

## Supplementary data

**Table S1.** List of used primer sequences.

Primer	Sequence (5' > 3')	Gene
Construction of mutants		
0257_LF_KpnI	AAGGTACCTATCGCCATAAGCTCTAA	<i>dps3</i>
0257_LR_Sall	AAGTCGACGCTCCAATTGTGGATTAA	<i>dps3</i>
0257_RF_XbaI	AATCTAGAAAGAAGCTCCTTGACGTCGT	<i>dps3</i>
0257_RR_PstI	AACTGCAGGGTTTTGGAACTGCTTTA	<i>dps3</i>
0281_LF_BamHI	AAGGATCCAATCAGGATCACATTGAGTC	<i>dps1</i>
0281_LR_BglII	AAAGATCTGACTTTCTTCACGCTTGCG	<i>dps1</i>
0281_RF_KpnI	AAGGTACCATGAACTGAGCGGGG	<i>dps1</i>
0281_RR_Sall	AAGTCGACGCTTTCATAGCAAACC	<i>dps1</i>
2840_LF_KpnI	AAG GTA CCC GGG TGA ACA TCA GAT	<i>lysR</i>
2840_LR_Sall	AAG TCG ACC AGA ATC AAT TCG TCC T	<i>lysR</i>
2840_RF_XbaI	AAT CTA GAC TAC AGA CGA GAA CGG T	<i>lysR</i>
2840_RR_PstI	AAC TGC AGG TCT TAA GCC TCG GA	<i>lysR</i>
To check for streptomycin related genes		
Dgeo_2335_F	TGATGGCGGTGGTTGTCTT	<i>rsmG</i>
Dgeo_2335_R	ATTTTCAGTGGATGGCGCG	<i>rsmG</i>
Dgeo_1873_F	GAACACCTTCAGCGGCTCTT	<i>rpsL</i>
Dgeo_1873_R	CCCTCCGGCATCCTTGC	<i>rpsL</i>
Dgeo_0447_F	AGGGTCACTTTCCTGGTCTGT	<i>mthA</i>
Dgeo_0447_R	GGCGAGCCACTGGTTGGAA	<i>mthA</i>
Dgeo_0776_F	GACCCATCCCGACCACTGA	<i>mthA</i>
Dgeo_0776_R	GCAGGCCTGGTCCTTCACA	<i>mthA</i>
Dgeo_0534_F	AGGTGATATTGCGAGTGCCT	<i>znuA</i>
Dgeo_0534_R	AGACGATGGTGGTGAACGC	<i>znuA</i>
Dgeo_0915_F	CATGAGTGACAACATCGGCG	<i>nuoG</i>
Dgeo_0915_R	TGTACGCAAAGGTCGTCAGC	<i>nuoG</i>
Dgeo_1583_F	CGGAGCGTACTTTGAGTGG	<i>trkH</i>
Dgeo_1583_R	CATCTCATAGAGGGTGGTCG	<i>trkH</i>
16S_rRNA_F1	GCGCAAGCTTGGATTAAACG	<i>dgeo_R0002</i>
16S_rRNA_R1	CAGCTCCCAAAGCGCACA	<i>dgeo_R0002</i>
16S_rRNA_F2	GACAGGGTGGTACACGCG	<i>dgeo_R0008</i>
16S_rRNA_R2	GCGGGAAATGCAGCAAGATG	<i>dgeo_R0008</i>
16S_rRNA_F3	GATCATTGTGCTGGGGGAC	<i>dgeo_R0013</i>
16S_rRNA_R	GGCGCAAGTGACGATGACA	<i>dgeo_R0013</i>
16S_rRNA_F4	GAAGCAAAGGGCCACCTGA	<i>dgeo_R0067</i>
16S_rRNA_R4	CGGTGTGTGTACCCGTCT	<i>dgeo_R0067</i>
Dgeo_1841_F	TGGGATTCCCGATGGTGAC	<i>rpsD</i>

Dgeo_1841_R	AGAAGAAGTTCCGCGCCTAAA	<i>rpsD</i>
Quantitative Real Time PCR		
Dgeo_1202_F	AGCTCGGTGCGGTAGGTAAA	<i>aadA</i>
Dgeo_1202_R	CGTTCGGGTATCTCGAGGG	<i>aadA</i>
Dgeo_0646_F	TGAAGGTAATCGCTGCCGT	EF-Tu
Dgeo_0646_R	GCAAAAGGAACGTTTGAGCG	EF-Tu
Dgeo_1869_F	GTGAAGGTAATCGCTGCTGT	EF-Tu
Dgeo_1869_R	CAAAAGGAACGTTTGAGCGGA	EF-Tu

---

## Figure legends

**Figure. S1.** Comparison of growth pattern among wild-type,  $\Delta dgeo\_0257$ , and  $\Delta dgeo\_0281$  parent and Sm-resistant mutants in antibiotics-free TGY medium (A) and TGY+Sm 50  $\mu\text{g/mL}$  medium (B).

