Cooperation of RAD51 and RAD54 in regression of a model replication fork

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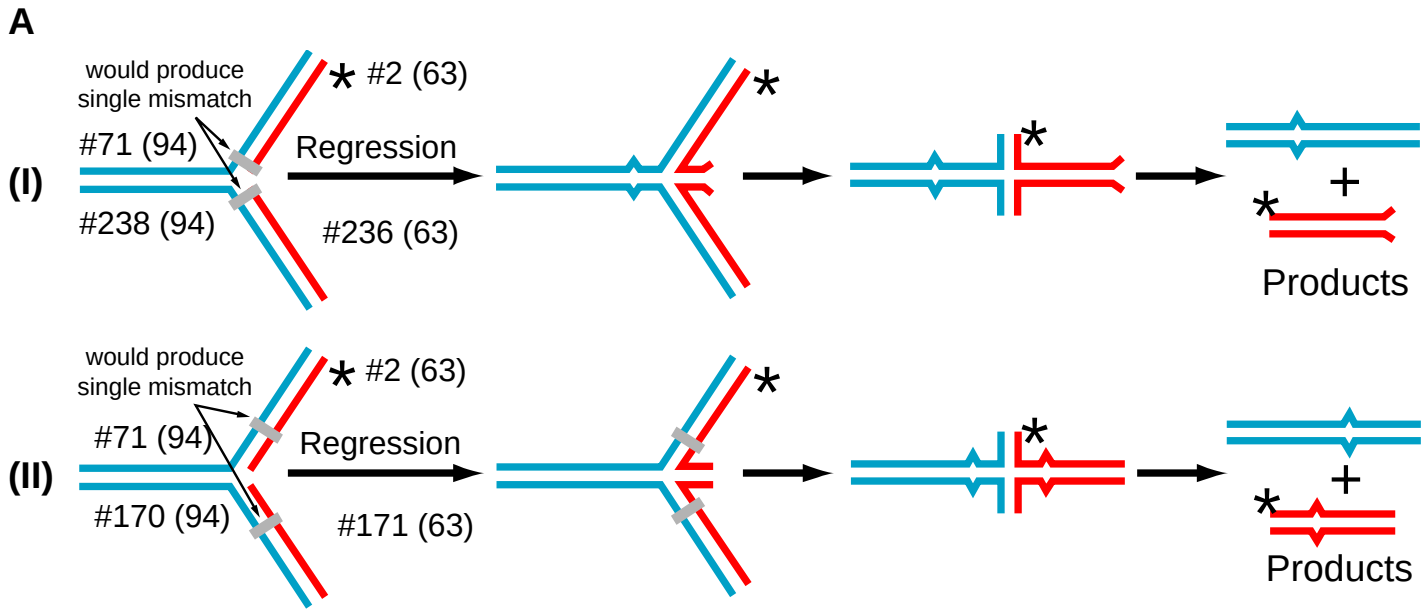
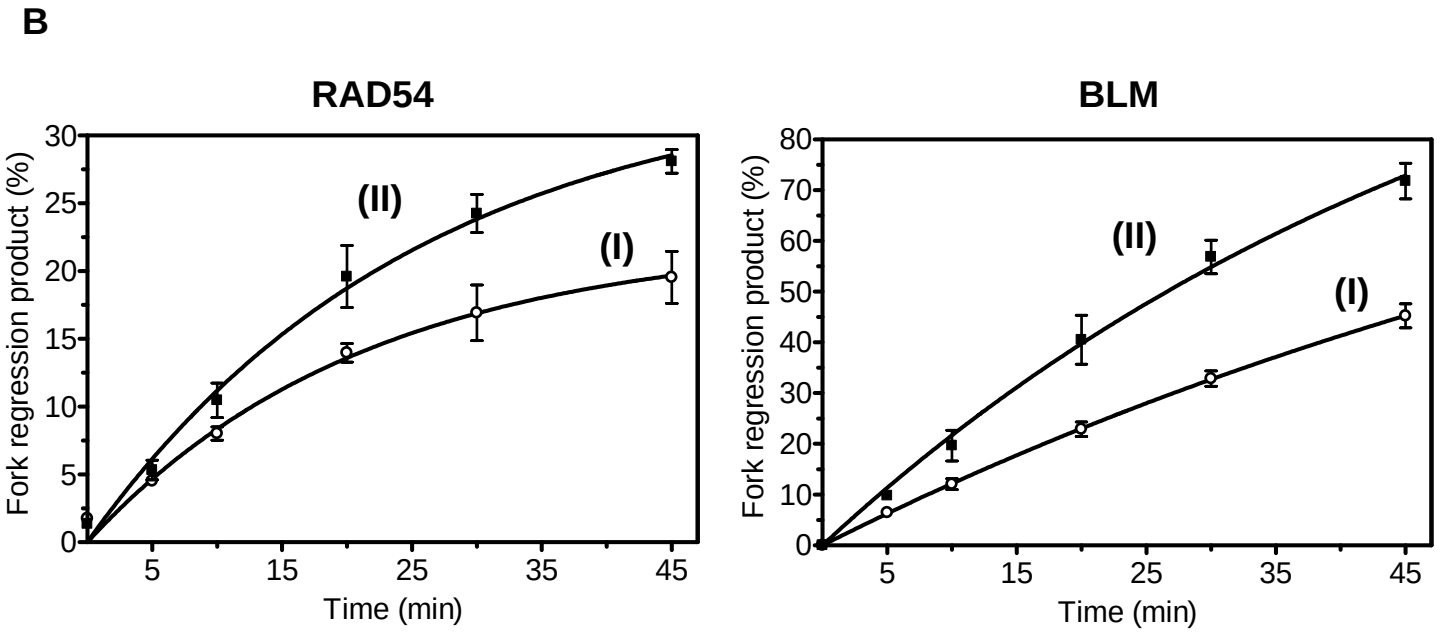
This Supplementary Material contains:

