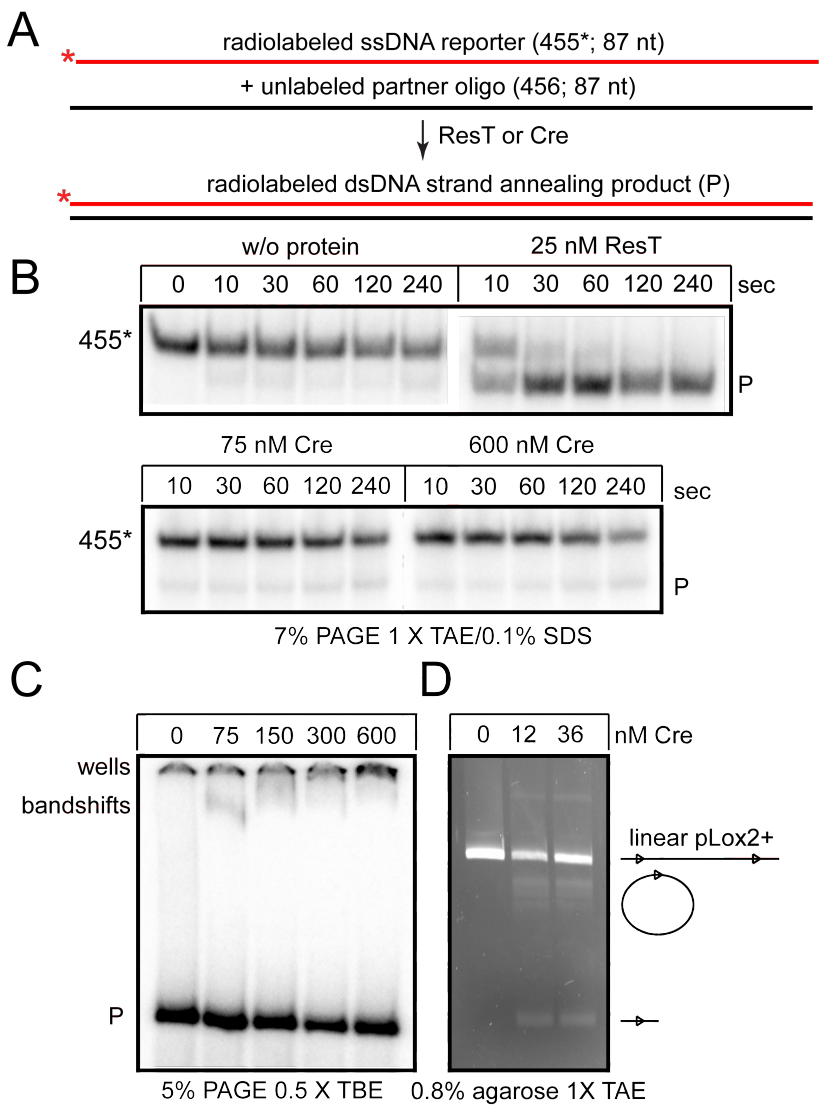

Figure S1. Specificity of SSA: Cre, a prototypical tyrosine recombinase does not promote SSA.
A) Schematic of the Single-Strand Annealing (SSA) assay for a model substrate that anneals into an 83 bp duplex with 26% GC-content (455* labeled strand and 456 unlabeled strands used in Figures 1 & 8). SSA assays were run under protein-free conditions, 25 nM ResT and with concentrations of Cre recombinase ranging between 37.5 and 600 nM.
B) 7% native TAE-SDS PAGE analysis of timecourses of spontaneous, ResT and representative Cre-containing SSA assays detailed in A). 455* marks the migration position in the gel of the radiolabeled 455* reporter oligonucleotide; P marks the migration position of the duplex product of annealing of 455* with the unlabeled 456 strand. Electrophoresis was for 2:30 h at 13.3 V/cm on a 20 X 20 cm gel.
C) 5% native 0.5 X TBE PAGE electrophoretic mobility shift assays (EMSA) of various concentrations of Cre using heat renatured 455*/456 duplex DNA to test the ability of Cre to bind 455*/456 duplex DNA. Briefly, substrate DNA (15 nM) was incubated with the indicated concentrations of Cre at 0^{o}C for 20 min in the buffer used for SSA assays (see Material and Methods) supplemented with 100 μg/ml BSA and 1.5 μg/ml salmon sperm DNA. P marks the migration position of the heat renatured 455*/456 substrate DNA; bandshifts marks the migration position on the gel of Cre-DNA complexes. Electrophoresis was for 1:30 h at 13.3 V/cm on a 16 X 16 cm gel run at 8^{o}C.
D) 0.8% agarose 1X TAE gel analysis of a plasmid recombination assay performed with the indicated concentrations of Cre and linearized pLox2+ plasmid (see Material and Methods section). To the right of the gel are schematics of the linear pLox2+ substrate plasmid and the recombinant products.

