Volume 17 Number 22 1989 Nucleic Acids Research

PCR amplification of DNA microdissected from a single polytene chromosome band: a comparison with conventional microcloning

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Received September 4, 1989; Accepted October 10, 1989

ABSTRACT

A novel alternative to microcloning for the production of region specific chromosomal DNA is described. In this method, 'microamplification', single bands are dissected from polytene chromosomes and digested with *Sau*3A. Oligonucleotide adaptors are ligated to these fragments to provide convenient priming sites for polymerase chain reaction amplification. In this way, as much as 1μg of DNA can be amplified from a single band. Probes made from PCR amplified DNA from two such dissections have been used to probe cloned DNA form a 100kb chromosome walk. Whereas conventional microcloning has generated cloned *Eco*RI fragments corresponding to 3−4kb of the walk, the PCR probes cover greater than 90% of this chromosomal region. Thus microamplification is significantly more effective than microcloning in providing probes for establishing chromosomal walks.

INTRODUCTION

The polytene chromosomes of the *Drosophila melanogaster* larval salivary glands represent a linear array of the euchromatic portion of the genome, laterally reduplicated approximately one thousand fold, resulting in a reproducible pattern of banding. The *D. melanogaster* genome is approximately 165,000kb (1), of which approximately 30% appears heterochromatic in larval neuroblast chromosomes. Using the many chromosomal rearrangements available, in conjunction with the well documented cytology of the polytene chromosomes (2,3), genetic loci can be accurately mapped, to within a few tens or hundreds of kilobases. These cytogenetic maps subdivide the polytene chromosome map into 102 divisions, each of which contains DNA representing approximately 1Mb of the haploid genome.

Microdissection of chromosomal fragments, and cloning the DNA extracted from them, has proven to be a powerful method of establishing startpoints for chromosomal walking in *D. melanogaster*. This technique was developed by Scalenghe et al (4). Essentially, microcloning procedures involve the microdissection of chromosome bands, and the extraction and subsequent manipulation of the DNA carried out in nanolitre volumes in an oil chamber mounted on a microscope. The purified DNA is finally cloned within a bacteriophage lambda insertion vector.

While the technique of microcloning has been extended to condensed mammalian chromosomes (5,6), this approach does not give the resolution possible with polytene chromosomes and many dissected fragments, often more than 100, must be pooled to obtain sufficient material. In order to circumvent these problems, Ludecke and colleagues have applied the polymerase chain reaction to amplify DNA microdissected from human chromosome 11, estimated to contain 10,000kb of DNA (8). This amplified DNA was cloned into a plasmid vector, and 20,000 recombinants recovered.

We have developed a technique capable of amplifying as little as 50−100kb of DNA dissected from a single polytene band of *Drosophila* salivary gland chromosomes, and with the potential of amplifying even smaller segments. Our method differs from that of Ludecke *et al.* (8) in several respects, notably the means by which sequences complementary to the primers are ligated to the genomic DNA fragments, and in the use of a thermostable DNA polymerase in the amplification reaction. Amplified material need not be cloned before use as a probe for genomic library screening, although cloning is permitted by the experimental design.

MATERIALS AND METHODS

Microcloning from polytene chromosomes

Microcloning was performed essentially as described (9). Polytene chromosome squashes were prepared by fixing salivary glands dissected from third instar larvae for no more than two minutes in 45% acetic acid, before spreading and squashing between siliconised and unsiliconised 22mm×40mm coverslips. The coverslips were frozen in liquid nitrogen, popped apart, and dehydrated through ethanol. All micromanipulations were performed in an oil chamber filled with Paraffin oil (Merck IR grade),using a De Fonbrune micromanipulator. Instruments were constructed using a Narashige MF-9 microforge. Dissected fragments were transferred to a 1nl microdrop of extraction buffer (0.5mgml^{-1} proteinase K (Sigma),10mM Tris.HCl pH7.5, 10mM NaCl, 0.1% SDS) hanging from a siliconised coverslip, and incubated at room temperature for 30 minutes. The drop was extracted three times with phenol (BRL molecular biology grade) equilibrated with R-buffer (10mM Tris.HCl pH7.5, 100mM NaCl, 10mM $MgCl_2$, 10mM 2-mercaptoethanol), and once with chloroform. *Eco*RI (200 units per μl, Anglian Biotechnology) was diluted with an equal volume of 4×R buffer, and an equal volume was added to the reaction drop. After incubation for 90 minutes at 37°C, the enzyme was inactivated with a 20 minute incubation at 65°C. An equal volume of *Eco*RI digested NM1149 DNA (200 μgml^{-1}, in 10mM Tris.HCl pH7.5, 10mM $MgCl_2$, 5mM 2-mercaptoethanol, 2mM ATP) was added, followed by approximately 1nl T4 DNA Ligase (Boehringer, 1 unit per μl), and the reaction was incubated at 4°C overnight. The ligated DNA was removed from the coverslip using a Gilson pipetman after flipping the coverslip in a Petri dish of paraffin oil. The DNA was packaged using commercially available packaging extracts (Amersham International), and plated on *E.coli* strain NM514 (a selective *hfl* strain). An aliquot was also plated on Q358, to estimate the total pfu produced in the reaction.

