Abstract Submitted
for the MAR12 Meeting of
The American Physical Society

Picoliter droplet-based digital peptide nucleic acid clamp PCR and dielectric sorting for low abundant K-ras mutations HUIDAN ZHANG, RALPH SPERLING, ASSAF ROTEM, LIANFENG SHAN, JOHN HEYMAN, YIZHE ZHANG, DAVID WEITZ, School of Engineering and Applied Sciences, Department of Physics, Harvard University — Colorectal cancer (CRC) remains the second leading cause of cancer-related mortality in the US, and the 5-year survival of metastatic CRC (mCRC) is less than 10%. Although monoclonal antibodies against epidermal growth factor receptor (EGFR) provide incremental improvements in survival, approximately 40% of mCRC patients with activating KRAS mutations won't benefit from this therapy. Peptide nucleic acid (PNA), a synthetic non-extendable oligonucleotides, can bind strongly to completely complementary wild-type KRAS by Watson-Crick base pairing and suppress its amplification during PCR, while any mutant allele will show unhindered amplification. The method is particularly suitable for the simultaneously detection of several adjoining mutant sites, just as mutations of codons 12 and 13 of KRAS gene where there are totally 12 possible mutation types. In this work, we describe the development and validation of this method, based on the droplet-based digital PCR. Using a microfluidic system, single target DNA molecule is compartmentalized in microdroplets together with PNA specific for wild-type KRAS, thermocycled and the fluorescence of each droplet was detected, followed by sorting and sequencing. It enables the precise determination of all possible mutant KRAS simultaneously, and the precise quantification of a single mutated KRAS in excess background unmutated KRAS.

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Date submitted: 23 Nov 2011 Electronic form version 1.4