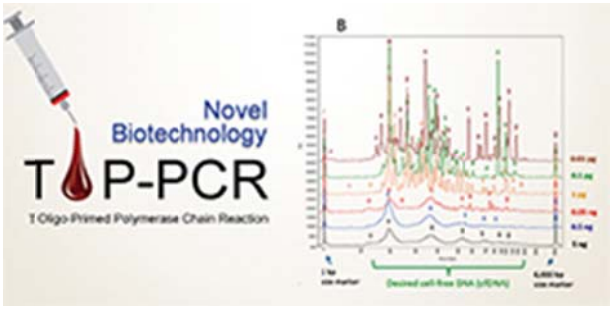
New PCR Method Allows Sequencing of Minute DNA Fragments from Body Fluids



Sequencing DNA from body fluids is a powerful noninvasive approach for the diagnosis of genetic defects, infectious agents and diseases, but its success relies on the quality and quantity of the DNA samples. Numerous DNA samples are either at low quality or of poor quality. Dr. Kuo-Ping Chiu, an Associate Research Fellow at the Genomics Research Center (GRC) has recently developed a method that can amplify minute quantities of DNA fragments found in body fluids for DNA sequencing, an advance that will potentially be very useful for DNA-based diagnosis. His innovative approach was reported in the January 17, 2017 issue of *Scientific Reports*.

Dr. Chiu's team came up with an improved Polymerase Chain Reaction (PCR) method to make copies of tiny traces of cell-free DNA molecules circulating in body fluids by remodeling the technique they used in a collaborative study on Early Onset Breast Cancer (EOBC) with Dr. Alice L. Yu, Distinguished Visiting Chair at the GRC. The redesigned PCR method is called "T Oligo-Primed Polymerase Chain Reaction" (TOP-PCR).

Conventional PCR uses two primers (short strands of RNA or DNA that serve as a starting point for DNA synthesis); however, TOP-PCR only uses one. The difference plays an important role in amplification efficiency. Conventional methods of PCR fail to deal with low abundance DNA because the large losses in target sequence information incurred during the ligation process invalidate the PCR. Dr. Chiu's team overcame this problem by successfully adopting a so-called "half adaptor", generated by annealing P oligo and T oligo, with subsequent PCR primed by the T oligo alone.

Using this approach, the team has demonstrated made a big improvement as far as sample requirement is concerned. For only 0.01 picograms of DNA from body fluid, that is 1/50,000 of the conventional requirement, they are able to trap the DNA and amplify it to a quantity suitable for research work.

Dr. Chui's method outperforms the method regularly adopted by the next-generation sequencing platform Illumina, and can therefore be expected to improve sensitivity in that area. And it is easily foresee how TOP-PCR may be applied for early diagnosis of diseases. This method has the potential to be used to monitor cancer metastasis, viral infections or even for prenatal genetic diagnosis.

"TOP-PCR makes two major breakthroughs: First of all, the minimum quantity requirement (0.5 ng) for DNA sequencing is demolished; furthermore, even above 0.5 ng, the sensitivity of TOP-PCR is about two times better than the traditional method. Thus, it creates tremendous

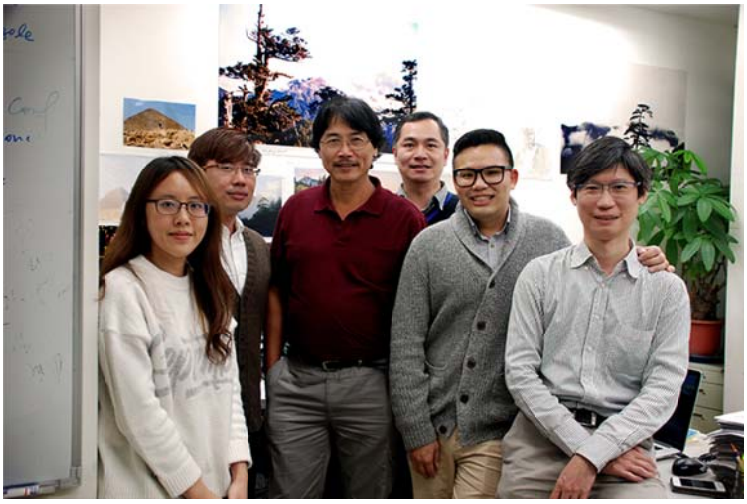
potential for diagnostics. The next step is to use the technology in clinical applications via collaborations with diagnostics industry and sequencing industry." Dr. Chiu said.

In short, TOP-PCR facilitates DNA quantity and quality assessment and improves sequence data analysis of healthy and diseased body fluid DNA samples.

The method has been patented in Taiwan and applications have been submitted in other countries.

For more information, please visit:
http://www.genomics.sinica.edu.tw/index.php/en/news/latest-news/503-top-pcr-dna

The full research article can be found at: http://www.nature.com/articles/srep40767



Team members (from left): Yenju Chen, Tzu-Han Chen, Dr. Kuo-Ping Chiu, Dr. Hsin-Chieh Shiau, Dr. Yu-Shin Nai, Dr. Yu-Feng Huang.