Supplementary Material

CRISPR/Cas9-regulated dual-ring topological allosteric probe for detection of CTCs EGFR L858R resistance mutation

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Table S1. The sequences used in the reaction

Name	Sequence (5'-3')
L858R	TTTTGGGCGGGCCAAACTGCTGG
WT	TTTTGGGCTGGCCAAACTGCTGG
Recognition ring	P-TTTTTTCACGATTGAGGTGAGTGTGAGCATTTTTTTTTT CTGATTGAGGTGAGTGTGAGCAGGCCAGCAGTTTGGCCC GCCCAAAATAATTCCACGCCCG
Reporter ring	P- CACCTCAATCAGCTTTTCTCTCTCTCTCTCTCTCTCCTA CGCCTACGTTCACTATGTCTTTCTTTCTTTCTTTCTTTCCC TGCTCACACT
Fluorescent probe	TACGCCTACGTTCACTAT-Cy5
Quencher probe	CGTAGGCGTA-BHQ2
sgRNA	UUUUGGGCGGGCCAAACUGCGAGCUAGAAAUAGCAAG UUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUU
RCA primer	GAAAGAAAGAAAGAAAGAAAG
Circularization primer	TGAAAAAACGGGCGTG
Forward primer	GCCACCTCCTTACTTTGCCTCCTTCTGCAT
Reverse primer	GCATGAACTACTTGGAGGACCGTCGCTTGG

Table S2. Comparison of Single-Nucleotide Mutation Detection Methods

Detection methods	Source of sample	LOD	Mutation type	Reference
specific PCR combined with CRISPR/Cas12a assays	tissue	0.1%	TP53 R248W	[1]
CRISPR/Cas9 mediated triple signal amplification platform combined with RCA, RPA and hGNPs	ctDNA	0.01%	KRAS-G12D	[2]
SPEAR: CRISPR-mediated ultrasensitive, specific and rapid one-pot detection strategy for cancer-related SNPs	ctDNA	0.1%	KRAS-G12D	[3]
single-molecule fluorescence imaging technique of ctDNA detection, SIMUL	ctDNA	0.05%	KRAS, G12C, G12D, and G12V	[4]
All-in-one approaches for rapid and highly specific quantification of single nucleotide polymorphisms based on ligase detection reaction using molecular beacons as turn-on probes	tissue	0.1%	T790M	[5]
NGS (Next-generation sequencing)	tissue/ plasma	5-10%	Multiple EGFR and other driver mutations	[6]

[1] H. Wang, R. Liu, K. Dong, L. Zhang, J. Zhang, X. Zhang, J. Zhang, X. Xiao, W. Zhang, X. Wang, A universal and sensitive gene mutation detection method based on

CRISPR-Cas12a, Anal Chim Acta 1246 (2023) 340886. https://doi.org/10.1016/j.aca.2023.340886.

[2] M. Zhou, H. Wang, C. Li, C. Yan, P. Qin, L. Huang, CRISPR/Cas9 mediated triple signal amplification platform for high selective and sensitive detection of single base mutations, Anal Chim Acta 1230 (2022) 340421. https://doi.org/10.1016/j.aca.2022.340421.

[3] L. Bai, Y. Pang, T. Wang, S. Wang, K. Guo, T. Xuan, Z. Zhang, D. Liu, F. Qian, Y. Zheng, G. Jin, R. Wang, SPEAR: CRISPR-mediated ultrasensitive, specific and rapid one-pot detection strategy for cancer-related SNPs, Theranostics 15 (2025) 3275–3288. https://doi.org/10.7150/thno.107488.

[4] S. Shin, S. Han, J. Kim, Y. Shin, J.-J. Song, S. Hohng, Fast, sensitive, and specific multiplexed single-molecule detection of circulating tumor DNA, Biosens Bioelectron 242 (2023) 115694. https://doi.org/10.1016/j.bios.2023.115694.

[5] W. Zhang, K. Liu, P. Zhang, W. Cheng, Y. Zhang, L. Li, Z. Yu, M. Chen, L. Chen, L. Li, X. Zhang, All-in-one approaches for rapid and highly specific quantifcation of single nucleotide polymorphisms based on ligase detection reaction using molecular beacons as turn-on probes, Talanta 224 (2021) 121717. https://doi.org/10.1016/j.talanta.2020.121717.

