Supplemental Figure Legends

Supplemental Figure S1. Analysis of 2 μm hyperamplification. (A) DNA from *siz1Δ siz2Δ* cells was prepared either embedded in agarose (Schwartz and Cantor, 1984) (left panel) or in solution (Holm *et al.*, 1986) (right panel). Agarose-embedded DNA was either melted and loaded directly (lane 1) or treated with β-agarase (lane 2). DNA in solution was loaded directly (lane 4) or brought to 0.6% low melting agarose before loading (lane 3). (B) Uncut DNA from the indicated strains was analyzed by Southern blotting as in Figure 3. Lanes were normalized to contain equal amounts of 2 μm DNA, although the *xrs2Δ* sample was underloaded. Designations as in Figure 3. (C) Indicated strains containing plasmids expressing thymidine kinase (TK) and a nucleoside transporter (hENT) (Vernis *et al.*, 2003) were grown to log phase or arrested with nocodazole (noc) for three hours prior to labeling for 1 hour with bromo-deoxyuridine (BrdU) as described (Vernis *et al.*, 2003). DNA was left uncut or digested with the indicated restriction enzyme, as indicated, and analyzed by agarose gel electrophoresis, followed by immunoblotting with an Ab against BrdU. Note the absence of a 6.3 kb 2 μm band in the noc-arrested samples digested with *Pst*I, which cuts 2 μm once.

Supplemental Figure S2. Diagram of locus-specific QAOS (Booth *et al.*, 2001). A "tagging primer" with 8 b of homology with a particular locus is incubated with target DNA sample, Taq polymerase and dNTPS at low temperature. If ssDNA is present at this locus, the primer will anneal and be extended. The primer will not anneal to dsDNA because the DNA has not been denatured. Next qPCR is performed using one primer specific for the tag sequence in the tagging primer (in red) and one primer to a sequence flanking the target locus. This reaction will only generate a product if the tagging primer was extended during the first reaction because the tagging primer cannot anneal at the temperatures used during PCR. Thus, it cannot initiate amplification of DNA that was originally double-stranded, but which is denatured during the PCR reaction. Unlike in Booth et al, our tagging primers contained only 8 b of homology with the

target sequence. Longer sequences gave high background signals, presumably because the tagging primer could prime to some degree during the PCR portion of the protocol.

