Supplementary Material

Supplementary Figure 1. Reversible strand exchange reaction between short oligonucleotides in the presence of ATPγS. (Original data for Fig.1B)

(A) Controls of zero points and stability of ds-oligonucleotides under electrophoretic conditions. Lane 1, single stranded dye labeled oligonucleotide. Lanes 2-5, the reaction substrates for direct (2,3) and reverse (4,5) strand exchange reaction were prepared as described in Material and Methods, mixed at 0^{O}C, deproteinized with addition of SDS and electrophoresed as described. Substrates with perfect homology (2,4) and containing one base pair substitution in the ds-oligonucleotide (3,5) were used.

(B) The time course of the direct RecA promoted strand exchange reactions. Lanes 1,3,4,7, mismatch containing substrates, incubation time 3, 8, 20, 40 min. correspondingly (curve 3 in Fig.1B). Lanes 2,5,6,8, substrates of perfect homology, incubation time 3, 8, 20, 40 min. correspondingly (curve 4 in Fig.1B).

(C) The time course of the reverse RecA promoted strand exchange reactions. Lanes 1,3,5,7, mismatch containing substrates, incubation time 3, 8, 20, 40 min. correspondingly (curve 1 in Fig.1B). Lanes 2,4,6,8, substrates of perfect homology, incubation time 3, 8, 20, 40 min. correspondingly (curve 2 in Fig.1B). The reaction time, direction ("+" for the direct and "-" for the reverse exchange) and configuration of substrates (P for perfect homology between the substrates; M, mismatch containing substrates) are indicated.

Supplementary Figure 2. Scans of electrophoretic lanes of the gels presented in Supplementary Figure 1. The scans are grouped to facilitate visualization of the effect of the convergence of the direct and the reverse strand exchange reactions. Data for strand exchange between mismatch containing substrates (A) and the substrates of perfect homology (B) are presented. The direction of reaction is designated by "+" for direct and "-" for the reverse reactions. Each scan is designated by the number of the corresponding panel and the gel lane presented in Supplementary Figure 1. For example, scan A1 corresponds to lane 1 of the gel presented in panel A; scan C3 - to lane 3 of the gel presented in panel C etc. Near the right end of each scan the corresponding reaction yield (in percent of ds-oligonucleotide) is also indicated.

Supplementary Figure 3. RecA promoted strand exchange reactions. The influence of the order of the protein addition (Original data for Fig.4A)

The time course of the forward (A) and reverse (B;C) RecA promoted strand exchange between mismatch containing oligonucleotides substrates. (A) Direct strand exchange reaction. Lanes 1,3,5,7, conventional reaction scheme (with preincubation of RecA with the dye labeled oligonucleotide), incubation time 1.5, 5, 15, 45 min. correspondingly (curve 1 in Fig.4A). Lanes 2,4,6,8, no preincubation of RecA with the dye labeled oligonucleotides, incubation time 1.5, 5, 15, 45 min. correspondingly (curve 3 in Fig.4A). (B) Reverse strand exchange reaction, conventional reaction scheme. Lanes 1,2,3,4, incubation time 1.5, 5, 15, 45 min. correspondingly (curve 2 in Fig.4A). (C) Reverse strand exchange reaction, no preincubation of RecA with the dye labeled oligonucleotides. Lanes 1,2,3,4, incubation time 1.5, 5, 15, 45 min. correspondingly (curve 4 in Fig.4A). The reaction time and reaction scheme (C for conventional reaction scheme and N for no premix of RecA protein with fluorescent labeled oligonucleotides) are indicated.

Supplementary Figure 4. Scans of electrophoretic lanes of the gels presented in Supplementary Figure 3. The scans are grouped to facilitate visualization of the effect of the convergence of the direct and the reverse strand exchange reactions in the experiment with the conventional reaction scheme (A) and the absence of such convergence in experiments with no preincubation of RecA with the dye labeled oligonucleotide (B). Each scan is designated by the number of the corresponding panel and the gel lane presented in Supplementary Figure 3. See legend for Supplementary Fig.2 for other designations.

