SHORT NOTE

Microdissection of Human Chromosomes by a Laser Microbeam

S. MONAJEMBASHI,[1,2,]* C. CREMER,[2] T. CREMER,[3]
J. WOLFRUM[1], and K. O. GREULICH[1]

[1]*Physikalisch-Chemisches Institut,* [2]*Institut für angewandte Physik, and* [3]*Institut
für Anthropologie und Humangenetik, Ruprecht-Karls-Universität,
D-6900 Heidelberg, FRG*

A laser microbeam apparatus, based on an excimer laser pumped dye laser is used to microdissect human chromosomes and to isolate a single chromosome slice. © 1986 Academic Press, Inc.

In studies on human genetic diseases, investigators are confronted with a steadily increasing number of cases where, although a gene of interest can be pinpointed to a defined region on a chromosome by linkage studies, neither the gene nor its gene product is known (see e.g. ref. [1]). Present methods to isolate marker sequences in the vicinity of the gene or even the gene of interest itself rely on the use of gene libraries of the whole human genome or sorted individual chromosomes [2]. In cases where patients with small deletions, including the gene of interest, are known, phenol-enhanced reassociation kinetics between the patient's DNA and normal genomic DNA have been exploited to clone pieces of DNA from the deleted region [3]. Where such deletions are not available, microcloning of the chromosome region in question could be used.

This requires fine microdissection of human chromosomes. Fine glass needles and a micromanipulator have been used for the mechanical microdissection of *Drosophila* chromosomes [4, 5]. Mouse chromosomes have been microdissected into two pieces [6, 7]. An even finer microdissection is desirable for the study of particular chromosome bands. We present an optical method using a laser microbeam which allows chromosomes to be microdissected to slices of less than 0.5 μm (corresponding to roughly 30 Mb) thickness and to isolate single slices which can be used for microcloning and construction of slice-specific gene libraries.

Laser microdissection is based on the fact that at high photon densities light can liquefy, evaporate or break down optically biological material [8]. The material is heated locally to a few thousand degrees for some microseconds [9] so that even strong chemical bonds can be cleaved.

Materials and Methods

The laser microbeam apparatus (fig. 1) is similar to that described previously [10, 11], but uses an excimer laser (Lambda Physik EMG 103 MSC) as a primary source of laser light, which pumps a dye

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* To whom offprint requests should be sent. Address: Ruprecht-Karls Universität, Physikalisch-Chemisches Institut, Im Neuenheimer Feld 253, D-6900 Heidelberg, FRG.
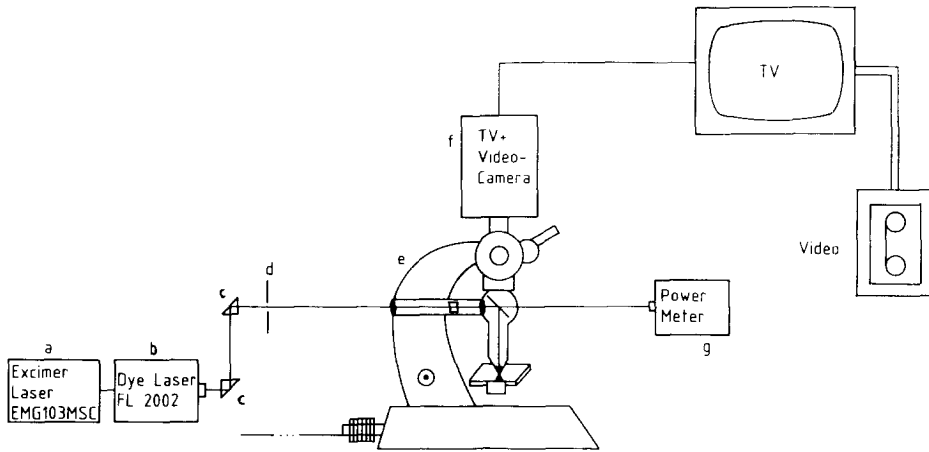
Fig. 1. Schematic diagram of the laser microbeam apparatus. (*a, b*) Lasers; (*c*) suprasil prisms; (*d*) aperture; (*e*) beam expansion optics; (*f–i*) video system, for final documentation replaced by a camera; (*g*) power meter.

laser (Lambda Physik FL 2002) for tunability (i.e.. free choice of wavelength) and improvement of beam quality. This system provides pulse energies above 1 mJ at virtually any wavelength between 320 and 800 nm and up to 10 mJ at optimum wavelengths for several dyes and is particularly suitable for working with ultraviolet light.

The pulses of the laser, 20 nsec in length, are directed into a microscope using optics similar to those of a fluorescence microscope [11]. By focusing through the objective lens with a numerical aperture of 1.25, the pulses can be focused to the diffraction limit. The cross-section of the laser pulse in the focal plane, which is close to the object plane, is a circular disc with a diameter of the wavelength being used, which at high pulse energies is surrounded by a system of concentric rings with a radial distance corresponding to the wavelength. Energy densities of more than 10^{14} W/cm^2 can be achieved, so that the energy densities in the ring system are sufficient to cut biological material. From studies on polymer materials it is known that, particularly in ultraviolet light, the damaging effect of the laser is strictly defined, i.e. that few nanometres away from the cutting region secondary damage should be negligible. We suggest, therefore, that DNA situated in chromosome slices dissected by this procedure will retain its biological integrity and be highly suitable for subsequent microcloning procedures. Damage of DNA by stray light is only expected for wavelengths well below 300 nm when the DNA molecule absorbs directly.