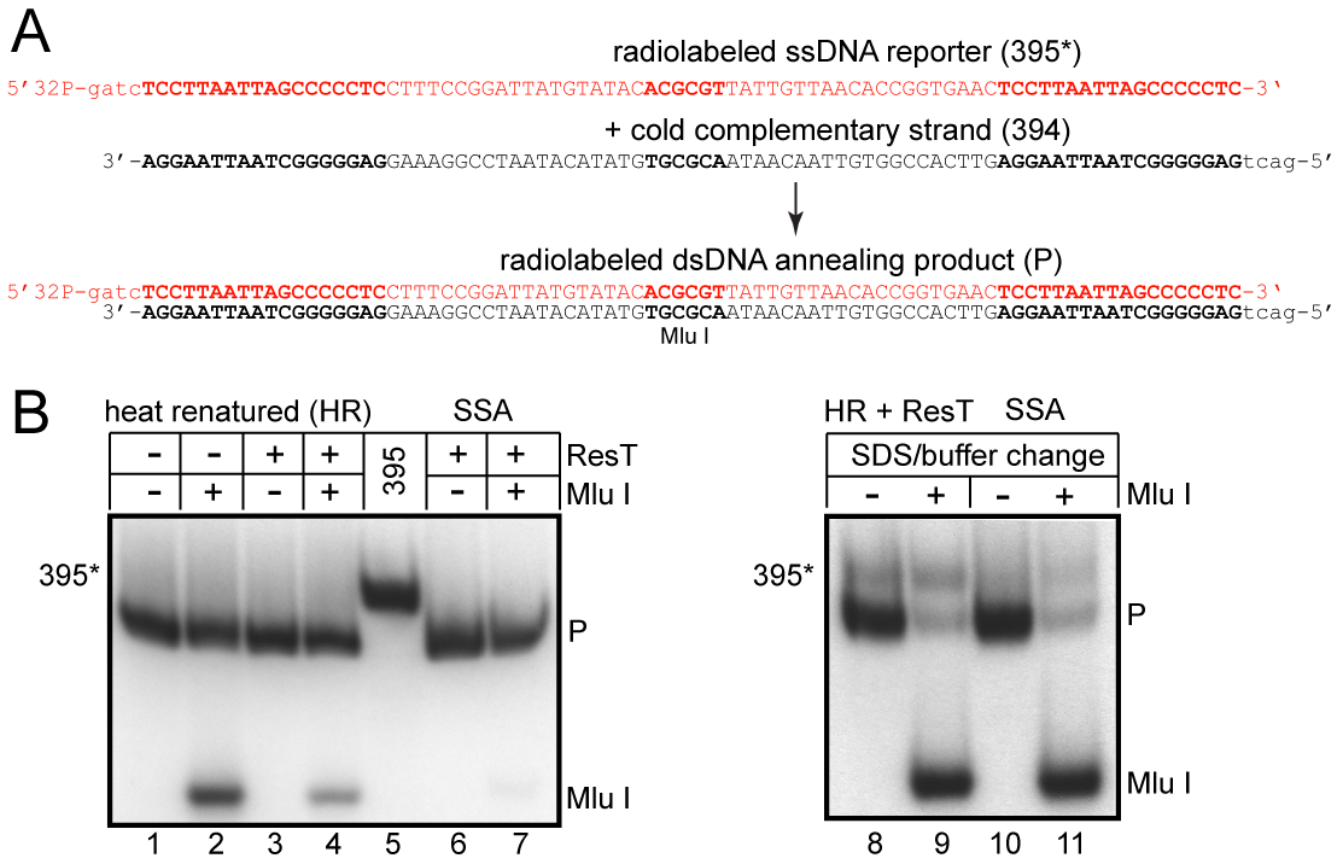

Figure S2. ResT remains associated with the annealed product of the *vls* model substrate.
A) Schematic of the SSA assay for a model substrate that assembles into an 81 bp duplex bounded by 17 bp direct repeats derived from the boundaries of the variable region of the *vls* locus that undergoes antigenic variation by gene conversion. The 5'-^{32}P endlabeled reporter strand (395*) is shown in red script while the complementary, unlabeled, strand is shown in black (394). Also, represented is the 81 bp duplex product of Single-Strand Annealing (SSA).
The sequence is designed to reconstitute an Mlu I restriction site in the annealed product (highlighted in bold letters) used to test if ResT remains associated with the product of SSA.
B) 7% native TAE-SDS PAGE analysis of Mlu I restriction digestion (partial) of duplex product produced by heat denaturation/renaturation (HR) vs. ResT-promoted SSA of the 395* and 394 strands detailed in A). 5 units of Mlu I were incubated for 60 min at 30^{o}C with HR duplex after pre-incubation of the duplex at 30^{o}C without ResT for 10 minutes (lane 2), after pre-incubation of the HR duplex at 30^{o}C for 10 minutes with 150 nM concentration of ResT (lane 4) or with the product of ResT-promoted SSA, formed with 150 nM ResT in a 10 min 30^{o}C incubation (lane 7). HR duplex incubated with 150 nM ResT at 30^{o}C for 10 minutes and the duplex product of ResT-promoted SSA were deproteinated by addition of SDS to 0.1%, applied to a G-25 sephadex spin column to change the buffer and then treated with 5 units of Mlu I for 60 min at 30^{o}C prior to electrophoresis (lanes 9 & 11). Gel labels: 395* marks the migration position of the labeled 395 reporter, P marks the migration position of 395*/394 duplex, Mlu I marks the migration position of the radiolabeled half of the products of Mlu I digestion of P.

