Gene Deletion with Drug Resistant Cassettes.

To make a deletion of a gene with a drug resistant cassette, the cassette is amplified from a vector by PCR. There are three drug resistant cassettes that are used: kanMX4 (resistance to Geneticin,) hphMX4 (resistance to Hygromicin B,) and natMX4 (resistance to clonNAT-Nourseothricin,) – they are amplified from pFA6-KanMX4, pAG32, and pAG25 respectively. Oligos for cassette amplification are designed in such a way that they contain 2 parts: the first part contains 45 bp of sequence just upstream and downstream from the ORF (including the ATG start codon and the stop codon) of a gene of interest, the second contains common sequence homologous to the sequence of the cassette.

1. Design primers:

For the upstream oligo: 45 bp of directly upstream sequence of the yeast open reading frame (including the ATG)+CGTACGCTGCAGGTCGAC

For the downstream oligo: 45 bp of directly downstream sequence of the yeast open reading frame (including the stop codon)+ATCGATGAATTCGAGCTCG

These oligos are universal for all three cassettes as described in "Three New Dominant Drug Resistance Cassettes for Gene Disruption in Saccharomyces cerevisiae"Alan L. Goldstein and John H. McCusker. (Yeast **15:**1541-1553, 1999).

2. Amplify a cassette from a plasmid by PCR.
PCR reaction mix:
Plasmid template-0.5-1.0 ul
10X polymerase buffer-10 ul
DMSO-5-8 ul*
Forward primer (10uM)-2 ul
Reverse primer (10mM)-2 ul
dNTP Mix (2.5mM)-4 ul
Taq polymerase-0.5 ul
H_2O- to the total of 100 ul

*- Add DMSO when natMX4 cassette is amplified due to the GC-rich content.

PCR conditions:
94^0-4 min
25 cycles:
    94^0-30 sec
    55^0-1 min.
    72^0-1 min 30sec
72^0-5min
4^0-hold

3. Purify the PCR product with the Qiagen PCR Purification Kit.
4. Transform yeast cells with about 800 ng of cassette PCR product. Refer to the Yeast Transformation protocol. Spread transformations on YPD and incubate for 2-3 days at 30^0C. Replica plate the transformation lawns to plates containing drugs (Geneticin-200ug/ml, Hygromicin B-300ug/ml, and clonNAT-100 ug/ml) and incubate for 2-3 days at 30^0C. Check the colonies growing on selective media for the gene deletion by PCR. The deletion is scored by difference of PCR products (oligos to amplify full gene length are used), or by presence and absence of PCR product (one oligo is selected outside of the gene and the other oligo is selected inside of the cassette).

For more information you can visit the Yeast Deletion Project web pages at
http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html