**Figure. S2.** Amino acid alignment of RpsL (ribosomal protein S12) among *E. coli*, *D. geothermalis*, and *T. thermophilus* (A). Two streptomycin interacting regions were marked red color such as P42, K43, K44, and P45, and K88 and 91P amino acid residues. Scheme indicates streptomycin interacting region using Mol\* viewer (Sehna *et al.*, 2021) in 30S small ribosomal subunit from PDB 4DV7 (B). The decoding center region composed of ribosomal proteins S12, S4, and helices h18, h27, and h44 of 16S rRNA. Light green indicates the streptomycin interacting surface.

**Figure. S3.** PCR detection of additional Sm-resistant related genes such as *znuA* (*dgeo\_0534*), *nuoG* (*dgeo\_0915*), *trkH* (*dgeo\_1583*) and *dgeo\_1841* for ribosomal protein S4. Lanes: M, size marker; 1, wild-type; 2-5, WT S1-S4; 6,  $\Delta dgeo\_0257$  S1; 7-9,  $\Delta dgeo\_2840$  S1, S2, and *SrsmG*; 10,  $\Delta dgeo\_0281$  S1.

**Figure. S4.** PCR detection of four 16S rRNAs. Lanes: M, size marker; 1-4, wild-type S1-S4; 5  $\Delta dgeo\_0257$  S1; 6-8,  $\Delta dgeo\_2840$  S1, S2, and *SrsmG*; 9,  $\Delta dgeo\_0281$  S1.

**Figure. S5.** Differential antibiotics-resistant phenotypes from complement strain. Streptomycin-resistant MIC levels among wild-type,  $\Delta dgeo\_2840$ , and a complement (C2840) (A). Nine streptomycin-resistant mutants from the complement strains (C2840) exhibit different streptomycin MIC values following K43R, K88R, K88E, and P91H comparing with parent strains (B).

**Figure. S6.** Phenotypic recovery of cell growth in complement strain. Both  $\Delta dgeo\_2840/\text{pRADgro}$  and  $\Delta dgeo\_2840/\text{pRADgro}::dgeo\_2840$  were grown on TGY medium containing 3  $\mu\text{g/mL}$  of chloramphenicol.

**Figure. S7.** Phenotypic recovery of hydrogen peroxide sensitivity in complement strain. Wild-type,  $\Delta dgeo\_2840$ , and a complement (C2840) strains were cultivated till  $\text{OD}_{600}$  2.0, then exposed 80-120 mM hydrogen peroxide of final concentration for one hour. Then the diluted samples were spotted on TGY agar plates and incubated for 2 days at 48 °C.