A. Supplementary Figure 1-2

B. Supplementary Table 1-3.

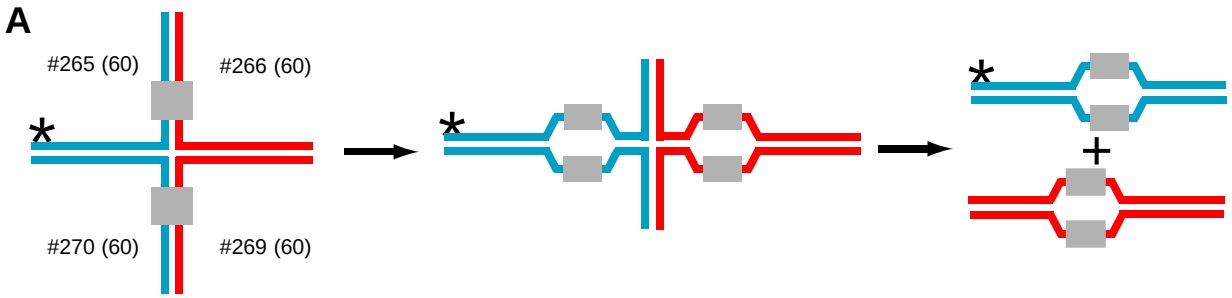
A. Supplementary Figure 1

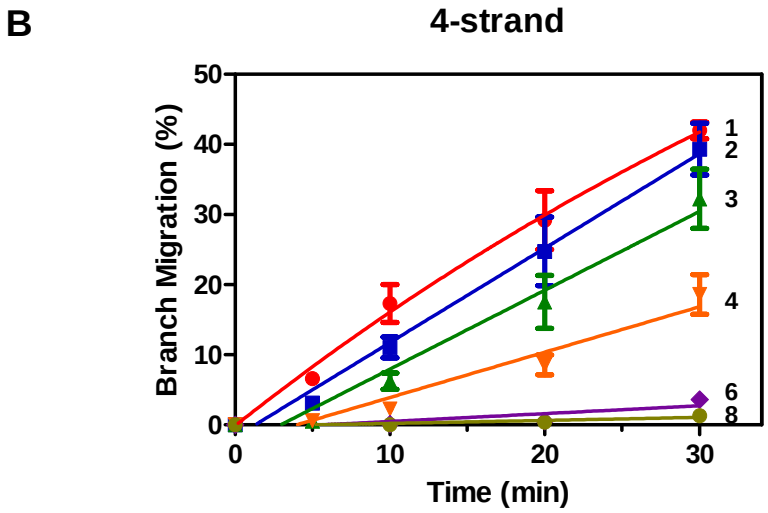


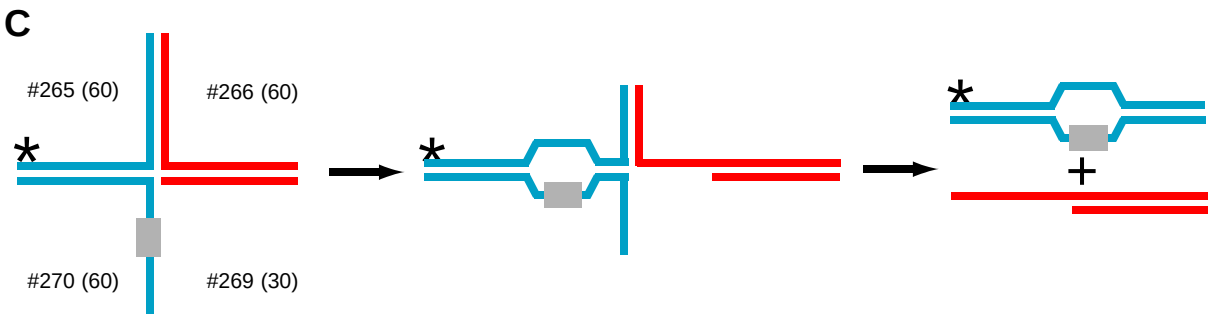


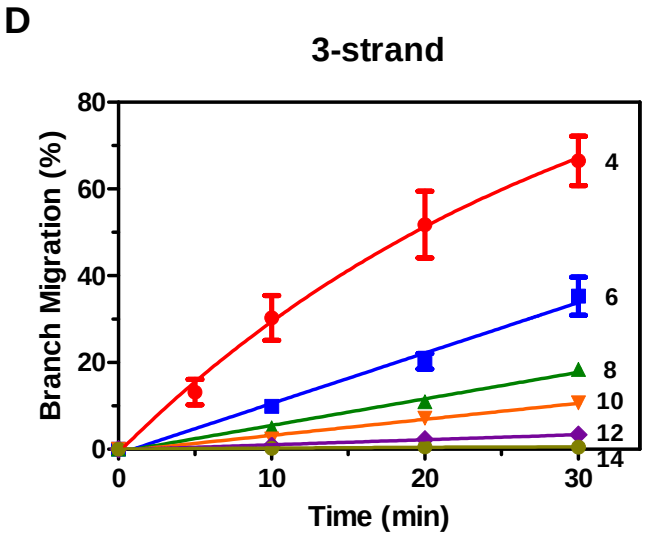
Supplementary Figure 1. The effect of the heterology position on fork regression. (A) The experimental scheme. The asterisk indicates the ^{32}P label. Positions of heterologous bases that would produce mismatches are shown by grey boxes. Replication forks substrates were prepared by annealing tailed DNA intermediates #71/2* (32 nM, molecules) with either #238/236 (48 nM, molecules) to produce the fork I, or with #170/171 (48 nM, molecules) to produce the fork II. Annealing was carried out in buffer containing 25 mM Tris acetate, pH 7.5, 2 mM ATP, 5 mM magnesium acetate, 2 mM DTT, BSA (100 μg/ml), 15 mM phosphocreatine and creatine phosphokinase (30 units/ml) for 15 min at 37 °C. Fork regression was initiated by addition of RAD54 (100 nM) or BLM (10 nM) and was carried out at 37 °C. At indicated time points aliquots were withdrawn, the reaction was stopped by addition of 1.5% SDS and proteinase K (800 μg/ml), mixed with a 0.10 volume of loading buffer (70% glycerol, 0.1% bromophenol blue) and analyzed by electrophoresis in 8% polyacrylamide gels in TBE buffer (89 mM Tris-borate, pH 8.3, and 1 mM EDTA) at 135 V for 1.5 hr. Gels were dried on DEAE-81 paper (Whatman) and the products were quantified using a Storm 840 PhosphorImager (GE Healthcare). (C) The kinetics of fork regression by RAD54 and BLM using fork (I) and fork (II) as substrates. The error bars indicate s.e.m.

