Supplemental information:

Figure S1:

The results from Figure 5 show that including two EcoRV roadblocks separated by 21 bp (between the Dam sites) significantly increases Dam processivity. To further challenge our interpretation that this increase is due to roadblock mediated DNA bending, we employ a system involving a roadblock that minimally bends the DNA, yet is as similar as possible to the system from Figure 5 (Figure S1). It has been previously shown that EcoRV is able to bind specifically to 5'-GAATTC-3' sites, which is coincidentally the site for EcoRI (1). Importantly, this binding has been demonstrated to involve drastically less DNA bending than what is seen for EcoRV on its cognate site (1) (Figure S1C). We use this roadblock system to test for Dam processivity (Fig S1B). When the EcoRI sites are separated by 21bp, Dam's processivity is slightly less than with no roadblock (Figure S1B), and certainly much less that what was seen with 2rV21+ (Figure 5). This further suggests that the increase in processivity seen in figure 5C is due to the bending of the DNA by the roadblock (Figure S1D).

A) DNA constructs; red squares are EcoRI sites. B) Processivity values for Dam in the absence and presence of EcoRV bound to EcoRI sites. Mean and SD of 3 independent experiments are shown. C) EcoRV bends DNA on its cognate site, but does not bend DNA on an EcoRI site. D) Processivity enhancements are likely due to the roadblock induced bending of the DNA.

Figure S1:

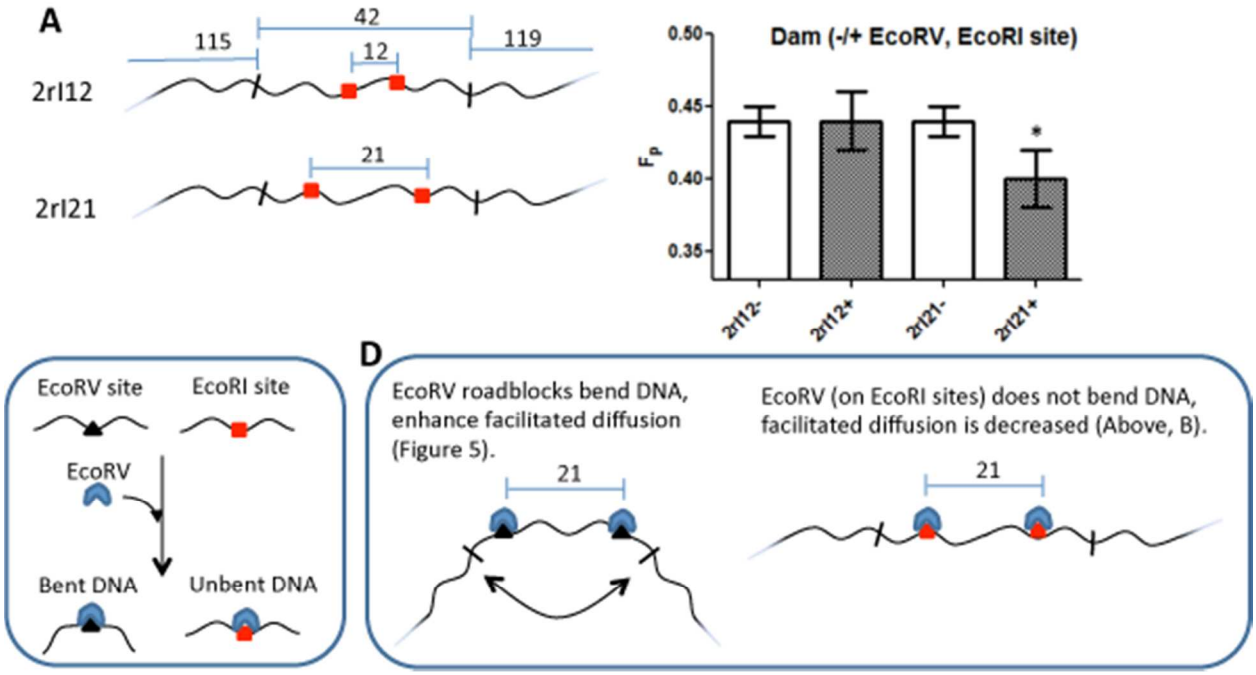
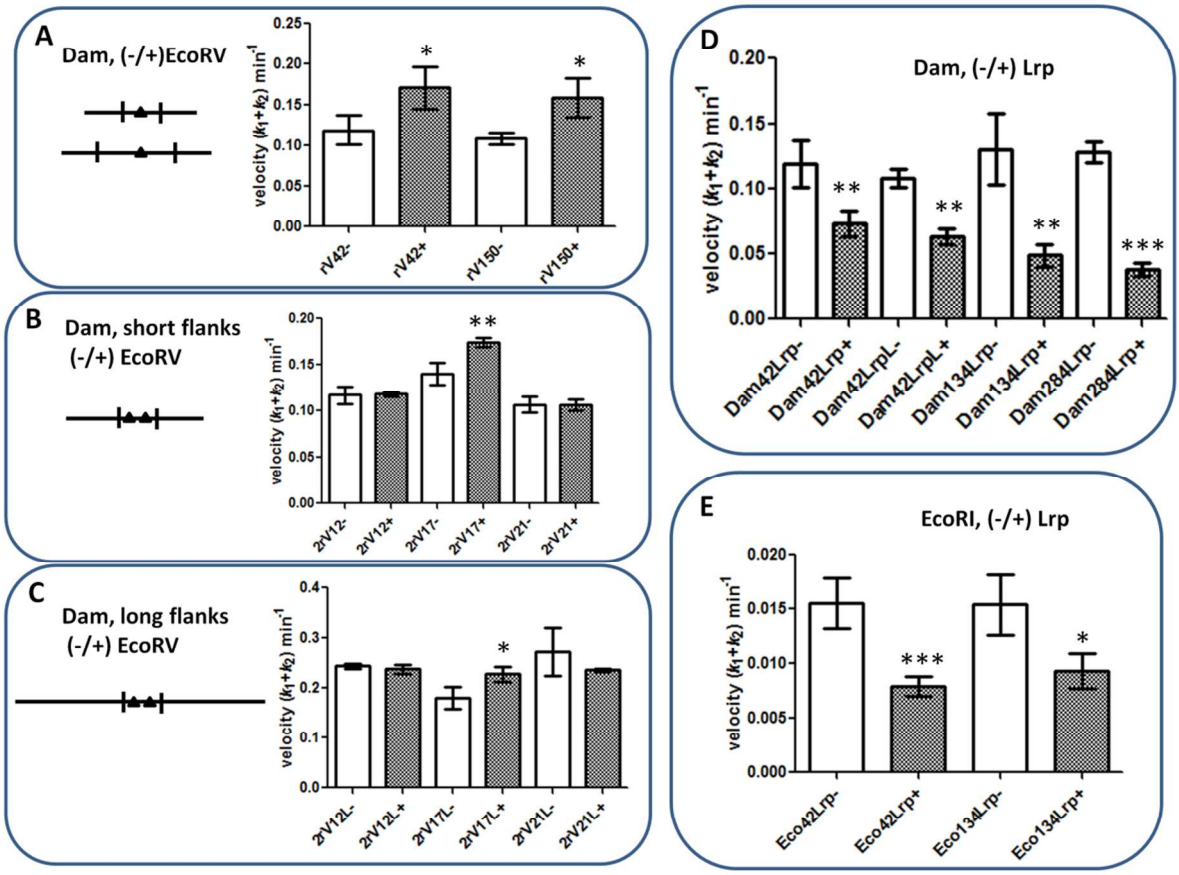

A
115
42
119
12
2rI12
21
2rI21
Dam (-/+ EcoRV, EcoRI site)
0.50
0.45
0.40
0.35
Fp
*
2rI12-
2rI12+
2rI21-
2rI21+
EcoRV site
EcoRI site
EcoRV
Bent DNA
Unbent DNA
D
EcoRV roadblocks bend DNA, enhance facilitated diffusion (Figure 5).
21
EcoRV (on EcoRI sites) does not bend DNA, facilitated diffusion is decreased (Above, B).
21

Figure S2: Reaction velocities ($k_1 + k_2$) from Figure 8. **A**) Single site EcoRV roadblock with Dam enzyme (Fig.4). **B**) Two site EcoRV roadblocks with Dam enzyme (Fig. 5C). **C**) Two site EcoRV roadblocks (with longer flanking DNA amounts) with Dam enzyme (Fig. 5D). **D**) Lrp roadblock with Dam enzyme (Fig. 6,7). **E**) Lrp roadblock with EcoRI enzyme (Fig. 6,7). Mean and S.D. of ≥4 independent experiments are shown. P values (-/+ roadblock) are displayed or are n.s.