A

## Growth curve



### Growth curve

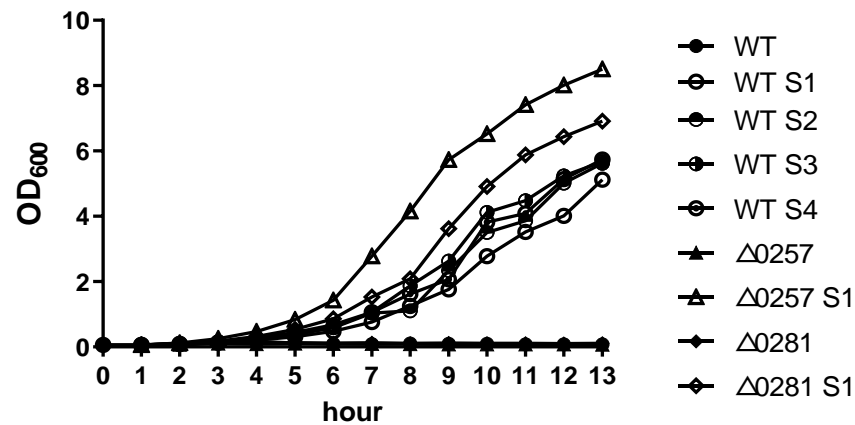
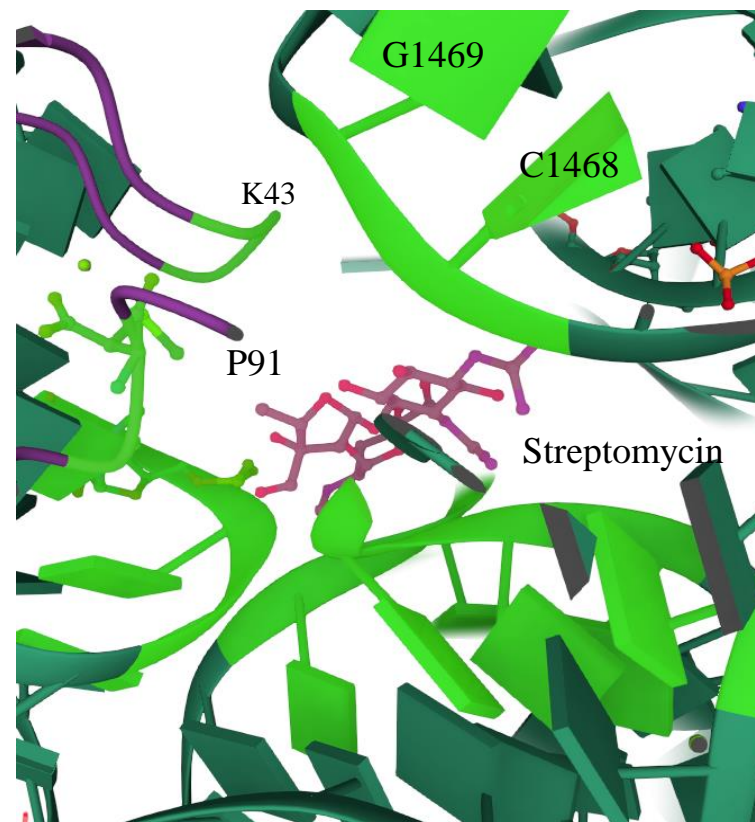
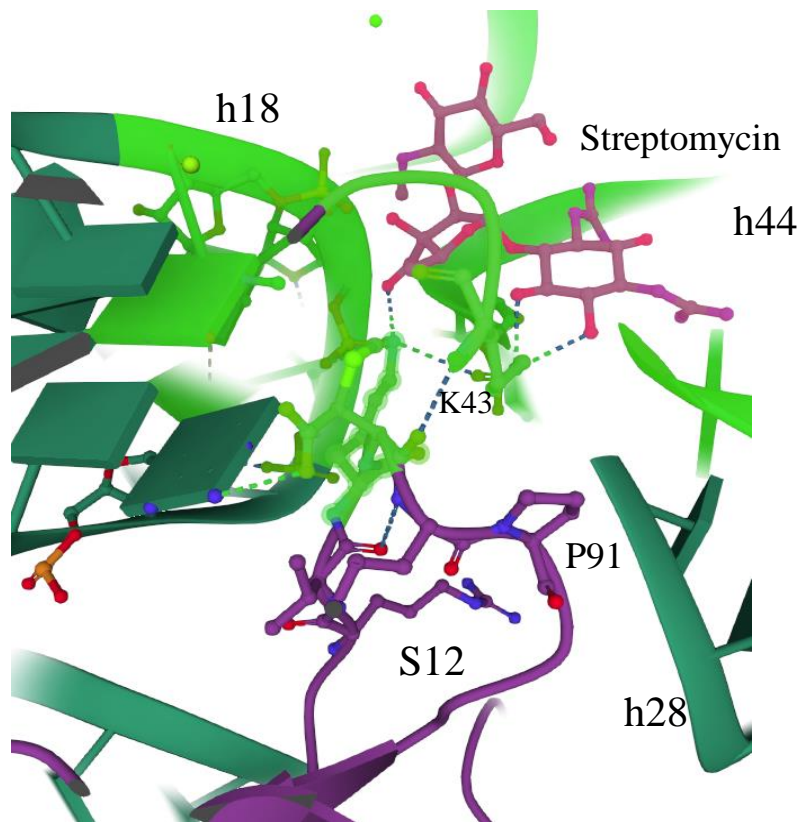


