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A Molecularly Defined Duplication Set for the X Chromosome of *Drosophila melanogaster*

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ABSTRACT

We describe a molecularly defined duplication kit for the X chromosome of *Drosophila melanogaster*. A set of 408 overlapping P[acman] BAC clones was used to create small duplications (average length 88 kb) covering the 22 Mb sequenced portion of the chromosome. The BAC clones were inserted into an *attP* docking site on chromosome 3L using ΦC31 integrase, allowing direct comparison of different transgenes. The insertions complement 92% of essential and viable mutations and deletions tested, demonstrating that almost all *Drosophila* genes are compact and that the current annotations of the genome are reasonably accurate. Moreover, almost all genes are tolerated at twice the normal dosage. Finally, we more precisely mapped two regions at which duplications cause diplo-lethality in males. This collection comprises the first molecularly defined duplication set to cover a whole chromosome in a multicellular organism. The work presented removes a longstanding barrier to genetic analysis of the Drosophila X chromosome, will greatly facilitate functional assays of X-linked genes *in vivo*, and provides a model for functional analyses of entire chromosomes in other species.

INTRODUCTION

The X chromosome of *Drosophila melanogaster* contains approximately 2,300 protein-coding genes or about 15% of such genes in the genome. It contains 22 Mb of euchromatic DNA (Adams *et al.* 2000). About one third of these genes are predicted to be mutable to a phenotype that can be scored, e.g., lethality, sterility or abnormal behavior (Peter *et al.* 2002). However, most molecularly recognized X-linked genes have not been associated with mutations or studied in any detail (http://flybase.org/) (Drysdale 2008). Indeed, one hallmark of the X chromosome in *Drosophila melanogaster* and many other species is that it is haploid in males. In addition, the presence of one copy of the X in an otherwise diploid animal leads to the phenomenon of dosage compensation, a process that essentially doubles the expression of X-linked genes in *Drosophila* males (Gelbart & Kuroda 2009).

The presence of a single X chromosome in males facilitates screens for behavioral or visible mutant phenotypes in the hemizygous male progeny of a single-generation cross. For this reason, the X chromosome has been well saturated for viable mutations. However, many of these mutations have not been mapped since existing methods are tedious. Moreover, mutations in essential genes and genes required for male fertility cannot be propagated and genetically characterized unless they are complemented with a duplication maintained in the male. Hence, the X chromosome has been significantly less studied than the autosomes for mutations in essential and male fertility genes. For many of those mutations, the genes associated with these phenotypes have been

elusive due to the lack of appropriate genetic reagents. Thus, X-linked genes in critical developmental and regulatory pathways are under-represented in reported analyses as compared to similar classes of genes on the autosomes.

Mutations in essential and male fertility genes on the X chromosome can be mapped using a variety of techniques. One approach is to rely on recombination in females and perform meiotic mapping against visible markers (Lindsley & Zimm 1992), *P* element insertions (Zhai *et al.* 2003) or SNPs (Berger *et al.* 2001; Martin *et al.* 2001; Hoskins *et al.* 2001; Nairz *et al.* 2002; Chen *et al.* 2008), all of which are labor-intensive strategies or require specialized infrastructure. An alternative is complementation mapping using deficiencies, which only requires a single cross. This approach is possible for viable mutations but not for X-linked lethal and sterile mutations since those cannot be propagated through males. Instead, complementation rescue tests need to be carried out using a segregating duplication e.g., an X chromosome fragment on the Y chromosome (Dp(1;Y)), an autosome (Dp(1;A)) or a free duplication (Dp(1;f)) (Lindsley & Zimm 1992). Currently, duplications are available that encompass approximately 90% of the X chromosome. Only three cytological regions at 13A to 13F (~1 Mb), 16D7 to 16F4 (~0.3 Mb) and 18A to 18F (~0.8 Mb) are not covered. Unfortunately, these duplications are typically very large (~1 to 1.5 Mb) (http://flybase.org/) (Drysdale 2008), limiting their utility for fine mapping. Moreover, most available duplications were isolated following x-ray mutagenesis, and their breakpoints are poorly defined.

Hence, a complete set of small molecularly defined duplications of the X chromosome would be extremely useful for identifying mutations in essential and male fertility genes and for fine-scale mapping of any mutation, including recessive viable mutations. In addition to promoting new genetic screens, a duplication set would allow one to map and assess the numerous, poorly characterized X-linked lethal mutants. Moreover, if molecularly defined genomic DNA clones are used to create the duplication set, then epitope tagging using recombineering would permit determination of expression patterns of genes included in the duplications (Venken *et al.* 2008; Venken *et al.* 2009; Ejsmont *et al.* 2009). Finally, such defined duplications would allow one to carry out structure-function analyses of genes through recombineering, by introducing point mutations and small deletions into a gene of interest, at unprecedented speed (Sharan *et al.* 2009).

Previously, we created the P[acman] (P/ΦC31 artificial chromosome for manipulation) transgenesis platform (Venken & Bellen 2005; Venken *et al.* 2006; Venken & Bellen 2007) for retrieval and manipulation of large DNA fragments in a conditionally amplifiable BAC (Wild *et al.* 2002). Genomic clones inserted into this vector can be subjected to recombineering (Sharan *et al.* 2009) and used for transformation of these fragments (up to at least 146 kb) into the genome of flies that carry a defined *attP* docking site using the ΦC31 integrase system (Groth *et al.* 2004; Venken *et al.* 2006; Bischof *et al.* 2007; Markstein *et al.* 2008). In a next step, we constructed two genomic BAC libraries, one with an average insert size of 21 kb (CHORI-322) and another with an average insert size of 83 kb (CHORI-321) (Venken *et al.* 2009). These BAC libraries

were end-sequenced and mapped onto the genome sequence, and are publicly available (http://pacmanfly.org) and distributed (http://bacpac.chori.org/). Here we bring these resources to a next level: BAC TransgeneOmics (Poser *et al.* 2008) of an entire chromosome *in vivo*. The 8.2-fold coverage of the X chromosome in mapped clones from the CHORI-321 library allowed us to select a tiled path of overlapping BACs containing almost all of the annotated genes on this chromosome. Here we describe the creation of the first set of molecularly defined duplications covering an entire chromosome of a multicellular organism, and we illustrate its utility for X-chromosome genetics in several experimental paradigms.

MATERIALS AND METHODS

Clone verification: Selected BACs were streaked on LB plates (12.5 µg/ml chloramphenicol). Single colonies were used to produce primary working glycerol stocks. An aliquot of primary culture was used to inoculate a secondary culture, induce high plasmid copy number with CopyControl solution (Epicentre), perform paired end sequencing, and analyze sequences to determine BAC end coordinates in the genome, as previously described (Venken *et al.* 2009). The sequence data were curated to verify the identity of each BAC clone and ensure precise mapping of BAC end coordinates in the genome sequence.

BAC DNA preparation: Working glycerol stocks were re-streaked on LB plates (12.5 µg/ml chloramphenicol). A single colony was grown in 1 ml LB (12.5 µg/ml chloramphenicol) for 17 hours at 37°C. The plasmid copy number was induced for 5 additional hours at 37°C by adding 9 ml LB (12.5 µg/ml chloramphenicol) containing 2 µl CopyControl solution (Epicentre). The culture was spun down and the bacterial pellet frozen at -20°C. BAC DNA was isolated with the PureLink™ HiPure Plasmid Kit (Invitrogen) according to the manufacturer's instructions with the following modifications. The bacterial pellet was resuspended in 0.4 ml R3 buffer and transferred to a microcentrifuge tube. Lysis was performed with 0.4 ml L7 buffer, gentle inversions (10 times) and incubation at room temperature for no longer than 4 minutes. Neutralization was performed with 0.4 ml N3 buffer, gentle inversions (10 times) and incubation on ice for 4 minutes. Precipitation was performed by centrifugation for 10 minutes at 4°C at full

speed. 2 ml EQ1 buffer was added to the gravity purification column for equilibration. After centrifugation, the supernatant was loaded onto the column. The column was washed twice with 2.5 ml W8 buffer. The DNA sample was eluted with 850 µl E4 buffer, prewarmed to 50°C, into a microcentrifuge tube. DNA was precipitated with 595 µl isopropanol and centrifugation for 20 minutes at 4°C at full speed. The DNA pellet was washed with 800 µl 70% ethanol and centrifugation for 2 minutes at 4°C at full speed. The DNA pellet was air dried for 4 minutes at room temperature and rehydrated in 20 µl EB Buffer (Qiagen: 10 mM Tris-Cl, pH 8.5). The DNA sample was allowed to dissolve overnight at 4°C. The sample was then centrifuged for 2 minutes at 4°C at full speed and transferred to a new microcentrifuge tube, avoiding the remaining pellet. 2 µl of the sample was used for an O.D. measurement. 1 µl of the sample was used to assess the yield and supercoiled quality of the DNA preparation using a 0.7% agarose gel. The remaining 17 µl of the DNA sample was adjusted to a concentration of 15 ng/µl for each 10 kb of plasmid length, a concentration that was decided upon after extensive testing. The diluted DNA was stored at -20°C.