Supplementary Figure 2









Supplementary Figure 2. Effect of the length of mismatches on branch migration by RAD54. The experimental scheme of 3-strand (A) and 4-strand (C) branch migration. The asterisk indicates the ^{32}P label. Positions of heterologous bases that would produce mismatches are shown by grey boxes. Partial X-junction (PX) and X-junction substrates were prepared by annealing tailed DNA intermediates (Supplementary Tables. 2 and 3). Annealing was carried out in buffer containing 25 mM Tris acetate, pH 7.5, 2 mM ATP, 10 mM magnesium acetate, 2 mM DTT, BSA (100 μg/ml), 15 mM phosphocreatine and creatine phosphokinase (30 units/ml) for 15 min at 30 °C. Branch migration was initiated by addition of RAD54 (100 nM) and was carried out at 30 °C. At indicated time points aliquots were withdrawn, the reaction was stopped by addition of 1.5% SDS and proteinase K (800 μg/ml), mixed with a 0.10 volume of loading buffer (70% glycerol, 0.1% bromophenol blue) and analyzed by electrophoresis in 8% polyacrylamide gels in TBE buffer (89 mM Tris-borate, pH 8.3, and 1 mM EDTA) at 135 V for 1.5 hr. Gels were dried on DEAE-81 paper (Whatman) and the products were quantified using a Storm 840 PhosphorImager (GE Healthcare). The kinetics of branch migration by RAD54 using PX-junctions (B) and X-junctions (D) as substrates. The error bars indicate s.e.m.

C. Supplementary Table 1. Sequences of the oligonucleotides used in the fork regression portion of this study*

Number	Length, nt	Sequence, 5'→3'
#1	63	ACA GCA CCA GAT TCA GCA ATT AAG CTC TAA GCC ATC CGC AAA AAT GAC CTC TTA TCA AAA GGA
#2	63	TCC TTT TGA TAA GAG GTC ATT TTT GCG GAT GGC TTA GAG CTT AAT TGC TGA ATC TGG TGC TGT
#71	94	CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TAC AGC ACC AGA TTC AGC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A
#117	94	T CCT TTT GAT AAG AGG TCA TTT TTG CGG ATG GCT TAG AGC TTA ATT GCT GAA TCT GGT GCT GTA GGT CAA CAT GTT GTA AAT ATG CAG CTA AAG
#170	94	T CCT TTT GAT AAG AGG TCA TTT TTG CGG ATG GCT TAG AGC TTA ATT GCT AAA TCT GGT GCT GTA GGT CAA CAT GTT GTA AAT ATG CAG CTA AAG
#171	63	ACA GCA CCA GAT TTA GCA ATT AAG CTC TAA GCC ATC CGC AAA AAT GAC CTC TTA TCA AAA GGA
#236	63	GCA GCA CCA GAT TCA GCA ATT AAG CTC TAA GCC ATC CGC AAA AAT GAC CTC TTA TCA AAA GGA
#238	94	T CCT TTT GAT AAG AGG TCA TTT TTG CGG ATG GCT TAG AGC TTA ATT GCT GAA TCT GGT GCT GCA GGT CAA CAT GTT GTA AAT ATG CAG CTA AAG
#290	81	CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TTC AGT A/isodC/A ATC TGC TCT GAT GCC GCA TAG TGT CAT GCC AGA GCT TTG TAC
#340	81	CGG GTG TCG GGG CGC ATG ACA CTA TGC GGC ATC AGA GCA GAT TGT ACT GAA GGT CAA CAT GTT GTA AAT ATG CAG CTA AAG
#341	50	TC AGT ACA ATC TGC TCT GAT GCC GCA TAG TAT CAT GCG CCC CGA CAC CCG