Supplementary Figure 5. Reverse strand exchange reactions. The influence of the order of protein addition and an excess of single stranded oligonucleotides. (Original data for Fig.4B)

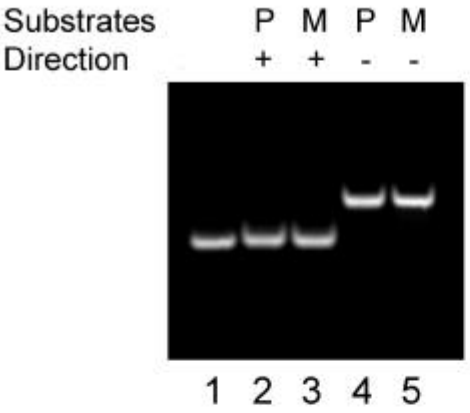
(A) The time course of the reverse strand exchange reactions. Lanes 1,3,5,7, RecA promoted strand exchange; conventional reaction scheme; incubation time 3, 10, 30, 90 min. correspondingly (curve 1 in Fig.4B). Lanes 2,4,6,8, spontaneous strand exchange (RecA is absent); incubation time 3, 10, 30, 90 min. correspondingly (curve 5 in Fig.4B).

(B) The time course of the reverse strand exchange reactions in the presence of an excess of heterologous oligonucleotide. Lanes 1,3,5,7, no preincubation of RecA with the dye labeled oligonucleotide, incubation time 3, 10, 30, 90 min. correspondingly (curve 4 in Fig.4B). Lanes 2,4,6,8, conventional reaction scheme, incubation time 3, 10, 30, 90 min. correspondingly (curve 2 in Fig.4B).

(C) Lanes 1,2,3,4, RecA promoted strand exchange with an excess of homologous oligonucleotides (+16mer); conventional reaction scheme; incubation time 5, 10, 30, 90 min. correspondingly (curve 3 in Fig.4B). The reaction time, presence of RecA protein and the reaction scheme are also indicated (R, RecA promoted exchange; S, spontaneous exchange; C, conventional reaction scheme; N, no preincubation of RecA with fluorescent dye labeled oligonucleotides).

Supplementary Figure 6. Scans of electrophoretic lanes of the gels presented in Supplementary Figure 5. (A) RecA promoted and spontaneous reverse strand exchange. (B) RecA promoted reverse strand exchange reactions in the presence of an excess of heterologous oligonucleotides. Data for conventional reaction scheme and experiments with no preincubation of RecA with the dye labeled oligonucleotides are presented. (C) RecA promoted strand exchange with an excess of homologous oligonucleotides; conventional reaction scheme. Each scan is designated by the number of the corresponding panel and the gel lane presented in Supplementary Figure 5. The reaction time for all three panels is indicated in the left part of the Figure. See legend for Supplementary Fig.5 for other designations.

