Supplementary Material

Trapping and breaking of *in vivo* nicked DNA during pulsed-field gel electrophoresis

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Short title: PFGE converts nicks into double-strand breaks

Subject Category: Electrophoretic Techniques

Abbreviations: PFGE, pulsed-field gel electrophoresis; LLFS, long run at low field strength; SHFS, short run at high field strength; CZ, compression zone; UV, ultraviolet light; HMW, high molecular weight; LMW, low molecular weight; DBA, different batch of agarose.

Table S1. Summary of results.

Strains	Experiments	Conclusions	Respective Figures
AB1157, SK129 AK107, SRK312 SRK322	Spontaneous and Clastogen- induced fragmentation at 3V and 6V	HMW DNA is trapped and broken during PFEG, Number of nicks determine the extent of chromosomal fragmentation	1, 2, and S1, S2
SK129, SRK312 SRK322	Kinetics of UV-induced fragmentation at 3V and 6V	Increase in fragmentation is due to release of DNA from wells, nick-dependent breakage is FS-independent	3, S4, S5 and S6
SK129, SRK312 SRK322	Field strength Vs. time	Both time of electrophoresis and FS are important for trapping and nick-dependent fragmentation increase, especially in the DBA experiments	4, 5 and S7
SK129, SRK301	Excision- and FS-dependent breakage of *Not*I fragments at 3V and 6V	Nick-dependent breakage is FS-independent, number of nicks determines the final size of DNA fragments during PFGE	6, S8 and S9

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Effect of electrophoretic conditions on the extent of spontaneous and DNA damage-induced chromosomal fragmentation. Samples were run on a 1% agarose gel at 12°C in 0.5X TBE with 60-120 seconds switch time, either at 6 V/cm for 24 H, or at 3 V/cm for 72 H.
(A) Quantification of spontaneous fragmentation in AB1157, *recBC*(Ts), *recBC*(Ts) *polA12*, *recBC*(Ts) *ligA251* and *recBC*(Ts) *dut-1* after growth of the strains at 37°C for 2 hours.
(B) The corresponding gel from which the quantification in "A" was derived. CZ, compression zone.
(C) Quantification of chromosomal fragmentation in *recBC*(Ts) cells upon exposure to a variety of DNA damaging agents. Cells were treated with clastogens as described in materials and methods and transferred to 37°C for 2 hours before the preparation of plugs.
(D) The corresponding gel from which the quantification in "C" was derived. CZ, compression zone.

Figure S2. Effect of electrophoretic conditions on the amount of signal staying within the wells (A and B); and total signal (well+lane) (C and D). Values are derived from the gels in Fig. S1, ran in a "different batch of agarose".
(A) Differences in the absolute amount of signal (arbitrary units) within wells in strains undergoing spontaneous fragmentation.
(B) Differences in the absolute amount of signal (arbitrary units) within wells in *recBC*(Ts) cells exposed to a variety of DNA damaging agents.
(C) Decrease in the total signal in strains undergoing spontaneous fragmentation, presented as ratios of the total signal in the LLFS conditions divided by the total signal from the same plug in the SHFS conditions.
(D) Decrease in the total signal in strains exposed to various clastogens (calculated as in "C").

Figure S3. Comparison of migration of the yeast chromosome markers and lambda concatemer markers in various electrophoretic conditions.

Figure S4. UV dose-dependence of chromosomal fragmentation in *recBC*(Ts) or its derivatives harboring additional mutations in *polA* or *ligA* genes. Agarose plugs were made after incubating the UV-exposed cultures at 37°C for 10 minutes. Plugs were run either at 6 V/cm for 24 H or at 3 V/cm for 72 H as described in Fig. 1, but using the "different batch of agarose". Panels A, C and E are radiograms of the gels. Panels B, D and F show quantificatin of fragmentation from the corresponding gels. CZ, compression zone.

Figure S5. Effect of UV exposure and electrophoretic conditions on the amount of total signal, as well as the signal staying within wells. *recBC*(Ts), *recBC*(Ts) *polA12*, and

recBC(Ts) *ligA251* were exposed to various UV doses, and plugs were electrophoresed at 6 V/cm for 24 H or at 3 V/cm for 72 H.
(A) Quantification of changes in the signal inside the wells (calculated as percent of the total signal (well+lane).
(B) Decrease in the amount of total signal. Fold decrease is calculated as total signal (well+lane) upon 3 V/cm electrophoresis ÷ total signal (well+lane) upon 6 V/cm electrophoresis.

Figure S6. Dissection of UV-induced chromosomal fragmentation.
(A) Comparison of migration of the yeast chromosome markers and lambda concatemer markers in various separation conditions.
(B, C, D) Distribution of UV dose-dependent fragmented DNA as observed upon SHFS and LLFS electrophoresis. The gels shown in Fig. S3BDE were used for quantification. The signal entering the lanes was subdivided into chromosomal fragments shorter than 0.7 MB and longer than 0.7 MB, as described in the text. **(B)** The *recBC*(Ts) single mutant. **(C)** The *recBC*(Ts) *polA12* double mutant. **(D)** The *recBC*(Ts) *ligA251* double mutant.

