

Figure 4. Alternate pathways of DNA DSB repair.

Homologous recombination (left) and nonhomologous end joining (right) are the two major modes of repair at a DNA DSB. In order to enter the homologous recombination pathway, a DSB must undergo extensive 5'-end resection to leave free 3'-ends that can invade the repair template, to form a D-loop (left). In the predominate homologous recombination sub-pathway in somatic cells, synthesis-dependent strand annealing, the invading 3' end then primes limited replication using the homologous DNA as template. The newly extended end is then released from the D-loop and anneals with the other end of the processed DSB. Synthesis-dependent strand annealing transfers relatively short stretches of DNA sequence. After ligation, the resulting heteroduplex is resolved either by the mismatch repair pathway or replication and segregation to generate a corrected gene. Alternatively, a DNA DSB may enter the nonhomologous end-joining pathway (right), in which DNA ends are bound by the Ku70-Ku80 heterodimer. Limited processing occurs, which may be accompanied by small insertions or deletions, and generates DNA ends that can be ligated by the XRCC4-LIG4 complex. The factors that play important roles at various stages of these DSB repair pathways are shown in boxes. A color version of this figure is available online.

Figure 5. Variations of targeted genome engineering.

This diagram illustrates some of the outcomes that can result when a targeted DNA double-strand break (DSB) is repaired by homologous recombination or nonhomologous end joining. Homologous recombination (left) requires a donor homologous at least in part with the cleaved allele. Depending on details of that donor, it may be used to effect gene correction, site-directed mutagenesis, or site-specific insertion driven by matching arms of homology. Nonhomologous end joining (right) will occur in the absence of donor DNA. If cleavage is followed by excision, genetic information may be lost (or gained) at the site of the break, and gene disruption can result. Two DSBs occurring simultaneously on the same chromosome can cause loss of the intervening stretch of DNA, resulting in a defined deletion. Targeted cleavage may also lead to site-specific insertion of a transgene at a defined DSB. A color version of this figure is available online.

Supplementary Table 1. Targeted genome engineering applications