Supplementary Information

PoreGlow: A Split Green Fluorescent Protein-Based System for Rapid Detection of *Listeria monocytogenes*

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Name	Sequence (5′ to 3′)
GH 1-157 (sfGFP (1-157) – FcBP – His6)	CATATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCAC AAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCAACAATCGGAAAACTTACCCTTAAATTTATTTGCACTACTGGA AAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACTTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACAT GAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGG GAAATACAAGACGCGTGCTGTAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTACTGATTTT AAAGAAGATGGAAACATTCTCGGACACAAACTCGAATACAACTTTAACTCACACAATGTATACATCACGGCAGACAA ACAAAAGAGCGGAAGCGAGACACCGGGAACCAGCGAGAGCGCGACCCCTGAGAGCGACTGTGCATGGCAC CTGGGAGAACTGGTCTGGTGCACCGCGGCCGC
GH 158-238 (sfGFP (158-238) – FcBP – His6)	CATATGAATGGAATCAAAGCTAACTTCACTGTTCGCCACAACGTTGAAGATGGCTCCGTTCAACTAGCAGACCATTAT CAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAAACTGTTCTTTCGA AAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATG AGCTCTACAAAAGCGGAAGCGAGACACCGGGAACCAGCGAGAGCGCGACCCCTGAGAGCGACTGTGCATGGCACC TGGGAGAACTGGTCTGGTGCACCGCGGCCGC
HG 158-238 (His6-FcBP-sfGFP (158-238))	CATATGCACCACCACCACCACCACGGTGGAGATGTGCATGGCACCTGGGAGAACTGGTCTGGTGCACCAGCGGAAG CGAGACACCGGGAACCAGCGAGAGCGCGACCCCTGAGAGCAATGGAATCAAAGCTAACTTCACTGTTCGCCACAAC GTTGAAGATGGCTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAG ACAACCATTACCTGTCGACACAAACTGTTCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGT TTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAAGCGGCCGC

Fig. S1. Oligonucleotide sequences used in split green fluorescent protein (GFP) fragment design.

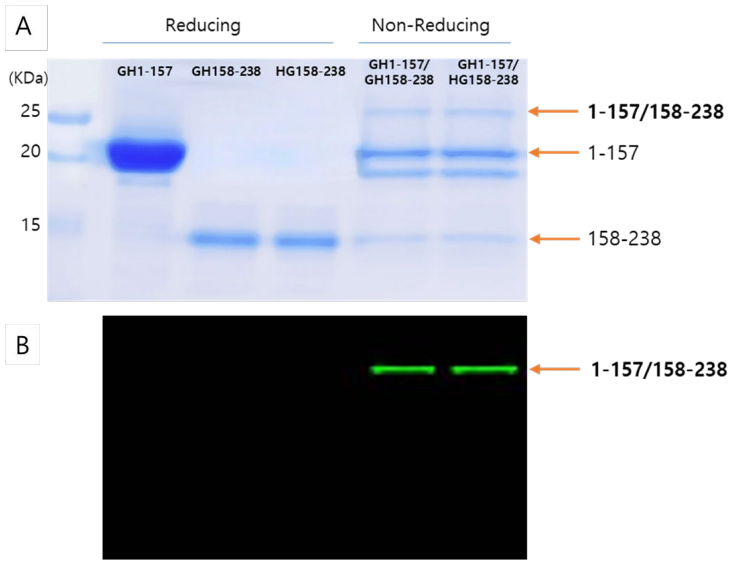

Fig. S2. Assembly of the GFP complex formation. (A) Analysis of purified GFP fragments and their complex formation through SDS-PAGE analysis using Coomassie blue staining. (B) Determination of functional GFP formation by fluorescent image analysis using a fluorescent image analyzer (Typhoon, Cytiva) with excitation/emission at 480/520 nm.

