A Yeast Artificial Chromosome Clone Map of the Drosophila Genome

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ABSTRACT

We describe the mapping of 979 randomly selected large yeast artificial chromosome (YAC) clones of Drosophila DNA by *in situ* hybridization to polytene chromosomes. Eight hundred and fifty-five of the clones are euchromatic and have primary hybridization sites in the banded portions of the polytene chromosomes, whereas 124 are heterochromatic and label the chromocenter. The average euchromatic clone contains about 211 kb and, at its primary site, labels eight or nine contiguous polytene bands. Thus, the extent as well as chromosomal position of each clone has been determined. By direct band counts, we estimate our clones provide about 76% coverage of the euchromatin of the major autosomes, and 63% coverage of the *X*. When previously reported YAC mapping data are combined with ours, euchromatic coverage is extended to about 90% for the autosomes and 82% for the *X*. The distribution of gap sizes in our map and the coverage achieved are in good agreement with expectations based on the assumption of random coverage, indicating that euchromatic clones are essentially randomly distributed. However, certain gaps in coverage, including the entire fourth chromosome euchromatin, may be significant. Heterochromatic sequences are underrepresented among the YAC clones by two to three fold. This may result, at least in part, from underrepresentation of heterochromatic sequences in adult DNA (the source of most of the clones analyzed), or from clone instability.

THE yeast artificial chromosome (YAC) cloning technique (BURKE *et al.* 1987) allows the cloning and faithful propagation in yeast cells of fragments of exogenous DNA hundreds of kilobases in length. In this report, we apply this cloning technology to the mapping of the Drosophila genome. Our approach has been a very simple one: random large YAC clones of Drosophila DNA are mapped by *in situ* hybridization to polytene chromosomes. Since the average YAC clone mapped contains about 211 kb and the average polytene chromosome band contains about 22 kb per chromatid, most YAC clones label eight or nine contiguous bands, or approximately a lettered unit on BRIDGES (1935) map [see also LEFEVRE (1976) and SORSA (1988)]. Thus, the cytological extent as well as chromosomal position of each clone can be determined by *in situ* hybridization. With the use of biotinylated probes, the resolution of this method is remarkably good. In finely banded regions, resolution likely can be within 5–10 kb (SPIERER *et al.* 1983).

A major advantage of our cytological approach is that continuity of the clone map is provided at all stages by the polytene chromosomes themselves. Thus, at least for a crude map, clones need only be mapped by their site of *in situ* hybridization, and do not need to be mapped relative to one another and placed into contigs. In addition, the cytological mapping of clones is little affected by the presence of repeats; with few exceptions, the primary site of hybridization is unambiguous even when repetitive sequences are present. Indeed, the method can provide substantial information about the repeats themselves. A major drawback is that the method is useful only for mapping euchromatic clones and provides little information about the approximately 25% of the genome composed of heterochromatin. This material either does not replicate in polytene cells (α-heterochromatin) or replicates but has a diffuse poorly banded morphology (β-heterochromatin) (GALL *et al.* 1971) [for reviews see SPRADLING and RUBIN (1981) and ASHBURNER (1989)]. Although many heterochromatic clones are identified in our study by hybridization to the chromocenter, their relative chromosomal locations have not been determined.

In this report, we present the *in situ* hybridization pattern of 979 Drosophila YAC clones. For 855 of these, the primary site of hybridization is euchromatic, whereas for 124, the primary site is the chromocenter. The euchromatic clones appear to be essentially randomly distributed, and, as estimated by direct band counts, provide about 76% coverage of the autosomes and 63% coverage of the *X*. When combined with the YAC clones reported by AJIOKA *et al.* (1991), these should extend coverage of the autosomal euchromatin to about 90% and the *X* chromosome euchromatin to about 82%.

MATERIALS AND METHODS

Construction of Drosophila YAC clones: Genomic DNA was prepared from a Canton-S strain isogenic for chromosomes *2* and *3* as described (BINGHAM *et al.* 1981). Adult flies (mixed males and females) were collected, flash frozen in

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liquid nitrogen and ground to a fine powder. The frozen powder was then homogenized in cold nuclear isolation buffer (BINGHAM *et al.* 1981) using a Dounce B pestle. Bulk fly parts were removed by brief centrifugation and discarded. The suspension of nuclei and other cellular debris was washed 1–2 times in cold nuclear isolation buffer and then lysed in 2% Sarkosyl. CsCl was gently dissolved to 1 g/ml in the lysate. The mixture was then centrifuged at 45,000 rpm in a Vti65 vertical rotor for 16 hr at 25°. DNA was collected from the CsCl gradient, dialyzed extensively against 1 × TE (10 mM Tris, 1 mM EDTA, pH 8.0), and fractions were checked for nuclease contamination by incubating with 1 × *Eco*RI buffer at 37° for 20 min followed by field inversion gel electrophoresis (see below). Fractions showing significant degradation of high molecular weight DNA after incubation in *Eco*RI buffer were discarded. Fractions devoid of nuclease activity were then pooled and aliquots containing 2–5 μg DNA were used in test *Eco*RI partial digestions. Test digestions were done for 10 min at 37° with *Eco*RI concentrations ranging from 0.5 to 20 units/ml. Digestion conditions were identified that lowered the average size of DNA by 200 kb or more depending on the quality of the input DNA. Partial digestions were scaled up to 200–500 μg DNA and were stopped by adding EDTA to 50 mM and placing the reaction on ice. Digestion products were then size fractionated over 5–12.5% sucrose velocity gradients and fractions containing DNA greater than 200 kb were pooled, concentrated and dialyzed in UH100 collodion bags (Schleicher & Schuell). The vector pYAC4 (BURKE *et al.* 1987) was digested to completion with *Eco*RI and *Bam*HI and treated with calf intestinal alkaline phosphatase (Boehringer). After treatment at 75° for 10 min to inactivate phosphatase, followed by phenol extraction, vector DNA was added to partially digested high molecular weight Drosophila DNA at a 3:1 weight ratio and ligated for 1–2 hr at 25°. Ligation reactions were used directly to transform yeast strain AB1380 (BURKE *et al.* 1987) essentially as described by BURGERS and PERCIVAL (1987). Transformants were selected on –Ura plates containing 1 M sorbitol (Fisher Biotech.). Both top and bottom agars were 2.5% agar.

Transformant selection: All YAC-containing cells were grown on YCD medium, which lacks uracil and tryptophan. The recipe is modified from the AHC$^-$ medium of BROWNSTEIN *et al.* (1989) and is as follows: for 1 liter, 20 g glucose, 6.7 g Bacto yeast nitrogen base without amino acids (Difco), 10 g casein hydrolysate (Sigma), 20 mg adenine, 50 mg lysine, 20 mg histidine, 40 mg arginine, 60 mg isoleucine, 60 mg leucine, 20 mg methionine, 50 mg phenylalanine, 200 mg threonine, and 50 mg tyrosine. Primary transformants were picked onto YCD/2% agar plates and grown for 2–3 days at 30°. Patches showing strong red growth were harvested into 96-well microtiter plates containing 15% glycerol in water and stored at -80°. A Replaclone 96-prong transfer device (L.A.O. Enterprises) was used to replicate cultures from these master plates into 1-ml YCD cultures in micro-test tubes in racks of 96 (Bio-Rad 223–9395). Cultures were grown at 30° with vibration (Bellco mini-orbital shaker, setting 4). These 1-ml cultures were used for initial size screening.

Initial sizing: When the 1-ml cultures had reached 1–3 × 10^8 cells/ml, agarose plugs of the YAC clones were prepared as described by CARLE and OLSON (1984). Cells were pelleted gently and incubated 1 hr at 37° in 0.05 ml of 1 mg/ml zymolyase (100T, ICN) in SCE (1 M sorbitol, 0.1 M citric acid, 10 mM EDTA, pH 5.8) plus 25 mM β-mercaptoethanol to form spheroplasts. The suspension was then mixed with an equal volume of 1.2% molten low melting temperature agarose and poured into plug molds (Pharmacia LKB Biotechnology). Plugs were digested in 1.5 ml 1 mg/ml proteinase K (Boehringer) in 1% Sarkosyl, 0.45 M EDTA, 10 mM Tris·HCl, pH 9.0, for 24–48 hr at 55°. Digested plugs were equilibrated in 0.5 × TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) by multiple buffer changes for 6–8 hr followed by field inversion gel electrophoresis (FIGE) (CARLE *et al.* 1986) using a Bio-Rad Pulsewave 760 switching module. One percent agarose gels were run at 10–15° at a constant voltage of 250 V. Throughout electrophoresis, the 0.5 × TBE buffer was continuously recirculated and cooled through a closed system similar to that described by CARLE and OLSON (1984). To insure temperature uniformity, electrophoresis tanks were kept in Styrofoam chests during electrophoresis runs. For the first 12 hr, a pulse ramp of 3–30 sec forward time and a constant pulse ratio of 3:1 was used. To increase separation in the 50–200-kb range, a second ramp of 3–60 sec forward time with changing pulse ratio of 3–12:1 followed for 6 hr. DNA was visualized by ethidium bromide staining/deionized water destaining after electrophoresis.

Verification sizing and DNA purification: Master plate cultures showing clones of 180 kb or larger were streaked to single colonies on YCD plates. A single colony was then grown in 5 ml YCD on a roller drum for 3 days at 30°. One milliter of this culture was embedded in agarose, processed as above and subjected to FIGE as for the initial sizing (except the two ramps were each for 8 hr). Agarose gel bands containing YAC clones were excised, and the DNA was purified using the sodium iodide-glass powder method (Geneclean, Bio 101) (VOGELSTEIN and GILLESPIE 1979). For long term maintenance, the settled pellet from the remaining 4 mls of culture was resuspended in 1.0 ml 15% glycerol in water and stored at -80° in cryovials (Nalge 5000–0020).

Polytene chromosome squashes: Polytene chromosome squashes were prepared essentially as described in a protocol generously provided by JOHNG LIM. Slides were cleaned in Nochromix (Godax Laboratory)/sulfuric acid according to manufacturer's instructions, rinsed well in deionized water, and air dried. Slides were then subbed by dipping in a solution of 0.1% gelatin/0.01% chrome alum in water, followed by air drying. Most slides were further treated by incubating in 3 × SSC/1 × Denhardt's solution (MANIATIS *et al.* 1982) for 2–3 hr at 65°. Slides were then rinsed in deionized water, dipped in 3:1 ethanol/acetic acid and dried. Coverslips were siliconized under vacuum in a desiccator using dimethyldichlorosilane as described by MANIATIS *et al.* (1982). Slides and coverslips were kept as dust-free as possible during processing. Climbing third instar larvae from uncrowded cultures were collected, washed in water and dissected in a drop of 45% acetic acid on a siliconized slide. Two or three glands were transferred to a drop of 1:2:3 solution (lactic acid:water:acetic acid) on a subbed slide, covered with a siliconized coverslip and squashed by tapping the coverslip 10–20 times with blunt forceps. Excess 1:2:3 solution was then blotted off the slide, the edge of the coverslip held with a folded Kimwipe and the tip of blunt forceps dragged over the coverslip in a serpentine pattern. Most slides were then flattened in a custom slide press fabricated from a small arbor press by the Washington University Biology machine shop. Slides were then left at 4° for 3–4 hr to promote flattening, followed by freezing on dry ice, flipping off the coverslip with a razor blade and dehydration in 95% ethanol. Finally, slides were air dried and examined by phase contrast microscopy. Only slides with well flattened, nonrefractile, chromosomes were chosen for *in situ* hybridization.

