Supplementary Data

Construction of JM

A single *cer* sequence was amplified from plasmid pSD115 using primers A and B (supplementary Table 1 and supplementary Figure 1) to generate PCR-cer1 that was cloned directionally into pRS411 via Kpn1 and XhoI restriction sites to create pRS411-cer1. The *HIS3* marker was amplified by PCR using primers C and D and plasmid pRS313 as a template to generate fragment PCR-HIS3 (supplementary Table 1 and supplementary Figure 1). A second *cer* sequence was amplified by PCR using primers E and F and plasmid pSD115 as a template to generate fragment PCR-cer2 (supplementary Table 1 and Figure 1). pRS411-cer1 that had been linearised by XhoI was co-transformed with PCR-HIS3 and PCR-cer2 fragments into *dnl4Δ* cells. The three linear DNA fragments contained overlapping regions of homology that facilitated recombination-mediated assembly of the three fragments into JM which could be selected for by screening for both methionine and histidine prototrophy (Supplementary Figure 1). Plasmid DNA from independent clones was transformed into an *xerD*$^{-}$ strain of *E.coli* (JW2861-2) and the construction of JM confirmed using a combination of restriction digestion and sequence analysis.

Induction and purification of JM-HJ

To generate the JM-HJ molecule in which a HJ is created between the two *cer* sites thereby segregating the CEN-ARS and *HIS3* marker into distinct circular domains, JM was transformed into RM40 cells which contains XerC under the control of the lac promoter (Figure 2A). *xerC* expression was induced for 2h in a 5l log-phase culture of

RM40/JM by the addition of 1mM IPTG. Induction of Xer-mediated intra-molecular recombination between the two *cer* sites generates two circular products, R1 and R2 (Supplementary Figures 3 and 2B, lanes 1 and 5). As has previously been reported in this system, a supercoiled intermediate containing a single HJ was also found to accumulate, which we termed JM-HJ (Supplementary Figures 3 and 2B, lanes 1 and 5) (1). This intermediate, which we termed JM-HJ, is covalently closed and supercoiled making it indistinguishable by gel electrophoresis from the parental supercoiled JM molecule that had not undergone XerC-mediated recombination. However, the presence of JM-HJ could be detected by its conversion to either α or χ structures via the linearization of either one or both circular domains of JM-HJ by digestion with BamHI or Bbs1, respectively (Supplementary Figure 2B, lanes 6 and 7). As predicted, these structures had retarded gel mobilities compared to the linear fragments generated by restriction digestion of the parental JM molecule and the XerC-mediated R1 and R2 circles (Supplementary Figure 2B, compare lanes 2 and 3 with lanes 6 and 7). To purify JM-HJ from the mix of molecules generated by XerC induction, we followed the scheme in supplementary Figure 3. Plasmids prepared from the XerC-induced culture were digested overnight with the nicking enzyme Nt.BbvCI (NEB) at 37°C to relax the R2 domain of JM-HJ via a unique BbvCI site. Nt.BbvCI thus converts JM-HJ into a partially supercoiled molecule in which relaxed R2 is connected via a HJ to supercoiled R1 (Supplementary Figure 3). Nt.BbvCI digestion of plasmid material prepared from XerC-induced cultures generated two species that had electrophoretic mobilities that were intermediate to that of supercoiled and nicked JM, consistent with the notion that these species represented partially supercoiled JM-HJ (Supplementary Figure 2B, lane 8, red

box). Moreover both these species were absent from the plasmid preparation made from the uninduced culture where XerC-mediated recombination was not expected to have occurred (Supplementary Figure 2B, compare lanes 4 and 8). Both molecules were enriched following CsCl density gradient centrifugation, confirming that these species contained a supercoiled domain (Supplementary Figure 3). Both molecules underwent two rounds of gel purification on a 0.7% TAE agarose gel before being subjected to restriction digestion analysis to confirm the presence of a HJ. Although a HJ was confirmed to be present in both molecules (Figure 1 and data not shown), only the upper species was used in all subsequent analyses. Herein, we refer to this molecule as JM-HJ.

Supplementary Figure 1

Construction of JM. Schematic diagram outlining the construction of JM. Coloured boxes indicate regions of homology that facilitate recombination-mediated circularisation of pRS411-cer1 to form JM. Green arrows indicate primers used in PCR. See text for details.

