# Supporting information

# Genome replication, synthesis and assembly of the bacteriophage T7 in a single cell-free reaction

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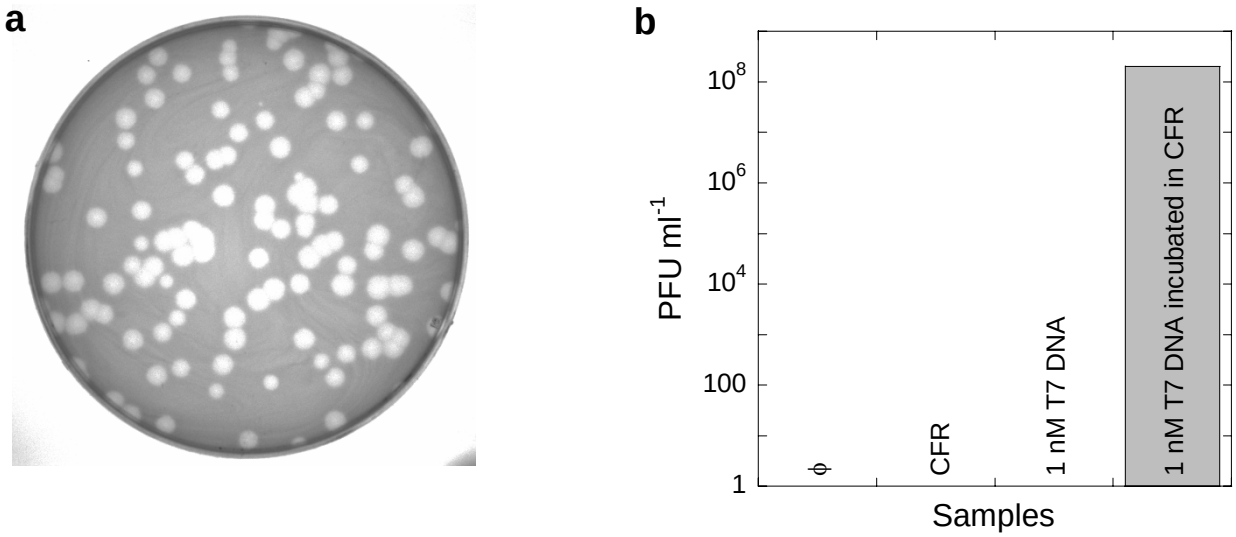


**Figure S1.** Bacteriophage titration by plaque formation. (a) A petri dish showing the plaques formed by T7 phages synthesized in a test tube. A concentration of 1 nM T7 genome was incubated in a cell-free reaction for 15 hours at 29°C. (b) Number of plaques formed for four different samples. No plaques were formed for the blank (∅), for a cell-free reaction (CFR) supplemented with a plasmid (5 nM) encoding for the reporter protein eGFP incubated for 15 hours at 29°C, and for 1 nM of T7 DNA (resuspended in a cell-free reaction with no incubation). Between $10^8$ and $10^9$ plaques were measured from a cell-free reaction supplemented with the T7 DNA genome (1 nM), incubated for 15 hours at 29°C.

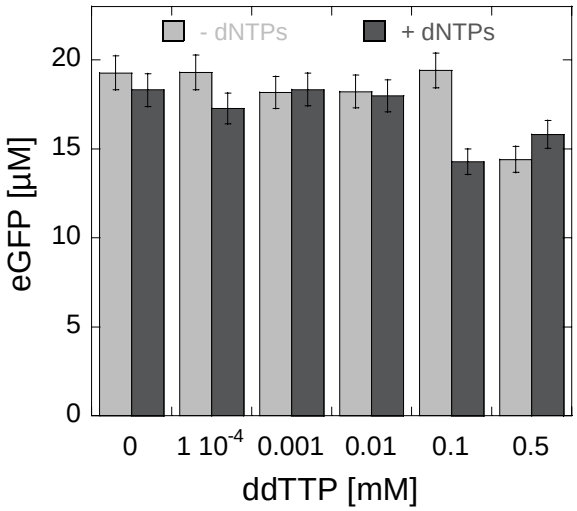


**Figure S2.** Cell-free gene expression of the reporter protein eGFP as a function of ddTTP added to the reaction. A cell-free reaction supplemented with 5 nM of plasmid encoding for eGFP was incubated at 29°C for 12 hours with various concentrations of the DNA synthesis chain terminator ddTTP, with and without dNTPs (0.5 mM of each) in the reaction.