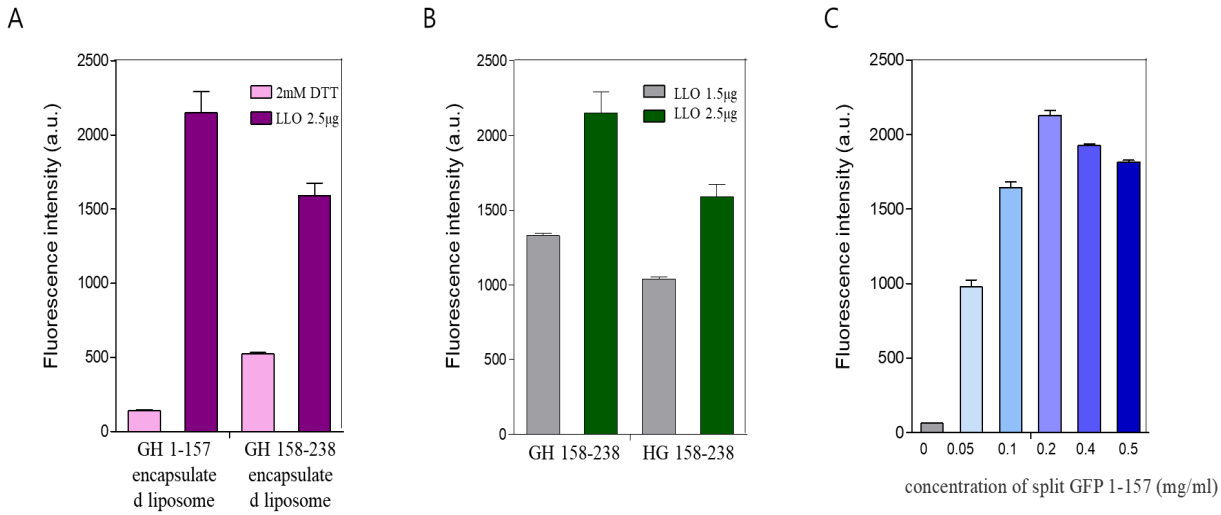

Fig. S3. Optimization of the PoreGlow system for enhanced performance (A) Determination of the fragment for liposome encapsulation. (B) Optimal split GFP combination for pairing with encapsulated GH 1-157. (C) Impact of different concentrations of liposome-encapsulated split GFP 1-157 on fluorescence intensity at λ_{ex} = 480 nm and λ_{em} = 520 nm. Data are presented as the mean ± SD of triplicate measurements.