Number	Length, nt	Sequence, 5'→3'
#342	43	**GTA CAA AGC TCT GGC ATG ATA CTA TGC GGC ATC AGA GCA GAT T**
#376	53	**TCC TTT TGA TAA GAG GTC ATT TTT GCG GAT GGC TTA GAG CTT AAT TGC TGA AT**
#377	94	**CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TAC AGC ACC AAA TTC AGC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A**
#378	94	**CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TAC AGC ACC GAA TTC AGC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A**
#379	94	**CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TAC AGC AAA GAA TTC AGC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A**
#386	94	**CTT TAG CTG CAT ATT TAC AAC ATG TTG ACC TAC CCG GAA GAA TTC AGC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A**
#417	30	**GTA CAA AGC TCT GGC ATG ATA CTA TGC GGC**

***Bold Red letters** indicate the nucleotides that form mismatched pairs in the branch migration products.

Supplementary Table 2. Sequences of the oligonucleotides used in the branch migration portion of this study *

Number	Length, nt	Sequence, 5'→3'
#265	60	CCT GCA TAC AGA TGT TGA CCC AGC ACT GAC TAC TGT CGT CAA TCA TCG TGC ATC ACA GTG
#266	60	CAC TGT GAT GCA CGA TGA TTG ACG ACA GTA GTC AGT GCT GCA GTG GTC AGG TGT CAT CAC
#269	20	GTG ATG ACA CCT GAC CAC TG
#270	60	CAC TGT GAT GCA CGA TGA TCA GTG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#273	60	CAC TGT GAT GCA CGA TGA TCA GTT CCA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#274	60	CAC TGT GAT GCA CGA TGA TCA GTT CAC GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#275	60	CAC TGT GAT GCA CGA TGA TCA GTT CAC AGA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#276	60	CAC TGT GAT GCA CGA TGA TCA GTT CAC AGC TTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#277	60	CAC TGT GAT GCA CGA TGA TCA GTT CAC AGC TGG AGT GCT GGG TCA ACA TCT GTA TGC AGG
#337	60	CAC TGT GAT GCA CGA TGA TCG ACG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#338	60	GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CGT CGA TCA TCG TGC ATC ACA GTG
#345	60	CAC TGT GAT GCA CGA TGA TCA ACG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#346	60	GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CGT TGA TCA TCG TGC ATC ACA GTG

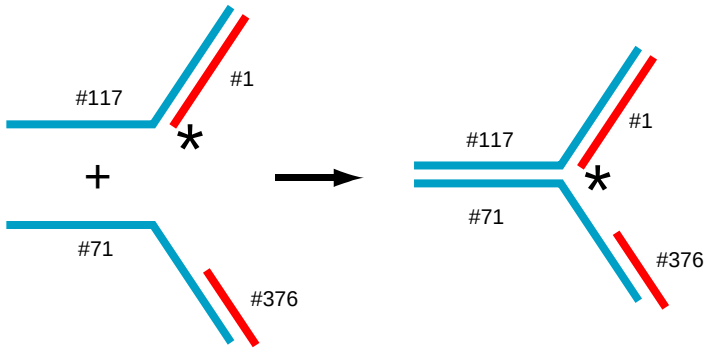
Number	Length, nt	Sequence, 5'→3'
#347	60	**CAC TGT GAT GCA CGA TGA TCA GCG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG**
#348	60	**GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CGC TGA TCA TCG TGC ATC ACA GTG**
#349	60	**GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CAC TGA TCA TCG TGC ATC ACA GTG**
#350	60	**GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGG AAC TGA TCA TCG TGC ATC ACA GTG**
#351	60	**GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC GTG AAC TGA TCA TCG TGC ATC ACA GTG**

***Bold red letters** indicate the nucleotides that form mismatched pairs in the branch migration products.

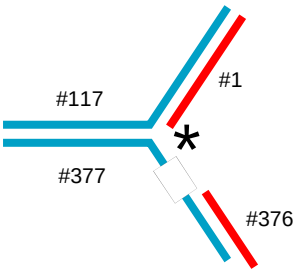
Supplementary Table 3. Scheme of replication forks substrates containing heterologous bases used in this study.

