Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Basic evaluation of CRISPR/Cas system stability for the application of paper-based analytical devices

Yohei Tanifuji, Hikaru Suzuki, Guodong Tong, Yuki Hiruta, Daniel Citterio*

Department of Applied Chemistry, Faculty of Science and technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku,

Yokohama 223-8522, Japan. E-mail: citterio@applc.keio.ac.jp; Tel: +81 45 566 1568

*To whom correspondence should be addressed.

Email: citterio@applc.keio.ac.jp

Table of Contents

Table 1 Sequences of nucleic acids used in the current work.

Name	Sequence (5'→3')
Target dsDNA (tgDNA)	TTTTTTTTTTAGTACATTGCAAGATACTAAATGTGAGGTACCA
crRNA	UAAUUUCUACUAAGUGUAGAUGUACAUUGCAAGAUACUAAA
ssDNA FQ reporter	56-FAM*1/TTATTATTATTATTA/3IABkFQ*2

*1: A single isomer derivative of fluorescein. Maximum absorbance and emission are at 495 and 520 nm.

*2: Iowa Black® fluorescence quencher which has a broad absorbance spectrum ranging from 420 to 620 nm with peak absorbance at 531 nm.

Table S2 Characteristics of used filter papers.

	Whatman No.1	Whatman No. 541	Advantec 5C
Pore size (μm)	10.8	22	1
Thickness (μm)	180	155	220
Filtration speed (sec/100 mL)	150	34	570
Material	Cellulose	Cotton linter	Cotton α-cellulose

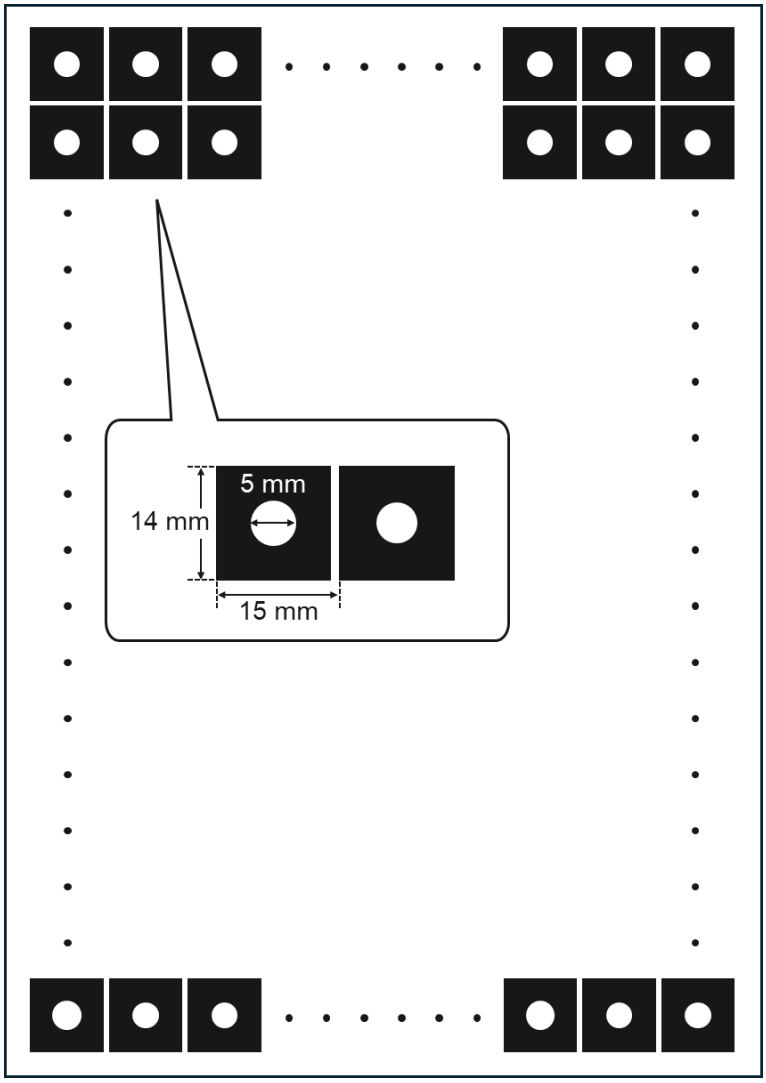

Fig. S1 Wax printing pattern designed using Adobe Illustrator CC software. Black area was printed using black cartridges of wax-printer.