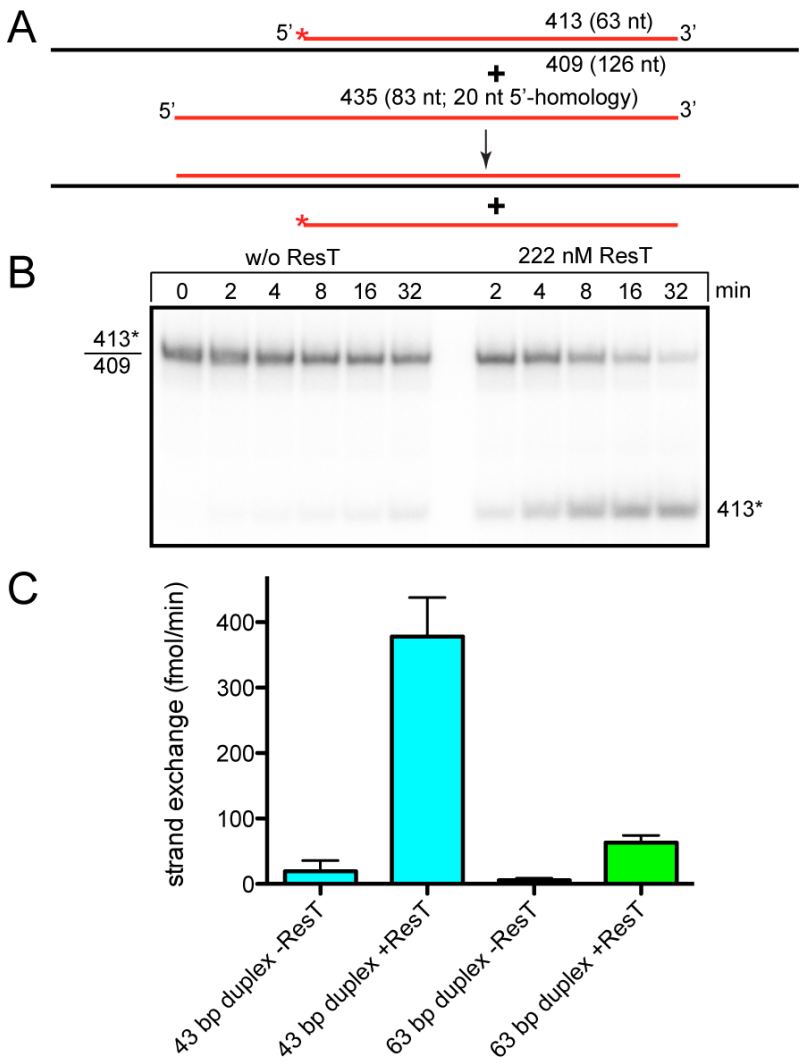

Figure S3. ResT promotes DNA strand exchange with short partial duplex substrates.
A) Schematic representation of the DNA strand exchange reaction between a 63 bp partial duplex target DNA (413*/409) and an 83 nt ssDNA donor (435) with 20 nt of 5'-flank homology with the bottom strand of the partial duplex target. The top strand sequence is represented with red script and the bottom strand with black; the red asterisk indicates a 5'-^{32}P endlabel. DNA strand exchange results in displacement of the 413* strand.
B) 7% native TAE-SDS PAGE analysis of representative timecourses of spontaneous and ResT-promoted DNA strand exchange reactions detailed in A). Gel labels: 