Biotinylation and *in situ* hybridization: DNA was labeled with biotin-dCTP (ENZO Biochem Inc.) by the random hexamer labeling method (FEINBERG and VOGELSTEIN 1983) for 24–48 hr at 25°. *In situ* hybridization to polytene chromosome squashes was carried out as described in LANGER-SAFER *et al.* (1982) using streptavidin-horseradish peroxidase (Detek-kit,

ENZO Biochem Inc.) and diaminobenzidine (DAB) (Sigma) to visualize the hybridization. For most slides, hybridization signal was intensified using 0.08% $NiCl_2$. All hybridizations were to polytene chromosomes from a Canton-S strain isogenic for chromosomes *2* and *3*.

Error minimization: During pilot experiments, it became apparent that errors could be a significant problem. Since most errors in these experiments occurred during transfers of cultures or solutions to new tubes, our procedures were revised so as to minimize transfers and consequent tube relabelings. Use of a centrifuge that accommodates 96-tube boxes eliminated one particularly error-prone tube transfer. An additional important change was to keep all tubes involved in processing each clone until completion of the *in situ* slides, so that the slides and tubes could be checked against one another. These changes appear to have helped, since no errors were detected among the approximately 5% of clones for which in situs were repeated.

Other Drosophila YAC clones used in mapping: Clones designated with the prefix R or Rx were produced as described above using DNA from our strain of Canton-S. Clones designated Rt were derived by the same method from a *T(Y;2)CB25, cn/y;cn bw* strain (provided by TERRY LYTTLE). Clones denoted DY are from GARZA *et al.* (1989) and were made from random-sheared Oregon RC adult DNA using the pYACP-1 vector. Clones designated with the prefix N were constructed by ANDREW LINK from *Not*I fragments of Oregon RC embryo DNA as described by DANILEVSKAYA *et al.* (1991).

RESULTS

Construction of YAC libraries: Production of large YAC clones of Drosophila DNA has proven to be rather difficult, apparently because of high nuclease levels. After a number of attempts, we succeeded in producing two sets of clones, designated DYR and DYRx. The DYR library is the major source of clones used in our mapping, and consists of nearly 1,000 clones of average insert size about 200 kb. The DYRx library, although large (6,000 clones), has an average insert size of only about 140 kb and contributed relatively few clones to the mapping. Also mapped were a few clones (designated DYRt) constructed from DNA from males of a *T(Y;2)CB25, cn/y;cn bw* stock. In an attempt to circumvent nuclease contamination, we also tried (without success) to prepare YAC clones from the double nuclease mutant *DNase-2^{n1} DNase-1^{lo}* (GRELL 1976).

Euchromatic clones: The primary sites of hybridization of 855 euchromatic clones are listed in the APPENDIX (see Table 2). The average size of these clones is 211 kb. The appearance of a typical *in situ* hybridization is shown in Figure 1a. Although many of the clones contain repetitive sequences, and label numerous sites, the primary site of hybridization is almost always clear by its extent and strength of staining (see Figure 1b). It is difficult to convey using black and white photographs just how easily the primary site can usually be recognized. In our preparations, the primary site is almost always jet black, whereas secondary sites have a grayish or purplish cast. Of the 855 euchromatic clones, at least 39 are chimeric and show two (34 clones) or three (five clones) primary sites of hybridization. Additional chimeric clones are likely present, since chimeras in which one fragment predominates in size or in which heterochromatic fragments are present would be difficult to detect. In assigning band numbers, the maps of SORSA (1988) were used. These differ from the commonly used maps of LEFEVRE (1976) at several locations, including 18C, 28A, 39A, 43DE, 72C, 74CD, 78D-79A, 97A and 99A.

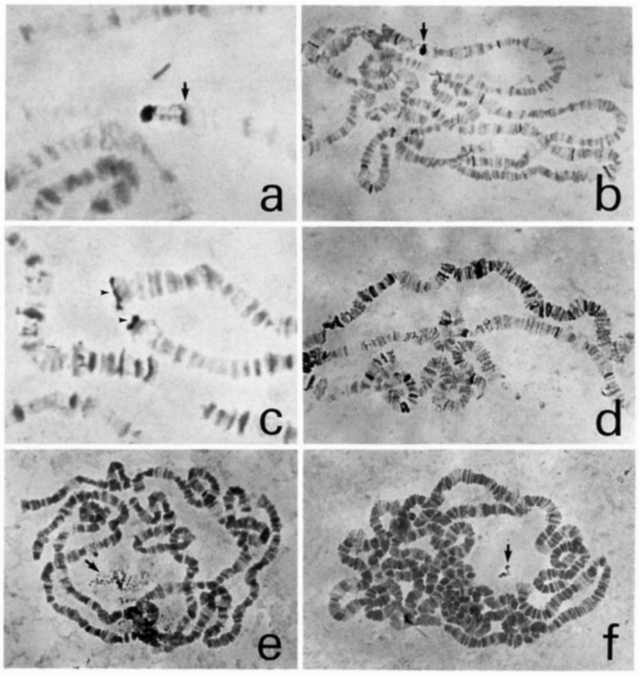

FIGURE 1.—*In situ* hybridizations to polytene chromosomes. The reproducibility of the following hybridization patterns has not been tested. (a) Typical appearance of an *in situ* hybridization signal. DY609 (260 kb) is shown hybridized to its locus at 28C1-2; D3-4. Note that the large heavy band indicated by the arrow (actually a cluster of five or six bands including 28B and 28C1, 2) is only partially labeled. The importance of examining well stretched chromosomes is illustrated here, since in unstretched chromosomes DY609 often appears to label this entire complex of bands. (b) *In situ* hybridization of a clone (DY902) containing dispersed repeats. Note that the primary site of hybridization (42C3-5; D3) (see arrow) is easily distinguished from dispersed repeats by the strength of hybridization. Confirmation is provided by well stretched chromosomes, which show a continuous block of labeling at the primary site. (c) *In situ* hybridization of DY628, one of five clones that labels all telomeres. Shown is hybridization signal at the tips of *3*R (top) and *2*R (bottom) (see arrowheads). All such clones also label the chromocenter and dispersed euchromatic repeats. DY628 is likely derived from heterochromatin, since it shows no major euchromatic site of hybridization. (d) A clone (DYN17-71) containing a repeat or repeats enriched on the *X* chromosome. The *X* (at top) shows substantially more label than the autosomal arms, including *2*L (in the middle) and *3*R (at the bottom). Although the primary site of this clone is on the *X* (at 11D1; D10-E1), similar labeling was also seen with certain autosomal clones. (e) Labeling of the nucleolus (arrow) by DYN15-24. Eleven other clones show similar speckled labeling of the nucleolus. (f) Labeling of an island within the nucleolus by DYN27-08. This labeling pattern is unique among our clones, and is not seen in all nuclei. DYN27-08 also labels the base of the *X* at 20AB.

TABLE 1

Clone coverage by chromosome arm

	Chromosome arm					
	X	2L	2R	3L	3R	*4*
Total bands:[a]	1,120	927	1,152	1,073	1,233	50
Bands *not* covered:	414	252	262	269	258	50
Percent coverage by band:	63	72.8	77.3	74.9	79.1	0
Sites mapped/arm:	147	174	163	191	224	0
Sum of clone sizes (kb):[b]	28,960	36,180	32,090	37,930	45,340	0

[a] Taken from SORSA (1988). These numbers assume all BRIDGE's doublets are real.
[b] Calculated assuming chimeric clones split equally among different sites.

Using the SORSA map, we estimated by direct counts the number of bands in each chromosome arm covered by our clones. These counts and the percent of bands included in clones for each arm are summarized in Table 1. We find that coverage of the euchromatin of the autosomal arms is, on average, about 76%, whereas coverage of the *X* chromosome is about 63%. Reduced coverage of the *X* results from a lower number of *X* chromosomal clones: there are only 147 primary sites mapped on the *X*, as compared to an average of 188 for the autosomal arms. A deficit of *X* chromosomal clones is to be expected because the DNA used for cloning was from a mixed population of males and females, in which *X* chromosomal DNA is present at only 75% the level of autosomal DNA (AJIOKA *et al.* 1991). Quite unexpected, however, is the complete absence of clones from chromosome *4*, which constitutes the largest gap in coverage (see below). Also unexpected is an apparent deficit in sites mapped in the right arm of chromosome *2*. *2*R is second only to *3*R in band count and estimated DNA content (SORSA 1988), and yet has the fewest sites mapped of any major autosomal arm. We have no ready explanation for this deficit, which is not seen in the YAC mapping data of AJIOKA *et al.* (1991).

Based on our observed band coverage of 63% for the *X*, and 76% for the major autosomes, the Poisson distribution can be used to calculate that our clones should contain 0.99 equivalents of *X* chromosome euchromatic DNA and 1.44 equivalents of autosomal euchromatic DNA, assuming random coverage. These estimates of DNA content agree reasonably well with physical measurements. The generally accepted figure for the size of the Drosophila genome is 1.65×10^5 kb (RASCH *et al.* 1971). From 25 to 30% of this is heterochromatic DNA and is not present in the banded regions of polytene chromosomes [see SPRADLING and RUBIN (1981) for review]. Of the remaining DNA, about a fifth (SORSA 1988), or $0.23–0.25 \times 10^5$ kb, is located in the *X* euchromatin, and four fifths, or $0.92–0.99 \times 10^5$ kb, in autosomal euchromatin. Our clones contain about 28,960 kb of *X* euchromatic DNA, and 151,530 kb of autosomal euchromatic DNA (assuming chimeric clones are equally subdivided), corresponding to 1.16–1.26 equivalents of *X* euchromatic DNA, and 1.5–1.65 equivalents of autosomal euchromatic DNA. The reasonably good agreement between *X* and autosomal equivalents calculated by the Poisson distribution and by physical measurement indicates that the mapped YAC clones are essentially randomly distributed. The slightly higher DNA equivalent values estimated by physical measurements than by the Poisson distribution may indicate that the Drosophila genome is slightly larger, or the fraction of heterochromatic sequences slightly lower, than is commonly thought. Alternatively, coverage may not be entirely random.

The size distribution (in band numbers) of gaps in our map of the major autosomal arms is summarized in Figure 2. These gaps were estimated by assuming clone limits lay at the midpoint of the band uncertainties listed in the APPENDIX. Most of the gaps are relatively small, and could be spanned by cosmids, P1 clones (STERNBERG 1992), or small YACs. Also shown in Figure 2 are the numbers of gaps expected based on the assumption of random clone placement. These expectations were calculated using the exponential distribution, as described in the legend to Figure 2. There is reasonably good agreement between observed and expected values ($\chi^2 = 12.38$, degrees of freedom = 8, $p \cong 0.14$), with the exception that we see fewer very small (1 or 2 band) gaps than expected. This disparity is not surprising, however, because many small gaps lying within the uncertainties of adjacent YACs would be missed. The good agreement between gaps observed and gaps expected according to the exponential distribution provides additional evidence that the YACs are essentially randomly distributed, at least on the major autosomes.

