SUPPORTING ONLINE INFORMATION

Polarity and bypass of DNA heterology during branch migration of Holliday junctions by human RAD54, BLM, and RECQ1

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Running title: Branch migration activity of human DNA translocases

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This supplement contains:

Supplementary Figure Legends

Supplementary Figures S1-S4

Supplementary Table S1.

SUPPLEMENTARY FIGURE LEGENDS

FIGURE S1. **RuvAB promotes BM of JMs preferentially with a 5' → 3' polarity.** The JM (0. 5 nM) with 3'- or 5'-ssDNA displaced strand (3'- and 5'-JMs) (Fig 1A) were used for branch migration by *E. coli* RuvAB (140 nM RuvA and 360 nM RuvB) at 37 °C. Aliquots (10 μl) were withdrawn from the reaction mixture at the indicated time points and the DNA products were analyzed by gel electrophoresis in 1.5% agarose gels.

FIGURE S2. **BLM and RECQ1 promote the four-stranded DNA BM with the 3' → 5' polarity.** (A and B) The kinetics of BM by BLM and RECQ1 on the 3'- and 5'-JMs (Fig 1A). BM was initiated by addition of BLM (20 nM) or RECQ1 (200 nM) to the JMs (0.9 nM) and carried out at 37 °C. Protein-independent (spontaneous) BM controls (denoted as "sp") are shown in (A), (lanes 11 and 20); and (B) (lanes 13 and 23). Wild type BLM was replaced with its ATPase-deficient K695R mutant (A) (lanes 12 and 21). BM in the absence of ATP is shown for RECQ1 (B) (lanes 14 and 24). Aliquots (10 μl) were withdrawn from the reaction mixtures at the indicated times and the DNA products were analyzed by electrophoresis on 1.5% agarose gels.

FIGURE S3. **Bypass of DNA heterologies during RAD54-, BLM-, and RECQ1-promoted four-strand branch migration**. (A) Experimental design**.** The 3'-JMs containing 2-96 bp heterologous DNA sequences (denoted by the black box) were prepared by carrying out RAD51-promoted DNA strand exchange between gapped DNA and ^{32}P-labeled pBSK (+) linear dsDNA. (B-D) The kinetics of BM by RAD54 (200 nM), BLM (20 nM) or RECQ1 (200 nM) on 3'-JMs (0.9 nM) containing DNA heterologous sequences of the indicated lengths. The DNA heterology provided a thermodynamic block to BM proteins resulted in JMs in which the branch point had

migrated up to the region containing the heterology and then stalled. These JMs produced a smeared band visible above the original JMs' band in an agarose gel.

FIGURE S4. **The effect of RAD54 and BLM concentration on the bypass of DNA heterology.** (A and B) RAD54 and BLM in indicated concentrations were added to the 3'-JMs (0.3 nM), either fully homologous or containing DNA heterology of the indicated length.