Figure. S2A

	1	43	60
Dgeo	MPTTQQLLRKGR	TTLQKKS	KVPAL
Tth	MPTINQLVRKGR	EKVRKKS	KVPAL
Ecoli	MATVNQLVRKPR	ARKVAKS	NPAL
	*.* :*:** * **:*****:..* :***** * *.*****:..*:**:*.*		
	61	88 91	120
Dgeo	FEVTAYIPGEGH	NLQEH	SVVLIRGGRV
Tth	YEVTAYIPGEGH	NLQEH	SVVLIRGGRV
Ecoli	FEVTSYIGGEGH	NLQEH	SVILIRGGRV
	:***:** *****:***** ***** * *****:.:*****.*		
	121	134	
Dgeo	KPKAGAAA	AAGAKKK	
Tth	KPKEAAK	TAAKK-	
Ecoli	RPKA-	-----	
	: **		

Figure. S2B



Resource : PDB 4DV7 (*T. thermophilus* 30S subunit)

Figure. S3

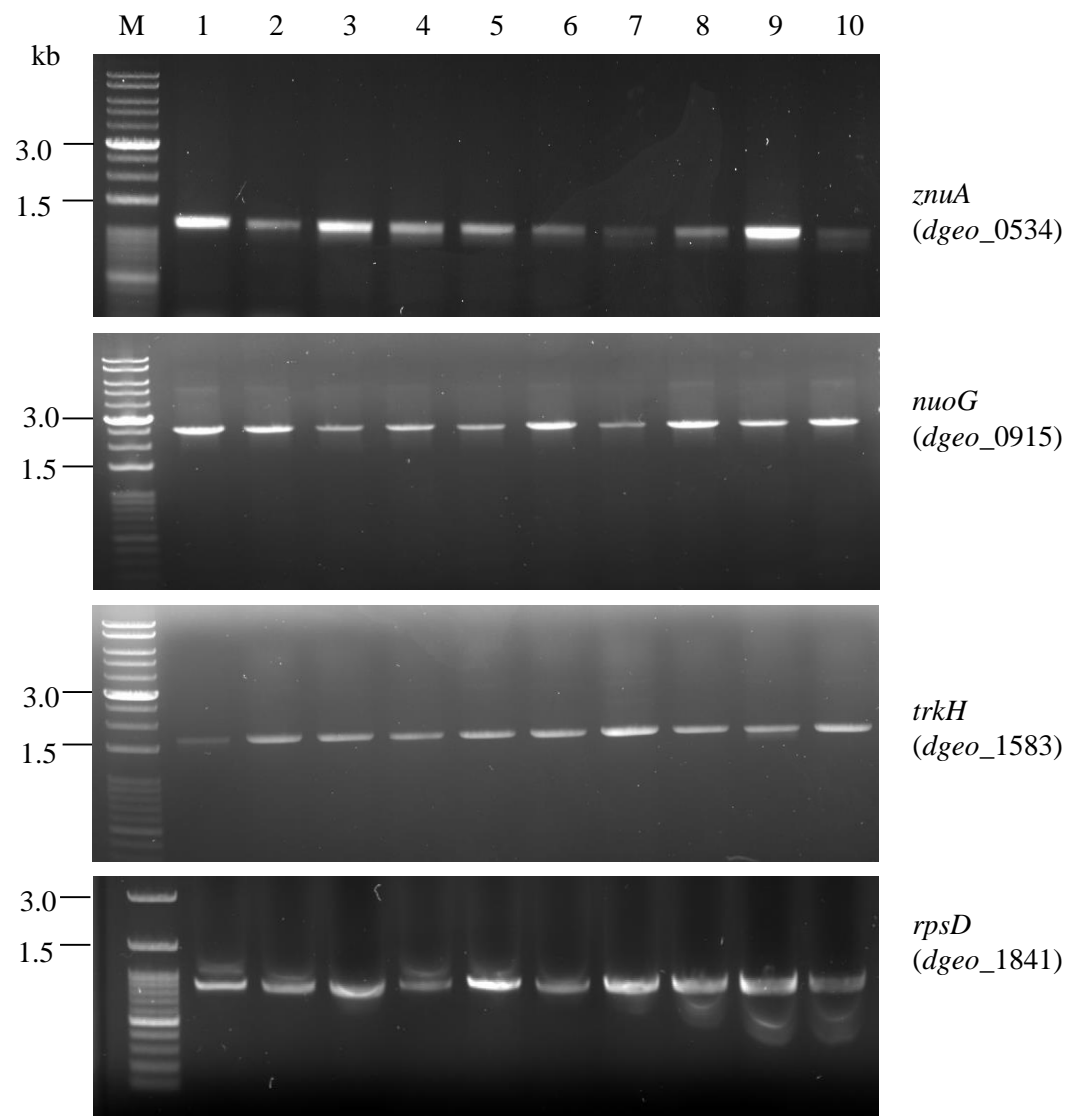


Figure. S4

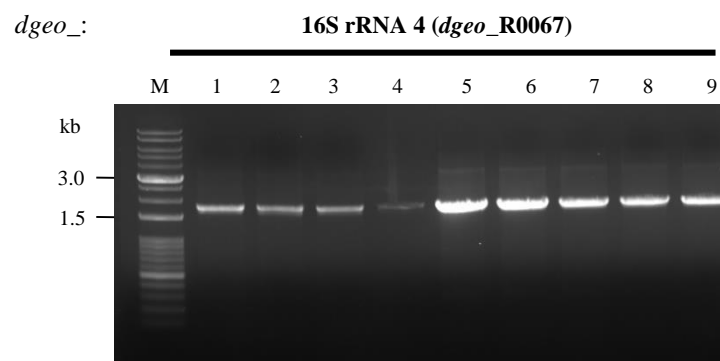
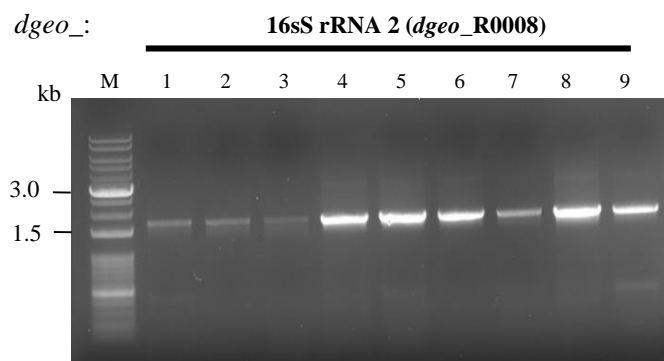
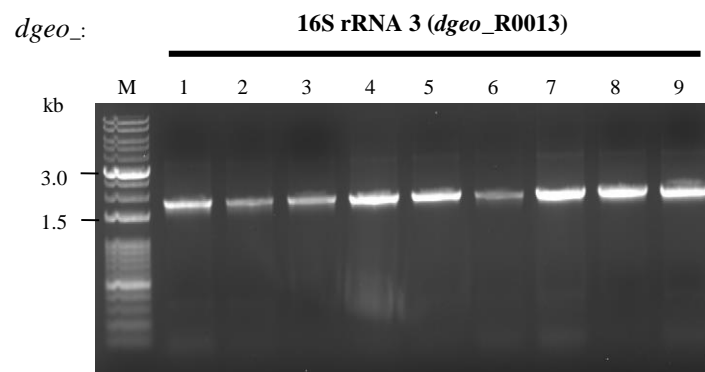
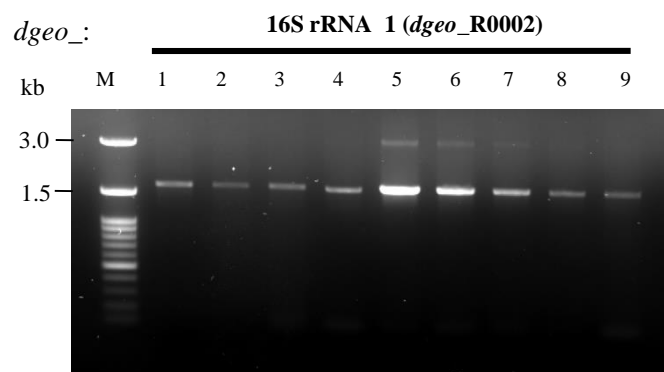
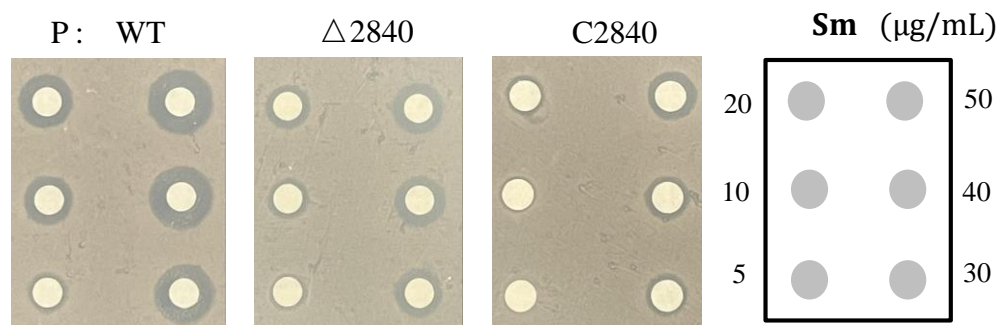




