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TECHNOLOGY

The sequencing game

A new approach to pooling DNA samples promises to increase the number of templates that can be sequenced in a single experiment from a few dozen to over 100,000. This 'DNA Sudoku' method, named after the logic puzzle, will offer substantial cost savings to high-throughout sequencing and efficient medical genotyping.

The pooling of DNA sequencing samples is not new, but current protocols rely on bar coding each sample with a short oligonucleotide, which is then used to associate a read to the correct sample. This approach is laborious, however, as a unique tag has to be created for each sample. The new method creates pools of samples, and then associates a bar code to each pool, rather than to each individual sequence. Because the samples are pooled in a particular pattern, a Sudoku-like approach is needed to assign each read to a particular pool, and then to a particular sample. The reliability of the method (97%) was established *in silico* and in two experimental trials: to detect cystic fibrosis carrier mutations in 18,000 individuals and the presence of particular short hairpin RNA inserts in over 40,000 bacterial clones.

Because new-generation sequencing technologies produce only short reads, DNA Sudoku is most useful in detecting the presence of medically relevant mutations in defined genomic regions; although Prabhu and Pe'er also describe a further use of pooling and combinatorial genotyping for detecting very rare variants. As sequencing reads get longer, so will the list of applications.

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ORIGINAL RESEARCH PAPERS Erlich, Y. *et al.* DNA Sudoku — harnessing high-throughput sequencing for multiplexed specimen analysis. *Genome Res.* **19**, 1243–1253 (2009) | Prabhu, S. & Pe'er, I. Overlapping pools for high-throughput targeted resequencing. *Genome Res.* **19**, 1254–1261 (2009)

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