Supporting Information

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SI Materials and Methods

Plasmid Construction. Plasmid pFHC2973 (1) was the basic plasmid and carried the genes for CFP-P1Δ30ParB and yGFP-pMT1Δ23ParB. These tagged constructs were a generous gift from Stuart J. Austin. The CFP site was exchanged to mCherry, which was introduced into pFHC2973 as a PCR fragment carrying a Bgl II site in two steps. First, Fw-NarI and Rev-Bgl II primers were used in one reaction and Rev-EcorI and Fw-Bgl II primers were used in a second reaction to amplify fragments from the plasmid pFHC2973. The resulting PCR products were mixed and used as a template for a third PCR that was amplified with Fw-NarI and Rev-EcorI primers. The PCR product of the second step and pFHC2973 backbone were cut with the restriction enzymes Nar I and EcorI, and the cut insert was ligated into the backbone plasmid. A PCR mCherry fluorescent protein (RFP) fragment carrying the restriction sites Eco RI and Bgl II was amplified with Fw-mCherry and Rev-mCherry primers from the plasmid pFPV-mCherry (Addgene). The purified PCR product of the mCherry insert and the pFHC2973 plasmid carrying the Bgl II site were cut with EcorI and Bgl II, and the insert was ligated into the pFHC2973 backbone. The modified pFHC2973 plasmid carrying mCherry-P1Δ30ParB and yGFP-pMT1Δ23ParB is termed p2973. The plasmids were transformed into DH5α *Escherichia coli* competent cells (Invitrogen) and colony PCR was performed to check for correct fragment insertion by using one primer annealing to the insert and the other to the plasmid backbone sequence. Clones were sequenced and plasmids were transformed into the MG1655 derivatives used in the paper. All PCR primers are listed in Table S1.

Preparation of λ Stock. Bacteria were grown overnight in LBMM (LB supplemented with 0.2% maltose and 10 mM $MgSO_4$). Cells were diluted 1/10 in 2 mL fresh TB (10 g/L Bacto-tryptone, 5 g/L NaCl, pH 7.2) containing 0.2% maltose, 10 mM $MgSO_4$ and incubated at 32 °C for 2.5 h. Then 0.25 mL of the cells were mixed with 0.3 mL from a single phage plaque suspended in 1 mL of TM (10 mM Tris base, 10 mM $MgSO_4$, pH 7.4) and incubated at room temperature for 10 min. Top Agar (10 g/L Bacto-Tryptone, 5 g/l NaCl, 7 g/L agar, pH 7.2) at 50 °C was then added to the cells and poured on TB plates supplemented with 0.3% glucose, 75 μM $CaCl_2$, and 2 mM $MgSO_4$ and incubated for 3.5 h at 37 °C. When plaques on the plates began to exhibit a lacy appearance, they were harvested by adding 5 mL TM to the surface of each plate. Plates were put overnight in 4 °C, and the next day, the TM from the surface of each plate was collected together with a scrape of the top surface of the agar. Forty microliters of chloroform was added, and the lysate was centrifuged at 8000 × *g* for 10 min at 4 °C. The supernatant, which contains high-titer, pure phage particles, was collected and stored at 4 °C.

Image Segmentation. All image processing and data analysis were performed using Matlab (MathWorks). Cell recognition was performed on phase contrast images of cells using a program developed in our laboratory. The program applies morphological operations, using the Matlab image processing toolbox. The program's output was checked manually in all experiments and corrected for errors in recognition.

Fluorescent Spot Recognition and Tracking. The fluorescent spots were identified and tracked using a version of the IDL Particle Tracking software (www.physics.emory.edu/~weeks/idl/) (2), adapted to Matlab by D. Blair and E. Dufresne (http://physics.georgetown.edu/matlab). Briefly, the images were first spatially filtered with a band-pass filter to eliminate pixel noise. Fluorescent spots were then identified to pixel resolution by finding the maximum of each spot, followed by subpixel centroid identification of the spot. Then the tracking procedure was used to track each spot throughout the experiment.

