# New method for sequencing genome in a single cell

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(Phys.org)—The traditional genome sequencing process requires thousands of cells (or more) to provide sufficient DNA, and this means that variations that are only present in a small number of cells—such as early cancer cells—are missed. Now a new technique has been developed for effectively sequencing the DNA in an individual cell.

The genome of single cells has been sequenced before using a technique known as a polymerase chain reaction (PCR) to produce enough copies of the DNA for sequencing. This procedure enables researchers to sequence the genome with about 40-70 percent accuracy, but greater precision is difficult because of amplification bias, in which some parts of the genome tend to be copied more readily than others. Not only does this reduce the extent of the genome that can be sequenced, but it also means segments of DNA that are copied are difficult to detect and may be missed.

In a paper published in *Science* a US research team describe a new way of getting around the amplification bias problem. The team, led by Professor X. Sunney Xie of Harvard University in Massachusetts, has developed a new technique which they have named "Multiple Annealing and Looping-Based Amplification Cycles" (MALBAC), in which the genome from a cell is first isolated and then "primers" consisting of short segments of DNA are added. When the DNA with the added primers is copied, up to 93 percent of the genome can be sequenced because the common segments incorporated into the copies loop back on themselves.

The primers are pieces of DNA with a common section of 27 nucleotides and a variable section of eight nucleotides. The shorter section binds to the cell's DNA and the longer, common section reduces amplification bias by preventing the DNA from being copied too often.

The researchers used the new technique to sequence the DNA in three closely related cells, and also the DNA of 99 sperm from a single Asian male (as described in a second paper in the same journal). They were able to identify variations in individual nucleotides and observed no false positives.

The improvement in single cell genome sequencing is a big advance, but variations in single nucleotides could still be missed. The copying method can also introduce occasional copying errors.

The ability to sequence the genome in a single cell could help in cancer and other research since it would allow comparisons between individual cells. It could also prove useful in applications where only a small sample of DNA is available, such as in forensic science.

**More information:** Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell, *Science*, 21 December 2012: Vol. 338 no. 6114 pp. 1622-1626. DOI: 10.1126/science.1229164

**ABSTRACT**
Kindred cells can have different genomes because of dynamic changes in DNA. Single-cell sequencing is needed to characterize these genomic differences but has been hindered by whole-genome amplification bias, resulting in low genome coverage. Here, we report on a new amplification method—multiple annealing and looping-based amplification cycles (MALBAC)—that offers high uniformity across the genome. Sequencing MALBAC-amplified DNA achieves 93% genome coverage ≥1x for a single human cell at 25x mean sequencing depth. We

detected digitized copy-number variations (CNVs) of a single cancer cell. By sequencing three kindred cells, we were able to identify individual single-nucleotide variations (SNVs), with no false positives detected. We directly measured the genome-wide mutation rate of a cancer cell line and found that purine-pyrimidine exchanges occurred unusually frequently among the newly acquired SNVs.