There are four exceptionally large (>33 band) gaps in our coverage: 6B3-4; 6F5-7 (43 bands), 16A1-2; 16F1 (42 bands), 31B1;32A1 (47 bands), and all of the euchromatin of chromosome 4 (50 bands). The gap in section 31 is likely due to chance, since AJIOKA *et al.* (1991) report four YAC clones from within this region. However, the remaining gaps may be significant, as they are also seen in the data of AJIOKA *et al.*; these authors found no clones covering 6C1-2; 7A and 16A1-2; 16F, and observed an underrepresentation of clones from the fourth chromosome. One explanation could be that DNA from these regions is not clonable in YACs. Alter-

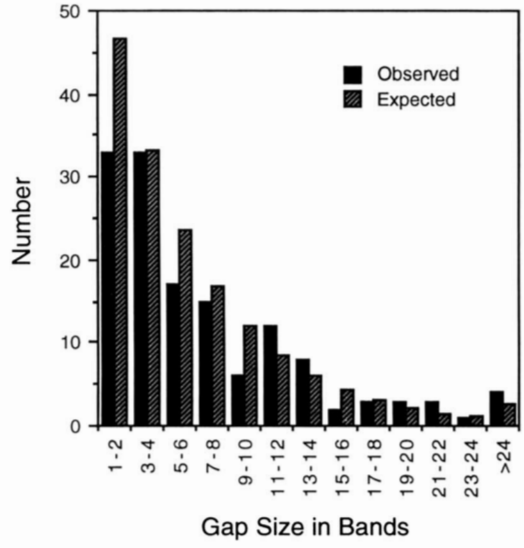

FIGURE 2.—Distribution of gaps in coverage of the major autosomes. Black columns represent the observed numbers of gaps of the indicated sizes. Hatched columns represent the expected numbers calculated according to the exponential distribution. Expected values were calculated using the following parameters: total number of autosomal bands = 4385; number sites mapped on the autosomes = 752; average coverage at each site = 201.5 kb; average size of band = 22.5 kb. Note that, because chimeric clones have been taken into account, the number of sites mapped exceeds the number of clones mapped, and the average coverage at each site is less than the average clone size. To calculate the expected distribution, each mapped site was initially treated as a point and the distribution of gaps calculated by evaluating the exponential distribution integral $-e^{-0.171x}$ (where 0.171 is the number of sites mapped per band). Since the average coverage at each mapped site was about nine bands (201.5/22.5 = 8.96), gaps in the distribution so derived would be expected to be shortened by this same distance. Accordingly, the distribution was truncated at nine bands to produce the expected distribution shown. The goodness of fit of the observed gap distribution to the expected distribution was tested by χ^2. Classes in the tail of the distribution were pooled in this test so that the expected number in each group always exceeded five. The P value obtained ($\cong$0.14) indicates the observed distribution is not significantly different from expectation. Note we have not attempted to incorporate variability in clone size or band size into our calculations. However, doing so could only improve the fit of the observed results to expectation.

natively, the presence of many dispersed repetitive sequences in these regions could obscure the primary site of hybridization. If repeats abundant in the chromocenter were present at these sites, clones from them could have been misclassified as heterochromatic. The possibility of such misclassification is very real for the euchromatin of chromosome *4*, which is strongly enriched in repeats (MIKLOS *et al.* 1988). Misclassification is most likely to occur with smaller (<150 kb) clones, since the primary sites of large clones are normally quite unambiguous, even when many dispersed repeats are labeled. Indeed, a few smaller clones initially classified as heterochromatic were found, upon reexamination, to be euchromatic clones containing repeats abundant in the chromocenter. Finally, it is possible that the large size in terms of band counts of the four gaps above is misleading; these regions may be no larger physically than many other gaps in the map. This would appear to be the case at least for the section 31 gap. AJIOKA *et al.* (1991) report that a single clone of 220 kb covers most of four lettered divisions in this finely banded region.

About 38% of the mapped euchromatic YACs clearly label dispersed middle repetitive sequences in addition to the primary site. If such sequences were randomly distributed, then based on their abundance in the genome [see SPRADLING and RUBIN (1981) and RUBIN (1983) for reviews), one would expect a far higher percentage of clones to contain repeats. Indeed, 38% is almost certainly an underestimate, since the labeling of repeats in our *in situs* is highly dependent on hybridization conditions. The same clone can show no trace of mobile elements in one slide, and strong labeling of such elements in another. A number of our slides show weak *X*-specific labeling in addition to the primary site. Hybridization sites are clustered in numbered sections 1–14, and resemble the distribution of a repetitive sequence family described by WARING and POLLACK (1987) (see Figure 1d). About 28% of all euchromatic YACs, and 66% of those showing dispersed repeats, label the chromocenter. Presumably such clones hybridize to mobile elements present in heterochromatin. About 3% of the clones in Table 1 labeled the chromocenter in addition to a euchromatic primary site, but did not label dispersed repeats. Very likely, dispersed repeats are present in most or all of these clones, but went undetected in euchromatin because the *in situ* signal was too weak. AJIOKA *et al.* (1991) report a very low percentage of euchromatic YACs labeling dispersed repeats (6%) and the chromocenter (7%). This difference with our data is not understood, but may result from weak labeling of their slides, or incomplete data collection.

Heterochromatic clones: Of the 979 YAC clones examined by *in situ* hybridization, 124, or about 13%, were classified as heterochromatic since they labeled the chromocenter and had no obvious euchromatic primary site. As described above, these assignments are probably not totally accurate, since euchromatic clones very rich in repeats could be misclassified as heterochromatic. The chromocentric staining patterns for heterochromatic clones are remarkably diverse, and include strong, almost uniform labeling; granular staining; weak, fibrous staining; staining of jumbled bands; and staining of dots that vary widely in size according to clone. All label β-heterochromatin. Since most *in situ* hybridizations were done only once, for only a few clones have these patterns been shown to be reproducible. Many heterochromatic clones show weak labeling along the entire fourth chromosome, in addition to strong labeling of β-heterochromatin, a pattern described previously by MIKLOS *et al.* (1988). One hundred and four of

the heterochromatic clones labeled dispersed repetitive sequences in euchromatin in addition to the chromocenter. This is probably an underestimate, since *in situ* labeling was so weak for ten of the remaining clones that euchromatic repeats would not have been seen if present. Ten of the heterochromatic clones show stippled labeling of the nucleolus (see Figure 1e). Two clones with euchromatic primary sites (N11-66, N27-12) also label the nucleolus, perhaps because they are chimeric, or contain repeats also present in the nucleolus organizer. Clone N27-08, whose primary site includes the most proximal portion of the *X* euchromatin (20A3-5;B1–3) labels an island within the nucleolus (see Figure 1f).

The fraction of YAC clones that is heterochromatic is almost certainly less than the 13% estimated above, since the DY and DYN libraries are badly contaminated with multiple isolations of identical clones. Duplicate clones of euchromatic DNA are easily identified by their identical *in situ* localizations and extents, and have been removed from the data in the APPENDIX. Synonymous clones of heterochromatic DNA are not so easily identified, and many duplicates or triplicates of DY and DYN clones are likely present among our heterochromatic clones. Based on the frequency of reisolations of euchromatic clones (duplicates comprised about 25% of the DY and 10% of the DYN clones mapped), it is probable that only about 11% of unique YAC clones are heterochromatic.

Four of the clones classified as heterochromatic label all telomeres (see Figure 1c) as well as the chromocenter and dispersed euchromatic repeats. These clones presumably contain He-T sequences (RUBIN 1977; YOUNG *et al.* 1983; RENKAWITZ-POHL and BIALOJAN 1984; TRAVERSE and PARDUE 1989; BEISSMANN *et al.* 1990), which are located at all telomeres and in the pericentric heterochromatin, as well as one or more mobile elements present in euchromatin. Clone R19-83, whose primary site (1A1; B2-3) is at the tip of the *X*, also labels all telomeres, the chromocenter, and euchromatic repeats.

Finally, in addition to the 979 clones described above, we have identified a number that, after repeated attempts, do not label polytene nuclei. These clones could contain sequences that are severely underreplicated, or perhaps deleted (KARPEN and SPRADLING 1990; GLASER *et al.* 1992), during polytenization. Alternatively, these clones may contain DNA from some source other than Drosophila.

Clone stability: The large majority of Drosophila YAC clones appear completely stable. However, a few instances of instability have been seen. Many of our initial cultures, picked directly from the transformation plates, were mixed, and showed more than one clone on sizing gels. By streaking to single colonies, almost all of these cultures could be resolved into separate clones, which were given lettered designations. In most cases, separated clones labeled completely different sites and appear unrelated. In nine cases, however, subclones of different size labeled the same site by *in situ*. Presumably, these have undergone some type of rearrangement in yeast. In two of these cases, smaller derivatives labeled a visibly shorter region, and appear to have arisen by terminal deletion. In our entire analysis, we found only one clone (Rt05-24A; primary site 13A5-6 + 13B4; C4-7) that showed a gap in a primary hybridization site, and that may, therefore, have undergone a large internal deletion. Initial instability of heterochromatic clones would have been rather difficult to detect in our work, since rearranged heterochromatic clones would probably show the same chromocentral labeling as their progenitors. Nonetheless, we find no evidence of size instability upon subculturing of heterochromatic clones. The frequency of apparent rearrangement of YAC clones seen here (about 1%) is comparable to that seen by others working on Drosophila and other systems (VILAGELIU and TYLER-SMITH 1992).

DISCUSSION

We report the mapping of 979 randomly selected large YAC clones by *in situ* hybridization to polytene chromosomes. Of these, 855 clones of average size 211 kb map to euchromatic sites. By direct band counts, we estimate that these clones provide about 76% coverage of the euchromatin of the major autosomes, and 63% coverage of the *X* euchromatin. Two lines of evidence indicate that the euchromatic clones are essentially randomly distributed. First, coverage (as estimated by band counts) is in good agreement with the Poisson expectation, calculated using the generally accepted figure of 165,000 kb as the genome size. Second, the distribution of gaps in the coverage matches well the distribution expected if coverage were random (*i.e.*, the distribution predicted by the exponential distribution). Although euchromatic coverage appears essentially random, three of the largest gaps (6B;6F, 16A;16F, and all of chromosome *4*) may be significant, as similar gaps are present in the YAC mapping data of AJIOKA *et al.* (1991).

In the study of AJIOKA *et al.* (1991), 500 euchromatic YAC clones were mapped. Thirty-eight of these clones were also mapped in our study, and are described again here because, in most cases, our mapping is somewhat different. When the clones from the two projects are pooled, they contain about 42,700 kb of DNA from *X* euchromatin and 228,000 kb of DNA from autosomal euchromatin. Assuming a genome size of 165,000 kb and a heterochromatin fraction of 25%, these amounts correspond to about 1.7 equivalents of *X* euchromatic DNA and about 2.3 equivalents of autosomal euchromatic DNA. Assuming random distribution of clones, the combined sets of YACs should, according to the Poisson distribution, provide about 82% coverage of the *X*, and 90% coverage of the major autosomes.