413*/409 marks the migration position of the 63 bp partial duplex target labeled on the 413* strand; 413* marks the migration position of the displaced 413* strand.
C) Comparison of the initial rates of spontaneous and ResT-promoted DNA strand exchange using the substrates detailed in A) and an example of the reaction with a 43 bp partial duplex target and a 63 nt ssDNA donor with 20 nt of 5'-flank homology (see Figure S4A for details). The mean and standard deviation of 3 independent timecourses for each donor DNA and reaction condition are shown.

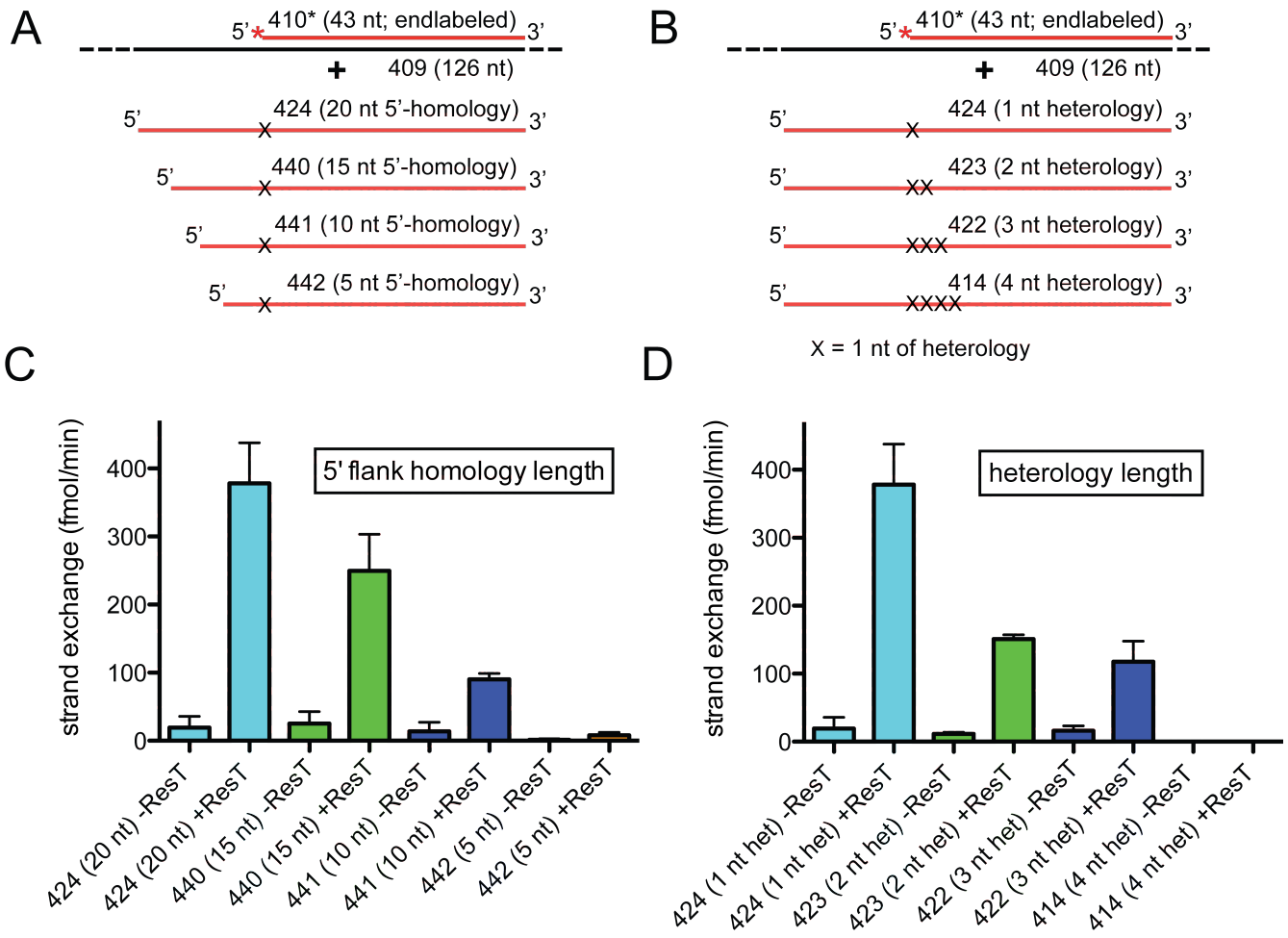

Figure S4. Flank homology dependence and sensitivity to heterology of DNA strand exchange (5'-flank homology).

