METHODS

BAC library construction

Two large-scale ligation/ transformation experiments were carried out using *E. coli DH10B* cells (Invitrogen) for electroporation (Osoegawa et al. 1999). Over 90,000 recombinant clones were produced in nine 12.5 aliquots. A sample of clones from each ligation experiment was spread on Petri dishes and their insert size estimated by PFGE of *NotI* digested DNA. Given the somewhat larger insert size of the clones derived from the first large-scale ligation experiment, cells from the first five aliquots were spread on large trays with LB agar medium and colonies picked and arrayed in 48 384-well plates using an automatic colony-picking robot. This library segment (CHORI-225), containing 18,353 clones, is the one used here for map construction. The remaining four aliquots, derived from the second large-scale ligation experiment and containing an estimated number of over 65,000 clones, were stored as an additional resource.

Screening the BAC library with gene clones

Gridded filters containing 18,353 clones spotted in duplicate were produced using an automatic colony gridding robot (*Bio*Grid, *Bio*Robotics). Two of these filters were used to screen the library with six euchromatic gene probes. The first filter was hybridized with a pool of three plasmid clones corresponding to two fragments of *D. buzzatii* gene *labial* (*lab-5'* and *lab-3'*) (Negre et al. 2003) and a cDNA clone of gene *proboscipedia* (*pb*) (B. Negre, pers. comm.). The second filter was hybridized with a pool of three 40-bp overlaping oligonucleotide (overgo) probes (McPherson 1999): two overgo probes were designed from the sequences of *D. buzzatii α-Esterase 3* (*α-Est3*) and *α-Esterase 10* (*α-Est10*) (Robin et al. 2000) and the third one from exon 8 of the *D. virilis abdominal-A* gene *(abd-A)* (kindly provided by Barret Pfeiffer, Lawrence Berkeley

National Laboratory). Plasmid probes were labeled by incorporation of P^{32} dCTP by random primer. Filter hybridization, washing and visualization of positive clones were carried out using standard protocols (see http://bacpac.chori.org/highdensity.htm). Positive clones were analyzed by southern blot with individual probes. This procedure was used to confirm positive clones and distinguish which clones were hybridizing with each probe (Supplemental Table S3). Out of 48 clones recovered from the first filter, only 29 could be confirmed as true positives whereas all 72 clones recovered from the second filter were corroborated as true positives. The average number of positive clones per probe was 23 (SD = 9.5). This value must be taken with caution because of the small number of (non-independent) hybridizations carried out. However, it points to a high genome representation in the arrayed *D. buzzatii* library.

Fingerprinting and contig assembly

BAC clones from plates 1-26 of CHORI-225 were fingerprinted using an agarose gel-based methodology (Marra et al. 1997, Schein et al. 2004) and the restriction enzyme *EcoRI*. All successful fingerprints (9,555) were imported into FPC (Soderlund et al. 1997, 2000, Ness et al. 2002) for assembly and further analysis. Default FPC assembly parameters were used with the exception of the cutoff value for Sulston score (Sulston et al. 1988), which was set at 10^{-11} in order to avoid false-positive clone overlaps and thus minimize the assembly of contigs containing clones from more than one genomic region. This resulted in a conservative assembly consisting of many contigs, each containing highly related clones. The DQ function of FPC was used to subject contigs containing a large proportion of Q (questionable) clones to a higher-stringency assembly. Clone order within contigs was refined using Coral software (Flibotte et al. 2004). Fingerprint comparisons using only clones at the ends of contigs which had been previously *in situ* localized to the chromosomes of *D. buzzatii* were performed using a

higher (less-stringent) cutoff score of 10^{-5}. This allowed identification of singleton clones that extended contigs as well as potential contig merges. Each potential merge was manually reviewed in order to ensure that merges were only made if the fingerprint data were consistent and supported the join. Joins were incorporated into the map if the fingerprinting data was logically consistent with the cytological data.

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