Transformation: DNA was injected into *y*^{1} M{vas-int.Dm}ZH-2A *w**;PBac{*y*$^{+}$-attP-3B}VK00033 embryos. Adult flies were crossed to five *w*^{1118};TM2/TM6C,*Sb*. Initially we transferred the adults to fresh vials three times and screened the G_1 progeny for mini-white expressing transformants once a week for three weeks. Analysis of 150 transformation experiments showed that 89% of those producing transformants did so in the first week. Therefore, to save time, we transferred adults to fresh vials once and screened all flies simultaneously when both vials were producing adult progeny. We

ceased screening once two independent lines, one transgenic from two independent vials, were identified. If two independent lines were not identified, two lines were maintained from sibling transformants where possible. Individual balanced G_1 transformed flies were backcrossed to *w*^{1118}*;*TM2/TM6C,*Sb*. A single G_2 male was backcrossed to *w*^{1118}*;*TM2/TM6C,*Sb* and a sibling was used for PCR confirmation of proper integration. Sometimes, transgenic progeny were obtained from a female injected animal and the integrase containing X chromosome may still have been present. Hence, these flies were screened for absence of dsRed fluorescence in the eye at this stage. Virgin G_3 females and males were crossed to establish the balanced line (TM6C*,Sb*). Homozygous viability and fertility were assessed in the G_4 and homozygous lines established when possible. The six male lethal or subvital lines were propagated through virgin females and lines established with *dsx*^{D}*,e*^{1}*,Sb*^{1} and TM2.

PCR confirmation of integration: PCR confirmation of insertion into the docking site was performed on DNA isolated from single flies using the “squish” method (Engels *et al.* 1990). PCR primers and conditions are described (Venken *et al.* 2009). When possible we tested at least two lines for each clone injected. Of the 408 transformed clones tested, 382 gave the appropriate PCR pattern in at least one line. 44 of these 382 also produced an incorrect PCR pattern in another line indicating a low percent of defective integration.

Complementation testing: Rescue experiments were performed with standard Drosophila crossing protocols using the alleles described in **Supplemental Table 2**.

Accession numbers: BAC end sequences have been deposited in GenBank under accession numbers **XXX** to **XXX**.

RESULTS

Selection of a tiling path of P[acman] clones spanning the X chromosome: Using BAC end sequence coordinates (Venken *et al.* 2009) and FlyBase gene annotations (Misra *et al.* 2002; Drysdale 2008), we selected a tiling path of P[acman] BAC clones from the CHORI-321 library spanning the sequenced portion of the *Drosophila melanogater* X chromosome: Release 5 armX (22,423 kb) (www.fruitfly.org) and XHet (153 kb) (Hoskins *et al.* 2007) sequences. The clones are contained within a vector backbone harboring an *attB* site for ΦC31-mediated transgenesis (Groth *et al.* 2004; Bischof *et al.* 2007) and the dominant mini-white eye marker for the identification of transgenic animals (Venken *et al.* 2006; Venken *et al.* 2009). Our aim was to minimize the number of clones in the tiling path while maximizing coverage of complete gene annotations and unannotated 5' control regions. As some portions of the X chromosome are not represented by mapped 80 kb CHORI-321 clones we selected six clones from the 21 kb-insert CHORI-322 library to cover some of these regions. We selected 582 clones that were streaked from 384 well plates (Venken *et al.* 2009) for single colonies, and the DNA sequence was verified for each. This resulted in 566 verified clones with an average insert length of 87,710 bp and an average overlap of 47,774 bp. The resulting tiling path covers the X chromosome from the telomeric to pericentric heterochromatin. Of the 2,210 annotated protein-coding genes present on the X chromosome (http://flybase.org/, FlyBase release 5.12) (Adams *et al.* 2000; Drysdale 2008), all but a small number are contained within at least one clone in the tiling path. We were not able to find appropriately mapped clones for 18 genes (**Supplemental**

Table 1). Some of these genes may be better represented by clones in the unmapped fraction of the CHORI-321 library. Other genes are very large and will not be covered by any clone in this library. In addition, twelve regions encompass a minimal overlap between clones (7) or a gap in mapped clone coverage (5) (**Supplemental Table 1**). Four of these gaps are not represented in any other mapped BAC library (Celniker *et al.* 2002; Hoskins *et al.* 2007), suggesting that these regions of the Drosophila genome cannot be stably cloned in *E. coli*.

Generation of transgenic Drosophila lines: The P[acman] clones in the tiling path were injected into embryos that carried the VK33 *attP* docking site at polytene location 65B2 on chromosome arm 3L (Venken *et al.* 2006) and a Drosophila codon optimized ΦC31 integrase driven in the germline by the *vasa* promoter (Bischof *et al.* 2007). The VK33 integration site was chosen because it is homozygous viable, isogenic since it was obtained as a single balanced transgenic animal (Venken *et al.* 2006), and had been shown to be a reliable site for the recovery of transgenic inserts (Venken *et al.* 2009). When the VK33 insert was originally recovered and mapped, there were no annotated genes in the genomic interval. Subsequently, it has been shown that the interval does contain a gene, *CG42747* (http://flybase.org/, FlyBase release 5.30) (Adams *et al.* 2000; Drysdale 2008) and that the VK33 insertion lies in a 5' intron of this gene. The gene is transcribed at relatively low levels, and its function is unknown. The fact that the VK33 insert is homozygous viable and fertile indicates that if *CG42747* has an essential role then the insert does not compromise that function. Transgenic progeny expressing mini-white were identified as described (Venken *et al.* 2006), and balanced

and tested for proper integration into the docking site using a multiplex PCR procedure as described (Venken *et al.* 2009). Transgenic lines were tested for homozygous viability and fertility. Homozygous transgenic lines were established whenever possible.

We have injected 461 P[acman] clones and obtained transgenics for 408 of them (88%). Multiplex PCR revealed that 382 (94%) of the recovered lines integrated into the proper docking site. The improper events resulted in three different types of PCR patterns: an empty docking site pattern or no pattern at all, both results suggesting an insertion at one of several pseudo-*attP* integration sites located within the fly genome (Groth *et al.* 2004); or differently sized bands, which suggests an imprecise integration event at the docking site. All duplications are stably maintained since loss of the mini-*white* marker has not been observed over many generations. The 382 correctly targeted duplications cover approximately 96% of the euchromatic portion of the X chromosome and extend into pericentric heterochromatin (**Fig. 1**, **Supplemental Figure 1** and **Supplemental Table 2**), with the largest contig being 6,121,885 bp in length and the second largest being 3,269,101 bp. The transgenic flies are currently available as the “Duplication Consortium X Chromosome” Duplications from the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu/Browse/dp/DC-Dps.php).

There are twenty-two small gaps in our current coverage (**Fig. 1**). Five of these gaps are not covered by clones in the CH321 and CH322 BAC libraries (**Fig. 1**, red bars and **Supplemental Table 2**). The other seventeen gaps for which CH321 clones do exist (**Fig.1**, pink bars) are being injected. The gaps in the current path are likely covered by

larger, Y chromosome linked BSC duplications (Kevin Cook, **accompanying manuscript**). This is illustrated for a 3 megabase region extending from 14B to 18A (**Fig. 2**).

The average transformation efficiency was one transformant-producing fly for every 54 fertile G_0 animals. For the 382 clones that produced transgenic animals we recovered two independent lines for 214 (56%), while the remainder only produced a single transgenic line. We recovered correctly inserted clones in 66% of cases during a first injection round. Re-injections yielded 80% of appropriate transformants. Hence we conclude that our failure to recover lines for 53 clones is likely due to the small number of fertile G_0 flies screened.

Interestingly, while multiple lines from an individual clone exhibit the same orange eye color from the mini-*white* marker gene, lines of different clones can vary in the degree of mini-*white* expression (from yellow to red). Since all clones are incorporated into the same docking site, the variation in eye color is most likely due to the sequences of the genomic insert, as previously reported (Venken *et al.* 2006).

Preliminary characterization of the duplication stocks: Of the 367 current, characterized duplication lines, 302 (82%) are homozygous viable and fertile with no obvious phenotype. However, viability is often reduced in homozygotes. Mendelian ratios of homozygous adult progeny from crossing heterozygotes are generally decreased to 10% from the expected 33%. Nevertheless, most of the transgenic

duplications are tolerated in three copies in males and four copies in females. This is somewhat surprising as dosage compensation in males does occur for most X-linked material duplicated on autosomes (Alekseyenko *et al.* 2008). Homozygotes of the remaining 65 transgenic lines (18%) either exhibit an obvious phenotype or have severely reduced viability (**Supplemental Table 3**). The main phenotypes observed are *Minute*-like, similar to a dominant ribosomal protein deficiency phenotype, wings out, similar to the *held out wings* (*how*) phenotype, male lethality, and sex-specific sterility. Interestingly, in about 50% of these stocks males are more severely affected than females, suggesting that dosage compensation is responsible for the severity of these phenotypes. Similarly, the eye color of males is in general darker than that of females, suggesting that dosage compensation acts on the mini-*white* marker of the P[acman] transgene.

