8/28/96

Christina's quickie notes on FcRn constructs in Bluescript

All Louis Gastinel's original wild-type FcRn and β2m constructs were cloned into the pBSSK- version of the pBluescript vector, as stated in his 1992 PNAS paper. (Therefore all the mutants made from them are in SK- as well.)

There are maps of the four Bluescript vectors, pBSSK +/- and pBSKS +/- in the Stratagene catalog. ("Bluescript II" is basically the same but contains an extra BssHII site on either end of the polylinker. As far as I know we don't have it in the lab.)

The FcRn inserts are in the opposite orientation to the beta-gal gene, i.e. if you sequence with the T3 primer you'll read the sense strand of beta-gal, but the anti-sense strand of FcRn. Stratagene calls the sense strand of beta-gal the plus (+) strand, meaning that the sense strand of FcRn is the minus (-) strand.

Since the vector is SK- (rather than SK+), the minus strand will be rescued when making single-stranded DNA with helper phage, which will be the sense strand of FcRn. Therefore to make mutants using this ssDNA you will need ANTI-SENSE OLIGOS.

You can't confirm that a Bluescript vector is minus or plus except by making the ssDNA, but many FcRn (and beta2m) mutants have been made with anti-sense oligos - they work!

The FcRn constructs go: 5' Xho site - FcRn or beta2 - NotI site 3'. Note that to make them Louis Gastinel first cloned the DAF sequence into the Bluescript polylinker downstream of the FcRn gene, and then either did a deletion to fuse the DAF in frame for DAF-linked constructs, or simply inserted a stop codon to create the secreted (soluble) form. This means that the soluble forms still have the DAF sequence downstream, and you'll see it when sequencing with the T3 primer. So the soluble constructs actually go:
5' Xho-FcRn/Beta2m-stop-some polylinker-DAF-NotI 3'.

For mutagenesis it doesn't matter if the vector is SK or KS, but if you're deciding on restriction sites to use it does.

To confirm that a construct is in SK (as opposed to KS) is simple: just look at sequences from primers T3 and T7 and compare them to the BSSK map from the Stratagene catalog (1995 p. 334). Stretches of sequence including the T3 and T7 primers are printed below the map, and you can match the sequence 3' to these primers with yours. Note that the T7 primer reads the minus strand 5' to 3', which the way it's printed in the catalog means reading the bottom line of sequence right to left.

The difference is in the orientation of the multiple cloning site, i.e. what order the restriction sites are in relative to the T3/T7 primers and the beta-gal gene. You may not be able to see the Sal and Kpn sites which the "SK" and "KS" refer to because they are too close to the primers, but you'll be able to see the following (3') sites. The BSKS map will show the opposite set of sites next to T3 and T7.