Chapter 4

Techniques in Karyology: The Bone Marrow Extraction Method

Deanna K. Tolliver and Lynn W. Robbins

Department of Biology
Southwest Missouri State University
Springfield, Missouri 65804

Deanna K. Tolliver is a Laboratory Supervisor in the Department of Biology at SMSU where she coordinates and teaches laboratory sections of General Biology and Cell Biology. She received an M.S. in Biology from Fort Hays State University. Her research interests include mammalian systematics and population genetics/ecology.

Lynn W. Robbins is an Associate Professor in the Department of Biology at SMSU where he teaches Cell Biology, Mammalogy, and Wildlife Management. He received a Ph.D. from Texas Tech University. He and his graduate students are involved in research in the areas of small mammal population genetics, ungulate behavior, natural history and management of endangered species, and systematics of salamanders.

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Contents

Introduction

In this laboratory exercise, chromosomes are isolated from white blood cells in the bone marrow of white mice. This procedure has been used in research for several years. In general, we follow the procedure as given in Baker et al. (1982) and Lee and Elder (1980). We have found it to be easily adaptable to a 2-hour laboratory period, and it is included as part of the curriculum for our Introductory Cell Biology class. It is equally suitable for classes in Zoology and/or Genetics.

This procedure replaces that of tissue culture, which is time-consuming, expensive, and requires that the procedure be performed wholly in the laboratory. The bone marrow extraction method can be performed in the field, if desired.

Some preparation of the mice is required before the class meets: about 15 minutes 2 days prior to laboratory, and 15 minutes 2 hours before the laboratory. Very little equipment is needed, except for microscopes and a centrifuge. An incubator is optional; we have the students hold the tubes in their hands to keep them warm for the required amount of time. We have used a manually-operated centrifuge in the field with equally good results.

Notes for the Instructor

This exercise requires that the mice be sacrificed. Cervical dislocation is the method of choice, and we feel this is best performed by the instructor. We have not had good preparations when the mice were anesthetized, although we don't know why. We suggest that once you have gone through the procedure using cervical dislocation, and have obtained good preparations, that you try anesthetizing the mice if you feel more comfortable with that method.

Of the approximate 400 students who have participated in this exercise, about six have refused to be involved because they objected to the idea of killing the mice. Some refused to attend the laboratory altogether. We talked with these students and respected their wishes, although we did require them to view the microscope slides and complete the required laboratory hand-ins. This exercise could be viewed as an opportunity to discuss bioethical issues in science, perhaps in a lecture. Also, if the sequence of topics in this course's schedule, or another course's schedule, are timed right, the mice could be used as part of a dissection laboratory or as practice for preparing museum specimens.

The resulting microscope slides show standard chromosomes, as opposed to banded chromosomes. Although not included here, if facilities and equipment allow, photographs of the chromosomal spreads can be taken, enlarged, and used to construct a karyotype. A simple Polaroid camera set-up can be used.

Appendix A contains recipes for making the various buffers and other solutions, and contains sources for materials.

Materials

In our class, students work in pairs. Each pair receives one mouse. Each student can remove the marrow from one leg. See Appendix A for ingredients of solutions.

1. Microscope (oil immersion enhances the prep but is not essential)
2. Microscope slides (2–4 per pair). The type of slides having a frosted end is desirable because students can mark their slides with a grease pencil.
3. Microscope coverslips (2–4 per pair)—necessary for oil immersion
4. Grease pencil (1 per pair)
5. 3-cc syringe with 23 gauge, 1-inch needle (one per pair)
6. Sharp scissors (2 per pair)
7. Paper towels (for dissecting mice and absorbing excess fluid on slides)
8. Centrifuge (1500 rpm minimum). In a small class (less than 10 students) one will suffice, but note that each pair will centrifuge four times. The more centrifuges available, the less time each pair will have to wait to use one. *Note:* Hand operated centrifuges work fine. Not only are they less expensive, they can be used in the field or anywhere where electricity is not available.
9. Incubator (optional). We have our students hold the tubes in their hands.
10. Pasteur pipets with bulbs (one per pair)
11. Coplin jars (will hold 8–10 slides, more if slides are inserted back-to-back)
12. 2% Giemsa (about 45 ml per Coplin jar)
13. Yeast solution (0.5 ml per mouse)
14. Colchicine solution (0.1 ml per mouse)
15. Matches
16. 0.075 KCl (3 ml per pair)
17. Carnoy's fixative (about 15 ml per pair)

Student Outline

Introduction

Karyology is the study of chromosomes. The morphology and number of chromosomes can be used to (1) determine the species of an organism in cases where identification is questionable; (2) construct phylogenies when karyotypes are compared across taxa; and (3) diagnose diseases associated with chromosomal aberrations.

Every species of plant and animal has a specific number of chromosomes. This is referred to as the 2N, or diploid, number. For example, humans (*Homo sapiens*) have 2N = 46, fruit flies (*Drosophila melanogaster*) have 2N = 8, and corn (*Zea mays*) has 2N = 20. Some very different species may have the same 2N number, yet be very different. For example, the diploid numbers of both lilies and yellow pines is 24. What makes these two species different, of course, is the genes on the chromosomes.

