The recombineering strategy:

read the methods to Yu et al. PNAS (2000) 97:5978. this walks you through designing primers. It also contains the sequences you can use in your primers for several antibiotic resistance cassettes.

Finding the sequences to your gene:

http://genolist.pasteur.fr/Colibri/

enter your gene name in the search box.
this site is nicely organised graphically and very clickable. The disadvantages are that it only has E.coli genes and t hasnt been updated in a while.

http://www.ncbi.nlm.nih.gov/

go to the nucletide database then enter keywords related to your gene in the search box.
you can find any organism or known genes at this site and uses a much more robust search function. The disadvantages here are that it has so much information, until you get the hang of it, you can sometimes get lost trying to find what you're looking for.

Basic Design outline:

Target Gene to Knockout on the chromosome

Antibiotic Resistence Gene

Forward primer: uses the sequence directly from the database

List 40 bp upstream of the first codon,
then 20 bp upstream from the upstream region of the promotor for the antibiotic resistence

5'NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN**ATGXXXXXX**nnnnnnnnnnnnnnn**nnnnnn3'**

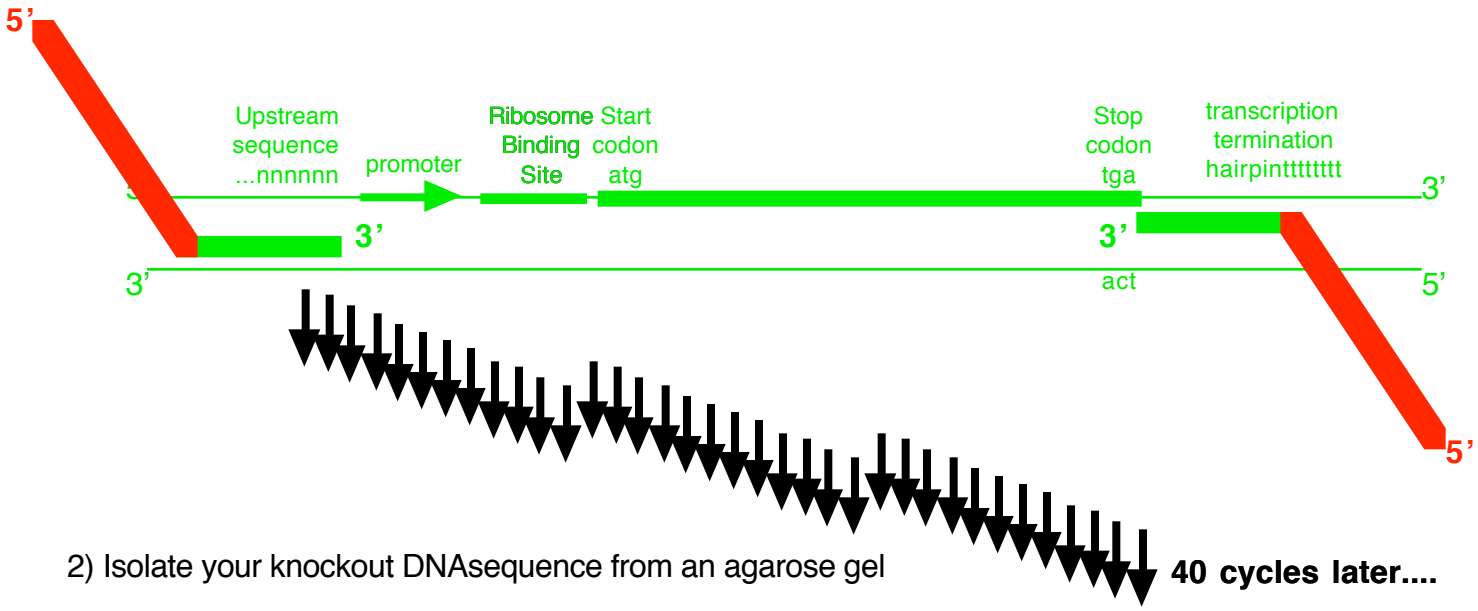
Reverse primer: uses the reverse complement of the sequence downloaded from the database

Looking at the reverse complement sequence in a 5' to 3' direction
List 40 bp downstream of the gene, ending with the stop codon,
then list 20 bp downstream from the end of the gene for the antibiotic resistence

5'NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCAnnnnnnnnnnnnnnnnnnn3'

Making your Knockout DNA

1) Use your hybrid primers in a PCR reaction to amplify the antibiotic resistence gene sequence



2) Isolate your knockout DNAsequence from an agarose gel

40 cycles later....

3) Transform your knockout DNA sequence into the hyper-recombinagenic E. coli