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果蝇实验指南

Drosophila Protocols

（影印版）

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内容简介

果蝇是遗传学、发育学研究的重要模式生物之一,对于发育生物学、基因组学领域中的探索具有极为重要的意义。本书是冷泉港实验室出版社 *Drosophila Protocols* 的影印版,共提供了近40个未来10年内最可能用到的以果蝇为实验对象的分子生物学、生物化学和细胞生物学研究的实验方案。每一个方案都经过专家的精心挑选和雕琢,实验设计严谨、准确、简洁、规范,可操作性强,值得称道。方案涉及染色体、细胞、基因组和发育等方面,既包含了初学者需要了解的基础知识,也涵盖了资深研究者所需的细节。本书的版式设计侧重于方便读者使用,正文中穿插了丰富的图表作为实验设计的辅助说明,附录中还列出了果蝇研究所需的重要资料如基本溶液、缓冲液的配方和配制方法等。

本书适合于从事发育生物学、细胞生物学、基因组学、遗传学、分子生物学、药物设计和开发以及功能基因学组研究的相关教学科研人员以及相关学科的本科生、研究生参考使用。

正文内彩图集中列于书末彩色图版,请读者对应参考。

书名原文:*Drosophila* protocols

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Preface

LARRY SANDLER WOULD OFTEN TELL HIS STUDENTS "I can make a living pushing flies, but you can't." He was referring to the fact that during his days as a graduate student and post-doc, formal genetic analysis yielded many of the most exciting findings in *Drosophila*. However, since that time, *Drosophila* has become an organism of choice for molecular, biochemical, and cellular studies, and many key insights rely on a combination of approaches. For students just beginning to work with *Drosophila*, mastering techniques and approaches to be used in combination with "fly pushing," Larry correctly concluded, would be an essential element of their training.

With new techniques being generated at an ever increasing rate, Larry's advice is even more appropriate. Although the variety of approaches currently available to the fly community makes *Drosophila* research particularly exciting, it is also daunting. It requires all of us to step outside the comfort zone of tried-and-true procedures we learned as graduate students and post-docs. Establishing a new technique in the lab is often a leap of faith and requires a large commitment of time and resources. Therefore, reducing this activation barrier was foremost in our minds when we developed a strategy for revising the Laboratory Manual that accompanied Michael Ashburner's Drosophila: *A Laboratory Handbook* (1989), known to the community as the Grey Book. Unlike the original edition, this book makes no attempt to be comprehensive. We felt liberated from this responsibility because there exists a number of other excellent *Drosophila* protocol manuals, including Ashburner's original more comprehensive work (Drosophila: *A Laboratory Manual* 1989). Instead, we chose to provide in-depth descriptions of a select set of protocols that are most likely to be used by the *Drosophila* community in the next decade. Each protocol includes enough basic information to be useful to the uninitiated, in sufficient detail to serve as a useful reference for the more experienced.

We arrived at a "Top 37" list by soliciting the advice of the *Drosophila* community and surveying the literature for the most frequently used protocols. Some of the protocols, such as RNA interference, are currently used by only a few laboratories, but are likely soon to become more routine. Some widely used protocols, such as transformation, are not included because there already exist a number of excellent published descriptions of this technique.

The protocols are grouped into six sections: Chromosomes, Cell Biology, Molecular Biology, Genomics, Biochemistry, and The Organism. Several of the protocols assume a knowledge of basic molecular techniques and may require users to refer to one of the many excellent sources of such methods. Very little genetics is included in this volume, because much of this material is covered in the Grey Book. New advances in *Drosophila* genetic techniques will be included in a planned second edition of the Grey Book. In addition to an introduction describing the purpose of each procedure, the chapters include many figures, tables, illustrations, and examples of the kinds of data that can be produced. We believe that these features make for a more useful and interesting reference. It is our hope that it will be read between time-points for ideas and new approaches.

