

CRISPR-mediated strand displacement logic circuits with toehold-free DNA

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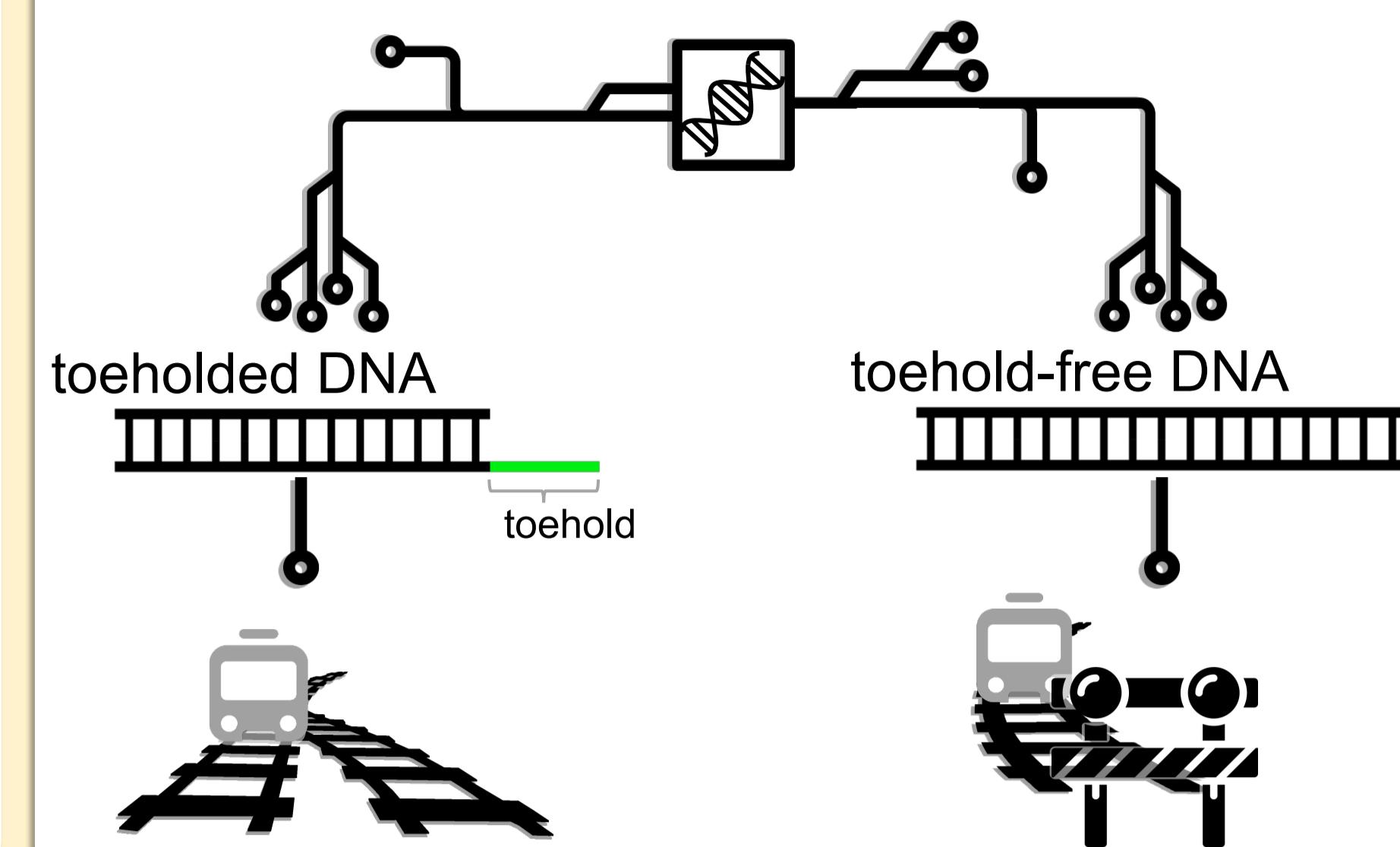
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Summary:

- DNA computing using DNA strand displacement has only been successful with ssDNA or dsDNA containing a toehold (overhanging region).
- The use of regular DNA, double strands perfectly complementary, has remained elusive in nanotechnology^{1,2}.
- We report the exploitation of CRISPR-Cas systems to engineer logic circuits based on isothermal DNA strand displacement that perform with toehold-free DNA.