Supplementary Figure 2

Induction of JM-HJ. (A) Schematic diagram showing the generation of JM-HJ via the Xer-mediated recombination at the *cer* sequences on JM. See text for details. (B) Gel electrophoresis analysis of plasmids prepared from RM40 cells carrying JM, which have or have not been subjected to XerC induction, as indicated. DNA has been digested with restriction enzymes as indicated. Species that arise following XerC induction have been boxed, as indicated. See text for details.

Supplementary Figure 3

Purification of JM-HJ. Upper panel: Flow diagram outlining process for the purification of JM-HJ. Molecules expected to be enriched at each step are shown to the right (although R2 does not contain an origin of replication, R2 is still present since the period of XerC induction is too short for the circular R2 to be lost from the population). The HJ in JM-HJ is depicted in red. Lower panel: Agarose gel electrophoresis of DNA from steps (A) and (B) shows the enrichment of JM-HJ following CsCl density gradient centrifugation.

Supplementary Table 1

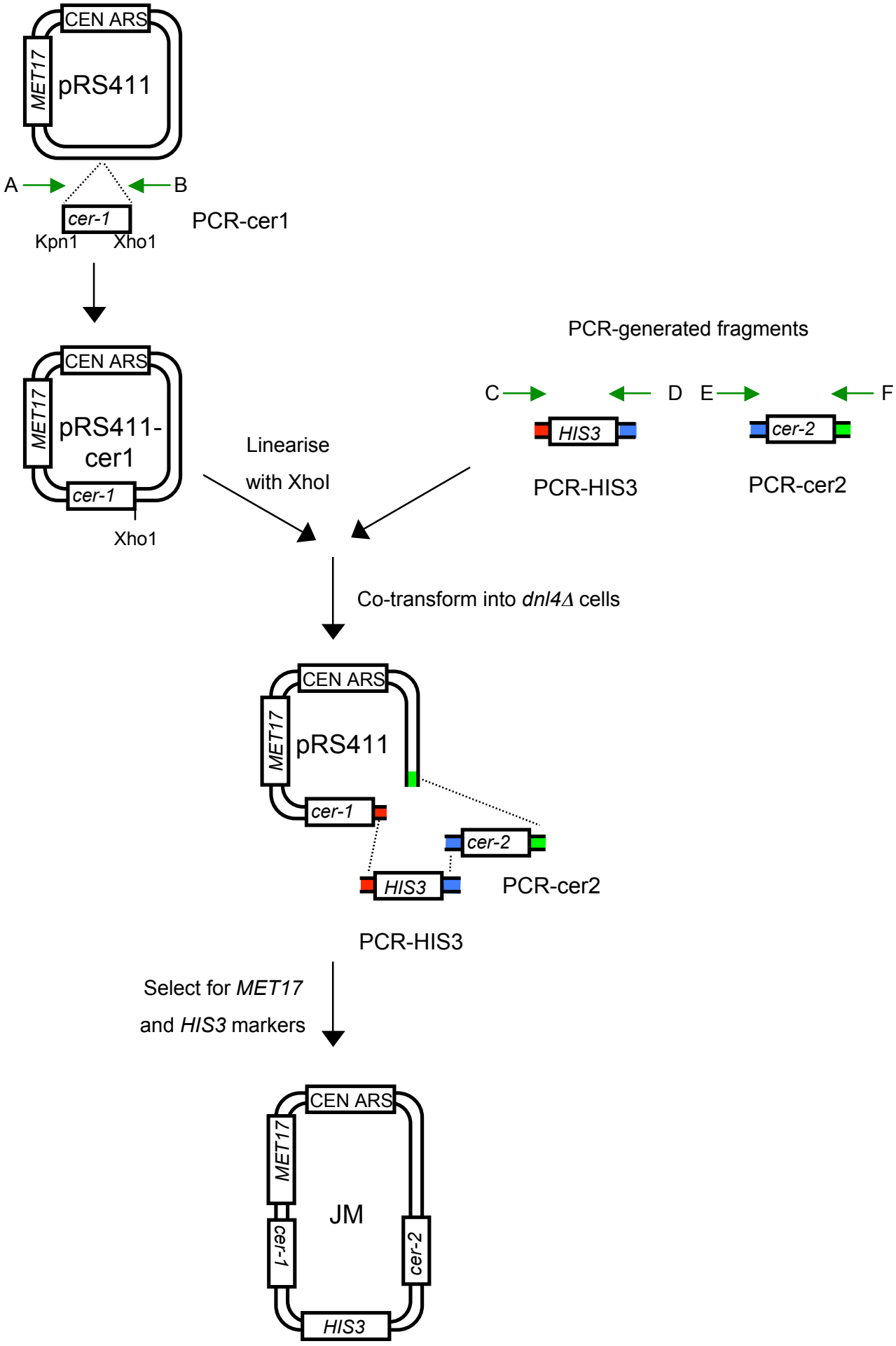
Oligonucleotides used in the construction of JM and the analysis of JM-HJ resolution products. Restriction sites relevant to this study that were introduced during the construction of JM have been highlighted: BamHI sites are indicated in bold; BbvCI site has been underlined.

