

Methods in Chiropteran Mitotic Chromosomal Studies

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1. INTRODUCTION

Karyotypic information is one of the valuable data sets used in systematic and genetic studies of bats. It is important in assessing relationships between taxa (Patton and Baker, 1978; Baker et al., 1979, 1982, 1984; Haiduk and Baker, 1982; Koop et al. 1984), identifying undescribed species (Baker, 1984), and in studying the processes of speciation and evolution (Bickham and Baker, 1978; Baker and Bickham, 1980; Baker, 1981; Baker et al., 1982; Greenbaum, 1981; Baker et al., 1987).

This paper explains the techniques used for obtaining karyotypic preparations from both bone marrow and primary cell cultures.

2. BONE MARROW PREPARATIONS

The best sources of bone marrow from bats are the humeri. For bone marrow preparations, live bats can be processed without prior treatments such as with mitotic inhibitors and mitogens. However, good health of the bat is important in order to achieve a sufficiently high mitotic index for study. For medium and large bats one humerus is sufficient, for small bats (less than 15 g), both humeri should be used. The humerus should be quickly dissected from the sacrificed animal by clipping through the humerus near the elbow and removing the proximal portion with the head and tuberosity intact (Fig. 1A). Muscle is cleaned from the humerus and the proximal end is removed (Fig. 1B). At least 1-2 mm of the shaft and the trochlear region of the humerus should be left articulating with the radius and ulna, as removal of the entire humerus from the specimen affects the length of the forearm, which can be critical in

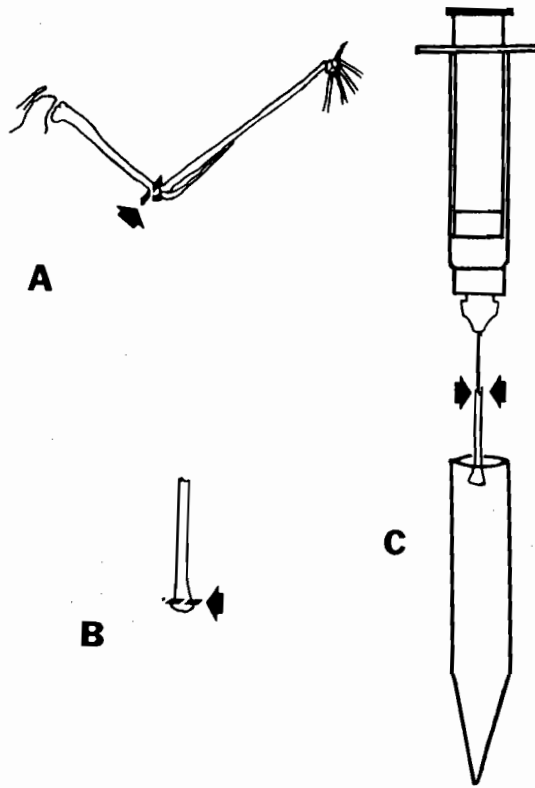


Figure 1. Method of extracting bone marrow from the humerus of a bat. (A) The humerus is cut and dissected out of freshly killed bat. (B) Then the proximal tuberosity is chipped. (C) The bone marrow is forced out of the humerus using a needle full of the hypotonic solution. The thumb and the index finger are used to seal the area around the needle tip and bone (arrows) when forcing the hypotonic solution through the shaft.

identifying the specimen. The bone marrow is flushed into a centrifuge tube with a syringe containing a 0.075 M KCl hypotonic solution (see Appendix 2) at 37°C (Fig. 1C). The bone marrow is gently aspirated with a Pasteur pipette until all the cells are dispersed in the hypotonic solution, then incubated for 27 min at 37°C.

