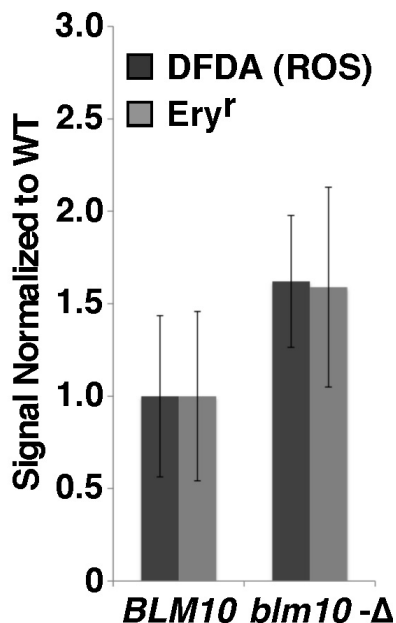


(B). Loss of *BLM10* has minor effects on ROS formation and mitochondrial genome mutation



Isogenic strains with or without *BLM10* (8127-7-4, 8634-9-1) were grown to log phase in rich medium and then tested for production of reactive oxygen species (ROS) or mutation of the mitochondrial genome as detected by production of erythromycin resistant clones, essentially as described (Malc et al., 2009). Briefly, for the ROS assay, cells were collected by centrifugation, washed, then multiple aliquots were suspended in a solution containing 10 μ M 2', 7'-Dichlorofluorescein diacetate (Sigma, DFDA). After incubating 30 minutes at 30° the cells were washed again, suspended in detergent and lysed by agitation with glass beads. The fluorescence of the supernatant was then tested at 520 nm with excitation at 485 nm. Signal in this assay depends on the intracellular level of ROS (Doudican et al., 2005). Dilutions of the same cultures were plated on rich medium with glycerol as the sole carbon source and containing 4 mg/ml of erythromycin. The yield of erythromycin resistant clones was then determined as an indication of the frequency of mutation of the mitochondrial rDNA locus, which determines sensitivity to this antibiotic. Three independent cultures were tested in each assay, normalized to the value obtained for the WT samples, and the average and standard deviation (error bars) presented here. The average values for the WT were 1.4 fluorescence units/A660 value, and 24 erythromycin resistant colonies per 10⁷ viable cells on glycerol medium lacking the drug. Loss of *BLM10* in this and other assays consistently caused slightly higher levels of ROS production and mitochondrial genome mutation, but the effect is small and not statistically significant in any single assay.