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New research resources at the Bloomington Drosophila Stock Center

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Abstract

The Bloomington Drosophila Stock Center (BDSC) is a primary source of Drosophila stocks for researchers all over the world. It houses over 27,000 unique fly lines and distributed over 160,000 samples of these stocks this past year. This report provides a brief overview of significant recent events at the BDSC with a focus on new stock sets acquired in the past year, including stocks for φC31 transformation, RNAi knockdown of gene expression, and SNP and quantitative trait loci discovery. We also describe additions to sets of insertions and molecularly defined chromosomal deficiencies, the creation of a new Deficiency Kit, and planned additions of X chromosome duplication sets.

Keywords

Bloomington Drosophila Stock Center; stocks; insertions; deletions; deficiency kit; duplications; C31; RNAi; Minos; sequenced inbred strains

The Bloomington Drosophila Stock Center (BDSC), now in its 23rd year of operation, is a research resource that collects, maintains and distributes genetically defined lines of *Drosophila melanogaster*. The collection has grown to over 27,000 unique stocks—thanks to the fly community's creativity and commitment to sharing. In this article, we provide a brief status report on the Center and highlight recent acquisitions that should be of interest to a wide range of fly researchers.

The most notable event of the past year was the arrival of Annette Parks, Ph.D. to join the Bloomington management team. Annette has extensive experience in the area of cell signaling, having studied the *Notch* receptor pathway for several years. She brings a wealth of genetic knowledge to the BDSC from her work at Boston College and Exelixis, Inc. As a collections manager, she will lead an effort to strengthen the Center's support of scientists using Drosophila to model human disease. Watch for improvements to both the collection holdings and web support of this area in coming months.

Another recent milestone was the renewal of the Bloomington Stock Center's grant for operational support, which is funded by the National Science Foundation, the National Center for Research Resources, the National Institute of General Medical Sciences and the National Institute of Child Health and Human Development. Grant funds cover roughly half of our costs and the remainder must come from user fees. The continued support of NSF,

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NIH and our users allows us to maintain the level of service the fly community has come to rely on while expanding our services in key areas.

Close to 3,200 new stocks were donated to the collection last year, a 13% increase from December 2008 holdings. Some of this increase was balanced by the retirement of approximately 550 stocks that had largely outlived their usefulness, resulting in a collection of 27,376 stocks at the end of 2009. Drosophila researchers continue to request stocks at a brisk pace. They submitted nearly 16,000 stock requests during 2009, resulting in shipment of over 166,000 samples to 1,660 research groups in 46 countries.

The variety and depth of the Bloomington collection would not exist without the work of large-scale community efforts designed to improve research resources. Here, we provide a quick overview of recent acquisitions from ongoing projects generating important tools for fly geneticists. Table 1 lists links to web pages with more detail about each set of stocks.

φ C31 Transformation System Stocks

The φC31 transformation system, introduced to Drosophila by Groth et al.[1] uses the phage φC31 integrase to facilitate recombination between two specific DNA sequences, *attP* and *attB*. This system offers two significant advantages over *P-element* transformation. First, it targets integration of new *attB*-bearing constructs to previously generated *attP* "docking sites", allowing researchers to avoid mapping transformants and to minimize position effects by comparing multiple insertions in the same chromatin context. Different docking sites allow different levels of expression permitting researchers to pick expression levels appropriate for a particular experiment.[2] Second, Venken et al.[3] have successfully integrated large constructs into *attP* sites—including a 146 kb piece—demonstrating that φC31 transformation is not limited by the size constraints of *P-element* transformation. The ability to introduce larger constructs has allowed researchers to rescue lethal mutations and deletions of large genes and gene complexes and study the effects of engineered mutations in homozygous deletion backgrounds.[3–5] Endogenous protein expression patterns have also been recapitulated with proteins tagged via recombineering techniques in genomic clones and then integrated via φC31 transformation.[5,6]