Following incubation in hypotonic solution, the suspension is centrifuged at 1000 rpm for 1 min and the supernatant is gently decanted, leaving two or three drops of liquid in the centrifuge tube. The pellet should be *very gently* but thoroughly resuspended in the

remaining small amount of hypotonic solution. The treatment of the cells at this stage is extremely critical and care must be taken to avoid rupturing the mitotic cells. Five milliliters of Carnoy's fixative (one part glacial acetic acid: three parts absolute methanol) is gently introduced down the side of the centrifuge tube. It is important that the fixative be prepared fresh each time (we generally make 40 ml in a 50 ml graduated cylinder) and that the methanol and acetic acid are absolutely free of water. The cells are very gently suspended and the tube centrifuged as before. This time all the supernatant is decanted or removed with a Pasteur pipette, and the cells are gently resuspended in new fixative and are recentrifuged. After one more washing with fixative, the cells are suspended in a small amount of fixative (0.5-3.0 mm depending on the number of cells), and a slide is made as described below. The slide is then either scanned with phase contrast microscopy or, if phase is not available, it can be Giemsa stained and observed with bright field microscopy. Under phase contrast, fixed chromosomes look black and contrast with the background, but if yellowish or surrounded by a bright yellow haze, they should be fixed one or more times as described above. Under bright field microscopy, well fixed cells appear to have little or no cytoplasm, which will appear as purplish haze, remaining associated with the chromosomes. At this stage, slides can be made, or the cell suspension can be frozen in liquid nitrogen (Baker et al., 1982). Freezing in liquid nitrogen is particularly useful on long field trips where bats cannot be transported alive to the laboratory. Cell suspensions can then be thawed after returning to the laboratory. We have made slides for G- and C-banding in the field and transported them to the laboratory, but these efforts generally produce poor G-bands and also pose some problems in transporting large numbers of slides on sustained field trips. Conical eppendorf or microcentrifuge tubes work well for storing cell suspensions and for additional fixation of cells by centrifugation, thus avoiding loss of

cells in larger glass tubes. If a microcentrifuge is not available, eppendorf or NUNC tubes can be used by placing them directly inside larger glass tubes.

2.1. Standard Karyotypes

For each specimen, it is desirable to have a standard karyotype for reference, and air dried slides generally do not provide the best quality spreads for determining centromere position on the chromosomes. Therefore, for each specimen, we prepare one or more slides by the blaze dry technique (Scherz, 1962). This technique consists of dropping three or four drops of the cell suspension onto a slide and immediately igniting the fixative. After the fire extinguishes itself, the residue solution on the slide is removed by tapping the slide gently on its side on a paper towel. Blotting the material with filter paper may destroy some of the cells so this technique is not generally recommended.

2.2. Preparations of Air-dried Slides for Banding

Two, three, or more drops of the cell suspension are dropped from a distance of two feet into water on a slide. The method is illustrated in Baker et al. (1982). Slides are then drained of excess water and can be used for the various banding techniques. For a review of the available banding techniques see Pathak (1976).

2.3. Problems

2.3.1. Low Mitotic Index

Even with healthy animals, the mitotic index may be too low. Yeast stress (Lec and Elder, 1980), however, significantly increases the mitotic index. A subcutaneous injection of 0.1 ml/10 g body mass of the yeast solution (3 g yeast: 2 g dextrose: 12 ml H₂O) is given to the animal on two or three consecutive days before sacrificing. We inject the specimen subcutaneously on the dorsal part of the fore-

arm because too often individuals died when injected on the main body areas. It is important to keep the bats in good condition for the duration of the treatment. This will allow the animals to respond fully to the yeast infection. Although an injection with a mitotic inhibitor (e.g., Velban or Colchicine) causes an increase in the number of cells in the metaphase stage, this also results in shorter chromosomes which are not desirable for the banding techniques. When we do use a mitotic inhibitor, we add it to the hypotonic solution (see Baker et al., 1982) rather than injecting the animal. We have found that for some species of bats it is very difficult to obtain banded chromosomes from bone marrow preparations because of the very low mitotic index. This is true for very small and/or less hardy species of bats, as well as bats taken from hibernation. For such bats, cell cultures can be initiated to obtain the needed data.

2.3.2. Inadequate Spreading of Chromosomes

If a high number of prophase and early metaphase cells are observed, but the chromosomes are not spread sufficiently so that each chromosome is separate, this can be corrected by increasing the time in hypotonic solution. Incubation time is critical because cells will rupture if incubated for too long a time, and the chromosomes will not be spread enough if the incubation time is too short. For most bats the optimum time is around 27 min. Some adjustment of this time may be in order and our times generally range from 25-40 min. The temperature of the hypotonic solution is also critical, and if room temperature is too cool, we keep the temperature elevated by keeping the centrifuge tube inside a belt next to the body, where it is warmed by body heat or in an incubator if available.