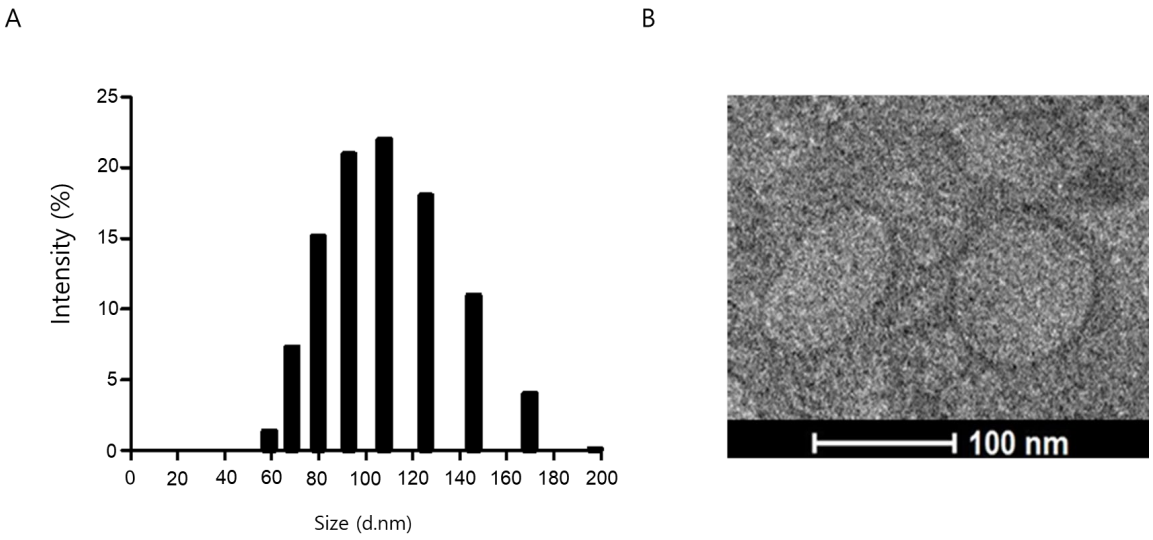

Fig. S4. Characterization of liposomes containing GH 1-157 split GFP. (A) Representative dynamic light scattering profile of the GH 1-157 split GFP-liposome size. (B) Transmission electron microscopy (TEM) image of GH 1-157 split GFP-Liposomes. Scale bar, 100 nm.

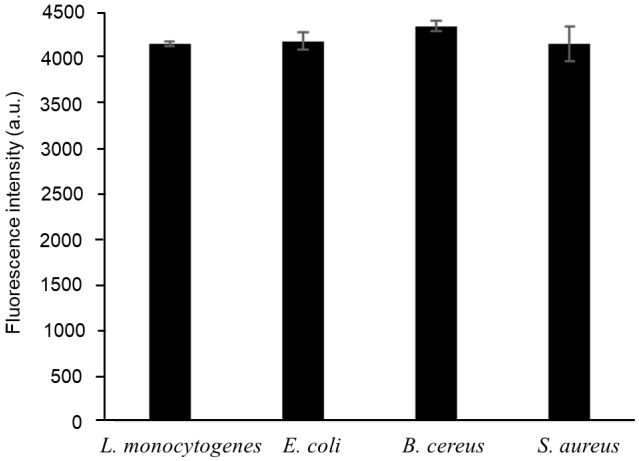

Fig. S5. Cross-reactivity testing using fluorescent dye- encapsulated liposomes. The release of 5(6)-carboxyfluorescein from liposomes after treatment with various bacterial strains is represented as fluorescence intensity (λ_{ex} = 480 nm and λ_{em} = 520 nm). Data are presented as the mean ± SD of triplicate measurements.

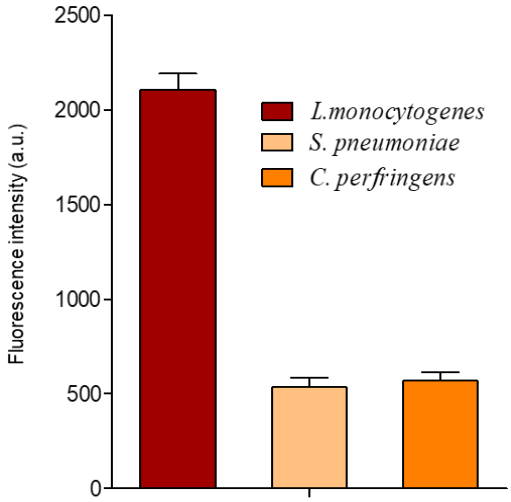

Fig. S6. Cross-reactivity Testing of CDC Family bacteria using the PoreGlow System. The Fluorescence Intensity plot represents the results of the PoreGlow reaction conducted on different CDC family bacteria. Fluorescence intensity is measured at λ_{ex} = 480 nm and λ_{em} = 520 nm. The data are presented as the mean ± SD of triplicate measurements.

Table S1. Pathogen Detection in Food matrices: PoreGlow and Reference culture Method.

Matrix	N	PoreGlow			Reference		
		X	PODc	95%CI	X	POD_R	95%CI
milk	10	9	0.9	0.9 ± 0.26 (0.74,1.096)	10	1	1 ± 0
vanilla ice cream	10	8	0.8	0.8 ± 0.26 (0.539,1.061)	10	1	1 ± 0
chocolate milk	10	9	0.9	0.9 ± 0.26 (0.74,1.096)	10	1	1 ± 0
cottage cheese	10	9	0.9	0.9 ± 0.26 (0.74,1.096)	10	1	1 ± 0

N=Number of test portions
X= Number of positive test portions
PODc= PoreGlow method confirmed positive outcomes divided by the total number of trials
POD_R = Reference method confirmed positive outcomes divided by the total number of trials