A) Schematic representation of the substrate DNAs used in DNA strand exchange reactions between a 43 bp partial duplex target DNA (410*/409) and a series of single-stranded DNA donors with decreasing lengths of 5'-flank homology with the bottom strand of the partial duplex target. The top strand is coloured red and the bottom strand black; the red asterisk indicates a 5'-^{32}P endlabel.

B) Schematic representation of the substrate DNAs used in DNA strand exchange reactions between a 43 bp partial duplex target DNA (410*/409) and a series of single-stranded DNA donors with the indicated length of sequence heterology introduced at what would be the initial branch point with the 5'-ss/dsDNA junction of the partial duplex target.

C) Comparison of the initial rates of spontaneous and ResT-promoted DNA strand exchange using the 410*/409 partial duplex substrate and a series of single-stranded DNA donors with the indicated length of 5'-flank homology (detailed in A)). All donors incorporate the single base heterology at the initial branch point present in 424 to reduce background branch migration, which was found to be higher than in reactions utilizing donor DNAs with 3'- flank homology. Shown is the mean and standard deviation of 3 independent timecourses for each donor DNA and reaction condition.

D) Comparison of the initial rates of spontaneous and ResT-promoted DNA strand exchange using the 410*/409 partial duplex substrate and a series of single-stranded DNA donors with the indicated length of sequence heterology introduced at what would be the initial branch point with the 5'-ss/dsDNA junction of the partial duplex target (detailed in B)). The mean and standard deviation of 3 independent timecourses for each donor DNA and reaction condition are shown.