Polymerase Chain Reaction amplification of microdissected chromosomes fragments

Chromosomes were prepared and dissected as described above. Dissected fragments were placed in extraction buffer and extracted as above. *Sau*3A (50 units per μl, Amersham International) was diluted with an equal volume of 4×R buffer, and an equal volume was added to the reaction drop. The reaction was incubated for 90 minutes at 37°C, and the enzyme inactivated by incubation at 65°C for 20 minutes. An equal volume of 10μM adaptor in 10mM Tris.HCl, 10mM $MgCl_2$, 5mM 2-mercaptoethanol, 2mM ATP was added. After addition of approximately 1nl T4 DNA Ligase (1 unit per μl, Boehringer), the ligation was incubated at 4°C overnight. The ligation was removed, and digested in a 10μl volume, with *Bcl*I (Boehringer). The digested DNA was used as template in a 100μl PCR reaction, using the Perkin-Elmer Cetus Amplitaq kit. Primer (see below) was added to a final concentration of 1 μM. Amplifications were performed in a Hybaid Intelligent Heating Block using one cycle of 94°C, 1 minute; 37°C, 1 minute; 30 cycles of 94°C, 1 minute, 55°C, 1 minute, 72°C, 3 minutes; and one cycle of 55°C, 1 minute, 72°C, 10 minutes.

Preparation of the adapter

Two oligonucleotides, a 24-mer of sequence 5'GATCAGAAGCTTGAATTCGAGCAG, and a 20-mer of sequence 5'CTGCTCGAATTCAAGCTTCT were synthesized. The 24-mer was 5'phosphorylated with T4 polynucleotide kinase, using conditions described (11). The phosphorylated 24-mer was mixed with an equimolar amount of the 20-mer, and allowed to anneal.

In situ hybridisation to polytene chromosomes

Polytene chromosomes were dissected from third instar larvae in 0.7% NaCl, and fixed for 30 seconds in 45% acetic acid, and then for three minutes in 1:2:3 fix (three parts acetic acid to two parts water to one part lactic acid), before being spread between a siliconised coverslip and a glass slide. After fixation overnight, the preparations were squashed, frozen in liquid nitrogen, and the coverslip removed. The slides were dehydrated through ethanol and air dried. Before hybridisation, the chromosomes were incubated in 2×SSC, 65°C for 30 minutes, then denatured in 70mM NaOH for 2 minutes, and dehydrated through ethanol. Probes were synthesized by the random priming method (12) either as described, or using the 20-mer as primer, in the presence of 1nmole of biotin-16-UTP (Boehringer). The probes were passed through a Sephadex G50 column, then ethanol precipitated and resuspended in hybridisation buffer (4×SSC, 1×Denhardt's, 10% Dextran sulphate, 0.4% sonicated salmon sperm DNA). Probes were boiled for three minutes and cooled on ice before hybridising under a coverslip at 58°C overnight. The slides were washed in three changes of 2×SSC, 58°C, then passed through two changes (five minutes each) of PBS (55mM sodium phosphate pH7.2; 165mM NaCl), and PBS plus 0.1% Triton X-100. After a final rinse in PBS, the chromosomes were overlaid with 100μl of a 1/250 dilution of streptavidin-horseradish peroxidase (Enzo biochemicals), covered with a coverslip, and incubated at 37°C for 30 minutes. The slides were washed in PBS as before, then 100μl of 0.5mgml^{-1} diaminobenzidine, 0.01% hydrogen peroxide in PBS was pipetted over the chromosomes. The signal was allowed to develop for up to 60 minutes, although signals were generally visible after 10 minutes. The chromosomes were stained with a 5% dilution of Giemsa stain in 10mM $NaPO_4$, pH6.8.

In situ hybridisations to polytene chromosomes using tritiated probes were performed essentially as described (12). Probes were synthesized as described (11), with two labelled nucleotides, ^{3}HTTP and ^{3}HdCTP (Amersham International).

Southern blots and restriction enzyme digests were performed as described (10). Enzymes were used as recommended by the manufacturers unless otherwise noted.

