Isolation of Intact Plastids and Mitochondria from *Chromera velia*

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Chromerids are alveolate algae that are the closest known phototrophic relatives to apicomplexan parasites such as *Plasmodium* or *Toxoplasma*. While genomic and transcriptomic resources for chromerids are in place, tools and experimental conditions for proteomic studies have not been developed yet. Here we describe a rapid and efficient protocol for a simultaneous isolation of plastids and mitochondria from chromerid alga *Chromera velia*. This procedure involves enzymatic treatment and breakage of cells, followed by differential centrifugation. While plastids sediment in the first centrifugation step, mitochondria remain in the supernatant. Subsequently, intact plastids can be purified from the crude pellet by centrifugation on a discontinuous 60 % / 70 % sucrose density gradient, while mitochondria can be purified by centrifugation on a discontinuous 33 % / 80 % Percoll density gradient. Isolated plastids are autofluorescent and their intact state was confirmed by transmission electron microscopy. Isolated mitochondria are intact and maintain membrane potential, as confirmed by staining with mitochondrion-specific MitoTracker dyes. Total proteins were extracted from isolated organellar fractions and the purity of isolated organelles was confirmed using immunoblotting. Antibodies against the beta subunit of the mitochondrial ATP synthase and the plastid protochlorophyllide oxidoreductase did not cross-react on immunoblots, suggesting that the each organellar fraction is largely free from the residues of the other. The presented protocol represents an essential step for further proteomic studies on the organellar biochemistry and cell biology of *C. velia* and can be employed, with minor optimizations, in other thick-walled unicellular algae.