

Table 1. Methods to assess mitochondria biogenesis

<u>Method</u>	<u>Measure</u>	<u>Reagents/Materials Needed</u>
1. Microscopy		
a) fluorescent microscopy using dyes	relative area occupied by mitochondria	a) Fluorescent microscope b) Membrane potential independent fluorescent dye such as Mito-tracker probes from Molecular Probes (Eugene, Oregon)
b) transmission electron microscopy followed by morphometric analysis	relative and/or absolute area occupied by mitochondria, mitochondria pathology, general cell and extracellular morphology	a) transmission electron microscope b) ultramicrotome c) fixation. Embedding, and staining reagents d) image analyzer or transparent grid for quantification

<p>2. Mitochondria DNA determination</p>	<p>Biochemical measure of mitochondria number-amount of mtDNA is proportional to the number of mitochondria</p>	<p><u>Real-time PCR</u></p> <ul style="list-style-type: none">a) mtDNA primers selected from small arc of the circular DNA strand between oriH and oriLb) primers that correspond to genomic DNA to correct for cell numberc) thermocycler <p><u>Southern Blot</u></p> <ul style="list-style-type: none">a) mitochondria DNA probe and genomic DNA probe (eg. 18S RNA)b) agarose gel electrophoresis apparatusc) radioactive ^{32}P α-dCTP to label probe OR non-radioactive
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		<p>biotinylated probes can be produced</p> <p>d) X-Ray film or phosphoimager</p>
<p>3. Western Blotting for proteins upregulated in I biogenesis:</p> <p>a) PGC1-α</p> <p>b) NRF-1 and NRF-2</p> <p>c) mtTFA</p> <p>d) I proteins</p> <ol style="list-style-type: none"> 1. Cytochrome C oxidase subunit II 2. ATP synthase subunit c 	<p>Increased protein levels of each suggest more mitochondria are present. Increased PGC1-α, NRF-1, NRF-2, and mtTFA protein levels suggest that the I biogenesis gene program is turned on. Increase level of mtiochondrial proteins suggest more mitochondria.</p>	<p>a) SDS-PAGE system (eg. Bio-Rad Inc, Richmond, CA)</p> <p>b) Membrane transfer system (tank or semi-dry transbot apparatus from BioRad Inc, Richmond, CA)</p> <p>c) Antibodies (primary) to each protein are commercially available from several suppliers</p> <p>d) Secondary antibody against the primary antibody linked to horse-radish peroxidase or some other marker.</p>

4. Radiolabelling	translation rates of mtDNA-encoded proteins	a) radioactive amino acid such as (4, 5- ³ H) leucine b) scintillation counter
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