2.3.3. Improper Fixation

The stage of fixation also affects the spreading of cells as well as quality of banding. If

chromosomes appear under-fixed (see above) and the chromosomes are not well spread on the slide, then one or more additional washes in new fixative is appropriate. However, each time that cells are washed in fixative, some of the best cells may be ruptured during handling. If additional washes do not provide adequate fixation, this may indicate that a new supply of absolute methanol and glacial acetic acid should be obtained.

2.3.4. Clumping after Fixation

For some species, cells may become clumped after centrifugation in hypotonic solution. Aspirating the clump until it dissociates can cause the fragile mitotic cells to rupture. Clumping can be reduced by introducing a few drops of fixative to the hypotonic solution and gently mixing the solutions immediately prior to the initial centrifugation.

3. G-BANDING PROCEDURE

G-bands are useful tools for identifying and matching homologous chromosomes (Fig. 2) even between distant taxa (see as examples Patton and Baker, 1978; Dutrillaux, 1979; Baker et al., 1982, 1983; Haiduk and Baker, 1982, 1984; Koop et al., 1984; Yunis and Prakash, 1982; Qumsiyeh and Baker 1985). Preparation of G-banded chromosomes is not easily done in the field and requires some experience before the material can be used in systematic or genetic studies. The following procedure is a modification of the techniques used by Seabright (1971) as modified in Patton and Baker (1978).

- 1) Place slides on a 60°C slide warmer for 12-24 h.
- 2) The G-banding set-up consists of 6 Copeland jars as follows:
 - A. 7 ml of 0.25% trypsin solution and 43 ml Hanks buffer (see Appendix 2).
 - B. 50 ml of Hanks buffer
 - C. 70% ethanol
 - D. 95% ethanol
 - E. 95% ethanol



Figure 2. A partial spread of correctly banded chromosomes of a bat (*Uroderma bilobatum*).

F. 2% Giemsa stain in phosphate buffer (see Appendix 2).

- One slide at a time is prepared and then studied for the quality and stage of banding.
- 3) Place the slide in the trypsin solution (jar A) for 4-8 min. If the slides have been on a warmer for less than 20 h, then start at 4 min. Rinse in each of the next four jars and allow the slide to dry.
- 4) Stain in Giemsa for 7-8 min.
- 5) Dry and scan under light microscope for spreads then check under oil for the quality of the G-bands. If the chromosomes are fuzzy, faint, and destroyed (see Fig. 4), then decrease the time in trypsin. The slides can be left on the slide warmer longer if the chromosomes are still too soft or the bands are not sharp. If, on the other hand, the spreads look like standards by showing no banding or little banding (Fig. 3), more time is needed in trypsin.



Figure 3. A partial spread of chromosomes showing their appearance when undertreated in trypsin (*Rhinolophus* sp.).



Figure 4. A partial spread of chromosomes showing their appearance when overtreated in trypsin (*Rhinolophus* sp.).

Usually 4-6 slides are adequate to get good G-band preparations using these techniques.

4. C-BANDING PROCEDURE

When chromosomes are treated in acid and the chromatin material is dissociated (usually in barium hydroxide) followed by a reassociation, a characteristic banding pattern can be observed. The bands are usually localized around the centromeres or in some cases elsewhere on the arms. Such bands represent heterochromatin material (usually highly repetitive DNA). Following is a modification of the techniques described by Stefos and Arrighi (1971) for obtaining C-bands.

1) Air dried slides are stored in covered slide

boxes at room temperature generally for 1-8 weeks before treatment. However, we have obtained consistently adequate results from slides aged for up to five months.

