Supplementary Information

Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine

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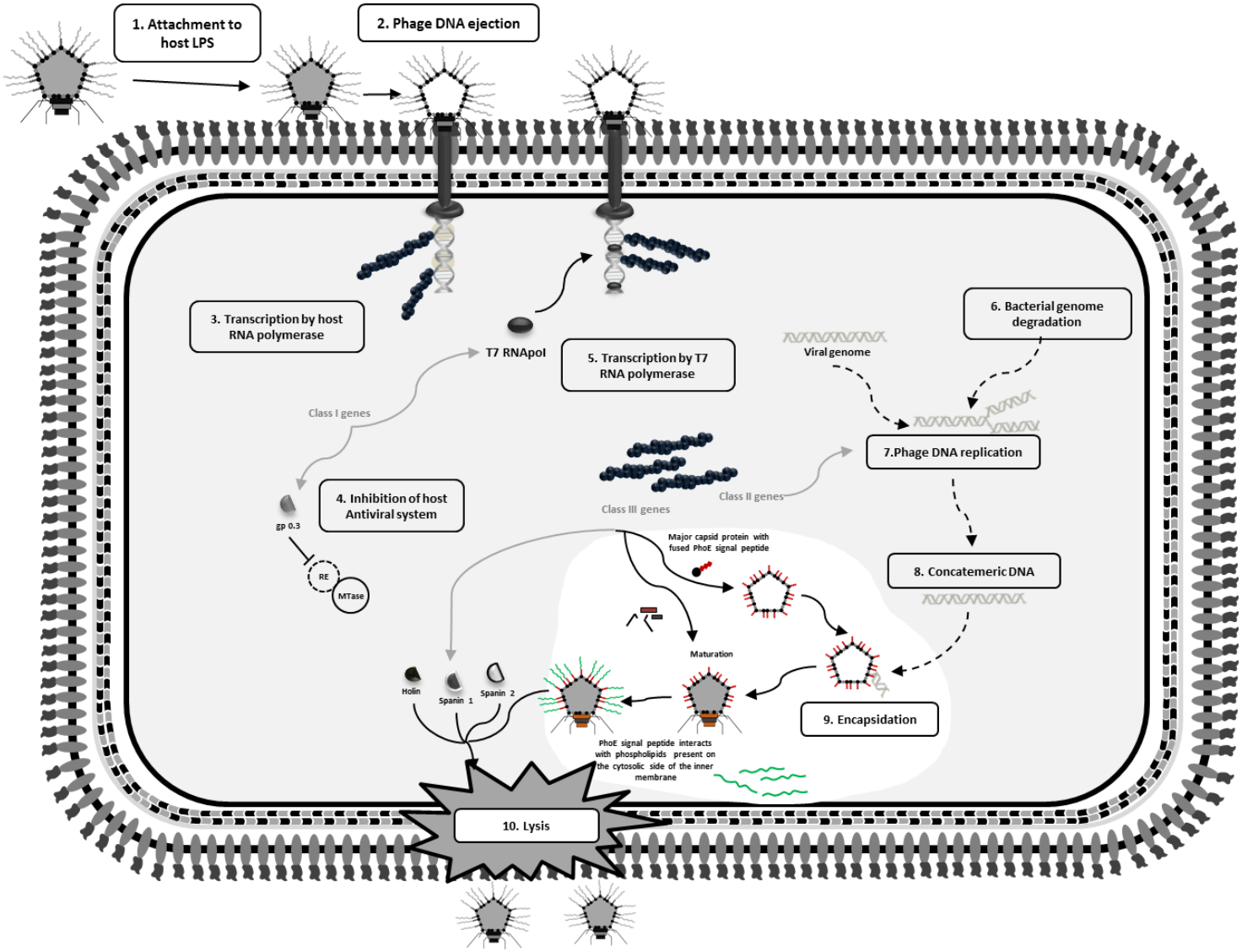
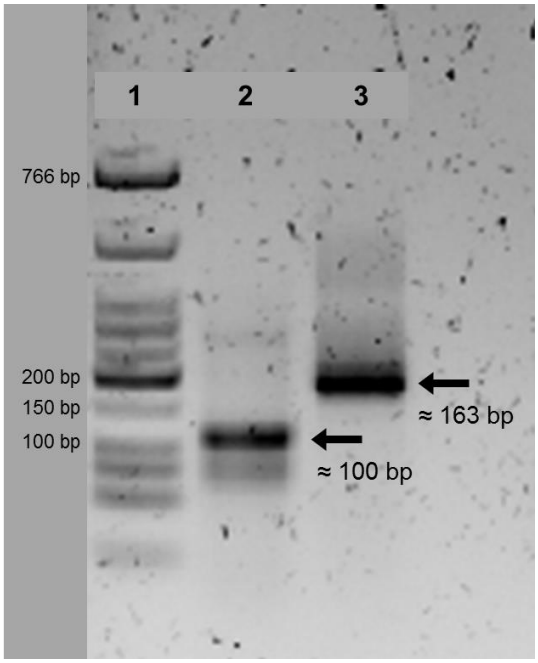
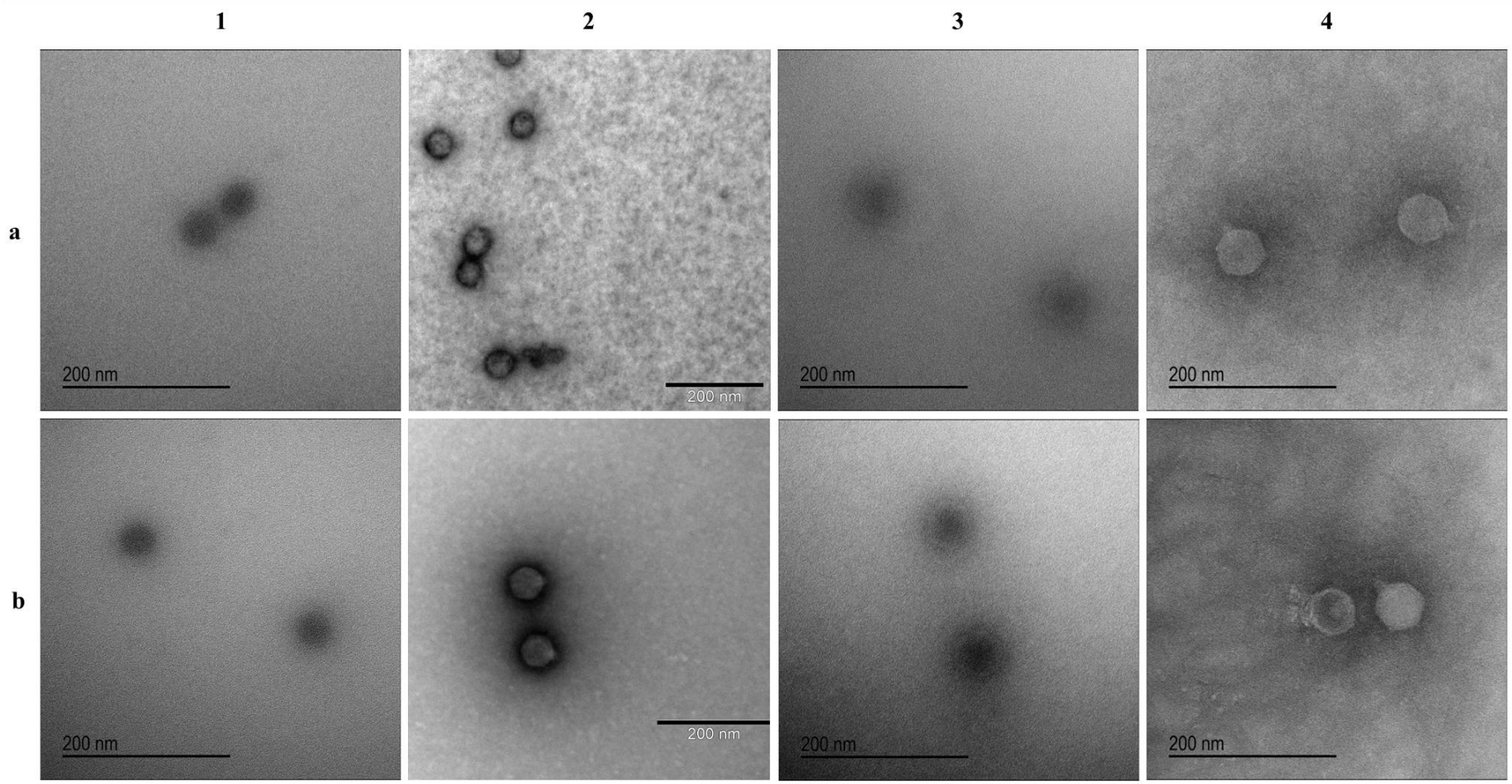


