**Liver Mitochondria Isolation Procedure**

Excise liver and remove gall bladder

Weigh liver and place in 10 volumes of LIVER ISOLATION SOLUTION in beaker

Mince liver with scissors

Homogenize liver with Potter-Elvehjem PTFE pestle and glass tube – 2 strokes

Centrifuge homogenate @ 500g for 10 minutes

Transfer supernatant through cheesecloth/gauze

Centrifuge @ 3500g for 10 minutes

Discard supernatant

Resuspend mitochondrial pellet in a volume equal to original in LIVER ISOLATION SOLUTION

Centrifuge @ 3500g for 10 minutes

Pour off supernatant and suspend final mitochondrial pellet in 0.35 ml of mannitol/sucrose

Gently work pellet off side of tube

Transfer to Dounce homogenizer and resuspend with 4-5 passes

Place mitochondrial suspension in labeled microcentrifuge tubes