Start this procedure after the wash of mitochondria from percoll gradient in wash buffer without BSA.

The supernatant is discarded and the loose pellet is resuspended in 10 mls of Buffer D (lysis buffer) preheated to 37C; carefully resuspend mitochondrial pellet. Try to place all mitochondrial lysis suspensions in a single Oakridge (with seal) tube. Allow lysis to occur for 1 hour at 37 degrees with occasional gentle mixing (every 10-15 minutes).

Add equal volume of phenol and invert gently 40 times, and spin in HS-4 rotor for 20 minutes at 6,000 rpm

Add equal volume of chloroform:isoamyl (24:1) Invert gently 40 times, and spin in HS-4 rotor for 20 minutes at 6,000rpm

Remove top (aqueous) phase, and place in clean oakridge tube and repeat step 21 at least once more... if solution is still cloudy do again (Need to do at least 2 extractions with phenol and/or chloroform)

Add 0.1 volume 3.0 M NaOCl and gently mix. Then add twice the volume (DNA with NaOCl) of ice-cold 100% alcohol mix gently inverting 50 times, and place at -20 C overnight.

Collect precipitated DNA by centrifugation. Spin in HS-4 rotor for 30 minutes at 7,000 rpm

Decant EtOH, allow tube to dry. Add a small amount (10 ml) of 76% EtOH + 10mM NH_4OAc, allow to sit for 5 minutes, and decant (be careful NOT to lose pellet). Place tubes in front of Laminar flow hood for 20 minutes to dry.

Resuspend pellet in a small amount of TE 8.0. It helps to pre-heat TE 8.0 to 60 C and allow DNA to incubate in TE at 60 C for 1 hour, this should dissolve DNA. Some proteins still may be present, but don't worry about these. Transfer solution to microfuge tube and a quick spin in the microfuge will pellet debris.

Measure DNA concentration on nanodrop.

Buffer D (Lysis solution)
For 1L final volume:

- 25 g SDS
- 0.5 g Proteinase K
- 50 ml 1.0 M solution of TRIS pH 8.0 (50 mM/L)
- 20 ml of 0.5 M solution of EDTA pH 8.0 (10 mM/L)