Figure. S5  
A



B

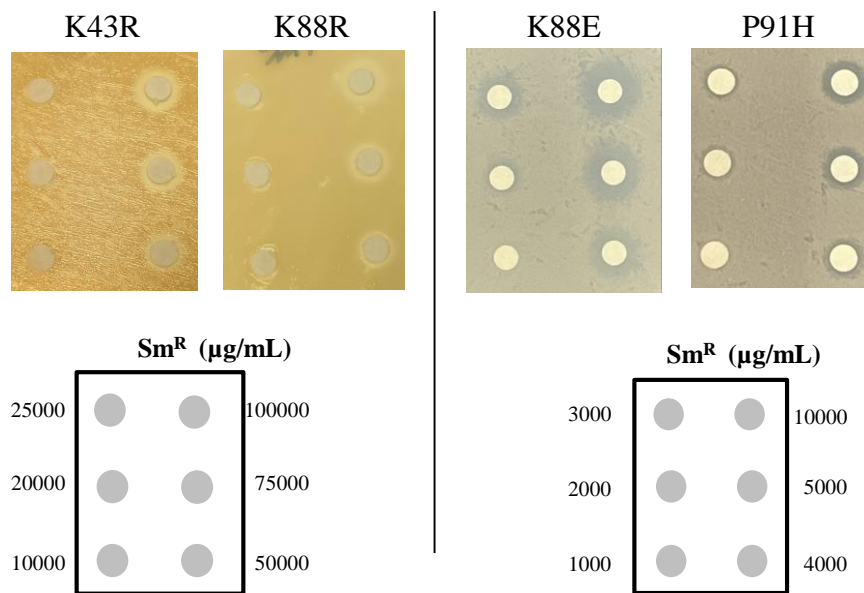


Figure. S6

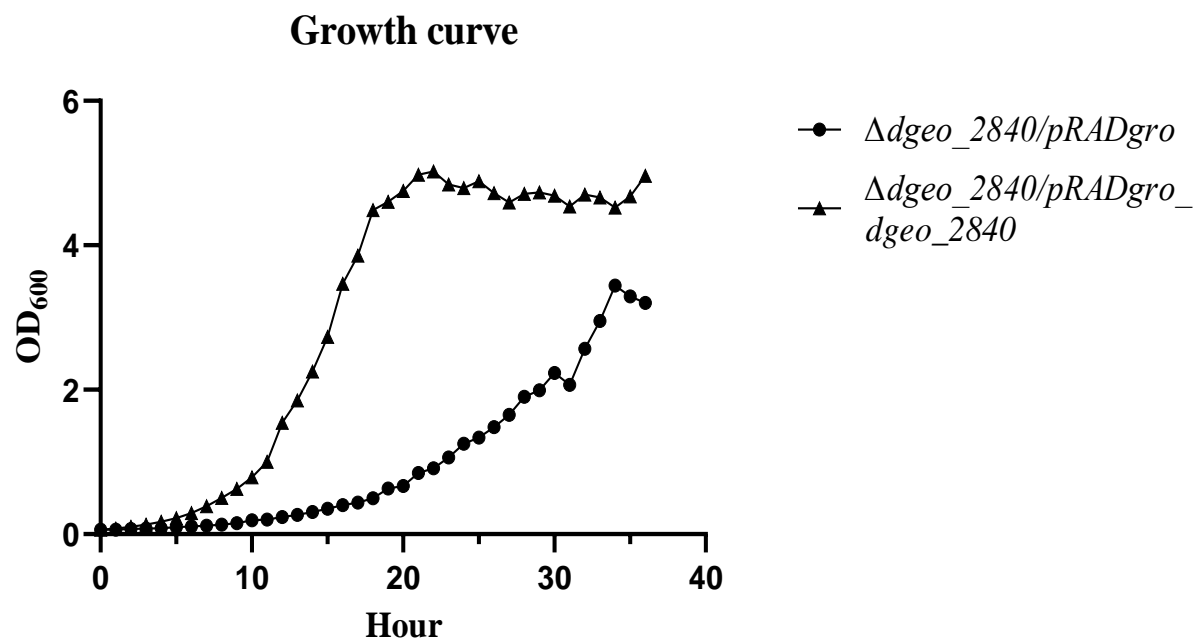


Figure. S7

