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Escherichia coli O Antigen Serotype O16 Is a Restriction Factor for Bacteriophage T4 Infection

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SUPPLEMENTAL MATERIAL

TABLE S1 *E. coli* strains used in this study.

***E. coli* strain**	**Relevant Genotype**	**Reference**
MG1655	K-12 strain. F- λ- *rfb-50 rph-1* (CGSC7740)	(9)
DFB1655 L9	MG1655 with pJP5603/ *wbbL* integrated into the *rfb* gene cluster. O antigen producing strain.	(9)

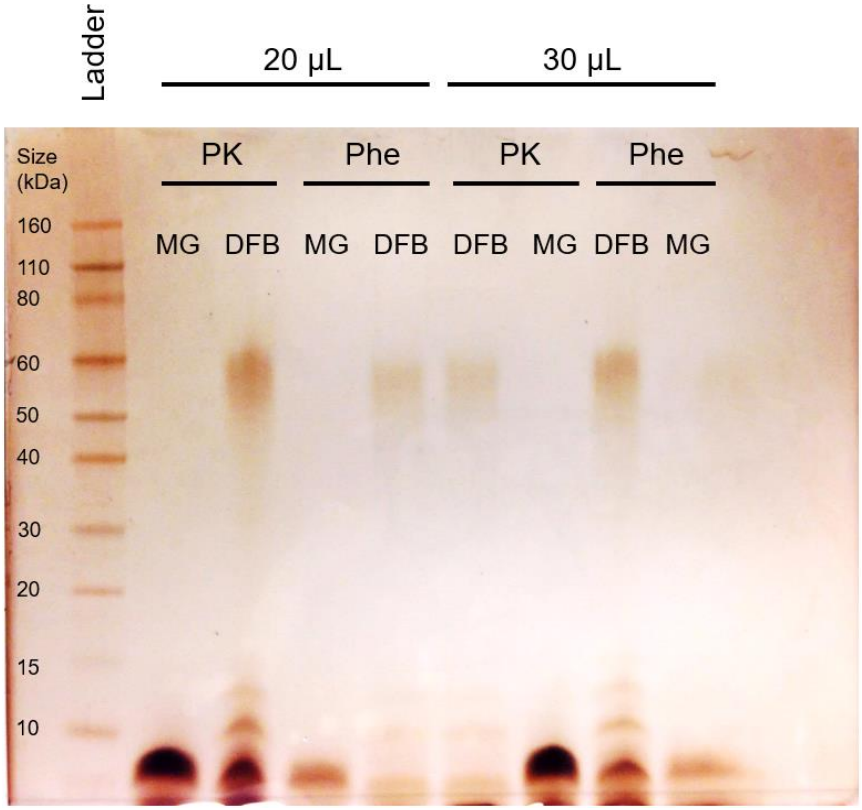

FIG. S1 Verification of *E. coli* K-12 substrain DFB1655 L9-specific synthesis of O antigen. LPS extracts of MG1655 (MG) and DFB1655 L9 (DFB) were generated by Proteinase K or aqueous phenol methods, as denoted. Methods were adapted from Davis and Goldberg (13). Briefly, overnight cultures were pelleted and resuspended in SDS sample buffer. All samples incubated with Proteinase K; one set of samples was not processed further (PK). Another set of samples were vortexed with ice-cold buffer-saturated phenol, incubated with ethyl acetate, centrifuged, and aliquoted into microcentrifuge tubes (Phe). With either method of extraction, O antigen is seen as a 50-60 kDa smear in DFB1655 L9 and not in MG1655.