Chromosomes can be isolated from cells for diagnostic work and research. Until several years ago, the only method available for karyology of small mammals was that of tissue culture, where blood cells were cultured for several weeks in a sterile medium. For research involving surveys of small mammals, for example, this method was expensive and time-consuming, and the animals had to be transported back to the laboratory because special equipment was required (a laminar flow hood, for example).

The technique presented in this exercise, however, allows the preparation of microscope slides while in the field (if necessary), is inexpensive, and requires little in the way of equipment. The animal is sacrificed and chromosomes are isolated from the white blood cells (WBCs) in the bone marrow of the hind legs. In this exercise, white laboratory mice will be used.

Procedure

2 days prior to the laboratory: Inject each mouse subcutaneously with 0.5 ml of the yeast solution. This will provoke an immune response (infection) and will cause an increase in the production of WBCs.

2 hours prior to the laboratory: Inject each mouse intraperitoneally with 0.1 ml of 0.01% colchicine, a mitotic inhibitor. Colchicine is an alkyloid derived from a plant, the autumn crocus. It interferes with spindle formation during mitosis, and replicated chromosomes cannot migrate to their respective poles.

1. Sacrifice the mouse in a humane manner (cervical dislocation).
2. Open the abdominal cavity with a pair of scissors, being careful to not cut into the viscera.
3. (a) Remove the hind leg bones (femur and tibia) by cutting through the bones at the ankle and as near the pelvis as possible.
   (b) Trim off as much muscle and fat from the bones as possible.
   (c) Separate the two bones by cutting through the knee joint.
   (d) After the bones are cut, there should be an opening into the bone marrow cavity at both ends of each bone.
4. Read through all of step 4 before you begin.
   (a) Fill a 3-cc syringe with 0.075M KCl (it is easier to fill the syringe with the needle removed).
   (b) Insert the tip of the needle into the more narrow end of one bone, and, holding onto the bone, flush the marrow into a centrifuge tube.
   (c) Repeat this procedure for the rest of the bones, flushing the bone marrow from each into one centrifuge tube.
   (d) Use only a portion of the KCl in the syringe for each bone (i.e., do not use more than the total of 3-cc of KCl in the syringe for one mouse).
5. *Gently* aspirate the solution with a Pasteur pipet until a more or less homogenous cellular suspension is produced. *Be gentle!* If all the cell clumps don't break apart, that will be okay at this point.
6. Incubate the cell suspension for 15 minutes at about 37°C, in an incubator, or hold the tube in your hand for 15 minutes.
7. Centrifuge the suspension for 2 minutes at 1500 rpm.
8. (a) There should be a small button of cells at the bottom of the tube. Remove most—not all—of the supernatant with the pipet, without disturbing the cell button.
   (b) When you have removed all but about 0.5 ml of the supernatant, gently break up the cell button with the tip of the pipet.

9. (a)Fill the pipet with *fresh, cold* Carnoy's fixative, and add it to the tube by letting it flow down the sides.
   (b) Let the solution sit for about 30 seconds, then gently aspirate.
   (c)Centrifuge as before.
10. Remove all the supernatant using the pipet, without disturbing the cell button.
11. Repeat step 9 twice, then go to step 12.
12. Resuspend the cells in about 1.0 ml of fixative, then aspirate.
13. (a)Place two microscope slides on the edge of a table. Put the frosted end of the slide toward you, so it will be easy to pick up off the table.
   (b) Be sure there are no papers or flammable materials near the slides.
14. Place 2–4 drops of the suspension on each slide, and quickly ignite the cell suspension with a match. Have someone else light the slide for you immediately after you put the drops on the slide.
15. (a)Hold the slides vertically on a paper towel and let the excess liquid run off. Do **not** wipe the slides.
   (b) Let the slides air dry.
16. Stain the slides in a Coplin jar with 2% Giemsa stain for 10 minutes.
17. (a)Gently rinse the slides over a sink with a distilled water rinse bottle.
   (b) Let the slides air dry.
18. Examine the slides under high power and oil immersion.

Literature Cited

Baker, R. J., M. W. Haiduk, L. W. Robbins, A. Cadena, and B. F. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Special Publication Pymatuning Laboratory of Ecology, No. 6: 303–327.

Lee, M. R., and F. F. B. Elder. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenetics and Cell Genetics, 26:36–40.

APPENDIX A
Solution Ingredients

Yeast solution

3 g baking yeast
2 g dextrose
12 ml distilled H_2O
Mix in a test tube by shaking.

Colchicine

0.01% solution (use distilled H_2O)
Refrigerate.
Available from: Sigma Chemical Company, Stock no. C-9754 (1-800-325-3010)

Carnoy's Fixative

3 parts absolute methanol
1 part glacial acetic acid
Must be freshly made no earlier than 1 hour before use.

2% Giemsa stain

2 ml Giemsa stock solution (0.4% w/v)
98 ml PO_4 buffer

PO_4 buffer

0.469g NaH_2PO_4
0.937g Na_2HPO_4
1000 ml distilled H_2O