Supplementary Table 1:

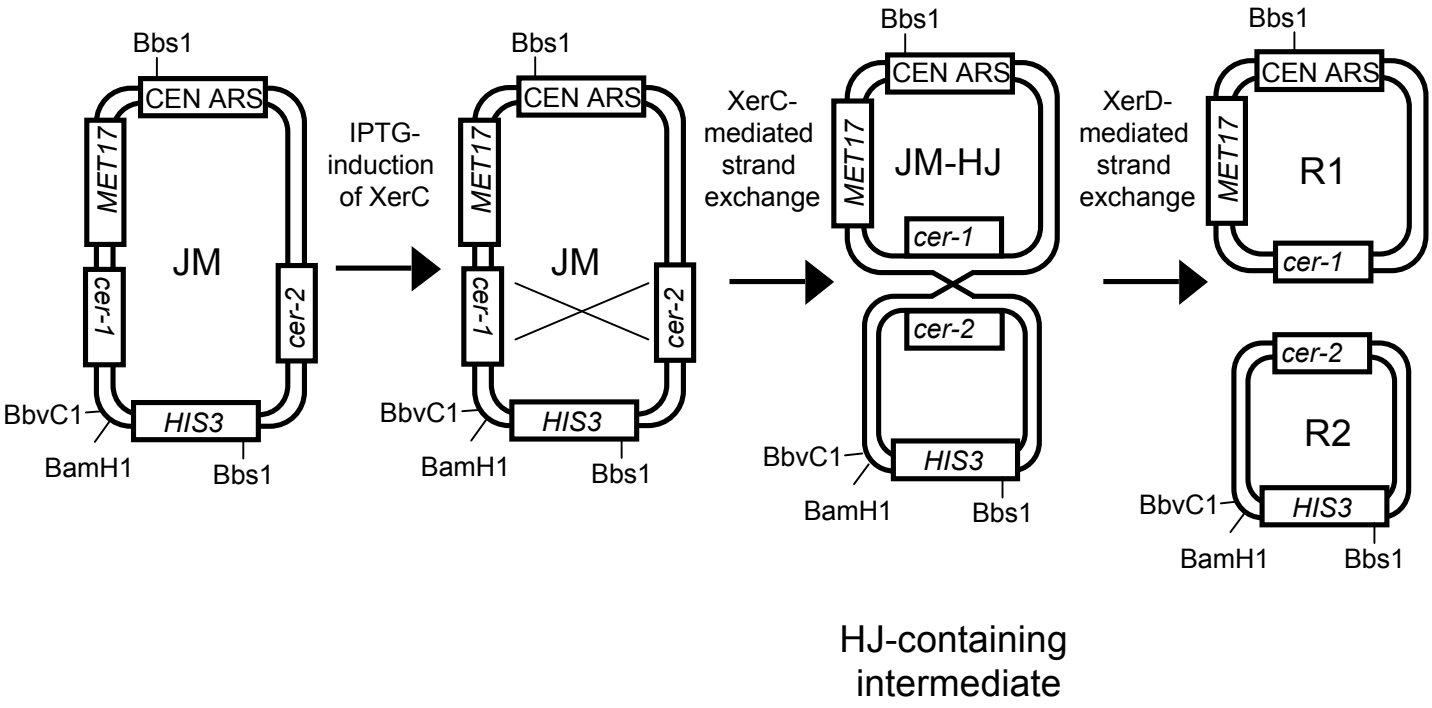
Oligonucleotide	Sequence
primer A	TTGGGTACCGAGGAACGGCTGATACA
primer B	ACCTCGAGCGCATTCACCGCAGCAAA
primer C	CCAGCAGCCTGGATTTTTTCGGGGTAAGGTCTTTGCTGCGG TGAATGCGCTCGAGGTCGACCGTTTTAAGAGCT
primer D	TCTGCTCGCTCAAGTT**GGATCC**GCCGG<u>CTGAGG</u>GCTAGCT CAGGCGTACGGCTTAGGTCACACCGCATATGATCCGTCGA GTTCAAGAG
primer E	CCGGC**GGATCC**AACTTGAGCGAGCAGACAGAACGCTTGA GCCAGTTATACAGCGAGAACTGGAGATAAGAGAGGAACG GCTGATACAG
primer F	CGGGGTAAGGTCTTTGCTGCGGTGAATGCGCCAGCTTTTGT TCCCTTTAGTGAGGGCTGCATTAATGAATCGG
primer G	CGGTGCGGGCCTCTTCGCTA
primer H	AGAGTGTACTAGAGGAGGCCAAGA
primer I	AGCGAGCAGACAGAACG
primer J	CTTTGAGTGAGCTGATACCGC

