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Label-free quantification of diagonostic biomarkers of microRNA's using ligase-assisted sandwich hybridization on a DNA microarray

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MicroRNAs (miRNAs) can be used as biomarkers for cancer and other human diseases; therefore, high-throughput and reliable miRNA-quantification methods are required to exploit these markers for diagnostic testing. Sandwich hybridization, based on DNA microarray technology and where two kinds of probes hybridize on different sites of an mRNA, is a method that can potentially be used for label-free, high-throughput quantification of target mRNAs. However, it is difficult to apply the sandwich hybridization method to miRNA because its length of ~22 nucleotides is too short for stable hybridization with both a non-attached fluorescent probe and a microarray-attached probe. To overcome this limitation, we constructed a platform for miRNA-quantification using ligase-assisted sandwich hybridization (LASH). T4 DNA ligase was used to compensate for the low affinity between miRNAs and two complementary DNA probes, and it improved the hybridization yield 50,000 times and enabled miR-143 to be quantified at concentrations of 30 fM to 30 pM. The LASH assay could also quantify miR-143 contained in total miRNA exported from living cells. Furthermore, multi-color detection enabled us to distinguish homologous miRNAs, which was an inherent limitation of the sandwich platform. This simple label-free quantification technique can be used in other approaches for the diagnosis of disease.

Biography

Taro Ueno received his BS, MS and PhD degrees from Waseda University in 2009, 2001 and 2004, respectively. He studied as a postdoctoral fellow at Stanford University in 2008. Since 2010, he has been a postdoctoral fellow of Graduate School of Pharmaceutical Sciences, The University of Tokyo.

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