

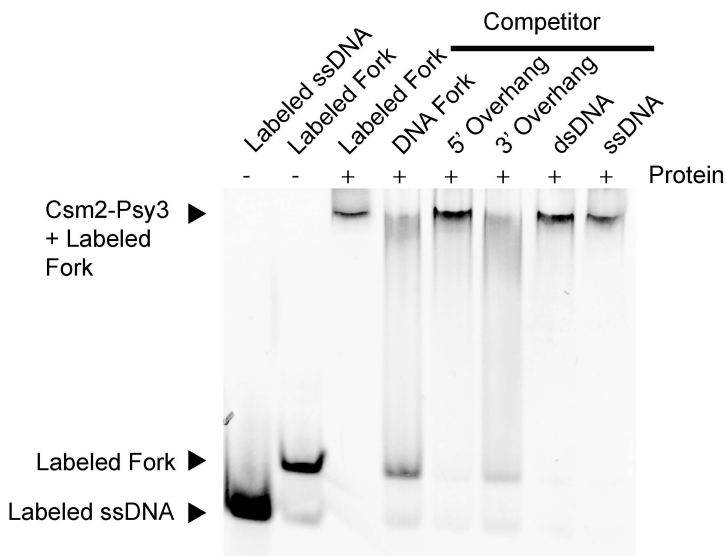
SUPPLEMENTARY TABLE 1: Strains and plasmids

Name	Description
W1588-4A	<i>MATα</i> <i>ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5</i>
W5909-1B	<i>MATa ADE2 TRP1 lys2Δ</i> <i>MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
PJ69-4A	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ</i> <i>MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
PJ69-4 α	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ</i> <i>MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
KBY 217	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ rad51::natMX4</i> <i>MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
KBY 222	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ rad51::natMX4</i> <i>MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
KBY 212	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ rad55::natMX4</i> <i>MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ</i>
KBY 221	<i>gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3</i> <i>met2::GAL7-lacZ rad55::hphMX4</i>
W3770-4D	<i>MATa leu2-ΔEcoRI::URA3::leu2-ΔBstEII</i>
KBY57-1B	<i>MATa leu2-ΔEcoRI::URA3-HO::leu2-ΔBstEII</i> <i>psy3::KanMX4</i>
KBY38-3B	<i>MATa leu2-ΔEcoRI::URA3-HO::leu2-ΔBstEII</i> <i>csm2::KanMX4</i>
KBY65-21C	<i>MATa leu2-ΔEcoRI::URA3-HO::leu2-ΔBstEII rad55Δ</i>

KBY65-1C	<i>MATa leu2-ΔEcoRI::URA3-HO::leu2-ΔBstEII</i> <i>csm2::KanMX4 rad55Δ</i>
KBY65-12B	<i>MATa csm2::KanMX rad55Δ leu2-ΔEcoRI::URA3-</i> <i>HO::leu2-ΔBstEII</i>
KBY15-2A	<i>MATa csm2::KanMX</i>
KBY89-2B	<i>MATα rad51::LEU2 ade2-n::TRP1::ade2-I-SceI</i> <i>lys2::GAL-I-SceI</i>
W4121-9B	<i>MATa rad55Δ bar1::LEU2</i>
W3791-2B	<i>MATa rad57::LEU2 YFP-8ala-RAD55 bar1::LEU2</i>
KBY107-5C	<i>MATa csm2::KanMX</i>
KBY108-1C	<i>MATa psy3::KanMX bar1::LEU2</i>
W9100-2D	<i>MATα ADE2 leu2-3,112 his3-11,15 ura3-1 TRP1 lys2Δ</i>
W3778-2B	<i>MATa YFP-8ala-RAD55 bar1::LEU2</i>
KBY108-5A	<i>MATα psy3::KanMX4 YFP-8ala-Rad55</i>
KBY107-4A	<i>MATα csm2::KanMX YFP-8ala-Rad55</i>
pWJ1481	<i>pGBD-CSM2 (TRP, KAN^R)</i>
pKBB5	<i>pGBD-PSY3 (TRP, KAN^R)</i>
pGBD-C1	<i>pGBD-C1 (TRP, KAN^R)</i>
pGAD-C2	<i>pGAD-C2 (LEU, AMP^R)</i>
pGAD-RAD51	<i>pGAD-C2-RAD51 (cloned SmaI to Sall; LEU, AMP^R)</i>
pGAD-RAD55	<i>pGAD-RAD55 (LEU, AMP^R)</i>
pGAD-RAD57	<i>pGAD-RAD57 (LEU, AMP^R)</i>
pWJ1921	<i>pCDF-DUET1 with CSM2-TEV in MCS1 (PstI to</i> <i>HindIII) and TEV-PSY3 in MCS2 (BglIII to KpnI)</i>
pRS313-RAD51	<i>pRS313-RAD51 (CEN, HIS, AMP^R)</i>
pRS416	<i>pRS416 (CEN, URA, AMP^R)</i>
pRS413	<i>pRS413 (CEN, HIS, AMP^R)</i>
pRS313-RAD57	<i>pRS313-RAD57 (CEN, HIS, AMP^R)</i>
pRS316-RAD55	<i>pRS316-RAD55 (CEN, URA, AMP^R)</i>

All yeast strains are W303 background derivatives and *RAD5* (23) W1588 (24) except for PJ69-4A and PJ69-4 α (25). The KBY217 and KBY212 were constructed in PJ69-4A strain background whereas KBY222 and KBY221 were constructed in PJ69-4 α strain background. The strains are listed in the order they appear in the figures and text.

Supplemental Figure 1



Supplemental Figure 1. The forked DNA and 3' overhang DNA substrates are preferentially bound by the Csm2-Psy3 heterodimer by electrophoretic mobility shift assay. Fluorescein-labeled DNA fork (2.6 pmol) was incubated on ice for 20 minutes with the Csm2-Psy3 heterodimer (30 pmol) either alone or in the presence of 13 pmol unlabeled DNA competitors (DNA fork, 5' overhang, 3' overhang, dsDNA, and ssDNA). Protein-free fluorescein-labeled ssDNA and fluorescein-labeled fork are shown on the left-hand side of the gel.

SUPPLEMENTARY METHODS

Competition electrophoretic mobility shift assay (EMSA). Fluorescein-labeled DNA fork (260 nM), detailed in Table 1, with or without an unlabeled competitor DNA oligonucleotide (1300 nM), was incubated on ice for 30 minutes with purified Csm2-Psy3 complex (3.0 μ M) in EMSA reaction buffer (10 mM Tris pH 8.0, 50 mM NaCl, 2 mM DTT, 4 mM $MgCl_2$) in a reaction volume of 10 μ l. Equilibrated samples were loaded on a pre-cooled and pre-run 5% native polyacrylamide gel containing 0.5X TBE and run at 200 volts for 2 hours at 4°C. The resulting gel was visualized by fluorescence using a FLA-5100 Fluorescent Image Analyzer (FujiFilm).