Figure S2:



DNA substrates:

All restriction endonucleases for cloning were purchased from NEB. All synthetic DNA substrates and primers were purchased from IDT and were re-suspended in TE buffer (10mM Tris, pH 7.5, 1mM EDTA). When necessary, DNA was annealed with its reverse complement in a 1:1 mixture for 5 minutes at 95 degrees, and allowed to slow cool to room temperature (~5 hours) to generate

oligonucleotides. Annealing was verified by PAGE. The production of substrates used the following general strategy: A synthetic oligonucleotide with two GATC sites (below), and two restriction sites between the GATC sites was cloned into plasmid pBR322 (NEB), making a new plasmid, pBR322GATC (Figure S3). The same strategy was used to generate the EcoRI ENase substrates making another plasmid, pBR322GAATTC (Figure S4). Spacers (PCR derived and synthetic oligonucleotides) were cloned into pBR322GATC and pBR322GAATTC, generating several different plasmids with different distances between the GATC or GAATTC sites and different amounts and locations of EcoRV sites for roadblocks. These plasmids were PCR amplified with different primers to adjust the spacings from the GATC or GAATTC sites to the ends of the DNA. PCR amplicons were purified using the Agilent PCR clean-up kit.

The following oligonucleotide (described above) was cloned into the plasmid pBR322 at the EcoRI and HindIII sites, 5'- aattcggtgatctcgtcga*cccggg*agctggtagtatgc*ccatgg*ttcgatcggatgcca-3'; 5'-agcttggcatccgatcgaa*ccatggg*catactaccagct*cccggg*tcgacgagatcaccg-3', making, pBR322GATC (Figure S3). The following oligonucleotide was cloned into the plasmid pBR322 also at the EcoRI and HindIII sites, 5'-agcttggcattgaattctaccatgggcatactaccagctcccgggtcggctcgatcaccg-3' ; 5'-aattcggtgatcgagccgacccgggagctggtagtatgcccatggtagaattcaatgcca-3', making pBR322GAATTC (Figure S4). The cloned, synthetic oligonucleotides (above) used to make pBR322GATC and pBR322GAATTC had additional cloning sites within each: XmaI and NcoI (italicized) (Figure S3,S4). These sites were used to insert PCR purified spacers or oligonucleotides (listed below) between the two (Dam or EcoRI) sites (underlined).

Annealed oligonucleotide spacers cloned into pBR322GATC at XmaI and NcoI sites (Figure S3,S4):

rV42 (Figures: 2B,3,4B-D,6A-B,7C,8)

Top: 5'-ccgggtacaacttggatatcggttacacgcc-3'

Bottom: 5'-catgggcgtgtaaccgatatccaagttgtac-3'

2rV12 (Figures: 5A,5C-E,8)

Top: 5’- ccgggtacgatatcaccatagatatctggac

Bottom: 5’catggtccagatatctatggtgatatcgtac

2rV17 (Figures: 2C,5A,5C-E,8)

Top: 5’-ccgggtgatatctactcaggttagatatcac 3’

Bottom: 5’- catggtgatatctaacctgagtagatatcac

2rV21 (Figures: 5A-E,8)

Top: 5’-ccgggatatctcgactcagagtctagatatc-3’

Bottom: 5’-catggatatctagactctgagtcgagatatc-3’

PCR amplicon spacers cloned into pBR322GATC at XmaI and NcoI sites. Upon PCR amplification, the following spacers were digested with XmaI and NcoI to generate overhangs. The spacers were generated by PCR from the plasmid pBR322 (not shown).

rV150mer (Figures: 4D,8)

Forward: 5’-attcccgggtcatcctcggcaccgtcacc-3’

Reverse: 5’-taatccatggtccgagaacgggtgcgcata-3’

Dam134Lrp and Eco134Lrp (cloned into pBR322GAATTC): (Figures: 7C-D,8)

Forward: 5’- att*cccgggg*ggctaccctgtggaacacct - 3:

Reverse: 5’- taatccatggtactggaacgttgtgagggt-3’

Dam284Lrp: (Figures: 7C,8)

Forward: 5’- att*cccgggg*gctaccctgtggaacacct - 3:

Reverse: 5’- taatccatggtgataaagcgggccatgtta-3’

Note: Eco42Lrp: (Figures: 7C,8) used pBR322GAATTC without the insert of a spacer.