We have characterized differences between isolates for 269 of the 367 transgenic strains. Fifty exhibited differences in homozygous viability or fertility between independent lines (37) containing the same P[acman] clone as well as between transgenic siblings from the same G_0 parent (13). This suggests that some chromosomes may carry a second site lethal or sterile mutation resulting from either a cryptic mutation that originated in the injection stock after isogenization several years ago (Venken *et al.* 2006), or mutations caused during the trangenesis procedure. ΦC31 integrase has been shown to induce DNA damage and chromosome rearrangements (Ehrhardt *et al.* 2006; Liu *et al.* 2006; Liu *et al.* 2009) although this has been reported to be relatively rare in *Drosophila* (Bischof *et al.* 2007). We therefore outcrossed 18 of the

37 insertion stocks to a wild-type isogenized 3rd chromosome for two to three generations. In this short period of time, 10 of 18 cases produced viable and fertile homozygotes, suggesting that distantly linked second site mutations, and not the insertions were the cause of the observed phenotypes.

P[acman] duplications rescue 90% of mutants tested: To ensure that integrated P[acman] clones are functional duplications of the X chromosome, we tested 112 different transgenic lines for their ability to rescue known molecularly mapped mutations, including both lethal and viable mutant alleles. As shown in **Table 1** and **Supplemental Table 2** the rescue experiments demonstrate that the duplications complement 92% of the tested mutations. This indicates that the majority of Drosophila genes have their required regulatory elements in the vicinity of the currently annotated transcripts (http://flybase.org/) (Drysdale 2008). Lack of rescue of lethal mutations, however, does not necessarily mean that the transgene does not contain the full-length gene with all its regulatory elements. It is possible that the chromosome bearing the mutation being tested also carries unidentified second site lesions. This is illustrated for genes for which multiple alleles were tested, e.g., *squash*, *TATA box binding protein-related factor 2* and *cut up*: some alleles are complemented, whereas others are not by the same transgene, indicating that second site mutations are present on some of these chromosomes (**Supplemental Table 2**). However, a few examples of transgenes that fail to complement the corresponding mutations are noteworthy. Based on the current gene annotation, mutations in *roughest* (*rst*) should have been rescued by two independent duplications, Dp(1;3)DC052 (CH321-04A01) and Dp(1;3)DC108 (CH321-

65P11). However, these duplications do not rescue, suggesting that essential distant regulatory regions are lacking in the duplications. Indeed, recent RNA-Seq data (http://www.modencode.org and Rui Chen, personal communication) have shown that *rst* has an unannotated 5' exon 13.8 kb upstream from the currently annotated transcription start site that is absent in both clones. Similarly, mutations in *cut* (*ct*) are not rescued by duplication Dp(1;3)DC178 (CH321-62C02). This was anticipated as the known regulatory elements of *ct* extend over more than 80 kb from the annotated gene (Jack & DeLotto 1995) and thus beyond the extent of the duplication.

We expected Dp(1;3)DC572 (CH321-82G19) which encompasses *ocelliless (oc)* to rescue mutations in that gene. We found that the duplication modifies the ocelliless phenotype of oc^1 but does not restore the missing ocelli. The dorsal region that normally contains the ocelli is more like wild-type but ocelli are still absent. In one instance, however a single ocellus was restored. These results are consistent with an increase in, but not a normal level of *oc* gene expression. This supports a report that increasing the levels of *oc* expression with a heat-shock driven transgene in an *oc* mutant background improved the ocellarless phenotype (Royet & Finkelstein 1995). A second, slightly smaller duplication, Dp(1;3)DC195 (CH321-05H15) also failed to rescue oc^1. Notably, larger duplications containing significantly more sequence 5' of the gene, such as Dp(1;Y)BSC39 fully rescue the ocelliless phenotype of oc^1 (Kevin Cook, **accompanying manuscript**), suggesting that *oc* requires a very large upstream regulatory region (>41 kb) for normal transcription.

The molecularly defined duplications can also be used to rescue molecularly defined deletions previously generated by Flp/*FRT* mediated recombinational excision (Parks *et al.* 2004; Ryder *et al.* 2004; Ryder *et al.* 2007). For example, Dp(1;3)DC134 (CH321-18K02) rescues two *paralytic* (*para*) alleles as well as Df(1)FDD-0230908 (Ryder *et al.* 2004; Ryder *et al.* 2007) (**Fig. 3**). Similarly, the duplications Dp(1;3)DC130 (CH321-70G03), Dp(1;3)DC205 (CH321-25D20), Dp(1;3)DC243 (CH321-74F04) and Dp(1;3)DC273 (CH321-23B06) rescue Df(1)BSC823, Df(1)Excel9049, Df(1)Excel9050 and Df(1)BSC546, respectively (**Table 1** and **Supplemental Table 2**).

Finally, 11 Minute loci are present on the X chromosome (Marygold *et al.* 2007). Minutes display a variety of cellular and developmental defects associated with a dominant haplo-insufficient phenotype due to a ribosomal protein deficiency. Two *Minutes* were tested for complementation: Dp(1;3)DC009 (CH321-46B03), Dp(1;3)DC010 (CH321-04A18) and Dp(1;3)DC011 (CH321-11D11) rescue *RpL36* while Dp(1;3)DC325 (CH321-64E02) rescues *RpS5a* (**Table 1** and **Supplemental Table 2**).

Aneuploid-sensitive loci associated with obvious visible phenotypes: Twelve duplication lines exhibit an obvious aneuploid-associated phenotype in animals heterozygous for the duplication (**Supplemental Table 3**). Five of the lines have transgenes that encompass aneuploid-sensitive loci associated with obvious visible phenotypes, whereas two lines are associated with known diplo-lethal regions and five lines are not associated with known diplo-lethal regions (see below). Flies that carry one or two extra copies of Dp(1;3)DC006 (CH321-32O15), which encompasses the *achaete*

(*ac*) and *scute* (*sc*) genes, display a *Hairy wing* (*Hw*) phenotype. Dp(1;3)DC097 (CH321-82N07) encompasses *sc* but not *ac* and homozygous adults exhibit a much weaker *Hw* phenotype. *Hw* mutations have been associated with over-expression of *ac* or *sc* (Balcells *et al.* 1988), and our data suggest that just one extra copy of *ac* and/or *sc* is sufficient to cause a *Hw* phenotype in females. This phenotype is also observed in other small duplications that cover this region, including Dp(1;Y)y+ which only duplicates *ac* and not *sc* (Muller 1948; Lindsley & Zimm 1992), suggesting that the *Hw* phenotype is not due to ectopic expression but to elevated expression levels of *ac* and/or *sc* within their normal expression domains.

Flies that carry an extra copy of Dp(1;3)DC109 (CH321-91P23) exhibit a *Confluens* (*Co*) phenotype (Lyman & Young 1993). This wing vein phenotype is typically associated with an extra copy of *Notch* (*N*), and this clone includes four genes: *Notch, Follicle cell protein 3C, CG18508* and a portion of *kirre.* Interestingly, the *Co* phenotype is also dose dependent as two extra copies of Dp(1;3)DC109 cause a more extreme phenotype than a single extra copy.

Similarly, an extra copy of Dp(1;3)DC329 (CH321-85I09), which encompasses the *BarH1* gene, causes a *Bar* eyed phenotype in males and females. This result is consistent with the fact that the dominant *Bar* mutations are associated with unequal cross-over events and an increase in the copy number of *BarH1* and/or *BarH2* genes (Sturtevant & Morgan 1923; Gabay & Laughnan 1973). Interestingly, Dp(1;3)DC328 (CH321-04D11) which encompasses *BarH2* but does not contain *BarH1* does not

exhibit a Bar phenotype, therefore supporting the idea that the Bar eye phenotype is due to extra copies of the *BarH1* gene alone (Kojima *et al.* 1991; Kojima *et al.* 1993).

Males homozygous for Dp(1;3)DC327 (CH321-56I13) which encompasses *forked* (*f*) exhibit bent macrochaete and microchaete, a phenotype similar to that observed in flies containing four copies of a *f* transgene in a f^{+} background (Petersen *et al.* 1994; Tilney *et al.* 1998; Tilney *et al.* 2004). Homozygous females do not exhibit this bristle phenotype suggesting that dosage compensation causes an increased expression of *f* in these Dp males. Males homozygous for this Dp could be expressing the equivalent of 6 doses of *f* resulting in the bent bristle phenotype. Notably, the original transgene causing the bent bristle phenotype (Tilney *et al.* 2004) encodes only four of the six *f* transcripts. Dp(1;3)DC327 encompasses all six transcripts. Thus the additional transcripts may contribute to the bent bristle phenotype even if the level of expression from the duplication is not quite equivalent to six doses.