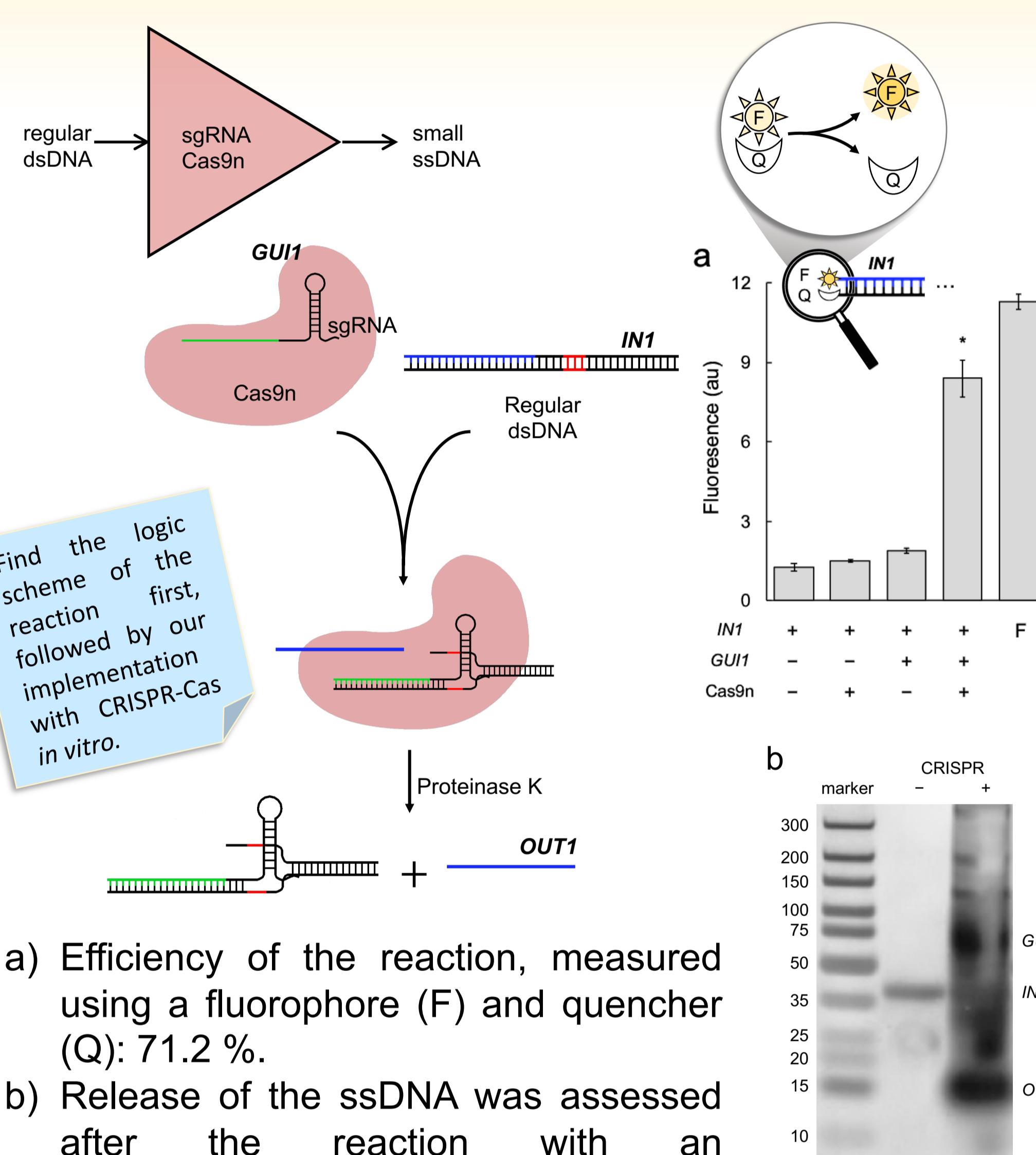
State of the art:



Can we surmount this barrier?