Amplicons with 115/119 base pairs flanking GATC sites were amplified from each plasmid using primers: (forward) 5’- gggttccgcgcacatttccc-3’ and (reverse) 5’-(Fl) ccagggtgacggtgccgagg-3’(From Figures (2,4,5C,6,7C,8).

Amplicons with 300 base pairs flanking GATC sites were amplified from each plasmid using primers: (forward) 5’- gcatcttttactttcaccagcg-3’, and (reverse) 5’- (Fl)ggctccaagtagcgaagcgagc-3’. (From Figures (3,5D,7C,8)

rV chase DNA (Figure 2):

Forward:5’-Ccgggtacaacttggatatcggttacacgcccatg

Reverse:5’-catgggcgtgtaaccgatatccaagttgtacccgg

Figure S3: Schematic of the construction of DNA substrates for Dam processivity experiments. Substrate 2rVxx refers to substrates 2rV12, 2rV17 and 2rV21.

Figure S3:

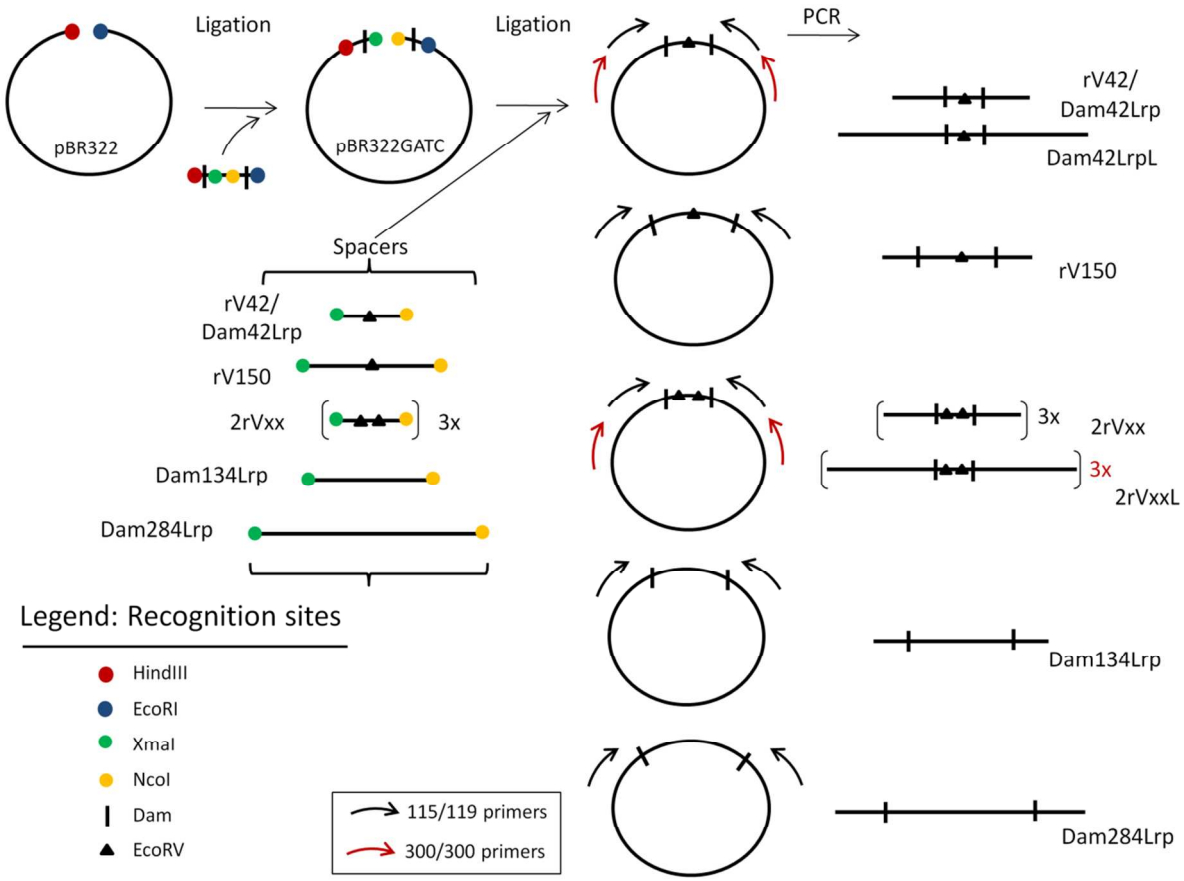
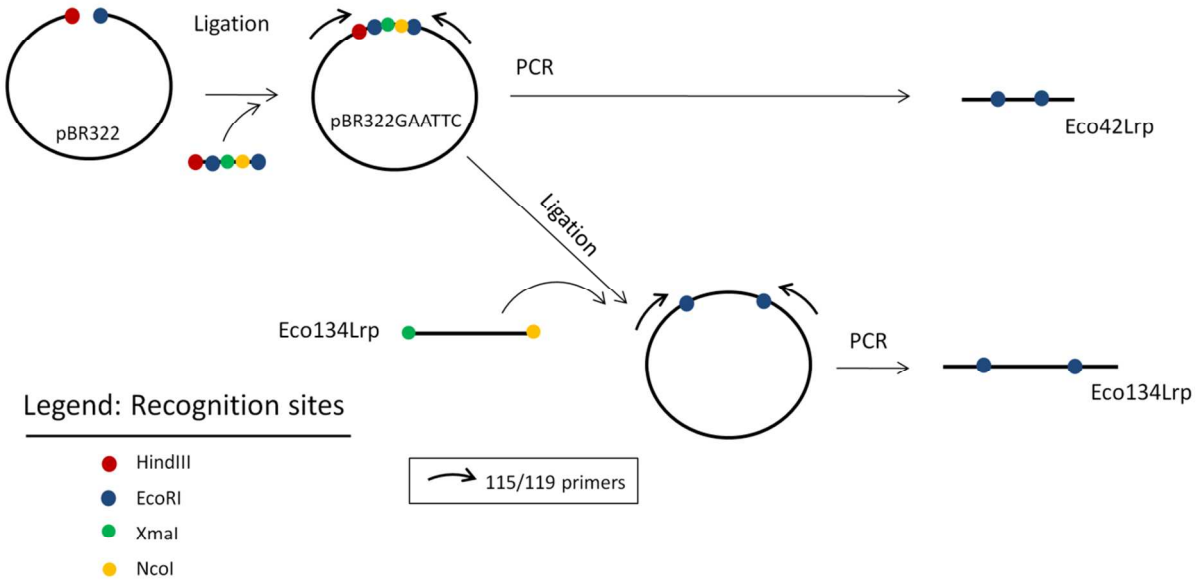