The BDSC supplies molecularly characterized *attP* docking sites distributed around the genome. We have three *attP* docking sites within *P-element* insertions,[1,2] ten *attP* sites within *mariner* insertions[7] and 34 *attP* sites within *piggyBac* insertions.[3] Four additional *P-element*-based docking sites consist of a pair of *attP* sequences flanking the *mini-white* marker.[8] This "*attP* w^+ *attP*" cassette can be replaced by another sequence flanked by *attB* sites in a φC31 integrase-mediated event termed Recombination-Mediated Cassette Exchange (RMCE).[8–10] We also distribute several stocks expressing φC31 integrase in the germ line under the control of *nanos* or *vasa* regulatory sequences.[7] In many stocks, the integrase construct is already combined with a docking site for ease of transformation.

Molecular resources complimentary to the φC31 system stocks at the BDSC are available from other providers. High-copy *attB*-containing vectors,[1,11] including vectors for RMCE,[8] have been deposited at Addgene. BAC-based *attB*-containing vectors (*attB*-P[acman])[3] for cloning large pieces of DNA are available at the Drosophila Genomics Resource Center (DGRC). In addition, three genomic libraries have been constructed in *attB* vectors: two in *attB*-P[acman][5] (clones available from the BACPAC Resources Center) and one in a fosmid-based vector[6] (clones available from the TransgeneOme Project).

TRiP Stocks

Bloomington began distributing RNAi lines from the Transgenic RNAi Project (TRiP)[12] in November 2008 and the size of the collection quadrupled in 2009. TRiP stocks express RNAi hairpins under the regulation of the *GAL4-UAS* system, allowing controlled knockdown of the expression of targeted genes in any tissue except the female germline. The RNAi constructs are integrated into an *attP* site on chromosome arm 3L that permits high levels of GAL4-induced expression.[2] Over 1,330 lines targeting 1,313 genes were available from the BDSC at the end of 2009 and new lines are arriving every few weeks. In addition to RNAi lines, Bloomington distributes "toolbox" stocks from the TRiP and from Barry Dickson's laboratory[13] for creating and working with transgenic RNAi lines. These include *UAS* stocks expressing *Dicer-2* to potentiate RNAi effects.

Lists of genes targeted by TRiP constructs are available from the TRiP and BDSC web sites. Community nomination is one way the TRiP selects genes to target with RNAi constructs. You may make the case for targeting a new gene by writing to TRiP@flyrnai.org.

Gene Disruption Project Stocks

1,222 stocks were accessioned in 2009 from the Gene Disruption Project (GDP), which has been a major contributor of stocks to the Bloomington collection for over 16 years.[14,15] The GDP's goal is to place a transgenic insertion in or near (within 500 bp 5′ of the transcription start site) every *D. melanogaster* gene to either disrupt gene function directly or provide starting material for making gene knockouts by imprecise excision. To that end, the GDP generates and analyzes its own insertions as well as sets of insertions from other groups. Recent donations to Bloomington via the GDP pipeline include *P{EP}* insertions created by the Korean company GenExel and lethal *P{lacW}* insertions generated in Steven Hou's lab. [16] For its own screening, the GDP switched several years ago from *P-element* vectors to a *Minos* vector[17] to take advantage of the more random insertion site distribution of *Minos*. Thus far the GDP has donated almost 14,000 stocks to the BDSC with insertions that tag over 9,000 genes.

In 2010, the GDP will begin to donate insertions of their new *Minos* vector MIMIC (Minos-Mediated Integration Cassette) (Venken K, Hoskins R and Bellen HJ, personal communication). MIMIC includes a pair of φC31 *attP* sites that allow replacement of sequences within the *Minos* ends using RMCE.[18,19] This feature provides a new way to take advantage of intronic insertions to manipulate genes. RMCE can be used to introduce sequences that will be spliced into mRNAs, such as stop codons, hairpin sequences for RNAi, fluorescent markers, epitope tags and other experimentally useful features.