Our estimates of coverage are conservative relative to those of AJIOKA *et al.* (1991), who claim their 500 clones

of average size 198 kb comprise one euchromatic genome equivalent. This claim is highly suspect, however, since their central argument to support it is incorrect. Assuming random clone distribution, the proportion of sequences not covered by clones should be equal to the zero term of the Poisson distribution, e^{-G}, where G is the number of genome equivalents of DNA present in the clones analyzed. AJIOKA *et al.* consider the fraction of lettered divisions not labeled by any of their YACs (212/600 for the major chromosome arms) to be equal to the proportion of all sequences not covered, and calculate G as 1.04. However, it is clear from their own data that many of the lettered divisions that are labeled are only partially so. Thus, their calculation substantially overestimates the coverage achieved. We calculate that their clones constitute about 0.8 euchromatic DNA equivalents, rather than the 1.04 claimed, and their euchromatic coverage at about 55%. Unfortunately, it is not possible to estimate their coverage by direct band counts, since many of their localizations are only to lettered division, not to band.

One hundred and twenty-four of the 979 YAC clones (13%) mapped in this report are heterochromatic and label the chromocenter as the primary site. As described above, because of contamination of the DY and DYN clone sets with duplicate clones, probably only about 11% of unique YACs are heterochromatic. Since 25–30% of the genome is composed of heterochromatin, there would appear to be an underrepresentation of heterochromatic sequences in YAC clones by a factor of two or three. Taken together, the heterochromatic YACs contain only about 0.6 equivalents of heterochromatic sequences in the genome. A number of factors could contribute to this apparent underrepresentation. First, it could be that the distribution of *Eco*RI and *Not*I sites precludes the efficient cloning of some heterochromatic sequences using these enzymes. Second, it is possible that many heterochromatic clones went unrecognized because they contain sequences not replicated in polytene cells, and fail to label any site by *in situ* hybridization. Third, it is quite possible that many heterochromatic clones are highly unstable, and break down before they can be analyzed. We note, however, that the heterochromatic YACs in our collection appear stable and are of even larger average size (216 kb *vs.* 211 kb) than the euchromatic clones.

The deficit of heterochromatic clones may be more apparent than real. Because most of our YACs were constructed using DNA from adults, the expected fraction of heterochromatic clones is not entirely clear. BLUMENFELD and FORREST (1972) found one satellite of *Drosophila melanogaster* to be underrepresented in total adult DNA by about 50%. Similarly, HAMMOND and LAIRD (1985) found the 1.705 g/cm^3 satellite to be underrepresented in nurse and follicle cells from adults. These instances presumably result from underreplication in polytene (*e.g.*, Malpighian tubules) or polyploid (*e.g.*, midgut, hindgut, salivary gland, ovary) tissues of the adult. In *Drosophila virilis*, it would appear that different satellites are under independent replication control, since specific satellites are underrepresented to different levels in different adult tissues (BLUMENFELD and FORREST 1972; ENDOW and GALL 1975). Thus, although underrepresentation of heterochromatic YACs is to be expected among clones made from adult DNA, the extent of this underrepresentation is difficult to predict, and is probably sequence-specific.

The fraction of YAC clones labeling the nucleolus (about 1.3%) is close to the fraction of adult DNA composed of ribosomal DNA repeats (about 2%) (SPRADLING and RUBIN 1981; TARTOF 1973). Some nucleolar clones were probably incorrectly scored as chromocentral, since nucleolar labeling would likely have been missed in weakly labeled slides.

On first impression, it would seem reasonable to use the data the APPENDIX to estimate clone overlaps and define contigs. Indeed, this is exactly what was done to generate the gap distribution shown in Figure 1. However, we believe it would be a mistake to take the details of such an analysis too seriously, since several factors limit the resolution of our cytological data. Because of duplicate clones in the DY and DYN libraries, we unintentionally did a fairly large test of the reproducibility of our cytological localizations. Although independent mappings of the same clone were always approximately the same, they usually differed in detail. Similarly, although rough agreement is seen for the 38 clones mapped by both AJIOKA *et al.* (1991) and ourselves, when considered in detail, direct contradictions exist for 24 of the 33 of these clones mapped to band by AJIOKA *et al.* In practice, the greatest limitation to the accuracy of mapping was the variable quality of *in situ* slides. Every effort was made to examine highly stretched chromosomes, since localizations precise to a fraction of a band can often be made in such preparations. However, for some clones, stretched chromosomes could not be found, rendering the mapping much less accurate. Almost always in such cases clones appear to cover a greater area than is real. The magnitude of this effect was a surprise, and caused us to reexamine most of our *in situs*. As a result, we significantly downsized the coverage of many, perhaps most, of our clones described in preliminary reports (HARTL *et al.* 1992; ASHBURNER *et al.* 1991). Another important variable is the strength of the *in situ* signal. If slides are overdeveloped, stain obscures the underlying banding pattern and can spread beyond the primary site. Conversely, if staining is weak only major bands are labeled, and coverage can be underestimated. Even with excellent slides, localizations in "difficult" regions, including sections 35 and 36 and regions near β-heterochromatin, are likely to contain errors. Finally, the extents of clones containing mobile elements can easily be overestimated, since adjacent repeats may

be mistakenly included within the primary site. For all of these reasons, we believe determination of clone overlaps and generation of contigs cannot be done convincingly from cytological data [although see HARTL (1992) for such an attempt using our preliminary data], and must await molecular studies.

A major motivation for our mapping has been to provide materials to aid other Drosophila researchers in their cloning efforts. As shown by VAN DER BLIEK and MEYEROWITZ (1991) in their cloning of the *shibire* locus from one of our YACs, gel-purified YAC DNA can be rapidly subcloned into cosmids. These subclones can then be ordered to generate a complete "walk" and, if appropriate mutations exist, the gene of interest identified. A detailed protocol for subcloning from YACs is presented by WHITTAKER *et al.* (1993). An alternate approach, useful for YACs not containing repeats, has been to use YACs as probes to screen existing phage or cosmid libraries [as, for example, in the cloning of *buttonhead* (E. A. WIMMER, personal communication)]. YACs should be particularly useful in cloning genes for which breakpoint alleles exist, since it should be relatively easy, by *in situ* hybridization to polytene chromosomes, to identify subclones that span such breakpoints. Although the techniques required for subcloning from YACs are relatively straightforward, YACs have not received wide use in the Drosophila community, probably because most labs are not set up to run the pulse field gels required to purify the clones. To encourage the use of YACs, detailed protocols describing the culturing of YAC clones and the purification of YAC DNA by field inversion gel electrophoresis are included with all YAC shipments.

Obviously, of greater utility for gene cloning would be the development of a complete genome map using more convenient clones, such as cosmids or P1 clones. The YACs reported here are potentially of great use in the development of such finer scale maps, and have already played a significant role in the isolation and ordering of *X* chromosomal cosmids in the mapping project of KAFATOS *et al.* (1991) and SIDÉN-KIAMOS *et al.* (1990). As in the nematode genome project (COULSON *et al.* 1988)), YACs may also help by bridging gaps in P1 or cosmid maps caused, for example, by sequences not clonable in bacteria. The large size and stability of heterochromatic YACs suggest that YACs will be of central importance in the analysis of heterochromatin. A particularly exciting prospect in this regard is the cloning of a Drosophila centromere, as this could facilitate the development of yeast-Drosophila shuttle vectors, and allow application of the powerful techniques of yeast genetics to Drosophila.

This work was initiated as a collaboration with DANIEL L. HARTL, and we would like to thank him for generously providing clones from the DY and DYN libraries. We are also grateful to ANDREW LINK for providing the DYN library to our groups for mapping. DAN GARZA gave us much advice on YAC cloning in the early phases of the project, and MAYNARD OLSON and GEORGES CARLE were very helpful in setting up the pulse field gel apparati. We thank JOHNG LIM for generously providing an important unpublished protocol for *in situ* hybridization, and TERRY LYTTLE and ED GRELL for providing fly stocks. Finally, we thank ALAN TEMPLETON and JOY BERGELSON for advice on statistics, DIANNE MATTSON for discussions, and DIANNE MATTSON, FLAVIANO GIORGINI and the anonymous reviewers for comments on the manuscript. Our work was supported by a grant from the National Institutes of Health.

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APPENDIX

The primary sites of hybridization of 855 euchromatic clones are listed in Table 2.

TABLE 2

Primary sites of hybridization of 855 euchromatic clones

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromocenter
1A1; B2-3	R19-83	200	+	+
1B1; B10-11	N13-23	360	+	+
1B8-9; C2-4	R14-34	180	–	–
1B14-C1; C3-4	N11-18	175	–	–
1D2-3; E3-4	N24-25	180	–	–
1E1; E4-5	Rt04-67	220	+	+
1E3-4; F1-2	N18-90	270	+	–
1F4; 2B1-2	723	150	+	+
2A1; B8-10 + 87C	N27-20	220	+	+
2A3-B1; B7-8	N10-31	160	+	–
2B3-4; B12-16	645	230	+	+
2B15-17; C1-2	N23-03B/AB	110	–	–
2D1-2; E1-2 + 38B	736	160	–	–
2E3-F1; 3A2-3	N17-29	150	–	+
2F4; 3A1-2	N15-58	170	–	–
3A3; A5	774	200	–	–
3A3-4; 3C2-4	R22-11	180	–	+
3B5-6; C1-2 + 67D	N13-61	300	–	–
3C1-2; C5-7	R07-58	320	–	–
3C4-5; C10-12	R01-60	150	+	–
3D1-2; E2-3	N11-88	220	+	+
3E1; E2 + 67F	N09-07	150	+	+
3F1-2; 4A2-3	R07-40	250	–	–
3F3-6; 4A2-3 + 62F	N26-15	210	–	–
3F5-6; 4A3-5	N12-95	145	–	–
4B1; B4	354	230	+	+
4B1; C4-5	N21-40	220	–	–
4B1; C4-5	Rt10-29	250	+	+
4B1-2; B3-4 + 15F; 16A	N25-12	240	–	–
4B4-5; C1-2 + 64BC	N26-96	190	–	–
4D1; D6-7	R17-02	280	–	–
4D1; D6-7	R21-74	220	+	+
4D1; E1	R17-24	210	–	–
4D1-2; E1-2	494	230	–	–
4D3-4; E2-3	623	200	–	–
4D3-5; E2-3	N24-66	190	–	–
4D5-7; E2-3	N25-78	240	–	–
4E1-2; F4-5 + 89E; 90A + 96EF	N22-12	310	–	–
4E2; F3-4	R22-68	220	+	+
4F9; 5A2-3	N26-03B/AB	160	+	+
5A10-11; C2	N28-47	150	–	–
5B1; C1 + 21BC	N27-15	270	–	–
5C3-6; D2	588	200	–	–
5D3-5; E4-6	N11-80	175	–	–
5D5; D6	574	140	–	–
6A1; A2	N23-89	150	+	+
6A1; B3-4	R15-21	180	+	+
6F5-7; 7A6-8	N20-66	200	–	–
7A1; B2-3	R01-53	320	–	–
7A6-7; B2-3	N26-01	170	–	–
7B1; B4-5	N12-93	260	–	–
7C1; C3-4	N09-15	240	–	–