Supplementary Figures

Figure S1

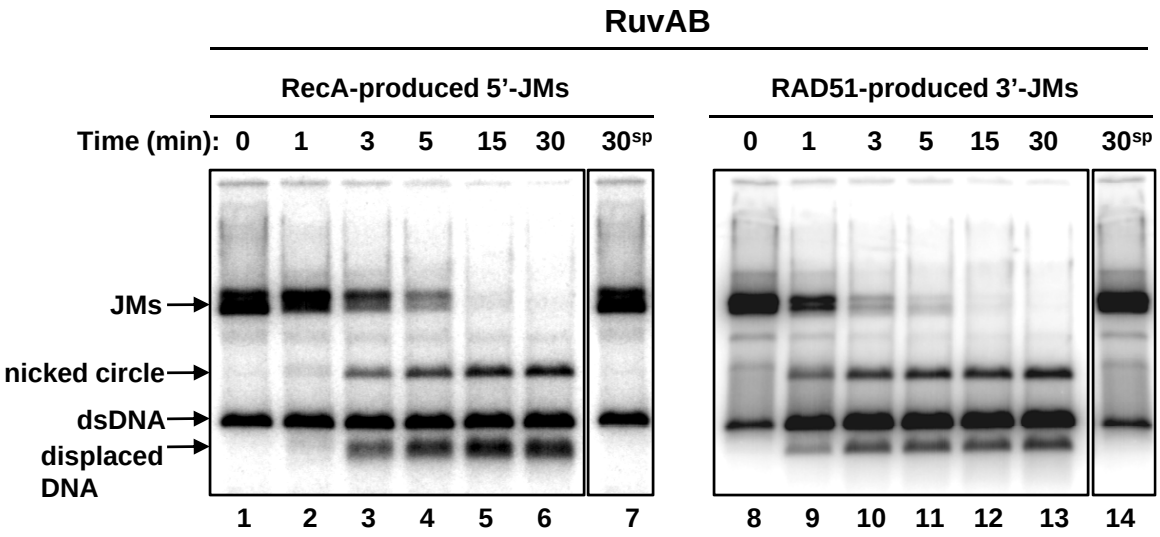


Figure S2

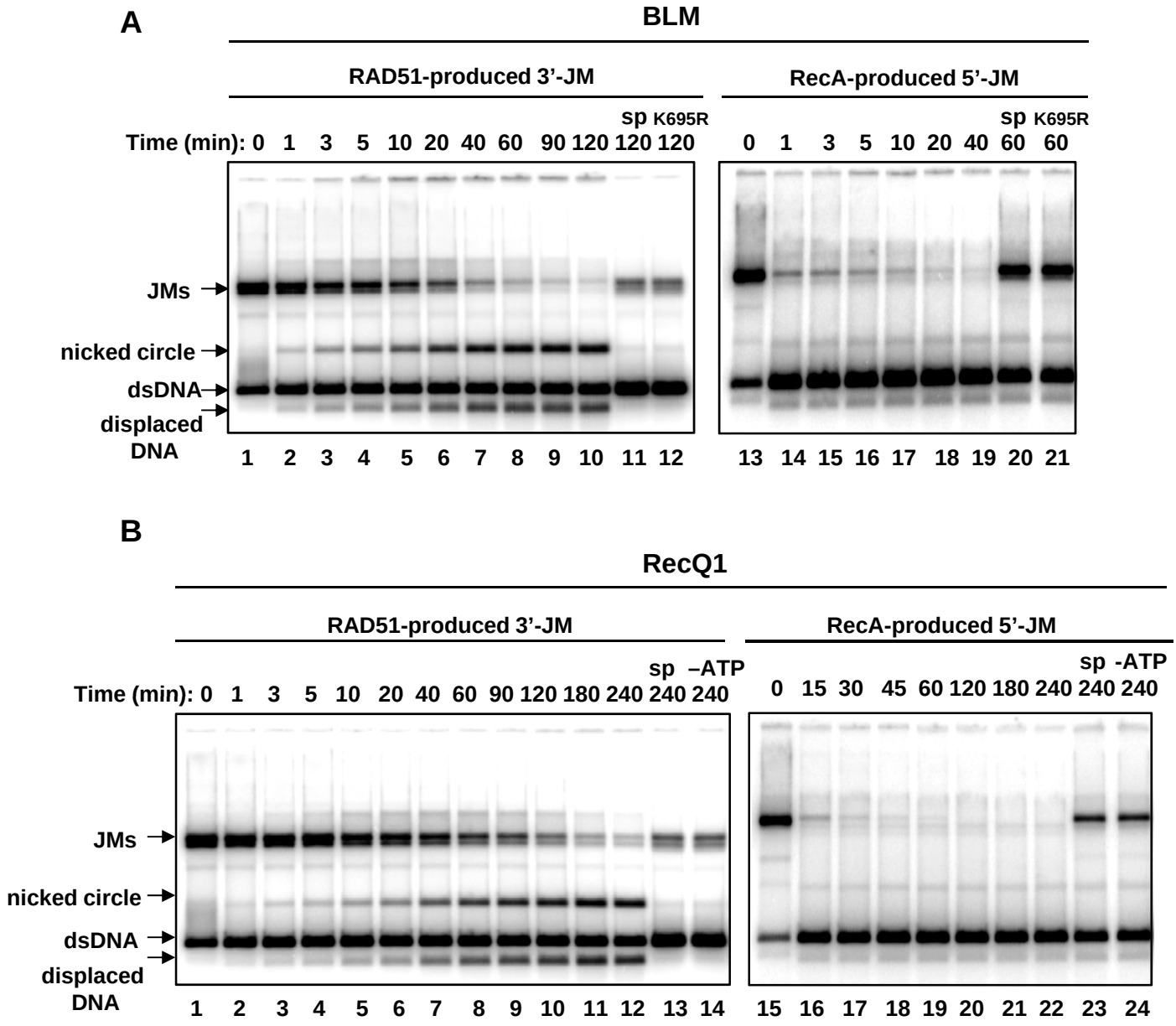