Image Registration. To calculate the distance between two fluorescent foci of different wavelengths, images were registered into the same coordinate system. Control points that were evenly spread across the RFP image were mapped to their appropriate points in the yGFP image. Local weighted mean mapping was then used to correct for registration errors that arise locally without allowing their influence to extend to the rest of the space. This mapping is a weighted sum of second-order polynomials determined locally around each point (3).

Calculation of the Radius of Confinement from MSD Measurement. Two fluorescent spots are diffusing in a confined space. To cancel out drift of the frame with time, bacterial movement and growth effects, we use the distance between the spots to calculate the MSD. We denote r_1 and r_2 as the position of the two spots, and the MSD is defined as:

$$\mathrm{MSD}(\tau) = \left\langle \left((r_1(t+\tau) - r_2(t+\tau)) - (r_1(t) - r_2(t)) \right)^2 \right\rangle.$$

For long enough τ, there is no correlation between the position of the spots and the MSD reaches a plateau that depends on the radius of confinement of the spots. Because there is no correlation, the probability of a spot to be in a volume dV inside the confining volume V is dV/V, and the joint probability of all of the spots is simply the multiplication of the single spot probability:

$$\mathrm{MSD}(\infty) = \frac{1}{V^4} \int \iiint_V \left[(r_1(\infty) - r_2(\infty)) - (r_1(t) - r_2(t)) \right]^2 dV$$

$$\mathrm{MSD} = \frac{1}{V^4} \int \iiint_V \Big[(r_1(\infty) - r_2(\infty))^2 + (r_1(t) - r_2(t))^2 - 2(r_1(\infty) - r_2(\infty))(r_1(t) - r_2(t)) \Big] dV.$$

Because the integral of r is 0, the last term cancels out. The first two terms are equal:

$$\mathrm{MSD} = \frac{2}{V^2} \iint_V (r_1 - r_2)^2 dV$$

$$\mathrm{MSD} = \frac{2}{V^2} \iint_V \left[(x_1 - x_2)^2 + (y_1 - y_2)^2 \right] dV = \frac{4}{V^2} \iint_V (x_1 - x_2)^2 dV$$

$$\mathrm{MSD} = \frac{4}{V^2} \iint_V \left(x_1^2 + x_2^2 - 2x_1 x_2 \right) dV.$$

Again, the last term equals 0 and the first two terms are equal:

$$\mathrm{MSD} = \frac{8}{V} \int_V (x_1^2) dV = \frac{8}{V} \iiint r^2 \cos^2\varphi \sin^2\theta * \mathrm{r}^2 \sin\theta \, dr d\theta d\varphi = \frac{8}{5} R^2.$$

We find that the plateau in the MSD is equal to $8/5*R^2$ with the confinement radius R.

Sequence S1: The P1 *parS-kan* Cassette in λ Phage. The λ sequences used as targeting homology are in blue. The *kan* is in green, the P1 *parS* fragment is in black.

TGAATGAACTGGCCGCAGCGCTCGGGAATGATCCAGA-TTTTGCTACCACCCAC AGCTTTAGAGCGTTTTGCGATG-ACCTGCAGGGGGGGGGGGGAAAGCCACGTT GTGTCT-CAAAATCTCTGATGTTACATTGCACAAGATAAAAATAT-ATCATCATG AACAATAAAACTGTCTGCTTACATAAAC-AGTAATACAAGGGGTGTTATGAGCC ATATTCAACGG-GAAACGTCTTGCTCGAGGCCGCGATTAAATTCCAACA-TGGAT GCTGATTTATATGGGTATAAATGGGCTCGCG-ATAATGTCGGGCAATCAGGTGC GACAATCTATCGATT-GTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAAC ATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGA-GATGGTCAGACTAAA CTGGCTGACGGAATTTATGCCT-CTTCCGACCATCAAGCATTTTATCCGTACTCC TGATGA-TGCATGGTTACTCACCACTGCGATCCCCGGGAAAACA-GCATTCCAGG TATTAGAAGAATATCCTGATTCAGGTG-AAAATATTGTTGATGCGCTGGCAGTG TTCCTGCGCCG-GTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAG-CGAT CGCGTATTTCGTCTCGCTCAGGCGCAATCACGA-ATGAATAACGGTTTGGTTGA TGCGAGTGATTTTGAT-GACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGA AAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCA-GTCGTCACTCATGGT GATTTCTCACTTGATAACCTTAT-TTTTGACGAGGGGAAATTAATAGGTTGTATT GATGTT-GGACGAGTCGGAATCGCAGACCGATACCAGGATCTTG-CCATCCTATG GAACTGCCTCGGTGAGTTTTCTCCTTCA-TTACAGAAACGGCTTTTTCAAAAATA TGGTATTGATA-ATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTC-GATGA GTTTTTCTAATCAGAATTGGTTAATTGGTTGT-AACACTGGCAGAGCATTACGCT GACTTGACGGGACGG-CGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAA

GGA TCAGA TCACGCA TCTTCCCGACAACGCAGA-CCGTTCCGTGGCAAAGCAA AAGTTCAAAATCACCAA-CTGGTCCACCTACAACAAAGCTCTCATCAACCGTGG CTCCCTCACTTTCTGGCTGGATGATGGGGCGATTCAG-GCCTGGTATGAGTCAG CAACACCTTCTTCACGAGGC-AGACCTCAGCGCCCCCCCCCCCTGCAGGTCA T CTGC-AATACGATTCTGGTATTCGCGTAAGTTACTCAATTTAC-TCTTCCTTAAAC ACAAGGGCACTTGTGTGAATCCCTT-TTCGGAAGAGATTTTATTGAAAGTCACT TGTTGACTT-TCTCGTGACAAGCAATTTTTTTATTTTTTTCGGGAGGA-GGGGAGG AAGGTAAAATCCAAGGTGAAATCGTGGC-GATTTCACCTTGAAATTTTAGAGTA ATTTACTTTAAA-AACAGTCAGTTAATAGTGAAATTTGAATGGCGAAAG-TTTAA GGCTTCGGCTTTTTATCGTCGTTTTGTGATAT-GCCGCAGAAACGTTGTATGAAA TAACGTTCTGCGGT

Sequence S2: The pMT *parS* Cassette. The cat gene is in green, the pMT *parS* fragment is in black. The primer pairs will add homology at the 5′ and 3′ end of this cassette to target it for recombination at a specific site on the bacterial genome.

GAGGTTGAAAAGCGTGGTGATTTCAGTGTGAAAGAA-TTTTCAATTTTCCTTTA TAATCAAACAAATAACCATG-AAATTGGCGTGGTGAAAAACATCTAGACGTTT AAGG-GCACCAATAACTGCCTTAAAAAAATTACGCCCCGCCC-TGCCACTCATCG CAGTACTGTTGTAATTCATTAAGCA-TTCTGCCGACATGGAAGCCATCACAGAC GGCATGAT-GAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCC-TTGCGTAT AATATTTGCCCATGGTGAAAACGGGGGC-GAAGAAGTTGTCCATATTGGCCAC GTTTAAATCAAA-ACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAA-AAAC ATATTCTCAATAAACCCTTTAGGGAAATAGGCC-AGGTTTTCACCGTAACACGC CACATCTTGCGAATATA-TGTGTAGAAACTGCCGGAAATCGTCGTGGTATTCAC TCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAA-AACGGTGTAACAAGG GTGAACACTATCCCATATCAC-CAGCTCACCGTCTTTCATTGCCATACGGAATTC CGGA-TGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAG-GCCGGATAAAAC TTGTGCTTATTTTTCTTTACGGTCTT-TAAAAAGGCCGTAATATCCAGCTGAACG GTCTGGTTA-TAGGTACATTGAGCAACTGACTGAAATGCCTCAAAAT-GTTCTTT ACGATGCCATTGGGATATATCAACGGTGGT-ATATCCAGTGATTTTTTTCTCCAT TTTAGCTTCCTTAGC-TCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTA GTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTT-ACGTGCCGATCAACGT CTCATTTTCGCCAAAAGTTGGC-CCAGGGCTTCCCGGTATCAACAGGGACACCA GGATTT-ATTTATTCTGCGAAGTGATCTTCCGTCACA

1. Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG (2006) The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Mol Microbiol* 62(2):331–338.
2. Crocker JC, Grier DG (1996) Methods of digital video microscopy for colloidal studies. *J Colloid Interface Sci* 179(1):298–310.
3. Goshtasby A (1988) Image registration by local approximation methods. *Image and Vision Computing* 6(4):255–261.