A. Zero point controls



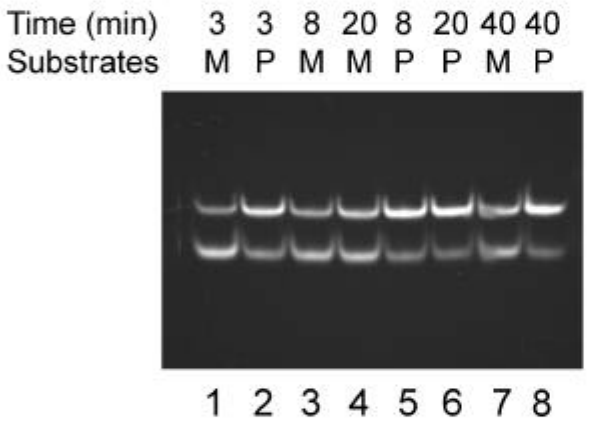
+ - direct exchange

– - reverse exchange

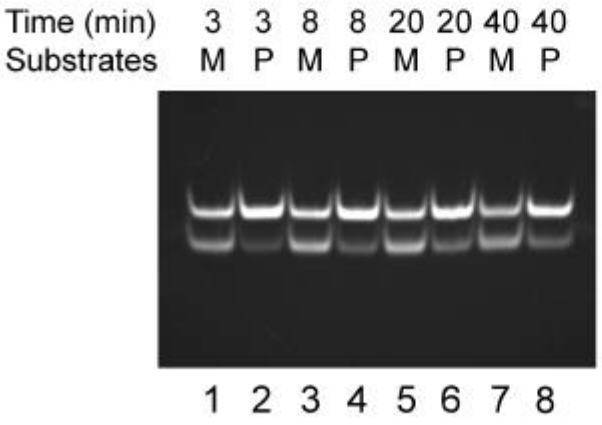
P - perfect homology

M - mismatched substrates

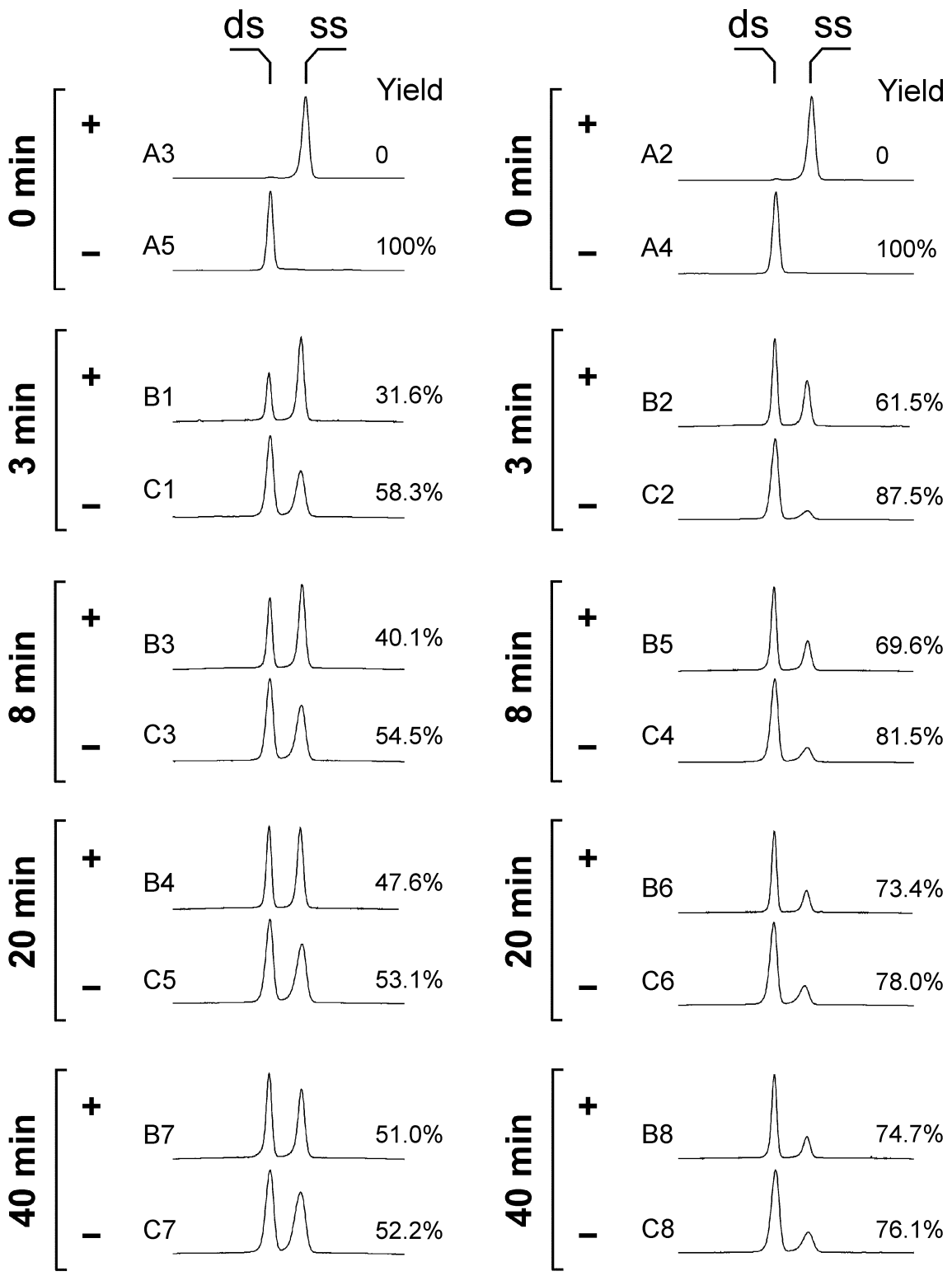
B. Direct exchange



C.Reverse exchange

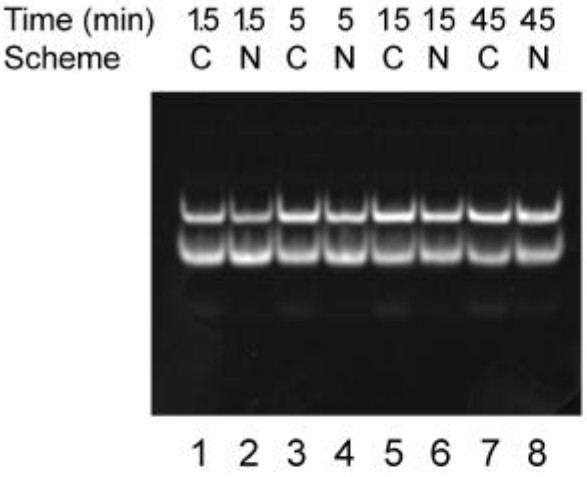