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
7E1; E2	N09-26	160	–	–
7E1; E2 + 79E	N21-23	150	+	–
7F1; F2 + 73A	665	160	–	–
7F1-2; F8-10	N12-74	150	–	–
7F3-4; 8B1-2	867	300	+	–
7F7-9; 8A5	N10-55	170	–	–
8A1; C2-3	R07-36	350	–	–
8D9-11; E3-4	N28-60	260	–	–
8D10-11; E6-9	R16-01	210	+	–
8E1-2; F1-2	R19-61	230	+	+
8E3-4; F9-10	N17-61	180	+	+
9A1; A2-3 + 21CD	R23-73	280	–	–
9A1; A2-3 + 36D	R19-57	220	+	–
9A1; A3-4	N23-10	210	–	–
9A1; A3-4	R25-03	200	+	+
9A2; A3	860	180	+	–
9A3; B1	N13-46	150	+	–
9B1; B14-15	886	160	–	–
9B1; C2-3	N28-76	200	+	+
9B6-7; D2-4	R07-57	260	–	–
9B8-10; C1-2	R15-64	210	+	–
9C2-3; E1	R20-59	180	+	+
9C3-5; D3-4	N26-20	150	–	–
9D1-2; E1-2	806	280	+	–
9D3-4; F5-10	R13-74	190	+	+
9E1; E2	600	180	–	–
9E1; E2 + 61E	N09-57	150	–	–
9E1; F10-13	805	205	+	–
9F5-6; 10A2-3	R16-45	200	–	–
9F8-11; 10B2-3	288	280	–	–
10A2-3; A10-11	R18-90	220	–	–
10B7-8; C2-4	N19-75	240	+	–
10B8-9; C1-2	N09-72	170	+	–
10D2-3; 11A1	N24-12	230	–	–
11A5-6; A10-12	Rt09-48	205	+	+
11A6; A10-11	RX01-96	150	+	–
11A7-8; B1-2	N24-20	240	–	–
11B17-19; D1-2	R21-50	190	+	+
11D1; D10-E1	N17-71	150	+	+
11D1; D8-E1	N10-08	220	+	–
11F1-2; 12A5-6	R20-11	190	+	+
12A1; A1-2	483	150	–	–
12A2-3; A9-10	766	280	–	–
12A8-10; D2-3	R16-51A	280	+	+
12B1; C7-8	R19-07	220	+	+
12B2-4; D1-2	N12-25	210	+	–
12C1-2; D2-3	R13-12	200	–	–
12E5-8; F2-3	N19-21	280	–	–
12E8-9; F2-3	R15-34	220	+	+
12F3-4; 13A10-12	R16-37	210	–	–
13A1; B4-5	R03-06	230	–	–
13A5-6 + 13BC + 22B	Rt05-24A	220	–	–
13B3; C1-2	581	190	+	–
13B4; C4-7 + 13A + 22B	Rt05-24A	220	–	–
13D3-6; E4-5	751	190	–	–
13E1-2; F7-15	822	300	+	+
13E10-11; 14A4-5	922	275	–	–
14B1; B15-18	699	220	+	–
14B1; B3-4	802	200	+	–
14B3-4; C1-2	R17-25	210	–	–
14B5-6; C6-7	R16-08	190	–	–
14F1-3; 15B1	N10-89	240	–	–
15D1; F1-2	N12-19	150	+	+
15E7; 16A1-2	N12-21	170	–	–
15F1-3; 16A4-6 + 4B	N25-12	240	–	–
15F1-4; 16A1-2	N18-70	150	+	–
15F2-4; F5-6	N16-96	150	–	–
16F1; 17A1-3	R23-40	210	–	–
16F1; F7-17A1	451	250	+	–
17A1-2; A10-12	755	220	–	–
17A4-5; A5-7	875	150	–	–
17A5; A6 + 22CD	N26-54	190	–	–
17A5-6; B2-3	N12-44B/AB	200	–	–
17A7-8; C2-3	R25-10	180	–	–
17A7-9; B2-3	N25-33	170	–	–
17D4-5; F1-2	N16-71	160	–	–
17D5-6; 18A2-3	877	170	–	–
18A1; A3-4	678	190	–	–
18A2-3; B1-2	705	180	+	–
18A5-7; C4-5	R03-28	240	–	–
18B10-11; C4-5	N18-82	235	–	–
18C1; C4	N11-04	205	–	–
18F4-5; 19B2-3	N15-63	280	–	–
19A1; A4-5	R23-39	200	+	+
19B1-2; E5-6	R07-48	350	–	–
19B2-3; E1-2	R01-63	345	–	–
19B3-C1; C3-6	N14-15	180	–	+
19D1-2; E3-4	R04-37	140	–	–
19D1-2; E4-5	N27-47	200	+	+
19E1; E4	N27-32	150	+	+
19E2-3; E7-8	R21-76	230	–	–
19E9-F1; F4-6	R17-03	240	+	+
19F3-4; 20A2-3	N19-79	170	–	+
20A3-5; B-C	R19-85	240	+	+
20A3-5; B1-3	N27-08	240	+	+
20A5-B1; C2-3	R18-12	240	+	+
21A3-B1; C1	Rt12-15	280	+	+
21B1; B2 + 77E	N11-56	360	–	–
21B2-3; B4-7	R14-43	250	–	–
21B4-7; C1-2	N13-91	140	–	–
21B5-7; C1 + 5BC	N27-15	270	–	–
21C1-2; C4-5	N28-95	150	–	–
21C3-4; D2	N13-64	240	–	–
21C5; C7-8 + 82E	N15-42	240	–	–
21C6-7; D1-2	N25-92	150	+	+
21C7-8; D2	N19-86	150	–	–
21C7-8; D3-4 + 9A	R23-73	280	–	–
21D1; D2	818	150	–	–
21E1-2; F3-4	584	200	–	–
21E2-3; 22A1-2	N10-22	150	–	–
22A1-2; A5-8	Rt03-28	260	–	–
22A3-4; A5-7	778	175	+	–
22A5-7; B6-7	Rt04-22	210	+	+
22A8-B1; B7-8	R22-78	230	+	+
22B1; B2 + 13A + 13BC	Rt05-24A	220	–	–
22B8-C1; E2-3	R02-46	265	+	–
22C1; D1 + 17A	N26-54	190	–	–
22C1; D4-6	R11-45	190	–	–
22D1-2; E3-4	202	170	+	–
22E1; E3-4	757	150	–	–
22E1; F1-2	666	180	+	–
22E1; F1-2	N24-80	240	–	–
22E1; F2-3	R19-63	180	+	+
22E2-3; F3-4	R16-92	230	–	–
22F1; 23A2-3	R22-72	180	+	+
22F1-2; 23A1-2	N09-42	330	–	–
22F1-2; 23A5-7	730	360	+	+
22F3-4; 23A2-3	612	150	–	–
22F3-4; 23B2-3	R24-21	210	–	–
22F3-4; 23C2-3	Rt02-64	280	+	+
23A1; A1-2	R23-60	220	–	–
23A2-3; C1	RX02-10B	200	–	–
23D1-2; E3-4	R21-72	240	+	+
23F2; 24A2-3	R25-27	230	–	–
24B1; C1-2	685	145	–	–
24C1; D2-3	R11-16	210	–	–
24C1; D2-3	Rt14-75	280	+	+
24C1-2; D1-2	801	160	+	–
24D1; E1-2	617	240	+	+
24D1-2; E1	R25-91	240	–	–
24E1; 25A3-4	Rt06-05	270	–	–
24E1-2; 25A1	N22-78	240	–	–
24E2; 25A2-3	N17-23	240	+	–
25A1; A4	N23-06	165	–	–
25A1; A5-8	R17-30	200	–	–

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
25A2-3; B5-6	R16-25	240	–	–
25C4-7; D2-3	R17-64	220	+	+
25D1-2; E2-3	R25-59	240	–	–
25E1; 26A1-2	N20-72	370	+	+
25F1; F5-26A1	N22-11	200	–	–
25F3-4; 26A1-2	RX02-16	210	–	–
26B7-8; C3-4	R19-62	240	–	–
26C2-3; D2-3	R22-41	190	+	+
26D1; E2	817	240	+	+
26D10-E1; F5-7	R19-84	200	–	–
26D10-E1; 27B1-2	N12-06	260	+	+
27A1; C5-6	R18-64	290	–	–
27A1-2; C1-2	R18-09	210	–	–
27C1; C2 + 49C + 58BC	N26-34	175	–	–
27C8-9; F1	R24-23	250	–	–
27E1; E7-8	RX04-95	200	–	–
27E3-4; F2-3	R16-84	220	–	–
27F4-7; 28B3-C1	R15-17	240	–	–
28A1; B3-4	R25-53	210	–	–
28C1-2; D3-4	609	260	+	–
28D1; D4-5	RX01-64	190	+	–
28E2-4; 29A1-2	R12-23	200	–	–
28F3-5; 29D1-2	N13-72	260	–	–
29D1; E2	N18-37	240	–	–
29D1; E2-3	N13-38A/AB	200	–	–
30A3-4; A8-9	893	190	+	+
30A3-4; A9-B2	R18-83	210	–	–
30A3-5; A7	Rt01-43	280	–	–
30A3-5; A7-8	R14-91B	200	+	+
30A6-7; B2-3	R15-73	220	–	–
30A7; A8	752	175	–	–
30C1; C5-6	918	230	+	+
30C3-5; F3-6	N14-84	310	+	–
31A1; B1 + 33A	R25-57	240	+	+
32A1; C2-3	Rt10-61	200	–	–
32A2-3; C1-2	R12-90	230	–	–
32E3-4; 33A2-3	R14-08	210	–	–
32F1; 33A8-B1	R17-44	220	–	–
32F1; F1-2	827	50	–	–
32F2-3; 33A6-8	N10-65	210	+	–
33A1; A2 + 31AB	R25-57	240	+	+
33A4-7; B13-C6 + 56AB + 69DEF	N10-58B/AB	240	–	–
33A5-7; B2-3	731	160	+	+
33B1; C1-2	N25-47	260	–	–
33B1; C5-6	N10-58A/AB	260	+	+
33C4-6; D2-4	N09-89C/AC	170	–	–
33D1-2; D3-4	919	220	–	–
33D1-2; D4-E2	N26-65	200	+	–
33D2-3; E7-9	R23-58	190	–	+
33D3-E1; E3-5 + 89B	N21-75	180	–	–
33E6-8; F2-3	R04-79	235	–	–
33F1; 34A5-7	R24-35	210	+	+
34A1; A9-11	R15-05	270	–	–
34A1-2; A2	R12-28	180	–	–
34A5-6; B2-3	N20-14	220	+	–
34A5-6; B4-5	R11-31	170	+	+
34A5-7; B7-8	R03-65	240	–	–
34A6; B2-3	R05-80	260	–	–
34B8-11; C5-6	Rt05-50	180	+	+
34C3-4; C5-6	N15-74B/AB	180	–	–
34D1-2; D7-8 + 91CDE	RX04-23	200	–	–
34D3-5; D8-E1 + 79EF	RX03-16	200	+	+
34F1-2; F3-4	R14-68	210	–	–
35B2-3; B6-7	R15-19	220	+	–
35B3-4; B7-9	R22-88	220	+	+
35B6; B7	N12-56	170	–	–
35B6-8; B9-10	641	160	+	+
35B8-9; B10-C1	Rt04-01	240	–	–
35C1-2; C3-4	928	230	–	–
35D1; D4-5	Rt03-48	190	+	+
35D1; D5-7	R18-51	200	–	–
35D1-2; D3-4	N09-47	170	–	–
35D5-7; E2-F2	N11-95	170	–	–
35D5-7; F1-2	784	200	+	+
35E1; E4-F4	668	220	+	+
35E1-2; E7-F4	R21-44	240	+	+
35E1-2; F2-3	R14-44	200	+	+
35F2; F9-12	RX04-60	280	–	–
36A3; A7-8	N18-56	160	–	–
36A5-6; B2-3	R05-78	280	–	–
36A9-10; C2-3	R18-23	230	–	–
36B1; C1	R23-55	180	+	+
36B8-C1; D1	R14-83	220	+	+
36C1; C2	R07-11	220	–	–
36D1; D2	R07-63	240	–	+
36D1; D2	R21-12	255	+	+
36D1; D2 + 9A	R19-57	220	+	–
36D1; D2-3	Rt12-17	240	–	–
36D1; D3-E1	R18-38	240	+	+
36D1; E2-3	R22-45	280	+	+
36D3-E1; E3-4	N11-41	290	–	–
36E1; E3-4	N24-19	130	–	–
36E1; E4	R07-37	280	–	–
36E2-3; F7-8	Rt15-10	250	+	+
36E3-4; F1-2	N24-54	240	+	–
36F1-2; 37A2-3	R19-59	280	+	+
36F6-7; 37B1-2	R16-27	220	–	–
37A1; B4-5	RX02-37	160	–	–
37A1-2; B1-2	N09-63	150	–	–
37C1-3; D1-2	700	150	+	+
37C5-7; D5-E2	N19-85	240	–	–
37D1; D2-3 + 82F; 83A + 94B	526	190	–	–
37F6-38A1; 38A7-8	R17-14	210	–	+
38A1; A2-3	N15-73	200	–	–
38A1; A3-4	R15-74	200	+	–
38A1; A5-8	N13-28	180	–	–
38A3-4; B4-6	N17-30	240	+	+
38A4-5; A8-B1	R15-50	190	–	+
38B1-2; C2-3	679	260	+	+
38B3-4; B5-6 + 2DE	736	160	–	–
38C1-2; D1-2 + 78AB	N22-63	180	–	–
38C2; D2-3	R17-34	240	+	+
38D1; E7-10	R11-02	230	+	–
38D1-2; E4-7	N21-29	240	–	–
38F2-5; 39A1-2	R18-74	190	–	–
39A1; B1-2	589	270	+	+
39C1; C2	917	70	–	–
39E3-4; 40A2-3	R23-29	270	–	–
39E5-6; 40A3-4	N12-87A/AB	440	+	+
40A	N28-84	280	+	+
40A1; A4	N18-93	270	–	–
40A1; A4-5	Rt02-72	230	+	+
40B1-2; B3-4	N09-89A/AB	280	–	–
40BC	N15-72	200	–	+
40CD	607	190	+	+
41E3-5; F1-2	N10-43	230	–	+
41E3-5; F5-8	N21-20	150	+	+
41F1; F2	R16-15	280	+	+
41F1; F2-3	N16-79	170	+	+
41F1; F3	N10-17	250	+	+
41F1; F9-11	201	250	+	+
41F1-2; F6-8	N28-07	180	+	+
41F2-3; F10-11	R19-21	210	–	+
42A1; A2	RX02-33	230	–	–
42A1; A7-9 + 62BC	N23-77	210	+	+
42A1-2; A4-5	R17-37	170	+	+
42A1-2; A18-B3	N13-83	160	+	+
42A2; A8-9	849	170	–	–
42A4-5; A8-10	N15-67	155	–	–
42A8-10; A18-B1	N14-37	150	+	–
42A14-19; B1-2	R18-65	180	–	–
42A15-17; B3-4	N16-37	200	–	+
42B1-2; B4-C6	798	210	+	+
42C3-5; D3	902	190	+	+

