The genome of the eukaryote is divided into several numbers of chromosomes that can be viewed in an orderly manner at mitosis. Molecular cytogenetics enable us to locate cloned DNA fragments on systematic chromosomal bands. Conversely, DNA fragments from any specific bands or regions of chromosomes can be obtained by chromosome microdissection and microcloning. These isolated clones are useful for studying chromosomal region-specific genes or structural domains of chromosomes (centromere, telomere, heterochromatin, fragile site, etc.). Recent developments in positional cloning methods have become a powerful tool for finding many genetic disease-related genes. Genetic linkage analysis by using restriction fragment length polymorphisms (RFLPs) as probes has allowed the mapping of many disease loci to specific chromosomal regions. Despite these technical advances, obtaining DNA sequence markers close to any particular defined region of the genome remains tedious and time consuming. Physical dissection of a specific region of a chromosome and recovery of DNA fragments have a great advantage in acquiring a large numbers of clones from a specific chromosome area in a short time.

In 1981, chromosome microdissection was first applied to the polytene chromosomes of *Drosophila* (Sca-lenghe et al. 1981). Then, mouse (Rohme et al. 1984) and human (Bates et al. 1986) chromosomes were also used to clone chromosome region-specific DNA sequences. In these experiments, these authors used a manual micromanipulator to collect parts of chromosomes with a glass needle. About 100 dissected chromosomes were required to purify and ligate chromosomal DNA to a cloning vector.

Lu¨decke et al. (1989, 1990) developed a modified technique for precise dissection from GTG-banded human chromosomes with a glass needle. GTG-banding facilitates the location of the chromosome and improves the quality of microdissection. Furthermore, these authors first used the polymerase chain reaction (PCR) to amplify dissected DNA (microligation-PCR method). They digested purified DNA with a restriction enzyme (Rsal), which produces blunt ends. After ligation to a cloning vector, PCR amplification was performed with primers that have a complementary sequence to the vector. By this method, various parts of chromosome-specific libraries were constructed (Butting et al. 1990, Davis et al. 1990, Fiedler et al. 1991, Hampton et al. 1991, Puech et al. 1992, Bardenheuer et al. 1994).

Although the number of dissected chromosomes can be reduced to 20–30 by using PCR, this technique still requires extremely time-consuming and labour-intensive steps, such as manual microdissection, purification of DNA, restriction enzyme digestion and microligation. Moreover, for microligation-PCR amplification, dissected DNA is required to have a Rsal recognition sequence for cloning, and digested DNA longer than 3 kb is very difficult to amplify.

To overcome these problems, degenerated primers (Wesley et al. 1990, Telenius et al. 1992) were used for PCR. The *Alu* repetitive family sequence, which is dispersed among the human genome, was also used as a primer to amplify dissected DNA (Nelson et al. 1989). Even though these two methods succeeded in reducing the steps for cloning, it was difficult to obtain enough clones to cover the dissected region of the chromosome.

Our group has developed a laser-based chromosome microdissection technique that includes DNA amplification with a single unique primer, SUP-PCR, in order to construct chromosome region-specific DNA libraries from a single metaphase spread (Hadano et al. 1991).

Instead of manual microdissection, we used the argon ion laser chromosome dissector, which can be purchased from Hamamatsu Photonics (Hamamatsu Photonics C3144, Figure 1). Chromosome dissection begins by scanning Giemsa-stained metaphase chromosomes with the 488-nm laser beam at an energy density sufficient to ‘burn down’ or rapidly disintegrate all the area excluding the chromosome part of interest (Figure 2). Laser chromosome dissection processes automatically by computerized program.

After dissection, chromosomal DNA fragments are recovered from the glass slide as follows: a well of silicon caulking is made surrounding the microdissec-
Figure 1. Photograph of the argon ion laser chromosome dissector, which can be purchased from Hamamatsu Photonics.

Figure 2.

Figure 3.