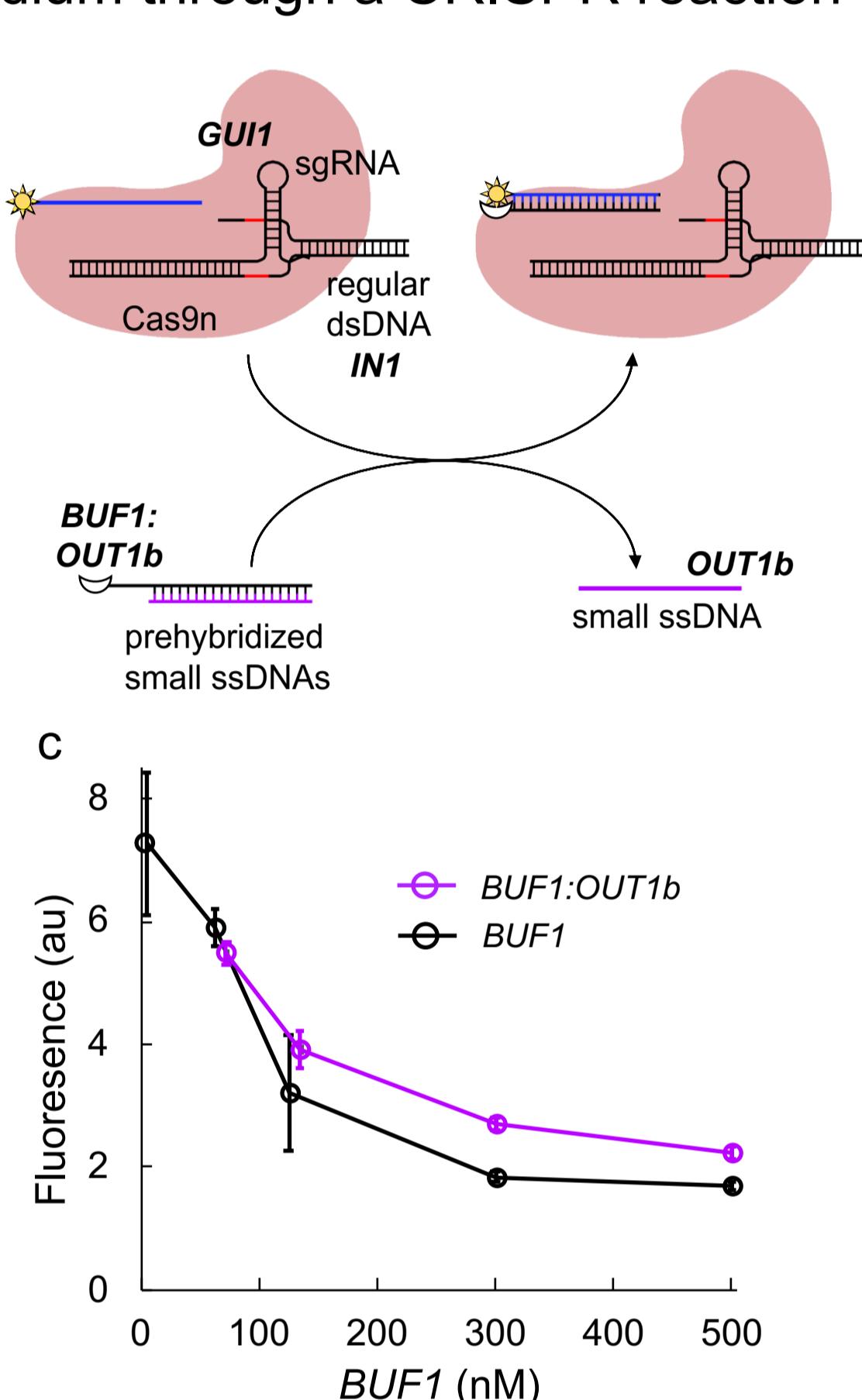


Engineering a molecular converter from dsDNA to ssDNA based on CRISPR-mediated DNA strand displacement.

