**Banding patterns**

*Introduction*

The genetic instructions for an organism are located in the chromosomes of the cells of the organism and are transmitted to the offspring by inheritance. A logical prediction of evolutionary theory is that closely related species should have similar chromosomes. Techniques of chromosome banding have now been available for a long enough period of time that some trends have been discovered, and the results can be examined profitably.

Comparisons of karyotypes (sets of chromosomes) can be based upon differing levels of detail (White 1978:47). The first comparisons were made on the basis of the number of chromosomes. In some cases the number of one-armed (acrocentric) and two-armed (metacentric) chromosomes were included in the comparison, and the sex chromosomes were identified. Extensive lists of chromosome counts can be found in Matthey (1973a,b) for placental mammals and in Sharman (1973) and Hayman (1977) for marsupials. However, attempts to infer relationships based upon unbanded karyotypes have not been satisfactory (Atchley 1972). Frequently, individual chromosomes could not be identified, making comparisons of uncertain validity. Differences in arm number due to gain or loss of heterochromatin (tightly condensed chromatin, generally considered to have little genetic activity) were not correctly interpreted using conventional staining (Duffy 1972).

The development of banding techniques overcame these difficulties and made comparisons more meaningful. Structural changes in chromosomes (chromosomal rearrangements) can now be identified precisely. However, much remains to be learned about the meaning of banding and the structure of chromatin (the chromosomal material), and further developments can be expected to add to the value of comparative karyology.

**Chromosome Staining and Banding Techniques** (Cytogenetics Laboratory of the Laboratory of Pathology in Seattle, Washington, USA.)

**Conventional Staining**
- Conventional Giemsa Stain 1
- Conventional Giemsa Stain 2
- Leishman's Stain

**G-Banding**
- GTL-Banding
C-Banding  
Q-Banding  
R-Banding  
  Giemsa Reverse Banding (RHG)  
  R Banding by Fluorescence Using Acridine Orange (RHG)  
T-Banding  
NOR-Staining  
  Silver Nucleolar Organizing Region Staining  
DAPI/Distamycin A Staining

**History**

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**Conventional Staining Methods**

**Principle**

Conventional staining techniques are used to uniformly stain chromosomes and leave the centromeres constricted, thus enabling the measurement of chromosome length, centromeric position, and arm ratio.
Background

Prior to 1960, when Moorehead and Nowell described the use of Giemsa in their chromosome preparations, conventional cytologic stains such as acetoorcein, acetocarmine, gentian violet, hematoxylin, Leishman's, Wright's, and Feulgen stains were used to stain chromosomes. The Romanovsky dyes (which include Giemsa, Leishman's, and Wright's stain) are now recommended for conventional staining, because the slides can be easily destained and banded by most banding procedures. Orcein-stained chromosomes cannot be destained and banded; therefore, orcein is generally not used in routine chromosome staining. Giemsa stain is now the most popular stain for chromosome analysis (Gustashaw, 1991).

**Conventional Giemsa Stain 1**

**Solutions**

- Giemsa stain
- pH 6.8 phosphate buffer
- Working stain: 4 mL Giemsa; 96 mL pH 6.8 buffer

**Conventional Giemsa Stain 2**

**Solutions**

- 5N HCl
- Distilled water
- pH 6.8 phosphate buffer (Gurr's tablets, Biomedical Specialties cat. # 33199)
- Giemsa stain: 2 mL stock Giemsa; 4 mL pH 6.8 buffer; 92 mL distilled water

**Leishman's Stain**

**Solutions**

- Leishman's stain
- pH 6.8 phosphate buffer (Gurr's tablets, Biomedical Specialties cat. # 33199)
- Working stain: Leishman's stain diluted 1:4 with buffer
- Xylene
**G-Banding (GTL)**

Technique for producing banding patterns in eukaryotic chromosomes. Bands are produced by staining with Giemsa stain after pretreating chromosomes with trypsin. Each homologous chromosome pair has a unique pattern of g-bands, enabling recognition of particular chromosomes.