Supplementary Figure S1. Replication of T7::PhoE inside *Escherichia coli*. During replication inside the bacteria, phage T7::PhoE, due to the presence of the PhoE signal peptide on the capsid, signals the incorporation of phospholipids synthesized at the cytoplasmic side of the inner membrane, creating a phospholipid coating.



Supplementary Figure S2. PCR confirmation of PhoE signal peptide sequence insertion in phage T7 genome. Lane 1 – Low molecular weight ladder (NEB); Lane 2 – Wild-type T7; and Lane 3 – T7::PhoE. Expected size of approximately 100 bp for wild type T7 and 163 bp for T7::PhoE, run in a 1% SGTB agarose gel.



Supplementary Figure S3. Transmission electron microscopy images of the phages. a) wild-type T7 and b) mutant T7::PhoE phages. Images of the phages in a 400 mesh copper grid with a carbon film with 1) no treatment, 2) uranyl acetate negative staining, 3) osmium tetroxide vapours for 1.5h and 4) 10 min glutaraldehyde/paraformaldehyde fixation with with osmium tetroxide vapours for 1.5h.

Supplementary Table S1. Primers used to construct the recombineering substrate for BRED and to confirm the mutation.

PRIMER	SEQUENCE (5' – 3') [a,b]	
PhoE.fw	GTGGTCTTCGCCCAGAAGCTGCTGGTGCAGTGGTTTT CAAAGTGGAGTAAATGAAGAAATCTACGTTGGCACTT GT**CGTGATGGG**	To construct PhoE recombineering substrate for BRED
PhoE.rv	AACACTCGCCTCTTCGGGACTAGCAGCGACCGTTGAG GCCACCCCCAGCAAGCCTGAACAGACGCACTTGCCAC GATG**CCCATCACG**	
PhoE.conf.fw	GTGGTCTTCGCCCAGAA	To confirm the presence of the PhoE signal peptide on the mutant phages
PhoE.conf.rv	AACACTCGCCTCTTCGG	

[a] Underlined are the homology regions for the T7 major coat protein

[b] **Bold** is the overlapping region of the primers