Suppl. Figure 1



Suppl. Figure 2

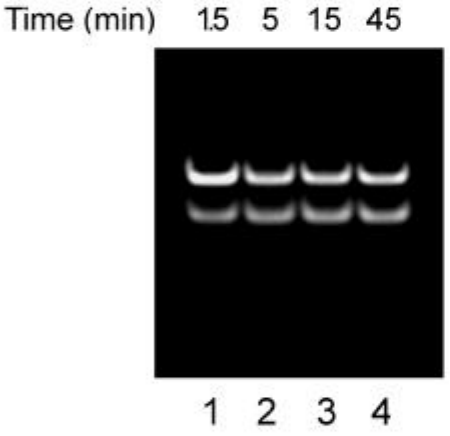
A. Direct exchange. Conventional scheme and No premix



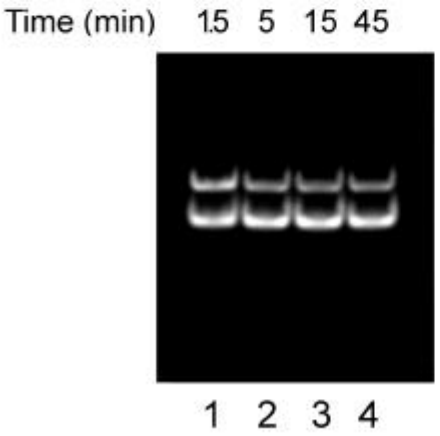
C - conventional scheme

N - no premix

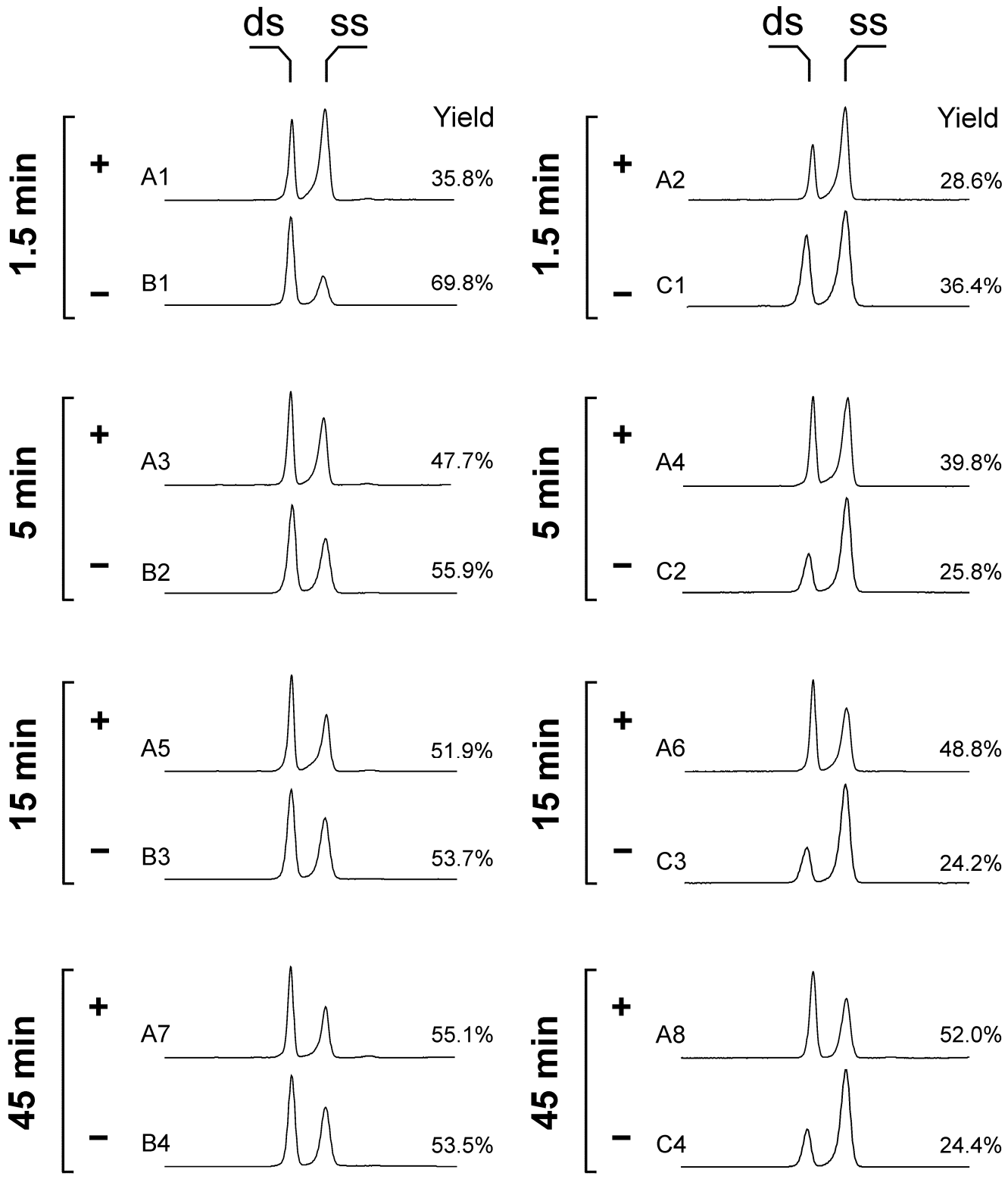
B. Reverse exchange. Conventional scheme