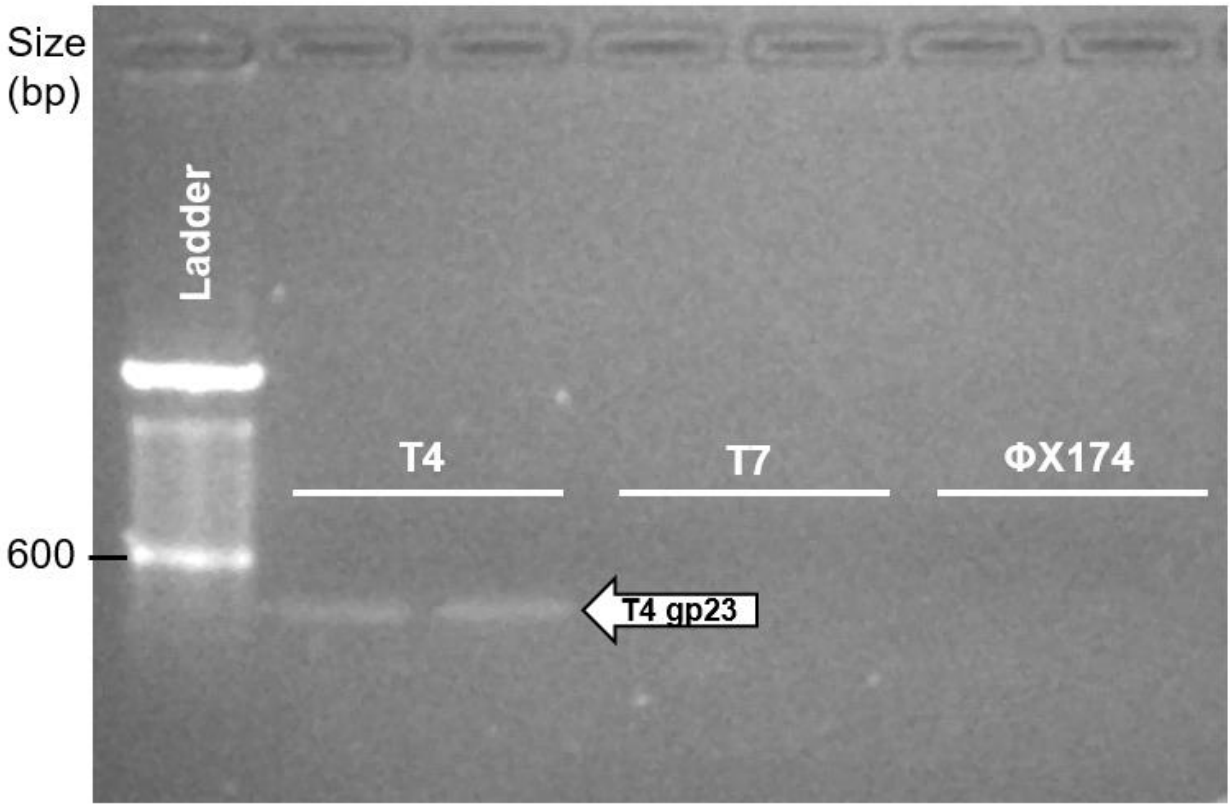

FIG. S2 Validation of bacteriophage T4 purity. Following extraction of propagated bacteriophage T4, selected phage genes were PCR amplified as described in Materials and Methods using primers specific for T4, T7, and ΦX174 as indicated (Table 1). The ~400 bp amplicon (denoted by the white arrow) indicates the presence of the T4 gp23 gene. Importantly, bacteriophage T4 extracts did not appear to be contaminated with bacteriophages T7 or ΦX174, common laboratory contaminants.

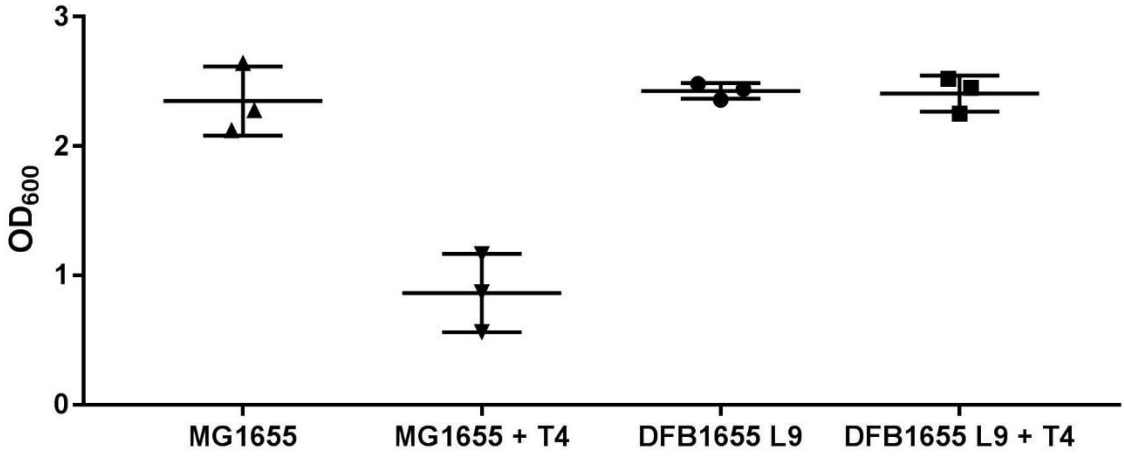

FIG. S3 T4 inoculation results in incomplete lysis of *Escherichia coli* K-12 substrain MG1655 after 12 hours of incubation. Liquid cultures of MG1655 and DFB1655 L9 were inoculated with bacteriophage T4, as denoted. OD_{600} readings were taken at 12 hours post-inoculation. Incomplete bacterial lysis of MG1655, as denoted by a mean OD_{600} of 0.84, is observed 12 hours post-inoculation.