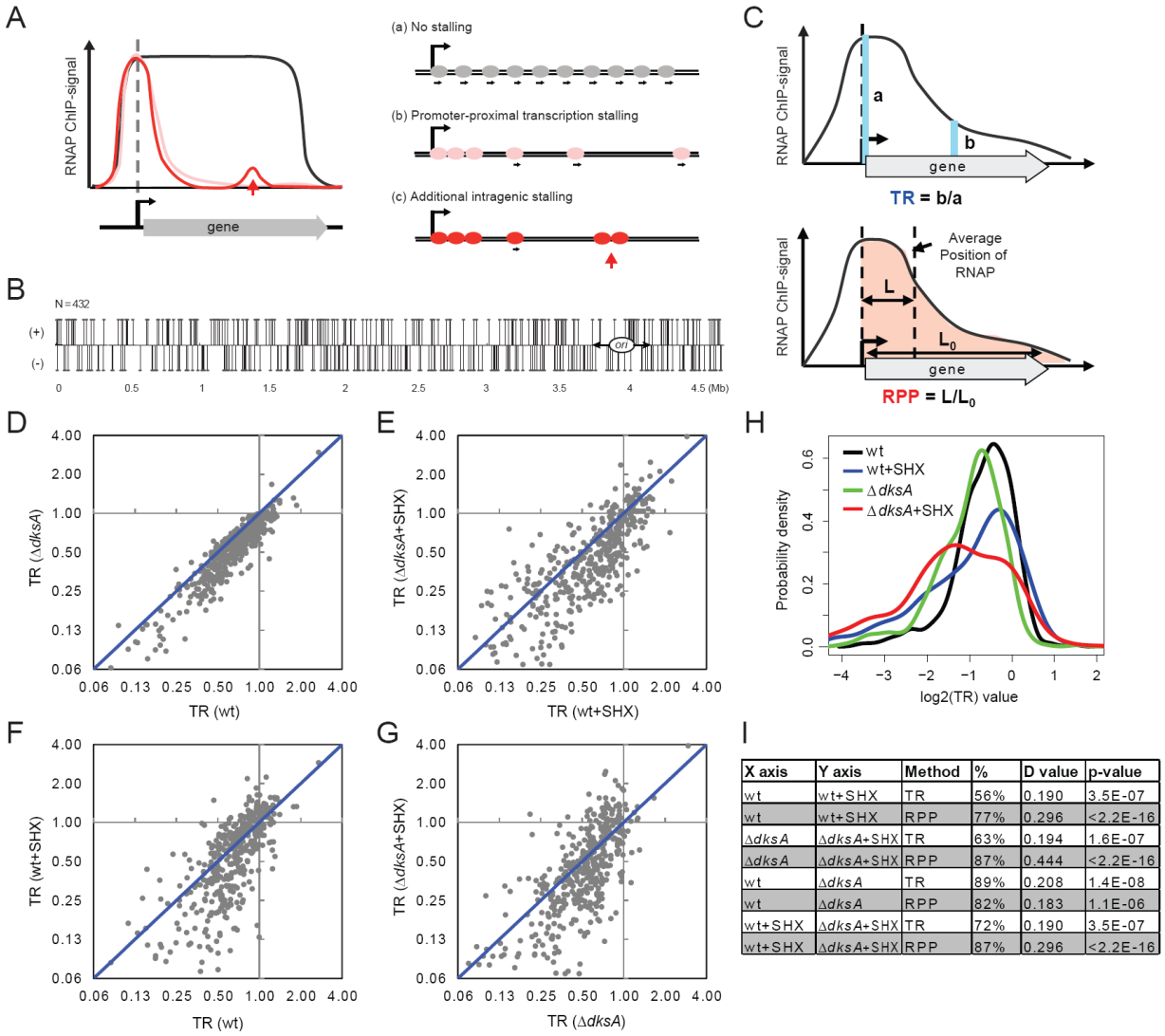

X axis	Y axis	Method	%	D value	p-value
wt	wt+SHX	TR	56%	0.190	3.5E-07
wt	wt+SHX	RPP	77%	0.296	<2.2E-16
ΔdksA	*ΔdksA*+SHX	TR	63%	0.194	1.6E-07
ΔdksA	*ΔdksA*+SHX	RPP	87%	0.444	<2.2E-16
wt	*ΔdksA*	TR	89%	0.208	1.4E-08
wt	*ΔdksA*	RPP	82%	0.183	1.1E-06
wt+SHX	*ΔdksA*+SHX	TR	72%	0.190	3.5E-07
wt+SHX	*ΔdksA*+SHX	RPP	87%	0.296	<2.2E-16

Figure S1, related to Figure 2. RPP (Relative Polymerase Progression), a robust algorithm for calculating polymerase progression into genes using ChIP signals

(A) Schematics of the RNAP ChIP signals. Three types of expected ChIP signals are shown. Black (a): If there is no change of transcription elongation rate, the ChIP signals would be constant across the gene. Pink (b): Promoter-proximal stalling or premature termination results in higher ChIP signals near the promoter. Red (c): Promoter-distal polymerase arrest results in intragenic peaks (red arrows). (B) Genomic locations and orientations of selected 432 TUs for transcription analysis. X axis: genomic coordinates of selected TUs; *ori*: origin of replication; Y

axis: transcription orientation (+ or -). (C) Sampling windows for calculating RNAP processivity with Traveling Ratio (TR; blue) or Relative Polymerase Progression (RPP; red) algorithms. (D-G) Scatter plots of TR values (shown in $\log_2$ scale) calculated from ChIP signals of 432 TUs from indicated samples. Values were averages of two independent replicates. (H) Distributions of $\log_2$(TR) values of 432 TUs from untreated (black), starved (blue) wild-type cells and untreated (green), starved (red) Δ*dksA* cells. (I) Statistics of TR and RPP comparison between indicated samples. X, Y axis: two samples compared; Method: TR or RPP values; %: percentage of TUs (out of 432) having lower TR/RPP values in sample Y than sample X (below diagonal lines); D-values and p-values were calculated from K-S test (see Experimental Procedures).