Flies containing one copy of Dp(1;3)DC197 (CH321-38K07) exhibit necrotic wings and slightly mis-shaped eyes. The only annotated gene contained within this duplication is *Lim1*. Interestingly, a second independent transgenic line, Dp(1;3)DC500 (CH321-51H02) that encompasses *Lim1* as well, exhibits the same phenotype. However, larger duplications that contain significantly more material 5' of the *Lim1* gene do not have this phenotype, e.g., Dp(1;Y)BSC41 and Dp(1;Y)BSC42 (Kevin Cook, **accompanying manuscript**). Therefore it would appear that the observed defects in animals carrying the two small Dp's is not caused by aneuploidy alone. The phenotype thus may be due

to abnormal expression of *Lim1* either from a loss of normal regulatory sequences further upstream or from a position effect. In summary, all of these clones with obvious visible phenotypes can now be used as new dominant markers.

Aneuploid sensitive loci associated with known diplo-lethal regions: The set of duplications reported here has allowed a more precise localization of dosage dependent lethal regions of the X chromosome, which are typically difficult to identify and map. The X chromosome was originally reported to contain only one hyperploid-sensitive locus (*Beadex* at 17A-C), and one locus associated with visible phenotypes when present in excess (triplo-abnormal) or when reduced to a single copy in a female (haplo-abnormal) (*Notch* at 3C7) (Lindsley *et al.* 1972). Subsequently, a duplication of the 11E-12B region was discovered to be lethal in males (Stewart & Merriam 1975). The cause of the lethality was hypothesized but not demonstrated to be due to mutations in *upheld* (*up*), which was proposed to be both diplo- and haplo-lethal, lethal as two copies in males or one copy in females respectively (Homyk, Jr. & Emerson, Jr. 1988).
Consistent with this prior mapping Dp(1;3)DC271 (CH321-77D16), which covers polytene region 12A4-7, is associated with diplo-lethality in males. It encompasses eight loci, including *up* (**Fig. 4A**). However, partial loss-of-function mutations of *up*, up^{1} and up^{101} (null alleles and deficiencies do not exist) do not suppress the diplo-lethality, suggesting that *up* hyperploidy may not be causing the lethality or that the partial loss-of-function alleles do not affect hyperploid male lethality. Interestingly, Dp(1;Y)BSC185 also extends into this interval but is not associated with male diplo-lethality (Kevin Cook, **accompanying manuscript** and **Fig 4A**). Complementation analysis using this

duplication with *up* alleles has shown that these lesions are complemented, similar to results using Dp(1;3)DC271 (Kevin Cook, **accompanying manuscript**). This result appears to rule out *up* or any of the genes mapping to its right as the cause of the observed diplo-lethatlity (**Fig 4A**, pink box) and instead implicates one of the loci mapping to the left of *up* (**Fig 4A**, blue box). The fact that deficiencies including the region bounded by the pink box in Fig 4A have not been recovered implicates that region as the cause of the haplo-lethality. Thus the combined behavior of the recovered duplications and deficiencies in this region has shown that the haplo- and diplo-lethality mapped to this interval are likely caused by two different albeit tightly linked loci. We have not resolved which of the potential 4 diplo- and 8 haplo-lethal genes is responsible but the analysis has dramatically narrowed the search and points to the resolving power afforded by these new reagents.

Several labs have reported that duplication of a region in 3F causes male lethality (a male-specific diplo-lethal region) (Cline 1988; Oliver *et al.* 1988). Here we provide molecular data that refine the mapping to a small number of loci. Dp(1;3)DC068 (CH321-33A07), which covers much of the 3F cytological region, is essentially diplo-lethal in males. Overlapping duplications that do not exhibit this phenotype allowed us to exclude some of the resident loci and suggest that any of the following three loci or a combination thereof cause male lethality when present in two copies in males: *Vacuolar H^+-ATPase C39 subunit* (*VhaAc39*), *CG15239* and/or *CG42541* (**Fig. 4B**). *VhaAc39* has recently been shown to impinge on Notch signaling (Yan *et al.* 2009). Since *N* is one of very few loci that are haplo-insufficient and hyperploid sensitive, an additional copy of

VhaAc39 could possibly lead to a gain-of-function phenotype of Notch signaling. However, complementation crosses involving a mutant allele of *VhaAc39* and Dp(1;3)DC068 demonstrate that the duplication rescues the recessive lethality of this locus in females but that the *VhaAc39/Y; Dp(1;3)DC068/+* genotype is male lethal. Hence, either *CG15239* or *CG42541*, or an unannotated feature in this region, is associated with the male diplo-lethality.

Aneuploid sensitive duplications not associated with known diplo-lethal regions: There are 5 duplications that affect male viability but do not map to known dosage sensitive regions. In all five cases larger duplications encompassing the DC duplications do not exhibit an effect on male viability. The male lethal Dp(1;3)DC194 (CH321-69A10) is covered by a larger duplication that does not affect male viability (Dp(1;Y)BSC39) suggesting that the lethality may be due to truncation of a gene carried at one of the ends of the duplication, creating a dominant negative protein, either *Neuroglian* (*Nrg*) or *ocelliless* (*oc*), or by a position effect of the DNA surrounding the docking site on chromosome arm 3L (**Fig. 5A**).

The male lethal Dp(1;3)DC334 (CH321-16L02) is contained within the larger duplication, Dp(1:Y)BSC67, that does not affect male viability (Kevin Cook, **accompanying manuscript**) (http://flystocks.bio.indiana.edu/Browse/dp/BDSC-Dps.php). Dp(1;3)DC334 contains *CG8188*, *par-6*, *CG8173*, *CG42684*, *unc-4*, and part of *CG32556* (**Fig. 5B**). Overlapping duplications do not affect male viability and encompass all but the *CG42684* gene. Hence, the male lethality may be due to mis-

expression of *CG42684* or a position effect associated with the insertion that becomes neutralized within the larger duplication Dp(1:Y)BSC67.

Males carrying a single copy of Dp(1;3)DC087 (CH321-01B20) are reduced in number (approximately 1/2 to 2/3 the expected number of males in the balanced stock). In addition, homozygotes are rare. There are several cytologically mapped viable duplications that cover this region (http://flystocks.bio.indiana.edu/Browse/dp/BDSC-Dps.php). This duplication contains three annotated genes, two of which are contained within Dp(1;3)DC088 (CH321-25A14) which does not affect male viability (**Fig. 5C**). This leaves *runt* (*run*) as the candidate for the cause of the male sub-viability by this duplication. Further testing will be required to confirm that hyperploidy for *run* is indeed associated with reduced viability.

Males with one copy of Dp(1;3)DC312 (CH321-48H12) are reduced in number with very reduced fertility. Again, this region is contained within a larger duplication that does not affect male viability (Dp(1;Y)BSC228) (Kevin Cook, **accompanying manuscript**). It is unclear what is causing the male viability problems associated with this duplication. It contains 21 genes, 11 of which are not covered by any other DC duplication (**Fig. 5D**). Both Dp(1;3)DC312 and Dp(1;3)DC311 (CH321-93B12) encompass *disco* but male viability is unaffected in DC311. However, males with two copies of Dp(1;3)DC311 are sterile. Hence, it is possible that *disco* is involved in the reduced male fertility. It is unclear which genes could be involved in the reduced male viability. Again further analysis will be required to determine the cause of both affects.

Finally, flies homozygous for Dp(1;3)DC097 (CH321-82N07) are viable and both sexes are fertile but males are extremely rare. This duplication contains *sc, l(1)sc, pcl, ase, Cyp4gi* (**Fig. 5E**). The homozygous male lethality is probably not due to *pcl, ase* or *Cyp4gi* since these are contained within Dp(1;3)DC007 (CH321-34A23) which is homozygous viable and fertile. Both male and female homozygotes of Dp(1;3)DC006 (CH321-32O15) which contains *sc* are rare. It is possible that *l(1)sc* or a combination of *sc* and *l(1)sc* cause the observed lethality of homozygous males in this duplication. Alternatively, the lethality could be caused by truncation or altered expression of CG32816, which extends the length of the duplication.

DISCUSSION

We describe the creation of a collection of molecularly defined duplications that will allow a much better characterization of more than 95% of the genes on the X chromosome of *Drosophila melanogaster*. Several conclusions can be drawn from our analyses of these duplication lines. First, the efficiency of transformation with large-insert P[acman] clones is quite high: 66% upon a first injection attempt, better than achieved previously (Venken *et al.* 2006; Venken *et al.* 2009). Subsequent re-injection of clones that failed on the first attempt led to 80% transformation efficiency, suggesting that more than 90% of large-insert clones can be integrated into the fly genome provided that about 100 fertile injected G_0 animals are obtained. Second, second-site mutations are created during the transformation process at a frequency of 9%. Similar observations were reported previously in other experimental paradigms using ΦC31 integrase (Ehrhardt *et al.* 2006; Liu *et al.* 2006; Liu *et al.* 2009). Hence, we suggest outcrossing any transgenic chromosome that produces an unanticipated phenotype before attributing that phenotype to the inserted DNA, unless two independently generated transgenic lines produce the same phenotype. Third, our data show that most fly genes are quite compact: enhancers and other regulatory elements are generally located near the transcription units, as illustrated for several large genes such as *para* and *shakB* that are rescued by clones containing little additional genomic DNA on either end of the current transcript annotation. Although we have identified a few exceptions, we conclude that a majority of the current FlyBase gene annotations (http://flybase.org/) are an excellent guide for selecting rescue constructs. Fourth,

aneuploidy of more than 90% of the genes is well tolerated, even when four copies are present in females. Similarly, males with three copies of most genes, which may effectively correspond to six copies due to dosage compensation (Gelbart & Kuroda 2009), are often viable and fertile, and display no obvious abnormal phenotypes, except for reduced Mendelian ratios in their progeny. It will be interesting to establish how dosage compensation is affected in these males. Fifth, consistent with previous work, very few small duplications of the X chromosome cause diplo-lethality in males, and this set of duplications has allowed the refined mapping of two diplo-lethal regions on the X chromosome. Overlapping duplications have identified potential culprits that may cause these phenotypes: two genes at cytological band 3F and four genes at cytological band 12F.

