Nuclei Fractionation from whole cochlea (with bone)

Acronyms or shorthands:

HB
GB1
RB

1. Put whole cochleae in a dounce homogenizer containing 2 mL of ice-cold HB.
2. Grind cochlea with pestle A 20 times.
3. Filter the suspension through a 40-micron mesh.
4. Transfer the filtered suspension into microcentrifuge tubes and spin at 2500x g for **1 min** at **4°C**.
5. Transfer the supernatant (including whitish pellets along the side walls) into a 15-mL falcon tube, leaving only the pellet at the bottom. Add 3.2 mL of cold HB.
6. Add 5 mL GB1 and mix gently.
7. Layer gently on top of 5 mL of GB2 in a centrifugation tube.
8. Spin at 7500x g for **30 min** at **4°C**.
9. Remove supernatant.
10. Dissolve pellet in RB containing DAPI at 1:1000 dilution.
11. Put a few drops on a glass slide and observe under the microscope.