A fluorescence-based high-throughput screening method for determining activity of diguanylate cyclases and c-di-GMP phosphodiesterases

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Table 1: Gene construction of WspR and RocR expression plasmids.

Name	Sequence (5'-3') [a]
WspR	CATATGCACAACCCTCATGAGAGCAAGACCGACCTGGGCGCCCCTCTGGACGGCGCGG TCATGGTACTGCTTGTCGATGATCAGGCCATGATCGGAGAGGCTGTGCGTCGCTCACTG GCGAGCGAGGCGGGCATCGACTTCCATTTCTGTTCCGATCCGCAGCAGGCGGTGGCGGT GGCCAACCAGATCAAGCCGACGGTGATCCTCCAGGACCTGGTGATGCCCGGCGTCGAC GGCCTCACGCTGCTCGCTGCCTACCGCGGCAACCCGGCGACCCGCGACATCCCGATCAT CGTCCTGTCGACCAAGGAAGAGCCGACGGTGAAGAGCGCGGCGTTCGCCGCCGGCGCC AACGACTACCTGGTCAAGCTGCCGGACGCCATCGAGCTGGTGGCGCGGATCCGCTACCA CTCGCGTTCGTACATCGCCCTGCAGCAACGCGACGAGGCCTATCGGGCGCTGCGCGAGA GCCAGCAGCAGTTGCTGGAGACCAACCTGGTGCTGCAGCGGCTGATGAACTCCGACGG CCTGACCGGGCTCTCCAACCGTCGTCACTTCGACGAATACCTGGAGATGGAGTGGCGCC GCTCGCTGCGCGAGCAGTCGCAACTGTCGCTGCTGATGATCGACGTGGACTATTTCAAG AGCTACAACGACACCTTCGGCCACGTCGCCGGCGACGAGGCCCTGCGCCAGGTCGCCG GGGCCATCCGCGAGGGCTGCAGTCGCTCCTCGGACCTGGCGGCTCGGTACGGCGGCGA GGAGTTCGCCATGGTCCTCCCGGGCACCTCGCCGGGCGGCGCACGGTTGCTGGCGGAGA AGGTCCGGCGCACGGTGGAGAGCCTGCAGATCAGCCACGACCAGCCACGCCCGGGCTC GCACCTGACCGTCAGCATCGGCGTCAGCACCCTGGTGCCGGGCGGCGGCGGGCAAACC TTCCGGGTTCTCATCGAGATGGCCGACCAGGCGCTCTACCAGGCCAAGAACAATGGCCG CAACCAGGTGGGCCTGATGGAACAGCCGGTGCCGCCGGCCCCGGCGGGCTGAGAATTC
RocR	CATATGAATGATTTGAATGTTCTGGTGTTGGAGGATGAGCCTTTTCAGCGCCTGGTAGC CGTCACAGCCCTGAAGAAAGTGGTGCCCGGATCGATCCTCGAGGCGGCGGACGGCAAG GAGGCGGTGGCGATCCTGGAGTCCTGCGGACACGTCGACATCGCCATCTGCGACCTGCA GATGAGCGGCATGGACGGCCTGGCGTTCCTTCGCCACGCAAGCCTGAGCGGCAAGGTC CATTCGGTGATCCTCAGCAGCGAGGTGGATCCCATCCTGCGCCAGGCCACCATTTCGAT GATCGAGTGCCTGGGCCTCAATTTTCTCGGCGACCTCGGCAAGCCATTCAGCCTGGAGC GGATCACCGCCCTGTTGACCCGCTACAACGCTCGTCGCCAGGACCTTCCGCGACAGATC GAAGTCGCCGAGCTGCCCTCCGTGGCGGACGTGGTGCGCGGCCTCGACAATGGCGAGTT CGAAGCCTACTACCAGCCCAAGGTGGCCCTGGATGGCGGCGGCCTGATCGGCGCCGAG GTCCTGGCACGCTGGAACCACCCGCATCTCGGCGTATTGCCGCCGTCGCATTTCCTCTAT GTGATGGAAACCTACAACCTGGTCGACAAGCTGTTCTGGCAACTGTTCAGCCAGGGGCT GGCGACGCGCAGGAAGCTGGCGCAGTTGGGGCAGCCGATCAACCTGGCGTTCAACGTC

	CATCCTTCGCAACTGGGTTCGCGTGCCCTGGCCGAGAACATCTCGGCGTTGCTGACCGA GTTCCACCTGCCGCCCAGTTCGGTGATGTTCGAGATCACCGAGACCGGGTTGATCAGCG CTCCTGCCAGCAGCCTGGAGAACCTCGTGCGTCTGCGGATCATGGGCTGCGGCCTGGCG ATGGACGATTTCGGCGCCGGCTATTCGTCCCTCGACCGGCTCTGCGAGTTTCCCTTCAGC CAGATCAAGCTGGACAGAACCTTCGTCCAGAAGATGAAGACCCAGCCCAGGAGTTGCG CCGTCATCAGCAGTGTCGTGGCGTTGGCGCAGGCGCTGGGAATTTCCCTGGTGGTGGAA GGGGTGGAGAGCGACGAACAACGGGTACGCCTGATCGAACTCGGTTGCTCCATAGCCC AGGGCTATCTGTTCGCCCGGCCGATGCCGGAACAGCACTTTCTCGACTATTGCTCCGGA TCCTGAGAATTC

[a] The restriction sites at both ends of the gene are 5'-NdeI and 3'- EcoRI respectively.

