Pepper-tagged reporter plasmid manual

The $(F30\text{-}2xPepper)_{10}$ tag is a fluorogenic RNA aptamer tag for imaging mRNA in live cells. When inserted to the 3'UTR of mRNAs, the $(F30\text{-}2xPepper)_{10}$ tag can bind to and stabilize an unstable fluorogenic protein $(mNeonGreen)_4$-tDeg (Addgene #129402 or #129404). In this way, the green fluorescence of $(mNeonGreen)_4$-tDeg is only turned on when bound to the mRNAs with the $(F30\text{-}2xPepper)_{10}$ tag for fluorescence imaging.

The CMV-mCherry-$(F30\text{-}2xPepper)_{10}$ plasmid encodes an *mCherry* reporter mRNA with the $(F30\text{-}2xPepper)_{10}$ tag in the 3'UTR. The *mCherry* gene can be swapped out with any gene of interest by digesting the plasmid with HindIII and XhoI. When cloning in a new gene of interest, please make sure to include a Kozak sequence (e.g. GCCACC) after the HindIII restriction site and before the AUG start codon. Repeat-containing DNA sometimes poses difficulties for typical sequencing protocols. We recommend using the "Difficult Template" protocol offered by GENEWIZ for sequencing the $(F30\text{-}2xPepper)_{10}$ tag in the 3'UTR.

For imaging protocol, please refer to the Supplementary protocol in "*Live imaging of mRNA using RNA-stabilized fluorogenic proteins*" or on Protocol Exchange (DOI: 10.21203/rs.2.11494/v1)