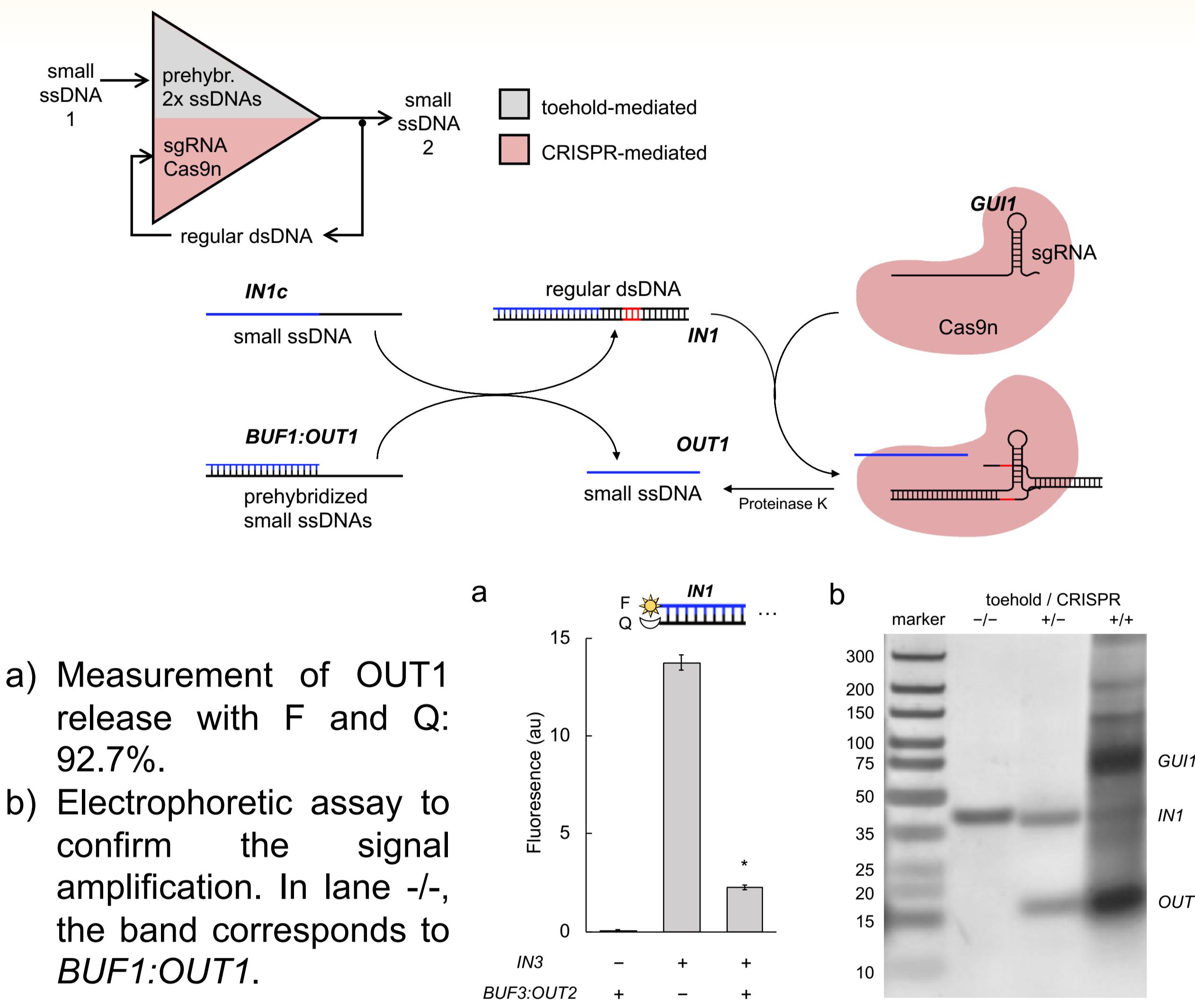


- a) Efficiency of the reaction, measured using a fluorophore (F) and quencher (Q): 71.2 %.
b) Release of the ssDNA was assessed after the reaction with an electrophoretic assay.