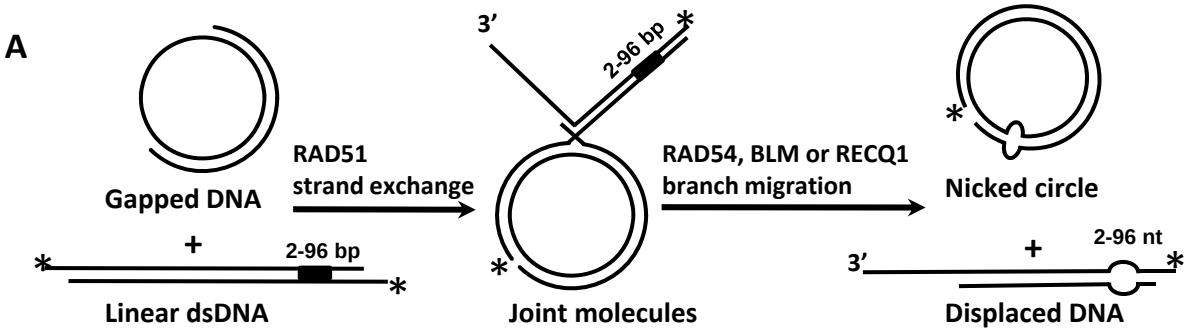
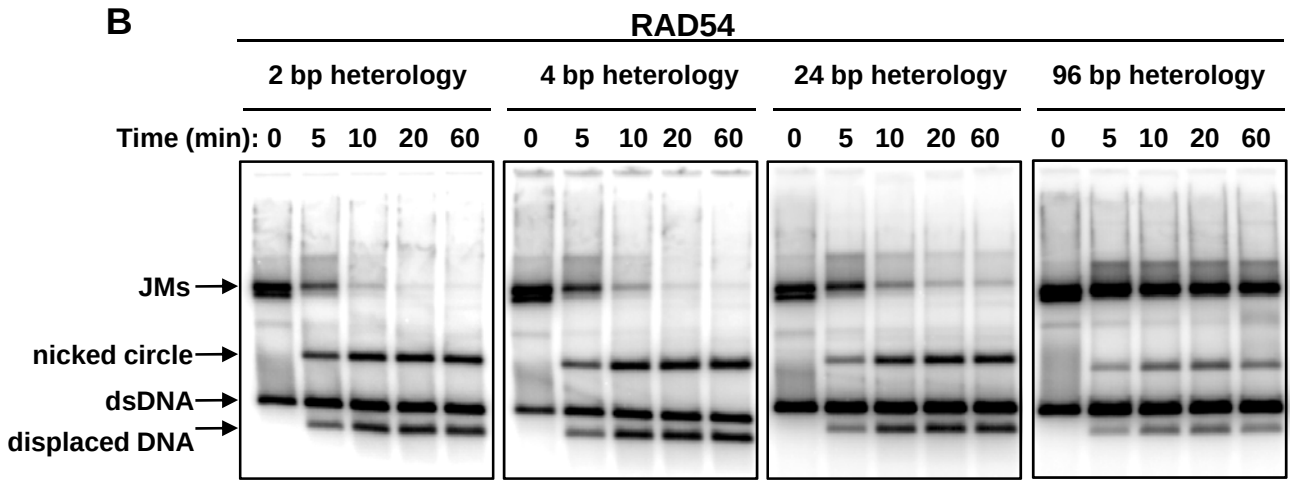
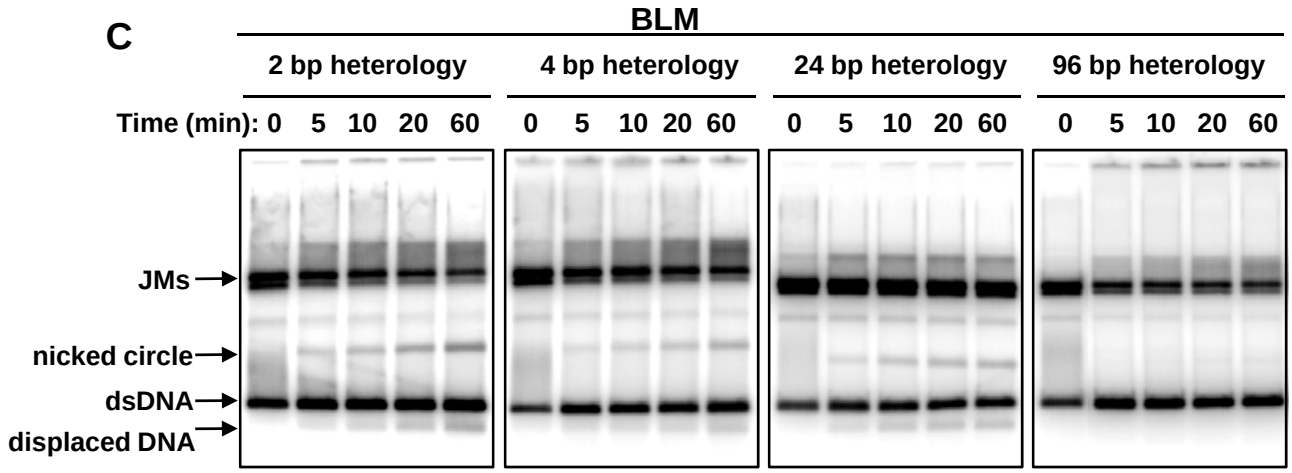

