Procedure

1. 20 large plates of confluent CHO cells grown in MEMα + 10% fetal calf serum. Aspirate media and replace with medi containing 10 µg/ml nocodazole and 5 µg/ml cytoochalaisin B (or 1µg/ml CD). Return to incubator for 90 minutes.

All subsequent procedures carried out at 4 degrees

2. Cells are washed and lysed directly on the plate.

   Aspirate the media and wash sequentially with:

   - PBS
   - PBS/10 + 8% sucrose w/w
   - 8% sucrose w/w
   - LB
   - 10 mls LB + 0.5% NP40

   Each plate is carried through all the washes individually (< 1 minute/plate) then placed on a rotating platform in LB +NP40 for 10'.

3. The cell lysates are then washed off with a pipette, transferred to four 50 ml conical tubes and 1/50 volume of 50x PE is added.

4. Spin out the nuclei 1500g for 3' (3K in the HS4).

5. Transfer the supernatent to 8 30 ml corex tubes and underlayer each with 2 mls of 20% w/w ficoll.

6. Spin down 25,000g 15' (12.5K in an HB4) to concentrate the centrosomes on the ficoll cushion.

7. Aspirate the supernatant to 2 mls above the cushion, then collect the centrosomes from the interface using a pipette held so that the tip is just above the interface and moved back and forth.