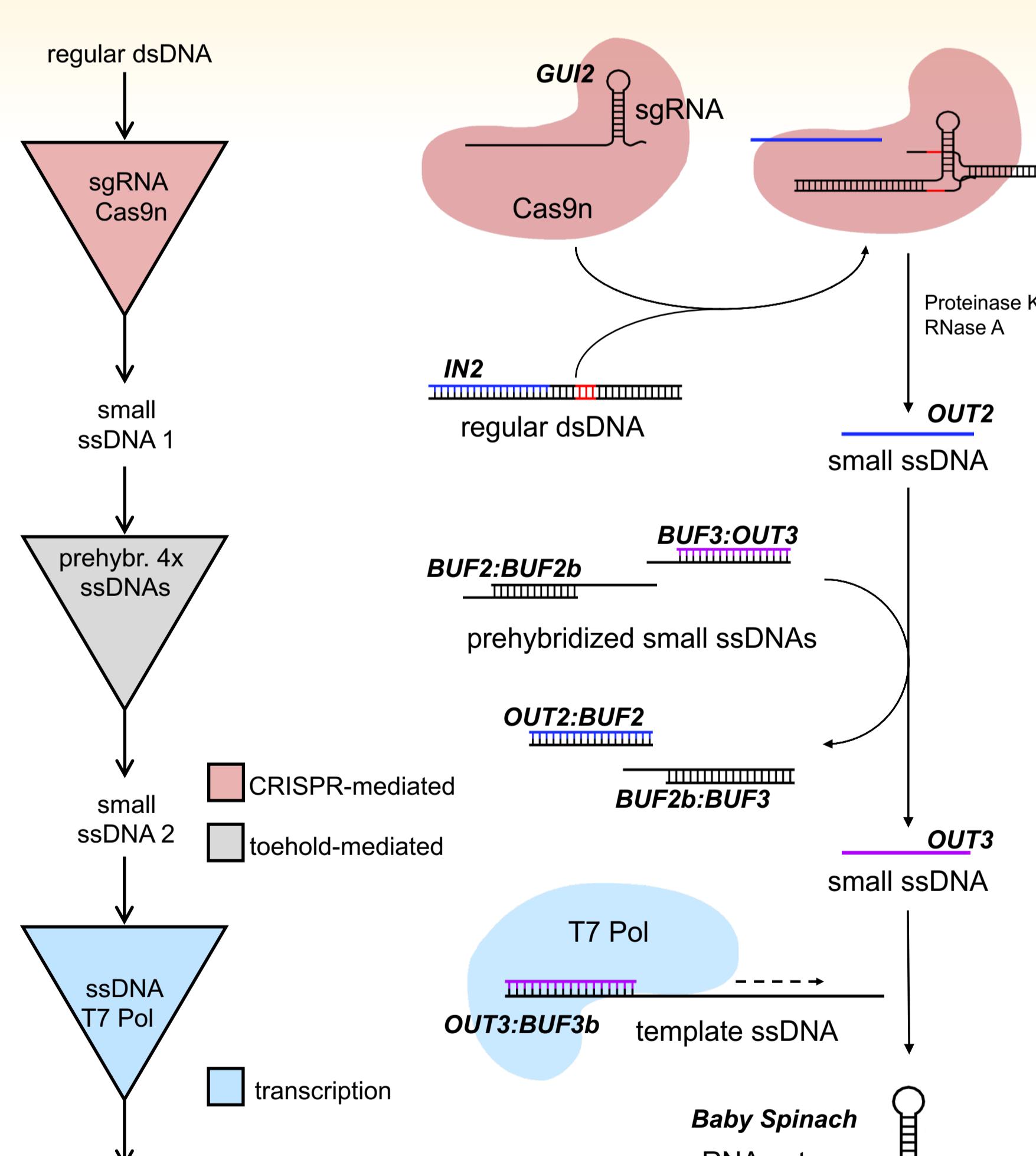
c) ssDNA species can interact with the nontarget strand. An ssDNA species in a complex (OUT1b) can be released to the medium through a CRISPR reaction



Engineering a close-loop molecular amplifier based on CRISPR- and toehold-mediated DNA strand displacement. Exploiting the formation of a dsDNA “waste” product.