- 2) Treat in 0.1 molar HCl for 20 min.
- 3) Rinse in distilled water and air dry.
- 4) Incubate individual slides in saturated $\text{Ba}(\text{OH})_2$ at 46°C for 0.5, 1, 2, and 3 min. There is a direct correlation between how long slides have been aged and the proper treatment time in barium hydroxide (older slides require longer treatment times). If more slides are available, then more times can be covered (between 0.5-4 min). This will give one or more slides that are at the correct dissociation time.
- 5) Immediately rinse in 0.1 molar HCl, give two rinses in distilled water and let air dry.
- 6) Set up humidity chambers (these can be

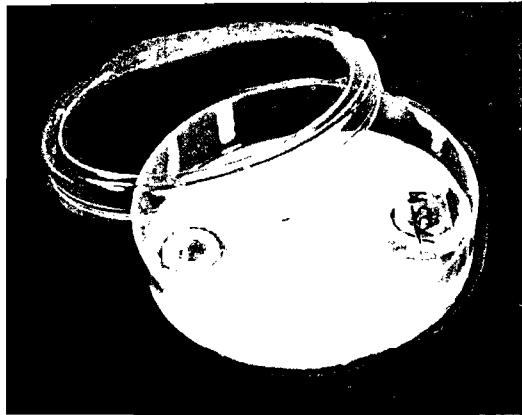


Figure 5. A petri dish with a filter paper lining and slide supporters used as a humidity chamber for C-banding.

simply constructed out of petri dishes lined with filter paper and provided with slide supporters, see Fig. 5). Put 4-8 drops of 2X SSC solution (see Appendix 2) on the slide and cover with a cover slip and soak the filter paper well with 2X SSC. Cover and incubate overnight (or 10-14 h) at 60°C. Make sure that the humidity chamber remains moist and does not dry out during the incubation. If needed, more layers of moist filter paper can be added.

- 7) Rinse slides in water to remove cover-slips.
- 8) Rinse in 70% then 95% ethanol then air dry.
- 9) Stain for 10-12 min in 2% Giemsa.

If the chromosomes do not take up the stain, and both the chromosomes and the non-mitotic cells show faint (ghost) images, then the slides have been overtreated in the barium hydroxide. If the chromosomes are dark and look like standard (nondifferentially banded) chromosomes, then the slides have been under-treated and a longer time in barium hydroxide is needed.

5. CELL CULTURE AND HARVESTING TECHNIQUES

Cell culture is a complex technique commonly used in hospital and university labora-

tories. The general process is beyond the scope of this chapter and there are general references (Kruse and Patterson, 1973; Paul, 1975; Pollack, 1973 and Adams, 1980) dealing with various aspects of the process. Many colleges and universities offer courses in these general techniques and they should be mastered before attempting to use cell cultures to karyotype bats. We will discuss here only the aspects of cell culture that are modified in our laboratory or are unique to obtaining G- and C-banded chromosomal data from cultures of bat cells.

5.1. Procedures for Taking Tissue for Culture

The major concern in taking tissue for culture is avoiding contamination. The tissue sample should be taken under a sterile hood or, if this is not available (in the field), inside a clear plastic bag that has been sprayed thoroughly with 70% ethanol. All instruments used to take the sample should be sterilized with alcohol and, if possible, flamed before use. We usually place instruments in a Copeland jar of 70% ethanol. Plastic disposable centrifuge tubes containing 10 ml of sterile medium are taken to the field to keep biopsy samples alive until returned to the laboratory. Once aseptic procedures have been followed, the bat is sacrificed and the tissues are obtained immediately as follows:

- 1) Spray entire animal with 70% ethanol until soaked.
- 2) To take ear biopsies, saturate a piece of cheesecloth with 70% ethanol and clean the ear thoroughly by rubbing with the cloth, even to the extent of removing outer layers of skin. Hold ear out with forceps and clip with scissors. Place in tube containing media as below (see step 8 below).
- 3) To take lung biopsies, slit skin along chest with scissors, peel back, and secure (hemostats work well for this).
- 4) Spray chest and dip scissors in ethanol before opening chest cavity.
- 5) Open chest cavity to expose lungs.