1. McCulloch, R., Coggins, L. W., Colloms, S. D., and Sherratt, D. J. (1994) *EMBO J* **13**, 1844-1855

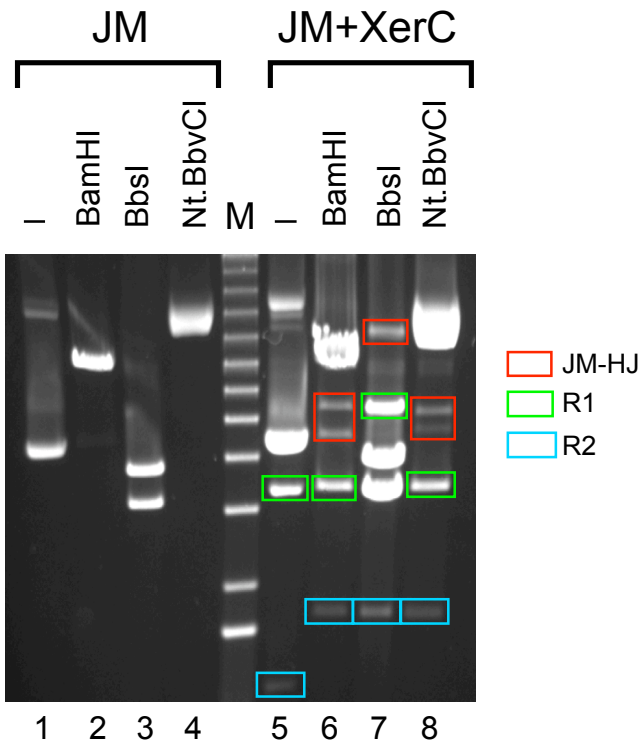
Supplementary Figure 1

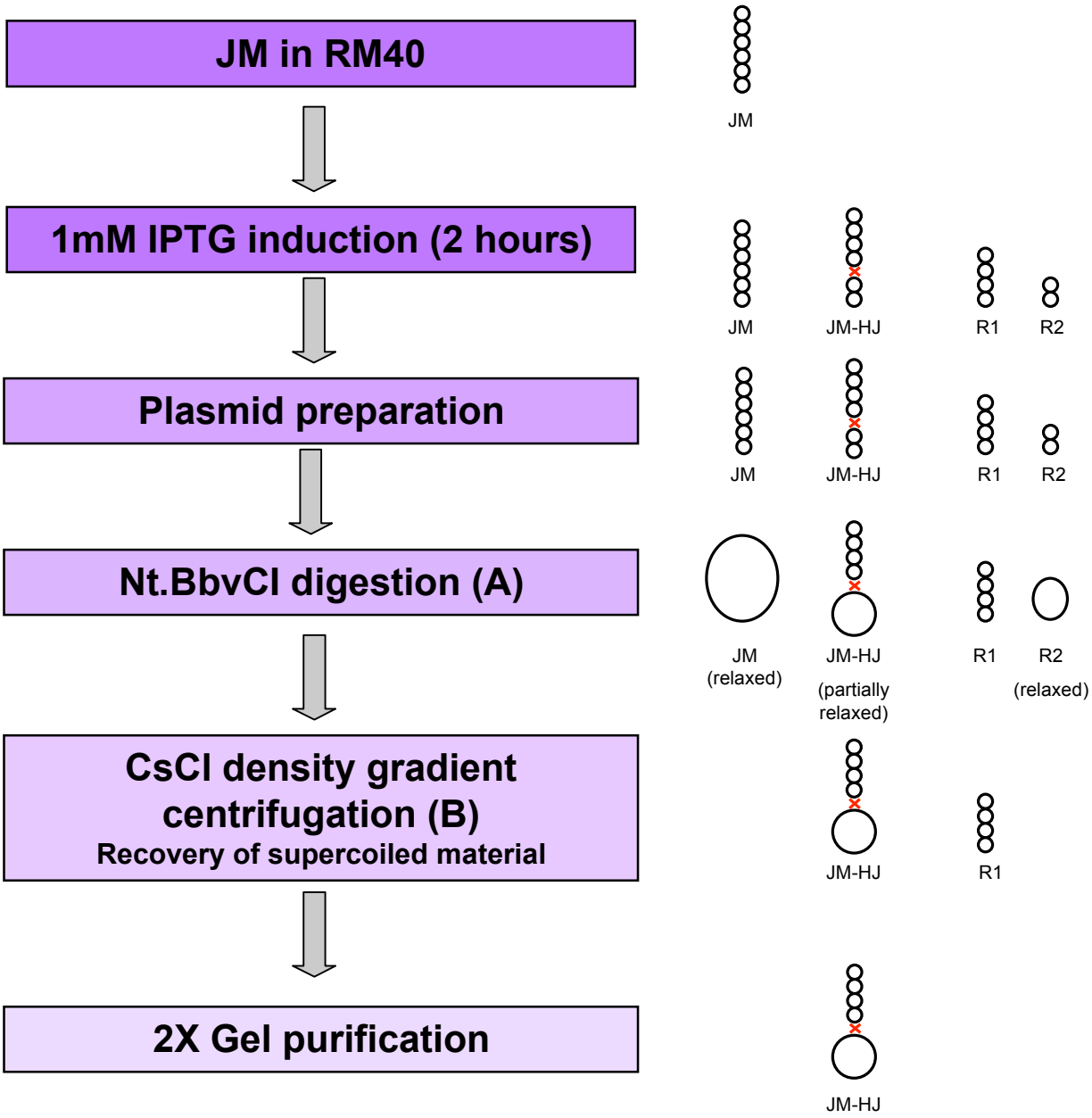


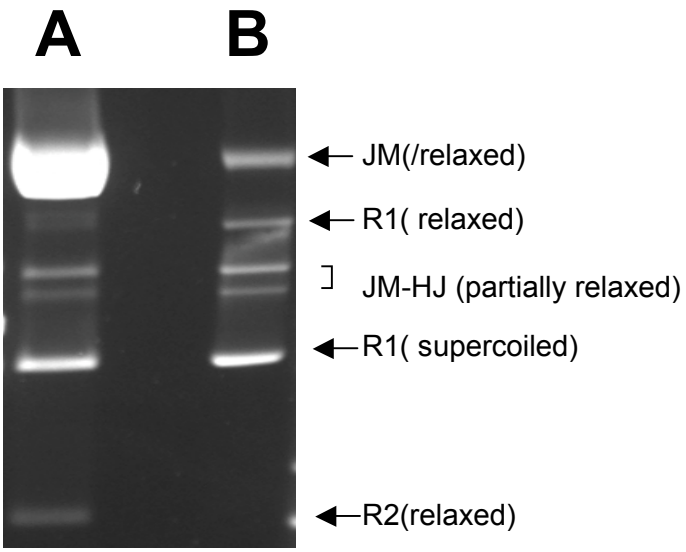
A


Bbs1
CEN ARS
MET17
cer-1
JM
cer-2
BbvC1
HIS3
BamH1
Bbs1
IPTG-induction of XerC
XerC-mediated strand exchange
JM-HJ
XerD-mediated strand exchange
R1
R2
HJ-containing intermediate

B


JM
JM+XerC
–
BamHI
BbsI
Nt.BbvCI
M
JM-HJ
R1
R2
1 2 3 4 5 6 7 8


JM in RM40
1mM IPTG induction (2 hours)
Plasmid preparation
Nt.BbvCI digestion (A)
CsCl density gradient centrifugation (B)
Recovery of supercoiled material
2X Gel purification
JM
JM-HJ
R1
R2
JM (relaxed)
JM-HJ (partially relaxed)
R2 (relaxed)


A
B
JM(/relaxed)
R1(relaxed)
JM-HJ (partially relaxed)
R1(supercoiled)
R2(relaxed)