**UW Medicine - Pathology**

400-03-01-04

C-Banding by Barium Hydroxide Using Wright's (CBW)

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| Adopted Date: 09/04/91  Review Date: 06/12/09  Revision Date: 04/13/11 |

PURPOSE

This technique selectively stains chromosomal areas known to contain constitutive heterochromatin, which is a fraction of chromatin usually located around the centromere or in blocks in the chromosomal arms. It is generally genetically silent (not transcribed) and contains highly repetitive DNA sequences (G-C or A-T rich satellite DNAs). Human chromosomes stain darkly in the pericentric areas of all chromosomes, the secondary constriction of chromosomes 1, 9 and 16, the distal segment of the long arm of the Y chromosome and the acrocentric short arm segments. This technique is very useful for identification of polymorphisms and in investigating chromosome rearrangements near centromeres, as well as the presence of centromeric material in small rings or marker chromosomes.

PROCEDURE

### Materials and Equipment

1. Waterbath at 37°C.
2. Waterbath at 65°C.
3. Light microscope

### Reagents and Solutions

* + 1. Glacial acetic acid (Baker, Cat.# 9508-33).
    2. 4 M/L hydrochloric acid HCl.
    3. Barium hydroxide, 8-hydrate, Ba(OH)28H20 (Baker, Cat. #1006-04).
    4. 2X SSC, refer to 20X and 2X SSC Stock procedure.
    5. Distilled H2O.
    6. Wright's stain
    7. pHydrion buffer, pH 6.8 (VWR Cat. #34175-231)
    8. Make 200 ml of 0.2 M/L HCl by diluting 10 ml of 4 M/L HCl in 190 ml distilled H2O. Expires in 1 yr at room temperature.
    9. Make 0.035 M/L Ba(OH)2 by weighing 11.04 g/L of distilled H20. Expires in 1 yr at room temperature.
    10. Xylene (Baker, Cat. #9490-01) or Xylene Substitute (Shandon, Cat. #9990505).

### Procedure

1. Treat the aged slides with 0.2 M/L HCl at room temperature for 30 min.
2. Rinse in distilled H20 for 1 min.
3. Treat slides with heated (37°C) 0.035 Ml/L Ba(OH)2 for 7 min.
4. Rinse briefly in a Coplin jar of H20 containing 1 ml of glacial acetic acid; then rinse in plain H20.
5. Incubate the slides in 2X SSC at 65°C for 2 hr.
6. Rinse briefly in a Coplin jar of H2O.
7. Stain for 3 min in a 1:4 solution of Wright's stain in pHydrion buffer.
8. Rinse with H20 briefly and air dry.

***Note****:* Barium hydroxide solution should be stored in a tightly sealed container. If a precipitate forms, a fresh solution should be prepared, as the precipitate will deposit on the slide.

### Short C-Banding Method after G-Bands

After a slide has been G-banded, the following procedure will produce C-bands.

* + - 1. Soak G-banded slide in two consecutive Coplin jars of Xylene (or 3 jars of Xylene substitute) until immersion oil is removed. Air dry.
      2. De-stain slide completely by flooding repeatedly with fixative. Air dry.
      3. Treat slide for 15 min in 0.2 M/L HCl.
      4. Rinse in H2O.
      5. Treat slide for 1.5-2 min in 0.035 mol/L Ba(OH)2 at 37ºC.
      6. Rinse briefly in a Coplin jar of H2O containing 1 ml of glacial acetic acid. Rinse in plain H2O.
      7. Treat slide for 30 min in 2X SSC at 65ºC.
      8. Rinse in H2O.
      9. Stain for 3 min in 1:4 solution of Wright's stain in buffer.
      10. Rinse in H2O. Air dry.

REFERENCES

1. Salamanca F, Armendares S, C-bands in human metaphase chromosomes treated with barium hydroxide. *Ann. Genet*., 17:135-136, 1974.
2. Benn PA, Perle MA, Human Cytogenetics. A Practical Approach. Vol. I, Chapter 4, eds. Rooney DE, Czepulkowski BH, IRL Press, 1992.
3. Miller OJ, Therman E, Human Chromosomes, 4th ed., pp. 81-83, Springer, 2001.
4. 20X and 2X Stock procedure, 400-03-01-16

Written By: Director Approval:

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Cytogenetics Supervisor