Genomic Libraries

Clones were isolated from the following genomic libraries, as indicated in the text. The lambda vector library was constructed by M. Freeman, in the vector lambda dash (Stratagene), using Oregon R DNA. One cosmid library was constructed in the SmartII vector (13), by M. Speek. A second cosmid library, was a gift of John Tamkun and Matt Scott. All libraries were screened following amplification.

Bacterial and phage strains

NM514: *hsd*R *lyc*7 (14)
Q358: *hsd*R *sup*E, $\Phi 80^r$ (15)
Q359: Q358(P2) (15)
NM1149: *imm*^{434} lambda insertion vector (14)

Drosophila stocks

Chromosomes for dissection were prepared from the Oregon R wild type strain of *Drosophila melanogaster*, and from a wild type stock of *Drosophila simulans*.

The *ats* inversion chromosome (In(3L)63C;72E; In(3R)84E;88A; In(3R)89C;96A21-25 (16) contains the defining breakpoint in 96A21-25, and the Y;3 translocation T(Y;3)B197 (17) contains the defining breakpoint in 96B1-10.

RESULTS

Microcloning DNA from 96A21-25 to establish a 100kb walk

Microcloning procedures rely upon a system whereby recombinant phage can be efficiently generated and reliably detected. Such a system is provided by bacteriophage lambda immunity insertion vectors, such as NM1149 (14). NM1149 can accept DNA fragments generated by either *Eco*RI or *Hin*dIII. DNA is extracted from chromosome fragments obtained by microdissection, digested with *Eco*RI and ligated with *Eco*RI cleaved NM1149. DNA is packaged *in vitro*, and selection is achieved by plating the phage on an *hfl* host strain, for example NM514 (14).Since a typical microcloning reaction with DNA from a single polytene band may produce up to about thirty recombinant phage in 2000 pfu, a low background is critical. It is therefore important to select a stock of vector that gives an adequately low background of false recombinants when cut and religated.

We (RDCS and DMG) wish to isolate the locus *abnormal spindle*, *asp*, which lies in a region defined by breakpoints in 96A21-25 and 96B1-10 (18,19), and have used conventional microcloning techniques towards this goal. A dissection of chromosome bands 96A21-25 from *D. melanogaster* chromosomes was performed, and the DNA extracted for microcloning. A total of 1280 pfu were recovered, of which 29 (2.3%) were cI^-, and thus putative recombinants. These 29 phage were probed with a total *D. melanogaster* genomic DNA probe. One of them gave a very strong signal, presumably because it contained repetitive *Drosophila* DNA sequences. Four phage gave moderately strong signals and contained large single copy *Eco*RI fragments (2.2 to 6kb). Of the 24 phage giving little or no hybridisation signal, four contained single copy *Drosophila Eco*RI fragments; three others contained inserts from an unidentified source, while the remaining 17 appeared not to contain an insert (although very small inserts due to *Eco*RI* activity would not have been detected). Thus eight detectable single copy *Drosophila* DNA fragments were represented in this collection. These 8 inserts were pooled and used as probe for *in situ*

Table 1

Total pfu	Number of cI^- phage	Expected Background[1]	Signal intensity[2]		*Eco*R1 inserts[3]	Insert in walk[4]
1280	29 (2.3%)	3–4	+++	1	1	
			++	1	1	
			+	3	3	
			(+)	24	7	2

Notes

1: Expected background is the number of spontaneously occurring cI^- phage following religation with no insert.

2: Signal intensity represents the signal strength when probed with ^{32}P labelled total *D.melanogaster* genomic DNA. Units are arbitrary.

3: Numbers of phage bearing visible *Eco*RI fragments.

4: The number of phage bearing *Eco*RI inserts derived from DNA corresponding to the 100kB walk in Fig. 1.

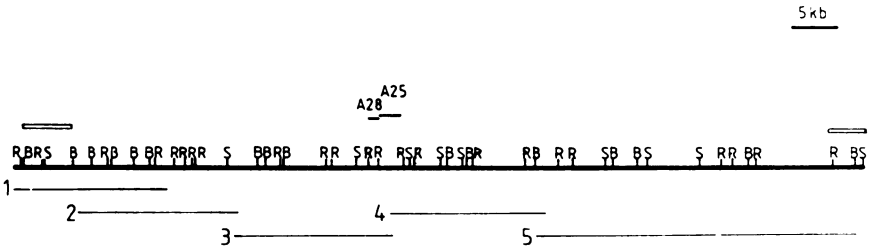


Figure 1 A chromosome walk constructed following microcloning from 96A21-25;96B1-10. Clones are 1:B3A.25, 2:B3A.8, 3:A28.3, 4:A56.12, 5:A56.14. Clones A56.14 is a cosmid, while the other clones were isolated from a lambda library. Solid bars labelled A25 and A28 indicate the location of microclones. Open bars indicate the location of fragments referred to in the text, and used as probes for *in situ* hybridisations.
R, *Eco*RI; B, *Bam*HI; S, *Sal*I.

hybridisation to polytene chromosomes (Fig. 3a). Signals were visible in 96A10-20, 96A21-25, and in 96B1-10.