[6] R.R. Singh, Next-Generation Sequencing in High-Sensitive Detection of Mutations in Tumors, The Journal of Molecular Diagnostics 22 (2020) 994–1007. https://doi.org/10.1016/j.jmoldx.2020.04.213.

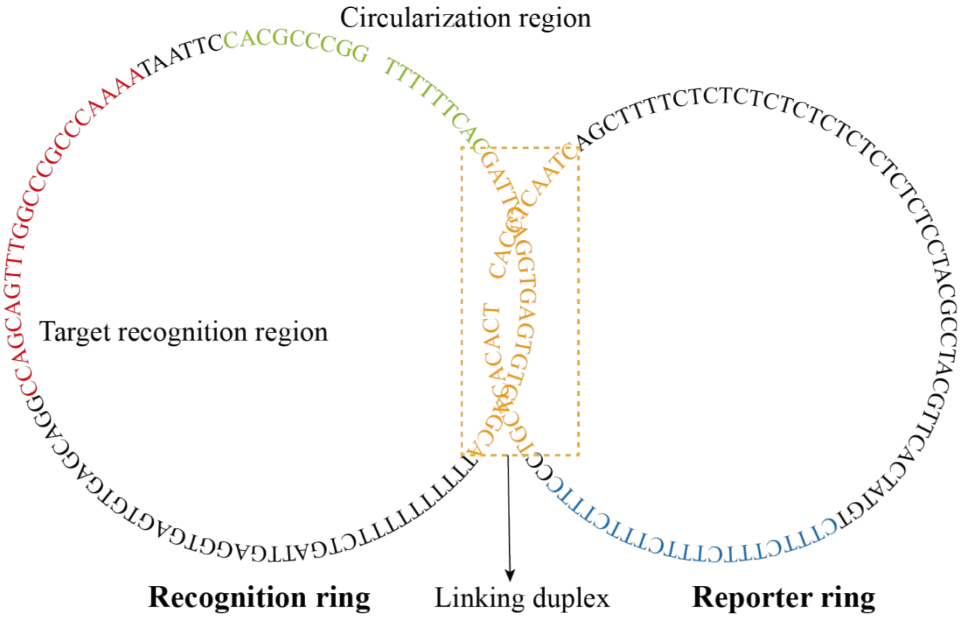

Figure. S1. Schematic representation of the DNA dual-ring structure consisting of a recognition ring and a reporter ring. Nucleotides in boxes represent the complementary hybridization regions connecting the two rings.

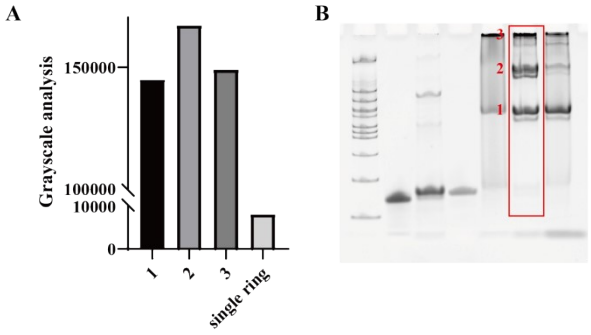

Figure. S2. Grayscale analysis of native PAGE images of DNA dual-rings structures.

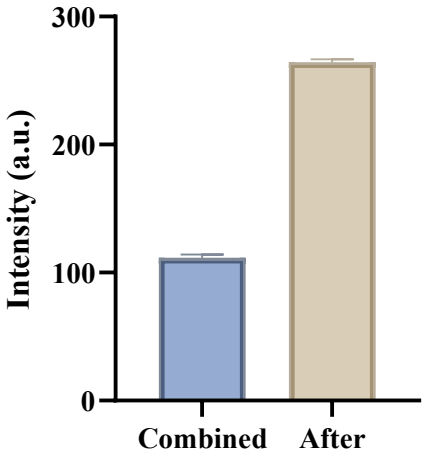

Figure. S3. Fluorescence signal comparison of the probe added simultaneously during the RCA reaction versus added after completion of the RCA reaction.