Very few genes on the X chromosome are not covered by mapped P[acman] clones. Some genes such as *Tenascin accessory* (*Ten-a*) and *dunce* (*dnc*) are simply too large to be contained within a single P[acman] clone from the library used here. For others we were unable to find an appropriate clone due to the finite number of clones mapped and their non-random distribution. We are therefore planning to upgrade existing low-copy number, large-insert BAC clones from available mapped BAC libraries (Hoskins *et al.* 2000; Benos *et al.* 2001) by insertion of a retrofitting plasmid that contains the required elements for P[acman] transgenesis; this technique was recently used to retrofit clones from available fosmid and BAC libraries (Kondo *et al.* 2009). However, it remains to be determined whether retrofitted BACs from the large-insert RPCI-98 library (Hoskins *et al.* 2000), with an average insert size of 165 kb, can be integrated into *attP* docking sites

in the fly genome. Alternatively, fragment size can be reduced through gap-repair (Venken *et al.* 2006) or BAC trimming (Hill *et al.* 2000). In addition to the gene size problem, entire annotated genes in four regions of the X chromosome are not represented in mapped P[acman] clones due to minimal clone overlap or gaps in mapped clone coverage. Three of these regions are represented in large-insert BAC clones from other mapped libraries, and these regions should be amenable to our molecularly-defined duplication strategy. The one remaining region containing annotated genes within the sequenced portion of the X chromosome, at polytene location 9A, is not represented in any available large-insert genomic library (www.fruitfly.org) (Hoskins *et al.* 2007), apparently due to problems associated with bacterial cloning of certain regions of the fly genome in an *E.coli* bacterial host. One potential solution is to switch cloning organism such as *Saccharomyces cerevisiae*. Recombinogenic technologies, such as transformation-associated recombination cloning (Kouprina & Larionov 2008), have been used numerous times to retrieve large genomic regions through cotransformation mediated gap-repair directly from high-molcular-weight DNA into a linearized vector backbone (Kouprina & Larionov 2006). We conclude that very few genes are not represented in the selected tiling path of P[acman] clones. We are continuing to work on the remaining unrepresented regions to complete the duplication kit.

We note that it may be possible to create large transgenic duplications using our set of small *Drosophila* X chromosome duplications. Since all the clones have been integrated into the same *attP* docking site, a large duplication could potentially be generated

through *in vivo* meiotic recombination between the overlapping regions of two smaller duplications. We are currently testing this possibility.

The collection of duplications we have described will be useful for many purposes. It will be very valuable for rapidly mapping mutants, including the numerous publicly available and poorly mapped X-linked viable and lethal mutations, at high resolution. This should greatly accelerate mutation identification on the X chromosome: a first set of crosses with about 20 to 30 large duplications (Kevin Cook, **accompanying manuscript**) (http://flystocks.bio.indiana.edu/Browse/dp/BDSC-Dps.php) will in general allow mapping to an interval of a few 100 kb. A second set of crosses with a few P[acman] duplications will allow mapping to a 20-30 kb interval encompassing on average 2 to 3 genes. This can be followed by Sanger sequencing of the annotated protein-coding sequences in the region (H.J.B., unpublished data). Based on our current experience, this strategy is much cheaper and more effective than whole genome sequencing or gene capture sequencing technologies (Metzker 2010; Mamanova *et al.* 2010). Indeed, the large number of SNPs and the bioinformatic burden associated with next-generation sequencing technologies make this approach much more expensive than setting up 30 fly crosses. Moreover, any sequencing-based strategy will still require a rescue strategy at the end of the sequencing process, as 10-100 SNPs per chromosome arm are identified depending on the concentration of the chemical mutagens used to generate the mutations. It is therefore much more efficient to set up duplication crosses to map the mutation to a small genomic interval and then to carry out Sanger sequencing or targeted next generation sequencing to identify the causal mutation.

The rescue of mutations will also provide an extremely useful framework for tagging and manipulating genes of interest using P[acman] clones and recombineering technology (Venken *et al.* 2008; Ejsmont *et al.* 2009; Venken *et al.* 2009). Thus, the collection of molecularly defined duplications that we have described will allow new experimental designs and strategies and will significantly expand the repertoire of manipulations of the X chromosome in *Drosophila*. Finally, we propose that the strategy we have used is a model for future analysis of other chromosomes in *Drosophila* and other species.

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FIGURE LEGENDS

Figure 1. Overview of the molecularly defined X chromosome duplication kit. The hash marked line at the top of the three segments (A, B and C) of the X chromosome represents the coordinates (Mb) from the telomeric (top left) to the pericentromeric heterochromatin (bottom right). Each panel overlaps the next by 10 kb, the region of overlap is indicated in gray at the right end of each panel. The distribution of annotated gene spans (light blue arrows) is shown. Below are shown the estimated extents of the polytene chromosome bands from 1A to 20D. The extent of molecularly mapped duplications generated in this work is shown: duplications that complement molecularly defined mutations are indicated in green, others are indicated in black. Below, the current gaps in coverage are indicated by pink and red bars with the size of the gaps indicated above each bar. The pink gaps are represented by identified P[acman] BACs while the 5 red gaps are not represented by mapped clones in the CH321 and CH322 libraries. The map was derived from the GBrowse representation of the X chromosome in FlyBase.

Figure 2. Overview of the polytene chromosome bands 14B to 18A illustrating the complementary nature of the two new sets of X-chromosome duplications. The region illustrates the density of the DC duplications (this work) and compares them to the larger BSC duplications (Kevin Cook, **accompanying manuscript**) in the same interval. This region contains 4 gaps in DC duplication coverage that are well covered by the

BSC duplications. The tiers are the same as in Fig.1 with the BSC duplications added at the bottom in dark blue.

Figure 3. Example of gene and deficiency complementation with a molecularly defined duplication. Dp(1;3)DC134 (CH321-18K02) complements the large gene *paralytic* (*para*) demonstrating that all the regulatory sequences necessary for its function reside within the duplicated sequence and illustrating the compactness of the gene. Additionally, the duplication complements the recessive lethality associated with Df(1)FDD-0230908. The gene spans of the loci covered by both the deficiency and the duplication are indicated. Annotated gene spans are indicated in light blue, molecularly defined deficiencies are in red, the DC duplications are in black. The map was derived from the GBrowse representation of the X chromosome in FlyBase.

Figure 4. Aneuploid sensitive loci associated with known diplo-lethal regions. (A) The haplo- and diplo-lethal region in cytological division 12A. The annotated genes potentially associated with diplo-lethality (blue) and the genes associated with haplo-lethatlity (red) are indicated. (B) The diplo-lethal interval in cytological division 3F. Two genes that are potentially associated with the lethality are indicated. The tiers and derivation of the maps are the same as in the previous figures.

Figure 5. Aneuploid sensitive duplications not associated with known diplo-lethal regions. (A) Truncation of the *Nrg* and/or *oc* genes. (B) Position effects associated with *CG42684*. Only Dp(1;3)DC334 (CH321-16L02) results in male lethality indicating that

CG42684 is the culprit. (C) Position effects on the *run* gene may cause the male viability defects seen with Dp(1;3)DC087 (CH321-01B20). (D) Position effects on any of fourteen different genes may cause the male viability defects of Dp(1;3)DC312 (CH321-48H12). (E) The male lethality observed with Dp(1;3)DC097 (CH321-82N07) could be caused by truncation of *CG32816* or by position effects on *l(1)sc* and/or *sc*. The tiers and derivation of the maps are the same as in the previous figures.

TABLES

TABLE 1.

