

SUPPORTING INFORMATION

Cloning of the dual-tag EGFR-ECD

The dual-tag EGFR-ECD plasmid was generated in 3 steps:

- (1) Amplification of EGFR-ECD from the full length receptor sequence (first 621aa N-terminal),
- (2) Cloning eGFP C-terminal to EGFR-ECD to create EGFR-ECD-eGFP, and
- (3) Cloning mRFP N-terminal to EGFR-ECD-eGFP to create mRFP-EGFR-ECD-eGFP (dual-tag EGFR-ECD).

EGFR-ECD cDNA was amplified using PCR. It was built up from pEGFR513 (14), containing the first 513 amino acids of EGFR-ECD and the remaining 108 aa were added by PCR using the full length EGFR plasmid as a template (25). The forward primer was designed to amplify upstream of EGFR513 to incorporate the unique enzyme site *PsiI*. The reverse primer was designed to incorporate EGFR621 with *KpnI* and *AgeI* for in-frame subcloning. The PCR product was cloned into the pPCR-Script Amp vector (Stratagene), for easy amplification of the fragment before sub cloning into pEGFR513 using the *PsiI* site and the *KpnI* site and creating pEGFR621.

In order for eGFP to be fused C-terminally to EGFR-ECD, pEGFR621 was subcloned into an eGFP containing plasmid (pEGFP-N1, Clontech) via a *Sall/XhoI* ligation and *AgeI* to create EGFR-ECD-eGFP.

Insertion of mRFP N-terminally to EGFR-ECD-eGFP was achieved by Gene Splicing using Overlap Extension Method. This was done to eliminate the need for compatible restriction enzymes. cDNA encoding monomeric Red Fluorescent Protein (mRFP) was a kind gift from Dr. Mark Prescott (Monash University, Australia). The mRFP was inserted directly after the EGFR signal peptide with no linker between the two. Three sets of primers were designed to amplify three fragments where the ends of each

product contained complementary sequences so that when they were mixed, denatured and re-annealed, the strands had matching sequences to serve as primers in the presence of DNA polymerase.

The first set of primers amplified 97bp containing the signal peptide of EGFR and the first 9bp of mRFP (fragment 1). The second set of primers amplified 756bp containing mRFP (fragment 2), and the third set amplified 242bp containing EGFR from the first amino acid to the EcoRI site (fragment 3). Fragments 1 and 2 were spliced together using the forward primer from fragment 1 and the reverse primer for fragment 2. Fragments 2 and 3 were spliced together using the forward primer from fragment 2 and the reverse primer for fragment 3. These two resulting products were added in a PCR reaction as a template and using the forward primer for fragment 1 and the reverse primer for fragment 3 the three fragments were all joined. This final product was ligated into pPCR Script (Stratagene) and subcloned into the EGFR-ECD-eGFP using the BglIII site in pEGFP-N1 and BamHI in pPCRScrip and the EcoRI site in the receptor. The final cDNA sequence, dual-tag EGFR-ECD, contained 3.2 kbp that translate into 1085 amino acid protein with estimated molecular weight of 123 kDa.