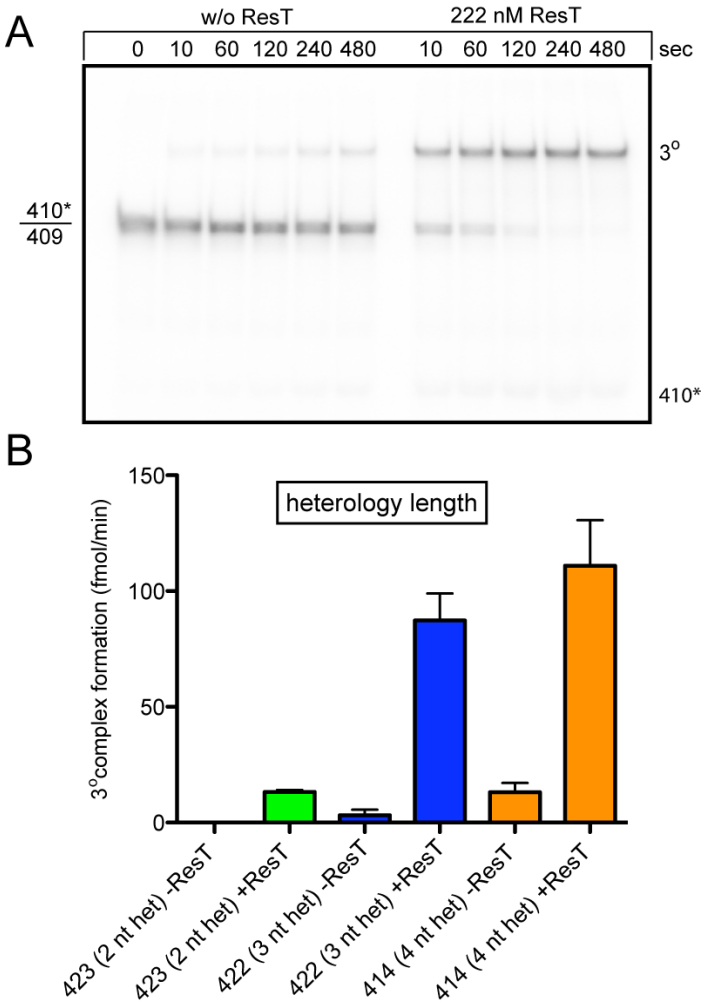

Figure S5. Sequence heterology in the exchanging segment of the single-stranded donor DNA promotes the formation of ternary complexes (5'-flank homology).
A) 7% native TAE-SDS PAGE analysis of representative timecourses of spontaneous and ResT-promoted DNA strand exchange reactions between a 43 bp partial duplex target DNA (410*/409) and a 63 nt ssDNA donor (414) with 20 nt of 5'-flank homology with the bottom strand of the partial duplex target and a contiguous 4 nt block of heterology at the branch point with the 5'-ss/dsDNA junction of the partial duplex target. Gel labels: 410*/409 marks the migration position of the 43 bp partial duplex target labeled on the 410* strand; 410* marks the migration position of displaced 410* strand; 3^{o} marks the migration position of the ternary complex.
B) Comparison of the initial rates of spontaneous and ResT-promoted ternary complex formation using the 410*/409 partial duplex substrate detailed in A) and a series of single-stranded DNA donors with the indicated length of sequence heterology introduced at what would be the branch point with the 5'-ss/dsDNA junction of the partial duplex target. The mean and standard deviation of 3 independent experiments for each donor DNA and reaction condition are shown.

