New highly efficient molecular probe for real-time PCR monitoring and genetic testing

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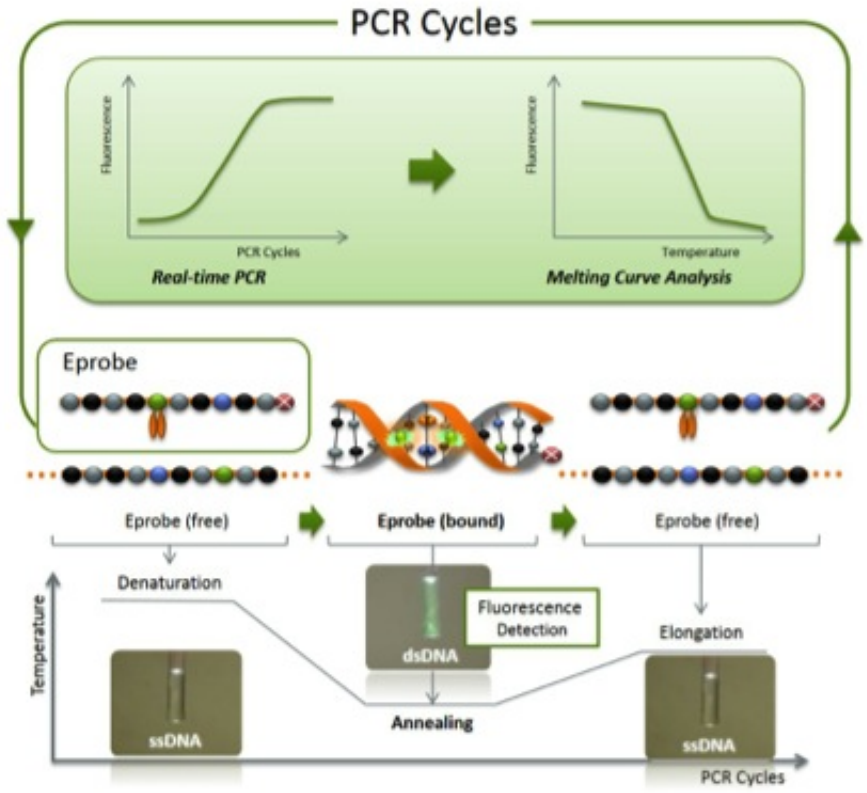

Eprobe®, a highly efficient and reliable fluorescent probe for PCR DNA amplification techniques and DNA analysis in hybridization experiments, has been developed by researchers from RIKEN and Japanese firm K.K.DNAFORM. This technology will enable the development of new, advanced assays for DNA-based genetic testing and help to bring the benefits of genome-wide sequencing studies to patients in the clinic. Credit: RIKEN

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Takeshi Hanami, Diane Delobel and colleagues from the RIKEN Center for Life Science Technologies, the RIKEN Preventive Medicine & Diagnosis Innovation Program, and K.K.DNAFORM report on the development of the new molecular probe today in the journal *PLOS ONE*.

PCR, for Polymerase Chain Reaction, is a simple and inexpensive DNA amplification technique, widely used to analyse DNA and RNA in life science laboratories. PCR is further applied in hospitals to diagnose diseases, identify bacteria and viruses, or in forensic medicine. During PCR, small amounts of target DNA molecules are copied and rapidly amplified, thus enabling researchers to analyze the DNA, test it or clone it.

Eprobes are short DNA oligonucleotides labeled with two fluorescent dye moieties attached to the same nucleotide. During the DNA amplification reaction, the probes bind to the newly synthesized DNA fragments, emitting a strong fluorescence signal upon binding, which enables researchers to monitor the reaction in real-time. In the study, the researchers used Eprobes for the detection of genetic variations in the human EGFR and KRAS tumor genes by combining real-time PCR with a hybridization technique. They show that Eprobes provide decisive advantages over commonly used hybridization probes because of their unique background signal reduction, enhanced DNA-binding affinity and very low false positives rate.

"Eprobe® enables real-time PCR methods, which are gaining importance for medical diagnostics and many life science applications, because they can provide quantitative results and increased reliability as compared to standard PCR methods," explains Kengo Usui, the leader of the Genetic Diagnosis Technology Unit at RIKEN Center for Life Science Technologies.

"This new technology will enable the development of advanced assay formats for the simultaneous detection of multiple target genes, as needed for example in the diagnosis of tumors" explains Takeshi Hanami, first author of the paper.

"We are very excited about the potential of the new Eprobes", comments Matthias Harbers, Visiting Scientist to the Division of Genomic Technologies at the RIKEN Center for Life Science Technologies and supervisor of the Eprobe development project. "In the reactions, Eprobes acted like sequence-specific fluorescent dyes, which gives them great potential for use as hybridization probes not only in PCR and melting curve analysis but also in other important applications like for instance in Fluorescent in situ Hybridization or FISH."

More information: Hanami et al. "Eprobe mediated real-time PCR monitoring and melting curve analysis" *PLOS ONE*, 2013, dx.plos.org/10.1371/journal.pone.0070942

Provided by RIKEN