

Electronic Supplemental Information to Analyst

**Surface Invasive Cleavage Assay on a Maskless Light-directed Diamond DNA Microarray for
the Genome-wide Human SNP Mapping**

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Photolithographic DNA synthesis on diamond chip. As illustrated in Fig. S1, the DNA synthesis is a multiple cycling process, which stepwise develops oligonucleotide strand, base by base. Light-directed photolithographic synthesis was performed on a digital controlled micromirror light system connected to a Perseptive Biosystems Expedite Nucleic Acid Synthesis instrument (Framingham, MA, USA). The removal of photolabile NPPOC (30-nitrophenylpropyloxycarbonyl) protection group was achieved by irradiation with 3.95 J/cm² of 365nm uv-light from a 200W Hg/Xe arc lamp (Newport, Stratford, CT, USA). The optimum dose for was studied and described in experimental section of manuscript. Oligonucleotide synthesis reagents and oxidizer were purchased from Sigma-Proligo. All NPPOC-protected phosphoramidites were manufactured by Proligo Biochemie GmbH (Hamburg, Germany). The on-chip synthesis protocol is described below, step by step:

- 1) Quartz synthesis flow cell synthesis flow cell (~100 ml) is flushed with 500 ml of exposure solvent;
- 2) A digital image is specifically designed and illuminates the pixel where the next base will be attached. During the irradiation, the exposure solvent remains the flow rate of 100 ml/0.5J/cm², maintaining a sufficient basic concentration;
- 3) The array is rinsed up with dry acetonitrile to remove residual exposure solvent, dry washing to eliminate trace water, and followed by flow of activator solution .
- 4) Coupling of the next base is achieved by filling the flow cell with a 1:1 solution of the desired phosphoramidite and activator. The 50-NPPOC-

protected amidites reacts on oligonucleotide stub with a single 40s coupling step. 5) After amidite coupling, the array is washed with acetonitrile and either oxidized by flushing the cell with oxidizer solution (THF, pyridine, iodine, water) or subjected to the next phosphoramidite addition. The non-acidic conditions of deprotection allow for oxidation of the backbone phosphite groups only after every fourth coupling step and at the end of the synthesis. 6) Upon the successful synthesis, the nucleoside bases are deprotected in the bath of ethylene diamine and ethanol mixture (v:v 1:1) for 2~4h under ambient conditions.

Tables

Table S1. Overall optimizing half-life time and mismatch study on diamond DNA chip^a

| Half-lives | Arbitr. Intensity (Mean \pm Std.) | | | |
|------------|-------------------------------------|------------------|-----------------|----------------|
| | Perfect match | 1 mismatch | 2 mismatch | 3 mismatch |
| 6 | 103.2 \pm 10.0 | 69.4 \pm 12.8 | 42.9 \pm 4.6 | 21.1 \pm 2.0 |
| 8 | 188.5 \pm 9.3 | 92.8 \pm 15.3 | 48.5 \pm 3.2 | 19.2 \pm 2.1 |
| 10 | 205.5 \pm 7.4 | 102.8 \pm 3.8 | 52.8 \pm 2.8 | 18.5 \pm 2.9 |
| 12 | 216.8 \pm 5.2 | 110.3 \pm 5.1 | 56.0 \pm 2.9 | 16.3 \pm 2.7 |
| 14 | 226.5 \pm 6.0 | 115.6 \pm 7.5 | 57.9 \pm 15.9 | 15.8 \pm 2.0 |
| 16 | 226.1 \pm 6.2 | 116.2 \pm 6.8 | 55.3 \pm 2.3 | 16.0 \pm 6.6 |
| 18 | 225.0 \pm 7.7 | 123.4 \pm 14.0 | 57.8 \pm 2.7 | 17.9 \pm 1.6 |

^a The arbitrative fluorescence intensity is roughly calculated based on Fig. 3 after converting RGB value to gray level.