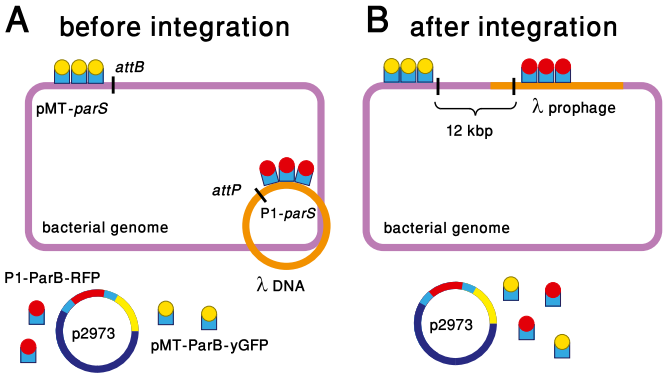

Fig. S1. Labeling scheme of λ phage *attP* and bacterial *attB* sites. An *E. coli* strain carrying the pMT *parS* site near the *attB* integration site on the bacterial genome and a modified bacteriophage λ containing the P1 *parS* near *attP* were constructed by recombineering methods. These *parS* sites serve as specific and unique binding sites for the two different fluorescently tagged ParB proteins, P1 ParB-RFP and pMT ParB- yGFP, expressed from the same plasmid (p2973) from an inducible promoter. (*A*) After infection but before integration, the expressed ParB proteins label their respective *parS* sites, allowing monitoring of the labeled loci through the microscope. (*B*) After λ phage DNA integration at the *attB* site, the genomic distance between *parS* sites is ∼12 kbp and the physical distance is much less than the optical resolution.

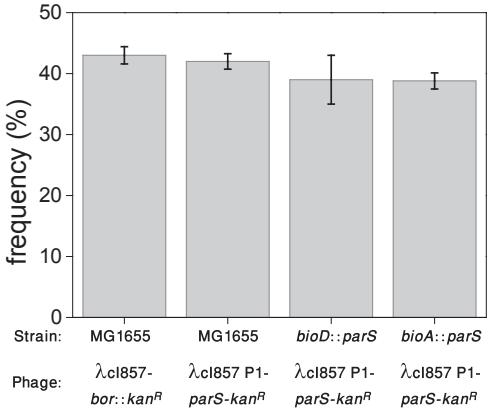

Fig. S2. Labeling with *parS*/ParB systems does not affect integration frequency. Comparison of the integration frequency of λcI857 P1 *parS kan*^{R} phage DNA into MG1655 strains containing a pMT1-*parS* site inserted either in the *bioD* or *bioA* genes, with the integration frequency of λ cI857 *bor*::*kan*^{R} (1) into a MG1655 strain that does not contain a *parS* site. The modified MG1655 strains were transformed with plasmid p2973 (see *Materials and Methods*). The frequency of integration is defined as 100 times the ratio of lysogens to the number of surviving cells. The methods are the same as for Fig. 4 (see *Materials and Methods*), except for the addition of 100 μM Isopropyl β-D-1-thiogalactopyranoside to induce ParB expression from the plasmid p2973. Error bars denote SEs from three independent experiments. This control shows that ParB polymerization does not interfere with λ DNA integration. Note that strain λ cI857 *bor*::*kan*^{R} has been shown to have similar integration frequency as a wild-type λ (1). As in the experiments reported in Fig. 4, within each experiment, the number of colonies grown on LB plates for the different strains did not vary by more than 9%, i.e., the number of survivor cells per total cell input at the start is strain independent.

1. St-Pierre F, Endy D (2008) Determination of cell fate selection during phage lambda infection. *Proc Natl Acad Sci USA* 105(52):20705–20710.