Figure S7. Effect of time of electrophoresis on signal within wells.
(A) Plugs made from UV exposed cultures of *recBC*(Ts) or its derivatives were run for 20 H or 72 H, either at 6 V/cm, or at 3 V/cm. Data is presented as fold change, which is the ratio of the amount of signal within wells obtained after 72 H electrophoresis over the signal obtained after 20 H electrophoresis under the same field strength.
(B) Plugs made from *recBC*(Ts), *recBC*(Ts) *polA12* and *recBC*(Ts) *ligA251* after growth of the strains at 37°C for 2 hours (spontaneous fragmentation). Calculations were done as described in (A).

Figure S8. Quantification of UV-induced chromosomal fragmentation in non-replicating cells. Stationary cultures of *recBC*(Ts) and *recBC*(Ts) *uvrA* were exposed to the indicated UV doses, and plugs were made after 10 min incubation in spent culture medium, so not to "awaken" the cells. One set of plugs was digested with *Not*I and electrophoressed, together with non-*Not*I digested plugs, at 6 V/cm for 20H and 3 V/cm for 70H. The *Not*I-digested lanes are shown in Fig. 6BD.
(A) Dose-dependence of chromosomal fragmentation in undigested samples electrophoressed at 6V/cm for 20H.
(B) Percent signal in wells in *Not*I-digested samples upon 3 V/cm and 6 V/cm electrophoresis.
(C) Percent signal in the 360 kbp *Not*I doublet (fragment II) upon 3V electrophoresis.

Figure S9. *Not*I digestion and chromosomal fragmentation in non-replicating cells exposed to high UV doses. Stationary cultures of *recBC*(Ts) and *recBC*(Ts) *uvrA* mutants were exposed to 48 J/m^2 UV, and plugs were made after 10 min incubation in spent culture medium. One set of plugs were digested with *Not*I and electrophoressed, together with non-*Not*I digested plugs, at 6 V/cm for 20 H and 3 V/cm for 70 H.
(A) Chromosomal fragmentation in non *Not*I-digested samples at 6 V/cm.
(B) Radiogram of the gel from which the data in (A) are derived. CZ, compression zone.

(C) Percent signal in the 1 Mbp *Not*I fragment (6 V/cm) and compression zone (3 V/cm).
(D) Percent signal in the 360 kbp *Not*I doublet (fragment II) upon 3 V electrophoresis.
(E) Radiogram of the gel from which the data in (D) is derived. CZ, compression zone, FII, fragment II.