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
42C6-9; D5-6	N28-13	190	–	–
42C7-10; D5-6	R12-56	150	+	+
42C8-9; E1	N23-37	180	+	+
42E3-5; 43A2-3	N22-62	200	–	–
42F1-2; 43A2-3	R16-60	200	–	–
43E1-3; E7-8	N10-80	210	–	–
43E8-14; F1-2	714	190	+	+
43E14-17; 44A2-3	Rt01-41	190	+	+
44C1; C2-3	619	190	–	–
44C1-2; D1-2	N10-10	280	–	–
44D1; D3-4	898	185	–	–
44E1-2; F3-4	N22-41	180	+	+
44E1-3; F1-2	N12-63	190	+	–
44F2-3; 45A2-3	N26-90	170	–	–
45A2; C1-2	R19-72	250	–	–
45C1; E1-2	N20-67	240	–	–
45D1-2; F1-2	R25-46	240	+	+
45D7-9; 46A3-4	N14-48	280	+	–
46A1; A3-4	N24-84	200	+	+
46A1; A4	N17-62	270	–	–
46B3-4; D2-3	626	220	–	–
46C11-D1; F6-7	N16-40	240	–	–
46E7-9; 47A2	R15-68	190	–	–
46F10-47A1; A5-8	R14-41	210	+	–
47B3-4; D3-4	R25-07	220	+	+
47B4; B7-8	R03-20	140	–	–
47B4; D1	728	200	+	+
47B5-6; C6-7	912	200	+	–
47D1-2; D5-6	R17-69	270	–	–
47E6-F1; 48A1	R19-34	200	+	+
47F3-5; 48A2-3	R24-76B	180	–	–
47F10-14; 48A4	R17-63	160	–	–
48A1; B4-6	R12-87	160	+	–
48A3-4; B6-7	N14-62	170	–	–
48A3-4; C1	829B	180	+	–
48A5-B1; C5-6	R17-49	270	–	–
48B1-2; C2-3	R19-41	200	–	–
48C1; C3	N09-89B/AB	190	–	–
48C1; C4-7	R03-43	220	–	–
48C1; C5-8	N10-34	170	–	–
48C1; D1 + 88EF	N09-13	175	–	–
48D1; E2-3	N15-20	180	+	–
48D2-3; E1	N12-62	180	–	–
48D6-7; F2-3	R23-77	200	–	–
48E2-3; F6-7	R19-66	180	+	+
49A3-4; B10-12	R16-41	220	–	–
49A9-11; B1-2	878	150	–	–
49C1; C2-3 + 27C + 58B	N26-34	175	–	–
49C1-2; E1-2	R06-67	190	+	–
49D1; E7-F1	N12-92A/AB	260	–	–
49D1-2; E6-7	N25-60	170	+	+
49E5-7; F4-6	R05-85	230	–	–
49F3-4; 50A3-4	R12-14	195	–	–
50A5-7; B6-9	N10-95	220	–	–
50A10-11; C3-4	R16-07	220	–	–
50A12-14; C4-5	N09-10	170	+	–
50B1; C1-2	N17-88	160	–	–
50B1-2; C3-4	N27-48	180	+	+
50C1; C10-12	N21-05	210	+	–
50C1; C4-5	N18-25	170	+	–
50C3; C4 + 59D	N13-78	240	–	–
50C8-9; C23-D1	N17-22	140	–	–
50C9-11; D2-3	N14-27	150	+	–
50C16-18; D6-7	Rt01-57	240	–	–
50D1; E6-7	N17-51	270	–	–
50F1-4; 51A5-6	R07-34	180	+	–
50F8; 51A4-5 + 92F; 93A	686	210	+	–
51A6-7; C1	N09-90	160	+	+
51B1; C2-3	N28-28	170	+	+
51C1; D2-3	R14-72	240	–	–
51D9-11; E2-3	N20-27	200	–	–
51D9-11; E6-7	R15-51	230	+	+
51E1; E5-6	N17-08	140	–	–
51E4-5; E10-F1	N20-75	150	–	–
51F1-2; 52A5-7	R07-31	255	+	+
51F7-10; 52B3-4	N22-16	290	–	–
52A4; B2-3	N16-65A/AB	150	+	–
52B1-2; C6-9	N28-02	180	–	–
52C1; D1-2	551	200	–	–
52C1-2; D1-2	R07-60	190	–	–
52C1-4; C8-D2	N27-23	240	–	–
52C8-D1; E1-2	510	200	+	+
52D1-2; E1-2	R17-20	250	–	–
52D7-8; E9-11	R15-76	220	+	–
52E1; 53A2-3	664	260	–	–
53B3-C1; C7-11	R22-59	200	–	–
53C1; C8-11	R24-56	180	+	+
53C1-2; D2-3	R21-11	240	+	–
53D10-11; E2-3	R18-80	230	–	–
53D10-13; F1-2	R14-17	210	–	–
53D12-14; E9-11	N13-66	220	–	–
53E1; F11-13 + 69AB	853	350	+	+
54A1; B2-3	N12-75	260	–	–
54A2-B1; B16-18	N28-01	150	–	–
54B1-2; D1-2	R06-45	240	–	–
54B10-16; D2-3	R24-60	190	–	–
54B16-18; D2-3	Rt09-29	200	–	–
55A1; A4-B3	843	260	–	–
55B3-4; C6-7	R06-51	210	–	–
55B5-10; C5-8	N22-40	140	–	–
55C1; C5	R17-59	180	–	–
55C6-8; E2-3	R03-14	260	–	–
55F1-2; 56B1-2	N19-69	160	–	–
56A1-2; B1-2	139	280	+	–
56A1-2; B1-2 + 33AB + 69DEF	N10-58B/AB	240	–	–
56A2-3; B1-2	848	100	–	–
56D1; E3-5	N10-14	290	–	–
56D11-E2; E5-6	N25-59	150	–	–
56F2-3; F7-8	R18-15	240	+	+
56F4-6; F13-15	N26-70	140	+	+
56F8-9; F16-17	R21-33	180	+	+
57A1; A4	RX01-81	200	–	–
57A5-6; B3-4	R17-43B	220	+	–
57B1-2; B5-6	586	175	+	–
57B4-5; B10-13	R18-77	220	–	–
57B4-5; C1-2	N17-58	220	–	–
57B8-11; D2-3	R24-34	280	+	+
57C2-4; E2-4	R23-92	300	+	+
57D10-11; E4-5	R21-93	140	–	–
57D11-E1; F2-3	R15-93	210	–	–
57E1; E2	N28-96	190	–	–
57E1; F4-7	R17-75	260	+	+
57E5-7; F6-8	N14-34	240	–	+
57F8-9; 58A3-4	N24-26	240	–	–
58A2-3; B6-7	RX02-27	160	+	–
58B2-3; B9-C4 + 27C + 49C	N26-34	175	–	–
58B3-4; D2-3	741	280	+	+
58B6-7; D2-3	739	150	+	–
58C1-2; D2-4	R12-33	180	+	–
58D1; D7-8	Rt02-43	180	+	+
58D3-4; D7-8	R23-17	240	–	–
58D6-E2; F4-7	N19-77	240	–	–
59A1; A3-4	N11-66	180	–	+
59B4-6; C3-5	R22-60	230	–	–
59C4-5; D3-4	R25-101	190	–	–
59D1-2; D3-4 + 50C	N13-78	240	–	–
59D7-10; F2-3	N18-87	210	–	–
60A8-11; B10-12	R25-80	190	–	–
60A10-13; B12-13	N13-25	155	–	–
60A15-16; B11-12	N11-96	170	–	–
60D9-10; D14-15	N21-10	150	–	–
60D14-16; E7-9	N28-18	210	+	–
60F1-2; F3	R11-32	210	–	–
60F2; F3	909	150	–	–
61A1; A5-6	N23-66	150	–	+
61C4-7; D1-2	N28-17B/AB	240	+	–
61D1; D2	R07-14	210	+	+

