

Figure 4.3: pGFP-Kif5c and pGFP-Kif5cmut Vectors and Diagnostic Digestions

Panel A shows pGFP-Kif5c, constructed using the pEGFPC3 vector and the rat *kif5c* heavy chain inserted as a *Bam*HI fragment. Panel B shows pGFP-Kif5cmut constructed as in Figure 4.2. Overlap PCR introduced the T93N mutation and also a novel *Spel* site (1652). Panel C shows diagnostic digestions of pGFP-Kif5c and pGFP-Kif5cmut. *Bam*HI excises the ~3 kbp *kif5c* or *kif5cmut* fragments from the ~4.7 kbp pEGFPC3 vector. *Spel* cuts only pGFP-Kif5cmut, linearising the vector to ~7.7 kbp.

KHC (from Scott Brady) originally received as BamH1 fragment in pBSK+ (from nt144 to nt3162 lacking the first 2 amino acids of KHC was cloned into pEGFP C3 to make an N-terminal fusion.