New Single-Cell Genome Amplification Method Aims to Reduce Biases to Detect Micro CNVs

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Premium

SAN FRANCISCO (GenomeWeb) – A Harvard and Peking University team has developed a method for single-cell whole-genome amplification that seeks to reduce biases introduced by other whole-genome amplification methods.

The researchers described the linear amplification via transposon insertion (LIANTI) method today in *Science*, reporting that it enables micro-copy number variation detection with kilobase resolution. Sunney Xie, senior author on the study, said that he is interested in using the method for preimplantation genetic screening and diagnosis, which typically rely on the analysis of single or very few cells to screen embryos for chromosomal alterations prior to implantation.

Xie's group previously developed another method for single-cell amplification called MALBAC, for multiple annealing and looping based amplification cycles, that they have been using in an *in vitro* fertilization clinical trial at Peking University and Third Hospital to screen embryos for aneuploidies.

Xie, who has joint appointments at Harvard and Peking University, said that while MALBAC enabled more accurate CNV measurement compared to previous methods like multiple displacement amplification (MDA), its ability to call SNVs is limited due to high allele dropout. In addition, he wanted a method that would have even greater resolution to detect sub-chromosomal CNVs.

In the LIANTI method, the team uses transposon insertions to enable linear amplification, as opposed to exponential amplification. Exponential amplification magnifies discrepancies in amplification — regions that amplify well will exponentially multiply such that the final product consists mostly of that region, even if it actually represents only a small fraction of the genome. And, any errors introduced during amplification will also be magnified, decreasing accuracy.

In LIANTI, the researchers designed what they called a LIANTI transposon, which consists of a 19-base pair double-stranded transposase binding site and a single-stranded T7 promoter loop. That is mixed with the Tn5 transposase and the two bind and randomly fragment genomic DNA from a single cell via transposition. Next, DNA polymerase converts the single-stranded T7 promoter loop into double-stranded T7 promoters, which are now at each end of the DNA fragment. Then *in vitro* transcription is

performed, which linearly amplifies the fragments into RNAs. Finally, after reverse transcription, RNase digestion, and second strand synthesis, the amplicons are tagged with a unique molecular barcode. Library prep and sequencing is then performed.

The authors compared their method to other commonly used methods, including MDA, MALBAC, and degenerate oligonucleotide primed-PCR (DOP-PCR), as well as to bulk sequencing.

First, the authors used LIANTI to amplify DNA from single cells from a skin fibroblast cell line. The researchers sequenced the cells to 30x coverage and achieved 97 percent coverage of the genome with a 17 percent allele dropout rate.

To evaluate how uniform the amplification was, they plotted the data in 1-megabase bins across the genome and then zoomed in to a specific region on chromosome 1. The researchers found that LIANTI significantly outperformed all methods in this regard.

To increase LIANTI's spatial resolution, the researchers took advantage of the fact that the LIANTI amplicons are essentially tagged: amplicons with the same ends originate from the same DNA fragment. Using this feature, the researchers were able to get a more accurate count of how many fragments were at each genomic position, enabling better resolution for CNV detection. They showed in the study that the method could detection subchromosomal alterations down to about 10 kilobases in size. Previous methods are limited to around a 1-megabase resolution.

Next, the researchers evaluated how well SNVs could be called using the LIANTI method. Initially, the researchers noted that while LIANTI still had an improved false-positive rate compared to the other methods, it was still higher than what would be expected with linear amplification. On further examination, the researchers found that false positives were dominated by C-to-T calls, which they attributed to an artifact caused by C-to-U deamination.

To account for this artifact, the researchers treated genomic DNA with uracil-DNA glycosylase prior to LIANTI amplification, which eliminates cytosine-deaminated uracil bases. That reduced the number of false positives to around 5,000 from just under 16,000. The MDA method, by contrast, resulted in 41,000 false positives and bulk sequencing resulted in just 89 false positives. Aside from reducing the sheer number of false positives, after UDG treatment, the spectra of false positives was more similar to that seen from bulk sequencing, as opposed to being dominated by C-to-T false positives.

Iwijn De Vlaminck, an assistant professor of biomedical engineering at Cornell University who was not involved with the study, said that the method "addresses a timely problem related to single-cell sequencing." De Vlaminick works in the single-cell genomics field and previously was an author of a study that compared the various single-cell whole-genome amplification methods.

As next-generation sequencing costs have come down, researchers have been increasingly interested in analyzing genomes of single cells, De Vlaminck said. "Single-cell technology is starting to shed a lot of light on genetic heterogeneity," for instance. Whole-genome amplification is needed for such studies, but current methods introduce lots of errors, he said. The *Science* study authors seek to address those problems by proposing a new method based on a modified version of transposase chemistry, which is "robust" and has "proved its worth in many other applications," he added.

Xie said that going forward, he will first be interested in applying this method within the context of PGD and PGS applications. "The ability to call micro CNVs will be extremely useful," he said. For instance, the known pathogenic microdeletions have a higher combined incidence than trisomy 21, he said. "But they have not been detectable on a single-cell basis," he said. The first step for this will be to first analyze patients with known microdeletions to verify that the single-cell approach yields the same result as bulk sequencing, Xie said.

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