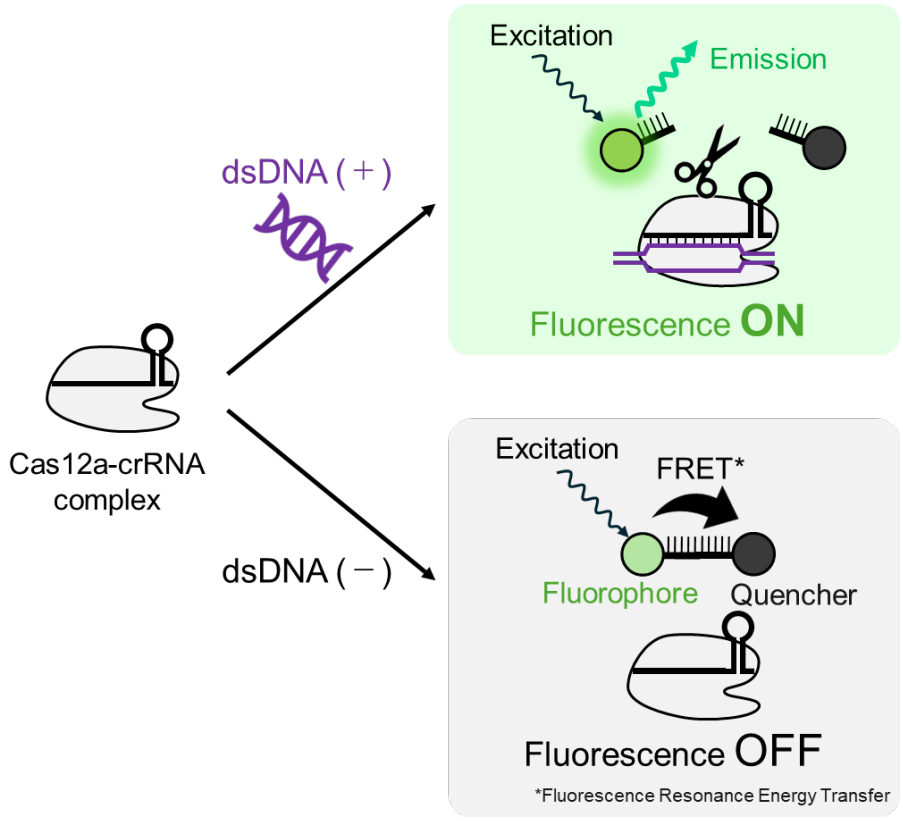

Fig. S2 Mechanism of fluorescence emission induced by the trans-cleavage activity of CRISPR/Cas12a with ssDNA FQ reporter.