Fig. S1

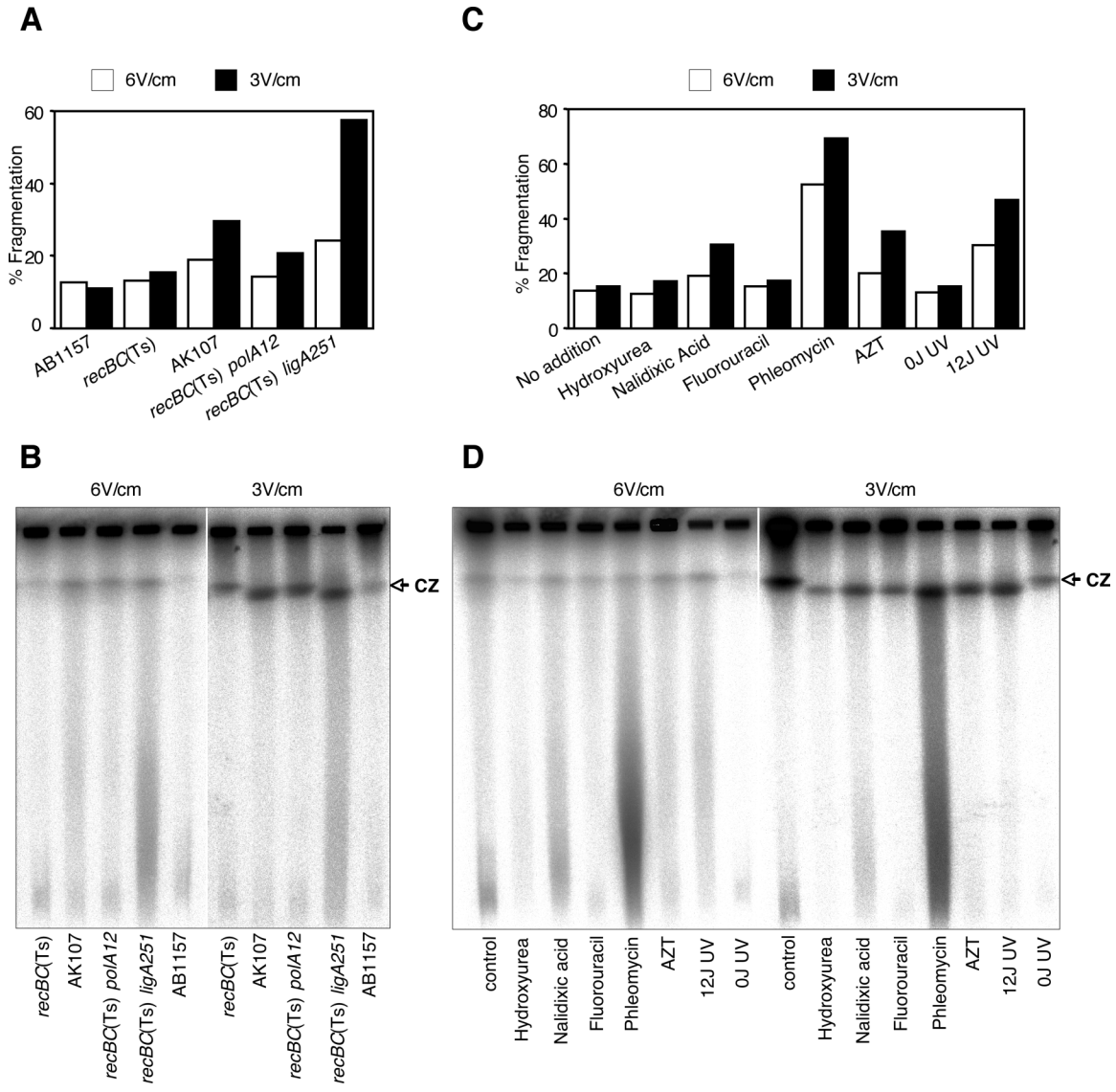

A
6V/cm
3V/cm
% Fragmentation
0
20
40
60
AB1157
recBC(Ts)
AK107
recBC(Ts) polA12
recBC(Ts) ligA251
C
6V/cm
3V/cm
% Fragmentation
0
20
40
60
80
No addition
Hydroxyurea
Nalidixic Acid
Fluorouracil
Phleomycin
AZT
0J UV
12J UV
B
6V/cm
3V/cm
CZ
recBC(Ts)
AK107
recBC(Ts) polA12
recBC(Ts) ligA251
AB1157
recBC(Ts)
AK107
recBC(Ts) polA12
recBC(Ts) ligA251
AB1157
D
6V/cm
3V/cm
CZ
control
Hydroxyurea
Nalidixic acid
Fluorouracil
Phleomycin
AZT
12J UV
0J UV
control
Hydroxyurea
Nalidixic acid
Fluorouracil
Phleomycin
AZT
12J UV
0J UV

