

Cloning Strategy Template

(Use CloneManager to map current plasmids, intermediate and final products)
(Also use CloneManager to select restriction enzymes)

- I. Cloning xxx fragment into yyy plasmid
 - a. Cut xxx vector with specific restriction enzymes
 - i. Cut and gel purify the fragment of interest (? bp)
 - ii. Describe other fragment that will be discarded
 - b. Cut yyy plasmid with specific restriction enzymes
 - i. Cut and gel purify the fragment of interest (? bp)
 - ii. Describe other fragment that will be discarded
 - c. Ligate xxx fragment into yyy plasmid
 - d. Transform bacteria, plate on specific antibiotic and select clones
 - e. Mini-culture bacteria clones and isolate the DNA (MiniPrep Kit)
 - f. Digest with specific restriction enzymes to check incorporation
 - i. Expected Product (fragment sizes)
 - ii. Product if only original plasmid (fragment sizes)
 - iii. Product if only original Insert (fragment sizes)
 - g. Run on a gel to check fragments.
 - h. Midi-culture bacteria clones and isolate the DNA (MiniPrep Kit)
 - i. Quantitate DNA on spectrophotometer
 - j. Linearize with specific restriction enzyme
 - k. Transfect into ES cells and others

Example:

- II. Cloning IRES puro fragment into pTIE triple plasmid
 - a. Cut pTIE triple vector with NotI and XbaI
 - i. Cut and gel purify larger fragment (~7900bp)
 - ii. Other fragment will be very small (12bp) and undetectable
 - b. Cut pTie AcGFP IRES puro plasmid with NotI and XbaI
 - i. Cut and gel purify smaller (insert) fragment (~1676bp)
 - ii. vector fragment discarded (~4929bp)
 - c. Ligate IRES-puro fragment into pTie-triple
 - d. Transform and select with Kanamycin
 - e. Mini-culture bacteria clones and isolate the DNA (MiniPrep Kit)
 - f. Digest with to check Not I, XbaI
 - i. Right Product (1676, 7900)
 - ii. Original pTie triple (only one visible at 7900bp)
 - iii. Original pTie AcGFP IRES puro (1676, 4929)
 - g. Run on a gel to check fragments.
 - h. Midi-culture bacteria clones and isolate the DNA (MiniPrep Kit)
 - i. Quantitate DNA on spectrophotometer
 - j. Linearize with Eco47III
 - k. Transfect into ES cells and others