MgCHO Chromosome Prep

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Things You'll Need:

Buffers:

Swelling Buffer (PME):

5 mM PIPES, pH 7.2
5 mM NaCl
5 mM MgCl2
1 mM EGTA

Recipe:

1.25 ml 0.2 M Pipes pH7.2
50 ul 5 M NaCl
250 ul 1 M MgCl2
0.625 ml 0.4 M EGTA
47.8 ml water
store at 30 deg C
Lysis Buffer: PMED (PME + 0.1% digitonin) + 5 ug/ml LPC. Store on ice; add LPC and digitonin just before use.

Sucrose step gradients : 30%, 40%, 50% and 60% in PME + 5 ug/ml LPC

30% :
2.2 ml 2M sucrose
2.3 ml water
0.5 ml 10X PME
40% :
2.9 ml 2M sucrose
1.6 ml water

0.5 ml 10X PME
50% :
3.6 ml 2M sucrose
0.9 ml water
0.5 ml 10X PME
60% :
4.4 ml 2M sucrose
0.1 ml water
0.5 ml 10X PME
store on ice; add LPC just before pouring gradient (see below)

Other items required:

1. Chilled 15 ml dounce with tight pestle (B for Kontes; A for Wheaton)
2. 2 15 ml Corex tubes on ice
3. HB-4 rotor at 4 C and clinical at RT

Protocol:

1. Arrest CHO cells (~70% confluent) using 8-10 ug/ml vinblastine for 8-10 hours
2. Make all buffers and store at appropriate temperature before starting prep
3. Collect mitotics by blowoff
4. Spin in clinical at #5 for 3' to collect cells
5. Swell cells by resuspending in 10 ml Swelling buffer (30deg.C), adding additional 40 ml swelling buffer and incubating at 30 deg.C for 5'.
6. Pellet swollen cells in clinical at #5 for 3'. During steps 4 &5 do the following :
    1. add digitonin to lysis buffer and LPC to lysis buffer and sucrose gradient solutions
    2. pour step gradient :
        - 3 mls 60%
        - 2 mls 50%
        - 2 mls 40%
        - 2 mls 30%
    3. Ensure that dounce is ice cold with pestle in.
7. **CRITICAL STEP**
    1. Aspirate supe and vigorously pipet 7 mls of ice-cold lysis buffer

onto cell pellet.

2. Rapidly pipet up and down once and pipet the cell suspension into the dounce
3. Snap the pestle up immediately and quickly apply 3-4 strokes being careful not to 'pop'. **This is critical to increase yield of single chromosomes; delay at this step will cause the chromosomes to cluster**
4. Apply 15 strokes total waiting ~2 secs between each stroke before looking at the lysate by mixing with a little Hoechst

8. Transfer lysate to a 15 ml conical on ice
9. Spin in HB-4 for 1' at 900 rpm (to pellet nuclei) at 4 deg.C
10. Layer supe onto sucrose step gradient and spin 5K 15' 4 deg.C with brake off.
11. Aspirate till the middle of the 40% step and collect flocculent white material at the 40-50 % and 50-60 % interfaces using a pasteur pipet.
12. Mix well to resuspend the chromosomes and aliquot into 10 ul aliquots. Store -80.