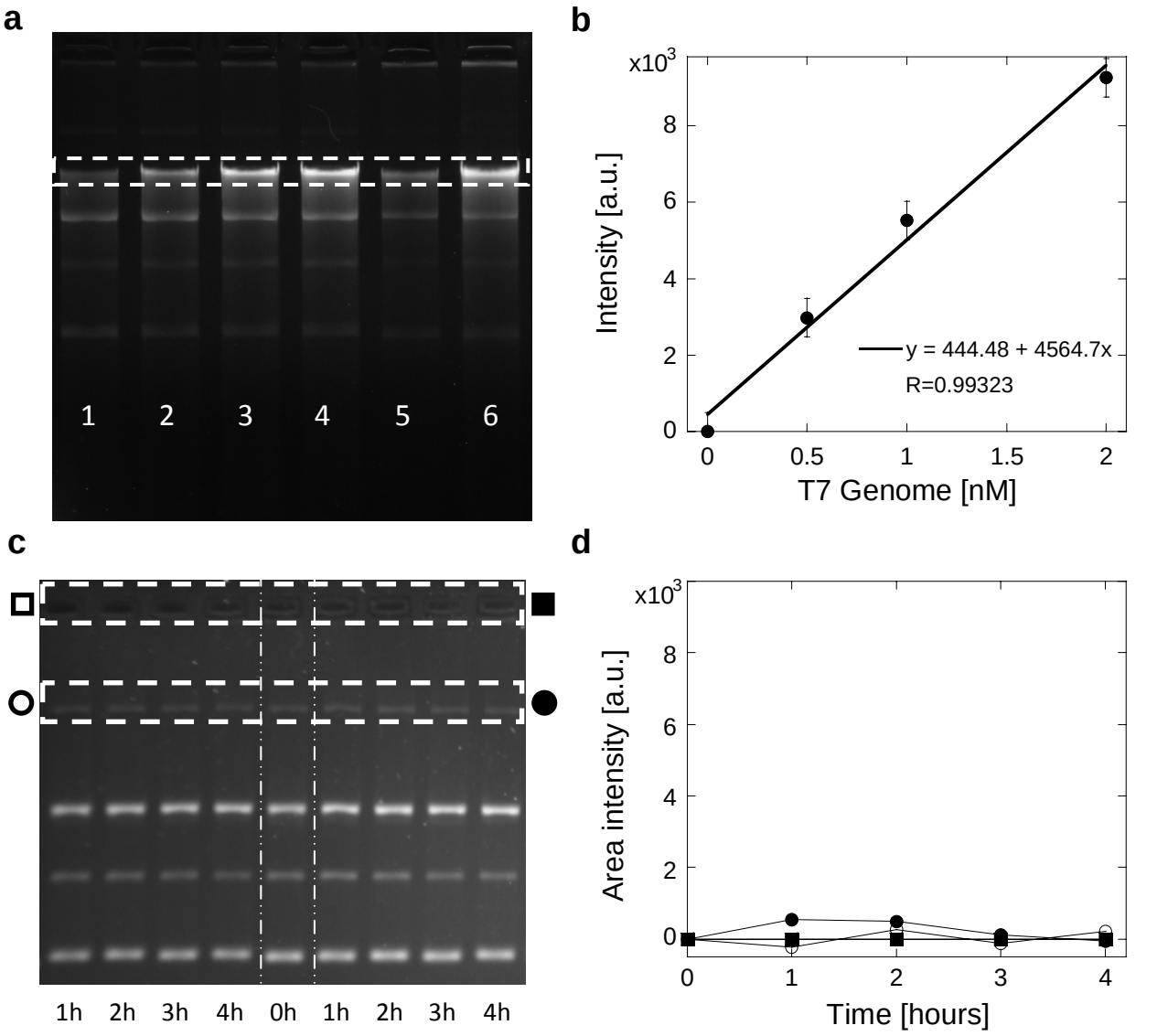


**Figure S3.** Quantification of T7 DNA genome by gel electrophoresis. (a) Picture of the gel (labeled with ethidium bromide). The T7 DNA band is indicated by the white dotted frame. Lane 1 to 4: 0, 0.5, 1 and 2 nM of T7 DNA genome added to a cell-free reaction (no incubation) used for calibration. Lane 5: cell-free reaction initially containing 1 nM of T7 DNA, incubated for 15 hours at 29°C. Lane 6: cell-free reaction supplemented with 0.5 mM of each dNTP and initially containing 1 nM of T7 DNA, incubated for 15 hours at 29°C. (b) The calibration graph constructed from the gel (lane 1 to 4) by measuring the intensity of the T7 DNA band. More than twice of the initial quantity of T7 DNA (1 nM) is measured when dNTPs are present in the reaction (lane 6, ≈ 2.2 nM of T7 DNA genome). (c) Picture of the gel for the negative control (labeled with ethidium bromide). A cell-free reaction with no DNA was incubated in the same conditions as for the T7 DNA. Samples were taken every hours up to four hours. Four lanes on the left: no dNTPs were added to the reaction. Four lanes on the right: dNTPs were