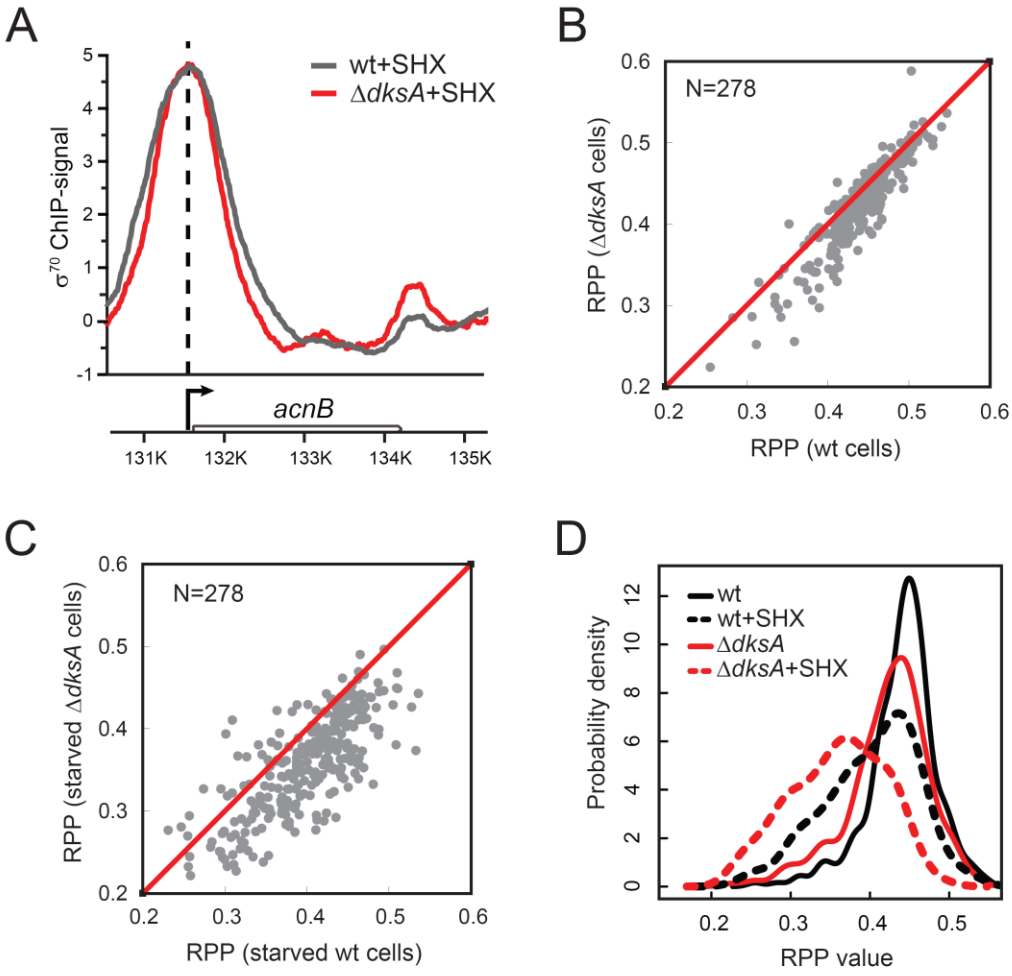

Figure S2, related to Figure 3. RPP analysis of 278 TUs in which DksA does not affect transcription initiation upon starvation

(A) σ^{70} ChIP signals of an example (*acnB*) of 278 TUs for the second round of RPP analysis. Starved wild-type cells (grey) are similarly enriched with σ^{70} compared to starved Δ*dksA* cells (red) at the promoter. (B) Scatter plot comparing the RPP values of 278 selected genes in untreated wild-type (X axis) and Δ*dksA* (Y axis) cells. RPP values of both samples were calculated based on average of two independent replicates. p=3.49e-30, paired student's t test. (C) Scatter plot comparing the RPP values of 278 selected genes in starved wild-type (X axis)

and Δ*dksA* (Y axis) cells. p=1.26e-45, paired student's t-test. (D) Distributions of RPP values of untreated (solid black), starved (dashed black) wild-type cells and untreated (solid red), starved (dashed red) Δ*dksA* cells. N=278.