A
BLM
RAD51-produced 3'-JM
RecA-produced 5'-JM
Time (min): 0 1 3 5 10 20 40 60 90 120 sp 120 K695R 120
0 1 3 5 10 20 40 sp 60 K695R 60
JMs
nicked circle
dsDNA
displaced DNA
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21
B
RecQ1
RAD51-produced 3'-JM
RecA-produced 5'-JM
Time (min): 0 1 3 5 10 20 40 60 90 120 180 240 sp 240 –ATP 240
0 15 30 45 60 120 180 240 sp 240 -ATP 240
JMs
nicked circle
dsDNA
displaced DNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24

Figure S3


A
Gapped DNA
RAD51
strand exchange
3'
2-96 bp
RAD54, BLM or RECQ1
branch migration
Nicked circle
+
2-96 bp
Linear dsDNA
Joint molecules
3'
+
2-96 nt
Displaced DNA


B
RAD54
2 bp heterology
4 bp heterology
24 bp heterology
96 bp heterology
Time (min): 0 5 10 20 60 0 5 10 20 60 0 5 10 20 60 0 5 10 20 60
JMs
nicked circle
dsDNA
displaced DNA


C
BLM
2 bp heterology
4 bp heterology
24 bp heterology
96 bp heterology
Time (min): 0 5 10 20 60 0 5 10 20 60 0 5 10 20 60 0 5 10 20 60
JMs
nicked circle
dsDNA
displaced DNA