Fig. S2

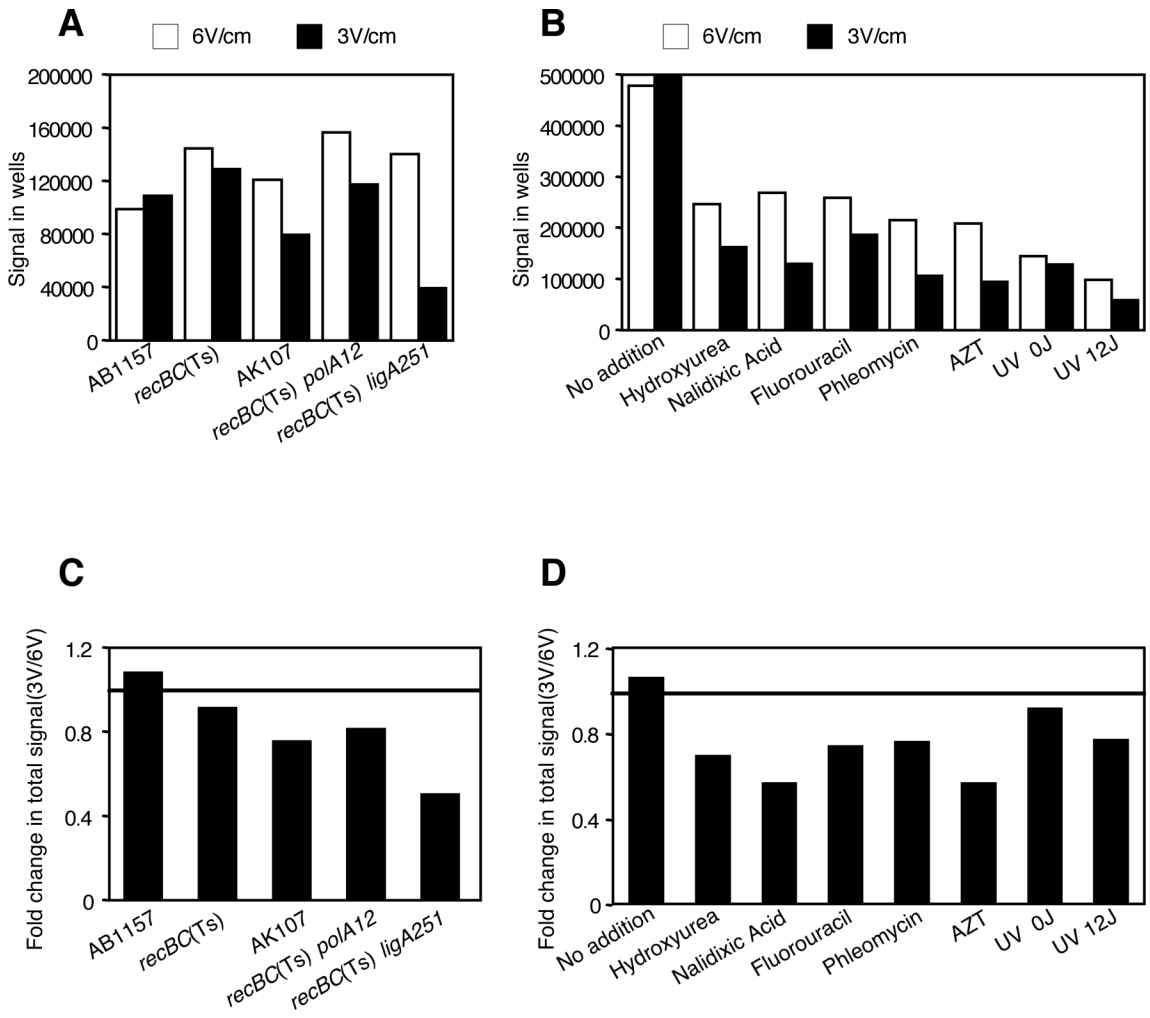

A
6V/cm
3V/cm
Signal in wells
200000
160000
120000
80000
40000
0
AB1157
recBC(Ts)
AK107
recBC(Ts) polA12
recBC(Ts) ligA251
B
6V/cm
3V/cm
Signal in wells
500000
400000
300000
200000
100000
0
No addition
Hydroxyurea
Nalidixic Acid
Fluorouracil
Phleomycin
AZT
UV 0J
UV 12J
C
Fold change in total signal(3V/6V)
1.2
0.8
0.4
0
AB1157
recBC(Ts)
AK107
recBC(Ts) polA12
recBC(Ts) ligA251
D
Fold change in total signal(3V/6V)
1.2
0.8
0.4
0
No addition
Hydroxyurea
Nalidixic Acid
Fluorouracil
Phleomycin
AZT
UV 0J
UV 12J

Fig. S3

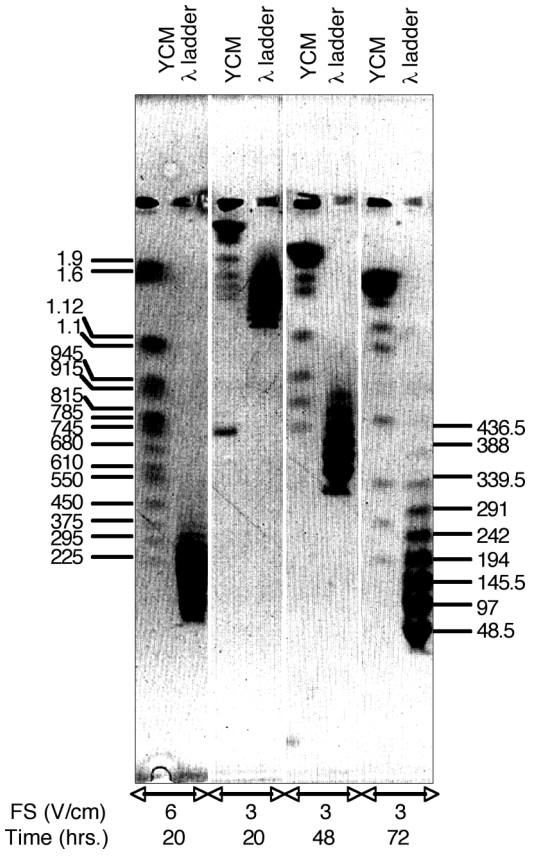

YCM
λ ladder
YCM
λ ladder
YCM
λ ladder
YCM
λ ladder
1.9
1.6
1.12
1.1
945
915
815
785
745
680
610
550
450
375
295
225
436.5
388
339.5
291
242
194
145.5
97
48.5
FS (V/cm)
Time (hrs.)
6
20
3
20
3
48
3
72