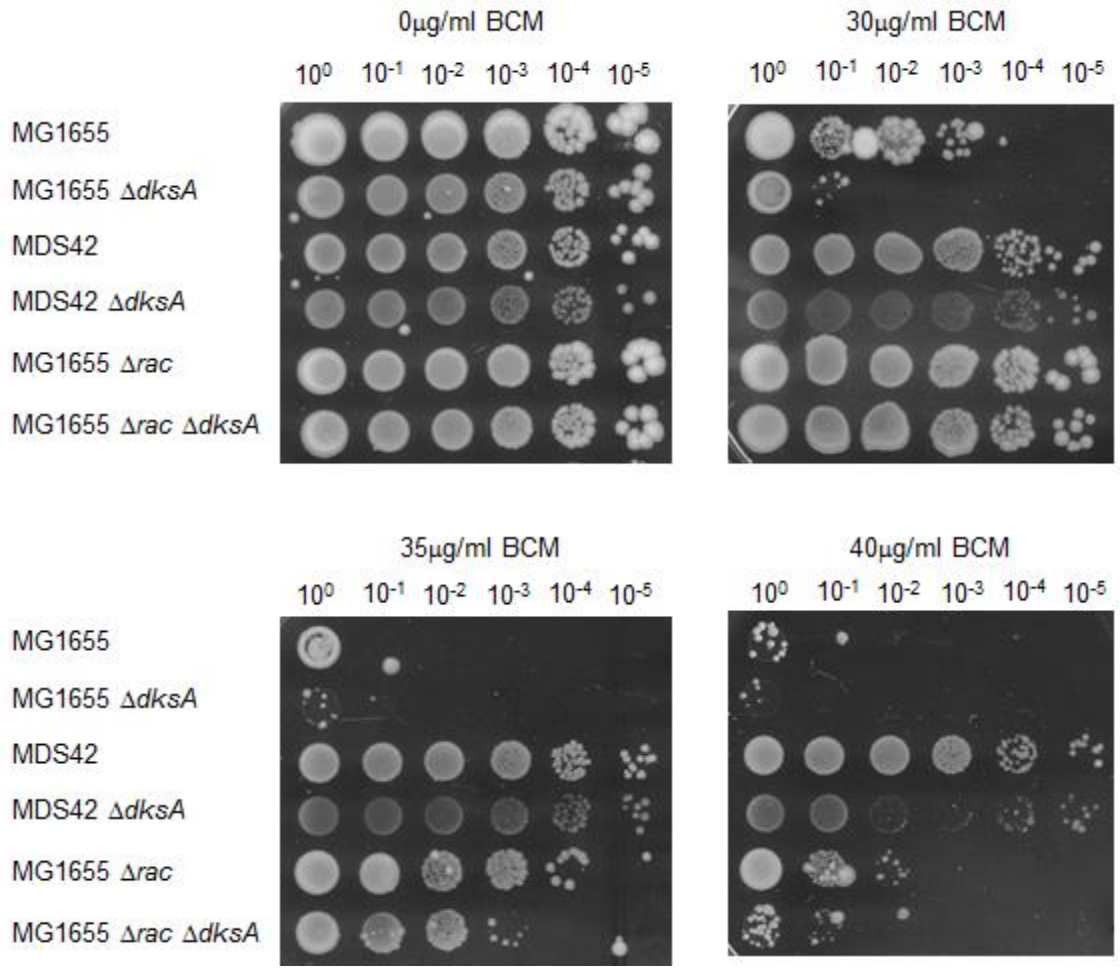

Figure S3, related to Figure 4. Bicyclomycin (BCM) sensitivity of *dksA*$^{+}$ and *dksA*$^{-}$ cells with different backgrounds

Serial diluted cells were spotted on LB plates with different concentration of BCM and incubated at 37 °C for 2 days. MG1655 Δ*dksA* cells were more sensitive to BCM compared to wild-type cells, but this sensitivity was diminished in the Δ*rac* background and diminished further in the MDS42 strain background, in which 14% of *E. coli* genome was deleted (including all IS elements and cryptic prophages). Therefore, the effect of BCM on Δ*dksA* cells appeared to be dependent on only a small subset of non-essential genes in *E. coli*, *e.g*. IS elements and cryptic prophage genes.

Table S1, related to Figure 2. 432 TUs selected from the *E. coli* genome for RPP analysis (Excel file)

Table S2, related to Figure 3. 278 TUs selected from the 432 TUs satisfying an additional criterion that DksA has no effect on σ^{70} enrichment at promoters upon starvation, for a second round of RPP analysis (Excel file)

Table S3, related to Figure 4. Genetic interaction between DksA and several other factors involved in replication-transcription conflict

Mutant *x*	Mutant *y*	W_x[a]	W_y[b]	W_x x W_y	W_{xy}[c]	p-value[d]
dksA	*rep*	0.91	0.85	0.77	0.72	0.51
dksA	*uvrD*	0.91	0.95	0.86	0.88	0.78
dksA	*rnhA*	0.91	0.80	0.73	0.69	0.58
dksA	*invBE*	0.91	0.77	0.70	0.75	0.54
rnhA	*invBE*	0.80	0.77	0.62	0.27	0.01[e]

[a] Fitness (W) of a strain with gene *x* deleted, as defined in (St Onge et al., 2007)

[b] Fitness (W) of a strain with gene *y* deleted

[c] Fitness (W) of a strain with both gene *x* and gene *y* deleted

[d] P-values were calculated based on student's t-test (two-tailed, 3 independent replicates).