Figure S4: Schematic of the construction of DNA substrates for EcoRI processivity experiments.

Figure S4:



Supplementary Materials and Methods.:

DNA substrates:

Substrates 2rI12 and 2rI21 were generated by annealing and then cloning the following oligonucleotides into pBR322 at the EcoRI and HindIII sites.

2rI12:

Forward: 5'- aattgatctcgtcgacccgggtacgaattcaccatagaattctggaccatggttcgatca -3'

Reverse: 5'- agcttgatcgaaccatggtccagaattctatggtgaattcgtacccgggtcgacgagatc -3'

2rI21:

Forward: 5'- aattgatctcgtcgacccgggaattctcgactcagagtctagaattccatggttcgatca -3'

Reverse: 5'- agcttgatcgaaccatggaattctagactctgagtcgagaattcccgggtcgacgagatc-3'

The same primers from above were used to generate the amplicons with 115/119bp of flanking DNA. Note that the EcoRI sites used for the cloning are altered upon ligation.

Processivity assay: The processivity assay was performed exactly as described in the primary manuscript, except 1μM of EcoRV was used as the roadblock.

Supplemental References:

1. Hiller, D. A., Rodriguez, A. M., and Perona, J. J. (2005) Non-cognate enzyme–DNA complex: structural and kinetic analysis of EcoRV endonuclease bound to the EcoRI recognition site GAATTC. *J. Mol. Biol.* 354, 121-136.