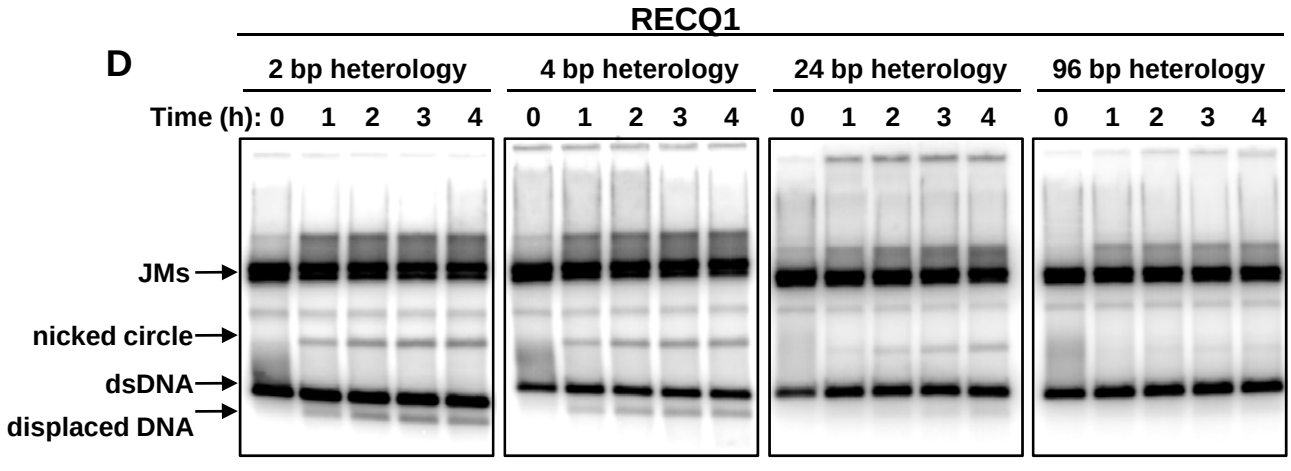
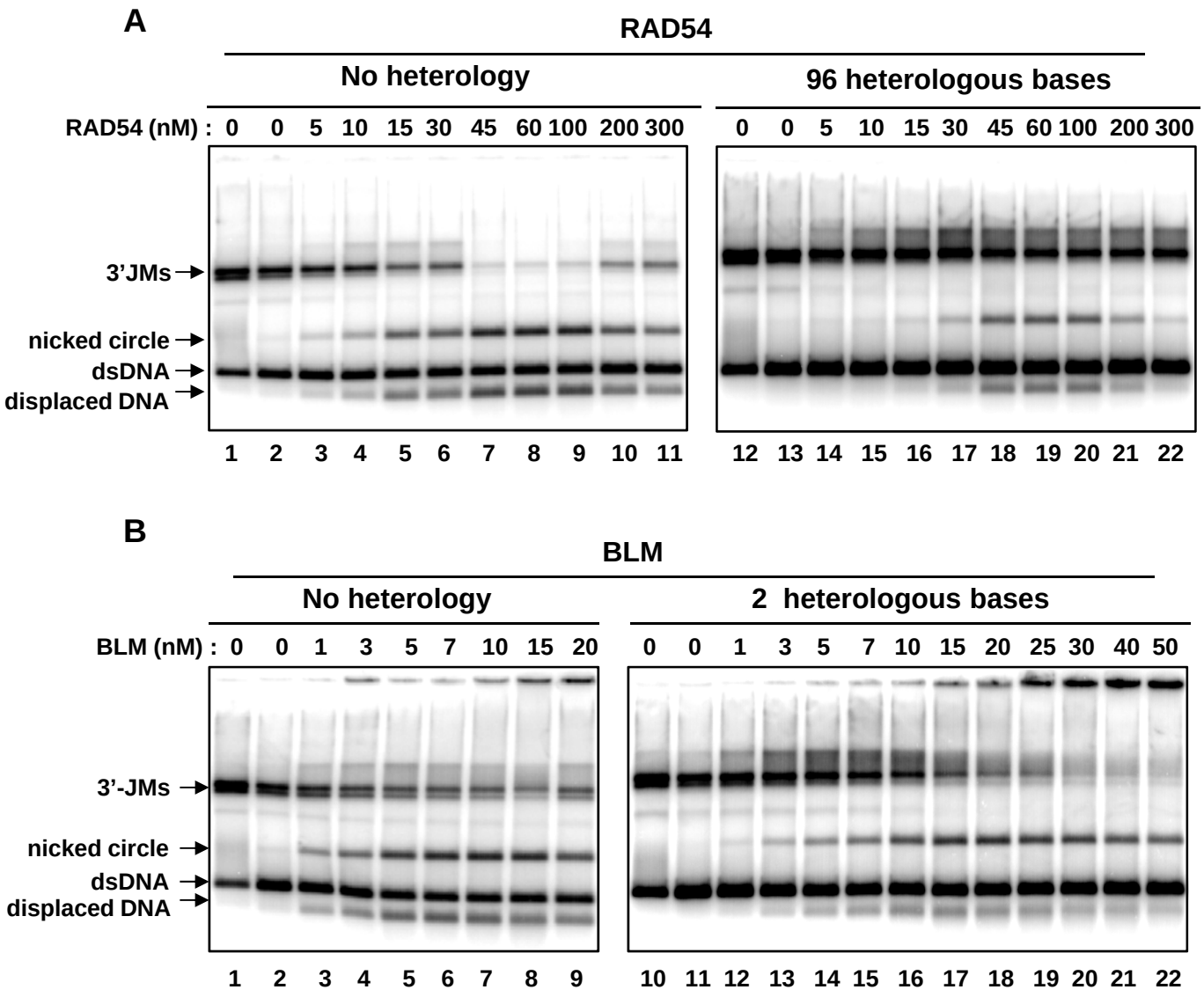