**Principle**

Chromosomes are G-banded to facilitate the identification of structural abnormalities. Slides are dehydrated, treated with the enzyme trypsin, and then stained.

**Procedure**

1. Place fixed, dry slides on slide rack in 95 °C oven and bake for 20 minutes. Cool.
2. Immerse slide in 0.025% trypsin for 10 to 120 seconds.
3. Remove slide from trypsin and immediately immerse in Fisher phosphate buffer to stop trypsin action.
4. Place slide cell side up on staining rack and flood with solution of 1 part Leishman's stain and 3 parts pHydrion working solution. Stain for 2 minutes.
5. Rinse slides thoroughly with distilled water.
6. Allow slides to drain, then place on 60 °C slide warming tray until completely dry.

**C-Banding**

**Principle**

To specifically stain the centromeric regions and other regions containing constitutive heterochromatin, i.e., the secondary constrictions of human chromosomes 1, 9, 16, and the distal segment of the Y chromosome long arm.

**Procedure**

1. G-band slides to facilitate identification of chromosomes.
2. Remove oil from G-banded slide thoroughly with at least two rinses of fresh Xylene substitute.
3. Destain slide by dipping in 3:1 fix; wipe bottom of slide, place on 40-60 degree C slide warming tray until beads of solution are formed: then blot gently with bibulous paper. These four steps should be repeated until beads are clear.

4. Place dry, destained slide in 0.2 N HCL for one hour. After 1/2 hour turn on pre-set waterbath and start to filter BA(OH)2 through #1 Whatman filter paper into Coplin jar.

5. Rinse slide (treated in 0.2 N HCl) in Coplin jar filled with distilled water.

6. Place rinsed slide in freshly filtered BA(OH)2 solution for two minutes.

7. Rinse with distilled water in squirt bottle (some force is required to remove BA(OH)2 crystals).

8. Place rinsed slide in Coplin jar (in waterbath) filled with 2 X SSC at approximately 62.5 degrees C for one hour.

9. Remove slide slowly and rinse gently in Coplin jar filled with freshly distilled water.

10. After drying, the slide should be stained as follows:
    - For peripheral blood specimen, stain 90 seconds with 1:5 Wright's stain.

**Q-Banding**

A staining technique in which metaphase chromosomes are stained with quinacrine mustard to produce temporary fluorescent Q bands on the chromosomes.

**Principle**

Chromosomes are treated with quinacrine mustard solution, a fluorescent stain, to identify specific chromosomes and structural rearrangements. It is especially useful for distinguishing the Y chromosome (also Y bodies in interphase nuclei) and various polymorphisms involving satellites and centromeres of specific chromosomes.

**Procedure**  Same as C banding.
**Giemsar Reverse Banding (RHG)**

**Principle**

R-banding methods are useful for analyzing deletions or translocations that involve the telomeres of chromosomes.

**Background**

Reverse banding using heat and Giemsa (RHG) was first described by Dutrillaux and Lejeune. This technique involves the incubation of slides in hot phosphate buffer with subsequent Giemsa staining. The resulting chromosome pattern shows darkly stained R bands and pale G bands. R bands are GC-rich and the AT-rich regions are selectively, or more readily, denatured by heat, but the GC-rich regions remain intact. This is consistent with the fact that GC-specific fluorochromes also produce a reverse chromosome banding pattern. In many laboratories, RHG methods have been abandoned in favor of a fluorescent R-banding technique (Gustashaw, 1991).

