Publication on the use of Arrayer 2000 (MicroArrayer ezJet)

Advanced Technology Inc.

Jung, S. *et al*. Extensible Multiplex Real-time PCR of MicroRNA Using Microparticles.
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ABSTRACT

Multiplex quantitative real-time PCR (qPCR), which measures multiple DNAs in a given sample, has received significant attention as a mean of verifying the rapidly increasing genetic targets of interest in single phenotype. Here we suggest a readily extensible qPCR for the expression analysis of multiple microRNA (miRNA) targets using microparticles of primer-immobilized networks as discrete reactors. Individual particles, 200~500 μm in diameter, are identified by two-dimensional codes engraved into the particles and the non-fluorescent encoding allows high-fidelity acquisition of signal in real-time PCR. During the course of PCR, the amplicons accumulate in the volume of the particles with high reliability and amplification efficiency over 95%. In a quick assay comprising of tens of particles holding different primers, each particle brings the independent real-time amplification curve representing the quantitative information of each target. Limited amount of sample was analyzed simultaneously in single chamber through this highly multiplexed qPCR; 10 kinds of miRNAs from purified extracellular vesicles (EVs).

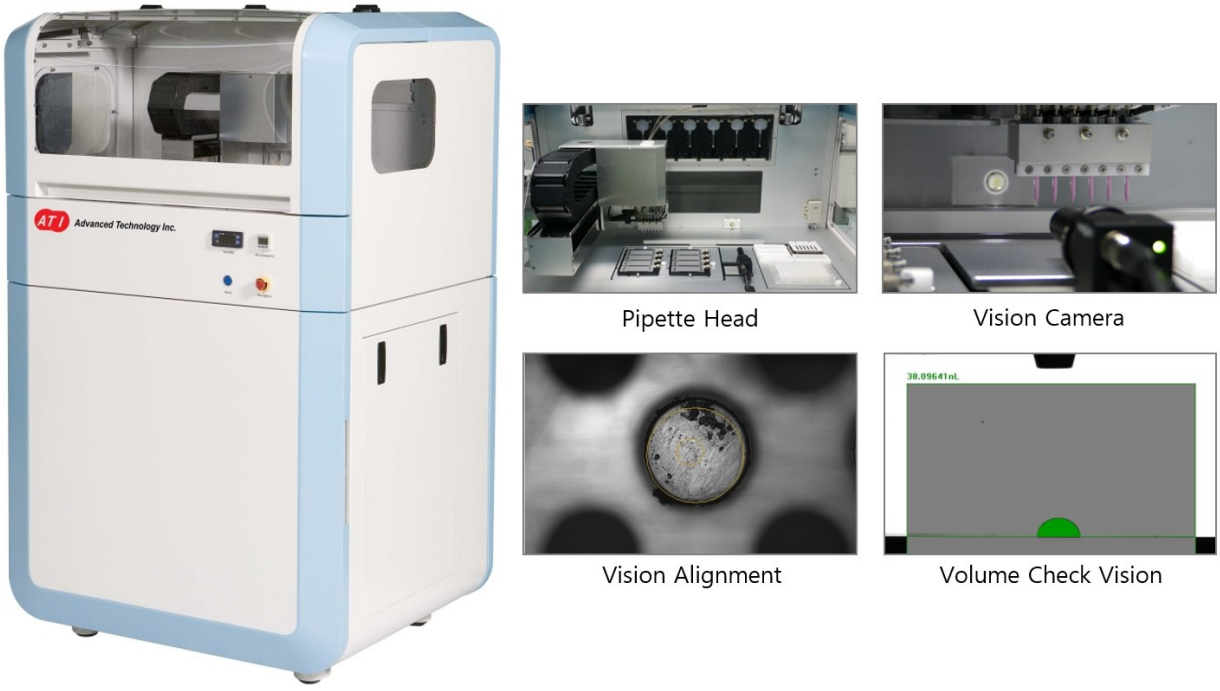

Figure 1: ATI MicroArray Spotter (ezJet Series) and functions

Use of Advanced Technology Inc., *MicroArray Spotter (ezJet Series).*

Dr. Jung (Center for BioMicrosystems, Korea Institute of Science and Technology) and his colleagues employed the *Arrayer 2000 (ezJet Series)* equipment to array lithographically encoded microparticles of primer-immobilized networks (LEM-PINs) onto the pattern-engraved spots on a hydrophobic substrate for particle-based qPCR.

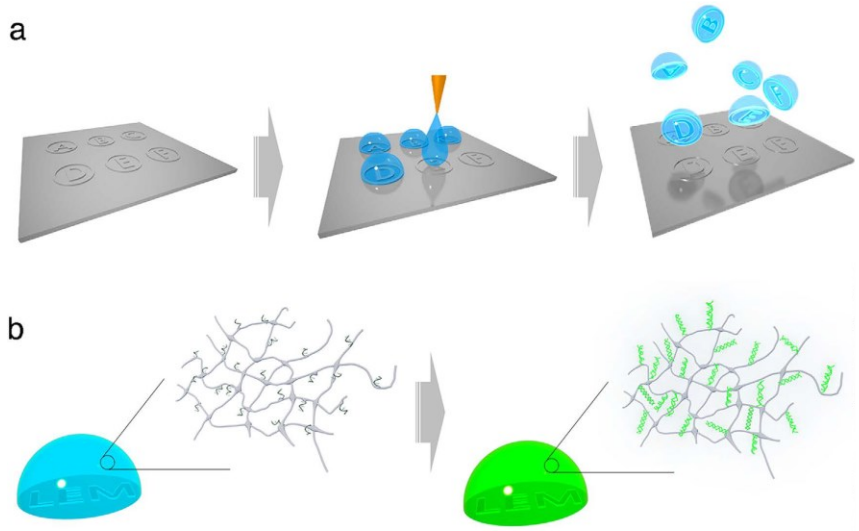

Figure 2 (a) Schematic of LEM-PIN fabrication composed of jetting (*Arrayer 2000*) the solution on the pre-patterned PDMS. (b) Accumulation of PCR amplicons and its intercalating fluorescence inside LEM-PIN.

The LEM-PINs were produced by dropping pre-polymer solution on the pre-patterned polydimethysiloxane (PDMS) using a jetting system *(Arrayer 2000)* followed by a UV cross-linking of 35 mJ/cm2. The aqueous solution of pre-polymer drop array was automatically generated after the manual recognition of three aligning marks on the PDMS. Volume of several nl was dropped over pattern-engraved spots on a hydrophobic substrate. In order to control the volume of the drops, the opening time was tuned from 150 μsec to 500 μsec and the volumes of 100 drops were averaged to obtain a mean value. The volume of LEM-PINs could be adjusted from 3nl to 1μl with a standard deviation of less than 10%. The LEM-PINs could be produced precisely with a high throughput of about 15,000 particles/hr per channel, guaranteeing the particles' uniformity requisite for quantitative analysis.

The two-dimensional codes on the particle can be read clearly which showed an accumulation of fluorescence during PCR without diffusion of the signal to the surrounding solution or to other particles. This particle qPCR provided superior specificity and quantitative resolution, and the number of targets detectable in single well is easily extended by virtue of lithographically encoded patterns. Multiple LEM-PINs simultaneously analyzed cDNAs which originated from target miRNAs and in the future can be transformed into a multiplex qPCR of other genetic contents such as RNA and DNA. This approach offers substantial opportunities for the realization of extensive genetic profiling on limited clinical specimens such as tumor tissue or extracellular body fluids.