REFERENCES

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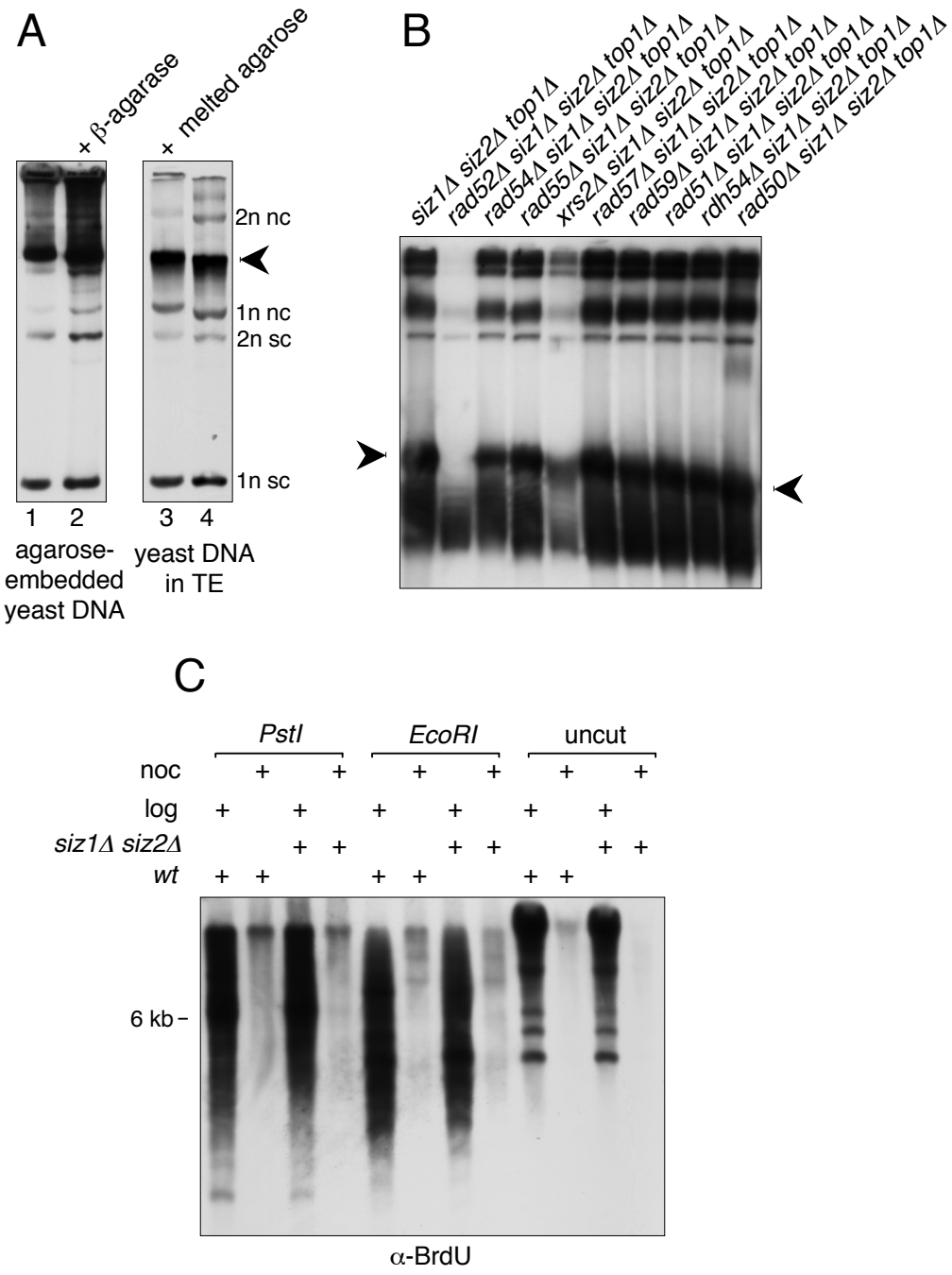
Schwartz, D.C., and Cantor, C.R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell *37*, 67-75.

Vernis, L., Piskur, J., and Diffley, J.F. (2003). Reconstitution of an efficient thymidine salvage pathway in *Saccharomyces cerevisiae*. Nucleic Acids Res *31*, e120.


A
+ β-agarase
+ melted agarose
2n nc
1n nc
2n sc
1n sc
1 2 3 4
agarose-embedded yeast DNA
yeast DNA in TE
B
siz1Δ siz2Δ top1Δ
rad52Δ siz1Δ siz2Δ top1Δ
rad54Δ siz1Δ siz2Δ top1Δ
rad55Δ siz1Δ siz2Δ top1Δ
xrs2Δ siz1Δ siz2Δ top1Δ
rad57Δ siz1Δ siz2Δ top1Δ
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rad51Δ siz1Δ siz2Δ top1Δ
rdh54Δ siz1Δ siz2Δ top1Δ
rad50Δ siz1Δ siz2Δ top1Δ
C
PstI
EcoRI
uncut
noc
log
siz1Δ siz2Δ
wt
6 kb
α-BrdU