A genomic walk was established using as startpoints, four of these microclones, clones A16, A23, A25 and A28, which lay within the region of interest. Microclone inserts were used to isolate clones from a genomic library constructed in the vector lambda dash (Stratagene). This is a lambda replacement vector in which inserted DNA is flanked by T3 and T7 RNA polymerase promoters, facilitating the production of termini-specific probes. Probes synthesised in this way were used to identify a series of overlapping clones comprising a 100kb cloned region (Fig. 1). Among the eight microclones with single copy inserts, A23 and A25 proved identical, and only these together with A28 hybridise to the contiguous 100kb walk (see Fig. 1). *In situ* hybridisation to polytene chromosomes using probes synthesised from the indicated 5.4kb *Bam*HI fragment of phage B3A.25 (phage 1 in Fig 1) revealed that the proximal breakpoint, (in 96A21-25) is located within this leftmost end of the walk. Microclone A28 also hybridises to 96A21-25. A probe from the extreme right end of the walk hybridises to the interband between 96A21-25 and 96B1-10 (data not shown), while clone A16 derived from 96B1-10, proximal to the distal defining breakpoint. Thus the distal chromosomal breakpoint that defines the *asp* region has not yet been reached.

Amplification of Microdissected DNA

Within the 100kb chromosome walk established using the conventional approach described above, only 3−4kb is represented in the original microclones. As described below, a dramatically increased efficiency of recovery of DNA from microdissected regions is possible utilising PCR amplification of chromosomal material.

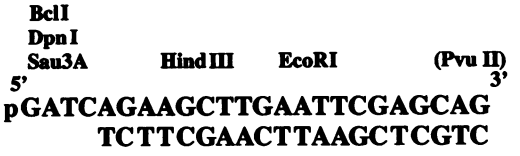


Figure 2 The oligonuceotide adapter used to provide priming sites for PCR amplification of genomic *Sau*3A sites. Relevant restriction sites are indicated. Only the 24-mer strand is phosphorylated.