The manual also includes six appendices. Reprinted in Appendix 1 is the Table of Contents of the original edition of the protocol manual. This has been included because the revision required the sacrifice of many useful protocols from that book. (Although it was published more than a decade ago, most labs still have a copy and the taped covers attest to its continued value as a reference.) Appendix 2 consists of line drawings of many key aspects of *Drosophila* biology and development. These have been plucked from a number of sources and slightly modified to serve as templates for figures and transparencies. Appendix 3 includes a number of commonly used solutions, buffers, and recipes used by the *Drosophila* community. Appendix 4 lists the toxic and dangerous reagents used in these protocols. Also included are Suppliers and Trademarks in Appendices 5 and 6, respectively.

This book is in print only because of the enthusiastic support of both the *Drosophila* community and the good folk at Cold Spring Harbor Laboratory. To all of the contributing authors, we owe a hearty thanks. Without exception the authors put a great deal of time, effort, and thought into the project. The majority of the chapters required only minor changes, and the authors diligently responded to any of our suggestions. This made our job relatively painless and sometimes even pleasant. (And to those authors who were late returning manuscripts and wondering if they were the very last, rest easy, the person concerned atoned with an excellent chapter and is forgiven.)

The publishing group at Cold Spring Harbor was a pleasure to work with. Throughout the entire project, it was clear that quality was their top priority. John Inglis and his team had an extraordinary ability to focus our rather vague ideas about the book and marshal the resources and personnel to carry them out. We thank Dotty Brown for the wonderful job she did with the copy editing, drawings, figures, appendices, and indexing. We also want to thank Marilu Hoeppner for cheerfully carrying out that most difficult task of ensuring the accuracy and consistency of the book. Finally, we wish to give a special thanks to Mary Cozza for her exceptional organizational skills and equally important her firm, but gentle, prodding.

W. Sullivan
M. Ashburner
R. Scott Hawley

Abbreviations

Acetyl CoA	acetyl coenzyme A
ACh	acetylcholine
ADH	alcohol dehydrogenase
ADP	adenosine diphosphate
AEL	after egg laying
AL	antennal lobe
ALH	after larval hatching
AP	alkaline phosphatase
BAC	bacterial artificial chromosome
BCIP	5-bromo-4-chloro-3-indolyl phosphate (see X-phosphate)
BDGP	Berkeley *Drosophila* Genome Project
BES	*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
BFD	Berkeley Fly Database
BLAST	basic local alignment search tool
BrdU	5-bromo-2′-deoxyuridine
BSA	bovine serum albumin
BSS	balanced salt solution
b/w	black/white
BWSV	black widow spider venom
CALI	chromophore-assisted laser inactivation
CAT	chloramphenicol acetyltransferase
CCD	charge-coupled device
CDS	coding sequence
CF	chemical fixation
CHEF	contour-clamped homogeneous electric field
CLSM	confocal laser-scanning microscopy
CMFDG	5-chloromethylfluorescein di-β-D-galactopyranoside
CNS	central nervous system
ConA	concanavalin A
CPD	criticial point dried
CPITC	coumarin phenylisothiocyanate
CPRG	chlorophenol red β-D-galactopyranoside
DAB	3,3′-diaminobenzidine
DABCO	1,4-diazabicyclo-(2,2,2)-octane
DAPI	4′,6-diamidino-2-phenylindole

