Table S1. Repeatability and precision of dual-plex qPCR assay for antibiotic resistant genes and taxon-specific genes using serial dilutions of plasmid and genome DNA from two genetically modified *Escherichia coli* (*E.coli*) strains.

Strains	Targets	True DNA copy	Mean Cq	Mean DNA copy	SD	RSDr %	Bias %	Total DNAs (ng)
pJ281 (Plasmid insertion)	*KmR* (Single-plex)	100000000	15.31	84518734	0.05	0.35	-15.48	0.842
		10000000	18.25	12411955	0.13	0.70	24.12	0.0842
		1000000	21.86	1181000	0.20	0.92	18.10	0.00842
		100000	25.94	82138	0.12	0.47	-17.86	0.000842
		10000	29.36	8783	0.12	0.41	-12.17	0.0000842
		1000	32.52	1119	0.37	1.14	11.86	0.00000842
	dxs (Dual-plex)	100000000	15.84	80220382	0.08	0.58	-19.78	500
		10000000	18.69	12134034	0.09	0.54	21.34	50
		1000000	22.36	1066923	0.08	0.38	6.69	5
		100000	25.94	99389	0.09	0.38	-0.61	0.5
		10000	29.18	11565	0.37	1.39	15.65	0.05
		1000	33.14	838	0.05	0.17	-16.25	0.005
BW25113 (Genome insertion)	*nptII* (Dual-plex)	100000000	15.48	82806062	0.12	0.75	-17.19	500
		10000000	18.43	11623493	0.11	0.61	16.23	50
		1000000	21.95	1118459	0.09	0.40	11.85	5
		100000	25.60	98926	0.19	0.73	-1.07	0.5
		10000	29.00	10330	0.06	0.22	3.30	0.05
		1000	32.65	911	0.42	1.27	-8.92	0.005
	dxs (Dual-plex)	100000000	15.50	81514281	0.05	0.31	-20.00	500
		10000000	18.51	11838679	0.15	0.81	16.42	50
		1000000	22.17	1131299	0.15	0.69	11.52	5
		100000	25.89	103641	0.12	0.45	2.42	0.5
		10000	29.32	11536	0.22	0.73	14.25	0.05
		1000	33.42	829	0.12	0.36	-17.69	0.005

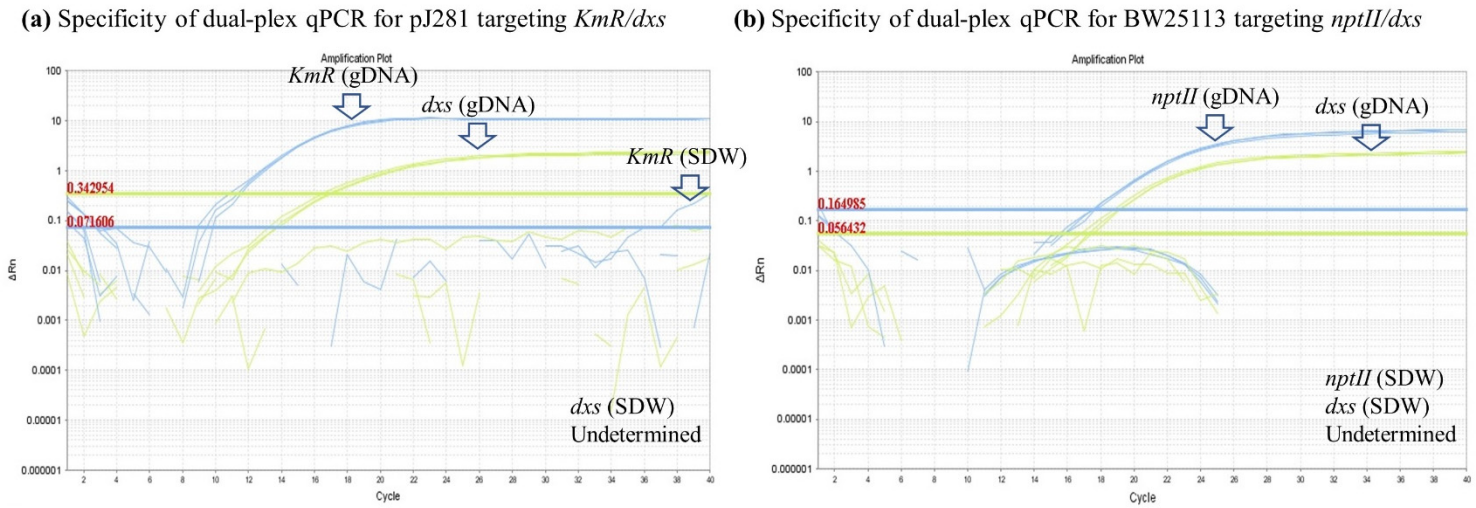

Figure S1. Dual-plex qPCR using genome DNA of two genetically modified *Escherichia coli* (*E.coli*) strains to verify specificity of primer and probe combinations. (a) qPCR for pJ281 targeting *KmR*/*dxs*; (b) qPCR for BW25113 targeting *nptII*/*dxs*; Sterile distilled water (SDW) was used as negative control.