Genetic Reference Panel Stocks

Another set of stocks now available at Bloomington is the Genetic Reference Panel. This panel is composed of a large and fully sequenced set of inbred strains derived from a wild Drosophila population in Raleigh, North Carolina. The stocks serve as a source of characterized SNPs and indels for mapping traits relative to sequence polymorphisms and as a resource for the identification of quantitative trait loci. They are useful for any experimental purpose where well-characterized genetic variability is important and should provide invaluable genetic tools for investigating complex, multigenic traits such as longevity and behavior. An excellent overview of the project is given in the Drosophila community White Paper where the panel was originally proposed.[20]

Bloomington Deletion Project Stocks

The most heavily used stocks in the Bloomington collection are chromosomal deletions. Deletions are most commonly used for gene mapping and complementation analyses and in screens for genetic enhancers and suppressors of mutant phenotypes. The BDSC itself has sponsored a long-term, large-scale research resource project funded by the National Center for Research Resources to improve the selection of deletions. Similar to projects by Exelixis[21] and the DrosDel consortium,[22] we are using *FLP-FRT* technology to generate deletions with breakpoints characterized at single nucleotide resolution. Our goals are to maximize deletion coverage of the genome and, by combining deletions from all three projects, to subdivide the genome with breakpoints spaced no more than a dozen genes apart. We added 213 new deletions to the Bloomington collection in 2009 to make a total of 760 BDSC-generated deletions. We plan to add three dozen more deletions from our final screens in early 2010. Our *FLP-FRT* deletions combined with 444 Exelixis deletions, 355 DrosDel deletions and 55 deletions we generated by Hybrid Element Insertion[21] have introduced molecular precision into mapping experiments and have enabled Drosophila workers to localize mutations to very small gene clusters, or even single genes, with ease.

In July 2009, we used the molecularly defined deletions to assemble a new Bloomington Deficiency Kit that replaced the old Deficiency Kit, which had been composed primarily of deletions with roughly mapped breakpoints based on polytene cytology. Like the previous kit, the new Deficiency Kit provides maximal genome coverage with the fewest deletions, but unlike the old kit the new one uses precisely mapped deletions wherever possible, resulting in a larger but more informative Kit. Most Stock Center users who order the Deficiency Kit as a whole do so to screen for dosage-dependent modifiers of mutant phenotypes. The improved kit is much better for screening because most of the deletions were generated in uniform genetic backgrounds. By reducing background effects, modifier screens are more likely to detect real dosage-dependent interactions. At release, the new Kit covered 97.8% of euchromatic genes. Upon completion, our project will have increased coverage to approximately 99%, leaving gaps only around haplolethal and haplosterile genes.

X Chromosome Duplication Stocks

Analyzing the functions of genes on the X chromosome can be challenging, because crosses with X-linked mutations often necessitate the use of chromosomal duplications. Basic experiments like complementation tests and mapping crosses can be impossible if the gene lies in a region lacking duplication coverage. Even when duplication coverage is present, existing duplications can be difficult to use experimentally. Two large-scale projects are working to remedy this situation. The first is a project sponsored by the BDSC and funded by the National Center for Research Resources. Our goal is to generate a comprehensive set of large X chromosome duplications. Because megabase-scale duplications cannot be made by transgenesis, we are generating them by in vivo chromosomal manipulation methods. Our approach involves generating X inversions on compound-XY chromosomes via FLP-FRT technology and irradiating these chromosomes to isolate Y-linked duplications of X segments. The molecular extents of the duplicated segments are being mapped by comparative genomic hybridization microarrays and PCR mapping relative to transposon insertion sites. Our screens will ultimately produce an “X Chromosome Duplication Kit”—a tiling path of duplicated segments providing maximal X coverage with the fewest stocks—as well as numerous smaller duplicated segments to provide extensive breakpoint subdivision of the X chromosome. These large duplications will allow gene mapping at both coarse- and medium-level resolution.