Complementation data

Duplication	Clone	Predicted Cytology	Rescue	No rescue
Dp(1;3)DC003	CH321-12B18	1A1;1A1	*ewg, cin*	
Dp(1;3)DC004	CH321-46A16	1A1;1A3	*ewg*	*cin*
Dp(1;3)DC006	CH321-32O15	1A3;1A8	*ac, sc*	
Dp(1;3)DC007	CH321-34A23	1B1;1B5	*Exp6*	
Dp(1;3)DC009	CH321-46B03	1B9;1B13	*RpL36*	
Dp(1;3)DC010	CH321-04A18	1B11;1C3	*skpA, RpL36*	
Dp(1;3)DC011	CH321-11D11	1B11;1B13	*RpL36*	
Dp(1;3)DC012	CH321-32G05	1C3;1C5	*Rbf*	
Dp(1;3)DC029	CH321-35P01	2B4;2B8	*dor*	
Dp(1;3)DC030	CH321-02D20	2B7;2B9	*l(1)G0284*	
Dp(1;3)DC033	CH321-17O06	2B12;2B14	*l(1)G0355*	
Dp(1;3)DC034	CH321-22J19	2B13;2B17	*l(1)G0355, arm*	
Dp(1;3)DC037	CH321-24E10	2C4;2D2	*Unc-76, usp, csw*	
Dp(1;3)DC038	CH321-04O23	2D1;2E1	*ph-d, Pgd*	*wapl*
Dp(1;3)DC039	CH321-43N04	2D5;2F2	*crn, pn*	
Dp(1;3)DC045	CH321-25N08	3A4;3A6	*wds, egh*	
Dp(1;3)DC048	CH321-34G02	3A8;3B2	*sgg*	
Dp(1;3)DC050	CH321-23A22	3B4;3C1	*w*	
Dp(1;3)DC052	CH321-04A01	3C2;3C5		*rst*
Dp(1;3)DC060	CH321-42E18	3D1;3D3	*dm*	
Dp(1;3)DC067	CH321-32O23	3F1;3F4	*ec*	
Dp(1;3)DC068	CH321-33A07	3F3;3F7	*VhaAC39*	

Dp(1;3)DC081	CH321-29F10	4C4;4C7	*CHOp24*	*rb*
Dp(1;3)DC087	CH321-01B20	19E1;19E3	*run*	
Dp(1;3)DC090	CH321-27E22	19E3;19E4	*shakB*	
Dp(1;3)DC092	CH321-05L19	19E3;19E4		*l(1)19Ec*
Dp(1;3)DC096	CH321-23M15	19E7;19F1	*unc*	
Dp(1;3)DC104	CH321-90H13	2B3;2B6	*dor*	
Dp(1;3)DC106	CH322-76B11	2C1;2C5	*east*	
Dp(1;3)DC108	CH321-65P11	3C3;3C5		*rst*
Dp(1;3)DC109	CH321-91P23	3C6;3C9	*N*	
Dp(1;3)DC114	CH321-85H19	4A3;4B2	*brn*	
Dp(1;3)DC120	CH321-61L05	4C11;4C16	*rap, ctp*	
Dp(1;3)DC126	CH321-22I01	4E1;4E2		*ovo*
Dp(1;3)DC130	CH321-70G03	4F3;4F5	Df(1)BSC823	
Dp(1;3)DC131	CH321-60D21	10F6;11A1	*cac*	
Dp(1;3)DC132	CH321-77E01	13A1;13A5	*eag*	
Dp(1;3)DC134	CH321-18K02	14C4;14E1	*CG4420, Rbp2, para,* Df(1)FDD-0230908	
Dp(1;3)DC143	CH321-73A03	5A13;5C2		*l(1)G0060*
Dp(1;3)DC146	CH321-64D18	5C5;5C10	*Act5C*	
Dp(1;3)DC149	CH321-84E21	5D2;5D5	*rux*	
Dp(1;3)DC152	CH321-17L08	5E1;5E6	*sqh*	
Dp(1;3)DC158	CH321-50D09	6B1;6C1	*dx*	
Dp(1;3)DC166	CH321-65P16	6E3;6F1	*cm*	
Dp(1;3)DC172	CH321-80M12	7A7;7B2	*brk*	
Dp(1;3)DC178	CH321-62C02	7B4;7B6		*ct*
Dp(1;3)DC180	CH321-59L01	7B6;7B8	*Tom40*	
Dp(1;3)DC184	CH321-60P23	7D2;7D6	*mys*	*fs(1)h*
Dp(1;3)DC185	CH321-24M24	7D5;7D16	*mys, Smox, CG2263*	*fs(1)h*

Dp(1;3)DC186	CH321-18C02	7D12;7D18	*Smox*	
Dp(1;3)DC187	CH321-74I15	7D17;7E1	*sdt*	
Dp(1;3)DC190	CH321-75D02	7E2;7E8	*CG10555*	
Dp(1;3)DC191	CH321-09O19	7E6;7F1	*CG10555, Trf2*	
Dp(1;3)DC192	CH321-28F18	7E9;7F1	*otu*	
Dp(1;3)DC193	CH321-80I04	7F1;7F6	*otu*	*Nrg*
Dp(1;3)DC196	CH321-64J01	8A1;8A5	*l(1)G0020*	
Dp(1;3)DC205	CH321-25D20	8D2;8D7	*amx, lz, i*	
Dp(1;3)DC209	CH321-01K05	8E3;8E10	*l(1)G0320*	
Dp(1;3)DC212	CH321-44B04	8F8;9A1	*CG15321, btd*	
Dp(1;3)DC221	CH321-69O03	9B2;9B5	*Hk, l(1)G0230*	
Dp(1;3)DC223	CH321-67L01	9B6;9C1		*l(1)G0289*
Dp(1;3)DC224	CH321-64N06	9B12;9C6	*flw*	
Dp(1;3)DC229	CH321-18K03	9E1;9E4	*ras*	
Dp(1;3)DC232	CH321-47A13	9F8;10A1	*v*	
Dp(1;3)DC235	CH321-50P18	10A6;10B1	*ran, rtv, Dlic*	
Dp(1;3)DC237	CH321-64M10	10B2;10B5	*dsh, l(1)10Bb, Kap3*	
Dp(1;3)DC238	CH321-67L16	10B3;10B12	*dsh, hop, dlg1*	
Dp(1;3)DC241	CH321-05O16	10C2;10D1	*Rpll215, Kmn1*	
Dp(1;3)DC243	CH321-74F04	10D4;10E2	Df(1)Excel9050	
Dp(1;3)DC244	CH321-26N14	10E1;10E6	*m, dy*	
Dp(1;3)DC246	CH321-76G11	10F1;10F7	*wisp, pot*	
Dp(1;3)DC247	CH321-60H11	10F11;11A2	*gd, tsg, fw*	*Usp7*
Dp(1;3)DC257	CH321-47M02	11B3;11B10	*CkIalpha*	
Dp(1;3)DC264	CH321-38B18	11D9;11E3	*hep, lic*	
Dp(1;3)DC266	CH321-03G21	11E3;11E8	*sno, mew*	
Dp(1;3)DC267	CH321-48B18	11E6;11E11	*comt*	
Dp(1;3)DC271	CH321-77D16	12A3;12A8	*up*	

Dp(1;3)DC273	CH321-23B06	12B2;12C1	*g,* Df(1)BSC546	*CG9940, CG32627*
Dp(1;3)DC274	CH321-75E20	12B4;12C6	*l(1)dd4, Rtc1, rdgB*	
Dp(1;3)DC275	CH321-70M13	12C5;12C7	*Clic*	*mamo*
Dp(1;3)DC282	CH321-60I22	12E5;12E8	*na*	
Dp(1;3)DC294	CH321-38B08	13A5;13B1	*CG5599, drd*	
Dp(1;3)DC297	CH321-60I24	13B4;13B6	*Top1, dah*	
Dp(1;3)DC300	CH321-59E06	13C3;13D1	*shtd*	*Gmap*
Dp(1;3)DC302	CH321-25M17	13D3;13E3	*sog*	
Dp(1;3)DC305	CH321-82A12	13E12;13F4	*l(1)G0136, Tcp-1zeta, mRpL3*	
Dp(1;3)DC309	CH321-12N23	14A5;14A8	*exd*	
Dp(1;3)DC313	CH321-28P07	14B9;14C3	*sl*	
Dp(1;3)DC316	CH321-89E23	14E1;14F2	*Arp14D*	
Dp(1;3)DC317	CH321-20I24	14F2;14F4		*rok*
Dp(1;3)DC319	CH321-08C14	15A3;15A9	*if*	
Dp(1;3)DC320	CH321-49N08	15A6;15B2	*CG9609*	
Dp(1;3)DC322	CH321-62B18	15B5;15D1	*wus*	
Dp(1;3)DC325	CH321-64E02	15E3;15F1	*RpS5a, xmas-2*	
Dp(1;3)DC327	CH321-56I13	15F3;16A1	*f*	
Dp(1;3)DC330	CH321-57P05	16A5;16B4		*CG8557*
Dp(1;3)DC331	CH321-09C05	16B1;16B8	*CG8557, l(1)G0222*	
Dp(1;3)DC337	CH321-16E18	16E1;16F1	*CG32557*	
Dp(1;3)DC341	CH321-90A16	16F5;16F7	*scu*	
Dp(1;3)DC344	CH321-35O24	17A3;17A5	*os*	
Dp(1;3)DC345	CH321-91O05	17A4;17A9	*os*	
Dp(1;3)DC346	CH321-32C05	17A7;17B1	*por*	*CrebB-17a*
Dp(1;3)DC347	CH321-90G02	17B1;17C1	*Aats-his*	
Dp(1;3)DC353	CH321-59N15	17D6;17F2	*Pvf*	