[e] P-value lower than 0.05 indicates a significant genetic interaction between *x* and *y*.

Table S4. Strains and Plasmids

Strain	Genotype	Reference
MG1655	$F^- \lambda^-$ *ilvG*$^-$ *rfb-50 rph*$^+$	(Blattner et al., 1997)
CH580	MG1655 *lacZ-U118* Δ*dksA*::FRTKan^{R}FRT	(Tehranchi et al.)
CH2137	MG1655 *rpsD12* Δ*acrF::Tn10* Δ*lacA::gfp* FRTCm^{R}FRT	this study
CH2139	MG1655 *rpsL141* Δ*lacA::gfp* FRTCm^{R}FRT	this study
CH3643	MG1655 Δ*attλ::PsulAΩgfp-mut2 rpsL*[SmP] *tet*	this study
CH4250	MG1655 Δ*attλ::PsulAΩgfp-mut2 rpsL*[SmP] *tet* Δ*dksA*::FRTKan^{R}FRT	this study
CH4341	MG1655 *rpsD12* Δ*acrF::Tn10* Δ*lacA::gfp* FRTCm^{R}FRT Δ*dksA*::FRTKan^{R}FRT	this study
CH4343	MG1655 *rpsL141* Δ*lacA::gfp* FRTCm^{R}FRT Δ*dksA*::*tet*	this study
JDW610	MG1655 *lacZ-U118*	(Brooker and Wilson, 1985)
JDW618	MG1655 Δ*lac* Δ*dksA::tet*	(Blankschien et al., 2009)
JDW902	MG1655 Δ*lac*	(Blankschien et al., 2009)
JDW1011	MG1655 Δ*lac* [pBR322]	(Tehranchi et al.)
JDW1012	MG1655 Δ*lac* [pDNL278]	(Tehranchi et al.)
JDW1013	MG1655 Δ*lac* Δ*dksA::tet* [pBR322]	(Tehranchi et al.)
JDW1014	MG1655 Δ*lac* Δ*dksA::tet* [pDNL278]	(Tehranchi et al.)
JDW1067	MG1655 Δ*lac* Δ*rnhA::cat*	this work
JDW1070	MG1655 Δ*lac* Δ*rnhA::cat* Δ*dksA::tet*	this work
JJC4349	InvBE ΔCm^R	(Boubakri et al.)
JDW1113	InvBE ΔCm^R Δ*dksA::tet*	this work
JDW1163	MDS42	(Posfai et al., 2006)
JDW1164	MDS42 Δ*nusG* with Kan^R insertion	(Cardinale et al., 2008)
JDW1165	MDS42 Δ*dksA::tet*	this work
JDW1270	MG1655 Δ*lac* Δ*tolC::*Kan^R Δ*dksA::tet*	this work
JDW1271	MG1655 Δ*lac* Δ*tolC::*Kan^R	this work
JDW1390	InvBE ΔCm^R Δ*rnhA::cat*	this work
JDW1526	MG1655 Δ*dksA::tet*	this work
JDW1527	MG1655 Δ*lac* Δ*rep::*Kan^R	this work
JDW1528	MG1655 Δ*lac* Δ*uvrD::*Kan^R	this work
JDW1545	MG1655 Δ*lac* Δ*rep::*Kan^R Δ*dksA::tet*	this work
JDW1546	MG1655 Δ*lac* Δ*uvrD::*Kan^R Δ*dksA::tet*	this work