Fig. S3. Time-lapse images of an infected cell in the lysis pathway. The images are an overlay between the fluorescent channel of the λ DNA (red) and the phase contrast image. Time indicated is the time from infection. As the λ DNA replicate, the fluorescent focus becomes diffuse and eventually the cell lyses.

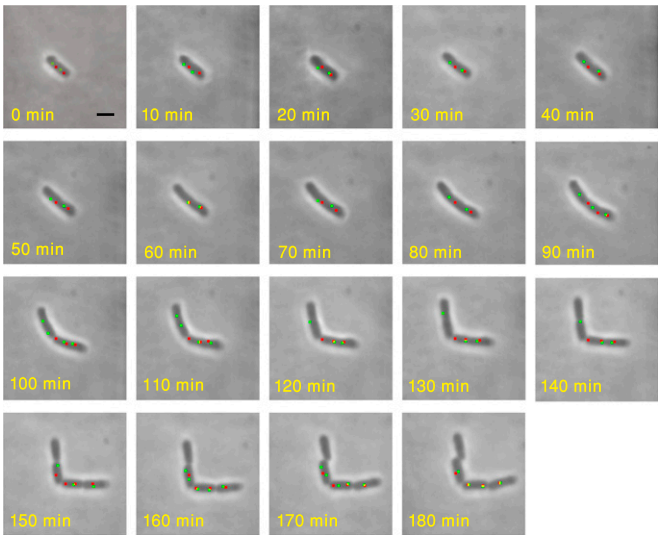

Fig. S4. Time-lapse images of infection of a cell leading to lysogeny. The images, taken every 10 min, show the ParB-RFP locus (red) marking the *attP* site and the ParB-yGFP locus (yellow) marking the *attB* site on the phage DNA. The ParB-RFP and the ParB- yGFP loci are separated by ~12 kbp when lysogeny is established. The scale bar at $t = 0$ denotes 3 μm.

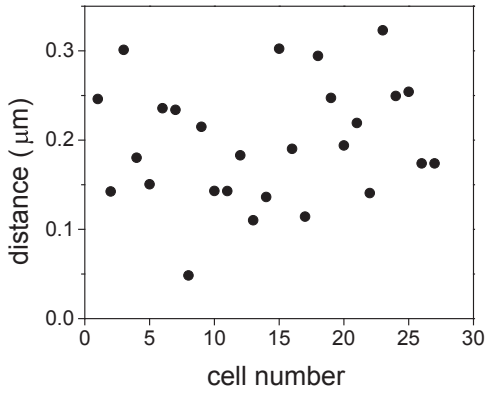

Fig. S5. Measurements of the distance between the yGFP and RFP foci in lysogenic cells. The distance was measured in lysogenic cells expressing the ParB proteins and grown under the same conditions as in the experiments. The mean distance 197 ± 67 nm reflects the accuracy limit of our experimental setup.

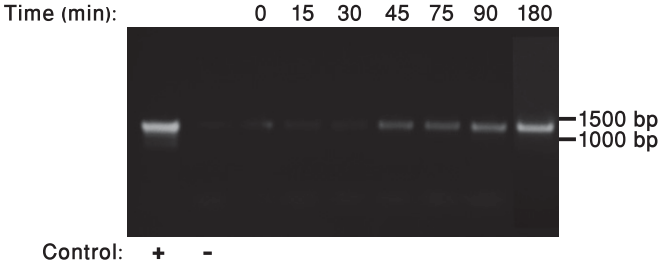

Fig. S6. Measurement of integration time in bulk cultures using PCR methods. Cells were grown following the same procedure as for the experiments performed under the microscope, except that DNA samples were extracted at the indicated times with TRIzol (Ambion), following the manufacturer's instructions. Time is measured after a 10-min incubation of cells with bacteriophages. DNA was extracted from a lysogen culture (+) or from uninfected cells (-) as controls. Insertion of the λ phage into the *attB* site was verified using PCR with primers B-1 and AT-FWR that amplify the boundary between the phage and the bacterial genomic DNA. The expected size of the PCR product is 1150 bp. For primer sequences, see Table S1.