Replication Forks

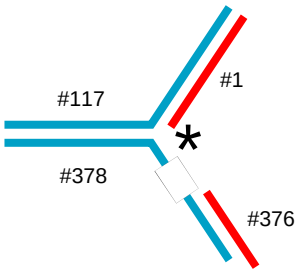
No Heterology



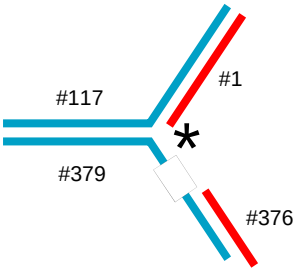
1 mismatch



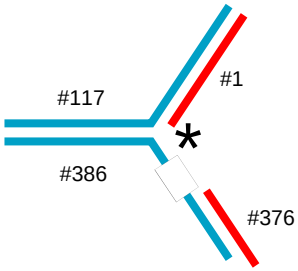
2 mismatches



4 mismatches

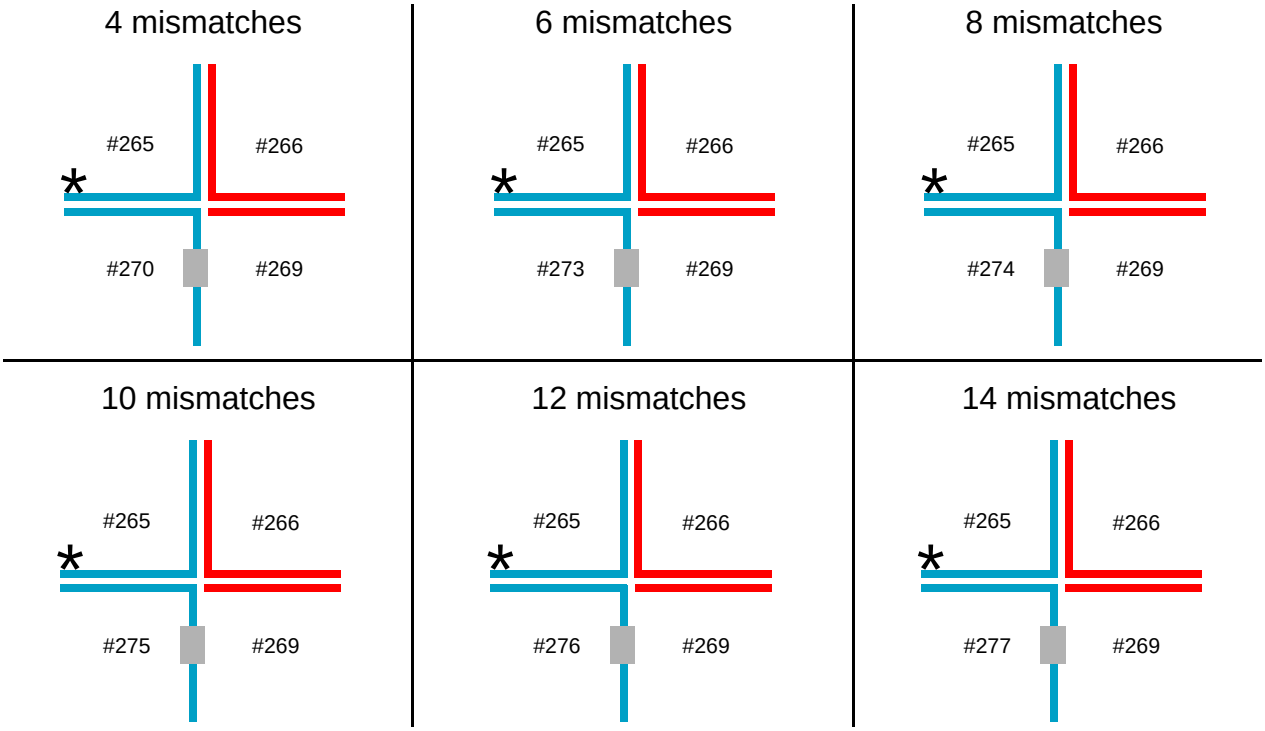


8 mismatches



Supplementary Table 4. Scheme of partial X-junction and X-junction substrates containing heterologous bases used in this study.

Parital X-junctions



X-junctions