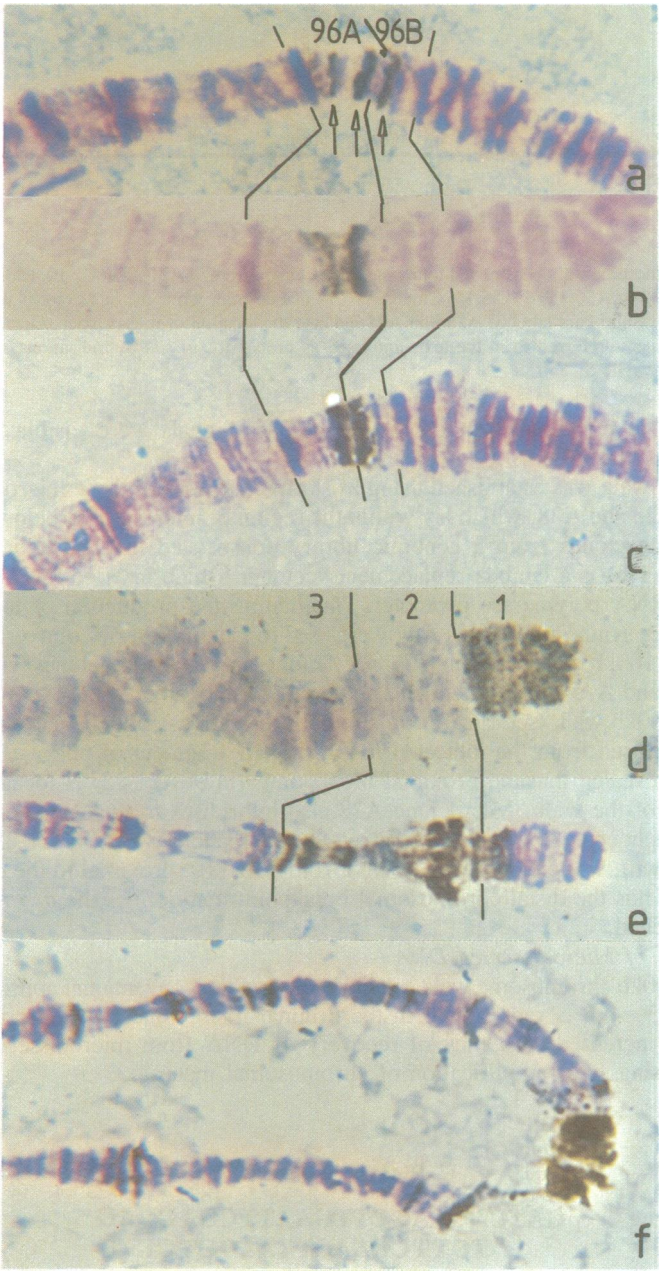


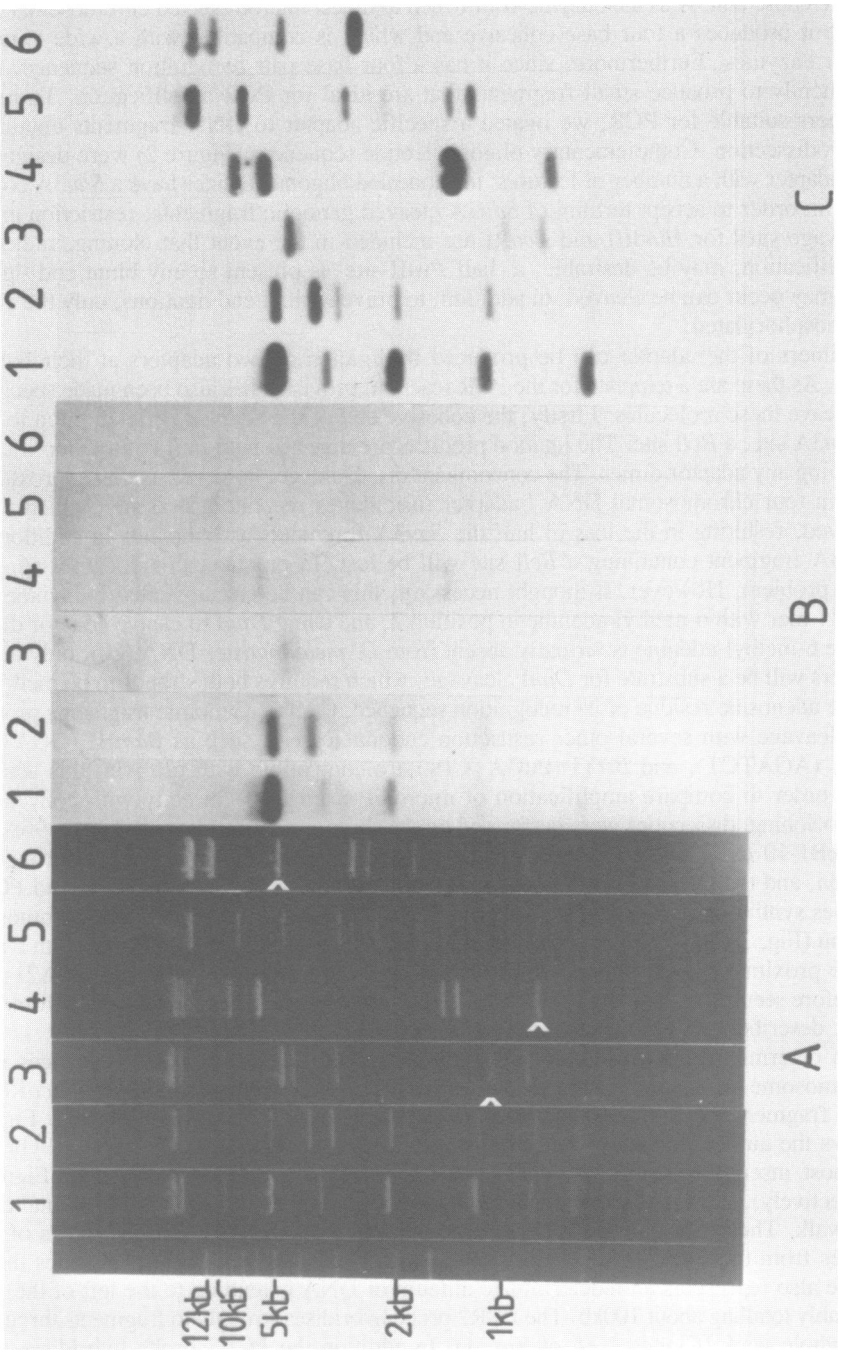
Figure 3 In situ hybridisation to *D.melanogaster* polytene chromosomes, probed with (a) mixed *Eco*RI insert fragments from microcloning, (b) DNA amplified from material dissected from 96A21-25 (PCR1), (c) DNA amplified from materia dissected from 96A21-25;96B1-10 (PCR2), (d,f) DNA amplified from a dissection of the X-chromosome division 1, (e) DNA amplified from a dissection of the X-chromosome division 2.

