An Optical Deoxyribonucleic Acid-based Half-subtractor

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Experimental procedures

All oligonucleotides (dual-gate, Input I_A and I_B) were purchased from Yao-Hong Biotechnology Inc. (HPLC grade, New Taipei City, Taiwan). Sodium chloride (A. R. grade), potassium chloride, potassium dihydrogen phosphate (KH₂PO₄, >99.9%), and sodium phosphate dibasic (Na₂HPO₄, >99%) were purchased from J.T. Baker (Phillipsburg, NJ). Deionized water used in the preparation of phosphate buffered saline (PBS, 110 mM NaCl, 2 mM KCl, 8 mM Na₂HPO₄, 2 mM KH₂PO₄, pH=7.4) and for rinse solutions was 18.2 M Ω , produced by PURELAB Ultra (ELGA, Albania). All chemicals were used as received unless otherwise mentioned.

Two microliters of 50 μ M dual-gate molecules were dissolved in PBS and pipetted on a screen-printed gold substrate (4 mm diameter, DropSens, Spain) for immobilization for 30 min. The dual gate–coated Au surface was then rinsed with 500 μ L PBS to remove unbound molecules. Hybridization of the hairpin probes on Au was performed at room temperature for 30 min^{ref*} after the same number (i.e. 2 μ L 50 μ M) of I_A and I_B molecules were applied under the same buffer conditions. All processes including dual-gate immobilization and hybridization were covered with aluminum foil in order to prevent photobleaching.

The fluorescence intensities were monitored with a fluorescence spectrophotometer (F7000, Hitachi

High-Technologies, Japan) equipped with a solid sample holder. The emission wavelengths were 520

nm and 694 nm for fluorescein and Cy5, respectively. The relative intensities of samples were

calculated by $(I_{hybrid} - I_{blank})/I_{blank}$, where I_{hybrid} and I_{blank} were the fluorescence intensities of

dual-gate on gold after and before hybridization with the target sequences.

ref*: see ref 6 in the text.