**Solutions**

- **Buffer:**
  10.0 mL Earle's balanced salt solution
  0.1 mL 7.5% Sodium bicarbonate
  89.9 mL distilled water
  Place buffer in water bath, and heat to 88 degrees to 89 degrees C.
- **tap water**
- **2% Giemsa in distilled water**

**Procedure**

1. Incubate slides in hot EBSS for 10 to 15 minutes. (Fresh slides require 1 to 2 hours. One-day-old slides require 25 minutes. One-week-old slides require 7 minutes. In general, older slides require less time.)
2. Cool quickly in tap water. Do not dry.
3. Stain in 2% Giemsa for 10 to 20 minutes.
4. Rinse in xylene.
5. Rinse in tap water. Air dry.
**R Banding by Fluorescence Using Acridine Orange (AO)**

**Principle**

R-banding methods are useful for analyzing deletions or translocations that involve the telomeres of chromosomes.

**Background**

Acridine orange was originally used to stain untreated chromosomes, both human and mouse. Bobrow et al. and Baserga and Castoldi independently reported the use of acridine orange to obtain a reverse banding pattern of chromosomes. Acridine orange (AO) is a base composition-independent fluorochrome that binds to DNA by intercalation and which gives relatively uniform fluorescence along the length of the chromosome arms. The dye binds very little to non-nucleic acid cell components, but it fluoresces orange-red when bound to single-stranded nucleic acids and yellow-green when bound to double-stranded nucleic acids. Following hot phosphate buffer treatment, R bands are yellow-green, and G/Q bands are orange-red. The major factor that contributes to R banding is the relative GC-richness of the R bands. In many laboratories, RHG methods have been abandoned in favor of a fluorescent R-banding technique (Gustashaw, 1991).

**Solutions**

- Phosphate Buffer:
  
  - 32 ml of 0.07N Na2HPO4 . 12 H2O
  - 68 mL of 0.07 mol/L KH2PO4
  - Adjust pH to 6.5 by adding 0.07N Na2HPO4 . 12 H2O to the solution.

- Acridine Orange: 0.01%, prepared in the phosphate buffer

**Procedure**

1. Prewarm the buffer to 85 degrees C.
2. Incubate one slide for 10 to 30 minutes in hot phosphate buffer.
3. Stain with 0.01% acridine orange for 4 to 6 minutes.
4. Rinse in phosphate buffer (pH 6.5) for 1.5 to 3 minutes.
5. Mount with the same buffer, without sealing the coverslip.
6. Examine by fluorescence microscopy using the appropriate filter combination (e.g., excitation: 450-490 nm; suppression: 515 nm).
**T-Banding**

**Principle**

T-banding is used to stain the telomeric regions of chromosomes for cytogenetic analysis. Also used in the study of translocations of juxta-telomeric break points.

**Background**

Telomeric (or terminal) banding was first reported by Dutrillaux, who used two types of controlled thermal denaturation followed by staining with either Giemsa or acridine orange. The T bands apparently represent a subset of the R bands because they are smaller that the corresponding R bands and are more strictly telomeric. (Gustashaw, 1991).

**T Banding by Thermal Denaturation: Method 1**

1. Bring 94 mL of distilled water and 3 mL of phosphate buffer (pH 6.7) to 87 degrees C in a Coplin jar.
2. Add 3 mL of Giemsa stain.
3. Add slides to jar; stain for 5 to 30 minutes.
4. Rinse in distilled water, air dry, and examine.

   For Fluorescent Observation

5. Destain, rehydrate through a series of alcohols, rinse in distilled water.
6. Stain in acridine orange (5mg/100mL) for 20 minutes.
7. Rinse in phosphate buffer, mount, and examine with a fluorescence microscope (excitation: 450-490 nm; suppression: 515 nm).

**T Banding by Thermal Denaturation: Method 2**

1. Bring a Coplin jar containing Earle's BSS, PBS, or phosphate buffer to 87 degrees C. The pH must be adjusted to 5.1.
2. Stain with Giemsa or acridine orange as in Method 1, steps 2 to 7.

**NOR-staining (Silver Nucleolar Organizing Region Staining)**

**Principle**

Chromosomes are treated with silver nitrate solution which binds to the Nucleolar Organizing Regions (NOR), i.e., the secondary constrictions (stalks) of acrocentric chromosomes.
**Procedure**

1. Place unstained slide in coplin jar filled with distilled water. Place in 37°C water bath for 2 hours. Remove slide and allow to air dry.