We chose *Sau*3A as the enzyme with which to digest microdissected chromosomal DNA, since it produces a four base cohesive end which is compatible with a wide variety of other enzymes. Furthermore, since it has a four base pair recognition sequence, it cuts frequently to produce small fragments that are ideal for PCR amplification. To provide primers suitable for PCR, we ligated a specific adapter to DNA fragments obtained by microdissection. Complementary oligonucleotide sequences (Figure 2) were designed for the adapter with a number of features: the annealed oligonucleotides have a *Sau*3A cohesive end, in order to accept termini of *Sau*3A cleaved genomic fragments; restriction enzyme cleavage sites for *Hin*dIII and *Eco*RI are included in the event that cloning, rather than amplification, may be desirable; a 'half *Pvu*II site' is present so any blunt end ligations that may occur can be cleaved; in addition, to prevent blunt end ligations, only the 24-mer is phosphorylated.

Dimers of the adapter can be produced by ligation of two adapters at their cohesive ends. As these are a template for the PCR reaction, provision has also been made specifically to cleave these molecules. Firstly, the cohesive end of the adaptor is, in addition to being a *Sau*3A site, a *Bcl*I site. The ligation products are digested with *Bcl*I before amplification, cleaving any adaptor dimer. The concomitant disadvantage, however, is that approximately one in four chromosomal DNA / adapter fusion sites recreate a *Bcl*I site, which will be cleaved, resulting in the loss of half the *Sau*3A fragments as template. In addition, any *Sau*3A fragment containing a *Bcl*I site will be lost. In practice, this does not appear to be a problem. However, if thought necessary, this can be circumvented by synthesizing the 24-mer with 6-methyl-adenine in position 2, and using *Dpn*I to cleave adaptor dimers. Since 6-methyl-adenine is virtually absent from *D. melanogaster* DNA (20), only adaptor dimers will be a substrate for *Dpn*I cleavage, which requires both strands to be methylated at the adenosine residue of its recognition sequence, GATC. Genomic fragments produced by cleavage with several other restriction endonucleases , such as *Bam*HI (GGATCC), *Bgl*II (AGATCT), and *Bst*YI (PuGATCPy),are compatible with use with this adapter.

In order to compare amplification of microdissected DNA directly with conventional microcloning, dissections were performed on the chromosome bands in the region 96A21-25 to 96B1-10 of *D.melanogaster* chromosomes. Two serial cuts were made through this region, and the DNA was extracted and amplified (here referred to as PCR1 and PCR2). Probes synthesised from this material hybridise specifically to the pertinent chromosomal region (Fig. 3b and c respectively). PCR1 hybridises primarily to 96A21-25, with a minor more proximal signal. PCR2 hybridises primarily to 96B1-10, but also to 96A21-25. It therefore seems that these two probes, in addition to covering the region of the chromosome walk described above, extend both to the left and right respectively.

To determine how extensively the microamplified DNA represents sequences in the chromosome walk, representative clones from the walk were digested with *Eco*RI, and their fragments separated by agarose gel electrophoresis for Southern blotting. Figure 4 shows the autoradiogram of this experiment. It is clear that the PCR1 probe hybridises to most insert fragments of clones B3A.25 and B3A.8 (clones 1 and 2 of Figure 1, respectively), i.e. throughout a region of approximately 30−40kb at the proximal end of the walk. The probe also hybridises, although less strongly, to insert fragments of other clones from the walk. *In situ* hybridisation with the PCR1 (see above) indicates that the probe also represents an indeterminate amount of DNA extending to the left of the walk, probably totalling about 100kb. The PCR2 probe hybridises strongly to fragments throughout the whole walk (Clones 1−5 of Fig. 1). In addition the PCR2 probe hybridises to the


1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
A
B
C
12kb
10kb
5kb
2kb
1kb

majority of *Eco*RI fragments from a recombinant cosmid not linked to the walk. This cosmid was selected by microclone A16, which hybridises *in situ* to 96B1-10. This result is consistent with the pattern of *in situ* hybridisation given by PCR2 indicating that the probe extends to the right of the chromosomal walk (Fig. 3c). Together the two probes hybridise to EcoRI fragments corresponding to greater than 90% of the total walk. In strong contrast to this extensive coverage of the walk by the microamplified probes, the *Eco*RI fragments corresponding to the inserts of the original microclones described above are few, widely spaced, and correspond to 3−4kb of the 100kb walk (indicated by arrowheads in Fig. 4). Comparable lengths of chromosome were microdissected in both experiments. We conclude that a dramatically better representation of the dissected region is obtained when microdissected DNA is amplified rather than cloned by conventional microcloning techniques.

