13) Transfer supernatant to a clean 1.5 mL microfuge tube containing 600 µL of isopropanol.
14) Gently mix by inversion.
15) Centrifuge for 1 minute at 13,000-16,000 ×g.
16) Carefully remove isopropanol with a pipette and discard.
17) Add 600 µL of 70% ethanol and gently mix by inversion.
18) Centrifuge for 1 minute at 13,000-16,000 ×g.
19) Carefully remove ethanol with a pipette and discard.
20) Add 100 µL 45% acetic acid and mix gently; let stand for 2 minutes.
21) Add 300 µL Wizard Rehydration Solution and 1/10 volume 7.5 M ammonium acetate and mix gently.
22) Add 2 volumes 95% ethanol and mix gently.
23) Incubate at 70ºC for 5 minutes.
24) Centrifuge for 1 minute at 13,000-16,000 ×g.
25) Carefully remove supernatant with a pipette and discard.
26) Add 1000 µL ethanol, mix gently, and centrifuge for 1 minute at 13,000-16,000 ×g.
27) Carefully remove supernatant with a pipette and discard.
28) Invert tube on a paper towel and air-dry the pellet for 10–15 minutes.
29) Add 40 µL of Wizard DNA Rehydration Solution and incubate at 65°C for 1 hour.
30) Store DNA at 2–8°C.

PCR was performed on these extracts using GoTaq® (Promega) and primers that amplify an approximately 744 nt segment at the beginning of the *mal* mRNA (primers: 5'CAGCTGTATGTGTAGGCTATCGTC3' and 5'CCGCATGATCCAGGTAAACACTCT3'). Longer extension times were used (94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 3 minutes for 33 cycles) and hot start PCR was performed using AmpliWax® PCR Gem wax beads (Applied Biosystems). A typical reaction used 10.5 µL of DNA in the lower layer and 12.5 µL of GoTaq and 1 µL of each primer in the upper layer. DNA amplified from two slides using these primers was indistinguishable on agarose gels from similarly amplified DNA from wild-type flies. PCR products were cloned using the TOPO® TA method (Invitrogen) and sequenced (Macrogen, Korea). As shown in Figure 1, this amplified DNA is from the *mal* locus of *Drosophila melanogaster*. All clones from the two slides gave similar results.

It is my hope that this report will make such archived *Drosophila* DNA more accessible.

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An organizational strategy for deficiency mapping: A computational approach.

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Introduction

Deficiency mapping provides *Drosophila* geneticists with the unique ability to physically map mutations by studying a single generation of animals. By crossing a lethal mutation in an unknown

gene with deficiency lines possessing a deletion of a known region of a chromosome, the presence or absence of lethality in the offspring can reveal whether or not the mutation resides in the same location as the chromosomal deficiency. In contrast, researchers studying other model organisms including *Caenorhabditis elegans* and *Mus musculus* must rely on the tedious process of recombination mapping, which involves multiple generations of crosses, scoring large numbers of offspring, and computational analyses. Deficiency mapping, combined with the potential for rapid generation, isolation, and phenotypic analysis of random chemically-induced mutations makes *Drosophila* the organism of choice for rapid forward genetics.

Purpose

Deficiency mapping is often an arduous task involving a multitude of crosses between the deficiency stocks and the specific mutation to be mapped. If, for example, one is attempting to map a mutation on the second chromosome, they may need to do upwards of 109 crosses. A major problem is that these deficiency stocks contain deletions that often overlap, leading to crosses that may be unnecessary. Thus, it is logical to use the stocks that cover the largest deficiencies, but the problem is that the deficiency stocks are not organized according to the order of deletions along the chromosome. If one attempts to simply carry out this task by creating genetic crosses in order of the deficiency kit stock numbers, this will lead to numerous crosses that are unnecessary, and often unorganized. We have created an efficient method of visually organizing deficiency crosses according to the deletions that they cover on the chromosome.

Methods

Sorting the deficiency stocks by chromosomal position

To illustrate this organizational strategy we have chosen to present information on the second chromosome molecularly defined deficiencies for the *Drosophila* genome, although in principle the same organizational approach could readily be applied to molecularly defined deficiencies covering the X and third chromosomes as well. Importantly, this organizational strategy relies upon known molecularly defined sequence breakpoints for each of the chromosomal deficiencies, such as those generated in the DrosDel deficiency kit (Ryder *et al.*, 2007) available through the Bloomington Stock Center (Bloomington, Indiana).

