Supporting Information

Application of Surface Selective site-directed crystallization in visual assay of

DNA

Jin R. Chen^{a,b}, Ru W. Xie^a, Rui Liu^b, Li S. Liu^a*, Shu S. Zhang^a*

a. Shandong Province Key Laboratory of Detection Technology of Tumor Markers, Linyi University, Linyi 276005, China.

b. Department of Biotechnology, College of Engineering, The University of Suwon, Hwaseong 18323, Korea.

 Table S1.
 The DNA oligonucleotides used in SSSC DNA assay method.

Oligonucleotides used	sequence
Capture DNA (cDNA)	5' ΑΤΤΤΑΑCΑΑΤΑΑΤCCAAAAAAAAAA 3'
Target DNA (tDNA)	5' GGATTATTGTTAAATATTGATAAGGAT 3'
Probe DNA (pDNA)	5' COOH-AAAAAAAAAAATCCTTATCAAT 3'
FAM-probe DNA	5' FAM-AAAAAAAAAAATCCTTATCAAT 3'
Random	5' GAGTAATTAGGTTAATAGAGTAGTTGG 3'
HVB	5' TTGGCTTTCAGTTATATGGATGATGTGGTA 3'
HIV	5' AGAAGATATTTGGAATAACATGACCTGGATGCA 3'



Figure S1. Modification process of glass slide.



Figure S2. X-ray diffraction(XRD) pattern of CaCO₃ crystals formed on functionalized slides.



Figure S3. Raman spectroscopy of $CaCO_3$ crystals formed on functional slides, that 1085, 281 and 153 cm⁻¹ are typical calcite peaks, and the double peaks of 1090 and 1075 cm⁻¹ indicate vaterite crystal.



Figure S4. Optical microscopy images of functionalized slide surface after CaCO3 crystallization experiment.



Figure S5. Crystal growth was detected by QCM: the concentration of the inhibitory group was fixed to 10nM.



Figure S6. Optimization of DNA hybridization temperature used in SSSC DNA assay method, the concentration of tDNA: 1 nM, 100 pM, 10 pM, 1 pM, 100 fM, 10 fM, 1 fM, 0 M. (The concentration of Ca²⁺: 3 mM; crystallization time: 1 h).



Figure S7. Optimization of Ca²⁺ concentration used in SSSC DNA assay method, the concentration of tDNA: 1 nM, 100 pM, 10 pM, 1 pM, 100 fM, 10 fM, 1 fM, 0 M. (DNA hybridizition temperature: 45 °C; crystallization time: 1 h).



Figure S8. Optimization of the crystallization time used in SSSC DNA assay method, the concentration of tDNA: 1 nM, 100 pM, 10 pM, 1 pM, 100 fM, 10 fM, 1 fM, 0 M. (Ca²⁺ concentration: 3 mM; DNA hybridization temperature: 45 °C).



Figure S9. Confocal imaging of DNA hybridized with FAM-labeled probe DNA. (tDNA concentration: 1μ M, 100 nM, 10 nM).



Figure S10. X-ray diffraction (XRD) pattern of CaCO₃ crystals formed on glass surface in SSSC DNA assay method.



Figure S11. Picture of the 20× to 400× and 20× to 200× universal tip scope used in SSSC

DNA assay method.



Figure S12. CVs of analyzing area. The result shows that the counting accuracy increases with the increment of analyzing area.



Figure S13. User interface of the mobile app "Count Things".



Figure S14. The comparison of two counting programs. The readouts by the app "Count Things" correlate well with that by Image J.

tDNA(mol/L)	cv
10 ⁻¹⁵	6%
10 ⁻¹⁴	14%
10 ⁻¹³	5%
10-12	12%
10 ⁻¹¹	7%
10-10	15%

Table. S2. CVs of crystal counts in SSSC DNA assay method assay of DNA.

10 ⁻⁹	16%
Average CV	11%

Video S1. Growth of $CaCO_3$ crystals on a section of the glass surface recorded by conventional microscope (20× magnification, 1 frame per 30 s, Play at 20× speed).

The video file is provided separately in the attachment