Results and Discussion

Fig. 2 shows a chromosome of a human lymphocyte (prepared by standard procedures onto a cover slide) treated by the ring system of a single laser pulse. The thickness of each slice is 0.5 μm. By treating the chromosome first with the ring system and then with the central disc of an attenuated laser pulse. a single slice of a chromosome can be prepared (fig. 3). First, the chromosome is cut into four slices by the diffraction ring (fig. 3 *A*), then the pulse is attenuated so that the ring system no longer causes lesions in a chromosome. The remaining central disc is then used to destroy all parts of the chromosome except the slice needed for microcloning experiments. This slice can be taken up by a microdrop and used as described in [4]. The preparation of a single chromosome slice takes about 2 min. Cutting chromosomes as shown in figs 2 and 3 into equidistant slices is desirable
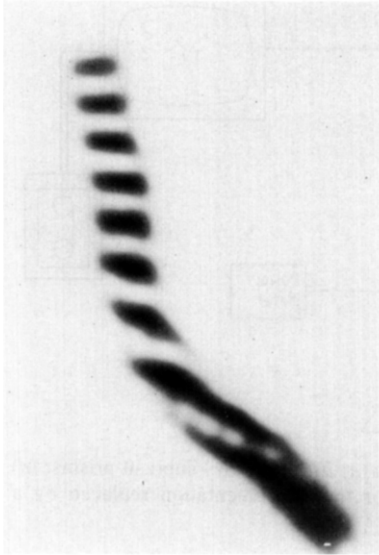
17+−868341

Fig. 2. Human lymphocyte chromosome, microdissected into nine parts by the diffraction rings of a laser microbeam.

if one is interested in a systematic analysis of a chromosome via slice-specific libraries. This approach is possible with homogeneously stained as well as with unstained chromosomes if, in the latter case, one uses a contrast enhancing video camera (e.g., the handy and low-priced Panasonic WV1600 CCD camera).

If one is interested in isolating a particular portion of a banded chromosome one has to work solely with the central disc of an attenuated beam. As a consequence of this, preparation of a single band takes some tens of minutes rather than just a few minutes. Fig. 4 shows how bands can be prepared.
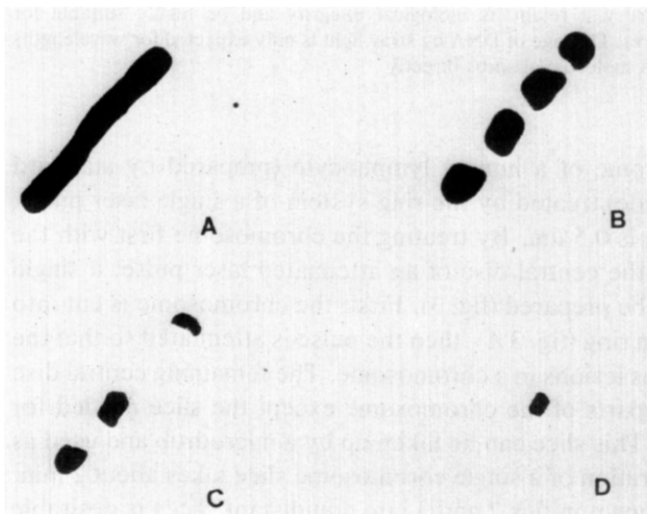

Fig. 3. Preparation of a single chromosome slice by combined use of the diffraction rings and the central disc of the laser microbeam. (*A*) Untreated; (*B*) after a single shot using diffraction rings; (*C*) part of the chromosome removed by the central disc; (*D*) isolated slice.
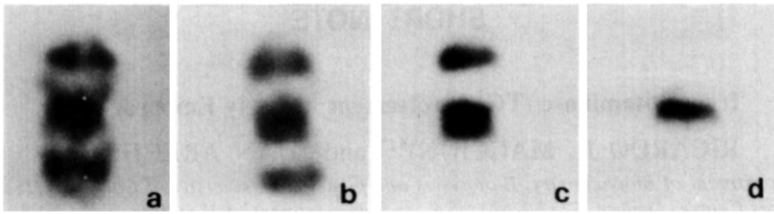
Fig. 4. Preparation of a single band from a G-banded chromosome. (*a*) Untreated chromosomes; (*b*) after first pre-cutting with the central disc of the beam; (*c*) removal of lower part of the chromosome; (*d*) isolated band in the centromeric region.

So far it has been demonstrated that microcloning is possible with pieces of unstained chromosomes [11]. However, based on present experience with sorting of chromosomes it seems likely that certain banding procedures using fluorescent dyes should not interfere with the microcloning procedure.

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