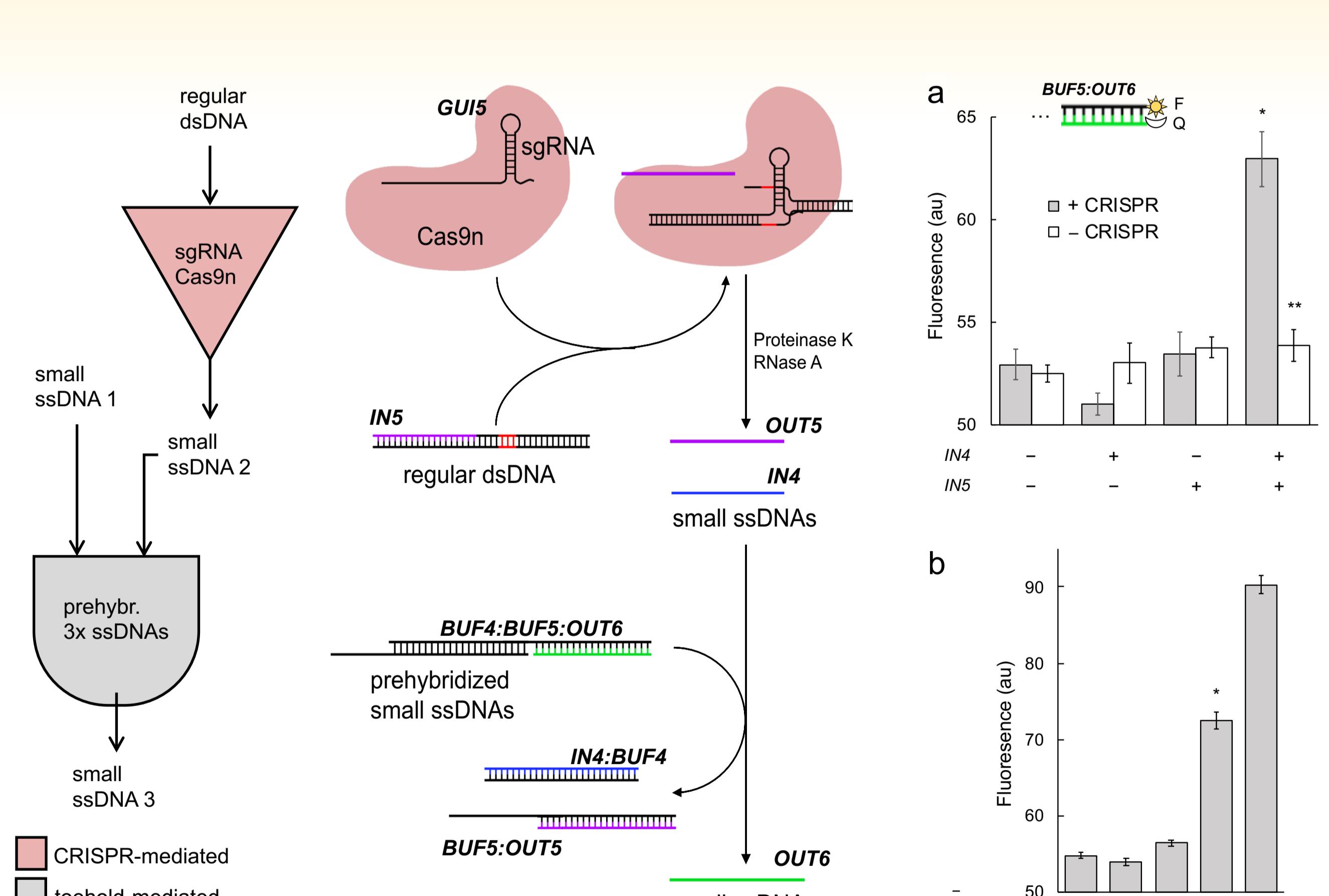


Engineering a serial cascade based on CRISPR- and toehold-mediated DNA strand displacement and in vitro transcription.



- a) Release efficiency of OUT3 in response to IN2, using F and Q: 21.3%.
b) Fluorescent RNA aptamer production after the cascade, measured by addition of DFHBI, is fully functional.

Engineering a combinatorial device working as an AND gate based on CRISPR- and toehold-mediated DNA strand displacement.



- a) Efficiency measurement of the reaction using F and Q: 28.3%.
b) Efficiency measurement of the toe-hold mediated part (AND GATE): 47.1%.

CONCLUSIONS:

- A regular DNA fragment, without toehold, can be used as a substrate in strand displacement reactions to engineer logic circuits.
- CRISPR-mediated strand displacement leads to the generation of defined, individual ssDNA molecules, which can then trigger downstream nonenzymatic DNA reactions. In turn, waste dsDNA products from conventional toehold-mediated strand displacement can be recycled through the use of CRISPR ribonucleoproteins.
- The excess of Cas9:sgRNA required with respect to dsDNA (5:5:1 at least) for the reaction to successfully work increases the monetary cost significantly, which may limit widespread implementation.
- Our logic circuits might be of utility in the development of novel strategies for (pre)clinical diagnostics, such as to detect viral infections, like what is already being developed with other CRISPR-based systems³.

References:

1. Montagud-Martínez, R., Heras-Hernández, M., Goiriz, L., Daròs, J. A., & Rodrigo, G. CRISPR-Mediated Strand Displacement Logic Circuits with Toehold-Free DNA. *ACS synthetic biology* (2021) 10(5):950-956.
2. Seelig, G., Soloveichik, D., Zhang, D.Y., Winfree, E. Enzyme-free nucleic acid logic circuits. *Science* (2006) 314:1585-1588.
3. Chen, Y.J., Dalchow, N., Srinivas, N., Phillips, A., Cardelli, L., Soloveichik, D., Seelig, G. Programmable chemical controllers made from DNA. *Nat. Nanotechnol.* (2013) 8:755-762.
4. Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., Doudna, J.A. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* (2018) 360:436-439.