D
RECQ1
2 bp heterology
4 bp heterology
24 bp heterology
96 bp heterology
Time (h): 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4
JMs
nicked circle
dsDNA
displaced DNA

Figure S4


A
RAD54
No heterology
96 heterologous bases
RAD54 (nM) : 0 0 5 10 15 30 45 60 100 200 300
0 0 5 10 15 30 45 60 100 200 300
3'JMs
nicked circle
dsDNA
displaced DNA
1 2 3 4 5 6 7 8 9 10 11
12 13 14 15 16 17 18 19 20 21 22
B
BLM
No heterology
2 heterologous bases
BLM (nM) : 0 0 1 3 5 7 10 15 20
0 0 1 3 5 7 10 15 20 25 30 40 50
3'-JMs
nicked circle
dsDNA
displaced DNA
1 2 3 4 5 6 7 8 9
10 11 12 13 14 15 16 17 18 19 20 21 22

Supplementary Tables

Supplementary Table S1. Sequences of the oligonucleotides used in this study*

Number	Length, nt	Sequence, 5'→3'
PX junction substrate with 4 bp heterology		
#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**
#169	93	**TCC TTT TGA TAA GAG GTC ATT TTT GCG GAT GGC TTA GAG CTT AAT TGC TGA ATC TGG TGC TGT TTT TTT TTT TTT TTT TTT TTT TTT TTT**
#201	94	**T CCT TTT GAT AAG AGG TCA TTT TTG CGG ATG GCT TAG AGC TTA ATT ATA AAA TCT GGT GCT GTA GGT CAA CAT GTT GTA AAT ATG CAG CTA AAG**
#460	63	**ACA GCA CCA GAT TTT ATA ATT AAG CTC TAA GCC ATC CGC AAA AAT GAC CTC TTA TCA AAA GGA**
PCR primers to construct pBSK (+)-derived plasmids, containing 2, 4, 8, 16, and 24 bp heterology		
#P34	40	**AGA GCT TGA CGG GGA AAG CTA GCG AAC GTG GCG AGA AAG G**
#P35	40	**CCT TTC TCG CCA CGT TCG CTA GCT TTC CCC GTC AAG CTC T**
#P36	40	**AGA GCT TGA CGG GGA AAG GTA CCG AAC GTG GCG AGA AAG G**
#P37	40	**CCT TTC TCG CCA CGT TCG GTA CCT TTC CCC GTC AAG CTC T**
#P38	40	**AGA GCT TGA CGG GGA ACA GTA CAC AAC GTG GCG AGA AAG G**
#P39	40	**CCT TTC TCG CCA CGT TGT GTA CTG TTC CCC GTC AAG CTC T**
#P61	48	**CCC GAT TTA GAG CTT GGT ACT ACT CAG TAC TCA ACG TGG CGA GAA AGG**
#P62	48	**CCT TTC TCG CCA CGT TGA GTA CTG AGT AGT ACC AAG CTC TAA ATC GGG**

Number	Length, nt	Sequence, 5'→3'
#P65	61	CCC GAT TTA GAG CTT GGT ACT ACT CAG TAC TCC TGT ATC AGA GAA AGG AAG GGA AGA AAG C
#P66	61	GCT TTC TTC CCT TCC TTT CTC TGA TAC AGG AGT ACT GAG TAG TAC CAA GCT CTA AAT CGG G
Oligonucleotides for construction of pBSK (+)-derived plasmids, containing 48 and 96bp heterology		
#S75	101	GTG 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 ACT AG
#S76	108	CCG GCT AGT 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 CAC GTA
#S77	101	GTG AAC CAT CAC CCT AAT CAA GTT TTT TGG GGT CGA GGT GCC GTA AAG CAC AAT TAA GCT CTA AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG ACT AG

#S78	108	CCG GCT AGT CCT TTT GAT AAG AGG TCA TTT TTG CGG ATG GCT TAG AGC TTA ATT GTG CTT TAC GGC ACC TCG ACC CCA AAA AAC TTG ATT AGG GTG ATG GTT CAC GTA

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