2. Prepare moist chamber by putting two 12.5cm circles of Whatman #2 filter in bottom of glass petri dish. Saturate paper with distilled water. Be sure to remove trapped air bubbles in paper. Place plastic lids or rings on wet filter paper to support each slide at both ends. Cover dish.

3. Attach 18 gauge needle to 1cc syringe.

4. Remove silver-nitrate solution from refrigerator and place bottle in beaker to prevent tipping (see "Precautions" below).

5. Put on gloves.

6. Draw 1cc of silver-nitrate solution into syringe.

7. Remove needle and replace with acrodisc filter.

8. Attach unused 18 gauge needle to acrodisc filter.


10. Using forceps, lower one end of pre-cleaned coverslip into drops of silver nitrate solution on slide and gently lower coverslip to avoid trapping air bubbles.

11. Transfer treated slide to moist chamber and support on lids above wet filter paper. Cover dish.

12. Carefully place moist chamber in 37 degrees C incubator. Check to see that treated slide is horizontal after closing inner glass door of incubator.

13. Incubate for 7 hours at 37 degrees C.


15. Check treated slide under 10X phase and 40X phase to judge effectiveness of first treatment.
16. If stained NOR's are unapparent, repeat steps 3-15 for one hour.

17. Repeat steps 14 and 15.

18. Repeat treatment at on hour intervals two or three times if necessary.

19. Counterstaining is not necessary, but may be desired. Counterstaining with Quinacrine is ideal; however, a pale counterstain with Wright's stain (see "G-Banding" procedure) may work satisfactorily. If Wright's stain is too dark, it is difficult to distinguish between satellites and silver staining on NOR's.

**DAPI/Distamycin A Staining**

**Principle**

The DAPI/Distamycin A staining technique is useful in identifying pericentromeric breakpoints in chromosomal rearrangements and in identifying chromosomes that are too small for standard banding techniques. Also, DAPI/DA is the method of choice for Yqh chromosome material in suspected Y autosome translocations.

**Background**

The DAPI/distamycin A fluorescent staining technique was first described by Schweizer, Ambros, and Andrle as a method for labeling a specific subset of C bands. (Gustashaw, 1991).

**Procedure**

1. Flood a slide with distamycin solution. Coverslip and incubate the slide, in the dark, at room temperature for 5 to 15 minutes.
2. Remove the coverslip and rinse briefly with pH 7.0 buffer.
3. Flood with DAPI working solution. Coverslip and incubate the slide, in the dark, at room temperature for 5 to 15 minutes.
4. Remove the coverslip and rinse the slide briefly with pH 7.0 buffer.
6. Photograph as for other fluorescent techniques.

**Standardization in karyotyping**

Advance in techniques and improvement in the quality of chromosomal preparations have necessitated adoption of a standard nomenclature. Initially each research team had devised its own
system of chromosome nomenclature. This resulted in considerable confusion in the identification and classification of chromosomes. Due to the absence of internationally adoptable system in nomenclature of farm species, the pattern adopted by the medical cytogeneticists was followed. It is pertinent to record that human cytogeneticists have made several attempts to develop an internationally acceptable uniform nomenclature. As and when new discoveries emerge, a conference is held for updating the nomenclature. The first conference of human chromosomes was held at Denvour in April, 1960. at this conference an attempt was made to find a uniform classification of chromosomes. The next conference was held in London in 1963 at which description of secondary constriction was taken into consideration. The Chicago conference in 1966 was followed by Paris conference in 1971. the Paris conference made several new recommendations on the basis of advances made in the fluorescent banding techniques. Since then a number of conference have taken up standardization based on various banding technique.

The criteria for identification and classification of chromosomes of farm livestock have not been completely standardized except for a preliminary attempts at Reading (U.K.) in 1976 and another conference held in 1989 at Jouy en Josas (France). A standing committee was constituted to take care of various recommendations.

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References


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