added to the reaction. The symbols on the side of the gel are used in Figure S3d. The wells are indicated by the top white dotted frame (Square symbols). The T7 unit-length genome band is indicated by

the white dotted frame below (Circle symbols). (d) Kinetic of the intensity of the bands present in the wells and at the same position as the T7 unit-length genome.

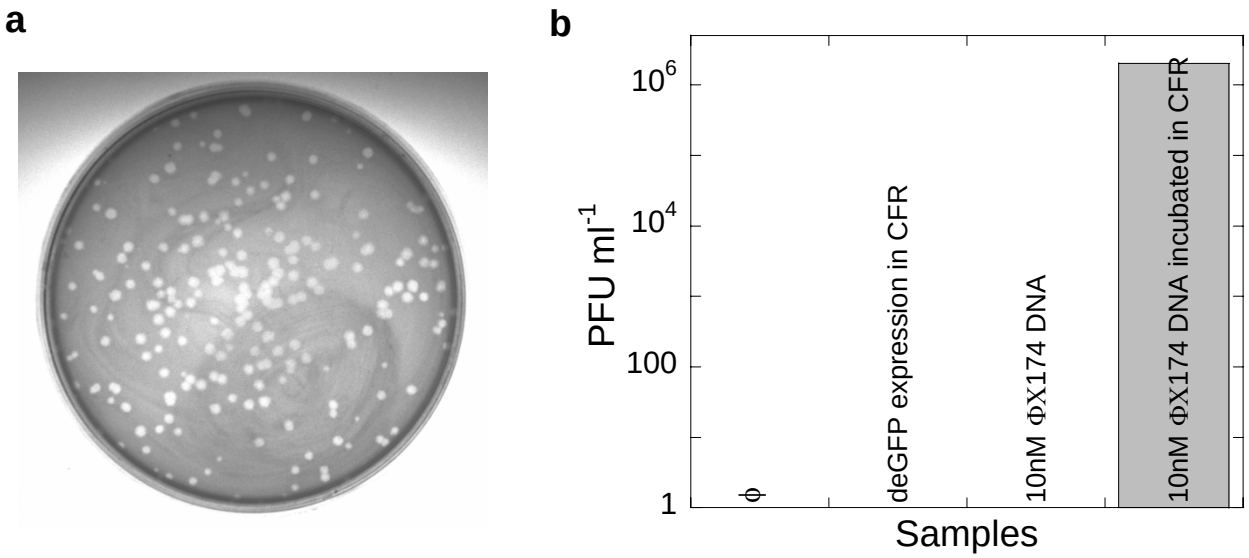


**Figure S4.** Bacteriophage titration by plaque formation for the phage ΦX174. (a) A petri dish showing the plaques formed by ΦX174 phages synthesized in a test tube. A concentration of 30 nM ΦX174 genome was incubated in a cell-free reaction for 15 hours at 29°C. (b) Number of plaques formed for four different samples. No plaques were formed for the blank (∅), for a cell-free reaction (CFR) supplemented with a plasmid (5 nM) encoding for the reporter protein eGFP incubated for 15 hours at 29°C, and for 10 nM of ΦX174 DNA (resuspended in a cell-free reaction with no incubation). Approximately $10^6$ plaques were measured from a cell-free reaction supplemented with the ΦX174 DNA genome (10 nM), incubated for 15 hours at 29°C.