About the plasmid constructs that have an IgG1/Fc tag, in the vector pEF-BOS/IgG:

Plasmids already submitted: 24422, 24423
We intend to submit as many as six more in this vector.

Medline reference for the IgG1 Fc sequence: J00228.1

The vector, pEF-BOS/IgG was constructed in the Rosen lab by transferring the IgG1/Fc fragment from the vector pIgG (Rosen lab plasmid No. 27) to the vector pEF-BOS (Rosen lab plasmid No. 57).

The pIgG vector was received from David Simmons and is based on a very early pCDM8 vector. We don't have a lot of specific information about this vector.

The pEF-BOS vector was received from the Weiss lab here at UCSF and is described in *Mizushima and Nagata, Nucleic Acids Research, Vol. 18, No. 17*. We don't have any additional information about this vector as received from the Weiss lab.

pEF-BOS/IgG vector was constructed as follows;

Vector pIgG was cut with NotI.
Vector pEF-BOS was cut with SalI.
Both vectors were incubated in the presence of the enzyme PfuI to polish the ends.

Both vectors were cut with BamHI, thus excising the IgG fragment from pIgG. The liberated IgG fragment was purified away from the pIgG vector and ligated into pEF-BOS.

Resulting construct was verified by restriction analysis and shown to contain unique sites for BamHI and XbaI.

The resulting construct was partially sequenced (Claudia, 2001) and sequence corresponding to the 3' end of the J00228.1 sequence was found to be present.

Referencing the nucleotide sequence for J00228.1:

Coding sequence	Amino Acid sequence
212-502	(STKGP….NTKVDKKV)
891-935	(EPKSCDKTHTCPPCP)
1054-1383	(APPELL….EKTISKAK)
1480-1800	(GQPREP…SLSPGK)

Rev primer for sequencing insert: 5'-gggatgctgccaggcaggagcg-3' (anneals nt's 574-553)
The sequence from nt 1823 - 2000 was found to be present by sequencing in 2001.

Characteristics of the pEF-BOS/IgG vector:

Sites available for cloning: BamHI and XbaI.
This allows fro PCR-mediated cloning with: Bam HI, XbaI, BclI (XbaI) and NheI (BamHI). If BclI and NheI are used, subsequent restriction excision of insert is not possible.

The IgG/Fc fragment in the vector contains a splice acceptor sequence at its 5' end, ie, immediately 3' of the inserted fragment.

This means that the inserted fragment must have a splice donor sequence incorporated and this is accomplished in the design of the PCR primer.

The PCR primer at the 5' end of the insert (forward primer) must have an XbaI or a BclI restriction sequence.

The PCR primer at the 3' end of the insert (reverse primer) must have a BamHI or an NheI restriction sequence.

The PCR primer at the 3' end of the insert (reverse primer) must have a splice donor sequence "upstream" of the restriction sequence.
The 3' reverse (antisense) PCR primer therefore should include the following sequence:

5'-NN-GGATCC-ACTTACCTGT-(gene-specific-sequence)-3'

Where

NN = any nt's
GGATTC = BamHI site (could also be TGATCA = BclI site)
ACTTACCTGT = splice donor sequence.