

**Figure 1: Southern blot analysis of mtDNA.** Total DNA was extracted from parental MDA-MB-435 cells (Lanes 1A, 1B) and the clone 7 mtDNA-depleted variant of MDA-MB-435 cells (Lanes 2A, 2B), (A): Total DNA was then digested with PvuII (which cuts a single site in mtDNA generating a single 16.6 Kb fragment), separated by electrophoresis on agarose gel containing ethidium bromide. (B) DNA was transferred to nylon membrane, and the membrane was hybridized to a <sup>32</sup>P-labeled mtDNA-specific DNA probe. The 16.6 Kb mtDNA is present in parental MBA-MD-435 cells (Lane 1B), but absent in Clone 7 (Lane 2B). The 16.6 Kb genome is indicated by arrow.

**FIGURE 2: Rho<sup>0</sup> cells do not express mtDNA-encoded COX II protein.** Equal amounts of total cell lysates from parental MDA-MB-435 (Lane 1) and Clone 7 Rho<sup>0</sup> variant (Lane 2) cells were separated by SDS-PAGE and transferred to a membrane. (A) Blot was hybridized with a monoclonal antibody against cytochrome oxidase II (COXII), and (B) then stripped and rehybridized with a monoclonal antibody against  $\beta$ -actin. COXII staining demonstrates the loss of expression of the mtDNA-encoded COXII (Lane 2A). Actin staining shows that protein loading is approximately equal (Lanes 1B, 2B).

**Figure 3: Rho<sup>0</sup> cells do not grow in galactose medium.** Rho<sup>+</sup> (MDA-MB-435) cells and Rho<sup>0</sup> cells were grown in custom-made medium containing galactose (instead of glucose, purchased from Sigma). While MDA-MB-435 cells continue to grow in this medium (A), Rho<sup>0</sup>clone 7 cells began to die after 48 hours (B).

**Figure 4: Lipid peroxidation is increased due to dysfunction in mitochondria:** Subconfluent cultures were collected by trypsinization, washed with ice cold PBS and lysed by freeze/thaw cycles in sterile deionized water. Lipid peroxidation was measured as described in material methods. .

**Figure 5: RT-PCR analysis:** RT-PCR analysis of up-regulated or down regulated genes was carried out as described in material methods.