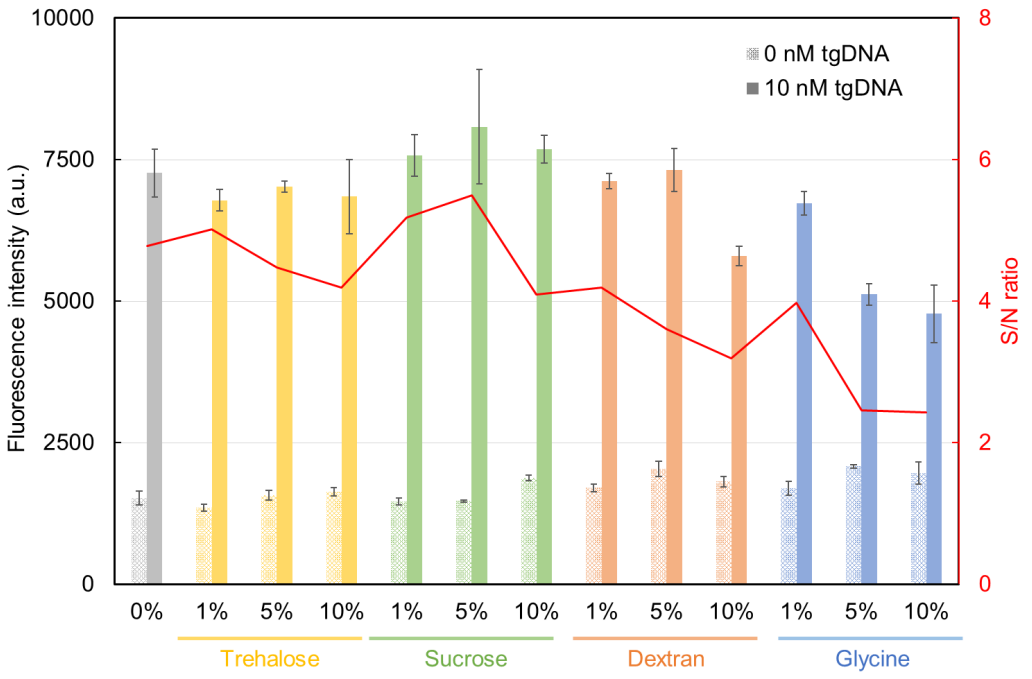

Fig. S3 Fluorescence emission intensity (left vertical axis, bars) and S/N ratio (right vertical axis; red line) observed for micro-spots with CRISPR-related reagents spontaneously dried at room temperature on a WF1 paper substrate in the presence of various concentrations of different stabilizers; the CRISPR assay was proceeded just after drying reagents; all percentages are weight ratios; error bars represent mean values ± 1σ (n = 3).