The first step was to separate the deficiencies between the two chromosomal arms, in this case 2L and 2R. Using Microsoft Excel®, we created two spreadsheets, each sheet used specifically for one chromosomal arm. Next, we simply went through all of the DrosDel deficiency kit stocks and listed the molecularly defined start and end sequence breakpoints and the corresponding stock number in three separate columns as illustrated in Figure 1.

Starting Breakpoint	Ending Breakpoint	Stock Number
159063	285763	8901
67365	72671	9180
160605	285763	9177
67365	161120	9353

Figure 1. Organizing deficiency kit stocks into a spreadsheet

The next step is to use an ascending sort function. First, highlight the full data set and then under the "Data" pull-down tab select the "Sort" function followed by the "Sort by starting breakpoint" option which can be summarized as follows: *Data* → *Sort* → *Sort by starting breakpoint.* The two screenshots shown below in Figure 2a and 2b visually demonstrate the steps described above. As shown in Figure 2c, the chromosome deficiency crosses are now sorted according to the starting breakpoint.

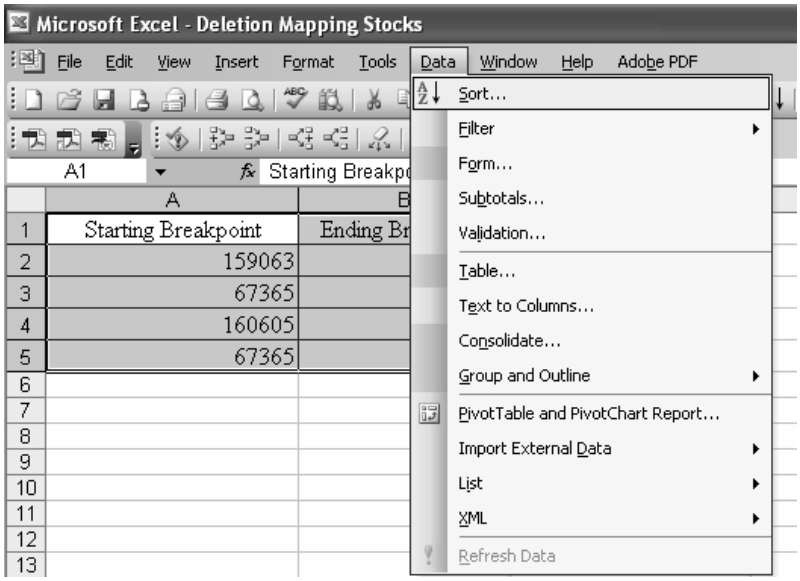

Figure 2a. Accessing the sort function through the Data pull-down tab.

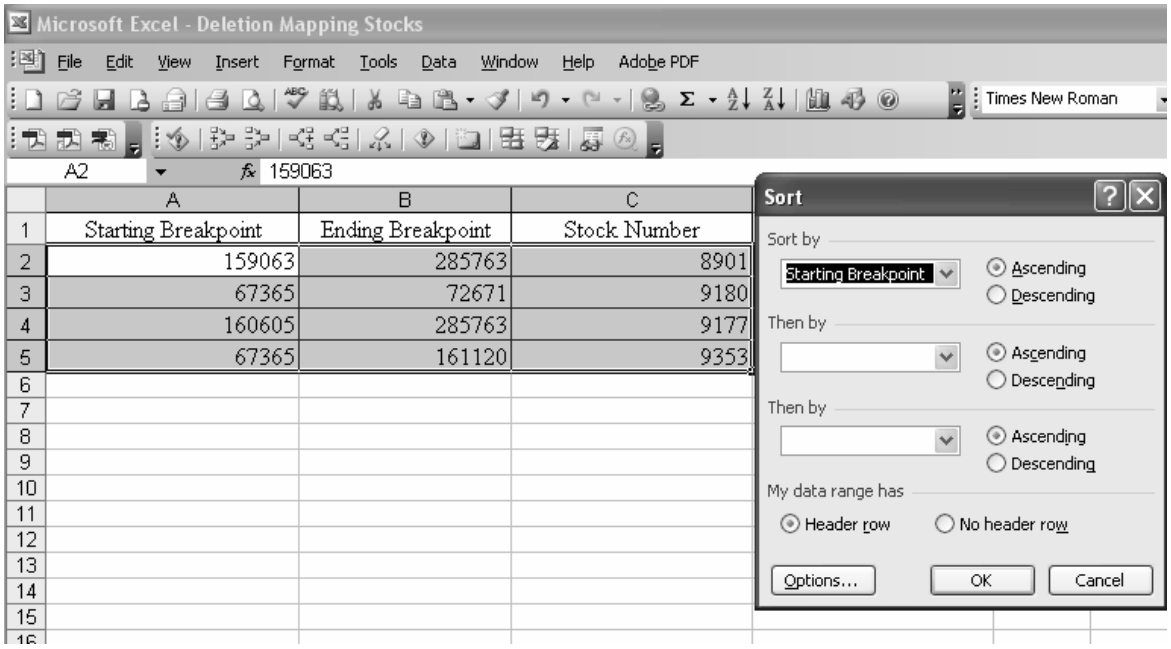

Figure 2b. Sorting the starting breakpoint values in ascending order.

