BBa_K1607011:

The anti-p185*her2/neu* antibody chA21 mediates specific inhibitory effects on p185*her2/neu*-overexpressed cancer cells, as well as human breast and ovarian cancer xenograft [1]

BBa_K1607010 is the CDS of the scFv of chA21. **This part, BBa_K1607011, is the CDS of the chA21scFv-eGFP fused protein**. In this part the scFv CDS is connected to the eGFP CDS by a (Gly4Ser)3 linker.

To get the CDS of chA21scFv, we found the AA sequence of it on NCBI first (PDB: 3H3B_B). The AA sequence was then reverse translated into DNA sequence. The DNA sequence was analyzed by gene2oligo and 36 oligos were given as a result. We synthesized the 36 oligos and obtained the 601bp fragment of SCFV coding sequence by LCR assembly using these oligos.

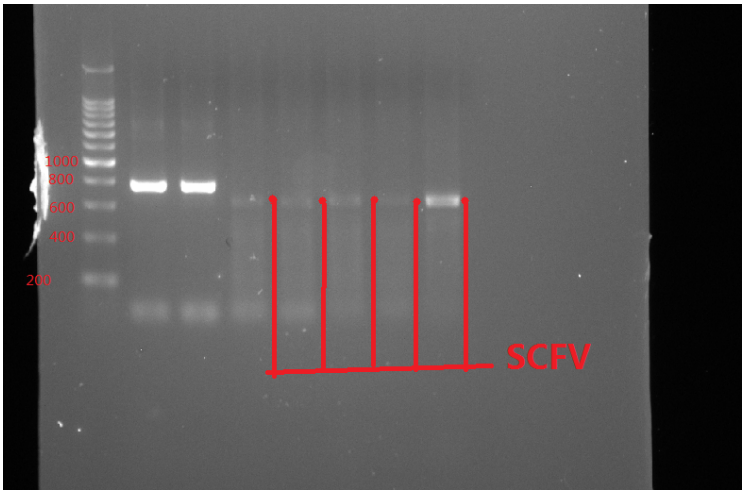

Fig: 601bp scFv sequence (CDS together with restriction sites at both ends) obtained by LCR assembly followed by a round of PCR amplification.

We then connected the scFV CDS to the eGFP CDS using SOE PCR.

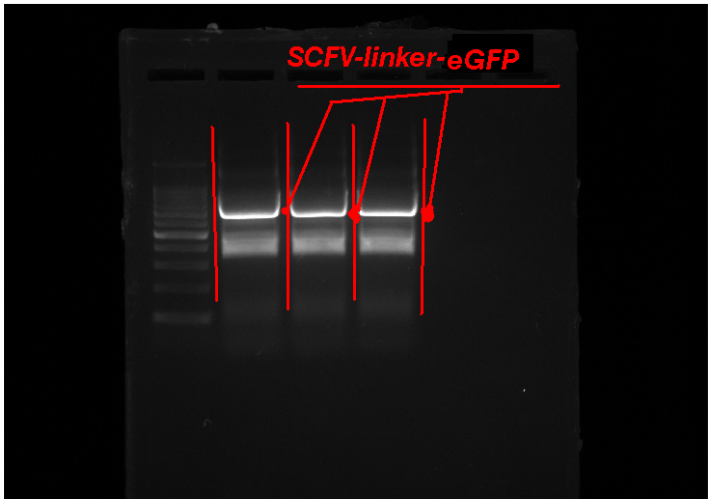

Fig: 1347bp SCFV-linker-eGFP sequence constructed by SOE PCR (This gel was for gel extraction)

Finally, we add standard Biobrick prefix and suffix to this scFv-linker-eGFP fused protein CDS and cloned the standard part to PSB1C3. The plasmid was sequenced and then submitted.

[1] Zhou H, Zha Z, Liu Y, et al. Structural Insights into the Down-regulation of Overexpressed p185her2/neu Protein of Transformed Cells by the Antibody chA21[J]. Journal of Biological Chemistry, 2011, 286(36): 31676-31683.