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
61E1; E1 + 9E	N09-57	150	–	–
61E1; F1	N11-70	160	–	–
61E1; F2-3	N10-01	230	+	+
61E1; F2-3	N16-88	160	–	–
61F1-2; F4-5	N14-05	165	–	–
62A1; A11-12	N13-38B/AB	140	–	–
62A1-2; B3-6	N14-43A/AB	270	–	–
62B1-2; B10-11	N28-67	180	+	–
62B2-4; C2-3	N18-02	150	–	–
62B3-4; B11-12	R18-25	230	–	–
62B10-11; C2-3 + 42A	N23-77	210	+	+
62D4-5; E2-3	660	210	+	+
62D5; E2-3	903	130	–	–
62D5-6; E2-3	R07-10	220	–	–
62D5-6; E4-7	RX04-38	190	+	+
62E5-7; F2-3	R11-20	190	–	–
62F1; 63A1-2	N13-19	195	–	–
62F3; F4-5 + 3F; 4A	N26-15	210	–	–
62F4-5; 63A1	753	150	–	–
62F4-5; 63B2-3	N21-67	205	+	–
63C1; D2-3	R16-17	220	–	–
63D2-3; E5-6	N13-93	190	–	–
63E1; E3-4	R18-53	220	–	–
63E1; E3-4	R21-49	200	–	–
63E1; E5-6	527	250	–	–
63E2-3; E5-6	R11-21	190	–	–
63E4-5; 64A2-3	R22-28	220	–	–
63E4-5; F6-7	839	170	+	+
63E6-8; F2-3	785	210	+	–
63F1; 64A4-5	N09-76	270	–	–
63F1-2; 64A5-6	RX02-87	230	–	–
64A3-4; A10-B1	895	175	+	–
64A6-8; A10-12 + 88E	836	100	–	–
64A7-9; B10-11	Rt03-73	240	+	+
64B1; B9-10	R04-91	160	–	–
64B12; B16-17	N25-23	150	–	–
64B12-14; B17-C2 + 4BC	N26-96	190	–	–
64C4; C5	R15-89	220	+	+
64D1; D2	824	150	+	+
64E2; E12-13	604	170	–	–
64F1-2; 65A5-6	Rt12-36	240	+	–
64F3-5; 65A2-3	R13-33	210	–	–
65A7-8; B3-4	R25-40	230	–	–
65B1-2; C3-5	R13-64	220	+	–
65C3-4; E1-2 + 96D	N13-56	190	+	+
65C3-4; E2-3	Rt09-17	200	–	–
65C5-D1; E2-4	RX02-68	200	+	–
65D1; D3-4	N20-69	200	–	–
65D1; E2-3	405	240	–	–
65E10-11; 66A2-3	N13-52	230	–	–
65E10-11; 66A1	845	200	–	–
65E11-12; 66A1-2	R24-41	220	+	+
66A1; A5	R15-75	200	–	–
66A1; A5-6	N28-81	190	+	+
66A1; A6-9	N25-66	160	–	–
66A5; A8-9	R01-57	150	+	+
66A16-18; B2-3	R11-19	200	+	+
66A17; B7-9	147	240	+	+
66B1; C2-3	549	205	+	–
66B1-2; C2-3	R15-22	180	+	+
66C4-5; D2-3	N11-47	175	+	–
66C8; D4-6	712	170	+	–
66D13-15; E2-3	N12-20	300	–	–
66D14-15; E2-3	N23-90	240	–	–
66F1; F5-6	697	160	–	–
67A1; B1-2	543	300	–	–
67A1; B5-8	N12-72	170	+	–
67A6-9; B4-5 + 100AB	924	240	–	–
67A7-9; B7-9	N09-40	150	–	–
67B9-11; C3-4	R20-44	190	+	+
67B10-11; C3-5	N09-41	260	–	–
67C7-9; D7-8	Rt05-60	180	+	+
67D3-4; D11-13	R04-93	270	–	–
67D8-9; D12-13	N28-09B/AB	220	–	–
67D9; D12-13	745	170	+	+
67D9-11; D9-11 + 3BC	N13-61	300	–	–
67D13-E1; E4-5	854	460	–	–
67E2-3; F4	R21-35	190	–	–
67E3; E4	N27-12	150	+	+
67E6-7; F3-4	N11-06	190	–	–
67F1; F2 + 3E	N09-07	150	+	+
67F2-3; 68A2	R25-35	220	+	–
68A1; A3-4	R12-60	240	–	–
68C7-8; D3-4	904	210	+	+
68D4; E2-3	740	180	+	+
68D5-6; E3-4	R12-07	240	+	–
69A1; B1 + 53EF	853	350	+	+
69A2-3; B1-2	716	160	–	–
69B1; C7-10	N12-70	200	–	–
69D2-3; F4-6	N15-52	180	+	–
69D3-4; F2-3	N27-17	190	–	–
69D4-6; F2-3 + 33AB + 56AB	N10-58B/AB	240	–	–
69E8-F1; F7-70A1	R15-23	220	+	–
69F2-3; 70A3-7	726	260	+	+
69F2-3; F4-7	N17-42B/AB	90	–	–
69F3-5; 70A1-2	R19-03	240	–	–
70A1; A4-5	R17-38	200	+	+
70B1-2; C1	R12-01	270	–	–
70C1; C2	R23-14	240	–	–
70C1; C11-13	N20-11	280	–	–
70C2; C3	R19-51	210	–	–
70C2; C15-D1	N16-13	250	–	–
70F5-6; 71C1	267	270	–	–
70F5-7; 71A3-4	R14-37	240	–	–
71A1; B1	R25-17	200	–	–
71A2; B1	R15-65	210	+	–
71A3-4; B1-2	N11-87	170	–	–
71B2; C1-2	R24-54	190	–	–
71B2; C2-3	R07-23	280	–	–
71B4-8; C1-2 + 84D	N24-27	150	+	–
71C1; C3-4	N12-44A/AB	320	–	+
71E1-3; F2-3	N20-96	260	–	–
71E3-5; F4-5	R18-89	240	–	–
72A1; A2	894	140	–	–
72A2; D2-10	R19-02	220	–	–
72C1; E2-3	R21-03	265	–	–
72D3-5; E2-3	N12-64	170	–	–
72D4-8; E2-3	N10-66	170	–	–
72D5-7; E1-2	N27-06	190	–	–
72D8-10; E4-5	R23-13	200	–	–
72E1; E3-4	R14-26	200	–	–
72F3-4; 73C1-2	R17-06	280	+	+
73A4-5; A4-5 + 7F	665	160	–	–
73B3-4; C2-3	R15-53	200	–	–
73C2-3; D5-7	R15-57	240	+	+
73D2-3; E4-6	N13-77	170	+	–
73E3-4; 74A2-3	847	275	–	–
73E5-F2; 74A2-3	R24-32	190	+	–
73F3-4; 74A6-B3	Rt01-02	255	+	+
74A1; A5	N26-11	240	+	+
74A2-3; E2-4	Rt10-53	210	+	+
74A2-4; D1-2	R19-43	220	–	–
74C1; F1	R25-94	210	–	–
74C2-3; F2-4	R15-67	180	–	–
74D1; E3-5	N14-88	160	–	–
74D1; 75A1-2	R18-01	240	–	–
74D3-5; 75A4-7	R11-28	230	–	–
75A1; B5-8	R24-22	220	+	+
75A1-2; B4-7	RX04-81	180	+	+
75A7-8; B8-10	N15-29	190	–	–
75B1; B5-7	N09-21A/ABC	210	+	+
75B5-7; C1-2	R12-85	240	–	–
75B10-12; C2-3	R17-53	240	–	–
75C1; C3-5	657	260	+	+
75C2; C5-7	R24-53	200	+	+
75C4; C6-7	749	140	–	–
75D3-5; F3-5	N15-10A/AB	240	–	–