Fig. S4

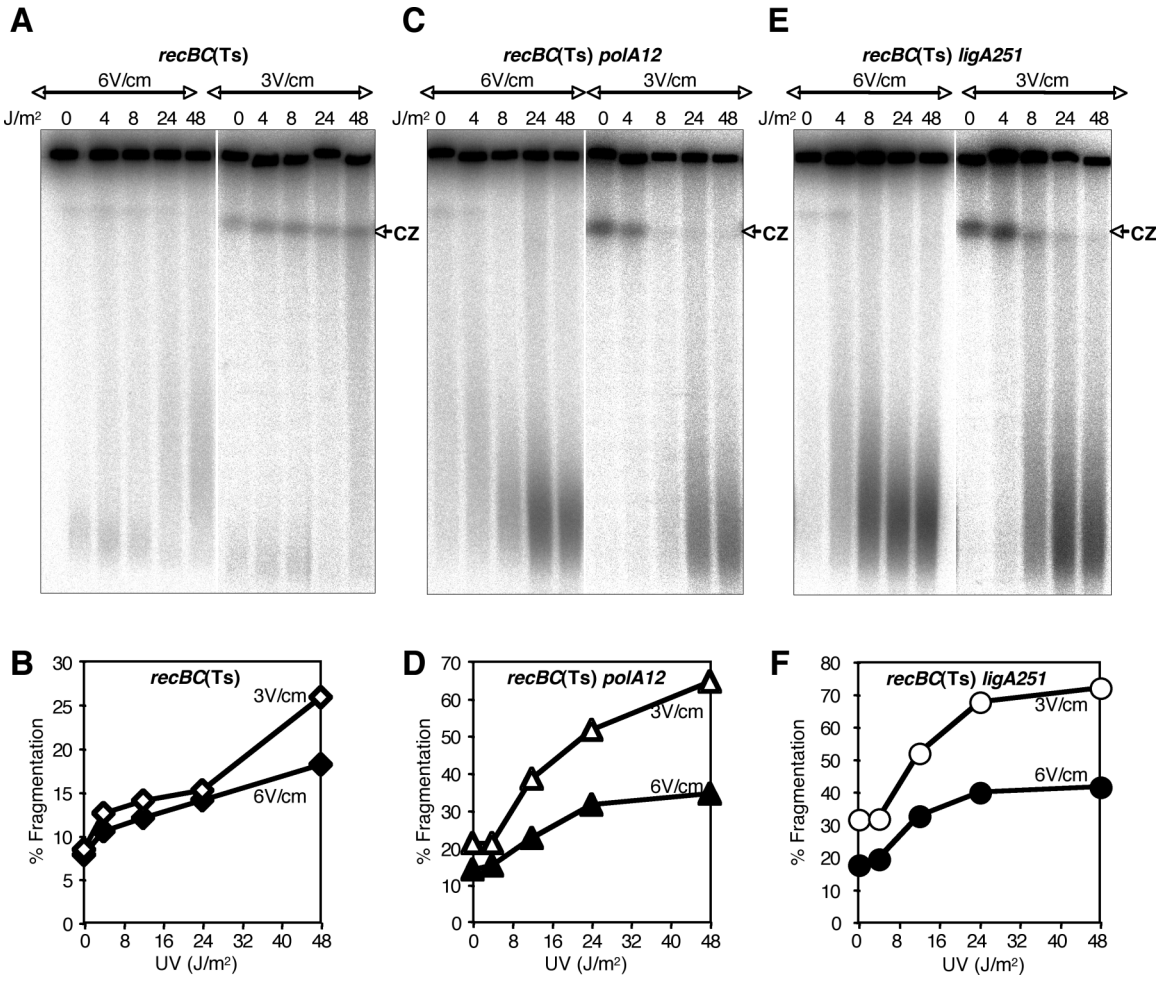

A
recBC(Ts)
6V/cm
3V/cm
J/m² 0 4 8 24 48 0 4 8 24 48
CZ
C
recBC(Ts) polA12
6V/cm
3V/cm
J/m² 0 4 8 24 48 0 4 8 24 48
CZ
E
recBC(Ts) ligA251
6V/cm
3V/cm
J/m² 0 4 8 24 48 0 4 8 24 48
CZ
B
recBC(Ts)
3V/cm
6V/cm
% Fragmentation
UV (J/m²)
D
recBC(Ts) polA12
3V/cm
6V/cm
% Fragmentation
UV (J/m²)
F
recBC(Ts) ligA251
3V/cm
6V/cm
% Fragmentation
UV (J/m²)