We have also applied microamplification to entire divisions of the X-chromosome. We expected that amplification of material dissected from *D. melanogaster* would contain a large proportion of repetitive sequences. As our ultimate aim is to utilise amplified sequences as probes to screen a genomic library in order to identify overlapping cosmids (23), we wished to avoid amplifying *D. melanogaster* repeats. We therefore dissected whole divisions from the polytene chromosomes of *Drosophila simulans*. This sibling species of *D. melanogaster* has polytene chromosomes that have been well characterised, differing from those of *D. melanogaster* by several minor and one major inversions (21,22). While *D. simulans* does possess many repetitive elements of its own, it has a different spectrum of repeats. PCR amplification yielded approximately 1μg of DNA, from each division, of fragment length 150bp to 1kb, visualised as a smear by gel electrophoresis.The amplified DNA was checked by *in situ* hybridisation to *D. melanogaster* polytene chromosomes.

In situ hybridisation to polytene chromosomes with material amplified from divisions 1 and 2 of the X-chromosome is shown in Fig. 3d−e. As expected, signal is only seen over the entire corresponding division. No dispersed repetitive sequences were seen with prolonged development of the peroxidase catalysed reaction in the case of the division 2 probe. However, prolonged development of the division 1 probe reveals additional signal over approximately 60 secondary bands (Fig. 3f). In this case, it is interesting to note that, in addition to the signal over the whole of division 1, there is hybridisation to an autosomal telomere, and to chromatin strands ectopically linking the two telomeres. Similar telomeric hybridisation patterns have been previously reported with a probe from a cloned repetitive telomeric sequence (24). We are now using these microamplified probes from *D. simulans* to select corresponding *D. melanogaster* sequences from cosmid libraries.

DISCUSSION

We have been using conventional microcloning of small regions of *D.melanogaster* salivary gland polytene chromosomes to generate probes for chromosomal walking in cytogenetically defined regions containing genes of interest to us. In addition, as part of a project to produce

Figure 4 (a) A 0.6% agarose gel stained with ethidium bromide.
(b) Autoradiograph of a Southern blot of the gel, probed with amplified DNA dissected from 96A21-25. The lanes are representative clones from the chromosomal walk, digested with *Eco*RI. Lane numbers correspond to clones as abbreviated in Fig. 1. Lane 6 contains the digest of cosmid A16.21 selected by one of the microclones. This cosmid is not part of the walk, and hybridises to 96B1-10. Bands with homology to *Eco*RI fragments identified in microclones are indicated with arrowheads. The markers are the BRL 1kb ladder.

a complete physical map of the euchromatic portions of the *D. melanogaster* genome, we have been making libraries of *Eco*RI fragments from DNA microdissected from entire single divisions of the polytene chromosome map. These 'microlibraries' are being used as probes in order to identify cosmids derived from particular regions of the genome. The cosmids are subsequently ordered using the methods described by Coulson and Sulston (23).A detailed description of this strategy will be presented elsewhere (manuscript in preparation).

Problems have been encountered in routinely generating sufficient numbers of microclones, having adequate representation of single copy genomic sequences, whilst minimising the presence of repeated sequences. The *Eco*RI inserts are frequently biased in favour of the smaller fragments generated by digestion of the microdissected DNA. Some *Eco*RI fragments will exceed the cloning capacity of the vector used, approximately 10−11kb. In addition, the biological amplification of the cloning procedure itself may perturb the representation of the clone collection, indicated by the variability in plaque size. In addition, clones derived from *Eco*RI* fragments occur frequently and may, in some cases, represent the largest class of clones present. Such small inserts are difficult to work with, and to characterise. For all these reasons, we thought it necessary to devise an alternative means of generating division-specific probes.

We have developed an efficient method of obtaining such probes by microamplification, i.e.microdissection of the desired chromosomal region followed by PCR amplification of its DNA. The DNA is extracted from the microdissected chromosomal fragment, and is then digested with *Sau*3A, to generate a number of short fragments, which are ligated to adapter molecules that provide priming sites for PCR amplification. Following our procedure, as much as 1μg of DNA can be amplified from as little as one band dissected from a polytene chromosome. Because of the sensitivity of the technique, less dissected material is required than in conventional microcloning, and DNA can be amplified from very precise dissections. The use of probes from a sibling species is advantageous in suppressing detection of *D. melanogaster* repeats. We have applied our new method to a 100kb region of the *Drosophila* genome. This region was obtained by using conventional microclones as probes with which to initiate a chromosome walk. The 100kb region is represented by a series of overlapping phage and cosmid clones, within which are just two *Eco*RI fragments of total size 3−4kb, that were isolated by the original microcloning experiment. In contrast sequences present in virtually all of the *Eco*RI fragments from this region are represented in the products of two microamplifications. Each of these reactions was carried out with microdissected chromosomal regions which we estimate to correspond to 80−100kb of the *Drosophila* genome present in approximately 1000 copies in the polytene chromosomes. Together this is approximately equivalent to the amount of chromosomal material also used for conventional microcloning. The amplified material thus gives markedly better representation of the dissected region than provided by conventional microcloning methods. We have not reached the lower limit of material required for successful amplification. It should be possible to achieve further accuracy in microdissection, by applying this technique to stretched polytene chromosomes. Furthermore, we expect full representation of all genomic sequences to be achieved if *Bcl*I is replaced with *Dpn*I to cleave the adaptor dimers, in the manner suggested above, since all ligated *Sau*3A fragments will then be substrates for amplification.