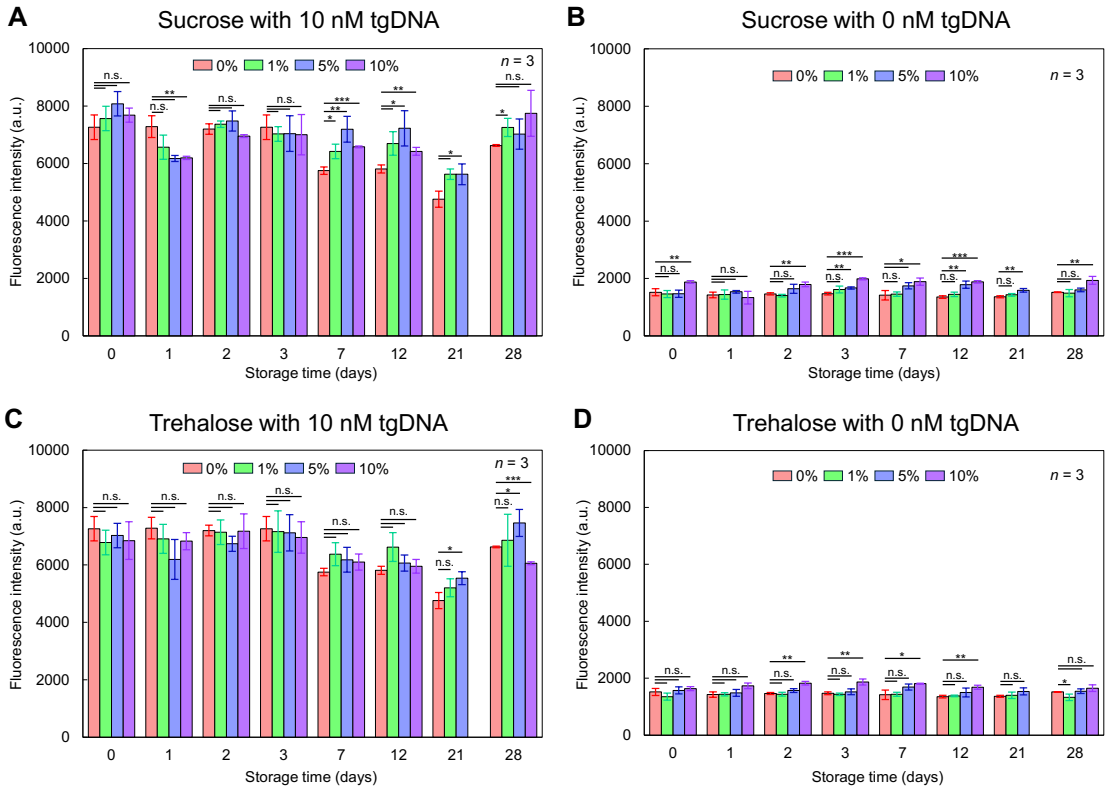

Fig. S4 Statistical comparison over influence of type and concentration of disaccharide on the storage stability of CRISPR-related reagents spontaneously dried at room temperature on WF1 paper substrates stored at −20 °C: (A, B) absolute fluorescence emission intensities with sucrose, and (C, D) that with trehalose; error bars represent mean values ± 1 σ (n = 3); n.s., not significant with $p > 0.05$; the asterisks (*, **, and ***) represent significant differences with p values (*: $0.01 < p \leq 0.05$, **: $0.001 < p \leq 0.01$, ***: $p \leq 0.001$).

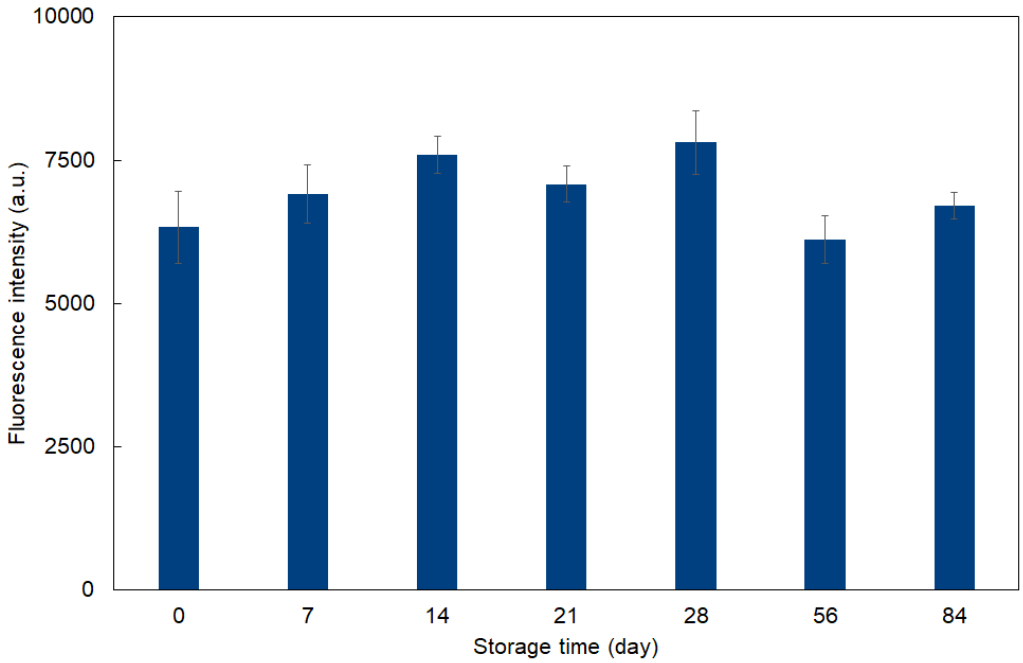

Fig. S5 Fluorescence intensity response to 10 nM tgDNA after long term storage of CRISPR-related reagents (1% sucrose) spontaneously dried at room temperature on WF1 paper substrates stored at −20 °C; error bars represent mean values ± 1σ (*n* = 3).

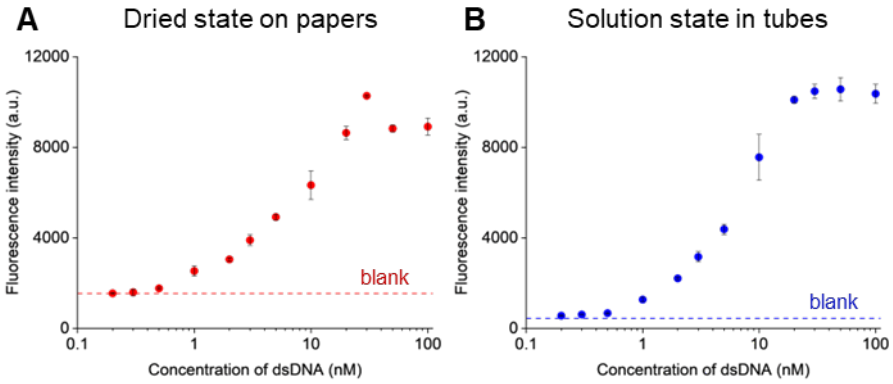

Fig. S6 Target dsDNA concentration-dependent (0–100 nM) fluorescence emission intensities obtained with all CRISPR-related reagents stored at −20 °C: (A) in dried state on WF1 paper substrates, and (B) in frozen state in microtubes; error bars represent mean values ± 1σ (*n* = 3).

