REFERENCE:

Earnshaw, W.C., Ratrie, H. III & Stetten, G. (1989). Visualization of centromere proteins CENP-B and CENP-C on a stable dicentric chromosome in cytological spreads. *CHROMOSOMA (BERL.)* 98:1-12.

SOLUTIONS:

10x PBS-azide

10x final concentration	*stock soln*	*add/1 liter final*
100 mM Na PO_4 pH 7.4	0.5 M	200 ml
1.5 M NaCl	5 M	300 ml
10 mM EGTA	100 mM pH 7.5 (NaOH)	100 ml
0.1% NaN_3	10 %	10 ml

10x TEEN

10x final concentration	*stock soln*	*add/1 liter final*
10 mM Triethanolamine:HCl pH 8.5	1 M	10 ml
2 mM NaEDTA	100 mM pH 9.0 (NaOH)	20 ml
250 mM NaCl	5 M	50 ml

10x KB^-

10x final concentration	*stock soln*	*add/1 liter final*
100 mM Tris:HCl pH 7.7	1 M	100 ml
1.5 M NaCl	5 M	300 ml
1% BSA 30 % Pentex grade	33 ml	

3% formaldehyde in 1X TEEN (Made from paraformaldehyde. Dissolve by refluxing, then filter through a 0.2 μm filter.

30% BSA, Pentex grade (from Sigma)

10% Triton X-100 in H_2O

MOWIWOL in 10 mM Tris:HCl pH 7.7, 150 mM NaCl

0.8% Na Citrate in water

Methanol:HOAc 3:1

colcemid - 0.1 mg/ml in H_2O filtered sterile

PROCEDURES:

Prepare the following solutions:

0.8% Na Citrate - 100 ml.
1X PBS-azide - 100 ml.
1X TEEN + 0.1% Triton X-100 + 0.1% BSA - 200ml
1X KB^- - 500ml.

1. Grow cultures of fibroblasts or amniocytes on glass cover-slips in 2.5 cm petri dishes.
2. Block cells with colcemid as usual.
3. Aspirate medium.
4. Pipette on 2 ml of 0.8% Na Citrate (you can also use 75 mM KCl). (Gently!)
   ☛ 20-25 minutes at room temperature.
5. Add 2 ml of a solution of MeOH/HOAc (3:1).
   ☛ 2 minutes at room temperature.
6. Remove this solution by aspiration.
7. Add 2 mls of MeOH/HOAc (3:1).
   ☛ 5 minutes at room temperature.
8. Aspirate the MeOH/HOAc.
9. Vigorously bang the (inverted) petri dishes with cover-slips on a paper towel on the bench top three times to remove most of the remaining fixative.
10. Immediately dry the cover slip with an aquarium pump (Hagen 800 or 800D - using a cut 1 ml plastic pipette as nozzle), with the nozzle ~1-3 inches above the cover-slip. (Move the nozzle over the surface rapidly and at random. (Process two or three slides simultaneously, depending on the size of your hand.)
11. <u>As soon as</u> the surface is dry, remove the cover-slips from the petri dishes (to get forceps under the cover-slips, bend the petri dishes by pressing down on a rubber stopper). Immediately immerse the cover-slips in a 1X solution of PBS-azide.
    ☛ 5 minutes at room temperature (or as long as it takes to process the rest of the cover-slips.
12. Wash cover-slips 3x with 1X TEEN + 0.1% Triton X-100 + 0.1% BSA.
    ☛ 1 minute each at room temperature.
13. Add antibody (diluted appropriately in 1X TEEN + 0.1% Triton X-100 + 0.1% BSA) to cover-slips.
    ☛ 30 minutes at 37^o C.
14. Wash three times with 1X KB^-.
    ☛ 2 minutes, 5 minutes, 3 minutes, at room temperature.
15. Add preadsorbed biotinylated anti-human antibody (Vector Labs) diluted 1:100 (or as appropriate) in 1X KB^-.
    ☛ 30 minutes at 37^o C.
16. Wash three times with 1X KB^-.
    ☛ 2 minutes, 5 minutes, 3 minutes, at room temperature.
17. Add streptavidin:Texas Red (BRL) diluted 1:800 (or as appropriate) in 1X KB^-.
    ☛ 30 minutes at 37^o C.

18. Wash 2 minutes with 1X KB^- at room temperature.

19. Stain with DAPI (0.5 μg/ml in 1X KB^-) for 5 minutes at room temperature.

20. Wash 3 minutes with 1X KB^- at room temperature.

21. Mount cover slip inverted on Mowiwol in 1X KB^- minus BSA.

11a. ***Optional****: Fix with 3% formaldehyde in TEEN for 5 minutes at room temperature. (Good spread morphology has been obtained in the absence of this fixation.)*

12a. ***Optional****: If you have background problems with the fluorescence images, at this point immerse the cover-slips in 10% fetal bovine serum in TEEN + 0.1% Triton X-100 + 0.1% BSA.*

☛ *10 minutes at 37^o C.*

Rinse the blocked cover-slips with 1X TEEN + 0.1% Triton X-100 + 0.1% BSA.

☛ *3 times for 2 minutes each at room temperature.*