Fig. S5

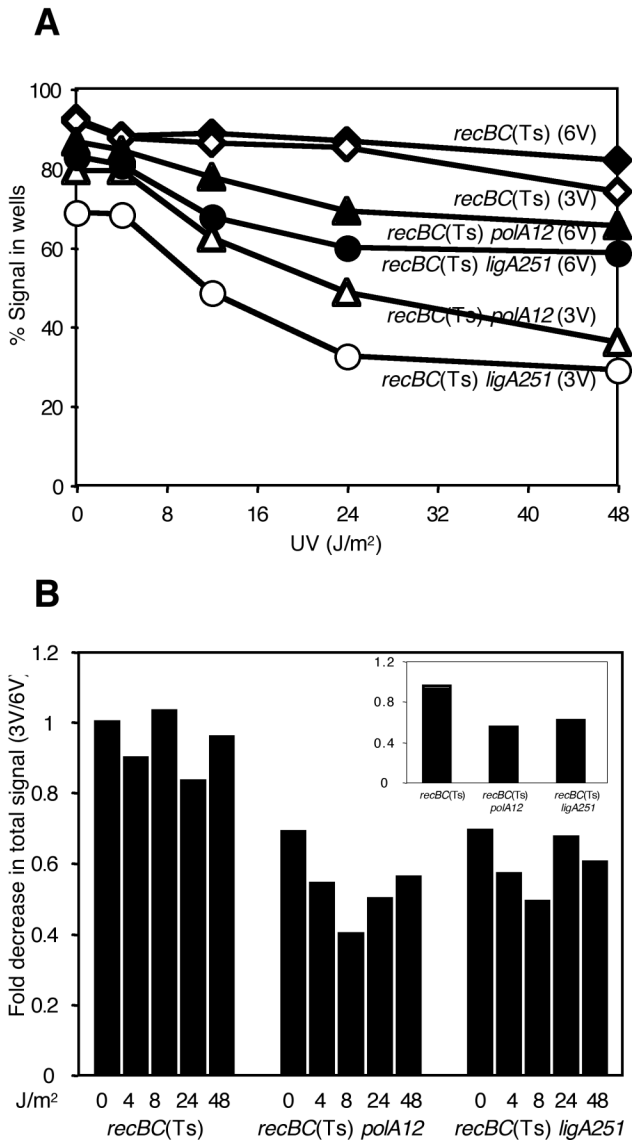

A
100
80
60
40
20
0
% Signal in wells
recBC(Ts) (6V)
recBC(Ts) (3V)
recBC(Ts) polA12 (6V)
recBC(Ts) ligA251 (6V)
recBC(Ts) polA12 (3V)
recBC(Ts) ligA251 (3V)
0 8 16 24 32 40 48
UV (J/m²)
B
1.2
1
0.8
0.6
0.4
0.2
0
Fold decrease in total signal (3V/6V)
J/m² 0 4 8 24 48 0 4 8 24 48 0 4 8 24 48
recBC(Ts) recBC(Ts) polA12 recBC(Ts) ligA251
1.2
0.8
0.4
0
recBC(Ts)
recBC(Ts) polA12
recBC(Ts) ligA251

Fig. S6

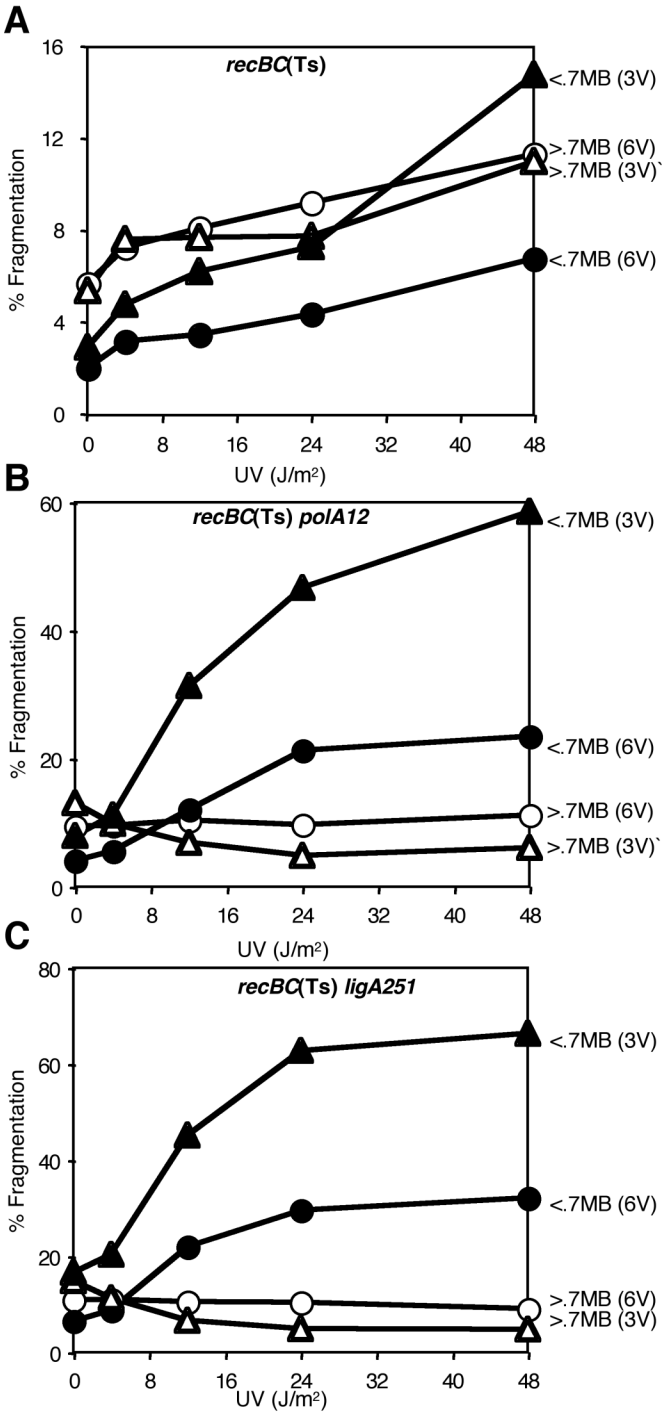

A
recBC(Ts)
% Fragmentation
UV (J/m²)
<.7MB (3V)
>.7MB (6V)
>.7MB (3V)`
<.7MB (6V)
B
recBC(Ts) polA12
% Fragmentation
UV (J/m²)
<.7MB (3V)
<.7MB (6V)
>.7MB (6V)
>.7MB (3V)`
C
recBC(Ts) ligA251
% Fragmentation
UV (J/m²)
<.7MB (3V)
<.7MB (6V)
>.7MB (6V)
>.7MB (3V)