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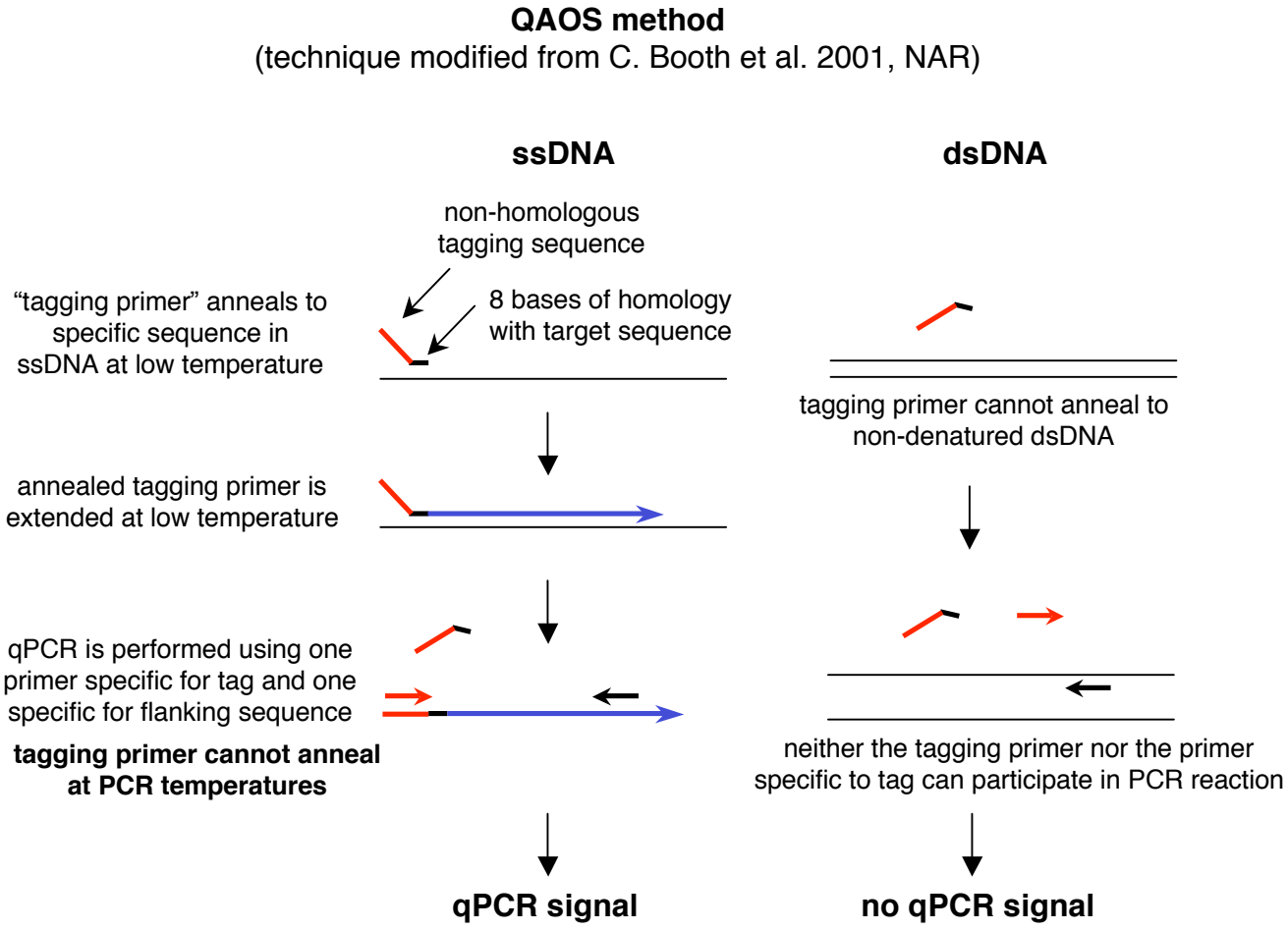