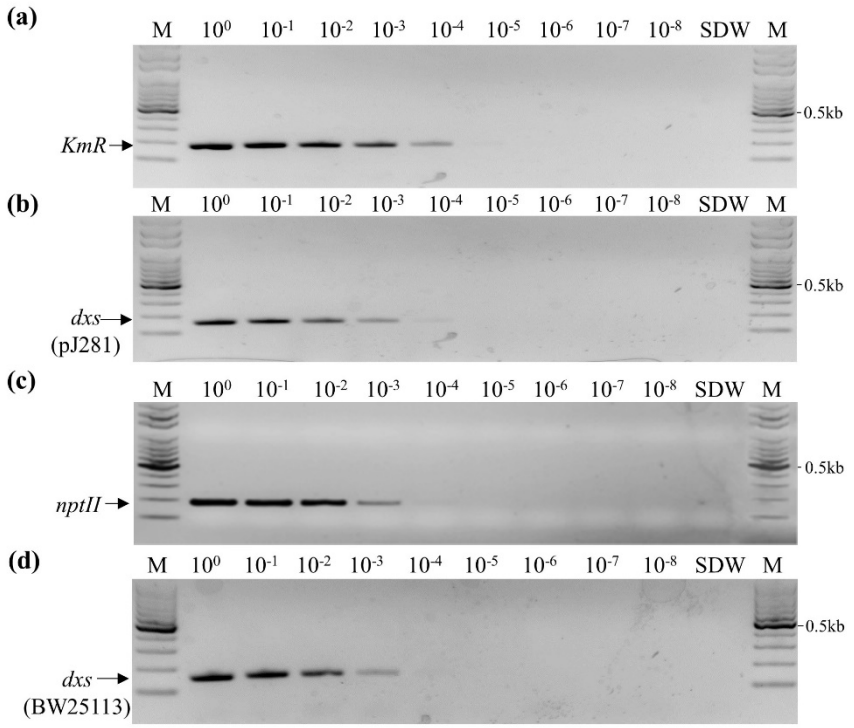

Figure S2. Primer specificity confirmation by **c**onventional PCR analysis using serially diluted cell suspension as template for two genetically modified *Escherichia coli* (*E.coli*) strains. 10^0 indicates overnight cell culture with a mean CFU of 2.39×10^6 per microliter (μL) for the strain pJ281 and 2.62×10^6 per microliter (μL) for the strain BW25113; SDW indicates nuclease-free water as the negative control.

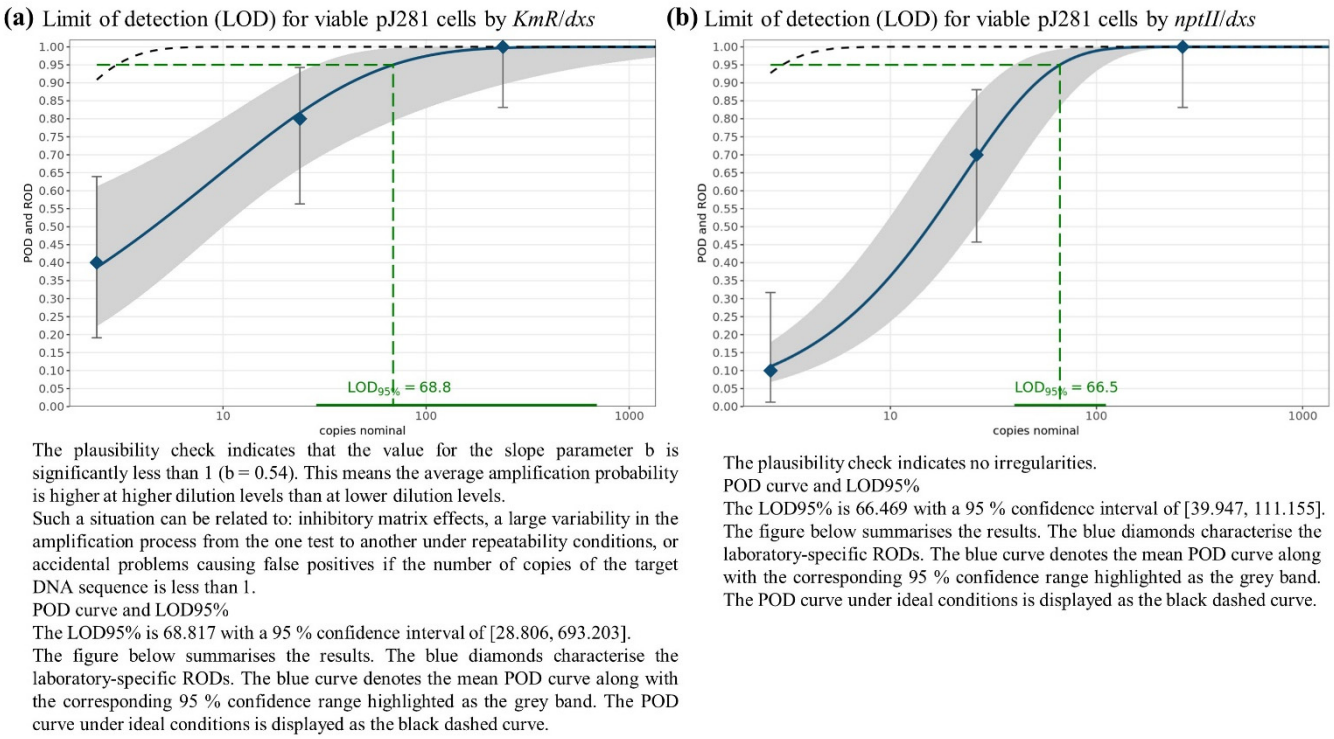

Figure S3. Plausibility check of limit of detection (LOD) at 95% confidence interval for viable *E.coli* cells harboring *KmR*- and *nptII*-resistant genes analyzed by Quodata web application.