Fig. S7

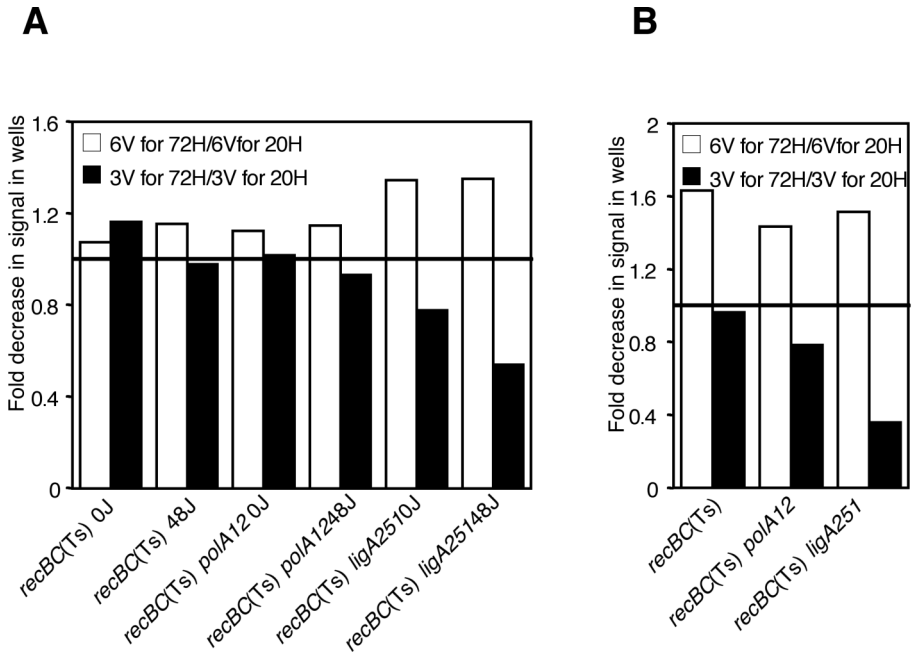

A
B
Fold decrease in signal in wells
6V for 72H/6Vfor 20H
3V for 72H/3V for 20H
0
0.4
0.8
1.2
1.6
2
recBC(Ts) 0J
recBC(Ts) 48J
recBC(Ts) polA120J
recBC(Ts) polA1248J
recBC(Ts) ligA2510J
recBC(Ts) ligA25148J
recBC(Ts)
recBC(Ts) polA12
recBC(Ts) ligA251