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
75E1-2; F7-9	N19-60	230	–	–
75F3-4; 76A4-5	R22-39	270	–	–
75F3-5; 76A5-6	R17-60	180	–	–
76A1-2; B2-3	N26-35	200	–	–
76B1-2; B8-10	R19-39	210	+	–
76B8-11; D2-4	N09-46	240	+	+
76C1; C3-4	636	180	–	–
76C1-2; D5-8	N09-31	160	+	+
76E1; F2-3	R24-57	200	+	+
76F1; 77A2-3	R20-43	210	+	+
76F1; 77A3-4	485	220	+	+
76F1; 77B1-2	Rt02-69	240	+	+
76F3-77A2; B1-2	R04-13	240	–	–
77A1-2; C5-7	317	220	+	+
77D3-4; F3-5	Rt04-04	180	–	–
77E1; F2-5	N20-51	210	–	–
77E1-2; E7-8 + 21B	N11-56	360	–	–
77E1-2; F4-5	653	190	–	–
77F3-4; 78A1	837	100	–	–
78A1; A3	N18-35	200	–	–
78A1; B1-2 + 38CD	N22-63	180	–	–
78A1; B3-4	R15-38B	280	+	–
78A2-3; B3-4	R15-37	240	+	–
78B1; C1-2	N24-46	150	–	–
78B1; C2-3	N18-28	180	–	–
79A1; C1	N18-84	190	–	–
79A1-2; A4-B3	N25-50	170	–	–
79A2-3; C1-2	N17-72	220	–	–
79C1; D1	R14-49	150	–	–
79C2-3; E1-2	Rt15-11	180	+	+
79D2-3; E4	R16-55	240	+	–
79E1; E4	N13-65	270	+	+
79E1; E4 + 87A + 87C	N09-52	180	+	–
79E1; E6-8	442	240	+	+
79E1; F1-2	R04-69B	260	–	+
79E3; E4 + 7E	N21-23	150	+	–
79E3-4; F2-3 + 34D	RX03-16	200	+	+
80A3; B2-3	797	210	+	+
80A4-B1; B3-chromocenter	N17-13	180	–	+
80B1; B2-chromocenter	N10-90	200	+	+
80B1; B2-chromocenter	N21-82	200	+	+
82C1; C2	N11-39	200	–	–
82D5-7; E4-6	N10-45	160	–	–
82D7-8; E3-5	N16-86	160	–	–
82E1; E3-4 + 21C	N15-42	240	–	–
82E3-4; E7-8	N16-72	140	–	+
82E4-5; E7-8	N12-31B/BC	150	–	–
82E6-7; F2-3	N20-40	170	–	–
82E6-7; F5-6	N16-56	175	–	–
82F1; F8-9	710	170	+	+
82F1; F8-9	N24-50	200	–	–
82F10-11; 83A5-6 + 37D + 94B	526	190	–	–
83A1; A8-B2	207	210	–	–
83A1; B4-8	R24-73	230	–	–
83C1; C6-8 + 88C	N20-52A	200	+	+
83C4-7; E2-3	N09-14A/AB	360	+	+
83C7-8; D2-3	N15-43	235	–	–
83C8-9; D5-E1	N09-14B/AB	150	–	–
83D2-4; E2-3	R21-59	210	–	–
83D4; D5	N12-12	170	–	–
83D4-5; E3-5	Rt01-08	270	+	+
83E2-3; 84A3-4	622	200	–	–
83E4-7; 84A2-3	N28-05	180	+	+
83F3-4; 84A5-B1	N20-63	240	–	–
84A1; A2-3	R13-09	200	–	–
84A1; A5-6	N28-59	240	–	–
84A5-6; B2-3	N10-64	240	–	+
84B1; C3-4	R19-87	210	–	+
84B1-2; D1-2	R06-93	220	+	+
84B2-3; B4-6	896	175	–	–
84B2-3; D1-2	R19-94	230	–	–
84D1; D5-8	N19-33	230	+	+
84D3-4; D11-12	R22-23	240	+	+
84D8-9; E2-3	R14-02	190	+	+
84D9-10; D10-11 + 71BC	N24-27	150	+	–
84D11-12; E4-6	N28-44	180	–	+
84F1; F2	N24-53	50	–	–
84F2-3; 85A3-4	N18-68	270	–	–
85A1; B1-2	841	220	–	–
85A1; B5-6	N19-19	290	–	–
85A2-3; A10-11	863	200	–	–
85B1-2; C2-3	R20-65	230	–	+
85B8-C1; C12-D1	N09-14BS	210	–	–
85C3-4; C11-D1	N12-60	210	–	–
85C8-11; D2-3	R18-68	230	–	–
85D1; D2	N19-46	190	+	+
85D1; D6	914	180	–	–
85D1; D7-10	388	310	+	+
85D1; D9-10	R14-96	270	–	–
85D11-12; E2-3	R19-73	240	–	–
85E12-14; F2-3	782	150	–	–
86B3-4; C2-3	R22-20	240	+	+
86B4-5; C8-11	Rt01-39	360	+	–
86D1; D2	R18-81	220	–	–
86D1; D2	R23-85A	210	+	+
86E1; E2-3	N27-13	195	–	–
86E3-4; E11-15	N13-92	190	–	–
86E7; E10-12	N27-45	170	–	–
86E15-18; 87A1-2	R24-11	200	–	–
86F2; 87A5-6	N27-05	200	–	–
87A4; A10 + 79E + 87C	N09-52	180	+	–
87A8-9; B3-4	R21-15	190	+	+
87B1; B5-8	N11-44	205	–	–
87C1; C1 + 79E + 87A	N09-52	180	+	–
87C1; C2-3 + 2AB	N27-20	220	+	+
87C1; C7-9	R17-51	280	–	–
87C1-2; D1	136	240	+	–
87C4-5; D4-5	N28-86	270	+	–
87C6-7; D4-5	N25-54	300	–	–
87D1-2; D11-14	814	340	+	+
87E4-5; F2-3	R23-78	190	–	–
87F1; F3	N12-76	180	–	–
87F3-4; F14-15	R18-36	200	–	–
87F4-7; 88A9-12	807	350	+	+
87F11-12; 88A1	N21-08	170	+	–
87F11-12; F14-15	N15-82	170	–	–
87F12-13; 88A1	913	150	–	–
88A10-11; B8-9	R22-54	180	+	+
88B1; B6-8	857	160	–	–
88B7-9; C9-10	R25-06	180	+	–
88C3-4; C9-D2	568	210	–	–
88C6-7; C10-D1 + 83C	N20-52A	200	+	+
88C6-7; D5-6	R19-52	240	–	–
88D8; E2-3	N24-29	240	–	–
88D10-E1; E3-4	N20-94	140	–	–
88E1; E1 + 64A	836	100	–	–
88E3-4; F6-9 + 48CD	N09-13	175	–	–
88E4-5; F1-2	N15-89	180	–	–
88F5-8; 89A1-2	R12-08	280	–	–
88F5-8; 89A2 + 92B	N27-34	200	–	–
88F8-9; 89A1-2	N11-16	170	–	–
89A1; A3-4	N15-10B/AB	100	+	+
89A2-3; A10-13	N10-42	180	–	–
89A8-11; B7-8	R14-86	180	+	–
89B1-2; B9-11	R15-24A	240	–	–
89B2-3; B16-18	R20-47	240	–	–
89B6-7; B10-14 + 33DE	N21-75	180	–	–
89B14-16; C2-3	N26-85	200	–	–
89B17-19; C6-7	713	160	+	+
89C1; D3-4	R23-66	220	–	–
89D1-2; D3-4	N10-59A/AB	150	–	–
89D1; E3-4	N25-72	170	–	–
89D2-3; E2-4	Rt10-67	190	–	–

TABLE 2—Continued

Cytology	Clone number	Size (kb)	Dispersed repeats	Chromo-center
89D3-4; E1-2	N10-13	180	–	–
89D3-4; E1-2	R19-19	220	–	–
89D8-9; E3-4	R21-81	240	–	–
89E9-10; 90A2-3 + 4EF + 96EF	N22-12	310	–	–
90B1; C4-6	R23-64	230	+	–
90B1; C7	R18-79	220	+	+
90C1-2; E2-3	N13-62	220	–	+
90D1-2; E2-3	N09-86	170	–	–
90D2-3; E2-3	R22-17	200	+	+
90D3-5; E3-5	R06-17	190	+	–
90E1; F1-2	702	200	–	–
90F1-2; 91A2-3	N14-14	260	–	–
90F9-11; 91A2-3	N26-17	160	+	–
91B2-3; B5-6	N24-21B/AB	190	+	+
91B4-6; D2-3	Rt09-61	230	+	+
91C1-2; E1-2 + 34D	RX04-23	200	–	–
91C4-5; E4-5	565	330	–	–
91D1-2; E1-2	N09-75	260	–	–
91D2; F1	891	190	–	–
91D2-3; F1-2	R21-05	180	+	+
91D3-5; F2-3	R18-05	240	–	–
91F1; F11-12	R11-07	170	–	–
91F1-2; 92A1	N13-74	240	–	–
91F4-5; 92A4-5	R16-29	190	–	–
92A1; A7-8	R11-05	240	+	+
92A3; A6	605	170	+	–
92A9-11; B3-4	N09-29	220	–	–
92B1; B8-10 + 88F; 89A	N27-34	200	–	–
92B9-10; C3-4	N17-84	140	–	–
92C2-3; D7-9	R16-23	220	+	+
92C3-5; E1	876	310	–	–
92D1; D2	N21-58	160	–	–
92D2-3; E2-3	N18-32	190	–	+
92E1; E8-9	N25-57	160	+	+
92F1; F5-7	R07-35	150	–	+
92F1; F8-10	N19-48	170	–	+
92F1-2; 93A2-3	R14-74	200	+	+
92F5-6; 93A4-5	R14-11	190	–	–
92F5-6; 93A4-5 + 50F; 51A	686	210	+	–
93A2-3; B9-12	R23-50	230	+	–
93B6-C1; D1-7	N26-73	160	+	+
93D3-4; D9-10	R05-89B	290	–	–
93E1; F1	R11-33	200	–	–
93E1-2; F1	R12-43	240	–	–
93E2-3; E9-F1	R18-47	280	–	–
93E4-5; F4-5	N09-35	220	–	–
93F8-9; 94A3-4	RX04-08	190	+	+
93F9-10; 94A3-4	655	270	–	–
94A1; A7-10	N24-49	200	–	–
94B1-2; B5-6 + 37D + 82F; 83A	526	190	–	–
94B8-10; D4-5	Rt01-44	270	–	–
94C1; D2-3	643	310	+	–
94C6-8; D4-5	N17-28B/AB	145	–	–
94D1; D10-E5	N19-81	160	–	–
94D8-9; F1	R22-79	240	–	–
94F1-2; 95A8-10	Rt11-60	230	+	+
95C3-4; E1-2	852	300	–	–
95C10-13; D8-10	N27-18	165	–	–
95E1; F1-2	N26-74	140	–	–
95E5; F8-10	R17-87	220	–	–
95F1-2; 96A2-3	Rt02-35	280	+	+
96A3-5; A20-21	Rt02-47	225	+	+
96A8-9; B5-7	Rt12-13	280	+	+
96A13-15; A19-20	N21-80B/AB	150	–	–
96A22-24; B20-C1	N13-63	150	–	–
96B9-10; C1-2	R25-45	220	–	–
96B14-18; C7-9	804	230	–	–
96C8-9; D4-6	N13-54A/AB	260	+	+
96C8-9; E1	N28-08	250	+	+
96D1; D2	715	280	–	–
96D1; D2 + 65CDE	N13-56	190	+	+
96D1; E1	R15-48	210	–	–
96E4; F2-3 + 4EF + 89EF; 90A	N22-12	310	–	–
96F7-8; 97A6-7	R21-90	210	–	–
96F7-8; 97A7	R22-90	240	–	–
97B1; B8-9	R24-65	200	–	–
97B1; C5-D1	Rt13-25	330	–	–
97B6; C2-3	N13-03	180	+	–
97B6; C4-5	R25-38	200	+	+
97E1; F1-2	R24-44	210	+	+
98A1; A12-B1	Rt13-03	180	–	–
98A1; A14-15	N18-11	250	–	–
98A1; A4	N18-08	150	–	–
98A5-8; B2-3	RX01-49	170	–	–
98A10-11; B2-3	R02-69	240	+	+
98A13-15; B8-C1	R25-42	180	+	+
98B1; B2-3	899	70	–	–
98B1; B8-C1	N15-15	340	–	–
98B1; C1-2	N23-56	300	–	–
98C1; C2 + 99F; 100A	R13-71	200	–	–
98C1; D1-2	Rt09-69	190	+	+
98C1; D2-3	528	240	–	–
98C5-D1; D5-7	R13-80	200	–	–
98D1-2; E3-6	123	320	+	–
98E1-2; F4-5	N22-18	180	+	–
99A1; A8-9	851	270	+	–
99A3-4; A10-11	R15-41	240	+	–
99A4-5; A7-8	R15-12	200	–	–
99B3-4; C1-2	R11-22	200	–	–
99B4-5; C1	Rt01-01	180	+	+
99B9-10; D3-4	R16-22	230	–	–
99D1-2; E3-4	R21-61	210	–	–
99F8-10; 100A2-3	N13-50	300	–	–
99F8-11; 100A1-2 + 98C	R13-71	200	–	–
99F9-11; 100B1	R25-37	190	+	+
100A1; A5-6	704	250	+	–
100A2-3; A3	N27-59	230	–	–
100A5-6; B1-2 + 67AB	924	240	–	–
100A7-B1; B4	R15-71	230	–	+
100A7-B1; B4-5	R13-77	220	–	–
100B4; B5	N25-44	160	–	+
100B4-5; C2-3	R07-38A	360	–	–
100B4-5; C4-5	N17-87	290	+	–
100B7-9; C2-3	R25-84	200	–	–
100C3-4; D3-4	Rt03-80	190	–	–
100C4-5; D3-4	R16-72	220	–	–
100C7; E1	R21-17	240	–	–
100D1; E2-3	Rt02-03	200	+	+
100D3-4; E2-3	N19-58	170	–	–
100D3-4; E2-F2	R16-53	180	–	–
100E2-3; E3-F2	R14-90	190	–	–
100F1; F2-3	RX04-85	360	+	–