- 6) With sterile forceps, bring one lobe of the lung into a position that makes it easy to remove a small portion of tissue from the outer rim.
- 7) Clip a small piece (approximately 5 mm²) of tissue from this area of the lung.
- 8) Remove cap from media tube and drop tissue sample into it with forceps. Be careful not to touch the lip of the tube with your hands or the forceps. Replace the cap immediately, being careful not to pass your hand over the open mouth of the tube.
- 9) Take a second sample as above but place the tissue in a separate tube of media.
- 10) Label all samples immediately as to sex, tissue type, species, specimen number and locality. Voucher specimens of all bats should be preserved in the usual ways (in formalin, alcohol, or as skins and skeletons) for reference (see Chapter 26).
- 11) If possible, immediately mail tissue samples with proper permits and instructions to customs officials identifying the contents, asking them not to open the tubes. In the United States permits available from the Center for Disease Control (Atlanta, Georgia) should be obtained so that customs officials will not open the containers in transit.

Contamination usually arises from handling the tubes, from the original specimen itself, and from air if the medium or sample receive prolonged exposure to it. It is best to open the culture tube only briefly and with one hand hold the cap on top of the tube while introducing the sample with sterile forceps in the other hand to the inside of the tube.

5.2. Procedure for Initiating Primary Cultures

The following is a summary of techniques used in our laboratory. For a more detailed and alternate techniques of cell and tissue culture see Pollack (1973), Paul (1975) and Adams (1980). Most of the cell culture work is done under a horizontal laminar flow hood equipped with HEPA (high efficiency particulate

ulate air) filters. Spray the inside walls of the hood with 70% ethanol, wipe, and turn the hood on to allow the airflow to stabilize for one-half hour before proceeding.

HAMS F-10 (or HAMS F-12) supplemented with 15-20% fetal bovine serum (FBS) is a good medium for growing primary cell cultures of mammals. HAMS F-10 is best purchased in powdered form, which occupies little space in the freezer. It is diluted into a 10X stock solution and then sterilized by filtration (see Adams, 1980). The 1 X solution is prepared by introducing 50 ml of the sterile 10X solution into a bottle containing 450 ml sterile deionized water. Next, 75-100 ml of fetal calf serum are added aseptically, and a small sample of the medium is poured into a T-25 flask for a sterility check. Antibiotics are not generally used in this procedure because they can result in lax aseptic procedures and selection for resistant bacterial and fungal strains that could spread extensively to other flasks. However, if a particularly important cell line becomes contaminated, it can be cleared by washing the monolayer with Hanks buffer several times (see steps 1-3 below) and then introducing a medium that contains antibiotics. Antibiotics that are commonly used and their recommended concentrations are listed in Table 1.

Bacteria and fungi can be easily recognized both microscopically and macroscopically (cloudiness or colonies in flasks). Mycoplasmas are harder to detect but they cause a slowdown in cell growth and can be tested for by using PPLO agar gels (see Adams, 1980).

Table 1. Antibiotics commonly used against culture contaminants and their optimum concentrations in the culture medium (from Paul, 1975).

Antibiotic	Concentration ($\mu\text{g/ml}$)	Contaminant
Penicillin- Streptomycin	50	Bacteria
Gentamycin	50	Bacteria
Mycostatin	20	Fungi
Fungizone (Amphotericin B)	2.5	Fungi
Kanamycin	100-200	Mycoplasmas
Gentamycin	200	Mycoplasmas

5.3. Initiating and Maintaining Primary Cell Lines

It is important that all equipment and material that will touch the tissue or medium be absolutely sterile. Forceps and scalpels are placed in a Copeland jar with 70% ethanol and flamed lightly before use. The contents of the tube with the animal explant are poured into a petri dish, then the explant is transferred to another petri dish (or the petri dish cover). The explant may be rinsed quickly by dipping it into some fresh medium. The tissue is then minced into small pieces using a sterile scalpel. The chopped tissue is then transferred to a growing flask for initiating the culture with cell culture medium. There are many kinds of culture vessels on the market; the easiest to use are disposable T-25 or T-75 flasks (size of surface area available for cell growth) which are designed for best attachment of cells. For mammalian cells, 5 ml of HAMS F-10 are required for the T-25 flasks and 15 ml are required for the T-75 flasks. Flasks containing tissue are then incubated horizontally at 37°C. Generally pieces of explants will attach to the surface and begin explanting fibroblast cells in 2-10 days. Fibroblast type cells are not the only type of cells that will be initiated, but they generally outgrow epithelial and other types of cells.