Dp(1;3)DC363	CH321-63C20	18C8;18D3	*car*	
Dp(1;3)DC409	CH321-05J07	19C5;19D1	*l(10G0004*	
Dp(1;3)DC414	CH321-80H02	14D1;14E4	*Arp14D*	
Dp(1;3)DC362	CH321-71B04	18C5;18C8	*l(1)G0156*	
Dp(1;3)DC364	CH321-35A10	18D1;18D13	*e(y)3, car*	*RpS10b*
Dp(1;3)DC365	CH321-35O18	18D7;18E3	*e(y)3, dome, Mer, Cdc42*	
Dp(1;3)DC413	CH321-72K03	2C1;2D1	*east, Actn, usp, Unc-76*	
Dp(1;3)DC550	CH321-51D08	18D13;18F1	*dome, Mer, Cdc42*	

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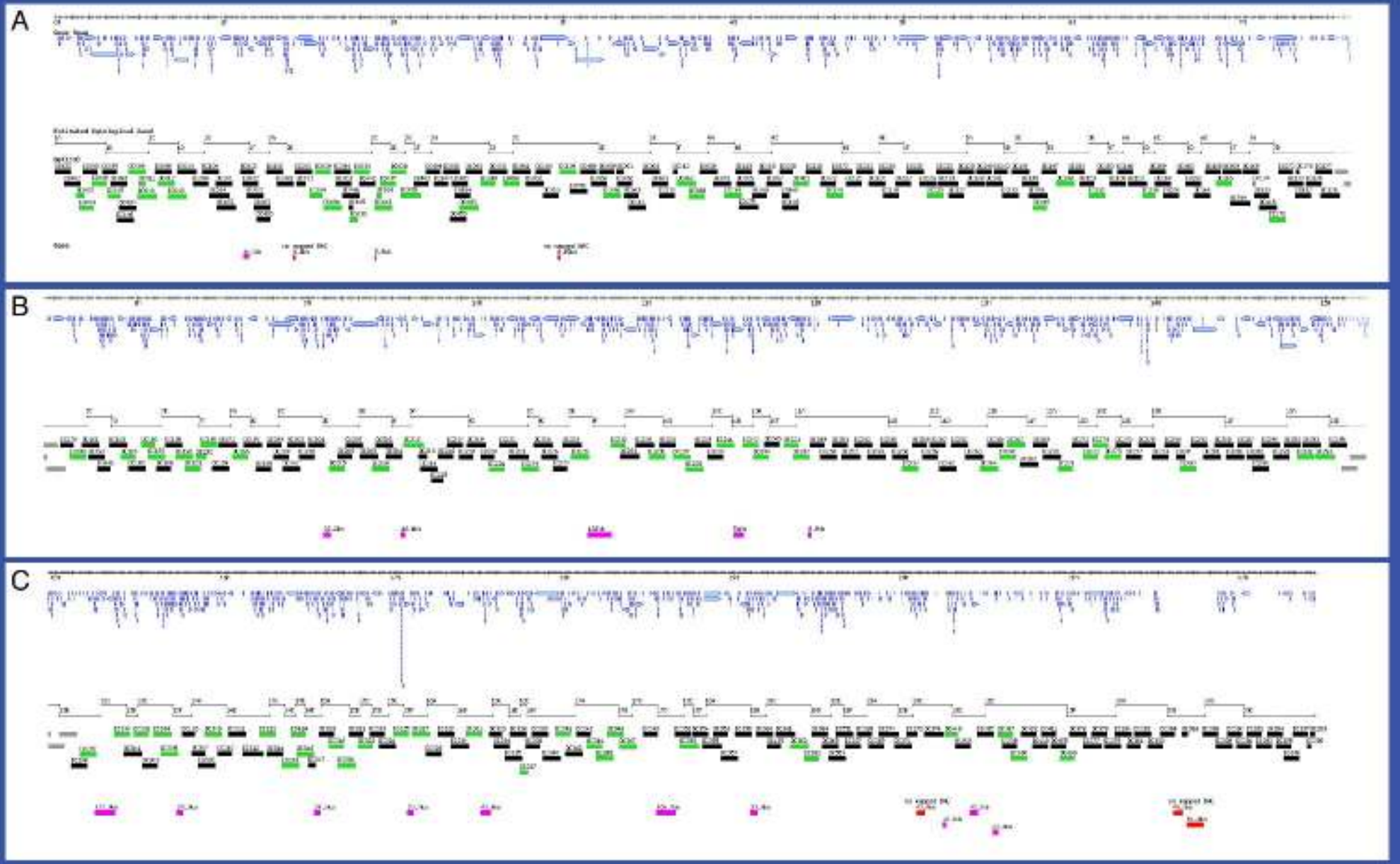
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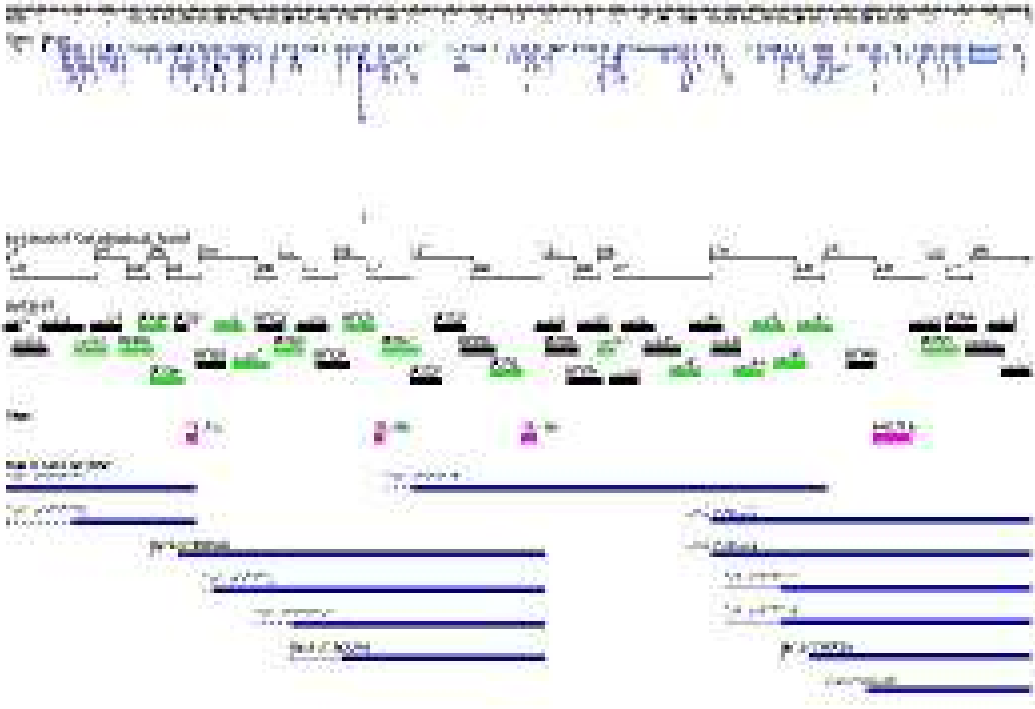