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DCV	*Drosophila* C virus
DDBJ	DNA Database of Japan
DEPC	diethyl pyrocarbonate
DHFR	dihydrofolate reductase
DIC	differential interference contrast
DIG	digoxigenin
DLM	dorsal longitudinal flight muscle
DMF	*N,N*-dimethylformamide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphates
DOP-PCR	degenerate oligonucleotide-primed PCR
DRES	*Drosophila*-related EST sequences
DSHB	The Developmental Studies Hybridoma Bank (University of Iowa)
dsRNA	double-stranded RNA
DT-A	diphtheria toxin A subunit
DTT	dithiothreitol
ECL	enhanced chemiluminescence
EDAC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EDGP	European *Drosophila* Genome Project
EGFP	"enhanced" GFP
EJC	excitatory junctional current
EJP	excitatory junctional potential
EM	electron microscopy
EMBL	European Molecular Biology Laboratory
EMS	ethyl methane sulfonate
ENU	ethyl nitrosourea
EP	enhancer promoter
ERG	electroretinogram
EST	expressed sequence tag
FAQs	frequently asked questions
FCS	fetal calf serum
FETi	fast extensor tibiae motor neuron
FIGE	field inversion gel electrophoresis
FISH	fluorescent in situ hybridization
FITC	fluorescein isothiocyanate
FLP-FRT	site-specific FLP recombinase–FLP recombination target
FRT	FLPase recombination target
FLUOS	5(6)-carboxyfluorescein-*N*-hydroxysuccinimide ester
FTP	file transfer protocol
GF	giant fiber
GFP	green fluorescent protein
GIFTS	Gene Interaction in the Fly Transworld Server
GST	glutathione-*S*-transferase
GTPase	guanosine triphosphatase
HAME	*p*-hydroxyl benzoic acid methyl ester
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)
HGT	high gelling temperature
HHMI	Howard Hughes Medical Institute
HL	hemolymph-like

HMDS	hexamethyldisilazane
HMW	high molecular weight
HPF	high-pressure freezing or high-pressure freezer
HPF-FS	high-pressure freezing/freeze-substitution
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
hsp	heat shock promoter
HU	hydroxyurea
iACT	inner antenno-cerebral tract
IdU	5-iodo-2′-deoxyuridine
iEM	immunoEM
ip	intraperitoneal
IPTG	isopropyl-β-D-thiogalactopyranoside
IR	infrared
kb	kilobase
KC	Kenyon cell
LB medium	Luria-Bertani medium
LM	light microscopy
LMW	low molecular weight
LocI	local interneuron
MAb	monoclonal antibody
Mb	megabase
MB	mushroom body
mEJCs	miniature EJCs
$[Mg^{++}]$	magnesium ion concentration
MM3 medium	modified M3 medium
MRP	mechanoreceptor potential
MT	microtubule
Mt	metallothionein
$[Na^{+}]$	sodium ion concentration
NA	numerical aperature
ND	neutral density
NBT	nitroblue tetrazolium
NIDA	National Institute on Drug Abuse
NGS	normal goat serum
NMJ	neuromuscular junction
NPG	*n*-propyl gallate
NOR	nucleolus organizer region
n-syb	neuronal synaptobrevin
NVOC-C1	6-nitroveratryl chloroformate
OD	optical density
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDMN	posterior dorsal mesothoracic nerve
PEG	polyethylene glycol
PFGE	pulsed-field gel electrophoresis
PI	propidium iodide
PIPES	piperazine-*N,N′*-bis(2-ethanesulfonic acid)
PLT	progressive lowering of temperature

PMSF	phenylmethylsulfonyl fluoride
PNS	peripheral nervous system
PPF	paired-pulse facilitation
ppm	peripodial membrane
PPD	*p*-phenylenediamine
PTP	post-tetanic potentiation
Rac	ricin A chain
RH	relative humidity
Ril	relay interneurons
RNA	ribonucleic acid
RNA-i	dsRNA genetic interference
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SEM	scanning electron microscopy
SETi	slow extensor tibiae motor neuron
SIT	silicon intensifier target
SNF	soluble nuclear fraction
STF	short-term facilitation
STS	sequence tagged site
syb	synaptobrevin
TBS	Tris-buffered saline
TdT	terminal deoxynucleotidyl transferase
TEM	transmission electron microscopy
TEMED	*N,N,N',N'*,-tetramethylethylenediamine
TEP	transepithelial potential
TES	*N*-Tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid
TeTxLC	tetanus toxin light chain
TEVC	two-electrode voltage-clamp
TLC	thin-layer chromatography
TRITC	tetramethylrhodamine isothiocyanate
TSA	tyramide signal amplification system
TTM	tergotrochanteral jump muscle
TTMn	tergotrochanteral motor neuron
TUNEL	terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick end-labeling
UV	ultraviolet
UAS	upstream activation sequence
URL	uniform resource locator
UTR	untranslated regions
VNC	ventral nerve cord
WGA	wheat germ agglutinin
WPP	white prepupal stage
WWW	World Wide Web
X-gal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside
X-phosphate	5-bromo-4-chloro-3-indolyl phosphate
YAC	yeast artifical chromsome

