

## Supporting Information

# Highly sensitive mutation detection based on digital amplification coupled with hydrogel bead-array

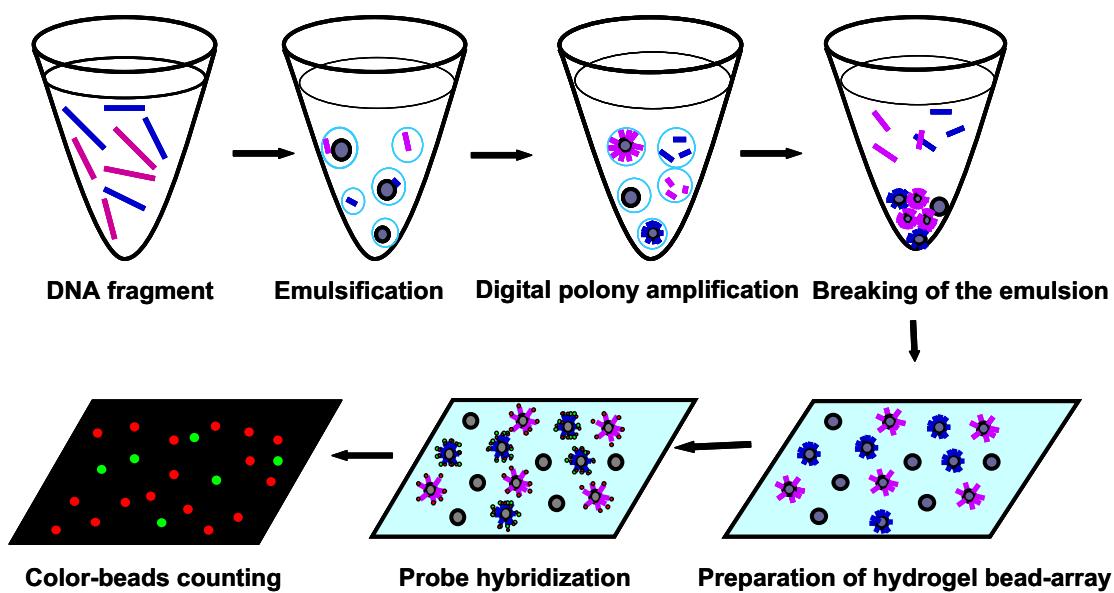
Huan Huang<sup>1, 2</sup>, Zongtai Qi<sup>1, 2</sup>, Lili Deng<sup>1, 2</sup>, Guohua Zhou<sup>1, 3, \*</sup>, Tomoharu Kajiyama<sup>4</sup> and Hideki Kambara<sup>4</sup>

1 Huadong Research Institute for Medicine and Biotechnics, Nanjing 210002, China.  
E-mail: ghzhou@nju.edu.cn; Fax: +86 25 84514223; Tel: +86 25 84514223

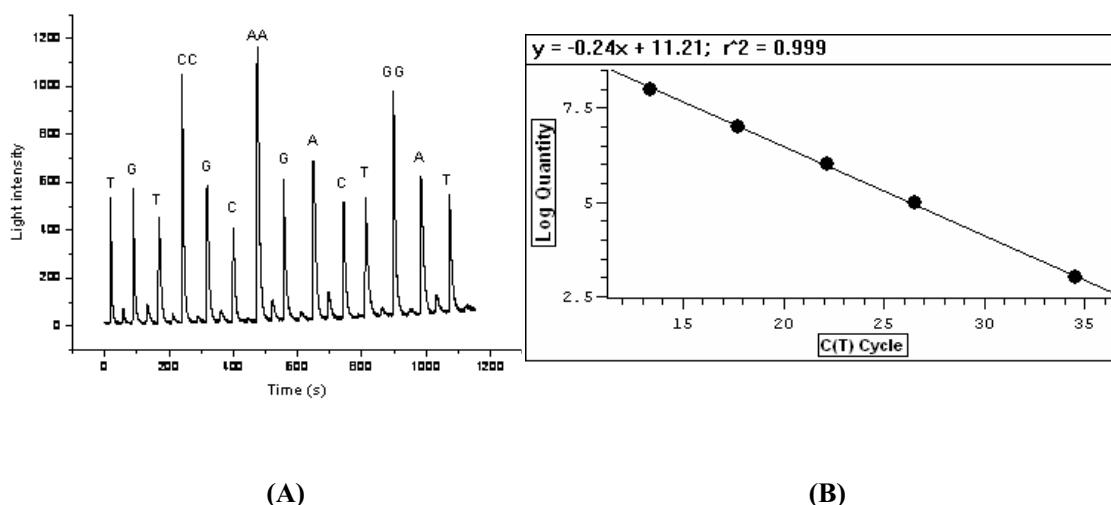
2 China Pharmaceutical University, Nanjing 210009, China