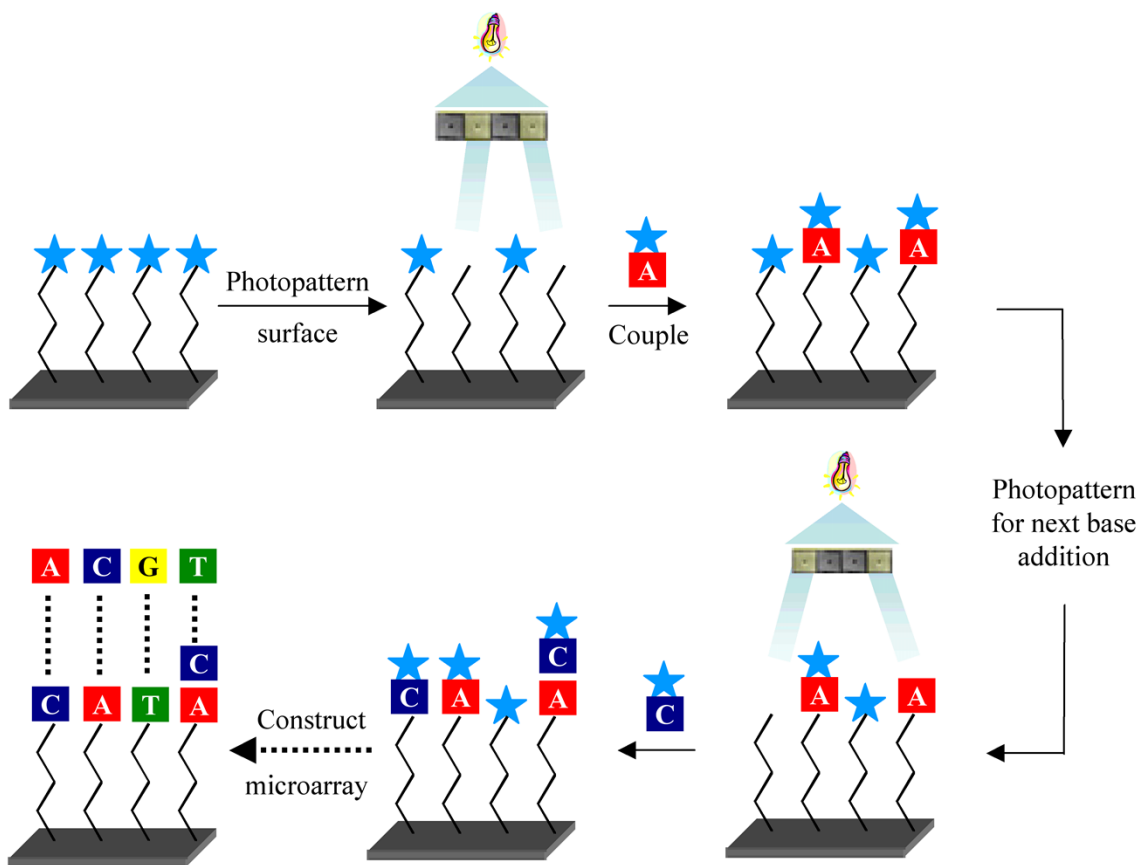


Figure S1. Schematic illustration of the photolithographic growth of DNA microarrays.