The flasks can be rapped after the initial growth to allow explants to resettle and initiate new colonies of cells. Once 5-10 colonies of rapidly growing cells are initiated, the cells must be dispersed. To disperse cells in medium, proceed as follows:

- 1) Gently pour medium into a T-25 flask and save for possible growth of other explants or as a bacterial check.
- 2) Add 10 ml of sterile Hanks buffer (see Appendix 2), close the flask, and let it sit horizontally for 10 min.
- 3) Decant and repeat step 2 two more times.
- 4) Decant and introduce 1-2 ml of 0.25% sterile trypsin (see Appendix 2), cover and let sit horizontally for 1-2 min.
- 5) Scan using inverted microscopy for the

number of cells detaching from the substrate. If cells are not responding very well, then rap the flask gently. If this does not work, incubate for 5-15 min at 37°C. For some bat cell lines, longer periods of trypsin treatment may be required, but for most, 5-15 minutes are sufficient. Selection for cells that are more resistant to the action of trypsin can be done if the cell line is kept for several generations.

Trypsin acts as a proteolytic enzyme by dissociating glycoproteins of cell membranes, making cells separate from one another and from the substrate. Action of trypsin is inhibited by calcium and magnesium ions which are present in most cell culture media. Accordingly, it is essential to wash the attached cells with Hanks buffer (Ca⁺⁺ and Mg⁺⁺ free) before adding the trypsin. It should be emphasized here that if cells respond to trypsin, by rounding up and then detaching from the surface, the next step should be performed quickly by not allowing cells to remain any longer in the trypsin. Over-exposure to the trypsin can destroy cellular membranes and result in cell death.

- 6) Introduce fresh culture medium when about 80% of cells are in suspension in the trypsin. This will inhibit further trypsin action.
- 7) To subculture dispersed cells, simply add double the amount of medium (i.e., 30 ml for T-75 flasks) and then remove half the medium with cells into a new flask. Some rapidly growing cells can be subcultured using a split ratio of 1:3 (i.e., one flask is subcultured into 3 flasks) or even higher.

5.4. Karyotyping from Cultured Cells

Generally we karyotype a cell line only if three or more flasks of the same animal are apparently confluent. To reduce the risk that all cells from the same animal are contaminated, we do not feed or treat all flasks from the same animal at the same time. Flasks that show an abundance of cells undergoing mitosis are chosen for karyotyping. An active culture

should show a number of cells rounded up, and many of those should be in the telophase stage. Proceed to disperse the cells as described above for maintaining cell lines, but save all media (F-10 and Hanks) and centrifuge for 5 minutes to collect any floating cells. When cells have dispersed into the trypsin, pour out the suspension into a tube and centrifuge. Before centrifugation, one or two drops of Velban (0.00025%) can be added to the tubes if the chromosomes are long and thin and as such show much overlap. After centrifugation for 5 min at 1000 rpm, decant the supernatant leaving but 2-3 drops in which the cells are resuspended. A hypotonic solution of one part medium: four parts deionized water is added, and the tube is incubated for 25 min at 37°C. After this step, follow the procedure as for fixing and making slides from bone marrow. Cells obtained from culture are generally more fragile than bone marrow cells, and are obviously less in volume. These disadvantages are outweighed by the higher mitotic index obtained in cell culture preparations. Flasks may be saved after harvesting cells for karyotyping by simply adding new media.

Living cells may be frozen in liquid nitrogen and revived when needed again (Baker and Haiduk, 1984). For freezing, cells are dispersed as above but in a small amount of sterile medium rather than the hypotonic solution. Suspended cells are introduced into sterile vials (NUNC tubes), and Dimethyl Sulfoxide (DMSO) is added to a concentration of 7%. The vials are closed tightly and correctly labeled. Slow freezing and fast thawing of cells gives best results. Vials ready to freeze are put on ice for 30 min, then into a -70°C freezer overnight, and then into the liquid nitrogen (-196°C).