Plasmid	Genotype	Reference
pBR322	Ap^R	(Bolivar et al., 1977)
pDNL278	pTrc99/*greA*, Ap^R	(Feng et al., 1994)

Supplemental Experimental Procedures

Strains and growth condition

To prepare cells for ChIP-chip experiments, MG1655 and MG1655 Δ*dksA* cells were grown overnight to stationary phase (OD_{600}~1.5) in MOPS medium supplemented with 0.2% glucose and 0.4% casamino acid, and diluted back to OD_{600}~0.01 in MOPS medium with 0.2% glucose, leucine, isoleucine, valine, glycine, phenylalanine, threonine (40 μg/ml) and uracil (50 μg/ml) the next day. Cells were grown with vigorous shaking at 37 °C to mid-log phase (OD_{600}~0.4) and each culture was split into two flasks, one treated with SHX (0.5 mg/ml) and the other left untreated. After growing for another 20 min, sodium phosphate (1/100 vol. of 1M, pH 7.6; 10mM final) was added to the cultures followed by formaldehyde to 1% final, and shaking was continued for 5 min. To stop the crosslinking, 2.5M cold glycine was added to 100mM and the mixture was kept at 4°C for 30 min. Cells were spun at 5000 x *g* for 10min, washed with ice-cold PBS twice and flash-frozen in liquid nitrogen.

Preparation of DNA for ChIP-chip

Cell pellets were thawed and resuspended in 500 μl of IP buffer (100 mM Tris pH 8, 300 mM NaCl, 2% TritonX-100) and sonicated using a Misonix sonicator (S-4000) with a cup horn (431C) set at 60% output, 10 sec ON and 10 sec OFF, for a total sonication time of 16 min. Cells were then treated for one hour at 4 °C with RNase A (2 ng/ml; USB, Inc.) and the samples were centrifuged at 20,000 x *g* for 10 min at 4˚C to remove cell debris. The lysate was then incubated with a 50/50 slurry of Sepharose protein A beads (Upstate; now Millipore) and protein G beads (GE Healthcare) in IP buffer for 3 hours at 4 °C. The beads were removed by centrifugation (1000 x *g* for 2 min at 4 ˚C) and antibodies were added to the pre-cleared lysate for an overnight

incubation. For ChIP, we used anti-RNAP β subunit NT63 monoclonal antibodies (Neoclone W0002), anti-RNAP σ^{70} subunit monoclonal antibodies (Neoclone W0004), or anti-DksA rabbit polyclonal antisera (a kind gift from Diana Downs). The next day, 30 µl of a 50/50 slurry of Sepharose protein A and G beads in 250 µl IP buffer was added to the lysate to capture antibody-protein-DNA complex for one hour at 4 °C. Beads were then washed once with 1 ml of 250 mM LiCl wash buffer (100 mM Tris pH 8, 250 mM LiCl, 2% Triton X-100), twice with 1 ml 600 mM NaCl wash buffer (100 mM Tris pH 8, 600 mM NaCl, 2% SDS), twice with 1 ml 300 mM NaCl wash buffer (100 mM Tris pH 8, 300 mM NaCl, 2% SDS), and twice with 1 ml TE (10 mM Tris pH 8, 1 mM EDTA). Elution buffer (50 mM Tris pH 8, 10 mM EDTA, 1% SDS) was added after the final wash step, and beads were incubated at 65 °C for 30 minutes to remove the crosslinked protein-DNA complexes from the beads. After centrifugation (1000 x *g* for 2 min at 25˚C) to remove the beads, the samples were incubated overnight at 65 °C to reverse the protein-DNA formaldehyde crosslinks. DNA was purified using Qiagen's PCR Purification kit and eluted in a final volume of 65 µl with 10 mM Tris pH 8.

