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10 years of rolling the minicircles: RCA assays in DNA diagnostics

'...RCA has become increasingly popular due to its simplicity and high specificity in the detection of nucleic acids, proteins and other biomarkers.'

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DNA amplification is the starting point of most DNA diagnostics and related assays [1,2]. Although temperature-cycling PCR still remains the dominant amplification technique, some robust isothermal alternatives to PCR have successfully invaded the area and significantly increased the potential of DNA diagnostics [2]. Among these techniques, an approach that was launched 10 years ago based on rolling-circle DNA amplification (RCA) has become increasingly popular due to its simplicity and high specificity in the detection of nucleic acids, proteins and other biomarkers [3–6].

The RCA approach is quite simple, being based on the ability of certain DNA polymerases to repetitively replicate small single-stranded circular DNA molecules at a constant temperature. This linear process can be exponentially branched to yield the ramification amplification (also known as hyperbranched or cascade RCA). As a result, up to several billion linear copies of circular DNA probes can be obtained in approximately 1 h, without elaborate temperature-cycling instrumentation, thus providing fast and ultrasensitive detection of corresponding analytes that rivals PCR [7].

The idea of using DNA minicircles for isothermal amplification originated in the pioneering studies of several research groups who filed independent patent applications in the early 1990s [101–104]. Although these groups focused on different aspects of the technology (for example, on its ability to mediate target vs. signal amplification, or on its exquisite sensitivity), their cumulative efforts resulted in the development of the seminal concept of

RCA so that it could be applied in real-life experiments [8,9]. In the following 10 years, RCA technology found numerous applications in identification of pathogens, oncogenes, hot spot mutations and single nucleotide polymorphisms (SNPs), as well as in multiplexed genomic and proteomic profiling with microarrays [3–5,10–16].

RCA has the potential to detect single molecules, to be monitored in real time and, using peptide nucleic acid (PNA) openers, to be performed directly on duplex DNA [10,17–23]. In addition, RCA can be used for creating artificial telomeres, DNA vaccines and ribozymes [24,25]. Based on this impressive and promising progress, several biotech companies are currently engaged in the development of RCA-based diagnostics: Molecular Staging (USA; now acquired by Qiagen), Hamilton Thorne Biosciences (USA), Amersham Biosciences (UK; now part of GE Healthcare), Olink Bioscience (Sweden) and Biokit (Spain), to name a few worldwide.

On the verge of its second decade, as an enthusiast of RCA technology, I hope that rolling-off DNA copies will successfully be continued, and that commercial RCA-based diagnostic kits will soon be on the market. I also hope that certain apparent tensions between some of the RCA-engaged companies related to the admittedly complex patent situation (that, in my opinion, somewhat hamper RCA development) will eventually be resolved to the satisfaction of all parties involved. Then, these companies could unite their forces in a faster commercialization of this powerful technology to finally bring it to practical use.

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