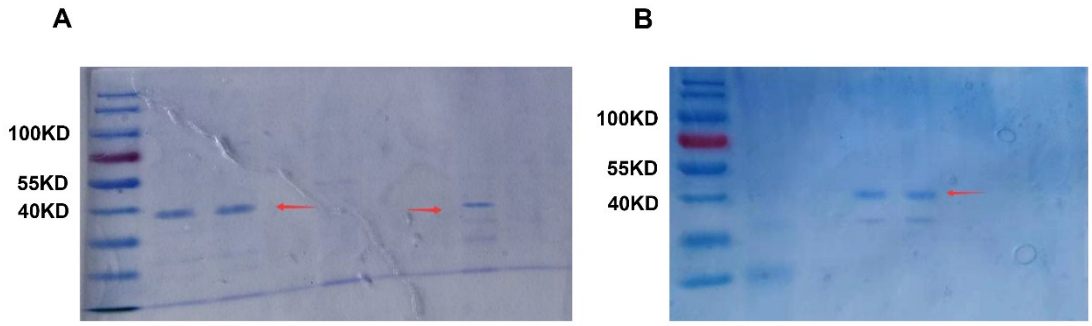

Figure S1. SDS-PAGE results of purified (A) WspR and (B) RocR enzymes. The experiments were run at a working voltage of 130 V for 1.2 h.

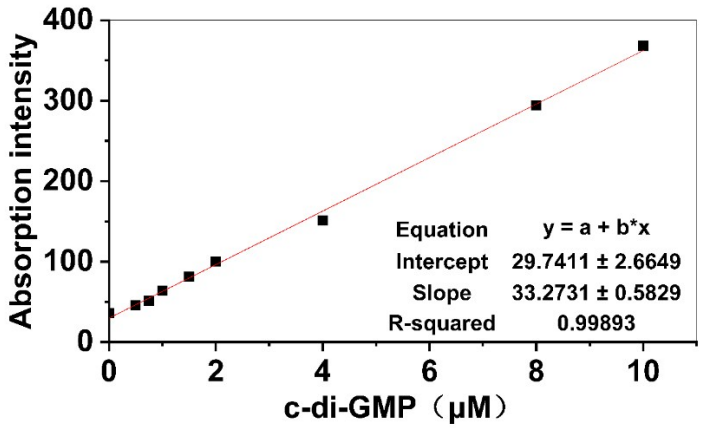

Figure S2. The relationship between the fluorescence intensity detected at the fifth hour and the corresponding c-di-GMP concentration. Detected by fluorescence spectrophotometer. [**A18**] = 2.5 μM, buffer B: 10 mM Tris-HCl, 2 mM MgCl2, 1 M KCl pH 7.5. Ex. 485 nm, Em. 500-700 nm.Incubation by improved method. Incubation temperature: -20°C. Incubation time: 12 hours.

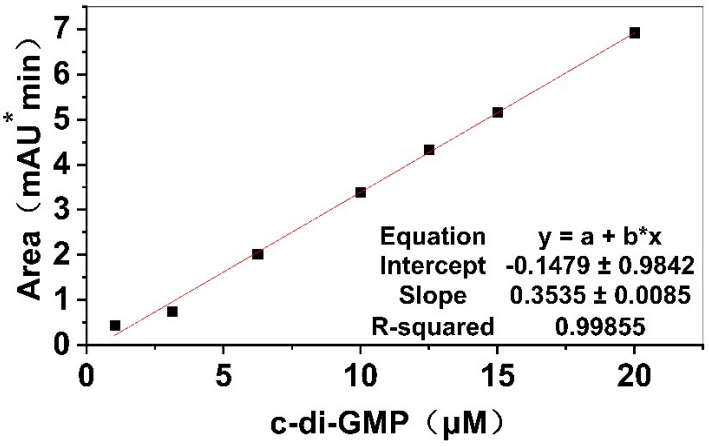

Figure S3. HPLC Peak areas corresponding to different concentrations of c-di-GMP standard solution. Detection wavelength was 252 nm, and HPLC conditions: A: 100 mM TEAA, B: CH3CN, B%: 2-2 %/ 3 min, 2-10 %/ 15 min, 10-30 %/ 25 min, 30-100 %/ 30 min.