The second project is being conducted in the laboratories of Thom Kaufman at Indiana University, Hugo Bellen at Baylor College of Medicine and Roger Hoskins at Lawrence Berkeley National Laboratory. They are using the φC31 transformation system to introduce 70 to 120 kb X chromosome fragments from one of the *attB*-P[acman] genomic libraries[5] mentioned above into an *attP* site on the third chromosome. These smaller duplications will allow fine-level gene mapping.

The first duplications from these complementary projects will be placed into public distribution in early 2010 and both projects will likely be concluded by mid-2011. When complete, these sets will allow X-linked mutations to be localized to roughly three gene intervals by successive crosses to large-, intermediate- and small-sized duplications. These duplication sets can also be used to identify dosage-dependent modifiers of mutant phenotypes. Such experiments have not been practical in the past, because the selection of duplications has not been adequate for comprehensive screening or for fine-scale mapping of modifiers.

Stocks from Independent Researchers

In addition to acquisitions from large-scale community resource projects, we added 425 stocks from individual investigators in 2009. These valuable additions to the Bloomington collection include mutant alleles, *GAL4* drivers, *UAS* responders, tissue-specific reporter constructs and other broadly applicable components. Of the 50 new stocks used most heavily last year, 17 were donated by individual laboratories. For example, stocks donated by Yang Hong's group introduce a virginizing scheme[23] into the gene replacement methods[24] developed by Kent Golic's lab, an innovation that makes screening for gene knockouts dramatically more efficient.

We can provide access to the latest genetic technologies and a broad range of stocks because individuals involved in hypothesis-driven research have been willing to share useful strains with their colleagues. Indeed, this ethic of sharing has helped place Drosophila research at the forefront of model organism biology and has allowed us to assemble a strong and relevant collection. We urge investigators to donate important stocks for public distribution to assure the continued vigor of the Drosophila community and the Bloomington Stock Center.

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Abbreviations

BDSC	Bloomington Drosophila Stock Center
DGRC	Drosophila Genomics Resource Center
FLP	FLP recombinase
FRT	FLP recognition target
GDP	Gene Disruption Project
MIMIC	Minos-mediated integration cassette
PCR	polymerase chain reaction

RMCE	recombination-mediated cassette exchange
RNAi	RNA interference
TRiP	Transgenic RNAi Project
SNP	single nucleotide polymorphism
UAS	upstream activating sequence

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Table 1

Links to websites with additional information about resource projects and stocks

Website content	Website URL
BDSC homepage	http://flystocks.bio.indiana.edu/
BDSC stock donations	http://flystocks.bio.indiana.edu/Inst/acquisition.htm
ϕC31 transformation stocks	http://flystocks.bio.indiana.edu/Browse/phiC31.htm
FlyC31 homepage	http://flyc31.frontiers-in-genetics.org/
attB-P[acman] genomic library	http://pacmanfly.org/
BACPAC Resource Center	http://bacpac.chori.org/home.htm
The TransgeneOme Project	http://transgeneome.mpi-cbg.de/
attB vectors at the DGRC	https://dgrc.cgb.indiana.edu/
attB vectors at Addgene	http://www.addgene.org/pgvec1
TRiP homepage	http://www.flyrnai.org/TRiP-HOME.html
TRiP and other RNAi lines	http://fly.bio.indiana.edu/Browse/RNAi.php
TRiP toolbox stocks	http://fly.bio.indiana.edu/Browse/TRiPtb.htm
Dickson RNAi toolbox stocks	http://fly.bio.indiana.edu/Browse/VDRCtb.htm
GDP homepage	http://flypush.imgen.bcm.tmc.edu/pscreen/
GDP insertion stocks	http://flystocks.bio.indiana.edu/Browse/in/GDPtop.htm
Genetic Reference Panel	http://service004.hpc.ncsu.edu/mackay/Good_Mackay_site/DBRP.html