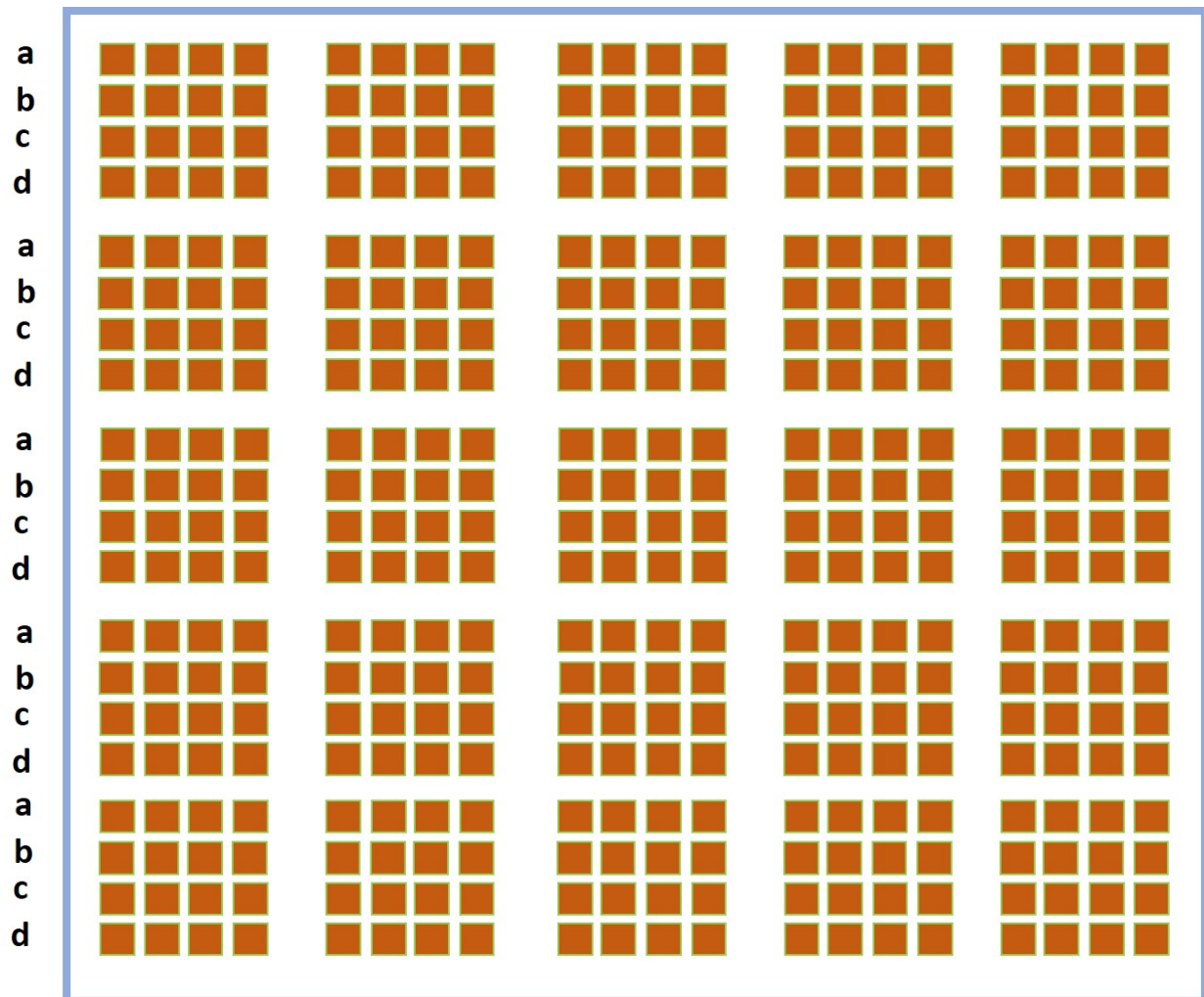


Figure S2. The design of dual probe diamond DNA array. The sequences used in this template are: Sequence 1, 25mer, 5'-GTC-ATC-ATC-ATG-AAC-CAC-CCT-GGT-C-(T)₁₄-3'; Sequence 2; 34mer, 5'-(T)₁₄-GCT-CAC-CTG-TGG-TAT-CAC-TCC-AAA-GGC-TTT-CCT-A-3'. DNA strands are accurately synthesized on 500 μ m x 500 μ m features, where, a) row #1 contains solo Sequence 1; b) row #2 comprises of Sequence 2 only; c) row #3 contains 50:50 mixture of both sequences, following the synthesis strategy I; d) row #4 defines both sequences using "Y-linker" synthesis strategy II.

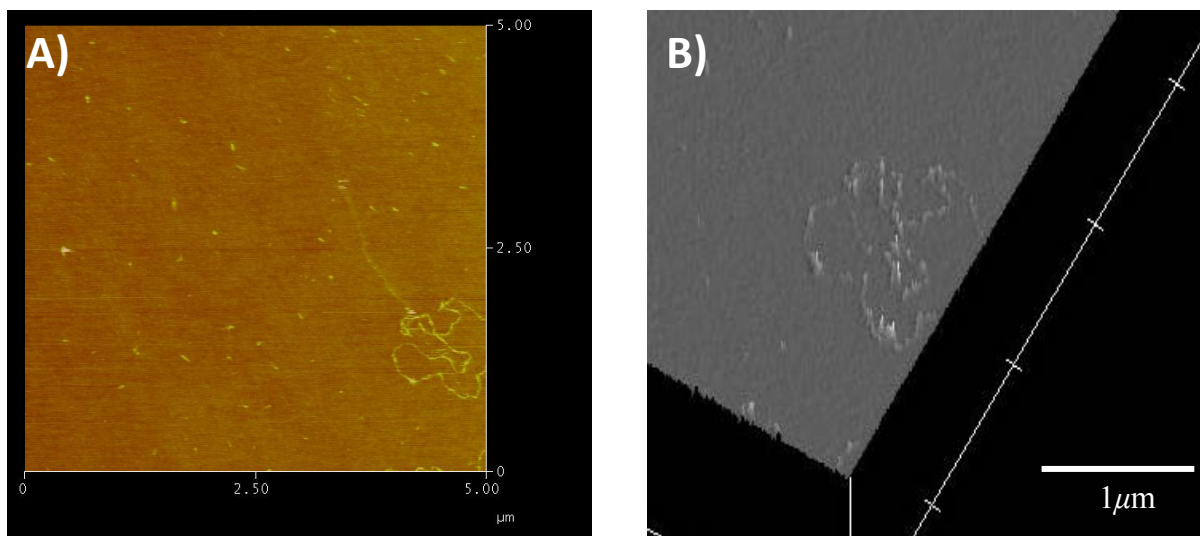


Figure S3. The atomic force microscopic image of single-stranded rolling circle-amplification product on planar glass slide. A) plane-view two-dimensional AFM micrograph; B) three-dimensional contour from same sample. The approximately length of RCA product is $6\sim 8\mu\text{m}$ upon stretching by a laminar flow on plain surface.