Contents

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APPENDICES

Chromosomes

CONTENTS

1

Preparation and Analysis of *Drosophila* Mitotic Chromosomes

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MITOTIC CHROMOSOME CYTOLOGY HAS AN IMPORTANT ROLE in many areas of *Drosophila* research. It is routinely needed for characterization of the mitotic phenotypes elicited by mutations affecting chromosome structure and/or behavior (for review, see Gatti and Goldberg 1991). In addition, mitotic cytology has proven to be essential for cytogenetic analysis of heterochromatin (for review, see Gatti and Pimpinelli 1992). Heterochromatin cannot be cytologically dissected by polytene chromosome analysis, because the bulk of this material is included in the chromocenter. However, heterochromatin breakpoints can be precisely determined in mitotic chromosomes processed with high-resolution banding techniques, such as quinacrine, Hoechst, and N-banding (for review, see Gatti and Pimpinelli 1992). Finally, good mitotic preparations are essential for fine mapping of repetitive DNA sequences along heterochromatin by in situ hybridization (see, e.g., Palumbo et al. 1994; Pimpinelli et al. 1995; Dernburg et al. 1996) and for immunolocalization of chromosomal proteins (see, e.g., Pak et al. 1997; Fanti et al. 1998; Platero et al. 1998).

In this chapter, we present the protocols routinely used in our laboratories for mitotic chromosome preparation, chromosome banding, and fluorescent in situ hybridization (FISH). In addition, we describe our fixation and immunostaining procedures for protein localization along mitotic chromosomes.

PREPARATION AND STAINING OF LARVAL BRAIN MITOTIC CHROMOSOMES

Although mitotic chromosome preparations can be obtained from embryonic and gonial cells of both sexes, the tissue that provides the best mitotic figures is the larval brain. This tissue contains two major types of dividing cells: the neuroblasts and the ganglion mother cells (Hofbauer and Campos-Ortega 1990). The neuroblasts divide either symmetrically, producing two neuroblast stem cells, or asymmetrically, producing another neuroblast and a smaller cell called the ganglion mother cell. The ganglion mother cell divides only once, producing two daughter cells that differentiate into neurons. Several squashing techniques have been developed for preparation of larval brain mitotic chromosomes (Ashburner 1989; Gonzalez and Glover 1993). Below, we present a series of squashing protocols that are routinely used in our laboratories for a variety of experimental purposes. These procedures are minor modifications of a basic technique developed 25 years ago (Gatti et al. 1974) and can be successfully used for preparing mitotic chromosomes of various *Drosophila* and mosquito species (Gatti et al. 1976; Pimpinelli et al. 1976; Bonaccorsi et al. 1980, 1981). To characterize various aspects of mitotic chromosome morphology and behavior, larval brains can be squashed either in aceto-orcein to obtain orcein-stained chromosomes (Protocol 1.1) or in 45% acetic acid to obtain unstained preparations (Protocol 1.2). Unstained material can be then stained with Giemsa to obtain permanent preparations, processed with a variety of banding techniques, or used for in situ hybridization.