C. Reverse exchange. No premix

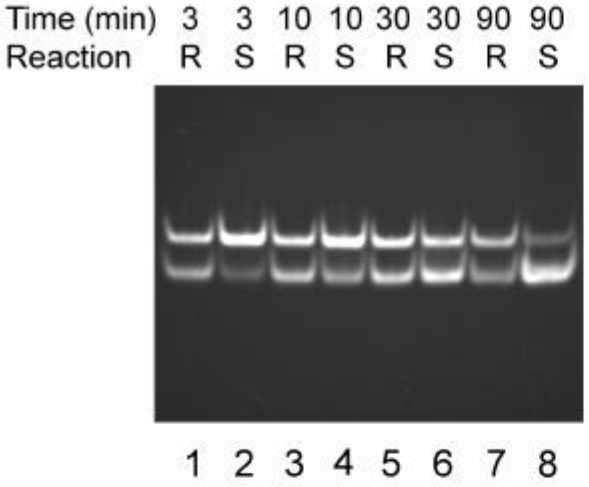


Suppl. Figure 3



Suppl. Figure 4

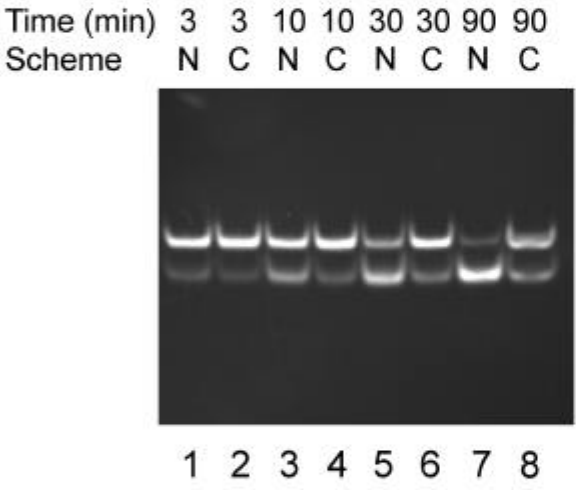
A. Reverse exchange. RecA promoted and spontaneous



R - RecA promoted

S - spontaneous

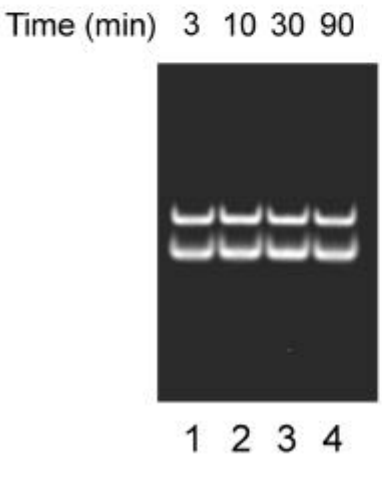
B. Excess of heterologous oligonucleotide