6. SUMMARY

Methods for obtaining mitotic chromosomal preparations from bone marrow or cultured cells for systematic and evolutionary studies of bats are presented. We discuss methods for

nondifferentially stained, G-, and C-banded chromosomal preparations. G-bands are produced on chromosomes treated with trypsin and stained in giemsa. C-bands are obtained by subsequent treatments in hydrochloric acid, saturated barium hydroxide, reassociation in humidity chambers with 2x SSC buffer, and stained with giemsa.

Problems of low mitotic index, inadequate spreading of chromosomes, improper fixation, and clumping after fixation are addressed and possible solutions presented. Procedures are presented for obtaining samples, initiating and maintaining primary cell cultures, and karyotyping from cultured cells. We also present formulas for making growth media, buffers, trypsin, and hypotonic solution, and provide the names and addresses of sources for the chemicals and supplies.

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Appendix 1. Chemicals, Equipment, and Their Suppliers

This list gives only the supplies that are not commonly available in universities (i.e., it does not include the common chemicals). This list gives only the most common American suppliers. For European suppliers, see Adams (1980).

Chemicals and Media

Nutrient F-10 medium (Ham's)

Penicillin-Streptomycin
(10,000 mcg/ml each)

Neomycin-Sulfate
(10,000 mcg/ml)

Hanks base

Fetal Bovine or Fetal Calf Serum

Trypsin

Chemicals and media can be obtained from one of the following suppliers:
KC Biological, Irvine
Scientific, Grand Island
Biological Company

Equipment and Supplies

Plastic petri dishes, 100x20 mm

Falcon, Corning, NUNC

15 mm Polystyrene conical tubes
with screw caps

Falcon, Corning, NUNC

25 cm and 75 cm culture flasks

Filtration set-up with 0.22 μ pore
size, 47 mm filter size

Millipore

Addresses of Suppliers

Corning Glass Works
Corning, New York 14830

Falcon
Cockeysville, Maryland 21030

Grand Island Biological Company
Grand Island, New York 14072

Irvine Scientific
2511 Daimler Street
Santa Ana, California 92705

KC Biological, Inc.
Lenexa, Kansas 66215

Millipore Corporation
Bedford, Massachusetts 01730

NUNC (Denmark) distributed by
Southland Cryogenics, Inc.
1212 Tappan Circle, Box 627
Carrollton, Texas 75006

Appendix 2. Media and Recipes for Chromosomal Studies**Standard F-10 Medium**

450 ml sH_2O (sterile water)

50 ml 10 X F-10 medium

75 ml FBS (Fetal bovine serum)

adjust pH with sterile $NaHCO_3$ to golden amber,
and refrigerate

Field F-10 medium

as above but use 100 ml FBS and add

2 ml Penicillin-Streptomycin

2 ml Neomycin-Sulfate

0.25 ml Fungizone

Hanks Buffer

450 ml sH_2O

25 ml 20 X Hanks Salts

25 ml 20 X Glucose

20 X Hanks Salts

80 g NaCl

5 g KCl

0.6 g Na_2HPO_4

0.6 g KH_2PO_4

Mix in 500 ml dH_2O (distilled water) and
auto-clave

20 X Hanks Glucose

20 g glucose in 500 ml dH_2O and autoclave

Phosphate buffer for Giemsa stain

0.469 g NaH_2PO_4

0.937 g Na_2HPO_4

1000 ml dH_2O

pH=7

Phenol Red

1 gm Phenol Red

100 ml H_2O

Autoclave

0.075 M Potassium Chloride (Hypotonic Solution)

2.79 g KCl

500 ml dH_2O

2X SSC (for C-banding)

5.47 g NaCl

4.32 g Sodium Citrate

500 ml H_2O

0.25% Trypsin

10 ml Stock 2.5% Trypsin

90 ml Hanks Solution

adjust pH with sterile $NaHCO_3$ until color is
pinkish.

For G-banding we use 8 ml of this Trypsin
solution and dilute it further by adding 42 ml
of Hanks solution (to a Copeland jar, jar A in
page 428)