Array hybridization and data analysis

Both the immunoprecipitated (IP) DNA and input DNA (chromosomal DNA purified prior to IP) were amplified by ligation-mediated PCR (LM-PCR) as described previously (Mooney et al., 2009). IP samples were compared to control samples of input DNA by labeling with Cy5 and Cy3 dyes and hybridizing to a tiling microarray. The custom microarray (Nimblegen) contained 378,238 T_m-matched ~50mer oligonucleotides that tile the *E. coli* K-12 chromosome (Genbank accession NC_000913.2) on alternated strands with ~12-bp spacing designed by Y. Dufour using chipD (Dufour et al., 2010).

The Cy5 (for IP DNA) and Cy3 (for input DNA) signal intensities for all probes were

converted to $\log_2$(IP/input) values that represent the ChIP signals. Each ChIP signal was associated with a genome position of the midpoint of the corresponding probe. To smooth the association curve across the genome and eliminate the bias due to different hybridization efficiency of probes, the rolling average of ChIP signals within a 300bp window was calculated. We utilized the smoothed $\log_2$ (IP/input) curves of RNAP, σ^{70} or DksA for data analysis. ChIP signals across the genome were visually compared using the viewing software MochiView (http://johnsonlab.ucsf.edu/mochi.html). Gene annotation and promoter locations were identified based on the databases RegulonDB (http://regulondb.ccg.unam.mx/) and EcoCyc (http://ecocyc.org/).

Supplemental references

Blankschien, M.D., Lee, J.H., Grace, E.D., Lennon, C.W., Halliday, J.A., Ross, W., Gourse, R.L., and Herman, C. (2009). Super DksAs: substitutions in DksA enhancing its effects on transcription initiation. The EMBO journal *28*, 1720-1731.

Blattner, F.R., Plunkett, G., 3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., *et al.* (1997). The complete genome sequence of Escherichia coli K-12. Science (New York, N.Y *277*, 1453-1462.

Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L., Boyer, H.W., Crosa, J.H., and Falkow, S. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene *2*, 95-113.

Boubakri, H., de Septenville, A.L., Viguera, E., and Michel, B. The helicases DinG, Rep and UvrD cooperate to promote replication across transcription units in vivo. The EMBO journal *29*, 145-157.

Brooker, R.J., and Wilson, T.H. (1985). Isolation and nucleotide sequencing of lactose carrier mutants that transport maltose. Proceedings of the National Academy of Sciences of the United States of America *82*, 3959-3963.

Cardinale, C.J., Washburn, R.S., Tadigotla, V.R., Brown, L.M., Gottesman, M.E., and Nudler, E. (2008). Termination factor Rho and its cofactors NusA and NusG silence foreign DNA in E. coli. Science (New York, N.Y *320*, 935-938.

Feng, G.H., Lee, D.N., Wang, D., Chan, C.L., and Landick, R. (1994). GreA-induced transcript cleavage in transcription complexes containing Escherichia coli RNA polymerase is controlled by multiple factors, including nascent transcript location and structure. The Journal of biological chemistry *269*, 22282-22294.

Posfai, G., Plunkett, G., 3rd, Feher, T., Frisch, D., Keil, G.M., Umenhoffer, K., Kolisnychenko, V., Stahl, B., Sharma, S.S., de Arruda, M., *et al.* (2006). Emergent properties of reduced-genome Escherichia coli. Science (New York, N.Y *312*, 1044-1046.

St Onge, R.P., Mani, R., Oh, J., Proctor, M., Fung, E., Davis, R.W., Nislow, C., Roth, F.P., and Giaever, G. (2007). Systematic pathway analysis using high-resolution fitness profiling of combinatorial gene deletions. Nature genetics *39*, 199-206.

Tehranchi, A.K., Blankschien, M.D., Zhang, Y., Halliday, J.A., Srivatsan, A., Peng, J., Herman, C., and Wang, J.D. The transcription factor DksA prevents conflicts between DNA replication and transcription machinery. Cell *141*, 595-605.