentrifuge tube in which samples were prepared. They resolved the problem, says Hansen, "by a whiff of soap."

When the team began trying to measure single genetic variants at high dilutions, they initially thought they were plagued by contamination because they saw too many instances of the rare allele. It turned out that the signal they were detecting was actually the error rate of DNA polymerase itself. In fact, by looking for signals from wild-type and rare alleles in the same chamber, the researchers actually could lower the limit of detection below what they had previously thought possible. "Once we knew the technology was working, implementing this idea took about a week," says Hansen.

Overall, the million-chamber device can be used to detect rare variants at dilutions of 10^5 and discriminate as little as a 1% difference in chromosome copy number. Fluidigm is working to commercialize this technology, and researchers in several labs are working on other technologies to make digital PCR more precise as well. That means researchers will soon have ready ways to quantify DNA reliably, predicts Hansen, and that will allow digital PCR to begin to replace several techniques based on quantitative PCR, which estimates the amount of DNA in a sample by counting the number of cycles necessary to reach a threshold and is highly susceptible to artifacts.

"A lot of the important applications are probably yet to come." —Carl Hansen

Sensitive digital PCR will also usher in unanticipated uses, he says. "In many cases, we don't really know how to use digital PCR because the precision and sensitivity of measures have been limited. A lot of the important applications are probably yet to come."

Monya Baker

Heyries, K.A. *et al.* Megapixel digital PCR. *Nat. Methods* **8**, 649–651 (2011).

Lecault, V. *et al.* High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. *Nat. Methods* **8**, 581–586 (2011).