Fig. S8

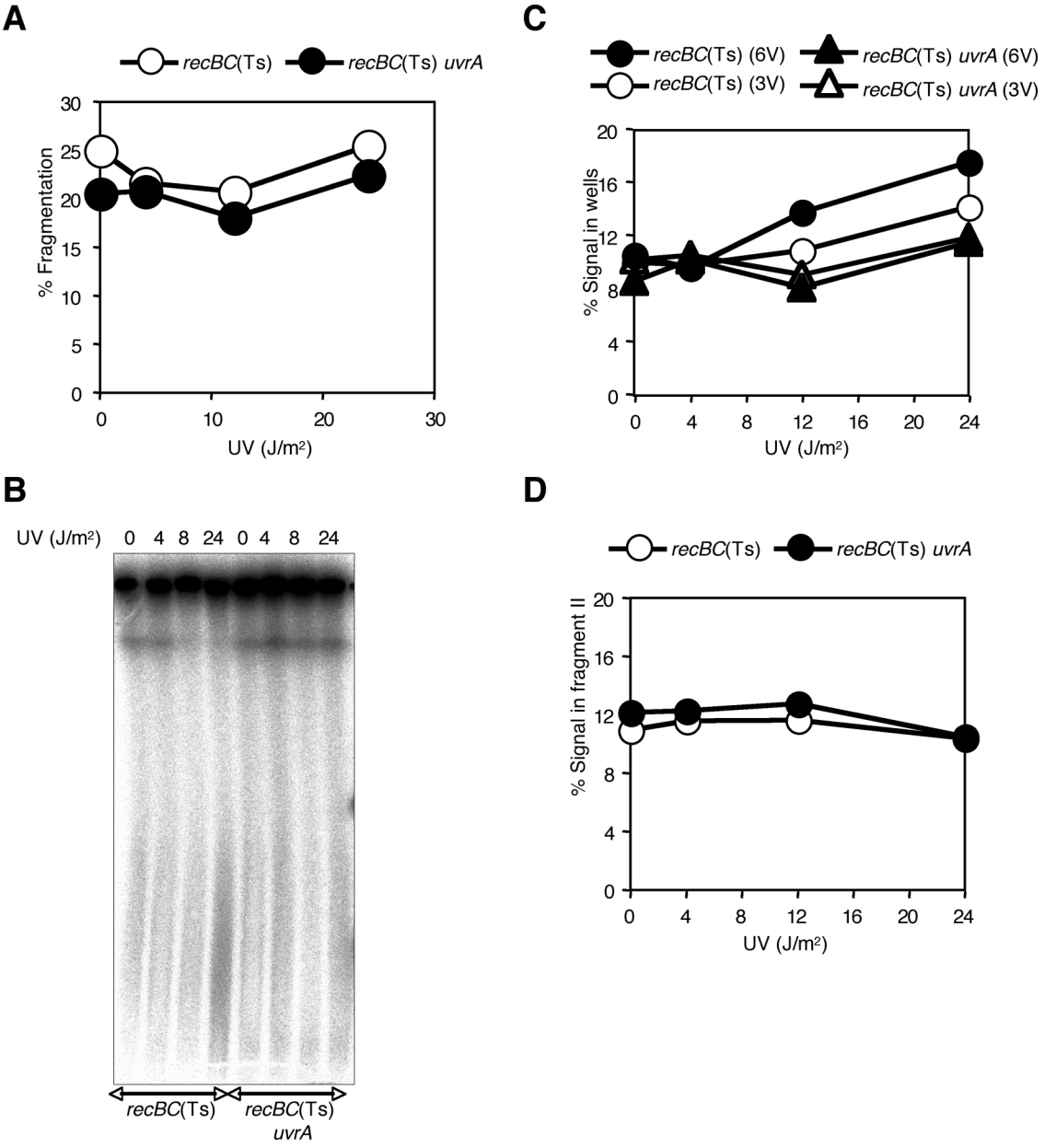
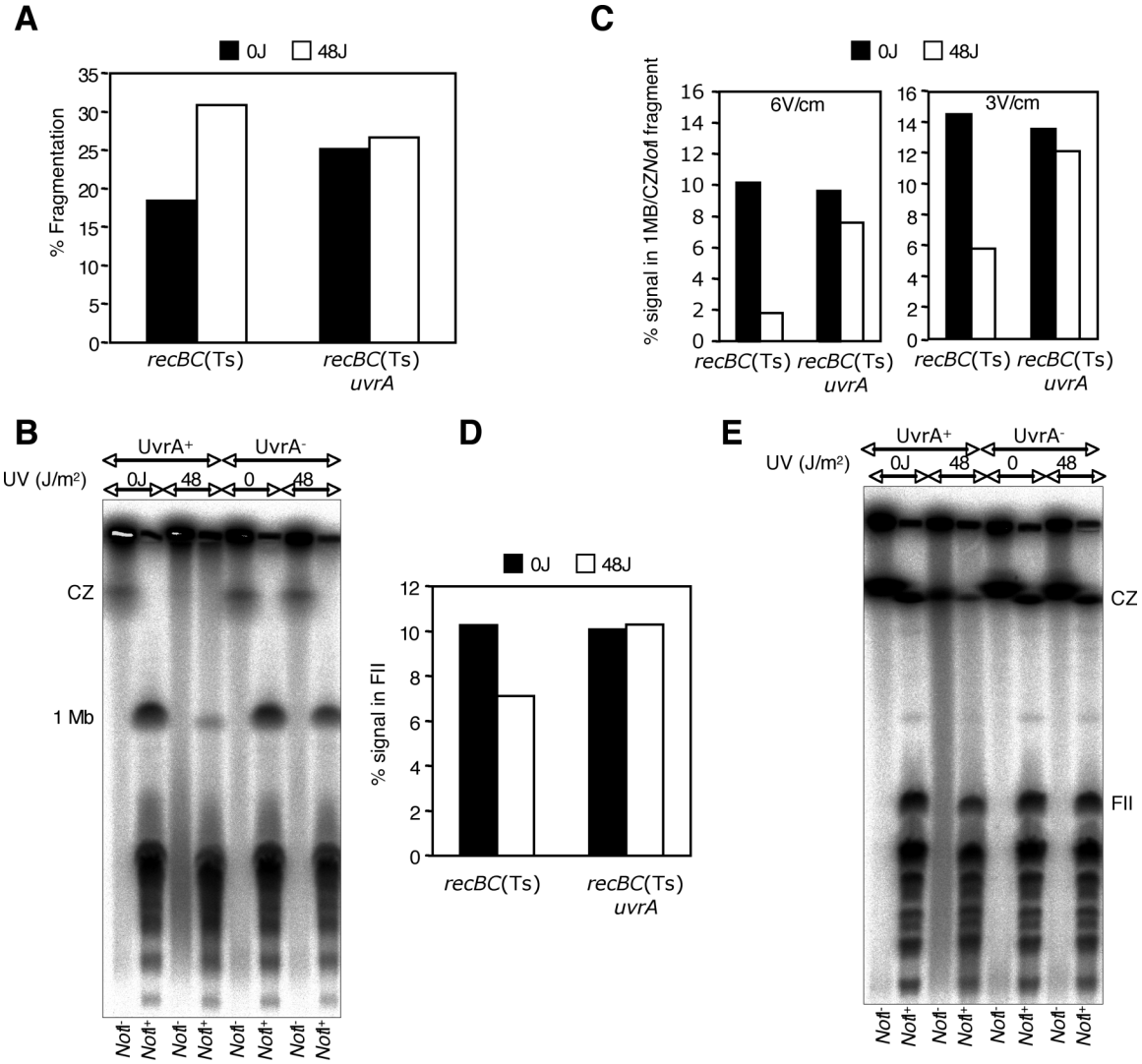

A
recBC(Ts)
recBC(Ts) uvrA
% Fragmentation
UV (J/m²)
B
UV (J/m²) 0 4 8 24 0 4 8 24
recBC(Ts)
recBC(Ts) uvrA
C
recBC(Ts) (6V)
recBC(Ts) uvrA (6V)
recBC(Ts) (3V)
recBC(Ts) uvrA (3V)
% Signal in wells
UV (J/m²)
D
recBC(Ts)
recBC(Ts) uvrA
% Signal in fragment II
UV (J/m²)

Fig. S9


A
0J
48J
% Fragmentation
recBC(Ts)
recBC(Ts) uvrA
C
% signal in 1MB/CZNotI fragment
6V/cm
3V/cm
B
UvrA+
UvrA-
UV (J/m²)
CZ
1 Mb
NotI-
NotI+
D
% signal in FII
E
FII