Starting Breakpoint	Ending Breakpoint	Stock Number
67365	72671	9180
67365	161120	9353
159063	285763	8901
160605	285763	9177

Figure 2c. Deficiency stocks now organized in ascending order according to the starting breakpoint.

Visually mapping the deficiency stocks

After sorting the crosses in ascending order, the next step was to visually map the base pair deletions. This visual map does not correlate with physical distance; instead, it shows relative positions of the respective breakpoints. After creating a separate column indicating the visual map of breakpoints, we used the left side of the column to indicate the starting breakpoint and the right side to indicate the ending breakpoint. Next, we simply advanced through the spreadsheet, placing the breakpoints in sequential order as seen in Figure 3. The breakpoints are placed in cells based on ascending numerical value. Each unique number occupies a given column. This creates a visual map with the deficiencies spanning their relative positions. By highlighting the positions occupied by the deficiencies one can indicate the area covered between the breakpoints. The end result is that we can now look at the visual map and immediately see that stocks 9353 and 9177 cover the breakpoints between base pairs 67365 and 285763 (Figure 3). The other two stocks represent sub-deficiencies within this region of the chromosome and are not necessary for the initial deficiency cross mapping. Thus, in this example of four crosses, we have reduced our workload by 50%.

Stock Number	Visual Map of Breakpoints					
9353	67365				161120	
9180	67365	72671				
8901			159063			285763
9177				160605		285763

Figure 3. Visually mapping the deficiency stocks.

Discussion

Thanks to the accumulated efforts of the *Drosophila* community, including Exelixis (Parks *et al.,* 2004) and the recent DrosDel consortium (Ryder *et al.,* 2007), researchers now possess collections of *Drosophila* stocks with deficiencies covering greater than 90% of the fly genome (BDSC; http://flystocks.bio.indiana.edu/Browse/df-dp/dfkit-info.html). The size of each deficiency ranges from the maximum tolerable size of approximately 1 Mb (Ashburner and Bergman, 2005) to relatively small deficiencies of ~100 kb.

We have created a strategy that simplifies the logistics of deficiency mapping. By visually arranging deficiency stocks according to breakpoints, one can create a map representing the relevant information for mapping an unknown mutation. In the example shown in Figure 2, the workload

became reduced by 50 percent after implementing our technique. Thus if one has to scan the entire second chromosome using the DrosDel deficiency kit that has 109 deficiency stocks, our method can reduce the workload by 43 percent, meaning that one only needs to use 62 deficiency stocks for the first round of crosses.

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Drosophila Proteome Atlas.

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Since the first report of the *Drosophila* genome project in 2000 (Adams *et al.*, 2000), DNA sequence information became a valuable asset to research projects using *Drosophila* as a model organism. However, the knowledge of *Drosophila* proteins that are expressed and thereby manifest the function of the genome is far behind the success of its genetics and genomics counterparts. We would like to draw the attention of *Drosophila* researchers to our effort to provide information on proteins based on proteomics inquiries in which proteins are the immediate subject matter. In our recent paper (Takemori *et al.*, 2007) we reported 1) that proteomics profiling of *Drosophila* compound eyes in comparison to brain is a powerful tool to investigate post-translational modification of proteins using tissue-specific calmodulin methylation as an example, and 2) that such proteome information will be a useful asset to the *Drosophila* community. In our protocol, microdissection of the compound eyes and brain from dehydrated tissues provides clean and sufficient materials for analysis on a two-dimensional (2-D) gel electrophoresis (Matsumoto *et al.*, 1982; Matsumoto and Pak, 1984). Furthermore, a multi-stage mass spectrometric analysis of a 2-D gel spot allows us to determine the structure of the modified amino acids at a microscale (at ~100 fmol levels) (Takemori *et al.*, 2006). With a belief that the information obtained in this work and that to be obtained in our future work will benefit other *Drosophila* researchers, we initiated an open access protein database "*Drosophila Proteome Atlas* (DPA)" posted at The University of Oklahoma (Drosophila Information Service) and at Kyoto Institute of Technology (Drosophila Genetic Resource