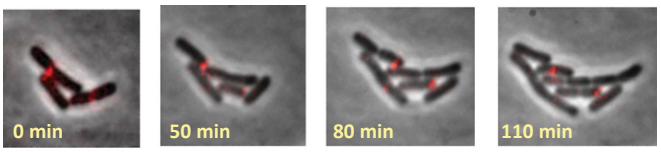

Fig. S7. Infection of a cell with an immunity region present. What happens to λ DNA when a phage infects a lysogenic cell? To address this question, we cloned the cI gene and its control region into the bacterial chromosome *lac* operon (1), and followed the fate of λ DNA following infection. As shown, the λ DNA does not replicate with the bacterial DNA, and is diluted away by being passed to one of the daughter cells following cell division. On long time scales, the λ DNA foci disappear. We assume that eventually they are degraded. Time-lapse images of immune cells that are infected with λ phage. The images are an overlay between the fluorescent channel of the λ DNA (red) and the phase contrast image. Time indicated is the time from infection. The λ DNA does not replicate, and cells carrying the λ DNA are diluted through divisions, when only one of the daughter cells receives the λ DNA.

1. Svenningsen SL, Costantino N, Court DL, Adhya S (2005) On the role of Cro in lambda prophage induction. *Proc Natl Acad Sci USA* 102(12):4465–4469.

Table S1. Sequences of primers used for clone and plasmid preparation

Primer	Sequence (5′ to 3′)
Fw-mCherry	GGAAGATCTATGGTGAGCAAGGGCGAGG
Rev-mCherry	CCGGAATTCCTTGTACAGCTCGTCCAT
Fw-NarI	CCCTGGCGCCCAATACGCAAACC
Rev-EcorI	AATACCTGCTCGACACTGAGCTCGAATTCTTTATACAGC
Fw-Bgl II	CACACAGGAAACAGACCAGATCTAAAGGAGAAGAACTT
Rev-Bgl II	AAGTTCTTCTCCTTTAGATCTGGTCTGTTTCCTGTGTG
RG3	TGAATGAACTGGCCGCAGCGCTCGGGAATGATCCAGATTTTGCTACCACCCACAGCTTTAGAGCGTTTTGC
RG4	ACCGCAGAACGTTATTTCATACAACGTTTCTGCGGCATATCACAAAACGACGAT AAAAAGCCGAAGCCTTA
RG11	GGCGACCGGTTTATAACCTGCCGTCCGGTAGCCTGCTGC CTTTGCGGCTTTGTGACGGAAGATCACTTCG
RG12	CGGAACGGATACCGAAGTGGGGAAAACTGTCGCCAGTTG TGCACTTTTACGATTTATTCAAGAGGTTGAAAAGC
RG15	CGGGCAAGAATGTGAATAAAGG
RG17	CGTTTGCTGCCGCGCAGGCCAGCGGATTGCCCATAAAAGTTGGCCCATGCTGTGACGGAAGATCACTTCGC
RG18	ACGCGCGAGGTTGCAGAAACCATCAGTAACGGTGAAGCCGGTTGCTTTATGATTTATTCAAGAGGTTGAAAAGC
RG21	TTTTTACCTTCCCGTTTCGCTCAAGTTAGTACCGGTAAGCAGGCTTCAACGGATTCATTTTTCTATTTCA
RG25	TAATAAGAAAACTGTTTCTTTCAATAGGAAAATTATCATCATCATATTTAAAGCCTGCTTTTTTATACTAACTTGA
RG26	AAAAGGGGTTTAATAAACTTCGATTTTTAGCAATTAGTGCTATTCTTTGTCTTTATGATTTATTCAAGAGGTTGAAAA
RG29	TAACATAACATAACTTTGCATCAGATAATTCGCAATGACCCTTATAAATAAAGC CTGCTTTTTTATACTAACTTGA
RG30	CAAGAGCAGGGATCCGTCATTTATCCGAGCATTTTACCCCAAAAACCTTTCTTTATGATTTATTCAAGAGGTTGAAAA
AT-FWR	GTCATTCCAACCATCTGCTCG
B-1	GGCGTTTTGCAGACGCGTAC