## The development of a light-up red-emitting fluorescent probe based

## on a G-quadruplex specific cyanine dye

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Name	Sequence(5' to 3')
с-тус	TGGGGAGGGTGGGGGAGGGTGGGGAAGG
bcl-2	GGGCGGGCGCGGGAGGAAGGGGGGGGGGG
kit l	AGGGAGGGCGCTGGGAGGAGGG
Kras	AGGGCGGTGTGGGAAGAGGGGAAGAGGGGGGGGGG
HT 22	AGGGTTAGGGTTAGGGTTAGGG
Hras	TCGGGTTGCGGGCGCAGGGCACGGGCG
TBA	GGTTGGTGTGGTTGG
ssDNA1	TAACATGTTCGTCGATTAGGTACGACT
ssDNA2	AGTCGTACCTAATCGACGAACATGTTA
dsDNA	ssDNA1+ ssDNA2
27	
ct-DNA	Calf thymus DNA

Table S1 The oligonucleotides used in this study.







Fig. S2 The <sup>13</sup>C NMR expansion 1 of Dir.



Fig. S3 The <sup>13</sup>C NMR expansion 2 of Dir.



Fig. S4. The ESI-MS spectrum of Dir.



Fig. S5. The purity of Dir was determined by UPLC.



Fig. S6 Comparison on the photostability of Dir and Cy5. All samples were continuously irradiated using a 500 W xenon lamp.



Fig. S7. The excitation spectra of Dir (0.5  $\mu$ M) alone and Dir with DNA (15 $\mu$ M).

The emission wavelength was set at 651 nm.



Fig. S8. Fluorescence titration assay of Dir (0.5  $\mu$ M) with *c-myc* (A, 0.5–15  $\mu$ M), *bcl-*2 (B, 0.5–15  $\mu$ M), *kit 1* (C, 0.5–15  $\mu$ M), *Kras* (D, 0.5–15  $\mu$ M), *HT 22* (E, 0.5–15  $\mu$ M) and *Hras* (F, 0.5–15  $\mu$ M) in 20 mM Tris-HCl buffer with 100 mM KCl. Each insert figures represented the determination of apparent binding equilibrium constant  $K_a$ .



Fig. S9 Fluorescence quantum yield determination of Dir in the absence (A) and presence of G-quadruplex (A, B) in 20 mM Tris-HCl buffer with 100 mM KCl.



Fig. S10. CD spectra of 4  $\mu$ M G-quadruplex-forming oligonucleotides *c-myc* (A), *HT22* (B) and *Hras* (C) in the absence and presence of Dir (8  $\mu$ M) in 20 mM Tris-HCl buffer, pH 7.4.