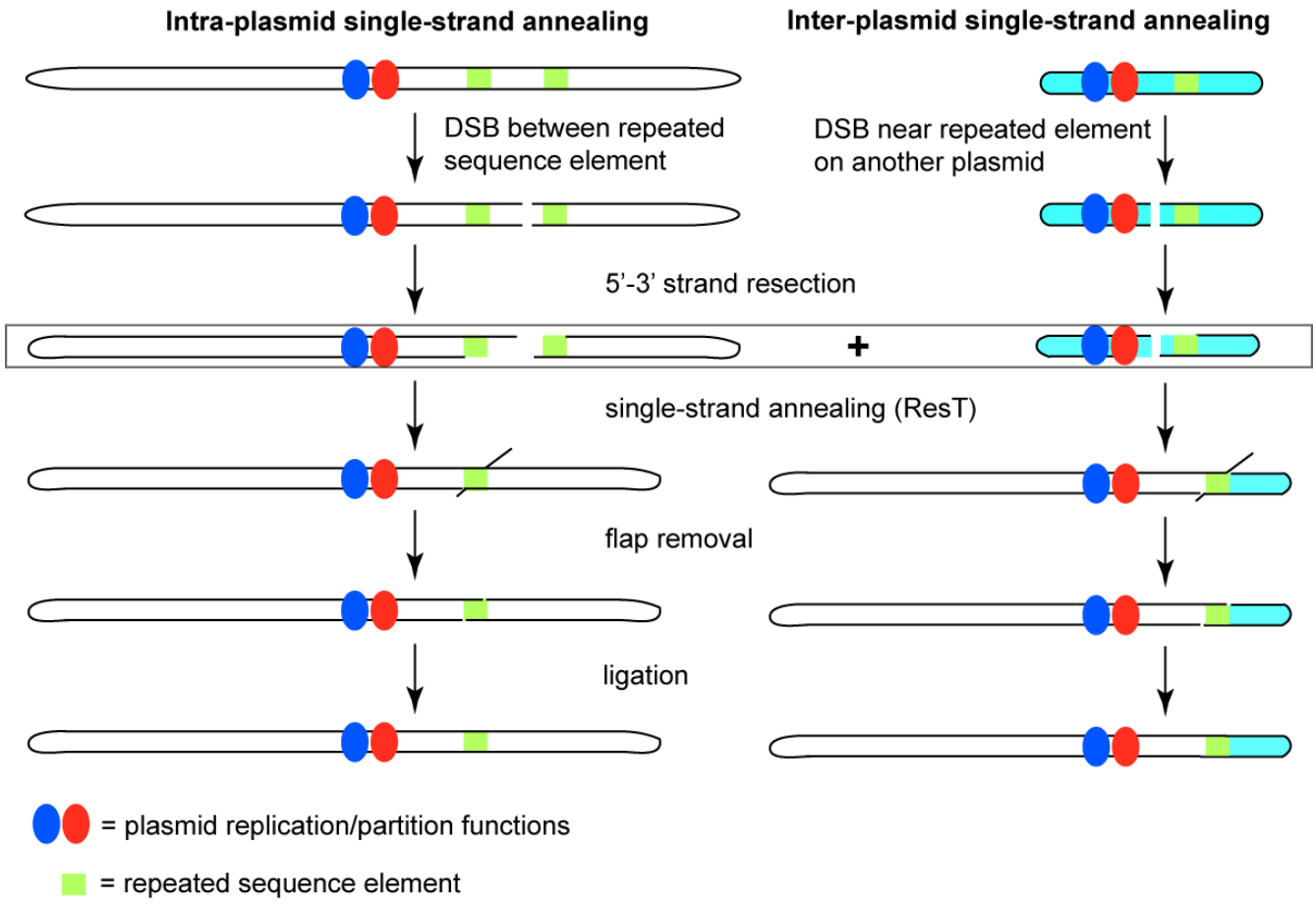

Figure S6. Model of ResT-promoted linear plasmid rearrangement by Single-Strand Annealing. Schematic representation of SSA repair of DNA double-strand breaks (DSBs). In the intra-plasmid pathway a DSB between a directly repeated sequence element is repaired by the action of a 5'-3' exonuclease to reveal the sequences that can be annealed (shaded in green). Such exonucleases are often partnered with bacterial Single-Strand Annealing Proteins (41). Flap removal and ligation rejoins the strands resulting in a variant of the plasmid harbouring a deletion of the sequence found between the repeated element and deletion of one of the repeated elements. The inter-plasmid pathway depicted on the right involves non-allelic DSBs (on different plasmids as depicted in the figure) with SSA repair via a fortuitous sequence match able to be annealed by ResT. Provided a viable molecule results from this repair a new mosaic linear plasmid results.

Supplementary Material and Methods

Oligonucleotides used to make the substrates used in this study.

Figures 1, 8A and Figure S1 used OGCB455 (5'-gatcATATCCTTTCTTTAAACTTCTATCATT GATTCTTACTAGTCTTTACCTTACTATACTTCTATCAGTTTATCGATTCTTCTTTA-3') and 456 (5'-gatcTAAAGAAGAATCGATAAACTGATAGAAGTATAGTAAGGTAAAGACTAGTAAGA ATCAATATAGAAGTTTAAAGAAAGGATAT-3'). The lower case gatc's indicate 5'-overhangs in the annealed duplex product. These oligos were previously reported in a study of strand exchange by Rad51 (53). Figure 2 and Figure S2 used OGCB394 (5'-gatcGAGGGGGCTAATTAAGGAGTTCACCGGTGTTAACAA TAACGCGTGTATACATAATCC GGAAAGGAGGGGGCTAATTAAGGA-3') and 395 (5' gatcTCCTTAATTAGCCCCCTCCTTTC CGGATTATGTATACACGCGTTATTGTTAAC ACCGGTGAACTCCTTAATTAGCCCCCTC-3')

Figure 3 used OGCB121 (5'-GATCtcctctaaccattgcACTTTATACTAAATAAATATTATAT ATAT AATTTTTAATTAGTATAGAAT-3') for the reporter, 120 (5'-GATCATTCTATACTAATTAAAAA TTATAT ATATAATATTTATTTAGTATAAAGTgcaatggttagagga-3') for oligo 1, OGCB 302 (5'-GATCATTCTATACTAATTAAAAATTAATA TATTAATATTTATTTAGTATAAAGTgcaatggttaga gga-3') for oligo 2, OGCB392 (5'-GATCATTCTATACTAATTAAAAAAATATA TATATTTATTT ATTTAGTATAAAGTgcaatggttagagga-3') for oligo 3, OGCB393 (5'-GATCATTCTATACTAAT TAATTTAATATA TATATTATATTATTTAGTATAAAGTgcaatggttagagga-3') for oligo 4, OGCB403 (5'-GATCATTCTATACTAATATTTTTAATATA TATATTATAAATTTTAGTATAAA GTgcaatggttagagga-3') for oligo 5, OGCB 404 (5'-GATCATTCTATACTTTAATTTTTAATATA TATATTATAAATAAAAGTATAAAGTgcaatggttagagga-3') for oligo 6, OGCB 405 (5'-GATCAT TCTATTGATTAATTTTTAATATA TATATTATAAATAAATCAATAAAGTgcaatggttagagga-3'), for oligo 7, OGCB406 (5'-GATCATTCATATGATTAATTTTTAATATA TATATTATAAATAAA TCATATAAGTgcaatggttagagga-3') for oligo 8 and OGCB478 (5'-GATCTAAGATATGATTAA TTTTTAATATA TATATTATAAATTTTAGTATAAAGTgcaatggttagagga-3') for the bubble to flap transition marker.

