A Ground-Breaking Method for Distinguishing Needles from Hay

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As scientists continue to elucidate differences between the genomes of cells in healthy and diseased conditions, therapies get progressively more targeted. The benefits are obvious: increased knowledge about disease states, including cancer-associated mutations and drug resistance, leads to greater possibilities for early diagnosis and efficient treatment. However, cells exhibit large mutational variations, leading to mixed populations of subclones that may respond differently to therapeutics and radiation. Although an individual cancer consists of billions of cells, conventional DNA sequencing approaches merely generate an "average" of the genomic DNA of most of the cells. Detection of rare mutations requires much more sensitive sequencing, but available techniques have been unable to sufficiently distinguish true variation from method-related errors. Worse yet, they are not just unsatisfactory for the task, they are also exceedingly expensive.

To overcome these issues, Drs. Michael Schmitt and Jerry Radich from the Clinical Research Division teamed up with colleagues at the University of Washington to develop a sequencing approach based on a novel technique termed "duplex sequencing". In 2012, Dr. Schmitt published findings showing that results could be greatly improved by separately analyzing both strands of the DNA molecule. This was in contrast to current technologies, so called next-generation DNA sequencing, which look at a single strand, generating an unacceptable amount of technical errors in comparison to the rate of low-frequency mutations. Duplex sequencing overcomes those limitations by only scoring mutations that appear in the same position in both complementary DNA strands, ignoring amplification and sequencing errors found in just one strand. In a follow-up study, the Fred Hutch investigators combined this new approach with an iterative capturing method to enrich targeted genes by more than a million-fold. The result: jackpot. "Our approach improves the accuracy of DNA sequencing by 100,000-fold or more, and allows us to readily identify rare mutations that are undetectable by other approaches," Dr. Schmitt said. The technique is described in *Nature Methods*, demonstrating not only high capacity and accuracy, but also scaling ability for multiple targets.

The field of oncology harbors numerous applications for this technique, according to Dr. Schmitt. Besides facilitating early cancer diagnosis and design of targeted treatments, another possibility is

continuous screening of patients undergoing therapy. "For example, we could monitor for early resistance to a drug by monitoring the levels of cancer-associated mutations in blood, and then rapidly switch the patient to a new drug before their cancer progresses significantly," Dr. Schmitt said. However, there is more good news: the extreme accuracy of the technique allows sequencing of sample sizes as small as a single cell. The new technology could therefore be applied in settings where only tiny amounts of DNA are available, yet accuracy is critical, such as forensics and studies of extinct organisms (paleogenomics).

Still, the dinosaurs will have to wait. The investigators are currently using the technique for exploring drug resistance mutations in blood cancers, and assessing genetic variability within various human cancer types. Methods are also under development for identification of occasional cancer cells that may persist after conventional treatment. To avoid relapse, patients who have those cells in their blood might need further aggressive treatment, whereas those who are free of circulating tumor cells could be treated less aggressively, with lower risks of negative side effects.

The take-home message is clear: finding a needle in a haystack just got a million times easier.

Schmitt MW, Fox EJ, Prindle MJ, Reid-Bayliss KS, True LD, Radich JP, Loeb LA. 2015. Sequencing small genomic targets with high efficiency and extreme accuracy. *Nat Methods*. 12(5):423-5.

See also: Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. 2012. Detection of ultra-rare mutations by next-generation sequencing. *Proc Natl Acad Sci U S A*. 109(36):14508-13.

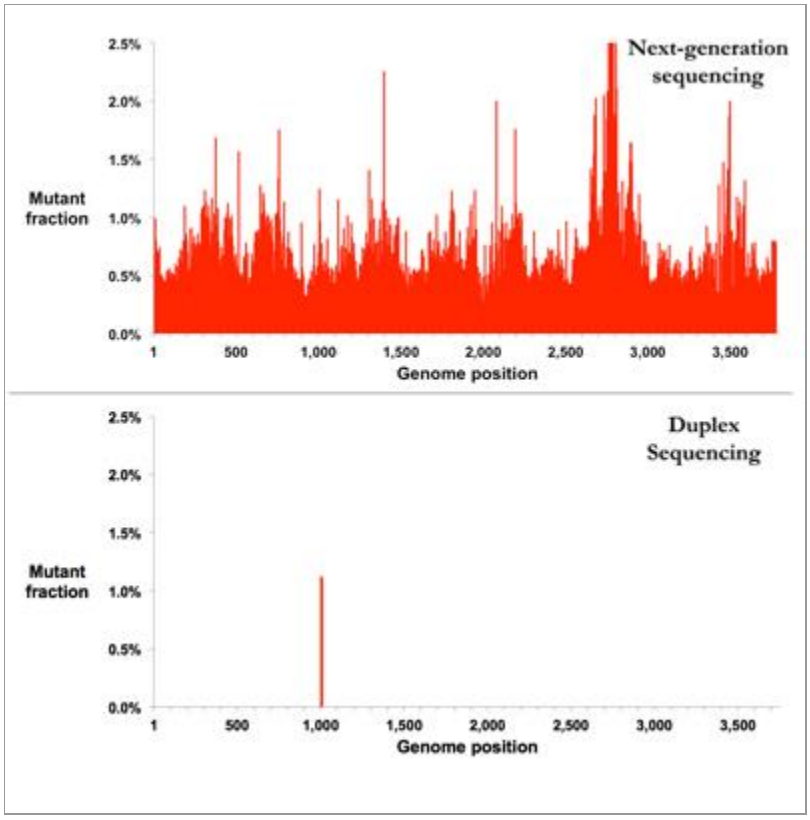

Image provided by Dr. Michael Schmitt

Duplex sequencing (lower panel) efficiently removed background errors, revealing a single point mutation that could not be discerned using conventional high-throughput sequencing (upper panel).