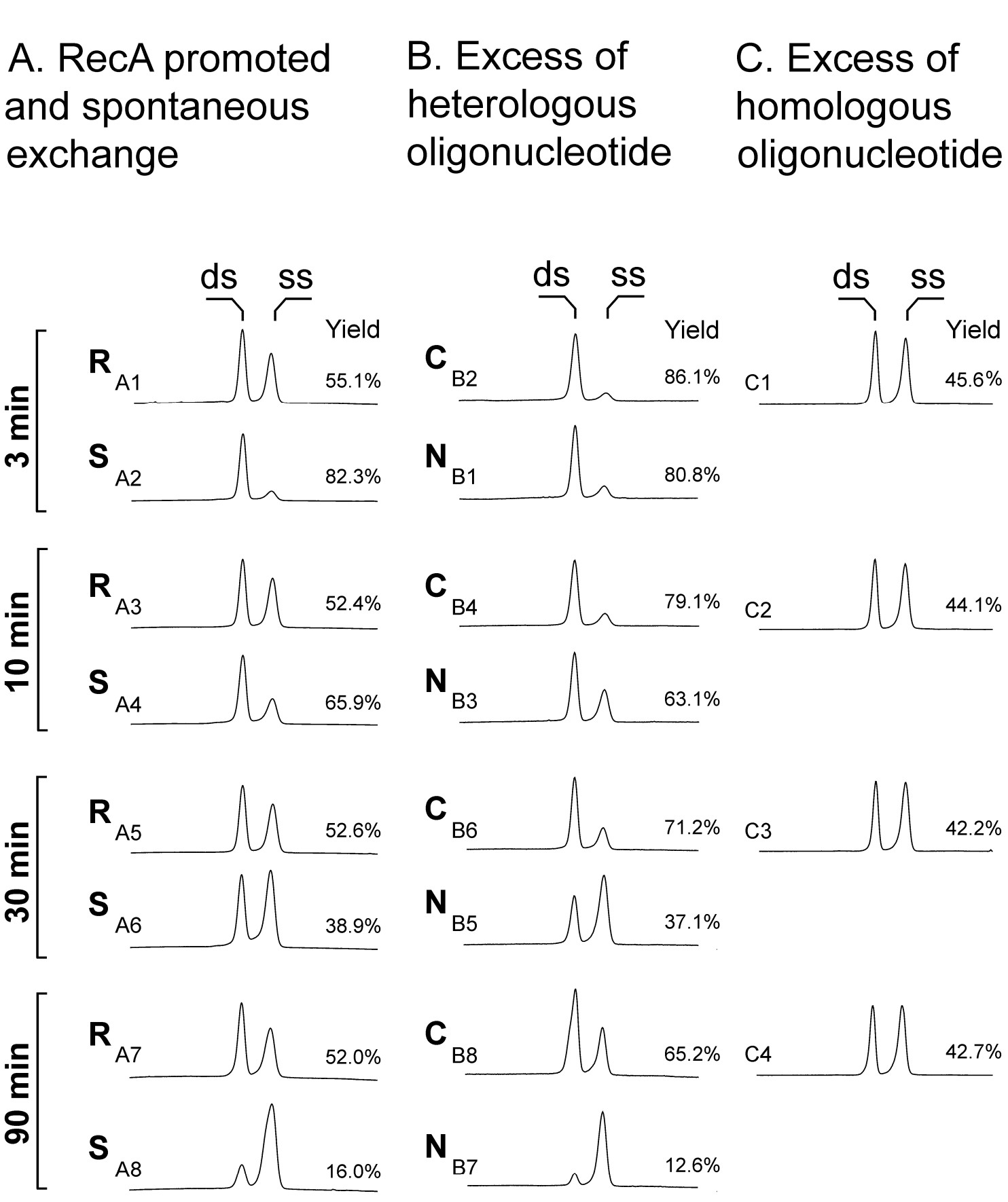
C - conventional scheme

N - no premix

C. Excess of homologous oligonucleotide



Suppl. Figure 5



Suppl. Figure 6