Oligos used to for the strand exchange assays presented in Figures 4-7 and 8B and Figures S3-5 are OGCB409 (5'-TCTGCGCCTCGTTCCGGCTAAGTAACATG GAGCAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAATCTCCGTTGTACTTTGTTTCGCGCTT GGTATAATCGCTGGGGGTCAAAGAT-3'), OGCB410 (5'-CCTGATAAA TTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB411 (5'-CCTGATAAATTGT GTCGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGCGCAGA-3'), OGCB412 (5'-CCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTCGTGTAGCCG GAACGAGGCGCAGA-3'), OGCB413 (5'-AACGGAGATTTGTATCATCGCCTGATAAATTGT GTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB414 (5'-AACGGAGATTTGTATCA

TCGTTCAATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB422 (5'-AACGGAGATTTGTATCATCGTTCGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB423 (5'-AACGGAGATTTGTATCATCGTTTGATAAATTGTGTCGAAATCCG CGACCTGCTCCATGTTACT-3'), OGCB424 (5'-AACGGAGATTTGTATCATCGTCTGATAAA TTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB426 (N_{63}), OGCB428 (5'-CC TGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACGTAGCCGGAACGAGGCGCAGA-3'), OGCB435 (5'-CCAAGCGCGAAACAAAGTACAACGGAGATTTGTATCATCGCCTG ATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB440 (5'-AGATTTGT ATCATCGTCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB441 (5'-TGTATCATCGTCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB442 (5'-CATCGTCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACT-3'), OGCB467 (5'-CCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTATGTAGCCGG AACGAGGCGCAGA-3'), OGCB468 (5'-CCTGATAAATTGTGTCGAAATCCGCGACCTGCT CCATGTTGTGTAGCCGGAACGAGGCGCAGA-3'), OGCB469 (5'-CCTGATAAATTGTGTC GAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGC-3'), OGCB470 (5'-CCTG ATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAAC-3'), OGCB471 (5'-CCTGATAAATTGTGTCGAAATCCGCGACCTGCTCCATGTTACTTAGCC-3') based on the substrates used to study strand exchange by the λ Beta protein (39).

Cre recombinase activity assay

Cre recombinase was obtained from New England Biolabs in concentrated form (8.7 μM; cat # M0298M). An activity assay with the supplied pLox2+ plasmid confirmed activity of the supplied Cre. The assay was with 250 ng pLox2+ in 50 μL of buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 33 mM NaCl and the indicated concentrations of Cre that represent 1 and 3 units of specific activity, respectively (optimal activity for these conditions should be with the 1 unit reaction). The reactions were incubated at 37^{o}C for 30 min and heat inactivated at 70^{o}C for 10 min prior to gel loading and electrophoresis.

Mlu I protection assays

Heat renatured 395*/394 duplex DNA was assembled by mixing 5'-^{32}P endlabeled oligonucleotide 395 with unlabeled 394 to a final concentration of 300 nM in 25 mM HEPES (pH = 7.6), 0.1 mM EDTA (pH = 8.0), 50 mM NaCl, heating in a boiling water bath followed by slow cooling to room temperature. For the Mlu I protection assays SSA duplex product was assembled with 14 nM 5'-^{32}P endlabeled oligonucleotide 395, 14 nM unlabeled 394 and 148 nM ResT incubated in 25 mM HEPES (pH = 7.6), 0.1 mM EDTA (pH = 8.0), 100 mM NaCl at 30^{o}C for 10 min. 14 nM HR duplex was incubated with or without 148 nM ResT using the same conditions. $MgCl_2$ was then added to the reaction tubes to a final concentration of 10 mM followed by addition of 5 units of Mlu I and incubation at 30^{o}C for 60. For conditions without Mlu I treatment the reactions were handled

identically, excepting the addition of Mlu I. For the versions of the Mlu I challenge with prior deproteinization SDS was added to a concentration of 0.1% and the buffer changed to 25 mM HEPES (pH = 7.6), 10 mM $MgCl_2$, 100 mM NaCl by application to a pre-equilibrated GE G-25 sephadex spin column. The products of the Mlu I protection assays were separated by addition of SDS-load dye to a 1X concentration (10 X contains 200 mM EDTA, 32% glycerol, 1% SDS, 0.024% bromophenol blue) and application of the reactions to a 7% polyacrylamide, 1 X TAE/0.1% SDS gel run for 2 h at 13.3 V/cm.

Supplementary References

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41. Iyer, L.M., Koonin, E.V. and Aravind, L. (2002) Classification and evolutionary history of the single-strand annealing proteins, RecT, Redbeta, ERF and RAD52. *BMC Genomics*, **3**, 8.

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