

This plasmid expresses the thiol redox-sensitive ratiometric fluorescent sensor roGFP2 in the mitochondrial matrix, under the control of the CMV promoter. The roGFP was generated starting with the eGFP expression plasmid by Clontech. Mutations corresponding to the roGFP2 sensor were introduced using a Stratagene Quikchange kit. The mitochondrial matrix targeting signal (MTS) from cytochrome c oxidase subunit IV was appended to the 5' end of the roGFP2 cDNA. The MTS-roGFP2 construct was then ligated into the VQ Ad5CMV K-NpA vector between the KpnI (5') and NotI (3') restriction sites. That vector contains an ampicillin-resistance gene. Expression of this construct in the mitochondrial matrix was confirmed by immunofluorescence and by Immunogold electron microscopy. The roGFP2 sensor was originally described by S. James Remington (J Biol Chem 279:13044, 2004).