Table S1. *S. cerevisiae* strains

Name	Relevant genotype	Source
JD52[a]	*MAT***a** *trp1-Δ1 ura3-52 his3-Δ200 leu2-3,112 lys2-801* [cir$^+$]	J. Dohmen
EJY326	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1* [cir$^+$]	Johnson and Gupta (20
EJY341	*MAT***a** [cir°]	Chen et al (2005)
EJY346	*MAT***a** [cir$^+$ *FLP1-HA-His$_8$::HIS3*]	Chen et al (2005)
EJY349	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1* [cir$^+$ *FLP1-HA-His$_8$::HIS3*]	Chen et al (2005)
EJY356	*MAT***a** [cir$^+$ *FLP1(K375R)-HA-His$_8$::HIS3*]	Chen et al (2005)
EJY359	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1* [cir$^+$ *FLP1(K375R)-HA-His$_8$::HIS3*]	Chen et al (2005)
EJY416	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3* [cir°]	Chen et al (2007)
EJY420	*MAT***a** *rad52Δ::kanMX siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3* [cir°]	Chen et al (2007)
EJY509	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3* [cir$^+$]	this study
EJY510	*MAT***a** *rad52Δ::kanMX siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3* [cir$^+$]	this study
EJY511	*MAT***a** *rad52Δ::kanMX* [cir$^+$]	this study
EJY512	*MAT***a** *rad52Δ::kanMX siz1Δ::LEU2 siz2Δ::TRP1* [cir$^+$]	this study
EJY513	*MAT***a** *hex3Δ::kanMX* [cir$^+$]	this study
EJY514	*MAT***a** *hex3Δ::kanMX rad52Δ::kanMX* [cir$^+$]	this study
EJY515	*MAT***a** *slx8Δ::kanMX* [cir$^+$]	this study
EJY516	*MAT***a** *slx8Δ::kanMX rad52Δ::kanMX* [cir$^+$]	this study
EJY517	*MAT***a** *RAD52-GFP::URA3* [cir°]	this study
EJY518	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 RAD52-GFP::URA3* [cir°]	this study

EJY519	*MAT***a** *hex3Δ::kanMX RAD52-GFP::URA3* [cir°]	this study
EJY520	*MAT***a** *nup60Δ::kanMX RAD52-GFP::URA3* [cir°]	this study
EJY521	*MAT***a** *RAD52-GFP::URA3* [cir$^+$]	this study
EJY522	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 RAD52-GFP::URA3* [cir$^+$]	this study
EJY523	*MAT***a** *hex3Δ::kanMX RAD52-GFP::URA3* [cir$^+$]	this study
EJY524	*MAT***a** *nup60Δ::kanMX RAD52-GFP::URA3* [cir$^+$]	this study
EJY525	*MAT***a** *hex3Δ::kanMX* [cir$^+$ *FLP1(K375R)-HA-His$_8$::HIS3*]	this study
EJY526	*MAT***a** *slx8Δ::kanMX* [cir$^+$ *FLP1(K375R)-HA-His$_8$::HIS3*]	this study
EJY527	*MAT***a** [cir$^+$ *FLP1(Y343F)-HA-His$_8$::HIS3*]	this study
EJY528	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1* [cir$^+$ *FLP1(Y343F)-HA-His$_8$::HIS3*]	this study
EJY529	*MAT***a** *hex3Δ::kanMX* [cir$^+$ *FLP1(Y343F)-HA-His$_8$::HIS3*]	this study
EJY530	*MAT***a** *slx8Δ::kanMX* [cir$^+$ *FLP1(Y343F)-HA-His$_8$::HIS3*]	this study
EJY531	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 pol32Δ::kanMX* [cir$^+$]	this study
EJY532	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rad51Δ::kanMX* [cir$^+$]	this study
EJY533	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rad59Δ::kanMX* [cir$^+$]	this study
EJY534	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rad51Δ::kanMX rad59Δ::kanMX* [cir$^+$]	this study
EJY535	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rad54Δ::kanMX* [cir$^+$]	this study
EJY536	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rdh54Δ::kanMX* [cir$^+$]	this study
EJY537	*MAT***a** *siz1Δ::LEU2 siz2Δ::TRP1 top1Δ::HIS3 rad54Δ::kanMX rdh54Δ::kanMX* [cir$^+$]	this study

[a] Strains are in the JD52 background.