Preparation of Orcein-stained Chromosomes

Depending on the experimental purpose, aceto-orcein squashes can be prepared by three different experimental regimes, which are summarized in Protocol 1.1. First, dissected brains can be squashed in aceto-orcein without colchicine treatment and hypotonic shock (i.e., Protocol 1.1, with the omission of steps 3 and 4). This procedure allows observation of all phases of mitosis and permits evaluation of the mitotic index and the frequency of anaphases (Gatti and Baker 1989). However, chromosome morphology is poorly defined in these preparations.

In the second regime, colchicine treatment is omitted, but brains are incubated in hypotonic solution (i.e., Protocol 1.1, with the omission of step 3). Hypotonic treatment improves metaphase chromosome spreading and causes sister chromatid separation, allowing examination of chromosome condensation and detection of hyperploid and polyploid metaphases. However, hypotonic shock disrupts anaphase (Brinkley et al. 1980), and anaphase figures are almost absent in hypotonically treated brains.

In the third regime (Protocol 1.1), brains are incubated in vitro with colchicine, treated with hypotonic solution, fixed, and squashed. This procedure provides a large number of well-spread metaphase figures (200–400 per brain) that can be analyzed for chromosome morphology, the presence of chromosome aberrations, and the degree of ploidy. However, because colchicine disrupts spindle microtubules, inducing metaphase arrest followed by chromosome overcontraction, colchicine treatment must be omitted if the degree of chromosome condensation has to be evaluated.

Preparation of Unstained Chromosomes

Unstained brain chromosomes can be prepared according to the same experimental regimes described above for aceto-orcein squashes. The procedure for this type of preparation is given in Protocol 1.2.

Giemsa Staining

Although well-sealed aceto-orcein squashes remain in good condition for 1–2 months, there are cases in which permanent chromosome preparations are needed. This can be done by staining preparations obtained according to Protocol 1.2 with 2% Giemsa in a phosphate buffer at pH 7.0. We routinely use Giemsa from Merck, but other Giemsa brands work just as well. The timing of Giemsa staining varies with the Giemsa brand and should be adjusted to obtain the desired staining. After Giemsa staining, chromosome preparations are differentiated by washing the slides in tap water. Giemsa stain is additive, and thus if chromosomes are not sufficiently stained, the slides can be stained again in 2% Giemsa, until the desired stain is obtained. After washing in tap water, the slides are air-dried and then mounted in Euparal (Carolina Biological Supply) or similar medium.

PROTOCOL 1.1*

Aceto-Orcein Squashes of Larval Brains

Materials

Supplies and Equipment

Siliconized slides, used only as a support for drops of either saline or hypotonic solution (for preparation, see Protocol 12.6 [Dernberg] or Protocol 6.1 [Kennison] in this volume.)

Dissecting tools

- dissecting microscope
- forceps (2 pairs; e.g., Dumont #5 Biologie)

Petri dish (35 x 10 mm) with cover

Nonsiliconized slides and coverslips (20 x 20 mm or 22 x 22 mm)

Blotting paper

Depilatory wax (found in most cosmetic shops) or nail polish

Solutions and Reagents

Saline (0.7% NaCl in H_2O)

(*Optional*) **Colchicine** (10^{-3} M) in H_2O

(*Optional*) Hypotonic solution (0.5% sodium citrate • $2H_2O$ in H_2O)

Fixative

Acetic acid/**methanol**/H_2O (ratio 11:11:2)

Use freshly prepared fixative.

Preparation of 2% Aceto-orcein

Boil synthetic orcein powder (Gurr, BDH Laboratory Supplies, Poole, Dorset BH15 1TD England; Phone: +44 1202 660444; Fax: +44 1202 666856; Web site: http://www.bdh.com) in 45% **acetic acid** for 45 minutes in a reflux condenser. We usually prepare 5% orcein, which is subsequently diluted to 2% with 45% acetic acid.

Before use, remove particulate matter from aceto-orcein either by filtration through blotting paper or by centrifugation in a microcentrifuge.

CAUTION: acetic acid, colchicine, methanol (see Appendix 4)

*In all protocols, H_2O indicates glass distilled and deionized.

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