3 Medical School, Nanjing University, Nanjing 210093, China

4 Central Research Laboratory, Hitachi, Ltd., 1-280 Higashi-Koigakubo,  
Kokubunji-shi, Tokyo 185-8601, Japan



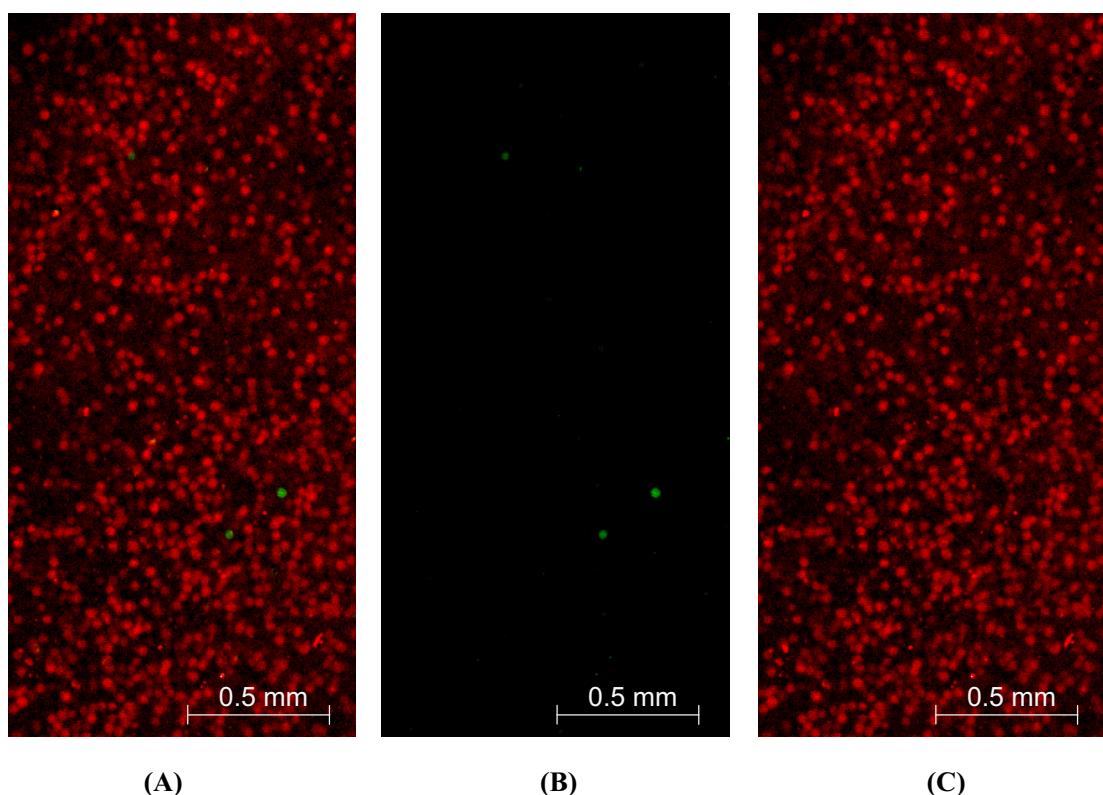
**Supplemental Fig. S1.** Schematic illustration for DABA. The procedure of DABA is as follows: emulsification, single molecule amplification, breaking of the emulsion, preparation of hydrogel bead-array, probe hybridization and color-beads counting. Based on the concepts of data analysis from Digital-PCR and the sample processing of emulsion-PCR, single molecule amplification (single DNA copy per bead) with an improved emulsion quality is performed to produce the samples for preparing the hydrogel-chip which can trap the beads with single DNA sequences. The chip was further treated with electrophoresis to remove the free and mismatched probes before allele-specific probe hybridization and chip-scan.



(A)

(B)

Supplemental **Fig. S2.** (A) The pyrosequencing result of the amplicons on the surface of the beads. The tested sequences are TGTCCGCAAGACTGGAT. (B) The standard curve of real-time PCR for detecting the average copies of amplicons on the surface of each bead.



**Supplemental Fig. S3.** The images by scanning the gel-chip immobilized with beads from emulsion-PCR using the DNA sample from the tumor tissue of CRC patient as the template for detecting mutants at codon 1406 of APC gene. (A) Cy3+Cy5, (B) Cy3, (C) Cy5. Only a part of the scanning area was shown for the illustration. Every red bead originates from one wild-type molecule and every green bead is from one mutant molecule. The number of beads scored is about  $2.4 \times 10^3$ .