ACKNOWLEDGMENTS

This work was supported by an Operation contract of the Stimulation Programme of the European Economic Community number ST2P-0477-C(TT). The early work on the *asp* region was supported by an MRC project grant to RDCS and DMG. DMG also wishes to acknowledge a career development award from the Cancer Research Campaign. We wish to acknowledge the following colleagues: P.F.R. Little for much helpful discussion, and pointing out the useful characteristics of *DpnI*, C.Gonzalez for help with the cytogenetics of the *asp* region, and H.Jaeckle for advice concerning microcloning, and A. Cheshire for help with photography.

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REFERENCES

1. Rasch,E.M., Barr,H.J. and Rasch,R.W. (1971) Chromosoma, **33**, 1–18.
2. Bridges,C.B. (1935) J. Hered., **26**, 60–64.
3. Lefevre,G.,Jr. (1976) In Ashburner,M. and Novitski,E. (ed), The Genetics and Biology of Drosophila. Academic Press, NY, vol. 1a, pp.31–66.
4. Scalenghe,F., Turco,E., Edström,J.E., Pirrotta,V. and Melli,M. (1981) Chromosoma I82ıI, 205–216.
5. Rohme,D. , Fox,H., Herrman,B., Frischauf,A-M., Edstrom,J-E., Mains,P., Silver,L.M., and Lehrach,H. (1984) Cell **36**, 783–788.
6. Weith,A., Winking,H., Brackman,B., Boldyreff,B., and Traut,W. (1987) EBMO Journal **6**, 1295–1300.
7. White,T.J., Arnheim,N. and Erlich,H.A. (1989) Trends Genet. **5**, 185–189.
8. Ludecke,H-J., Senger,G., Claussen,U., and Horsthemke,B., (1989) Nature, **338**, 348–350.
9. Pirrotta,V. (1986) In Roberts,D.B. (ed) Drosophila–A Practical Approach. IRL Press, Oxford, pp.83–110.
10. Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
11. Feinberg,A.P. and Vogelstein,B. (1983) Anal. Biochem., **132**, 6–13.
12. Pardue,M-L. (1986) In Roberts,D.B. (ed) Drosophila–A Practical Approach. IRL Press, Oxford, pp.111–137.
13. Speek,M., Raff,J.R., Harrison-Lavoie,K., Little,P.F.R. and Glover,D.M. (1988) Gene, **64**, 173–177.
14. Murray,N.E.M. (1983) In Hendrix,R.W., Roberts,J.W., Stahl,F.W. and Weisberg,R.A. (ed) Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 395–432.
15. Gough,J. and Murray,N.E.M. (1983) J. Mol. Biol., **166**, 1–19.
16. Sierra, L.M., and Comendador,M.A. (1984) Drosophila Information Service **60**, 244–245.
17. Lindsley,D.L., Sandler,L., Baker,B.S., Carpenter,A.T.C., Denell,R.E., Hall,J.C., Jacobs,P.A. Miklos,G.L.G., Davis,B.K., Gethmann,R.C., Hardy,R.W., Hessler,A., Miller,S.M., Nozawa,H. Parry,D.M., and Gould-Somero,M. (1972) Genetics, **71**, 157–184.
18. Ripoll et al, (1985) Cell, **41**, 907–912.
19. Gonzalez,C., Casal,J., Molina,I. and Ripoll, P, Genetics, In press.
20. Urieli-Shoval,S., Gruenbaum,Y., Sedat,J. and Razin,A. (1982) FEBS Letts. **146**, 148–152.
21. Horton,I.H. (1939) Genetics, **24**, 234–243.
22. Lemeunier,F. and Ashburner,M. (1976) Proc. R. Soc. Lond. B, **193**, 275–294.
23. Coulson,A., and Sulston, J. (1987) reference to be added.
24. Rubin,G.M. (1978) Cold Spring Harbor Symp. Quant. Biology **62**, 221–226.