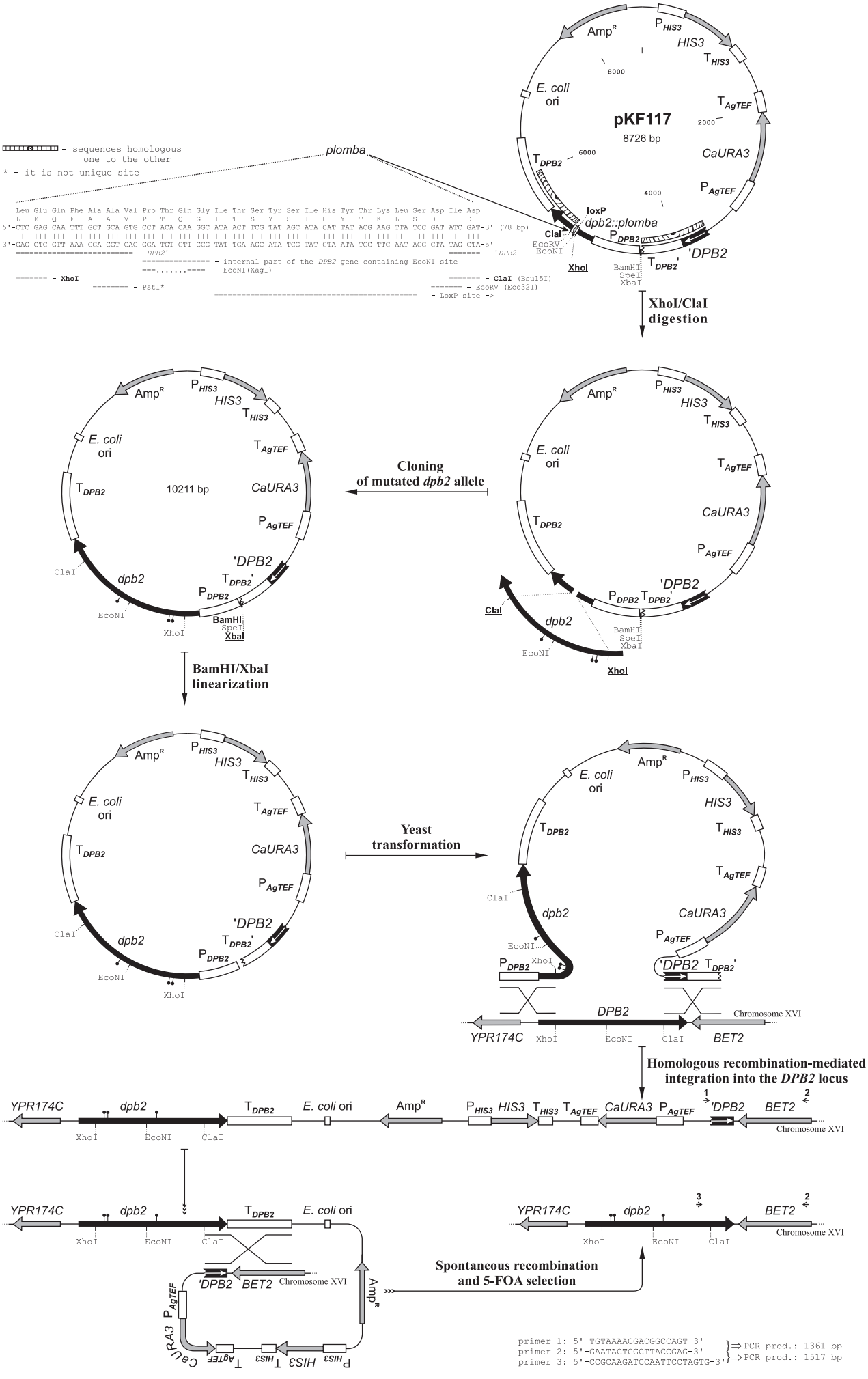

Supplemental Figure S1. — Cloning of *dpb2* alleles and integration of *dpb2* alleles into the yeast chromosome (for description see the next page)**.**

Supplemental Figure S1. — Cloning of *dpb2* alleles and integration of *dpb2* alleles into the yeast chromosome (for illustration see previous page)**.**

The pKF117 integrative plasmid serves as an acceptor vector for introduction of *dpb2* alleles into the *DPB2* locus. Construction of the pKF117 plasmid is described in Materials and Methods and its features are shown in the upper panel. Filled arrows mark locations and orientations of the ORFs, while other important regions are shown as boxes. Restriction sites, important for a particular step of the procedure, are underlined. Noteworthy is the presence of a truncated *DPB2*-derivative sequence, i.e. '*DPB2*-T_{DPB2}' (apostrophes indicate the sides of truncation), upstream of the complete P_{DPB2}-*dpb2*::*plomba*-T_{DPB2} cassette. *DPB2* is flanked on the chromosome by two divergently transcribed genes, *YPR174C* and *BET2*. The *BET2* gene is essential and its ORF is positioned immediately downstream of *DPB2* ORF. Therefore, the 3'-terminal region of *DPB2* probably serves as transcriptional terminator of *BET2*. After integration, this feature is provided by the '*DPB2*-T_{DPB2}' fragment. Each *dpb2* variant (examples of mutations are shown as pins) was cloned into the pKF117 vector in the place of *plomba* using *Xho*I and *Cla*I sites, naturally occurring in *DPB2*, or other flanking sites. The resulting plasmid was linearized with *Bam*HI, *Spe*I and/or *Xba*I (double *Bam*HI/*Xba*I digestion preferred) allowing for direct transplacement of the pKF117-derivative plasmid into the *DPB2* locus using selection for Ura^+. Since the *dpb2* allele is not duplicated, a stable single-copy transformant is obtained. The integration into the *DPB2* locus was confirmed by PCR with primers 1 and 2, as indicated in the scheme by thin arrows. Although vector sequences were not cured from the strains in the current study, subsequent loss of vector sequences could be selected for in the presence of 5-FOA using *CaURA3* as a counterselection marker (GOLDSTEIN *et al.* 1999) as shown, if desired. The curing of the vector sequence could be subsequently checked by PCR with primers 2 and 3 as indicated on the scheme. In control studies, where we removed vector sequences, we did not observe any difference in the frequency of mutagenesis between *DPB2* and *DPB2*::vector alleles.

More details available on request (mail to kflis@bp.onet.pl).

GOLDSTEIN, A. L., X. PAN and J. H. MCCUSKER, 1999 Heterologous *URA3MX* cassettes for gene replacement in *Saccharomyces cerevisiae*. Yeast **15:** 507-511.