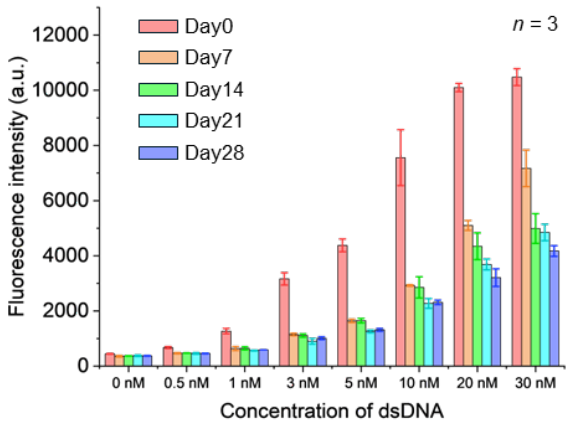

Fig. S7 Target DNA concentration-dependent fluorescence intensities after various periods of storage at −20 °C observed with CRISPR-related reagents (1% sucrose) stored in frozen state in microtubes; error bars represent mean values ± 1σ (*n* = 3).

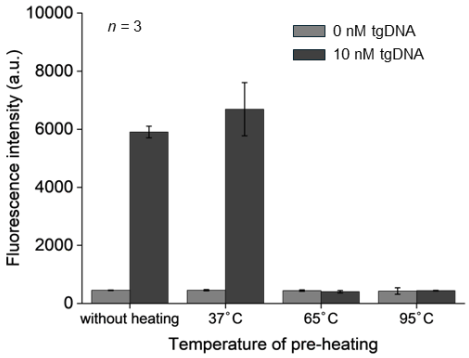

Fig. S8 Fluorescence intensities obtained with assays performed after pre-heating at various temperatures involving CRISPR-related reagents in solution state in microtubes; error bars represent mean values ± 1σ (*n* = 3).

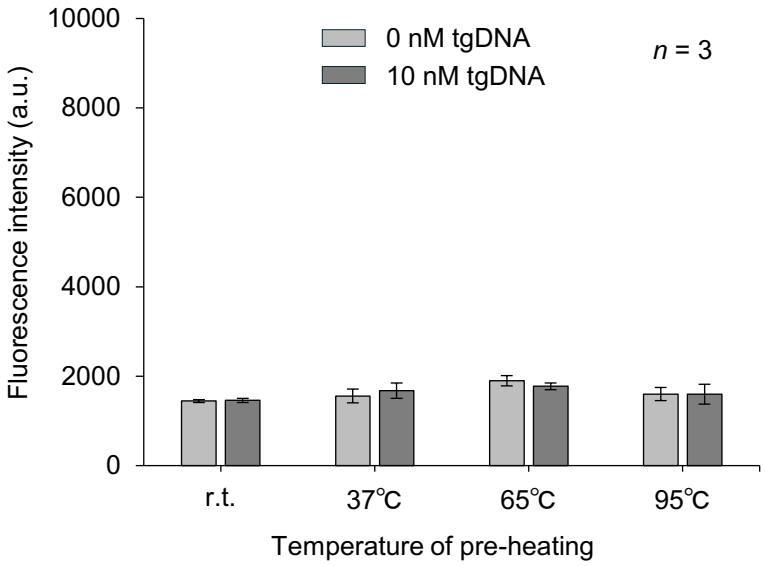

Fig. S9 Fluorescence intensities obtained with assays performed after pre-heating at various temperatures involving CRISPR-related reagents dried at room temperature spontaneously on WF1 paper substrates without BSA blocking pre-treatment; error bars represent mean values ± 1σ (*n* = 3).