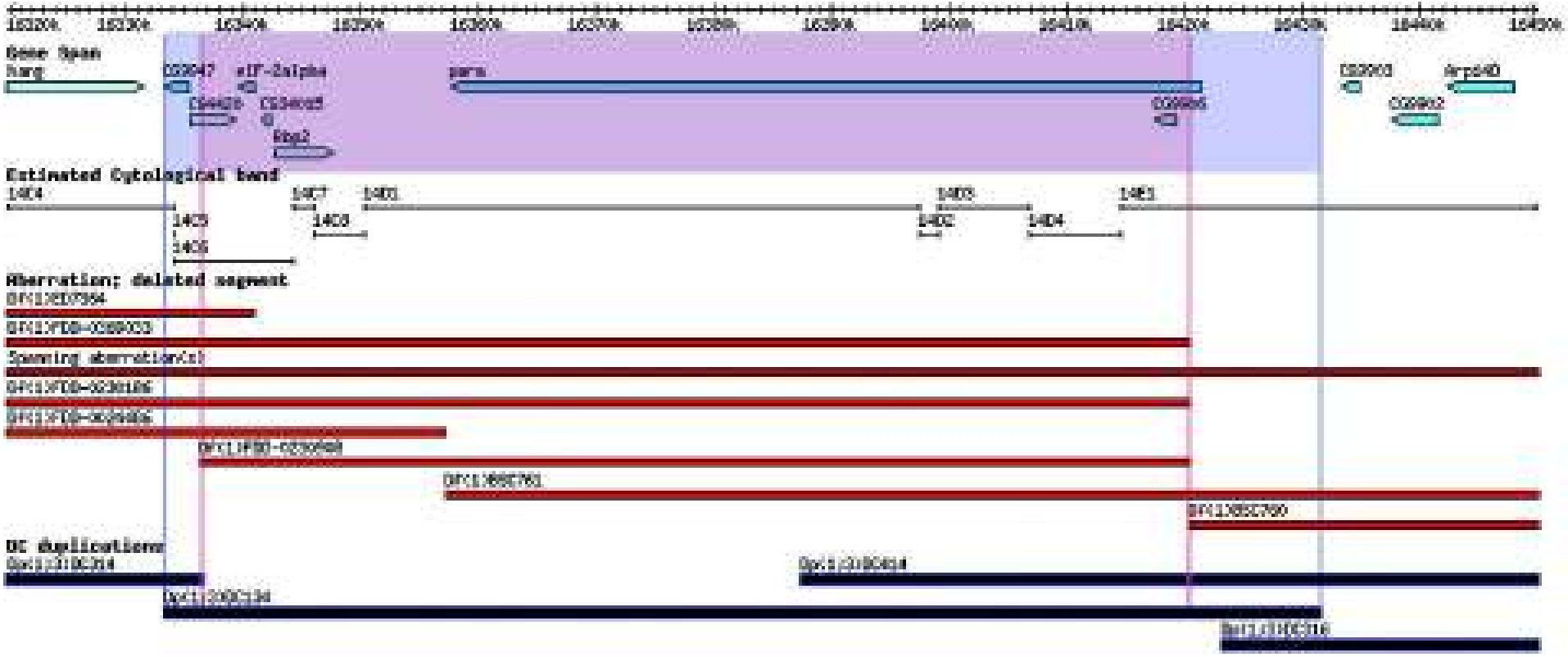
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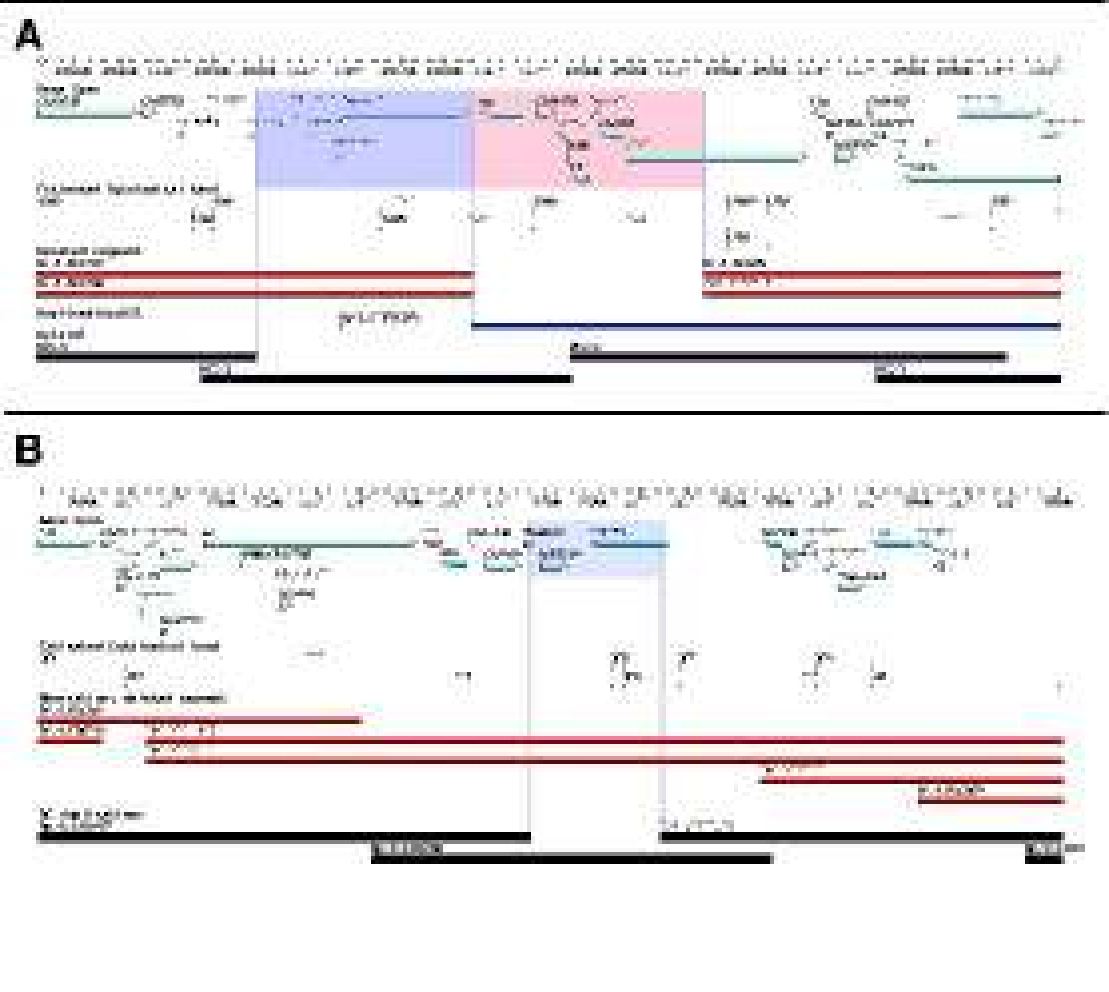
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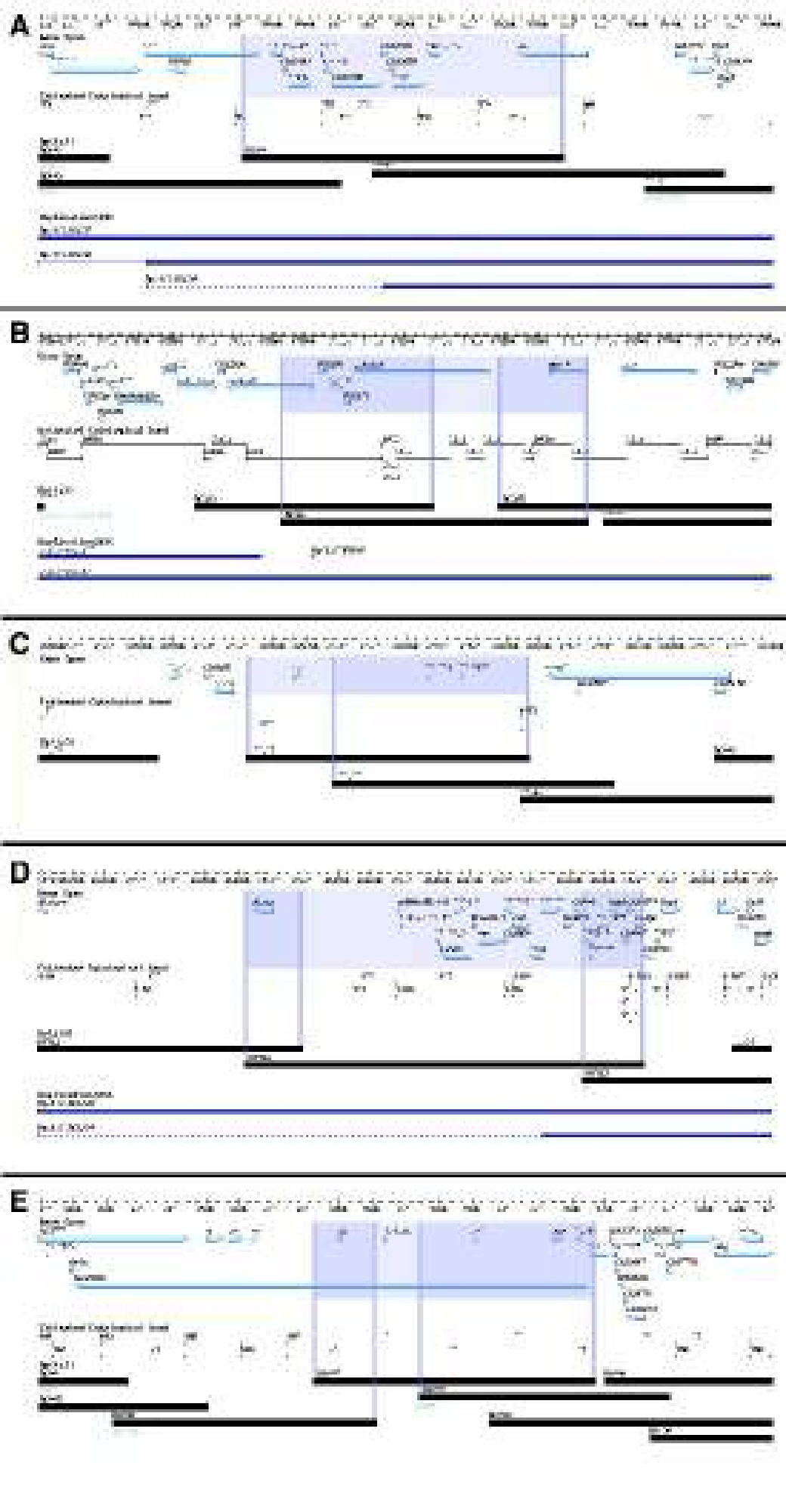
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